Asymmetric Synthesis of 2,2'-Bimorpholine and its 5,5'-Substituted Derivatives

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Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for any academic degree.

/Kristin Lippur/

2,2'-bimorfoliini ja selle 5,5'-asendatud derivaatide asümmeetriline süntees

KRISTIN LIPPUR

Contents

List of publications

The thesis is based on the following papers referred to in the text by the Roman numerals:

I   Lippur, K.; Kanger, T.; Kriis, K.; Kailas, T.; Müürisepp, A.-M.; Pehk, T.; Lopp, M. Synthesis of (2*S*,2'*S*)-bimorpholine *N,N'*-quaternary salts as chiral phase transfer catalysts. *Tetrahedron: Asymmetry* **2007**, *18*, 137-141.

II   Lippur, K.; Elmers, C.; Kailas, T.; Müürisepp, A.-M.; Pehk, T.; Kanger, T.; Lopp, M. Synthesis of 5,5'-disubstituted bimorpholines. *Synth. Commun.* **2010**, *40*, 266-281.

III   Kriis, K.; Laars, M.; Lippur, K.; Kanger, T. Bimorpholines as Alternative Organocatalysts in Asymmetric Aldol Reaction. *Chimia* **2007**, 232-235.

IV   Noole, A.; Lippur, K.; Metsala, A.; Lopp, M.; Kanger, T. Enantioselective Henry Reaction Catalyzed by Cu[II] Salt and Bipiperidine. *J. Org. Chem.* **2010**, *75*, 1313-1316.

Author's contribution:

The contribution by the author to the papers included in the thesis is as follows:

I: Carrying out all of the experiments, major role in writing.

II: Responsible for project planning. Carrying out most of the experiments, major role in writing.

III: Carrying out the experiments with 2,2'-bimorpholines, minor role in writing.

IV: Carrying out the experiments with 2,2'-bimorpholines, minor role in writing.

Abbreviations

AD	asymmetric dihydroxylation
Am	amyl
AZT	azidothymidine
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
Bn	benzyl
Boc	*tert*-butoxycarbonyl
CSA	camphorsulfonic acid
dba	dibenzylideneacetone
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DET	diethyl tartrate
DIBAL-H	diisobutylaluminium hydride
DIPEA	*N,N*-diisopropylethylamine
DIPT	diisopropyl tartrate
DME	1,2-dimethoxyethane
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
Dpe-phos	bis[2-(diphenylphosphino)phenyl]ether
dr	diastereomeric ratio
ee	enantiomeric excess
EI	electron ionization
FCS	fetal calf cerum
Fmoc	9-fluorenylmethyloxycarbonyl
FT	Fourier transform
GCMS	gas chromatography-mass spectrometry
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HPLC	high pressure liquid chromatography
IFN	interferon
IMDM	Iscove's Modified Dulbecco's Medium
IMS	industrial methylated spirits
IR	infrared
L	ligand
LDA	lithium diisopropylamide
M	metal
mp	melting point
Ms	methanesulfonyl
MS	molecular sieves
MTBE	*tert*-butyl methyl ether
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide – tetrazole
NIS	*N*-iodosuccinimide

NK-1	neurokinin 1
NMR	nuclear magnetic resonance
pen/strep	penicillin/streptomycin
PTC	phase transfer catalyst or phase transfer catalysis
Py	pyridine
Red-Al	sodium bis(2-methoxyethoxy)aluminum hydride
RLU	relative light units
RNA	ribonucleic acid
rt	room temperature
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
TBHP	*tert*-butylhydroperoxide
TEBA	benzyltriethylammonium chloride
Tf	trifluoromethanosulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP	tetrahydropyranyl
Thr	threonine
TLC	thin layer chromatography
TMS	trimethylsilyl
Tris	2,4,6-triisopropylbenzenesulfonyl
Ts	*p*-toluenesulfonyl
TsIm	*N*-(*p*-toluenesulfonyl)imidazole
VLPs	virus like particles

Introduction

A simple heterocycle morpholine has been widely used in organic synthesis as a weak base for a long time. Since the pioneering work of Stork[1], morpholine has been used as a reagent for the formation of enamines. Applications of its derivatives were substantially widened due to the explosive growth of the asymmetric synthesis in the second half of the last century. Morpholine itself is an achiral compound, but *C*-substitution creates a asymmetry and opens new perspectives using morpholine derivatives in the asymmetric synthesis. Two heteroatoms in the ring make morpholine a bidentate ligand that can be used in metal-catalyzed reactions. A secondary nitrogen atom makes it a potential organocatalyst used in aminocatalysis. In addition, H-bonding of N-H-moiety allows weak interaction catalysis.

On the other hand, substituted morpholine subunits are found in various biologically and therapeutically active compounds.[2] The World Drug Index contains well over 100 drugs incorporating this structural feature (as of 2003), including its presence as a side-chain and scaffold, and within fused-ring systems.

The morpholine moiety has been utilized extensively by the pharmaceutical industry in drug design, often because of the improvement in pharmacokinetic properties it can confer. The biological utility of molecules containing the morpholine moiety is wide-ranging, including antidepressant activity,[3,4] hypolipidemic action,[5,6,7] as a tachykinin receptor antagonist, serotonin agonist and NK-1 receptor antagonist,[8,9,10] and in antifungal activity.[11,12] They are also novel selective norepinephrine and dual serotonin/norepinephrine reuptake inhibitors.[13,14]

The applications of biologically relevant carbon-substituted morpholines vary from medicinal use to agricultural use. For example, reboxetine[15] is an antidepressant drug used in the treatment of clinical depression, panic disorder, attention deficit disorder and attention deficit hyperactivity disorder; fenpropimorph[16,17] is a widely used leaf fungicide,[18] whose major use is to control fungal diseases in cereals; aprepitant[19] is used for chemotherapy-induced nausea and vomiting (commercially available as Emend) (Figure 1).
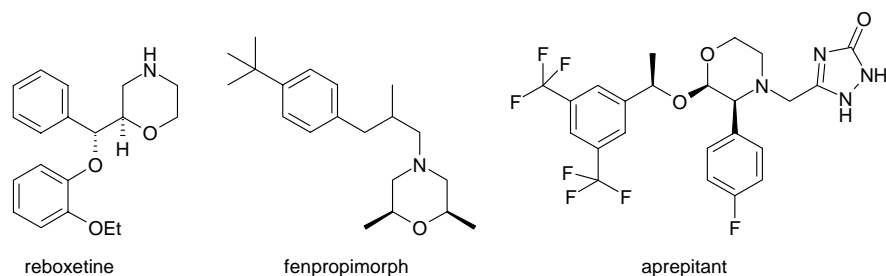

Figure 1. The structures of reboxetine, fenpropimorph and aprepitant.

The synthesis of (2*S*,2'*S*)-bimorpholine **1** and (3*R*,3'*R*)-bimorpholine **2** (Figure 2) has been previously published.[20,21,22] These bimorpholines and their derivatives have been used as chiral ligands in many asymmetric reactions[23], such as in organocatalysis[24,25,26,27,28,29] and in metal-catalysis[30,31].

(2*S*,2'*S*)-bimorpholine **1**   (3*S*,3'*S*)-bimorpholine **2**   5,5-substituted (2*S*,2'*S*)-bimorpholines

3 R = Me, R' = H
4 R = Bn, R' = H
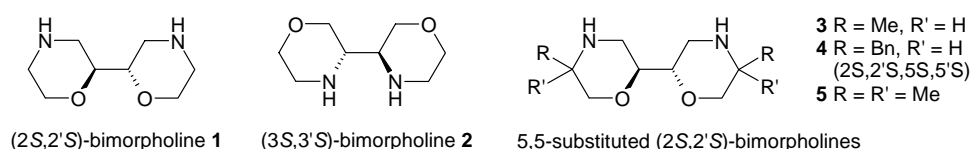(2S,2'S,5S,5'S)
5 R = R' = Me

Figure 2. (2*S*,2'*S*)-bimorpholine **1**, (3*S*,3'*S*)-bimorpholine **2** and 5,5'-substituted (2*S*,2'*S*)-bimorpholines **3**, **4** and **5**.

In the present work, we limited our object of investigation to C_2-symmetric 2,2'-bimorpholine **1** and its 5,5'-substituted derivatives **3** and its diastereoisomer, **4**, and **5** in order to evaluate their catalytic and some biological properties. We felt that the generality of the strategy would give rise to easy access to various substituted bimorpholines. The unique properties of C_2-symmetric compounds make its derivatization easier. The ideal starting material for that is the tartaric acid ester. The substituent can be introduced by a reaction with an appropriate amino alcohol, and one-step cyclization should afford the desired morpholine derivative. The stereochemistry of all stereogenic centers is defined by tartaric acid and amino alcohol. Both compounds are derived from the natural pool and are in both enantiomeric forms. Thus, all possible diastereoisomers can be obtained via the same scheme.

The use of tartaric acid and its derivatives has many advantages in synthetic chemistry: these inexpensive chirons provide convenient access to an enantiomerically pure material. They allow two stereocenters to be unambiguously set in a target molecule. This provides absolute stereochemical assignment of complex natural products and for the synthesis of structural analogs.[32]

The study of the improved synthesis of (2*S*,2'*S*)-bimorpholine **1** is realized and described in Article I. Article II itemizes the synthesis of 5,5'-substituted bimorpholines **3** and its diastereoisomer and **4**. The use of (2*S*,2'*S*)-bimorpholine **1** and its derivatives as organocatalysts is presented in Articles I, III and IV.

11

1. Literature overview

1.1. Asymmetric synthesis of enantiomeric *C*-substituted morpholines

The first synthesis of an enantiomerically pure *C*-substituted morpholine was reported in 1956 by W. G. Otto[33]. L-Ephedrine **6** was reacted with chloroethanol **7** to give phendimetrazine **8** (Scheme 1).
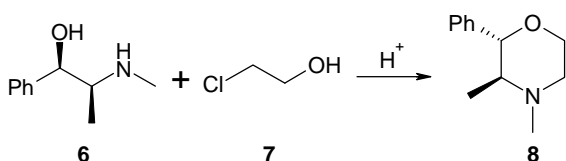
Scheme 1. The formation of phendimetrazine **8**.

The synthesis of substituted morpholines differs substantially from the synthesis of five- or six-membered heterocycles with one nitrogen atom (pyrrolidines and piperidines, respectively). There are two main reasons for this: firstly, there are no aromatic derivatives of morpholine and reactions characteristic of the aromatic compounds followed by their dearomatization can not be applied to obtain morpholines. Secondly, widely used deprotonation of *N*-Boc protected heterocycles by organolithium and quenching the anion with an electrophile[34] do not work in the case of morpholines because the electronegative oxygen atom causes the breakage of the C-O bond and the fragmentation of the heterocycle.

The literature overview is classified by cyclization methods, which provide enantiomeric *C*-substituted morpholine rings. There are several possibilities for the cyclization, and the following will be discussed in the Thesis:

- base-mediated cyclization
- acid-mediated cyclization
- morpholine ring formation by metal-catalysis
- formation of morpholine rings via carboxylic acid derivatives (lactams and lactoles)
- miscellaneous cyclization methods

1.1.1. Synthesis of *C*-substituted morpholines by base-mediated cyclization

In base-mediated cyclizations, the base is used to increase the nucleophility of the attacking *N*- or *O*-nucleophile. In this approach, typically enantiomerically pure starting materials (amino alcohols, amino ethers, aziridines, epoxides etc) are used to obtain enantiomerically pure targets. Special care should be taken in working with amino acid derivatives to avoid their racemization.

Prisinzano *et al.*[35] completed the synthesis of (*R,R*)- and (*S,S*)-reboxetine derivatives, whose structures contain a carbon substituted morpholine, from cinnamyl alcohol (Scheme 2).
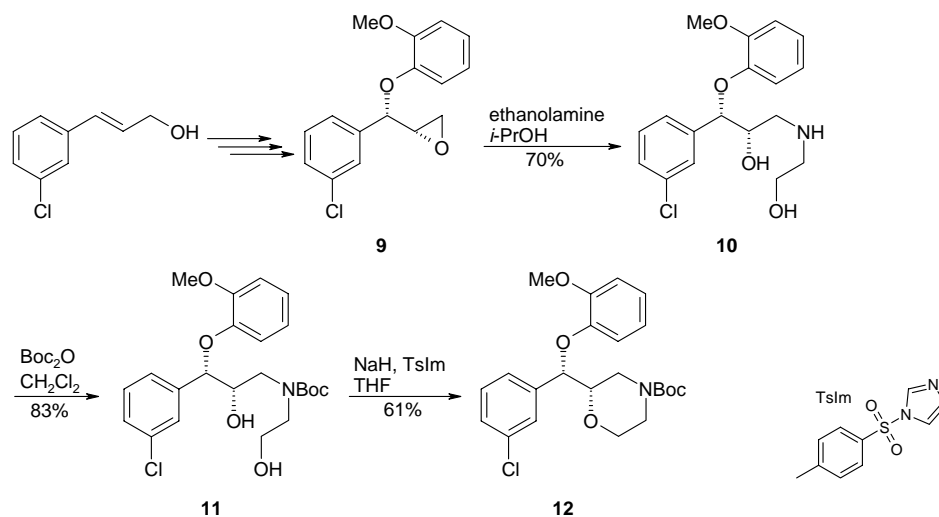
Scheme 2. Enantioselective route to (*S,S*)-reboxetine, over the base-mediated cyclization, affording morpholine derivative **12**.

Cinnamyl alcohol was converted to epoxide **9** via several steps, which included Sharpless asymmetric epoxidation. Subsequently, ethanolamine was reacted with the epoxide **9**, and after Boc-protection of the secondary amine **10**, ring closure was achieved in a one-pot reaction, which involved deprotonation and cyclization of compound **11** after the addition of *p*-toluenesulfonyl imidazole (TsIm). TsIm reacts with the primary hydroxyl group, affording a good leaving group. After the nucleophilic attack by the deprotonated secondary hydroxyl group, the morpholine ring in compound **12** is formed. The (*S,S*)-reboxetine can be easily achieved after the *N*-Boc deprotection of **12** with trifluoroacetic acid.

In the above-mentioned example, the *O*-nucleophile was used to form the morpholine ring. In this example, the target was achieved via *N*-nucleophile promoted ring closure. Sasaki *et al.*[36] started the synthesis of *C*-functionalized morpholine alcohols from a commercially available optically active serine derivative (Scheme 3).
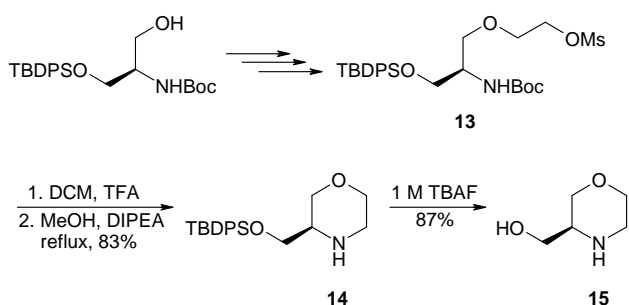
Scheme 3. Synthesis of (*S*)-3-(hydroxymethyl)morpholine **15** by Sasaki *et al*., starting with a serinol derivative.

First, a serinol derivative was coupled with *tert*-butyl bromoacetate to give the ester, which was then reduced to alcohol. Morpholine derivative was then achieved by a three-step sequence of *O*-mesylation, the removal of the Boc-group to create a stronger nucleophile, which led to cyclization under basic conditions. After the removal of the silyl group in **14**, the desired morpholine **15** was achieved in a 50% overall yield. A similar synthetic route was used for the synthesis of other *C*-functionalized morpholine alcohols, such as 3- and 3,5-substituted morpholines.

The same group has previously reported a facile route to 3,5-disubstituted morpholines.[37] *C*$_2$-symmetric *trans*-3,5-bis(benzyl/*tert*-butyldiphenylsilyl-oxymethyl)-morpholines with excellent optical purities were synthesized by employing commercially available chiral serine and solketal.

Breuning *et al*.[38] presented a straightforward one-pot procedure that allows fast and efficient access to enantiomerically pure 2-(hydroxymethyl)morpholines **16** (Scheme 4) with a widely variable substitution pattern.
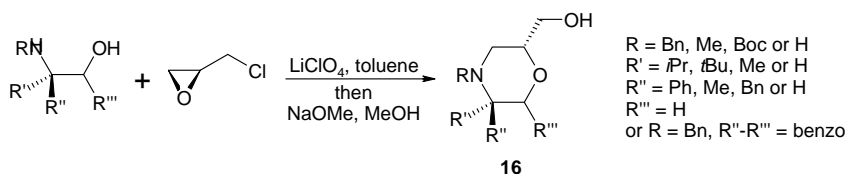
Scheme 4. Enantioselective preparation of morpholines **16** from β-amino alcohols and (*S*)-epichlorohydrin.

The one-pot approach is comprised of three steps: 1) the ring opening of enantiomerically pure epichlorohydrin with nucleophilic amino alcohol in the presence of Lewis acid to give the chloro alcohol, 2) its base-induced cyclization to epoxide, and 3) the final ring closure, delivering the desired morpholine **16**. The target molecules were obtained in a 55–77% yield. In two cases, either a complex product mixture or no reaction was detected, depending on the substituents in the amino alcohol. In some cases, the moderate yields were caused by the formation of

14

oxazepanes, which is explained by the competing 7-*endo* cyclization at the less-substituted terminal carbon atom in basic conditions. The intramolecular cyclization of epoxide to morpholine **16** requires a 6-*exo* ring closure (i.e. a reaction at the more highly substituted inner position of the epoxide).

They extended the method for the synthesis of 2-substituted 9-oxabispidines, which are constructed from substituted morpholine ring.[39]

Fish and Mackenny *et al.*[40] prepared the (*R*)- and (*S*)-*N*-Boc-morpholine-2-carboxylic acids **19** and **20**, using an enantioselective synthesis employing a highly selective enzyme-catalyzed kinetic resolution of racemic *n*-butyl 4-benzylmorpholine-2-carboxylate **18** as the key step (Scheme 5).
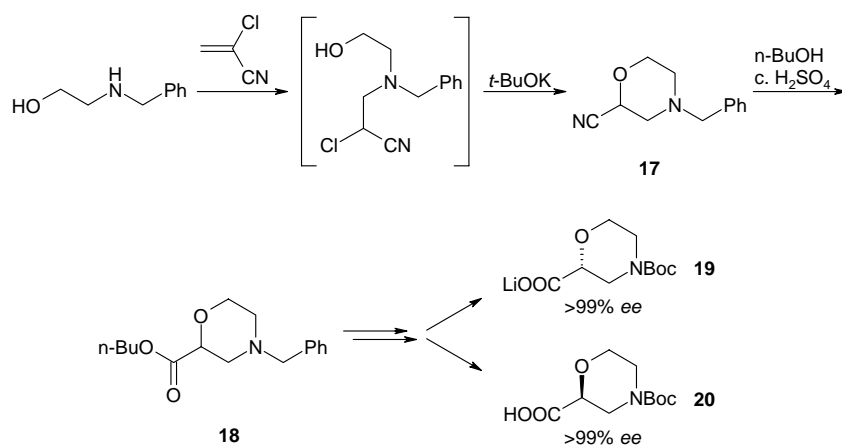
Scheme 5. Preparation of the (*R*)- and (*S*)-*N*-Boc-morpholine-2-carboxylic acids **19** and **20**.

Michael addition of *N*-benzylethanolamine with 2-chloroacrylonitrile followed by *t*-BuOK-promoted cyclization gave morpholine nitrile **17**, which then underwent alcoholysis with *n*-BuOH in the presence of conc. H_2SO_4 to give a racemic ester **18**. Lipase candida rugosa was completely stereoselective, with the enzyme catalyzing the hydrolysis of the (*S*)-ester to give (*S*)-acid, while leaving the (*R*)-ester untouched. After the exchange of the *N*-benzyl-protecting group of both compounds to the corresponding *N*-Boc amines, and the base hydrolysis of the n-butyl ester, this gave the morpholine derivatives **19** and **20**, which were further used for the synthesis of reboxetine analogs.

Myers *et al.*[41] used enantiopure epoxides and amino alcohols as starting materials for the synthesis of *trans*-2,5-disubstituted morpholines **22** (Scheme 6). Due to the steric hindrance in the 5-position (α-position to the nitrogen atom), two variations of the synthetic scheme were required. Formerly they had used one of the cyclization methods[42] for the preparation of structural analogues of (-)-Saframycin A.

15
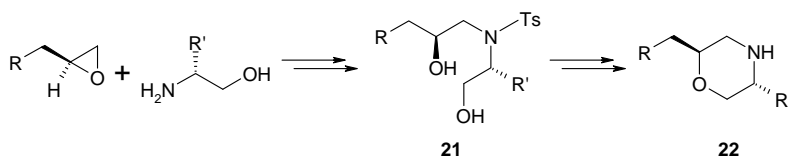
**Scheme 6.** The enantio- and diastereoselective synthesis of *trans*-2,5-disubstituted morpholines **22**.

