DISSERTATION

Synthesis of Bioactive 3-Amino-Glycyrrhetinic Acid Derivatives

ausgeführt zum Zwecke der Erlangung des akademischen Grades eines Doktors der technischen Wissenschaften unter der Leitung von

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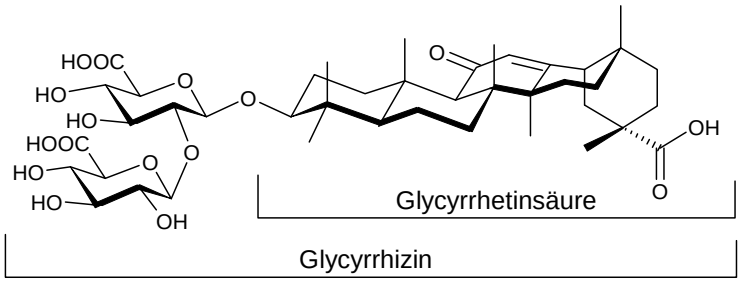
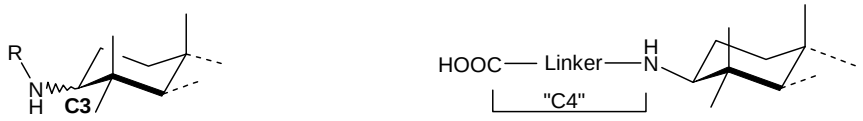
Kurzfassung der Dissertation

Glycyrrhizin (GN), ein Saponin, und sein Aglykon Glycyrrhetinsäure (GA) sind die bestuntersuchten biologisch aktiven Substanzen des Extrakts der Süßholzwurzel mit breiter Verwendung in der traditionellen Medizin. Unter anderem sind entzündungshemmende Wirkung, antivirale Aktivität sowie auch inhibierende Wirkung der Glukocorticoid-regulierenden 11β-Hydroxysteroid Dehydrogenasen beschrieben.



Auf Basis von GN und GA als *Lead*-Verbindungen wurde eine Vielzahl pharmakologisch relevanter Derivate der 3-Amino-3-deoxy-glycyrrhetinsäure (Amino-GA) ausgewählt, synthetisiert und für pharmakologische Tests zur Verfügung gestellt. Die Substanzen wurden einerseits auf ihre antivirale Wirkung in mit Influenza A Virus (IAV) infizierten Zellen hin untersucht sowie auch als potentielle selektive 11β-Hydroxysteroid Dehydrogenase Inhibitoren (11β-HSD1- und 11β-HSD2-Subtypen) getestet. Es wurden sowohl Strukturen mit als auch ohne Zuckerfunktionalitäten hergestellt. So wurden Analoga einerseits des GN und andererseits auch der GA und dereen Hemisuccinat Carbenoxolon erhalten.

3-Amino-glycyrrhetinsäure (+/- hydroxamsäure modifikation)



R=H, OMe, Ac, Succinyl, SO_2R

"Amidocarbenoxolon"
"Sulfonamido-carbenoxolon"

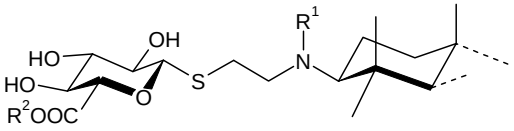
HSD1/HSD2-Inhibitoren

Durch Variation der *N*-Substitution beider C3-epimerer Amine wurden Derivate der GA erzeugt, die sich stark in Acidität (respektive Nukleophilie) der Aminfunktionalität unterscheiden. Des Weiteren wurden zusätzliche Carbonsäurefunktionalitäten sowie Zuckerstrukturen eingeführt um Glycyrrhizin zu mimikrieren. Im 11β-HSD1/-HSD2-Inhibierungsexperiment zeigte eine große Anzahl *N*-Acyl- und *N*-Sulfonylderivate erfreuliche

Aktivität wie auch Selektivität für den 11β-HSD2 Subtyp. Auf Basis dieser ersten positiven Ergebnisse wurden dieser Verbindungen formal mit dem C30 Hydroxamsäuremotiv – einem weiteren erfolgreichen Strukturelement – kombiniert, um eine zweite Generation von 3-Aminoglycyrrhetinsäureverbindungen zu erhalten.

Der zweite Teil der Arbeit befasste sich mit der Synthese *N*-basierter Neoglycoside der Glycyrrhetinsäure. Es wurde eine ganze Subfamilie von überbrückten Thioglucuroniden synthetisiert, die alle wesentlichen Strukturelemente des Glycyrrhizin aufweisen, abgesehen von der natürlichen *O*-glykosidischen Bindung, die durch eine physiologisch stabilere thioglycosidische Bindung ersetzt wurde. Die ganze Substanzklasse war inaktiv in den Enzym-Tests wies aber vielversprechende Ergebnisse im IAV-Test auf.

Überbrückte Thioglucuronide

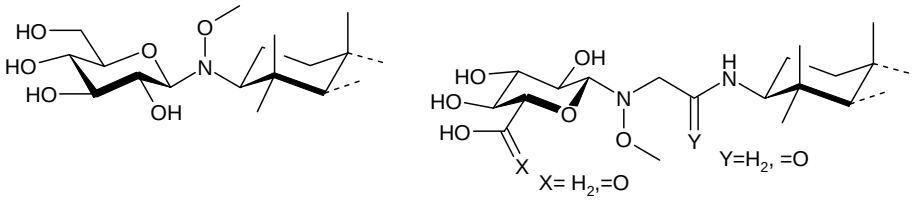


R^2=H, Me R^1=H, Ac, Succinyl

activ im IAV-Test

Zuletzt beschäftigte sich die Arbeit mit der möglichen Anwendung der Methode der *direct glycosylation* von *O*-substitutierten Hydroxylaminen mit reduzierenden Zuckern auf den Grundkörper der Glycyrrhetinsäure.

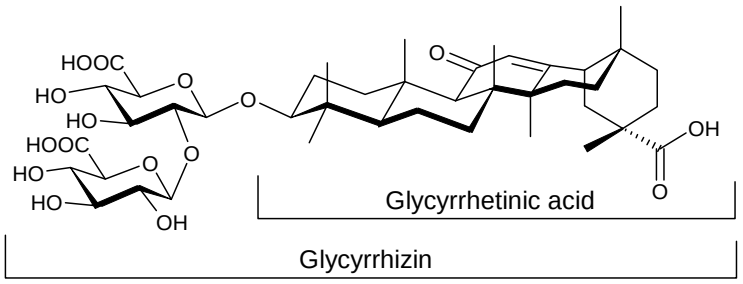
Methoxyaminoglycoside **Überbrückte Methoxyaminoglycoside**



Die ursprünglich angestrebten direkten 3β-Methoxyaminoglycoside der GA konnten zwar nicht mittels *direct glycosylation* hergestellt werden, jedoch wurde eine prototypische Verbindung mittels konventioneller Glycosylierungschemie hergestellt um die Stabilität dieser Verbindungsklasse zu belegen. Im Gegensatz dazu konnten überbrückte Methoxyaminoglycoside der GA mittels *direct glycosylation* erfolgreich synthetisiert werden. Im Sinne eines *Proof-of-concepts* wurden zwei Beispielverbindungen hergestellt, um diese Substanzklasse im Rahmen einer Diplomarbeit weiterzubetreuen.

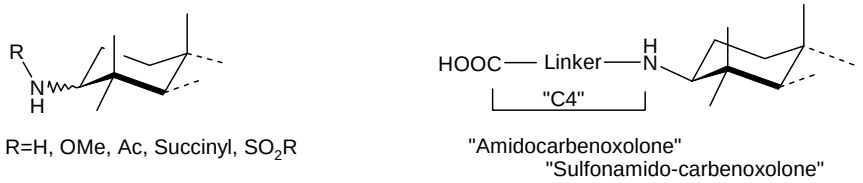
Abstract

The saponin glycyrrhizin (GN) and its aglycon glycyrrhetinic acid (GA), the main biological active compounds of licorice root, have a long tradition in natural medicine. The broad spectrum of reported activities comprises anti-inflammatory, cytotoxic, antiulcer, antiproliferative, anti-oxidative and endocrine activities. Starting from GN and GA as lead structures a variety of pharmacologically interesting compounds based on the 3-amino-3-deoxy-glycyrrhetinic acid (both diastereomers) has been envisioned, prepared, characterized and submitted for pharmacological screening.



The compounds have been evaluated as antiviral compounds (influenza A virus, IAV) and as selective 11β-hydroxysteroid dehydrogenase inhibitors. The synthesized compounds include structures with and without sugar moieties, thus mimicking the parent structures, glycyrrhizin, the corresponding aglycon glycyrrhetinic acid and its hemisuccinate ester carbenoxolone, a licensed drug with anti-inflammatory activities. Target compounds were selected in order to vary the relative stereochemistry at the 3-position, the acidity and nucleophilicity of the NH-group by installation of different residues at the amino moiety.

3-Amino-glycyrrhetinic acids (+/- hydroxamic acid modifications)



R=H, OMe, Ac, Succinyl, SO_2R

"Amidocarbenoxolone"
"Sulfonamido-carbenoxolone"

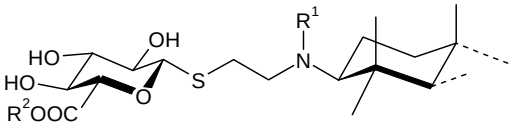
active in the HSD1/HSD2-assay

Furthermore, additional carboxylic acid functionalities and monosaccharides were attached to the core structure to mimic glycyrrhizin and carbenoxolone. A large number of “sugar-free” 3-amino-GA derivatives, including “amido-carbenoxolone” and “sulfonamido-carbenoxolone”, gave promising results in the 11β-HSD1/11β-HSD2 enzyme assay as potent

and selective 11β-HSD2 inhibitors. This positive response from biology triggered the selection of several candidates and their decoration with the C30 hydroxamic acid motif, another promising structural modification developed within the ASPEX-project, bringing forward a second generation of 11β-HSD inhibitors.

The second part of the thesis was focused on neoglycosides of the glycyrrhetinic acid. A small compound family of spacered aminoethylthioglucuronides was prepared, having all structural features of glycyrrhizin except for the natural O-glycosidic bond which was formally replaced by the physiologically more stable thioglycosidic bond. The whole set of compounds was inactive in the enzymatic assay but gave promising results in the antiviral screening against influenza A virus.

Spacered thioglucuronides

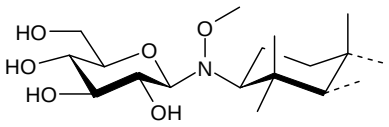


R^2=H, Me R^1=H, Ac, Succinyl

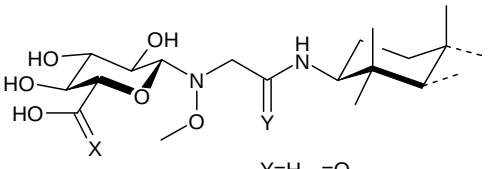
active in the antiviral screening

The last two fields of interest were dealing with the applicability of the direct glycosylation methodology of alkoxyamines with unprotected, unactivated reducing sugars on methoxyamino-derivatives of the glycyrrhetinic acid.

Methoxyaminoglycosides



Spacered methoxyaminoglycosides (ethylamino)



X= H_2,=O Y=H_2, =O

The initially targeted 3β-methoxyaminoglycosides of GA could not be prepared by means of direct glycosylation of the 3β-methoxyamino-GA, but one prototype was synthesized *via* conventional glycosylation chemistry to prove the principal stability of such compounds. In contrast, the chemistry towards ethylamino-spacered methoxyaminoglycosides, was established in a small scope (glucose and glucuronic acid) as a proof-of-concept study and was extended into a supervised diploma thesis.

Dedicated to my grandfather Kurt Wachter

First of all, I would like to express my deep gratitude to Prof. Paul Kosma; not only for the interesting and challenging topic but also for having revitalized my passion for science and research and for his support and trust beyond the borders of this thesis.

I am very grateful to the people currently in the lab, Daniel Artner, David Baum, Markus Blaukopf, Willi Herok, Ralph Hollaus, Bernhard Müller, Alex Doppelreiter and Alexander Gadinger, but also to my former colleagues Hassan Amer, Carmen Ruiz-Ruiz, Georg Sixta, Iris Koller and Kurt Wimmer and our technician Maria Hobel for the incredible atmosphere we share in our lab; it is a pleasure to go to work every day.

Special thanks go to all members of the ASPEX-team and especially the steady ones; Bernhard Küenburg, Dirk Classen-Houben, Andrea Wolkerstorfer, Prof. Kosma, Hassan Amer, Carmen Ruiz-Ruiz, Prof. Jordis, Laszlo Czollner and Igor Beseda for inspiring discussions and a fruitful collaboration. I am very grateful for the discussions with Prof. Andrea Vasella and Prof. Hugo Kubinyi within the ASPEX days.

I thank Andreas Hofinger for recording the 400MHz-NMR spectra and his support with non-routing NMR-experiments, Markus Blaukopf for recording 400MHz-NMR spectra. I thank Prof. Stephan Hann for his admission to use their HPLC-HRMS facilities at the Dep. of Analytical Chemistry and Ralph Hollaus for performing the HPLC-HRMS analysis.

I want to thank Karin Hofbauer, David Baum and Martin Eisinger for technical support and "my" diploma students Iris Koller and Alexander Doppelreiter for a fruitful and enjoyable cooperation. Furthermore I want to thank our technician Maria Hobel for all the effort she takes to make our everyday life as comfortable as it is.

I am grateful for the scientific exchange with all colleagues but I want to especially thank Bernhard Müller for many valuable discussions about chemistry, economic questions from our MBA-classes and for sharing his experience in the field of analytical and preparative chromatography with the rest of us.

My special thanks belong to my parents, my sister and my grandparents for their love and their continuous support in all my life and especially during the years of this thesis. Furthermore I want to thank my father for being one of my chemical teachers as well.

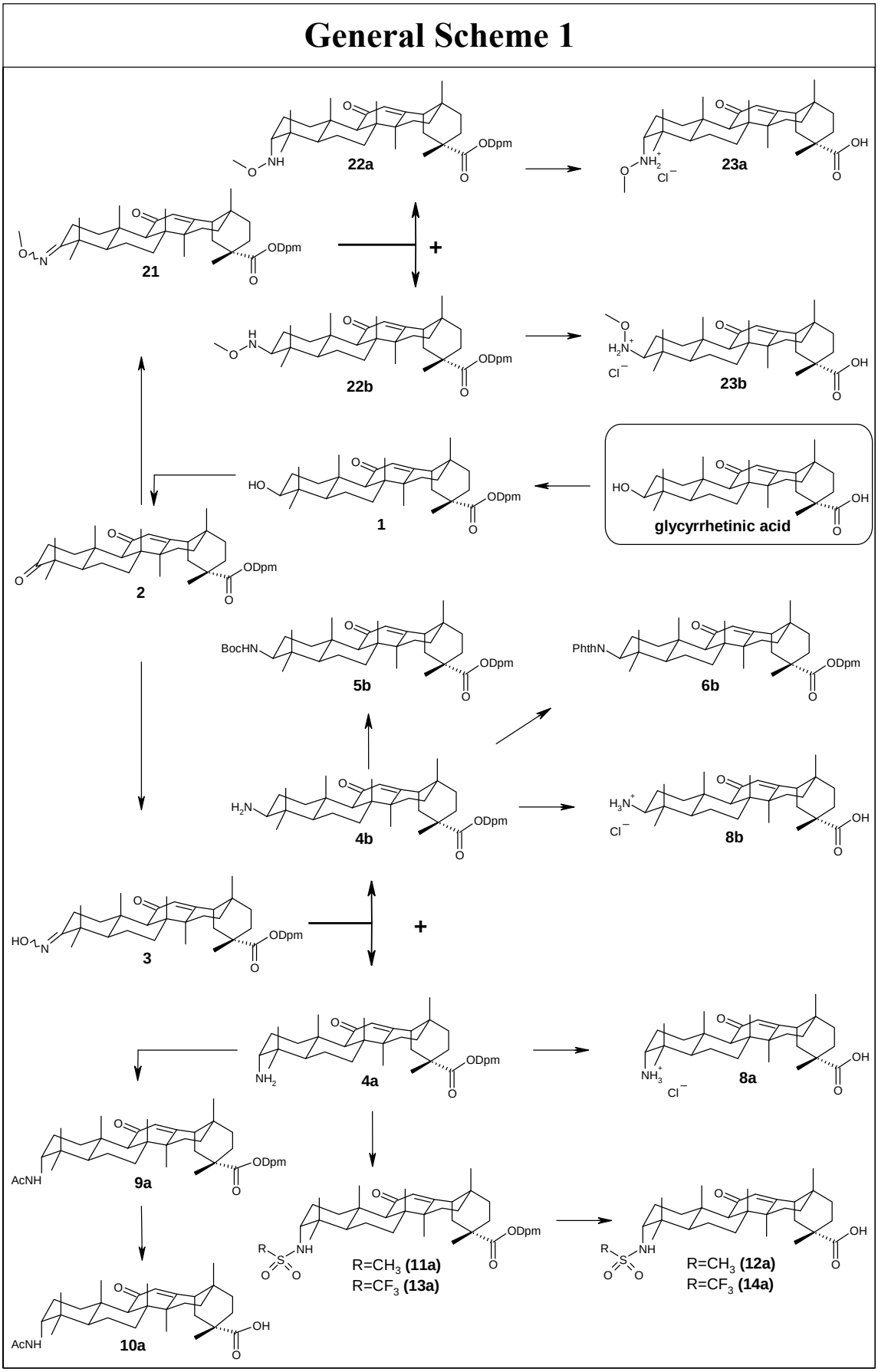
Table of contents

Abbreviations

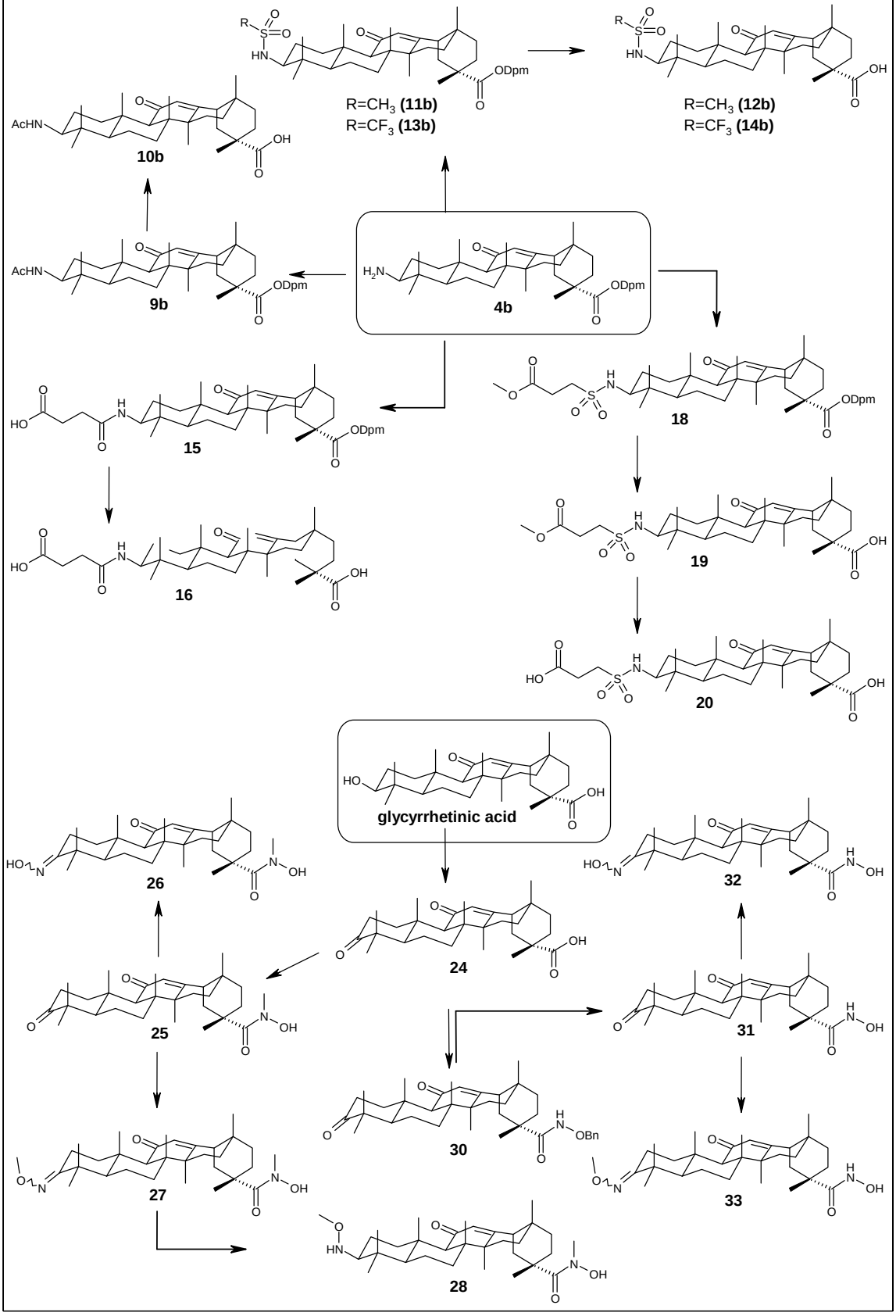
APT	attached proton test (NMR)
ATP	adenosine triphosphate
Ac	acetyl
Ac_2O	acetic anhydride
ASPEX	antiviral spot of excellence
b.p.	boiling point
Bn	benzyl
Boc	*tert.*-butyloxycarbonyl
COSY	H,H-correlated spectroscopy (NMR)
CPE	cytopathic effect
d	doublet
dr	drop
δ	chemical shift (NMR)
DCM	dichloromethane
DEPT	distortion-less enhancement by polarization transfer (NMR)
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
Dpm	diphenylmethyl
Hex	n-hexane
equiv.	equivalent
EtOAc	ethyl acetate
Et_2O	diethyl ether
EtOH	ethanol
GA	18β-glycyrrhetinic acid
GN	glycyrrhizin
HPLC	high performance liquid chromatography
HMBC	hetero multiple bond coherence (NMR)
HRMS	high resolution mass spectrometry
11β-HSD1	11β-hydroxy steroid dehydrogenase typ 1
11β-HSD2	11β-hydroxy steroid dehydrogenase typ 2
HSQC	hetero single quantum coherence (NMR)

IAV	influenza A virus
MeOH	methanol
m.p.	melting point
MsCl	methanesulfonyl chloride
NMR	nuclear magnetic resonance
Pd/C	Palladium on charcoal (10%)
PCC	pyridinium chloro chromate
q	quartet
rt	room temperature
s	singlet
t	triplet
Tf	trifluoromethylsulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TEA	triethylamine
TLC	thin layer chromatography
TOCSY	total correlated spectroscopy [NMR]

General Scheme 1



General Scheme 2



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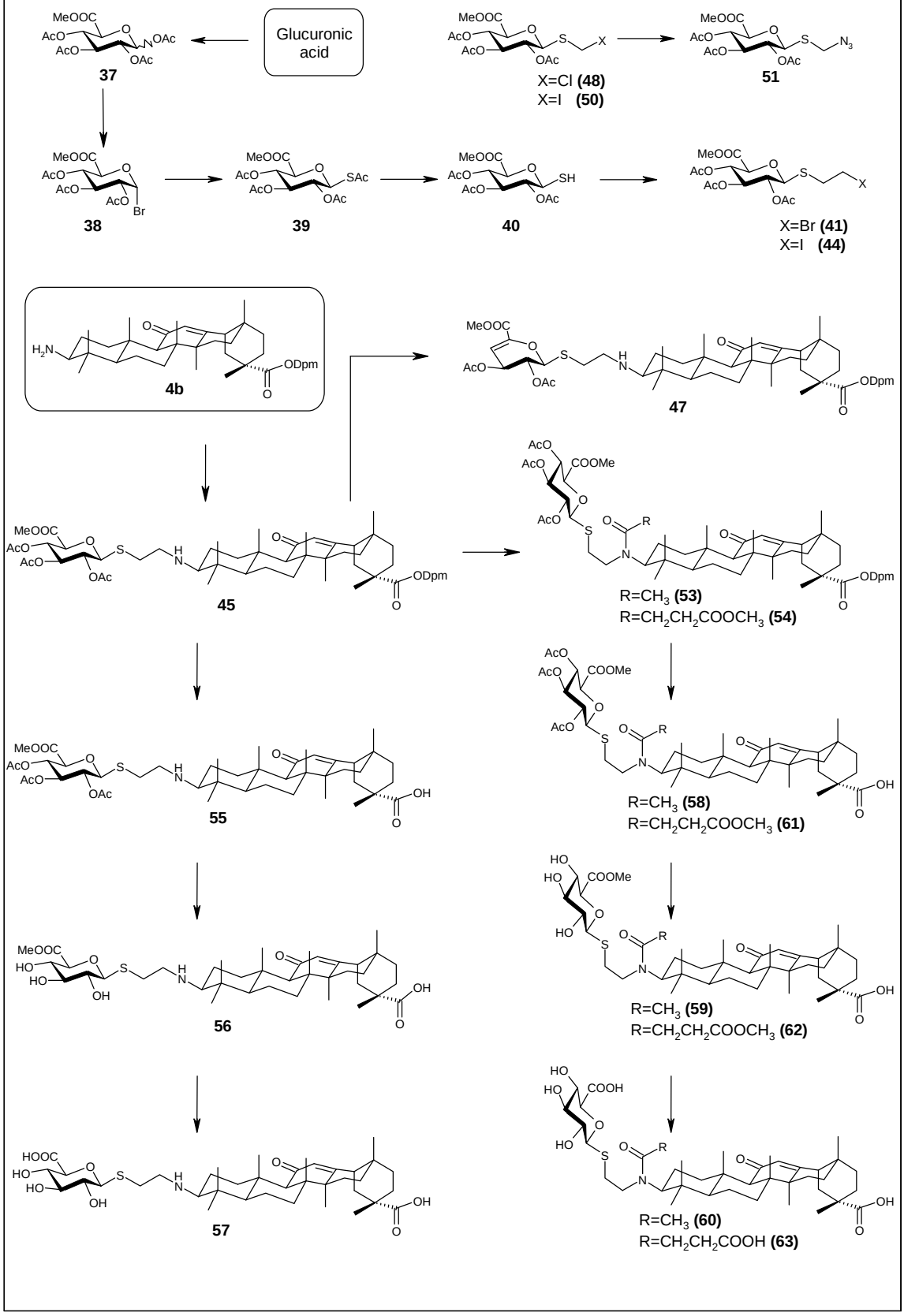
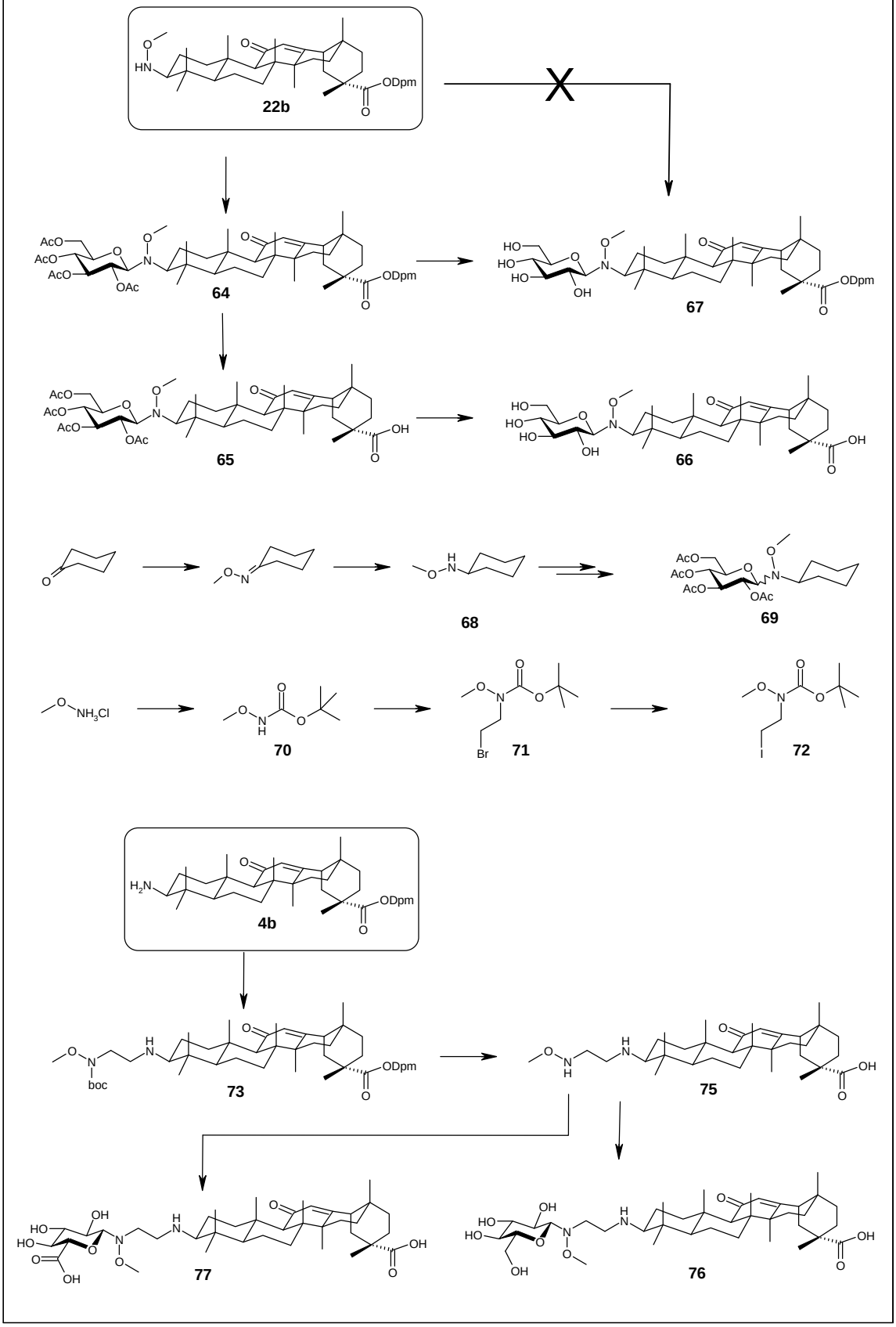


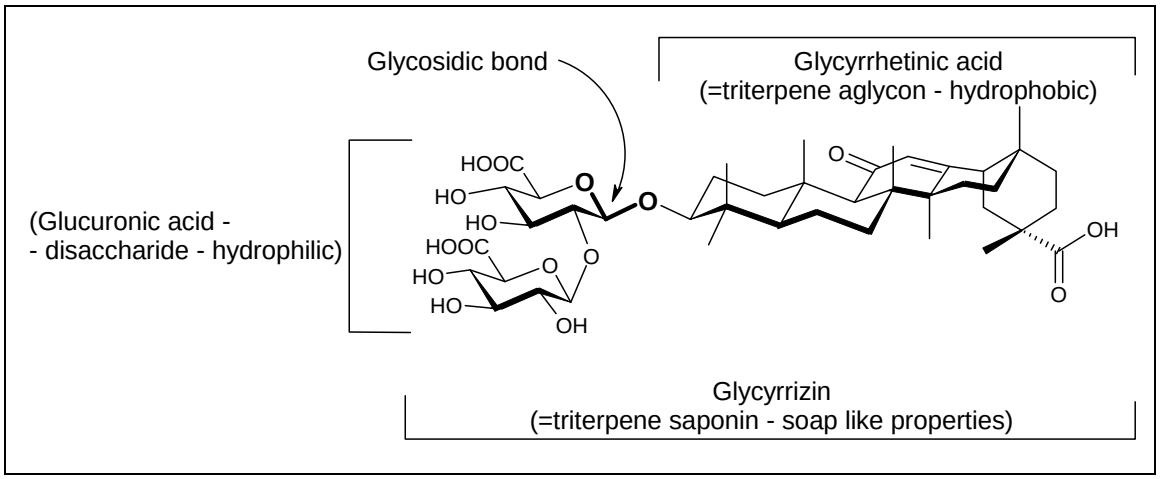
General Scheme 4



1 Introduction

1.1 Saponins

The term saponin (Latin word *sapo* for soap) refers to the most prominent physical feature of this compound family, namely that most saponins form stable foams when shaken in water. While earlier this chemo-physical characteristic has been the major criterion for the classification of a substance as a saponin, based on expanded knowledge about the structural identity of saponins, today saponins are characterized by their chemical structure. The structural characteristic of a saponin is to be either a triterpene- or a steroid-glycoside, thus having both a hydrophobic aglycon part and a hydrophilic sugar moiety (Scheme 1).



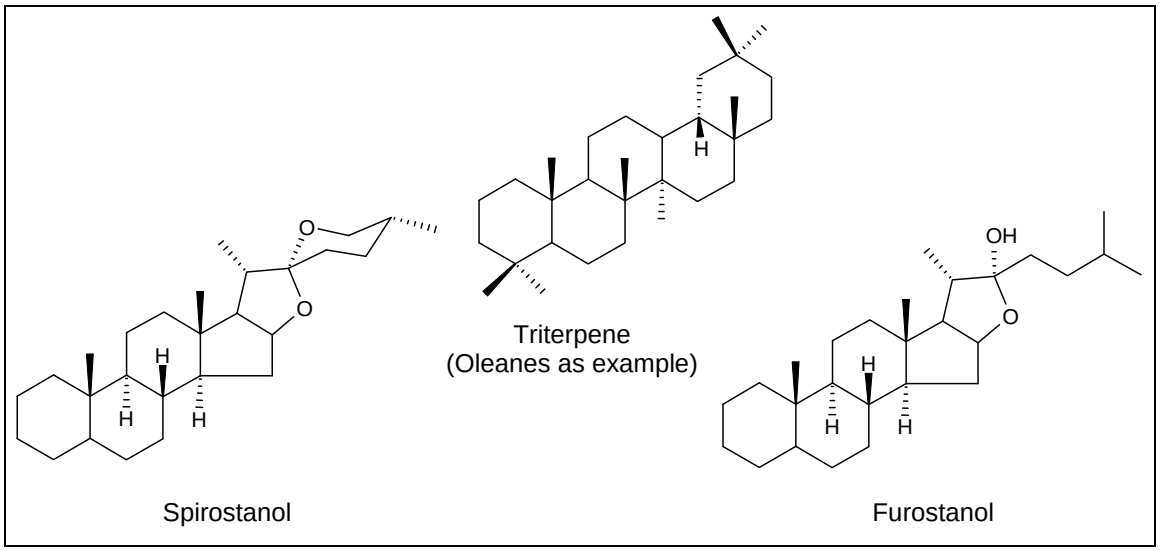
Scheme 1: Principle structural features of a saponin using the example of glycyrrhizin (GN)

This ambivalent chemical nature is the reason for the name-giving physical property as well as the base for a number of pharmacological properties.

1.1.1 Structural motifs and sub-classification of saponins

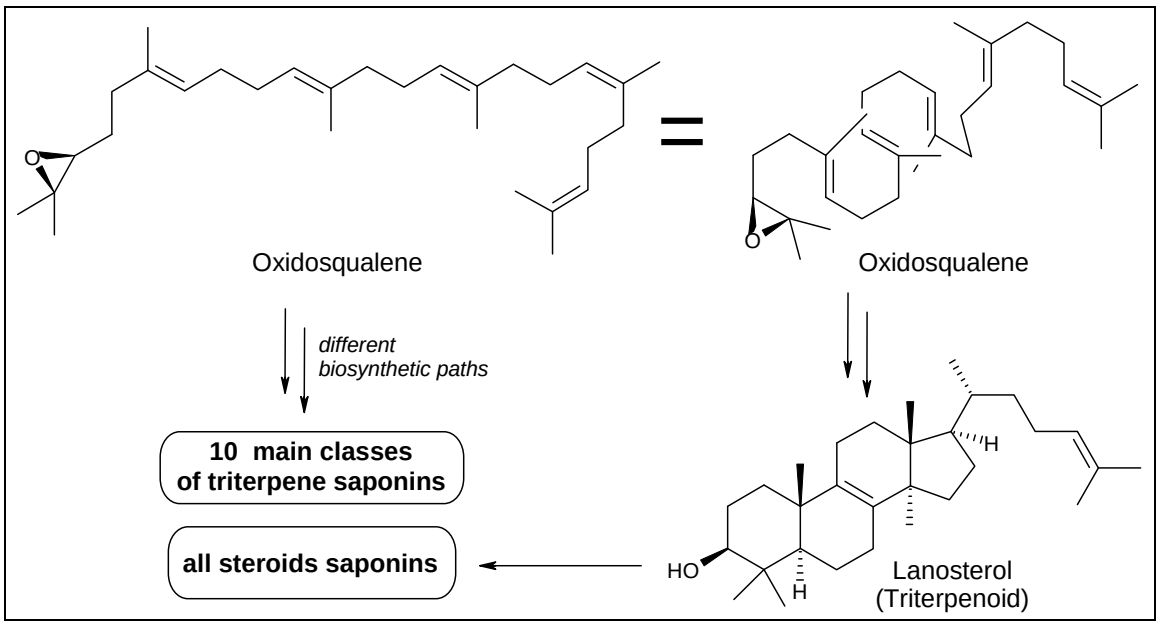
The family of saponins is further sub-classified based on the chemical structure of their members. Like all glycosides, saponins consist of one or more sugar moieties and the aglycon part, which is also called sapogenin. It is this sapogenin part which is the basis for further sub-classifications. Following the classical approach, saponins are divided into triterpenoid and steroid saponins or more detailed into triterpenoid, spirostanol and furostanol saponins[1] (the latter two are steroid subtypes) (Scheme 2). The most obvious difference between the triterpene and the steroid scaffold is the number of carbon atoms.

While steroids are missing at least three methyl groups, triterpenoid sapogenins generally contain all 30 carbon atoms from their common biosynthetic precursor oxidosqualene (Scheme 3).



Scheme 2: Classification of saponins based on the nature of their aglycon

A sophisticated differentiation of saponins into eleven subsets based on their biochemical pathways was attempted more recently[1].

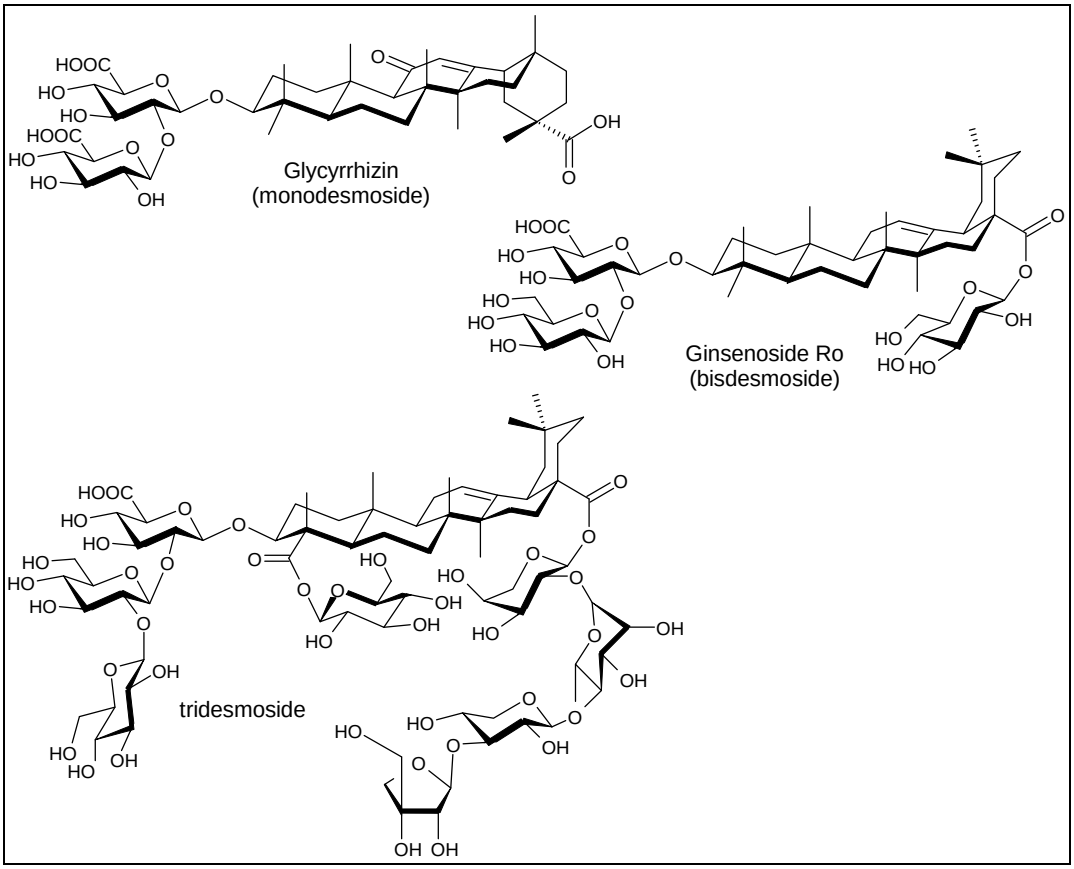


Scheme 3: The biosynthetic relation between oxidosqualene steroid and triterpenoid saponins

As stated above both main classes of saponins are biosynthetically derived from oxidosqualene. *Via* several biosynthetic steps oxidosqualene is converted to lanosterol, which is still a triterpene but is the biosynthetic precursor for cholesterol, the parent-compound for

all steroid saponins[1]. On the other hand ten different triterpenoid saponin subclasses are derived from oxidosqualene *via* different biosynthetic pathways[1].

Although saponins are generally sub-classified based on the nature of their aglycon part there is a sorting-criterion for saponins related to the saccharide moiety. Since a wide range of different neutral and acidic mono- and oligosaccharides are found in naturally occurring saponins, the nature of the sugar moiety is generally not used for sorting. However, saponins are classified based on the amount of glycosidic linkages between the sapogenin part and different (oligo)saccharide moieties.



Scheme 4: Examples for mono-, bis- and tridesmosides

While monodesmosides like glycyrrhizin carry only one saccharide (mono- or oligosaccharide) moiety, bidesmosides have two separate saccharide parts (Ginsenoside Ro) and rarely occurring tridesmosides (Scheme 4) have three separately attached sugar moieties in their structure[2]. Generally, in saponins the sugars are attached to the sapogenin part either *via* a glycosidic bond with an alcohol (Glycyrrhizin) or with a carboxylic acid (Ginsenoside Ro) moiety.

1.2 Licorice and its constituents

Licorice species are perennial herbs native to the Mediterranean region, central to southern Russia and Asia Minor to Iran and are now widely cultivated throughout Europe, the Middle East and Asia[3]. Licorice or *Glycyrrhizia radix* is one of the most ancient medical plants with a long history in the Chinese, Tibetian and Indian medicine for the treatment of allergic, pulmonary and hepatic ailments and inflammatory processes [4-7].

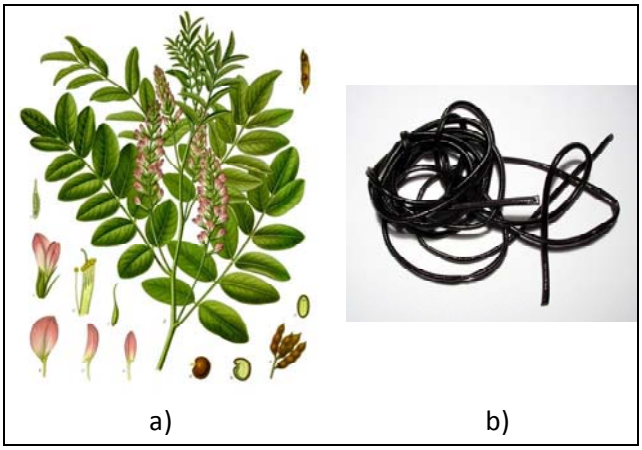
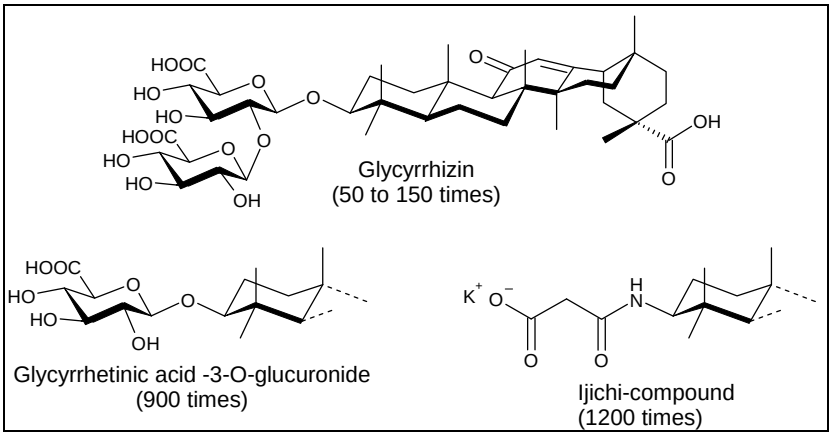


Figure 1: a) *Glycyrrhizia radix* b) licorice sweets

Besides the long tradition of licorice products as medicine it is also a popular source of sweetness. The root of *Glycyrrhizia radix* is traditionally extracted; the extracts are concentrated and refined to give licorice sweets (Figure 1), which are especially popular in the northern European countries.

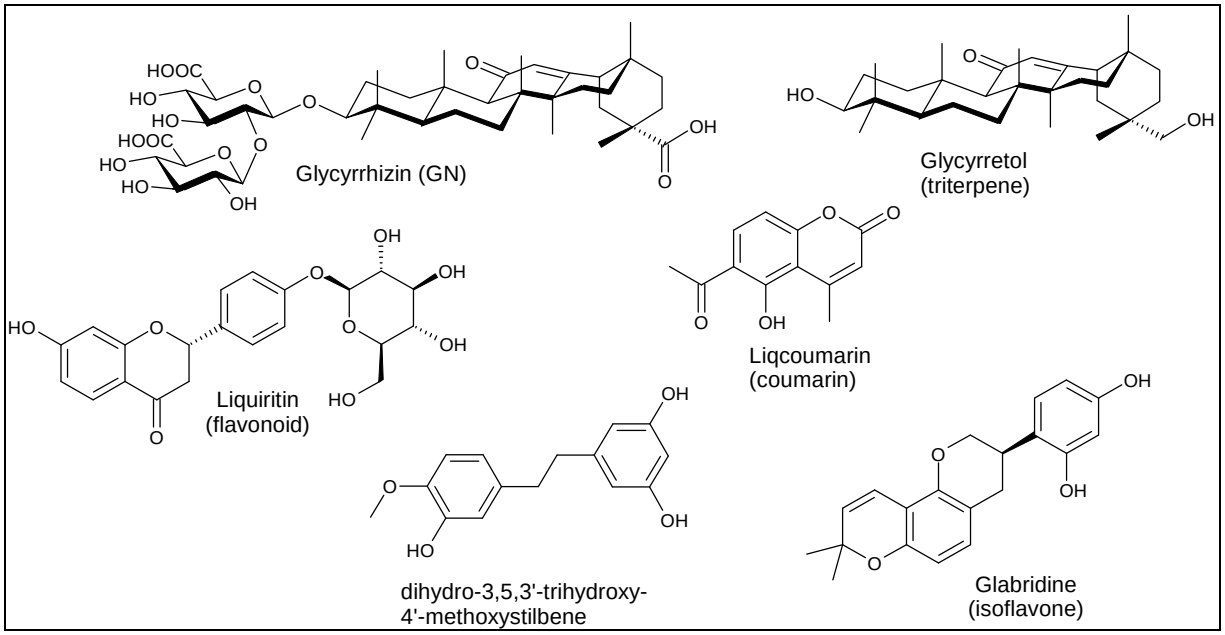


Scheme 5: GN-based compounds exhibiting strong sweetness (compared to sucrose)

Isolated glycyrrhizin, the main component in licorice, however, was reported to be observed by the human tongue as up to 150 times sweeter than sucrose[8]. Nevertheless, it is still outperformed regarding in this property by the corresponding monoglucuronide

species (900 fold sweeter) and other synthetic derivatives carrying 3-carboxyalkylamido groups of optimized lengths (1200 times sweeter)[8] (Scheme 5).

Besides its major and best studied bioactive components glycyrrhizin (GN) and its corresponding aglycon 18β-glycyrrhetinic acid (GA) a large number of other (active) constituents has been identified in licorice extracts[4]. Besides other saponins and triterpenes members of different other compound families like flavonoids, isoflavones, coumarins or stilbenoids have also been reported and studied.



Scheme 6: Glycyrrhizin and other components isolated from the *Glycyrriza radix* family

However, an interesting observation for the manufacturers, who isolate glycyrrhizin from the licorice root, was recently reported. A group closely studied the optimization of growing conditions for licorice investigating the influence of elevated CO_2 levels and different wavelengths used for irradiation during cultivation on the levels of GN in the plants. The authors underline the possibility to reach GN levels within several months under optimized conditions which are typical for field plants after several years of cultivation[9].

1.3 Pharmacological activities of glycyrrhizin and glycyrrhetinic acid

A wide range of pharmacological activities of extracts from different species out of the *Glycyrrhizia radix* family has been described and many activities of different identified constituents of these extracts have been evaluated in pharmacological studies[4]. The broad spectrum of reported activities comprises anti-inflammatory, cytotoxic, antiulcer, antiproliferative, anti-oxidative and endocrine activities[10-15]. This introduction, however, will focus on pharmacologic properties of glycyrrhizin and glycyrrhetinic acid which are related to the biological tests that have been carried out with the prepared compounds of this thesis.

1.3.1 Antiviral activities of glycyrrhizin

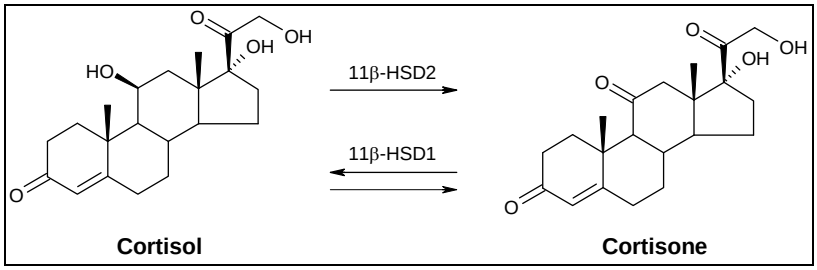
Mainly within the last decade glycyrrhizin was evaluated against a variety of viruses[16,17]. It was found to be active against important pathogenic viruses like herpes[18,19], flaviviruses[20] and HIV[21]. Glycyrrhizin has already been used to treat patients with hepatitis C[22] and upper respiratory tract infections[23] and was in the center of international scientific concern in 2003 when it was found to be among the first compounds to be active against SARS-coronavirus (SARS-CoV) *in vitro*[24] and was later used in a study for treatment of SARS in patients[25,16,26]. A study was reported in which more than 10000 compounds were analyzed for anti-SARS-CoV activity which delivered a small set of potent compounds including two more derivatives of glycyrrhizin, thus making the family of GN-derivatives an interesting target.

More related to the topics of this thesis are the results in the influenza A virus (IAV) field.

A strong protective effect of glycyrrhizin in a mouse model was reported, stating that all mice treated with GN prior to infection and during infection with IAV survived a period of 21 days while in the reference group of untreated mice the mean survival time was 10.5 days without any survivors[27]. Based on further investigations the authors stated that the protective effect of glycyrrhizin in mice treated with lethal dose of IAV might be caused by induction of interferon-gamma production by T cells, a hypothesis which is supported by the independent finding of Abe *et al*[28]., who also considered that the induction of interferon production by GN and GA in mice as a possible mechanism of action against viral infection.

1.3.2 The glucocorticoids cortisol and cortison and their regulation by 11β-hydroxysteroid dehydrogenases

Glucocorticoids are steroidal hormons which are important regulators of various physiological processes including immunomodulation, cell growth and energy metabolism[29-31]. In humans the most important glucocorticoid is cortisol (Scheme 7) which antagonizes the effects of insulin and raises the glucose levels in blood by up-regulating gluconeogeneses[29,32]. Chronic glucocorticoid excess has been associated with many diseases including muscle wasting osteoporosis, cataract formation, cognitive disorders and metabolic syndrome like diabetes type 2 and obesity[29,33,29]. However, the local concentration of cortisol can be regulated by oxidation to the corresponding 11-dehydro product cortison, which has lower affinity to the glucocorticoid receptors and is therefore the inactive form of cortisol. This interconversion is modulated by the 11β-hydroxysteroid dehydrogenases enzymes (11β-HSDs)[34] (Scheme 7). 11β-Hydroxysteroid dehydrogenases Type 1 and Type 2 are microsomal oxidoreductases which catalyze the interconversion of cortison and cortisol. The two isozymes, however, are functionally quite different and have only 18% identical amino acid sequences[35].



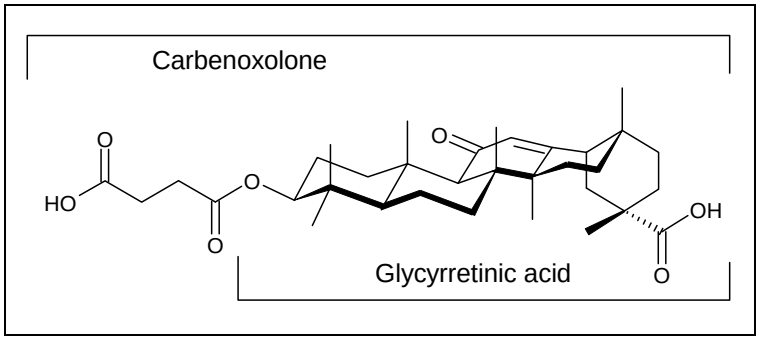
Scheme 7: The activity of the oxidoreductases 11β-HSD1 and 11β-HSD2

While 11β-HSD2 is a NAD^+-dependent unidirectional enzyme and catalyzes only the oxidation of cortisol to cortison, 11β-HSD1 is a bidirectional enzyme which primarily reduces cortison to active cortisol (with NADPH as cofactor)[34]. Therefore selective inhibition of 11β-HSD1 leads to reduction of cortisol levels and is an interesting therapeutic target for the treatment of metabolic diseases. Inhibition of 11β-HSD2, on the other hand, could be used to potentiate the anti-inflammatory effects of glucocorticoids, leading to a reduction in the amount of glucocorticoid required for anti-inflammatory therapy and in the severity of very significant side effects that can result from this type of medication[36]. Nevertheless inhibition of 11β-HSD2 also leads to activation of the corresponding mineralocorticoid receptors in the kidney, leading to hypernatremia, hypokalemia and finally hypertension[37].

Other studies have highlighted the correlation between 11β-HSD2 activity and increased proliferation[38-44]. These observations validate the need for selective inhibitors of 11β-HSD1 and 11β-HSD2 both as therapeutic agents but also as tools for further mechanistic studies[45].

1.3.3 Inhibition of 11β-HSD enzymes by glycyrrhetinic acid

Glycyrrhetinic acid and its hemisuccinate ester carbenoxolone (Scheme 8) are non-selective potent inhibitors of the 11β-HSD enzymes[45,33,46,47], which explains the undesirable side effect hypertension which is observed upon licorice abusive consumption of licorice.



Scheme 8: GA and Carbenoxolone, two non-selective 11β-HSD inhibitors

For the above potential applications the glycyrrhetinic acid scaffold is regarded a good starting point for an investigation towards 11β-HSD1 and 11β-HSD2 selective inhibitors.

1.4 Pharmacological assays used for the evaluation of the synthesized compounds

The compounds prepared within this thesis were pharmacologically evaluated as antiviral compounds against Influenza A Virus (IAV) and were tested as selective 11β-HSD1 and 11β-HSD2 enzyme, respectively. The basic principles of both test systems are briefly explained in the next chapters, whereas development of the assays and carrying out of the pharmacological screenings was not part of this work.

1.4.1 Antiviral assay

The antiviral potential against influenza A virus (IAV) of the prepared compounds was tested in a cell based *in vitro* assay using Mardin-Darby canine kidney (MDCK) cells. Upon infection, IAV induces a cytopathic effect (CPE) in MDCK cells resulting in reduced cell viability. Details of this assay are reported by Wolkerstorfer *et al.*[48], who was responsible for the evaluation of the antiviral activity of the compounds prepared within this thesis.

1.4.1.1 Preparation of virus stocks

Virus stocks were prepared by propagation of the virus in MDCK cells (Figure 2).

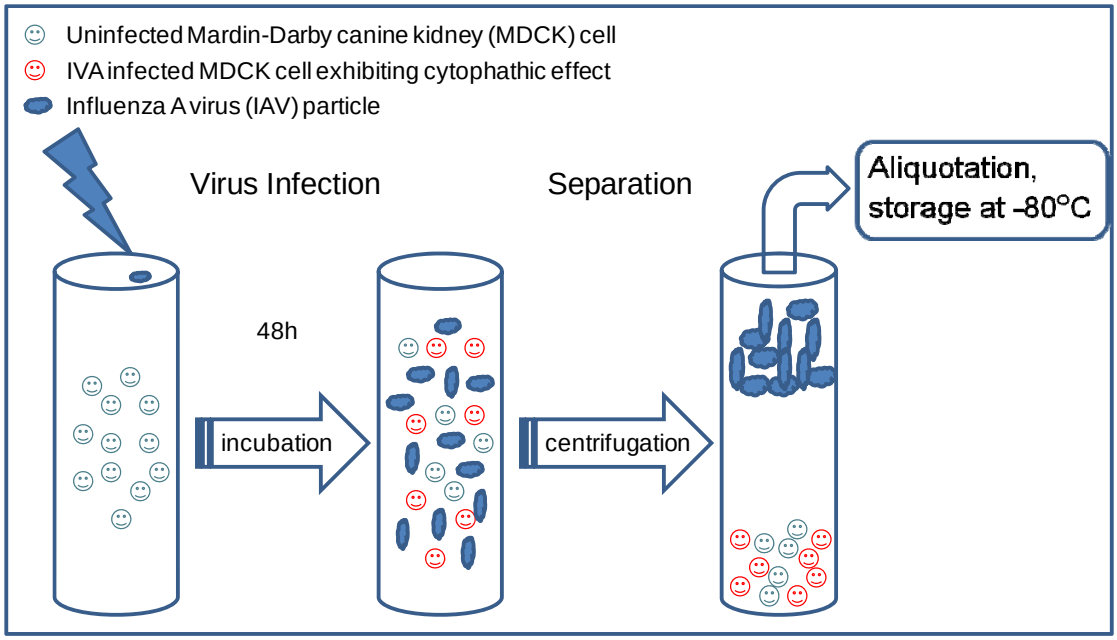


Figure 2: Preparation of influenza A virus (IAV) stocks for the antiviral screening

Approximately 48h post infection, cell supernatants were harvested, clarified by centrifugation, aliquoted and stored at -80°C. In general, if cells are infected the virus is applied at a multiplicity of infection (MOI) of 0.05 meaning that the amount of virus particles equals 5% of the amount of cells.

1.4.1.2 Experimental setup of the anti-influenza assay

The principle of the antiviral assay used for the evaluation of the antiviral activity of a test compound is depicted in Figure 3. Mardin-Darby canine kidney (MDCK) cells were seeded in 96-well plates and incubated in a culture medium allowing growth and formation of a cell monolayer on the bottom of each well (approximately 20000 cells). Within one experiment antiviral activity and cytotoxicity of the compounds was tested in a cell-based assay with cell viability as primary endpoint. Cell viability was determined using an ATP-based cell viability assay (Promega Corp.). The ATP of viable cells is used to generate a luminescent signal directly proportional to the number of viable cells in the well. Compounds with antiviral activity result in reduced CPE leading to increased cell viability compared to untreated infected samples. Four different wells (A, B, C, D) of one experiment are depicted in Figure 3 and are explained below.

A: Untreated uninfected cells (100 % viable)

Untreated uninfected cells cultured in infection medium are used as reference for the determination of cytotoxicity and calculation of CPE.

B: Treated uninfected cells (cytotoxicity)

Cells are treated with the test compound alone (at several concentrations) to determine its cytotoxicity, independently of its antiviral activity.

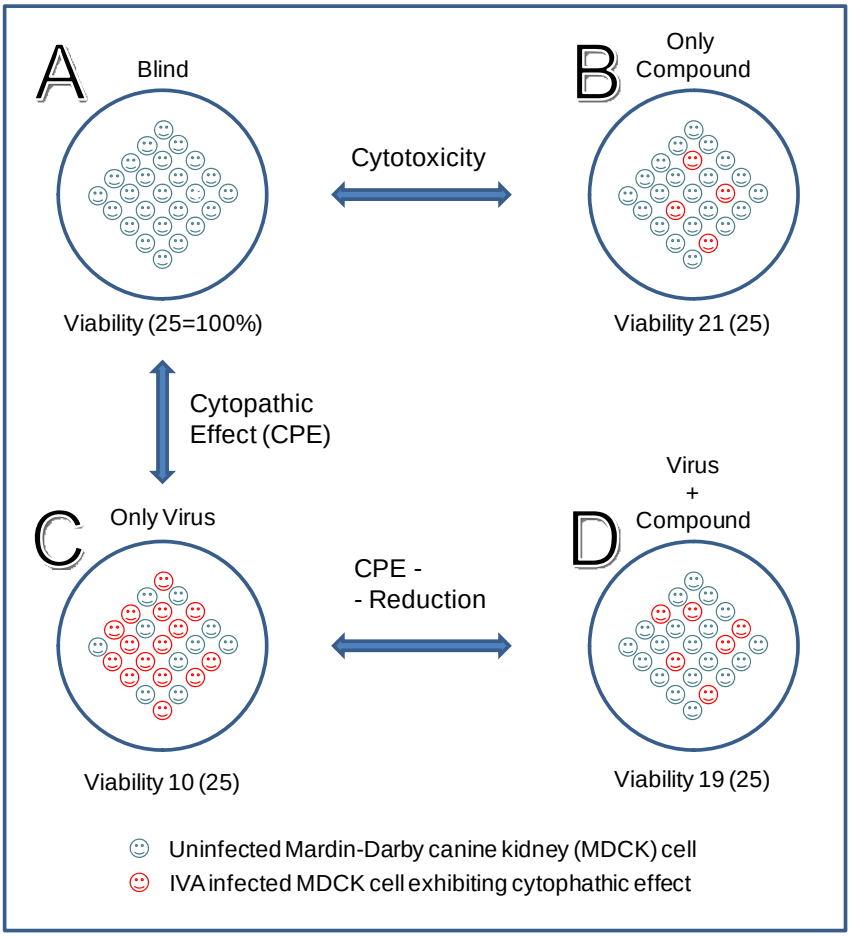


Figure 3: Principle of the *in vitro* IAV assay characterizing the antiviral activity of a certain compound

C: Untreated infected cells (cytopathic effect)

Untreated cells are infected with IAV and cell viability is determined reflecting the virus induced cytopathic effect (CPE).

D: Treated infected cells (Reduction of CPE)

Treated cells are infected with IAV and cell viability is determined reflecting the reduction of CPE caused by the treatment with the compound.

1.4.1.3 Interpretation and analysis of the antiviral experiments

Based on the numbers of the example in Figure 3 the cytotoxicity and the reduction of the cytopathic effect can be calculated.

Cytotoxicity

The cytotoxicity of a compound at a certain concentration (c_1) was determined based on the relative cell viability of uninfected treated cells (B) versus uninfected untreated cells (A). Relative cell viabilities below 80% indicate cytotoxicity and would mask any antiviral activity. Hence cytotoxic compounds were retested at lower concentrations.

$$\textit{Cytotoxicity of a compound } (c_1)\text{: } \frac{B}{A} < 80\% \quad \rightarrow \quad \text{cytotoxic at concentration } c_1$$

Reduction of CPE

For the determination of the reduction of the CPE by a test compound at a certain concentration (c1) the difference of cell viability of infected treated cells (D) and infected untreated cells (C) was divided through the cell viability of uninfected untreated cells (A).

$$\textit{Reduction of CPE } (c_1) = \frac{(D-C)}{A}$$

Dependence of CPE-reduction on concentration

For active compounds the above experiment was carried out at several concentrations from 0 to 250µM to investigate the dependence of the CPE-reduction on the concentration of test compound. From the results of these experiments IC_{50} values can be derived, which is not included into this introduction since only the principle parameters of the antiviral assay and their interpretation should be outlined.

1.4.2 Inhibition assay for 11β-HSD1 and 11β-HSD2 enzymes

The 11β-HSD1- and 11β-HSD2-inhibition of test compounds was investigated by a functional activity assay using radiolabeled cortisol or cortisone, depending on whether enzymatic oxidation or reduction was examined. For every test compound the inhibition potency was determined by measuring the conversion of tritiated [1,2,6,7-^{3}H]-cortisol or [1,2,6,7-^{3}H]-cortisone at several concentrations. The principle setup of the 11β-HSD-assay is explained in the following chapter while the details of this test are reported by Odermatt *et al*[49,50], who's lab carried out the enzymatic evaluation of the compounds prepared within this thesis.

1.4.2.1 Preparation of 11β-HSD1 and 11β-HSD2 enzymes

The 11β-HSD1 and 11β-HSD2-enzyme respectively, has been expressed in Human Embryonic Kidney (HEK-293, HEK) cells which have been stably transfected with a plasmid., prepared mainly based on RT-PCR.

