PhD Thesis

Selective functionalization of alicyclic β-amino acids by 1,3-dipolar cycloaddition of nitrile oxides

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1. Introduction and aims

Alicyclic β-amino acids, such as the naturally occurring, antifungal cispentacin [(1*R*,2*S*)-2-aminocyclopentane-1-carboxylic acid] and Icofungipen [(1*R*,2*S*)-2-amino-4-methylidenecyclopentane-1-carboxylic acid], and the antibacterial oryzoxymycin [(*S*)-2-((5*R*,6*R*)-6-amino-5-hydroxycyclohexa-1,3-dienecarbonyloxy)propanoic acid], have attracted considerable interest in the past twenty years because of their pharmacological potential. Cyclic β-amino acids are also of paramount importance in peptide chemistry, since they can be used as building blocks for the preparation of modified biologically active peptide analogues. Carbocyclic β-amino acids are additionally used as starting substances for the synthesis of heterocyclic compounds, potential pharmacons and natural product analogues. Their enantiomerically pure forms can serve as chiral auxiliaries in asymmetric transformations.

One of the main research topics at the Institute of Pharmaceutical Chemistry, University of Szeged, is the synthesis of highly functionalized cyclic β-amino acids. A number of scientific articles have been published in recent years on the selective formation of new functional groups (hydroxy, dihydroxy, amino, azido and fluoro) on cyclic β-amino acids. The 1,3-dipolar cycloaddition of nitrile oxides is a powerful technique for the functionalization of a C-C double bond. The olefinic double bond in the protected cyclic β-amino acids may be utilized as a dipolarophile in a 1,3-dipolar cycloaddition in order to synthetize different functionalized derivatives.

Accordingly, my work focused on the regio- and stereoselective 1,3-dipolar cycloaddition of nitrile oxides to cyclic β-amino acid derivatives. The primary aim was to study the regio- and stereoselectivity of the cycloaddition of nitrile oxides to protected five- or six-membered cyclic β-amino esters, and the transformation of the resulting isoxazoline-fused cyclic β-amino ester carboxylates by reductive ring opening of the isoxazoline skeleton. Moreover, the preparation of cycloadducts in enantiomerically pure form through appropriate enzymatic resolution of bicyclic β-lactams was proposed.

2. Applied methods

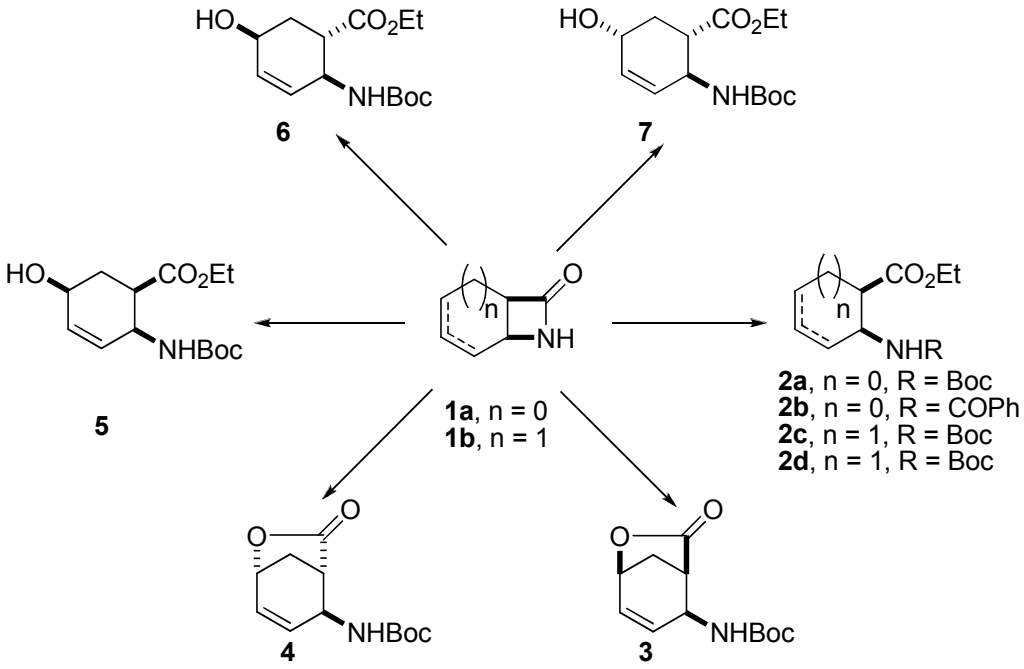
The synthetized compounds were separated and purified by column chromatography on silica gel and by crystallization. The newly prepared products were characterized by

melting point measurements, IR, NMR, mass spectroscopy and elemental analysis. The *ee* values of the optically active compounds were determined by gas chromatography and 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

