University of Szeged

Faculty of Pharmacy

Institute of Pharmaceutical Chemistry

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

Ph.D. thesis

Dániel Ozsvár

Supervisor

Prof. Dr. Zsolt Szakonyi

2024

TABLE OF CONTENTS

PUBLICATION LIST

Papers related to the thesis

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

IF: 5.923

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

IF: 6.208

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

IF: 4.600 (2022)

Publications not related to the thesis

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

IF: 3.119

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

IF: 4.029

Scientific lectures

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

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

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

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

List of abbreviation

tert-BuOOH: *tert*-Butyl hydroperoxide

PDC: Pyridinium Dichromate

TsCl: Tosyl chloride

TsOH: *p*-Toluenesulfonic acid

THF: Tetrahydrofuran

DMF: Dimethylformamide

DMAP: 4-Dimethylaminopyridine

TEA: Triethylamine

IC_{50}: Half maximal inhibitory concentration

*m*CPBA: *meta*-Chloroperoxybenzoic acid

DBU: 1,8-Diazabicyclo(5.4.0)undec-7-ene

DIPEA: *N*,*N*-Diisopropylethylamine

MIC: Minimum inhibitory concentration

EDCI: 1-Ethyl-3-(3-methylaminopropyl)carbodiimide

PCC: Pyridinium chlorochromate

DDQ: 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone

DMSO: Dimethyl sulfoxide

TEMPO: (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl

TBAB: Tetrabutylammonium bromide

NBS: *N*-Bromosuccinimide

DCM: Dichloromethane

rt: room temperature

DMDO: Dimethyldioxirane

Et_2Zn: Diethylzinc

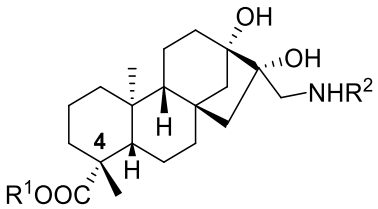
1. Introduction

A severe worldwide health problem is posed by the increasing number of cancer cases, with an expectation to reach 24 million by 2035.[1] Despite modern therapies, several limitations are encountered in current cancer treatment, including side effects and the high costs of anticancer agents.[2,3,4] Therefore, the development of new compounds, such as cheaper anticancer agents with higher bioactivities and weaker side effects, is deemed imperative. Natural products and their modifications have been considered as vital as anticancer agents, with metabolites of diterpenoids increasingly recognised as a significant part of anticancer drug research.[5,6]

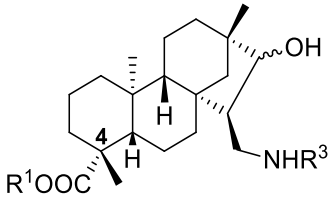
In recent times, particular attention has been paid to the glycosides of the plant *Stevia rebaudiana*, not only because of their sweeter taste compared to sucrose and their application as a zero-calorie artificial sweetener[7], but also because of their wide range of biological activities, including antibacterial, antiviral and anticancer properties.[8,9] Additionally, isosteviol, a derivative of the stevioside aglycone steviol, has also been found to exhibit several biological activities.

In recent years, the significance of aminodiols and their *N*-heterocyclic analogues as crucial building blocks for complex bioactive molecules with notable biological activities has been underscored.[7,8] Various aminodiol-based nucleoside analogues synthesised recently have been found to possess cardiovascular, cytostatic and antiviral effects.[10,11,12] The Abbott aminodiol, recognised as a valuable building block for synthesizing the potent renin inhibitors Zankiren® and Enalkiren®, has been integrated into hypertension therapy.[13,14]

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



I



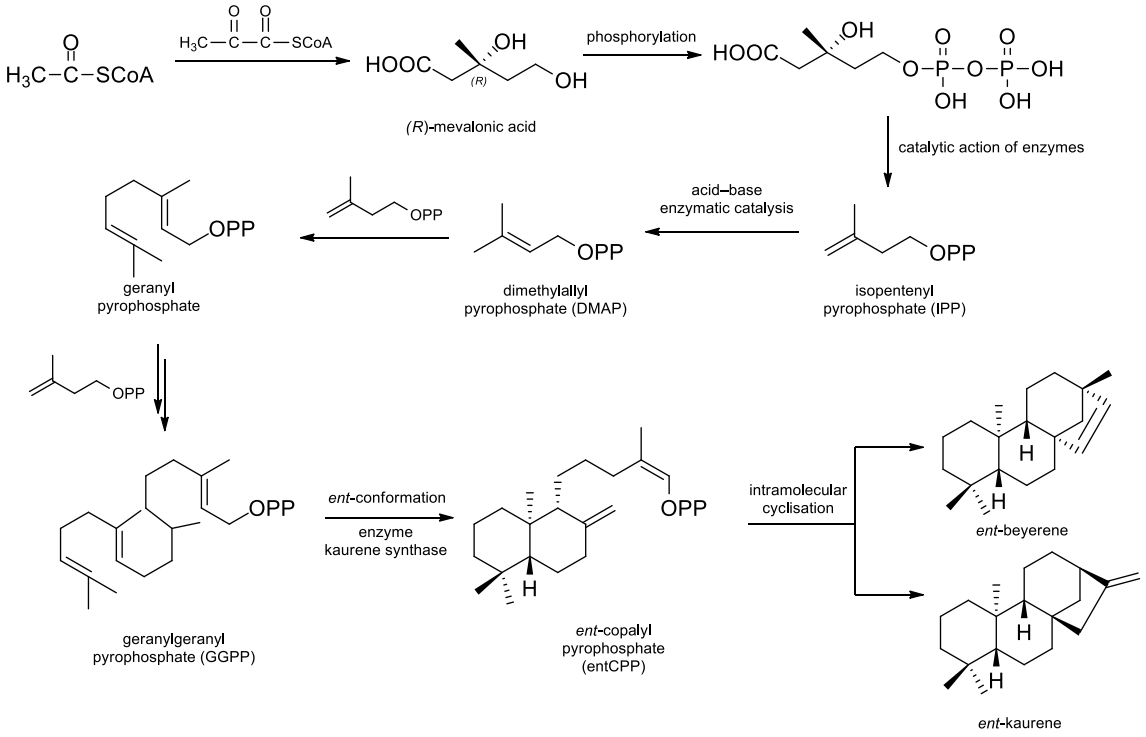
II

2. Literature survey

2.1 General information of diterpenoids

Diterpenoids are a diverse group of natural compounds that are characterised by their hydrocarbon skeleton and various degrees of cyclisation.[15] They can be found in a wide range of organisms including plants, fungi, insects, sea organisms and more.[16,17] According to the degree of cyclisation of the hydrocarbon skeleton, diterpenoids can be divided into acyclic (Phytanes and others), monocyclic (Cembrene A), bicyclic (Sclarenes, Labdanes, Clerodanes, etc.), tricyclic (Pimaranes, Abietanes, Taxanes, etc.) and tetracyclic (Beyeranes, Kauranes, Atisiranes, Stemarene etc.) products. Despite the wide variety of structures and natural sources of diterpenoids, they all originate from the same biosynthetic precursor, the isoprenoid 2*E*,6*E*,10*E*-geranylgeranyl pyrophosphate (GGPP).[18] At present, three pathways have been found, which lead to the formation of primary isoprenyl fragments, from which all isoprenoids are built up (Scheme 1). These are mevalonate, mevalonate-independent and amino acid pathways, with the first one ruling in nature. The mevalonate pathway is one of three pathways for the formation of primary isoprenyl fragments, which are the building blocks for all isoprenoids. The starting step in this pathway is the reaction between pyruvic acid and a thiol group of Coenzyme A (CoA) in the presence of NAD^+. This reaction produces acetyl coenzyme A, which then interacts with another molecule to produce acetoacetylcoenzyme A. Another reaction with acetylcoenzyme A produces mevalonic acid, which is a precursor for nearly all isoprenoids. Phosphorylation of mevalonic acid leads to the release of CO_2 and water and the formation of 3-isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAP). These are the primary "isoprene" blocks for biosynthesis of almost all known isoprenoids. The biosynthesis of isoprenoids begins with the interaction of IPP and DMAP through the nucleophilic substitution of the pyrophosphate group in DMAP. This reaction and subsequent steps involve the enzyme geranylgeranyl diphosphate synthase. Geranyl pyrophosphate, the product of this reaction, is a precursor to all monoterpenoids. Its reaction with IPP leads to the formation of farnesyl pyrophosphate, which is a precursor to triterpenoids and steroids. The reaction of farnesyl pyrophosphate with another IPP molecule produces geranylgeranyl pyrophosphate (GGPP). GGPP is a precursor to a wide range of isoprenoids including diterpenoids. Among all GGPP biotransformation pathways in nature, we only consider those leading to tetracyclic diterpenoids. In this pathway, cyclisation is initiated by protonation the C-14–C-15 double bond

followed by nucleophilic attacks of C-10 on C-15 and C-7 on C-11, which lead to four products with structures dependent on the conformation of GGPP. We are only interested in the biotransformation pathway that leads to the formation of the diterpene *ent*-kaurene. This pathway, known as the "enantio" (*ent*) conformation, involves the cyclisation of geranylgeranyl pyrophosphate (GGPP) into *ent*-copalyl pyrophosphate (*ent*-CPP) through the participation of the enzyme kaurene synthase. The intramolecular cyclisation of *ent*-CPP leads to the formation of a carbocation, which reacts further in two pathways, leading to the formation of either *ent*-kaurene or *ent*-beyerene.

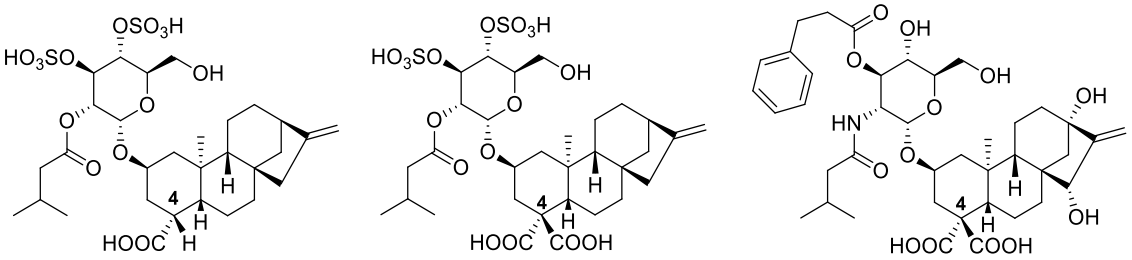


Scheme 1. Biosynthesis of *ent*-kaurene and *ent*-beyerene.

2.2 General information of *ent*-kaurane glycosides

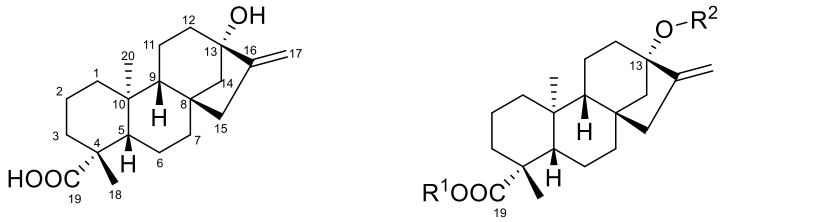
The glycosides of *ent*-kaurane-type diterpenes form an important class of natural substances, which have been studied because of their obvious biological activity. Atractyloside **1** (Scheme 2) is a naturally occurring glycoside that has been studied for its biological activity.[19] It was first isolated from *Atractylis gummifera* and later found in *Wedelia glauca* herbs. It has toxic effects in animals, causing hypoglycemia, respiratory depression, neurotoxicity and cell damage. In humans, atractyloside **1** can cause hepatic failure and necrosis because of its ability to inhibit oxidative phosphorylation in mitochondria.[20] *Atractylis gummifera* plants contain the glycoside

carboxyatractyloside **2** (Scheme 2), which is about ten times more toxic than atractyloside **1**.[21] The toxicity is due to the presence of a second carboxyl group at C-4 of the *ent*-kaurene skeleton. The glycoside also has cancer-preventive activity. Wedeloside **3** (Scheme 2), an aminoglycoside isolated from *Wedelia asperrima*, is the first example of a glycoside with an amide-bound sugar moiety in diterpenoids. This aminoglycoside inhibits the mitochondrial ADP/ATP transport.[22]



Scheme 2. Glycosides **1** and **2** isolated from *Atractylis gummifera* and glycoside **3** isolated from *Wedelia asperrima*.

Stevioside and its derivatives found in the leaves of *Stevia rebaudiana* are the most widely known *ent*-kaurene glycosides (Rebaudiosides), because they are used as highly effective low caloric sugar substitutes (sweeteners) worldwide.[23,24,25] The following beneficial features explain these properties: (1) low caloric sweeteners are usable for diabetics;[26] (2) stevioside is nontoxic and nonmutagenic;[27] (3) it also has therapeutic properties such as antihypertensive,[28,29,30] antihyperglycemic,[31,32,33] immunomodulatory,[34,35,36] antiinflammatory,[37,38] cancer-preventive[39,40,41] and insulinotropic activity.[42,43]



Scheme 3. The structure and numbering of sugar moieties of steviol and steviol glycosides.

The sweetness of *S. rebaudiana* leaves is due to glycosides, with stevioside being the major component.

In addition to stevioside, this plant also contains steviol glycosides, such as Rebaudiosides A-F and Dulcoside. All steviol glycosides have steviol **4** as a common aglycone and they differ from

each other by the structure and number of sugar moieties (Scheme 3, Table 1). Most Rebaudiosides are sweeter than sucrose and the sweetness of these glycosides increases with the number of glucose derivatives bound to the aglycone (steviol **4**).[44,45]

Table 1. Structures of different steviol glycosides.

Rebaudiosides	R^1 (C-19)	R^2 (C-13)	m_{tot} (%)*
Stevioside	Glc(β1)-	Glc(β1-2)Glc(β1)-	2.0
Rebaudioside A	Glc(β1)-	Glc(β1-2)[Glc(β1-3)]Glc(β1)-	5.0
Rebaudioside B	H-	Glc(β1-2)[Glc(β1-3)]Glc(β1)-	0.5
Rebaudioside C	Glc(β1)-	Rha(α1-2)[Glc(β1-3)]Glc(β1)-	2.0
Rebaudioside D	Glc(β1-2)Glc(β1)-	Glc(β1-2)[Glc(β1-3)]Glc(β1)-	3.3
Rebaudioside E	Glc(β1-2)Glc(β1)-	Glc(β1-2)- Glc(β1)-	ND
Rebaudioside F	Glc(β1)-	Xyl(β1-2)[Glc(β1-3)]Glc(β1)-	ND
Dulcoside A	Glc(β1)-	Rha(α1-2) Glc(β1)-	1.0

*According to Jaworski et al.: Analysis of dried *Stevia rebaudiana* leaves (100 g dry weight basis)[46]
ND: not determined
Glc: D-glucose; Rha: L-rhamnose; Xyl: D-xylose

2.3. Biological activity of *ent*-kaurane diterpenoids

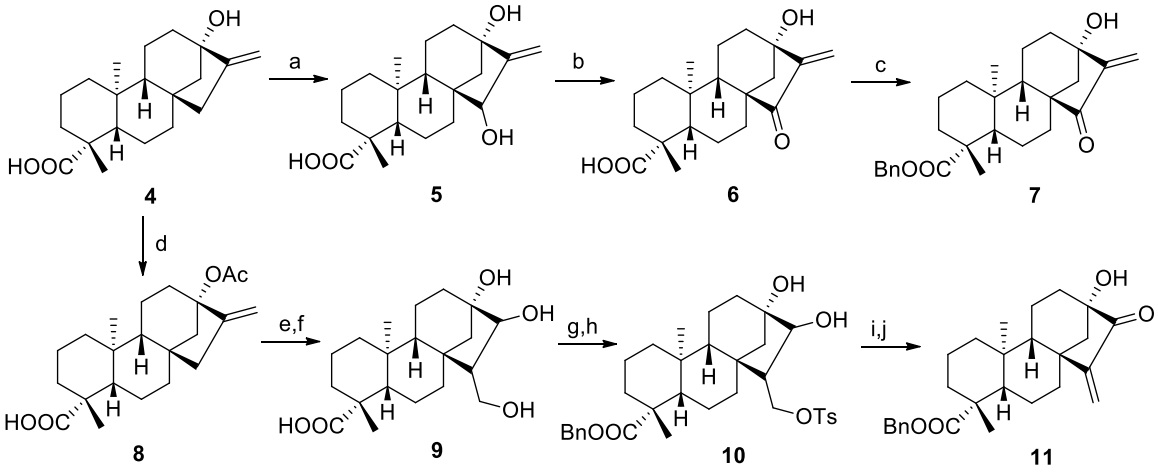
Natural products have been valuable sources of inspiration for researchers, and they have made a significant impact on chemical, biological and drug discovery. *Ent*-kaurane diterpenoids, found in liverworts[47,48,49] and other higher plants[50,51], have demonstrated various pharmacological activities such as cancer-preventive,[52] antituberculosis,[53] antibacterial[54] and acetylcholinesterase inhibitor[55] properties. Moreover, some of them have been developed as cancer therapeutic agents, further emphasizing their potential in the field. The biological activity of *ent*-kauranes is discussed separately in the following chapters.

2.3.1 *Ent*-kaurane diterpenoids with anticancer properties

Jing Li et al. tried to build some steviol derivatives to establish novel anticancer compounds preserving crucial structural fragments of *exo*-methylene cyclopentanone in the ring D of steviol.[56]

They worked on developing novel anticancer compounds based on steviol derivatives. They have demonstrated previously that certain modification of steviol can increase its cytotoxicity. In this work, they synthesised four different tetracyclic diterpenoids and evaluated their biological activities. Steviol was treated with SeO_2 and *tert*-BuOOH to form 16-hydroxysteviol **5**. PDC oxidation of this compound gave the desired compound **6**,[57] which was then successfully esterified with benzyl

bromide to form **7**. In order to achieve the structural isomer of product **7**, another reaction path was used, where steviol **4** was transformed into *ent*-13-acetoxyl-16-oxo-17-dimethyl kaurane-19-acid **8**. This was achieved by protecting the 13-hydroxy group, while the double bond was oxidised with the Ac_2O/O_3 system.[58] To protect the primary alcohol function in **9** before oxidizing the 15-hydroxy group, TsCl was used, because of its large steric effect. Oxidation of **10** utilising PDC and Ts deprotection resulted in target compound **11** (Scheme 4). Antiproliferative activities were evaluated on the synthesised compounds against human gastric carcinoma MGC-803, HepG-2 and breast carcinoma MDA-MB-231 cell lines. Derivatives **7** and **11** were found to be the strongest and most selective anticancer agents, with IC_{50} values of 1.80 and 0.95 μM, respectively, against the two cancer cell lines. In summary, a beneficial attribute on the anticancer effect is observed with the formation of the *exo*-methylene cyclopentanone moiety of steviol. Significant values were achieved by the products containing the benzyl ester function. The activity of compounds containing free carboxylic acid groups, in turn, was not detectable.

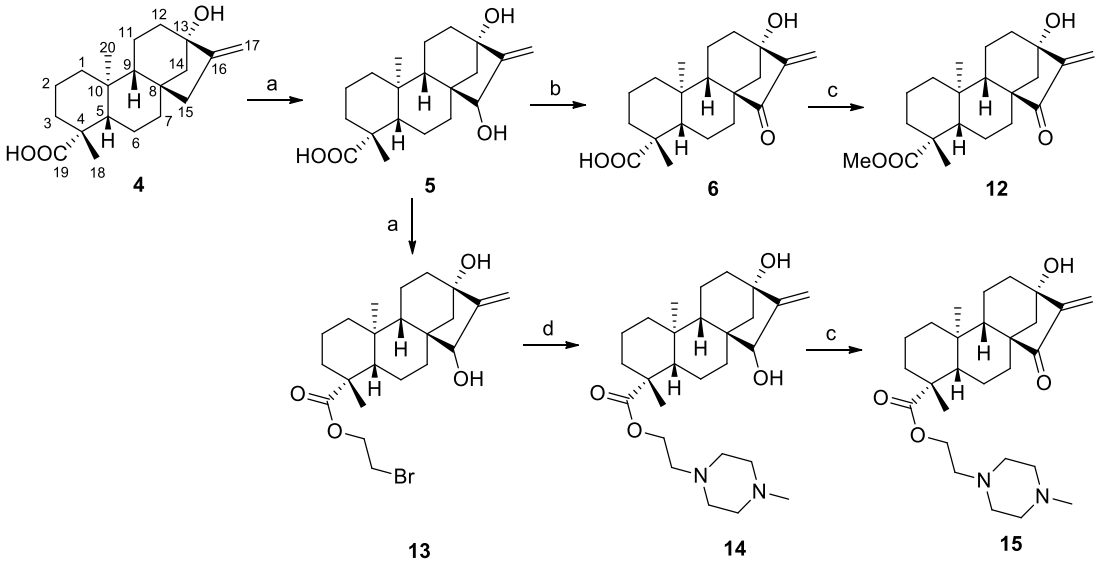


Scheme 4. Reagents and conditions: (a) SeO_2, *tert*-BuOOH, THF; (b) PDC, DMF; (c) BnBr, K_2CO_3, DMF, KI; (d) Ac_2O, DMAP, TEA, THF; (e) O_3/O_2, DCM, Ph_3P; (f) HCHO, NaOH, C_2H_5OH, 75 °C, 3 h; (g) BnBr, K_2CO_3, DMF, KI; (h) TsCl, pyridine, DMAP, rt; (i) PDC, DMF; (j) pyridine, DMAP, reflux. Yield for product **7**: 71.5%. Yield for product **10**: 53.6%.

