University of Szeged

Doctoral School of Pharmaceutical Sciences

Educational Programme:	Pharmaceutical Chemistry and Drug Research
Programme director:	Prof. Dr. Ferenc Fülöp
Institute:	Institute of Pharmaceutical Chemistry
Supervisors:	Prof. Dr. Ferenc Fülöp
	Dr. Loránd Kiss

Maria Cherepanova

A stereocontrolled access to functionalized cispentacins and acyclic $\beta^{2,3}$-amino acids through oxidative ring cleavage protocol

Final examination committee:

Head:	Dr. Árpád Molnár, DSc
Members:	Dr. György Dombi, PhD
	Dr. Géza Tóth, DSc

Reviewer committee:

Head:	Dr. Judit Hohmann, DSc
Reviewers:	Dr. Éva Frank, PhD
	Dr. Csaba Tömböly, PhD
Members:	Dr. Tamás Janáky, DSc
	Dr. Dóra Rédei, PhD

1. Introduction and aims

β-Amino acids are in the limelight of current interest due to their valuable pharmacological properties. They serve as essential structural units of a number of biologically active compounds, found in natural products. Naturally occurring cyclopentane-based β-amino acids, such as cispentacin and icofungipen, are strong antifungal agents, while the cyclohexane amino acid Tamiflu and the *O*-heterocyclic amino acid Zanamivir exert notable antiviral activities. These compounds serve as building blocks of β-lactams, antibiotics and peptides. Open-chain β-amino acids, and particularly $\beta^{2,3}$-amino acids, a subclass of β-amino acids, are also structural elements of natural products with activity against leukemia, e.g. Dolastatin 11, 12, 16 and D, Majusculamide C and Onchidin, natural antitumoural agents such as Guineamide C and D, Ulongapeptin and Malevamide C.

The Institute of Pharmaceutical Chemistry at the University of Szeged has extensive experience in the field of the synthesis and transformation of β-amino acids. The research is focused on the development of stereoselective approaches towards highly functionalized alicyclic and open-chain β-amino acid derivatives. The regio- and stereoselective syntheses of mono- and dihydroxylated, mono- and difluorinated, protected amino, epoxy, azido, isoxazoline alicyclic β-amino acid derivatives, starting from the appropriate alicyclic unsaturated β-lactams have been successfully accomplished by C-C ring double bond functionalization.

Although numerous methods have been reported for the synthesis of β-amino acid derivatives, the development of new regio-, stereocontrolled, efficient approaches towards highly functionalized enantiomerically pure species still remains an important goal.

The present PhD work was focused on the development of stereocontrolled synthetic strategies to novel enantiomerically pure disubstituted alicyclic β-amino esters derived from norbornene-based *diendo*- and *diexo*-β-amino carboxylates and acyclic $\beta^{2,3}$-substituted amino acid derivatives from *cis* or *trans* cyclopentene and cyclohexene β-amino acids. The method is based on functionalization of the ring C-C double bond of the β-lactam through dihydroxylation, and oxidative C-C bond cleavage of the vicinal diol, followed by Wittig transformation with different phosphoranylides and reduction of the olefinic bond. All products were obtained in both racemic and optically pure forms.

