UNIVERZITA PALACKÉHO V OLOMOUCI

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Laboratoř růstových regulátorů

Novel approach to sugar conjugates of gibberellin A₃ and A₄

BAKALÁŘSKÁ PRÁCE

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Abstrakt	Bakalářská práce se zabývá rostlinnými hormony, gibereliny. Teoretická část se zaměřuje na popis jejich vlastností a zejména pak popis znalostí ohledně giberelinových cukerných derivátů. Praktická část se pak zaměřuje na vývoj nových syntetických postupů vhodných pro přípravu C3 cukerných derivátů giberelinů A_3 a A_4 a zabývá se jejich charakterizací a popisem jejich fyzikálně-chemických vlastností.
Klíčová slova	gibereliny; cukerné deriváty giberelinů; rostlinné růstové regulátory; biologická aktivita; organická syntéza
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Abstract	The bachelor thesis deals with plant hormones, gibberellins. The theoretical part focuses on the description of their properties and special focus is paid to the description of current knowledge about gibberellin sugar derivatives. The practical part then focuses on the development of new synthetic procedures suitable for the preparation of C3 sugar derivatives of gibberellins A_3 and A_4, deals with their characterization and description of their physico-chemical properties.

Keywords	gibberellins; gibberellin sugar conjugates, plant growth regulators; biological activity; organic synthesis
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„Prohlašuji, že jsem předloženou bakalářskou práci vypracovala samostatně za použití citované literatury.“

V Olomouci dne 20.05.2022   *Anna Chesnokova*

Acknowledgment

Contents

List of abbreviations

Ac_2O – acetic anhydride

DMAP - 4-(dimethylamino)pyridine 98 %

Py – pyridine

DMB - 2,4-dimethoxybenzyl alcohol

EDC*Cl - N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride

DCM - dichloromethane

MeOH – methanol

MECN – acetonitrile

TBAI - tetrabutylammonium iodide

TBSCl - *tert*-butyldimethylsilyl chloride

NaOMe - sodium methoxide

DMF - N,N-dimethylformamide

DCC - N'N-dicyclohexylcarbodiimide

DIPEA - N,N-diisopropylethylamine

EA - ethyl acetate

PE - petroleum spirit

$CDCl_3$ – deuterochloroform

GA_3 – gibberellic acid

GA_4 – gibberellin A_4

GA_7 – gibberellin A_7

GAs - gibberellins

Ppm – parts per million

RT – room temperature

NMR – nuclear magnetic resonance

TLC – thin-layer chromatography

δ - chemical shift

s - singlet

d – duplet

m – multiplet

q – quadruplet

dd – doublet of doublets

HPLC - high performance liquid chromatography

CKs - cytokinins

ABA - abscisic acid

GGDP - trans-geranylgeranyl diphosphate

CPS - *ent*-copalyl diphosphate synthase

KS - *ent*-kaurene synthase

MEP - methylerythritol phosphate

ent-CDP - *ent*-copalyl diphosphate

KO - *ent*-kaurene oxidase

KAO - *ent*-kaurenoic acid oxidase

GID1 - Gibberellin Insensitive Dwarf1

TPR - corepressor topless related

1. Introduction

Gibberellins (GAs) are plant hormones that regulate different developmental processes in plants such as stem elongation, flowering, seed dormancy, germination and senescence inhibiting. The history of gibberellin discovery starts in Japan back in 1926. Back in time when, unenticingly, first gibberellin, later on named as gibberellin A_3, was isolated. At that time Japanese scientists noticed that the rice disease, that causes hyper elongation of rice stems and leaves, can be artificially caused by special extract of ill rice plants. Only much later it was understood that this disease was caused by fungi *Gibberella fujikuroi*, which produced a gibberellic acid (GA_3). In time it was also discovered that gibberellin biosynthesis occurs not only in fungi, but also in bacteria and plants[1] and that fungi and bacteria produce GAs to stimulate the growth of the host plant and to promote bacterial infection. The plants, on the other way, use GAs to regulate the DELLA protein presence via interaction with specific GID1 receptor.

In our days gibberellins are widely used in agriculture with various application mechanisms. The most common method is to spray them on plants during or shortly after blooming. GAs are mainly used for producing seedless bigger grapes with nice form and also for delaying maturing of citruses. Also the mixtures of gibberellins are used in (mostly GA_4/GA_7 mixture) to improve apple shape by increasing the length of the fruit relative to the diameter.

At this moment 137 endogenic gibberellins are isolated. Problem is that almost all of them are not active, which directs scientists to prepare various modifications and test the GA activity. The bioactive GAs are GA_1, GA_4, GA_7 and GA_3. Only GA_1 is not commercially available, that's why almost all experiments have been done with GA_3, GA_4, GA_7, which are produced by fermentation of microorganisms[2]. From additional bioactivity view point, some of GAs interesting biological activity against various cells and organisms. However, the point of my bachelor work is to tackle another important point in gibberellin research. In my work I would like to address the question of the GA transport and storage in plants. The field that is mostly abandoned and left alone. To start with the goal, first we need to prepare the sugar conjugates of GAs that are believed to be a "storage" molecules of GAs in plants.

2. Goals of the thesis

The goals of my theses are:

1) to carry out literature search that would focus on plant hormones gibberellins and on their sugar conjugates.
2) To develop the synthetic method towards the synthesis of C3-hydroxy sugar conjugates of gibberellin A_4 and A_3.
3) To characterize all prepared compounds with help of available physical-organic methods.

3. Theoretical part

3.1 Plant hormones

Plant hormones are a group of naturally occurring, organic substances which exercise their biological function at very low concentrations. They are responsible for growth, differentiation and development, though other processes, such as stomatal movement, may also be affected. Plant hormones have also been referred to as 'phytohormones' though this term is less often used[1]. But not every chemical structure can be considered as plant hormone, it must execute several characteristics. Their universal characteristics are that they are small organic molecules, are natural constituents in plants, affect physiological processes at concentrations far below those where either nutrients or vitamins would affect these processes and have a specific receptor. It should be noted that different hormones are responsible for different plant functions, here are the main ones: differentiation and elongation of cells; formation of leaves, flowers, and stems; wilting of leaves; ripening of fruit; seed dormancy (Table 1)[1].

Gibberellins (GAs) is a class of diterpenoid phytohormones that are produced by plants and some fungus. They play an important role in modulating diverse processes throughout plant growth and development. Bioactive GAs promote plant growth and development by promoting the degradation of the DELLA proteins, a family of nuclear growth repressors[2]. The functions are described in more detail in Table 1.

Table 1. List of and functions of main plant hormones[1]

Name of hormone	Function
Auxin	- Cell enlargement - Cell division - Vascular tissue differentiation - Root initiation - Tropistic responses - Apical dominance - Leaf senescence - Leaf and fruit abscission - Fruit setting and growth - Assimilate partitioning - Fruit ripening - Flowering - Growth of flower parts
Gibberellins (GAs)	- Stem growth - Induction of seed germination - Enzyme production during germination - Fruit setting and growth - Induction of maleness in dioecious flowers.
Cytokinins (CKs)	Cell division

	• Morphogenesis - in tissue culture and crown gall CKs promote shoot initiation. In moss, CKs induce bud formation. • Growth of lateral buds • Leaf expansion • CKs delay leaf senescence. • CKs may enhance stomatal opening in some species. • Chloroplast development
Ethylene	• Release from dormancy. • Shoot and root growth and differentiation. • Adventitious root formation. • Leaf and fruit abscission. • Flower induction in some plants. • Induction of femaleness in dioecious flowers. • Flower opening. • Flower and leaf senescence. • Fruit ripening.
Abscisic acid (ABA)	• Stomatal closure-water shortage brings about an increase in ABA which leads to stomatal closure. • ABA induces transport of photosynthate towards developing seeds, and its subsequent uptake by growing embryos. • ABA induces storage protein synthesis in seeds. • ABA counteracts the effect of gibberellin on £-amylase synthesis in germinating cereal grains. • ABA affect the induction and maintenance of dormancy in seeds and buds.
Polyamines	• Regulate growth and development at micromolar concentrations. • Involved in modulating senescence of organs. • Increase the activity of various antioxidant enzymes in plants.

3.2 Gibberellins

3.2.1 History and distribution

Gibberellin research has its origins in Japan in the 19th century when a disease of "silly" rice was first observed[3]. The symptoms of the disease, including overgrowth of the seedling and sterility, were later shown to be due to secretions of the fungus *Gibberella fujikuroi* (now reclassified as *Fusarium fujikuroi*), from which the name gibberellin was derived for the active component. After World War 2, when the scientific information gathered in Japan become available to US and

British scientists, the importance of those compounds to plant growth and development become apparent, the active research programs in gibberellin field were initiated (early 1950s). US and UK approach hand in hand with original Japanese data resulted in the isolation and structural determination of the main active compound from the fungus. It was first independently named gibberellic acid (UK), gibberellin-X (USA) and gibberellin A_3 (GA$_3$, Japan) to later on finished up to be known as gibberellin A_3 (GA$_3$) worldwide.

Gibberellins are usually located in vascular plants, but are seldomly present in primitive plants. The moss *Physcomitrella patens* produces the GA precursor *ent*-kaurenoic acid, which is a biologically active in it's organism[4]. The most common GA in such plants is GA$_1$, although its 13-deoxy analog, GA$_4$ is usually also present, however at lower concentrations in vegetative tissues. In some species, such as *Arabidopsis thaliana* and members of the *Cucurbitaceae*, GA$_4$ is the predominant form and indeed has more growth stimulating activity than GA$_1$.[4] The fungal product GA$_3$ is often reported to be a minor component of some plant species, but the clear evidence that GA$_3$ is produced by plants is still missing. It should be mentioned that gibberellins do not have a physiological function in fungi, but are used to enter the host plant and are able to cheat upon its immunity. Several rhizobacterial species also produce GAs; these include endophytes, such as some nitrogenfixing *Rhizobia* species as well as certain *Azospirillum* species[4]. The GAs are there to stimulate the growth of the host plant, but on the other hand they do promote bacterial infection. The bioactive GAs are always accompanied by their biosynthetic precursors and catabolites, which are present in higher concentrations than the active forms of GA (GA$_1$. GA$_3$, GA$_4$ and GA$_7$, Figure 1). Bioactive gibberellins are normally present at lower concentrations than in shoots.
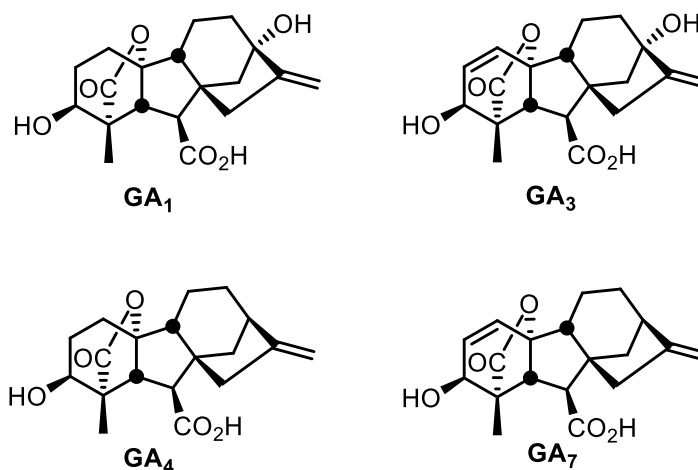

Figure 1. Biologically active gibberellins

3.2.2 Gibberellin's diversity and structure

Gibberellins, commonly abbreviated GA, are member of a group of naturally occurring tetracyclic diterpenoid carboxylic acids, based on the *ent*-gibberellane (C20) or *ent*-20-norgibberellane (C19) carbon skeleton[4] (Figure 2).
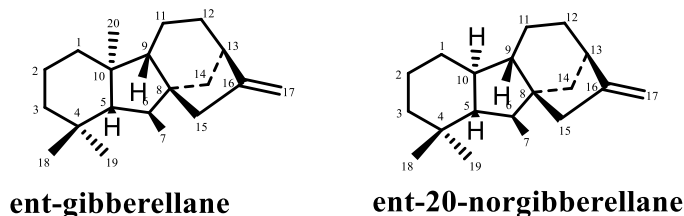

ent-gibberellane   **ent-20-norgibberellane**

Figure 2. Structures of ent-gibberellane (C20) or ent-20-norgibberellane (C19) carbon skeletons

As each individual endogenic gibberellin that was isolated, has been assigned a number. Thus, gibberellic acid is gibberellin A_3 (GA_3). GA_3 is the first isolated gibberellin from the fungi, which is not in plants. The gibberellins that have been isolated fall into two classes: those that retain the full diterpenoid compliment of 20 carbon atoms, such as GA_{13}, and those like gibberellic acid (GA_3) that are C19 compounds and have lost C-20 carbon atom[5]. Currently 137 different endogenic GAs have been identified from higher plants, fungi, or bacteria. Only four of them (Figure 1) are biologically active in higher plants where they regulate the plant growth and development, and mediate developmental and environmental signals. Their function includes promotion of organ growth through stimulation of cell elongation and/or division, and activation of developmental switches, such as between seed dormancy and germination, juvenile and adult growth phases, and vegetative and reproductive development. GAs with phytohormonal activity possess a C19 skeleton, with a C-19–C-10 lactone, a 7-carboxylic acid group and, for stimulation of vegetative growth, a 3b-hydroxy function, as exemplified by two of the most common endogenous GA growth regulators, GA_1 and GA_4. Except GA_1, GA_4 exist GA_3 and GA_7 bioactive GAs (Figure 1).

Due to a low concentration of GAs in plants, structural characterization of novel GAs required the isolation of large quantities of pure material, and the structure determination was based on the chemical degradation of the original compound. As more chemically characterized GAs and related compounds became available, it was often possible to use conversion to known compounds in relatively few steps to confirm novel structures[3]. In our days, the use of nuclear magnetic resonance reduced the required amounts of material to mg, which allows us to determine the structure of isolated molecules faster and also with more precision.[6]

The most abundant gibberellin present in *F. fujikuroi* and the first structurally characterized gibberellin, was gibberellin A_3, also known as gibberellic acid[7]. This gibberellin is nowadays produced on an industrial scale in fungal cultures for application in agriculture, with the largest use in the production of seedless grapes. However, although it is believed that GA_3 is present in some higher plant species as a minor gibberellin, there is little evidence that GA_3 itself plays an important role in plants. It is more likely a compound that mimic the real biologically active gibberellin GA_1 taking in account their structural similarity. GA_3 possess a double bond between C1 and C2 (Figure 2) otherwise is the same. C1-C2 olefin presumably serves as GA_3-desactivater protector since it blocks possible 2β-hydroxylation pathway, a major mechanism for inactivating GAs in higher plants. An inability to regulate the concentration of GA_3. The production of GA_3 by the phytopathogenic *F. fujikuroi* is presumably giving the special benefit to fungus in compromising the plant host's ability to protect itself from a high GA dosage.

Plant growth effects could also be obtained with plant extracts, providing a strong indication that gibberellins were endogenous plant metabolites. This was confirmed by the isolation of gibberellin A_1 (GA_1) from immature seeds of runner bean, *Phaseolus coccineus*, in 1958[7]. Accumulation of GA_1 in the cytosol would disrupt the membrane pH gradient and stressed the importance of metabolism to more polar metabolites that could be stored in the vacuole.

From among the exogenous gibberellins so far used for induction of flowering, the best results have been obtained with $GA_{4/7}$.[8] Spraying plants with such gibberellin mixture has increased the number of male and/or female flowers[9].

3.2.3 Biosynthesis pathway of gibberellins

Gibberellins are formed from *trans*-geranylgeranyl diphosphate (GGDP) via the diterpene hydrocarbon *ent*-kaurene, which is converted to GAs by a series of oxidative reactions (Figure 3)[4]. Formation of GGPP occurs in plastids, predominantly via the methylerythritol phosphate pathway. It is converted within the plastid to *ent*-kaurene in two steps via *ent*-copalyl diphosphate, requiring the action of two enzymes, *ent*-copalyl diphosphate synthase (CPS) and *ent*-kaurene synthase (KS)[4]. The most common methylerythritol phosphate (MEP) pathway in higher plants we can split into eight steps[2]:
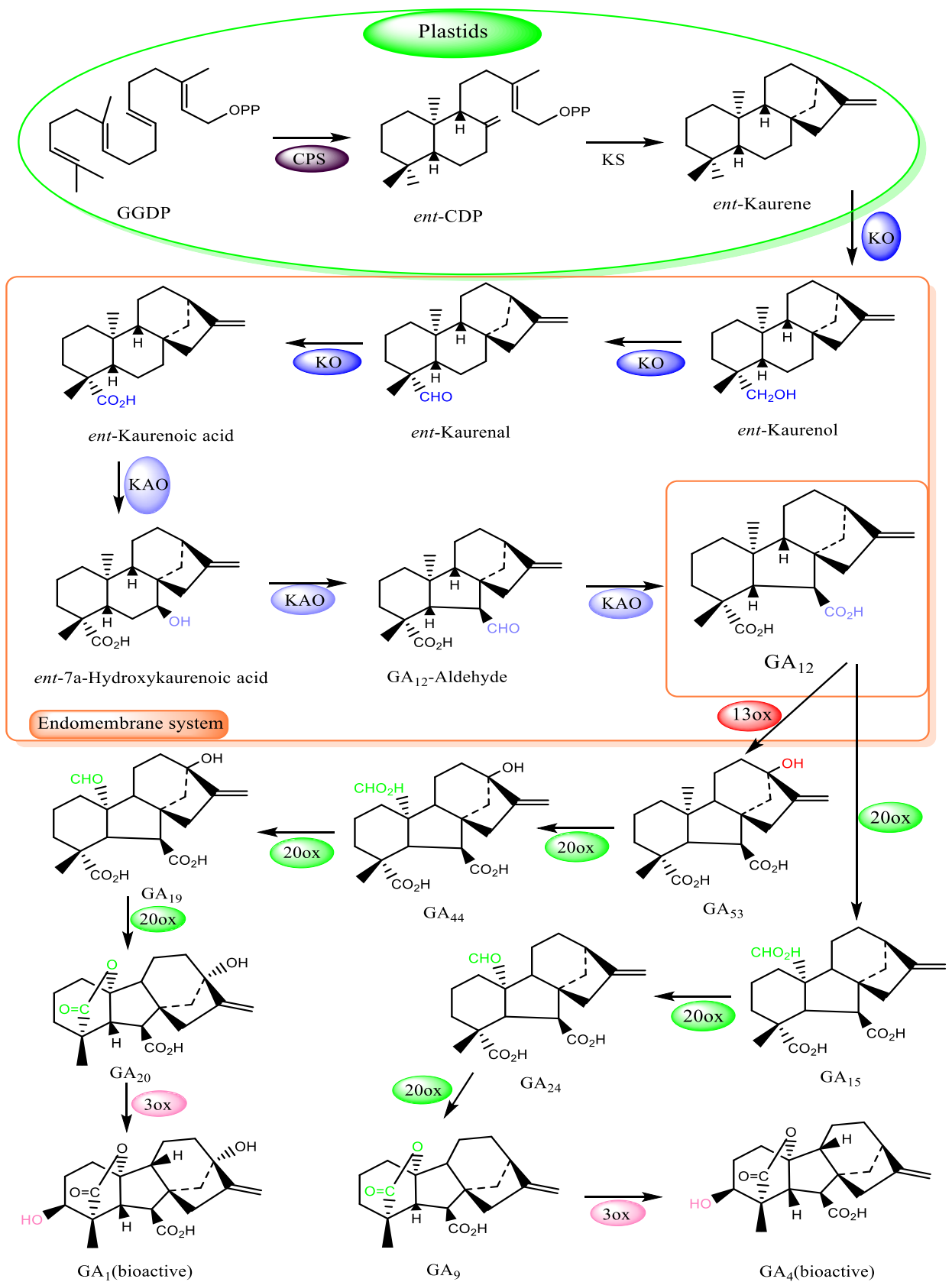
Figure 3. Biosynthesis pathway of gibberellins with intermediates

1) GGDP is converted to *ent*-copalyl diphosphate (*ent*-CDP) by *ent*-copalyl diphosphate synthase.