It is generally accepted that the cytotoxicity of *ent*-kaurane diterpenoids is due to the formation of reactive oxygen species (ROS) through Michael modification of protein thiols and depletion of glutathione. The *α,β*-unsaturated carbonyl moiety is considered to be a key factor in this process. Lin et al. isolated 32 new and 12 known *ent*-kaurane diterpenoids from two Chinese liverworts.[59]

The study conducted by Lou et al. confirmed these findings by screening newly isolated and

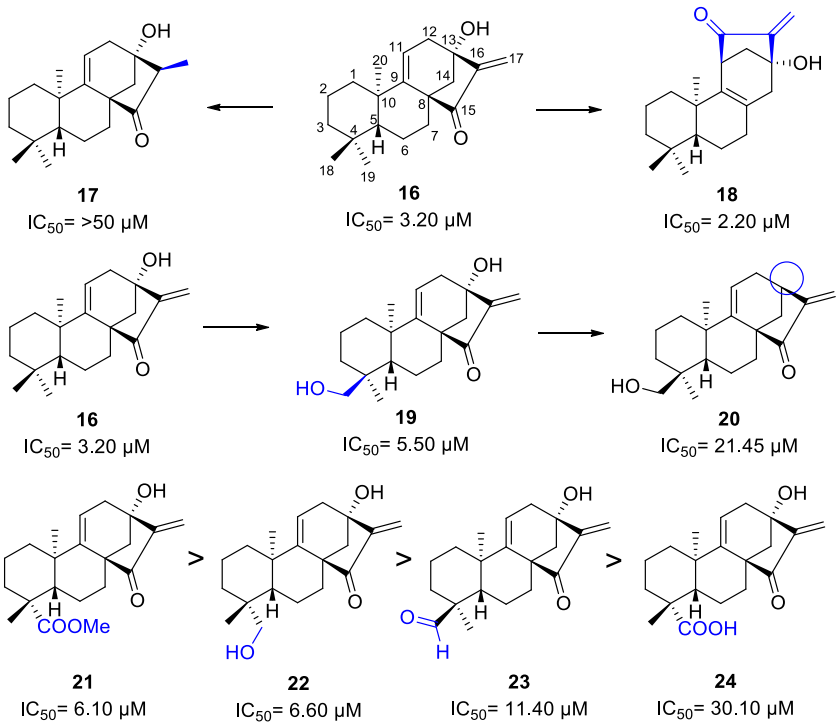
known *ent*-kaurane diterpenoids and found that they showed anticancer activities through ROS formation. The authors started the synthesis with steviol **4**, which was oxidised using SeO_2 and *tert*-BuOOH to form compound **5** (Scheme 5). PDC oxidation of **5** resulted in the desired compound **6**, which was esterified with methyl iodide to form **12**. To build up the carboxyl group (C-19), the esterified product **14** was synthesised from **5** applying 1,2-dibromoethane and *N*-methylpiperazine. The final step involved PDC oxidation of **14** to give the desired compound **15**, which has the *α*,*β*-unsaturated carbonyl moiety. Derivatives **12** and **15** had remarkable anticancer activity [**12**: IC_{50} = 1.50 μM, **15**: IC_{50} = 3.00 μM against HL-60 cancer cell lines (myelogenous leukemic cell lines)].



Scheme 5. Reagents and conditions: (a) SeO_2, *tert*-BuOOH, THF; (b) PDC, DMF; (c) CH_3I, DMF, rt; (d) $BrCH_2CH_2Br$, K_2CO_3, DMF, rt; (e) *N*-methylpiperazine, DMF, reflux. Yield for product **14**: 68%. Yield for product **17**: 29%.

In summary, the structure–activity relationship (SAR) investigation revealed that the presence of an *exo*-methylene cyclopentanone system in the D ring is crucial for the cytotoxicity of the compounds (Scheme 6: **16**→**17**).[24] Any modification that disrupts this system leads to a complete loss of cytotoxicity. The rearrangement of the *exo*-methylene cyclopentanone system causes no influence in cytotoxic activity (Scheme 6: **16**→**18**). The comparison of the IC_{50} values of some compounds indicated that the presence of a hydroxyl group at the C-13 position can enhance cytotoxicity and compounds with a hydroxyl group at C-18 also showed high activity (Scheme 6: **16**→**19**→**20**). However, the biological activity decreased, when the hydroxymethyl group (**22**) was oxidised to a formyl (**23**) or a carboxylic acid group (**24**). This activity loss could be partially reversed by esterification of the carboxylic acid, especially through methyl esterification (**21**). These findings

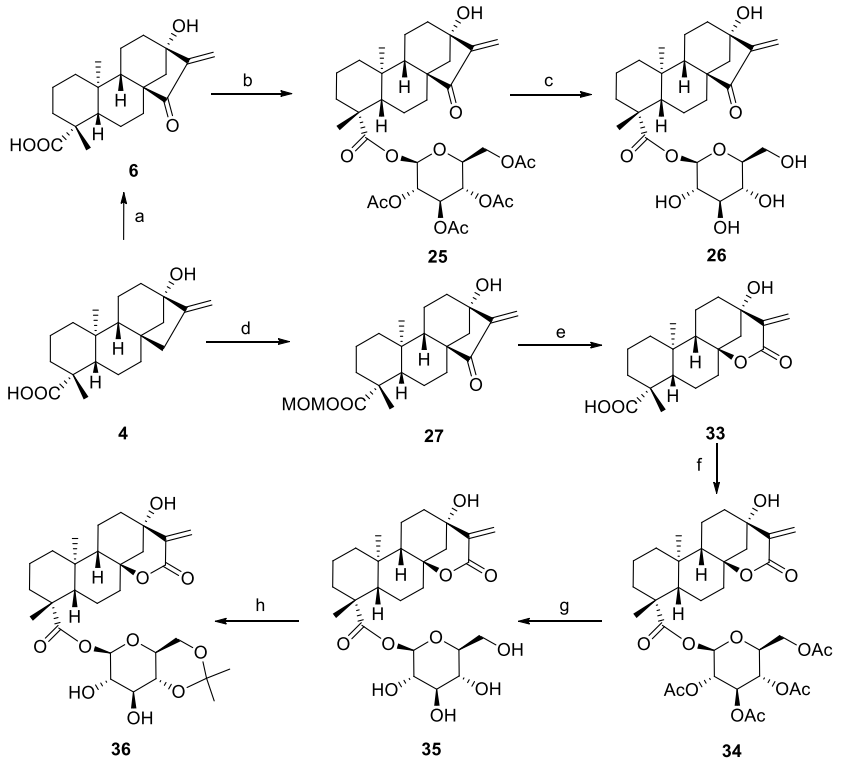
provide valuable information for future drug design and development efforts (Scheme 6).



Scheme 6. The structure–activity relationship summary for cytotoxicity. IC_{50} values were measured against PC3 cells.

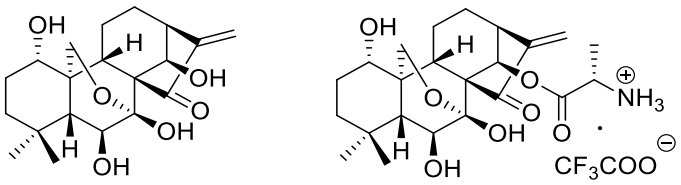
In connection with the previous study, Zou et al. synthesised steviol derivatives with either the *exo*-methylene cyclopentanone or the *α*-methylene lactone structure, where some glycoside moieties were connected to the carboxyl group (Scheme 7).[60] The starting material was stevioside, which was converted to steviol. In the first pathway, the *exo*-methylene cyclopentanone derivative was prepared. Steviol **4** was treated with SeO_2 and *tert*-BuOOH to produce 16-hydroxy steviol **5**, which was then oxidised with PDC to form the desired compound **6** with the *exo*-methylene cyclopentanone system. Compound **6** was treated with 2,3,4,6-tetra-*O*-acetylglucopyranosyl bromide in the presence of K_2CO_3 and KI in DMF to form **25**.[61] In the next step, deacetylation of **25** was performed in the presence of 10% $KHCO_3$ in methanol to obtain the required compound **26**. The second pathway described in the study involves the treatment of steviol **5** with chloromethyl methyl ether (MOMCl) and *N*,*N*-diisopropylethylamine as base to produce compound **27**. Then, compound **27** was reacted with SeO_2 and *tert*-BuOOH to produce compound **28**. Oxidation of **28** with PDC produced compound **29**. Then the *α*-methylene unit was protected with the phenylthio group. The addition of *p*-thiocresol on compound **29** produced *β*-thioketone **30**, which was then transformed into sulfone lactone **31** through Baeyer–Villiger oxidation with excess *m*CPBA. Finally, the sulfonyl

group of **31** was removed via desulfonylation with DBU in THF under mild conditions, producing the desired compound **32**. Compound **33** was then prepared from **32** by removing the methoxymethyl group with 10% HCl in THF. The synthesis of compound **36** involved the treatment of compound **33** with 2,3,4,6-tetra-*O*-acetylglucopyranosyl bromide in the presence of K_2CO_3 and KI in DMF as solvent. This reaction resulted in compound **36** an acetylated glucopyranoside derivative. In the next step, deacetylation of compound **34** was performed in the presence of 10% $KHCO_3$ in methanol, resulting in the formation of compound **35**. Finally, compound **35** was reacted with 2,2-dimethoxypropane with a catalytic amount of pyridinium *p*-toluenesulfonate (PPTs) in DMF to obtain compound **36**. Compound **26** was found to be strong against numerous cancer cell lines, including HepG-2, Bel-7402 (human hepatocellular carcinoma), A549 (human lung cancer), MCF-7 and MDA-MB-231 (breast carcinoma) with IC_{50} values between 0.07 and 0.91 μM. Compound **36** showed even more potent and selective antiproliferative activity against the HepG-2 cell line, with an IC_{50} value of 0.01 μM. This indicates that compound **36** has strong potential for use as an anticancer agent specifically against HepG-2 cells.



Scheme 7. Reagents and conditions: (a) 1) SeO_2, *tert*-BuOOH, THF; 2) PDC, DMF; (b) 2,3,4,6-tetra-*O*-acetyl-glucopyranosyl bromide, K_2CO_3, DMF, KI; (c) 10% $KHCO_3$, CH_3OH; (d) 1) MOMCl, DIPEA, DMF; 2) SeO_2, *tert*-BuOOH, THF; 3) PDC, DMF; (e) 1) *p*-thiocresol, Et_3N, THF; 2) 85% *m*CPBA, $NaHCO_3$, DCM; 3) DBU, THF; 4) 10% HCl, THF, H_2O; (f) 2,3,4,6-tetra-*O*-acetyl-glucopyranosyl bromide, K_2CO_3, DMF, KI; (g) 10% $KHCO_3$, CH_3OH; (h) 2,2-dimethoxypropane, DMF, PPTs.

Due to its cytotoxic effect, it is worth mentioning oridonin the skeleton of *ent*-kaurane and its derivatives.[62,63] Oridonin **37** is a natural product with cancer-preventive properties, but its limitations in terms of potency, solubility and bioavailability have limited its potential use in cancer treatment (Scheme 8).[64,65,66] This has led to the development of derivatives of oridonin, such as L-alanine-(14-oridonin) ester trifluoroacetate **38** (HAO472), to enhance its pharmacological properties and advance its clinical development.[67] It is important to note that HAO472 is in Phase I human clinical trial in China by Hengrui Medicine Co. Ltd. However, more research is needed to determine its safety and efficacy in humans.

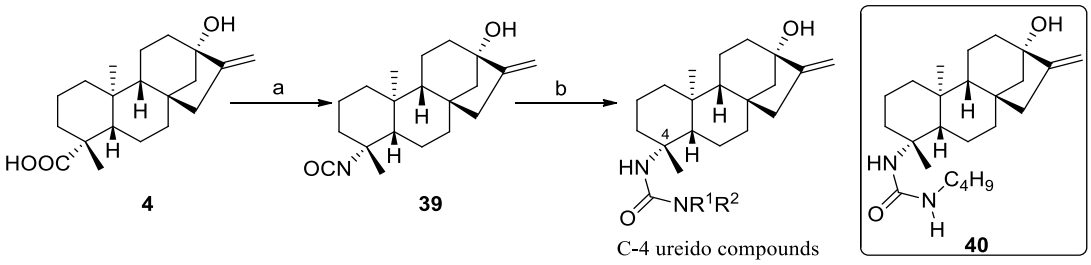


Scheme 8. The structure of oridonin **37** and its derivative L-alanine-(14-oridonin) ester trifluoroacetate **38** (HAO472).

2.3.2 *Ent*-kaurane diterpenoids with antimycobacterial and antituberculotic properties

Two series of steviol derivatives were synthesised by Lin and co-workers using similar methods.[68] Steviol **4** was converted to intermediate **39** by reacting it with diphenylphosphoryl azide (DPPA) in benzene (Scheme 9). C-4 ureido compounds with specific substituents were obtained by reacting **39** with various primer or secondary amines in DMF in the presence of triethylamine at room temperature.

Compound **40** with $R^1 = H$ and $R^2 = C_4H_9$ substituents showed excellent results in suppressing HBV DNA replication, with an IC_{50} value of 16.9 μM and a selectivity index (SI) of 57.7, which were better than those of the reference molecule lamivudine (3-TC). The anti-HBV mode of action of **40** was examined to find a particularly decreased viral gene levels.

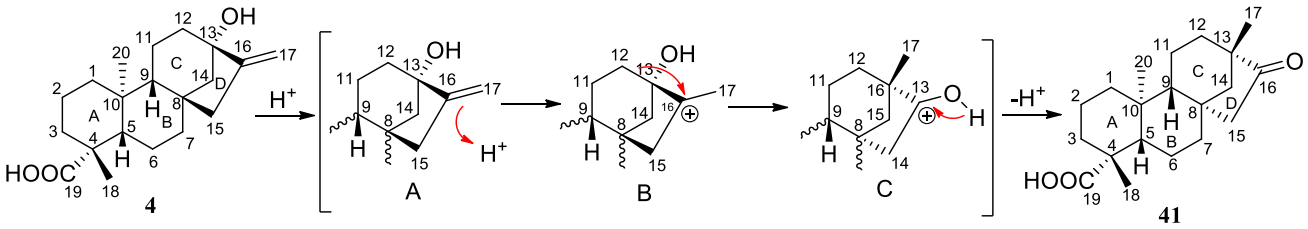


Scheme 9. Synthesis of C-4 ureido libraries. Reagents and conditions: (a) DPPA, benzene, reflux; (b) RNH_2 or R^1R^2NH, Et_3N, DMF, rt.

2.1. Biological activity of *ent*-beyerane diterpenoids

Steviol glycosides (Table 1) contain an *ent*-kaurene core, which is a diterpene molecule consisting of a cyclopentane ring (D) and 3 cyclohexane rings (A, B, C). Three glucose molecules are attached to this core, making it a glycoside.

Enzymatic hydrolysis or alkaline hydrolysis of the glycosidic bonds in steviol glycosides leads to the formation of steviol **4**. It has the same *ent*-kaurene core as steviol glycosides but lacks the glucose molecules,[69] while isosteviol **41** belongs to the *ent*-beyerane series of compounds. It has a tetracyclic skeleton that is similar in appearance to the kaurene skeleton. Isosteviol is one of the most well-known molecules in the *ent*-beyerane series. It can be obtained from steviol glycosides or steviol through an acid-catalysed Wagner–Meerwein rearrangement (Scheme 10).[70,71] The rearrangement involves the protonation of the C-16–C-17 double bond in steviol, which leads to the formation of a C-16 tertiary carbocation with the *ent*-kaurane structure (State A). This carbocation then undergoes a 1,2 sigmatropic shift, which cleaves the C-12–C-13 bond and results in an *ent*-beyerane structure (State B). Finally, a deprotonation occurs, resulting in the formation of isosteviol (State C).



Scheme 10. Wagner–Meerwein rearrangement of steviol **4** into the isosteviol **41**.

Isosteviol has been found to exhibit a wide range of biological activities, including antihyperglycemic,[32] antihypertensive,[72,30] cancer-preventive,[39,41] and immunomodulatory actions[35] beside several other biological activities.[73,74]

2.4.1 *Ent*-beyerane diterpenoids with anticancer properties

The ongoing study of the relationship between the structure and activity of isosteviol derivatives has indicated that components previously used with steviol were not effective. This may be due to many reasons. First, the change in structure from *ent*-kaurane to *ent*-beyerane; second, the lack of an OH group in the C-13 position, resulting in a more apolar molecule, etc. Scheme 12 displays some ineffective isosteviol derivatives that are remarkably similar to steviol derivatives

found to possess high cytotoxic activity. These derivatives have a common feature: they are connected to the A or D ring in some way.

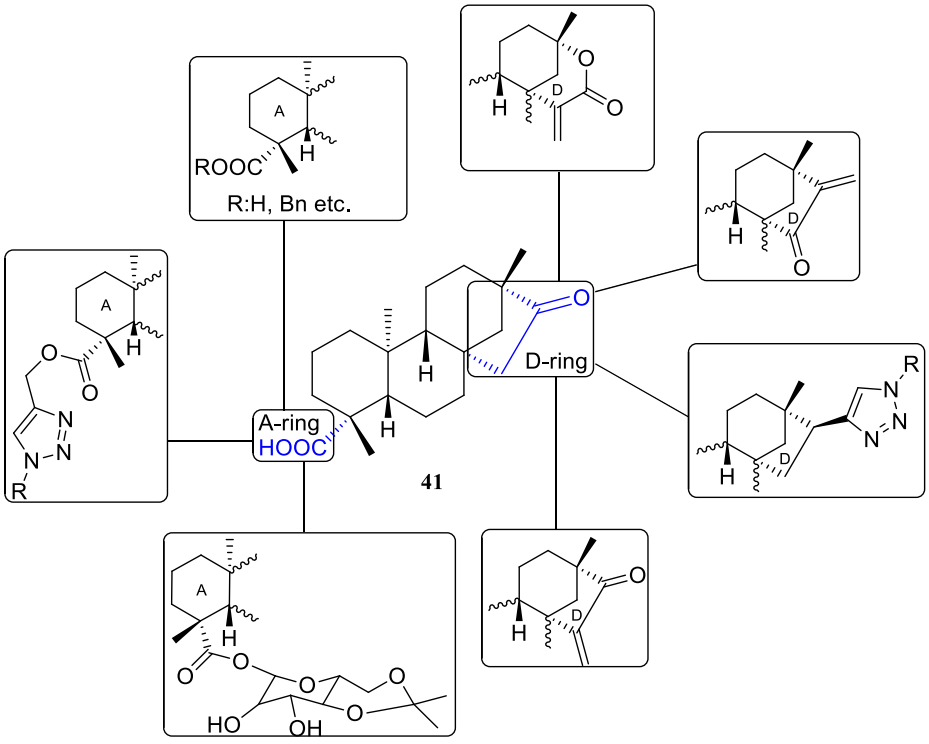
(1) Most of the derivatives glycosylated at the C-19 carboxyl group on the A ring did not exhibit potent antiproliferative activities (related cell lines: HepG2, Bel-7402, A549, U251, MCF-7, MDA-MB-231).[60]

(2) None of the products, except for a single one with an *exo*-methylene cyclopentanone structure on the D ring, had positive effects on the cytotoxic activity of isosteviol derivatives (related cell lines: MDA-MB-231, Hep-G2, MGC-803).[56]

(3) Similarly, products containing the α-methylenelactone moiety did not show any activity against cancer cell lines (related cell lines: PC-3, HCT-116, MDA-MB-231, K562, HepG2, MGC803).[75]

(4) Several cell lines, including A549, ASPC-1, MDA231, PC-3, HCT116 and HeLa, were tested with a significant number of isosteviol triazole derivatives.[76]

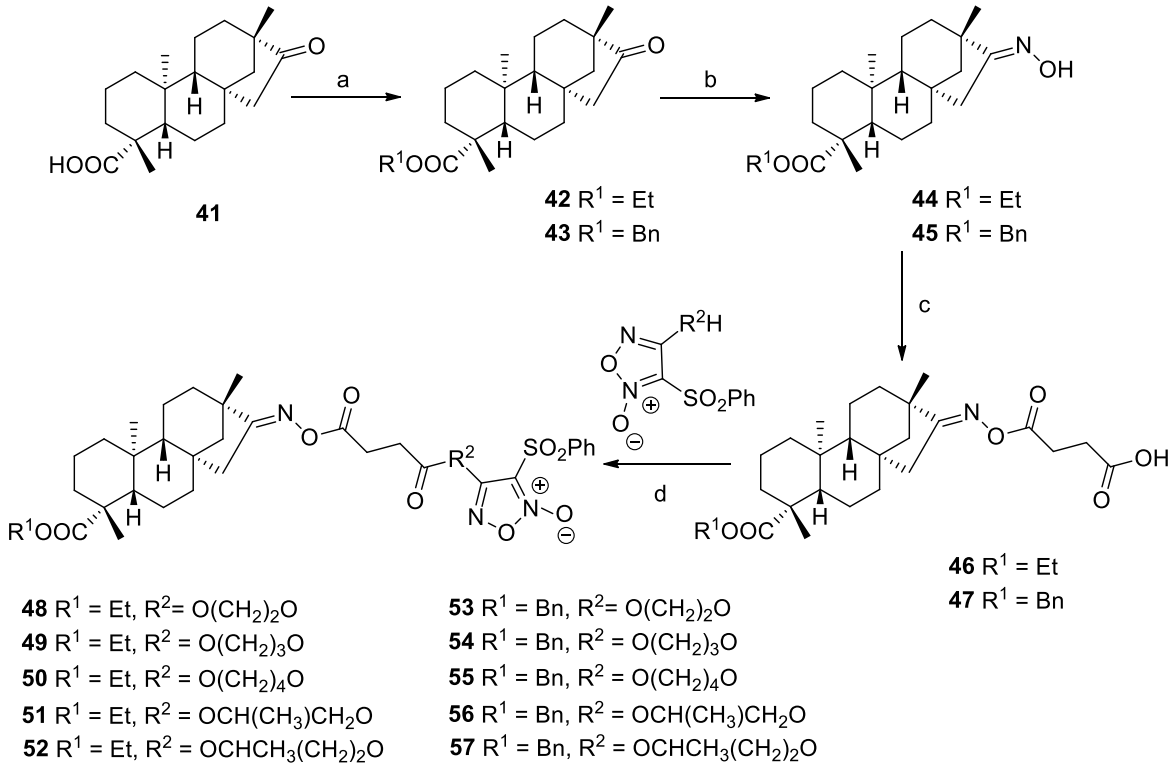
Derivatives that had the triazole moiety connected to position C-15 were found to be almost ineffective. However, it is interesting to note that compounds with an OH group at the C-15 position showed better inhibitory activities. This suggests that the OH group is a crucial moiety, as confirmed by numerous previous studies.



