University of Szeged
Faculty of Pharmacy
Institute of Pharmacognosy

# Semisynthesis from of *α,β*–unsaturated oxo-terpenes: studies on monoterpene aminodiol catalysts and bioactive ecdysteroid derivatives

Ph.D. Thesis Summary

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# Semisynthesis from of *α,β*–unsaturated oxo-terpenes: studies on monoterpene aminodiol catalysts and bioactive ecdysteroid derivatives

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Szeged
2025

# Introduction

*α,β*-Unsaturated carbonyl compounds represent a distinct class of natural products. Their unique structural features make them suitable for diverse chemical transformations. Due to their conjugated system, one of the most common reactions they undergo is nucleophilic addition. Our scientific interest has focused on the structural modification of natural compounds containing this motif, including the monoterpenoid perillaldehyde, the ecdysteroid 20-hydroxyecdysone, and cinnamic acid as a representative phenolic carboxylic acid.

Monoterpenoids are $C_{10}$ terpenes that consist of two isoprene units, with more than 1,000 naturally occurring representatives identified to date. They can be classified into volatile essential oil components and non-volatile iridoids. Essential oils can be extracted by steam distillation, a technique suitable for obtaining perillaldehyde, one of the starting materials of this dissertation, from the aerial parts of *Perilla frutescens*. Our research group has extensive experience in the catalytic application of monoterpenoids in organocatalytic reactions involving aromatic aldehydes. Because of their availability and inherent chirality, monoterpenoids are excellent starting materials for asymmetric synthesis. The development of chiral catalysts remains a key focus in pharmaceutical chemistry. In this context, our objective was to synthesise novel chiral monoterpenoid-based catalysts. In previous studies conducted by our group, chiral 3-amino-1,2-diols were synthesised from limonene-derived perillaldehyde and pulegone. The use of these substances as catalysts provides varying degrees of *ee* in the reaction of benzaldehyde and diethylzinc, depending on the properties of the substituents, thus helping to understand the reaction mechanism. In addition, aminodiol functionalities are present in several important drug candidates. These structures can be accessed through various synthetic strategies; in the present work, we used the dihydroxylation of allylamines for their preparation.

Ecdysteroids are steroidal compounds of triterpenoid origin, with natural representatives that are polar compounds containing multiple hydroxyl groups. A characteristic structural feature of these compounds is the pharmacophore *α,β*-unsaturated ketone moiety (7-ene-6-one) in the B ring. Ecdysteroids exert a variety of beneficial effects in mammals, including anabolic, adaptogenic, antidiabetic, and wound-healing activities. For the chemical and biological characterisation of these compounds, the online database *Ecdybase* is available, which currently contains information on 590 naturally occurring ecdysteroids (as of April 2025). In recent years, there has been a marked increase in interest in this class of bioactive compounds. In particular, 20-hydroxyecdysone (20E) has been included in the World Anti-Doping Agency (WADA) monitoring program for six consecutive years. Advanced clinical trials are also underway,

which show that 20E is effective and safe in the treatment of sarcopenia in the elderly and as a life-saving lung protective agent in severely ill SARS-CoV-2 infected patients.

Natural and semisynthetic ecdysteroids have been subjected to relatively few studies regarding their antibacterial and antifungal properties, and the available data indicate only modest activity against various pathogenic microorganisms. As part of our research, our objective was to evaluate the antifungal potential of 20E and selected nitrogen-containing derivatives. Several natural compounds, including certain steroid derivatives, have been reported to exhibit activity against *Trypanosoma cruzi*. Previous studies have shown that ecdysone affects *Rhodnius prolixus*, one of the main vectors of Chagas disease, and also influences the development and differentiation of the protozoan parasite within the insect host. Based on these observations, we considered it worthwhile to initiate the screening of a structurally diverse ecdysteroid library against *T. cruzi*.

## Objectives

We aimed to expand the chemical space of novel limonene-based chiral monoterpenoids, investigate the anticryptococcal and antitrypanosomal activity of ecdysteroids, and develop new, more potent derivatives. Accordingly, the objectives of our work were as follows:

**1. Stereoselective synthesis of aminodiol derivatives from perillaldehyde.** We aimed to prepare a diastereo- and regioisomeric library of 3-amino-1,2-diols from perillaldehyde, using reductive amination and the Overman rearrangement, followed by dihydroxylation.

**2. Investigation of the catalytic activity of aminodiols in the reaction of diethylzinc with aromatic aldehydes.** The catalytic activities were planned to be evaluated in the enantioselective addition of diethylzinc to benzaldehyde and, in some selective cases, on further aromatic aldehydes.

**3. Semisynthesis of imine and cinnamate ester derivatives from 20-hydroxyecdysone.** The designed derivatives included 20-hydroxyecdysone cinnamates, oximes, and oxime ethers, as well as a B-ring-extended lactam.

**4. Ecdysteroids as bioactive agents: evaluation of natural or semisynthetic derivatives.** A structurally versatile library of natural and semisynthetic ecdysteroids' *in vitro* anticryptococcal and trypanocidal activities was aimed to be investigated in research collaborations.

## Materials and Methods

Starting materials: (–)-(*S*)-perillalldehyde was purchased from Merck Co. (Merck Co., Darmstadt, Germany), 20-hydroxyecdysone (20E) was acquired from Shaanxi KingSci Biotechnology (Xi'an, China) with a purity of 90%, and was recrystallised applying an EtOAc/MeOH:2/1 (*v/v*) mixture, achieving >97% purity as determined by RP-HPLC. Perillaldehyde and 20E were applied as starting materials in subsequent synthetic work.

Chromatographic procedures for purifications: The synthesised monoterpenoids were purified using normal phase column chromatography on silica gel. The semisynthetic ecdysteroid derivatives were purified via normal-phase flash chromatography and reversed-phase high-performance liquid chromatography (RP-HPLC).

Procedures for structure elucidation: The structural determination of the gained products was carried out by applying multiple spectroscopic techniques such as HR-MS, 1D and 2D-NMR and X-ray crystallography on compound **7a**.

Computational analysis using DFT methods: DFT (Density Functional Theory) calculations were carried out in research collaboration. Gaussin 09 Revision A 02 software was used for the analyses.

Procedure for the reaction of aromatic aldehydes with diethylzinc in the presence of chiral catalysts: The catalytic activity of aminodiols was measured in the reaction of diethylzinc with benzaldehyde and further aromatic aldehydes. To determine the enantiomeric excess, the obtained products were derivatised by acetylation and analysed via chiral phase GC or HPLC.

Antifungal investigation of ecdysteroid derivatives: Bioactivity testing was conducted in a research collaboration. The mode of interaction was assessed using the Abbot formula. The inhibitory activity assay of some selected ecdysteroids against *Cryptococcus neoformans* was performed on a 96-well microtiter plate. The optical density of the cultures was measured on a nano plate reader. The viability of *C. neoformans* IFM 5844 cells was assessed using the calcein-AM assay and analysed by flow cytometry. Selected ecdysteroid derivatives were subjected to a combination treatment with efflux pump inhibitors, employing the checkerboard titration method. The outcomes were evaluated using a nano plate reader.

Trypanocidal investigation of ecdysteroid derivatives: The measurement of antitrypanosomal activity was carried out in research collaboration. The cytotoxic or antiproliferative effects of ecdysteroids on mammalian cells were assessed using the MTT assay with CHO and

RAW264.7 cell lines. The cells were cultured in 96-well plates, and the absorbance values were recorded using a microplate reader. Trypanocidal activity against epimastigotes was evaluated by XTT assay. The Trypanosoma cruzi epimastigotes were cultured in 96-well plates. Benznidazole was applied as the positive control. XTT and PMS solution was added and the plates were incubated. After fixation of the parasites, absorbance was measured using a plate reader. The $IC_{50}$ values were calculated using GraphPad Prism version 8.0. The fluorescence-activated cell sorting-based (FACS) quantitation of released parasites from infected cells was carried out in 24-well plates with the host RAW264.7 cells. For microscopic measurements, host CCL39 cells were seeded in 24-well plates and treated for four days. Subsequently, the cells were fixed with paraformaldehyde and scanned on a microscope.

