# Stereoselective synthesis and application of pinane-based bi- and trifunctional chiral ligands

**PhD Thesis**

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# Stereoselective synthesis and application of pinane-based bi- and trifunctional chiral ligands

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## Introduction and aims

It has been found that several natural products, like monoterpenes, appear to be excellent sources for producing chiral bi- and trifunctional compounds, such as aminoalcohols and aminodiols, as well as heterocycles. In organic chemistry, chiral synthons are increasingly used in asymmetric heterogeneous and homogeneous catalysis.

Apart from their chemical value, aminoalcohols and aminodiols possess significant biological properties. In particular, aminodiols are well known for their antibiotic and anticancer properties. Furthermore, these moieties have proven to be highly efficient building blocks, and they have been used as starting materials for the stereoselective synthesis of compounds of pharmacological importance, for example, 1,3-oxazines, 1,3-thiazines, or 2-iminothiazolidines. Aside from their application in synthetic chemistry, aminodiols may also be used as chiral ligands and auxiliaries in enantioselective reactions. As a result, the preparation of new chiral aminodiols has been a topic of increasing interest in recent years. Several chelating ligands have been prepared, but new types synthesised from inexpensive starting materials are still needed.

Accordingly, monoterpene-based 1,2- and 1,3-aminoalcohols have been demonstrated to be excellent chiral auxiliaries in a variety of stereoselective transformations, notably, in intramolecular radical cyclization and intramolecular [2+2] photocycloadditions. However, there are only a few examples of 1,4-aminoalcohols, derived from monoterpenes used successfully as chiral catalysts with high catalytic activity. Furthermore, the 1,4-aminoalcohol moiety has proven to be a privileged structural motif found in a wide range of biologically relevant molecules, including Terfenadine and Ibutilide.

Considering the advantages of monoterpenes, my aim in this PhD work was to synthesise a variety of bi- and tri-functionalised pinane building blocks starting from commercially available enantiopure natural monoterpenes (−)-α- and β-pinene, (−)-myrtenol, and (−)-apopinene. Additionally, I studied their ring-closure processes with aldehydes and 2-phenylisothiocyanate and also performed a coupling between pinane-based aminoalcohols with a variety of pyrimidine compounds to obtain pyrimidine derivatives. Aminodiols and their ring-closed derivatives were planned to be applied as chiral catalysts in the enantioselective addition of $Et_2Zn$ to benzaldehyde.

On the other hand, we also combined pinane-based aminoalcohols and a representative aminodiol reported previously with diaminopyrimidine moieties to study the antiproliferative

activity of the prepared compounds on multiple human cancer cell lines along with our newly developed pinane-derived 1,4-aminoalcohol as well as their antimicrobial activity against Gram-negative and Gram-positive bacteria.

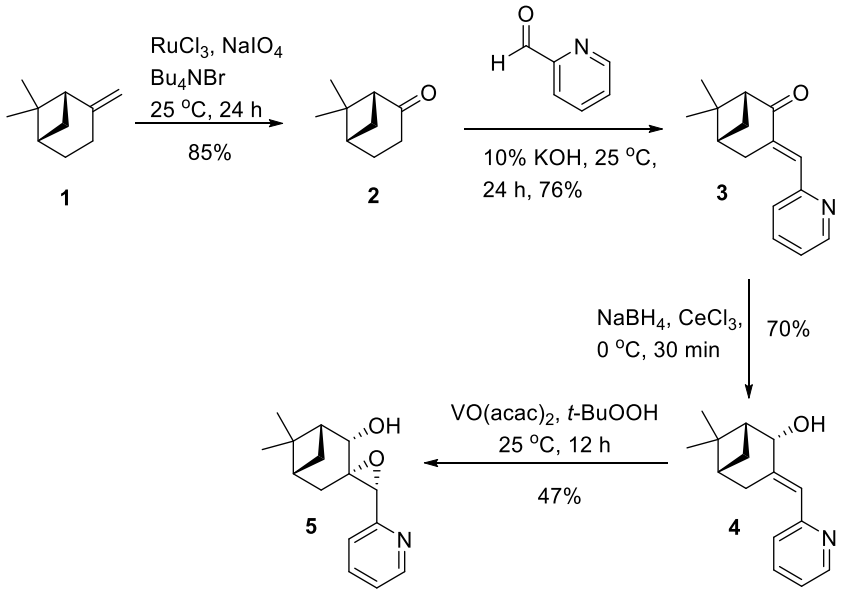
## Methods

Reactions were performed on a mmol scale, and products were purified by column chromatography on silica gel or by crystallisation. All new compounds were characterised by their melting point, with the ue of 1D- and 2D-NMR spectroscopy, elemental analysis, optical rotation and, in a few cases, X-ray crystallography. The enantiomeric excess was determined by chiral GC. The antiproliferative properties against human tumour cell lines (A2780, SiHa, HeLa, MCF-7, and MDA-MB-231) were determined by a microplate reader (BMG Labtech, Ortenberg, Germany). Calculations were performed by means of the GraphPad Prism 5.01 software (GraphPad Software Inc., San Diego, CA, USA). Antimicrobial activities were measured spectrophotometrically using the SPECTROstar®Nano microplate reader system (BMG Labtech, Ortenberg, Germany).