Scheme 11. Some ineffective isosteviol derivatives, which were very similar to steviol derivatives except the triazol derivatives.

Liu et al. synthesised a variety of isosteviol derivatives that were modified with nitric oxide (NO) donors and tested them for their ability to inhibit the growth of four human cancer cell lines.[77] Nitric oxide has a complex role in physiological and pathophysiological processes,[78] as it can promote tumour angiogenesis at low concentrations and inhibit at high concentrations.[79] NO donors are compounds that can release NO via enzymatic or non-enzymatic mechanisms. Furoxans are a type of NO donors that can generate high levels of NO through a known release process.[80]

In many studies, in situ synthesis of important intermediate compounds of furoxan was accomplished. First, isosteviol **41** was submitted to esterification with ethyl bromide or benzyl chloride to obtain the corresponding esters **42** and **43**.Then these underwent a condensation reaction between the ketone functional group and hydroxylamine hydrochloride to produce **44** and **45** (Scheme 15). Next, compounds **46** and **47** were prepared by reacting **44** and **45** with succinic anhydride through *O*-acylation. Finally, compounds **46** and **47** were combined with furoxan derivatives in the presence of 4-dimethylaminopyridine (DMAP) and 1-ethyl-(3-dimethylamino-propyl)carbodiimide hydrochloride (EDCI) to form the target compounds. Among the isosteviol derivatives synthesised, **48** and **52** exhibited the most potent antitumor activities (**48**, IC_{50} = 0.48 μM; **52**, IC_{50} = 0.94 μM), which were higher than that of the positive control. In conclusion, from these results some structure–effect relationship could be established. Namely, ethyl esters from **48** to **66** were found to be effective, while benzyl esters **53**–**57** were not. This suggests that esters with less large groups at the C-19 position are more favourable, whereas compounds **52** and **57** with branched linker derivatives are more effective than straight linker with the same number of carbons (**50** and **51**). Derivatives containing the longest straight linker showed the lowest cytotoxic activity, which indicates that the aliphatic chain extension weakened the activity. The researchers then investigated whether the antitumor activity of these derivatives was affected by the release of NO from furoxan derivatives. Derivatives with higher potency in releasing NO demonstrated stronger antitumor activity as well, indicating a positive correlation between antitumor activity and the release of NO from furoxan-based derivatives.



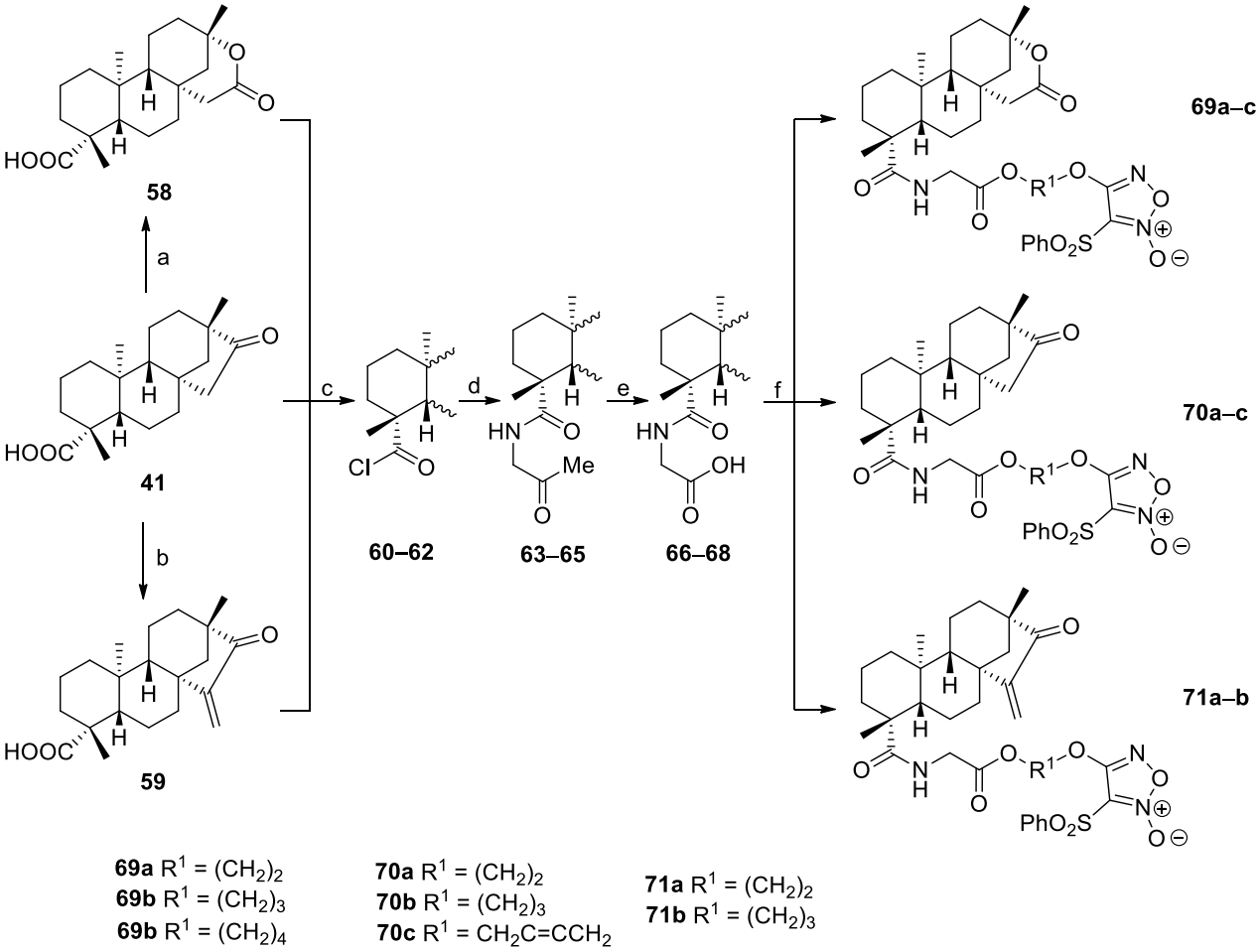
Scheme 12. Introduction of furoxan derivatives **48**–**57**. Reagents and conditions: (a) EtBr or BnCl, KOH, DMSO, rt; (b) $NH_2OH{*}HCl$, pyridine, rt; (c) succinic anhydride, DMAP, DCM, reflux; (d) EDCI, DMAP, dry DCM, rt.

Liu et al. also synthesised different types of furoxan-isosteviol derivatives by connecting them to the C-19 carboxylic acid (Scheme 13).[81] They prepared isosteviol **41** using the acid hydrolysis method, while derivative **58** containing an *α*-lactone function was synthesised by Baeyer–Villiger reaction from isosteviol.[56]

Moreover, the *exo*-methylene cyclopentanone **59** compound was synthesised using the method applied for the steviol derivative (product **6**). Thionyl chloride was used to treat the three starting compounds to create the corresponding acyl chloride derivatives **60**–**62**. Glycine methyl ester was then added with TEA to prepare compounds **63**–**65** and the methyl ester group was removed using methanol solution of sodium hydroxide. Finally, compounds **66**–**68** were reacted with furoxan derivatives, 4-dimethylamino pyridine (DMAP) and 1-ethyl-(3-dimethylamino-propyl) carbodiimide hydrochloride (EDCI) to produce several furoxan-isosteviol derivatives. All derivatives underwent testing for cytotoxicity against two cancer cell lines, HepG2 (hepatocellular liver carcinoma) and B16F10 (highly metastatic melanoma), with *Comptothecin* chosen as the positive control drug. Most of the derivatives exhibited greater cytotoxicity against the B16F10 cells than the control drug. However, these derivatives were ineffective against HepG2, as indicated by the information in

summaries on page 17.

Application of linkers with two or three carbons were found to be more potent than longer-chain linkers. Compound **70c**, which contains an allyl moiety, exhibited greater efficiency than compound **69c**, which has a straight-chain linker with the same number of carbon atoms. Finally, the derivative containing an *exo*-methylene cyclopentanone moiety (**71b**) proved to be the most effective with an IC_{50} value of 0.02 μM against B16F10 cells.

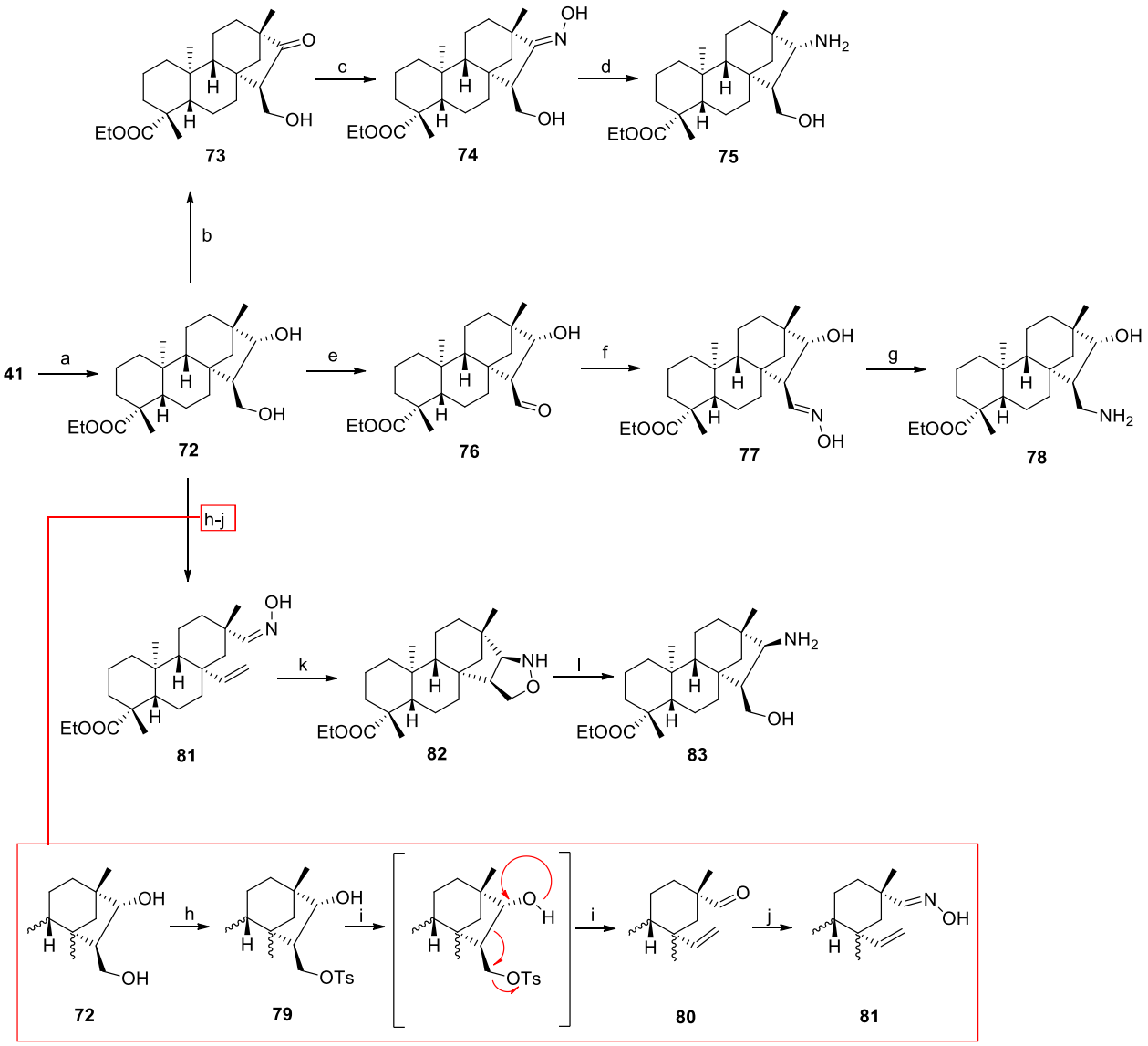


Scheme 13. Introduction of furoxan derivatives (C-19) **69a**–**71b**. Reagents and conditions: (a) *m*CPBA, H_2SO_4, 0 °C to rt; (b) four steps by the known method; (c) $SOCl_2$, ice bath, 2 h; (d) Glycine methyl ester, TEA, rt; (e) NaOH, MeOH, rt; (f) EDCI, DMAP, dry DCM, rt.

It is challenging to find isosteviol derivatives with an aminoalcohol moiety in the literature. This is also true for derivatives that contain thiourea moieties. Aminoalcohols are significant components of many natural or artificial products, and they exhibit a broad range of cytotoxic activities. Similarly, thiourea derivatives exhibit good inhibitory activity against numerous proteins.[82,83]

Liu et al. synthesised several isosteviol derivatives that contain aminoalcohol and thiourea fragments and investigated their activity against three human cancer cell lines.[84] First, the researchers synthesised the ethyl ester derivative using isosteviol and then 1,3-diol key intermediate **72** via a one-pot aldol-Cannizzaro reaction process was obtained (Scheme 14).[85] Afterwards, they successfully produced hydroxy keto product **73** by selectively oxidizing the OH group at the C-16 position using trimethylammonium chlorochromate.[86]

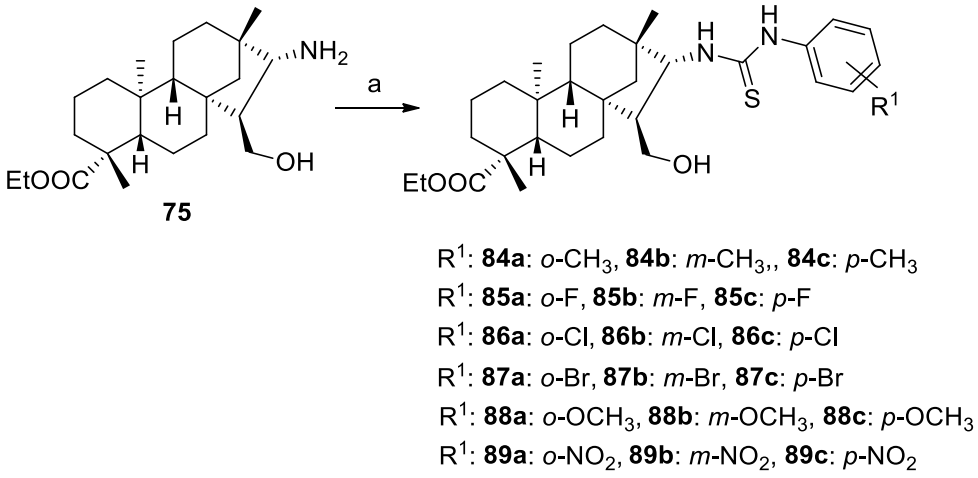
When **73** was treated with hydroxylamine hydrochloride hydroxyoxime derivative **74** was obtained, which was then converted into the 1,3-aminoalcohol derivative **75** by reducing it with $NaBH_4$ in the presence of MoO_3 as catalyst. Moreover, hydroxyaldehyde **76** was also successfully synthesised by the selective oxidation of the primary OH group in a TEMPO-catalysed oxidation process.[87] The resulting product (**77**) was then subjected to an oximation reaction with hydroxylamine hydrochloride and subsequently converted to another 1,3-aminoalcohol (**78**), through hydrogenation catalysed by Raney Ni. An alternative method for the preparation of 1,3-aminoalcohol was also reported, where the corresponding toluenesulfonate ester **79** was first obtained by converting the key intermediate 1,3-diol **72**, applying 4-methylphenylsulfonyl chloride in the presence of pyridine, which absorbed the liberated hydrogen chloride.[88] Additionally, allyl aldehyde **80** was formed by transforming compound **79** through a process called Grob fragmentation using NaOH in MeCN.[89] The reaction of **80** with hydroxylamine hydrochloride and $NaHCO_3$ in EtOH resulted in the formation of only the allyl oxime isomer **79**. Compound **82** was produced by an intramolecular 1,3-dipolar cycloaddition of compound **81** with a catalytic amount of $BF_3 \bullet OEt_2$. Furthermore, catalytic hydrogenolysis of the N–O bond with Raney Ni/H_2 produced compound **83**, which is an epimer of **75**. The obtained derivatives were tested against several human cancer cell lines comparing them with cisplatin as the positive control. It was observed that there was no significant difference in cytotoxicity between the two epimers (**75** and **83**) and regioisomer **78** (**75**: IC_{50} values of 4.49 μM, 5.83 μM and 3.54 μM, **83**: IC_{50} values of 4.90 μM, 7.25 μM and 2.47 μM, **78**: IC_{50} values of 12.25 μM, 4.01 μM and 5.02 μM against HCT-116, EC9706, Eca109 cells). Note, however, that any modification of the aminoalcohol moiety resulted in decreased activity.[90,91] Based on the information presented, it can be concluded, that the cytotoxic activity of the compounds was affected by both the aminoalcohol function and the chirality of the molecules.



Scheme 14. Introduction of aminoalcohol derivatives (**75**, **78** and **83**). Reagents and conditions: (a) 1) CH_3CH_2Br, DMSO, KOH, rt; 2) HCHO, Na, EtOH, 55 °C; (b) TCC, DCM, rt; (c) $NH_2OH{*}HCl$, $NaHCO_3$, EtOH, reflux; (d) $NaBH_4$, MoO_3, MeOH, rt; (e) TEMPO, NBS, DCM/H_2O, TBAB, reflux; (f) $NH_2OH{*}HCl$, $NaHCO_3$, EtOH, reflux; (g) Raney Ni/H_2, THF, 50 °C; (h) TsCl, pyridine, rt; (i) NaOH, MeCN, rt; (j) $HONH_2{*}HCl$, $NaHCO_3$, EtOH, 60 °C; (k) toluene, $BF_3{*}OEt_2$, 110 °C; (l) Raney Ni/H_2, 1MPa, MeOH, 60 °C.