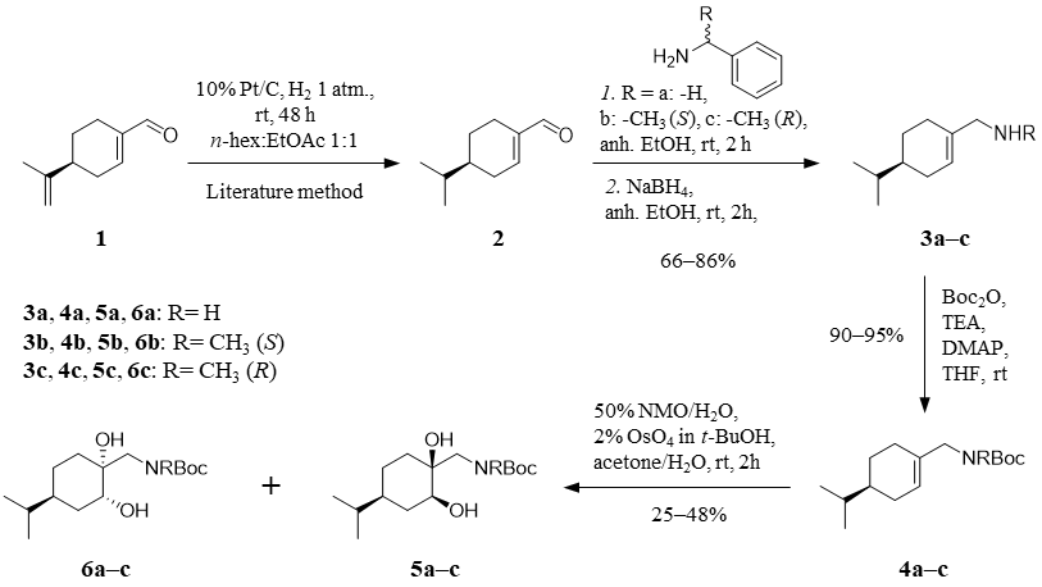
## Results and Discussion

### *Monoterpenoids*

#### Preparation of aminodiols starting from perillaldehyde

To synthesise the target aminodiols, commercially available (–)-(*S*)-perillaldehyde,a limonene-based compound with limited prior evaluation as a catalytic scaffold, was selected as the starting material. Given our objective to comprehensively investigate its catalytic potential, we designed two synthetic approaches to obtain distinct sets of 3-amino-1,2-diol derivatives from perillaldehyde. In both synthetic routes, the preparation of dihydroperillaldehyde proved to be a critical step. The presence of the *exo*cyclic double bond in the starting material was anticipated to contribute to undesired side-product formation during subsequent dihydroxylation. Therefore, in the initial step, we established optimised conditions for the selective hydrogenation of the *exo*cyclic double bond, on literature protocols, employing Pt/C as the catalyst. The progress of the reaction and the formation of compound **2** were monitored by $^1$H NMR spectroscopy. In the first approach, a series of aromatic allylamines were synthesised via Schiff base formation using various benzylamines, as well as both (*R*)- and (*S*)-phenylethylamines. The resulting intermediates (**3a–c**) were subsequently protected with a Boc group and then subjected to dihydroxylation using $OsO_4$ and NMO as the oxidising system. This transformation yielded two diastereomeric series of Boc-protected aminodiols (**5a–c** and **6a–c**). The corresponding reaction sequence is illustrated in **Scheme 1**.

Following the dihydroxylation step, the Boc protecting group was removed using hydrochloric acid, yielding the free aminodiols **7a–c**. Structural identification of the compounds was performed by HR-MS and 1D and 2D NMR spectroscopy.




**Scheme 1.** Preparation of protected aminodiols through reductive amination

The absolute configuration of compound **7a** was determined through NOESY NMR spectroscopy and single-crystal X-ray diffraction analysis, as illustrated in the accompanying **Figure 1**. The X-ray diffraction analysis was performed in research collaboration by *Prof. Dr. Matti Haukka* at the University of Jyväskylä.

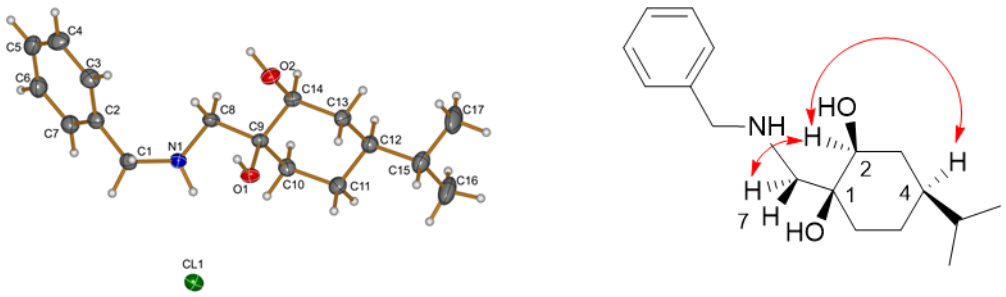
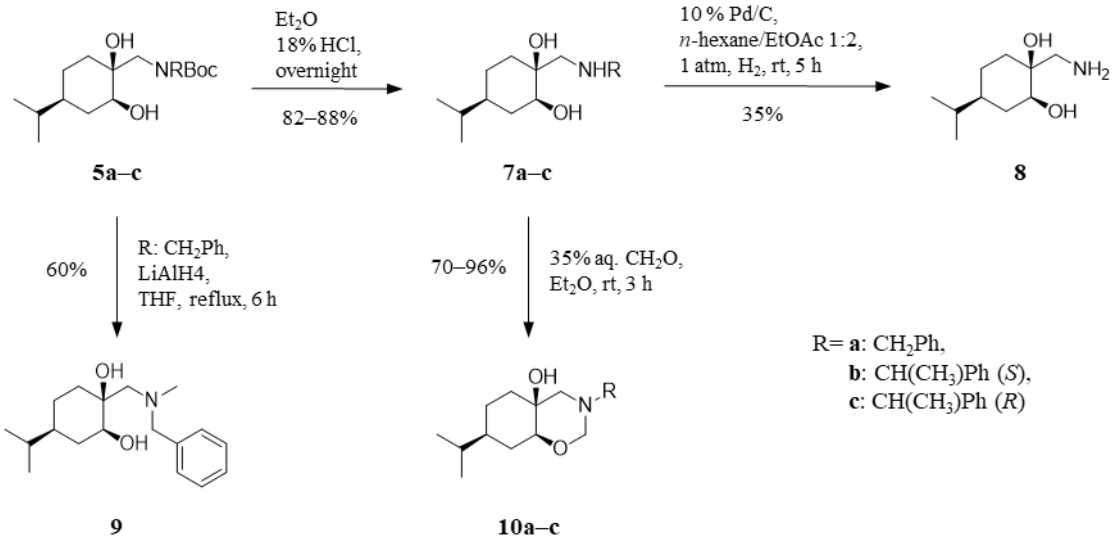



**Figure 1.** Absolute configuration of compound **7a** (**A**) as determined by single-crystal X-ray diffraction and supported by NOESY interactions (**B**)

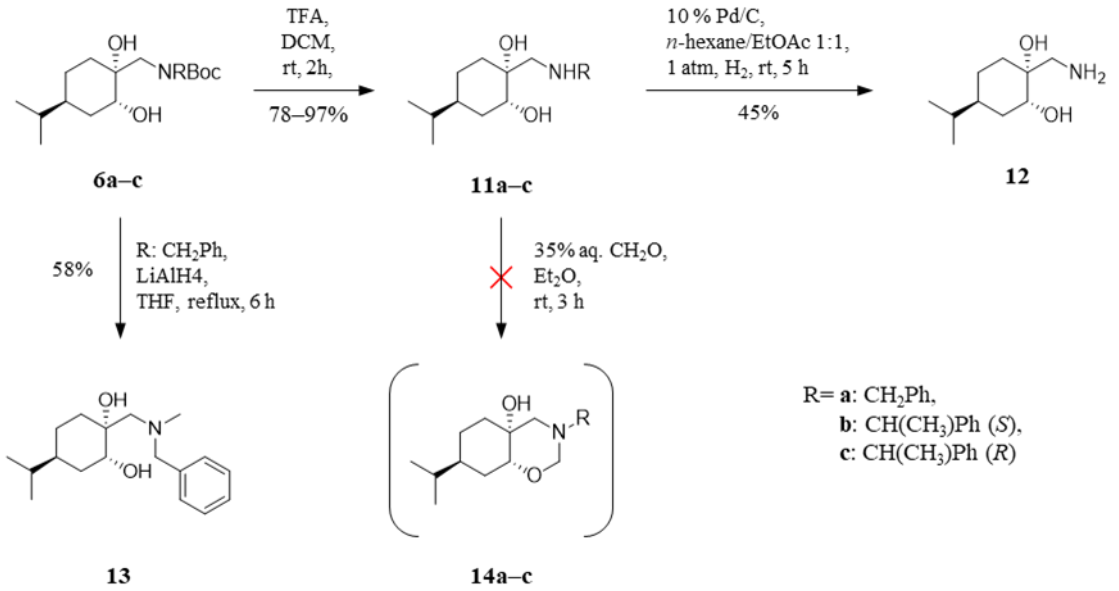
Based on these results, the relative configurations of compounds **6a–c** were also assigned indirectly, given that they were obtained via the *syn*-selective Upjohn dihydroxylation pathway. From compound **7a**, the primary aminodiol **8** was prepared via a debenzylation reaction. Additional derivatives were also synthesised: compound **5a** was reduced using $LiAlH_4$ to produce the *N*-methyl-*N*-benzyl derivative. Furthermore, formaldehyde-mediated cyclisation reactions were carried out on the **7a–c** series to investigate their propensity for ring closure. These transformations exclusively led to the formation of six-membered bicyclic oxazine

derivatives (**10a–c**), indicating a strong preference for this ring size. The corresponding synthetic steps are summarised in **Scheme 2**.