The synthesis began with the reaction of the enantiopure (*S*)-epoxide with a 4-fold excess of enantiopure D-alaninol, providing only the monoalkylated product, with the amino group bonding to the less hindered carbon atom of the epoxide ring. The amino group was then selectively tosylated and the product **21** was cyclized in a one-step procedure, involving deprotonation and the addition of *p*-toluenesulfonyl imidazole – cyclization, affording the *N*-tosyl morpholine derivative. Cleavage of the *N*-protective group gave the desired morpholine derivative **22**.

With sterically more demanding substituents (phenyl and benzyl), selective *O*-tosylation occurred during attempted *N*-protection with tosyl chloride. Thus it was necessary to protect the hydroxyl groups of the amino diol product by silylation. Also, a two-step activation-closure sequence – firstly, the formation of primary tosylate and secondly, ring closure with potassium carbonate – was needed, instead of the one-step procedure in order to achieve the cyclization of diol.

Ghorai *et al.*[43] described a highly regio- and diastereoselective strategy for the synthesis of substituted morpholines **23**. The reaction proceeded via an S_N2-type ring opening of activated aziridines and azetidines by suitable halogenated alcohols in the presence of Lewis acid, followed by the base-mediated intramolecular ring closure (Scheme 7).
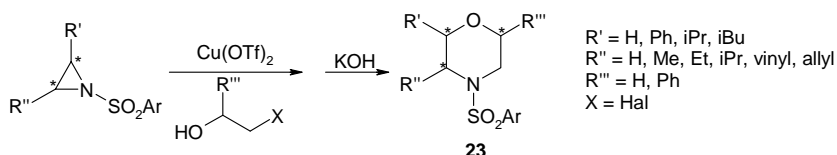
Scheme 7. Synthesis of substituted morpholines **23**.

While optimizing the reaction conditions, $Cu(OTf)_2$ was found to be the best Lewis acid, giving the highest regioselectivity in the aziridine ring opening. Morpholine **23** was achieved under basic conditions with a strong base (KOH), which was needed for the alkylation of sulfonamide. The reaction was found to be highly efficient with chloroethanol as the solvent. Morpholines **23** were synthesized from *N*-arylsulfonyl aziridines in these reaction conditions with good yields (72-90%) and enantioselectivities (68-80%). In the one-pot synthesis via the ring opening of aziridine with chloroethanol in the presence of 20 mol% $Cu(OTf)_2$ followed by KOH-assisted intramolecular cyclization, morpholines **23** were obtained in excellent yields (83-92%). The same reaction conditions were applied for the

synthesis of other 2-substituted morpholines, 2,3-disubstituted morpholines, and 2,6- disubstituted morpholines.

Henegar[44] developed a method for process chemistry for the synthesis of *N*-Boc-2-hydroxymethylmorpholine **30** (Scheme 8) and *N*-Boc-morpholine-2-carboxylic acid from epichlorohydrin. These compounds have been extensively used for the preparation of pharmacologically active compounds.
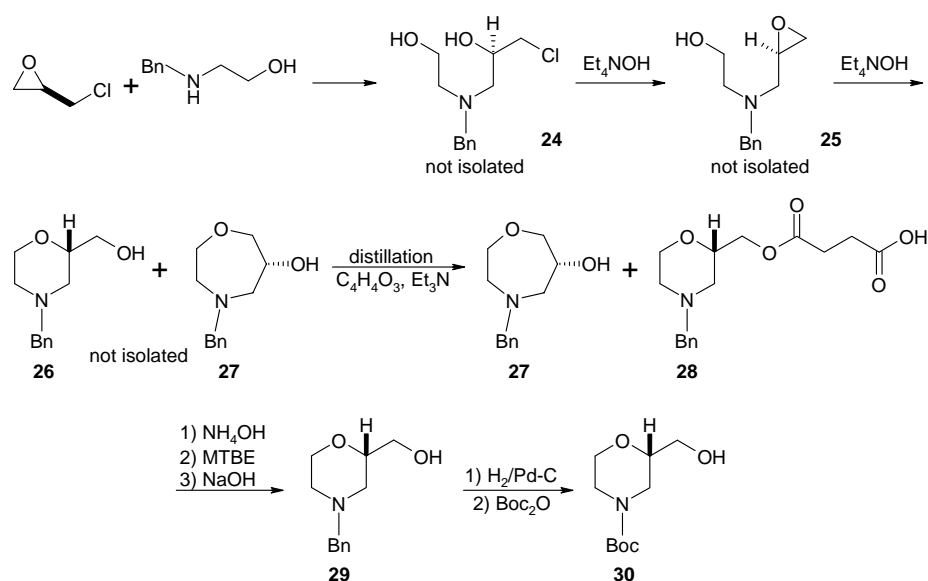
Scheme 8. The synthesis of *N*-Boc-2-hydroxymethylmorpholine **30**.

The reaction of (*R*)-epichlorohydrin with *N*-benzylethanolamine yielded the chlorohydrin **24**. After the formation of the unstable epoxide **25**, different reaction conditions were tested for the cyclization reaction. Lewis acids showed either a slow reaction with many reaction products or no reaction, lanthanide triflates also produced no reaction. The reaction with aq Et$_4$NOH yielded two main products: the desired morpholine **26** and a seven-membered 1,4-oxazepane **2**7. The ratio was invariably about 70:30, regardless of the reaction conditions used. Et$_4$NOH was chosen as the hydroxide due to its hydrophilic properties (needed for the extraction). The selective succinoylation of the primary alcohol moiety of the morpholine **26** to hemisuccinate **28** allowed nonchromatographic separation of the mixture of **27** and **28** by distillation. The succinate **28** was hydrolyzed, and after hydrogenation and Boc-protection, the desired *N*-Boc-2-hydroxymethylmorpholine **30** yielded >99% *ee* in a 41% overall yield.

Gandour *et al.*[45] synthesized the stereoisomers of 6-(carboxylatomethyl)-2-(hydroxymethyl)-2,4,4-trimethylmorpholinium from morpholine derivatives **33** and **34** (Scheme 9) in the search for carnitine acetyltransferase inhibitors.
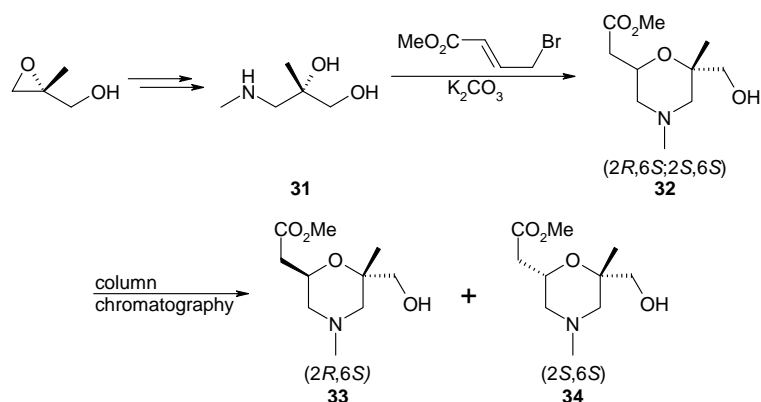
Scheme 9. Synthesis of morpholines **33** and **34**.

2-methylglycidol was used as a starting material to make compound **31**. Two approaches yielded morpholine **32**. The first was a two-step procedure, where DBU promoted the ring closure, but afforded only 40% of the desired product **32**. It turned out that the morpholine **32** fragmented on silica gel in the presence of DBU. The second approach was a one-pot procedure (Scheme 9), where morpholine **32** was achieved directly by the reaction of diol **31** with methyl 4-bromo-2-butenoate and a subsequent intramolecular Michael addition as a 6:1 diastereomeric mixture in a quantitative yield. The diastereomers **33** and **34** were separated by column chromatography. The morpholiniums were further synthesized from morpholines **33** and **34**.

1.1.2. Synthesis of *C*-substituted morpholines via cyclization under acidic conditions

In cyclization under acidic conditions, asymmetry is also introduced into the target molecules by starting with enantiomerically pure compounds. An acid plays a role in the activation of the electrophile (epoxide, carbonyl compound etc).

Albanese *et al.* reported[46] a concise, high-yielding synthesis of enantiopure 2,6-disubstituted morpholines **37** by the cyclization of epoxy alcohols (Scheme 10).
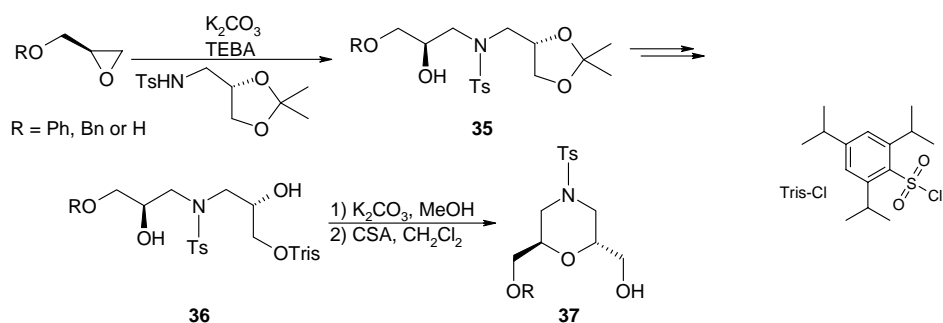
Scheme 10. Synthesis of 2,6-disubstituted morpholines **37**.

First, tosylsulfonamide was used for the ring opening of epoxide under solid-liquid-PTC conditions[47]. Removal of the diol protecting group in compound **35**, followed by the selective sulfonylation of the primary hydroxy group, afforded the sulfonate ester **36**. The latter was converted into epoxide and cyclized under acidic conditions. Enantiomerically pure morpholines **37** were obtained in high yields, up to 87%.

Guarna *et al.*[48] synthesized a morpholine derivative **40** from a readily available amino acid derivative (Scheme 11).
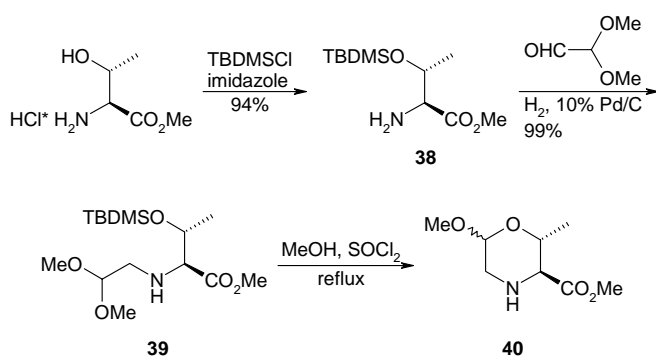
Scheme 11. Synthesis of morpholine derivative **40** from threonine.

First, the hydroxyl group was protected with TBDMSCl affording compound **38**. After a reductive amination step, the hydroxyl group was deprotected under acidic conditions and simultaneous trans-acetalization occurred. The obtained morpholine derivative **40** was further used for the preparation of dihydroxazine and, thereafter, for cyclopropanation.

The same group had previously reported a method for the synthesis of Fmoc-protected morpholine-3-carboxylic acid over a dihydroxazine derivative.[49]

1.1.3. Synthesis of *C*-substituted morpholines via cyclization by metal-catalysis

This approach differs in principle from the previous ones. In metal-catalyzed cyclizations, prochiral (or achiral) starting materials are used and the chirality is created by a catalytic amount of ligand.

Hayashi *et al.*[50] reported a catalytic asymmetric construction of morpholines **41** by palladium-catalyzed tandem allylic substitution reactions (Scheme 12).

a: X = OCOCH$_3$ a: R = CH$_2$Ph
b: X = OCO$_2$CH$_3$ b: R = SO$_2$C$_6$H$_4$CH$_3$-*p*
c: X = OCO$_2$C$_4$H$_9$-*t*

Pd(0) / L*
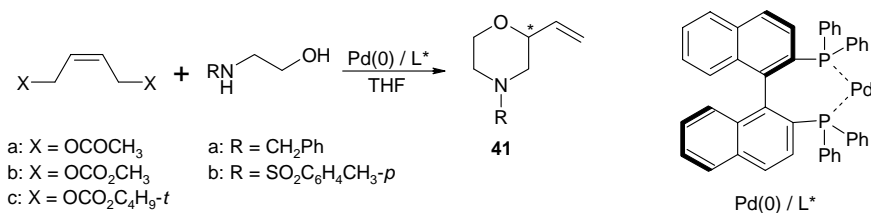
Scheme 12. Palladium-catalyzed asymmetric synthesis of morpholines **41**.

They established a method that was realized by the use of tandem allylic substitution reactions via π-allylpalladium intermediates having an optically active phosphine ligand. Different ligands were tested on compound **a**. The most stereoselective ligand was (*R*)-BINAP. The palladium catalyst was prepared in situ by mixing tris(dibenzylideneacetone)dipalladium (Pd$_2$(dba)$_3$*CHCl$_3$) and BINAP. The cyclization gave the product **41** with 61% *ee* in a 72% yield as a single stereoisomer. The asymmetric induction was controlled by the thermodynamic equilibrium of the π-allylpalladium intermediates before the second nucleophilic attack giving the heterocycle. The use of bis(BINAP)palladium(0) or palladium catalyst generated from [PdCl(π-C$_3$H$_5$)]$_2$ and BINAP increased the enantioselectivity to 65%, though the yield was only 22%. Palladium complexes with other phosphine ligands were less catalytically active and/or less stereoselective.

The method was extended to a one-step synthesis of 2,5-disubstituted morpholines, but mixtures of cis/trans-isomers were obtained, with enantiopurities varying from 45% to 73%

This initial work was followed by Achiwa and Yamazaki[51] (*ee* 83%), Ito and Katsuki *et al.*[52] (*ee* 81%) and Nakano *et al.*,[53] who achieved the highest *ee* – 94% – with a *P,N*-xylofuranose-based ligand.

Wilkinson[54] further developed the asymmetric route to a morpholine intermediate via a palladium-catalyzed allylic substitution. They examined several reaction conditions in order to improve the outcome of the reaction. The improvements led to an increase in isolated yield to 80% at 90% *ee* on a 10 g input, using only 1 mol% of catalyst.

Shi et al.[55] developed a novel access to ketal skeletons through a highly regio- and diastereoselective intermolecular addition of alcohols to alkynyl epoxides catalyzed by gold(I) (Scheme 13). Gold salts are powerful soft Lewis acids and can readily activate alkynes, allenes and olefins toward attacks by a variety of nucleophiles. Gold(I) can also be an efficient catalyst for the rearrangement of oxirane.

42   **43**

X = TsN or o-NO$_2$C$_6$H$_4$SO$_2$N or p-BrC$_6$H$_4$SO$_2$N
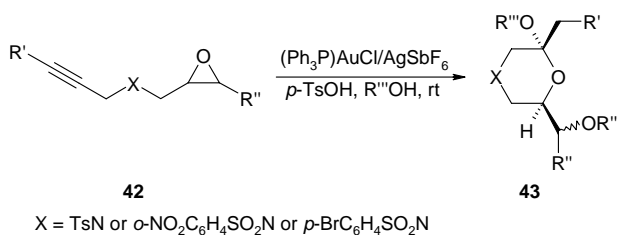
Scheme 13. Gold- and acid-catalyzed diastereospecific addition of alcohols to alkynyl epoxides.

The procedure involves a domino three-membered ring-opening, 6-*exo*-cycloisomerization, and a subsequent intermolecular nucleophilic addition to a double-bond sequence (Scheme 14). The corresponding 2,6-substituted morpholines **43** were obtained in 44-80% yields with high diastereoselectivities.
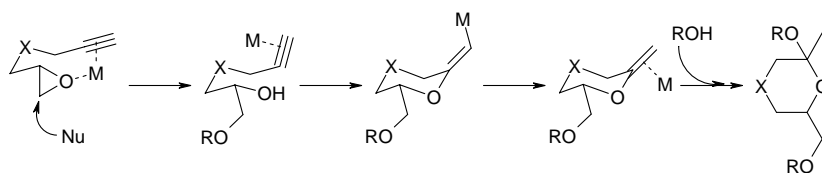
Scheme 14. The proposed mechanism of gold(I) catalyzed morpholine formation.

Dai et al.[56] discovered that three types of morpholine products **44**, **45** and **46** can be obtained from an allylic amine by a slight variation of the Li$_2$PdCl$_4$-CuCl$_2$ reagent system (Scheme 15).
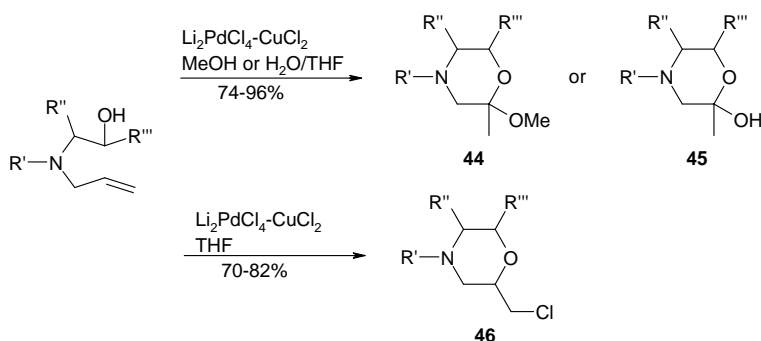
Scheme 15. Palladium(II)- and copper(II)-catalyzed synthesis of optically active morpholine derivatives **44**, **45** and **46**.

21

Ring closure toward the morpholines proceeded through a Wacker-type reaction, in which both Pd(II) and Cu(II) were necessary for the reaction to be successful. The regiochemistry was probably controlled by the ring size of the product. Compounds **44** and **45** were obtained from the intermediate via a nucleophilic attack of water and MeOH, respectively, after the β-elimination. In the absence of the nucleophile, the intermediate reacted with $CuCl_2$ to give the product **46**. The nucleophile prefers to attack the inner carbon atom of the double bond in order to form a six-membered morpholine ring rather than to attack the external carbon to form a seven-membered heterocycle. The diastereoselectivities varied from 71% to 99%, with the exception of two substrates having no selectivity. The yield values ranged from 70 to 96%.

Wolfe *et al.*[57] described a four-step synthesis of *cis*-3,5-disubstituted morpholines from enantiomerically pure amino alcohols (Scheme 16). The morpholine products were generated as single stereoisomers in moderate to good yields.

Scheme 16. Synthesis of *cis*-3,5-disubstituted morpholines.

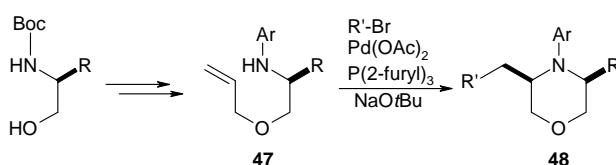The substrates for the Pd-catalyzed carboamination reactions were synthesized in three steps from commercially available starting materials. The treatment of the *N*-protected amino alcohols with NaH and allyl bromide afforded allyl ethers. Cleavage of the Boc-group followed by Pd-catalyzed arylation of the resulting amine trifluoroacetate salts provided moderate to good yields (57-73%). The optimal conditions for the 3,5-disubstituted morpholine-forming carboamination reactions were: the catalyst was composed of $Pd(OAc)_2$ and $P(2\text{-furyl})_3$, using toluene as a solvent and NaO*t*Bu as a base. Other ligands (e.g., PPh_3, Dpe-phos) were also tested, but they did not improve the results of the reaction. Several different 2-substituted *O*-allylethanolamines **47** were effectively converted to the desired heterocycles **48**, the yields of the reactions were low to modest (21-66%), and the diastereoselectivities were uniformly high (>20:1 dr). The same reaction conditions were used for the synthesis of 2,3-disubstituted and 2,5-disubstituted morpholines, but the products were obtained with only 2:1 diastereoselectivity and with yields of 45% and 48%.

The key intermediate in the reaction is a palladium(aryl)(amido) complex, which is produced by oxidative addition of the aryl bromide to Pd(0) followed by Pd-N bond formation. The relative stereochemistry of the substituted morpholine products **48** is most consistent with a pathway involving syn-aminopalladation through a boat-like transition state, whose reductive elimination provides the morpholine products **48**.

1.1.4. Formation of morpholine rings via carboxylic acid derivatives

This method is widely used for the synthesis of enantiomeric *C*-substituted morpholines, since the carboxylic acid derivatives are easily reduced into morpholines. The carboxylic acid derivatives are also mainly derived from natural amino alcohols and amino acids. Therefore, introduction of chirality is achieved by starting with enantiopure compounds.

Tamagnan *et al.*[58] reported an efficient synthesis leading directly to (*S,S*)-reboxetine via a new (*S*)-2-(hydroxymethyl)morpholine **15** preparation, starting with commercially available (*S*)-3-amino-1,2-propanediol (Scheme 17).
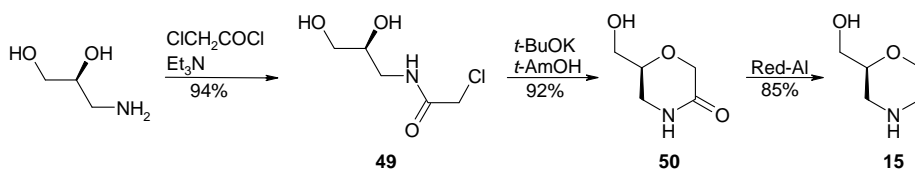
Scheme 17. Synthesis of (*S*)-2-(hydroxymethyl)morpholine **15**.