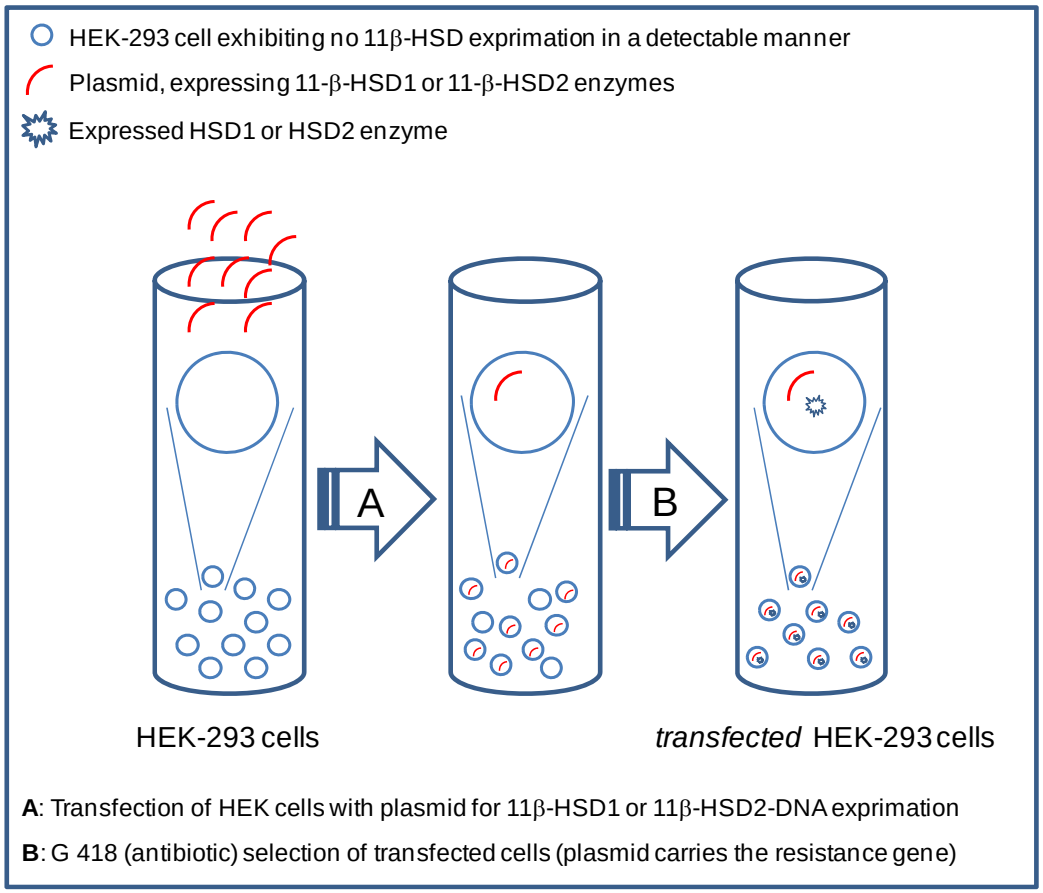


Figure 4: Preparation of stably transfected HEK-293 cells expressing 11β-HSD1 and 11β-HSD2 respectively

This plasmid contains not only the genetic sequence encoding for the 11β-HSD1 or 11β-HSD2-enzyme but also a FLAG-tag for determination by immuno-fluorescence analysis and a

resistance gene against G418, an antibiotic which is used for the selection of successfully transfected (resistant) over nontransfected cells (Figure 4). It was proven that nontransfected HEK-293 cells do not deliver any detectable 11β-HSD1 or 11β-HSD2 activity and thus the measured conversion of cortisol to cortisone and reverse is only based on the 11β-HSD1 and 11β-HSD2-enzyme activity induced by the transfection process. Growing the stably transfected HEK cells and lysis of the cells by sonification delivers the lysate used in the screening for inhibitors of 11β-HSD1 or 11β-HSD2-enzyme.

1.4.2.2 Determination of the inhibition of a test compound

A small amount of tritiated [1,2,6,7-^{3}H]-cortisol (NAD^{+} as cofactor) or tritiated [1,2,6,7-^{3}H]-cortisone (NADPH as cofactor) is converted by the enzyme for one hour before the reaction was "stopped" by addition of large excess of unlabeled substrate.

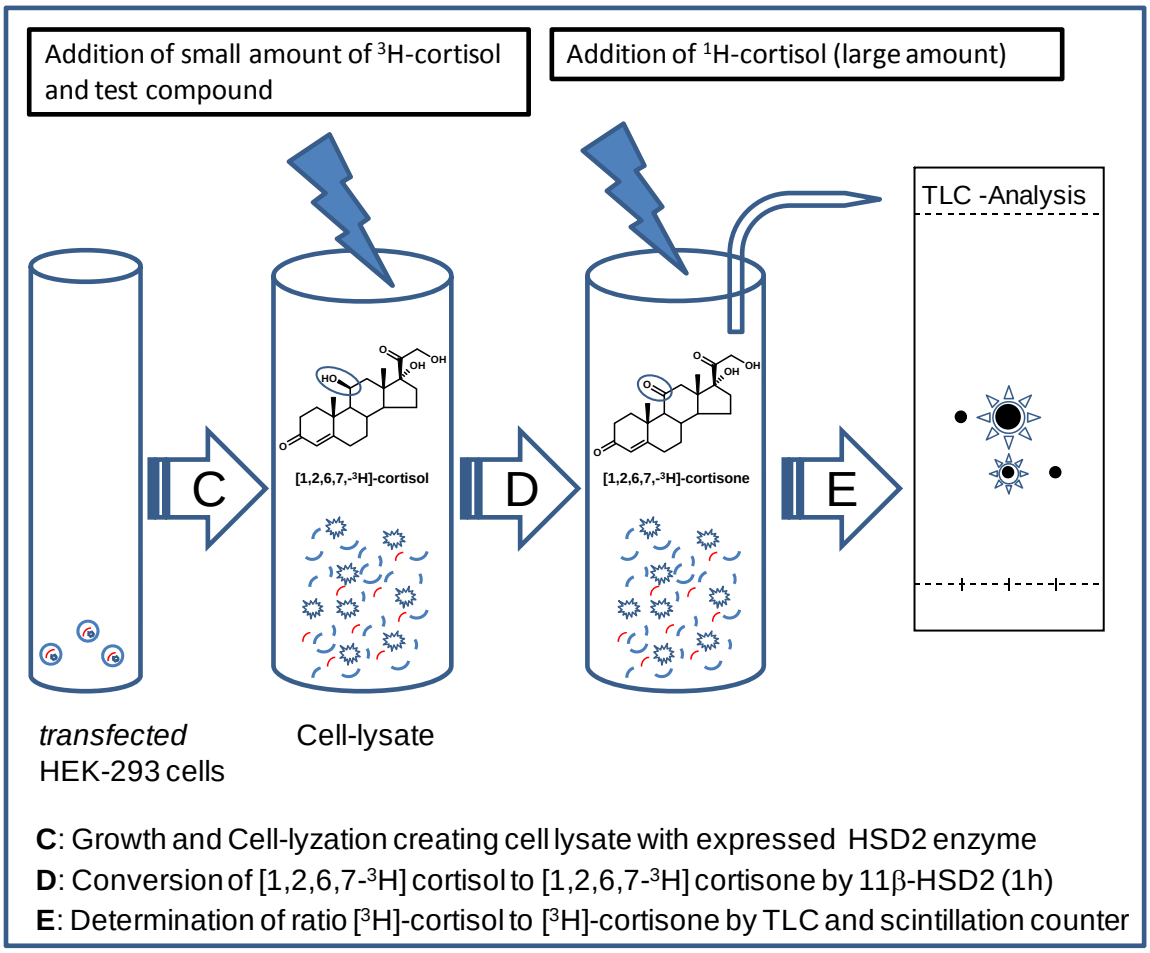


Figure 5: Principle of the determination of conversion of ^{3}H-cortisol to ^{3}H-cortisone by 11β-HSD2

Subsequently, the ratio of [1,2,6,7-^{3}H]-cortisol and [1,2,6,7-^{3}H]-cortisone was analyzed by TLC and scintillation counting of the corresponding spots. These experiments have to be

carried out in absence and in presence of a test compound at certain concentrations. In Figure 5 the process is outlined for the determination of the enzymatic oxidation of cortisol to cortisone in presence of a test compound. As a first indicator for a potential inhibitor the synthesized compounds have been evaluated at only one concentration to compare resulting enzymatic activity as % of control (low numbers indicating a good inhibition) at this concentration.

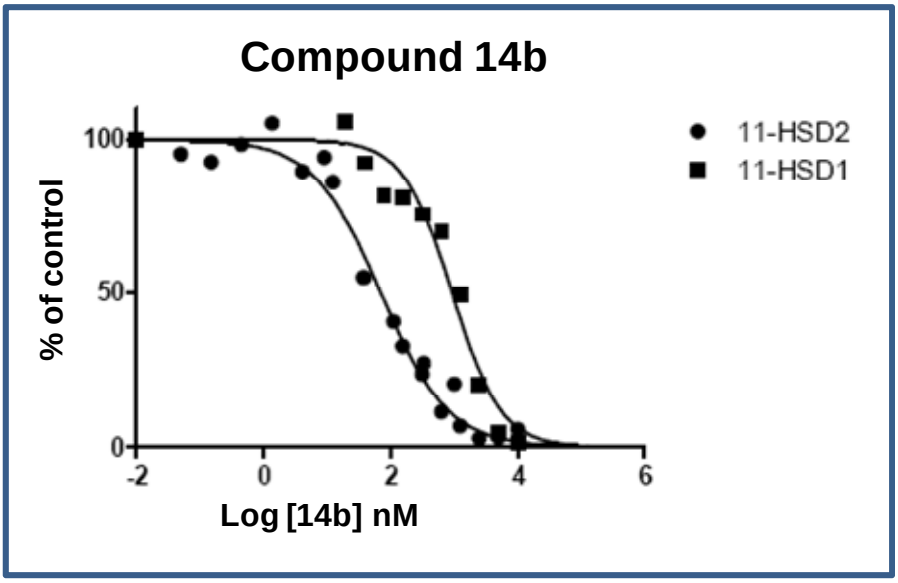
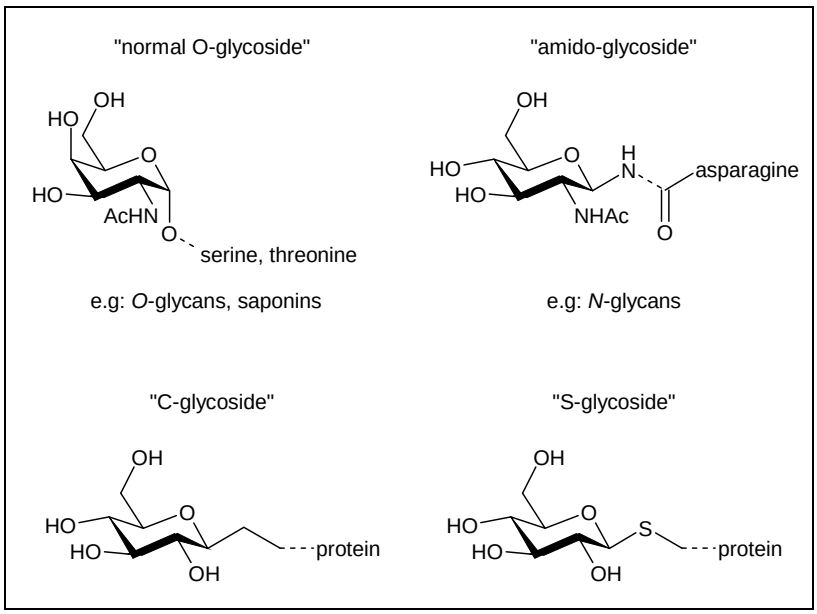


Figure 6: Concentration dependent inhibition of 11β-HSD1 and 11β-HSD2-enzyme by compound [14b]

For the interesting compounds EC_{50} values were determined by examination of inhibition at various concentrations and repeated measurements (Figure 6), thus causing a lot of experimental work. For this reason and limited financial resources only selected compounds have been evaluated in regard to their inhibitory properties (one concentration for 11β-HSD1 and 11β-HSD2) and even fewer in more depth (EC_{50} values for 11β-HSD1 and 11β-HSD2).

1.5 Neoglycosylation approaches

In all living species, the attachment of saccharide moieties to other bioactive biopolymers or small molecules is ubiquitous in nature, leading to DNA, RNA, glycoproteins[51], glycolipids or triterpene and steroid saponins to name only a few. In general the sugars are either attached *via* an *O*-glycosidic bond like in *O*-glycans (glycoproteins with sugars attached *via* a serine or threonine residue) or saponins, or *via* an *N*-glycosidic bond like in DNA or *N*-glycans (glycoproteins with sugars attached *via* the amide of an asparagine residue).



Scheme 9: Usual natural and unnatural glycosidic linkages

In living systems a wide range of enzymes can generate or cleave these *O*-glycosidic or *N*-glycosidic bonds both in selective as well as in an unselective manner[52].

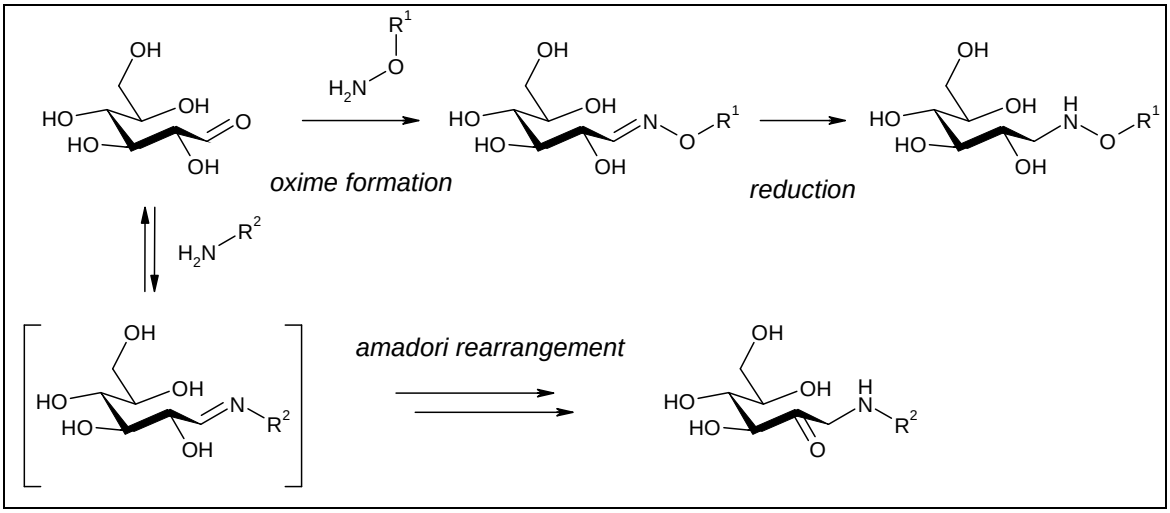
For this reason it is a logical approach to mimic *O*-glycosides with unnatural linkages between a sugar and an aglycon moiety in order to come up with physiologically more stable enzyme inhibitors. Over the years different types of unnatural neoglycosides have been examined both regarding their synthesis as well as their pharmacological evaluation[53,54].

Among other less frequently described derivatives the *S*-glycosides[55-57] and *C*-glycosides[58,55,59] , which are exact mimics of the natural *O*-glycosides, shall be mentioned.

1.5.1 Neoglycosylation approaches with nitrogen involved

Among the oldest methods developed for the attachment of sugars to other molecules several reactions of amines or hydroxylamines with the aldehyde moiety of reducing sugars are found, although many of them are not exact mimics of the natural *O*-glycosides.

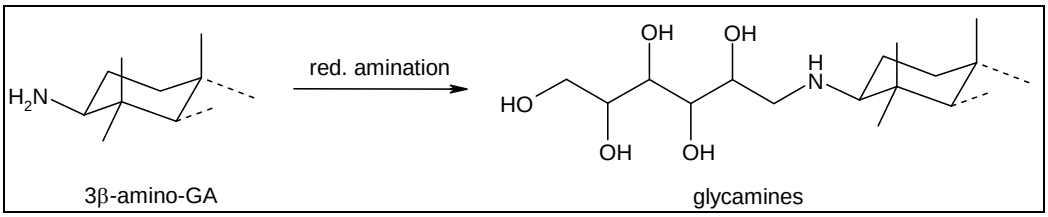
The reaction of reducing sugars with aliphatic amines (Scheme 10 with a primary amine as example) for example leads to the long-known Amadori rearrangement[60] of the primarily formed imine species[61].



Scheme 10: Attachment of sugars *via* Amadori rearrangement or oxime formation

This cascade of reactions is actually the first step of the so called Maillard-reaction of amino acids and sugars which is responsible for the darkening of food which is observed upon heating. The Amadori rearrangement is occurring only with aliphatic amines – aromatic amines give stable *N*-glycosides in ring-form analogous to the natural *N*-glycans or DNA and RNA. The reaction of *O*-substituted hydroxylamines with reducing sugars affords the intermediate oximes, which occur predominantly in the ring opened form and can be reduced to the corresponding hydroxylamines[62].

Last but not least sugars can also be attached to amines by simple reductive amination protocols, a method which was for example used in 1979 to convert this thesis' lead structure glycyrrhetinic acid to several so called glycamines[63] (Scheme 11).

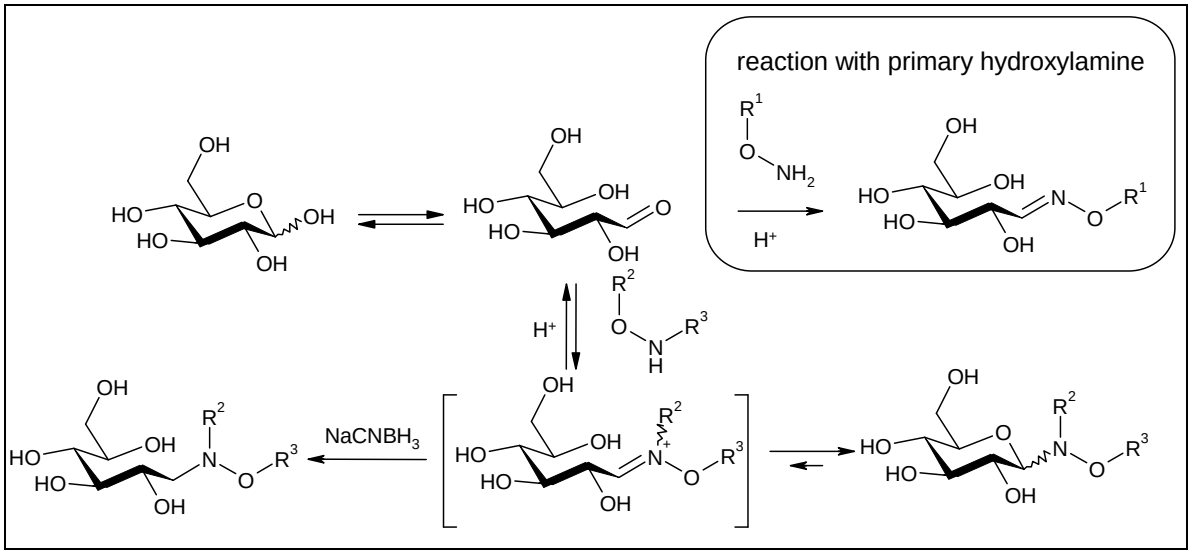


Scheme 11: Coupling of sugars to 3β-amino-glycyrrhetinic acid by reductive amination

1.5.2 Direct glycosylation of alkoxyamines with reducing sugars

Based on methods described above, in the last years some effort was undertaken in the field of alkoxyaminoglycosides. These are stable glycosides which are obtained predominantly in the ring form[64] and are therefore close analogues of natural *O*-glycosides.

In the literature conventional glycosylations of alkoxyamines as glycosyl acceptors with protected, anomerically activated glycosyl donors are reported[65,66]. However, a much more appealing approach towards alkoxyaminoglycosides in their ring form is the direct formation starting from unprotected, unactivated reducing sugars and the corresponding secondary alkoxyamines (Scheme 12).



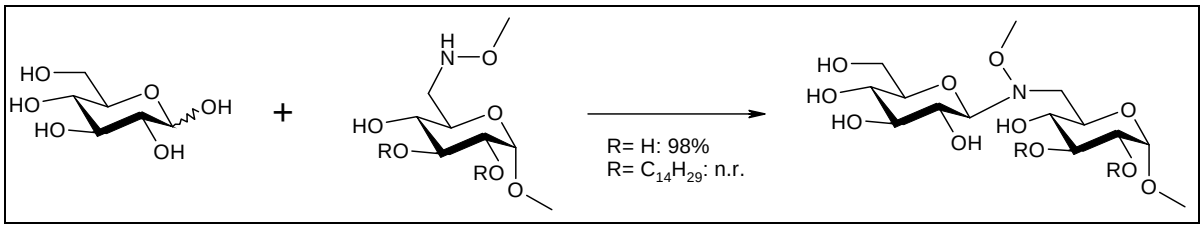
Scheme 12: Reported mechanism of the reaction of reducing sugars with hydroxylamines

The course of this reaction is in clear contrast to the established mode of reaction of primary alkoxyamines and reducing sugars generating generally ring opened oximes[67,68] (Scheme 12 in the upper part). Since the direct glycosylation of secondary alkoxyamines does not require

any protecting group manipulation or activating methodology for the sugar, this modern approach can be helpful to save a lot of synthetic effort thus allowing building up larger libraries of neoglycosides in short periods of time.

In their first reports Peri *et al*[64] already decribed the broad applicability regarding both sugars as well as *O*,*N*-disubstituted hydroxylamines, good to excellent conversions, predominance of the pyranose form over the furanose form as well as high diastereoselectivity for the 1,2-*trans* configuration. The depicted mechanism (Scheme 12) was proven to involve the corresponding cationic iminium-species by trapping with $NaCNBH_3$ reduction to give the corresponding tertiary hydroxylamine[64]. Very recently, the stability of several alkoxyaminoglycoside model compounds in an aqueous medium depending on time and pH has been studied in great detail[69]. The authors showed that a very reasonable stability of model compounds at pH above 5 was exhibited, thus further encouraging the use of glycosides of this type for rapid pharmacological evaluation of different glycoconjugates.

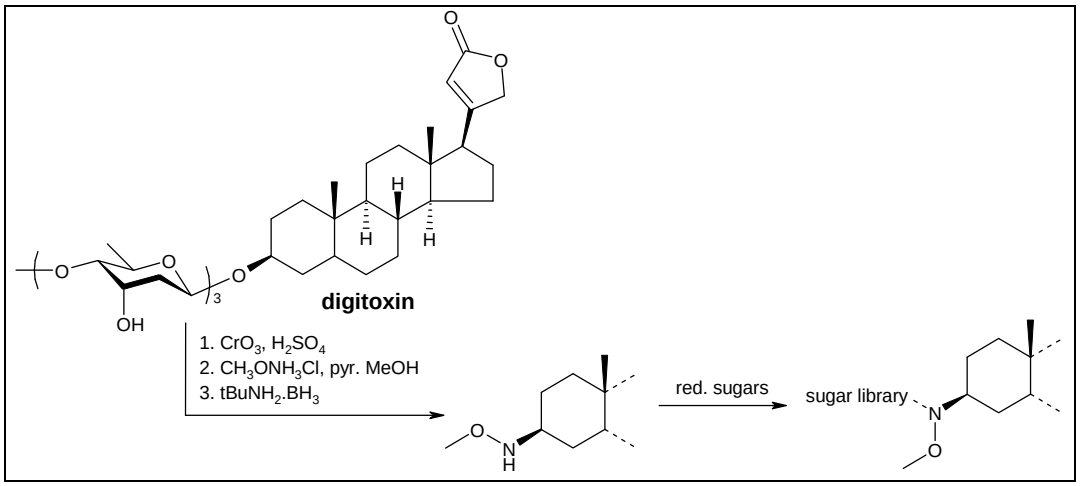
In the last decade several groups used the methodology of direct glycosylation to prepare libraries of pharmacologically interesting neoglycoconjugates. Reducing sugars have been attached to modified peptides or modified pre-activated amino acids, respectively, which were designed to be inserted into oligopeptides[70,71]. Peri *et al.* have used the methodology to prepare a disaccharide as intermediate for the synthesis of Lipid A antagonists[66] (Scheme 13).



Scheme 13: Example for the effect of steric demand in the direct glycosylation approach

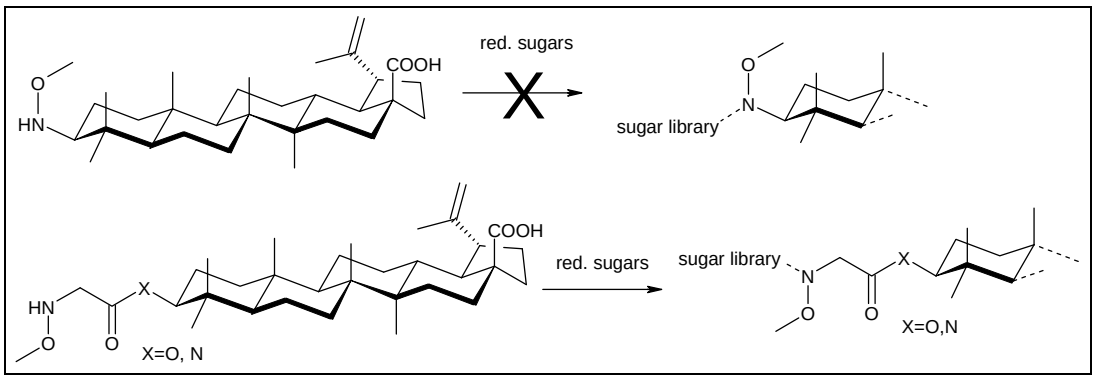
In their work they also showed that steric demand of nearby substituents can lead to complete loss of conversion. They have further shown that in such a case the target compounds can be prepared by conventional glycosylation methods using for example glycosyl bromides under the catalysis of Ag salts.

Within the reports about direct glycosylation there is also a very impressive example of a saponin-type natural compound being transformed into a large library of neoglycoconjugates. Starting from natural digitoxin, a cardiac glycoside, a large library of neoglycoconjugates was prepared and its members have been evaluated as anticancer agents (Scheme 14)[72].



Scheme 14: Preparation of a large number of neoglycoconjugates starting from digitoxin

Recently, the first example of a direct glycosylation by a neoglycorandomization approach starting from a triterpene saponin, betulinic acid, has been reported. The unsuccessful attempt to directly glycosylate the 3-methoxyamino moiety and the preparation of spacered neoglycosides are reported[73] (Scheme 15).

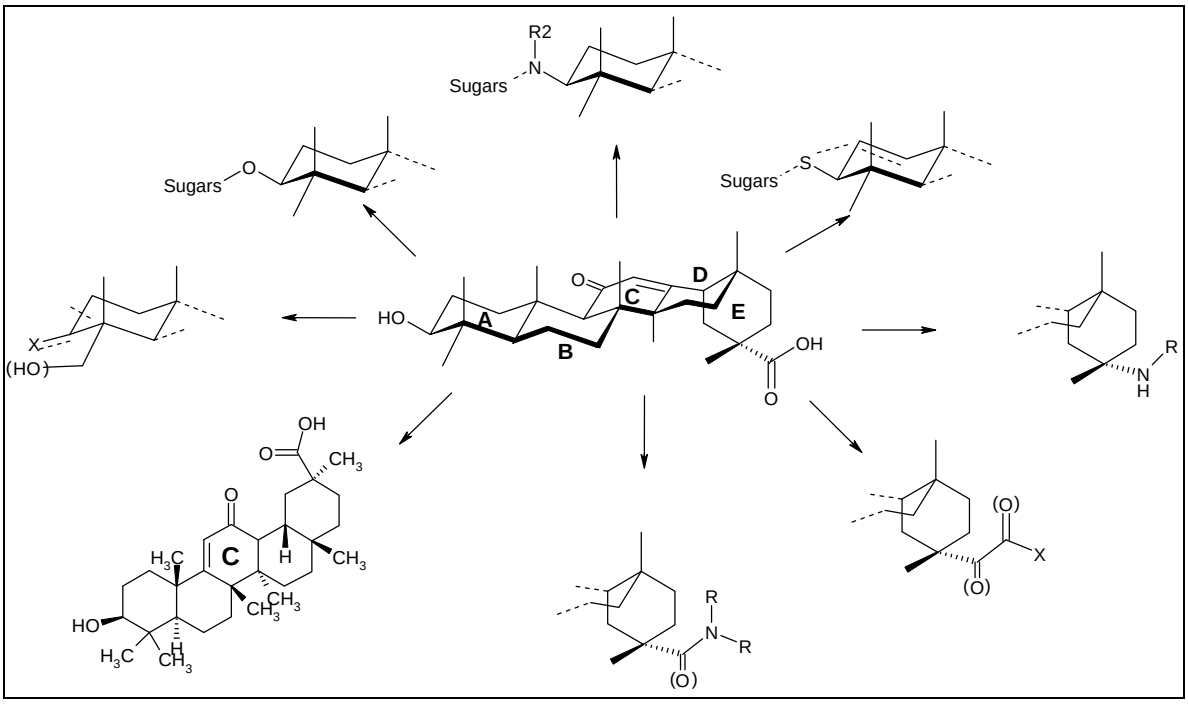


Scheme 15: Approaches towards triterpene neoglycoconjugates of betulinic acid

A similar circumvention making use of spacered precursor molecules allowing for the direct glycosylation protocol was used in a colchicine neoglycorandomization approach earlier[74]. However, a good entry into this field is offered in recent reviews[75,76] summarizing the reported approaches of direct glycosylation in the light of general neoglycorandomization.

1.6 The ASPEX-project and the role of this thesis therein

The work presented in this thesis was performed within the ASPEX-project (Antiviral spot of Excellence) which was raised by the Onepharm GmbH and funded by the ZIT[i] and was initially aiming at glycyrrhizin- and glycyrrhetinic acid derivatives with antiviral activities. However, during the course of the project the field of interest was expanded to evaluate the potency and selectivity of test compounds as selective inhibitors of the 11β-11β-HSD1 and 11β-11β-HSD2-enzymes. In the initial phase of the project, a wide range of small variations of the parent structures should be prepared synthetically to be evaluated in biological testing. Different aspects like the influence of the triterpenoid structure, the linkage between sapogenin part and sugar moiety as well as modification of the most prominent positions of glycyrrhetinic acid (3, 11, 12, 13 and 30) have been considered as possible changes to the lead structures GA and GN. Scheme 16 gives an overview of the activities within the project including the results described in this thesis.



Scheme 16: Different types of modifications of the lead structures glycyrrhizin and glycyrrhetinic acid

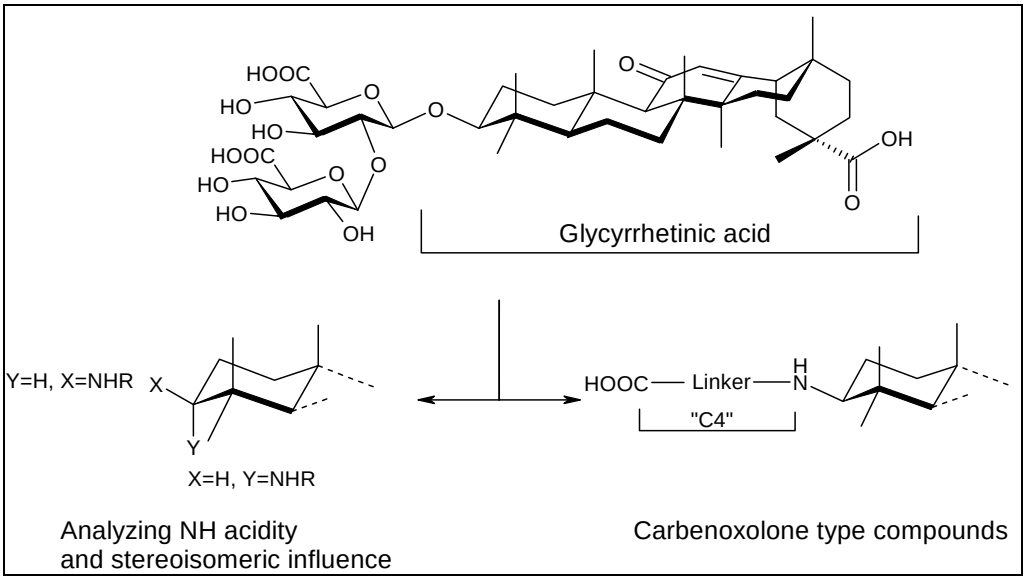
The team of Prof. Kosma was mainly focused on variations in the A-Ring substitution pattern and the linkage of the sapogenin to sugars (upper part of Scheme 16). These approaches included the preparation of a large number of *O*-glycosides[77,78] as well as approaches towards unnaturally linked glycosides, including the preparation of sulfur containing compounds[79]

[i] Zentrum für Innovation und Technologie GmbH: Vienna Spot of Excellence, Grant Number 182081

as well as nitrogen containing compounds, which this thesis was focused on. The team of Prof. Jordis was focused on modifications of other parts of the parent structure. Among other achievements the researchers of this team were focused on various modifications of position 30, including degradation as well as elongation reactions. Furthermore, efforts towards the modification of ring C and A (including side-chain oxidation at the methyl groups of ring A) have been undertaken (lower part of Scheme 16)[80]. From the retrospective it can be stated that derivatives carrying sugar moieties are interesting for antiviral screening only, while derivatives of glycyrrhetinic acid showed interesting activities both in the antiviral screening and as 11β-HSD1/11β-HSD2 inhibitors. While in the first phase of the project single modifications have been performed, based upon first pharmacological results, these modifications have been combined to give tentatively more potent bioactive compounds.

1.6.1 Subject of the thesis

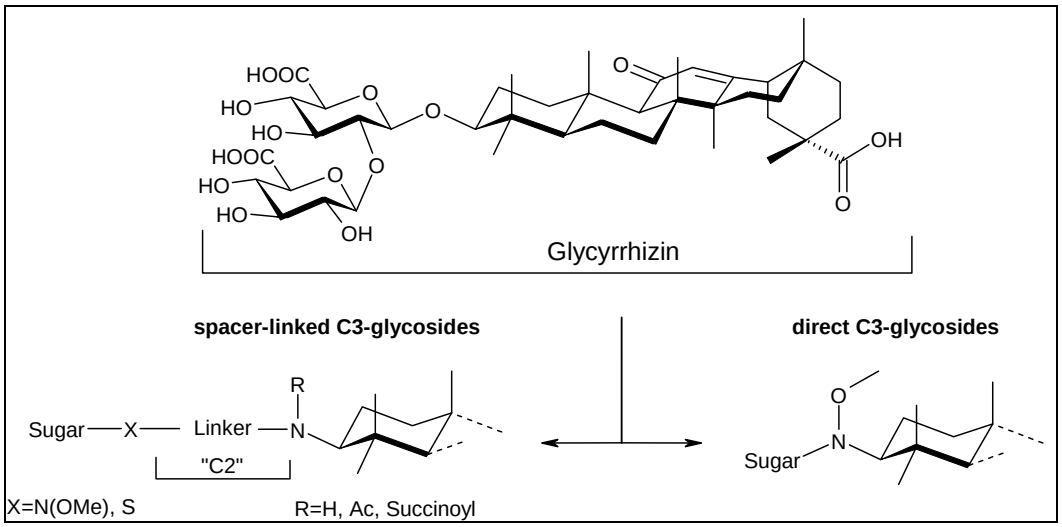
The major aim of this thesis was to prepare, characterize and deliver 3-amino derivatives of glycyrrhetinic acid and glycyrrhizin for pharmacological evaluation as antiviral compounds and 11β-HSD1/11β-HSD2-selective inhibitors. Based on the two lead structures the target compounds can as well be separated into two classes of compounds. First, 3-amino-glycyrrhetinic acid derivatives should be investigated (Scheme 17).



Scheme 17: Synthetic targets in the field of 3-amino-glycyrrhetinic acid derivatives

By versatile decoration (amides, sulfonamides, and alkylation) of both epimeric 3-amino-positions, compounds carrying NH moieties with a wide range of acidity should be prepared. This set of compounds should allow to define structure activity relationships concerning the

relative stereochemistry of the amino-group at the 3-position on the one hand and concerning the acidity/basicity of the adjacent NH group on the other hand. Furthermore, 3β-amino-derivatives mimicking carbenoxolone, should be prepared to be compared with the parent structure. By this first more random-like approach towards new 3-amino-GA derivatives structure activity relationships should be obtained from the biological tests. Based on the biological results of compounds described within this thesis but also related results from compounds prepared by other members of the ASPEX-project more tailor-made compounds should be envisioned and prepared. The second targeted group of compounds is consisting of glycyrrhizin derivatives, thus carrying sugar moieties, attached to the 3-amino-GA triterpene scaffold. Aiming at mimicking the first sugar moiety of the parent structure it should be investigated if a library approach towards neoglycosides, carrying the glycosidic bond directly at the 3-position of the triterpene scaffold, can be established (Scheme 18, right path).



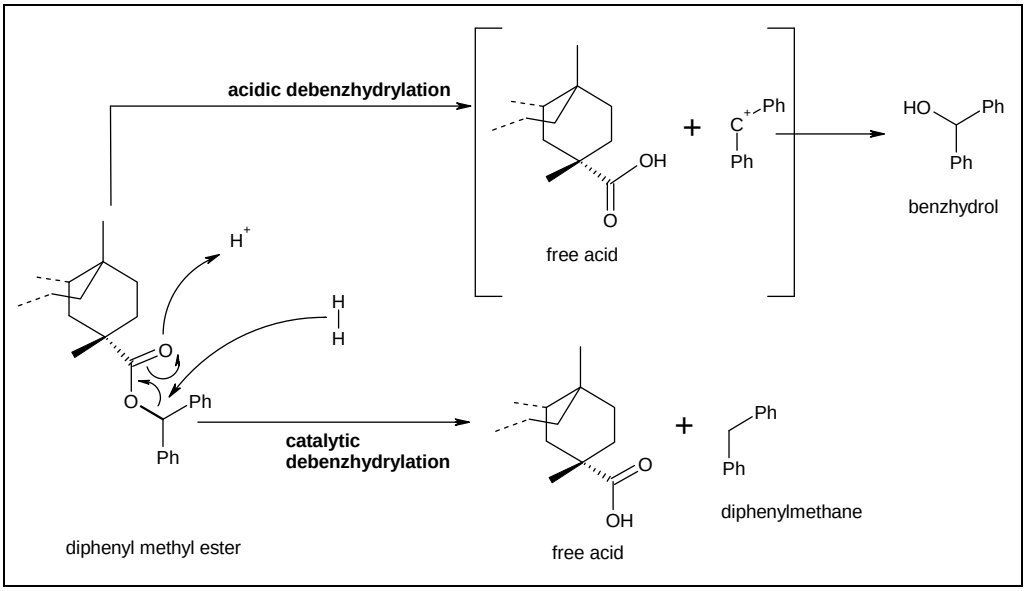
Scheme 18: Synthetic targets in the field of 3-amino-glycyrrhizin derivatives

Furthermore, spacer-linked neoglycosides, in a very simple way mimicking the second sugar moiety in glycyrrhizin, should be prepared. For both synthetic directions the applicability of the direct glycosylation of secondary alkoxyamines with reducing sugars should be examined. The set of compounds should be chosen in a way to allow evaluation of the role of charges or acidic moieties in the neighborhood of the 3-position of the triterpene core. By attaching glucuronic acid to the triterpene structure the influence of the free carboxylic acid can be evaluated, preparing both esters and free carboxylic acid derivatives. Furthermore, similarly to the approach above, the amino-moiety can further be decorated with succinyl residues (carbenoxolone-analogue) to establish a second acidic position, coming up with compounds very similar to glycyrrhizin regarding to number and position of carboxylic acid functions.

2 Results and Discussion

2.1 Strategy for the preparation of 3-amino-GA derivatives

Attempting the preparation of a wide range of test compounds for the investigation of selectivity between 11β-HSD1- and 11β-HSD2-enzymes and their antiviral activity, diastereomerically pure amines had to be prepared. We were especially interested in the pure 3β-amino-GA derivatives. Although the parent structure 3-amino-3-deoxy-glycyrrhetinic acid[6,63,8] and the corresponding methyl esters are known[63], no detailed report for a convenient large scale synthesis of especially the 3β-amine was available. Furthermore, using the 3-amino-GA as most atom-economic precursor would mean to deal with amino acids in workup, purification and characterization (salt forms). It was therefore decided to use a protected precursor molecule for the initial preparation of many different compounds in small amounts. The diphenylmethyl ester (Dpm) was selected as carboxylic acid protecting group, which is very stable under basic and nucleophilic conditions but can be cleaved easily either by catalytic hydrogenation (double-activated benzylic position) or by strong acids (stabilized carbocation), both in excellent yields[81].

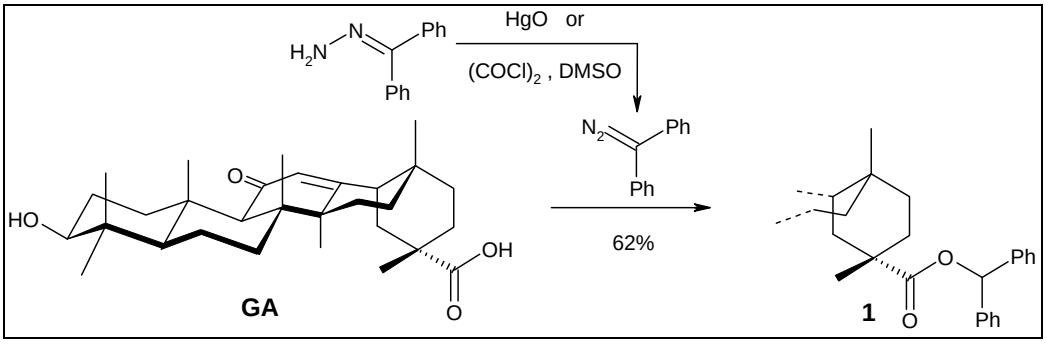


Scheme 19: Two possible approaches for the cleavage of the diphenylmethyl ester moiety

One disadvantage of the Dpm-ester is that during deprotection either non volatile benzhydrol or diphenylmethane are formed, which have to be separated from the acid. The diphenylmethyl ester as protection for GA was claimed early[82] and was used more recently in the preparation of *O*-glycosides[83,84].

2.2 Preparation of the 3-amino-glycyrrhetinic acid, Dpm esters

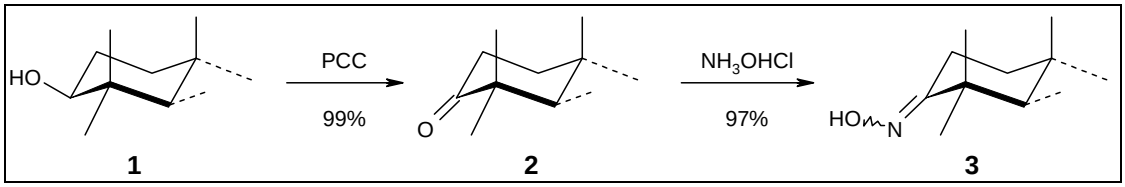
The synthesis of both 3-epimers of 3-aminoglycyrrhetinic acid, diphenylmethyl ester was started from commercial GA, which was first protected as diphenylmethyl ester by reaction with diphenyl diazomethane (Scheme 20).



Scheme 20: Protection of GA as diphenylmethyl ester [1]

The preparation of diphenyl diazomethane by oxidation of benzophenone hydrazone can either be achieved by the classical and very old procedure with HgO[85] or alternatively by a green chemistry approach based on the oxidation with oxalyl chloride and DMSO, which was published recently[86,87]. Although the prevention of the use and disposal of hazardous material like mercury is clearly a praiseworthy and knightly thing to do, from the practical point of view a high price is paid due to much more sophisticated reaction conditions, including deep temperatures under inert gas atmosphere.

Starting from the diphenylmethyl ester of GA **1** the corresponding 3-amino-GA derivatives have then been prepared by the reduction of the corresponding oxime, which was obtained from the alcohol **1** *via* PCC oxidation to give ketone **2** and subsequent oxime formation (**3**) (Scheme 21).

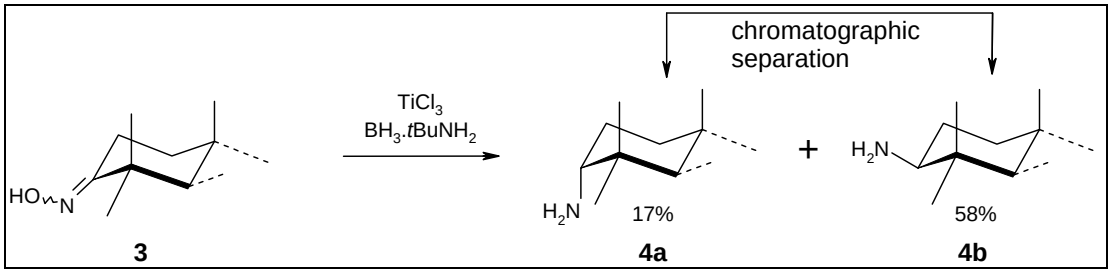


Scheme 21: Preparation of 3-keto glycyrrhetinic acid derivative [2] and its corresponding oxime [3]

The reduction of **3** to the diastereomeric mixture of amines **4a** and **4b** was achieved with $NaCNBH_3/TiCl_3$ or $BH_3.tBuNH_2/TiCl_3$ in EtOH-H_2O, based on literature protocols. The latter method was established as a reproducible and scaleable procedure (Scheme 22).

Although the reduction proceeded fast giving mainly the targeted amines, some practical details complicated the isolation of larger amounts of diastereomerically pure 3-amino-GA

Dpm esters. On the one hand in order to keep reaction mixtures homogenous (which is especially important on larger scale), oxygen should be excluded from the reaction mixture (stripping with argon is sufficient) since otherwise a slimy white solid (most probable some Ti(IV) species from the oxidation of $TiCl_3$ with O_2) is hindering stirring and complicating the aqueous workup. During work up it is important to first distribute the reaction mixture between satd. NH_4Cl and DCM in order to separate most of the titanium species, which would precipitate from alkaline solutions.



Scheme 22: Reduction of oxime [3] to the two diastereomeric amines [4a] (α-epimer) and [4b] (β-epimer)

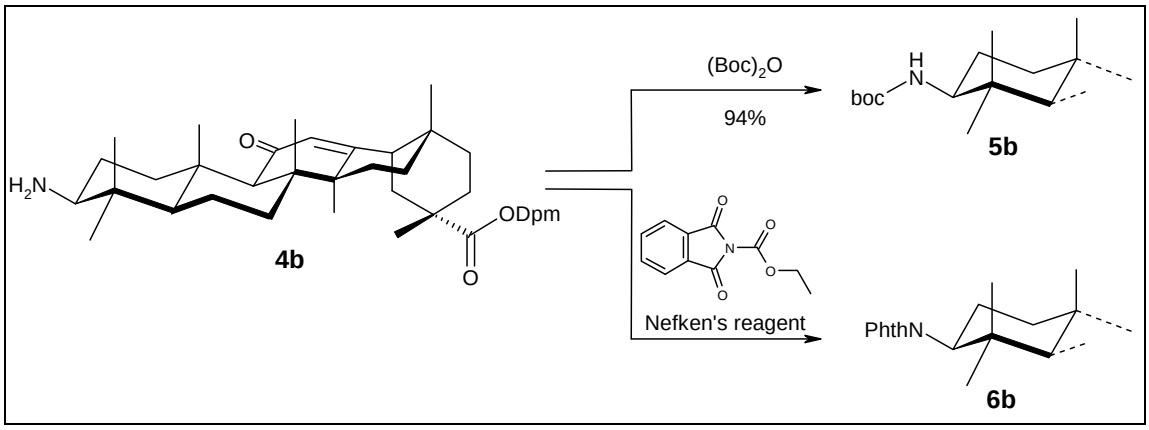
Due to the hydrophobic part of the GA scaffold (especially when protected as Dpm ester) hardly any material is lost in the aqueous phase despite the large amounts of EtOH in the reaction mixture. For the separation of the diastereomers **4a** and **4b** column chromatography of the corresponding acetates on SiO_2 by a stepwise gradient based on $CHCl_3$:MeOH with 1% AcOH as modifier was the methode of choice. In order to obtain the free base and also get rid of SiO_2 washed out from the column with the polar solvent mixture, target compound containing fractions were concentrated and treated with satd. $NaHCO_3$, dried and evaporated before storage.

Unfortunately, a separation based on crystallization could not be established for the protected amines, which remained solid foams under all conditions attempted. Within a closely supervised diploma thesis a more efficient synthesis towards both the protected as well as the unprotected amines was elaborated[88] in the meantime.

2.2.1 Support of the of absolute stereochemistry 3β-amine [4b]

All literature precedence covering the reduction of other 3-hydroximino or 3-imino derivatives of GA with BH_3 or $NaCNBH_3$ reported the reaction outcome to be in favor of the 3β-configuration. However, since especially the 3β-amino-GA Dpm ester **4b** was a central intermediate for most of the following syntheses the β-configuration was further supported by NMR-analysis of the corresponding *N*-Boc-derivative **5b** and the *N*-Phthalyl-derivative **6b,** respectively, which were prepared by standard methods (Scheme 23).

While the ^{1}H-spectra of the two diastereomeric amines did not allow determining the vicinal proton couplings of H3 in an unambiguous way this could be achieved in case of the protected derivatives **5b** and **6b** and did further support and confirm the assigned stereochemistry.



Scheme 23: Derivatization of β-amine [4b] for the determination of the stereochemistry at C3

For both species **5** and **6** the H3 signal showed a large coupling of ~12Hz explained by the vicinal axial-axial coupling with the axial H2-proton, thus supporting the equatorial orientation of the amino-substituent. For easier interpretation the carbamate NH-proton of **5** was exchanged with D_2O prior to NMR-recording (Figure 7).

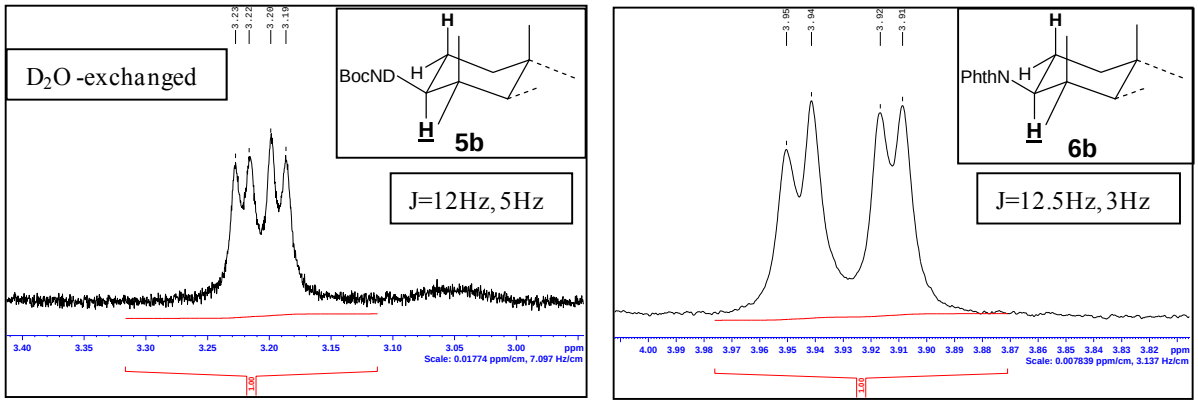


Figure 7: Proton signals of H3 in *N*-Boc-3β-amino-GA- [5b] and *N*-Phth-3β-amino-GA derivatives [6b]

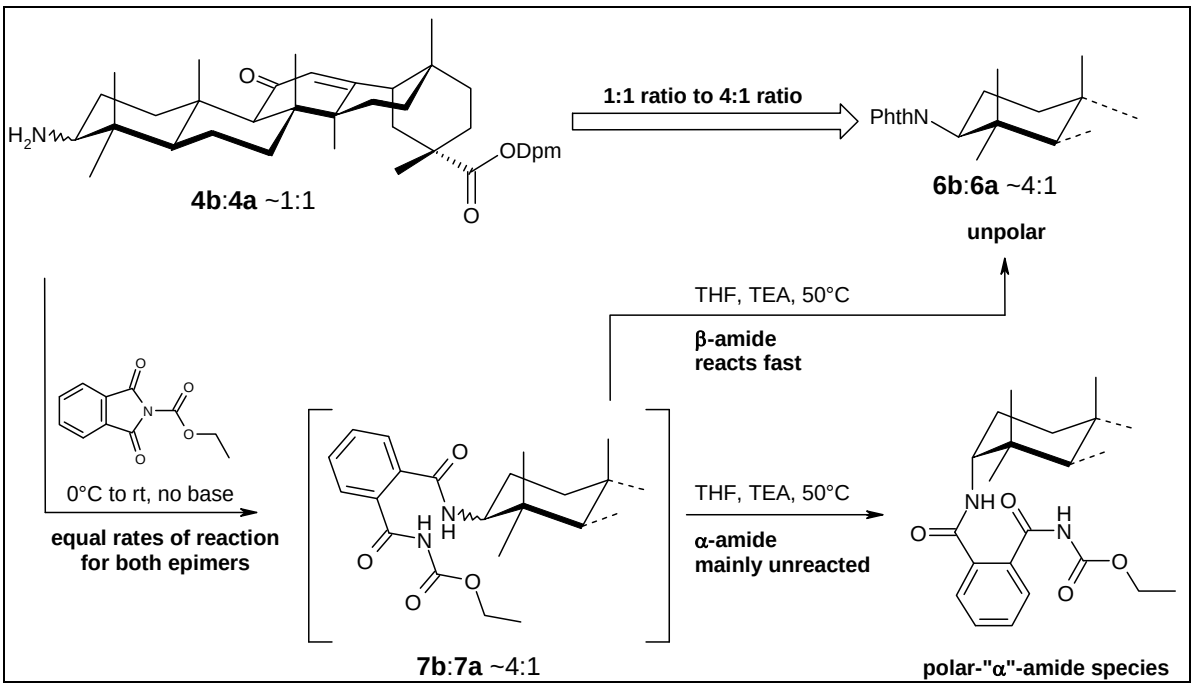
The correct stereochemical assignment was further supported by the NMR-spectra of several pairs of derivatives, which have been prepared later in the project.

In this discussion it is obviously assumed, that the whole ring system did not flip into another conformation, which would allow for the α-amine and its derivatives to take an axial position as well. This assumption is supported by a lot of spectral and other data gained within the whole project.

2.2.2 Chemical discrimination of amines [4a] and [4b]

Although the preparation and purification of the two epimers **4a** and **4b** was finally established in very good yield the large amounts of time and solvent (10L of eluent for 7g of amines, recycling of solvents already included) necessary for the chromatographic separation prompted the evaluation of possible chemical discrimination between the two diastereomeric amines. The main idea was to address the potentially higher reactivity of the equatorial amine **4b** compared to the axial amine **4a**. It was expected that the axial amino-group should be more affected by the adjacent, sterically demanding methyl groups.

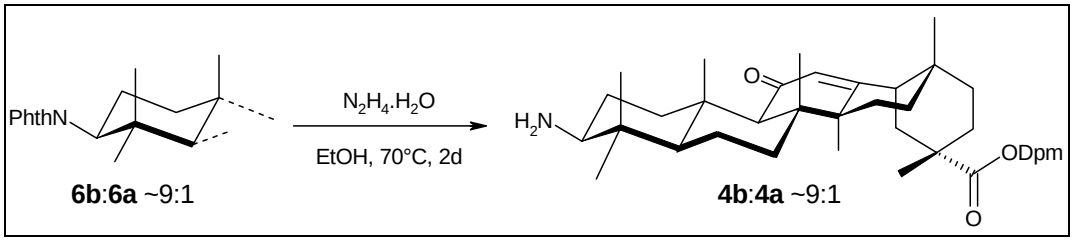
Preliminary results had shown that both epimers can be transformed to corresponding Boc-carbamates and that those are not easily separable. To further enhance the steric stress, the phthalyl group was chosen for temporary protection. For the introduction of the phthalyl protection the Nefken's reagent can be used, reactive enough to convert primary amines to phthalimides under much milder conditions than the classic conditions with phthalic anhydride (Scheme 24).



Scheme 24: Superior reactivity of β-amine [4b] over its α-epimer [4a] in phthalimide formation

Similarly to the equal reactivity shown towards Boc_2O the initial reaction with the Nefken's reagent was equally fast for the two epimers, even at the mild conditions applied (Scheme 24). However, when the mixture of intermediates **7a** and **7b** was treated with catalytic amounts of TEA at 50°C in THF the β-intermediate reacted faster to the corresponding phthalimide **6b**, thus proving the initial hypothesis. To challenge this method a 1:1 mixture of the amines

4a/**4b** was converted to the corresponding phthalimides **6a**/**6b** in a diastereomeric ratio of β:α~4:1. The successful deprotection of the mixture of the phthalimides (β:α 9:1) was also proven to be possible with $N_2H_4.H_2O$ in EtOH at elevated temperatures and prolonged reaction time. Both epimers were re-converted to the corresponding amines (Scheme 25).



Scheme 25: Successful re-formation of 3-amino-GA ester [4b] from its phthalimide [6b]

Although this methodology was actually not used to prepare larger amounts of β-amine **4b** it is regarded as valuable information, which could very probably be further optimized in case that really larger amounts of **4b** are needed in the future.

2.2.3 Determination of the epimeric ratio of the 3-amino-GA, Dpm esters [4a/4b]

The 3-amino-GA diphenylmethyl esters **4a** and especially **4b** were starting materials for the majority of compounds prepared within this thesis. It was therefore mandatory to prepare diastereomerically pure **4a** and **4b** in a reproducible manner and have a tool to quantify the ratio between the two epimers in a simple and fast way.

Since reliable quantification can not be done on TLC (tailing behavior, similar R_f-values) an HPLC-method was developed to address this issue. Optimizing mainly the flow rate and gradient of CH_3CN and water (both with 0.1% HCOOH as modifier, which is the standard setup in the lab) a method was elaborated (Method **C** in the experimental part), which can reliably distinguish between amines **4a** and **4b** down to a ratio of 95:5. Since base-line resolution was only achieved at very low concentration of analytes the method was calibrated by recording samples with different ratios **4a**/**4b**. In order to allow reliable quantification a total concentration of amines of approximately 0.1µmol/mL should be applied (Figure 8).

The developed method was used during the optimization of the conditions for the preparative chromatographic separation and for the determination of epimeric ratios of crude mixtures and isolated fractions or pools of fractions.

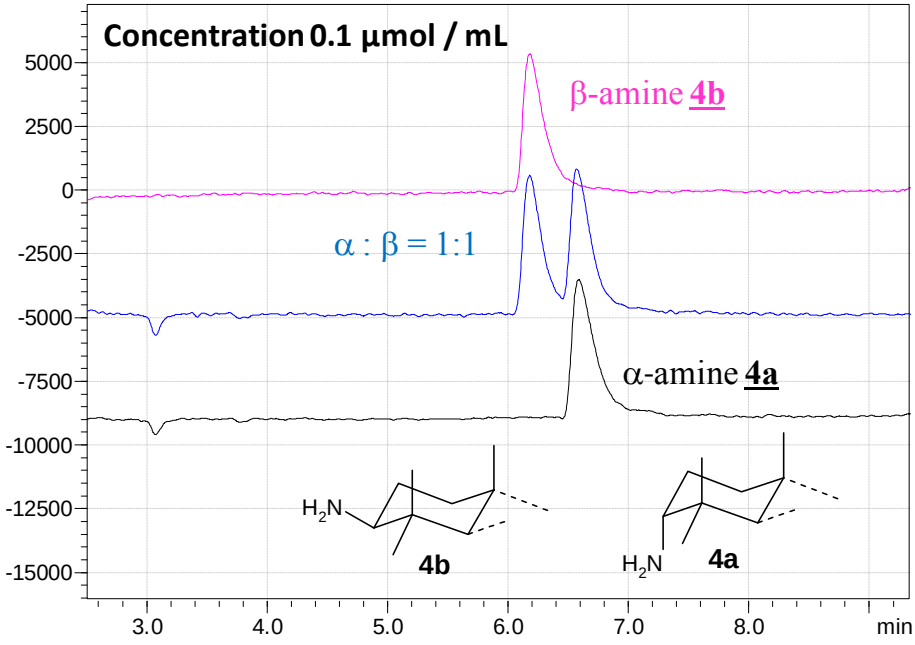
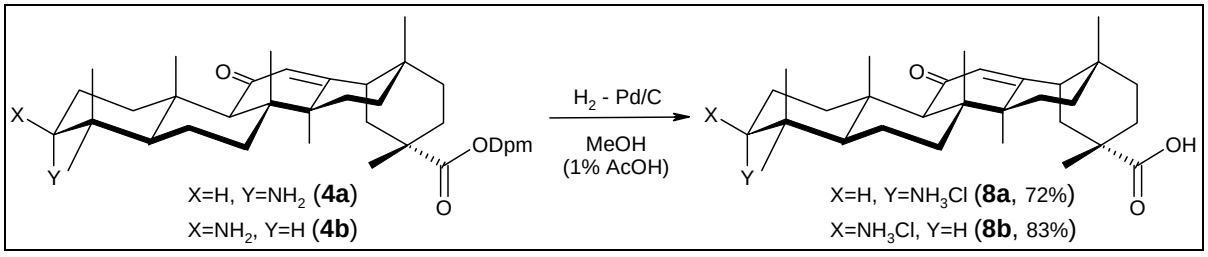


Figure 8: HPLC-analysis of the epimeric ratio of amines [4a] and [4b]

2.2.4 Deprotection of the 3-amino-GA, Dpm esters [4a/4b]

Starting from the protected 3-amino-GA derivatives (mainly from the 3β-amine **4b**) a variety of related compounds should be prepared and deprotected to be evaluated as selective 11β-HSD1/11β-HSD2 inhibitors or as antiviral compounds, respectively. It was therefore clear that successful debenzhydrylation will be a frequent and important synthetic transformation within this investigation.



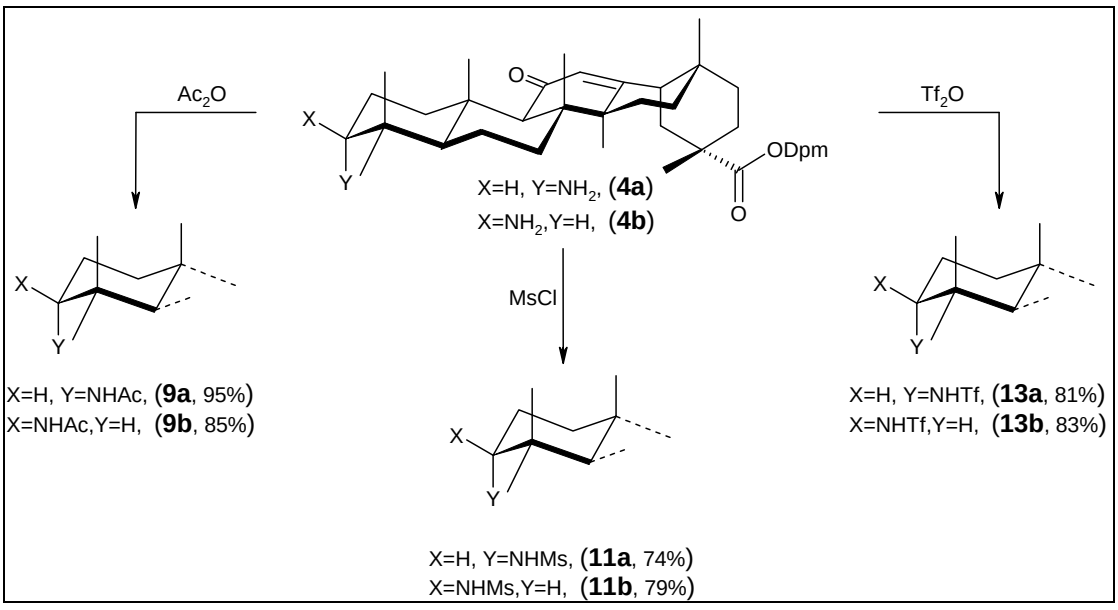
Scheme 26: Hydrogenolyis of 3-amino-glycyrrhetinic acid, Dpm esters [4a] and [4b]

For this reason the cleavage of the diphenylmethyl ester was investigated in the preparation of the free amine acid hydrochlorides **8a** and **8b**, known compounds which can be prepared also directly from the unprotected GA[8,63]. The 3-amino-GAs **8a** and **8b** have been prepared as

hydrochlorides by hydrogenolytic deprotection under standard conditions with Pd/C in MeOH (with 1% AcOH) at rt. It is important to add some AcOH in the hydrogenation step to obtain a clean and fast conversion, since without AcOH the reaction is sluggish and needs several days at elevated temperature to reach full conversion. Initially, the targeted amino acids were partly lost during workup due to their almost complete insolubility in common solvents as zwitterionic species. It is therefore advisable to convert them to the corresponding hydrochloride already before separation of crude materials from Pd/C. The conversion to hydrochlorides is also beneficial for stable storage and measurement of defined physical data.

2.3 Preparation of 3-amido- and 3-sulfonamido-GA derivatives

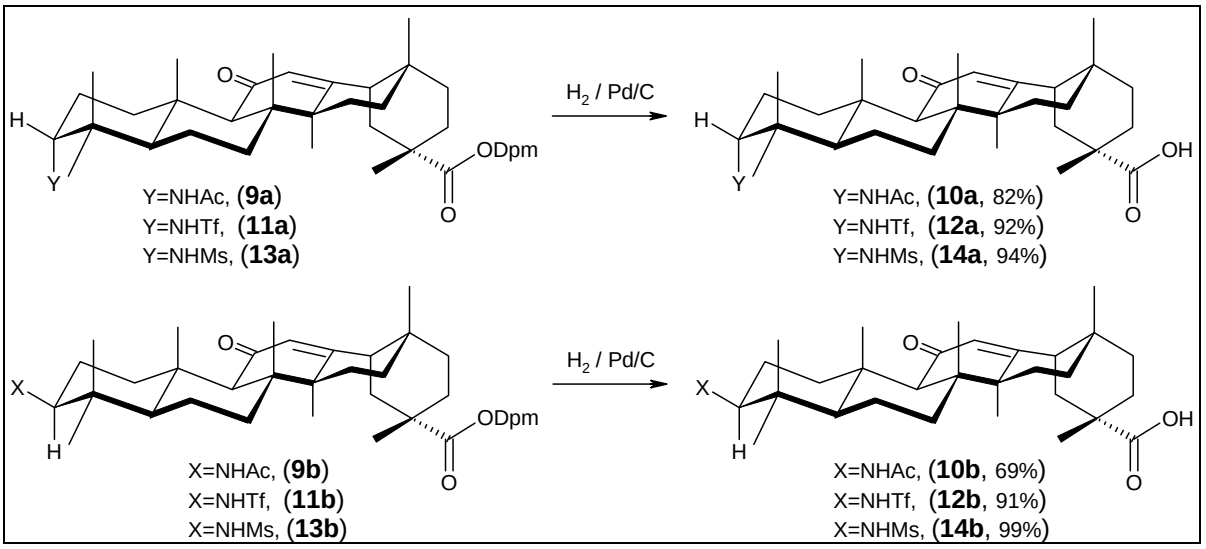
Starting from the 3-amino-GAs **4a** and **4b** a series of acetamides and sulfonamide species of different chemical and physical properties was prepared, to evaluate the effect of different acidities of the NH moiety on the potency of the compounds in the pharmacological assays. Although these compounds were clearly accessible without the use of a protecting group, ease in preparation and purification was favored over synthetic elegance in order to afford the small amounts needed for biological screening reliably and in short time. Therefore, the following test compounds have all been prepared in a two step manner by acetylation or sulfonylation of the protected amines followed by hydrogenolytic deprotection (Scheme 27).



