Ph.D. Thesis

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

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University of Szeged

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LIST OF PUBLICATIONS RELATED TO THE 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.

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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.

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

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LIST OF ABBREVIATIONS

^{13}C NMR	Carbon-13 nuclear magnetic resonance
1D	One-dimensional
^{1}H NMR	Proton nuclear magnetic resonance
20E	20-Hydroxyecdysone
2D	Two-dimensional
Ac_2O	Acetic anhydride
anh.	Anhydrous
Boc	*tert*-Butyloxycarbonyl
CCl_3CN	Trichloroacetonitrile
CHO	Chinese hamster ovary
DCM	Dichloromethane
DFT	Density functional theory
DMAP	4-Dimethylaminopyridine
DMSO	Dimethyl sulfoxide
ee	Enantiomeric excess
EDC·HCl	*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
EtOH	Ethanol
GC	Gas chromatography
HPLC	High-performance liquid chromatography
HR-MS	High-resolution mass spectrometry
IC_{50}	Fifty percent inhibitory concentration
$LiAlH_4$	Lithium aluminium hydride
MeOH	Methanol
MIC	Minimum inhibitory concentration
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Na_2CO_3	Sodium carbonate
NH_4Cl	Ammonium chloride
$NaBH_4$	Sodium borohydride
NMO	4-Methylmorpholine *N*-oxide
NOE	Nuclear overhauser effect
OsO_4	Osmium(VIII) oxide
PTSA	4-Methylbenzene-1-sulfonic acid
RP-HPLC	Reversed-phase high-performance liquid chromatography
rt	Room temperature
t-CA	*trans*-Cinnamic acid
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TsCl	4-Toluenesulfonyl chloride
XTT	2,3-Bis(2-Methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide

1. INTRODUCTION

α,β-Unsaturated carbonyl compounds represent a unique category of organic molecules, defined by the presence of a carbonyl group conjugated to a neighbouring double bond. This conjugation results in distinctive chemical properties, such as electrophilic behaviour at the *β*-carbon and nucleophilic tendencies at the *α*-carbon, making these compounds exceptionally adaptable for various synthetic applications.[1] Their chemical structure is demonstrated in the figure below (**Figure 1**). The groups R′, R″, and R‴ represent alkyl or aryl functional groups. Depending on the R group, they may be enals (R= H), enones (R= alkyl), or unsaturated carboxylic acids (R= OH). This doctoral thesis will discuss some representatives of these functional groups in more detail.

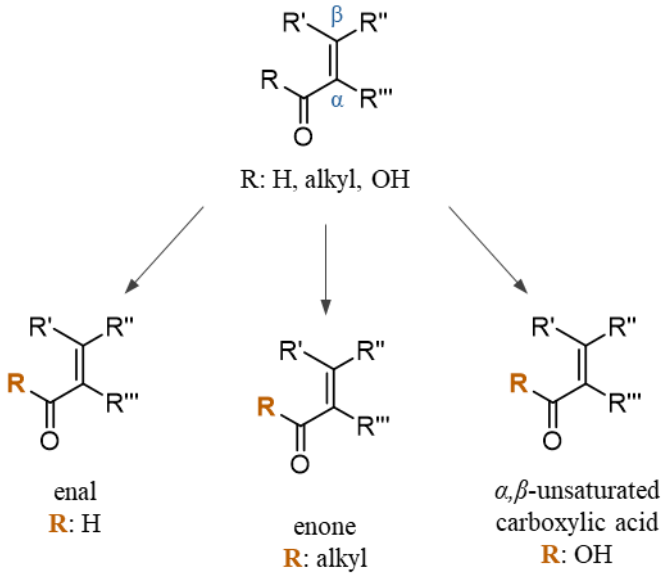


Figure 1. General formula of the *α,β*-unsaturated carbonyl compounds, which are the subject of this doctoral thesis

α,β-Unsaturated carbonyl compounds serve as building blocks of many natural compounds and are readily available from diverse natural sources.[2] Their unique structure makes them suitable starting materials for the semisynthesis of novel derivatives. Consequently, the following topic as the focus of my doctoral dissertation was starting materials containing *α,β*-unsaturated carbonyl functions selected for semisynthetic transformations, such as monoterpene perillaldehyde and ecdysteroid 20-hydroxyecdysone (20E). Furthermore, cinnamic acid, applied in 20E is classified as an *α,β*-unsaturated carboxylic acid.

1.1. Monoterpenoids

1.1.1. Perillaldehyde as our starting material

Monoterpenoids are abundant in fauna and flora. To date, more than 1000 monoterpenes have been identified, spanning over 38 distinct structural categories.[3] These compounds are biosynthesised in nature from two C_5 isoprene units, namely dimethylallyl pyrophosphate and isopentenyl pyrophosphate, and are found in plant kingdoms predominantly as components of essential oils or as iridoids. Monoterpenoids may occur either in acyclic or cyclic forms. Essential oil components can often be isolated from plants in significant quantities, frequently in enantiomerically pure form, typically by steam distillation.[4] This accessibility makes them promising starting materials for the synthesis of chiral compounds with diverse structures in considerable quantities.[5] Our study focuses on the synthesis of monoterpenes that have an aminodiol functional group, for which a suitable starting material was required. In this context, perillaldehyde was identified as an appropriate starting material, whose derivatives have been relatively underexplored in the field of catalysis, offering a versatile framework for the development of novel aminodiol derivatives. Perillaldehyde is a volatile green-yellow liquid, abundant in the leaves and aerial parts of *Perilla frutescens* (L.)[6] and features a limonene-derived scaffold (**Figure 2**). Notably, it is a monocyclic monoterpenoid and possesses an enal moiety that plays a key role in terms of synthetic perspective.

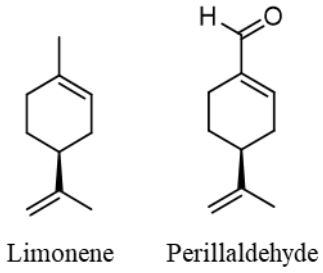


Figure 2. Structures of monoterpene limonene and perillaldehyde (**1**)

1.1.2. Preparation of aminodiol derivatives of monoterpenoids

Monoterpenes serve as valuable precursors for chiral building blocks and catalysts[7]. We aimed to prepare 3-amino-1,2-diols because they possess both amino and hydroxyl groups within a single-molecule structure, which serve as potentially active catalytic substituents. The importance of this family is further underscored by the numerous catalytic applications of such

compounds reported in the literature and their presence as structural elements in various biologically active molecules.[8, 9]

Numerous well-established and thoroughly studied methods for preparing 3-amino-1,2-diols are described in the literature. For example, these compounds can be obtained with good yields through aminolysis of β-hydroxy-epoxydes,[10] dihydroxylation of allylamines,[11] hydrolysis of epoxyamines,[12] or nucleophilic substitution of a good leaving group (e.g., OMs, OTs).[13] **Figure 3** illustrates these main synthetic pathways.

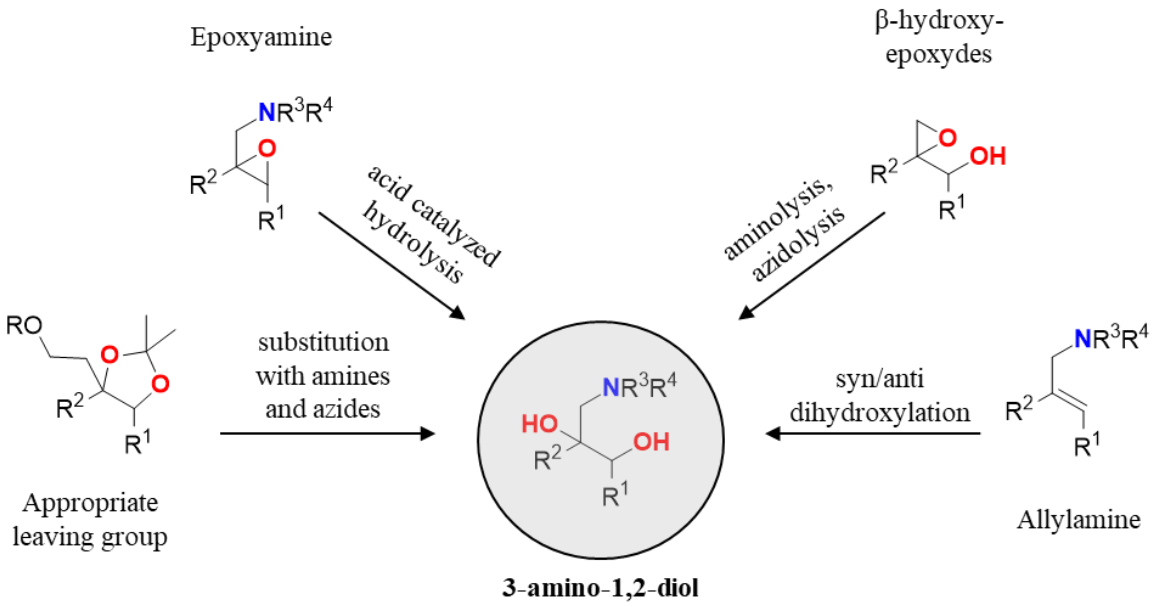


Figure 3. Preparative strategies for the synthesis of 3-amino-1,2-diols

Monoterpenes can be excellent starting materials for the asymmetric syntheses of aminodiols such as (+)-and (−)-α-pinene,[14, 15] (−)-nopinone,[16] (+)-carene,[17, 18] (+)-sabinol,[19] (−)-pulegone,[20] or camphor and fenchon[21].

(*S*)-(–)-Perillaldehyde and (*R*)-(+)-pulegone are excellent starting materials for the preparation of monoterpene-based 3-amino-1,2-diols because they are commercially available in sufficient quantities in enantiopure form. The α,β-unsaturated carbonyl group is highly capable of being converted by reduction to allyl alcohol or by reductive amination to the corresponding allylamine. In a previous study of our research group, diastereoisomeric aminodiols were prepared from (*R*)-pulegone.[22] In the first step, pulegone was reduced to pulegol, followed by the Overmann rearrangement with trichloroacetonitrile and DBU to *N*-protected allylamine. Dihydroxylation of the double bond led to the appropriate aminodiols. The resulting diastereomers were successfully separated, and analogous aminodiol libraries were formed. An interesting ring-ring tautomerism was observed during secondary aminodiol

ring closure that involved the interconversion between the resulting oxazolidine and 1,3-oxazine (**Figure 4**).

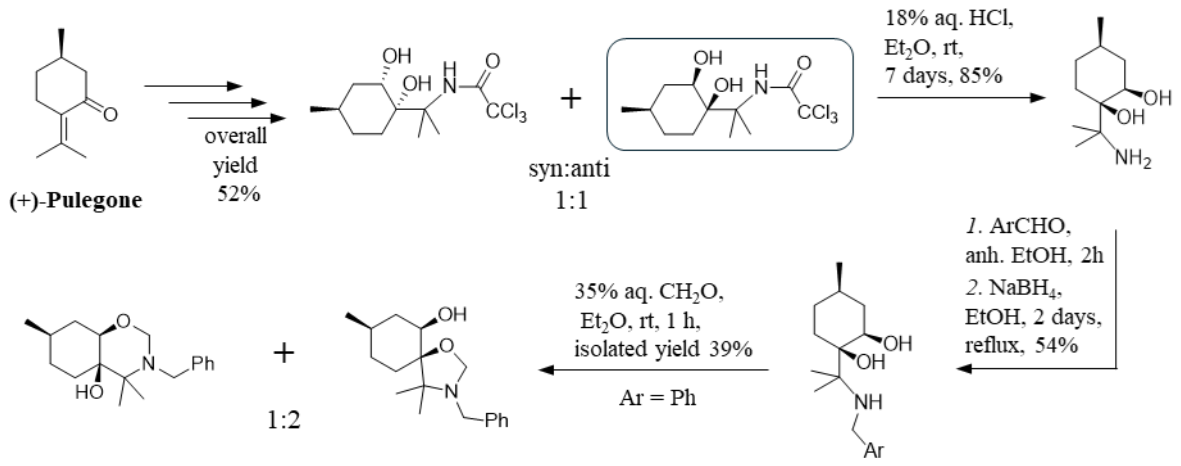


Figure 4. Preparation and ring closure of pulegone-based aminodiols

In another previous work, a stereoselective preparation of 3-amino-1,2-diols from (*S*)-(–) perillaldehyde was also achieved using a carane-based aldehyde.[18] Starting from perillaldehyde, a bicyclic aldehyde was synthesised through a two-step, one-pot process. The first step involved reductive amination with primary amines, followed by the addition of a protective group (Boc or Cbz) to yield bicyclic allylamines. This was followed by a key step, a stereoselective dihydroxylation, which provided enantiopure products. The ring closure of 3-amino-1,2-diol with formaldehyde exclusively led to the formation of a fused 1,3-oxazine ring (**Figure 5**). The catalytic activity of the synthesised aminodiols was investigated in the enantioselective addition of diethylzinc to the benzaldehyde model reaction. These derivatives were prepared to evaluate their catalytic efficiency in the enantioselective addition of diethylzinc to a benzaldehyde model reaction, where enantiomeric excesses (*ee*) ranging from low to excellent values were achieved depending on the substituents.

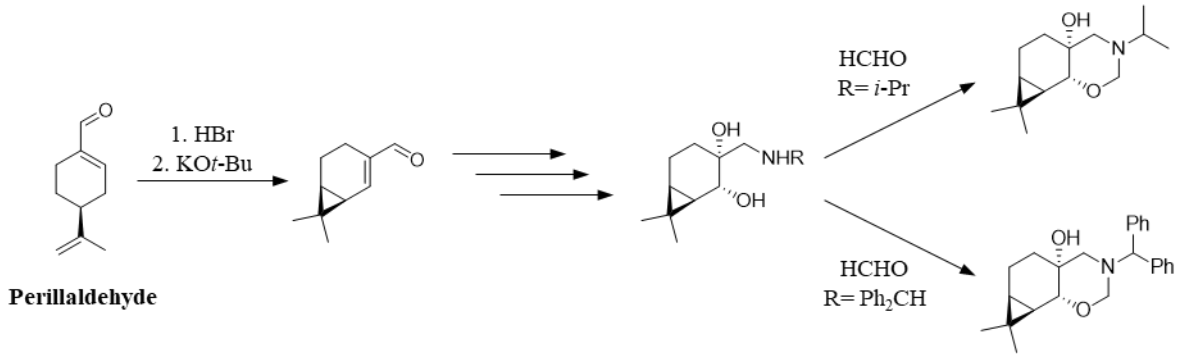


Figure 5. Preparation of aminodiols starting from perillaldehyde

1.1.3. Pharmacological potential of aminodiols

Recent studies have shown that several aminodiol-based nucleoside analogues exhibit cardiovascular, cytostatic, and antiviral properties.[23-28] A notable example is Abbott aminodiol, which has been used as a key intermediate in the synthesis of potent renin inhibitors such as Zankiren® and Enalkiren®, both of which possess antihypertensive activity.[29, 30] The structure of reboxetine, a selective norepinephrine reuptake inhibitor marketed as *Edronax*®, contains an aminodiol unit, which is approved in numerous countries for the treatment of unipolar depression.[31] Additionally, aminodiols may serve as precursors for the synthesis of biologically active natural products. An example is cytoxazone, a microbial metabolite derived from Streptomyces species, which acts as a selective modulator of TH2 cytokine secretion.[32, 33]

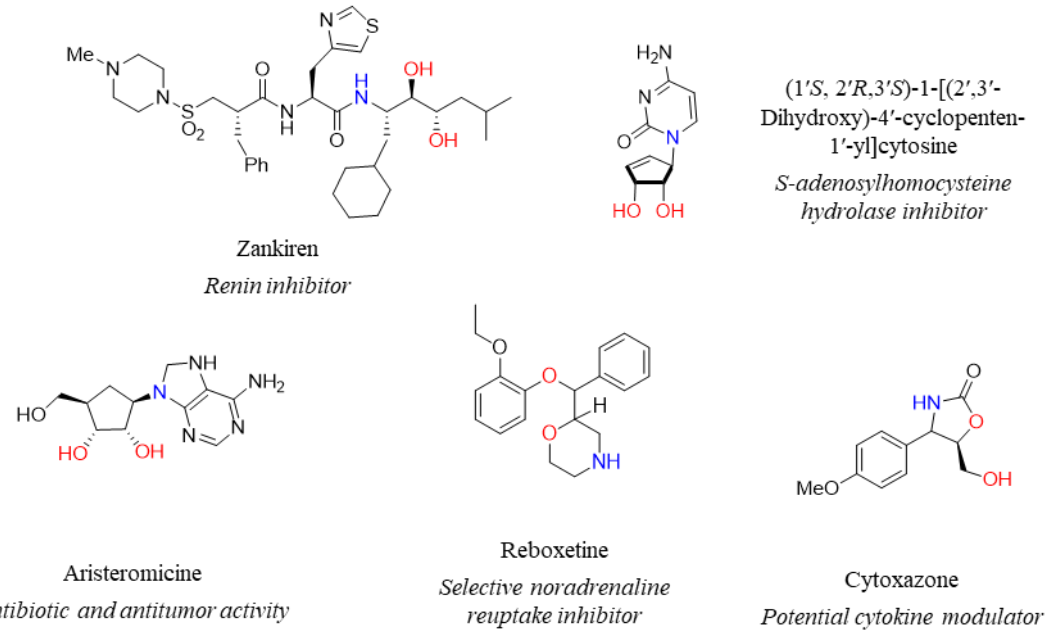


Figure 6. Bioactive molecules containing aminodiol moieties

1.1.4. Application of monoterpene-based aminodiols as chiral catalysts

The development and use of new chiral catalysts remains a prominent and enduring focus in organic, applied, and pharmaceutical chemistry.[34-37] One of the key application areas of chiral catalysts is the asymmetric formation of carbon–carbon bonds. In the present study, we aimed to investigate our synthesised chiral aminodiols in addition of an organometallic compound to aromatic aldehydes, namely, the model reaction of diethylzinc addition to benzaldehyde. The favorability of this model reaction arises not only from its relative ease of carrying out but also from the straightforward analysis of the resulting enantiomers, specifically (*R*)- or (*S*)-1-phenyl-

1-propanol (**Figure 7**). The enantiomeric excess (*ee*) of the products can be measured using chiral GC or HPLC techniques.