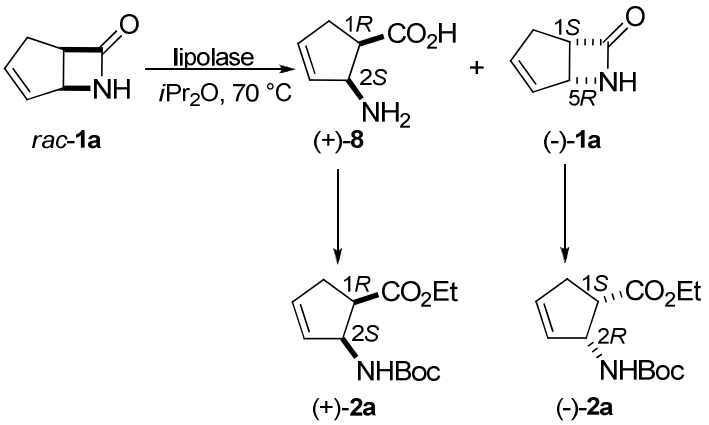
The starting materials **2-7**, as dipolarophiles, were synthetized from bicyclic β-lactams **1a**,**b** (Scheme 1). Dipolarophiles **2a-d** were obtained by lactam ring opening of **1a**,**b**, followed by N-protection (Boc or COPh protocol; Scheme 1).



Scheme 1.

The preparation of compounds **3** and **4** was based on regio- and stereoselective iodolactonization, and a HI elimination reaction. Hydroxylated amino esters **5**, **6** and **7** were synthetized by lactone ring opening of **3** or **4** (Scheme 1).

Enantiomerically pure starting materials (+)-**2a** and (-)-**2a** were also used for the preparation of isoxazoline-fused cyclic β-amino ester carboxylates. Enantiomers (+)-**8a** and (-)-**1a** were synthetized by enzymatic resolution of racemic β-lactam **1a** with *Candida antartica* lipase B (Scheme 2).