Scheme 27: Acetylation and sulfonylation of α- and β-amino-GA derivatives [4a] and [4b]

In order to supply biology with a set of similar compounds not only the β-amine **4b** (with natural configuration) but both 3-epimers have been submitted to these conversions in order to allow evaluation of the influence of the configuration of the 3-position on the

pharmacological potency. Applying standard methods the 3-acetamide, 3-methylsulfonamide and the 3-trifluoromethylsulfonamide compounds were prepared as diphenylmethyl esters (**9a/9b, 11a/11b** and **13a/13b**) in good to excellent yields.



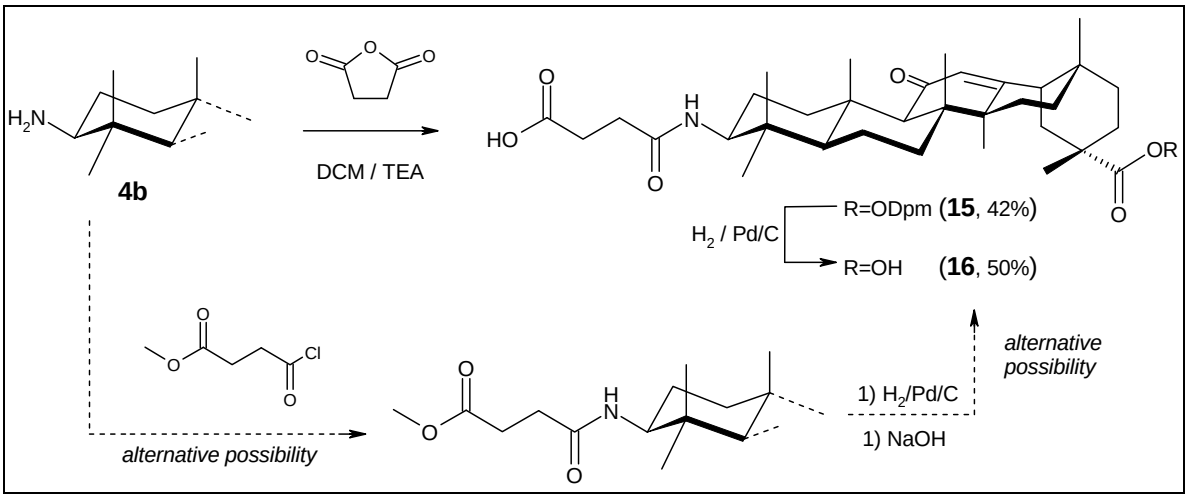
Scheme 28: Hydrogenolytical deprotection of N-acetyl and N-sulfonylamino-GA esters to the corresponding acids

The deprotection of the acetylated and sulfonylated compounds by hydrogenolysis was fast and high yielding without any need for optimization. The only modification that was done from the standard protocol in MeOH was the addition of EtOAc for solubility and 1% of AcOH for reactivity reasons.

2.3.1 Preparation of aminocarbenoxolone analogues

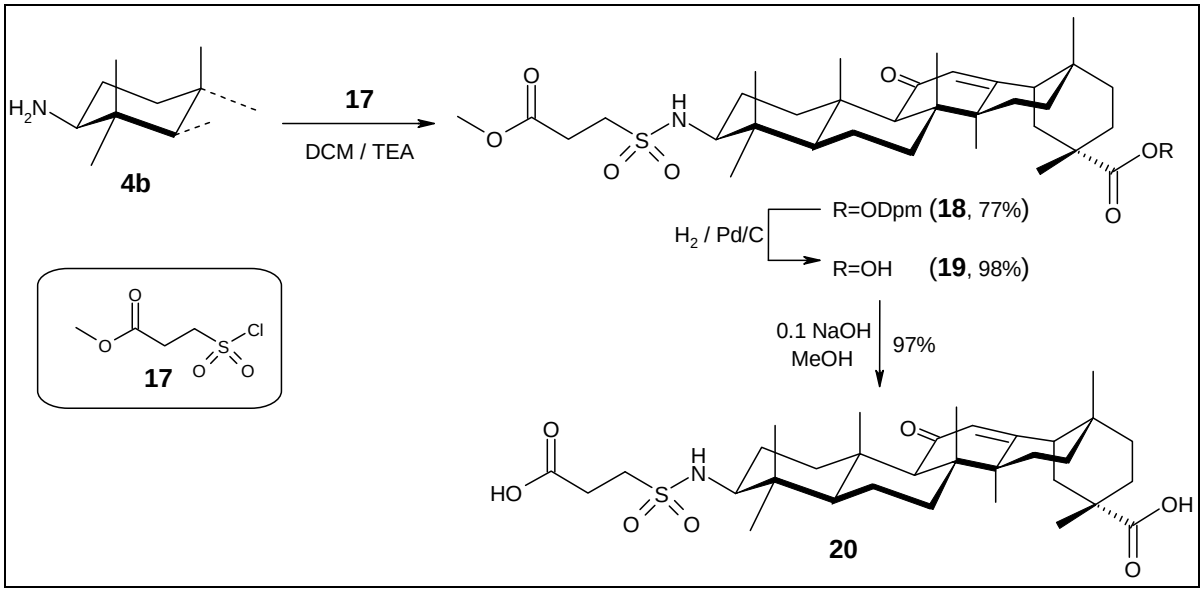
In addition to the above described amide and sulfonamide compounds in the β-series three more test compounds, closely related to carbenoxolone, have been prepared. Their pharmacological evaluation should give insight into the effect of the increased physiological stability compared to carbenoxolone and the effect of the different pK-values of the introduced NH moieties.

Starting from the protected amine **4b**, intermediate **15** was prepared by acylation with succinic anhydride in moderate yields and was deprotected to give amino-carbenoxolone **16**[8] (Scheme 29).



Scheme 29: Preparation of amino-carbenoxolone [16]

The moderate yield of this approach could very probably be increased by optimization of reaction conditions or by using methyl succinyl chloride, and thus a two step process for the acylation. Methyl succinyl chloride was used with excellent results in the acylation of secondary amines of the GA structure (Chapter 2.6.3) later in the project.



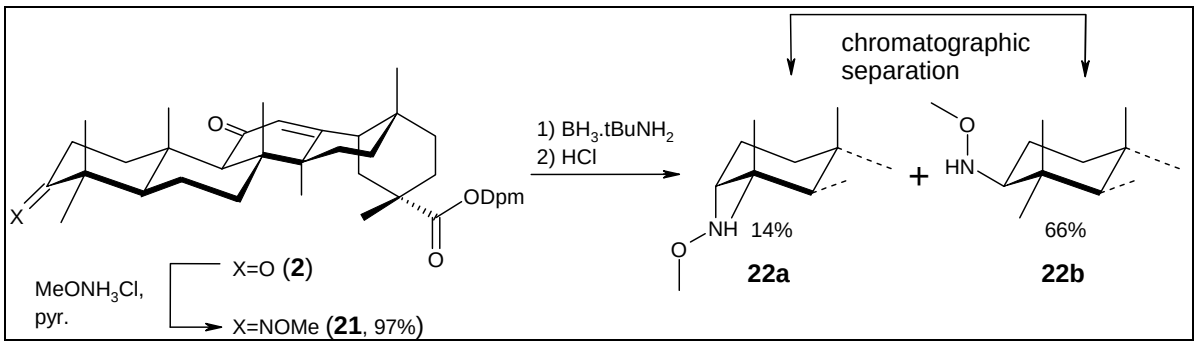
Scheme 30: Preparation of sulfamide- carbenoxolone derivative [20]

For the preparation of the sulfonamido-carbenoxolone derivative **20,** amine **4b** was reacted with commercial sulfonyl chloride **17** applying standard reaction conditions to give intermediate **18** in good yield. Compound **18** was deprotected in a two step process in almost

quantitative yield to give the methyl ester **19** and finally the corresponding carboxylic acid **20** (Scheme 30). It was decided to prepare both methyl ester **19** and free carboxylic acid **20** to allow for the evaluation of the effect of the acidic moiety. This strategy of stepwise deprotection was followed throughout this thesis whenever it was easily possible to prepare two test compounds instead of one.

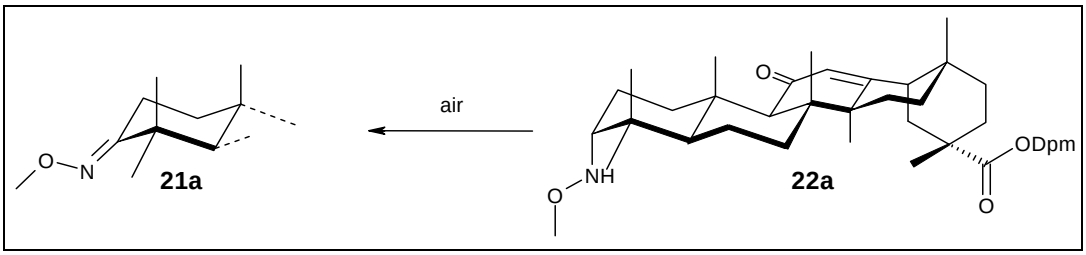
2.4 Synthesis of 3-methoxyamino-glycyrrhetinic acid derivatives

In analogy to the preparation of the 3-amino-GA epimers **4a/4b**, the 3-methoxyamino-GA derivatives **23a** and **23b**, which were initially planned as precursors for libraries prepared by direct glycosylation (Chapter 2.7.1), have been prepared *via* the corresponding oxime ether **21** (Scheme 31).



Scheme 31: Preparation of 3-methoxyamino-GA, diphenylmethyl esters [22a/22b]

Oxime ether **21** was prepared from ketone **2** with methoxylamine hydrochloride in quantitative yield and was reduced with $BH_3.tBuNH_2$-HCl to the two protected diastereomeric 3-methoxyamines **22a** and **22b** (α:β roughly 1:4.5), separable by column chromatography. Pure β-epimer **22b** can alternatively be obtained by repeated recrystallization of the mixture from hot EtOAc.



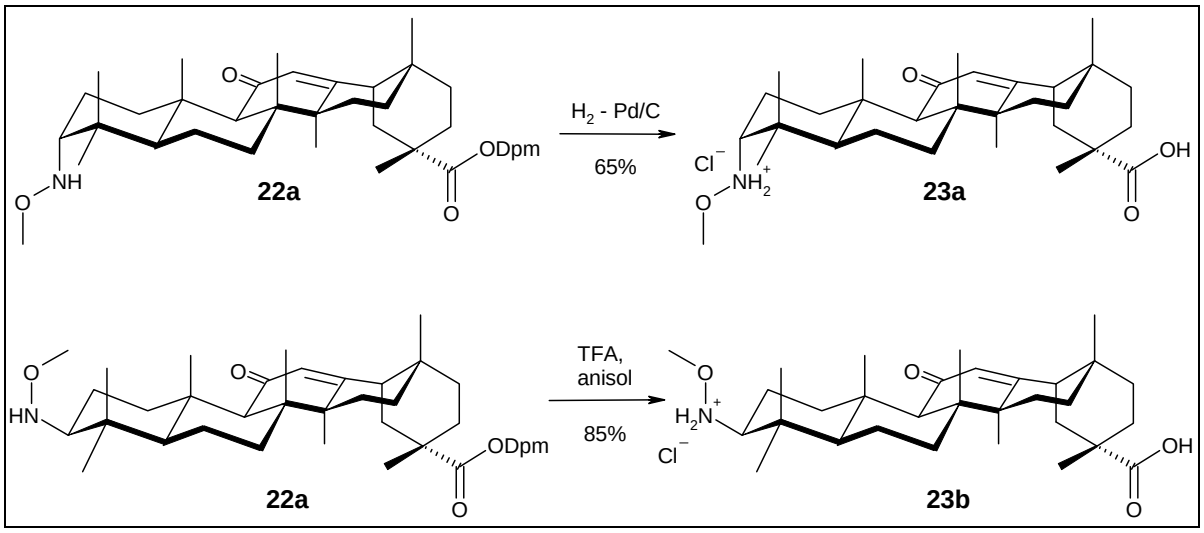
Scheme 32: Reoxidation of α-methoxyamine [22a] on air

Upon storage significant difference in the stability of the two epimers was observed.

While the β-epimer **22b** was stable even for months at rt, α-epimer **22a** faced partial reoxidation on air to the starting material **21**, a behavior, which was more generally described for hydroxylamines recently[89] (Scheme 32). However, attempted use of this difference in stability towards air for purification of the diastereomers **22a/22b** by bubbling air through crude mixtures in DCM, MeOH and $CHCl_3$ did not deliver the expected selective and complete conversion of the α-methoxyamine **22a**.

To evaluate the methoxyamine epimers as inhibitors of 11β-HSD1 and 11β-HSD2 the diphenylmethyl esters **22a** and **22b** were deprotected under hydrogenolytic or acidic conditions, respectively.

Although an explanation cannot be offered, the hydrogenolytic deprotection towards β-methoxyamino-GA **23b** was repeatedly sluggish leading to byproducts, although the parallel experiment with the α-epimer **22a** was completely uneventful and gave the targeted carboxylic acid **23a** in a clean conversion. Therefore, β-methoxyamine **22a** was deprotected under acidic conditions with TFA (with anisol as scavenger) to give **23a** in excellent yield (Scheme 33).

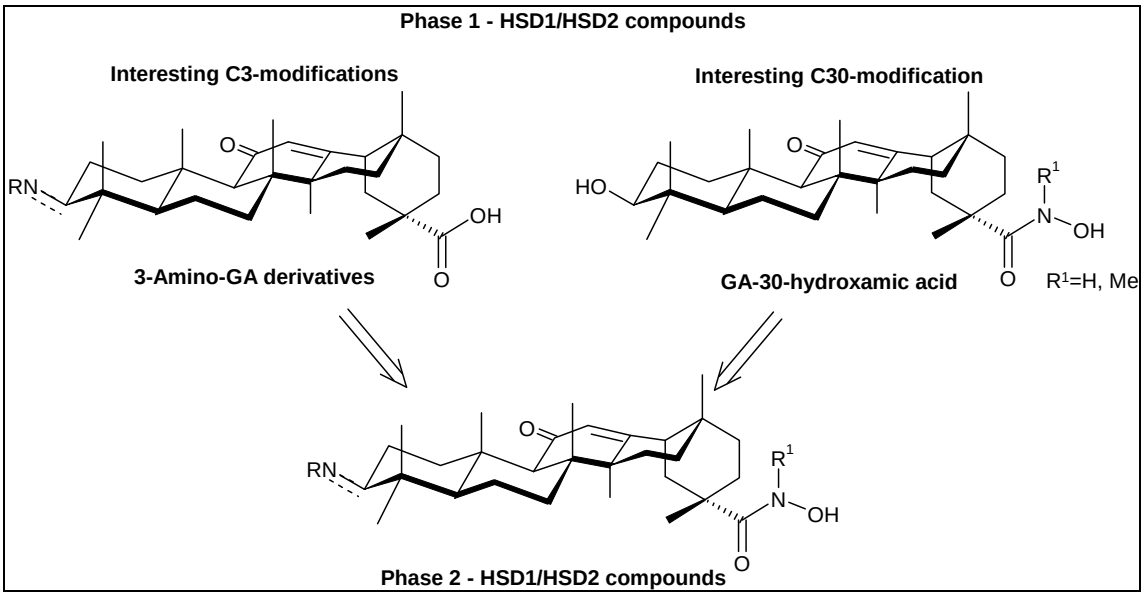


Scheme 33: Different behavior of the 3-methoxyamino-GA derivatives upon deprotection

For stability and solubility reasons in the biologic assay **23a/23b** were converted to the corresponding hydrochlorides during work-up.

2.5 Selection of 2nd generation 3-amino-GA derivatives

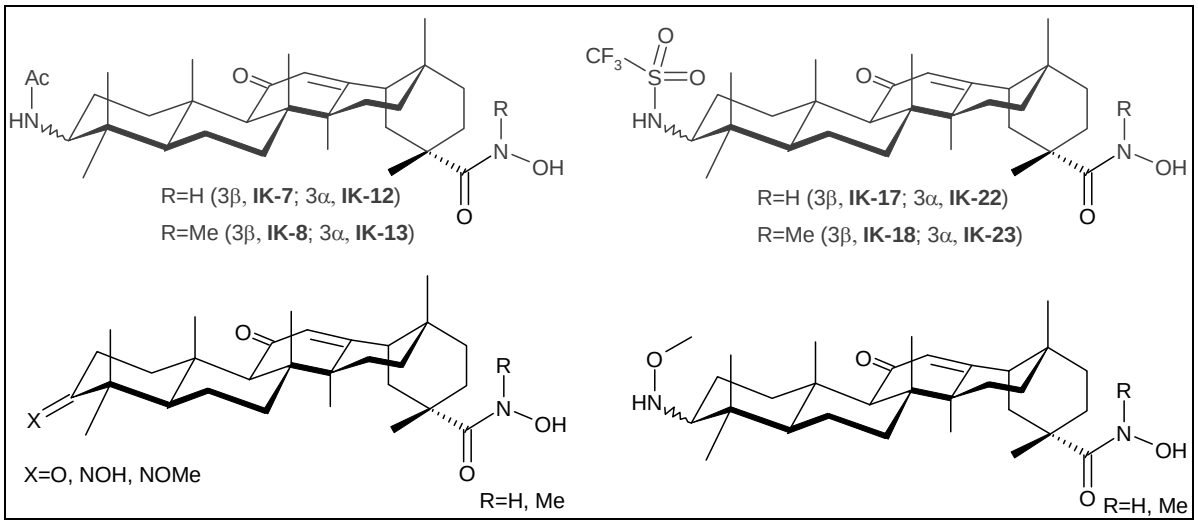
Based on pharmacological results from compounds prepared in the first phase of the project several structural motifs were identified, leading either to noteworthy potency in inhibition or selectivity between 11β-HSD1 and 11β-HSD2 or both. Among these successful structural modifications was on the one hand the introduction of hydroxamic acid moieties at C30, both unsubstituted and *N*-substituted, and on the other hand several amino-based modifications at the 3-position of GA, reported within this thesis.



Scheme 34: Rationale for the selection of 2nd generation 3-amino-GA derivatives

Based on these first positive results from the biology, it was decided to combine these putative pharmacophoric structural motifs within new test compounds. As far as this thesis is concerned, 3-acetylamino-GA, 3-trifluoromethylsulfonylamino-GA and 3-methoxyamino-GA were especially interesting and it was decided to use both 3-amino-epimers and to include the 3-keto- and 3-hydroxyimino derivative as well to come up with a reasonable subset of related compounds to allow structure activity relationships in the enzymatic assay (Scheme 34).

In Scheme 35 the selected compounds from the first part of this thesis are summarized which should all be decorated as *N*-methyl-hydroxamic acids and free hydroxamic acids, respectively.



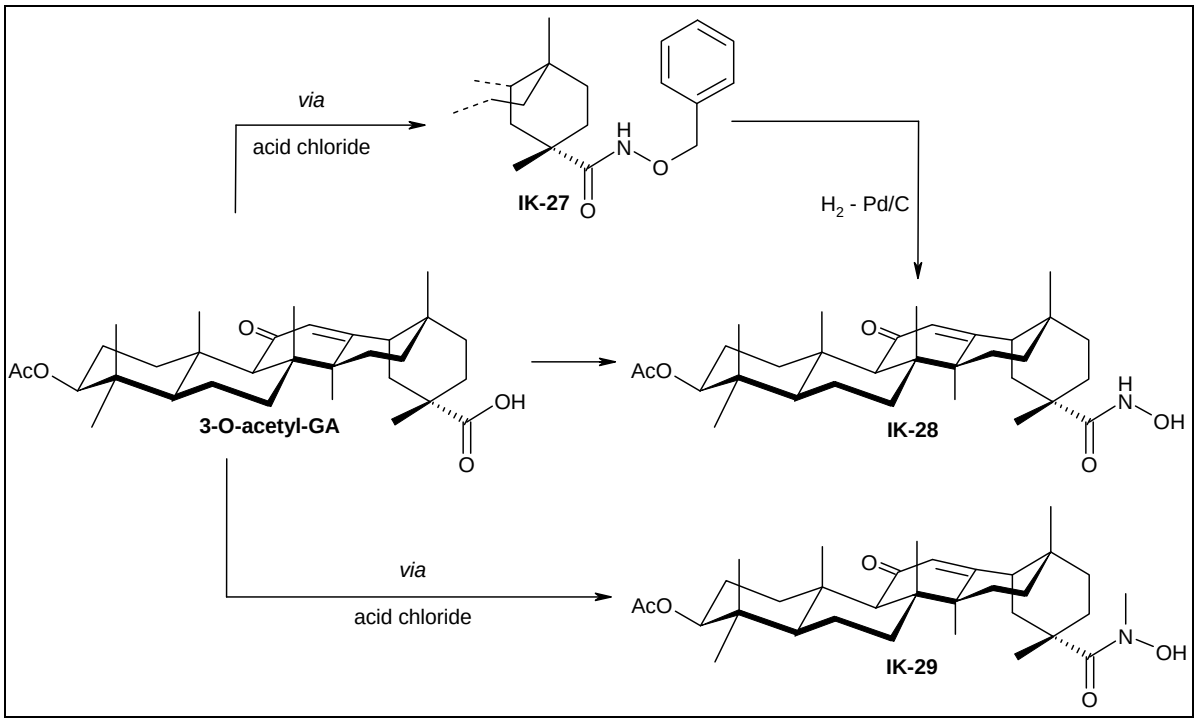
Scheme 35: Selected compounds for decoration as free- and *N*-methyl-hydroxamic acids

The syntheses of these hydroxamic acids was partly carried out within this thesis but was also rolled-out into the supervised diploma thesis of Iris Koller (IK)[90]. The syntheses of 3-acetylamino-GA epimers (**10a**/**10b**) and the 3-trifluoromethylsulfonylamino-GA epimers (**14a**/**14b**) have been scaled up and the compounds were converted to the corresponding hydroxamic acids (**IK-7**, **IK-12**, **IK-17**, **IK-22**), and *N*-methyl-hydroxamic acids (**IK-8**, **IK-13**, **IK-18**, **IK-23**), respectively. The syntheses of these compounds were carried out by Iris Koller and details of their preparation together with a detailed discussion of the synthetic strategies towards and physical properties of hydroxamic acids have been described there[90].

The preparation of *N*-methyl-hydroxamic acids of the 3-keto-, 3-hydroxyimino, 3-methoxyimino and 3-methoxyamino-GA derivatives is described below.

Based on earlier results in the ASPEX-project[91], a close investigation of the applicability of different general methods for the preparation of hydroxamic acids starting from 3-*O*-acetyl-GA was undertaken[90]. The main conclusion of this investigation was to use classical and strong activation for the carboxylic acid as acid chloride, since milder coupling methods did not lead to successful and high-yielding conversion of C30 carboxylic acids to the corresponding hydroxamic acids.

Furthermore, it was elaborated that for free hydroxamic acids (like **IK-28)** a two step process *via* the corresponding *O*-benzyl-hydroxamic acid species **IK-27** is most generally applicable. For the synthesis of *N*-methyl-hydroxamic acids, however, the acid chloride species may be transformed directly to the target compounds[92] (like **IK-29)**.



Scheme 36: Synthetic strategies outlined for 3-AcO-GA (*N*-methyl) hydroxamic acid

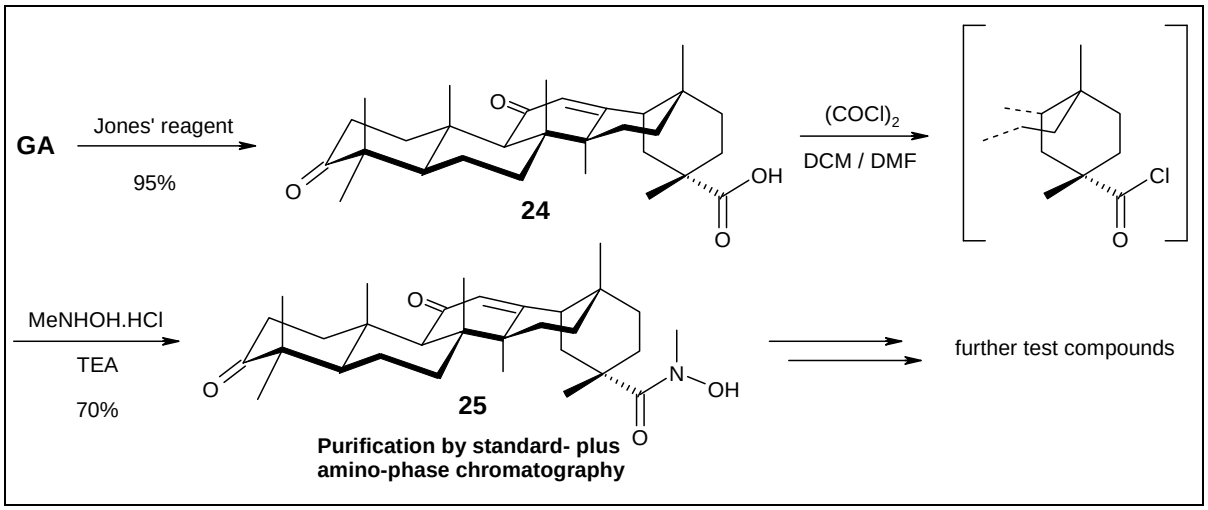
The selected synthetic strategies are depicted in Scheme 36, exemplified on the *O*-acetyl GA, which was used as readily available model compound[90].

2.5.1 Preparation of *N*-methyl-hydroxamic acids

In contrast to the synthetic strategy applied for the *N*-acetyl and *N*-triflyl-GA derivatives for the following compounds the hydroxamic acid moiety was introduced very early in the synthetic route. The synthesis was started from the unprotected 3-keto-GA **24**, which was prepared from GA according to a literature protocol[12].

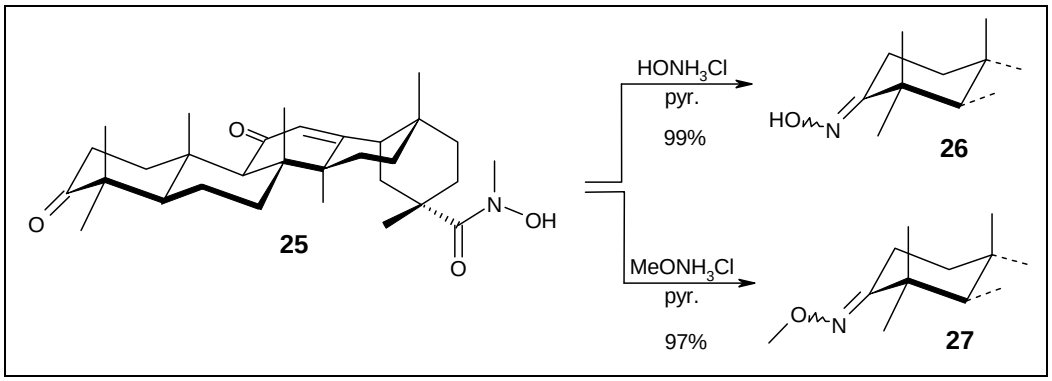
It turned out, that for the conversion of 3-keto-GA **24** to the corresponding acid chloride it was crucial to use the mild oxalyl chloride method catalyzed by DMF in DCM. Only under these conditions reliable and scalable results were achieved. Initial attempts with $SOCl_2$, conditions which were successful with other starting materials, could not be reproduced on a larger scale. During conversion of acid chloride to the N-methyl-hydroxamic acid **25** always substantial amounts of starting acid **24** were re-formed. This observation is especially troublesome because acid **24** and the corresponding *N*-methyl-hydroxamic acid **25** turned out

to be inseparable in all attempted chromatographic eluent systems, due to very similar R_f-values and the tailing behavior on SiO_2.



Scheme 37: Synthesis of *N*-methyl-hydroxamic acid derivatives of GA

However, it was found out that it is easy to separate the two compounds on amino-phase, which is silica gel end-capped with dimethylaminopropyl groups[i]. The purification with standard chromatography was followed by passing the isolated product through a very short bed of amino-phase (3g of material were purified in portions over a bed of 15g amino-phase) in order to afford pure hydroxamic acid **25,** free of any carboxylic acid **24**. (Scheme 37)

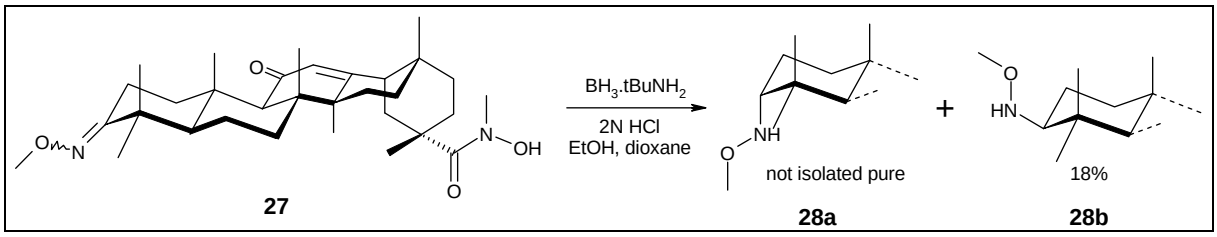


Scheme 38: Synthesis of 3-hydroxyimino and 3-methoxyimino-GA *N*-methyl-hydroxamic acids [26] and [27]

From the 3-keto-derivative **25** the corresponding 3-hydroxyimino-GA derivative **26** and 3-methoxyimino-GA **27** were prepared under standard conditions in pyridine in quantitative yields (Scheme 38). Reduction of 3-methoxyimino-GA-hydroxamic acid derivative **27** with borane *t*$BuNH_2$ was optimized concerning the necessary amount of reducing agent (2equiv. is ideal) and delivered the epimeric methoxyamines **28a** and **28b** (β:α ratio approximately 3:1)

[i] *private communication* of Bernhard Müller is gratefully acknowledged

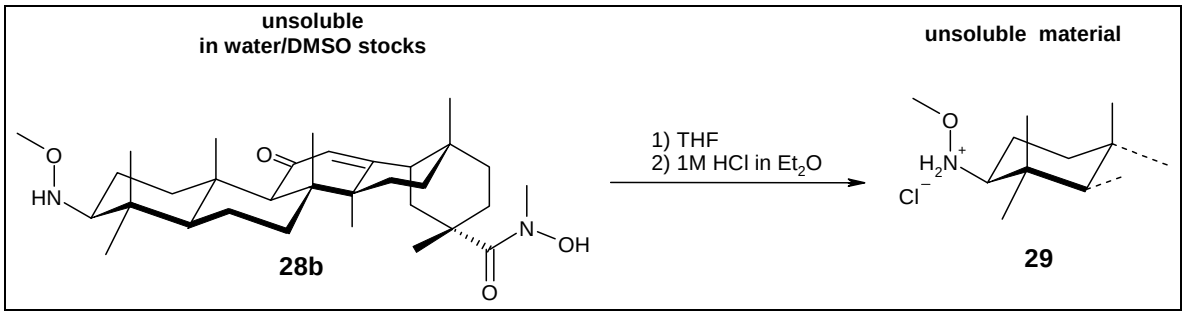
as only detected reaction products. No side reactions at the hydroxamic acid moiety were observed according to TLC and ^{1}H-NMRs of the crude materials, which was a pleasant observation (Scheme 39).



Scheme 39: Reduction to epimeric 3-methoxyamino-GA-*N*-methyl-hydroxamic acids [28a] and [28b]

Unfortunately, the separation of the epimeric 3-methoxyamino-GA-*N*-methyl-hydroxamic acid derivatives **28a**/**28b** turned out to be very difficult in contrast to the corresponding Dpm esters **22a**/**22b**. After excessive chromatography and re-precipitation pure β-isomer **28b** was isolated, although in low yield, **28a** could not be isolated in pure form at all.

Due to the fact, that both structural modifications of the GA in **28b** – formation of *N*-methyl hydroxamic acid on the one hand and introduction of 3β-methoxyamino-moiety on the other hand – have shown especially promising results in the 11β-HSD1/11β-HSD2-enzyme assay, their formal combination in compound **28b** was a key-target for the biology (Scheme 40).
Following *Murphy's Law* the initially submitted material **28b** was not soluble for the biological screening. Only successful transformation to the corresponding hydrochloride **29** allowed the evaluation as 11β-HSD1/11β-HSD2 selective inhibitor.

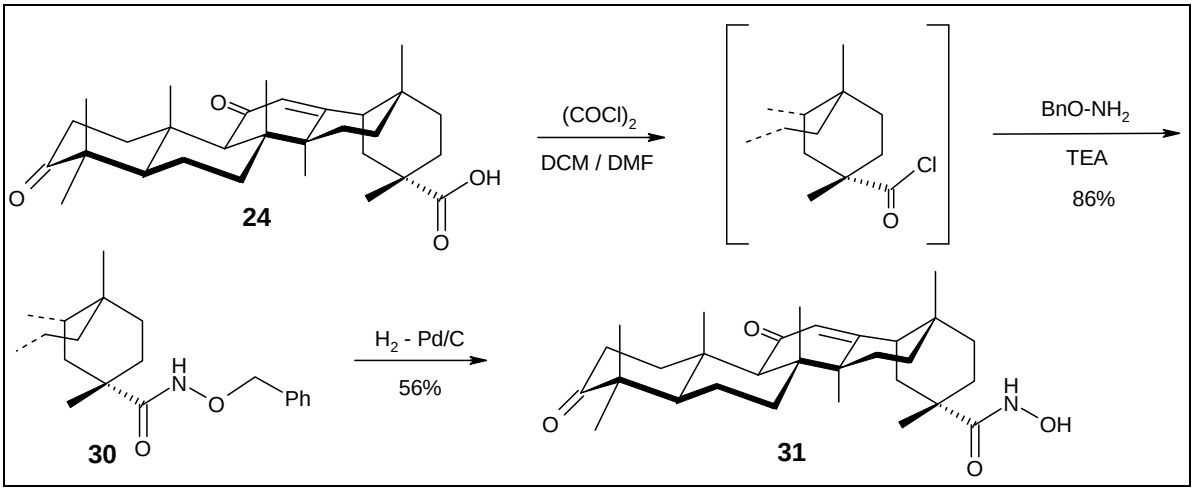


Scheme 40: Conversion of 3-methoxyamino-GA derivative [28b] to hydrochloride [29] to increase solubility

Compound **28b** showed one more interesting and challenging feature: Its ^{1}H-NMR spectrum was extremely sensitive to traces of acid, thus passing $CDCl_3$ over a short bed of neutral Al_2O_3 prior to use is crucial for reproducible results. Until this was recognized, analysis of crude materials and pre-purified fractions was a stunning experience.

2.5.2 Preparation of unsubstituted hydroxamic acids

As explained before, preliminary experiments have shown that the transformation of a C30 carboxylic acid to the unsubstituted C30 hydroxamic acid is most reliably achieved by a two step process *via* the corresponding *O*-benzyl-hydroxamic acid as intermediate. In analogy to the approach above starting from 3-keto-GA **24** the acid chloride (oxalyl chloride/DMF) was formed and trapped with *O*-benzylhydroxylamine to give almost exclusively the targeted intermediate **30** (Scheme 41).



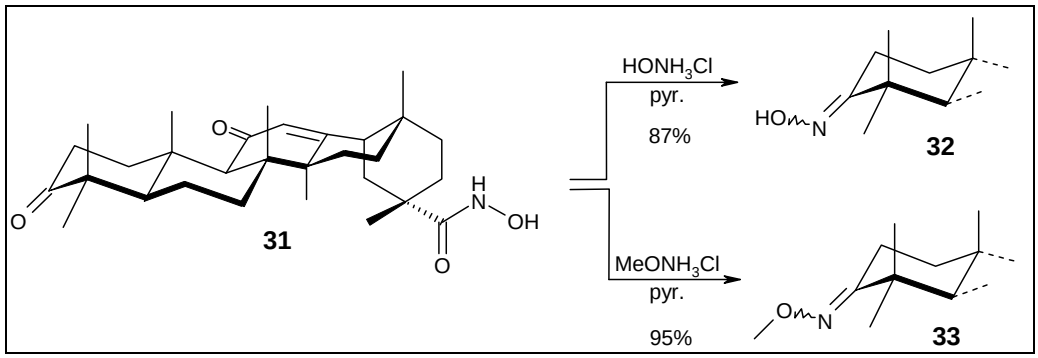
Scheme 41: Transformation of 3-keto-GA [24] to the corresponding hydroxamic acid [31]

Purification by column chromatography and a short bed of amino-phase to separate the remaining trace amounts of acid **24** gave *O*-benzyl-hydroxamic acid **30**, which was debenzylated by catalytic hydrogenation to give hydroxamic acid **31**. Although no by-products were observed, final purification on SiO_2 led to only moderate isolated yields of 56%, a drawback which was also observed in the corresponding acetamide- and sulfonamide series[90].

Due to the lower stability of free hydroxamic acid derivatives compared to their *N*-methyl-analogues a milder oxime-formation method with NaOAc in $CHCl_3$/MeOH at slightly elevated temperatures was applied for the preparation of the 3-hydroxyimino-GA derivative **32** and 3-methoxyimino-GA derivative **33**. This method was optimized for a similar conversion within the diploma thesis of Alexander Doppelreiter[88], based on a literature protocol[73].

In order to prevent the necessity to purify by column chromatography (with expected loss of compound analogous to the purification of **31**) complete conversion was especially important in these two oxime formations. Due to very similar R_f-values on TLC and a tendency to exhibit tailing spots of all three hydroxamic acids **31**, **32** and **33** other means of reaction

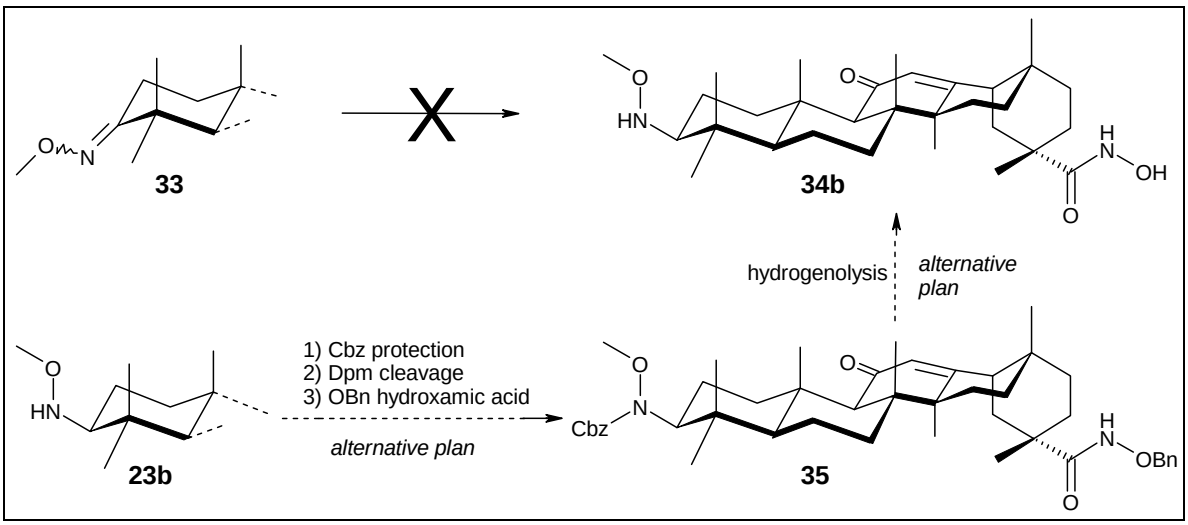
monitoring had to be applied. While the formation of oxime **32** could easily be followed and quantified *via* HPLC, this was not achieved for the conversion of **31** to **33** (Scheme 42).



Scheme 42: Synthesis of 3-hydroxyimino and 3-methoxyimino-GA hydroxamic acids [32] and [33]

Therefore the conversion of **31** to **33** was monitored by ^{1}H-NMR, based on the integration of the H9 singlet.The conversion of oxime **33** to the corresponding 3β-methoxyamine **34b** could not be achieved analogous to the preparation of *N*-methyl-hydroxamic acid **28b**. An unidentified mixture of compounds, unseparable on SiO_2 was obtained; not really unexpected after the tedious separation of **28b** and the low stability of free hydroxamic acid **31** on SiO_2.

It is planned to prepare pure **34b** *via* Cbz-protection of diastereomerically pure 3β-methoxyamino-GA **23b**, introduction of *O*-benzyl-hydroxamic acid (**35**), purification and double deprotection by catalytic hydrogenation (Scheme 43).



Scheme 43: Alternative strategy towards the synthesis of pure β-3-methoxyamino-GA hydroxamic acids

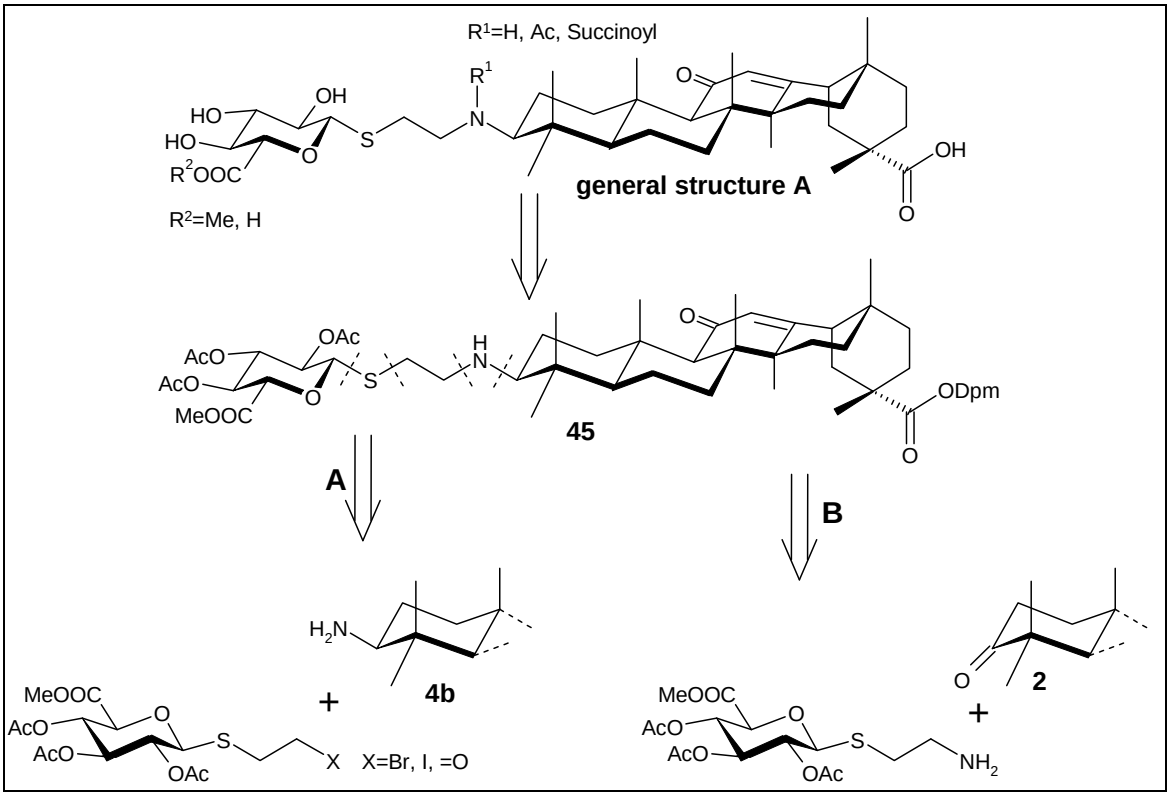
Unfortunately, this series of reaction could not be included within this thesis any more, and the considerations above are a kind of outlook into envisioned improvements towards the synthesis of hydroxamic acid derivatives carrying alkoxyamino-substitution at the 3-position of the GA scaffold.

2.6 Preparation of neoglycosides

In the chapters above all effort was focused at derivatives of the aglycon glycyrrhetinic acid. Nevertheless, a second part of the work within this thesis was dealing with derivatives containing a sugar moiety, thus rather mimicking glycyrrhizin (GN) than glycyrrhetinic acid (GA) as lead structure.

2.6.1 Preparation of 3-aminoethylthioglucuronide neoglycosides of GA

Within the survey covering neoglycosides of glycyrrhetinic acid a whole compound family of general structure A (Scheme 44) was prepared. In compounds with general structure A the glucuronic acid is in a very simplified way mimicking the second sugar moiety of the parent glycyrrhizin structure, whereas the amino group provides the attachment site of a second carboxylic group as a surrogate of the first sugar moiety



Scheme 44: Retrosynthetic analysis for the preparation of key aminoethylthioglucuronide [45]

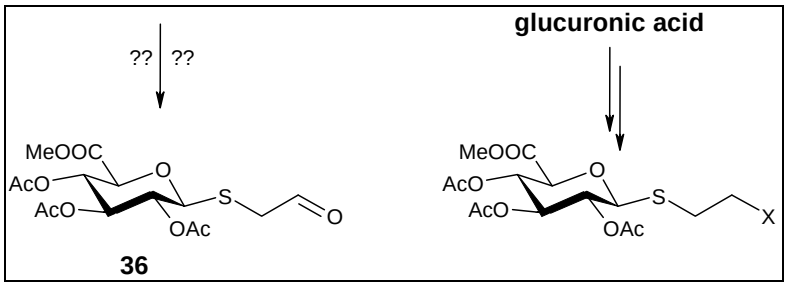
. In order to be able to decorate the amino moiety without any chemoselectivity issues arising from free hydroxyl or carboxyl groups in the molecule, it was decided to prepare completely protected intermediate **45** as branching point for further decoration of the amino-moiety. Considering the retrosynthesis, the formation of the thioglycosidic bond in presence of an

amino-moiety was expected to be a difficult task to achieve. Therefore, only two different strategies were evaluated towards intermediate **45**. Path **A** would use 3β-amino-GA **4b** which is alkylated with a 2-haloethylthioglucuronide or reductively alkylated with the corresponding aldehyde (derived from an allyl thioglycoside). Path **B** would involve reductive amination of 3-ketoglycyrrhetinc acid derivative with an aminoethylthioglucuronide.

The decision towards path A was based on the following conciderations: First, the already successfully implemented preparation of diastereomerically pure 3-amino-GA, diphenylmethyl ester **4b** and second, the relative rareness of reported aminoethylthioglycopyranosides. Furthermore, the use of an aminoethylthioglucuronide as intermediate would probably abolish the use of standard acetyl protection and maybe also the methyl ester of the glucuronic acid.

2.6.1.1 Preparation of 2-haloethylthioglucuronides

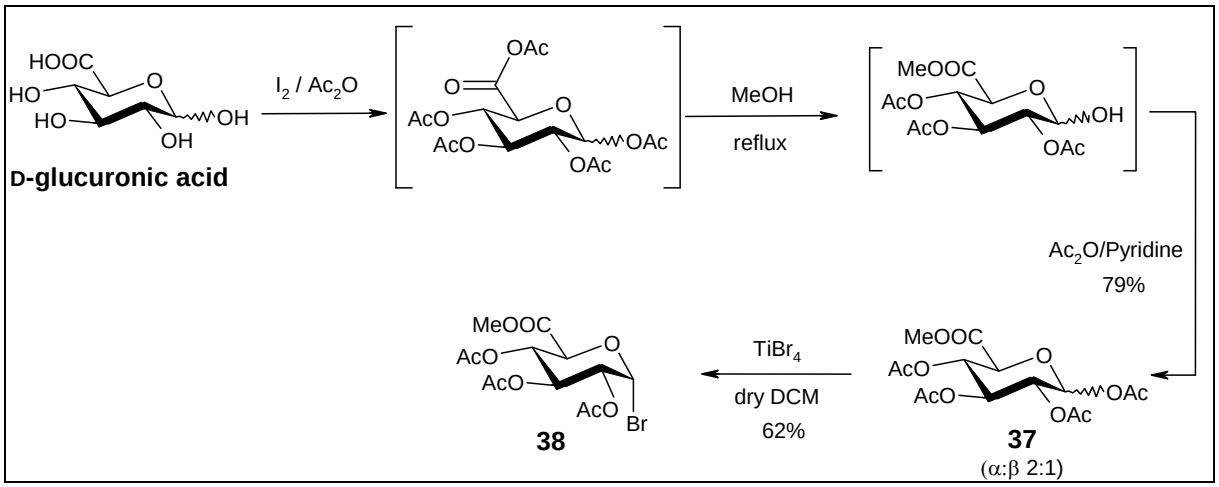
Due to the fact that neither ozonolysis of an allyl-thioglycopyranoside nor any other preparation procedure for a formylmethylthioglycopyranoside such as **36** was reported in the literature, classical Hoffmann alkylation was clearly favored over an approach based on reductive amination. Therefore 2-haloethylthioglucuronides (like in Scheme 45) were the logic precursors to be prepared.



Scheme 45: Evaluated thio-glycoside-precursors to be attached to 3-amino-GA

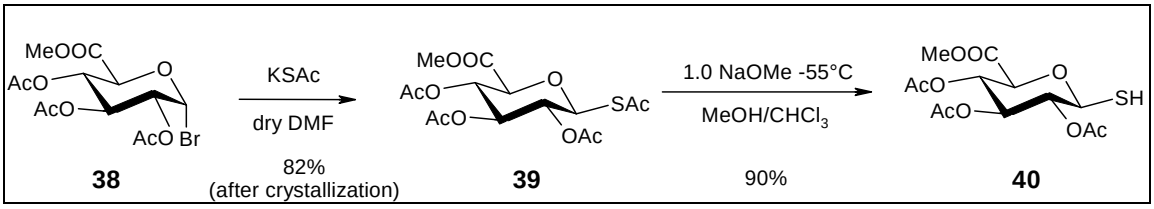
Although related 2-haloethylthioglycosides are known in the literature[93] (they are used as selective glycosyl donors), these kind of compounds were unprecedented in the glucuronic acid family. Starting from D-glucuronic acid, methyl 1,2,3,4-tetra-*O*-acetyl-D-glucuronate **37** (as anomeric mixture) was prepared in an elegant three step-one-pot synthesis according to the literature[94] *via* an intermediate mixed anhydride species (Scheme 46).

Peracetate **37** was transformed to the corresponding α-glycosyl bromide **38** with $TiBr_4$ in DCM. Interestingly, under the mild conditions with $TiBr_4$ only the β-anomer **37b** is reacted at a reasonable reaction rate, while the anomeric α-acetate **37a** is converted by far slower and is not completely consumed even when treated with a high excess of $TiBr_4$ and at elevated temperatures for several days.



Scheme 46: Preparation of glycosyl bromide [38] as starting material[94]

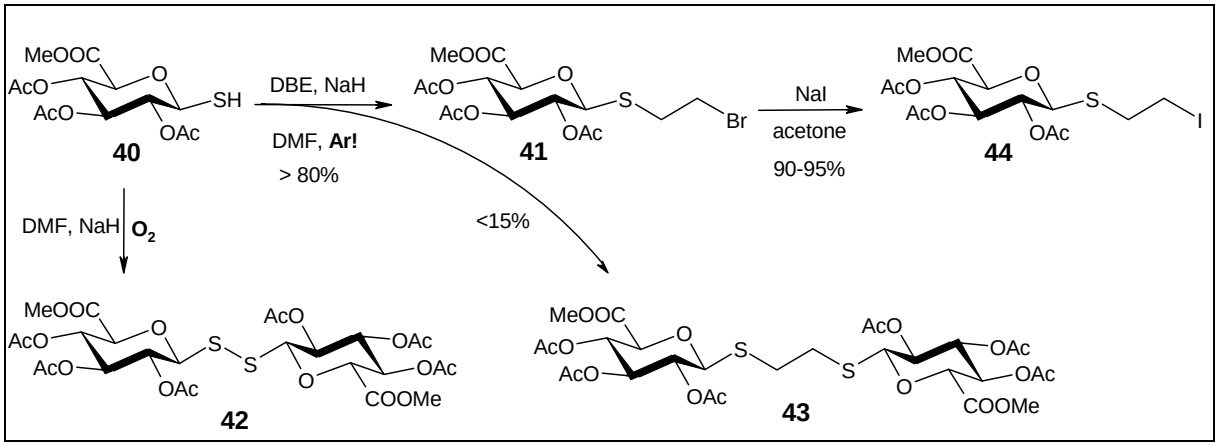
The course of the reaction was monitored by ^{1}H-NMR, based on integration of the well resolved signals of the anomeric protons of the anomeric acetates **37a/37b** and the glycosyl bromide **38**. However, **38** can be easily separated from remaining **37a** by chromatographic separation. Glycosyl bromide **38** was then first transformed to the corresponding anomeric thioacetate **39**[95,96] with KSAc in DMF in excellent yield and scalable manner (only crystallization is needed for purification) and subsequent selective deacetylation at the anomeric position with 1.0 equiv. of NaOMe at low temperature gave compound **40** in excellent yield (Scheme 47).



Scheme 47: Improved synthesis of methyl 2,3,4-tri-*O*-acetyl-1-thio-glucopyranosyl uronate [40]

The used method for the selective cleavage of the anomeric thioacetate was superior in yield and reagent handling to the published methods with NaSMe[95] or Et_2NH[96]. It is important to exclude oxygen in the anomeric de-S-acetylation step to avoid formation of the corresponding

disulfide **42** which is also formed in the subsequent alkylation steps if oxygen is not properly excluded. Thio-glucose **40** is not exceptionally stable and should be stored at -25°C and best only be prepared prior to use. Alkylation with dibromoethane (DBE) in dry DMF with NaH gave methyl 2,3,4-tri-*O*-acetyl-2-bromoethyl-1-thio-glucuronate **41** in good to excellent yields. By exclusion of air the formation of initially observed disulfide **42** was avoided. Nevertheless, bis-alkylated compound **43** was observed in all experiments in up to 15% yield as easily separable more polar byproduct (Scheme 48). The structure of compound **43** was confirmed by NMR (only one signal for the ethylene-bridge indicating symmetry in the molecule) and combustion analysis.

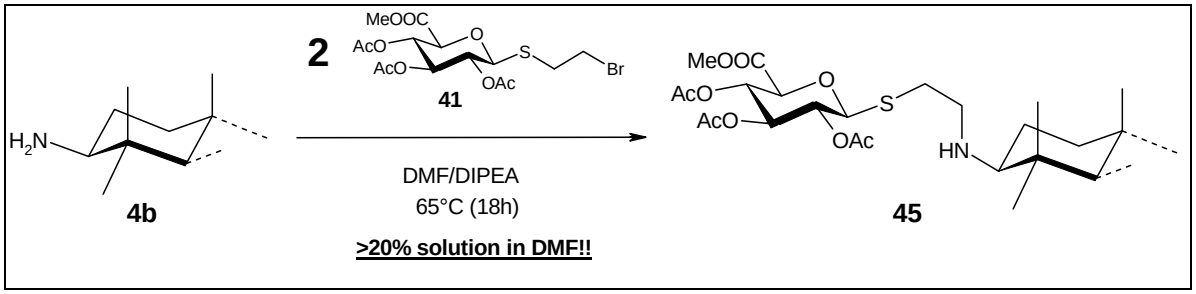


Scheme 48: Preparation of haloethylthioglucuronides [41] and [44]

Disulfide **42** was never obtained in pure form but only contaminated with compound **43**. The reason was that **42** and **43** have a very similar R_f-value in the used eluents and **43** is always formed; under argon atmosphere but also in presence of air, the conditions favoring the formation of **42**. However, bromide **41** was converted to the corresponding 2-iodoethylthio-glucuronate **44**, a stronger alkylating reagent, needed in the planned alkylation of 3β-amine **4b**.

2.6.1.2 Preparation of key-intermediate [45]

With the haloethylthioglucuronides in hands the alkylation reaction towards **45** was optimized. During the optimization process it was found out that in order to obtain a complete conversion high concentrations of reagents in the reaction mixture were crucial (Scheme 49).



Scheme 49: Alkylation of amine [4b] with bromide [41] giving key intermediate [45]

A series of parallel reactions of amine **4b** with the initially used bromoethylthioglucuronide **41** in 5%, 10% and 20% solutions at 65°C in DMF was closely monitored by HPLC and made evident the necessity of keeping high concentration of 20% or higher.

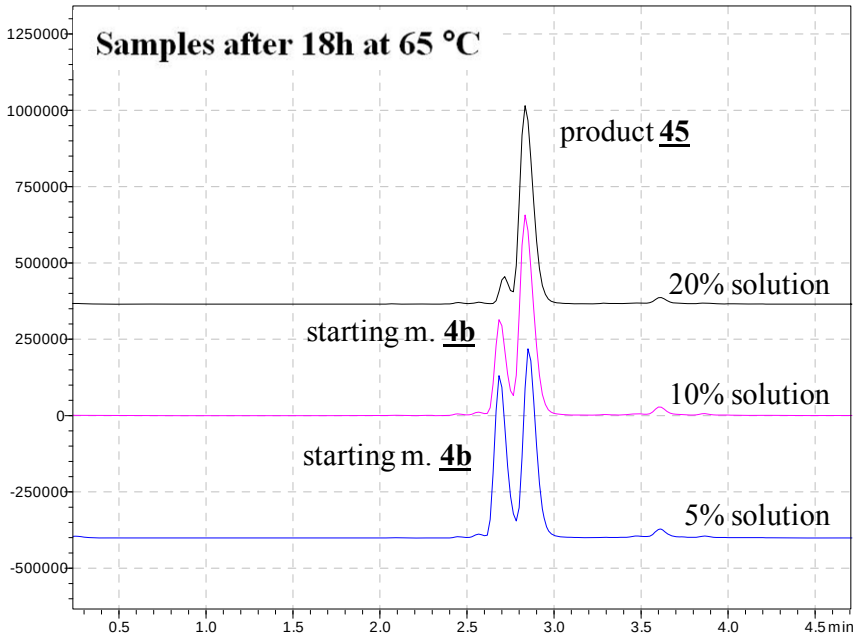
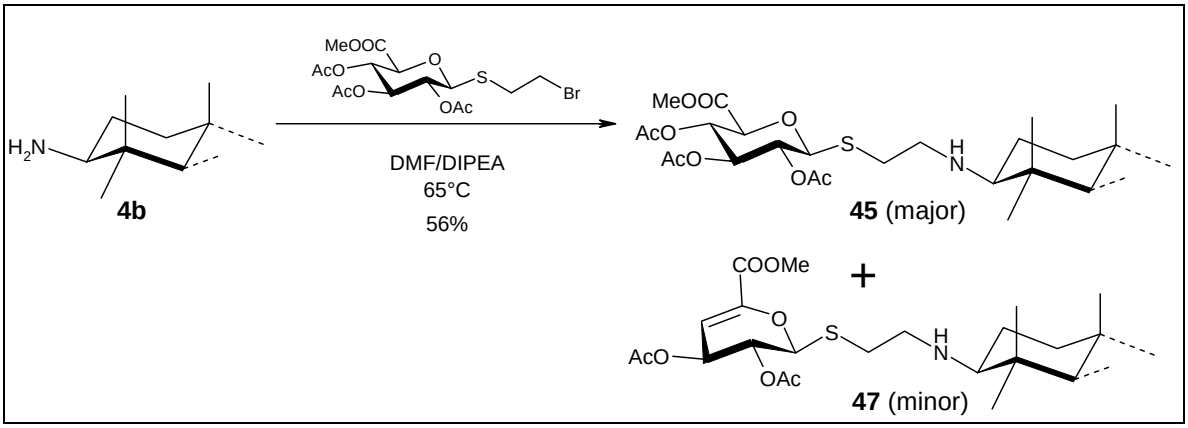


Figure 9: Comparison of reaction mixtures with different concentrations after 18h

More diluted reaction mixtures need to be treated at elevated temperatures for longer periods of time to reach reasonable conversion (Figure 9), favoring the formation of byproducts and thus complicating purification of intermediate **45**. Interestingly, the corresponding bis-alkylated tertiary amine **46** was never observed under any of the reaction conditions, even when an excess of alkylating agent was applied.

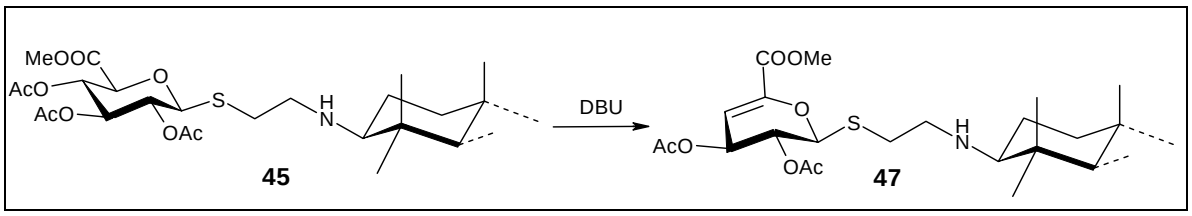
2.6.1.3 Occurrence and identification and of side-product [47]

It is noteworthy that for the alkylation of **4b** with bromoethylthioglucuronide **41** with DIPEA as base elevated temperatures (~65°C) were necessary. Although good conversion was observed with high concentration of reagents in the reaction mixture, the elevated temperature resulted in the formation of a initially not identified side-product **47**, which was very hard to separate by chromatography, leading to low isolated yields of **45** (Scheme 50).



Scheme 50: Formation of hexenuronic acid byproduct [47] at elevated temperature

However, side-product **47** was only obtained in small amounts in the mixed fractions with target compound as major component (Figure 10, upper NMR), thus contaminating a lot of valuable intermediate **45**. Because of prominent signals in the ^{1}H-NMR (olefinic region) a hexenuronic acid species was considered as possible chemical identity.



Scheme 51: Support of the suggested structure of byproduct [47] by intentional synthesis out of [45]

To prove or at least support the suggested structure of side-product **47**, isolated intermediate **45** containing some side product **47** was treated with DBU analogous to a literature protocol developed for the formation of hexenuronic acid species by elimination of AcOH from 4-*O*-acetyl glucuronic acid derivatives[97]. The identical compound was formed according to TLC and was isolated. Comparison of the ^{1}H-NMR showed consistent spectral data with byproduct **47**, confirming the suggested structure (Figure 10). However, to prevent the formation of **47** the alkylating agent **41** was replaced by the more reactive iodide **44** so that the reaction temperature could be lowered from 65°C to 45°C.

Under these conditions the formation of side-product **47** could be avoided and high isolated yields (80%) of key-intermediate **45** were realized.

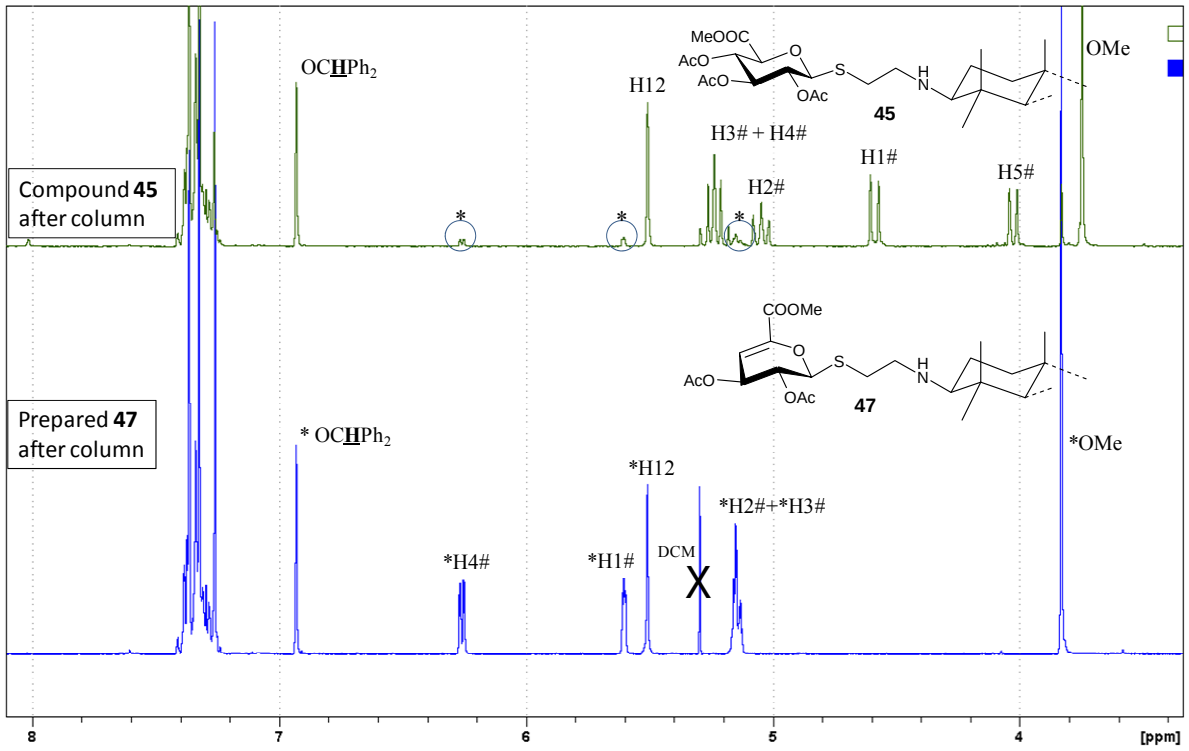
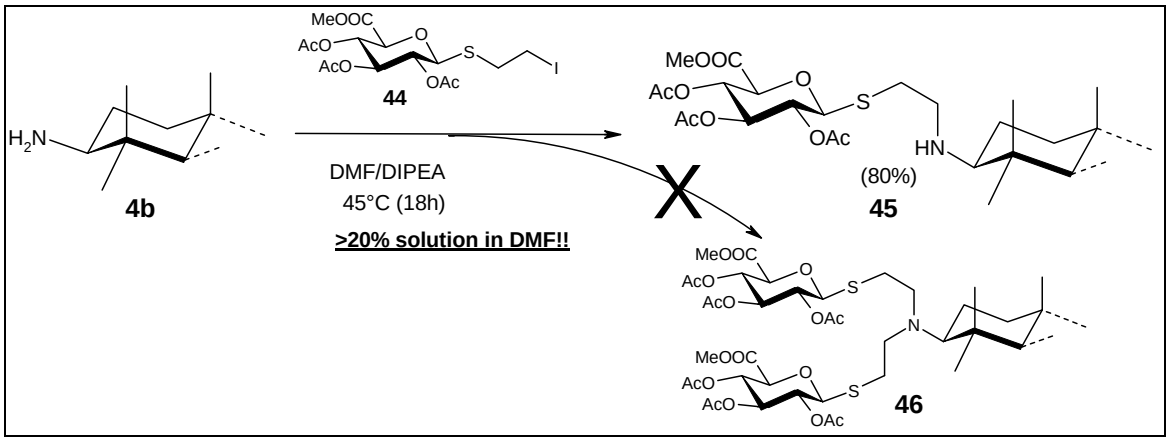


Figure 10: Comparision of the prepared compound [47] and the impurity of intermediate [45] in ^{1}H-NMR

A twofold excess of alkyl iodide **44** turned out to be ideal to reach almost complete conversion with minimal side reactions (Scheme 52).

