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PhD thesis

STEREOSELECTIVITY IN ASYMMETRIC PROCESSES AND MOLECULAR ASSEMBLIES

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Preface

This thesis is divided into the following chapters:

Chapter 1: *Regio- and Stereoselective Carbolithiations of 1-Aryl-1-Alkenyl N,N'-Diethylcarbamates*. In this chapter the carbolithiation reaction is described, reporting the most relevant examples present in the literature. Moreover, the results obtained by the investigation of the carbolithiation of functionalised styrene derivatives, considering 1-aryl-1-alkenyl *N,N'*-diethylcarbamates as model substrates, are presented. A particular attention is also focused on the extension of the carbolithiation reaction to the preparation of optically active products. Therefore, a synthetic methodology in order to prepare trisubstituted benzyl carbamates, generating a quaternary centre in α to oxygen, also in enantioselective manner, is described.

Chapter 2: *Asymmetric Synthesis of the Fungal Phytotoxins Colletochlorin A and Radicinin*. In this chapter the synthesis of naturally occurring bioactive compounds, such as fungal metabolites with phytotoxic activity suitable as bioherbicides against invasive weeds, is reported, with the aim to study the structure-activity relationship. In particular, the first asymmetric total synthesis of both enantiomers of the natural products colletorin A, colletochlorin A and the synthesis of their halogenated analogues in optically active form is presented. Moreover, a novel synthetic strategy for obtainment of (±)-3-deoxyradicinin, the biosynthetic precursor of the phytotoxin radicinin, is described.

Chapter 3: *Cyclochirality Emerging from Hydrogen-Bonding Networks in Cyclic Oligoureas*. In this chapter the synthesis of cyclic oligoureas based on TACN structure having the intriguing, under-explored and potentially highly valuable property of cyclochirality is reported. In this case, the cyclochirality emerges only by virtue of the presence of a cyclic hydrogen-bonding arrangement. The new class of compounds decribed here could be utilised in the future as organocatalysts in asymmetric synthesis or as enantiospecific host molecules, opening a new field of organic chemistry.

Abbreviations

°C	celsius degrees	DMF	*N*,*N*-dimethylformamide
^{13}C	carbon (NMR)	DMSO	dimethylsulfoxide
^{19}F	fluorine (NMR)	e.e.	enantiomeric excess
^{1}H	hydrogen (NMR)	NMR	nuclear magnetic resonance
Aib	aminoisobutyric acid	EtOAc	ethyl acetate
Ar	aryl	Et_2O	diethyl ether
b	broad	ECD	electronic circular dichroism
BOX	bis(oxazoline) ligands	(+)-$Eu(hfc)_3$	Europium tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate]
Bn	benzyl	Fmoc	9-Fluorenylmethyloxycarbonyl
Bu	n-butyl	h	hour
n-and *t*-BuLi	normal- and tert-butyl lithium	hexacyclen	1,4,7,10,13,16-hexaazacyclooctadecane
C	concentration	HPLC	high-performance liquid chromatography
$CHCl_3$	chloroform	HCl	hydrochloric acid
cyclen	1,4,7,10-tetraazacyclododecane	HMPA	hexamethylphosphoramide
d	doublet	Hz	hertz
DCE	1,2-dichloroethane	H_2SO_4	sulfuric acid
DCM	dichloromethane	LDA	Lithium diisopropylamide
DIPEA	*N*,*N*-diisopropylethylamine	LiTMP	Lithium tetramethylpiperidide
DMPU	*N*,*N*′-dimethylpropyleneurea	Me	methyl

GC-MS	Gas chromatography mass spettroscopy	MEM	β-Methoxyethoxymethyl ether
m	meter, multiplet, medium	MeOH	methanol
M	molar	SiO_2	silica gel
mol	mole	SO_2Cl_2	sulfuryl chloride
MOM	methoxymethyl	T	temperature
NaOMe	sodium methoxide	t	triplet
OR	optical rotation	TACN	1,4,7-triazacyclononane
PCC	pyridinium chlorochromate	*t*-Bu	tert-butyl
PE	petroleum ether	TBAF	tetra-*n*-butylammonium fluoride
Ph	phenyl	TEA	triethylamine
PhCHO	benzaldehyde	TFA	trifluoroacetic acid
pm	parts per million	THF	tetrahydrofuran
PMDTA	*N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine		
PPY	4-pyrollidinopyridine	TLC	thin layer chromatography
q	quartet	TMS	trimethylsilyl
R_f	retention factor	TMEDA	tetramethylethylenediamine
RT	room temperature	UV	ultraviolet
SEM	trimethylsilylethoxymethyl	VT-NMR	variable-temperature nuclear magnetic resonance
s	singlet	δ	chemical shift

Abstract

"The universe is dissymmetrical; for if the whole of the bodies which compose the solar system were placed before a glass moving with their individual movements, the image in the glass could not be superimposed on reality. . . . Life is dominated by dissymmetrical actions. I can foresee that all living species are primordially, in their structure, in their external forms, functions of cosmic dissymmetry."

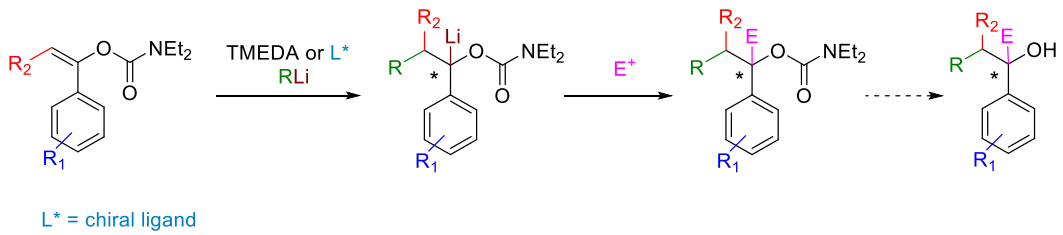
Louis Pasteur

These visionary words of Pasteur, written more than 100 years ago, have profoundly influenced the development of stereochemistry, becoming clear that many fundamental phenomena and laws of nature result from dissymmetry. In modern chemistry, an important term to describe dissymmetry is *chirality*, which is defined as the geometric property of a three-dimensional object of being non-superimposable on its mirror image. This particular property play a fundamental role both in nature and in synthetic organic chemistry since most of the biological macromolecules of living systems occur in nature in one enantiomeric form only. It is suffice to consider that the three most important and most abundant natural products, i.e. peptides, nucleic acids and carbohydrates, are all chiral molecules. In general, the different stereoisomers of any biologically active chiral compound interact with receptors, transport proteins, membranes etc. of living organisms in a chiral manner. Thus, it is not surprising that the enantiomers of a drug, interacting differently with their receptor site, may lead to different effects. Consequently, it is very important to keep the idea of chiral discrimination in mind when designing the synthesis of biologically active molecules.

Since chirality is such an important feature of organic chemistry, this thesis was aimed to explore different aspects of chirality, spanning from the stereochemical aspects of the

synthetic processes to novel and unusual type of chirality like the cyclochirality due to hydrogen bonding networks in molecular assemblies.

In particular, a methodological study on the carbolithiation reaction, namely the *syn* addition of organolithium compounds to a double or triple bonds, was carried out in order to synthesise trisubstituted benzyl carbamates, which are direct precursors of tertiary benzylic alcohols, common structural subunits in naturally occurring bioactive compounds. An enantioselective version of this transformation was also investigated, employing chiral ligands with the aim to provide asymmetric induction, which allows the preparation of enantioenriched tertiary benzyl carbamates. The stereoselectivity of the tandem one-pot carbolithiation-electrophile trapping process of 1-aryl-1-alkenyl *N*,*N'*-diethylcarbamates, as model substrates, was studied, as well as the influence of substituents on the aromatic ring of starting materials subjected to the carbolithiation step (Scheme I).



Scheme I. Carbolithiation-trapping process of 1-aryl-1-alkenyl *N*,*N'*-diethylcarbamates.

As previously mentioned, the stereochemistry is a fundamental concept that needs to be considered in the study of the structure-activity relationship of bioactive compounds. This topic was explored in the asymmetric synthesis of naturally occurring fungal metabolites, which were found to possess phytotoxic activity. This biological property made such compounds suitable candidates as natural herbicides for the control of invasive weeds.

The first asymmetric total synthesis of both enantiomers of the natural products colletorin A, colletochlorin A and the synthesis of their halogenated analogues in optically active form was described (Figure I). Moreover, a novel synthetic strategy for the preparation of the chiral natural compound (±)-3-deoxyradicinin, the biosynthetic precursor of the phytotoxin radicinin, was reported (Figure II).

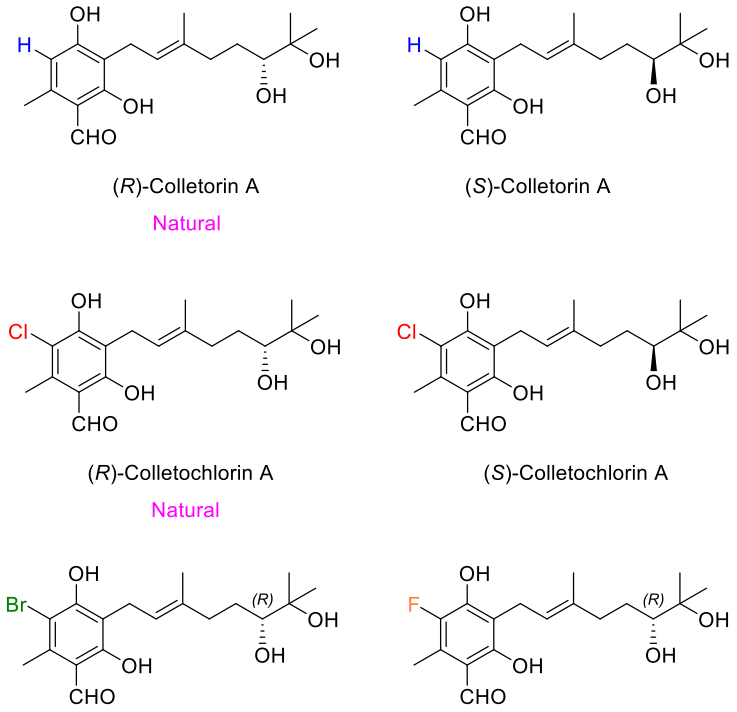


Figure I. Structure of both enantiomers of colletorin A and colletochlorin A and the unnatural halogenated analogues, prepared in optically active form.

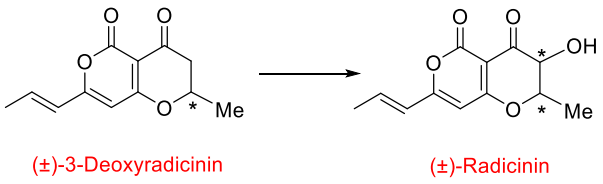


Figure II. Stuctures of (±)-3-deoxyradicinin and (±)-radicinin.

Interestingly, the poorly explored concept of cyclochirality was also investigated. Cyclochirality was defined as an isomerism arising when cyclic arrangements of stereocenters are associated with ring systems. In this thesis, such intriguing property was

evaluated by the construction of a novel conformational motif obtained by synthesising cyclic oligoureas based on TACN structure (Figure III). It is noteworthy to highlight that in these kind of molecules, the cyclochirality emerges only by virtue of the presence of cyclic intramolecular hydrogen-bonding network, whose establishment was detected by means of NMR experiments. This new class of compounds could be utilised in the future as organocatalysts in asymmetric synthesis or as enantiospecific host molecules, opening a new field of organic chemistry.

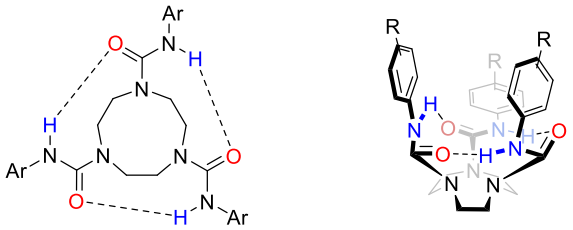


Figure III. Cyclochiral triureas based on TACN structure.

Chapter 1

Regio- and Stereoselective Carbolithiations of 1-Aryl-1-Alkenyl N,N'-Diethylcarbamates

1.1. Introduction

1.1.1. Organolithium Compounds

Organolithium reagents are organometallic compounds containing a direct carbon-lithium bond. Lithium is strongly electropositive and therefore the electron density of the bond is mostly localised on the carbon atom, effectively creating a carbanion. Due to the polar nature of the C-Li bond, organolithium reagents are both good nucleophiles and strong bases. These compounds are much more reactive than the Grignard reagents and, for this reason, they are incompatible with water, oxygen and carbon dioxide and must be handled in an atmosphere of nitrogen or argon. Organolithiums, except methyllithium and phenyllithium, are also remarkably soluble in hydrocarbon solvents.[1] Indeed, simple organolithiums used as starting materials are readily available in the form of stable hydrocarbon solutions. On the contrary, methyllithium and phenylithium are stable at RT in the presence of ethers. Hydrocarbon solutions of *n*-BuLi, *s*-BuLi and *t*-BuLi constitute the main source for most organolithiums, while other bases are widely used to generate organolithiums, from more acidic substrates. Among these, there are LDA, LiTMP, other more hindered lithium amide bases and hindered aryl lithiums such as mesityl lithium and triisopropylphenyl lithium.

Organolithium reagents are present in solution as aggregate structures, where lithium is coordinated to more than one carbon atom, and carbon is coordinated to more than one lithium atom.[2] The electron-deficient lithium atom of an organolithium compound requires greater stabilisation than can be provided by a single carbanionic ligand, and freezing-point measurements indicate that in hydrocarbon solution organolithiums are invariably aggregated as hexamers, tetramers or dimers.[3] Aggregation is influenced by three main

factors: the electrostatic interaction between opposite charges, the composition of the coordination sphere of lithium that can contain solvent molecules or Lewis bases, and the steric hindrance of the hydrocarbon part.[4]

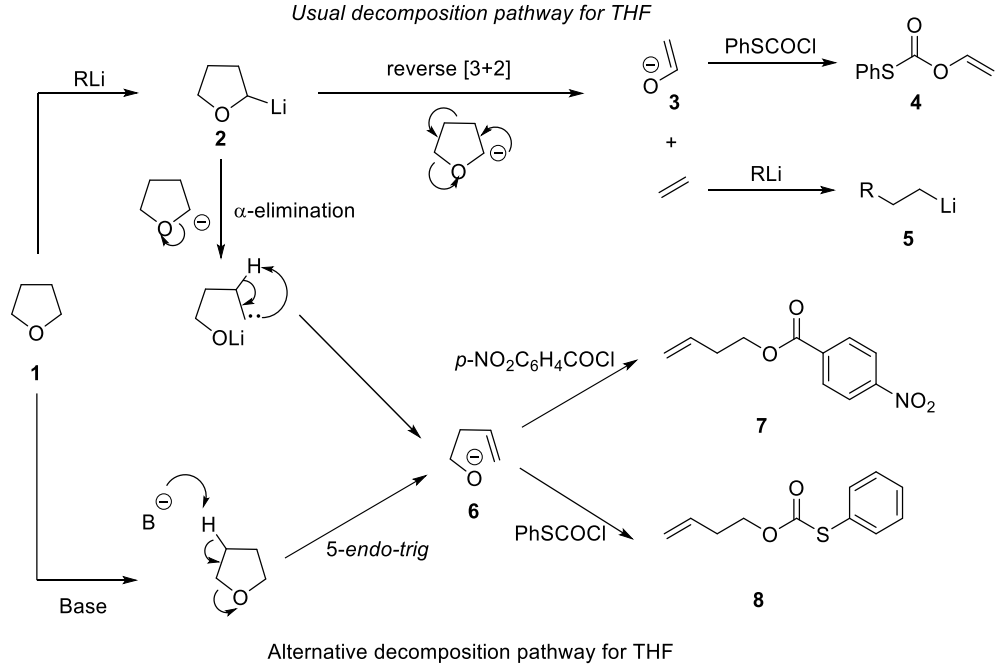
Primary organolithiums are hexamers in hydrocarbons, except when they are branched β to the lithium atom, when they are tetramers. Secondary and tertiary organolithiums are tetramers, while benzyllithium and very bulky alkyllithiums are dimers.[1,3]

Coordinating ligands, such as ethers or amines, or even metal alkoxides, can provide an alternative source of electron density for the electron-deficient lithium atoms.

These ligands can stabilise the aggregates by means of the coordination to the lithium atoms, causing a decrease in the degree of aggregation. The presence of ethers typically reduces the aggregation state, but only occasionally leads to a complete deaggregation to the monomer. Methyllithium, ethyllithium and butyllithium remain tetramers in Et_2O, THF,[5] or dimethoxyethane (DME),[6] with some dimer forming at low temperatures;[7] *t*-BuLi becomes dimeric in Et_2O and monomeric in THF at low temperatures.[8] In the presence of TMEDA alone, however, *s*-BuLi remains a tetramer.[9] Coordinating solvents also greatly increase the reactivity of the organolithiums due to deaggregation, and an ether or an amine solvent is essential in almost all organolithium reactions. The most important coordinating solvents commonly employed in organolithium reactions are HMPA, PMDTA, (-)-sparteine, DMPU, DME, TMEDA, THF, *t*-BuOMe e Et_2O, listed in an approximate empirical ordering of decreasing activating power.

However, the aforementioned solvents can show a certain tendency to react with organolithiums, precluding their use at temperatures above ambient, and in some cases limiting them to 0 °C or below. For example, Et_2O is stable over a period of days in the presence of *n*-BuLi at RT, but THF (**1**) is readily decomposed by organolithiums at this temperature by reverse cycloaddition of the anion derived from **2** (Scheme 1.1).[10] The products of this decomposition, ethylene and the lithium enolate of acetaldehyde **3**, can be trapped. For example, acylation with phenylthiochloroformate of a solution of *n*-BuLi in THF gives good yields of the thiocarbonate **4** and carbolithiation of ethylene affords products **5**, arising from secondary and tertiary organolithiums. THF occasionally decomposes by an alternative mechanism to give but-3-en-1-oxide (**6**), which can react with with *p*-nitrobenzoyl chloride to yield compound **7**[11] or with phenylthiochloroformate, in the

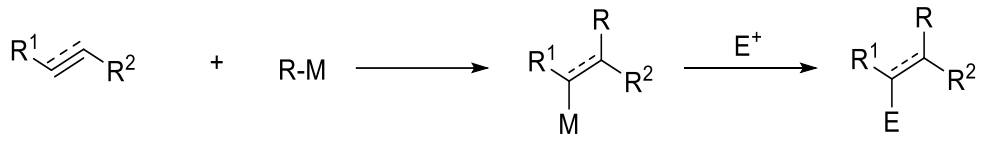
presence of HMPA, to give **8**.[12] On the contrary, *s*-BuLi and *t*-BuLi decompose ethereal solvents much more rapidly than *n*-BuLi.[13] Generally, a temperature increase of 20 °C shortens the lifetime of an organolithium in ethereal solvents by a factor of 10. Therefore, *n*-BuLi turns out to be stable in THF for 24 h at about 0 °C (–15 °C if TMEDA is present), whilst *t*-BuLi is stable in THF for 24 h only at –50 °C or below. Concentrated solutions of *n*-BuLi undergo slow β-elimination to give 1-butene and LiH. *t*-Butyl methyl ether is a less widely used as alternative to Et_2O offering better solubility for some compounds. The common cosolvents TMEDA, DMPU and HMPA are all susceptible to decomposition on extended exposure to organolithiums. HMPA freezes at +7 °C and is a suspected carcinogen, but as a cosolvent in THF, at low temperature, it can have profound effects on organolithium reactivity. Toluene is liquid to a much lower temperature, but it is slowly lithiated by alkyllithiums.[10]



Scheme 1.1. Decomposition of THF in the presence of alkyllithium (RLi).

1.1.2. Carbolithiation Reaction

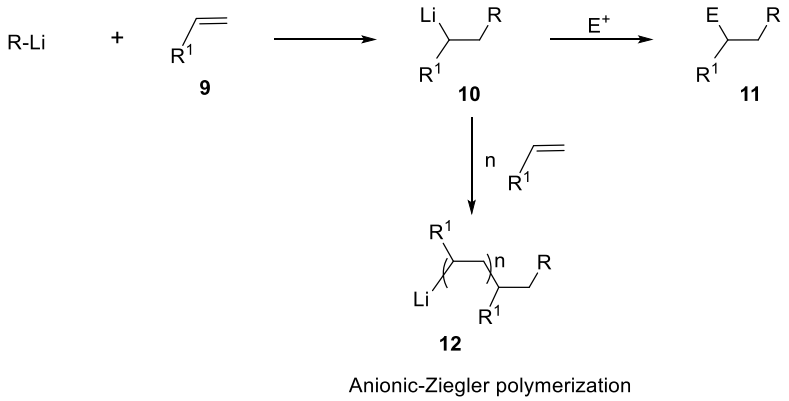
Since the discovery of organomagnesium reagents by Barbier and Grignard, organometallics have been one of the most versatile reagents for the carbon-carbon bond formation. Among the several reactions of organometallic nucleophiles with polar carbon electrophiles the carbometalation reaction, i.e. the addition of organometallic reagents to a C-C double or triple bond, is particularly appealing, leading to the simultaneous formation of a new C-C bond and a new organometallic compound which may give rise to further synthetic transformations. Moreover, if the carbometallation is performed on a 1,2-disubstituted double bond, two regioisomers can be obtained and, after reaction with a suitable electrophile, two contiguous stereogenic centres can be created (Scheme 1.2).[14,15]



Scheme 1.2. Carbometalation reaction.

A subset of the broader family of carbometalations is carbolithiation, i.e. the addition of organolithiums to unsaturated compounds. This reaction displays considerable interest in synthetic organic chemistry, providing an attractive pathway for the efficient construction of new C-C bonds by addition of an organolithium reagent to non-activated alkenes or alkynes, also offering the possibility of introducing further functionalisation on the molecule by trapping the reactive organolithium intermediates with electrophiles.[16,17] The organolithium addition to unsaturated substrates is a thermodynamically driven process, which proceeds if the newly formed organolithium is more stable than the starting organometallic reagent. For this reason, alkyllithiums are the most reactive nucleophiles, while more stable aryl-, vinyl, and methyl lithium derivatives are poorly reactive. A potential limitation to the application of carbolithiation reaction as synthetic method derives from the difficulty to control the high reactivity of the organolithium intermediate towards the unsaturated starting substrate. Indeed, the lithiated intermediate can react with a second molecule of the olefinic substrate, triggering an anionic polymerisation process. In fact, the

intermolecular nucleophilic addition of alkyllithium to an alkene represents the first step in anionic polymerisation and *n*-BuLi is commonly used as catalyst to start the polymerisation of styrene, butadiene and isoprene.[18] It follows that, for achieve a synthetically useful carbolithiation, reaction conditions are required to facilitate the initial carbolithiation but not to favour further addition by the generated organolithium. In other words, the new organometallic **10** produced in the addition must be less reactive than the starting organolithium (R-Li) in order to avoid further addition to the starting olefin (Scheme 1.3).[14,19,20]

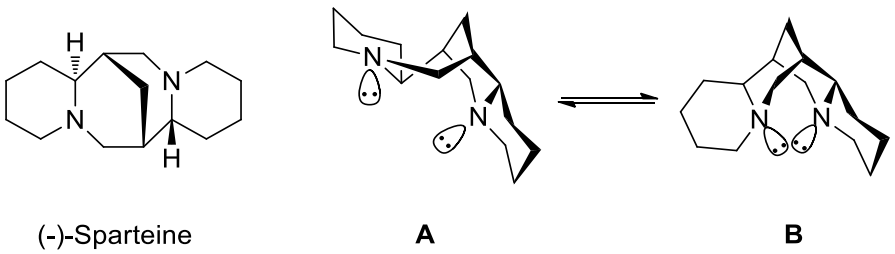


Scheme 1.3. Carbolithiation reaction.

In order to stop the double bond addition at the first step, avoiding the formation of polymers, a special stabilisation of the organolithium intermediate **10** is required. Such a stabilisation can be obtained by *intra* or *inte*r-molecular organolithium coordination processes which leads to anion stabilisation thus preventing the polymerisation and, conversely, promoting carbolithiation. This coordination can occur in the presence of coordinating groups bearing heteroatom Lewis-base moieties proximal to the reacting alkene, which can give rise to pre-coordination of the alkyllithium on the olefinic substrate. This phenomenon is termed *Complex-Induced Proximity Effect* (CIPE), a process in which a pre-complexation step between the reagents, usually a heteroatom and an organometallic, leads to the formation of unexpected kinetic products, instead of the apparently available thermodynamic alternatives, or also induces a dramatic acceleration of normally unfavourable reactions. It must be to emphasize that unusual or unexpected products attributable to a CIPE process appear to arise

in a wide variety of reactions of organolithium compounds.[21] Indeed, this concept is also used to explain the mechanism of the deprotonation reactions using alkyllithium reagents and several applications of CIPE to synthetic goals have been particularly well-developed for directed *ortho*-metalation (D*o*M) methodologies.[22]

The carbolithiation reaction can also be facilitated by the presence of coordinating ligands such as tertiary diamines, in particular TMEDA, which enhance the reactivity of organolithium derivatives by reducing their aggregation (*vide supra*).[10] Moreover, the use of diamines has favored the development of methods to make this reaction also stereoselective. Indeed, the presence of a chiral diamine ligand coordinating the organolithium intermediate, promotes an asymmetric carbolithiation. Among these, (–)-sparteine has proven to be the most powerful promoter of asymmetric carbolithiation and it has been also the most widely used chiral ligand to control the enantioselectivity of organolithium reactions.[23] (–)-Sparteine exists predominantly in the **A** conformation, but the **B** conformation, which is only slightly higher in energy, is the favorite one when it acts as bidentate ligand, coordinating metallic ions (Figure 1.1). This alkaloid is readily available and it can be isolated in significant quantities from the seeds of a variety of legumes.[10,24]



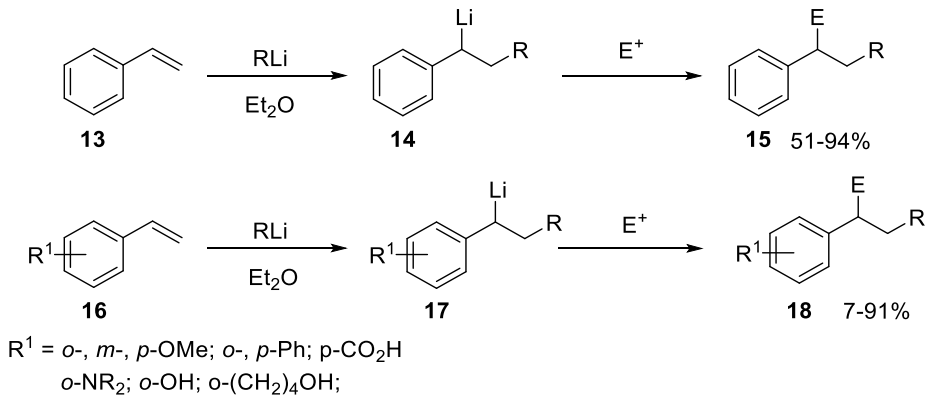
(-)-Sparteine **A** **B**

Figure 1.1. (–)-Sparteine and its conformations.

In the following paragraphs the most representative and relevant examples of the intermolecular carbolithiation processes were described, considering the main substrates employed divided in unfunctionalised and functionalised aryl alkenes.

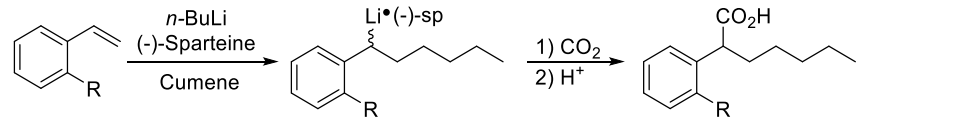
1.1.2.1. Carbolithiation of Unfunctionalised Aryl Alkenes

Styrene (**13**) represents the simplest aryl alkene useful as substrate for carbolithiation reactions. Styrene undergoes facile polymerisation in THF, even at −78 °C, when it reacts with alkyllithium,[25] while the reaction is smoothly controllable in diethyl ether, leading to the stabilised benzylic organolithium (**14**) and then to **15** in good yields. Such difference in reactivity indicates that in Et_2O the resulting lithiated intermediate (**14**) is sufficiently stabilised to minimize the polymerisation, whereas in THF it is not. Taylor and co-workers[26,27] reported that styrene (**13**) and several aryl-substituted styrene derivatives (**16**) undergo efficient tandem carbolithiation-trapping reactions in Et_2O at −78 °C or at −25 °C (Scheme 1.4). In particular, *t*-BuLi and *s*-BuLi react with most of the aryl-substituted styrene derivatives at −78 °C, but primary alkyllithium reagents usually need higher temperatures (ca. −25 °C). Therefore, the reactivity of different types of alkyllithium reagents was found to be: tertiary, secondary > primary >> alkenyl, methyl, phenyl. As expected, electron-donating groups, such as methoxy and dialkylamino, at the *ortho*- or *para*-positions of the benzene ring, deactivate the double bond towards alkyllithium addition, thus slowing down the reaction. However, in these cases, their reactions with *n*-BuLi can be facilitated by using TMEDA as co-solvent, which activate the alkyllithium for an efficient transformation.



Scheme 1.4. Carbolithiation-trapping reaction of styrene (**13**) and aryl-substituted styrenes (**16**).

The alkyllithium addition to styrene (**13**) and its *o*-substituted derivatives (**16**), followed by electrophilic trapping, has been investigated in the enantioselective version in the presence of (-)-sparteine (Scheme 1.5).[28] A low enantiomeric excess (up to 30 %) was observed with styrene at −78 °C in cumene as solvent. Among the styrene derivatives, the best result (72 % e.e.) was obtained using 2-methoxystyrene (**16**) in cumene at −95 °C. In both cases 2 equivalent of the chiral diamine and CO_2 as electrophile were used. Probably, the presence of an auxiliary binding site in 2-methoxy-substituted systems enables to stabilize the benzyllithium intermediate (**19**) by coordination, resulting in a greater stereoselectivity.



13 R= H
16 where R= OMe

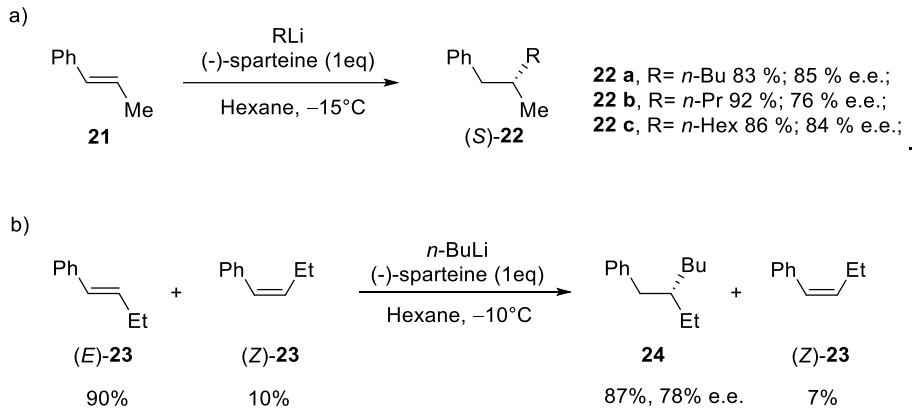
19

20a R= H; 30% e.e.; 76% yield
20b R= OMe; 72% e.e.; 58% yield

Scheme 1.5. Enantioselective carbolithiation-trapping reaction of styrene (**13**) and *o*-substituted styrenes (**16**).

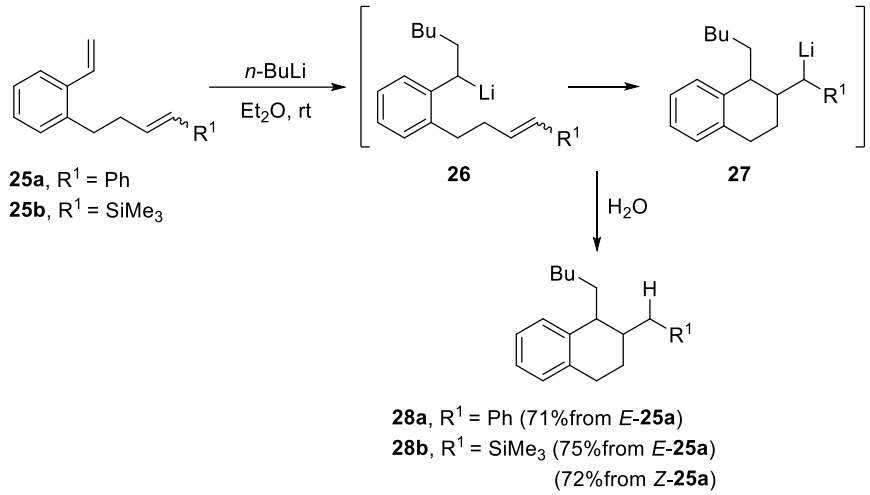
The enantioselective carbolithiation of β-substituted non-functionalised styrenes was also investigated by Normant, Marek and co-workers (Scheme 1.6).[29,30] Hence, the addition of various alkyl lithium to (*E*)-β-methyl styrene (**21**) in hexane and in the presence of (-)-sparteine at −15 °C led, after hydrolysis, to the corresponding alkylated products (**22a-c**) in good yield and good enantioselectivity (76-85 % e.e.) without polymerisation (Scheme 1.6a). A slightly lower 70 % e.e. was obtained for **22a** when the reaction was performed in the presence of a catalytic amount of (-)-sparteine (10 %) at 0 °C. As shown in Scheme 1.6b, the stereochemistry of the olefin is crucial for the enantioselectivity of the carbolithiation. Indeed, whereas the carbolithiation of the (*E*)-β-methyl styrene (**21**) gave the (*S*)-alkylated product in 4 h at −15 °C, the same reaction on the (*Z*)-β-methyl styrene led to the opposite enantiomer (*R*) with a lower e.e. (28 %) in 50 % yield after 8 h at 0 °C. Since the addition of alkyllithium on the (*Z*)-isomer differs notably in rate from the one on the (*E*)-isomer, a kinetic resolution of other β-alkylated styrene derivatives, such as β-ethyl styrene (**23**), was possible. Therefore, carbolithiation with *n*-BuLi of **23** in *E*/*Z* ratio of 90/10, after hydrolysis,

led to the alkylated derivative (**24**), in 87 % yield and 78 % e.e., while the (*Z*)-isomer was recovered intact (Scheme 1.6b).

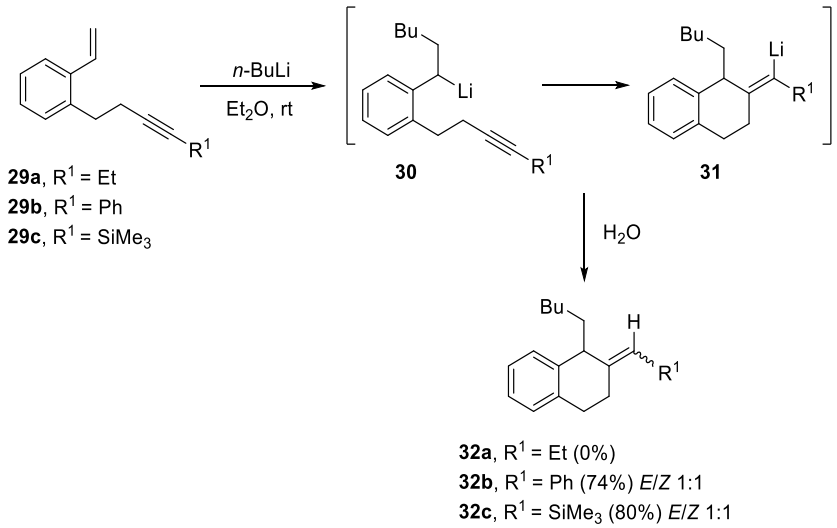


Scheme 1.6. Enantioselective carbolithiation of β-substituted non-functionalised styrenes (**21** and **23**).

Further investigation have shown that styrenes bearing unsaturated alkene or alkyne side chains at the *o*-position (**25** or **29**) undergo regioselective carbolithiation on the styrene unit, followed by *6-exo-trig* (Scheme 1.7) or *6-exo-dig* (Scheme 1.8) cyclisation to produce 1,2-disubstituted tetralins (**28**) or their unsaturated analogues (**32**) respectively.[31] The whole strategy represents an example of tandem intermolecular/intramolecular carbolithiation process, which can also be stereoselective in the case of the corresponding vinylsilanes *E*-**25b** and *Z*-**25b**, where only the 1,2-*trans*-isomer of tetralin **28b** was isolated regardless of which isomer of the silane was employed. Instead, the cyclisation did not occur using **29a** as starting material indicating that 6-*exo*-cyclisation is not facile with an unactivated alkyne.



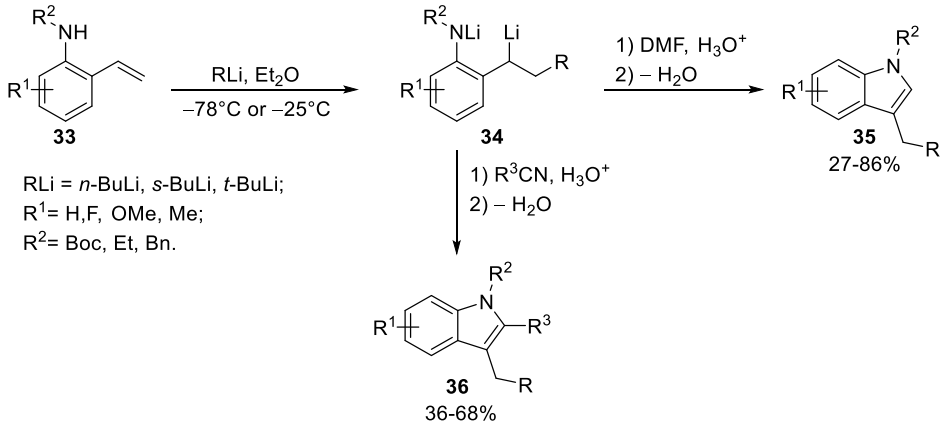
Scheme 1.7. Regioselective carbolithiation of styrenes bearing unsaturated alkene side chains at the *o*-position (**25**) followed by *6-exo-trig* cyclisation.



Scheme 1.8. Regioselective carbolithiation of styrenes bearing unsaturated alkyne side chains at the *o*-position (**29**) followed by *6-exo-dig* cyclisation.

The intermolecular carbolithiation reactions of the *o*-aminostyrenes (**33**) resulted to be efficient and, interestingly, allowed the preparation of functionalised indole ring systems (**35** and **36**) by means of a one-pot process.[32,33] This methodology involves a cascade reaction

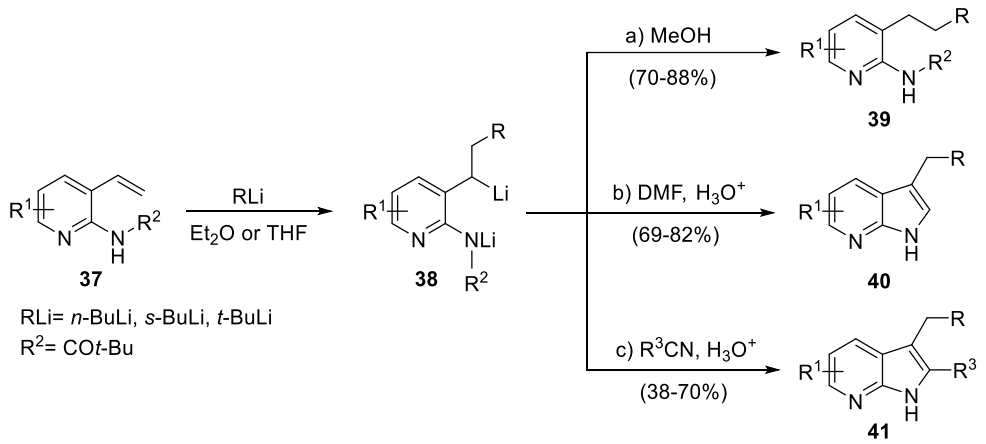
sequence formed by initial NH deprotonation, alkyllithium addition to the styrene double bond and subsequent trapping of the organolithium intermediate with a suitable electrophile able to generate a carbonyl moiety, followed by an *in situ* ring closure and dehydration to form the indole ring (Scheme 1.9). This is an efficient and versatile method because it allows to introduce diverse substituents at all positions on the indole scaffold. Indeed, the procedure was shown to be successful with a range of both C and N substituents on the *o*-aminostyrenes and it was, moreover, tolerant to the reactivity range of alkyllithiums such as *t*-, *s*-, and *n*-BuLi. The electrophiles used were DMF, which generated indole products unsubstituted at C-2 (**35**), and nitriles, which allowed to incorporate the nitrile substituent at C-2 position of the indole (**36**) (Scheme 1.9).



Scheme 1.9. Carbolithiation-trapping reactions of *o*-aminostyrenes (**33**).

The same procedure was extended to the synthesis of 7-azaindoles starting from 3-vinylpyridin-2-ylamines (**37**), which undergo efficient carbolithiation/protonation reaction (Scheme 1.10). As mentioned earlier, the methodology involves a reaction sequence consisting of a controlled carbolithiation of the vinyl double bond, subsequent trapping of the formal di-anion intermediate with a suitable electrophile, again DMF and substituted nitriles, followed by an *in situ* ring closure and dehydration (Scheme 1.10).[34] The reaction sequence allows for aryl, heteroaryl, alkyl and keto substituents to be included at different positions around the heterocycle. This reaction sequence proved to be tolerant for different

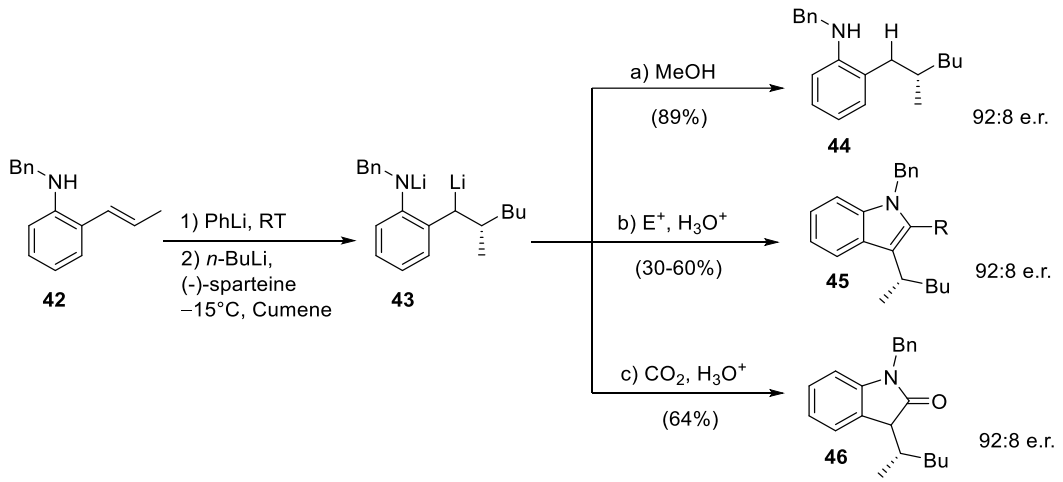
alkyllithiums (*t*-, *s*-, or *n*-butyl), leading to the preparation of the 7-azaindoles (**40** and **41**) in good to excellent yields (69-82%) when DMF was used as electrophile (pathway b), and in moderate to good yields (38-70%) using nitriles, which again allowed for the introduction of functional group diversity at C-2 position of the azaindole scaffold (pathway c).



Scheme 1.10. Carbolithiation/protonation reaction of 3-vinylpyridin-2-ylamines (**37**).

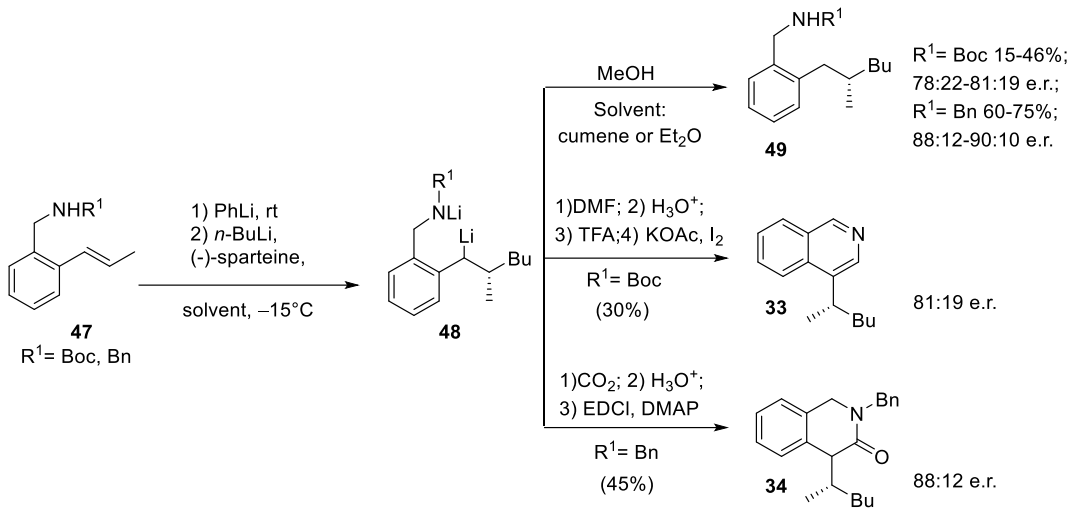
The procedure described above was also applied to *o*-substituted β-methylstyrenes (**42**) and in an enantioselective version. The enantioselective carbolithiation of (*E*)-2-propenylarylamines (**42**), mediated by (-)-sparteine, allowed to generate chiral lithiated intermediates (**43**) which have wide synthetic potential (Scheme 1.11).[35] Indeed, these intermediates (**43**) can undergo a series of further *in situ* transformations, reacting with electrophiles to afford a collection of products each containing a common stereogenic centre. The stereogenic centre, formed in high enantiomeric ratio in the first carbolithiation step, is carried through the cascade reaction sequence to the final products (**44**, **45** and **46**) and it is independent of electrophile used. This methodology provided access to the synthesis of structurally diverse chiral anilines (**44**), indoles (**45**), and indolones (**46**) all with an e.r. of 92:8. The heterocyclic syntheses involve an enantioselective alkene carbolithiation and subsequent trapping of the intermediate organolithium with a suitable electrophile, followed by an *in situ* ring closure and dehydration to generate the indole or indolone rings. Moreover, a study of the quantity of (-)-sparteine additive employed revealed its crucial role in gaining

selectivity. The best results were obtained when an equal ratio of organolithium (PhLi + *n*-BuLi) to (-)-sparteine was used.[36] After the carbolithiation step, the organolithium intermediate formed (**43**) was reacted with MeOH or other several electrophiles (E^+ = DMF, 2,2-diethoxypropionitrile and γ-butyrolactone) leading to chiral aniline (**44**) or indole (**45**), respectively. If CO_2 is used as electrophile, it is also possible to obtain a chiral indolone (**46**).

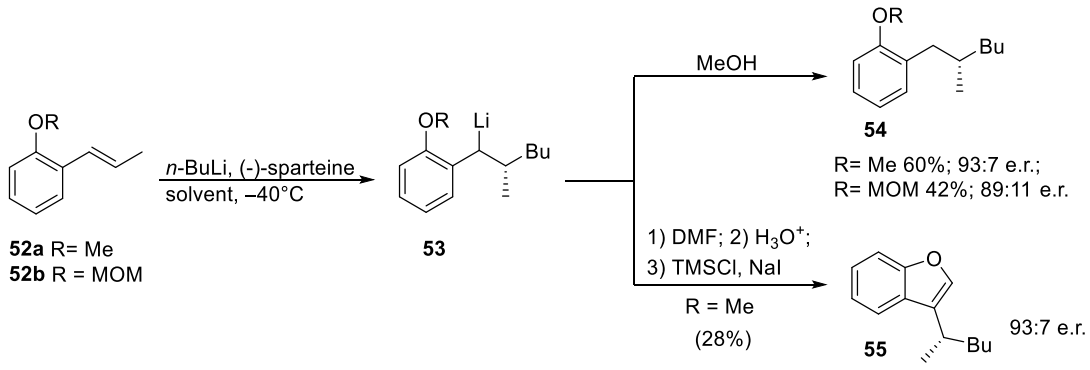


Scheme 1.11. Enantioselective carbolithiation of (*E*)-2-propenylarylamines (**42**) (for E^+ in route B see text).

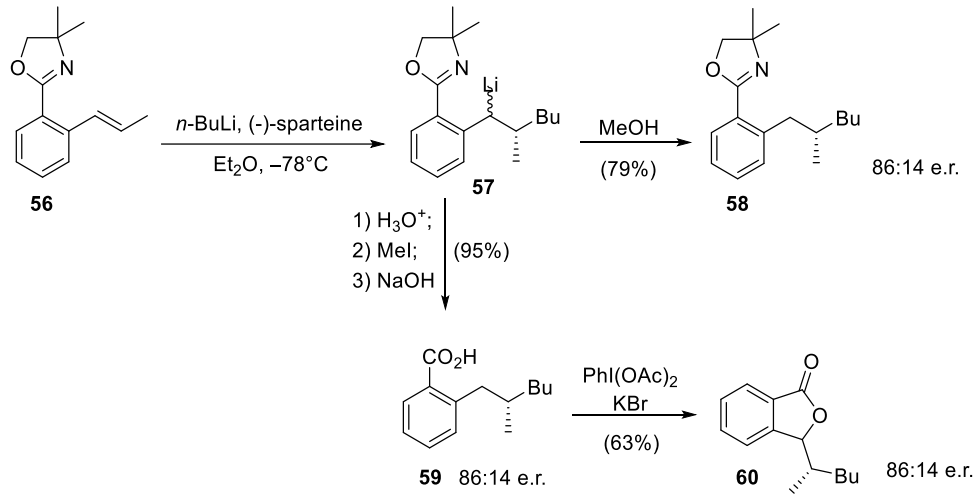
The same protocol allowed an easy access to four further important heterocyclic classes, namely the isoquinolines (**50**) (Scheme 1.12), isoquinolinones (**51**) (Scheme 1.12), benzofurans (**55**) (Scheme 1.13), and isobenzofuranones (**60**) (Scheme 1.14), starting from the corresponding prochiral β-methylstyrene substrates containing *o*-aminomethyl (**47**), ether (**52**) and oxazoline (**56**) substituents, respectively. As the chiral centre, generated during the C-C bond formation in the first carbolithiation step, is carried through the electrophile reaction sequence to the final products, the enantioselectivity is solely dependent upon the alkyllithium addition.[37] Moreover, this approach was shown to be tolerant of several alkyllithium and both electron donating and withdrawing substituents on the aryl ring.[35,36] It is noteworthy that the *o*-methoxy-substituted β-methylstyrene (**52a**) gave a better selectivity (94:6 e.r.) and yield (60%) than the MOM-substituted (**52b**), which would be considered a stronger coordinating group (Scheme 1.13).[37]



Scheme 1.12. Synthesis of isoquinolines (**50**) and isoquinolinones (**51**) by using carbolithiation-trapping-cyclisation sequence.

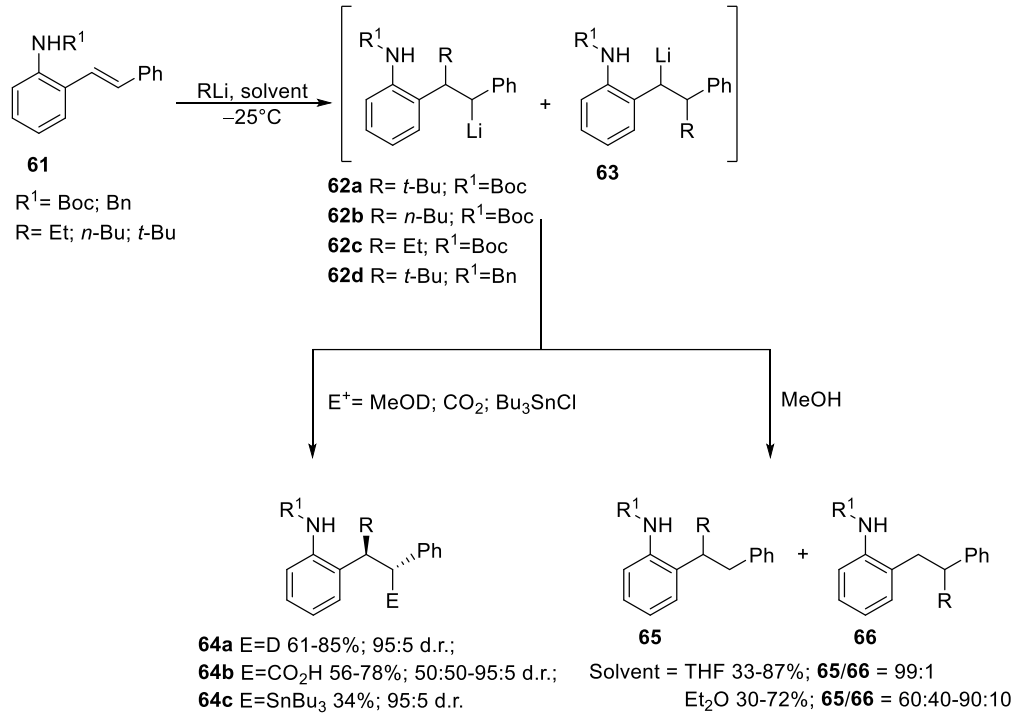


Scheme 1.13. Synthesis of benzofurans (**55**) by using carbolithiation-trapping-cyclisation sequence.



Scheme 1.14. Synthesis of isobenzofuranones (**60**) by using carbolithiation-trapping-cyclisation sequence.

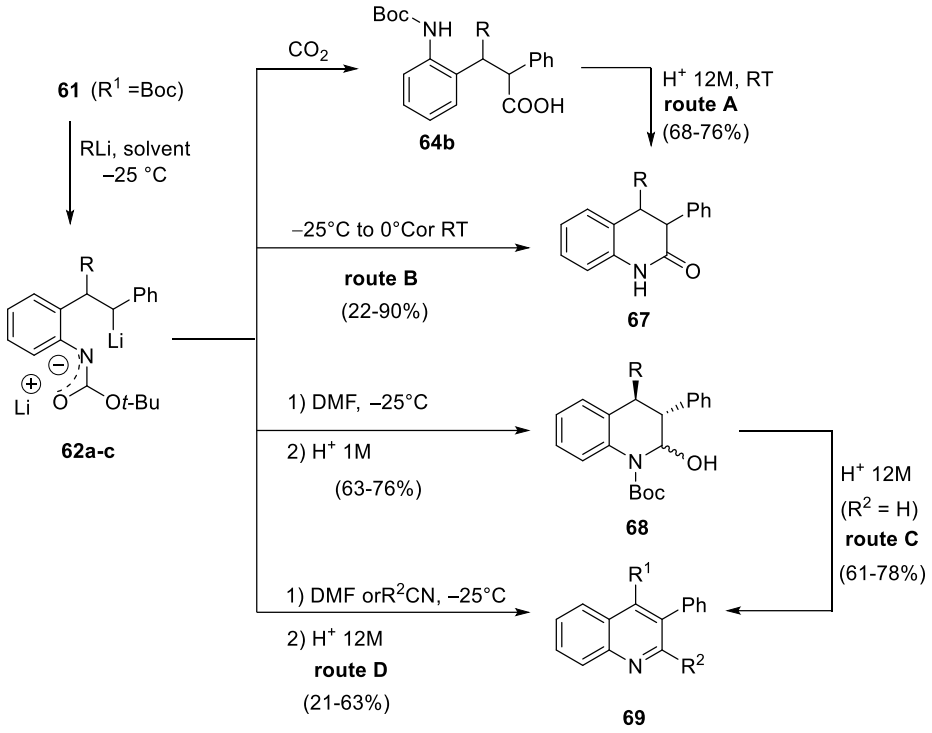
As previously shown, carbolithiation of styrenes and β-alkylstyrenes is regiospecific with the more stabilised benzylic lithiated regioisomer generated exclusively in each case. In contrast, by addition of alkyllithium to unsymmetrical stilbenes two different benzyllithium intermediates could be formed. However, interestingly, the carbolithiation of *o*-amino substituted (*E*)-stilbenes (**61**) performed in THF led to a single regioisomer in which the lithiated carbon is α to the phenyl ring (**62**). Instead, the use of other solvents, such as diethyl ether, led to a pronounced loss in regioselectivity, giving to mixtures of both possible regioisomers (Scheme 1.15).[38,39] Hence, in this case, the regiochemistry can be controlled by using the appropriate solvent. Furthermore, the diastereoselectivity of carbolithiation/electrophile substitution sequence was also investigated (Scheme 1.15).[39] Indeed, the carbolithiation in THF of unsymmetrical 1,2-disubstituted alkenes, such as stilbenes **61**, generated an organolithium species (**62**) with two contiguous stereocentres, which can be trapped with several electrophiles, leading to two possible diastereomers. In the specific case, high levels of diastereoselectivity have been obtained in THF using different electrophiles such as MeOD, CO_2, and Bu_3SnCl, with the major diastereomer being the one in which the electrophile and alkyl group are *anti* to each other (**64**).



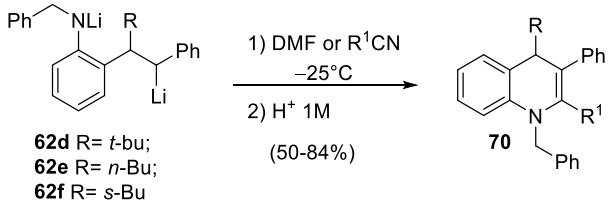
Scheme 1.15. Carbolithiation of *o*-amino substituted (*E*)-stilbenes (**61**).

Futhermore, the regioselective carbolithiation of substituted *o*-amino-(*E*)-stilbenes (**61**) was exploited in a cascade methodology involving a regiospecific alkyllithium addition to the alkene, subsequent trapping of the organolithium intermediate with a suitable electrophile, followed by an *in situ* ring closure and dehydration to provide the quinoline scaffold (Scheme 1.16).[38,39] By using this method it was possible to synthesize substituted 3,4-dihydro-quinolin-2-ones (**67**), 1,2,3,4-tetrahydroquinolines (**68**), di- or tri-substituted quinolines (**69**), and di- or tri-substituted *N*-benzyl- 1,4-dihydroquinolines (**70**) (Scheme 1.17). It was found that the treatment of the acyclic carboxylic acids **64b** with aqueous 12 M HCl led to removal of the Boc group and intramolecular cyclisation, allowing isolation of 3,4-dihydro-1*H*-quinolin-2-ones (**67**) (Scheme 1.16, route A). The same product (**67**) can be obtained by raising the reaction temperature to either 0 °C or RT, after the carbolithition step at –25°C (Scheme 1.16, route B). Indeed, the increasing in temperature causes an intramolecular cyclisation, due to an intramolecular acyl nucleophilic substitution of the benzylic lithium centre at the Boc group. In both cases, the predominant isomer was the 3,4-

trans. The treatment of lithiated intermediates with DMF followed by acidification with aqueous acid provided a versatile synthesis of the 1,2,3,4-tetrasubstituted-tetrahydroquinolines (**68**), isolated as mixture of the two diastereomeric products, 2,3-*cis*-3,4-*trans* and 2,3-*trans*-3,4-*trans* (Scheme 1.16, route C). Dehydration and *in situ* aromatisation of **68**, where R= Et or *n*-Bu, by treatment with aqueous HCl (12 M) allowed the synthesis of 3,4-disubstituted quinoline rings (**69**, R^2=H). Surprisingly, the *t*-Bu substituted analogue (**68**, R=*t*-Bu) yielded only the mono-substituted 3-phenylquinoline (**69**, R^1=R^2=H), indicating that the aromatic ring was generated by loss of the *t*-Bu group. The same result was also obtained when milder acidification conditions were employed (5 M HCl in THF). Compounds **69** could also be prepared directly from lithiated intermediates **62a-c** without the isolation of **68**, using the electrophiles DMF or nitriles, which also provide the opportunity to introduce another substituent on the quinoline ring (R^2) (Scheme 1.16, route D). Moreover, the reaction of *N*-benzyl substituted lithiated intermediates **62d-f** with DMF or nitriles as electrophiles provided a direct entry into the 1,4-dihydroquinoline class (**70**) (Scheme 1.17).[38]



Scheme 1.16. Trapping of the organolithium intermediate derived from carbolithiation of *o*-amino-(*E*)-stilbenes (**61**) with a suitable electrophile, followed by an *in situ* ring closure and dehydration to provide the quinoline scaffold (**69**).

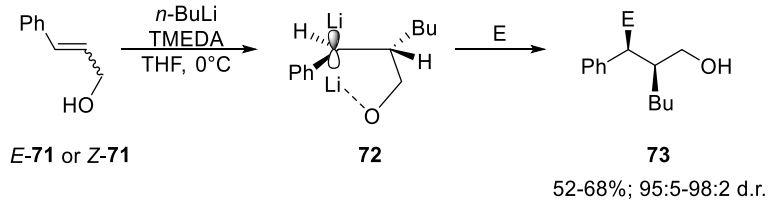


Scheme 1.17. Reaction of *N*-benzyl substituted lithiated intermediates **62d-f** with the electrophiles DMF or nitriles.

1.1.2.2. Carbolithiation of Functionalised Aryl Alkenes

Among the functionalised aryl alkenes, the most representative examples were described, considering cinnamyl derivatives, aryl-vinyl carbamates, aryl-vinyl ureas and naphthalene derivatives as substrates prone to undergo carbolithiation reaction.

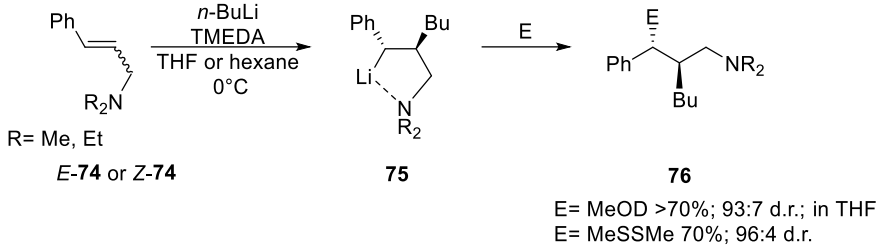
Carbolithiation reaction of cinnamyl alcohol (**71**) (Scheme 1.18) was reported for the first time in 1990 by Kuwajima and coworkers.[40] The addition of *n*-BuLi was followed by electrophilic substitution using several electrophiles. The whole tandem reaction was found to proceed in highly diastereoselective manner, with the preferential formation of the *syn*-isomer in every investigated case. Surprisingly both (*E*)-**71** and (*Z*)-**71** olefin substrates generated the same *syn*-product, showing that the selectivity is not affected by the stereochemistry of the starting alkene. In order to rationalize the *syn*-selectivity the authors supposed the formation of two five-membered cyclic benzyllithium intermediate species (**72**) having a sp^2-like carbon to which two lithium atoms coordinate from both upper and lower sites. Among these, dilithium intermediate **72** seems to be energetically favored and the electrophile selectively substitutes the lithium atom from the upper site to afford **73**, with retention of configuration.



Scheme 1.18. Carbolithiation reaction of cinnamyl alcohol (**71**).