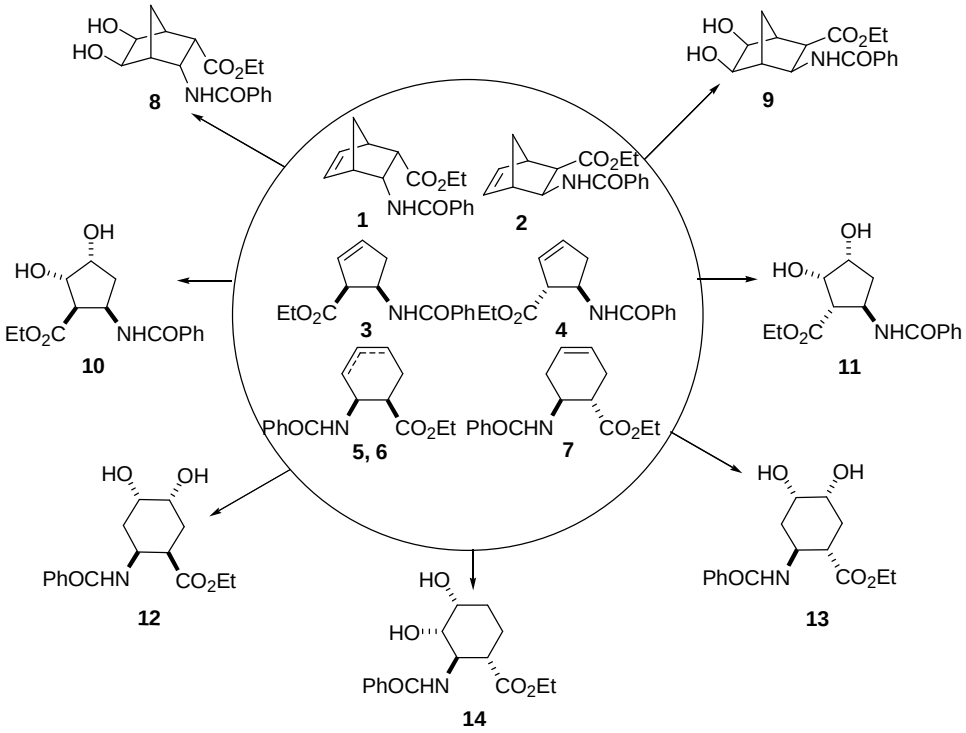
2. Applied methods

The synthesized compounds were separated and purified by column chromatography on silica gel and by crystallization. The newly prepared products were characterized by melting point measurements, IR, NMR, mass spectroscopy and elemental analysis. The *e.e.* values of the optically active compounds were determined by gas chromatography or HPLC. For determination of the stereochemistry of the compounds, 2D NMR spectroscopy (COSY, HSQC, HMBC and NOESY) and X-ray diffraction were also used.

3. Results and discussion

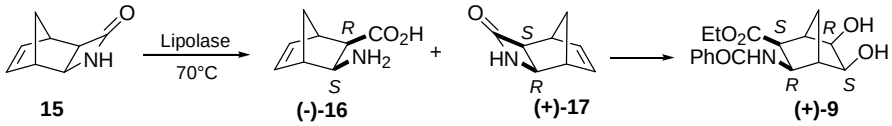
3.1. Synthesis of starting materials

Racemic β-amino esters **2-7** as required starting materials were synthesized by a β-lactams ring opening, followed by *N*-protection. Bicyclic *diendo*-β-amino ester **1** was obtained from bicyclic *diendo*-anhydride via amide formation, followed by Hoffmann degradation, esterification and *N*-Bz protection. Stereoselective dihydroxylation of these compounds by means of NMO and catalytic amount of OsO_4 furnished dihydroxylated β-amino esters **8-14** (Scheme 1).



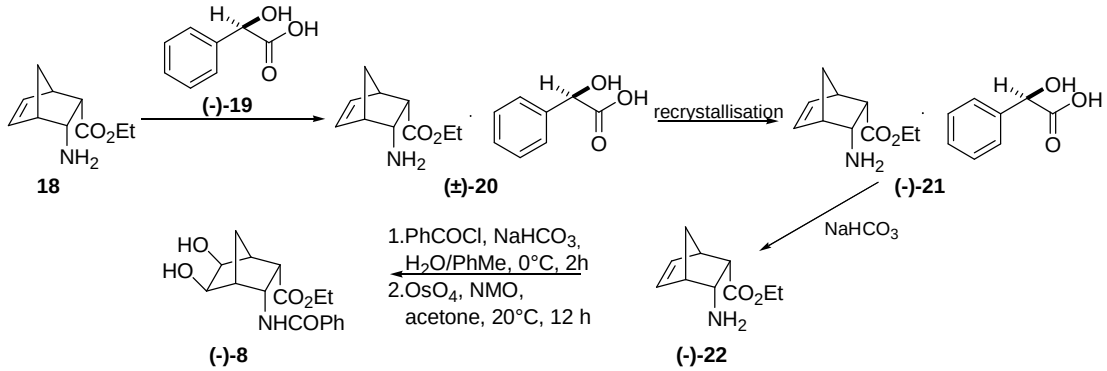
Scheme 1.

The well-established protocol for the synthesis of dihydroxylated β-amino esters allowed the preparation of starting materials in enantiomerically pure form. Racemic β-lactam **15** was subjected to enzyme-catalysed enantioselective ring opening resulting in enantiomerically pure amino acid **(-)-16** and unreacted β-lactam **(+)-17**. Then enantiomer **(+)-17** was transformed to optically pure diol **(+)-9** through ring-opening reaction, *N*-Bz protection and dihydroxylation (Scheme 2).