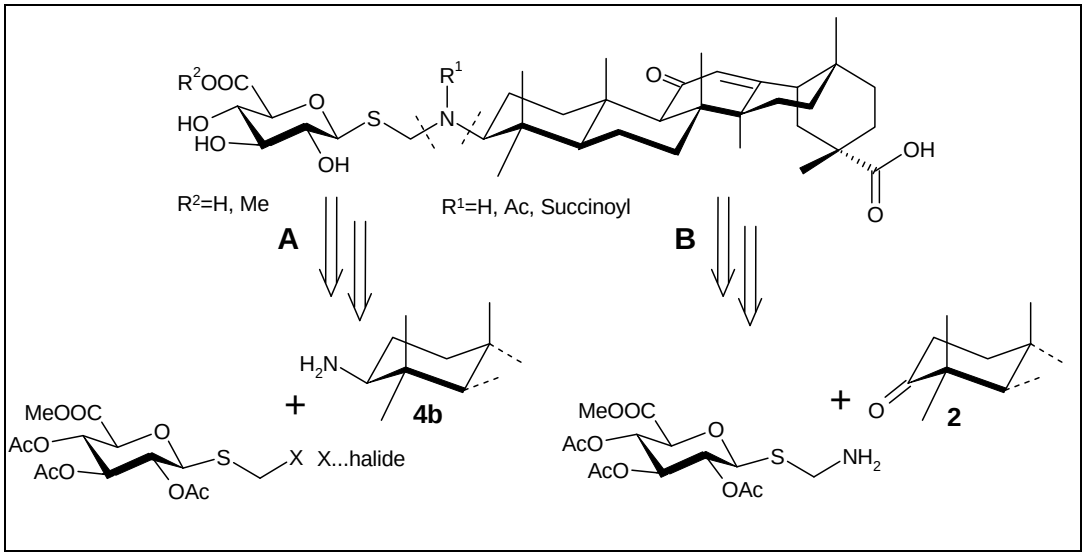


Scheme 52: Alkylation of 3-amino-GA derivative [4b] with iodoethylthioglucuronides [44]

However, even with the stronger alkylating agent **44** no tertiary amine **46** was ever detected. With key-intermediate **45** in hands decoration of the amino-moiety was attempted (proceed to Chapter 2.6.3). Prior to this, the results of the unsuccessful approach towards the analogous series of C1-spacered compounds are briefly summarized on the following pages.

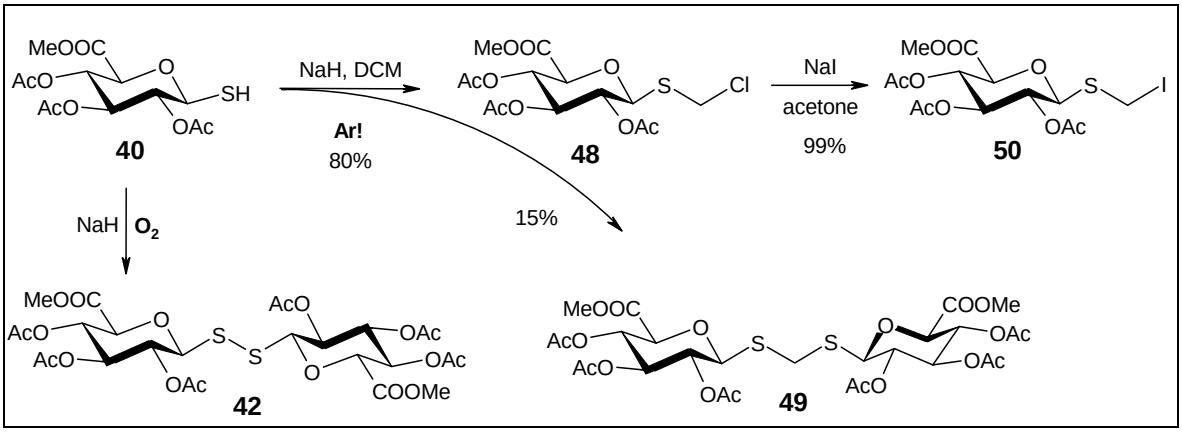
2.6.2 Attempted preparation of C1-spacered neoglycosides

Prompted by the successful preparation of key-intermediate **45** and an interesting literature precedence for a stable aminomethylthiofucoside[98] the possibility to synthesize the analogous C1 series was briefly investigated (Scheme 53).



Scheme 53: Retrosynthetic analysis for the preparation of aminomethylthioglucuronide

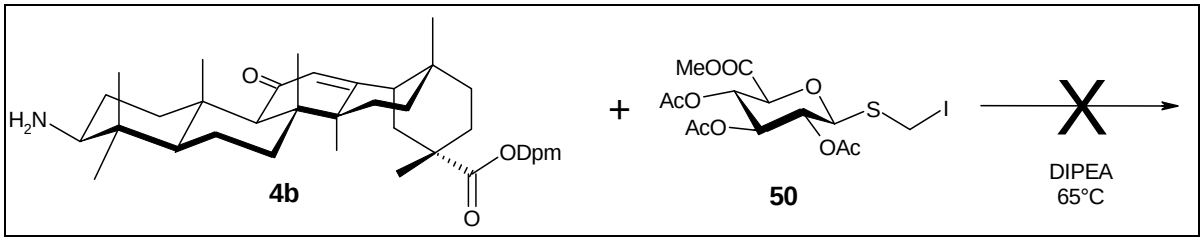
For the preparation of the necessary sugar precursors, thiol **40** was alkylated with DCM to give chloromethylthioglucuronide **48** (similar conversions have been reported with DBU[99,100]).



Scheme 54: Preparation of halomethylthioglucuronides [48] and [50]

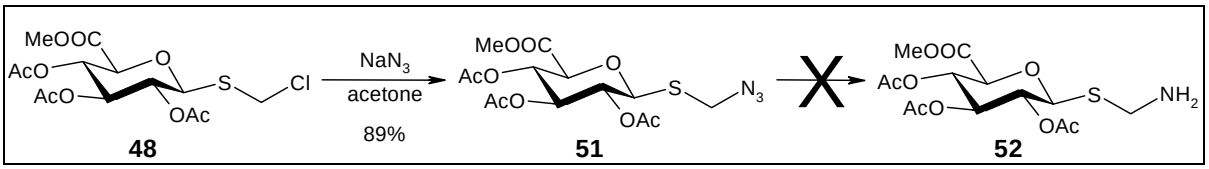
In analogy to the synthesis of bromoethylthioglucuronide **41** exclusion of air was important to avoid disulfide formation and the analogous bis-alkylated product **49** was the only side-product detectable under argon atmosphere. Finkelstein reaction to the corresponding iodo species **50** was established in excellent yield (Scheme 54).

In contrast to the successful alkylation of **4b** with haloethylglucuronide **41** and **44** the analogous reaction within the C1 series remained unsuccessful. As indicated in Scheme 55 the analogous alkylation reaction could not be established; under comparable and even harsher conditions. Methylene-bridged compound **49** was the only isolated and identified compound. Its formation supports the relative instability of the chloromethylthioglycoside **48**, which must have been hydrolyzed under the reaction conditions, a behavior which was not observed at all in the haloethyl-analogues **41** and **44**.



Scheme 55: Attempted alkylation towards aminomethylthioglucuronide compounds

Upon failure in this initially favoured alkylation approach **A**, a short investigation towards alternative route **B** (reductive amination) was also undertaken. Azidomethylglucuronide **51** was prepared in excellent yield from **48** with NaN_3 and was tried to be reduced to the amine **52**. The reduction of the azide to the corresponding amino-moiety was attempted under different conditions (Pd/C-H_2, Ph_3P and propanedithiol) but the target compound could not be isolated from or identified in complex crude mixtures (Scheme 56).

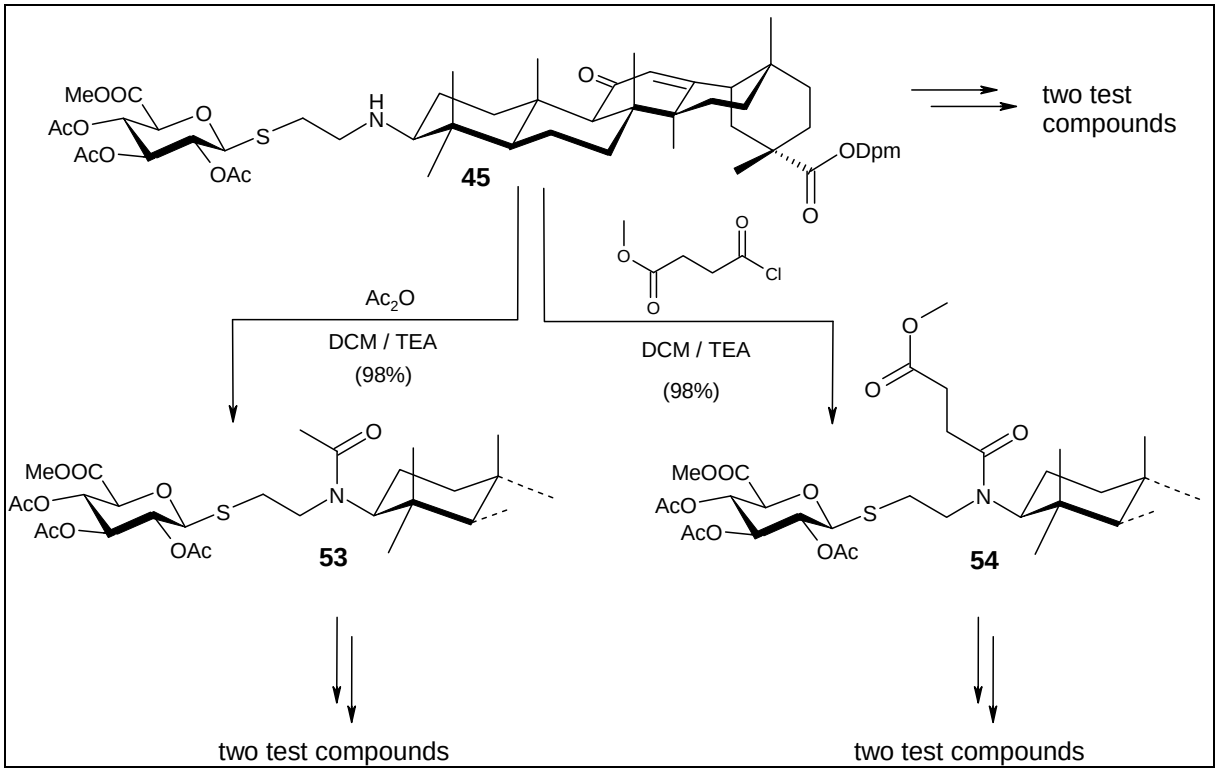


Scheme 56: Preparation and attempted reduction of azidomethylthioglucuronide [51] to corresponding amine [52]

In regard to the application of the prepared compounds in biological assays this route was no longer supported by the project and was not further followed within the thesis.

2.6.3 Preparation of aminoethylthioglucuronide-based test compounds

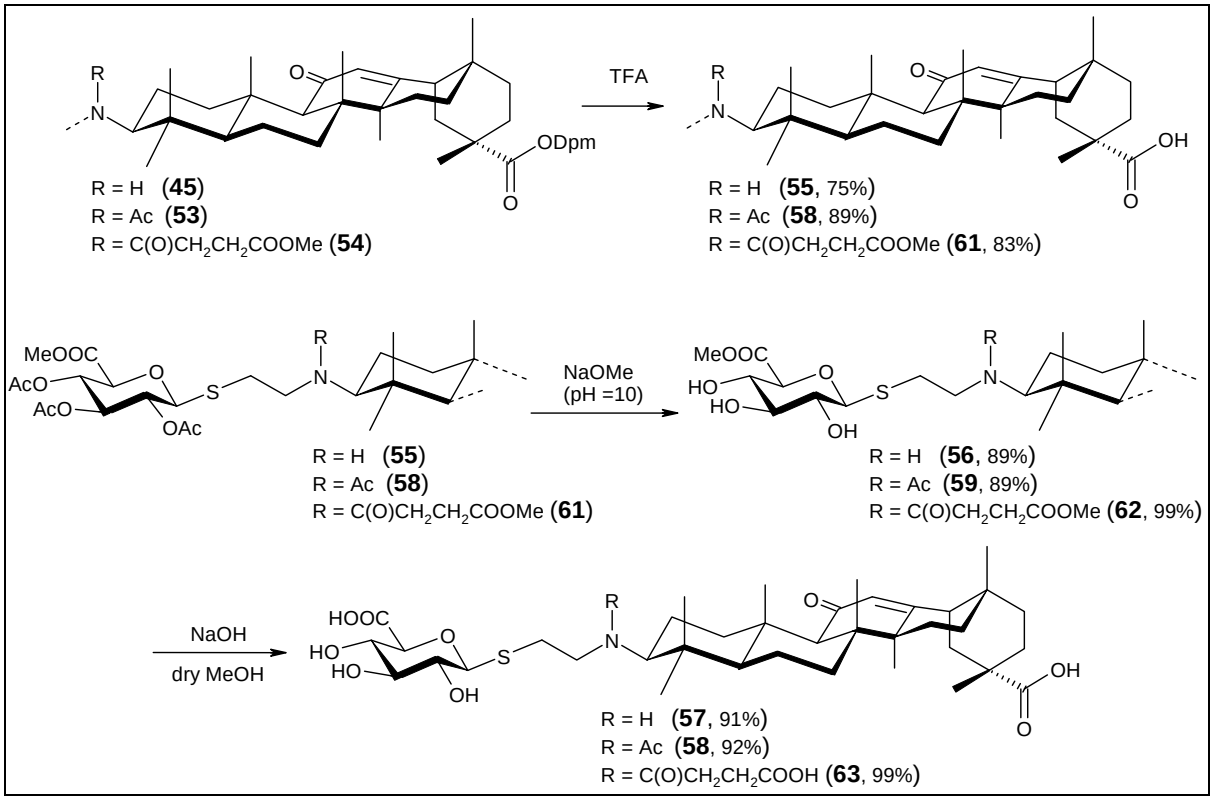
With intermediate **45** in hands straightforward and standard chemistry was applied to prepare a small family of compounds with general structure A. *N*-Acetylation and *N*-succinylation, respectively, led to compounds **53** and **54**, which were subsequently deprotected by the same series of reaction steps like the parent structure **45** yielding two valuable test compounds each (Scheme 57), the free glucuronic acid derivatives and the corresponding methyl esters.



Scheme 57: Decoration of intermediate [45] as *N*-acetamide [53] and *N*-succinylamide [54]

The appearance of a thioglycosidic bond had two consequences for the deprotection steps. On the one hand it hinders the use of catalytic hydrogenolysis conditions (poisoning of the catalyst by sulfur) for the ester cleavage but allows on the other hand the alternative deprotection under acidic conditions with TFA/anisole, which would not be recommended for corresponding *O*-glycosides. For the whole compound family the acidic deesterification was uneventful and high yielding giving GA derivatives **55** (NH), **58** (NAc) and **61** ($NCO(CH_2)_2COOMe$). These peracetates were submitted to Zemplén deacetylation conditions to give the corresponding glucuronic acid methyl esters **56**, **59** and **62**, which were subsequently hydrolyzed with NaOH in dry MeOH to give the free glucuronic acid derivatives **57**, **60** and **63**. Applying this two step ester-deprotection for each core-structure two test compounds were generated, which thus allowed the evaluation of structure activity

relationship concerning the effect of the free acid in the glucuronic acid moiety compared to an ester group in this position. The deprotection steps were in general high yielding. Due to the relatively high number of polar groups (and their changes during the single reaction steps) the final deprotection steps were monitored by HPLC additional to TLC in order to reach complete conversion (Scheme 58).

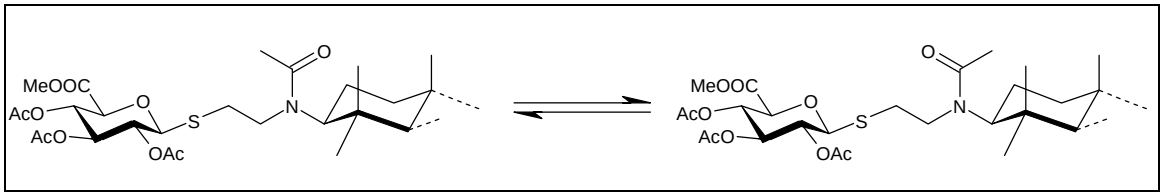


Scheme 58: Stepwise deprotection of the three aminoethylthioglucuronides of GA

A chromatographic purification of completely deprotected materials was in general not necessary if properly purified materials were subjected to the Zemplén deacetylation step. It is noteworthy that while for handling of the compounds (purification, solubility), the acylation of the secondary amine moiety was beneficial; however, NMR-analysis was complicated as will be explained on the following pages.

2.6.3.1 NMR analysis of the *N*-acylated compounds

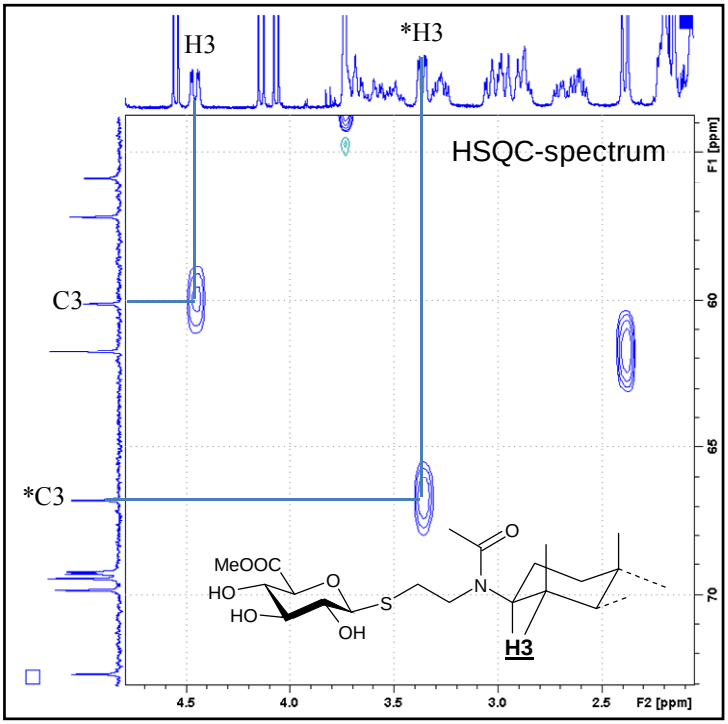
NMR spectra of the whole class of glycyrrhetinic acid derivatives are not easily completely and reliably assigned but the order of complexity was raised tremendously by the introduction of the acetyl or succinyl group at the secondary 3-amino-group (Scheme 59).



Scheme 59: Acylated compounds like [58] are observed as two rotamers in ^{1}H- and ^{13}C-NMR

The reason for the increased complexity is the observation of two rotamers (~4:6 ratio) for each compound (obviously due to the hindered rotation of the amide bond), resulting in two different sets of signals (^{1}H-NMR and ^{13}C-NMR) thus making a complete assignment troublesome. Interestingly, this phenomenon was not observed in case of acetylated primary amines **10a** and **10b**. The effort towards a complete assignment for the crowded aliphatic region of these compounds was considered unnecessary and only all signals above 45ppm (in ^{13}C-NMR) – including the high-field quaternary and the CH carbons of the triterpene and the complete sugar moiety - were assigned together with their corresponding ^{1}H-NMR signals, respectively. For one exemplary compound **58** a complete assignment was undertaken in order to make sure, that the observed spectra can be correlated with the expected structures, which was the case. Among all the protons and carbons which were observed as a set of two signals the most impressive difference was obtained at the H3/C3 position of the triterpene, carrying the amide moiety.

The two signals for H3 are separated by more than 1.0 ppm, the minor signal (0.4H) at 4.46 and major signal at 3.37ppm in $CDCl_3$), the corresponding ^{13}C-signals have a difference of around 7ppm in their chemical shift (Scheme 60).



Scheme 60: Significant difference in the chemicals shifts of H3/C3 of the two rotamers of [58]

Furthermore, for compound **58** ^{1}H-NMR spectra and COSY spectra have been recorded at different temperatures (27°C, 50°C, 75°C and 100°C in DMSO-d_6) in order to study the changes of the ^{1}H-NMR signals. In Figure 11 the ^{1}H-NMR spectra for temperatures between 27 and 100°C are aligned and show the typical coalescence phenomenon of a molecule with a bond with restricted rotation. The sugar region was chosen because it is comparably easy to interpret compared to the crowded aliphatic region and the simplifying effect of raising the temperature up to 100°C is impressive. At rt two rotamers are observed in a ratio of approximately 4:6, related to two signals for almost each proton- and carbon-type. The shape and the shifts change with the elevated temperatures to give only one set of sharp signals for most of the signals.

It is noteworthy that not only the signals related to the sugar moiety but also those of the triterpene scaffold (only 7 methyl-groups signals instead of 12 at rt) are simplified at 100°C.

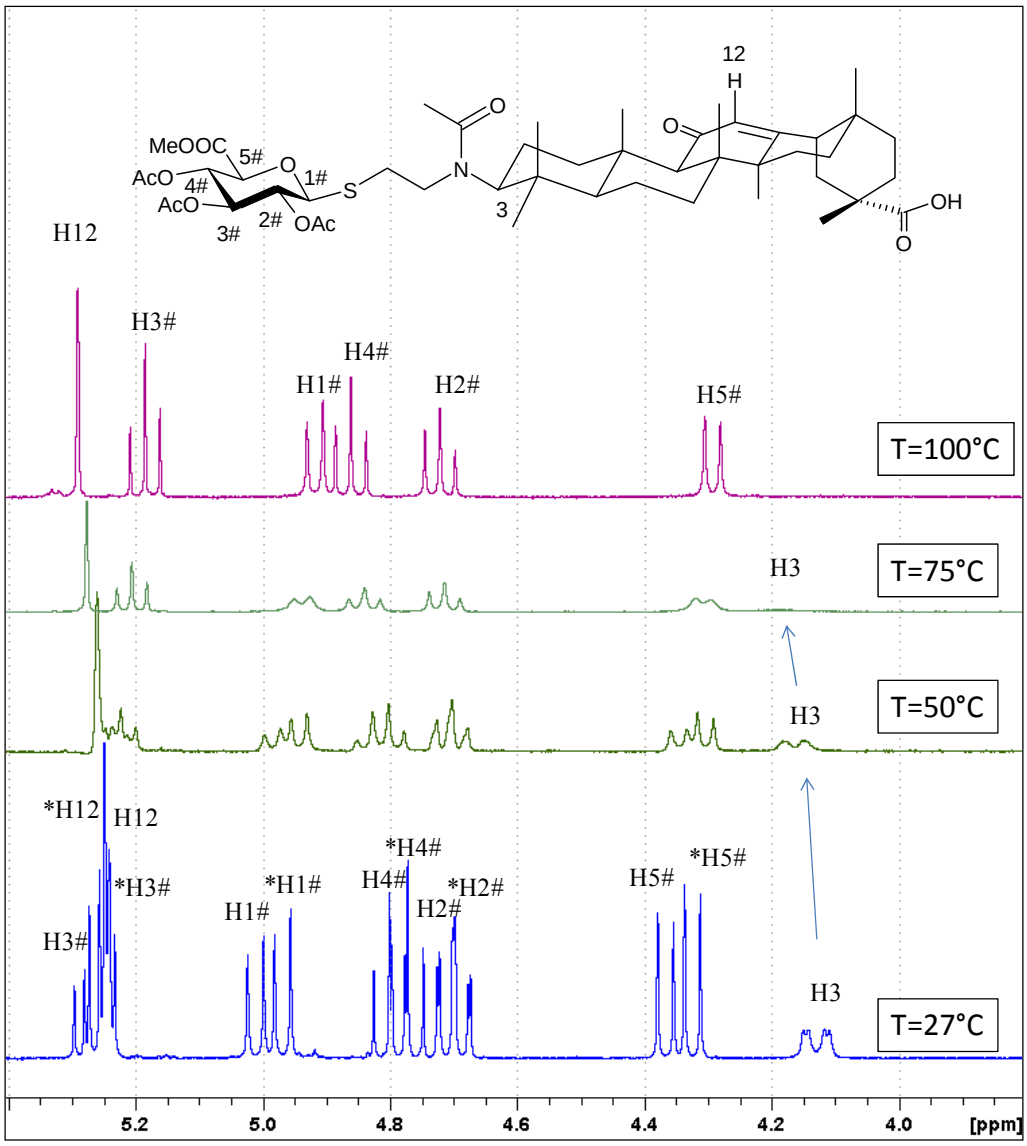


Figure 11: Temperature dependent ^{1}H-NMR of the sugar region of secondary acetamide [58]

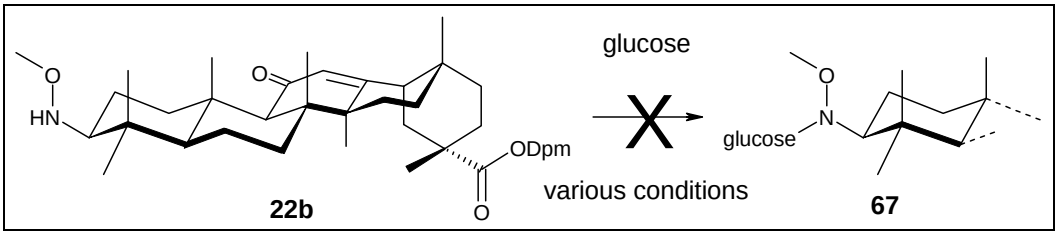
On the other hand the signals related to the ethylene-spacer as well as the H3 position, get broader with rising temperature and cannot be observed at 100°C any more. Upon cooling of the sample another ^{1}H-NMR was recorded at rt in order to prove that reversible processes have been the cause for the changes in the NMR-spectra and not irreversible conversion to some new species. This new rt spectrum and the very first match completely in shifts and relative integrals between the two subspectra, thus further supporting a reversible NMR-phenomenon *versus* the formation of stable atropisomers during the course of the acetylation of **45**.

2.7 Approaches towards methoxyaminoglycosides

The initial plan was to apply the modern concept of direct glycosylation of a secondary alkoxyamines with reducing sugars (see Chapter 1.5.2 in the introduction) to the glycyrrhetinic acid structure. This synthetic approach had already been successfully applied to the preparation of a library of neoglycosides of digitoxin[72], a cardiac glycoside and had not yet been validated for the sterically demanding 3-position of a triterpene structure (reported in early 2009 by Goff *et al.*[73]), when the following experiments were carried out.

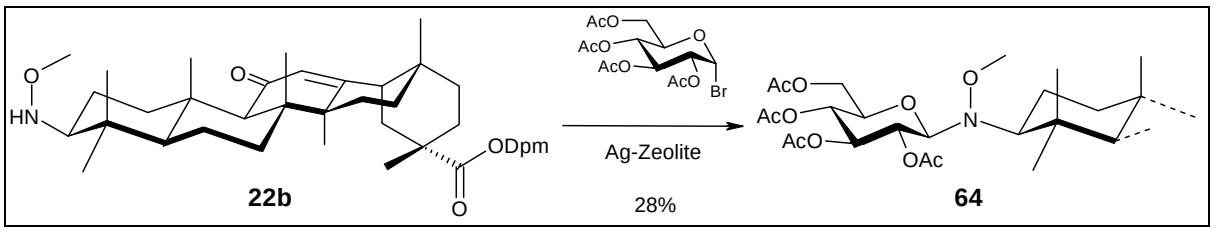
2.7.1 Towards 3β-methoxyaminoglycosides of glycyrrhetinic acid

With 3-methoxyamino-GA derivative **22b** in hands it was tried to reproduce various literature conditions both in buffered aqueous (NaOAc/AcOH) conditions as well as in polar organic solvents applying an excess of glucose as model reducing sugar. Glucose was chosen because direct glycosylation with glucose gave good results in all reported investigations (Scheme 61).



Scheme 61: Attempted application of the concept of direct glycosylation on the GA structure

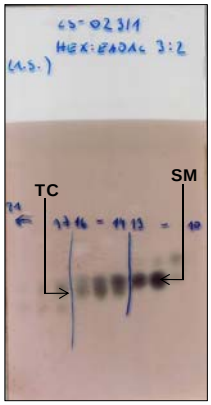
However, with the compound **22b** no conversion at all was observed in different solvents and at elevated temperatures and really extended reaction times (14 days). Upon the first negative results, attempts were undertaken to prepare the target compound **66** *via* a conventional glycosylation approach to check if this yields a stable compound at all (Scheme 62).

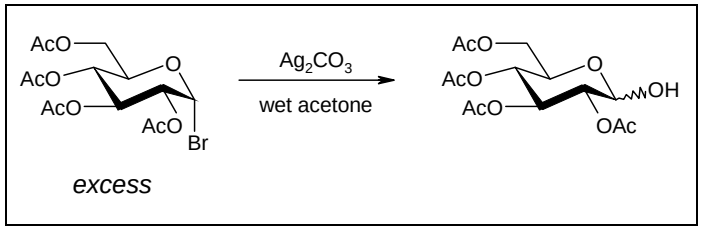


Scheme 62: Conventional glycosylation of 3-methoxyamino-GA [22b]

Methoxyamine **22b** was successfully reacted with an excess of peracetylglucosyl bromide as glycosyl donor under the catalysis of silver zeolite as promoter system, conditions which have been elaborated within the ASPEX-project by Carmen Ruiz-Ruiz for the *O*-glycosylation of GA with various glycosyl bromides[78]. The purification of *N*-glycoside **64**, however, was

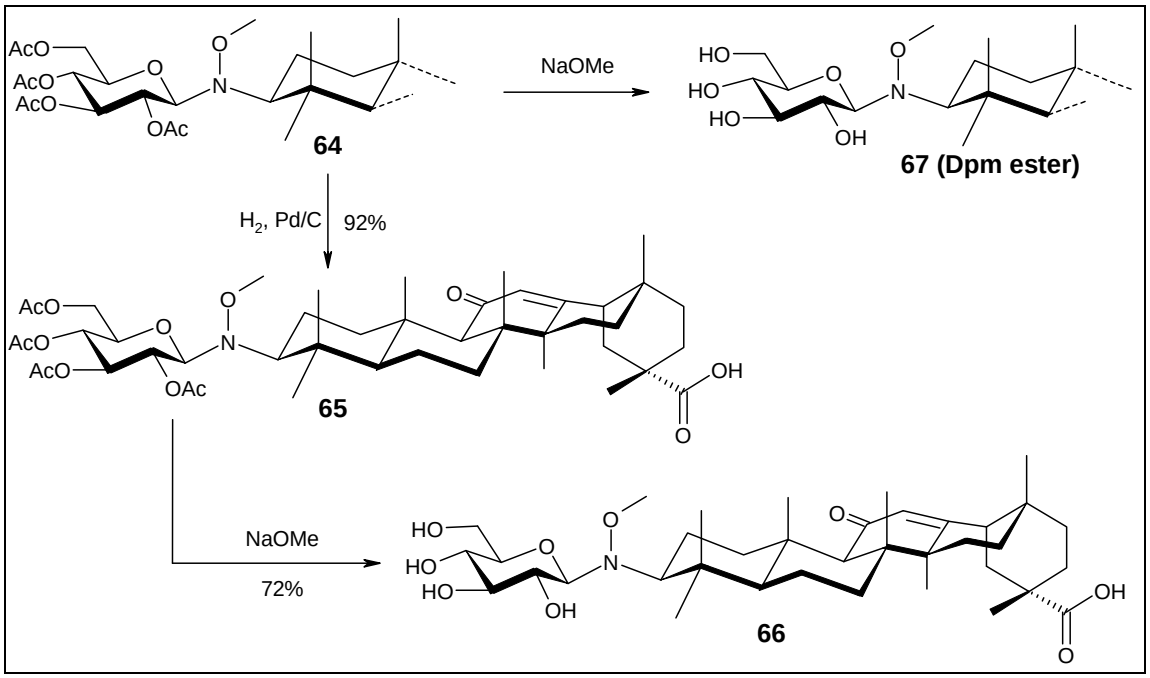
tricky because the glycosyl bromide (excess) could not be separated easily from the target compound due to similar R_f-values (TLC-scan). Therefore the crude material was treated with Ag_2CO_3 in wet acetone[101], thus hydrolyzing the glycosyl bromide to the corresponding more polar reducing sugar, which was easily separated (Scheme 63).





Scheme 63: Hydrolysis of excess glycosyl bromide for facilitating purification

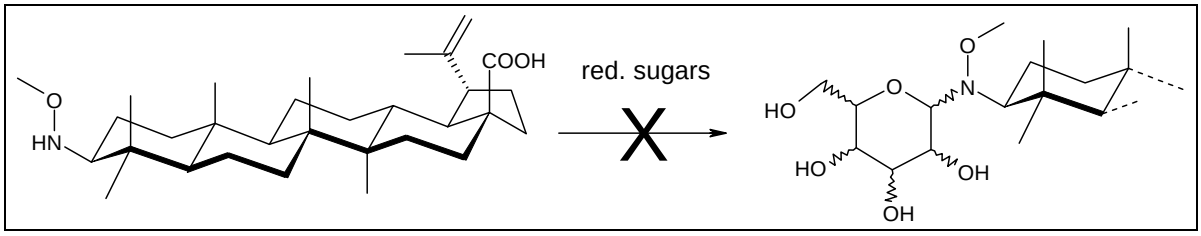
Nevertheless, it is difficult to get compound **64** free from small amount of other byproducts and it is therefore recommended to submit compound **66** after a short column directly to hydrogenolysis and to purify the free acid **65**, which is far easier accomplished. Zemplén deacetylation of **65** gave the initially targeted compound **66**, which was evaluated as antiviral compound (Scheme 64).



Scheme 64: Deprotection towards test compound [66] and TLC standard [67] for the direct glycosylation

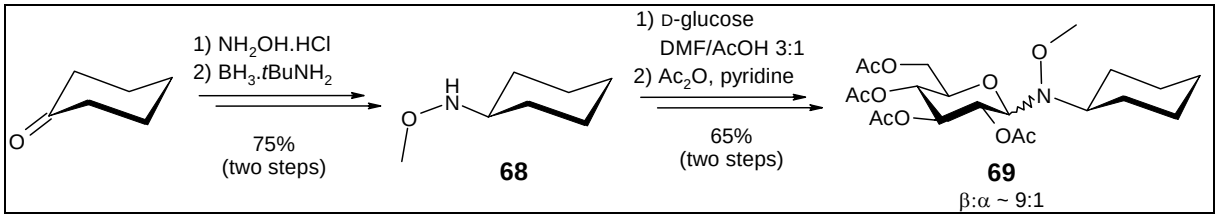
Furthermore, a small amount of diphenylmethyl ester **67** was prepared under Zemplén conditions in order to obtain TLC-reference material for the initial approach of the direct glycosylation reaction.

Nevertheless, also with this material in hands no formation of neoglycoside **67** was observed. These negative results in the direct glycosylation efforts found some literature precedence in early 2009[73]. Goff and Thorson[73] have described unsuccessful attempts towards the direct glycosylation on the very similar betulinic acid system and did as well argue with the steric hindrance of the adjacent methyl groups.



Scheme 65: Unsuccessful attempt to directly glycosylate 3-methoxyamino-betulinic acid[73]

Lacking this literature precedence it was tried to prove, that the unreactivity is caused by the sterical hindrance of the two methyl groups in the adjacent 4-position. Therefore, the *N*-methoxycyclohexylamine **68**[102] was prepared in two steps from cyclohexanone in good yield and was evaluated in the direct glycosylation reaction. In contrast to the results with 3-methoxyamino-GA derivative **22b**, conversion of *N*-methoxycyclohexylamine **68** with glucose under several conditions was observed. Anhydrous conditions in DMF/AcOH mixtures were clearly superior over the aqueous buffered conditions with AcOH/NaOAc buffer and within several days at rt almost complete conversion was obtained (Scheme 66).

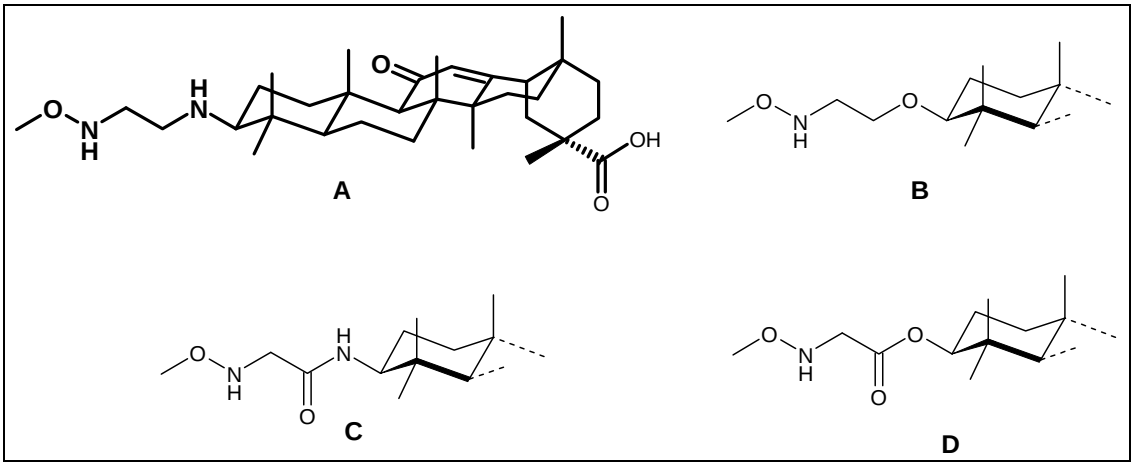


Scheme 66: Synthesis and direct glycosylation of methoxyaminocyclohexane [68]

For the ease of purification and characterization, the crude material was peracetylated under standard conditions and purified as peracetate **69** by column chromatography. The isolated material was obtained as α/β mixture with the β-isomer as major compound, which is absolutely consistent with the literature reports[64,66,103,76,72]. Accepting that unspacered methoxyamino-neoglycosides of GA will not be accessible *via* direct glycosylation of **22b** a short and final investigation whether spacered methoxyamino-neoglycosides are accessible *via* direct glycosylation chemistry was initiated, thus allowing in principle the preparation of compound libraries with reasonable synthetic effort.

2.7.2 Spacered alkoxyaminoglycosides

In principle four different precursor molecules were considered for the direct glycosylation with reducing sugars (Scheme 67). Ester linked intermediate **D** was in principle abolished towards the amide linker **C** for its relative chemical and especially physiological instability.



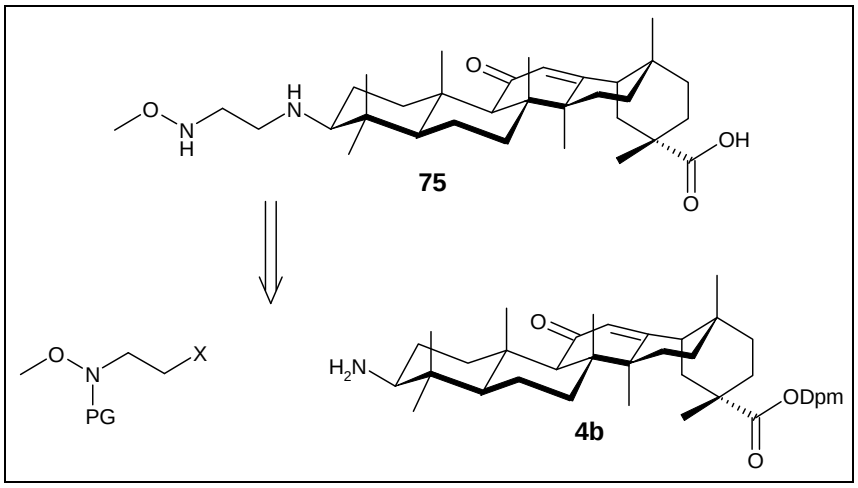
Scheme 67: Considered types of C2-spacered methoxyamino-GA precursors

Amine **A** and ether-linked precursor **B** were also both considered as useful, nevertheless, due to the interesting preliminary results of the amine linked thioglucuronides in the antiviral assay the amine approach was favored over the ether linkage, which would be more convenient regarding physical properties. Nevertheless, the choice of amino-linker in **A** would allow further decoration analogous to the aminoethylthioglucuronide family (**53** to **63**). Intermediate **A** was therefore the first choice and should at least be developed until a first proof-of-concept regarding its potential to develop compound libraries based on direct glycosylation with reducing sugars.

2.7.3 Ethylamino-linked neoglycosides of glycyrrhetinic acid

Based on the positive results of the alkylation approach in the thioglucuronide series, it was decided to choose an alkylation approach for this purpose as well. Anticipating side reactions at the free carboxylic acid moiety (which were later confirmed within the diploma thesis of Alexander Doppelreiter[88]) 3-amino-GA, diphenylmethyl ester **4b** was chosen as starting material (Scheme 68).

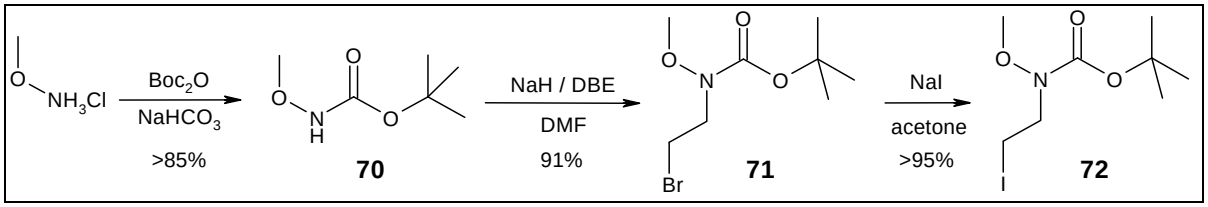
For the necessary 2-(methoxyamino)-ethylhalide, Boc-protection was chosen in order to be able to deprotect under the conditions appropriate for the cleavage of the diphenylmethyl ester, thus allowing complete deprotection in one reaction step.



Scheme 68: Retrosynthetic strategy for methoxyaminoethylamino-GA

2.7.3.1 Synthesis of the *N*-Boc-*N*-haloethylmethoxylamine reagents

For the synthesis of *N*-Boc-*N*-haloethyl-*O*-methylhydroxylamine literature protocols of similar compounds could be successfully adopted.

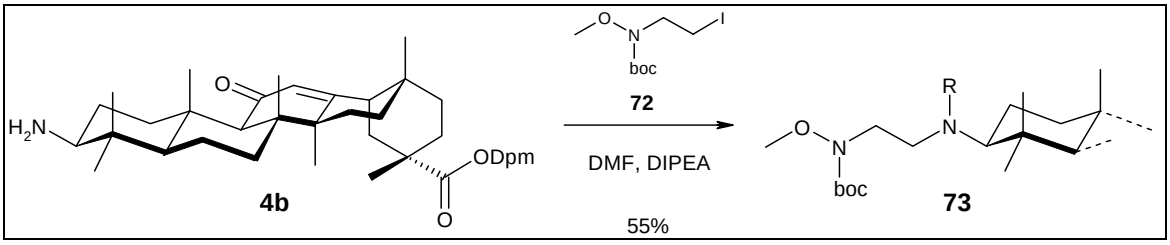


Scheme 69: Preparation of the protected methoxyaminoethyl halides [71] and [72]

By adopting a protocol for the Boc-protection of *O*-benzyl-hydroxylamine[104] the yield for the Boc-protection of *O*-methylhydroxylamine was improved compared to the existing literature protocol[105] and the product was distilled *in vacuo* at large scale. The alkylation of **70** with excess of dibromoethane delivered *N*-Boc-*N*-bromoethylmethoxylamine **71** which was submitted to a Finkelstein reaction (both based on a literature protocol for the analogous reaction of *N*-Alloc-*O*-methyhydroxylamine[106]) to give the iodoethyl compound **72**, which was used for the following alkylation reaction (Scheme 69). While bromide **71** was purified by *Kugelrohr* distillation, thus allowing easy access to larger amounts, attempted distillation of iodoethyl compound **72** led to decomposition.

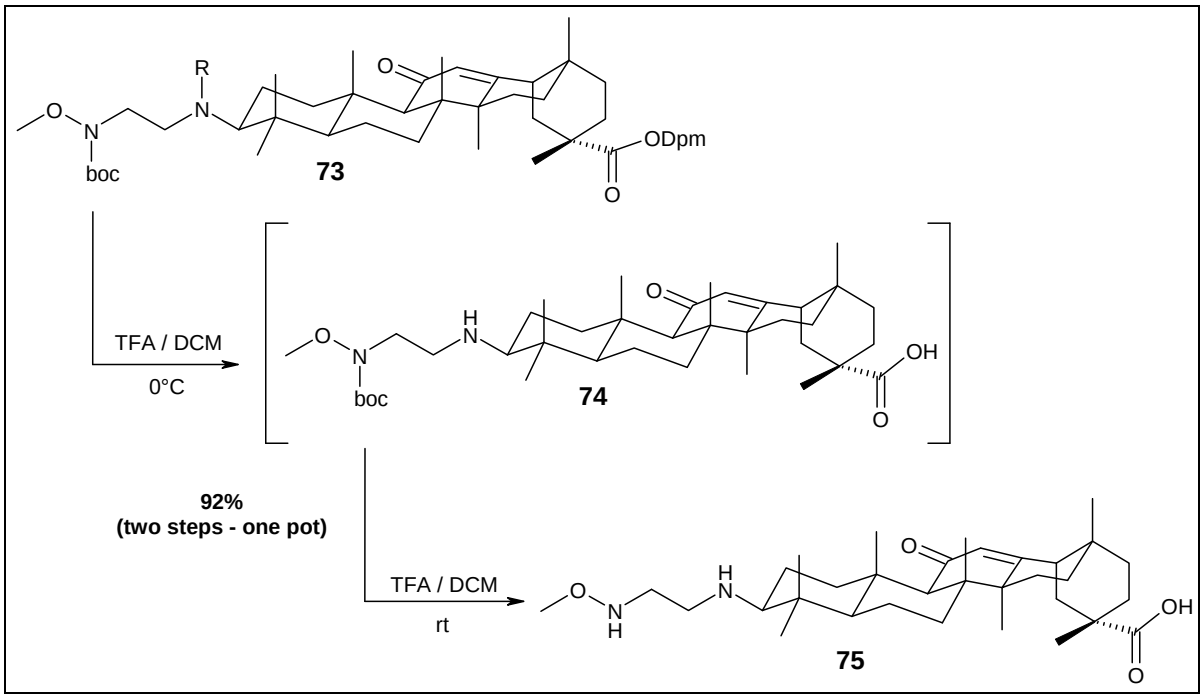
2.7.3.2 Methoxyaminoethylamino-GA and first direct glycosylations

With the iodoethyl linker **72** in hands the alkylation of amine **4b** was performed under conditions developed for the alkylation reactions in the aminoethylthioglucuronide series with similar course of the reaction and moderate yield (Scheme 70).



Scheme 70: Alkylation of amine [4b] with Boc-methoxyaminoethyl iodide [72]

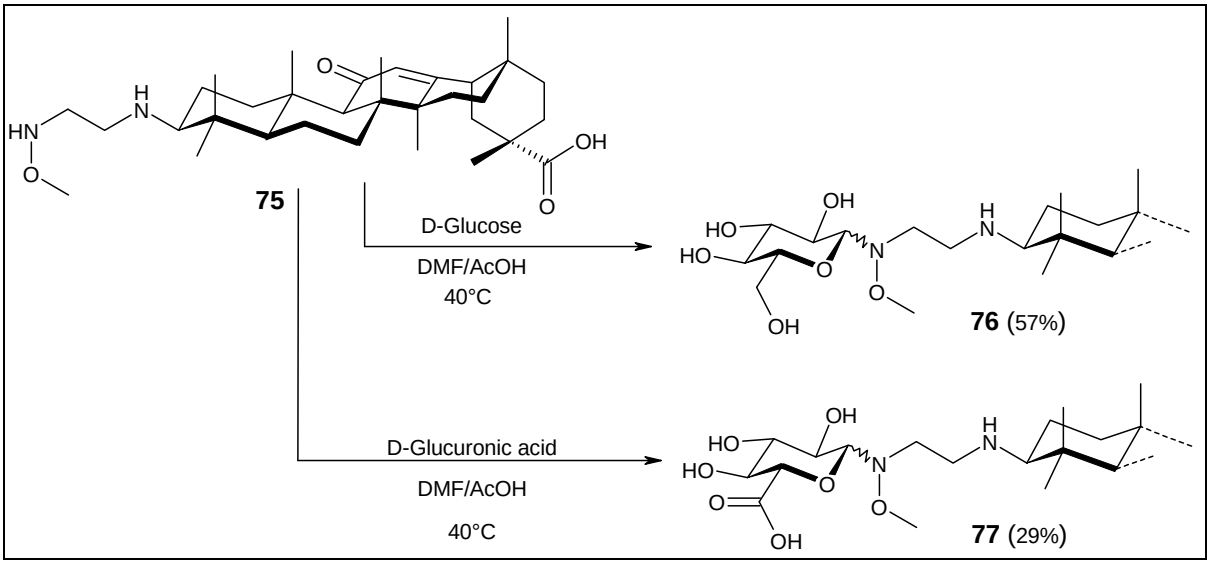
The protected intermediate **73** was fully deprotected under acidic conditions with TFA/anisole to give target compound **75** in excellent yield.



Scheme 71: Difference in the reactivity of the Boc-carbamate and the diphenylmethyl ester under TFA conditions

Interestingly, while at temperatures below 0°C almost exclusively the diphenylmethyl ester was cleaved, leaving the Boc-protection intact (intermediate **74**), by raising the temperature to rt the completely deprotected precursor **75** could be obtained in a one pot procedure and in excellent yield. With precursor **75** in hands, the principal applicability of the direct glycosylation with reducing sugars was evaluated with glucose and glucuronic acid as model compounds (Scheme 71).

For the proof-of-concept study regarding the direct glycosylation of precursor **75** the anhydrous conditions in DMF/AcOH that had proven most successful in the test reactions with the *N*-methoxy-cyclohexylamine, were applied. Similar to the experiments with *N*-methoxy-cyclohexylamine both conversions did reach completion within several days at rt or within 24h at 40°C. The course of these reactions is best monitored by HPLC and TLC in parallel, due to large differences of stainability and UV-activity between starting material, target compounds and byproducts (Scheme 72).



Scheme 72: First successful neoglycosylation reactions with a GA precursor molecule

It is noteworthy that similarly to the oxidations of 3-methoxyamino-GA derivatives **22a/22b** the precursor **75** is oxidized with air in solutions. It is therefore advisable to prevent oxygen in the reaction mixture. Although reaction monitoring indicates complete conversion the compounds are easily lost during workup and purification. The crude materials are best purified by distribution between DCM:*i*PrOH as organic layer and diluted brine to wash out the excess of sugar (TLC monitoring of extraction is recommended). The crude materials are further purified over a short bed of SiO_2 to give the pure target compounds.

With this proof-of-concept in hands it was decided to roll-out this part of the project into a closely supervised diploma thesis[88] to come up with a small library of this type of neoglycosides together with the corresponding amide-linked compounds (Type **C** in Scheme 67), thus allowing structure activity relationships (antiviral activity) concerning the sugar moiety and the type of the linkage.

2.8 NMR analysis of the synthesized compounds

2.8.1 General comments

Since the aliphatic region of glycyrrhetinic acid derivatives is generally very crowded, assignment of NMR signals is not trivial, if attempted properly and in a complete manner, and was a substantial fraction of the effort, that was undertaken for the results in this thesis. This appraisal is supported by the fact that publications exist, which exclusively contain the assignment of the parent structures glycyrrhetinic acid and glycyrrhizin based on 600MHz ^{1}H-spectra and 150MHz ^{13}C-spectra[107]. It is warned to assign the ^{13}C-spectra relying on analogy: Although especially the ^{13}C shifts are quite conserved for many GA derivatives, the single signals are close enough to each other and shift enough to make a proper analysis based on a full set of 1D-and 2D-NMR measurements necessary in order to deliver reliable assignments.

All assignments within this thesis are based on at least COSY, HSQC and HMBC spectra (in the optimum case accompanied by DEPT and TOCSY) additional to the ^{1}H and APT spectra. In the following chapter it is described how a typical set of spectra is handled to come up with a reliable but still time-efficient assignment of all carbon and proton shifts including the conversion of data into the format used for a publication of a thesis or a publication.

Details of assignments do differ from molecule to molecule but the general strategy laid out at the example of the 3-keto-GA-hydroxamic acid **31** as example is generally highly recommended to follow. Compound **31** was chosen because its spectra were among the easiest to interpret (mainly due to the clearly separated signals of the methyl groups in the proton spectrum) thus allowing the reader to follow the typical assignment process stepwise without too many “if that is that, and cannot be this...” considerations. The full set of spectra used in the assignment process is for **31** collected in the Appendix.

In order to handle the assignment of molecules of the complexity and size of glycyrrhetinic acid it is clearly stated that assignment by just drawing and writing on printouts, as it might be appropriate for smaller molecules is extremely unreliable in a complex case like here. All NMR analysis and assignments of GA derivatives were therefore documented on MS-Excel sheets which can finally be automatically translated into lines of texts by the help of small macro routines. The use of automation helps avoiding both unnecessary work as well as mistakes during the transfer. Adopting a general behavior like this has several advantages

towards more usual hand-written assignments on spectra or lists. Using MS-Excel tables (for example) the finished assignments can easily be used for comparisons between sets of signals from similar structures in order to find irregularities in assignment but also to uncover systematic changes in ^{13}C or ^{1}H data correlating with chemical changes.

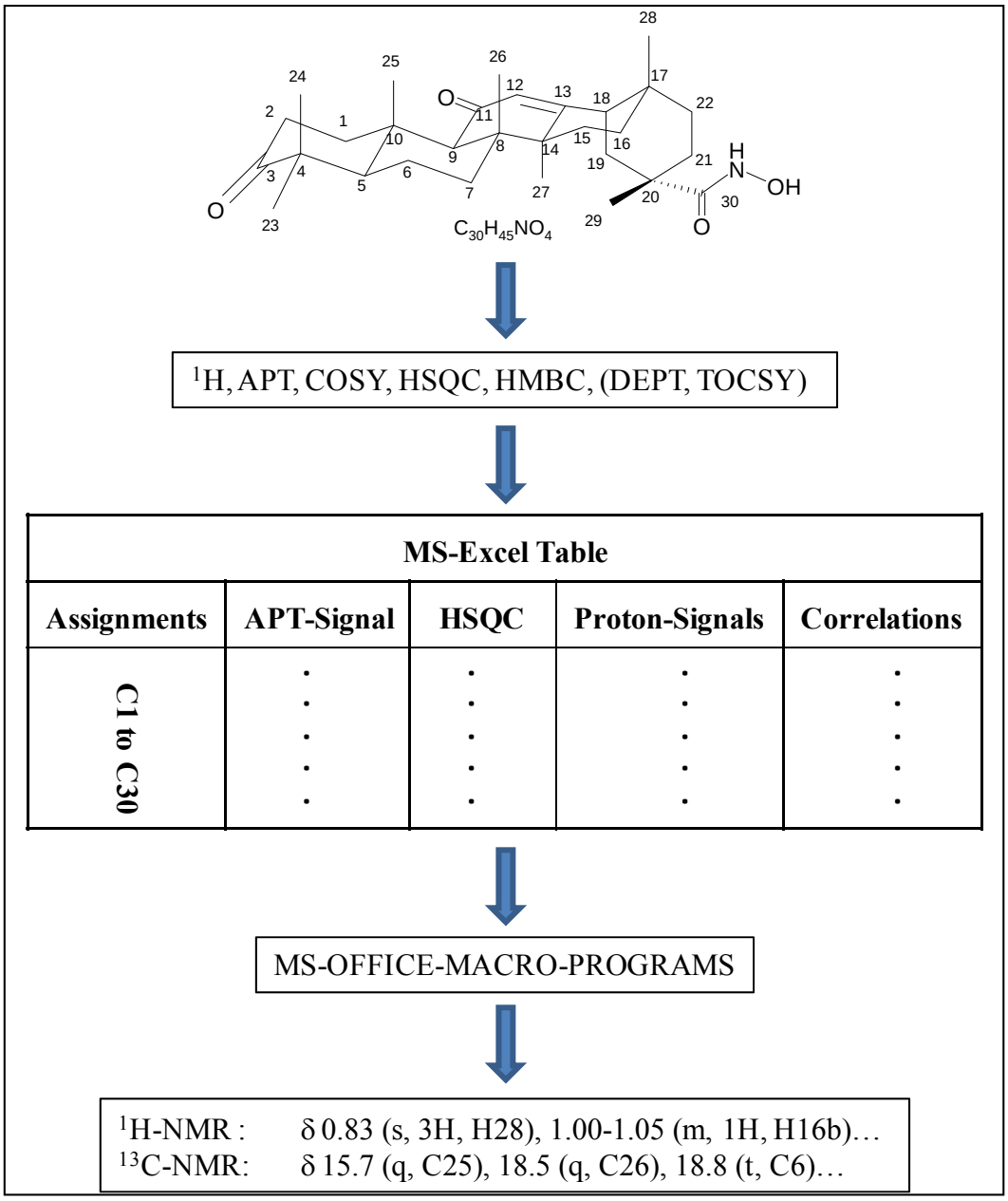


Figure 12: General strategy for the effective assignment and documentation of NMR data of GA derivatives

Throughout the following chapter and the experimental part the assignments of NMR signals to the corresponding positions in the triterpene scaffold is done according to the structure of compound **31** in Figure 12 or the example in the general methods of the experimental part (Chapter 3.3).

2.8.2 Stepwise assignment and processing of NMR data of compound [31]

2.8.2.1 Analysis of APT and HSQC spectra

The best way to start NMR analysis is to process and peakpick the APT spectrum, have a look at the Topspin™-peaklist, eliminate residual solvent peaks based on reported solvent peaklist[108]. By simple counting of signals it has to be made sure that also signals which are very close or do overlap and appear as one (short glimpse on the HSQC, comparison of relative height of equal types of signal is helpful) are identified. These signals have to be added manually either in Topspin™ or at the latest in the Excel-table after importing the peaks from Topspin to Excel.

As next step the HSQC spectrum (Figure 13) is used to simply correlate all carbon signals with the corresponding ^{1}H-shifts (a phase sensitive HSQC discriminating CH_2 from other signals is very helpful, Figure 13).

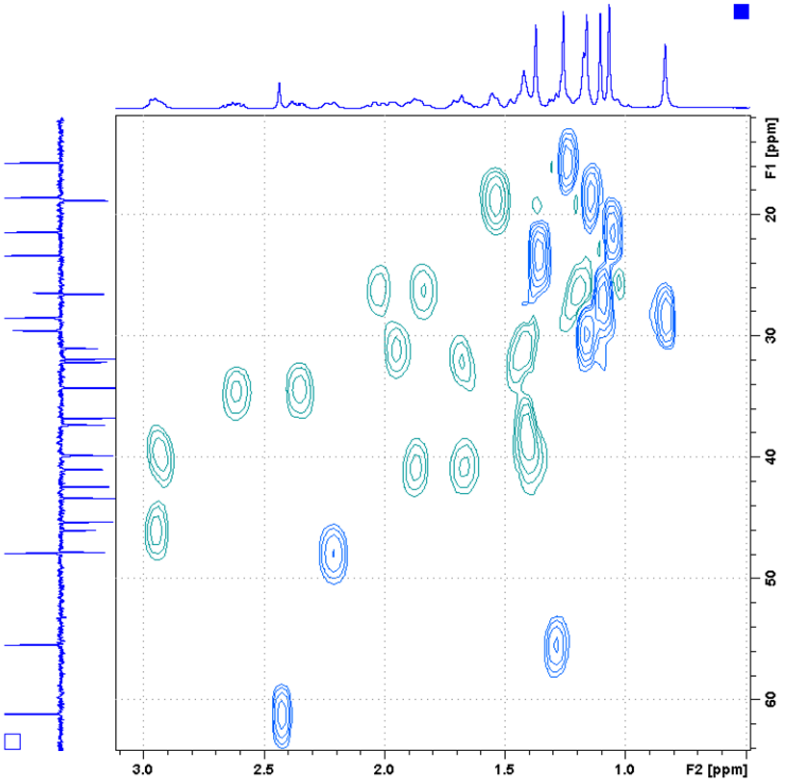


Figure 13: HSQC of all signals except the olefinic C12 at 125ppm

The early and **complete** correlation of all ^{1}H and ^{13}C signals prior to any real assigning of signals allows assignment of each carbon either based on a correlation of the carbon shift or one of the corresponding ^{1}H shifts.

As stated before analysis is simplified tremendously if not only an APT spectrum but also a DEPT 135 spectrum is recorded to be able to differentiate reliably between CH_2 and quaternary carbon signals with very similar shifts.

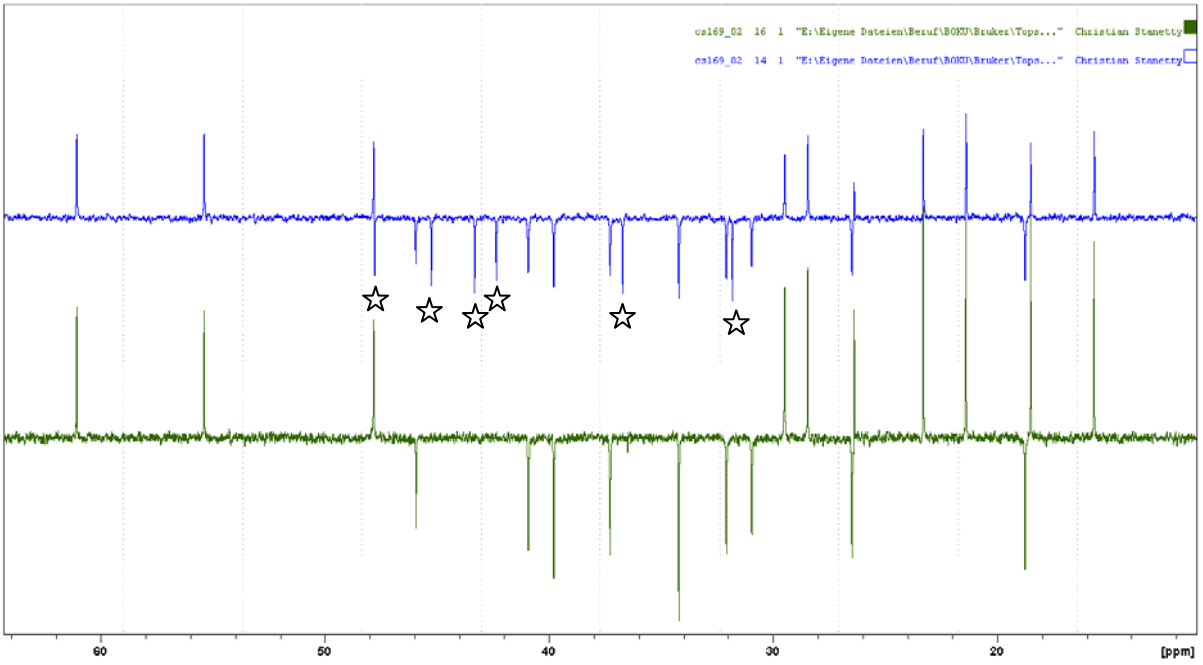


Figure 14: Comparision of the APT and DEPT spectra to discriminate CH_2 and quaternary carbon signals (stars)

Quite often carbon signals are too close to be discriminated against each based only on existing or not existing correlation signals in HSQC spectra (due to the low resolution in the F1-axes).

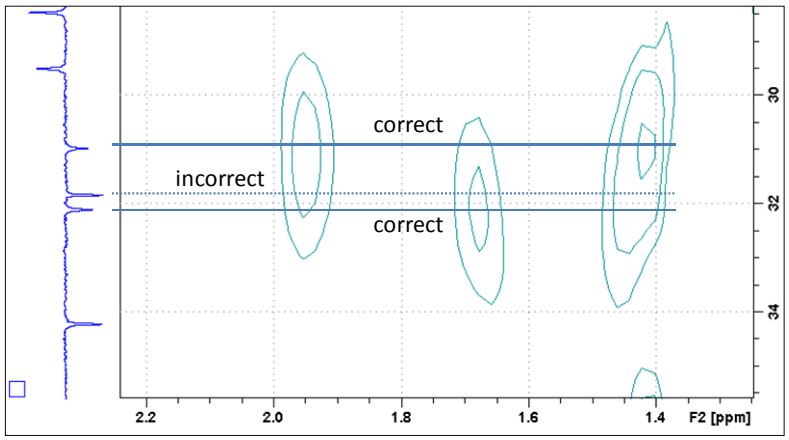


Figure 15: Example for a difficult discrimination between a CH_2 and a quaternary carbon only based on HSQC

In this example this holds especially true for the three signals (2×CH_2, 1×quaternary) between 30 and 32ppm, which would be hard to assign based only on HSQC, but are easily discriminated by DEPT and APT spectra.

Following the described path should end with an overview similar to the following screenshot (Figure 16). In some cases (not the case in the example) isolated regular multiplets can be identified in ^{1}H-NMR exact shift and coupling constants can be already be included in the table.