**Scheme 2.** Synthesis of aminodiol derivatives from **5a–c**

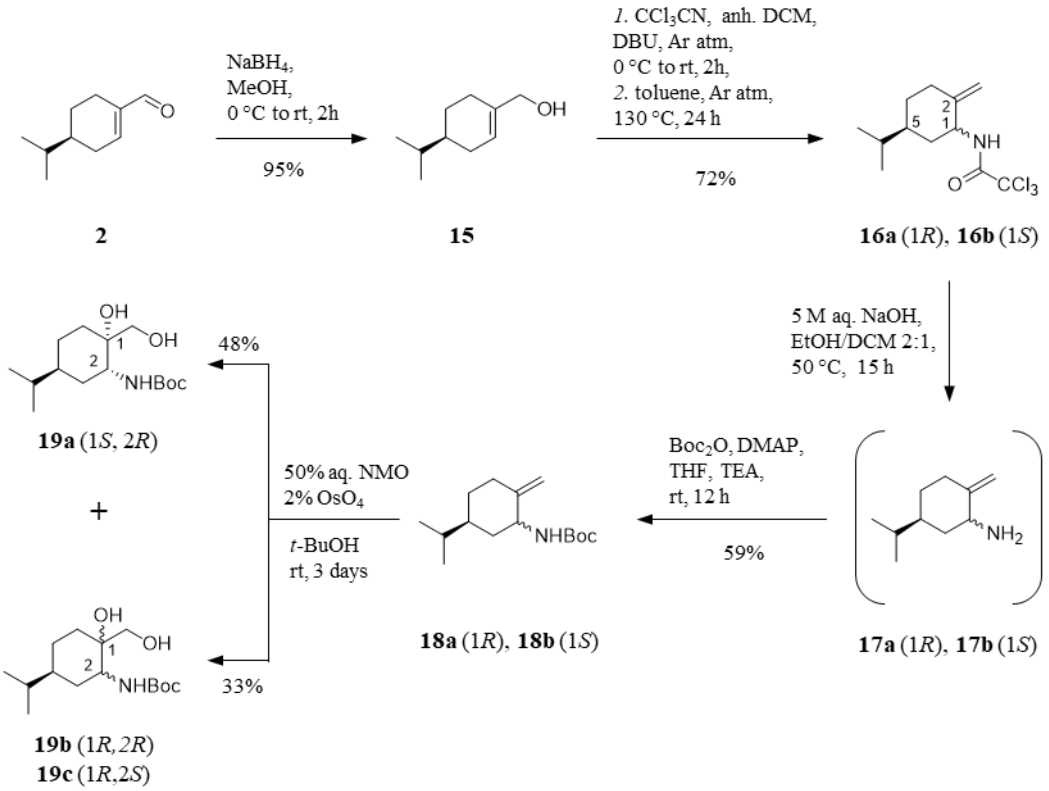
Analogous transformations were performed using **6a–c** diastereomers. Boc deprotection yielded compounds **11a–c**, while compound **12** was obtained as a primary aminodiol derivative. From compound **6a**, derivative **13** was also synthesised. However, under the applied reaction conditions, attempts to induce ring closure from **11a–c** did not result in the formation of the corresponding bicyclic products (see **Scheme 3**).




**Scheme 3.** Synthesis of aminodiol derivatives starting from **6a–c**

To investigate this phenomenon, *in silico* DFT calculations were performed at Eötvös Loránd University in collaboration with *Prof. Dr. Antal Csámpai*. The computational results

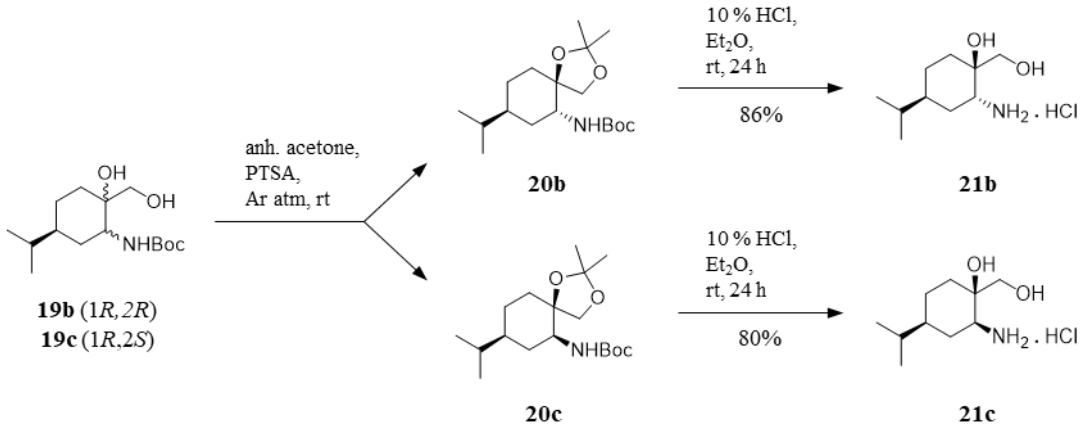
indicated that ring formation is thermodynamically feasible for both diastereomeric series, namely the conversion of **7a** to **10a** and of **11a** to the hypothetical compound **14a**. Although the target compounds **14a–c** could not be isolated, a faint spot consistent with product formation was observed on TLC for the reaction involving **11a**, suggesting transient formation of the desired ring-closed species. Several factors may explain the failure to isolate compounds **14a–c**. These include the potential acid-catalysed degradation of the products on silica gel, as well as the sterically hindered ring inversion required for cyclisation in the **11a–c** series. Both factors could significantly limit the stability and accessibility of the bicyclic products under the applied experimental conditions.




**Scheme 4.** Preparation of aminodiols via Overman rearrangement

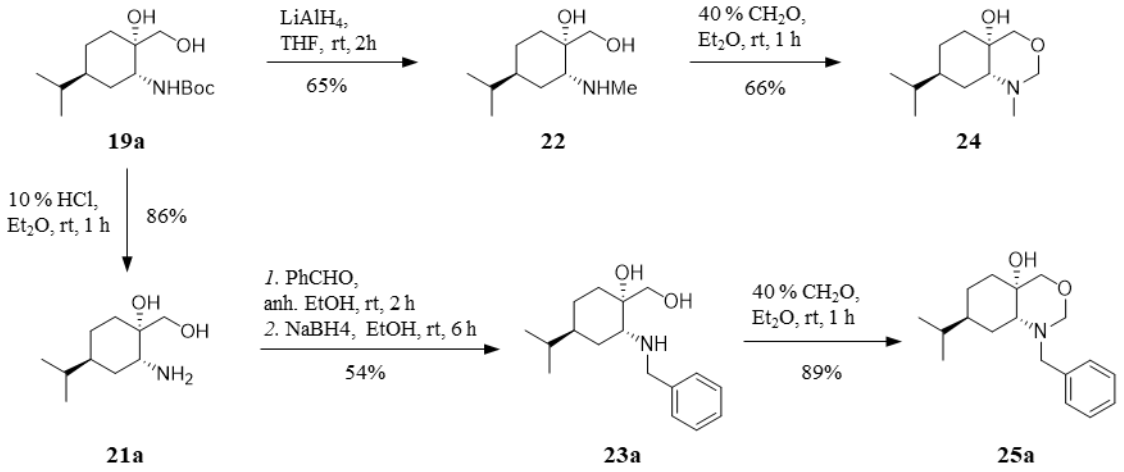
To synthesise the regioisomeric 3-amino-1,2-diols, we also started from dihydroperillaldehyde (**2**). In the first step, the corresponding allyl alcohol was prepared via $NaBH_4$ reduction (**15**). This was followed by a two-step reaction involving an intermediate that underwent an Overman rearrangement to obtain a trichloroacetyl-protected allylamine mixture with the ratio **16a**:**16b** = 85:15. As the resulting diastereomers could not be separated using normal phase chromatography, the trichloroacetyl group was replaced with a Boc protecting group. However, even after this modification, the diastereomeric mixture remained inseparable (**18a** and **18b**). Therefore, the final step, dihydroxylation to yield the aminodiols, was carried

out on the unseparated mixture, following the same methodology as previously described, yielded products in the following ratio **19a**:**19b**:**19c** = 71:16:13. After chromatographic purification, only one aminodiol could be isolated in pure form at this stage (**19a**), while the other two remained in the mixture (**19b** and **19c),** as demonstrated in **Scheme 4**. To facilitate separation, the vicinal diol substituents on **19b** and **19c** were converted into their corresponding acetal derivatives. This process enabled successful chromatographic separation of the protected aminodiols, which, upon acidic hydrolysis using hydrochloric acid, furnished the corresponding primary aminodiols. These transformations are illustrated in **Scheme 5**.