Several compounds containing thiourea function at C-16 were synthesised by condensation of compound **75** with various substituted phenyl isothiocyanates in DCM (Scheme 15).[84] The substituent effect in the *ortho*, *para* or *meta* positions of the aromatic ring of the thiourea fragments was also studied and the inclusion of substituted thiourea moieties into the structure further increased the cytotoxic activities of the compounds. Among the compounds, those containing electron-withdrawing groups in the *para* position exhibited greater activity compared to those with groups in the *ortho* and *meta* positions. Specifically, *para*-nitro-substituted derivative **89c** showed the strongest

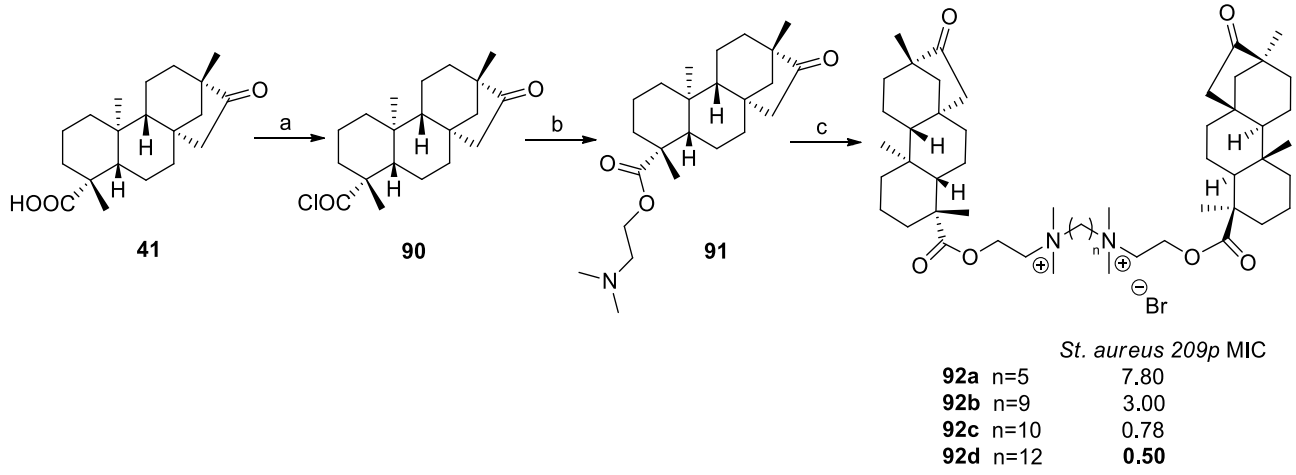
cytotoxic activity with IC_{50} values of 1.45 μM, 2.36 μM and3.54 μM against HCT-116, HGC-27 and JEKO-1 cells.



Scheme 15. Introduction of thiourea derivatives (**84a–89c**). Reagents and conditions: (a) isothiocyanates, DCM, rt.

2.4.2 *Ent*-beyerane diterpenoids with antibacterial activity

Kataev et al. prepared several isosteviol derivatives substituted at the 19-position.[92] All products were macromolecules based on isosteviol, in which two units were connected by a polymethylene chain containing quaternary *N* atoms. First, isosteviol **41** was treated with thionyl chloride to obtain the corresponding acyl chloride derivative **90**, which was then reacted with *N*,*N*′-dimethylaminoethanol without purification in an *O*-acylation reaction, to produce **91** containing the linker (Scheme 16).[93] Bis-quaternary ammonium derivatives **92a–d** were obtained by connecting two identical **91** products with 1,ω -dibromoalkanes of various lengths.



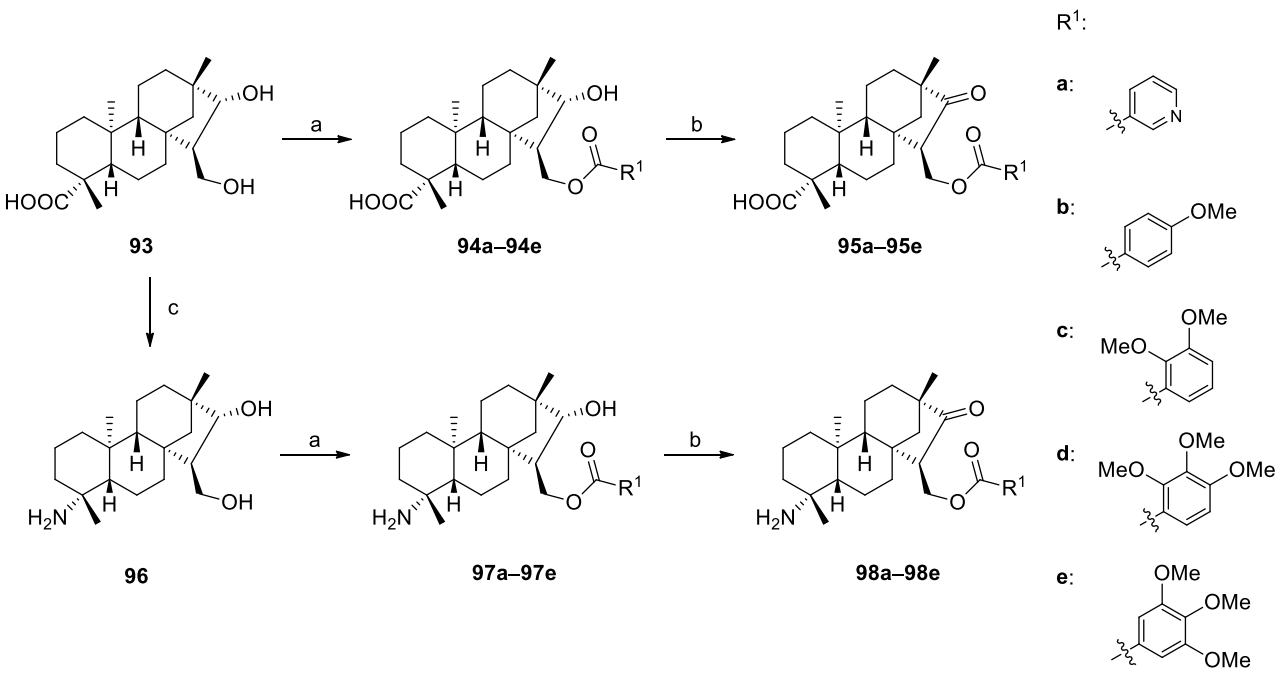
Scheme 16. Introduction of antimicrobacterial derivatives (**92a–d).** Reagents and conditions: (a) $SOCl_2$, (b) $Me_2N(CH_2)_2OH$, (c) $Br(CH_2)_nBr$.

All compounds were tested for bacteriostatic, fungistatic, bactericidal and fungicidal activities. The results indicated that the length of dibromoalkanes determined the antimicrobial activity, which, in all cases, was stronger when the chain was lengthened. The connected macromolecule **92d** exhibited the strongest activity and could be compared with positive control drugs (Ciprofloxacin and Clotrimazole). The strongest effect was measured with an MIC value of 0.50 μg/mL against the *Staphylococcus aureus 209p* Gram-positive bacteria.

2.4.3 *Ent*-beyerane diterpenoids with cardioprotective activity

Over the past few decades, zebrafish has become a popular animal model for *in vivo* compound screening in the early phase of drug discovery.[94] At the anatomical, cellular and membrane-biology levels, cardiovascular physiology are highly similar between humans and zebrafish. Several cardiovascular drugs have been found to exhibit the same effects on zebrafish physiology as they do on humans.[95,96] Isosteviol has been reported several times to have remarkable cardioprotective activity. To discover new cardioprotective derivatives, many compounds were synthesised and evaluated *in vivo* using the easy-to-handle and efficient zebrafish model.[97]

The first step of the synthetic route was the hydroxylation of isosteviol **41** via aldol-Cannizzaro reaction with a large amount of paraformaldehyde and NaOH in ethanol, which produced 1,3-dihydroxy derivative **93** in high yield (Scheme 17). Selective acylation of the hydroxymethyl group of **93** with acetic anhydride (Ac_2O), nicotinic acid, 4-methoxybenzoic acid, 2,3-dimethoxybenzoic acid, 2,3,4-trimethoxybenzoic acid or 3,4,5-trimethoxybenzoic acid gave **94a–e**. Further oxidation of the 16-OH with PDC produced their keto derivatives **95a–e**. Starting from 1,3-dihydroxy derivative **93**, the 19-COOH group was modified to the **96** primary NH_2 group via Curtius rearrangement and it was reacted with the same acid derivatives mentioned above. These acylations gave compounds **97a–e**. Finally, keto products **98a–e** were obtained via an oxidation reaction with PDC reagents. A cardioprotective effect was demonstrated by the products produced according to preliminary screening results. The most effective products contained the *O*-2,3,4-trimethoxybenzoyl (**95d**) and *O*-3,4,5-trimethylbenzoyl (**95e**) unit. The survival rate was increased from 77% to 82% and from 68% to 80% by the oxidation of the 16-OH group. Mortality was increased by replacing the 19-COOH group with an NH_2 group. Moderately active derivatives were obtained by acylating compound **96** with different acids. Further oxidation of the 16-OH group resulted in the corresponding keto derivatives where the **98d** and **98e** were found to be the most potent. At 40 μM, the survival rate was increased to approximately 98% and 87%.



Scheme 17. Introduction of cardioprotective derivatives. Reagents and conditions: (a) acid, DMAP, EDCI, DCM; (b) PDC, DMF; (c) DPPA, Et_3N, *tert*-BuOH, reflux.

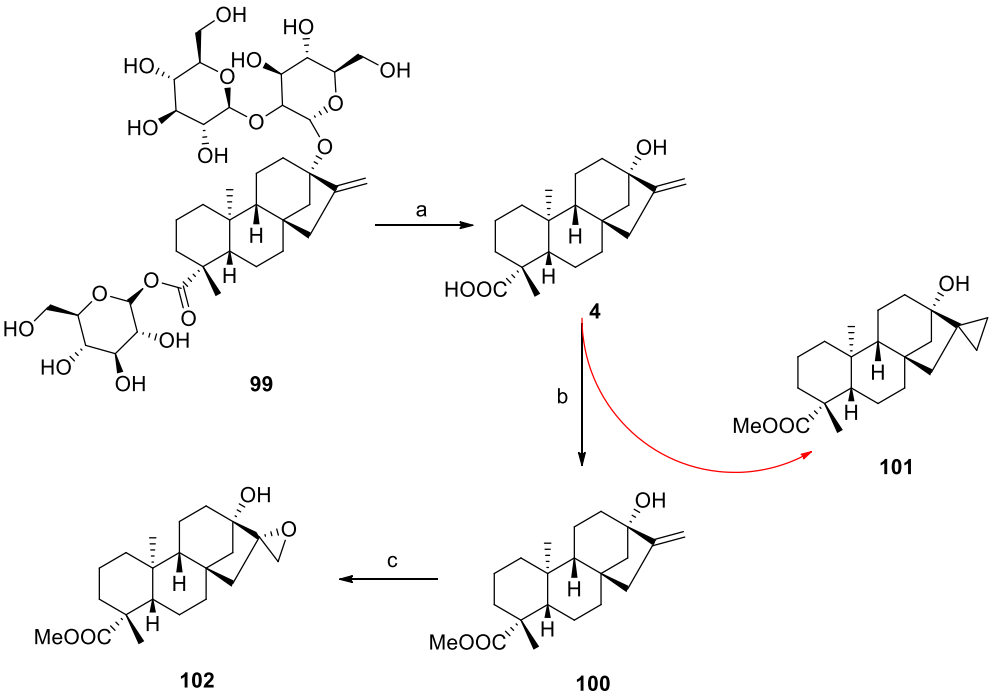
3. Results and discussion

3.1. Steviol-based trifunctional chiral derivatives

3.1.1 Steviol-based epoxyalcohol key intermediates

In our study, we undertook the preparation of steviol **4** utilizing natural stevioside **99**, which can easily be obtained from the market. To synthesise steviol, we employed two literature methods, as described in previous studies.[98,99] Diazomethane was used to esterify compound **4**, resulting in the formation of steviol methyl ester **100**.[98] The preparation of diazomethane was conducted in situ by reacting *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald) and NaOH was used immediately. It was necessary to use diazomethane carefully, as it could react with the allyl group, resulting in the formation of a cyclopropane product **101**, which is an unusable by-product in the synthesis.

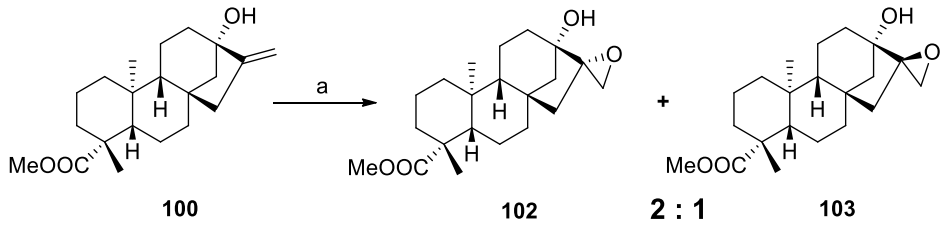
Next, we subjected compound **100** to epoxidation using *tert*-BuOOH and vanadyl acetylacetonate ($VO(acac)_2$) as the catalyst resulting in *cis*-epoxyalcohol **102**. The reaction proceeded in a stereospecific manner and the stereochemistry of the product was known in advance, as shown in Scheme 18.[100,101]



Scheme 18. Reagents and conditions: (a) 1) $NaIO_4$, H_2O, 25 °C, 16 h, 2) KOH, H_2O, 100 °C, 2 h, 60%; (b) CH_2N_2, Et_2O, 25 °C, 3 min, 72%; (c) $VO(acac)_2$, 70% *tert*-BuOOH, dry toluene, 25 °C, 12 h, 65%.

Various methods have been attempted to synthesise diastereoisomeric *trans*-epoxyalcohol

103. However, in most cases, *cis*-epoxyalcohol **102** was isolated as the sole product. Despite the challenges, a few attempts were made to produce diastereoisomer **103** through epoxidation reactions. Notably, application of dimethyldioxirane (DMDO) as a mild epoxidation reagent led to the formation of diastereoisomer **103** as a minor component (**102**:**103** = 2:1 ratio) (Scheme 19). The two isomers were separated by preparative column chromatography.[102]

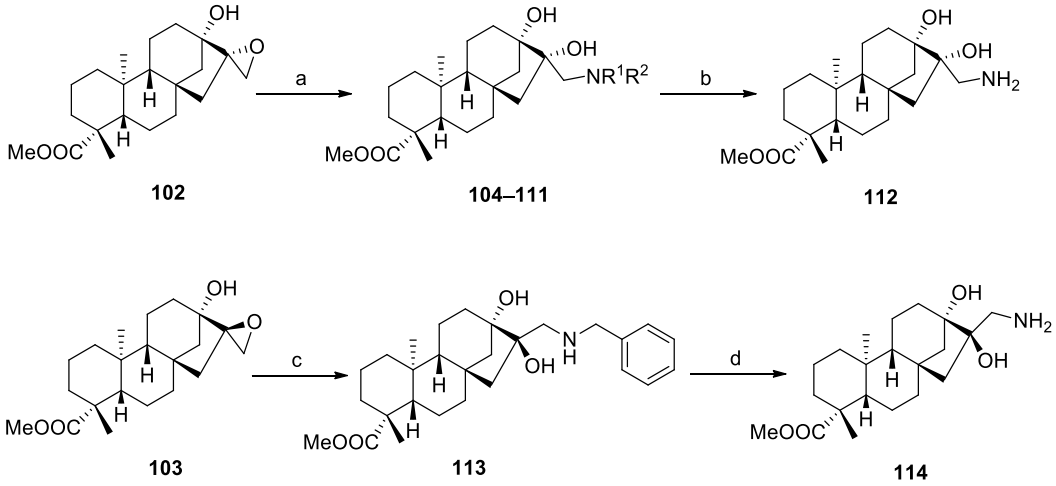


100 102 103

Scheme 19. Reagents and conditions: (a) Dimethyldioxirane (DMDO), acetone/H_2O, 25 °C, 12 h, 64%.

3.1.2 Synthesis of steviol-based aminodiol derivatives

A highly diverse library of 3-amino-1,2-diols can be efficiently prepared through aminolysis of **102** and **103**, which involves the nucleophilic addition of amines to epoxyalcohols. Previous studies have demonstrated the effectiveness of this method.[103,104,105] The reaction involves the opening of the oxirane ring of compound **102** using primary and secondary amines as well as $LiClO_4$ as catalyst. The resulting diastereomers were transformed into 3-amino-1,2-diols in moderate yield through hydrogenolysis over Pd/C. Products **104** and **113** as well as **112** and **114** are diastereomers of each other. To minimize by-product formation, reactions were performed at room temperature rather than at higher temperatures (Scheme 20).



102 104–111 112

103 113 114

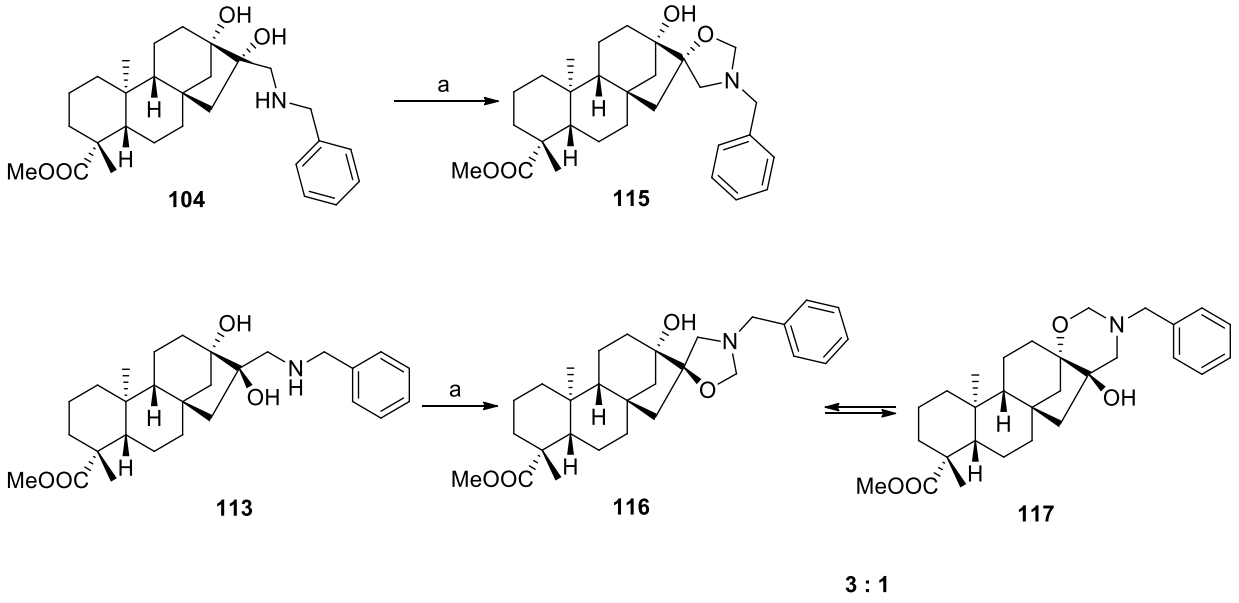
Scheme 20. Reagents and conditions: (a) R^1R^2NH, $LiClO_4$, MeCN, 25 °C, 3 d, 32–75%; (b) Product **104**, 5% Pd/C, H_2 (1 atm), MeOH, 25 °C, 12 h, 57%; (c) $BnNH_2$, $LiClO_4$, MeCN, 25 °C, 3 d, 77%; (d) 5% Pd/C, H_2 (1 atm), MeOH, 25 °C, 12 h, 58%.

Table 2 presents a library of tridentate aminodiol derivatives (**104**–**114**) resulting from the reaction. The relative configurations of the compounds were not experimentally determined as the configuration of the epoxyalcohol **102** products was already known from the literature.[106]

Table 2. Library of tridentate aminodiol derivatives.

Entry	Compound	R^1	R^2	Yield (%)
1	**104**	H	Benzyl	67
2	**105**	Methyl	Benzyl	75
3	**106**	H	(*R*)-*α*-Methylbenzyl	52
4	**107**	H	(*S*)-*α*-Methylbenzyl	59
5	**108**	H	*i*-Propyl	57
6	**109**	Ethyl	Ethyl	63
7	**110**	H	Propargyl	72
8	**111**	H	3,5-bis(trifluoromethyl)benzyl	32

Ring closure of diterpene-based 3-amino-1,2-diols can increase their catalytic potential. Treatment of aminodiol **104** with formaldehyde at room temperature afforded spiro-oxazolidine derivative **115** through a highly regioselective ring-closing reaction. In contrast, an interesting phenomenon was observed when **113** was treated with formaldehyde. Namely, another product was observed in small amounts in the solution during thin-layer chromatography tests (TLC). After the reaction, the product was purified by column chromatography (Scheme 21).