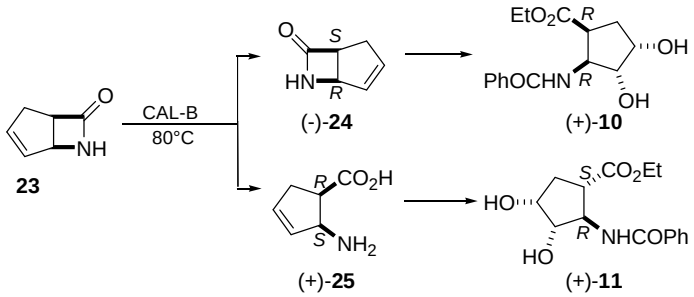
Scheme 2.

The preparation of enantiomerically pure β-amino ester **(-)-8** was attained through diastereomeric salt recrystallization. The reaction between racemic *diendo*-β-amino ester **(±)-18** and 1 equivalent of enantiomerically pure *D*-(-)-mandelic acid **(-)-19** yielded the corresponding diastereomeric salt mixture **(±)-20.** This mixture was then twice recrystallized from EtOAc-EtOH 10:1, the diastereomeric ratio was monitored by ^{1}H NMR. Pure diastereomer was filtered off and treated with a saturated $NaHCO_3$ solution giving optically pure *diendo*-β-amino acid **(-)-22** with *e.e.* = >99%. Following benzoylation and stereoselective dihydroxylation resulted in enantiomer **(-)-8** (Scheme 3).



Scheme 3.

Cyclopentene *cis* and *trans* β-amino esters were gained through enzyme-catalysed kinetic resolution of β-lactam. Enantiomerically pure amino acid **(+)-25** and unreacted β-lactam **(-)-24** were obtained and separated. Next, **(-)-24** was treated with HCl/EtOH solution, and following benzoylation and dihydroxylation led to optically pure diol (1*R*,2*R*)-**(+)-10.** The amino acid **(+)-25** was transformed to dihydroxylated *trans* β-amino carboxylate (1*S*,2*R*)-**(+)-11** via esterification, group protection, base-induced epimerization and dihydroxylation procedures (Scheme 4).

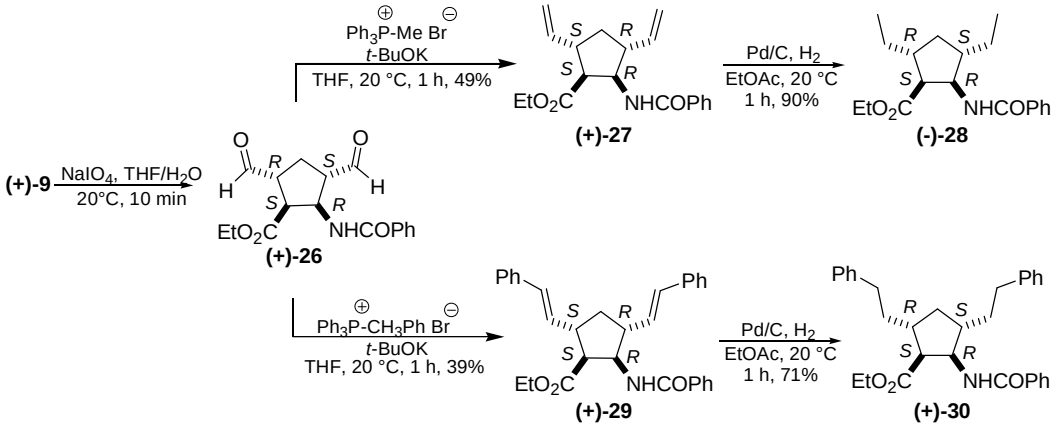


Scheme 4.

