Università degli Studi di Firenze

PH. D. THESIS IN "SCIENZE CHIMICHE"

XXI CICLO

New strategies for the enantioselective synthesis of morpholines and β-hydroxy-α-amino acids

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Contents

“It is not titles that honor men, but men that honor titles.”

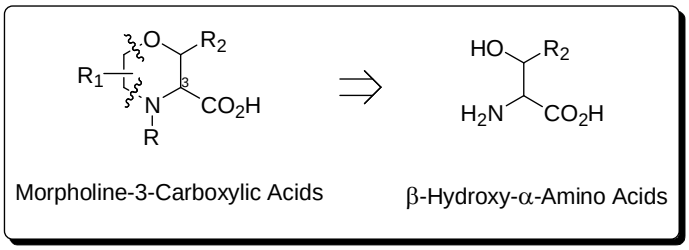
Niccolò Machiavelli

“I have been impressed with the urgency of doing. Knowing is not enough; we must apply. Being willing is not enough; we must do.”

Leonardo da Vinci

Concept

The term “privileged structure” is used in medicinal chemistry to define a single molecular framework able to provide ligands for diverse receptors. Among the different classes of compounds that have been proposed as privileged scaffolds for the drug discovery process we can find morpholines. These six membered heterocycles are widely used in medicinal chemistry, not only as simple secondary amines during the diversification of combinatorial libraries, but also as proper platforms on which a diversification process itself is developed. Many bioactive compounds are based on carbon-substituted morpholines and out of these several are already marketed drugs such as Reboxetine, Aprepitant and many others (a complete overview is given in Chapter 1). The strong attention of the medicinal chemist community for the morpholine core prompted us to investigate new and more efficient routes for the preparation of this heterocycle. An interesting subclass of carbon-substituted morpholines that attracted our attention are those presenting a carboxylic moiety in position three of the ring (morpholine-3-caroxylic acids).

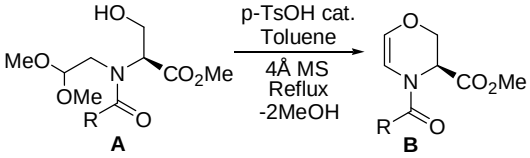


This set of morpholines is an interesting case of study since it represents a group of cyclic, non natural α-amino acids that can be applied in the field of peptidomimetics and, again, as nucleus of various bioactive compounds (a detailed discussion of such structures is given in Chapter 2, Section 1). This PhD work was aimed to develop new synthetic strategies towards the production of diversely functionalized morpholine-3-carboxylic acids and towards the application of these structures in the field of peptidomimetics. The idea for the preparation of our target structures was that of using enantiomerically pure β-hydroxy-α-amino acids as building blocks for the creation of the morpholine ring. Initially, it was decided to develop a new methodology for the synthesis of morpholines using natural occurring β-hydroxy-α-amino acids, namely serine and threonine. Once this new methodology was succesfully developed, it appeared clear that the major drawback was represented by the limited avaibility of enantiomerically pure β-hydroxy-α-amino acids. In order to overcome this problem and increase the diversity of morphloines that can be obtained with our methodology we required a new and reliable source of β-hydroxy-α-amino acids. Although some methods for the preparation of enantiomerically pure β-hydroxy-α-amino acids are reported (Chapter 5), we found it challenging to develop our own strategy. Moreover β-hydroxy-α-amino acids themselves are attracting targets and their asymmetric preparation is a current hot topic in organic synthesis because these compounds are constituents of many natural products such as vancomicyn and other natural antibiotics. An innovative synthetic strategy towards this class of compounds,

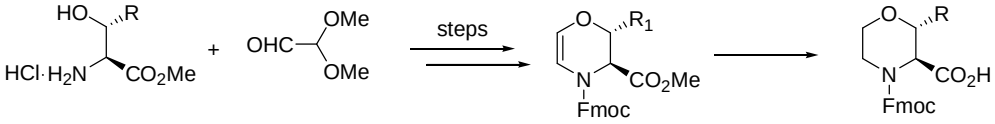
needed the development of a catalytic asymmetric synthesis, instead of using approaches which are based on chiral auxiliaries.
Therefore we decided to develop a new methodology for the asymmetric preparation of β-hydroxy-α-amino acids and do scientific collaboration with the group of professor Darren J. Dixon (Manchester University), which is specialized in the area of asymmetric catalysis and could provide the required “know how” to face our objective.
This thesis discusses both concepts presented in this section and is divided into two parts. First part introduces a new strategy for the preparation of morpholines strating from β-hydroxy-α-amino acids which is described together with an application of morpholine-3-carboxylic acid as β-turn inducer. The second part discusses the development of a new catalytic system for the preparation of optically active β-hydroxy-α-amino acids.

Abstract

This thesis is divided into two parts. The first part describes the development and the application of a new methodology for the synthesis of morpholines starting from β-amino alcohols. In particular the focus is on the construction of diversified morpholine-3-carbixylic acids (**F** and **H**), which can be obtained using β-hydroxy-α-amino acids as precursors. This methodology is based on an acid catalysed transactelysation of a linear precursor (**A**) and a subsequent elimination of MeOH, in order to obtain dihydroxazine scaffolds **B** that can be further functionalyzed leading to diversified, biologiacally relevant morpholines.



In Chapter 2 the dihydroxazine scaffolds are reduced by means of a catalytic hydogenation, providing an easy access to Fmoc-protected morpholine-3-carboxylic acids **F**, unnatural cyclic amino acids used in poptidomimetics and drug design. The synthesis proved particularly efficient, providing access to the target products in less steps and better yields than previously reported approaches.



C

R=H, Me

D

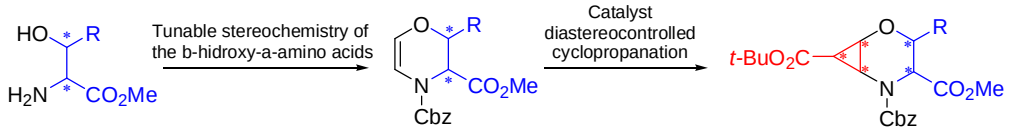
E

F

For R = H
74% yield
ee > 96%

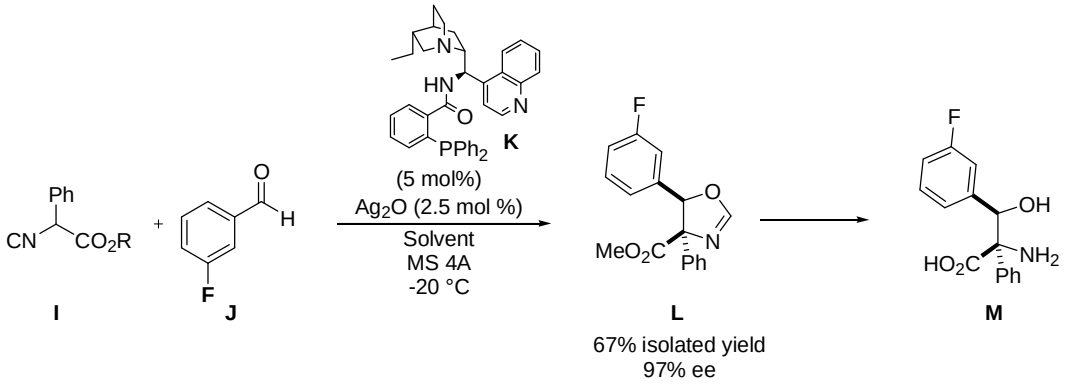
In Chapter 3, it is fully described how the previously synthesised Fmoc-morpholine-3-carboxylic acid **F** has been inserted into model tetrapeptide sequences, in order to study the attitude of acting as a proline surrogate. The peptides conformation has been studied by means of NMR, IR and molecular modeling techniques and the tendency to nucleate into folded structures such as β-turns has also been investigated.

Chapter 4 describes the diastereoselective cyclopropanation of dihydroxazines with diazo-acetates to access geometrically diversified and orthogonally protected scaffolds to be used in peptidomimetics and combinatorial chemistry (**H**). The stereochemical diversity is obtained starting from β-hydroxy-α-amino acids with different configuration and also by means of a transition metal-catalyzed cyclopropanation step, where the stereochemical outcome is governed by PyBOX chiral ligands.



.Introduction of conformational costrain
.Introduction of 3 stereocenters
.Inroduction of a third diversification site

The second part of the thesis is focused on the development of a new synthetic strategy to access enantiopure β-hydroxy-α-amino acids, the starting materials used for the preparation of dihydroxazine scaffolds. A reliable source of these compounds guarantees the possibility of further increase the diversity of the morpholine platforms introduced in the first part. In order to develop a new methodology for the preparation of β-hydroxy-α-amino acids, it was decided to exploit a catalytic, enantioselective version of the aldol reaction of α-isocyanoacetates. The products of this reaction are oxazolines **L**, which are a protected form of β-hydroxy-α-amino acids. Chapter 6 describes the design and the synthesis of a small library of amino-phosphines based on cinchona alkaloid-derived scaffolds. The synthesised ligands are then screened for the enantio- and diastereoselective addtion of α-isocyanoaacetates to aldehydes, in the presence of different transition metals. It is found that ligand **K** is particularly effective in the control of enantio- and diastereo-selectivity, providing the desired products with ee up to 97%.



The new catalytic system is also efficient in the presence of α-substituted isocyanides **J**, thus allowing the simultaneous creation of a quaternary and a tertiary stereogenic center next to each other. Finally, an efficient hydrolysis procedure for the conversion of oxazolines to the corresponding β-hydroxy-α-amino acids is disclosed.

Acknowledgments

First I wish to remember the people that gave me the possibility of working and made all this possible, through funding, teaching, supervising. So I really need to acknowledge Professor Antonio Guarna and Dr Andrea Trabocchi and Dr Darren J. Dixon.
Second I want to mention the people I have met and I have been working together in these three years, many of which are now good friends. Among all I remember, Claudia Lalli, Nicoletta Cini Massimo Rolla and all members of the Guarna group. I also like to thank all the members of the Dixon group in Manchester. It is a pleasure for me to remember the nice time I had with Pavol, Wolfgang, Alessandro, Ting, Kevin, Dane, Kath, Caroline, Chloe, Tom, Michael, John, Adam, Karen, Mei-Ling and Haifei.

Abbreviations

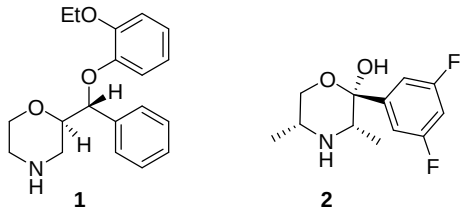
°C	degrees Celsius
Å	ångström
Ac	acetyl
Ar	aromatic group, not phenyl
Atm.	standard atmosphere
Bn	benzyl
Boc	Di-tert-butyl dicarbonate
Bs	broad signal
Bu	butyl
C	cyclo / concentration
Cat	catalyst
Cbz	Carboxybenzyl
Conv	conversion
COSY	correlation spectroscopy
D	doublet
Dd	doublet of doublets
De	diastereomeric excess
DFT	density functional theory
DIAD	diisopropyl azodicarboxylate
DKR	dynamic kinetic resolution
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DPPA	diphenylphosphoryl azide
Dr	diastereomeric ratio
EDG	electron donating group
Ee	enantiomeric excess
eq.	equivalents
ESI	electrospray ionisation
Et	ethyl
EWG	electron withdrawing group
Fmoc	9H-(f)luoren-9-yl(m)eth(o)xy(c)arbonyl
H	hour(s)
HPLC	high performance liquid chromatography
HRMS	high-resolution mass spectrometry
Hz	hertz
I	iso
IPA	isopropylalcohol
IR	infrared
M	molar
m	meta
m	multiplet
m/z	mass-to-charge ratio
Me	methyl

Mg	milligram
MHz	megahertz
Min	minute
mL	millilitre
µL	microlitre
mmol	millimole
Mol	mole
MP	melting point
MS	molecular sieves / mass spectrometry
n	normal, straight chain
nm	nanometre
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
Nu	nucleophile
o	ortho
p	para
Ph	phenyl
Pr	propyl
ppm	parts per million
q	quartet
quat	quaternary
R	any alkyl group
Ref	reference
rt	room temperature
s	singlet
t	retention time (HPLC) / triplet (NMR) / tertiary
TBME	tert-butyl methyl ether
TBS	tertiary butyldimethylsilyl
Temp	temperature
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
Ts	p-toluenesulfonyl
p-TsOH	toluene sulfonic acid

1 Introduction to part I: carbon-substituted morpholines

1.1 Biological Relevance of Morpholines

Carbon-functionalized morpholines find a wide application as constituent of many pharmacological relevant compounds and are also a relatively common moiety among natural products. Many morpholine-based structures are under development or are currently marketed for treatment of various diseases.
Reboxetine **1** is an antidepressant drug used in the treatment of clinical depression, panic disorder and attention-deficit hyperactivity disorder, acting as a a norepinephrine reuptake inhibitor.[1] Its mesylate salt is sold under different tradenames including Edronax, Norebox, Prolift, Solvex, Davedax or Vestra.



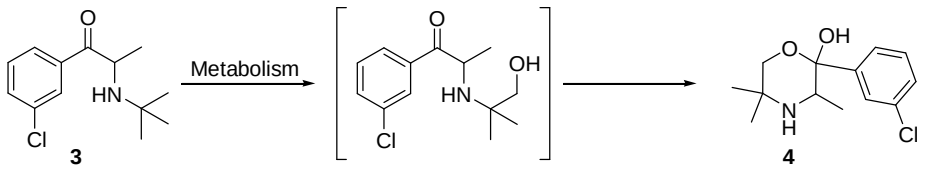
1
Reboxetine

2

Figure 1.1

Racemic bupropion **3** has been used in the treatment of depression and has been approved as an aid in smoking cessation treatment. It is believed that its biological activity is related to an oxidized form (morpholine **4**) which is formed when bupropion is metabolized.

Scheme 1.1 Bupropion mechanism of action



3
Bupropion

4

On the basis of this work, many analogues of morpholine **4** have been synthesized and compound **2** was identified as novel antidepressant and selective inhibitor of

[1] Hajos, M.; Fleishaker, J. C.; Filipiak-Reisner, J. K.; Brown, M. T.; Wong, E. H. F., *CNS Drug Reviews*, **2004**, 10, 23-44 and references therein.

noerepinephrine uptake.[2] Morpholines are also found as many weight loss pescriptions. One example is the widely prescribed phendimetrazine **6** which is metabolized as phenmetrazine **7**.[3]

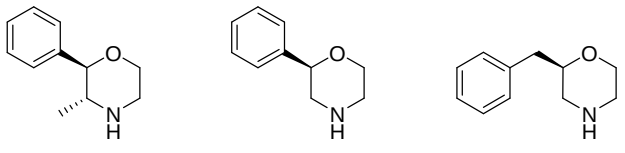


Figure 1.2

2-Benzyl-morpholine **7** is another appetite suppressant. Tests on animals proved that the active enatiomer is the one having positive optical rotation, which was then assigned as the R-enantiomer by D'Arrigo and co-workers.[4]

From structure activity research, the orally active compound **8** was found to have potent antibacterial activity against Pseudomonas aeruginosa, and Helicobacter pylori,[5] the primary cause of chronic gastritis and highly associated with peptic ulcer.[6] The S-enantiomer proved to be thirty times more active then the corresponding R-enantiomer. Further studies on the same scaffold led to the development of Levofloxacin **9**, a third-generation fluoroquinolone antibiotic, marketed by Ortho-McNeil under the trade name Levaquin in the United States. In Europe, it is marketed by Sanofi-Aventis under the trade name Tavanic. Levofloxacin is one of the so-called respiratory quinolones, which are effective against a number of Gram-positive and Gram-negative bacteria and, specifically, against the organisms that cause atypical pneumonia. Because of its broad spectrum of action, levofloxacin is frequently prescribed empirically for a wide range of infections (such as pneumonia and urinary tract infection) before the causal organism is known.

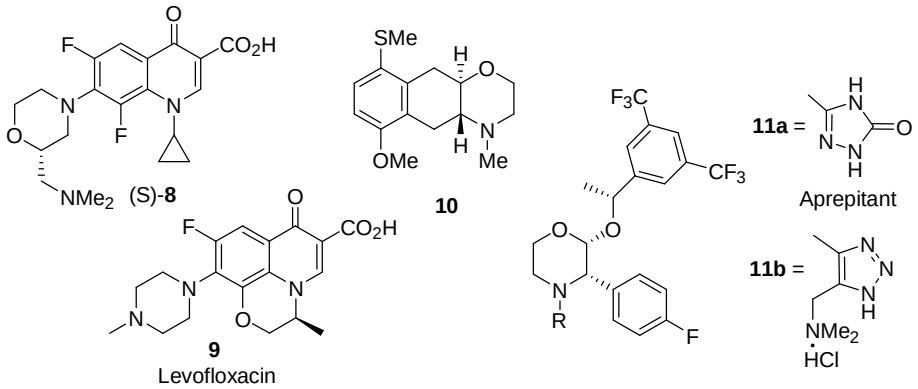


Figure 1.3

[2] Kelley, J.L.; Musso, D.L.; Boswell, G.E.; Soroko, F.S.; Cooper, B.R., *J. Med. Chem.* **1996,** *39,* 347-349.
[3] Rothman, R.B.; Katsnelson, M.; Vu, N.; Partilla, J.S.; Dersch, C.M.; Blough, B.E.; Baumann, M.H. *Eur. J. Pharmacol.* **2002,** 51.
[4] D'Arrigo, P.; Lattanzio, M.; Fantoni, G.P.; Servi, S.; *Tetrahedron: Asymmetry* **1998**, 9, 4021-4026.
[5] Araki, k.; Kuroda, t.; Uemori, S.; Moriguchi, A.; Ikeda, Y.; Hirayama, F.; Yokoyama, Y.; Iwao, E.; Yakushiji, T. *J. Med. Chem.* **1993**, 36, 1356-1363.
[6] Sakurai, N.; Sano, M.; Hirayama, F.; Kuroda, T.; Uemori, S.; Moriguchi, A.; Yamamoto, K.; Ikeda, Y.; Kawakita, T. Bioorg. Med. Chem. Lett. **1998,** 8, 2185-2190.

Centrally acting α_1-agonist may be of therapeutic value in dementia and other CNS disorders characterized by symptoms of noradrenergic insufficiency. Compound **10** was found to be a potent selective α_1-agonist in a study conducted by Sandoz Ltd.[7]
In a study targeted to discover new neurokinin-1 receptor antagonist, Merck laboratories identified the series of morpholine-based structures **11**.[8] Compound **11a** was found to be a orally active, water-soluble neurokinin-1 receptor antagonist and is currently marketed under the trade name of Aprepitant.
Kim and co-workers synthesized many analogs of Camptothecin **12**, a natural compound isolated from the Chinese tree Camptotheca Acuminata, which was reported to have antitumor activity. The morpholine analogue **13** showed promising in vitro antitumor activity.[9]

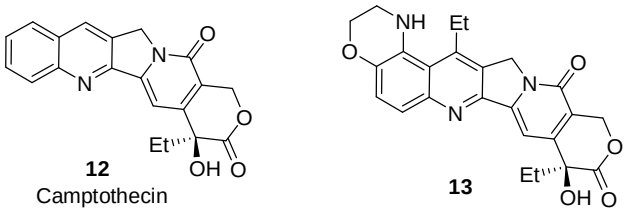


Figure 1.4 Camptothecin and its synthetic analogue **13**

Many morpholine-derivatives were found to have antioxidant activity. The Morpholine-derivatives of lipoic acid illustrated in figure 1.5 were found to have enhanced antioxidant activity when compared to lipoic acid itself.[10] Moreover some of these new compounds were able to cross the blood-brain barrier, an important feature for antioxidant candidates, since the brain is the most susceptible organ to oxidative stress.

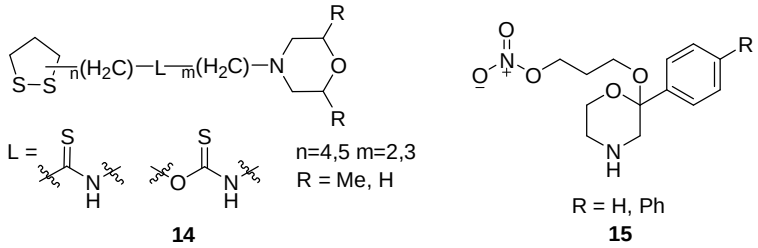


Figure 1.5

The morpholine-acetal **15**, prepared by Chrysellis and co-workers, displayed antioxidant activity and also was able of releasing NO, thing which was considered advantageous for the inhibition of atherogenic mechanisms.[11]

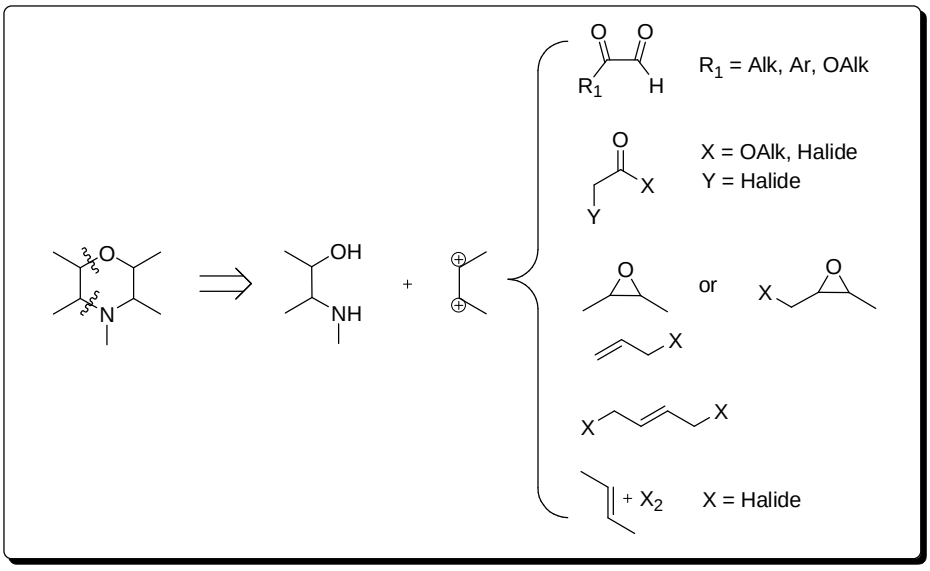
.

[7]Nozulak, J.; Vigouret, J.M.; Jaton, A.L.; Hofmann, A.; Dravid, A.R.; Weber, H.P.; Kalkman, H.; Walkinshaw, M.D. *J. Med. Chem.* **1992,**35,480-489.
[8]Timothy Harrison, T.; Owens, A.P.; Williams, B.J.; Swain, C.J.; Williams, A.; Carlson, E.J.; Rycroft, W.; Tattersall, F.D.; Cascieri, M.A.; Chicchi, G.G.; Sadowski, S.; Rupniak, N.M.J.; Hargreaves, R.J. *J. Med. Chem.* **2001,** 44, 4296-4299.
[9] Kim, D.K.; Ryu, D.H.; Lee, J.Y.; Lee, N.; Kim, Y.W.; Kim, J.S.; Chang, K.; Im, G.J.; Kim, T.K.; Choi, W.S. *J. Med. Chem.* **2001,** *44,* 1594-1602.
[10]Claude Guillonneau, C.; Charton, Y.; Ginot, Y.M.; Fouquier-d'Herouel, M.V.; Bertrand, M.; Lockhart, B.; Lestage, P.; Goldstein, S. *Eur. J. Med. Chem.* **2003**, 38, 1-11.
[11]Chrysellis, M.C.; Rekka, E.A.; Kourounakis, P.N.; *J. Med. Chem.* **2000**, 43, 609-612.

1.2 Synthesis of Morpholines

Many approaches have been reported so far. In this section the most significant strategies for the synthesis of the morpholine-ring will be reviewed and illustrated through the presentation of some selected significant examples. The focus will be on the key reactions used to create the morpholine ring and not on further chemical modifications made once the morpholine ring itself has been formed.
Due to the presence of the two heteroatoms, the morpholine ring suggests obvious disconnections between the α-carbons and the heteroatoms. The most of these disconnections are based on the nucleophilic character of nitrogen and oxygen, which can easily cyclize on electrophilic carbons and form the heterocyclic ring. So the most evident disconnection is that shown in scheme 1.2, where the morpholine ring is derived from an amino alcohol and a 1,2-dielectrophile. Many synthetic equivalents are available and have been applied as 1,2-dielectrophile. The most common of them are illustrated in scheme 1.2.

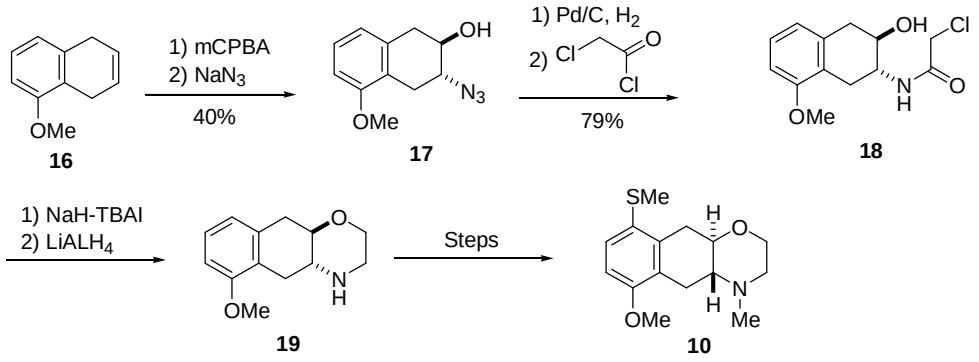
Scheme 1.2



Usually the ring formation consists of two steps and the two electrophilic centers are differentiated. Many times the 1,2-dielectrophile presents explicitly the two electrophilic centers when it is reacted with the amino alcohol and none of the two is in a “masked form”. This is the case, for example, of the use of chloroacetyl chloride. This is a very common strategy and is illustrated by the preparation of compound **10.**[12]

[12] Nozulak, J.; Vigouret, J.M.; Jaton, A.L.; Hofmann, A.; Dravid, A.R.; Weber, H.P.; Kalkman, H.; Walkinshaw, M.D. *J. Med. Chem.* **1992,**35,480-489.

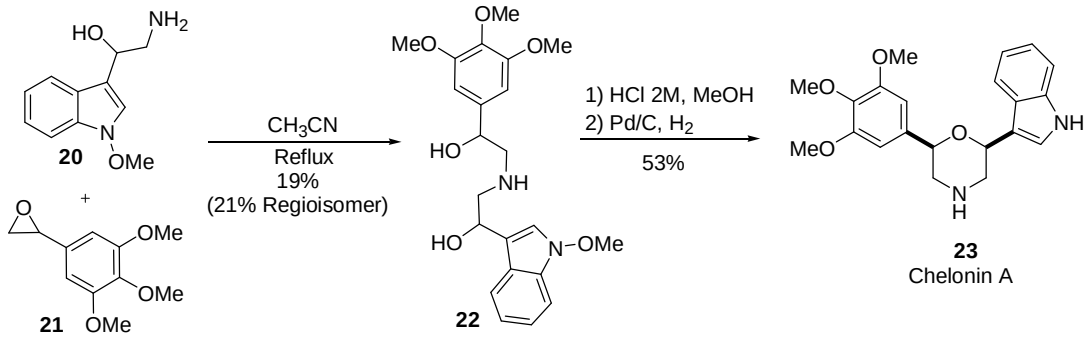
Scheme 1.2 Use of chloroacteyl chloride for the synthesis of morpholines



Other electrophiles that act in a similar way to chloroacetil chloride are the α-halo-esters, α-halo-ketons/aldehydes etc.

In some other cases one of the two electrophilic centers is masked or requires a further activation in order to create to final ring. This is the case of the use of epoxides in the reaction with amino alcohol. Once the epoxide has reacted with the amine, the cyclization is usually induced by a further activation of one of the two alcoholic groups. The kind of activation, in this case, depends on the nature of the two hydroxyl groups. If one of them is in a particularly reactive position (for example is a benzylic alcohol such in Scheme 1.3[13]) acid treatment and/or heating is enough to obtain the cyclization. If the hydroxyl group is not activated, common cyclization strategies are the use of Mitsunobu conditions[14] or the use of mesyl/tosyl chloride to activate one of the alcohols followed by treatment with a base.[15]

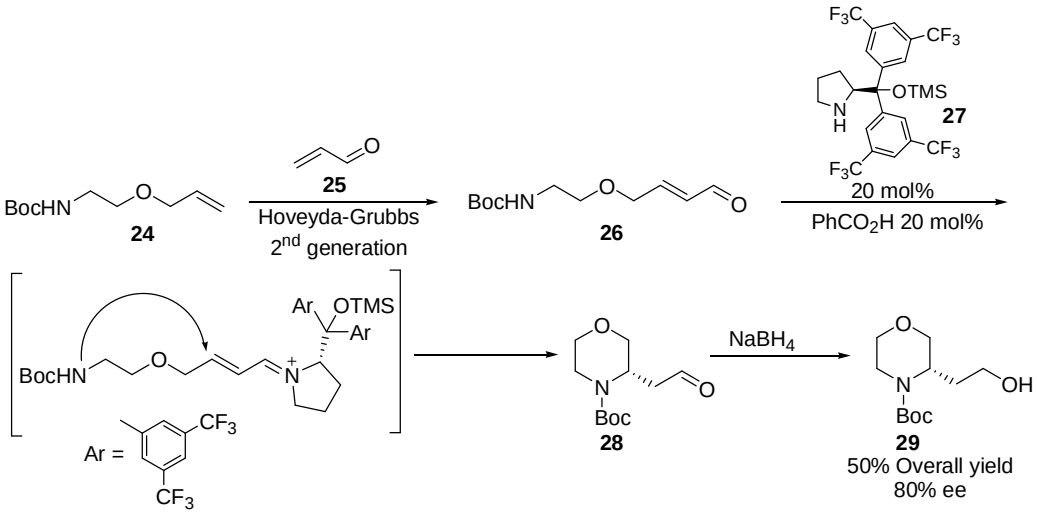
Scheme 1.3



Also when allyl halides are used, the first electrophilic center is readily reacted with the amino alcohol and then the alkene requires a further activation, unless it is not a Michael acceptor[16] as in scheme 1.4

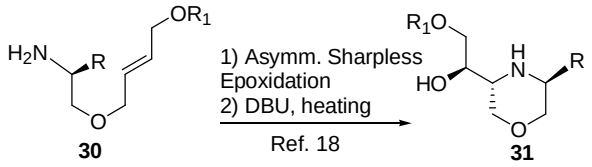
[13] Somei, M.; Aoki, K.; Nagaham, Y.; Nakagawa, K.; *Heterocycles*, **1995**, 41, 5-8.
[14] Nishi, T.; Ishibashi, K.; Takemoto, T.; Nakajima, K.; Fukazawa, T.; Iio, Y.; Itoh, K.; Mukayama, O.; Yamaguchi, T. *Biorg. Med. Chem. Lett.* **2000**, 10, 1665.
[15] Lanman, B.A.; Myers, A.G. *Org. Lett.* **2004**, 6, 1045-1047.
[16] Fustero, S.; Jimenez, D.; Moscardo', J.; Catalàn, S.; Del Pozo, C. *Org. Lett.*, **2007**, 5283-5286.

Scheme 1.4 Oranocatalytic, enantioselective synthesis of morpholine ring through intramolecular aza-Michael reaction. The olefin is activated by imminium ion catalysis.

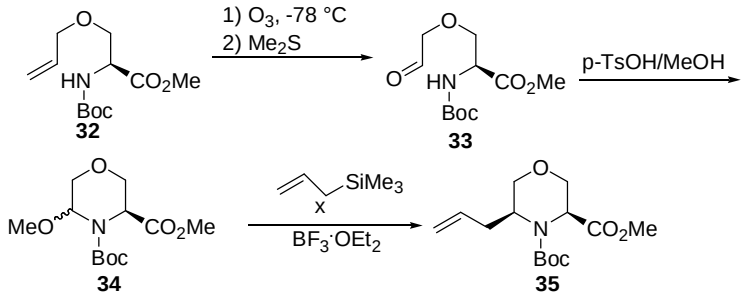


The second electrophilic activation of the alkene can be obtained with different methods. Common are the use of transition metal catalysts, reaction with bromine/iodine,[17] epoxidation[18] (Scheme 1.5), oxidation of the alkene to aldehyde (scheme 1.6).[19]

Scheme 1.5 Sharpless Asymmetric Epoxidation strategy for the morpholine ring closure.



Scheme 1.6 Cyclization through alkene ozonolysis and N-acyl-iminium functionalization

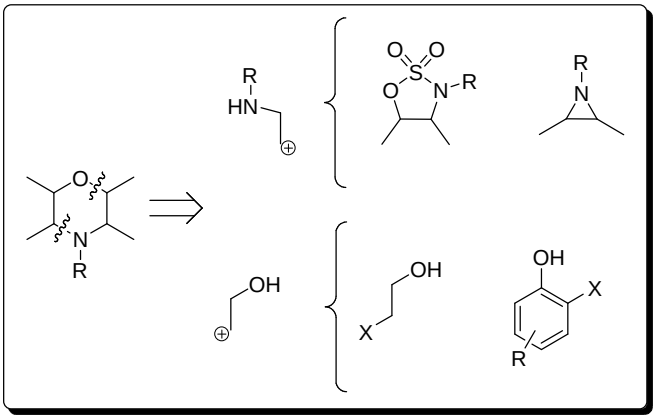


A second possible disconnection is breaking between atoms 1-2 and 3-4, as shown in scheme 1.7. This second possibility is less common compared to the previously reported but is a valuable alternative. The major drawback is the availability of synthetic

[17] D'hooghe, M.; Vanlangendonck, T.; Tornroos, K.W.; De Kimpe, N. *J. Org. Chem.* **2006**, 71, 4678-4681.
[18] Kozlowski M.C.; Bartkett, P.A. *J. Org. Chem.* **1996**, 61, 7681-7696.
[19] Steven V. O'Neil, S.V.; Wang, Y.; Laufersweiler, M.C.; Oppong, K.A.; Soper, D.L.; Wos, J.A.; Ellis, C.D.; Baize, M.W.; Bosch, G.K.; Fancher, A.N.; Lu, W.; Suchanek, M.K.; Wang, R.L.; De, B.; and Demuth, Jr. J.P. *Biorg. Med. Chem. Lett.* **2005**, 5434-5438.

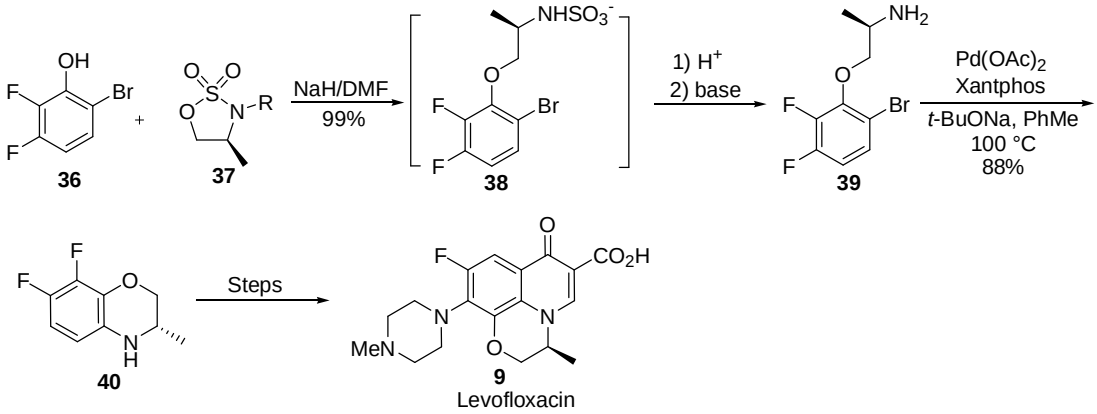
equivalents. The nitrogen containing synthons are usually represented by an aziridine[20] or a cyclic sulfamidate which often needs to be prepared from an independent route.

Scheme 1.7



A recent example of this disconnection is based on a regiospecific nucleophilic cleavage of enantiopure sulfamidates with 2-bromo-phenols (cleavage occurs on the carbon next to oxygen) and a subsequent Pd(0) mediated amination to provide the final morpholine ring. The strategy has been applied to the synthesis of the blockbuster antibiotic Levofloxacin and is illustrated in scheme 1.8.[21]

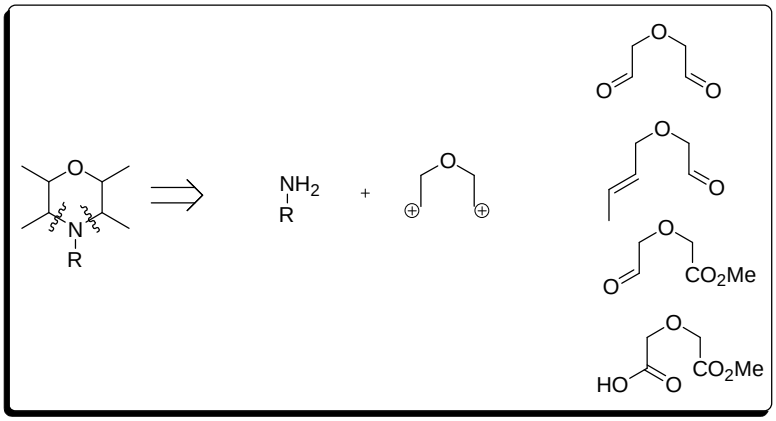
Scheme 1.8 Application of sulfamidates to the synthesis of Levofloxacin



A third possible disconnection is the cleavage between the nitrogen and the two α-carbons. This cleavage has been used mainly to prepare morpholines from furanosides[22] and it has been proved particularly suitable for the preparation of morpholines bearing stereogenic centers next to the oxygen, as illustrated in scheme 1.10.

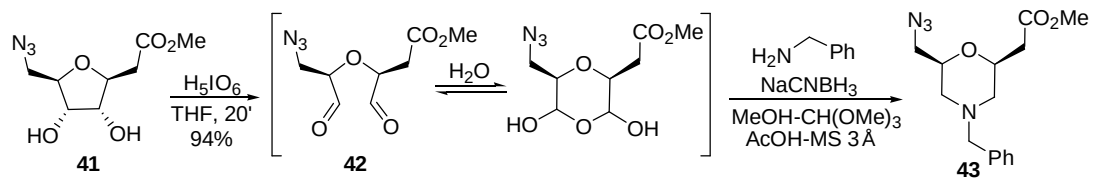
[20] Kogami Y., Okawa K., *Bull. Chem. Soc. Jpn.* **1987**, 2963-2965.
[21] Bower, J.; Szeto, P.; Gallagher, T. *Org. Lett.* **2007**, 3283-3286.
[22] Grotenbreg, G.M.; Christina, A.E.; Buizert, A.E.M.; Van der Marel, G.A.; Overkleeft, H.S.; Overhand, M. *J. Org. Chem.* **2004**, 69, 8331-8339.

Scheme 1.9



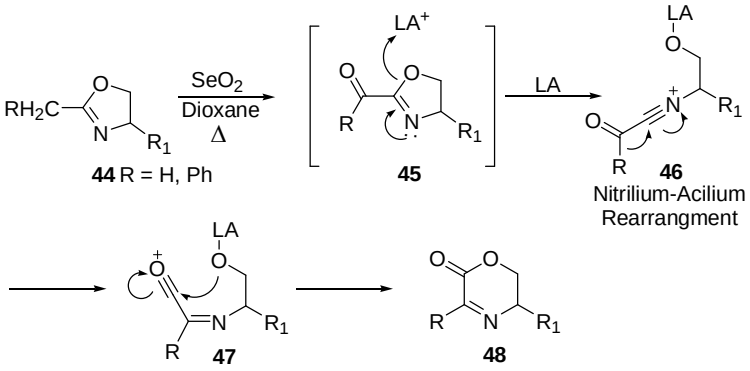
In this case an cleavage of the cis diols with H_5IO_6 is used to obtain the dialdehyde **42**, which is then converted into the morpholine **43** using a double reductive amination with a primary amine.

Scheme 1.10



Finally some non-conventional disconnections will be briefly reviewed. Many of these strategies are based on rearrangements or multicomponent reactions. Molinski reported an oxidative rearrangement of oxazoline to morpholine-2-ones, induced by SeO_2. The proposed reaction mechanism is illustrated in scheme 1.11 and it is supposed to proceed through a first oxidation of the oxazoline α-carbon to the corresponding aldehyde that in presence of the Lewis acid gives rise to a "nitrilium to acylium" rearrangement that originates the morphilone-2-one **48**.[23]

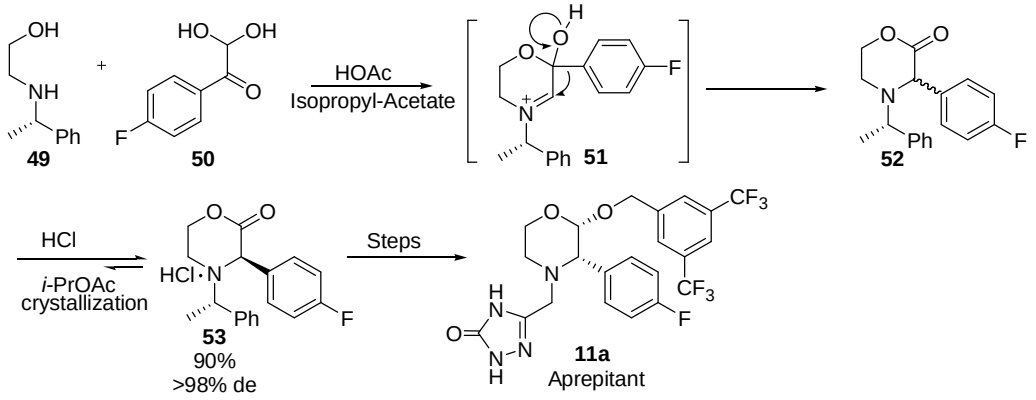
Scheme 1.11 Oxazoline to morpholine-2-one rearrangement in the presence of SeO_2



[23] Shafer, C. M.; Molinski, T. F. *J. Org. Chem.* **1995**, 61, 2044-2050.

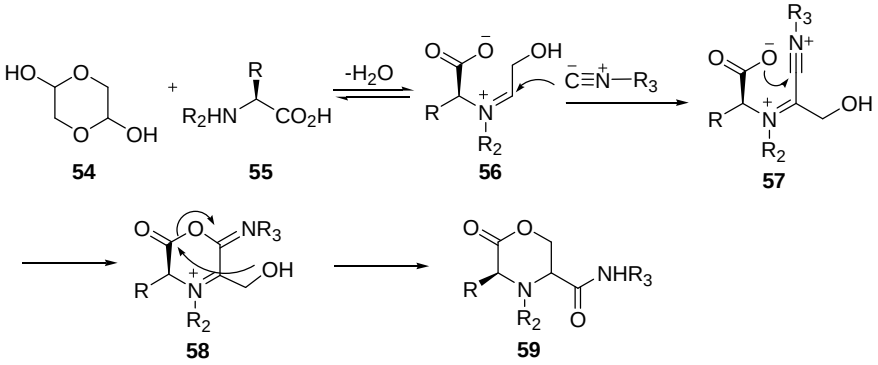
A similar rearrangement has been reported by Agami[24] and has been applied to the synthesis of aprepitant by Merck.[25] Both papers report the condensation of phenylglyoxals with amino alcohol. The reaction proceeds through the formation of a hemiacetal that rearranges to morpholinone species, most presumably by the mechanism reported below, where a phenyl cation migrates from the acetal carbon to the adjacent iminium moiety.

Scheme 1.12



Recently the group of Kim described an approach to morpholine-2-one derivatives based on a multicomponent Ugi reaction using glycoladehyde dimer, amino acids and an isocyanide.[26]

Scheme 1.13 Ugi 5-centers-3-components reaction to access morpholine-2-one derivatives.

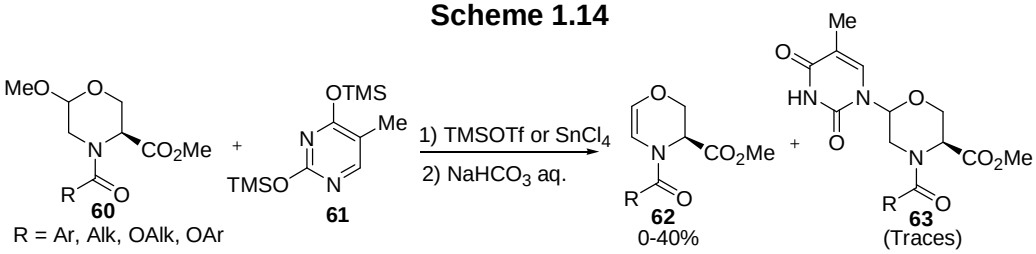


[24] Agami, C.; Couty, F.; Prince, B.; Venier, O. *Tetrahedron. Lett.***1993**, 34, 7061-7062.
[25] Zhao, M.M. McNamara, J.M.; Ho, G.J.; Emerson, K.M.; Song, Z.J.; Tschaen, D.M.; Brands, K.M.J.; Dolling, U.H.; Grabowsky, E.J.J.; Reider, P. *J. Org. Chem.* **2002**, 67, 6743-6747.
[26] Kim, Y.B.; Choi, E.H.; Keum, G.; Kang, S.B.; Lee, D.h.; Koh, H.Y.; Kim, Y. *Org. Lett.* **2001**, 3, 4149-4152.

1.3 Aims & Concept

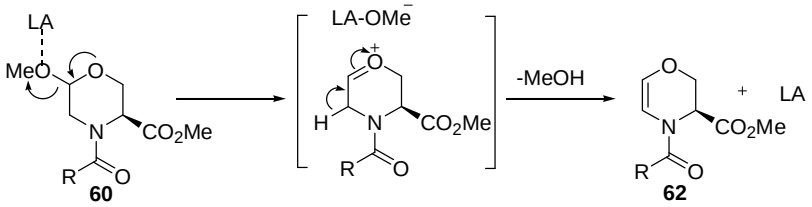
The first part of this thesis work will describe a new approach to the construction of the morpholine ring. This strategy is briefly summarized and introduced in this section and then it will be illustrated and discussed in detail in the next two chapters.
The starting point for the development of a new strategy for the synthesis of enantiopure morpholines was the isolation of an elimination by-product (**62**), during some reactivity studies involving morpholine-acetals of the kind reported below (**60**).
During these studies, an activation of the acetal with Lewis acids was attempted, in order to obtain a condensation product in the presence of the protected thymine **61**.

Scheme 1.14



Unfortunately all attempts to convert acetal **60** to the desired product were unfruitful or very low yielding. Anyway, from the crude reaction mixture it was evident the presence of dihydroxazine **62**. Compound was also isolated in low to moderate yields. The formation of the dihydroxazine occurred presumably through the mechanism reported in scheme 1.15. In a first step the methyl-acetal is cohordinated by the lewis acid and the resultant cation does not react with the nucleophile but readily rearranges to the stable dihydroxazine **62**.

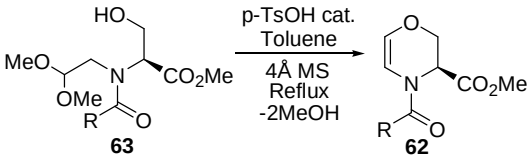
Scheme 1.15 Mechanism for the Lewis acid catalysed formation of dihydroxazine **62**



Dihydoxazines **62** are attractive scaffolds because of the presence of the electron-rich olefin which can be used as a reactive intermediate, in order to access a wide range of medicinally relevant structures and many cyclic unnatural amino acids. We decided to consider the formation of dihydroxazines as a starting point and we planned to optimise the reaction conditions in order to obtain the desired scaffolds in higher yield.
A quick conditions screen took us to identify the use of a catalytic amount of p-TsOH as the ideal acid to obtain the elimination of MeOH. Heating in toluene was beneficial, in order to remove methanol from the reaction mixture and drive the equilibrium towards dihydroxazines **62**. Finally, the use of 4Å molecular sieves to trap MeOH and a Dean Stark apparatus allowed reaching yields higher then 80%.
In a second optimisation stage we considered that the acetal precursor **60** was also formed in an acid catalysed trans-acetalysation and we envisaged the possibility of carrying both steps (trans-acetalysation, MeOH elimination) together. This intuition proved

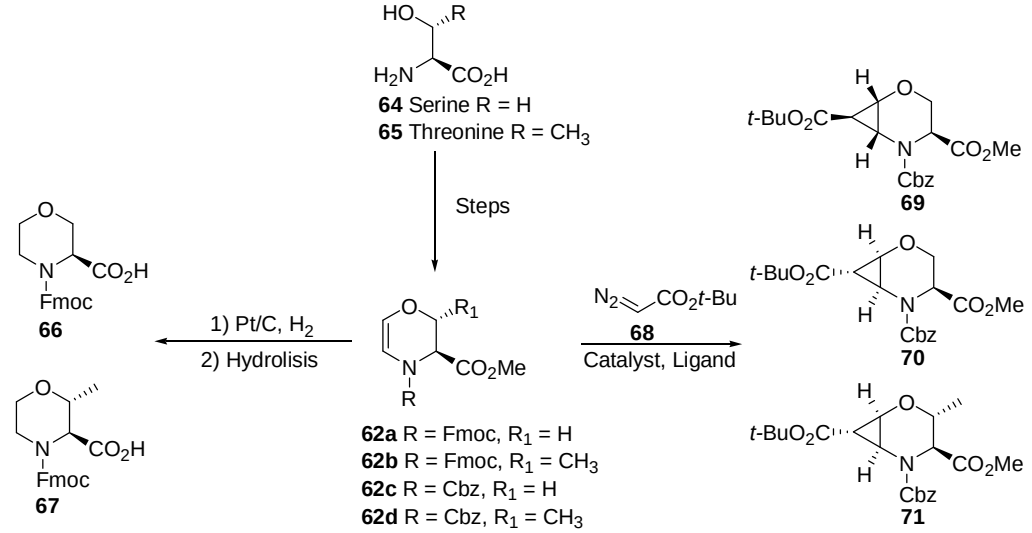
feasible and allowed the synthesis of dihydroxazines **62** from linear precursors **63** in a single step by simply refluxing in toluene in the presence of a catalytic amount of p-TsOH.