## Results and discussion

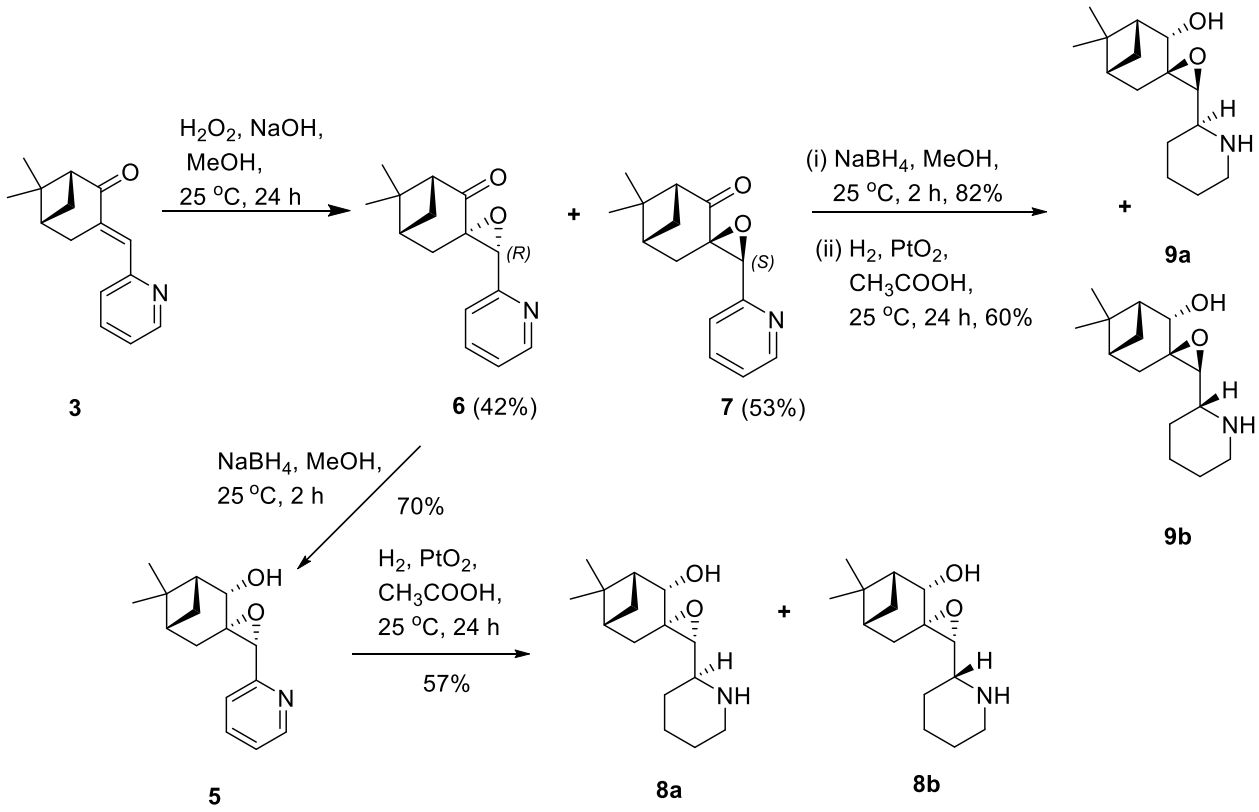
### 1. Synthesis of pinane-based 1,4-aminoalcohol and analogue

The key intermediate (+)-nopinone **2** was prepared from (−)-β-pinene **1** by following the literature method. Diastereoselective aldol condensation of 2-pyridinecarboxaldehyde with (+)-nopinone **2** under alkaline conditions provided α,β-unsaturated ketone **3**. Stereoselective reduction of **3** in Luche condition led to compound **4** in high yield. Epoxidation of this latter compound with *t*-BuOOH catalysed with $VO(acac)_2$ gave diendo-epoxy alcohol **5** in moderate yield.



Scheme 1

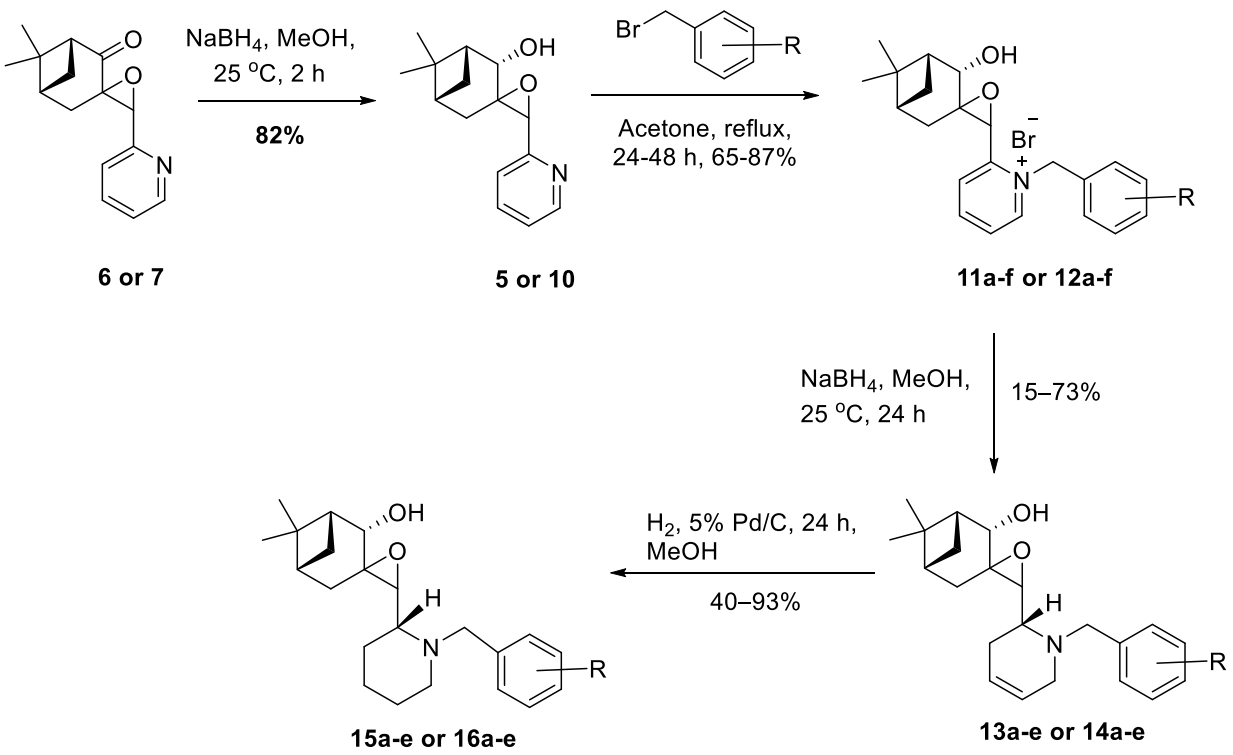
The mixture of epoxides **6** and **7** were obtained by the epoxidation of α,β-saturated ketone **3** using $H_2O_2$ in alkaline conditions. After the formation and separation of diastereomers **6** and **7**, their reduction led to inseparable mixtures **8a,b** and **9a,b**, respectively.