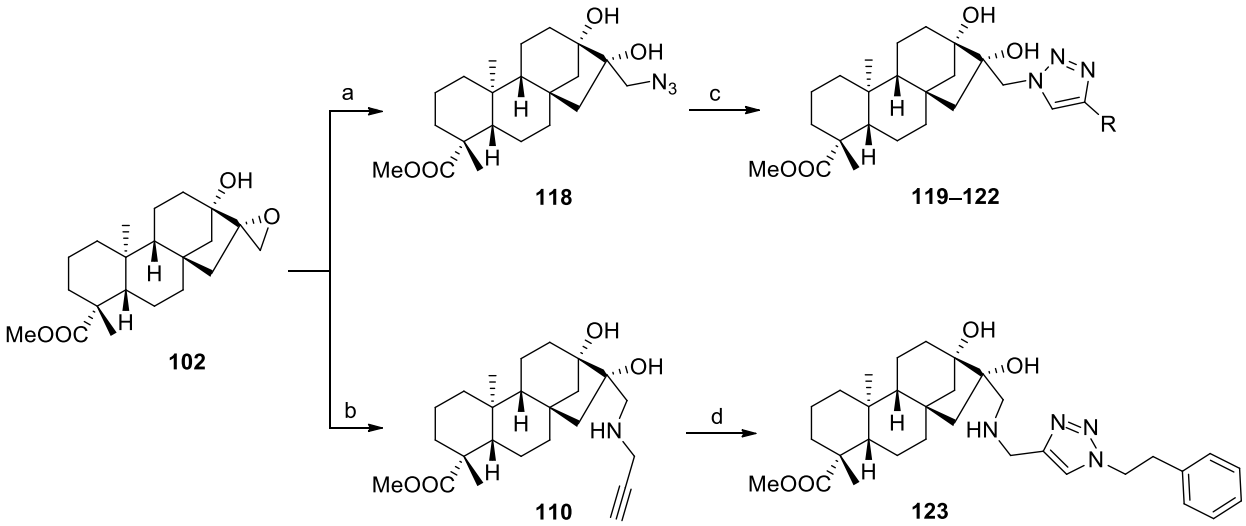


Scheme 21. Reagents and conditions: (a) 35% HCHO, Et_2O, 25 °C, 2 h, 88–91%.

After purification, the above-mentioned product also appeared in small quantities. During ^{1}H-NMR measurements, it was observable that there were two similar products in a 1:3 ratio. Thanks to the ratio, the structures of the two products were easily determined. It can be declared that the new product that appeared next to spiro-oxazolidine product **116** was a so-called 1,3-oxazine product **117**. After repeated purification, the amount of 1,3-oxazine product **117** decreased, but after a certain time, it increased again until the 1:3 ratio was reached at 25 °C. Afterwards, the ratio of the two products did not change.

3.1.3 Synthesis of steviol-based dihydroxytriazoles via azidodiol

Reaction of *cis*-epoxyalcohol **102** with sodium azide resulted in azidodiol **118** in the presence of ammonium chloride in an EtOH/water mixture (Scheme 22). Applying "click" reaction, triazoles **119**–**122** were synthesised from **118** with various substituted alkynes.[107,108] Additionally, a triazol-type tridentate product (**123**) was synthesised with "click" reaction, starting from *N*-propargyl-substituted aminodiol **110** and 2-phenylethyl azide. However, the Huisgen 1,3-dipolar cycloaddition reaction between alkynes and azides requires higher temperatures and usually gives mixtures of the two regioisomers. Therefore, copper-catalysed azide–alkyne cycloaddition was used instead, which operates at room temperature and produces specifically 1,4-disubstituted regioisomers. In this reaction, Cu(I) is the active agent, and it is generated from the Cu(II) salt using sodium ascorbate. Table 3 presents a library of triazole-type tridentate derivatives (**119**–**123**).



Scheme 22. Reagents and conditions: (a) NaN_3, NH_4Cl, EtOH/H_2O, 70–80 °C, 6 h, 57%; (b) Propargylamine, $LiClO_4$, MeCN, 25 °C, 3 d, 72%; (c) Alkyne, 2 mol% $CuSO_4$*$5H_2O$, 10 mol% sodium ascorbates, DCM, 25 °C, 16 h, 74–91%; (d) 2-(Azidoethyl)benzene, 2 mol% $CuSO_4$*$5H_2O$, 10 mol% sodium ascorbates, DCM, 25 °C, 16 h, 62%.

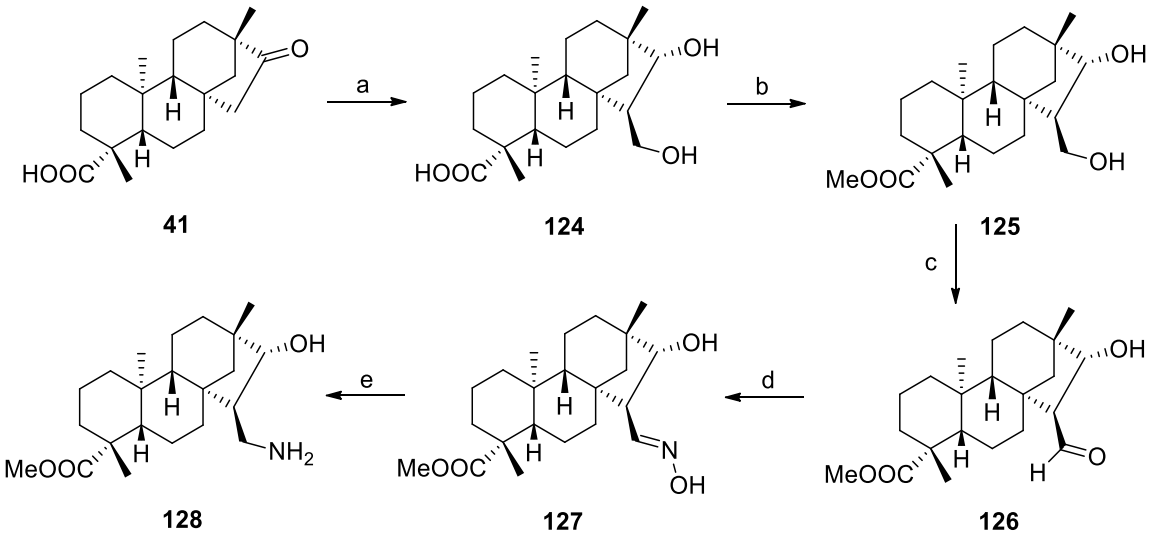
Table 3. Library of triazol-type tridentate derivatives.

Entry	Compound	R	Yield (%)
1	**119**	Phenyl	83
2	**120**	Ferrocenyl	91
3	**121**	Pyridyl	89
4	**122**	Cyclopropyl	74

3.2 Isosteviol-based bifunctional chiral derivatives

3.2.1 Synthesis of isosteviol-based 1,3-aminoalcohols

Starting from commercially available stevioside or mixtures of steviol glycosides, key intermediates 3-hydroxyaldehyde **126** and 1,3-aminoalcohol **128** were synthesised through a four- and six-step synthesis, respectively (Scheme 23). To obtain isosteviol **41**, stevioside **99** was subjected to acid-catalysed hydrolysis and rearrangement.[109] The stereoselective synthesis of diol **124** was accomplished with good yield in a one-pot aldol-Cannizzaro process in two steps using literature methods.[110,111] The esterification of **124** was then carried out with diazomethane in Et_2O, which resulted in methyl ester **125**.[85] The TBAB-catalysed oxidation of **125** using TEMPO and NCS led to the regioselective formation of **126** in excellent yield.[87] Subsequently, compound **127** was synthesised by oximation of **126** with hydroxylamine hydrochloride in the presence of $NaHCO_3$ in ethanol. Compound **127** was then hydrogenated using Raney-Ni in THF to successfully obtain 1,3-aminoalcohol **128** with good yield.

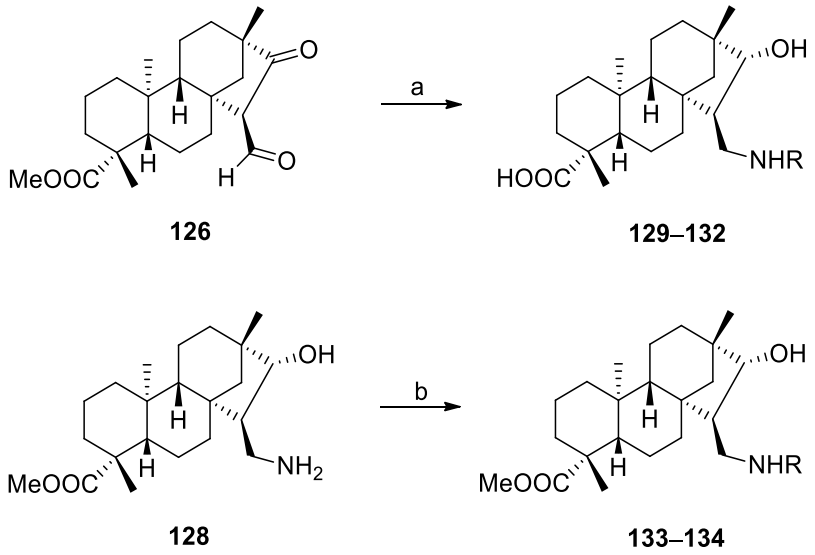


Scheme 23. Introduction of isosteviol-based 1,3-aminoalcohols. Reagents and conditions: (a) HCHO, NaOH, EtOH,1 h, 60 °C, 70%; (b) CH_2N_2, Et_2O, 5 min, 25 °C, 72%; (c) 10 mol% TEMPO, NCS, TBAB, DCM/H_2O, 12 h, reflux, 90%; (d) NH_2OH-HCl, EtOH, 12 h, reflux, 76%; (e) Raney-Ni, H_2 (10 atm), THF, 12 h, 25 °C, 83%.

3.2.2. Synthesis of isosteviol-based 1,3-aminoalcohols via Schiff bases

Based on the results of our previous study on *N*-substituted steviol-based aminodiols and related literature data on the antiproliferative activity of primary 1,3-aminoalcohols based on isosteviol, we anticipated that *N*-substituted 1,3-aminoalcohols would be of interest. Therefore, we synthesised a small library of 1,3-aminoalcohols to investigate the structure–bioactivity relationship of *N*-substitution and antiproliferative activity.[112,113]

Two pathways were used to accomplish the synthesis of these compounds: reductive amination of hydroxy aldehyde **126** with primary amines and reductive alkylation of primary 1,3-amino alcohol **128** with various aldehydes through the formation of Schiff bases, followed by reduction with $NaBH_4$ under mild conditions. The desired *N*-substituted 1,3-aminoalcohols (**129**–**134**) were isolated in acceptable yields. The reaction conditions and yields are presented in Scheme 24 and Table 4.



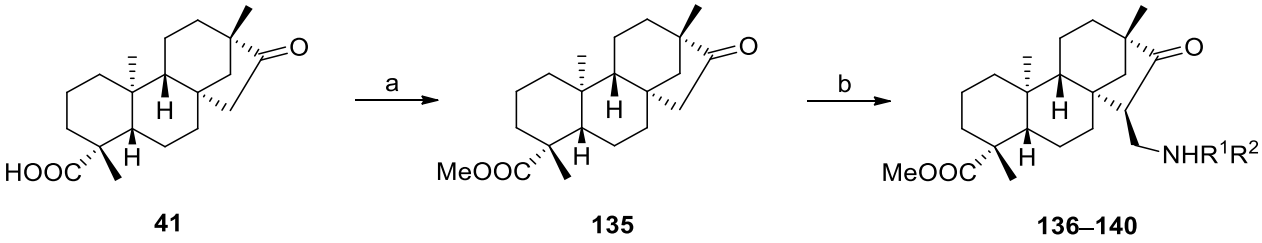
Scheme 24. Synthesis of isosteviol-based 1,3-aminoalcohols. (a) 1) RNH_2, dry EtOH, 3 h, 25 °C; 2) dry MeOH, $NaBH_4$, 3–4 h, 25 °C, 64–83%; (b) 1) aldehydes, dry EtOH, 3 h, 25 °C; 2) dry MeOH, $NaBH_4$, 3–4 h, 25 °C, 64–65%.

Table 4. Library of isosteviol-based 1,3-aminoalcohols.

Entry	Compound	R	Yield (%)
1	**129**	Methyl	77
2	**130**	Benzyl	83
3	**131**	(*S*)-*α*-Methylbenzyl	70
4	**132**	(*R*)-*α*-Methylbenzyl	64
5	**133**	4-Methoxybenzyl	64
6	**134**	4-Fluorobenzyl	65

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

Isosteviol methyl ester **135** was prepared with excellent yield from **41** by treating it with diazomethane.[114] The Mannich condensation of **135** was carried out in glacial acetic acid with paraformaldehyde and various secondary amine hydrochloride salts, resulting in a library of aminoketones with good to moderate yields (Scheme 25, Table 5).[85] Mannich condensation, i.e., amino alkylation, was possible in the case of isosteviol, because it has an acidic proton adjacent to the carbonyl (C=O) functional group. The final product is a *β*-amino carbonyl compound, also known as a Mannich base. The condensation reaction proceeded with exclusive stereoselectivity, resulting in the formation of a single diastereoisomer with a (7*R*) configuration of the new stereocenter at C-15. The results are summarised in Table 5.



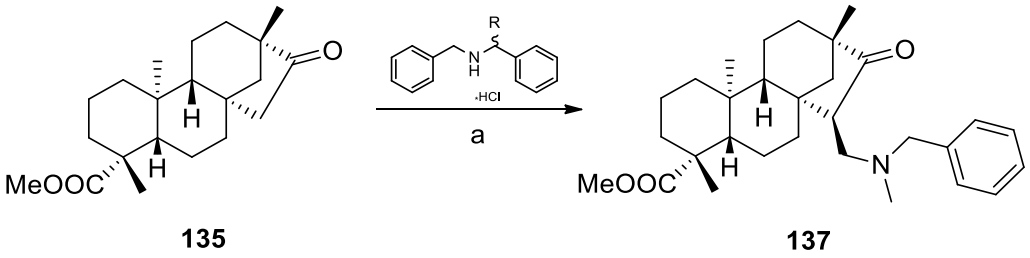
41 **135** **136–140**

Scheme 25. Synthesis of isosteviol-based Mannich base. (a) CH_2N_2, Et_2O, 5 min, 25 °C, 79%; (b) NHR^1R^2 ∗HCl, $(CH_2O)n$, AcOH, 24 h, reflux, 13–68%.

Table 5. Library of isosteviol-based 1,3-aminoalcohols.

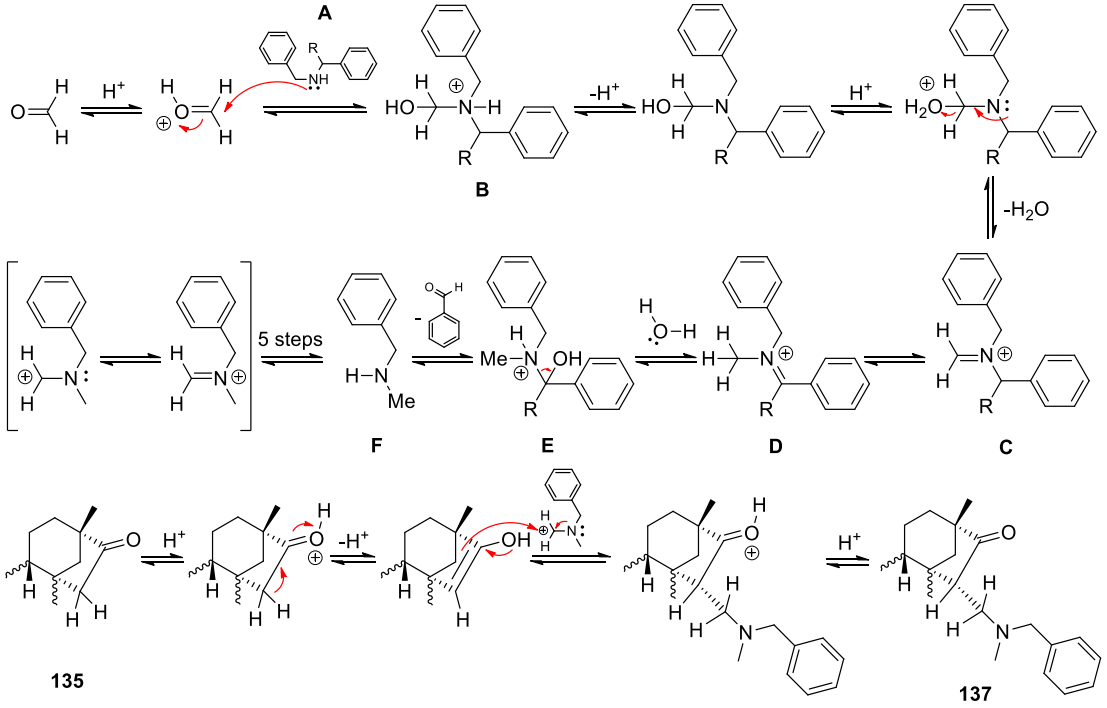
Entry	Amine HCl	Compound	R^1	R^2	Yield (%)
1	Morpholine	**136**	Ethoxyethylene		68
2	*N*-Methyl-*N*-benzylamine	**137**	Methyl	Benzyl	47
3	Pyrrolidine	**138**	Butylene		59
4	Dimethylamine	**139**	Methyl	Methyl	62
5	Diethylamine	**140**	Ethyl	Ethyl	56
6	Dibenzylamine	**137**	Methyl	Benzyl	13

Table 5 indicates that the reaction of **135** with dibenzylamine hydrochloride produced the unexpected *N*-methyl-*N*-benzyl derivative **137** in a low yield of 13%, instead of the anticipated *N*, *N*-dibenzyl-substituted product. To investigate this unexpected result, the reaction was performed again with *N*-benzyl-*N*-(*S*)-*α*-methylbenzylamine, and the corresponding (7*R*) enantiomer and the isolated product was found to be **137** as a single compound (Scheme 26).



Scheme 26. Unexpected substituent exchange in Mannich condensation. R = H, Me(*S*), Me(*R*). (a) $(CH_2O)n$, AcOH, 24 h, reflux, 13%.

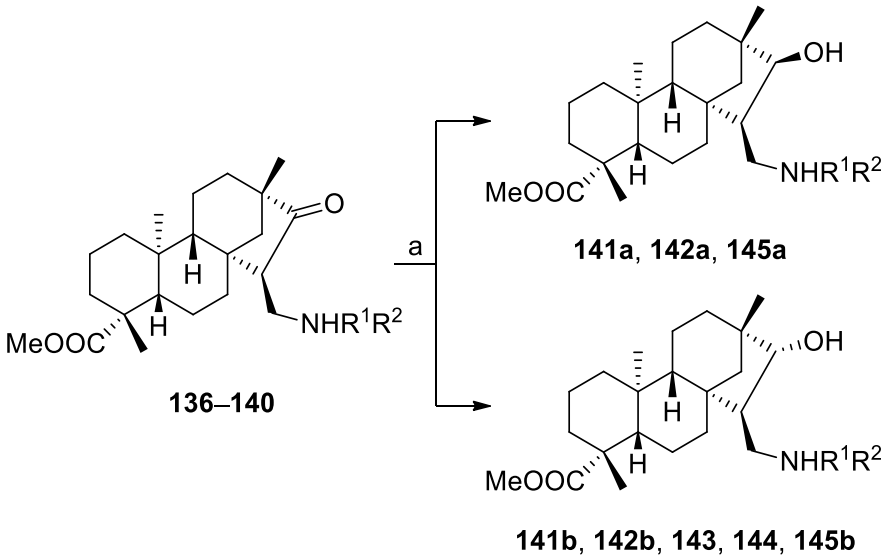
The reason behind the intriguing exchange of *N*-benzyl → *N*-methyl substituent can be attributed to the specific steric hindrance of the diterpenoid skeleton. In this ring system (Scheme 27), *N*-methyl-*N*-benzylamine represents the extreme of the Mannich condensation limit. As per the standard Mannich condensation mechanism, the first step involves the creation of an iminium ion by reacting dibenzylamine with formaldehyde (Scheme 31). Due to steric hindrance, iminium species **C** fails to respond to the ketone enolate. Instead, under the given circumstances, an isomeric iminium salt **D** is produced. This step is then followed by the addition of water and the elimination of benzaldehyde, which results in the formation of *N*-methyl-*N*-benzylamine (**F**). This final product is then ready for the condensation process to yield **137**. The steps allude to the intricate process involved in this chemical reaction.



Scheme 27. Proposed mechanism in Mannich condensation of dibenzylamine and reaction with isosteviol methyl ester.

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

The reduction of aminoketones **136**–**140** with $NaBH_4$ under mild reaction conditions produced a diastereomeric mixture of 1,3-aminoalcohols, as shown in Scheme 28. The reduction was highly selective in the case of pyrrolidinoaminoketone (**138**) or dimethylaminoketone (**139**) derivatives, resulting in single diastereoisomers **143** and **144**.