3.2. Functionalization of cispentacins through C-C oxidative cleavage of *diexo*-and *diendo*-norbonene β-amino acids

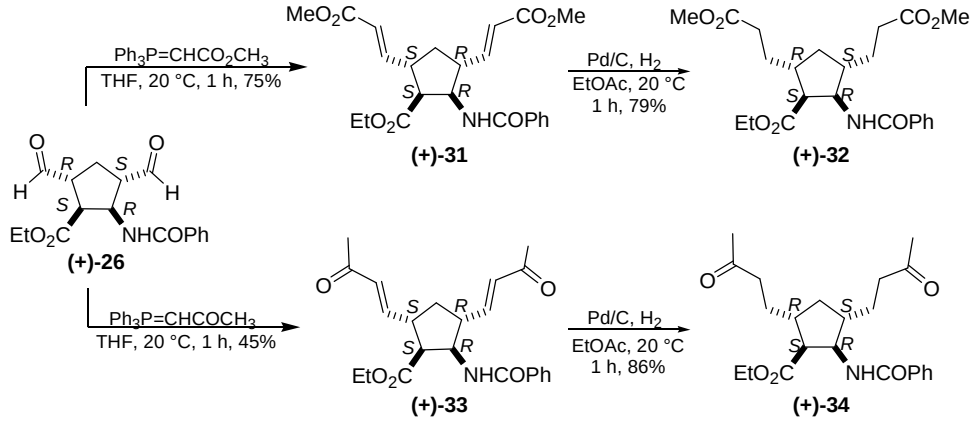
The concept of the synthetic route included the functionalization of the ring C-C double bond of *diexo*-norbornene β-lactam and *diendo*-norbornene β-amino acid through stereoselective dihydroxylation, conversion of vicinal diol by oxidative ring cleavage in order to generate the corresponding dialdehyde key intermediate, followed by the Wittig transformation with different phosphoranylides and hydrogenation of formed double bond. Preliminary experiments were conducted with racemic substances, and then the newly developed synthetic strategy allowed the preparation of novel cispentacin derivatives in enantiomerically pure form.

The C-C bond cleavage of optically active dihydroxylated β-amino carboxylate **(+)-9** with $NaIO_4$ gave access to dialdehyde **(+)-26.** The *in situ* Wittig reaction allowed the preparation of dialkenylated compounds **(+)-27** and **(+)-29**, and further saturation of the olefinic bonds led to optically pure substituted cispentacins **(-)-28** and **(+)-30** in good yields (Scheme 5).



Scheme 5.

The diformyl β-amino ester was further transformed into the corresponding enantiomers **(+)-31** and **(+)-33** in reaction with methyl (triphenylphosphoranylidene)acetate and (triphenylphosphoranylidene)-2-propanone, and catalytic reduction of double bonds furnished saturated cispentacin derivatives **(+)-32** and **(+)-34** (Scheme 6).

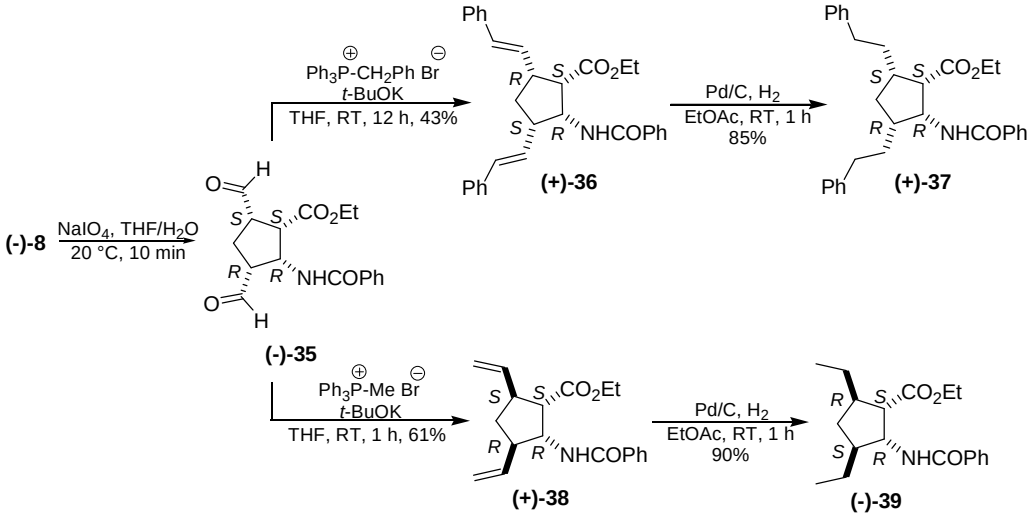


Scheme 6.