Scheme 2

An alternative pathway was based on reduction of amines **6** and **7** followed by rection with a variety of benzyl bromides and subsequent quaternisation of the pyridine ring. Finally, the stereoselective reduction of the pyridine ring to piperidine was successfully accomplished.

The resistance of the oxirane ring during the nucleophile-initiated opening reaction was interpreted by professor Antal Csámpai via a systematic series of comparative Hartree–Fock modeling study using 6-31+G(d,p) basis set.

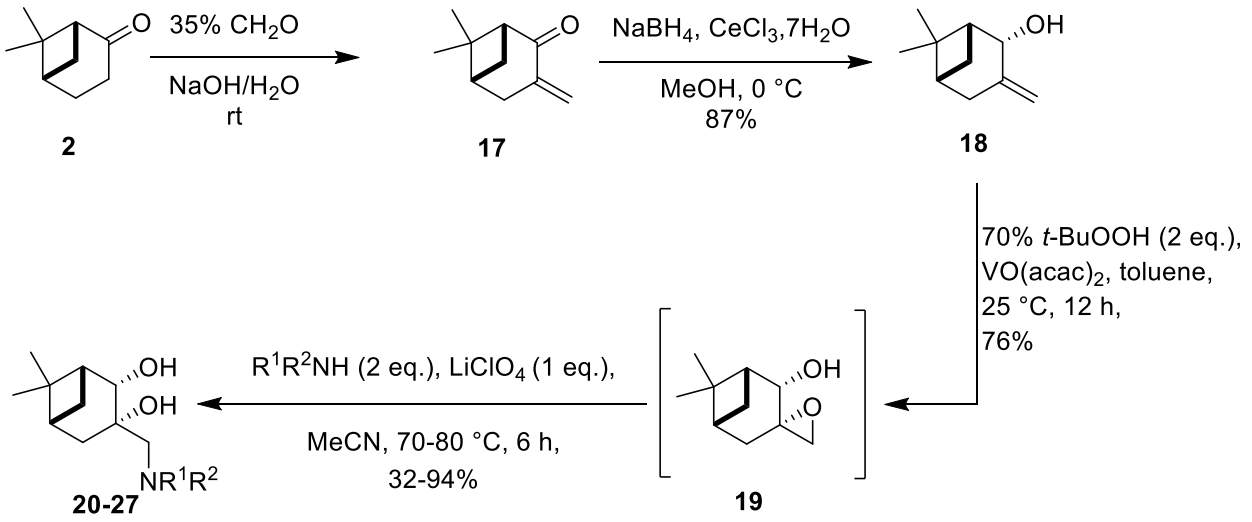



**11a, 12a, 13a, 14a, 15a, 16a** : R = H
**11b, 12b, 13b, 14b, 15b, 16b**: R = *p*-Trifluoromethoxybenzyl
**11c, 12c, 13c, 14c, 15c, 16c** : R = *m*-Methylbenzyl
**11d, 12d, 13d, 14d, 15d, 16d**: R = *m*-Methoxybenzyl
**11e, 12e, 13e, 14e, 15e, 16e** : R = 3,5-Ditrifluoromethylbenzyl
**11f, 12f** : R = 2,5-Ditrifluoromethylbenzyl

Scheme 3

## 2. Synthesis of pinane-based 3-amino-1,2-diol and its analogues

Starting from nopinone **2**, a library of 3-amino-1,2-diols was prepared by aldolisation of **2** with formaldehyde. Subsequent reduction of ketone **17** followed by stereospecific epoxidation led to epoxyalcohol **18**. Opening of the oxirane ring with primary and secondary amines afforded the required aminodiols **20–27**.