   The initial step is the conversion of GGDP by using a common C20 precursor for diterpenoids into *ent*-kaurene by *ent*-copalyl diphosphate (*ent*-CDP) synthase (CPS) in the plastids.

2) *ent*-CDP is converted to *ent*-kaurene by *ent*-kaurene synthase (KS)

   Second step occurs by *ent*-kaurene synthase in the plastids like initial step. Loss-of-function mutations of CPS or KS in *Arabidopsis* and rice cause severe GA-deficient dwarf phenotypes, whereas overexpression of both CPS and KS greatly increases levels of the early intermediates *ent*-kaurene and *ent*-kaurenoic acid, with only slight increases in later metabolites. As a result, these overexpression lines have wild-type levels of bioactive GAs and do not exhibit any GA overdose phenotypes[8]. Therefore, plants have developed the ability to maintain GA homeostasis despite the accumulation of early intermediates[2].

3) *ent*-kaurene is converted to *ent*-kaurenol by *ent*-kaurene oxidase (KO)

   In the third step of the GA biosynthesis pathway, the conversion of *ent*-kaurene to GA_{12} via stepwise oxidation is catalyzed by *ent*-kaurene oxidase (KO), steps 3,4, and *ent*-kaurenoic acid oxidase (KAO), step 5. The single AtKO protein is a multi-functional cytochrome P450 that catalyzes the sequential oxidation of *ent*-kaurene from $-CH_3 \rightarrow -CH_2OH \rightarrow -CHO \rightarrow -COOH$ on C-19 to produce the intermediates *ent*-kaurenol and *ent*-kaurenal and the end product *ent*-kaurenoic acid through three steps of reactions[10].

4) *ent*-kaurenol is converted to *ent*-kaurenal by KO
5) *ent*-kaurenal is converted to *ent*-kaurenoic acid by KAO
6) *ent*-kaurenoic acid is converted to *ent*-7a-hydroxykaurenoic acid by *ent*-kaurene acid oxidase (KAO)

   Last three steps of the GA biosynthesis pathway (steps 6-8) from *ent*-kaurenoic acid, *ent*-7-hydroxykaurenoic acid, and GA_{12}-aldehyde to GA_{12}, are catalyzed by the action of KAO[11]. These reactions occur on the outer membranes of the plastid and require the transport of *ent*-kaurene from the organelles by an unknown mechanism.

7) *ent*-7a-hydroxykaurenoic acid is converted to GA_{12}-aldehyde by KAO

8) GA_{12}-aldehyde is converted to GA_{12} by KAO.

GA_{12} is processed to the bioactive GA_4 by oxidations on C-20 and C-3, which is accomplished by 2 soluble ODDs: GA 20-oxidase and GA 3-oxidase. With a 3- or 4-step process, GA_{12} is converted into various GA intermediates and bioactive GA_4 through the non-13-hydroxyl step. GA_{12} is also a substrate for the 13-hydroxylation branch in the production of GA_{53} (13-OH GA_{12}), which is a precursor for GA_1[2]. The GA20ox enzymes are encoded by multigene families that are responsible for the production of C19-GAs using C20-GAs as substrates. GA_9 and GA_{20} are precursors of bioactive GAs, which are converted by GA20ox via oxidation of C-20 to an aldehyde followed by the removal of this C atom and the formation of a lactone[12]. The inactive precursors GA_9 and GA_{20} are hydroxylated by GA3ox enzymes to form the biologically active hormones GA_4 and GA_1 through 3β-hydroxylation[13]. GA3ox enzymes are also encoded by multiple genes in all plant species. There are four GA3ox enzymes in Arabidopsis and two GA3ox enzymes in rice.

3.2.4 The role of GAs as plant hormones

GAs play different roles in plant development. Levels of bioactive GAs are maintained via feedback and feedforward regulation of GA metabolism, including regulation of the transcription of core GA signaling components, such as the GA receptor GID1 (Gibberellin Insensitive Dwarf1), which is nuclear-localized GA receptor and repressor of DELLA[2].

GID1 is related to hormone-sensitive lipases[4], which are esterases involved in lipid metabolism, although replacement of the histidine in the catalytic triad of GID1 with valine or isoleucine precludes esterase activity. The presence of GA (GA_4 has the highest affinity of tested GAs) in the binding pocket causes the flexible N-terminal strand to be fixed over the pocket, promoting association with DELLA proteins. The GID1–DELLA complex associates with an F-box protein, allowing recruitment of an E3 ubiquitin ligase, by which the DELLA is polyubiquitinated and thus targeted for degradation in the 26S proteasome. Although DELLA degradation is the main mechanism by which GID1-GA regulates its activity in higher plants, association with GID1-GA may prevent DELLA binding to its target transcription factors. Rice, in common with other cereals, has a single GID1 and DELLA protein (SLR1), whereas *Arabidopsis* has three GID1 and five DELLA paralogs, allowing considerable redundancy, although there is some specificity with regards to physiological function[4].

Also exist negative feedback regulation. The end point of GA signaling is a change in the level of expression of specific genes that results in the developmental changes that are characteristic to GA responses. DELLA proteins increase expression of at least as many genes as they downregulate. Their negative activity results from sequestering transcription factors and thus preventing them

from binding to their target site on gene promoters[4]. The AtGA3ox1 gene is under negative feedback regulation during *Arabidopsis* seed germination[2]. Bioactive GAs regulates AtGA3ox1 transcript abundance in a dose-dependent manner, AtGA20ox1 and AtGA3ox1 genes were highly upregulated in plants, which have lower endogenous GA_4 levels.

There are 3 main steps of gibberellin regulation:

1. Binding of GA causes a conformational change in the receptor GID1, promoting association with DELLA, which then, following interaction with an E3 ubiquitin ligase, is polyubiquitinated and targeted for degradation by the 26S proteasome[3].
2. DELLA function through sequestration of a transcription factor (PIF) to repress transcription.
3. DELLA function as coactivator of gene expression, in this case with the indeterminate domain transcription factor GAF1. The absence of DELLA allows association of GAF1 with the corepressor topless related (TPR) and transcription is inhibited[4].

3.3 Gibberellin conjugates

GA conjugation is considered as a ubiquitous process in the metabolism of GAS in plants[14]. The most common GA conjugates isolated from plants are those in which the GAs are bonded to glucose. These conjugates can be divided into two groups: ethers, where a hydroxy group of the GA skeleton is linked to glucose (Table 2), and glucosyl esters, in which glucose is bonded to the 7- carboxyl groups of the GAs (Table 3)[14].

Table 2. Prepared GA-O-glucosides[14]

$$\boxed{\text{Sugar}} - O - \boxed{\text{Gibberellin A}} - CO_2H$$

Number	Name of GA-O-glucoside
1	GA_1-3-O-glucoside
2	GA_1-13-O-glucoside
3	3-epiGA_1-3-O-glucoside
4	3-epiGA_1-13-O-glucoside
5	$16\xi,17$-H_2-GA_1-3-O-glucoside
6	$16\xi,17$-H_2-GA_1-13-O-glucoside
7	GA_3-3-O-glucoside
8	GA_3-13-O-glucoside
9	isoGA_3-3-O-glucoside
10	isoGA_3-13-O-glucoside
11	GA_4-3-O-glucoside- $16,17$-H_2,16,17-dihydroxy-
12	GA_4-17-O-glucoside
13	GA_5-13-O-glucoside
14	GA_7-3-O-glucoside
15	GA_8-2-O-glucoside
16	GA_8-13-O-glucoside
17	GA_{20}-13-O-glucoside
18	GA_{29}-2-O-glucoside
19	GA_{29}-13-O-glucoside
20	GA_3-3,13-di-O-glucoside

Table 3. Prepared GA glucosyl esters (glycosides)[14]
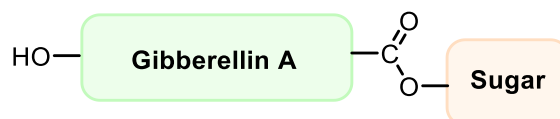
Number	Name of GA glycosyl ester
1	GA_1 β-D-glucopyranosyl ester
2	GA_3 β-D-glucopyranosyl ester
3	GA_3 β-D-galactopyranosyl ester
4	GA_3 β-D-xylopyranosyl ester
5	GA_3 α-L-arabinopyranosyl ester
6	isoGA_3 β -D-glucopyranosylester
7	isoGA_3 β -D-xylopyranosylester
8	GA_4 β-D-glucopyranosyl ester
9	GA_5 β-D-glucopyranosyl ester
10	GA_7 β-D-glucopyranosyl ester
11	GA_8 β-D-glucopyranosyl ester
12	GA_9 β-D-glucopyranosyl ester
13	GA_{20} β-D-glucopyranosyl ester
14	GA_{37} β-D-glucopyranosyl ester
15	GA_{38} β-D-glucopyranosyl ester
16	13-O-β-D-glucopyranosyl-GA_5 β-D-glucopyranosyl ester

3.3.1 Synthesis of gibberellin conjugates

3.3.1.1 Synthesis of gibberellin glucosides (ethers)

GA_8-2-O-β-D-glucoside has been isolated from pods of *Phaseolus coccineus* as the first conjugate of gibberellins[15]. The structural elucidation of this glucoside and of subsequently identified gibberellin glucosides from plant was based on spectroscopical data of the intact compounds, their derivatives or on investigations with parts of them after hydrolysis.

Typical procedures for their synthesis:

GA_{20}[16] (50 mg, 0.15 mmol) in 4 ml dichloroethane reacted with 65 mg (0.16 mmol) of χ-acetobromoglucose in the presence of 95 mg (0.17 mmol) Ag_2CO_3/Celite at boiling temperature. After 10 min filter the mixture. After evaporation chromatograph on 15 ml DEAE-Sephadex A 25. Elute the column with 15 ml aliquots of methanol, 0.5 N HOAc/methanol, 1.0 N HOAc/methanol; collect 5 ml fractions. Fractions 3-5 contain the neutral component which was rechromatographed on 8 g silica gel (petrol ether/ethyl acetate) resulting in 48 mg GA_{20}-β-D-(2.3.4.6-tetra-O-acetyl)-glucopyranosyl ester (49 % yield, mp. 142-144ºC, $C_{33}H_{42}O_{14}$. Fractions 11-12 contain near 25 mg (48 %) starting material.

28 mg GA_{20}-β-D-(2,3,4,6-tetra-0-acetyl)-glucosyl ester (0.04 mmol) within 1 ml methanol treat with 30 μl 0.5 N sodium methoxide for 5 min at room temperature. Stop the reaction by 30 μl

HOAc and separate the crude reaction mixture by silica gel chromatography with chloroform/methanol affording 12 mg GA_{20}-β-D-glucopyranosyl ester (57 % yield, amorphous, H-NMR)[15].

3.3.1.2 Synthesis of gibberellin glucosyl esters (glycosides)[16]
Typical procedures for their synthesis:

Free gibberellin (150 mg) should be dissolved in dry dioxane (10 ml) and α-bromoacetoglucose (190 mg), Ag_2O (250 mg) and add a few pieces of molecular sieve to the soln. Stir the mixture at 25ºC in darkness. After 20 hr, filter solids off and wash the cake with EtOAc (20 ml) several times, after that extract combined filtrate twice with aq. $NaHCO_3$. Dry and evaporate the organic phase. Crystallize the solid from EtOAc-hexane to give gibberellin acetylglucosyl ester (120-180 mg) as fine needles. Physical data for the gibberellin acetylglucosyl esters are as follows (GA_{37} acetylglucosyl ester is not crystallized) A_1 M^+ 678 m.p. 186.5-188º; A_3 M^+ 676 m.p. 216-217º. A_4 M^+ 662 m.p. 185-187º. Next dissolve gibberellin acetylglucosyl ester (60 mg) in MeOH (7 ml) and cool the solution to -10º. Add to this solution 0,05 M NaOMe (5 ml) and stir the mixture at - 10º for 1 hr. Add 0,1 M HCl (2.5 ml) and evaporate the solution under red. pres. at 30º. Extract thus obtained solid with Me_2CO-EtOH (1:1) at 35-40º several times. On evaporation of solvent obtain crude gibberellin glucosyl ester (40 mg). Prep. TLC ($CHCI_3$-MeOH, 3: 1) to afford the pure gibberellin glucosyl ester (25 mg).

3.3.2 Functions

Glucosyl fragment of GA conjugates causes a distorted orientation of the GA molecule within the membrane, prohibiting appropriate binding to an assumed receptor[17]. Because of their polar properties, GA glucosyl conjugates also have been considered to maintain for the transport and compartmentation of GA metabolites within the cells. A possible function of GA glucosyl conjugates in long distance transport has been suggested due to the occurrence of these types of compounds in sap of trees[14]. With respect to their preferential formation and accumulation during seed maturation, GA glucose conjugates are also considered to be a storage form of GAs[18]. However, this applies only to conjugates of biologically active GAs, where reconversion during early stages of seed germination gives raise to free GAs prior to *de novo* GA biosynthesis.

2β-hydroxylated GAs are biologically inactive metabolites, which can be a step in the GA metabolism[14]. The GA_1-O-3-glucoside is also almost inactive in the dwarf-pea and the dwarf-maize bioassays, whereas the corresponding GA_1-O-13-glucoside showed a low but significant activity[19]. In general, it may be concluded that conjugation of GAs either by glucosylation or by formation of glucosyl esters and amides with amino acids may lead to a reversible deactivation of

the GA. The activity of GA conjugates represents the ability of hydrolytic plant enzymes, and of possible microbial activities, to hydrolyze glycosidic, glucosyl-ester and amide-like linkages[19].

3.3.3 Impact of Gibberellin to agriculture

The gibberellins are a demonstrative example of the influence plant scientists have to human and animal health and nutrition. The gibberellins help us to get a control over plants, because they supplement natural growth-promoters that occur in plants. Their "triggering" effect seems to start up well established biochemical mechanisms within seeds and plants, causing normal or characteristic growth. Otherwise inhibited because of light, temperature, other environmental factors, or other substances in the plant that have net effects opposite to those of gibberellins, such growth or continued development is often important for crop and fruit production[20].

When the first efforts were made by Paleg (1960) and MacLeod (1964) to elucidate the function of gibberellic acid by studying the changes it was able to impose to germinating barley, it became evident that one important aspect of its behavior was its ability to increase the quantities of enzymes secreted by the germinating barley corn[21]. Overall, Gibberellic acid (GA_3) causes increasing production of the ribonucleic acids, can trigger a sequence of events culminating in the rapid release of sugars in the plant by increasing the production of α-amylase, and offers an explanation for many of the apparent reactions caused by exogenous supply of isolated growth material. Some use of GA_3 in agricultural production are listed below:

Grapes. When we hear about gibberellin use in agriculture, the first thing that should be mentioned are grapes without seeds. Gibberellins must be sprayed during or shortly after blooming of grapes. Spraying gibberellins on grapes not only deprives them of seeds, but also increase berry size and modify their shape. Applications at full bloom cause the berries to elongate, while an additional prebloom application of GA_3 increases the length of the rachis, producing a looser cluster that is less susceptible to fungal infection, and decreases pollen viability and ovule fertility, so reducing fruit numbers and increasing the weight of remaining fruit[4].

Citrus. Application of GA_3 to citrus fruit is used to delay maturing and to improve the appearance of the peel. The color change from green to yellow, or green to orange, which becomes due to loss of chlorophyll, is also delayed by the treatment[21].

Apples. Poor pollination of apple trees due to unfavorable weather conditions at flowering time can lead to poor yields. In these conditions fruit can grow parthenocarpically by application of GA_3; the GA must be used in combination with an auxin and cytokinin to receive optimal fruit growth. Mixtures of GA_4/GA_7 and benzyladenine, a cytokinin, usually applied to apple trees immediately after flowering to improve fruit shape by increasing the length of the fruit relative to

the diameter. These treatments also reduce the incidence of russeting, a browning of the peel that decreases the value of the fruit. GA_4 is the more active GA component and preparations containing GA_4 virtually free of GA_7 are commercially available[4].

Potatoes. Gibberellins accelerate the late and slow-sprouting potatoes so it can give more harvest. Gibberellins spur seed potatoes to sprout, resulting in more uniform emergence and promising yield increases up to 30%[20].

Since recently, a lot of research was done on 16,17-dihydro-gibberellins, which is the generic name for a class of gibberellins (GAs) bearing the 16,17-dihydro moiety. The most promising GA compound of such origin is *exo*-16,17-dihydro-gibberellin A_5-13-acetate (DHGA5)[22]. It is assumed that DHGA5 can delay plant growth and development, reduce plant elevation to resist lodging and regulate plant shape.

3.3.4 Goals of the Thesis

The aim of this bachelor thesis not only elaborate a literature search on the topic of sugar derivatives of gibberellins but synthesize sugar conjugates with GA_3, GA_4, and possibly GA_7. My goal is to find a new synthetic path to such molecules since the previously published proved to be inefficient and not reliable in our hands. In details, my goal is to find the right method to synthesize GA_3-3-O-β-D-glucopyranoside and GA_4-3-O-β-D-glucopyranoside (figure 4). And about my efforts will be nest part of my theses (Figure 4).

GA$_3$-3-O-β-D-glucopyranoside   GA$_4$-3-O-β-D-glucopyranoside
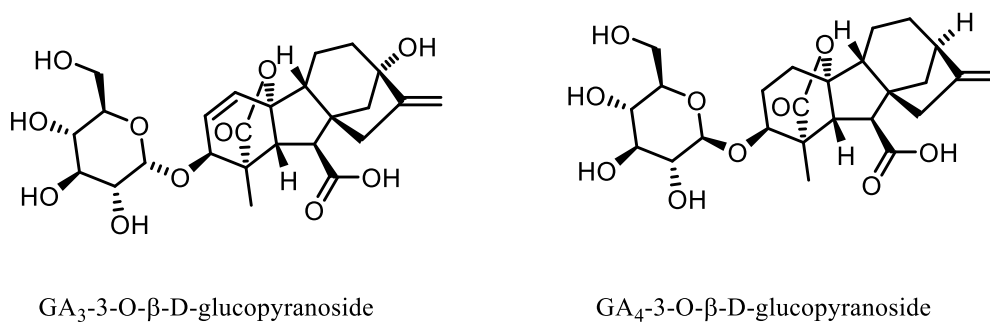
Figure 4. Targeted sugar conjugates with GA$_3$ and GA$_4$.

4. Experimental part

4.1 Chemicals

The following chemicals were used for each step: acetic anhydride (Ac₂O), 4-(dimethylamino)pyridin 98 % (DMAP), pyridine (Py), 2,4-dimethoxybenzyl alcohol (DMB), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC*HCl), dichloromethane (DCM), potassium carbonate (K₂CO₃), methanol (MeOH), silver(I) oxide (Ag₂O), silver carbonate (Ag₂CO₃), 2,3,4,6-tetra-O-acetyl-alpha-D-glucopyranosyl bromide (C₁₄H₁₉BrO₉), acetonitrile (MeCN), tetrabutylammonium iodide (TBAI), gibberellin A3 (GA₃), gibberellin A4 (GA₄), gibberellin A7 (GA₇), ammonia (NH₃), *tert*-butyldimethylsilyl chloride (TBSCl), imidazole, N,N-dimethylformamide (DMF), sodium methoxide (NaOMe), N,N'-dicyclohexylcarbodiimide (DCC), oxalyl chloride, N,N-diisopropylethylamine (DIPEA), penta-O-acetyl-β-D-glucopyranose 98%, hydrazine acetate, zinc chloride (ZnCl₂), ethyl acetate (EA), chloroform-d (CDCl₃), petroleum spirit (PE), acetone, acetic acid. All chemicals were used in the same purity as provided unless otherwise stated.

4.2 Methods and material

4.2.1 General information

Thin layer chromatography (TLC) on aluminum plates coated with silica gel 60 SIL G / UV254 with a fluorescent indicator (Machery-Nagel or Sigma-Aldrich) were used for monitoring progress of the reactions, the approximate purity of the intermediates and products. The individual compounds present in the reaction mixture were visualized using visualization solutions, using vanillin solution and 1M H₂SO₄. The final products were purified by column chromatography (CC) on silica gel 34 from Sigma Aldrich (particle size is 230-400 mesh; pore size is 60 Å). The eluted fractions were combined in 10-30 ml portions and monitored by TLC. Fractions containing the same product were combined, concentrated under reduced pressure and subsequently analyzed by spectral methods.