13C-Information					1H-Information				
Peak	Assign.	13C-Shift	Integral	HSQC / DEPT	Multiplett	1H-Shift (middle)	1H-Shift (middle)	J [Hz]	Correlation peaks
1		217.3	5	quartenary					
2		199.8	8	quartenary					
3		173.8	5	quartenary					
4		169.9	8	quartenary					
5		128.5	-10	CH	s	5.78			
6		61.1	-12	CH	s	2.43			
7		55.4	-12	CH	m	1.29			
8		47.82	-11	CH	m	2.22			
9		47.77	9	quartenary					
10		45.2	10	quartenary					
11		43.3	11	quartenary					
12		42.3	9	quartenary					
13		40.9	8	CH2	2xm	1.87	1.67		
14		39.8	10	CH2	2xm	2.94	1.39		
15		37.3	8	CH2	2xm	1.40	1.40		
16		36.7	11	quartenary					
17		34.2	12	CH2	2xm	2.61	2.35		
18		32.1	9	CH2	2xm	1.67	1.45		
19		31.8	12	quartenary					
20		31.0	7	CH2	2xm	1.95	1.41		
21		29.5	-9	methyl	s	1.17			
22		28.4	-12	methyl	s	0.83			
23		26.5	8	CH2	2xm	2.03	1.03		
24		26.38	-5	methyl	s	1.10			
25		26.40	overlapp	CH2	2xm	1.85	1.20		
26		23.3	-13	methyl	s	1.37			
27		21.4	-15	methyl	s	1.07			
28		18.8	9	CH2	2xm	1.55	1.55		
29		18.5	-11	methyl	s	1.16			
30		15.7	-13	methyl	s	1.26			

Figure 16: NMR table including all CH correlations but no assignments yet

2.8.2.2 Assignment of the low-field carbons (Cq + CH)

The actual assignment is best started by the assignment of C11, C12, C13, C30 and C3 based on the chemical shift and the phase in APT. Only the discrimination of C13 and C30 has to be supported by 2D data, either by correlation of C13 to the very prominent proton singlet of H12 (>5 ppm), or to the H27 methyl signal, which is always the most low-field methyl group in ^{1}H-NMR. Identification of C3 is particularly easy in case of 3-keto-compounds although also in case of a sp^{3}-C3 not too difficult, since only 4 CH groups exist. In the next step the three CH groups C5, C9, C18 are assigned which are by the way highly conserved in ^{13}C NMR shifts, based on the number of HMBC-correlations to methyl groups. C9 has two correlation signals to methyl groups (H25, H26) in the HMBC spectrum while C5 has three correlations to methyl groups (H23, H24, H25) and C18 has only one correlation to methyl

group H28, which is by the way in most of the cases most high-field methyl group at around 0.8 ppm. Furthermore, C9 is clearly identified *via* its proton signal (singlet at ~2.4 ppm). Using these pieces of information normally the low-field quaternary carbons and the CH groups are assigned in a reliable way.

2.8.2.3 Assignment of the methyl groups C23 to C29

Based on these first eight assignments all methyl groups can be identified *via* their proton singlets and their HMBC correlations (see Table 1 and Figure 17).

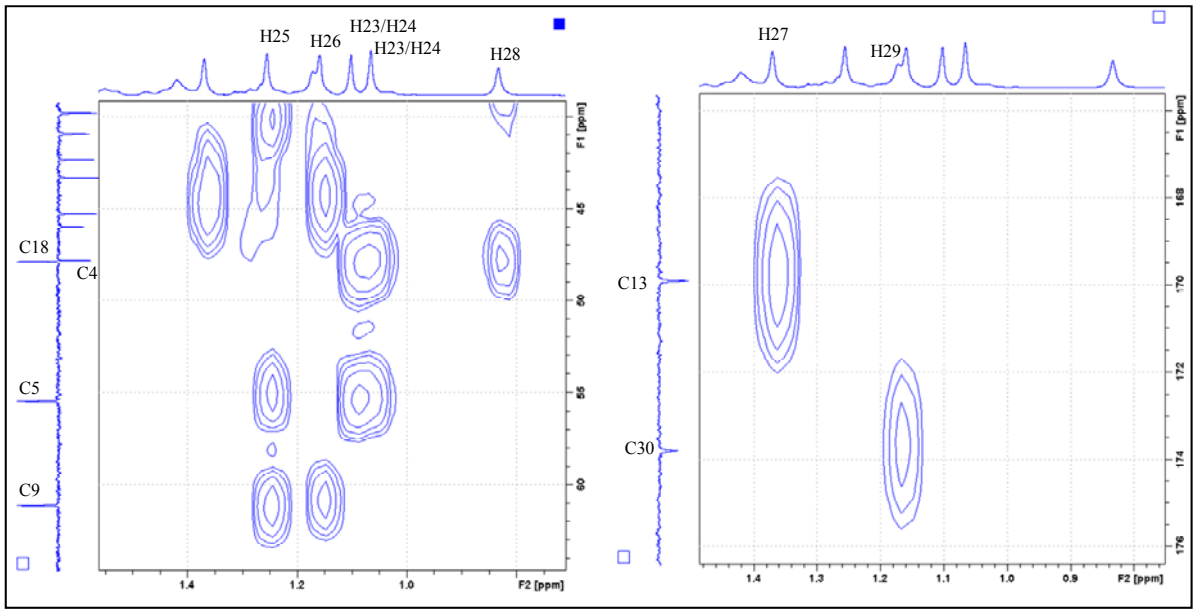


Figure 17: HMBC-based assignment of methylgroups C23-C29

In this special case the C18-signal overlaps with the quaternary C4, (equal correlations like C5) which is only the case in C3-carbonyl compounds.

HMBC C→H		Assignment
C9	**H25**, H26	**H25**, H26
C5	H23, H24, **H25**	2×H23/H24
C18	H28	H28
C30	H29	H29
C13	H27	H27

Table 1: Assignment of methyl groups based on HMBC signals to prominent carbon signals

It is crucial for the whole assignment process to find a solvent in which the seven methyl-

singlets in the ^{1}H-NMR spectrum discriminate against each other as far as possible. In case of 3-amino-GA derivatives it might even make sense to decide whether to characterize the compounds as hydrochlorides or as free amine species, depending on which species gives the better resolved methyl groups in ^{1}H-spectrum.

In Figure 18 the so far assigned carbon signals are summed-up together with the documentation of the correlation peaks leading to the assignments.

13C-Information					1H-Information				
Peak	Assign.	13C-Shift	Integral	HSQC / DEPT	Multiplett	1H-Shift (middle)	1H-Shift (middle)	Correlation peaks	Comment
1	**3**	217.3	5.3	quartenary				shift	
2	**11**	199.8	7.6	quartenary				shift	
3	**30**	173.8	4.8	quartenary				shift and exclusion	
4	**13**	169.9	7.6	quartenary				shift, phase and HMBC H12	
5	**12**	128.5	-10.3	CH	s	5.78		shift and phase	
6	**9**	61.1	-12	CH	s	2.43		1H-singlet, 2xHMBC to methyl	
7	**5**	55.4	-12.4	CH	m	1.29		3xHMBC to methyl	
8	**18**	47.82	-10.9	CH	m	2.22		1xHMBC to methyl	
9		47.77	8.7	quartenary					
10		45.2	10	quartenary					
11		43.3	11	quartenary					
12		42.3	9.4	quartenary					
13		40.9	8	CH2	2xm	1.87	1.67		
14		39.8	9.9	CH2	2xm	2.94	1.39		
15		37.3	8.3	CH2	2xm	1.40	1.40		
16		36.7	10.8	quartenary					
17		34.2	11.7	CH2	2xm	2.61	2.35		
18		32.1	8.9	CH2	2xm	1.67	1.45		
19		31.8	12	quartenary					
20		31.0	7	CH2	2xm	1.95	1.41		
21	**29**	29.5	-9.2	methyl	s	1.17		HMBC C30	
22	**28**	28.4	-11.8	methyl	s	0.83		HMBC C18	
23		26.5	8.2	CH2	2xm	**2.03**	**1.03**		
24	**23/24**	26.4	-5.2	methyl	s	1.10		HMBC C5	
25		26.4	overlapp	CH2	2xm	**1.85**	**1.20**		
26	**27**	23.3	-12.8	methyl	s	1.37		HMBC C13	
27	**23/24**	21.4	-15	methyl	s	1.07		HMBC C5	
28		18.8	8.9	CH2	2xm	1.55	1.55		
29	**26**	18.5	-10.7	methyl	s	1.16		HMBC H9, HMBC C9	
30	**25**	15.7	-12.6	methyl	s	1.26		HMBC C9	

Figure 18: Assignment of low-field carbons, all CH and all CH_3 groups

2.8.2.4 Assignment of the CH_2 groups

The next step is to identify the 5 existing isolated proton spin systems which can very often be done by very careful interpretation of the COSY spectrum taking into account the already known geminal CH_2 relationships from the HSQC spectrum.

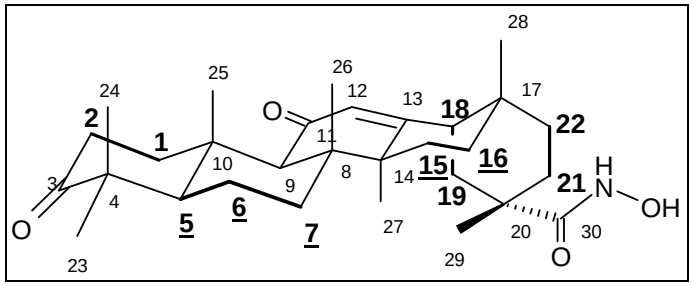


Figure 19: Highlighted isolated spin-systems in GA structure

Unambiguous COSY signals can sometimes be verified by the corresponding HMBC signal, although HMBC signals other than from methyl-proton signals are not observed frequently.

Spin System 1: H1 – H2 – (H3)

The spin system 1-2 is normally relatively easily identified because of the extreme splitting up of the two ^{1}H-signals in the proton NMR, due to the anisotropic effect of the C11 carbonyl on one of the H1-signals, resulting in a signal in the relatively empty region of about 3ppm.

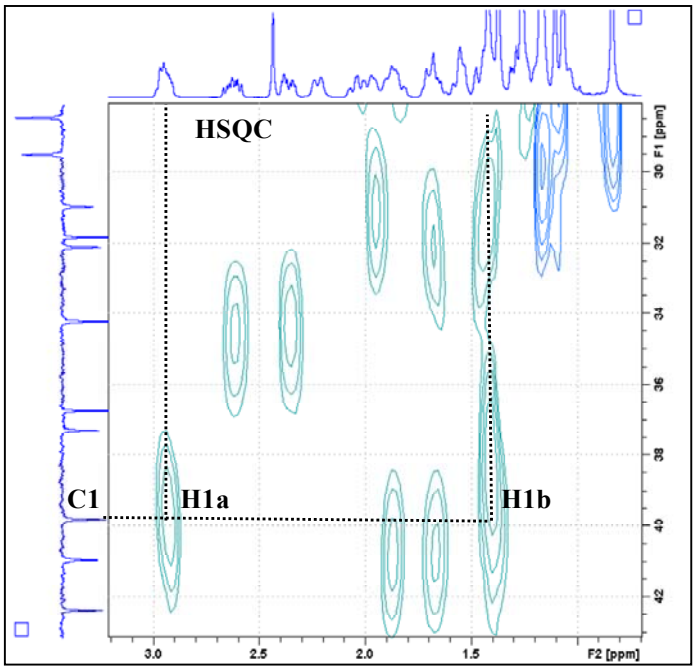


Figure 20: Typical strong discrimination between H1a and H1b

Based on the COSY correlations of this isolated H1a signal the identification of H1, H2 (and H3) protons and therefore carbons is generally easy. Already identified H3/C3 can further

help to identify or confirm this system. The final identification as H1-H2 system can be supported *via* HMBC from C5 to H1.

Spin system: H5-H6-H7

Starting from already identified H5, this system can sometimes be identified based on COSY alone but needs normally additional correlation signals from HMBC (C5-H6, or H5-C6). It is helpful to know that the C6 is generally the most high-field CH_2 group.

Spin system: H18-H19

The COSY correlation from H18 to H19a and H19b are in general observed although the H18 signal is not always a well isolated one. Nevertheless, in a repetitive process over the CH and CH_2 groups the correlation can normally be reliably identified.

Spin system 4: H15-H16

The H15-H16 spin system is characterized by two pairs of ^{1}H-signals which both exhibit a large difference between the two geminal protons. However, the corresponding ^{13}C-signals are always a pair of very close signals in the range of 26ppm (in general within 0.2ppm).

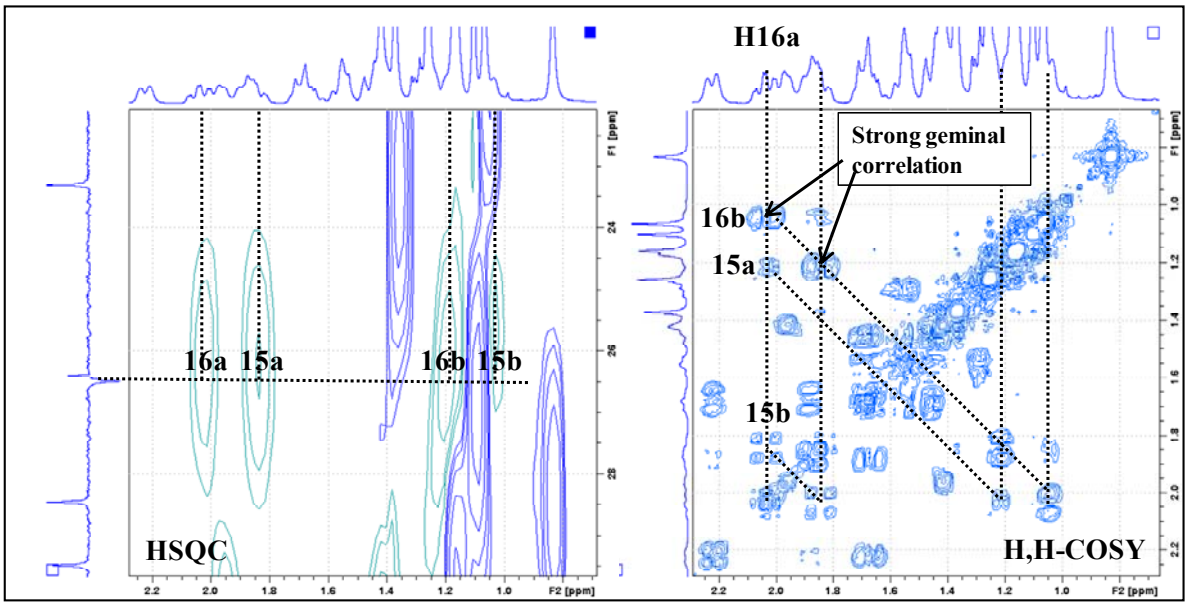


Figure 21: Identification of the two CH_2-pairs of 15 and 16 *via* their strong geminal COSY correlation

Therefore, the corresponding carbon signal can normally not be assigned reliably, however the strong COSY signals were interpreted as geminal correlation to identify pairs of geminal protons (Figure 21). This interpretation of the COSY signal was supported in cases with slightly more separated carbon signals and possible differentiation of the four HSQC signals

into two pairs of two. Generally, there is a strong HMBC correlation from H27 and H28 to this pair of carbons, thus identifying it as H15-H16.

Spin system 5: H21-H22

The H21-H22 spin system is identified at the carbon level as the only remaining CH_2 carbons and is confirmed by the relevant COSY-correlation peaks, that can normally be identified based on the known HSQC relationships and the prior identified correlation peaks from the other spin systems. The differentiation between C21 and C22 can usually be done *via* HMBC correlation signals to H28 and/or H29.

13C-Information					1H-Information				
Peak	Assign.	13C-Shift	Integral	HSQC / DEPT	Multiplett	1H-Shift (middle)	1H-Shift (middle)	Correlation peaks	Comment
1	3	217.3	5.3	quartenary				shift	
2	11	199.8	7.6	quartenary				shift	
3	30	173.8	4.8	quartenary				shift and exclusion	
4	13	169.9	7.6	quartenary				shift, phase and HMBC H12	
5	12	128.5	-10.3	CH	s	5.78		shift and phase	
6	9	61.1	-12	CH	s	2.43		1H-singlet, 2xHMBC to methyl	
7	5	55.4	-12.4	CH	m	1.29		3xHMBC to methyl	
8	18	47.82	-10.9	CH	m	2.22		1xHMBC to methyl	
9		47.77	8.7	quartenary					
10		45.2	10	quartenary					
11		43.3	11	quartenary					
12		42.3	9.4	quartenary					
13	**19**	40.9	8	CH2	2xm	1.87	1.67	COSY H18	
14	**1**	39.8	9.9	CH2	2xm	2.94	1.39	HSQC-split up, HMBC-C3, Spinsystem 1-2	
15	**22**	37.3	8.3	CH2	2xm	1.40	1.40	HMBC H28	
16		36.7	10.8	quartenary					
17	**2**	34.2	11.7	CH2	2xm	2.61	2.35	COSY H1	
18	**7**	32.1	8.9	CH2	2xm	1.67	1.45	Cosy 6	
19		31.8	12	quartenary					
20	**21**	31.0	7	CH2	2xm	1.95	1.41	Cosy, HSQC Spinsystem 21- 22 no HMBC to C28	
21	29	29.5	-9.2	methyl	s	1.17		HMBC C30	
22	28	28.4	-11.8	methyl	s	0.83		HMBC C18	
23	**15/16**	26.5	8.2	CH2	2xm	2.03	1.03	COSY + HMBCH27	
24	23/24	26.4	-5.2	methyl	s	1.10		HMBC C5	
25	**15/16**	26.4	**overlapp**	CH2	2xm	1.85	1.20	COSY + HMBCH27	
26	27	23.3	-12.8	methyl	s	1.37		HMBC C13	
27	23/24	21.4	-15	methyl	s	1.07		HMBC C5	
28	**6**	18.8	8.9	CH2	2xm	1.55	1.55	COSY 5	
29	26	18.5	-10.7	methyl	s	1.16		HMBC H9, HMBC C9	
30	25	15.7	-12.6	methyl	s	1.26		HMBC C9	

Figure 22: Assignment including all the CH_2 groups, assigned by COSY and HMBC

With all the CH_2 groups assigned the table is completed for all signals but the quaternary aliphatic signals. Figure 22 gives a typical table at this stage of the assignment process.

2.8.2.5 Assignment of the high-field quaternary carbon signals

Starting from assigned ^{1}H-signals of the methyl group C23 to C29, their corresponding quaternary carbons can be identified *via* their HMBC correlation signals. It is beneficial, that the HMBC signals of the methyl groups are significantly stronger than all other correlation signals and have a very good resolution in the ^{1}H-axis. Nevertheless, the reliable identification of a cross-peak towards one carbon signal very often relies on relative more than absolute shift. It is extremely helpful to be able to exclude nearby CH_2-signals which have already been assigned and can be excluded or identified as correlation partner of a specific methyl-proton signal.

Generally, it is possible to identify the following HMBC correlations which are the basis for the assignments of the quaternary carbons 4, 8, 10, 14, 17 and 20 (Table 2).

HMBC H→C		Assignment
2×H23/H24	C4	C4
H28	C17	C17
H25, H9	C10	C10
H9	C8	C8
H29	C20	C20
H27	C14	C14

Table 2: Assignment of the quaternary carbons based on HMBC correlations

Frequently, quaternary carbons C8 and C14 can not be assigned unambiguously, due to the fact that both of them have HMBC correlations to both their direct methyl group as well as to the neighboring methyl group. After these last assignments a full table of carbon and proton shifts is obtained (Figure 23).

2.8.3 Semiautomatic transformation of the ^{13}C-NMR data to text lines:

In order to avoid unnecessary work on the one hand and mistakes during transformation into text lines for publication or thesis a system to semi-automatically transform the MS-Excel table (like in Figure 23) to text lines was developed.

13C-Information					1H-Information				
Peak	Assign.	13C-Shift	Integral	HSQC / DEPT	Multiplett	1H-Shift (middle)	1H-Shift (middle)	Correlation peaks	Comment
1	3	217.3	5.3	quartenary				shift	
2	11	199.8	7.6	quartenary				shift	
3	30	173.8	4.8	quartenary				shift and exclusion	
4	13	169.9	7.6	quartenary				shift, phase and HMBC H12	
5	12	128.5	-10.3	CH	s	5.78		shift and phase	
6	9	61.1	-12	CH	s	2.43		1H-singlet, 2xHMBC to methyl	
7	5	55.4	-12.4	CH	m	1.29		3xHMBC to methyl	
8	18	47.82	-10.9	CH	m	2.22		1xHMBC to methyl	
9	**4**	47.77	8.7	quartenary				HMBC 23, 24	
10	**8**	45.2	10	quartenary				HMBC 9	
11	**14**	43.3	11	quartenary				HMBC-H27, H26	
12	**20**	42.3	9.4	quartenary				HMBC-H26, H29	
13	19	40.9	8	CH2	2xm	1.87	1.67	COSY H18	
14	1	39.8	9.9	CH2	2xm	2.94	1.39	HSQC-split up, HMBC-C3, Spinsystem 1-2	
15	22	37.3	8.3	CH2	2xm	1.40	1.40	HMBC H28	
16	**10**	36.7	10.8	quartenary				HMBC H9, HMBC H25	
17	2	34.2	11.7	CH2	2xm	2.61	2.35	COSY H1	
18	7	32.1	8.9	CH2	2xm	1.67	1.45	Cosy 6	
19	**17**	31.8	12	quartenary				HMBC H28	
20	21	31.0	7	CH2	2xm	1.95	1.41	Cosy, HSQC Spinsystem 21- 22 no correlation to C28	
21	29	29.5	-9.2	methyl	s	1.17		HMBC C30	
22	28	28.4	-11.8	methyl	s	0.83		HMBC C18	
23	15/16	26.5	8.2	CH2	2xm	**2.03**	**1.03**	COSY + HMBCH27	
24	23/24	26.4	-5.2	methyl	s	1.10		HMBC C5	
25	15/16	26.4	overlapp	CH2	2xm	**1.85**	**1.20**	COSY + HMBCH27	
26	27	23.3	-12.8	methyl	s	1.37		HMBC C13	
27	23/24	21.4	-15	methyl	s	1.07		HMBC C5	
28	6	18.8	8.9	CH2	2xm	1.55	1.55	COSY 5	
29	26	18.5	-10.7	methyl	s	1.16		HMBC H9, HMBC C9	
30	25	15.7	-12.6	methyl	s	1.26		HMBC C9	

Figure 23: Completed assignments of all carbons and protons of an exemplary GA derivative

To use such a systematic approach of assigning and processing the data into text format is recommended to avoid mistakes other ways probable in retyping the data from tables into text lines.

The relevant columns *chemical shift*, *multiplicity* and *assignment* are copied from the assignment table into a MS-Excel mask (in Table 3 only some signals have been transferred) already including all necessary brackets, semicolons and discriminators of assignment.

13C-shifts		Multiplicity			Assignment		
217.3	(	quartenary	,	C	3	)	,
199.8	(	quartenary	,	C	11	)	,
173.8	(	quartenary	,	C	30	)	,
169.9	(	quartenary	,	C	13	)	,
128.5	(	CH	,	C	12	)	,
61.1	(	CH	,	C	9	)	,
55.4	(	CH	,	C	5	)	,
47.82	(	CH	,	C	18	)	,

Table 3: Selected ^{13}C information fitted into transformation mask in MS-Excel

The multiplicity discriminators are transformed to the usual s, d, t, q nomenclature and the data can be resorted from low numbers to high numbers.

13C-shifts		Multiplicity			Assignment		
47.82	(	d	,	C	18	)	,
55.4	(	d	,	C	5	)	,
61.1	(	d	,	C	9	)	,
128.5	(	d	,	C	12	)	,
169.9	(	s	,	C	13	)	,
173.8	(	s	,	C	30	)	,
199.8	(	s	,	C	11	)	,
217.3	(	s	,	C	3	)	,

Table 4: Processed transformation mask in MS-Excel

The whole mask is copied to MS-Word and by applying a macro-routine, which mainly transforms tables into text and eliminates all residual blanks and tab-stops is finally transformed to end up with the ^{13}C-NMR data in exactly the form it is presented in this thesis.

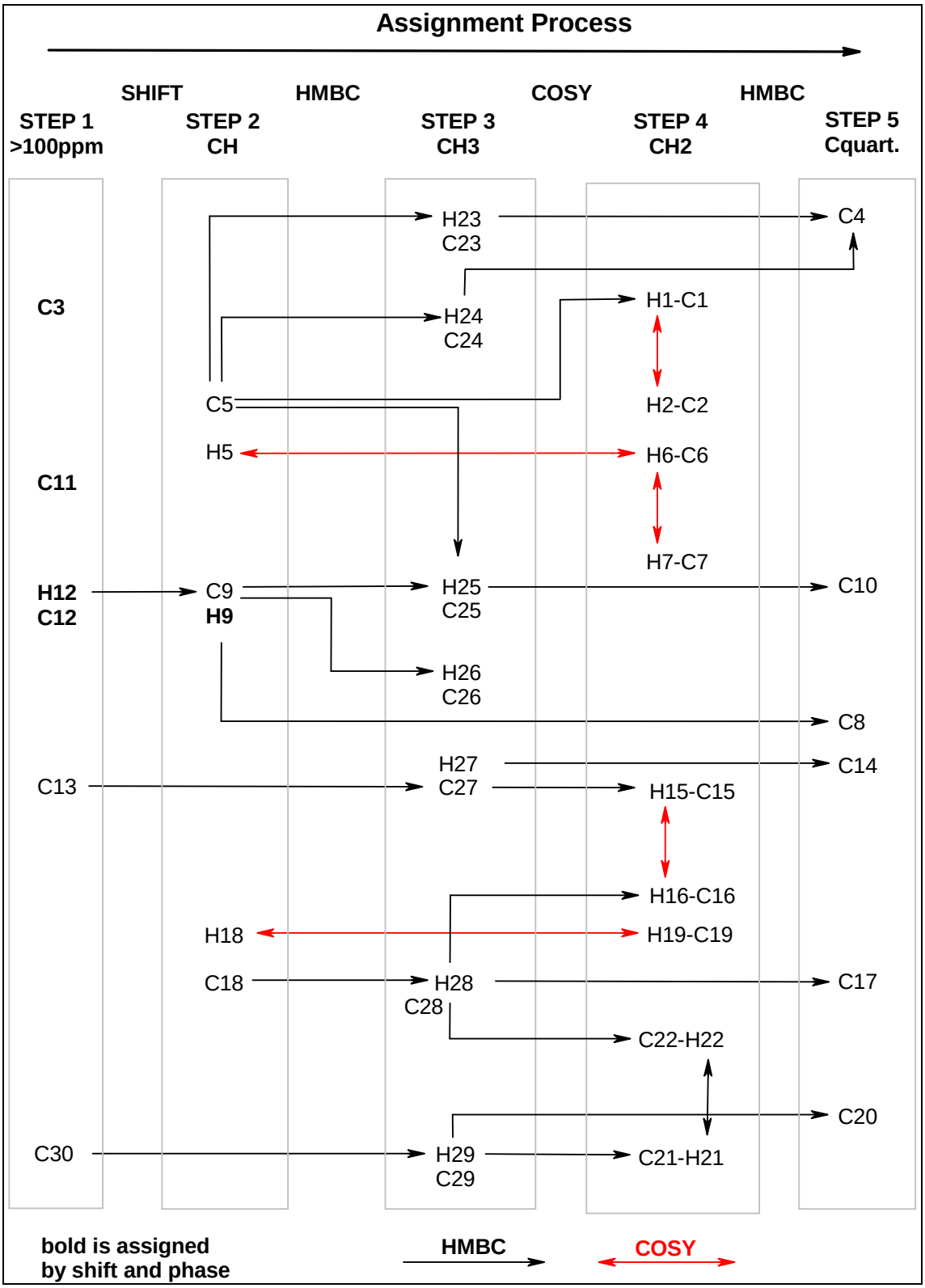
Result of the application of macro routine:

… 47.82 (d, C18), 55.4 (d, C5), 61.1 (d, C9), 128.5 (d, C12), 169.9 (s, C13), 173.8 (s, C30), 199.8 (s, C11), 217.3 (s, C3)

By similar transformations which need a little more handling and are harder to present, the proton signals are also translated from the MS-Excel table to lines of text.

2.8.4 Summing up the process of assignments

In the following pictogram the generally recommended path of assignments is summarized.



2.9 Biological results of the prepared compounds

The compounds prepared within the ASPEX-project in general and within this thesis in particular have been evaluated mainly in two biological fields: On the one hand as inhibitors of the 11β-HSD1- and 11β-HSD2-enzymes, striving for compounds with selectivity towards 11β-HSD2 and at the other hand as compounds exhibiting antiviral activities against influenza A virus (IAV).

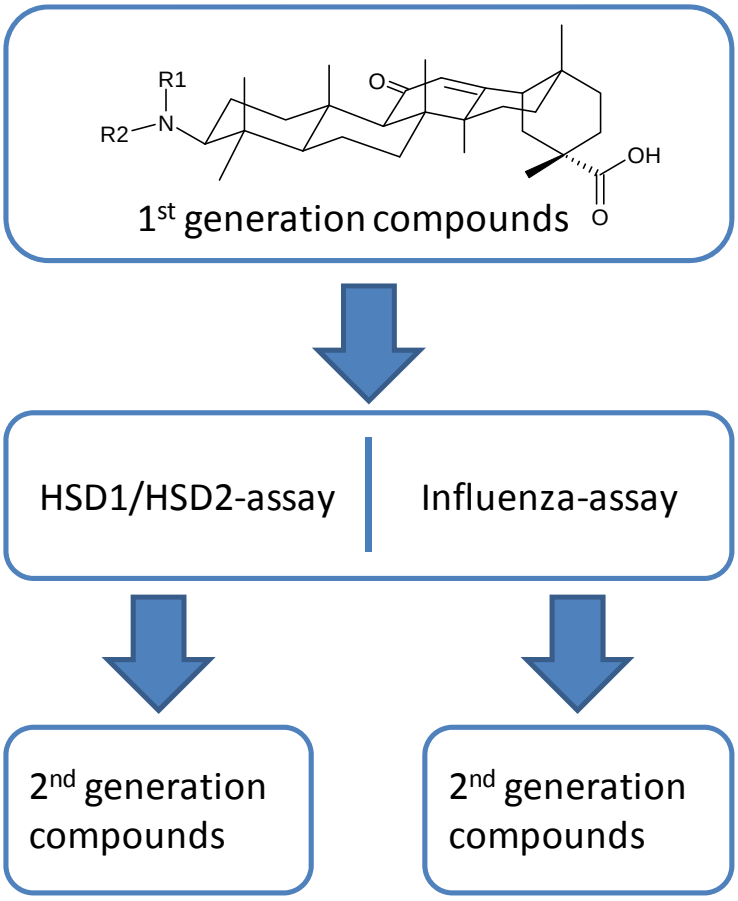


Figure 24 Biological evaluation of prepared ASPEX-compounds

Luckily in both parts of the project positive feedback from biology allowed more focused efforts. Based on first results from the 11β-HSD1/11β-HSD2 assay promising 3-amino-derivatives could be identified and were formally combined with other interesting structural modifications to come up with second-generation derivatives. In the antiviral screening a group of similar compounds was found to exhibit interesting cytoprotective properties and did allow refocusing in this field as well.

2.9.1 Enzymatic 11β-HSD1 and 11β-HSD2 assay

Within the ASPEX-project around 100 compounds, natural and synthetic ones have so far been evaluated as inhibitors against 11β-HSD1- and 11β-HSD2-enzymes. The following tables show the inhibition of 11β-HSD1 and 11β-HSD2 for each compound *via* the retained enzymatic activities (determined at 1000nM or 200nM concentration, the latter being the standard as soon as stronger inhibitors were at hands). Strong inhibition is highlighted (dark red: below 10% of control, orange: below 30% of control) and the last column indicates selectivity between the inhibition of 11β-HSD1 and 11β-HSD2.

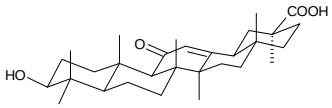
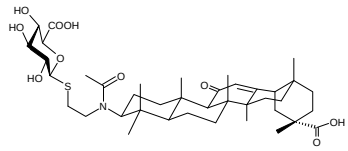
Compound	Structure	Conc. [nM]	11β-HSD2 [%]	11β-HSD1 [%]
glycyrrhetinic acid (GA)		1000	6.4	16.1
3α-GA		1000	3.0	2.6
3β-acetyl-GA		1000	14.3	52.9
3β-18α-glycyrrhetinic acid		1000	50.7	50.3
glycyrrhizin		1000	71.9	36.8
60		1000	71.1	25.5

Table 5: General SAR regarding compounds with principally different structural features

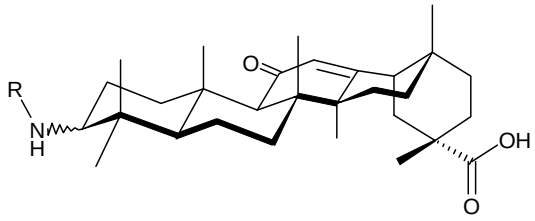
Table 5 is underlining some more general trends of the observed SAR regarding inhibition of 11β-HSD2 and 11β-HSD2-enzyme. It was shown that the inversion of stereochemistry at the 3-position (3α-GA) or the acetylation of 3β-GA has no or even beneficial effect on the inhibitory potential of the 3β-GA lead structure.

Changes in the triterpene structure, however, like for example the close relative 18α-glycyrrhetinic acid but also other compounds based on different types of triterpenes (data not shown) are clearly unfavorable. Furthermore, very generally the formal addition of a sugar moiety, like in the natural glycyrrhizin (GN) or in compound **60** or its corresponding methyl

ester **59** led to further decrease in inhibition compared to the already rather low inhibition of the lead compound glycyrrhetinic acid (GA).

2.9.1.1 Evaluation of the 3-acetyl- and 3-sulfonylamino-GA derivatives

It was a very pleasant and motivating finding that actually all the prepared 3-amino-GA derivatives exhibited a relatively strong inhibition of 11β-HSD1 and 11β-HSD2 activity, which was clearly superior to the lead structure GA. Furthermore, several compounds exhibited a significant selectivity towards the inhibition of 11β-HSD2 over 11β-HSD1.



Compound	Structure	Conc. [nM]	11β-HSD2 [%]	11β-HSD1 [%]
10b	3β-AcNH-GA	**1000**	**0.2**	**35.8**
10a	3α-AcNH-GA	**1000**	**3.8**	**4.2**
12b	3β-MsNH-GA	**200**	**4.3**	**57.7**
12a	3α-MsNH-GA	**200**	**3.5**	**32.1**
14b	3β-TfNH-GA	**200**	**22.7**	**87.1**
14a	3α-TfNH-GA	**200**	**50.2**	**89.5**
23b	3β-MeONH-GA	**200**	**1.8**	**33.1**
23a	3α-MeONH-GA	**200**	**4.6**	**42.5**
16	3β-HOOC-$(CH_2)_2$-CONH-GA	**1000**	**13.3**	**5.6**
19	3β-MeOOC-$(CH_2)_2$-SO_2NH-GA	**1000**	**3.3**	**1.3**
20	3β-HOOC-$(CH_2)_2$-SO_2NH-GA	**1000**	**39.9**	**5.3**

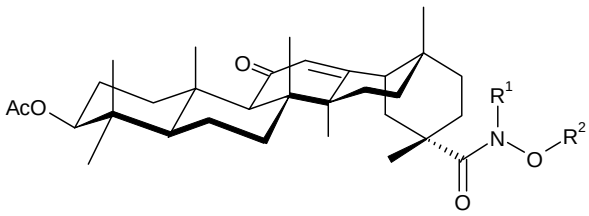
Table 6: Inhibitory potential for the first generation of 3-amino-GA derivatives

Interestingly, the Aminocarbenoxolone **16** and the sulfonamido-carbenoxolone type derivatives **19** and **20** gave weaker and unspecific inhibition and were not considered as

starting point for further structural optimization. The smaller derivatives **10**, **12** and **14** and the 3-methoxyamino-GA derivatives **23**, however gave strong inhibition for the 11β-HSD2-enzyme while 11β-HSD1-enzyme was only moderately inhibited at the new standard concentration of 200nM. The free amine **4b**, however was only a weak and rather unspecific inhibitor, even at the initially applied 1000nM concentration (data not shown).

2.9.1.2 Hydroxamic acids as powerful modification at the C30-position

Among the many modifications of the C30-position, which were elaborated by the team of Prof. Jordis[i], the hydroxamic acids delivered a very interesting pharmacological response.



Compound	Structure	Conc. [nM]	11β-HSD2 [%]	11β-HSD1 [%]
PS-168-1-2	AcO-GA-N(Me)OH	**200**	**4.2**	**96.7**
PS-167-1-2	AcO-GA-NHOH	**1000**	**1.2**	**48.3**
PS-178-1-3	AcO-GA-NHOMe	**1000**	**35.7**	**37.9**
PS-160-1-3	AcO-GA-N(Me)OMe	**200**	**71.7**	**94.9**
LCz-1049-1-3	AcO-GA-N$(CH_2)_2$OH	**1000**	**59.3**	**97.1**

Table 7: Performance of the hydroxamic acid family in the 11β-HSD1/11β-HSD2-enzyme assay

While the free hydroxamic acid and the *N*-methyl-hydroxamic acid derivative of 3β-AcO-GA exhibited excellent selectivity for and strong inhibition of the 11β-HSD2-enzyme, *O*-alkyl hydroxamic acids like (**PS-160-1-3**) or amide structures like the hydroxyethylamide of GA acid were inactive in both enzyme assays.

Based on the positive feedback from biology for the reported C3 and C30 modifications, targets with the formal combination of both were selected. The *N*-Acetyl- **10**, the *N*-Triflyl- **14** and the 3-methoxylamino-GA derivatives **23** were selected for decoration as *N*-methyl- and free hydroxamic acids (Chapter 2.5). Due to the promising results of the compounds in

[i] Laszlo Czollner and Priti Sha have to be named in regard to the hydroxamic acid topic

the first round, the first results of compounds containing both putative pharmacophoric structural motifs were of high interest. On closer investigation (determination of EC_{50}-values – additional columns in Table 8) the *N*-methyl-hydroxamic acid motif had been proven to be superior over the free hydroxamic acid moiety, thus only *N*-methyl-hydroxamic acids were further considered in the enzyme assay.

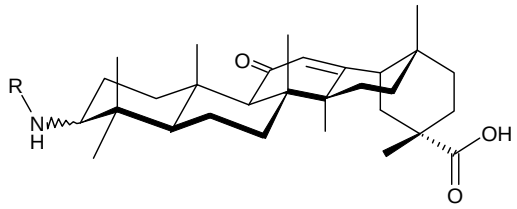
Compound	Structure	Conc. [nM]	11β-HSD2 [%]	11β-HSD1 [%]	11β-HSD2 EC_{50} [nM]	11β-HSD1 EC_{50} [nM]
GA	Glycyrrhetinic acid	**1000**	**6.4**	**16.1**	**256**	**778**
PS168-1-2	AcO-GA-N(Me)OH	**1000**	**2.6**	**50.5**	**7**	**1012**
PS167-1-2	AcO-GA-NHOH	**1000**	**1.2**	**48.3**	**60**	**627**
10b	3β-AcNH-GA	**1000**	**0.2**	**35.8**	**458**	**826**
IK-7	3β-AcNH-GA-N(Me)OH	**200**	**3.6**	**71.6**	**n.d.**	**n.d.**
14b	3β-TfNH-GA	**200**	**22.7**	**87.1**	**n.d.**	**n.d.**
IK-17	3β-TfNH-GA-N(Me)OH	**200**	**44.3**	**70.6**	**n.d.**	**n.d.**
23b	3β-MeONH-GA	**200**	**1.8**	**33.1**	**15**	**76**
28	3β-MeONH-GA-N(Me)OH	**200**	**29.5**	**93.3**	**547**	**40000**

Table 8: Comparison of the inhibitory properties of corresponding carboxylic acid and *N*-Me-hydroxamic acids

However, the compounds of the 2nd generation which have so far been examined did not really fulfill the expectations regarding their enzymatic properties. While the inhibitory activity of 3β-acetylamino-GA-N-methyl-hydroxamic acid **IK-7** was at least in the range of the corresponding carboxylic acid **10b,** the 3β-*N*-triflyl-GA-N-methyl-hydroxamic acid **IK-17** was inferior to both parent compounds. The 3β-methoxyamino-GA-N-methyl-hydroxamic **28** acid, however, exhibited significantly less inhibition of 11β-HSD2-enzyme (also confirmed in the EC_{50}-values) but has on the other hand an extremely high EC_{50}-value for the 11β-HSD1-enzyme, thus exhibiting very good selectivity between the two enzymes.

2.9.2 Antiviral assay

The majority of the prepared compounds was tested for their antiviral properties in the cell-based assay described in Chapter 1.4.1. Within a concentration range of 250 µM and 5 µM the compounds were tested for CPE reduction as surrogate marker for antiviral activity. Most promising compounds would inhibit virus replication leading to up to 100 % CPE reduction at non-toxic concentrations. In analogy to the data presented from the enzyme-assay the antiviral properties of the acylated and sulfonylated 3-amino-GA derivatives are presented first (Table 9).

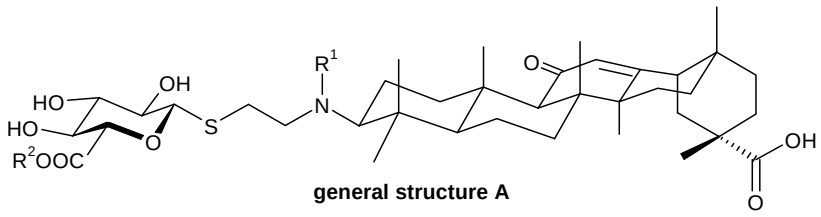


Compound	Structure	Antiviral activity	Toxicity
10b	3β-AcNH-GA	**inactive**	
10a	3α-AcNH-GA	**weak**	
12b	3β-MsNH-GA	**inactive**	
12a	3α-MsNH-GA	**inactive**	
14b	3β-TfNH-GA		**toxic**
14a	3α-TfNH-GA	**inactive**	
16	3β-HOOC-$(CH_2)_2$-CONH-GA	**inactive**	
19	3β-MeOOC-$(CH_2)_2$-SO_2NH-GA	**inactive**	
20	3β-HOOC-$(CH_2)_2$-SO_2NH-GA	**inactive**	
23b	3β-MeONH-GA	**weak**	
23a	3α-MeONH-GA	**inactive**	

Table 9: Antiviral properties of the prepared 3-amino-GA derivatives (weak<50% CPE reduction)

It can be stated, that the members of this class of compounds generally exhibited no antiviral activity or were toxic at the concentration tested. There were only two noteworthy exceptions.

The 3α-acetylamino-GA derivative **10a** exhibited weak antiviral activity at low concentration (25µM) but was also already slightly cytotoxic at this concentration. 3β-methoxyamino-GA **23b**, on the other hand, exhibited antiviral activity at very low concentration (5µM) without any indication for cytotoxicity. However, the extent of CPE reduction is low. In contrast to the results above the thioethylglucuronide family delivered much more promising results as depicted in Table 10.



general structure A

Compound	Structure	Antiviral activity	Toxicity
56	R^1=H, R^2=Me	**weak**	
57	R^1=H, R^2=H	**strong**	
59	R^1=Ac, R^2=Me	**strong**	
60	R^1=Ac, R^2=H	**strong**	
62	R^1=MeSucc, R^2=Me	**strong**	
63	R^1=Succ, R^2=H	**Weak**	

Table 10: Promising results of the thioglucuronide family in the antiviral assay (weak<50% CPE-reduction; strong>50% CPE-reduction)

Actually all members of general structure **A** exhibited at least weak antiviral properties (250µM) and some compounds exhibited complete CPE reduction. No indication for cytotoxicity was observed at these concentrations.

The above compounds have been evaluated in more depth and concentration dependent CPE for compound **60** as an example is shown in Figure 25.

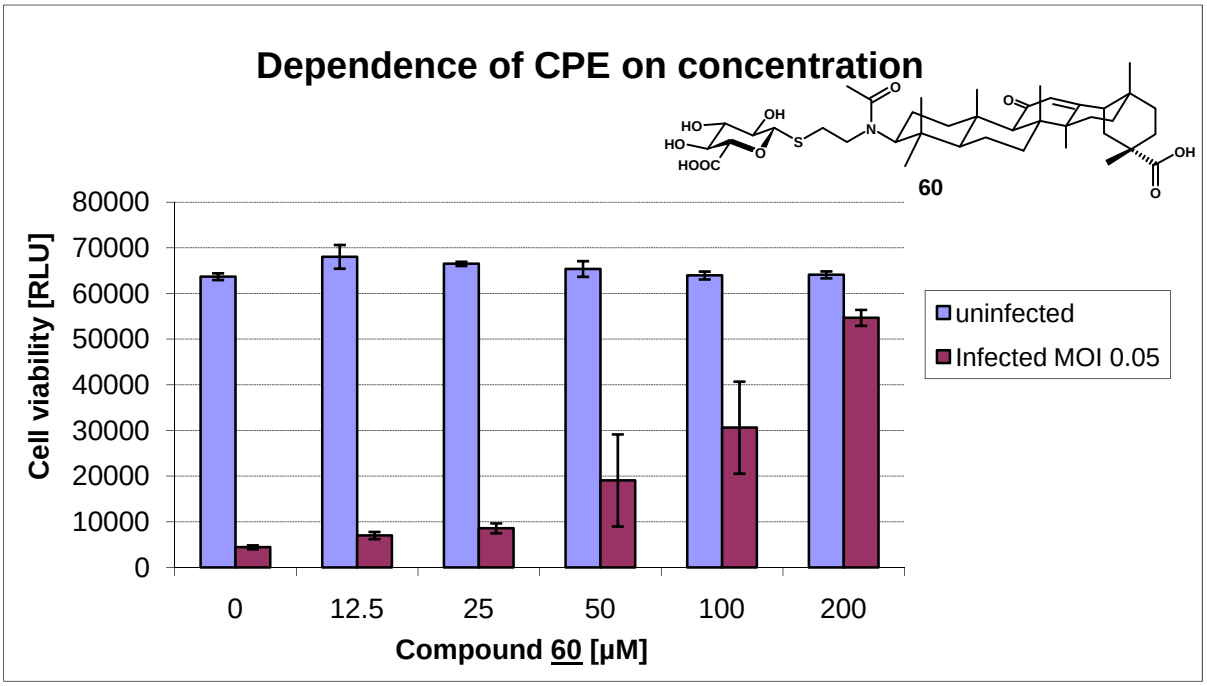


Figure 25: Increase of the cytoprotective effect of compound [60] with its concentration

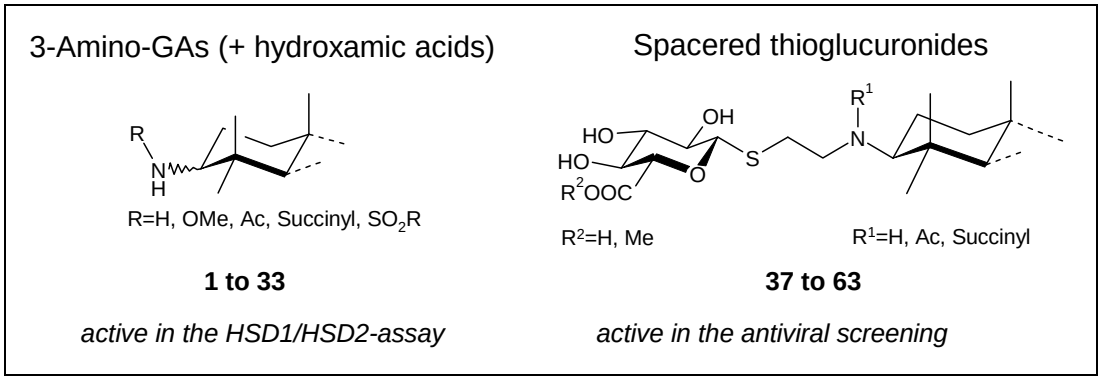
The blue bars show that the compound is not cytotoxic over the range of concentrations, which have been analyzed whereas the ruby bars show the increasing reduction of cytopathic effect (increasing cell viability) with increasing concentrations from 0 to 200µM. Thus, the first ruby bar refers to the experiment with untreated IAV infected cells.

Based on these first findings of antivirally active compounds with spacered neoglycosides of glycyrrhetinic acid, the decision was taken to expand the analogous series of methoxyaminoglycosides (prototype compounds **76** and **77)** into the supervised diploma thesis of Alexander Doppelreiter[88] with the aim to prepare a small library of similar spacered neoglycosides (general structures **A** and **C** in Scheme 67), thus allowing structure activity relationships regarding the sugar moiety and the linker.

2.10 Summary and Outlook

A variety of pharmacologically interesting compounds based on the 3-amino-glycyrrhetinic acid (both epimers) was envisioned, prepared, characterized and submitted for the evaluation of their biological properties with respect to anti-influenza activity and as selective inhibitors of 11β-HSD1 and 11β-HSD2, respectively. The synthesized compounds include structures with and without sugar moieties, thus mimicking the parent structures, glycyrrhizin, glycyrrhetinic acid and carbenoxolone.

The large family of "sugar-free" 3-amino-GA derivatives (Scheme 73, left side) gave promising results in the 11β-HSD1/11β-HSD2 enzyme assay as potent and selective 11β-HSD2 inhibitors and selected compounds were developed into a second generation of compounds - the 3-amino-glycyrrhetinic acid hydroxamic acids, an investigation which was partly extended into a diploma thesis[90]. Unfortunately so far the compounds with these combined putative pharmacophoric structural elements did not fulfill the expectations in the 11β-HSD1/11β-HSD2 assay.

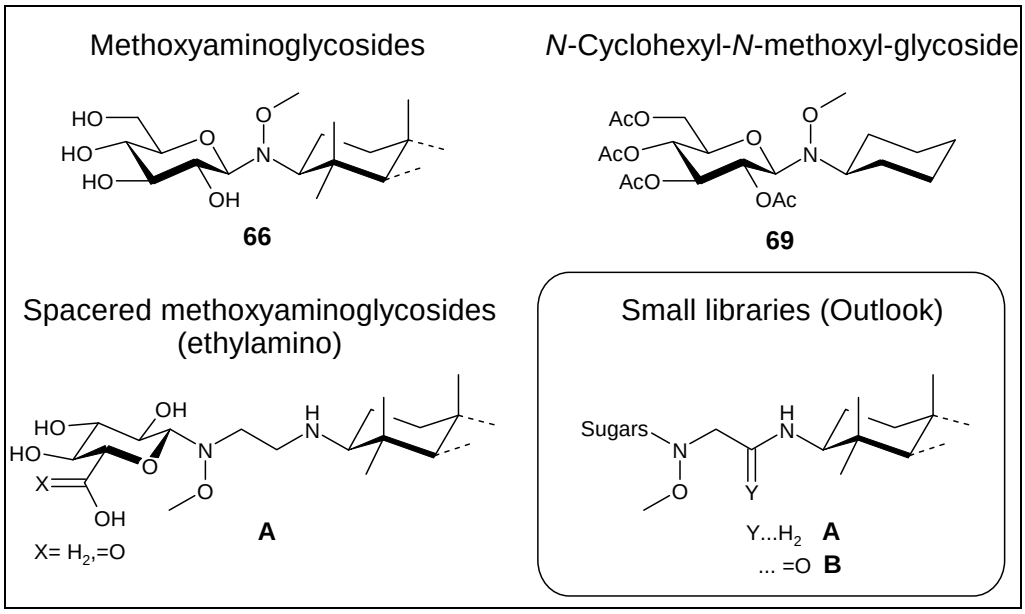


Scheme 73: Bioactive 3-amino-glycyrrhetinic acid based compounds

In the second part of the thesis – the neoglycosides of the glycyrrhetinic acid – a small compound family of spacered thioglucuronides was prepared which was inactive in the enzymatic assay but gave promising results in the antiviral screening against influenza A virus (Scheme 73, right side).

The last two parts of the thesis were dealing with the applicability of the direct glycosylation methodology on methoxyamino-derivatives of the glycyrrhetinic acid with unprotected reducing sugars. The initially targeted 3β-methoxaminoglycosides of GA could not be prepared by means of direct glycosylation of the 3β-methoxyamino-GA, but a role-model **66** was synthesized *via* conventional glycosylation chemistry to prove the principle stability of such compounds (Scheme 74).

The hypothesis that the sterically demanding adjacent methyl groups were causing the lack of reactivity was supported by the successful synthesis of the analogous *N*-cyclohexyl-*N*-methoxy-glucopyranoside **69** under analogous conditions.



Scheme 74: Spacered and direct methoxyamino-glycosides of glycyrrhetinic acid

The chemistry towards spacered methoxyaminoglycosides, which represent the closest chemically stable amino analogues of the antivirally active ethylthioglucuronides (Scheme 73), was established in a small scope (glucose **76** and glucuronic acid **77**) as a proof-of-concept study. Based on the promising results of the spacered thioglucuronides in the antiviral screening and the given proof-of concept in the direct glycosylation towards general structures **A**, it was decided to roll-out further efforts in this field into a diploma thesis[88], which is currently carried out aiming at small libraries of neoglycosides of structure **A** and structure **B** in order to allow closer insight into the structure activity relationships regarding attached sugars and linker on the antiviral activity of the compounds.

Apart from pharmacological considerations, it is noteworthy that the complete NMR-analysis and unambiguous assignment of triterpene structures was a substantial fraction of the work. Therefore a detailed description of and a recommendation for the systematic and efficient NMR-analysis of GA derivatives is included in the discussion.

The *N*-acylated members of the thioglucuronide family (Scheme 73, right side) showed especially complex NMR-spectra due to the observation of two rotamers. However, the closer analysis by NMR measurements at different temperatures brought forth an “out of text textbook” coalescence-phenomenon for a compound with restricted bond rotation.

3 Experimental Part

3.1 General methods

Melting points (m.p.)

Melting points were determined using a Kofler-type Reichert Thermovar micro hot stage microscope and are uncorrected. Melting points were only determined from crystallized compounds. Compounds described as solids or solid foams were amorphous and no melting points are reported for those compounds.

Optical rotation

Optical rotation was measured with a Perkin–Elmer 243 B polarimeter; $[\alpha]_D^{20}$ values are given in units of deg $dm^{-1}cm^3\ g^{-1}$.

Combustion analysis

Combustion analysis was performed at the micro analytical laboratory of the Faculty of Chemistry (Mag. Johannes Theiner), University of Vienna. The used equipment was a "2400 CHN Elemental Analyzer" from Perkin Elmer or a "EA 1108 CHNS-O" from Carlo Erba (Thermo-Quest).

HPLC-HRMS analysis

HPLC-HRMS analysis was carried out from CH_3CN solutions (concentration: 1mg/L) using an HTC PAL system autosampler (CTC Analytics AG, Zwingen, Switzerland), an Agilent 1100/1200 HPLC with binary pumps, degasser and column thermostat (Agilent Technologies, Waldbronn, Germany) and Agilent 6210 ESI-TOF mass spectrometer (Agilent Technologies, Palo Alto, United States).

The mass spectrometer was previously tuned with the Agilent tune mix and further reference masses were added to the method to provide a mass accuracy below 2 ppm. Data analysis was performed with Mass Hunter software from Agilent Technologies.

Thin layer chromatography (TLC)

Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} precoated glass plates (Merck) or on silica gel 60 F_{254} HPTLC precoated glass plates with 2.5 cm concentration zone (Merck). The substances were visualized by ultraviolet light (254 nm), by dipping into anisaldehyde-H_2SO_4 reagent and subsequent heating or by placement into an iodine-chamber.

HPLC analysis

HPLC analysis was done by injection of 5µL-20µL of 0.1% to 1% solutions (CH_3CN+1drop of water) on a Shimadzu LC-10A system with two gradient pumps, degasser and UV-detector (254nm, 195nm). The three methods reported in this thesis used gradients of CH_3CN (0.1% HCOOH) as mobile **A** and water (0.1% HCOOH) as mobile **B** with 0.1% HCOOH as modifyer on an Atlantis dC_{18}-3µm precolumn (3.9×20mm) as stationary phase:

Method A: Flow: 2mL/min (0-5min)

Gradient: 0-0.3min: 5% **B**, 0.3-2.3min: 5-90% **B**, 2.3-3.3min: 90-95% **B**, 3.3-4.3min 95% **B**

Method B: Flow: 2mL/min (0-10min)

Gradient: 0-0.3min: 5% **B**, 0.3-7.3min: 5-90% **B**, 7.3-8.3min: 90-95% **B**, 8.3-9.3min 95% **B**

Method C: Flow: 1mL/min (0-1min), 1.5mL/min (1-12 min)

Gradient: 0-1min: 40% **B**, 1-8min: 40-55% **B** , 8-8.5min: 55-95%

Nuclear magnetic resonance spectra (NMR)

NMR spectra were recorded at 27°C (unless otherwise stated) with a Bruker DPX 300 spectrometer (^1H at 300.13 MHz, ^{13}C at 75.47 MHz) and with a Bruker DPX 400 spectrometer (^1H at 400.13 MHz, ^{13}C at 100.61 MHz) using standard Bruker NMR software. ^1H-NMR spectra were referenced to tetramethylsilane, ^{13}C-NMR spectra were referenced to $CDCl_3$ (δ 77.00) or CD_3OD (49.00). In case mixtures of solvents had to be used the excess solvent was used. Coupling constants are reported in Hz and chemical shifts (δ) in ppm using TMS as internal standard.

3.2 Reagents and solvents

Unless otherwise noted, chemicals and solvents were purchased from commercial suppliers and used without further purification. Ion exchange treatment was performed on Dowex 50 WX8 resin, H+ form, 50-100 mesh, which was washed with the respective solvent prior to use. Water free solvents were distilled from an adequate desiccant and stored under Ar. Et_2O, THF and dioxane were distilled from sodium/benzophenone, DCM from CaH_2 prior to use. Other solvents were stored over molecular sieve for at least one day.

3.3 Numbering of the compounds in nomenclature and NMR

In the triterpenes literature two slightly different approaches are found to number the positions of substituents along the ring system. On the one hand the substituent with the higher priority according to systematic IUPAC-nomenclature can be assigned the lower number; on the other hand, the substituent below the plane of the ring-system gets the lower number and the substituent above-plane the higher number (C23-C24). Throughout this thesis the latter approach was chosen and the carboxylic acid is referred to as C30 while the adjacent methyl group is referred to as C29 (H29).

To allow uniform description of the corresponding positions along the course of the synthesis in all compounds carrying a triterpene scaffold the positions were labelled analogous to the following example.

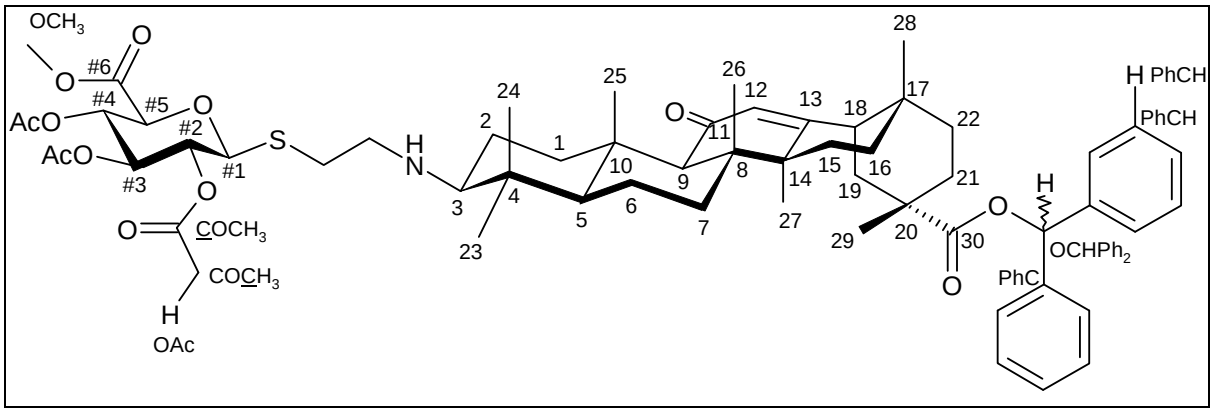
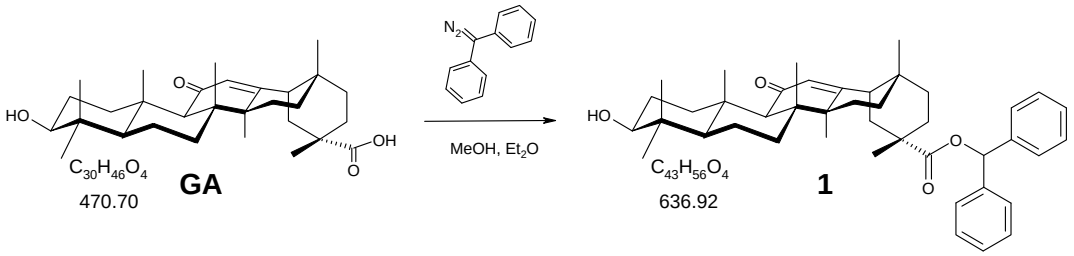


Figure 26: Consistent assignment of positions within this thesis

Carbon signals were assigned as singlet (s) for quaternary carbons, doublet (d) for CH, triplet (t) for CH_2 and quartet (q) for CH_3 groups respectively. This traditional assignment based on the appearance in CH-coupled ^{13}C-spectra was chosen to deliver additional information although the information was derived from APT-phase, shift and HSQC-correlation signals and is therefore kind of an artificial one. In proton NMR the singlets from methyl groups were always indicated as singlets even if they were not completely resolved from multiplets (hardly ever the case). However, the documentation of the exact shifts of the methyl groups was regarded important for the identification of the specific compound by the interested reader.

3.4 Synthetic procedures and analytical data

3.4.1 (*3S*, *18R*, *20S*)-3-Hydroxy-11-oxo-olean-12-en-30-oic acid, diphenylmethyl ester [1]



Procedure: Formation of diphenyldiazomethane

Freshly distilled oxalyl chloride (0.94mL, 10.9mmol, 1.37equiv.) was added in a dropwise manner to a stirred solution of DMSO (0.894g, 11.4mmol, 1.43equiv.) in dry THF (104mL) at -55 °C under a nitrogen atmosphere. The reaction was maintained at -55°C until gas evolution ceased (~20 min). The reaction mixture was cooled to -78 C and a solution of benzophenone hydrazone (2.04 g, 10.4mmol, 1.3equiv.) and Et_3N (3.04mL, 21.8mmol, 2.73equiv.) in THF (10mL) was added dropwise. The resulting pink-purple solution was maintained at -78 °C for 1 h and was then directly filtered with Ar-pressure in a a closed filter vessel. The residue was washed twice with dry THF (20mL) and the filtrate was evaporated. Diphenyldiazomethane was obtained as red oil and used directly for the diphenylmethyl ester formation.

Procedure: Formation of diphenylmethyl ester

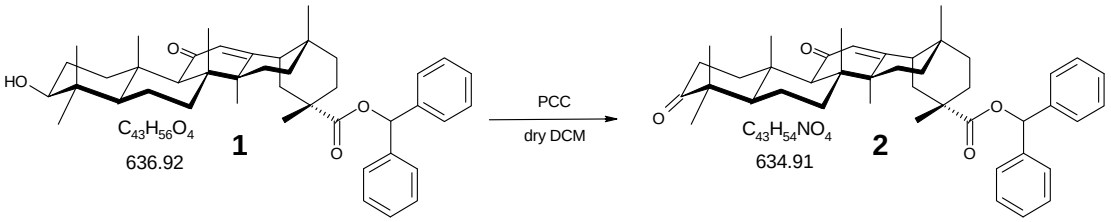
Glycyrrhetinic acid was dissolved in dry MeOH (40mL) and heated to 40°C. A solution of diphenyldiazomethane in Et_2O (32mL), which was filtered through little cotton (to remove a substantial amount of white solid; TEA.HCl), was added dropwise at this temperature within 1h. The reaction mixture was checked for starting materials by TLC (DCM:MeOH 20:1) and was stirred overnight. The target compound was purified by column chromatography (SiO_2, Hex:EtOAc 7:1 to 4:1) and trituration with Et_2O to give target compound as white solid with spectral data consistent with the literature[84].

Yield: 3.17g (62.2%) as white solid

Physical properties

R_f-value = 0.25 (Hex:EtOAc 3:1)

3.4.2 (*18R*, *20S*)-3,11-Dioxo-olean-12-en-30-oic acid, diphenylmethyl ester [2]



Procedure: PCC oxidation

PCC (10.8 g, 50.3mmol, 1.50equiv.) was added to a stirred solution of alcohol **1** (21.34g, 33.5mmol, 1.00equiv.) in DCM (600mL) and the reaction mixture was stirred at reflux under TLC monitoring (Hex:EtOAc 3:1). Upon complete conversion (2.5h) the reaction mixture was cooled to rt within 30min and filtered through a bed of dry SiO_2 (350 g). Fractioned elution with Et_2O (500mL) and evaporation of appropriate fractions gave pure target compound as white solid foam in a yield of 21.1 g (99.3%) after drying *in vacuo*. An analytical sample was prepared by recrystallization from Et_2O.

Yield: 21.1g (99.3%) as white solid foam

Physical properties

R_f-value = 0.35 (Hex:EtOAc 3:1)

m.p. =172-174 °C (Et_2O)

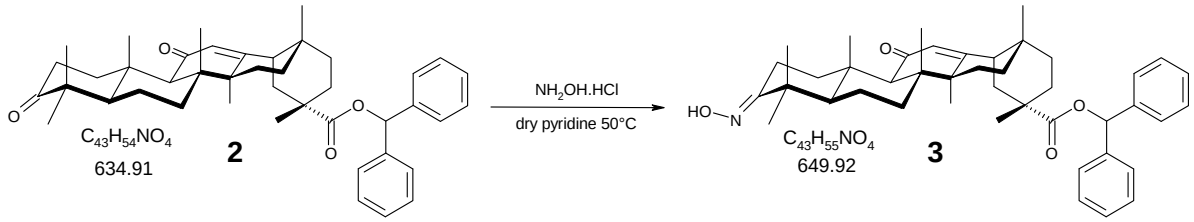
Optical rotation: $[\alpha]_D^{20}$ = +142.0 (c=1.0 in $CHCl_3$)

HRMS: calcd. [M+1]: 635.4095
found [M+1]: 635.4113, dev.: -2.83ppm

^{1}H-NMR ($CDCl_3$) δ 0.68 (s, 3H, H28), 0.95-1.04 (m, 1H, H15b), 1.07 (s, 3H, H23/24), 1.10 (s, 3H, H23/24), 1.13 (s, 3H, H26), 1.15-1.24 (m, 4H, H16b), 1.17 (s, 3H, H29), 1.24-1.38 (m, 4H, H5, H21b, H22b, H22a), 1.27 (s, 3H, H25), 1.37 (s, 3H, H27), 1.40-1.49 (m, 2H, H1b, H6b), 1.49-1.60 (m, 2H, H7b, H7a), 1.60-1.75 (m, 2H, H6a, H19b), 1.74-1.89 (td, 1H, H15a), 1.94-2.13 (m, 4H, H16a, H18, H19a, H21a), 2.30-2.41 (m, 1H, H2b), 2.42 (s, 1H, H9), 2.56-2.70 (m, 1H, H2a), 2.91-3.02 (m, 1H, H1a), 5.55 (s, 1H, H12), 6.93 (s, 1H, $OCHPh_2$), 7.24-7.42 (m, 10H, 10×PhCH)

^{13}C-NMR ($CDCl_3$) δ 15.6 (q, C25), 18.5 (q, C26), 18.8 (t, C7), 21.4 (q, C23/24), 23.3 (q, C27), 26.36 (t, C15/16), 26.39 (q, C23/24), 26.5 (t, C15/16), 28.2 (q, C29), 28.3 (q, C28), 31.2 (t, C21), 31.8 (s, C17), 32.1 (t, C6), 34.2 (t, C2), 36.7 (s, C10), 37.5 (t, C22), 39.8 (t, C1), 41.2 (t, C19), 43.3 (s, C20), 44.0 (s, C8), 45.2 (s, C14), 47.8 (s, C4), 48.1 (d, C18), 55.4 (d, C5), 61.0 (d, C9), 76.6 (d, $OCHPh_2$), 127.0 (d, PhCH), 127.3 (d, PhCH), 127.8 (d, PhCH), 128.1 (d, PhCH), 128.41 (d, C12), 128.46 (d, PhCH), 128.6 (d, PhCH), 140.06 (s, PhC), 140.12 (s, PhC), 169.3 (s, C13), 175.1 (s, C30), 199.3 (s, C11), 217.1 (s, C3)

3.4.3 (*18R*, *20S*)-3-(Hydroxyimino)-11-oxo-olean-12-en-30-oic acid, diphenylmethyl ester [3]



Procedure: Oxime formation

A solution of ketone **2** (3.98g, 6.23mmol, 1.00equiv.) and hydroxylamine hydrochloride (2.18g, 31.34mmol, 5.00equiv.) in dry pyridine (30mL) was stirred for 2h at 50°C under TLC (Hex:EtOAc 3:1) monitoring. Upon complete conversion (2h) the reaction mixture was allowed to cool to rt, diluted with DCM (200mL) and washed with 2N HCl (3x 100mL), water and brine and dried over Na_2SO_4 and was evaporated to give the target compound as white solid after grinding and drying *in vacuo*.