The synthesis of *all-cis*-disubstituted cispentacin derivatives was based on similar strategy as for previously described *trans*-disubstituted analogues, namely the transformation of C-C double bond of carbocycle by dihydroxylation, oxidative ring cleavage and the Wittig transformation of the formed dialdehyde. Preliminary experiments were performed by utilizing racemic substances. The synthetic protocol described above was further extended to the preparation of optically pure substances.

all-cis-Dialdehyde **(-)-35** was synthesized from **(-)-8** by oxidative ring cleavage in optically pure form. The absolute configurations of stereocenters in **(-)-35** are 1*R*,2*S*,3*R*,4*S*, which was determined by chemical correlation.

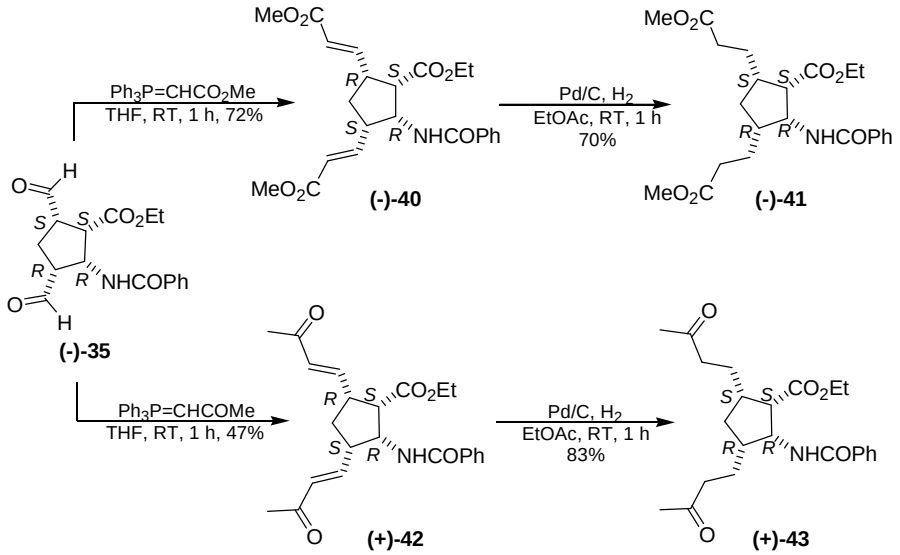
Optically active dialdehyde **(-)-35** was transformed into dialkenylated Wittig product **(+)-36** in reaction with benzyltriphenylphosphonium bromide generated phosphorane. After C-C bond reduction, saturated compound **(+)-37** was attained (Scheme 7).



Scheme 7.

The reaction of diformyl derivative **(-)-35** with commercially available phosphoranes methyl (triphenylphosphoranylidene)acetate and triphenylphosphoranylidene-2-propanone

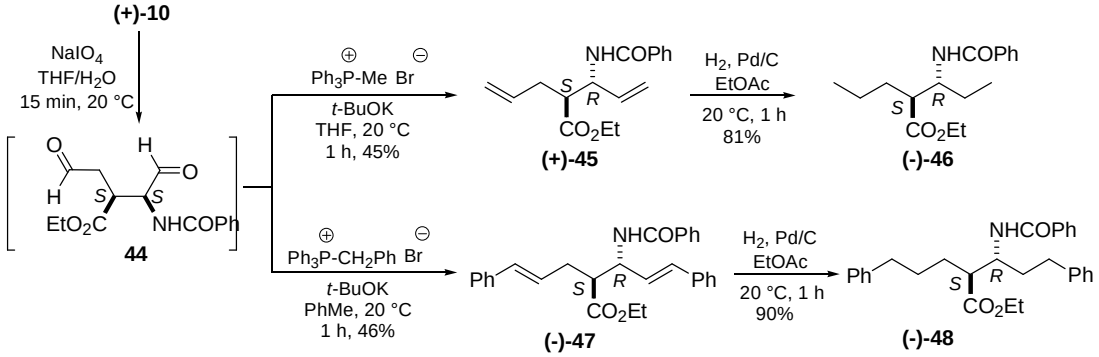
gave access to optically pure **(-)-40** and **(+)-42**, reduction of these products led to **(-)-41** and **(+)-43** in enantiomerically pure form (Scheme 8).



Scheme 8.