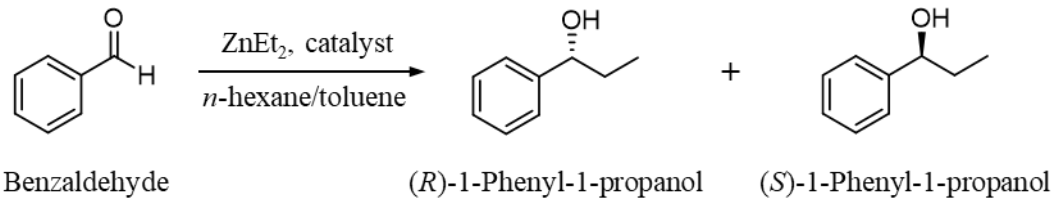


Figure 7. The addition of diethylzinc to benzaldehyde

The catalytic activity of amino alcohols (and later aminodiols) was first reported by Noyori et al., using (2*S*)-(−)-3-(dimethylamino)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol (DAIB) successfully at 10 mol%. Their results demonstrate that the reaction occurs entirely (and rapidly) when the reactant-catalyst ratio exceeds 1. An interpretation of the mechanism is given in **Figure 8**. In the first step, zinc coordinates with the amine and alcohol groups of DAIB accompanied by the release of ethane gas. Subsequently, a second diethylzinc molecule coordinates to the oxygen atom. The resulting activated species participate later in the nucleophilic addition to aldehyde, while the remaining part of the complex governs the stereoselectivity of the reaction through asymmetric induction.[38]

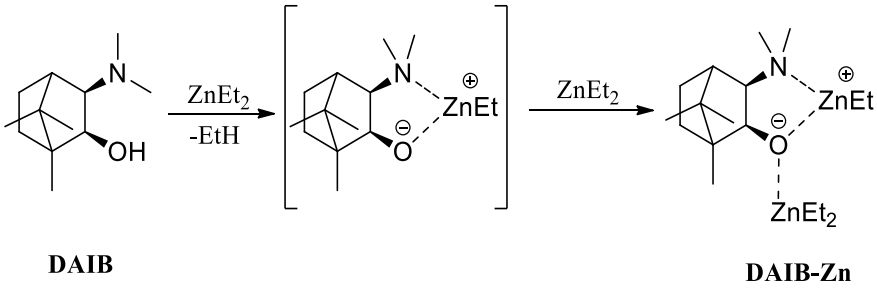


Figure 8. Mechanism of the catalytic reaction of diethylzinc.

1.2. Ecdysteroids

1.2.1. Ecdysteroids – Natural occurrence and chemical characterisation

Ecdysteroids are a distinct group of steroidal compounds biosynthetically originating from triterpenoids. 20-Hydroxyecdysone (20E) is one of the most widespread representatives of ecdysteroids, and observing this compound allows us to better understand ecdysteroids' structural characteristics. Natural derivatives are polar because of their various hydroxyl moieties. The OH groups are typically attached with the *R* configuration to C-3, C-20, and C-22, and with *S* configuration to C-2, C-14, and C-20. Ecdysteroids bear methyl groups at C-10 and C-13, and the most typical structural characteristic is the *α,β*-unsaturated ketone function

in ring B. The ring annellation is characteristic, with the A/B ring junction typically occurring in the *cis* configuration, while the C/D ring junction is almost always *trans*. In general, they contain a side chain at position C-17; nevertheless, derivatives with shorter side chains or without a side chain also occur (e.g., poststerone, rubrosterone).[39] Ecdysteroids are widely distributed in the Animal and Plant Kingdoms, and based on these, they can be divided into zoo- and phytoecdysteroids. Their first representative, α-ecdysone, was successfully isolated from the pupae of *Bombyx mori* in 1954, performed by Karlson and Butenandt.[40] Subsequently, ecdysteroids were discovered from plant sources (e.g. *Podocarpus nakaii*) as well.[41] Early research revealed that ecdysteroids serve as hormones regulating moulting in arthropods. Ecdysteroids have since been recognised for their diverse biological roles and versatile chemical properties.[42] As a result of 70 years of research effort since their first isolation, 590 natural ecdysteroids are currently known as of April, 2025, according to the *Ecdybase*.[43] This continuously updated online database provides comprehensive chemical, biological, and structural descriptive data on ecdysteroids. Notably, as a commercial perspective, *Cyanotis*, *Rhaponticum* and *Serratula* species are primarily used as a source of 20E, which serves as the starting material in our semisynthesis (**Figure 9**). Considering the structural complexity and multiple hydroxyl groups of ecdysteroids, which provide numerous opportunities for semisynthetic modifications and preparation of novel derivatives with potential biological activity, one of the aims of this study was the semisynthesis of new bioactive compounds.

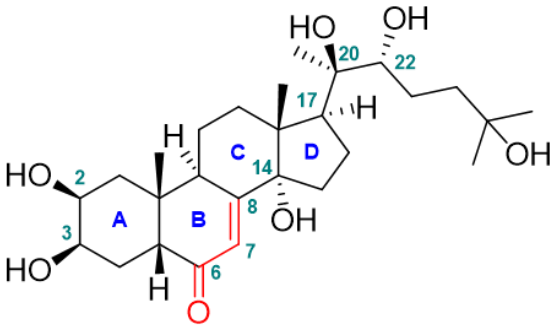


Figure 9. Chemical structure of 20E

1.2.2. General bioactivities and possible applications

Ecdysteroids act as moulting hormones in arthropods and play a protective role in plants against herbivorous invertebrates; additionally, they also exert significant effects on mammals.[42, 44] Ecdysteroids, such as 20E and turkesterone, are known for their anabolic effects, promoting muscle growth and strength without the hormonal side effects of traditional anabolic steroids. They enhance protein synthesis[45] and have shown growth-stimulating effects in various animal studies.[46-49] Due to concerns about potential misuse in sports, the World Anti-

Doping Agency (WADA) included 20E in its monitoring program in 2020, and, as of 2025, it is still being considered for potential doping control.[50, 51, 52] Ecdysteroids possess adaptogenic effects: they improve stress resistance and overall endurance, and increase resistance to fatigue.[53] Animal studies show that orally administered 20E reduces hyperglycemia induced in rats,[54, 55] stimulates the conversion of glucose into glycogen in mouse liver,[56] and generally enhances glucose utilisation in tissues[57] through a mechanism that increases insulin sensitivity.[58] Ecdysteroids are associated with numerous other effects, including antiosteoporotic activity, wound healing properties, and cholesterol-lowering effects.[45] Their activity on tumour drug resistance is well known and has been extensively studied by our research group.[59-66] Their potential clinical applications are highlighted by a phase 2 trial reporting *per os* 20E treatment as safe and efficient against sarcopenia,[67, 68] and by a phase 2/3 trial reporting 20E as a life-saving agent in critical-state COVID-19 patients.[69] Ecdysteroids do not appear to bind to any of the nuclear steroid hormone receptors. As a result, their bioactivities can be distinguished from those of conventional steroids, leading to a much more favourable side effect profile and lower toxicity. While their mechanism of action remained unclear for years, a recent study revealed that activating the MAS receptor may be key to their multiple bioactivities.[70]

Within the framework of the present PhD work, both natural and semisynthetic ecdysteroid derivatives were evaluated for their antimicrobial and antitrypanosomal properties. Therefore, in the following, I give a brief overview of these bioactivities.

1.2.3. Antimicrobial activities

Ecdysteroids have shown some slight antimicrobial activities; however, this research field has not yet been sufficiently explored. Five isolated ecdysteroids from *Diplopterygium rufopilosum* demonstrated a modest antimicrobial effect against *Streptococcus mutans* and *S. viridans*. Their antifungal effects were mild against *Candida albicans*, *C. glabrata* and *C. tropicalis*.[71] The antimicrobial activities of two ecdysteroids, 22-*epi*-ajugasterone C and ajugasterone C isolated from *Serratula cichoracea*, were studied against *Staphylococcus aureus,* a Gram-positive bacterium, as well as several Gram-negative bacteria such as: *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia sp.*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and the fungus *Candida albicans*. The compounds exhibited antimicrobial activity against *S. aureus*, *K. pneumoniae*, *E. coli*, *Serratia sp*. and *C. albicans*.[72] In another study, 20E, 25(*S*)-inokosterone and ecdysone, isolated from *Serratula coronata,* were tested for their antimicrobial activity on various bacteria and fungi. Furthermore, it was investigated how the

incorporation of acyl group into the molecule, thereby increasing its lipophilicity, influences the antimicrobial potential of ecdysteroids. This modification was carried out in 20E by introducing acetyl groups, which significantly enhanced activity against most of the tested strains.[73] Three additional ecdysteroids, isolated from *Dioscorea dumetorum*, (20*R*)-5β,11α,20-trihydroxyecdysone, ajugasterone and herkesterone were tested against *Candida albicans*, *C. glabrata,* and *C. tropicalis*, but showed no antimicrobial activity.[74]

Trypanosoma cruzi is a pathogenic protozoan responsible for one of the neglected tropical diseases, called Chagas disease. It can be transmitted mainly through the bites of vector-spreading kissing bugs, *Rhodnius prolixus*, but transmission can also take place via blood transfusion, transplantation, or ingestion of contaminated food. The disease consists of an acute and a chronic phase. The acute phase remains asymptomatic or characterised by mild, non-specific symptoms, whereas the chronic phase may involve severe clinical manifestations (gastrointestinal, neurological and cardiac) which can also be fatal.[75] The disease exhibits a high prevalence in Central and South America, and globally up to 7 million people are affected by the disease, which leads to several thousand deaths each year.[76, 77] Given that only benznidazole and nifurtimox are currently available for treatment and they are associated with undesirable side effects and commonly encountered resistance, there is a high need to discover new and effective therapeutic agents.[75] Natural compounds have provided valuable leads in the search for new trypanocidal chemical frameworks.[78-83] Although the antitrypanosomal potential of ecdysteroids is an unexplored research field, it is noteworthy that 20E has been shown to influence the reproduction cycle of *Rhodnius prolixus*.[84] Our work was inspired by previous findings that ecdysone not only affects the development of *T. cruzi*, but also promotes the differentiation of the protozoon in the midgut of *R. prolixus*.[85] Building on these observations and considering the extensive chemical diversity of ecdysteroids, we aimed to systematically screen these compounds for antitrypanosomal activity and to perform semisynthetic modifications to optimise the structures of any hits for enhanced efficacy. The potential trypanocidal activity of ecdysteroids may also be supported by findings that a steroidal scaffold compound, dehydroepiandrosterone, exhibited moderate activity against *T. cruzi*.[86] Moreover, semisynthetic modifications of epiandrosterone – including the introduction of halogen, sulfonate, ether, and ester groups – enhanced the selectivity of the compounds against *T. cruzi*.[87] Additional semisynthetic approaches, starting from natural steroids and incorporating substituents such as phenylpropane, thiosemicarbazide, or amide groups, also yielded active derivatives.[88] Nevertheless, ecdysteroids may represent a more favorable choice for drug development, as they are free from the side effects characteristic for hormones.[87]

2. OBJECTIVES:

Inspired by the findings of the literature discussed above, the following aims were established for this Ph.D. research.

MONOTERPENOIDS

1. Stereoselective synthesis of aminodiol derivatives starting from perillaldehyde. We planned to obtain a diastereomeric and regioisomeric library of 3-amino-1,2-diol using two different methods. In the first case, our goal was to synthesise the products by reductive amination of perillaldehyde, followed by dihydroxylation. In the second case, the preparation of aminodiols was aimed via the Overman rearrangement of perillyl alcohol.

2. Investigation of catalytic activity in the reaction with diethylzinc in aromatic aldehydes. The synthesised products were intended to serve as chiral catalysts in the enantioselective addition of diethylzinc to benzaldehyde. Additionally, further aromatic aldehydes, including 4-methyl-, 4-methoxy-, and 3-methoxybenzaldehyde, were planned to be tested with the most effective catalysts.

ECDYSTEROIDS

3. Semisynthesis of imine and cinnamate ester derivatives starting from 20-hydroxyecdysone. Our goal was to prepare bioactive cinnamic acid esters of 20-hydroxyecdysone and related nitrogen-containing oximes and oxime ethers, and a lactam.

4. Ecdysteroids as bioactive agents: evaluation of a focused library of natural and semisynthetic derivatives. Various natural and semisynthetic ecdysteroid derivatives were planned to be evaluated for their anticryptococcal and antitrypanosomal activity in research collaborations.

3. MATERIALS AND METHODS

All reagents and solvents applied for both chemical and microbiological purposes were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Molar (Molar Chemicals Kft. Halásztelek, Hungary).

Melting point measurements were performed with a Kofler apparatus (Nagema, Dresden, Germany) or an RK Tech melting point apparatus with a microscope. Optical rotations were determined using a PerkinElmer 341 polarimeter (PerkinElmer Inc., Shelton, CT, USA) or a JASCO P-2000 polarimeter (JASCO International Co. Ltd, Hachioji, Tokyo, Japan) in MeOH or $CHCl_3$.

The one- and two-dimensional NMR spectra were determined with a Bruker Avance DRX 400 or 500 (Bruker Biopsin, Karlsruhe, Baden-Württemberg, Germany). The *J* values were given in Hz. The NMR evaluations of the compounds discussed in this thesis can be found in the publications indicated. HR-MS spectra were recorded on an Agilent 1100 LC-MS instrument (Agilent Technologies, Santa Clara, CA, USA) coupled with a Thermo Q-Exactive Plus orbitrap analyser (Thermo Fisher Scientific, Waltham, MA, USA) used in positive ionisation mode. Chemical reactions were monitored by thin layer chromatography on Kieselgel $60F_{254}$ precoated TLC silica plates purchased from Merck (Merck KGaA, Darmstadt, Germany) and characteristic spots of the compounds were examined under UV light at 254 and 366 nm.

3.1. Monoterpenoids [II]

3.1.1. Source of the monoterpene starting materials

(–)-(*S*)-Perillaldehyde **1** was acquired from Merck Co. (Merck Co., Darmstadt, Germany). (*S*)-4-isopropylcyclohex-1-ene carbaldehyde (**2**) and (*S*)-4-isopropylcyclohex-1-ene-1-ylmethanol (**15**) were prepared according to procedures from the literature, and their spectroscopic data were in agreement with the literature.[89]

Chromatographic separations were performed with Merck Kieselgel 60 (230–400 mesh ASTM, Merck Co., Darmstadt, Germany).

GC measurements were made with a PerkinElmer Autosystem KL GC consisting of a flame ionisation detector and a Turbochrom Workstation data system (PerkinElmer Corporation, Norwalk, CT, USA). Separation of the enantiomers of the *O*-acetyl derivatives of 1-phenyl-1-propanol was performed on a CHIRASIL-DEX CB column (2500 × 0.265 mm inner diameter,

Agilent Technologies, Inc., Santa Clara, CA, USA. Chiral HPLC analysis was performed on a Chiralcel OD-H column (250 × 4.6 mm). UV detection was monitored at 210 nm or 254 nm.