Yield: 3.98g (97.7%) as white solid

Physical properties

R_f-value = 0.24 (Hex:EtOAc 3:1)

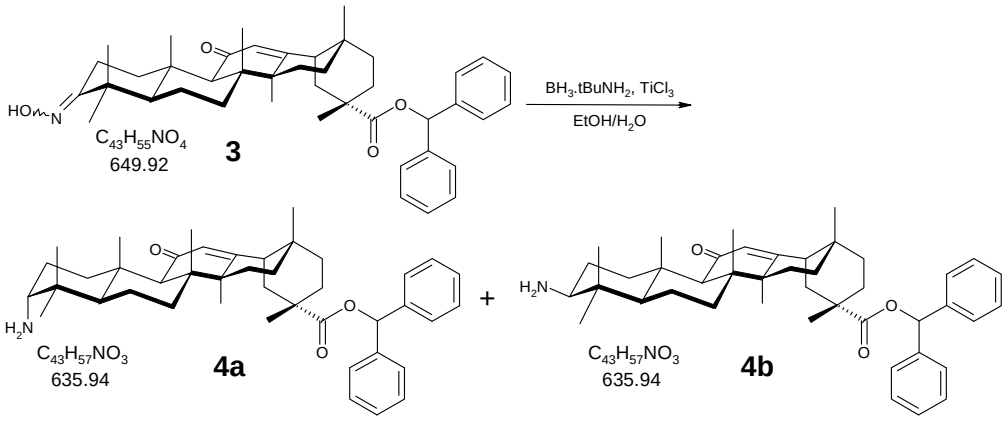
Optical rotation: $[\alpha]_D^{20}$ =+85.2 (c=1.0 in $CHCl_3$)

HRMS: calcd. [M+1]: 650.4204
found [M+1]: 650.4206, dev.: -0.25ppm

^{1}H-NMR ($CDCl_3$) δ 0.67 (s, 3H, H28), 0.93-1.07 (m, 2H, H5, H15b), 1.07-1.14 (m, 1H, H1b), 1.08 (s, 3H, H23/24), 1.12 (s, 3H, H26), 1.14-1.22 (m, 1H, H16b), 1.17 (2xs, 6H, H23/24, H29), 1.22-1.42 (m, 3H, H21b, H22b, H22a), 1.25 (s, 3H, H25), 1.34 (s, 3H, H27), 1.38-1.57 (m, 2H, H6b, H7b), 1.57-1.73 (m, 3H, H6a, H7a, H19b), 1.73-1.89 (m, 1H, H15a), 1.93-2.14 (m, 4H, H16a, H18, H19a, H21a), 2.20-2.34 (m, 1H, H2b), 2.36 (s, 1H, H9), 2.82-2.94 (m, 1H, H1a), 3.00-3.13 (m, 1H, H2a), 5.53 (s, 1H, C12), 6.93 (s, 1H, $OCHPh_2$), 7.23-7.43 (m, 10H, 10×PhCH)

^{13}C-NMR ($CDCl_3$) δ 15.7 (q, C25), 17.1 (t, C2), 18.2 (t, C7), 18.6 (q, C26), 23.24 (q, C27), 23.27 (q, C23/24), 26.36 (t, C16), 26.43 (t, C15), 27.1 (q, C23/24), 28.2 (q, C29), 28.3 (q, C28), 31.2 (t, C21), 31.7 (s, C17), 32.4 (t, C6), 37.0 (s, C10), 37.5 (t, C22), 39.1 (t, C1), 40.4 (s, C4), 41.1 (t, C19), 43.2 (s, C20), 44.0 (s, C8), 45.3 (s, C14), 48.1 (d, C18), 55.6 (d, C5), 61.3 (d, C9), 76.6 (d, $OCHPh_2$), 127.0 (d, PhCH), 127.3 (d, PhCH), 127.8 (d, PhCH), 128.1 (d, PhCH), 128.4 (d, C12, PhCH), 128.6 (d, PhCH), 140.1 (s, PhC), 140.12 (s, PhC), 166.9 (s, C3), 169.0 (s, C13), 175.2 (s, C30), 199.6 (s, C11)

3.4.4 (*3R*, *18R*, *20S*)-3-Amino-11-oxo-olean-12-en-30-oic acid, diphenylmethyl ester [4a] and (*3S*, *18R*, *20S*)-3-Amino-11-oxo-olean-12-en-30-oic acid, diphenylmethyl ester [4b]



Procedure: Oxime reduction with BH_3.*t*-$BuNH_2$/$TiCl_3$

NaOAc (12.35g, 150.48mmol, 16.3equiv.) was added portionwise to a $TiCl_3$ solution (12% in 5-10% HCl, 47.6g, 36.9mmol, ~ 4equiv.) and was allowed standing until a clear solution was obtained. The solution was stripped with argon. This solution was added dropwise over a period of 30min to a solution of oxime **3** (6.00g, 9.23mmol, 1.00equiv.) and BH_3.*t*$BuNH_2$

(2.00g, 23.08mmol, 2.5equiv.) in dry EtOH at -9°C, which had been stripped with argon for approximately 15min. During addition the solution turned to a dark blue to black suspension. After 1.5h a small sample was worked up and analyzed by TLC indicating complete conversion of starting material to the target compound. First satd. NH_4Cl (600mL) and then DCM (600mL) were added at rt, the phases were separated, the aqueous layer was extracted three times with DCM, the combined organic layers were washed with satd. NH_4Cl, satd. $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated. The crude material was purified by column chromatography (SiO_2; 680g, $CHCl_3$:MeOH 8:1 + 0.1% AcOH to $CHCl_3$:MeOH 4:1 + 0.1% AcOH to give the two diastereomeric amines in pure form together with minor amounts of mixed fractions.

All identical amine-containing fractions were pooled and treated with satd. $NaHCO_3$, brine, dried over Na_2SO_4 and evaporated to give pure α-amine (980mg, 16.7%) as white solid foam and pure β-amine (3.40g, 57.9%) as white solid foam.

Yield (α-amine): 980mg, (16.7%) as white solid foam

Yield (β-amine): 3.40g, (57.9%) as white solid foam

Physical properties (α-amine)

R_f-value = 0.33 ($CHCl_3$:MeOH 9:1 + 0.1% AcOH)

Optical rotation: $[\alpha]_D^{20}$ = +111.6 (c = 1.0 in $CHCl_3$)

^{1}H-NMR ($CDCl_3$): δ 0.67 (s, 3H, H28), 0.87-0.93 (m, 6H, H23, H24), 0.93-1.02 (m, 1H, H2b), 1.09 (s, 3H, H26), 1.15 (s, 3H, H25), 1.12-1.16 (m, 1H, H15b), 1.17 (s, 3H, H29), 1.21-1.27 (m, 1H, H3), 1.39 (s, 3H, H27), 1.27-1.41 (m, 6H, H1b, H6b, H16b, H21b, H22b, H22a), 1.41-1.52 (m, 2H, H7b, H7a), 1.53-1.77 (m, 3H, H1a, H16a, H19b), 1.76-1.86 (m, 1H, H15a), 1.93-2.15 (m, 4H, H2a, H18, H19a, H21a), 2.46 (s, 1H, H9), 2.47-2.56 (m, 1H, H6a), 2.62-2.96 (m, 1H, H5), 5.52 (s, 1H, H12), 6.93 (s, 1H, $OCHPh_2$), 7.22-7.43 (m, 10H, 10xPhCH)

^{13}C-NMR ($CDCl_3$): δ 16.5 (q, C25), 17.4 (t, C7), 18.7 (q, C26), 23.5 (q, C23/24), 23.6 (q, C27), 25.6 (t, C16), 26.4 (2xt, C2, C15), 28.3 (2xq, C28, C29), 28.8 (q, C23/24), 31.2 (t, C21), 31.7 (s, C17), 32.7 (t, C6), 33.6 (t, C1), 36.8 (s, C4), 37.4 (s, C10), 37.5 (t, C22), 41.1 (t, C19), 43.2 (s, C20), 44.0 (s, C8), 45.5 (s, C14), 48.0 (2xd, C3, C18), 56.2 (d, C5), 61.8 (d, C9), 76.6 (d, $OCHPh_2$), 127.0 (d, PhCH), 127.3 (d, PhCH), 127.8 (d, PhCH), 128.1 (d, PhCH), 128.4 (d, PhCH), 128.58 (d, C12), 128.63 (d, PhCH), 140.10 (s, PhC), 140.13 (s, PhC), 168.8 (s, C13), 175.2 (s, C30), 200.3 (s, C11)

Physical properties (β-amine)

R_f-value = 0.23 ($CHCl_3$:MeOH 9:1 + 0.1% AcOH)

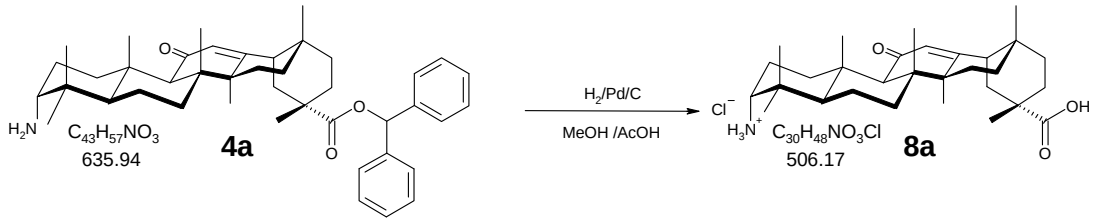
Optical rotation: $[\alpha]_D^{20}$ = +129.8 (c = 1.0, $CHCl_3$)

HRMS: calcd. [M+1]: 636.4411
found [M+1]: 636.4423, dev.: -1.91ppm

^{1}H-NMR ($CDCl_3$): δ 0.66 (s, 3H, H28), 0.69-0.77 (m, 2H, H5, H16b), 0.84 (s, 3H, H23/24), 0.91-1.07 (m, 3H, H1b, H7b, H16a), 1.04 (s, 3H, H23/24), 1.09 (s, 3H, H26), 1.13 (s, 3H, H25), 1.17 (s, 3H, H29), 1.22-1.53 (m, 4H, H6b, H21b, H22b, H22a), 1.36 (s, 3H, H27), 1.55-1.73 (m, 5H, H2b, H2a, H6a, H7a, C19b), 1.73-1.87 (m, 1H, H15b), 1.92-2.10 (m, 4H, H15a, H18, H19a, H21a), 2.33 (s, 1H, H9), 2.50-2.59 (m, 1H, H3), 2.75-2.86 (m, 1H, H1a), 5.51 (s, 1H, H12), 6.93 (s, 1H, $OCHPh_2$), 7.22-7.42 (m, 10H, 10×PhCH)

^{13}C-NMR ($CDCl_3$): δ 16.05 (q, C25), 16.13 (q, C23/24), 17.7 (t, C7), 18.7 (q, C26), 23.3 (q, C27), 26.4 (3xt, C2, C15, C16), 28.2 (2xq, C28, C29), 28.5 (q, C23/C24), 31.2 (t, C21), 31.7 (s, C17), 32.7 (t, C6), 37.2 (s, C10), 37.5 (t, C22), 38.0 (s, C4), 39.6 (t, C1), 41.1 (t, C19), 43.2 (s, C20), 44.0 (s, C8), 45.2 (s, C14), 48.0 (d, C18), 55.4 (d, C5), 60.0 (d, C3), 61.7 (d, C9), 76.6 (d, $OCHPh_2$), 127.0 (d, PhCH), 127.3 (d, PhCH), 127.8 (d, PhCH), 128.1 (d, PhCH), 128.4 (d, PhCH), 128.5 (d, C12), 128.6 (d, PhCH), 140.07 (s, Dpm), 140.11 (s, Dpm), 168.7 (s, C13), 175.2 (s, C30), 199.9 (s, C11)

3.4.5 (*3R*, *18R*, *20S*)-3-Amino-11-oxo-olean-12-en-30-oic acid hydrochloride [8a]



Procedure: Hydrogenolysis

Ester **4a** (200mg, 0.314mmol, 1.00equiv.) was dissolved in MeOH/AcOH 100:1 and the reaction mixture was evacuated and purged with argon three times, before Pd/C was added. The atmosphere was exchanged to H_2 and the reaction mixture was stirred at rt overnight.
Upon complete conversion ($CHCl_3$:MeOH 3:1 + 0.5% AcOH) the atmosphere was exchanged to argon and the mixture was filtered over a short bed of Celite®, washed with MeOH and the filtrate was concentrated until first precipitation. HCl in Et_2O (1M, 0.6mmol) was added dropwise to the solution and precipitation was completed by addition of Et_2O (10mL). The suspension was stirred for several hours before the target compound was collected by filtration and washed excessively with Et_2O to provide the target compound in pure form.

Yield: 115mg (72.2%) as white solid

Physical properties

R_f-value = 0.12 ($CHCl_3$:MeOH 9:1 + 0.5% AcOH)

m.p.: >350°C (MeOH/Et_2O)

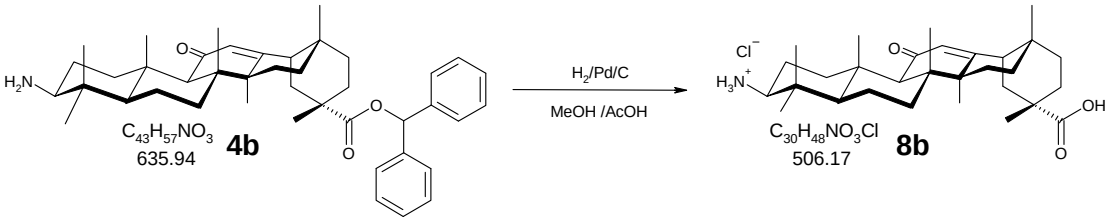
Optical rotation: $[\alpha]_D^{20}$ = +152.4° (c = 1.0 in MeOH)

HRMS: calcd. [M+1]: 470.3629
found [M+1]: 470.3625 dev.: 0.88ppm

^{1}H-NMR (DMSO): δ 0.78 (s, 3H, H28), 0.89 (s, 3H, H23/24), 0.91 (s, 3H, H23/24), 0.93-1.03 (m, 1H, H15b), 1.03 (s, 3H, H26), 1.05 (s, 3H, H25), 1.08 (s, 3H, H29), 1.15-1.50 (m, 9H, H1b, H5, H6a, H6b, H7b, H16b, H21b, H22a, H22b), 1.4 (s, 3H, H27), 1.55-1.85 (m, 6H, H2b, H7a, H16a, H19a, H19b, H21a), 1.95-2.15 (m, 3H, H2a, H15a, H18), 2.41-2.53 (m, 1H, H1a), 2.61 (s, 1H, H9), 2.94 (bs, 1H, H3), 5.45 (s, 1H, H12)

^{13}C-NMR (DMSO): δ 16.5 (q, C25), 17.3 (t, C6), 18.7 (q, C26), 21.6 (t, C2), 22.8 (q, C23/24), 23.9 (q, C27), 26.2, 26.5 (2×t, C15, C16), 28.20, 28.28 (2×q, C23/24, C29), 28.8 (q, C28), 30.8 (t, C21), 31.9 (s, C17), 32.1 (t, C7), 32.5 (t, C1), 35.4 (s, C8), 36.9 (s, C10), 37.9 (t, C22), 41.1 (t, C19), 43.5 (s, C20), 43.6 (s, C8), 45.4 (s, C14), 46.7 (d, C5), 48.5 (d, C18). 56.9 (d, C3), 60.6 (d, C9), 127.5 (d, C12), 170.5 (s, C13), 178.0 (s, C30), 199.6 (s, C11)

3.4.6 (*3S*, *18R*, *20S*)-3-Amino-11-oxo-olean-12-en-30-oic acid hydrochloride [8b]



Procedure: Hydrogenolyis

Ester **4b** (467mg, 0.734mmol, 1.00equiv.) was dissolved in MeOH/AcOH 100:1 (8mL) and the reaction mixture was evacuated and purged with argon three times. Then Pd/C (100mg) was added, the reaction atmosphere was exchanged to H_2.The suspension was stirred at rt under TLC monitoring ($CHCl_3$:MeOH 9:1 + 0.5% AcOH). Upon complete conversion (2.5h), the reaction atmosphere was exchanged to argon, 2N HCl (2equiv.) was added and the reaction mixture was stirred for 30 min, filtered over a bed of Celite® and evaporated. The crude material was purified by column chromatography (SiO_2, $CHCl_3$: MeOH 10:1 + 0.1% AcOH). The target compound containing fractions were concentrated, HCl in Et_2O (1M, 1.4mmol) was added dropwise to the solution and precipitation was completed by addition of Et_2O. The suspension was stirred for several hours before the target compound was collected by filtration and washing with Et_2O in pure form.

Yield: 307mg (82.6%) as white solid

Physical properties

[CAS-73047-74-2]

R_f-value = 0.07 ($CHCl_3$:MeOH 9:1 + 0.1% AcOH)

m.p.: 287-290°C (toluene, MeOH)

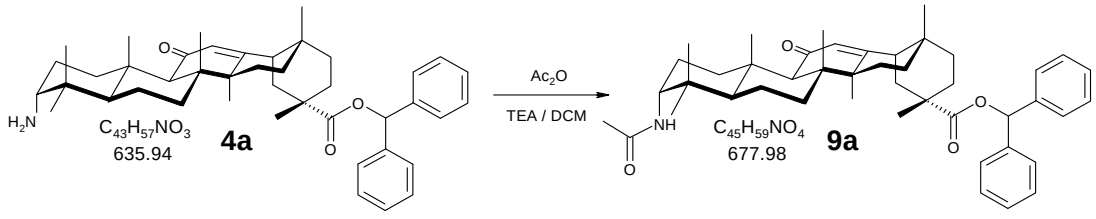
Optical rotation: $[\alpha]_D^{20}$ = +125.9 (c = 1.0, MeOH:$CHCl_3$ 1:1)

HRMS: calcd. [M+1]: 470.3629
found [M+1]: 470.3625 dev.: 0.86ppm

^{1}H-NMR (MeOD): δ 0.84 (s, 3H, H28), 0.91 (s, 3H, H23/24), 0.90-0.99 (m, 1H, H5), 1.00-1.20 (m, 3H, H1b, H15b, H16b), 1.09 (s, 3H, H23/24), 1.12-1.18 (bs, 9H, H25, H26, H29), 1.30-1.60 (m, 5H, H6b, H7b, H21b, H22a, H22b), 1.60-2.02 (m, 8H, H2a, H2b, H6a, H7a, H15a, H19a, H19b, H21a), 2.08-2.30 (m, 2H, H16a, H18), 2.52 (s, 1H, H9), 2.76-2.89 (m, 1H, H1a), 2.90-3.00 (m, 1H, H3), 5.59 (s, 1H, H12)

^{13}C-NMR (MeOD): δ 17.33, 17.43 (2×q, C23/24, C25), 19.3 (t, C6), 20.1 (q, C26), 24.7 (q, C27), 25.0 (t, C2), 28.19, 28.42 (2×t, C15, C16), 29.2, 29.6, 30.1 (3×q, C23/24, C28, C29), 32.8 (t, C21), 33.8 (s, C17), 34.4 (t, C7), 38.6 (s, C4), 39.0 (s, C10), 39.9 (t, C22), 40.3 (t, C1), 43.2 (t, C19), 45.5, 45.8, 47.5 (3×s, C8, C14, C20), 50.8 (d, C18), 56.7 (d, C5), 62.1 (d, C3), 63.5 (d, C9), 129.7 (d, C12), 174.0 (s, C13), 181.2 (s, C30), 203.0 (s, C11)

3.4.7 (*3R*, *18R*, *20S*)-3-(Acetylamino)-11-oxo-olean-12-en-30-oic acid, diphenylmethyl ester [9a]



Procedure: *N*-Acetylation

To a solution of amine **4a** (500mg, 0.79mmol, 1.00equiv.) in dry DCM (10mL) TEA (0.76mL, 5.5mmol, 7.00equiv.) followed by Ac_2O (0.370mL, 3.93mmol, 5.00equiv.) were added at 0°C. The reaction mixture was stirred at 0°C monitored by TLC (Hex:EtOAc 1:2,

$CHCl_3$:MeOH 9:1 + 0.5% AcOH). After 1h the reaction mixture was diluted with EtOAc, washed twice with diluted HCl, with satd. $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated to give a crude material which was purified by column chromatography (SiO_2: 60g, Hex:EtOAc 1:3) to give pure target compound as white solid.

Yield: 509mg (95.5%) as white solid

Physical properties

R_f-value = 0.20 (Hex:EtOAc 1:2)

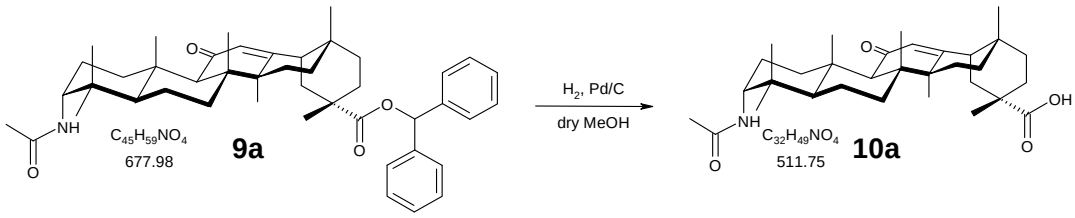
Optical rotation: $[\alpha]_D^{20}$ = +99.0 (c = 1.0 in $CHCl_3$)

HRMS: calcd. [M+1]: 678.4517;
found [M+1]: 678.4523; dev.: -0.83ppm

^{1}H-NMR ($CDCl_3$): δ 0.68 (s, 3H, H28), 0.72-0.79 (m, 1H, H5), 0.88 (s, 3H, H23/24), 0.94-0.99 (m, 4H, H1b, H23/24), 0.99-1.04 (m, 1H, H16b), 1.10 (s, 3H, H26), 1.12-1.22 (m, 7H, H15b, H25, H29), 1.23-1.26 (m, 1H, H22b), 1.28-1.38 (m, 3H, H2b, H22a, H21b), 1.38-1.44 (m, 2H, H2a, H6b), 1.41 (s, 3H, H27), 1.45-1.57 (m, 2H, H7a, H7b), 1.59-1.73 (m, 2H, H6a, H19b), 1.74-1.89 (m, 1H, 15a), 1.95-2.12 (m, 4H, H16a, H18, H19a, H21a), 2.03 (s, 3H, NHAc), 2.38 (s, 1H, H9), 2.66-2.77 (m, 1H, H1a), 3.82-3.89 (m, 1H, H3), 5.51 (s, 1H, H12), 6.93 (s, 1H, $OCHPh_2$), 7.24-7.42 (m, 10H, 10×PhCH)

^{13}C-NMR ($CDCl_3$): δ 16.4 (q, C25), 17.3 (t, C7), 18.7 (q, C26), 13.1 (q, C23/24), 23.2 (t, C2), 23.6 (q, C27), 23.8 (q, NHCO**C**H_3), 26.4 (t, C16), 26.4 (t, C15), 28.2 (q, C29), 28.3 (q, C28), 28.4 (q, C23/24), 31.2 (t, C21), 31.7 (s, C17), 32.6 (t, C6), 35.0 (t, C1), 36.6 (s, C4), 37.3 (s, C10), 37.5 (t, C22), 41.2 (t, C19), 43.2 (s, C20), 44.0 (s, C8), 45.5 (s, C14), 48.0 (d, C18), 51.2 (d, C5), 53.6 (d, C3), 61.8 (d, C9), 76.6 (d, $OCHPh_2$), 127.0 (d, PhCH), 127.2 (d, PhCH), 127.9 (d, PhCH), 128.1 (d, PhCH), 128.5 (d, PhCH, C12), 128.6 (d, PhCH), 140.07 (s, PhC), 140.11 (s, Dpm), 169.0 (s, NH**C**OCH_3), 169.2 (s, C13), 175.2 (s, C30), 199.7 (s, C11)

3.4.8 (*3R*, *18R*, *20S*)-3-(Acetylamino)-11-oxo-olean-12-en-30-oic acid [10a]



Procedure: Hydrogenolyis

Diphenylmethyl ester **9a** (170mg, 0.251mmol, 1.00equiv.) was dissolved in MeOH:EtOAc:AcOH 50:50:1 (6mL) The atmosphere was exchanged to argon, before Pd/C (17mg, 10%) was added. Then the atmosphere was exchanged to H_2 and the reaction was stirred at rt monitored by TLC (Hex:EtOAc 1:2). Upon complete conversion (17h) the reaction atmosphere was exchanged to argon, the reaction mixture was filtered over Celite® and washed with MeOH. The solvents were evaporated and the crude material was purified by trituration with hot EtOAc to give pure target compound as white solid.

Yield: 105mg (81.8%) as white solid

Physical properties

R_f-value = 0.27 (DCM:MeOH 8:1)

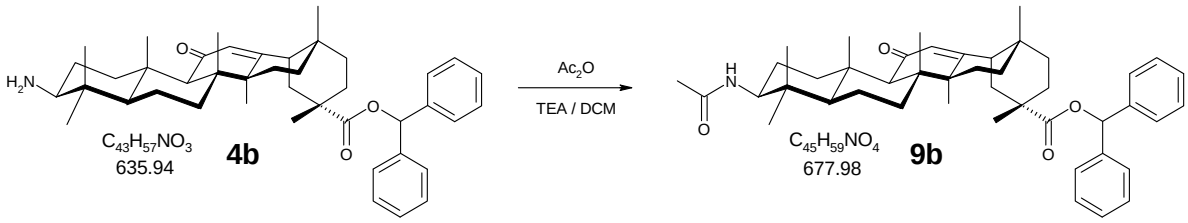
Optical rotation: $[\alpha]_D^{20}$ = +95.0 (c = 1.0 in $CHCl_3$:MeOH)

HRMS: calcd. [M+1]: 512.3734
found [M+1]: 512.3737; dev.: -0.44ppm

^{1}H-NMR ($CDCl_3$): δ 0.84 (s, 3H, H28), 0.86 (s, 3H, H23/24), 0.93-1.29 (m, 4H, H1b, H5, H15b, H16b), 0.98 (s, 3H, H23/24), 1.15 (s, 3H, H26), 1.16 (s, 3H, H25), 1.19 (s, 3H, H29), 1.28-1.59 (m, 8H, H2a, H2b, H6b, H7a, H7b, H21b, H22a, H22b), 1.46 (s, 3H, H27), 1.58-1.76 (m, 2H, H6a, H19b), 1.84-2.15 (m, 4H, H10a, 15a, 19a, 21a), 2.02 (s, 3H, NHAc), 2.15-2.27 (m, 1H, H18), 2.56 (s, 1H, H9), 2.62 (bs, 1H, H1a), 3.79 (s, 1H, H9), 5.66 (s, 1H, H12)

^{13}C-NMR ($CDCl_3$): δ 16.1 (q, C25), 17.0 (t, C7), 18.3 (q, C26), 22.2 (q, NHCO**C**H_3), 22.8 (q, C27), 22.9 (t, C2), 23.0 (q, C23/24), 26.0 (t, C15), 26.1 (t, C16), 27.8 (q, C23/24), 28.1 (q, C29), 28.2 (q, C28), 30.7 (t, C21), 31.5 (s, C17), 32.1 (t, C6), 33.9 (t, C1), 36.0 (s, C4), 36.9 (s, C10), 37.4 (t, C22), 40.9 (t, C19), 43.1 (s, C20), 43.4 (s, C8), 45.4 (s, C14), 48.1 (d, C18), 49.6 (d, C5), 53.6 (d, C3), 61.2 (d, C9), 127.7 (d, C12), 170.6 (s, NH**C**OCH_3), 171.0 (s, C13), 179.2 (s, C30), 201.2 (s, C11)

3.4.9 (*3S*, *18R*, *20S*)-3-(Acetylamino)-11-oxo-olean-12-en-30-oic acid, diphenylmethyl ester [9b]



$C_{43}H_{57}NO_3$ 635.94 **4b** → $C_{45}H_{59}NO_4$ 677.98 **9b**

Procedure: *N*-Acetylation

To a solution of amine **4b** (440mg, 0.692mmol, 1.00equiv.) in dry DCM (10mL) first TEA (0.490 g, 4.8mmol, 7.00equiv.) followed by Ac_2O (350mg, 3.46mmol, 5.00equiv.) were added at 0°C. The reaction mixture was stirred at 0°C monitored by TLC (Hex:EtOAc 1:2, $CHCl_3$:MeOH 9:1 + 0.1% AcOH). After 1h the reaction mixture was diluted with EtOAc, washed twice with diluted HCl, satd. $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated to give a crude material as white solid which was recrystallized from DCM/EtOAc to give white crystals (395mg, 84.2%) of pure target compound.

Yield: 395mg (84.2%) as white crystals

Physical properties

R_f-value = 0.22 (Hex:EtOAc 1:2)

m.p.: 162-165 °C (EtOAc/DCM)

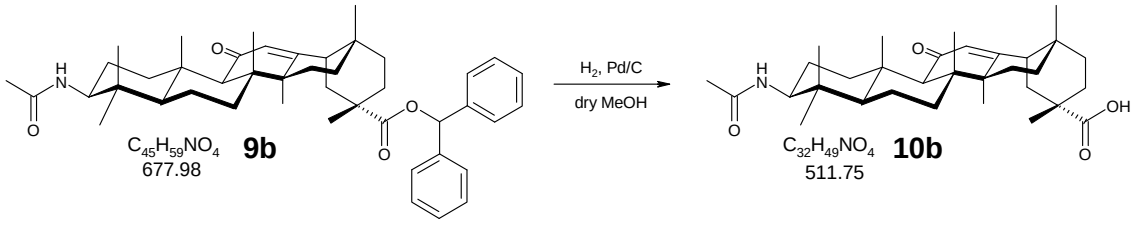
Optical rotation: $[\alpha]_D^{20}$ = +101.2 (c = 1.0 in $CHCl_3$)

HRMS: calcd. [M+1]: 678.4517;
found [M+1]: 678.4522; dev.: -0.81ppm

^{1}H-NMR ($CDCl_3$): δ 0.66 (s, 3H, H28), 0.79 (s, 3H, H23/24), 0.81-0.93 (m, 1H, H5), 0.91 (s, 3H, H23/24), 0.93-1.07 (m, 2H, H7b, H16b), 1.09 (s, 3H, H26), 1.10-1.16 (m, 1H, H1b), 1.13 (s, 3H, H25), 1.17 (s, 3H, H29), 1.22-1.27 (1H, H16a), 1.27-1.34 (m, 3H, H21b, H22b, H22a), 1.36 (s, 3H, H27), 1.38-1.45 (m, 1H, H6b), 1.45-1.56 (2H, H2b, H2a), 1.56-1.60 (m, 1H, H7a), 1.60-1.69 (m, 2H, H6a, H19b), 1.69-1.85 (1H, H15b), 1.63-2.10 (m, 4H, H15a, H18, H19a, H21a), 2.00 (s, 3H, NHAc), 2.36 (s, 1H, H9) 2.73-2.82 (m, 1H, H1a), 3.65-3.77 (1H, H3), 5.50 (s, 1H, H12), 6.93 (s, 1H, $OCHPh_2$), 7.22-7.42 (m, 10H, 10×PhCH)

^{13}C-NMR ($CDCl_3$):δ 16.2 (q, C25), 16.6 (q, C23/24), 17.7 (t, C7), 18.6 (q, C26), 23.2 (q, C27), 23.7 (q, NHCO**C**H_3), 25.4 (t, C2), 26.4 (t, C16), 26.4 (t, C15), 28.2 (q, C29), 28.3 (q, C28), 28.4 (q, C23/24), 31.1 (t, C21), 31.7 (s, C17), 32.7 (t, C6), 36.9 (s, C10), 37.5 (t, C22), 38.0 (s, C4), 39.7 (t, C1), 41.1 (t, C19), 43.2 (s, C20), 44.0 (s, C8), 45.3 (s, C14), 48.1 (d, C18), 55.4 (d, C5), 56.4 (d, C3), 61.7 (d, C9), 76.6 (d, $OCHPh_2$), 126.9 (d, PhCH), 127.2 (d, PhCH), 127.8 (d, PhCH), 128.1 (d, PhCH), 128.2 (d, C12), 128.4 (d, PhCH), 128.6 (d, PhCH), 140.07 (s, PhC), 140.13 (s, PhC), 168.9 (s, NH**C**OCH_3), 169.6 (s, C13), 175.2 (s, C30), 200.0 (s, C11)

3.4.10 (*3S*, *18R*, *20S*)-3-(Acetylamino)-11-oxo-olean-12-en-30-oic acid [10b]



Procedure: Hydrogenolyis

Diphenylmethyl ester **9b** (200mg, 0.295mmol, 1.00equiv.) was dissolved in MeOH:EtOAc:AcOH 50:50:1 (10mL) The atmosphere was exchanged to argon, before Pd/C (20mg, 10%) was added. Then the atmosphere was exchanged to H_2 and the reaction was stirred at rt and monitored by TLC (Hex:EtOAc 1:2). Upon complete conversion the reaction atmosphere was exchanged to argon, the reaction mixture was filtered through a bed of Celite® and washed with MeOH. The solvents were evaporated and the crude material was purified by separation between 0.5N NaOH and Et_2O and trituration with hot EtOAc to give pure target compound as white solid.

Yield: 104mg (68.9%) as white solid

Physical properties

R_f-value = 0.48 (Hex:EtOAc 1:5)

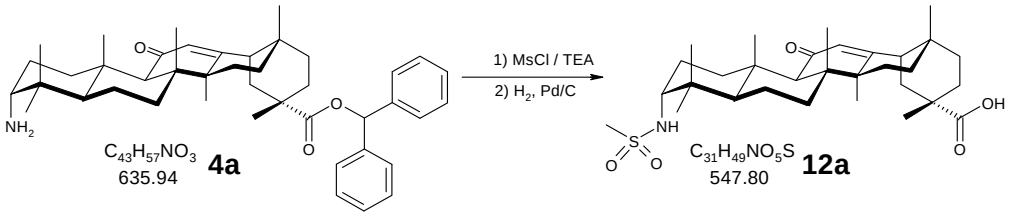
Optical rotation: $[\alpha]_D^{20}$ = +113.0 (c = 1.0 in $CHCl_3$:MeOH 3:1)

HRMS: calcd. [M+1]: 512.3734
found [M+1]: 512.3739; dev.: -0.82ppm

^{1}H-NMR ($CDCl_3$): δ 0.82 (s, 3H, H25), 0.83 (s, 3H, H28), 0.85-0.94 (m, 1H, H5), 0.89 (s, 3H, H23/24), 0.99-1.17 (m, 2H, H1b, H16b), 1.14 (2xs, 6H, H23/24, H26), 1.17-1.28 (m, 1H, H15b), 1.20 (s, 3H, H29), 1.30-1.54 (m, 6H, H2b, H6b, H7b, H21b, H22a, H22b), 1.41 (s, 3H, H27), 1.55-1.74 (m, 4H, H2a, H6a, H7a, H19b), 1.79-2.15 (m, 4H, H15a, H16a, H19a, H21a); 1.99 (s, 3H, NHAc), 2.15-2.26 (m, 1H, H18), 2.44 (s, 1H, H9), 2.69-2.80 (m, 1H, H1a), 3.58-3.70 (m, 1H, H3), 5.65 (s, 1H, H12)

^{13}C-NMR ($CDCl_3$): δ 15.8 (q, C23/24), 16.0 (q, C25), 17.3 (t, C7), 18.2 (q, C26), 22.3 (q, NHCO**C**H_3), 22.8 (q, C27), 24.6 (t, C2), 26.0 (2xt, C15, C16), 27.9-28.1 (3xq, C23/24, C28, C29), 30.6 (t, C21), 31.5 (s, C17), 32.2 (t, C6), 36.6 (s, C10), 37.3 (t, C22), 37.9 (s, C4), 39.4 (t, C1), 40.7 (t, C19), 43.0 (s, C20), 43.3 (s, C8), 45.1 (s, C14), 48.6 (d, C18), 55.0 (d, C5), 56.3 (d, C3), 61.4 (d, C9), 127.6 (d, C12), 170.8 (2xs, NH**C**OCH_3, C13), 179.0 (s, C30), 201.0 (s, C11)

3.4.11 (*3R*, *18R*, *20S*)-3-Methylsulfonylamino-11-oxo-olean-12-en-30-oic acid [12a]



First step: Mesylation of amine 4a

Amine **4a** (250mg, 0.393mmol, 1.00equiv.) was dissolved in dry DCM (8mL), TEA (0.164ml, 1.18mmol, 3.0equiv.) was added and the reaction mixture was chilled to -10°C (EtOH/ice bath). Mesyl chloride (0.52mL, 0.67mmol, 1.7equiv.) was dissolved in dry DCM (<2mL) and was added dropwise to the reaction mixture within 30min using a syringe pump, and checking the consumption of starting material by TLC ($CHCl_3$:MeOH 9:1 + 0.5% AcOH; Hex:EtOAc 2:1 + 0.5% AcOH). Upon complete addition almost complete conversion of the starting amine was detected and the reaction mixture was diluted with DCM (50mL), evaporated onto SiO_2 (700mg) and purified by column chromatography (SiO_2: 30g, Hex:EtOAc 2:1) to give pure target compound **11a** as white solid.

Yield: 208mg (74.0%) as white solid

Second step: Hydrogenolyis

Ester **11a** (187mg, 0.262mmol, 1.00equiv.) was dissolved in MeOH:EtOAc 2:1 + 1% AcOH and the atmosphere was exchanged to argon. Pd/C was added and the atmosphere was exchanged to H_2 and the reaction mixture was stirred at rt for 1h. According to TLC (Hex:EtOAc 3:4) the complete conversion was achieved and the reaction atmosphere was exchanged to argon. The reaction mixture was filtered through a syringe-filter, evaporated onto SiO_2 (650mg) and submitted to column chromatography (SiO_2, 10g, Hex:EtOAc 1:1 + 0.1% AcOH) to give pure target compound **12a**.

Yield: 0.134g (93.7%)

Physical properties (Dpm-ester)

R_f-value = 0.31 (Hex:EtOAc 2:1)

Physical properties (acid)

R_f-value = 0.42, (Hex:EtOAc 3:4 + 0.5% AcOH)

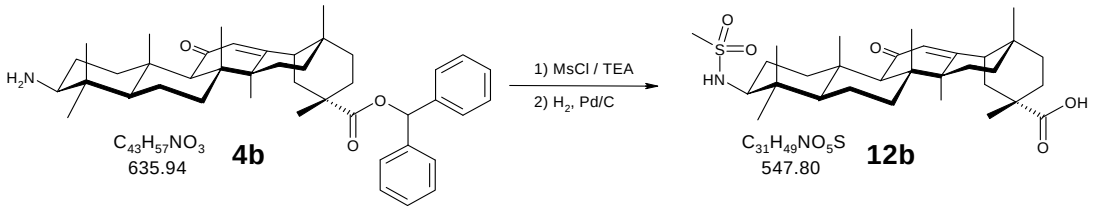
Optical rotation: $[\alpha]_D^{20}$ = +96.3 (c=1.0, MeOH:$CHCl_3$ 5:1)

HRMS: calcd. [M+1]: 548.3404
found [M+1]: 548.3415; dev.: -1.94ppm

^{1}H-NMR ($CDCl_3$): d 0.77-0.83 (m, 1H, H5), 0.80 (s, 3H, H28), 0.97 (s, 3H, H23/24), 0.97-1.05 (m, 1H, H16b), 1.01 (s, 3H, H23/24), 1.10 (s, 3H, H26), 1.10-1.20 (m, 1H, H15b), 1.19, 1.20 (2×s, 2×3H, H25, H29), 1.24-1.45 (m, 6H, H1b, H6b, H7b, H21b, H22a, H22b), 1.40 (s, 3H, H27), 1.41-1.51 (m, 4H, H2b, H6a, H7a, H19b), 1.76-1.86 (m, 2H, H15a, H19a), 1.94-2.06 (m, 4H, H2a, H18, H21a, H16a), 2.50 (s, 1H, H9), 2.72-2.81 (m, 1H, H1a), 2.96 (s, 3H, SO_2CH_3), 3.16-3.23 (m, 1H, H3), 5.45 (s, 1H, H12), 6.21 (d, 10.0Hz, 1H, NH)

^{13}C-NMR ($CDCl_3$): δ 16.4 (q, C25), 17.2 (t, C6), 18.5 (q, C26), 23.1 (q, C23/24), 23.9 (t, C2), 24.4 (q, C27), 26.25, 26.38 (2×t, C15, C16), 28.4 (q, C29), 28.6 (q, C28), 29.4 (q, C23/24), 31.0 (t, C21), 31.9 (s, C17), 32.6 (t, C7), 33.2 (t, C1), 36.8, 37.1 (2×s, C4, C10), 38.1 (t, C22), 41.1 (t, C19), 41.9 (q, SO_2CH_3), 43.3 (s, C20), 43.8 (s, C14), 45.6 (s, C8), 48.1 (d, C18), 49.9 (d, C5), 59.6 (d, C3), 61.2 (d, C9), 128.1 (d, C12), 168.0 (s, C13), 180.4 (s, C30), 198.7 (s, C11)

3.4.12 (*3S*, *18R*, *20S*)-3-Methylsulfonylamino-11-oxo-olean-12-en-30-oic acid [12b]



$C_{43}H_{57}NO_3$ 635.94 **4b** → $C_{31}H_{49}NO_5S$ 547.80 **12b**

First step: Mesylation of amine

Amine **4b** (252mg, 0.396mmol, 1.00equiv.) was dissolved in dry DCM (8mL), TEA (0.165ml, 1.19mmol, 3.00equiv.) was added and the reaction mixture was chilled to -10°C (EtOH/ice bath). Mesyl chloride (0.67mL, 0.859mmol, 2.17equiv.) dissolved in dry DCM (<2mL) was added dropwise to the reaction mixture within 30min using a syringe pump, and checking the consumption of starting material by TLC ($CHCl_3$:MeOH 9:1 + 0.5% AcOH; Hex:EtOAc 2:1 + 0.5% AcOH). Upon complete addition almost complete conversion of the starting material was detected and the reaction mixture was diluted with DCM (50mL), evaporated onto SiO_2 (600mg) and purified by column chromatography (SiO_2: 30g, Hex:EtOAc 2:1 to Hex:EtOAc 3:2) to give the pure target compound **11b** as white solid

Yield: 224mg, (79.2%) as white solid

Second step: Hydrogenolyis

Ester **11b** (196mg, 0.274mmol, 1.00equiv.) was almost completely dissolved in MeOH:EtOAc 2:1 + 1% AcOH (55mL) and the atmosphere was exchanged to argon. Pd/C was added, the atmosphere exchanged to H_2 and the reaction mixture was stirred at rt for 1h. According to TLC (Hex:EtOAc 2:1) complete conversion of starting material was obtained. The reaction mixture was filtered through a syringe-filter, evaporated onto SiO_2 (600mg) and submitted to column chromatography (SiO_2, 23g, Hex:EtOAc 3:4 + 0.1% AcOH) to give pure target compound **12b**.

Yield: 148mg (98.6%) as white solid

Physical properties (Dpm-ester)

R_f-value = 0.12 (Hex:EtOAc 2:1)

Physical properties (acid)

R_f-value = 0.35 (Hex:EtOAc 3:4 + 0.5% AcOH)

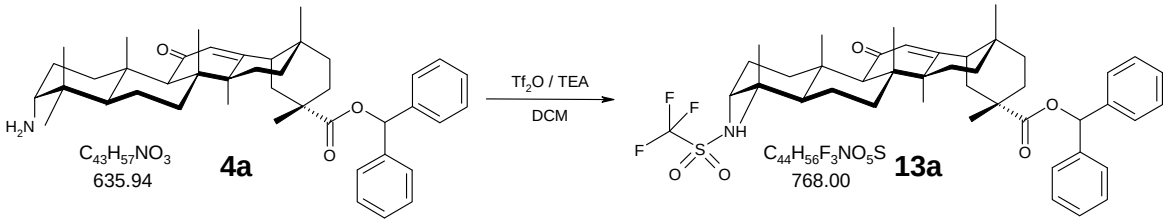
Optical rotation: $[\alpha]_D^{20}$ = +122.6 (c=1.0, MeOH:$CHCl_3$ 5:1)

HRMS: calcd. [M+1]: 548.3404
found [M+1]: 548.3405; dev.: -0.17ppm

^{1}H-NMR ($CDCl_3$:MeOD 5:1): δ 0.80 (s, 3H, H23/24), 0.80-0.87 (m, 1H, H5), 0.83 (s, 3H, H28), 1.00-1.12 (m, 2H, H1b, H16b), 1.04 (s, 3H, H23/24), 1.14 (bs, 6H, H25, H26), 1.19-1.24 (m, 1H, H15b), 1.19 (s, 3H, H29), 1.30-1.54 (m, 5H, H6b, H7b, H21b, H22a, H22b), 1.40 (s, 3H, H27), 1.57-1.82 (m, 5H, H2a, H2b, H6a, H7a, H19b), 1.83-2.03 (m, 3H, H15a, H19a, H21a), 2.00-2.13 (m, 1H, H16a), 2.20 (dd, *J*=13.4Hz, *J*=3.3Hz, 1H, H18), 2.40 (s, 1H, H9), 2.79 (dt, *J*=13.0Hz, *J*=4.0Hz, 1H, H1a), 2.94-3.02 (m, 1H, H3), 2.97 (s, 3H, SO_2CH_3), 5.66 (s, 1H, H12)

^{13}C-NMR ($CDCl_3$:MeOD 5:1): δ 15.8 (q, C25), 15.9 (q, C23/24), 17.7 (t, C6), 18.3 (q, C26), 22.9 (q, C27), 26.04 (t, C15/16), 26.15 (t, C2+C15/16), 28.02, 28.09, 28.15 (3×q, C23/24, C28, C29), 30.7 (t, C21), 31.6 (s, C17), 32.4 (t, C7), 36.6 (s, C10), 37.4 (t, C22), 38.3 (s, C4), 39.6 (t, C1), 40.8 (t, C19), 41.1 (q, SO_2CH_3), 43.1 (s, C14), 43.4 (s, C20), 45.2 (s, C8), 48.1 (d, C18), 55.6 (d, C5), 61.5 (d, C9), 61.9 (d, C3), 127.7 (d, C12), 170.8 (s, C13), 179.0 (s, C30), 200.8 (s, C11)

3.4.13 (*3R*, *18R*, *20S*)-11-Oxo-3-trifluoromethylsulfonylamino-olean-12-en-30-oic acid, diphenylmethyl ester [13a]



Procedure: Triflation of amine

To a solution of amine **4a** (250mg, 0.393mmol, 1.00equiv.) and TEA (119mg, 1.18mmol, 3.00equiv.) in dry DCM (25mL) a precooled solution of Tf_2O (133mg, 0.427mmol, 1.20equiv.) in dry DCM (1mL) was added at -10°C with a syringe pump. Upon complete addition the reaction mixture was stirred at -10°C and monitored by TLC (Hex:EtOAc 2:1, $CHCl_3$:MeOH 9:1 + 0.5% AcOH). Upon complete conversion, the reaction mixture was directly evaporated onto SiO_2 and purified by column chromatography (SiO_2: 40g, Hex:EtOAc 10:1) to give pure target compound **13a** as white solid.

Yield: 243mg (80.5%) as white solid

Physical properties:

R_f-value = 0.51 (Hex:EtOAc 2:1)

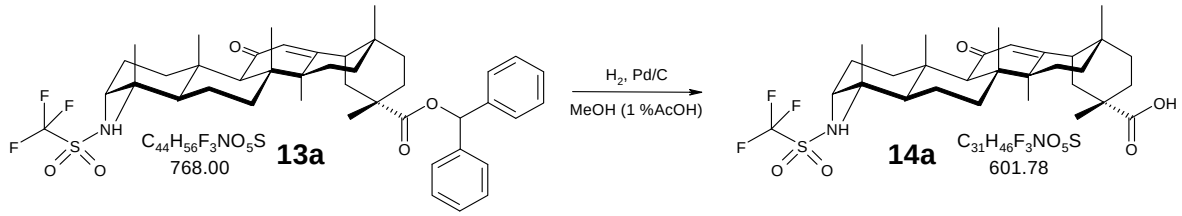
Optical rotation: $[\alpha]_D^{20}$ = +86.8 (c = 1.0 in $CHCl_3$:MeOH 3:1)

HRMS: calcd. [M+1]: 768.3904
found [M+1]: 768.3904

^{1}H-NMR ($CDCl_3$): δ 0.66 (s, 3H, H28), 0.74-0.83 (m, 1H, H5), 0.99 (s, 3H, H23/24), 1.00 (s, 3H, H23/24), 0.92-1.05 (m, 2H, H1b, H16b), 1.09 (s, 3H, H26), 1.15 (s, 3H, H25), 1.18 (s, 3H, H29), 1.12-1.22 (m, 1H, H15b), 1.39 (s, 3H, H27), 1.22-1.45 (m, 5H, H6b, H7b, H21b, H22a, H22b), 1.45-1.85 (m, 5H, H2b, H6a, H7a, H15a, H19b), 1.04-2.21 (m, 5H, H2a, H16a, H18, H19a, H21a), 2.39 (s, 1H, H9), 2.73-2.84 (m, 1H, H1a), 3.32-3.41 (m, 1H, H3), 5.47-5.56 (d, *J*=10.0Hz, NH), 5.27-5.46 (s, 1H, H12), 6.93 (s, 1H, $OCHPh_2$), 7.23-7.42 (m, 10H, 10×PhCH),

^{13}C-NMR ($CDCl_3$): δ 16.5 (q, C25), 17.2 (t, C7), 18.6 (q, C26), 23.0 (q, C23/24), 23.5 (q, C27), 23.8 (t, C2), 26.3 (2×t, C15, C16), 26.3 (t, C15), 28.2 (q, C29), 28.3 (q, C28), 28.9 (q, C23/24), 31.2 (t, C21), 31.7 (s, C17), 32.4 (t, C6), 34.1 (t, C1), 37.0 (s, C10), 37.0 (s, C4), 37.5 (t, C22), 41.1 (t, C19), 43.3 (s, C20), 44.0 (s, C8), 45.4 (s, C14), 48.1 (d, C18), 50.3 (d, C5), 61.5 (d, C9), 61.8 (d, C3), 76.6 (d, $OCHPh_2$), 127.0 (d, PhCH), 127.3 (d, PhCH), 127.8 (d, PhCH), 128.1 (d, PhCH), 128.3 (d, C12), 128.5 (d, PhCH), 128.6 (d, PhCH), 140.1 (s, PhC), 140.1 (s, PhC), 169.4 (s, C13), 175.2 (s, C30), 199.7 (s, C11)

3.4.14 (*3R*, *18R*, *20S*)-11-Oxo-3-trifluoromethylsulfonylamino-olean-12-en-30-oic acid [14a]



Procedure: Hydrogenolyis

Diphenylmethyl ester **13a** (140mg, 1.82mmol, 1.00equiv.) was dissolved in MeOH:AcOH 100:1 (12mL), the atmosphere was exchanged to argon, before Pd/C (14mg, 10%) was added. The atmosphere was exchanged to H_2 and the reaction was stirred at rt and monitored by TLC (Hex:EtOAc 2:1, $CHCl_3$:MeOH 9:1 + 0.5% AcOH). Upon complete conversion the reaction atmosphere was exchanged to argon, the reaction mixture was filtered through hydrophobic filter, washed with MeOH, evaporated and purified by column chromatography (SiO_2: 30g, Hex:EtOAc 3:1 to Hex:EtOAc 2:1 + 0.1% AcOH) to give pure target compound **14a** as white solid. The material was collected from a suspension in Et_2O.

Yield: 101mg (92.1%) as white solid

Physical properties

R_f-value = 0.41 ($CHCl_3$:MeOH 9:1 + 0.5% AcOH)

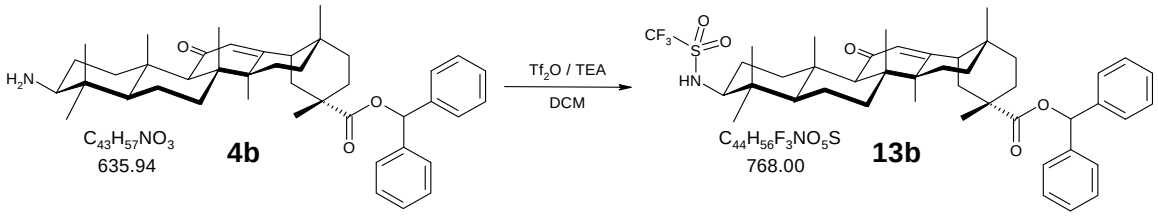
Optical rotation: $[\alpha]_D^{20}$ = +82.0 (c = 1.0 in $CHCl_3$:MeOH 3:1)

HRMS: calcd. [M+1]: 602.3122;
found [M+1]: 602.3121; dev.: 0.16ppm

^{1}H-NMR ($CDCl_3$:MeOD 5:1): δ 0.83 (s, 3H, H28), 0.98 (s, 3H, H23/24), 0.99 (s, 3H, H23/24), 0.95-1.10 (m, 2H, H16b, H5), 1.14 (s, 3H, H26), 1.16 (s, 3H, H25), 1.19 (s, 3H, H29), 1.10-1.28 (m, 2H, H1b, H15b), 1.42 (s, 3H, H27), 1.28-1.47 (m, 5H, H6b, H7b, H21b, H22a, H22b), 1.50-1.75 (m, 4H, H2b, H6a, H7a, H19b), 1.75-1.94 (m, 2H, H15a, H19a), 1.94-2.16 (m, 3H, H2a, H16a, H21a), 2.20 (dd, 1H, *J*=13.6Hz, *J*=3.6Hz, H18), 2.58 (s, 1H, H9), 2.62 (dt, 1H, *J*=14.2Hz, *J*=3.7Hz, H1a), 3.29-3.36 (m, 1H, H3), 5.66 (s, 1H, 12)

^{13}C-NMR ($CDCl_3$:MeOD 5:1): δ 16.3 (q, C25), 17.0 (t, C6), 18.4 (q, C26), 22.7 (q, C23/24), 22.9 (q, C27), 23.9 (t, C2), 26.1 (t, C15), 26.2 (t, C16), 28.1 (q, C29), 28.2 (q, C28), 28.5 (q, C23/24), 30.8 (t, C21), 31.6 (s, C17), 32.2 (t, C7), 33.3 (t, C1), 36.67 (s, C10), 36.74 (s, C4), 37.5 (t, C22), 41.0 (t, C19), 43.2 (s, C20), 43.5 (s, C8), 45.4 (s, C14), 48.2 (d, C18), 48.9 (d, C5), 61.0 (d, C9), 61.4 (d, C3), 127.8 (d, C12), 170.8 (s, C13), 179.1 (s, C30), 201.1 (s, C11)

3.4.15 (*3S*, *18R*, *20S*)-11-Oxo-3-trifluoromethylsulfonylamino-olean-12-en-30-oic acid, diphenylmethyl ester [13b]



Procedure: Triflation of amine 4b

To a solution of amine **4a** (250mg, 0.393mmol, 1.00equiv.) and TEA (119mg, 1.18mmol, 3.00equiv.) in dry DCM (7mL) a cooled solution of Tf_2O (122mg, 0.432mmol, 1.10equiv.) in dry DCM (1mL) was added at -10°C, keeping the temperature below -5°C. Upon complete conversion observed by TLC (Hex:EtOAc 2:1, $CHCl_3$:MeOH 9:1 + 0.5% AcOH), the reaction mixture was directly evaporated onto $_{SiO2}$ (1g) and purified by column chromatography (Hex:EtOAc 7:1) to give pure target compound **13b** as white solid foam.

Yield: 250mg (82.8%) as white solid foam

Physical properties

R_f-value = 0.46 (Hex:EtOAc 2:1)

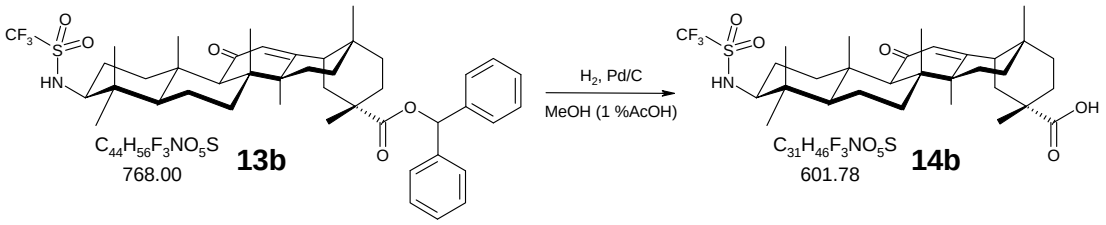
Optical rotation: $[\alpha]_D^{20}$ = +82.8 (c = 1.0 in $CHCl_3$:MeOH 3:1)

HRMS: calcd. [M+1]: 768.3904;
found [M+1]: 768.3907; dev.: -0.36ppm

^{1}H-NMR ($CDCl_3$): δ 0.67 (s, 3H, H28), 0.83 (s, 3H, H23/24), 0.79 -0.85 (m, 1H, H5), 1.02 (s, 3H, H23/24), 1.10 (s, 3H, H26), 1.14 (s, 3H, H25), 0.95-1.19 (m, 3H, H1b, H15b, H16b), 1.20 (s, 3H, H29), 1.38 (s, 3H, H27), 1.23-1.50 (m, 5H, H6b, H7b, H21b, H22a, H22b), 1.59-1.84 (m, 6H, H2a, H2b, H6a, H7a, H15a, H19b), 1.94-2.09 (m, 4H, H16a, H18, H19a, H21a), 2.38 (s, 1H, H9), 2.79 (td, 1H, *J*=13.4Hz, *J*=3.4Hz, H1a), 3.13 (dd, 1H, *J*=12.6Hz, *J*=4.1Hz, H3), 5.51 (s, 1H, H12), 6.93 (s, 1H, $OCHPh_2$), 7.25-7.41 (m, 10H, 10×PhCH)

^{13}C-NMR ($CDCl_3$): δ 15.9 (q, C25), 16.0 (q, C23/24), 17.7 (t, C6), 18.3 (q, C26), 22.9 (q, C27), 25.3 (t, C2), 26.0 (t, C15), 26.1 (t, C16), 27.8 (q, C28), 27.96 (q, C29), 27.99 (q, C23/24), 30.8 (t, C21), 31.5 (s, C17), 32.4 (t, C7), 36.6 (s, C10), 37.2 (t, C22), 38.4 (s, C4), 39.7 (t, C1), 40.8 (t, C19), 43.0 (s, C20), 43.8 (s, C8), 45.1 (s, C14), 47.9 (d, C18), 55.3 (d, C5), 61.4 (d, C9), 63.9 (d, C3), 76.7 (d, PhCH), 126.7 (d, PhCH), 127.0 (d, PhCH), 127.6 (d, PhCH), 127.9 (d, C12, PhCH), 128.2 (d, PhCH), 128.3 (d, PhCH), 139.7 (s, PhC), 139.8 (s, PhC), 170.0 (s, C13), 175.4 (s, C30), 200.4 (s, C11)

3.4.16 (*3S*, *18R*, *20S*)-11-Oxo-3-trifluoromethylsulfonylamino-olean-12-en-30-oic acid [14b]



Procedure: Hydrogenolyis

Diphenylmethyl ester **13b** (140mg, 0.182mmol, 1.00equiv.) was dissolved in MeOH:AcOH 100:1, the atmosphere was exchanged to argon, before Pd/C (12mg, 10%) was added. The atmosphere was exchanged to H_2 and the reaction was stirred at rt overnight. According to TLC (Hex:EtOAc 2:1) complete conversion of starting material was obtained. The reaction atmosphere was exchanged to argon, the reaction mixture was filtered through a hydrophobic filter, and washed with MeOH. The filtrate was evaporated and the crude material purified by column chromatography (SiO_2: 23g, Hex:EtOAc 2:1 to 1:1 + 0.1% AcOH) to give pure target compound **14b**, which was triturated with Et_2O, filtered and dried.

Yield: 100mg (91.2%) as white solid

Physical properties

R_f-value = 0.40 ($CHCl_3$:MeOH 9:1 + 0.5% AcOH)

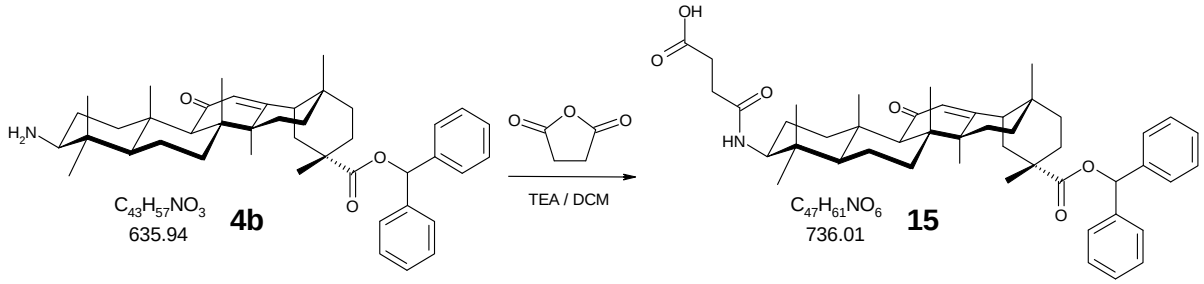
Optical rotation: $[\alpha]_D^{20}$ = +96.4 (c = 1.0 in $CHCl_3$:MeOH 3:1)

HRMS: calcd. [M+1]: 602.3122;
found [M+1]: 602.3130; dev.: 1.36ppm

^{1}H-NMR ($CDCl_3$:MeOD 5:1): δ 0.83 (s, 6H, H23/24, H28), 0.76-0.87 (m, 1H, H5), 1.03 (s, 3H, H23/24), 0.97-1.11 (m, 2H, H1b, H16b), 1.14 (s, 6H, H25, H26), 1.19 (s, 3H, H29), 1.22-1.30 (m, 1H, H15b), 1.39 (s, 3H, H27), 1.30-1.56 (m, 5H, H6b, H7b, H21b, H22a, H22b), 1.56-1.73 (m, 5H, H2a, H2b, H6a, H7a, H19b), 1.74-2.14 (m, 4H, H15a, H16a, H19a, H21a), 2.21 (dd, 1H, *J*=13.5Hz, *J*=3.8Hz, H18), 2.41 (s, 1H, H9), 2.79 (dt, 1H, *J*=13.4Hz, *J*=3.3Hz, H1a), 3.13 (dd, 1H, *J*=12.5Hz, *J*=4.2Hz, H3), 5.66 (s, 1H, H12)

^{13}C-NMR ($CDCl_3$:MeOD 5:1): δ 15.8 (q, C25), 15.9 (q, C23/24), 17.7 (t, C6), 18.2 (q, C26), 22.9 (q, C27), 25.3 (t, C2), 26.0 (t, C15), 26.1 (t, C16), 28.0 (q, C29), 27.9 (q, C28), 28.1 (q, C23/24), 30.7 (t, C21), 31.5 (s, C17), 32.3 (t, C7), 36.5 (s, C10), 37.4 (t, C22), 38.4 (s, C4), 39.6 (t, C1), 40.8 (t, C19), 43.1 (s, C20), 43.4 (s, C8), 45.1 (s, C14), 48.1 (d, C18), 55.3 (d, C5), 61.4 (d, C9), 63.8 (d, C3), 127.7 (d, C12), 171.0 (s, C13), 179.0 (s, C30), 200.8 (s, C11)

3.4.17 (*3S*, *18R*, *20S*)-11-Oxo-3-succinylamino-olean-12-en-30-oic acid, diphenylmethyl ester [15]



Procedure: *N*-Acylation

To a solution of amine **4b** (400mg, 0.629mmol, 1.00equiv.) in dry DCM (9.5mL) first TEA (0.61mL, 4.40mmol, 7.00equiv.) followed by succinic anhydride (0.315g, 3.15mmol, 5.00equiv.) were added at 0°C. The reaction mixture was allowed to reach rt and was stirred over the weekend. The reaction mixture turned to be a dark solution, which remained slightly inhomogeneous also during dilution and aqueous workup with diluted HCl. The organic layers were filtered and again washed with diluted HCl, water and brine, dried over Na_2SO_4 and evaporated. The crude material was purified by column chromatography (SiO_2: 20g, Hex:EtOAc 1:1 + 0.1% AcOH to Hex:EtOAc 1:3 + 0.1% AcOH) and (SiO_2: 7g, DCM:MeOH 70:1 + 0.1% AcOH) to give pure target compound **15**.