Scheme 1.16



In chapters 2 and 4 the development and the application of the new strategy will be discussed. According to the way the double bond of the dihydroxazine structure is transformed, a various number of morpholine-derivatives can be obtained.
In the next chapter a simple catalytic reduction of the double bond is considered, in order to obtain an easy, high yielding access to the pharmacologically relevant Fmoc-3-morpholine carboxylic acid. In chapter 4, the diastereoselective cyclopropanation of the double bond with diazo-acetates is explored, in order to access new, geometrically diversified and orthogonally protected scaffolds to be applied as peptidomimetics and templates for combinatorial synthesis.

Scheme 1.17 Some applications of the new strategy to access the morpholine ring

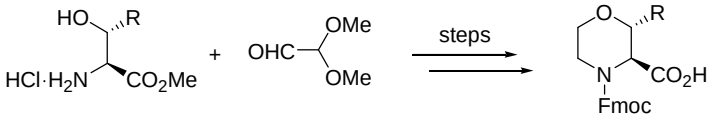


In chapter 3, the previously synthesised Fmoc-3-morpholin-carboxylic acid is included, by means of solid phase synthesis, in the middle of two model tetrapeptides in order to study its attitude to act as a β-turn inducer. The conformational folding of the two tetrapeptides is studied with NMR techniques, IR and molecular modelling.

2 Fmoc-Morpholine-3-Carboxylic Acids from Serine and Threonine

"Sladojevich, F.; Trabocchi, A.; Guarna, A.
Journal of Organic Chemistry, **2007**, 72, 4254-4257"
&
"Guarna, A.; Trabocchi, A.; Menchi, G.; Lalli, C.; Sladojevich, F.; Cini, N.
WO Patent 2008129004, 2008"

ABSTRACT



R=H, Me

For R = H
74% yield
ee > 96%

Enantiopure Fmoc-protected morpholine-3-carboxylic acid was synthesized from dimethoxyacetaldehyde and serine methyl ester through a short and practical synthetic route. The preparation consisted of a five-step process based on reductive amination, intramolecular acetalization and concomitant elimination of the anomeric methoxy substituent, followed by hydrogenation of the double bond and final acidic ester hydrolysis. The optical purity of both enantiomers of the title amino acid was demonstrated by HPLC analysis of the corresponding amide derivatives obtained from coupling with chiral (*S*)-(–)-1-phenyl-ethylamine. Moreover, the synthesis of a model tripeptide showed full compatibility of the title Fmoc-amino acid with Solid-Phase Peptide Synthesis, thus allowing the application of Fmoc-morpholine-3-carboxylic acid in peptidomimetic chemistry on solid-phase. The new synthetic approach was then extended to the synthesis of Fmoc protected 2-methyl-morpholine-3-carboxylic acid using threonine methyl ester in place of serine as starting material.

2.1 Introduction

Over the years the synthesis and applications of cyclic amino acids has attracted considerable attention from synthetic and medicinal chemists, especially in the area of peptidomimetics.[27] The incorporation of cyclic secondary amino acids[28] has profound effects on the conformation of peptides, due to the inability of the nitrogen atom to act as a hydrogen bond donor unless it is located at the *N*-terminal position of the molecule, and to the conformational strain imparted by the cyclic structure. Also, *cis*/*trans* isomerism of the tertiary amide bond formed by cyclic amino acids is responsible for the modulation of the conformational preferences. Cyclic secondary amino acids have been applied in several biological issues, and their incorporation into bioactive peptides has been reported over the years.[29] In particular, morpholine-3-carboxylic acid has been used to synthesize several bioactive molecules, such as TACE,[30] MMP and TNF inhibitors,[31] and a potent orally active VLA-4 antagonist.[32] Also, it has been included in the core structure of tricyclic benzodiazepines,[33] 6-methylidene penems as -lactamase inhibitors,[34] *β*-carbolines as IKK-2 inhibitors,[35] 8,6-fused bicyclic peptidomimetic compounds as interleukin-1*β* converting enzyme inhibitors,[36] and in the structure of benzoxazepines as stimulators of AMPA receptor.[37]

[27] (a) Giannis, A.; Kolter, T. *Angew. Chem. Int. Ed. Engl*. **1993**, *32*, 1244-1267. (b) Gante, J. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699-1720. (c) Gibson, S. E.; Thomas, N.; Guillo, N.; Tozer, M. J. *Tetrahedron* **1999**, *55*, 585-615.

[28] Hruby, V. J.; Al-Obeidi, F.; Kazmierski, W. *Biochem. J.* **1990**, *268*, 249-262.

[29] Goodman, M.; Ro, S. In *Burger's Medicinal Chemistry and Drug Design, Vol. 1*; Wolff, M. E., Ed.; Wiley: New York, 1995.

[30] Levin, J. I.; Chen, J. M.; Laakso, L. M.; Du, M.; Du, X.; Venkatesan, A. M.; Sandanayaka, V. ; Zask, A.; Xu, J.; Xu, W.; Zhang, Y.; Skotnicki, J. S. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 4345-4349.

[31] (a) Almstead, N. G.; Bradley, R. S.; Pikul, S.; De, B.; Natchus, M. G.; Taiwo, Y. O.; Gu, F.; Williams, L. E.; Hynd, B. A.; Janusz, M. J.; Dunaway, C. M.; Mieling, G. E. *J. Med. Chem*. **1999**, *42*, 4547-4562. (b) Piscopio, A. D.; Rizzi, J. P. WO 96/33172, October 24, 1996.

[32] Chiba, J.; Machinaga, N.; Takashi, T.; Ejima, A.; Takayama, G.; Yokoyama, M.; Nakayama, A.; Baldwin, J. J.; McDonald, E.; Saionz, K. W.; Swanson, R.; Hussain, Z.; Wong, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 41-45.

[33] Matthews, J. M.; Dyatkin, A. B.; Evangelisto, M.; Gauthier, D. A.; Hecker, L. R.; Hoekstra, W. J.; Liu, F.; Poulter, B. L.; Sorgi, K. L.; Maryanoff, B. E. *Tetrahedron: Asymmetry* **2004**, *15*, 1259-1267.

[34] Venkatesan, A. M.; Agarwal, A.; Abe, T.; Ushirogochi, H.; Yamamura, I.; Ado, M.; Tsuyoshi, T.; Dos Santos, O.; Gu, Y.; Sum, F. –W.; Li, Z.; Francisco, G.; Lin, Y. –I.; Petersen, P. J.; Yang, Y.; Kumagai, T.; Weiss, W. J.; Shlaes, D. M.; Knox, J. R.; Mansour, T. S. *J. Med. Chem*. **2006**, *49*, 4623-4637.

[35] (a) Hepperle, M. E.; Liu, J. F.; Soucy, F.; Raman, P.; Little, J. D.; Fleming, P. E.; Reynolds, D.; Harriman, G. C. B. US2005/0239781 A1, October 27, 2005. (b) Hepperle, M. E.; Liu, J. F.; Soucy, F.; Ye, Y.; Murray, R. S.; Prakash, R.; Little, J. D.; Castro, A.; Mazdiyasni, H.; Fleming, P. E.; Reynolds, D. WO2004/092167 A1, October 28, 2004.

[36] O'Neil, S. V.; Wang, Y.; Laufersweiler, M. C.; Oppong, K. A.; Soper, D. L.; Wos, J. A.; Ellis, C. D.; Baize, M. W.; Bosch, G. K.; Fancher, A. N.; Lu, W.; Suchanek, M. K.; Wang, R. L.; De, B.; Demuth, Jr., T. P. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 5434-5438.

[37] Grove, S. J. A.; Zhang, M.; Shahid, M.; WO02/100865 A1, December 19, 2002.

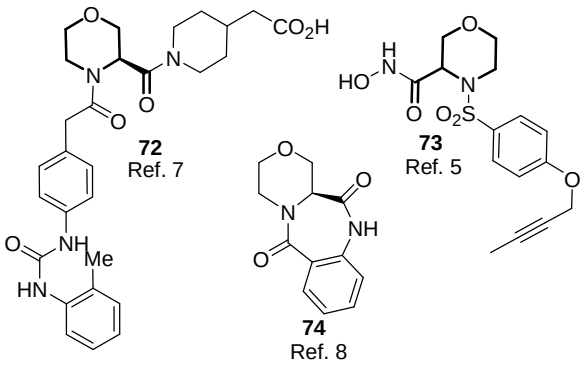


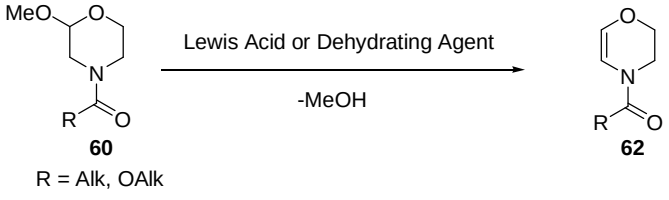
Figure 2.1 Selected examples of bioactive compounds incorporating the morpholine-3-carboxylic acid backbone.

The first syntheses of morpholine-3-carboxylic acid were reported in 1981 by Anteunis et al. as a racemate,[38] and by Brown et al. describing the preparation of the cyclic amino acid as the *N*-benzyl, ethyl ester derivative from serine in 6% overall yield.[39] In the same years, Kogami and Ogawa reported the most convenient synthesis of morpholine-3-carboxylic,[40] which employed chiral benzyl *N*-Cbz-2-aziridine-carboxylate, derived from serine, to give the title amino acid after three steps in 66% overall yield. Recently, Dave and Sasaki reported the synthesis of enantiomerically pure Boc-morpholine-3-carboxylic acid from the corresponding alcohol derivative in 81% yield,[41] which in turn was obtained from a protected serinol derivative in six steps and in 50% overall yield. In addition, an enzymatic synthesis of cyclic amino acids, including morpholine-3-carboxylic acid, has been proposed recently.[42]

2.2 proposed synthesis

During our ongoing research scheme in the field of cyclic acetals derived from amino acids precursors, we observed that morpholine-acetals **60**, with the nitrogen atom protected as amide or carbamate, could undergo elimination of alcohol when treated with Lewis acids or dehydrating agents, to give 1,4-dihydro-oxazine species **62** (Scheme 2.2).

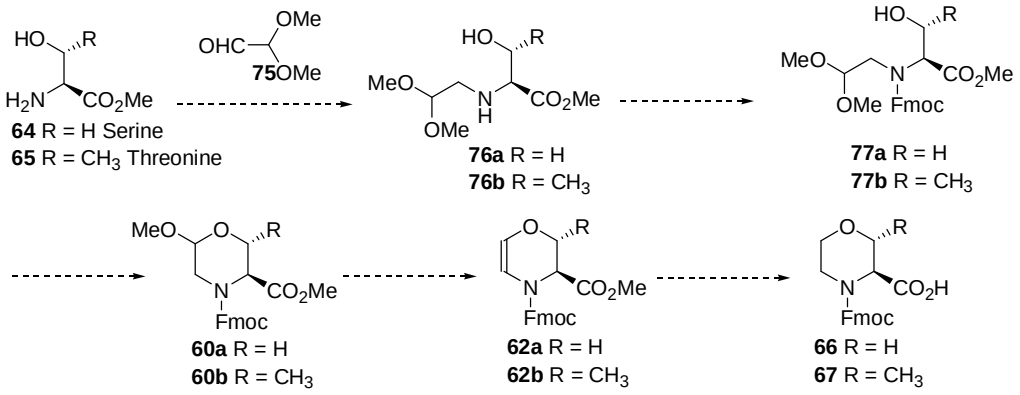
Scheme 2.2: Synthesis of 1,4-dihydro-oxazine from morpholine-acetals.



[38] Asher, V.; Becu, C.; Anteunis, M. J. O.; Callens, R. *Tetrahedron Lett*. **1981**, *22*, 141-144.
[39] Brown, G. R.; Foubister, A. J.; Wright, B. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2577-2580.
[40] Kogami, Y.; Okawa, K. *Bull. Chem. Soc. Jpn*. **1987**, *60*, 2963-2965.
[41] Dave, R.; Sasaki, N. A. *Tetrahedron: Asymmetry* **2006**, *17*, 388-401.
[42] Yasuda, M.; Ueda, M.; Muramatsu, H.; Mihara, H.; Esaki, N. *Tetrahedron: Asymmetry* **2006**, *17*, 1775-1779.

We envisaged the possibility of exploiting this new reactivity in the asymmetric synthesis of Fmoc protected morpholine-3-carboxylic acid **66** and Fmoc protected 2-methyl-morpholine-3-carboxylic acid **68**. Our approach consisted in using serine and threonine methyl esters as precursor of morpholine-acetals **60a** and **60b**, which could then undergo elimination of alcohol to give 1,4-dihydro-oxazines **62**. The latter are then easily converted to the target materials upon hydrogenation of the alkene and hydrolysis of the methyl ester.

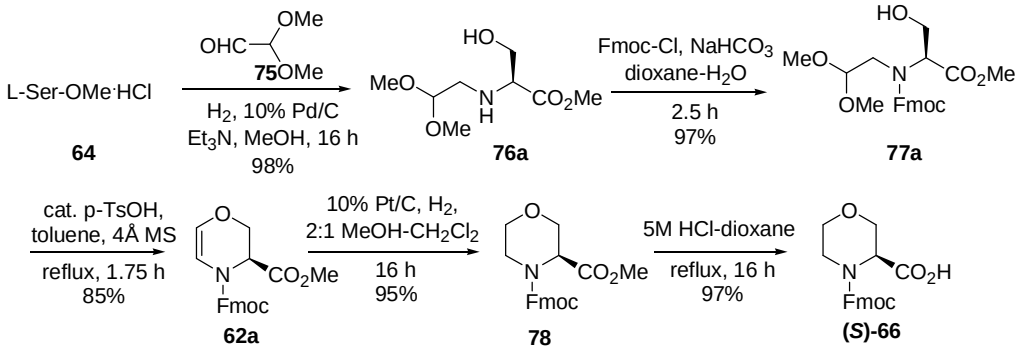
Scheme 2.3: Proposed synthesis of Fmoc-morpholine-3-carboxylic acids **66** and **67**



2.3 Synthesis of Fmoc-morpholine-3-carboxylic acid

L-serine methyl ester hydrochloride was treated with dimethoxyacetaldehyde in a reductive amination step carried out under a hydrogen atmosphere in the presence of catalytic amounts of 5% Pd/C, giving compound **76a** in 50% yield. Optimization of the reaction conditions consisted in the addition of one molar equivalent of triethylamine, thus furnishing clean adduct **76a** in 98% yield after overnight stirring at room temperature (Scheme 2.4).

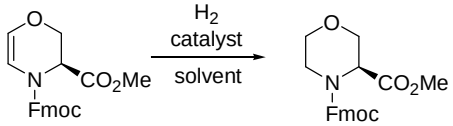
Scheme 2.4



Subsequent amine protection as Fmoc-urethane was achieved using Fmoc-Cl in water-dioxane as solvent system, and in the presence of $NaHCO_3$ as base, giving **77a** in 97% yield. Acid-catalyzed cyclization by acetalization and *in-situ* elimination of the methoxy group was achieved in one-pot, by refluxing compound **77a** in toluene in the presence of

catalytic quantities of *p*-toluenesulfonic acid, thus giving **62a** as a single stereoisomer in 85% yield. When the reaction was carried out without using molecular sieves, we observed a decrease of the yield to 67%, and the reaction time was prolonged to 4 h in order to achieve complete conversion of the starting material. Hydrogenation of the double bond at C-5 and C-6 carbon atoms of **78** was initially attempted by treatment with H_2 and catalytic 10% Pd/C, leading to partial deprotection of the amine function (Table 2.1). The extent of such deprotection was found to be dependent on the choice of the solvent, as when 2:1 MeOH – CH_2Cl_2 was used as the solvent mixture the amount of deprotected amino ester increased, compared to MeOH (Table 2.1, entries 1 and 2). The use of Raney-Ni as catalyst was unsuccessful, as only starting material was recovered from the reaction mixture.

Table 2.1 Optimization of reaction conditions for the double bond hydrogenation of **62a**



62a **78**

Entry	Catalyst	Solvent	Yield
1	10% Pd/C	MeOH	67[a]
2	10% Pd/C	2:1 MeOH-CH_2Cl_2	25[a]
3	Raney Ni	MeOH	-[b]
4	10% Pt/C	MeOH	91
5	10% Pt/C	2:1 MeOH:CH_2Cl_2	95

[a]Partial deprotection of the Fmoc group was observed. [b]Only starting material was recovered.

Finally, 10% Pt/C catalyst gave clean reduction of the double bond and allowed preservation of the amine protecting group. The reaction proceeded in 91% using MeOH as solvent, and upon application of 2:1 MeOH – CH_2Cl_2 as solvent system, the yield was further optimized to 95% (Table 2.1, entry 5). Hydrolysis of the ester function to give the title Fmoc-protected amino acid was carried out in an acidic medium in order to preserve the base-labile Fmoc group. Optimization of the reaction conditions required several attempts in order to achieve high yield. Hydrolysis of **78** in 2:1 acetonitrile – 4M HCl with overnight stirring at room temperature resulted in poor conversion, giving **66** in 15% yield. Replacement of acetonitrile with dioxane, without altering the other reaction conditions, produced **66** in 29% yield. A marked improvement was obtained by raising the reaction temperature. In fact, the hydrolysis was carried out by refluxing the Fmoc-amino ester **78** in a 0.07 M 1:1 dioxane – 4M HCl solution for 2.5 h, giving **66** in 69% yield. Further optimization gave **66** in 97% yield by overnight refluxing a 0.2 M solution of **78** in a 1:1 mixture of 5M HCl – dioxane. Consequently, compound **(*S*)-66** was obtained in five steps and an overall yield of 74%. Also, the synthesis of the corresponding enantiomeric (*R*)-Fmoc-morpholine-3-carboxylic acid [**(*R*)-66**] was carried out starting from D-serine methyl ester, giving the title amino acid in 73% overall yield.

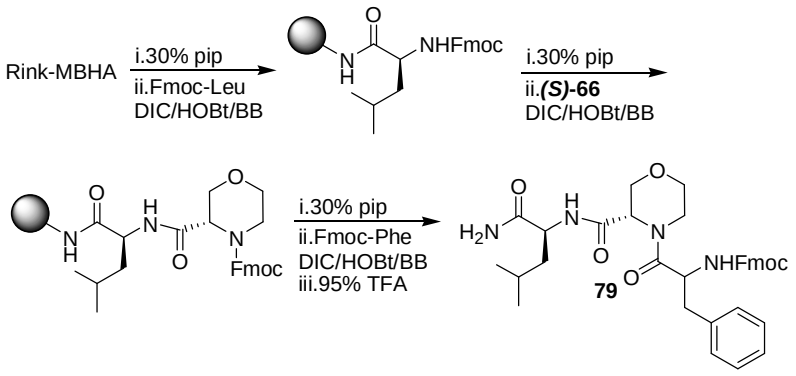
2.4 Enantiomeric purity

Determination of the enantiomeric purity of ***(R)*-66** and ***(S)*-66** was performed by preparing and analyzing the corresponding diastereomeric amides obtained by reaction with a chiral amine, followed by HPLC analysis,[43] according to methods reported for similar compounds.[44] Thus, coupling between (*S*)-(–)-1-phenyl-ethylamine (98% pure) and ***(S)*-66** or ***(R)*-66** was carried out using HBTU/HOBt and DIPEA in CH_2Cl_2 at room temperature for 3 h, giving the corresponding diastereomeric amides in quantitative yields. Since the 1:1 mixture of the two diastereoisomers could not be separated by reverse-phase HPLC due to similar retention times, we found it more convenient to deprotect a 1 mg/mL analytical sample of each of the two diastereomeric amides in acetonitrile with a drop of piperidine. The two deprotected samples showed different retention times, and the enantiomeric purity of the final product was measured by HPLC, giving a de>96%, without any detectable traces of the parent isomer in both cases.

2.5 Compatibility with solid phase peptide synthesis

Fmoc-3-morpholine carboxylic acid was applied in the Solid-Phase synthesis of a model tripeptide using Rink-HMBA resin, and DIC/HOBt as the activating mixture. The internal colorimetric Bromophenol Blue test, as reported by Krchnák et al.,[45] was used to monitor the coupling efficiency on both amino and carboxylic functions of morpholine-3-carboxylic acid. Thus, Fmoc-Phe-morpholine-3*S*-CO-Leu-NH_2 **79** was synthesized. The coupling reactions proved to proceed slower on both functions compared to acyclic □-amino acids. The crude tripeptide was obtained in 90% yield after cleavage from the resin, and in 86% purity as determined by HPLC, thus showing full compatibility with solid-phase peptide chemistry.

Scheme 2.5 Solid phase peptide synthesis using Fmoc-morpholine-3-carboxylic acid

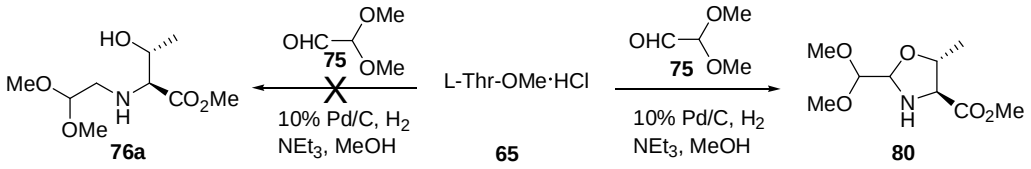


[43] NMR analysis of the diastereomeric amides derived from **(*S*)-66** and **(*R*)-66** for assessing the enantiomeric purity of the Fmoc-amino acids was complicated by the presence of rotamers at the urethane bond.
[44] Souers, A. J.; Ellman, J. A. *J. Org. Chem.* **2000**, *65*, 1222-1224.
[45] Krchnák, V. ; Vágner, J. ; Säfar, P. ; Lebl, M. *Coll. Czech. Chem. Commun.* **1988**, *53*, 2542-2548.

2.6 Strategy extension to threonine: synthesis of Fmoc-2-methyl-3-morpholine-caroxylic acid

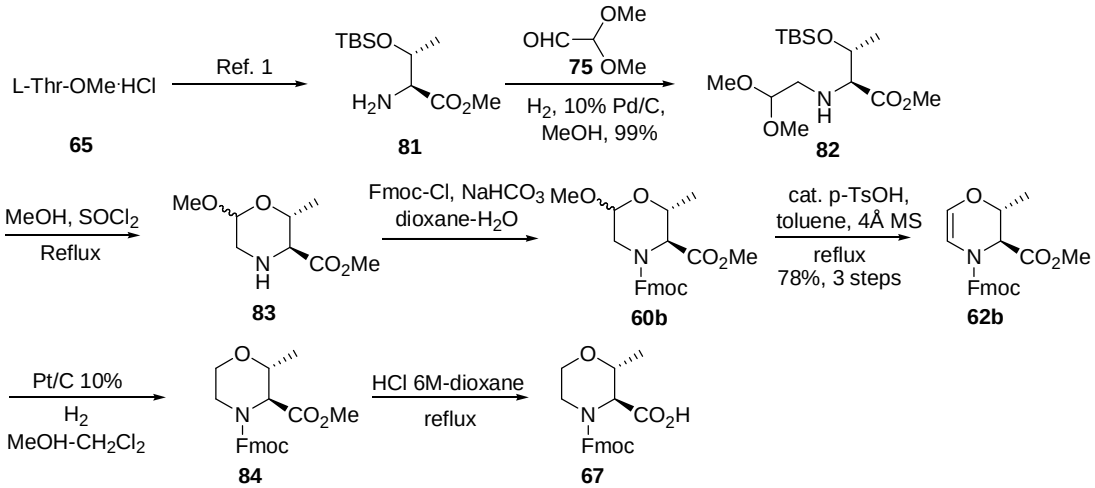
The strategy described in this section and the strategy described in section 2.3 are both based on the key step of an acid catalyzed cyclization and a subsequent elimination of MeOH, in order to obtain the dihydroxazine structure. However, the synthesis of the dihydroxazine derived from threonine is not a mere extension of the strategy developed for serine and required much more experimental research, demonstrating the difference in reactivity of serine compared to threonine When the strategy developed with serine was extended to threonine, problems initially arose in the reductive amination step with dimethoxyacetaldehyde. In fact, upon treatment of threonine methyl ester hydrochloride with Pd/C-NEt_3 under a hydrogen atmosphere, the only observed product was not the expected **76a**, but **80** (Scheme 2.6).

Scheme 2.6 Attempted synthesis of compound **80**



Protection of the hydroxyl group of threonine with TBSCl[46] before performing the reductive amination proved essential and allowed the synthesis of the **82** in almost quantitative yield. Unfortunately amine **82** was found to be completely unreactive upon treatment with Fmoc-Cl under various reaction conditions. We reasoned that this poor reactivity was caused by high steric hindrance and so we decided to subject amine **82** to more harsh acid conditions, in order to deprotect the hydroxyl group and simultaneously obtain the trans-acetalyzation.

Scheme 2.7 Synthesis of Fmoc-2-methyl-3-morpholine carboxylic acid.



[46] Niu, Chuansheng; Pettersson, Teresia; Miller, Marvin J. *J. Org. Chem*. **1996**, 61, 1014-1022.

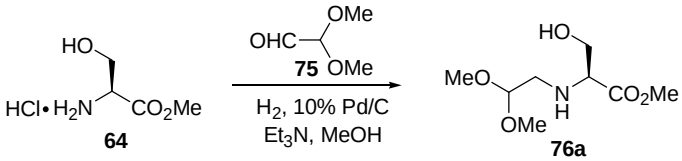
The amino group of the resultant cyclic acetal **83** proved to be much more reactive than linear precursor **82** and the crude product deriving from trans-acetalyzation was easily protected upon treatment with Fmoc-Cl/$NaHCO_3$ aq. Subsequent treatment of cyclic acetal **60b** with p-TSOH/MS 4Å in refluxing toluene furnished the dihydroxazine scaffold **62b** in good overall yield, requiring only one purification through flash chromatography (Scheme 2.7). Schemes 2.4 and 2.7 are both based on the key step of an acid catalyzed cyclization and a subsequent elimination of MeOH, in order to obtain the dihydrozazine structure. However, the synthesis of the dihydroxazine derived from threonine is not a mere extension of the strategy developed for serine and required much more experimental research, demonstrating the difference in reactivity of serine compared to threonine. Final conversion of compound **62b** to acid **67** was then achieved through catalytic hydrogenation using Pt/C, H_2 and acid hydrolysis in refluxing dioxane-HCl 6M, using similar conditions to those previously described for the synthesis of **66**

2.7 Experimental for chapter 2

General experimental methods

Melting points are uncorrected. Chromatographic separations were performed on silica gel using flash-column techniques. R_f values refer to TLC carried out on 25 mm silica gel plates (Merck F_{254}) with the same eluant indicated as for column chromatography. All the solid-phase reactions were carried out on a shaker, using solvents of HPLC quality. HPLC analyses were carried out on a HPLC system equipped with analytical C-18 10 μm, 250 × 4.6 mm, reverse-phase column, using H_2O – CH_3CN eluant buffered with 0.1% TFA. ^1H and ^{13}C NMR spectra were recorded with NMR instruments operating at 400 MHz for proton and at 50 MHz for carbon, respectively, and using $CDCl_3$ solutions unless otherwise stated. EI mass spectra were carried out at 70 eV ionizing voltage, and ESI-MS was carried out for peptide **79** using a linear ion-trap LC-MS spectrometer.

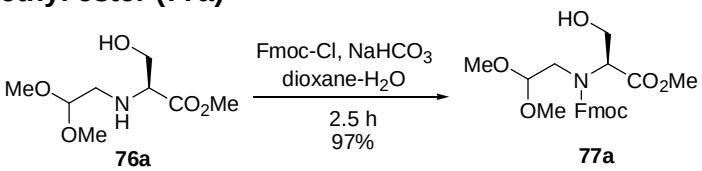
(2*S*)-2-(2,2-Dimethoxy-ethylamino)-3-hydroxy-propionic acid methyl ester (76a)



L-serine methyl ester hydrochloride (1.00 g, 6.47 mmol) was dissolved in MeOH (20 mL), then triethylamine (902 µL, 6.47 mmol), 60% aqueous solution of dimethoxyacetaldehyde (1.11 g, 6.47 mmol) and 10% Pd/C (90 mg) were successively added, and the resulting mixture was stirred overnight at room temperature under a hydrogen atmosphere. Then, the suspension was filtered on Celite and the organic solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2 – MeOH 12:1, R_f 0.43) to yield **76a** as a colorless oil (1.31 g, 98%). $[\alpha]^{24}_D$ -28.5 (*c* = 1.0, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$) δ 4.44 (t, *J* = 4.5 Hz, 1 H), 3.77 (dd, *J* = 11.2, 4.5 Hz, 1 H), 3.74 (s, 3 H), 3.59 (dd, *J* = 12.5, 8.0 Hz, 1 H), 3.40 (t, *J* = 4.5 Hz, 1 H), 3.36 (s, 6 H), 2.84 (dd, *J* = 12.5, 4.5 Hz, 1 H), 2.65 (dd, *J* = 12.5, 4.5 Hz, 1 H), 2.39 (br, 1 H). ^{13}C NMR

(50 MHz, $CDCl_3$) δ 173.1 (s), 103.5 (d), 62.7 (d), 62.5 (t), 53.9 (q), 53.1 (q), 52.0 (q), 49.1 (t). MS *m/z* 207 (M^+, 26), 149 (13), 133 (18). Anal. calcd for $C_8H_{17}NO_5$: C, 46.37; H, 8.27; N 6.76. Found: C, 46.40; H, 8.31; N 6.69.

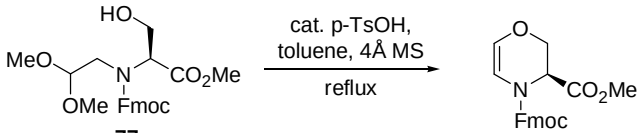
(2*S*)-2-[(2,2-Dimethoxy-ethyl)-(9*H*-fluoren-9-ylmethoxycarbonyl)-amino]-3-hydroxy-propionic acid methyl ester (77a)



76a **77a**

To a solution of **76a** (1.14 g, 5.5 mmol) in 2:1 water-dioxane (15 mL) was added $NaHCO_3$ (0.92 g, 11.0 mmol), and the mixture was cooled to 0 °C with an ice bath, then a solution of Fmoc-Cl (1.42 g, 5.5 mmol) in dioxane (15 mL) was added dropwise over 15 min. The ice bath was removed, and the reaction mixture was left stirring for 2.5 h. Successively, the mixture was partitioned between EtOAc (40 mL) and water (20 mL), and the organic phase was washed with 1M HCl, brine, and dried over Na_2SO_4. The organic solvents were then removed under reduced pressure, and the crude product was purified by flash column chromatography (EtOAc – hexanes 3:2, R_f 0.53), thus giving pure **77a** as a colorless oil (2.29 g, 97%). $[\alpha]^{22}_D$ -31.6 (*c* = 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) 3:2 Mixture of rotamers δ 7.76 (d, *J* = 7.2 Hz, 2 H), 7.60 (d, *J* = 6.0 Hz, 1 H), 7.55-7.53 (m, 1 H), 7.42-7.39 (m, 2 H), 7.35-7.29 (m, 2 H), 4.76-4.69 (m, 2 H), 4.61-4.47 (m, 2 H), 4.24-4.21 (m, 1 H), 3.97-3.94 (m, 1 H), 3.86-3.80 (m, 1 H), 3.69 and 3.61 (s, 3 H), 3.69-3.59 (m, 1 H), 3.49 and 3.43 (2s, 2.4 H), 3.16 and 3.11 (2s, 3.6 H), 3.21-3.11 (m, 0.4 H), 2.97 (dd, *J* = 15.2, 7.2 Hz, 0.6 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.0 (s), 156.6 (s), 143.4 (s, 2 C), 141.2 (s, 2 C), 127.6 (d, 2 C), 127.1 and 126.9 (d, 2 C), 124.6 and 124.5 (d, 2 C), 119.9 (d, 2 C), 103.3 and 103.0 (d), 67.7 and 66.6 (t), 62.9 and 62.1 (d), 60.7 and 60.2 (t), 55.6 (q), 54.7 (q), 52.2 (q), 49.1 and 48.9 (t), 47.3 and 47.0 (d). MS *m/z* 367 [0.2, M^+- $(OCH_3)_2$], 324 (0.2). Anal. calcd for $C_{23}H_{27}NO_7$: C, 64.32; H, 6.34; N, 3.26. Found: C, 64.30; H, 6.39; N, 3.21.

(3*S*)-2,3-Dihydro-[1,4]oxazine-3,4-dicarboxylic acid 4-(9*H*-fluoren-9-ylmethyl) ester 3-methyl ester (62a)

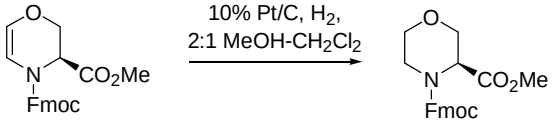


77a **62a**

A solution of compound 4 (1.1 g, 2.56 mmol) in toluene (25 mL) containing a catalytic amount of *p*-toluenesulfonic acid monohydrate (49 mg, 0.26 mmol) was placed in a single-necked round-bottomed flask equipped with a reflux condenser and dropping funnel containing approximately 13 g of 4Å molecular sieves. The mixture was refluxed for 1.75 h. Then it was cooled to room temperature and filtered through a thin layer of $NaHCO_3$. Toluene was removed under reduced pressure, and the crude product was purified by flash column chromatography (hexanes-EtOAc 3:1, R_f 0.55) to yield compound 5 as a white foam (795 mg, 85%). $[\alpha]^{23}_D$ +6.2 (*c* = 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) 3:2 Mixture of rotamers δ 7.77 (t, *J* = 8.0 Hz, 1 H), 7.61 (t, *J* = 8.0 Hz, 1 H), 7.50 (m, 1 H), 7.41

and 7.32 (m, 2 H), 6.42 (d, *J* = 5.2 Hz, 0.4 H), 6.36 (d, *J* = 5.2 Hz, 0.6 H), 6.02 (d, *J* = 5.2 Hz, 0.4 H), 5.98 (d, *J* = 5.2 Hz, 0.6 H), 4.97 (s, 0.4 H), 4.68 (d, *J* = 11.2 Hz, 0.4 H), 4.60-4.40 (m, 3.2 H), 4.32 (t, *J* = 7.2 Hz, 0.6 H), 4.23 (t, *J* = 7.2 Hz, 0.4 H), 3.99 (dd, *J* = 11.2, 3.2 Hz, 0.6 H), 3.87 (dd, *J* = 11.2, 3.2 Hz, 0.4 H), 3.86 (s, 1.8 H), 3.71 (s, 1.2 H). ^{13}C NMR (50 MHz, $CDCl_3$) *δ* 168.2 (s), 151.9 and 151.2 (s), 143.4 and 143.2 (s, 2 C), 141.0 (s, 2 C), 129.7 and 128.8 (d), 127.6 (d, 2 C), 126.9 (d, 2 C), 124.9 (d), 124.8 (d), 124.8 and 124.5 (d), 119.9 (d, 2 C), 105.9 and 105.3 (d), 68.3 and 67.8 (t), 65.4 and 64.9 (t), 54.5 and 53.9 (d), 52.8 (q), 47.0 and 46.9 (d). MS *m/z* 365 (M^+, 7), 306 (0.4, M^+-CO_2Me), 179 (100). Anal. calcd for $C_{21}H_{19}NO_5$: C, 69.03; H, 5.24; N, 3.83. Found: C, 69.16; H, 5.31; N, 3.80.

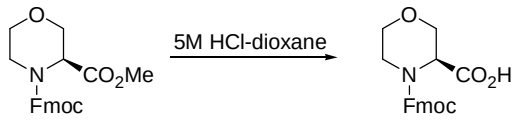
(3*S*)-Morpholine-3,4-dicarboxylic acid 4-(9*H*-fluoren-9-ylmethyl) ester 3-methyl ester (78)



62a **78**

Compound **62a** (1.30 g, 3.3 mmol) was dissolved in a 2:1 mixture of MeOH-CH_2Cl_2 (30 mL), and 10% Pt/C (166 mg) was added. The suspension was hydrogenated overnight at room temperature, and then filtered over Celite. The organic solvents were removed under reduced pressure and the crude product was purified by flash column chromatography (hexanes-EtOAc 2:1, R_f 0.48) to yield pure **78** as a white foam (1.15 g, 95%). $[\alpha]^{24}_D$ -51.5 (*c* = 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers *δ* 7.78-7.75 (t, *J* = 6.9 Hz, 2 H), 7.60 (t, *J* = 6.5 Hz, 1 H), 7.50 (t, *J* = 7.1 Hz, 1 H), 7.43-7.38 (m, 2 H), 7.35-7.28 (m, 2 H), 4.65 (m, 0.5 H), 4.56-4.46 (m, 1.5 H), 4.44-4.37 (m, 1 H), 4.33-4.28 (m, 1.5 H), 4.24-4.20 (m, 0.5 H), 3.91-3.84 (m, 1.5 H), 3.78 and 3.73 (s, 3 H), 3.66 (dd, *J* = 12.0, 2.4 Hz, 1.5 H), 3.58 (dd, *J* = 12.0, 2.4 Hz, 0.5 H), 3.50-3.40 (m, 1.5 H), 3.22 (td, *J* = 12.0, 4.0 Hz, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$) *δ* 169.9 (s), 143.7 and 143.5 (s, 2 C), 141.1 (s, 2 C), 127.5 (d, 2 C), 126.9 (d, 2 C), 124.8 (d), 124.6 and 124.5 (d), 119.8 (d, 2 C), 67.8 and 67.5 (t), 67.5 and 67.2 (t), 66.5 and 66.1 (t), 54.6 and 54.3 (d), 52.5 (q), 47.0 (d), 41.5 and 41.0 (t). MS *m/z* 367 (M^+, 0.6), 278 (0.8), 178 (9), 57 (100). Anal. calcd for $C_{21}H_{21}NO_5$: C, 68.65; H, 5.76; N, 3.81. Found: C, 68.70; H, 5.77; N, 3.82.

(3*S*)-Morpholine-3,4-dicarboxylic acid 4-(9*H*-fluoren-9-ylmethyl) ester [(*S*)-66]



78 **(S)-66**

Ester **78** (1.70 g, 4.6 mmol) was dissolved in dioxane (12 mL) and 5M HCl (12 mL) was added. The reaction was refluxed for 18 h and then diluted with 5% Na_2CO_3 (120 mL). The resulting solution was washed with diethyl ether and then the aqueous layer was acidified to pH=1 with concentrated HCl and the organic phase was extracted with CH_2Cl_2. The organic extracts were combined, dried over Na_2SO_4 and concentrated under reduced pressure to yield compound **(*S*)-66** as a white solid (1.57 g, 97%). Mp = 128-130 °C. $[\alpha]^{24}_D$ -56.9 (*c* = 1, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers *δ* 8.44 (br, 1 H), 7.69-7.62 (m, 2 H), 7.52-7.50 (m, 1 H), 7.44-7.39 (m, 1 H), 7.33-7.17 (m, 4 H), 4.62 (s, 0.5

H), 4.51-4.41 (m, 1.5 H), 4.38-4.32 (m, 1 H), 4.25-4.11 (m, 2 H), 3.83-3.81 (m, 1 H), 3.71 (d, *J* = 13.0 Hz, 0.5H), 3.65-3.50 (m, 1 H), 3.45 (dd, *J* = 11.8, 3.8 Hz, 0.5 H), 3.42-3.31 (m, 1.5 H), 3.22-3.16 (td, *J* = 12.5, 3.4 Hz, 0.5 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.7 and 173.5 (s), 156.1 and 155.5 (s), 143.5 and 143.3 (s, 2 C), 141.0 (s, 2 C), 127.5 (d, 2 C), 126.8 (d, 2 C), 124.7 (d), 124.5 and 124.4 (d), 119.7 (d, 2 C), 67.9 and 67.4 (t), 67.4 and 67.0 (t), 66.4 and 66.0 (t), 54.5 and 54.1 (d), 47.0 (d), 41.5 and 41.0 (t). ESI-MS *m/z* 354.09 (M^++H, 8), 376.18 (M^++Na, 100), 392.18 (M^++K, 46). Anal. calcd for $C_{20}H_{19}NO_5$: C, 67.98; H, 5.42; N, 3.96. Found: C, 67.90; H, 5.43; N, 3.98.

(2*R*)-2-(2,2-Dimethoxy-ethylamino)-3-hydroxy-propionic acid methyl ester [(*R*)-76a]. D-serine methyl ester hydrochloride (2.50 g, 16 mmol) was treated as described for **76a**, giving pure **(*R*)-76a** as a colorless oil (3.25 g, 98%), with spectroscopic data as for the enantiomer. $[\alpha]^{24}_D$ +27.7 (*c* = 1.0, CH_2Cl_2). Anal. calcd for $C_8H_{17}NO_5$: C, 46.37; H, 8.27; N 6.76. Found: C, 46.30; H, 8.41; N 6.79.

(2*R*)-2-[(2,2-Dimethoxy-ethyl)-(9*H*-fluoren-9-ylmethoxycarbonyl)-amino]-3-hydroxy-propionic acid methyl ester [(*R*)-77a]. Compound **(*R*)-76a** (2.33 g, 11.2 mmol) was treated as described for **77a**, giving pure **(*R*)-77a** as a colorless oil (4.57 g, 95%), with spectroscopic data as for the enantiomer. $[\alpha]^{22}_D$ +32.5 (*c* = 1.0, CH_2Cl_2). Anal. calcd for $C_{23}H_{27}NO_7$: C, 64.32; H, 6.34; N, 3.26. Found: C, 64.36; H, 6.31; N, 3.22.

(3*R*)-2,3-Dihydro-[1,4]oxazine-3,4-dicarboxylic acid 4-(9*H*-fluoren-9-ylmethyl) ester 3-methyl ester [(*R*)-62a]. Compound **(*R*)-62a** (2.10 g, 4.89 mmol) was treated as described for **62a**, giving pure **(*R*)-62a** as a white foam (1.54 g, 86%), with spectroscopic data as for the enantiomer. $[\alpha]^{23}_D$ -6.9 (*c* = 1.0, CH_2Cl_2). Anal. calcd for $C_{21}H_{19}NO_5$: C, 69.03; H, 5.24; N, 3.83. Found: C, 69.06; H, 5.21; N, 3.78.

(3*R*)-Morpholine-3,4-dicarboxylic acid 4-(9*H*-fluoren-9-ylmethyl) ester 3-methyl ester [(*R*)-78]. Compound **(*R*)-78** (1.05 g, 2.87 mmol) was treated as described for **78**, giving pure **(*R*)-78** as a white foam (1.00 g, 95%), with spectroscopic data as for the enantiomer. $[\alpha]^{24}_D$ +51.1 (*c* = 1.0, CH_2Cl_2). Anal. calcd for $C_{21}H_{21}NO_5$: C, 68.65; H, 5.76; N, 3.81. Found: C, 68.60; H, 5.79; N, 3.77.

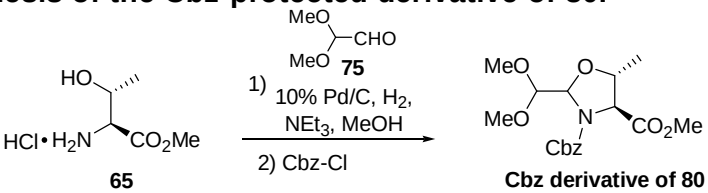
(3*R*)-Morpholine-3,4-dicarboxylic acid 4-(9*H*-fluoren-9-ylmethyl) ester [(*R*)-66]. Compound **(*R*)-66** (0.90 g, 2.45 mmol) was treated as described for **(*S*)-66**, giving pure **(*R*)-66** as a white solid (0.83 g, 96%), with spectroscopic data as for the enantiomer. MP = 128-129 °C. $[\alpha]^{24}_D$ +57.4 (*c* = 1, CH_2Cl_2). Anal. calcd for $C_{20}H_{19}NO_5$: C, 67.98; H, 5.42; N, 3.96. Found: C, 67.92; H, 5.39; N, 3.91.

Enantiomeric purity. In two separate vessels, **(*S*)-66** and **(*R*)-66** (50 mg, 0.14 mmol) were each coupled with (*S*)-(–)-1-phenyl-ethylamine (36 μL, 0.28 mmol) using HBTU (106 mg, 0.28 mmol) and HOBt (38 mg, 0.28 mmol) in CH_2Cl_2 (2 mL) and in the presence of DIPEA (96 μL, 0.56 mmol). After 3 h reacting at room temperature, the mixture was diluted with EtOAc and the organic phase was washed with 1M HCl, 5% Na_2CO_3, and brine. The organic phase was evaporated to yield the corresponding diastereomeric amides. A 1 mg/mL analytical sample of the Fmoc-amides from **(*S*)-66** and **(*R*)-66** were further deprotected by addition of a drop of piperidine, followed by HPLC analysis using the

following gradient: 10% acetonitrile/5 min, then 10 – 25% acetonitrile/20 min, then 25 – 30% acetonitrile/5 min, then 30 – 90% acetonitrile/15 min. Co-injection of the 1:1 mixture of diastereomeric amides from **(*S*)-66** and **(*R*)-66** resulted in two peaks of t_R = 22.9 and 21.8 min, respectively.

Fmoc-Phe-(S)-Morph-Leu-NH_2 (79). Solid-phase peptide synthesis was carried out using Fmoc-Leu, **(*S*)-66**, Fmoc-Phe, and Rink-HMBA resin (122 mg, 0.71 mmol/g). All amino acids and coupling reagents (DIC/HOBt) were used in 3-fold excess as 0.17 M DMF solutions. Coupling reactions were monitored using the internal Bromophenol Blue colorimetric indicator, as reported.[20] Fmoc-deprotection was performed using a 30% solution of piperidine in DMF. Couplings on both functions of morpholine-3-carboxylic acid were completed within 24 h. After each coupling, the indicator was washed from the resin using 5% DIPEA in DMF, and successively the resin was washed thoroughly with DMF, CH_2Cl_2, and MeOH. Peptide was cleaved from the resin using 95% TFA in the presence of 2.5% TIS and 2.5% H_2O as scavengers. Peptide **79** was precipitated using cooled methyl-*t*-butyl ether, and it was isolated after centrifugation. The crude product (48 mg) was analyzed by ESI-MS and by reverse-phase HPLC, resulting in 90% yield and 86% purity (10% acetonitrile/5 min, then 10 – 90% acetonitrile/20 min as gradient, 223 nm, t_R = 18.80 min). ESI-MS *m/z* 613.09 (M^++H, 7), 635.36 (M^++Na, 77), 651.27 (M^++K, 62).

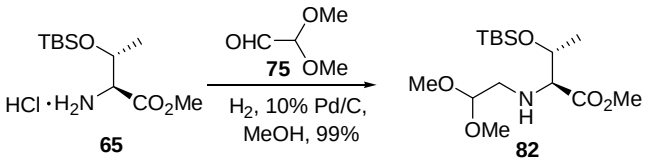
(2*R*/*S*,4*S*,5*R*)-2-Dimethoxymethyl-5-methyl-oxazolidine-4-carboxylic acid methyl ester (80): synthesis of the Cbz-protected derivative of 80.



65 **Cbz derivative of 80**

Compound **80** was obtained as a by-product starting from L-threonine methyl ester (**65**) (2.42 g, 14.3 mmol) according to the reported procedure for the preparation of **76a** (Chapter 2 and ref. 14). The crude product **80** was then characterized as the corresponding Cbz-protected derivative, after treatment of crude **80** with Cbz-Cl according to procedure as for **87** (Chapter 4). Pure Cbz-protected compound (2.76 g, 12.4 mmol) was obtained after chromatographic purification (Hexanes-EtOAc 1:3) in 87% yield over two steps. (Found: C, 57.96; H, 6.61; N, 3.74. $C_{17}H_{23}NO_7$ requires C, 57.78; H, 6.56; N, 3.96%); ^{1}H-NMR (200 MHz, $CDCl_3$) mixture of diastereomers 7.31 (m, 5 H, *Ph*), 5.31 [s, 1 H, C*H*$(OCH_3)_2$], 5.15 (s, 2 H, CH_2Ph), 4.58 (m, 1 H, OC*H*N), 4.46 (m, 1 H, C*H*CH_3), 4.07 (m, 1 H, NC*H*CO_2), 3.69 (s, 3 H, CO_2CH_3), 3.46 (s, 6 H, OCH_3), 1.39 (d, J = 5.6 Hz, 3 H, CHCH_3); ^{13}C-NMR (50 MHz, $CDCl_3$) mixture of diastereomers 169.6 (s, *C*O_2CH_3), 135.6 (s, *i-Ph*), 128.2 (d, 2 C, *Ph*), 127.9 (d, 2 C, *Ph*), 127.5 (d, *Ph*), 104.2 [d, *C*H$(OCH_3)_2$], 88.6 (d, O*C*HN), 67.5 (t, *C*H_2Ph), 64.4 (d, *C*HCH_3), 55.7 (d, NCHCO_2), 52.3 (q, CO_2CH_3), 19.5 (q, CH*C*H_3).