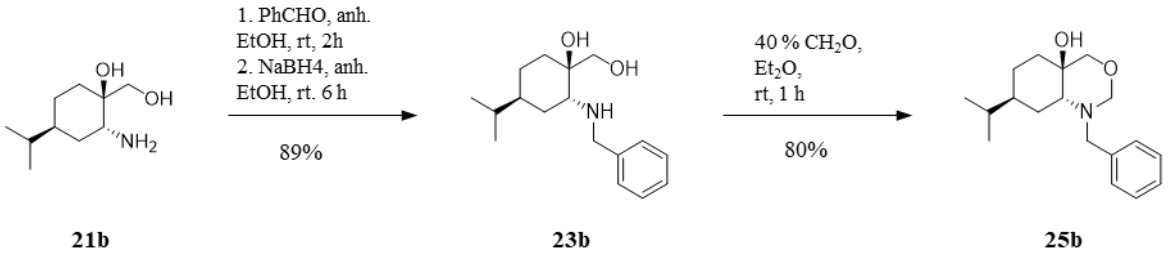

**Scheme 5.** Isolation of aminodiol diastereomers via formation of acetonides

Aminodiol **19a** was reacted with $LiAlH_4$ to form the *N*-methyl derivative (**22**). The primary aminodiol was formed **21a** using acid hydrolysis of **19a**. *N*-Benzyl (**23a**), and bicyclic derivatives (**24**, **25a**) were also obtained (see **Scheme 6**).




**Scheme 6.** Synthesis of aminodiol derivatives

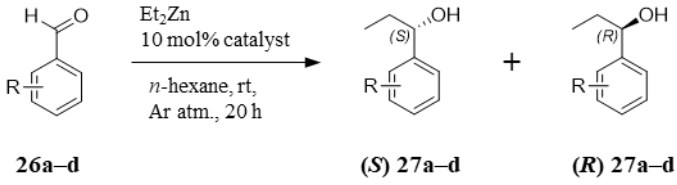
The *N*-benzyl derivative **23b** was also synthesised from the primary amine **21b**, followed by a ring closing reaction using formaldehyde to produce **25b** (see **Scheme 7**). Interestingly, in all cases, the cyclisation resulted in the formation of six-membered 1,3-oxazine ring systems.




**Scheme 7.** Preparation of *N*-benzyl and ring-closed derivatives

**Application of aminodiols in the enantioselective reaction on aromatic aldehydes**

We tested twenty-two 3-amino-1,2-diols as chiral catalysts in the model reaction of diethylzinc with aromatic aldehydes. Benzaldehyde was used as the primary substrate; however, **10a** (the most active compound), and, for comparative purposes, **7a** were also applied to other aromatic aldehydes (4-methylbenzaldehyde, 4-methoxybenzaldehyde, and 3-methoxybenzaldehyde). The resulting (*R*)- and (*S*)-1-aryl-1-propanols were determined by chiral GC or HPLC. The reaction procedure is shown in **Scheme 8**.




R= **a**: H, **b**: $CH_3$ (C′-4), **c**: $OCH_3$ (C′-4), **d**: $OCH_3$ (C′-3)

**Scheme 8.** Enantioselective addition of diethylzinc to aromatic aldehydes; yields: 51–92%

From these outcomes, it can be concluded that aminodiols with the (1*S*,2*S*,4*S*) configuration bearing free dihydroxy groups predominantly promote the formation of the *S* enantiomer. Interestingly, the condensed bicyclic derivatives (**10a–c**) exhibited excellent, but opposite (*R*) selectivity. Extended reactions (**entries 2–4** and **11–13**) further demonstrate that the same diastereomers can facilitate the formation of either the *R* or *S* enantiomer. Compounds **7a–c** and **10a–c**, which incorporate a condensed 1,3-oxazine ring, displayed high catalytic selectivity, likely a consequence of their rigid conformational structures. It is noteworthy, however, that the regioisomeric condensed derivatives showed only modest activity, although with retention of *R*-selectivity. The results are compiled in **Table 1**. Selectivity ranged from poor to excellent.

**Table 1**. Addition of diethylzinc to aromatic aldehydes using the obtained aminodiols as catalysts

| Entry | Catalysts[I] | R group of aromatic aldehyde | Isolated yield[II] (%) | Enantiomeric excess[III] (%) | Configuration of major isomer[IV] |
|---|---|---|---|---|---|
| **1** | **7a** | H | 87 | 68 | *S* |
| **2** | **7a** | 4-Me | 80 | 89 | *R* |
| **3** | **7a** | 4-MeO | 85 | 52 | *S* |
| **4** | **7a** | 3-MeO | 87 | 42 | *S* |
| **5** | **7b** | H | 80 | 60 | *S* |
| **6** | **7c** | H | 80 | 35 | *S* |
| **7** | **8** | H | 90 | 70 | *S* |
| **8** | **9** | H | 89 | 60 | *R* |
| **9** | **10a** | H | 85 | 94 | *R* |
| **10** | **10a** | 4-Me | 90 | 95.5 | *R* |
| **11** | **10a** | 4-MeO | 89 | 95 | *R* |
| **12** | **10a** | 3-MeO | 86 | 99 | *R* |
| **13** | **10b** | H | 88 | 81 | *R* |
| **14** | **10c** | H | 84 | 86 | *R* |
| **15** | **11a** | H | 77 | 26 | *R* |
| **16** | **11b** | H | 85 | 68 | *R* |
| **17** | **11c** | H | 86 | 42 | *S* |
| **18** | **12** | H | 88 | 10 | *S* |
| **19** | **13** | H | 89 | 28 | *S* |
| **20** | **21a** | H | 92 | 4 | *S* |
| **21** | **21b** | H | 91 | 54 | *S* |
| **22** | **21c** | H | 67 | 12 | *S* |
| **23** | **22** | H | 75 | 20 | *R* |
| **24** | **23a** | H | 51 | 0 | - |
| **25** | **23b** | H | 71 | 18 | *R* |
| **26** | **24** | H | 82 | 20 | *R* |
| **27** | **25a** | H | 78 | 62 | *R* |
| **28** | **25b** | H | 89 | 8 | *R* |

[I] 10 mol%. [II] Yields were calculated after chromatographic purification. [III] Evaluation of the crude product by GC or HPLC[IV]. Determined by comparing the $t_R$ of the GC analysis and the optical rotations with the data from the literature.

Our current work complements the thematic efforts of our group. We have investigated a series of monoterpene-derived aminodiols as chiral catalysts for the asymmetric addition of diethylzinc to aromatic aldehydes. Aminodiols bearing a pinane scaffold (derived from myrtenol) afforded catalysts with low to moderate enantioselectivities. On the contrary, carane-based derivatives delivered moderate to excellent enantioselectivities. Starting from pulegone, we were able to prepare catalysts that induce up to 90 % enantiomeric excess. Aminodiols derived from isopulegol exhibited mild to moderate catalytic selectivities, while those derived from sabinol and from β-pinene gave only moderate and low to moderate selectivities, respectively.

## *Ecdysteroids*

### Chemistry of the discussed compounds

As stated in the introduction, to the best of our knowledge, the trypanocidal potential of ecdysteroids have not been studied earlier, and very limited data is available on their antifungal effects. Therefore, we set out to assemble a structurally diverse library of ecdysteroids for a comprehensive evaluation of their aforementioned biological activities. The assembled ecdysteroid library is presented in **Figure 2**.