X-ray crystallography on compound **7a** was carried out by Prof. Matti Haukka from the University of Jyväskylä, Department of Chemistry (Finland). For the structural determination, a Rigaku Oxford Diffraction Supernova diffractometer (Rigaku Oxford Diffraction, Yarnton, UK) was applied using Cu Kα radiation. The CrysAlisPro software package (version: 1.171.37.35) was used for cell refinement and data reduction.

Table 1. The following table lists the chromatographic methods used to purify monoterpenes. The procedure was carried out on atmospheric columns, the stationary phase being, in all cases, silica gel.

Compound	Chromatographic procedure
3a	toluene/EtOAc 9:1
3b	toluene/EtOAc 9:1
3c	toluene/EtOAc 9:1
4a	*n*-hexane/EtOAc 9:1
4b	*n*-hexane/EtOAc 19:1
4c	*n*-hexane/Et_2O 19:1
5a	*n*-hexane/EtOAc 4:1
6a	*n*-hexane/EtOAc 4:1
5b	*n*-hexane/EtOAc 4:1
6b	*n*-hexane/EtOAc 4:1
5c	*n*-hexane/EtOAc 4:1
6c	*n*-hexane/EtOAc 4:1
9	DCM/MeOH 19:1
13	*n*-hexane/EtOAc 2:1

Compound	Chromatographic procedure
10a	*n*-hexane/EtOAc 9:1
10b	*n*-hexane/EtOAc 4:1
10c	*n*-hexane/EtOAc 4:1
18a and 18b	*n*-hexane/Et_2O 9:1
19a	*n*-hexane/Et_2O 2:1
19b and 19c	*n*-hexane/Et_2O 2:1
20b	*(1)* *n*-hexane/EtOAc 1:1 (*2*) *n*-hexane/EtOAc 9:1
20c	*(1)* *n*-hexane/EtOAc 1:1 (*2*) *n*-hexane/EtOAc 9:1
23a	$CHCl_3$/MeOH 9:1
23b	$CHCl_3$/MeOH 9:1
24	toluene/EtOH 4:1
25a	toluene/EtOH 4:1
25b	toluene/EtOH 4:1

3.1.2. Preparation of aminodiols by reductive alkylation starting from 1

3.1.2.1. Reductive alkylation of 2 using aromatic amines

A 4.00 g (26.28 mmol) aliquot of compound **2** (prepared from 1 according to literature method) was dissolved in 200 ml of anh. EtOH. Under stirring, 27.6 mmol of aromatic amines were added to the solution: benzylamine in case **3a**, (*S*)-1-phenylethylamine in case **3b** and (*R*)-1-phenylethylamine in case of **3c** was added to the solution. The reaction mixture was stirred at rt for 1 h. Subsequently, the solvent was evaporated under reduced pressure and the residue was dissolved in 200 ml of anh. EtOH again. After stirring it for an additional hour, 2.98 g

(78.84 mmol) $NaBH_4$ was carefully added in multiple portions to the mixture and was stirred at rt (for compound **3a**) or under reflux (for compounds **3b** and **3c**). After 2 h of stirring, the anh. EtOH was evaporated on a rotary evaporator. Ice-cold H_2O (70 ml) was poured into the crude product and extracted with DCM (3 x 100 ml). The combined organic fractions were dried (Na_2SO_4), filtered, and evaporated. The crude products were purified by column chromatography (see **Table 1**)**,** and then the hydrochloride salts of the compounds were formed to gain: **3a** (6.32 g, 86%); **3b** (5.64 g,73 %); **3c** (5.10 g, 66%).

3.1.2.2. Preparation of the *tert*-butyloxycarbonyl-protected amines from 3a–c

A 12 mmol aliquot of the liberated bases of compounds **3a–c** was dissolved in 30 ml of anh. THF. Under stirring, di-*tert*-butyl dicarbonate (2.88 g for **4a**, 5.76 g for **4b**, and 3.56 g mmol for **4c**), TEA (3.64 g), and DMAP (0.15 g) were added to the appropriate solution. The reaction mixture was stirred overnight at rt. When the TLC indicated, the THF was removed under reduced pressure. The crude products were subjected to column chromatographic purification (see **Table 1**) to obtain **4a** (3.92 g, 95%); **4b** (3.99 g, 93%); **4c** (3.86 g, 90%).

3.1.2.3. Preparation of the dihydroxylated derivatives of 4a–c

A 10 mmol aliquot of **4a–c** was dissolved in 50 ml of acetone. In one portion, an 8.5 ml aliquot of 50% aqueous solution of NMO and 4.5 ml of OsO_4 in 2% *tert*-BuOH were added to the solution. The reaction mixture was stirred at rt overnight. When the reaction was complete, indicated by means of TLC, an 80 ml aliquot of saturated aqueous solution of Na_2SO_3 was added to quench the reaction. The mixture was extracted with EtOAc (3 x 60 ml) and the combined organic phase was dried, filtered and evaporated. The products were purified by column chromatography (see **Table 1**) to obtain: **5a** (0.95 g, 25%); **6a** (1.72 g, 46%); **5b** (1.68 g, 43%); **6b** (1.58 g, 38%); **5c** (1.92 g, 48%); **6c** (1.88 g, 48%).

3.1.2.4. Preparation of the Boc-deprotected form of 5a–c and 6a–c

A 3 mmol aliquot of **5a-c** and **6a-c** was dissolved in 25 ml of Et_2O, and then a 70 ml aliquot of 18% aqueous solution of HCl was added to the mixture and stirred overnight. After completion of the reaction, Et_2O was evaporated under reduced pressure. The hydrochloride salt formed was filtered off and washed with Et_2O to form **7a** (0.83 g, 88%); **7b** (0.81 g, 82%); **7c** (0.73 g, 84%); **11a** (0.91 g, 97%); **11b** (0.78 g, 79%); **11c** (0.77 g, 78%).

3.1.2.5. General procedure for debenzylation of 7a and 11a

A 1.8 mmol aliquot of the compounds was dissolved in 24 ml of the *n*-hexane/EtOAc mixture (1:2 mixture for **7a**; 1:1 mixture for **11a**). The solution was added to a stirred suspension of 0.10 g of 10% Pd/C and stirred under an H_2 atmosphere at rt and normal pressure. After the reaction was completed, indicated by TLC, the mixture was filtered using a short Celite pad, the solvent was removed under reduced pressure and the hydrochloride salts were formed: **8** (0.14 g, 35%); **12** (0.18 g, 45%).

3.1.2.6. Preparation of the *N*-methyl derivatives of compounds 9 and 13

A 1.00 g aliquot of **5a** and **6a** was dissolved in 12 ml of anh. THF was carefully added at 0 °C to a stirred suspension of 0.30 g of $LiAlH_4$ in 15 ml of anh. THF. The reaction mixture was stirred under reflux for 6 h. Subsequently, a mixture of 1 ml of H_2O and 16 ml of THF was added in small portions, applying cooling. The precipitate formed was filtered off and washed with THF. The liquid phase was dried over Na_2SO_4, filtered and evaporated. The crude products were purified by column chromatography (see **Table 1**) to form **9** (0.46 g, 60%) and **13** (0.45 g, 58%).

3.1.2.7. Preparation of 1,3 oxazines from 7a–c

A 0.15 g aliquot of **7a**–**c** was dissolved in 6 ml of Et_2O and 4.5 ml of 35% aqueous formaldehyde solution was added to the solution and stirred vigorously at rt for 3 h. Subsequently, the mixture was extracted with 10 ml of 10% aqueous KOH solution. The aqueous phase was extracted with Et_2O (3 x 25 ml). The combined organic phase was washed with brine (3 x 25 ml) and then dried (Na_2SO_4), filtered and concentrated to dryness. The crude products were purified via column chromatography (see **Table 1**) to obtain **10a** (0.11 g, 70%); **10b** (0.15 g, 96%) and **10c** (0.13 g, 83%)

3.1.3. Preparation of aminodiols by Overman rearrangement of 2

3.1.3.1. Preparation of 2,2,2-trichloro-*N*-((1*R*,5*S*)-5-isopropyl-2-methylenecyclohexyl)acetamide 16a and 2,2,2-trichloro-*N*-((1*S*,5*S*)-5-isopropyl-2-methylenecyclohexyl)acetamide 16b

A 10.48 g aliquot of **15** was dissolved in 320 ml of anh. DCM and then 12.2 ml of DBU was added under an Ar atmosphere. To the reaction mixture, 10.3 ml of trichloroacetonitrile was added in small quantities and after 2 h of stirring at rt, the solvent was concentrated to dryness. Column chromatography was applied to purify the crude product on silica gel by applying DCM. The upper third of the column was anh. Na_2SO_4 and the bottom two-thirds of the column

was silica gel. The isolated slightly yellow oil product was dissolved in 300 ml of anh. toluene and reacted in an autoclave at 130 °C in an Ar atmosphere for 1 day. After the completion of the reaction, the mixture was extracted with a cold 5% aqueous solution of HCl (3 x 100 ml) and the organic phase was dried, filtered and evaporated under reduced pressure. On the basis of ^{1}H NMR measurements and GC determination (Chirasil-DEX CB column), the diastereomers formed a mixture with a ratio of 85:15. Despite the various column chromatography methods attempted, the diastereomers remained inseparable and yielded a mixture of isomers of **16a** and **16b** (14.52 g, 72%).

3.1.3.2. Preparation of *tert*-butyl ((1*R*,5*S*)-5-isopropyl-2-methylenecyclohexyl*)* carbamate 18a and *tert*-butyl ((1*S*,5*S*)-5-isopropyl-2-methylenecyclohexyl) carbamate 18b

An 11.11 g aliquot of **16a** and **16b** was dissolved in 66 ml of EtOH/DCM 2:1 and then 525.6 ml of 5M aqueous solution of NaOH was added. Following this, the reaction mixture was stirred at 50°C for 15 h. When the TLC indicated, the reaction mixture was cooled to rt and then extracted with DCM (3 x 150 ml). The combined organic layer was washed with 100 ml of brine, dried, and evaporated. The residue was redissolved in 100 ml anh. THF, 11.29 g of triethylamine, 0.45 g of DMAP, and 8.93 g di-*tert*-butyl dicarbonate were added and stirred at rt for 12 h. Following this, the solvent was removed, and the crude product was subjected to chromatographic purification (see **Table 1**). The diastereomers remained inseparable, and based on the GC determination, the ratio of the obtained products was the same at 85:15. The yield of the **18a** and **18b** mixtures was 5.56 g (59%).

3.1.3.3. Preparation of the dihydroxylated products from the mixture of 18a and 18b

A 21.3 mmol aliquot of **18a** and **18b** was dissolved in 100 ml of acetone. In one portion, a 22.5 ml aliquot of 50 % aqueous solution of NMO and 1.5 ml of OsO_4 in 2% *tert*-BuOH were added to the solution. After 3 days of stirring the reaction mixture at rt, 20 ml of saturated aqueous solution of Na_2SO_3 was applied to quench the reaction, then extracted with EtOAc (3 x 100 ml). The combined organic layer was dried, filtered, and evaporated. Based on the ^{1}H NMR spectra, **19a**, **19b**, and **19c** were formed in a ratio of 71:16:13. The crude product was purified via column chromatography (see **Table 1**). **19a** was successfully isolated (2.94 g, 48%), although **19b** and **19c** remained a mixture (2.02 g, 33%).

3.1.3.4. Preparation of acetals starting from 19a and 19b

A 1.20 g aliquot of the diastereomeric mixture **19a** and **19b** was dissolved in 100 ml of anh. acetone, then 0.10 g of 4-methylbenzene-1-sulfonic acid was added to the solution and stirred

at rt. As the reaction was completed (monitored by TLC), acetone was evaporated under reduced pressure. The crude product was purified by column chromatography (see **Table 1**) to obtain **20b** (0.77 g, 56%) and **20c** (0.24 g, 18%).

3.1.3.5. Preparation of the deprotected form of 19a, 20b, and 20c

0.70 mmol of **19a**, **20b** or **20c** was dissolved in 6 ml of Et_2O; after this, 10 ml of 10% aqueous solution of HCl was added. After 1 day of intensive stirring at rt, when the reaction was complete, the mixture was extracted with Et_2O (2 x 10 ml). The compounds were obtained and further purified as hydrochloride salts. Compound **21a** (0.133 g, 86%); **21b** (0,131 g, 86%) and **21c** (0.124 g, 80%) were formed.

3.1.3.6. Preparation of the *N*-benzyl derivatives of 21a and 21b

A 0.19 g aliquot of **21a** or **21b** was dissolved in 20 ml of anh. EtOH, 0.11 g of benzaldehyde were added. After 1 h of stirring, when the reaction was complete (indicated by TLC), the solvent was evaporated, and the residue was dissolved in 20 ml of anh. EtOH was added and stirred for an additional hour. Subsequently, a 0.11 g aliquot of $NaBH_4$ was added in small quantities. After completion of the reaction (monitored by means of TLC), the EtOH was removed under reduced pressure. Subsequently, a 15 ml aliquot of H_2O was poured into the residue, and DCM (3 x 25 ml) was used for extraction. The combined organic phase was dried over Na_2SO_4, filtered, and evaporated. The crude product was subjected to column chromatography (see **Table 1**) to form **21a** (0.150 g, 54%) and **23b** (0.246 g, 89%).

3.1.3.7. Preparation of the *N*-methyl derivatives of 19a

A 0.18 g aliquot of $LiAlH_4$ was suspended in 5 ml of anh. THF. Subsequently, a 0.45 g aliquot of **19a** was dissolved in 5 ml of anh. THF and was added to the suspension drop-by-drop. The reaction mixture was stirred for 2 h, then the excess of $LiAlH_4$ was decomposed using 0.36 ml of H_2O and 5 ml of THF at 0 ° C. After 1 h of stirring, the suspended suspension formed at rt was filtered, and then the non-organic residue was washed using THF (3 x 30 ml). The organic phase was dried (Na_2SO_4), filtered, and evaporated in vacuo. The crude product was purified by forming its hydrochloride salt, applying a 10% HCl solution in EtOH and Et_2O to gain **22** (0.246 g, 65%).

3.1.3.8. Preparation of 1,3 oxazine derivatives of 22, 23a, and 23b with formaldehyde

A 0.58 mmol of **22**, **23a** or **23b** was dissolved in 10 ml of Et_2O and 5 ml of 40% aqueous solution was added. After 1 h of stirring at rt, 5% aqueous solution of NaOH was added to make

the reaction mixture alkaline and extracted with Et_2O (3 x 20 ml). The organic layers were combined, dried, filtered, and evaporated under reduced pressure. The crude products were purified by column chromatography (see **Table 1**) to yield: **24** (0.081 g, 66%); **25a** (0.149 g, 89%) or **25b** (0.134 g, 80%).

3.1.4. Enantioselective addition of diethylzinc to aldehydes facilitated by chiral aminodiols

To the suitable 10 mol% chiral aminodiol catalyst, 4.5 ml of 1M solution of $ZnEt_2$ under an Ar atm at rt. After 20 minutes of stirring at rt, 153 ul of aromatic aldehyde was added to the solution. The reaction mixture was stirred for an additional 20 h at rt. Subsequently, 50 ml of saturated aqueous solution of NH_4Cl was introduced to quench the reaction, then extracted with EtOAc (2 x 30 ml). The organic phase was dried, filtered, and concentrated to dryness. The *ee* value and absolute configuration of the obtained secondary alcohol **27a** each case was determined by chiral phase GC by using a CHIRASIL-DEX CB column at 90 °C after *O*-acetylation in an Ac_2O/DMAP/pyridine system. The determination of **27b–d** was carried out by a chiral HPLC Chiralcel OD-H column; the retention times were: 1-(4-tolyl)-1-propanol **27b**, t_R(*R*) = 16.0 min, t_R(*S*) = 22.2 min, V(*n*-hexane)/V(2-propanol) = 95:5, 0.5 ml/min, 210 nm; 1-(4-methoxyphenyl)-1-propanol **27c**, t_R(*R*) = 15.9 min, t_R(*S*) = 18 min; V(*n*-hexane)/V(2-propanol) = 95:5, 0.7 ml/min, 210 nm; 1-(3-methoxyphenyl)-1-propanol 27d; t_R(*R*) = 74.9 min, t_R(*S*) = 77.8 min, V(*n*-hexane)/V(2-propanol) = 98:2, 0.4 ml/min, 210 nm.

3.1.5. Computational analysis using DFT methods on compounds 10a and 14a

All DFT calculations were carried out by Prof. Dr. Antal Csámpai from Eötvös Loránd University (ELTE) Department of Organic Chemistry. Gaussian 09 Revision A.02 software was used for DFT calculations (Gaussian Incorporation, Pittsburgh, PA, USA), package [Gaussian 09], using the M06-2X global hybrid DFT functional[90] and 6-31+G(d,p) basis set.[91] The IEFPCM solvent model parameterised with the dielectric constant of water ($\varepsilon = 78.4$)[92] was applied to support structural optimisations and subsequent frequency calculations to represent the approximate polarity of the experimental reaction conditions. The Gibbs free-energy values of optimised structures were obtained by correcting the calculated total energy with zero-point vibrational energy (ZPE) and the computed thermal corrections.