The reaction of amine with chloroacetyl chloride provided amide **49** in a high yield. The cyclization to morpholinone **50** was realized directly (without the protection of the primary alcohol) by the addition of a base, giving exclusively the desired product **50**. After a hydride reduction of amide **50**, the (*S*)-2-(hydroxymethyl)morpholine **15** was synthesized in only three steps, with a 73% overall yield. (*S,S*)-reboxetine was achieved after five steps, starting with (*S*)-2-(hydroxymethyl)morpholine **15**.
Previous to Tamagnans work, Berg *et al.*[59] had used a similar route for the synthesis of both enantiomers of 2-(hydroxymethyl)morpholine.

Henegar and Cebula[60] developed an enantioselective route to (*S,S*)-reboxetine succinate **55** (Scheme 18) for process chemistry. The lactam ring **54** in reboxetine was achieved by base-mediated cyclization.

23
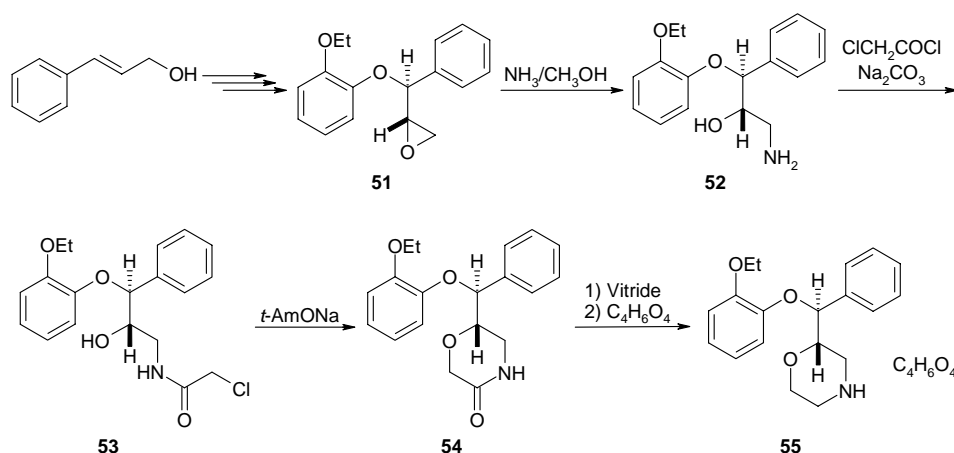
Scheme 18. An enantioselective route to (*S,S*)-reboxetine succinate **55**.

The synthesis started with a Sharpless asymmetric oxidation of cinnamyl alcohol, establishing two chiral centres. After the formation of epoxide **51**, aminolysis and acylation afforded compound **53**, which was converted to lactam **54** in basic conditions. The last step afforded (*S,S*)-reboxetine succinate **55** in a 19% overall yield, with high chemical and enantiomeric purity (>99%).

Kumar *et al.*[61] and Srinivasan *et al.*[62] had previously used a similar route for the synthesis of (*S,S*)-reboxetine.

Similar methods have been used for the synthesis of substituted morpholines via lactam derivatives by Cossy *et al.*[63] and Quirion *et al.*[64]

Kazmierski *et al.*[65] used a morpholinone derivative **57** in the synthesis of an HIV-1 protease inhibitor. Their initial target was the synthesis of substituted morpholinone (Scheme 19).
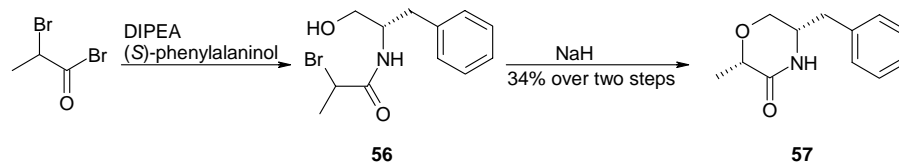
Scheme 19. Synthesis of (2*S*,5*S*)-2-methyl-5-(benzyl)-morpholin-3-one **57**.

After obtaining the diastereomeric mixture of **56** from the starting materials, the Williamson cyclization resulted in the exclusive formation of a pure (2*S*,5*S*)-2-methyl-5-(benzyl)-morpholin-3-one **57** in a 34% yield. The morpholinone **57** was further modified to the desired HIV-1 protease inhibitor.

Fang and Senanayake *et al.*[66] reported a stereospecific nucleophilic substitution of α-hydroxy ketones with alkylamines, utilizing α-ketotriflate intermediates to prepare enantiomers of hydroxybupropion **61** (Scheme 20). Hydroxybupropion **61**

is the major active metabolite of bupropion, which is used in the treatment of depression (Wellbutrin®) and as an aid to smoking cessation (Zyban®).
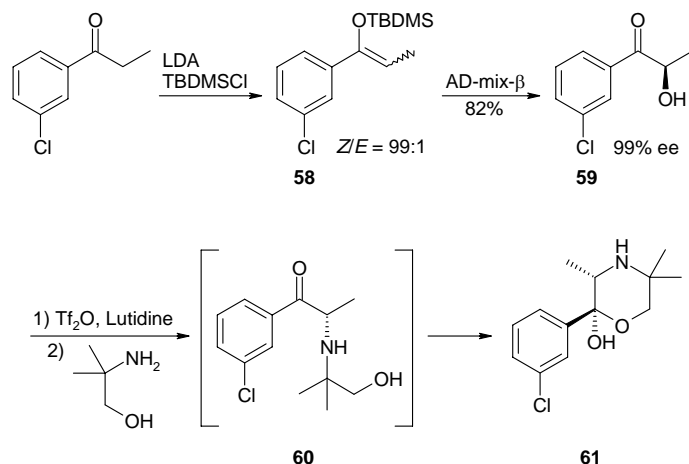
Scheme 20. The synthesis of (2*S*,3*S*)-hydroxybupropion **61**.

After the formation of a silyl enol ether **58**, asymmetric dihydroxylation with AD-mix-β as a catalyst afforded (*R*)-hydroxyketone **59**, with 98% *ee*. The compound was converted to ketotriflate and then reacted with aminoalcohol, affording (2*S*,3*S*)-hydroxybupropion **61** via lactolization as a single isomer. (2*R*,3*R*)-hydroxybupropion was similarly obtained from (*S*)-hydroxyketone.

Ashwood *et al.*[67] described a method for the preparation of 1,4-oxazine **65**, via the synthesis of lactone **63** (Scheme 21). They intended to use the amine for the synthesis of compounds that have been shown to be active at the NK-1 receptor.
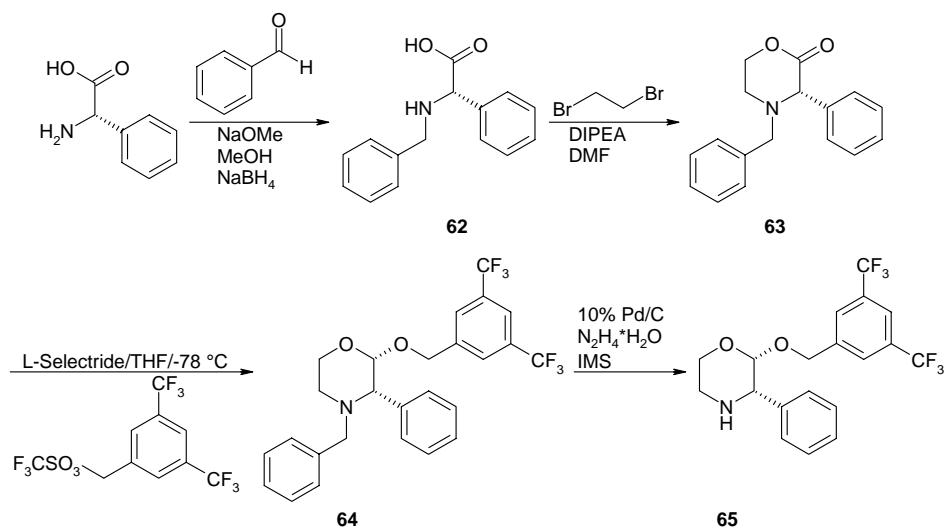
Scheme 21. The preparation of 1,4-oxazine **65**, via the synthesis of lactone **63**.

Reductive amination of benzaldehyde with (*S*)-phenylglycine afforded compound **62**, which was reacted with 1,2-dibromoethane, resulting in chiral (*ee* >99%) morpholone **63** after crystallization in a 57% yield. The stereoselective reduction of **63**, followed by *in situ* alkylation with a 3,5-bis(trifluoromethyl)benzyl triflate gave 2*S*,3*S*-acetal **64**. After transfer hydrogenation of the triflate **64**, the desired secondary amine **65** was obtained as a single enantiomer.

Bandichhor *et al.*[19] described a new approach for the synthesis of enantiopure aprepitant **70**, which is a potent antagonist for the human NK-1 receptor (Scheme 22). The synthesis proceeds over the preparation of racemic morpholinone **68**. The synthesis is carried out without any chiral starting materials, the stereogenic centres of aprepitant **70** are obtained by resolution with L-(-)-CSA.
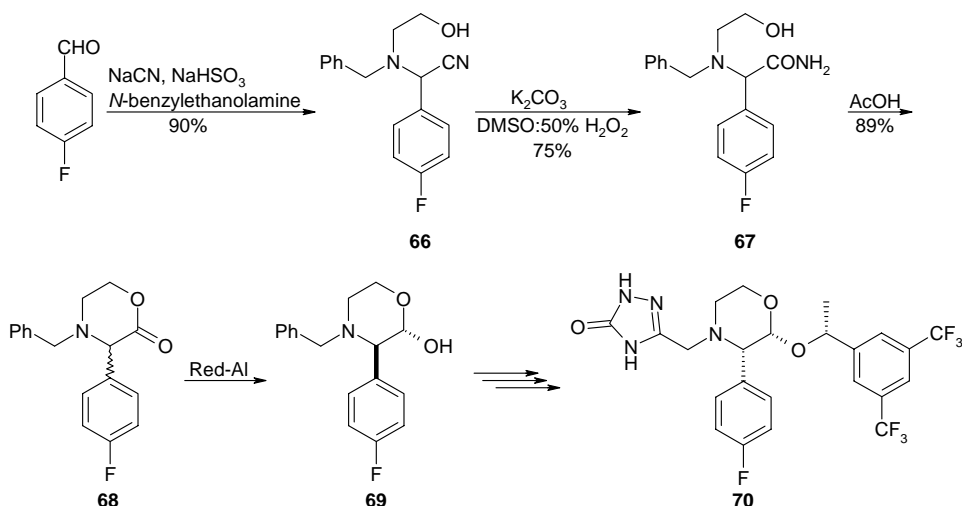
Scheme 22. The synthesis of aprepitant **70**.

p-Fluorobenzaldehyde was converted to *a*-aminonitrile **66**, which was further hydrolyzed with alkaline hydrogen peroxide to amide **67**. Cyclization of amide **67** in acetic conditions afforded racemic morpholinone **68**. Conversion of **68** to morpholinol **69** was obtained by reduction with Red-Al. Aprepitant **70** was achieved by further steps, which also included the resolution of enantiomers with L-(-)-CSA.

1.1.5. Miscellaneous methods for the synthesis of *C*-substituted morpholines

Aggarwal *et al.*[68] generated a method for the synthesis of six- and seven-membered rings from enantiomerically pure 1,2-/1,3-aminoalcohols in a reaction with bromoethylsulfonium salt. The reactions proceed through the generation of a vinyl

sulfonium salt, followed by annulation, to give 1,4-heterocycles such as morpholines **71**, in a simple procedure (Scheme 23).
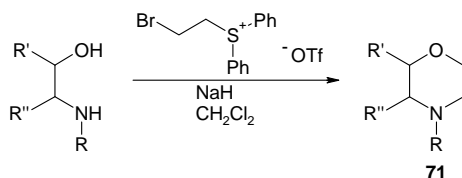
Scheme 23. The synthesis of morpholine derivatives **71**.

They had previously used a diphenyl vinyl sulfonium salt for the formation of β-heteroamino compounds, but the salt was very air sensitive. Later, they reported a new method for the reaction, using bromoethylsulfonium salt, which is converted to vinyl sulfonium salt *in situ* by the base in the reaction. NaH was found to give the best results in the annulation reaction. Several different amino alcohols were converted to morpholines **71**, in good to excellent yields (68-94%) as single enantiomers. The proposed mechanism of the reaction is shown in scheme 24. The cascade events are initiated by a nucleophilic attack of the amide on the electrophilic sulfonium salt **a**, which forms an intermediate sulfur ylide **b**. A proton transfer unmasks a second electrophilic centre and creates a potent nucleophile, leading to the heteroatom displacing the sulfide from **c** and forming the desired heterocyclic product **71**.
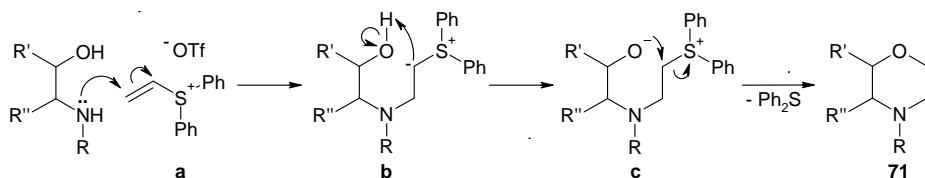
Scheme 24. Proposed mechanism for morpholine **71** synthesis using vinyl sulfonium salt.

De Kimpe et al.[69] reported a diastereoselective approach to *cis*-3,5-disubstituted morpholine derivatives **73** based on a ring enlargement of a 2-(allyloxymethyl)aziridine **72** via an electrophile-induced ring closure (Scheme 25).
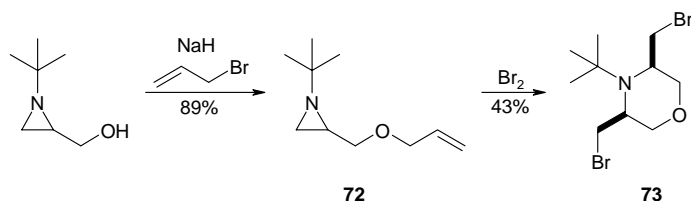
Scheme 25. Synthesis of *cis*-3,5-disubstituted morpholine derivative **73**.

1-(*tert*-butyl)-2-(hydroxymethyl)aziridine was converted to the corresponding 2-(allyloxymethyl)aziridine **72** by Williamson ether synthesis. The presence of a double bond in the ε,γ-position with respect to the nitrogen atom allowed intramolecular reactions upon activation of this alkene moiety. Activation of the double bond by bromine afforded a morpholine derivative **73** as one stereoisomer. They broadened the synthesis with different substituents on the nitrogen atom, although mostly with low yields. The disadvantage of the method is that it provides symmetrically disubstituted (meso) products.

Bartlett *et al.*[70] synthesized heteroatoms containing tricyclic structure, the route to this compound started with the synthesis of the morpholine derivative **78** (Scheme 26).
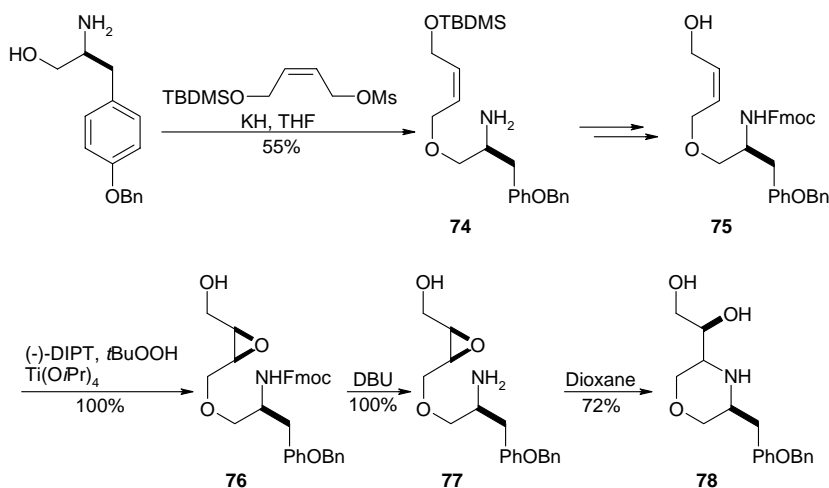
Scheme 26. Synthesis of morpholine derivative **78**.

cis-2-butene-1,4-diol was previously mono-protected as *tert*-butyldimethylsilyl ether, then converted to mesylate and coupled with *O*-benzyl-L-tyrosinol in the presence of potassium hydride, which resulted in the desired ether as an exclusive isomer. Subsequently, *N*-acylation, *O*-deprotection, Sharpless epoxidation and *N*-deprotection gave the epoxide **76**. Attempts to form the morpholine ring **78** under basic conditions failed, presumably due to the steric hindrance. However, the *N*-deprotected amino epoxide **77** was cyclized to morpholine **78** after refluxing in dioxane for nine hours.

Nishi *et al.*[71] designed a route for the synthesis of 2-substituted morpholine **82**, which was further modified for testing tachykinin receptor binding affinity. It included such key steps as Sharpless asymmetric dihydroxylation and the Mitsunobu reaction (Scheme 27).
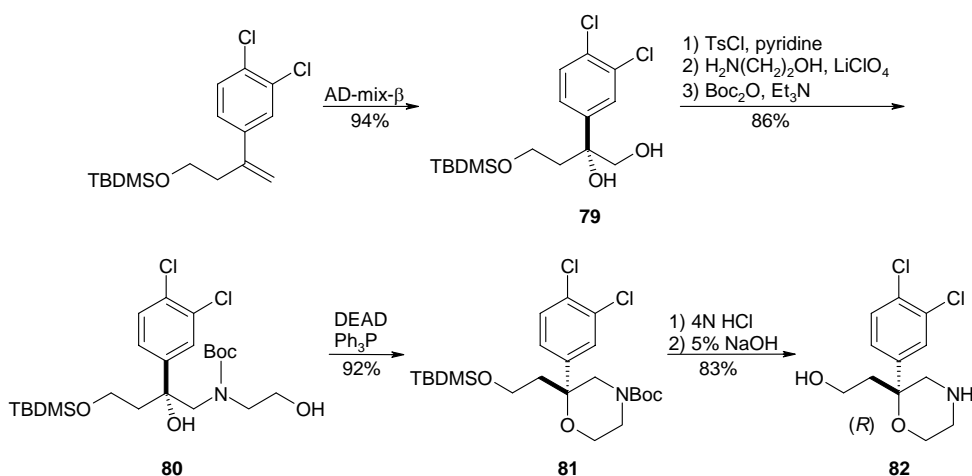
Scheme 27. Synthesis of **82** using Sharpless AD and Mitsunobu reaction.

First, olefin was treated with AD-mix-β to obtain diol **79** with >97% *ee*. After selective formation of primary tosylate, a substitution with aminoethanol was performed in the presence of LiClO$_4$, and the protection of the resulting secondary amine gave **80** in a good yield. The treatment of **80** with DEAD and Ph$_3$P provided the compound **81** in a 92% yield. The deprotection provided enantiomerically pure (*R*)-2-substituted morpholine **82**.

Zhou and Xiao *et al.*[72] found *t*-BuOK to be an effective promoting reagent for tandem ring-opening/closing reactions of various *N*-Ts aziridines and aryl propargyl alcohols to afford the dihydroxazine derivatives **83**, which can further be hydrogenated to the morpholine derivatives **84** (Scheme 28).
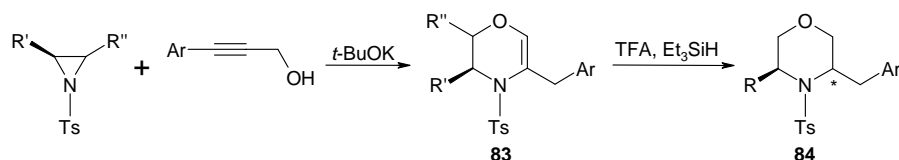
Scheme 28. Reaction of *N*-Ts aziridines and aryl propargyl alcohols promoted by *t*-BuOK at 40 °C.

Depending on the substituents in the aziridine ring and in propargyl alcohols, the yield of the reaction varied from 22 to 78%. They have proposed a possible reaction mechanism for the preparation of dihydroxazine derivatives **83**, where the aryl propargyl alcohol was deprotonated by *t*-BuOK to form the oxygen nucleophile, which then attacked the *N*-Ts aziridine to generate a new nucleophilic intermediate. Then, the nucleophilic intermediate underwent isomerization of the triple bond to form the allene species. The intramolecular nucleophilic attack therefore formed the product **83**. The synthetic utility of this methodology was demonstrated in a highly efficient route to morpholine derivatives **84** by

hydrogenation. In the case of isopropyl-substituted dihydroxazines **83**, only cis morpholine derivatives **84** were obtained. However, for the methyl- and isobutyl-substituted dihydroxazines **83**, a diastereomeric mixture of the desired morpholine derivatives **84** was obtained.

