University of Szeged

Faculty of Pharmacy

Institute of Pharmaceutical Chemistry

Stereoselective syntheses and application of steviol- and isosteviol-based bi- and trifunctional chiral ligands

Summary of Ph.D. Thesis

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Introduction and aims

A severe health problem worldwide is posed by the increasing number of cancer cases, with an expectation to reach 24 million by 2035. Despite modern therapies, several limitations are encountered in current cancer treatment, including side effects and the high costs of anticancer agents. Therefore, the development of new compounds, such as cheaper anticancer agents with higher bioactivities and weaker side effects, is deemed imperative. Natural products and their modifications have been deemed vital as anticancer agents, with metabolites of diterpenoids increasingly recognized as a significant part of anticancer drug research.

In recent times, particular attention has been paid to the glycosides of the plant *Stevia rebaudiana*, not only due to their sweeter taste compared to sucrose and their application as a zero-calorie artificial sweetener, but also because of their wide range of biological activities, including antibacterial, antiviral, and anticancer properties. Additionally, isosteviol, a derivative of the stevioside aglycone steviol, has also been found to exhibit several biological activities.

In recent years, the significance of aminodiols and their *N*-heterocyclic analogues as crucial building blocks for complex bioactive molecules with notable biological activities has been underscored. Various recently synthesized aminodiol-based nucleoside analogues have been found to possess cardiovascular, cytostatic, and antiviral effects. The Abbott aminodiol, recognized as a valuable building block for synthesizing the potent renin inhibitors Zankiren® and Enalkiren®, has been integrated into hypertension therapy.

In our study, a series of isosteviol and steviol-based aminodiols and aminoalcohols were synthesized, highlighting the advantage of the antiproliferative activity exhibited by the prepared compounds. Our objective is to observe the structure–activity relationship through an examination of the effects of *N*-substitution, as well as variations in the ester function at position 4, including conversion to a free carboxylic acid function or other ester groups, such as benzyl or methyl functionalities.

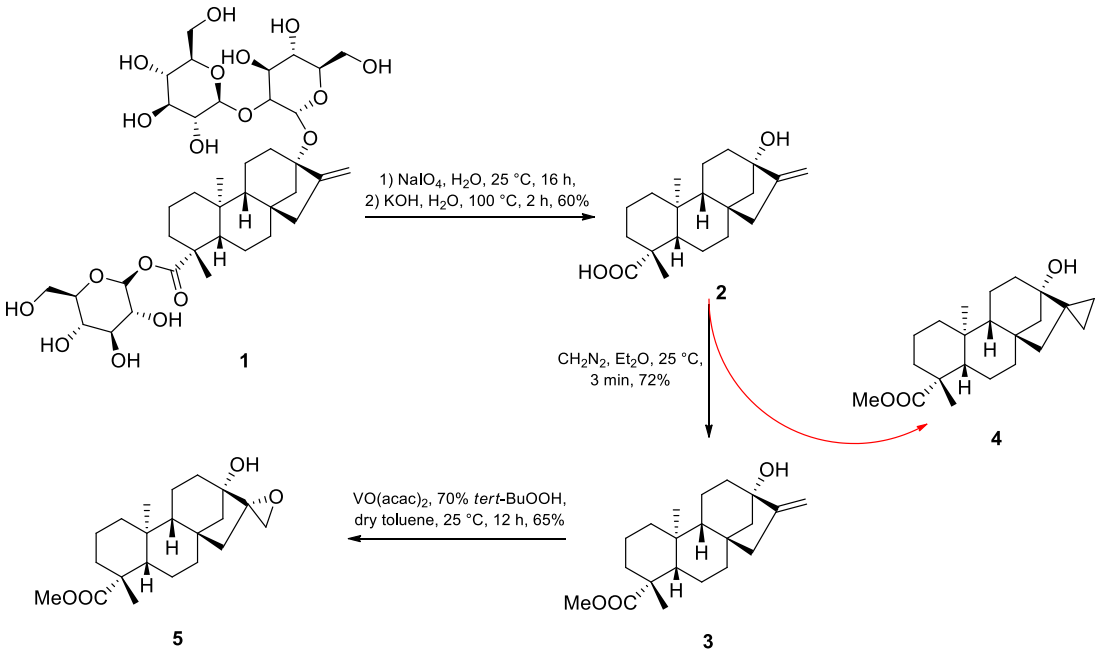
Applied investigation methods

Reactions were performed on a mmol scale, and products were separated and purified by silica gel column chromatography or by recrystallization. All new compounds were characterized by their melting points, NMR, optical rotations and elemental analysis or HRMS. Enantiomeric excess of 1-phenyl-1-propanols in catalytic model reactions was checked by chiral stationary-phase GC. Complex structures were identified by means of two-dimensional NMR techniques (COSY, HSQC, HMBC and NOESY).

Results and discussion

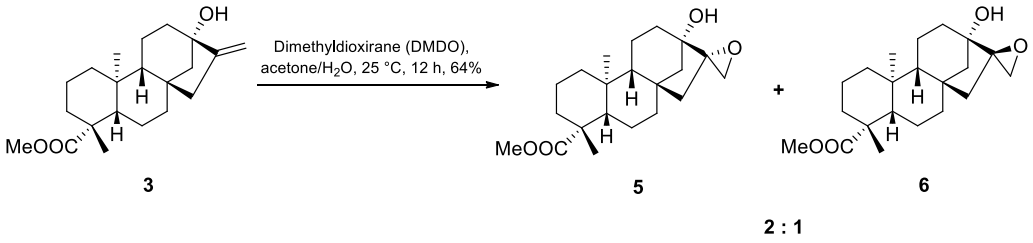
1. Preparation of steviol-based epoxyalcohol key intermediates

To synthesize steviol **2**, we employed two literature methods. In situ prepared diazomethane was used to esterify compound **2**, resulting in the formation of steviol methyl ester **3**. Next, we subjected compound **3** to epoxidation applying *tert*-BuOOH and vanadyl acetylacetonate ($VO(acac)_2$) as the catalyst resulting in *cis*-epoxyalcohol **5**. The reaction proceeded in a stereospecific manner, and the stereochemistry of the product was determined according to literature data (Scheme 1).