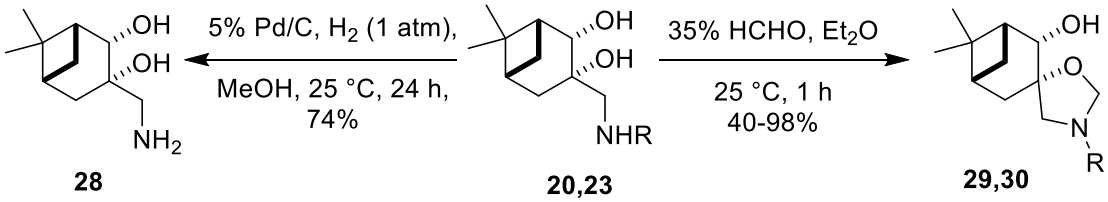



**20**: $R^1$ = H, $R^2$ = Bn, **21**: $R^1$ = H, $R^2$ = CH(Me)Ph (*R*), **22**: $R^1$ = H, $R^2$ = CH(Me)Ph (*S*)
**23**: $R^1$ = H, $R^2$ = $CH(Me)_2$, **24**: $R^1$ = Bn, $R^2$ = Bn, **25**: $R^1$ = Bn, $R^2$ = CH(Me)Ph (*R*),
**26**: $R^1$ = Bn, $R^2$ = CH(Me)Ph (*S*), **27:** $R^1$=$R^2$=-$(CH_2)_2$-CH(Bn)-$(CH_2)_2$-

Scheme 4

Primary aminodiol **28** was obtained by debenzylation of the corresponding *N*-benzyl aminodiol **20** under the hydrogenation process over Pd/C.

The ring-closing reaction of **20** and **23** with formaldehyde was also investigated. When these aminodiols were treated with formaldehyde under mild conditions, spiro-oxazolidines **29** and **30** were obtained in highly regioselective ring closure.

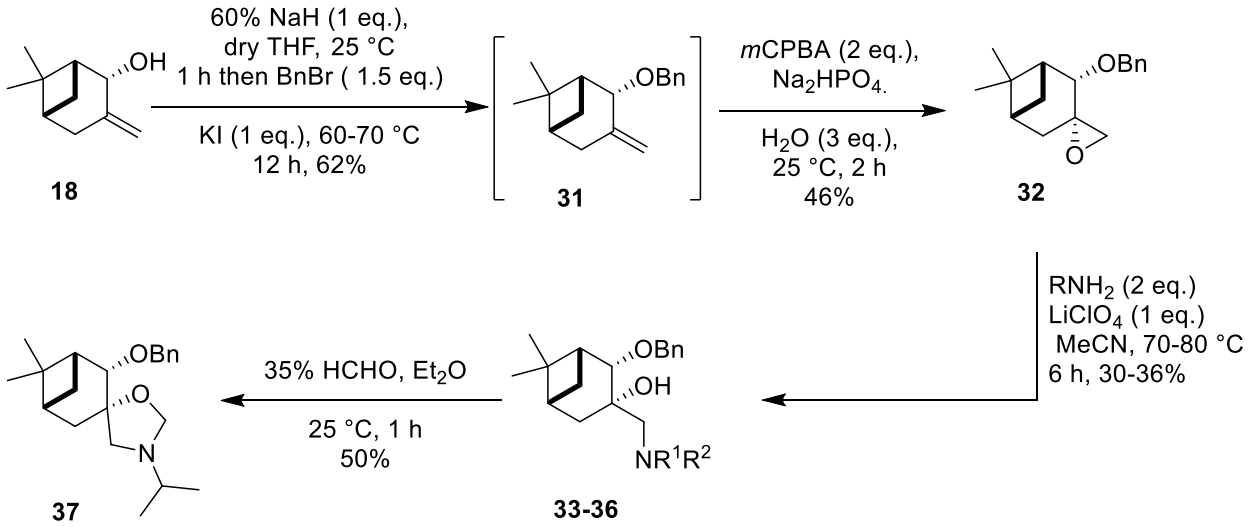



**20,29**: R = Bn, **23,30**: R = $CH(Me)_2$

Scheme 5

On the other hand, to assess the importance of the secondary hydroxyl group in the catalytic application, allylic alcohol **18** was transformed into *O*-benzyl derivative **31**. which Then, without

isolation, it underwent stereoselective epoxidation to epoxide **32**. Aminolysis of the oxirane ring resulted in the *O*-benzyl aminodiols **33–36**. And after The treatment of **36** with formaldehyde gave the spiro-oxazolidine **37** in a yield of 50%.

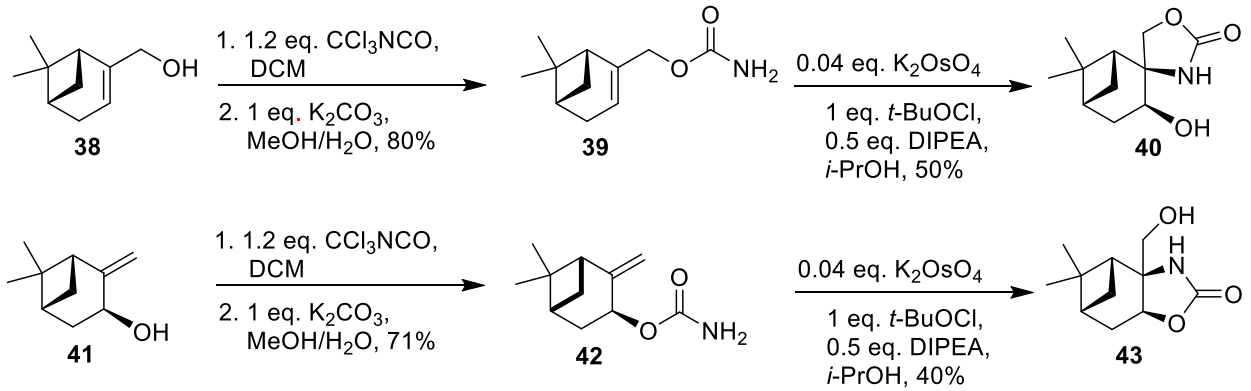



**33**: $R^1$ = H, $R^2$ = Bn, **34**: $R^1$ = H, $R^2$ = CH(Me)Ph (*R*), **35**: $R^1$ = H, $R^2$ = CH(Me)Ph (*S*)
**36**: $R^1$ = H, $R^2$ = $CH(Me)_2$