The stereochemical outcome of the carbolithiation-trapping with electrophiles of cinnamylamines (**74**) (Scheme 1.19) was later described by Normant and co-workers.[41] Also in this case, the high diastereoselectivity observed in the reaction can be ascribed to thermodynamic control of the organolithium intermediate **75**, since the same *anti*-product starting from both (*E*)-**74** and (*Z*)-**74** cinnamylamine was obtained. According to the authors, this can be explained considering that, after thermodynamic equilibration, the tertiary amino

group (NEt_2 or NMe_2) on the γ position blocks the configuration of the benzyllithium through coordination. This allows a diastereoselective introduction of electrophiles with retention of configuration. Moreover, the diastereomeric ratio is dependent on the reaction temperature in hexane, raising from 80:20 to 95:5 with increasing temperature from −30 °C to RT.

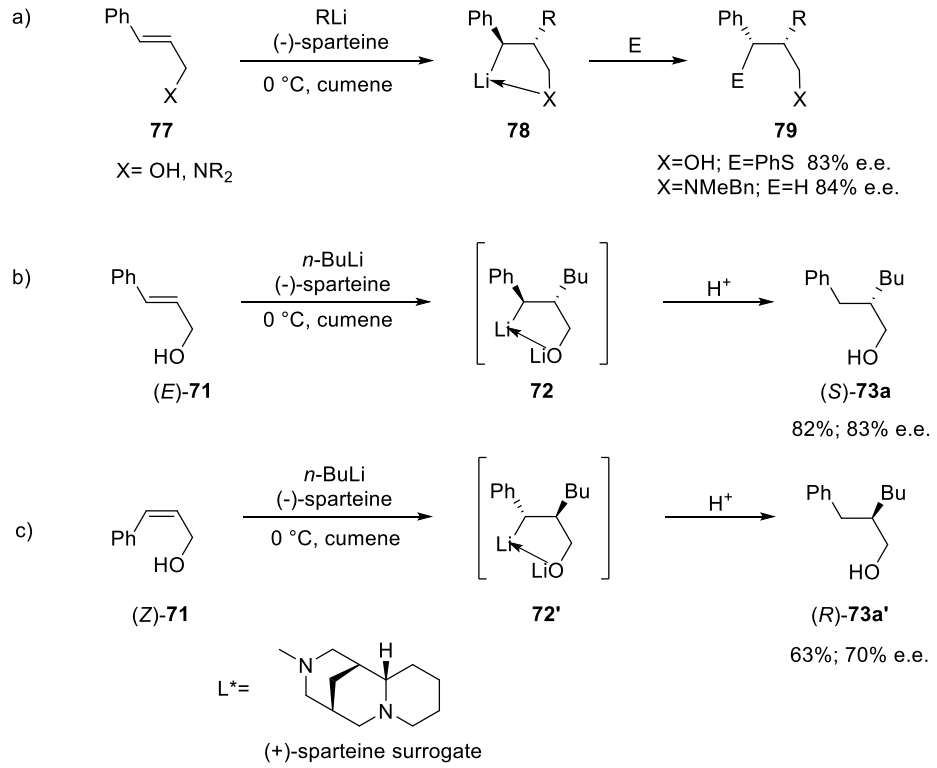


Scheme 1.19. Diastereoselective carbolithiation-trapping reaction of cinnamylamines (**74**).

Summarizing, cinnamyl amines gave *anti*-products while the alkoxide compounds were shown to yield mainly the *syn*-substituted diastereomers. This result was attributed by the authors to the oligomeric nature of the carbolithiation products of cinnamyl alcohol (**72**), which exist as dimer and higher aggregates under the reaction conditions.[42,43]

Normant, Marek and co-workers also described the asymmetric carbolithiation of cinnamyl alcohol or amines **77** promoted by stoichiometric amounts of the chiral ligand (-)-sparteine and using primary and secondary alkyllithium (Scheme 1.20a).[14,30,44] Contrary to what was described above, the authors reported that the benzylic organolithium (**78**), obtained from both types of substrates, undergoes electrophilic substitution with inversion of configuration, in this case, leading to *syn*-isomers of the products with up to 85 % e.e. and a high (> 96 %) diastereomeric purity (**79**). Therefore, such a simple one-pot process allows to introduce two contiguous stereogenic centres on an acyclic system with a good level of stereoselectivity. The best results were obtained using cumene as solvent, since sparteine shows the most pronounced effect in the absence of coordinating solvents, such as ether or THF, and using primary alkyl lithium (*n*-BuLi), being the RLi-sparteine interaction stronger with sterically less demanding organolithium derivatives. Moreover, the stereochemical outcome of the reaction was found to be closely dependent on the stereochemistry of the starting olefin. Indeed, whereas the asymmetric carbolithiation of (*E*)-**71** cinnamyl alcohol gave the (*S*)

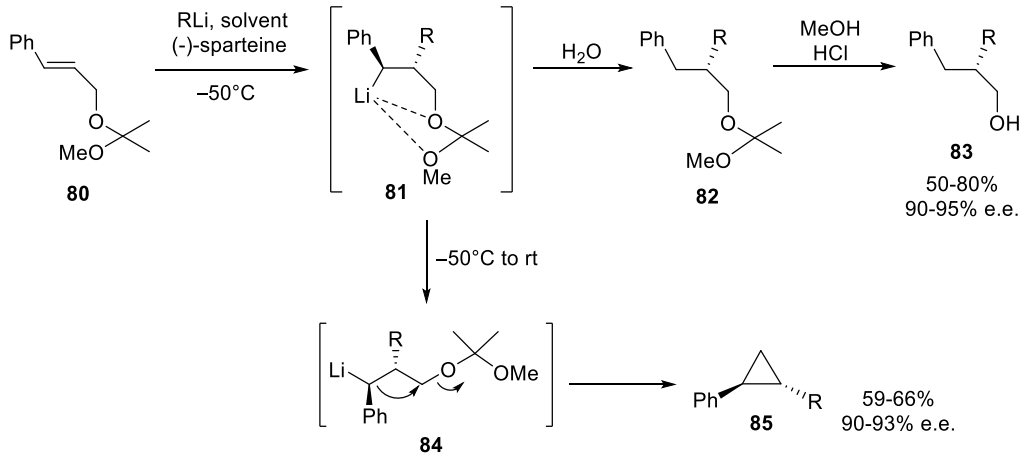
alkylated product (**73a**) with 83 % e.e. (Scheme 1.20b), the reaction of the (*Z*)-**71** isomer led mainly to (*R*)-enantiomer (**73a'**) (Scheme 1.20c). Interestingly, when the last reaction was performed using catalytic amounts (5 %) of (–)-sparteine products with comparable e.e. were obtained. Furthermore, the high stereospecificity of this process allows obtaining both enantiomers of the product by means of the same enantiomer of the ligand. Subsequently, enantioselective carbolithiation of cinnamyl alcohol was also reinvestigated by using (+)-sparteine surrogate (**L*** in Scheme 1.20). Therefore, the addition of the complex *n*-butyllithium/**L*** to cinnamyl alcohol (*E*)-**71** in cumene at 0 °C gave alcohol (*R*)-**73a'** in 71 % yield with 74 % e.e., that is with essentially opposite stereoinduction in respect to that obtained by Normant with (–)-sparteine (82% yield, 83 % e.e. in favour of (*S*)-**73a**).[45]



Scheme 1.20. Asymmetric carbolithiation of cinnamyl alcohol or amines **77**.

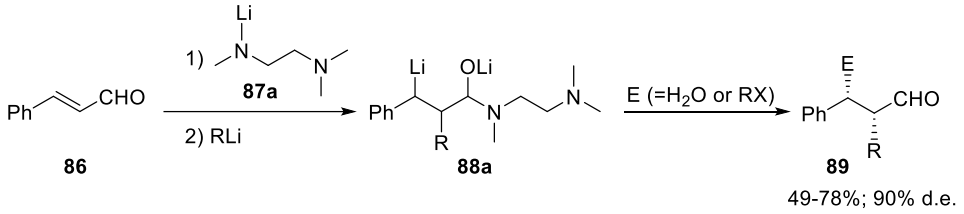
Highly enantioselective carbolithiation of the dimethyl acetal of the (*E*)-cinnamyl alcohol (**80**), mediated by a stoichiometric or catalytic (1 or 10 %) amount of (–)-sparteine, was also

reported (Scheme 1.21).[46] The acetal group gives the advantage to enforce the proximity effect and consequently to increase the e.e. Through this strategy it was possible to obtain, after hydrolysis at low temperature, the corresponding alkylated alcohols (**83**) in 90-95 % e.e. and in 50-80 % yields using stoichiometric amounts of the chiral ligand and comparable 92 % ee in the presence of 10 % of (-)-sparteine, whereas a slight loss of enantioselectivity (85 % ee) was observed with only 1% of chiral ligand. The use of the acetal allows the reaction to proceed at −50 °C instead of 0 °C, as for the carbolithiation of the corresponding alcohol (**71**). Furthermore, simply by warming the reaction mixture to RT, the benzylic organolithium intermediate (**81**), formed by the carbolithiation step, undergoes a 1,3-elimination, affording the chiral disubstituted cyclopropane (**85**) in 90-93% e.e. and in 59-66 % yields. In summary, when the chelating moiety is a dimethyl-methoxymethyl ether, the formed benzylic organolithium species is not stabilised and thermally labile at RT, leading to a chiral disubstituted cyclopropane, *via* an internal nucleophilic substitution, in which the alkyl and phenyl groups were found to be *anti* to each other. That is because in this transformation, the initially formed stereogenic centre C-R is invariant, whereas the benzylic carbon is free to epimerize and to promote the formation of the thermodynamically more stable *trans* cyclopropane.[14,44,46]



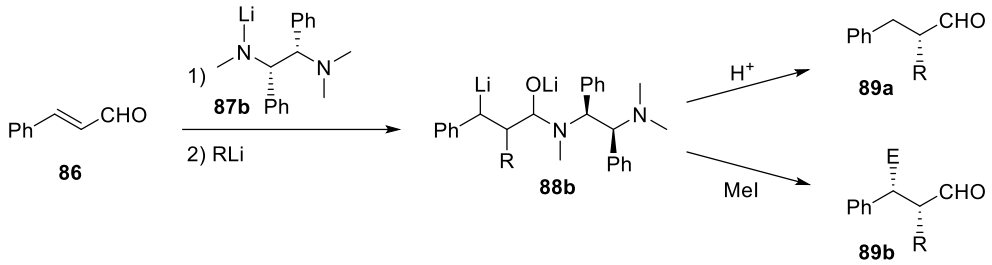
Scheme 1.21. Enantioselective carbolithiation of the dimethyl acetal of the (*E*)-cinnamyl alcohol (**80**).

Among the cinnamyl derivatives, cinnamaldehyde (**86**) was also shown to be a suitable substrate for the carbolithiation reaction. Indeed cinnamaldehyde, protected as a lithium aminoalkoxide by means of the lithium amide of *N*,*N*,*N'*-trimethylethanediamine (**87**), undergoes a regioselective addition of alkyllithiums on the C-C double bond to give the benzyllithium intermediate (**88**). This was reacted diastereoselectively with several electrophiles, such as alkyl, allyl and benzyl halides, giving the α,β-disubstituted 3-phenylpropanals (**89**), as *syn*-isomers with good yields (49-78 %) and a good diastereoselectivity (90 % d.e.) (Scheme 1.22).[47]



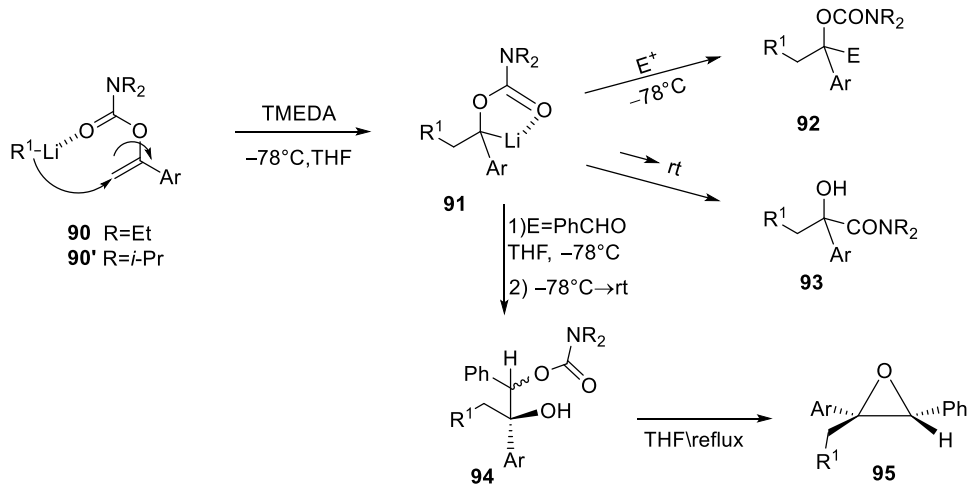
Scheme 1.22. Carbolithiation-trapping reaction of cynnamaldehyde (**86**).

An asymmetric version of the above reported reaction was also developed.[48] In the presence of the chiral lithium amides derived from (*R*,*R*) or (*S*,*S*)-1,2-diphenyl-*N*,*N*,*N'*-trimethylethanediamine (**87b**), used to protect the aldehyde function, it was possible to obtain an enantiomerically enriched organolithium intermediate. Subsequent hydrolysis or trapping with MeI led to α-mono- or α,β-disubstituted 3-phenylpropanals (**89a** or **89b**) with 76-96 % e.e. (Scheme 1.23). An excess of chiral lithium amide (2 equivalent) is preferable because a lower amount provides similar yields but lower e.e. Contrary to the carbolithiation of cinnamyl derivatives by RLi/(–)-sparteine, which has to be run in hydrocarbons, diethylether is the best solvent for this reaction. Moreover, primary, secondary and tertiary alkyllithiums can be used with good yields and enantioselectivities.



Scheme 1.23. Enantioselective carbolithiation-trapping reaction of cynnamaldehyde (**86**).

In 1996 Snieckus and co-workers[49] described an efficient carbolithiation of α-aryl *O*-vinyl-*N,N'*-diethyl carbamates **90** (R = Et) (Scheme 1.24). After the carbolithiation step, promoted by CIPE, the organolithium **91** was reacted with several electrophiles, obtaining trisubstituted aryl carbamates (**92**) or, after subsequent [1,2]-Wittig rearrangement, mandelic amides (**93**). When PhCHO was used as electrophile, a 3:1 mixture of diastereomers of the product **94** is formed (3:1 by ^{1}H-NMR), and after heating at reflux an intramolecular cyclisation occurred, leading to isolation of only the epoxide **95** with *cis* stereochemistry.



Scheme 1.24. One-pot tandem carbolithiation-α-alkylation and -[1,2]-Wittig rearrangement process of α-aryl *O*-vinyl-*N,N'*-diethyl carbamates **90** and synthesis of *cis* epoxide **95**.

Subsequently Hoppe[50] reported that also 1-aryl-1-alkenyl *N*,*N'*-diisopropyl carbamates **90'** (R = *i*-Pr) (Scheme 1.25) undergo intermolecular carbolithiation reactions easily. The *syn* addition of alkyllithium, in the presence of TMEDA, led to the formation of lithiated benzyl carbamates **91**, which could be trapped by several different electrophiles. Moreover, when the reaction was carried out in the presence of a chiral diamine, such as (–)-sparteine (**L1**) or (–)-α-isosparteine (**L2**) (Figure 1.2), moderate enantiofacial differentiation was observed (Scheme 1.25).[10,50] The best results were obtained by using *n*-BuLi/(–)-α-isosparteine (58 % e.e.) and *i*-PrLi/(–)-sparteine (40 % e.e.). This process can also provide a route to enantiomerically enriched benzyl alcohols.

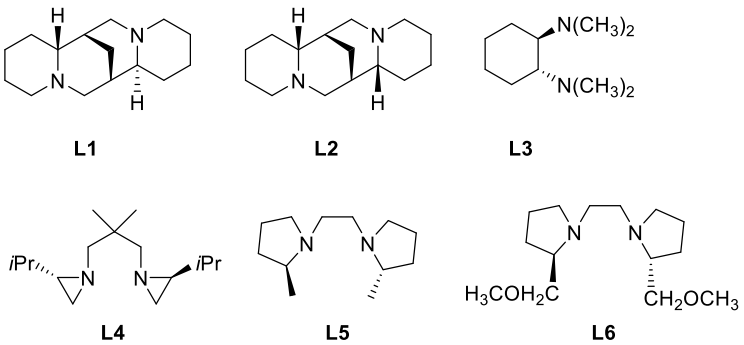
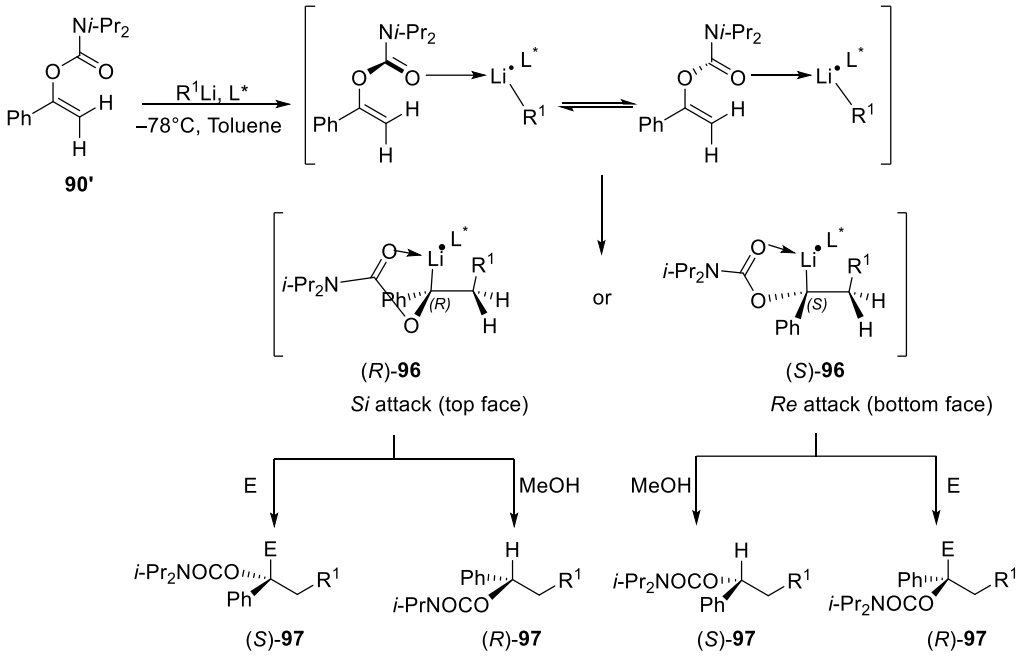


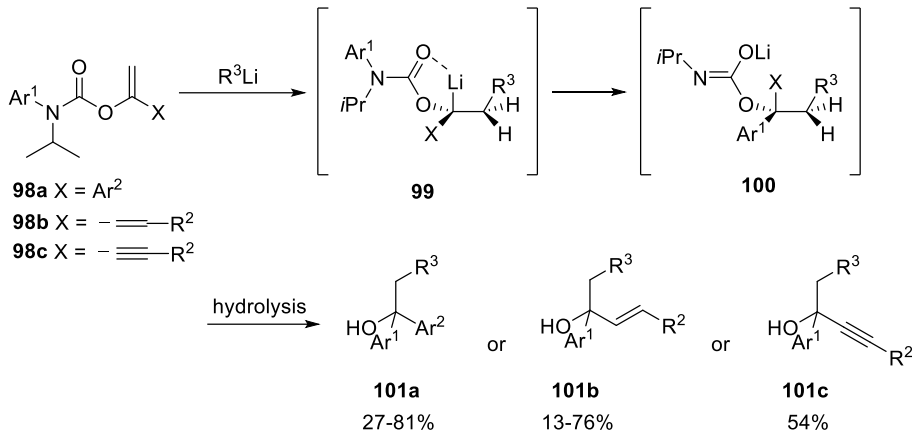
Figure 1.2. Chiral diamine used as chiral ligands in the carbolithiation-trapping reaction of 1-Aryl-1-alkenyl *N*,*N'*-diisopropyl carbamates (**90'**).

According to the asymmetric induction mechanism proposed by the authors[50] the diastereoisomeric complexes, formed by the coordination between the alkyllithium and the chiral ligand, react with the double bond in an intramolecular *syn*-addition to give the enantiomeric benzyllithium derivatives (*R*)-**96** and (*S*)-**96**, which are configurationally stable and can be trapped with electrophiles. In particular, the lithium/hydrogen exchange occurs with retention of configuration, giving the enantiomeric products (*R*)-**97** and (*S*)-**97**. On the contrary, using others electrophiles an inversion of the configuration was observed (Scheme 1.25).



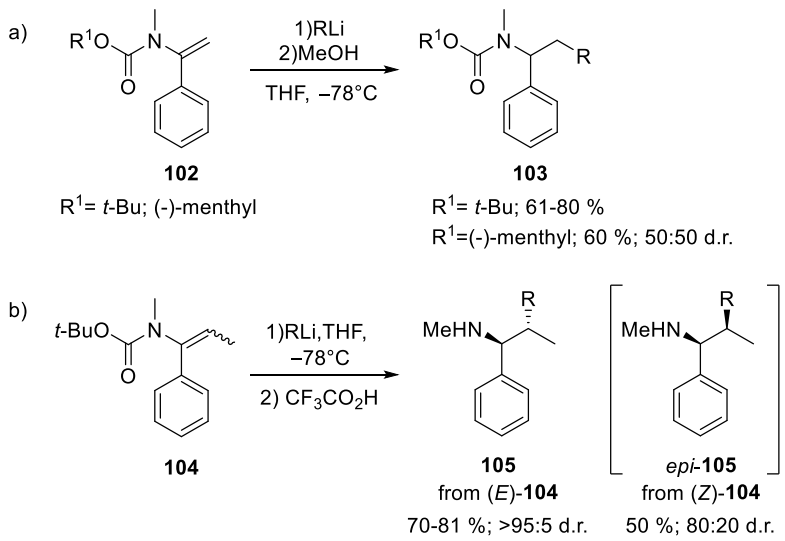
Scheme 1.25. Asymmetric carbolithiation-trapping reaction of 1-Aryl-1-alkenyl *N*,*N'*-diisopropyl carbamates (**90'**).

Clayden[51] described the carbolithiation of aryl, alkenyl and alkynyl enol carbamates (*O*-vinyl carbamates) bearing an *N*-aryl substituent (**98a-c**) (Scheme 1.26). After the *syn* addition of the alkyllithium to the vinyl double bond, in the presence of DMPU, the resulting organolithium carbamate intermediate (**99**) rearranges with N-C migration of the *N*-aryl substituent, creating a novel quaternary carbon centre α to oxygen (in **100**). Such migration occurs with inversion of configuration. The products obtained can be hydrolysed to give benzyl, allyl and propargyl branched tertiary alcohols (**101a-c**) by means of a one-pot tandem process.



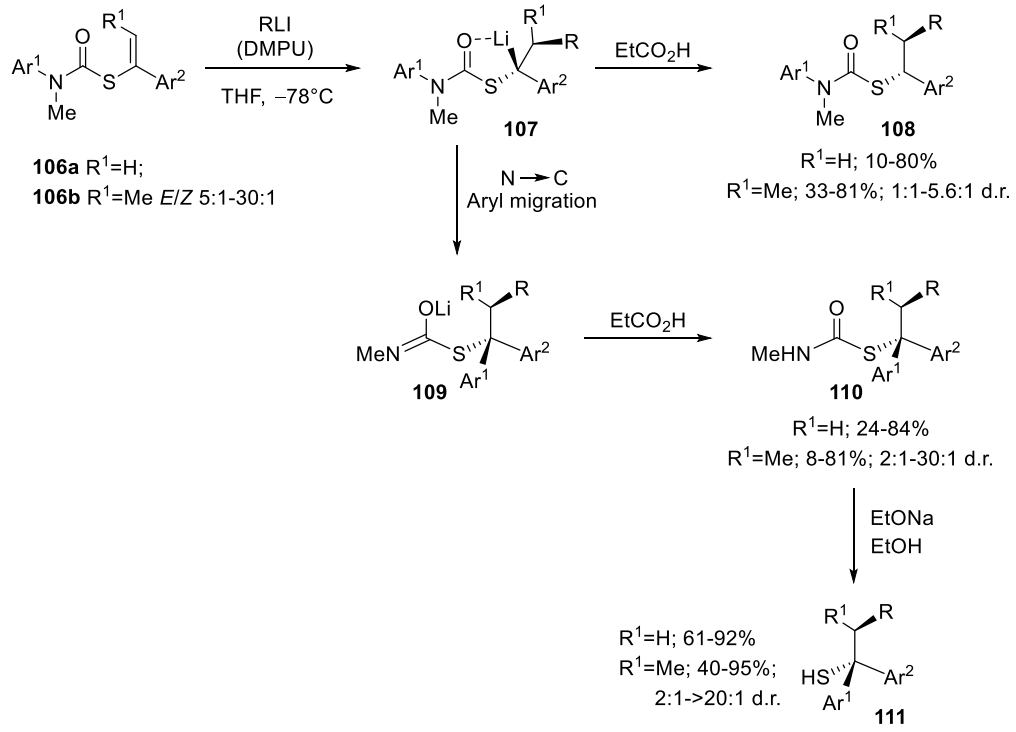
Scheme 1.26. Carbolithiation of aryl, alkenyl and alkynyl enol carbamates (*O*-vinyl carbamates) bearing an *N*-aryl substituent (**98a-c**).

Additionally, *N*-alkenyl carbamates (**102**) have been shown to be useful substrates for carbolithiation with primary, secondary, and tertiary alkyllithium reagents (Scheme 1.27a).[52] No diastereoselectivity was observed using (-)-menthyl carbamate as substrate. *N*-*t*-butoxycarbonyl vinyl carbamates (**104**) may be carbolithiated, protonated and deprotected in a one-pot synthesis of amines (Scheme 1.27b). The *Z* isomer of the β-substituted vinyl carbamate **104** is less reactive than its *E*-isomer, giving rise to a slower carbolithiation step, occurring in 24 hours instead of 1 hour. After deprotection with trifluoroacetic acid, the epimeric amine (*epi*-**105**) was obtained as an 8:2 mixture of diastereomers in lower yield. According to the authors, the loss of diastereospecificity was explained by the long reaction time during which the *syn*-carbolithiation is followed by a partial epimerisation of the organolithium intermediate.



Scheme 1.27. Carbolithiation-trapping reaction of *N*-alkenyl carbamates (**102** and **104**).

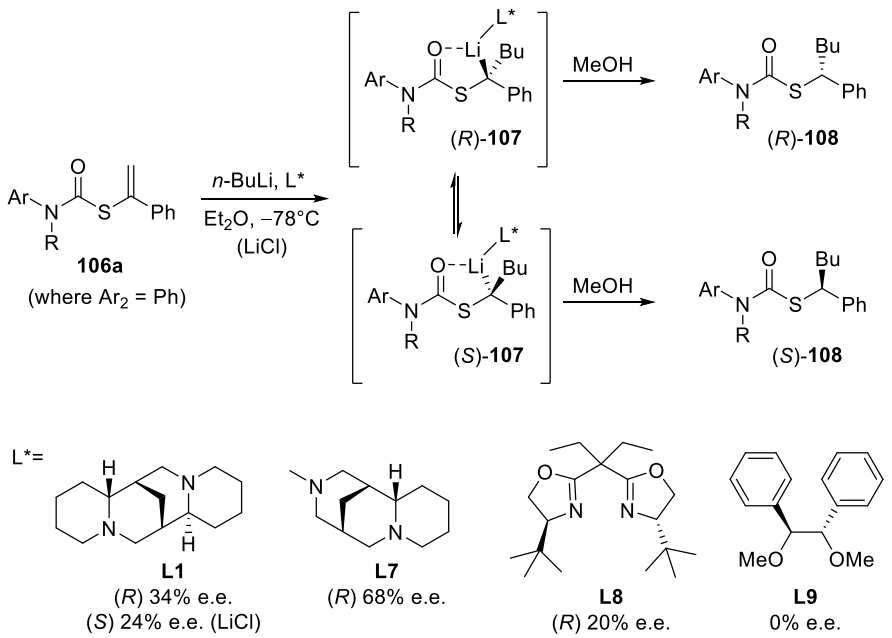
The carbolithiation of *S*-alkenyl-*N*-arylthiocarbamates (**106**) (Vinyl thiocarbamates), with a range of alkyllithium reagents, stereospecifically generates benzyllithium intermediates (**107**), which may also undergo intramolecular arylation, due to the N to C aryl migration, providing tertiary thiocarbamates (**110**). The latter are in turn precursors to new families of tertiary functionalised thiols (**111**), obtained in many cases with diastereoselective control (Scheme 3.16).[53]



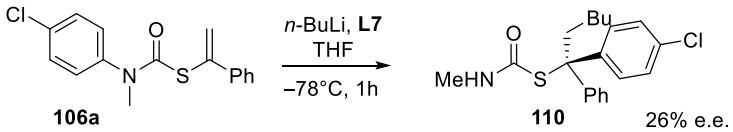
Scheme 1.28. Carbolithiation of *S*-alkenyl-*N*-arylthiocarbamates (**106**).

The first asymmetric version of the carbolithiation of *S*-Alkenyl-*N*-arylthiocarbamates (**106a**) was also developed (Scheme 1.29).[54] The addition of *n*-BuLi to alkenylthiocarbamates in the presence of chiral diamine ligands, such as (–)-sparteine (**L1**) or (+)-sparteine surrogate (**L7**), followed by protonation, generates enantiomerically enriched thiocarbamate derivatives of secondary thiols. Remarkably, the two pseudoenantiomeric chiral ligands do not always give enantiomeric products in the absence of additives. However, when LiCl was added to the carbolithiation mediated by (–)-sparteine, a sign inversion of the asymmetric induction was observed. In order to explain these results the authors proposed two possible mechanisms, hence two reaction pathways. In the first one, kinetic control induces facial selectivity in the addition step to give a configurationally stable organolithium-ligand complex, the stereospecific protonation of which yields to enantiomerically enriched compounds. In the second pathway, the addition step may or may not be enantioselective, but thermodynamic control over the equilibration of the resulting

diastereoisomeric organolithium complexes gives enantiomerically enriched products, again by stereospecific protonation. Moreover, by using THF as solvent and **L7** (in Scheme 1.29) as chiral ligand, a tandem enantioselective carbolithiation/rearrangement process, due to an *in situ* N-C aryl migration, occured, leading to enantiomerically enriched tertiary thiols precursors (Scheme 1.30).



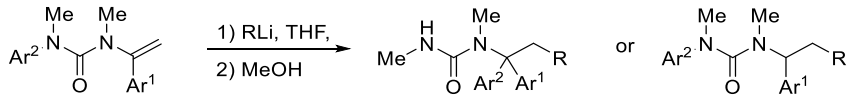
Scheme 1.29. Asymmetric carbolithiation of *S*-Alkenyl-*N*-arylthiocarbamates (**106a**).



Scheme 1.30. Enantioselective carbolithiation/rearrangement process of *S*-Alkenyl-*N*-arylthiocarbamates.

N-alkenyl ureas (*N*-carbamoyl enamines) (**112**) were also shown to exhibit umpolung reactivity undergoing facile addition of alkyllithium at their β-carbons (Scheme 1.31).[52]

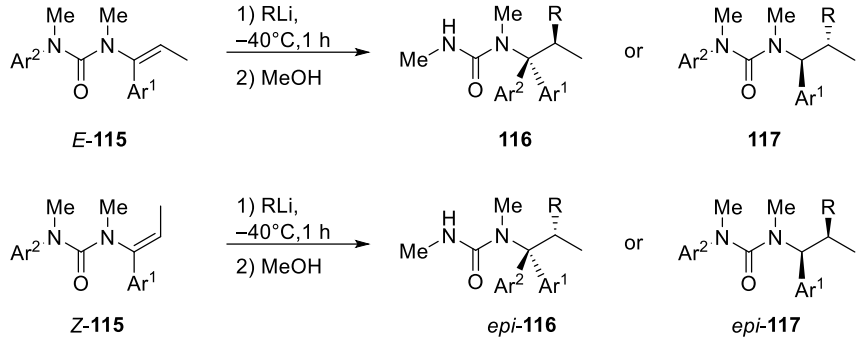
When the reaction was performed in THF at −78 °C or, more easily, by using hindered alkyllithium, the carbolithiation products (**114**) were obtained in moderate to good yields (47-77 %).[52] The carbolithiation reaction of *N*-alkenyl ureas bearing an *N*-aryl substituent in THF at −50 °C was coupled with N→C aryl transfer within the lithiated urea intermediate, giving rise to rearranged products **113**, in good to excellent yields (72-96 %).[55] Migration of a phenyl ring is generally faster and cleaner than migrations of other substituted aryl groups, such as the electron-rich ones, and using less hindered alkyllithium. This tandem β-alkylation-α-arylation protocol allows two new C-C bonds at α and β carbons of the urea-substituted alkene to be formed in a single pot, and at the same time it shows the ability of urea moieties in the α position to a double bond to promote carbolithiation by CIPE.



112 **113** **114**

Scheme 1.31. Carbolithiation of *N*-alkenyl ureas (**112**).

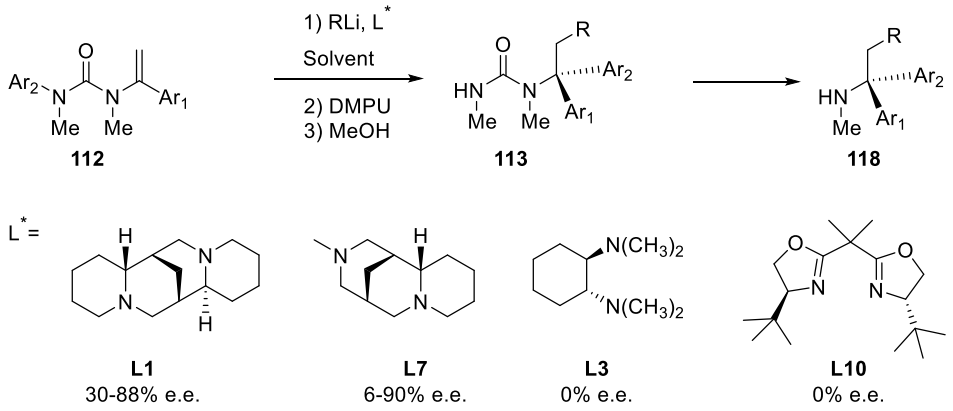
In the case of β-substituted vinyl ureas (Scheme 1.32),[52,55] the addition of alkyllithium to *E*-isomer (*E*-**115**) at −40 °C in toluene, followed by protonation with retention of configuration, led to the formation of products **81,** as single diastereomers. A noncoordinating solvent was used in order to suppress rearrangement. The tandem carbolithiation/protonation process is completely diastereospecific. In fact using the *Z*-isomer (*Z*-**115**) as starting material, under the same reaction conditions, the other diastereomer *epi*-**117** is obtained selectively. When the carbolithiation of β-methyl vinyl ureas (*E*-**115** or *Z*-**115**) was carried out in THF, a more coordinating solvent, or in toluene and in the presence of DMPU as coordinating co-solvent, a rearrangement occurred, due to the N→C aryl migration, giving **116** or *epi*-**116** compounds. Contrary to as shown before for the *O*-vinyl carbamates, the migration of the *N*-aryl groups in *N*-alkenyl ureas occurred with retention of configuration. Both the carbolithiation and aryl migration steps are then stereospecific, since changing the stereochemistry of the double bond in the starting material, the relative configuration of the products changes.



Scheme 1.32. Carbolithiation of β-substituted vinyl ureas (**115**).

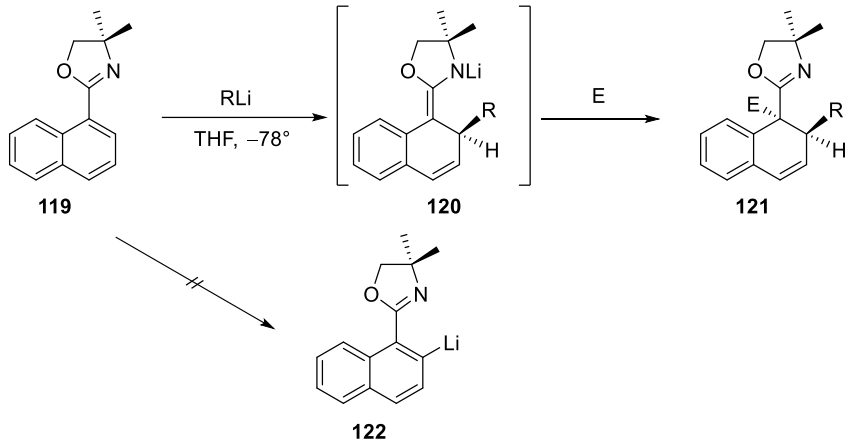
In the presence of chiral diamine ligands, such as (–)-sparteine (**L1**) and (+)-sparteine surrogate (**L7**), enantioselective carbolithiation of *N*-alkenyl-*N'*-arylureas (**112**) occurred, leading to benzyl organolithiums which, in the presence of DMPU, undergo rearrangement with the N→C aryl transfer, giving **113** (Scheme 1.33).[56]

The tandem asymmetric carbolithiation/rearrangement process allowed to generate urea derivatives of enantiomerically enriched amines bearing tertiary substituents (α,α-diarylamines) (**118**), providing a synthetic route to compounds with quaternary stereogenic centres α to the nitrogen. In the asymmetric version, the aryl migration is also stereochemically retentive.



Scheme 1.33. Enantioselective carbolithiation of *N*-alkenyl-*N'*-arylureas (**112**).

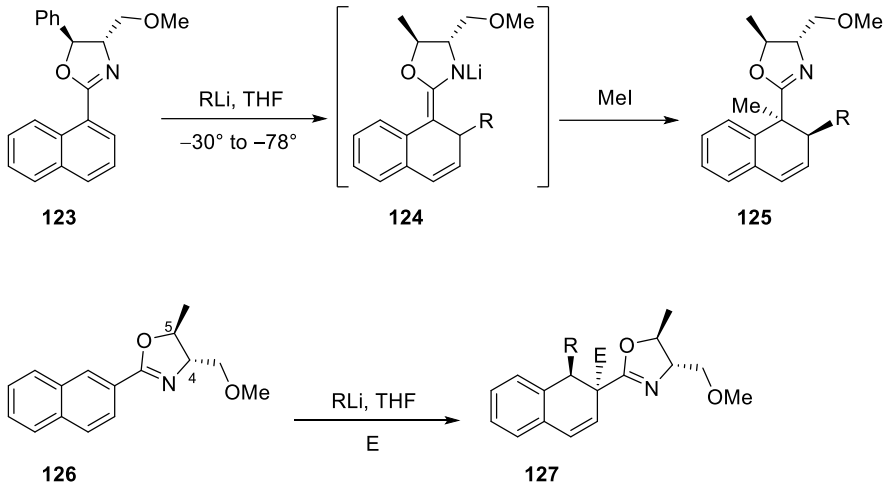
Finally, it is worthy to mention the addition of organolithiums to naphtalene derivatives described by Meyers.[57] During the investigation of the *o*-metalation of naphthyloxazolines (to provide **112**), it was observed that the conjugatively addition of organometallics to such achiral naphthalene compounds (**119**) followed by electrophilic trapping led to the doubly alkylated product **121** in good yield (Scheme 1.34). The addition of alkylithium reagents into the naphthalene π system to give tandem 1,2-addition products similar to **121** had been observed earlier, with generally poor success. It was noted that the effect of the oxazoline substituent made this reaction considerably more effective[58] than earlier efforts and opened up the way to a possible asymmetric version of this process.



Scheme 1.34. Carbolithiation of naphtalene derivatives (**119**).

Indeed, the asymmetric additions of alkylithiums were successful both onto 1- and 2-naphthyloxazolines (**123** and **126** respectively). The addition step on **123** led to the formation of the intermediate azaenolate **124**, which was trapped by various electrophilic reagents (e.g., MeI) at low temperatures providing the doubly alkylated dihydronaphthalene **125** in > 95:5 diastereoisomeric ratios. The products were exclusively *trans* disubstituted (R and Me) and none of the *cis* isomers were detected (Scheme 1.35).[57] In the same way, the tandem addition-trapping reaction on **126** gave the trisubstituted dihydronaphthalenes **127** in generally good yields and high diastereomeric ratios (Scheme 1.35). In this case, it was observed that the stereochemistry at the methoxymethyl groups (C-4) in the oxazolines was the major factor

responsible for the stereochemical outcome of the alkyllithium addition. On the contrary, it was found that the stereochemistry and size (Ph, Me, H) of the substituent at C-5 on the oxazoline had very little effect on the stereochemical addition to the naphthalene π system.[57]



Scheme 1.35. Enantioselective carbolithiation of naphtalene derivatives (**123** and **126**).

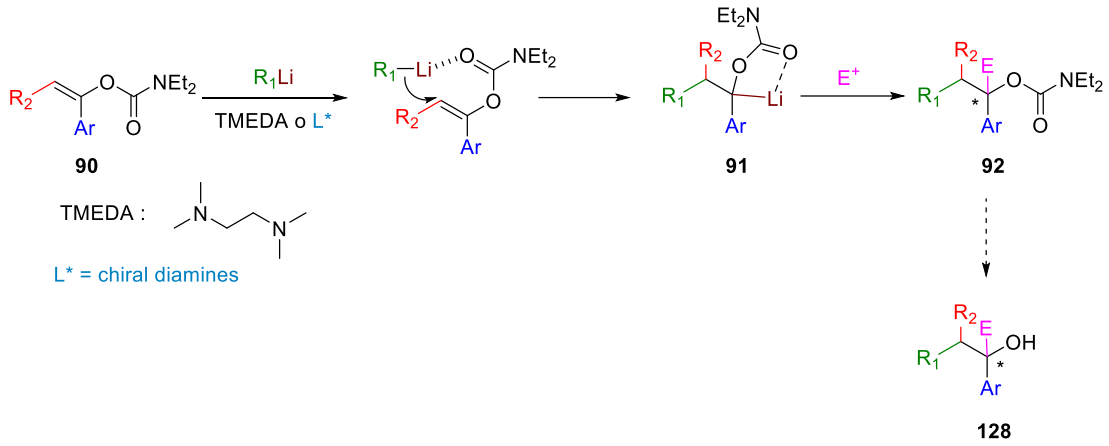
1.1.3. Aims of the project

Aim of this project was to investigate the carbolithiation of 1-aryl-1-alkenyl *N,N'*-diethylcarbamates (**90**), taken as model substrates of functionalised styrene derivatives (Scheme 1.36). In particular, the attention was also focused on the extension of the carbolithiation reaction to the preparation of optically active products.

Carbolithiation reactions with styrenyl olefins represent an attractive synthetic methodology, offering pathways for the preparation of highly functionalised aromatic compounds. This transformation generates a benzyllithium species, which, through a tandem carbolithiation-trapping reaction, can be reacted with electrophiles, allowing the efficient regioselective formation of two new bonds and of one or two new stereocentres. As previously mentioned, a potential limitation to the application of carbolithiation as a synthetic method derives from the difficulty of tempering the reactivity of the organolithium intermediate towards the

unsaturated substrate. In fact, the lithiated intermediate can react with a second molecule of the olefin substrate, triggering an unwanted anionic polymerisation process (Scheme 1.3). Hence, despite its synthetic appeal, the potential of this class of reactions has not been fully explored.[49,50] As early described, for synthetically useful carbolithiation reactions, a special stabilisation of the benzyl lithium intermediate is required. Such a stabilisation can be obtained by either intra- or intermolecular organolithium coordination, which occurs in the presence of chelating groups proximal to the reacting alkene and/or by means of bidentate ligands such as diamines.

In the case of this project, the presence of the carbamoyl coordinating group on the double bond of 1-aryl-1-alkenyl *N*,*N'*-diethylcarbamates (**90**) is essential to prevent polymerisation and promote the carbolithiation reaction, through precoordination of the lithium reagent with the heteroatom, exploiting the aforementioned *Complex Induced Proximity Effect*. Therefore, this project was aimed to develop a synthetic methodology to obtain trisubstituted benzyl carbamates, direct precursors of tertiary benzylic alcohols (**128**), by carbolithiation-electrophile trapping, also in enantioselective manner. For this reason, the addition of alkyllithium on the styrenyl double bond was studied, performing the tandem reaction in the presence of a bidentate ligand, such as TMEDA. After the carbolithiation step, the organolithium intermediate (**91**) was reacted with several electrophiles, leading to trisubstituted benzyl carbamates (**92**) as direct precursors of tertiary benzylic alcohols (**128**), common structural subunits in naturally occurring bioactive compounds. The stereoselectivity of the reaction as well as the presence of substituents on the aromatic ring are important aspect to be considered. The enantioselective tandem carbolithiation-trapping process was also investigated in the presence of chiral diamines, allowing the preparation of enantioenriched tertiary benzyl carbamates.



Scheme 1.36. Carbolithiation-trapping process of 1-aryl-1-alkenyl *N*,*N'*-diethylcarbamates (**90**).

Moreover, in this kind of reaction, 1-aryl-1-alkenyl *N*,*N'*-diethylcarbamates are synthetic equivalents of an *umpolung* synthon. In fact, the β carbon, which undergoes the addition of alkyllithium, is electrophilic and not nucleophilic as in enolates. On the contrary, the α carbon, which can react with electrophiles, is nucleophilic and not electrophilic like in carbonyls (Figure 1.3).

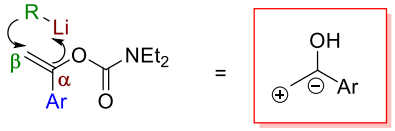
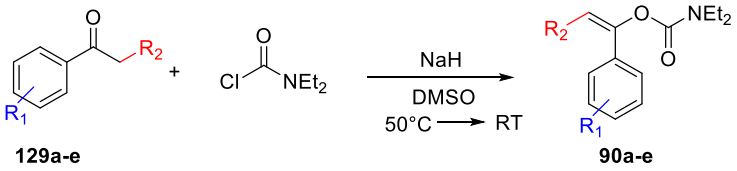


Figure 1.3. 1-Aryl-1-alkenyl *N*,*N'*-diethylcarbamates (**90**) act as an *umpolung* synthon.

1.2. Results and Discussion

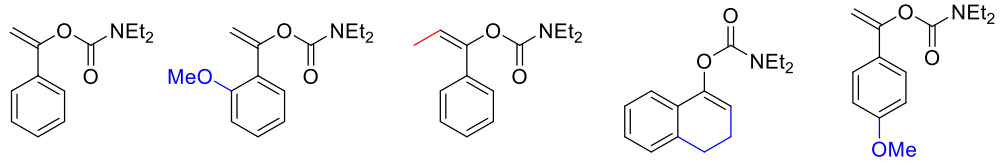
1.2.1. Preparation of 1-Aryl-1-Alkenyl N,N'-Diethylcarbamates

In order to perform the carbolithiation- electrophile trapping process, it was necessary to synthesize the starting substrates, namely the 1-aryl-1-alkenyl *N,N'*-diethylcarbamates (Scheme 1.37). Accordingly, compounds **90a-e** (Figure 1.4) were obtained by reacting the aryl ketones **129a-e** with diethylcarbamoyl chloride in the presence of an excess (3.5 eq.) of NaH and using DMSO as solvent.[59]



129a-e **90a-e**

Scheme 1.37. Preparation of 1-aryl-1-alkenyl *N,N'*-diethylcarbamates (**90a-e**).

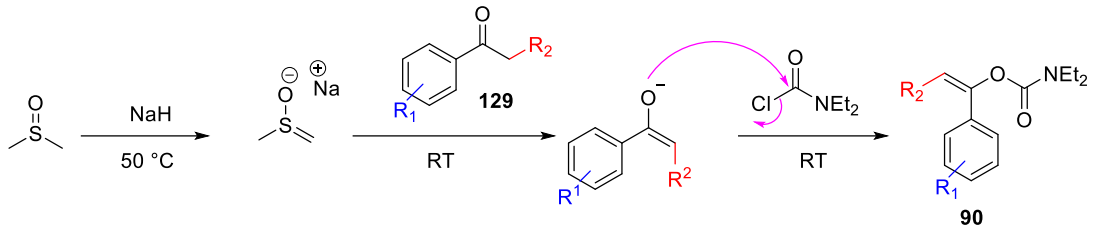


90a	**90b**	**90c**	**90d**	**90e**
31%	25%	53%	59%	17%

Figure 1.4. 1-Aryl-1-alkenyl *N,N'*-diethylcarbamates synthesised.

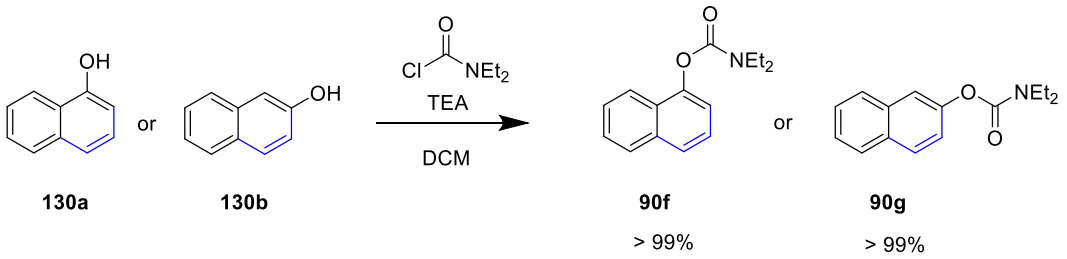
The reaction mechanism (Scheme 1.38) involves the initial formation of the enolate of DMSO by the reaction with NaH. This enolate appears to be a sufficiently strong base to give rise, in turn, to the generation of the enolate of aryl ketones **129a-e**. The latter affordes the desired compound **90a-e** by reacting with diethylcarbamoyl chloride through a nucleophilic acyl substitution reaction. The presence of a coordinating solvent such as

DMSO, which complexes the sodium counterion of enolate, favors *O*-alkylation of enolate compared to *C*-alkylation.



Scheme 1.38. Mechanism of the reaction of preparation of 1-aryl-1-alkenyl *N,N'*-diethylcarbamates (**64**).

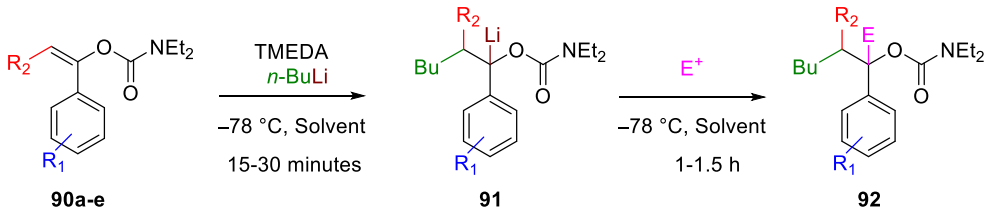
Additionally, inspired by the study into the addition of organolithiums to naphtalene derivatives reported by Meyers (*Vide* paragraph 1.1.2.3),[57] the two naphthyldiethyl carbamates **90f** and **90g** were prepared and used as substrates for the tandem carbolithiation-electrophile trapping reaction. Therefore, by reacting 1- (**132a**) and 2-naphthol (**132b**) with diethylcarbamoyl chloride in the presence of TEA and using DCM as solvent, both **90f** and **90g** were synthesised in quantitative yields.



Scheme 1.39. Synthesis of naphthyldiethyl carbamates (**64f** and **64g**).

1.2.2. Carbolithiation of 1-Aryl-1-Alkenyl N,N'-Diethylcarbamates

The tandem carbolithiation-electrophile trapping reaction was performed adding *n*-BuLi (1.1 eq.) to a solution of 1-aryl-1-alkenyl *N,N'*-diethylcarbamate (**90a-g**) and TMEDA at –78 °C in dry and inert atmosphere. After the addition of the alkyllithium to the styrenyl double bond, an excess of electrophiles (3.0 eq.) was added affording the trisubstituted benzyl carbamates (Scheme 1.40). The carbolitiation step, leading to the organolithium intermediated **91**, proceeded rapidly. In fact, in a range of 15-30 minutes a complete conversion of the starting substrate was generally observed. The reaction of the organolithium intermediate **91** with electrophiles was also found to proceed quite rapidly, with a maximum reaction time of 1.5 h using benzaldehyde as electrophile. The tandem process was carried out in the presence of diamine TMEDA, which, by a coordination with the alkyllithium, reduce its aggregation, thus increasing thus its reactivity. The trisubstituted benzyl carbamates synthesised are highlighted in Figure 1.5 and the results of carbolithiation followed by reaction with electrophiles are collected in Table 1.1.



90a-e **91** **92**

Scheme 1.40. Tandem carbolithiation-electrophile trapping reaction.

The coordinating solvent THF was preferred over toluene, by comparing the results obtained with these two solvents by adding *n*-BuLi to the substrate **90a** and using MeOH as electrophile (Table 1.1, entries 1 and 2). It can be deduced that MeOH, MeI and allyl bromide were suitable electrophiles in this kind of process starting from the substrate **90a**, as the yields of the tandem reaction were excellent (Table 1.1, entries 1, 3, 4). On the contrary, the use of DMF and PhCHO as electrophiles after the addition of *n*-BuLi to the substrate **90a** led to low yields (Table 1.1, entries 5 and 6).

In particular, when the addition of *n*-BuLi to substrate **90a** was followed by addition of PhCHO, a diastereomeric mixture (3:1 from ^{1}H-NMR) of the product **92ae'** was formed, due to the migration of the carbamoyl group, favored by an increase in temperature. Presumably, it was speculated that the *erythro* diastereomer could be the major isomer owing to the steric hindrance. The formation of compound **92ae'** was deduced by the ^{1}H-NMR spectrum, where the signal ascribed to the proton H_b, proximal to the carbamoyl group in the product **92ae'**, was detected to be more downfield with respect to the signal expected for the proton H_a nearby the hydroxyl group of the compound **92ae**, actually not detected (Scheme 1.41).

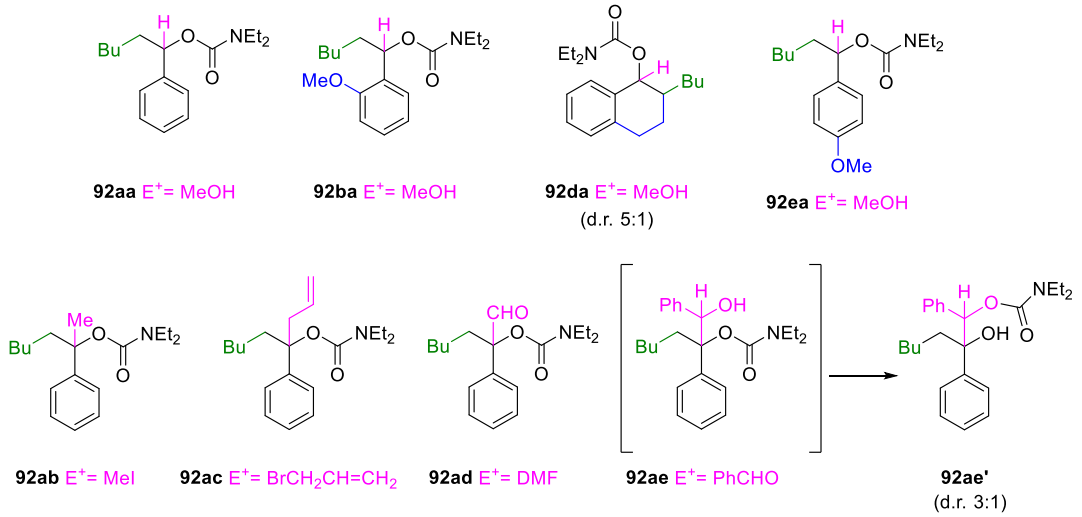
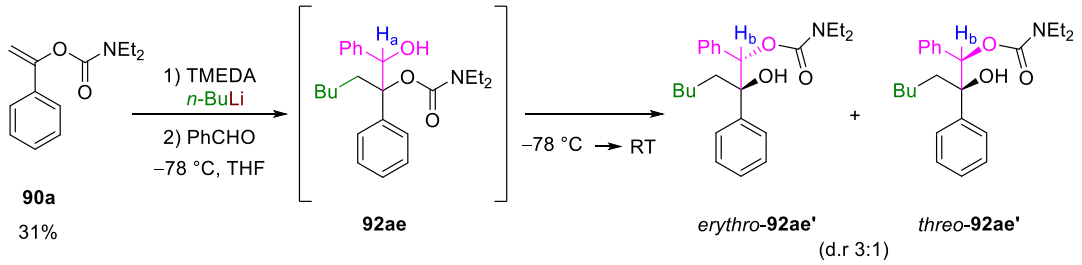


Figure 1.5. Trisubstituted benzyl carbamates synthesised.



Scheme 1.41. Carbolithiation of **90a** followed by reaction with PhCHO.

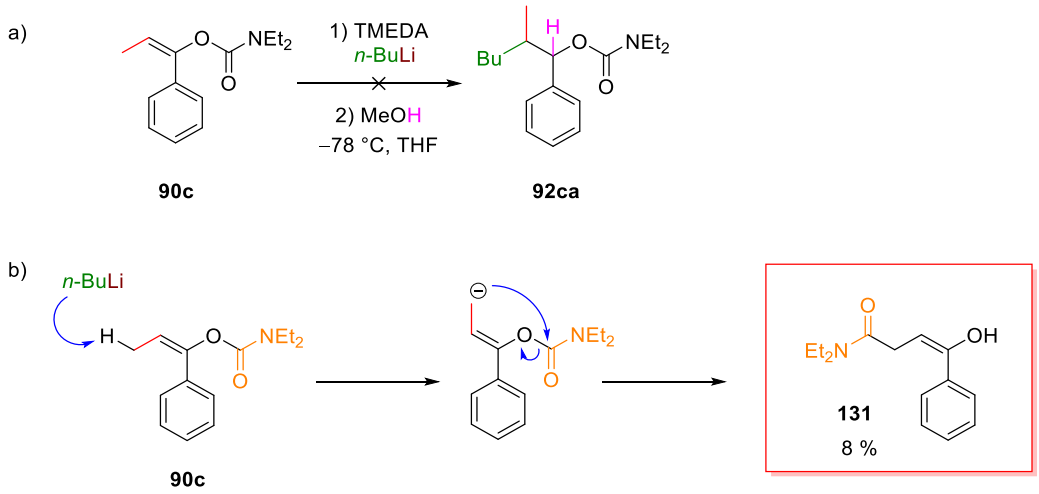
By using propionaldehyde as electrophile starting from the substrate **90a** (Table 1.1, entry 7), it was not possible to obtain compound **92af**, because a mixture of products difficult to identify was recovered probably due to aldol reactions of the starting aldehyde triggered by the lithiated species acting as base. Attempts to prepare compounds **92ag**, **92ah** and **92ai** starting from the substrate **90a** and using EtI, I_2 and Br_2 as electrophiles, respectively, were in vain (Table 1.1, entries 8, 9 and 10). Indeed, in all three cases compound **92aa** (Figure 1.5) was recovered.

Table 1.1. Tandem carbolithiation-trapping reaction with electrophiles.

Entry	Substrate	Electrophile	Solvent	E	Product	Yield (%)
1	**90a**	MeOH	THF	H	**92aa**	95[a]
2	**90a**	MeOH	Toluene	H	**92aa**	80[a]
3	**90a**	MeI	THF	Me	**92ab**	92[a]
4	**90a**	Allyl bromide	THF	$CH_2CH{=}CH_2$	**92ac**	83[a]
5	**90a**	DMF	THF	CHO	**92ad**	18[c]
6	**90a**	PhCHO	THF	PhCHOH	**92ae'**[d]	26[b]
7	**90a**	CH_3CH_2CHO	THF	CH_3CH_2CHOH	**92af**	–
8	**90a**	EtI	THF	Et	**92ag**[f]	–
9	**90a**	I_2	THF	I	**92ah**[f]	–
10	**90a**	Br_2	THF	Br	**92ai**[f]	–
11	**90b**	MeOH	THF	H	**92ba**	68[b]
12	**90b**	MeI	THF	Me	**92bb**	–
13	**90c**	MeOH	THF	H	**92ca**	–
14	**90d**	MeOH	THF	H	**92da**[e]	32[b]
15	**90e**	MeOH	THF	H	**92ea**	83[a]
16	**90f**	MeOH	THF	H	**92fa**	–
17	**90g**	MeOH	THF	H	**92ga**	–

[a] Yield of isolated product without purification; [b] Product obtained after chromatographic purification; [c] Yield determined by ^{1}H NMR spectrum; [d] Product **92ae** was not isolated. It was instead obtained the rearranged product **92ae'** as diastereomeric mixture (3:1); [e] Obtained as diastereomeric mixture (5:1); [f] product **92aa** was recovered.

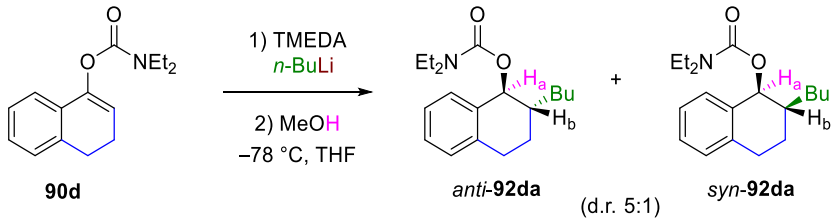
The carbolithiation reaction of substrate **90b** followed by trapping with MeOH led to the desired compound **90ba** (Figure 1.5) in good yield (Table 1.1, entry 11). On the contrary, it was not possible to obtain product **92bb** starting from **90b** and using MeI as electrophile (Table 1.1, entry 12). Only compound **92ba** and a mixture of products difficult to identify were instead recovered. The addition of *n*-BuLi to substrate **90c** followed by reaction with MeOH did not lead to the desired compound **92ca** (Table 1.1, entry 13) (Scheme 1.42a). Conversely, a different product (**131**) was isolated (Scheme 1.42b) together with starting material **90c** and other products difficult to identify. It was speculated that in this case *n*-Buli, acting as a base, caused the deprotonation of the hydrogen in allylic position. Therefore, the new formed nucleophilic species could lead to the migration of the carbamoyl group on γ carbon through an intramolecular acyl substitution reaction.



Scheme 1.42. a) Carbolithiation-trapping reaction of **90c** and b) synthesis of compound **131**.

The tandem carbolithiation-trapping reaction starting from substrate **90d** and using MeOH as electrophile led to the desired compound **92da**, obtained in low yield as a mixture of diastereomers (5:1) (Table 1.1, entry 14) (Scheme 1.43). Even in this case, the *anti* diastereomer was supposed to be the major isomer due to the steric hindrance. This hypothesis can also be confirmed by the evidence of the values of the H_a-H_b ^{1}H-NMR coupling constants, which were found to be J = 6.0 Hz, a more typical value for *anti*-isomer,

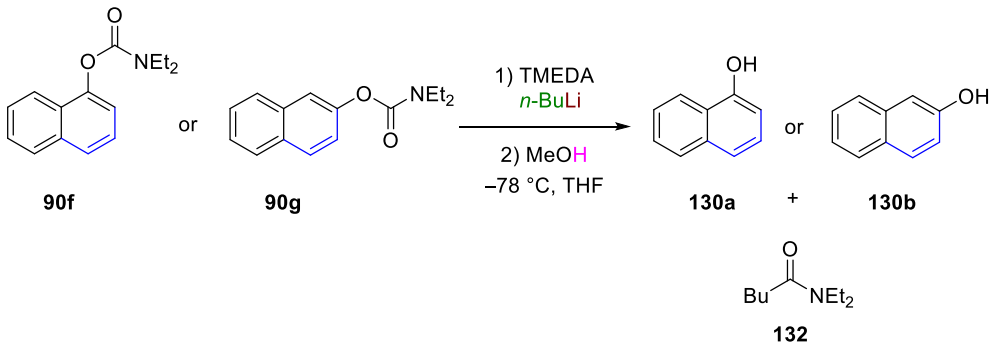
for the major diastereomer, and J = 2.0 Hz, a more typical value for *syn*-isomer, for the minor diastereomer.