Nuclear magnetic resonance (NMR) analysis was carried out on a JEOL 500 JNM-ECA 500 MHz spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C). The measurements were occurred at room temperature using deuterated solvents such as chloroform (CDCl₃) and acetone (CD₃COCD₃) for the measurements. Chemical shifts (δ, ppm) and spin-spin coupling constants (Hz) of the ^{1}H NMR spectra are described in the standard way relative to the remaining CHCl₃ present in CDCl₃ (δH = 7.26 ppm) and the intermediate signal in CHD₂C(O)CD₃ present in acetone-*d6* (δH = 2.05 ppm). ^{13}C NMR chemical shifts (δ, ppm) are described relative to CDCl₃ (δC = 77.16 ppm, middle signal) and CD₃C(O)CD₃ (δC = 29.84 ppm, middle signal). Proton

interactions are represented as singlet (s), doublet (d), doublet of doublet (dd), triplet (t), triplet triplet (tt), pentet (p) and multiplet (m). HRMS values were obtained using a quadrupole / ion trap mass analyzer. Analysis and assignments were held by comparison with literature spectroscopic data or by 2D-COSY, HMQC, HMBC experiments. The purity of the final compounds was determined as follows: 1 mg of the compound was dissolved in 1 ml of 1% methanol and injected (10 μl) onto a reverse phase column (Symmetry C18, 5 μm, 150 mm x 2.1 mm; Milford, MA, USA), incubated at 25 ° C. Solvent (A) was done from 15 mM ammonium formate adjusted to pH 4.0. Solvent (B) was methanol. At a flow rate of 200 μl / min was binary gradient used: 0 min, 10% B; 0-24 min. linear gradient to 90% B; 25-34 min. isocratic elution 90% B; 35-45 min. linear gradient to 10% B. The eluted phase was then bring into the PDA detector (sensing range 210-700 nm with resolution 1.2 nm) and electrospray source (source temperature 120 ° C, desolvation temperature 300 ° C, capillary voltage 3 kV, cone voltage 20 V). Nitrogen was used as cone (50 l / h) and desolvation gas (500 l / h). Data collection was performed in full mode scan (50-1000 Da), scan time 0.5 s and a collision energy was 6 V. Analyzes were performed in positive mode (ES$^+$) or in negative mode (ES$^-$) and were therefore collected as quasimolecular ions [M + H]$^+$ and [M-H]$^-$

The products were weighed on analytical scales of the AS 220.R2 brand. All reactions were performed using a Heidolph MR 3001 K laboratory magnetic stirrer (Heidolph, Germany). The chemicals were evaporated and concentrated using a Interface I-300 vacuum rotary evaporator (Büchi, Switzerland).

## 4.2.2 Visualization solutions for TLC

The following visualization methods were used:

- An acidic H_2SO_4 solution was prepared by dissolving 13.62 ml 98% H_2SO_4 in 250 ml water.
- Vanillin solution was prepared dissolving 15g of vanillin in 250 ml ethanol and 2.5 ml conc. sulfuric acid

4.3 Organic synthesis

4.3.1 Preparation of GA$_3$ sugar derivatives

4.3.1.1 Acetylation of gibberellin A$_3$
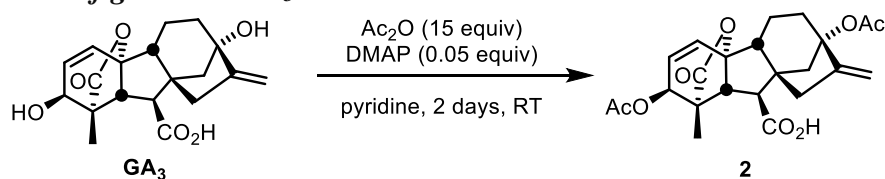
Crystalline substance 1 (10 g, 28.9 mmol, 1 equiv) was dissolved in dry pyridine (93.5 ml, 28.6 mmol, 1 equiv) and cooled to 0°C. After that acetic anhydride (40.3 ml, 429 mmol, 15 equiv) was added over 35 min, followed by 98 % 4-(dimethylamino)pyridine (0.178 g, 1.43 mmol, 0.05 equiv). The reaction mixture was stirred at RT for 45 h and was complete by TLC (PE-EtOAc 1:1 + 1 drop of AcOH). Quenched by addition of methanol (50 ml) with ice water cooling, then stirred for 20 min at RT. All the volatiles were evaporated in vacuum (up to 15 mbar, 50°C).

Then impurity product was added to the residue water (150 ml) and it was sonicated for 1 min acidified by 6M aq. HCl (8 ml). Extracted by DCM (2×50 ml; 40 ml), organic extract was washed with 2M HCl (3×25 ml), brine (30 ml), dried over Na_2SO_4 and evaporated in vacuum. Yield is 98%, mass is 12.3 g.

$[\alpha]_D^{19.0} = +147.4°$ (c 1.0, MeOH)

^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 11.26 br s (1H, COOH), 6.58 (d, J = 10.4 Hz, 1H), 5.87 (dd, J = 9.3, 3.8 Hz, 1H), 5.27 (dd, J = 3.8, 0.8 Hz, 1H), 5.17 (dd, J = 3.1, 1.6 Hz, 1H), 4.98 (s, 1H), 3.26 (d, J = 11.0 Hz, 1H), 2.76 (d, J = 11.0 Hz, 1H), 2.45–2.15 (m, 6H), 2.10 (s, 3H), 1.98 (s, 3H), 1.94-1.87 (m, 1H), 1.83–1.72 (m, 3H), 1.13 (s, 3H).

^{13}C NMR (125 MHz, $(CD_3)_2CO$) δ 177.5, 172.9, 170.4, 170.0, 155.1, 135.7, 129.5, 107.9, 90.8, 84.6, 70.7, 53.9, 52.8, 51.41, 51.35, 51.22, 42.8, 40.7, 37.1, 32.3, 23.3, 21.9, 20.69, 17.4, 14.7.

LC-MS Rt 20.37 min.

ESI-MS (intensity, assignment): 448.3 (100, $[M+H_2O]^+$); 431.3 (7, M^+); 327.3 (10, $[M-CO_2-AcOH]^+$); 267.2 (20, $[M-CO_2-2AcOH]^+$).

HRMS (ESI) calcd. for $C_{23}H_{26}O_8$ M^+ 430.1628, found 430.1629.

4.3.1.2 Esterification of GA$_3$ acetyl
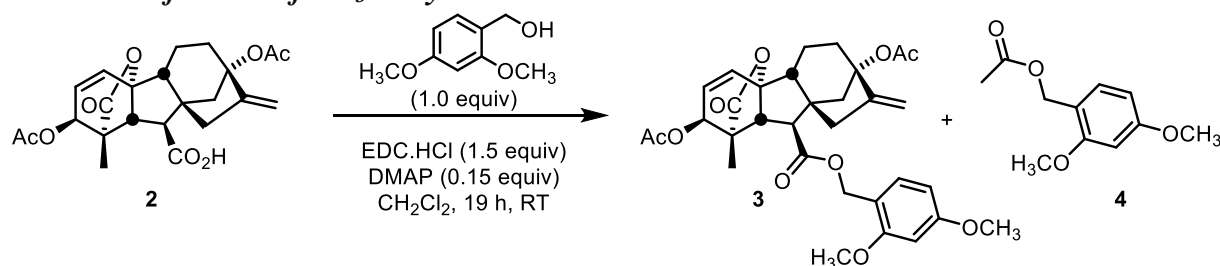
Under argon atmosphere, to a solution of **2** (12.3 g, 28.6 mmol, 1 equiv) and 4-(dimethylamino)pyridine (0.535 g, 4.29 mmol, 0.15 equiv) in dry DCM (115 ml, 1.755 mol) at 0 °C was added N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (8.22 g, 42.9 mmol, 1.5 equiv) over 10 min. Reaction mixture was stirred for 10 min at this temperature and a solution of 2,4-dimethoxybenzyl alcohol (4.81 g, 28.6 mmol, 1 equiv) in DCM (8 ml) was added

dropwise over 10 min. Then the reaction mixture was stirred at RT for 19 h, washed with 1M aq. HCl (3×120 ml), aq. layer was extracted with DCM (3×60 ml). Combined organic layer was washed with brine (50 ml) and dried over Na_2SO_4, evaporated in vacuum. Crude product was purified by column chromatography (SiO$_2$; petroleum ether:EtOAc = 4:1->7:3->13:7) and yielded compound **4** (2.64g, 19 %) and desired product **3** (9.79 g, 80%).

Product **3**

$[\alpha]_D^{19.5} = +110.5°$ (c 1.0, CHCl$_3$)

^{1}H NMR (500 MHz, (CDCl$_3$) δ (ppm) 7.23 (d, J = 8.1 Hz, 1H), 6.47-6.43 (m, 3H) 6.36 (d, J = 9.3 Hz, 1H), 5.85 (dd, J = 9.3, 3.78 Hz, 1H), 5.31 (d, J = 4.3 Hz, 1H), 5.19 (dd, J = 3.1, 1.5 Hz, 1H), 5.12 (d, J = 3.3 Hz, 2H), 3.85 – 3.76 (m, 1H), 4.95 (s, 1H), 3.81 (s, 3H), 3.77 (s, 3H), 3.32 (d, J = 11.03, 1H), 2.77 (d, J = 11.04, 1H), 2.42 – 2.37 (m, 2H), 2.21 (dt, J = 14.5, 2.7), 2.09 (s, 3H), 2.00 (s, 3H), 1.88 (dd, 12.3, 5.5, 1H), 1.78 – 1.72 (m, 1H), 1.69 – 1.64 (m, 1H), 1.13 (s, 3H).

^{13}C NMR (125 MHz, (CDCl$_3$) δ (ppm) 177.32, 171.99, 170.30, 169.93, 161.79, 159.25, 154.04, 134.50, 132.05, 129.28, 116.08, 108.40, 104.04, 98.64, 90.19, 84.34, 70.41, 63.16, 55.62, 55.51, 53.49, 52.32, 51.26, 50.59, 42.41, 40.21, 36.16, 22.21, 21.02, 17.10, 14.45.

ESI-MS m/z [M+H]$^+$ (%): 582 (25).

HRMS (ESI) for $C_{32}H_{36}KO_{10}$ [M+K]$^+$: calcd. 619.1940, found 619.1943.

Product **4**

^{1}H NMR (500 MHz, (CDCl$_3$) δ (ppm) 7.24 (d, J = 9.0 Hz, 1H), 6.48-6.45 (m, 2H), 5.09 (s, 2H), 3.82 (s, 3H), 3.81 (s, 3H), 2.07 (s, 3H).

^{13}C NMR (125 MHz, (CDCl$_3$) δ 171.3, 161.4, 159.1, 131.5, 116.8, 104.1, 98.7, 61.8, 55.6, 55.5, 21.2.

4.3.1.3 Selective deacetylation of GA$_3$ ester
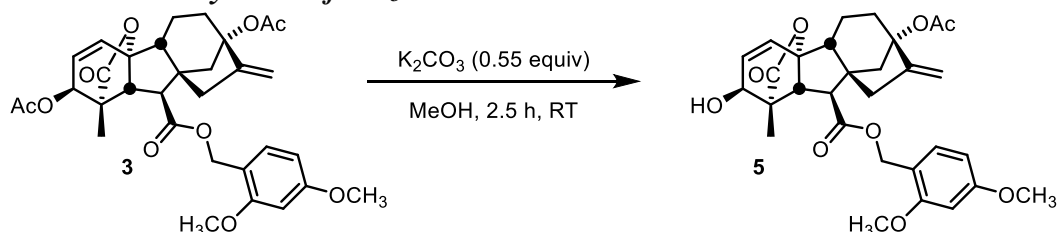
To a solution of compound **3** (9.79 g, 16.9 mmol, 1 equiv) in methanol (420 ml, 10379 mmol) was slowly added solid K$_2$CO$_3$ (1.28 g, 0.55 equiv) portionwise until the pH value of the mixture reached 9–10. The solution was stirred at RT for approximately an hour, then acidified until it reached pH 7 (1 M HCl).

To the residue was partitioned between water and DCM (60+60 ml) and resulting phases were separated. Water phase was extracted with DCM (3x15 ml). Combined organic extracts were washed with brine (30 mL), water (30 ml), then with brine (20 ml), dried over Na_2SO_4, filtered and filtrate was evaporated Mass of the crude product was 9,37 g. The crude was purified by column chromatography (SiO_2; 4:1->2:1->1:1) and yielded product **5** (8,85 g; 90%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ = 7.22 (d, *J* = 8.1 Hz, 1H), 6.49 – 6.39 (m, 2H), 6.29 (d, *J* = 10.0 Hz, 1H), 5.88 (dd, *J* = 9.3, 3.7 Hz, 1H), 5.17 (dd, *J* = 3.0, 1.4 Hz, 1H), 5.12 (d, *J* = 11.7 Hz, 1H), 5.08 (d, *J* = 11.7 Hz, 1H), 4.94 (s, 1H), 3.80 (s, 3H), 3.76 (s, 3H), 3.19 (d, *J* = 10.9 Hz, 1H), 2.77 (d, *J* = 10.9 Hz, 1H), 2.43 – 2.35 (m, 2H), 2.21 (dt, *J* = 15.3, 2.9 Hz, 1H), 2.13 – 2.06 (m, 2H), 2.00 (s, 2H), 1.98 – 1.92 (m, 0H), 1.94 – 1.82 (m, 2H), 1.79 – 1.69 (m, 1H), 1.68 – 1.62 (m, 1H), 1.21 (s, 2H) ppm.

^{13}C NMR (126 MHz, Chloroform-*d*) δ = 178.44, 172.06, 169.86, 161.67, 159.18, 154.01, 133.22, 132.44, 132.03, 116.06, 108.24, 103.91, 98.56, 90.36, 84.33, 69.95, 63.10, 55.55, 53.58, 52.85, 51.30, 50.63, 42.40, 40.22, 36.11, 32.02, 22.17, 17.07, 14.46 ppm.

ESI-MS *m/z* [M+H]$^+$ (%): 540 (12).

HRMS (ESI) for $C_{30}H_{34}KO_9$ [M+K]$^+$: calcd. 577.1834, found 577.1830.

4.3.1.5 Glycosylation of GA₃Ac ester
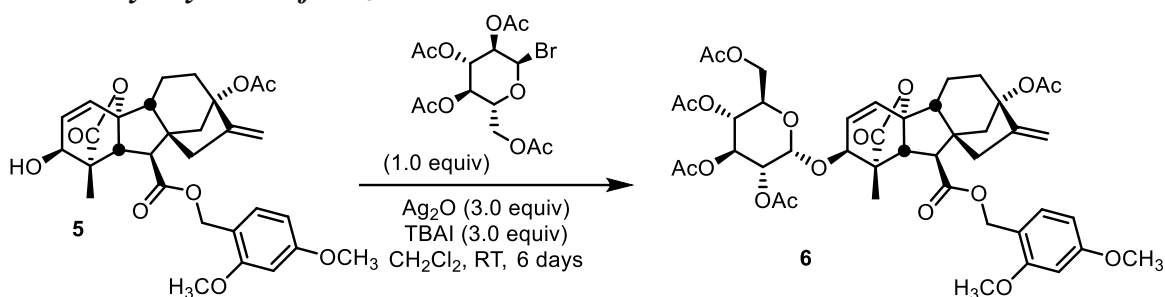
Compound **5** (500 mg, 0.861 mmol, 1 equiv) and 2,3,4,6-tetra-O-acetyl-alpha-D-glucopyranosyl bromide (1.0 g, 2.58 mmol, 3 equiv) were dissolved in DCM (3 ml) at 0 °C, and TBAI (953 mg, 2.58 mmol, 3 equiv) was added. After additional 5 min at 0 °C, Ag_2O (604 mg, 2.58 mmol, 3 equiv) was added and the resulting solution was stirred at RT for next 6 days. The whole mixture was loaded on column (SiO_2; petroleum ether:EtOAc = 3:1->2:1) to yield the desired product **6** (431 mg, 58%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ = 7.24 (d, *J* = 8.1 Hz, 1H), 6.65 (s, 1H), 6.47 (d, *J* = 2.3 Hz, 1H), 6.44 (t, *J* = 2.4 Hz, 1H), 6.36 (dd, *J* = 9.2, 0.8 Hz, 1H), 5.86 (dd, *J* = 9.3, 3.7 Hz, 1H), 5.57 (d, *J* = 4.4 Hz, 1H), 5.32 (dd, *J* = 3.7, 0.8 Hz, 1H), 5.24 (dd, *J* = 5.7, 4.4 Hz, 1H), 5.19 (dd, *J* = 3.2,

1.7 Hz, 1H), 5.17 – 5.06 (m, 2H), 4.98 – 4.94 (m, 2H), 4.44 (dd, J = 11.9, 6.7 Hz, 1H), 4.41 – 4.34 (m, 1H), 4.24 (dd, J = 11.9, 3.4 Hz, 1H), 3.82 (s, 3H), 3.78 (s, 3H), 3.32 (d, J = 11.0 Hz, 1H), 2.78 (d, J = 11.0 Hz, 1H), 2.52 – 2.35 (m, 1H), 2.27 – 2.19 (m, 1H), 2.11 (d, J = 1.1 Hz, 6H), 2.11 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.01 – 1.92 (m, 1H), 1.89 (dd, J = 12.2, 5.3 Hz, 1H), 1.75 (dddd, J = 12.2, 10.7, 7.6, 1.5 Hz, 1H), 1.67 (dddd, J = 13.1, 7.3, 5.4, 1.5 Hz, 1H), 1.14 (s, 3H) ppm.

^{13}C NMR (126 MHz, Chloroform-d) δ = 177.31, 171.99, 170.72, 170.35, 170.28, 169.90, 169.82, 169.71, 161.79, 159.26, 154.05, 139.48, 134.50, 132.05, 129.29, 127.59, 116.09, 108.39, 104.05, 98.65, 90.18, 84.34, 74.33, 70.41, 67.66, 66.53, 63.15, 61.16, 55.62, 55.52, 53.50, 52.32, 51.26, 50.60, 42.42, 40.22, 36.17, 22.21, 21.02, 20.98, 20.94, 20.71, 17.10, 14.45 ppm.

ESI-MS m/z [M+H]$^+$ (%): 870 (19).

HRMS (ESI) for $C_{44}H_{52}KO_{18}$ [M+K]$^+$: calcd. 907.2785, found 907.2780.

4.3.1.6 Deacetylation of GA$_3$-glucoside ether
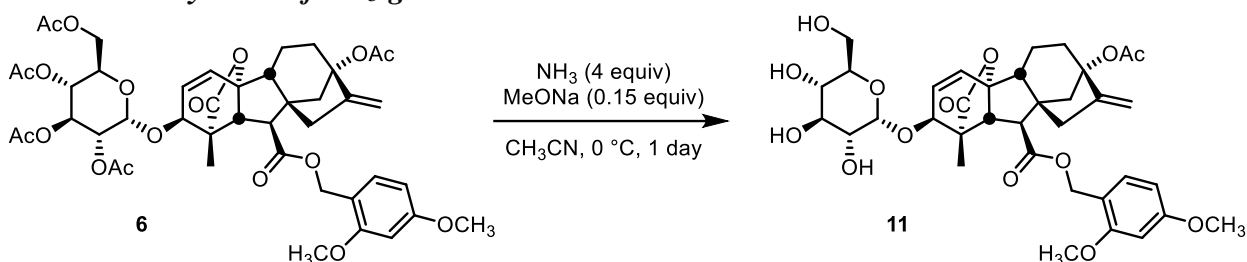
A solution of ammonia (0.23 mmol, 4 equiv, 1.0M in CH$_3$CN) in MeCN (2.5 ml, 47.6 mmol, 4 equiv) was added to a solution of compound **6** in MeCN (25 ml) at 0°C, the reaction mixture was warmed to RT and stirred for 30 min, monitored by TLC. **No product formation**. Then was added freshly preapred NaOMe (0.1 M, 0,15 equiv) at 0 °C (generated from Na and MeOH). After addition of NaOMe mixture was left for 3 h, monitored by TLC, starting compound disappeared. The whole mixture was evaporated under reduced pressure, and the residue was partitioned between DCM and water (10 + 10 ml). Aq. phase was washed with DCM (3x7 ml). Organic phase was washed with water (2x20 ml), brine (10 ml), dried over Na$_2$SO$_4$, filtered and the evaporation yielded the desired compound **11** (28 mg,70 %).