Carboni et al.[73] applied the Petasis[74,75] three-component reaction for the synthesis of substituted morpholines **85** (Scheme 29).
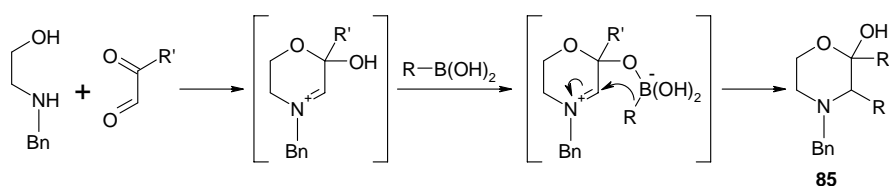
Scheme 29. Synthesis of 2-hydroxymorpholines **85**.

In a one-pot cyclization procedure benzyl-protected amino alcohol reacted with a glyoxal derivative to give imine, which reacted with the boronic acid via a tetra-coordinated boron complex to give the 2-hydroxymorpholines **85**. The reaction proceeded in a 50-92% yield with diastereoselectivities varying from 10-78%, the diastereomeric ratio depended on the substituents.

1.2. Asymmetric synthesis of *C₂*-symmetric bimorpholines

Kriis *et al.* achieved a synthesis of (2*S*,2'*S*)-bimorpholine **1**[20,21] (Scheme 30) and (3*S*,3'*S*)-bimorpholine **2**[21,22] (Scheme 31), starting with a commercially available (*R*,*R*)-tartaric acid derivative.
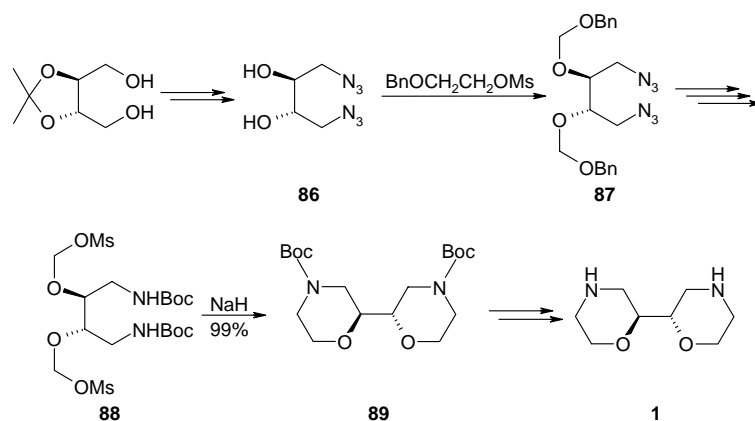
Scheme 30. The synthesis of (2*S*,2'*S*)-bimorpholine **1**.

For the synthesis of (2*S*,2'*S*)-bimorpholine **1**, a multi-step procedure was conducted. First, the introduction of nitrogen-containing functionality into the tartaric acid derivative was conducted by a two-step procedure, which involved mesylation of the hydroxyl groups, followed by their azidation with sodium azide, affording compound **86**. After chain lengthening via *O*-alkylation of hydroxyl groups, intramolecular cyclization of compound **88** afforded bimorpholine **89**. After the removal of the boc-protecting group, bimorpholine **1** was achieved. Starting with an (*R*,*R*)-tartaric acid ester, the target compound **1** was obtained in a series of 10 steps, with a 15% overall yield and high enantiomeric purity (>98%).
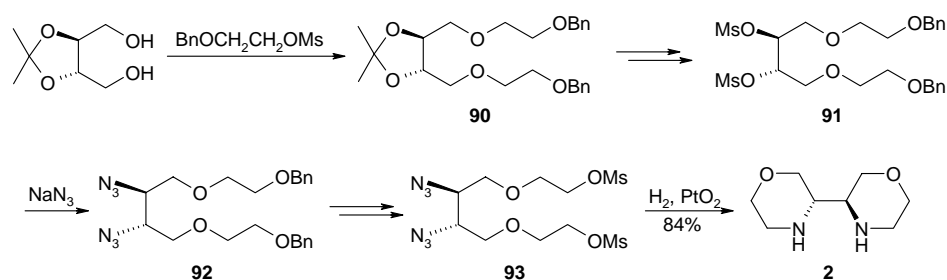
Scheme 31. Synthesis of (3*S*,3'*S*)-bimorpholine **2**.

(3*S*,3'*S*)-bimorpholine **2** was synthesized starting with diol, which was alkylated in order to elongate the chain by two carbon units. After that, deacetalization and mesylation were conducted. The nitrogen functionality was introduced with sodium azide. After debenzylation of diol **92**, *O*-mesylate **93** was synthesized and in the final step, bimorpholine **2** was achieved. The synthesis of (3*S*,3'*S*)-bimorpholine **2** was realized in seven steps, with a 26% overall yield and high enantiomeric purity (>98%).

1.3. Summary of the literature overview

There is an obvious need for enantiomerically pure *C*-substituted morpholines, due to their biological activity and the range of their uses as chiral ligands or catalysts. Several methods have been developed for their synthesis (Scheme 32). The formation of the morpholine ring has been achieved through many synthetic approaches, e.g. by base- or acid-mediated cyclization, by metal-catalysis, via formation of carboxylic acid derivatives (lactams, lactoles and lactones) or by several miscellaneous reactions. The routes vary from one-step to multi-step procedures, giving substituted morpholine derivatives. There are many possibilities for the introduction of asymmetry into the morpholine derivatives. The easiest way is to use enantiomerically pure starting materials, such as amino alcohols, epoxides, aziridines etc. This method is mainly used in the base and acid-induced cyclizations, as well as in the synthesis of carboxylic acid derivatives. Another possibility is to create chirality from a prochiral (or achiral) starting material in

metal-catalyzed cyclizations. Other methods include Sharpless oxidation, dihydroxylation or epoxidation (usually conducted with an achiral starting material). The synthesis of enantiomerically pure bimorpholines has only been accomplished by Kriis *et al.*, and the topic of *C*-substituted C_2-symmetric bimorpholines has never before been explored.
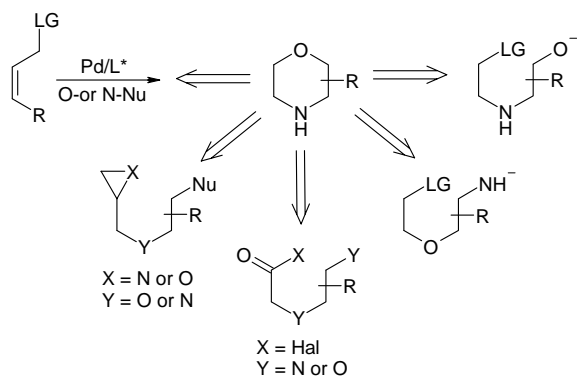
Scheme 32. Retrosynthetic routes towards *C*-substituted morpholines.

2. Aims of the present work

There is a plethora of methods providing substituted morpholines, but the synthetic strategy for every target is specified and determined by the target structure (substitution pattern, absolute and relative stereochemistry etc). Our special interest lies in C_2-symmetric bimorpholines. Despite their potential in organocatalysis or in metal-catalyzed reactions, there are no efficient synthetic sequences leading to these compounds. In order to investigate systematically their catalytic properties or structure-activity relationship, an easy and straightforward access to 2,2'-bimorpholines is needed.

In the present work, our main goal was to work out an efficient and general strategy for the asymmetric synthesis of the skeleton 2,2'-bimorpholine. More specific aims were the following:

- synthesis of substituted 2,2'-bimorpholines (5,5'-substituted and *N*-substituted derivatives)
- determination of the enantiomeric purity of the synthesized compounds
- investigation of the influence of the substituents at the C-5 on the cyclization reaction
- determination of the catalytic properties of the obtained bimorpholines in organo- and metal-catalytic reactions
- screening of their biological properties

3. Results and discussion

The retrosynthetic analysis of the synthesis of 2,2'-bimorpholines is outlined in scheme 33.
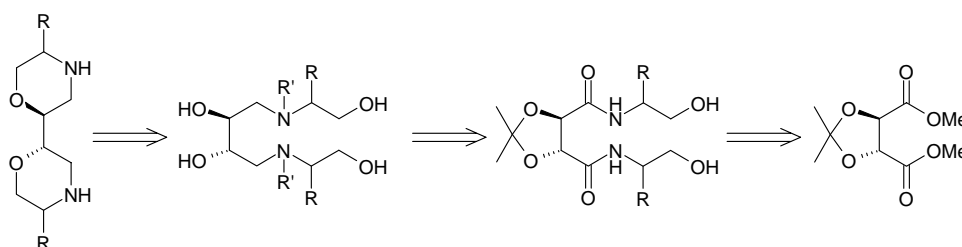
Scheme 33. A retrosynthetic route for (2*S*,2'*S*)-bimorpholines.

The key step of the synthesis is the cyclization of tetraol. A one-step procedure using TsIm is the most straightforward for that. It should be noted that a potential side reaction will lead to an undesired heterocyclic bicyclo[5.5.0] system. The introduction of nitrogen-containing functionality into the skeleton of bimorpholine is achieved by the amidation of ester groups of the tartaric ester acetal with amino alcohol. The stereogenic centers of the tartaric acid and amino alcohol are incorporated into the target compound and, thus, the stereochemistry is strictly defined. Since tartaric acid is a natural compound and amino alcohol is easily derived from amino acid, both enantiomers are available and all possible diastereoisomers can be synthesized.

3.1. Synthesis of (2*S*,2'*S*)-bimorpholine 1 (Article I)

Article I deals with the synthesis of an unsubstituted bimorpholine and its ammonium salts. The general idea of the synthetic scheme depicted in scheme 33 was realized.

We started the synthesis of (2*S*,2'*S*)-bimorpholine **1** from tartaric acid ester acetal and 2-aminoethanol. The cyanide-catalyzed amidation[76,77] enabled us to introduce nitrogen functionality into the molecule in a one-step procedure. The reaction proceeded in a 97% yield (Scheme 34).
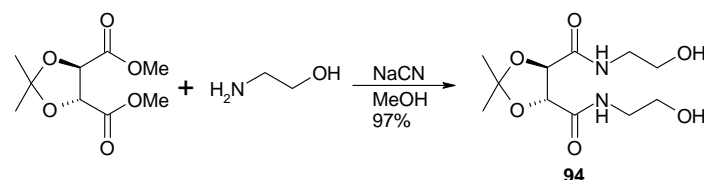
Scheme 34. Amidation of (*R*,*R*)-tartaric acid ester acetal.

The reduction of amide **94** with LiAlH$_4$ in THF afforded the desired amine **95** in an 82% yield. Subsequently, the secondary amine **95** was *N*-benzylated with benzyl bromide to achieve tertiary amine **96** in a 93% yield. The protection is needed to avoid *N*-tosylation in the cyclization step. After deacetalization of compound **96** with 6 N HCl, tetraol **97** was synthesized in an 82% yield. (Scheme 35)
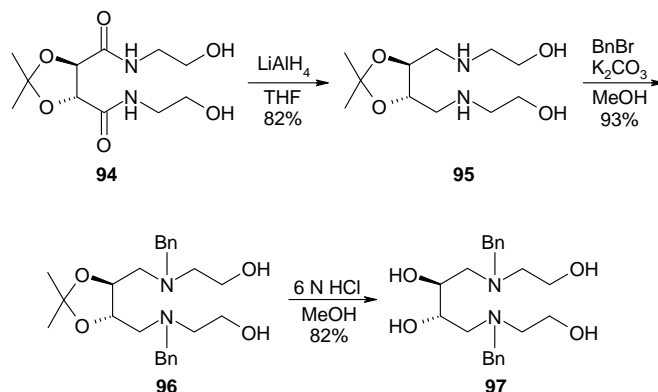
Scheme 35. Three-step synthesis of tetraol **97** from amide **94**.

Since there are many references[78,79,80,81] in which diols have been cyclyzed into morpholine rings with either sulphuric acid or hydrochloric acid, the same reaction was expected with tetraol **97**. Unfortunately, by heating tetraol **97** with concentrated sulphuric acid in 1,2,3,4-tetrahydronaphthalene at 150 °C for 11 h, a multi-component mixture was obtained.

In order to perform a two-step cyclization, mesylation and tosylation of primary hydroxyl groups in tetraol **97** were conducted (with the intention of preparing a good leaving group for the cyclization), but the reactions also resulted in a multi-component mixture.

The use of TsIm can provide the desired product easily in a one-pot reaction. TsIm allows for simultaneous hydroxyl activation – ring closure of suitably substituted 1,5-diols.

The cyclization of tetraol **97** with TsIm proceeded as expected in one step (Scheme 36). TsIm reacts with the primary hydroxyl groups in tetraol **97**, affording good leaving groups. After the nucleophilic attack by the deprotonated secondary hydroxyl groups, the morpholine rings in compound **98** are formed. The yield of the reaction is highly dependent on the concentration. By decreasing it from 0.1 to 0.013 M, the yield of bimorpholine **98** increased from 23% to 82%. The low yield of the reaction was most likely caused by intermolecular reactions.
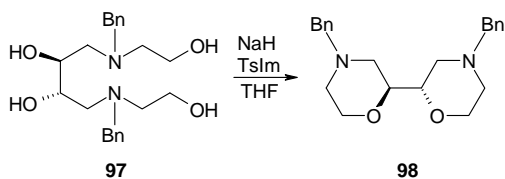
Scheme 36. Cyclization of tetraol **97**.

For the final step in the synthesis of (2*S*,2'*S*)-bimorpholine **1**, debenzylation of compound **98** was attempted with H_2 in the presence of $Pd(OH)_2$ catalyst but, after a week of reaction time, only 49% of the product was obtained. When ammonium formate was used as a hydride source in the presence of Pd/C catalyst, (2*S*,2'*S*)-bimorpholine **1** was obtained in 7 h, in a 70% yield (Scheme 37).
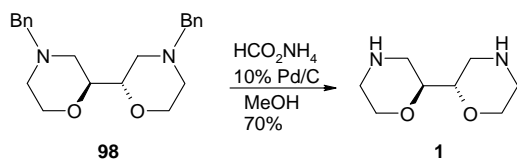
Scheme 37. Formation of (2*S*,2'*S*)-bimorpholine **1**.

In order to determine the enantiomeric purity of the obtained (2*S*,2'*S*)-bimorpholine **1**, its enantiomer (2*R*,2'*R*)-bimorpholine was synthesized according to the same route, starting with (*S*,*S*)-tartaric acid ester acetal. Bimorpholine **98** and its enantiomer were analyzed with chiral HPLC on a Chiracel OD-H column, which revealed their high enantiomeric purity – *ee* 99%.

Thus, enantiomerically pure (2*S*,2'*S*)-bimorpholine **1** and its enantiomer were obtained over six steps in a 35% overall yield. The same synthetic route was expected to work in order to achieve the synthesis of substituted bimorpholine derivatives.

3.2. Synthesis of 5,5'-disubstituted bimorpholine derivatives (Article II)

In order to broaden the scope of the synthetic route that was used to obtain (2*S*,2'*S*)-bimorpholine **1**, we intended to synthesize substituted bimorpholines with the substituents at the α-position of the nitrogen atoms, according to the same procedure, just by modifying the aminoalcohol moiety used in the amide formation. Article II deals with the synthesis of these disubstituted bimorpholines.

3.2.1. Synthesis of (2*S*,2'*S*,5*S*,5'*S*)-5,5'-dimethyl-2,2'-bimorpholine

First, (*S*)-alaninol was used as the substituted amino alcohol in the cyanide-catalyzed amidation of (*R*,*R*)-tartaric acid ester acetal (Scheme 38). The reaction proceeded with a 80% yield. After the reduction of amide **99** to amine **100** in a 95% yield, the protection of secondary amine **100** with benzyl bromide in the presence of K_2CO_3 gave a mixture of the starting material, monobenzylated and dibenzylated amine in 20 h at 40 °C. When DIPEA was used, an 80% yield for the benzylated amine **101** was achieved in 18 h at the same temperature. After the removal of the acetal group with a 90% yield, tetraol **102** was cyclized by using TsIm. Only 18% of the desired product **103** was isolated. (Scheme 38)
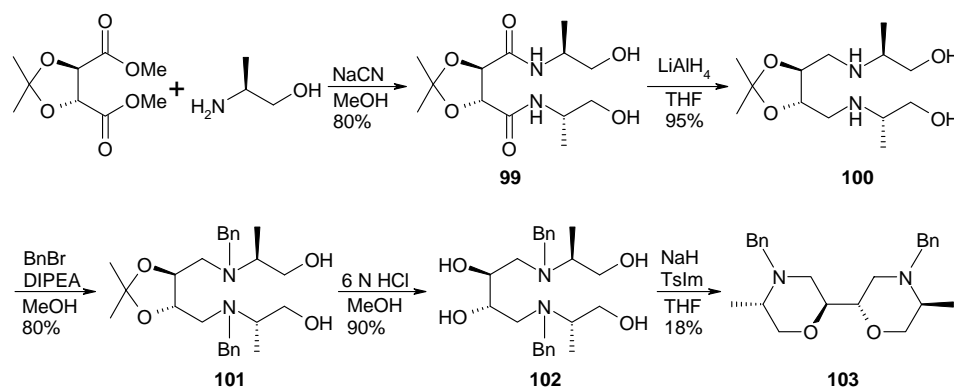
Scheme 38. The synthesis of bimorpholine **103**.

The reason for the low yield of the cyclization of tetraol **102** is explained in Article II. The proposed mechanism[41] of the formation of the aziridinium ion and its nonregioselective opening is shown in scheme 39. The possible products of the reaction are also demonstrated in the scheme 39. After deprotonation of the primary hydroxyl group with sodium hydride, intermediate **a** is tosylated by p-toluenesulfonyl imidazole, affording intermediate **b.** It forms an aziridinium ion **c**, which can be opened, affording **d**, which has the methyl-group in the β-position of the nitrogen atom. After the cyclization of compound **d**, bimorpholine **103a** can be obtained. If only one half of the molecule undergoes the aziridinium ion's unpreferred opening, bimorpholine **103b** could be obtained. We have not been able to isolate these proposed molecules from the reaction mixture, but their formation could explain the low yield of the reaction.
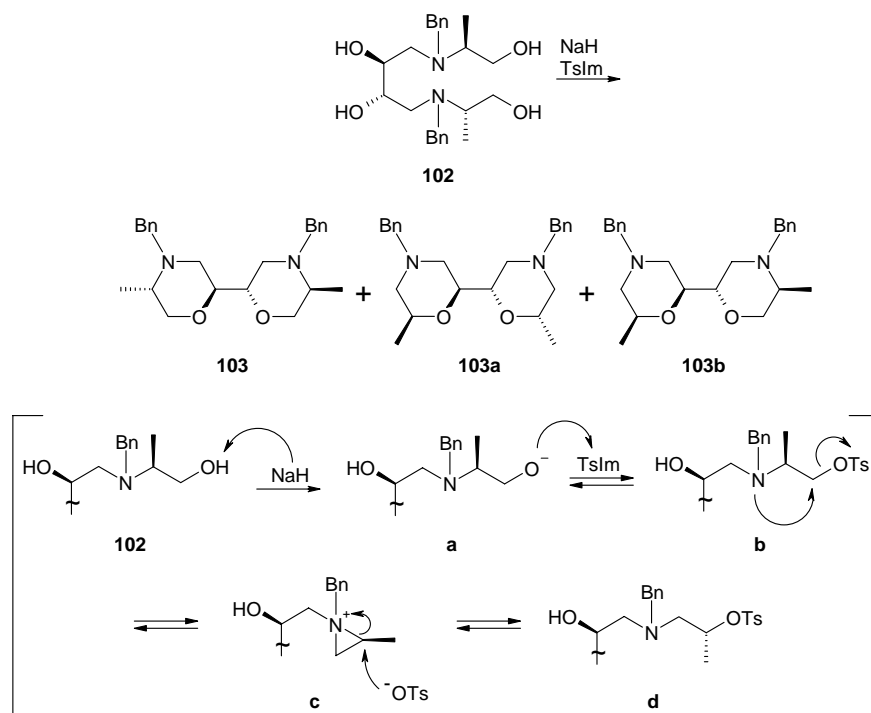
Scheme 39. Possible products of the cyclization of tetraol **102** and the proposed mechanism of the formation of the reaction side products **103a** and **103b**.

While using a tosyl-protecting group on the *N*-atom, the formation of the aziridinium ion is not favorable, because of the high destabilizing charge distribution (two positive charges at the neighboring atoms) in the mesomeric form. Therefore, the yield of the reaction should be much higher. For that, amine **100** was protected with tosylchloride in the presence of MgO with a 52% yield. The next steps were conducted as described earlier, by removing the acetal group with a 92% yield and the cyclization of the tetraol **105** with TsIm, which afforded bimorpholine **106** in a 71% yield. The tosyl-groups were removed with LiAlH$_4$ and the desired 5,5'-dimethyl-2,2'-bimorpholine **3** was synthesized in six steps, with an overall yield of 18% (Scheme 40).
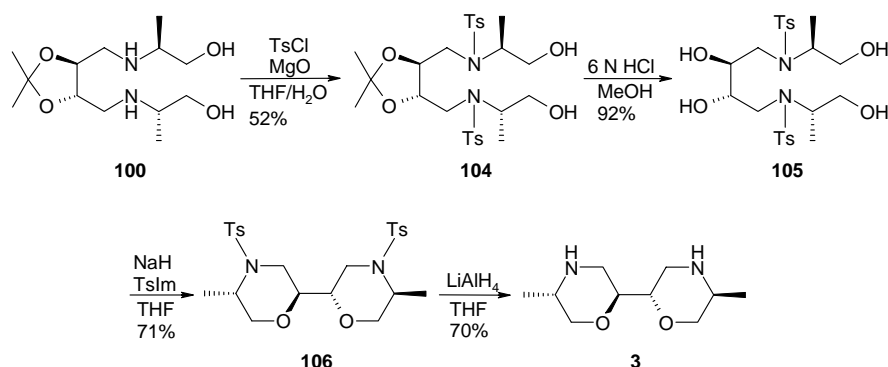
Scheme 40. The synthesis of 5,5'-dimethyl-2,2'-bimorpholine **3**.