Scheme 1.43. Carbolithiation of **90d** followed by reaction with MeOH.

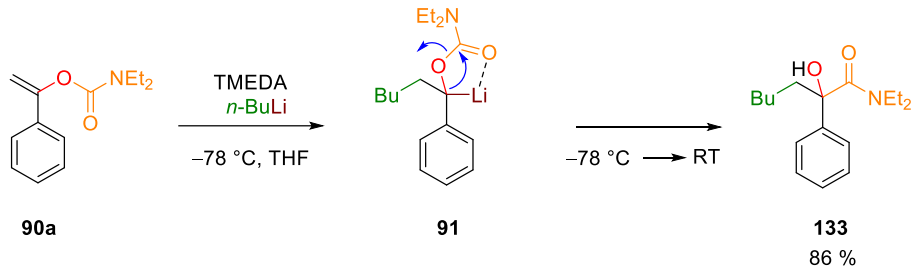
The carbolithiation reaction of substrate **90e** followed by trapping with MeOH also led to the desired compound **92ea** (Figure 1.5) in 83 % yield (Table 1.1, entry 15).

Unfortunately, it was not possible to synthesise the desired carbolithiation products **92fa** and **92ga**, starting from the naphthyl carbamates **90f** and **90g**, respectively (Table 1.1, entry 16 and 17). Indeed, in both cases starting materials, **90f** and **90g**, 1- (**130a**) or 2-naphthol (**130b**), depending on the substrate, and *N*,*N*-diethylpentanamide (**132**) were recovered (Scheme 1.44). Therefore, it can be thought that with these substrates *n*-BuLi, acts as nucleophile, promoting the acyl substitution on the carbamoyl moiety rather than the *syn* addition on the activated double bond of the naphthalene derivatives.



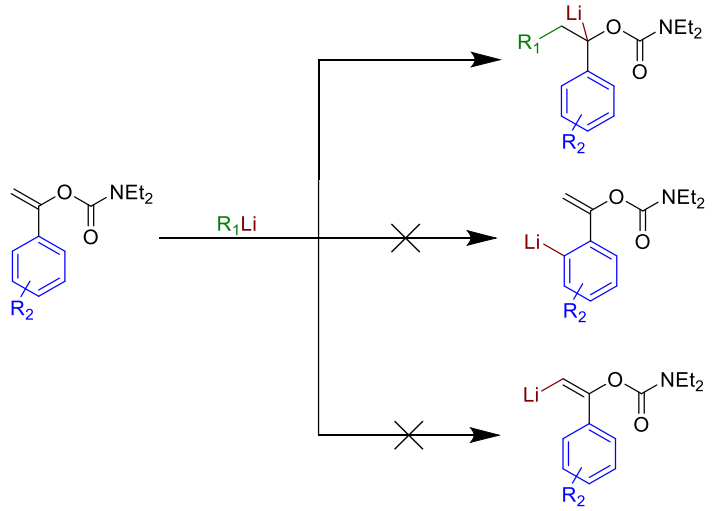
Scheme 1.44. Reaction of **90f** and **90g** with *n*-BuLi.

Moreover, when after the addition of *n*-BuLi to the solution of substrate **90a** in anhydrous THF, the reaction mixture was left slowly warming to the RT, the α-hydroxy benzylamide (**133**) was formed, resulting from the carbamoyl migration on the benzyl position through the [1-2]-Wittig rearrangement (Scheme 1.45).



Scheme 1.45. Carbolithiation and [1,2]-Wittig rearrangement process of **90a**.

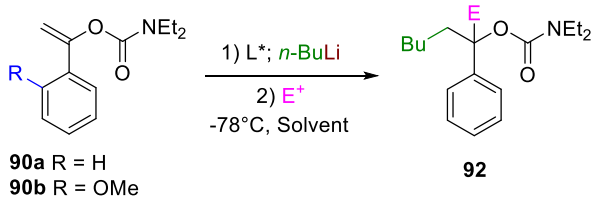
It is interesting to note that in the addition reaction on arylenol carbamates, the alkyllithium acts as a nucleophile, giving addition to the double bond, and not as a base, since no deprotonation was observed neither on the aromatic ring nor on the vinyl carbon (Scheme 1.46).



Scheme 1.46. Alkyllithium acts as a nucleophile in the carbolithiation reaction of 1-aryl-1-alkenyl *N*,*N'*-diethylcarbamates.

1.2.3. Enantioselective Carbolithiation of 1-Aryl-1-Alkenyl N,N'-Diethylcarbamates

The tandem carbolithiation-electrophile trapping was also performed in enantioselective manner using chiral diamines in place of TMEDA, with the aim to obtain enantioenriched tertiary benzyl carbamates (Scheme 1.47). The chiral diamines used were (+)-sparteine (**L1**), 2,2′-isopropylidenebis[(4*S*)-4-*tert*-butyl-2-oxazoline] (**L10**), 2,2-Bis((4*S*)-(–)-4-isopropyloxazoline)propane (**L11**) and (*S*,*S*)-2,2-bis(4-phenyl-2-oxazolin-2-yl)propane (**L12**) (Figure 1.6). In particular, this class of BOX ligands have never been used in this kind of reactions. In the enantioselective version, the reaction was carried out adding the substrate (**90a-b**) (1.0eq.) to a solution of *n*-BuLi (1.1 eq.) and the chiral ligand (1.1 eq.) at –78 °C in dry and inert atmosphere. After the carbolithiation step, the electrophile reagent (3.0 eq.) was added and the reaction mixture was stirred for ca. 1 h, affording the desired product. The obtained results, varying chiral ligand, solvent and electrophile, are collected in Table 1.2.



Scheme 1.47. Enantioselective carbolithiation-trapping process.

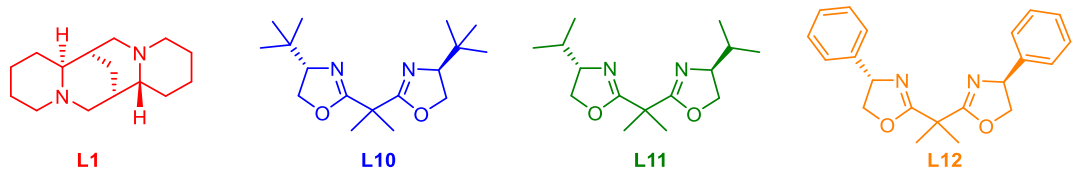


Figure 1.6. Chiral diamines employed as ligands in the enantioselective carbolithiation-trapping process.

Table 1.2. Enantioselective carbolithiation-electrophile trapping reaction.

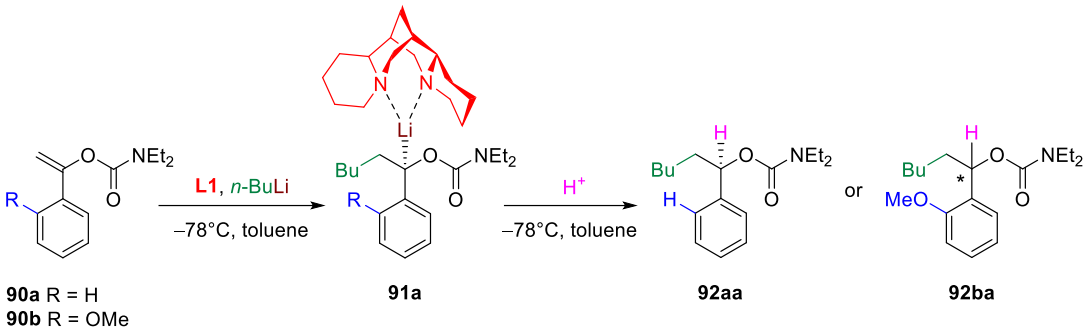
Entry	Substrate	Electrophile	Solvent	L*	E	Product	Yield (%)	e.e. (%)
1	**90a**	MeOH	THF	**L1**	H	**92aa**	96[a]	0[d]
2	**90a**	MeOH	Toluene	**L1**	H	**92aa**	13[b]	20[d](*S*)[f]
3	**90a**	MeOH	Toluene	**L11**	H	**92aa**	71[b]	22[d](*S*)[f]
4	**90a**	MeOH	Toluene	**L10**	H	**92aa**	11[b]	16[d](*S*)[f]
5	**90a**	MeOH	Toluene	**L12**	H	**92aa**	8[b]	6[d](*S*)[f]
6	**90a**	MeI	THF	**L1**	Me	**92ab**	61[c]	0[e]
7	**90a**	MeI	Toluene	**L1**	Me	**92ab**	–	–
8	**90a**	MeI	Toluene	**L11**	Me	**92ab**	52[b]	22[e]
9	**90b**	MeOH	Toluene	**L1**	H	**92ba**	55[b]	14[d]

[a] Yield of isolated product without purification; [b] Product obtained by chromatography purification; [c] Yield determined by ^{1}H NMR spectrum; [d] e.e. determined by chiral HPLC after transformation of the carbamoyl group into the corresponding secondary alcohol; [e] e.e. determined by ^{1}H NMR shift experiments with (+)-Eu(hfc)$_3$ in deuterated toluene; [f] Absolute configuration determined comparing HPLC elution order on chiral stationary phase with literature data.[60]

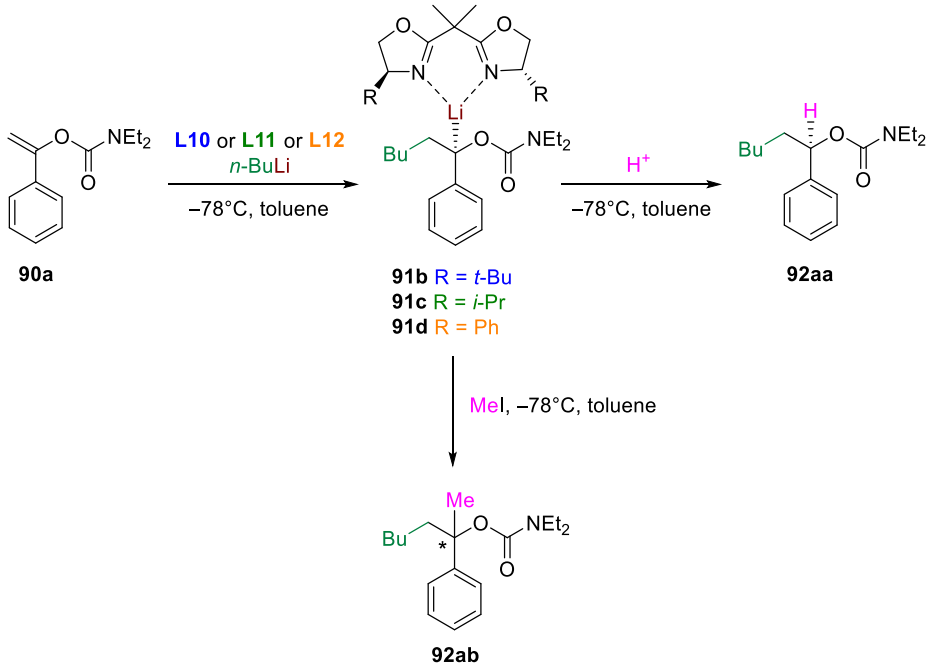
As inferred from Table 1.2, when the carbolithiation of **90a** was carried out using THF as solvent and (+)-sparteine (**L1**) as ligand a racemic mixture was obtained using both MeOH (Table 1.2, entry 1) and MeI (Table 1.2, entry 6) as electrophile reagents. This evidence confirms that coordinating solvents, such as THF, compete with the chiral ligands in the coordination with the substrate, preventing the asymmetric induction. When the reactions were carried out in the presence of toluene/**L1** the carbolithiation step was found to proceed very slowly. In fact, after 3 h of stirring at –78 °C only a partial conversion of the starting substrate **90a** was observed (Table 1.2, entry 2 and 7). Therefore, a further portion of *n*-BuLi was added. However, the large excess of alkyllithium gave rise to the formation of products difficult to identify, compromising the yield of the reactions. In particular, product **6aa** was obtained in only 13 % yield and 20 % e.e. (Table 1.2, entry 2) using MeOH as alectrophile, whilst it was not possible to isolate compound **92ab** (Table 1.2, entry 7). The best results were obtained using **L11** in dry toluene, providing 22% e.e. using both MeOH and MeI as electrophiles and starting from substrate **90a**. In fact, products **92aa** and **92ab** were isolated in 71 and 52 % yield, respectively (Table 1.2, entries 3 and 8). Compound **92aa** was prepared

in very low yields and e.e., 16 % and 6 %, respectively, by performing the reaction in dry toluene with both the chiral ligands **L10** and **L12** (Table 1.2, entries 4 and 5). The enantioselective carbolithiation of compound **90b** was also investigated with the aim to observe an increase in the stereoselectivity due to the presence of an auxiliary binding site, namely the 2-methoxy group. However, carbolithiation of **90b** in the presence of toluene/**L1** followed by reaction with MeOH afforded compound **92ba** in 55 % yield and only 14 % e.e (Table 1.2, entry 9). The products obtained by using the different ligands are highlighted in Schemes 1.48 and 1.49. As shown, in all performed attempts a low asymmetric induction was observed, thus different kind of chiral diamines should be used with the aim to increase the enantioselectivity. The asymmetric induction mechanism proposed in literature[50] suggests that the diastereoisomeric conformers, generated by the coordination of the alkyllithium to the chiral ligand, react with the double bond in an intramolecular *syn* addition to give the enantiomeric benzyllithium derivatives, which are configurationally stable and can be trapped with electrophiles (Scheme 1.25).

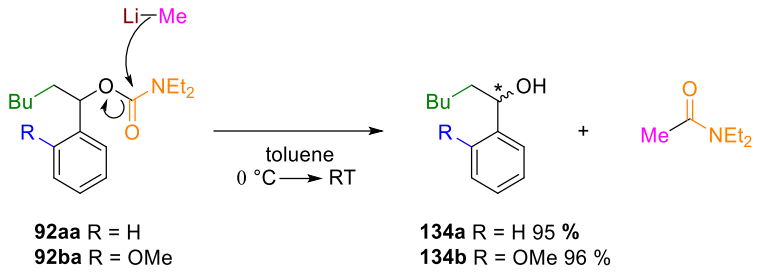
Unfortunately, it was not possible to determine the e.e. for all **92aa**, **92ab** and **92ba** by means of chiral HPLC analysis. Indeed, even employing different chiral stationary phases and several eluent mixtures, the complete resolution of enantiomers has never been achieved. For this reason, compounds **92aa** and **92ba** were first transformed into the corresponding benzyl alcohols and then subjected to chiral HPLC analysis. Thus, the trisubstituted benzyl carbamates **92aa** and **92ba** were treated with an excess of MeLi at 0 °C in dry toluene to give 1-phenylhexanol (**134a**) and 1-(2-methoxyphenyl)hexanol (**134b**) in excellent yields, respectively and acetamide through a nucleophilic acyl substitution (Scheme 1.50). The two enantiomers of **134a** and **134b** were separated on Chiralcel OB chiral stationary phase, giving the enatiomeric excesses reported in Table 1.2.



Scheme 1.48. Enantioselective carbolithiation-trapping process using chiral ligand **L1**; absolute configuration of **92ba** was not determined.



Scheme 1.49. Enantioselective carbolithiation-trapping process using chiral ligand **L10**, **L11** and **L12**; absolute configuration of **92ab** was not determined.



92aa R = H
92ba R = OMe

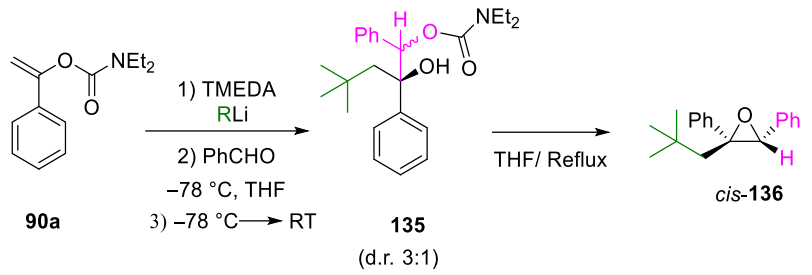
134a R = H 95 %
134b R = OMe 96 %

Scheme 1.50. Synthesis of benzyl alcohols by removal of carbamate group.

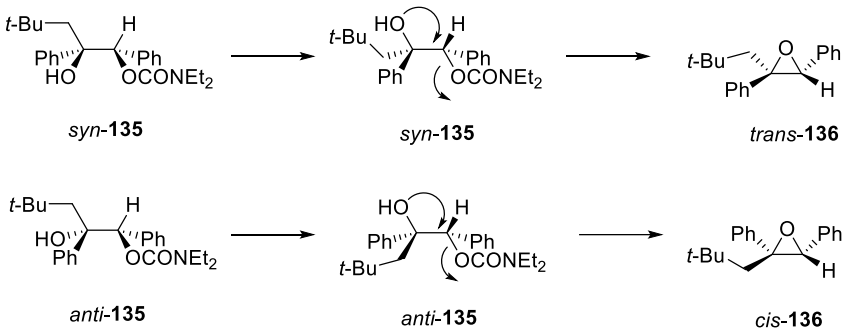
On the contrary, the enantiomeric excess of **90ab** was determined by ^{1}H-NMR shift experiments using the chiral shift reagent (+)-Eu(hfc)$_3$ in deuterated toluene.

1.2.4. Synthesis of cis Epoxides

As reported in literature,[49] when the *t*-BuLi addition to the carbamate **90a** was followed by reaction with PhCHO, a diastereoisomeric mixture (3:1 from ^{1}H-NMR) of **135** is formed, due to a Wittig rearrangement. When this mixture is heated at reflux before workup epoxide **138** with *cis* stereochemistry is the only isolated product (Scheme 1.51). The formation of the epoxide occurrs through an intramolecular substitution mechanism, which leads to the cyclic molecule. In order to give intramolecular cyclisation an antiperiplanar mutual position between the two groups is required. Consequently, the cyclisation of *anti* diastereomer leads to *cis* epoxide, while the cyclisation of *syn* diastereomer affords the *trans* epoxide (Scheme 1.52).

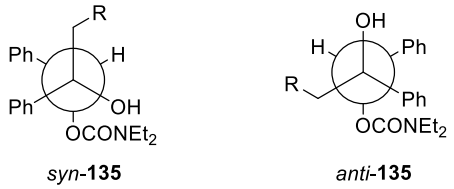


Scheme 1.51. Carbolithiation-trapping reaction of **90a** using *t*-BuLi and PhCHO as electrophile followed by cyclisation leading to epoxide *cis*-**136**.



Scheme 1.52. Cyclisation of *anti*-**135** leads to *cis*-**136**, while the cyclisation of *syn*-**135** affords the *trans*-**136**.

In order to justify the formation of only *cis* epoxide (*cis*-**136**), a conformational analysis of *syn* and *anti* diastereomers (**135**) was carried out in the literature (Figure 1.7).[49] As shown, in the most stable conformer of *anti*-**135** the carbamate and hydroxyl groups are in antiperiplanar mutual position, which is the suitable conformation for the substitution reaction, whilst in the most stable conformer of *syn*-**135** the same groups are in *gauche* conformation, which prevents the cyclisation.



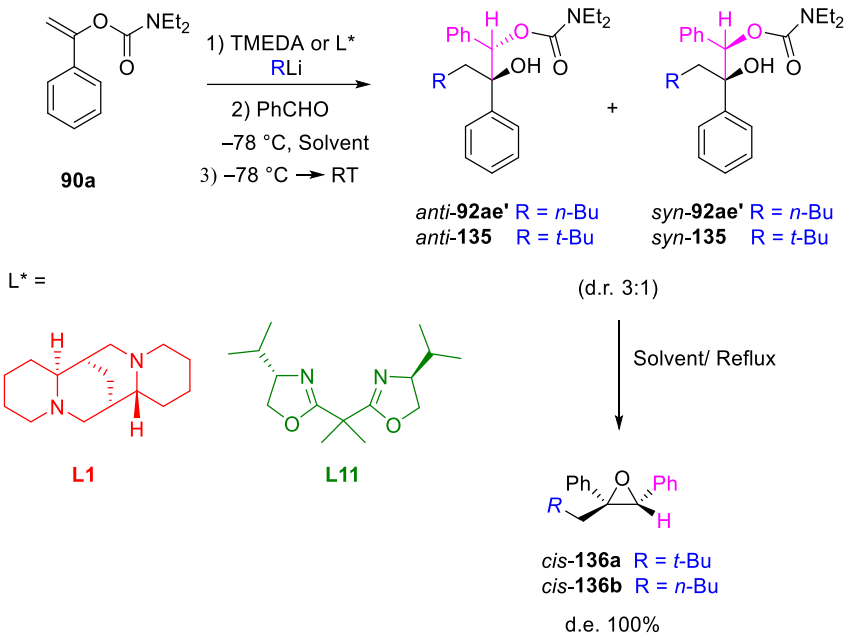
syn-**135** *anti*-**135**

Figure 1.7. Most stable conformers of *syn*-**135** (on the left) and *anti*-**135** (on the right).

This transformation appeared to be a very interesting synthetic methodology, but it was poorly described in literature, lacking experimental details.

Therefore, it was decided to investigate further this reaction in order to provide an original approach to the synthesis of 2-alkyl-2,3-diarylepoxides with *cis* stereochemistry, extending the method also to the preparation of enantioenriched *cis* epoxides by performing the reaction in enantioselective manner using chiral ligands.

Thus, substrate **90a** was subjected to carbolithiation reaction using both *t*-BuLi and *n*-BuLi. This step was followed by trapping with PhCHO (1.4 eq.) at –78 °C, in the presence of TMEDA or a chiral diamine. The subsequent warming up at RT stirring for 15 h afforded the compounds **92ae'** and **135** as a mixture of diastereomers (3:1 from ^{1}H-NMR), which were directly heated at reflux to give *cis* epoxides **136a** and **136b** in 20 and 10 % yield, respectively (Scheme 1.53) (Table 1.3, entries 1 and 2). Unfortunately, in both cases low conversion was achieved, recovering precursors (**92ae'** and **135**) and other side products. The reaction was also attempted varying the solvent, using toluene and hexane (Table 1.3, entries 3 and 4), but the desired product was not observed. Attempts to make enantioselective the procedure, performing the reaction with *t*-BuLi in the presence of **L1** and **L11** as chiral diamine, were also in vain (Table 1.3, entries 1 and 2). The *cis* stereochemistry for both the epoxides (**136a** and **136b**) was confirmed by comparing the experimental ^{1}H-NMR spectra with data reported in the literature.[61]



Scheme 1.53. Carbolithiation-trapping reaction of **90a** followed by cyclisation leading to epoxide *cis*-**136a** and *cis*-**136b**.

Table 1.3. Synthesis of *cis*-epoxides

Entry	RLi	L	Solvent	R	Product	Yield%
1	*n*-BuLi	TMEDA	THF	*n*-Bu	**136b**	10[a]
2	*t*-BuLi	TMEDA	THF	*t*-Bu	**136a**	20[b]
3	*t*-BuLi	TMEDA	Toluene	*t*-Bu	**136a**	–
4	*t*-BuLi	TMEDA	Hexane	*t*-Bu	**136a**	–
5	*t*-BuLi	**L1**	Toluene	*t*-Bu	**136a**	–
6	*t*-BuLi	**L11**	Toluene	*t*-Bu	**136a**	–

[a] Yield determined by ^{1}H-NMR spectrum; [b] Product obtained by chromatographic purification.

1.3. Conclusions

In this project the tandem carbolithiation reaction of 1-aryl-1-alkenyl *N*,*N'*-diethylcarbamates followed by subsequent trapping with electrophiles was investigated. In particular, the use of different electrophiles has been studied and the reaction conditions have been optimised. This procedure provided a method for construction of trisubstituted benzyl carbamates, direct precursors of tertiary benzyl alcohols, in a single pot, creating a chiral quaternary carbon α to oxigen.

The carbolitiation-trapping reaction was also performed in an enantioselective manner using the chiral diamines (+)-sparteine (**L1**), 2,2′-isopropylidenebis[(4*S*)-4-*tert*-butyl-2-oxazoline] (**L10**), 2,2-Bis ((4S)-(-)-4-isopropyloxazoline)-propane (**L11**) and (*S*,*S*)-2,2-bis(4-phenyl-2-oxazolin-2-yl)propane (**L12**), obtaining enantiomeric excesses up to 22%. In particular, the class of BOX ligands have never been used in this kind of reactions. Despite the modest enantioselection the studied reaction showed interesting potential and needed for further optimization.

Future developments of the studied process may include investigations on other diamine ligands, in an attempt to observe a better asymmetric induction.

Moreover, by reacting with benzaldehyde the organolithium intermediate derived from the carbolithiation step, α-hydroxycarbamates were obtained which, by intramolecular cyclisation, led to *cis*-2-alkyl-2,3-diphenyl epoxides with absolute diastereoisomeric control.

1.4. Experimental section

1.4.1.General Information

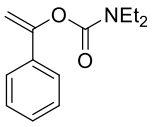
^{1}H (500 or 400 MHz) and ^{13}C (125 or 100 MHz) NMR spectra were recorded in $CDCl_3$ on a Varian INOVA 500 or a Varian INOVA 400 spectrometer, using tetramethylsilane (TMS) as an internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), integration and coupling constants (Hz). GC-MS spectra were recorded on a Hewlett Packard 6890 gas chromatograph, equipped with a mass spectrometric detector HP- 5973 type and a capillary column HP-5MS 30m×0.25 mm. HPLC analyses were performed on a JASCO PU-1580 intelligent HPLC pump equipped with a Varian 2550 UV detector and a CHIRALCEL OBa column. Analytical thin layer chromatography (TLC) was performed using silica gel 60 Macherey-Nagel plates and visualised by ultraviolet radiation and/or spraying the chromatograms with a potassium permanganate solution. Column chromatography separations were carried out using silica gel 60 (70-230mesh). Toluene and THF were freshly distilled before their use on sodium benzophenone ketyl under nitrogen atmosphere. DMF was dried by distillation over calcium hydride and stored under a nitrogen atmosphere. Benzaldehyde was freshly distilled before its use under nitrogen atmosphere and at controlled pressure. Allyl bromide was washed with a saturated solution of $NaHCO_3$ and then dried by distillation over calcium chloride under a nitrogen atmosphere before its use. Commercially available *n*-butyllithium (Aldrich) was a 1.6M solution in hexane. The other analytical grade solvents and commercially available reagents were used without further purification.

1.4.2. Preparation of 1-Aryl-1-Alkenyl N,N'-Diethylcarbamates.

General procedure A

Under dry and inert atmosphere, a round-bottomed flask was charged with anhydrous DMSO (C = 0.4 M) and NaH (60% in oil, 3.5 eq.). The solution was stirred for 2 h at 50 °C until hydrogen evolution ceased and the mixture was then cooled to RT. To the gray solution the aryl ketone (1.0 eq.) in DMSO (C = 3.8 M) was added dropwise in 15 min, the addition being slightly exothermic and changing the color of the solution. This solution was left stirring for 15 min before diethylcarbamoyl chloride (1.1 eq.) in DMSO (C = 4.0 M) was added dropwise in 15 min, while maintaining RT. After stirring for an additional 1 h, water was carefully added to the solution. The mixture was extracted with *n*-hexane and the combined extracts were washed with brine and dried over anhydrous $NaSO_4$. The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2; *n*-hexane:Et_2O 8:2), to afford the product.

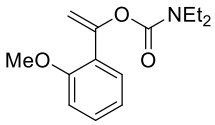
1-Phenylvinyl diethylcarbamate, 90a



Following the general procedure A, starting from acetophenone, the product was isolated as a yellow oil (4.10g, 18.7 mmol, 31%).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.19 (t, 3H, J = 6.5 Hz), 1.28 (t, 3H, J = 7.0 Hz), 3.36 (q, 2H, J = 6.8 Hz), 3.45 (q, 2H, J = 6.8 Hz), 5.06 (s, 1H), 5.45 (s, 1H), 7.30-7.37 (m, 3H), 7.51 (d, 2H, J = 7.5 Hz). **^{13}C-NMR** (125 MHz, $CDCl_3$) δ (ppm): 13.4, 14.4, 37.6, 41.8, 42.1, 124.9, 128.5, 128.7, 135.3, 153.5, 153.9. **GC-MS** (EI) m/z: 219 ([M^+], 12), 103 (8), 100 (100), 91 (5), 77 (11), 72 (54), 44 (12).

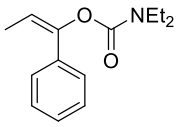
1-(2-methoxyphenyl)Vinyl diethylcarbamate, 90b



Following the general procedure A, starting from 2'-methoxyacetophenone, the product was isolated as a yellow oil (457 mg, 1.84 mmol, 25%).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.14 (t, 3H, J = 6.5 Hz), 1.22 (t, 3H, J = 7.0 Hz), 3.31 (brd, 2H, J = 6.5 Hz), 3.41 (brd, 2H, J = 7.0 Hz), 3.86 (s, 3H), 5.17 (s, 1H), 5.49 (s, 1H), 6.91 (d, 1H, J = 10 Hz), 6.94 (t, 1H, J = 5 Hz), 7.28 (t, 1H, J = 5 Hz), 7.37 (d, 1H, J = 10 Hz). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm):13.5, 14.0, 41.2, 41.6, 55.4, 71.1, 110.5, 120.4, 126.0, 128.0, 130.8,155.4, 156.0. **GC-MS** (EI) m/z: 249 ([M^+], 15), 100 (100), 72 (47), 44 (7).

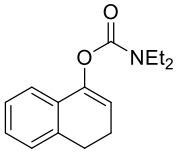
(*Z*)-1-Phenylprop-1-en-1-yl diethylcarbamate, 90c



Following the general procedure A, starting from propiophenone, the product was isolated as a yellow oil (3.72 g, 16.0 mmol, 53%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.17 (t, 3H, J = 7.0 Hz), 1.30 (t, 3H, J = 7.0 Hz), 1.75 (t, 3H, J = 6.8 Hz), 3.36 (q, 2H, J = 6.8 Hz), 3.50 (q, 2H, J = 6.8 Hz), 5.87 (q, 1H, J = 6.8 Hz), 7.24 (t, 1H, J = 8.0 Hz), 7.31 (t, 2H, J = 8.0 Hz), 7.40 (d, 2H, J = 8.4). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 11.4, 13.4, 14.4, 41.7, 42.1, 112.5, 124.2, 127.7, 128.3, 136.0, 147.3, 153.4. **GC-MS** (EI) m/z: 233 ([M^+], 17), 115 (8), 105 (6), 100 (100), 91 (4), 77 (8), 72 (42), 44 (6).

3,4-Dihydronaphthalen-1-yl diethylcarbamate, 90d

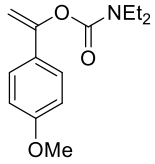


Following the general procedure A, starting from 1-tetralone, the product was isolated as a brown oil (4.35 g, 17.7 mmol, 59%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.19 (t, 3H, J = 7.0 Hz), 1.28 (t, 3H, J = 6.8 Hz), 2.43 (q, 2H, J = 8.4 Hz), 2.86 (t, 2H, J = 8.2 Hz), 3.37 (q, 2H, J = 6.8 Hz), 3.47 (q, 2H, J = 7.0 Hz), 5.72 (t, 1H, J = 4.6 Hz), 7.10-7.17 (m, 4H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.4, 14.3, 22.1, 27.6, 41.8, 42.2, 114.9, 120.7, 126.3, 127.4,

127.6, 131.4, 136.5, 146.0, 154.1. **GC-MS** (EI) m/z: 277 ([M^+], 2), 178 (14), 177 (100), 105 (16), 72 (10), 43 (6). **GC-MS** (EI) m/z: 245 ([M^+], 24), 128 (6), 115 (13), 100 (100), 72 (44), 44 (6).

1-(4-methoxyphenyl)Vinyl diethylcarbamate, 90e



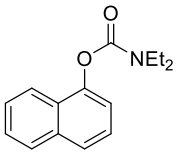
Following the general procedure A, starting from 4′-methoxyacetophenone, the product was isolated as a yellow oil (638 mg, 2.56 mmol, 17%). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 1.17 (t, 3H, J = 6.8 Hz), 1.26 (t, 3H, J = 6.8 Hz), 3.35 (q, 2H, J = 6.8 Hz), 3.45 (q, 2H, J = 6.8 Hz), 4.92 (s, 1H), 5.29 (s, 1H), 6.86 (d, 2H, J = 9.0 Hz), 7.41 (d, 2H, J = 9.0 Hz). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.3, 14.3, 41.7, 42.0, 55.3, 99.6, 113.8, 126.3, 127.9, 153.2, 153.9, 159.9. **GC-MS** (EI) m/z: 249 ([M^+], 11), 133 (6), 100 (100), 72 (49), 44 (9).

1.4.3. Preparation of Naphthyl N,N'-Diethylcarbamates.

General procedure B

Under dry and inert atmosphere, a round-bottomed flask was charged with anhydrous THF (C = 1.5 M) and NaH (60% in oil, 2.5 eq.) at 0 °C. The solution was stirred until solid was completely dissolved. To this mixture a solution of 1-naphtol or 2-naphtol (1.0 eq.) in THF (C = 2.8 M) was added dropwise. The pale yellow solution was stirred at 0 °C for 1 h. Then, a solution of diethylcarbamoyl chloride (1.1 eq.) in THF (C = 4.0 M) was slowly added and the mixture was stirred at RT for 2 h. After this time, a saturated solution of NH_4Cl was added and the mixture was extracted with EtOAc and the combined organic layers were washed with brine and dried over anhydrous $NaSO_4$. The solvent was evaporated under reduced pressure to afford the product as a pink solid, which was used without further purification.

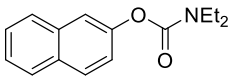
Naphthalen-1-yl diethylcarbamate, **90f**



Following the general procedure B, starting from 1-naphtol the product was isolated as a pink solid (3.83 g, 15.0 mmol, > 99%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.25 (t, 3H, J = 7.0 Hz), 1.38 (t, 3H, J = 7.0 Hz), 3.45 (q, 2H, J = 7.0 Hz), 3.61 (q, 2H, J = 7.0 Hz), 7.28 (d, 1H, J = 7.6 Hz), 7.43-7.52 (m, 3H), 7.70 (d, 1H, 8.0 Hz), 7.82 (d, 1H, J = 8.0 Hz), 7.92 (d, 1H, J = 6.8 Hz). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.4, 14.5, 42.0, 42.3, 118.1, 121.3, 125.3, 125.5, 126.2, 127.5, 127.9, 1346, 147.3, 154.2. **GC-MS** (EI) m/z: 243 ([M^+], 21), 144 (7), 127 (7), 115 (25), 100 (100), 72 (48), 44 (7).

Naphthalen-2-yl diethylcarbamate, 90g



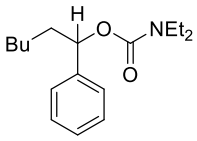
Following the general procedure B, starting from 2-naphtol the product was isolated as a pink solid (4.17 g, 17.0 mmol, > 99%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.22 (t, 3H, J = 6.8 Hz), 1.27 (t, 3H, J = 6.8 Hz), 3.41 (d, 2H, J = 7.0 Hz), 3.49 (d, 2H, J = 7.0 Hz), 7.28 (d, 1H, J = 8.8 Hz), 7.40-7.48 (m, 2H), 7.58 (s, 1H), 7.78 (d, 1H, J = 7.6 Hz), 7.82 (d, 1H, J = 8.8 Hz). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.4, 14.3, 41.9, 42.3, 118.4, 121.7, 125.3, 126.3, 127.5, 127.7, 129.1, 131.1, 133.8, 149.2, 154.3. **GC-MS** (EI) m/z: 243 ([M^+], 31), 144 (9), 127 (6), 115 (25), 100 (100), 72 (47), 44 (7).

1.4.4.Addition of n-BuLi to 1-Aryl-1-Alkenyl N,N'-Diethylcarbamates followed by electrophile trapping. General procedure

Under dry and inert atmosphere, to a solution of 1-aryl-1-alkenyl *N,N'*-diethylcarbamate (1.0 eq.) and TMEDA (1.1 eq.) in anhydrous THF (C = 0.07 M) at -78 °C a solution of *n*-BuLi (1.6 M in *n*-hexane, 1.1 eq.) was added. After 15-30 minutes of stirring at -78 °C, the electrophile (3.0 eq.) was added and the reaction mixture was stirred at the same temperature for 1 h. After this time water was added to quench the reaction. The mixture was extracted with Et_2O and the combined organic layers were washed with a saturated solution of NH_4Cl, then brine and dried over anhydrous $NaSO_4$. The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2; *n*-hexane:Et_2O 8:2), to afford the product.

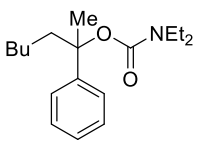
1-Phenylhexyl diethylcarbamate, 92aa



Following the general procedure, starting from 1-phenylvinyl diethylcarbamate (**90a**), the product was isolated as a yellow oil (180 mg, 0.65 mmol, 95 %) without purification.

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.85 (t, 3H, J = 7 Hz), 1.05-1.15 (m, 6H), 1.25-1.28 (m, 6H), 3.10-3.30 (m, 4H), 5.65 (dt, 1H, J = 1.5 Hz, J = 6.5 Hz), 7.29-7.32 (m, 5H). **^{13}C-NMR** (125 MHz, $CDCl_3$) δ (ppm): 13.9, 22.5, 25.1, 31.5, 36.8, 76.6, 126.3, 127.4, 128.2, 141.9, 155.4. **GC-MS** (EI) m/z: 277 ([M^+], 3); 161 (14); 117 (8); 105 (18); 100 (10); 91 (100); 72 (6); 44 (4).

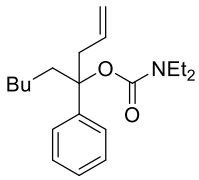
2-Phenylheptan-2-yl diethylcarbamate, 92ab



Following the general procedure, starting from 1-phenylvinyl diethylcarbamate (**90a**), the product was isolated as a yellow oil (182 mg, 0.62 mmol, 92 %).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.83 (t, 3H, J = 7 Hz), 1.05-1.15 (m, 6H), 1.21-1.26 (m, 6H), 1.85 (s, 3H), 3.20-3.40 (m, 4H), 7.29-7.33 (m, 5H). **^{13}C-NMR** (125 MHz, $CDCl_3$) δ (ppm): 13.9, 22.5, 23.4, 25.6, 31.9, 41.5, 43.0, 83.1, 124.5, 126.4, 128.0, 146.3. **GC-MS** (EI) m/z: 291 ([M^+], 2), 175 (32), 131 (14), 118 (30), 105 (100), 91 (52), 72 (12), 44 (6).

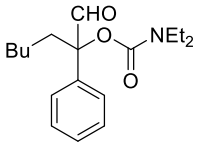
4-Phenylnon-1-en-4-yl diethylcarbamate, 92ac



Following the general procedure, starting from 1-phenylvinyl diethylcarbamate (**90a**), the product was isolated as a yellow oil (178 mg, 0.56 mmol, 83 %) without purification.

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.82 (t, 3H, J = 6.5 Hz), 1.05-1.14 (m, 6H), 1.16-1.27 (m, 6H), 2.01 (dt, 1H, J = 2 Hz, J = 11.5 Hz), 2.38 (dt, 1H, J = 2.5 Hz, J = 10 Hz), 3.30 (m, 4H,), 5.00 (ddd, 2H, J = 1 Hz, J = 10 Hz, J = 17.5), 5.53 (ddt, 1H, J = 7.5 Hz, J = 10.5 Hz), 7.26-7.32 (m, 5H).

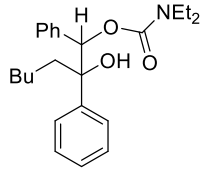
1-Oxo-2-phenylheptan-2-yl diethylcarbamate, 92ad



Following the general procedure, starting from 1-phenylvinyl diethylcarbamate (**90a**), the product was obtained (37 mg, 0.12 mmol, 18 %, determined by ^{1}H-NMR).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.76 (t, 3H, J = 10.5 Hz); 1.00-1.21 (m, 6H); 1.23-1.29 (m, 6H); 1.90-196 (m, 1H); 2.02-2.06 (m, 6H); 3.30 (m, 4H); 7.45-7.55 (m, 5H); 9.46 (s, 1H).

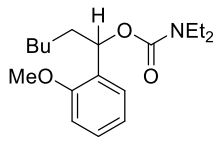
2-Hydroxy-1,2-diphenylheptyl diethylcarbamate, 92ae'



Following the general procedure, starting from 1-phenylvinyl diethylcarbamate (**90a**), the product was isolated as a yellow oil (68 mg, 0.18 mmol, 26 %).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 0.77 (t, 3H, J = 7 Hz), 0.97 (t, 3H, J = 7 Hz), 1.10-1.30 (m, 6H), 1.84-1.96 (m, 2H), 3.09-3.19 (m, 4H), 5.90 (s,1H, major diastereomer), 5.94 (s,1H, minor diastereomer), 7.24-7.41 (m, 10H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.9, 22.5, 25.1, 32.0, 37.6, 41.8, 42.2, 80.5, 84.7, 127.3,127.9, 128.1, 128.3, 128.5, 136.2, 143.3,155.7. **GC-MS** (EI) m/z: 207 (94), 177 (35), 148 (35), 116 (70), 100 (86), 91(100), 72 (29), 43 (21).

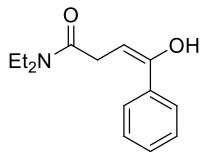
1-(2-methoxyphenyl)Hexyl diethylcarbamate, 92ba



Following the general procedure, starting from 1-(2-methoxyphenyl)vinyl diethylcarbamate (**90b**), the product was isolated as a yellow oil (83 mg, 0.27 mmol, 68 %).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.86 (t, 3H, J = 7.2 Hz), 1.14 (brd, 6H, J = 29.5 Hz), 1.26-1.35 (m, 6H), 1.77 (m, 2H), 3.30 (brs, 4H), 3.83 (s, 3H), 6.01 (t, 1H, J = 6.5 Hz), 6.85 (d, 1H, J = 8.5 Hz), 6.93 (t, 1H, J = 7.5 Hz), 7.21 (t, 1H, J = 8.5 Hz), 7.27 (d, 1H, J = 10.5 Hz). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.7, 14.0, 21.2, 22.5, 25.0, 29.7, 31.6, 35.9, 55.4, 71.1, 110.5, 120.4, 126.0, 128.0, 130.9, 155.4, 156.0. **GC-MS** (EI) m/z: 307 ([M^+], 2), 191 (34), 147 (4), 121 (100), 91 (14).

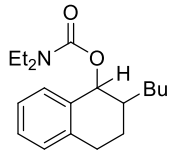
***N*,*N*-diethyl-4-hydroxy-4-phenylbut-3-enamide, 131**



Following the general procedure, starting from 1-Phenylprop-1-en-1-yl diethylcarbamate (**90c**), the side-product was obtained as a yellow oil (12 mg, 0.05 mmol, 8 %).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.15 (t, 3H, J = 7.0 Hz), 1.27 (t, 3H, J = 6.8 Hz), 2.36 (d, 2H, J = 6.8 Hz), 3.34 (q, 2H, J = 6.8 Hz), 3.46 (q, 2H, J = 6.8 Hz), 5.84 (t, 1H, J = 6.8 Hz), 7.24 (t, 1H, J = 7.2 Hz), 7.31 (t, 2H, J = 7.2 Hz), 7.42 (d, 2H, J = 7.2 Hz). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.4, 14.4, 25.5, 41.7, 42.0, 117.0, 124.4, 127.7, 128.3, 135.9, 147.0, 153.4. **GC-MS** (EI) m/z: 215 ([M^+ –18], 4), 173 (4), 115 (3), 100 (100), 72 (29), 44 (6).

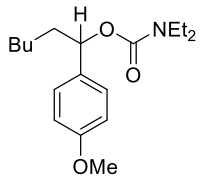
2-Butyl-1,2,3,4-tetrahydronaphthalen-1-yl diethylcarbamate, 92da



Following the general procedure, starting from 3,4-dihydronaphthalen-1-yl diethylcarbamate (**90d**), the product, a yellow oil, was obtained as a mixture of diastereoisomers (d.r. 5:1) (43 mg, 0.14 mmol, 23 %).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.89 (t, 3H, J = 9.6 Hz, major diastereomer), 0.90 (t, 3H, J = 9.6 Hz, minor diastereomer), 1.04 (brs, 3H), 1.16 (brs, 3H), 1.31 (brs, 4H), 1.43 (brs, 2H), 1.64 (m, 1H), 1.80 (m, 1H), 2.06 (m, 1H), 2.80 (t, 2H, J = 8.5 Hz), 3.28 (brd, 4H, J = 55 Hz), 5.70 (d, 1H, J = 6.0 Hz, major diastereomer), 5.95 (d, 1H, J = 2.0 Hz, minor diastereomer), 7.10 (d, 1H, J = 9.0 Hz), 7.13-7.23 (m, 2H), 7.28 (d, 1H, J = 7.0 Hz). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.6, 14.2, 22.9, 24.0, 26.6, 28.8 (minor diastereomer), 29.1 (major diastereomer), 30.3 (major diastereomer), 31.0 (minor diastereomer), 38.6 (minor diastereomer), 38.7 (major diastereomer), 41.1, 41.9, 72.0 (minor diastereomer), 74.8 (major diastereomer), 125.8 (minor diastereomer), 125.9 (major diastereomer), 127.4 (major diastereomer), 127.8 (minor diastereomer), 128.6 (major diastereomer), 128.7 (minor diastereomer), 129.5 (major diastereomer), 130.4 (minor diastereomer), 135.5 (major diastereomer), 136.1 (minor diastereomer), 137.3 (minor diastereomer), 137.4 (major diastereomer), 156.2. **GC-MS** (EI) m/z: 303 ([M^+], 3), 144 (7), 187 (68), 143 (12), 131 (49), 117 (100), 100 (12), 72 (6).

1-(4-methoxyphenyl)Hexyl diethylcarbamate, 92ea

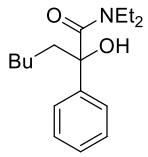


Following the general procedure, starting from 1-(4-methoxyphenyl)vinyl diethylcarbamate (**n**), the product was isolated as a yellow oil (158 mg, 0.51 mmol, 83 %) without purification.

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 0.85 (t, 3H, J = 6.0 Hz), 1.10 (brs, 6H), 1.27 (brs, 6H), 1.73 (m, 1H), 1.89 (m, 1H), 3.27 (brs, 4H), 3.79 (s, 3H), 5.60 (t, 1H, J = 7.2 Hz), 6.86 (d, 2H, J = 8.8 Hz), 7.25 (d, 2H, J = 8.0 Hz). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.5, 14.0, 14.2, 22.5, 25.2, 31.5, 36.7, 41.2, 41.7, 55.2, 113.6, 127.7, 134.0, 155.4, 158.9.

1.4.5. Carbolithiation reaction followed by [1,2] Wittig Rearrangement

***N*,*N*-diethyl-2-hydroxy-2-phenylheptanamide, 133**



Under dry and inert atmosphere, to a solution of 1-phenylvinyl diethylcarbamate (**90a**) (150 mg, 0.68 mmol, 1.0 eq.) and TMEDA (112 µL, 0.75 mmol, 1.1 eq.) in anhydrous THF (10 mL) at -78 °C, a solution of *n*-BuLi (1.6 M in *n*-hexane, 0.5 mL, 0.75 mmol, 1.1 eq.) was added. After 10 minutes of stirring at -78 °C, the solution was slowly wormed up to RT. After 2 h of stirring at RT, water was added to quench the reaction. The mixture was extracted with Et_2O and the combined organic layers were washed with a saturated solution of NH_4Cl, brine and dried over anhydrous $NaSO_4$. The solvent was evaporated under reduced pressure, affording the product as a yellow oil (162 mg, 0.58 mmol, 86 %).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 0.89 (t, 3H, J = 7.6 Hz), 1.08-1.18 (m, 6H), 1.25-1.36 (m, 6H), 2.17 (ddd, 2H, J = 5 Hz, J = 12 Hz, J = 28 Hz), 3.28-3.47 (m, 4H), 3.73 (t, 1H, J = 6.4 Hz), 7.30-7.36 (m, 5H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 12.5, 14,0, 22.6, 23.2, 32.1, 36.4, 41.3, 76.8, 125.8, 127.6, 128.6, 143.5, 173.4. **GC-MS** (EI) m/z: 277 ([M^+], 2), 178 (14), 177 (100), 105 (16), 72 (10), 43 (6).

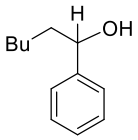
1.4.6. Enantioselective Carbolithiation: Asimmetric synthesis of benzyl carbamates.General procedure

Under dry and inert atmosphere, to a solution of *n*-BuLi (1.6 M in *n*-hexane, 1.1 eq.) and chiral ligand (1.1 eq.) in anhydrous toluene (C = 0.07 M) at -78 °C 1-aryl-1-alkenyl *N,N'*-diethylcarbamate (n1 or n2) (1.0 eq.) was added. After 15-30 minutes of stirring at -78 °C, the electrophile (3.0 eq.) was added and the reaction mixture was stirred at the same temperature for ca. 1 h. After this time, water was added to quench the reaction. The mixture was extracted with Et_2O and the combined organic layers were washed with a saturated solution of NH_4Cl, then brine and dried over anhydrous $NaSO_4$. The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2; *n*-hexane:Et_2O 8:2), to afford the desired product (**92aa**, **92ab** and **92ba**, yields and e.e. reported in Table 1.2).

1.4.7. Removal of Carbamate group. General procedure

Under dry and inert atmosphere, the carbamate (1.0 eq.) was dissolved in anhydrous toluene (C = 0.08 M). The solution was cooled to 0 °C and MeLi (1.6 M in *n*-hexane, 2.5 eq.) was added. The reaction mixture was stirred for 1 h at RT. After this time, NH_4Cl was added to quench the reaction. The mixture was extracted with Et_2O, the combined organic layers were washed with brine and dried over anhydrous $NaSO_4$. The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2; *n*-hexane:Et_2O 8:2), to afford the desired product.

1-phenylhexan-1-ol, 134a

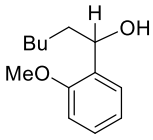


Following the general procedure, starting from 1-phenylhexyl diethylcarbamate (**92aa**), the product was isolated as a yellow oil (18 mg, 0.10 mmol, 95 %).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.89 (t, 3H, J = 7.5 Hz), 1.27-1.32 (m, 6H), 1.68-1.78 (m, 1H), 1.80-1.86 (m, 1H), 2.4 (s, 1H), 4.68 (dd, 1H, J = 1.5 Hz, J = 6 Hz), 7.34-7.40 (m, 5H). **^{13}C-NMR** (125 MHz, $CDCl_3$) δ (ppm): 14.1, 22.6, 29.7, 31.7, 39.1, 74.7, 125.9, 127.5, 128.4, 144.9.

The enantiomers were separated by HPLC on Chiralcel-OB column, hexane:2-propanol 95:5, 254 nm: t_R (*S*) = 10.2 min, t_R (*R*) = 12.5 min. The alcohol **134a** was found to possess 20 % e.e. (with ligand **L1**), 22 % e.e. (with ligand **L11**), 16 % e.e. (with ligand **L10**), 6 % e.e. (with ligand **L12**).

1-(2-methoxyphenyl)hexan-1-ol, 134b



Following the general procedure, starting from 1-(2-methoxyphenyl)vinyl diethylcarbamate (**92ba**), the product was isolated as a yellow oil (31 mg, 0.15 mmol, 96 %).

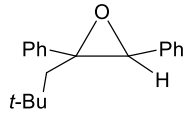
^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 0.88 (brs, 3H), 1.12-1.20 (m, 1H), 1.21-1.39 (m, 4H), 1.40-1.54 (m, 1H), 1.69-1.85 (m, 2H), 3.85 (s, 3H), 4.85 (t, 1H, J = 6.8 Hz), 6.87 (d, 1H, J = 8.0 Hz), 6.95 (t, 1H, J = 7.4 Hz), 7.23 (t, 1H, J = 8.2 Hz), 7.29 (d, 1H, J = 7.2 Hz). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 14.0, 22.6, 25.8, 31.7, 37.2, 55.2, 71.0, 110.5, 120.7, 126.9, 128.1, 132.7, 156.5.

The enantiomers were separated by HPLC on Chiralcel-OB column, hexane:2-propanol 98:2, 254 nm: t_R = 14.0 min, t_R = 17.6 min. The alcohol 96b was found to possess 14 % e.e., using ligand **L1**.

1.4.8. Synthesis of cis-Epoxides. General procedure

Under dry and inert atmosphere, to a solution of 1-phenyl-1-alkenyl *N,N'*-diethylcarbamate (1.0 eq.) and TMEDA (1.1 eq.) in anhydrous THF (C = 0.07 M) a solution of *n*-BuLi (1.6 M in *n*-hexane, 1.1 eq.) or *t*-BuLi (1.7 M in pentane, 1.1 eq.) was added at -78 °C. After 15-30 minutes of stirring at -78 °C, benzaldehyde (1.4 eq.) was added and the reaction mixture was stirred at the same temperature for 1 h. After this time, the reaction mixture was allowed to warm to RT over 16 h and then was stirred at reflux. The solution was cooled down to RT and water was added to quench the reaction. The mixture was extracted with Et_2O, the combined organic layers were washed with a saturated solution of NH_4Cl, brine and dried over anhydrous $NaSO_4$. The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2; *n*-hexane:Et_2O 8:2), to afford the product.

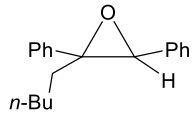
2-neopentyl-2,3-diphenyloxirane, 136a



Following the general procedure, using *t*-BuLi, the product was obtained after 24 h of stirring at reflux and isolated as a yellow solid (37 mg, 0.14 mmol, 20 %).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.84 (s, 9H), 1.81 (d, 1H, J = 14 Hz), 2.29 (d, 1H, J = 15 Hz), 4.05 (s, 1H), 7.00-7.20 (m, 10H). **^{13}C-NMR** (125 MHz, $CDCl_3$) δ (ppm): 30.5, 31.9, 52.0, 64.7, 67.8, 126.4, 126.8, 127.1, 127.4, 127.5, 128.0, 135.5, 137.6.

2-pentyl-2,3-diphenyloxirane, 136b



Following the general procedure, using *n*-BuLi, the product was obtained after 6 days of stirring at reflux (18 mg, 0.07 mmol, 10 %, determined by ^{1}H-NMR spectrum).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.86 (t, 3H, J = 6.5 Hz), 1.25-1.30 (m, 6H), 1.82-187 (m, 1H), 2.125 (dt, 1H, J = 4 Hz, J = 10.5 Hz) 4.13 (s, 1H) 7.08-7.18 (m, 10H).

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Chapter 2

Asymmetric Synthesis of the Fungal Phytotoxins Colletochlorin A and Radicinin

2.1. Introduction

2.1.1. The Importance of Chirality in Bioactive Compounds

Chirality is ubiquitous in living organisms. The three most important and most abundant class of natural products, peptides, nucleic acids and carbohydrates, are all chiral. Consequently, chirality plays a fundamental role in the interaction of all living matters with their environment. In general, the different stereoisomers of any biologically active compound interact differently with the receptors, transport proteins, membranes etc. of living organisms. Therefore, the two enantiomers of a chiral substance show in general different pharmacology, different pharmacokinetics as for all ADME parameters (absorption, distribution, metabolism, excretion), and different toxicology.[1] While the desired biological activity is often associated with only one enantiomer, the other enantiomer may be less effective, or totally ineffective, or have unwanted effects, or even be an antagonist of its antipode. For this reason, an increasingly larger portion of chiral drugs is patented as single enantiomer (about 90% between 2004-2006 in USA).[2] A recent report by Global Industry Analysts has foreseen that nearly 95% of all drugs will be chiral by 2020.[3] This indicate how much the control of chirality is a feature to be considered when the synthesis of a natural bioactive compound is faced. Moreover, especially when structure-activity studies are carried out, the complete structure elucidation of bioactive product, including a full stereochemical assignement, is necessary.

2.1.2. Fungal Bioactive Compounds as Biological Herbicides

Fungi constitute an essentially endless source of bioactive metabolites, often showing phytotoxic activity. Their large structural diversity reflects in a wide variety of bioactivities and potential applications in both medicine and agriculture. One of the most appealing application of fungal phytotoxins lies in the development of bioherbicides, which show lower or no toxicity, and thus a lower environmental and ecological impact, than the traditional synthetic pesticides. For this reason, the studies on the production, identification and chemical and biological characterisation of fungal secondary metabolites is receiving great attention.

The isolation of phytotoxins from weed pathogens constitutes the starting point for the search of new and selective bioherbicides. Recently, from the culture filtrates of the fungus *Colletotrichum gloeosporiodes* was isolated for the first time and identified the known phytotoxin colletochlorin A (**1a**). This compound was found to show significantly phytotoxic activity against the host plant *Ambrosia artemisiifolia*, revealing its potential employment as a natural bioherbicide against this weed.[4] *A. artemisiifolia*, also known as common ragweed, is a widespread invasive weed native to North America, which is very competitive and forms large populations in natural or uncultivated areas, gardens, railway ballast, yards, roadsides and cultivated fields after harvest.[5] This plant is then becoming a major weed causing severe crop losses and troubles for its control.[6] Moreover, the major concern regarding *A. artemisiifolia* is its highly allergenic pollen that causes serious allergies in humans, generating huge medical costs. For ragweed, its chemical herbicides and mechanical control are effective as short-term measures in small infested areas, but are rather insufficient in larger areas and in natural environments. Therefore, biological control, employing microorganisms or their toxic metabolites, has been considered a potential alternative approach for managing this weed.[7] As described before, colletochlorin A (**1a**) appeared as an ideal compound to develop bioherbicides against *A. artemisiifolia* and the development the total enatioselective synthesis of this phytotoxin represents an alternative for its large-scale preparation in respect to the inefficient fermentative production.

Another promising compound to be used as bioherbicides is radicinin (**2a**). Radicinin (**2a**) was isolated as a phytotoxic metabolite by *Pennisetum ciliare* or *Cenchrus ciliaris* foliar

pathogen *Cochliobolus australiensis*. Radicinin (**2a**) was already known being first isolated from the fungus *Stemphylium radicinum* and later by many other fungal species. Radicinin (**2a**) demonstrated high target-specific toxicity on *P. ciliare*, low toxicity to native plants, and no teratogenic, sub-lethal, or lethal effects on zebrafish (*Brachydanio rerio*) embryos, thus its use as herbicide for the biocontrol of this plant could be envisaged.[8] *P. ciliare*, whose common name is buffelgrass, is a perennial grass-weed native to Africa, the Mediterranean area, and Middle-East, which has become highly invasive in North America and Australia, where it is a menace for the native vegetation, infesting roadsides and urban landscapes and promoting wildfires. Therefore, buffelgrass control is an emerging concern in those countries, where it is now carried out by broad-spectrum herbicides which, however, can heavily damage non-target native plants. Total synthesis of radicinin (**2a**) and/or its analogues could be a very useful tool to provide suitable amounts of such compounds necessary to manage naturally that invasive weed. In fact, by the synthetic preparation, it would be possible to have amounts of the target compounds much greater than those obtainable from natural sources.

2.1.3. Aims of the project

The aim of this project was to study the structure-activity relationship for the fungal metabolites colletorin A (**1a**), colletochlorin A (**1b**) (Figure 2.1), radicinin (**2a**) and its natural precursor 3-deoxyradicinin (**2b**) (Figure2.2). In order to assay the biological properties and in particular, to develop target-specific bioherbicides for invasive plants, namely *Ambrosia artemisifolia* and buffelgrass, it is necessary to have amounts of the compounds much larger than those obtainable from natural sources. For this reason, the development of an efficient total synthesis of these products has appeared mandatory. Of course, in order to investigate the biological properties and define an appropriate structure-activity relationship, a full knowledge of the molecular structures, including the stereochemistry, of the compounds is necessary. Indeed the knowledge of the absolute configuration of a chiral molecule is a fundamental prerequisite for the understanding and prediction of its interactions with chiral systems both at molecular and supramolecular

levels. In fact, the role of the absolute configuration on the bioactive properties, well known for the majority of chiral compounds,[9] has also been clearly recognised in fungal metabolites.[10]

Therefore, a new stereoselective synthesis of both enantiomers of **1a** and **1b**, as well as their brominated (**1c**) and fluorinated (**1d**) analogues, in optically active form (Figure 2.1), was designed, with the aim to investigate the effect of both absolute stereochemistry and halogen nature on the biological properties of this class of phytotoxins.[11]

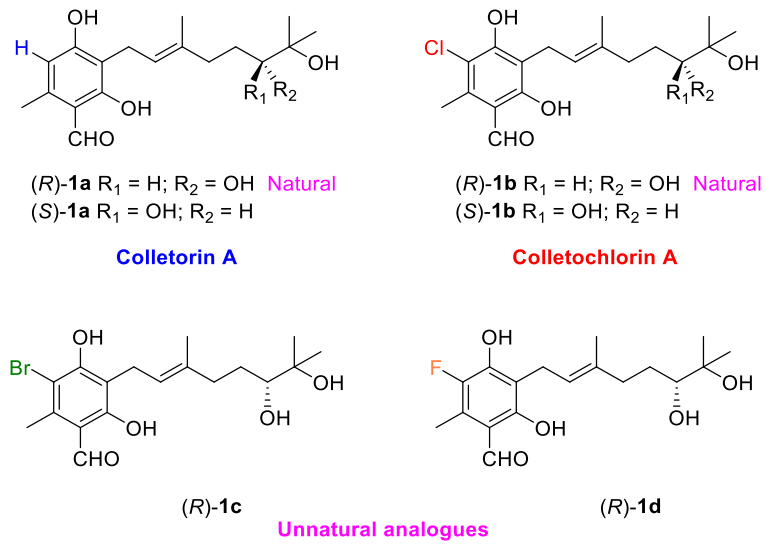
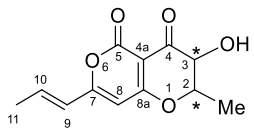


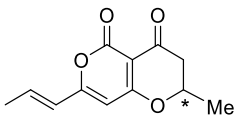
Figure 2.1. Structure of colletorins A (**1a**), colletochlorins A (**1b**) and the unnatural analogues, the brominated (**1c**) and fluorinated (**1d**).