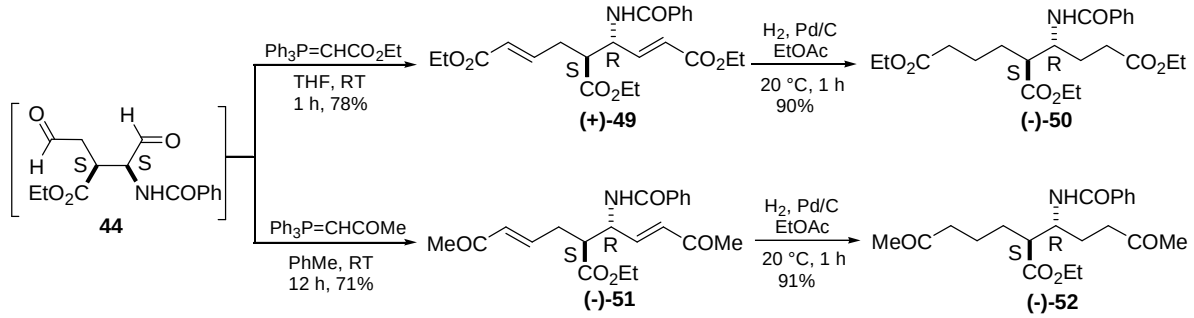
3.3. Synthesis of disubstituted acyclic $\beta^{2,3}$-amino acids from 2-amino-3-cyclopentene carboxylic acid and 2-amino-3-cyclohehexene carboxylic acid

The synthesis of $\beta^{2,3}$-disubstituted open chain amino acid derivatives was approached by the transformation of *cis* and *trans* cyclopentene β-amino acids and cyclohexene β-amino carboxylates through above described strategy. Preliminary experiments were carried out by using racemic substances, and the protocol was further extended in order to get enantiomerically pure products. Optically pure dialkenylated products **(+)-45** and **(-)-47** were prepared from dialdehyde **44** via Wittig reaction. The saturation of these products furnished *anti* β-amino carboxylates **(-)-46** and **(-)-48** (Scheme 9).



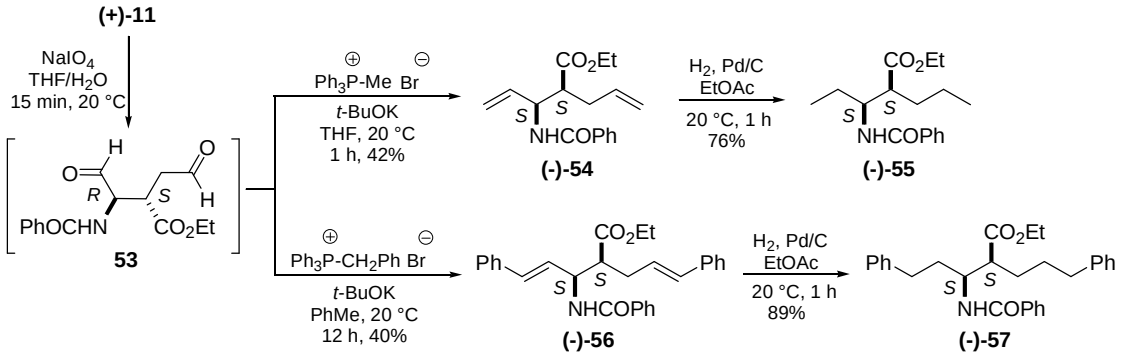
Scheme 9.

Further *anti* β-amino acid derivatives **(+)-49** and **(-)-51** were synthesized by treating the diformyl compound **44** with commercially available phosphoranes. Subsequent catalytic hydrogenation led to enantiomers **(-)-50** and **(-)-52** (Scheme 10).



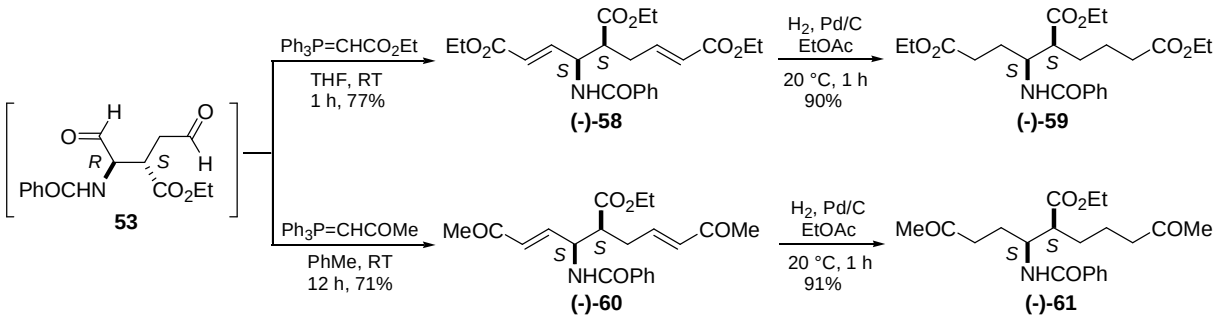
Scheme 10.