^{1}H NMR (500 MHz, Chloroform-d) δ = 7.24 (dd, J = 8.1, 1.4 Hz, 1H), 6.48 – 6.43 (m, 2H), 6.31 (dt, J = 9.3, 1.2 Hz, 1H), 5.97 – 5.84 (m, 1H), 5.19 (dt, J = 3.0, 1.5 Hz, 1H), 5.16 – 5.05 (m, 2H), 4.98 – 4.94 (m, 1H), 4.14 (ddd, J = 7.1, 5.3, 2.3 Hz, 1H), 3.82 (d, J = 1.4 Hz, 3H), 3.78 (d, J = 1.3 Hz, 3H), 3.20 (dd, J = 10.9, 1.3 Hz, 1H), 2.79 (dd, J = 10.8, 1.3 Hz, 1H), 2.41 (ddd, J = 14.2, 9.6, 2.1 Hz, 2H), 2.27 – 2.20 (m, 1H), 2.15 – 2.07 (m, 2H), 2.02 (d, J = 1.4 Hz, 3H), 2.01 – 1.94 (m,

1H), 1.92 – 1.84 (m, 2H), 1.75 (dddd, J = 12.3, 10.7, 7.6, 1.4 Hz, 1H), 1.67 (dtd, J = 12.4, 6.0, 3.2 Hz, 1H), 1.23 (d, J = 1.4 Hz, 3H) ppm.

^{13}C NMR (126 MHz, Chloroform-d) δ = 178.49, 172.12, 169.93, 161.76, 159.27, 154.10, 133.33, 132.51, 132.11, 116.16, 108.31, 104.00, 98.65, 90.42, 84.40, 70.04, 63.16, 55.62, 55.52, 53.65, 52.94, 51.38, 51.34, 50.71, 42.48, 40.30, 36.19, 22.23, 17.14, 14.52 ppm.

ESI-MS m/z [M+H]$^+$ (%): 702 (11).

HRMS (ESI) for $C_{36}H_{44}KO_{14}$ [M+K]$^+$: calcd. 739.2363, found 739.2368.

4.3.17 Deesterification of GA₃-glucoside
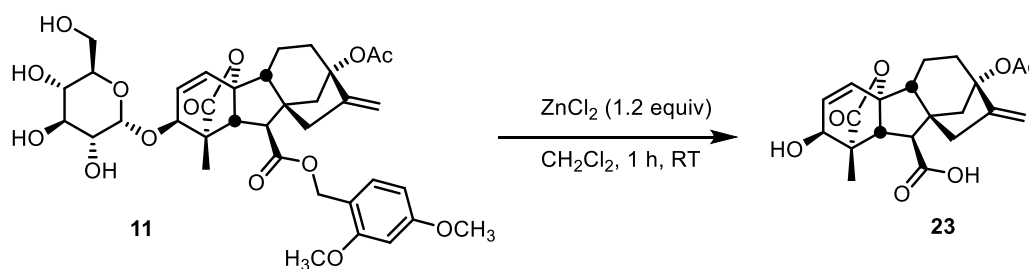
A solution containing compound **11** (60 mg, 0.856 mmol, 1 equiv) and ZnCl₂ (14 mg, 0.103 mmol, 1.2 equiv) was dissolved in DCM (3 ml) stirred at RT for 1 h. The mixture was filtered through a pluck of Celite® and filtercake was washed with MeOH. Filtrates were concentrated under reduced pressure and the residue was purified with help of semipreparative HPLC (MeCN-water, gradient 2% to 90% MeCN in 9 min, duration 10 min). Even though the product was present in HPLC of the crude reaction mixture, only product **23** was isolated after HPLC (22 mg).

^{1}H NMR (500 MHz, Acetone-d_6) δ = 6.38 (dd, J = 9.3, 1.0 Hz, 1H), 5.89 (dd, J = 9.3, 3.7 Hz, 1H), 5.15 (dd, J = 3.2, 1.6 Hz, 1H), 5.05 – 4.93 (m, 1H), 4.04 (dd, J = 3.7, 0.9 Hz, 1H), 3.24 (d, J = 10.9 Hz, 1H), 2.71 (d, J = 10.9 Hz, 1H), 2.49 – 2.29 (m, 3H), 2.29 – 2.20 (m, 2H), 2.08 – 2.06 (m, 1H), 2.03 – 1.98 (m, 1H), 1.97 (s, 3H), 1.95 – 1.86 (m, 1H), 1.79 – 1.71 (m, 2H), 1.20 (s, 3H) ppm.

^{13}C NMR (126 MHz, Acetone-d_6) δ = 179.01, 173.18, 170.01, 155.21, 134.03, 132.81, 107.84, 90.99, 84.72, 70.00, 54.30, 53.36, 51.73, 51.54, 51.49, 43.12, 40.83, 37.14, 21.90, 17.52, 15.05 ppm.

ESI-MS m/z [M]$^-$ (%): 387 (16).

HRMS (ESI) for $C_{21}H_{24}KO_7$ [M+K]$^+$: calcd. 427.1154, found 427.1158.

4.3.2 Separation of GA₄/GA₇ mixture

4.3.2.1 Acetylation of GA₄ and GA₇
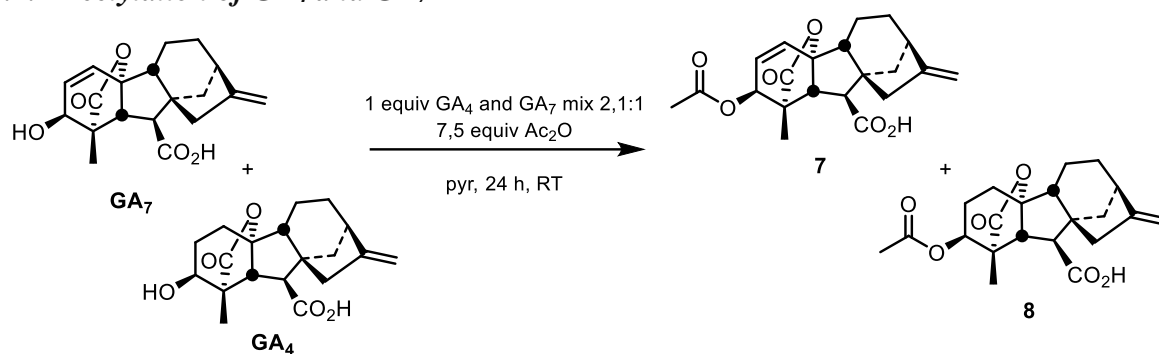
GA₇ and GA₄ mixture (4:7 mass ratio) (2 g, 5.9 mmol, 1.03 equiv) was dissolved in dry pyridine (19.1 ml, 5.72 mmol, 1 equiv), cooled to 0°C. Acetic anhydride (4.03 ml, 42.9 mmol, 7.5 equiv) was added over 35 min, followed by 4-(dimethylamino)pyridin 98 % (0.0356 g, 0.286 mmol, 0.05 equiv). The reaction mixture was stirred at RT for 24 h and was complete by TLC (PE-EtOAc 1:1 + 1 drop of AcOH). Quenched by addition of methanol (50 ml) with ice water cooling, then stirred for 20 min at RT. All the volatiles were evaporated in vacuum (up to 15 mbar, 50°C).

To the residue water (150 ml) was added, it was sonicated for 1 min and acidified by 6M aq. HCl (1.5 ml). Water suspension was extracted by DCM (3×20 ml), organic extract was washed with 2M HCl (3×25 ml), brine (30 ml), dried over Na₂SO₄, evaporated in vacuum, monitored by NMR. Estimated mass of pure product (compound 7 and 8) is 2,03 g. Yield is 95%

(for mixture of products characterization, see[23])

4.3.2.2 Esterification of GA₄Ac and GA₇Ac
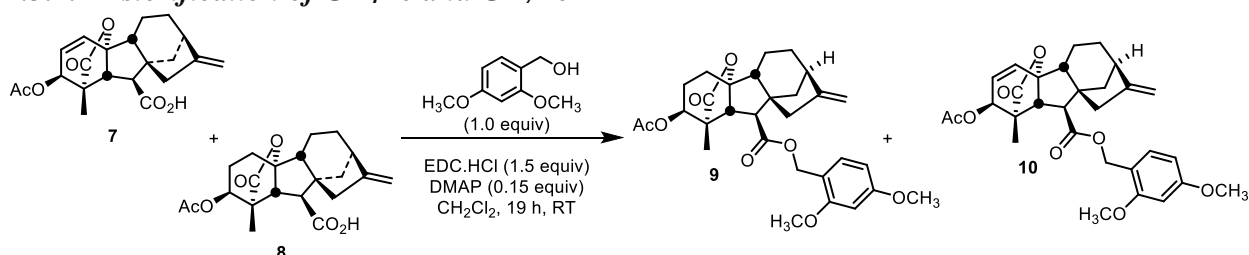
Under argon atmosphere, to a solution of compounds **7** and **8** (2.26 g, 5.43 mmol, 1 equiv) and DMAP (0.101 g, 0.813 mmol, 0.15 equiv) in dry DCM (22.5 ml) at 0 °C, EDC.HCl (1.56 g, 8.13 mmol, 1.5 equiv) was added over a period of 10 min. Reaction mixture was stirred for 10 min at 0 °C before a solution of DMB-OH (0.912 g, 5.42 mmol, 1 equiv) in DCM (4 ml, 351 mmol) was added dropwise over next 10 min. Then the reaction mixture was stirred at RT for 19 h, washed with 1M aq. HCl (3×30 ml) and aq. layer was back extracted with DCM (3×15 ml). Combined organic layer was washed with brine (20 ml), dried over Na₂SO₄, filtered and evaporated in vacuum. Unfortunately, the separation of products **9** and **10** proved to be fruitless and therefore no pure product was obtained.

4.3.2.3 Deacetylation of GA₄-GA₇ esters
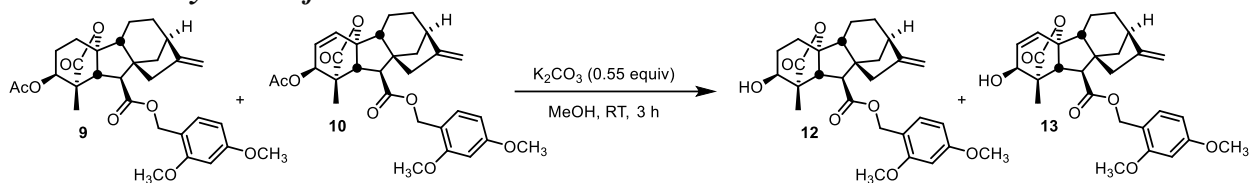
To a solution of compounds **9** and **10** (1706 mg, 3.26 mmol, 1 equiv) in methanol (100 ml) was slowly added saturated K_2CO_3 (248 mg, 1.8 mmol, 0.55 equiv) until the pH value of the mixture reached 9–10. The solution was stirred at RT for approximately an hour, then acidified until it reached pH 7 (1 M HCl). To test pH, a drop of reaction mixture was placed on wet indicator paper (specified for pH 7-10). pH was controlled after addition of ≈0.4-0.5 eq of K_2CO_3 solution.

After stirring for 40 min, the reaction was not complete. The lumps of salts at the bottom were crushed and the reaction mixture was stirred for 30 min (almost all dissolved), fully complete after 3 hours. The reaction mixture was neutralized by 1M HCl (2 ml consumed) and left for half an hour at RT.

Resulting mixture was diluted with water and DCM (30+30 ml), and resulting phases were separated. Aqueous phase was extracted with DCM (3x10 ml). Combined organic extracts were washed with brine (30 mL), water (30 ml), brine (20 ml), dried over Na_2SO_4, filtered and evaporated in vacuum. Inseparable mixture of **12** and **13** (**12:13** =1:1.21, 1.21g, 71%) was obtained.

4.3.2.4 Separation of GA₄ and GA₇ esters
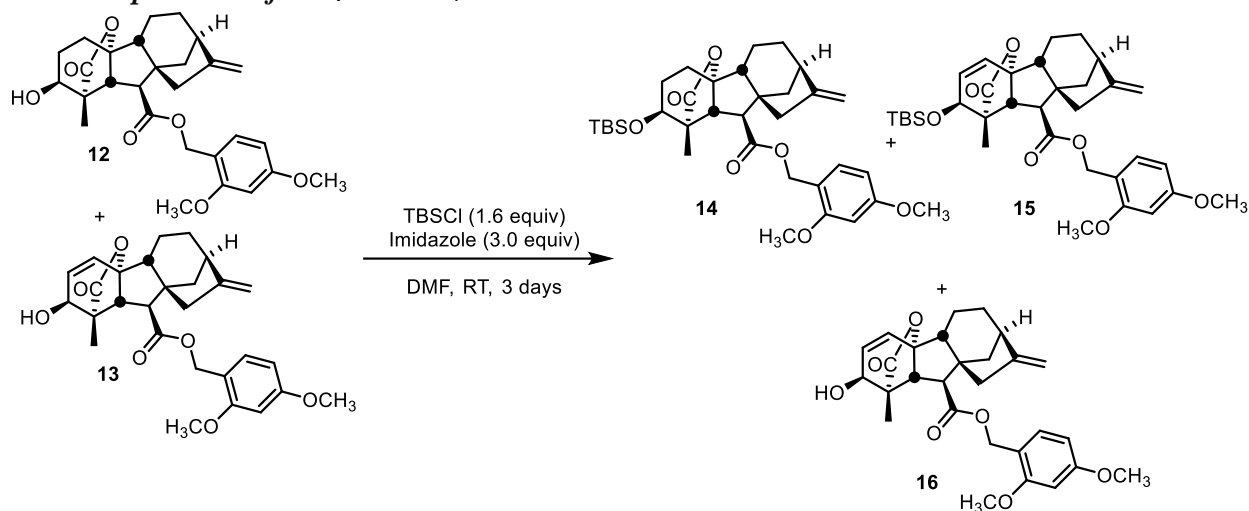
To a solution of compound **12** and **13** (1.21 g, 2.32 mmol, 1 equiv) in DMF (6 ml) was added imidazole (0.474 g, 6.96 mmol, 3 equiv). After imidazole dissolved, TBSCl was added (0.577 g, 3.71 mmol, 1.6 equiv) at RT and the reaction mixture was stirred for 3 days at RT. AcOH (25 ml) and H_2O (35 ml) were added to stop the reaction and the whole mixture was placed to ice bath for

30 min. White solid precipitated and then dried before it was purified by column chromatography (SiO$_2$; petroleum ether:EtOAc = 10:1->4:1->2:1) yielded compound **16** (593 mg, 53%).

$[\alpha]_D^{24.2}$ = -21.1° (c 0.65, CHCl$_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ = 7.22 (dd, *J* = 7.8, 0.7 Hz, 1H), 6.45 (d, *J* = 7.9 Hz, 2H), 5.13 (d, *J* = 11.7 Hz, 1H), 5.04 (d, *J* = 11.7 Hz, 1H), 4.96 (dd, *J* = 2.9, 1.5 Hz, 1H), 4.81 (d, *J* = 2.4 Hz, 1H), 3.85 – 3.82 (m, 1H), 3.82 (s, 3H), 3.77 (s, 3H), 3.19 (d, *J* = 10.9 Hz, 1H), 2.70 (d, *J* = 10.9 Hz, 1H), 2.64 – 2.55 (m, 1H), 2.13 – 1.91 (m, 4H), 1.88 – 1.73 (m, 3H), 1.72 – 1.56 (m, 4H), 1.51 (dddd, *J* = 13.7, 7.6, 5.9, 1.5 Hz, 1H), 1.42 – 1.30 (m, 1H), 1.14 (s, 3H) ppm.

^{13}C NMR (126 MHz, Chloroform-*d*) δ = 178.51, 172.92, 161.70, 159.26, 157.58, 132.01, 116.38, 107.33, 103.95, 98.63, 94.27, 70.46, 62.91, 55.61, 55.49, 54.64, 53.95, 53.65, 51.63, 51.60, 51.12, 44.43, 39.00, 36.67, 31.63, 28.20, 27.59, 16.29, 14.82 ppm.

ESI-MS *m/z* [M+H]$^+$ (%): 482 (19).

HRMS (ESI) for C$_{28}$H$_{32}$KO$_7$ [M+K]$^+$: calcd. 519.1780, found 519.1784.

4.4 Biological testing of prepared chemicals for their anthelmintic activity

All test were carried out by Dr. Alena Kadlecová and their co-workers (Department of Experimental Biology, UP) according to the following protocol.

The short-term toxicity of selected prepared substances and their effect on the diversity of worms *C. elegans* was tested using a chitinase test and visual evaluation of worms under a microscope. Age worm synchronization was performed prior to testing. Freshly hatched L1 larvae of *C. elegans* were counted and diluted to the required concentration of 200-300 worms per 1 ml of medium. For screening (testing at two concentrations), 10 ml of suspension was required per 1 test dish with 3 mg/ml bacteria. A solution of larvae in S-complete medium together with ZR bacteria in LB medium was transferred to the wells of a microtiter plate at a concentration of 15-30 worms per well. The substances were tested in triplicate at two concentrations: 500 µmol/l and 50 µmol/l. Ivermectin (a substance used as a highly effective anthelmintic) at concentrations of 10 µmol/l and 1 µmol/L was used as a positive control. 1% DMSO was used as a negative control. The plate was covered with foil and allowed to incubate on a shaker (150 rpm) at 20 ° C for 4 days, during which time the worms reach adulthood and begin to multiply. At the end of the incubation period, the worms in each well were visually evaluated under a light microscope (Leica DM IL LED) and then the chitinase test itself was performed to determine chitinase activity. 1.5 µl of 0.8µmol/l solution of 4-methylumbelliferyl β-D-N, N´, N´´-triacetylchitotrioside, which was the substrate for chitinase, was added to each well, and the plates were incubated at 38 ° C for 1 hour. The reaction was then stopped by the addition of 30 µl of chitinase assay buffer (per 1000 ml: 1000 ml H$_2$O; 40 g NaOH; 75 g glycine; pH 10.6) and the fluorescence at 360 nm excitation and 460 nm emission was measured on a Tecan (Tecan Infinite). 200 PRO, Switzerland).