Moreover, an efficient synthetic strategy in order to prepare (±)-radicinin (**2a**) and its immediate biosynthetic precursor, (±)-3-deoxyradicinin (**2b**) (Figure2.2), in high yield, starting from commercially available products was ideated. Such a synthetic method was also designed with the aim to be versatile for the synthesis of analogues of **2a** and **2b** with different side-chains at C-7. The availability of racemic **2a** as well as of the deoxygenated derivative **2b** could allow to carry out detailed structure-activity studies on the role of both the absolute and relative stereochemistry on the bioactivity of these compounds. In particular, the synthesis of racemic (±)-3-deoxyradicinin (**2b**) can be useful to define the effect of the presence of the α-hydroxyl moiety at C-3. In fact, a recent structure-activity

relationship study carried out on **2a** demonstrated that the presence of an α,β-unsaturated carbonyl group at C-4 was essential for the phytotoxic activity and that the double bond of the side chain at C-7 also played a role to impart activity.[12] However, the effect on phytotoxicity of the hydroxyl group at C-3 was not ascertained with certainty. The absolute configuration at C-3 seemed to have some role, because 3-*epi*-radicinin was found to be less active, but the effect on the absolute stereochemistry at C-2 was not investigated.[12]
Therefore, it appears clear that the synthesis of these fungal metabolites is essential to establish unequivocally the relationship that exists between the structure of these molecules and their biological properties, especially seeking their use as natural bioherbicides.



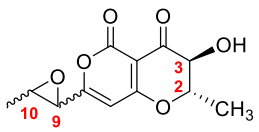
(±)-Radicinin (**2a**)



(±)-3-Deoxyradicinin (**2b**)

Figure 2.2. Stuctures of (±)-radicinin (**2a**) and (±)-3-deoxyradicinin (**2b**).

Moreover, a new isolated compound, namely cochliotoxin (**2a'**), has also shown phytotoxic activity against buffelgrass and consequently it could potentially be used as natural herbicide in the search for novel control strategies against this weed. However, while the structure of radicinin (**2a**) was completely defined, including its absolute stereochemistry, the absolute configuration at C-9 and C-10 of cochliotoxin (**2a'**) has not yet been assigned (Figure 2.3). For this reason, it was decided to employ the method of quantum mechanical simulations of chiroptical properties, such as electronic circular dichroism (ECD) and optical rotation (OR), for the assignment of the absolute configuration[13] of cochliotoxin.



Cochliotoxin (**2a'**)

Figure2.3. Structure of cochliotoxin (**2a'**).

2.2. Results and Discussion

2.2.1. The Fungal Metabolites Colletochlorin A and Colletorin A

As previously mentioned, fungi are able to produce a large number of bioactive secondary metabolites, thus representing an excellent source of pharmaceutical, antifungal, and herbicidal compounds. Among these, colletorins and colletochlorins are two classes of interesting bioactive metabolites isolated for the first time from *Colletotrichum nicotianae*, a fungus causing anthracnose disease in tobacco plants (Figure 2.4).[14] Such compounds share as common structural feature a multi-substituted polyphenolic ring joined to a prenyl or diprenyl side chain. Some of them have this terpene moiety partly oxidised at the terminal double bond, displaying one or two hydroxylated stereogenic centres. This is the case of colletorin A (**1a**) and colletochlorin A (**1b**) (Figure 2.4), which possess a chiral diol moiety. Recently, colletochlorin A (**1b**) has also been isolated from *Colletotrichum higginsianum*[15] and *Colletotrichum gloeosporioides*.[4] These compounds showed different interesting biological properties. In particular, the structurally-related colletochlorin B (Figure 2.4) displayed promising pharmaceutical activity being able to readily induce the differentiation of human promyelocytic cells (HL-60),[16] associated with the onset of diseases such as acute promyelocytic leukemia and neutrophilic leukopenia. Colletochlorin B also showed inhibitory activity toward the enzymes acetylcholinesterase (AChE) and β-glucuronidase, as well as some toxicity toward human lung fibroblasts.[17] Moreover, a recent study has also revealed the phytotoxic activity of **1b** against *Ambrosia artemisiifolia*,[4] allowing to envisage its possible use as natural herbicide for the control of this weed responsible for serious allergies in humans. In this latter study, the (*R*) absolute configuration at the hydroxylated stereocentre of naturally occurring (+)-**1b** has also been determined by application of the modified Mosher's method,[4] while the absolute configuration of **1a** was still unknown. However, only a few studies have been undertaken to investigate their biological properties due to the scarce availability of these compounds from natural sources, as fungi produce these metabolites in very small amounts. Solely few approaches for the synthetic preparation of **1a** and **1b** were reported in the literature, none providing them in an optically active form. In particular, only a single synthesis of racemic **1b** was described,[18] employing, as a key

step, a lithium-mediated C-alkylation of 1,5-dimethoxy-3-methyl-1,4-cyclohexadiene with the dihydroxylated side chain, in turn obtained from geranyl acetate. Nevertheless, this strategy provided the desired compound in a very low yield and was not applicable neither to the synthesis of **1a** nor to the preparation of any halogenated analogues (Scheme 2.1).

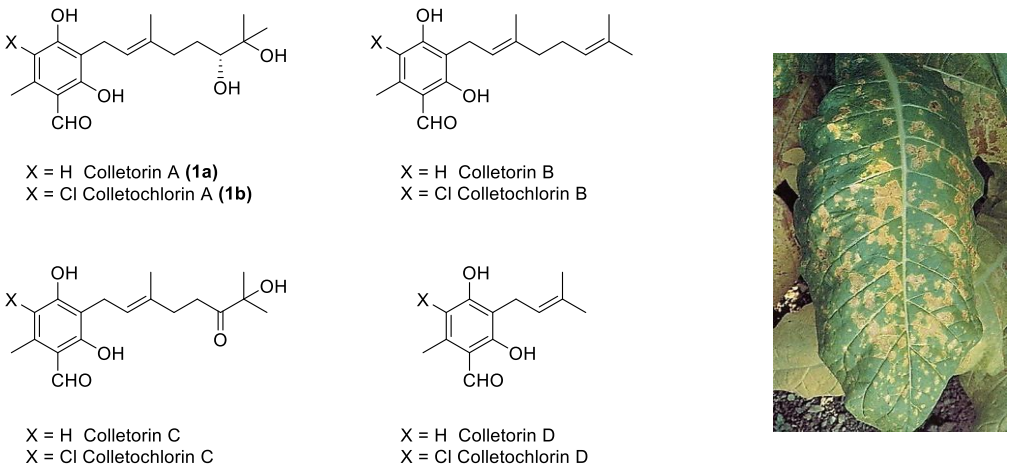
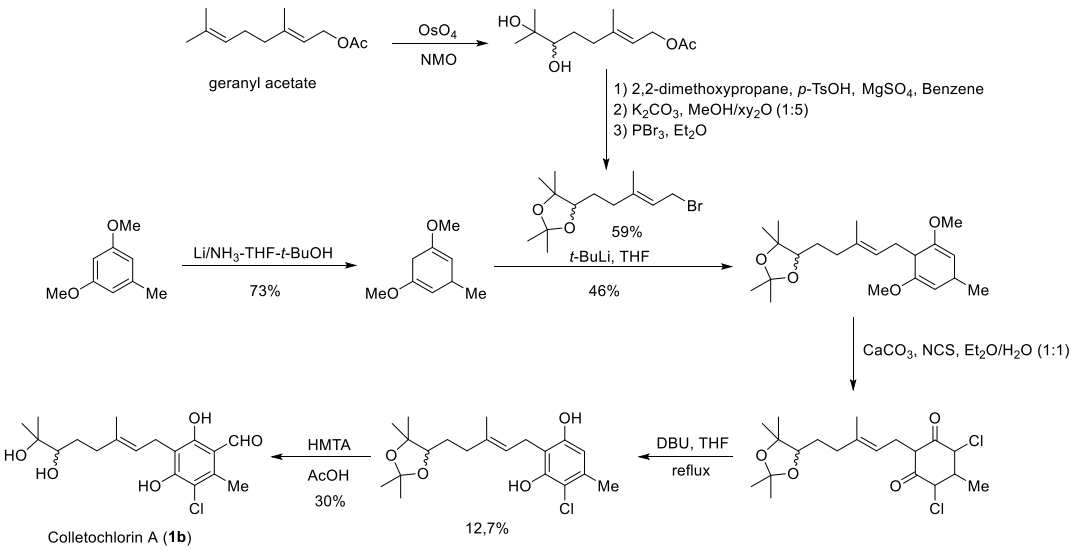


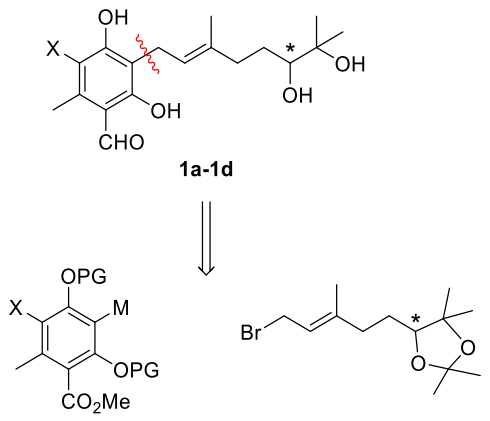
Figure 2.4. Structure of colletorins A (**1a**), B, C and D, colletochlorins A (**1b**), B, C and D and a representation of a tobacco leaf attacked by the fungus *Colletotrichum nicotianae*.



Scheme 2.1. Synthesis of racemic colletochlorin A (**1b**) reported in literature.[18]

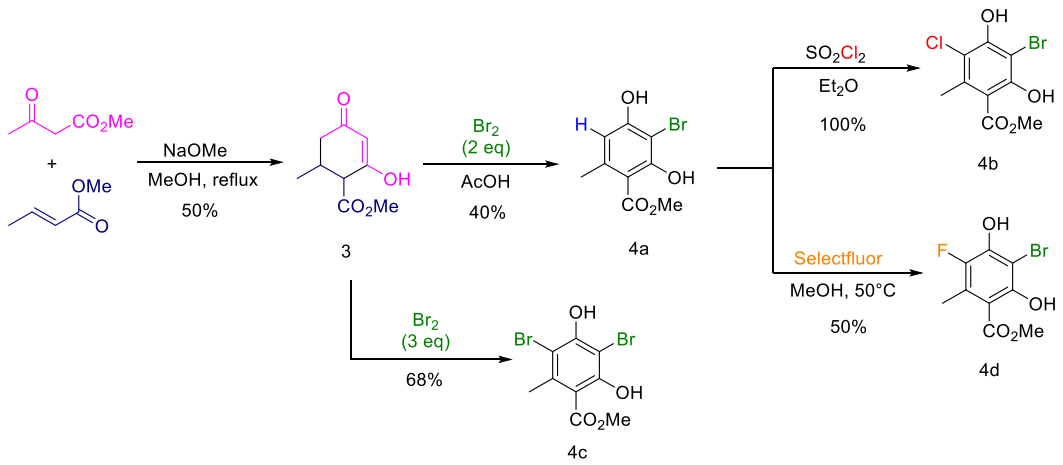
2.2.2. Colletochlorin A, Colletorin A and their Unnatural Halogenated Analogues: Asymmetric Synthesis and Structure-Activities Studies

In order to synthesize colletorin A (**1a**), colletochlorin A (**1b**) and their halogenated analogues and to study the correlation between the structure and the activities for such molecules, the synthetic approach highlighted in Scheme 2.2 was proposed.[19] This retrosynthetic analysis is based on the disconnection of the structures of **1a-d** into an aromatic synthon and an optically active side chain, which, subsequently, could be combined through a coupling reaction. In such design, both of the precursors should have all the hydroxyl groups protected and the aromatic formyl group should be replaced by a less reactive group, such an ester, with the aim to preserve the functionality in the following reaction steps. For the preparation of unsaturated analogues colletorin B and colletochlorin B (Figure 2.4), a similar approach has been reported, by alkylation of an aromatic mixed cuprate precursor with geranyl bromide.[20] On the contrary, herein the possibility to obtain the chiral side chain in optically active form, by a regio- and stereoselective Sharpless asymmetric dihydroxylation of geranyl acetate, is also envisaged.[21] Moreover, to avoid oxidizing conditions to the polyphenolic aromatic precursor, the diol function is introduced on the side chain before the cross-coupling reaction between the two moieties.



Scheme 2.2. Retrosynthetic approach.

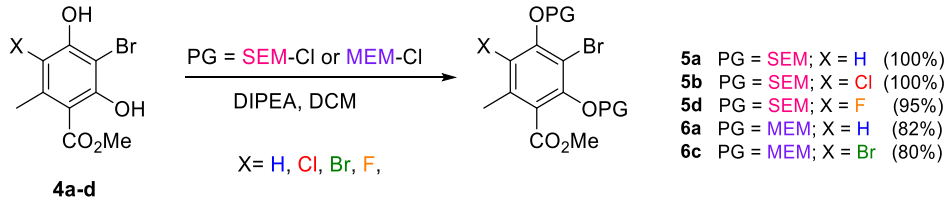
At first, the preparation of the functionalised aromatic precursors was performed (Scheme 2.3). Cyclohexenone **3**, prepared by Robinson annulation from readily available starting materials,[22] was brominated and aromatised with either 2 or 3 equivalents of bromine to achieve benzoates **4a** and **4c**, respectively. Then, the unsubstituted position of **4a** was smoothly chlorinated by tratment with a sulfuryl chloride solution. Pure **4b** was obtained in quantitative yield after solvent removal under controlled pressure and temperature to prevent sublimation of the product. Conversely, the introduction of fluorine on the aromatic ring was rather challenging. Several unsuccessful attempts in different experimental conditions have been tried, by employing well known reagents for electrophilic fluorination of phenols, such as *N*-fluoropyridinium triflate[23] and *N*-chloromethyl-*N'*-fluorotriethylendiammonium- bis (tetrafluoborate) (Selectfluor®).[24] No conversion was obtained with the pyridinium salt even at high temperature and for long reaction time. Finally, a moderate 50% conversion of **4a** in **4d** was achieved using Selectfluor® in MeOH at 50 °C. Higher temperatures and different solvents led to lower conversions and/or to the formation of unwanted quinones-type by-products. Through this synthetic sequence, it was possible to obtain all the aromatic precursors **4a-d** with all substituents in the correct position.



Scheme 2.3. Preparation of functionalised aromatic moieties **4a-d**.

Next, to compare the effect of protecting groups on the following synthetic steps, the phenolic groups of compounds **4a-d** were protected as either SEM- or MEM-ethers **5** and **6**,

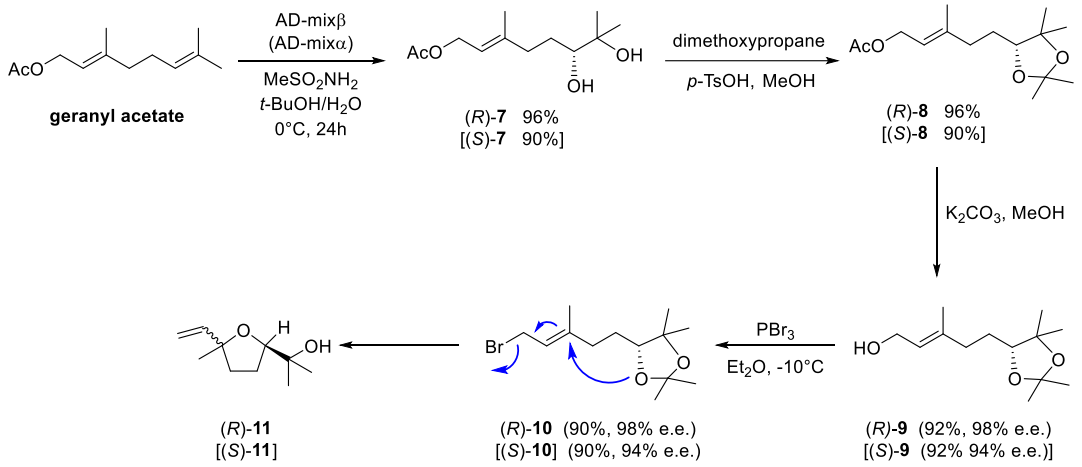
respectively (Scheme 2.4), by the reaction with SEM-Cl or MEM-Cl in the presence of DIPEA and in DCM. The SEM-derivatives **5a**, **5b**, and **5d** were obtained in quantitative yield, while the protection with the cheaper MEM-chloride provided products **6a** and **6c** in a lower yield.



Scheme 2.4. Protection reaction of the phenolic moieties.

Both enantiomers of the side chain were prepared in high yield, high stereoselectivity and full regioselectivity by Sharpless asymmetric dihydroxylation, starting from geranyl acetate to give the appropriate diols (*R*)- and (*S*)-**7** (Scheme 2.5).[21] In this reaction, the temperature control is extremely important because at T < 0 °C a very low conversion occurs, whilst at T > 4 °C a partial oxidation of the inner double bond was also observed. The absolute configuration of the obtained diols was preliminarily assigned[21] on the basis of the predictions supplied by the empirical Sharpless rule.[25] Accordingly, the oxidation reaction was expected to provide diol (*R*)-**7** when carried out with the ADmix-β and diol (*S*)-**7** when ADmix-α was instead employed. Then, diols (*R*)- and (*S*)-**7** were transformed into ketals (*R*)- and (*S*)-**8**, respectively, by treatment with dimetoxypropane, and the subsequent hydrolysis of ester group yielded alcohols (*R*)- and (*S*)-**9**. Subsequently, the treatment of alcohols (*R*)-**9** and (*S*)-**9** with PBr_3 (0.5 eq.) at 7 °C in anhydrous diethyl ether allowed to obtain the corresponding bromides (*R*)-**10** and (*S*)-**10**, isolated in 90% yield. It was found out that bromides **10** were very light sensitive slowly transforming into a diastereoisomeric mixture of tetrahydrofuran **11**, even if stored in the dark at low temperature and under inert atmosphere (Scheme 2.5).[21] For this reason those compounds had to be used within 3-4 days of their preparation. Notably, all steps of this synthetic sequence provided very high yields (≥ 90%). The enantiomeric excess of the optically active side chains was evaluated through chiral HPLC analysis, by converting both enantiomers of alcohol **9** into the corresponding

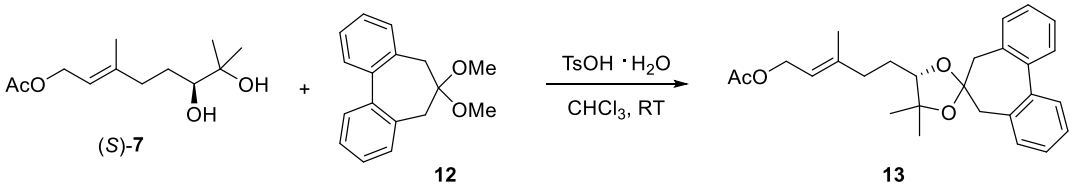
benzoates, which were separated on Chiralcel OD chiral stationary phase, giving 98% e.e. for (*R*)-**9** and 94% e.e. for the (*S*)-**9** enantiomer.



Scheme 2.5. Asymmetric synthesis of the side chain.

Although the empirical Sharpless rule[25] is commonly employed to predict the absolute configuration of diols obtained by alkene dihydroxylation with osmium tetraoxide as oxidant and either dihydroquinine or dihydroquinidine derivatives as chiral ligands, some cases have been described in which such a rule fails to predict the correct assignment.[26] Therefore, for a more reliable configurational assignment, Electronic Circular Dichroism (ECD) spectroscopy was utilised. In particular, flexible biphenyl chiroptical probes were employed for this purpose, following an approach described by the group where this PhD thesis was performed. Such a method proved to be very straightforward and reliable for the absolute configuration assignment to chiral diols,[27] acids,[28] and amines.[29] Following this approach, to assign the absolute configuration to a chiral diol, it must be transformed in the corresponding biphenyl dioxolane and the ECD spectrum of the latter analyzed. A positive Cotton effect at 250 nm (A band) in the ECD spectrum reveals a (*R*) absolute configuration of the diol, while a negative band is attributed to an (*S*) configuration. Therefore, (–)-**7**, obtained from ADmix-α, was transformed to the corresponding biphenyl dioxolane[27] **13** (Scheme 2.6) and its ECD spectrum was recorded. The appearance of a clear negative Cotton effect at 250 nm in the ECD spectrum (Figure 2.5) allowed to assign (*S*) absolute configuration to the diol (-)-**7**,

thus independently confirming the tentative assignment based on the empirical Sharpless rule. Through this method, it was also possible to determine the absolute configuration of compounds **8**-**10** derived from **7**. Therefore, the (R)/(-), (S)/(+) relationship was established for the ketals **8** and **9**, while the instability of bromide **10** did not allow measurement of its optical rotation. Moreover, this result allowed to correct the optical rotation sign of (*R*)-**9** erroneously reported in the literature.[30]



Scheme 2.6. Synthesis of the biphenyl dioxolane derivative **13**.

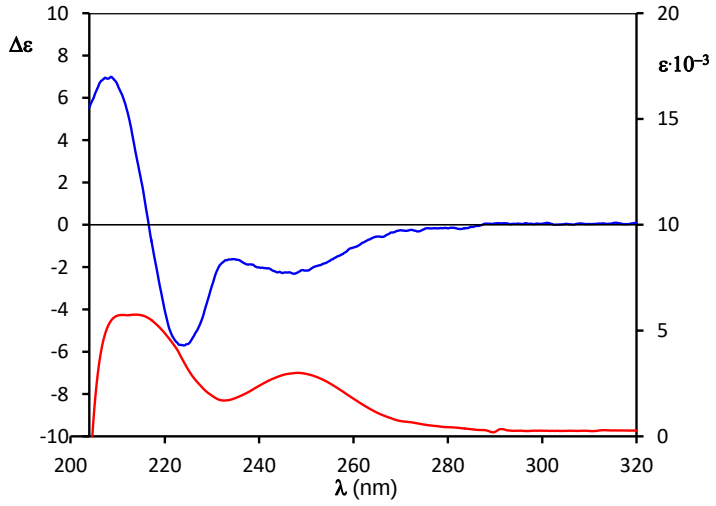
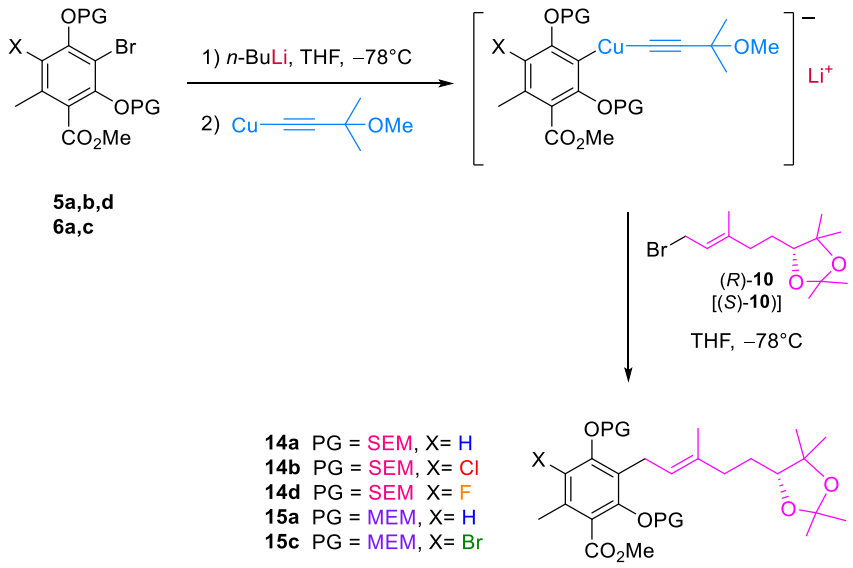


Figure 2.5. ECD (blue line) and UV (red line) spectra of biphenyldioxolane **13** in THF.

The coupling reaction between 3-bromo-benzoates **5** and **6** and the optically active bromides **10** was first attempted by Suzuki-Miyaura cross-coupling. However, attempts to transform **5** and **6**, or even the unprotected analogues **4a-d**, into the boronic esters required for the

Suzuki-Miyaura coupling, were inconclusive, probably due to high steric hindrance at the halogenated position on the aromatic moieties. Therefore, it was thought to employ mixed cuprate chemistry for the coupling reaction. Accordingly, SEM-protected benzoates **5** were lithiated at C-3 with *n*-BuLi at –78 °C in dry THF and treated, at the same temperature, with a freshly prepared solution of (3-methyl-3-methoxy-1-butynyl)copper in THF and HMPA to provide the mixed cuprate.[31] Then, (*R*)- or (*S*)-bromide **10** in THF was added to this solution and the mixture was slowly warmed at RT stirring for 18-20 h (Scheme 2.7). After quenching with a pH 8 solution of ammonia/ammonium chloride the crude product was purified by column chromatography affording benzoates **14** in 40-72% yield (Scheme 2.7). The same procedure provided benzoates **15** starting from the MEM-protected bromoesters **6**. As it can be deduced from Table 2.1, the reactions have not appeared to be influenced by the nature of the protecting group on the aromatic ring, since the SEM-benzoate **5a** and its MEM-analogue **6a** gave the coupling products **14a** and **15a**, respectively, in comparable yields (entries 1 vs 2). It was also noted that a slight excess of bromides **10** could increase the yield of the coupling reaction. In fact, in this case the 5-haloesters **15c** and **14b** were obtained in 70% yield, starting from **6c** and **5b**, respectively (entries 3 and 4). No significant influence by the nature of the halogen atom on the aromatic ring was also found. A larger amount of electrophile did not improve the yield but made the cross-coupling side reaction between the butynyl copper and bromide **10** more significant. Notably, in these reaction conditions the metalation of 3,5-dibromobenzoate **6c** is completely regioselective, since no trace of products resulting from either lithiation at C-5 position of the substrate or from a double coupling reaction was detected. Conversely, a lower scale seemed to have negative effects on yield, because the same substrate **5b** provided the product **14b** in only 40% yield when the reaction was carried out on 0.3 mmol solution (entry 5). From the coupling of 5-chlorobenzoate **5b** with both bromides (*R*)- and (*S*)-**10**, both enantiomers of the precursor **14b** of colletochlorin A (**1b**) were prepared, while the fluorinated analogue was also obtained in moderate yield (entry 6) from 5-fluorobenzoate **5d**.



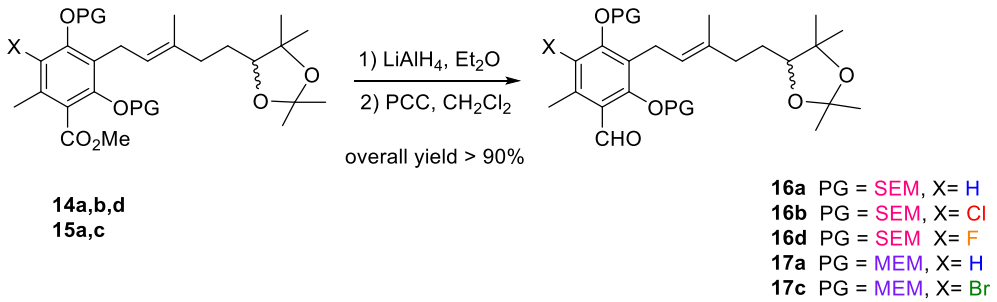
Scheme 2.7. Cross-coupling reaction.

Table 2.1. Coupling reactions: synthesis of benzoates **14-15**

Entry[a]	Substrate	X	PG	10 (eq.)	Product	Yield[b] (%)
1	**5a**	H	SEM	(*S*)-**10** (1.0)	(*S*)-**14a**	42
2	**6a**	H	MEM	(*R*)-**10** (1.0)	(*R*)-**15a**	40
3	**6c**	Br	MEM	(*R*)-**10** (1.2)	(*R*)-**15c**	70
4	**5b**	Cl	SEM	(*S*)-**10** (1.2)	(*S*)-**14b**	72
5[c]	**5b**	Cl	SEM	(*R*)-**10** (1.2)	(*R*)-**14b**	40
6	**5d**	F	SEM	(*R*)-**10** (1.2)	(*R*)-**14d**	46

[a] All reactions were carried out using 0.5 mmol of substrate; [b] Isolated yield; [c] Performed with 0.3 mmol of **5b**.

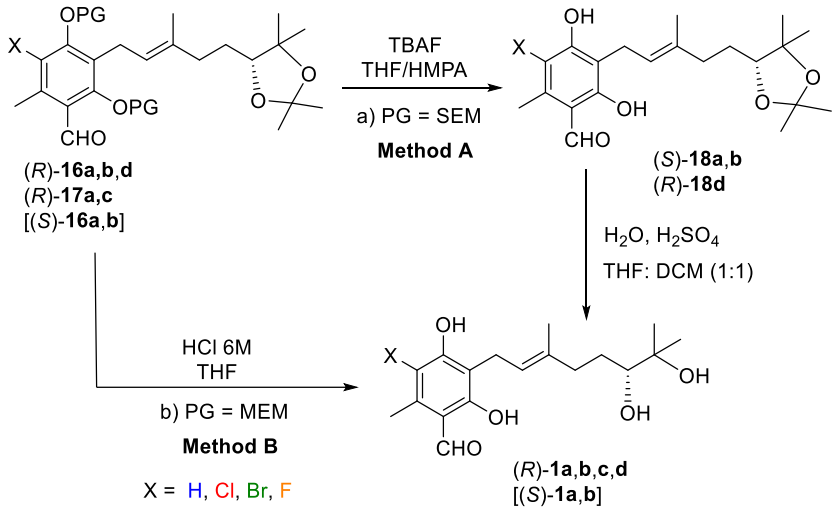
Finally, the ester group of all compounds **14** and **15** was converted into the aldehyde in high overall yields, performing a two-steps reaction, which is a reduction with $LiAlH_4$ followed by oxidation of the resulting alcohol with pyridinium chlorochromate (PCC) (Scheme 2.8).



Scheme 2.8. Conversion of the ester group into aldehyde.

The final step of the total synthesis involved the deprotection of the phenolic and diol groups (Scheme 2.9). First attempts to cleave SEM and MEM protecting groups by using P_2I_4 in DCM, mild conditions commonly employed for resorcinols, were unsuccessful, providing no reaction at RT and a mixture of unidentified products when the reaction was carried out at higher temperature or for longer time. Instead, reaction of **16a** with TBAF in THF/HMPA provided the removal of both SEM groups, giving ketal **18a** (Scheme 2.9). The latter was then hydrolised with H_2SO_4/H_2O, affording colletorin A (*S*)-**1a** in 49% overall yield (Table 2.2, entry 1, method A). Concerning the deprotection of MEM ethers, the use of the most common reagents, namely Lewis acids such as zinc or magnesium bromide, was discarded. Indeed, it is known that acidic conditions can induce ring closing and chroman or chromene formation and thus could not be applicable to highly functionalised phenol ethers such as **17**.[20,32] Therefore, it was thought to test the same procedure used above to cleave the SEM groups, even if only a single example reported its use to remove MEM.[33] However, TBAF was found to be unsuitable to remove both MEM protecting groups simultaneously, providing only the 4-monoprotected 2-hydroxybenzaldehyde. Finally, the contemporary deprotection of both the phenol and diol moieties was obtained by treatment of MEM ethers **17a**,**c** with 6M HCl in THF. This method yielded colletorin (*R*) ((*R*)-**1a**) as well as the brominated analogue (*R*)-**1c** in moderate to good yields (Table 2, entries 2-3, method B). In order to compare the two synthetic routes, the enantiomeric benzaldehydes (*S*)-**16b** and (*R*)-**16b** were deprotected according to the method A and B, respectively, providing both enantiomers of colletochlorin A, ((*S*)-**1b** and (*R*)-**1b**), (entries 4-5). Although method B was simpler and shorter, providing the simultaneous cleavage of both SEM protecting groups

and ketal moiety, a better overall yield was achieved using the two-step sequence based on the selective deprotection of phenolic and diol moieties (method A). For this reason method A was also used as protocol to synthesize the fluorinated analogue (*R*)-**1d** (entry 6).



Scheme 2.9. Final steps of total synthesis.

Table 2.2. Deprotection of phenol and diol moieties.

Entry	Substrate	X	PG	Method	Product	Total Yield (%)
1	(*S*)-**16a**	H	SEM	**A**	(*S*)-**1a**	49
2	(*R*)-**17a**	H	MEM	**B**	(*R*)-**1a**	30
3	(*R*)-**17c**	Br	MEM	**B**	(*R*)-**1c**	55
4	(*S*)-**16b**	Cl	SEM	**A**	(*S*)-**1b**	46
5	(*R*)-**16b**	Cl	SEM	**B**	(*R*)-**1b**	37
6	(*R*)-**16d**	F	SEM	**A**	(*R*)-**1d**	30

The enantioselective synthesis of **1a-d** allowed to establish to all these compounds the (R)/(+), (S)/(-) relationship between the absolute configuration and the optical rotation sign, thus providing a reliable absolute configuration assignment to the naturally occurring colletorin A (**1a**) and colletochlorin A (**1b**). In the latter case such assignment agrees with that carried out by application of the Mosher's method.[4]

Finally, the synthesised compounds were subjected to bioactivity assays in order to investigate their biological properties. In particular, phytotoxicity and insecticidal activity were evaluated in collaboration with the Institute of Sciences of Food Production, CNR, in Bari (Italy) and the Emerging Pathogens Institute, Department of Entomology and Nematology, at University of Florida (U.S.A.), respectively. The phytotoxic activity was assayed on *Ambrosia artemisifolia* and *Sonchus arvensis* (Figure 2.6), two weedy plants, on which the toxicity of the naturally occurring **1b** was recently reported with comparable results,[4,15] by using a leaf puncture assay. Droplets of solutions (10 mL) containing the metabolites (20 mg) were added to detached leaves previously wounded with a needle, kept in moistened chambers, thus observing the eventual appearance of necrotic lesions. Moreover, the effect on chlorophyll content was tested on an aquatic model plant, *Lemna minor* (Figure 2.6), by adding the test solutions to wells containing fronds of the plant, and then determining by spectrophotometer the eventual reduction of the chlorophyll content in comparison with an untreated control. The results, summarized in Table 3, indicated that both enantiomers of **1b** exhibited the strongest phytotoxicity on *S. arvensis* and, among them, the natural enantiomer (*R*)-**1b** showed a slightly higher toxicity than its enantiomer on *A. artemisifolia* plants. Conversely, the other tested compounds, namely both (*R*)- and (*S*)-**1a** and the halogenated unnatural analogues (*R*)-**1c** and (*R*)-**1d** caused only weak effects.
In addition, on the *L. minor* assay, all the tested molecules caused a reduction in chlorophyll content, even though to a different extent, with (*S*)-**1a** being the most active compound and the fluorinated analogue (*R*)-**1d** the least. The presence of a chlorine atom seemed to impart to these componds higher phytotoxicity against weeds, in respect to the other halogens. The same enhanced phytotoxicity was observed with the naturally occurring (*R*) absolute configuration at the hydroxylated stereocentre. Conversely, halogenated compounds displayed lower phytotoxicity against *L. minor*. These results highlighted the relevant effects both the halogen nature and the absolute configuration on the bioactivity of these fungal metabolites.

Sonchus arvensis ***Ambrosia artemisiifolia*** ***Lemna minor***

Figure 2.6. Representative pictures of plants *S. arvensis*, *A. artemisifolia* and *L. minor*.

Table 2.3. Phytotoxic activity assays.

Compound	*Sonchus arvensis*	*Ambrosia artemisiifolia*	*Lemna minor*[a]
(*R*)-**1a**	++	+	62.7
(*S*)-**1a**	+	+	71.6
(*R*)-**1b**	+++	++++	59.1
(*S*)-**1b**	+++	+++	43.5
(*R*)-**1c**	++	+	59.5
(*R*)-**1d**	++	+	16.0

[a] Necrotic symptoms expressed as diameter of the necrosis in the leaf punctureassay, by using a visual scale from "-" = no symptoms, to "++++" = necrosis around 1 cm diameter; [b] Reduction of chlorophyll content expressed as percentage in comparison with the untreated control.

Insecticidal activity of both enantiomers of **1b** and the brominated analogue (*R*)-**1c** was examined *via* topical application and injection on *Aedes aegypti* (Figure 2.7), the yellow fever mosquito, which is a vector for transmitting several tropical fevers.[34] The results (Table 4) show that all three compounds exhibited negligible insecticidal activity against the adult mosquitos in both test methods. Except for (*R*)-**1b** at 2 mg/mosquito dose (3.3 ± 3.3% mortality), none of the compounds showed any mortality *via* topical application. Moreover, (*R*)-**1b** showed greater mortality in the injection assay (13 ± 3.3%), but this value is not statistically significant compared to controls. A pre-treatment with piperonyl butoxide (PBO) slightly increased toxicity of all three tested compounds, but again the effect was found to be not statistically significant in a t-test compared to compound injected alone.

Thus, mixed-function oxidases did not seem to be a major route of metabolism in the tested compounds in *A. aegypti*, and the result suggested that the observed low toxicity was due to limited intrinsic insecticidal potency.

Figure 2.7. Representative pictures of *Aedes aegypti* mosquito.

Table 2.4. Insecticidal activity assays on *Aeades aegypti*.

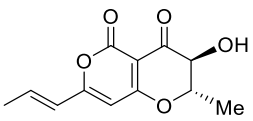
Compd.	Topical application			Injection		
	dose (μg/insect)	knock-down (% ± SE)[a]	mortality (% ± SE)[a]	dose (μg/insect)	knock-down (% ± SE)	Mortality (% ± SE)
(*R*)-**1b**	2	0.0 ± 0.0	3.3 ± 3.3	0.1	6.7 ± 3.3	13.3 ± 3.3
	1	0.0 ± 0.0	0.0 ± 0.0	0.1 + PBO[b]	20.0 ± 5.8	33.3 ± 8.8
(*S*)-**1b**	2	0.0 ± 0.0	0.0 ± 0.0	0.1	10.0 ± 5.8	6.7 ± 3.3
	1	0.0 ± 0.0	0.0 ± 0.0	0.1 + PBO	16.7 ± 8.8	30.0 ± 10.0
(*R*)-**1c**	2	0.0 ± 0.0	0.0 ± 0.0	0.1	6.7 ± 3.3	10.0 ± 5.8
	1	0.0 ± 0.0	0.0 ± 0.0	0.1 + PBO	13.3 ± 3.3	23.3 ± 3.3
control		0.0 ± 0.0	0.0 ± 0.0	without PBO	10.0 ± 5.8	10.0 ± 5.8
				with PBO	6.7 ± 3.3	10.0 ± 5.8

[a] Knock-down effect was recorded at 1 h post-treatment, and 24 h for mortality; [b] For assay with a mono-oxygenase synergist, mosquitoes were pre-treated with piperonyl butoxide (PBO, at 0.5 mg/insect) via a topical application, and left for 4 h.

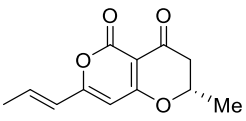
2.2.3. The Fungal Metabolites Radicinin and Deoxyradicinin

Radicinin (**2a**) is a fungal natural product first isolated in 1953 from *Stemphylium radicinum* (*Alternaria radicina*),[35] the fungus responsible for black rot disease of carrots and, since then, it has also been found to be produced by a variety of other plant-associated fungi (Figure 2.8). Subsequently, its structure was elucidated by chemical and spectroscopic studies[36] while the absolute configuration was established by X-ray analysis of its 4-*p*-bromobenzoate.[37] Such secondary metabolite displays different phytotoxic and antibiotic properties. In fact, compound **2a** demonstrated high target-specific toxicity on buffelgrass, low toxicity to native plants, and no teratogenic, sub-lethal, or lethal effects on zebrafish (*Brachydanio rerio*) embryos.[8] Previous studies has also shown its antifungal, insecticidal and plant growth regulatory activity, as well as antibiotic activity against Gram-positive bacteria, including *Staphylococcus aureus*.[38] More recently, the ability of **2a** to inhibit *Xylella fastidiosa*, the phatogen agent of many devastating plant diseases, including Pierce′s Disease of grapevine, phony peach disease, alfalfa dwarf disease, plum leaf scald, citrus variegated chlorosis, and leaf scorch of almond, coffee, elm, oak, oleander, pear, and sycamore was reported.[39] Although the high and specific bioactivity of **2a** against buffelgrass made this phytotoxin very promising for the development of bioherbicides for the control of this weed, the low quantity of **2a** available from fungal sources constituted a real bottleneck for such development, do not allowing open field studies.[40] Moreover, the large-scale production of fungal natural products requires the use of fermenters from which critical problems derive, strongly affecting the yield of the desired compounds.[41] Only one approach for the total synthesis of (±)-**2a** and its biosynthetic precursors, (±)-deoxyradicinin (**2b**) has been reported (Figure 2.8), and only another one to prepare solely (±)-**2b** have been reported in the literature so far. However, the first procedure, reported by Kato *et al.* in 1969[42] (Scheme 2.10), appears unpractical, providing (±)-**2a** and (±)-**2b** in seven and five steps, respectively, and < 10% overall yields, but from not commercially available precursors which, in turn, required some additional steps for preparation (Scheme 2.10).[43] On the other hand, the second procedure by Suzuki *et al.*,[44] although leading to (±)-**2b** in only four steps from triacetic lactone methyl ether, still provided only a 1% of overall yield (Scheme 2.11).

In addition, both approaches appear to be poorly versatile to the synthesis of other pyrones differently substituted at C-7.



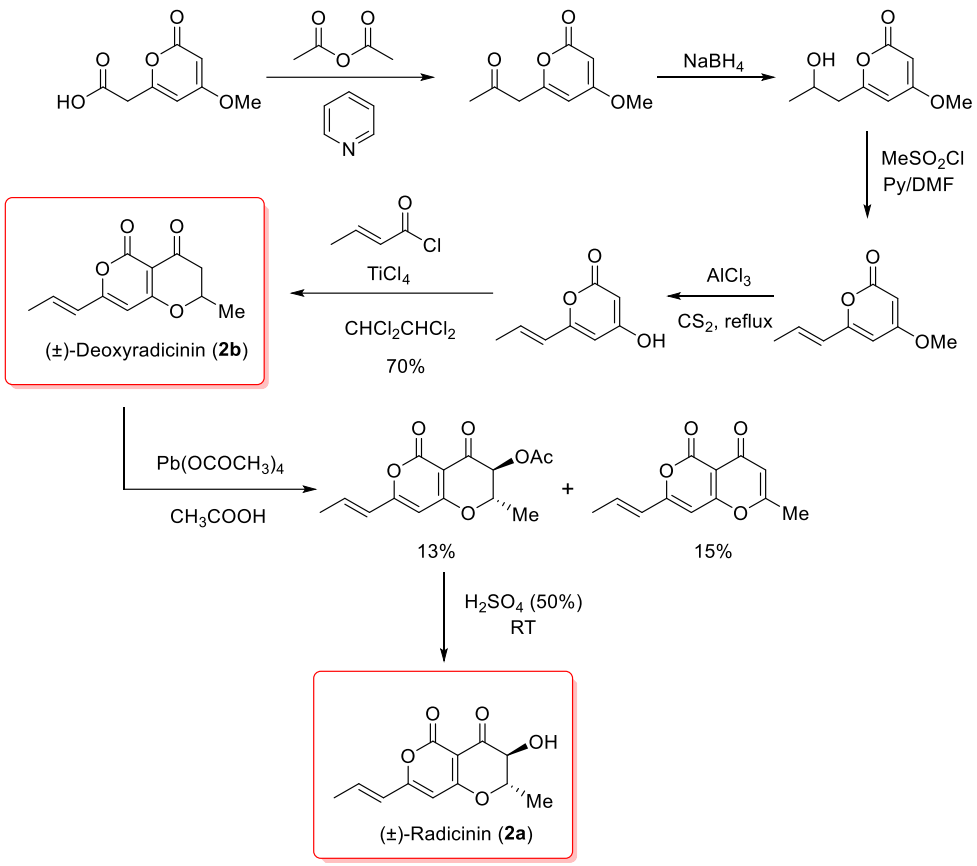
(–)-Radicinin (**2a**)



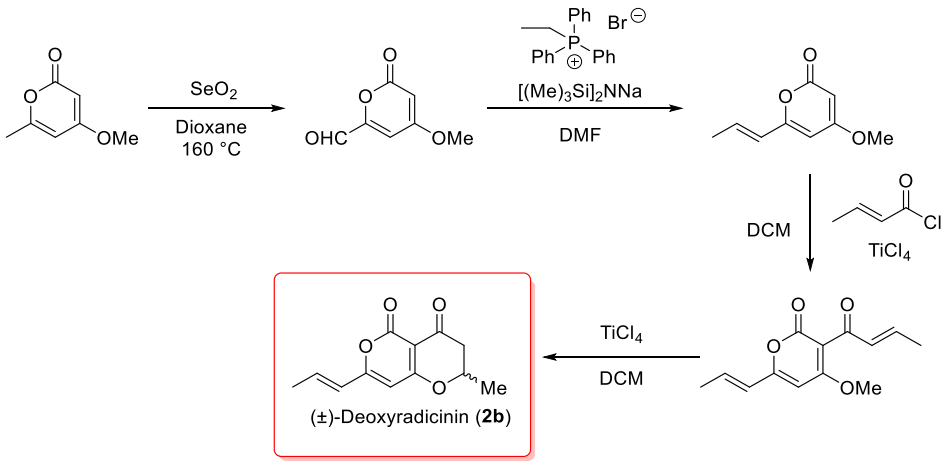
(–)-Deoxyradicinin (**2b**)

Figure 2.8. Structure of (–)-radicinin (**2a**) and (–)-deoxyradicinin (**2b**) (on the left) and a representative picture of carrots attacked by the fungus *Stemphylium radicinum* (*Alternaria radicina*) (on the right).

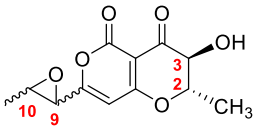
Moreover, from the liquid culture of the fungus *Cochliobolus australiensis*, the new dihydropyran-pyran-4,5-dione, named cochliotoxin (**2a'**) (Figure 2.9), together with the known phytotoxin radicinin (**2a**), was recently isolated. This compound was found to show a strong phytotoxicity against buffelgrass, thus resulting as another possible candidate as a natural herbicide against this invasive weed. Interestingly, the presence of the epoxy group in cochliotoxin, also seemed to be an important feature involved in the activity of these class of phytotoxins.[45]



Scheme 2.10. Synthesis of (±)-radicinin (**2a**) and (±)-deoxyradicinin (**2b**) reported in literature.[42]



Scheme 2.11. Synthesis of (±)-deoxyradicinin (**2b**) reported in literature.[44]

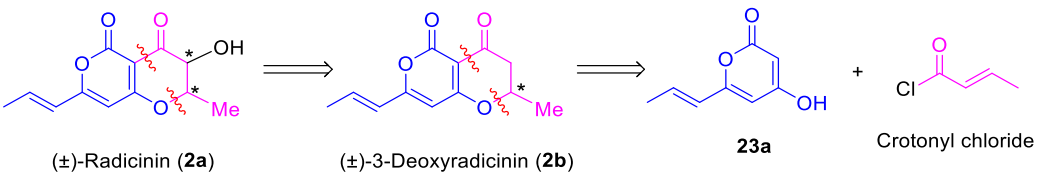


Cochliotoxin (**2a'**)

Figure2.9. Structure of cochliotoxin, on the left, and a representative picture of a rice plant attacked from the fungus *Cochliobolus australiensis*.

2.2.4. Deoxyradicinin: Synthesis and Herbicidal Activity

The synthetic approach proposed to prepare (±)-radicinin (**2a**) and its biosynthetic precursor, (±)-deoxyradicinin (**2b**), was based on the disconnection of their structure into pyranone **23a**[46] and crotonyl chloride (Scheme 2.12).[47]

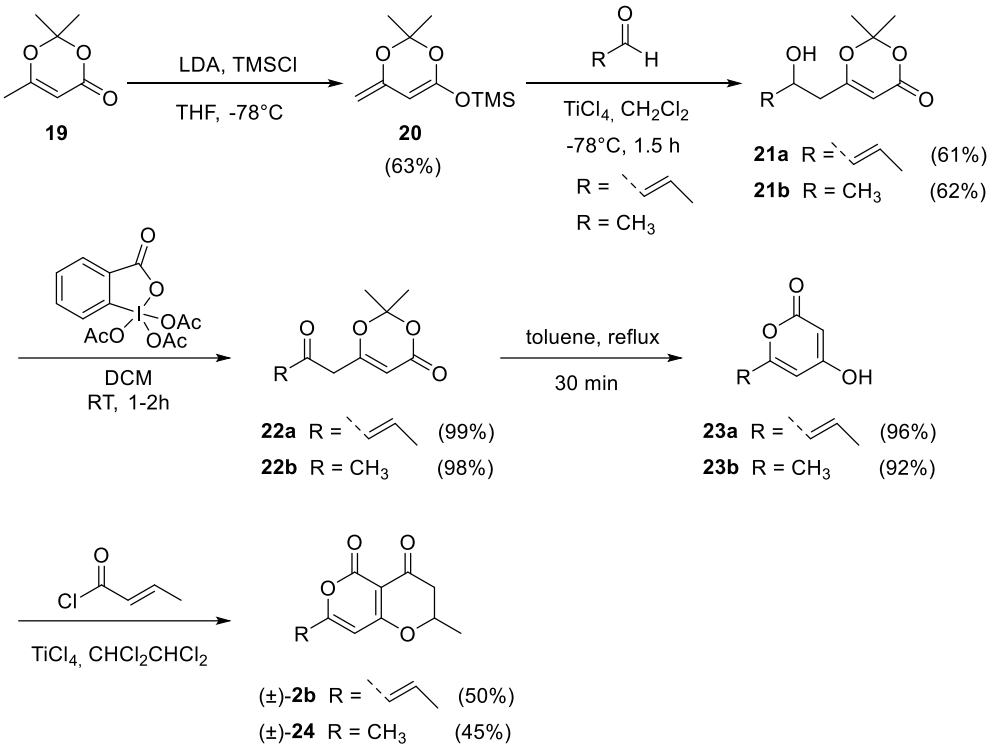


Scheme 2.12. Retrosynthetic analysis.

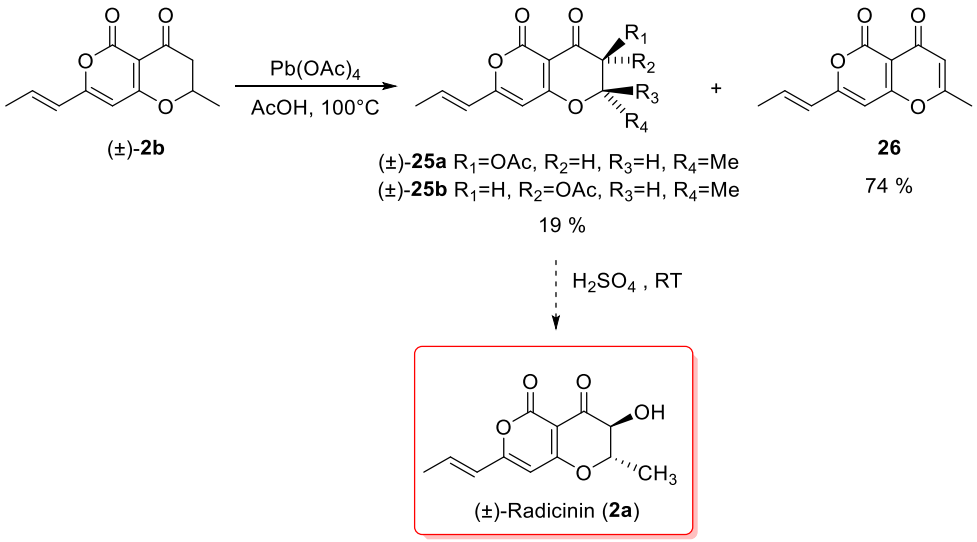
The synthetic procedure reported for the preparation **2a** and **2b** are highlighted in Scheme 2.13 and Scheme 2.14. Accordingly, the commercially available 2,2,6-trimethyl-4H-1,3-dioxin-4-one (**19**) was enolized with lithium diisopropylamide (LDA) at –78 °C in dry THF and trapped with trimethylsilyl chloride (TMSCl), providing the silyl dienolether **20** in 63% yield, after high vacuum distillation (pressure lower than 1.0 mmHg) of the crude product. This yield may be considered satisfactory, taking into account that the purification of **20** by distillation should be carried out on strictly controlled temperature conditions because it decomposes when heated at temperatures higher than 50 °C. Then the silyl enol ether **20** was reacted with crotonaldehyde through Mukaiyama aldol condensation reaction in the presence

of $TiCl_4$ in DCM at –78 °C, affording the alcohol **21a** in 61% yield. Oxidation of **21a** with Dess-Martin periodinane gave rise to the corresponding ketone **22a** in quantitative yield. The latter compound spontaneously eliminated acetone and cyclised upon heating in toluene, providing the hydroxy-pyrone **23a** in 96% yield. Finally, acylation of **23a** with crotonyl chloride, followed by in situ intramolecular Michael addition of the hydroxy moiety, provided the desired deoxyradicinin ((±)-**2b**) in 50% yield (overall yield 18%) (Scheme 2.13). Notably, such synthetic strategy appears to be versatile towards the synthesis of radicinin analogues with different substituents at C-7 and C-2. In fact, by simply employing a different aldehyde in the Mukaiyama reaction with compound **20**, a different side-chain at C-7 could be introduced, while the use of a different, α,β-unsaturated acid chloride in the last step would allow to insert a different substituent at C-2. This potentiality was verified by the synthesis of the methyl-substituted dihydropyran-pyran-4,5-dione (±)-**24**, which was obtained following the same synthetic procedure described for (±)-**2b**. In fact, the Mukaiyama reaction of **20** with acetaldehyde provided alcohol **21b** which, after oxidation to **22b** and subsequent cyclisation, afforded the pyrone **23b**. The acylation with crotonyl chloride finally provided (±)-**24** in similar overall yield (17%) (Scheme 2.13).

With (±)-**2b** in hand, its conversion to (±)-**2a** was attempted, following the oxyacetylation procedure described in the literature by Kato *et al*.[42] Accordingly, (±)-**2b** was treated with lead tetracetate in acetic acid, stirring at 100 °C for 2 h (Scheme 2.14). Contrary to what reported in the literature, where the obtainment of only 3-acetoxy radicinin was claimed, a 6:4 *trans*:*cis* mixture of (±)-radicinin acetate **25a** and 3-*epi*-(±)-radicinin acetate **25b** (19% overall) together with the unsaturated pyranopyrandione **26** (74%), as the main product of the reaction, were obtained (Scheme 2.14). It was speculated that compound **26** was probably formed through spontaneous acetic acid elimination starting from (±)-**25**. The low yield obtained, the low diastereoselectivity observed and, most importantly, the large formation of **26**, as a by-product in the present reaction, suggested that a further optimization of such synthetic procedure is necessary, possibly trying in the future different reagents for the α-oxidation of 3-deoxyradicinin (**2b**).



Scheme 2.13. Synthesis of (±)-3-deoxyradicinin (**2b**) and analogue (±)-**24**.



Scheme 2.14. Acetoxylation of (±)-3-deoxyradicinin (**2b**) with $Pb(OAc)_4$ (bold and dashed lines in figure refer to the relative configuration).

Despite it was not possible to synthesize (±)-radicinin (**2a**), it was however considered worthwhile to test both the available prepared compounds, (±)-deoxyradicinin (**2b**) and the radicinin derivative **26**, in the phytotoxicity assays on buffelgrass (*C. ciliaris*).

Therefore, the compounds (±)-**2b** and **26** were subjected to phytotoxic activity assays in collaboration with ENEA C.N.R., SSPT-BIOAG-PROBIO, in Rome. Thus, (±)-**2b** and **26** were tested by buffelgrass (*C. ciliaris*) leaf puncture assay at 2.5×10^{-3} M and their activity was evaluated in comparison with that shown by the natural metabolite radicinin (**2a**) previously reported[8] (Figure 2.10). As it can be deduced from Figure 2.10, the activity of compound (±)-**2b** is comparable to that of the natural radicinin (2*S*,3*S*)-**2a**, while the activity of the pyranopyrandione **26** is slightly lower. The lack of the hydroxy group on C-3 in **2b** seemed to suggest, contrary to previous observations,[12] that this moiety is not essential for the compounds phytotoxicity. Moreover, the comparable activity of racemic **2b** and non-chiral compound **26** with optically active (2*S*,3*S*)-**2a**, indicated that the absolute configuration of the methyl group at C-2 also has a minor influence on phytotoxicity. Concerning the absolute configuration at C-3, previous results have shown that in the presence of a hydroxy moiety on this stereocentre its stereochemistry results very important to impart a strong activity considering the significant reduction in phytotoxicity showed by (2*S*,3*R*)-3-epi-radicinin in comparison with (2*S*,3*S*)-**2a**.[12]

The obtained results indicated that derivatives like (±)-**2b** and **26**, much simpler to be prepared than optically active radicinin (**2a**), can be considered a valid alternative as possible bioherbicides against buffelgrass. Moreover, the possibility to employ, with similar results, racemic or non-chiral compounds in place of enantiopure ones represents a great advantage for the preparation of the large amounts of compounds necessary for the field test of bioherbicides. Indeed, it is obviously much simpler to prepare on large scale a racemic compound than an optically active one, which usually require utilising expensive chiral reagents or catalysts.

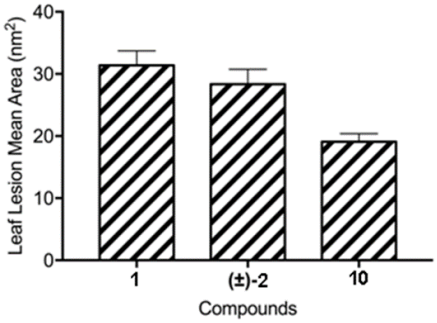


Figure 2.10. Results of leaf puncture bioassay on buffelgrass (*Cenchrus ciliaris*) at concentration of 2.5 × 10−3 M for the synthetic derivatives (±)-2 and 10 and the natural compound 1, on the left, and a representative picture of buffelgrass, on the right.

2.2.5. Absolute Configuration Assignment of Cochliotoxin

The absolute configuration at C-9 and C-10 of cochliotoxin (**2a'**) was assigned by exploiting quantum mechanical computational methods for prediction of chiroptical properties, in particular electronic circular dichroism (ECD) and optical rotatory dispersion (ORD).[48]

Nowadays, quantum mechanical calculations of chiroptical properties have become the most popular method for the assignment of absolute configuration of organic compounds, including natural products. This method possesses the advantage of making it possible to assign absolute configurations without the need for any reference system or any chemical derivatization. In addition, the development and the efficiency of quantum mechanical theory TD-DFT (Time Dependent Density Functional Theory), allows the simulation of the chiroptical spectra of a medium-size molecule on a desktop PC in a reasonable time.[49]

In general, the assignment of the molecular absolute configuration by chiroptical spectroscopy relies on the comparison between the experimental spectrum and predicted spectrum for a given enantiomer. If both spectra agree, then the absolute configuration of the molecule corresponds to the one chosen *a priori* for the spectral prediction.[50] The quantum mechanical calculation of chiroptical spectra is based on a well-established procedure highlighted in flow-chart in Figure 2.11. Starting from a geometry of the molecule with a known relative configuration (RC), obtained from NMR spectroscopy, a complete computational treatment requires: 1) conformational search, using molecular mechanics

(MM) with a good force field (FF), for example MMFF; 2) geometry optimization of all conformers, using density functional theory (DFT); 3) calculation of final energies; 4) assessment of the Boltzmann populations; 5) check of the consistency of obtained results so far; 6) calculation of the chiroptical properties for each conformer, using TD-DFT for ECD calculation and DFT for ORD; 7) and 8) Boltzmann averaging of the chiroptical spectra; 9) comparison with the experimental data.[51]

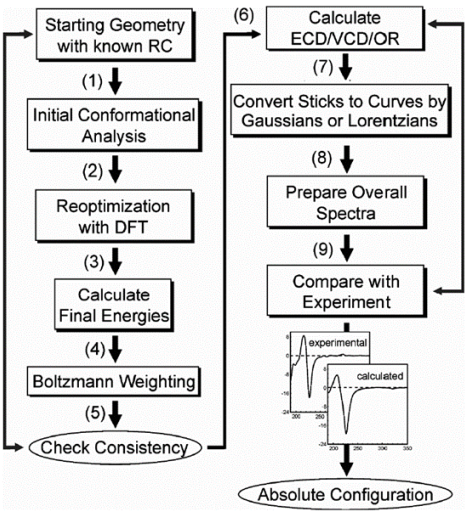


Figure 2.11. Flowchart showing the typical steps in quantum mechanical calculations of chiroptical properties (see Ref. 49).

Therefore, applying the above-described method, the absolute configuration of all the stereocentres of natural cochliotoxin (**2a'**) was established. The same method was also applied to (–)-radicinin (**2a**) with the aim to confirm the absolute configuration reported in the literature and, eventually, to test the reliability of the approach on this class of natural products. First, the UV and ECD spectra of **2a** and **2a'** were measured on acetonitrile solutions in the 190-400 nm range, while the experimental ORD's for the two molecules were measured in chloroform solutions. The UV spectra exhibited two bands centred at about 200 and 220 nm respectively, a weak band at ca. 270 nm and a broad intense band at ca. 340 nm for **2a** and at ca. 320 nm for **2a'**. This low-lying transition was ascribed to enedione transition; the tri-enedione transition of radicinin (**2a**) was clearly found at higher

wavelength with respect to the di-enedione transition of cochliotoxin due to the more extended conjugation. The experimental ECD spectra of the two compounds showed quite similar profiles: three Cotton effects (CEs) in the 190-250 nm range (negative at 197 nm, positive at 212 nm, and negative at 230 nm), a weak negative band centred at ca. 270 nm and a broad negative band, which can also be attributed to the low lying enedione transition. Then the computational analysis for both natural radicinin (**2a**) and cochliotoxin (**2a'**) was carried out, assuming (2*S*,3*S*) absolute configuration for the common pyranone moiety. Regarding the 9,10-oxyrane ring of cochliotoxin, only the *trans*-relative configuration was established by ^{1}H-NMR data,[45] whilst the absolute configuration of this moiety was still unknown. Therefore, the calculations were performed on both possible diastereoisomers (2*S*,3*S*,9*R*,10*S*) and (2*S*,3*S*,9*S*,10*R*) of cochliotoxin (**2a'**) (Figure 2.12), having the oxyrane moiety in *trans* conformation. The conformational analysis at the Molecular Mechanics (MM) level provided 6 distinct populated conformers for all three molecules (2*S*,3*S*)-radicinin (**2a**), (2*S*,3*S*,9*R*,10*S*)- and (2*S*,3*S*,9*S*,10*R*)-cochliotoxin (**2a'**). In order to take into account possible solvation effects, all conformations found by MM were fully optimised in acetonitrile, for ECD-UV calculations, and chloroform, for ORD calculations, using IEFPCM solvation model by density functional theory (DFT) at TD-DFT/CAM-B3LYP/aug-cc-pVDZ for ECD-UV and TD-DFT/B3LYP/aug-cc-pVDZ for ORD calculations. Moreover, the geometry optimizations provided two most populated conformers for (2*S*,3*S*)-radicinin (**2a**), with one of them at almost 90% of the overall population, two conformers for (2*S*,3*S*,9*R*,10*S*)-cochliotoxin (**2a'**) and three for the (2*S*,3*S*,9*S*,10*R*)-isomer (**2a'**). In the last two cases just one conformer was found, at ca. 99% of the overall population. The comparison between the experimental and computed UV and ECD spectra is reported in Figure 2.13, for **2a**, and in Figure 2.14, for **2a'**. For a better comparison with experimental spectra, the calculated ones were divided by a factor 3. As inferred from Figure 2.13, calculation of radicinin ECD-UV spectra provided a good prediction of the experimental ones in signs and relative positions of the bands. The comparison of ECD-UV calculated spectra for the diastereomers (9*R*,10*S*) and (9*S*,10*R*) of cochliotoxin (**2a'**) (Figure 2.14) showed that the calculated UV spectra of the two isomers are practically coincident, while the ECD profiles are slightly different especially with regard to the low-lying transition at ca. 320 nm. In particular, (9*R*,10*S*) isomer displays a

positive calculated Cotton effect at ca. 330 nm, while its (9*S*,10*R*) counterpart has a negative ECD band, consistent with experimental measure (Figure 2.14). As reported above, this transition was attributed to the di-enedione-like chromophore, which appeared to be sensitive to the chirality at C-9, thus becoming a highly discriminating electronic transition for the absolute configuration assignment of cochliotoxin (**2a'**). The absolute configuration of radicinin (**2a**) and cochliotoxin (**2a'**) was also investigated by calculating optical rotation (OR) values at several wavelength and then comparing to the corresponding experimental values. It was found that the experimental negative trend for **2a** was well predicted by calculation of optical rotatory power at four different wavelengths (Figure 2.15) confirming the (2*S*,3*S*)-**2a**. On the other hand, the (9*S*,10*R*) diastereomer of cochliotoxin (**2a'**) had negative ORD trend sign, which was consistent with the experimental measured in chloroform solution, while calculated ORD for (9*R*,10*S*) isomer of **2a'** showed positive trend (Figure 2.16). Therefore, it is possible to conclude that the agreement of both calculated ECD and ORD with the experimental data supports the (2*S*,3*S*,9*S*,10*R*) absolute configuration for cochliotoxin (**2a'**).