Next, enantiomerically pure dialdehyde **53** was transformed into acyclic *syn* Wittig products **(-)-54** and **(-)-56**. Reduction of the olefinic bond afforded final products **(-)-55** and **(-)-57** in 99% enantiomeric purity (Scheme 11).



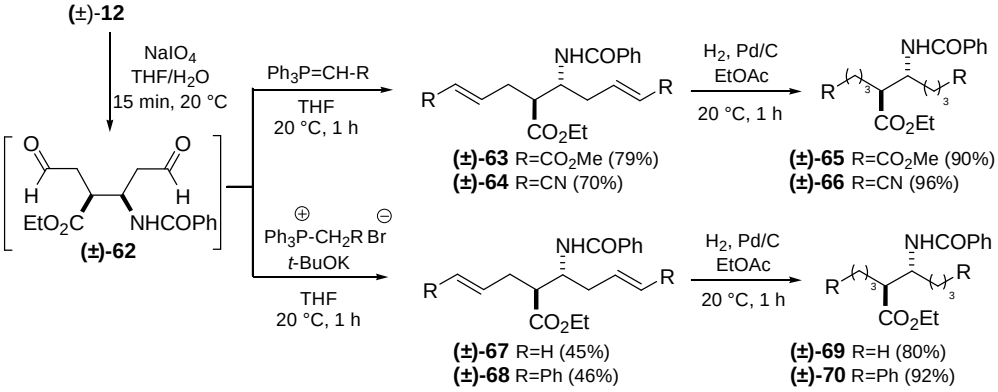
Scheme 11.

Finally, similar synthetic procedures afforded the dialkenylated β-amino acid derivatives **(-)-58** and **(-)-60** in enantiomerically pure form. Catalytic hydrogenation of these products in the presence of Pd/C gave access to *syn* saturated final products **(-)-59** and **(-)-61** (Scheme 12).



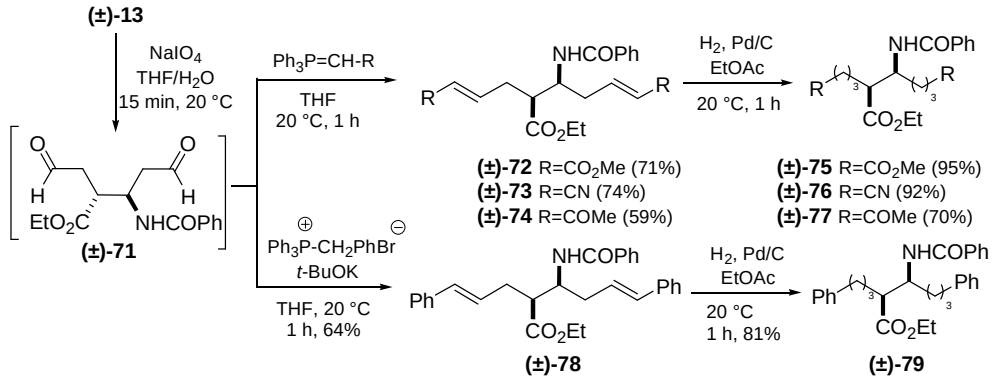
Scheme 12.

Next, diformyl derivative **62** was synthesized from dihydroxylated β-amino ester **12** through oxidative ring cleavage protocol. The Wittig reaction between **62** and methyl (triphenylphosphoranylidene)acetate or (triphenylphosphoranylidene)acetonitrile resulted in *anti* open-chain dialkenylated products **63** and **64**. Following hydrogenation under catalytic conditions furnished saturated β-amino esters **65** and **66** in good yields. Further *anti* disubstituted compounds **67** and **68** were obtained via *in situ* Wittig reaction. Subsequent double bond reduction yielded β-amino acid derivatives **69** and **70** (Scheme 13)



Scheme 13.