3.2. Ecdysteroids [I,III]

3.2.1 Previously prepared ecdysteroids

20-hydroxyecdysone (**20E**) **28** was acquired from Shaanxi KingSci Biotechnology (Xi'an, China) with a purity of 90%. It was recrystallised using an EtOAc/MeOH mixture (2/1), achieving >97% purity as determined by RP-HPLC. Ecdysteroids **29–32** were isolated in our previous phytochemical work from *Cyanotis arachnoidea* extracts.[93, 94] Compounds **34–43**, **46–55**, **57–64** were obtained by semisynthesis in our previous research.[60, 63, 64, 66, 95, 96]

The following chromatographic methods were used to separate and purify ecdysteroids:

- medium-pressure chromatography: Flash chromatography was conducted using a CombiFlash Rf+ Lumen instrument (TELEDYNE Isco, Lincoln, NE, USA), which was characterised by diode array detection (DAD) and evaporative light scattering (ELS) detection. Chromatographic separations were performed by applying commercially obtained prefilled RediSep NP-silica flash columns 24g gold (Teledyne Isco),
- high-pressure chromatography: RP-HPLC methods:
    - analytical HPLC was performed on a Jasco HPLC instrument (Jasco International Co., Ltd., Hachioji, Tokyo, Japan) equipped with an MD-2010 Plus photo diode array detector. The Gemini NX-C18 250 x 4.6 mm column (Phenomenex Inc., Torrance, CA, USA), Kinetex Biphenyl 250 x 4.6 mm column (Phenomenex Inc.) and Kinetex XB-C18 250 x 4.6 mm column were applied for measurements using a 1 ml/min flow rate with different ratios of aqueous MeCN, MeOH or THF,
    - for preparative purposes, an Armen Spot Prep II integrated HPLC system (Gilson, Middleton, WI, USA) with dual-wavelength detection was used. A Gemini NX-C18 250 x 21.2 mm column (Phenomenex Inc.), a Kinetex Biphenyl 250 x 21.2 mm column (Phenomenex Inc.) and a Kinetex XB-C18 250 x 21.2 mm column were used for the separations with a flow of 15 ml/min, applying the eluents mentioned above.

Table 2. The applied chromatographic conditions to obtain the pure ecdysteroid derivatives related to this thesis. In the case of stationary phase: 24 g silica (plain) refers to the readily available RediSep (Teledyne Isco) NP flash chromatography columns; XB-C18: Kinetex® 5 µm XB-C18 100 Å 250 x 21.2 mm HPLC column (Phenomenex Inc.); NX-C18: Gemini® 5 µm NX-C18 110 Å 250 x 21.2 mm HPLC column (Phenomenex Inc.); Biphenyl: Kinetex® 5 µm Biphenyl 100 Å 250 x 21.2 mm HPLC column (Phenomenex Inc.). Numbers in parentheses, such as "(*x*)" denote the chromatographic purification steps, respectively. All the eluent ratios are expressed as v:v.

Compound	Stationary phase	Flow rate	Elution
44 and **45**	(*1*) 24 g silica (*2*) XB-C18	(*1*) 35 ml/min (*2*) 15 ml/min	(*1*) DCM:MeOH (A:B) 0 → 35% B (30 min) (*2*) H_2O:CH_3CN 16:84
56	(*1*) 24 g silica (*2*) XB-C18	(*1*) 35 ml/min (*2*) 15 ml/min	(*1*) DCM:MeOH (A:B) 0 → 35% B (30 min) (*2*) H_2O:MeOH 45:55
65	(*1*) 24 g silica (*2*,*3*) Biphenyl	(*1*) 35 ml/min (*2*,*3*) 15 ml/min	(*1*) DCM:MeOH (A:B) 0 → 5% B (10 min), 5% B (40 min) (*2*) H_2O:CH_3CN 48:52 (*3*) H_2O:CH_3CN 70:30
66	(*1*) 24 g silica (*2*,*3*) Biphenyl	(*1*) 35 ml/min (*2*,*3*) 15 ml/min	(*1*) DCM:MeOH (A:B) 0 → 5% B (10 min), 5% B (40 min) (*2,3*) H_2O:CH_3CN 48:52
67	(*1*) 24 g silica (*2*) Biphenyl	(*1*) 35 ml/min (*2*) 15 ml/min	(*1*) DCM:MeOH (A:B) 0 → 5% B (10 min), 5% B (40 min) (*2*) H_2O:CH_3CN 48:52
68	(*1*) 24 g silica (*2*,*3*) Biphenyl	(*1*) 35 ml/min (*2*,*3*) 15 ml/min	(*1*) DCM:MeOH (A:B) 0 → 5% B (10 min), 5% B (40 min) (*2*) H_2O:CH_3CN 48:52 (*3*) H_2O:CH_3CN 50:50
69	NX-C18	15 ml/min	H_2O:(CH_3CN:MeOH 9:1) A:B 65:35
70	NX-C18	15 ml/min	H_2O:(CH_3CN:MeOH 9:1) A:B 65:35
71	(*1*,*2*) Biphenyl	15 ml/min	(*1*) H_2O:(CH_3CN:THF 3:1) A:B 26:74 (*2*) H_2O:(CH_3CN:THF 3:1) A:B 48:52
72	(*1*,*2*) Biphenyl	15 ml/min	(*1*) H_2O:(CH_3CN:THF 3:1) A:B 26:74 (*2*) H_2O:(CH_3CN:THF 3:1) A:B 56:44
73	(*1*,*2*) Biphenyl	15 ml/min	(*1*) H_2O:(CH_3CN:THF 3:1) A:B 26:74 (*2*) H_2O:(CH_3CN:THF 3:1) A:B 70:30
74	(*1*,*2*) Biphenyl	15 ml/min	H_2O:(CH_3CN:THF 3:1) A:B 45:55

3.2.3 Semisynthesis of ecdysteroid oximes 44 and 45 starting from 28

An 807.4 mg aliquot of KOH was dissolved in EtOH and added to a freshly distilled ethanolic solution of hydroxylamine hydrochloride (1.00 g 14.39 mmol) to liberate the free base form of the hydroxylamine, then 1.00 g of **28** was added to the solution. The mixture was stirred for 14 days under reflux. Subsequently, the mixture was evaporated to 4 g of silica and purified by flash chromatography (see **Table 2**) to obtain **44** (181 mg, 18%) and **45** (509 mg, 49%).

3.2.4 Semisynthesis of lactam 56 starting from 45

To a solution of **45** (300 mg, 0.61 mmol) in acetone (20 ml), 83.3 mg aliquot of Na_2CO_3 and 461.6 mg of TsCl were added, and the reaction mixture was stirred for 1 day at rt. Subsequently, 3 g of silica was added to the solution; then, the solvent was evaporated under reduced pressure, preparing the sample for dry loading flash chromatographic purifications (see **Table 2**) to obtain **56** (183.9 mg, 61.3 %).

3.2.5 Semisynthesis of cinnamate esters 65–68 starting from 28

A 1.00 g aliquot of **28** was suspended in 250 ml of anh. DCM, then 0.64 g of DMAP, 1.30 g of *trans*-cinnamic acid, and 1.20 g of EDC·HCl were added. The reaction mixture was stirred at rt and monitored by TLC. As the reaction proceeded, the starting suspension became a solution. After 4.5 days, upon completion of the reaction, an aliquot of aqueous $NaHCO_3$ (saturated) solution and brine were added to the reaction mixture and stirred for five minutes. The mixture was extracted by DCM (3 x 50 ml). The organic phases were combined and washed with brine, dried (Na_2SO_4), filtered, and removed by vacuum. The crude product was then subjected to chromatographic separations (see **Table 2**) to obtain **65** (21.3 mg, 1.7 %); **66** (52.4 mg, 3.4 %); **67** (0.1603 g, 10.4%) and **68** (0.0446 g, 2.9 %). Ecdysteroids **65** and **67** were also obtained from other research groups. The spectroscopic data matched those reported in the literature.[97, 98]

3.2.6 Semisynthesis of stachysterone B 6-*O*-*tert*-butyl oxime ethers (69, 70)

A 0.500 g aliquot of **28** was dissolved in 25 ml of anh. pyridine, followed by the addition of 0.500 g of *o*-*tert*-butylhydroxylamine hydrochloride. The reaction mixture was stirred at 70 °C for 48 hours. When the reaction was completed (indicated by TLC), it was cooled to rt and quenched by adding 10 ml of aqueous 0.40 mM KOH solution. The solvent was concentrated to dryness and then 20 ml of H_2O was added to the residue and extracted with EtOAc (3 x 30 ml). The organic layers were combined, washed with brine, dried, filtered, and evaporated. The

crude product was subjected to reverse phase chromatographic purifications (see **Table 2**) to yield **69** (250.31 mg, 45.1%) and **70** (75.28 mg, 13.6%).

3.2.7 Semisynthesis of 69 cinnamic esters

A 0.150 g aliquot of **69** was suspended in 20 ml of anh. DCM, then 0.089 g of DMAP, 0.167 g of *trans*-cinnamic acid, and 0.162 g of EDC·HCl were added. The reaction mixture was stirred at rt and monitored by means of TLC. As the reaction proceeded, the starting suspension became a solution. After 3 days of stirring, a 10 ml aliquot of H_2O was poured into the reaction mixture and stirred for 5 minutes. Subsequently, a saturated aqueous NH_4Cl solution was added, and EtOAc (3 x 20 ml) was used to extract the reaction mixture. The combined organic phases were washed with brine, dried, filtered, and removed using a rotary evaporator. The crude product was then purified using RP-HPLC (see **Table 2**) to obtain **71** (10.22 mg, 5.5%), **72** (26.86 mg, 12.1%) and **73** (11.78 mg, 4.5%).

3.2.8 Semisynthesis of a cinnamic ester of 70

A 59.33 mg aliquot of **70** was suspended in 10 ml of anh. DCM, then 35 mg of DMAP, 65.8 mg of *trans*-cinnamic acid, and 63.9 mg of EDC·HCl were added. The reaction mixture was stirred at rt and monitored by means of TLC. As the reaction progressed, the starting suspension became a solution. After 4 days of stirring, a 5 ml aliquot of H_2O was poured into the reaction mixture and stirred for five minutes. Subsequently, a saturated aqueous ammonium chloride solution was added and then EtOAc (3 x 15 ml) was used to extract the reaction mixture. The combined organic phases were washed with brine, dried, filtered, and removed using a rotary evaporator. The crude product was then purified using RP-HPLC (see **Table 2**) to obtain **74** (16.6 mg, 8.9%).

3.3. Biological activity measurements of ecdysteroid derivatives

3.3.1. Anticryptococcal activity assay

The anticryptococcal activity testing was carried out by the research group of Prof. Dr. Csaba Vágvölgyi from the University of Szeged Institute of Biology (Hungary).

Stock solutions for bioactivity tests were made using methanol at a concentration of 40 mg/ml. Verapamil and quinidine were dissolved in DMSO at concentrations of 5.0 mg/ml and 2.5 mg/ml, respectively. At the same time, the indomethacin stock solution was prepared in EtOH at a concentration of 7.0 mg/ml.

The mode of the interaction was assessed using the Abott formula[99] with Microsoft Excel Software version 16.0. A given interaction's expected inhibition (Ie) was calculated as $I_e = X + Y - (XY/100)$. X and Y represent the percentage of inhibition observed for each compound when tested individually. The observed inhibition was denoted as I_0. Interaction ratio (IR) = I_0/I_e. An IR value between 0.5 and 1.5 indicates an additive interaction, $I_R>1.5I_R > 1.5I_R>1.5$ signifies synergism, and $I_R<0.5I_R < 0.5I_R<0.5$ denotes antagonism.

3.3.1.1. Antifungal Activity Assay

The inhibitory activity of ecdysteroids against strains of *C. neoformans* was tested. The MIC was determined using the microdilution method in 96-well microtiter plates. Briefly, 100 µl of the ecdysteroid solution, diluted serially twice, was mixed with 100 µL of cell suspension. The final concentration of ecdysteroids in the wells ranged from 2.00 to 0.125 mg/ml. After 72 h of incubation at 30 °C, the optical density of the cultures was measured at 620 nm using a SPECTROstar Nano plate reader (BMG LabTech, Offenburg, Germany). MIC was defined as the concentration exerting a growth inhibition of ≥90% compared to the 100% growth of the untreated control. Each experiment was performed in three independent biological replicates, each in triplicate.

Additionally, 5 µl samples from untreated cultures and those exposed to **45** or **44** were diluted 10 and 100-fold in 95 µl of sterile distilled water. From each dilution, 5 µl was placed in a solid YPD medium, and the growth of the strains was detected after 48 h of incubation at 30 °C.

3.3.1.2. Yeast Viability Assay

The viability of *C. neoformans* IFM 5844 cells was assessed using the calcein-AM assay and analysed by flow cytometry. Briefly, 2×10^5 cells were treated with 1.00 mg/ml **44** in RPMI 1640 medium at 30 °C for 3 h. After treatment, cells were washed twice with sterile distilled water and suspended in 100 µl RPMI 1640 medium. Calcein-AM (Invitrogen, Waltham, MA, USA) was added at a concentration of 10 µM concentration, and the suspension was incubated in the dark at 30 °C for another 3 h. Subsequently, cells were washed twice with sterile distilled water, and the fluorescence intensity was measured with a flow cytometer (FlowSight®, Amnis-EMD Millipore) using a 488 nm excitation laser. Untreated cells stained with calcein-AM and cells stained with calcein-AM after exposure to 96% ethanol for 30 min were used as controls. The experiment was carried out four times independently, with fluorescence intensities measured for 10,000 cells per sample in each run.

3.3.1.3. Combined treatment of *C. neoformans* with 44, 45 and transporter inhibitors

The interaction between efflux pump inhibitors (indomethacin, quinidine, and verapamil) and **44** or **45** was evaluated using the standard chequerboard titration method.[99] The efflux pump inhibitors were tested at concentrations ranging from 200 to 12.50 µg/ml, while the ecdysteroid concentration ranged from 2.00 to 0.125 mg/ml. The initial cell concentration in each well was 1×10^5 cells/ml. After 72 hours of incubation at 30 °C, the optical density of the cultures was measured at 620 nm using a SPECTROstar Nano plate reader (BMG LabTech, Offenburg, Germany). Inhibitory concentrations were determined for each compound as well as for their combinations. The experiments were carried out at least three times.

3.3.2. Antitrypanosomal activity

The antitrypanosomal activity investigations were carried out by the research group of Prof. Dr. Jürg Gertsch from the University of Bern Institute of Biochemistry and Molecular Medicine (Switzerland).

3.3.2.1. Host Cell Cytotoxicity Assessment

The cytotoxicity or antiproliferative effects of ecdysteroid against mammalian cells were assessed using MTT CHO or RAW264.7 cells were applied to the screening of the compounds. In summary, cells were seeded at a density of 2000/well in 96-well plates. Compound **71** was added to cells at various concentrations after 24 h of incubation at 37 °C and 5% CO_2. Podophyllotoxin was used as a positive control in all experiments. Vehicle controls were included in all experiments, and the final DMSO concentration was below 0.5%. After 72 h, the plates were examined under an inverted microscope for sterility and growth of controls after incubation. The spent medium was removed and 100 µl of fresh medium containing 0.5 mg/ml of the final concentration of MTT was added to the cells. The plates were incubated for 4 h at 37 °C in 5% CO_2. The formazan crystals were solubilised by adding 200 µl of DMSO and thoroughly mixed. The absorbance was determined at 550 nm by using a Tecan plate reader. The values were corrected for blank medium and vehicle control (DMSO only). Results were expressed as a percentage of cell viability relative to vehicle control. Each assay was performed using technical triplicate in three independent experiments.

3.3.2.2. Trypanocidal Activity

Trypanocidal activity against epimastigotes was evaluated by XTT assay. Briefly, 1.5×10^6 epimastigotes per well were seeded in 96-well plates. Compound **71** was added at eight concentrations ranging from 25 to 0.2 µM for 72 h at 28 °C. Benznidazole was used as the

positive control in all experiments. After the incubation period, plates were examined under a microscope for sterility and growth of controls. To the plates, 50 µl of XTT and PMS (phenazine methosulfate, Sigma-Aldrich, MO, USA) solution (XTT and PMS at 0.5 and 0.025 mg/ml, respectively) was added, and the plates were then incubated at 28 °C for 3 h. Parasites were fixed by adding 50 µl of MeOH for 15 min before absorbance was measured at 490 nm on a Tecan plate reader. The results were expressed as percentage cell viability relative to the vehicle control or IC_{50} values calculated by GraphPad Prism version 8.0. All evaluations were performed in triplicates in three independent experiments.