5. Results

5.1 Synthesis of key GA₃ sugar conjugate (compound 22)

According to the procedures given in chapter 3.3. conjugate **22** synthesis was targeted. The success was only partial and the result is disappointing since we failed to obtain the conjugate **22**. As can be seen (Figure 5), developed and obtained with partially success. The closest intermediate to compound 22 - 11 was successfully synthesized. The last step of the synthesis (deesterification) failed (Figure 5). All described synthetic steps were optimized to lead to the highest yields and purities obtained substances. The actual work was based on commercially available gibberellic acid (GA₃, **1**) that served as a starting point in our synthesis. As far as intermediate **11** the synthesis proceeded well, however, the last step, DMB protecting group removal, failed. The desired compound **23** was never obtained. In this chapter only display of selected important results is showed. The detailed discussion is in the next chapter, chapter 6.
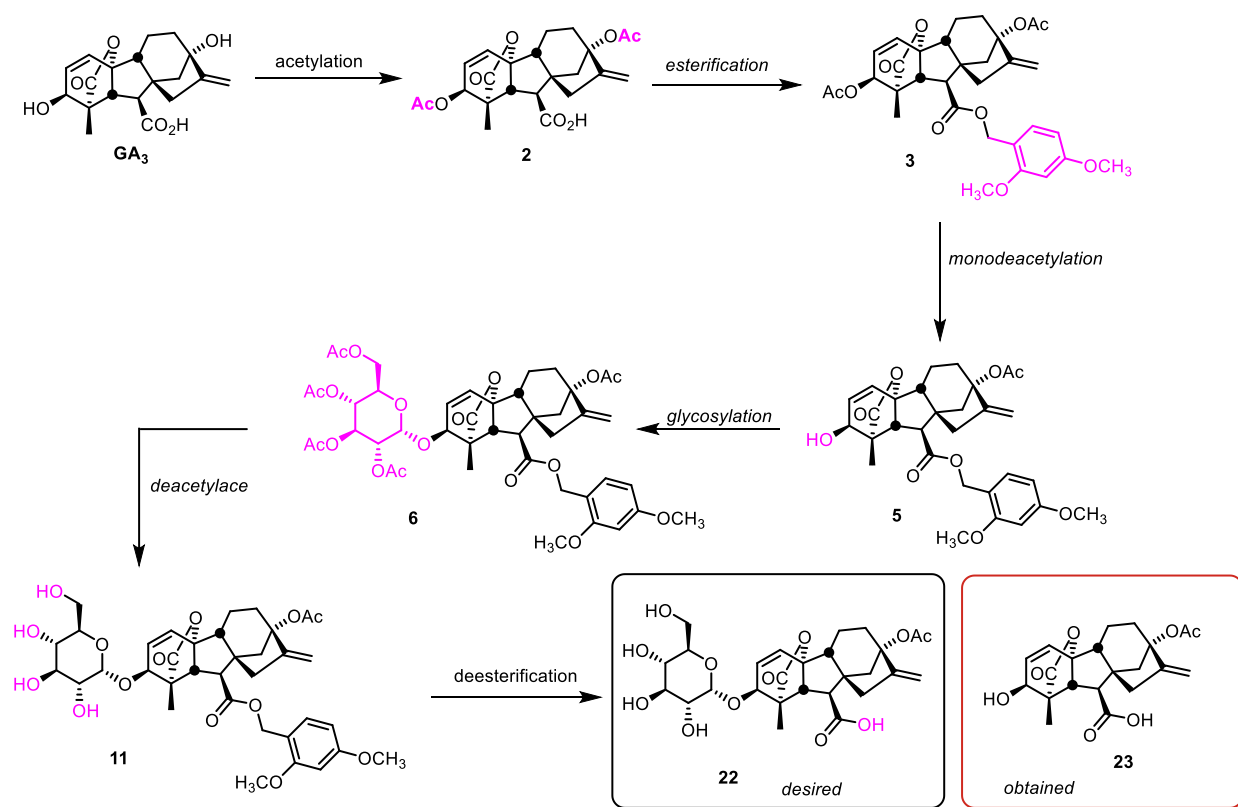
Figure 5. Synthesis of key conjugate 22: key steps and final disappointments.

The first step in the synthesis that should pave the way from GA₃ to conjugate **22** was the deacetylation reaction (Scheme 1). Compound 3,13-diacetylated GA₃ (**2**) was then the product of this reaction. The reaction was performed in pyridine as solvent with help of Ac_2O. The result of the reaction was the acetylation of the positions C-3 and C-13, while C7 carboxylic acid was left untouched. The reaction proceeded with very high yield (98%).
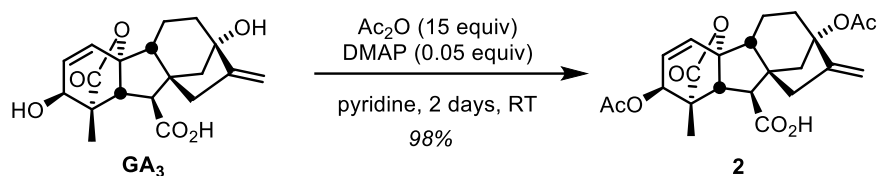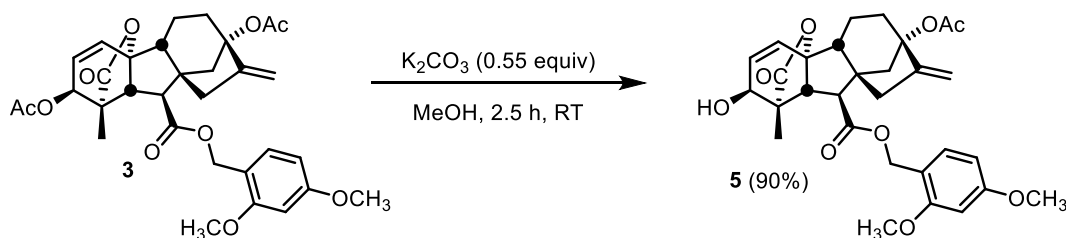
Scheme 1

Next, GA$_3$-dimethoxybenzyl ester (**3**) was prepared (Scheme 2). In this reaction, diacetylated compound **2** was reacted with DMB-alcohol using standard coupling agent (EDC·HCl) and DMAP to catalyze the transformation. The C-7 carboxylic group protection, formation of compound **3**, was achieved with 90% isolated yield. Interestingly, side product **4** was also formed.
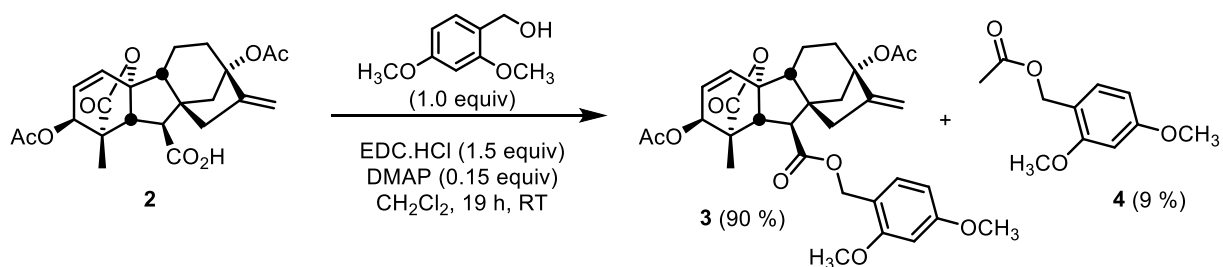

Scheme 2

The third step of synthesis was selective deacetylation of compound **3** at C3 position (Scheme 3). This reaction was performed in methanol with help of sub-stoichiometrical amount of K$_2$CO$_3$. Reaction was fast and successful and yielded compound **5** in 90%.

Scheme 3

Having in hands compound **5**, glycosylation of C3 hydroxy group in it was attempted (Table 4). First the reaction was performed in acetonitrile in presence of silver(I) oxide and silver carbonate. After 48 hours no product **6** was detected (TLC and NMR). The same transformation was then attempted with Ag$_2$O and TBAI in DCM. After some optimalization, the compound **6** was obtained in 58% yield.

Table 4. Synthesis of compound 6 – reaction optimization
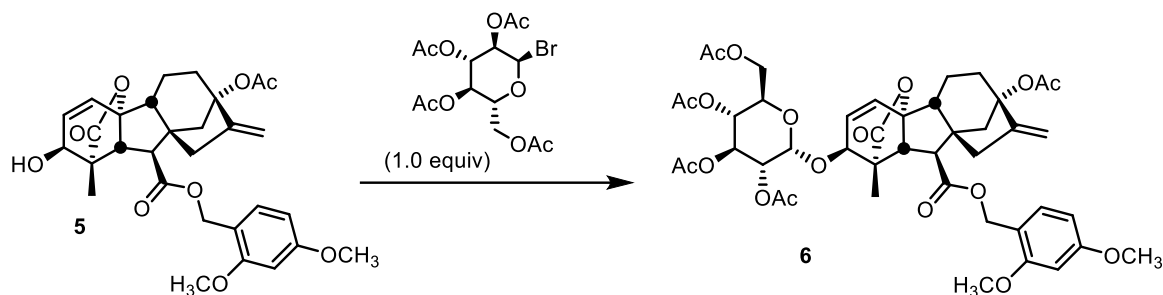
Number	Conditions	Yield (%)	Comp. 5 (mg)
1	Ag$_2$O (1.5 equiv), Ag$_2$CO$_3$ (3.0 equiv), CH$_3$CN, 2 days, RT	-	100
2	Ag$_2$O (1.5 equiv), Ag$_2$CO$_3$ (3.0 equiv), CH$_2$Cl$_2$, 2 days, RT	-	100
3	Ag$_2$O (1.5 equiv), TBAI (1.5 equiv), CH$_2$Cl$_2$, RT, 6 days	33	100
4	Ag$_2$O (3.0 equiv), TBAI (3.0 equiv), CH$_2$Cl$_2$, RT, 6 days	58	500

Next, the acetyl group removal was attempted (Table 5). Several conditions were attempted, but the use of NH$_3$ followed by freshly generated MeONa proved to be the best.

Table 5. Optimalization of preparation compound 11
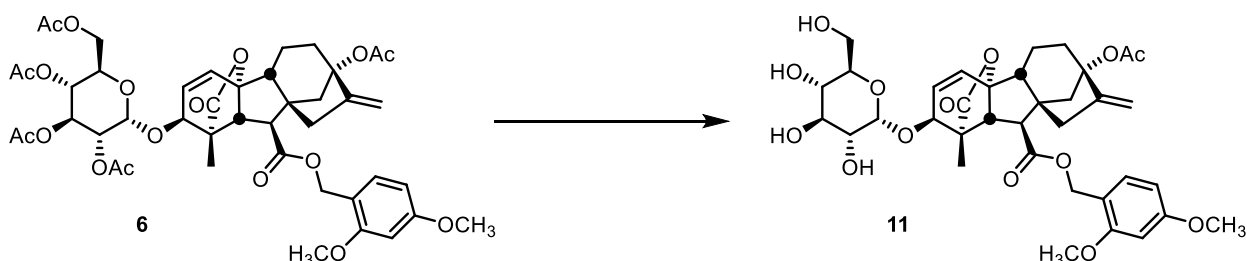
Number	Conditions	Yield (%)	Mass (mg)
1	NH$_3$ (4 equiv), CH$_3$CN, 5 days, RT	-	50
2	NH$_3$ (4 equiv), NaOMe (0.15 equiv), acetonitrile, 3 h, RT	70	50
3	NaOMe (0.15 equiv), CH$_3$CN, 3 days, RT	-	395
4	NaOMe (1.3 equiv), CH$_3$CN, 2 h, RT	54	395

The last step of the synthesis that should lead to conjugate **22**, was the C7 ester hydrolysis in compound **11**. Reaction was carried out using the standard laboratory protocol using $ZnCl_2$ I CH_2Cl_2. Unfortunately it was observed that the reaction yielded not only free acetic acid, but also the product of glucose hydrolysis, compound **23** (Scheme 4).
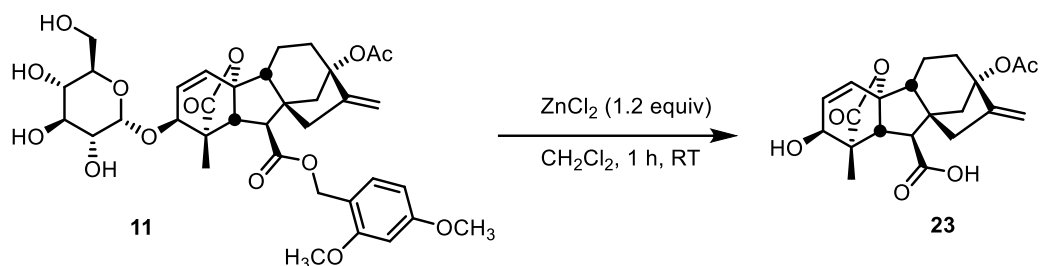
*Scheme 4. C7 ester hydrolysis of **11** that yielded undesired product **23**.*

5.2 Towards the synthesis of GA₄ sugar conjugate

According to the procedures given in chapter 4.3. I was trying to develop a method suitable to the synthesis of GA_4 conjugate with glucose (Figure 6). First, GA_4, a starting material of the synthesis, had to be prepared since only GA_4/GA_7 mixture of compounds is commercially available at reasonable prize. In the following steps, similar coupling/protection/deprotection sequence as was used in the case of GA_3 conjugate preparation was used. Unfortunately, the deprotection steps failed and therefore the desired product was not obtained.

The first step of the synthesis was the GA_4 isolation from the GA_4/GA_7 mixture. Thus, mixture of those two compounds was reacted with Ac_2O and C3 hydroxy group was transformed to the corresponding acetylated product (Scheme 5). Acetylation was performed in pyridine and the desired products **7** and **8** were isolated in 95% yield.
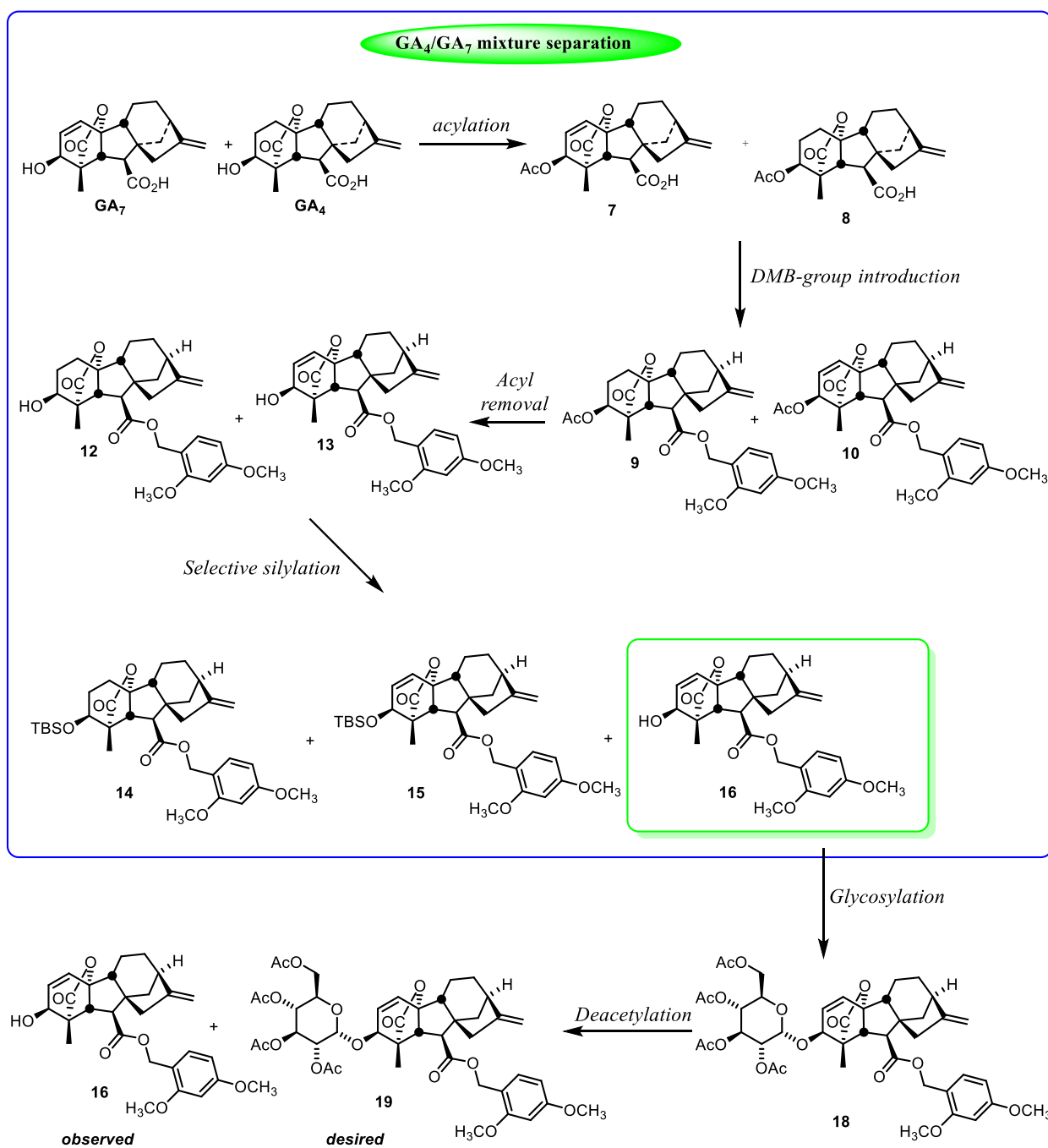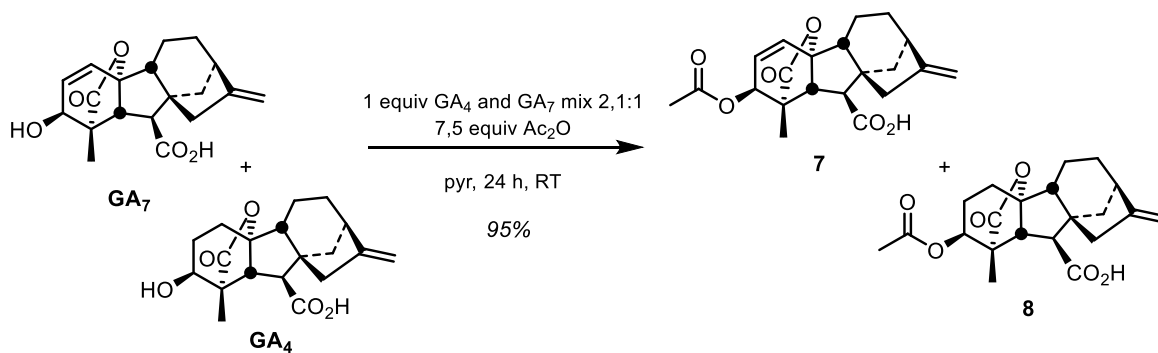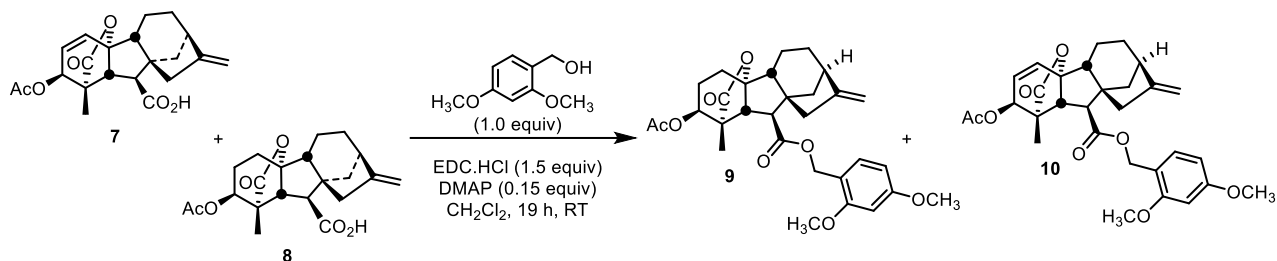
*Figure 6. Towards the synthesis of GA₄ bioconjugate.*

Scheme 5

In the next step, C7 carboxylic acid in **7/8** compound mixture was transformed (esterification) to the corresponding DMB-ester and yielded the desired compounds **9** and **10** in 83% overall yield (Scheme 6). The reaction was performed in DCM with help of EDC·HCl coupling agent and DMAP catalyst.
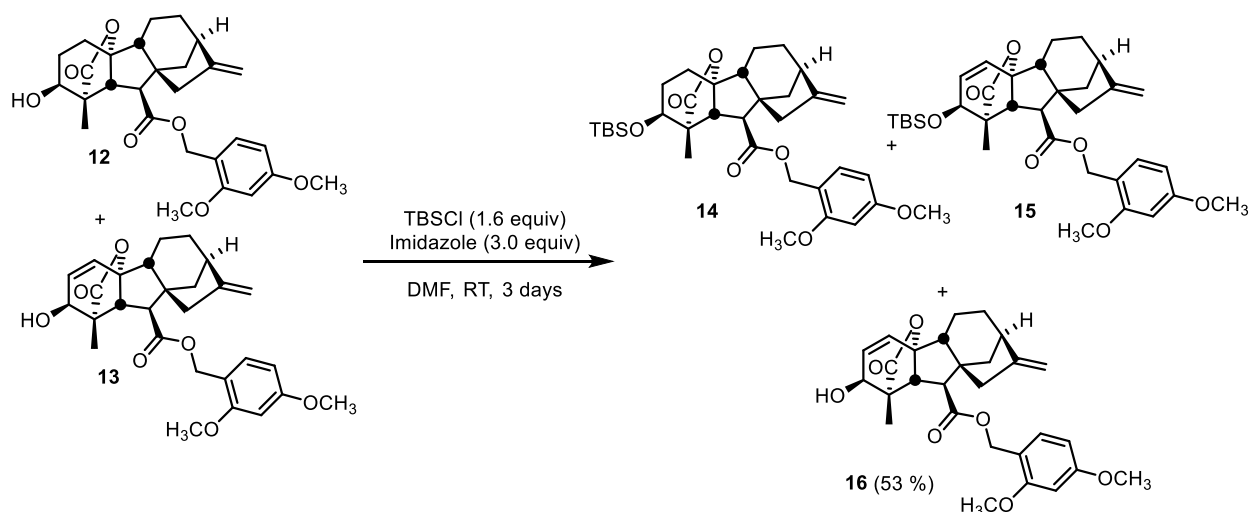

Scheme 6

At this point we tried to separate chromatographically compounds **9** and **10**. However, regardless the separating conditions (eluents) and sorbents (SiO_2, basic Al_2O_3) tried, no success was achieved.