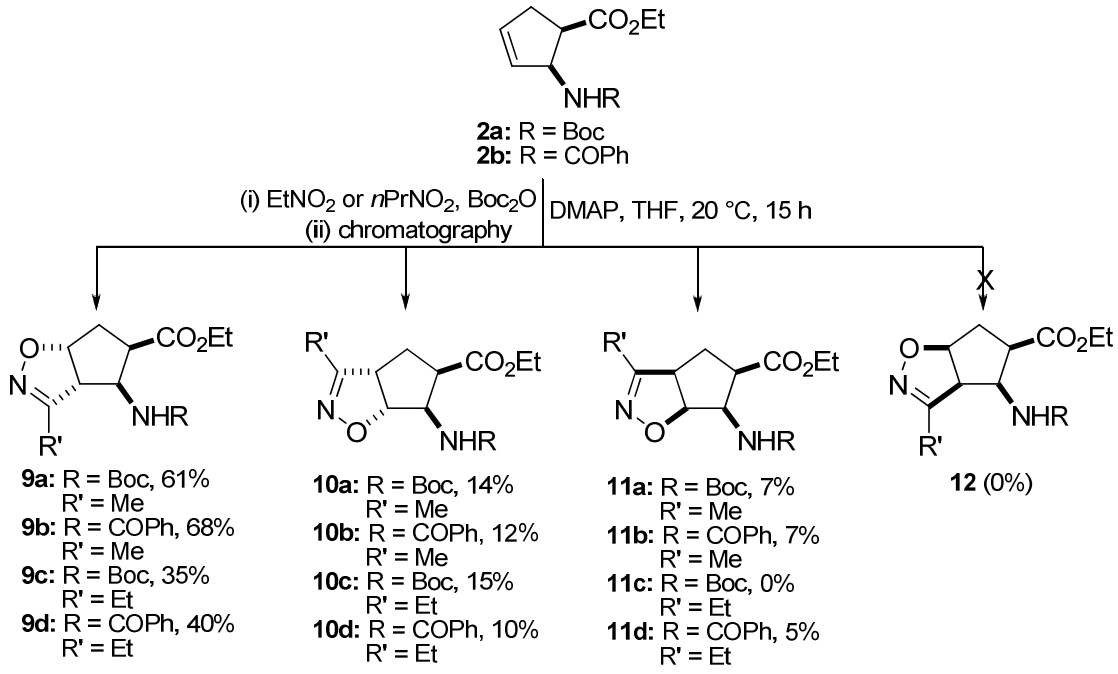


Scheme 2.

The amino acid (+)-**8** and β-lactam (-)-**1a** enantiomers prepared were then transformed to the corresponding enantiomerically pure starting materials (+)-**2a** and (-)-**2a** (Scheme 2).

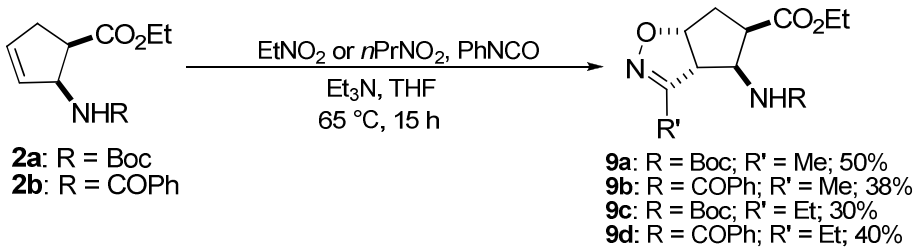
3.2. Synthesis of isoxazoline-fused amino ester carboxylates

Novel isoxazoline-fused *cis*-2-aminocyclopentenecarboxylate stereoisomers **9a-d**, **10a-d**, **11a-d** were synthetized and isolated in good or moderate overall yields by the 1,3-dipolar cycloaddition of nitrile oxide (generated from $EtNO_2$ or $nPrNO_2$, with Boc_2O as dehydrating agent in the presence of DMAP) to *cis*-2-aminocyclopentenecarboxylates (**2a,b**) (Scheme 3).



Scheme 3.

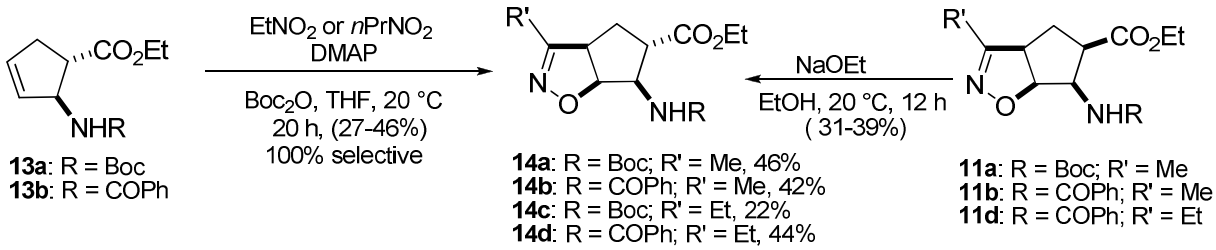
On change of the dehydrating agent Boc_2O to PhNCO, high selectivity was observed in the cycloaddition reaction. Only the previously detected major product **9a-d** was formed and isolated, in moderate yield (Scheme 4).



Scheme 4.

The stereoselectivity of these reactions can be explained by H-bonding steric interactions between the carbamate moiety and the nitrile oxide. The regioselectivity is determined by the electron-withdrawing effect of the N-atom of the amide or carbamate group, favouring the attack of the nitrile oxide O-atom on C-4, distant from the carbamate.