3.3.2.3. FACS-Based Quantitation of Released Parasites from Infected Cells

The host RAW264.7 cells were seeded in 24-well plates at a density of 20,000 cells/ml. Cells were allowed to adhere for 24 h and infected with trypomastigotes at a multiplicity of 10. The next day, non-internalised trypomastigotes were washed away and fresh RPMI with 2% hiFBS was added to the wells along with **71** at various concentrations. All experiments included a non-infected control, a DMSO control, and a benznidazole control at 20 µM. Three independent experiments were performed to determine the activity of the ecdysteroid.

3.3.2.4. Microscopy

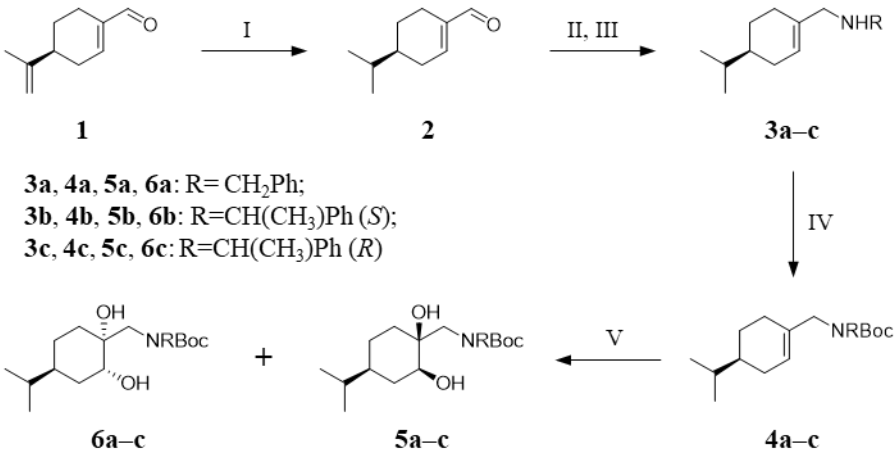
The host CCL39 cells were seeded in 24-well plates at a density of 20,000 cells/ml. Cells were allowed to adhere for 24 h and infected with trypomastigotes at a multiplicity of 10. The next day, non-internalised trypomastigotes were washed away, and fresh RPMI with 2% hiFBS was added to the wells and the test substance. On the fourth day after infection, the cells were fixed with 4% paraformaldehyde for 1 h and scanned on an epi-fluorescence Nikon eclipse TS2 microscope.

4. Results

4.1. Monoterpenoids

4.1.1. Preparation of aminodiol library by reductive alkylation of perillaldehyde (1)

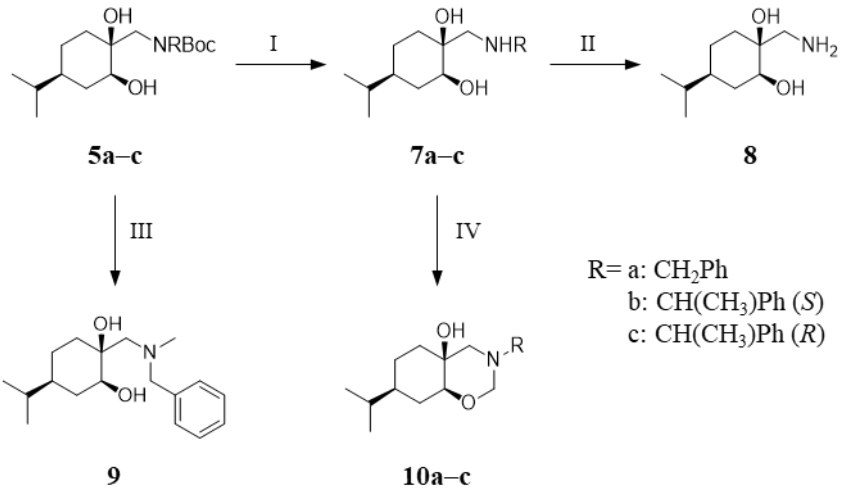
The first step in the synthesis of aminodiols was the selective hydrogenation of perillaldehyde (**1)** with a Pt/C catalyst in an *n*-hexane and EtOAc solvent system to obtain compound **2**, as described in the literature.[89] The reduction of the exo double bond was followed by ^{1}H-NMR monitoring to identify the proper conditions for the reaction. Subsequently, allylamines were prepared in two steps using benzylamine and (*R*) and (*S*)-1-phenylethylamine. After chromatographic purifications, compounds **3a**–**c** were obtained. The obtained products were subjected to Boc protection, forming **4a**–**c**. The protected allylamino groups were subjected to dihydroxylation using the OsO_4 and NMO systems to produce *cis*-vicinal aminodiols. The compounds were successfully separated by column chromatography, and the observed diastereomer ratio was 1:1. These reactions and their conditions are shown in **Scheme 1**.



Scheme 1. Preparation of protected aminodiols through reductive alkylation of perillaldehyde. I: Lit.[89]; II: R-NH_2, (R= a: CH_2Ph, b: $CH(CH_3)Ph$ (*S*), c: $CH(CH_3)$ (*R*)), anh. EtOH, rt, 2 h; III: $NaBH_4$, anh. EtOH, rt, 2 h, 66–86%; IV: Boc_2O, TEA, DMAP, THF, rt, 90–95%; V: 50% NMO/H_2O, 2% OsO_4 in *tert*-BuOH, acetone/H_2O, rt, 2 h. **5:6** = 1:1, 25–48%

The deprotection of **5a**–**c** was carried out using an aqueous 18% HCl solution to gain **7a**–**c**. Primary aminodiol **8** was prepared by removing the benzyl group of **7a**. From **5a**, the reduction with $LiAlH_4$ led to the formation of the *N*-methyl-*N*-benzyl derivative **9**. Furthermore, the cyclisation ability of compounds **7a**–**c** in the presence of formaldehyde was also studied. It is essential to mention that six-membered 1,3-oxazine derivatives (**10a–c**) were yielded in the

three compounds, while five-membered spirooxazolidine derivatives were not even formed as a by-product (**Scheme 2**).



Scheme 2 Synthesis of aminodiol derivatives from 5a–c. I: Et2O, 18% HCl, overnight; 82–88%; II: 10% PD/C, *n*-hexane/EtOAc 1:2 mixture, 1 atm, H_2, rt, 5 h, 35%; III: R= CH_2Ph, $LiAlH_4$, THF, reflux, 6 h, 60%; IV: 35% aq. CH_2O, Et_2O, rt, 3 h, 70–96%

NOESY experiments and X-ray crystallography (which was carried out in collaboration with the University of Jyväskylä) were applied to elucidate the relative configuration of chiral aminodiol **7a**. NOE interactions were detected across H-2 and H-4, H-2 and H-7, along with OH-1 and OH-2 protons (**Figure 10**).

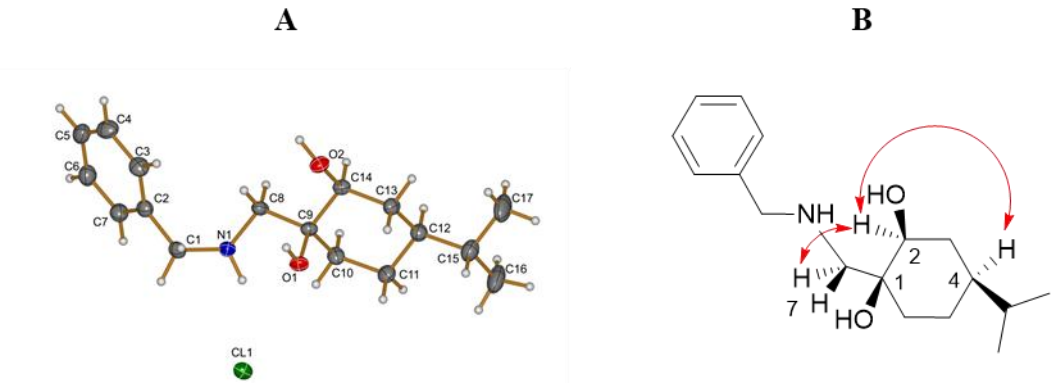
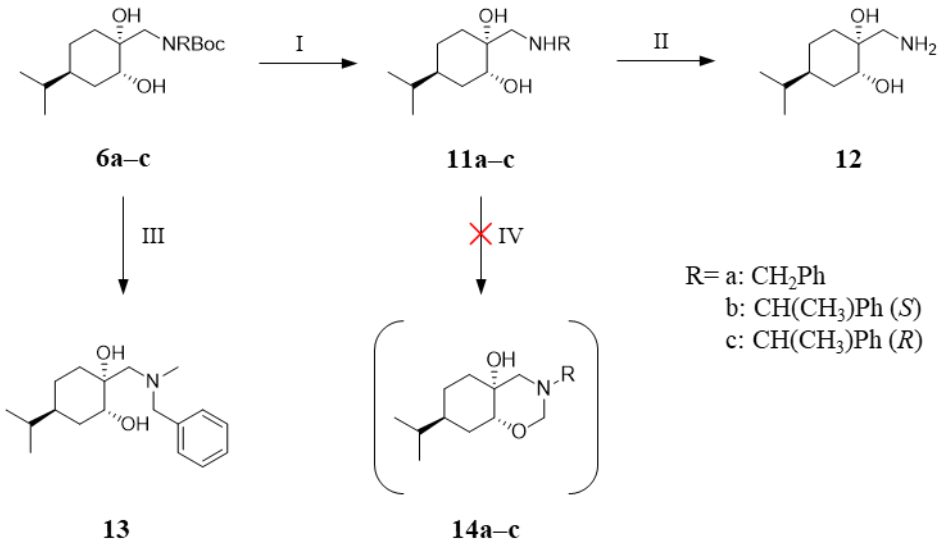


Figure 10. Structural elucidation of compound **7a**. **A**: X-ray crystallography, **B**: NOESY interactions

Similar reactions, starting from diastereomeric **6a–c** compounds, were also performed to compare reactivity and cyclisation ability. Analogous deprotected derivatives **11a**–**c**, *N*-methyl-*N*-benzyl aminodiol **13** and primary aminodiol **12** were prepared similarly. An attempt was made to prepare a ring-closed product from **11a**–**c** using formaldehyde to yield **14a**–**c**; however, under the applied conditions, no ring-closed product could be isolated (**Scheme 3**).



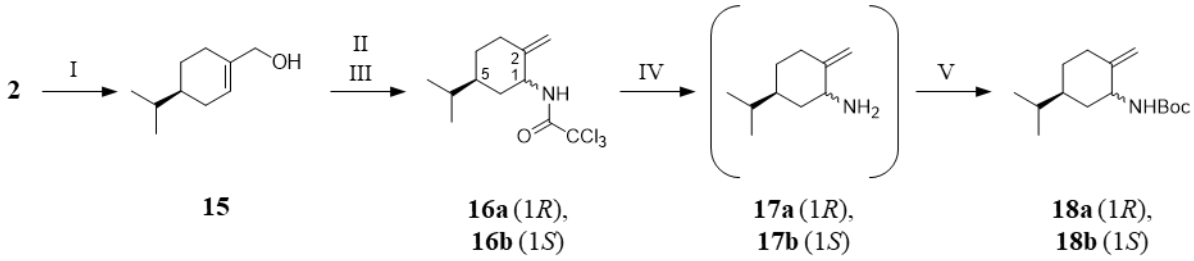
Scheme 3. Synthesis of aminodiol derivatives starting from **6a–c**: I: TFA, DCM, rt, 2 h, 78–97%; II: 10 % Pd/C, *n*-hexane/EtOAc 1:1 mixture, 1 atm, H_2, rt, 5 h, 45%; III: R= CH_2Ph, $LiAlH_4$, THF, reflux, 6 h, 58%; IV 35% aq. CH_2O, Et_2O, rt, 3 h – no product could be isolated

4.1.2. DFT computational analysis performed on compounds 10a and 14a

Based on series of comparative theoretical modelling analyses in collaboration with ELTE we found that both cyclisation processes (**7a** to **10a** and **11a** to **14a**) are thermodynamically viable based on the calculated Gibbs free energy changes. However, no fused 1,3-oxazine **14a** product was obtained. Although the target product was not obtained, a spot likely corresponding to this compound was detected during TLC monitoring.

4.1.3. Preparation of the diastereomeric aminodiol library via Overman rearrangement

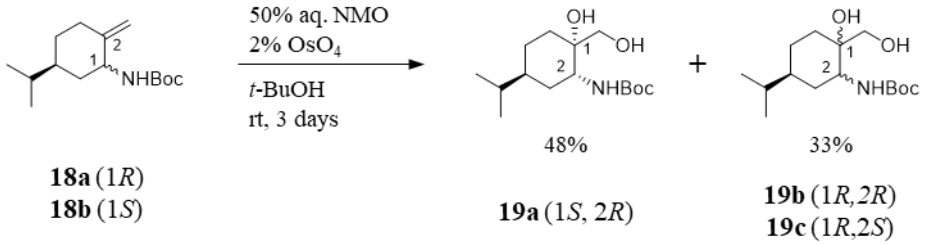
An alternative synthetic route was applied to obtain the 3-amino-1,2-diol regioisomer from perillaldehyde. The allyl alcohol **15** was prepared from compound **2** by applying $NaBH_4$. Following this, CCl_3CN was used to carry out the Overman rearrangement. Therefore, a protected allylamine diastereomer mixture was obtained with a **16a**:**16b** = 85:15 as investigated by gas chromatography (Chirasil-DEX CB column). The relative configuration was consistent with that observed in a similar reaction in the literature.[100] Separation of the diastereoisomers was not feasible, so the trichloroacetonitrile protection group was replaced by the easily handled Boc protecting group. Despite further chromatographic attempts, the resulting diastereomers could not be separated either; therefore, we proceeded with this mixture in the next step. The relative configuration was determined by NOESY experiments.



15 **16a** (1*R*), **16b** (1*S*) **17a** (1*R*), **17b** (1*S*) **18a** (1*R*), **18b** (1*S*)

Scheme 4. Preparation of allyamines via Overman rearrangement: I: $NaBH_4$, MeOH, 0 °C to rt, 1 h, 95%; II: CCl_3CN, anh. DCM, DBU, Ar atm, 0 °C to rt, 2 h; III: toluene, Ar atm, 130 °C, 1 day, 72%, de = 70% for **16a**; IV 5M NaOH/H_2O, EtOH/DCM 2/1, 50 °C, 15 h; V Boc_2O, DMAP, THF, TEA, rt, 12 h, 59%.

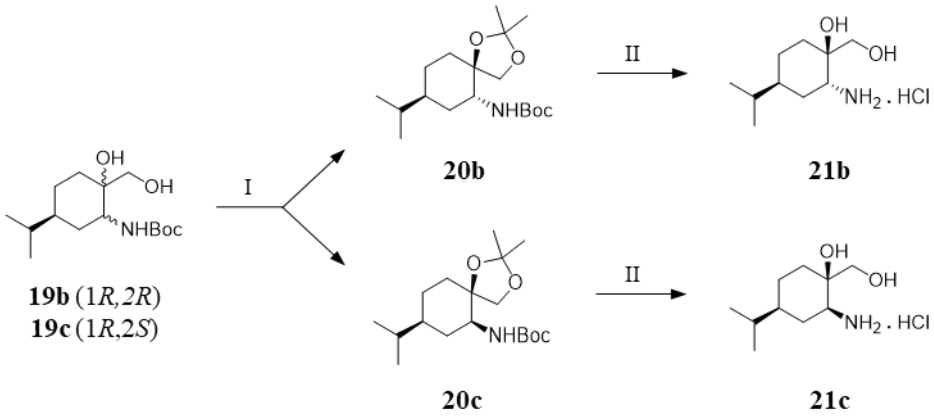
One of the most important steps for the preparation of aminodiols was the dihydroxylation of the allyl double bond in the presence of an osmium(VIII) tetroxide oxidising agent and NMO as the catalyst, which was carried out with high selectivity. The reaction yielded products in the following ratio, **19a**:**19b**:**19c** = 71:16:13. Compound **19a** could be isolated with a chromatographic process, while the other two products remained inseparable.



18a (1*R*) **18b** (1*S*) **19a** (1*S*, 2*R*) **19b** (1*R*,2*R*) **19c** (1*R*,2*S*)

Scheme 5. Dihydroxylation of the Boc -protected allylamine mixtures **18a** and **18b**

To separate compounds **19b** and **19c**, acetal was prepared from a mixture of Boc-protected aminodiols with acetone in the presence of PTSA catalysis (**20b** and **20c**). In this case, we were able to separate the products by chromatography.