Scheme 1.

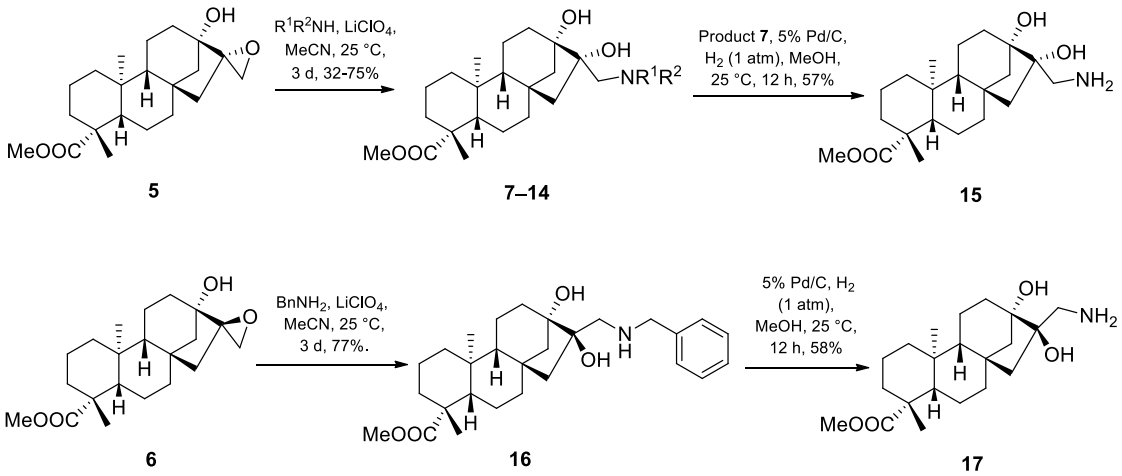
Various methods have been attempted to synthesize diastereoisomeric *trans*-epoxyalcohol **6**. However, in most cases, *cis*-epoxyalcohol **5** was isolated as the sole product. Notably, applying dimethyldioxirane (DMDO) as a mild epoxidation reagent led to the formation of diastereoisomer **6** as a minor component (**5**:**6** = 2:1 ratio), as shown in Scheme 2.



Scheme 2.

2. Synthesis of steviol-based aminodiol derivatives

A diverse library of 3-amino-1,2-diols was efficiently prepared through aminolysis of **5** and **6**. The reaction involved the opening of the oxirane ring of compound **5** with primary and secondary amines in the presence of $LiClO_4$ as a catalyst. The resulting diastereomers were transformed into primary 3-amino-1,2-diols **15** and **17** through hydrogenolysis over Pd/C.

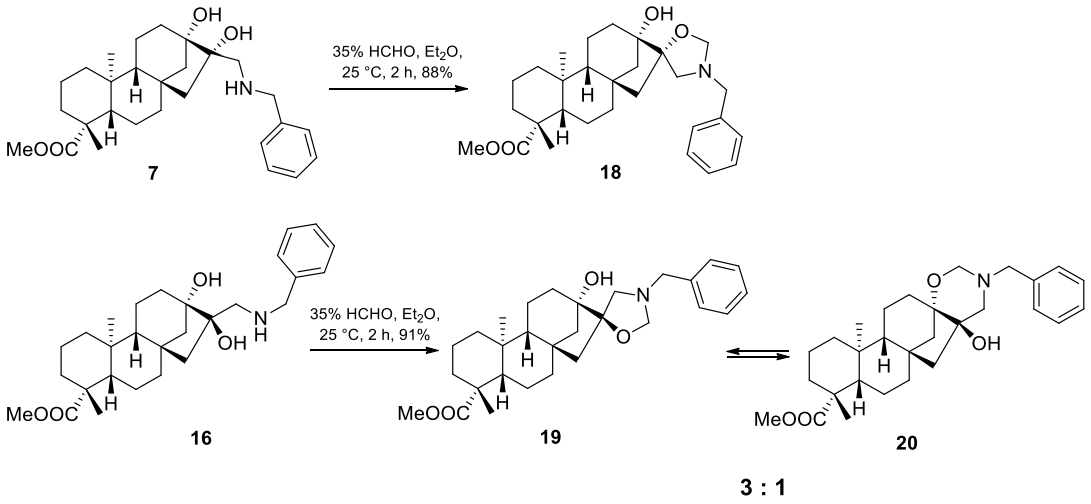


7: R^1 = H, R^2 = Bn, **8**: R^1 = Me, R^2 = Bn, **9**: R^1 = H, R^2 = CH(Me)Ph (*R*), **10**: R^1 = H, R^2 = CH(Me)Ph (*S*), **11**: R^1 = H, R^2 = *i*-Pr, **12**: R^1 = Et, R^2 = Et, **13**: R^1 = H, R^2 = $CH_2C{\equiv}CH$, **14**: R^1 = H, R^2 = 3,5-*Bis*$(CF_3)_2C_6H_3CH_2$

Scheme 3.