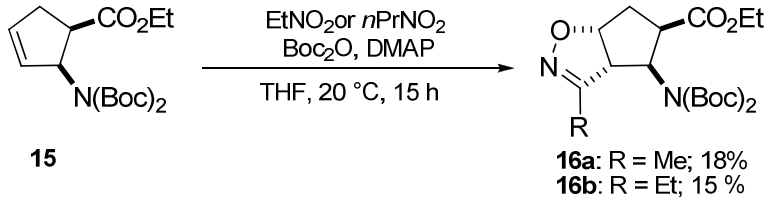
The 1,3-dipolar cycloaddition of nitrile oxides (derived from $EtNO_2$ and $nPrNO_2$, Boc_2O and DMAP) to *trans*-2-aminocyclopentenecarboxylates (**13a**,**b**) resulted 100% regio- and stereoselectively in only one cycloadduct, **14a-d**, which could also be prepared in moderate yield by epimerization of the very minor products **11a-d** at C-5 with NaOEt in EtOH (Scheme 5).



Scheme 5.

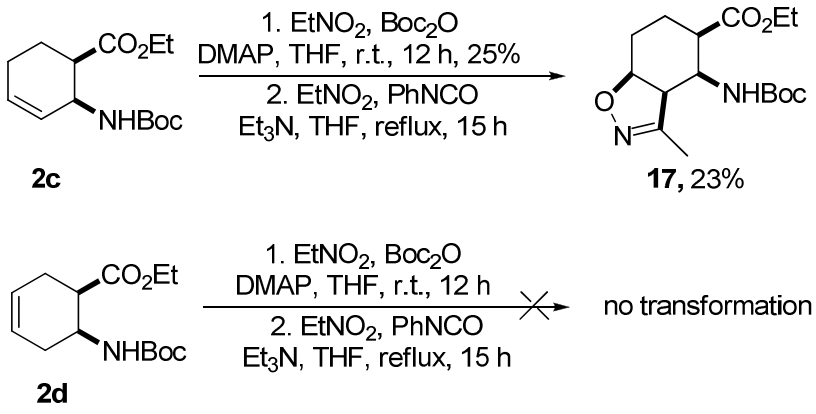
The selectivity in the formation of **14a-d** is explained by steric and H-bonding interactions. Steric repulsion in the transition state between the nitrile oxide and the ester functional group and H-bonding interaction between the carbamate and the nitrile oxide are responsible for the observed selectivity, but it is also determined by the H-bonding effect in the transition state of the reaction.

High selectivity was observed in the dipolar cycloaddition of nitrile oxides when Boc-diprotected *cis* amino ester **15** was used as a starting material (Scheme 6). The yield was rather low: together with the cycloaddition product **16a**,**b**, unreacted starting material **15** was isolated and recovered during the purification. This selectivity may be explained by electronic and steric factors; H-bonding interactions did not arise between the diprotected amino function and the nitrile oxide.



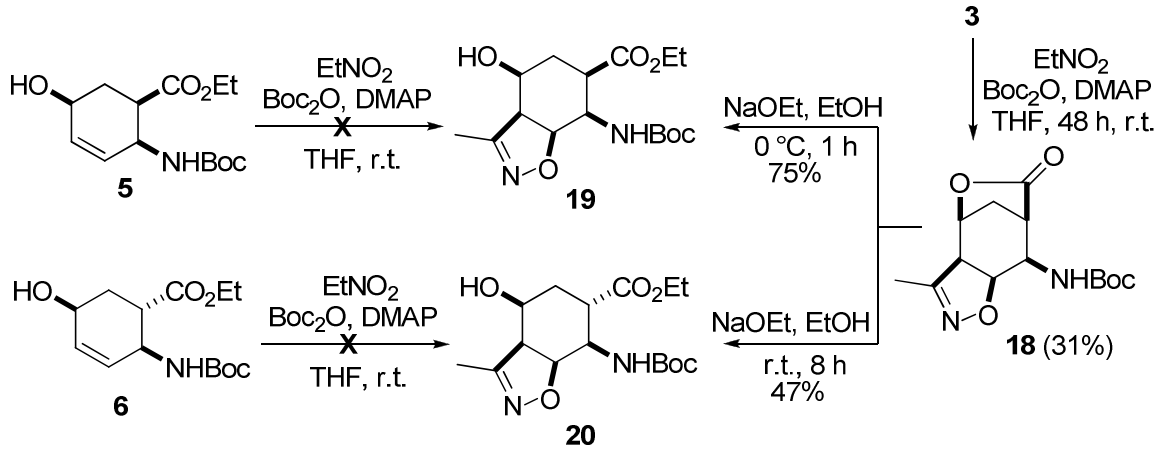
Scheme 6.