Scheme 6

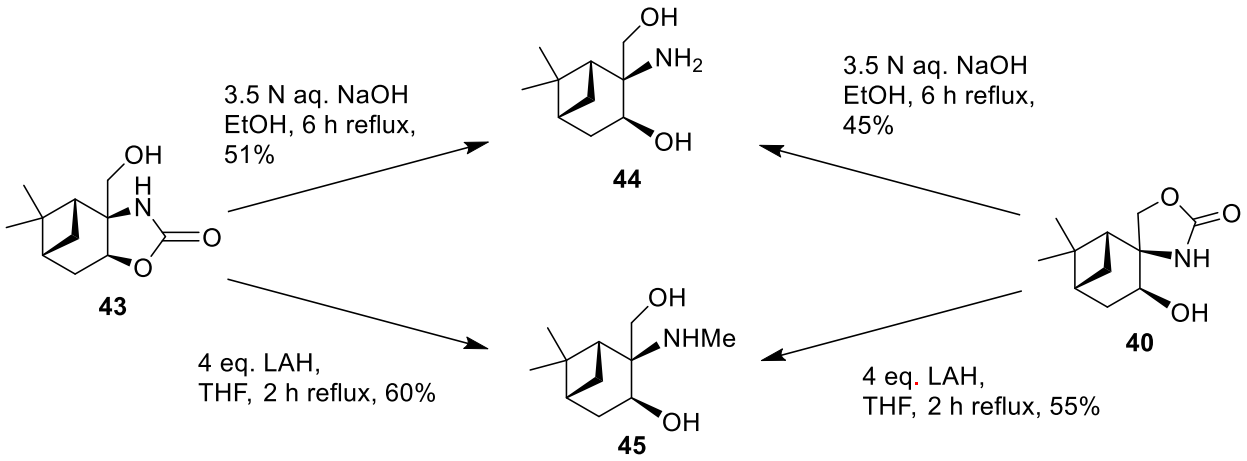
### 3. Synthesis of pinane-based 2-amino-1,3-diol and analogues

A small library of pinane-based 2-amino-1,3-diols was synthesised in a stereoselective manner starting from (1*R*)-(−)-myrtenol **38** and isopinocarveol **41** prepared from *α*-pinene. Pinane-condensed or spiro-oxazolidine-2-ones **40** and **43**, respectively, were formed in three steps by a stereoselective hydroxyamination process.



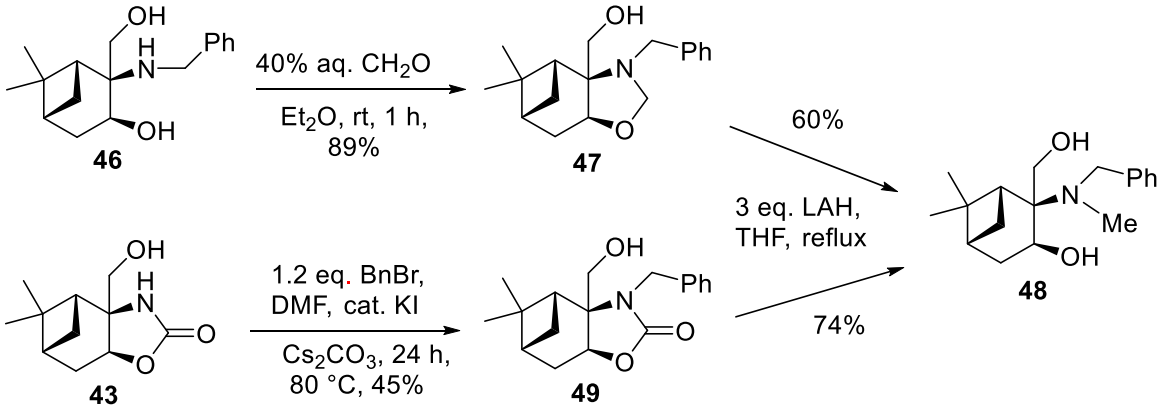

Scheme 7

To obtain a library of pinane-based 2-amino-1,3-diols, oxazolidine-2-ones **40** and **43** were applied as starting materials. Alkaline hydrolysis of both resulted in the same primary aminodiol **44**. In a similar manner, LAH reduction of both oxazolidines gave the same *N*-methylaminodiol **45** with modest yield.