Treatment of aminodiol **7** with formaldehyde at room temperature afforded spiro-oxazolidine derivative **18** through a highly regioselective ring-closing reaction. In contrast, when **16** was treated with formaldehyde an interesting phenomenon was observed. Another

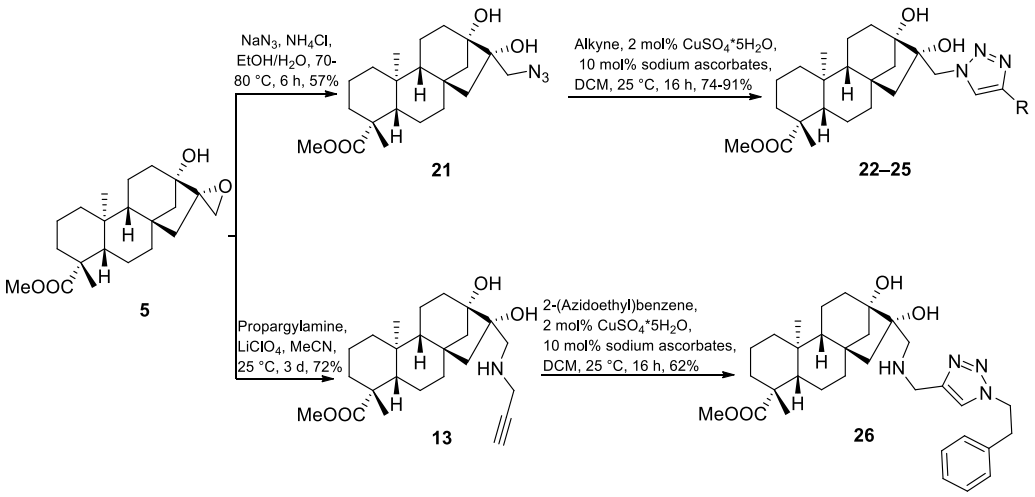
product was observed in small amounts. The new product was a so-called 1,3-oxazine product **20**. After repeated purification, the amount of 1,3-oxazine decreased, but after a certain short time, it increased again until the 1:3 ratio was reached at 25 °C, caused by tautomeric equilibrium process (Scheme 4).



Scheme 4.

3. Synthesis of steviol-based dihydroxytriazoles via “Click” reaction

Reaction of *cis*-epoxyalcohol **5** with sodium azide resulted in azidodiol **21** (Scheme 5). Applying “Click" reaction, triazoles **22–25** were synthesized from **21** with various substituted alkynes. Additionally, a triazol-type tridentate product **26** was synthesized by opposite functionalization, starting from *N*-propargyl-substituted aminodiol **13** and 2-phenylethyl azide.

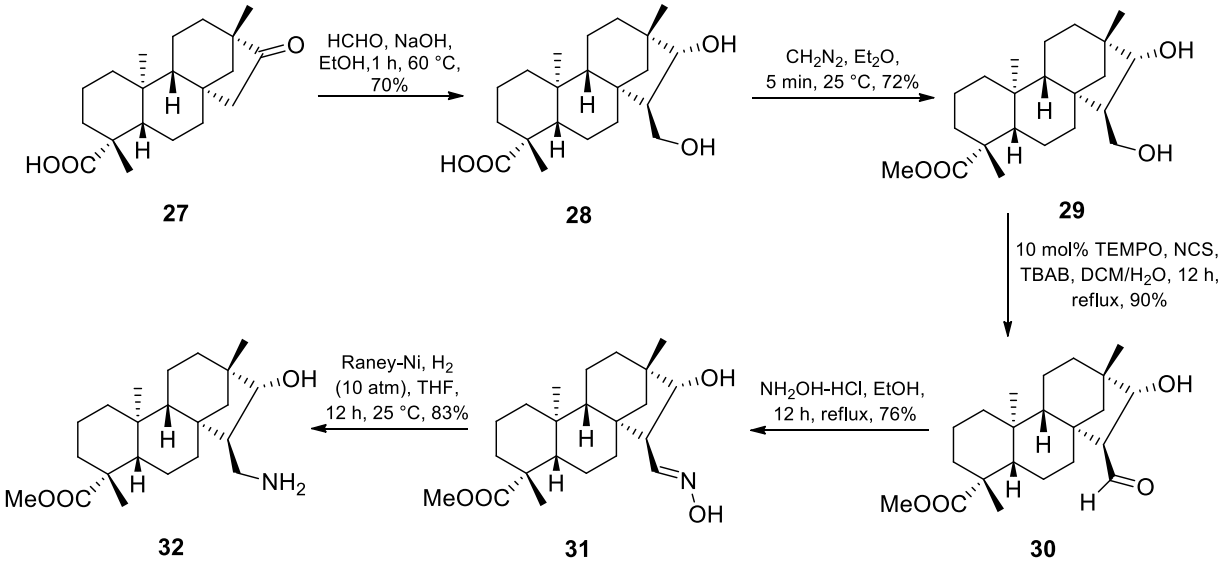


22: R = Ph, **23**: R = $Fe(C_5H_5)_2$, **24**: R = C_5H_5N, **25**: R = C_3H_5

Scheme 5.