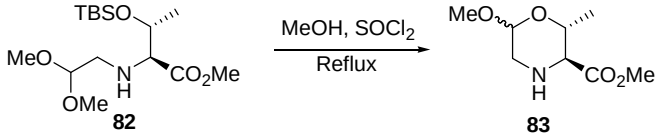
(2*S*,3*R*)-3-(*t*-Butyldimethylsilanyloxy)-2-(2,2-(dimethoxy)ethylamino)butyric acid methyl ester (82)



65 **82**

Compound **65** (3.70 g, 14.9 mmol) was dissolved in MeOH (45 mL), then 60% aqueous solution of dimethoxyacetaldehyde (**75**) (2.59 g, 14.9 mmol) and 10% Pd/C (329 mg) were successively added, and the resulting mixture was stirred overnight at room temperature under a hydrogen atmosphere. Then, the suspension was filtered on Celite and MeOH was removed under reduced pressure. The resulting mixture was partitioned between water and Et2O. The combined organic layers were washed with brine, dried over Na2SO4 and concentrated under reduced pressure to yield compound **82** as a colourless oil (4.95 g, 99%). (Found: C, 53.86; H, 10.00; N, 4.22. $C_{15}H_{33}NO_5Si$ requires C, 53.70; H, 9.91; N, 4.17%); $[\alpha]_{25}^{D}$ −11.4 (*c* 1.1, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.53 (t, *J* = 5.2 Hz, 1 H, C*H*$(OCH_3)_2$], 4.18 (qui, *J* = 5.3 Hz, 1 H, OC*H*CH_3), 3.73 (s, 3 H, CO_2CH_3), 3.37 (s, 6 H, OCH_3), 3.37 (m, 1 H, NC*H*CO_2), 2.94 (dd, *J* = 12.2, 5.8 Hz, 1 H, CH_2CH), 2.73 (dd, *J* = 12.2, 5.0 Hz, 1 H, CH_2CH), 1.25 (d, *J* = 6.4 Hz, 3 H, CHCH_3), 0.85 [s, 9 H, $(CH_3)_3$CSi], 0.44 (s, 3 H, CH_3Si), 0.14 (s, 3 H, CH_3Si); ^{13}C NMR (50 MHz, $CDCl_3$) δ171.9 (s, *C*O_2CH_3), 102.9 [d, *C*H$(OCH_3)_2$], 69.1 (d, O*C*HCH_3), 66.8 (d, N*C*HCO_2), 54.4 (q, O*C*H_3), 53.6 (q, O*C*H_3), 52.0 (q, CO_2CH_3), 48.9 (t, *C*H_2CH), 25.7 [q, 3 C, $(CH_3)_3$CSi], 20.8 (q, CH*C*H_3), 17.9 [s, $(CH_3)_3$*C*Si], −4.2 (q, *C*H_3Si), −5.1 (q, *C*H_3Si); MS *m*/*z* 304 (M+ − CH3O, 8), 291 (15), 278 (6), 246 (13), 159 (38), 73 (100).

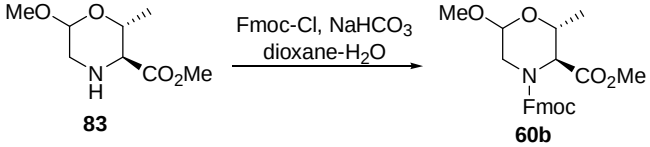
(2*R*,3*S*,6*R*/*S*)-6-Methoxy-2-methyl-morpholine-3-carboxylic acid methyl ester (83)



82 **83**

$SOCl_2$ (511 *μ*L, 7 mmol) was added dropwise, at 0 °C, to 7 mL of MeOH. The resulting solution was used to dissolve compound **82** (600 mg, 1.79 mmol). The resulting mixture was refluxed for 4 h, and then concentrated under reduced pressure. The crude material was dissolved again in MeOH, neutralized with Amberlist A-21, and the solvent was evaporated to dryness. The product was directly used without further purification for the subsequent protection step. An analytical pure sample was obtained after purification through flash column chromatography (EtOAc). (Found: C, 50.86; H, 8.08; N, 7.22. $C_8H_{15}NO_4$ requires C, 50.78; H, 7.99; N, 7.40%); ^{1}H-NMR (400 MHz, $CDCl_3$) 3:2 mixture of diastereomers *α* and *β:* *δ* 4.47 (s, 0.4 H, *H*-6 β), 4.40 (dd, *J* = 8.8, 2.4 Hz, 0.6 H, *H*-6 *α*), 3.88 (qd, *J* = 5.0, 1.8 Hz, 0.4 H, *H*-2 *β*), 3.74 and 3.73 (s, 3 H, CO_2CH_3), 3.65 (qd, *J* = 4.2, 1.2 Hz, 0.6 H, *H*-2 *α*), 3.50 (s, 1.8 H, OCH_3 *α*), 3.39 (s, 1.2 H, OCH_3 *β*), 3.27 (d, *J* = 9.4 Hz, 0.4 H, *H*-3 *β*), 3.18 (d, *J* = 9.4 Hz, 0.6 H, *H*-3 *α*), 3.04 (dd, *J* = 12.4, 2.4 Hz, 0.6 H, *H*-5 *α*), 2.92-2.90 (m, 0.8 H, *H*-5 *β*), 2.59 (dd, *J* = 12.4, 8.8 Hz, 0.6 H, *H*-5 *α*), 1.75-1.95 (br, 1 H, N*H*), 1.25 (d, *J* = 6.4 Hz, 1.8 H, CHCH_3 *α*), 1.15 (d, *J* = 6.0 Hz, 1.2 H, CHCH_3 *β*); ^{13}C NMR (50 MHz, $CDCl_3$) mixture of diastereomers δ 171.1 (s, *C*O_2CH_3), 100.6 and 95.6 (d, *C*-6), 73.7 and 65.4 (d, *C*-2), 63.6 and 62.8 (d, *C*-3), 56.1 and 54.5 (q, OCH_3), 52.1 (q, CO_2CH_3), 47.9 and 47.2 (t, *C*-5), 18.2 (q, CH*C*H_3).

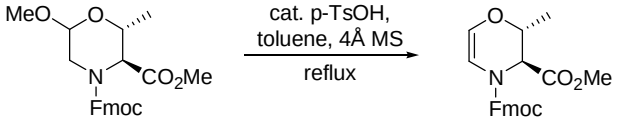
6-Methoxy-2-methyl-morpholine-3,4-dicarboxylic acid 4-(9H-fluoren-9-ylmethyl) ester 3-methyl ester (60b)



83 **60b**

The crude cyclic acetal **83** was dissolved in H_2O (13 mL) and $NaHCO_3$ (1.33 g, 15.9 mmol) was added. The mixture was stirred until complete dissolution of the salt and then dioxane (20 mL) was added. The flask was cooled at 0°C with an ice bath and solid Fmoc-Cl (1.17 g, 4.55 mmol) was added portion wise. After 10 minutes the ice bath was removed and the reaction mixture was stirred 16 h at room temperature. After that EtOAc (30 mL) and water (20 mL) were added. The aqueous layer was discarded and the organic phase was washed with 5% citric acid, brine and dried over Na_2SO_4. The solvents were removed under reduced pressure and the crude material was purified through flash column chromatography (hexanes/AcOEt) to yield 1.778 g (95%) of **60b**. (Characterization data refers to the first eluted diastereomer) $[\alpha]^{26}_D$ -3.40 (*c* = 1, CH_2Cl_2). ^{1}H-NMR (200 MHz, $CDCl_3$) (mixture of rotamers) δ 7.77 (d, *J* = 7.2 Hz, 2 H), 7.54 (bs, 2 H), 7.44-7-23 (m, 4 H), 4.81-4.68 (m, 1 H), 4.45-4.00 (m, 6 H), 3.74 and 3.67 (2 overimposed singlets, 3 H), 3.41 (s, 3 H), 3.20-3.97 (m, 1 H), 1.37 (s, 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$) (mixture of rotamers) δ 170.6 (s), 155.1 (s), 143.3 (s), 141.1 (s), 127.5 (d, 2 C), 126.9 (d, 2 C), 124.7 (d, 2 C), 119.8 (d, 2 C), 97.0 (d), 96.4 (d), 67.8 (t), 64.9 (d), 64.4 (d), 61.5 (d), 61.0 (d), 55.3 (q), 52.3 (q), 47.1 (d), 42.9 (t), 42.0 (t), 19.1 (q). MS *m/z* 411 (M^+, 8), 189 (3), 178 (100), 165 (10), 152 (6).
Anal. calcd for $C_{23}H_{25}NO_6$: C, 67.14, H, 6.12, N, 3.40. Found: C, 67.65, H 6.18, N, 3.50.

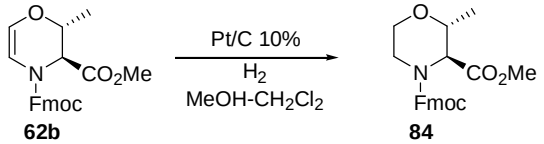
2-Methyl-2,3-dihydro-[1,4]oxazine-3,4-dicarboxylic acid 4-(9H-fluoren-9-ylmethyl) ester 3-methyl ester (62b)



60b **62b**

A solution of compound **60b** (736 mg, 1.79 mmol) in toluene (15 mL) containing a catalytic amount of *p*-toluenesulfonic acid monohydrate (34 mg, 0.18 mmol) was placed in a single-necked round-bottomed flask equipped with a reflux condenser and dropping funnel containing approximately 10 g of 4Å molecular sieves. The mixture was refluxed for 1.45 h. Then it was cooled to room temperature and filtered through a thin layer of $NaHCO_3$. Toluene was removed under reduced pressure, and the crude product was purified by flash column chromatography (hexanes-EtOAc 7:2, R_f 0.50) to yield compound **62b** as a white foam (598 mg, 88%). $[\alpha]^{27}_D$ 6.3 (*c* = 1.15, $CHCl_3$). ^{1}H-NMR (200 MHz, $CDCl_3$) (mixture of rotamers) δ 7.77 (t, *J* = 7.2 Hz, 2 H), 7.74 (t, *J* = 4.4 Hz, 2 H), 7.53-7.26 (m, 4 H), 6.37-6.32 (m, 1 H), 6.29-6.83 (m, 1 H), 4.88 (q, *J* = 6.6 Hz, 1 H), 4.72 (s, 1 H), 4.60-4.21 (m, 4 H), 3.70 and 4.25 (2 singlets, 3 H), 1.33 (d, *J* = 6.6 Hz, 2 H), 1.24 (d, *J* = 6.6 Hz, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$) Mixture of rotamers *δ* 168.3 (s), 152.7 and 151.9 (s), 143.5 and 143.2 (s, 2C), 141.1 (s, 2C), 127.6-124.5 (d, 7 C), 119.9 (d, 2C), 104.5 and 104.0 (d), 69.8 and 69.2 (d), 68.3 and 67.8 (t), 57.9 and 57.4 (d), 52.7 (q), 47.1 and 47.0 (d) 17.3 (q). MS *m/z* 379 (M^+, 10), 179 (100), 157 (20), 98 (28).
Anal. calcd for $C_{22}H_{21}NO_5$: C, 69.64, H, 5.58, N, 3.69. Found: C, 69.65, H 5.98, N, 3.70.

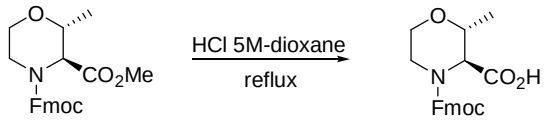
2-Methyl-morpholine-3,4-dicarboxylic acid 4-(9H-fluoren-9-ylmethyl) ester 3-methyl ester (84)



62b **84**

Compound **62b (**530 mg, 1.40 mmol) was dissolved in a 2:1 mixture of MeOH-CH_2Cl_2 (15 mL), and 10% Pt/C (63 mg) was added. The suspension was hydrogenated overnight at room temperature, and then filtered over Celite. The organic solvents were removed under reduced pressure and the crude product was purified by flash column chromatography (hexanes-EtOAc 3:1, R_f 0.41) to yield pure **84** as a white foam (480 mg, 90%). $[\alpha]^{23}_D$ -33.6 (*c* = 1.1, $CHCl_3$). ^{1}H-NMR (200 MHz, $CDCl_3$) (mixture of rotamers) δ 7.76 (d, *J* = 7.0 Hz, 2 H), 7.59 (bs, 2 H), 7.40-7.26 (m, 4 H), 4.60-4.40 (m, 3 H), 4.40-4.00 (m, 2 H), 3.75 (s, 3 H), 3.80-3.20 (m, 4 H), 1.33 (d, *J* = 6.4 Hz, 3 H).^{13}C NMR (50 MHz, $CDCl_3$) Mixture of rotamers *δ* 169.8 (s), 156.3 (s), 143.4 and 143.3 (s, 2C), 141.0 (s, 2C), 127.4 (d, 2C), 126.8 (d, 2C), 124.5 (d, 2C), 119.7 (d, 2C), 69.4 (d), 67.6 (t), 58.6 (1d and 1t, 2C), 52.4 (q), 47.1 (d), 41.1 (t), 16.6 (q). MS *m*/*z* 322 (3), 178 (100), 165 (6), 152 (4). Anal. calcd for $C_{22}H_{23}NO_5$: C, 69.28, H, 6.08, N, 3.67. Found: C, 69.45, H 5.98, N, 3.70.

2-Methyl-morpholine-3,4-dicarboxylic acid 4-(9H-fluoren-9-ylmethyl) ester (67)



84 **67**

Ester **84** (420 g, 1.1 mmol) was dissolved in dioxane (4 mL) and 5M HCl (4 mL) was added. The reaction was refluxed for 18 h and then diluted with 5% Na_2CO_3 (30 mL). The resulting solution was washed with diethyl ether and then the aqueous layer was acidified to pH=1 with concentrated HCl and the organic phase was extracted with CH_2Cl_2. The organic extracts were combined, dried over Na_2SO_4 and concentrated under reduced pressure to yield compound **67** as a white solid (390 mg, 96%). $[\alpha]^{24}_D$ -26.7 (*c* = 2, $CHCl_3$). ^{1}H-NMR (200 MHz, $CDCl_3$) (mixture of rotamers) δ 10.0 (bs, 1 H), 7.75 (d, *J* = 7.4 Hz, 2 H), 7.57 (bs, 2 H), 7.40-7.25 (m, 4 H), 4.60-4.45 (m, 3 H), 4.40-4.26 (m, 2 H), 4.00-3.21 (m, 4 H), 1.36 (d, *J* = 5.2 Hz, 3 H).^{13}C NMR (50 MHz, $CDCl_3$) Mixture of rotamers *δ* 174.0 (s), 156.7 and 156.0 (s), 143.4 (s, 2C), 141.0 (s, 2C), 127.5 (d, 2C), 126.9 (d, 2C), 124.7 (d, 2C), 119.7 (d, 2C), 69.3 and 68.4 (d), 67.7 (t), 58.2 (1d and 1t, 2C), 47.0 (d), 41.0 (t), 16.4 (q). MS *m*/*z* 321 (2), 178 (100), 152 (16), 88 (19), 76 (24).
Anal. calcd for $C_{21}H_{21}NO_5$: C, 68.65, H, 5.76, N, 3.81. Found: C, 68.40, H, 5.66, N, 3.90.

3 L- or D-morpholine-3-carboxylic acids as β-turn nucleators

"Trabocchi, A.; Sladojevich, F.; Guarna, A.
Chirality, Early View"

ABSTRACT

The conformational analysis by NMR, IR and molecular modeling of tetrapeptides containing morpholine-3-carboxylic acid (Mor) as a proline surrogate is presented. The relationhip between the chirality of the cyclic amino acid at position *i*+1 and the turn propensity was maintained with respect to the reference proline-containing peptides, although marked differences in the type of folded structures were observed. The conformational profile of morpholine-containing turn peptides as a function of the chirality of the cyclic amino acid indicated that the heterochiral tetrapeptide containing the D-isomer of the cyclic amino acid is more prone to nucleate compact folded structures, although with no resemblance to the β-turn structures of D-proline-containing peptides.
Also, the solvation system proved to influence the organization of folded structures, as in the more interactive CD_3CN the model peptides showed more compact conformations. L-morpholine-3-carboxylic acid displayed two rotamers at the Val-Mor amide bond. The *trans* isomer of **85** did not experience any turn structures, nor any intramolecular hydrogen-bonds, whereas the *cis* isomer showed a strong preference for a type VI β-turn structure, thus providing a different conformational asset with respect to the β-turn structure as reported for the reference L-proline model peptide.

3.1 Introduction

One of the relevant aspects in medicinal chemistry is related to the development of peptides and peptidomimetics as drugs, and to the comprehension of the role of small modified peptides in the development and cure of diseases. β-Turns play a crucial role in proteins and bioactive peptides for folding and generating compact structures,[47] often being involved in molecular recognition processes,[48] and so is for γ-turns, which are considered as rare turns among the types of secondary structures able to reverse the chain direction.[49] B-Turns are a subset of reverse turns and consist of a tetrapeptide sequence in a non-helical region in which the chain direction is reversed. These turns are often stabilized by an ten-membered ring intramolecular hydrogen-bond between the carbonyl oxygen of the first residue (*i*) and the amide proton of the fourth one (*i*+3) (Figure 3.1, left). γ-Turns, which are less common than β -turns, are three residue sequences containing a seven-membered ring intramolecular hydrogen-bond between the carbonyl group of the first residue (*i*) with the amide proton of the third residue (*i*+2) (Figure 3.1, right). Further classification into classical or inverse γ-turns depends on the position of the side chain of the *i*+1 residue, being equatorial for the inverse type and axial for the classical γ-turn.[50]

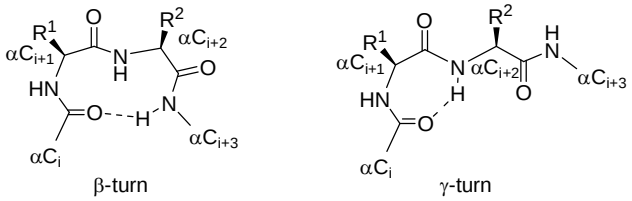


Figure 3.1: β- (left) and γ-turn (right) structures.

β- And γ-turns mimetics have been taken into account for peptidomimetic design, as they allow to present in a stereocontrolled fashion two to four side chains of amino acids involved in biological interactions. Among the mimetics of γ-turns reported in the literature, the mimicry of such conformation by suitable γ-turn inducers is of special interest, which provide stabilization of the turn motif by an intramolecular hydrogen-bond.[51] The majority of β -turn mimetics is based upon the replacement of the *i*+1 - *i*+2 central dipeptidic sequence of a turn with dipeptide isosteres capable to preserve the intramolecular 10-membered ring hydrogen-bond.[52] Also, the use of proline-mimetics has

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been pursued and several scaffolds have been proposed, either having bicyclic structures or four to six-membered ring proline homologues, as among the naturally occurring amino acids, proline is known to play a central role in the nucleation of reverse turn structures like β -turns and β -hairpins, due to its ability to form *cis* peptide bonds and to undergo *cis*/*trans* isomerization.[53] In fact, *cis* geometry of proline amide bond causes peptide backbone to fold in a type VI β -turn, in which proline occupies the *i*+2 position, although proline is often observed at the *i*+1 position of β -turn structures, generating a *trans* amide bond with the preceding amino acid at position *i*.[54] In addition, it has been demonstrated that the *cis*/*trans i*somerization at Xaa-Pro peptide bond is accelerated by changing the proline ring in size and structure, and as an example, pipecolates have served as proline substitutes in structure-activity studies of biologically relevant peptides.[55] Finally, whereas L-proline as the second residue (*i* + 1) is known to nucleate type I/II, β -turns in peptides involving an intramolecular hydrogen-bond between the C=O of the first (*i*) and NH of the fourth (*i* + 3) residue, its D-isomer favors antiparallel β-sheet formation via type I'/II' β -turns.[56,57] As an additional unexplored proline analogue, morpholine-3-carboxylic acid (Mor) is of interest due to the oxygen atom in the ring which can modulate the polarity and the chair conformation profiles with respect to pipecolic acid. The relevance of such secondary amino acid in medicinal chemistry is remarkable, as morpholine-3-carboxylic acid has been used to synthesize several bioactive molecules, such as TACE,[58] MMP and TNF inhibitors,[59] and a potent orally active VLA-4 antagonist.[60] In the previous chapter, we reported a new method for the synthesis of enantiopure Fmoc-protected morpholine-3-carboxylic acid **(66)** from dimethoxyacetaldehyde and serine methyl ester through a short and practical synthetic route.[61] This preliminar results opened the way to the synthesis of a new class of cyclic amino acids containing the morpholine nucleus, and also, with the aim to gain informations for the design of turn peptides containing the

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[56] One of the earliest examples of the turn-forming ability of *N*-acyl proline dipeptides composed of amino acids of opposite configurations ("heterochiral sequences") may be found in the structure of gramicidin: Hull, S.E.; Karlsson, R.; Main, P.; Woolfson, M.M.; Dodson, E.J. *Nature* **1978**, 275, 206-275.

[57] Deber, C.M.; Madison, V.; Blout, E.R. *Acc. Chem. Res.* **1976**, 9, 106-113.

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[59] (a) Almstead, N.G.; Bradley, R.S.; Pikul, S.; De, B.; Natchus, M.G.; Taiwo, Y.O.; Gu, F.; Williams, L.E.; Hynd, B.A.; Janusz, M.J.; Dunaway, C.M.; Mieling, G.E. *J. Med. Chem*. **1999**, 42, 4547-4562; (b) Piscopio, A.D.; Rizzi, J.P. WO 96/33172, October 24, **1996**.

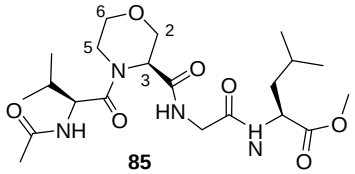
[60] Chiba, J.; Machinaga, N.; Takashi, T.; Ejima, A.; Takayama, G.; Yokoyama, M.; Nakayama, A.; Baldwin, J.J.; McDonald, K.W.; Hussain, R.; Wong, A; *Bioorg. Med. Chem. Lett.* **2005**, 15, 41-45.

[61] Sladojevich, F.; Trabocchi, A.; Guarna, A. *J. Org. Chem.* **2007**, 72, 4254-4257.

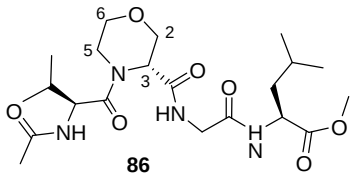
morpholine nucleus, we were interested in exploring the conformational role of such amino acid in model peptides. Specifically, in this chapter is reported the conformational analysis of model tetrapeptides containing either L- or D-morpholine-3-carboxylic acid **(66)** at position *i*+1, with the aim to assess the role of such amino acids in determining the conformational preferences of the peptide with respect to the parent peptide sequence bearing proline at the same position, as reported by Gellman et al.[11g]

3.2 Synthesis of model tetrapeptides

The synthesis of tetrapeptides **85** and **86** was achieved by means of Solid-Phase techniques using standard Fmoc protocol and HMBA (4-hydroxymethylbenzoic acid) resin, which allowed to perform a nucleophilic cleavage at the end of the synthesis, thus obtaining the title peptides with the C-terminus protected as methyl ester. Amino acid couplings were performed using DIPC (*N,N'*-diisopropylcarbodiimide)/HOBt (1-hydroxybenzotriazole) as activating mixture and DMF as solvent. Completion of coupling reaction was monitored using bromophenol blue as internal standard, as described by Krchnák et al.[62] In the case of valine coupling to the morpholine nucleus, reaction times were prolonged due to lowered reactivity at nitrogen atom of the latter. Despite the harsh conditions employed during the synthesis, the preparation of peptides afforded a mixture of the desired compound and Ac-Mor-Gly-Leu-OMe by-product, due to incomplete coupling. Truncated and title compounds were separated by semi-preparative HPLC, and peptides **85** and **86** were characterized by analytical HPLC, ESI-MS and NMR spectroscopy.



85



86

Figure 3.2 Model tetrapeptides containing L- (top) and D- (bottom) morpholine-3-carboxylic acids.

3.3 Conformational analysis

The conformational analysis of peptides **85** and **86** was carried out by NMR and IR spectroscopy.[63] As an additional aspect of the conformational study, the influence of

[62] Krchnák, V.; Vágner, J.; Sáfar, P.; Lebl, M. *Collect. Czech. Chem. Commun.* **1998**, 53, 2542-2548.
[63] (a) Belvisi, L.; Gennari, C.; Mielgo, A.; Potenza, D.; Scolastico, C. *Eur. J. Org. Chem.* **1999**, 389-400; (b) Yang, J.; Gellman, S.H. *J. Am. Chem. Soc.* **1998**, 120, 9090-9091; (c) Liang, G.B.; Rito, C.J.; Gellman, S.H. *J.*

different solvents on the structural organization of morpholine-containing turn peptides was investigated. The behaviour of amide protons was studied in $CDCl_3$, a relatively non-polar solvent, which is well-suited for evaluating intrinsic conformational propensities of small oligoamides, and CD_3CN, being a moderate hydrogen-bond acceptor with enhanced solvating properties, was used to test the strength of intramolecular hydrogen-bonds of amide protons. The conformational analysis consisted in the examination of the hydrogen-bonding pattern through the behaviour of amide proton chemical shifts as a function of the temperature and of increasing quantities of $[D_6]$DMSO as a competitive solvent, and in the analysis of the 3D topology by 2D NMR experiments.

3.3.1 Tetrapeptide containing L-morpholine-3-carboxylic acid

The model tetrapeptide containing the L-isomer of morpholine-3-COOH [**(*L*)-66**] resulted in a 2:3 mixture of *cis* and *trans* rotamers at the Val-morpholine amide bond. The *cis* rotamer was more populated (55%) in the more interactive CD_3CN solvent, in analogy with literature data indicating a growing amount of *cis* isomer of proline-containing peptides with increasing polarity of the solvent, as reported.[64] Temperature dependence experiments in $CDCl_3$ for amide protons belonging to the *trans* isomer showed medium temperature coefficients in absolute value (Table 3.1 and Figure 3.3, bottom left) and low chemical shift values (< 7 ppm), which suggested the absence of significant hydrogen-bonded structures in such solvent. The termodinamic profile of amide proton chemical shifts in CD_3CN showed little differences (Table 3.1 and Figure 3.3, bottom right), as Gly NH and Leu NH experienced lower temperature coefficient, and the chemical shifts suggested a non hydrogen-bonded profile for all the amide protons. This evidence was also supported by significant chemical shift deviations of the amide protons in $CDCl_3$ with increasing amounts of $[D_6]$DMSO as a competitive solvent (Figure 3.4, right), thus proving undoubtedly the absence of significant intramolecular hydrogen-bonding interactions for peptide **85**, *trans* isomer.

Table 3.1 Chemical shifts and temperature-dependent ^{1}H-NMR data for amide protons of peptide **85**.[a]

	Amide Proton	$CDCl_3$ δ	Δδ/ΔT	CD_3CN δ	Δδ/ΔT
Cis	ValNH	6.23	-5.57	6.67	-2.7
	GlyNH	8.03	-0.4	7.99	-4.3
	LeuNH	6.88	-4.76	7.17	-3.1
Trans	ValNH	6.32	-4.77	6.90	-4.1
	GlyNH	6.70	-3.23	6.90	-1.8
	LeuNH	6.39	-3.68	6.80	-0.7

[a] Chemical shifts (δ) are reported in ppm, and temperature coefficients (Δδ/ΔT) in ppb/K.

Am. Chem. Soc. **1992**, 114, 4440-4442; (d) Gellman, S.H.; Dado, G.P.; Liang, G.B.; Adams, B.R. *J. Am. Chem. Soc.* **1991**, 113, 1164-1173; (e) Boussard, G.; Marraud, M. *J. Am. Chem. Soc.* **1985**, 107, 1825-1828.

[64] (a) Higashijima, T.; Tasumi, M.; Miyazawa, T. *Biopolymers* **1977**, 16, 1259-1270; Madison, V.; Kopple, K.D. *J. Am. Chem. Soc.* **1980**, 102, 4855-4863.

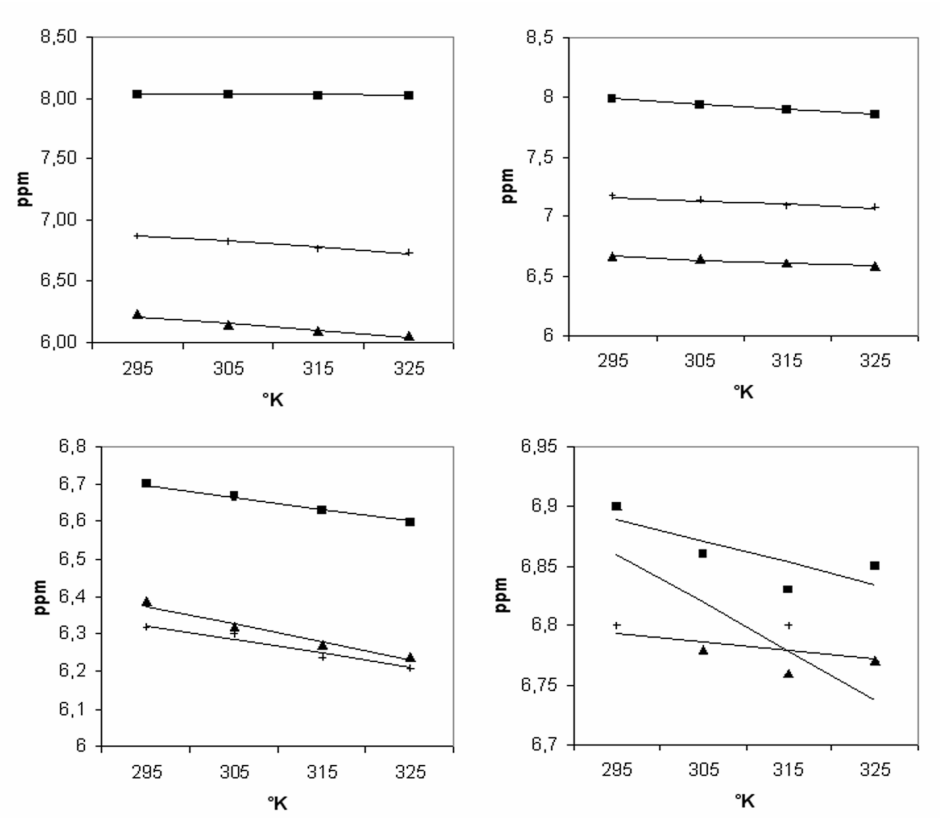


Figure 3.3 Variable temperature NH chemical shifts for L-peptide **85**, *cis*-rotamer in $CDCl_3$ (top left) and CD_3CN (top right), and *trans*-rotamer in $CDCl_3$ (bottom left) and CD_3CN (bottom right).

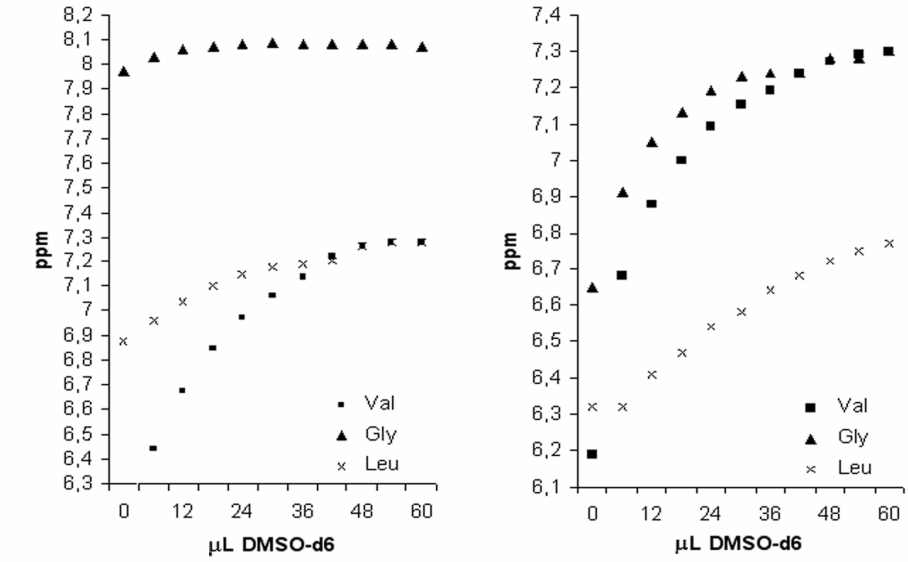


Figure 3.4 $[D_6]$DMSO titration of 1-2 mM $CHCl_3$ solutions of peptide **85** (left: *cis* rotamer; right: *trans* rotamer).

The indication that the *trans* isomer of peptide **85** does not nucleate into ordered structures was confirmed by ROESY experiments, which showed the absence of any relevant cross peaks diagnostic of reverse turn motifs. ROESY peaks between Val H-α (4.90) and H-5 (3.87) of the morpholine ring allowed to assign the *trans* geometry at the Val-Mor amide bond. Thus, experimental data indicated that replacing L-proline with L-morpholine causes the peptide to lose the equilibrating γ- and β-turn conformations, as suggested by Gellman et al. for the same rotamer of the model peptide Ac-Val-Pro-Gly-Leu-OMe,[11g] and to fall into random conformations.
The *cis* isomer of **85** displayed a more organized structure. 1D NMR data showed a deshielded chemical shift for Gly NH, which, in conjunction with a low temperature coefficient (Table 3.1), suggested the existence of a stable hydrogen-bonded structure in the less polar $CDCl_3$, and of equilibrating hydrogen-bonded and non hydrogen-bonded conformations in the more interactive CD_3CN, as indicated by higher $\Delta\delta/\Delta T$ values (Table 3.1, and Figure 3.3, top). Also, Leu NH proved to experience a slight deshield in CD_3CN, indicating a greater tendency to experience equilibrating hydrogen-bonded conformations, whereas Val NH showed chemical shift values typical of non hydrogen-bonded conformations in both solvents. 1D experiments in the presence of increasing amounts of $[D_6]$DMSO confirmed Gly NH as a strong intramolecular hydrogen-bond donor, as the chemical shift of such proton was not altered significantly within the range of 0.2 ppm (Figure 3.4, left). Also, Leu NH displayed a smaller deviation of the chemical shift as a function of increasing quantities of $[D_6]$DMSO compared to the parent *trans* isomer, as a consequence of equilibrating hydrogen-bonding states. Although Val H-α and H-3 of the morpholine scaffold were almost isocronous in the ROESY spectrum, the absence of cross peaks between Val H-α and H-5 was diagnostic of the *cis* geometry at the Val-Mor amide bond. Nevertheless, ROESY data in CD_3CN clearly showed a cross peak between Val H-α (4.38) and H-3 (4.61) which allowed to assign the *cis* rotamer. Also, this contact was consistent with the existence of a type VI β-turn stabilized by a ten-membered ring hydrogen bond between the acetyl group and Gly NH (Figure 3.5). This hydrogen-bond was in agreement with 1D experiments indicating a strong preference of Gly NH for hydrogen-bonded states, thus shifting the morpholine-based amino acid to the *i*+2 position of the turn. The equilibrating nature of Leu NH in hydrogen-bonding interactions was in agreement with interactions with either Mor C=O to generate a γ-turn or the acetyl group (Figure 3.5).

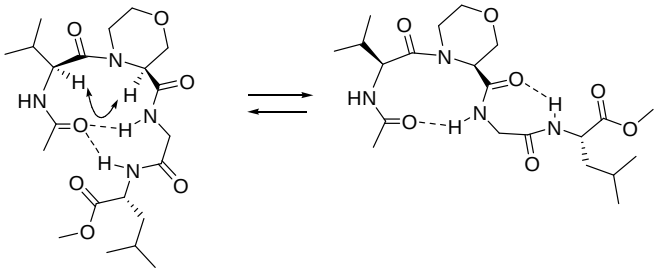


Figure 3.5 Reverse turn conformations of peptide **85**, *cis* isomer: the arrows indicate diagnostic ROESY peaks.

3.3.2 Tetrapeptide containing D-morpholine-3-carboxylic acid

1D NMR data of peptide **86** showed a single set of signals, indicating the existence of a unique rotamer. Sequential ROESY peaks between Val H-α and H-5 protons of the morpholine nucleus allowed to assign this structure as the *trans* isomer. Temperature dependence data showed a different behaviour of amide protons of peptide **86** with respect to the *trans* rotamer of diastereoisomer **85**, (Table 3.2 and Figure 3.6). The analysis of the chemical shifts of amide protons suggested marked differences with respect to the reference D-proline-containing peptide as reported by Gellman et al.,[11g] as Gly NH showed higher deshield of its chemical shift and higher propensity to establish hydrogen-bonds with respect to Leu NH, whereas Val NH did not display any hydrogen-bonding preference, in analogy with both rotamers of peptide **85**.

Table 3.2 Chemical shifts and temperature-dependent ^{1}H NMR data for amide protons of peptide **85**[a]

Amide	$CDCl_3$ δ	Δδ/ΔT	CD_3CN Δ	Δδ/ΔT
ValNH	6.68	-3.14	7.01	-4.2
GlyNH	7.45	-4.57	7.74	-3.3
LeuNH	6.78	-2.36	6.82	-1.1

[a] Chemical shifts (δ) are reported in ppm, and temperature coefficients (Δδ/ΔT) in ppb/K.

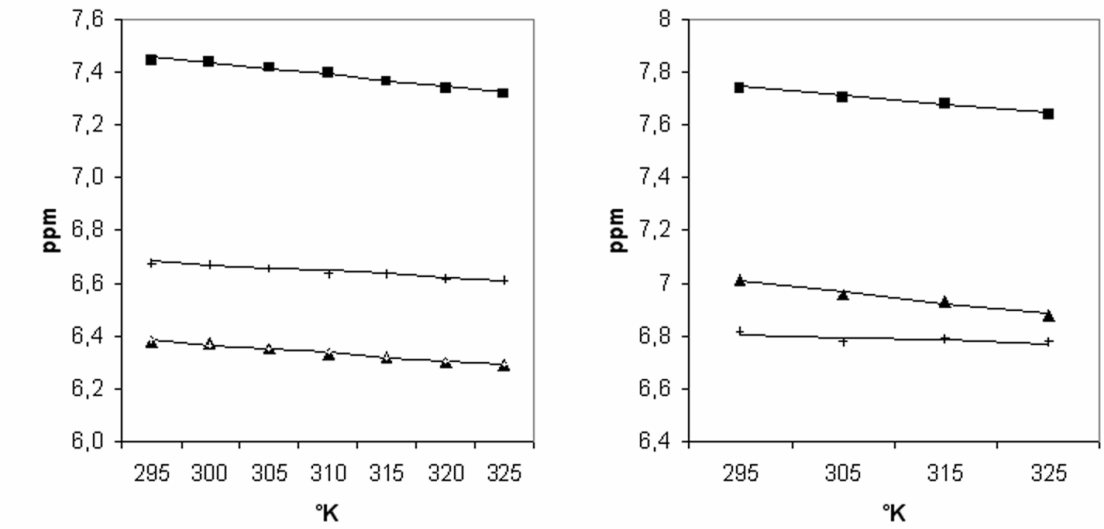


Figure 3.6 Variable temperature NH chemical shifts in $CDCl_3$ (left) and CD_3CN (right) for peptide **86**.

Temperature coefficient and chemical shift values of Gly NH indicated an equilibrium between hydrogen-bonded and non hydrogen-bonded states, suggesting the capability of nucleating reverse turn structures. [D_6]DMSO competition studies confirmed the marked tendency of Gly NH to act as a hydrogen-bond donor, as the conservation of the chemical shift within 0.2 ppm upon increasing quantities of the competitive solvent was observed (Figure 3.7). Also, Leu NH, although displaying lower chemical shift values compared to

Gly NH, showed a similar stability of the chemical shift in the presence of increasing amounts of [D_6]DMSO, whereas Val NH showed high deviation of the chemical shift in the presence of [D_6]DMSO, in accordance with δ and Δδ/ΔT values suggesting the absence of any involvement in intramolecular hydrogen-bonds. These data proved peptide **86** to fold into equilibrating γ- and β-turn structures stabilized by seven- and ten-membered ring intramolecular hydrogen-bonds established by Gly NH and Leu NH.

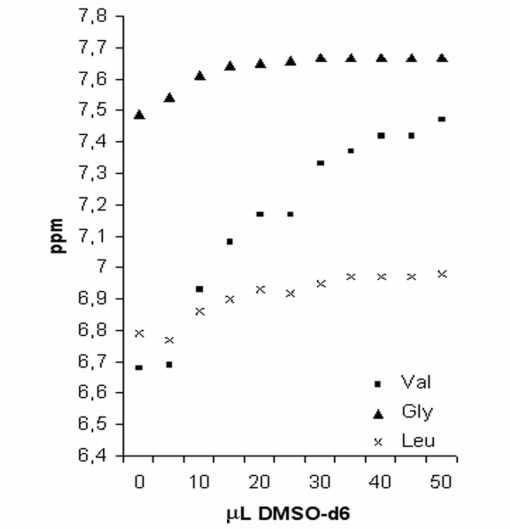


Figure 3.7 [D_6]DMSO titration of peptide **86**.

ROESY data of **86** in $CDCl_3$ showed a correlation between Val H-α (4.37) and H-5 (3.94), diagnostic of the *trans* configuration at the Val-Mor amide bond. Taking into account the behaviour of amide protons in 1D experiments, the absence of other relevant ROESY peaks were in agreement with turn conformations having the morpholine scaffold at *i*+2 position to give a type II β-turn, and at *i*+1 giving a type I' β-turn, as a consequence of the absence of the correlation between Gly NH with H-3 (Figure 3.8), in analogy with **85**, *cis* rotamer.

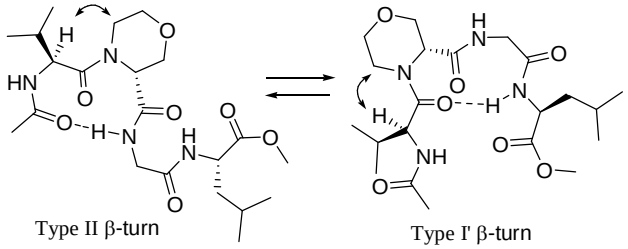


Figure 3.8 Reverse turn conformations of peptide **86** in $CDCl_3$: the arrows indicate selected ROESY peaks.

Thus, the replacement of D-pro with D-morpholine-3-COOH proved to alter completely the conformational preferences of the peptide, by destabilizing completely the β-hairpin structure typical of peptides containing D-proline at *i*+1 position of the turn in favour of conformations stabilized by Gly NH as a hydrogen-bond donor.
2D NMR data in CD_3CN suggested peptide **86** to assume a more compact structure, as a consequence of the modulation of the conformational asset moving towards a more

interactive solvent. Temperature dependence experiments showed higher hydrogen-bonding propensity for Leu NH, suggested by the smaller temperature coefficient in CD_3CN (Table 3.2 and Figure 3.6, right), whereas Val NH data did not exhibit any hydrogen-bonding preference, in analogy with both rotamers of peptide **85**. ROESY experiments in CD_3CN showed cross peaks typical of β-turn structures, as outlined in Figure 3.9.

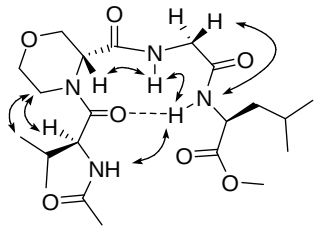


Figure 3.9 Reverse turn conformation of peptide **86** in CD_3CN: the arrows indicate selected ROESY peaks.

In particular, the correlation between Gly NH (7.72) and Leu NH (6.82), although weak, and the strong H-3 (4.80) / Gly NH cross peak suggested the existence of a β-turn stabilized by a ten-membered ring hydrogen-bond between Val carbonyl group and Leu NH in equilibrium with a γ-turn stabilized by a 7-membered ring hydrogen-bond between Gly NH and Val C=O. Also, a key cross strand ROESY peak between Leu NH and Gly NH was observed, indicating the proximity of the two amides as a consequence of the folded b-turn conformations. The conformational preferences of peptide **86** for turn structures in CD_3CN were thus different than in $CDCl_3$, probably by virtue of the more interactive solvent, and this conformational profile was not completely similar to the reference D-proline-containing peptide, as the replacement with D-morpholine-3-COOH caused the peptide to lose the β-hairpin structure and to equilibrate between γ- and β-turn structures as a function of the solvent.
The conformational preferences of D-morpholine-3-carboxylic acid-containing tetrapeptide **86** were also studied in a $[D_6]$DMSO solution, so as to detect any intramolecular hydrogen-bond in a solvent able to disrupt the intramolecular interactions in flexible peptidomimetics. 1D NMR data showed a minimal amount of the *cis* isomer, consistent with the higher polarity of the solvation system. Also, 2D spectra indicated the existence of equilibrating reverse turn structures in line with the conformational behaviour as observed in CD_3CN (see Figure 3.9). Specifically, ROESY data suggested tetrapeptide **86** to fold into a β-turn conformation having D-Mor at *i*+1 position of the turn, as showed by the key correlations of Gly NH (7.93) with H-3 (4.75) and Leu NH (8.10), and of Gly H-α with Leu NH.

3.3.3 IR data

IR experiments supported the evidences that D-Mor is more prone to nucleate folded structures with respect to the corresponding enantiomer. In fact, peptide **85** showed a sharp peak at 3400 cm^{-1} typical of non hydrogen-bonded states, and exhibiting a small shoulder in the 3300 cm^{-1} attributable to the well organized hydrogen-bonded cis isomer (Figure 3.10). On the contrary, peptide **86** showed a stronger stretch peak in the region

typical of hydrogen-bonded states, with a minor shoulder attributable to Val NH proton. Thus, IR experiments confirmed the different conformational profile of the same model peptide having embedded two morpholine-based amino acids of opposite configurations.

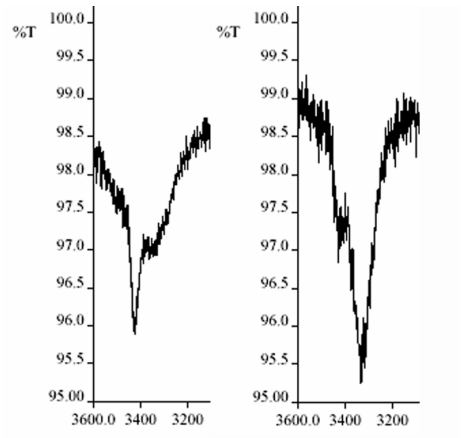


Figure 3.10 IR expansion of the amide proton stretch regions for peptide **85** (left) and **86** (right).

3.4 Molecular modelling

Initially, the conformational preference of the monomeric acetyl-morpholine-3-methylamide as a model compound was investigated by computational methods to assess the most stable structures of the morpholine nucleus, thus both *cis* and *trans* configuration at the acetyl-morpholine amide bond were evaluated in the case of the 3-methylamide group at equatorial or axial position. AM1 semiempirical method was used to optimize the global minimum conformer. The geometry of the most abundant minimum energy conformer was successively subjected to *ab initio* single point energy calculation at the 6-31G*/ HF level of quantum chemical theory. In both *cis* and *trans* structures, the axial orientation of the methylamide group was found to be more stable than the corresponding equatorial of about 8 kcal/mol, indicating a favourable stereoelectronic effect irrespective of the type of rotameric structure. A lower energetic difference of about 3.2 kcal/mol in absolute value was observed between the *cis* and *trans* structures as a consequence of strain relief for the latter case. These calculations indicated the morpholine ring to show a lower steric hindrance in the conformation having the carboxamido group at the axial position by virtue of the relief of diaxial destabilizing interactions given by the presence of the oxygen atom at position 4.

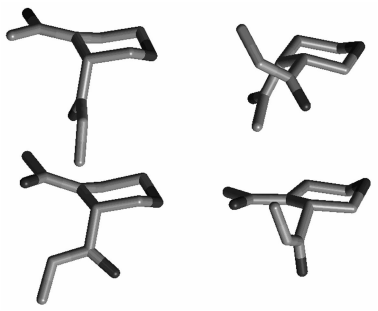


Figure 3.11 Top right: axial, *cis* conformer, E_{rel} = +3.16 kcal/mol; Top left: equatorial, *cis* conformer, E_{rel} = +12.14 kcal/mol; axial, *trans* conformer, E_{rel} = 0 kcal/mol; equatorial, *trans* conformer, E_{rel} = +8.89 kcal/mol.

Molecular mechanics calculations using OPLSA*[65] as a force field were carried out to gain further insights into the conformational space accessible for peptides **85** and **86**, using full unconstrained Monte Carlo conformational search.[66] The *cis* rotamer of compound **85** was sampled using the implicit $CDCl_3$ GB/SA solvation system. The global minimum conformer (Figure 3.11) resulted in a folded structure having the morpholine nucleus shifted at position *i*+2 of a β-turn and bearing the C=O group at position 3-axial, in analogy with the folding propensity of pipecolic and azapipecolic acid-containing peptides, as reported.[67] The β-turn structure was stabilized by a 10-membered ring hydrogen-bond between Gly NH and the acetyl group, in accordance with NMR data for such amide proton, and also an additional hydrogen-bonding interaction with acetyl C=O was found for Leu NH, in agreement with NMR data of this amide proton indicating a partial hydrogen-bonding character. Also, dihedral angles of the global minimum conformer corresponding to φ and ψ values at positions *i*+1 and *i*+2 suggested L-morpholine-3-COOH to induce a type-VIa β-turn (see Table 3.3, entries 1 and 9).

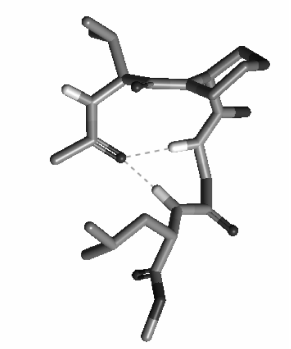


Figure 3.12 Global minimum conformer of **85**, *cis* isomer.

[65] Jorgensen, W.L.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1988**, 110, 1657-1666.
[66] Chang, G.; Guida, W.C.; Still, W.C. *J. Am. Chem. Soc.* **1989**, 111, 4379-4386
[67] Didierjean, C.; Aubry, A.; Wyckaert, F.; Boussard, G. *J. Peptide Res.* **2000**, 55, 308–317.

Table 3.3 Dihedral angle values for **85** and **86**, low-energy conformers of different GB/SA solvation systems and reference values of β-turn types

Entry		φ*i*+1	ψ*i*+1	φ*i*+2	ψ*i*+2
1	**85**, $CHCl_3$	-65.5	140.4	-99.0	28.8
2	**86**, $CHCl_3$[a]	66.0	3.6	105.0	-14.3
3	**86**, $CHCl_3$[b]	-71.6	115.7	66.0	3.6
4	**86**, H_2O[c]	74.3	-128.5	-114.1	14.3
5	**86**, H_2O[d]	-67.3	129.8	87.9	-8.2
6	Type I'	60	30	90	0
7	Type II	-60	120	80	0
8	Type II'	60	-120	-80	0
9	Type Via	-60	120	-90	0

[a] Lowest-energy conformer having D-Mor at position *i*+1. [b] Global minimum conformer having D-Mor at position *i*+2. [c] Global minimum conformer having D-Mor at position *i*+1. [d] Lowest-energy conformer having D-Mor at position *i*+2.

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Conformational preferences of the *trans* isomer of tetrapeptide **86** were evaluated using both the chloroform and water GB/SA solvation system. The calculation using the chloroform parameters resulted in a global minimum conformer displaying a compact organization stabilized by multiple hydrogen-bonds.