3.2.2. Synthesis of (2*R*,2'*R*,5*S*,5'*S*)-5,5'-dimethyl-2,2'-bimorpholine

(2*R*,2'*R*,5*S*,5'*S*)-5,5'-dimethyl-2,2'-bimorpholine was prepared using the same route as its diastereoisomer, starting with (*S*,*S*)-tartaric acid ester acetal and (*S*)-alaninol, the only changes being in the reaction times. In this case, another deprotection method of tosylated bimorpholine was also tried, using sodium and naphthalene in THF, but the yield obtained in 1 h was only 36%, with no starting material detected on TLC. When LiAlH$_4$ was used as a deprotecting reagent, the yield of the reaction was 78%. The overall yield of bimorpholine **107** over 6 steps was 23% (Scheme 41).
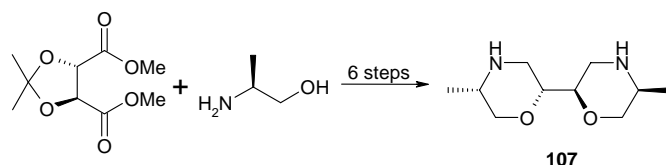
Scheme 41. The synthesis of 5,5'-dimethyl-2,2'-bimorpholine **107**.

3.2.3. Synthesis of (2S,2'S,5S,5'S)-5,5'-dibenzyl-2,2'-bimorpholine

The synthesis of (2*S*,2'*S*,5*S*,5'*S*)-5,5'-dibenzyl-2,2'-bimorpholine **4** started with (*R*,*R*)-tartaric acid ester acetal and (*S*)-phenylalaninol (Scheme 42). After two successful steps of amidation and reduction, the *N*-tosylation was conducted. The tosylation of amine **109** with tosyl chloride in the presence of MgO resulted in compound **110** in only a 35% yield, even after a four-day reaction.
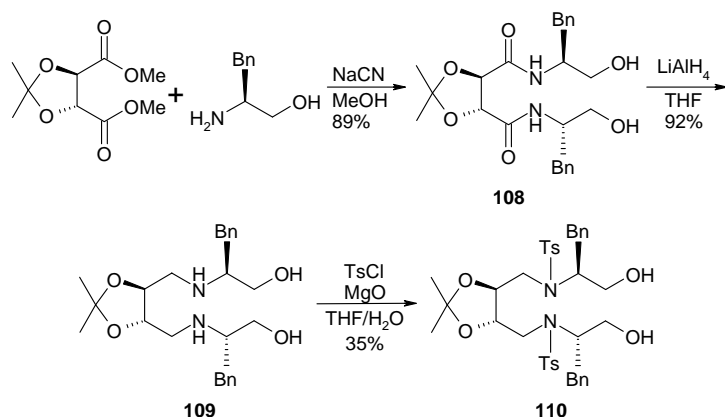
Scheme 42. The first part of the synthesis of 5,5'-dibenzyl-2,2'-bimorpholine **4**.

Since the tosylation of amine **109** gave such a low yield, *N*-benzylation of amine **109** was conducted. It was not known what the selectivity in the cyclization step would be in the case of having a benzyl-substitution at the α-position of the nitrogen atom. After the formation of tertiary amine **111** and deacetalization, the cyclization step afforded tetrabenzyl bimorpholine **113** in a 63% yield. Presumably, even though the aziridinium ion had formed in this step, its opening mainly afforded the desired compound, probably due to the steric effect of the benzyl group in the α-position of the nitrogen atom. Debenzylation of bimorpholine **113** afforded the desired 5,5'-dibenzyl-2,2'-bimorpholine **4** in six steps, with a 38% overall yield (Scheme 43).
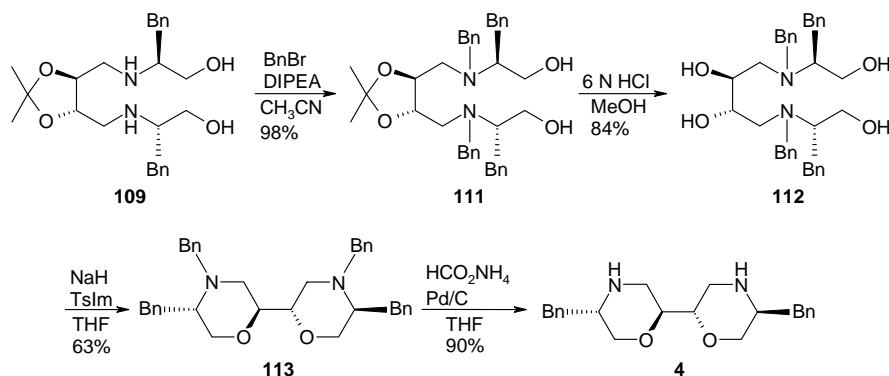
Scheme 43. The synthesis of 5,5'-dibenzyl-2,2'-bimorpholine **4**.

3.3. Synthesis of (2*S*,2'*S*)-5,5'-tetramethyl-2,2'-bimorpholine

To achieve disubstitution at the α-position of the nitrogen atom, 2-amino-2-methyl-1-propanol was used as the aminoalcohol in the cyanide-catalyzed amidation of (*R*,*R*)-tartaric acid ester acetal (Scheme 44). The reaction afforded amide **114** in an

82% yield. The reduction of amide **114** with LiAlH$_4$ in THF afforded amine **115** in only a 40% yield.
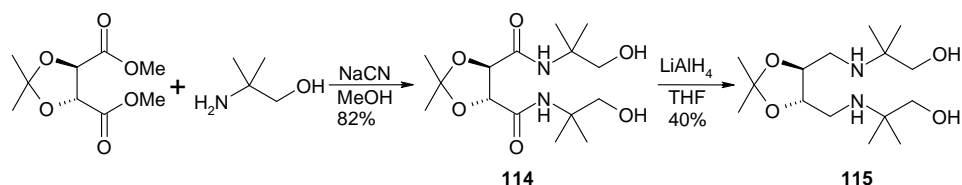
Scheme 44. The formation of amine **115**.

Since 5,5'-dibenzyl-2,2'-bimorpholine **4** was synthesized over the benzyl-protected amine, it was assumed that the benzyl-protected tetramethyl compound would also afford the desired product. Therefore, the protection of amine **115** with benzyl bromine in the presence of K$_2$CO$_3$ as a base was conducted, but its use did not lead to the completion of the reaction; in the case of CsOH, a multicomponent mixture was obtained. When DIPEA was used, protected amine **116** was obtained in a 92% yield (Scheme 45). The deprotection of secondary hydroxyl groups in amine **116** led to tetraol **117** in a high yield.
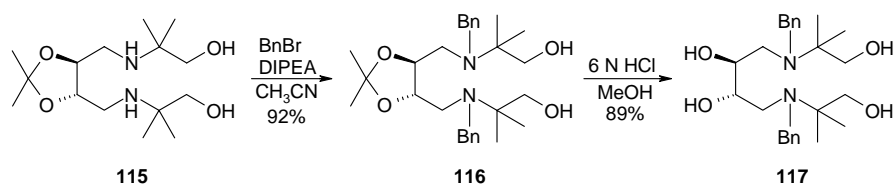
Scheme 45. The formation of tetraol **117**.

Unfortunately, after several attempts to cyclize tetraol **117** with TsIm, substituted bimorpholine was not obtained from the reaction mixture. Also, the attempts to mesylate tetraol **117** resulted in a multicomponent mixture. When tetraol **117** was heated with 6 N HCl or conc. H$_2$SO$_4$ at 60 °C, no reaction was detected: the TLC plate showed only the starting material.

In order to reduce the number of possible reaction products in the cyclization with TsIm, the amine protective group was to be changed. As the methyl-substituted bimorpholine synthesis showed, it was necessary to prepare the tosyl-protected tetraol for the cyclization stage. A direct tosylation of amine **115** with tosyl chloride and MgO resulted in a multicomponent mixture. The reaction did not run as expected because of the steric hindrance in the α-position of the nitrogen atom. Since there are also two primary hydroxyl groups in the molecule, it could be assumed that *O*-tosylation also occurred. Since there was only one equivalent of tosyl chloride for each nitrogen atom in the reaction mixture, both *N*- and *O*-tosylation could not be completed, so a mixture of products was obtained.

For the selective tosylation of nitrogen atoms in amine **115**, the primary hydroxyl groups had to be protected as silyl ethers in order to exclude *O*-tosylation (Scheme 46). The silylation of compound **115** proceeded with a high yield and, after that, tosylation of amine **118** was successfully completed with an 87% yield.
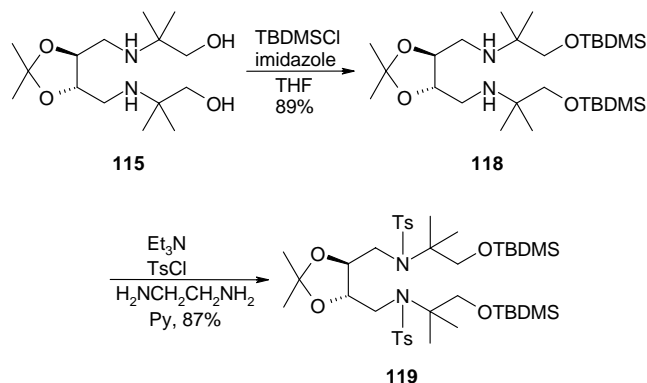
Scheme 46. The synthesis of compound **119**.

The deacetalization of compound **119** with 6 N HCl in methanol did not give the desired tetraol **122**. Instead, a cleavage of the N-C bond occurred (Scheme 47), and the product **120** was obtained in an 89% yield. The formation of the undesired product **120** was caused by the elimination of the stable tertiary carbocations (it is well known that silicon stabilizes a positive charge at the β-carbon[82]), which were formed after the protonation of nitrogen atoms.
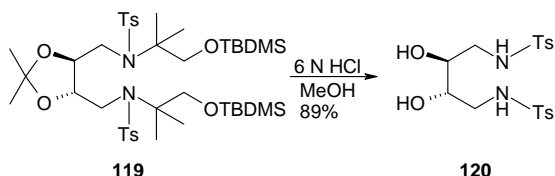
Scheme 47. The formation of undesired product **120**.

To exclude the undesired elimination of the stable tertiary carbocations during the deacetalization step, the silyl groups in compound **119** had to be removed first (Scheme 48). The reaction was carried out with tetrabutylammonium fluoride in an 83% yield. After that, deacetalization of compound **121** with 6 N HCl in methanol resulted in the formation of tetraol **122** in an 85% yield. Unfortunately, tetraol **122** was not received as a pure compound, traces of tosyl amide **120** were also detected.
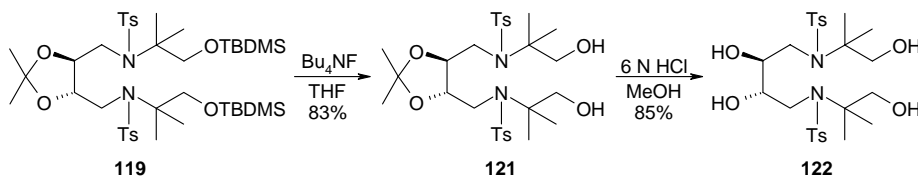
Scheme 48. The synthesis of tetraol **122**.

The cyclization of tetraol **122** with TsIm resulted in a mixture of several products. After a five-day reaction, at least four reaction products were detected on TLC, one of which was proven by NMR to be epoxide **123** (Scheme 49). The formation of tetrasubstituted bimorpholine could not be completed under the same conditions as the other bimorpholines **1**, **3**, **4** and **107**.
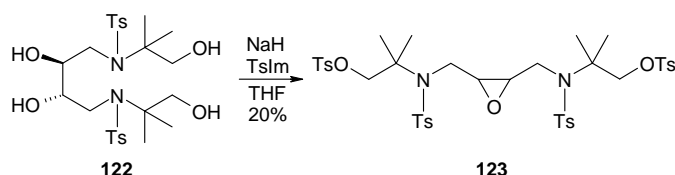
Scheme 49. The cyclization of tetraol **122**.

3.4. Catalytic properties of (2*S*,2'*S*)-bimorpholine and its derivatives in asymmetric reactions (Articles I, III and IV)

The broad utility of synthetic chiral molecules as single-enantiomer pharmaceuticals, in electronic and optical devices, as components in polymers with novel properties and as probes of biological function has made asymmetric catalysis a prominent area of investigation.

Chiral amines are extensively used as catalysts in asymmetric reactions.[83,84,85,86,87,88,89,90] They are widely studied by many groups as chiral auxiliaries, chiral reagents, or chiral external ligands. Both organo- and metal-catalytic reactions take advantage of the properties that these molecules possess. Diamines have shown, since their first use, much success in many important and useful transformations.

The catalysis by primary and secondary amines of electrophilic substitution reactions in the α-position of carbonyl compounds and related reactions via enamine intermediates is enamine catalysis. An enamine is generated by reacting a carbonyl compound with an amine under dehydration conditions. Reaction of the enamine can proceed via an addition or substitution route, depending on the nature of the electrophile. In either case, iminium ions are usually formed, which are then hydrolyzed to afford the products.
The most versatile and most often used method of formation of enamines involves the condensation between an aldehyde or ketone and a secondary amine. Primary amines form imines with carbonyl compounds. The rate of enamine formation depends on several factors: the basicity of the amine, the degree of steric hindrance in either the amine or the carbonyl compound, the rate of loss of the hydroxyl group, and the rate of loss of a proton. The enamine, once formed, may be isolated or used *in situ* for subsequent reactions.

Bimorpholines have several properties that are useful for their application in asymmetric reactions as catalysts or ligands. They have good chelating ability due to the heteroatoms in the molecule. Bimorpholines are C_2-symmetric, which makes them highly useful auxiliaries. The combination of C_2-symmetry and the position of heteroatoms in the chiral ligand can lead to conformational restriction in the chelation with metal, which increases the stereodifferentiation for various metal mediated reactions.

We explored the catalytic properties that our synthesized bimorpholines might possess. Various organo- and metal-catalytic reactions were examined. The results of this research are included in the following sections.

3.4.1. PTC alkylation of glycine imine ester (Article I)

Asymmetric phase-transfer catalyzed (PTC) alkylation of glycine imines is a highly effective method for the enantioselective synthesis of amino acids.[91,92,93,94,95] The most commonly used catalysts in this reaction are *Cinchona* alkaloids and spiro ammonium salts, which are sterically very bulky. We have designed two new dicationic catalysts, where nitrogen atoms are fixed into a rigid cyclic two-centred diammonium structure.

First, quaternary ammonium salts were prepared from *N,N'*-dibenzyl (2*S*,2'*S*)-bimorpholine **98**. *N*-alkylation with methyl iodide resulted in compound **124** in a quantitative yield, and *N*-alkylation with benzyl bromide afforded compound **125** in a 60% yield (Scheme 50).
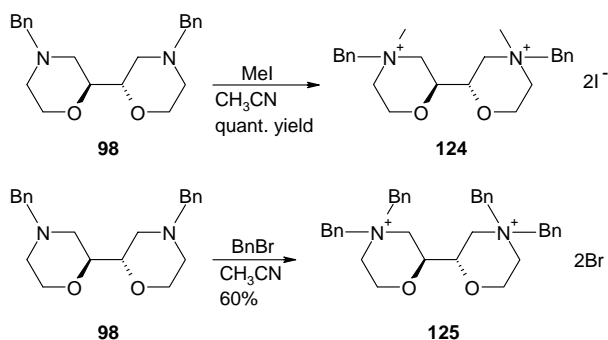
Scheme 50. Formation of quaternary ammonium salts **124** and **125**.

The PTC alkylation of glycine imine ester **126** in the presence of catalyst **124** gave a racemic product **127** and, in the presence of catalyst **125**, the product **127** was obtained with only 18% *ee* (Scheme 51).
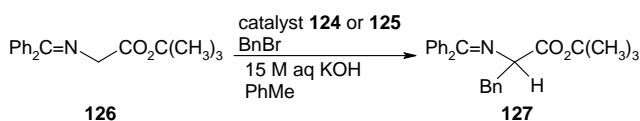
Scheme 51. PTC alkylation of glycine imine ester **126**.

3.4.2. Aldol reaction (Article III)

The aldol reaction is one of the most honoured reactions in organic chemistry.[96,97] This useful transformation allows the formation of a C–C bond by reaction of an enolizable carbonyl compound acting as a nucleophile, with itself or another carbonyl compound acting as an electrophile to give a β-hydroxycarbonyl compound known as aldol. When the aldol product undergoes a subsequent dehydration step to give the related α,β-unsaturated carbonyl compound, the process is called aldol condensation. The reaction can be catalyzed by either basic or acidic compounds. Besides the new C–C bond formed, one or more stereogenic centres can also be created.

Bimorpholines are cyclic secondary amines that have additional donor sites in their rings (O-atoms). Our bimorpholines are 1,4-diamines that have C_2-symmetric skeleton and four donor sites. It is known that structurally very similar 3,3'-bimorpholines are efficient catalysts for intramolecular, as well as intermolecular, aldol reactions.[25,26,27] It was shown with 3,3'-bimorpholines that trifluoroacetic acid salts were the most reactive. Therefore we decided to use TFA as well. Acid is needed for the acceleration of the condensation and also for the formation of the fixed conformation of the catalyst via hydrogen bonding, thus providing higher stereoselectivity.

We tested our bimorpholines in an intramolecular aldol condensation, specifically in the cyclization of triketone **128** in the presence of TFA. Unfortunately, while bimorpholine **1** was quite reactive, it afforded a racemic product **129**, and bimorpholine **3** turned out to be completely inactive in this reaction (Table 1).

Table 1. Cyclization of triketone **128**.
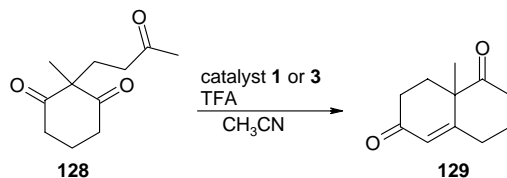
Catalyst	Reaction conditions	Yield, %	*ee*, %
1	80 °C, 42 h	99	0
3	80 °C, 24 h	-	-

A monosalt of 3,3'-binorpholine **2** gives strictly fixed conformation via hydrogen bonding.[23] In the case of 2,2'-bimorpholines in which the *N*-atoms are at the 1,4-position, such H-bonding is not favourable. Thus, the aminocatalyst does not have a preferable conformation, and the flexibility of two rings leads to a nonselective reaction.

3.4.3. Aziridination and epoxidation of enones

Aziridines are the nitrogen analogues of epoxides and exhibit similar reactivity patterns as electrophilic reagents. They undergo highly regio- and stereoselective transformations and, therefore, are useful building blocks for organic synthesis. In addition, aziridines may exhibit antitumor or antibiotic activity or other biological properties, which makes them attractive synthetic targets.

We chose the aziridination of *trans*-chalcone **130** as a model reaction,[98] and tested two of our bimorpholines for the determination of their catalytic properties (Scheme 52). Since secondary amines do not promote aziridination, we chose two of our tertiary bimorpholines for this reaction. With catalyst **98**, aziridine **131** was obtained in an 86% yield in three hours, but the enantioselectivity was only 7%. In the case of catalyst **103**, aziridine **131** enantioselectivity rose to 35%, but the reactivity dropped, and only 11% of the yield was obtained in three hours.
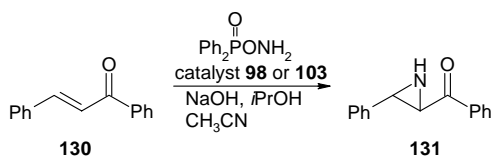
Scheme 52. Amine-promoted aziridination of chalcone **130**.

A proposed catalytic[99] cycle for the aziridination of *trans*-chalcone **130** is shown in scheme 53. The tertiary amine reacts with *O*-(diphenylphosphinyl)hydroxylamine to form a hydrazinium salt, which then reacts with NaOH to give the N-N nitrogen ylide. The ylide then undergoes conjugate addition to the chalcone **130**, followed by cyclization, to form the aziridine **131** and regenerate the tertiary amine.
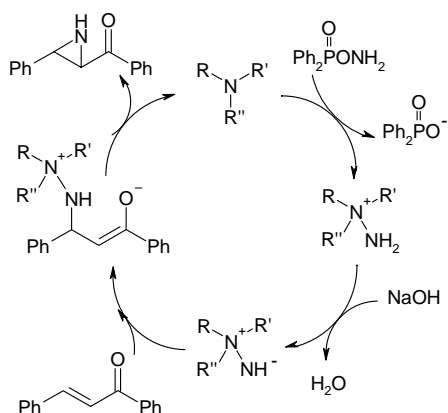
Scheme 53. A proposed catalytic cycle for the aziridination of *trans*-chalcone **130**.