On 1,3-dipolar cyloaddition to ethyl *cis*-2-aminocyclohexenecarboxylate **2c**, folloving the use of $EtNO_2$ for the generation of the nitrile oxide, with Boc_2O and PhNCO as dehydrating agent in the presence of base, only one isoxazoline derivative **17** was selectively formed and isolated. When compound **2d** was used as a dipolarophile in the 1,3-dipolar cycloaddition of nitrile oxides, no cycloaddition product was detected (Scheme 7), probably because the C-C double bond is more isolated from the carbamate and ester functions.



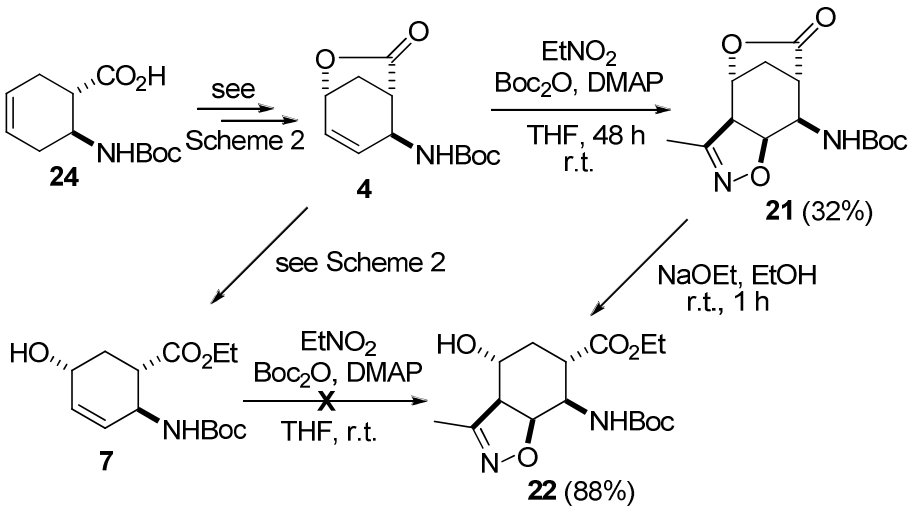
Scheme 7.

Cycloaddition to hydroxylated aminocarboxylates **5**, **6** and **7** was unsuccessful (Schemes 8 and 9). The reactions were attempted under different reaction conditions, such as the Huisgen (from aldoxime) and Mukaiyama (from primary nitroalkanes) methodologies, but only the starting material was recovered.



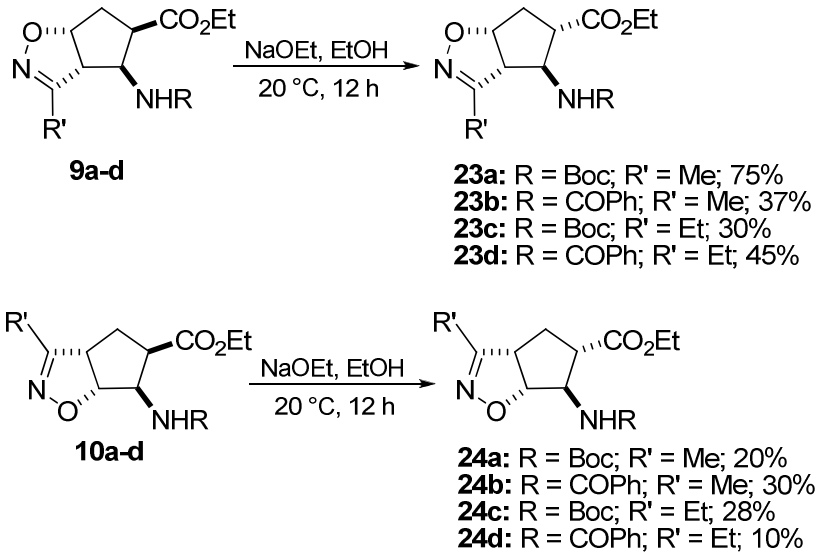
Scheme 8.