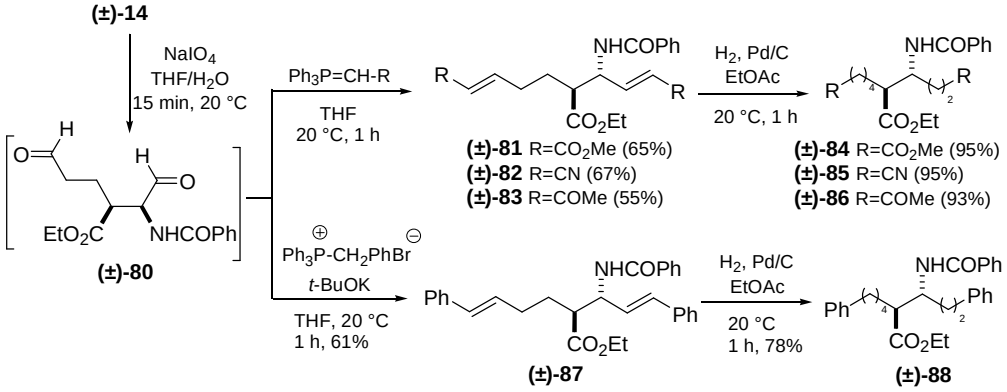
The above synthetic protocol was subsequently extended to the preparation of open-chain *syn* β-amino esters. Difunctionalized Wittig products **72**, **73** and **74** were formed by treating the dialdehyde **71** with commercially available phosphoranes. Following catalytic hydrogenation resulted in saturated acyclic compounds **75**, **76** and **77** in good yields. Then the reaction of dialdehyde **71** with ylide generated from benzyltriphenylphosphonium bromide afforded the disubstituted Wittig product **78**, which was further reduced to derivative **79** (Scheme 14).



Scheme 14.

The dialdehyde **80** was obtained from the dihydroxylated β-amino carboxylate **14** via oxidative ring cleavage. The dialkenylated species **81**, **82**, **83** and **87**, were gained via Wittig

reaction. Subsequent olefinic bond reduction furnished novel *anti* β-amino acid derivatives **84**, **85**, **86** and **88** (Scheme 15).



Scheme 15.

List of publications and lectures

Full papers related to the thesis

I. Loránd Kiss, **Maria Cherepanova**, Enikő Forró, Ferenc Fülöp

A novel access to functionalized cispentacins from norbornene β-amino acids

Chem. Eur. J. **2013**, *19*, 2102. **IF: 5.83***

II. **Maria Cherepanova**, Loránd Kiss, Reijo Sillanpää, Ferenc Fülöp

Synthesis of novel functionalized cispentacins through C-C oxidative cleavage of *diendo*-norbornene β-amino acid

RCS Advances, **2013**, *3*, 9757. **IF: 2.56***

III. **Maria Cherepanova**, Loránd Kiss, Enikő Forró, Ferenc Fülöp

A *de novo* stereocontrolled approach to *syn* and *anti* disubstituted acyclic $\beta^{2,3}$-amino acid enantiomers

Eur. J. Org. Chem. **2014**, 403. **IF: 3.34***

IV. **Maria Cherepanova**, Loránd Kiss, Ferenc Fülöp

Stereocontrolled transformation of cyclohexene β-amino esters into *syn* or *anti* difunctionalized acyclic $\beta^{2,3}$-amino acid derivatives

*Tetrahedron***,** accepted for publication **IF: 2.89***

*2012 impact factors

Scientific lectures related to the thesis

I. **Maria Cherepanova**

Synthesis of novel highly functionalized cyclic β-amino acids

XXXIV. Kémiai Előadói Napok

Szeged, 2011. November 2-4

II. **Maria Cherepanova**

Synthesis of novel highly functionalized cispentacins

A Szegedi ifjú Szerves Kémikusok Támogatásáért Alapítvány Tudományos Előadóülése

Szeged, 2012. May 8.

III. **Maria Cherepanova**, Loránd Kiss, Ferenc Fülöp

Synthesis of novel highly functionalized cispentacins
MTA Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése
Balatonszemes, 2012. June 6-8.

IV. **Maria Cherepanova**, Loránd Kiss, Ferenc Fülöp
Synthesis of novel highly functionalized cyclic β-amino acids
BOSS XII – 13th Belgian Organic Synthesis Symposium,
Leuven, Belgium, 2012. July 15-20.

V. **Maria Cherepanova**, Loránd Kiss, Enikő Forró, Ferenc Fülöp
A *de novo* stereocontrolled approach to *syn* and *anti* disubstituted acyclic $\beta^{2,3}$-amino acids
MTA Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése
Balatonszemes, 2013. June 5-7.