Scheme 28. Synthesis of aminoalcohols from Mannich bases. (a) $NaBH_4$, MeOH, 2–3 h, 25 °C, 10–75%

However, in other instances, a mixture of diastereomers were produced, as indicated in Table 6. The observed diversity in stereoselectivity during the reduction of aminoketones can be explained by the different steric hindrances of their *N*-substituents. It is possible that in the case of aminomethyl substituents with less hindrance, a cyclic complex can form with the protic solvent, allowing the hydride to attack only from the less hindered side. However, this complex cannot form in the case of bulky *N*-substitution. Hence, the hydride can attack both sides of the carbonyl function, resulting in a mixture of diastereoisomers. This explanation is based on previous research.[115]

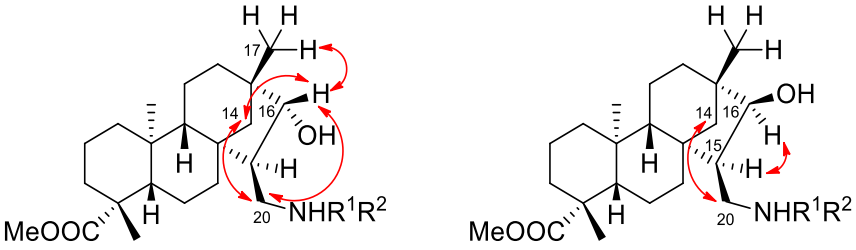
Table 6. Library of isosteviol-based 1,3-aminoalcohols.

Entry	Aminoketones	Product	dr (a:b)*	Yield (a:b) (%)
1	136	**141a, 141b**	1:1	28:24
2	137	**142a, 142b**	1:5	10:42
3	138	**143**	0:1	70
4	139	**144**	0:1	75
5	140	**145a, 145b**	1:1	10:10

*Ratio of diastereomeric pairs

3.2.4. Determination of the relative configuration

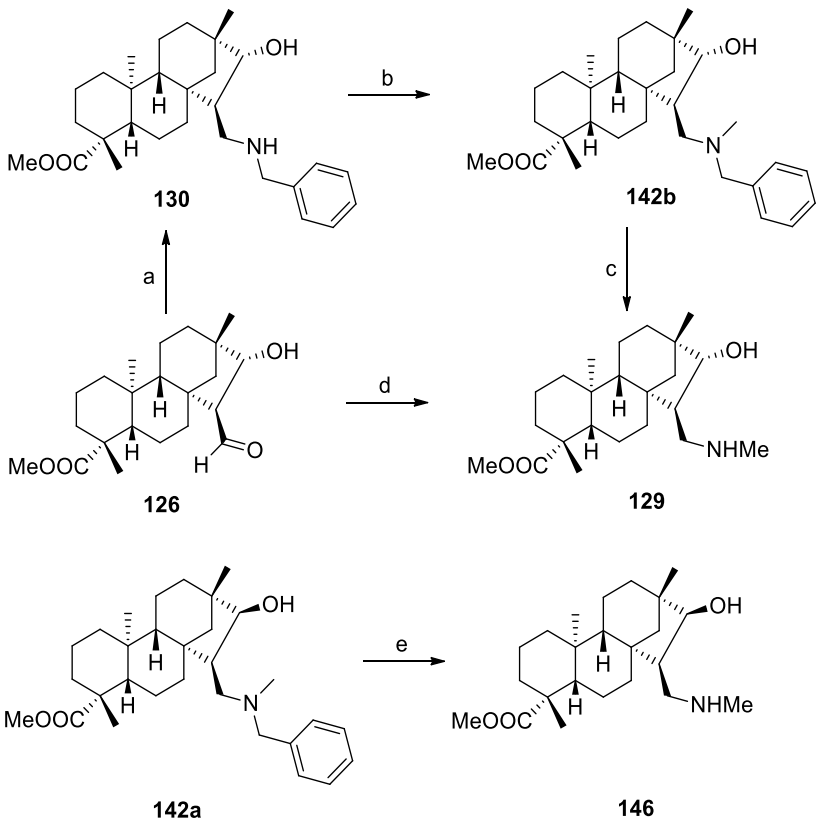
NMR spectroscopy with NOESY spectral analysis was used to determine the relative and, therefore, the absolute configuration of the new stereocenters of aminoalcohols **141**–**145** at positions C-15 and C-16. The analysis was based on the observed NOE effects between H–C (14) and H–C (16), H–C (16) and H–C (20), H–C (16) and Me–C (17), as well as between H–C (14) and H–C (20). Therefore, the structure of **141b**, **142b**, **143**, **144** and **145b** were determined, as outlined in Scheme 29. Similarly, NOE effects were observed in the case of **141a**, **142a** and **145a** between H–C (14) and H–C (20) and H–C (15) and H–C (16). The NMR analysis and NOE effects provided valuable information regarding the configurations of the new stereocenters of aminoalcohols.



141b, **142b**, **143**, **144**, **145b** **141a**, **142a**, **145a**

Scheme 29. The most important NOE ^{1}H-^{1}H correlations.

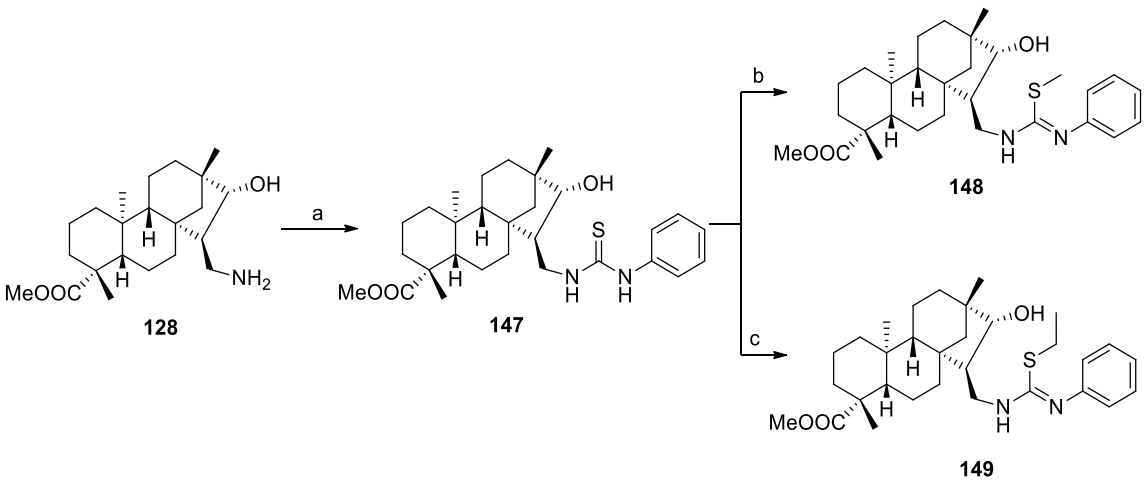
In addition to the NOESY experiments, the configurations of the newly formed stereocenters of 1,3-aminoalcohols were determined through two synthetic pathways, as depicted in Scheme 30. The reductive amination of known **126** with benzylamine, followed by the methylation of **130** with iodomethane, produced a product identical to **142b**, which was the major product obtained from the reduction of aminoketone **137.**[97] Alternatively, debenzylation of **142b** using a 5% Pd/C catalyst in methanol resulted in an *N*-methyl aminoalcohol, which was identical to **129** produced by the reductive amination of **126** with methylamine. Diastereoisomer **146** was also prepared by debenzylation of **142a** over 5% Pd/C catalyst, as shown in Scheme 30.



Scheme 30. Determination of the structure of 1,3-aminoalcohols. (a) 1) $BnNH_2$, dry EtOH, 3 h, 25 °C; 2) dry MeOH, $NaBH_4$, 3–4 h, 25 °C, 83%; (b) DCM, Et_3N, MeI, 4 h, 25 °C, 77%; (c) MeOH, 5% Pd/C, H_2 (1 atm), 12 h, 25 °C, 24%; (d) 1) $MeNH_2$, EtOH, 3 h, 25 °C; 2) MeOH, $NaBH_4$, 3–4 h, 25 °C, 77%; (e) MeOH, 5% Pd/C, H_2 (1 atm), 12 h, 25 °C, 28%.

3.2.5. Ring closure of isosteviol-based thiourea derivative

In our previous research, we found that monoterpene-fused 2-phenylimino[1,3]oxazines and -[1,3]thiazines exhibited significant cytotoxic activity against human cancer cell lines.[111,113] Based on this finding, we aimed to synthesise 1,3-oxazine and 1,3-thiazine derivatives of primary 1,3-aminoalcohol **128**. Treatment of **128** with phenyl isothiocyanate in DCM at room temperature resulted in the formation of hydroxy-thiourea **147** with high yield (Scheme 31). We attempted to convert **147** to the target compound through a well-known two-step procedure involving treatment with methyl iodide followed by alkaline-induced elimination of methyl mercaptol, but only methylthio ether intermediate **148** was formed. Alternatively, acid-promoted dehydrative cyclisation of **147** with EtOH containing 18% hydrochloric acid yielded only ethylthio ether **149** in moderate yield instead of the expected 2-phenylimino[1,3]thiazine.[114] This unexpected result can be explained by the steric hindrance of the diterpene skeleton, which hindered the attack of sulphur on the H–C (16) carbon. Additionally, the reaction of HCl with EtOH under the applied conditions generated EtCl in situ, which reacted with thiourea **147** similar to iodomethane.



Scheme 31. Ring closure of hydroxy-thiourea derivative **147.** (a) PhNCS, DCM, 2 h, 25 °C, 91%; (b) MeI, EtOH, 2 h, 25 °C, 69%; (c) 18% HCl, EtOH, 12 h, reflux, 46%.

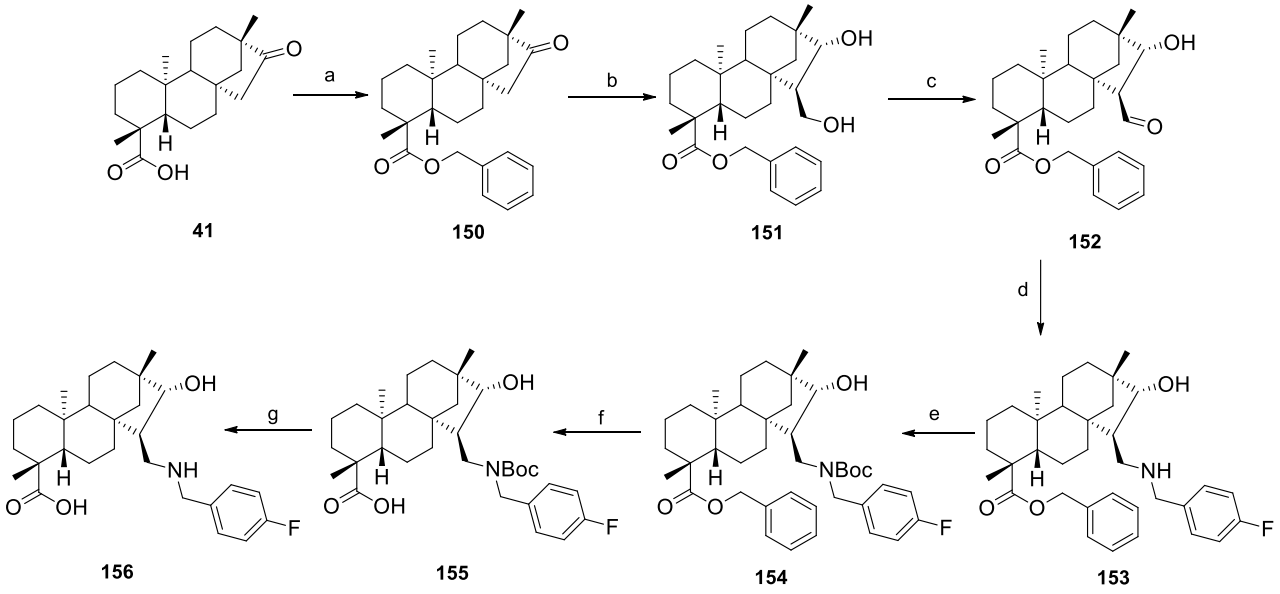
3.2.6. Allyl and acetylene derivatives of isosteviol-based 1,3-aminoalcohols

The antiproliferative investigation revealed that 1,3-aminoalcohol **134**, containing the 4-fluorobenzyl moiety, was the most effective derivative against cancer cell lines (see in section 3.4.2). Consequently, this compound was chosen for further modifications. Two possibilities were considered: several new moieties (mainly benzyl-type functions) were contemplated to be built on the secondary amino group, or an alternative functional group was considered to be formed on the carboxyl function at position C-19. The second substitution of the amino function was excluded since the NH group was found in our previous study to be essential for cytotoxic activity. Therefore, only an additional modification of the benzylamino function seemed to be promising. In the literature, the carboxyl group of isosteviol was not found to play any role in the cytotoxic activity, making it a good starting point. We aimed to prepare a derivative containing a free carboxyl group by the removal of the methyl ester group. However, in our experiments, this was proven to be unsuccessful, and this led to a change in the entire synthesis route. The use of a free carboxyl group in the synthesis was not feasible because of cross-reactions during the building of the amino functionality, and the resulting products were not purifiable. The benzyl ester functional group was chosen, since it could be easily removed by a debenzylation reaction (giving the possibility to introduce a further ester function). Furthermore, the purification of the resulting intermediates seemed to be easier by column chromatography. The desired key intermediate **155** was produced in six steps with an acceptable yield (Scheme 32).

Isosteviol **41** was obtained through acid-catalysed hydrolysis and the Wagner–Meerwein rearrangement of a natural mixture of stevioside was carried according to a literature process.[84] The

benzyl ester derivative of isosteviol **150** was prepared with benzyl bromide in a good yield. Benzyl ester diol **151** was prepared with a good yield applying a one-pot aldol-Cannizzaro process, a well-known stereoselective method as described in the literature.[85] Compound **152** was prepared in a regioselective manner with an excellent yield by the TBAB-catalysed oxidation of **151** with TEMPO and NBS.[87] The synthesis of *N*-4-fluorobenzyl-substituted benzyl ester **152** was carried out through a two-step synthesis. First, the reductive amination of hydroxyaldehyde **151** with 4-fluorobenzylamine formed a Schiff base, followed by its reduction with $NaBH_4$ under mild conditions. To circumvent the unavoidable *N*-debenzylation of **156** during the catalytic debenzylation of the ester function, the nitrogen was protected with the Boc group. Boc-protected **155** with a free carboxyl function was obtained in an excellent yield during the debenzylation of **154** by hydrogenolysis over Pd/C in a 1:1 mixture of EtOAc/*n*-hexane without the elimination of the 4-fluorobenzyl function.

Removal of the *N*-Boc protecting group under acidic conditions was achieved by TFA treatment, resulting in the corresponding **156** salt, which was dissolved in DCM, neutralised with TEA to yield free base **156** in a pure form (Scheme 32).

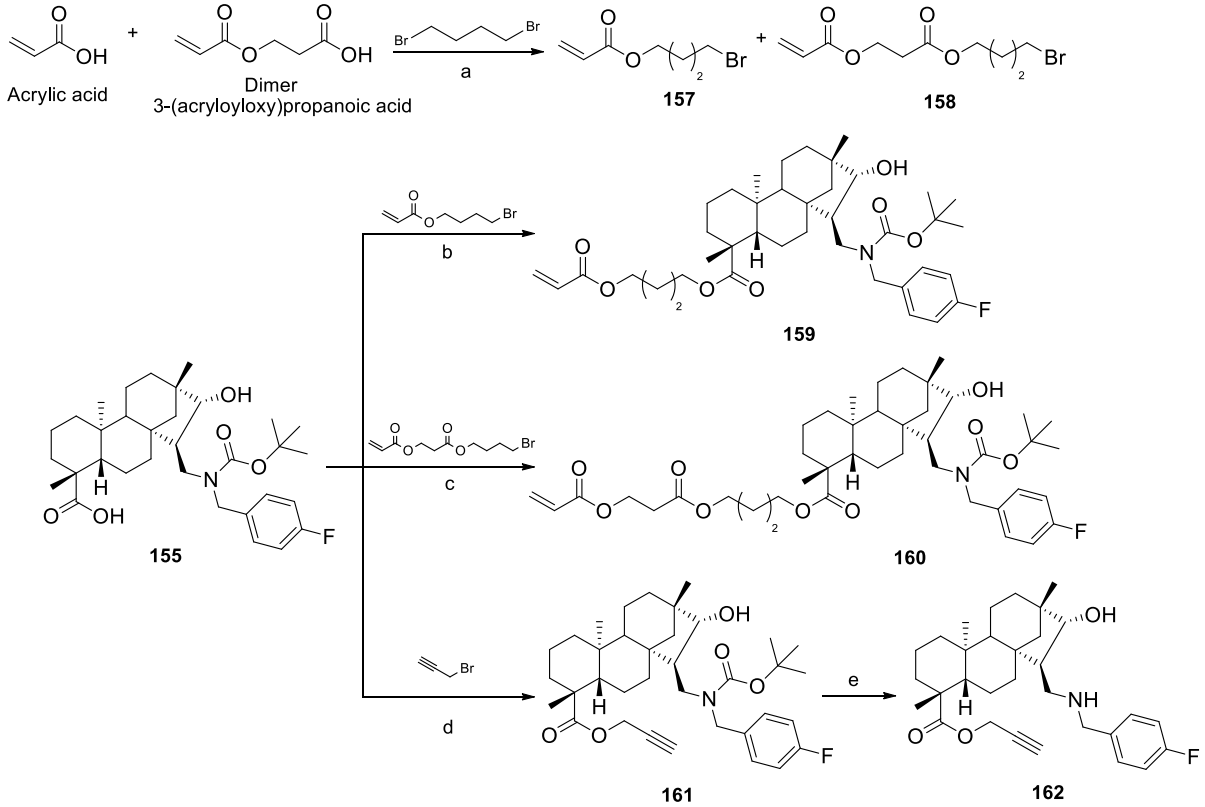


Scheme 32. Synthesis of isosteviol-based 4-fluorobenzyl 1,3-aminoalcohol **134**. (a) BnBr, K_2CO_3, dry acetone, 4 h, 60 °C, 87%; (b) HCHO, NaOEt, dry EtOH, 1 h, 60 °C, 78%; (c) 10 mol% TEMPO, NBS, TBAB, DCM/H_2O, 12 h, reflux, 83%; (d) 1) 4-Fluorobenzylamine, dry EtOH, 3 h, 25 °C; 2) MeOH, $NaBH_4$, 4 h, 25 °C, 70%; (e) Boc_2O, dry DCM, 1 h, 25 °C, 79%; (f) EtOAc/*n*-hexane, 5% Pd/C, H_2 (1 atm), 24 h, 25 °C, 82%; (g) 1) TFA, dry DCM, 3 h, 25 °C; 2) Et_3N, dry DCM, 3 min, 25 °C. 77%.

In the next step of the synthesis, a linker containing an acrylic acid unit was prepared through

a simple esterification reaction between acrylic acid and 1,4-dibromobutane. The use of 1,4-dibromobutane in excess was deemed necessary to suppress diester formation. However, due to the fast dimerisation of acrylic acid and the extended reaction time, both esterified monomer and dimer products were formed and isolated in the esterification reaction (Scheme 33). This provided an opportunity to explore the contribution of carbon chain length to the development of cytotoxic effects. During the synthetic process, acrylic acid esters containing one or two acrylic units were coupled to product **155** via a simple ester formation, resulting in **159** and **160**. *N*-Boc protection was necessary to prevent the *N*-alkylation reaction. Unfortunately, the removal of the Boc groups could not be achieved due to the occurrence of many side reactions. It was assumed that these products were formed in inter- or intramolecular *aza*-Michael reactions between the free NH group and the acryloyloxy function (Scheme 33).

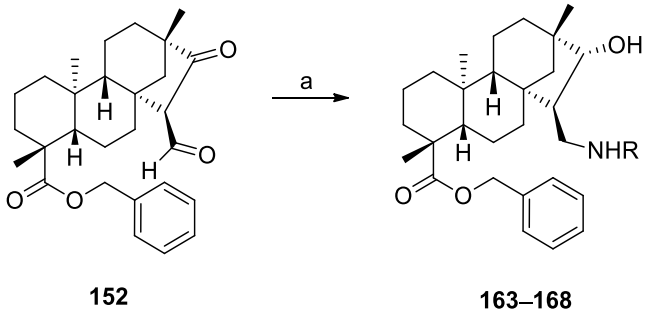
Finally, compound **162** with an alkyne function on C-19 was prepared by reacting **155** with propargyl bromide, followed by the removal of the Boc-protecting group.