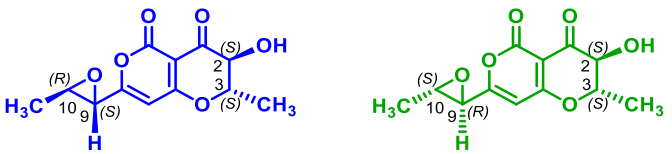


Figure 2.12. Structures of the two diastereomers of cochliotoxin considered for quantum mechanical calculations.

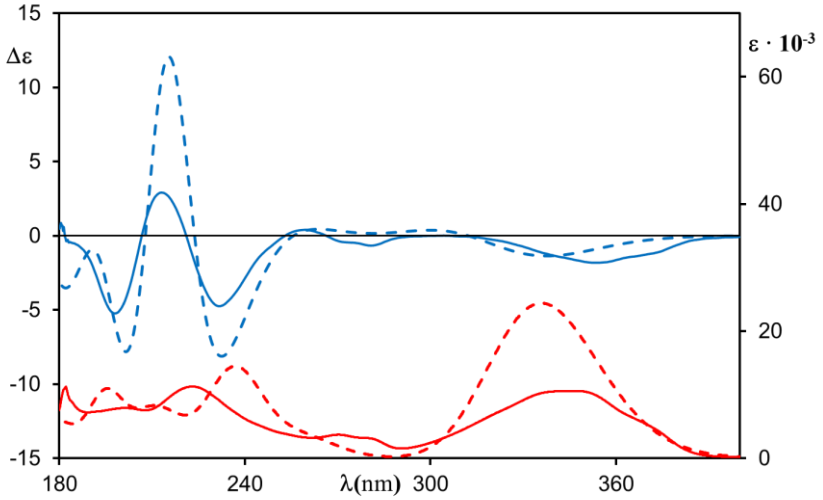


Figure 2.13. Comparison of experimental (solid lines) and calculated (dotted lines) ECD (blue lines) and UV (red lines) spectra of radicinin. TD-DFT/CAM-B3LYP/aug-cc-pVDZ/PCM(ACN) level of theory. Calculated spectra are red-shifted by 15 nm and divided by factor 3.

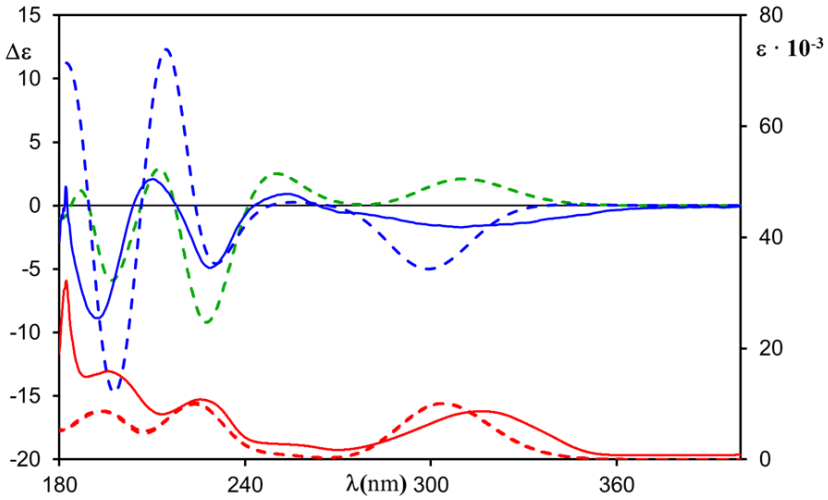


Figure 2.14. Comparison of experimental (solid lines) and calculated (dotted lines) ECD (blue line for experimental and calculated (2*S*,3*S*,9*S*,10*R*)-isomer and green line for (2*S*,3*S*,9*R*,10*S*)-isomer) and comparison of experimental (red solid lines) and calculated (red dotted lines) UV spectra of cochliotoxin. The two possible diastereomers have been calculated at TD-DFT/CAM-B3LYP/aug-cc-pVDZ/PCM(ACN) level of theory. Calculated spectra are red-shifted by 15 nm and divided by factor 3.

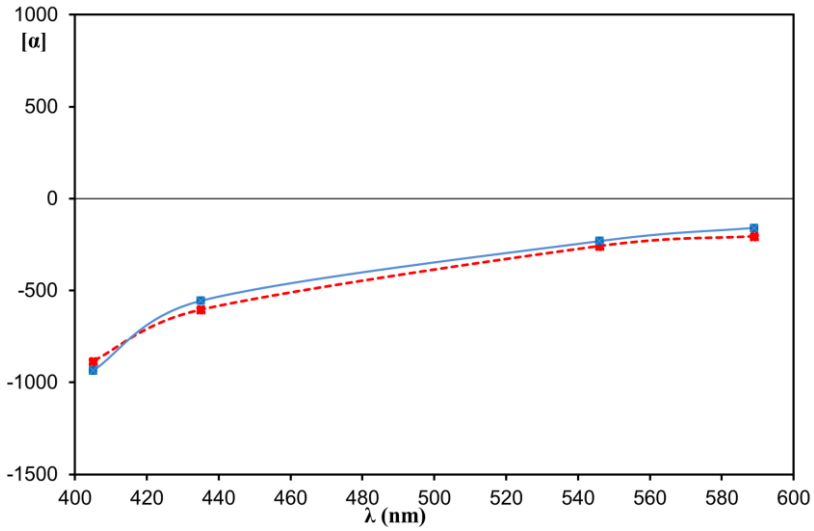


Figure 2.15. Comparison of experimental (blu solid line) and calculated (red dotted line) specific OR values at four different wavelengths for radicinin (**2a**). Experimental ORD was measured in chloroform solvent. TD-DFT/B3LYP/aug-cc-pVDZ/PCM($CHCl_3$) level of theory.

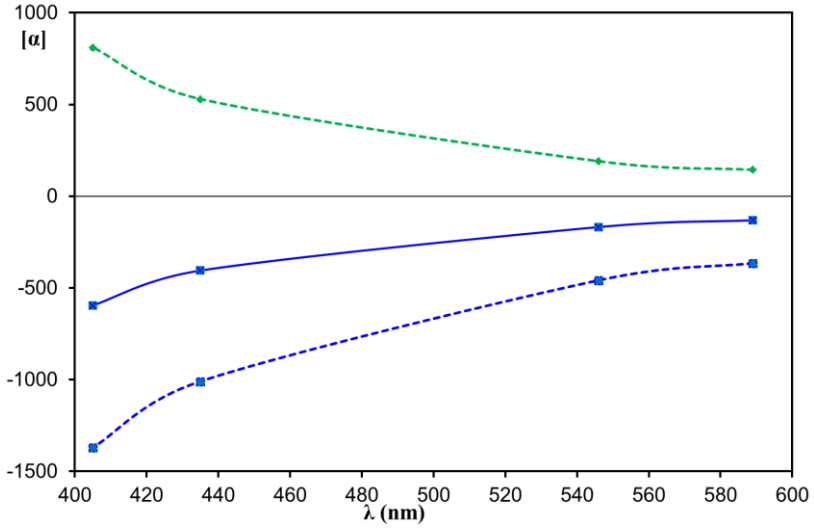


Figure 2.16. Comparison of experimental (blu solid line) and calculated (blue line for (2*S*,3*S*,9*S*,10*R*)-isomer and green line for (2*S*,3*S*,9*R*,10*S*)-isomer) specific OR values at four different wavelengths for cochliotoxin. Experimental ORD was measured in chloroform solvent. TD-DFT/B3LYP/aug-cc-pVDZ/PCM($CHCl_3$) level of theory.

2.3. Conclusions

In this project, the first asymmetric total synthesis of colletorin A (**1a**) and colletochlorin A (**1b**) was reported. The same efficient methodology was used for the highly enantioselective synthesis of the brominated (**1c**) and fluorinated (**1d**) synthetic analogues. This protocol allowed to carry out the most critical steps of the whole synthetic pathway, namely the coupling and deprotection reactions, in medium to good yields (40-72%). Moreover, two different procedures for the deprotection of both phenolic and ketal moieties have been tested, depending on the nature of the phenolic protecting group. A high regio- and stereo-control was also guaranteed by the Sharpless asymmetric dihydroxylation of geranyl acetate, which constitutes the enantioselective step of the total synthesis. It is believed that this strategy could also be successfully applied to other highly functionalised phenolic compounds bearing terpenoid side chains. In addition, the enantioselective synthesis of **1a-d** allowed to reliably assign the absolute configuration to the natural compounds **1a** and **1b**. Moreover, the preliminary tests of biological activity carried out as herbicides and insecticides highlighted the potential bioactivity of these compounds and allowed sorting out the dependence on the absolute stereochemistry and on the halogen nature of the compound bioactivity.Moreover, a novel synthetic strategy for obtainment of (±)-3-deoxyradicinin (**2b**) was described. This synthetic methodology was found to be more efficient than those previously reported in the literature and showed higher versatility towards the introduction of different side-chains at both C-7 and C-2. The prepared compound (±)-**2b** displayed phytotoxicity against the grass-weed buffelgrass comparable to that of the natural phytotoxin radicinin (**2a**). Therefore, (±)-**2b** could constitute a more practical synthetic alternative to **2a** as bioherbicide for buffelgrass control. Finally, the absolute configuration at C-9 and C-10 of the oxyrane-ring of cochliotoxin (**2a'**), a novel compound isolated from *C. australiensis* showing phytotoxic activity against buffelgrass, was assigned, by means of quantum mechanical computational methods for prediction of chiroptical properties, in particular ECD and ORD. The same method was utilised to confirm the absolute configuration of radicinin (**2a**) previously reported in literature.

2.4. Experimental section

2.4.1. General Information

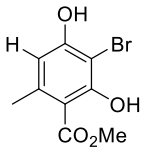
^{1}H (500 or 400 MHz) and ^{13}C (125 or 100 MHz) NMR spectra were recorded in $CDCl_3$ or DMSO-*d*6 on a Varian INOVA 500 or a Varian INOVA 400 spectrometer, using tetramethylsilane (TMS) as an internal standard. ^{19}F (376 MHz) NMR spectra were recorded in $CDCl_3$ on a Varian INOVA 400 spectrometer, using $CFCl_3$ as an internal standard. GC-MS spectra were recorded on a Hewlett Packard 6890 gas chromatograph, equipped with a mass spectrometric detector HP- 5973 type and a capillary column HP-5MS 30m×0.25 mm. Optical rotations were measured on a JASCO DIP-370 or a Jasco P-2000 polarimeters, with a values expressed in $deg \cdot cm^3 \cdot g^{-1} \cdot dm^{-1}$ and concentration c in $g \cdot (100\ mL)^{-1}$. ECD spectra were taken from 400 to 185 nm on a Jasco 815SE spectropolarimeter. HPLC analyses were performed on a JASCO PU-1580 intelligent HPLC pump equipped with a Varian 2550 UV detector and a CHIRALCEL OD column. Analytical thin layer chromatography (TLC) was performed using silica gel 60 Macherey-Nagel plates and visualised by ultraviolet radiation and/or spraying the chromatograms with a potassium permanganate solution. Column chromatography separations were carried out using silica gel 60 (70-230mesh). Et_2O and THF were freshly distilled before their use on sodium benzophenone ketyl under nitrogen atmosphere. *N*,*N*-diisopropylethylamine (DIPEA), Diisopropylamine (DIPA), trimethylamine, and DCM were dried by distillation over calcium hydride and stored under a nitrogen atmosphere. Commercially available *n*-BuLi (Aldrich) was a 1.6M solution in hexane. 3-Hydroxy-4-carbomethoxy-5-methylcyclohexenone **3** was prepared starting from methyl crotonate and methyl acetoacetate as described.[52] The ketal **12** was prepared as described[27] and (3-methyl-3-methoxy-1-butynyl)copper was obtained starting from the commercially available (Aldrich) cuprous acetylide (2-methylbut-3-yn-2-ol) as already described elsewere.[31] Trimethylsilyl chloride (TMSCl), crotonaldehyde and crotonoyl chloride were distilled and stored under an argon atmosphere before their use.

The other analytical grade solvents and commercially available reagents were used without further purification.

2.4.2. Colletochlorin A, Colletorin A and their Halogenated Unnatural Analogues

*2.4.2.1. Syntesys of the aromatic precursors (**4a-d**)*

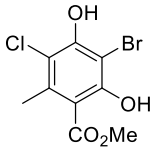
Methyl 3-bromo-2,4-dihydroxy-6-methylbenzoate, (4a)



To a solution of cyclohexenone **3** (1.0 g, 5.4 mmol. 1.0 eq.) in acetic acid (10 mL) bromine (0.56 mL, 10.8 mmol, 2.0 eq.) was added. The mixture was first stirred at RT for 30 min and then heated at 100 °C for further 30 min. After cooling to RT, the mixture was poured into water/ice and extracted with ethyl acetate. The organic layer was washed with a saturated sodium metabisulphite solution and, then, brine. The crude product was purified by column chromatography affording compound **4a** as a white solid (SiO2; hexane:diethyl ether 7:3) (772 mg, 55%).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 2.49 (s, 3H), 3.96 (s, 3H), 6.00 (brs, 1H), 6.47 (s, 1H), 12.56 (s, 1H). **^{13}C-NMR** (125 MHz, $CDCl_3$) δ (ppm): 24.2, 52.3, 96.6, 106.2, 110.7, 142.6, 156.8, 161.1, 172.0. **MS (EI)**: m/z 262 (M^+, 30), 260 (M^+, 30), 230 (98), 228 (100), 149 (20).

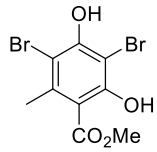
Methyl 3-bromo-5-chloro-2,4-dihydroxy-6-methylbenzoate, (4b)



To a solution of the monobromide **4a** (0.524 g, 2.0 mmol) in diethyl ether (4 mL) a solution of sulfuryl chloride (4 mL, 1M in DCM, 2.0 eq.) was added and the mixture was stirred at RT for 3 h. By solvent removal under reduced pressure (300-400 mbar, 40 °C) the pure product was obtained (560.5 mg, 95%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.61 (s, 3H), 3.98 (s, 3H), 6.46 (brs, 1H), 12.23 (s, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 19.8, 52.7, 96.6, 107.3, 113.8, 138.7, 153.3, 159.0, 171.3. **MS (EI)**: m/z 298 (M^+, 6), 296 (M^+, 24), 294 (M^+, 18), 266 (26), 364 (100), 262 (78).

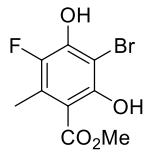
Methyl 3,5-dibromo-2,4-dihydroxy-6-methylbenzoate, (4c)



To a solution of cyclohexenone **3** (1.0 g, 5.4 mmol, 1.0 eq.) in acetic acid (10 mL) 3 equivalents of bromine (0.84 mL, 16.2 mmol, 3.0eq.) were added. The mixture was first stirred at RT for 30 min and then heated at 100 °C for further 30 min. After cooling to RT, the mixture was poured into water/ice and extracted with ethyl acetate. The organic layer was washed with a saturated sodium metabisulphite solution and brine. The crude product was purified by crystallization from hexane (826 mg, 45%).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.66 (s, 3H), 3.98 (s, 3H), 6.48 (s, 1H), 12.19 (s, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 23.3, 52.8, 96.3, 105.5, 107.7, 140.5, 153.9, 159.4, 171.2. **MS (EI)**: m/z 342 (M^+, 12), 340 (M^+, 24), 338 (M^+, 12), 310 (50), 308 (100), 306 (50).

Methyl 3-bromo-2,4-dihydroxy-5-fluoro-6-methylbenzoate, (4d)



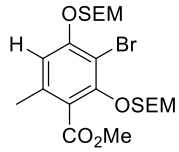
To a solution of the monobromide **4a** (0.930 g, 3.58 mmol, 1.0 eq.) in methanol (25 mL) Selectfluor® (1.65 g, 1.3 eq.) was added and the mixture was heated to 50 °C for 24 h. The reaction was monitored by GC-MS analysis and quenched, when no further conversion occurred (about 50%), by cooling at RT and removing the solvent (300 mbar, 40 °C). Then the residue was treated with water and extracted with diethyl ether. The pure product was obtained by column chromatography (SiO2; DCM) (448 mg, 45%).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 2.68 (s, 3H), 3.98 (s, 3H), 6.48 (s, 1H), 12.19 (s, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.6 (d, J = 7.5 Hz), 52.5, 96.8 (d, J = 2.2 Hz), 104.9, 126.7 (d, J = 14.4 Hz), 143.2 (d, J = 231.4 Hz), 147.0 (d, J = 21.7 Hz), 157.0 (d, J = 2.3 Hz), 171.6 (d, J = 3.0 Hz). **^{19}F-NMR** (376 MHz, $CDCl_3$) δ (ppm): -145.4. **MS (EI)**: m/z 280 (M^+, 26), 278 (M^+, 27), 248 (98), 246 (100).

2.4.2.2. General procedure for the protection of phenolic groups

2,4-dihydroxybenzoates **4a-d** (1.0 mmol) were dissolved in 4mL of dry DCM and DIPEA (5.0 mmol, 1.04 mL) was added, followed by the slow addition of the SEM-Cl or MEM-Cl (4.0 mmol). The mixture was stirred at RT for 2-4 h and then poured into water/ice and extracted with diethyl ether. The crude products were purified by column chromatography (SiO2; hexane:diethyl ether, 7:3 for SEM-ethers and 1:1 for MEM-protected compounds).

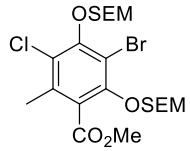
Methyl 2,4-bis[2-(trimethylsilyl)ethoxymethoxy]-3-bromo-6-methylbenzoate, (5a)



Following the general procedure, the product was isolated as a colourless oil (521 mg, > 99%).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.01 (s, 9H), 0.04 (s, 9H), 0.96 (t, 2H, J = 8.5 Hz), 1.01 (t, 2H, J = 8.5 Hz), 2.29 (s, 3H), 3.79 (t, 2H, J = 8.5 Hz), 3.85 (t, 2H, J = 8.5 Hz), 3.90 (s, 3H), 5.15 (s, 2H), 5.30 (s, 2H), 6.83 (s, 1H).

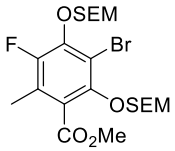
Methyl 2,4-bis[2-(trimethylsilyl)ethoxymethoxy]-3-bromo-5-chloro-6-methylbenzoate, (5b)



Following the general procedure, the product was isolated as a colourless oil (556 mg, > 99%).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.04 (s, 18H), 1.01 (m, 4H), 2.30 (s, 3H), 3.83 (t, 2H, J = 8.5 Hz), 3.92 (s, 3H), 4.00 (t, 2H, J = 8.5 Hz), 5.14 (s, 2H), 5.21 (s, 2H).

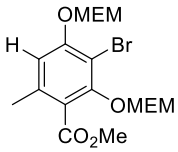
Methyl 2,4-bis[2-(trimethylsilyl)ethoxymethoxy]-3-bromo-5-fluoro-6-methylbenzoate, (5d)



Following the general procedure, the product was isolated as a colourless oil (512 mg, 95%).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.04 (s, 18H), 1.19 (m, 4H), 2.34 (s, 3H), 3.85 (t, 2H, J = 8.5 Hz), 3.92 (s, 3H), 4.02 (t, 2H, J = 8.5 Hz), 5.15 (s, 2H), 5.21 (s, 2H).

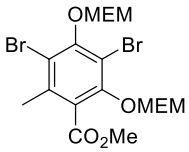
Methyl 2,4-bis[(2-methoxyethoxy)methoxy]-3-bromo-6-methylbenzoate, (6a)



Following the general procedure, the product was isolated as colourless oil (357 mg, 82%).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 2.34 (s, 3H), 3.37 (s, 3H), 3.40 (s, 3H), 3.72 (m, 4H), 3.79 (m, 2H), 3.85 (m, 2H), 3.89 (s, 3H), 5.24 (s, 2H), 5.33 (s, 2H), 6.93 (s, 1H).

Methyl 2,4-bis[(2-methoxyethoxy)methoxy]-3,5-dibromo-6-methylbenzoate, (6c)

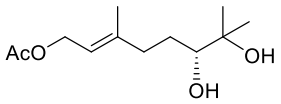


Following the general procedure, the product was isolated as colourless oil (413 mg, 80%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.32 (s, 3H), 3.38 (s, 3H), 3.39 (s, 3H), 3.58 (m, 4H), 3.70 (m, 2H), 3.91 (s, 3H), 4.09 (m, 2H), 5.18 (s, 2H), 5.24 (s, 2H).

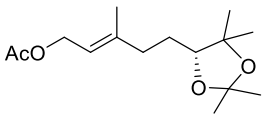
2.4.2.3. Asymmetric synthesis of the side chain

(*R*,*E*)-6,7-Dihydroxy-3,7-dimethyloct-2-en-1-yl acetate, ((*R*)-7)



AD-mix-β (14 g, 10 mmol, 1.0 eq.) and methanesulfonamide (951 mg, 10 mmol, 1.0 eq.) were added to a 1:1 mixture of *t*-BuOH:H_2O (100 mL) and vigorously stirred for 15 min at RT. After cooling to 0 °C, geranyl acetate (2.13 mL, 10 mmol, 1.0 eq.) was added, the mixture was stirred at that temperature for 24 h. Then the reaction was quenched with solid sodium metabisulfite (13.5 g), the mixture was extracted several times with DCM and the combined organic layers were washed with a 10% aq. NaOH solution and brine. The crude product was purified by column chromatography (SiO2; hexane:ethyl acetate 7:3) to afford the diol (*R*)-**7** as a colourless oil (2.20 g, 9.60 mmol, 96%). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = +26.1 (c = 0.7, $CHCl_3$), lit.[53] $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{23}}$ = +26.8 (c = 1.0, $CHCl_3$). Following the same procedure with AD-mix-α, the enantiomeric diol (*S*)-**7** was isolated (2.07 g, 9.00 mmol, 90%). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = –23.9 (c = 0.9, $CHCl_3$), lit.[54] $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ = –25.1 (c = 0.7, EtOH).
^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.17 (s, 3H), 1.22 (s, 3H), 1.45 (m, 1H), 1.62 (m, 1H), 1.73 (s, 3H), 1.91 (brs, 2H), 2.06 (s, 3H), 2.12 (m, 1H), 2.32 (m, 1H), 3.45 (dd, 1H, J = 10.5, 2.0 Hz) 4.59 (d, 2H, J = 7.0 Hz), 5.40 (brt, 1H, J = 7.0 Hz). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 16.4, 21.01, 23.2, 26.5, 29.4, 35.6, 61.3, 73.0, 78.0, 118.7, 142.0, 171.1. **MS (EI)**: m/z 170 (M^+ - AcOH, 1), 152 (3), 111 (18), 94 (32), 81 (27), 68 (72), 59 (100), 43 (73).

(*R*,*E*)-3-Methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl acetate, ((*R*)-8)

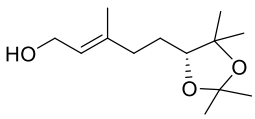


To a solution of the diol (*R*)-**7** (2.06 g, 9 mmol, 1.0 eq.) in MeOH (5 mL) dimethoxypropane (15 mL, 12.2 mmol, 1.35 eq) and *p*-toluensulfonic acid monohydrate (10 mg, 0.05 mmol, 0.05% mol) were sequentially added and the mixture was stirred at RT for 3 h. The mixture was quenched with pyridine (51 µL, 0.63 mmol) and concentrated under reduced pressure. The residue was dissolved in ethyl acetate and filtered on a silica gel pad to give the pure ketal (*R*)-**8** (2.26 g,

8.40 mmol, 93%). $[\alpha]_D^{25}$ = –2.0 (c = 1.0, $CHCl_3$). Following the same procedure, the ketal (*S*)-**8** was also isolated (2.19 g, 8.11 mmol, 90%). $[\alpha]_D^{25}$ = +2.0 (c = 1.0, $CHCl_3$).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.10 (s, 3H), 1.24 (s, 3H), 1.33 (s, 3H), 1.42 (s, 3H), 1.50 (m, 1H), 1.65 (m, 1H), 1.73 (s, 3H), 2.06 (s, 3H), 2.10 (m, 1H), 2.29 (m, 1H), 3.65 (dd, 1H, J = 9.5, 3.0 Hz), 4.59 (d, 2H, J = 7.5 Hz), 5.39 (dt, 1H, J = 7.0, 1.5 Hz). **^{13}C-NMR** (125 MHz, $CDCl_3$) δ (ppm): 16.4, 20.9, 22.7, 25.8, 26.7, 27.1, 28.3, 36.4, 61.1, 79.9, 82.5, 106.4, 118.6, 141.3, 170.8. **MS (EI)**: m/z 255 (M^+ - 15, 52), 153 (54), 135 (60), 85 (50), 81 (65), 71 (84), 43 (100).

(*R*,*E*)-3-Methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-ol, ((*R*)-9)



To a solution of the ketal (*R*)-**8** (2.16 g, 8.00 mmol, 1.0 eq.) in MeOH (27 mL) potassium carbonate (510 mg, 3.70 mmol, 0.5 eq.) was added and the mixture was stirred at RT. After 24 h the mixture was quenched with 2 mL of a saturated aq. solution of NH_4Cl and MeOH was removed under reduced pressure. The residue was dissolved in ethyl acetate and the organic phase was washed with brine. The pure alcohol (*R*)-**9** was isolated by evaporation of the solvent as a colourless oil (1.68 g, 7.40 mmol, 92%). $[\alpha]_D^{25}$ = –2.8 (c = 1.0, $CHCl_3$). Following the same procedure, the alcohol (*S*)-**9** was also obtained (1.68 g, 7.40 mmol, 92%). $[\alpha]_D^{25}$ = +3.1 (c = 1.0, $CHCl_3$). Both alcohols **9** were stored at –20 °C under argon atmosphere.

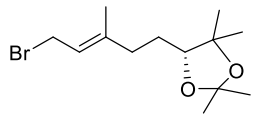
^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.10 (s, 3H), 1.25 (s, 3H), 1.33 (s, 3H), 1.42 (s, 3H), 1.52 (m, 1H), 1.64 (m, 1H), 1.70 (s, 3H), 2.04 (s, 1H), 2.07 (m, 1H), 2.27 (m, 1H), 3.65 (dd, 1H, J = 9.2, 3.2 Hz), 4.17 (d, 2H, J = 7.0 Hz), 5.46 (brt, 1H, J = 7.0 Hz). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 16.3, 22.9, 26.0, 26.9, 27.5, 28.6, 36.6, 59.3, 80.1, 82.9, 106.6, 123.6, 139.1. **MS (EI)**: m/z 213 (M^+ - 15, 46), 142 (48), 109 (22), 81 (100), 71 (92), 59 (60), 43 (88).

In order to determine the enantiomeric excesses by chiral HPLC analysis, a small portion of both the enantiomers of the alcohol **9** was transformed into the corresponding benzoate. Anhydrous triethylamine (21 µL, 0.15 mmol, 1.5 eq.) and benzoyl chloride (12 µL, 0.10 mmol, 1.0 eq.) were sequentially added to a solution of the alcohol **9** (23 mg, 0.10 mmol,

1.0 eq.) in anhydrous diethyl ether (2 mL) at 0 °C. The mixture was then stirred at RT for 16 h, poured into icy water and extracted with ethyl acetate to give the corresponding benzoate (25.8 mg, 0.08 mmol, 80%).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.12 (s, 3H), 1.25 (s, 3H), 1.33 (s, 3H), 1.43 (s, 3H), 1.55 (m, 1H), 1.68 (m, 1H), 1.81 (s, 3H), 2.14 (m, 1H), 2.33 (m, 1H), 3.68 (dd, 1H, J = 9.5, 3.5 Hz), 4.87 (d, 2H, J = 7.5 Hz), 5.54 (brt, 1H, J = 7.5 Hz), 7.44 (t, 2H, J = 8.0 Hz), 7.56 (t, 1H, J = 8.0 Hz), 8.06 (d, 2H, J = 8.0 Hz). The enantiomers were separated by HPLC on Chiralcel-OD column, hexane:2-propanol 98:2, 254 nm: t_R (*S*) = 9.6 min, t_R (*R*) = 11.7 min. The alcohol (*R*)-**9** was found to possess 98% e.e., while its enantiomer (*S*)-**9** gave 94% e.e..

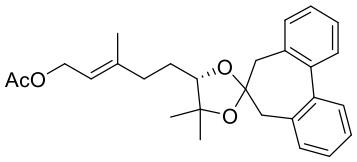
(*R*,*E*)-5-(5-bromo-3-methylpent-3-en-1-yl)-2,2,4,4-tetramethyl-1,3-dioxolane, ((*R*)-10)



To a solution of the alcohol (*R*)-**9** (230 mg, 1.0 mmol, 1.0eq.) in anhydrous diethyl ether PBr_3 (48 mL, 0.51 mmol, 0.5 eq.) was slowly added at –7 °C. After 20 minutes the mixture was diluted with diethyl ether and poured into a cooled 10% aq. $NaHCO_3$ solution. The separated organic phase was washed with $NaHCO_3$ and brine and the solvent removed under reduced pressure to give the pure bromide (*R*)-**10** (262 mg, 0.90 mmol, 90%). Bromide (*S*)-**10** was obtained following the same procedure, starting from alcohol (*S*)-**9**. (262 mg, 0.90 mmol, 90%). These compounds are stable for about one week only if stored at –20 °C under argon atmosphere.

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.10 (s, 3H), 1.25 (s, 3H), 1.33 (s, 3H), 1.42 (s, 3H), 1.50 (m, 1H), 1.63 (m, 1H), 1.75 (s, 3H), 2.12 (m, 1H), 2.31 (m, 1H), 3.66 (dd, 1H, J = 9.5, 2.5 Hz), 4.03 (d, 2H, J = 8.3 Hz), 5.59 (brt, 1H, J = 8.3 Hz).

(*S*,*E*)-5-(4',4'-dimethyl-5,7-dihydrospiro[dibenzo[a,c][7]annulene-6,2'-[1,3]dioxolan]-5'-yl)-3-methylpent-2-en-1-yl acetate, (*S*)-13



To a solution of the ketal **12** (91.6 mg, 0.36 mmol, 1.0eq.) in anhydrous $CHCl_3$ (3 mL) the diol (*S*)-**7** (83 mg, 0.36 mmol, 1.0 eq.) and traces of ptoluenesulfonic acid monohydrate were added in the presence of 4 Å molecular sieves. After 8 h of stirring atRT, the reaction mixture was filtered and the solvent removed under reduced pressure. The crude product was purified by column chromatography(SiO_2; diethyl ether:petroleum ether 1:3), giving the biphenyl dioxolane **13** as a yellow oil (43.8 mg, 0.10 mmol, 29% yield). $[\alpha]_D^{25}$ = –70.0 (c = 1.75, $CHCl_3$).

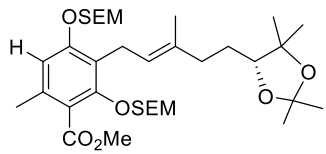
^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.22 (s, 3H), 1.28 (s, 3H), 1.59 (m, 1H), 1.69 (m, 1H), 1.75 (s, 3H), 2.06 (s, 3H), 2.18 (m, 1H), 2.32 (m, 1H), 2.65 (m, 1H), 2.72 (m, 2H), 2.86 (m, 1H), 3.75 (brs, 1H), 4.64 (brs, 2H), 5.46 (brs, 1H), 7.24 (t, 1H, J = 7 Hz), 7.30 (m, 3H), 7.36 (t, 2H, J = 7 Hz), 7.44 (d, 2H, J = 7.0 Hz). **^{13}C-NMR** (125 MHz, $CDCl_3$) δ (ppm): 10.9, 20.0, 21.1, 26.8, 35.9, 36.6, 43.9, 50.4, 61.4, 80.3, 82.5, 116.2, 119.2, 126.9, 127.3, 128.1, 128.5, 136.1, 140.2, 145.7, 171.1.

*2.4.2.4 General procedure for coupling reactions: synthesis of benzoates **14** and **15***

A solution of 3-methoxy-3-methyl-1-butyne (57 mg, 0.57 mmol, 1.15 eq.) in THF (0.5 mL) was treated with *n*-BuLi (1.6M in hexane, 356 mL, 0.57 mmol, 1.15 eq.) at 0 °C and stirred for 5 minutes. This solution was added to a suspension of copper iodide (109 mg, 0.57 mmol, 1.15 eq.) in THF (0.5 mL), precooled to 0 °C, giving a red-orange solution, which was stirred at that temperature for 30 minutes. Then HMPA (175 mL) was added and the resulting solution of the cuprous acetylide was transferred to a solution of the desired lithium-aryl reagent previously prepared as follows: the benzoates **5** or **6** (0.5 mmol, 1.0 eq) were dissolved in THF (3 mL), the solution was cooled at –78 °C, treated with *n*-BuLi (0.57 mmol, 1.15 eq.) and, after 15 minutes, the solution of cuprous acetylide was added. The resulting clear solution was stirred at –78 °C for 30 min and the optically active bromide **10** (0.57

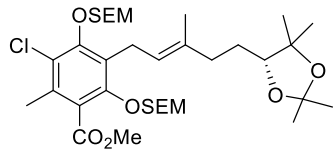
mmol, 1.15 eq.) solution in THF (0.7 mL) was added. The mixture was slowly warmed to RT and stirred for 16-20 h. The mixture was poured in a pH 8 aqueous ammonia/saturated ammonium chloride solution and extracted with diethyl ether. The organic phase was filtered on Celite and the crude products were purified by column chromatography on silica gel.

(*S*,*E*)-methyl 6-methyl-3-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)-2,4 -bis((2-(trimethylsilyl)ethoxy)methoxy)benzoate, (*S*)-14a



The product was isolated by column chromatography (SiO_2; hexane:diethyl ether 3:1) (137 mg, 0.21 mmol, 42%). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 0.00 (s, 9H), 0.02 (s, 9H), 0.97 (m, 4H), 1.06 (s, 3H), 1.21 (s, 3H), 1.25 (m, 1H), 1.27 (s, 3H), 1.39 (s, 3H), 1.58 (m, 1H), 1.77 (s, 3H), 1.98 (m, 1H), 2.18 (m, 1H), 2.28 (s, 3H), 3.37 (d, J ¼ 6.8 Hz, 2H), 3.61 (dd, J = 9.4, 3.4 Hz, 1H), 3.75 (m, 4H), 3.88 (s, 3H), 4.99 (s, 2H), 5.20 (br t, J = 7.0 Hz, 1H), 5.22 (s, 2H), 6.75 (s, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): -1.4, 16.3, 18.0, 18.1, 19.9, 22.9, 23.2, 26.0, 26.7, 27.7, 28.5, 36.7, 52.0, 66.3, 67.5, 80.0, 82.9, 92.6, 99.0, 106.4, 111.8, 122.0, 123.2, 134.3, 135.2, 153.6, 157.0, 168.7.

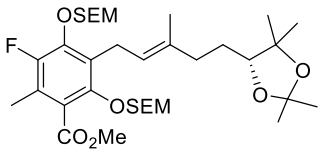
(*S*,*E*)-methyl 3-chloro-2-methyl-5-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)-4,6-bis((2-(trimethylsilyl)ethoxy)methoxy)benzoate, (*S*)-14b



The product was isolated by column chromatography (SiO_2; hexane: diethyl ether 3:1) (247 mg, 0.36 mmol, 72%). By the same procedure, starting from 0.3 mmol of substrate, the enantiomer (*R*)-**14b** was obtained (82 mg, 0.12 mmol, 40%). **^{1}H-NMR** (500 MHz, $CDCl_3$) δ (ppm): 0.02 (s, 9H), 0.03 (s, 9H), 0.98 (m, 4H), 1.06 (s, 3H), 1.21 (s, 3H), 1.27 (s, 3H), 1.39 (s, 3H), 1.43 (m, 1H), 1.58 (m,1H), 1.77 (s, 3H), 2.00 (m, 1H), 2.19 (dt, 1H, J = 10.5, 4.5 Hz), 2.29 (s, 3H), 3.37 (d, 2H, J = 6.8 Hz), 3.61 (dd, 1H, J = 9.5, 3.5 Hz), 3.76 (m, 2H), 3.89 (m, 2H), 3.91 (s, 3H), 4.99 (s, 2H), 5.09 (s, 2H), 5.25 (brt,

1H, J = 6.0 Hz). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): -1.4, 16.5, 17.6, 18.1, 22.9, 24.4, 26.0, 26.7, 27.7, 28.5, 36.6, 52.4, 67.6, 67.8, 80.0, 82.8, 97.8, 99.1, 106.4, 122.8, 124.9, 126.9, 129.2, 132.7, 135.0, 151.6, 153.6, 168.0.

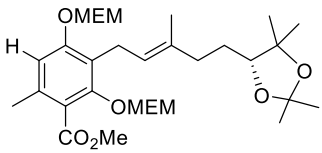
(*R*,*E*)-methyl 3-fluoro-2-methyl-5-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en -1-yl)-4,6-bis((2-(trimethylsilyl)ethoxy)methoxy)benzoate, (*R*)-14d



The product was isolated by column chromatography (SiO_2; hexane:diethyl ether 3:1) (154 mg, 0.23 mmol, 46%) followed by further column chromatography (SiO_2; DCM).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.03 (s, 9H), 0.04 (s, 9H), 0.99 (m, 4H), 1.08 (s, 3H), 1.22 (s, 3H), 1.29 (s, 3H), 1.41 (s, 3H), 1.43 (m, 1H), 1.61 (m, 1H), 1.78 (s, 3H), 2.02 (m, 1H), 2.20 (s, 3H), 2.21 (m, 1H), 3.44 (d, 2H, J = 6.0 Hz), 3.63 (brd, 1H, J = 9.5 Hz), 3.78 (t, 2H, J = 8.5 Hz), 3.85 (t, 2H, J = 9.0 Hz), 3.91 (s, 3H), 4.98 (s, 2H), 5.18 (s, 2H), 5.24 (m, 1H). **^{13}C-NMR** (125 MHz, $CDCl_3$) δ (ppm): -1.4, 18.0, 18.1, 22.9 (d, J = 12.5 Hz), 24.0, 26.0 (d, J = 11.5 Hz), 26.7 (d, J = 9.5 Hz), 27.7, 28.5 (d, J = 7.6 Hz), 30.9, 31.0, 36.6, 52.3 (d, J = 20.0 Hz), 67.5, 67.6, 80.0, 82.9 (d, J = 19.0 Hz), 97.3 (d, J = 7.6 Hz), 99.2, 106.4, 121.9 (d, J = 19.5 Hz), 122.8 (d, J = 24.0 Hz), 125.0, 128.4, 134.9, 149.2 (d, J = 119.3 Hz), 151.6, 167.5, 206.9.

(*R*,*E*)-methyl 2,4-bis((2-methoxyethoxy)methoxy)-6-methyl-3-(3-methyl-5-(2,2,5,5tetramethyl- 1,3-dioxolan-4-yl)pent-2-en-1-yl)benzoate, (*R*)-15a

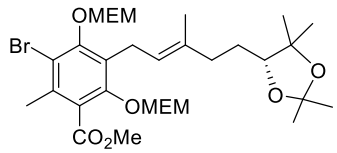


The product was purified by column chromatography (SiO_2; hexane:ethyl acetate 1:1). A further chromatographic purification (SiO_2; $CHCl_3$), afforded the pure product (114 mg, 0.20 mmol, 40%).

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.08 (s, 3H), 1.21 (s, 3H), 1.27 (s, 3H), 1.30 (m, 1H), 1.40 (s, 3H), 1.44 (m, 1H), 1.58 (m, 1H), 1.77 (s, 3H), 2.00 (m, 1H), 2.18 (s, 3H), 3.32 (d,

2H, J = 7.0 Hz), 3.37 (s, 3H), 3.39 (s, 3H), 3.55 (m, 4H), 3.62 (dd, 1H, J = 9.5, 3.0 Hz), 3.72 (m, 2H), 3.79 (m, 2H), 3.88 (s, 3H), 4.83 (s, 2H), 5.03 (brt, 1H, J = 6.8 Hz), 5.22 (s, 2H), 6.84 (s, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 16.2, 16.5, 22.9, 24.9, 26.0, 26.7, 27.6, 28.5, 36.6, 52.1, 59.0, 67.4, 67.7, 71.4, 71.5, 71.7, 80.0, 82.8, 92.3, 93.5, 94.0, 100.1, 106.4, 122.7, 123.4, 134.2, 135.2, 152.7, 156.1, 169.1.

(*R*,*E*)-methyl 3-bromo-4,6-bis((2-methoxyethoxy)methoxy)-2-methyl-5-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)benzoate, (*R*)-15c



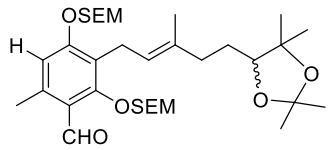
The product was isolated by column chromatography (SiO_2; hexane:diethyl ether 1:1) (226 mg, 0.35 mmol, 70%). **^{1}H-NMR** (500 MHz, $CDCl_3$) δ (ppm): 1.07 (s, 3H), 1.21 (s, 3H), 1.27 (s, 3H), 1.39 (s, 3H), 1.43 (m, 1H), 1.57 (m, 1H), 1.74 (s, 3H), 1.99 (m, 1H), 2.18 (dt, 1H, J = 10.0, 4.5 Hz), 2.32 (s, 3H), 3.38 (s, 3H), 3.39 (s, 3H), 3.48 (d, 2H, J = 6.0 Hz), 3.58 (m, 4H), 3.60 (dd, 1H, J = 10, 3.2 Hz), 3.83 (m, 2H), 3.90 (s, 3H), 3.97 (m, 2H), 5.04 (s, 2H), 5.14 (s, 2H), 5.22 (brt, 1H, J = 6.4 Hz). **^{13}C-NMR** (125 MHz, $CDCl_3$) δ (ppm): 16.4, 20.8, 22.9, 24.6, 26.0, 26.7, 27.6, 28.5, 36.5, 52.4, 59.1, 66.7, 69.3, 69.6, 71.6, 71.7, 80.0, 82.7, 95.6, 98.7, 99.7, 106.4, 122.7, 127.1, 129.2, 134.5, 135.1, 152.3, 154.6, 167.9.

*2.4.2.5. General procedure for the synthesis of aldehydes **16** and **17***

To a suspension of $LiAlH_4$ (18 mg, 0.47 mmol, 2.35 eq.) in diethyl ether (4 mL) a solution of benzoates **14** or **15** (0.2 mmol, 1.0 eq.) in diethyl ether (5 mL) was added at 0 °C and the mixture was stirred at RT for 2 h. Then it was diluted with diethyl ether (15-20 mL), treated with a saturated aqueous sodium sulphate solution (167 µL), filtered and the solvent removed under reduced pressure. The crude alcohol was dissolved in DCM (7 mL), treated with PCC (2 eq.) and stirred at RT for 1 h. Then, 2 more equivalents of PCC were added and the mixture was stirred for further 2-3 h, when a complete conversion to aldehyde was detected

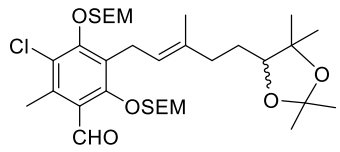
by TLC analysis. The mixture was diluted with diethyl ether, filtered on Celite and subsequently on a silica gel pad, eluting with diethyl ether. By removing the solvent at reduced pressure, all pure aldehydes were recovered in about 90% overall yield.

(*R* or *S*,*E*)-6-methyl-3-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)-2,4-bis((2-(trimethylsilyl)ethoxy)methoxy)benzaldehyde, (*R*)- and (*S*)-16a



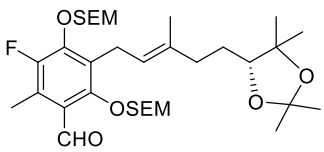
(111 mg, 0.18 mmol, 89%). **^{1}H-NMR** (500 MHz, $CDCl_3$) δ (ppm): -0.03 (s, 9H), 0.02 (s, 9H), 0.97 (m, 4H), 1.07 (s, 3H), 1.20 (s, 3H), 1.24 (m, 1H), 1.27 (s, 3H), 1.39 (s, 3H), 1.59 (m, 1H), 1.79 (s, 3H), 2.01 (m, 1H), 2.21 (m, 1H), 2.59 (s, 3H), 3.39 (d, 2H, J = 6.5 Hz), 3.63 (dd, 1H, J = 9.5, 4.0 Hz), 3.76 (m, 2H), 3.84 (m, 2H), 5.06 (s, 2H), 5.22 (brt, 1H, J = 6.8 Hz), 5.30 (s, 2H), 6.78 (s, 1H), 10.36 (s, 1H).

(*R* or *S*,*E*)-(3-chloro-2-methyl-5-(3-methyl-5-(2,2,5,5- tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)-4,6-bis((2-(trimethylsilyl)ethoxy)methoxy)benzaldehyde, (R)- and (S)-16b



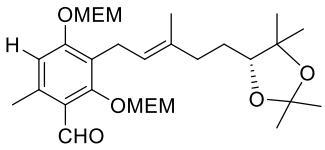
(121 mg, 92%). **^{1}H-NMR** (500 MHz, $CDCl_3$) δ (ppm): 0.03 (s, 18H), 0.99 (m, 4H), 1.07 (s, 3H), 1.21 (s, 3H), 1.27 (s, 3H), 1.40 (s, 3H), 1.44 (m, 1H), 1.59 (m, 1H), 1.79 (s, 3H), 2.02 (m, 1H), 2.21 (m, 1H), 2.63 (s, 3H), 3.47 (d, J ¼ 6.5 Hz, 2H), 3.63 (dd, J ¼ 9.2, 3.2 Hz, 1H), 3.81 (m, 2H), 3.91 (m, 2H), 5.02 (s, 2H), 5.18 (s, 2H), 5.26 (br t, J ¼ 7.0 Hz, 1H), 10.36 (s, 1H).

(*R*,*E*)-(3-fluoro-2-methyl-5-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)-4,6-bis((2-(trimethylsilyl)ethoxy)methoxy)benzaldehyde, 16d



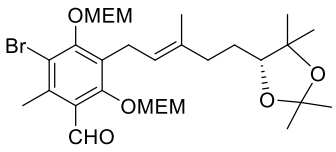
(113 mg, 88%). **^{1}H-NMR** (500 MHz, $CDCl_3$) δ (ppm): 0.07 (s, 18H), 0.97 (m, 4H), 1.07 (s, 3H), 1.20 (s, 3H), 1.26 (s, 3H), 1.39 (s, 3H), 1.43 (m, 1H), 1.60 (m, 1H), 1.79 (s, 3H), 2.02 (m, 1H), 2.20 (m, 1H), 2.47 (s, 3H), 3.43 (d, 2H, J = 5.5 Hz), 3.62 (dd, 1H, J = 9.5, 3.5 Hz), 3.81 (m, 4H), 5.01 (s, 2H), 5.23 (brt, 1H, J = 6.0 Hz), 5.26 (s, 2H), 10.34 (s, 1H).

(*R*,*E*)-(2,4-bis((2-methoxyethoxy)methoxy)-6-methyl-3-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)benzaldehyde, (*R*)-17a



(112.5 mg, 88%). **1H NMR** (500 MHz, $CDCl_3$) δ (ppm): 1.07 (s, 3H), 1.20 (s, 3H), 1.25 (s, 3H), 1.39 (s, 3H), 1.45 (m,1H), 1.58 (m, 1H), 1.78 (s, 3H), 2.01 (m, 1H), 2.18 (dt, 1H, J = 10.0, 4.5 Hz), 2.53 (s, 3H), 3.36 (d, 2H, J = 7.0 Hz), 3.38 (s, 3H), 3.39 (s, 3H), 3.57 (m, 4H), 3.61 (dd, 1H, J = 9.0, 3.5 Hz), 3.79 (m, 2H), 3.84 (m, 2H), 4.83 (s, 2H), 5.02 (brt, 1H, J = 6.8 Hz), 5.33 (s, 2H), 6.85 (s, 1H), 10.54 (s, 1H).

(*R*,*E*)-(3-bromo-4,6-bis((2-methoxyethoxy)methoxy)-2-methyl-5-(3-methyl-5-(2,2,5,5-tetra methyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)benzaldehyde, (*R*)-17c



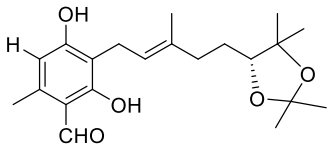
(111 mg, 90%). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 1.07 (s, 3H), 1.21 (s, 3H), 1.27 (s, 3H), 1.39 (s, 3H), 1.44 (m, 1H), 1.59 (m, 1H), 1.77 (s, 3H), 2.01 (m, 1H), 2.18 (m, 1H), 2.67 (s, 3H), 3.37 (s, 3H), 3.39 (s, 3H), 3.48 (d, 2H, J = 6.0 Hz), 3.55 (m, 2H), 3.60 (m, 2H), 3.63 (dd, 1H, J = 10.0, 3.6 Hz), 3.86 (m, 2H), 3.98 (m, 2H), 5.09 (s, 2H), 5.21 (s, 2H), 5.23 (brt, 1H, J = 6.5 Hz), 10.34 (s, 1H).

2.4.2.6. Deprotection reactions: synthesis of colletorin A, colletochlorin A and analogues

2.4.2.6.1. Method A: step 1 (general procedure)

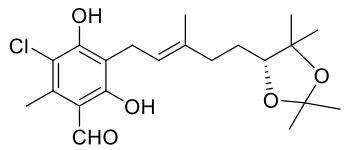
To a solution of SEM-protected 2,4-dihydroxybenzaldehydes **16** (0.16 mmol) in HMPA (1,6 mL) a TBAF solution (1M in THF, 10 eq., 1.6 mL) was added at RT. Then the reaction mixture was warmed up to 70 °C and stirred for 4-5 h. After removal of THF, the residue was poured in a mixture of ethyl acetate and brine. The solution was extracted with ethyl acetate and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (SiO2; hexane: ethyl acetate 3:1) affording the corresponding ketals **18**.

(*R*,*E*)-2,4-dihydroxy-6-methyl-3-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)benzaldehyde, (*S*)-18a



Following the general procedure the product was isolated as a pale yellow oil (70.8 mg, 70%). **1H NMR** (500 MHz, $CDCl_3$) δ (ppm): 1.08 (s, 3H), 1.22 (s, 3H), 1.28 (s, 3H), 1.39 (s, 3H), 1.50 (m, 1H), 1.63 (m, 1H), 1.84 (s, 3H), 2.11 (m, 1H), 2.26 (m, 1H), 2.49 (s, 3H), 3.40 (d, 2H, J = 7.0 Hz), 3.63 (dd, 1H, J = 10.0, 3.5 Hz), 5.30 (brt, 1H, J = 7.0 Hz), 6.05 (brs, 1H), 6.21 (s, 1H), 10.08 (s, 1H), 12.74 (s, 1H).

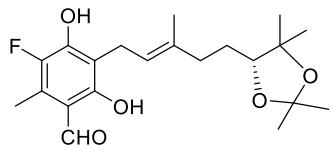
(*S*,*E*)-3-chloro-4,6-dihydroxy-2-methyl-5-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl) pent-2-en-1-yl)benzaldehyde, (*S*)-18b



Following the general procedure the product was as a pale yellow oil (44.5 mg, 70%). **^{1}H-NMR** (400 Hz, $CDCl_3$) δ (ppm): 1.07 (s, 3H), 1.19 (s, 3H), 1.23 (s, 3H), 1.39 (s, 3H),

1.47 (m, 1H), 1.61 (m, 1H), 1.80 (s, 3H), 2.03 (m, 1H), 2.19 (dt, 1H, J = 12.5, 5.5 Hz), 2.60 (s, 3H), 3.40 (d, 2H, J = 9.0 Hz), 3.61 (dd, 1H, J = 11.5, 4.5 Hz), 5.26 (brt, 1H, J = 9.0 Hz), 6.40 (brs, 1H), 10.13 (s, 1H), 12.69 (s, 1H).

(*R*,*E*)-3-fluoro-4,6-dihydroxy-2-methyl-5-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl) pent-2-en-1-yl)benzaldehyde, (*R*)-18d

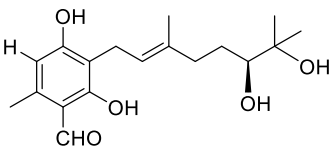


Following the general procedure the product was isolated as pale yellow oil (42.6 mg, 70%). **^{1}H-NMR** (500 MHz, $CDCl_3$) δ (ppm): 1.06 (s, 3H), 1.20 (s, 3H), 1.25 (s, 3H), 1.38 (s, 3H), 1.44 (m, 1H), 1.60 (m, 1H), 1.79 (s, 3H), 2.01 (m, 1H), 2.18 (dt, 1H, J = 10.0, 3.5 Hz), 2.40 (s, 3H), 3.35 (d, 2H, J = 7.0 Hz), 3.62 (dd, 1H, J = 10.5, 3.0 Hz), 4.16 (brs, 1H), 5.28 (brt, 1H, J = 7.0 Hz), 9.91 (s, 1H), 12.61 (s, 1H).

2.4.2.6.2. Method A: step 2 (general procedure)

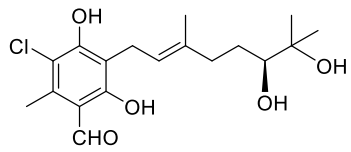
To a solution of ketals **18** (0,11 mmol) in THF: DCM (1:1) mixture (3 mL) H_2O (123 µL) and conc. H_2SO_4 (84 µL) were added sequentially. The reaction mixture was stirred at RT for 30 minutes and then was quenched adding dropwise saturated aqueous $NaHCO_3$ up to pH 7. The resulting mixture was extracted with ethyl acetate and the organic layer was washed with brine. The solvent was evaporated under reduced pressure and the obtained residue was purified by column chromatography (SiO_2; hexane:ethyl acetate 1:1) affording the desired products (*S*)-**1a**, (*S*)-**1b** and (*R*)-**1d**.

(*S*)-Colletorin A, (*S*)-1a



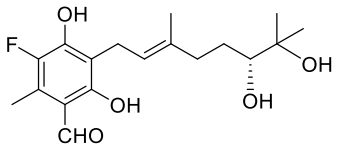
Following the general procedure, starting from (*S*)-**18a**, the product was isolated as a white foamy solid (24.8 mg, 70%). $[\alpha]_D^{25}$ = –8.7 (c 0.3, $CHCl_3$). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 1.15 (s, 3H), 1.19 (s, 3H), 1.43 (m, 1H), 1.69 (m, 1H), 1.82 (s, 3H), 2.04 (bs, 2H), 2.14 (m, 1H), 2.28 (m, 1H), 2.49 (s, 3H), 3.34 (d, 1H, J = 10.0 Hz), 3.39 (d, 2H, J = 6.8 Hz), 5.30 (brt, 1H, J = 6.8 Hz), 6.21 (s, 1H), 10.07 (s, 1H), 12.74 (s, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 16.1, 18.0, 21.1, 23.2, 26.4, 29.3, 36.9, 73.2, 78.3, 110.6, 111.9, 113.2, 121.9, 138.3, 141.9, 162.2, 163.6, 192.9. **Elemental analysis: found:** C, 67.15, H, 8.06, O, 24.79. **Calc. for $C_{18}H_{26}O_5$:** C, 67.06, H, 8.13, O 24.81%.

(*S*)-Colletochlorin A, (*S*)-1b



Following the general procedure, starting from (*S*)-**18b**, the product was isolated as a white foamy solid (25.5 mg, 65%). $[\alpha]_D^{25}$ = –18.6 (c 0.5, $CHCl_3$). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 1.13 (s, 3H), 1.17 (s, 3H), 1.41 (m, 1H), 1.58 (m, 1H), 1.79 (s, 3H), 2.02 (bs, 2H), 2.07 (m, 1H), 2.22 (m, 1H), 2.59 (s, 3H), 3.32 (dd, 1H, J = 12.0, 1.6 Hz), 3.39 (d, 2H, J = 6.7 Hz), 5.27 (brt, 1H, J = 6.7 Hz), 10.12 (s, 1H), 12.68 (s, 1H). **^{13}C-NMR** (100 MHz, CDCl3) δ (ppm): 14.4, 16.0, 22.0, 23.2, 26.3, 29.5, 36.9, 73.0, 78.3, 113.2, 113.5, 114.2, 121.6, 136.4, 137.7, 156.4, 162.1, 193.2. **Elemental analysis: found:** C, 60.50, H, 7.09, Cl, 9.87, O, 22.54. **Calc. for $C_{18}H_{25}ClO_5$:** C, 60.59, H, 7.06, Cl, 9.94, O, 22.42%.

(*R*)-Colletofluorin, (*R*)-1d

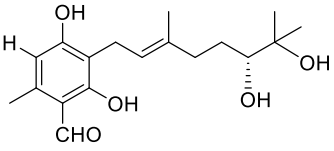


Following the general procedure starting from (*R*)-**18d**, the product was isolated as a white foamy solid (16.1 mg, 43%). $[\alpha]_D^{25}$ = +14.5 (c 0.4, $CHCl_3$). **^{1}H-NMR** (500 MHz, $CDCl_3$) δ (ppm): 1.14 (s, 3H), 1.18 (s, 3H), 1.43 (m, 1H), 1.59 (m, 1H), 1.80 (s, 3H), 2.05 (bs, 2H), 2.10 (m, 1H), 2.21 (m, 1H), 2.45 (s, 3H), 3.34 (d, 1H, J = 10.5 Hz), 3.37 (d, 2H, J = 6.5 Hz), 5.29 (brt, 1H, J = 6.5 Hz), 10.02 (s, 1H), 12.53 (s, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 16.0, 21.4 (d, J = 1.5 Hz), 23.2, 26.3, 29.4, 29.7, 36.9, 73.0, 78.3, 111.2, 114.4, 121.8, 124.2 (d, J ¼ 14.4 Hz), 136.5, 143.2 (d, J = 227.6 Hz), 150.5 (d, J = 16.7 Hz), 159.8, 192.7. **^{19}F-NMR** (376 MHz, $CDCl_3$) δ (ppm): -152.3. **Elemental analysis: found:** C, 63.48, H, 7.45, F, 5.85, O, 23.22. **Calc. for $C_{18}H_{25}FO_5$:** C, 63.51, H, 7.40, F, 5.58, O, 23.50%.

2.4.2.6.3. Method B (general procedure)

6M HCl (407 mL, 24,4 eq) was slowly added to a stirred solution of **17a**, **17c** or **16b** (0,10 mmol, 1.0 eq.) in THF (500 µL) at RT. After 16-18 h, the reaction was quenched adding dropwise saturated aqueous $NaHCO_3$ solution up to pH 7. Then the solution was diluted with ethyl acetate and the organic layer was washed with brine. The solvent was evaporated *in vacuo* and the obtained residue was purified by column chromatography (SiO_2; hexane:ethyl acetate 1:1), giving the pure compounds (*R*)-**1a**, (*R*)-**1b** and (*R*)-**1c**.

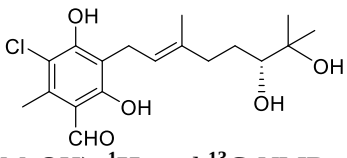
(*R*)-Colletorin A, (*R*)-1a



Following the general procedure, starting from (*R*)-**17a**, the product was isolated as a white foamy solid (9.7 mg, 30%). $[\alpha]_D^{25}$ = +9.8 (c 0.4, $CHCl_3$). **^{1}H-** and **^{13}C-NMR** spectra were

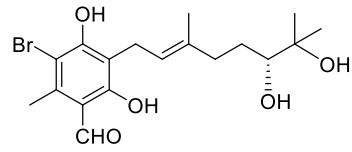
identical to those of (*S*)-**1a**. **Elemental analysis: found:** C, 67.02, H, 8.15, O, 24.83. **Calc. for $C_{18}H_{26}O_5$:** C, 67.06, H, 8.13, O, 24.81%.

(*R*)-Colletochlorin A, (*R*)-1b



Following the general procedure, starting from (*R*)-**16b**, the product was isolated as a white foamy solid (13.2 mg, 37%). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = +13.0 (c 0.4, $CHCl_3$), lit.[55] $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = +11.6 (c 10, MeOH). **^{1}H-** and **^{13}C-NMR** spectra were identical to those of (*S*)-**1b**. **Elemental analysis: found:** C, 60.62; H, 7.01; Cl, 9.97; O, 22.40. **Calc. for $C_{18}H_{25}ClO_5$:** C, 60.59; H, 7.06; Cl, 9.94; O, 22.42%.

(*R*)-Colletobromin, (*R*)-1c



Following the general procedure, starting from (*R*)-**17c**, the product was isolated as a pale yellow foamy solid (22.0 mg, 55%). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = +10.9 (c 0.5, $CHCl_3$). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 1.14 (s, 3H), 1.18 (s, 3H), 1.42 (m, 1H), 1.59 (m, 1H), 1.81 (s, 3H), 2.05 (bs, 2H), 2.09 (m, 1H), 2.24 (m, 1H), 2.65 (s, 3H), 3.36 (brd, 1H, J = 10.0 Hz), 3.43 (d, 2H, J = 7.2 Hz), 5.28 (brt, 1H, J = 7.0 Hz), 6.42 (bs, 1H), 10.16 (s, 1H), 12.73 (s, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 16.1, 17.6, 22.2, 23.3, 26.3, 29.5, 36.9, 72.9, 78.3, 106.1, 114.2, 114.3, 121.6, 136.6, 139.7, 156.9, 162.7, 193.5. **Elemental analysis: found:** C, 53.83; H, 6.32; Br, 19.89; O, 19.96. **Calc. for $C_{18}H_{25}FO_5$:** C, 53.87; H, 6.28; Br, 19.91; O, 19.93%.