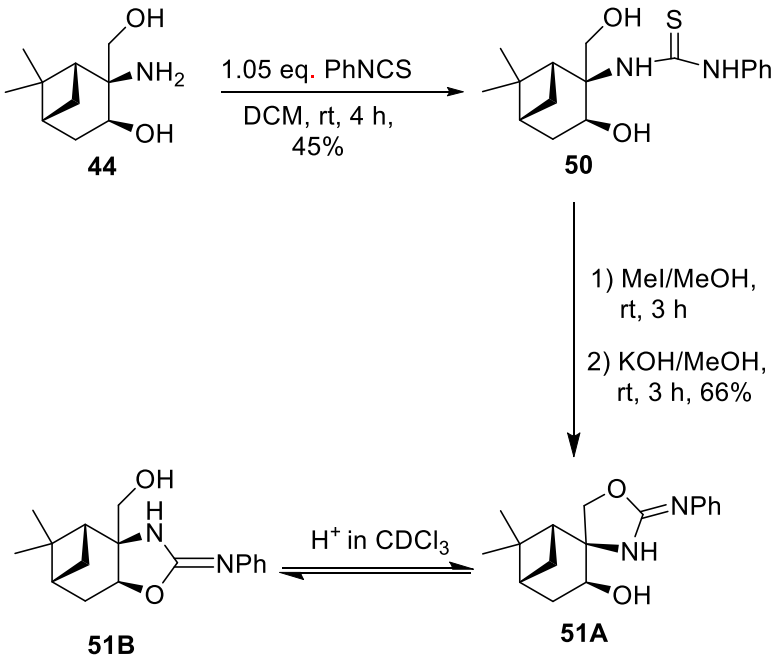

Scheme 8

The resulting secondary 2-amino-1,3-diols **46** underwent a regioselective ring closure with formaldehyde producing pinane-condensed oxazolidines **47,** which under LAH reduction gave *N*-benzyl,*N*-methyl analogue **48**. Alternatively, **48** was prepared directly from 2-oxazolidinone **43** in 2 steps via *N*-benzylation followed by LAH reduction.




Scheme 9

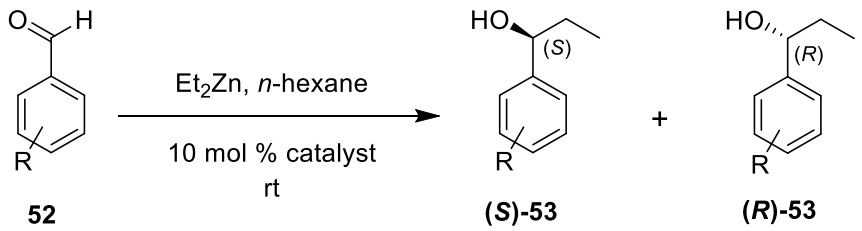
When **44** was reacted with phenylisothiocyanate, thiourea **50** was obtained, which underwent regioselective ring closure resulting in **51A**, and an interesting ring–ring tautomerism was observed in $CDCl_3$ between **51A** and **51B**.




Scheme 10

## 4. Application of pinane-based chiral aminoalcohols/aminodiols as chiral catalysts

The catalytic value of aminoalcohols, aminodiols, and their ring-closed derivatives were tested in the enantioselective addition of diethylzinc to aldehydes **52** to form (*S*)-1-phenyl-1-propanol [(S)-**53**] and (*R*)-1-phenyl-1- propanol [(*R*)-**53**] (Scheme 11).




Scheme 11

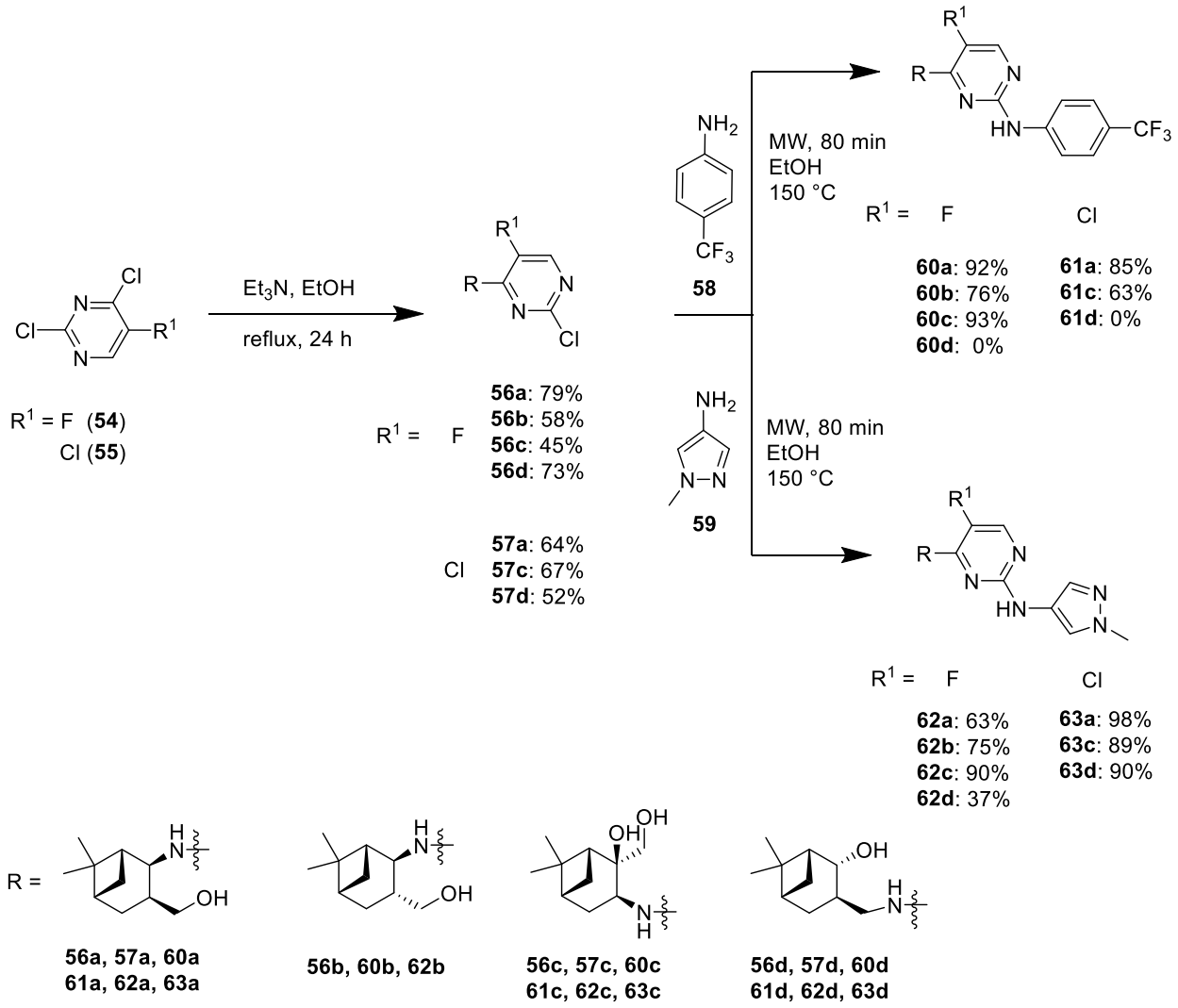
Aminodiol **24** and **35** afforded the best *ee* value of 80% with an (*R*)-selectivity with benzaldehyde, whereas *O*-benzyl aminodiol **34** afforded (*S)*-selectivity up to *ee* = 92%, when used with 4-methoxybenzaldehyde. However, the pinane-based 1,4-aminoalcohols showed poor selectivity with the best, but still moderate value of *ee*= 33%.