Scheme 33. Synthesis of allyl and acetylene derivatives. (a) Acrylic acid, K_2CO_3, 1,4-dibromobutane, dry acetone, 24 h, 25 °C, 61%, 26%; (b) 4-bromobutyl acrylate (**157**), K_2CO_3, dry acetone, 24 h, 25 °C, 83%; (c) 3-(4-bromobutoxy)-3-oxopropyl acrylate (**158**), K_2CO_3, dry acetone, 24 h, 25 °C, 77%; (d) Propargyl bromide, K_2CO_3, dry acetone, 24 h, 25 °C, 88%; (e) 1) TFA, dry DCM, 3 h, 25 °C; 2) Et_3N, dry DCM, 3 min, 25 °C. 79%.

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

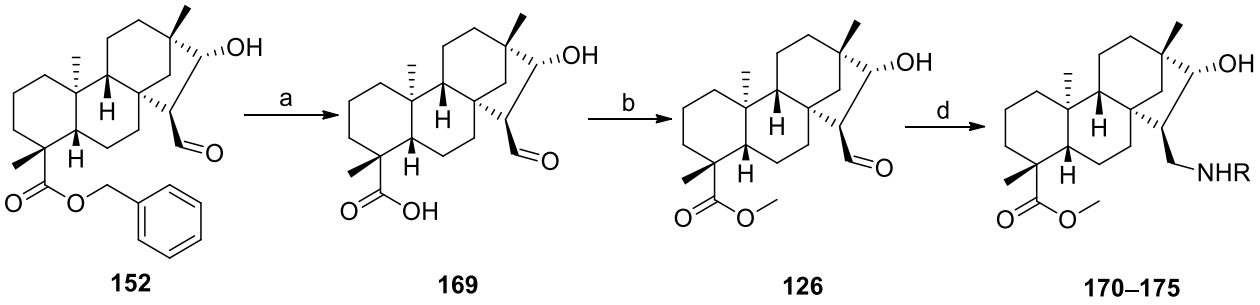
Isosteviol-based 1,3-aminoalcohols were determined to be the most effective products against cancer cell lines among those synthesised to date (see in Chapter 3.4.2). Therefore, the decision was made to expand the library of 1,3-aminoalcohols by reacting hydroxyaldehyde benzyl ester **152** with six different primary amines. The syntheses were conducted in two parts. Initially, hydroxyaldehyde **152** underwent reductive amination with primary amines to form Schiff bases. The second step involved reduction with $NaBH_4$ under mild conditions. The desired *N*-substituted 1,3-aminoalcohols **160**–**165** were obtained in moderate yields. All products were purified by crystallisation, resulting in aminoalcohols with a purity of 98% with low product recovery. Scheme 34 and Table 7 display the synthesised products and the corresponding yields.



152 **163–168**

Scheme 34. Synthesis of isosteviol-based 1,3-aminoalcohols. (a) 1) R^1NH_2, dry EtOH, 3 h, 25 °C; 2) MeOH, $NaBH_4$, 4 h, 25 °C, 7–44%.

To determine the extent to which the development of the cytotoxic effect was correlated with various NH-linked units, a 6-membered 1,3-aminoalcohol library (**170**–**175**) was prepared, starting from benzyl ester **152** as a key intermediate. Acid **169** was derived from **152** through debenzylation over 5% Pd/C in a 1:1 mixture of *n*-hexane/EtOAc, followed by re-esterification with diazomethane to obtain **126** (Scheme 33). The 1,3-aminoalcohol library **170**–**175** was prepared by reacting hydroxyaldehyde **126** with six primary amines, followed by the reduction of the resulting Schiff bases (Scheme 35 and Table 7).



152 **169** **126** **170–175**

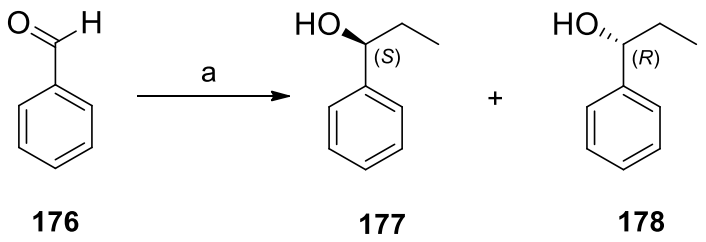
Scheme 35. Synthesis of isosteviol-based 1,3-aminoalcohols. (a) EtOAc/*n*-hexane 1:1, 5% Pd/C, H_2 (1 atm), 24 h, 25 °C, 63%; (b) CH_2N_2, Et_2O, 5 min, 25 °C, 81%; (c) (1) RNH_2, dry EtOH, 3 h, 25 °C; (2) MeOH, $NaBH_4$, 4 h, 25 °C, 38–88%.

Table 7. Library of isosteviol-based 1,3-aminoalcohols.

Entry	R	Product	Yield (%)
1	(*R*)-1-(4-fluorophenyl)ethyl	**163**, **170**	21, 50
2	(*R*)-1-phenylpropyl	**164**, **171**	52, 88
3	(*S*)-1-phenylpropyl	**165**, **172**	68, 48
4	(*S*)-1-(naphthalen-1-yl)ethyl	**166**, **173**	40, 42
5	(*R*)-1-(naphthalen-1-yl)ethyl	**167**, **174**	46, 38
6	3-(1*H*-imidazol-1-yl)propyl	**168**, **175**	33, 58

3.3. Application of steviol-based chiral aminodiols as chiral catalyst in the model reaction

The catalytic effect of aminodiol derivatives **104**–**123** was investigated using them as chiral catalysts in the formation of (*S*)- or (*R*)-1-phenyl-1-propanol **177** and **178** by the enantioselective addition of diethylzinc to benzaldehyde **176** (Scheme 36).



Scheme 36. Model reaction of enantioselective catalysis. (a) Et_2Zn, *n*-hexane, 10 mol% catalyst, 25 °C.

The enantiomeric purity of the major product 1-phenyl-1-propanol enantiomers **177** and **178** was determined using literature methods[116,117] through GC analysis on a Chirasil-DEX CB column. Low to moderate enantioselectivities were observed. It was clearly shown by the obtained results that the formation of the (*R*)-enantiomer was favoured by all aminodiol derivatives, whereas triazol-type compounds had only a weak catalytic effect on the transformation. Table 8 displays a selection of the best results.

Table 8. Addition of diethylzinc to benzaldehyde, catalysed by aminodiol derivatives.

Entry	Ligand[a]	Yield[b] (%)	*ee*[c] (%)	Configuration[d]
1	**104**	78	23	(*R*)
2	**105**	85	52	(*R*)
3	**110**	77	33	(*R*)
4	**111**	85	31	(*R*)
5	**115**	79	30	(*R*)

[a] 10 mol%. [b]After silica column chromatography. [c] Determined on the crude product by GC (Chirasil-DEX CB column). [d] Determined by comparing the t_R of GC analysis and optical rotations with literature data.

The best *ee* value (*ee* = 52%) with an (*R*)-selectivity was achieved by aminodiol **105**, which still showed moderate results. Contrary to our earlier findings, the catalytic activity did not increase by ring closure of **104** and **113** towards oxazolidines **114** and **115**, or by decreasing the temperature (0 °C) or by changing the solvent (from *n*-hexane to toluene). All other compounds were also examined as chiral ligands, but their selectivity was less than 10%.

3.4. Antiproliferative activity of the prepared new compounds

3.4.1. Antiproliferative activity of steviol-based trifunctional chiral derivatives

The antiproliferative activities of the prepared diterpene analogues were determined via an MTT assay on a range of human adherent cancer lines, including cells derived from cervical (HeLa, SiHa), breast (MDA-MB-231, MCF-7) and ovarian (A2780) cancers, as shown in Table 9.

Table 9. Antiproliferative properties of the tested diterpene analogues.

Compound	Calculated IC_{50} (μM)			
	A2780	**HeLa**	**SiHa***	**MDA-MB-231***
104	6.68	9.37	24.68	26.16
105	17.34	23.49	-	-
106	4.19	4.79	6.07	4.32
107	4.91	3.96	6.54	4.39
111	6.25	5.73	7.84	4.76
115	1.07	1.05	1.62	1.25

*Cell growth inhibition values less than 20% were considered negligible and are not given numerically.

No relevant effect was elicited by a few of them, while the activities of others were comparable to those of the reference agent cisplatin (Table 9).[115] Based on these data, conclusions concerning structure–activity relationships could be drawn. It was observed that **103**, **112** and **118** were ineffective, suggesting that the presence of the aromatic ring is a requirement for the cell-growth inhibiting effect of the compounds. Testing the set of aminodiol analogues (**104**–**111**) with secondary or tertiary amino function, it was consistently found that the *N*-benzyl substituent is an essential part of the molecule. When **104** and **105** were compared, it was evident that the secondary amino function was favoured over the tertiary group.

3.4.2. Antiproliferative activity of isosteviol-based 1,3-aminodiol derivatives

The antiproliferative activities of the prepared diterpene analogues were determined on a panel of human adherent cancer cell lines, similar to the previous investigation. Some conclusions could be arrived at with respect to structure–activity relationships based on the obtained activities. It was concluded that the simultaneous presence of the ester function and the basic secondary amino function was essential for the inhibition of cell growth, as no relevant effect on the growth of cancer cells was elicited by diol (**125** and **151**), hydroxyaldehyde analogues (**126** and **152**), *N*-Boc-protected aminoalcohols (**154**, **155**, **159** and **160**), the corresponding oxime (**127**), or even compound **156** with a free carboxylic function. Similar pronounced antiproliferative action was exerted by primary amine **128** as well as secondary amine derivatives **129**–**134**, and the calculated IC_{50} values of these compounds are comparable to or lower than those of cisplatin. It seems to be clear from the results presented in Table 10 that both the aminoalcohol function and the *N*-benzyl-type substitution (**130**–**134**) are essential for the remarkable antiproliferative activity. No consistent and substantial difference was recognised comparing the activity of the benzyl esters (**163**–**168**) and their methyl analogues (**170**–**175**), indicating that the behaviour of the ester function had no crucial impact on the antiproliferative properties. The further change in the 4-fluorobenzyl substituent into other aromatic systems did not increase the activity. Surprisingly, compound **168** substituted with the *N*-(1*H*-imidazol-1-yl)propyl group was proven to be the most active derivative (best IC_{50} value: 1.37 μM for MCF-7 cells), despite our previous observation that an *N*-alkyl substitution reduced the antiproliferative activity.

Table 10. Antiproliferative properties of the tested diterpene analogues.

Compound	Calculated IC_{50} (μM)				
	HeLa	**SiHa**	**MDA-MB-231**	**MCF-7**	**A2780**
128	4.11	4.73	5.25	4.13	6.52
130	5.47	6.43	5.37	7.44	7.96
131	3.09	4.75	7.34	4.36	4.21
132	2.92	4.95	8.28	4.34	4.29
133	2.55	4.37	5.58	2.51	4.04
134	2.75	4.19	4.40	2.14	3.81
168	1.99	-*	1.78	1.37	3.74

*Cell growth inhibition values was not measurement.

4. Summary

In my thesis work, systematic synthesis and the study of new diterpene-based chiral aminoalcohols and aminodiols derivatives derived from natural diterpenes such as steviol and isosteviol were performed.

We undertook the preparation of steviol **4** by utilizing stevioside **99**. Compound **4** was esterified resulting in the formation of steviol methyl ester. Compound **100** was subjected to epoxidation, yielding *cis*-epoxyalcohol **102** and its diastereoisomeric pair, *trans*-epoxyalcohol **103** as a minor component. A wide range of 3-amino-1,2-diols can be effectively synthesised, a process entailing the nucleophilic addition of amines to epoxyalcohols. Treatment of aminodiol **104** with formaldehyde afforded spiro-oxazolidine derivative **115** through a highly regioselective ring-closing reaction. In contrast, primary aminodiol **113** yielded a mixture of spiro-oxazolidine **116** and 1,3-oxazine derivative **117**. *cis*-Epoxyalcohol **102** reacted with sodium azide to give azidodiol **118**. Using "click" reaction with various substituted alkynes, triazoles **119**–**122** were synthesised. Copper-catalysed azide–alkyne cycloaddition can be used, and it produces specifically 1,4-disubstituted regioisomers.

To obtain isosteviol **41**, natural stevioside **99** was subjected to acid-catalysed hydrolysis and rearrangement. The stereoselective synthesis of diol **124** was accomplished in two steps. The esterification of **124** was then carried out and oxidation of the ester led to the regioselective formation of **126**. Subsequently, compound **127** was synthesised by oximation of **126** and compound **127** was then hydrogenated to successfully obtain 1,3-aminoalcohol **128**.

A small library of 1,3-aminoalcohols were synthesised using two pathways: reductive amination of hydroxyaldehyde **126** with primary amines, and reductive alkylation of primary 1,3-aminoalcohol **128** with various aldehydes.

Isosteviol methyl ester **135** was prepared in excellent yield from **41**. The Mannich condensation of **135** was carried out with various secondary amine hydrochloride salts, resulting in a library of aminoketones. The reduction of aminoketones **136**–**140** were produced the corresponding diastereomeric mixture of 1,3-aminoalcohols. Treatment of **128** with phenyl isothiocyanate resulted in the formation of hydroxy-thiourea **147**. We attempted to convert **147** to the target compound, but only hydroxy-thiomethyl ether intermediate **148** was formed. Alternatively, acid-promoted dehydrative cyclisation of **147** yielded hydroxy-thioethyl ether **149** instead of the expected 2-phenylimino- [1,3]-thiazine.

Additionally, benzyl ester diol **151** was synthesised from the benzyl ester derivative **150**.

Compound **152** was obtained through a regioselective oxidation of **151**. The preparation of *N*-(4-fluorobenzyl)-1,3-aminoalcohol benzyl ester **153** involved the reductive amination of hydroxyaldehyde **152**. To prevent any further undesired reactions, nitrogen was protected. Boc-protected **155**, featuring a free carboxyl function, was obtained through the debenzylation of **154**. The removal of the *N*-Boc-protecting group under acidic conditions was achieved.

In subsequent synthesis, a linker incorporating an acrylic acid moiety was synthesised. Within the synthetic pathway, acrylic acid esters, featuring one or two acrylic units, were linked to yield products **159** and **160** through an ester formation reaction with product **155**. Furthermore, compound **162**, containing an alkyne functionality at C-19, was generated by reacting **155** with propargyl bromide, followed by the removal of the Boc-protecting group.

Diversification of the 1,3-aminoalcohols involved the reaction of hydroxyaldehyde benzyl ester **152** with six different primary amines. To determine the influence of different NH-linked units on the development of cytotoxic effects, a library of 1,3-aminoalcohols **170**–**175** were synthesised, substituting the benzyl ester with a methyl ester functional group.

Aminodiols were applied as chiral catalysts in the enantioselective addition of diethylzinc to benzaldehyde. Product **105** proved to be the best catalyst from the library (*ee* = 80%) with (*R*)-selectivity, which still showed moderate results.

The antiproliferative activities of aminoalcohols and aminodiols were explored and the structure–activity relationships were studied. The resulting products (**115**, **168**) exert marked antiproliferative action on a panel of human cancer cell lines. The in vitro pharmacological studies have clearly shown that both the aminoalcohol function and the *N*-benzyl substitution are essential for the remarkable antiproliferative activity.

5. Acknowledgements

I am grateful to **Prof. Dr. Zsolt Szakonyi**, my supervisor and **Prof. Dr. István Szatmári**, head of the Institute of Pharmaceutical Chemistry, for giving me an opportunity to work in the Institute of Pharmaceutical Chemistry as well as for their encouragement and scientific support in my work.

I would like to thank **Prof. Dr. Zupkó István, Dr. Viktória Nagy** and **Dr. Noémi Bózsity** for antiproliferative activity investigations. Furthermore, I am grateful to my colleagues for their advice and the inspiring working atmosphere.

Finally, I would like to give my special thanks to my family and my friends for their love and inexhaustible spiritual support during my Ph.D. years.

6. References

1 McGuire S. World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. *Adv Nutr* 2016; **7**: 418–419.

2 Vera-Badillo FE, Al-Mubarak M, Templeton AJ, Amir E. Benefit and Harms of New Anti-Cancer Drugs. *Curr Oncol Rep* 2013; **15**: 270–275.

3 Chen EH LD. Anticancer Drug Development, a Matter of Money or a Matter of Idea? *J Postgenomics Drug Biomark Dev* 2015; **05**.

4 Wefel JS, Schagen SB. Chemotherapy-Related Cognitive Dysfunction. *Curr Neurol Neurosci Rep* 2012; **12**: 267–75.

5 Newman DJ, Cragg GM. Natural Products as Sources of New Drugs over the Last 25 Years. *J Nat Prod* 2007; **70**: 461–477.

6 Gordaliza M. Natural products as leads to anticancer drugs. *Clin Transl Oncol* 2007; **9**: 767–776.

7 El Alami MSI, El Amrani MA, Agbossou-Niedercorn F, Suisse I, Mortreux A. Chiral Ligands Derived from Monoterpenes: Application in the Synthesis of Optically Pure Secondary Alcohols via Asymmetric Catalysis. *Chem – Eur J* 2015; **21**: 1398–1413.

8 Grajewska A, Rozwadowska MD. Stereoselective synthesis of cytoxazone and its analogues. *Tetrahedron Asymmetry* 2007; **18**: 803–813.

9 Sanches Lopes SM, Francisco MG, Higashi B, et al. Chemical characterization and prebiotic activity of fructo-oligosaccharides from *Stevia rebaudiana* (Bertoni) roots and in vitro adventitious root cultures. *Carbohydr Polym* 2016; **152**: 718–725.

10 Jacobson KA, Tosh DK, Toti KS, Ciancetta A. Polypharmacology of conformationally locked methanocarba nucleosides. *Drug Discov Today* 2017; **22**: 1782–1791.

11 Sadler JM, Mosley SL, Dorgan KM, Zhou ZS, Seley-Radtke KL. Synthetic strategies toward carbocyclic purine–pyrimidine hybrid nucleosides. *Bioorg Med Chem* 2009; **17**: 5520–5525.

12 Wróblewski AE, Głowacka IE, Piotrowska DG. 1′-Homonucleosides and their structural analogues: A review. *Eur J Med Chem* 2016; **118**: 121–142.

13 Kleinert HD, Rosenberg SH, Baker WR, et al. Discovery of a Peptide-Based Renin Inhibitor with Oral Bioavailability and Efficacy. *Science* 1992; **257**: 1940–1943.

14 Chandrasekhar S, Mohapatra S, Yadav JS. Practical synthesis of Abbott aminodiol: A core unit of the potent renin inhibitor Zankiren. *Tetrahedron* 1999; **55**: 4763–4768.

15 Kataev E, Khaybullin RN, Sharipova RR, Strobykina IYu. Ent-kaurane diterpenoids and glycosides: Isolation, properties, and chemical transformations. *Rev J Chem* 2011; **1**: 93–160.

16 Nizamov IS, Al'metkina LA, Gabdullina GT, et al. Chiral phosphorus dithio acids derived from (1S,2S,3S,5R)-(+)-isopinocampheol. Synthesis and fungicidal activity. *Russ Chem Bull* 2012; **61**: 2370–2371.

17 Williams CA, Grayer RJ. Anthocyanins and other flavonoids. *Nat Prod Rep* 2004; **21**: 539.

18 Kawaide H, Sassa T, Kamiya Y. Functional Analysis of the Two Interacting Cyclase Domains in ent-Kaurene Synthase from the FungusPhaeosphaeria sp. L487 and a Comparison with Cyclases from Higher Plants. *J Biol Chem* 2000; **275**: 2276–2280.

19 Schteingart CD, Pomilio AB. Atractyloside, Toxic Compound from Wedelia glauca. *J Nat Prod* 1984; **47**: 1046–1047.

20 Stewart MJ, Steenkamp V. The Biochemistry and Toxicity of Atractyloside: A Review: *Ther Drug Monit* 2000; **22**: 641–649.

21 Turgut M, Alhan CC, Gürgöze M, et al. Carboxyatractyloside poisoning in humans. *Ann Trop Paediatr* 2005; **25**: 125–134.

22 Klingenberg M, Appel M, Oelrichs PB. Wedeloside, a powerful inhibitor and ligand of the mitochondrial ADP/ATP carrier. *FEBS Lett* 1985; **189**: 245–249.

23 Geuns JMC. Stevioside. *Phytochemistry* 2003; **64**: 913–921.

24 Lin Z, Guo Y, Gao Y, et al. *ent* -Kaurane Diterpenoids from Chinese Liverworts and Their Antitumor Activities through Michael Addition as Detected in Situ by a Fluorescence Probe. *J Med Chem* 2015; **58**: 3944–3956.

25 Hann MM, Oprea TI. Pursuing the leadlikeness concept in pharmaceutical research. *Curr Opin Chem Biol* 2004; **8**: 255–263.

26 Ilić V, Vukmirović S, Stilinović N, Čapo I, Arsenović M, Milijašević B. Insight into anti-diabetic effect of low dose of stevioside. *Biomed Pharmacother* 2017; **90**: 216–221.