2.4.2.7. Plant biological assays

2.4.2.7.1. Leaf puncture assay

The metabolites were tested at 2 mg/ml concentration on *A. artemisiifolia* and *Sonchus arvensis* (family: Asteraceae). Droplets (20 mL) of the solution containing the compound were applied to detached leaves previously punctured with a needle. Five replications were used for each plant species tested. Leaves were kept in a moistened chamber under continuous fluorescent lights at 25 °C. The eventual appearance of symptoms, consisting in circular necrosis, was observed three days after droplet application. Control treatments were carried out by applying droplets of a methanol solution (MeOH 1%). Effects were expressed on a visual scale from "-" = no symptoms, to "++++" = necrosis around 1 cm diameter.

2.4.2.7.2. Lemna minor assay

Pure compounds were tested against *L. minor* at 2 mg/mL concentration, by adapting a protocol already described.[56] Briefly, the wells of sterile, polystyrene 96-well microtiter plates were filled with a 100 mL aliquot of solutions containing the metabolites to be tested at 2 mg/mL concentration. One frond of an actively growing axenic L. minor plant was placed into each well. Control wells were included in each plate. Four replications were prepared for each compound. The plates were incubated in a growth chamber with 12/24 h fluorescent lights and observed daily up to 7 days. One day after the application of the test solution, 100 mL of distilled H_2O was added to each well. The chlorophyll contents of the fronds were determined by extracting the fronds from each well with 95% EtOH. A 2.0 mL aliquot of EtOH was added to the test tube containing the fronds, and incubated in the dark for 6 h. This aliquot was removed, and a second 1.5mL aliquot was added and incubated in the dark overnight. The EtOH extracts were combined and the chlorophyll content in each sample was determined spectrophotometrically from the absorbance at 649 and 665 nm by using the equation by Marr et al.[57]

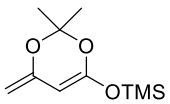
2.4.2.7.3. Mosquitos biological assays

Topical and injection bioassays were performed essentially as described.[58] Adult females were reared from *Ae. aegypti* larvae provided by the United States Department of Agriculture, Agricultural Research Service (USDA, ARS, Gainesville, FL, USA). After emergence, adult female mosquitoes were maintained at 28 °C under a 12 h light/dark cycle, and fed a diet of 10% sucrose solution. Compounds were dissolved in EtOH. Mosquitoes (2-7 days old) were briefly anaesthetised on ice, and 200 nL of the treatment solution applied to the dorsal thorax with a microdispenser. Control treatments were vehicle alone. Mosquitoes were, then, held for 24 h in paper cups with 10% sucrose solution for sustenance. For toxicity by injection, each compound was dissolved in EtOH and diluted into mosquito saline (5% EtOH final concentration). After cold anesthesia, mosquitoes were placed on their sides, and a 0.2 mL aliquot was injected into the thorax with a glass capillary needle attached to a micro-syringe. After treatment, the mosquitoes were held in paper cups and fed sugar water, with mortality recorded at 24 h (n = 10/dose, replicated three times) in all cases. For synergism against monooxygenase metabolism, 500 ng of PBO dissolved in EtOH was applied topically to the mosquitoes 4 h before topical or injected treatment with test material.

2.4.3. Radicinin and Deoxyradicinin

*2.4.3.1 Synthesis of (±)-Deoxyradicinin (**2b**)*

((2,2-dimethyl-4-methylene-4H-1,3-dioxin-6-yl)oxy)-trimethylsilane, 20

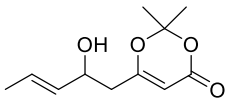


A solution of DIPA (5.4 mL, 38.5 mmol,1.1 eq) in THF (26 mL) was stirred under a nitrogen atmosphere and cooled to 0 °C. To this solution *n*-BuLi (15.4 mL, 38.5 mmol, 1.1 eq.) was slowly added in about 10 minutes and the mixture was stirred at the same temperature for another 45 minutes. The pale yellow solution obtained was, then, cooled to -78 °C and 2,2,6-trimethyl-4H-1,3-dioxin-4-one **19** (4.65 mL, 35 mmol, 1.0 eq.) was added in 5 minutes. After 1h, a freshly distilled TMSCl

(5.33 mL, 1.2 eq) was added in about 10 minutes. The dark orange mixture obtained was stirred for 1 h and then slowly heated to RT. The mixture was filtered under reduced pressure, the solid was washed with hexane and the filtrate concentrated by distillation under a reduced pressure. The crude product was purified by distillation under a high vacuum, using a rotary pump (pressure less than 1 mmHg) and heating to 50 °C. The pure silyl dienolether **20**[59] (4.76 g, 22.2 mmol, 63%) corresponded to the collected fraction having bp = 32-34 °C.

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 0.27 (s, 9H,), 1.55 (s, 6H), 3.88 (s, 1H), 4.07 (s, 1H), 4.65 (s, 1H). **^{13}C-NMR** (100 MHz, CDCl3) δ (ppm): 0.24, 24.5, 76.6, 84.9, 102.5, 151.8, 153.3.

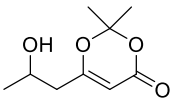
(*E*)-6-(2-hydroxypent-3-en-1-yl)-2,2-dimethyl-4H-1,3-dioxyn-4-one, 21a



A solution of crotonaldehyde (2.02 mL, 24.4 mmol, 1.1 eq) in DCM (90 mL) was cooled to –78 °C under a nitrogen atmosphere. Then $TiCl_4$ (2.43 mL, 22.2 mmol, 1.0 eq), and silyl dienolether **20** (4.76 g, 22.2 mmol, 1.0 eq.) were slowly added in sequence. After 1.5 h of stirring at the same temperature, the mixture was quenched with 40 mL of a saturated aqueous solution of $NaHCO_3$ and the organic phase separated. The aqueous phase was again extracted with DCM and the combined organic layers were dried over anhydrous $NaSO_4$ and the solvent evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2; first *n*-hexane:ethyl acetate 7:3 and then 1:1), obtaining pure alcohol **21a**[60](2.87 g, 13.5 mmol 61%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.69 (s, 3H), 1.70 (s, 3H), 1.71 (d, 3H, J = 7.2 Hz), 2.40-2.48 (m, 2H), 4.36 (brq, 1H, J = 6.4 Hz), 5.32 (s, 1H), 5.51 (dd, 1H, J = 15.2 Hz, 7.2 Hz), 5.71-5.78 (m, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 17.6, 24.9, 25.3, 41.5, 69.8, 95.2, 106.6, 128.5, 132.3, 161.1, 168.5.

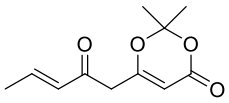
6-(2-Hydroxypropyl)-2,2-dimethyl-4H-1,3-dioxyn-4-one, (21b)



Following the same procedure as above, but starting from freshly distilled acetaldehyde, alcohol **21b**[61] was obtained in 62% yield.

^{1}H-NMR (400 MHz, CDCl3) δ (ppm): 1.27 (d, 3H, J = 6.0 Hz), 1.70 (s, 6H), 1.86 (brs, 1H), 2.38 (d, 2H, J = 6,4 Hz), 4.09-4.13 (m, 1H), 5.33 (s, 1H). **^{13}C-NMR** (100 MHz, CDCl3) δ (ppm): 23.5, 24.8, 25.2, 43.2, 65.2, 95.0, 106.6, 161.3, 169.0.

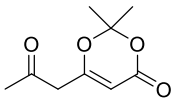
(*E*)-2,2-dimethyl-6-(2-oxopent-3-en-1-yl)-4H-1,3-dioxyn-4-one, 22a



Dess-Martin periodinane (2.50 g, 5.90 mmol, 1.3 eq) was added in small portions to a solution of alcohol **21a** (940 mg, 4.43 mmol, 1.0 eq.) in DCM (20 mL)and the solution was stirred at RT, monitoring the conversion by TLC analysis. After 1.5 h, the mixture was diluted with 120 mL of diethyl ether and 24 mL of a saturated solution of $NaHCO_3$ containing 5% sodium thiosulfate and the mixture was stirred for further 20 minutes. The organic layer was separated, dried over anhydrous $NaSO_4$ and then concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2; first *n*-hexane:EtOAc 7:3 and then 1:1), to afford pure ketone **22a**[60] (912 mg, 4.34, 98% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.71 (s, 6H), 1.95 (d, 3H, J = 6.8 Hz), 3.46 (s, 2H), 5.36 (s, 1H), 6.17 (d, 1H, J = 15.6 Hz), 6.90-6.97 (m, 1H). **^{13}C-NMR** (100 MHz, CDCl3) δ (ppm): 18.4, 25.0, 44.6, 96.6, 107.1, 130.8, 145.4, 160.7, 165.0, 192.3.

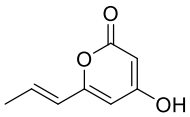
2,2-dimethyl-6-(2-oxopropyl)-4H-1,3-dioxyn-4-one, 22b



Following the same procedure as above ketone **22b**,[61] was obtained in 98% yield.

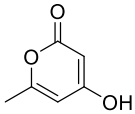
^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.72 (s, 6H), 2.25 (s, 3H), 3.35 (s, 2H), 5.36 (s, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 25.0, 30.1, 47.9, 96.7, 107.2, 160.6, 164.3, 184.3.

(*E*)-4-hydroxy-6-(propen-1-yl)-2H-piran-2-one, 23a



Ketone **22a** (912 mg, 4.34 mmol, 1.0 eq.) was dissolved in toluene (40 mL) and the solution was heated to 100 °C for 0.5-1 h. During this time, a very thin white precipitate was formed. After cooling to room temperature, toluene was removed *in vacuo* and the solid was washed with diethyl ether and a small amount of $CHCl_3$, giving the pure pyranodienone **23a**[60] (632 mg, 4.16 mmol, 96%). **^{1}H-NMR** (400 MHz, DMSO-*d*6) δ (ppm): 1.82 (d, 3H, J = 6.4 Hz), 5.22 (s, 1H), 5.98 (s, 1H), 6.15 (d, 1H, J = 16.8 Hz), 6.41–6.49 (m, 1H), 11.63 (s, 1H). **^{13}C-NMR** (100 MHz, DMSO-*d*6) δ (ppm): 23.2, 94.6, 104.9, 128.5, 138.9, 164.1, 168.1, 175.5. **MS (EI)**: m/z 152 (M^+, 51), 137 (50), 124 (34), 69 (100), 55 (11).

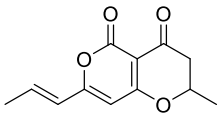
4-hydroxy-6-(methyl)-2H-piran-2-one, 23b



Following the same procedure as above, 4-hydroxypyranone **23b**[62] was obtained in 92% yield.

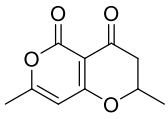
^{1}H-NMR (400 MHz, DMSO-*d*6) δ (ppm): 2.12 (s, 3H), 5.17 (s, 1H), 5.92 (s, 1H), 11.58 (s, 1H). **^{13}C-NMR** (100 MHz, DMSO-*d*6) δ (ppm): 35.9, 93.3, 105.4, 168.5, 169.1, 175.7.

(E)-2-methyl-7-(prop-1-en-1-yl)-2,3-dihydropyrane[4,3-b]pyran-4,5-dione ((±)-3-deoxyradicinin), (±)-2b



Under dry and inert atmosphere, $TiCl_4$ (0.234 mL, 213 mmol, 1.8 eq) and crotonoyl chloride (0.170 mL, 1.77 mmol, 1.5 eq) were dropwise added in sequence to a solution of pyranodienone **23a** (180 mg, 1.18 mmol, 1.0 eq.) in $CHCl_2CHCl_2$ (2 mL). The mixture was first stirred for 15 minutes at RT and then heated to 100 °C for 3 h. After cooling to RT, the mixture was poured into water/ice (10 mL) and extracted with ethyl acetate. The organic layer was washed with brine and concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2; *n*-hexane:acetone 1:1), affording (±)-**2**[44] (130 mg, 0.59 mmol, 50%). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 1.54 (d, 3H, J = 6.4 Hz), 1.95 (d, 3H, J = 6.8 Hz), 2.59-2.70 (m, 2H), 4.73-4.80 (m, 1H), 5.83 (s, 1H), 6.02 (d, 1H, J = 15.6 Hz), 6.89-6.97 (m, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 18.7, 20.3, 43.7, 53.4, 98.1, 100.2, 122.7, 140.0, 157.2, 163.4, 175.9, 186.4. **MS (EI)**: *m/z* 220 (M^+. 44), 205 (100), 179 (24), 111 (17), 69 (77).

2,7dimethyl-2,3-dihydropyrane[4,3-b]pyran-4,5-dione, (±)-24



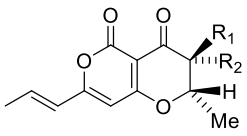
Following the same procedure as above, the compound analogue (±)-**24**[63] was obtained in 45% yield.

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.54 (d, 3H, J = 6.8 Hz), 2.27 (s, 3H), 2.59-2.68 (m, 2H), 4.74-4.79 (m, 1H), 5.90 (s, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 20.2, 20.7, 29.2, 43.7, 95.1, 99.6, 157.8, 168.7, 175.9, 186.4. **MS (EI)**: m/z 194 (M^+, 28), 179 (100), 153 (16), 69 (61), 41 (16).

2.4.3.2. Oxidation of (±)-3-deoxyradicinin (2b) with $Pb(OAc)_4$

A mixture of compound **2b** (40 mg, 0.18 mmol, 1.0 eq.), $Pb(OAc)_4$ (120 mg, 0.27 mmol, 1.5 eq) and acetic acid (3 mL) was stirred and heated to 100 °C for 2h. The reaction mixture was then cooled to RT, poured into water/ice and extracted with DCM. The organic layer was washed with a 5% $NaHCO_3$ solution and brine and concentrated under reduced pressure. The crude was purified by column chromatography (SiO_2; *n*-hexane: acetone 1:1) and two fractions were eluted, corresponding to a 6:4 mixture of *trans*:*cis* (±)-radicinin acetate **25**[39] (9.5 mg, 0.034, 19%) and the pyranopyrandione **26**[42] (29 mg, 0.13 mmol, 74%).

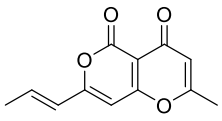
(*E*)-2-methyl-4,5-dioxo-7-(prop-1-en-1-yl)-2,3,4,5-tetrahydropyrano[4,3-b]pyran-3-yl acetate, 25a+25b mixture



(±)-**25a** R_1=OAc, R_2=H
(±)-**25b** R_1=H, R_2=OAc

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.45 (d, 3H, J = 6.4 Hz, **25b**), 1.55 (d, 3H, J = 6.4 Hz, **25a**), 1.97 (d, 3H, J = 7.2 Hz, **25a** + **25b**), 2.17 (s, 3H, **25b**), 2.21 (s, 3H, **25a**), 4.69-4.75 (m, 1H, **25a**), 4.85-4.92 (m, 1H, **25b**), 5.25 (d, 1H, J = 10.8 Hz, **25a**), 5.49 (d, 1H, J = 3.6 Hz, **25b**), 5.85 (s, 1H, **25a** + **25b**), 6.04 (dd, 1H, J = 15.2 Hz, 1.6 Hz, **25a** + **25b**), 6.93-7.00 (m, 1H, **25a** + **25b**).

(*E*)-2-methyl-7-(prop-1-en-1-yl)pyrano[4,3-b]pyran-4,5-dione, 26



^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.97 (d, 3H, J = 7.2 Hz), 2.30 (s, 3H), 6.06 (s, 1H), 6.07 (d, 1H, J = 15.6 Hz), 6.17 (s, 1H), 6.90-6.99 (m, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 18.7, 19.6, 96.7, 105.7, 115.7, 122.3, 139.8, 156.9, 162.2, 163.6, 169.3, 174.3.

2.4.3.3. Leaf Puncture Bioassays

The synthetic derivatives (±)-**2b** and **26** were assayed at 2.5×10^{-3} M for phytotoxicity on leaves of buffelgrass (*Cenchrus ciliaris*) following the procedure previously reported[40,12] and their activity compared with that of natural **2a**.[8] Accordingly, compounds were first dissolved in MeOH (final concentration 4%) and stock solutions at the two concentrations using sterile distilled water were then prepared. An incision of ca. 3 mm was made on the adaxial surface of each leaf section of 3 cm with an insulin needle. The leaf sections were placed in groups of six on the surface of a water-saturated filter paper in each of four petri dishes. Five leaf sections in each petri dish were tested with the solution containing the compound, while one leaf section was used as a negative control (4% MeOH only). A droplet (10 μL) of the appropriate solution was applied over each needle incision using a micropipette. The dishes were sealed with parafilm and incubated at 24 °C for 3 days in a temperature-regulated chamber under a photoperiod of 14-10 h (light/dark). After three days of treatment, necrotic lesion development was evaluated by removing the petri dish cover, placing a glass disc on the leaf sections to flatten them into a single plane, and photographing each dish with its leaf sections. Each acquired image was then analyzed with the software ImageJ to measure the necrotic area caused by the solution.

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Chapter 3

Cyclochirality Emerging from Hydrogen-Bonding Networks in Cyclic Oligoureas

3.1. Introduction

3.1.1. Molecular Assemblies displaying Cyclochirality

Chirality is the geometric property of a three-dimensional object of being non-superimposable on its mirror image. This particular property plays a fundamental role both in nature and in synthetic organic chemistry since most of the biological macromolecules of living systems occur in nature in one enantiomeric form only. A biologically active chiral compound interacts with its receptor site in a chiral manner, and enantiomers may be discriminated by the receptor in very different ways. Thus, it is not surprising that the two enantiomers of a drug may interact differently with the receptor, leading to different effects. Consequently, it is very important to keep the idea of chiral discrimination in mind when designing biologically active molecules. Different types of chirality can arise (Figure 3.1): *point or central chirality*, due to a single stereogenic atom, having four different substituents arranged in a tetrahedron; *axial chirality*, for a system with two planar groups arranged about an axis, where the two planes are non-coplanar. The system is asymmetric when the groups on each side of the axis are different; *planar chirality*, which is a stereoisomerism resulting from the arrangement of out-of-plane groups with respect to a plane (chirality plane);[1] *helical chirality or helicity*, which is a special case of chirality in which molecules are shaped as a right- or left-handed spiral like a screw or spiral stairs. The configurations are designed *M* and *P*, respectively, according to the helical direction. Viewed along the axis, a clockwise helix is definened as *P*, whereas a counterclockwise orientation is defined as *M*.

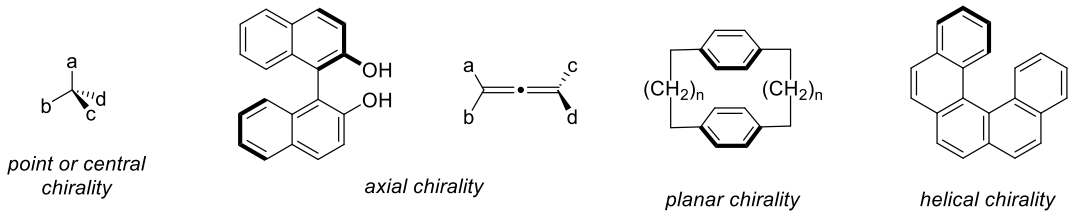


Figure 3.1. Different types of chirality.

However, with the growing complication of molecular systems that are characterised by chemists, it has been realised that the classical understanding of molecular chirality is not always sufficient. For instance, classical chirality elements like centre, axis or plane are not adequate to describe the chirality of rotaxanes, catenanes, fullerenes, cavitands or capsular assemblies.[2] New definitions such as *inherent chirality* and *cyclochirality* or *cicloenantiomerism* are therefore necessary. Inherent chirality is defined as chirality arising from the introduction of curvature in an ideal planar structure that is devoid of perpendicular symmetry planes in its bidimensional representation.[2] The term "cyclochirality" was introduced in 1964 by Prelog and Gerlach for cyclopeptides.[3] In their paper, they described *cyclostereoisomers* as composed of an equal number of enantiomeric building blocks ("Bauelemente"), possessing the same arrangement of stereocentres, i.e., the same cyclic distribution pattern of configurational descriptors ("Verteilungsmuster"), and differ only in the sense of direction of the ring ("Ringrichtung").[4] In this contest *cyclostereoisomerism* is considered as an isomerisms that arise when cyclic arrangements of stereocenters are associated with ring systems, and the stereocenters can be attached externally to the ring or incorporated in it. In particular, it refers to a clockwise or anti-clockwise array of chiral building blocks in the cyclopeptide that imply directionality of the ring. An example of cyclostereoisomerism is highlighted in Figure 3.2.

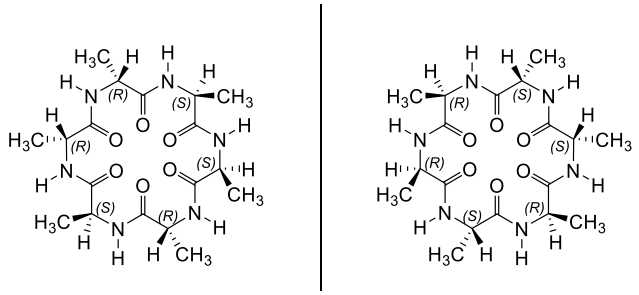


Figure 3.2. Cycloenantiomeric cyclohexaalanyls.

Cyclochirality can be also observed in supramolecular structures such as catenanes, namely molecules consisting of two or more interlocked rings, (Figure 3.3a) and rotaxanes, molecules consisting of stoppered axle(s) components threaded through one or more macrocyclic rings, (Figure 3.3b).

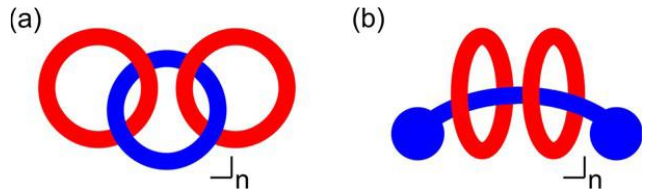


Figure 3.3. Schematic representations of: a) [n+2]catenane, and b) [n+2]rotaxane.

A catenane or rotaxane may be made chiral by the inclusion of a classical chiral motif (Figure 3.4a) or, alternatively, chirality may arise as a consequence of the mechanical bond (Figure 3.4b).[5] A straightforward way to create a chiral catenane or rotaxane is by incorporating a classical chiral element, such as a chiral centre, axis or plane into at least one of the components of the interlocked molecule. However, chirality may arise in catenanes and rotaxanes due to the presence of the mechanical bond, even when the interlocked components are achiral. This may be described as "mechanical chirality", a term that has recently been defined specifically by Bruns and Stoddart as "a non-classical form of chirality resulting from the spatial arrangements of component parts connected by mechanical bonds".[6]

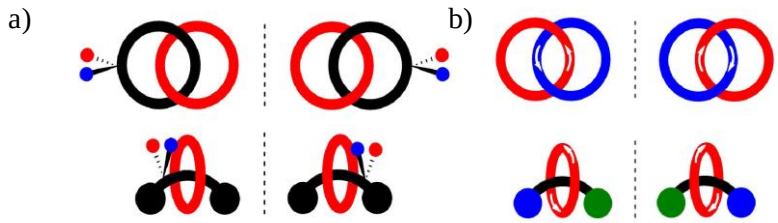


Figure 3.4. Chirality arising from: a) classical chiral elements and b) the mechanical bond.

As mentioned above, such mechanically interlocked molecules present a range of novel structural and chiroptical properties and non-classical types of chiralities.

Cycloenantiomerism of such molecules was foreseen theoretically by Frisch and Wassermann in 1961.[7] Nevertheless the first example of an enantioenriched cycloenantiomeric [2]rotaxane, was reported in the 1990's, and consisted of a dumbbell and a wheel, which are achiral. Cycloenantiomerism of rotaxanes occurs when, for example, one enantiomer has a clockwise orientation of the wheel with respect to the unsymmetrical axle, whereas the other enantiomer is arranged anti-clockwise (Figure 3.5).[8] The covalent connection of the wheel and the axle of such a chiral rotaxane with suitable bridges leads to cycloenantiomeric [1]rotaxanes as shown in Figure 3.6. The racemates of the [1]rotaxanes were separated by chiral HPLC and the ECD spectra obtained for each of the enantiomers were in a mirror image relationship over the whole spectral region.[10]

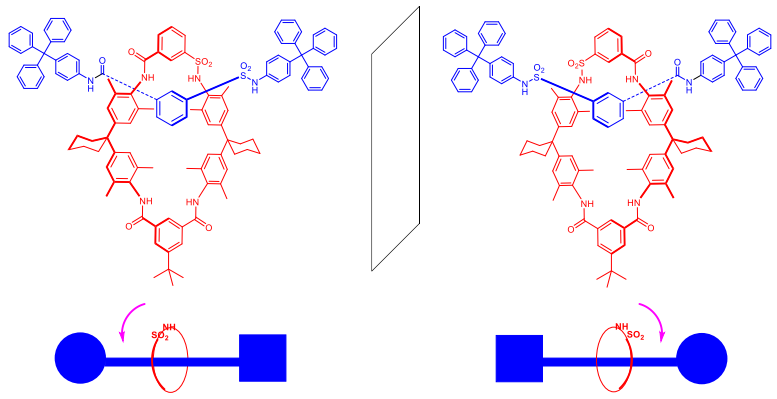


Figure 3.5. Cycloenantiomeric [2]rotaxane (mirror plane grey shaded, arrows indicate the sequence of the atoms).

The synthesis of a cyclochiral [3]rotaxanes was also reported (Figure 3.7).[9] By using two achiral macrocycles threaded on a symmetric, achiral axle, diastereomeric [3]rotaxanes were formed. Alternatively, if there were only one oriented macrocycle on the symmetrical axle, a [2]rotaxane with no stereoisomerism would result. For rotaxanes bearing two macrocycles (Figure 3.7), the atomic sequences can be arranged clockwise or anti-clockwise, and therefore should occur in either a *meso* form or a C_2-symmetric racemic form (Figure 3.8). The orientation of the enantiomers is caused by a different sequence of the three amide groups and X = *N*-methyl sulphonamide group. The two macrocycles can be oriented parallel or antiparallel. In the parallel case, the *meso* isomer is obtained, whilst the antiparallel orientation led to *R*-**A** and *S*-**A**. Separation of enantiomer was successfully carried out using chiral HPLC and analysis of both enantiomers by circular dichroisms (CD) showed our significant Cotton effects as well as optical rotation at different wavelengths, (Figure 3.9) whereas the *meso* form was not optically active. Thus, the remarkable property of these [3]rotaxanes is that the mechanical bonding of their achiral components, axles, and macrocycles, leads to cyclodiastereomeric species.[10]

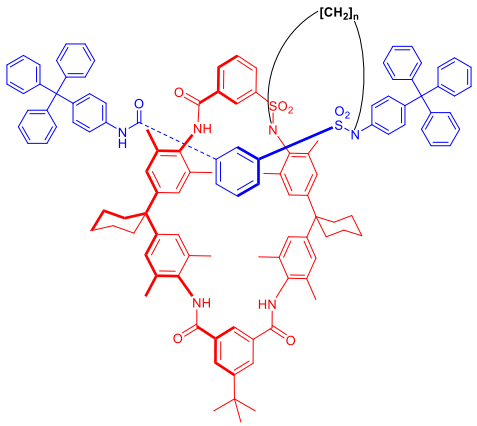


Figure 3.6. Cycloenantiomeric or cyclochiral [1]rotaxane.

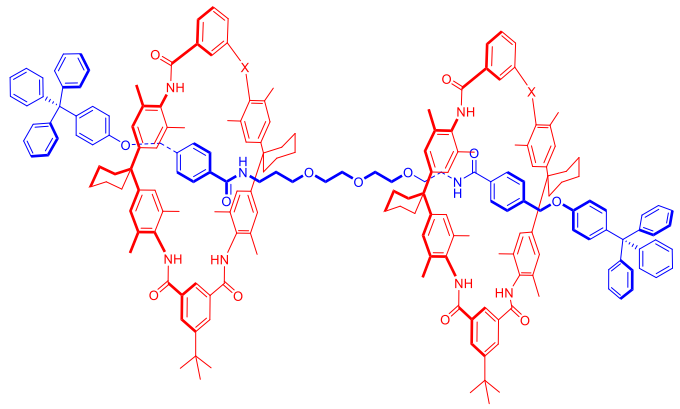


Figure 3.7. Cycloenantiomeric or cyclochiral [3]rotaxane.

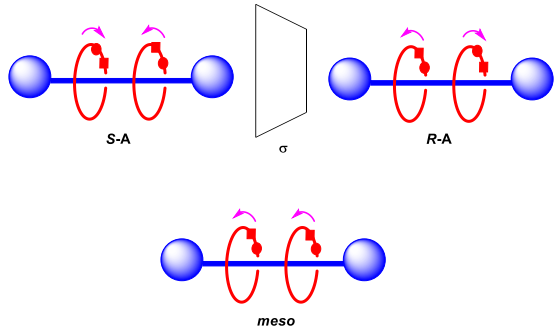


Figure 3.8. Cyclodiastereomeric [3]rotaxanes.

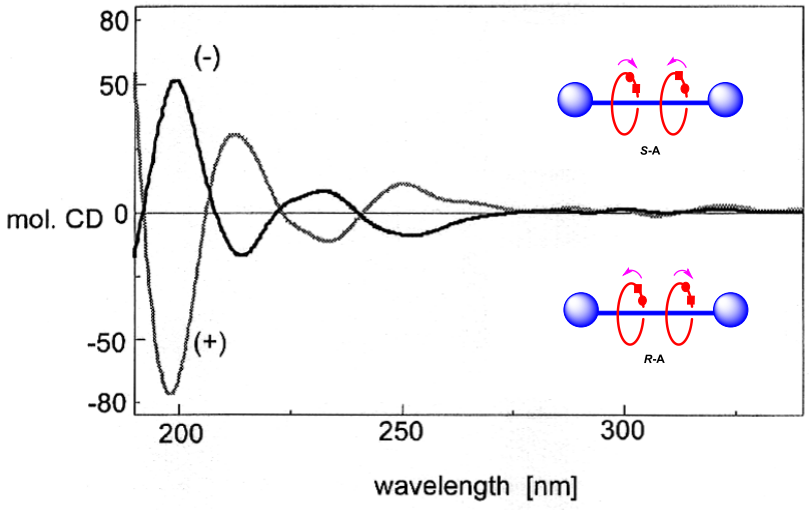


Figure 3.9. Circular dichroism spectra of the cycloenantiomers of [3]rotaxanes.

A chiral arrangement can be also achieved using directional non-covalent interactions, such as hydrogen bonds mounted on a non-planar skeleton. The formation of hydrogen bonds is one of the most important interaction in supramolecular chemistry.[11] Consequently, interest in the investigation of hydrogen-bonded systems is still growing. Higher-order structures of proteins, such as α-helices and β-sheets, are supported by a continuous hydrogen-bonding network constructed from alternating sequences of N−H and C═O moieties in a series of amide groups on the protein backbone. For this reason, synthetic chemists exploit non-covalent interactions such as hydrogen-bonding to create artificial systems with unique properties. Molecules with suitable conformations are able to form intramolecular interactions between non-contiguous atoms of the same molecule. When a hydrogen bond donor and acceptor group are brought into the correct geometry, intramolecular hydrogen bonds can form. The first examples of chirality due to hydrogen-bonding systems were reported by the group of Rebek, which described self-folding cavitands, synthetic hosts in which intramolecular hydrogen bonding and solvophobic effects play important roles in maintaining their unique conformation. Rebek and co-workers reported that resorcin[4]arene-based (Figure 3.10) cavitands bearing four aromatic rings, each having two amide groups, fold into a deep open-ended cavity by means of intramolecular (C=O· · · H-N) hydrogen bonds in non-polar solvents.[13] The belt of the hydrogen-bonded amide groups makes two possible cycloenantiomers, with clockwise and anti-clockwise orientations of the amide bonds (Figure 3.11).[13] In solutions at room temperature, the interconversion between these two conformational enantiomers occurs through the reorientation of the hydrogen-bonding arrays.[12]

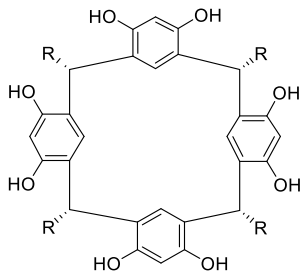


Figure 3.10. Resorcinarene: a bowl-shaped cyclic resorcinol tetramer with four alkyl tails oriented in the same direction.

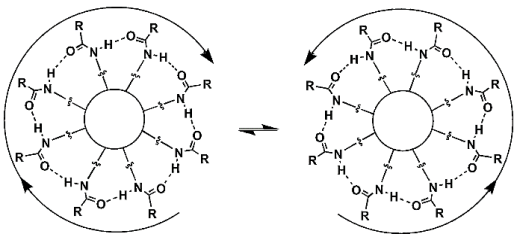


Figure 3.11. Head-to-tail hydrogen-bonding array formed cooperatively by eight amide groups of Rebek's self-folding cavitand. Interconversion of two cycloenantiomers, with clockwise and anti-clockwise orientation of the amide bonds, is shown. The large circles represent the resorcinarene skeleton; R is an alkyl group.[13]

Not only the amide group, but also urea moieties act as strong hydrogen bond donors and acceptors. In fact, ureas have been frequently used as functional groups for the formation of supramolecular architectures by virtue of their ability to form hydrogen bonds. Chiral macrocycles with eight (*R*)- and (*S*)-homobenzyl urea residues on the resorcinarene skeleton linked through a hexyl or dodecyl spacer and having amide linkages have been prepared by the reactions of the corresponding octaamine derivative with (*R*)- and (*S*)-α-methylbenzyl isocyanate, respectively (Figure 3.12).[13] In chloroform, the urea-functionalised resorcinarenes with hexyl spacers formed intramolecular hydrogen bonds by bundling the urea and amide residues in a cyclic fashion to give a self-folding cavitand. The urea and amide residues were cooperatively oriented in the same direction to result in asymmetric hydrogen-bonding belts. Unique ECD bands were induced in the absorption wavelength ranges of the macrocyclic skeleton, caused by a remote chirality transmission from their chiral urea termini through the hexyl spacers in the asymmetrically self-folded conformation. On the other hand, urea-functionalised resorcinarenes with a longer dodecyl spacer did not show such unique ECD bands on the macrocycle, because of their weaker propensity for hydrogen bond formation. The characteristic ECD bands of the urea-functionalised self-folding macrocycles disappeared upon complexation with anions such as chloride and bromide, or in the presence of protic solvent reflecting the rupture of the intramolecular hydrogen-bonding belts.

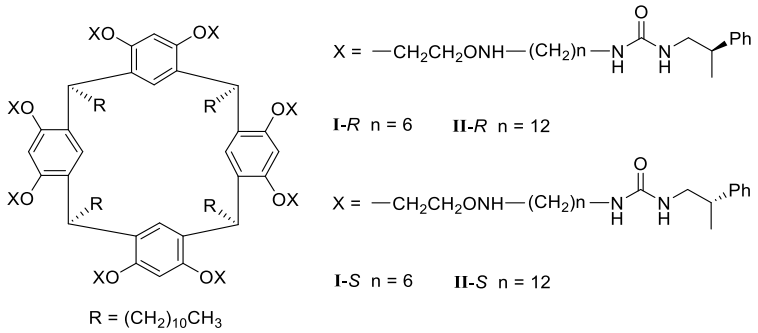


Figure 3.12. Resorcinarenes bearing eight (*R*)- and (*S*)-homobenzyl urea residues, **I**-R and **I**-S (n = 6) and **II**-R and **II**-S (n = 12).

Another example of cyclochiral resorcinarenes that maintain their cyclochirality by means of hydrogen bonds was reported by Szumna (Figure 3.13).[14] It was shown that the isomerisation process for that molecule is characterized by the relatively high racemization barrier (61.1-77.4 kJ·mol^{-1}) and thus it can be concluded that the transformation of one cycloconformer into the other requires the simultaneous rupture of all eight hydrogen bonds. For derivatives with additional stereogenic centres, two cyclodiastereoisomeric conformations were detected. Both molecular and crystallographic data have shown that the molecule highlighted in Figure 3.13 has C_4 symmetry and adopts a cyclochiral kite conformation that is stabilised by the presence of eight hydrogen bonds. The most pertinent feature of this structure is the presence of four eight-membered hydrogen bonded rings that bond concurrently and unidirectionally, giving rise to the cyclochiral conformation. Compounds devoid of other types of chirality exist in the solution as mixtures of (*P*)- and (*M*)-cycloenantiomeric conformations. However, for compounds with additional stereocentres, for example with *R* stereochemistry, two diastereomeric cyclochiral conformers (*P*)-(*R*)- and (*M*)-(*R*)-can be expected and are presumed to show different spectra and populations.

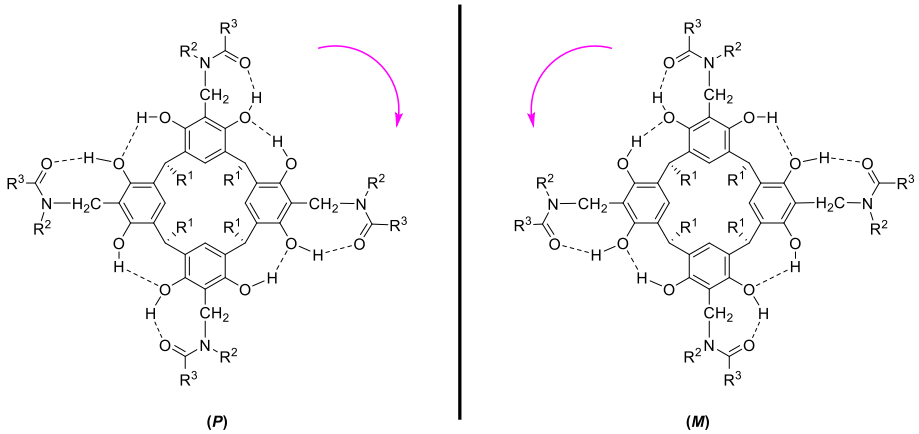


Figure 3.13. Conformationally cyclochiral resorcinarenes

As already described, cyclic hydrogen bonding systems can give rise to conformational chirality in a molecule, only depending on its directionality. Unfortunately, the hydrogen-bonding stabilisation energy is often not high enough to preserve the chiral conformation and, for this reason, racemisation barriers of these molecules tend to be too low to permit separation of the enantiomers.[2]

However, Kawabata and co-workers[15] succeeded in developing the first example of a stable and isolable cyclochiral structure, namely a compound that is chiral by virtue of its cyclic hydrogen-bonding array only. In particular, they synthesised a molecule based on the 4-pyrrolidinopyridine (PPY) structure, bearing four identical amide substituents on the pyrrolidine. These moieties were approximately coplanar thus forming a series of alternating 6- and 8-membered hydrogen-bonding rings (Figure 3.14). It was found out that the four amide protons appeared in the ^{1}H-NMR spectrum at room temperature as two sets of non-equivalent signals, suggesting that all four amide groups were involved in a strong intramolecular hydrogen-bonding network. This result also indicated that the structure was in a C_2-symmetrical conformation, instead of C_{2v}-symmetrical. Heating the compound caused the coalescence of the amido protons into one signal showing C_{2v} symmetry. Thus, at room temperature the compound was chiral despite the lack of any classical element of chirality, but only due to the strength and directionality of its cyclic hydrogen-bonding arrangements. That molecule existed as racemic mixture, with one enantiomer of the

structure orientating its hydrogen-bonds in a clockwise fashion, and its antipode in an anti-clockwise fashion. Full line shape analysis of VT- NMR spectra and subsequent Eyring plot analysis allowed determination of the racemisation barrier, which was found to be ca. 84 kJ mol^{-1} where R = Ph. The relatively high energy value suggested that the compound was not racemising quickly at room temperature and, in fact, the enantiomers of the PPY derivative could be separated by preparative chiral HPLC at 0 °C. Different PPY derivatives with a variety of amide substituents were prepared, all showing similarly large barriers to racemisation, with a maximum of 100 kJ mol^{-1} when R = dicyclohexylmethyl, suggesting that the introduction of a more sterically bulky group can increase the racemisation barrier. When chiral amide substituents, such as (*R*)-1-(1-naphthyl)ethylamino, were introduced in the structure, a conversion of enantiomeric conformers to the diastereomeric isomers occurred, with a ratio of 73:27 when R = (*R*)-1-(1-naphthyl)ethyl. Finally, one enantiomer of the PPY derivative with R = dicyclohexylmethyl was used as a chiral nucleophilic catalyst in an acylative kinetic resolution of a chiral secondary alcohol. With a 10 mol% loading of the PPY, enantioselective acylation occurred with a 59% e.e. and without racemisation of the catalyst. Kawabata and co-workers showed that it is possible exploit cyclochiral molecules, whose asymmetry arises only by virtue of the strength of its anisotropic cyclic hydrogen-bonding network despite not having any classical elements of chirality. Such cyclochiral compounds can provide a new class of functional molecules useful in asymmetric organocatalysis, enantiospecific host-guest chemistry, and as functional materials, opening a new field of organic chemistry.

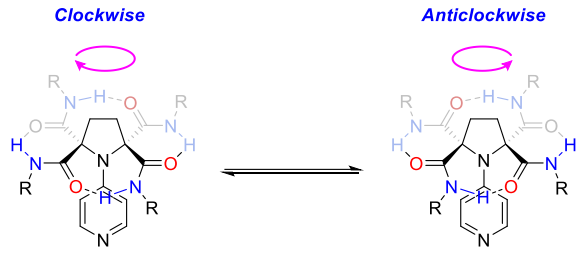


Figure 3.14. Kawabata's cyclochiral 4-pyrollidinopyridine derivatives.

3.1.2 Background on Cyclochiral Ureas

In the Group of J. Clayden, where part of this Ph.D thesis work has been carried out, "*dynamic foldamers*", namely extended oligomeric molecules with well-defined but switchable conformation, have been extensively studied and the development of a new class of structures with fluxional character has been pioneered. Molecules with fluxional stereochemistry, that is switchable between alternative stereoisomers, have a variety of potential applications in catalysis, signalling and analytical chemistry. In fact, Clayden and co-workers reported that helical structures, capable of switching between a left- and right-handed screw sense, can be used as mimics of membrane bound receptors[16] photoresponsive mimics of rhodopsin,[17] photoswitchable catalysts,[18] and as models of competitive biological interactions.[19] Clayden and co-workers have also recently introduced a new class of polarity-switchable dynamic foldamers based on the urea function, which is commonly used in foldamer chemistry. This is because ureas possess the rigidity and ability to form hydrogen bonds like amides, but in addition the ureido proton allows for synthesis of unique structures. In such new class of foldamers, based on *cis*-1,2- cyclohexanediamine, a linear network of hydrogen bonds allows concerted rotation about a whole series of C–N bonds to be induced by a single terminal influence (Figure 3.15).[20]

However, inspired by Nowick's study into ethylene-bridged triureas,[21] Clayden and co-workers have found out that much simpler oligoureas, derived from readily available or easily synthesised poly(ethylenediamines), also possess similar properties (Figure 3.16).[22,23] More recently, taking inspiration by Kawabata's work on cyclochiral molecules, the possibilities arising if the linear hydrogen-bonded oligourea represented in Figure 3.15 was converted into a cyclic hydrogen-bond network (Figure 3.17) were explored. In fact, these cyclic oligoureas could have the intriguing, under-explored and potentially highly valuable property of cyclochirality. In this case, the property would emerge only by virtue of the presence of the cyclic hydrogen-bonding arrangement.

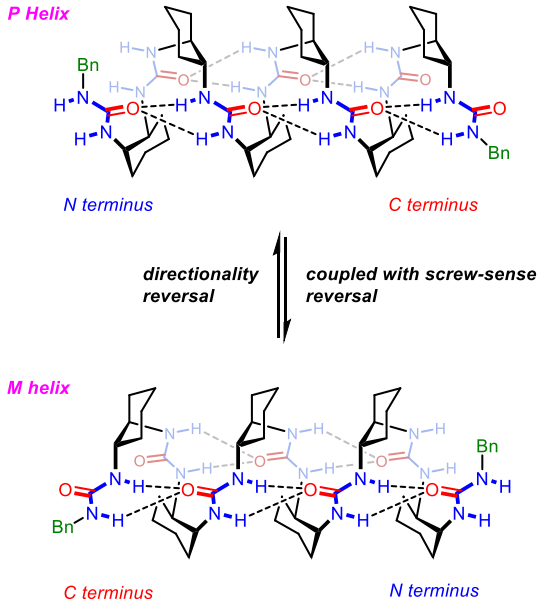


Figure 3.15. Schematic representation of the screw-sense inversion coupled with hydrogen-bond directionality-reversal in the *meso* helical dynamic oligo(cyclohexane-1,2-diamine)urea foldamers.

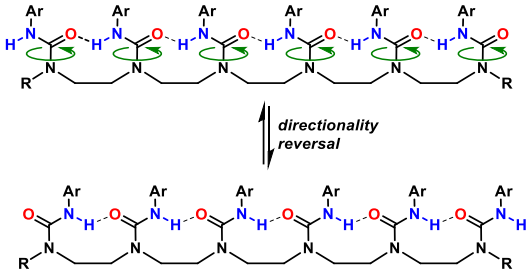


Figure 3.16. Linear dynamic oligourea foldamers with reversible directionality.

In a previous work, cyclic oligoureas based on the structures of 1,4,7-triazacyclononane (TACN), cyclen and hexacyclen have been prepared by reacting the cyclic polyamines with the corresponding isocyanates (1.5 equivalents for each secondary amine) through a one-pot addition reaction, following the reported procedures (Figure 3.17).[24,25]

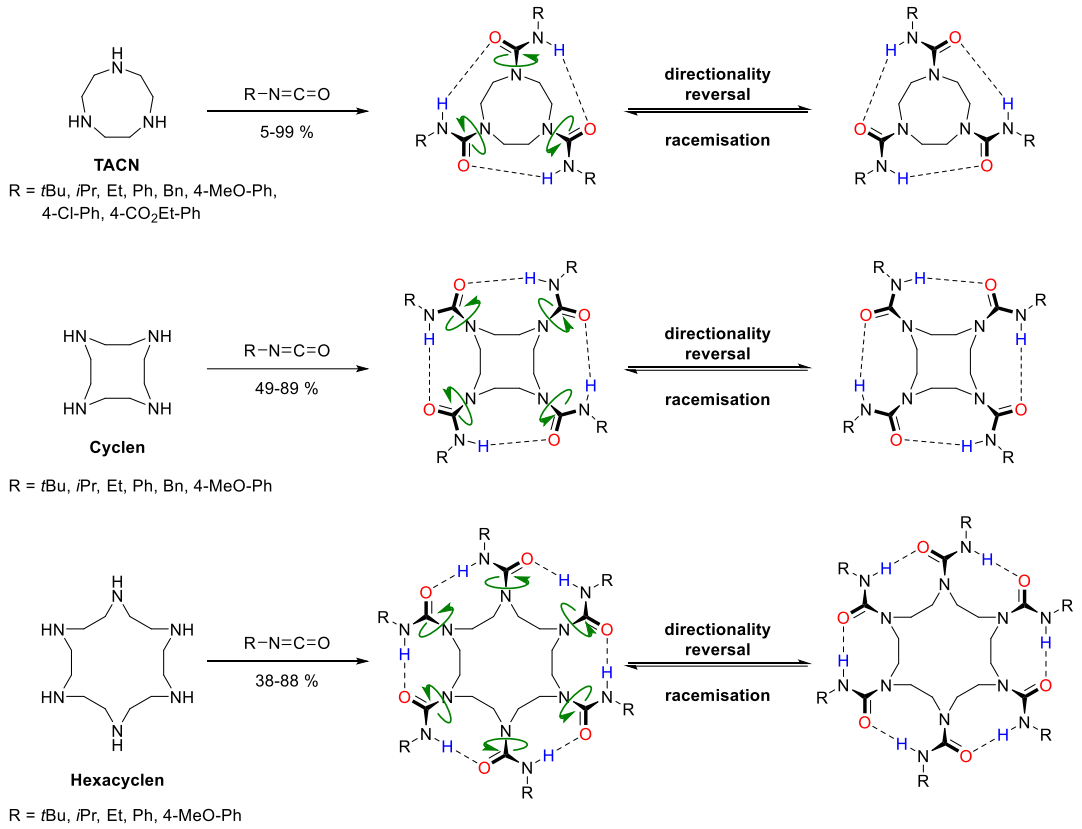


Figure 3.17. Cyclic oligoureas synthesised in the Clayden Group.[23]

As highlighted in Figure 3.17, in the correct conformation, the ureas are able to form intramolecular hydrogen bonds due to the presence of a carbonyl group, acting as an acceptor, and an ureido proton, which can act as a donor.

If the molecules are cyclochiral the methylene protons on the ethylene bridges of the amine skeleton become diastereotopic, experiencing distinct chemical environments, thus displaying different chemical shift in the ^{1}H-NMR spectrum. Therefore, the cyclic hydrogen-bonding network, as well as the cyclochirality, can be detected and analysed by NMR spectroscopy. Moreover, the use of VT-NMR techniques make it possible to conduct kinetic studies into cyclochiral structures, whose atoms are in dynamic exchange by virtue of the interconversion of the two enantiomers when the rate of exchange is comparable to the NMR timescale. The detection of the individual chemical environments as distinct peaks in a NMR spectrum at room temperature indicates that the molecule is undergoing slow exchange. It is

expected that increasing the temperature of the molecule would speed up the exchange rate and thus would result in signal broadening and finally coalescence.

When undergoing slow exchange, the NMR spectra of the oligoureas synthesised (Figure 3.17) should display four distinct signals for each of the methylene protons on the polyamine ring. Indeed, such protons are in different environments depending on the orientation of the hydrogen-bonding array, if it is clockwise or anti-clockwise, that is if one enantiomer or its antipode is present. This occurs because the two carbons on the ethylene bridges of the oligourea are different. In fact, depending on the enantiomer, one carbon is close to the oxygen of urea and the other is close to the nitrogen, thus the four methylene protons are different in chemical shift and develop a very complex spin system. It can be supposed that the mechanism of racemisation occurs either *via* bowl-to-bowl inversion of the ring or *via* the reversal of the directionality of the hydrogen-bonding network, resulting by the 180° rotation of the side-chains. Any of these mechanisms could result in the formation of the opposite enantiomer. Hence, in one enantiomer H_1 will be proximate to the ureido proton of one side-chain, whilst H_2 will be proximate to the carbonyl of the adjacent side-chain, vice versa will occur in the other enantiomer (Figure 3.18). Thus, cyclochirality can be revealed by the presence of four distinct proton peaks at low temperature, which broaden and coalesce into a singlet at high temperature.

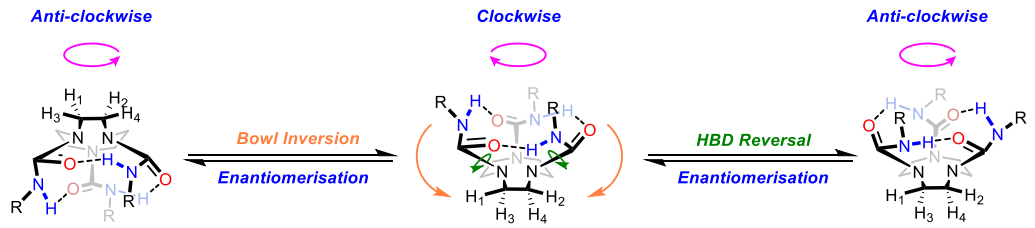


Figure 3.18. Suggested mechanisms of racemisation for cyclic oligoureas based on TACN structure.

Among the cyclic oligoureas previously synthesised by Clayden and co-workers (Figure 3.16), those derived from Cyclen and Hexacyclen showed low energy barrier to racemisation as detected by their ^{1}H-NMR at room temperature and VT-NMR analysis.

On the contrary, the investigated analogous cyclic triureas derived from TACN, with an aromatic ring as its substituent and directly conjugated to the urea group (Figure 3.17), displayed different signals corresponding to the aliphatic protons in the room temperature ^{1}H-NMR spectrum, therefore showing greater promise in exploiting cyclochiral structures. In particular, the line shape analysis of the VT-NMR and the Eyring plots for these molecules allowed calculating the corresponding energy barrier of racemisation. Such energies were found to be $\Delta G^{\ddagger}$ (298 K) = 64.0 kJ mol^{-1} when R = Ph, $\Delta G^{\ddagger}$ (298 K) = 64.5 kJ mol^{-1} when R = 4-MeO-Ph, $\Delta G^{\ddagger}$ (298 K) = 63.2 kJ mol^{-1} when R = 4-Cl-Ph, $\Delta G^{\ddagger}$ (298 K) = 62.7 kJ mol^{-1} when R = 4-CO_2Et-Ph. This also highlights the importance of the urea's conjugation to the arene in promoting cyclochirality and especially in providing a higher barrier to racemization in the oligourea products. Moreover, X-ray crystallography for the cyclic triurea with R = 4-MeO-Ph (Figure 3.19) proved that the molecule was in a *C*$_3$-symmetrical stable conformation, having a structure where the 4-methoxy phenyl urea side-chains were arranged on the same face of the amine skeleton, forming the bowl-like structure. In this way, a stable slow-exchanging cyclic hydrogen-bonding network was allowed to form, providing a cyclochiral structure.

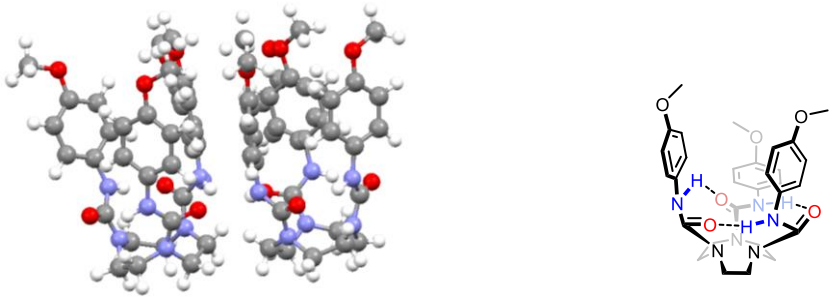


Figure 3.19. X-ray crystallograph of the cyclic triurea based on TACN structure where R = 4-MeO-Ph.

3.1.3 Aims of the project

The aim of this thesis work was to synthesise cyclic oligoureas as isolable and stable cyclochiral products in order to provide a new class of compounds, which could be utilised in the future as organocatalysts in asymmetric synthesis or as enantiospecific host molecules, opening a new field of organic chemistry.

As previously reported, among the cyclic oligoureas prepared by Clayden and co-workers (Figure 3.17),[22] the most promising cyclochiral structures were found to be the cyclic triureas, based on TACN, with an aromatic ring directly conjugated to the urea function (Figure 3.20). Indeed, this guaranteed the establishment of a cyclic intramolecular hydrogen-bonding network, detectable by NMR spectra at room temperature.

With this in mind, this project was aimed at the preparation of analogoues cyclochiral triureas, possessing a sufficiently strong hydrogen-bonding array to prevent racemisation at room temperature, and thus making the two enantiomers separable and useful for the aforementioned functions. Moreover, varying the substituent on the arene would make it possible to evaluate the electronic influence of the aromatic group on the strength of the hydrogen-bonding array responsible for the cyclochirality. For this reason, several cyclic triureas differently *para*-substituted on the phenyl ring were to be synthesised (Scheme 3.1). Then the obtained compounds will be analysed through NMR spectroscopy, in particular using VT-NMR in order to determine the energy barrier in the process of interconversion between the enantiomers.

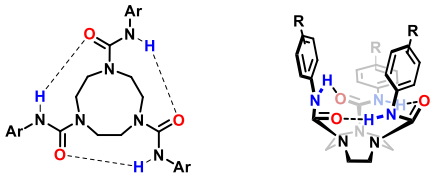
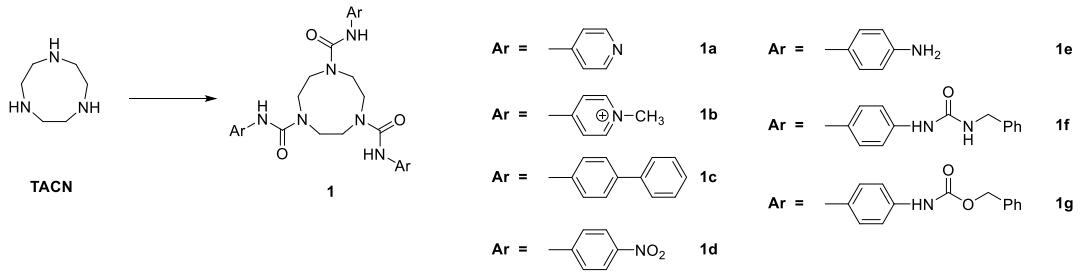


Figure 3.20. Cyclochiral triureas based on TACN structure.



Scheme 3.1. Cyclic triureas differently *para*-substituted on the phenyl ring.

In addition, the synthesis of cyclochiral triureas bearing substituents on the ethylene bridges of amine skeleton have to be attempted because their NMR analysis would be useful in understanding to what extent the two proposed mechanisms, bowl-to-bowl inversion or hydrogen-bonding directionality reversal, contribute into the process of interconversion between the enantiomers. In fact, when two vicinal methyl groups are on one of the ethylene bridges of the cyclic triamine, the bowl-to-bowl inversion converts one enantiomer in a diastereomer whilst, on the contrary, the hydrogen-bonding directionality reversal transforms it in its antipode (Figure 3.21). Since diastereomers are distinguishable by use of NMR spectroscopy, it will then be clear which mechanism prevails over the other. Further information about the mechanism of racemisation may allow the design of new molecular structures in order to increase the barrier of racemisation. Therefore, in this project, the synthesis of compounds with geminal or vicinal methyl groups on the skeleton of cyclic triamine was attempted (Figure 3.22).

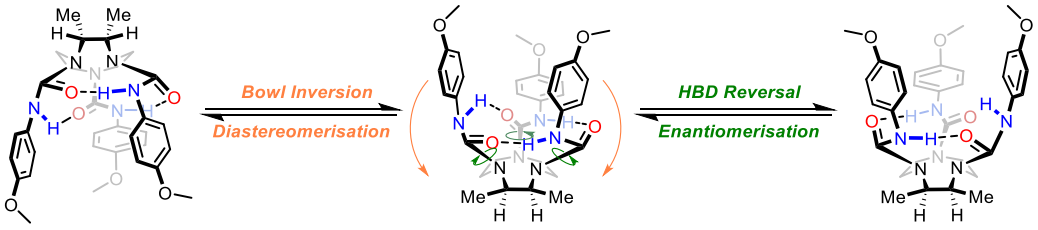


Figure 3.21. Proposed mechanisms of enantiomerisation and diastereomerisation.

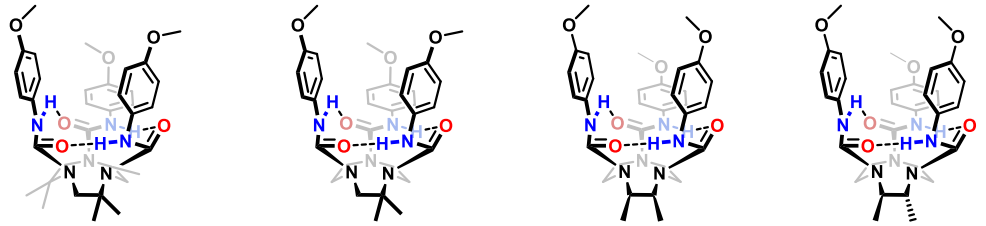


Figure 3.22. Triureas bearing geminal or vicinal methyl groups on the amine skeleton.

Furthermore, it was speculated that the reduction of the conformational freedom obtained by mechanically forcing the triureas into more restricted structures, in which the ureas are held together forming the hydrogen-bonding array, should increase the barrier to racemisation. With this in mind, the synthesis of cyclochiral cages based on the TACN and calix[4]arene structures (Figure 3.23) was also attempted.

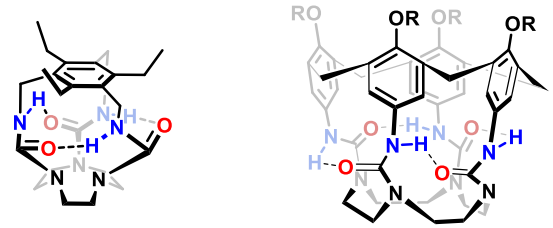
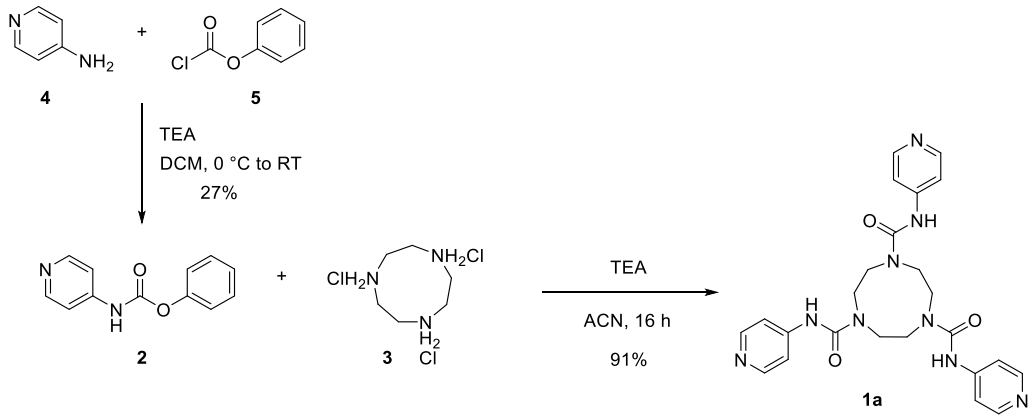


Figure 3.23. Cyclochiral cages based on the TACN and calix[4]arene structures.

3.2. Results and Discussion

3.2.1 Synthesis of Tris(ureido)triazacyclononanes

At first, it was thought to introduce a 4-pyridyl substituent into the triurea in order to equip the cyclochiral structure with an electron-withdrawing moiety also suitable to act as a site for asymmetric organocatalysis. Accordingly, compound **1a** was prepared in 91% yield by reaction of 1,4,7-triazacyclononane trihydrochloride (TACN·3HCl) (**3**) with an excess (4.5 eq.) of carbamate **2** in anhydrous ACN and in the presence of TEA at RT. The triurea precipitated out of the solvent and then was washed with cold acetonitrile to give pure **1a**. In turn compound **2** was obtained in 27% yield by reacting 4-aminopyridine (**4**) with phenyl chloroformate (**5**) at RT in the presence of TEA, added at 0 °C, in dry DCM (Scheme 3.2).



Scheme 3.2. Synthesis of compound **1a**.