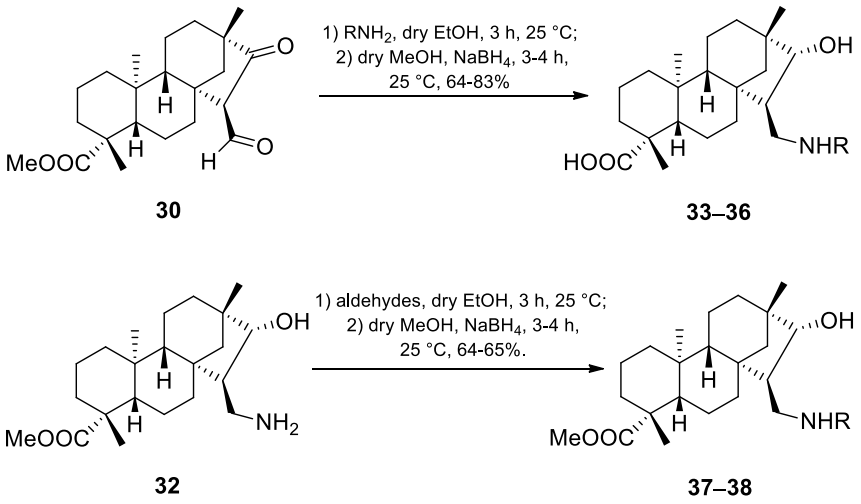
4. Synthesis of isosteviol-based 1,3-aminoalcohols

Key intermediates, 3-hydroxyaldehyde **30** and 1,3-aminoalcohol **32** were synthesized through a four- and six-step synthesis, respectively (Scheme 6). The stereoselective synthesis of diol **28** was accomplished in a one-pot aldol-Cannizzaro process. The esterification of **28** was then carried out with diazomethane, which resulted in methyl ester **29.** The TBAB-catalyzed oxidation of **29** led to the regioselective formation of **30**. Subsequently, compound **31** was synthesized by oximation of **30** with hydroxylamine hydrochloride. Compound **31** was then hydrogenated over Raney-Ni catalyst obtaining 1,3-aminoalcohol **32**.



Scheme 6.

A small library of 1,3-aminoalcohols was also synthesized to investigate the structure-bioactivity relationship of *N*-substitution and antiproliferative activity (Scheme 7). Two pathways were applied to synthesize these compounds (**33–38**): reductive amination of hydroxyaldehyde **30** with primary amines or reductive alkylation of primary 1,3-aminoalcohol **32** with various aldehydes through the formation of Schiff bases, followed by reduction with $NaBH_4$ under mild conditions.

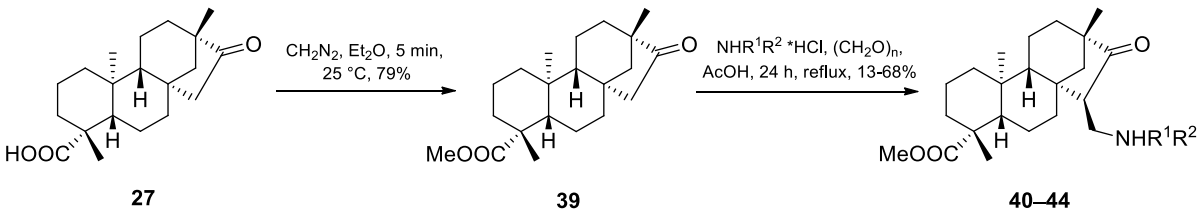


33: R = Me, **34**: R = Bn, **35**: R = CH(Me)Ph (*S*), **36**: R = CH(Me)Ph (*R*), **37**: *p*-Methoxybenzyl, **38**: *p*-Fluorobenzyl

Scheme 7.

5. Syntheses and reduction of isosteviol-based 1,3-aminoketones obtained via Mannich condensation

Isosteviol methyl ester **39** was prepared with an excellent yield from **27** by diazomethane treatment. The Mannich condensation of **39** was than carried out in glacial acetic acid with paraformaldehyde and various secondary amine hydrochloride salts, resulting in a library of aminoketones with good to moderate yields (Scheme 8).



40: $R^1 = R^2 = (CH_2)_2\text{-O-}(CH_2)_2$, **41**: R^1 = Me, R^2 = Bn, **42**: $R^1 = R^2 = (CH_2)_5$,

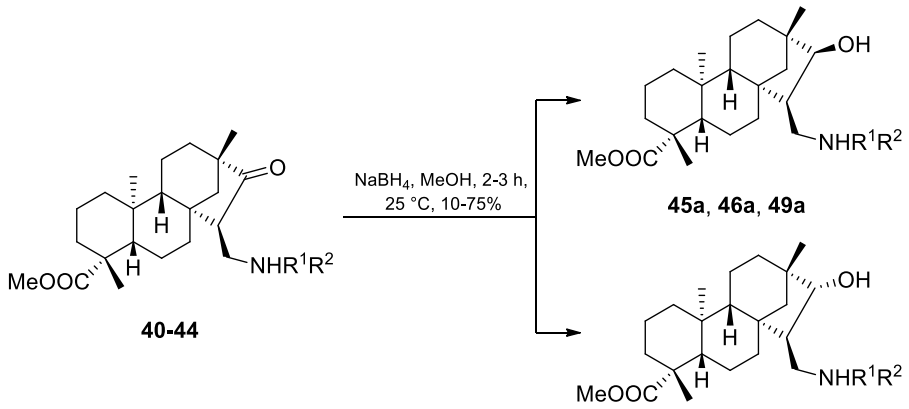
43: R^1 = Me, R^2 = Me, **44**: R^1 = Et, R^2 = Et

Scheme 8.

6. Synthesis of isosteviol-based 1,3-aminoalcohols from 1,3-aminoketones

The reduction of aminoketones **40**–**44** with $NaBH_4$ produced a diastereomeric mixture of 1,3-aminoalcohols in most cases, as shown in Scheme 9. The reduction was selective in the case of **42** or **43** derivatives, resulting in single diastereoisomers, **47** or **48**. However, in other instances, a mixture of diastereomers was produced. The observed diversity in stereoselectivity

during the reduction of aminoketones can be explained by the different steric hindrances of their *N*-substituents.