Asymmetric epoxidation of α,β-enones is very important, since optically active epoxy ketones are among the most versatile building blocks for access to several natural products and pharmaceuticals.[100,101]

The epoxidation of *trans*-chalcone **130** with *t*-BuOOH, in the presence of our catalyst **3**, gave, after a three-day reaction time, only traces of the product **132**, which was racemic (Scheme 54). This outcome was anticipated, since the epoxidation of chalcones is usually catalyzed by amino alcohols, where the hydroxyl group (which in our case was absent) activates and orientates chalcone through hydrogen bonding.
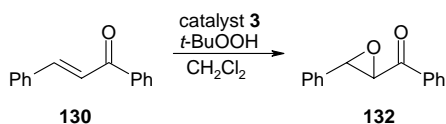
Scheme 54. Amine-promoted epoxidation of chalcone **130**.

3.4.4. Michael addition

C-C bond formations by conjugate addition of nucleophiles to the β-position of α,β-unsaturated carbonyl compounds (Michael reaction) are frequently used in organic synthesis.

Michael addition of nucleophiles formed from aldehydes to nitroolefins is one of the reactions that is catalyzed by simple chiral amines. 3,3'-bimorpholine derivatives catalyze Michael addition of enamine intermediates formed from aldehydes to nitroolefins, with dr up to 95:5 and *ee* up to 90%.[29]

We tested our catalyst **3** in the asymmetric conjugate addition of pentanal **133** to β-nitrostyrene **134**. After a 24 h reaction time, the reaction product **135** was obtained in a 97% yield, but with poor selectivity (dr 85:15 (syn:anti), *ee* 35% for both isomers) (Scheme 55).
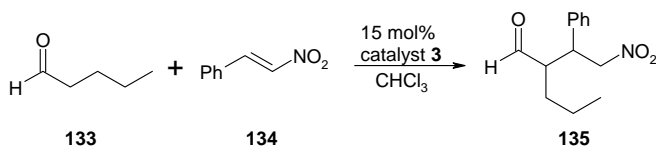
Scheme 55. Conjugate addition of pentanal **133** to β-nitrostyrene **134**.

We also tried the addition of ketones (pentane-3-one and cyclohexanone) to β-nitrostyrene **134**, but after eight days, only the starting materials were detected.

3.4.5. Henry reaction (Article IV)

Among the various C–C bond-forming reactions, the nitroaldol or Henry reaction is one of the classical named reactions in organic synthesis. Essentially, the coupling of the nucleophile generated from a nitroalkane with a carbonyl electrophile is a widely used transformation, since its discovery in 1895. The resulting product of this reaction is a β-nitro alcohol, which is a versatile intermediate in synthetic organic chemistry.[102,103,104] Both approaches – metal- and organocatalysis – have been successfully applied in nitroaldol reactions.

Chiral complexes of copper have found wide application in the general context of catalytic asymmetric transformations. The first application of these types of organometallics to the asymmetric nitroaldol reaction was reported by Jørgensen.[105]
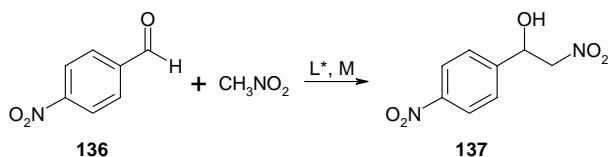
Scheme 56. Henry reaction of *p*-nitrobenzaldehyde **136** with nitromethane.

We investigated Henry reaction catalyzed by bimorpholine-metal salt catalysts. The reaction of *p*-nitrobenzaldehyde **136** with nitromethane was catalyzed by our different complexes (Scheme 56). We envisioned that the bimorpholine ligands would have sufficient basicity and co-ordination ability to influence the catalysis of product **137**. While bimorpholines **3**, **4**, **103** and **106** proved to be quite reactive under certain reaction conditions, they showed no selectivity (Table 2).

Table 2. The bimorpholines catalytic properties in the Henry reaction.

Entry	L*	M	Time, h	Yield, %	*ee*, %
1	**106**	$Cu(OAc)_2*H_2O$	20	18	0
2	**106**	$Cu(OTf)_2$		nr	-
3	**106**	$(CuOTf)_2*C_6H_6$	22h	77	0
4	**103**	$Cu(OAc)_2*H_2O$	18h	46	0
5	**3**	$Cu(OAc)_2*H_2O$	18h	93	0
6	**3**	$(CuOTf)_2*C_6H_6$	18h	70	0
7	**3**	$Pd(OAc)_2$	22	6	0
8	**4**	$Cu(OAc)_2*H_2O$	21	61	0

Reaction conditions in MeOH: 5 mol% M, 5 mol% L*, RT, 10 eq CH_3CN. In the case of $(CuOTf)_2*C_6H_6$, MS were added to the reaction mixture.

Since bimorpholine **113** was insoluble in methanol, we used it for the screening of other solvents. The addition of nitromethane to *p*-nitrobenzaldehyde **136** in the presence of 5 mol% of ligand and 5 mol% of $Cu(OAc)_2*H_2O$ was again used as a model reaction. The results are presented in table 3. As seen in table 3, there was virtually no solvent effect on the selectivity of the reaction while using bimorpholine **113** as a catalyst.

Table 3. Solvent effect on the selectivity of the reaction in the presence of bimorpholine **113**.

Entry	L*	Solvent	Time, h	Yield, %	*ee*, %
1	**113**	CH_2Cl_2	93	23	0
2	**113**	CH_3CN	116	50	0
3	**113**	Toluene	139	5	5 (R)
4	**113**	$CH_3CN:H_2O$ 1:1	116	88	0
5	**113**	THF	90	22	0
6	**113**	THF:MeOH 1:1	90	41	11 (R)
7	**113**	DMF	90	50	1 (S)
8	**113**	Et_2O	114	traces	0

Reaction conditions: 5 mol% $Cu(OAc)_2*H_2O$, 5 mol% bimorpholine **113**, RT, 10 eq CH_3CN.

3.5. Biological activity of (2*S*,2'*S*)-bimorpholine derivatives

The biological activity of bimorpholines was determined at the University of Tartu Institute of Technology. The work was performed in collaboration with Baltic Technology Development Ltd. Bimorpholines **1**, **3**, **4**, and **107** were tested for HIV and HCV models. Compounds **A** and **B** were used as the control substances (**A** – negative control, **B** – positive control). All biological experiments are described in the appendix.

First, the cytotoxicity of DMSO for cells used in subsequent assays was examined. Then, the MTT cytotoxicity assay was performed to determine the cytotoxicity of bimorpholines **1**, **3**, **4**, and **107** to HeLa cells. It was found that none of the substances was toxic at any of the used concentrations.

Next, the ability of bimorpholines to suppress HIV reverse transcription was tested using HIV-1-based viral-like particles (VLPs) ("ViraPower Lentiviral Expression Systems", Invitrogen). The normalized values (normalized to the amount of VLPs) of the repeated experiments are shown on graph 1.

Graph 1. Average activity values of bimorpholines on HIV-1-based VLPs.
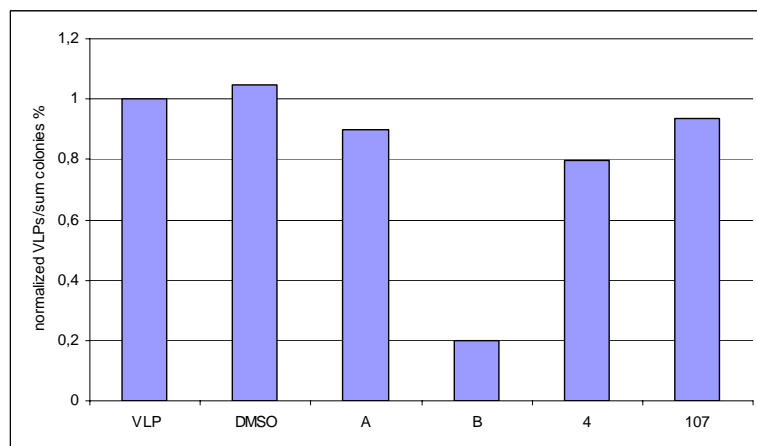
The results are shown as a ratio of compound-treated cell colony number to infected and non-treated cell colony number (the sum of three experiments). The number of colonies in infected and non-treated cells is taken as 1. This data confirms that the compound **4** possesses moderate anti-HIV activity. However, the possible mechanism(s) of its action remain unknown.

Then, the preliminary tests on hepatitis C virus (HCV) were conducted with all of the substances. The main result of this assay was finding that bimorpholine **4**

50

potently inhibited HCV replication, while other substances were proven to be inactive. The results are shown in graph 2 in logarithmic scale.

Graph 2. The preliminary tests on HCV-replicon cell line, 56 h incubation.
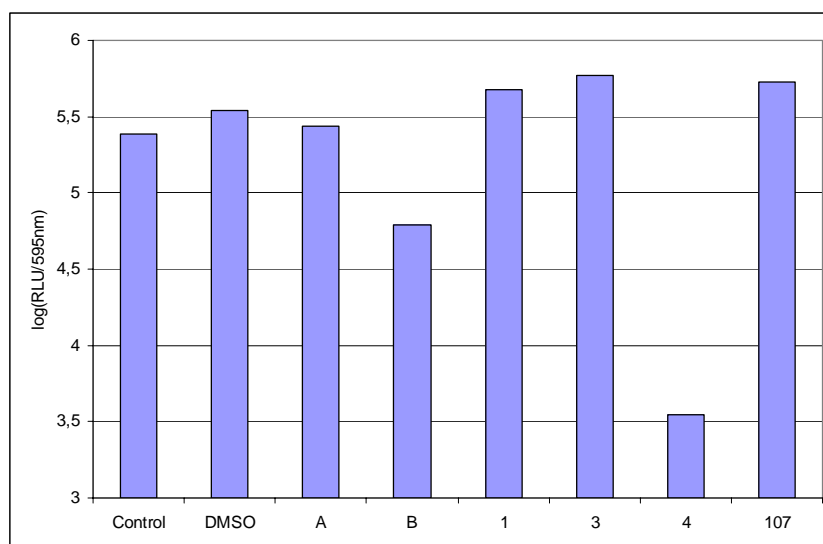
The results are shown as luciferase activity normalyzed to total protein concentration (RLU/OD_{595}).

Since compound **4** possessed unexpected effect on the HCV cell line (Huh7-Luc-neo/ET), its effect on these and other cells was analyzed in greater detail. The normalized values (normalized to the amount of cells) are presented in graph 3. Compound **4** was not toxic for cells not carrying HCV replicon (coherent with the finding that the compound is not cytotoxic for HeLa cells). Also, it can be concluded that it is toxic only for dividing cells harbouring HCV replicon RNA.

Graph 3. Normalized toxicity values for bimorpholine **4**.
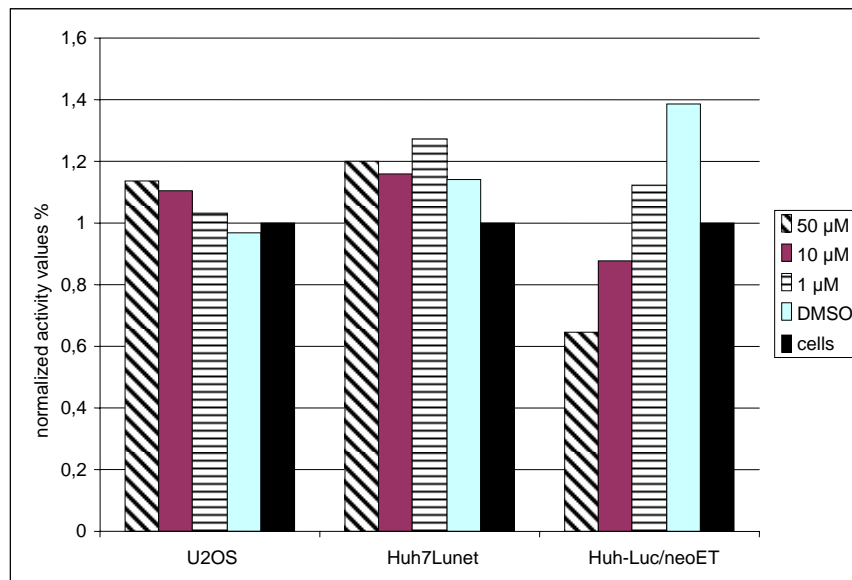
The results are shown as compound-treated cells OD_{595} to non-treated cells OD_{595}. OD_{595} of non-treated cells is taken as 1.

Conclusions

In the course of the present study, we worked out an efficient and general strategy for the asymmetric synthesis of the skeleton 2,2'-bimorpholine and screened the catalytic properties of the obtained molecules in both organo- and metal-catalysis.

The main results of the synthesis of bimorpholines were:
- (2*S*,2'*S*)-bimorpholine **1** was synthesized in six steps, according to the new route, which improved the overall yield of the product from 15% to 35%.
- The synthetic scheme is general, as the same synthetic route was applied to the synthesis of 5,5'-substituted bimorpholine derivatives, with slight changes due to the steric differences in the molecules.
- The main scheme included amidation of tartaric acid ester with amino alcohol, reduction of amide, introduction of an *N*-protective group, deprotection of secondary diol, cyclization of tetraol and, finally, deprotection of nitrogen atoms in the morpholine rings.
- The cyclization of tetraols to bimorpholines was efficiently and selectively performed in a one-step procedure with *N*-(*p*-toluenesulfonyl)imidazole (TsIm). One-step cyclization with TsIm is an efficient and selective reaction to produce bimorpholines. Only the tetra-substituted bimorpholine was not cyclized by this method.
- The reactions involved in the scheme were stereoselective. All of the obtained bimorpholines were prepared in high enantiomeric purity (>99%).

The main results of the screening of catalytic properties were:
- The synthesized bimorpholines showed high reactivity in some reactions, but poor or no stereoselectivity in all of them.
- The enantiomeric purity of the product received from the bimorpholine derivative induced reaction did not exceed 35%.

The main results concerning the investigations of the biological activity of bimorpholine and its derivatives were:
- MTT cytotoxicity assay was performed in order to determine the cytotoxicity of bimorpholines **1**, **3**, **4**, and **107** to HeLa cells. It was shown that none of the substances were toxic to the HeLa cells at any of the used concentrations.
- Bimorpholines **4** and **107** showed some activity towards HIV-1-based VLPs.
- (2*S*,2'*S*,5*S*,5'*S*)-5,5'-dibenzyl-2,2'-bimorpholine **4** was found to be highly cytotoxic for the stable HCV replicon when the concentration of the bimorpholine was 100 μM.

Experimental

Chemicals were purchased from Aldrich Chemical Co and were used as received. All solvents were distilled prior to use. All reactions sensitive to moisture or oxygen were carried out under an argon atmosphere in oven-dried glassware. Precoated silica gel 60 F_{254} plates from Merck were used for TLC, whereas for column chromatography silica gel KSK40-100 μm was used. Full assignment of ^{1}H and ^{13}C chemical shifts is based on the 1D and 2D FT NMR spectra on Bruker AMX500, Avance[III] 400 and Avance[III] 800 instruments. Solvent peaks (CHCl$_3$ δ=7.27, CHD$_2$OD δ=3.30, CDCl$_3$ δ=77.00, and CD$_3$OD δ=49.00) were used as chemical shift references. IR spectra were measured on a Perkin-Elmer Spectrum BX FTIR spectrometer. Mass spectra were recorded on a Shimadzu GCMS-QP2010 spectrometer, using EI (70eV). High resolution mass spectra were recorded on a Hitachi M80B spectrometer, using EI (70eV), or on an LTQ Orbitrap (Thermo Electron). Elemental analyses were performed on a Perkin-Elmer C, H, N, S–Analyzer 2400. Optical rotations were obtained using a Krüss Optronic GmbH Polarimeter P 3002.

The formation of bimorpholine 107
To a solution of ditozyl bimorpholine (300 mg, 0.59 mmol) in THF (10 mL) a solution of naphthalene (230 mg, 1.80 mmol) and sodium (41 mg, 1.80 mmol) in THF (5 mL) was added at -78 °C under an argon atmosphere. The mixture was stirred for 45 min. The reaction was quenched by dropwise addition of water (3 mL), after warming to rt, the mixture was extracted with EtOAc (4 × 10 mL). The organic phase was dried (Na$_2$SO$_4$) and solvent was evaporated. The residue was purified by column chromatography on silica gel (10% NH$_3$/MeOH in CH$_2$Cl$_2$), affording bimorpholine **107** (yield 42 mg, 36%).

The synthesis of tosyl amide 110
To a solution of amine **109** (118 mg, 0.28 mmol) in THF (2.8 mL) and H$_2$O (0.7 mL) MgO (56 mg, 1.38 mmol) was added and the mixture was stirred at RT for 30 min. TsCl (105 g, 0.55 mmol) was added and the mixture was stirred for 4 d. The mixture was filtered through celite and the filtrate washed with EtOAc, after the evaporation of solvents, the precipitate was dissolved in CH$_2$Cl$_2$ (5 mL), water (7 mL) was added and the mixture was extracted with CH$_2$Cl$_2$ (3 × 5 mL). The organic phase was dried (Na$_2$SO$_4$) and solvent was evaporated. The residue was purified by column chromatography on silica gel (30% EtOAc in petroleum ether), affording tosylamide **110** (yield 70 mg, 35%).

^{1}H NMR (400 MHz, CDCl$_3$) δ: 7.84 (d, J = 8.2 Hz, 4H, o-Ar), 7.34 (d, J = 8.1 Hz, 4H, m-Ar), 7.21 (m, 6H, o-Bn and p-Bn), 7.02 (d, J = 6.9 Hz, 4H, m-Bn), 4.28 (br s, 2H, CHO), 3.98 – 3.87 (m, 4H, CHN and CH$_2$N), 3.76 (d, J = 16.1 Hz, 2H, CH$_2$N), 3.63 (br s, 4H, C**H**$_2$OH), 3.09 (br s, 2H, OH), 2.93 (dd, J = 12.6, 11.1 Hz, 2H, Bn CH$_2$), 2.44 (s, 6H, Ar CH$_3$), 2.41 (m, 2H, Bn CH$_2$), 1.50 (s, 6H, C(CH$_3$)$_2$).
^{13}C NMR (100 MHz, CDCl$_3$) δ: 143.86 (p-Ar), 137.80 (i-Bn), 136.94 (i- Ar), 129.88 (m-Ar), 129.13 (m-Bn), 128.50 (o-Bn), 127.60 (o-Ar), 126.50 (p-Bn),

108.81 ($\mathbf{C}$(CH$_3$)$_2$), 78.26 (CHO), 62.29 (CH$_2$OH), 62.18 (CHN), 44.55 (CH$_2$N), 34.84 (Bn CH$_2$), 26.84 (C($\mathbf{C}$H$_3$)$_2$), 21.55 (Ar CH$_3$).

The synthesis of amide 114

2-Amino-2-methyl-1-propanol (10.06 mL, 105.38 mmol) and sodium cyanide (129 mg, 2.63 mmol) were added to a solution of (*R,R*)-tartaric acid ester acetal (5.749 g, 26.35 mmol) in MeOH (55 mL) at rt. The reaction mixture was refluxed for 24 h. MeOH was evaporated and the crude mixture was purified by column chromatography on silica gel (10% MeOH in CH$_2$Cl$_2$), affording amide **114** as white crystals (yield 7.139 g, 82%, mp 88-89 °C).

^{1}H NMR (400 MHz, CDCl$_3$) *δ*: 4.50 (s, 2H, CH), 3.62 (d, *J* = 2.0 Hz, 4H, CH$_2$), 1.50 (s, 6H, C(CH$_3$)$_2$), 1.33 (s, 12H, NHC($\mathbf{C}$H$_3$)$_2$).

^{13}C NMR (100 MHz, CDCl$_3$) *δ*: 170.05 (C=O), 112.59 ($\mathbf{C}$(CH$_3$)$_2$), 77.49 (CHO), 69.76 (CH$_2$OH), 56.26 (NH$\mathbf{C}$(CH$_3$)$_2$), 26.01 (C($\mathbf{C}$H$_3$)$_2$), 24.45 (NHC($\mathbf{C}$H$_3$)$_2$), 24.44 (NHC($\mathbf{C}$H$_3$)$_2$).

IR: ν = 3390, 2979, 2937, 1667, 1530, 1458, 1386, 1215, 1164, 1063, 666 cm^{-1}.

[α]$_D^{22}$= -14.1 (c 3.03, MeOH). MS m/z: 333 (M+1), 301, 283, 158, 128, 101, 86, 73, 58. Anal. Calcd for C$_{15}$H$_{28}$N$_2$O$_6$ (332.40): C 54.20, H 8.49, N 8.43; found: C 54.14, H 8.60, N 8.35.