The reason for this is probably the reduced reactivity of the isolated ring C-C double bond. In contrast, 100% regio- and stereoselectivity was found in the cycloaddition of nitrile oxide (generated from $EtNO_2$, Boc_2O and DMAP) to *cis* and *trans* lactones **3** and **4**. Only one product, **18** and **21**, respectively, was detected and isolated (Schemes 8 and 9) in each case, in which, as a result of the H-bonding interaction, the isoxazoline ring is *cis* to the carbamate, while the O-atom of the heterocycle is closest to the NHBoc. Next, these compounds were subjected to lactone opening with NaOEt in EtOH, whereby the hydroxylated isoxazoline-fused aminocyclohexanecarboxylates **19**, **20** and **22** were prepared.



Scheme 9.

The isoxazoline-fused *cis*-2-aminocyclopentanecarboxylates **9a-d** and **10a-d** were next transformed by epimerization with NaOEt in EtOH to the corresponding *trans* compounds **23a-d** and **24a-d** (Scheme 10).



Scheme 10.

The isoxazoline-fused β-aminocyclopenatencarboxylates **9a**,**c**, **10a**,**c**, **11a**,**c**, **14a**,**c** and **23a,c** were prepared in enantiomerically pure form, starting from Boc-protected ethyl-2-aminocyclopentene carboxylate enantiomers [(-)-**2a** and (+)-**2a**], which were synthetized by enzymatic resolution of racemic β-lactam (±)-**2a** with *Candida antarctica* lipase B (see Scheme 2, Figure 1). The reactions were performed in the same way as for the racemic compounds (see Schemes 3, 4, 5 and 10).

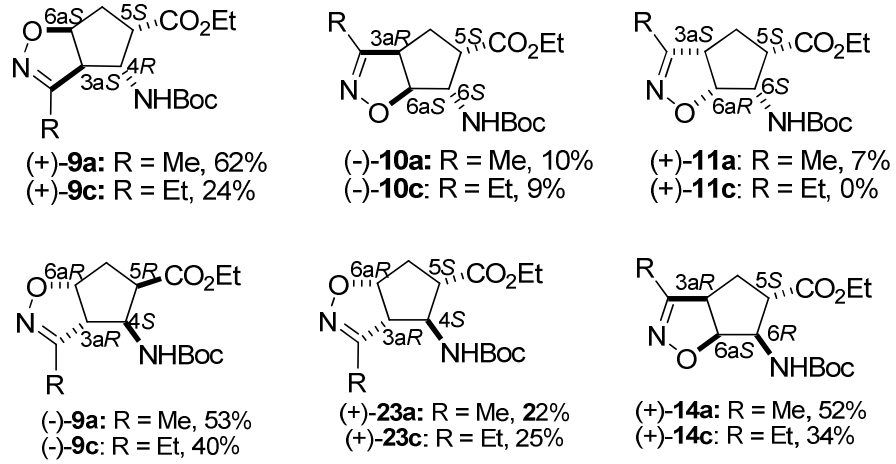
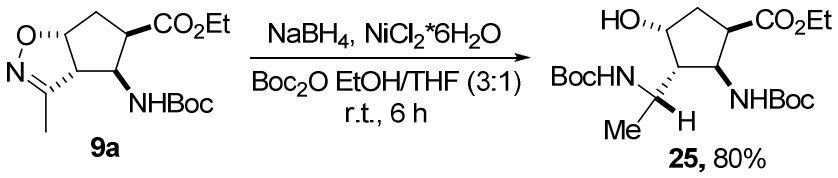


Figure 1.

3.3. Synthesis of highly functionalized β-amino acid derivatives

Highly functionalized β-amino acid derivatives **25-32** with four stereogenic centres were synthetized by reductive ring opening of the isoxazoline ring with $NaBH_4$ in the presence of $NiCl_2$, starting from the earlier prepared isoxazoline-fused β-amino acid derivatives **9a**, **10a**, **23a**, **24a**, **14a**, **19, 20** and **22** (Scheme 11, Figure 2).



Scheme 11.