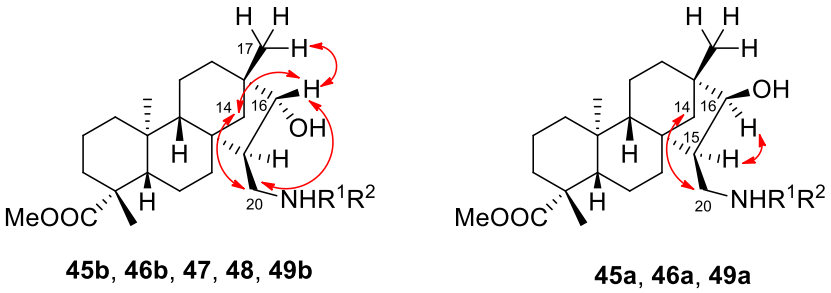
45: $R^1 = R^2 = (CH_2)_2\text{-O-}(CH_2)_2$, **46**: R^1 = Me, R^2 = Bn, **47**: $R^1 = R^2 = (CH_2)_5$,

48: R^1 = Me, R^2 = Me, **49**: R^1 = Et, R^2 = Et

Scheme 9.

7. Determination of the relative configuration

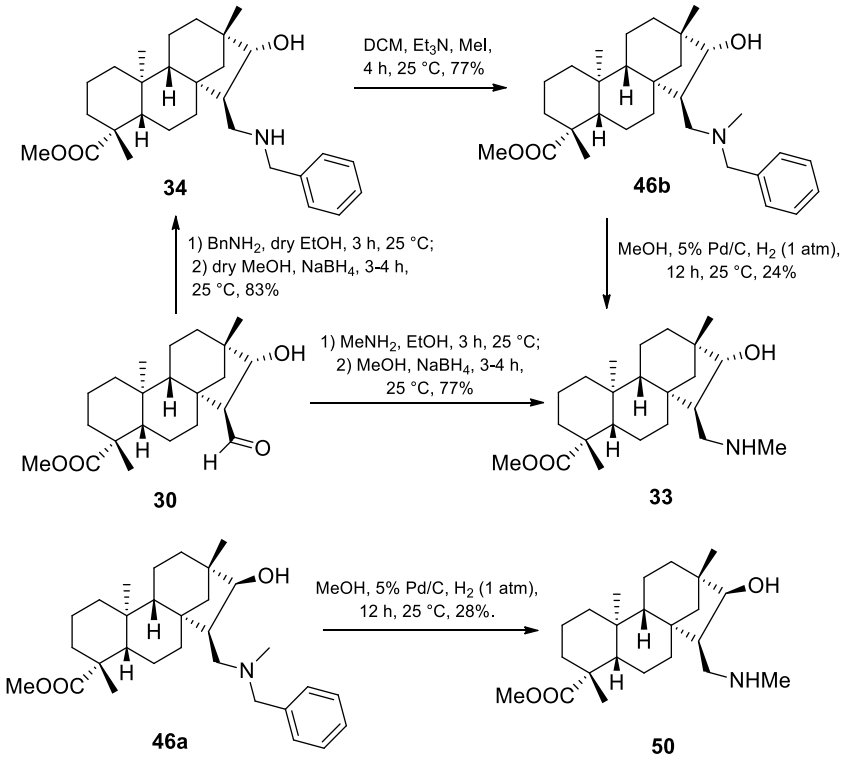
To determine the relative, and therefore the absolute configuration the newly formed chiral centres of aminoalcohols **45**-**49** at positions C-15 and C-16, NOESY spectral analysis was performed (Scheme 10). The analysis was based on the observed NOE effects.



Scheme 10.

In addition, the configurations of the newly formed stereocenters of 1,3-aminoalcohols were determined through synthetic pathways, too (Scheme 11). The reductive amination of known **30** with benzylamine, followed by the methylation of **34** with iodomethane, produced a product identical to **46b**, which was the major product obtained from the reduction of aminoketone **41.** Alternatively, debenzylation of **46a** resulted in *N*-methyl aminoalcohol, which was identical to **40** produced by the reductive amination of **30** with methylamine.

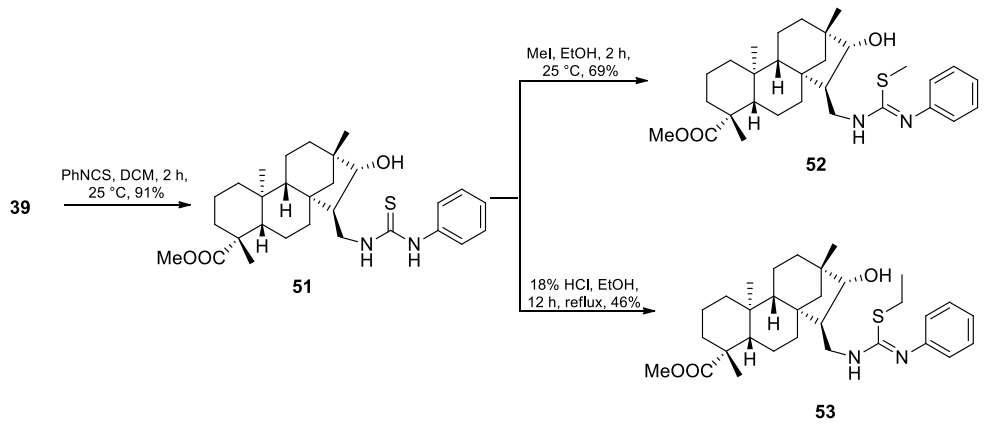
Diastereoisomer **50** was also prepared by debenzylation of **46a**.



Scheme 11.