The synthesis of amine 115

Amide **114** (9.630 g, 0.029 mol) in THF (90 mL) was added to a suspension of LiAlH$_4$ (8.796 g, 0.23 mol) in THF (200 mL) at 0 °C. After refluxing for 15 h, water (8.8 mL), 15% NaOH solution (8.8 mL) and water (26.4 mL) were added at 0 °C. The mixture was filtered and washed with EtOAc. The filtrate was dried over Na$_2$SO$_4$. Solvents were evaporated and the residue was purified by column chromatography on silica gel (5-15% MeOH in CH$_2$Cl$_2$), affording amine **115** as white crystals (yield 3.565 g, 40%, mp 52-53 °C).

^{1}H NMR (400 MHz, CDCl$_3$) *δ*: 3.89 – 3.80 (m, 2H, CHO), 3.36 – 3.25 (m, 4H, $\mathbf{C}$H$_2$OH), 2.78 – 2.64 (m, 4H, $\mathbf{C}$H$_2$NH), 1.40 (s, 6H, C(CH$_3$)$_2$), 1.07 (s, 6H, CH$_3$), 1.06 (s, 6H, CH$_3$).

^{13}C NMR (100 MHz, CDCl$_3$) *δ*: 108.69 ($\mathbf{C}$(CH$_3$)$_2$), 79.66 (CHO), 68.18 (CH$_2$OH), 53.54 (CNH), 44.22 (CH$_2$NH), 27.13 (C($\mathbf{C}$H$_3$)$_2$), 24.11 (CH$_3$), 23.86 (CH$_3$).

IR: ν = 3468, 3289, 3227, 2969, 2874, 1480, 1381, 1369, 1249, 1070 cm^{-1}. MS m/z: 305 (M+1), 273, 215, 144, 126, 114, 102, 86, 72, 58. Anal. Calcd for C$_{15}$H$_{32}$N$_2$O$_4$ (304.43): C 59.18, H 10.60, N 9.20; found: C 58.80, H 10.69, N 9.14.

The synthesis of dibenzyl amine 116

Amine **115** (1.290 g, 4.24 mmol) was dissolved in CH$_3$CN (43 mL), DIPEA (2.21 mL, 12.71 mmol) and benzyl bromide (3.02 mL, 25.42 mmol) were added. The reaction mixture was stirred at 50 °C for 5 h and at rt for 16 h. CH$_3$CN was evaporated, water (50 mL) was added and the mixture was extracted with EtOAc (2 × 20 mL). The organic phase was dried (Na$_2$SO$_4$) and solvent was evaporated. The

residue was purified by column chromatography on silica gel (6% NH_3/MeOH in CH_2Cl_2, affording benzylated amine **116** as yellow oil (yield 1.889 g, 92%).

^{1}H NMR (500 MHz, CDCl$_3$) δ: 7.36 (d, J = 7.3 Hz, 4H, o-Bn), 7.29 (dd, J = 13.8, 6.1 Hz, 4H, m-Bn), 7.22 (t, J = 7.2 Hz, 2H, p-Bn), 4.06 (d, J = 14.9 Hz, 2H, Bn C**H**$_2$), 3.64 (s, 2H, OH), 3.51 (d, J = 11.6 Hz, 2H, C**H**$_2$OH), 3.36 (d, J = 14.9 Hz, 2H, Bn C**H**$_2$), 3.11 (m, 4H, C**H**$_2$OH, C**H**O), 2.57 (dd, J = 14.6, 8.8 Hz, 2H, C**H**$_2$N), 2.12 (d, J = 14.4 Hz, 2H, C**H**$_2$N), 1.28 (s, 6H, C(C**H**$_3$)$_2$), 1.12 (s, 6H, NC(C**H**$_3$)$_2$), 0.91 (s, 6H, NC(C**H**$_3$)$_2$).

^{13}C NMR (125 MHz, CDCl$_3$) δ: 142.09 (i-Bn), 128.19 (m-Bn), 128.07 (o-Bn), 126.68 (p-Bn), 108.83 (**C**(CH$_3$)$_2$), 78.58 (**C**HO), 69.01 (**C**H$_2$OH), 58.74 (NC), 54.50 (Bn **C**H$_2$), 52.61 (**C**H$_2$N), 26.68 (C(**C**H$_3$)$_2$), 24.78 (NC(**C**H$_3$)$_2$), 18.23 (NC(**C**H$_3$)$_2$).

IR: ν = 3478, 2976, 1602, 1494, 1454, 1381, 1248, 1161, 1061, 945, 755, 721, 699, 515 cm^{-1}. $[\alpha]_D^{23}$= -35.8 (c 2.24, MeOH). MS m/z: 485 (M+1), 453, 363, 192, 162, 120, 91. Anal. Calcd for C$_{29}$H$_{44}$N$_2$O$_4$ (484.69): C 71.87, H 9.15, N 5.78; found: C 71.53, H 9.27, N 5.72.

The synthesis of tetraol 117

To a solution of N-benzyl amine **116** (90 mg, 0.19 mmol) in MeOH (3 mL), 6 N HCl solution (2 mL) was added and the mixture was heated at 40 °C for 29 h. 10N NaOH solution was added in an ice-water bath until pH~8-9. After the addition of brine (5 mL), MeOH was evaporated, and the mixture was extracted with CH_2Cl_2 (4 × 10 mL). The organic phase was dried (Na$_2$SO$_4$) and solvent was evaporated. The residue was purified by column chromatography on silica gel (5% MeOH/NH$_3$ in CH_2Cl_2), affording tetraol **117** as white crystals (yield 74 mg, 89%).

^{1}H NMR (400 MHz, CDCl$_3$) δ: 7.33 – 7.19 (m, 10H, Bn), 3.86 (d, J = 15.2 Hz, 2H, Bn C**H**$_2$), 3.49 (m, 4H, C**H**$_2$OH, Bn C**H**$_2$), 3.29 (d, J = 11.5 Hz, 2H, C**H**$_2$OH), 3.18 (br s, 4H, OH), 2.96 (dd, J = 8.5, 3.9 Hz, 2H, CH), 2.74 (dd, J = 13.9, 9.2 Hz, 2H, C**H**$_2$N), 2.28 (dd, J = 13.9, 4.1 Hz, 2H, C**H**$_2$N), 1.05 (s, 6H, CH$_3$), 0.98 (s, 6H, CH$_3$).

^{13}C NMR (100 MHz, CDCl$_3$) δ: 142.26 (i-Bn), 128.43 (m-Bn), 127.80 (o-Bn), 126.78 (p-Bn), 69.81 (**C**HO), 69.02 (**C**H$_2$OH), 58.91 (C), 55.23 (Bn **C**H$_2$), 53.98 (**C**H$_2$N), 23.19 (CH$_3$), 20.22 (CH$_3$).

IR: ν = 3370, 3061, 3026, 2970, 1602, 1494, 1363, 1168, 1123, 1050, 735, 698 cm^{-1}. MS m/z: 413 (M-CH$_2$OH), 222, 120, 91. Anal. Calcd for C$_{26}$H$_{40}$N$_2$O$_4$ (444.62): C 70.24, H 9.07, N 6.30; found: C 70.24, H 9.11, N 6.27.

The synthesis of disilyl diol 118

To a solution of diol **115** (94 mg, 0.31 mmol) in THF (3 mL), imidazole (84 mg, 1.24 mmol) and t-butyldimethylsilyl chloride (140 mg, 0.93 mmol) were added under an argon atmosphere. The reaction mixture was stirred for 3 h at rt. After the completion of reaction, water (5 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 5 mL). The organic phase was dried (Na$_2$SO$_4$) and solvent was evaporated. The residue was purified by column chromatography on silica gel (3%

NH$_3$/MeOH in CH$_2$Cl$_2$, affording compound **118** as colourless oil (yield 146 mg, 89%).

^{1}H NMR (400 MHz, CDCl$_3$) δ: 3.85 – 3.78 (m, 2H, CHO), 3.35 (dd, J = 34.8, 9.5 Hz, 4H, CH$_2$O), 2.67 (m, 4H, C**H**$_2$NH), 1.37 (s, 6H, C(CH$_3$)$_2$), 1.01 (s, 6H, NHC(C**H**$_3$)$_2$), 1.00 (s, 6H, NHC(C**H**$_3$)$_2$), 0.91 – 0.89 (m, 18H, SiC(CH$_3$)$_3$), 0.04 (s, 12H, Si(CH$_3$)$_2$).

^{13}C NMR (100 MHz, CDCl$_3$) δ: 108.65 (**C**(CH$_3$)$_2$), 80.10 (CHO), 69.16 (CH$_2$O), 53.36 (NHC), 45.05 (CH$_2$NH), 27.27 (C(**C**H$_3$)$_2$), 25.88 (SiC(**C**H$_3$)$_3$), 24.30 (NHC(**C**H$_3$)$_2$), 23.20 (NHC(**C**H$_3$)$_2$), 18.23 (Si**C**(CH$_3$)$_3$), -5.51 (Si(CH$_3$)$_2$), -5.54 (Si(CH$_3$)$_2$).

IR: ν = 3337, 2958, 2930, 2894, 2858, 1472, 1378, 1361, 1251, 1208, 1099, 837, 775 cm^{-1}. [α]$_D^{20}$= -19.0 (c 2.63, MeOH). MS m/z: 534 (M+1), 517, 417, 387, 329, 258, 121, 73. Anal. Calcd for C$_{27}$H$_{60}$N$_2$O$_4$Si$_2$ (532.96): C 60.85, H 11.35, N 5.26; found: C 60.78, H 11.60, N 5.16.

The synthesis of tosyl amide 119

Triethylamine (431 μl, 3.09 mmol) and *p*-toluenesulfonyl chloride (295 mg, 1.55 mmol) were added to a solution of amine **118** (103 mg, 0.19 mmol) in pyridine (2 mL) at rt. The mixture was stirred at rt for 24 h. Ethylene diamine (103 μl, 1.55 mmol) was added, and the resulting solution was stirred at rt for 2.5 h. The reaction solution was then diluted with ethyl acetate (20 mL). The resulting solution was washed sequentially with saturated aq. ammonium chloride solution (20 mL) and a 1:1 mixture of saturated aq. sodium bicarbonate solution and brine (20 mL). The organic layer was separated, and the aqueous washes were combined and extracted with EtOAc (30 mL). The combined organic extracts were dried (Na$_2$SO$_4$), and solvent was evaporated. The residue was purified by column chromatography on silica gel (4% EtOAc in petroleum ether), affording tosylamide **119** as a yellow oil (yield 141 mg, 87%).

^{1}H NMR (400 MHz, CDCl$_3$) δ: 7.77 (d, J = 8.3 Hz, 4H, *o*-Ar), 7.20 (d, J = 8.1 Hz, 4H, *m*-Ar), 4.14 – 4.08 (m, 2H, CH$_2$N), 3.98 (m, 2H, CHO), 3.93 (d, J = 10.0 Hz, 2H, CH$_2$O), 3.55 (d, J = 10.0 Hz, 2H, CH$_2$O), 3.48 (m, 2H, CH$_2$N), 2.37 (s, 6H, Ar CH$_3$), 1.41 (s, 6H, C(CH$_3$)$_2$), 1.39 (s, 6H, NC(CH$_3$)$_2$), 1.31 (s, 6H, NC(CH$_3$)$_2$), 0.87 – 0.84 (s, 18H, SiC(CH$_3$)$_3$), 0.03 (s, 6H, SiCH$_3$), 0.02 (s,6H, SiCH$_3$).

^{13}C NMR (100 MHz, CDCl$_3$) δ: 142.28 (*p*-Ar), 141.19 (*i*-Ar), 129.33 (*m*-Ar), 126.57 (*o*-Ar), 109.60 (**C**(CH$_3$)$_2$), 81.93 (CHO), 69.14 (CH$_2$O), 62.54 (N**C**), 48.93 (CH$_2$N), 27.15 (C(**C**H$_3$)$_2$), 25.81 (SiC(**C**H$_3$)$_3$), 24.93 (NC(**C**H$_3$)$_2$), 24.50 (NC(**C**H$_3$)$_2$), 21.37 (Ar CH$_3$), 18.14 (Si**C**), -5.50 (SiCH$_3$), -5.57 (SiCH$_3$).

The synthesis of compound 120

To a solution of *N*-tosyl amine **119** (125 mg, 0.15 mmol) in MeOH (5 mL), 6 N HCl solution (3 mL) was added and the mixture was heated at 60 °C for 23 h. 10 N NaOH solution was added in an ice-water bath until pH~7. After the addition of brine (5 mL), MeOH was evaporated, and the mixture was extracted with EtOAc (3 × 5 mL). The organic phase was dried (Na$_2$SO$_4$) and solvent was evaporated. The

residue was purified by column chromatography on silica gel (7% MeOH in CH_2Cl_2), affording diol **120** as a white solid (yield 57 mg, 89%, mp 138-140 °C).

^{1}H NMR (400 MHz, MeOD) δ: 7.72 (d, J = 8.3 Hz, 4H, o-Ar), 7.37 (d, J = 8.0 Hz, 4H, m-Ar), 3.58 – 3.53 (m, 2H, CH), 2.93 (dd, J = 13.1, 5.2 Hz, 2H, CH_2), 2.83 (dd, J = 13.1, 7.0 Hz, 2H, CH_2), 2.42 (s, 6H, CH_3).

^{13}C NMR (100 MHz, MeOD) δ: 144.71 (p-Ar), 138.68 (i-Ar), 130.77 (m-Ar), 128.10 (o-Ar), 71.24 (CH), 46.58 (CH_2), 21.45 (CH_3).

IR: ν = 3444, 3160, 1599, 1496, 1315, 1158, 811 cm^{-1}.

The synthesis of diol 121

Tetrabutylammonium fluoride solution in THF (5.73 mL, 5.73 mmol)) was added to a solution of compound **119** (802 mg, 0.95 mmol) in THF (10 mL) at rt. The mixture was stirred at rt for 45 min. After the addition of water (20 mL) the mixture was extracted with EtOAc (3 × 15 mL). The organic phase was dried (Na_2SO_4) and solvent was evaporated. The residue was purified by column chromatography on silica gel (30% EtOAc in petroleum ether), affording diol **121** as a white solid (yield 487 mg, 83%, mp 202-203 °C).

^{1}H NMR (400 MHz, MeOD) δ: 7.77 (d, J = 8.4 Hz, 4H, o-Ar), 7.31 (d, J = 8.0 Hz, 4H, m-Ar), 4.11 (dd, J = 5.3, 1.9 Hz, 2H, CHO), 4.03 (d, J = 16.4 Hz, 2H, CH_2N), 3.64 (s, 4H, C**H**$_2$OH), 3.53 (ddd, J = 16.5, 5.4, 2.2 Hz, 2H, CH_2N), 2.39 (s, 6H, Ar CH_3), 1.47 (s, 6H, $C(CH_3)_2$), 1.34 (s, 6H, $NC(CH_3)_2$), 1.23 (s, 6H, $NC(CH_3)_2$).

^{13}C NMR (100 MHz, MeOD) δ: 144.57 (p-Ar), 142.22 (i-Ar), 130.65 (m-Ar), 127.97 (o-Ar), 111.50 (**C**$(CH_3)_2$), 82.81 (CHO), 69.82 (CH_2OH), 63.96 (NC), 49.21 (CH_2N), 27.27 (C(**C**$H_3)_2$), 25.26 (NC(**C**$H_3)_2$), 24.16 (NC(**C**$H_3)_2$), 21.39 (Ar CH_3).

IR: ν = 3561, 3529, 2990, 2936, 2890, 1598, 1494, 1385, 1316, 1243, 1142, 1088, 814 cm^{-1}. $[\alpha]_D^{20}$ = -86.2 (c 2.37, MeOH). MS m/z: 581 (M-CH_2OH), 523, 222, 184, 155, 91. Anal. Calcd for $C_{29}H_{44}N_2O_8S_2$ (612.81): C 56.84, H 7.24, N 4.57; found: C 57.03, H 7.32, N 4.50.

The synthesis of tetraol 122

To a solution of N-tosyl amine **121** (467 mg, 0.76 mmol) in MeOH (10 mL), 6 N HCl solution (5 mL) was added and the mixture was heated at 60 °C for 1 h. 10 N NaOH solution was added in an ice-water bath until pH~7. After the addition of brine (5 mL), MeOH was evaporated, and the mixture was extracted with CH_2Cl_2 (4 × 15 mL). The organic phase was dried (Na_2SO_4) and solvent was evaporated. The residue was purified by column chromatography on silica gel (3% MeOH in CH_2Cl_2), affording tetraol **122** (yield 371 mg, 85%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (d, J = 8.3 Hz, 4H, o-Ar), 7.29 (d, J = 8.0 Hz, 4H, m-Ar), 4.18 (m, 2H, CH), 3.75 – 3.64 (m, 4H, CH_2, C**H**$_2$OH), 3.50 (m, 2H, C**H**$_2$OH), 3.46 – 3.33 (m, 2H, CH_2), 2.41 (s, 6H, Ar CH_3), 1.25 (s, 6H, CH_3), 1.20 (s, 6H, CH_3).

^{13}C NMR (100 MHz, CDCl$_3$) δ: 143.29 (*p*-Ar), 140.16 (*i*-Ar), 129.70 (*m*-Ar), 126.90 (*o*-Ar), 71.37 (CH), 70.11 (CH$_2$), 63.36 (C), 48.57 (CH$_2$OH), 24.68 (CH$_3$), 23.51 (CH$_3$), 21.46 (Ar CH$_3$).

Cyclization of tetraol 122

Tetraol **122** (160 mg, 0.28 mmol) in THF (15 mL) was added to a suspension of NaH (56 mg, 1.40 mmol) in THF (13 mL) at 0°C, under Ar atmosphere. The mixture was stirred at 0°C for 5 min and at RT for 1 h. The reaction mixture was cooled to 0°C and 1-(p-toluenesulfonyl)imidazole (124 mg, 0.56 mmol) was added. After stirring for 15 min at 0° the reaction mixture was allowed to warm to RT and then stirred at 50 °C for 90 h. The suspension was cooled to 0°C and the reaction was quenched by dropwise addition of sat. NH$_4$Cl solution (2 mL). THF was evaporated, sat. NaCl and sat. NaHCO$_3$ solutions were added and the mixture was extracted with EtOAc (4 × 10 mL). The organic phase was dried (Na$_2$SO$_4$) and solvent was evaporated. The residue was purified by column chromatography on silica gel (25% acetone in petroleum ether), affording epoxide **123** (yield 47 mg, 20%).

^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.75 (d, J = 8.3 Hz, 4H, O-Ts *o*), 7.67 (d, J = 8.3 Hz, 4H, N-Ts *o*), 7.46 (d, J = 8.1 Hz, 4H, O-Ts *m*), 7.34 (d, J = 8.1 Hz, 4H, N-Ts *m*), 4.15 (s, 4H, CH$_2$O), 3.98 (d, J = 15.7 Hz, 2H, CH$_2$N), 3.18 – 3.09 (m, 4H, CH, CH$_2$N), 2.41 (s, 6H, O-Ts CH$_3$), 2.38 (s, 6H, N-Ts CH$_3$), 1.24 (s, 6H, CH$_3$), 1.23 (s, 6H, CH$_3$).

^{13}C NMR (100 MHz, DMSO-d_6) δ: 145.08 (O-Ts *p*), 143.11 (N-Ts *p*), 139.58 (N-Ts *i*), 132.01 (O-Ts *i*), 130.15 (O-Ts *m*), 129.70 (N-Ts *m*), 127.65 (O-Ts *o*), 126.50 (N-Ts *o*), 74.36 (CH$_2$O), 59.94 (C), 57.40 (CH), 45.20 (CH$_2$N), 24.48 (CH$_3$), 24.36 (CH$_3$), 21.10 (O-Ts CH$_3$), 20.93 (N-Ts CH$_3$).

General procedure for the intramolecular aldol condensation

To a solution of triketone **128** (99 mg, 0.50 mmol) in CH$_3$CN (1 mL) catalyst **1** or **3** (0.025 mmol) and TFA (1.9 μl, 0.025 mmol) were added under argon atmosphere. The mixture was refluxed for suitable time. The conversions were measured by capillary gas chromatography and the enantiomeric excess by HPLC using a Chiralcel OD-H column (Hex:*i*PrOH 96:4, 1 ml/min, 254 nm).

General procedure for the aziridination of *trans*-chalcone

To a solution of DppONH$_2$ (38 mg, 0.16 mmol) in CH$_3$CN (2 mL) catalyst **98** or **103** (0.16 mmol) was added and the mixture was stirred at rt for 30 min. After that, NaOH (13 mg, 0.31 mmol), *trans*-chalcone **130** (33 mg, 0.16 mmol) and *i*PrOH (200 μl) were added. The mixture was stirred at rt for 3 h. Saturated NH$_4$Cl solution was added and the mixture was extracted with EtOAc (2 × 5 mL). The organic phase was dried (Na$_2$SO$_4$) and solvent was evaporated. The residue was purified by column chromatography on silica gel (5-10% EtOAc in petroleum ether), affording aziridine **131**. The enantiomeric excesses were measured by HPLC using a Chiralcel OD-H column (Hex:*i*PrOH 9:1, 1 mL/min, 254 nm).