## 5. Antiproliferative activity of pinane-based chiral aminoalcohols

The antiproliferative activities of the pinane-based 1,4-aminoalcohol library were explored, and structure–activity relationships were studied. The resulting 4-tetrahydropyridine-2,3-epoxy-1-ols exert markedly antiproliferative action on a panel of human cancer cell lines. The in vitro pharmacological studies have clearly shown that the 1,4-aminoalcohol function, together with the oxirane and tetrahydropyridine ring systems, seem to be essential for reliable antiproliferative activity. However, the stereochemistry of the oxirane ring and the *N*-substituents on the tetrahydropyridine function have no influence on the antiproliferative effect.

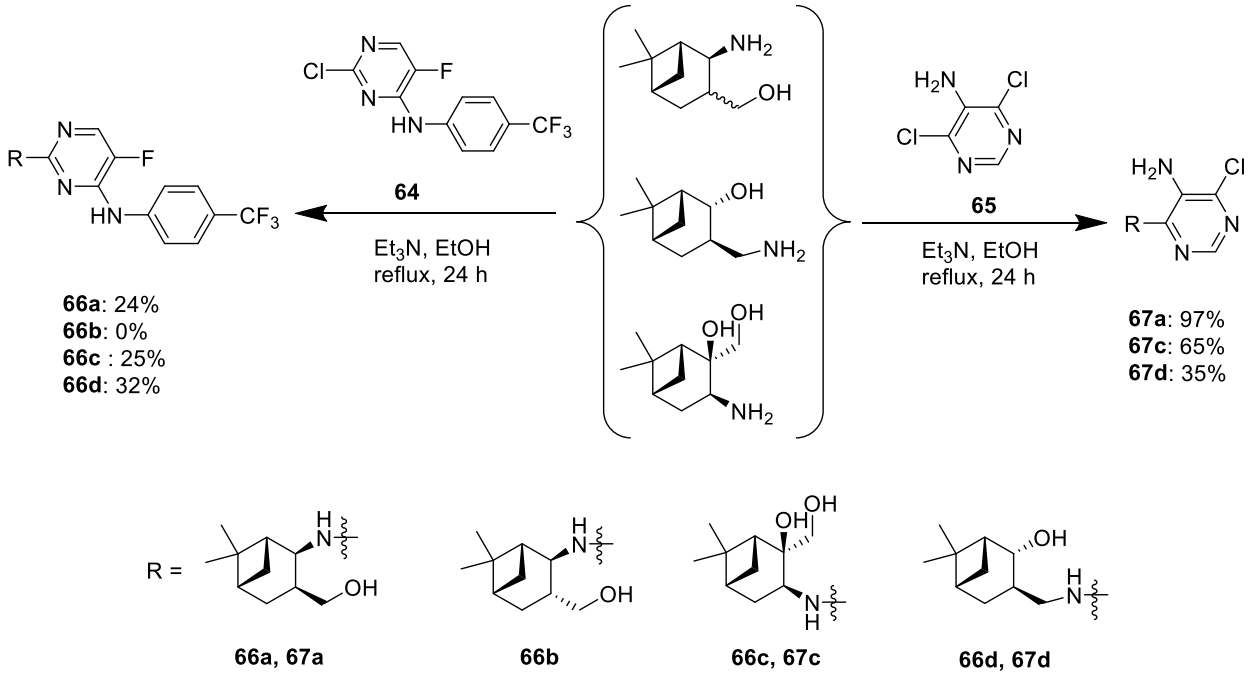
## 6. Synthesis of pinane-based diaminopyrimidines

Key intermediates **56** and **57** were prepared by condensation of pinane-based aminoalcohols / aminodiols according to the literature method with 2,4-dichloro-5-fluoropyrimidine **54** and 2,4,5-trichloropyrimidine **55**, respectively in the presence of $Et_3N$ in EtOH. The latter compounds were easily isolated by column chromatography and used for a microwave-assisted $S_NAr$ coupling reaction with 4-aminobenzotrifluoride **58** as well as 4-amino-1-methylpyrazole **59**, resulting in a new library of chiral, pinane-based 2,4- or 3,4-diaminopyrimidines.