The compilation encompass:

- *Natural derivatives*: 20-hydroxyecdysone (**28**), ajugasterone C (**29**), shidasterone (**30**), dacryhainansterone (**31**) and calonysterone (**32**)
- *Semisynthetic derivatives*:
    - Side-chain-cleaved derivatives (**33–35**)
    - Dioxolane derivatives: 2,3-monoacetonides (**37–40**, **42**) and 2,3;20,22-diacetonides (**36**, **41**, **43**)
    - Nitrogen-containing ecdysteroids: C-6 oximes (**44–46**, **52**), a C-20 oxime (**61**), C-6 oxime ethers (**47–51**, **53–55**, **69**, **70**), lactams (**56–58**) and an amide (**62**)
    - Fluorinated analogues (**63**, **64**)
    - Cinnamic esters of ecdysteroids (**65–68**) and oxime ethers with cinnamate moieties (**71–74**)

In the present work, we describe the synthesis of compounds **44**, **45**, **56** and **65–74** from precursor **28**. The C-6 oxime derivatives **44** and **45** were prepared by reaction of 20-hydroxyecdysone (**28**) with the free-base form of hydroxylamine hydrochloride, and the

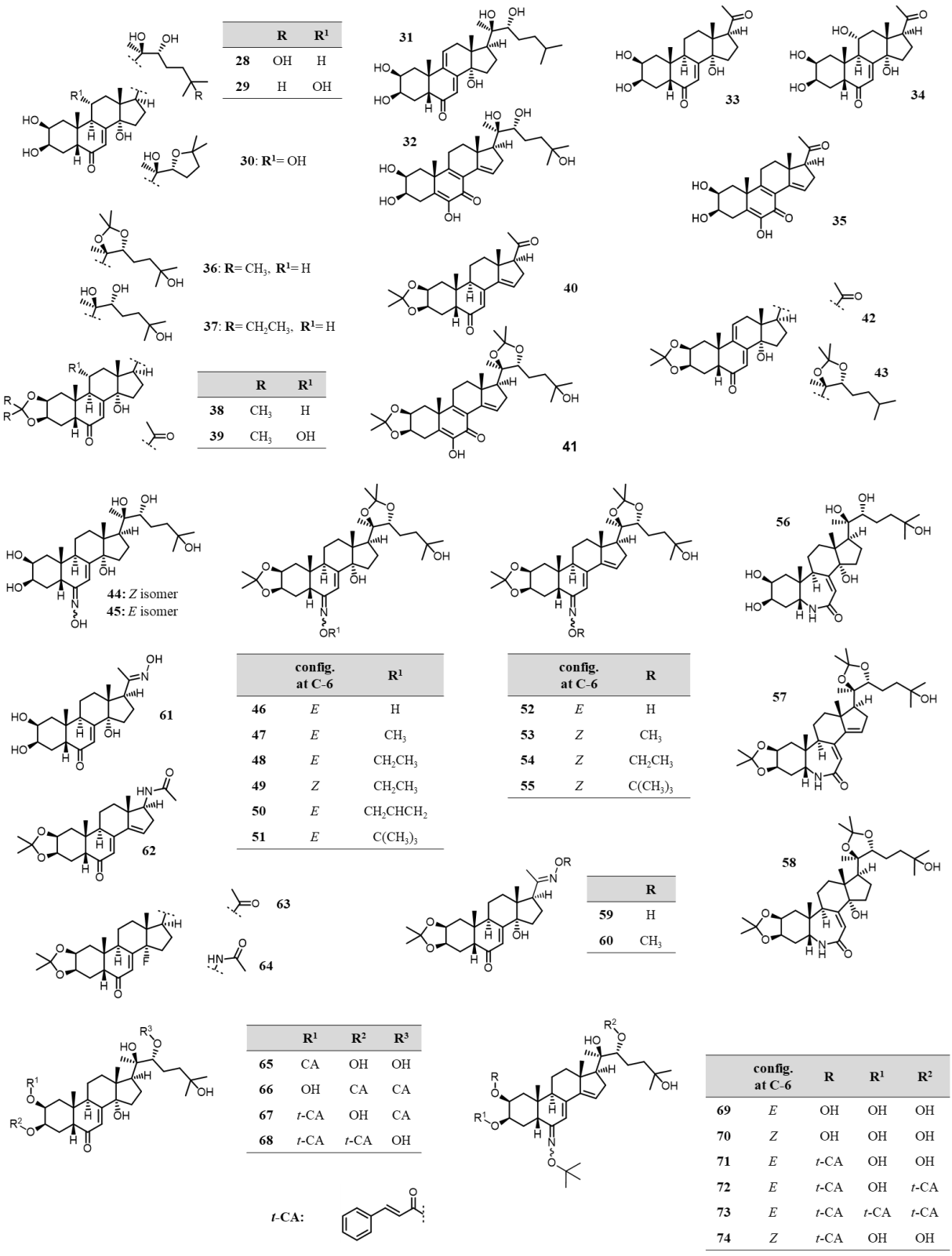
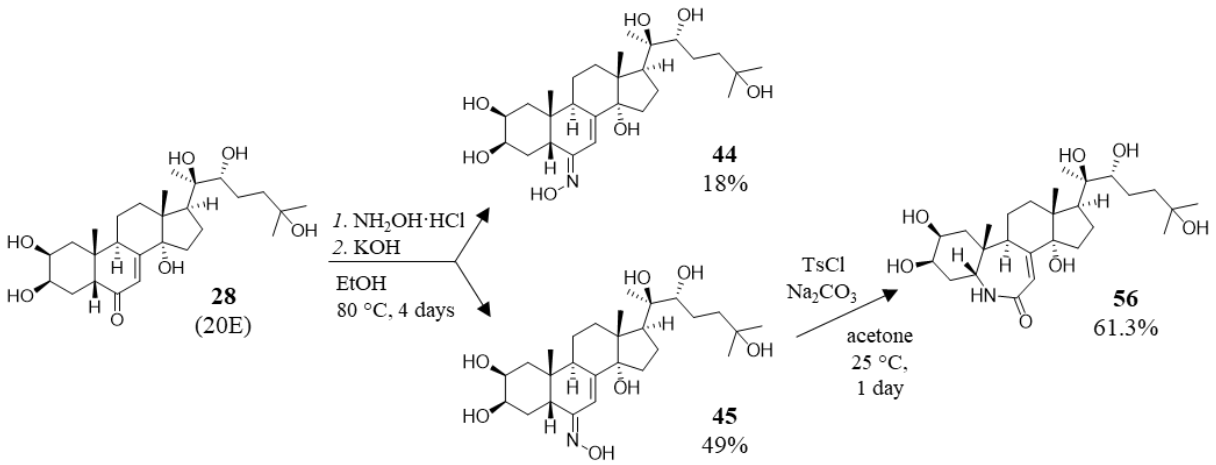


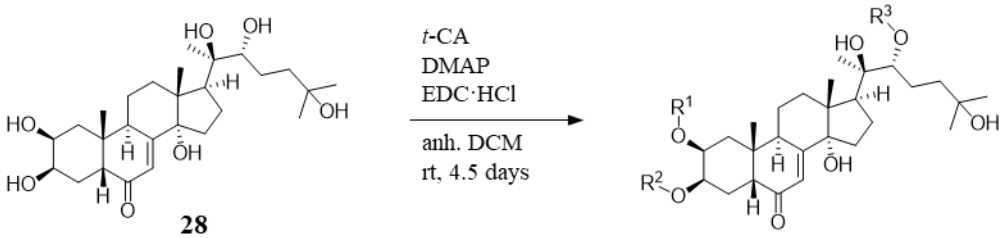

**Figure 2.** Structural variety of the tested ecdysterdoids discussed in this PhD thesis

resulting *E* and *Z* geometric isomers were isolated by flash chromatography followed by RP-HPLC. Lactam **56** was then obtained from the *E*-oxime ether isomer via Beckmann rearrangement (see **Scheme 10**), a transformation that proceeds exclusively from the *trans*-




**Scheme 9.** Semisynthesis of oximes **44**, **45** and a lactam **56**

configured oxime due to its required *anti*-orientation. Cinnamate esters **65–68** were obtained by coupling **28** with *trans*-cinnamic acid using EDC·HCl and DMAP (see **Scheme 10**).