General procedure for the epoxidation of *trans*-chalcone

To a solution of catalyst **3** (5 mg, 0.025 mmol) and *trans*-chalcone **130** (52 mg, 0.25 mmol) in CH_2Cl_2 *t*-BuOOH (98 μl, 0.60 mmol) and 4,4 M NaOH solution (136 μl, 0.60 mmol) were added. The mixture was stirred at rt for 3 days. The enantiomeric excess of **132** was measured by HPLC using a Chiralcel OD-H column (Hex:*i*PrOH 98:2, 1 mL/min, 254 nm).

General procedure for the Michael reaction

To a solution of catalyst **3** (10 mg, 0.05 mmol) in $CHCl_3$ (3 mL) β-nitrostyrene **134** (50 mg, 3.34 mmol) and pentanal **133** (356 μl, 3.34 mmol) were added. The mixture was stirred at rt for 24 h. 1 M HCl solution was added and the mixture was extracted with CH_2Cl_2 (3 × 5 mL). The organic phase was dried ($MgSO_4$) and solvent was evaporated. The residue was purified by column chromatography on silica gel (7% EtOAc in petroleum ether), affording the product **135** in 97% yield. The enantiomeric excess of **135** was measured by HPLC using a Chiralcel OD-H column (Hex:*i*PrOH 8:2, 1 mL/min, 254 nm).

General procedure for Henry reaction with *p*-nitrobenzaldehyde and nitromethane

A solution of catalyst (0.015 mmol) and metal salt (0.015 mmol) in an appropriate solvent (600 μl) was stirred for 1 h. After the addition of *p*-nitrobenzaldehyde (45 mg, 0.30 mmol) and nitromethane (163 μl, 3.00 mmol) the reaction was stirred at rt for the appropriate time. After completion of the reaction, the solvent was evaporated and the residue was purified by column chromatography on silica gel (25% EtOAc in petroleum ether). The enantiomeric excesses for **137** were measured by HPLC using a Chiralcel OD-H column (Hex:*i*PrOH 85:15, 1 mL/min, 254 nm).

Acknowledgements

Abstract

The morpholine motifs are extensively used in organic synthesis as bases or as alkylating agents. C_2-symmetric morpholines are widely known chiral auxiliaries. Since morpholines contain two heteroatoms, which make them bidentate ligands, they are useful for metal-catalyzed reactions. The nitrogen moiety alters morpholines to potential organocatalysts. Morpholines, especially C-substituted morpholines, are also found in many biologically active compounds that are used in areas such as medicine and agriculture. There are several methods for the synthesis of these kinds of molecules.

The main aim of the present work was to investigate the synthesis of C_2-symmetric 2,2'-bimorpholines. and to evaluate their catalytic and some biological properties. We intended to use enantiomerically pure starting materials for the introduction of asymmetry into the molecules.

The target bimorpholines were obtained with good overall yields and high enantioselectivities. The main scheme included amidation of tartaric acid ester with amino alcohol, reduction of amide, introduction of an N-protective group, deprotection of secondary diol, cyclization of tetraol and, finally, deprotection of nitrogen atoms in the morpholine rings. The cyclization of tetraols to bimorpholines was efficiently and selectively performed in a one-step procedure with TsIm. The same scheme was applied to the synthesis of 5,5'-substituted bimorpholine derivatives, with slight changes due to the steric differences in the molecules.

2,2'-bimorpholines were found to be unsuitable catalysts for the selected catalytic reactions. Though bimorpholines were quite reactive in some reactions, they showed poor enantioselectivity, the enantiomeric purity of the products did not exceed 35%.

The synthesized bimorpholines were tested on HIV-1-based VLPs and on three different HCV cell lines, and one of them ((2S,2'S,5S,5'S)-5,5'-dibenzyl-2,2'-bimorpholine **4**) was revealed to have high activity on the cells with stable HCV replicon (Huh-7-luc/neo-ET).

Kokkuvõte

Orgaanilises sünteesis on asendatud morfoliine väga laialdaselt kasutatud erinevates reaktsioonides. C_2-sümmeetrilised morfoliinid on tuntud kui kiraalsed mõjurid. Morfoliinide kaks heteroaatomit teevad neist bidentaatsed ligandid, mida kasutatakse metall-katalüütilistes reaktsioonides. Morfoliin kui sekundaarne amiin annab võimaluse tema derivaate kasutada organokatalüsaatoritena aminokatalüüsis. C-asendatud morfoliine leidub mitmetes bioloogiliselt aktiivsetes ühendites, mida kasutatakse erinevates valdkondades, näiteks meditsiinis ning põllumajanduses. Selliste molekulide sünteesiks on mitmeid võimalusi.

Antud töö peamiseks eesmärgiks oli C_2-sümmeetriliste 2,2'-bimorfoliinide sünteesi uurimine ning seejärel nende katalüütiliste ja mõningate bioloogiliste omaduste hindamine. Asümmeetriliste ühendite saamiseks plaaniti kasutada enantiomeerselt puhtaid lähteaineid.

Eesmärkühendid saadi kõrgete summaarsete saagistega ning kõrgete enantiomeersete puhtustega. Üldine sünteesiskeem hõlmas järgnevaid etappe: viinhappe estri amideerimine aminoalkoholiga, amiidi taandamine, aminorühma kaitsmine, sekundaarse diooli kaitsva rühma eemaldamine, tetraooli tsükliseerimine ning kaitsvate rühmade eemaldamine. Tetraoolide tsükliseerimine bimorfoliinideks saavutati selektiivselt ühes etapis, kasutades reagendina TsIm-i. Sarnast skeemi kasutati 5,5'-asendatud bimorfoliinide sünteesil, väikesed muudatused olid põhjustatud molekulide steerilistest erinevustest.

Leiti, et 2,2'-bimorfoliinid ei ole valitud reaktsioonide jaoks sobivad katalüsaatorid. Kuigi bimorfoliinid olid teatud reaktsioonides üsna reaktiivsed, ei saadud produkte kõrgema enantiomeerse puhtusega kui 35%.

Uuriti bimorfoliinide mõju HIV-1 baseeruval VLP-del ja kolmel erineval HCV raku liinil ning leiti, et üks sünteesitud bimorfoliinidest (($2S,2'S,5S,5'S$)-5,5'-dibensüül-2,2'-bimorfoliin **4**) omab kõrget aktiivsust stabiilse HCV replikoniga rakkudele (Huh-7-luc/neo-ET).

Appendix

Biological activity of bimorpholines

The cytotoxicity of DMSO for cells used in subsequent assays was examined, since all the substances were dissolved in DMSO. Based on these assays, it was decided to use no more than 0.5% of DMSO in cell culture media, since higher concentrations were considerably more toxic to the cell lines (data not shown).

Then, the MTT cytotoxicity assay was performed to determine the cytotoxicity of bimorpholines **1**, **3**, **4**, and **107** to HeLa cells. Compounds **A** and **B,** which were used as the control substances in subsequent virus inhibition assays (**A** (2',3'-dideoxythymidine) **B** (3'-azido-2'3'-dideoxythymidine) were also tested. It was shown that none of the substances was toxic to the HeLa cells at any of the used concentrations, as shown in table 4.

Table 4. MTT cytotoxicity assay on HeLa cells.

	50 μM, 0.5% DMSO, OD_{540}	10 μM, 0.1% DMSO, OD_{540}	1 μM, 0.1% DMSO, OD_{540}	0.1 μM, 0.1% DMSO, OD_{540}
1	1.403	1.279	1.455	1.315
3	1.526	1.355	1.294	1.302
4	1.583	1.614	1.404	1.351
107	1.426	1.394	1.428	1.361
A	1.63	1.625	1.457	1.376
B	1.572	1.559	1.557	1.525

name	OD_{540}
No treatment	1.481
0.5% DMSO	1.562
0.1% DMSO	1.509
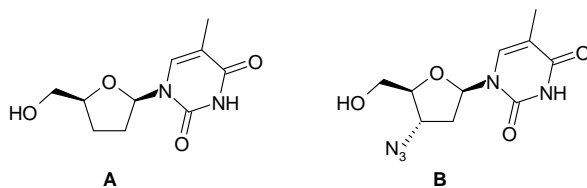
Scheme 57. Control substances **A** and **B**. **A** is a negative control for both HIV and HCV assays; **B** is a positive control for HIV assay (AZT was the first nucleoside drug used as an anti-HIV agent), but a negative control in HCV replication assay.

Next, the ability of bimorpholines to suppress HIV reverse transcription was tested using HIV-1-based viral-like particles (VLPs) ("ViraPower Lentiviral Expression

Systems", Invitrogen). Compounds **A**, **B** and **Lam** (Lamivudine, 2',3'-dideoxy-3'-thiacytidine) were used as the control substances (**A** – negative control, **B, Lam** – positive controls). The concentration of the substrates was 50 μM, and 70 cfu (colony forming units) of VLPs were used for each compound (Table 5). After 10 days, cells were stained with crystal violet and colonies were counted. If the compound had an inhibitory effect on the HIV reverse transcriptase, the number of colonies on the plate decreased. In the case of the positive controls (**B** and **Lam**), the number of the colonies was reduced 35 and 10 times, respectively. Of the bimorpholines, compounds **4** and **107** showed the highest activity. However, their effect was close to that of the negative control **A**. Therefore, the experiment was repeated with these molecules. In this case, the cells were infected with 150 cfu of VLP's and compounds **A** and **B** were used as the control substances; the experiment was performed in triplicate. The results of these experiments are shown in table 6.

Table 5. The initial activities of bimorpholines on HIV-1-based VLPs.

name, 50 μmol	number of colonies	inhibition, substrate ratios to Vir
1	68	0.944
3	54	0.750
4	50	0.694
107	47	0.653
DMSO	75	1.042
Vir	72	1.000
A	52	0.722
B	2	0.028
Lam	7	0.097

Vir – without the inhibitor
Lam – Lamivudine

Table 6. The substrate activities on HIV-1-based VLPs (the experiment was performed in triplicate).

	1	2	3	sum	ratio
VLP	138	163	140	441	1.000
DMSO	168	144	150	462	1.048
A	110	135	152	397	0.900
B	24	31	32	87	0.197
4	132	138	81	351	0.796
107	143	140	129	412	0.934

The preliminary tests on hepatitis C virus (HCV) were conducted with all of the substances, and in each case substances were used at a 100 μM concentration. In these assays, cells carrying stably replicating HCV RNA genome with inserted firefly luciferase gene (Huh7-Luc-neo/ET cells) were incubated with tested

substances for 56 h, then the cells were lysed and Firefly luciferase activity in the lysate was measured.[106,107] The total protein concentration in the lysate was determined using Bradford assay, and luminescence was normalized to total protein concentration. The results are shown in table 7. Compounds **A** and **B** were used as the control substances (note that both represent negative controls, since neither of them can inhibit HCV replication). The main result of this assay was finding that bimorpholine **4** potently inhibited HCV replication, while other subsances were proven to be inactive. It should, however, be noted that the inhibitory effect of bimorpholine **4** on the HCV cell lines was considered as „indirect" since treated cells had abnormal morphoplogy, indicating unexpected cytotoxic effect of the compound.

Table 7. The preliminary tests on HCV-replicon cell line, 56 h incubation.

	RLU	OD_{595}	RLU/OD_{595}	$\log(RLU/OD_{595})$
Control	99593	0.409	243504	5.387
DMSO	114556	0.333	344012	5.537
A	77674	0.283	274466	5.438
B	19892	0.320	62163	4.794
1	209067	0.441	474075	5.676
3	212574	0.360	590483	5.771
4	182	0.052	3500	3.544
107	187590	0.349	537507	5.730

OD_{595} – optical density at wave length 595 nm, RLU – relative light units

Since compound **4** possessed unexpected effect on the HCV cell line (Huh7-Luc-neo/ET), its effect on these and other cells were analyzed in greater detail. First, its cytotoxicity was tested at different concentrations; three different cell lines were used for the determination of cytotoxicity. Consistent with previous observation the highest cytotoxic activity was shown at a 50 μM concentration for Huh7-luc/neo-ET cell line; at the same time, no toxic effect for Huh7-Lunet cells (the parental cell line for Huh7-Luc-neo/ET cells) or U2OS was observed. Thus, the compound was not toxic for cells not carrying HCV replicon (coherent with the finding that the compound is not cytotoxic for HeLa cells, table 4).

HCV replication is sensitive both to the cytotoxic compounds as well as to the cytostatic ones (the later do not kill the cell, just inhibit its division). To find out does the bimorpholine **4** act as a cytostatic compound, corresponding assays were performed with Huh7-Luc-neo/ET cells. Indeed, it was found that the compound was active only at a low confluency of cells (i.e. on actively dividing cells). When the cells had stopped growing (the culture had become overconfluent), compound **4** was no longer toxic (data not shown). Thus, it can be concluded that it is toxic only for dividing cells harbouring HCV replicon RNA. This data is consistent with the view that the bimorpholine **4** has cytostatic function.

Experimental data

The following cell lines were used:
1) Huh7-Lunet (Huh-7 cell clone that was generated by treating luc-ubi-neo cells with IFN-α (Interferon-α). After several passages in the presence of IFN-α and the absence of G418, no replicon RNA could be detected. Compared to naive Huh-7 cells, these „cured" cells supported higher HCV RNA levels and had a more stable permissiveness).[108]
2) Huh-7-luc/neo-ET (Huh-7 cell clone, that carries autonomously replicating subgenomic Hepatitis C Virus replicon, expressing Firefly luciferase as a reporter protein).
3) U2OS (Human Osteosarcoma Cells).
4) HeLa (cervical cancer cells).

MTT cytotoxicity assay

Cells were seeded on a 96-well plate at approximately 90% confluency. In determining DMSO cytotoxicity, cells were incubated for 24 h with different concentrations of DMSO in media (4%, 2%, 1%, 0.5%, 0.25%, and 0.1% DMSO). In the determination of cytotoxicity of substances, cells were incubated for 24h (U2OS, Huh7-Lunet, Huh7-Luc/neo-ET cells), or for nine days (HeLa), with the indicated amount of the substances in the media. Then MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to the media to 10% concentration, the cells were incubated at 37°C for 2 h and lysed in DMSO, and OD at 540 nm was measured (DMSO was used as a blank control), using ELISA reader Tecan Sunrise.

HIV

The experiment was done according to the protocol in „The ViraPower Lentiviral Expression System", Invitrogen. Briefly, U2OS were seeded on 60 mm plates at app 95% of confluency. They were infected with HIV-1-based VLPs (in 300 μl of serum-free media (IMDM+pen/strep+50 μM of tested/control substances, 6 μg/μl polybrene)); after 1 h 2 mL of full media (IMDM+pen/strep+10% FCS+50 μM of tested/control substances) were added. During the infection and 24 h after cells were incubated in media with 50 μM of the tested/control substances.
24 h post-infection Blasticidin selection was started (24 h after infection, the media was changed for complete culture media (IMDM+pen/strep+10% FCS) with 10 μg/mL Blastacidin). 10 days post-infection, the cells were stained with crystal violet and the antibiotic resistant colonies were counted.

HCV

Huh-7-luc/neo-ET cells were seeded on 60 mm plates at approximately 50% confluency. Then the media was changed for complete culture media without G418

(Geneticin) (DMEM, pen/strep, 10% FCS) with 100 µM tested substances or in control with 1% DMSO, or without anything (non-treated cells). Cells were incubated for 56h, and then Firefly luciferase activity was measured according to the protocol in Luciferase Assay Systems, Promega. Briefly, cells were lysed with 200µl of Passive Lysis Buffer, then 4 µl of lysate was mixed with 20 µl of the Luciferase Assay Reagent, vortexed briefly and luminescence was measured using Glomax 20/20 Luminometer, Promega. Protein concentration in lysates was measured using Bio-Rad Protein Assay kit. Briefly, 5 µl of lysate were mixed with 795 µl H_2O and 200 µl of Protein Assay Dye Reagent concentrate, vortexed, and incubated at room temperature for 5 minutes, and then absorbance at 595 nm was measured, using Helios β spectrophotometer (Thermo Electron Corporation).

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4. Täiendusõpe

Märts 2006   TTÜ doktorikooli „Uued tootmistehnoloogiad ja -protsessid"
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                suvekool „Uued suunad sünteetilises orgaanilises keemias"
Veebruar 2007   TTÜ doktorikooli „Uued tootmistehnoloogiad ja -protsessid"
                talvekool
Jaanuar 2008   TTÜ doktorikooli „Uued tootmistehnoloogiad ja -protsessid"
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5. Teenistuskäik

2001 – 2006   TTÜ keemiainstituut   laborant
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2009 –   TTÜ keemiainstituut   teadur

6. Teadustegevus

Teadustöö põhisuunad: asümmeetriline süntees, bioaktiivsete ühendite süntees, organokatalüüs

7. Kaitstud lõputööd

Magistritöö – "Bitsüklo[3.3.0]oktanooni derivaatide süntees" 2005
Bakalaureusetöö – "Asendatud bitsüklo[3.2.0]heptanoonide asümmeetriline Baeyer-Villiger'i oksüdatsioon" 2003

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3. Education

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Tallinn University of Technology   2003   B. Sc.
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4. Special courses

March 2006   TUT doctoral school "New production technologies and processes" winterschool "Advanced engineering and technologies"
August 2006   TUT doctoral school "New production technologies and processes" summerschool "New directions in synthetic organic chemistry"
February 2007   TUT doctoral school "New production technologies and processes" winterschool
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5. Professional Employment

2001 – 2006   Department of chemistry, TUT
2006 – 2009   Department of chemistry, TUT, extraordinary researcher
2009 –   Department of chemistry, TUT, researcher

6. Scientific work

Main areas of scientific work: asymmetric synthesis, synthesis of bioactive compounds, organocatalysis

7. Defended theses

Master thesis – "Synthesis of bicyclo[3.3.0]octanone derivatives" 2005
Bachelor thesis – "Asymmetric Baeyer-Villiger oxidation of substituted bicyclo[3.2.0]heptanones" 2003

8. List of original publications

1. Noole, A.; Lippur, K.; Metsala, A.; Lopp, M.; Kanger, T. Enantioselective Henry reaction catalyzed by Cu[II] salt and bipiperidine. *J. Org. Chem.* **2010**, *75*, 1313-1316.

2. Lippur, K.; Elmers, C.; Kailas, T.; Müürisepp, A.-M.; Pehk, T.; Kanger, T.; Lopp, M. Synthesis of 5,5'-disubstituted bimorpholines. *Synth. Commun.*, **2009**, accepted.

3. Lippur, K.; Kriis, K.; Kailas, T.; Müürisepp, A.-M.; Pehk, T.; Kanger, T. Synthesis of substituted (2*S*,2'*S*)-bimorpholines. In: 30th Estonian Chemistry Days, Tartu, Estonia, **2007**, p. 82-83.

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5. Kriis, K.; Laars, M.; Lippur, K.; Kanger, T. Bimorpholines as Alternative Organocatalysts in Asymmetric Aldol Reaction, *Chimia*. **2007**,232-235.

6. Lippur, K.; Kanger, T.; Kriis, K.; Kailas, T.; Müürisepp, A.-M.; Pehk, T.; Lopp, M. Synthesis of (2*S*,2'*S*)-bimorpholine *N,N'*-quaternary salts as chiral phase transfer catalysts. *Tetrahedron: Asymmetry*, **2007**, 18, 137-141.

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9. Kanger, T.; Raudla, K.; Aav, R.; Müürisepp, A.-M.; Pehk, T.; Lopp, M. Synthesis and derivatization of bis-nor Wieland-Miescher ketone. *Synthesis*, **2005**, 18, 3147-3151.

10. Raudla, K.; Lopp, M.; Aav, R.; Kanger, T.; Pehk, T. Os-Catalyzed Dihydroxylation: an Efficient Method for Desymmetrization of 2-Allyl-2-Methyl-1,3-Cyclopentanedione. In: 13th IUPAC International Symposium on Organometallic Chemistry Directed Towards Organic Synthesis, OMCOS13, Geneva, Switzerland, **2005**, P-478.

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12. Raudla, K.; Kanger, T.; Paju, A.; Pehk, T.; Müürisepp, A.-M.; Lopp, M. Asymmetric Baeyer-Villiger oxidation of bicyclo[3.2.0]heptanones and bicyclo[3.2.0]heptanediones. In: Komppa Centenary Symposium, Espoo, Finland, **2003**.

DISSERTATIONS DEFENDED AT
TALLINN UNIVERSITY OF TECHNOLOGY ON
NATURAL AND EXACT SCIENCES

1. **Olav Kongas**. Nonlinear dynamics in modeling cardiac arrhytmias. 1998.

2. **Kalju Vanatalu**. Optimization of processes of microbial biosynthesis of isotopically labeled biomolecules and their complexes. 1999.

3. **Ahto Buldas**. An algebraic approach to the structure of graphs. 1999.

4. **Monika Drews**. A metabolic study of insect cells in batch and continuous culture: application of chemostat and turbidostat to the production of recombinant proteins. 1999.

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