Yield: 192mg (41.5%) as white solid foam

Physical properties

R_f-value = 0.15 (Hex:EtOAc 3:1 + 0.5% AcOH)

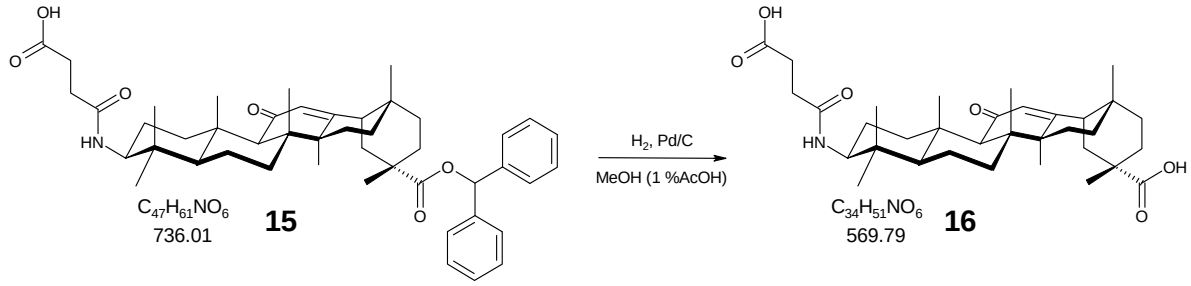
Optical rotation: $[\alpha]_D^{20}$ = +97.6° (c=0.3, $CHCl_3$)

HRMS: calcd. [M+1]: 736.4572;
found [M+1]: 736.4568; dev.: 0.41ppm

^{1}H-NMR ($CDCl_3$): δ 0.66 (s, 3H, H28), 0.80 (s, 3H, H23/24), 0.82-0.92 (m, 1H, H5), 0.91 (s, 3H, H23/24), 0.95-1.20 (m, 3H, H1b, H15b, H16b), 1.08 (s, 3H, H26), 1.12 (s, 3H, H25), 1.17 (s, 3H, H29), 1.21-1.50 (m, 5H, H6b, H7b, H21b, H22a, H22b), 1.37 (s, 3H, H27), 1.50-1.72 (m, 5H, H2a, H2b, H6a, H7a, H19b), 1.78 (td, *J*=13.6Hz, 4.6Hz, 1H, H15a), 1.95-2.10 (m, 4H, H16a, H18, H19a, H21a), 2.36 (s, 1H, H9), 2.53 (app. t, *J*=6.6Hz, 2H, CH_2), 2.71 (m, 2H, CH_2), 2.78 (td, *J*=13.9Hz, *J*=3.4Hz, 1H, H1a), 3.66-3.76 (m, 1H, H3), 5.51 (s, 1H, H12), 5.67 (d, *J*=9.9Hz, 1H, NH), 6.93 (s, 1H, $OCHPH_2$), 7.26-7.40 (m, 10H, PhCH)

^{13}C-NMR ($CDCl_3$): δ 16.2 (q, C25), 16.6 (q, C23/24), 17.7 (t, C6), 18.6 (q, C26), 23.3 (q, C27), 25.3 (t, C2), 26.36, 26.40 (2×t, C15, C16), 28.2 (q, C29), 28.3 (q, C28), 28.5 (q, C23/24), 30.2, (t, CH_2), 31.1, 31.2 (2×t, CH_2, C21, 31.8 (s, C17), 32.6 (t, C7), 36.9 (s, C10), 37.5 (t, C22), 38.0 (s, C4), 39.6 (t, C1), 41.1 (t, C19), 43.2, 44.0, 45.3 (3×s, C20, C8, C14), 48.1 (d, C18), 55.4 (d, C5), 57.0 (d, C3), 61.6 (d, C9), 76.6 (d, $OCHPh_2$), 127.0 (d, PhCH), 127.3 (d, PhCH), 127.8 (d, PhCH), 128.1 (d, C**12**), 128.4 (d, PhCH), 128.5 (d, PhCH), 128.6 (d, PhCH), 140.07 (s, PhC), 140.13 (s, PhC), 169.2 (s, C13), 172.1 (s, N**C**OCH_2), 175.0 (s, **C**OOH), 175.3 (s, C30), 200.1 (s, C11)

3.4.18 (*3S*, *18R*, *20S*)-11-Oxo-3-succinylamino-olean-12-en-30-oic acid [16]



Procedure: Hydrogenolysis

A solution of ester **15** (0.260g, 0.253mmol, 1.00equiv.) was evaporated and purged with argon three times, before Pd/C was added and the atmosphere was exchanged to H_2. The reaction was monitored by TLC ($CHCl_3$:MeOH 9:1 + 0.5% AcOH). Upon complete conversion the atmosphere was exchanged to argon, the reaction mixture filtered through a bed of Celite® and washed with MeOH. After evaporation the residue was distributed between Et_2O and 0.3M NaOH. The aqueous layer was acidified with 2N HCl and extracted with DCM. The combined organic layers were washed twice with water, once with brine, dried over Na_2SO_4 and evaporated. The crude material was purified by trituration with hot EtOAc and washing with EtOAc and Et_2O.

Yield: 100mg (49.7%) as white solid

Physical Properties:

[CAS-851509-95-0]

R_f-value = 0.32 ($CHCl_3$:MeOH 9:1 + 0.5% AcOH)

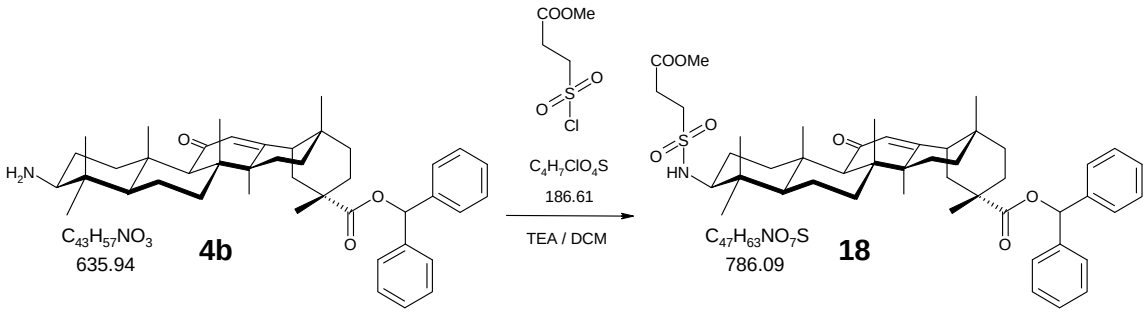
Optical rotation: $[\alpha]_D^{20}$ = +106.6° (c=1.0, $CHCl_3$:MeOH 5:1)

HRMS: calcd. [M+1]: 570.3789;
found [M+1]: 570.3799; dev.: -1.58ppm

^{1}H-NMR ($CDCl_3$:MeOD 5:1): δ 0.73 (s, 3H, H23/24), 0.74 (s, 3H, H28), 0.76-0.83 (m, 1H, H5), 0.79 (s, 3H, H23/24), 0.90-1.05 (m, 2H, H1b, H16b), 1.05 (s, 6H, H25, H26), 1.11 (s, 3H, H29), 1.09-1.43 (m, 7H, H2b, H6b, H7b, H15b, H21b, H22a, H22b) 1.32 (s, 3H, H27), 1.48-1.58 (m, 4H, H2a, H6a, H7a, H19b), 1.70-1.90 (m, 3H, H15a, H19a, H21a), 1.91-2.05 (m, 1H, H16a), 2.07-2.17 (m, 1H, H18) , 2.35 (s, 1H, H9), 2.36-2.46 (m, 2H, CH_2), 2.51-2.61 (m, 2H, CH_2), 2.60-2.71 (m, 1H, H1a), 3.50-3.58 (m, 1H, H3), 5.57 (s, 1H, H12), 6.78 (d, *J*=9.8Hz, 1H, NH)

^{13}C-NMR ($CDCl_3$:MeOD 5:1): δ 15.8 (q, C25), 16.0 (q, C23/24), 17.3 (t, C6), 18.2 (q, C26), 22.9 (q, C27), 24.6 (t, C2), 25.97, 26.07 (2×t, C15, C16), 27.97, 28.01 (2×q, C23/24, C29), 28.11 (q, C28), 29.5 (t, CH_2), 30.62, 30.70 (2×t, CH_2, C21) 31.5 (s, C17), 32.3 (t, C7), 36.6 (s, C10), 37.4 (t, C22), 37.9 (s, C4), 39.4 (t, C1), 40.8 (t, C19), 43.0 (s, C20), 43.4 (s, C14), 45.2 (s, C8), 48.1 (s, C18), 55.1 (s, C5), 56.3 (s, C3), 61.4 (s, C9), 127.7 (s, C12), 170.9 (s, C13), 172.1 (s, NH**C**O), 175.0 (s, **C**OOH), 179.0 (s, C30), 201.1 (s, C11)

3.4.19 (*3S*, *18R*, *20S*)-3-(2-Methoxycarbonyl-ethylsulfonylamino)-11-oxo-olean-12-en-30-oic acid, diphenylmethyl ester [18]



Procedure: Sulfonylation of amine

To a chilled solution of amine **4b** (400mg, 0.629mmol, 1.00equiv.) in dry DCM (14mL) and TEA (0.26ml, 1.89mmol, 3.0equiv.) a solution of 3-chlorosulfonyl propionic acid methylester (141mg, 0.755mmol, 1.2equiv.) in dry DCM (<2mL) was added dropwise to the reaction mixture within 20min keeping the temperature below 0°C. After complete addition the reaction mixture was stirred under TLC monitoring ($CHCl_3$:MeOH 9:1 + 0.5% AcOH; Hex:EtOAc 2:1 + 0.5% AcOH) and was allowed to reach rt. After one hour almost all starting material was consumed and the reaction mixture was diluted with DCM, evaporated onto SiO_2 (1.5g) and purified by column chromatography (SiO_2: 40g, Hex:EtOAc 2:1) to give pure target compound.

Yield: 0.381g (77.1%) as white solid

Physical properties

R_f-value = 0.31 (Hex:EtOAc 2:1)

Optical rotation: $[\alpha]_D^{20}$ = +100.9° (c=0.8, $CHCl_3$)

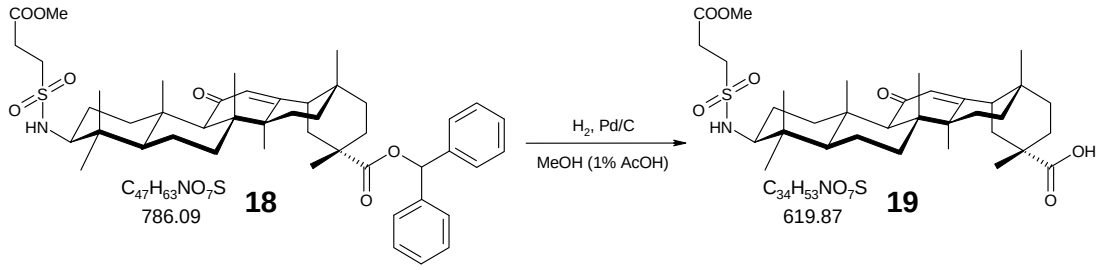
HRMS: calcd. [M+1]: 786.4398

found [M+1]: 786.4403; dev.: -0.61ppm

^{1}H-NMR ($CDCl_3$): δ 0.65 (s, 3H, H28), 0.78 (s, 3H, H23/24), 0.79-0.84 (m, 1H, H5), 0.93-1.08 (m, 2H, H1b, H16b), 1.03 (s, 3H, H23/24), 1.08 (s, 3H, H26), 1.12 (s, 3H, H25), 1.12-1.21 (m, 1H, H15b), 1.17 (s, 3H, H29), 1.22-1.47 (m, 5H, H6b, H7b, H21b, H22a, H22b), 1.34 (s, 3H, H27), 1.56-1.75 (m, 5H, H2a, H2b, H6a, H7a, H19b), 1.72-1.83 (m, 1H, H15a), 1.93-2.08 (m, 3H, H16a, H18, H19a, H21a), 2.33 (s, 1H, H9), 2.78-2.86 (m, 1H, H1a), 2.85 (t, *J*=7.6Hz, 2H, COC**H**$_2$), 2.99-3.08 (m, 1H, H3), 3.29-3.39 (m, 2H, SO_2C**H**$_2$), 3.73 (s, 3H, OCH_3), 4.07 (d, *J*=10.0Hz, 1H, NH), 5.51 (s, 1H, H12), 6.93 (s, 1H, $OCHPh_2$), 7.26-7.39 (m, 10H, 10×PhCH)

^{13}C-NMR ($CDCl_3$): δ 16.2 (q, C25), 16.4 (q, C23/24), 18.0 (t, C6), 18.6 (q, C26), 23.3 (q, C27), 26.37, 26.42 (2×t, C15, C16), 27.3 (t, C2), 28.2 (q, C28), 28.3 (q, C23/24), 28.5 (q, C29), 28.8 (t, CO**C**H$_2$), 31.2 (t, C21), 31.8 (s, C17), 32.7 (t, C7), 36.8 (s, C10), 37.5 (t, C22), 38.5 (s, C4), 39.8 (t, C1), 41.1 (t, C19), 43.2 (s, C14), 44.0 (s, C20), 45.2 (s, C8), 48.1 (d, C18), 49.3 (t, SO_2**C**H$_2$), 52.3 (q, OCH_3), 55.9 (d, C5), 61.6 (d, C9), 62.4 (d, C3), 76.6 (d, Ph_2CHO), 127.0 (d, PhCH), 127.3 (d, PhCH), 127.8, 128.1, 128.5 (3×d, 2×PhCH, C12) 128.6 (d, PhCH), 140.1 (s, PhC), 140.1 (s, PhC), 169.0 (s, C13), 171.0 (s, **C**OOCH$_3$), 175.2 (s, C30), 199.8 (s, C11)

3.4.20 (*3S*, *18R*, *20S*)-3-(2-Methoxycarbonyl-ethylsulfonylamino)-11-oxo-olean-12-en-30-oic acid [19]



Procedure: Hydrogenolyis

Ester **18** (350mg, 0.445mmol, 1.00equiv.) was dissolved in MeOH:AcOH 100:1 (22mL) and the atmosphere was exchanged to argon, Pd/C was added, the atmosphere was exchanged to H_2 and the reaction mixture was stirred at rt for 1h, while monitored by TLC (Hex:EtOAc 1:1). After 3h the reaction mixture was filtered through a bed of Celite®, washed with MeOH (+ 1% AcOH), the filtrate was evaporated onto SiO_2 (1g) and submitted to column chromatography (SiO_2, 25g, Hex:EtOAc 2:1 + 0.1% AcOH to Hex:EtOAc 1:1 + 0.1% AcOH). The residue was dissolved in EtOAc and washed twice with water, once with brine, dried over Na_2SO_4, evaporated and co-evaporated from DCM to afford pure target compound **19** as solid white foam.

Yield: 270mg (97.8%)

Physical properties

R_f-value = 0.25 (Hex:EtOAc 1:1 + 0.5% AcOH)

Optical rotation: $[\alpha]_D^{20}$ = +100.4 (c=1.0, MeOH:$CHCl_3$ 5:1)

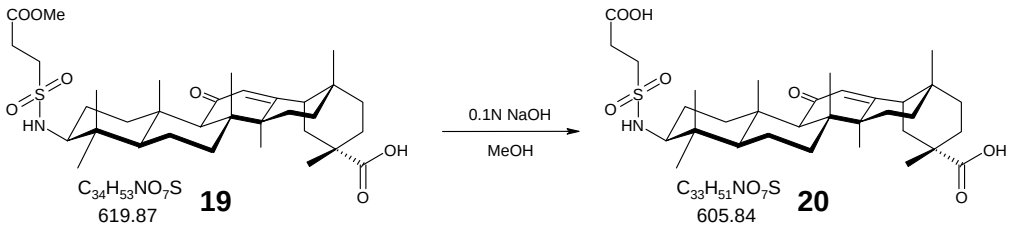
HRMS: calcd. [M+1]: 620.3616

found [M+1]: 620.3626; dev.: -1.77ppm

^{1}H-NMR ($CDCl_3$): δ 0.79-0.87 (m, 1H, H5), 0.79 (s, 3H, H23/24), 0.84 (s, 3H, H28), 0.98-1.11 (m, 2H, H1b, H16b), 1.04 (s, 3H, H23/24), 1.13 (bs, 6H, H25, H26), 1.15-1.23 (m, 1H, H15b), 1.23 (s, 3H, H29), 1.37 (s, 3H, H27), 1.38-1.53 (m, 5H, H6b, H7b, H21b, H22a, H22b), 1.56-1.77 (m, 5H, H2a, H2b, H6a, H7a, H19b), 1.81 (td, *J*=13.7Hz, *J*=3.8Hz, 1H, H15a), 1.88-2.09 (m, 3H, H16a, H19a, H21a), 2.19 (dd, *J*=13.2Hz, *J*=3.4Hz, 1H, H18), 2.36 (s, 1H, H9), 2.78-2.87 (m, 1H, H1a), 2.85 (app. t, *J*=7.6Hz, 2H, $COCH_2$), 2.99-3.09 (m, 1H, H3), 3.29-3.40 (m, 2H, SO_2CH_2), 3.73 (s, 3H, OCH_3), 4.31 (d, *J*=9.9Hz, 1H, NH), 5.71 (s, 1H, H12)

^{13}C-NMR ($CDCl_3$): δ 16.2 (q, C25), 16.4 (q, C23/24), 18.0 (t, C6), 18.6 (q, C26), 23.3 (q, C27), 26.37, 26.43 (2×t, C15, C16), 27.2 (t, C2), 28.42 (3×q, C23/24, C28, C29), 28.8 (t, CO**C**H_2), 30.9 (t, C21), 31.9 (s, C17), 32.7 (t, C7), 36.8 (s, C10), 37.7 (t, C22), 38.5 (s, C4), 39.8 (t, C1), 40.8 (t, C19), 43.2 (s, C14), 43.8 (s, C20), 45.4 (s, C8), 48.2 (d, C18), 49.3 (t, SO_2**C**H_2), 52.3 (q, OCH_3), 55.9 (d, C5), 61.6 (d, C9), 62.4 (d, C3), 128.4 (d, C12), 169.5 (s, C13), 171.0 (s, **C**$OOCH_3$), 181.5 (s, C30), 200.2 (s, C11)

3.4.21 (*3S*, *18R*, *20S*)-3-(2-Carboxyethylsulfonylamino)-11-oxo-olean-12-en-30-oic acid [20]



Procedure: Ester cleavage

Ester **19** (0210g, 0.339mmol, 1.00equiv.) was dissolved in MeOH (5mL), 0.2N NaOH in MeOH (5mL, 3equiv.) was added at 0°C and the reaction mixture was stirred at rt overnight. Since no conversion was observed (Hex:EtOAc 1:1 + 0.5% AcOH) first another 5mL of 0.2N NaOH in MeOH, later water (1mL) and finally 2N NaOH (0.5mL) were added and the reaction mixture was stirred at 6°C for another night to complete conversion. The reaction mixture was diluted with DCM, acidified with diluted AcOH (10%) and extracted with EtOAc under TLC monitoring.

The combined organic layers were washed once with diluted AcOH, with water, with brine, dried over Na_2SO_4 and evaporated. The crude material was evaporated onto SiO_2 (1g) and submitted to vacuum flash column chromatography (SiO_2: 10g, DCM:MeOH 30:1 + 0.1% AcOH to DCM:MeOH 15:1 + 0.1% AcOH) to give pure target compound **20**.

Yield: 199mg (97%) as white solid

Physical properties:

R_f-value = 0.18, (Hex:EtOAc 1:3 + 0.5% AcOH)

Optical rotation: $[\alpha]_D^{20}$ = +95.9 (c=1.0, MeOH:$CHCl_3$ 5:1)

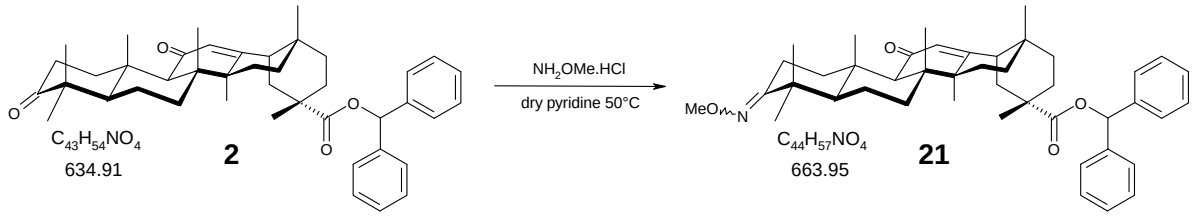
HRMS: calcd. [M+1]: 606.3459

found [M+1]: 606.3467; dev.: -1.38ppm

^{1}H-NMR ($CDCl_3$ + 3dr MeOD): δ 0.79 (s, 3H, H23/24), 0.80-0.87 (m, 1H, H5), 0.82 (s, 3H, H28), 0.98-1.09 (m, 2H, H1b, H16b), 1.03 (s, 3H, H23/24), 1.12 (bs, 6H, H25, H26), 1.19 (s, 3H, H29), 1.19-1.22 (m, 1H, H15b), 1.25-1.51 (m, 5H, H6b, H7b, H21b, H22a, H22b), 1.37 (s, 3H, H27), 1.56-1.77 (m, 5H, H2a, H2b, H6a, H7a, H19b), 1.83 (td, *J*=13.9Hz, *J*=4.2Hz, 1H, H15a), 1.88-2.08 (m, 3H, H19a, H21a, H16a), 2.19 (dd, *J*=13.3Hz, *J*=3.9Hz, 1H, H18), 2.37 (s, 1H, H9), 2.75-2.83 (m, 1H, H1a), 2.82 (app. t, *J*=7.6Hz, 2H, HOOCC**H**$_2$), 3.00 (dd, *J*=12.0Hz, *J*=4.6Hz, 1H, H3), 3.27-3.39 (m, 2H, SO_2CH_2), 5.20 (d, *J*=9.8Hz, 1H, NH), 5.66 (s, 1H, H12)

^{13}C-NMR ($CDCl_3$ + 3dr MeOD): δ 16.1 (q, C25), 16.2 (q, C23/24), 17.9 (t, C6), 18.5 (q, C26), 23.1 (q, C27), 26.23, 26.31 (2×t, C15, C16) 26.7 (t, C2), 28.28, 28.32, 28.36 (3×q, C23/24, C28, C29), 28.6 (t, HOOC**C**H_2), 30.9 (t, C21), 31.7 (s, C17), 32.5 (t, C7), 36.7 (s, C10), 37.6 (t, C22), 38.4 (s, C4), 39.7 (t, C1), 40.9 (t, C19), 43.2 (s, C14), 43.6 (s, C20), 45.3 (s, C8), 48.2 (d, C18), 49.1 (t, SO_2**C**H_2), 55.7 (d, C5), 61.5 (d, C9), 62.0 (d, C3), 128.0 (d, C12), 170.5 (s, C13), 172.9 (s, COOH), 179.4 (s, C30), 200.7 (s, C11)

3.4.22 (*18R*, *20S*)-3-Methoxyimino-11-oxo-olean-12-en-30-oic acid, diphenylmethyl ester [21]



$C_{43}H_{54}NO_4$ 634.91 **2** → $C_{44}H_{57}NO_4$ 663.95 **21**

Procedure: Oxime formation

A solution of ketone **2** (3.25g, 5.12mmol, 1.00equiv.) and methoxylamine hydrochloride (1.28g, 15.4mmol, 3.0equiv.) in dry pyridine (25mL) was stirred at rt. Upon complete conversion (1h) according to TLC (Hex:EtOAc 3:1) the reaction mixture was diluted with DCM (150mL) and washed with 2N HCl (3x100mL), with satd. $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated. This material was recrystallized from warm Et_2O to give white crystals (2.710g, 80%) and mother liquid (620mg, 18%), both pure target compound **21** according to TLC and NMR.

Yield: 3.30 (97.1%) as white solid

Physical properties:

R_f-value = 0.55 (Hex:EtOAc 3:1)

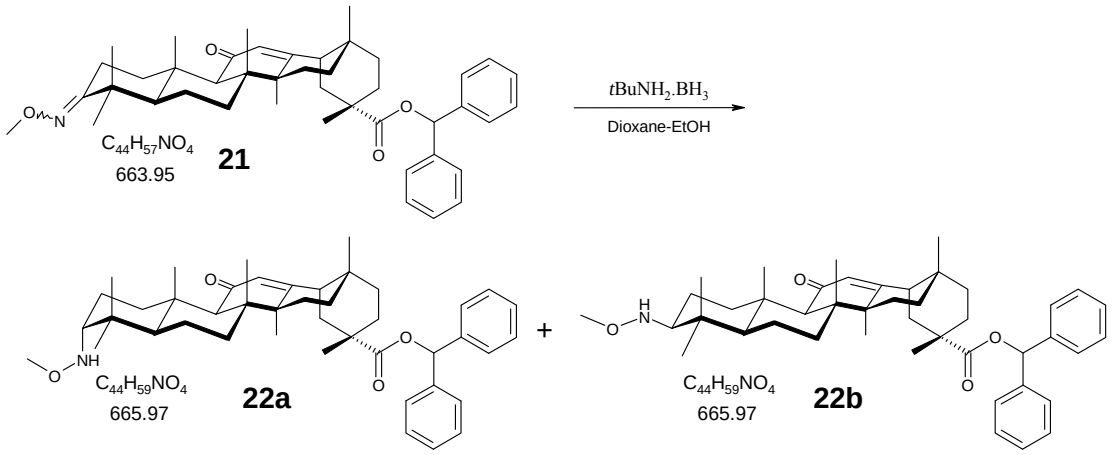
m.p.: 128-130°C (Et_2O)

Optical rotation: $[\alpha]_D^{20}$ = +103.2 (c=0.9 in $CHCl_3$)

^{1}H-NMR ($CDCl_3$): δ 0.67 (s, 3H, H28), 0.95-1.09 (m, 3H, H16b, H5, H1b), 1.06 (s, 3H, H23/24), 1.11 (s, 3H, H26), 1.12-1.16 (m, 1H, H15b), 1.17 (s, 6H, H23/24, H29), 1.23 (s, 3H, H25), 1.23-1.34 (m, 3H, H21b, H22a, H22b), 1.34 (s, 3H, H27), 1.36-1.50 (m, 2H, H6b, H7b), 1.57-1.69 (m, 3H, H6a, H7a, H19b), 1.79 (dt, *J*=13.6Hz, *J*=4.3Hz, 1H, H15a), 1.94-2.07 (m, 4H, H16a, H18, H19a, H21a), 2.15-2.25 (m, 1H, H2b), 2.35 (s, 1H, H9), 2.77-2.84 (m, 1H, H1a), 2.9-2.97 (m, 1H, H2a), 3.82 (s, 3H, OCH_3), 5.52 (s, 1H, H12), 6.93 (s, 1H, $OCHPh_2$), 7.25-7.41 (m, 10H, 10×PhCH)

^{13}C-NMR ($CDCl_3$): δ 15.6 (q, C25), 17.7 (t, C2), 18.2 (t, C6), 18.6 (q, C26), 23.3 (q, C27), 23.5 (q, C23/24), 26.4 (t, C16), 26.4 (t, C15), 27.2 (q, C23/24), 28.2 (q, C29), 28.3 (q, C28), 31.2 (t, C21), 31.7 (s, C17), 32.4 (t, C7), 36.9 (s, C10), 37.5 (t, C22), 39.1 (t, C1), 40.1 (s, C4), 41.1 (t, C19), 43.2 (s, C20), 44.0 (s, C8), 45.3 (s, C14), 48.1 (d, C18), 55.6 (d, C5), 61.0 (q, OCH_3), 61.3 (d, C9), 76.6 (d, $OCHPh_2$), 127.0 (d, PhCH), 127.3 (d, PhCH), 127.8 (d, PhCH), 128.1 (d, C12), 128.5 (d, PhCH), 128.6 (d, PhCH), 140.1 (s, PhC), 140.1 (s, PhC), 165.7 (s, C3), 169.1 (s, C13), 175.2 (s, C30), 199.8 (s, C11)

3.4.23 (*3R*, *18R*, *20S*)-3-Methoxyamino-11-oxo-olean-12-en-30-oic acid, diphenylmethyl ester [22a] and (*3S*, *18R*, *20S*)-3-Methoxyamino-11-oxo-olean-12-en-30-oic acid, diphenylmethyl ester [22b]



Procedure: Oxime reduction with BH_3.*t*BuNH$_2$

Borane tert-butylamine complex (196mg, 2.26mmol, 3.0equiv) was added to a solution of the methoxime **21** (664mg, 0.753mmol, 1.00equiv) in EtOH:dioxane (1:2, 5.5mL). The reaction mixture was cooled to 0°C and 10% HCl (2.6mL) was added dropwise keeping the temperature at 0°C. The reaction mixture was stirred at 0°C for 2.5h. After 1.5h almost all starting material was consumed according to TLC (Hex:EtOAc 9:1). Na_2CO_3 (~1g) was added portionwise and the mixture was distributed between satd. $NaHCO_3$ and DCM. The organic layer was dried over Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (SiO_2: Hex:EtOAc 20:1 to Hex:EtOAc 5:1) to give pure (3S)-methoxyamine **22b** (330mg, 65.8%) and pure (3R)-methoxyamine **22a** (70mg, 14%) as white solid foams.

Yield (α-methoxyamine): 70mg (14%) as white solid foam

Yield (β-methoxyamine): 330mg (65.8%) as white solid foam

Physical properties (α-methoxyamine):

R_f-value = 0.11 (Hex:EtOAc 5:1)

Due to the instability of the material it was deprotected and characterized as free acid only.

Physical properties (β-methoxyamine):

R_f-value = 0.17 (Hex:EtOAc 5:1)

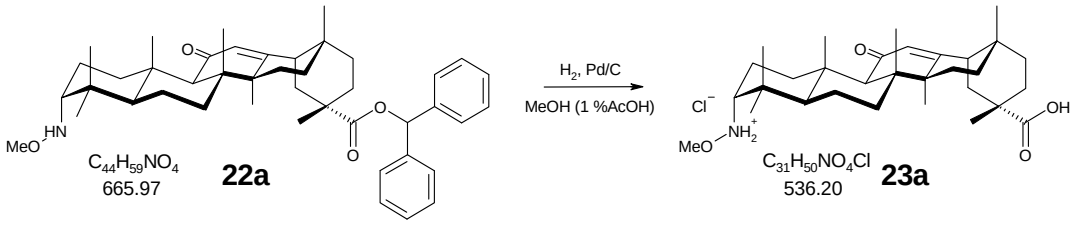
Optical rotation: $[\alpha]_D^{20}$ = +143.0 (c=1.0 in $CHCl_3$)

Combustion analysis:	calcd.	C 79.36%	H 8.93%	N 2.10%
	found	C 79.76%	H 8.55%	N 2.01%

^{1}H-NMR ($CDCl_3$): δ 0.66 (s, 3H, H28), 0.72 (s, 3H, H23/24), 0.69-0.77 (m, 1H, H5), 0.89-1.01 (m, 2H, H1b, H16b), 1.07 (s, 3H, H23/24), 1.09 (s, 3H, H26), 1.12-1.19 (m, 1H, H15b), 1.13 (s, 3H, H25), 1.17 (s, 3H, H29), 1.20-1.43 (m, 6H, H2b, H6b, H7b, H21b, H22a, H22b), 1.36 (s, 3H, H27), 1.55-1.71 (m, 3H, H6a, H7a, H19b), 1.79 (td, *J*=13.5Hz, *J*=3.8Hz, 1H, H15a), 1.86-1.94 (m, 1H, H2a), 1.94-2.08 (m, 4H, H16a, H18, H19a, H21a), 2.34 (s, 1H, H9), 2.51 (dd, *J*=11.8Hz, *J*=4.0Hz, 1H, H3), 2.75-2.83 (m, 1H, H1a), 3.50 (s, 3H, OCH_3), 5.34 (bs, 1H, NH), 5.51 (s, 1H, H12), 6.93 (s, 1H, $OCHPh_2$), 7.26-7.40 (m, 10H, 10×PhCH)

^{13}C-NMR ($CDCl_3$): δ 16.1 (q, C25), 16.7 (q, C23/24), 17.3 (t, C6), 18.7 (q, C26), 23.3 (q, C27), 23.5 (t, C2), 26.37, 26.40 (2×t, C15, C16), 28.23, 28.27, 28.6 (3×q, C23/24, C28, C29), 31.1 (t, C21), 31.7 (s, C17), 32.8 (t, C7), 36.9 (s, C4), 37.1 (s, C10), 37.5 (t, C22), 39.3 (t, C1), 41.1 (t, C19), 43.1 (s, C14), 44.0 (s, C20), 45.3 (s, C8), 48.0 (d, C18), 56.2 (d, C5), 61.6 (d, C9), 61.8 (q, OMe), 67.9 (d, C3), 76.6 (d, $OCHPh_2$), 127.0 (d, PhCH), 127.3 (d, PhCH), 127.8 (d, PhCH), 128.1 (d, PhCH), 128.4 (d, PhCH), 128.5 (d, C12), 128.6 (d, PhCH), 140.06 (s, PhC), 140.10 (s, PhC), 168.7 (s, C13), 175.2 (s, C30), 200.1 (s, C11)

3.4.24 (*3R*, *18R*, *20S*)-3-Methoxyamino-11-oxo-olean-12-en-30-oic acid hydrochloride [23a]



Procedure: Hydrogenolyis

Ester **22a** (200mg, 0.3mmol, 1.0equiv.) was dissolved in MeOH:EtOAc 1:1 (30mL) and 0.3mL of AcOH were added. The atmosphere was exchanged to argon, Pd/C was added, the atmosphere was exchanged to H_2 and the reaction mixture was stirred at rt while monitored by TLC (Hex:EtOAc 5:1). After 3h complete conversion of the starting material to one more polar compound was observed by TLC (Tol:EtOAc 2:1 + 0.5% AcOH). The atmosphere was exchanged to argon, the reaction mixture was filtered over a hydrophobic filter, washed with MeOH:EtOAc 1:1 + AcOH evaporated and dried. The crude material was purified by precipitation as hydrochloride by taking up in THF and addition of HCl in Et_2O (1M, 2equiv.). Final washing with Et_2O and collection by centrifugation gave pure target compound **23a** as white solid.

Yield: 104mg (64.6%) as white solid

Physical properties:

R_f-value = 0.29 (Hex :EtOAc 1:1 + 0.1% AcOH)

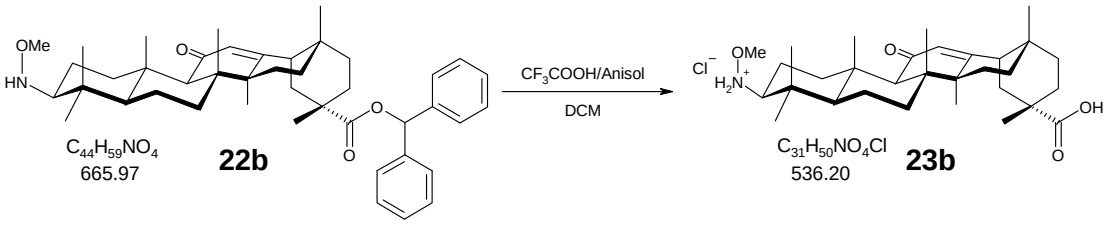
Optical rotation: $[\alpha]_D^{20}$ = +115.0 (c=0.3 in MeOH)

HRMS: calcd. [M+1]: 500.3734;
found [M+1]: 500.3734; dev.: -0.00ppm

^{1}H-NMR ($CDCl_3$:MeOD 1:5): δ 0.85 (s, 3H, H28), 0.98-1.08 (m, 1H, H16b), 1.09 (s, 3H, H23/24), 1.12 (s, 3H, H23/24), 1.15-1.25 (m, 1H, H15b), 1.16 (s, 3H, H26), 1.18 (s, 3H, H29), 1.22 (s, 3H, H25), 1.17-1.27 (m, 1H, H1b), 1.35-1.50 (m, 5H, H5, H7b, H21b, H22a, H22b), 1.45-1.65 (m, 2H, H6a, H6b), 1.49 (s, 3H, H27), 1.65-1.75 (m, 1H, H19b), 1.75-2.05 (m, 5H, H2b, H7a, H15a, H19a, H21a), 2.05-2.30 (m, 1H, H2a, H16a, H18), 2.61-2.70 (m, 1H, H1a), 2.69 (s, 1H, H9), 3.36-3.40 (m, 1H, H3), 4.01 (s, 3H, OCH_3), 5.64 (s, 1H, H12)

^{13}C-NMR ($CDCl_3$:MeOD 1:5): δ 17.4 (q, C25), 18.5 (t, C6), 18.9 (t, C2), 19.2 (q, C26), 23.7 (q, C23/24), 24.1 (q, 27), 27.2 (t, C16/15), 27.2 (q, C23/24), 27.4 (t, C15/16), 28.8 (q, C29), 29.1 (q, C28), 31.9 (t, C21), 32.8 (s, C17), 33.1 (t, C7), 34.9 (t, C1), 36.7 (s, C4), 37.9 (s, C10), 38.8 (t, C22), 42.3 (t, C19), 44.66, 44.69 (2×s, C14, C20), 46.6 (s, C8), 49.6 (d, C18), 50.0 (d, C5), 62.0 (q, OCH_3), 62.1 (d, C9), 67.8 (d, C3), 128.7 (d, C12), 173.1 (s, C13), 180.2 (s, C30), 202.0 (s, C11)

3.4.25 (*3S*, *18R*, *20S*)-3-Methoxyamino-11-oxo-olean-12-en-30-oic acid hydrochloride [23b]



Procedure: Acidic diphenylmethyl ester cleavage

To a solution of ester **22b** in dry DCM (13mL) anisole (1.60g, 15.02mol, 50equiv) and then TFA (4.5mL) was added dropwise at -10°C and the reaction mixture was stirred at 0°C until all starting material was consumed according to TLC (Hex:EtOAc 5:2). After 1h no starting material was detectable and the reaction mixture was diluted with DCM and treated with satd. $NaHCO_3$ (55mL) until the pH was slightly basic. The solution was buffered by addition of AcOH, the phases were separated and the aqueous layer was extracted three times with DCM (TLC: Tol:EtOAc 2:1 + 0.5% AcOH). The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated to give a crude material, which was submitted to column chromatography (SiO_2: 25g, Tol:EtOAc + 0.1% AcOH) to give the pure target compound (137mg, 85.1%) as white solid.

Upon storage the material turned out to be partly oxidized to the corresponding oxime, was again submitted to column chromatography and converted to hydrochloride **23b** by dissolving in MeOH and precipitation with 2N HCl in Et_2O (2-3equiv.) and Et_2O. Filtration and washing with Et_2O gave pure **23b**.

Yield: 137mg (85.1%) as free base white solid

Physical properties:

R_f-value = 0.29 ($CHCl_3$:MeOH 30:1)

m.p.: 249-251°C (Et_2O)

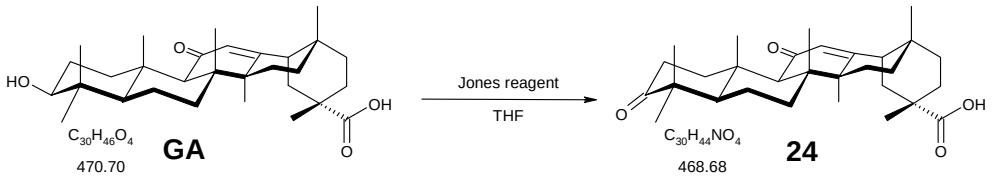
Optical rotation: $[\alpha]_D^{20}$ = +160.3 (c = 0.8, MeOH)

HRMS: calcd. [M+1]: 500.3735;
found [M+1]: 500.3734; dev.: -0.05ppm

^{1}H-NMR ($CDCl_3$:MeOD 2:5) δ 0.78-0.86 (m, 1H, H5), 0.83 (s, 3H, H28), 0.96-1.09 (m, 2H, H1b, H16b), 1.00 (s, 3H, H23/24), 1.15 (s, 3H, H26), 1.17 (s, 3H, H25), 1.19-1.26 (m, 1H, H15a), 1.19 (s, 3H, H29), 1.27 (s, 3H, H23/24), 1.29-1.45 (m, 3H, H21b, H22a, H22b), 1.39 (s, 3H, H27), 1.41-1.52 (m, 1H, H6b, H7b), 1.56-1.66 (m, 3H, H6a, H7a, H19b), 1.80-2.11 (m, 6H, H2a, H2b, H15b, H16a, H19a, H21a), 2.18-2.25 (m, 1H, H18), 2.39 (s, 1H, H9), 2.89-2.99 (m, 1H, H1a), 3.03-3.11 (m, 1H, H3), 4.06 (s, 3H, OCH_3), 5.67 (s, 1H, H12)

^{13}C-NMR ($CDCl_3$:MeOD 2:5): δ 15.5 (q, 25), 16.5 (q, C23/24), 16.9 (t, C6), 18.4 (q, C26), 19.4 (t, C2), 23.0 (q, C27), 26.1, 26.2 (2×t, C15, C16), 28.1, 28.16, 28.22 (3×q, C23/24, C28, C29), 30.7 (t, C21), 31.6 (s, C17), 32.2 (t, C7), 36.3, 36.6 (2×s, C4, C10), 37.4 (t, C22), 38.0 (t, C1), 40.9 (t, C19), 43.1 (s, C14), 43.5 (s, C20), 45.2 (s, C8), 48.2 (d, C18), 55.5 (d, C5), 61.1 (d, C9), 61.6 (q, OCH_3), 70.0 (d, C3), 127.8 (d, C12), 171.0 (s, C13), 179.0 (s, C30), 200.2 (s, C11)

3.4.26 (*18R*, *20S*)-3,11-Dioxo-olean-12-en-30-oic acid[12] [24]



Procedure: Jones oxidation

CrO_3 (3.25g, 21.4mmol) was dissolved in a mixture of H_2SO_4 (conc.) (3.3mL) and water (9.8mL) at 0°C within 30min and was stirred until used. Jones' reagent was added dropwise *via* a dropping funnel to a stirred solution (mechanical stirrer) of glycyrrhetinic acid (9.00g, 19.1mmol, 1.00equiv.) in THF (31mL) at -10°C (ice-EtOH) keeping the temperature below 0°C. After approximately 2/3 of the addition a lot of precipitate was formed. After complete addition of the reagent, the reaction mixture was allowed to reach rt and was stirred under TLC-monitoring (DCM:MeOH 20:1). Upon complete conversion (1h) water (90mL) was added and the mixture was stirred for 15min before the precipitated solid was filtered and washed with water several times.

The residue was taken up in THF/MeOH (150:10mL) and dried over Na_2SO_4, filtered and evaporated. The white solid was recrystallized from MeOH/DCM to give 8.1g (94.9%) as white crystals.

Yield: 8.1g (94.9%) (Lit.[12]: 98%) as white crystals

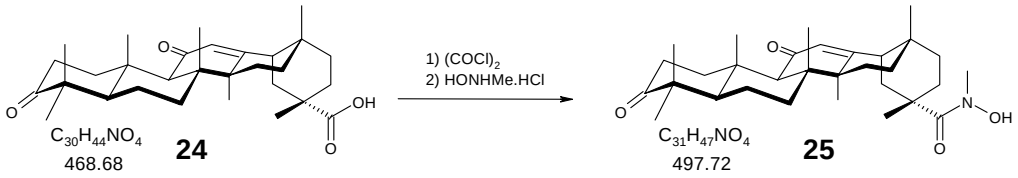
Physical properties:

[CAS-7020-50-0]

m.p: 281-283°C (MeOH/DCM) (Lit.[12]: 283-285°C (H_2O))

Spectral data: consistent with the literature[10]

3.4.27 (*18R*, *20S*)-*N*-Hydroxy-*N*-methyl-3,11-dioxo-olean-12-en-30-amide [25]



Procedure: Amide formation *via* acid chloride (oxalyl chloride method)

Acid **24** (2.00g, 4.27mmol, 1.00equiv.) was suspended in dry DCM (40mL) and DMF (20 drops, syringe-needle), oxalylchloride (0.49mL, 5.12mmol, 1.2equiv.) was added at 0°C, the reaction mixture was allowed to reach rt and was stirred under TLC-monitoring (sample from MeOH solution, SiO_2: Hex:EtOAc 2:1). The suspension turned to a clear solution and complete conversion was obtained after 40min. The reaction mixture was evaporated at rt, co-evaporated from DCM once and re-dissolved in DCM. The clear solution was cooled to 0°C and first TEA (2.38mL, 17.1mmol, 4.0equiv.) and then *N*-methyl-hydroxylamine hydrochloride was added in one portion. The reaction mixture had turned yellowish already at the addition of TEA and this colour was intensified during the reaction. After 10min complete conversion was detected (SiO_2: Hex:EtOAc 2:1 and amino-phase: DCM:MeOH 20:1). The reaction mixture was diluted with DCM and washed with 1N HCl, satd. $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated to give the crude material. Purification by column chromatography (SiO_2: 115g, DCM:MeOH 45:1 to 35:1) and column chromatography on amino-phase (15g, DCM to DCM:MeOH 50:1) gave pure target compound **25** as white solid.

Yield: 1.48g (69.7%) as white solid

Physical properties:

R_f-value = 0.14 (Hex :EtOAc 1:1 + 0.1% AcOH)

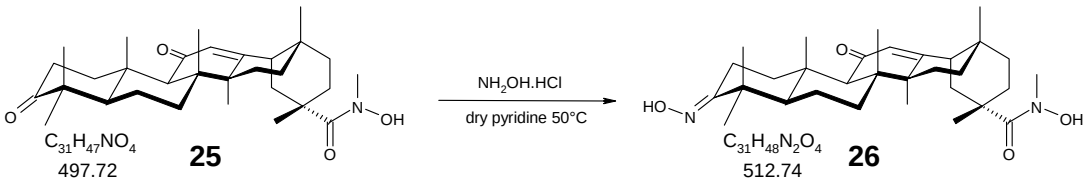
Optical rotation: $[\alpha]_D^{20}$ = +164.4 (c = 0.55, $CHCl_3$)

HRMS: calcd. [M+1]: 498.3578;
found [M+1]: 498.3572; dev.: 1.23ppm

^{1}H-NMR ($CDCl_3$): δ 0.83 (s, 3H, H28), 1.00-1.08 (m, 1H, H16b), 1.07 (s, 3H, H23/24), 1.10 (s, 3H, H23/24), 1.16 (s, 3H, H26), 1.2 (s, 3H, H29), 1.21-1.36 (m, 3H, H5, H15b, H21b), 1.26 (s, 3H, H25), 1.33-1.46 (m, 2H ,H1b, H22b), 1.37 (s, 3H, H27), 1.41-1.64 (m, 6H, H6a, H6b, H7a, H7b, H19b, H22a), 1.86 (td, *J*=13.6Hz, *J*=4.3Hz, 1H, H15a), 2.09 (td, *J*=13.6Hz, *J*=4.3Hz, 1H, H16a), 2.18-2.25 (m, 3H, H18, H19a, H21a), 2.37 (ddd, *J*=15.8Hz *J*=6.5Hz *J*=4.1Hz, 1H, H2b), 2.45 (s, 1H, H9), 2.62 (ddd, *J*=15.8Hz *J*=11.1Hz *J*=7.1Hz, 1H, H2a), 2.93 (ddd, *J*=13.5Hz, *J*=7.1Hz, *J*=4.1Hz, 1H, H1a), 3.36 (s, 3H, NCH_3), 5.69 (s, 1H, H12)

^{13}C-NMR ($CDCl_3$): δ 15.7 (q, C25), 18.5 (q, C26), 18.7 (t, C7), 21.4 (q, C23/24), 23.1 (q, C27), 26.3 (q, C29), 26.4 (q, C23/24), 26.5 (t, C15), 26.7 (t, C16), 28.6 (q, C28), 31.9 (s, C17), 32.1 (t, C6), 32.5 (t, C21), 34.2 (t, C2), 36.7 (s, C10), 37.7 (t, C22), 38.3 (q, NCH_3), 39.7 (t, C1), 42.5 (t, C19), 43.5 (s, C8/20), 43.6 (s, C8/20), 45.3 (s, C14), 47.8 (s, C4), 48.5 (d, C18), 55.4 (d, 5), 61.1 (d, C9), 128.1 (d, C12), 171.4 (s, C13), 174.0 (s, C30), 200.2 (s, C11), 217.4 (s, C3)

3.4.28 (*18R*, *20S*)-*N*-Hydroxy-3-hydroxyimino-*N*-methyl-11-oxo-olean-12-en-30-amide [26]



Procedure: Oxime formation

A solution of ketone **25** (320mg, 0.643mmol, 1.00equiv.) and hydroxylamine hydrochloride (223mg, 3.22mmol, 5.00equiv.) in dry pyridine (4mL) was stirred at rt under TLC-monitoring (DCM:MeOH 40:1) Upon complete conversion (3h), the reaction mixture was diluted with DCM and washed with cooled 10% HCl, satd. $NaHCO_3$, and brine, dried over Na_2SO_4, and evaporated to leave a white solid as crude material, which was dissolved in MeOH and evaporated to give a fine white solid, which was triturated with DCM to give pure target compound **26** as white solid.

Yield: 320mg (97%) as white solid

Physical properties:

R_f-value = 0.14 (Hex :EtOAc 1:1 + 0.1% AcOH)

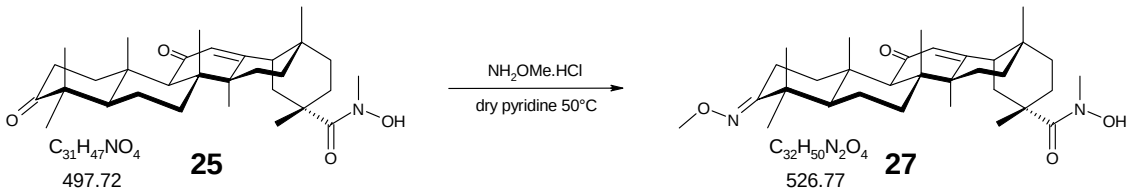
Optical rotation: $[\alpha]_D^{20}$ = +110.8 (c = 1.0 in $CHCl_3$)

HRMS: calcd. [M+1]: 513.3687;
found [M+1]: 513.3681; dev.: 1.15ppm

^{1}H-NMR ($CDCl_3$): δ 0.82 (s, 3H, H28), 0.97-1.16 (m, 3H, H1b, H5, H16b), 1.07 (s, 3H, H23/24), 1.15-1.27 (m, 2H, H15b, H21b), 1.15 (s, 3H, H26), 1.17 (s, 3H, H23/24), 1.2 (s, 3H, H29), 1.23 (s, 3H, H25), 1.31-1.40 (m, 2H, H6b, H22b), 1.35 (s, 3H, H27), 1.44-1.73 (m, 5H, H6a, H7a, H7b, H19b, H22a), 1.85 (td, *J*=13.6Hz, *J*=4.4Hz, 1H, H15a), 2.02-2.15 (m, 1H, H16a), 2.13-2.33 (m, 1H, H2b, H18, H19a, H21a), 2.41 (s, 1H, H9), 2.75-2.85 (m, 1H, H1a), 3 (dt, *J*=15.6Hz, *J*=4.6Hz, 1H, H2a), 3.26 (s, 3H, NCH_3), 5.70 (s, 1H, H12)

^{13}C-NMR ($CDCl_3$): δ 15.6 (q, C25), 17.1 (t, C2), 18.1 (t, C7), 18.5 (q, C26), 22.9 (q, C27), 23.2 (q, C23/24), 25.9 (q, C29), 26.4 (t, C16), 26.6 (t, C15), 27.2 (q, C23/24), 28.5 (q, C28), 31.8 (s+t, C17, C21), 32.3 (t, C6), 37 (s, C10), 37.7 (t, C22), 38 (q, NCH_3), 38.9 (t, C1), 40.2 (s, C4), 42.1 (t, C19), 43.5 (s, C20), 44.1, 45.3 (2×s, C8, C14), 48.7 (d, C18), 55.4 (d, C5), 61.3 (d, C9), 127.7 (d, C12), 166.8 (s, C3), 171.9 (s, C13), 175.5 (s, C30), 201.1 (s, C11)

3.4.29 (*18R*, *20S*)-*N*-Hydroxy-*N*-methyl-3-methoxyimino-11-oxo-olean-12-en-30-amide [27]



Procedure: Oxime formation

A solution of ketone **25** (500mg, 1.01mmol, 1.00equiv.) and methoxylamine hydrochloride (252mg, 3.01mmol, 3.00equiv.) in dry pyridine (5mL) was stirred at rt under TLC-monitoring (DCM:MeOH 40:1). Upon complete conversion (3h), the reaction mixture was diluted with DCM and washed with chilled 10% HCl, satd. $NaHCO_3$, and brine, dried over Na_2SO_4, and

evaporated. The crude material was purified by column chromatography (SiO_2: 25g DCM:MeOH 60:1 to DCM:MeOH 50:1) to give pure target compound **27** as white solid foam.

Yield: 536mg (quantitative) as white solid foam

Physical properties:

R_f-value = 0.23 (Hex :EtOAc 1:1 + 0.1% AcOH)

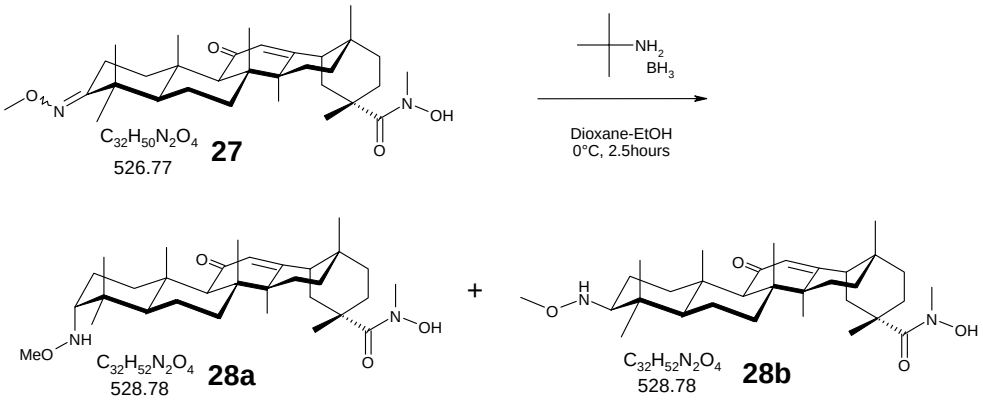
Optical rotation: $[\alpha]_D^{20}$ = +96.7 (c = 0.5 in $CHCl_3$)

HRMS: calcd. [M+1]: 527.3843;
found [M+1]: 527.3833; dev.:2.00ppm

^{1}H-NMR ($CDCl_3$): δ 0.82 (s, 3H, H28), 0.96-1.05 (m, 3H, H1b, H5, H16b), 1.07 (s, 3H, H23/24), 1.12-1.24 (m, 1H, H15b), 1.14 (s, 3H, H26), 1.18 (s, 3H, H23/24), 1.19 (s, 3H, H29), 1.22 (s, 3H, H25), 1.23-1.40 (m, 2H, H21b, H22b), 1.34 (s, 3H, H27), 1.38-1.68 (m, 6H, H6a, H6b, H7a, H7b, H19b, H22a), 1.83 (td, *J*=13.5Hz, *J*=4.1Hz, 1H, H15a), 1.99-2.14 (m, 1H, H16a), 2.11-2.24 (m, 4H, H2b, H18, H19a, H21a), 2.38 (s, 1H, H9), 2.72-2.82 (m, 1H, H1a), 2.86-2.96 (m, 1H, H2a), 3.36 (s, 3H, NCH_3), 3.82 (s, 3H, OCH_3), 5.66 (s, 1H, H12)

^{13}C-NMR ($CDCl_3$): δ 15.7 (q, C25), 17.7 (t, C2), 18.2 (t, C7), 18.6 (q, C26), 23.1 (q, C27), 23.4 (q, C23/24), 26.3 (q, C29), 26.5 (t, C15), 26.7 (t, C16), 27.3 (q, C23/24), 28.5 (q, C28), 31.9 (s, C17), 32.5, 32.7 (2×t, C6, C21), 37.0 (s, C10), 37.7 (t, 22), 38.3 (q, NCH_3), 39.1 (t, C1), 40.1 (s, C4), 42.4 (t, C19), 43.4, 43.5 (2×s, C8, C20), 45.4 (s, C14), 48.5 (d, C18), 55.6 (d, C5), 61 (q, OCH_3), 61.4 (d, C9), 128.2 (d, C12), 165.6 (s, C3), 170.9 (s, C13), 173.7 (s, C30), 200.5 (s, C11)

3.4.30 (*3S*, *18R*, *20S*)-*N*-Hydroxy-*N*-methyl-3-methoxyamino-11-oxo-olean-12-en-30-amide [28]



Procedure: Oxime reduction with BH_3.*t*BuNH$_2$

Oxime **27** (275mg, 0.522mmol, 1.0equiv.) was dissolved in dioxane:EtOH 2:1 (12mL). This solution was cooled to 0°C before BH_3.*t*BuNH$_2$ (91mg, 1.04mmol, 2.00equiv.) was added at 0°C and 5min later 3N HCl was added dropwise *via* a syringe. The reaction mixture was stirred at 0°C for several hours. After some time the reaction mixture became milky turbid but well stirrable. According to TLC almost all starting material was converted to the target compound. Addition of another equivalent of BH_3.*t*BuNH$_2$ and HCl did not lead to further conversion. The reaction mixture was worked up by pouring onto satd. $NaHCO_3$, and extraction with EtOAc (TLC-check), washing of the organic layers with brine, drying over Na_2SO_4 and evaporation to give crude material (280mg after drying *in vacuo* overnight). It was purified by column chromatography (MPLC, SiO_2: 30g, DCM:Et_2O 2:1) and precipitation from DCM:MeOH to give pure target compound (50mg, 18.1%) as white solid.
If needed the material, can be transformed to the corresponding hydrochloride **29** by taking up in THF, precipitating with HCl in Et_2O (1M, 2equiv.) and washing with Et_2O.

Yield: 50mg (18.1%) as white solid

Physical properties:

R_f-value = 0.31 (Tol : EtOAc 2:1 + 0.1% AcOH)

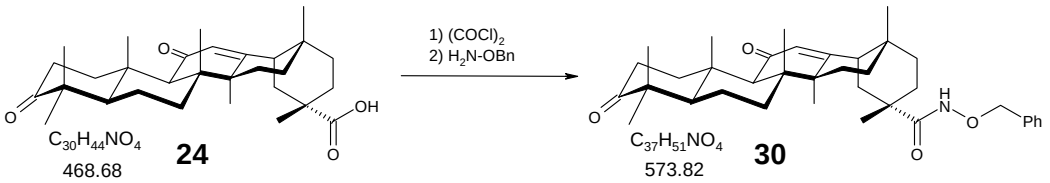
Optical rotation: $[\alpha]_D^{20}$ = 152.6°(c = 0.7 in $CHCl_3$:MeOH 5:1)

HRMS: calcd. [M+1]: 529.4000;
found [M+1]: 529.3993; dev.: 1.43ppm

^{1}H-NMR ($CDCl_3$:MeOD 5:1): δ 0.73 (s, 3H, H23/24), 0.73-0.79 (m, 1H, H5), 0.82 (s, 3H, H28), 0.90-0.99 (m, 1H, H1b), 0.98-1.05 (m, 1H, H16b), 1.07 (s, 3H, H23/24), 1.13 (s, 3H, H26), 1.14 (s, 3H, H25), 1.16-1.32 (m, 2H, H15b, H21b), 1.21 (s, 3H, H29), 1.32-1.52 (m, 5H, H2b, H6b, H7b, H22a, H22b), 1.39 (s, 3H, H27), 1.54-1.74 (m, 3H, H6a, H7a, H19b) 1.79-1.92 (m, 2H, H2a, H15a), 2.05 (td, *J*=13.6Hz, *J*=4.4Hz, 1H, H16a), 2.16-2.32 (m, 1H, H18, H19a, H21a), 2.40 (s, 1H, H9), 2.52 (dd, *J*=11.8Hz, *J*=4.1Hz, 1H, H3), 2.72 (dt, *J*=13.4Hz, *J*=3.4Hz, 1H, H1a), 3.25 (s, 3H, NCH_3), 3.52 (s, 3H, OMe), 5.69 (s, 1H, H12)

^{13}C-NMR ($CDCl_3$:MeOD 5:1): δ 15.8 (q, C25), 16.4 (q, C23/24), 17.0 (t, C6), 18.4 (q, C26), 22.8 (q, C27), 23.0 (t, C2), 25.6 (q, C29), 26.2 (t, C15), 26.4 (t, C16), 28.2, 28.3 (2×q, C28, C23/24), 31.6 (s, C17), 31.9 (t, C21), 32.5 (t, C7), 36.6 (s, C4), 37.0 (s, C10), 37.6 (t, C22), 37.7 (q, NCH_3), 38.9 (t, C1), 41.9 (t, C19), 43.2 (s, C20), 44.1 (s, C14), 45.2 (s, C8), 48.5 (d, C18), 55.9 (d, C5), 61.2 (q, OCH_3), 61.6 (d, C9), 67.5 (d, C3), 127.6 (d, C12), 171.6 (s, C13), 175.6 (s, C30), 201.5 (s, C11)

3.4.31 (*18R*, *20S*)-*N*-Benzyloxy-3,11-dioxo-olean-12-en-30-amide [30]



Procedure: Amide formation *via* acid chloride (oxalyl chloride method)

Carboxylic acid **24** (1.00g, 2.13mmol, 1.00equiv.) was suspended in dry DCM (20mL) and DMF (5 drops, syringe-needle) and oxalyl chloride (0.240mL, 2.55mmol, 1.2equiv.) was added at 0°C and the reaction mixture was allowed to reach rt and was stirred under TLC-monitoring. The suspension turned to a clear solution quickly and was analyzed by TLC. TLC

samples were prepared by quenching in dry MeOH and allowing standing for 10min before TLC analysis (SiO_2: Hex:EtOAc 2:1). After 1h the reaction mixture was evaporated, co-evaporated from DCM once and re-dissolved in DCM (precautions to exclude moisture were taken). The clear solution was cooled to 0°C and a solution of TEA (0.648g, 6.40mmol, 3.0equiv.) and $BnONH_2$ (0.289g, 2.35mmol, 1.1equiv.) was added over a period of 10min. The reaction was stirred at 0°C monitored *via* TLC (SiO_2: Hex:EtOAc 2:1 and amino-phase: DCM:MeOH 40:1). Upon complete conversion the reaction mixture was diluted with DCM and washed with 1N HCl, satd. $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated to give a white solid foam. The product was purified by column chromatography (SiO_2: 60g DCM:MeOH 50:1) and passing over a short bed of amino-phase gel (7g) to give pure target compound **30** as white solid foam.

Yield: 1.050g (85.8%) as white solid foam.

Physical properties:

R_f-value = 0.29 (Hex : EtOAc 1:1)
R_f-value = 0.45 (DCM : MeOH 30:1 + TEA)

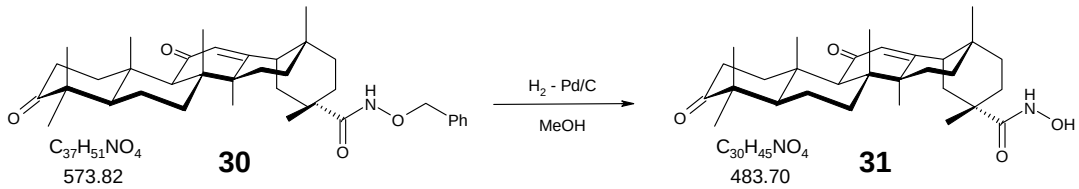
Optical rotation: $[\alpha]_D^{20}$ = +138.8 (c=1.0 in $CHCl_3$)

HRMS: calcd. [M+1]: 574.3891;
found [M+1]: 574.3886; dev.: 0.80ppm

^{1}H-NMR ($CDCl_3$): δ 0.81 (s, 3H, H28), 0.97-1.05 (m, 1H, H16b), 1.07 (s, 3H, H23/24), 1.1 (s, 3H, H23/24), 1.12 (s, 3H, H29), 1.16 (s, 3H, H26), 1.15-1.23 (m, 1H, H15b), 1.27 (s, 3H, H25), 1.25-1.32 (m, 1H, H5), 1.31 (s, 3H, H27), 1.32-1.42 (m, 4H, H1b, H21b, H22a, H22b), 1.4-1.48 (m, 1H, H6b), 1.49-1.60 (m, 2H, H7a, H7b), 1.60-1.69 (m, 3H, H6a, H19a, H19b), 1.78-1.87 (m, 1H, H15a), 1.84-1.92 (m, 1H, H21a), 2.00 (dt, *J*=13.6Hz, *J*=4.3Hz, 1H, H16a), 2.05-2.13 (m, 1H, H18), 2.3-2.40 (m, 1H, H2b), 2.39 (s, 1H, H9), 2.57-2.68 (m, 1H, H2a), 2.90-3.00 (m, 1H, H1a), 4.94 (bs, 2H, OCH_2Ph), 5.50 (s, 1H, H12), 7.35-7.45 (m, 5H, 5×Ph-H), 8.31 (s, 1H, NH)

^{13}C-NMR ($CDCl_3$): δ 15.6 (q, C25), 18.5 (q, C26), 18.7 (t, C7), 21.4 (q, C23/24), 23.3 (q, C27), 26.31 (t, C15/16), 26.35 (q, C23/24), 26.4 (t, C15/16), 28.4 (q, C28), 29.5 (q, C29), 31.2 (t, C21), 31.8 (s, C17), 32.1 (t, C6), 34.2 (t, C2), 36.7 (s, C10), 37.3 (t, C22), 39.8 (t, C1), 41.3 (t, C19), 43.0 (s, C20), 43.2 (s, C14), 45.1 (s, C8), 47.78 (s, C4), 47.84 (d, C18), 55.4 (d, C5), 61.0 (d, C9), 78.0 (t, O**C**H_2Ph), 128.4 (d, PhCH), 128.7 (d, PhCH), 128.9 (d, C12), 129.3 (d, PhCH), 135.3 (s, PhCH), 169.2 (s, C13), 173.6 (s, C30), 199.3 (s, C11), 217.2 (s, C3)

3.4.32 (*18R*, *20S*)-*N*-Hydroxy-3,11-dioxo-olean-12-en-30-amide [31]



Procedure: Hydrogenolytic debenzylation

O-Benzyl-hydroxamic acid **30** (500mg, 0.871mmol, 1.00equiv.) was dissolved in MeOH (50mL) and the atmosphere was exchanged to argon. Pd/C (50mg) was added and the atmosphere was exchanged to H_2. Since - according to TLC – the conversion stopped, additional Pd/C was added after several hours and after one day. After approximately 24h the reaction mixture was filtered over a bed of Celite®, a few drops of TEA were added to the filtrate, which was evaporated. The crude material was purified by column chromatography (vacuum-flash, SiO_2: 40g, DCM:MeOH 50:1 + 0.1% TEA to DCM:MeOH 30:1 + 0.1% TEA) to give the target compound in pure form according to TLC (DCM:MeOH 30:1 + TEA), accepting loss of some material due to small amounts of a by-product with very similar R_f-value. Pure target compound was collected as lyophilisate from dioxane:water solution.