Next, C3 acetyl group removal was carried out using the sub-stoichiometric amount of K_2CO_3 in MeOH. Gratifyingly, the reaction met with success and the desired product **12** and **13** mixture was obtained in 71 % yield (Scheme 7).
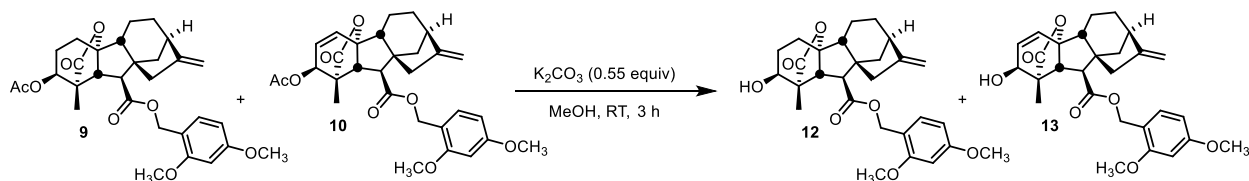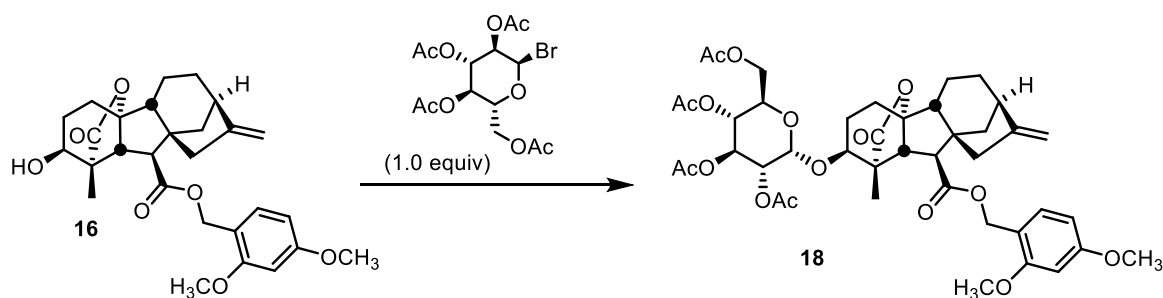

Scheme 7

Having free C3 hydroxy group, selective silylation of C3 hydroxyl was attempted. It was expected that the higher reactivity of allylic alcohol will lead to its etherification, while GA_4-placed C3 hydroxyl will be left untouched. The silylation was done in DMF in the presence of imidazole (base) and TBSCl (silylating agent). Using such conditions, GA_4-dimethoxybenzyl ester (**16**) was isolated in 53% yield (Scheme 8).
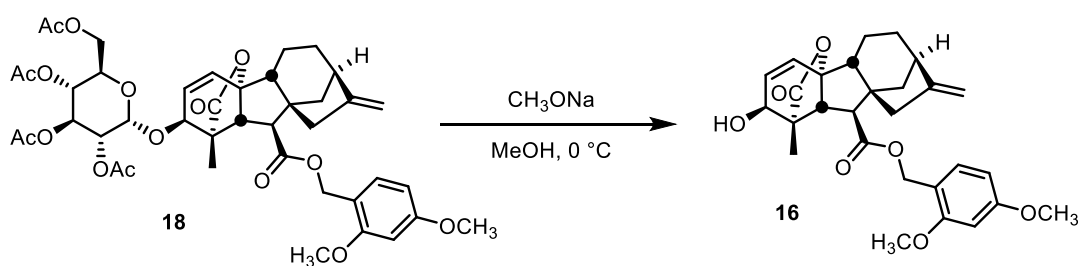
Scheme 8

Having protected GA$_4$ in hands (compound **16**), the glycosylation could be attempted (Table 6). In this case, the same conditions that were developed for the GA$_3$-conjugate **11** synthesis were employed. The reaction took 6 days and yielded the desired product in 43% yield.

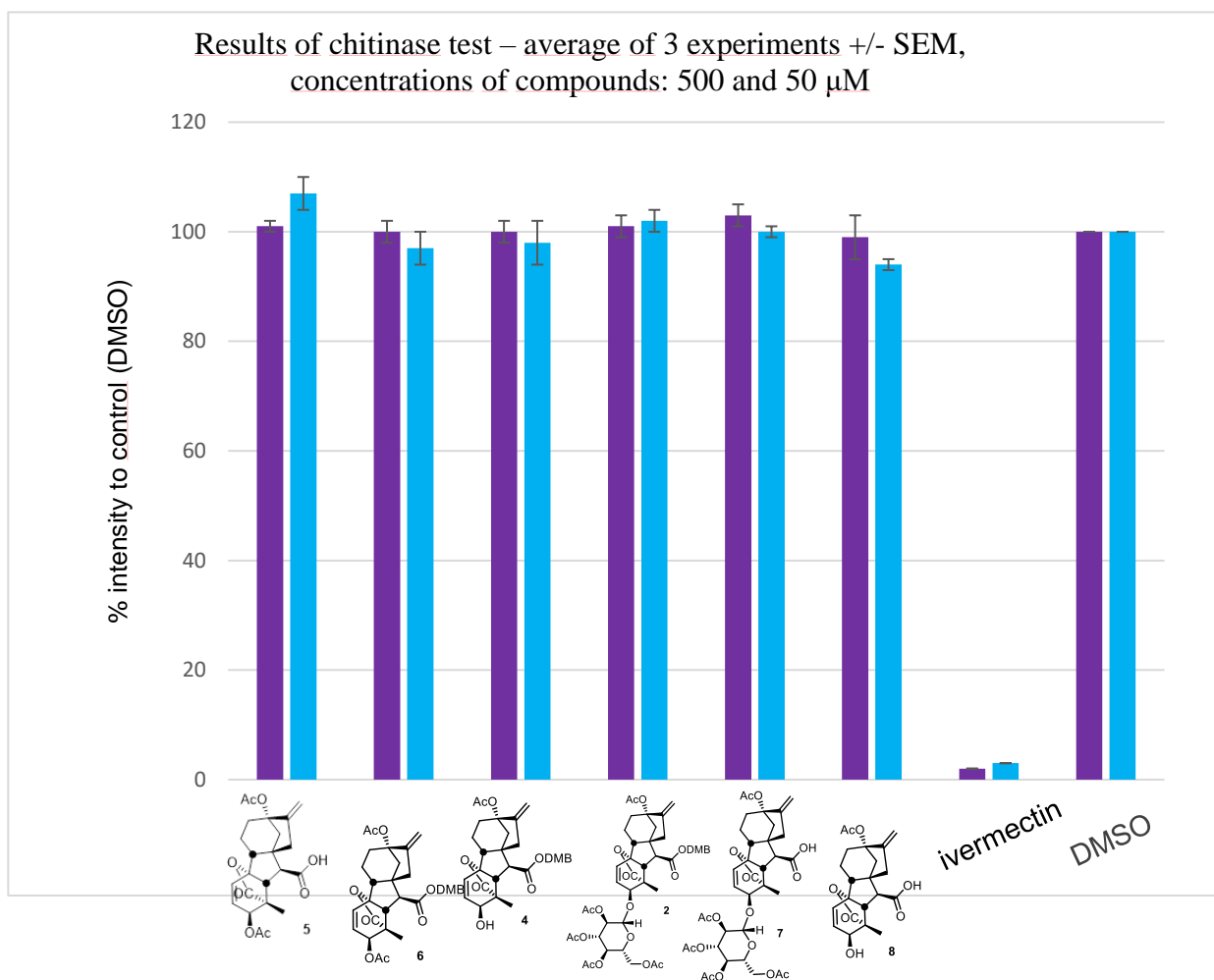*Table 6. Optimalization of synthesis compound 18*

Number	Conditions	Yield (%)	Mass (mg)
1	Ag$_2$O (3.0 equiv), TBAI (3.0 equiv), CH$_2$Cl$_2$, RT, 6 days	~35	593
2	Ag$_2$O (5.0 equiv), TBAI (5.0 equiv), CH$_2$Cl$_2$, RT, 6 days	43	261

Unfortunately, when the deacetylation step was attempted (Scheme 9), it was observed that the compound **18** undergoes to the deglucolysation as well. As a consequence, only product **16** was isolated after attempted deacetylation.

Scheme 9

40

5.3 Anthelmintic activity of prepared compounds

Results of chitinase test – average of 3 experiments +/- SEM, concentrations of compounds: 500 and 50 μM

6. Discussion

The aim of my bachelor thesis was the synthesis sugar conjugates **22** and **19**. Both compounds are already known in the literature, however, even after tremendous efforts in our group, previously published methods proved to be irreproducible in our hands (unpublished results). The goal of their synthesis is linked to their function in plants – commonly accepted "storage" function was never actually experimentally proved. In addition, we expect that their use in combination with additional experiments could allow us to shed more light into the transport of GAs in plants.

But to do so, compounds must be firstly prepared. The synthetic protocols (chapter 4) and my results (chapter 5) are gathered on several previous pages. In addition, selected prepared compounds were also tested on their anthelmintic activity[16] in the group of Dr. Kadlecová (Department of Experimental Biology, UP). In this chapter I would like to discuss obtained data in greater details.

6.1 Organic synthesis of GA$_3$ sugar conjugate

The main purpose of this part was to synthesize the conjugate **22**. Success of the synthesis depended mostly on the proper choice of the protecting groups. For GA$_3$ protection, acetyl groups were chosen as the protecting groups for C3 and C13 hydroxyl groups and 3,4-dimethoxybenzyl group for C7 carboxylic acid. The important criteria were the stability of C3 and C13 protecting group in term of synthetic transformations used prior their removal in lasts steps of the synthesis. C7 protecting group then had to be readily removable without the newly created glycosidic bond corruption.

Synthesis of key conjugate **22** begun from non-selective acetylation of GA$_3$ that yielded peracetylated GA$_3$, compound **2**. According to the literature[16,24] the most common and robust stable hydroxy protecting group in gibberellins is a acetyl group, thus the choice of it was clear. The attachment of the acetyl groups was done using simple acylation mediated by pyridine, and the desired product was obtained in 98 % yield. This reaction was previously developed and explored in our group and therefore the smooth progress and yield of the transformation were not surprising.

The second protecting group introduced to the C7 carboxylic acid was 2,4-dimethoxybenzyl. The reaction was carried out using standard esterification protocol based on the use of imidates that results in the formation of the urea derivatives as a side product along with the targeted ester function. The great advantage of this protocol are mild and quasi neutral reaction conditions employed for this transformation. I used EDC·HCl coupling agent and the whole process was catalyzed with DMAP. In overall manner, the desired product was isolated in 60% yield.

Interestingly and surprisingly, dimethoxybenzyl acetate **4** (30%) was isolated as a side product of the transformation. Such product was not in context of such type of coupling previously reported in the litterature[24] and it is not clear at the present stage how it could be formed.

The third step of synthesis was selective deacetylation of C3 hydroxyl group in compound **3**. Taking in account that C3 hydroxyl group is less sterically hindered and allylic, the base promoted hydrolysis of C3 ester function in the presence of C13 (tertiary alcohol) and C7 ester group (steric hindrance due to the rings presented) could be achieved. This reaction was performed in methanol with help of sub-stoichiometric amount of K_2CO_3. The desired product **5** was formed with 90% yield and complete regioselectivity since no product of C13 or C7 esters hydrolysis was observed.

Having free C3 hydroxy group, glycosylation reaction of it could be performed. Various conditions were attempted (Table 4), but soon it was evident that Ag_2O has to be used as a base. Silver(I)oxide allows the activation of the base present on peracetylated bromoglucose and at the same time is sufficiently basic to deprotonate C3 hydroxy group. If more powerful base would be used, a competitive A-ring opening (retroaldol reaction) in compound **5** would occur (previous results in the group). What was surprising was the use of TBAI. TBAI in the reaction served as a transhalogenating agent that replaced bromine atom in bromoglucose with more reactive (and less stable) iodine atom. As a consequence, faster formation of oxonium cation that was later on captured with C3 hydroxyl could occur.

Glycosylation was followed by deacetylation of GA_3-glucoside ether (**6**). Our first planned reaction was performed in acetonitrile in presence of ammonia at RT, but no reaction occurred. The reaction conditions were then changed[16] to more reactive *in situ* generated MeONa. After 3 hours the reaction was completed and yielded the desired product in 70%. At this point it should be noted that the sodium methoxide has to be freshly prepared by dissolving metallic sodium in MeOH.

The last step of the product **22** synthesis was the ester hydrolysis. Reaction was carried out using the standard group protocol using $ZnCl_2$ in DCM. The reaction proceeded smoothly, however, at the same time glycosidic bond was also broken and released undesired GA_3-monoacetyl **23**. This step failed presumably due to a week ether bond between C-3 hydroxyl and deacetylated unprotected glucose.

There are different possibilities how I could avoid it. E.g. I could try to inverse order of steps (ester hydrolysis prior to deacetylation). In this case glucose would still be protected during the ester hydrolysis and it could remain intact. The second possibility would be to change the protecting groups on glucose to benzyl groups. which would makes glucose more stable and at the

same time, readily removable using hydrogenation. Also, I could change primary protecting groups on C3 and C13 to dimethoxybenzyl ones which would make removing of protecting groups easier since it would proceed in one pot. Unfortunately, I had no time to explore such possibilities.

6.2 Organic synthesis of GA$_4$ sugar conjugate

The goal of this approach was to prepare the conjugate of glucose with GA$_4$. The overall approach was very the same as in the case of GA$_3$ conjugate. The main difference was the starting point: there is no pure GA$_4$ available. In general, GA$_4$ is available mainly as a part of the mixture GA$_4$ and GA$_7$. To separate is not an easy task since they do differ only by one olefinic bond in the A ring. Our idea was to protect hydroxy groups as acetyls and then C7 carboxyl in form of DMB esters. Unfortunately, it was observed that in all cases, no chromatographic or crystallization method tested for their separation was successful. Thus, another step, selective C3 hydroxy group deacetylation was carried out. It was thought that additional hydroxy group (H-bonding) interactions might increase the possibility of GA$_{4/7}$ derivatives separation. We were wrong, no attempted separation method met with success again.

At this point we have decided to take an advantage in different reactivity of C3 alcohol (allylic in case of GA$_7$) in GA$_4$ and GA$_7$ towards the silylation. Thus a mixture of GA derivatives was reacted with an access of TBSCl. In such case, pure GA$_4$ could be readily isolated and used in glycosylation step.

Glycosylation proved to be relatively easy and the same conditions as those used in case of GA$_3$ derivative could be used. Unfortunately GA$_4$ conjugate with glucose proved to be less stable than expected and glycosidic bond was cleaved during the deacetylation attempts.

6.3. Biological testing of prepared chemicals

None of tested compounds showed anthelmintic activity when compared to the positive (ivermectine) and negative control (DMSO).

7. Conclusion

In my bachelor thesis I was interested in the investigation of GA$_3$ and GA$_4$ conjugates. Synthetic methods I developed proved to be suitable for conjugate-derivatives synthesis, even though the glucose GA$_3$ and GA$_4$ were not prepared.

In the theoretical part biosynthetic pathways of gibberellins and their chemical modifications with the main focus on GA sugar conjugates were discussed. In the experimental part, I managed to synthesize all key intermediates of developed pathways. In both cases however the last step of deprotection failed. Clearly such facts bit hammer the synthesis, however it opens up a new possibility to the protecting group introduction. From the gathered data I believe that the best way to successfully accomplish the synthesis would be to change the protection group of sugar part if conjugate to benzyl or benzoyl group. Groups that can be readily removed with help either hydrogenation, Lewis acid or oxidative protocol.

Finally, several prepared compounds were selected for biological activity evaluation. They were tested in group of Dr. Kadlecová for their anthelmintic activity, however no activity was observed.