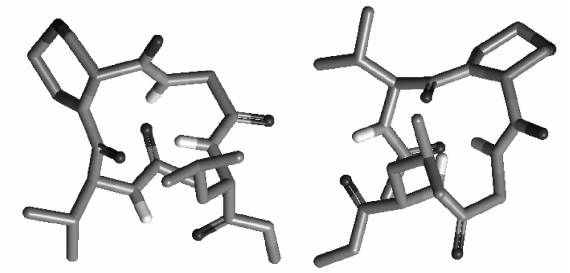

Figure 3.13. Global minimum conformer of **86**, *trans* isomer after MCMM calculation using $CHCl_3$ GB/SA solvation system (left), and 90° clockwise rotated view (right).

Dihedral angles of the global minimum conformer indicated a type II β-turn, whereas low-energy conformer having D-Mor at *i*+1 position folded into a type I' β-turn, in perfect accordance with the hypothesis, as shown in Figure 3.8. Interestingly, when the calculation was carried out using implicit water GB/SA solvation system, the global minimum conformer consisted in a β-turn structure having the morpholine nucleus at position *i*+1 of a 10-membered ring hydrogen-bonded β-turn, in accordance with NMR data in CD_3CN and [D_6]DMSO as solvent. Thus, the calculation was consistent with the observation that moving to more interactive solvents the heterochiral peptide folded in a β-turn conformation similar to D-proline β-turns.

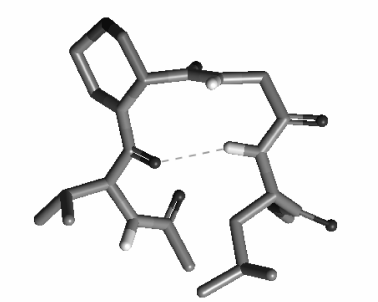

Figure 3.14 Global minimum conformer of **86**, *trans* isomer after MCMM calculation using H_2O GB/SA solvation system.

The dihedral angles corresponding to φ and ψ values at positions *i*+1 and *i*+2 suggested D-Mor to induce a distorted type-II' β-turn structure, as a consequence of the axial orientation of the carbonyl group at position 3 of the morpholine ring. The second low-energy conformer found in water GB/SA solvation system resulted in a structure having the morpholine ring at position *i*+2 of a β-turn stabilized by a 10-membered ring between Gly NH and the acetyl group, in analogy with the results found in $CHCl_3$

3.5 Conclusions

The conformational analysis of the diastereomeric peptides **85** and **86**, differing by the configuration of the morpholine-3-COOH component at the *i*+1 position of the designed model structure, indicated the nucleation of turn conformations to be influenced by the configuration of the cyclic amino acid acting as the turn inducer and by the solvent polarity. Although for peptides **85** and **86** the relationship between the configuration of the cyclic amino acid at position *i*+1 and the turn propensity was maintained with respect to the reference proline-containing peptides, marked differences in the type of folded structures were observed. Specifically, L-morpholine-3-carboxylic acid displayed two rotamers at the Val-Mor amide bond. The *trans* isomer of **1** did not experience any turn structures, nor any intramolecular hydrogen-bonds, whereas the *cis* isomer showed a strong preference for a type VI β-turn structure, thus providing a different conformational asset with respect to the β-turn structure as reported for the reference L-proline model peptide. Peptide **85**, having D-morpholine-3-carboxylic acid at the *i*+1 position, in analogy with the reference D-proline model peptide, proved to nucleate a more organized turn structure, although with significant differences. In fact, Val NH of peptide **86** never experienced any intramolecular hydrogen-bond necessary to stabilize a β-hairpin motif, as reported for the reference peptide. Moreover, peptide **86** showed equilibrating conformations ranging from γ- to β-turns as a function of solvent polarity, indicating D-morpholine-3-COOH as the more effective enantiomer in the turn inducing capability. The marked differences to D-proline in provoking structural organization of model peptides, as a consequence of the six-membered ring structure containing two heteroatoms, were not entirely predictable, and are of special importance as a guide for the design of reverse turn mimetics having the morpholine scaffold embedded in the peptide backbone

3.6 Experimental for chapter 3

IR methods. IR spectra were recorded on 1-2 mM chloroform solutions with a Perkin FT-IR spectrophotometer, using 128 scans. The pure solvent spectrum for a particular solution was subtracted from the sample spectrum prior to analysis. Peaks in the amide NH stretch region were baseline corrected, and analyzed without further manipulation.
NMR methods. NMR spectra were performed on a Varian Mercury 400Plus spectrometer operating at 400 MHz for ^{1}H. The spectra were obtained in 3-5 mM $CDCl_3$ or CD_3CN solutions where aggregation was not significant. One-dimensional ^{1}H NMR spectra for determining temperature coefficients were obtained at 295-325 K with increments of 5-10 K. Sample temperatures were controlled with the variable-temperature unit of the instrument. Proton signals were assigned via TOCSY spectra, and ROESY spectra provided the data used in the conformational analyses. TOCSY spectra were recorded with 2048 points in *t*1, 256 points in *t*2, and 8 scans per *t*2 increment. ROESY spectra were recorded with a similar number of *t*1 and *t*2 points unless otherwise noted, and 32 per *t*2 increment. Sample concentrations for two-dimensional spectra were respectively for **85** and **86**: 3.8 and 4.4 mM in $CDCl_3$, 4.4 and 6.6 mM in CD_3CN, and 4.4 mM in [D_6]DMSO for peptide **2**.
Computational methods. Molecular mechanics calculations were carried out on a SGI IRIX 6.5 workstation, using MacroModel (v6.5) molecular modelling software,[68] with OPLSA* as a force field[19] and the implicit chloroform GB/SA solvation system.[69] Monte Carlo conformational search[20] was carried out without imposing any constraint and including amide bonds among all rotatable bonds. A ring closure was defined for the six- and seven-membered ring of BGS and Bgs scaffolds. 2000 structures were generated and minimised until the gradient was less than 0.05 kJ/Å·mol using the TNCG gradient implemented in MacroModel.[70] All the conformers having an energy of 6 kcal/mol above the global minimum conformer were discarded. *Cis* and *trans* isomers of peptide **85** were separately analyzed. Calculations on the model compound were performed using SPARTAN version 5.147 running on a SGI IRIX 6.5 workstation. Conformational searches of Ac-Mor-NHEt were carried out using Monte Carlo method within MMFF94 force field,[71] and the AM1 semiempirical method[72] was used to optimize the global minimum conformer. The geometry of the most abundant minimum energy conformer was successively subjected to ab initio single point calculation of the electronic properties at the 6-31G*/HF level[73] of quantum chemical theory.

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Ac-Val-l-Mor-Gly-Leu-OMe (85). Pure peptide showed HPLC peak at t_R = 16.2 min (93% HPLC purity) using 0% acetonitrile/5 min, 0 – 10% acetonitrile/5 min, then 10 – 90% acetonitrile/20 min as gradient and ESI-MS peaks of *m*/*z* 457.09 (M^+ + 1, 8), 479.27 (M^+ + Na, 100), 495.27 (M^+ + K, 35). ^{1}H NMR data of the cis and trans conformers are shown in Table 3.4.

Ac-Val-d-Mor-Gly-Leu-OMe (86). Pure peptide showed HPLC peak at t_R = 16.7 min (96% HPLC purity) using 0% acetonitrile/5 min, 0 – 10% acetonitrile/5 min, then 10 – 90% acetonitrile/20 min as gradient and ESI-MS peaks of *m*/*z* 457.09 (M^+ + 1, 6), 479.27 (M^+ + Na, 100), 495.27 (M^+ + K, 41). ^{1}H NMR data of the unique conformer is shown in Table 3.5.

Table 3.4 Proton chemical shifts of **85**, *cis* and *trans* conformers in $CDCl_3$ and CD_3CN at 298 K

	Cis		*Trans*	
	δ ($CDCl_3$)	**δ (CD_3CN)**	**δ($CDCl_3$)**	**δ (CD_3CN)**
H-2	4.74, 3.49	4.55, 3.48	4.41, 3.65	4.30, 3.58
H-3	4.46	4.61	4.98	4.93
H-5	4.48, 3.05	4.35, 2.92	3.92, 3.87	4.42, 3.53
H-6	3.95, 3.43	3.85, 3.54	4.40, 3.56	4.38, 3.43
Gly NH	7.97	7.98	6.65	6.90
Leu NH	6.89	6.80	6.32	7.21
Val NH	6.19	6.91	6.37	6.72
Gly H-α	4.05, 4.01	3.85	4.10, 3.91	3.83
Leu H-α	4.63	4.47	4.63	4.45
Leu H-β	1.63, 1.53	1.65, 1.60	1.65, 1.55	1.64, 1.61
Leu H-γ	1.63	1.65	1.65	1.64
Leu H-δ	0.94	0.96	0.94	0.91
Val H-α	4.47	4.38	4.90	4.70
Val H-β	2.01	2.01	2.10	2.02
Val H-γ	1.06	1.05	1.01, 0.91	0.99
-OCH_3	3.70	3.66	3.74	3.68
CH_3CO	2.02	1.95	2.07	1.98

Table 3.5 Proton chemical shifts of **86**, *trans* conformer in $CDCl_3$ and CD_3CN at 298 K.

	δ ($CDCl_3$)	**δ (CD_3CN)**	**δ ($DMSO-d_6$)**
H-2	4.62, 3.58	4.48, 3.52	4.28
H-3	4.89	4.80	4.75
H-5	4.02, 3.72	3.94, 3.52	3.85, 3.22
H-6	3.84, 3.51	3.89, 3.46	3.82, 3.40
Gly NH	7.48	7.72	7.93
Leu NH	6.69	6.82	8.10
Val NH	6.79	7.00	8.18
Gly H-α	4.09, 3.91	3.81	3.85, 3.78
Leu H-α	4.60	4.40	4.44

Leu H-β	1.62	1.60	1.60, 1.46
Leu H-γ	1.62	1.60	1.60
Leu H-δ	0.91	0.94, 0.91	0.93, 0.90
Val H-α	4.42	4.37	4.22
Val H-β	2.07	1.99	1.96
Val H-γ	1.08, 1.03	1.05, 1.01	0.91
$-OCH_3$	3.72	3.68	3.78
CH_3CO	2.05	2.11	1.84

Table 3.6 Variable temperature 1D experiments for *Ac-Val-L-morph-Gly-Leu-OMe*

		$CDCl_3$			**CD_3CN**		
	Temp,°K	**Δ NH_V**	**Δ NH_G**	**δ NH_L**	**δ NH_V**	**δ NH_G**	**δ NH_L**
	295	6.39	6.70	6.32	6.90	6.90	6.80
	298	6.35	6.68	6.30	-	-	-
Trans	305	6.32	6.67	6.30	6.78	6.86	6.78
	315	6.27	6.63	6.24	6.76	6.83	6.80
	325	6.24	6.60	6.21	6.77	6.85	6.77
	295	6.23	8.03	6.88	6.67	7.99	7.17
	298	6.20	8.03	6.86	-	-	-
Cis	305	6.15	8.03	6.83	6.64	7.94	7.14
	315	6.10	8.02	6.77	6.61	7.90	7.10
	325	6.06	8.02	6.74	6.59	7.86	7.08

Calculation of Δδ(NH)/ΔT of trans-rotamer in $CDCl_3$
δ NH_V = -0.00477 (T) + 7.78 (R = -0.981) Δδ(NH)/ΔT = -4.77 ppb / °K
δ NH_G = -0.00323 (T) + 7.65 (R = -0.993) Δδ(NH)/ΔT = -3.23 ppb / °K
δ NH_L = -0.00368 (T) + 7.41 (R = -0.977) Δδ(NH)/ΔT = -3.68 ppb / °K
Calculation of Δδ(NH)/ΔT of trans-rotamer in CD_3CN
δ NH_V = -0.0041 (T) + 8.07 (R = -0.808) Δδ(NH)/ΔT = -4.1 ppb / °K
δ NH_G = -0.0018 (T) + 7.42 (R = -0.789) Δδ(NH)/ΔT = -1.8 ppb / °K
δ NH_L = -0.0007 (T) + 7.00 (R = -0.602) Δδ(NH)/ΔT = -0.7 ppb / °K
Calculation of Δδ(NH)/ΔT of cis-rotamer in $CDCl_3$
δ NH_V = -0.00557 (T) + 7.86 (R = -0.989) Δδ(NH)/ΔT = -5.57 ppb / °K
δ NH_G = -0.00040 (T) + 8.15 (R = -0.913) Δδ(NH)/ΔT = -0.40 ppb / °K
δ NH_L = -0.00476 (T) + 8.28 (R = -0.993) Δδ(NH)/ΔT = -4.76 ppb / °K
Calculation of Δδ(NH)/ΔT of cis-rotamer in CD_3CN
δ NH_V = -0.0027 (T) + 7.46 (R = -0.996) Δδ(NH)/ΔT = -2.7 ppb / °K
δ NH_G = -0.0043 (T) + 9.26 (R = -0.998) Δδ(NH)/ΔT = -4.3 ppb / °K
δ NH_L = -0.0031 (T) + 8.08 (R = -0.993) Δδ(NH)/ΔT = -3.1 ppb / °K

Table 3.7 Variable temperature 1D experiments for *Ac-Val-D-morph-Gly-Leu-OMe*

	$CDCl_3$			CD_3CN		
Temp, °K	**δ NH_V**	**δ NH_G**	**δ NH_L**	**δ NH_V**	**δ NH_G**	**δ NH_L**
295	6.38	7.45	6.68	7.01	7.74	6.82
300	6.37	7.44	6.67			
305	6.35	7.42	6.66	6.96	7.71	6.78
310	6.33	7.40	6.64			
315	6.32	7.37	6.64	6.93	7.68	6.79
320	6.30	7.34	6.62			
325	6.29	7.32	6.61	6.88	7.64	6.78

Calculation of Δδ*(NH)*/Δ*T* in $CDCl_3$
δ NH_V = -0.00314 (T) + 7.31 (R = -0.996) Δδ(NH)/ΔT = -3.14 ppb / °K
δ NH_G = -0.00457 (T) + 8.81 (R = -0.991) Δδ(NH)/ΔT = -4.57 ppb / °K
δ NH_L = -0.00236 (T) + 7.38 (R = -0.990) Δδ(NH)/ΔT = -2.36 ppb / °K
Calculation of Δδ*(NH)*/Δ*T* in CD_3CN
δ NH_V = -0.0042 (T) + 8.25 (R = -0.996) Δδ(NH)/ΔT = -4.2 ppb / °K
δ NH_G = -0.0033 (T) + 8.72 (R = -0.997) Δδ(NH)/ΔT = -3.3 ppb / °K
δ NH_L = -0.0011 (T) + 7.13 (R = -0.752) Δδ(NH)/ΔT = -1.1 ppb / °K

Table 3.8 1D competition studies with [D_6]DMSO for *Ac-Val-L-morph-Gly-Leu-OMe*

µL [D_6]DMSO	***Cis***			***Trans***		
	δ NH_V	**Δ NH_G**	**δ NH_L**	**δ NH_V**	**δ NH_G**	**δ NH_L**
0	6.19	7.97	6.88	6.19	6.65	6.32
6	6.44	8.03	6.96	6.68	6.91	6.32
12	6.68	8.06	7.04	6.88	7.05	6.41
18	6.85	8.07	7.1	7.00	7.13	6.47
24	6.97	8.08	7.15	7.09	7.19	6.54
30	7.06	8.09	7.18	7.15	7.23	6.58
36	7.14	8.08	7.19	7.19	7.24	6.64
42	7.22	8.08	7.2	7.24	7.24	6.68
48	7.26	8.08	7.26	7.27	7.28	6.72
54	7.28	8.08	7.28	7.29	7.28	6.75
60	7.28	8.07	7.28	7.30	7.30	6.77

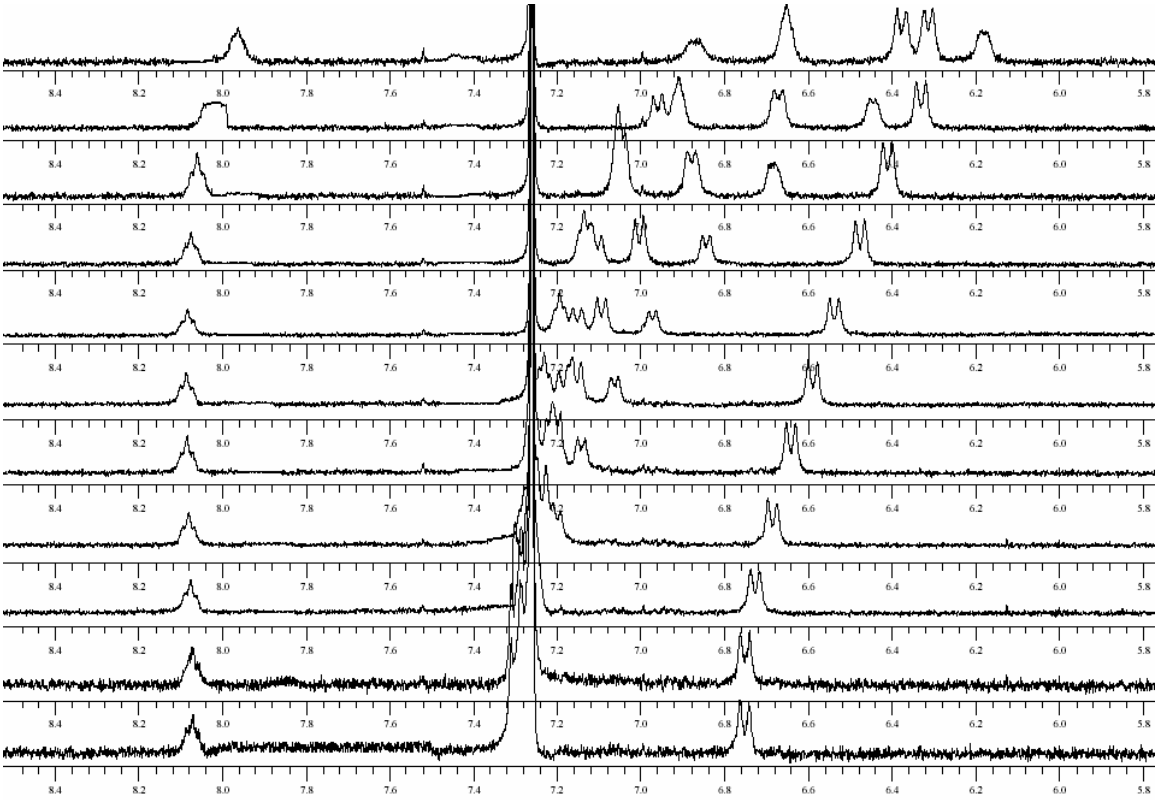


Figure 3.15 Stacked ^{1}H NMR plots of **85**.

Table 3.9 1D competition studies with [D_6]DMSO for *Ac-Val-D-morph-Gly-Leu-OMe*

µL [D_6]DMSO	δ NH_V	δ NH_G	δ NH_L
0	6.68	7.49	6.79
5	6.69	7.54	6.77
10	6.93	7.61	6.86
15	7.08	7.64	6.9
20	7.17	7.65	6.93
25	7.17	7.66	6.92
30	7.33	7.67	6.95
35	7.37	7.67	6.97
40	7.42	7.67	6.97
45	7.42	7.67	6.97
50	7.47	7.67	6.98

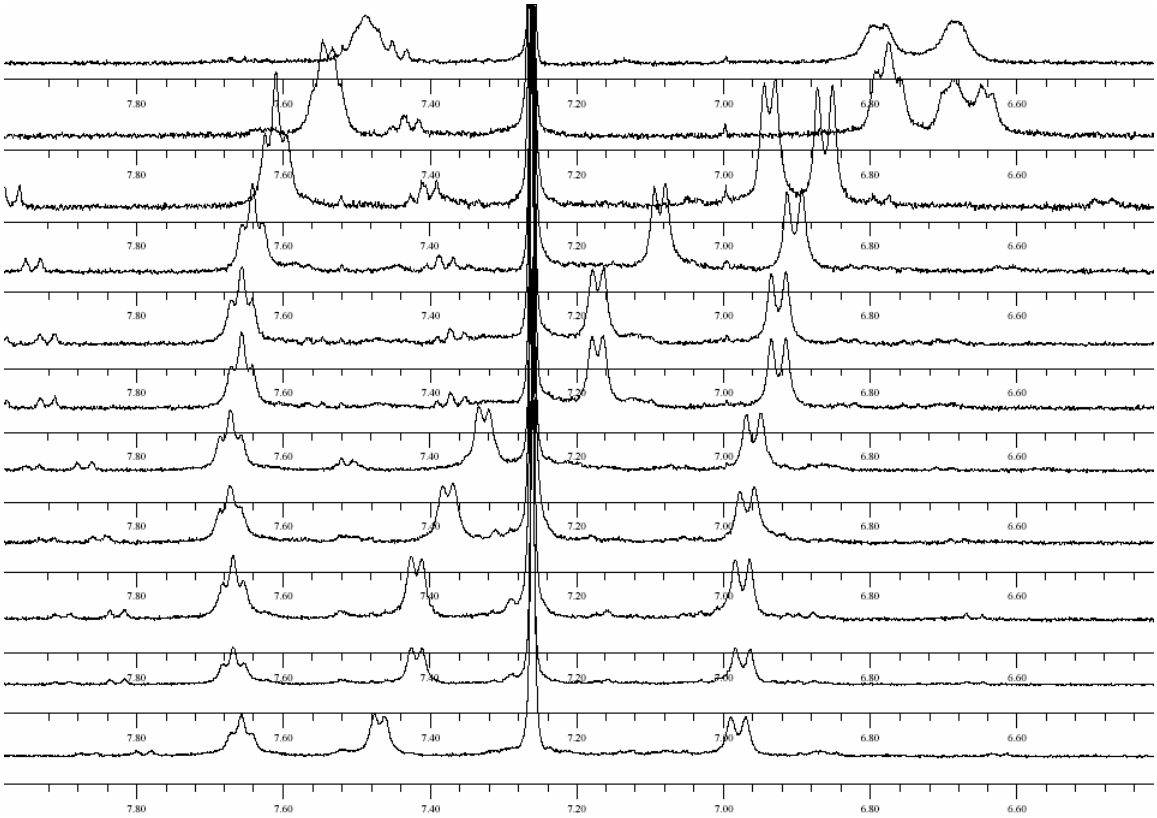
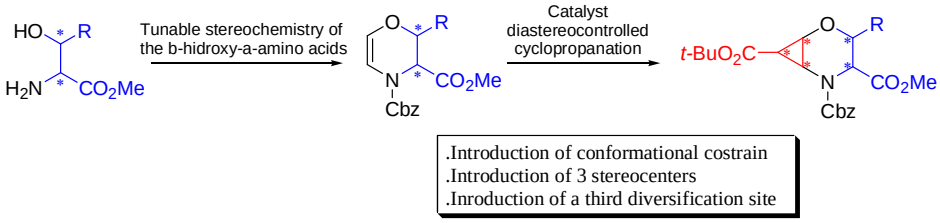


Figure 3.16 Stacked ^{1}H NMR plots of **86**.

4 Stereoselective cyclopropanation of oxazines to access new morpholine-based scaffolds

"Sladojevich, F.; Trabocchi, A.; Guarna, A.
Organic & Biomolecular Chemistry, **2008**, 6, 3328-3336"

ABSTRACT



A general strategy for the synthesis of novel, orthogonally protected scaffolds based on the unique 2-oxa-5-azabicyclo[4.1.0]heptane structure is presented. The described reaction sequence takes advantage of easily available starting materials such as serine and threonine and leads to stereochemically dense structures in few, high-yielding synthetic steps. We show how the stereochemistry can be easily tuned by starting from different β-hydroxy-α-amino acids and also by means of a transition metal-catalyzed cyclopropanation step. These compounds find application as constrained templates for the construction of geometrically diversified libraries of compounds.

4.1 Introduction

One of the initial steps in the drug discovery process is the identification of leads which bind to receptors or other targets of interest. To address this, a common and established approach is the screening of libraries of compounds. While combinatorial chemistry initially tended towards the synthesis of very large libraries of structurally similar products, nowadays this initial emphasis on creating mixtures of very large numbers of structures is giving way to a more measured approach based on arrays of fewer, well-characterized compounds.[74] This is particularly noticeable in the move towards the synthesis of complex and highly diversified mixtures of molecules that bear a structural resemblance to approved natural-product-based drugs[75] or to "privileged medicinal scaffolds".[76] There is a strong drive towards the generation of new chemo-types, displaying increasing complexity and possessing features that can be related to pharmacologically relevant structures.[77] Among the possible alternatives, nitrogen-containing-heterocycles with a saturated backbone have attracted considerable attention in the design of biologically active products.[78,79] Most marketed compounds and several promising leads fall into this category, and the discovery of small-molecular-weight scaffolds with a high degree of diversity belonging to this family is a tool of primary importance in the drug discovery process.[80] Among the various structures proposed by medicinal chemists, the morpholine ring represents a common motif.[81] Many carbon-substituted morpholines display biological

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For recent reviews, see:
(b) Duarte, Carolina D.; Barreiro, Eliezer J.; Fraga, Carlos A. M. Privileged structures: a useful concept for the rational design of new lead drug candidates. *Mini-Reviews in Medicinal Chemistry* **2007**, 7 (11), 1108-1119.
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activity and can find application as antidepressants,[82] appetite suppressants,[83] antioxidants,[84] etc. Some selected examples are reported in Figure 4.1

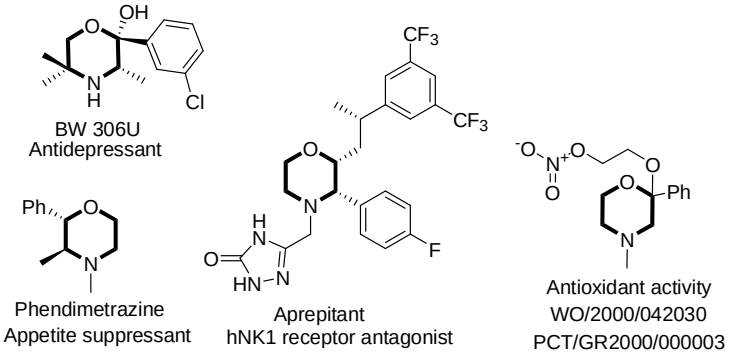

Figure 4.1 Selected, biologically relevant, morpholine-based compounds

During our ongoing research program toward the development of costrained, morpholine-based platforms for medicinal chemistry,[85] we developed the idea of rigidifying a chiral morpholine structure through fusion to a functionalized cyclopropane ring. This approach provides access to a stereochemically-rich and rigid backbone, allowing us to generate different scaffolds by means of geometric variation of the scaffold itself.[86] Herein we present the new template 2-oxa-5-aza-bicyclo[4.1.0]heptane heterocycle (Scheme 4.1) and we demonstrate a general synthetic strategy which can guarantee the introduction of diversification positions with a high degree of diastereocontrol. The concomitant presence of an oxygen and a nitrogen in the morpholine ring can be exploited in a wide range of retrosynthetical analysis which allow easy control of the stereochemistry of the carbon atoms, especially starting from readily available amino acid derivatives. In order to obtain the 2-oxa-5-aza-bicyclo[4.1.0]heptane skeleton we planned to use amino acid-derived dihydroxazine structures[87] (Scheme 4.1) as substrates for a diastereoselective, transition

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[83] Common appetite suppressants are Phendimetrazine and Phenmetrazine.

[84] (a) Guilloneau, C.; Charton, Y.; Ginot, Y.; Fouquier-d'Herouel, M.; Bertrand, M.; Lockhart, B.; Lestage, P., Goldstein, S.; *Eur. J. Med. Chem.* **2003**, 38, 1-11. (b) Chrysselis, M.C.; Rekka, E.A.; Siskou, I.C.; Kourounakis, P.N. *J. Med. Chem.* **2002**, 45, 5406. (c) Chrysselis, M.C.; Rekka, E.A.; Kourounakis, P.N. *J. Med. Chem.* **2000**, 43, 609-612.

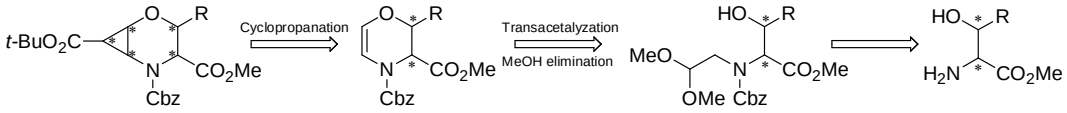
[85] (a) Cini, N.; Danieli, E.; Menchi, G.; Trabocchi, A.; Bottoncetti, A.; Raspanti, S; Pupi, A; Guarna, A. *Bioorganic & Medicinal Chemistry* **2006**, 14, 5110-5120. (b) Mannino, C.; Nievo, M.; Machetti, F.; Papakyriakou, A.; Calderone, V.; Fragai, M.; Guarna, A. *Bioorganic & Medicinal Chemistry* **2006** 14 (22), 7392-7403. (c) Guarna, A.; Cozzolino, F.; Torcia, M.; Garaci, E. Pharmaceutical compositions for the treatment of diseases related to neurotrophins. PCT Int. Appl. (2003). (d) Guarna, A.; Guidi, A.; Machetti, F.; Menchi, G.; Occhiato, E. G.; Scarpi, D.; Sisi, S.; Trabocchi, A. *J. Org. Chem.* **1999**, 64 , 7347-7364.

[86] For examples of the concept of geometrical diversity, see: (a) Burke, M.D., Berger E.M., Schreiber, S.L.; *J. Am. Chem. Soc*., **2004**, 126, 14095-14104. (b) Gierasch; T.M., Shi Z.; Verdine, G.L.; *Org. Lett.*, **2003**, 5, 621-624. (c) Paterson, I.; Temal-Laieb, T. *Org. Lett.* **2002**, *4*, 2473-2476. (d) Misske, A. M.; Hoffmann, H. M. R. *Chem. Eur. J.* **2000**, *6*, 3313-3320. (e) Paterson, I.; Scott, J. P. *J. Chem. Soc.*, *Perkin Trans. 1* **1999**, 1003-1014. Annis, D. A.; Helluin, O.; Jacobsen, E. N *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 1907-1909. Sutherlin, D. P.; Armstrong, R. W. *J. Org. Chem.* **1997**, *62*, 5267-5283.

[87] (*a*) F. Sladojevich, A.Trabocchi and A. Guarna, *J.Org. Chem.*, 2007, **72**, 110 4254; (*b*) A. Guarna, A. Trabocchi, G. Menchi, C. Lalli, F. Sladojevich and N. Cini, *Eur Pat. Appl.* PCT/EP2008/054750, April 18th 2008.

metal catalyzed cyclopropanation using diazo-acetates. The cyclopropanation creates three new adjacent stereocenters and at the same time introduces a strong conformational constraint. This approach allows the synthesis of structures bearing functionalizable groups in different, reciprocal stereochemical relationship. Chirality is first introduced using readily available, enantiomerically pure β-hydroxy-α-amino acids as starting materials and, in second instance, through a cyclopropanation step, whose stereochemical outcome is generally governed by the stereochemistry of the ligand used.

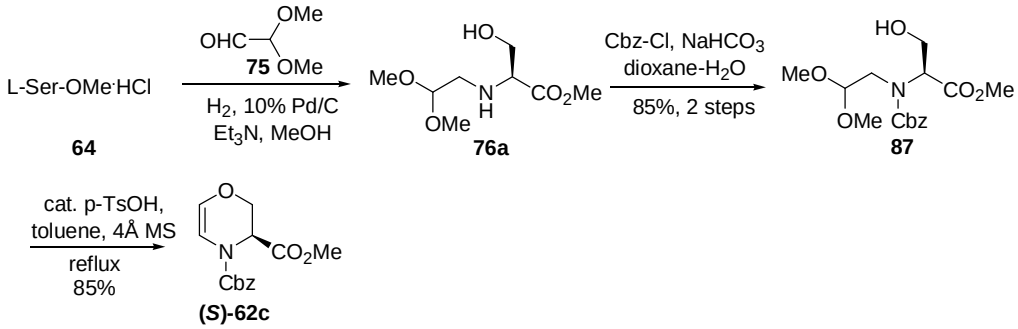
Scheme 4.1 Retrosynthetic analysis of 2-oxa-5-aza-bicyclo[4.1.0]heptane scaffolds



4.2 Preparation of dihydroxazine scaffolds

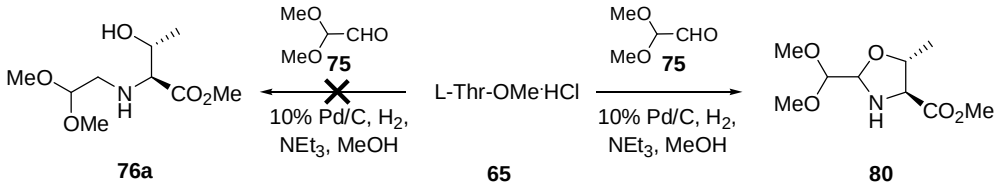
The strategy was developed using serine and threonine as starting materials and the Cbz as nitrogen protecting group. The serine derived dihydroxazine **62c** was prepared starting from amine **76a**,[14] which was protected using Cbz-Cl and then cyclized after treatment with p-TSOH/MS 4Å in refluxing toluene, in order to promote acid catalyzed trans-acetalyzation and subsequent elimination of MeOH (Scheme 4.2).

Scheme 4.2 Synthesis of serine-derived dihydroxazine **(*S*)-62c**



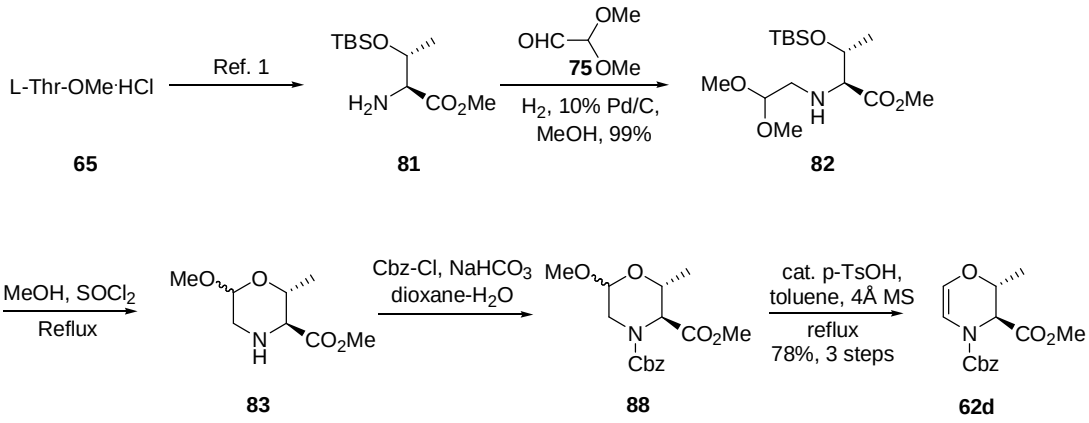
When the above reported strategy was extended to threonine, problems initially arose in the reductive amination step with dimethoxyacetaldehyde. In fact, upon treatment of threonine methyl ester hydrochloride with Pd/C-NEt_3 under a hydrogen atmosphere, the only observed product was not the expected **76a**, but **80** as reported in Scheme 4.3 (See discussion in Chapter 2, Section 2.6).

Scheme 4.3 Attempted synthesis of compound **76a**



Protection of the hydroxyl group of threonine with TBSCl[88] before performing the reductive amination proved essential and allowed the synthesis of the **82** in almost quantitative yield. Unfortunately amine **82** was found to be completely unreactive upon treatment with Cbz-Cl, in analogy to the attempted protection with Fmoc-Cl discussed in chapter 2, Section 2.6. The problem was solved using the same strategy developed in chapter 2, section 6, subjecting amine **82** to more harsh acid conditions, in order to deprotect the hydroxyl group and simultaneously obtain the trans-acetalyzation. The amino group of the resultant cyclic acetal **83** proved to be much more reactive than linear precursor **82** and the crude product deriving from trans-acetalyzation was easily protected upon treatment with Cbz-Cl/$NaHCO_3$ aq. Subsequent treatment of cyclic acetal **88** with p-TSOH/MS 4Å in refluxing toluene furnished the dihydroxazine scaffold **62d** in good overall yield, requiring only one purification through flash chromatography (Scheme 4.4).

Scheme 4.4 Synthesis of threonine-derived dihydroxazine **62d**



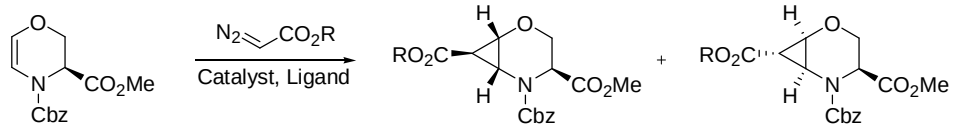
4.3 Cyclopropanation studies

We then focused on the study of the cyclopropanation reaction of the dihydroxazine scaffolds using diazoacetates. We performed some preliminary studies using ethyl diazoacetate, in order to determine the most effective metal catalyst and the best reaction conditions (Table 4.1). We found that $Rh_2(OAc)_4$ and Cu(I)(OTf) were both effective and gave comparable yields. Slow addition of ethyl diazo acetate was necessary to maintain a low "active carbene" concentration in solution, therefore preventing dimerization of the

[88] Niu, Chuansheng; Pettersson, Teresia; Miller, Marvin J. *J. Org. Chem*. **1996**, 61, 1014-1022.

carbene.[89] The best experimental conditions identified using ethyl diazoacetate were then extended to the cyclopropanation using *tert*-butyl diazoacetate, in order to obtain orthogonality between the two ester groups. The introduction of a more hindered ester did not affect yield or diastereoselectivity (Table 4.1, entry 6), and cyclopropanation products could be isolated in good yield. Low diastereoselectivity between the two diastereomers **69** and **70** was observed, and only traces of the other two possible diastereomers were detected. Although diastereomers **69** and **70** could be separated by standard chromatography, the use of suitable chiral ligands was taken into account to enhance the diastereocontrol.

Table 4.1 Cyclopropanation conditions for dihiydroxazine **(*S*)-62c**



(+)-(*S*)-62c

(-)-(1*S,4S,6R,7R*)-69a, R = Et **(+)-(1*R,4S,6S,7S*)-70a**, R = Et
(-)-(1*S,4S,6R,7R*)-69b, R = *t*-Bu **(+)-(1*R,4S,6S,7S*)-70b**, R = *t*-Bu

R	Catalyst	Ligand	EDA eq.	Time of Addition	Combined Yield 69+70	Ratio 69:70
Et	$Rh_2(OAc)_4$	-	2	10 min.	41 %	1.6:1
Et	$Rh_2(OAc)_4$	-	3	5 h	72 %	1.6:1
Et	$Cu(OTf)_2$/$PhNHNH_2$	-	3	5 h	75 %	1.5:1
Et	$Cu(OTf)_2$/$PhNHNH_2$	-	4.5	6 h	86 %	1.5:1
Et	$Cu(OTf)_2$/$PhNHNH_2$	**(S-S)**-*t*-BuBOX	4.5	6 h	73 %	1:6
t-Bu	$Cu(OTf)_2$/$PhNHNH_2$	**(S-S)**-*t*-BuBOX	4.5	5 h	67 %	1:5
t-Bu	$Cu(OTf)_2$/$PhNHNH_2$	**(S-S)**-*t*-BuBOX	4.5	6 h	80 %	1:6

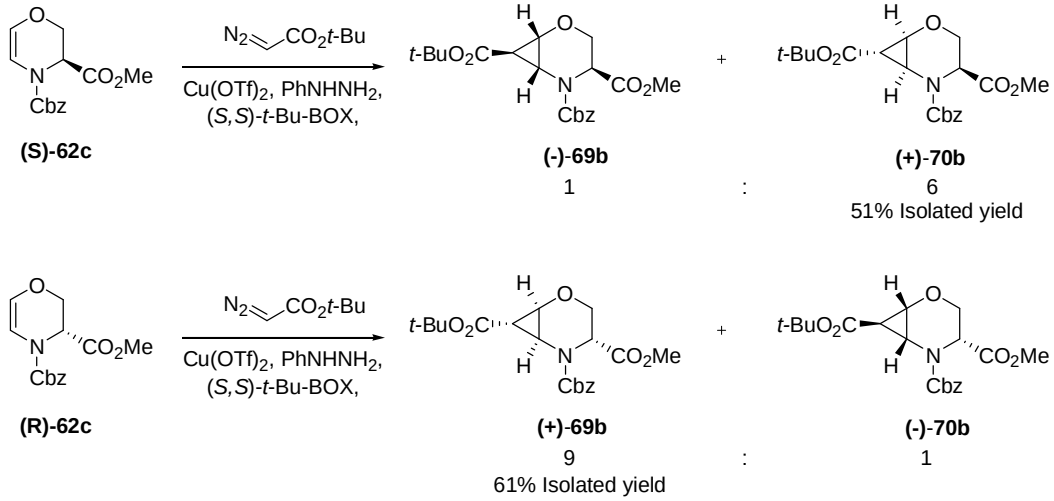
[a] Estimated by ^{1}H-NMR.

The bisoxazoline ligand (*S*,*S*)-2,2-methylenebis(4-*t*-butyl-2-oxazoline), (*S*,*S*)-*t*-BuBOX),[90] proved to be effective when used in combination with copper(I) triflate.We observed that the stereochemistry of the cyclopropanes formed was mainly controlled by the ligand chirality, regardless of the stereochemistry of the dihydroxazine scaffold, suggesting in each case that the chiral ligand orients the attacking carbene to the same alkene face. In fact, when dihydroxazine **(*S*)-62c** (derived from L-serine) was treated with *t*-butyl diazoacetate and (*S*,*S*)-*t*-BuBOX, the ratio between the diastereomers **69b** and **70b** was 1 : 6, whereas when the enantiomeric dihydroxazine (derived from D-serine) was used, the ratio of the two diastereomers [enantiomeric to **(-)**-**69b** and **(+)**-**70b**, respectively] was reversed, giving a 9:1 mixture in favour of compound **(+)**-**69b** (scheme 4.5). Comparison of these data with the diastereomeric ratios obtained in the absence of the chiral ligand (Table 4.1, entries 3, 4 and 6) suggested that the combination of (*S*,*S*)-*t*-BuBOX with

[89] Eric N. Jacobsen · Andreas Pfaltz · Hisashi Yamamoto, Comprehensive Asymmetric Catalysis vol. II, Springer-Verlag: Berlin-Heidelberg-New York 2000, pp 513-539.
[90] D. A. Evans, K. A. Woerpel, M. M. Hinman and M. M. Faul, *J. Am.Chem. Soc.*, 1991, **113**, 726.

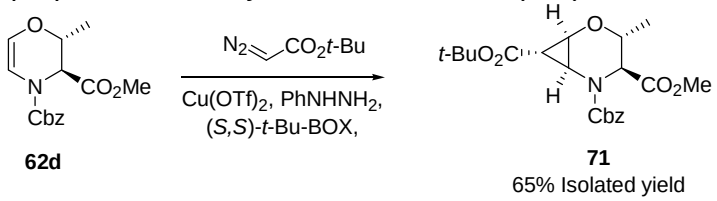
dihydroxazine **(*R*)-62c** is the matched pair, whereas the same chiral ligand in combination with **(*S*)-62c** is the mismatched pair.

Scheme 4.5 (S,S)-*t*-BuBOX-Cu(OTf) catalyzed cyclopropanation of dihydroxazines **(*R*)-62c** and **(S)-62c**



Cyclopropanation of dihydroxazine **62d**, derived from L-threonine methyl ester, with (*S*,*S*)-*t*-BuBOX and *t*-butyl diazoacetate resulted in compound **71** as the major stereoisomer and only traces of a second stereoisomer (Scheme 4.6), indicating an additional effect of the methyl group at C-2 of dihydroxazine **62d** on the stereoselectivity.

Scheme **4.6** Cyclopropanation of dihydroxazine **62d** with (S,S)-*t*-BuBOX-Cu(OTf)

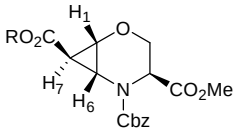


4.4 Structural assignament

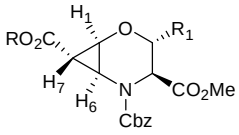
The structural assignment of the two major diastereomers was accomplished analyzing the values of the coupling constants for the hydrogens in the cyclopropane ring and by means of NOE experiments. We based on the assessment that coupling constants less than 7 Hz are associated with a trans relationship between two protons in a cyclopropane ring.[91] In all the isolated diastereomers, J couplings of proton H_7 (see Table 4.2) with the other two protons of the cyclopropane ring are comprised between 2.4 and 3.6 Hz (Table 4.2).

[91] Morris, D. G. Nuclear Magnetic Resonance and Infrared Spectra of Cyclopropanes and Cyclopropenes. In *The Chemistry of the Cyclopropyl Group;* Patai, S., Rappoport Z., Eds.; John Wiley and Sons: New York, 1987; Chapter 3, pp 101-172.

Table 4.2 Coupling constants for the hydrogens of the cyclopropane ring in the scaffolds synthesized



69a, R = Et
69b, R = *t*-Bu



70a, R = Et, R_1 = H
70b, R = *t*-Bu, R_1 = H
71, R = *t*-Bu, R_1 = CH_3

Product	$J_{6,7}$	$J_{1,7}$	$J_{1,6}$
69a	3.2	3.2	7.2
69b	3.6	2.4	7.2
70a	3.6	2.4	7.2
70b	3.2	3.2	7.2
71	3.2	3.2	7.2

A definitive structure elucidation was based on NOE spectra for the bicyclic morpholine-based scaffolds deriving from cyclopropanation 5 with *t*-butyl diazoacetate. In particular, a diagnostic NOE effect between H-4 and H-7 was observed for compound **69b** (Fig. 3). For compound **70b**,H-7 provided only a weak NOE effect with the methyl ester protons at C-4, and a strong NOE effect was observed between H-7 and one of the two methylenic protons at C-3 of the morpholine ring (Fig. 3).NOE spectra of compound **71** deriving from L-threonine resulted in NOE interactions between the protons of the methyl group at C-3 and the two protons H-1 and H-6. This strongly supported the structure in Fig. 3, with the two esters in a *trans* relationship.

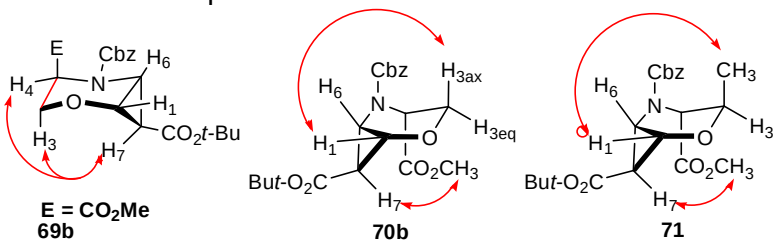


Figure 4.2 Most significant NOEs observed for structures **69b** and **70b** and **71**

4.5 Modeling studies

Molecular modeling calculations were carried out on compound **71** so as to assess the most stable conformation and to gain insight into the detailed structure of the bicyclic scaffold. Energy-minimized conformations of the 2-oxa-5-azabicyclo[4.1.0]heptane-based scaffold **71** were achieved using SPARTAN Version 5.11[92] running on a SGI IRIX 6.5 workstation. Conformational searches of **71** were carried out using a Monte Carlo method within the MMFF94 force field,[93] and the AM1 semiempiricalmethod[94] was used to optimize the globalminimum conformer. The geometries of the most abundant minimumenergy conformers were successively subjected to *ab initio* single-point energy calculation at the 3-21G*/HF level[95] of quantum chemical theory. The conformation having axial C-2 and C-3 substituents resulted in a twisted half-chair structure for the morpholine

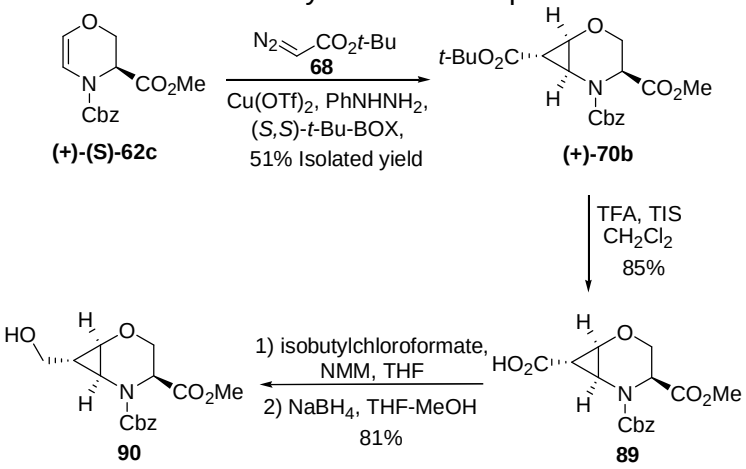
[92] *SPARTAN* Version 5.1, Wavefunction, Inc., Irvine, CA
[93] (*a*) T. A. Halgren, *J. Comput. Chem.*, 1996, **17**, 490; (*b*) T. A. Halgren, *J. Comput. Chem.*, 1996, **17**, 520; (*c*) T. A. Halgren, *J. Comput. Chem.*, 125, 1996, **17**, 553; (*d*) T. A. Halgren and R. B. Nachbar, *J. Comput. Chem.*, 1996, **17**, 587; (*e*) T. A. Halgren, *J. Comput. Chem.*, 1996, **17**, 616.
[94] M. J. S. Dewar, E. G. Zoebisch, E. F. Healy and J. J. P. Stewart, *J. Am. Chem. Soc.*, 1985, **107**, 3902.
[95] W. J. Hehre, L. Radam, P. R. Schleyer and J. A. Pople, *Ab initio* 130 *molecular orbital theory*, Wiley, New York, 1986

moiety, whereas a twisted half-boat structure was obtained for the conformation having the same substitutents in equatorial position. Also, the conformation with axial C-2 and C-3 substituents proved to be more stable by about 2.3 kcal mol−1 (*E*ax = −232.14 kcal mol−1; *E*eq = −229.86 kcal mol−1). Computation of the dihedral angle formed by H-2, C-2, C-3, H-3 atoms for the axial and equatorial conformations resulted in −73.6◦ and −144.6◦, respectively, which, in conjunction with ^{1}H-NMR data indicating absence of coupling between H-2 and H-3, suggested the axial conformer as the more favourable in chloroform solution (Fig. 4). Further corroboration of the preferential axial conformation for **71** was given by NOE experiments, which showed NOE correlation of H-7 with methyl ester protons (and not H-2), and of H-6 with the methyl group at C-2.