8. Ring closure attempting of isosteviol-based thiourea derivative

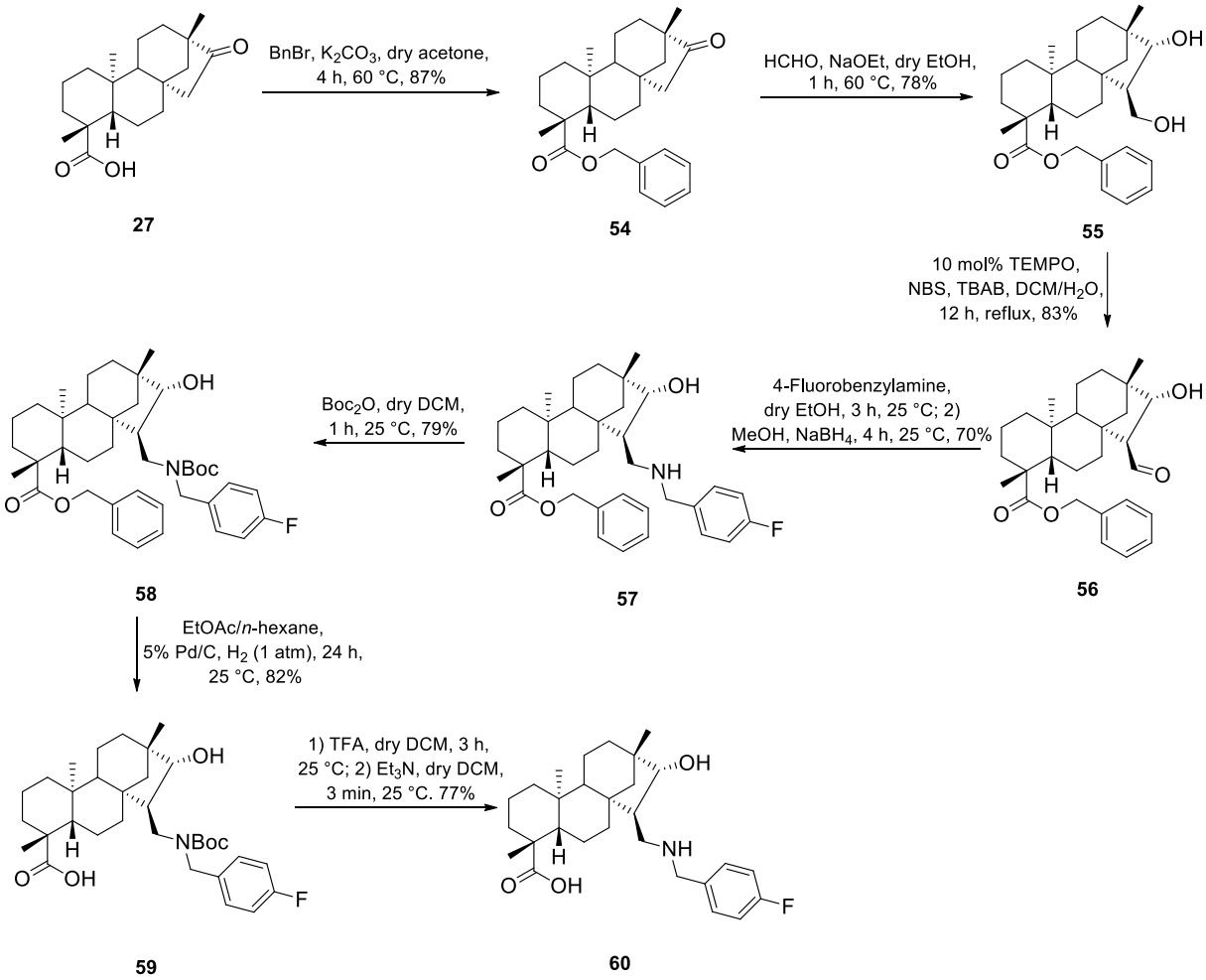
Treatment of **39** with phenyl isothiocyanate resulted in the formation of hydroxy-thiourea **51** (Scheme 12). We attempted to convert **51** to the target compound through a treatment with methyl iodide followed by alkaline-induced elimination of methyl mercaptol, but only methylthio ether intermediate **52** was formed. Alternatively, acid-promoted dehydrative cyclization of **51** yielded only ethylthio ether **53**.



Scheme 12.

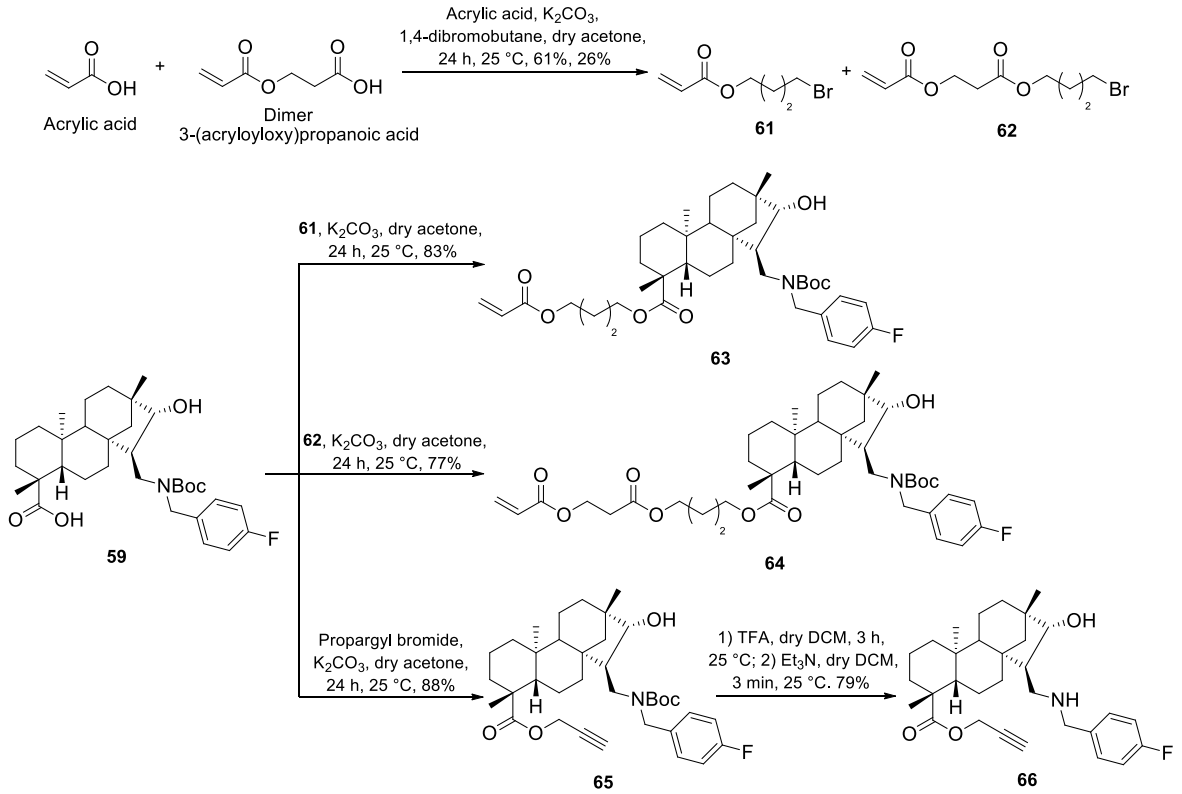
9. Allyl and acetylene derivatives of isosteviol-based 1,3-aminoalcohols