8. Literature

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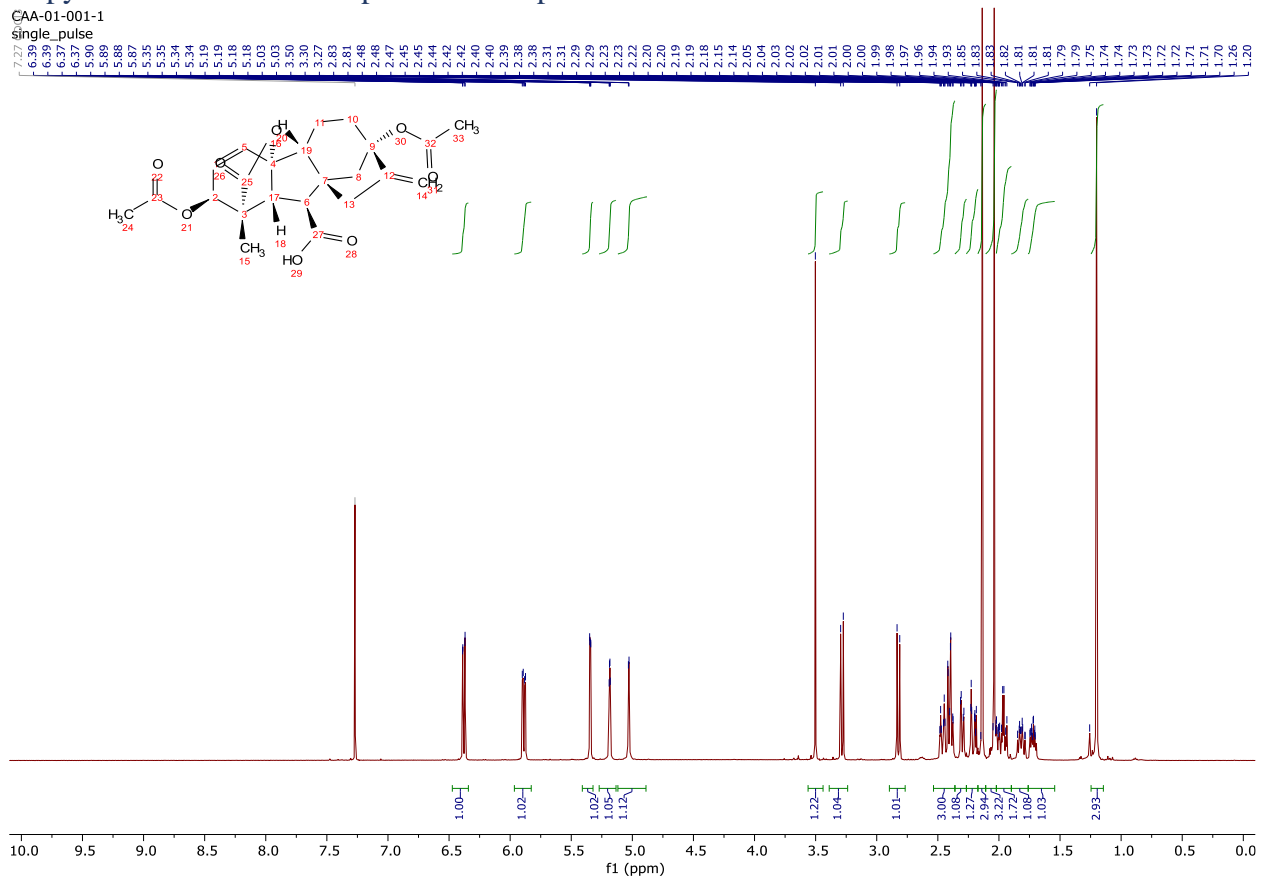
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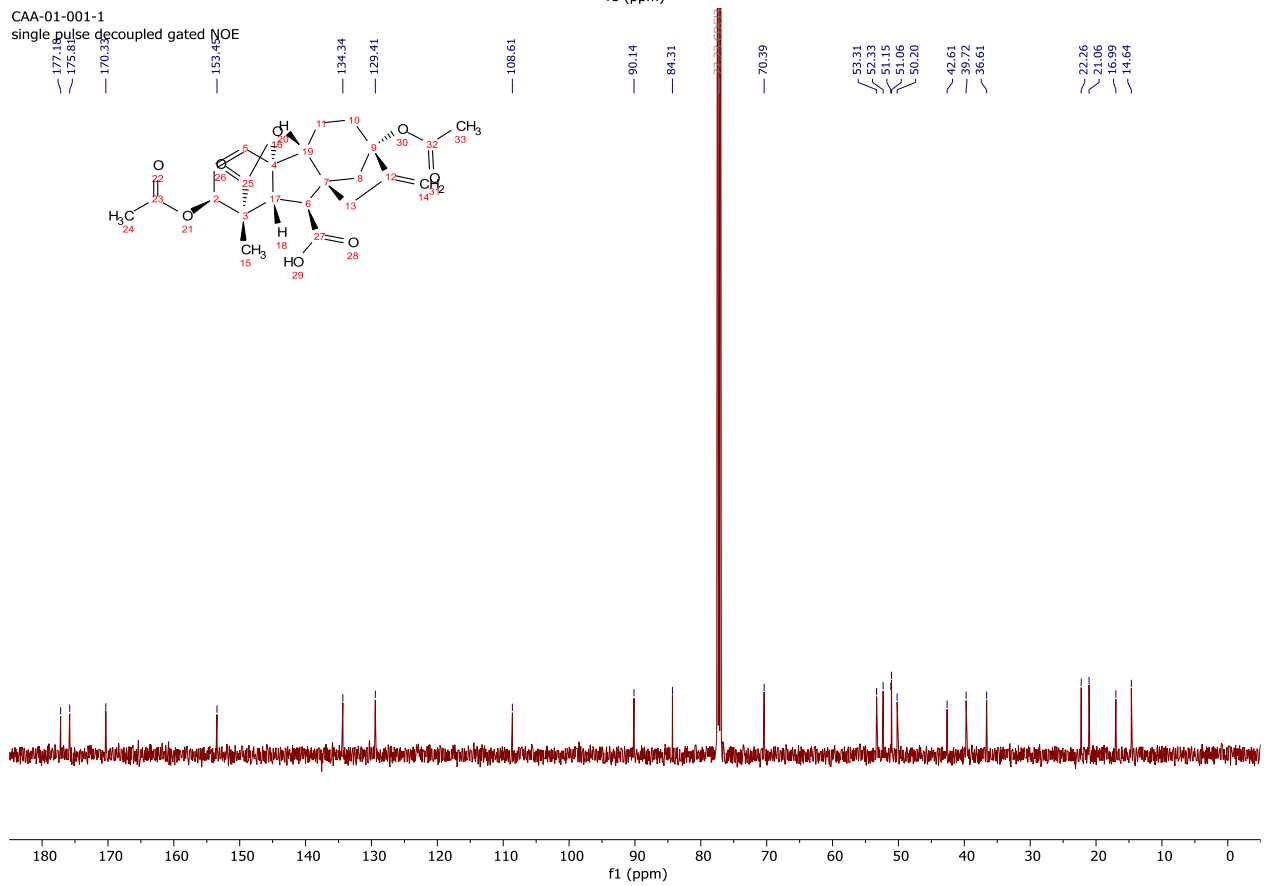
Attachments

Copy of ^{1}H and ^{13}C NMR spectra

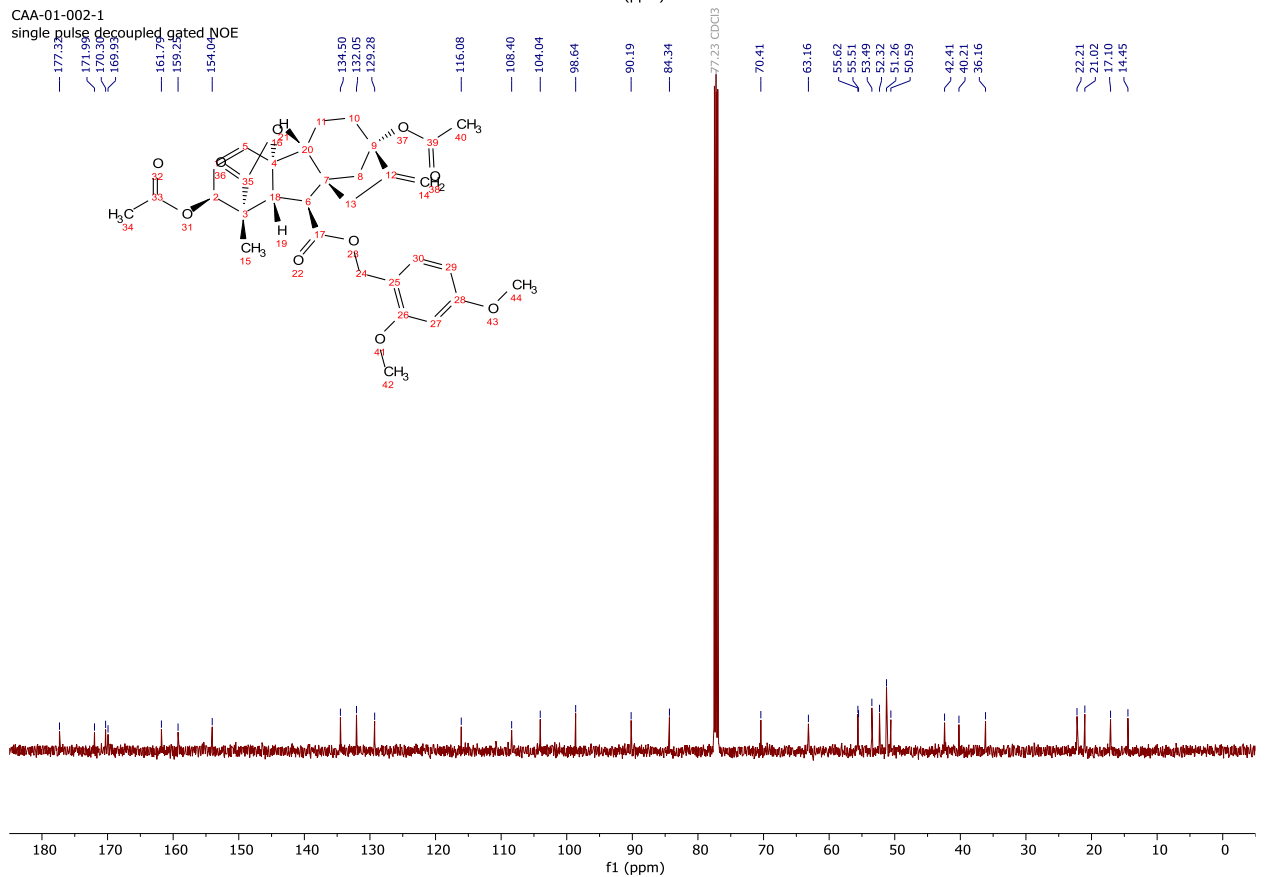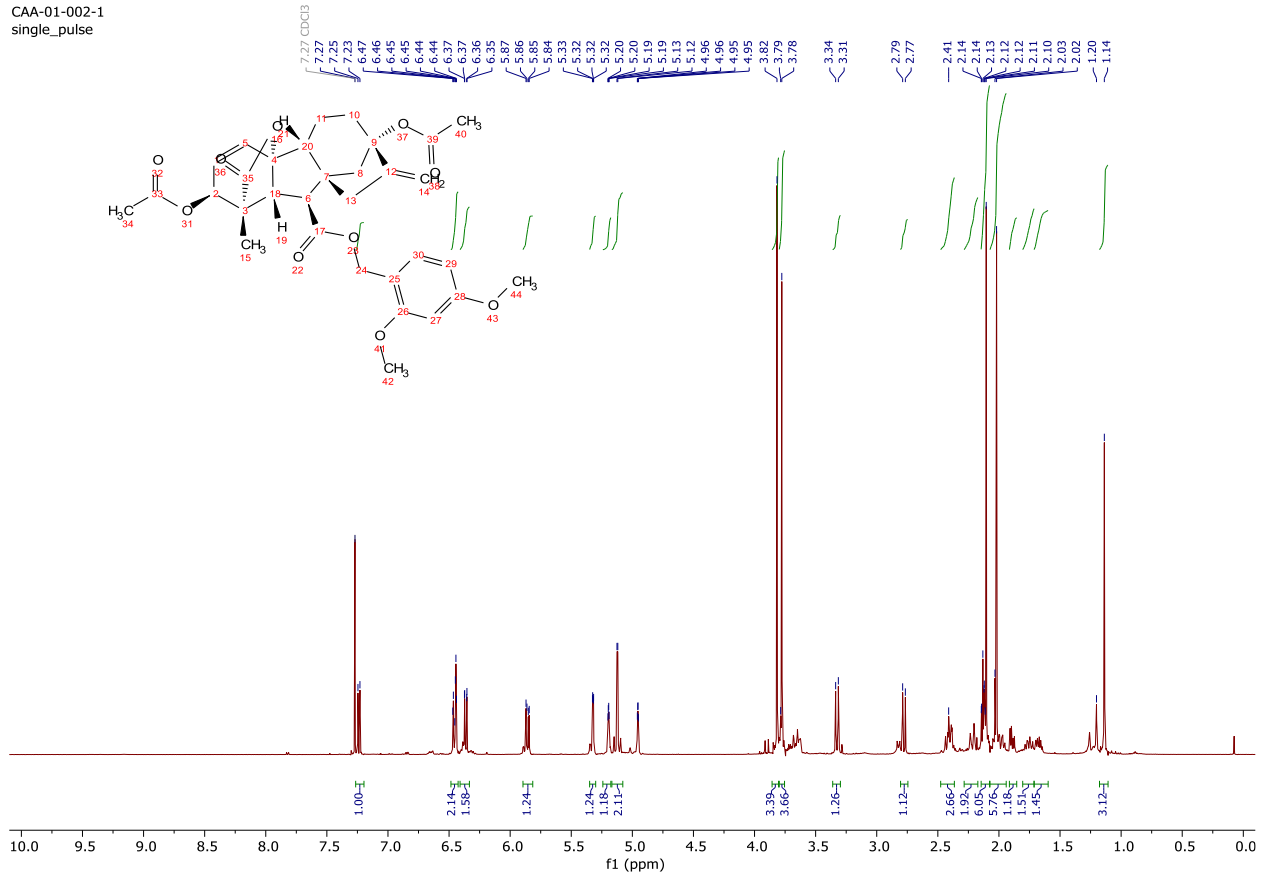## Copy of ^{1}H and ^{13}C NMR spectra of compound **2**

CAA-01-001-1
single_pulse

Copy of ^{1}H and ^{13}C NMR spectra of compound **3**

CAA-01-002-1
single_pulse

CAA-01-002-1
single pulse decoupled gated NOE

Copy of ^{1}H and ^{13}C NMR spectra of compound **5**

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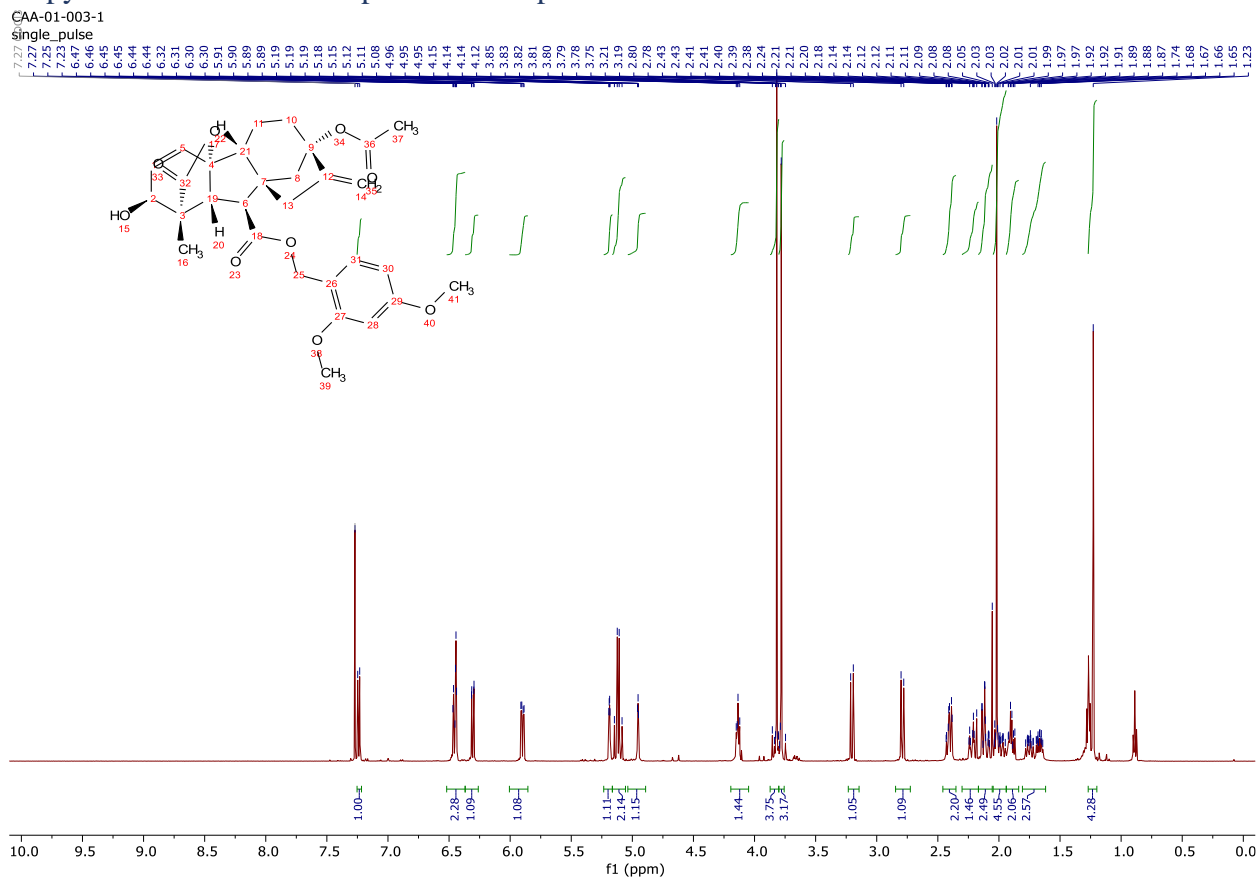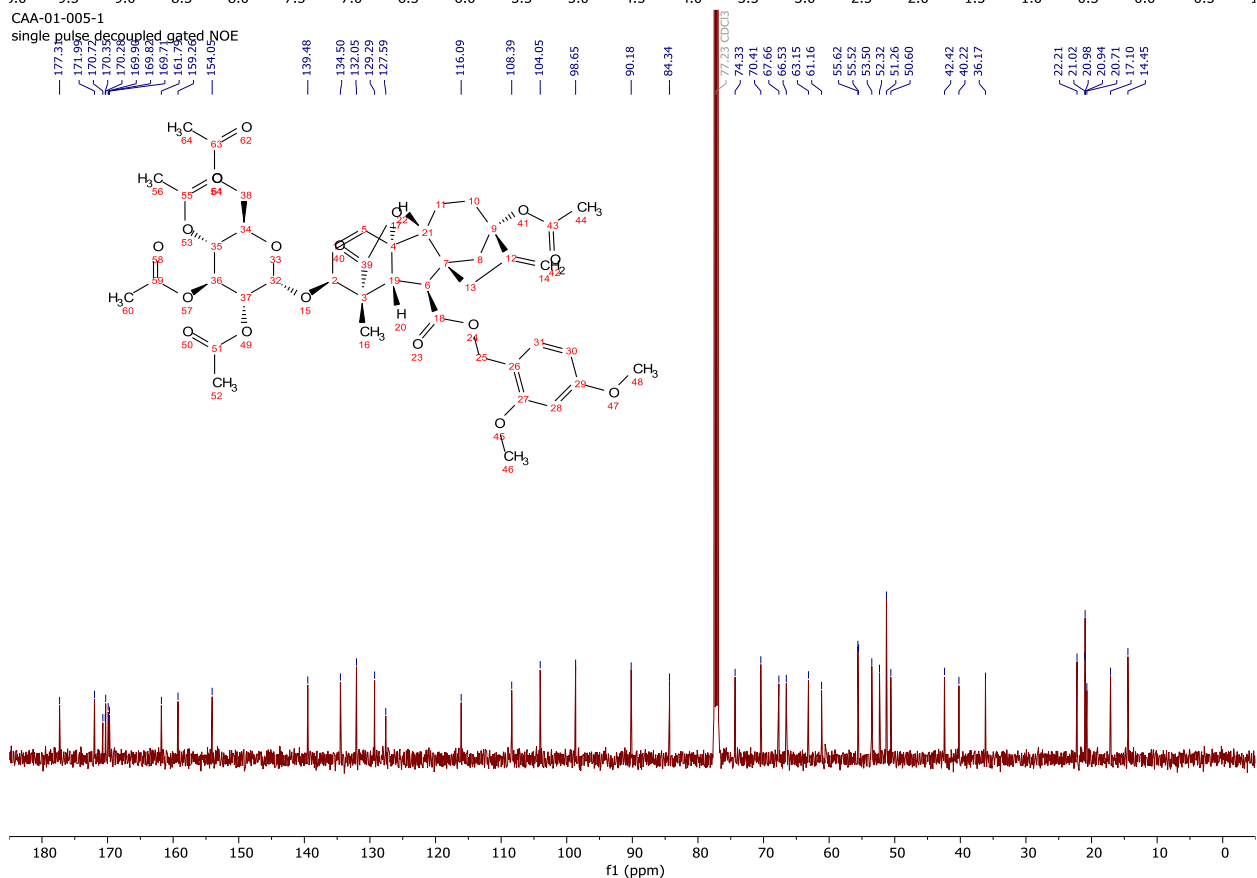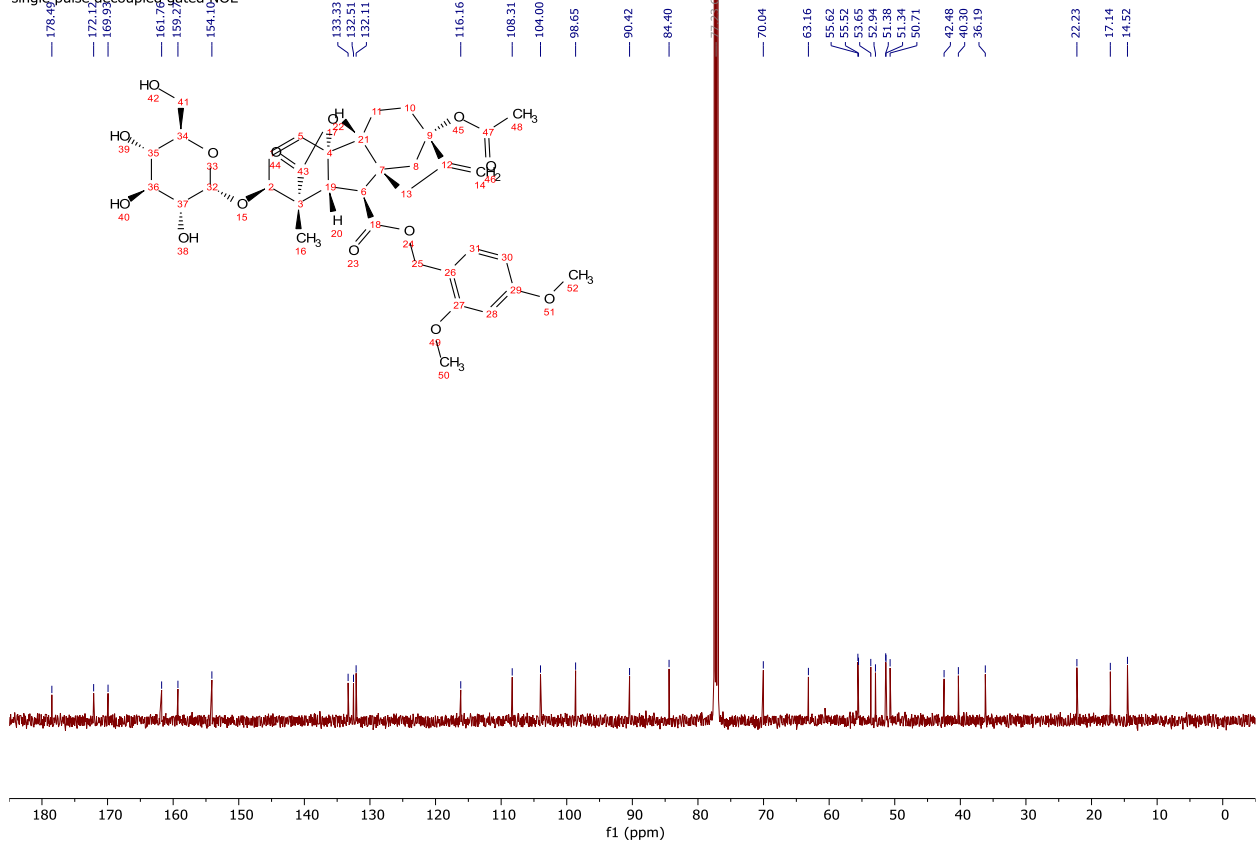single_pulse