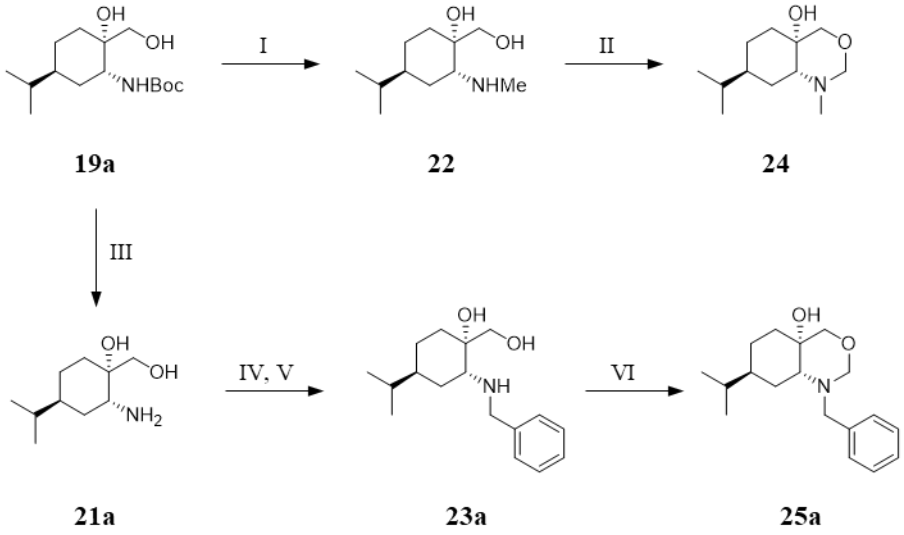


19b (1*R*,2*R*) **19c** (1*R*,2*S*) **20b** **21b** **20c** **21c**

Scheme 6. Isolation of aminodiol diastereomers via formation of acetonides. I: anh. acetone, PTSA, Ar atm, rt, flash chromatography, (**20b**: 56%, **20c**: 18%); II: 10% HCl, Et_2O, rt, 1 day, (**21b**: 86%, **21c**: 80%)

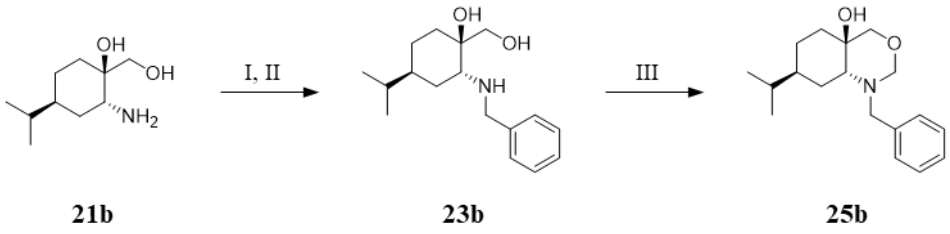
Subsequently, the acetal and the Boc protecting groups were removed in one step by hydrochloric acid hydrolysis to obtain the primary aminodiols **21b** and **21c**. Structure elucidation was carried out using 1D and 2D NMR techniques.

Similarly, derivatisation reactions were performed for the regioisomeric aminodiols. **19a** was reduced by applying $LiAlH_4$ to obtain the *N*-methyl derivative **22**. The acid hydrolysis of **19a** also yielded the primary aminodiol **21a**. The liberated base form of **21a** was reacted with benzaldehyde in the presence of $NaBH_4$, resulting in the **23a** *N*-benzyl derivative in two steps. Cyclisation reactions were also performed with formaldehyde. It is significant to note that, similar to diastereomeric aminodiols, only the six-membered ring system was formed to yield **24** and **25a**.



Scheme 7. Synthesis of aminodiol derivatives. I: $LiAlH_4$, THF, rt, 2 h, 65%; II: 40% CH_2O, Et_2O, rt, 1 h, 66%; III 10% HCl, Et_2O, rt, 24 h, 86%; IV benzaldehyde, anh. EtOH, rt, 2 h; V $NaBH_4$, EtOH, rt, 6 h, 54%; VI: 40% CH_2O sol., Et_2O, rt, 1 h, 89%

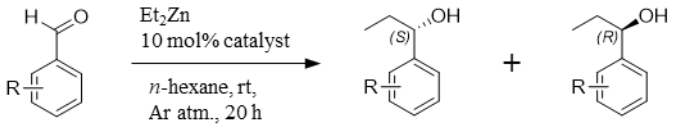
Subsequently, from the primary aminodiol **21b**, the *N*-benzyl derivative **23b** and the ring-closed derivative **25b** were prepared similarly to **21a**. In this case, the six-membered 1,3-oxazolidine ring system was also formed.



Scheme 8 Preparation of the *N*-benzyl and ring-closed derivatives. I: benzaldehyde, anh. EtOH, rt, 2 h; II: $NaBH_4$, anh. EtOH, rt, 6 h, 89%; III: 40% CH_2O, Et_2O, rt, 1 h, 80%

4.1.4. Use of aminodiols as catalysts in the reaction of addition of diethylzinc to aromatic aldehydes

Synthesised 3-amino-1,2-diols and their derivatives were investigated as chiral catalysts in the enantioselective addition of diethylzinc to benzaldehyde, and some selective cases with 3-methoxybenzaldehyde, 4-methoxybenzaldehyde and 4-methylbenzaldehyde, following literature procedure,[18, 101-103] whereas (*S*)- and (*R*)-1-aryl-1-propanol **27a**–**d** were formed. The reaction condition is described in **Scheme 9**.



26a–d **(*S*) 27a–d** **(*R*) 27a–d**

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

Scheme 9. Enantioselective addition of diethylzinc to aromatic aldehydes; yields: 51–92%

The results of the catalytic reactions are shown in **Table 3**. The isomer ratio was evaluated by a chiral GC[101, 104, 105] or chiral HPLC[18] column. These model reactions provided catalytic activities ranging from low to excellent. Notably, the aminodiol diastereomers gave opposite chiral induction. In case **10a,** the best catalytic activity was observed. Together with **7a**, the reaction was extended to further aromatic aldehydes.

Table 3. 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 Rt of the GC analysis and the optical rotations with the data from the literature.

4.2. Ecdysteroids

4.2.1. Selection of ecdysteroids for screening and preparation of new derivatives

Figure 11 shows all the structures of the ecdysteroid derivatives investigated in this study. These compounds represent a wide structural variety, encompassing natural and semisynthetic ecdysteroids. Compounds **28**−**74** are of natural origin, obtained during our previous phytochemical studies, or semisynthetic derivatives synthesised in this work. Among the natural compounds were the most studied ecdysteroid 20-hydroxyecdysone (**28**), ajugasterone C (**29**), shidasterone (**30**), dacryhainansterone (**31**), and calonysterone (**32**). Semisynthetic ecdysteroids included side chain-cleaved derivatives (**33**−**35**), dioxolane derivatives, such as 2,3 monoacetonides (**37**–**40** and **42**) and 2,3:20,22 diacetonides (**36**, **41** and **43**), nitrogen-containing ecdysteroids: C-6 oximes (**44**–**46** and **52**), C-20 oxime (**61**) C-6 oxime ethers (**47**–**51 and 53**–**55**, **69** and **70**), lactams (**56**–**58**) and an amide (**62**), fluorinated ecdysteroids (**63**, **64**), ecdysteroid cinnamic esters (**65**−**68**), and oxime ethers with cinnamic ester moieties (**71**−**74**). Compounds **44**, **45**, **56**, and **65**–**74** were prepared in the course of this study. These semisynthetic modifications are discussed in detail in the subsequent chapters.

4.2.2. Semisynthesis of 20E-6 oximes and a lactam starting from 28.

The C6 ketone function of 20E (**28**) was transformed into an oxime moiety using a previously reported semisynthetic method, in which 20E was reacted with the free base form of hydroxylamine.[64, 106] This process yielded a mixture of *E* and *Z* isomers of oximes, and two products (**44** and **45**) were isolated by flash chromatography followed by RP-HPLC. Subsequently, we aimed to synthesise a lactam by extending the B-ring. The transformation was performed using compound **45** in the presence of TsCl and Na_2CO_3. After purification by HPLC, compound **56** was successfully isolated.

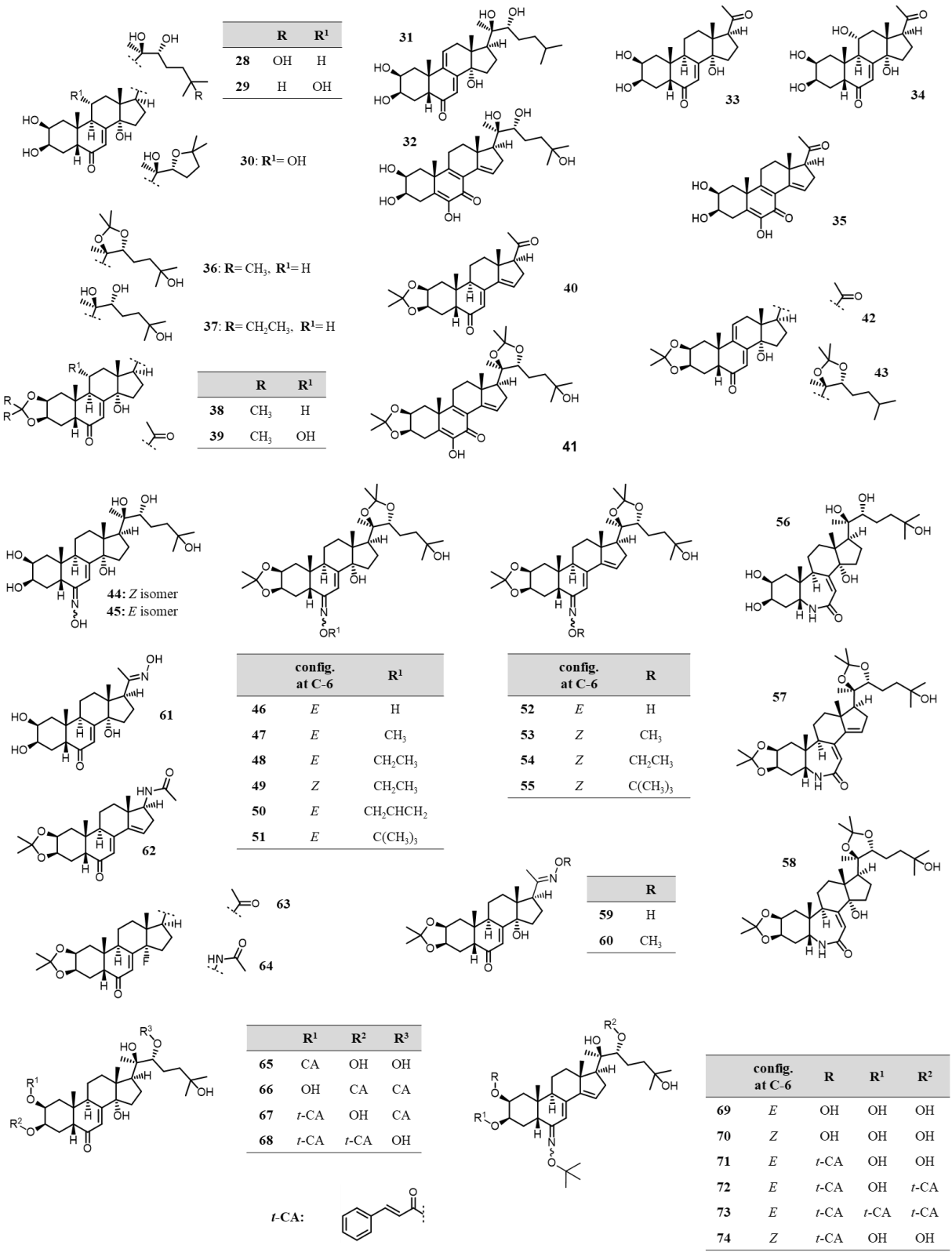
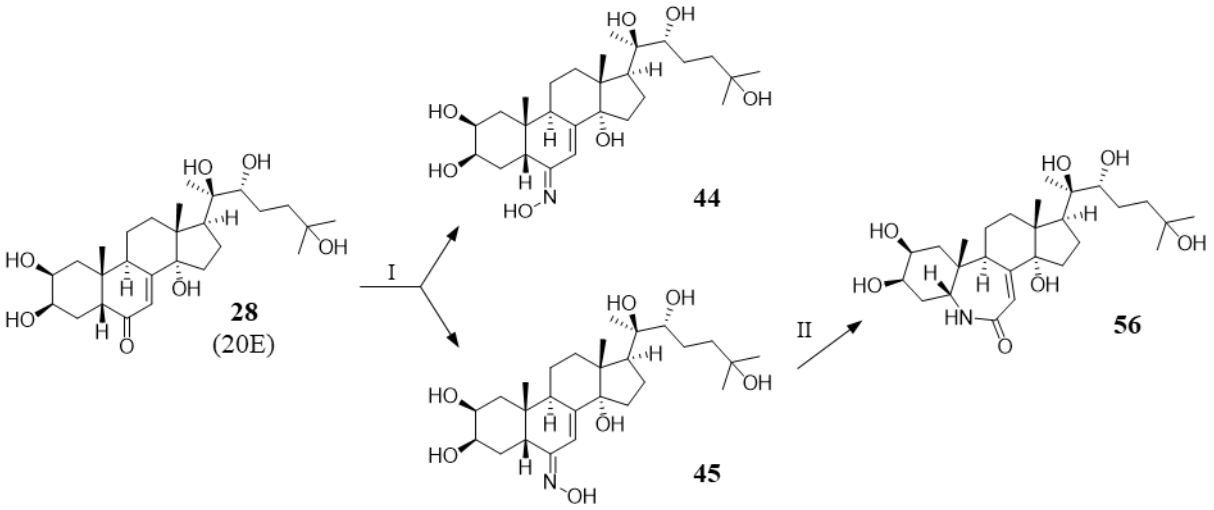


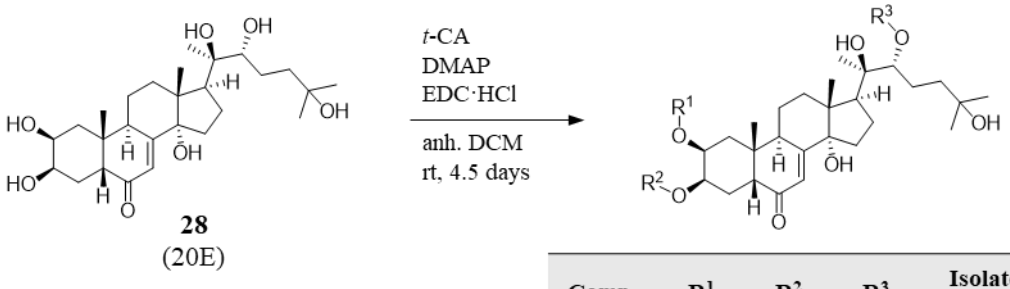
Figure 11. Structural variety of the tested ecdysteroids discussed in this doctoral thesis



Scheme 10. Semisynthesis of oximes **44**, 18%; **45**, 49%; and a lactam **56**, 61.3%;. I: $NH_2OH \cdot HCl$ in EtOH, KOH in EtOH, II: TsCl, Na_2CO_3, acetone

4.2.3. Preparation of the cinnamic ester derivatives of 28.

The synthesis of **28** (20E) cinnamic acid esters was carried out based on modified methodologies for the esterification of various di- and triterpenes with *trans*-cinnamic acid (*t*-CA) derivatives.[107-109] The reaction used EDC·HCl to form a reactive intermediate and DMAP as a catalytic agent, in anhydrous DCM as the solvent. The presence of multiple free hydroxyl groups resulted in the formation of various products. Subsequent purification applying flash chromatography and RP-HPLC enabled the successful isolation of compounds **65−68**.