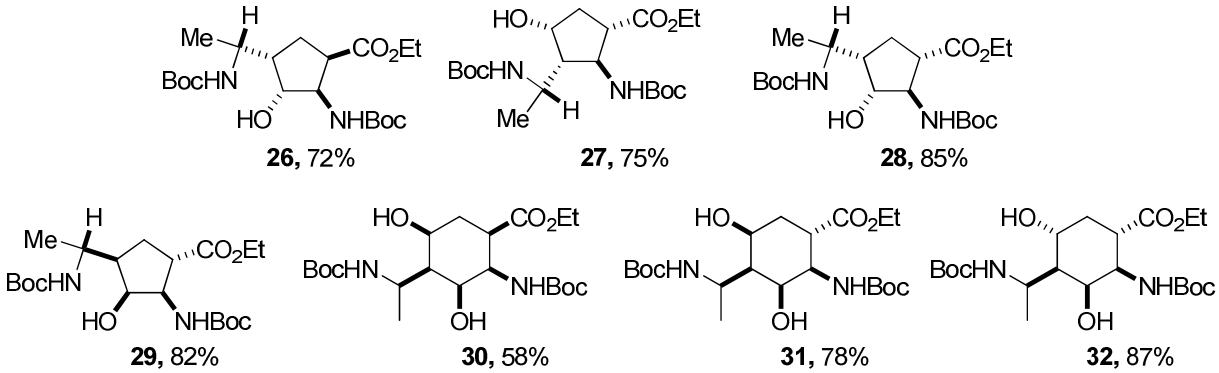


Figure 2.

4. List of publications and lectures

Full papers related to the thesis

I. Loránd Kiss, **Melinda Nonn**, Enikő Forró Reijo Sillanpää, Ferenc Fülöp
Synthesis of novel isoxazoline-fused cispentacin stereoisomers
Tetrahedron Lett., **2009**, *50*, 2605. **IF: 2.61**

II. **Melinda Nonn**, Loránd Kiss, Enikő Forró, Zoltán Mucsi, Ferenc Fülöp
Synthesis of novel isoxazoline-fused cyclic β-amino esters by regio- and stereoselective 1,3-dipolar cycloaddition
Tetrahedron, **2011**, *67*, 4079. **IF: 3.02***

III. **Melinda Nonn**, Loránd Kiss, Reijo Sillanpää, Ferenc Fülöp
Synthesis of highly functionalized β-aminocyclopentanecarboxylate stereoisomers by reductive ring opening reaction of isoxazolines
Beilstein J. Org. Chem., **2012**, *8*, 100. **IF: 2.51***

IV. **Nonn Melinda**, Kiss Loránd, Forró Enikő, Reijo Sillanpää, Mucsi Zoltán, Fülöp Ferenc
Izoxazolin gyűrűvel kondenzált ciszpentacin származékok szintézise
Magyar Kémiai Folyóirat, submitted for publication

V. **Melinda Nonn**, Loránd Kiss, Reijo Sillanpää, Ferenc Fülöp
Selective nitrile oxide dipolar cycloaddition toward the synthesis of highly functionalized β-aminocyclohexanecarboxylate stereoisomers
Tetrahedron, accepted for publication **IF: 3.02***

VI. Loránd Kiss, **Melinda Nonn**, Ferenc Fülöp
Syntheses of isoxazoline-based amino acids by nitrile oxide cycloaddition and their conversion to highly functionalized bioactive amino acid derivatives
Synthesis, **2012**, *44*, 1951. **IF: 2.46***

Other full papers

VII. László Sipos, István Ilisz, **Melinda Nonn**, Ferenc Fülöp, Zoltán Pataj, Daniel W. Armstrong, Antal Péter
High-performance liquid chromatographic enantioseparation of unusual isoxazoline-fused 2-aminocyclopentanecarboxylic acids on macrocyclic glycopeptide-based chiral stationary phases
J. Chromatogr. A., **2012**, *1232*, 142. **IF: 4.53***

VIII. Jessica A. Howard, **Melinda Nonn**, Ferenc Fülöp, Thomas J. Wenzel
Enantiomeric discrimination of isoxazoline-fused β-amino acid derivatives using (18-crown-6)-2,3,11,12-tetracarboxylic acid as a chiral NMR solvating agent
Chirality, accepted for publication **IF: 2.35***