4.6 Further diversifications of 2-oxa-5-azabicyclo[4.1.0]heptane

In order to extend the versatility of the bicyclic structures reported herein, the selective transformation of the *t*-butyl ester into a primary alcohol using compound **70b** as substrate was carried out. Specifically, the orthogonality of the two esters was used for selective deprotection of the *t*-butyl ester under standard acid conditions, followed by reduction of the resulting acid with isobutyl chloroformate/$NaBH_4$ (Scheme 4.7). Both transformations proved to be completely stereoselective, and no epimerization at C-7 was observed by ^{1}H-NMR, giving the corresponding alcohol **90** in overall 69% yield from **70b**, and demonstrating the feasibility of such scaffold as a template for subsequent appendage diversity.

Scheme 4.7 Synthesis of compound **90**



4.7 Conclusions

In summary we have developed an efficient strategy which gives access to a new series of scaffolds based on the unique heterocyclic structure of the 2-oxa-5-azabicyclo[4.1.0]heptane. The strategy allows the generation of compounds with up to five stereogenic centers in enantiopure form starting from readily available β-hydroxy-α-amino

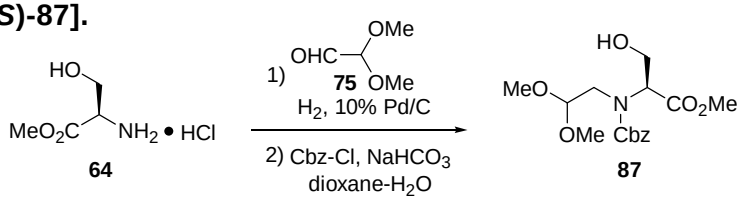
acids and by means of a diastereoselective cyclopropanation achieved using diazoacetates in conjunction with Cu(I)OTf and a chiral *t*-BuBOX ligand. The cyclopropanation outcome proved to be mainly controlled by the stereochemistry of the bis-isoxazolidine ligand. A variety of scaffolds can be obtained using this strategy, each of them differing for the spatial orientation of the orthogonally protected diversification sites. Some final manipulations of the synthesized structures have been presented, in order to prove the versatility and the orthogonal relationship between the various protecting groups introduced.

4.8 Experimental for chapter 4

General Experimental

Chromatographic separations were performed on silica gel using flash-column techniques. R_f values refer to TLC carried out on 25 mm silica gel plates (Merck F254) with the same eluant indicated as for column chromatography. ^1H and ^{13}C NMR spectra were recorded with NMR instruments operating at 200 MHz and 400 MHz for proton and at 50 MHz for carbon, and using $CDCl_3$ solutions unless otherwise stated. EI mass spectra were carried out at 70 eV ionizing voltage.

(*S*)-2-[Benzyloxycarbonyl-(2,2-dimethoxy-ethyl)-amino]-3-hydroxy-propionic acid methyl ester [(*S*)-87].

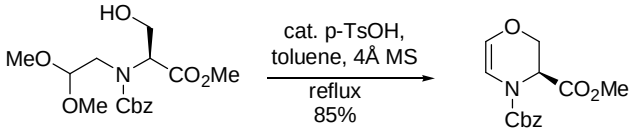


L-serine methyl ester hydrochloride (**64**) (5.34 g, 34.3 mmol) was dissolved in MeOH (110 mL), then triethylamine (4.79 mL, 34.3 mmol), 60% aqueous solution of dimethoxyacetaldehyde (**75**) (5.95 g, 34.3 mmol) and 10% Pd/C (477 mg) were successively added, and the resulting mixture was stirred overnight at room temperature under a hydrogen atmosphere. Then, the suspension was filtered over Celite and the organic solvent was removed under reduced pressure. The crude reaction mixture was dissolved in H_2O (60 mL) and $NaHCO_3$ (5.76 g, 68.6 mmol) was added. EtOAc (75 mL) was added and the mixture was cooled at 0 °C with an ice bath. Cbz-Cl (4.80 mL, 33.61 mmol) was added dropwise, then, after 1 h stirring, the ice bath was removed and the mixture was stirred overnight. The reaction was diluted with EtOAc (200 mL) and the aqueous layer was discarded. The organic phase was washed with 1M HCl, brine, dried over Na_2SO_4, concentrated and purified through flash chromatography (EtOAc-Hexanes 3:2) to provide compound **(*S*)-87** as a colourless oil (10.01 g, 85%). (Found: C, 56.40; H, 6.95; N 4.01. $C_{16}H_{23}NO_7$ requires C, 56.30; H, 6.79; N, 4.10%); $[\alpha]^{26}_D$ -42.9 (*c* 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz; $CDCl_3$) 1:1 mixture of rotamers 7.36-7.29 (m, 5 H, *Ph*), 5.21 (AB, part A, *J* = 6.0 Hz, 0.5 H, C*H*$_2$Ph), 5.13 (s, 1 H, C*H*$_2$Ph), 5.13 (AB, part B, *J* = 6.0 Hz, 0.5 H, C*H*$_2$Ph), 4.70 (dd, *J* = 7.2, 4.0 Hz, 0.5 H), 4.65-4.58 (m, 1 H), 4.49 (dd, *J* = 7.2, 4.0 Hz, 0.5 H), 4.05-3.77 (m, 2 H), 3.71 (s, 1.5 H, CO_2CH_3), 3.58 (s, 1.5 H, CO_2CH_3), 3.68-3.54 (m, 2 H) 3.45 (s, 1.5 H, OCH_3), 3.43 (s, 1.5 H, OCH_3), 3.31 (s, 1.5 H, OCH_3), 3.28 (s, 1.5 H, OCH_3), 3.26-3.21 (m, 1 H); ^{13}C-NMR (50 MHz; $CDCl_3$) mixture of rotamers 170.0 (s, CO_2CH_3), 156.3 and 156.1 (s, NCO_2), 135.7 (s, *i-Ph*), 128.2-127.6 (d, 5 C, *Ph*), 103.5 and 102.9

[d, *C*H(OCH$_3$)$_2$], 67.7 and 67.5 (t, Ph*C*H$_2$), 62.8 and 62.3 (d, N*C*H), 60.7 and 60.4 (t, *C*H$_2$OH), 55.1 and 54.8 (q, CO$_2$*C*H$_3$), 54.3 and 52.0 (q, 2 C, O*C*H$_3$), 49.2 (t, N*C*H$_2$); MS *m*/*z* 309 (M$^+$-CH$_3$OH, 1.9), 277 (0.7), 264 (0.7), 250 (0.6), 234 (0.5), 220 (0.1), 91 (100).

(*R*)-2-[Benzyloxycarbonyl-(2,2-dimethoxy-ethyl)-amino]-3-hydroxy-propionic acid methyl ester [(*R*)-87]. Compound **(*R*)-87** was prepared as for **(*S*)-87** starting from D-serine methyl ester hydrochloride and (**64**), with identical NMR data to the enantiomeric compound **(*S*)-87**. (Found: C, 56.20; H, 6.84; N 4.02. C$_{16}$H$_{23}$NO$_7$ requires C, 56.30; H, 6.79; N, 4.10%); [*α*]$^{25}_D$ 41.7 (*c* 1.0, CHCl$_3$).

(*S*)-2,3-Dihydro-[1,4]oxazine-3,4-dicarboxylic acid 4-benzyl ester 3-methyl ester [(*S*)-62c].

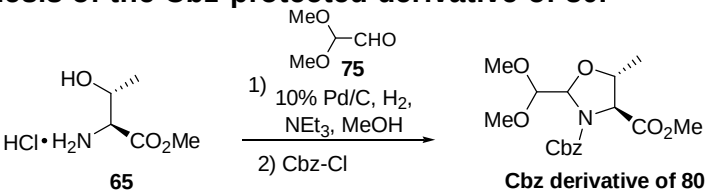


87 **62c**

A solution of compound **(*S*)-87** (1.12 g, 3.28 mmol) in toluene (45 mL) containing a catalytic amount of *p*-toluenesulfonic acid monohydrate (63 mg, 0.33 mmol) was placed in a single-necked round-bottomed flask equipped with a reflux condenser and a dropping funnel containing approximately 16 g of 4Å molecular sieves. The mixture was refluxed for 2.5 h, then cooled to room temperature and filtered through a thin layer of NaHCO$_3$. Toluene was removed under reduced pressure, and the crude product was purified by flash column chromatography (Hexanes-EtOAc 3:1) to yield compound **(*S*)-62c** as a colourless oil (729 mg, 78%). (Found: C, 60.81; H, 5.55; N, 5.01. C$_{14}$H$_{15}$NO$_5$ requires C, 60.64; H, 5.45; N, 5.05%); [α]$^{25}_D$ 8.6 (*c* 1.0, CHCl$_3$); ^{1}H-NMR (400 MHz, CDCl$_3$) 3:2 mixture of rotamers *α* and *β* 7.39-7.30 (m, 5 H, *Ph*), 6.45 (d, *J* = 2.4 Hz, 0.4 H, *H*-6 *β*), 6.33 (d, *J* = 2.6 Hz, 0.6 H, *H*-6 *α*), 6.03 (d, *J* = 2.4 Hz, 0.4 H, *H*-5 *β*), 5.90 (d, *J* = 2.6 Hz, 0.6 H, *H*-5 *α*), 5.29-5.15 (m, 2 H, C*H*$_2$Ph), 4.95 (s, 0.6 H, *H*-2 *α*), 4.83 (s, 0.4 H, *H*-2 *β*), 4.65 (dd, *J* = 10.8, 0.8 Hz, 0.6 H, *H*-2 *α*), 4.57 (d, *J* = 10.8, 0.8 Hz, 0.4 H, *H*-2 *β*), 3.97-3.92 (m, 1 H, *H*-3), 3.78 (s, 1.8 H, OC*H*$_3$ *α*), 3.71 (s, 1.2 H, OC*H*$_3$ *β*); ^{13}C-NMR (50 MHz, CDCl$_3$) mixture of rotamers 168.2 and 168.0 (s, *C*O$_2$CH$_3$), 151.7 and 151.0 (s, N*C*O$_2$), 135.4 (s, *i-Ph*), 129.4 and 128.2 (d, *C*-6), 128.1-127.6 (d, 5 C, *Ph*), 105.8 and 105.3 (d, *C*-5), 67.7 and 67.5 (t, *C*H$_2$Ph), 65.1 and 64.7 (t, *C*-2), 54.4 (q, CO$_2$*C*H$_3$), 53.7 and 52.5 (d, *C*-3); MS *m/z* 277 (M$^+$, 4), 249 (11), 91 (100).

(*R*)-2,3-Dihydro-[1,4]oxazine-3,4-dicarboxylic acid 4-benzyl ester 3-methyl ester [(*R*)-62c]. Compound **(*R*)-62c** was prepared as for **(*S*)-62c** starting from **(*R*)-87**, with identical NMR data to the enantiomeric compound **(*S*)-87**. (Found: C, 60.78; H, 5.51; N, 5.09. C$_{14}$H$_{15}$NO$_5$ requires C, 60.64; H, 5.45; N, 5.05%); [α]$^{25}_D$ -7.2 (*c* 2.5, CHCl$_3$).

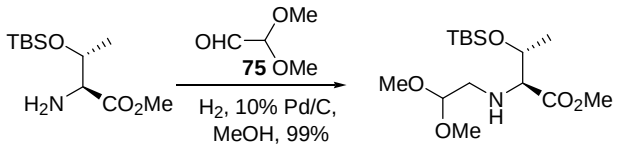
(2*R*/*S*,4*S*,5*R*)-2-Dimethoxymethyl-5-methyl-oxazolidine-4-carboxylic acid methyl ester (80): synthesis of the Cbz-protected derivative of 80.



65 **Cbz derivative of 80**

Compound **80** was obtained as a by-product starting from L-threonine methyl ester (**65**) (2.42 g, 14.3 mmol) according to the reported procedure for the preparation of **76a** (Chapter 2 and ref. 14). The crude product **80** was then characterized as the corresponding Cbz-protected derivative, after treatment of crude **80** with Cbz-Cl according to procedure as for **87**. Pure Cbz-protected compound (2.76 g, 12.4 mmol) was obtained after chromatographic purification (Hexanes-EtOAc 1:3) in 87% yield over two steps. (Found: C, 57.96; H, 6.61; N, 3.74. $C_{17}H_{23}NO_7$ requires C, 57.78; H, 6.56; N, 3.96%); ^{1}H-NMR (200 MHz, $CDCl_3$) mixture of diastereomers 7.31 (m, 5 H, *Ph*), 5.31 [s, 1 H, C*H*$(OCH_3)_2$], 5.15 (s, 2 H, CH_2Ph), 4.58 (m, 1 H, OC*H*N), 4.46 (m, 1 H, C*H*CH_3), 4.07 (m, 1 H, NC*H*CO_2), 3.69 (s, 3 H, CO_2CH_3), 3.46 (s, 6 H, OCH_3), 1.39 (d, *J* = 5.6 Hz, 3 H, CHCH_3); ^{13}C-NMR (50 MHz, $CDCl_3$) mixture of diastereomers 169.6 (s, *C*O_2CH_3), 135.6 (s, *i-Ph*), 128.2 (d, 2 C, *Ph*), 127.9 (d, 2 C, *Ph*), 127.5 (d, *Ph*), 104.2 [d, *C*H$(OCH_3)_2$], 88.6 (d, O*C*HN), 67.5 (t, *C*H_2Ph), 64.4 (d, *C*HCH_3), 55.7 (d, N*C*HCO_2), 52.3 (q, CO_2CH_3), 19.5 (q, CH*C*H_3).

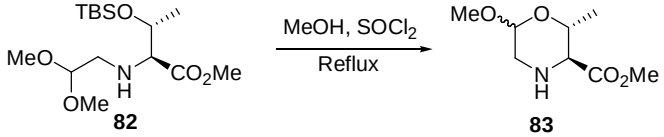
(2*S*,3*R*)-3-(*t*-Butyl-dimethyl-silanyloxy)-2-(2,2-dimethoxy-ethylamino)-butyric acid methyl ester (82).



81 **82**

Compound **81** (3.70 g, 14.9 mmol) was dissolved in MeOH (45 mL), then 60% aqueous solution of dimethoxyacetaldehyde (**75**) (2.59 g, 14.9 mmol) and 10% Pd/C (329 mg) were successively added, and the resulting mixture was stirred overnight at room temperature under a hydrogen atmosphere. Then, the suspension was filtered on Celite and MeOH was removed under reduced pressure. The resulting mixture was partitioned between water and Et_2O. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to yield compound **82** as a colourless oil (4.95 g, 99%). (Found: C, 53.86; H, 10.00; N, 4.22. $C_{15}H_{33}NO_5Si$ requires C, 53.70; H, 9.91; N, 4.17%); $[\alpha]^{25}_D$ -11.4 (*c* 1.1, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) 4.53 [t, *J* = 5.2 Hz, 1 H, C*H*$(OCH_3)_2$], 4.18 (qui, *J* = 5.3 Hz, 1 H, OC*H*CH_3), 3.73 (s, 3 H, CO_2CH_3), 3.37 (s, 6 H, OCH_3), 3.37 (m, 1 H, NC*H*CO_2), 2.94 (dd, *J* = 12.2, 5.8 Hz, 1 H, CH_2CH), 2.73 (dd, *J* = 12.2, 5.0 Hz, 1 H, CH_2CH), 1.25 (d, *J* = 6.4 Hz, 3 H, CHCH_3), 0.85 [s, 9 H, (CH_3)$_3$CSi], 0.44 (s, 3 H, CH_3Si), 0.14 (s, 3 H, CH_3Si); ^{13}C-NMR (50 MHz, $CDCl_3$) 171.9 (s, *C*O_2CH_3), 102.9 [d, *C*H$(OCH_3)_2$], 69.1 (d, O*C*HCH_3), 66.8 (d, N*C*HCO_2), 54.4 (q, O*C*H_3), 53.6 (q, O*C*H_3), 52.0 (q, CO_2CH_3), 48.9 (t, *C*H_2CH), 25.7 [q, 3 C, (*C*H_3)$_3$CSi], 20.8 (q, CH*C*H_3), 17.9 [s, (CH$_3$)$_3$*C*Si], -4.2 (q, *C*H_3Si), -5.1 (q, *C*H_3Si); MS *m*/*z* 304 (M^+-CH_3O, 8), 291 (15), 278 (6), 246 (13), 159 (38), 73 (100).

(2*R*,3*S*,6*R*/*S*)-6-Methoxy-2-methyl-morpholine-3-carboxylic acid methyl ester (83).

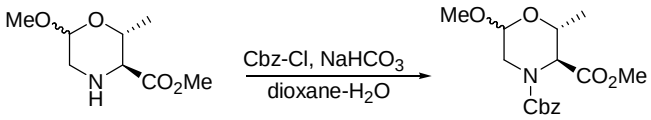


82 **83**

$SOCl_2$ (511 *µ*L, 7 mmol) was added dropwise, at 0 °C, to 7 mL of MeOH. The resulting solution was used to dissolve compound **82** (600 mg, 1.79 mmol). The resulting mixture was refluxed for 4 h, and then concentrated under reduced pressure. The crude material was dissolved again in MeOH, neutralized with Amberlist A-21, and the

solvent was evaporated to dryness. The product was directly used without further purification for the subsequent protection step. An analytical pure sample was obtained after purification through flash column chromatography (EtOAc). (Found: C, 50.86; H, 8.08; N, 7.22. $C_8H_{15}NO_4$ requires C, 50.78; H, 7.99; N, 7.40%); ^{1}H-NMR (400 MHz, $CDCl_3$) 3:2 mixture of diastereomers *α* and *β* 4.47 (s, 0.4 H, *H*-6 β), 4.40 (dd, *J* = 8.8, 2.4 Hz, 0.6 H, *H*-6 *α*), 3.88 (qd, *J* = 5.0, 1.8 Hz, 0.4 H, *H*-2 *β*), 3.74 and 3.73 (s, 3 H, CO_2CH_3), 3.65 (qd, *J* = 4.2, 1.2 Hz, 0.6 H, *H*-2 *α*), 3.50 (s, 1.8 H, OCH_3 *α*), 3.39 (s, 1.2 H, OCH_3 *β*), 3.27 (d, *J* = 9.4 Hz, 0.4 H, *H*-3 *β*), 3.18 (d, *J* = 9.4 Hz, 0.6 H, *H*-3 *α*), 3.04 (dd, *J* = 12.4, 2.4 Hz, 0.6 H, *H*-5 *α*), 2.92-2.90 (m, 0.8 H, *H*-5 *β*), 2.59 (dd, *J* = 12.4, 8.8 Hz, 0.6 H, *H*-5 *α*), 1.75-1.95 (br, 1 H, N*H*), 1.25 (d, *J* = 6.4 Hz, 1.8 H, $CHCH_3$ *α*), 1.15 (d, *J* = 6.0 Hz, 1.2 H, $CHCH_3$ *β*); ^{13}C-NMR (50 MHz, $CDCl_3$) mixture of diastereomers 171.1 (s, CO_2CH_3), 100.6 and 95.6 (d, *C*-6), 73.7 and 65.4 (d, *C*-2), 63.6 and 62.8 (d, *C*-3), 56.1 and 54.5 (q, OCH_3), 52.1 (q, CO_2CH_3), 47.9 and 47.2 (t, *C*-5), 18.2 (q, $CHCH_3$).

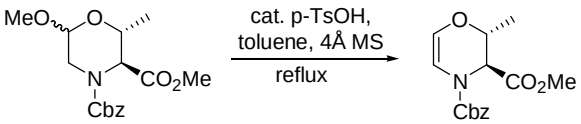
(2*R*,3*S*,6*R*/*S*)-6-Methoxy-2-methyl-morpholine-3,4-dicarboxylic acid 4-benzyl ester 3-methyl ester (88).



83 **88**

Crude cyclic acetal **83** was dissolved in H_2O (5 mL) and $NaHCO_3$ (297 mg, 3.54 mmol) was added. The mixture was stirred until complete dissolution of the salt, then dioxane (8 mL) was added. The flask was cooled at 0 °C with an ice bath and Cbz-Cl (253 mg, 1.77 mmol) was added dropwise. After 10 minutes the ice bath was removed and the reaction mixture was stirred 1 day at room temperature. Afterwards, EtOAc (25 mL) and water (10 mL) were added. The aqueous layer was discarded and the organic phase was washed with 1M HCl, brine, and dried over Na_2SO_4. The solvent were removed under reduced pressure, and the crude material was used without purification for the elimination reaction. An analytical pure sample was obtained after purification through flash column chromatography (Hexanes-EtOAc 3:1). (Found: C, 59.77; H, 6.78; N, 4.24. $C_{16}H_{21}NO_6$ requires C, 59.43; H, 6.55; N, 4.33%); ^{1}H-NMR (400 MHz, $CDCl_3$) mixture of diastereomers, mixture of rotamers 7.34-7.25 (m, 5 H, Ph), 5.23-5.01 (m, 2 H, CH_2Ph), 4.82-4.78 (m, 1 H, *H*-6), 4.70-4.63 (m, 1 H, *H*-2), 4.32-4.10 (m, 2 H, *H*-3 and *H*-5), 3.98-3.42 (m, 3 H, CO_2CH_3), 3.42-3.38 (m, 3 H, OCH_3), 1.43-1.35 (m, 3 H, $CHCH_3$); ^{13}C-NMR (50 MHz, $CDCl_3$) mixture of diastereomers, mixture of rotamers 170.2 (s, CO_2CH_3), 135.9 (s, *i-Ph*), 128.3-127.6 (d, 5 C, *Ph*), 97.0 (d, *C*-6), 69.1 (d, *C*-2), 67.6 (t, CH_2Ph), 59.5 (d, *C*-3), 55.3 (q, OCH_3), 52.3 (q, CO_2CH_3), 44.3 (t, *C*-5), 20.0 and 18.9 (q, $CHCH_3$).

(2*R*,3*S*)-2-Methyl-2,3-dihydro-[1,4]oxazine-3,4-dicarboxylic acid 4-benzyl ester 3-methyl ester (62d).



83 **62d**

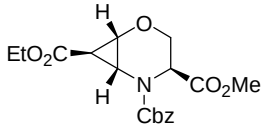
Crude protected acetal **83** was dissolved in toluene (10 mL) containing a catalytic amount of *p*-toluenesulfonic acid monohydrate (34 mg, 0.18 mmol) and placed in a single-necked round-bottomed flask equipped with a reflux condenser and a dropping

funnel containing approximately 10 g of 4Å molecular sieves. The mixture was refluxed for 2 h, then cooled to room temperature and filtered through a thin layer of $NaHCO_3$. Toluene was removed under reduced pressure, and the crude product was purified by flash column chromatography (Hexanes-EtOAc 7:2) to yield compound **62d** as colourless oil (406 mg, 78% over 3 steps from compound **82**). (Found: C, 61.80; H, 6.01; N, 4.91. $C_{15}H_{17}NO_5$ requires C, 61.85; H, 5.88; N, 4.81%); $[\alpha]^{26}_D$ -7.2 (*c* 0.2, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) 3:2 mixture of rotamers *α* and *β* 7.39-7.30 (m, 5 H, *Ph*), 6.39 (d, *J* = 4.8 Hz, 0.4 H, *H*-6 *β*) 6.27 (d, *J* = 4.8 Hz, 0.6 H, *H*-6 *α*), 5.88 (d, *J* = 4.8 Hz, 0.4 H, *H*-5 *β*) 5.75 (d, *J* = 4.8 Hz, 0.6 H, *H*-5 *α*), 5.25 (AB, part A, *J* = 12 Hz, 0.4 H, C*H*$_2$Ph *β*), 5.24 (s, 1.2 H, C*H*$_2$Ph *α*), 5.16 (AB, part B, *J* = 12 Hz, 0.4 H, C*H*$_2$Ph *β*), 4.83 (qd, *J* = 6.4, 1.2 Hz, 0.6 H, *H*-2 *α*), 4.72 (qd, *J* = 6.4, 1.2 Hz, 0.4 H, *H*-2 *β*), 4.68 (s, 0.6 H, *H*-3 *α*), 4.55 (s, 0.4 H, *H*-3 *β*), 3.77 (s, 1.8 H, CO_2CH_3 *α*), 3.69 (s, 1.2 H, CO_2CH_3 *β*), 1.31 (d, *J* = 6.4 Hz, 1.8 H, $CHCH_3$ *α*) 1.30 (d, *J* = 6.4 Hz, 1.2 H, $CHCH_3$ *β*); ^{13}C-NMR (50 MHz, $CDCl_3$) mixture of rotamers 168.4 (s, *C*O_2CH_3), 152.8 (s, N*C*O_2), 135.6 (s, *i-Ph*), 128.4-127.7 (d, 5 C, *Ph*), 126.8 and 125.7 (d, *C*-6), 104.7 and 104.3 (d, *C*-5), 69.7 and 69.1 (d, *C*-2), 68.0 and 67.7 (t, *C*H_2Ph), 58.1 and 57.3 (d, *C*-3), 52.6 (q, CO_2CH_3), 17.2 (q, $CHCH_3$); MS *m/z* 291 (M^+, 4.6), 188 (15.9), 91 (100).

Cyclopropanation with $Cu(OTf)_2$ and (*S*,*S*)-2,2'-isopropylidene-*bis*(4-*t*-butyl-2-oxazoline), general procedure A. To a solution of dihydroxazine **62c** (626 mg, 2.24 mmol) in dry CH_2Cl_2 (4 mL) cooled in an ice-salt bath were added $Cu(OTf)_2$ (16 mg, 0.045 mmol), (*S*,*S*)-2,2'-isopropylidene-*bis*(4-*t*-butyl-2-oxazoline) (16 mg, 0.056 mmol) and phenylhydrazine (4.4 *μ*L, 0.045 mmol). After 30 min, a 1.2 M solution of diazoacetate in dry CH_2Cl_2 was added (quantity and time according to Table 4.1). The reaction was then gently warmed to room temperature and stirred for 16 h. Then, the mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography (Hexanes-EtOAc 3:1) to yield the cyclopropanated products.

Cyclopropanation with $Cu(OTf)_2$, without chiral ligand, general experimental procedure B. To a solution of dihydroxazine scaffold **62c** (417 mg, 1.49 mmol) in dry CH_2Cl_2 (3 mL) cooled in an ice-salt bath were added $Cu(OTf)_2$ (11 mg, 0.030 mmol) and phenylhydrazine (2.9 *μ*L, 0.030 mmol). After 30 min, a 1.2 M solution of diazoacetate in dry CH_2Cl_2 was added (quantity and time according to Table 4.1). The reaction was then gently warmed to room temperature and stirred for 16 h. Then, the mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography (Hexanes-EtOAc 3:1) to yield the cyclopropanated products.

Cyclopropanation with $Rh_2(OAc)_4$, general experimental procedure C. To a solution of dihydroxazine scaffold **62c** (522 mg, 1.87 mmol) in dry CH_2Cl_2 (4 mL) cooled in an ice-salt bath $Rh_2(OAc)_4$ (2.5 mol %) and a 1.2 M solution of diazoacetate in dry CH_2Cl_2 were added (quantity and time according to Table 4.1). The reaction was then gently warmed to room temperature and stirred for 16 h. The mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography (Hexanes-EtOAc 3:1) to yield the cyclopropanated products.

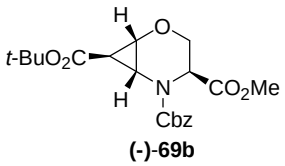


(-)-69a

(1*S*,4*S*,6*R*,7*R*)-2-Oxa-5-aza-bicyclo[4.1.0]heptane-4,5,7-tricarboxylic acid 5-benzyl ester 7-ethyl ester 4-methyl ester (-)-(69a). 626 mg (2.24 mmol) of compound **(*S*)-62c** were treated according to general procedure A using 4.5 equivalents of ethyl diazoacetate (6 h time of addition) to yield 81 mg (10%) of **(-)-69a** as the minor

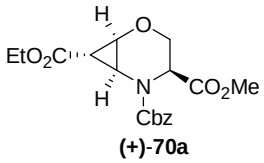
stereoisomer, or in higher amounts according to general procedure B. (Found: C, 60.02; H, 5.71; N, 3.70. $C_{18}H_{21}NO_7$ requires C, 59.50; H, 5.83; N, 3.85%); $[\alpha]^{25}_D$ -48.1 (*c* 1.2, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 2:1 mixture of rotamers *α* and *β* 7.35-7.29 (m, 5 H, *Ph*), 5.29 (AB, part A, *J* = 12.5 Hz, 0.66 H, C*H*$_2$Ph *α*), 5.17 (AB, part B, *J* = 12.5 Hz, 0.66 H, C*H*$_2$Ph *α*), 5.24-5.11 (m, 0.66 H, C*H*$_2$Ph *β*), 4.47 (t, *J* = 4.8 Hz, 0.66 H, *H*-4 *α*), 4.19 (t, *J* = 4.8 Hz, 0.33 H, *H*-4 *β*), 4.20-4.03 (m, 3 H, $CO_2CH_2CH_3$ and *H*-1), 4.03 (dd, *J* = 11.6, 5.2 Hz, 0.66 H, *H*-3 *α*), 3.94 (dd, *J* = 11.6, 5.2 Hz, 0.33 H, *H*-3 *β*), 3.84 (dd, *J* = 11.6, 4.8 Hz, 1 H, *H*-3), 3.78 (s, 2 H, CO_2CH_3 *α*), 3.67 (s, 1 H, CO_2CH_3 *β*), 3.54-3.49 (m, 1 H, *H*-6), 2.00 (t, *J* = 3.2 Hz, 1 H, *H*-7), 1.98 (t, *J* = 7.2 Hz, 2 H, $CO_2CH_2CH_3$); ^{13}C-NMR (50 MHz, $CDCl_3$) mixture of rotamers 169.4 (s, *C*O_2), 169.2 (s, *C*O_2), 155.9 (s, N*C*O_2), 135.7 (s, *i-Ph*), 128.2-127.1 (d, 5 C, *Ph*), 67.8 and 67.7 (t, *C*H$_2$Ph), 64.6 and 64.1 (t, *C*-3), 60.7 (t, $CO_2CH_2CH_3$), 59.1 (d, *C*-1) 53.1 and 52.6 (d, *C*-4) 52.0 (q, CO_2CH_3), 33.9 (d, *C*-6), 26.4 and 26.0 (d, *C*-7), 14.2 (q, $CO_2CH_2CH_3$); MS *m*/*z* 363 (M^+, 0.8), 318 (0.2), 290 (2), 228 (15), 182 (18), 91 (100).

(1*S*,4*S*,6*R*,7*R*)-2-Oxa-5-aza-bicyclo[4.1.0]heptane-4,5,7-tricarboxylic acid 5-benzyl ester 7-*t*-butyl ester 4-methyl ester (-)-69b. Compound **(-)-69b** was obtained from **(*S*)-62c** as the minor diastereomer according to general procedure A, or in higher amounts according to general procedure B. (Found: C, 61.06; H, 6.71; N, 3.55. $C_{20}H_{25}NO_7$ requires C, 61.37; H, 6.44; N, 3.58%); $[\alpha]^{25}_D$ -61.3 (*c* 1.1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 2:1 mixture of rotamers *α* and *β* 7.38-7.28 (m, 5 H, *Ph*), 5.28 (AB, part A, *J* = 12.4 Hz, 0.66 H, C*H*$_2$Ph *α*), 5.24 (AB, part A, *J* = 7.9 Hz, 0.33 H, C*H*$_2$Ph *β*), 5.14 (AB, part B, *J* = 12.4 Hz, 0.66 H, C*H*$_2$Ph *α*), 5.10 (AB, part B, *J* = 8.0 Hz, 0.33 H, C*H*$_2$Ph *β*), 4.45 (t, *J* = 4.8 Hz, 0.66 H, *H*-4 *α*), 4.38 (t, *J* = 4.8 Hz, 0.33 H, *H*-4 *β*), 4.10 (m, 0.33 H, *H*-1 *β*), 4.09 (dd, *J* = 7.2, 3.2 Hz, 0.66 H, *H*-1 *α*), 3.94 (dd, *J* = 11.6, 3.2 Hz, 0.66 H, *H*-3 *α*), 3.90 (dd, *J* = 11.6, 4.8 Hz, 0.33 H, *H*-3 *β*), 3.82 (dd, *J* = 11.6, 4.8 Hz, 1 H, *H*-3), 3.77 (s, 2 H, CO_2CH_3 *α*), 3.65 (s, 1 H, CO_2CH_3 *β*), 3.47 (dd, *J* = 7.2, 3.2 Hz, 0.33 H, *H*-6 *β*), 3.42 (dd, *J* = 7.2, 3.2 Hz, 0.66 H, *H*-6 *α*), 1.92 and 1.89 (t, *J* = 3.2 Hz, 1 H, *H*-7), 1.43 [s, 3 H, $CO_2C(CH_3)_3$ *β*], 1.37 [s, 6 H, $CO_2C(CH_3)_3$ *α*]; ^{13}C-NMR (50 MHz, $CDCl_3$) mixture of rotamers 169.4 (s, *C*O_2), 168.1 (s, *C*O_2), 156.0 (s, N*C*O_2), 135.6 (s, *i-Ph*), 128.3-127.2 (d, 5 C, *Ph*), 81.0 [s, *C*$(CH_3)_3$], 67.8 (t, CH_2Ph), 64.5 and 64.1 (t, *C*-3), 58.8 (d, *C*-1), 53.1 (d, *C*-4), 52.6 (d, *C*-6) 52.1 (q, CO_2CH_3), 33.5 (d, *C*-7), 28.0 and 27.4 [q, 3 C, C(*C*$H_3)_3$]; MS *m/z* 335 (M^+- *t*-Bu, 2), 318 (0.4), 291 (3), 200 (15), 91 (100).



(-)-69b

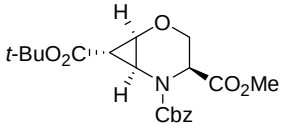
(1*R*,4*S*,6*S*,7*S*)-2-Oxa-5-aza-bicyclo[4.1.0]heptane-4,5,7-tricarboxylic acid 5-benzyl ester 7-ethyl ester 4-methyl ester (+)-70a. 626 mg (2.24 mmol) of compound **(*S*)-62c** were treated according to general procedure A using 4.5 equivalents of ethyl diazoacetate (6 h time of addition) to yield 512 mg (63%) of **(+)-70a**. (Found: C, 60.1; H, 5.11; N, 3.62. $C_{18}H_{21}NO_7$ requires C, 59.50; H, 5.83; N, 3.85%); $[\alpha]^{25}_D$ 5.5 (*c* 1.2, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 3:2 mixture of rotamers α and β 7.35-7.28 (m, 5 H, *Ph*), 5.27 (AB, part A, *J* = 12.5 Hz, 0.6 H, C*H*$_2$Ph *α*), 5.22 (AB, part B, J = 12.5 Hz, 0.6 H, C*H*$_2$Ph *α*), 5.21 (AB, part A, *J* = 13.1 Hz, 0.4 H, C*H*$_2$Ph *β*), 5.13 (AB, part B, *J* = 13.1 Hz, 0.4 H, C*H*$_2$Ph *β*), 4.27 (d, *J* = 3.2 Hz, 0.6 H, *H*-4 *α*), 4.24 (d, *J* = 3.2 Hz, 0.4 H, *H*-4 *β*), 4.20-4.05 (m, 4 H, $CO_2CH_2CH_3$, *H*-3, and *H*-1), 3.84 (dd, *J* = 11.6, 3.6 Hz, 0.6 H, *H*-3 *α*), 3.80 (dd, *J* = 11.6, 3.6 Hz, 0.4 H, *H*-3 *β*), 3.74 (s, 1.8 H, CO_2CH_3 *α*), 3.63 (s, 1.2 H, CO_2CH_3 *β*), 3.53 (dd, *J* = 7.2, 3.6 Hz, 0.4 H, *H*-6 *β*), 3.49 (dd, *J* = 7.2, 3.6 Hz, 0.6 H, *H*-6 *α*), 2.38 (dd, *J* = 3.6, 2.4 Hz, 0.6 H, *H*-7 *α*), 2.29 (dd, *J* = 3.6, 2.4 Hz, 0.4 H, *H*-7 *β*), 1.26 (t, *J* = 7.2 Hz, 1 H, CO_2CH_3 *β*), 1.21 (t, *J* = 7.2 Hz, 2 H, CO_2CH_3 *α*); ^{13}C-NMR (50 MHz, $CDCl_3$) mixture of rotamers



(+)-70a

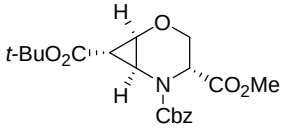
170.5 and 170.0 (s, *C*O_2), 169.9 and 169.8 (s, *C*O_2), 156.1 and 155.4 (s, N*C*O_2), 135.8 and 135.5 (s, *i-Ph*), 128.2-127.2 (d, 5 C, *Ph*), 67.7 and 67.6 (t, *C*H_2Ph), 65.9 and 65.5 (t, *C*-3), 60.6 (t, $CO_2CH_2CH_3$), 58.1 and 57.8 (d, *C*-1), 55.4 and 54.9 (d, *C*-4), 52.6 (q, CO_2CH_3), 35.3 and 35.2 (d, *C*-6), 27.5 and 27.3 (d, *C*-7), 14.2 (q, $CO_2CH_2CH_3$); MS *m/z* 363 (M^+, 1.2), 246 (15.2), 228 (17.4), 91 (100).

(1*R*,4*S*,6*S*,7*S*)-2-Oxa-5-aza-bicyclo[4.1.0]heptane-4,5,7-tricarboxylic acid 5-benzyl ester 7-*t*-butyl ester 4-methyl ester (+)-70b. 625 mg (2.24 mmol) of compound **(*S*)-62c** were treated according to general procedure A using 4.5 equivalents of *t*-butyl diazoacetate (6 h time of addition) to yield 447 mg (51%) of **(+)-70b**. (Found: C, 61.36; H, 6.31; N, 3.46. $C_{20}H_{25}NO_7$ requires C, 61.37; H, 6.44; N, 3.58%); $[\alpha]^{26}_D$ 14.1 (*c* 0.6, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 2:1 mixture of rotamers *α* and *β* 7.37-7.27 (m, 5 H, *Ph*), 5.30 (AB, part A, *J* = 13.1 Hz, 0.66 H, C*H*$_2$Ph *α*), 5.17 (AB, part B, *J* = 13.1 Hz, 0.66 H, C*H*$_2$Ph *α*), 5.22-5.12 (m, 0.66 H, C*H*$_2$Ph *β*), 4.25 (d, *J* = 2.8 Hz, 0.66 H, *H*-4 *α*), 4.23 (d, *J* = 2.8 Hz, 0.33 H, *H*-4 *β*), 4.13-4.06 (m, 0.66 H, *H*-1 *β* and *H*-3 *β*), 4.02 (dd, *J* = 7.2, 2.4 Hz, 0.66 H, *H*-1 *α*), 4.01 (d, *J* = 12.0 Hz, 0.66 H, *H*-3 *α*), 3.83 (dd, *J* = 12.0, 3.6 Hz, 1 H, *H*-3), 3.74 (s, 2 H, CO_2CH_3 *α*), 3.64 (s, 1 H, CO_2CH_3 *β*), 3.45 (dd, *J* = 7.2, 3.6 Hz, 0.33 H, *H*-6 *β*), 3.41 (dd, *J* = 7.2, 3.6 Hz, 0.66 H, *H*-6 *α*), 2.27 (dd, *J* = 3.6, 2.4 Hz, 0.66 H, *H*-7 *α*), 2.19 (dd, *J* = 3.6, 2.4 Hz, 0.33 H, *H*-7 *β*), 1.44 [s, 3 H, $CO_2C(CH_3)_3$ *β*], 1.37 [s, 6 H, $CO_2C(CH_3)_3$ *α*]; ^{13}C-NMR (50 MHz, $CDCl_3$) mixture of rotamers 170.1 (s, *C*O_2), 169.1 (s, *C*O_2), 156.2 (s, *C*O_2), 135.7 (s, *i-Ph*), 128.2-127.2 (d, 5 C, *Ph*), 80.9 [s, $C(CH_3)_3$], 67.7 (t, *C*H_2Ph), 65.9 and 65.4 (t, *C*-3), 57.6 (d, *C*-1), 54.9 (d, 2 C, *C*-4 and *C*-6) 52.5 (q, CO_2CH_3), 35.0 (d, *C*-7), 28.4 and 28.1 [q, 3 C, $C(CH_3)_3$]; MS (*m/z*) 335 (M^+- *t*-Bu, 2), 318 (0.3), 291 (1), 200 (16), 91 (100).



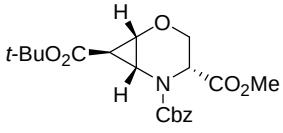
(+)-70b

(1*R*,4*R*,6*S*,7*S*)-2-Oxa-5-aza-bicyclo[4.1.0]heptane-4,5,7-tricarboxylic acid 5-benzyl ester 7-*t*-butyl ester 4-methyl ester (+)-69b. 390 mg (1.41 mmol) of compound **(*R*)-62c** were treated according to general procedure A using 4.5 equivalents of *t*-butyl diazoacetate (6 h time of addition) to yield 336 mg (61%) of **(+)-69b**, with identical NMR data as for **(-)-69b**. (Found: C, 61.11; H, 6.62; N, 3.54. $C_{20}H_{25}NO_7$ requires C, 61.37; H, 6.44; N, 3.58%); $[\alpha]^{25}_D$ 58.6 (*c* 1.0, $CHCl_3$).



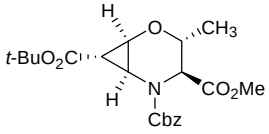
(+)-69b

(1*S*,4*R*,6*R*,7*R*)-2-Oxa-5-aza-bicyclo[4.1.0]heptane-4,5,7-tricarboxylic acid 5-benzyl ester 7-*t*-butyl ester 4-methyl ester (-)-70b. Compound **(-)-70b** was obtained from **(*R*)-62c** as the minor diastereomer according to general procedure A, or in higher amounts according to general procedure B, with identical NMR data as for **(+)-70b**. (Found: C, 61.31; H, 6.34; N, 3.49. $C_{20}H_{25}NO_7$ requires C, 61.37; H, 6.44; N, 3.58%); $[\alpha]^{25}_D$ -15.8 (*c* 1.0, $CHCl_3$).



(-)-70b

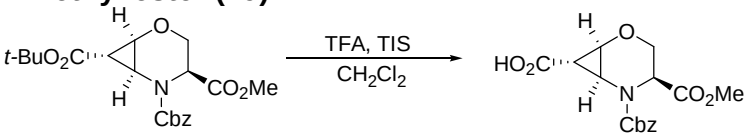
(1*R*,3*R*,4*S*,6*S*,7*S*)-3-Methyl-2-oxa-5-aza-bicyclo[4.1.0]heptane-4,5,7-tricarboxylic acid 5-benzyl ester 7-*t*-butyl ester 4-methyl ester (71). To a solution of dihydroxazine **62d** (450 mg, 1.54 mmol) in dry CH_2Cl_2 (4 mL) cooled in an ice-salt bath were added $Cu(OTf)_2$ (14 mg, 0.038 mmol), (*S*,*S*)-2,2'-isopropylidene-bis(4-*t*-butyl-2-oxazoline) (11 mg, 0.038 mmol) and phenylhydrazine (3.0 *μ*L, 0.031 mmol). After 30 min, a 1.2 M solution of *t*-butyl-diazoacetate (5 eq.) in dry



71

CH_2Cl_2 was added during 6 h. During the addition the volume was maintained constant, expelling CH_2Cl_2 by passing nitrogen through the flask. The reaction was then gently warmed to room temperature and stirred 16 h. The mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography (Hexanes-EtOAc 3:1) to yield 405 mg (65%) of **71**. (Found: C, 62.42; H, 6.93; N, 3.52. $C_{21}H_{27}NO_7$ requires C, 62.21; H, 6.71; N, 3.45%); $[\alpha]^{24}_D$ 34.6 (*c* 1.9, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 3:1 mixture of rotamers *α* and *β* 7.34-7.24 (m, 5 H, Ph), 5.29 (AB, part A, *J* = 12.3 Hz, 0.75 H, C*H*$_2$Ph *α*), 5.21 (AB, part A, *J* = 12.2 Hz, 0.25 H, C*H*$_2$Ph *β*), 5.13 (AB, part B, *J* = 12.6 Hz, 0.75 H, C*H*$_2$Ph *α*), 5.06 (AB, part B, *J* = 12.3 Hz, 0.25 H, C*H*$_2$Ph *β*), 4.34 (q, *J* = 6.8 Hz, 0.75 H, *H*-2 *α*), 4.27 (q, *J* = 6.8 Hz, 0.25 H, *H*-2 *β*), 4.08 (s, 0.75 H, *H*-4 *α*), 4.03 (s, 0.25 H, *H*-4 *α*), 3.79 (dd, *J* = 7.2, 3.2 Hz, 0.25 H, *H*-1 *β*), 3.75 (dd, *J* = 7.2, 3.2 Hz, 0.75 H, *H*-1 *α*), 3.68 (s, 2.25 H, CO_2CH_3 *α*), 3.58 (s, 0.75 H, CO_2CH_3 *β*), 3.54 (dd, *J* = 7.2, 3.2 Hz, 0.25 H, *H*-6 *β*), 3.48 (dd, *J* = 7.2, 3.2 Hz, 0.75 H, *H*-6 *α*), 2.27 (t, *J* = 3.2 Hz, 0.75 H, *H*-7 *α*), 2.17 (t, *J* = 3.2 Hz, 0.25 H, *H*-7 *β*), 1.44 (d, J= 6.4 Hz, 2.25 H, CHC*H*$_3$ *α*), 1.44-1.41 (m, 0.75 H, CHC*H*$_3$ *β*), 1.41 [s, 2.25 H, $CO_2C(CH_3)_3$ *β*], 1.35 [s, 6.75 H, $CO_2C(CH_3)_3$ *α*]; ^{13}C-NMR (50 MHz, $CDCl_3$) mixture of rotamers 170.5 (s, *C*O_2), 169.4 (s, *C*O_2), 156.8 (s, N*C*O_2), 135.7 (s, *i-Ph*), 128.3-127.2 (d, 5 C, *Ph*), 80.7 [s, *C*$(CH_3)_3$], 70.1 and 69.6 (d, *C*-3), 67.6 (t, *C*H_2Ph), 58.8 and 58.4 (d, *C*-1), 52.7 (q, CO_2CH_3), 52.3 (d, *C*-4), 34.9 and 34.7 (d, *C*-6), 27.9 and 27.6 [q, 3 C, C(*C*$H_3)_3$], 27.5 (d, *C*-7), 17.8 (q, CH*C*H_3); MS *m*/*z* 405 (M^+, 0.1), 305 (2), 260 (9), 214 (51), 91 (100).

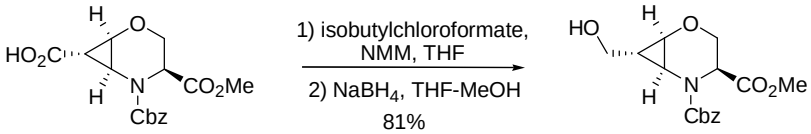
(1*R*,4*S*,6*S*,7*S*)-3-Methyl-2-oxa-5-aza-bicyclo[4.1.0]heptane-4,5,7-tricarboxylic acid 5-benzyl ester 4-methyl ester (19).



(+)-70b **89**

Compound **(+)-70b** (240 mg, 0.61 mmol) was dissolved in CH_2Cl_2 (2.8 mL) and TIS (125 *μ*L, 0.61 mmol) and TFA (1.2 mL) were added sequentially. The mixture was stirred 50 minutes at room temperature and then the solvents were removed under reduced pressure. The crude product obtained was redissolved in 5% Na_2CO_3 (20 mL) and the solution was extracted with Et_2O. The acqueous phase was acidified at pH 1-2 with concentrated HCl and extracted with CH_2Cl_2 (4x10 mL). The dichloromethane exctracts were combined, dried over Na_2SO_4 and concentrated under reduced pressure to obtain compound **89** (174 mg, 85%). (Found: C, 57.36; H, 5.21; H, 4.19. $C_{16}H_{17}NO_7$ requires C, 57.31; H, 5.11; N, 4.18%); $[\alpha]^{26}_D$ 5.8 (*c* 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 3:2 mixture of rotamers *α* and *β* 7.36-7.28 (m, 5 H, *Ph*), 5.27-5.10 (m, 2 H, C*H*$_2$Ph), 4.25 (d, *J* = 2.8 Hz, 0.6 H, *H*-4 *α*), 4.21 (d, *J* = 2.8 Hz, 0.4 H, *H*-4 *β*), 4.17-4.05 (m, 2 H, *H*-3), 3.85-3.78 (m, 1 H, *H*-1), 3.74 (s, 1.8 H, CO_2CH_3 *α*), 3.61 (s, 1.2 H, CO_2CH_3 *β*), 3.59-3.55 (m, 1 H, *H*-6), 2.39 (s, 0.6 H, *H*-7 *α*), 2.29 (s, 0.4 H, *H*-7 *β*); ^{13}C-NMR (50 MHz, $CDCl_3$) mixture of rotamers 175.6 and 175.0 (s, *C*O_2), 170.5 and 170.2 (s, *C*O_2), 156.3 and 155.8 (s, N*C*O_2), 135.8 and 135.4 (s, *i-Ph*), 128.4-127.3 (d, 5 C, *Ph*), 68.0 and 67.8 (t, *C*H_2Ph), 65.9 and 65.5 (t, *C*-3), 58.4 and 58.2 (d, *C*-4), 55.4 and 54.9 (d, *C*-1), 52.6 (q, CO_2CH_3), 36.0 and 35.8 (d, *C*-6), 27.4 (d, *C*-7); MS *m*/*z* 335 (M^+, 0.2), 290 (0.3), 246 (6), 232 (3), 200 (11), 91 (100).

(1*R*,4*S*,6*S*,7*R*)-7-Hydroxymethyl-3-methyl-2-oxa-5-aza-bicyclo[4.1.0]heptane-4,5-dicarboxylic acid 5-benzyl ester 4-methyl ester (20).