Scheme 12

Likewise, further coupling reactions were accomplished between 2-chloro-5-fluoro-*N*-(4-(trifluoromethyl)phenyl)pyrimidin-4-amine **64** with the aminoalcohols resulting in the formation of **66a-d** with moderate yield. On the other hand, another $S_NAr$-type coupling reaction was performed by utilising 5-amino-4,6-dichloropyrimidine as a coupling agent to form pinane-based pyrimidines **67a-d.**




Scheme 13

## 7. Biological properties of pinane-based diaminopyrimidines

Cytotoxic activity of compounds **56**, **57**, **60**, **61**, **62**, **63**, **66**, **67** against human tumour cell lines (A2780, SiHa, HeLa, MCF-7, and MDA-MB-231) was investigated. In our preliminary study, the 2,4-diaminopyrimidine and 1-methylpyrazol moieties seemed to be essential for a reliable antiproliferative activity. The antimicrobial activity of the prepared compounds against different bacterial and fungal strains was also evaluated. The 2,4-diaminopyrimidine moiety appeared to be essential but the effect of its aromatic or heteroaromatic substitution is opposite to that found in antiproliferative activity. Finally, the *N*-4-trifluorophenyl substitution showed a clear advantage over 1-methylpyrazol. The stereochemistry of the pinane ring system had a weak influence on the cytotoxic or antibacterial activity.

**Publication list**

[1] **Mounir Raji**, Tam Minh Le, Ferenc Fülöp, Zsolt Szakonyi

Synthesis and Investigation of Pinane-Based Chiral Tridentate Ligands in the Asymmetric Addition of Diethylzinc to Aldehydes

*Catalysts*, **2020**, 10, 474-491

IF: 4.14

[2] Ákos Bajtel, **Mounir Raji**, Matti Haukka, Ferenc Fülöp, Zsolt Szakonyi

Stereoselective synthesis and transformation of pinane-based 2-amino-1,3-diols

*Beilstein Journal of Organic Chemistry*, **2021**, 17, 983–990

IF: 2.88

[3] **Mounir Raji**, Tam Minh Le, Antal Csámpai, Viktória Nagy, István Zupkó, Zsolt Szakonyi

Stereoselective synthesis and applications of pinane-based chiral 1,4-aminoalcohol derivatives

*Synthesis*, **2022**, accepted, DOI: 10.1055/s-0040-1719887

IF: 3.157

[4] **Mounir Raji**, Tam Minh Le, Thu Huynh, András Szekeres, Viktória Nagy, István Zupkó, Zsolt Szakonyi

Divergent synthesis, antiproliferative and antimicrobial studies of 1,3-aminoalcohol and 3-amino-1,2-diol based diaminopyrimidines

*Chemistry & Biodiversity*, **2022**,19, accepted, DOI: 10.1002/cbdv.202200077

IF: 2.408

**Scientific lectures**

**Raji Mounir**

Synthesis of bi- and trifunctional pinane-based chiral synthons

*XLII. Kémiai Előadói Napok*

Szeged, 28-30 October 2019, oral presentation

**Raji Mounir**, Tam Minh Le, Szakonyi Zsolt

Synthesis and application of chiral 3-amino-1,2-diols derived from natural (-)-β-pinene

*MTA 2019, Szteroid- és Terpenoidkémiai Munkabizottság*

Szeged, 20-22 November 2019, oral presentation

**Raji Mounir**, Tam Minh Le, Ferenc Fülöp, Szakonyi Zsolt

Synthesis and investigation of pinane-based chiral tridentate ligands in the asymmetric catalytic reactions

*3rd International Conference on Pharmaceutical and Medical Sciences*

Martin, Kraków, Szeged, 24-26th of September 2020, oral presentation

**Raji Mounir**, Viktória Nagy, Istvan Zupkó, Szakonyi Zsolt.

Synthesis and *in vitro* Antiproliferative Studies of Pinane-Based Pyrimidine Derivatives

*XXIX European Colloquium on Heterocyclic Chemistry*

French, 26-28 April 2021, oral presentation

**Raji Mounir**

Synthesis and investigation of pinane-type, pyridine-based di- and trifunctional synthons

*A Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány 21. tudományos előadóülése*

Szeged, 25 May 2021, oral presentation

Tam Minh Le, **Mounir Raji**, Fatima Zahra Bamou, Thu Huynh, Szekeres András, Szakonyi Zsolt

Stereoselective synthesis and antimicrobial evaluation of monoterpene-based bi-, tri and tetrafunctionalized chiral synthons

*Gyógyszerkémiai és Gyógyszertechnológiai Szimpózium 21*

Herceghalom, 20-21 September 2021, oral presentation

Viktória Nagy, **Raji Mounir**, Zsolt Szakonyi, Gábor J. Szebeni, Zupkó István

Pharmacological investigation of a newly synthesised monoterpene derivatives on human cancer cell lines in vitro

*11th ISCTICO – HUPHAR – IUPHAR – Conference*, October 27-30, 2021 - PÉCS, HUNGARY, P84, oral presentation