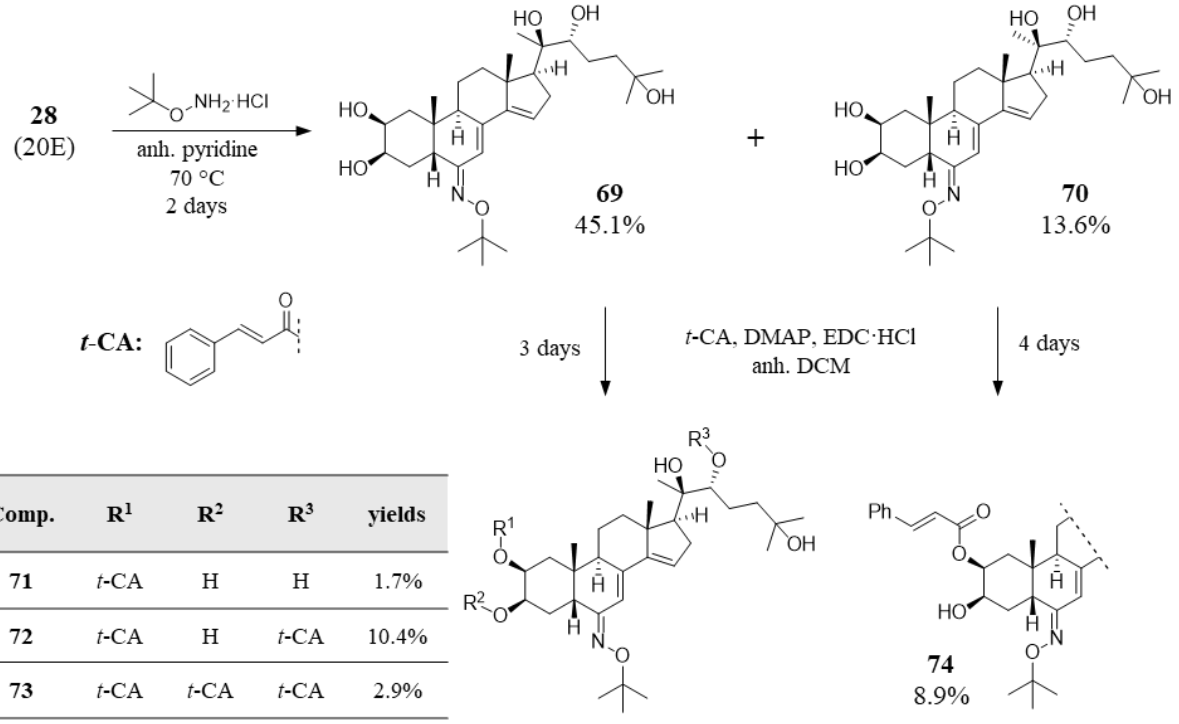



| Comp. | $R^1$ | $R^2$ | $R^3$ | Isolated yields (%) |
|---|---|---|---|---|
| **65** | *t*-CA | H | H | 1.7 |
| **66** | H | *t*-CA | *t*-CA | 3.4 |
| **67** | *t*-CA | *t*-CA | H | 10.4 |
| **68** | *t*-CA | *t*-CA | H | 2.9 |

**Scheme 10.** Preparation of cinnamate derivatives of **28** (20E)

Guided by preliminary biological screening, we subsequently introduced a *tert*-butyl oxime ether functionality alongside the cinnamate motif: C-6 *tert*-butyl oxime ethers (*E* and *Z*) **69** and **70** were formed from **28**, which were then esterified to furnish mono-, di- and triesters **71–74**. (see **Scheme 11**). During oxime ether formation, elimination of the C-14 hydroxyl moiety occurred, likely during the work-up procedure, yielding stachysterone B derivatives. The low yields observed in the esterification reactions are attributable to the presence of multiple hydroxyl groups; notably, esterification consistently occurred at C-2 in all products, indicating that this position is both the most reactive and sterically accessible. The resulting

series of mono-, di- and triesters provides a valuable platform for structure–activity relationship analysis.




| Comp. | $R^1$ | $R^2$ | $R^3$ | yields |
|---|---|---|---|---|
| **71** | *t*-CA | H | H | 1.7% |
| **72** | *t*-CA | H | *t*-CA | 10.4% |
| **73** | *t*-CA | *t*-CA | *t*-CA | 2.9% |

**Scheme 11.** Synthesis of 6 *tert*-butyl oxime ethers of stachysterone B and their cinnamate derivatives

## Biological evaluation of ecdysteroids

### Anticryptococcal activity of ecdysteroids

The emergence of resistance to existing antimicrobial agents has become an increasingly urgent challenge. Ecdysteroids have been scarcely investigated for their antimicrobial properties, and the few available reports describe only weak to moderate activity against pathogenic fungi and bacteria. In collaboration with the research group of *Prof. Dr. Csaba Vágvölgyi* from the University of Szeged Institute of Biology, we evaluated the antifungal activity of natural ecdysteroid **28** (20-hydroxyecdysone) and its semisynthetic derivatives **44**, **45** and **56** against *Cryptococcus neoformans* using a broth microdilution assay. Compound **28** did not exhibit an inhibitory effect at the concentrations tested. Conversion of the C-6 keto group into an oxime (compounds **44** and **45**) produced modestly more potent derivatives, with *Z*-oxime **44** proving to be the most active; the lactam **56** showed no significant activity, indicating that B-ring expansion does not enhance antifungal efficacy. Minimum inhibitory concentrations (MICs) were determined for compounds **44** and **45**, as depicted in **Figure 3**. Both oxime derivatives demonstrated fungicidal rather than merely fungistatic activity, and cotreatment with efflux-pump inhibitors produced only additive effects. These results suggest

that, despite structural modification, ecdysteroids are unlikely to serve as effective antimicrobial agents. Calcein-AM flow cytometry showed that *C. neoformans* IFM 5844 cells treated with **44** at its MIC for 3 h had lower fluorescence than the untreated control (**Figure 3**), indicating reduced viability after brief exposure.

**I**

| Strains | MIC (mg/ml) | | | |
|---|---|---|---|---|
| | **28** | **44** | **45** | **56** |
| IFM 5844 | >2.00 | 1.00 | 2.00 | >2.00 |
| IFO 410 | >2.00 | 1.00 | 2.00 | >2.00 |

**II**

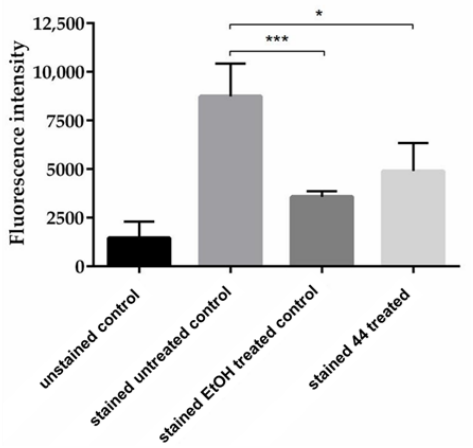



**Figure 3. A**: MIC of the investigated ecdysteroids. **B**: The activity of **44** on the viability of *C. neoformans* IFM 5844 cells. The values represent the mean ± standard deviation calculated from four independent experiments (*, $p \leq 0.05$, ***, $p \leq 0.001$, unpaired *t* test).

## Antitrypanosomal activity of ecdysteroids

In collaboration with the research group of *Prof. Dr. Jürg Gertsch* from the University of Bern Institute of Biochemistry and Molecular Medicine, ecdysteroids **28–74** were screened at 5 µM against *T. cruzi* epimastigotes (benznidazole as positive control).

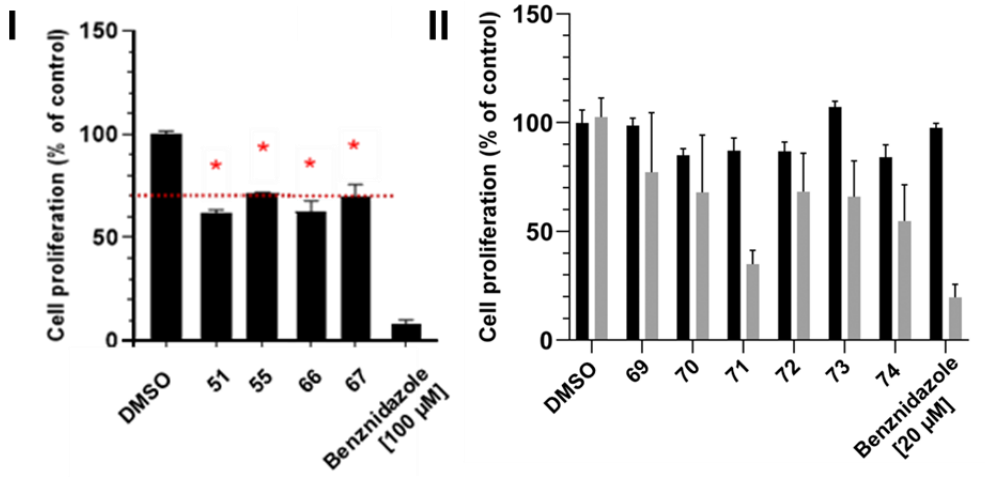



**Figure 4 I:** Screening of ecdysteroids against *T. cruzi* epimastigotes at 5 µM. Maximal *T. cruzi* epimastigote proliferation inhibitory activity of benznidazole (100 µM) shown as positive control. DMSO was the vehicle control. * ≥30% inhibition of epimastigote proliferation were considered as positive trypanocidal activity **II**: Antitrypanosomal and cytotoxic activity of compounds **69**−**74** (5 µM) against *T. cruzi* epimastigotes and Chinese hamster ovarian (CHO) cells measured by XTT and MTT, respectively, upon incubation of compounds for 72 h in the logarithmic proliferative phase. Benznidazole (20 µM) was used as a positive control.