89 **90**

N-Methylmorpholine (52 *μ*L, 0.47 mmol) and isobutyl chloroformiate (61 *μ*L, 0.45 mmol) were added, at 0°C, to a solution of compound **89** (144 mg, 0.43 mmol) in dry THF (4 mL). After 25 minutes, the white suspension was added dropwise at -78 °C to a suspension of $NaBH_4$ (32 mg, 0.86 mmol) in THF/MeOH 3:1 (4 mL). After 30 minutes at -78 °C a second portion of $NaBH_4$ (32 mg, 0.86 mmol) was added and the mixture was stirred another 30 minutes at -78 °C and then was gently warmed to -40 °C, until all the mixed anidride was consumed (TLC monitoring). The reaction was quenched with 10% AcOH/H_2O (2 mL), diluted with H_2O (8 mL), and extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to a residue which was purified by flash column chromatography (EtOAc-Hexanes 3:1, then EtOAc) to yield alcohol **90** (112 mg, 81%). (Found: C, 59.98; H, 5.71; N, 5.02. $C_{16}H_{19}NO_6$ requires C, 59.81; H, 5.96; N, 4.36%); $[\alpha]^{25}_D$ 74.6 (*c* 1.1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 1:1 mixture of rotamers *α* and *β* 7.40-7.29 (m, 5 H, Ph), 5.29-5.10 (m, 2 H, C*H*$_2$Ph), 4.16 (dd, *J* = 12.0, 3.2 Hz, 1 H, *H*-3), 4.03 (d, *J* = 12.0 Hz, 1 H, *H*-3), 3.83-3.72 (m, 2 H, C*H*$_2$OH), 3.74 (s, 1.5 H, CO_2CH_3 *α*), 3.64-3.59 (m, 1 H, *H*-4), 3.58 (s, 1.5 H, CO_2CH_3 *β*), 3.26-3.19 (m, 1 H, *H*-1), 2.76-2.70 (m, 1 H, *H*-6), 1.82-1.80 (m, 1 H, *H*-7); ^{13}C-NMR (50 MHz, $CDCl_3$) mixture of rotamers 170.8 and 170.5 (s, *C*O_2), 156.6 (s, N*C*O_2), 135.8 and 135.6 (s, *i-Ph*), 128.4-127.8 (d, 5 C, *Ph*), 67.8 (t, *C*H_2OH), 66.1 and 65.8 (t, *C*H_2Ph), 61.1 and 60.8 (t, *C*-3), 56.2 and 55.7 (d, *C*-1), 55.4 and 54.9 (d, *C*-4), 52.5 (q, CO_2CH_3), 30.3 and 30.0 (d, *C*-6), 28.6 and 28.2 (d, *C*-7); MS *m/z* 303 (M^+- OH, 1), 290 (4), 218 (2), 200 (2), 91 (100).

5 Introduction to part II: β-hydroxy-α-amino acids

5.1 Asymmetric synthesis of β-hydroxy-α-amino acids

In this section an overview of the most common and recent asymmetric synthesis of β-hydroxy-α-amino acids is presented. This section is not comprehensive of all the literature reported so far, but it just collects some highlights of the different approaches that appeared relevant to the author. The various synthetic strategies are presented on the basis of the key reaction used.

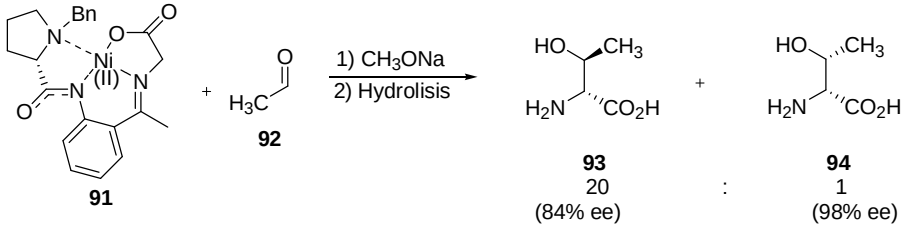
5.1.1 Asymmetric aldol reaction

A common approach towards the synthesis of β-hydroxy-α-amino acids is the aldol reaction of glycine equivalents with aldehydes. The different strategies will be described according to the different kind of glycine equivalent used.

Glycinate Schiff bases

One of the first notable and original approaches to β-hydroxy-α-amino acids using Schiff bases goes back to the beginning of the 80's and describes the use of glycine Schiff bases which are activated after complexation to a chiral Ni(II) or Cu(II) complex.[96] In this case the chiral complex is then reacted with aldehydes in the presence of NaOMe, yielding the correspronding β-hydorxy-amino acids after hydrolysis with respectable diastereo and enantioselectivities.

Scheme 5.1 Aldol reaction of chiral Ni(II) complexes of glycine Schiif bases.



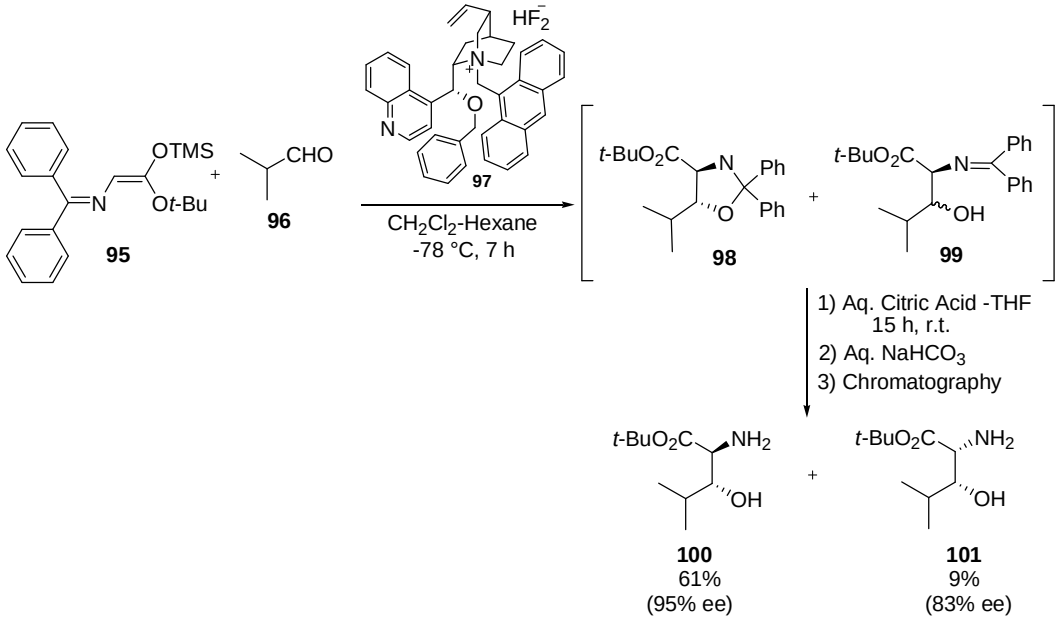
91 **92** **93** 20 (84% ee) : **94** 1 (98% ee)

Many efforts have been made to use glycinate Schiff bases in the presence of a suitable chiral catalyst. Among the first attempts of asymmetric catalytic synthesis (1999)

[96] Belokon, Y.N.; Bulychev, A.G.; Vitt, S.V.; Struchkov, Y.T.; Batsanov, A.S.; Tatiana Timofeeva, T.V.; Tsyryapkin, V.A.; Ryzhov, M.C.; Lysova, L.A.; Bakhmutov, V.I.; Belikov, V.M.; *J. Am. Chem. Soc.* **1984**, 107, 4252-4259. And References therein.

of β-hydroxy-α-amino acids using glycinate Schiff bases there is the work by the group of Corey[97] describing the addition of trimethylsilyl enol ether derivative of *tert*-butylglycinatebenzophenone Schiff base (**95**) to aldehydes using the chiral ammonium cinchonidine-derived bifluoride salt **97** as catalyst, under Mukayama-aldol conditions. Various branched aliphatic aldehydes were subjected to these reaction conditions, providing enantiomeric excesses in the range 72-95% for the syn diasteromer.

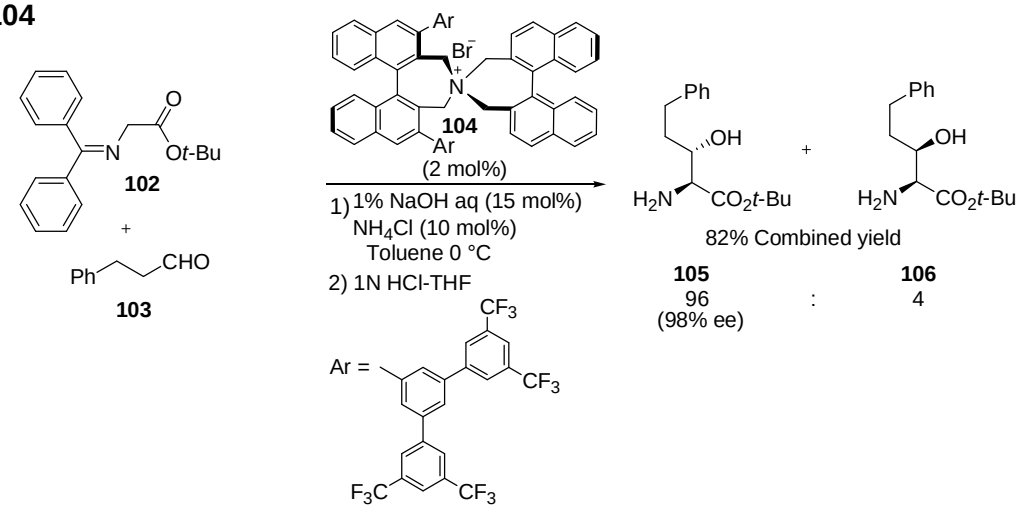
Scheme 5.2 Mukayama-aldol approach catalysed by a chiral bifluoride salt.



In 2002 the group of Shibasaki reported the addition of glycinate Schiff bases to aldehydes without preformation of enol silyl ethers using a heterobimetallic lanthanide catalyst.[98] In this case the major diasteromer obtained was mainly the anti even if diastereoselectivity and enantioslectivity were modest. In 2004 the cinchona alkaloid derived chiral ammonium salt developed by Park and Jew[99] was used by the Castle group[100] as a catalyst for the aldol reaction of unprotected glycinate Shiff bases to aldehydes under homogeneous conditions, in the presence of stoichiometric amounts of the organic base phosphazene base *tert*-butyliminotri(pyrrolidino)phosphorane (BTTP). The *syn* diastereomers were obtained as major compounds with ee in the range 52-83%. A big improvement in enantio- and diastereoselectivities with the direct aldol of glycinate Schiff bases was achieved by the group of Maruoka,[101] that reached very good enantioselectivities (up to 99% ee) and diastereoselectivities (up to >96:4 in favour of the anti diastereoisomer) using the axially chiral ammonium salt **104** under phase transfer catalysis conditions. Reaction conditions were particularly effective for the addition to aliphatic, greasy aldehydes.

[97] Horikawa, M.; Busch-Petersen, J.; Corey, E.J. *Tetrahedron Letters,* **1999**, 3843-3846.
[98] Yoshikawa, N.; Shibasaki, M. *Tetrahedron,* **2002**, 52, 8289-8298.
[99] Jew, S.-s.;Yoo, M.-S.; Jeong, B.-S.; Park, I.-Y.; Park, H.-g. *Org. Lett*. **2002**, *4*, 4245.
[100] Mettath, S.; Skrikanth, G.S.C.; Dangerfield, B.S.; Castle, S.L. *J. Org. Chem.*, **2004**, 69, 6489-6492.
[101] Ooi, T.; Kameda, T.; Maruoka, K. *J. Am. Chem. Soc.;* **2004**, 126, 9685-9694.

Scheme 5.3 Phase transfer catalysed aldol reaction using the axially chiral ammonium salt **104**

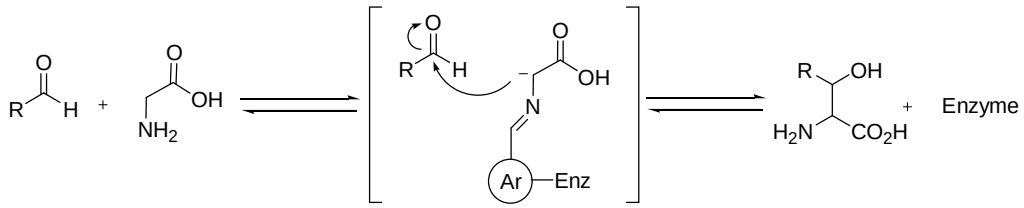


Enzymatic activation of glycine

Many approaches have been reported where enzymes are used to activate simple glycine towards aldehydes. In 1992 the enzyme serine hydroxymethyltransferase[102] (SHMT) has been used by Young[103] for the reaction of glycine with a variety of aldehyde substrates to form β-hydroxyaminoacids in reasonable yields. Anyway, although L-stereospecificity was observed at the α-centre, stereospecificity was not high at the β-centre. In the beginning of the 90's, many enzymatic approaches to β-hydroxy-α-amino acids based on the use of different threonine aldolases were presented.[104] In 1997 the group of Wong cloned and overexpressed in *E. coli* the genes coding for the *Escherichia coli* L-threonine aldolase (LTA; EC 2.1.2.1) and *Xanthomonus oryzae* D-threonine aldolase (DTA).[105] These enzymes accepted a wide range of aldehydes as acceptors, providing mixtures of diastereomeric products. LTA gave mainly *erythro-β*-hydroxy-α-L-amino acids from aliphatic aldehydes and the *threo* isomers from aromatic aldehydes, while DTA gave *threo-β*-hydroxy-α-D-amino acids as main kinetically controlled products. Diastereoselectivities ranged from modest to good but a careful control of experimental conditions and addition of pyridoxal phospate as cofactor were needed.

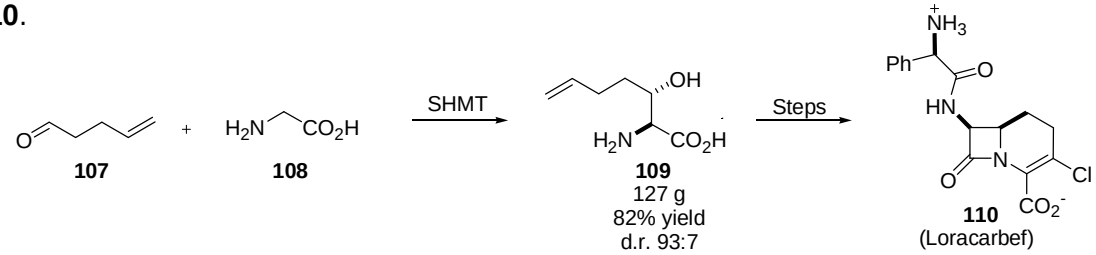
[102] For a review about SHMT see: Matthews, R.; Drummond, J.T. *Chem. Rev.*, **1990**, 90, 1275-1290.
[103] Saeed, A.; Young, W.D.; *Tetrahedron*, **1992**, 48, 2507-2514.
[104] (a) Ikemi, M.; Morikawa, T.; Miyoshi, T.; Shimizu, S.; Kataoka, M.; Yamada, H. *Biochem. Eng. 2001, Proc. Asia-Pac. Biochem. Eng. Conf.* **1992**, 96. (b) Vassilev, V. P.; Uchiyama, T.; Kajimoto, T.; Wong, C.-H. *Tetrahedron Lett.* **1995**, *36*, 4081. (b) Herbert, R. B.; Wilkinson, B.; Ellames, G. J.; Kunec, E. K. *J. Chem. Soc., Chem. Commun.* **1993**, 205.
[105] Kimura, T; Vassilve, V.P.; Shen, G.J.; Wong, C.H.; *J. Am. Chem. Soc.*, **1997**, 119, 11734-11742.

Scheme 5.4 Threonine Aldolase catalyzed addition of glycine to aldehydes



In 2000, the Ely Lilly R&D reported an efficient synthesis of oral carbacephalosporin antibiotic loracarbef **110** using SHMT catalysed aldol of glycine and 4-pentenaldehyde **107** to prepare the required intermediate L-erythro-2-amino-3-hydroxy-6-heptenoic acid **109** on a multigram scale.[106]

Scheme 5.5 Application of SHMT catalysed aldol reaction for the synthesis of Lorecarbef **110**.



107 **108** **109** 127 g 82% yield d.r. 93:7 **110** (Loracarbef)

In 2007 a protocol for the production of L-*allo*-threonine with a continuos process using the serine hydroxymethyltransferase GlyA form *Escherichia coli* was developed.[107] The authors obtained L-*allo*-threonine in an enzyme membrane reactor (EMR) with diastereoselectivity higher then 96% with a space time yield of 227 g L^{-1} d^{-1} without significant loss of enzyme activity over 120 h of operation.
In general, enzymatic methods present tha advantage of being environmentally friendly and use the simplest possible starting materials (glycine and aldehyde) but require strong efforts to obtain high levels of diastereoselectivities for every aldehyde which is used. Moreover the use of a cofactor or additives is needed. All this factors make enzyme-based startegies not the first choice in the common organic-laboratory practice, where a quick and simple procedure is needed. Anyway, enzymatic processes can be the method of choice for an industrial process where a single product is needed in batch quantities and a long optimization doesn't represent a substantial drawback.

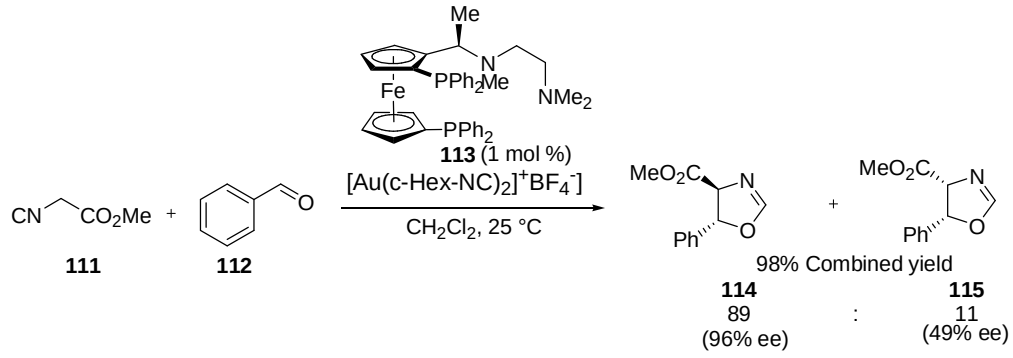
Isocyanoacetates

Addition of isocyanoacetates to aldehydes is a common reaction for the synthesis of oxazolines, which are direct precursors of β-hydroxy-α-amino acids after hydrolysis. The reaction is catalysed by many inorganic bases such as sodium cyanide in ethanol[108] and

[106] Jackson, B.G.; Pedersen, S.W.; Fisher, J.W.; Misner, J.; Gardner, J.P.; Staszak, M.A.; Doecke, C.; Rizzo, J.; Aikins, J.; Farkas, E.; Trinkle, K.L.; Vicenzi, J.; Reinhard, M.; Kroeff, E.P.; Higginbotham, C.A.; Gazak, R.J.; Zhang, T.Y. *Tetrahedron*, **2000**, 56, 5667-5677.
[107] Makart, S.; Bechtold, M.; Panke, S. *J. of Biotechnol.* **2007**, 130, 402-410.
[108]

by a combination of a tertiary amine and a transition metal salt, the most common of which are Au(I), Pd(II)[109], Cu(I).[110] Among the asymmetric versions of this reactions the most notable approach is that reported by the group of Ito[111] and successively studied by Togni[112] and co-workers in series of papers appeared from the second half of the 80's.

Scheme 5.6 Asymmetric aldol reaction of isocyanoacetates catalysed by a chiral ferrocenylphosphine-gold(I) complex **113**.



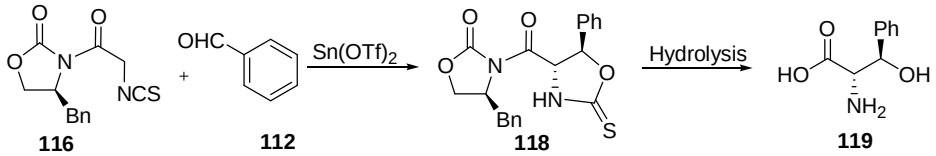
Ito designed one of the first bifunctional metal-organic catalyst,[16] based on a chiral ferrocenylphosphine-gold(I) complex prepared in situ by mixing a gold(I) salt and the ligand **113**, bearing a tertiary amine on a side chain. The mechanism of action of catalyst **113** has been deeply studied[113] and it has been reported that the chiral complex **113** acts as a bifunctional metal-organic catalyst, in which the gold cation serves as a Lewis acid and is binded by the isocyanide, enhancing the acidity of the α-protons of the isocyaoacetate which are deprotonated by the tertiary amine present on the side chain.
Less successful approaches are based on the use of chiral Pincer complexes of Pd(II) and Pt(II).[114] Different palladium complexes have been tested but enantioselectivities and diastereoselectivies are still modest.

Isothiocyanato-acetates

In 1986 Evans reported the highly syn diastereoselective aldol reaction of the chiral glycine synthon **116**, as its derived stannous enolate.[115] Yields ranging from 71 to 92% and diastereoselectivities up to 99:1 were obtained. Final cleavage of the chiral auxiliary allowed the isolation of the corresponding β-hydroxy-α-amino acids.

[109] Guillena, G.; Rodriguez, G.; Van Koten, G. *Tetrahedron Letters*, **2002**, 43, 3895-3898.
[110] (a) Ito, Y.; Matsuura, T.; Saegusa, T., . *Tetrahedron Letters*, **1985**, 26, 5781-5784. (b) Benito-Garagorri, D.; Bocokic, V.; Kirchner, K. *Tetrahedron Letters*, **2006**, 47, 8641-8644 and references therein.
[111] Ito, Y.; Sawamura, M.; Hayashi, T. *J. Am. Chem. Soc.*, **1986**, 108, 6405-6406.
[112] (a) Pastor, S.D.; Togni, A. *J. Am. Chem. Soc.*, **1989**, 111, 2333-2334. (b) Pastor, S.D.; Togni, A. *J. Org. Chem.*, **1990**, 55, 1649-1664.
[113] See Ref 16
[114] (a) Gosiewska,S.; Martinez Herreras, S.; Lutz, M.; Anthony L., Spek, A. L.; Havenith, R.W.A.; van Klink, G.P.M.; van Koten, G.; Klein Gebbink, R.J.M., *Organometallics*, **2008**, 27, 2549-2559. (b) Yoon, M.S.; Ramesh, R.; Kim, J.; Ryu, D.; Ahn, K.H., *J. Organomet. Chem.*, **2006**, 691, 5927-5934.
[115] (a) Evans, D. A.; Weber, A. E. *J. Am. Chem. Soc.* **1986**, *108*, 6757–6761. (b) Evans, D. A.; Weber, A. E. *J. Am. Chem. Soc.* **1987**, *109*, 7151–7157.

Scheme 5.7 Evans chiral oxazolidinone



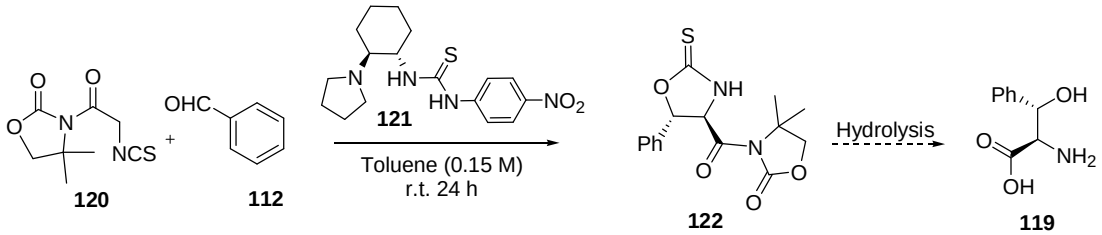
116 **112** **118** **119**

99:1 d.r.
91% Isolated yield
of major diastereomer

The mothod proved quite robust and general and has been applied to the synthesis of numerous β-hydroxy-α-amino acids.[116]

In 2008 an organocatalytic approach using α-isothiocyanato imides has been reported by the group of Seidel.[117] The isothiocyanato imide **120** was reacted in the presence of various aldehydes in the presence of the bifunctional thiourea catalyst **121**. Various aldehydes gave the resultant syn β-hydroxy-α-amino acids in high enantioselectivities (up to 96%) and good diastereoselectivities (in the range 82:18 to 97:3). Aromatic aldehydes proved to be the best substrates in terms of yield and enantioselectivity.

Scheme 5.8 Organocatalysed aldol reaction of isothiocyanato imides and aldehydes.



120 **112** **121** **122** **119**

93:7 d.r.
99% Combined yield
94% ee

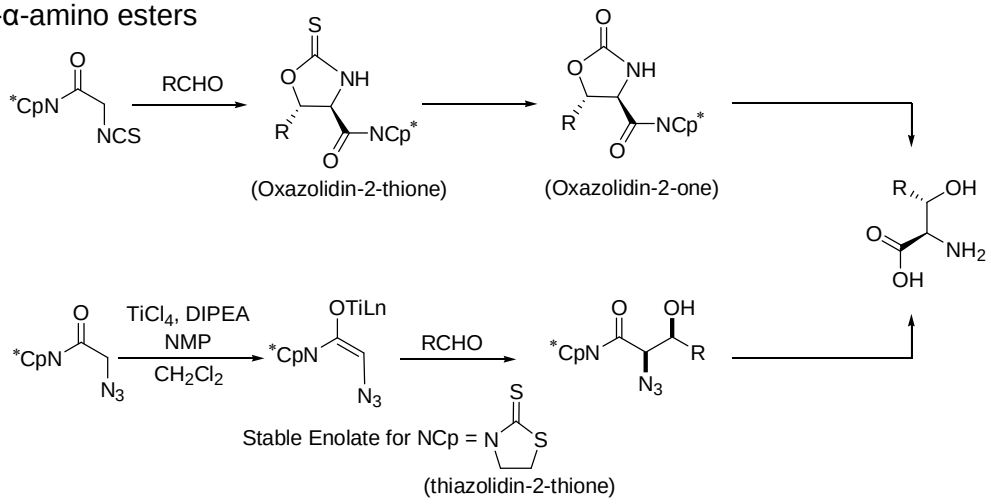
α-Azido enolates

The previously described strategy based on the use of Isothiocyanato-acetate has a major drawback in the hydrolysis step of the resulting oxazolidin-2-thione. This process, which is needed to recover the desired free amino alcol, is not a trivial step as prior transformation of the oxazolidin-2-thione into the more easily hydrolysed oxazolidin-2-one is needed.[118] In order to overcome this problem, the use of α-azido-enolates has been developed. The use of azide group as a masked amine would introduction the amine group with a simple and clean reduction step. Nevertheless it is well known that enolates of α-azido ketones or esters are not stable and that they spontaneously decompose into α-imino ketones or esters.[119] Recently (2008), the group of Franck an co-workers reported that α-azido

[116] (a) Evans, D. A.; Weber, A. E. *J. Am. Chem. Soc.* **1987**, *109*, 7151–7157. (b) Lago, M. A.; Samanen, J.; Elliott, J. D. *J. Org. Chem.* **1992**, *57*, 3493–3496. (c) Boger, D. L.; Colletti, S. L.; Honda, T.; Menezes, R. F. *J. Am.Chem. Soc.* **1994**, *116*, 5607–5618. (d) Boger, D. L.; Patane, M. A.; Zhou, J. *J. Am. Chem. Soc.* **1994**, *116*, 8544–8556. (e) Herbert, B.; Kim, I. H.; Kirk, K. L. *J. Org. Chem.* **2001**, *66*, 4892–4897.
[117] Li, L.; Klauber, E.G.; Seidel, D.; *J. Am. Chem. Soc.* **2008**, *130*, 12248–12249.
[118] (a) M. A. Lago, J. Samanen, J. D. Elliot, *J. Org. Chem.* **1992**, 57, 3493-3496; (b) B. Herbert, I. H. Kim, K. L. Kirk, *J. Org. Chem.* **2001**, 66, 4892-4897.
[119] (a) P. Martin, *Helv. Chim. Acta* **1989**, 72, 1554-1582; (b) T. Patonay, R. V. Hoffman, *J. Org. Chem.* **1995**, 60, 2368-377; (c) Y. Murakami, T. Watanabe, H. Suzuki, N. Kotabe, T. Takahashi, K. Toyanari, M. Ohno, K.

enolates of N-acyl-thiazolidin-2-thione substrates are stabilized by the presence of Ti(IV) species and react with aldehydes to give syn-β-hydroxy-α-azido esters in good yield. Moreover the thiazolidin-2-thiones can act as a chiral auxiliary. The authors proved that syn-β-hydroxy-α-azido esters can be obtained in high diastereoselectivities when the phenyl-glycine derived thiazolidin-2-thiones where used.

Scheme 5.9 Isothiocyanate vs azide as a masked amino group for the synthesis of -β-hydroxy-α-amino esters

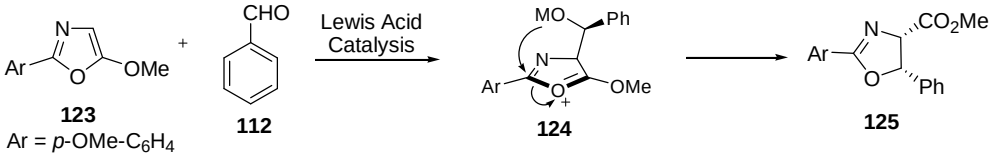


*CpN=oxazolidin-2-one, oxazolidin-2-thione, thiazolidin-2-thione

Alkoxyoxazoles

Suga and Ibata described an aldol addition/acyl transfer process wherein 5-methoxyoxazole **123** functions as a glycine enolate synthon.[120]

Scheme **5.10**



The reaction is catalysed by lewis acids, In particular aluminium-based lewis acids are efficient and have been applied in enantioselective versions by Suga and Ibata[120] and later by the Evans group in 2001.[121] It should be noted that the major diastereomer obtained is always the cis oxazoline, which can be epimerized to the more stable trans isomer by treatment with a catalytic amount of DBU. Evans reached enantiselectivities and diastereoselectivities up to >99:1 in the presence of aromatic aldehydes and the

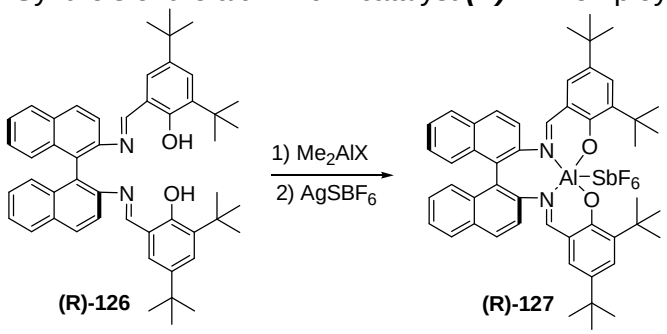
Takase, T. Suzuki, K. Kondo, *Chem. Pharm. Bull.* **1997**, 45, 1739-1744; (d) A. Padwa, M. M. SN, M. D. Weingarten, *Tetrahedron* **1997**, 53, 2371-2386; (e) G. Geen, C. J. Shaw, J. B. Sweeney, *Synlett* **1999**, 1444-1446; (f) T. Patonay, E. JuhNsz-TOth, A. BMnyei, *Eur. J. Org. Chem.* **2002**, 285-295.

[120] H. Suga, K. Ikai, T. Ibata, *J. Org. Chem.* **1999**, 64, 7040-7047, and references therein.

[121] Evans, D.A.; Janey, J.M.; Magomedov, N.; Tedrow, J.S.; *Angew. Chem. Int. Ed.* **2001**, 40, 1884-18888.

catalyst **127**, prepared from a diaminobinaphthyl-derived ligand **126** and dimethylaluminum chloride (Scheme 5.11).

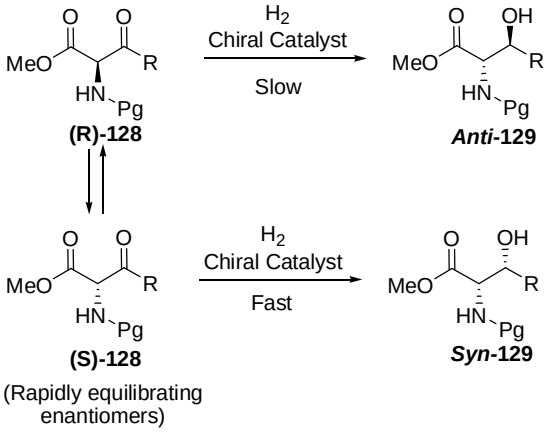
Scheme 5.11 Syntheis of the aluminium catalyst ***(R)*-127** employed by Evans.



5.1.2 Hydrogenation via dynamic kinetic resolution

A very elegant strategy is based on the hydrogenation via dynamic kinetic resolution of protected α-amino-β-keto esters (**128**), using chiral transition metal catalysts. The chiral lability of 2-substituted 3-oxo carboxylic esters of type **128**, coupled with the high chiral recognition ability of chiral hydrogenation complexes offers the possibility of stereoselective hydrogenation utilizing dynamic stereomutation as outlined in Scheme 5.12. If the racemization of the enantiomers ***(R)*-128** and ***(S)*-128** can be rapid enough with respect to the hydrogenation then, when rates of the reaction of the two enantiomers ***(R)*-128** and **(S)-128** are substantially different, the hydrogenation forms one isomer selectively among the four possible stereoisomeric products.

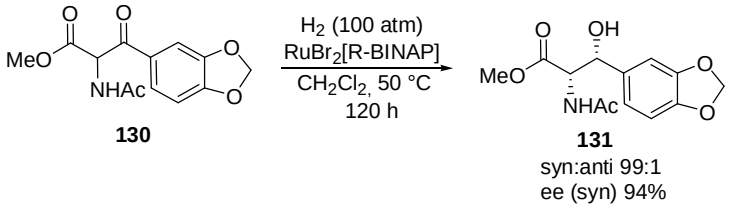
Scheme. 5.12 Concept for the Dynamic Kinetic Resolution approach to β-hydroxy-α-amino acids (the concept is exemplified in case the syn product is prevalent).



This concept has been successfully applied in 1989 by Noyori, who synthesised the hydroxyl amino ester **131**, useful intermediate for the preparation of L-DOPS, an anti-

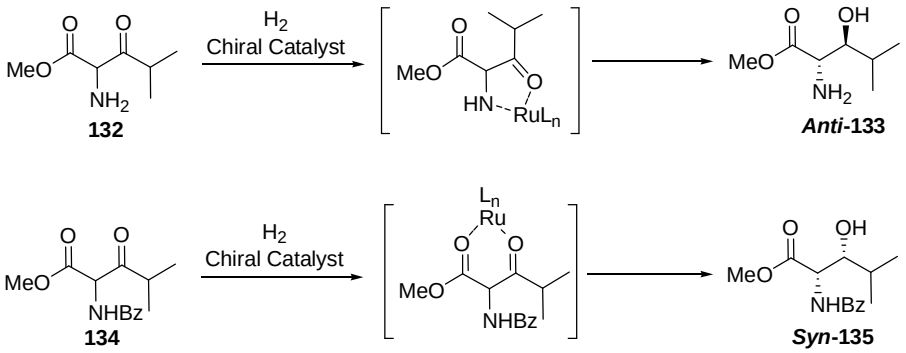
Parkinsonian agent.[122] The catalyst used was the simple $RuBr_2$[R-BINAP] and the syn diastereomer was the major as exemplified in scheme 5.13.

Scheme 5.13 Noyori synthesis of intermediate **131**.



The reaction developed by Noyori has been applied to the synthesis of various hydroxy-amino acids and different type of catalysts based on Ru(II) have been reported.[123]
Recently, in 2004, Hamada reported a new approach, in which instead of the syn diastereomer, the major product is the anti β-hydroxy-α-amino acid.[124] This difference in stereoselectivity is obtained using unprotected α-amino-β-keto esters hydrochloride salts, in the presence of Ru-BINAP catalysts. The anti-selectivity is attributed by the authors to a chelating effect of the amino group, which originates a 5 membered transition state in the presence of the chiral ruthenium catalyst. This five member transition state would then originate the anti diastereomer. In the case of the use of a benzoylated amino-group, the transition state is a 6 member ring and the successive hydrogenation gives rise to the syn diastereomer.

Scheme **5.14**

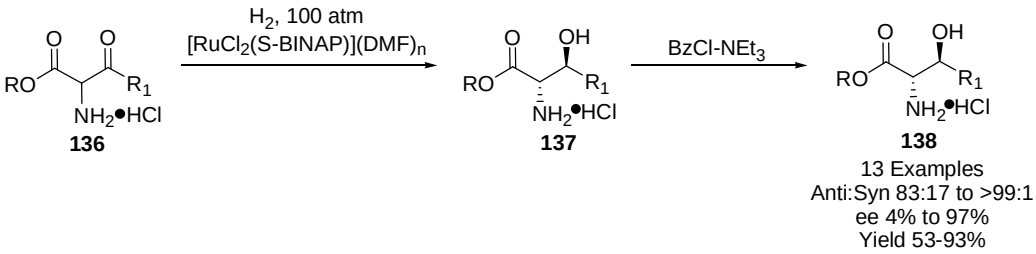


This methodology has been successfully applied by the authors cited in Ref. 29 to 13 different substrates, providing discrete to excellent enantioselectivities and excellent anti

[122] Suzuki, *T.;* Sakoda, S.; Ueji, M.; Kishimoto, ***S.;*** Hayashi, A.; Kondo, T.; Narabayashi, H. *Neurology* **1984,** 34, 1446.
[123] (a) K. Mashima, Y. Matsumura, K. Kusano, H. Kumobayashi, N. Sayo, Y. Hori, T. Ishizaki, S. Akutagawa, H. Takaya, *J. Chem. Soc. Chem. Commun.* **1991**, 609. (b) J.-P. Genet, C. Pinel, S. Mallart, S. Juge, S. Thorimbert, J. A. Laffitte, *Tetrahedron: Asymmetry* **1991**, 2, 555-567; (c) M. Kitamura, M. Tokunaga, R. Noyori, *J. Am. Chem. Soc.* **1993**, 115, 144-152; (d) K. Mashima, K. Kusano, N. Sato, Y. Matsumura, K. Nozaki, H. Kumobayashi, N. Sayo, Y. Hori, T. Ishizaki, S. Akutagawa, H. Takaya, *J. Org. Chem.* **1994**, 59, 3064-3076; (e) J.-P. Genet, M. C. C. de Andrade, V. Ratovelomanana-Vidal, *Tetrahedron Lett.* **1995**, 36, 2063-2066; (f) E. Coulon, M. C. C. de Andrade, V. Ratovelomanana-Vidal, J.-P. Genet, *Tetrahedron Lett.* **1998**, 39, 6467-6470; i) K. Makino, N. Okamoto, O. Hara, Y. Hamada, *Tetrahedron: Asymmetry* **2001**, 12, 1757-1762.
[124] Makino, K.; Goto, T.; Hiroki, Y.; Hamada, Y. *Angew. Chem. Int. Ed.* **2004**, 43, 882-882.

diastereoselectivities. Best substrates proved to be aliphatic ketones, while aromatic ketones gave good diastereoselectivities but low enantiomeric excesses.

Scheme 5.15 Anti-selective hydrogentation of α-amino-β-keto esters hydrochloride salts.



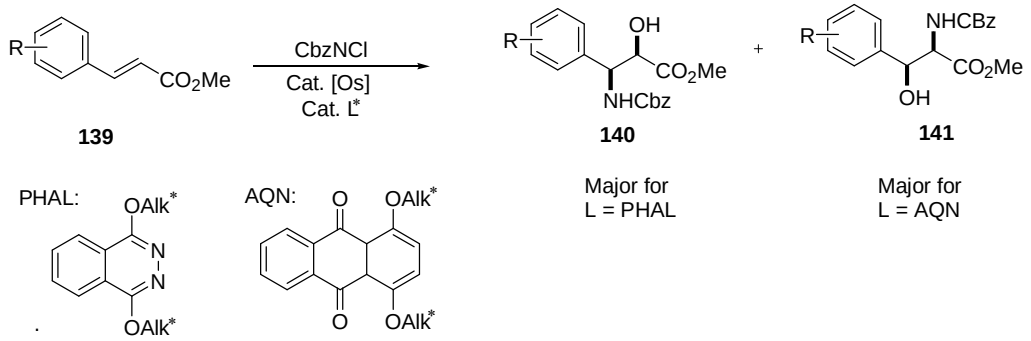
In 2004 also a different approach to anti-selective hydrogenation was reported using ruthenium catalysts. In this case the anti selectivity was obtained using α-phtalimido-β-keto esters as substrates for the hydrogenation and C_3-TunePhos as chiral ligands. The process was successfully applied to the high-yielding preparation of *allo*-threonine in 99% ee and >97:3 anti:syn ratio.[125] Other papers have then appeared, in which anti-selectivity is obtained in the presence of Ru-based catalysts.[126]
More recently, Hamada and co-workers have developed highly anti-selective and enantioselective catalysts for the hydrogenation of α-amino-β-keto esters based on iridium(I) complexes.[127] This new catalysts are effective with aromatic substituted keto esters, so they are complementary to the Ru(II) catalysts described by Hamade.

5.1.3 Sharpless asymmetric dihydroxylation, amino-hydroxylation and epoxidation

Asymmetric aminohydroxylation of α,β-unsaturated esters would provide an ideal strategy for the synthesis of syn β-hydroxy-α-amino esters, providing the desired products in one single step from easily available olefinic precursors. Unfortunately standard asymmetric aminohydroxylation of unsaturated esters with PHAL ligands system usually affords the corresponding β-amino esters. In 1998 Sharpless reported a modification in which the regiochemical outcome of the reaction could be reversed using AQN ligands in the presence of cinnamate esters.[128]

[125] Lei, A.; Wu, S.; He, M.;. Zhang, X. *J. Am. Chem. Soc*. **2004**, *12*6, 1626-1627.
[126] (a) Mordant, C.; Dunkelmann, P.; Ratovelomanana-Vidal, V.; Genet, J. P. *Chem. Commun*. **2004**, 1296-**1297**. (b) Mordant, C.; Dunkelmann, P.; Ratovelomanana-Vidal, V.; Genet, J.-P. *Eur. J. Org. Chem*. **2004**, 3017-3026.
[127] (a) Makino, K.; Hiroki, Y.; Hamada, Y. *J. Am. Chem. Soc.* **2005**, 127, 5784-5785. (b) Makino, K.; Iwasaki, M.; Hamada, Y.; *Org. Lett.* **2006**, 4573-4576.
[128] Tao, B.; Schlingloff, G.; Sharpless, K.B. *Tetrahedron Letters* **1998**, 39, 2507-2510.

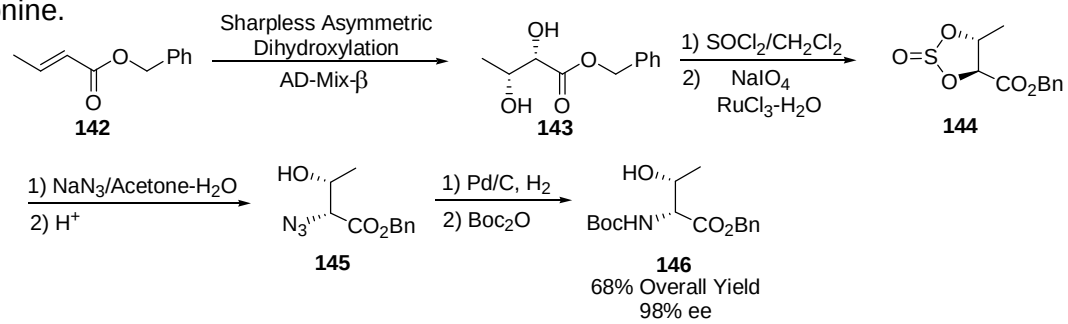
Scheme 5.16 Asymmetric aminohydroxylation approach to β-hydroxy-α-amino esters.



Alk* = dihydroquininyl (DHQ) or -quinidinyl (DHQD)

These finding opened the field to various application of asymmetric aminohydroxylation for the synthesis of β-hydroxy-α-amino esters.[129]
In 1996 Goodman and Shao reported a highly enantioselective synthesis of allo-thronine and β-hydroxy-valine using asymmetric Sharpless dihydroxylation.[130] The preparation of allo-threonine was based on the asymmetric dihydroxylation of benzyl crotonate; the diol was the converted to its 2,3-cyclic sulfite with $SOCl_2$ and oxidized to cyclic sulfate **X** in a one-pot synthesis. Nucleophilic displacement by NaN_3 at the α-C of cyclic sulfate **X** occurs with clean inversion of chirality, and acidic hydrolysis provides the desired α-azido ester that is then reduced and deprotected in a single hydrogenation step.

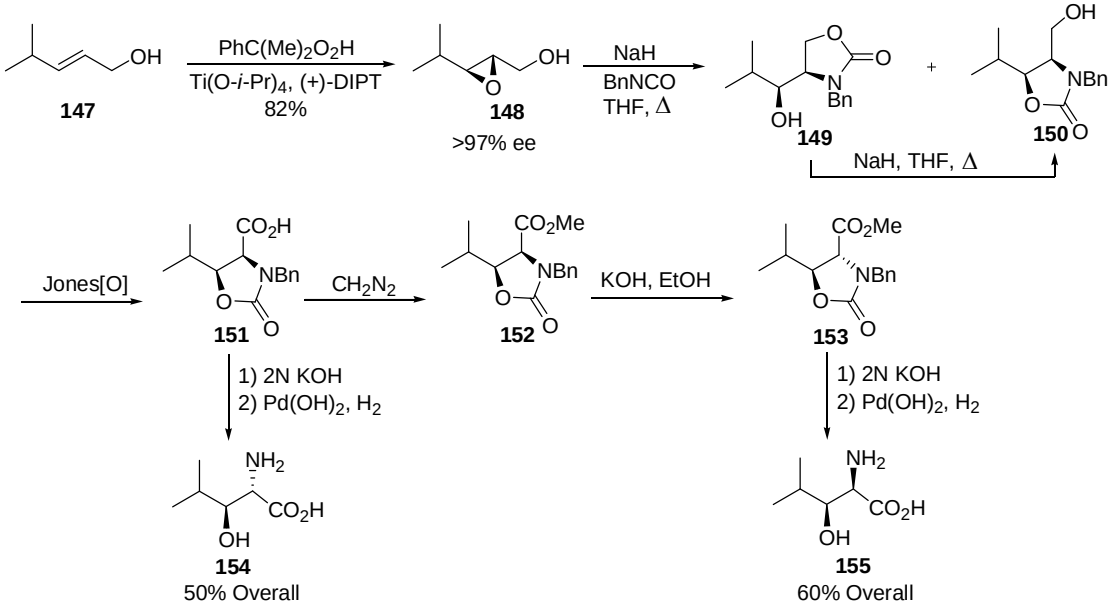
Scheme 5.17 Sharpless Asymmetric dihydroxylation strategy for the synthesis of allo-thronine.



During the enantioselective synthesis of (+)-Lacatcystin and its analogues by Omura and Amos B Smith III, it was required a scalable and flexible process to access to four possible stereoisomer of hydroxy-leucine.[131] The reported approach was based on a catalytic Sharpless epoxidation of olefin **147** and a benzyl isocyanate-induced opening of epoxide **148**. A final base-catalysed epimerization of the oxazolidinone ester **152** is used in case the syn diastereomer is needed. The process provides an access to all four possible diastereomer of hydoxy-leucine in high yield and high enantiomeric excess (>97%).

[129] (a) Park, H.; Cao, B.; Joullie, M. M. *J. Org. Chem.* **2001**, *66*, 7223. (b) Miller, M. J. *J. Org. Chem.* **2002**, *67*, 4759.
[130] Shao, H.; Goodman, M.; *J. Org. Chem.* **1996**, 61, 2582-2583.
[131] Nagamitsu, T.; Sunazuka, T.; Tanaka, H.;Omura, S.; Sprengeler, P.A.; Smith III, A.B. *J. Am. Chem. Soc.* **1996,** *118,* 3584-3590.

Scheme 5.18 Synthesis of hydroxyleucines based on asymmetric sharpless epoxidation.

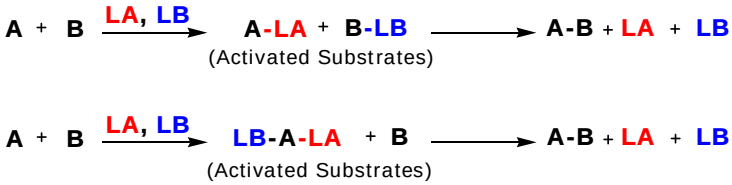


5.2 Bifucnctional metal-organic catlysis

A concept that has recently emerged in the wide field of catlysis is the part concerning the bifunctional metal-organic catalysis. "….There is a growing emphasis on multifunctional systems, in which multiple parts of a catalyst or multiple catalysts work together to promote a specific reaction. These efforts, in part, are resultdriven, and they are also part of a movement toward emulating the efficiency and selectivity of nature's catalysts, enzymes…." (Ethan Alden-Danforth and Thomas Lectka, Account Chemical Research, 2007, 41, 655-663). Two of the main approaches towards catalysis in organic chemistry are represented by metal activation of a substrate and by organic activation (organocatlysis). Very often the two strategies are complementary and, in some cases, they could even be used together, working in tandem in a single reaction.[132] These bifunctional systems can help to overcome some of the most recent challenges in current organic chemistry, providing a solution for the activation of system where both, orgnocatalysis and metal catalysis, have previously failed. Two mechanisms can be described for the activation provided by metal-organic bifunctional catalysis. In a first case the metal activates one of the reagents while the organic catalyst activates the second substrate. In a second case, both the metal catalyst and the organic catalyst cooperate to activate one of the substrates. The two systems are illustrated in scheme 5.19, where the metal catalyst is represented by a Lewis acid and the organic catalyst is represented by a Lewis base (for example a tertiary amine).