27 Yadav SK, Guleria P. Steviol Glycosides from Stevia: Biosynthesis Pathway Review and their Application in Foods and Medicine. *Crit Rev Food Sci Nutr* 2012; **52**: 988–998.

28 Chan P, Xu D-Y, Liu J-C, et al. The effect of stevioside on blood pressure and plasma catecholamines in spontaneously hypertensive rats. *Life Sci* 1998; **63**: 1679–1684.

29 Lee C-N, Wong K-L, Liu J-C, Chen Y-J, Cheng J-T, Chan P. Inhibitory Effect of Stevioside on Calcium Influx to Produce Antihypertension. *Planta Med* 2001; **67**: 796–799.

30 Liu J-C, Kao P-K, Chan P, et al. Mechanism of the Antihypertensive Effect of Stevioside in Anesthetized Dogs. *Pharmacology* 2003; **67**: 14–20.

31 Philippaert K, Pironet A, Mesuere M, et al. Steviol glycosides enhance pancreatic beta-cell function and taste sensation by potentiation of TRPM5 channel activity. *Nat Commun* 2017; **8**: 14733.

32 Bhasker S, Madhav H, Chinnamma M. Molecular evidence of insulinomimetic property exhibited by steviol and stevioside in diabetes induced L6 and 3T3L1 cells. *Phytomedicine* 2015; **22**: 1037–1044.

33 Chen T-H, Chen S-C, Chan P, Chu Y-L, Yang H-Y, Cheng J-T. Mechanism of the Hypoglycemic Effect of Stevioside, a Glycoside of *Stevia rebaudiana*. *Planta Med* 2005; **71**: 108–113.

34 Boonkaewwan C, Toskulkao C, Vongsakul M. Anti-Inflammatory and Immunomodulatory Activities of Stevioside and Its Metabolite Steviol on THP-1 Cells. *J Agric Food Chem* 2006; **54**: 785–789.

35 Boonkaewwan C, Ao M, Toskulkao C, Rao MC. Specific Immunomodulatory and Secretory Activities of Stevioside and Steviol in Intestinal Cells. *J Agric Food Chem* 2008; **56**: 3777–3784.

36 Sehar I, Kaul A, Bani S, Pal HC, Saxena AK. Immune up regulatory response of a non-caloric natural sweetener, stevioside. *Chem Biol Interact* 2008; **173**: 115–121.

37 Fengyang L, Yunhe F, Bo L, et al. Stevioside Suppressed Inflammatory Cytokine Secretion by Downregulation of NF-κB and MAPK Signaling Pathways in LPS-Stimulated RAW264.7 Cells. *Inflammation* 2012; **35**: 1669–1675.

38 Bunprajun T, Yimlamai T, Soodvilai S, Muanprasat C, Chatsudthipong V. Stevioside Enhances Satellite Cell Activation by Inhibiting of NF-κB Signaling Pathway in Regenerating Muscle after Cardiotoxin-Induced Injury. *J Agric Food Chem* 2012; **60**: 2844–2851.

39 Takasaki M, Konoshima T, Kozuka M, et al. Cancer preventive agents. Part 8: Chemopreventive effects of stevioside and related compounds. *Bioorg Med Chem* 2009; **17**: 600–605.

40 Paul S, Sengupta S, Bandyopadhyay TK, Bhattacharyya A. Stevioside Induced ROS-Mediated Apoptosis Through Mitochondrial Pathway in Human Breast Cancer Cell Line MCF-7. *Nutr Cancer* 2012; **64**: 1087–1094.

41 Ren H-P, Yin X-Y, Yu H-Y, Xiao H-F. Stevioside induced cytotoxicity in colon cancer cells via

reactive oxygen species and mitogen-activated protein kinase signaling pathways-mediated apoptosis. *Oncol Lett* 2017; **13**: 2337–2343.

42 Jeppesen PB, Gregersen S, Alstrup KK, Hermansen K. Stevioside induces antihyperglycaemic, insulinotropic and glucagonostatic effects in vivo: studies in the diabetic Goto-Kakizaki (GK) rats. *Phytomedicine* 2002; **9**: 9–14.

43 Lailerd N, Saengsirisuwan V, Sloniger JA, Toskulkao C, Henriksen EJ. Effects of stevioside on glucose transport activity in insulin-sensitive and insulin-resistant rat skeletal muscle. *Metabolism* 2004; **53**: 101–107.

44 Prakash I, Markosyan A, Bunders C. Development of Next Generation Stevia Sweetener: Rebaudioside M. *Foods* 2014; **3**: 162–175.

45 Prakash I, DuBois GE, Clos JF, Wilkens KL, Fosdick LE. Development of rebiana, a natural, non-caloric sweetener. *Food Chem Toxicol* 2008; **46**: S75–82.

46 Gupta, E., Purwa, S., Sundaram, S., & Rai, G.K. Nutritional and therapeutic values of *Stevia rebaudiana*: A review. *J. Med. Plants Res.* 2013; **46**: 3343-3353

47 Asakawa Y. Chemosystematics of the Hepaticae. *Phytochemistry* 2004; **65**: 623–669.

48 Asakawa Y. Liverworts-Potential Source of Medicinal Compounds. *Curr Pharm Des* 2008; **14**: 3067–3088.

49 Asakawa Y, Ludwiczuk A, Nagashima F. Phytochemical and biological studies of bryophytes. *Phytochemistry* 2013; **91**: 52–80.

50 Zheng H, Chen Q, Zhang M, et al. Cytotoxic *ent* -Kaurane Diterpenoids from *Salvia cavaleriei*. *J Nat Prod* 2013; **76**: 2253–2262.

51 Zhan R, Li X-N, Du X, et al. Bioactive *ent* -Kaurane Diterpenoids from *Isodon rosthornii*. *J Nat Prod* 2013; **76**: 1267–1277.

52 Ramos-Tovar E, Hernández-Aquino E, Casas-Grajales S, et al. Stevia Prevents Acute and Chronic Liver Injury Induced by Carbon Tetrachloride by Blocking Oxidative Stress through Nrf2 Upregulation. *Oxid Med Cell Longev* 2018; **2018**: 1–12.

53 Khaybullin RN, Strobykina IYu, Dobrynin AB, et al. Synthesis and antituberculosis activity of novel unfolded and macrocyclic derivatives of ent-kaurane steviol. *Bioorg Med Chem Lett* 2012; **22**: 6909–6913.

54 Lin L-H, Lee L-W, Sheu S-Y, Lin P-Y. Study on the Stevioside Analogues of Steviolbioside, Steviol, and Isosteviol 19-Alkyl Amide Dimers: Synthesis and Cytotoxic and Antibacterial Activity. *Chem Pharm Bull (Tokyo)* 2004; **52**: 1117–1122.

55 Korochkina MG, Nikitashina AD, Khaybullin RN, et al. Unfolded and macrocyclic ammonium derivatives of diterpenoids steviol and isosteviol having choline moieties. Synthesis and inhibitory activities toward acetylcholine- and butyrylcholinesterases. *MedChemComm* 2012; **3**: 1449–1454.

56 Li J, Zhang D, Wu X. Synthesis and biological evaluation of novel exo-methylene cyclopentanone tetracyclic diterpenoids as antitumor agents. *Bioorg Med Chem Lett* 2011; **21**: 130–132.

57 Fraga BM, González P, Guillermo R, Hernández MG, Perales A. The chemical and microbiological preparation of 15-oxo-gibberellin derivatives. *Tetrahedron* 1995; **51**: 10053–10064.

58 Chen Z, Chen H, Hu H, et al. Versatile Synthesis Strategy for Carboxylic Acid−functionalized Upconverting Nanophosphors as Biological Labels. *J Am Chem Soc* 2008; **130**: 3023–3029.

59 Lin Z, Guo Y, Gao Y, et al. *ent* -Kaurane Diterpenoids from Chinese Liverworts and Their Antitumor Activities through Michael Addition as Detected in Situ by a Fluorescence Probe. *J Med Chem* 2015; **58**: 3944–3956.

60 Zou M, Yu S-S, Wang K, Zhang D-Y, Wu X-M, Hua W-Y. Glycosylation of ent-kaurene derivatives and an evaluation of their cytotoxic activities. *Chin J Nat Med* 2013; **11**: 289–295.

61 DuBois GE, Stephenson RA. Diterpenoid sweeteners. Synthesis and sensory evaluation of stevioside analogs with improved organoleptic properties. *J Med Chem* 1985; **28**: 93–98.

62 Huang M, Lu J-J, Huang M-Q, Bao J-L, Chen X-P, Wang Y-T. Terpenoids: natural products for cancer therapy. *Expert Opin Investig Drugs* 2012; **21**: 1801–1818.

63 Bedard PL, Di Leo A, Piccart-Gebhart MJ. Taxanes: optimizing adjuvant chemotherapy for early-stage breast cancer. *Nat Rev Clin Oncol* 2010; **7**: 22–36.

64 Tian W, Chen S. Recent advances in the molecular basis of anti-neoplastic mechanisms of oridonin. *Chin J Integr Med* 2013; **19**: 315–320.

65 Li C, Wang E, Cheng Y, Bao J. Oridonin: An active diterpenoid targeting cell cycle arrest, apoptotic and autophagic pathways for cancer therapeutics. *Int J Biochem Cell Biol* 2011; **43**: 701–704.

66 Sun H-D, Lin Z-W, Niu F-D, et al. Diterpenoids from Isodon eriocalyx var. laxiflora. *Phytochemistry* 1995; **38**: 1451–1455.

67 Ding Y, Ding C, Ye N, et al. Discovery and development of natural product oridonin-inspired anticancer agents. *Eur J Med Chem* 2016; **122**: 102–117.

68 Lin S-J, Su T-C, Chu C-N, et al. Synthesis of C-4-Substituted Steviol Derivatives and Their Inhibitory Effects against Hepatitis B Virus. *J Nat Prod* 2016; **79**: 3057–3064.

69 Milagre HMS, Martins LR, Takahashi JA. Novel agents for enzymatic and fungal hydrolysis of stevioside. *Braz J Microbiol Publ Braz Soc Microbiol* 2009; **40**: 367–372.

70 Meerwein H. Über den Reaktionsmechanismus der Umwandlung von Borneol in Camphen; [Dritte Mitteilung über Pinakolinumlagerungen.]. *Justus Liebigs Ann Chem* 1914; **405**: 129–175.

71 Mosettig Erich, Beglinger Urs, Dolder Fred, Lichti Heinz, Quitt Peter, Waters JA. The Absolute Configuration of Steviol and Isosteviol. *J Am Chem Soc* 1963; **85**: 2305–2309.

72 Lee C-N, Wong K-L, Liu J-C, Chen Y-J, Cheng J-T, Chan P. Inhibitory Effect of Stevioside on Calcium Influx to Produce Antihypertension. *Planta Med* 2001; **67**: 796–799.

73 Singh N, Jaggi A, Sharma D, Puri M, Tiwary A. Antiamnesic effect of stevioside in scopolamine-treated rats. *Indian J Pharmacol* 2010; **42**: 164.

74 Holvoet P, Rull A, García-Heredia A, et al. Stevia-derived compounds attenuate the toxic effects of ectopic lipid accumulation in the liver of obese mice: A transcriptomic and metabolomic study. *Food Chem Toxicol* 2015; **77**: 22–33.

75 Zeng Y, Wu J, Shi L, et al. Synthesis and evaluation of cytotoxic effects of novel α-methylenelactone tetracyclic diterpenoids. *Bioorg Med Chem Lett* 2012; **22**: 1922–1925.

76 Khaybullin R, Zhang M, Fu J, et al. Design and Synthesis of Isosteviol Triazole Conjugates for Cancer Therapy. *Molecules* 2014; **19**: 18676–18689.

77 Liu Y, Wang T, Ling Y, et al. Design, synthesis and cytotoxic evaluation of nitric oxide-releasing derivatives of isosteviol. *Chem Biol Drug Des* 2017; **90**: 473–477.

78 Pervin S, Singh R, Chaudhuri G. Nitric oxide, Nω-hydroxy-l-arginine and breast cancer. *Nitric Oxide* 2008; **19**: 103–106.

79 Fukumura D, Kashiwagi S, Jain RK. The role of nitric oxide in tumour progression. *Nat Rev Cancer* 2006; **6**: 521–534.

80 Wu X, Tang X, Xian M, Wang PG. Glycosylated diazeniumdiolates: a novel class of enzyme-activated nitric oxide donors. *Tetrahedron Lett* 2001; **42**: 3779–3782.

81 Wang T, Liu Y, Chen L. Synthesis and cytotoxic activity of nitric oxide-releasing isosteviol derivatives. *Bioorg Med Chem Lett* 2014; **24**: 2202–2205.

82 Saeed S, Rashid N, Jones PG, Ali M, Hussain R. Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents. *Eur J Med Chem* 2010; **45**: 1323–1331.

83 Liu W, Zhou J, Zhang T, et al. Design and synthesis of thiourea derivatives containing a benzo[5,6]cyclohepta[1,2-b]pyridine moiety as potential antitumor and anti-inflammatory agents. *Bioorg Med Chem Lett* 2012; **22**: 2701–2704.

84 Liu C-J, Yu S-L, Liu Y-P, et al. Synthesis, cytotoxic activity evaluation and HQSAR study of novel isosteviol derivatives as potential anticancer agents. *Eur J Med Chem* 2016; **115**: 26–40.

85 Wu Y, Yang J-H, Dai G-F, et al. Stereoselective synthesis of bioactive isosteviol derivatives as α-glucosidase inhibitors. *Bioorg Med Chem* 2009; **17**: 1464–1473.

86 Zhang G-S, Shi Q-Z, Chen M-F, Cai K. Ammonium Chlorochromate Adsorbed on Silica Gel: A New Reagent for the Oxidation of Alcohols and Benzoins to Corresponding Carbonyl Compounds. *Synth Commun* 1997; **27**: 3691–3696.

87 Zhang T, Lu L-H, Liu H, et al. D-ring modified novel isosteviol derivatives: Design, synthesis and cytotoxic activity evaluation. *Bioorg Med Chem Lett* 2012; **22**: 5827–58232.

88 Wu Y, Liu C, Liu X, Dai G, Du J, Tao J. Stereoselective Synthesis, Characterization, and Antibacterial Activities of Novel Isosteviol Derivatives with *D* -Ring Modification. *Helv Chim Acta* 2010; **93**: 2052–2069.

89 Cossy J, ed. *Category 4, Compounds with Two Carbon Heteroatom Bonds: Ketones*. (1st edn). Stuttgart: Georg Thieme Verlag, 2005.

90 Cong-Jun Liu, Wang Y-F, Yao J-H, Liu Y-P, Jiang Q-J, Liu P-P. Cytotoxic Activities and QSAR Studies of Diterpenoid Isosteviol Derivatives as Anti-Esophageal Agents. *Russ J Bioorganic Chem* 2021; **47**: 288–298.

91 Liu C, Zhang T, Yu S, Dai X, Wu Y, Tao J. Synthesis, cytotoxic activity, and 2D- and 3D- QSAR studies of 19-carboxyl-modified novel isosteviol derivatives as potential anticancer agents. *Chem Biol Drug Des* 2017; **89**: 870–887.

92 Korochkina MG, Babaev VM, Strobykina IYu, Voloshina AD, Kulik NV, Kataev VE. Synthesis and antimicrobial activity of several bis-quaternized ammonium derivatives of the diterpenoid isosteviol. *Chem Nat Compd* 2012; **47**: 914–917.

93 Kataev VE, Strobykina IYu, Zakharova LYa. Quaternary ammonium derivatives of natural terpenoids. Synthesis and properties. *Russ Chem Bull* 2014; **63**: 1884–900.

94 Pott A, Rottbauer W, Just S. Streamlining drug discovery assays for cardiovascular disease using zebrafish. *Expert Opin Drug Discov* 2020; **15**: 27–37.

95 Asnani A, Peterson RT. The zebrafish as a tool to identify novel therapies for human cardiovascular disease. *Dis Model Mech* 2014; **7**: 763–767.

96 MacRae CA, Peterson RT. Zebrafish as tools for drug discovery. *Nat Rev Drug Discov* 2015; **14**: 721–731.

97 Zhang H, Liu B, Xu G, et al. Synthesis and in vivo screening of isosteviol derivatives as new cardioprotective agents. *Eur J Med Chem* 2021; **219**: 113396.

98 Ukiya M, Sawada S, Kikuchi T, Kushi Y, Fukatsu M, Akihisa T. Cytotoxic and Apoptosis-Inducing Activities of Steviol and Isosteviol Derivatives against Human Cancer Cell Lines. *Chem Biodivers* 2013; **10**: 177–188.

99 Shi L-Y, Wu J-Q, Zhang D-Y, Wu Y-C, Hua W-Y, Wu X-M. Efficient Synthesis of Novel

Jolkinolides and Related Derivatives Starting from Stevioside. *Synthesis* 2011; **2011**: 3807–3814.

100 Avent AG, Hanson JR, Hitchcock PB, De Oliveira BH. The influence of a 15-hydroxy group on the rearrangement reactions of steviol and its 16,17-epoxide. *J Chem Soc Perkin 1* 1990; 2661.

101 Mori K, Nakahara Y, Matsui M. Diterpenoid total synthesis—XIX. *Tetrahedron* 1972; **28**: 3217–3226.

102 Aggarwal VK, Yu Fang G. Highly regioselective and diastereoselective epoxidation of allylic amines with Oxone. *Chem Commun* 2005; 3448.

103 Szakonyi Z, Csillag K, Fülöp F. Stereoselective synthesis of carane-based aminodiols as chiral ligands for the catalytic addition of diethylzinc to aldehydes. *Tetrahedron Asymmetry* 2011; **22**: 1021–1027.

104 Le TM, Csámpai A, Fülöp F, Szakonyi Z. Regio- and Stereoselective Synthesis of Bicyclic Limonene-Based Chiral Aminodiols and Spirooxazolidines. *Chem – Eur J* 2018; **24**: 13607–13615.

105 Le TM, Szilasi T, Volford B, Szekeres A, Fülöp F, Szakonyi Z. Stereoselective Synthesis and Investigation of Isopulegol-Based Chiral Ligands. *Int J Mol Sci* 2019; **20**: 4050.

106 Khaibullin RN, Strobykina IYu, Kataev VE, et al. Wagner-Meerwein rearrangement of steviol 16α,17- and 15α,16-epoxides. *Russ J Org Chem* 2010; **46**: 1006–1012.

107 Kiss L, Forró E, Sillanpää R, Fülöp F. Diastereo- and Enantioselective Synthesis of Orthogonally Protected 2,4-Diaminocyclopentanecarboxylates: A Flip from β-Amino- to *β,γ*-Diaminocarboxylates. *J Org Chem* 2007; **72**: 8786–8790.

108 Szakonyi Z, Fülöp F. Carbocyclic nucleosides from enantiomeric, α-pinane-based aminodiols. *Tetrahedron Asymmetry* 2010; **21**: 831–836.

109 Hu X, Zhou Z, Zhang Z, et al. Reaction coupling separation for isosteviol production from stevioside catalyzed by acidic ion-exchange resin. *Bioprocess Biosyst Eng* 2021; **44**: 151–159.

110 Coates RM, Kang HY. Synthesis and evaluation of cyclobutylcarbinyl derivatives as potential intermediates in diterpene biosynthesis. *J Org Chem* 1987; **52**: 2065–2074.

111 Szakonyi Z, Zupkó I, Sillanpää R, Fülöp F. Stereoselective Synthesis and Cytoselective Toxicity of Monoterpene-Fused 2-Imino-1,3-thiazines. *Molecules* 2014; **19**: 15918–15937.

112 Shah BA, Kumar A, Gupta P, et al. Cytotoxic and apoptotic activities of novel amino analogues of boswellic acids. *Bioorg Med Chem Lett* 2007; **17**: 6411–6416.

113 Szakonyi Z, Zupko I, Fulop F. Stereoselective Synthesis and Antiproliferative Activity of Monoterpene-Fused 2- Imino-1,3-oxazines. *Curr Org Synth* 2017; **14**: 612–619.

114 Bernáth G, Szakonyi Z, Fülöp F, Sohá P. Synthesis of tert-Butylcyclopentane-fused 1,3-Oxazines and 1,3-Thiazines. *HETEROCYCLES* 1994; **37**: 1687.

115 Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; **65**: 55–63.

116 Tanaka T, Yasuda Y, Hayashi M. New Chiral Schiff Base as a Tridentate Ligand for Catalytic Enantioselective Addition of Diethylzinc to Aldehydes. *J Org Chem* 2006; **71**: 7091–7093.

117 Jimeno C, Pastó M, Riera A, Pericàs MA. Modular Amino Alcohol Ligands Containing Bulky Alkyl Groups as Chiral Controllers for Et_2Zn Addition to Aldehydes: Illustration of a Design Principle. *J Org Chem* 2003; **68**: 3130–3138.