Two *tert*-butyl oxime ethers (**51**, **55**) and two cinnamate esters of 20E (**66**, **67**) inhibited parasite proliferation by ≥ 30% while showing no or minimal cytotoxicity toward CHO cells (**Figure 4**). According to the literature, cinnamic esters have been reported to be effective against *T. cruzi*. Based on our preliminary screening, we incorporated both cinnamate and oxime pharmacophores into the ecdysteroid structure to obtain oxime ethers **69–70** and their cinnamate esters **71–74**.

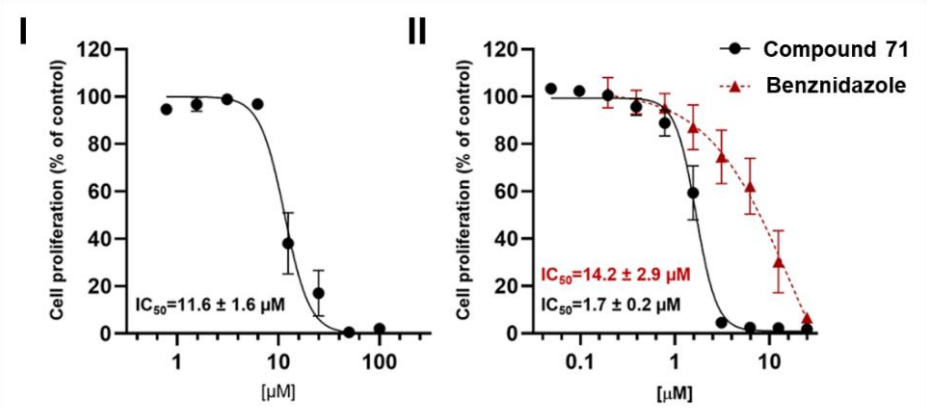



**Figure 5.** Selective trypanocidal effects of **71**. **I**: Effects on cell proliferation (MTT assay) were assessed in RAW264.7 cells after 72 h of incubation, **II**: XTT assays on epimastigote proliferation with benznidazole as positive control. Data show mean values ± SD of at least 3 independent experiments, each of which was performed in triplicates.

All hybrids bearing cinnamate esters and *tert*-butyl oxime ether motifs demonstrated selective antitrypanosomal activity, with compound **71** being the most potent. MTT and XTT assays gave an $IC_{50}$ of 1.7 ± 0.2 µM (see **Figure 5**) and **71** inhibited trypomastigote release by 95% ($IC_{50}$ 2.7 ± 0.1 µM), closely matching benznidazole ($IC_{50}$ 3.8 ± 0.7 µM) (**Figure 6**).

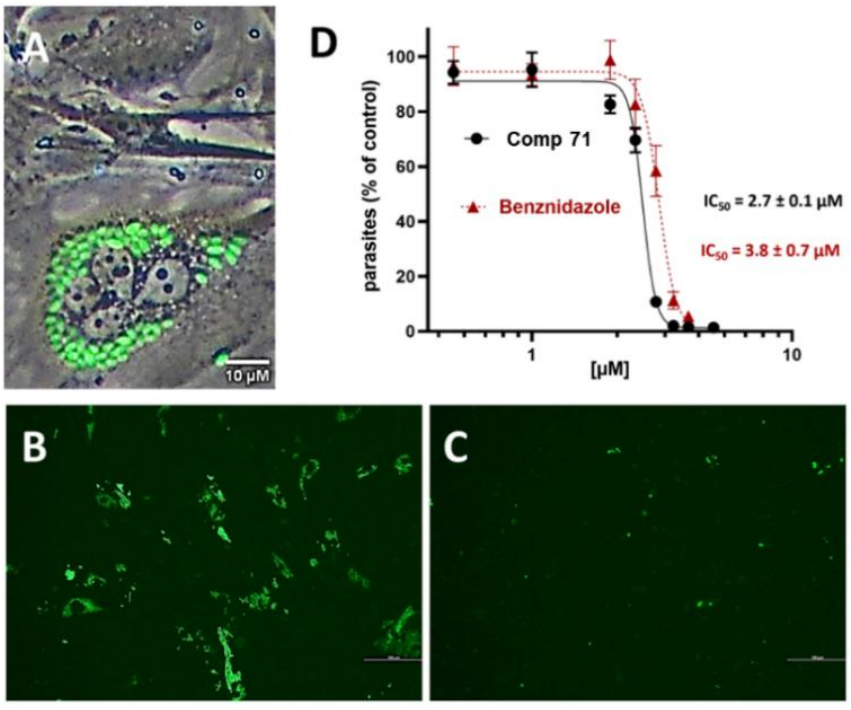



**Figure 6. A**: Representative brightfield/fluorescence microscopic image of CCL39 fibroblasts infected with *T. cruzi* showing amastigotes (green). **B**: Representative fluorescence microscopic images of infected cells showing the GFPY fluorescence signal of the *T. cruzi* amastigotes (vehicle control). **C**: Inhibition of amastigote replication (green) by **71** (5 µM) after 6 days of infection. **D**: The inhibition curves of **71** and the positive control benznidazole on releasing GFPY parasites in trypomastigote-infected RAW264.7 cells measured by FACS after 6 days. Data show mean values ± SD of three independent experiments, each performed in triplicates.

The following structure-activity relationships can be established:

- **A- and B-Ring Substituents**
    - An apolar group on A-ring (e.g. C-2 cinnamate) and a bulky nitrogen-containing substituent on B-ring (e.g. 6-*tert*-butyl oxime ether) are essential. The *E* oxime (**71**) is more active than the *Z* isomer (**74**), underscoring the role of stereochemistry.
- **Side Chain Integrity**
    - Compounds with an unmodified side chain (**71**, **74**) are more potent than those with additional C-22 cinnamate esters (**72**, **73**).
- **14α-OH vs. $\Delta^{14,15}$**
    - Conversion of 14α-OH to a $\Delta^{14,15}$ olefin (**50** → **55**) has negligible impact on trypanocidal activity, indicating this position is less critical.

The significance of our findings is underscored by the fact that we were the first to describe the promising antitrypanosomal activity of ecdysteroids.

## Summary

The objective of this doctoral thesis was to expand the chemical diversity of monoterpenoid aminodiols and ecdysteroids. In terms of monoterpenoids, we aimed to investigate their potential as catalysts in the enantioselective addition reaction of diethylzinc to aromatic aldehydes. Regarding ecdysteroids, we intended to evaluate their antimicrobial efficacy. The primary outcomes of our studies can be summarised as follows.

*Monoterpenoids*

**1. Synthesis of aminodiol library by reductive alkylation of perillaldehyde**

Following the reduction of the *exo* double bond in perillaldehyde, we synthesised a Boc-protected allylamine, which upon dihydroxylation yielded two diastereomeric libraries of 3-amino-1,2-diols. After removal of the protecting group, primary and various secondary aminodiol derivatives were prepared, and in some cases, their corresponding cyclic derivatives were also formed. Consequently, a total of twenty-six compounds were obtained, of which twenty-five represent novel structures.

**2. Computational analysis performed on compounds 10a and 14**

DFT studies showed structural differentiation between the diastereomers **7a** and **11a**, which significantly impacts their ring-forming reactions. Specifically, in compound **11a**, the

inversion of the cyclohexane ring and subsequent reorientation of the isopropyl group preferentially promote hydrolysis rather than recyclisation. On the contrary, compound **7a** structurally favours recyclisation accompanied by *O*-deprotonation, ultimately facilitating fused oxazine formation. The process circumvents iminium intermediates and proceeds instead through an *N*-hydroxymethylated intermediate stabilised by a crucial network involving four hydrogen-bonded water molecules. This proposed mechanism, supported by theoretical modelling, offers a credible rationale for the observed fused oxazine formation.