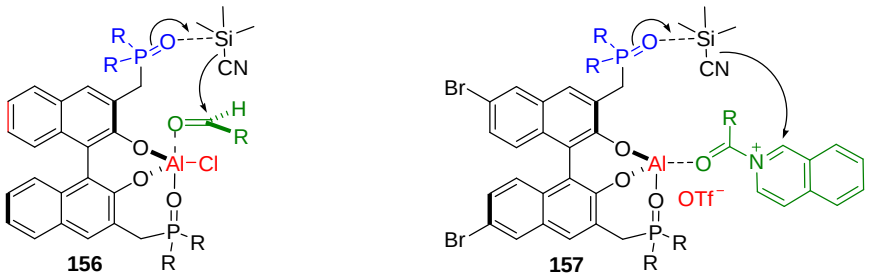
[132] For a review see: Alden-Danforth, E.; Lectka, T.; *Acc. Chem. Research*, **2007**, 41, 655-663.

Scheme **5.19**



A first problem that arises in the design of a practical bifunctional metal-organic system is the compatibility between the two catalysts that should act in synergy. In most cases the metal catalyst is a Lewis acid and the organic catalyst is a Lewis base. In this situation the two systems must be chosen carefully so that they don't react with each other. The large potential for the "self quenching" of the catalytic system can be avoided by a fine tuning of the two catalytic systems based on the "hard-soft" characters of the Lewis acid and the Lewis base. When an asymmetric catalyst is needed, a second point that should be considered is that often the LA and the LB are both linked together through a chiral scaffold, which must be properly chosen. A seminal example that can be used to illustrate how the concept is realised in practice is the Lewis base-BINOL-phosphine oxide catalyst for the asymmetric Strecker[133] and Reissert[134] reaction reported by Shibasaky and co-workers.

Scheme **5.20** Dual activation of Shibasaky's Strecker (left) and Reissert reaction (right)



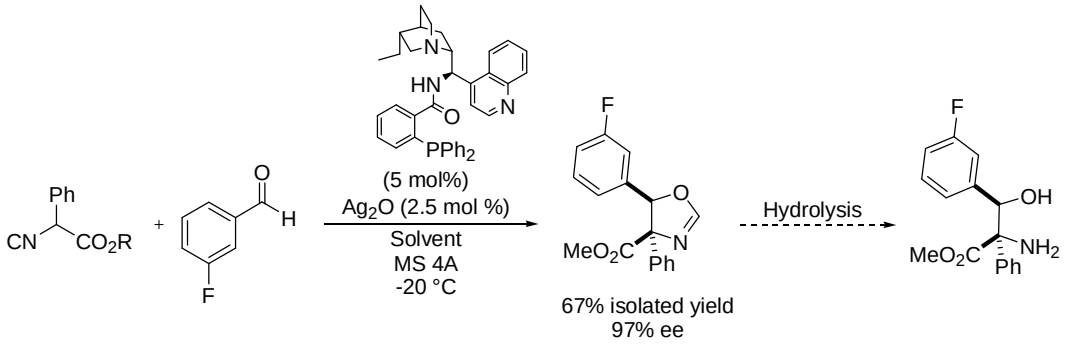
Using BINOL as a scaffold, they envisioned a catalyst-organized transition-state complex, where Al(III) activates the electrophile, while the tethered phosphine oxide interacts with TMS-CN.

5.3 Aims & Concept

In the first part, a new strategy for the synthesis of morpholines has been presented, using serine and threonine as cheap, enantiopure starting materials. This strategy requires the use of enantiopure β-hydroxy-α-amino acids building blocks and so is limited by the availability of suitable starting materials from the chiral pool. In order to extend the synthetic utility a reliable source of enantiopure β-hydroxy-α-amino acids was required. Although many different strategies for the asymmetric preparation of this starting materials have been reported so far, we found attractive and challenging the possibility of developing our own method. When we faced the problem we thought about the possibility

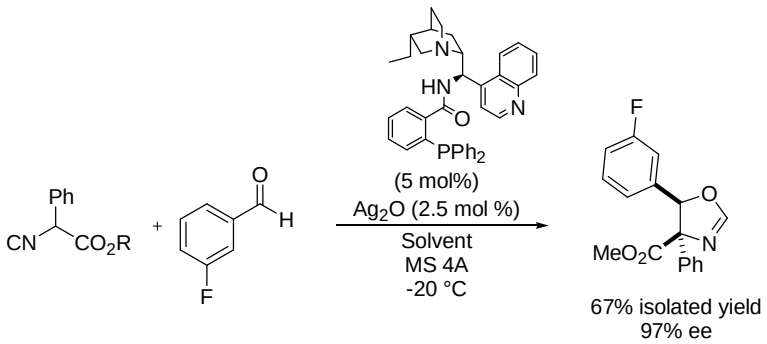
[133] Hamashima, Y.; Sawada, D.; Kanai, M.; Shibasaki, M. *J. Am. Chem. Soc.* **1999**, *121*, 2641-2642.
[134] Shibasaki, M.; Kanai, M.; Funabashi, K. *Chem. Commun.* **2002**, 1989-1999, and references therein.

of exploiting a catalytic method and we decided to carry on this new project in collaboration with the group of Professor Darren Dixon from the University of Manchester, which is specialised in the field of asymmetric catalysis. Together with Professor Darren Dixon we chose to focus our attention on the reaction of addition of α-isocyanoacetates to aldehydes. The reaction is catalysed by the cooperative action of transition metals and organic bases such as tertiary amines. The most common metals are palladium(II), copper(I), silver(I), gold(I). The products are oxazolines, which are a protected form of β-hydroxy-α-amino esters and can be readily converted to this latter after hydrolysis. Few attempts to obtain enantioselectivity in this reaction have been reported using Au(I) or Pd(II) complexes and are discussed in the previous section (5.1.1). The only remarkable catalyst has been reported by Ito and is based on the use of gold complexes. In this scenery we thought that the discovery of a new catalytic system based on a less expensive metal then gold could be a valuable contribute to the scientific community.
In this second part, we describe the design, synthesis and screen of a small library of bifunctional metal-organic catalysts for the enantio- and diastereoselective addition of isocyanoacetates to aldehydes. Optimization of this silver-catalysed reaction lead to the synthesis of syn β-hydroxy-α-amino esters with up to 96% enantiomeric excess. Moreover this new methodology allows the synthesis of β-hydroxy-α-amino acids bearing a fully substituted (quaternary) α-carbon with enantioselectivities up to 97%.



6 Highly enantio- and diastereo-selective addition of isocyanoacetates to aldehydes: development of a new catalytic system

ABSTRACT



A small library of amino-phosphine ligands has been designed and synthesized using cinchona alkaloid-derived scaffolds as source of chirality. This new ligands have been screened, together with various transition metals, as catalyst for the asymmetric addition of α-isocyanoacetates to aldehydes. Some of the ligands proved to be particularly effective in the presence of different silver salts. After a careful optimization, high diastereoselectivities (up to 98%) and enantioselectivities (up to 97%) were obtained. The new catalytic system was also efficient in the presence of α-substituted isocyanides, thus allowing the simultaneous creation of a quaternary and a tertiary stereogenic center next to each other. Finally, an efficient hydrolysis procedure for the conversion of oxazolines to the corresponding β-hydroxy-α-amino acids is disclosed.

6.1 Introduction

Optically active β-hydroxy-α-amino acids represent extremely important targets in organic chemistry. These structures are intermediates in the synthesis of many compounds such as β-lactams,[135] morpholines,[136] aziridines[137] and many bioactive natural products, especially glycopeptide antibiotics such as vancomycin and many others.[138] Moreover, in our case, β-hydroxy-α-amino acids are the precursors of the morpholine-based amino acids described in the first part of this thesis work and a reliable source of these starting materials was of primary importance in order to increase the diversity generated through the strategy described in the first part. Many approaches have been developed for their asymmetric preparation and the most of them have been introduced in Chapter 5. One of the most valuable strategies is the addition of α-isocyanoacetates to aldehydes. The reaction, first discovered by Schöllkopf[139] yields oxazolines, which are readily converted to β-hydroxy-α-amino esters/acids after mild hydrolysis.[140] Some asymmetric versions have been developed and are based on the use of pincer Pd(II) complexes and on the use ferrocenylphosphine gold(I) complexes. Attempts based on the use of palladium complexes only yielded modest enantioselectivities while the gold(I) complexes disclosed by Ito are more efficient and in the best cases can reach ee/de>90%. Unfortunately this approach requires the use of expensive gold complexes and a multistep synthesis is required for the preparation of pherrocenylphosphine ligands. We decided to develop our own catalytic system and we thought about using a bifunctional metal-organic system based on a combination of a chiral amine and a transition metal. This idea was suggested by literature papers that reported a cohoperative effect between the amine and the transition metal. The former would infact act as a base, able to create an enolate from the isocyanoacetate and the latter would act as a Lewis acid, being cohordinated by the isocyanide and thus enhancing the acidity of the α-protons of the isocyanoacetate, which are then more easily deprotonated.

6.2 New catalyst design

In order to develop a new metal-organic bifunctional catalytic system for the enantioselective addition of α-isocyanoacetate esters to aldehydes, we identified the cinchona alkaloid skeleton (Figure 6.1) as a versatile, powerful and readily accessible structure and we decided to plan our catalyst design using cinchona alkaloid derived scaffolds as source of chirality. We knew from previous work[141] that the addition of α-

[135] (a) Nagamitsu, T.; Sunazuka, T.; Tanaka, H.; Omura, S.; Sprengeler, P.A.; Smith, A. B., III. *J. Am. Chem. Soc.* **1996**, *118*, 3584. (b) Evans, D.A.; Weber, A. E. *J. Am. Chem. Soc.* **1986**, *108*, 6757.
[136] Sladojevich, F.; Trabocchi, A.; Guarna, A. *J. Org. Chem.* **2007**, 72, 4254-4257.
[137] Tanner, D. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 599
[138] For a review on Vancomicyn and related glycopeptide antibiotics see: Nicolaou, K.C.; Boddy, C.N.C.; Bräse, S.; Winssinger, N. *Angew. Chem. Int. Ed.* **1999**, 38, 2096-2152.
[139] Hoppe, D.; Schollkopf, U. *Justus Liebigs Ann. Chem.* **1972**, *763,* I.
[140] Ito, Y.; Sawamura, M.; Hayashi, T.; *J. Am. Chem. Soc.* **1986**, 108, 6406-6407.
[141] (a) Guillena, G.; Rodriguez, G.; Van Koten, G. *Tetrahedron Letters*, **2002**, 43, 3895-3898. (b) Ito, Y.; Matsuura, T.; Saegusa, T., . *Tetrahedron Letters*, **1985**, 26, 5781-5784. (c) Benito-Garagorri, D.; Bocokic, V.; Kirchner, K. *Tetrahedron Letters*, **2006**, 47, 8641-8644 and references therein. (d) Ito, Y.; Sawamura, M.; Hayashi, T. *J. Am. Chem. Soc.*, **1986**, 108, 6405-6406.

isocyanoacetate esters to aldehydes is catalyzed by a variety of late transition metals, in the presence of organic bases such as tertiary amines.

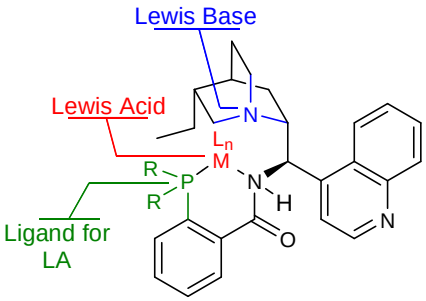


Figure 6.1 Catlyst design

Cinchona alkaloids combine a basic bridgehead nitrogen with a secondary alcohol, which can be used to add a further functionality, such as a strong-coordinating ligand, able to give strong binding with a late transition metal. Moreover the alcohol of cinchona alkaloids can be easily converted into a primary amine using a well established protocol consisting of "one pot" Mitsunobu-Staudinger[142], allowing further and simple catalyst differentiation. We identified phosphines and ureas/thioureas as ligands to be used to bind the transition metal required for our reaction. In this chapter the synthesis of this new catalyst will be described, together with the screening of optimal reaction conditions. Finally the scope of the reaction will be discussed.

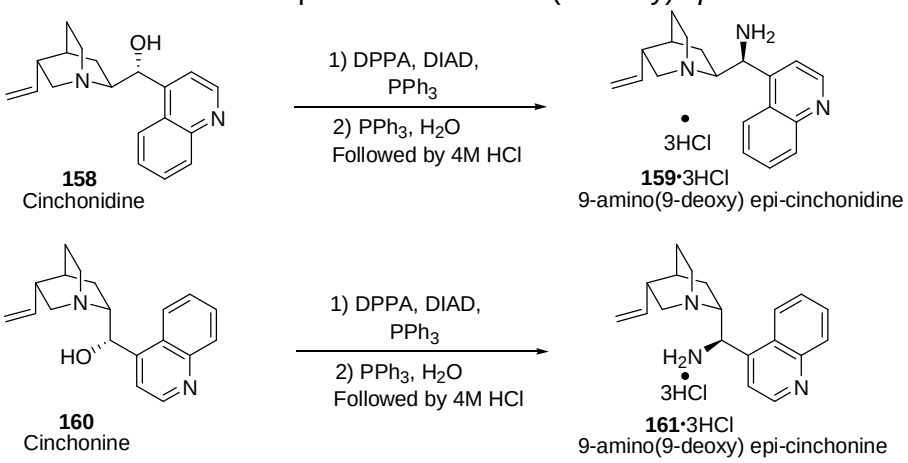
6.3 Ligand synthesis

6.3.1 Preparation of Chiral Scaffolds

The starting point in the synthesis of the new catalyst family was the preparation of chiral scaffolds. As chiral scaffolds we used commercially available cinchona alkaloids and the corresponding 9-amino(9-deoxy) *epi*-amines **159** and **161**.
The trihydrochloride salts of this chiral amines were synthesised on a multigram scale *via* a one-pot Mitsunobu/Staudinger reaction following a literature procedure (Scheme 6.1).[8] This reaction utilised naturally occurring alkaloid cinchonine and cinchonidine, diphenylphosphoryl azide (DPPA) as a source of azide instead of the more potentially hazardous hydrogen azide (HN_3). The ^{1}H-NMR indicated only one diastereomer signifying no retention of stereochemistry *via* neighbouring group participation.

[142] (a) Brunner, H.; Bügler, J.; Nuber, B. *Tetrahedron: Asymm.*, **1995**, *6*, 1699. 98) Brunner, H.; Bügler, J. *Bull. Soc. Chim. Belg.*, **1997**, *106*, 77. 99) Brunner, H.; Schmidt, P.; Prommesberger, M. *Tetrahedron: Asymm.*, **2000**, *11*, 1501. 100) Brunner, H.; Schmidt, P. *Eur. J. Org. Chem.*, **2000**, 2119. 101) Brunner, H.; Baur, M. A. *Eur. J. Org. Chem.*, **2003**, 2854.

Scheme 6.1 Preparation of 9-amino(9-deoxy) *epi*-amines



158
Cinchonidine

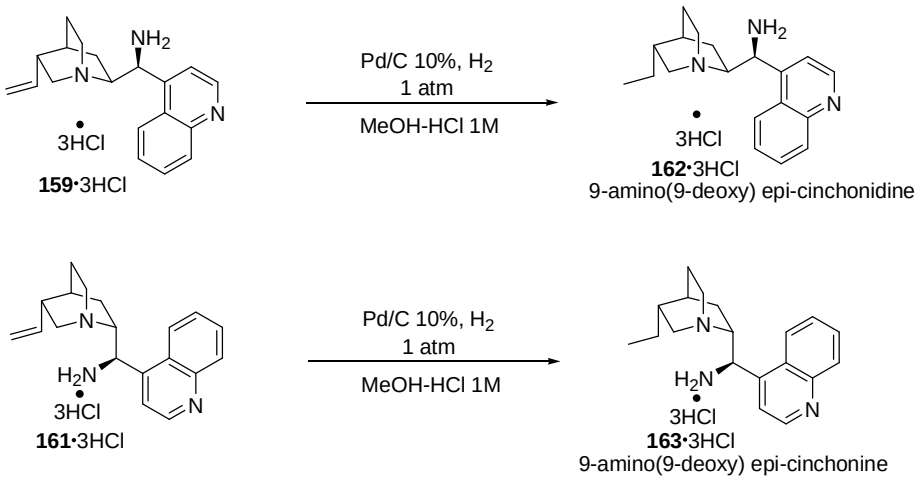
159·3HCl
9-amino(9-deoxy) epi-cinchonidine

160
Cinchonine

161·3HCl
9-amino(9-deoxy) epi-cinchonine

In the synthesis of catalysts, also the scaffolds deriving from the reduction of the double bonds were used. These were prepared from the corresponding amines trihydrochloride salts by catalytic hydrogenation using Pd/C in hydrogen atmosphere and a mixture MeOH-HCl 1M as solvent (Scheme 6.2). The crude products obtained proved to be NMR-pure and were directly used as substrates for the next coupling reactions without any purification.

Scheme 6.2 Hydrogenation of 9-amino(9-deoxy) *epi*-amines



159·3HCl

162·3HCl
9-amino(9-deoxy) epi-cinchonidine

161·3HCl

163·3HCl
9-amino(9-deoxy) epi-cinchonine

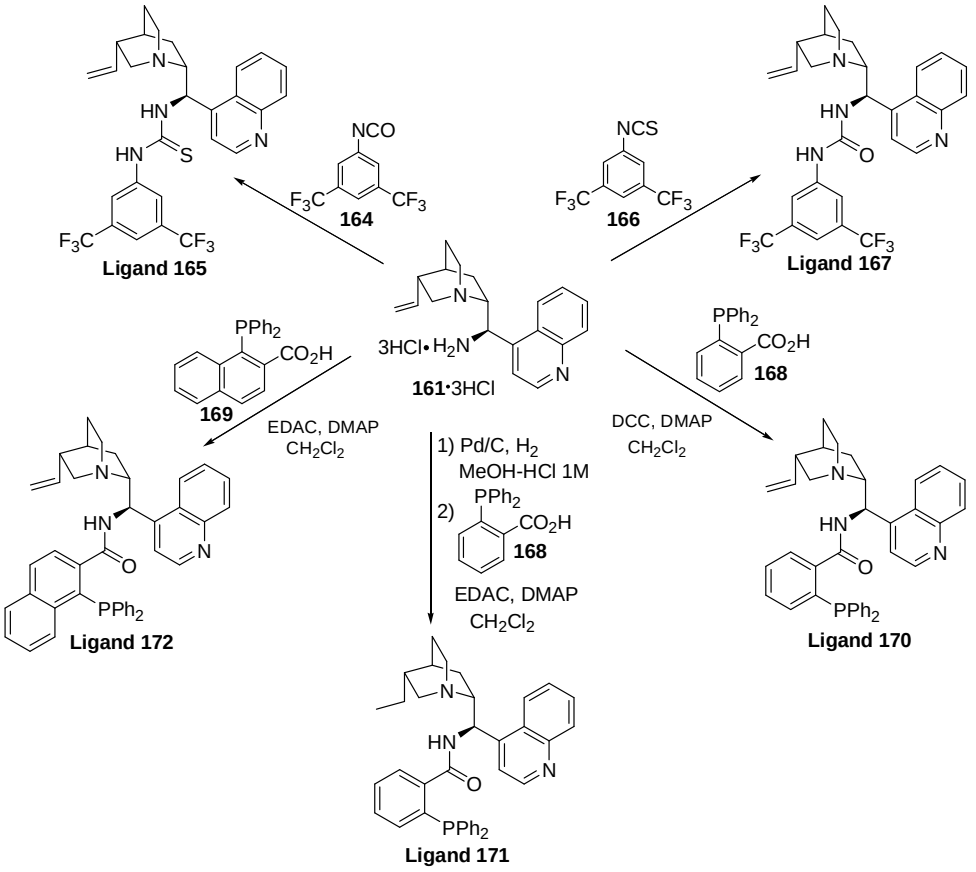
6.3.2 Functionalization of Cinchona Scaffolds

Ligands containing an urea or thiourea moiety were obtained by coupling of 9-amino(9-deoxy) *epi*-amines with *iso*(thio)cyanate R-CNX (X = S, O) and according to literature reported procedures.[143]

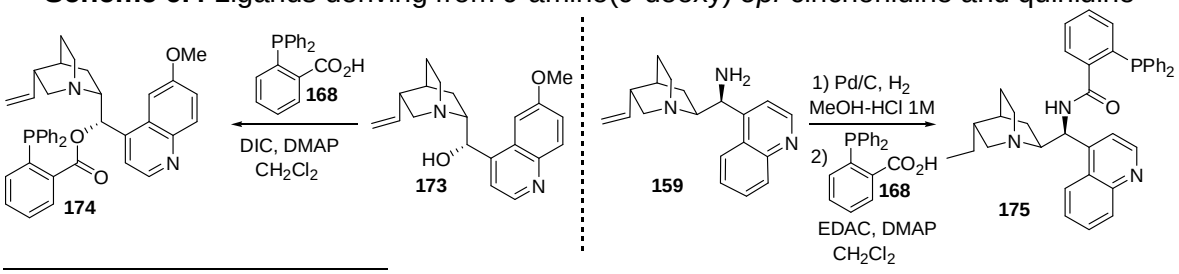
[143] Ye, J.; Dixon, D. J.; Hynes, P. S. *Chem. Commun*., **2005**, 4481-4483.

Ligands incorporating phosphines were synthesised after coupling of Cinchona scaffolds with 2-(diphenylphosphino)benzoic acid or 1-(diphenylphosphino)-2-naphthoic acid.[144] When natural alkaloids were used, the coupling was performed between the acid and the alcohol of the scaffold. When 9-amino(9-deoxy) *epi*-amines were used, the coupling was performed between the acid and the primary amine at C-9. In both cases the coupling reaction was performed using carbodiimides as activating system. Products were then isolated after flash chromatography purification.

Scheme 6.3 Synthesis of Ligands deriving from 9-amino(9-deoxy) *epi*-cinchonine



Scheme 6.4 Ligands deriving from 9-amino(9-deoxy) *epi*-cinchonidine and quinidine



[144] Trost, B. M..; Van Vranken, D. L.; Bingel, C. *J. Am. Chem. Soc.* **1992**, 114, 9327-9343.

6.4 Preliminary screen

Having some of the designed ligands in hands, the next step was the screen of the experimental conditions, in order to obtain reactivity and stereocontrol. The screening was performed mixing an equimolar amount of ligand and metal salt/complex in a given solvent, stirring for approximately 5 minutes in order to obtain the formation of the active catalyst *in situ*, and then adding the isocyanide and the aldehyde. Benzaldehyde and *t*-butyl-isocyanoacetate were selected as reagents for the screen.

Scheme 6.5

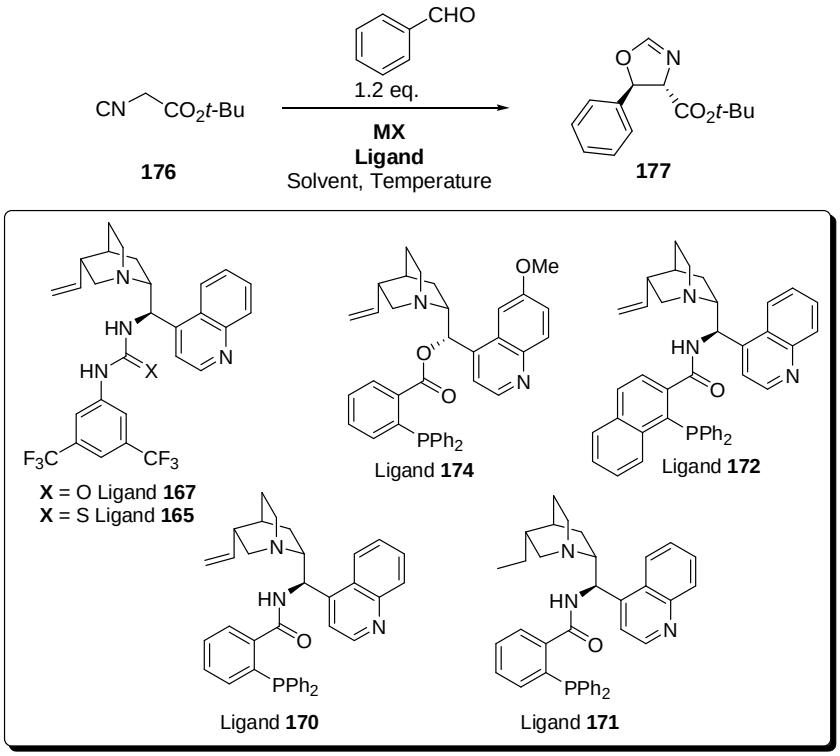


Table 6.1 Preliminary Screen

Ligand	Solvent	Metal Salt	T(°C)	Time (h)	Conv.[a] (%)	d.r.[a] Trans:cis	Ee[b] of trans (%)
167	DCM	AgOAc	25	2	100	5:1	29
165	DCM	AgOAc	25	4	25	-	25
174	DCM	AgOAc	25	24	100	2:1	-3
172	AcOEt	Ag_2O	-20	36	100	13:1	76
170	MTBE	Ag_2O	-20	24	100	11:1	76
171	MTBE	Ag_2O	-20	24	100	19:1	90
171	DCM	AgOAc	25	16	100	5:1	62
171	DCM	Ag_2O	25	2.5	100	6:1	71
171	DCM	Ag_2CO_3	25	3.5	100	5:1	64

171	DCM	$(PPh_3)_3AuCl$	25	48	100	2:1	44
171	DCM	AgF	25	48	100	4.5:1	67
171	DCM	CuCl	25	48	30	4:1	0

[a] Determined by ^{1}H-NMR. [b] Determined by HPLC analysis with a chiral column after conversion to derivative **182**

Many attempts were made and the first variable we decided to screen was the kind of metal. We focused on soft transition metals, which are known catalysts for the reaction. We found that:
1) Good yields of product could be achieved with many different cations. In detail Cu(I), Ag(I), Au(I) were found to be the most active species.
2) For a given metal ion, strong change in reactivity was observed according to the metal source that was used. For example for the series CuX (where X is a halogen), once a ligand was fixed, it was observed: CuCl>CuBr>CuI. The trend was not easy to rationalise and it was found to be dependent on many factors. In the case of silver salts a clear indication was that reactivity was improved by the increased basicity of the silver salt. It was observed: Ag_2O>Ag_2CO_3>AgOAc>AgOTf>$AgSBF_6$.
3) Urea and thiourea based ligands were less promising in terms of stereocontrol and in all cases gave enantioselectivities in the range 25-35% for the major diasteroisomer.
Thiourea ligand **167**, gave rise to a very slow reaction, probably due the poisoning of the silver cation caused by the thiourea moiety.
4) Most interesting results were obtained using phosphine-based ligands in the presence of silver salts. Silver oxide was found to be the most reactive and the best in inducing enantioselectivity.
Selected examples are summarized in Table 6.1.

6.5 Further optimization

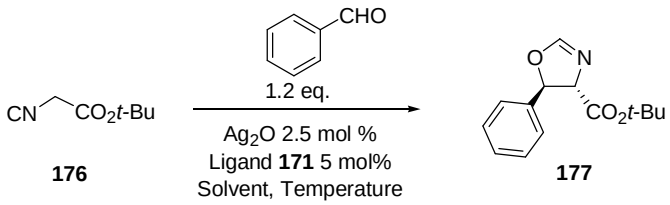
Having identified the system ligand **171**-Ag_2O as a promising catalytic system, we started a deep screen of reaction conditions with the aim of further optimize enantioselectivity and diastereoselectivity.

Temperature
Enantioselectivity and diastereoselecty in favour of the *trans* oxazoline improved with lowering the temperature. We identified a temperature of -20°C as an optimal compromise between reactivity-stereoselectivity. At this temperature the reaction reached completeness in 24 h, with 5 mol% of ligand **171** and 2.5 mol% of Ag_2O.
Lower reaction temperatures, such as -40°C gave rise to very long reaction times, even at higher catalyst loading and didn't provide a remarkably increase in enantioselectivity.

Solvent
The most common organic solvents were tested. MeOH and other protic solvents were known from preliminary studies with similar catalyst to give slow reactivity and poor enantioselectivities and were omitted from the screen. Usually reaction were first run at room temperature with different solvents, so that a quick data could be obtained (typical

reaction time at 25 °C was 2 hours). The interesting results were then repeated at -20 °C (the optimal temperature identified previously).

Scheme 6.6 Solvent screen



176 **177**

Table 6.2

Solvent	Temp (°C)	Time (h)	Conversion[a] (%)	d.r.[a] trans:cis	Ee[b] of trans (%)
DCM	25	2.5	100	6:1	71
Toluene	25	1.5	100	9:1	74
THF	25	1.5	100	13:1	80
DME	25	1.5	100	10:1	80
THF	-20	24	100	23:1	86
MTBE	-20	24	100	19:1	90
Et_2O	-20	24	100	18:1	86
Cyclohexane	25	24	100	17:1	60
AcOEt	-20	24	100	14:1	94

[a] Determined by ^{1}H-NMR. [b] Determined by HPLC analysis with a chiral column after conversion to derivative **182**

Ethereal solvents and AcOEt proved all very effective in inducing stereoselection. AcOEt and MTBE were selected as the best solvents.

Additives and Concentration effects

The addition of 4Å powdered MS sieves was beneficial and caused a further improvement in enantioselectivity. The reaction was found to be also sensitive to concentration of substrates. At concentrations higher then 1 M of isocyanide, a decrease in enantioselectivity was registered. A plausible explanation for this effect can be found in the change of coordination of the isocyanide around the silver ion. It has been reported that isocyanide coordination.[145] Optimal concentration range was found 0.3 M in isocyanide.

When trying to expand reaction scope, it was also found that the optimal concentration of aldehyde was substrate-dependent and two different values of aldehyde concentration were found effective (0.36 M and 0.51 M, corresponding to 1.2 and 1.7 equivalents of aldehyde with respect to the isocyanide when 500 µL of solvent are used).

When the pseudo-enantiomeric ligand **175** was employed the same experimental conditions, enantioselectivity resulted slightly lower (-91%).

145

Table 6.3

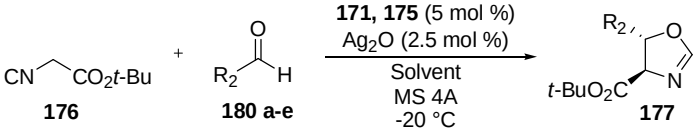
Ligand	Solvent	Time (h)	Conv[a] (%)	d.r.[a] trans:cis	Ee[b] of trans (%)
171	AcOEt (0.7 M)	24	100	13:1	94
171	AcOEt (0.3 M)	24	100	20:1	95
171	AcOEt (0.3 M) + MS 4A)	24	100	21:1	96
175	AcOEt (0.3 M + MS 4A)	24	100	12:1	-91

[a] Determined by ^{1}H-NMR. [b] Determined by HPLC analysis with a chiral column after conversion to derivative **182**

6.6 Reaction scope

Once identified the best reaction conditions we started to investigate the scope. The first isocyanide which was considered was *t*-butyl-isocyanoacetate. Many different aldehydes were used and both aromatic and aliphatic aldehydes proved to be reactive, even if branched aliphatic aldehydes required longer reaction time. Selected examples are reported in table 6.4.

Scheme 6.7



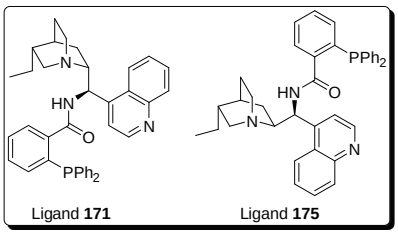


Table 6.4

Product	L	R_2	Solv.	Eq. of Ald.	t (h)	Conv (%)	d.r.[a]	Yield major (%)	Ee[b] of major (%)
177a	**171**	Ph	AcOEt	1.2	24	100	21:1	72[c]	96[d]
177a	**175**	Ph	AcOEt	1.2	24	100	12:1	76[c]	-91[d]
177b	**171**	4-F-Ph	AcOEt	1.7	24	100	15:1	77	92
177c	**171**	3-Br-Ph	AcOEt	1.7	24	100	10:1	67	92
177d	**171**	*t*-Bu	AcOEt	1.2	24	100	99:1	81[e]	91[f]
177e	**171**	4-Ome	AcOEt	1.7	24	100	13:1	60	89

[a]Determined by ^{1}H-NMR. [b]Determined by HPLC analysis with a chiral column. [c]Isolated yield after conversion to derivative **182**. [d]Determined after conversion to derivative **182**. [e]Isolated yield after hydrolysis to the corresponding formamide. [f]Determined after hydrolysis to the corresponding formamide.

Diastereoselectivity was mainly governed by steric factors when aliphatic aldehydes were reacted. When pivalaldehyde was used, the only diastereomer observed was the *trans* product. The enantioselectivity observed with aliphatic aldehydes was less pronounced compared to the reaction model and in many cases the ee of the *trans* diastreoisomer was in the range 50-70%. When aromatic aldehydes different from benzaldehyde were employed, reactivity proved to be increased by EWG groups on the aromatic ring. Diastereoselectivity was not only governed by steric factors (many orto-susbstituted benzaldehydes gave lower diastereoselectivity compared to the correspondent meta-and para-substituted). Enantioselectivities for aromatic aldehydes were variable, in the range 70-90+% but it was not possible to identify a general trend between enantioselectivity and electronic/steric properties of the aldehydes.
From our and previous work in the field of cinchona based catalysis, it appeared clear that enantioselectivity was enhanced by the presence of an aromatic moiety in the substrates. In order to realize these conditions, we decided to screen some isocyanide containing aromatic rings. We found an interesting substrate in the isocyanide **179**, deriving from phenylglicine methyl ester.[146]

Scheme 6.8

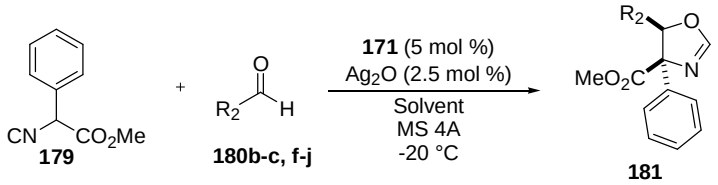


Table 6.5

Product	R_2	Solv.	Eq. of Ald.	T (h)	Conv[a] (%)	D.r.[a]	Yield major (%)	Ee[b] of major (%)
181b	4-F-Ph	MTBE	1.7	16	100	11:1	78	94
181c	3-Br-Ph	AcOEt	1.7	22	100	3.5:1	59	96
181f	3-OMe-Ph	MTBE	1.7	56	100	12:1	50	93
181g	3-F-Ph	MTBE	1.7	16	100	15:1	67	97
181h	4-Cl-Ph	MTBE	1.7	16	100	11:1	56	96
181i	4-CO_2Me-Ph	MTBE	1.7	16	100	8:1	57	93
181j	4-Br-Ph	AcOEt	1.7	22	100	5:1	55	90

[a]Determined by ^{1}H-NMR. [b]Determined by HPLC analysis with a chiral column.

The use of **179** as substrate was attractive also because it allows the simultaneous creation of a quaternary stereocennter next to a tertiary stereocenter, starting from two achiral substrates. When the isocyanide **179** was subjected to the optimized reaction conditions it proved to be reactive, especially when the aldehyde was aromatic and substituted with EWG groups. In many cases the use of MTBE gave the best results in terms of diastereoselectivity. Interestingly, the diastereomeric ratio outcome was hard to rationalize on the basis of simple steric effects and probably electronic reasons are very important in governing the diastereoselectivity. Unfortunately more experimental data need to be collected to reach a deep understanding of the reasons governing the diastereomeric ratio.

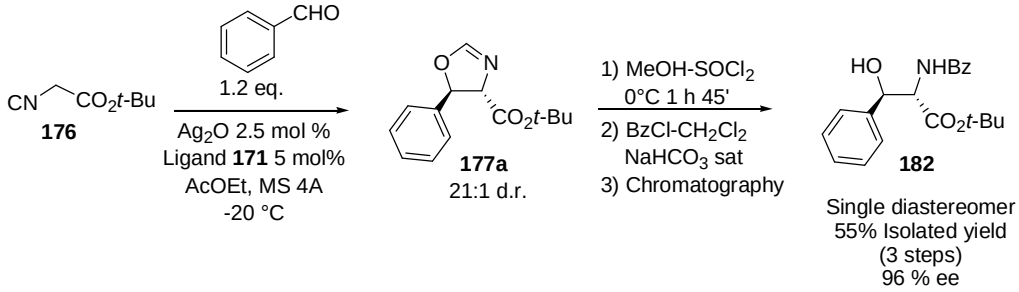
[146] Damien Bonne, Mouloud Dekhane, and Jieping Zhu, *J. Am. Chem. Soc*., **2005,** 127, 6926-6927.

As it was previously hypothesized, the enantiocontrol was particularly good and enantiomeric excesses up to 97 % for the major diastereoisomer were obtained. Some selected examples are reported in table 6.5.

6.7 Conversion of oxazolines to amino acids derivatives

The oxazolines synthesised represent direct precursors of β-hidroxy α-amino acids and many protocols have been reported for the conversion of this heterocycles to the corresponding amino acids or amino esters. Usually these procedures use oxazoline incorporating a methyl- or ethyl-ester moiety and are based on acid hydrolysis or alcoholisis step. Many of the oxazolines presented in this chapter contain a *t*-butyl ester which was not compatible with literature reported references. Moreover, treatment of oxazolines with strong acidic conditions caused a partial epimerization of the *trans* oxazolines. It was found that the best experimental conditions for the conversion of oxazolines to amino esters were based on a treatment with acidic methanol for 1 hour and 45 minutes at 0°C.

Scheme 6.9



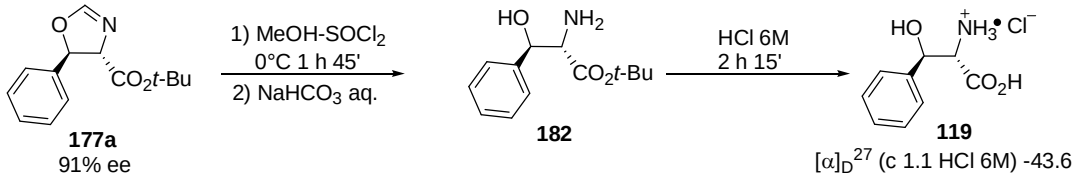
These conditions lead to the formation of the intermediate formamide and the subsequent methanolysis of the formamide resulted faster then the *t*-butyl ester cleavage. In an optimised procedure the oxazoline cleavage was performed on the crude oxazoline **177a** and the resultant amino ester hydrochloride was neutralized, benzoylated and purified by flash column chromatography to obtain compound **182** with 55% yield as a single diastereomer with 96% ee. This proved that the conversion of oxazoloines to the corresponding amino-ester under the conditions described herein is racemization-free.

6.8 Stereochemistry assignament

Diasatereomeric oxazolines derived from *t*-butylisocyanoacetate were assigned as *cis* or *trans* on the basis of the difference of the coupling constants between α- and β-protons. It is known that protons having a *trans* relationship have a smaller coupling constant then protons in a *cis* relationship. Absolute configuration of oxazoline deriving from benzaldehyde and *t*-butylisocyanoacetate was then assigned as (4S, 5R) converting the

oxazoline to the corresponding β-hidroxy α-amino acid and comparing the sign of the optical rotatory power with literature reported values.[147]

Scheme 6.10

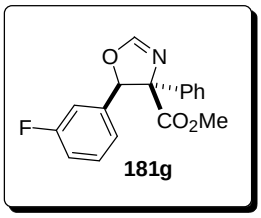


177a
91% ee

182

119
$[\alpha]_D^{27}$ (c 1.1 HCl 6M) -43.6

Absolute configuration of oxazolines deriving from different aromatic aldehydes was then assigned by analogy. Absolute configuration of the oxazoline deriving from pivalaldehyde and *t*-butylisocyanoacetate (**171d**) remains unknown.
Relative configuration of oxazolines derived from isocyano-phenyl-acetic acid methyl ester, **179**, was assigned according to the crystal structure of the major diastereomer obtained in the reaction with 3-fluoro-benzaldehyde.



181g

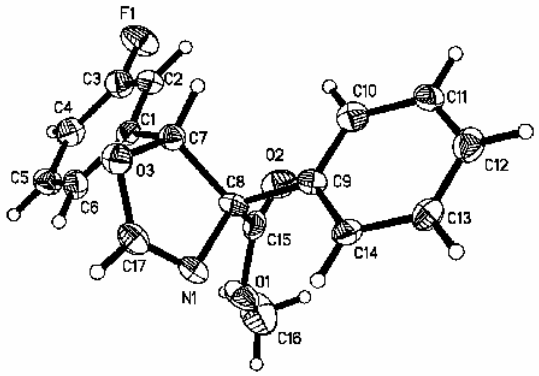


Figure 6.2 Crystal structure of compound **181g**

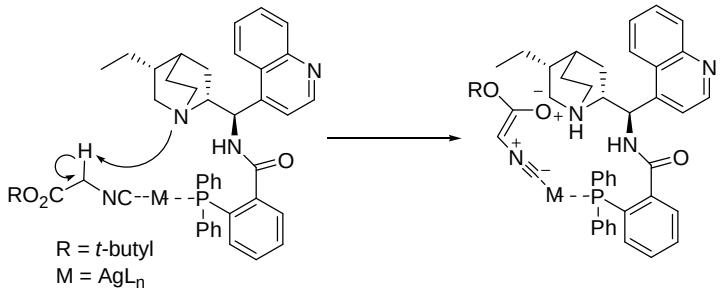
The absolute configuration of oxazolines **181b-c, f-j**, deriving from isocyano-phenyl-acetic acid methyl ester, remains unknown.

6.9 Mechanicistic hypothesis

Although a complete study has not carried out, a first mechanicistic hypothesis can be the one represented in scheme 6.11. The idea is that the isocyanide acts as a ligand and cohordinates the silver ion. This cohordination enhances acidity of the α-hydrogens of the isocyanide and brings the pro-nucleophile in a favorable position for the deprtotonation that can be carried on by the bridgehead nitrogen of the cinchona scaffold.

[147] Ito, Y.; Sawamura, M.; Hayashi, T.; Journal of the American Chemical Society; **1986**; 6405 – 6406.

Scheme 6.11 Activation of the isocyano-acetate by the catalyst developed in this chapter.



A simple experiment has been made, in order to prove the cohoperaitve mechanism between the silver salt and the ligand. The best reaction conditions were set using *tert*-butyl-isocyanoacetate and benzaldehyde. In a first vessel the reaction was run without ligand and only silver oxide. In a second vessel the reaction was run with the presence of the ligand but without silver oxide. The reaction run with only silver oxide took 5 days to reach complete conversion. The reaction run with only the ligand presented just traces of product after 5 days. Standard reaction conditions, obtained combining ligand and silver oxide, required 24 hours to reach full conversion. This simple test is a clear hint of the bifunctional nature of the catalytic system, prooving that both, tha amine and the silver salt, are required for the reaction to occur.
Further studies are needed to provide a model that can explain the origin of enantio- and diastereo-selectivity.

6.10 Conclusions

In this chapter the first silver-based catalytic system for the addition of α-isocyanoacetates to aldehydes has been developed. New amino-phosphine ligands based on cinchona-alkaloid derived chiral scaffolds have been designed and synthesised. These ligands have been successfully applied to the addition of *t*-butylisocyanoacetate and isocyano-phenyl-acetic acid methyl ester to aldehydes, obtaining enantioselectivities up to 97% and diastereoselectivities up to 98%. When the isocyanide derived from phenyl glycine is used, compounds with a quaternary next to a tertiary stereocenter are obtained in a single step, starting from two achiral substrates.
A method for the conversion of oxazoline to the corresponding amino ester without racemization and epimerization has been described. Finally a mechanicistic hypothesis, describing the catalytic system as a bifunctional metal-organic catalyst has been presented. Further experimental research is needed to give a complete explanation of the origin of enantio- and diastereoselectivity.

6.11 Experimental for chapter 6

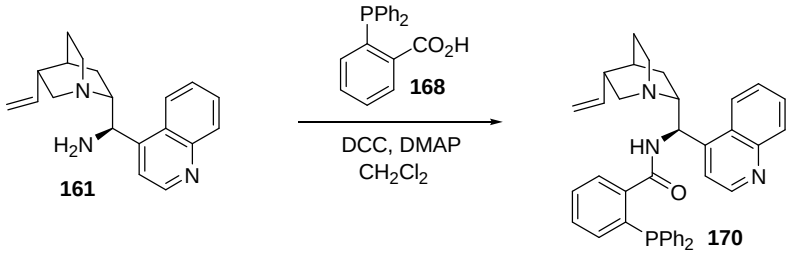
General Experimental

All reagents bought from commercial sources were used as sold. Organic solvents were concentrated under reduced pressure using a Büchi rotary evaporator. Syringes, needles

and cannulae were oven dried at 140 °C. Anhydrous dichloromethane, toluene, DIPA were purified by distillation over calcium hydride. Ethyl acetate used for the enantioselective preparation of oxazolines was dried and stored over 4Å MS. TLC analyses were performed using Merck aluminium-backed or glass-backed plates pre-coated with silica (0.25 mm, 60 F254) and visualized under UV light (254 nm) and/or by the use of p-anisaldehyde using the solvent system indicated. Systems using 'petroleum ether' refer to light petroleum 40-60 °C. Column chromatography was performed on silica Kieselgel 60 (40-60 µm). Infrared spectra were recorded on an ATI Matison: Genesis Series FTIR spectrometer from a thin film, with absorption maxima (ν_{max}) recorded in wavenumbers (cm^{-1}). NMR spectra were recorded using a Bruker Avance 400 MHz or 500 MHz spectrometer, chemical shifts (δ) are quoted in parts per million referenced to the residual solvent peak. The multiplicity of each signal is designated using the following abbreviations; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, bs, broad singlet. Coupling constants (*J*) are reported in Hertz (Hz). High resolution mass spectra (accurate mass) were recorded on a Thermo Finnigan Mat 95XP mass spectrometer. Melting points were obtained using a Griffin melting point apparatus and remain uncorrected.
Known compounds are indicated by a reference to a previous literature report in their title line.
Isocyano-phenyl-acetic acid methyl ester (**179**) was synthesized by dehydration of the corresponding formamide according to the procedure reported by Zhu et al. for the preparation of methyl 2-isocyano-2-(4-methoxyphenyl)acetate.

Practical Experimental

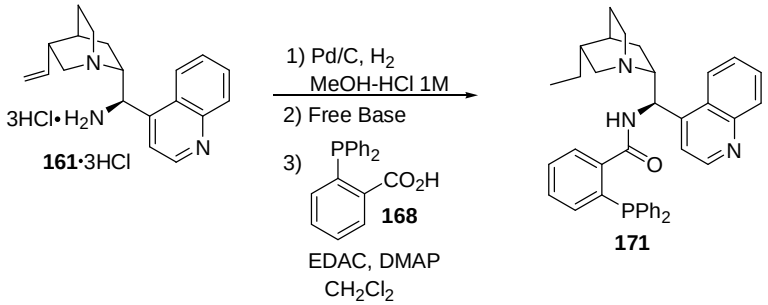
Synthesis and characterization of Catalyst 170



Epi-Amino-Cinchonine **161** (120 mg, 0.41 mmol) was dissolved in dry CH_2Cl_2 (2 mL). 2-(diphenylphosphino)benzoic acid (120 mg, 0.39 mmol), 4-dimethylaminopyridine (2.4 mg, 0.020 mmol) and *N,N'*-Dicyclohexylcarbodiimide (88 mg, 0.43 mmol) were sequentially added. The resultant mixture was stirred at room temperature for 24 hours, concentrated under reduced pressure and purified by flash column chromatography AcOEt/MeOH gradient 100:0 to 100:4 to yield 207 mg (83 %) of product. $[\alpha]_D^{28}$ = +86.0 (c 0.5, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.66 (d, *J* = 4.5 Hz, 1H), 8.28 (d, *J* = 8.5 Hz, 1H), 8.02 (d, *J* = 8.5 Hz, 1H), 7.62-7.56 (m, 2H), 7.49 (t, *J* = 7.5 Hz, 1H), 7.27-7.16 (m, 12H), 7.13-7.10 (m, 2H), 6.83 (dd, *J* = 7.40, 4.0 Hz, 1H), 5.79 (m, 1 H), 5.32 (br, 1H), 5.08 (d, *J* = 10.5 Hz, 1H), 5.03 (d, *J* = 17.5 Hz, 1H), 2.83 (br, 1H), 2.75-2.66 (m, 4H), 2.16 (dd, *J* = 15.0, 8.0 Hz, 1H), 1.52 (s, 1H), 1.42-1.40 (m, 1H), 1.36-1.30 (m, 1H), 1.21-1.16 (m, 1H), 0.81-0.76 (m, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) (C-P coupling constants not removed) δ 168.0, 149.0, 147.4, 145.8, 145.7, 140.3, 140.0, 139.0, 136.5, 136.4, 136.0, 135.9, 134.67, 134.45, 133.24, 132.88, 132.70, 132.50, 129.19, 127.92, 127.72, 127.60, 127.57, 127.52, 127.45, 126.32,

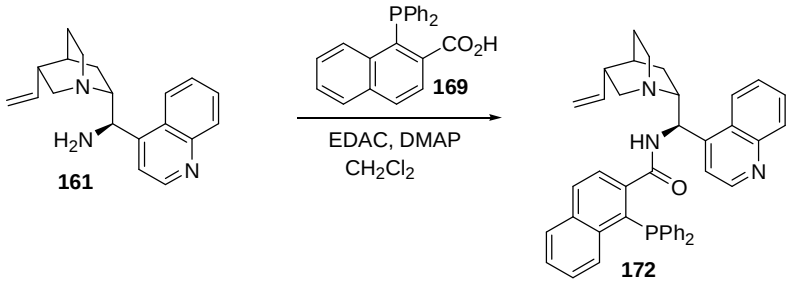
125.43, 124.92, 122.51, 114.05, 59.17, 50.79, 50.56, 47.92, 45.72, 38.02, 26.22, 25.36, 24.05; ^{31}P-NMR (162 MHz, $CDCl_3$) δ -10.5; IR ν_{max}/cm^{-1} 2934, 1648, 1508, 1478, 744, 696; MPT 99-101 °C; HRMS (ES+): calcd. for $C_{38}H_{37}N_3O_1P_1$ $[M+H]^+$ 582.2669, found 582.2658.