The room temperature ^{1}H-NMR spectrum (Figure 3.24) in $CDCl_3$ for **1a** showed that the aliphatic protons were in different environments, suggesting a stable non-C_{3v}-symmetrical structure. The four different aliphatic environments integrate to 3 (3.09 ppm), 3 (3.18 ppm), 3 (4.03 ppm) and 3 (4.43 ppm) respectively, indicating that the molecule was in a stable C_3-symmetrical conformation and thus displaying cyclochiral properties. The detection of individual environments for the methylene protons on the ring also indicated the molecule

was undergoing slow-exchange. On the contrary, in the ^{1}H-NMR spectrum (Figure 3.25) in deuterated acetic acid, the signal at 5.18 ppm integrated to 12, corresponding to all the aliphatic protons. This indicated a lack of cyclochirality in the molecule, due to the polar solvent, which prevented the formation of intramolecular hydrogen bonds in the cyclic triurea. The compound **1a** was then studied by VT-NMR using $Cl_2CDCDCl_2$ (d_4-1,1,2,2-tetrachloroethane) as solvent, whose high boiling point (145 °C) allowed to register the ^{1}H-NMR spectra varying the temperature. In addition, that solvent guaranteed the complete solubilisation of the compound **1a**. Upon heating the molecule, the aliphatic signals broadened into the baseline and at very high temperature (90-100 °C), coalesced into one very broad singlet, which corresponds to all methylene protons. This evidence further suggests that, at room temperature, the structure is in slow-exchange (Figure 3.26). Moreover, the room temperature ^{1}H-NMR using a 500 MHz spectrometer (Figure 3.27) revealed the presence of a signal at 9.29 ppm integrating three protons, which corresponds to the three ureido protons involved in the hydrogen-bonding array. This peak shifted upfield upon heating up the molecule, suggesting the weakening of the intramolecular hydrogen bonds of the triurea at 100 °C.

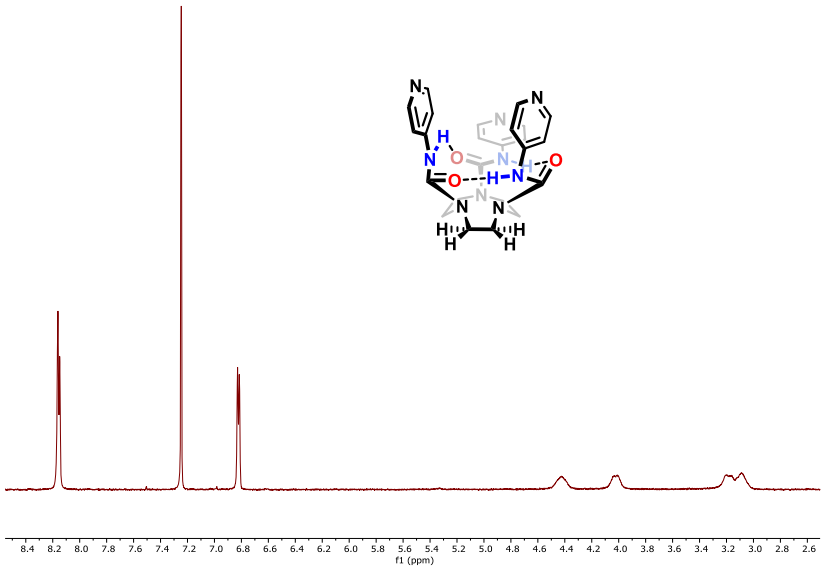


Figure 3.24. RT-^{1}H-NMR of **1a** (400 MHz in $CDCl_3$).

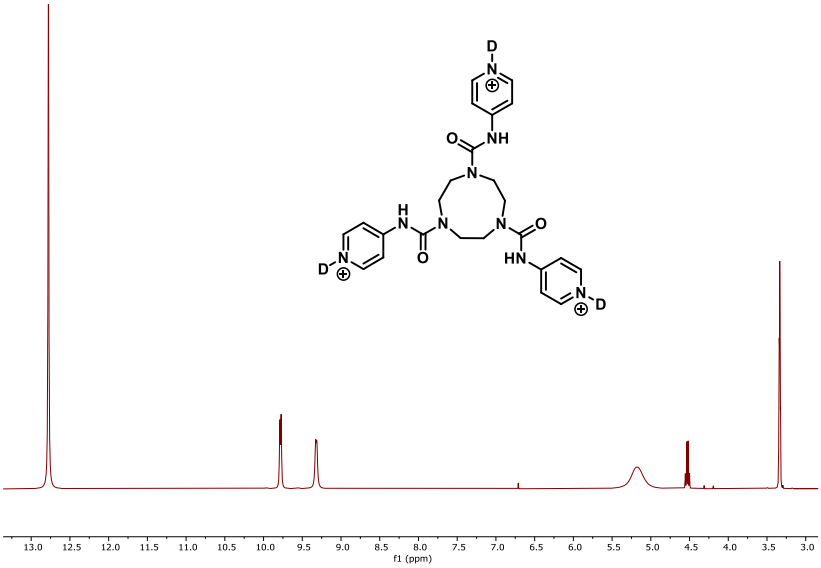


Figure 3.25. RT-^{1}H-NMR of **1a** (400 MHz in CD_3COOD).

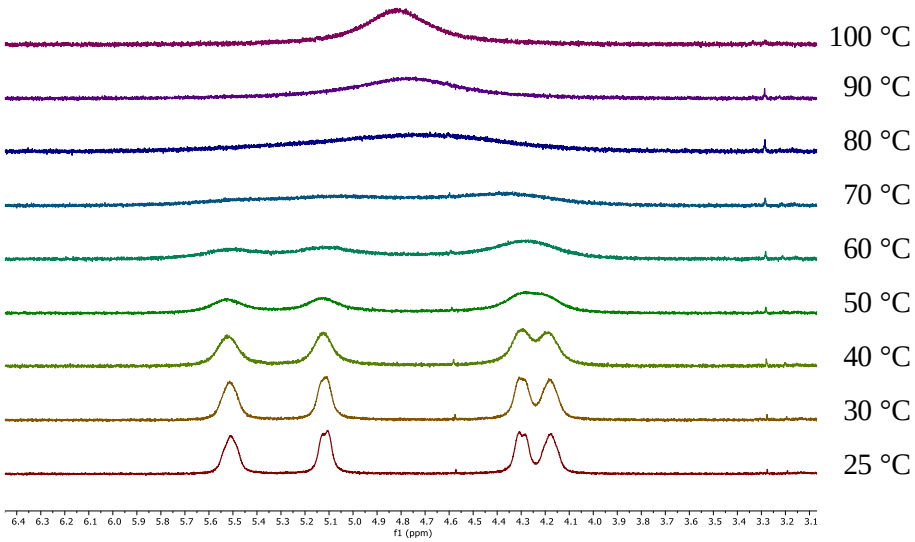


Figure 3.26. VT-^{1}H-NMR of **1a** (500 MHz in $Cl_2CDCDCl_2$) in 3.0-6.5 ppm range. Protons of the cycic triamine moiety.

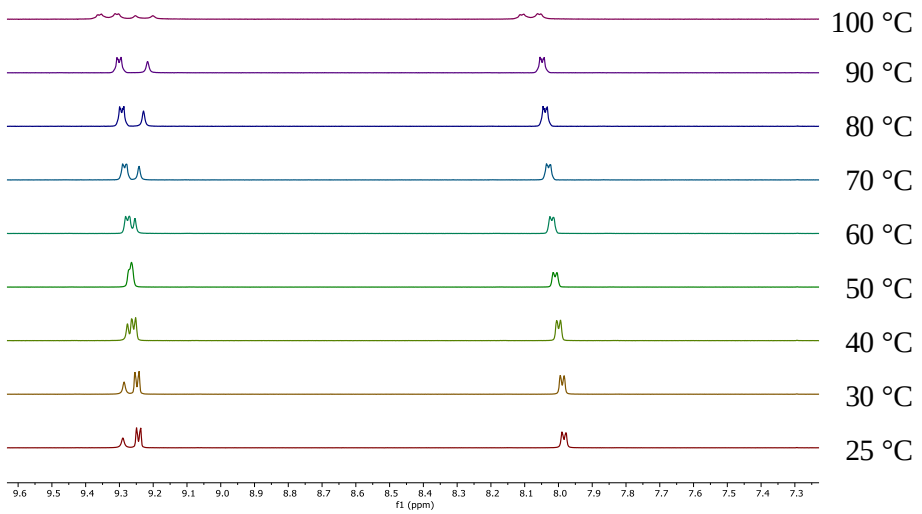


Figure 3.27. VT-^{1}H-NMR of **1a** (500 MHz in $Cl_2CDCDCl_2$) in 7.0-9.6 ppm range. Aromatic and ureido protons.

The racemisation barrier can be quantitatively expressed by means of the $\Delta G^{\ddagger}$ (Gibbs free energy of activation) associated with the equilibrium process of enantiomerisation. Thus $\Delta G^{\ddagger}$ can be used as a measure of the relative tendency of molecules to maintain their intramolecular hydrogen-bonding network and thus their cyclochiral conformation. Since the $\Delta G^{\ddagger}$ is directly correlated to the rate of exchange at a given temperature through the Eyring equation (Equation 3.1),[26] then the energy barrier of racemisation can be calculated, as well as the strength of intramolecular hydrogen-bonding array into the structure of the molecules.

$$k = \frac{k_B T}{h} e^{\frac{-\Delta G^{\ddagger}}{RT}}$$

Equation 3.1. The Eyring equation, where k is the rate constant, k_B is Boltzmann's constant, T is the absolute temperature, h is Planck's constant, $\Delta G^{\ddagger}$ is the Gibbs energy of activation and R is the molar gas constant.

Considering the Gibbs free energy equation, it is possible to separate the Gibbs free energy into its enthalpic and entropic contributions (Equation 3.2).

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\,\Delta S^{\ddagger}$$

Equation 3.2. The Gibbs free energy equation where $\Delta H^{\ddagger}$ is enthalpy of activation and $\Delta S^{\ddagger}$ is entropy of activation.

At this point, substituting the Gibbs free energy equation into the Eyring equation and applying natural logarithm, the linear form of Eyring equation is obtained (Equation 3.3).

$$ln\frac{k}{T} = \frac{-\Delta H^{\ddagger}}{R}\frac{1}{T} + ln\frac{k_B}{h} + \frac{\Delta S^{\ddagger}}{R}$$

Equation 3.3. The linear form of the Eyring-Polanyi equation.

The plot of ln(k/T) *versus* (1/T) provides a straight line whose gradient is equal to ($-\Delta H^{\ddagger}/R$) and whose intercept is equal to ($\ln(k_B/h) + \Delta S^{\ddagger}/R$). Therefore, the enthalpy, entropy, and consequently free energy of activation can be calculated for a defined dynamic process at each given temperature, by determining rate constants for the same given temperatures.

The rates of exchange at different temperature in the process of interconversion between two enantiomers can be obtained by the line shape analysis of the VT-NMR. In particular, SpinWorks 4 NMR simulation software was used here to model the shape of each experimental spectrum at each given temperature.

Then, line shape analysis of VT- NMR spectra and subsequent Eyring plot analysis, performed as previously described, allowed to determine the racemisation barrier for compound **1a**, which was found to be $\Delta G^{\ddagger} = 65.0$ kJ·mol^{-1}. Unfortunately, this energy value was much too low to allow the separation between the enantiomers, since the separation occurs if $\Delta G^{\ddagger} \geq$ 96-100 kJ·mol^{-1}.

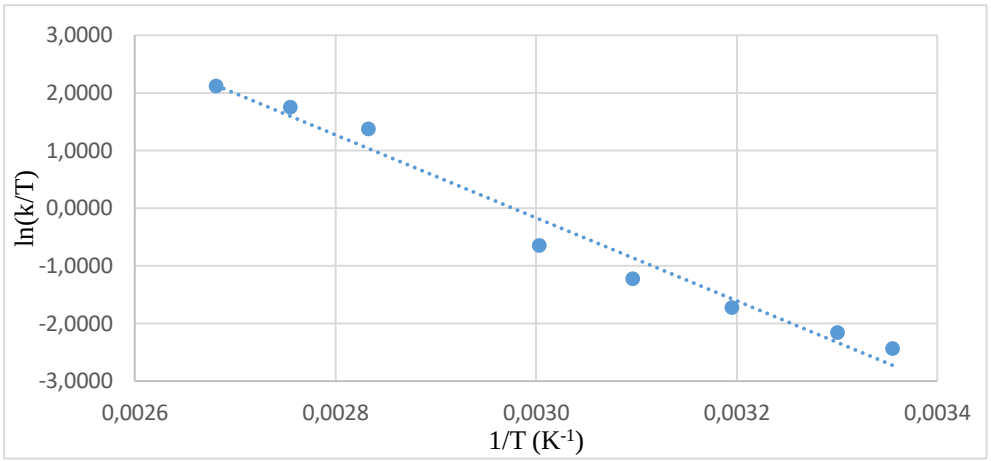
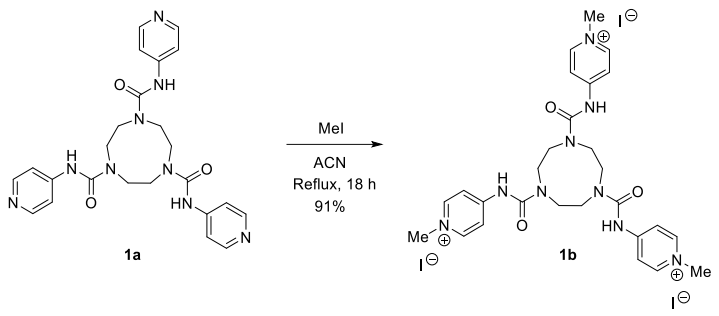


Figure 3.28. An Eyring plot or ln(*k*/T) vs 1/T, using the rate constants (k) obtained by simulations of the ^{1}H NMR spectra of **1a** (500 MHz in $Cl_2CDCDCl_2CD2Cl2$, 25-100 °C).

Compound **1a** was then treated with MeI in dry ACN at reflux for 18 h to afford the methylated compound **1b** in 91% yield (Scheme 3.3), after removal of the solvent under reduced pressure.[27] This compound was not soluble in the common organic solvents even after the addition of $NaBF_4$, in an attempt to increase the solubility. Therefore, compound **1b** was dissolved in D_2O in order to record the ^{1}H-NMR (Figure 3.29). The spectrum showed a very broad signal at 3.67 integrating for 12 protons and corresponding to the protons on the ethylene bridges of the triurea. Such a broadening of the peak could suggest a low rate of exchange in the compound and then the presence of cyclochirality. However, the presence of the polar solvent did not allow to reveal the different chemical environments for the aliphatic protons. Accordingly, due to the limited solubility of compound **1b**, it was not possible to study its dynamic properties using VT-NMR spectroscopy and to determine the energy barrier to racemisation.



Scheme 3.3. Synthesis of compound **1b**.

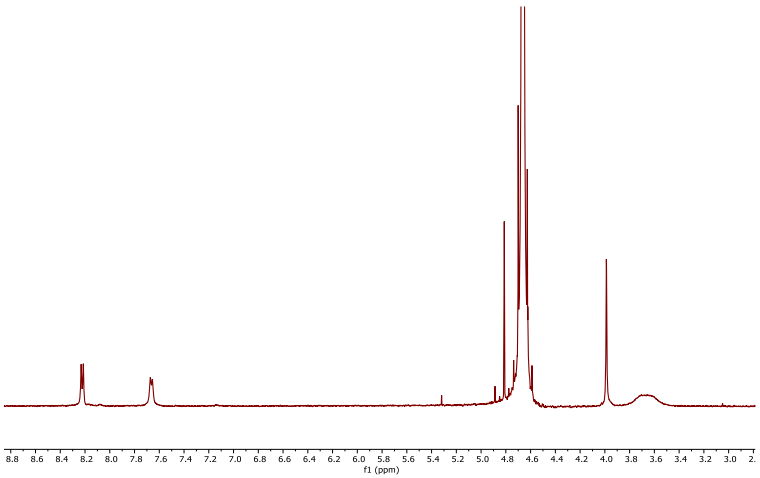
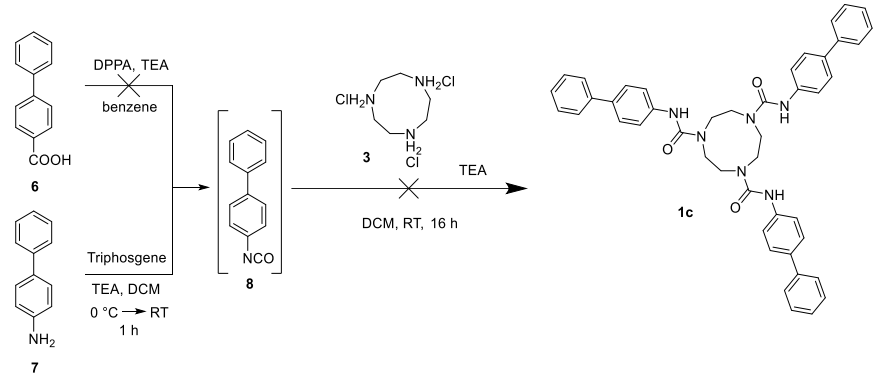


Figure 3.29. RT-^{1}H-NMR of **1b** (400 MHz in D_2O).

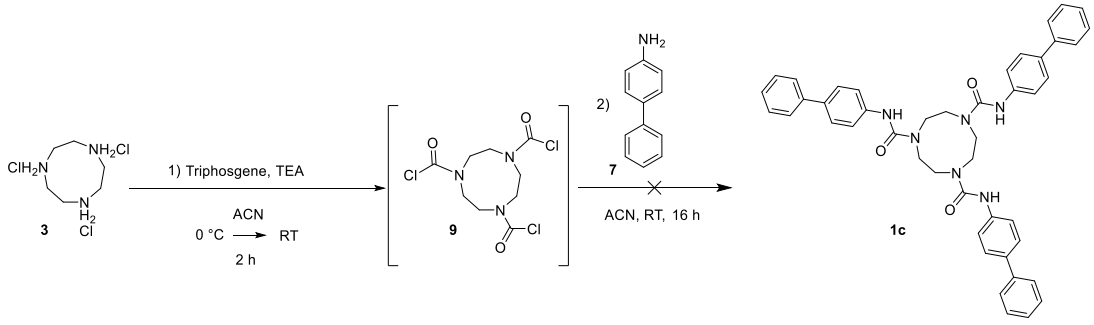
In order to obtain compound **1c**, the synthetic strategy represented in Scheme 3.4 was attempted at first. However, the attempts to synthesise 4-isocyanatobiphenyl (**8**) through a modified Curtius rearrangement,[28] treating 4-phenylbenzoic acid (**6**) with DPPA and TEA in anhydrous benzene, were in vain. Hence it was attempted to prepare **8** by reacting 4-aminobiphenyl (**7**) with triphosgene in dry DCM, but again it was not possible to recover the desired product, which was then assumed to be prone to degradation. Thus, to avoid the isolation of **8**, a one-pot reaction was performed, where to a solution of triphosgene and TEA in dry DCM at 0 °C, a solution of **7** in DCM was slowly added and allowed to stir at RT for 1 h. After this time, TACN·3HCl was added, followed by an additional portion of TEA and

the mixture was stirred at RT for 16 h. Unfortunately, compound **1c** was not recovered also in this case.



Scheme 3.4. Synthetic strategy proposed to obtain **1c**.

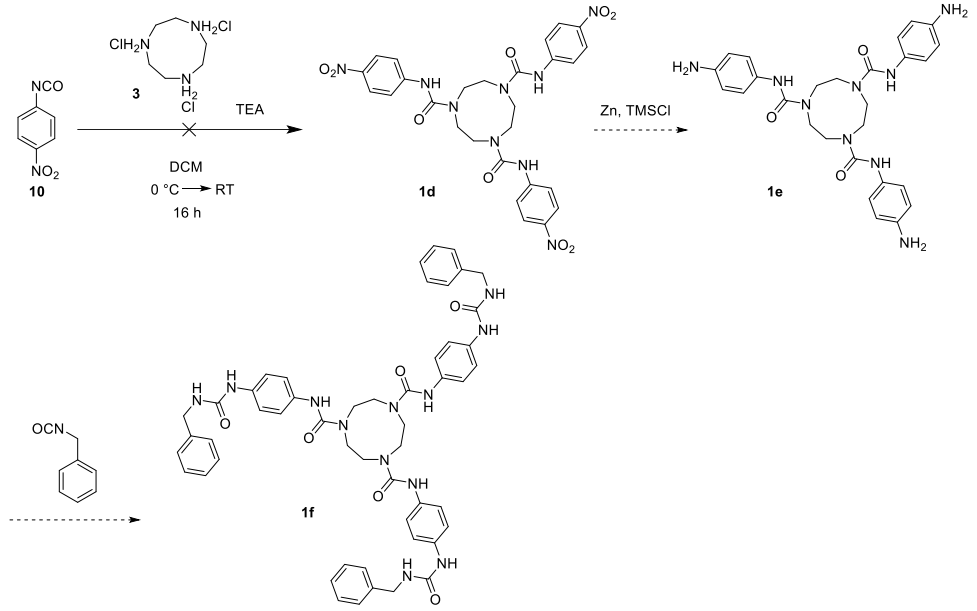
With the aim to obtain **1c**, another one-pot tandem reaction was then performed (Scheme 3.5), but again without success. TACN·3HCl was treated with triphosgene in dry ACN in the presence of TEA to obtain the carbamoyl chloride **9**. After 2 h of stirring, 4-aminobiphenyl (**7**) was added and the reaction mixture was stirred for 16 h at RT.



Scheme 3.5. Alternative synthetic strategy proposed to obtain **1c**.

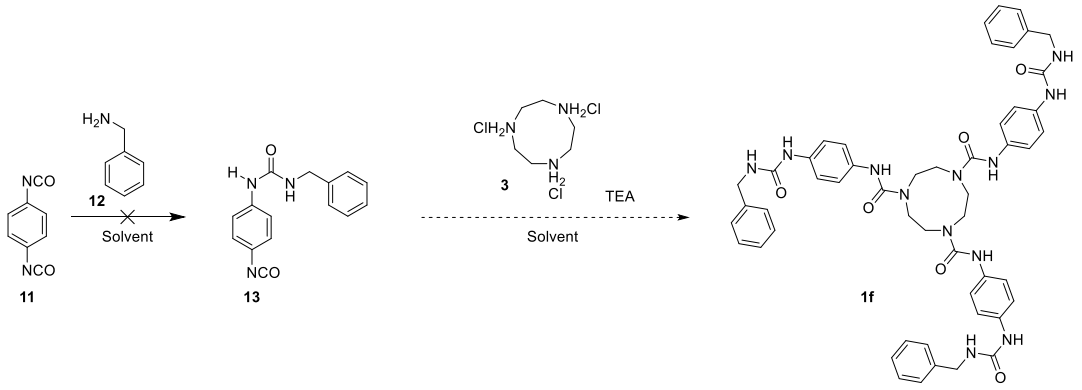
The synthetic strategy proposed to prepare compounds **1d**, **1e** and **1f** is displayed in the Scheme 3.6. Compound **1d** could be synthesised by the addition of 4-nitrophenyl isocyanate (**10**) to TACN·3HCl (**3**), in the presence of TEA. Then the reduction of nitro groups into the

corresponding amines could be conducted to obtain **1e**, which upon treatment with benzyl isocyanate would yield the product **1f**. Unfortunately, the reaction to prepare **1d**, following the described procedure, was unsuccessful.



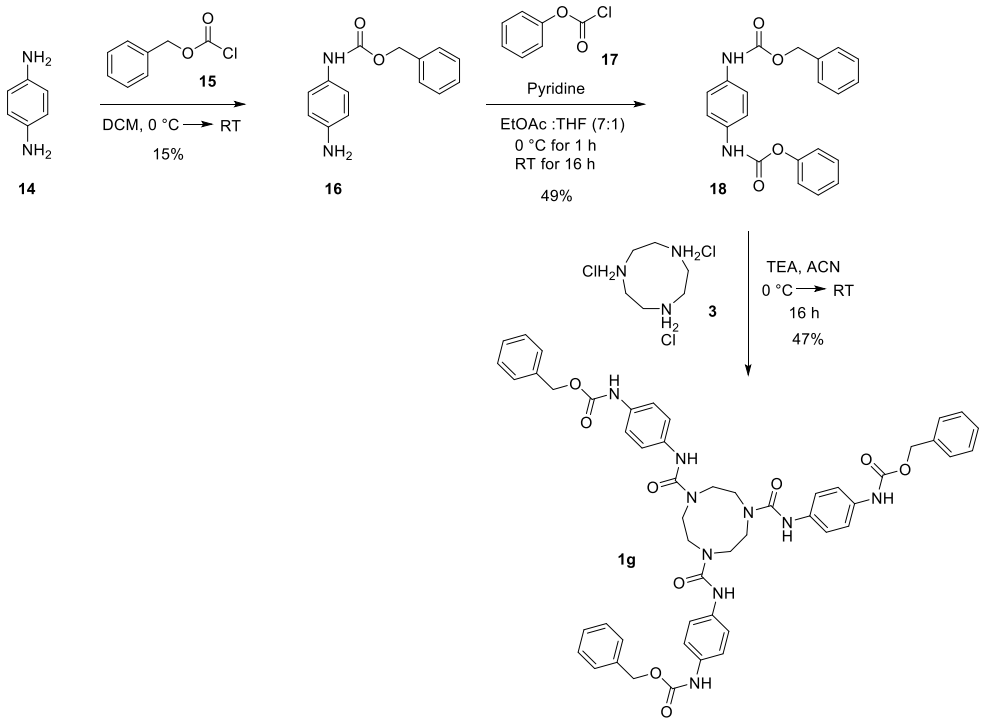
Scheme 3.6. Synthetic strategy proposed to obtain **1d**, **1e** and **1f**.

In a different approach the preparation of compound **1f** was attempted by reacting TACN·3HCl with the urea **13**. However the synthesis of **13**, though the reaction between 1,4-phenylene diisocyanate (**12**) and benzyl amine, was not successful (Scheme 3.7).



Scheme 3.7. Alternative synthetic strategy proposed to obtain **1f**.

The synthesis of compound **1g** is shown in Scheme 3.8. The reaction of 1,4-diamino benzene (**14**) in anhydrous DCM with benzyl chloroformate (**15**), added at 0 °C, gave rise to mono-Cbz protected amine **16** in 15% yield. Then **16** was reacted with a slight excess of phenyl chloroformate (**17**) in the presence of pyridine and in a mixture of THF and EtOAc (1:7).[29] This reaction was performed at 0 °C for 1 h and then at RT for 16 h and afforded the desired product **18** as a white solid in 49% yield. Subsequently an excess of **18** was reacted with 1,4,7-triazacyclononane trihydrochloride (**3**) in the presence of TEA in anhydrous ACN, leading to the preparation of the triurea **1g** obtained in 47% yield as determined by NMR on the crude.



Scheme 3.8. Synthesis of compound **1g**.

The room temperature ^{1}H-NMR in $CDCl_3$ for the crude of compound **1g** (Figure 3.30) displayed three signals at 3.04, 3.95 and 4.38 ppm integrating 6, 3 and 3 respectively and corresponding to the 12 aliphatic protons on the ethylene bridges of the triurea. Once again, these three different chemical environments indicated that the methylene hydrogens were in

slow-exchange and the molecule was in a stable C_3-symmetrical conformation. By this evidence, it was possible to conclude that **1g** was a cyclochiral compound at room temperature. Therefore, its dynamic properties were studied by VT-NMR. Again, $Cl_2CDCDCl_2$ was used as solvent for two reason: to ensure complete solubilisation of compound **1g** and to allow the NMR analysis at high temperatures. The VT-NMR from 3.70 to 6.00 ppm (Figure 3.31) showed that the signals corresponding to the aliphatic protons broadened into the baseline at 100 °C, confirming that the molecule was in slow-exchange status at room temperature. The VT-NMR from 7.40 to 10.00 ppm (Figure 3.32) revealed the presence of two peaks at 8.02 and 8.99 ppm, respectively, corresponding to the six ureido protons involved in the two hydrogen-bonding networks of the molecule. Such signals were shifted at 7.83 and 8.95 ppm respectively when the sample was heated up to 100 °C, indicating the weakening of the intramolecular hydrogen bonds. In this case, it was not possible to calculate the racemisation barrier because the coalescence temperature was not reached, indicating that energy barrier was too high to be reliably deduced by VT-NMR. In this case, VT- chiral HPLC analysis should probably be more useful to the purpose.

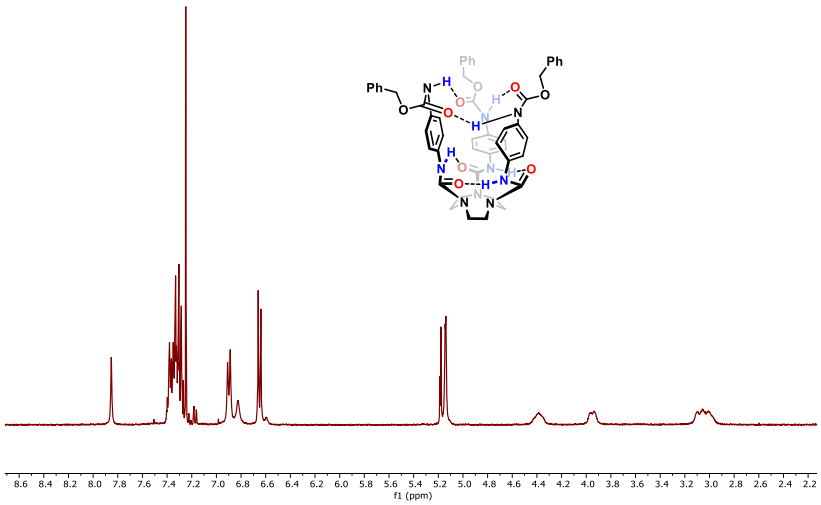


Figure 3.30. RT-^{1}H-NMR of **1g** (400 MHz in $CDCl_3$).

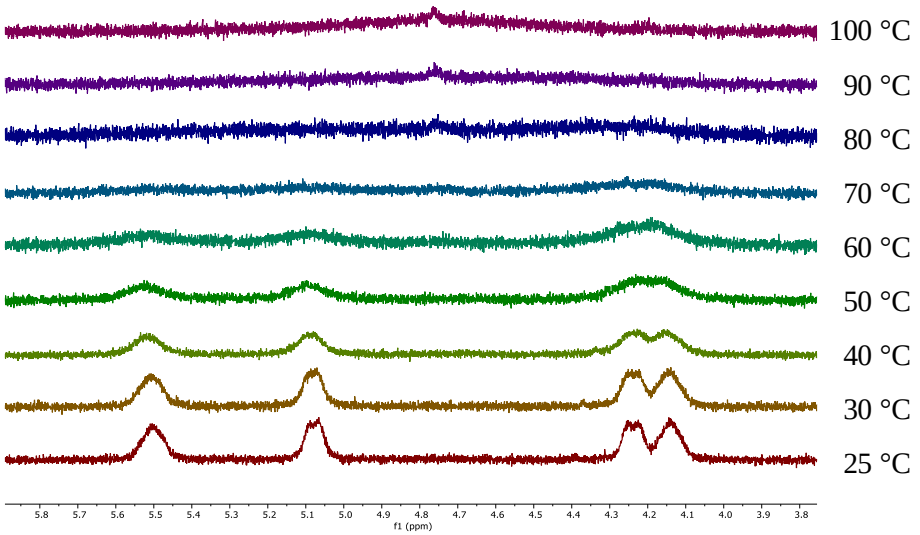


Figure 3.31. VT-^{1}H-NMR of **1g** (500 MHz in $Cl_2CDCDCl_2$) in 3.8-5.9 ppm range. Protons of the cycic triamine moiety.

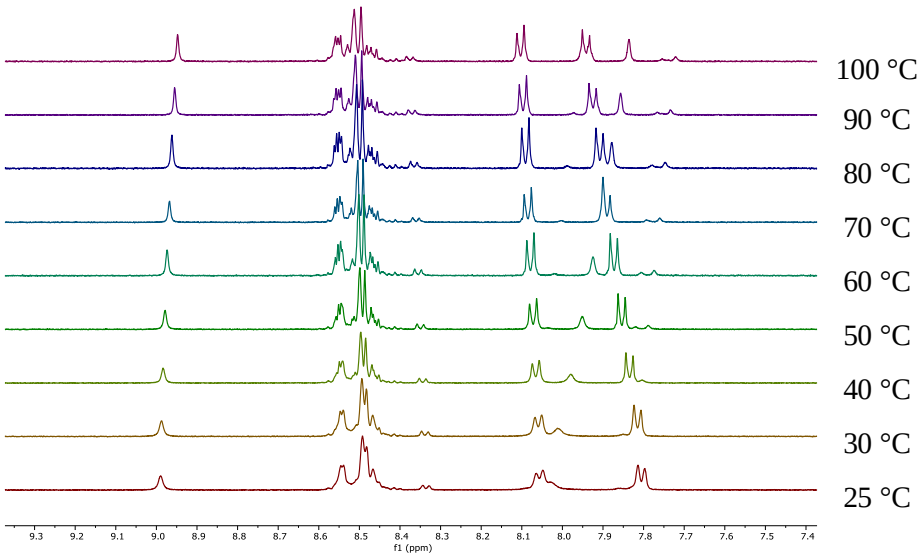
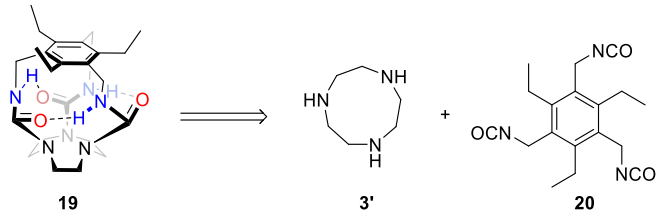


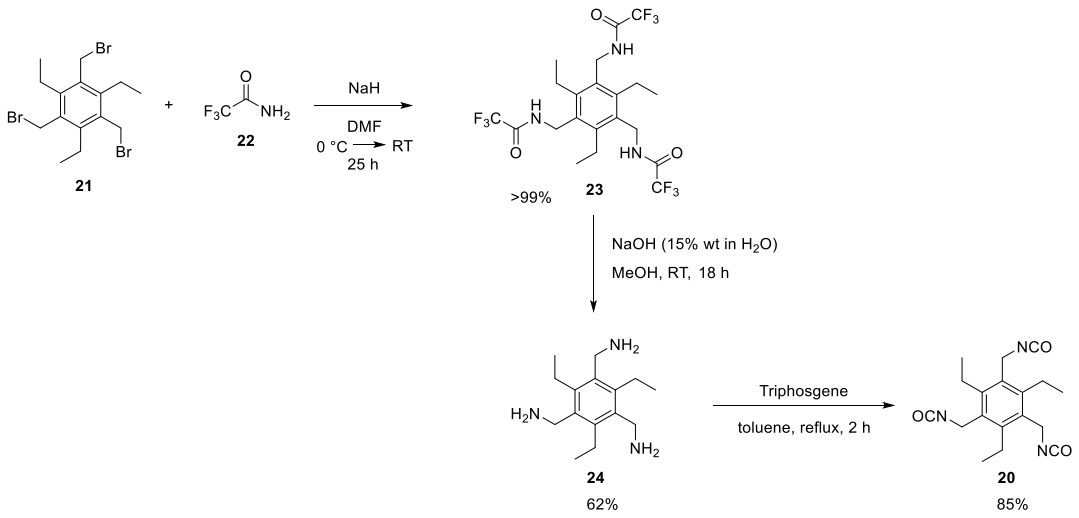
Figure 3.32. VT-^{1}H-NMR of **1g** (500 MHz in $Cl_2CDCDCl_2$) in 7.4-9.4 ppm range. Aromatic and ureido protons.

In order to stabilise the cyclochiral molecular assembly, thus rising its racemisation barrier, the synthesis of "cage" compound like **19**, based on the structure of 1,4,7-triazocyclononane, was attempted. Such a molecule could be derived by the reaction between TACN and triisocyanate **20** (Scheme 3.9).



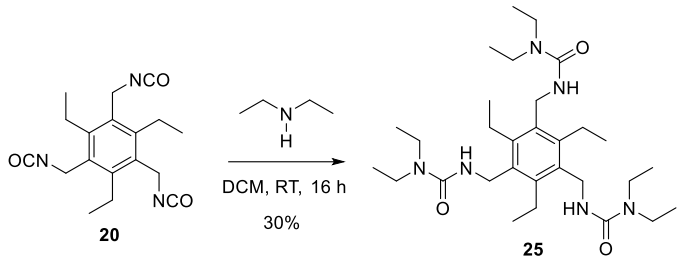
Scheme 3.9. Retrosynthetic scheme for preparation of **19**.

The synthesis of triisocyanate **20** was performed as illustrated in the Scheme 3.10. The reaction of tribromide **21** and trifluoroacetamide (**22**) in the presence of NaH in dry DMF afforded compound **23** in quantitative yield. In turn, **23** was hydrolysed, using NaOH in MeOH to give the triamine **24**, which was reacted with triphosgene in dry toluene at reflux in order to obtain compound **20** in 85% yield.



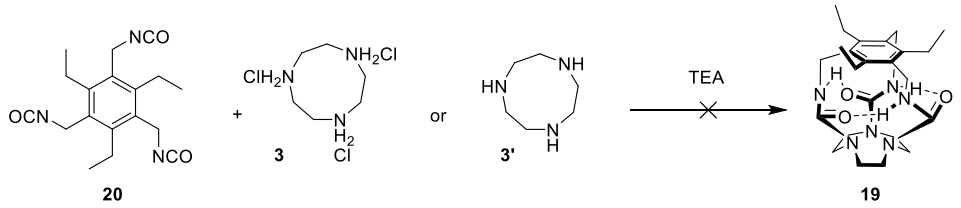
Scheme 3.10. Synthesis of triisocyanate **20**.

The reactivity of triisocyanate **20** was tested by the reaction with a simple secondary amine, diethylamine, leading to the formation of compound **25** (Scheme 3.11). The latter was found to be not cyclochiral, by the analysis of the ^{1}H-NMR spectra at room temperature and at -60 °C. Presumably the high conformational freedom of the molecule prevents the establishment of stable intramolecular hydrogen bonds, arranged in a ring system, necessary to give rise to cyclochirality.



Scheme 3.11. Synthesis of compound **25**.

All attempts to obtain the cyclochiral cage (**19**) by reacting the triisocyanate **20** with TACN (**3'**) or TACN·3HCl (**3**) were inconclusive (Scheme 3.12). In fact, even varying the solvent system, the concentration of the reaction solution and the time of the reaction, the desired product was never obtained (Table 1). In particular, the reaction was conducted using a syringe pump system in order to guarantee a very slow addition of both the reagents to the flask containing the solvent and consequently to avoid polymerisation.

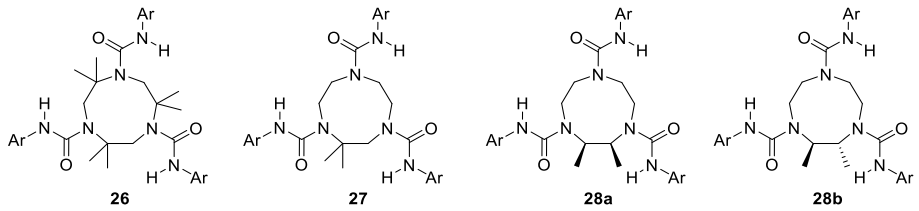


Scheme 3.12. Proposed synthetic strategy to prepare **20**.

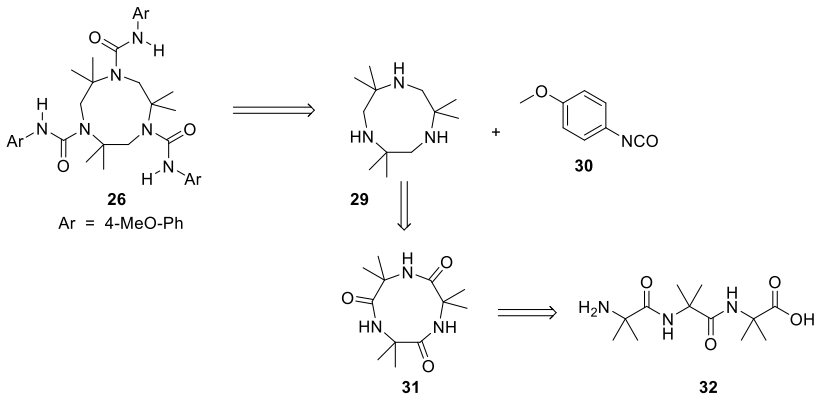
Table 1. Conditions for the reaction of triisocyanate **20** and triamine **3** or **3'**.

Substrate	Solvent	Conc. (M)	Time of reaction	Product Yield
TACN·3HCl	DCM	$5.0 \cdot 10^{-4}$	5 days	–
TACN·3HCl	ACN	0.1	3 days	–
TACN·3HCl	ACN/DMF	0.05	3 days	–
TACN·3HCl	Toluene	0.2	4 days	–
TACN	ACN	$5.0 \cdot 10^{-3}$	3 days	–

With the aim to investigate the mechanism of interconversion between the enantiomers of a cyclochiral triurea, the synthesis of compounds **26**, **27**, **28a** and **28b** was designed (Scheme 3.13). In particular, compound **26** could be synthesised by the reaction between 2,2,5,5,8,8-hexamethyl-1,4,7-triazonane (**29**) and 4-methoxyphenyl isocyanate (**30**). In turn, the cyclic triamine (**29**) could be obtained by the reduction of the trilactam **31**, which may be derived by the cyclisation of tripeptide **32** (Scheme 3.14).

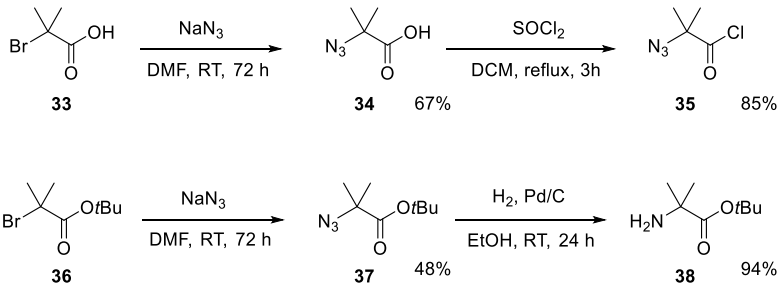


Scheme 3.13. Cyclic triureas bearing substituents on the ethylene bridges.



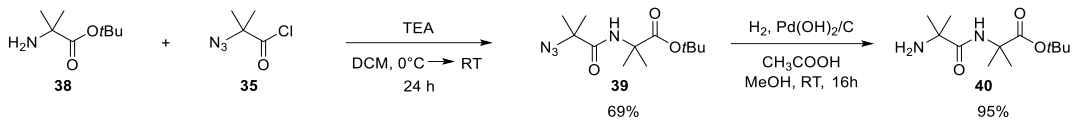
Scheme 3.14. Retrosynthetic scheme for preparation of **26**.

First of all, the preparation of tripeptide **32** was performed starting from the monomeric units, following a procedure established in the Clayden group.[30] The commercial product 2-bromo-2-methylpropionic acid (**33**) was converted into the azido-acid **34** in 67% yield by nucleophilic substitution with sodium azide in anhydrous DMF at room temperature for 72 h. Azido-acid **34** was then transformed into the corresponding acyl chloride **35** by treatment with an excess of thionyl chloride in dry DCM, allowing isolation of azido acyl chloride (**35**) in 85% yield, after purification by distillation (Scheme 3.14). The synthesis of the C-terminal *tert*-butyl ester-protected Aib residue (**38**) was achieved in two steps, similarly by reaction of commercially available *tert*-butyl 2-bromo-2-methylpropionate (**36**) with sodium azide under identical conditions. Azide **37** was then isolated in 48% yield and subsequently hydrogenated over Pd/C in ethanol, affording the deprotected amine **38** in 94% yield (Scheme 3.15).



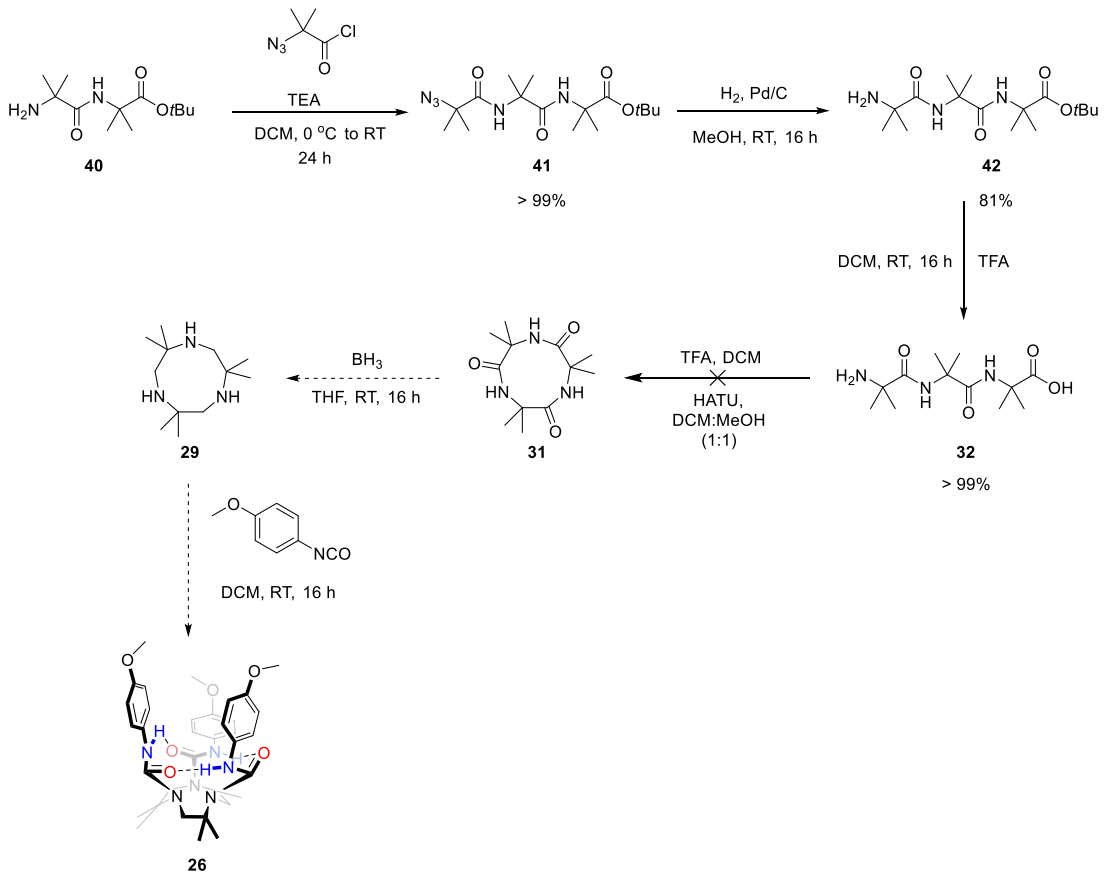
Scheme 3.15. The two steps synthesis of **35** and **38**.

Subsequently the azido acyl chloride (**35**) and *tert*-butyl ester-protected Aib residue (**38**) were combined by a coupling reaction, in the presence of TEA, leading to the formation of dimer N_3Aib_2OtBu (**39**) in 69% yield after 24 h. The subsequent hydrogenation of the azide-protected dimer (**39**), using 20% mol $Pd(OH)_2/C$ as catalyst in methanol, successfully afforded the free amine H_2N-Aib_2OtBu **40** in 95% yield (Scheme 3.16).



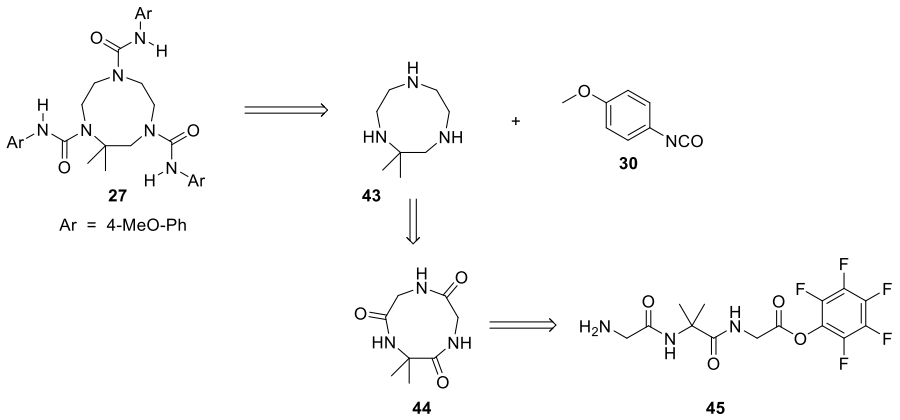
Scheme 3.16. Coupling reaction between **38** and **35** and following hydrogenation to give **40**.

As a result, the coupling of the amine **40** with azido acyl chloride (**35**) allowed the formation of the trimer N_3Aib_3OtBu (**41**) in quantitative yield. The subsequent hydrogenation of **41**, using 10% mol Pd/C in methanol, afforded the amine H_2N-Aib_3OtBu (**42**) in 81% yield. The *tert*-butyl ester group of H_2N-Aib_3-$OtBu$ was removed by treatment with TFA in dry DCM, leading to the free tripeptide H_2N-Aib_3OH (**32**) in quantitative yield (Scheme 3.16). Attempts were made to obtain the cyclic compound **31** starting from the trimer H_2N-Aib_3OH (**32**). Unfortunately, the treatment with both TFA in DCM and HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide-hexafluorophosphate) in a mixture of DCM and MeOH (1:1) were unsuccessful, even when the temperature and the time of reaction were increased (Scheme 3.17).



Scheme 3.17. Proposed synthetic strategy to obtain **26**.

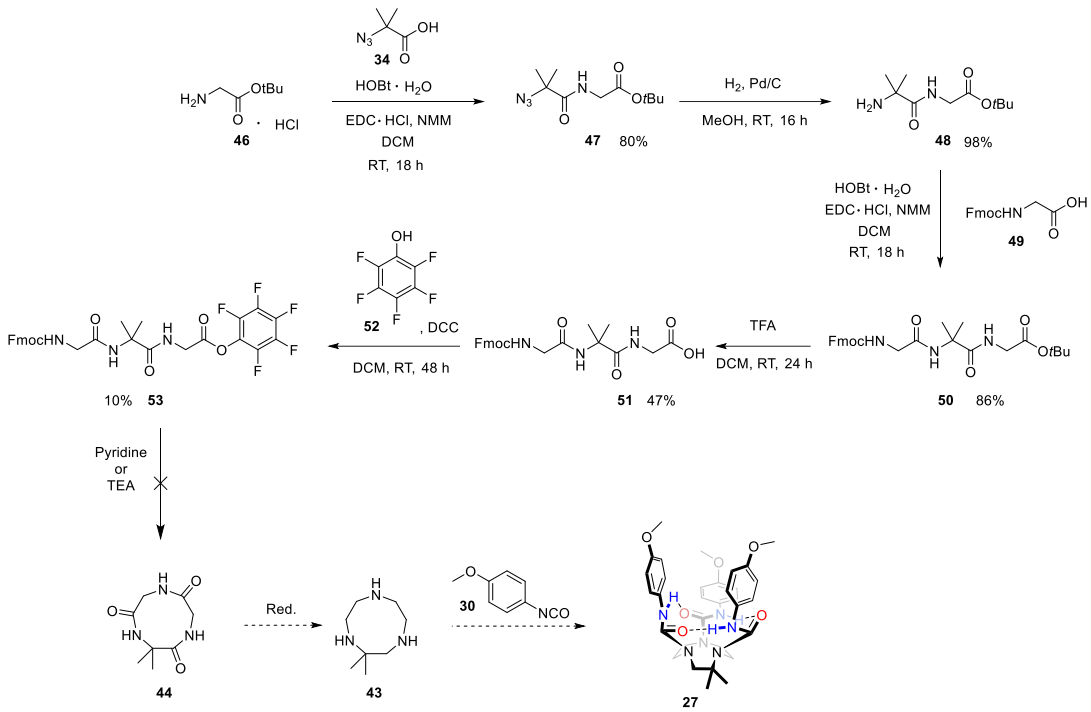
Due to the failure in the synthesis of the cyclic triamine with three pairs of geminal methyl groups, it was envisaged the possibility that the same information about the mechanism of racemisation could be deduced by analysing by NMR spettroscopy compound **27**, with only two geminal methyl groups on the structure of the molecule. Similarly, compound **27** could be synthesised by reacting 4-methoxyphenyl isocyanate with 2,2,-dimethyl-1,4,7-triazacyclonane (**43**) which, in turn, could be obtained by the reduction of the trilactam **44**, derived by the cyclisation of activated tripeptide **45** (Scheme 3.18).



Scheme 3.18. Retrosynthetic scheme for the preparation of **27**.

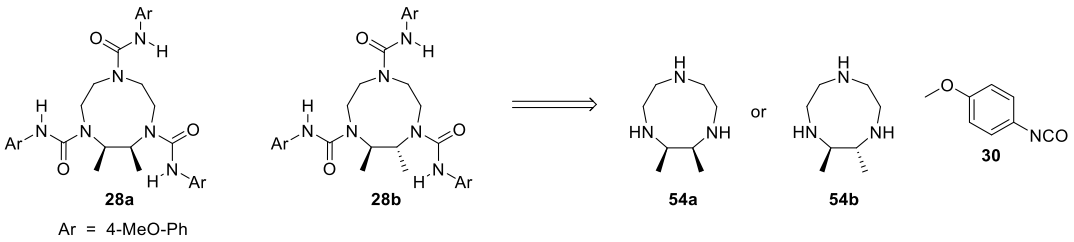
The preparation of tripeptide Gly-Aib-Gly activated as a pentafluorophenyl ester **45** was achieved starting from the coupling reaction between the azide **34** and glycine *tert*-butyl ester hydrochloride (**46**), in the presence of 1-hydroxybenzotriazole hydrate (HOBt·H_2O), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl) and 4-methylmorpholine (NMM) in DCM. This reaction led to the formation of the dimer **47**, which then was hydrogenated with 10% mol Pd/C in MeOH to give the free amine dimer NH_2Aib-Gly-O*t*Bu **48** in 98% yield. A further coupling step, under identical conditions, in order to combine **48** with Fmoc-glycine (**49**), afforded the trimer FmocNH-Gly-Aib-Gly-O*t*Bu **50** in 86% yield. The *tert*-butyl ester group of **50** was removed using TFA, as previously reported, giving the carboxylic acid FmocNH-Gly-Aib-Gly-OH **51** in 47% yield. The linear tripeptide was then activated with pentafluorophenol for the subsequent cyclisation step. In fact, treatment with pentafluorophenol (**52**) and *N*,*N'*-dicyclohexylcarbodiimide (DCC) allowed the formation of pentafluorophenyl ester **53** in only 10% yield. Unfortunately, all the attempts in order to obtain the trilactam were in vain. The reaction was performed under basic conditions, using pyridine or TEA as solvent, and high dilution to avoid polymerization, but only traces of Fmoc-deprotected tripeptide were recovered when TEA was used (Scheme 3.19). In fact, it was reported that the formation of α-cyclic tripeptides (CtPs) from linear natural amino acid sequences is very difficult due to the conformational preference of the tripeptide to exist in the linear form. Moreover, the cyclisation to CtPs can takes place only if all three amide bonds are in *cis* configuration.[31] It is well established that proline acts as a turn inducer to facilitate cyclisation through the

reduction of strain barriers, forming *cis*-amide bonds.[32] In addition, *N*-substituted amino acids are turn inducing.[33,34] Conversely, Gly is not a turn-inducing unit, since cyclo-(Gly-Gly-Gly) cannot be obtained form from linear Gly-Gly-Gly directly.[31] Therefore, even though tripeptide **53** is Gly-Aib-Gly type, the presence of the two methyl groups on the Aib unit is not sufficient to induce the cyclisation, exploiting the Thorpe–Ingold effect, observed when large substituents on a molecular structure favour ring closure and intramolecular reactions.[35,36]



Scheme 3.19. Proposed synthetic strategy to obtain **27**.

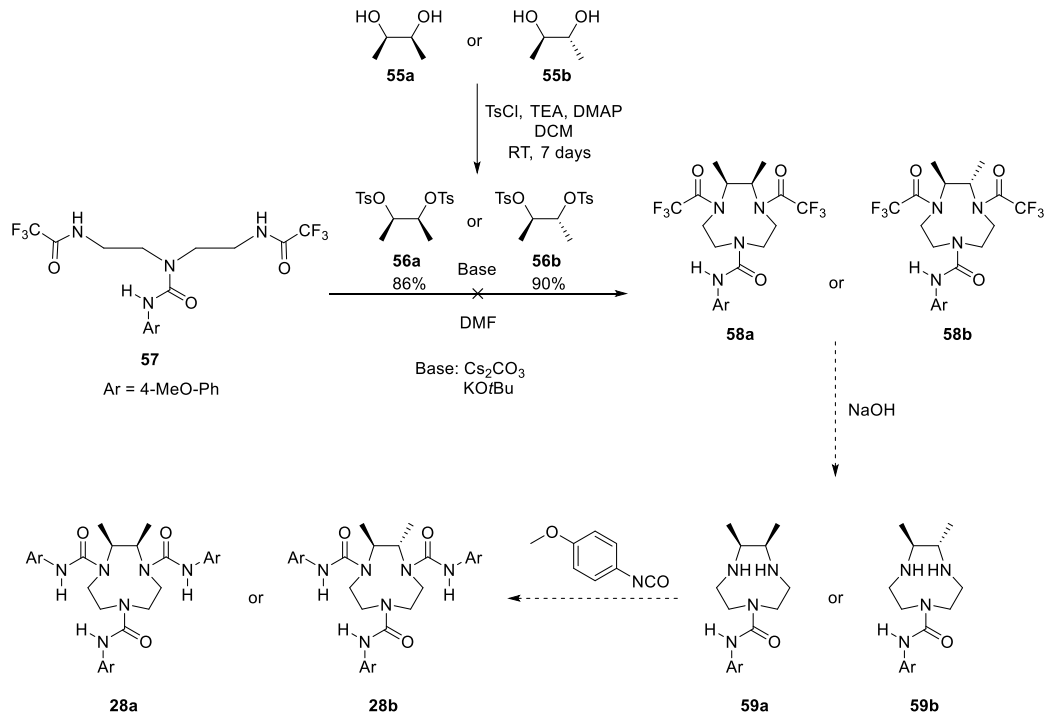
The synthesis of compound **28a** and **28b**, with two vicinal methyl groups on one of the ethylene bridges, could represent another useful way to understand the mechanism insight the enantiomerisation and diasteromerisation processes, by analysing the behaviour of the molecule through the NMR spectroscopy. Compound **28a** and **28b** might be obtained by reacting 4-methoxyphenyl isocyanate (**30**) with (2*S*,3*R*)-2,3-dimethyl-1,4,7-triazonane (**54a**) or (2*R*,3*R*)-2,3-dimethyl-1,4,7-triazonane (**54b**) (Scheme 3.20).



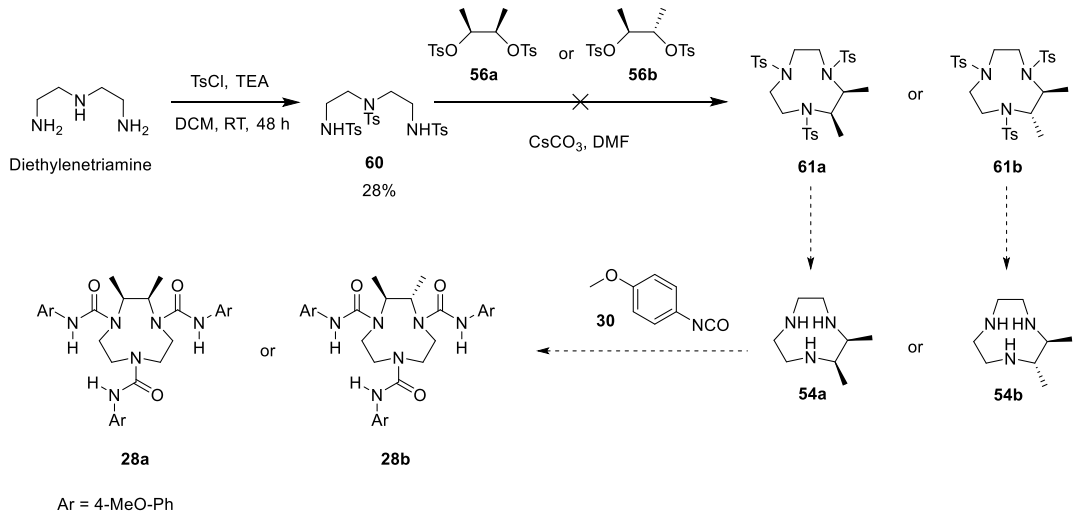
Scheme 3.20. Retrosynthetic scheme to prepare **28a** and **28b**.

The synthetic strategy designed to prepare compounds **28a** and **28b** is described in Scheme 3.21. The *meso* and *anti* diols **55a** and **55b** were converted into the two corresponding tosylated compounds **56a** and **56b** in 86% and 90% yield respectively, by treatment with TsCl in the presence of TEA and catalytic amount of DMAP in dry DCM. Unfortunately, attempts to synthesise the cyclic compounds **58a** and **58b**, by performing a macrocyclisation step in basic conditions, using Cs_2CO_3 or KO*t*Bu, were unsuccessful, with multiple conditions having been tried.[37,38] Due to the failure to obtain compounds **58a** and **58b**, it was not possible to perform the subsequent designed steps, namely the hydrolysis of the amide groups **59a** and **59b** and the following reaction with 4-methoxyphenyl isocyanate (**30**) with the aim to obtain the desired **28a** and **28b**.

Therefore, an alternative strategic synthesis in order to prepare **28a** and **28b** was proposed (Scheme 3.22). The synthesis of the two-substituted TACNs **54a** and **54b** by means of the hydrolysis of the triamines protected as tosylates (**61a** and **61b**) was envisaged. Accordingly, diethylenetriamine was reacted with TsCl in dry DCM to afford tri-tosyl-diethylenetriamine **60** in moderate yield. The subsequent macrocyclisation step also proved challenging in this case, trying different conditions without success, not allowing to obtain compounds **61a** and **61b** (Scheme 3.22).



Scheme 3.21. Proposed synthetic strategy to obtain **28a** and **28b**.

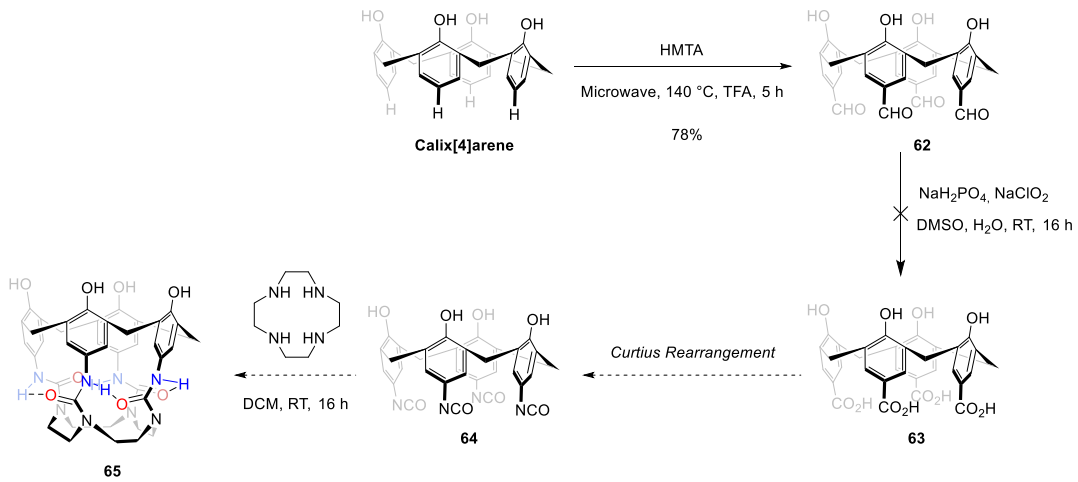


Scheme 3.22. Proposed synthetic strategy to obtain **54a** and **54b**.