**3. Preparation of a regioisomeric aminodiol library via Overman rearrangement**

Following the previously described synthetic method for aminodiols, three regioisomeric aminodiol libraries were prepared starting from perillaldehyde. After the reduction of the *exo* double bond in perillaldehyde, an alcohol intermediate was synthesised, which was subsequently converted to allylamine through the Overman rearrangement. After protection of the resulting amine, attempts to separate the diastereomers by employing various chromatographic eluent systems were unsuccessful. Therefore, dihydroxylation was performed on the diastereomeric mixture, leading to the corresponding protected aminodiols. In certain instances, acetals were prepared to facilitate the separation of aminodiols. Following the removal of the protecting groups, secondary and primary aminodiols, *N*-methyl-*N*-benzyl derivatives, and their corresponding cyclic analogues were synthesised.

**4. Use of monoterpene-based aminodiol derivatives as catalysts in the reaction of the addition of diethylzinc to aromatic aldehydes**

The synthesised aminodiols were evaluated as chiral catalysts in the reaction of diethylzinc with benzaldehyde; catalysts **7a** and **10a** were also tested with 4-methyl-, 3-methoxy-, and 4-methoxybenzaldehyde. Aminodiols that possess exclusively *S*-configuration stereocenters display *S*-selectivity. Conversely, bicyclic 1,3-oxazine derivatives (**10a–c**) exhibited *R*-selectivity. The excellent catalytic activity of the 1,3-oxazines can be attributed to their rigid molecular structure. Aminodiol regioisomers derived via the Overman rearrangement exhibited lower catalytic efficiency.

*Ecdysteroids*

**1. Selection of ecdysteroids for bioactivity screening and preparation of new semisynthetic derivatives**

In total, forty-seven ecdysteroids were examined in this dissertation. Among these compounds, five are natural, while the remaining are semisynthetically modified derivatives.

In this work, the preparation of thirteen semisynthetic ecdysteroids is described, including two oxime derivatives (**44**, **45**), one lactam derivative (**56**), four cinnamic acid esters (**65–68**), two *tert*-butyl oxime ethers (**69**, **70**) and four *tert*-butyl oxime ethers of ecdysteroid cinnamate esters (**71–74**). Nine of these ecdysteroids represent novel compounds.

**2. Antifungal activity of ecdysteroids**

In this study, the antifungal activities of 20E (**28**), two oxime derivatives (**44**, **45**), and one lactam derivative (**56**) were investigated. Compounds **44** and **45** did not exhibit antibacterial activity; however, they demonstrated effective fungicidal properties against *C. neoformans*, with compound **44** exhibiting a significantly stronger effect. When **44** and **45** were tested for interaction with efflux pump inhibitors, an additive effect was observed with verapamil, while no interaction was detected with indomethacin or quinidine.

**3. Antitrypanosomal investigation of ecdysteroids**

The antiprotozoal activity of the forty-seven ecdysteroids investigated in this dissertation was evaluated against *Trypanosoma cruzi*. On the basis of a preliminary screening, two promising chromophores were identified: *tert*-butyl oxime ether and cinnamate ester moieties. Incorporating these substituents led to the synthesis of six new derivatives, three of which exhibited notable antiparasitic activity. The best hit was **71**, for which the determined $IC_{50}$ value indicated a level of efficacy comparable to that of benznidazole, the positive control used in the assay.

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**LIST OF PUBLICATIONS RELATED TO THE PH.D. THESIS**

**I.** Szerencsés, B.; Vörös, M.; Bagi, K.; **Háznagy, M. B.**; Hunyadi, A.; Vágvölgyi, Cs.; Pfeiffer, I.; Vágvölgyi, M. Semi-Synthetic Ecdysteroid 6-Oxime Derivatives of 20-Hydroxyecdysone Possess Anti-Cryptococcal Activity. *Microbiology Research* **2022**, *13* (4), 985–994.

IF: **1.5** (2022); **Q3**

**II.** **Háznagy, M. B.**; Csámpai, A.; Ugrai, I.; Molnár, B.; Haukka, M.; Szakonyi, Zs. Stereoselective Synthesis and Catalytical Application of Perillaldehyde-based 3-Amino-1,2-diol Regioisomers. *International Journal of Molecular Sciences* **2024**, *25* (8), 4345.

IF: **4.9** (2024); **D1**

**III.** **Háznagy**, **M. B.**; Girst, G.; Vágvölgyi, M.; Cholke, K.; Krishnan, S. R.; Gertsch, J.; Hunyadi, A. Semi-Synthetic Ecdysteroid Cinnamate Esters and *tert*-Butyl Oxime Ether Derivatives with Trypanocidal Activity. *Journal of Natural Products* **2024**, *87* (10), 2478–2486.

IF: **3.6** (2024); **Q1**

## Presentations related to the Ph.D. thesis:

**I.** **Háznagy**, **M**. **B**.; Vágvölgyi, M.; Girst, G.; Krishnan, S. R.; Cholke, K; Gertsch, J.; Hunyadi, A.
Design and optimisation of ecdysteroid ester and oxime ether derivatives as potential trypanocidal agents
*Nordic Natural Products Conference 2025*, Tjärnö Marine Laboratory, Sweden, (2025) (Oral presentation)

**II.** **Háznagy**, **M**. **B**.; Vágvölgyi, M.; Girst, G.; Krishnan, S. R.; Cholke, K; Gertsch, J.; Hunyadi, A.
Bioactive ecdysteroid analogues: cinnamate esters and *tert*-butyl oxime ethers with antitrypanosomal properties
*TwiNSol-CECs International Conference on Environmental and Sustainable Research Solutions*, Novi Sad, Serbia, (2025) (Oral presentation)

**III.** **Háznagy**, **M**. **B**.; Vágvölgyi, M.; Krishnan, S. R.; Cholke, K; Gertsch, J.; Hunyadi, A.
Semi-synthetic ecdysteroids as promising new antichagasic agents
*International Congress on Natural Products Research,* Kraków, Poland, (2024) (Poster presentation)

**IV.** **Háznagy**, **M**. **B**.; Vágvölgyi, M.; Krishnan, S. R.; Cholke, K; Gertsch, J.; Hunyadi, A.
Application of high-performance separation techniques to the isolation of natural and semisynthetic antitrypanosomal ecdysteroids
*METT 13th Balaton Symposium on High-Performance Separation Methods*, Siófok, Hungary (2023) (Oral presentation)

**V.** **Háznagy**, **M**. **B**.; Vágvölgyi, M.; Krishnan, S. R, Gertsch, J.; Hunyadi, A.
Antitrypanosomal activity of natural and semi-synthetic ecdysteroids
*EUGLOH 4th International Symposium of Young Researchers on Medicinal Plants and Natural Product Research*, Szeged, Hungary (2023) (Oral presentation)

**VI.** **Háznagy**, **M**. **B**.; Vágvölgyi, M.; Krishnan, S. R.; Gertsch, J.; Graikioti, D.; Athanassopoulos, C. M.; Hunyadi, A.
Természetes és félszintetikus ekdiszteroid származékok antitripanoszóma aktivitása
*MTA Committee on Chemical Biology László Zechmeister Presenter's Competition*, Budapest, Hungary (2022) (Oral presentation)

**VII.** **Háznagy**, **M**. **B**.; Vágvölgyi, M.; Krishnan, S. R, Gertsch, J.; Hunyadi, A.
Antitrypanosomal activity of semisynthetic enone-type derivatives
*70th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA),* Thessaloniki, Greece, (2022) (Poster presentation)

**VIII.** **Háznagy, M. B.**
Ecdysteroid ester derivatives
*3rd Symposium of Young Researchers on Pharmacognosy*, Szeged, Hungary (2022) (Oral presentation)

**IX.** **Háznagy**, **M**. **B**.; Vágvölgyi, M.; Krishnan, S. R, Gertsch, J.; Hunyadi, A.
Semisynthetic ecdysteroid-cinnamic derivatives against *Trypanosoma cruzi*
*69th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA),* online, (2021) (Poster presentation)

**X.** **Háznagy, M. B.**
Preparation and investigation of ecdysteroid–cinnamic acid hybrid compounds
*2nd Symposium of Young Researchers on Pharmacognosy,* online, (2021) (Oral presentation)

**XI.** **Háznagy, M. B.**
*Preparation of nitrogen-containing ecdysteroids*
*1st Symposium of Young Researchers on Pharmacognosy*, Szeged, Hungary (2019) (Oral presentation)