To study the necessity of ester function at position 4, compound **60** with a free carboxylic function was prepared in six steps (Scheme 13). The benzyl ester derivative of isosteviol **54** was obtained by esterification of **27** with benzyl bromide. Benzyl ester diol **55** was prepared in a one-pot aldol-Cannizzaro process. In the next step, compound **56** was prepared by the TBAB-catalysed oxidation of **55**. The synthesis of *N*-4-fluorobenzyl substituted benzyl ester **57** was carried out through a two-step synthesis. A reductive amination of hydroxyaldehyde **56** resulted in a Schiff base, followed by its reduction with $NaBH_4$. To avoid the unavoidable *N*-debenzylation, the nitrogen was protected with the Boc group. Boc-protected **59** with a free carboxyl function was obtained without the elimination of the 4-fluorobenzyl function. The removal of the *N*-Boc-protecting group was achieved by TFA treatment, resulting salt of **60**, followed by neutralisation of the salt with TEA to yield the amphoter **60**.



Scheme 13.

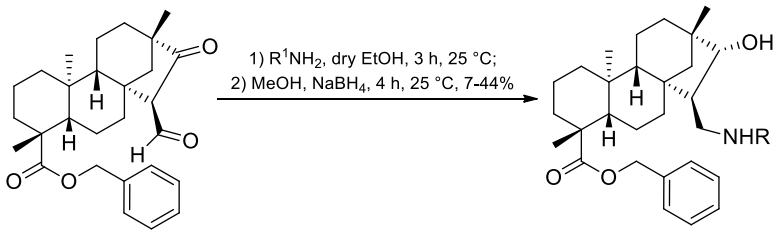
Linker containing an acrylic acid unit was prepared through a simple esterification reaction between acrylic acid and 1,4-dibromobutane (Scheme 14). Acrylic acid esters containing one or two acrylic units were coupled with **59**, resulting in **63** and **64**. *N*-Boc protection was deemed necessary to prevent the *N*-alkylation reaction. Unfortunately, the removal of the Boc groups could not be achieved without decomposition of products. Finally, compound **66** with an alkyne function was prepared by reacting **59** with propargyl bromide, followed by the removal of the Boc-protecting group.



Scheme 14.

10. A library of isosteviol-based 1,3-aminoalcohols with diverse ester groups

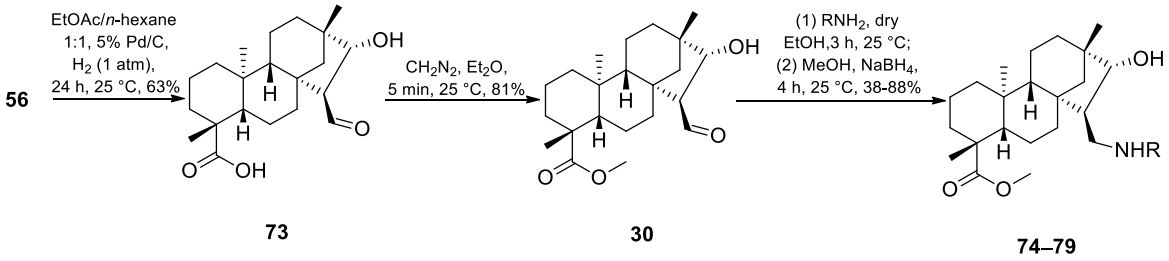
The library of 1,3-aminoalcohols was made to expand by reacting hydroxyaldehyde benzyl ester **56** with further six different primary amines. Hydroxyaldehyde **56** underwent condensation with primary amines to form Schiff bases. The second step involved reduction with $NaBH_4$. The desired *N*-substituted 1,3-aminoalcohols **67–72** were obtained in moderate yields (Scheme 15).



67: R = (*R*)-1-(4-fluorophenyl)ethyl, **68**: R = (*R*)-1-phenylpropyl, **69**: (*S*)-1-phenylpropyl, **70**: R = (*S*)-1-(naphthalen-1-yl)ethyl, **71**: (*R*)-1-(naphthalen-1-yl)ethyl, **72**: 3-(1H-imidazol-1-yl)propyl

Scheme 15.