Synthesis and characterization of catalyst 171



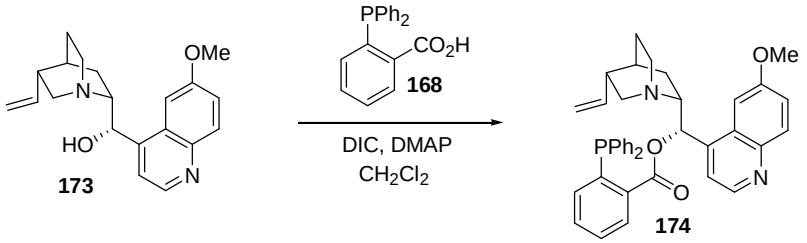
Epi-Amino-Cinchonine*3HCl salt [**161***3HCl] (2.024 g, 5.03 mmol) was dissolved in 30 mL of a mixture of 2:1 MeOH/HCl 1M and 10% Pd/C (303 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 7 h at room temperature and then filtered through Celite washing with water and MeOH. The filtrate was concentrated under reduced pressure and neutralized with sat. $NaHCO_3$. The resultant solution was extracted several times with CH_2Cl_2. The combined CH_2Cl_2 extracts were dried over Na_2SO_4 and concentrated under reduced pressure to yield a colourless oil which was dissolved in 20 mL of dry CH_2Cl_2. 2-(diphenylphosphino)benzoic acid (1.47 g, 4.80 mmol), 4-dimethylaminopyridine (59 mg, 0.48 mmol) were added. The mixture was cooled at 0 °C, *N*-(3-Dimethylaminoprpyl)-*N*'-ethylcarbodiimide hydrochloride (920 mg, 4.80 mmol) was added and then the ice bath was removed and the resultant solution was stirred at room temperature for 24 h. CH_2Cl_2 was then removed under reduced pressure and the crude was dissolved in Et_2O and washed with water, $NaHCO_3$ 10 %, brine and dried over Na_2SO_4. The organic phase was concentrated under reduced pressure and purified by flash column chromatography (CH_2Cl_2/MeOH 16:1). Impure fractions were further purified by flash column chromatography (AcOEt/CH_2Cl_2/MeOH 5:2:1) to yield 1.70 g (58 %) of product. $[\alpha]_D^{25}$ = +67.2 (c 0.5, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.76 (d, *J* = 4.8 Hz, 1H), 8.39 (d, *J* = 8.4 Hz, 1H), 8.12 (d, *J* = 8.4 Hz, 1H), 7.73-7.58 (m, 4H), 7.40-7.19 (m, 13H), 6.92 (dd, *J* = 7.2, 4.0 Hz, 1H), 5.36 (br, 1H), 3.00-2.52 (m, 4H), 2.49-2.44 (m, 1H), 1.51 (s, 1H), 1.49-1.30 (m, 5H), 1.29-1.16 (m, 1H), 0.90 (t, *J* = 7.0 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) (C-P coupling constants not removed) δ 169.1, 150.1, 148.4, 146.9, 141.4, 141.0, 137.7, 137.6, 137.2, 137.1, 135.9, 135.6, 134.3, 133.9, 133.8, 133.6, 133.5, 130.2, 127.4, 126.5, 123.5, 119.6, 60.3, 51.51, 49.0, 37.2, 27.1, 25.9, 25.7, 25.0, 12.1; ^{31}P-NMR (162 MHz, $CDCl_3$) δ -10.36;

Synthesis and characterization of Catalyst 172



Epi-amino-cinchonine **161** (270 mg, 0.92 mmol), 1-(diphenylphosphino)-2-naphthoic acid[148] (258 mg, 0.72 mmol) and 4-dimethylaminopyridine (8.8 mg, 0.072 mmol) were dissolved in dry CH_2Cl_2 (4 mL). The reaction mixture was cooled with an ice/salt bath and solid *N*-(3-Dimethylaminoprpyl)-*N*'-ethylcarodiimide hydrochloride (138 mg, 0.72 mmol) was added. The flask was gently warmed to room temperature and stirred for 16 h. The reaction mixture was diluted with AcOEt, washed with water, brine and dried over Na_2SO_4. The organic phase was concentrated under reduced pressure and purified by flash column chromatography (CH_2Cl_2/MeOH 13:1) to yield 248 mg (54 %) of product. $[\alpha]_D^{25}$ = +61.7 (c 1.75, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.80 (d, *J* = 4.4 Hz, 1H), 8.38 (d, *J* = 8.4 Hz, 1H), 8.06 (d, *J* = 8.4 Hz, 1H), 7.85 (d, *J* = 8.4 Hz, 1H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.62 (t, *J* = 7.2 Hz, 1H), 7.50 (d, *J* = 4.4 Hz, 2H), 7.34 (q, *J* = 6.8 Hz, 5H), 7.25 (t, *J* = 7.6 Hz, 1H), 7.18-7.10 (m, 7H), 7.01 (t, *J* = 8.4 Hz, 1H), 5.85-5.76 (m, 1H), 5.48 (br, 1H), 5.09 (d, *J* = 10.4, 1H), 5.03 (d, *J* = 17.6 Hz, 1H), 3.20-2.20 (m, 4H), 2.07 (s, 1H), 1.51 (s, 1H), 1.40-1.10 (m, 4H), 0.83 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.1, 150.1, 148.4, 146.9, 141.4, 141.0, 137.7, 137.6, 137.2, 137.1, 135.9, 135.6, 134.3, 133.9, 133.8, 133.6, 133.5, 130.2, 127.4, 126.5, 123.5, 119.6, 60.29, 51.51, 49.0, 37.2, 25.9, 25.7, 25.0; ^{31}P-NMR (162 MHz, $CDCl_3$) δ -12.6; IR ν_{max}/cm^{-1} 1654, 1508, 1482, 744, 696, 570; MPT 108-110 °C; HRMS (ES+): calcd. for $C_{42}H_{39}O_1N_3P_1$ $[M+H]^+$ 632.2825, found 632.2822.

Synthesis and characterization of Catalyst 174

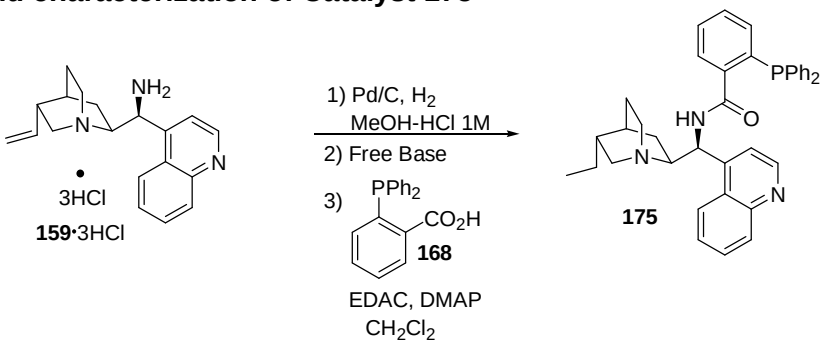


(+)-Quinidine **173** (550 mg, 1.70 mmol) was dissolved in dry CH_2Cl_2 (4 mL). 2-(diphenylphosphino)benzoic acid (528 mg, 1.72 mmol), 4-dimethylaminopyridine (10.0 mg, 0.085 mmol) and *N*,*N*'-Diisopropylcarbodiimide (300 µL, 1.85 mmol) were sequentially added. The resultant mixture was stirred at room temperature for 18 hours and then concentrated under reduced pressure. THF was added to precipitate the urea which was filtered off. The resultant clear solution was concentrated under reduced pressure and purified by flash column chromatography (CH_2Cl_2/MeOH) to yield 510 mg (55 %) of product. $[\alpha]_D^{28}$ = -5.1 (c 1.9, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.66 (d, *J* = 4.5 Hz, 1H), 8.17-8.14 (m, 1H), 8.00 (d, *J* = 8.0 Hz, 1H), 7.45-7.15 (m, 15H), 6.94-6.90 (m, 1H), 6.70 (d, *J* = 8.0, 1H), 5.99-5.90 (m, 1H), 5.08-5.03 (m, 2H), 3.94 (s, 3H), 3.36 (q, *J* = 8.7 Hz,), 2.90-2.66 (m, 4H), 2.25 (q, *J* = 8.7, 1H), 1.89-1.76 (m, 2H), 1.58-1.51 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) (C-P coupling constants not removed) δ 165.6, 157.7, 147.2, 144.4, 143.3, 141.2, 141.0, 140.0, 137.6, 137.5, 137.3, 137.2, 134.2, 133.8, 133.6, 133.3, 133.1, 132.2, 131.4, 130.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.1, 121.8, 118.9, 114.8, 101.4, 73.2, 59.1, 55.5, 49.3, 48.7, 39.5, 27.5, 26.1, 23.9; ^{31}P-NMR (162 MHz, $CDCl_3$) δ -

[148] Barry M. Trost, Richard C. Bunt, Rémy C. Lemoine, and Trevor L. Calkins, *J. Am. Chem. Soc*., **2000,** *122,* 5968-5976.

4.31; IR ν_{max}/cm^{-1} 1716, 1434, 1248, 1228, 1102, 1054, 744, 732, 698; MPT 83-85 °C. HRMS (ES+): calcd. for $C_{39}H_{37}N_2O_3P_1$ $[M+H]^+$ 613.2615, found 613.2604.

Synthesis and characterization of Catalyst 175



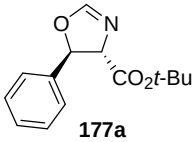
Epi-Amino-Cinchonidine*3HCl [**159***3HCl] salt (1.26 g, 3.13 mmol) was dissolved in 18 mL of a mixture of 2:1 MeOH/HCl 1M and 10% Pd/C (188 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 6 h at room temperature and then filtered through Celite washing with water and MeOH. The filtrate was concentrated under reduced pressure and neutralized with sat. $NaHCO_3$. The resultant solution was extracted several times with CH_2Cl_2. The combined CH_2Cl_2 extracts were dried over Na_2SO_4 and concentrated under reduced pressure to yield a colourless oil which was dissolved in 20 mL of dry CH_2Cl_2. 2-(diphenylphosphino)benzoic acid (961 mg, 3.14 mmol), 4-dimethylaminopyridine (38 mg, 0.31 mmol) were added. The mixture was cooled at 0 °C, *N*-(3-Dimethylaminoprpyl)-*N*'-ethylcarbodiimide hydrochloride (602 mg, 3.14 mmol) was added and then the ice bath was removed and the resultant solution was stirred at room temperature for 16 h. CH_2Cl_2 was then removed under reduced pressure and the crude was dissolved in Et_2O and washed with water, $NaHCO_3$ 10 %, brine and dried over Na_2SO_4. The organic phase was concentrated under reduced pressure and purified by flash column chromatography (AcOEt to AcOEt/CH_2Cl_2/MeOH 5:2:1) to yield 1.20 g (66%, 2 steps) of product. $[\alpha]_D^{28}$ = - 4.4 (c 1, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.78 (d, *J* = 4.4 Hz, 1H), 8.40 (d, *J* = 8.4 Hz, 1H), 8.10 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.70 (t, *J* = 6.8 Hz, 1H), 7.63 (dd, *J* = 6.8, 3.6 Hz, 1H), 7.58 (t, *J* = 7.2, 1H), 7.42-7.21 (m, 12H), 7.18 (td, *J* = 7.2, 1.6 Hz, 1H), 6.91 (s, 1H), 5.36 (br, 1H), 3.03 (dd, *J* = 13.6, 10 Hz, 2H), 2.85 (br, 1H), 2.49-2.61 (m, 1H), 2.11-2.25 (m, 1H), 1.80-1.60 (m, 4H), 1.25-1.08 (m, 3H), 0.91-0.84 (m, 1H), 0.76 (t, *J* = 7.6 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) (C-P coupling constants not removed) δ 169.1, 150.1, 148.5, 141.4, 141.2, 137.7, 137.5, 137.2, 137.1, 135.8, 135.6, 134.4, 133.8, 133.6, 130.2, 129.0, 128.8, 128.7, 128.6, 128.5, 127.4, 126.6, 123.7, 119.5, 60.2, 57.3, 51.5, 40.9, 37.0, 28.2, 27.3, 25.5, 25.1, 12.0; ^{31}P-NMR (162 MHz, $CDCl_3$) δ -10.87; IR ν_{max}/cm^{-1} 2360, 1648, 1508, 1458, 744, 696; MPT 107-109 °C.

General procedure for the enantioselective synthesis of Oxazolines

Ligand **171** (6.7 mg, 0.011 mmol) was dissolved in 0.7 mL of solvent (according to Table 6.4 & 6.5), Ag_2O (1.2 mg, 0.0051 mmol) was added. The heterogeneous mixture was stirred for approximately 2 minutes and the isocyanide (0.21 mmol, 1 eq.) and powdered 4Å MS were sequentially added. The flask was cooled in a dry ice/acetone bath and the aldehyde (equvailents according to Table 6.4 & 6.5) was added. The flask was then

quickly transferred in a -20 °C fridge and the reaction mixture was stirred for the amount of time specified in Table 6.4 & 6.5, until all the isocyanide was consumed (TLC, p-Anisaldehyde). The reaction mixture was then quickly filtered through a short pad of silica (in a glass pipette) and eluted with AcOEt. The filtrate was concentrated under reduced pressure and treated in the way described for each entry.

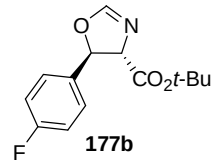
Synthesis and characterization of Compound 177a



177a

Obtained according to the general procedure, using tert-butylisocyanoacetate (0.21 mmol, 30 mg) and benzaldehyde (0.25 mmol, 26 µL) as starting materials and AcOEt as solvent. Crude was purified by flash column chromatography (E.P./AcOEt 3:1) to yield 31 mg (59 %) of product as a colourless oil. $[\alpha]_D^{25}$ = +99.6 (c 0.64, CH_2Cl_2); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.40-7.20 (m, 5 H, aromatic protons), 7.08 (d, *J* = 2.0 Hz, N=CH), 5.60 (d, *J* = 8.0, 1H), 4.51 (dd, *J* = 8.0, 2.0 Hz, 1H), 1.52 (s, 9H, ^{t}Bu); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.5 (s, 1C, C=O), 156.0 (d, 1C, C=N), 139.2 (s, 1C, Ph), 128.9 (d, 2C, Ph), 125.6 (d, 2C, Ph), 82.5 (s, 1C, ^{t}Bu), 82.3 (d, 1C), 76.2 (d, 1C), 28.00 (q, 3C, ^{t}Bu); IR ν_{max}/cm^{-1} 2978, 1732, 1688, 1626, 1370, 1156, 1106; HRMS (ES+): calcd. for $C_{14}H_{17}N_1O_3Na_1$ $[M+Na]^+$ 270.1101, found 270.1095. The ee (96%) was calculated after conversion to the N-benzoylated amino ester using the same procedure described in "direct conversion to N-benzoylated amino ester"

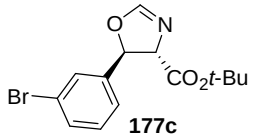
Synthesis and characterization of Compound 177b



177b

btained according to the general procedure, using tert-butylisocyanoacetate (0.21 mmol, 30 mg) and 4-fluoro- benzaldehyde (0.36 mmol, 38 µL) as starting materials and AcOEt as solvent. Crude was purified by flash column chromatography (E.P./AcOEt) 3.5:1 to yield 43 mg (77 %) of product as a colourless oil. The ee was determined by HPLC using a Ciralpak OD [hexane/iso-propanol 90:10, λ 220 nm, 1.0 mL/min] t (major) = 5.75 min., t (minor) = 7.37 min. (92%). $[\alpha]_D^{25}$ = +248.4 (c 1.5, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.49-7.45 (m, 2 H, aromatic protons), 7.28-7.24 (m, 3 H, aromatic protons and N=CH), 5.76 (d, *J* = 8.0, 1H), 4.65 (dd, *J* = 8.0, 2.0 Hz, 1H), 1.70 (s, 9H, ^{t}Bu); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.3 (s, 1C, C=O), 162.8 (d, $J_{C\text{-}F}$ = 247.7 Hz, 1C, C-F), 155.9 (d, 1C, C=N), 135.00 (d, $J_{C\text{-}F}$ = 3.01 Hz, 1C, Ph), 127.45 (d, $J_{C\text{-}F}$ = 8.2 Hz, 2C, Ph), 115.86 (d, $J_{C\text{-}F}$ = 21.74 Hz, 2C, Ph), 82.7 (s, 1C, ^{t}Bu), 81.6 (d, 1C), 76.2 (d, 1C), 27.9 (q, 3C, ^{t}Bu); IR ν_{max}/cm^{-1} 1732, 1626, 1512, 1156, 1104.

Synthesis and characterization of Compound 177c

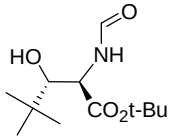


177c

Obtained according to the general procedure, using tert-butylisocyanoacetate (0.21 mmol, 30 mg) and 3-bromo-benzaldehyde (0.36 mmol, 42 µL) as starting materials and AcOEt as solvent. Crude was purified by flash column chromatography (E.P./AcOEt 3:1) to yield 47 mg (69 %) of product as a colourless oil. The ee was determined by HPLC using a Ciralpak OD [hexane/iso-propanol 90:10, λ 230 nm, 1.0 mL/min] t (major) = 6.23 min., t (minor) = 8.20 min. (92%). $[\alpha]_D^{25}$ = +168.2 (c 1, CH_2Cl_2); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.41-7.10 (m, 4 H, aromatic protons), 7.08 (d, *J* = 2.0 Hz, N=CH), 5.60 (d, *J* = 8.0, 1H), 4.51 (dd, *J* = 8.0, 2.0 Hz, 1H), 1.52 (s, 9H, ^{t}Bu); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.2 (s, 1C, C=O), 155.9 (d, 1C, C=N), 141.5 (s, 1C, Ph),

131.7 (d, 1C, Ph), 130.5 (d, 1C, Ph), 286.6 (d, 1C, Ph), 124.1 (d, 1C, Ph), 123.0 (s, 1C, Ph), 82.9 (s, 1C, ^{t}Bu), 81.3 (d, 1C), 76.2 (d, 1C), 28.00 (q, 3C, ^{t}Bu); IR ν_{max}/cm^{-1} 2978, 1732, 1626, 1368, 1154, 1102.

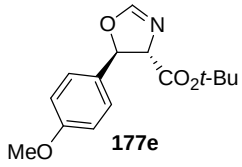
Synthesis and characterization of hydrolysed form of compound 177d



177d

Obtained according to the general procedure, using tert-butylisocyanoacetate (0.21 mmol, 30 mg) and pivalaldehyde (0.25 mmol, 27 µL) as starting materials and AcOEt as solvent. Crude reaction mixture was dissolved in 2.5 mL of AcOEt saturated with 5% solution of Citric acid and stirred until complete conversion of the starting material (TLC), about 6-8 hours. The organic phase was washed with 5% $NaHCO_3$ sol., dried over Na_2SO_4 and concentrated under reduced pressure. The crude was purified by flash column chromatography (E.P./AcOEt 1:1) to yield 42 mg (81 %) of product. The ee was determined by HPLC using a Ciralpak OD [hexane/iso-propanol 80:20, λ 210 nm, 1.0 mL/min] t (minor) = 4.62 min., t (major) = 6.66 min. (91%); $[\alpha]_D^{27}$ = -20.1 (c 0.25, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H, H-C=O), 6.59 (d, *J* = 8.4 Hz, N-H), 4.74 (d, 9.2 Hz, 1H, C_α-H), 3.72 (s, 1H, C_β-H), 2.42 (br, 1H, O-H), 1.46 (s, 9 H, ^{t}Bu-H), 0.96 (s, 9H, ^{t}Bu-H); ^{13}C-NMR (100 MHz, $CDCl_3$) 171.1 (s, 1C, ROC=O), 160.9 (d, 1C, HC(O)NH), 82.4 (s, 1C, Me_3C-O), 78.1 (d, 1C, C_β), 51.5 (d, 1C, C_α), 35.4 (s, 1C, Me_3C-C), 27.9 (q, 3C, ^{t}Bu), 26.0 (q, 3C, ^{t}Bu); IR ν_{max}/cm^{-1} 3384, 2978, 1714, 1656, 1368.

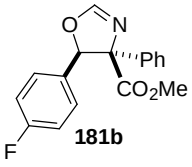
Synthesis and characterization of Compound 177e



177e

Obtained according to the general procedure, using tert-butylisocyanoacetate (0.21 mmol, 30 mg) and p-anisaldehyde (0.36 mmol, 43 µL) as starting materials and AcOEt as solvent. Crude was purified by flash column chromatography (E.P./AcOEt 2:1) to yield 35 mg (60 %) of product as a colourless oil. The ee was determined by HPLC using a Ciralpak OD [hexane/iso-propanol 90:10, λ 230 nm, 1.0 mL/min] t (major) = 7.74 min., t (minor) = 8.95 min. (89%). $[\alpha]_D^{27}$ = +118.4 (c 0.25, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.19-7.16 (m, 2 H, aromatic protons), 6.98 (d, *J* = 2.0 Hz, 1H, N=CH), 6.85-6.83 (m, 2H, aromatic protons), 5.48 (d, *J* = 8.0, 1H), 4.41 (dd, *J* = 8.0, 2.0 Hz, 1H), 1.44 (s, 9H, ^{t}Bu); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.6 (s, 1C, C=O), 159.9 (d, 1C, C=N), 156.0 (s, 1C, Ph), 131.1 (s, 1C, Ph), 127.2 (d, 2C, Ph), 114.2 (d, 2C, Ph), 82.4 (s, 1C, ^{t}Bu), 82.3 (d, 1C), 76.0 (d, 1C), 27.9 (q, 3C, ^{t}Bu); IR ν_{max}/cm^{-1} 1732, 1624, 1516, 1252, 1156, 1106.

Synthesis and characterization of Compound 181b

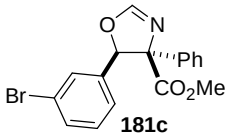


181b

Obtained according to the general procedure, using isocyano-phenyl-acetic acid methyl ester (0.21 mmol, 37 mg) and 4-fluoro-benzaldehyde (0.36 mmol, 38 µL) as starting materials and TBME as solvent. Crude was quickly purified by flash column chromatography (E.P./AcOEt 3:1) to yield 49 mg (78 %) of product. The ee was determined by HPLC using a Ciralpak OD [hexane/iso-propanol 90:10, λ 230 nm, 1.0 mL/min] t (major) = 10.37 min., t (minor) = 11.61 min. (94%); $[\alpha]_D^{27}$ = +158.0 (c 2.6, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.69-7.66 (m, 2H, aromatic protons), 7.44-7.33 (m, 5H, aromatic protons), 7.31 (s, 1H, N=CH), 5.75 (s, 1H, C_β-H), 3.25 (s, 3H, OCH$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.6 (s, 1C, C=O), 162.9 (d, $J_{C\text{-}F}$ = 248.50 Hz, 1C), 155.4 (d, C=N), 141.3 (s, 1C), 132.2 (d, $J_{C\text{-}F}$ = 3.38 Hz, 1C), 128.5 (d, 3C), 128.3 (d, $J_{C\text{-}F}$ = 17.40 Hz 2C), 126.6 (d,

2C), 115.5 (d, $J_{C\text{-}F}$ = 21.76 Hz, 2C), 89.1 (d, 1C, C_β), 84.2 (s, 1C, C_α), 52.3 (q, 1C, O<u>CH_3</u>); IR v_{max}/cm^{-1} 2362, 1740, 1638, 1512, 1228, 1104, 698; HRMS (ES+): calcd. for $C_{17}H_{15}O_3N_1F_1$ $[M+H]^+$ 300.1035, found 300.1032.

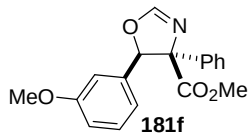
Synthesis and characterization of Compound 181c



181c

Obtained according to the general procedure, using isocyano-phenyl-acetic acid methyl ester (0.21 mmol, 37 mg) and 3-bromo-benzaldehyde (0.36 mmol, 42 µL) as starting materials and AcOEt as solvent. Crude was purified by flash column chromatography (E.P./AcOEt 3.5:1) to yield 45 mg (59 %) of product as colourless oil. The ee was determined by HPLC using a Ciralpak OD [hexane/iso-propanol 90:10, λ 230 nm, 1.0 mL/min] t (major) = 10.06 min., t (minor) = 12.53 min. (97%); $[\alpha]_D^{25}$ = +138.6 (c 1.12, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.31-7.59 (m, 2H, aromatic protons), 7.45-7.42 (m, 2H, aromatic protons), 7.37-7.19 (m, 6H, aromatic protons+N=<u>CH</u>), 5.62 (s, 1H, C_β-H), 3.21 (s, 3H, O<u>CH_3</u>); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.4 (s, 1C, C=O), 155.4 (d, 1C, C=N), 141.1 (s, 1C), 138.6 (s, 1C), 132.1 (d, 1C), 130.0 (d, 1C), 129.6 (d, 1C), 128.5 (d, 1C), 128.3 (d, 1C), 126.6 (d, 1C), 125.3 (d, 1C), 122.5 (s, 1C), 88.8 (d, 1C, C_β), 84.4 (s, 1C, C_α), 52.4 (q, 1C, O<u>CH_3</u>); IR v_{max}/cm^{-1} 1738, 1638, 1238, 1104, 694; HRMS (ES+): calcd. for $C_{17}H_{15}O_3N_1Br_1$ $[M+H]^+$ 360.0230, found 360.0231.

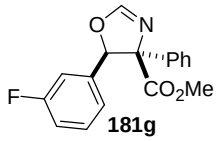
Synthesis and characterization of Compound 181f



181f

Obtained according to the general procedure, using isocyano-phenyl-acetic acid methyl ester (0.21 mmol, 37 mg) and 3-methoxy-benzaldehyde (0.36 mmol, 43 µL) as starting materials and TBME as solvent. Crude was purified by flash column chromatography (E.P./AcOEt 3:1) to yield 33 mg (50 %) of product as a colourless oil. The ee was determined by HPLC using a Ciralpak OD [hexane/iso-propanol 90:10, λ 230 nm, 1.0 mL/min] t (major) = 12.46 min., t (minor) = 14.15 min. (93%); $[\alpha]_D^{28}$ = +154.0 (c 1, CH_2Cl_2); ^{1}H-NMR (400 MHz, CD_2Cl_2) δ 7.60-7.58 (m, 2H, aromatic protons), 7.35-7.20 (m, 5H, aromatic protons+N=<u>CH</u>), 6.88-6.80 (m, 2H), 5.64 (s, 1H, C_β-<u>H</u>), 3.74 (s, 3H, O<u>CH_3</u>), 3.11 (s, 3H, O<u>CH_3</u>); ^{13}C-NMR (100 MHz, CD_2Cl_2) δ 170.1 (s, 1C, C=O), 160.1 (s, 1C), 155.7 (d, 1C, N=<u>C</u>H), 142.1 (s, 1C), 138.4 (s, 1C), 129.8 (d, 1C), 128.7 (d, 2C), 128.4 (d, 1C), 127.2 (d, 2C), 119.3 (d, 1C), 114.6 (d, 1C), 112.7 (d, 1C), 89.6 (d, 1C, C_β), 84.8 (s, 1C, C_α), 55.72 (q, 1C, OCH_3); IR v_{max}/cm^{-1} 1740, 1638, 1238, 1108, 742, 698; HRMS (ES+): calcd. for $C_{18}H_{17}O_4N_1Na_1$ $[M+Na]^+$ 334.1050, found 334.1050.

Synthesis and characterization of Compound 181g

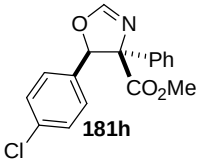


181g

Obtained according to the general procedure, using isocyano-phenyl-acetic acid methyl ester (0.21 mmol, 37 mg) and 3-fluoro-benzaldehyde (0.36 mmol, 38 µL) as starting materials and TBME as solvent. Crude was purified by flash column chromatography (E.P./AcOEt 3.5:1).to yield 42 mg (67 %) of product. The ee was determined by HPLC using a Ciralpak OD [hexane/iso-propanol 90:10, λ 230 nm, 1.0 mL/min] t (major) = 9.07 min., t (minor) = 10.61 min. (97%); $[\alpha]_D^{30}$ = +157.8 (c 1.9, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.70-7.68 (m, 2H, aromatic protons), 7.44-7.34 (m, 4H, aromatic protons), 7.32 (s, 1H, N=<u>CH</u>), 7.16 (d, *J* = 8.0 Hz, 1H, aromatic proton), 7.11-7.04 (m, 2H, aromatic protons), 5.73 (s, 1H, C_β-H), 3.26 (s, 3H, O<u>CH_3</u>); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.4 (s, 1C, C=O), 162.6 (d, $J_{C\text{-}F}$ = 247.23 Hz, 1C, <u>C</u>-F), 155.3 (d, 1C, C=N), 141.1 (s, 1C), 138.8 (d, $J_{C\text{-}F}$ =

7.12 Hz, 1C), 130.05 (d, J_{C-F} = 8.15 Hz, 1C), 128.5 (d, 2C), 128.3 (s, 1C), 126.6 (d, 2C), 122.3 (d, J_{C-F} = 2.93 Hz, 1C), 115.9 (d, J_{C-F} = 21.08 Hz, 1C), 113.7 (d, J_{C-F} = 22.75 Hz, 1C), 88.90 (d, 1C, C_β), 84.4 (s, 1C, C_α), 52.3 (q, 1C, O<u>C</u>H$_3$); HRMS (ES+): calcd. for $C_{17}H_{15}O_3N_1F_1$ $[M+H]^+$ 300.1035, found 300.1030.

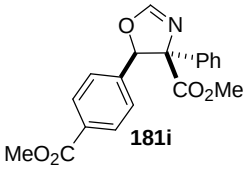
Synthesis and characterization of Compound 181h



181h

Obtained according to the general procedure, using isocyano-phenyl-acetic acid methyl ester (0.21 mmol, 37 mg) and 4-chloro-benzaldehyde (0.36 mmol, 50 mg) as starting materials and TBME as solvent. Crude was purified by quick flash column chromatography (E.P./AcOEt 3.5:1) to yield 37mg (56 %) of product. The ee was determined by HPLC using a Ciralpak OD [hexane/iso-propanol 95:5, λ 230 nm, 1.0 mL/min] t (major) = 13.99 min., t (minor) = 15.87 min. (96%); $[\alpha]_D^{30}$ = +222.6 (c 1.50, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.68-7.66 (m, 2H, aromatic protons), 7.43-7.30 (m, 8H, aromatic protons+N=C<u>H</u>), 5.73 (s, 1H, C_β-<u>H</u>), 3.25 (s, 3H, O<u>C</u>H$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.5 (s, 1C, C=O) 155.4 (d, C=N), 141.1 (s, 1C), 134.9 (s, 1C), 134.8 (s, 1C), 128.6 (d, 2C), 128.5 (d, 2C), 128.3 (s, 1C), 128.0 (d, 2C), 126.6 (d, 2C), 89.0 (d, 1C, C_β), 84.3 (s, 1C, C_α), 52.4 (q, 1C, O<u>C</u>H$_3$); IR v_{max}/cm^{-1} 1738, 1637, 1493, 1241, 1108, 1091; HRMS (ES+): calcd. for $C_{17}H_{14}O_3N_1Cl_1Na_1$ $[M+Na]^+$ 338.0554, found 338.0559.

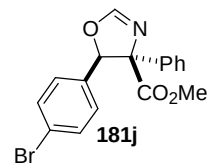
Synthesis and characterization of Compound 181i



181i

Obtained according to the general procedure, using isocyano-phenyl-acetic acid methyl ester (0.21 mmol, 37 mg) and 4-Formyl-benzoic acid methyl ester (0.36 mmol, 58 mg) as starting materials and TBME as solvent. Crude was redissolved in Et_2O, washed with $NaHSO_3$ 1M (3x), $NaHCO_3$ sat. (1x), dried over K_2CO_3 and quickly purified by flash column chromatography (Et_2O/E.P. 4:1) to yield 41 mg (57 %) of product. The ee was determined by HPLC using a Ciralpak OD [hexane/iso-propanol 90:10, λ 230 nm, 1.0 mL/min] t (major) = 15.39 min., t (minor) = 20.37 min. (93%); $[\alpha]_D^{24}$ = +173.3 (c 1.50, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 8.4, 2H, aromatic protons), 7.70-7.68 (m, 2H, aromatic protons), 7.46-7.36 (m, 5H, aromatic protons), 7.34 (s, 1H, N=C<u>H</u>), 3.93 (s, 3H, O<u>C</u>H$_3$), 3.19 (s, 3H, O<u>C</u>H$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.4 (s, 1C, C=O) 166.5 (s, 1C, C=O), 155.4 (d, 1C, C=N), 141.2 (s, 1C) 141.1 (s, 1C), 130.7 (s, 1C), 129.7 (d, 2C), 128.6 (d, 2C), 128.3 (d, 2C), 126.6 (d, 2C), 89.1 (d, 1C, C_β), 84.6 (s, 1C, C_α), 52.4 (q, 1C, O<u>C</u>H$_3$), 52.3 (q, 1C, O<u>C</u>H$_3$); IR v_{max}/cm^{-1} 1724, 1638, 1436, 1280, 1106, 700; HRMS (ES+): calcd. for $C_{19}H_{17}O_5N_1Na_1$ $[M+Na]^+$ 362.0999, found 360.0991.

Synthesis and characterization of Compound 181j

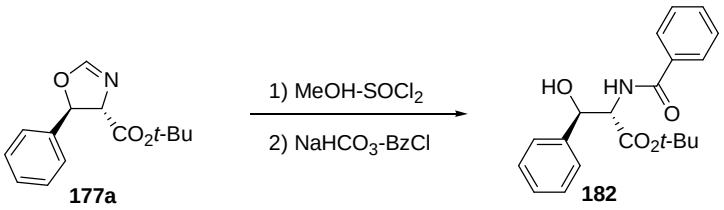


181j

Obtained according to the general procedure, using isocyano-phenyl-acetic acid methyl ester (0.21 mmol, 37 mg) and 4-bromo-benzaldehyde (0.36 mmol, 66 mg) as starting materials and AcOEt as solvent Crude was purified by flash column chromatography (E.P./AcOEt 3.5:1) to yield 42 mg (55 %) of product. The ee was determined by HPLC using a Ciralpak OD [hexane/iso-propanol 90:10, λ 230 nm, 1.0 mL/min] t (major) = 10.28 min., t (minor) = 11.53 min. (90%); $[\alpha]_D^{25}$ = +197.8 (c 1.60, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.61-7.59 (m, 2H, aromatic protons), 7.47-7.45 (m, 2H, aromatic protons), 7.36-7.17 (m, 6H, aromatic protons+N=C<u>H</u>), 5.64 (s, 1H, C_β-<u>H</u>), 3.18 (s, 3H,

O<u>CH</u>$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.5 (s, 1C, C=O) 155.4 (d, C=N), 141.1 (s, 1C), 135.3 (s, 1C), 131.6 (d, 2C), 128.5 (d, 2C), 128.3 (d, 2C), 126.6 (d, 2C), 123.1 (s, 1C), 89.0 (d, 1C, C_β), 84.2 (s, 1C, C_α), 52.4 (q, 1C, O<u>CH</u>$_3$); IR ν_{max}/cm^{-1} 1736, 1638, 1240, 1106, 1072, 700; HRMS (ES+): calcd. for $C_{17}H_{15}O_3N_1Br_1$ $[M+H]^+$ 360.0230, found 360.0233.

Direct conversion of Oxazoline 177a to N-benzoylated amino ester 182



177a **182**

Crude oxazoline **177a** prepared according to general procedure was dried in vacuo and dissolved in 1.5 mL MeOH dry. The reaction mixture was cooled at 0 °C and 1.5 mL of 1M solution of $SOCl_2$ in MeOH (2M HCl) are added drop wise. The solution was stirred 1 h 45' at 0 °C and then the solvent was removed under reduced pressure. The resultant oil was dissolved in CH_2Cl_2 (2 mL) and few drops of sat. $NaHCO_3$ solution were added under vigorous stirring until pH 8 (pH paper). The flask was cooled at 0 °C and 1 equivalent (0.21 mmol) of benzoyl chloride was added. After 20' the ice bath was removed and the reaction mixture was stirred further 40'. The suspension was partitioned between CH_2Cl_2 and water. The organic extracts were dried over Na_2SO_4 and purified by flash column chromatography (E.P./AcOEt 3.5:1) to yield 39 mg (55 % for 3 steps) of **182** as a single diastereomer. The ee was determined by HPLC using a Ciralpak AD [hexane/iso-propanol 80:20, λ 230 nm, 1.0 mL/min] t (minor) = 7.98 min., t (major) = 23.05 min. (96%); $[\alpha]_D^{25}$ = +60.4 (c 0.5, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.70 (d, *J* = 7.0 Hz, 2H, aromatic protons), 7.48 (t, *J* = 7.5 Hz, 1H, aromatic proton), 7.41-7.38 (m, 4H, aromatic protons), 7.32 (t, *J* = 7.0 Hz, 2H), 7.29-7.25 (m, 1H, aromatic proton), 6.92 (d, *J* = 8.0 Hz, N<u>-H</u>), 5.26 (d, 4.0 Hz, 1H), 4.96 (dd, *J* = 8.5, 4.0 Hz, 1H), 3.32 (br, 1H, O<u>-H</u>), 1.42 (s, 9H, ^{t}Bu); HRMS (ES+): calcd. for $C_{20}H_{23}O_4N_1Na_1$ $[M+Na]^+$ 364.1519, found 364.1510.
Spectroscopic data were consistent with previously reported data for this compound.[149]

Preparation of Racemic Compounds

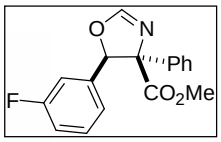
Racemic Oxazolines deriving from *tert*-butylisocyanoacetate
Racemic oxazoilnes derived from *tert*-butylisocyanoacetate were obtained by dissolving 1 equivalent of isocyanide and 1.2 equivalents of the corresponding aldehyede in dry CH_2Cl_2 (approximately 5 mL/mmol) at 0°C and adding 10 mol % of DBU as catalyst. The reaction mixture was then stirred at 0°C until TLC indicated complete consumption of the isocyanide (2-16 hours). The resultant solution was then diluted with CH_2Cl_2, washed with water, dried over Na_2SO_4 and concentrated under reduce pressure. The crude obtained was purified using the same method described for the enantioenriched examples.

Racemic Oxazolines deriving from Isocyano-phenyl-acetic acid methyl ester

[149] Hasegawa K., Arai S., Nishida A., *Tetrahedron*, **2006**, 62, 1390-1401.

Racemic oxazolynes derived from Isocyano-phenyl-acetic acid methyl ester were obtained by dissolving 1 equivalent of isocyanide and 1.2 equivalents of the corresponding aldehyde in dry CH_2Cl_2 (approximately 5 mL/mmol) at 0°C and adding 10 mol% of DBU and 5 mol% of Ag_2O. The reaction mixture was then gently warmed at room temperature until TLC indicated complete consumption of the isocyanide (2-16 hours). The resultant solution was then concentrated under reduced pressure and purified using the same method described for the enantioenriched examples.

X-Ray Crystal structure of Compound 181g



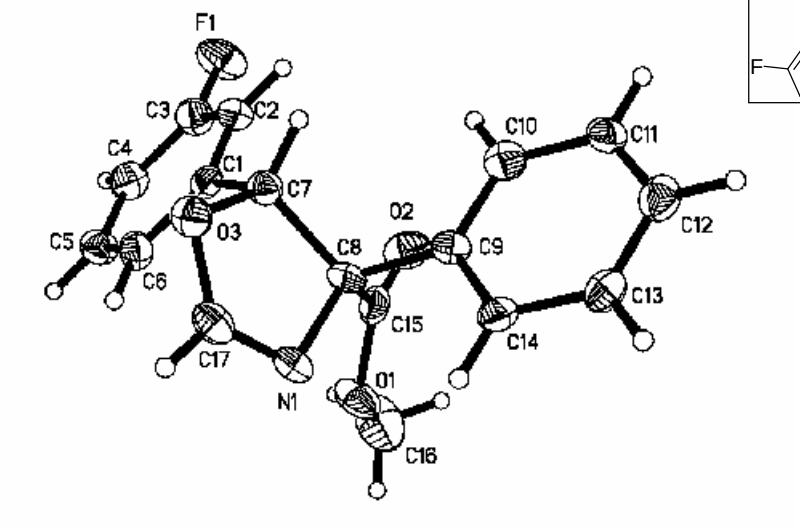


Table 1. Crystal data and structure refinement for s3049twa.

Identification code	z:\s3049\work\s3049twa
Empirical formula	C17 H14.67 F N O3.33
Formula weight	305.30
Temperature	100(2) K
Wavelength	0.71073 A
Crystal system, space group	Rhombohedral, R3
Unit cell dimensions	a = 25.205(8) A alpha = 90 deg. b = 25.205(8) A beta = 90 deg. c = 5.987(3) A gamma = 120 deg.
Volume	3294(2) A^3
Z, Calculated density	9, 1.385 Mg/m^3
Absorption coefficient	0.105 mm^-1

F(000)	1434
Crystal size	0.80 x 0.08 x 0.08 mm
Theta range for data collection	2.80 to 25.02 deg.
Limiting indices	-29<=h<=27, -30<=k<=30, 0<=l<=7
Reflections collected / unique	1288 / 1288 [R(int) = 0.0000]
Completeness to theta = 25.02	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. *trans*mission	0.9917 and 0.9207
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1288 / 1 / 184
Goodness-of-fit on F^2	1.072
Final R indices [I>2sigma(I)]	R1 = 0.0498, wR2 = 0.0937
R indices (all data)	R1 = 0.0625, wR2 = 0.0986
Largest diff. peak and hole	0.246 and -0.264 e.A^-3

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for s3049twa.
U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
F(1)	-460(2)	907(1)	-4715(5)	45(1)
O(1)	1022(2)	1928(2)	3513(6)	38(1)
O(2)	1150(2)	1853(2)	-150(6)	35(1)
O(3)	521(2)	3173(2)	740(6)	26(1)
N(1)	1058(2)	2987(2)	3370(7)	24(1)
C(1)	115(1)	2147(1)	-723(5)	23(1)
C(2)	65(1)	1817(1)	-2648(4)	26(1)
C(3)	-422(2)	1222(1)	-2893(5)	31(1)
C(4)	-858(1)	959(1)	-1213(6)	32(1)
C(5)	-808(1)	1289(1)	712(5)	30(1)
C(6)	-322(1)	1883(1)	957(4)	28(1)
C(7)	690(2)	2770(2)	-401(9)	22(1)
C(8)	1179(2)	2768(2)	1238(8)	22(1)

C(9)	1844(1)	3209(1)	462(4)	23(1)
C(10)	2036(1)	3140(1)	-1641(4)	28(1)
C(11)	2644(1)	3507(2)	-2274(4)	29(1)
C(12)	3061(1)	3943(1)	-804(6)	31(1)
C(13)	2870(1)	4012(1)	1299(5)	27(1)
C(14)	2262(1)	3645(1)	1932(4)	25(1)
C(15)	1112(2)	2125(2)	1401(9)	26(1)
C(16)	951(3)	1322(3)	3814(12)	58(2)
C(17)	725(2)	3207(2)	2875(9)	26(1)
O(1S)	0	0	8560(40)	69(5)
O(1T)	259(12)	389(11)	10360(40)	69(5)

Table 3. Bond lengths [A] and angles [deg] for s3049twa.

F(1)-C(3)	1.325(4)
O(1)-C(15)	1.335(6)
O(1)-C(16)	1.457(7)
O(2)-C(15)	1.186(6)
O(3)-C(17)	1.364(6)
O(3)-C(7)	1.452(6)
N(1)-C(17)	1.250(6)
N(1)-C(8)	1.482(6)
C(1)-C(2)	1.3900
C(1)-C(6)	1.3900
C(1)-C(7)	1.526(5)
C(2)-C(3)	1.3900
C(2)-H(2)	0.9500
C(3)-C(4)	1.3900
C(4)-C(5)	1.3900
C(4)-H(4)	0.9500
C(5)-C(6)	1.3900
C(5)-H(5)	0.9500
C(6)-H(6)	0.9500
C(7)-C(8)	1.577(7)
C(7)-H(7)	1.0000
C(8)-C(15)	1.548(7)
C(8)-C(9)	1.549(5)
C(9)-C(10)	1.3900
C(9)-C(14)	1.3900
C(10)-C(11)	1.3900
C(10)-H(10)	0.9500
C(11)-C(12)	1.3900
C(11)-H(11)	0.9500
C(12)-C(13)	1.3900
C(12)-H(12)	0.9500
C(13)-C(14)	1.3900
C(13)-H(13)	0.9500
C(14)-H(14)	0.9500
C(16)-H(16A)	0.9800
C(16)-H(16B)	0.9800

C(16)-H(16C)	0.9800
C(17)-H(17)	0.9500
O(1T)-O(1T)#1	1.50(4)
O(1T)-O(1T)#2	1.50(4)

C(15)-O(1)-C(16)	114.5(5)
C(17)-O(3)-C(7)	104.6(4)
C(17)-N(1)-C(8)	105.7(4)
C(2)-C(1)-C(6)	120.0
C(2)-C(1)-C(7)	118.5(3)
C(6)-C(1)-C(7)	121.2(3)
C(1)-C(2)-C(3)	120.0
C(1)-C(2)-H(2)	120.0
C(3)-C(2)-H(2)	120.0
F(1)-C(3)-C(2)	119.6(2)
F(1)-C(3)-C(4)	120.4(2)
C(2)-C(3)-C(4)	120.0
C(5)-C(4)-C(3)	120.0
C(5)-C(4)-H(4)	120.0
C(3)-C(4)-H(4)	120.0
C(6)-C(5)-C(4)	120.0
C(6)-C(5)-H(5)	120.0
C(4)-C(5)-H(5)	120.0
C(5)-C(6)-C(1)	120.0
C(5)-C(6)-H(6)	120.0
C(1)-C(6)-H(6)	120.0
O(3)-C(7)-C(1)	108.6(3)
O(3)-C(7)-C(8)	102.3(4)
C(1)-C(7)-C(8)	114.6(4)
O(3)-C(7)-H(7)	110.3
C(1)-C(7)-H(7)	110.3
C(8)-C(7)-H(7)	110.3
N(1)-C(8)-C(15)	113.7(4)
N(1)-C(8)-C(9)	110.0(4)
C(15)-C(8)-C(9)	106.4(3)
N(1)-C(8)-C(7)	103.1(4)
C(15)-C(8)-C(7)	111.5(4)
C(9)-C(8)-C(7)	112.3(4)
C(10)-C(9)-C(14)	120.0
C(10)-C(9)-C(8)	120.0(3)
C(14)-C(9)-C(8)	119.8(3)
C(11)-C(10)-C(9)	120.0
C(11)-C(10)-H(10)	120.0
C(9)-C(10)-H(10)	120.0
C(10)-C(11)-C(12)	120.0
C(10)-C(11)-H(11)	120.0
C(12)-C(11)-H(11)	120.0
C(13)-C(12)-C(11)	120.0
C(13)-C(12)-H(12)	120.0
C(11)-C(12)-H(12)	120.0
C(14)-C(13)-C(12)	120.0
C(14)-C(13)-H(13)	120.0

C(12)-C(13)-H(13)	120.0
C(13)-C(14)-C(9)	120.0
C(13)-C(14)-H(14)	120.0
C(9)-C(14)-H(14)	120.0
O(2)-C(15)-O(1)	125.1(4)
O(2)-C(15)-C(8)	123.9(5)
O(1)-C(15)-C(8)	111.0(4)
O(1)-C(16)-H(16A)	109.5
O(1)-C(16)-H(16B)	109.5
H(16A)-C(16)-H(16B)	109.5
O(1)-C(16)-H(16C)	109.5
H(16A)-C(16)-H(16C)	109.5
H(16B)-C(16)-H(16C)	109.5
N(1)-C(17)-O(3)	120.6(5)
N(1)-C(17)-H(17)	119.7
O(3)-C(17)-H(17)	119.7
O(1T)#1-O(1T)-O(1T)#2	60.001(11)

Symmetry transformations used to generate equivalent atoms:
#1 -x+y,-x,z #2 -y,x-y,z

7 Conclusions & Further Work

7.1 Conclusions

During a first part of this thesis work a new strategy for the synthesis of morpholines has been discovered and applied to the synthesis of relevant cyclic α-amino acids. In particular, the methodology has been applied to an efficient and high yielding synthesis of Fmoc-morpholine-3-carboxylic acid (Chapter 2) and for the preparation of costrained and geometrically diversified scaffolds to be used for peptidomimetics and new library templates (Chapter 4). Moreover, Fmoc-morpholine-3-caroxylic acid has been proved to be compatible with solid phase peptide synthesis by inserting it in small peptide sequences. Two of this tetrapeptides containing morpholine-3-carboxylic acid have been studied by means of NMR, IR and molecular modeling thecniques, in order to study the conformational folding induced by the presence of morpholine-3-carboxylic acid (Chapter 3). This new strategy is based on the use of enantiopure β-hydroxy-α-amino acids, which (apart from serine and threonine) are not easily available compounds and so we decided to focus our attention on the development of a new synthetic methodology for the asymmetric synthesis of β-hydroxy-α-amino acids. In the second part, the development of a new methodolgy to access this class of compounds is described. An highly enantio- and diastereo-selective addition of isocyanoacetates to aldehydes is described, in order to obtain enantioenriched oxazolines, which are then readily hydrolysed to β-hydroxy-α-amino esters. The catalytic system is based on a "de novo" designed and synthesised amino-phosphine ligand which works in the presence of silver salts. Although the mechanism has not been fully investigated a bifunctional metal-organic catalysis is hypothsised.
This thesis work has been published in 3 artcles in peer reviewed journals and is part of an European Patent Application. A further manuscript about the second part is in preparation.

7.2 Further work

To furhter expand this thesis work we believe that a conclusive study of the mechanism of action of the catalytic system discovered in the second part would be of great interest. We also believe that that the new ligand designed could be applied to different reaction where

a bifunctional metal-organic mechanism of action is postulated. Among the many examples there is the possibility of keep using the same nuchlophiles, isocyanoacetates, in the presence of different electrophiles such as imines. Finally, the new strategy for the preparation of β-hydroxy-α-amino esters could be exploited to prepare the starting materials required for the synthesis of new morpholine-3-carboxylic acids (using the methodology developed in the first part), in order to expand the number of new cyclic-amino acids available to the scientific community.