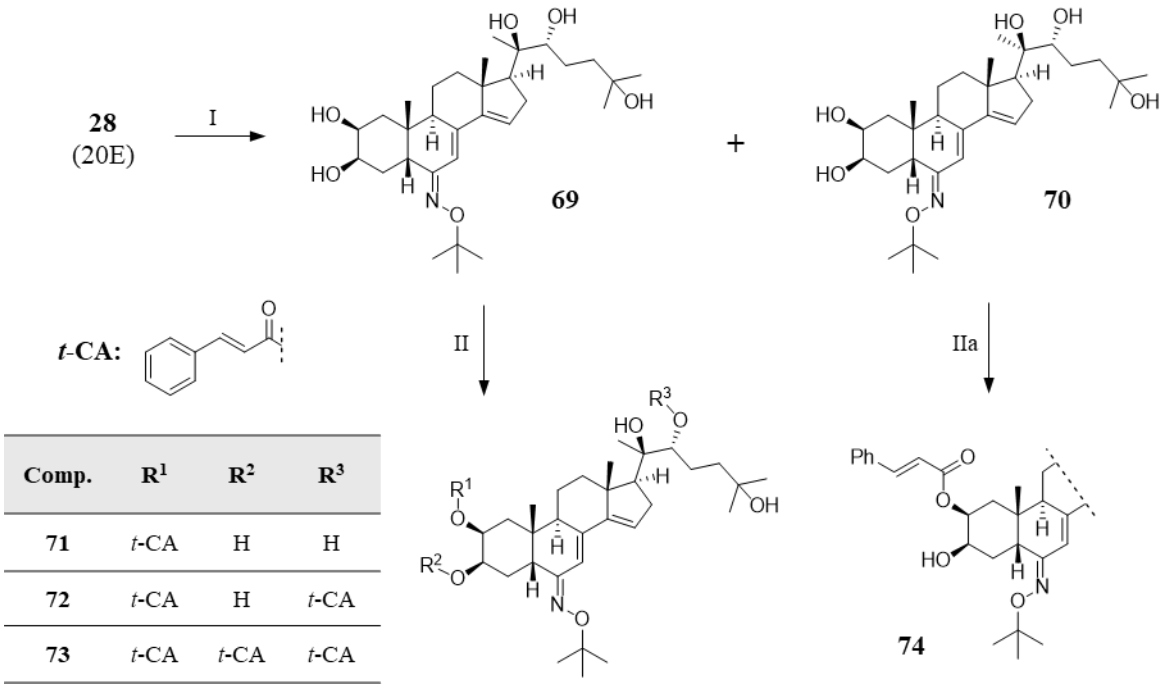


Comp.	R^1	R^2	R^3	Isolated yields (%)
65	*t*-CA	H	H	1.7
66	H	*t*-CA	*t*-CA	3.4
67	*t*-CA	*t*-CA	H	10.4
68	*t*-CA	*t*-CA	H	2.9

Scheme 11. Preparation of cinnamate derivatives of **28** (20E)

4.2.4. Preparation of Stachysterone B 6-*O-tert*-butyl oxime ethers and their cinnamic ester derivatives.

From **28**, treated with *o-tert*-butylhydroxylamine hydrochloride in anhydrous pyridine, the 6-*tert*-butyl oxime ether derivatives were formed. The reaction resulted in the formation of the corresponding oxime ether isomers. The oxime ether diastereomers were subsequently separated using RP-HPLC, yielding compounds **69** (*E* isomer) and **70** (*Z* isomer). Elimination of the 14-hydroxyl group was observed. The esterification of compounds **69** and **70** with cinnamic acid was carried out with some modifications to the procedure outlined in Section 4.2.1.3. This process yielded the mono- (**71**), di- (**72**) and triester (**73**) of compound **69**, while the monoester **74** was isolated from compound **70**.



Comp.	R^1	R^2	R^3
71	*t*-CA	H	H
72	*t*-CA	H	*t*-CA
73	*t*-CA	*t*-CA	*t*-CA

Scheme 12. Synthesis of 6 *tert*-butyl oxime ethers of stachysterone B and their cinnamate derivatives. I: *O-tert*-butylhydroxylamine hydrochloride, in anh. pyridine, at 70 °C 2 days (**69**: 45.1%, **70**: 13.6%); II: *t*-CA, DMAP, EDC·HCl in anh. DCM, at rt, for 3 days (**71**: 1.7%, **72**: 10.4%, **73**: 2.9%); IIa: *t*-CA, DMAP, EDC·HCl in anh. DCM, at rt, for 4 days (**74**: 8.9%)

4.2.5. Evaluation of the biological activity of ecdysteroids

4.2.5.1 Antifungal activities

In collaboration with the Institute of Biology, the evaluation of the impact of **28** (20E) and its oxime and lactam derivatives, **44**, **45** and **56**, on the growth of the opportunistic human pathogen yeast *C. neoformans* was carried out using a microdilution assay. Two-fold serial dilutions of the compounds, with concentrations ranging from 2.00 to 0.125 mg/ml, were prepared to determine the MICs. All compounds tested exhibited inhibitory activity against

both strains of *C. neoformans*. However, the MIC values were only determined for **44** and **45** since **28** (20E) and **56** showed only moderate effects on strain growth. The MIC of **45** was identified as 2.00 mg/ml, while **44** demonstrated greater potency with a MIC of 1.00 mg/ml (**Figure 11**). To evaluate whether the inhibitory effects of **44** and **45** are fungistatic or fungicidal, *C. neoformans* IFM 5844 cells treated with **44** and **45** were inoculated onto solid YPD medium. The results indicated that both compounds exhibit fungicidal activity at their MICs.

The effect of **44** on the viability of *C. neoformans* IFM 5844 cells was evaluated using a calcein-AM assay coupled with flow cytometry. Cells exposed to **44** at its MIC value for 3 hours exhibited reduced fluorescence compared to the untreated stained control (**Figure 12**), suggesting that **44** effectively decreases cell viability even after a short incubation period.

A

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

B

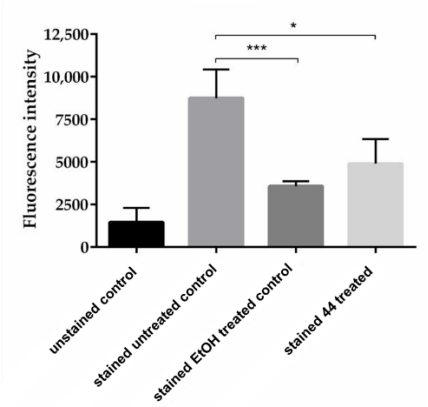


Figure 12. A: MIC of the investigated ecdysteroids. **B**: The activity of **44** on the viability of *C. neoformans* IFM 5844 cells. The values represent the mean ± standard deviation calculated from four independent experiments (*, $p \leq 0.05$, ***, $p \leq 0.001$, unpaired *t* test).

Combination treatment of the *C. neoformans* strain IFM 5844 with **28** and **56** was carried out but has showed only additive interaction. Similar outcomes were observed combining **44** with **28**, **45** and **56**; or **45** with **28** and **56**. The combination treatment of **44** and **45** with indomethacin, quinidine or verapamil (efflux pump inhibitors) were also studied, however only additive interactions were observed.

4.2.5.2. Antitrypanosomal investigation

4.2.5.2.1. Screening of ecdysteroids on *T. cruzi* epimastigotes

In collaboration with the University of Bern, the preliminary screening of ecdysteroids **28**−**74** was conducted against *T. cruzi* epimastigotes. at a concentration of 5 µM, with benznidazole as the positive control. From this ecdysteroid library, the *tert*-butyl oxime ethers of 20E diacetonide (**51**) and stachysterone B (**55**), along with two cinnamic esters of 20E (**66** and **67**), showed moderate selective toxicity. These compounds inhibited epimastigote proliferation by ≥30% while exhibiting no cytotoxicity (**51**, **55**, **66**) or only minimal cytotoxicity (**67**) against CHO cells, which served as a model for mammalian host cells.

Based on our initial findings involving compounds containing either a 6-*tert*-butyl oxime ether or at least one cinnamic ester group, we combined these potential pharmacophores into the structure of 20E (**28**). This led to the synthesis of compounds **71**–**74** and acetonide-free 6-*tert*-butyl oxime ethers **69** and **70**. The newly synthesised compounds were evaluated against the epimastigote stage of *T. cruzi* at a concentration of 5 µM.

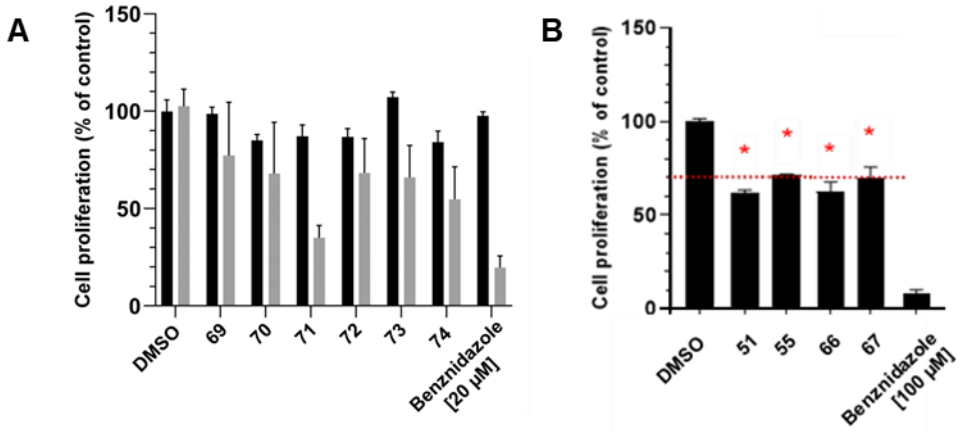


Figure 13. A: Antitrypanosomal and cytotoxic activity of compounds **69**−**74** (5 µM) against *T. cruzi* epimastigotes and Chinese hamster ovarian (CHO) cells measured by XTT and MTT, respectively, upon incubation of compounds for 72 h in the logarithmic proliferative phase. Benznidazole (20 µM) was used as a positive control. Data show mean values ± SD of three independent experiments, each performed in triplicate. **B**: 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. Data show mean values ± standard deviation of 3 independent experiments each performed in triplicates.

All semisynthesised derivatives, except for **69** and **70** (which lacked a cinnamic ester group), inhibited epimastigote proliferation by at least 30%. Among all the compounds tested in this study (**28**–**74**), compounds **71** and **74** showed the highest activity.

4.2.5.2.2. Trypanocidal evaluation of 71 by IC_{50} measurements and fluorescence techniques.

The trypanocidal effects of compound **71** are summarised in **Figure 2.** Compound **71** exhibited moderate cytotoxicity against host cells, with a selectivity index higher than 5. Compound **71** efficiently inhibited the proliferation of *T. cruzi* epimastigotes, with an IC_{50} value of 1.7 ± 0.2 µM (**Figure 11**).

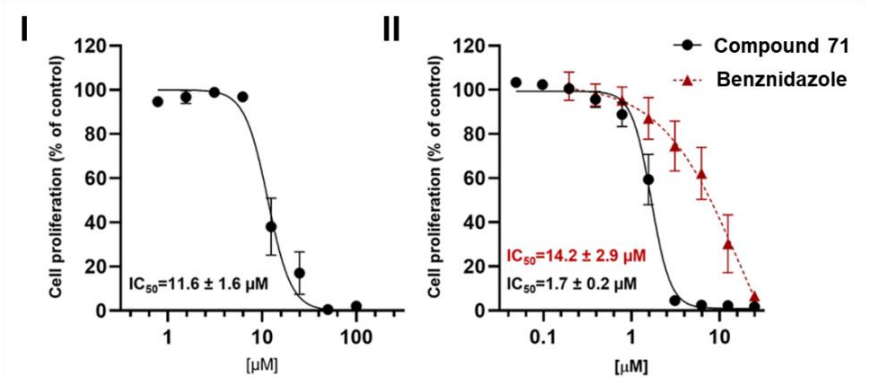


Figure 14. Selective trypanocidal effects of **71**. **I**: Effects on cell proliferation (MTT assay) were assessed in RAW264.7 cells after 72 h of incubation, **II**: XTT assays on epimastigote proliferation with benznidazole as positive control. Data show mean values ± SD of at least 3 independent experiments, each of which was performed in triplicates.

Furthermore, **71** achieved more than 95% inhibition of trypomastigote release, showing an IC_{50} value of 2.7 ± 0.1 µM. These results indicate that compound **71** has efficacy and potency similar to benznidazole, which showed an IC_{50} value of 3.8 ± 0.7 µM in the same assay.

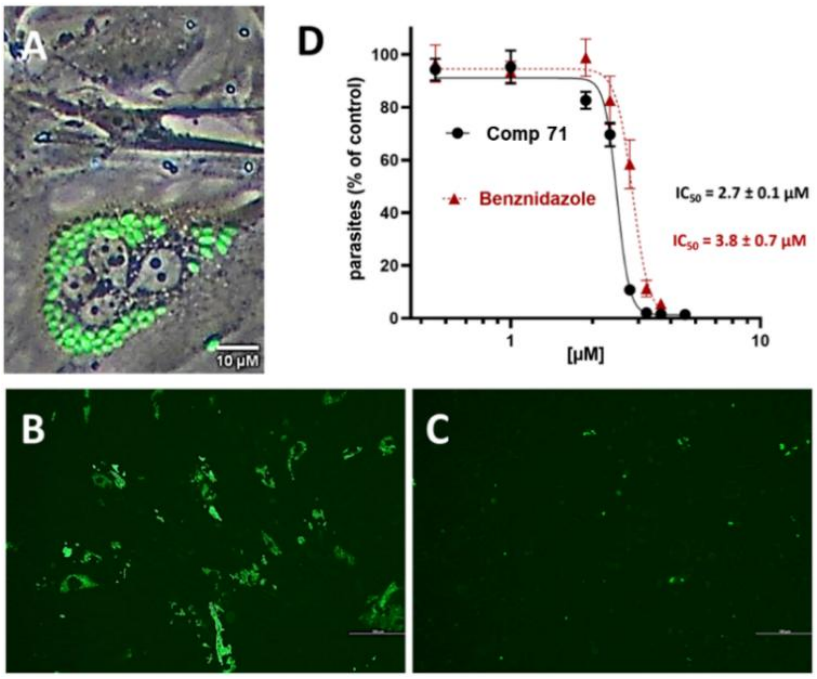


Figure 15. A: Representative brightfield/fluorescence microscopic image of CCL39 fibroblasts infected with T. cruzi showing amastigotes (green). **B**: Representative fluorescence microscopic images of infected cells showing the GFPY fluorescence signal of the T. cruzi amastigotes (vehicle control). **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.

5. Discussion

5.1. Monoterpenoids.

5.1.1. Preparation of chiral 3-amino-1,2-diols carried out by reductive alkylation of perillaldehyde and by Overman rearrangement

Starting from perillaldehyde (**1**), regioisomeric aminodiol libraries were synthesised via two distinct synthetic pathways. Reduction of the aliphatic double bond of **1** was necessary to prevent subsequent side-product formation. In the first approach, an allylamine function group was introduced through Schiff base formation (see **Schemes 1**–**3**), a method already established in our previous work.[18] In the second approach, the target intermediates were obtained via the Overmann rearrangement[100] (see **Schemes 4**–**8**), which is also documented in the literature.[15, 19, 22] The aminodiol functionality was introduced using the so-called Upjohn dihydroxylation.[110] Our research group also explored the introduction of the aminodiol functional group through an epoxide intermediate derived from allyl alcohol.[16, 17, 102, 111, 112]. We prepared the condensed bicyclic compounds using formaldehyde (**10a**–**c and 24**, **25a**, **25b**). It is important to note that only six-membered 1,3-oxazine rings were obtained in the cyclisation reactions. In previous studies by our research with monoterpenes, the formation of five-[15, 16, 19, 113] and six-membered[18, 113] rings was observed when using formaldehyde. Synthesising regioisomer aminodiols with the same core structure, allowing us to study the positions of the substituents and establish more comprehensive structure-activity relationships.

5.1.2. DFT calculations on compounds 11a and 7a

We propose a novel mechanism for the formation of 1,3-oxazines, involving a one-step ring closure of an *N*-hydroxymethylated intermediate. *In-silico* DFT modelling calculations demonstrated that both oxazine ring fission processes are thermodynamically possible, but only **11a–c** are formed. It probably occurs due to the decomposition of **14a–c** during silica-based chromatographic purification. As TLC monitoring revealed the transient formation of **14a**, we hypothesise that its isolation was hindered by the hydrolytic decomposition occurring on the acidic silica surface during the chromatographic purification of the crude product. This perspective is reinforced by the structural features and energetic insights obtained from additional comparative DFT modelling studies on the iminium-generating ring-opening processes of the diastereomeric O-protonated oxazines. Both ring-opening reactions (**10a**/OH^+→ **10a**/Im^+ and **14a**/OH^+ → **14a**/Im^+) are energetically favourable, but structural changes, such as inversion of the cyclohexane ring in the second process, create significant

spatial separation between key functional groups, limiting its recyclisation ability. The iminium intermediate **11a**/Im^+ is more likely to undergo hydrolytic decomposition to regenerate the precursor than to proceed with ring-inversion-enabled recyclisation to form **14a**/OH^+. A new mechanism is proposed to form fused 1,3-oxazines involving the one-step closure of the ring of an *N*-hydroxymethylated intermediate (**11a** / hm / $4H_2O$ → **14a** / $4H_2O$). This pathway relies on a hydrogen-bonded linear cluster of four water molecules, facilitating the reaction by activating both the nucleophilic and the leaving groups through concerted proton migration. The four-membered water cluster is identified as critical for creating the ideal molecular architecture necessary for the one-step intramolecular SN_2 reaction. The proposed pathway is supported by its ability to rationalise the formation of **10a** and by comparisons with other well-documented examples.[114, 115]

5.1.3. Application of aminodiols in the enantioselective reaction on aromatic aldehydes

The prepared aminodiols and their derivatives were used as chiral catalysts in the enantioselective addition of diethylzinc to benzaldehyde, yielding mild to remarkable catalytic activities (see **Table 3**). Among the regioisomers and diastereoisomers, aminodiols containing vicinal hydroxyl groups on the cyclohexane ring with a (1*S*,2*S*,4*S*) configuration emerged as the most efficient catalysts, predominantly providing *S*-selectivity. Interestingly, the bicyclic derivatives of these aminodiols, specifically the 1,3-oxazines **10a–c**, exhibited superior but opposite *R* selectivity in the same reaction. With two effective catalysts, **7a** and **10a**, the catalytic activity study was extended by including substituted benzaldehyde derivatives (4-methyl, 4-methoxy, 3-methoxy) (see **Table 3**). In particular, regarding 4-methylbenzaldehyde, aminodiol **7a** produced secondary alcohols with selectivity *R*. Remarkably, within the same enantiomeric library, catalysts capable of providing *S* and *R* selectivities were identified. Bicyclic 1,3-oxazines demonstrated exceptional catalytic activity in the addition of diethylzinc to benzaldehydes and their derivatives, likely due to their conformationally rigid structures. On the contrary, regioisomeric aminodiols and 1,3-oxazines exhibited reduced catalytic efficacy, but maintained similar selectivity patterns in the model reaction.