To determine the cytotoxic effect was contributed to by the various NH-linked units, a 6-membered 1,3-aminoalcohol library was prepared, starting from benzyl ester **56** as a key intermediate. Acid **73** was derived from **56** through debenzylation, followed by re-esterification with diazomethane to obtain **30** (Scheme 16). The 1,3-aminoalcohol library **74–79** was prepared by reacting hydroxyaldehyde **30** with six primary amines, followed by the reduction of the resulting Schiff bases.



74: R = (*R*)-1-(4-fluorophenyl)ethyl, **75**: R = (*R*)-1-phenylpropyl, **76**: (*S*)-1-phenylpropyl, **77**: R = (*S*)-1-(naphthalen-1-yl)ethyl, **78**: (*R*)-1-(naphthalen-1-yl)ethyl, **79**: 3-(*1H*-imidazol-1-yl)propyl

Scheme 16.

11. Application of steviol-based chiral aminodiols as chiral catalyst

The catalytic effect of aminodiol derivatives **7–26** was investigated using them as chiral catalysts on the formation of (*S*)- or (*R*)-1-phenyl-1-propanol by the enantioselective addition of diethylzinc to benzaldehyde. Low to moderate enantioselectivities were observed. The formation of the (*R*)-enantiomer was favoured by all aminodiol derivatives. The best *ee* value (*ee* = 52%) with an (*R*)-selectivity was achieved by aminodiol **8**, which still showed moderate results.

12. Antiproliferative activity of steviol-based 3-amino-1,2-diol and isosteviol-based 1,3-aminoalcohol derivatives

The antiproliferative activities of the prepared diterpene analogues were determined on a panel of human adherent cancer cell lines. Testing the set of aminodiol analogues (**7**–**14**) with secondary or tertiary amino function, it was consistently found that the *N*-benzyl substituent is an essential part of the molecule.

Antiproliferative action was exerted by primary amine **32** as well as secondary amine derivatives **33**–**38**, and the calculated IC_{50} values of these compounds are comparable to or lower than those of cisplatin. Aminoalcohol function and the *N*-benzyl type substitution (**34**–**38**) are essential for the remarkable antiproliferative activity. No consistent and substantial difference was recognized comparing the activity of the benzyl esters (**67**–**72**) and their methyl analogues (**74**–**79**), indicating that the behaviour of ester function had no crucial impact on the antiproliferative properties. Compound **72** substituted with the *N*-(1*H*-imidazol-1-yl)propyl group was proven to be the most active derivative (best IC_{50} value: 1.37 μM for MCF-7 cells).

Conclusions

During the experimental work, more than 70 new compounds were synthesized, purified, and characterized starting from commercially available diterpenes. The prepared compounds were tested as chiral catalysts in the nucleophilic addition of diethylzinc to benzaldehyde and their antiproliferative activity.

Publications related to the thesis

[1] **Dániel Ozsvár**, Viktória Nagy, István Zupkó, Zsolt Szakonyi
Stereoselective Synthesis and Antiproliferative Activity of Steviol-Based Diterpen Aminodiols *International Journal of Molecular Sciences*, 2020, *1*(1), 184-200.

IF: 5.923

[2] **Dániel Ozsvár**, Viktória Nagy, István Zupkó, Zsolt Szakonyi
Synthesis and Biological Application of Isosteviol-Based 1,3-Aminoalcohols
International Journal of Molecular Sciences, 2021, *22*(20), 11232-11241.

IF: 6.208

[3] **Dániel Ozsvár**, Noémi Bózsity, István Zupkó, Zsolt Szakonyi
Synthesis and Study of the Structure–Activity Relationship of Antiproliferative *N*-Substituted Isosteviol-Based 1,3-Aminoalcohols
Pharmaceuticals, 2024, *17*, 262-279.

IF: 4.600 (2022)

Publications are not related to the thesis

[1] Sándor B. Ötvös, Ádám Georgiádes, **Dániel Ozsvár**, Ferenc Fülöp
Continuous-flow synthesis of 3,5-disubstituted pyrazoles via sequential alkyne homocoupling and Cope-type hydroamination
Royal Society of Chemistry Advances, 2019, 9, 8197-8203.

IF: 3.119

[2] Bence Kutus, **Dániel Ozsvár**, Norbert Varga, István Pálinkó, Pál Sipos
ML and ML2 complex formation between Ca(II) and *D*-glucose derivatives in aqueous solutions
Royal Society of Chemistry Dalton Transactions, 2017,46, 1065-1074.

IF: 4.029

Total impact factor: 23.879

Scientific lectures

Szakonyi Zsolt, **Ozsvár Dániel**, Bai Dorottya, Nagy Viktória, Zupkó István
Stereoselective synthesis and antiproliferative activity of stevioland isosteviol-based bi- and trifunctionalized diterpenoids
Southern Brazilian Journal of Chemistry 2022: Suppl pp. 382-384., 3 p. (2022) oral presentation

Ozsvár Dániel, Nagy Viktória, Zupkó István, Szakonyi Zsolt
Ent-kauránvázas, diterpenoid királis aminodiolok sztereoszelektív előállítása, katalitikus alkalmazása és citotoxicitás vizsgálta
MTA Szteroid- és Terpenoidkémiai Munkabizottság,
Szeged, November 22, 2019, oral presentation

Dániel Ozsvár
Diterpénvázas királis aminodiolok sztereoszelektív előállítása és alkalmazása királis katalizátorként
XLII. Kémiai Előadói Napok
Szeged, 28th-30th October 2019, oral presentation

Dániel Ozsvár, Zsolt Szakonyi
Stereoselective synthesis and the application of diterpene-based chiral aminodiols
18th Blue Danube Symposium on Heterocyclic Chemistry
Ljubljana, Slovenia, 18th-21st September 2019, poster presentation