IX. László Sipos, István Ilisz, Anita Aranyi, **Melinda Nonn**, Ferenc Fülöp, Myung Hyun, Antal Péter
High-performance liquid chromatographic enantioseparation of unusual isoxazoline-fused 2-aminocyclopentanecarboxylic acids on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based chiral stationary phases
Chirality, **2012**, DOI: 10.1002/chir.22077 **IF: 2.35***

*2011 impact factors

Scientific lectures related to the thesis

X. **Nonn Melinda**, Kiss Loránd, Forró Enikő, Fülöp Ferenc
Izoxazolin gyűrűvel kondenzált ciszpentacin származékok szintézise
XIV. Nemzetközi Vegyészkonferencia
Cluj Napoca, Romania, 13-15, November, 2008, Abstr.: P52, poster presentation

XI. **Nonn Melinda**
Izoxazolin gyűrűvel kondenzált ciszpentacin származékok szintézise
XXXI. Kémiai Előadói Napok
Szeged, Hungary, 27-29, October, 2008, Abstr.: p. 105, oral presentation

XII. **Nonn Melinda**, Kiss Loránd, Forró Enikő, Fülöp Ferenc
Izoxazolin gyűrűvel kondenzált ciszpentacin származékok regio- és sztereoszelektív szintézise
MTA Heterociklusos Kémiai Munkabizottság Ülése
Balatonszemes, Hungary, 20-22, May, 2009, oral presentation

XIII. **Nonn Melinda**
β-Aminosav származékok funkcionalizálása 1,3-dipoláris cikloaddícióval
Magyar Tudomány Ünnepe − PhD hallgatóink eredményei
Szeged, Hungary, 10, November, 2009, oral presentation

XIV. **Melinda Nonn**, Loránd Kiss, Enikő Forró, Ferenc Fülöp
Regio- and stereoselective 1,3-dipolar cycloaddition of nitrile oxides to ethyl *cis*- or *trans*-2-aminocyclopent-3-enecarboxylates
COST Action CM0803. Foldamers: Building blocks, structure and function

Szeged, Hungary, 24-26, September, 2009, Abstr.: P03, p. 31, poster presentation

XV. **Nonn Melinda**, Kiss Loránd, Forró Enikő, Fülöp Ferenc
Izoxazolin gyűrűvel kondenzált ciklusos β-aminosav származékok szelektív szintézise
MTA Heterociklusos Kémiai Munkabizottság Ülése
Balatonszemes, Hungary, 19-21, May, 2010, oral presentation

XVI. Gert Callebaut, Sven Mangelinckx, **Melinda Nonn**, Loránd Kiss, Ferenc Fülöp, Norbert De Kimpe
Synthesis of α-hydroxy-β,γ-aziridino esters via stereoselective Mannich-type addition of Boc-protected glycolate esters across chiral *N*-sulfinyl α-chloroaldimines
14th SIGMA-ALDRICH Organic Synthesis Meeting
Sol Cress-Spa, Belgium, 2-3, December, 2010, poster presentation

XVII. **Melinda Nonn**, Gert Callebaut, Swen Mangelinckx, Loránd Kiss, Reijo Sillanpää, Ferenc Fülöp, Norbert De Kimpe
Stereoselective Mannich-type reaction of O-protected glycolate esters across N-sulfinyl α-chloro aldimines. Synthesis of α-hydroxy-β,γ-aziridino ester derivatives
Foldamers: Synthesis and Structure of Functional Materials
Barcelona, Spain, 7-9, April, 2011, Abstr.: OC8, p. 24, oral presentation

XVIII. **Melinda Nonn**, Loránd Kiss, Reijo Sillanpää, Ferenc Fülöp
Synthesis of highly functionalized β-aminocyclopentane- or cyclohexanecarboxylate stereoisomers via selective nitrile oxide dipolar cycloaddition
XIVth Conference on Heterocycles in Bio-organic Chemistry
Brno, Czech Republic, 4–8, September, 2011, Abstr.: P23 , poster presentation

XIX. **Nonn Melinda**, Gert Callebaut, Swen Mangelinckx, Kiss Loránd, Reijo Sillanpää, Norbert De Kimpe, Fülöp Ferenc
α-Hidroxi-β, γ-aziridin származékok szintézise
MTA Heterociklusos Kémiai Munkabizottság Ülése
Balatonszemes, Hungary, 26-28, September, 2011, oral presentation