3.2.2 Synthesis of Tetrakis(ureido)cyclens

With the aim to increase the barrier to racemization for cyclochiral molecules, the synthesis of a cyclochiral oligourea with tethering methylene bridges between the aromatic groups was attempted, with the aim to form in this way a molecular structure based on the calixarene moiety. In fact, it was speculated that limiting the conformational freedom by forming a mechanically constrained structure should force the hydrogen-bonding network to form by bringing the ureas together, thus raising the barrier to racemisation.

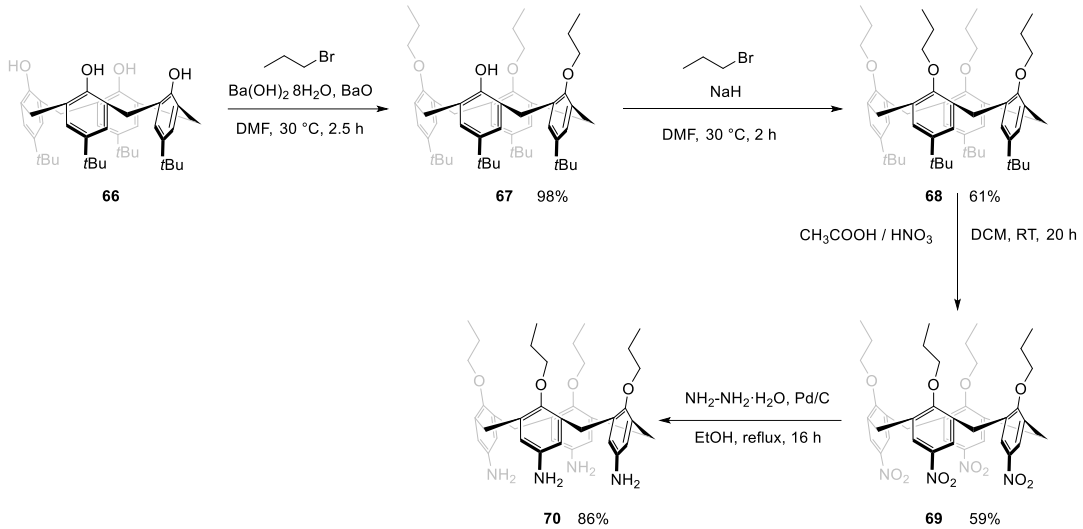
Preparation of a cyclochiral cage such as compound **65** was attempted by the synthetic route described in Scheme 3.23. Calix[4]arene was reacted with hexamethylenetetramine (HMTA) in trifluoroacetic acid and the reaction mixture was heated at 140 °C for 5 h under microwave irradiation, affording the corresponding tetra-formylated compound **62** in 78% yield. The latter one was then dissolved in DMSO and treated with aqueous solutions of sodium dihydrogen phosphate and sodium chlorite. Unfortunately, it was not possible to recover compound **63**, probably due to its high solubility in the aqueous phase.



Scheme 3.23. Proposed synthetic strategy to obtain **65**.

Therefore, another synthetic strategy [39] (Scheme 3.24) was used in order to prepare a cyclochiral cage based on calix[4]arene. A propyl group was introduced on the hydroxyls of

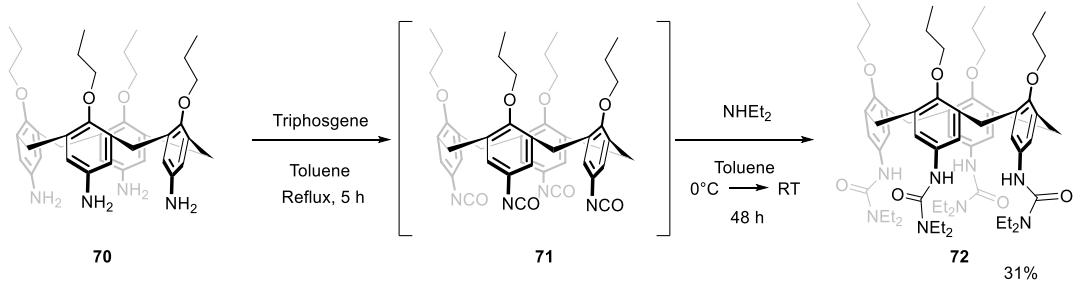
the 4-*tert*-butylcalix[4]arene **66** with the aim to reduce the solubility in water. Compound **66** was reacted with 1-bromopropane in the presence of barium oxide and barium hydroxide octahydrate in dry DMF, affording the tri-propyl *tert*-butylcalix[4]arene (**67**) in 98% yield. Compound **67** was then reacted with a stronger base, NaH, in dry DMF to give the tetra-propylate derivative **68** in 61% yield. The subsequent nitration reaction with acetic and nitric acids in dry DCM led to the formation of the tetra-nitro derivative **69** in a yield of 59%. The following reduction of nitro groups, performed with hydrazine hydrate and Pd/C in anhydrous EtOH, gave the calix[4]tetra-anilines **70** in 86% yield.



Scheme 3.24. Synthesis of calix[4]tetra-anilines **70**.

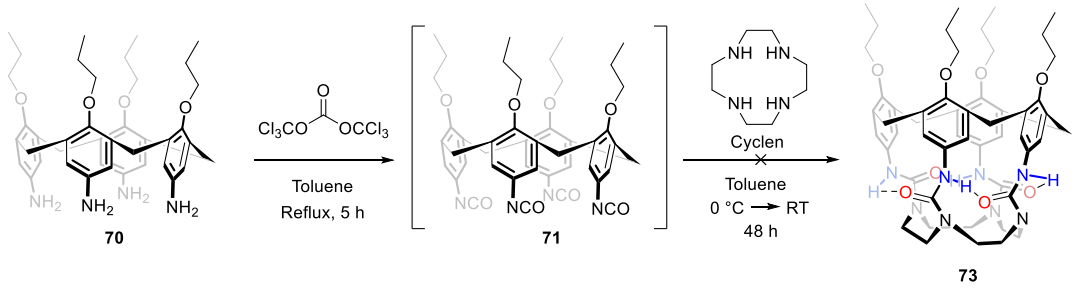
To test the reactivity of calix[4]tetra-anilines (**70**) in the formation of ureas, a tandem one-pot reaction was performed (Scheme 3.25). The synthesis of tetra-urea derivative **72** occurred starting from the compound **70**, which was converted into the corresponding tetra-isocyanate **71** by treatment with triphosgene in dry toluene. In turn, the tetra-isocyanate was reacted *in situ* with diethylamine ($NHEt_2$), leading to the formation of the tetraurea **72** in 31% yield. Unfortunately, no evidence of cyclochirality was observed by both ^{1}H-NMR spectra of **72** at room temperature and at –60 °C. Presumably, the establishment of sufficiently strong circular hydrogen bond array on the lower rim of calix[4]arene, which would make the molecule cyclochiral, was prevented by a not suitable position of urea

groups, maybe too far away from each other to interact. This could also be due to an alternate conformation preferred by the structure of calix[4]arene.[40]



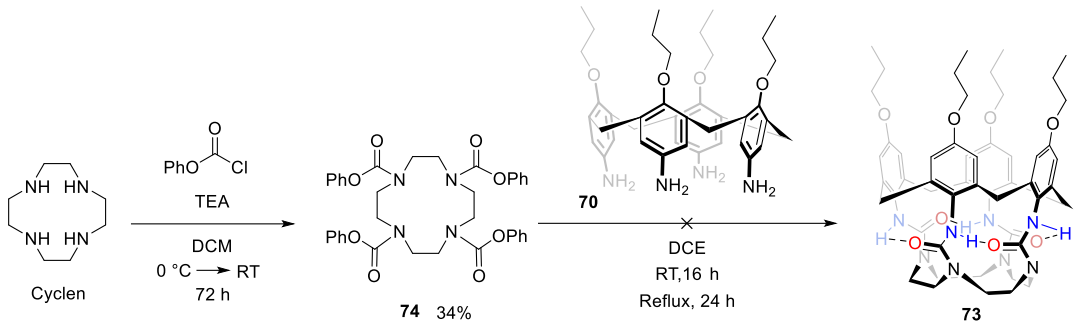
Scheme 3.25. Synthesis of compound **72**.

Finally, the same tandem reaction was performed in order to obtain compound **73**, using cyclen in place of diethylamine (Scheme 3.26). However, it was not possible to observe conversion of starting material due to lack of solubility. Indeed, the solid obtained after removal of the solvent was found to be insoluble in several apolar and polar solvents and it could not be characterised.



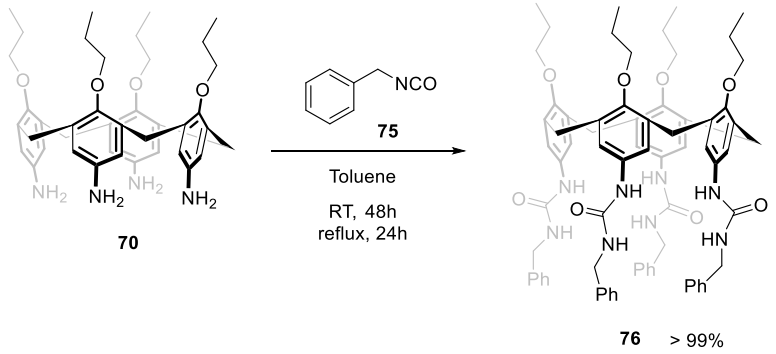
Scheme 3.26. Strategic synthesis proposed to obtain **73**.

Alternatively, preparation of cyclochiral tetraurea **73** was attempted by reaction of calix[4]tetra-anilines (**70**) with the tetra-carbamate **74**, which was obtained by treating cyclen with phenyl chloroformate in the presence of TEA in dry DCM. Unfortunately, no conversion was observed and instead only starting materials were recovered (Scheme 3.27).



Scheme 3.27. Alternative strategic synthesis proposed to obtain **73**.

With the aim to synthesise a cyclochiral compound based on the structure of calix[4]arene, the reaction of calix[4]tetra-anilines (**70**) with benzyl isocyanate (**75**) was performed in dry toluene, affording the tetra-urea derivative **76** in quantitative yield (Scheme 3.28). Indeed, it was speculated that the presence of two ureido protons, derived by using a primary amine, could lead to a stronger cyclic hydrogen bonding arrangement to create a cyclochiral compound. Nevertheless, **76** was found to be not cyclochiral by analysing its ^{1}H-NMR spectrum.



Scheme 3.28. Synthesis of **76**.

3.3. Conclusions

In this project, two new cyclochiral triureas (**1a** and **1g**) based on the TACN structure have been synthesised, enlarging the scope of new cyclochiral motif discovered by J. Clayden and coworkers. This unusual type of chirality is revealed in these structures by ^{1}H-NMR analysis, even if, at present, the barrier to racemisation found is still too low to allow the enantiomeric separation of the oligoureas prepared.

It is possible to speculate that an electron-withdrawing arene in the triurea should inhibit the electron density donation from the aniline nitrogen into the carbonyl, conversely promoting electron donation from the macrocycle nitrogen to carbonyl. This effect would increase the double bond character between the nitrogen of the cycle and the carbonyl. Consequently, the free rotation about the N-C bonds, causing the hydrogen-bonding directionality reversal, should be prevented and thus the racemisation energy barrier should be raised, if the directionality reversal is the mechanism of racemisation. However, the experimental results obtained show that the electronic effects of the urea substituents do not greatly influence the strength of the hydrogen-bonding network.[15] In fact, it was found that both the cyclic triurea bearing the 4-MeO electron-donating substituent on the phenyl ring and that having the 4-pyridyl electron-withdrawing moiety display very similar $\Delta G^{\ddagger}$ value. This suggests further that different effects influence the strength of the intramolecular hydrogen-bonding array in this kind of cyclochiral structures and thus their energy barriers to racemisation. Therefore, other methods to increase the racemisation barrier of the process shall to be investigated. In this regard, it will be greatly important to succeed in synthesising the cyclochiral triureas bearing substituents on the ethylene bridges with the aim to understand the mechanisms involved into the interconversion between the enantiomers.

Furthermore, it was found that the synthesis of the cyclochiral cages was very challenging probably due to polymerisation issues and/or to thermodynamic factors.

In conclusion, the discovery of the appropriate conditions to separate and isolate the enantiomers of these cyclochiral triureas could provide a new class of compounds, chiral by virtue of cyclic hydrogen-bonding array only, suitable for asymmetric organocatalysis and enantiospecific host-guest chemistry.

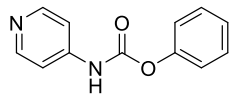
3.4. Experimental section

3.4.1.General Information

Where specified, procedures were performed using dried solvents and reagents under an atmosphere of nitrogen with glassware dried by flame-drying. Air and moisture-sensitive liquids/solutions were transferred to reaction vessels by syringe under an atmosphere of nitrogen. Anhydrous DCM, THF, MeCN and toluene were dried using an anhydrous Engineering Grubbs-type solvent system. All other solvents and reagents were purchased from commercial suppliers and were used without further purification. Agitation was achieved using Teflon coated stirrer bars by magnetic induction. All thin layer chromatography (TLC) experiments were conducted on pre-coated plastic plates (Macherey-Nagel polygram SIL G/UV_{254}) and visualised using ultraviolet light (254 nm) or staining. Flash chromatography was performed on an automated Biotage Isolera™ Spektra Four using gradient elution on pre-packed silica gel Biotage® SNAP Ultra or ZIP Sphere columns. All low-temperature VTNMR experiments were conducted using a JEOL ECS 300 spectrometer (300 MHz) or a Bruker AVANCE III HD 500 MHz NMR Spectrometer with 5 mm DCH ^{13}C–^{1}H/D Cryo Probe (500 MHz). All high-temperature VTNMR experiments were conducted using a Varian VNMRS 500 MHz Direct Drive Spectrometer with Agilent OneNMR probe (500 MHz). All room temperature NMR experiments were conducted using a Bruker Nano 400 Spectrometer (400 MHz) or a a Bruker AVANCE III HD 500 MHz NMR Spectrometer with 5 mm DCH ^{13}C–^{1}H/D Cryo Probe (500 MHz), with chemical shifts reported (δ in ppm) relative to the specified deuterated solvent. All ^{19}F are referenced relative to an internal standard. All NMR characterisation experiments were performed at 25 °C and 1 atm unless otherwise stated. Multiplicity is reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Reactions run in a microwave oven were completed on a Biotage Initiator+.

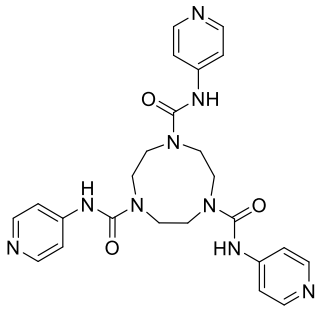
3.4.2 Synthesis of Tris(ureido)triazacyclononanes

Phenyl pyridin-4-ylcarbamate, 2



Under dry and inert atmosphere, a solution of 4-aminopyridine (10.6 mmol, 1.00 g, 1.0 eq.) in anhydrous DCM (60 mL) was cooled to 0°C. To this solution phenyl chloroformate (11.7 mmol, 1.47 mL, 1.1 eq.) was added dropwise and then TEA (14.9 mmol, 2.07 mL, 1.4 eq) was added in one portion. The resulting colourless solution became yellow after 2 h. The reaction mixture was stirred for 72 h at RT. After this time, the solution was washed with HCl (1M), NaOH (1M) and brine. The organic layer was dried on $MgSO_4$, filtered and concentrated *in vacuo* to give the crude product (1.1 g), which was purified by column chromatography to yield the title compound isolated as a white solid (581 mg, 2.71 mmol, 26%). **TLC** (SiO_2, 5:95 MeOH:DCM) R_f = 0.25.
^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.17 (d, 2H, J = 7.6), 7.25 (t, 1H, J = 7.6), 7.38-7.44 (m, 4H), 7.67(bs, 1H), 8.49 (d, 2H, J = 6 .0). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 112.7, 121.5, 126.2, 129.6, 145.2, 150.2, 150.6, 151.2.

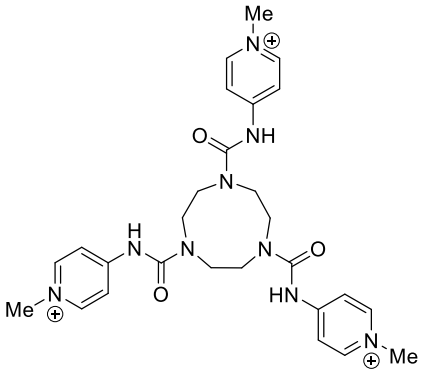
***N*^{1},*N*^{4},*N*^{7}-Tri(pyridin-4-yl)-1,4,7-triazonane-1,4,7-tricarboxamide, 1a**



Under dry and inert atmosphere, a solution of 1,4,7-triazacyclononane trihydrochloride (0.39 mmol, 93 mg,1.0 eq.) and TEA (1.17 mmol, 163 µL, 3.0 eq.) in anhydrous ACN (4 mL) was prepared. To this solution phenyl pyridin-4-ylcarbamate (**2**) (1.74 mmol, 373 mg, 4.5 eq.) was added as white solid and the resulting solution was stirred for 16 h at RT. After this time the obtained suspension was filtered and the residue was washed with cold ACN and air-dried to give the desired urea as a white solid (173 mg, 0.35 mmol, 91%). **TLC** – R_f = 0.10 (SiO_2, 10:90 MeOH:DCM). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 3.09 (brs, 3H), 3.18 (brs, 3H), 4.03 (brs, 3H), 4.43 (brs, 3H), 6.82

(d, 6H, J = 4.8), 8.15 (d, 6H, J = 4.8). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 46.7, 44.7, 114.2, 145.8, 150.1, 158.0.

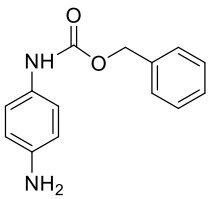
4,4',4''-((1,4,7-triazonane-1,4,7-tricarbonyl)tris(azanediyl))tris(1-ethylpyridin-1-ium), 1b



Under dry and inert atmosphere, **1a** (0.082 mmol, 40 mg, 1.0 eq.) was dissolved in anhydrous ACN (2 mL). To this solution CH_3I (0.98 mmol, 610 µL, 12 eq.) was added and the mixture was stirred at reflux for 18 h. After this time, it was cooled to RT and the solvent was removed under reduced pressure to afford the title product as a white solid (69 mg, 0.075 mmol, 91%).

^{1}H-NMR (400 MHz, D_2O) δ (ppm): 3.65 (bs, 12H), 3.99 (s, 9H), 7.66 (d, 6H, J = 7.2 Hz), 8.22 (d, 6H, J = 7.2 Hz).

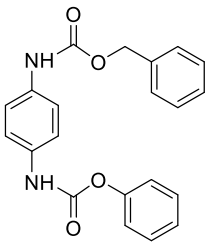
Benzyl (4-aminophenyl)carbamate, 16



To a stirred solution of 1,4 diamino benzene (18.5 mmol, 2.0 g, 1.0 eq.) in anhydrous DCM (20 mL), benzyl chloroformate (18.5 mmol, 2.64 mL, 1.0 eq.) was added at 0 °C. After 10 minutes, a precipitate formed and the reaction mixture was stirred with sonication at RT for 1 h. Water and DCM (20 mL) were then added and the reaction mixture was stirred for 1 h. The aqueous layer was separated and the organic layer was filtered and evaporated to dryness to afford the mono-Cbz protected product as a white solid (673 mg, 2.78 mmol, 15%).

^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 5.04 (s, 2H), 5.07 (s, 2H), 6.47 (d, 2H, J = 8.8 Hz), 6.69 (d, 2H, J = 8.8), 7.24-7.40 (m, 5H), 9.40 (s, 1H).

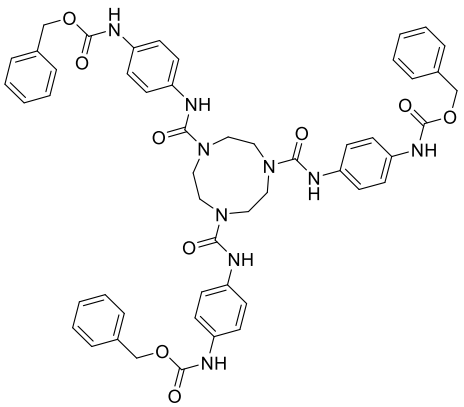
Benzyl phenyl 1,4-phenylenedicarbamate, 18



16 (2.78 mmol, 673 mg) was dissolved in pyridine (6 mL) and THF (8 mL). This solution was diluted in EtOA (57 mL) and cooled at 0°C. A solution of phenyl chloroformate (3.06 mmol, 0.384 mL, 1.1 eq.) was slowly added to the reaction mixture, which was stirred at 0°C for 1h and then for 16h at RT. After this time the reaction mixture was washed with water, HCl 1 M, with water again and dried over $MgSO_4$. The crude was triturated with DCM. The solid obtained as precipitate was filtered to give the desired product as a white solid (490 mg, 1.35 mmol, 49%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 5.18 (s, 2H), 6.57 (bs, 1H), 6.86 (bs, 1H), 7.31-7.40 (m, 14H).

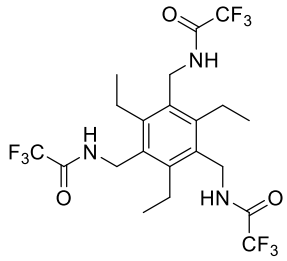
Tribenzyl (((1,4,7-triazonane-1,4,7-tricarbonyl)tris(azanediyl)) tris(benzene-4,1-diyl)) tricarbamate, 1g



Under dry and inert atmosphere, a solution of 1,4,7-triazacyclononane trihydrochloride (0.30 mmol, 72 mg, 1.0 eq.) and TEA (0.9 mmol, 125 µL, 3.0 eq.) in anhydrous ACN (3 mL) was prepared. To this solution **18** (1.35 mmol, 490 mg, 4.5 eq.) was added and the resulting solution was stirred for 16 h at RT. After this time the obtained suspension was filtered and the residue was washed with cold ACN and air-dried to give the corrisponding urea as a white solid (131 mg, 0.14 mmol, 47%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 3.05 (brt, 6H, J = 20 Hz), 3.95 (brd, 3H, J = 12 Hz), 4.39 (brs, 3H), 6.65 (d, 6H, J = 8.8 Hz), 6.90 (d, 6H, J = 8.8 Hz), 7.85 (s, 3H).

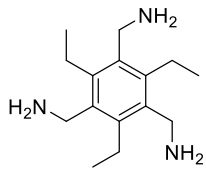
***N,N',N''*-((2,4,6-triethylbenzene-1,3,5-triyl)Tris(methylene))tris(2,2,2-trifluoroacetamide), 23**



Under dry and inert atmosphere, NaH (1.36 g, 56.7 mmol, 10 eq.) was placed in a round-bottomed flask. Anhydrous DMF (15 mL) was added at 0°C and trifluoroacetamide (5.77 g, 51.0 mmol, 9.0 eq.) was slowly added. The solution was allowed to stir at RT for 1 h. 1,3,5-Tris(bromomethyl)-2,4,6-triethylbenzene (2.5 g, 5.67 mmol, 1.0 eq.) was added and the reaction mixture was stirred under nitrogen at RT for 24 h. After this time, HCl (1M) was added to the stirring solution. The resulting white precipitate was filtered and dried under vacuum to give the desired product as a white solid (3.50 g, 6.51 mmol, > 99%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.15 (t, 9H, J = 7.6 Hz), 2.62 (q, 6H, J = 7.6 Hz), 4.53 (d, 6H, J = 4.0 Hz), 6.11 (s, 3H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 16.3, 23.4, 38.3, 130.9, 145.3, 164.1.

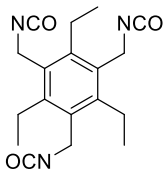
(2,4,6-triethylbenzene-1,3,5-triyl)Trimethanamine, 24



To a solution of *N,N',N''*-((2,4,6-triethylbenzene-1,3,5-triyl)tris(methylene))tris(2,2,2-trifluoroacetamide) (3.97 g, 7.40 mmol, 1.0 eq.) in MeOH (53 mL), an aqueous solution of NaOH (5%) (26 mL) was added. The resultant reaction mixture was stirred at RT for 18h and then concentrated *in vacuo*. The crude product was dissolved in a solution of NaOH 1M and washed with DCM. The combined organic extracts were dried on $MgSO_4$, filtered and concentrated under reduced pressure to give the corresponding triamine as a white powder (1.18g, 4.74 mmol, 64%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.17 (t, 9H, J = 7.4 Hz), 2.76 (q, 6H, J = 7.6 Hz), 3.81 (s, 6H).

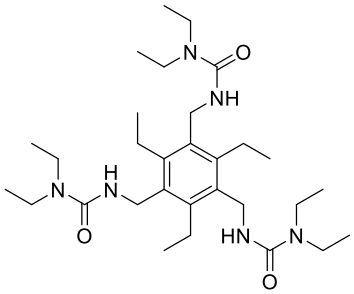
1,3,5-Triethyl-2,4,6-tris(isocyanatomethyl)benzene, 20



Under dry and inert atmosphere, a round bottom flask was charged with triphosgene (800 mg, 3.21 mmol, 3.0 eq.) and anhydrous toluene (30 mL). A solution of (2,4,6-triethylbenzene-1,3,5-triyl)trimethanamine (**n**) (250 mg, 1.00 mmol, 1.0 eq.) in anhydrous toluene (30 mL) was added dropwise to the solution of triphogene and the reaction mixture was heated to reflux then stirred for 2h. After this time the mixture was cooled to RT and the solvent was removed under reduced pressure. The crude product was triturated with cold toluene and the formation of a white precipitate was observed. Then it was filtered and the filtrate was dried under high vacuum to give the desired tris-isocyanate as a white solid (278 mg, 0.85 mmol, 85%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.04-1.30 (m, 9H), 2.55-2.86 (m, 6H), 4.41 (s, 6H).

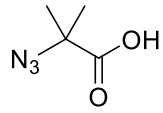
1,1',1''-((2,4,6-triethylbenzene-1,3,5-triyl)Tris(methylene))tris(3,3-diethylurea), 25



Under dry and inert atmosphere, a round-bottomed flask was charged with anhydrous DCM (10 mL) and 1,3,5-triethyl-2,4,6-tris(isocyanatomethyl)benzene (**20**) (33 mg, 0.1 mmol, 1.0 eq.). To this solution diethylamine was slowly added. The reaction mixture was stirred for16 h and then the solvent was evaporated *in vacuo*. The crude product (75 mg) was purified by column chromatography to yield the title compound isolated as a white solid (15 mg, 0.03 mmol, 30%). **TLC** – R_f = 0.4 (SiO_2, 5:95 MeOH:DCM).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.09 (t, 18H, J = 7.2 Hz), 1.21 (t, 9H, J = 7.6 Hz), 2.76 (q, 6H, J = 7.6 Hz), 3.22 (q, 12H, J = 7.2 Hz), 4.07 (bt, 3H, J = 4 Hz), 4.43 (d, 6H, J = 4 Hz).

2-Azido-2-methylpropanoic acid, 34

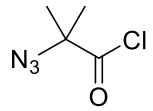


Under dry and inert atmosphere, 2-rromo-2-methylpropionic acid (**33**) (5.0 g, 30.0 mmol, 1.0 eq.) was dissolved in anhydrous DMF (23 mL). Sodium azide (3.0 g, 45.0 mmol, 1.5 eq.) was added and the mixture stirred at room temperature for 72 h. The reaction mixture was diluted with H_2O, acidified to pH = 2 with HCl (1 M) and then extracted with *tert*-butyl methyl ether. The combined organic phases were washed with HCl (1 M), dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield the title compound as a pale yellow oil, which was used without further purification (3.07 g, 24.0 mmol, 80%).

^{1}H-NMR (400 MHz, $CDCl_3$) 1.51 (s, 6H), 10.4 (bs, 1H).

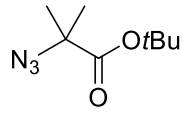
All other data are consistent with that reported in the literature.[41]

2-Azido-2-methylpropanoyl chloride, 35



Under dry and inert atmosphere, 2-azido-2-methylpropanoic acid (**34**) (2.57 g, 20.0 mmol, 1.0 eq.) was dissolved in dry DCM (5 mL). Thionyl chloride (3.00 mL, 40.0 mmol, 2.0 eq.) was added dropwise and the resulting solution was stirred at reflux for 3 h. The resulting yellow mixture was concentrated and then distilled under reduced pressure to yield the title compound as a colourless oil that was used immediately in the subsequent reaction (2.50 g, 17.0 mmol, 85%).

***tert*-Butyl 2-azido-2-methylpropanoate, 37**

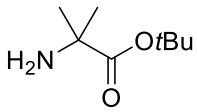


Under dry and inert atmosphere, *tert*-butyl-2-bromo-2-methylpropionate (**36**) (4.2 mL, 22.4 mmol, 1.0 eq.) was dissolved in dry DMF (23 mL) and sodium azide (2.20 g, 33.6 mmol, 1.5 eq.) was added. The obtained reaction mixture was stirred at RT for 72h, forming a cloudy white solution. The reaction mixture was diluted with H_2O (10 mL), acidified to pH = 2 with HCl (1 M) and then extracted with *tert*-butyl methyl ether. The combined organic layers were washed with HCl (1 M), dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield the pure product

as a pale yellow oil, which was used without further purification (2.0 g, 10.8 mmol, 48%). **TLC** SiO_2/Petrol:EtOAc (80:20) R_f = 0.80.

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.35 (s, 6H), 1.43 (s, 9H). **^{13}C-NMR** (100MHz, $CDCl_3$) 23.9, 28.2, 64.0, 81.8, 172.6.

***tert*-Butyl 2-amino-2-methylpropanoate, 38**

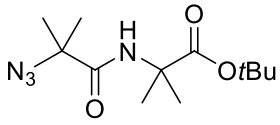


Under dry and inert atmosphere, *tert*-butyl 2-azido-2-methylpropanoate (**37**) (2.0 g, 10.8 mmol) was dissolved in anhydrous EtOH (54 mL). Pd/C (10% mol, 149 mg) was carefully added and the reaction mixture was stirred under an atmosphere of H_2 for 24 h. The mixture was washed through a pad of Celite with EtOAc and the filtrate concentrated under reduced pressure to yield the title compound as a pale yellow oil, which was used without further purification (9.174 g, 57.6 mmol, 94%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.23 (s, 6H), 1.39 (s, 9H), 1.81 (brs, 2H).

All other data are consistent with that reported in the literature.[42]

***tert*-Butyl 2-(2-azido-2-methylpropanamido)-2-methylpropanoate, 39**

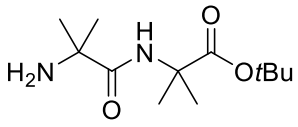


Under dry and inert atmosphere, *tert*-butyl 2-amino-2-methylpropanoate (**38**) (1.5 g, 9.40 mmol, 1.0 eq.) was dissolved in dry DCM (24 mL), cooled to 0 °C and Et_3N (2.0 mL, 14.1 mmol, 1.5 eq.) was added. A solution of freshly distilled 2-azido-2-methylpropanoyl chloride (**35**) (1.7 g, 11.3 mmol, 1.2 eq.) in dry DCM (3 mL) was added dropwise and the mixture was allowed to warm to RT whilst stirring for 24 h. The reaction mixture was concentrated under reduced pressure and dissolved in EtOAc, then washed with 5% aq. $KHSO_4$ solution, sat. $NaHCO_3$ solution and brine. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to afford the title compound as a pale yellow oil, which was used without further purification (1.74 g, 6.44 mmol, 69%). **TLC** SiO_2/Petrol:EtOAc (90:10) R_f = 0.40.

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.44 (s, 9H) 1.49 (d, 12H, J = 2.8 Hz), 7.1 (brs, 1H).

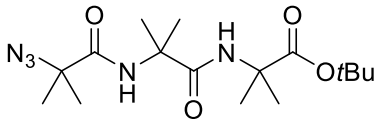
^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 24.29, 24.31, 27.8, 56.7, 64.2, 81.6, 171.1, 173.5.

***tert*-Butyl 2-(2-amino-2-methylpropanamido)-2-methylpropanoate, 40**



Under dry and inert atmosphere, *tert*-butyl 2-(2-azido-2-methylpropanamido)-2-methylpropanoate (**39**) (1.38 g, 5.11 mmol, 1.0 eq.) was dissolved in dry MeOH (26 mL). $Pd(OH)_2/C$ (20% mol, 100 mg) and 3 drops of CH_3COOH were added and the reaction mixture was stirred under an atmosphere of H_2 for 24 h. The mixture was washed through a pad of Celite with EtOAc and the filtrate was concentrated under reduced pressure to yield the title compound as a white solid, which was used without further purification (1.18 g, 4.84 mmol, 95%). **TLC** SiO_2/Petrol:EtOAc (80:20) R_f = 0.2. **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 1.32 (s, 6 H), 1.43 (s, 9H), 1.49 (s, 6 H), 8.04 (brs, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 24.6, 27.9, 29.0, 54.9, 56.3, 81.3, 174.0, 176.4.

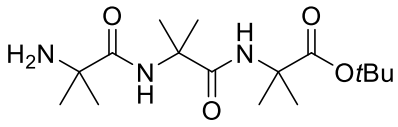
***tert*-Butyl 2-(2-(2-azido-2-methylpropanamido)-2-methylpropanamido)-2-methylpropanoate, 41**



Under dry and inert atmosphere, *tert*-butyl 2-(2- amino-2-methylpropanamido)-2-methylpropanoate (**40**) (549 mg, 2.25 mmol, 1.0 eq.) was dissolved in dry DCM (6 mL), cooled to 0 °C and Et_3N (940 µL, 6.75 mmol, 1.5 eq.) was added. A solution of freshly distilled 2-azido-2-methylpropanoyl chloride (**35**) (396 mg, 2.70 mmol, 1.2 eq.) in dry DCM (1 mL) was added dropwise and the mixture allowed to rise to RT whilst stirring for 24h. The reaction mixture was concentrated to dryness under reduced pressure and dissolved in EtOAc, then washed with 5% aq. $KHSO_4$ solution, sat. $NaHCO_3$ solution and brine. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to yield the title compound as a white solid, which was used without further purification (801 mg, 2.25 mmol, >99%). **TLC** SiO_2/Petrol:EtOAc (80:20) R_f = 0.20.

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.43 (s, 9H), 1.50 (s, 6H), 1.51 (s, 6H), 1.53 (s, 6H), 6.97 (brs, 1H), 7.19 (brs, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 24.0, 24.3, 24.8, 27.8, 59.9, 57.1, 64.3, 81.8, 171.1, 172.9, 174.1.

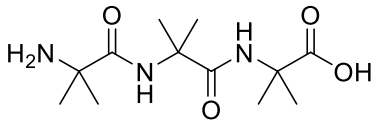
***tert*-Butyl 2-(2-(2-amino-2-methylpropanamido)-2-methylpropanamido)-2-methylpropanoate, 42**



Under dry and inert atmosphere, *tert*-butyl 2-(2-(2-azido-2-methylpropanamido)-2-methylpropanamido)-2-methylpropanoate (**41**) (801 mg, 2.25 mmol, 1.0 eq.) was dissolved in dry MeOH (12 mL). Pd/C (10% mol, 240 mg) was carefully added and the reaction mixture stirred under an atmosphere of H_2 for 24 h. The mixture was washed through a pad of Celite with EtOAc and the filtrate concentrated under reduced pressure to yield the title compound as a white solid, which was used without further purification (601 mg, 1.83 mmol, 81%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.34 (s, 6H), 1.43 (s, 9H), 1.49 (s, 6H), 1.52 (s, 6H), 7.45 (s, 1H), 8.15 (s, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 24.3, 25.2, 27.9, 28.9, 55.1, 56.7, 57.0, 81.4, 173.5, 174.1, 177.7.

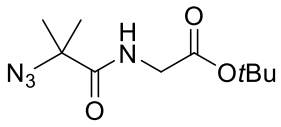
2-(2-(2-amino-2-methylpropanamido)-2-methylpropanamido)-2-methylpropanoic acid, 32



Under dry and inert atmosphere, **42** (100 mg, 0.30 mmol, 1.0 eq.) was dissolved in anhydrous DCM (1.5 mL). This solution was cooled to 0 °C and TFA (0.70 mL, 9.0 mmol, 30.0 eq.) was added dropwise. The reaction mixture was allowed to warm to RT and was stirred for 72 h. Upon completion, the solvent was removed under reduced pressure and Et_2O was added. The mixture was cooled to 0°C and the resulting precipitate was isolated by filtration, washed several times with cold Et_2O and dried under vacuum to give the pure product as a white solid (83 mg, 0.30 mmol, > 99%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.47 (s, 6H), 1.49 (s, 6H), 1.57 (s, 6H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 22.6, 23.3, 23.7, 56.2, 56.9, 57.1, 171.0, 174.2, 177.0.

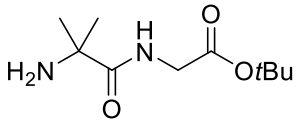
***tert*-Butyl (2-azido-2-methylpropanoyl)glycinate, 47**



To a solution of 2-azido-2-methylpropanoic acid (**34**) (774.72 mg, 6.0 mmol, 1.0 eq.) and glycine *tert*-butyl ester hydrochloride (**46**) (1.0 g, 6.0 mmol, 1.0 eq.) in DCM (60 mL), HOBt·H_2O (2.4 g, 18.0 mmol, 3.0 eq.), EDC·HCl (2.8 g, 18.0 mmol, 3.0 eq.) and NMM (1.82 g, 18.0 mmol, 3.0 eq.) were added. The reaction mixture was stirred for 18 h. After this time, water was added and the organic layer was washed with a 5% solution $KHSO_4$ and brine and then dried with $MgSO_4$. The solvent was evaporated *in vacuo* giving the pure compound as a white solid, which was used without further purification (1.16 g, 4.8 mmol, 80%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.46 (d, 9H, J = 1.6 Hz), 1.53 (d, 6H, J = 1.2), 3.88 (d, 1H, J = 1.6 Hz), 3.90 (d, 1H, J = 1.6 Hz), 6.94 (bs, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 24.5, 28.1, 41.8, 64.1, 82.2, 168.9, 172.5.

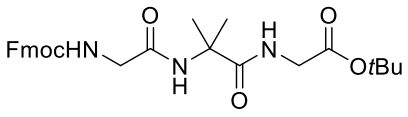
***tert*-Butyl (2-amino-2-methylpropanoyl)glycinate, 48**



Under dry and inert atmosphere, *tert*-butyl (2-azido-2-methylpropanoyl)glycinate (**47**) (1.16 g, 4.80 mmol, 1.0 eq.) was dissolved in dry MeOH (12 mL). Pd/C (10% mol, 512 mg) was carefully added and the reaction mixture stirred under an atmosphere of H_2 for 24 h. The mixture was washed through a pad of Celite with EtOAc and the filtrate concentrated under reduced pressure to yield the title compound as a white solid, which was used without further purification (1.02 g, 4.70 mmol, 98%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.35 (, 6H), 1.44 (, 9H), 3.88 (, 2H), 8.00 (brs, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 25.7, 28.7, 41.6, 61.1, 82.2, 169.5, 174.0.

***tert*-Butyl (2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)acetamido)-2-methylpropanoyl)glycinate, 50**

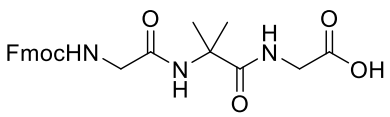


To a solution of *tert*-butyl (2-amino-2-methylpropanoyl)glycinate (**48**) (1.02 g, 4.7 mmol, 1.0 eq.) and Fmoc-Gly-OH (**49**) (1.40 g, 4.7 mmol, 1.0 eq.) in DCM (47 mL), HOBt·H_2O (1.90 g, 14.1 mmol, 3.0 eq.), EDC·HCl (2.2 g, 14.1 mmol, 3.0

eq.) and NMM (1.43 g, 14.1 mmol, 3.0 eq.) were added. The reaction mixture was stirred for 18h. After this time, water was added. The organic layer was washed with a 5% solution $KHSO_4$ and brine and then dried with $MgSO_4$. The solvent was evaporated in vacuo giving the pure compound as a white solid, which was used without further purification (2.00 g, 4.03 mmol, 86%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.45 (s, 9H), 1.59 (s, 6H), 3.84 (d, 2H, J = 5.2 Hz), 3.90 (d, 2H, J = 5.2 Hz), 4.21 (t, 1H, J = 6.8 Hz), 4.41 (d, 2H, J = 6.8), 5.48 (brs, 1H), 6.63 (s, 1H), 6.71 (brs, 1H), 7.30 (dt, 2H, J = 7.6 Hz, J = 1.2 Hz), 7.39 (t, 2H, J = 7.6 Hz), 7.58 (d, 2H, J = 7.2), 7.75 (d, 2H, J = 7.6 Hz). **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 25.2, 28.1, 42.4, 47.1, 57.4, 65.8, 67.3, 83.9, 120.1, 127.2, 127.8, 128.5, 141.4, 143.8, 156.2, 169.5, 170.4, 174.6.

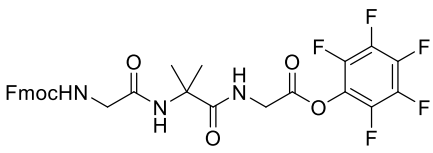
(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)acetamido)-2-Methylpropanoyl) glycine, 51



Under dry and inert atmosphere, **50** (500 mg, 1.0 mmol, 1.0 eq.) was dissolved in anhydrous DCM (4 mL). This solution was cooled to 0 °C and TFA (2.3 mL, 30 mmol, 30.0 eq.) was added dropwise. The reaction mixture was warmed to RT and was stirred for 72 h. Upon completion, the solvent was removed under reduced pressure and Et_2O was added. The mixture was cooled to 0 °C and the resulting precipitate was isolated by filtration, washed three times with cold Et_2O and dried under vacuum to give the pure product as a white solid (206 mg, 0.47 mmol, 47%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.51 (s, 6H), 3.79 (d, 2H, J = 5.6 Hz), 3.97 (d, 2H, J = 5.2 Hz), 4.17 (t, 1H, J = 6.8 Hz), 4.37 (d, 2H, J = 6.8 Hz), 5.90 (brs, 1H), 6.85 (brs, 1H), 7.26 (t, 2H, J = 8.0 Hz), 7.36 (t, 2H, J = 7.2 Hz), 7.55 (d, 2H, J = 8.0), 7.72 (d, 2H, J = 7.6 Hz). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 22.6, 43.2, 44.8, 47.2, 65.9, 67.2, 120.4, 125.3, 126.4, 126.9, 142.5, 143.3, 156.0, 170.3, 174.7.

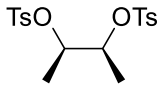
Perfluorophenyl (2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)acetamido)-2-methylpropanoyl)glycinate, 53



To a solution of acid-free tripeptide (**51**) (206 mg, 0.47 mmol, 1.0 eq.) in DCM (4.7 mL) pentafluorophenol (96 mg, 0.52 mmol, 1.1 eq.) and *N*,*N'*-Dicyclohexylcarbodiimide (DCC) (97 mg, 0.47 mmol, 1.0 eq.) were added at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and then at RT for 22 h. The solvent was evaporated and the residue was dissolved in EtOAc and filtered to remove *N*,*N'*-dicyclohexylurea (DCU). The filtrate was evaporated and the crude product was purified by column chromatography (SiO_2, 5:95 MeOH:DCM) to obtain the title pentafluorophenol-protected tripeptide (30 mg, 0.050 mmol, 10%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.55 (s, 6H), 3.78 (d, 2H, J = 5.6 Hz), 4.18 (t, 1H, J = 6.8 Hz), 4.31 (d, 2H, J = 5.6 Hz), 4.39 (d, 2H, J = 6.8 Hz), 5.69 (brs, 1H), 6.65 (s, 1H), 6.98 (brs, 1H), 7.27 (dt, 2H, J = 7.6 Hz, J = 1.2 Hz), 7.38 (t, 2H, J = 7.6 Hz), 7.54 (d, 2H, J = 7.2), 7.74 (d, 2H, J = 7.6 Hz). **^{19}F-NMR** (376 MHz, $CDCl_3$) δ (ppm): -161.75 (t, 2F, J = 20.7 Hz), -157.15 (t, 1F, J = 18.8 Hz), -152.25 (d, 2F, J = 18.4 Hz). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 25.2, 33.5, 44.8, 47.1, 59.6, 65.3, 120.1, 125.0, 126.7, 127.2, 139.3, 140.1, 142.0, 142.4, 143.6, 156.2, 168.0, 170.4, 174.5.

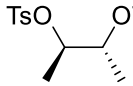
(2*R*,3*S*)-Butane-2,3-diyl bis(4-methylbenzenesulfonate), 56a



Under dry and inert atmosphere, (2*R*,3*S*)-butane-2,3-diol (**55a**) (2.0 g, 22.0 mmol, 1.0 eq.) was dissolved in dry DCM (187 mL) and TEA (18.0 mL, 132 mmol, 6.0 eq.) and 4-(dimethylamino)pyridine (DMAP) (269 mg, 2.20 mmol, 0.1 eq.) were added. To this mixture a solution of TsCl (9.20 g, 48.4 mmol, 2.2 eq.) in DCM (54 mL) was added dropwise. The reaction mixture was stirred at RT for 7 days. Then it was diluted with DCM and washed twice with water. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford the title compound as a brown solid, without further purification (7.5 g, 19.0 mmol, 86%). **TLC** SiO_2/Petrol:EtOAc (60:40) R_f = 0.50.

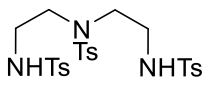
^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.15 (d, 6H, J = 6.8 Hz), 2.38 (s, 6H), 4.42-4.48 (m, 2H), 7.24 (d, 4H, J = 8.0 Hz), 7.64 (d, 4H, J = 8.4 Hz).
The other spectroscopic/analytical data agree with the literature.[43]

(2*R*,3*R*)-Butane-2,3-diyl bis(4-methylbenzenesulfonate), 56b



Under dry and inert atmosphere, (2*R*,3*R*)-butane-2,3-diol (**55b**) (1.83 mL, 20.0 mmol, 1.0 eq.) was dissolved in dry DCM (170 mL) and TEA (16.8 mL, 120 mmol, 6.0 eq.) and 4-(dimethylamino)pyridine (DMAP) (244 mg, 2.00 mmol, 0.1 eq.) were added. To this mixture a solution of TsCl (8.40 g, 44.0 mmol, 2.2 eq.) in DCM (48 mL) was added dropwise. The reaction mixture was stirred at RT for 7 days. Then it was diluted with DCM and washed twice with water. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford the title compound as a brown oil, without further purification (7.0 g, 18.0 mmol, 90%). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 1.11 (d, 6H, J = 6.4 Hz), 2.38 (s, 6H), 4.51 (m, 2H), 7.26 (d, 4H, J = 8.0 Hz), 7.67 (d, 4H, J = 8.3 Hz).
The other spectroscopic/analytical data agree with the literature.[44]

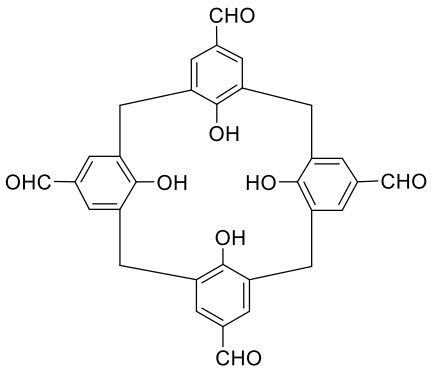
4-Methyl-*N*,*N*-bis(2-((4-methylphenyl)sulfonamido)ethyl)benzenesulfonamide, 60



Under dry and inert atmosphere, diethylenetriamine (1.83 mL, 20.0 mmol, 1.0 eq.) was dissolved in dry DCM (170 mL) and TEA (16.8 mL, 120 mmol, 6.0 eq.) was added. To this mixture a solution of TsCl (8.40 g, 44.0 mmol, 2.2 eq.) in DCM (48 mL) was added dropwise. The reaction mixture was stirred at RT for 48 h. Then it was diluted with DCM and washed twice with water. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was triturated with DCM and filtrated to afford the pure compound as a white solid (4.2 g, 7.40 mmol, 37%).
^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.42 (s, 9H), 3.08-3.19 (m, 9H), 5.17 (t, 2H, J = 5.8 Hz), 7.28-7.32 (m, 6H), 7.60 (d, 2H, J = 8.4), 7.74 (d, 4H, J = 8.4). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 21.6, 42.7, 50.6, 127.2, 129.9, 136.7, 143.7, 144.3.

3.4.3 Synthesis of Tetrakis(ureido)cyclens

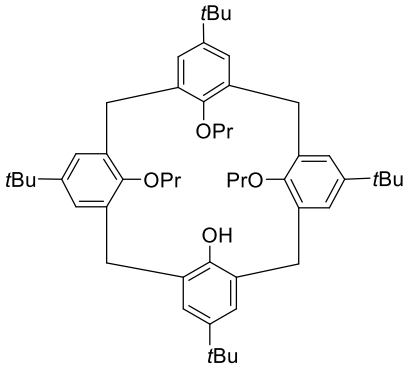
1^2,3^2,5^2,7^2-Tetrahydroxy-1,3,5,7(1,3)-tetrabenzenacyclooctaphane-1^5,3^5,5^5,7^5-tetracarbaldehyde, 62



Under dry and inert atmosphere, a 20 mL microwave reaction vial was charged with calix[4]arene (127 mg, 0.3 mmol, 1.0 eq.), TFA (12 mL) and HMTA (hexamethylenetetramine) (1.60 g, 11.4 mmol, 38 eq.). The reaction mixture was heated at 140 °C for 5 h under microwave irradiation. After cooling down the solution, the TFA was evaporated under reduced pressure. The crude product was washed with DCM and the filtrate was concentrated *in vacuo* to give the title compound (125 mg, 0.233 mmol, 78%).

^{1}H-NMR (400 MHz, DMSO-d_6) 3.87 (brs, 8H), 7.61 (s, 8H), 8.07 (brs, 4H), 9.59 (s, 4H).

1^5,3^5,5^5,7^5-tetra-tert-butyl-3^2,5^2,7^2-tripropoxy-1,3,5,7(1,3)-tetrabenzenacyclooctaphan-1^2-ol, 67[45]

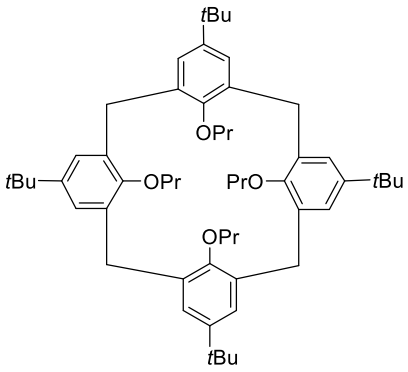


Under dry and inert atmosphere, *p*-*t*Bu-Calix[4]arene (1.0 g, 1.54 mmol, 1.0 eq.), $Ba(OH)_2 \cdot 8H_2O$ (792 mg, 4.62 mmol, 3.0 eq.) and BaO (1.11 g, 7.24 mmol, 4.7 eq.) were solved in anhydrous DMF (39 mL). To this solution 1-bromopropane (7.0 mL, 77 mmol, 50 eq.) was added and the reaction mixture was stirred for 2 h and 30 minutes at 30 °C. The mixture was concentrated under reduced pressure and dissolved in DCM. The organic layer was washed with H_2O and the combined aqueous layers were extracted with

DCM. The combined organic layers were concentrated under reduced pressure to yield compound **n** as a white solid, without further purification (1.17 g, 1.51 mmol, 98%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 0.80 (s, 18H), 0.90 (t, 3H, J = 7.6 Hz), 1.08 (t, 6H, J = 7.6 Hz), 1.31 (s, 9H), 1.32 (s, 9H), 1.81-1.98 (m, 4H), 2.32 (m, 2H), 3.15 (d, 2H, J = 12.4 Hz), 3.21 (d, 2H, J = 13.2 Hz), 3.73 (t, 4H, J = 7.2 Hz), 3.82 (t, 2H, J = 8.4 Hz), 4.33 (m, 4H), 5.57 (s, 1H), 6.49 (s, 4H), 7.03 (s, 2H), 7.12 (s, 2H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 9.7, 10.8, 22.5, 23.5, 31.1, 31.4, 31.8, 31.9, 33.7, 33.9, 34.2, 76.4, 77.9, 124.7, 124.8, 125.0, 125.7, 129.6, 131.9, 132.2, 136.1, 141.5, 145.1, 145.6, 150.8, 151.8, 154.0.

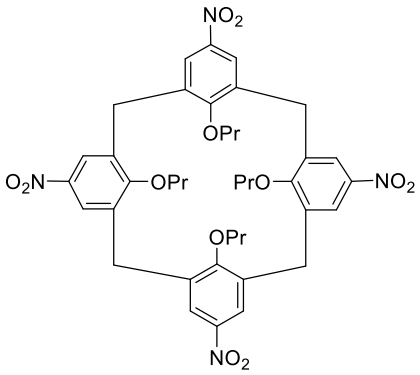
$1^5,3^5,5^5,7^5$-Tetra-tert-butyl-$1^2,3^2,5^2,7^2$-tetrapropoxy-1,3,5,7(1,3)-tetrabenzenacyclooctaphane, 68[45]



Under dry and inert atmosphere, compound **67** (1.17 g, 1.51 mmol, 1.0 eq.) was dissolved in anhydrous DMF (45 mL). To the solution NaH (60% in oil, 214 mg, 9.06 mmol, 6.0 eq.) and 1-bromopropane (2.7 mL, 30.2 mmol, 20 eq.) were added and the reaction mixture was stirred for 2h at 30 °C. The reaction mixture was concentrated under reduced pressure and dissolved in DCM. The organic layer was washed with H_2O and the combined aqueous layers were extracted with DCM. The combined organic layers were concentrated *in vacuo* to yield the desired compound **68** as a white solid (750 mg, 0.92 mmol, 61%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 0.98 (t, 12H, J = 7.6 Hz), 1.06 (s, 36H), 2.00 (m, 8H), 3.09 (d, 4H, J = 12.4 Hz), 3.80 (t, 8H, J = 7.6 Hz), 4.40 (d, 4H, J = 12.4 Hz), 6.76 (s, 8H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 10.4, 23.4, 31.1, 31.5, 33.8, 77.0, 124.9, 133.9, 144.2, 153.8.

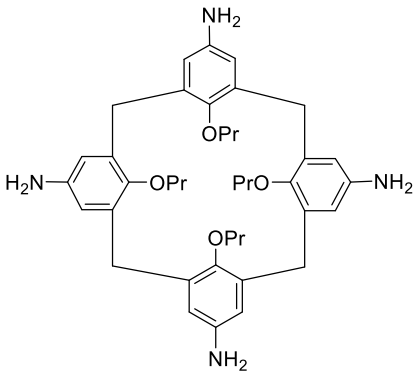
$1^5,3^5,5^5,7^5$-Tetranitro-$1^2,3^2,5^2,7^2$-tetrapropoxy-1,3,5,7(1,3)-tetrabenzenacyclooctaphane, 69[45]



Compound **68** (750 mg, 0.92 mmol, 1.0 eq.) was dissolved in DCM (31 mL) and a mixture of glacial CH_3COOH/fuming HNO_3 (70%) (1:1.42) (7.6 mL) was added at 0 °C. The resulting yellow solution gradually became purple over 2 h. Then the reaction mixture was stirred for 18 h at RT to yield again a yellow solution. The reaction mixture was concentrated under reduced pressure. The crude product was dissolved in DCM and washed with H_2O three times. The combined aqueous layers were extracted with DCM. The organic phase was concentrated *in vacuo*. The crude residue was washed with MeOH to yield the title compound as a pale yellow solid (420 mg, 0.54 mmol, 59%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 0.95 (t, 12H, J = 7.4 Hz), 1.84 (m, 8H), 3.34 (d, 4H, J = 14.0 Hz), 3.89 (t, 8H, J = 7.6), 4.46 (d, 4H, J = 13.6 Hz), 7.51 (s, 8H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 10.2, 23.3, 31.2, 77.8, 124.1, 135.5, 143.0, 161.7.

$1^2,3^2,5^2,7^2$-Tetrapropoxy-1,3,5,7(1,3)-tetrabenzenacyclooctaphane-$1^5,3^5,5^5,7^5$-tetraamine, 70

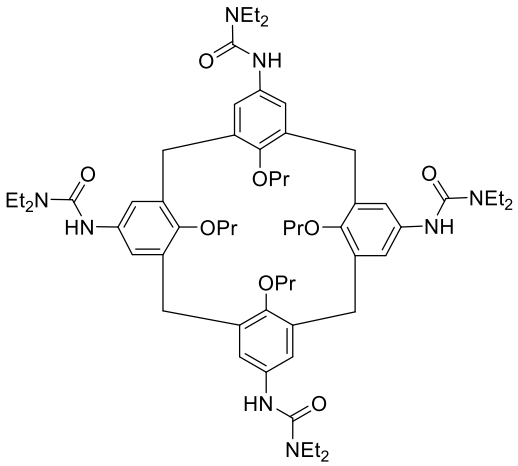


Compound **69** (420 mg, 0.540 mmol, 1.0 eq.) was dissolved in EtOH (16 mL) and Pd/C (10 mol%) (431 mg, 0.405 mmol, 0.75 equiv.) was added. Hydrazine hydrate (2.5 mL, 50.8 mmol, 94 eq.) was added and the reaction mixture was stirred for 16 h at reflux. The reaction mixture was filtered on Celite and the Celite was washed with EtOH and DCM. The filtrate was

concentrated under reduced pressure to yield the title compound as a pale yellow solid (304 mg, 0.466 mmol, 86%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 0.88 (t, 12H, J = 7.4 Hz), 1.79 (m, 8H), 2.84 (d, 4H, J = 13.2 Hz), 3.65 (t, 8H, J = 7.4 Hz), 4.24 (d, 4H, J = 13.2 Hz), 5.98 (s, 8H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 10.4, 23.2, 31.1, 76.7, 116.3, 135.8, 140.2, 150.4.

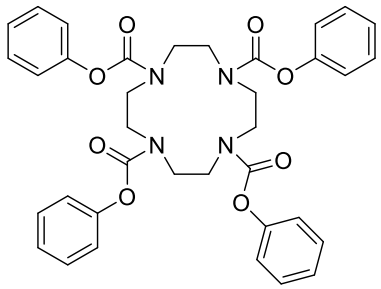
1,1',1'',1'''-(1^2,3^2,5^2,7^2-tetrapropoxy)-1,3,5,7(1,3)-tetrabenzenacyclooctaphane-1^5,3^5,5^5,7^5-tetrayl)tetrakis(3,3-diethylurea), 72



Under dry and inert atmosphere, triphosgene (91 mg, 0.306 mmol, 4.0 eq.) was dissolved in anhydrous toluene (2.5 mL). To this solution, a solution of the compound **70** (50 mg, 0.0766 mmol, 1.0 eq.) in dry toluene (2.5 mL) was added dropwise. The reaction mixture was heated to reflux for 5 h. The solution was cooled down to 0 °C and diethylamine was added. Then the reaction mixture was stirred at RT for 48 h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography to yield the title compound isolated as a white solid (25 mg, 0.0240 mmol, 31%). **TLC** (SiO_2, 5:95:1 MeOH:DCM:NEt_3) R_f = 0.50.

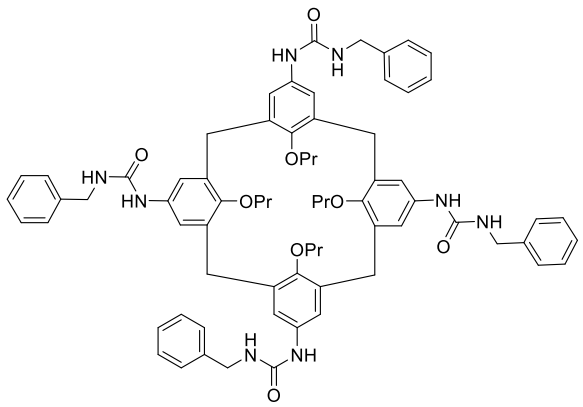
^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 0.94 (t, 12H, J = 7.6 Hz), 1.14 (t, 24H, J = 7.2 Hz) 1.88 (m, 8H), 3.07 (d, 4H, J = 13.2 Hz), 3.28 (q, 16H, J = 7.4 Hz), 3.77 (t, 8H, J = 8 Hz), 4.36 (d, 4H, J = 16 Hz), 6.32 (s, 4H), 6.71 (s, 8H).

Tetraphenyl 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracarboxylate, 74



Under dry and inert atmosphere, a solution of cyclen (250 mg, 1.45 mmol, 1.0 eq.) in anhydrous DCM (8 mL) was cooled to 0 °C. To this solution phenyl chloroformate (0.80 mL, 6.38 mmol, 4.4 eq.) was added dropwise and then TEA (1.13 mL, 8.12 mmol, 5.6 eq) was added in one portion. The reaction mixture was stirred for 72 h at RT. After this time it was washed with HCl (1M), NaOH (1M) and brine. The organic layer was dried on $MgSO_4$, filtered and concentrated *in vacuo* to give the crude product, which was purified by column chromatography to yield the title compound isolated as a white solid (327 mg, 0.501 mmol, 35%). **TLC** (SiO2, 5:95:1 MeOH:DCM:NEt_3) R_f = 0.40.
^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 3.81 (brs, 16H), 7.07 (d, 8H, J = 8 Hz), 7.19 (t, 4H, J = 7.6 Hz), 7.33 (t, 8H, J = 7.8 Hz). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 51.1, 93.5, 121.4, 126.6, 129.3, 150.8.

1,1',1'',1'''-(1^2,3^2,5^2,7^2-tetrapropoxy)-1,3,5,7(1,3)-Tetrabenzenacyclooctaphane-1^5,3^5,5^5,7^5-tetrayl)tetrakis(3-benzylurea), 76



Under dry and inert atmosphere , compound **n** (R54) (50 mg, 0.0766 mmol, 1.0 eq.) was dissolved in anhydrous toluene (2 mL). To this solution, benzyl isocyanate (57 µL, 0.460 mmol, 6.0 eq.) was added. The reaction mixture was allowed to stir at RT for 48 h and then at reflux for 24 h. The solvent was evaporated under reduced pressure to afford the pure title compound as white solid, without further purification (91 mg, 0.0766 mmol, > 99%).

^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 0.92 (t, 12H, J = 7.6 Hz), 1.85 (m, 8H), 2.98 (d, 4H, J = 12.8 Hz), 3.70 (t, 8H, J = 7.4 Hz), 4.26 (d, 4H, J = 12.8 Hz), 6.25 (t, 4H, J = 6.0 Hz), 6.70 (s, 8H), 7.13-7.32 (m, 20H), 8.08 (s, 4H). **^{13}C-NMR** (100 MHz, DMSO-d_6) δ (ppm): 10.7, 23.2, 31.3, 45.5, 76.9, 118.7, 127.1, 127.5, 128.7, 128.8, 141.0, 151.2, 155.8.

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