The present research aligns well with our previous investigations, in which we explored the applicability of monoterpene-based aminodiols as chiral catalysts in the asymmetric addition of diethylzinc to aromatic aldehydes. In our earlier studies, aminodiols derived from pinane-based (–)-myrtenol were synthesised and tested for catalytic activity, resulting in weak to moderate enantioselectivities providing *R*-configurations.[15] In subsequent work, we prepared aminodiols from perillaldehyde-based carane derivatives, achieving moderate to excellent

selectivities.[18] Using (+)-pulegone as a starting material, we attained enantioselectivities as high as 90% *ee*.[22] Similarly, aminodiols prepared from isopulegol also showed mild to moderate enantioselectivities.[113] Catalysts synthesised from (+)-sabinol exhibited moderate selectivities, whereas those derived from *β*-pinene provided low to moderate *ee* values.[16]

5.2 Ecdysteroids

5.2.1. The chemistry of the discussed ecdysteroids (28–74)

Ecdysteroids exhibit considerable structural diversity. Variations can occur in the number and position of double bonds within the steroid nucleus (e.g., dacryhainansterone), and oxidised derivatives are also known (e.g., calonysterone). Modifications of the side chain are common: it may be shortened (e.g., poststerone), absent (e.g., rubrosterone), or may undergo ring closure (e.g., ajugalactone). Ecdysteroids can also form conjugates with other natural substances, typically in the form of esters, such as acetate, cinnamic acid, or fatty acid esters. From a chemical transformation perspective, hydroxyl groups and the presence of an *α,β*-unsaturated ketone function are particularly important substituents. The number and position of hydroxyl groups vary widely among ecdysteroids, ranging from compounds with as few as two hydroxyl groups (e.g., 2,22,25-trideoxyecdysone) to those with as many as eight (e.g., integristerone B).[43] Since ecdysteroids' trypanocidal activity has not yet been studied, and data on their antifungal activity remain limited, we aimed to compile a structurally diverse selection that reflects the chemical variability of this compound class. The ecdysteroids included in this selection are presented in **Figure 11** and described in detail in section **4.2.1.** The series comprises both natural (compounds **28**–**32**) and semisynthetic (compounds **33**–**74**) derivatives. All semisynthetic compounds discussed in this study were prepared from 20E (**28**), which was available in sufficient quantity and is currently one of the most extensively studied ecdysteroids. Considering the desired pharmacological properties, it is justified to extend beyond the chemical space of natural ecdysteroids by incorporating additional heteroatoms, like nitrogen, or coupling with another natural compound, like cinnamic acid. First, we prepared *N*-containing derivatives and formed allyl oximes **44** (*Z*) and **45** (*E*) at C6 position from **28**. From the *E* oxime, a unique seven-membered ring was formed as a lactam derivative **56** (see **Scheme 10**). The conversion of an oxime to an amide is known as the Beckmann rearrangement.[64, 116] This reaction requires that the oxime's hydroxyl group occupies the *anti*-position, because the migration from the *syn*-position is sterically unfavourable. In the subsequent step, we prepared four esters with **28** and *trans*-cinnamic acid, to obtain derivatives that could also be referred to as hybrid molecules (**65**–**69**) (see **Scheme 11**). Based on biological studies, it was worthwhile

to incorporate the nitrogen-containing bulky *tert*-butyl oxime ether group into **28** and prepare esters with cinnamic acid. The semisynthesis resulted in *E* and *Z* oxime ether of a 20E derivative, stachysterone B (**69** and **70**), due to the elimination of the OH group at position C14. This observation is consistent with findings reported in earlier publications.[64, 106, 116] To preserve the 14-OH group at the end of the reaction, neutralisation of the mixture is required; otherwise, the anhydro derivative is formed. This neutralisation can be achieved using an alcoholic KOH solution.[64] An alternative approach is demonstrated in the formation described in Section **4.2.2**, where the use of KOH as a base facilitated the liberation of the free form of hydroxylamine from its hydrochloride salt prior to adding the ecdysteroid to the reaction mixture, thus preserving the 14-OH group. In this particular reaction, an aqueous KOH solution was employed, which likely contributed to the elimination process. The mono-, di-, and triester from **69**, and the monoester of **70** were obtained (**71**–**74**) (see **Scheme 12**), building on previous methodologies involving the synthesis of cinnamic acid derivatives of di- (e.g., oridinon) and triterpenoid compounds (e.g., oleanolic acid and glycyrrhetinic acid), a similar approach was applied using EDC·HCl and DMAP in DCM as the reaction medium. In these protocols, 1.0–1.1 equivalents of the aromatic acid were typically used, which may contribute to a higher degree of regioselectivity. However, in most cases, the target molecules possessed only a single reactive hydroxyl group.[107-109] The reactions afforded products in low yields, which may be attributed to steric hindrance, the presence of multiple hydroxyl groups and their varying reactivity, or suboptimal reaction conditions. It is noteworthy that esterification was consistently observed at the C-2 position in all esters (mono-, di-, and triesters), indicating that this hydroxyl group is likely the most reactive or sterically the most accessible. Diester **72** bears esters at C-2 and C-3, while triester **73** has them at C-2, C-3, and C-22. Accordingly, this approach allowed the simultaneous isolation of several compounds in a single reaction, providing a broader range of chemical scaffolds for analysing the structure-activity relationship.

5.2.2. Biological evaluation of ecdysteroids

5.2.2.1 Anticryptococcal activity of ecdysteroids

With the global increase in antibiotic resistance and the increasing prevalence of difficult-to-treat microbial infections, the need for new therapeutic compounds or the repurposing of existing drugs against resistant microbes has increased significantly.[117] There is limited literature data available on the antimicrobial activity of ecdysteroids. According to current knowledge, the ecdysteroids that were studied in this regard mostly exhibit mild to moderate activity against pathogenic bacteria or fungi. In our work, we evaluated the antifungal activity

of a natural ecdysteroid **28** and three of its *N*-containing semisynthetic derivatives (**44**, **45**, **56**) against *C. neoformans*, an opportunistic pathogenic yeast. Compound **28** showed negligible antimicrobial activity against the tested strains, as no inhibition was observed in the tested concentration range. The conversion of **28** to the two oxime derivatives, **44** and **45**, resulted in a modest enhancement of the antifungal activity, with **44** proving to be slightly more potent. The lactam derivative **56** obtained from **45** did not exhibit any significant activity. These findings indicate that the *Z* isomer (**44**) possesses a more advantageous chemical conformation, while the expansion of the B ring (**56**) does not improve anticryptococcal activity. Combination studies of the active compounds **44** and **45** with efflux pump inhibitors resulted only in additive interactions. This suggests that **44** and **45** do not serve as substrates for the tested transporters. Our results showed that these ecdysteroid derivatives, similarly to their natural counterparts, do not exhibit significant antimicrobial activity, and that ecdysteroids are likely not suitable starting materials for the development of antimicrobial agents.

5.2.2.2 Antitrypanosomal activity of ecdysteroids

The preliminary results of the screening of compounds **28**–**69** against *T. cruzi* epimastigotes at a concentration of 5 μM concentration proposed some ecdysteroids with moderate selective toxicity. Although the stages of amastigote and trypomastigote are more relevant to the life cycle, antitrypanosomal compounds often exhibit selective toxicity toward epimastigotes as an initial indicator of activity.[83] Our findings highlighted two structural elements as key contributors to antiparasitic activity: a *tert*-butyl oxime ether moiety at C6 and one or two cinnamic ester groups at positions C-2, C-3, or C-22.

In the literature, ester and amide derivatives of cinnamic acid and *p*-coumaric acid have been shown to be active against toxoplasma and leishmania.[118-120] Some free acid forms of cinnamic acid derivatives, although they show some activity against trypanosomes, were rather weak.[121] Therefore, it is unlikely that cinnamic acid esters of ecdysteroids could be used as prodrugs. Moreover, previous experimental evidence showed that among twelve *p*-coumaric aliphatic and aromatic esters, four aliphatic esters exhibited potent trypanocidal effects. Flow cytometric analyses of the best hits revealed that this effect was mediated by oxygen radicals and mitochondrial depolarisation of the parasite.[122]

Based on our preliminary results, we found incorporating the putative pharmacophore combination of *tert*-butyl oxime ether and cinnamic acid into the ecdysteroid scaffold worthwhile. Therefore, we synthesised oxime ethers **69**–**70** and then **71**–**74** of these, which were investigated against *T. cruzi*. All derivatives bearing a cinnamic acid moiety exhibited a

30% inhibition against epimastigote proliferation. Although the amastigote and trypomastigote stages are more relevant to the life cycle of *T. cruzi*, antitrypanosomal compounds often exhibit selective toxicity toward epimastigotes as an initial indicator of activity. Therefore, following the results of the entire tested ecdysteroid library, compounds **71** and **74** were deemed the most effective, both containing a cinnamic acid ester at C-2 and an *E* or *Z tert*-butyl oxime ether at C-6. Additionally, **71** was identified as the most active compound against the parasite. MTT and XTT assays were also performed with this derivative to determine IC_{50} values in combination with benznidazole used as a positive control. Unlike the nitroheterocyclic drug benznidazole, a prodrug that requires activation by trypanosomal type I nitroreductase (NTRI) and is therefore more effective against the amastigote stage,[123] compound **71** efficiently inhibited the proliferation of *T. cruzi* epimastigotes. Compound **71** exhibited similar activity to benznidazole on the release of trypomastigotes in this study. Based on our findings, some SAR insights could be provided for the development of ecdysteroid-based antitrypanosomal agents, such as the following.

Substituents on the A- and B-Rings:

- The presence of an apolar moiety on the A ring (e.g. cinnamic ester at C-2) and a bulky nitrogen-containing substituent on the B ring (e.g., 6-*tert*-butyl oxime ether) is crucial for activity.
- The *E* configuration of the oxime ether (compound **71**) outperforms the *Z* configuration (compound **74**), suggesting the importance of spatial arrangement for binding or activity.

Unaltered side chain:

- Derivatives with an intact ecdysteroid side chain (compounds **71** and **74**) were more active than those with additional cinnamate substitutions at C-22 (compounds **72** and **73**). This indicates that maintaining the natural side chain structure enhances potency.

14α-OH compared to $\Delta^{14,15}$ olefin:

- Retention of the 14α-OH group (compound **50**) or its conversion to a $\Delta^{14,15}$ olefin (compound **55**) did not significantly impact activity, suggesting that this modification is less critical for trypanocidal effects.

It is important to emphasise the novelty of our findings, as ecdysteroids have not previously been evaluated against *T. cruzi*, which could make them potential first-class therapeutic agents.

6. Summary

The goal of this Ph.D. thesis was to extend the chemical space of monoterpenoids and ecdysteroids. In the case of monoterpenoids, we planned to examine their catalytic activity in the enantioselective addition of diethylzinc to aromatic aldehydes. With respect to ecdysteroids, our objective was to study their antimicrobial and antiparasitic activity. We can summarise our results as follows.

Monoterpenoids

In total of forty-three derivatives were obtained, among which thirty-eight were new.

Preparation of an aminodiol library by reductive alkylation of perillaldehyde

Twenty-six derivatives were obtained, among which twenty-five were new based on the following. Starting from perillaldehyde, two subsets of diastereomeric 3-amino-1,2-diols were prepared via a reductive alkylation step after dihydroxylation of the protected allylamines (Boc). After the protective group was removed, the secondary and primary aminodiols, *N*-methyl-*N*-benzyl, and their ring-closed derivatives were formed.

Computational analysis performed on compounds 10a and 14

DFT modelling revealed structural differences between diastereomers **7a** and **11a** that influence their ring-closing reactions. In **11a**, the inversion of the cyclohexane ring and isopropyl group reorientation favour hydrolysis over recyclisation. On the contrary, **7a** facilitates recyclisation and *O*-deprotonation, allowing the formation of fused oxazine. The reaction bypasses iminium intermediates, proceeding via an *N*-hydroxymethylated intermediate and a critical chain of four hydrogen-bonded water molecules. This mechanism provides a plausible explanation for the formation of fused oxazine, supported by theoretical models.

Preparation of the aminodiol library via Overman rearrangement to obtain the regioisomer derivatives.

Seventeen derivatives were prepared this way, among which thirteen were new compounds. Using the Overman rearrangement, three diastereomeric 3-amino-1,2-diol subsets were prepared from perillaldehyde. The regioisomeric allylamine could not be separated chromatographically even with the use of two kinds of protection groups, so dihydroxylation of the mixture was performed to obtain the protected aminodiols. From the isolated products, secondary and primary derivatives were prepared.

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

The prepared aminodiols and their derivatives were evaluated as chiral catalysts in the enantioselective addition of diethylzinc to benzaldehyde, yielding mild to exceptional activities. Aminodiols with a configuration (1*S*,2*S*,4*S*) provided high *S* selectivity, while their bicyclic 1,3-oxazine derivatives (**10a–c**) showed superior *R* selectivity. The two best catalysts **7a** and **10a** were further tested with substituted benzaldehydes. Remarkably, the library included catalysts capable of delivering both *S*- and *R*-selectivities. The superior activity of 1,3-oxazines was attributed to their rigid conformations, while the regioisomeric derivatives exhibited lower efficiency but consistent selectivity.

Ecdysteroids

Selection of ecdysteroids for bioactivity screening and preparation of new semisynthetic derivatives

A total of forty-seven ecdysteroids are the subject of this dissertation. Among these, five are natural, and the rest are semisynthetically modified. During this Ph.D. work, thirteen ecdysteroids were prepared, among which nine were reported as new compounds. These were the following: two oxime derivatives (**44**, **45**); a lactam (**56**); four 20E-cinnamic acid esters (**65–68**); two *tert*-butyl oxime ethers of stachysterone B (**69**, **70**) and four cinnamic acid esters of these (**71–74**).

Antifungal activity of ecdysteroids

We evaluated the antimicrobial activity of natural ecdysteroid **28** and three of its semisynthetic derivatives (**44**, **45**, **56**). Among these, compounds **44** and **45** exerted mild fungicidal activity on *C. neoformans,* with **44** demonstrating superior activity. Both **44** and **45** exhibited additive effects when combined with verapamil.

Antitrypanosomal investigation of ecdysteroids

The biological activity of the forty-seven ecdysteroids mentioned above was investigated against *T. cruzi*. A preliminary screening, carried out with forty-one compounds, suggested that *tert*-butyl oxime ether and cinnamic acid moieties might act as potential pharmacophores. Based on this finding, we have designed and synthesised new derivatives bearing both of these moieties. Our results confirmed this approach as a valid strategy, and three of the new compounds possessed antitrypanocidal activity superior to that of the initial hits.

On the basis of our findings reported herein, it would be worthwhile preparing further aromatic ester derivatives of ecdysteroids to explore structure–activity relationships. In the future, we are planning to pursue this. It may be a reasonable strategy to couple ecdysteroids with cinnamic acid substituted with electron-donating and electron-withdrawing groups. Certainly, it would also be an important step forward in this regard to extend future studies towards ecdysteroids other than 20E, for example, ajugasterone C or dacryhainansterone, i.e., major natural ecdysteroids available from plant sources in reasonable amounts. In the long term, this work could hopefully pave the way towards the development of a much-needed, safe and efficient, first-in-class antitrypanosomal drug.

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