CAA-01-003-1
single pulse decoupled gated NOE

Copy of ^{1}H and ^{13}C NMR spectra of compound **6**

CAA-01-005-1
single_pulse

CAA-01-005-1
single pulse decoupled gated NOE

f1 (ppm)
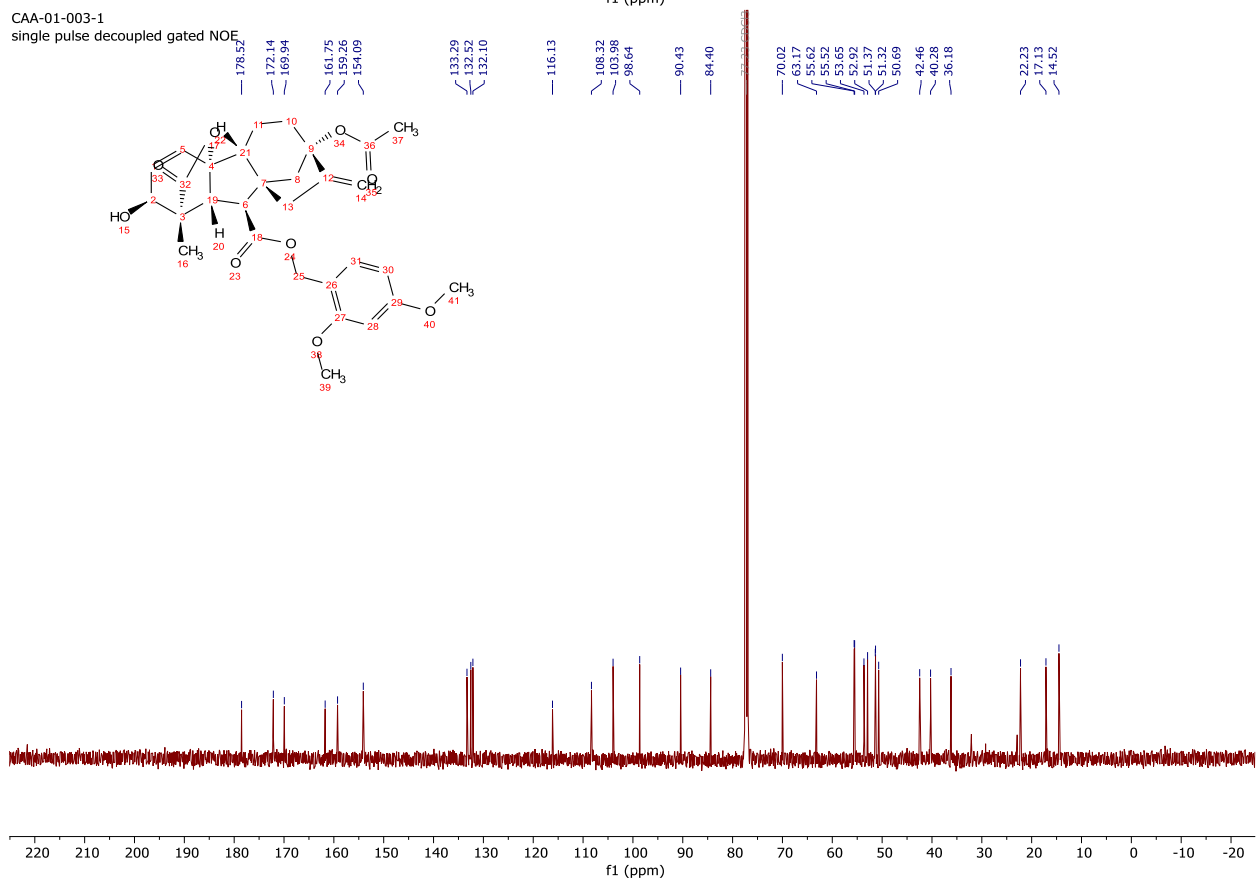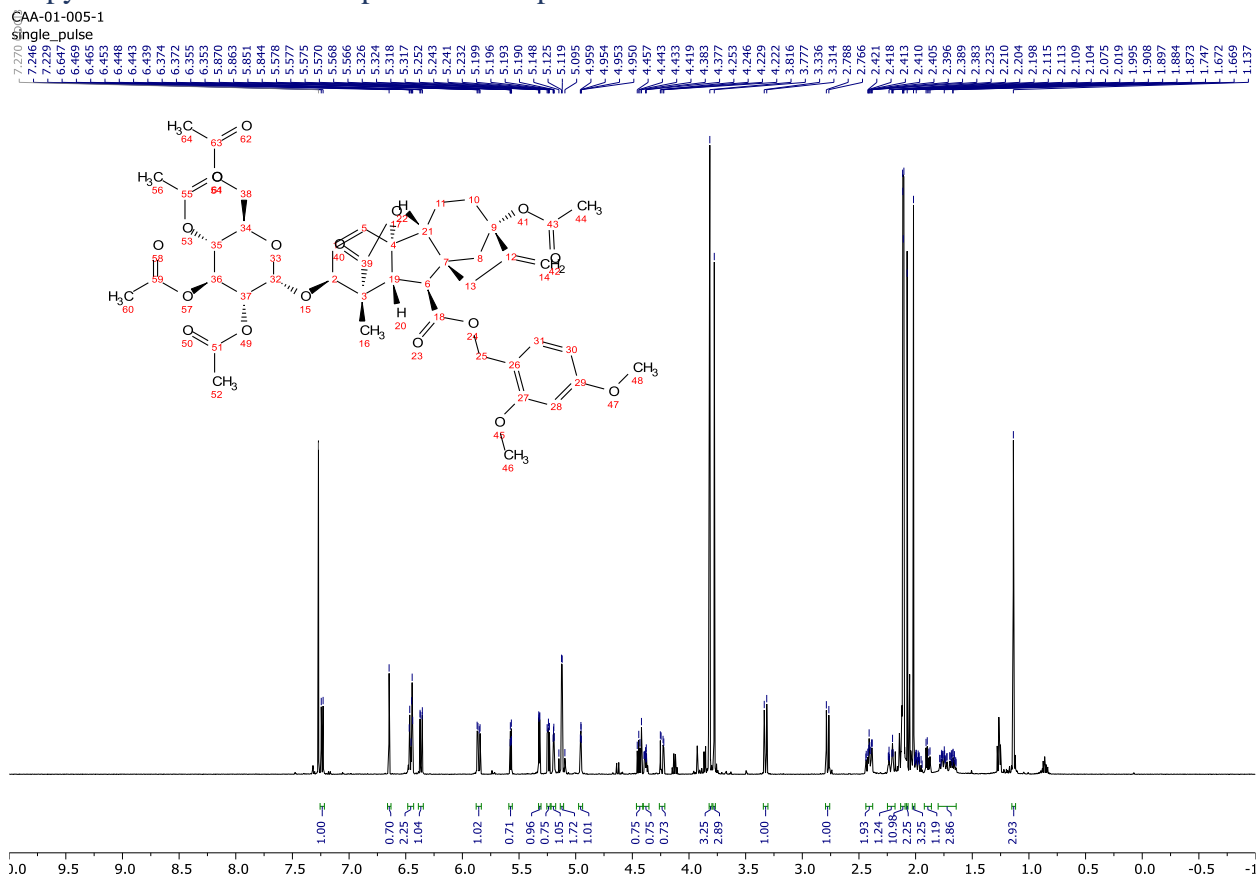
Copy of ^{1}H and ^{13}C NMR spectra of compound **11**

Copy of ^{1}H and ^{13}C NMR spectra of compound **16**
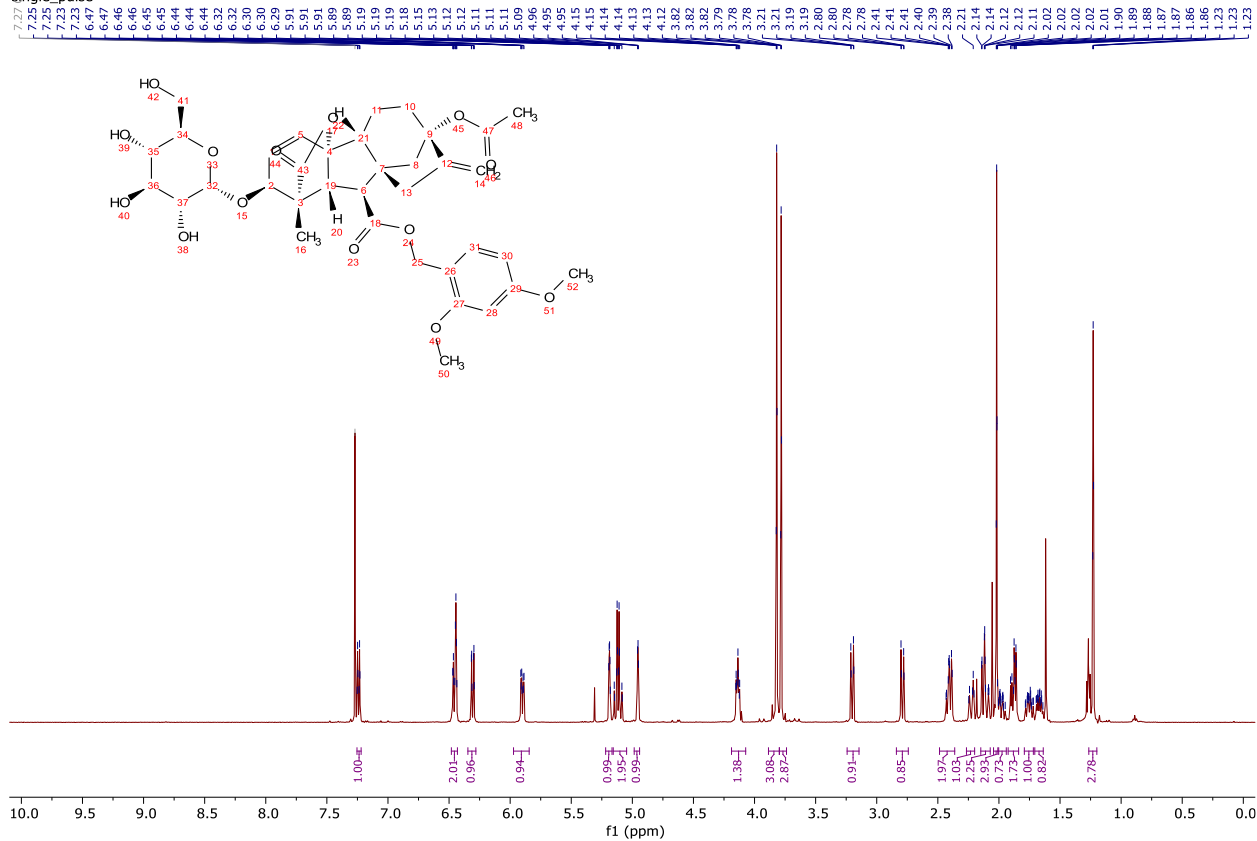
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single_pulse

CAA-01-011-2
single_pulse decoupled gated NOE
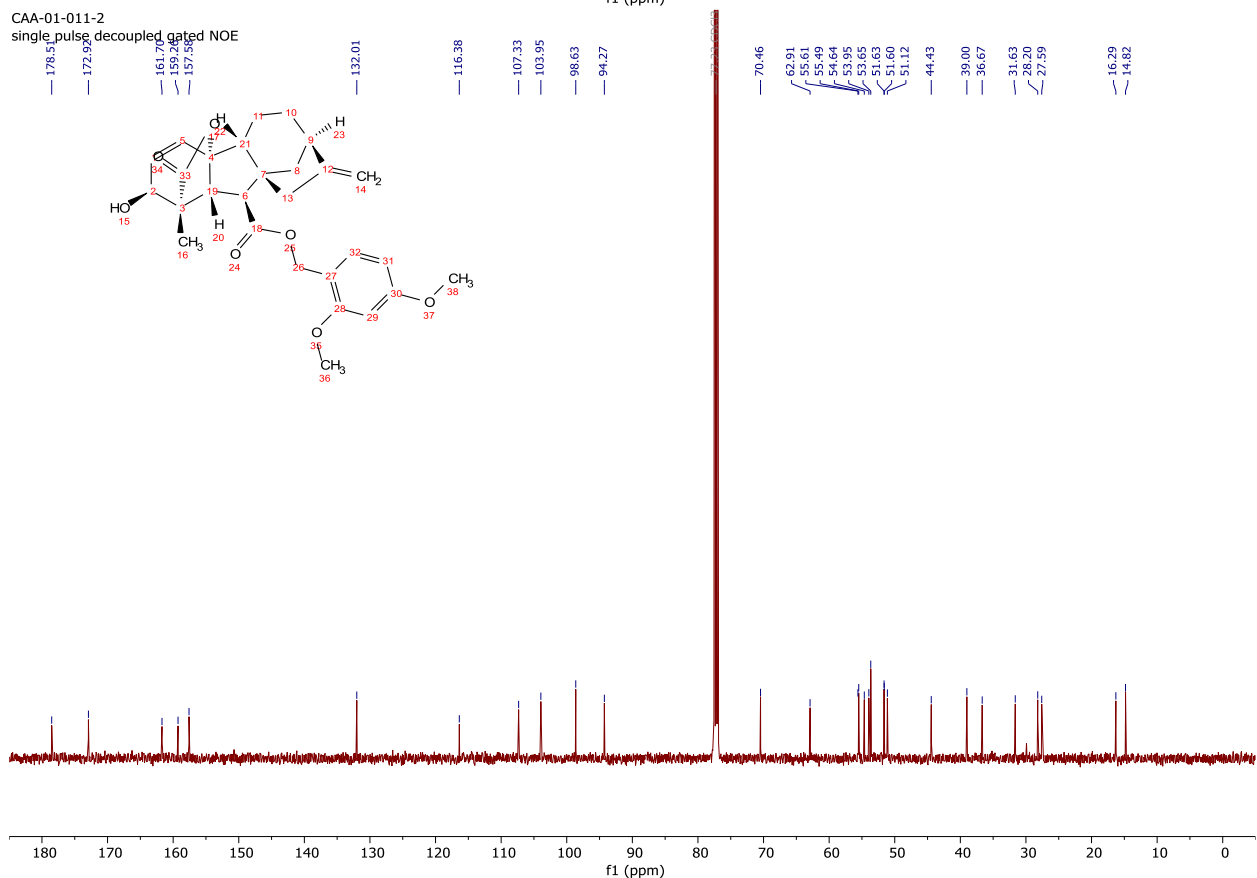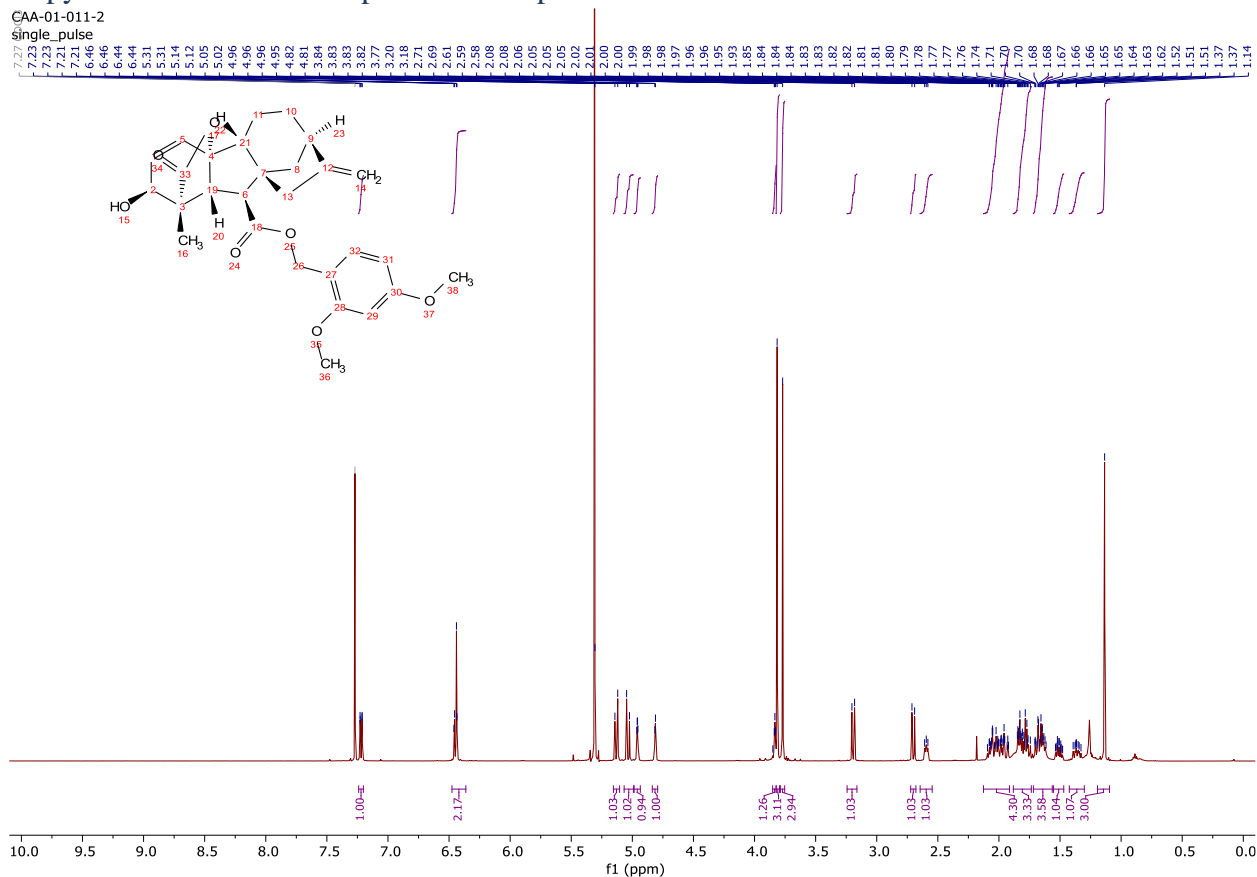
Copy of ^{1}H and ^{13}C NMR spectra of compound **23**

CAA-01-021-3
single_pulse

CAA-01-021-3
single pulse decoupled gated NOE