Yield: 236mg (56%) as white lyophilisate

Physical properties:

R_f-value = 0.09 (DCM : MeOH 30:1 + TEA)

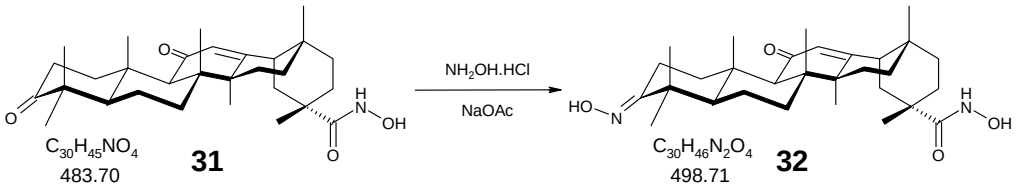
Optical rotation: $[\alpha]_D^{20}$ = +168.9 (c=1.1 in $CHCl_3$)

HRMS: calcd. [M+1]: 484.3421;
found [M+1]: 484.3423; dev.: -0.46ppm

^{13}C-NMR ($CDCl_3$): δ 15.7 (q, C25), 18.5 (q, C26), 18.8 (t, C6), 21.4 (q, C23/24), 23.3 (q, C27), 26.4 (q, C23/24), 26.4 (t, C15/16), 26.5 (t, C15/16), 28.4 (q, C28), 29.5 (q, C29), 31.0 (t, C21), 31.8 (s, C17), 32.1 (t, C7), 34.2 (t, C2), 36.7 (s, C10), 37.3 (t, C22), 39.8 (t, C1), 40.9 (t, C19), 42.3 (s, C20), 43.3 (s, C14), 45.2 (s, C8), 47.77 (s, C4), 47.82 (d, C18), 55.4 (d, C5), 61.1 (d, C9), 128.5 (d, C12), 169.9 (s, C13), 173.8 (s, C30), 199.8 (s, C11), 217.3 (s, C3)

^{1}H-NMR ($CDCl_3$): δ 0.83 (s, 3H, H28), 1.00-1.05 (m, 1H, H16b), 1.07 (s, 3H, H23/24), 1.10 (s, 3H, H23/24), 1.15-1.24 (m, 1H, H15b), 1.16 (s, 3H, H26), 1.17 (s, 3H, H29), 1.25-1.34 (m, 1H, H5), 1.26 (s, 3H, H25), 1.34-1.49 (m, 5H, H1b, H7b, H21b, H22a, H22b), 1.37 (s, 3H, H27), 1.47-1.61 (m, 2H, H6a, H6b), 1.61-1.73 (m, 2H, H7a, H19b), 1.77-2.00 (m, 3H, H15a, H19a, H21a), 1.97-2.08 (m, 1H, H16a), 2.16-2.27 (m, 1H, H18), 2.30-2.40 (m, 1H, H2b), 2.43 (s, 1H, H9), 2.55-2.66 (m, 1H, H2a), 2.88-2.98 (m, 1H, H1a), 5.78 (s, 1H, H12)

3.4.33 (*18R*, *20S*)-*N*-Hydroxy-3-hydroxyimino-11-oxo-olean-12-en-30-amide [32]



Procedure: Oxime formation

Ketone **31** (50mg, 0.103mmol, 1.00equiv.), NaOAc (30mg, 0.36mmol, 3.5equiv.) and $NH_3OH.Cl$ (25mg, 0.36mmol, 3.5equiv.) were dissolved in $CHCl_3$:MeOH 2:1 (2.5mL). In a closed reaction tube (15mL-volume) the reaction mixture was stirred at 60°C in a sandbath under HPLC monitoring. After 90min the reaction mixture was allowed to cool to rt and was stirred for further 2h. The reaction mixture was diluted with $CHCl_3$, washed three times with half satd. NaCl. The aqueous layers were extracted with $CHCl_3$ (some MeOH was added), the combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated (a drop of TEA was added for stability).The residue was taken up in distilled dioxane (5mL) and lyophilized to give the target compound in pure form only contaminated with small amounts of TEA and dioxane according to ^{1}H-NMR and ^{13}C-NMR.

Yield: 45mg (87%) as white lyophilisate

Physical properties:

R_f-value = 0.15 (DCM:MeOH 20:1 + 0.5% TEA)

Optical rotation: $[\alpha]_D^{20}$ = +93.9 (c=0.5 in $CHCl_3$)

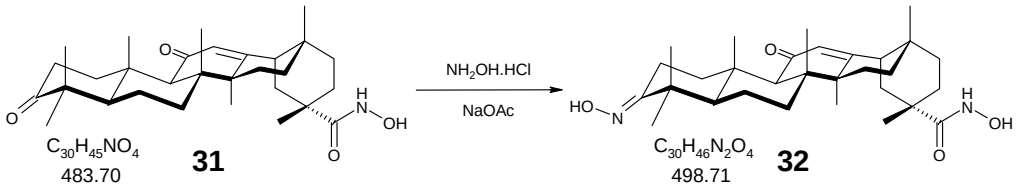
HRMS: calcd. [M+1]: 499.3530

found [M+1]: 499.3531; dev.: -0.08ppm

^{1}H-NMR ($CDCl_3$): δ 0.83 (s, 3H, H28), 0.98-1.10 (m, 3H, H1b, H5, H16b), 1.07 (s, 3H, H23/24), 1.15 (s, 3H, H26), 1.16 (s, 3H, H23/24), 1.17-1.25 (m, 1H, H15b), 1.18 (s, 3H, H29), 1.23 (s, 3H, H25), 1.34 (s, 3H, H27), 1.36-1.52 (m, 5H, H6b, H7b, H21b, H22a, H22b), 1.57-1.75 (m, 3H, H6a, H7a, H19b), 1.78-1.90 (m, 2H, H15a, H19a), 1.92-2.04 (m, 2H, H16a, H21a), 2.15-2.29 (m, 2H, H2b, H18, 2.37 (s, 1H, H9), 2.83 (app. dt, *J*=13.4, *J*=4.6, 1H, H1a), 3.04 (app. dt, *J*=15.5Hz, *J*=4.3Hz, 1H, H2a), 5.74 (s, 1H, H12)

^{13}C-NMR ($CDCl_3$): δ 15.7 (q, C25), 17.2 (t, C2), 18.2 (t, C6), 18.7 (q, C26), 23.2 (q, C23/24), 23.3 (q, C27), 26.38, 26.42 (2×t, C15,C16), 27.1 (q, C23/24), 28.4 (q, C28), 29.5 (q, C29), 31.0 (t, C21), 31.8 (s, C17), 32.4 (t, C7), 37.0 (s, C10), 37.3 (t, C22), 39.1 (t, C1), 40.4 (s, C4), 40.9 (t, C19), 42.3 (s, C17), 43.3, 45.4 (2×s, C8, C20), 47.9 (d, C18), 55.6 (d, C5), 61.4 (d, C9), 128.5 (d, C12), 167.0 (s, C3), 169.5 (s, C13), 173.8 (s, C30), 200.1 (s, C11)

3.4.34 (*18R*, *20S*)-*N*-Hydroxy-3-methoxyimino-11-oxo-olean-12-en-30-amide [33]



Procedure: Oxime formation

Ketone **31** (96mg, 0.198mmol, 1.00equiv.), NaOAc (65mg, 0.79mmol, 4.0equiv.) and $NH_2OMe.HCl$ (50mg, 0.60mmol, 3.0equiv.) were dissolved in $CHCl_3$:MeOH 2:1 (4.5mL) and stirred at 60°C in a sand-bath under an atmosphere of argon under ^{1}H-NMR monitoring. After 7h the reaction mixture was allowed to reach rt and was stirred overnight. Complete conversion was obtained according to ^{1}H-NMR. The reaction mixture was diluted with DCM, washed with halve saturated NaCl twice, diluted AcOH, satd. $NaHCO_3$ and brine (always with subsequent back-extraction with DCM:MeOH 10:1). The organic layer was dried over Na_2SO_4, 1 drop of TEA was added and evaporated, taken up in distilled dioxane (5mL) and lyophilized to give target compound in pure form according to NMR.

Yield: 97mg (95.3%) as white lyophilisate

Physical properties:

R_f-value = 0.19 (DCM:MeOH 20:1 + 0.5% TEA)

Optical rotation: $[\alpha]_D^{20}$ = +102.0 (c=0.5 in $CHCl_3$)

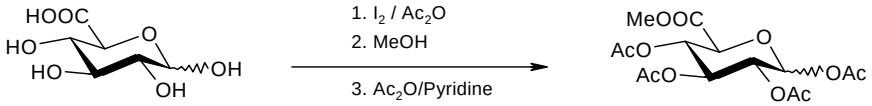
HRMS: calcd. [M+1]: 513.3687

found [M+1]: 513.3688; dev.: -0.23ppm

^{1}H-NMR ($CDCl_3$): δ 0.82 (s, 3H, H28), 0.99-1.10 (m, 3H, H1b, H5, H16b), 1.06 (s, 3H, H23/24), 1.14 (s, 3H, H26), 1.17 (bs, 6H, H23/24, H29), 1.15-1.30 (m, 2H, H15b, H21b), 1.22 (s, 3H, H25), 1.34 (s, 3H, H27), 1.34-1.55 (m, 4H, H6b, H7b, H22a, H22b), 1.56-1.76 (m, 3H, H6a, H7a, H19a), 1.76-1.90 (m, 2H, H15a, H19b), 1.90-2.10 (m, 2H, H16a, H21a), 2.12-2.26 (m, 2H, H2b, H18), 2.37 (s, 1H, H9), 2.75-2.85 (m, 1H, H1a), 2.85-2.98 (m, 1H, H2a), 3.81 (s, 3H, OCH_3), 5.75 (bs, 1H, H12)

^{13}C-NMR ($CDCl_3$): δ 15.6 (q, C25), 17.7 (t, C2), 18.2 (t, C6), 18.6 (q, C26), 23.28, 23.43 (2×q, C23/24, C27), 26.4 (t, C16), 26.4 (t, C15), 27.2 (q, C23/24), 28.4 (q, C28), 29.5 (q, C29), 31.0 (t, C21), 31.8 (s, C17), 32.4 (t, C7), 37.0 (s, C10), 37.3 (t, C22), 39.1 (t, C1), 40.1 (s, C4), 40.8 (t, C19), 42.3, 43.3, 45.4 (s, C8, C14, C20), 47.8 (d, C18), 55.6 (d, C5), 61.0 (q, OCH_3), 61.4 (d, C9), 128.6 (d, C12), 165.6 (s, C3), 169.4 (s, C13), 173.8 (s, C30), 200.2 (s, C11)

3.4.35 Methyl (1,2,3,4-tetra-*O*-acetyl-D-glucopyranosyl) uronate [37]



$C_6H_{10}O_7$
194.14

$C_{15}H_{20}O_{11}$
376.32

37

Procedure: Peracetylation – Methyl ester formation - Reacetylation

Glucuronic acid (9.79g, 50.4mmol, 1.00equiv) was dissolved in Ac_2O (140mL) and cooled to 0°C. I_2 was added gradually and the reaction mixture (suspension as white solid in dark solution) was stirred at 5°C for 2h and at rt overnight, monitored *via* TLC (DCM:MeOH 3:1). The reaction mixture was diluted with DCM (210mL) and washed with satd. $Na_2S_2O_3$ twice (100mL – 50mL) with subsequent back-extraction of the aqueous layer (phase separation is slow and poor). The organic layer was washed with water, brine, dried over $MgSO_4$, filtered and evaporated and co-evaporated from toluene to give 26.78g of a crude product as yellowish-white sticky solid, which was dissolved in dry MeOH (90mL) and stirred at reflux temperature under TLC-monitoring (DCM:MeOH 3:1, Hex:EtOAc 2:1). After two days only traces of starting material were observed in the mixture and the target compound was the major reaction product. The solvent was evaporated and co-evaporated twice with toluene to remove all MeOH. The residue (17.05g) was dissolved in pyridine, a small spatula tip of DMAP was added and the reaction mixture was cooled to 0°C. Ac_2O was added dropwise and the reaction mixture was stirred at rt overnight.

The reaction mixture was cooled to 0°C and quenched with MeOH (25mL). The solvents were evaporated by successive co-evaporation with toluene (three times) to leave a crude product of 24g which was purified by flash column chromatography (SiO_2, Tol:EtOAc 2:1 to 1:1) followed by recrystallization from hot dry EtOH to give crystalline fractions of a white solid 15.0g. ^{1}H-NMR of pure target compound **37**[94] indicated an anomeric ratio of α:β = 2:1.

Yield: 15.0g (79.0%) as white solid

Physical properties:

[CAS-7355-18-2]

α-anomer (out of mixture):

R_f-value = 0.3 (SiO_2, Hex:EtOAc 3:2)

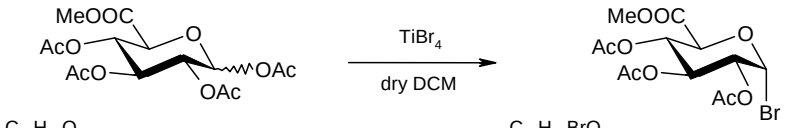
^{1}H-NMR ($CDCl_3$) δ 2.023, 2.051, 2.056, 2.20 (4×s, 12H, 4×OAc), 3.76 (s, 3H, OCH_3), 4.42 (d, $J_{4,5}$=10.2Hz, 1H, H5), 5.13 (dd, $J_{2,3}$=10.2Hz, $J_{1,2}$=3.7Hz, 1H, H2), 5.23 (app. t, J=9.9Hz, 1H, H4), 5.53 (app. t, J=9.9Hz, 1H, H3), 6.40 (d, $J_{1,2}$=3.6Hz, 1H, H1)

β–anomer (out of mixture):

R_f-value = 0.3 (SiO_2, Hex:EtOAc 3:2)

^{1}H-NMR ($CDCl_3$) δ 2.02-2.06 (3×s, 9H, 3×OAc), 2.12 (s, 3H, OAc), 3.75 (s, 3H, OCH_3), 4.19 (d, $J_{4,5}$=9.4Hz, 1H, H5), 5.10-5.35 (m, 3H, H2, H3, H4,) 5.77 (d, $J_{1,2}$=7.7Hz, 1H, H1)

3.4.36 Methyl (2,3,4-tri-*O*-acetyl–α-D-bromoglucopyranosyl) uronate [38]



$C_{15}H_{20}O_{11}$ 376.32 **37** $C_{13}H_{17}BrO_9$ 397.18 **38**

Procedure: Formation of glycosyl bromide with $TiBr_4$

$TiBr_4$ (5.57g, 15.1mmol, 5.7equiv.) was added in one portion to dry DCM (25mL) at temperatures below -10°C (cooling bath: EtOH/ice). Subsequently, a solution of **37** (α:β 2:1, 1.00g, 2.66mmol, 1.00equiv.) in dry DCM (10mL) was added within 15min keeping the temperature below -5°C, whereupon the orange solution turned to a deep red. The reaction mixture was allowed to warm to rt and was stirred at rt overnight and then for three days at reflux temperature under ^{1}H-NMR monitoring. As soon as no substantial additional conversion was detectable the reaction mixture was cooled to 0°C, diluted with DCM (50mL) and poured on ice (50g). The aqueous layer was extracted twice with DCM, the combined organic layers were washed twice with water, once with satd. $NaHCO_3$ and once with brine, dried over Na_2SO_4 and evaporated to give a crude product. Purification by column chromatography (SiO_2: 35g, Hex:EtOAc 3:1) gave the target compound in pure form.

Yield: 650mg (61.6%) as yellowish solid

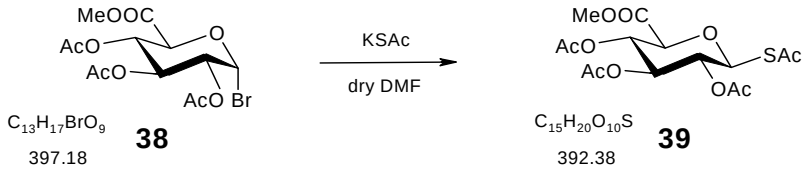
Physical Properties:

[CAS-21085-72-3]

R_f-value = 0.67 (SiO_2, Hex:EtOAc 1:1)

^{1}H-NMR ($CDCl_3$) δ 2.057, 2.063 (2×s, 6H, 2×OAc), 2.10 (s, 3H, OAc), 3.77 (s, 3H, OCH_3), 4.58 (d, $J_{4,5}$=10.2Hz, 1H, H5), 4.86 (dd, $J_{2,3}$=10.0Hz, $J_{1,2}$=4.0Hz, 1H, H2), 5.24 (app. t, J=10.0Hz, 1H, H4), 5.62 (app. t, J=9.7Hz, 1H, H3), 6.65 (d, $J_{1,2}$=4.0Hz, 1H, H1)

3.4.37 Methyl (2,3,4-tri–*O*-acetyl-1-*S*-acetyl-1-thio-β-D-glucopyranosyl) uronate [39]



$C_{13}H_{17}BrO_9$ **38** 397.18

$C_{15}H_{20}O_{10}S$ **39** 392.38

Procedure: Formation of anomeric thioacetate

To a chilled solution of glycosyl bromide **38** (10.00g, 25.18mmol, 1.0equiv.) in dry DMF (100mL) potassium thioacetate (5.32g, 46.58mmol, 1.85equiv.) was added in several portions within 10min at -10°C and the reaction mixture was stirred at temperatures below 0°C for 1h and at approximately 10°C for another hour, monitored by HPTLC (Hex:Et_2O 1:1). Then the reaction mixture was diluted with EtOAc (400mL) and treated with satd. $NaHCO_3$ (200mL) in a separatory funnel. The phases were separated, the aqueous layer was extracted two more times with EtOAc and the combined organic layers were washed five times with water, twice with brine, dried over Na_2SO_4 and evaporated to give a crude product of yellowish solid. Recrystallization from dry EtOH-EtOAc (60mL + 5mL) gave pure target compound **39**[95] as white crystals.

Yield: 8.15g (82.5%) as white crystals

Physical Properties:

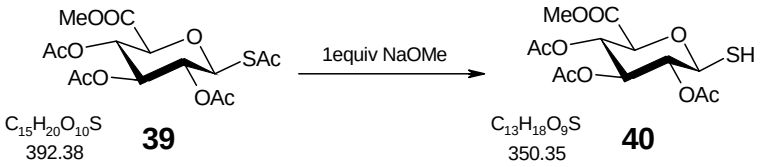
[CAS-314079-40-8]

R_f-value = 0.61 (SiO_2, Hex:EtOAc 1:1)

m.p.: 163-167°C (EtOH-EtOAc) (Lit[95].: 162°C (dry EtOH))

^{1}H-NMR ($CDCl_3$) δ 2.03 (2×s, 6H, 2×OAc), 2.04 (s, 3H, OAc), 2.39, (s, 3H, SAc), 3.74 (s, 3H, OCH_3), 4.17 (d, $J_{4,5}$=9.9Hz, 1H, H5), 5.11-5.23 (m, 2H, H2, H4), 5.29-5.37 (m, 2H, H1, H3)

3.4.38 Methyl (2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl) uronate [40]



$C_{15}H_{20}O_{10}S$ 392.38 **39** $C_{13}H_{18}O_9S$ 350.35 **40**

Procedure: Cleavage of anomeric thioacetate

A solution of NaOMe in dry MeOH (1M, 3.1mL, 3.1mmol, 1.0equiv) was added to a solution of thioacetate **39** (1.22g, 3.10mmol, 1.00equiv.) in dry MeOH:$CHCl_3$ 1:2 (15mL) at cooling bath temperature of -60 to -55°C (acetone/CO_2). The mixture was stirred at this temperature until precipitation hindered stirring. The reaction mixture was allowed to warm to -45°C and was analyzed by TLC (Hex:EtOAc 1:1). The reaction mixture was diluted with DCM (25mL) and quenched with satd. NH_4Cl and water. The phases were separated, the aqueous layer was extracted with DCM three more times, the combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated. The crude material was purified by column chromatography (SiO_2: 25g, Hex:EtOAc 1:1) to afford pure target compound **40**[95] as white solid. Storage under an argon atmosphere at -25°C is recommended.

Yield: 970mg (89.0%) as white solid

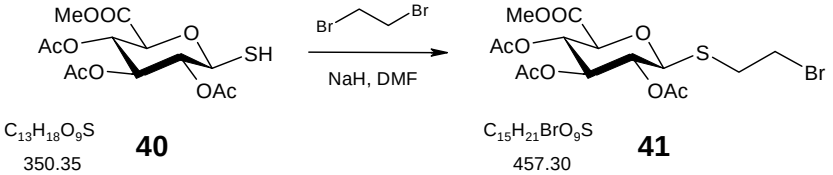
Physical Properties:

[CAS-68354-87-0]

R_f-value = tailing until 0.3 (SiO_2, Hex:EtOAc 1:1)

^{1}H-NMR ($CDCl_3$) δ 2.03 (2×s, 6H, 2×OAc), 2.06 (s, 3H, OAc), 2.40 (bs, 1H, SH), 3.76 (s, 3H, OCH_3), 4.02-4.09 (m, 1H, H5), 4.58 (d, $J_{1,2}$=10.0Hz, 1H, H1), 4.97-5.03 (m, 1H, H2), 5.19-5.29 (m, 2H, H3, H4)

3.4.39 Methyl (2-bromoethyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosid) uronate [41]



$C_{13}H_{18}O_9S$ 350.35 **40** $C_{15}H_{21}BrO_9S$ 457.30 **41**

Procedure: Alkylation of thiol

A solution of thiol **40** (500mg, 1.43mmol, 1.00equiv.) and dibromoethane (1.07g, 1.43mmol, 4.00equiv.) in DMF (17mL) was purged with Ar for 10min before NaH (40mg, 1.64mmol, 1.15equiv., washed with hexanes) was added portionwise under external ice/EtOH cooling. The reaction was monitored by HPTLC (easier to distinguish between starting material and by-product). After each addition the reaction turned slightly yellow and turbid and turned colorless again after ~15min. After addition of 40mg of NaH all starting material was consumed and the reaction mixture was quenched with halve satd. NH_4Cl (100mL) at 0°C and was extracted with DCM. The combined organic layers were washed with water (4x20mL) and brine, dried over Na_2SO_4, evaporated and co-evaporated from toluene twice to give a crude product which was purified by column chromatography (evaporated onto 1g of Celite®, SiO_2: 20g, Hex:EtOAc 3:1 to Hex:EtOAc 1:2) to give pure target compound **41** and small amounts of pure polar by-product **43** (33mg, 3.2%).

Yield: 525mg (80.4%) as white solid

Physical Properties:

R_f-value = 0.36 (Hex:EtOAc 2:1)

m.p.: 154-155.5°C (needles, dry EtOH, sweating from 150°C)

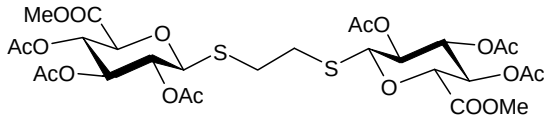
Optical rotation: $[\alpha]_D^{20}$ = -45.4 (c=0.4 in $CHCl_3$)

Combustion analysis:	calcd.	C 39.40%	H 4.63%	N 0.00%	S 7.01%
	found	C 39.24%	H.4.67%	N <0.05%	S 6.88%

^{1}H-NMR ($CDCl_3$) δ 2.03 (s, 6H, 2×OAc), 2.07 (s, 3H, OAc), 2.95-3.05 (m, 1H, SC**H**H), 3.16-3.26 (m, 1H, SCH**H**), 3.52-3.60 (m, 2H, CH_2Br), 3.76 (s, 3H, OCH_3), 4.05 (d, $J_{4,5}$=9.4Hz, 1H, H5), 4.59 (d, $J_{1,2}$=10.0Hz, 1H, H1), 5.05 (app. t, *J*=9.4Hz, 1H, H2), 5.21 (app. t, *J*=9.4Hz, 1H, H4), 5.28 (app. t, *J*=9.1Hz, 1H, H3)

^{13}C-NMR ($CDCl_3$) δ 20.5, 20.6, 20.7 (3×q, 3×CO**C**H_3), 30.7 (t, CH_2Br), 32.2 (t, SCH_2), 53.0 (q, OCH_3), 69.1 (d, C4), 69.3 (d, C2), 72.7 (d, C3), 76.2 (d, C5), 83.7 (d, C1) 166.7 (s, C6), 169.29, 169.34, 167.0 (3×s, 3×**C**OCH_3)

3.4.40 1,1'-Ethylenebis [methyl (2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl) uronate] [43]



$C_{28}H_{38}O_{18}S_2$
726.73 **43**

Physical Properties:

R_f-value = 0.14 (Hex:EtOAc 1:1)

m.p.: 171-174°C (EtOH:EtOAc)

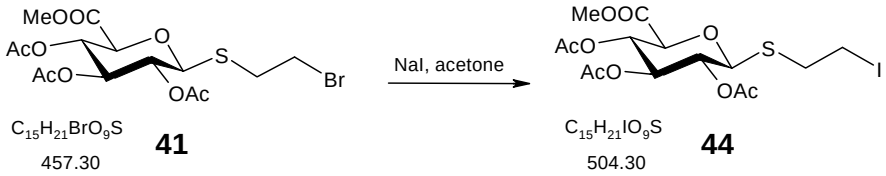
Optical rotation: $[\alpha]_D^{20}$ = -52.3 (c=0.9 in $CHCl_3$)

Combustion analysis: calcd.	C 46.28%	H 5.27%	N 0.00%	S 8.82%
	C 46.03%	H.5.30%	N <0.05%	S 8.66%

^{1}H-NMR ($CDCl_3$) δ 2.03 (s, 12H, 4×OAc), 2.08 (s, 6H, 2×OAc), 3.77 (s, 6H, OCH_3), 2.81-2.93 (m, 2H, 2×SC**H**H), 2.97-3.10 (m, 2H, 2×SCH**H**), 4.11 (d, $J_{4,5}$=9.7Hz, 2H, H5), 4.61 (d, $J_{1,2}$=10.0Hz, 2H, H1,), 5.06 (app. t, *J*=9.5Hz, 2H, H2), 5.23 (app. t, *J*=9.6Hz, 2H, H4), 5.29 (app. t, *J*=9.2Hz, 2H, H3)

^{13}C-NMR ($CDCl_3$) δ 20.5, 20.6, 20.7 (3×q, 3×CO**C**H_3), 30.4 (t, S**C**H_2CH_2S, SCH_2**C**H_2S), 52.9 (q, OCH_3), 69.3 (d, C4), 69.4 (d, C2), 73.0 (d, C3), 76.1 (d, C5), 83.6 (d, C1) 166.9 (s, C6), 169.3, 169.4, 170.0 (3×s, 3×**C**OCH_3)

3.4.41 Methyl (2-iodoethyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosid) uronate [44]



$C_{15}H_{21}BrO_9S$ **41** 457.30

$C_{15}H_{21}IO_9S$ **44** 504.30

Procedure: Finkelstein reaction

Bromide **41** (3.38g, 7.39mmol, 1.00equiv.) was dissolved in a 15% NaI solution (5.54g, 36.9mmol, 5.00equiv.) in acetone, the solution was stripped with argon for 15min and stirred in an argon atmosphere for 16h at 4°C in the dark, monitored by ^{1}H-NMR.

The reaction mixture was diluted with toluene (200mL) and washed with water, brine, dried over $MgSO_4$ and was evaporated to give a white solid which was evaporated onto 12g SiO_2 (12g). Purification by column chromatography (Hex:EtOAc 2:1 to Hex:EtOAc 1:1) gave pure target compound **44**.

Yield: 3.53g (94.7%) as white solid

Physical Properties:

R_f-value = 0.36 (Hex:EtOAc 2:1)

m.p.: 164-166°C (Hex:EtOAc)

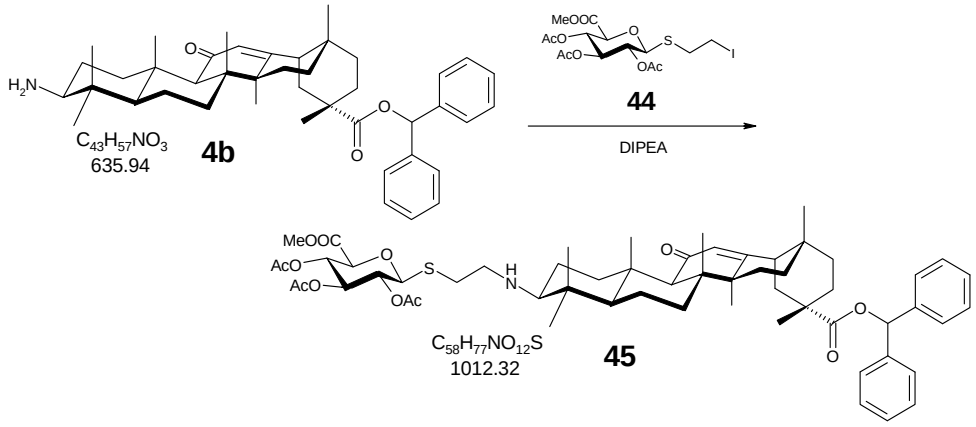
Optical rotation: $[\alpha]_D^{20}$ = -45.7 (c=0.9 in $CHCl_3$)

Combustion analysis:	calcd.	C 35.73%	H 4.20%	N 0.00%	S 6.36%
	found	C 35.64%	H.4.13%	N <0.05%	S 6.23%

^{1}H-NMR ($CDCl_3$) δ 2.03 (s, 6H, 2×OAc), 2.06 (s, 3H, OAc), 2.99-3.09 (m, 1H, SC**H**H), 3.18-3.31 (m, 1H, SCH**H**), 3.32-3.41 (m, 2H, ICH_2), 3.76 (s, 3H, OCH_3), 4.05 (d, $J_{4,5}$=9.4Hz, 1H, H5), 4.58 (d, $J_{1,2}$=10.0Hz, 1H, H1), 5.04 (app. t, *J*=9.4Hz, 1H, H2), 5.21 (app. t, *J*=9.4Hz, 1H, H4), 5.28 (app. t, *J*=9.1Hz, 1H, H3)

^{13}C-NMR ($CDCl_3$) δ 2.9 (t, ICH_2), 20.4, 20.5, 20.6 (3×q, 3×CO**C**H_3), 33.1 (t, CH_2), 52.9 (q, OCH_3), 69.2 (d, C4), 69.4 (d, C2), 72.8 (d, C3), 76.2 (d, C5), 83.7 (d, C1) 166.7 (s, C6), 169.2, 169.3, 169.9 (3×s, 3×**C**OCH_3)

3.4.42 (*3S*, *18R*, *20S*)-3-[2-[Methyl (2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl) uronate]-ethylamino]-11-oxo-olean-12-en-30-oic acid, diphenylmethyl ester [45]



Procedure: *N*-Alkylation in DMF

A solution of iodide **44** (3.50g, 6.94mmol, 2.00equiv.) was dissolved in DMF (11mL) and purged with argon for 15min at rt. Amine **4b** (2.20g, 3.47mmol, 1.00equiv.) and DIPEA (2.25g, 17.35mmol, 5.00equiv.) were added and the reaction mixture was stirred at 45-50°C external oil bath temperature overnight. Since after 16h more than 97% conversion of starting material was observed (according to HPLC) the reaction mixture was allowed to reach rt and was diluted with EtOAc, washed two times with satd. $NaHCO_3$, several times with water and once with brine. The organic layer was dried over $MgSO_4$ and was evaporated and co-evaporated from toluene. The crude material was purified by MPLC (SiO_2: 220g, DCM:MeOH 80:1 to DCM:MeOH 20:1. to give pure target compound **45** as white solid foam.

Yield: 2.79g (79.5%) as white solid foam

Physical properties:

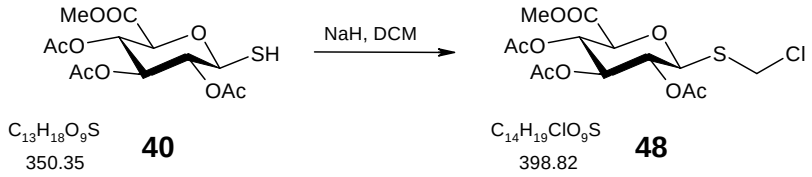
R_f-value = 0.30 ($CHCl_3$:MeOH 9:1 + 0.1% AcOH)

Optical rotation: $[\alpha]_D^{20}$ = +75.0 (c=0.9 in $CHCl_3$)

^{1}H-NMR ($CDCl_3$): δ 0.67 (s, 3H, H28), 0.70 (m, 1H, H5), 0.75 (s, 3H, H23/24), 0.87-1.00 (m, 1H, H1b, H16b), 1.00 (s, 3H, H23/24), 1.09 (s, 3H, H26), 1.13 (s, 3H, H25), 1.10-1.20 (m, 1H, H15b), 1.18 (s, 3H, H29), 1.20-1.50 (m, 6H, H2b, H6b, H7b, H21b, H22a, H22b), 1.36 (s, 3H, H27), 1.55-1.75 (m, 4H, H2a, H6a, H7a, H19b), 1.79 (td, *J*=13.6Hz, *J*=4.4Hz, 1H, H15a), 1.99 (m, 5H, H3, H16a, H18, H19a, H21a), 2.02 (s, 6H, 2×OAc), 2.06 (s, 3H, OAc), 2.33 (s, 1H, H9), 2.66-2.82 (m, 3H, H1a, SC**H**H, NC**H**H), 2.85-2.95 (m, 1H, SCH**H**), 3.00-3.10 (m, 1H, NCH**H**), 3.75 (s, 3H, OCH_3), 4.03 (d, $J_{4,5}$=9.6Hz, 1H, H5#), 4.60 (d, $J_{1,2}$=10.0Hz, 1H, H1#), 5.06 (dd, $J_{1,2}$=10.0Hz, $J_{2,3}$=9.1Hz, 1H, H2#), 5.20-5.29 (m, 2H, H3#, H4#), 5.51 (s, 1H, H12), 6.94 (s, 1H, $OCHPh_2$), 7.25-7.41 (m, 10H, 10×PhCH)

^{13}C-NMR ($CDCl_3$): δ 16.3 (q, C25), 16.5 (q, C23/24), 17.9 (t, C6), 18.7 (q, C26), 20.5 (q, CO**C**H_3), 20.6 (q, CO**C**H_3), 20.7 (q, CO**C**H_3), 23.3 (q, C27), 24.4 (t, C2), 26.39, 2.41 (2×t, C15, C16), 28.25 (q, C29), 28.29 (q, C23/24), 28.37 (q, C28), 31.2, 31.3 (2×t, SCH_2, C21), 31.7 (s, C17), 32.8 (t, C7), 37.2 (s, C10), 37.5 (t, C22), 38.5 (s, C4), 39.9 (t, C1), 41.1 (t, C19), 43.1 (s, C20), 44.0 (s, C8), 45.3 (s, C14), 47.8 (t, NCH_2), 48.0 (d, C18), 52.9 (q, OMe), 56.1 (d, C5), 61.9 (d, C9), 65.9 (d, C3), 69.4 (d, C2#), 69.7 (d, C4#), 73.1 (d, C3#), 76.3, 76.6 (d, $OCHPh_2$, C5#), 84.0 (d, C1#), 127.0, 127.3 (2×d, PhCH, C12), 127.8 (d, PhCH), 128.1 (d, PhCH), 128.5 (d, PhCH), 128.56 (d, PhCH), 128.63 (d, PhCH), 140.07 (s, PhC), 140.11 (s, PhC), 166.9 (s, C6#), 168.7 (s, C13), 169.27 (s, **C**OCH_3), 169.31 (s, **C**OCH_3), 170.1 (s, **C**OCH_3), 175.2 (s, C30), 200.2 (s, C11)

3.4.43 Methyl (chloromethyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosid) uronate [48]



$C_{13}H_{18}O_9S$ 350.35 **40** $C_{14}H_{19}ClO_9S$ 398.82 **48**

Procedure: Alkylation of thiosugar

A solution of thiol **40** (426mg, 1.22mmol, 1.00equiv.) in DCM/DMF 2:1 (15mL) was purged with Ar for 10min before NaH (29mg, 1.22mmol, 1.0equiv., washed with hexanes) was added portionwise under external ice/EtOH cooling. After each addition the reaction turned slightly red and turbid and turned colorless again after some minutes. The reaction mixture was stirred at -10°C under HPTLC monitoring. Upon complete conversion of starting material (1h) the reaction mixture was quenched with halve satd. NH_4Cl (25mL) and extracted with DCM. The combined organic layers were washed four times with water, once with brine, dried over Na_2SO_4 and evaporated to leave a crude product, which was purified by column chromatography (SiO_2: 20g, Hex:EtOAc 3:1 to 1:1) to give pure target compound **48** as white crystals followed by pure by-product **49 (**60mg, 15.4%) in smaller amounts.

Yield: 390mg (80.4%) as white solid

Physical Properties:

R_f-value = 0.43 (SiO_2, Hex:EtOAc 1:1)

m.p.: 91.5-92.5°C (dry EtOH)

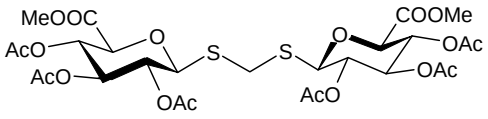
Optical rotation: $[\alpha]_D^{20}$ = -125.6 (c=0.9 in $CHCl_3$)

Combustion analysis:	calcd.:	C 42.16%	H 4.80%	N 0.00%	S 8.04%
	found	C 42.02%	H.4.78%	N <0.05%	S 7.85%

^{1}H-NMR ($CDCl_3$) δ 2.03 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.07 (s, 3H, OAc), 3.77 (s, 3H, OCH_3), 4.11 (d, $J_{4,5}$=9.8Hz, 1H, H5), 4.67 (d, J=12.0Hz, 1H, SCH**H**), 4.89 (d, $J_{1,2}$=10.2Hz, 1H, H1), 4.94 (d, J=12.0Hz, 1H, SC**H**H), 5.10 (dd, $J_{2,3}$=9.9Hz, $J_{1,2}$=10.1, 1H, H2), 5.24 (app. t, J=9.6Hz, 1H, H4), 5.34 (app. t, J=9.3Hz, 1H, H3)

^{13}C-NMR ($CDCl_3$) δ 20.4, 20.5, 20.6 (3×q, 3×CO**C**H_3), 45.3 (t, CH_2Cl), 53.0 (q, OCH_3), 69.2 (d, C4), 69.5 (d, C2), 72.8 (d, C3), 76.2 (d, C5), 81.4 (d, C1) 166.7 (s, C6), 169.3, (s, 2×**C**OCH_3), 169.9 (s, **C**OCH_3)

3.4.44 1,1'-Methylenebis[methyl (2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl) uronate] [49]



$C_{27}H_{36}O_{18}S_2$ **49**

712.71

Physical Properties:

R_f-value = 0.16 (SiO_2, Hex:EtOAc 1:1)

m.p.: 189-191°C (EtOH:EtOAc)

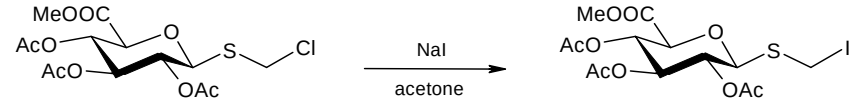
Optical rotation: $[\alpha]_D^{20}$ = -142.4 (c=0.9 in $CHCl_3$)

Combustion analysis:	calcd.	C 45.50%	H 5.09%	N 0.00%	S 9.00%
	found	C 45.20%	H 5.09%	N <0.05%	S 8.83%

^{1}H-NMR ($CDCl_3$) δ 2.027, 2.029 (2×s, 12H, 4×OAc), 2.11 (s, 6H, 2×OAc), 3.76 (s, 6H, OCH_3), 3.97 (s, 2H, SCH_2S), 4.07 (d, $J_{4,5}$=9.6Hz, 1H, H5), 4.75 (d, $J_{1,2}$=10.1Hz, 2H, H1), 5.09 (dd, $J_{2,3}$=9.0Hz, $J_{1,2}$=10.1, 2H, H2), 5.22 (app. t, J=9.4Hz, 2H, H4), 5.28 (app. t, J=9.1Hz, 2H, H3)

^{13}C-NMR ($CDCl_3$) δ 20.5, 20.6, 20.7 (3×q, 3×CO**C**H_3), 30.8 (t, SCH_2S), 53.0 (q, OCH_3), 69.2, 69.3 (2×d, C4, C2), 72.9 (d, C3), 76.1 (d, C5), 82.2 (d, C1) 166.8 (s, C6), 169.3, 169.4, 170.0 (3×s, 3×**C**OCH_3)

3.4.45 Methyl (iodomethyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosid) uronate [50]



$C_{14}H_{19}ClO_9S$ 398.82 **48**

$C_{14}H_{19}IO_9S$ 490.27 **50**

Procedure: Finkelstein reaction

Chloride **48** (120mg, 0.301mmol, 1.00equiv.) was dissolved in a 15% solution of NaI (225mg, 1.50mmol, 5.00equiv) in acetone and the solution was stirred at rt overnight. According to HPTLC (concentration zone, Hex:EtOAc 1:1) complete conversion of starting material was observed. The reaction mixture was diluted with EtOAc and washed with water and brine. The organic layer was dried over $MgSO_4$ and evaporated. The crude product was submitted to column chromatography (SiO_2: 10g, Hex: EtOAc 3:1) to give pure target compound **50**, which has to be stored below -20°C to prevent coloration.

Yield: 150mg (quantitative) as white solid.

Physical Properties:

R_f-value = 0.46 (SiO_2, Hex:EtOAc 1:1)

m.p.: 99-102°C (Hex:EtOAc)

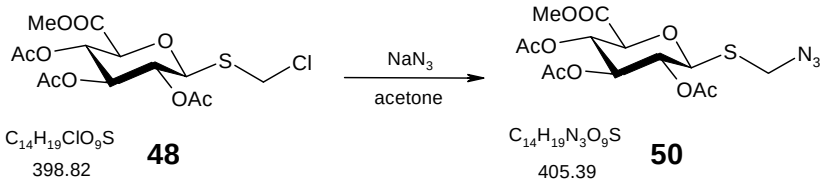
Optical rotation: $[\alpha]_D^{20}$ = -199.2 (c=0.9 in $CHCl_3$)

Combustion analysis: calcd.	C 34.30%	H 3.91%	N 0.00%	S 6.54%
found	C 34.23%	H.3.93%	N <0.05%	S 6.33%

^{1}H-NMR ($CDCl_3$) δ 2.035, (s, 3H, OAc), 2.041 (s, 3H, OAc), 2.06 (s, 3H, OAc), 3.77 (s, 3H, OCH_3), 4.13 (d, $J_{4,5}$=9.9Hz, 1H, H5), 4.37 (s, 2H, SCH_2I), 4.70 (d, $J_{1,2}$=10.1Hz, 1H, H1), 5.13 (dd, $J_{1,2}$=10.1Hz, $J_{2,3}$=9.2Hz, 1H, H2), 5.25 (app. t, *J*=9.7Hz, 1H, H4), 5.37 (app. t, *J*=9.3 Hz, 1H, H3).

^{13}C-NMR ($CDCl_3$) δ 0.3 (t, SCH_2I), 20.5, (q, $CO\underline{C}H_3$), 20.6 (q, 2×$CO\underline{C}H_3$), 53.0 (q, OCH_3), 69.1, 69.3 (2×d, C2, C4), 72.8 (d, C3), 76.2 (d, C5), 84.2 (d, C1) 166.7 (s, C6), 169.3 (s, 2×$\underline{C}OCH_3$), 170.0 (s, $\underline{C}OCH_3$)

3.4.46 Methyl (azidomethyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosid) uronate [51]



$C_{14}H_{19}ClO_9S$ 398.82 **48**

$C_{14}H_{19}N_3O_9S$ 405.39 **50**

Procedure: Azide formation

Chloride **48** (200mg, 0.501mmol, 1.00equiv.) and NaN_3 (39mg, 0.602mmol, 1.2equiv.) were dissolved in acetone/DMF (18mL, 5:2) and the solution was stirred at reflux temperature (60°C oil bath) monitored by HPTLC (Et_2O:Hex 1:1). After almost 36h complete consumption of starting material was observed and the reaction mixture was worked up by dilution with water (80mL) and extraction with EtOAc. The combined organic layers were washed twice with water, once with brine, dried over Na_2SO_4 and evaporated to give the crude product which was purified by column chromatography (SiO_2: 10g Hex:EtOAc 3:1) to give pure target compound.

Yield: 180mg (88.5%) as white solid

Physical Properties:

R_f-value = 0.30 (SiO_2, Hex:EtOAc 2:1)

m.p.: 119-120°C (dry EtOH)

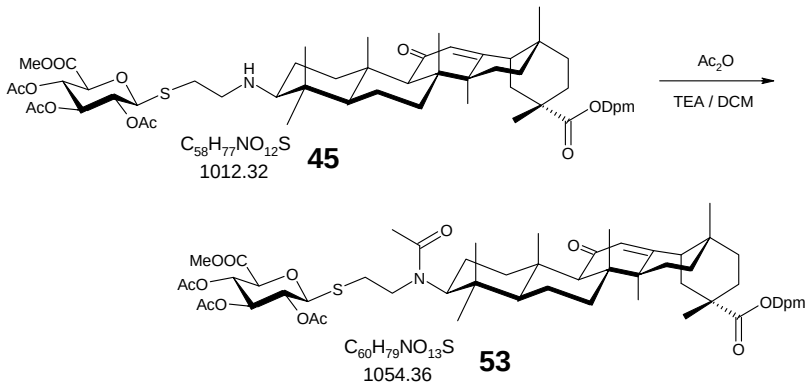
Optical rotation: $[\alpha]_D^{20}$ = -174.4 (c=0.9 in $CHCl_3$)

Combustion analysis:	calcd.	C 41.48%	H 4.72%	N 10.37%	S 7.91%
	found	C 41.64%	H.4.79%	N 9.59%	S 7.71%

^{1}H-NMR ($CDCl_3$) δ 2.03 (2×s, 6H, 2×OAc), 2.08 (s, 3H, OAc), 3.75 (s, 3H, OCH_3), 4.08 (d, $J_{4,5}$=9.6Hz, 1H, H5), 4.37 (d, *J*=13.5Hz, 1H, SCH**H**), 4.42 (d, *J*=13.6Hz, 1H, SC**H**H), 4.77 (d, $J_{1,2}$=10.1Hz, 1H, H1), 5.10 (dd, $J_{1,2}$=10.1Hz, $J_{2,3}$=9.0Hz, 1H, H2), 5.25 (app. t, *J*=9.6Hz, 1H, H4), 5.31 (app. t, *J*=9.1 Hz, 1H, H3).

^{13}C-NMR ($CDCl_3$) δ 20.45, 20.57, 20.58 (3×q, 3×CO**C**H_3), 51.0 (t, SCH_2N_3), 52.9 (q, OCH_3), 69.1 (d, C4), 69.8 (d, C2), 72.9 (d, C3), 76.1 (d, C5), 81.9 (d, C1), 166.9 (s, C6), 169.30, 169.34, 170.0 (3×s, 3×**C**OCH_3)

3.4.47 (*3S*, *18R*, *20S*)-3-[*N*-[2-[Methyl (2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl) uronate]-ethyl]-(acetylamino)]-11-oxo-olean-12-en-30-oic acid, diphenylmethyl ester [53]



Procedure: *N*-Acetylation

A solution of amine **45** (2.110g, 2.084mmol, 1.00equiv.) in dry DCM (30mL) was stripped with argon for 15min and then cooled to 0°C. After the addition of TEA (2.0mL, 14.6mol, 7.0equiv.) and slow addition of Ac_2O (0.98mL, 10.4mmol, 5.0equiv.) the reaction mixture was stirred for 2h at 0°C and then at rt and monitored by TLC and HPLC. Upon complete conversion the reaction mixture was diluted with EtOAc, washed two times with 10% AcOH, with water, satd. $NaHCO_3$ and brine, dried over $MgSO_4$ and evaporated. The crude material was purified by MPLC (SiO_2: 114g, Tol:EtOAc 2.5:1 to Tol:EtOAc 1.5:1 to give pure target compound **53**.

Yield: 2.10g (95.6%) as white solid foam

Physical properties

R_f-value = 0.27 (Tol:EtOAc 1:1)

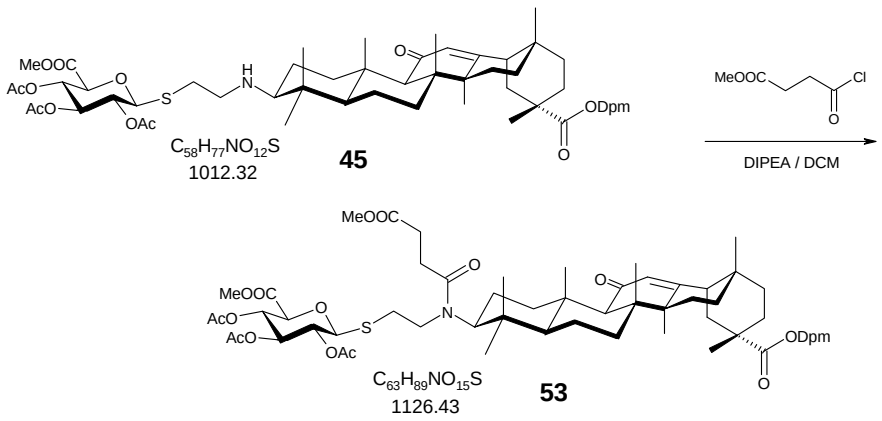
Optical rotation: $[\alpha]_D^{20}$ = +50.3 (c=0.8 in $CHCl_3$)

NMR-analysis: Two rotamers in a ratio of 6:4 are observed.

^{1}H-NMR ($CDCl_3$): δ 0.66, 0.67 (2×s, H28), 0.72-1.03 (m, 8H, 2xH23/24, H5, H16b), 1.03-1.20 (11H, H1b, H15b, H25, H26, H29), 1.20-1.93, (m, 13H, H2a, H6a, H6b, H7a, H7b, H15a, H19b, H21b, H22a, H22b, H27) 1.93-2.11 (m, 14H, 3×OAc, H2a, H21a, H16a, H18, H19a), 2.14 (s, 1.8H, NAc), 2.20 (s, 1.2H, NAc), 2.35 (s, 0.6H, H9), 2.38 (s, 0.4H, H9), 2.50-2.76, (m, 1H, SC**H**H), 2.82-3.10 (m, 2H, H1, SCH**H**), 3.18-3.32 (m, 1H, 0.6×H3, NC**H**H) 3.36 (dd, *J*=12.4Hz, *J*=3.0Hz, 0.6H, H3), 3.40-3.76 (m, 1H, NCH**H**), 3.74 (s, 1.8H, OCH_3), 3.74 (s, 1.2H, OCH_3), 4.06 (d, *J*=9.9Hz, 0.4H, H5#), 4.12 (d, *J*=9.8Hz, 0.6H, H5#), 4.47 (dd, *J*=12.8Hz, *J*=3.0Hz, 0.4H, H3), 4.55 (d, *J*=9.9Hz, 0.4H, H1#), 4.80 (d, *J*=10.1Hz, 0.6H, H1#), 5.00-5.09 (m, 1H, H2#), 5.05 (m, 1H, H2#), 5.17 (app. t, *J*=9.7Hz, 0.4H, H4#), 5.20 (app. t, *J*=9.7Hz, 1H, H4#), 5.28-5.34 (m, 1H, H3#), 5.51 (s, 0.4H, H12), 5.53 (s, 0.6H, H12), 6.93 (s, 1H, $OCHPh_2$), 7.26-740 (m, 10H, 10×PhH)

^{13}C-NMR ($CDCl_3$): δ 16.5, 17.5, 17.7, 18.6, 18.8, 20.45, 20.50, 20.59, 20.63, 20.72, 22.0 (q, NCO**C**H_3), 23.1 (q, NCO**C**H_3), 23.27, 23.33, 23.9, 26.3, 26.4, 28.2, 28.3, 28.5, 28.7, 29.0, 30.3, 31.1, 31.7, 32.6, 37.0, 37.2, 37.4, 40.4, 40.79, 40.84, 41.0, 41.08, 41.16, 43.15, 43.18, 44.0, 45.2, 45.3, 45.5, 47.3, 47.97 (d, C18), 48.04 (d, C18), 52.8 (q, O**C**H_3), 53.0 (q, O**C**H_3), 55.8 (d, C5), 57.1 (d, C5), 59.9 (d, C3), 61.7 (d, C9), 66.7 (d, C3), 69.1, 69.2, 69.4 (3×d, 2×C4#, C2#), 69.7 (d, C2#), 72.6 (d, C3#), 73.1 (d, C3#), 76.0 (d, C5#), 76.2 (d, C5#), 76.6 (d, $OCHPh_2$), 83.6 (d, C1#), 84.3 (d, C1#), 126.9 (d, PhCH), 127.0 (d, PhCH), 127.2 (d, PhCH), 127.80 (d, PhCH), 127.83 (d, PhCH), 128.09 (d, PhCH), 128.12 (d, C12), 128.5 (d, C12), 128.6 (d, PhCH), 140.00 (s, PhC), 140.05 (s, PhC), 140.08 (s, PhC), 140.13 (s, PhC), 166.6 (s, C6#), 167.0 (s, C6#), 169.0, 169.1, 169.25, 169.29 (4×s, 2×C13, 2×O**C**O**C**H_3), 169.35 (s, O**C**O**C**H_3), 169.42 (s, O**C**O**C**H_3), 169.9 (s, O**C**O**C**H_3), 170.0 (s, O**C**O**C**H_3), 171.6 (s, N**C**O**C**H_3), 175.16 (s, C30), 175.22 (s, C30), 199.7 (s, C11), 200.1 (s, C11)

3.4.48 (*3S*, *18R*, *20S*)-3-[*N*-[2-[Methyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl) uronate]-ethyl]-[(methylsuccinyl)amino]]-11-oxo-olean-12-en-30-oic acid, diphenylmethyl ester [54]



Procedure: *N*-Acylation

To a solution of amine **45** (0.350g, 0.346mmol, 1.00equiv.) in dry DCM (10mL) DIPEA (0.29mL, 1.73mmol, 5.00equiv.) was added and the reaction mixture was chilled to 0°C. methyl succinyl chloride (0.156g, 1.04mmol, 3.00equiv.) was added at 0°C gradually within 5min and the reaction mixture was stirred at 0°C under HPLC-monitoring. Upon complete conversion (1.5h) MeOH (2mL) was added and the reaction mixture was allowed to reach rt. The reaction mixture was distributed between satd. NH_4Cl and EtOAc (40mL), the phases were separated, the aqueous layer was extracted with EtOAc and the combined organic layers were washed with satd. NH_4Cl and brine, dried over Na_2SO_4 and evaporated. The crude material was purified by column chromatography (SiO_2: 17g, Hex:EtOAc 3:1 to Hex:EtOAc 3:2) to give pure target compound **53** as slightly yellowish solid foam.

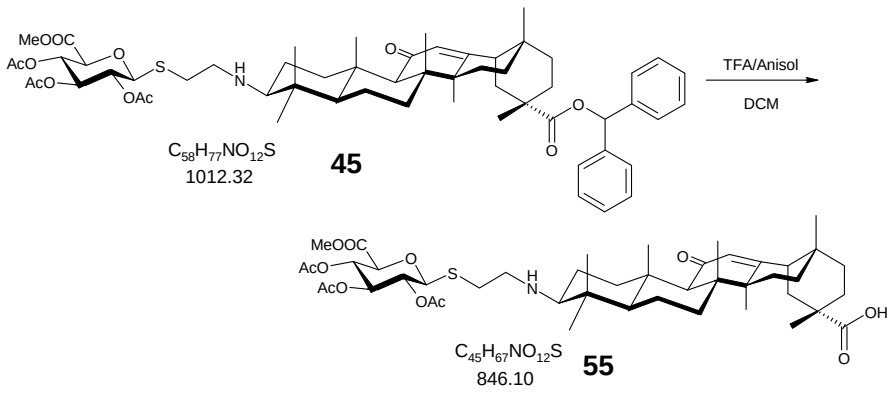
Yield: 379mg (97.3%) as slightly yellowish solid foam

Physical properties:

53 was converted to the corresponding acid **61** and only fully characterized at this stage

R_f-value = 0.34 (EtOAc:Hex 2:1)

3.4.49 (*3S*, *18R*, *20S*)-3-[2-[Methyl (2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl) uronate]-ethylamino]-11-oxo-olean-12-en-30-oic acid [55]



Procedure: Acidic diphenylmethyl ester cleavage

To a solution of ester **45** (400mg, 0.395mmol, 1.00equiv.) in dry DCM (20mL) anisole (0.43mL, 3.95mmol, 10.0equiv.) was added and the reaction mixture was cooled to 0°C. TFA (1.32mL, 17.8mmol, 45.0equiv.) was added dropwise and the reaction mixture was allowed to come to rt and was stirred under HPLC under TLC-monitoring ($CHCl_3$:MeOH 9:1 + 0.5% AcOH). After 3h all starting material was consumed. The reaction was neutralized with satd. $NaHCO_3$ (50mL) slowly at 0°C (pH ~8). The phases were separated, the aqueous layer was extracted four times with DCM (TLC check third time already empty) and the combined organic layers were washed with $NaHCO_3$, water and brine, dried over Na_2SO_4 and evaporated. The crude material was submitted to column chromatography (SiO_2: 20g, $CHCl_3$:MeOH 20:1 to 15:1) to give the target compound in pure form.

Yield: 250mg (74.8%) as white solid foam

Physical properties:

R_f-value = 0.08 ($CHCl_3$:MeOH 9:1 + 0.1% AcOH)

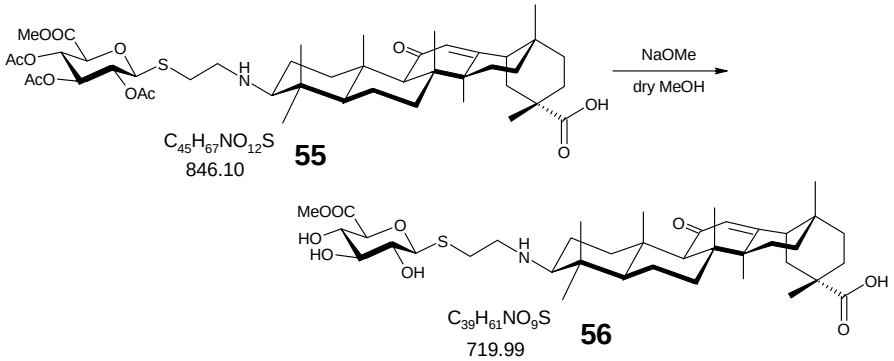
Optical rotation: $[\alpha]_D^{20}$ = +106.6° (c=1.0, $CHCl_3$)

HRMS: calcd. [M+1]: 846.4457;
found [M+1]: 846.4460; dev.: -0.38ppm

^{1}H-NMR ($CDCl_3$): δ 0.73 (m, 1H, H5), 0.82 (s, 3H, H28), 0.86 (s, 3H, H23/24), 0.85-0.95 (m, 1H, H1b), 0.95-1.05 (m, 1H, H16b), 1.07 (s, 3H, H23/24), 1.13 (s, 3H, H26), 1.14 (s, 3H, H25), 1.15-1.25 (m, 1H, H15b), 1.19 (s, 3H, H29), 1.30-1.52 (m, 5H, H6b, H7b, H21b, H22a, H22b), 1.37 (s, 3H, H27), 1.55-1.80 (m, 5H, H2a, H2b, H6a, H7a, H19b), 1.80-2.10 (m, 4H, H15a, H16a, H19a, H21a), 2.02 (bs, 6H, 2×OAc), 2.050 (s, 3H, OAc), 2.15-2.23 (m, 1H, H18), 2.25-2.35 (m, 1H, H3), 2.34 (s, 1H, H9), 2.80-3.05 (m, 4H, H1a, NC**H**H, SCH_2), 3.25-3.35 (m, 1H, 1×NCH**H**), 3.73 (s, 3H, OCH_3), 4.11 (d, *J*=9.9Hz, 1H, H5#), 4.66 (d, *J*=10.0Hz, 1H, H1#), 5.05 (app. t, *J*=9.6, 1H, H2#), 5.19 (app. t, *J*=9.8Hz, 1H, H4#), 5.28 (app. t, *J*=9.2Hz, 1H, H3#), 5.68 (s, 1H, H12)

^{13}C-NMR ($CDCl_3$): δ 16.21 (q, C25), 16.47 (q, C23/24), 17.71 (t, C6), 18.7 (q, C26), 20.5 (q, CO**C**H_3), 20.6 (q, CO**C**H_3), 20.7 (q, CO**C**H_3), 23.3 (t, C2), 23.4 (q, C27), 26.43 (t, C15), 26.50 (t, C16), 28.2 (q, C29), 28.5 (q, C23/24), 28.6 (q, C28), 29.6 (t, SCH_2), 31.1 (t, C21), 31.9 (s, C17), 32.8 (t, C7), 37.1 (s, C10), 37.8 (t, C22), 38.2 (s, C4), 39.4 (t, C1), 41.1 (t, C19), 43.2 (s, C20), 43.8, 45.4 (s, C8, C14), 47.0 (t, NCH_2), 48.3 (d, C18), 52.9 (q, OMe), 55.9 (d, C5), 61.7 (d, C9), 66.7 (d, C3), 69.3 (d, C4#), 69.5 (d, C2#), 73.0 (d, C3#), 76.0 (d, C5#), 84.0 (d, C1#), 128.5 (d, C12), 167.0 (s, C6#), 169.35, 169.43, 169.9 (s, C13, 2× **C**OCH_3) (s (s), 180.6 (s, C30), 200.0 (s, C11)

3.4.50 (*3S*, *18R*, *20S*)-3-[2-[Methyl (1-thio-β-D-glucopyranosyl) uronate]-ethylamino]-11-oxo-olean-12-en-30-oic acid [56]



Procedure: Zemplén deacetylation

Peracetate **55** (0.260g, 0.287mmol, 1.00equiv.) was dissolved in dry MeOH and 0.1M NaOMe was added at 0°C to adjust the pH to ~10.5. The reaction mixture was stirred at 0°C under TLC-monitoring ($CHCl_3$:MeOH 15:1 + 0.5% AcOH; $CHCl_3$:MeOH 3:1 + 0.5% AcOH).

Upon complete conversion the reaction mixture was neutralized (pH=6) by addition of freshly washed ion exchange resin. Filtration, washing of the resin with MeOH and evaporation gave target compound as white solid.

Yield: 200mg (89.4%) as white solid

Physical properties

R_f-value = 0.22 (EtOAc/MeOH 1:1 + 0.5% AcOH)

Retention time = 4.01min (HPLC-method B)

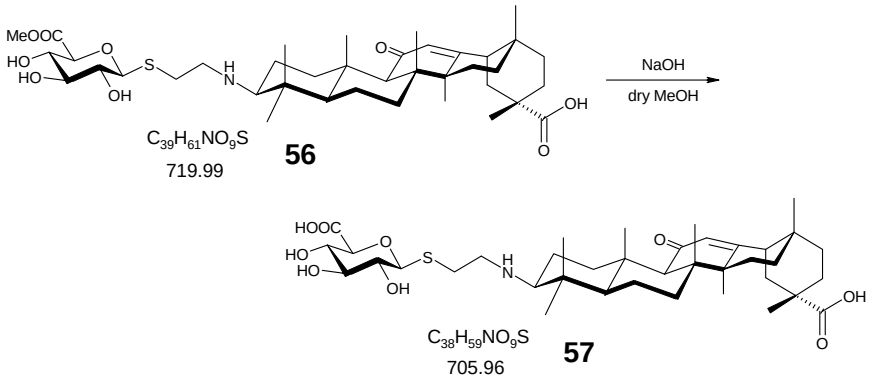
Optical rotation: $[\alpha]_D^{20}$ = +97.9° (c=1.0, MeOH)

HRMS: calcd. [M+1]: 720.4140;
found [M+1]: 720.4142; dev.: -0.23ppm

^{1}H-NMR (MeOD): δ 0.83 (s, 3H, H28), 0.85-0.95 (m, 1H, H5), 0.92 (s, 3H, H24), 0.98-1.08 (m, 2H, H1b, H16b), 1.11 (bs, 6H, H23, H29), 1.16 (s, 3H, H26), 1.18 (s, 3H, H25), 1.20-1.41 (m, 3H, H15b, H21b, H22b), 1.41-1.56 (m, 3H, H6b, H7b, H22a), 1.42 (s, 3H, H27), 1.60-2.00 (m, 8H, H2a, H2b, H6a, H7a, H15a, H19a, H19b, H21a), 2.15 (td, *J*=13.4Hz, *J*=4.1Hz, 1H, H16a), 2.28 (dd, *J*=13.0Hz, *J*=3.5Hz, 1H, H18), 2.48 (s, 1H, H9), 2.70 (m, 1H, H3), 2.83 (m, 1H, H1a), 2.95-3.05 (m, 2H, SCH_2), 3.28 (m, 1H, H2#), 3.10-3.30 (m, 2H, NCH_2), 3.40 (app. t, *J*=8.6Hz, 1H, H3#), 3.53 (app. t, *J*=9.0Hz, 1H, H4#), 3.78 (s, 3H, OCH_3), 3.92 (d, *J*=9.5Hz, 1H, H5#), 4.54 (d, *J*=9.2Hz, 1H, H1#), 5.70 (s, 1H, H12)

^{13}C-NMR (MeOD): δ 16.7 (q, C24), 16.8 (q, C25), 18.6 (t, C6), 19.3 (q, C26), 22.2 (t, C2), 23.8 (q, C27), 27.5 (t, C15), 27.7 (t, C16), 28.2 (t, SCH_2), 28.3 (q, C23), 29.28, 29.31 (2×q, C28, C29), 32.7 (t, C21), 33.0 (s, C17), 33.7 (t, C7), 38.2 (s, C10), 38.8 (s, C4), 39.4 (t, C22), 39.8 (t, C1), 43.3 (t, C19), 44.7 (s, C20), 45.8, 46.6 (s, C8, C14), 47.6 (t, NCH_2), 50.0 (s, C18), 53.0 (q, O**C**H_3), 56.5 (s, C5), 62.8 (s, C9), 67.5 (s, C3), 72.9 (s, C4#), 73.5 (s, C2#), 78.8 (s, C3#), 80.1 (s, C5#), 87.7 (s, C1#), 128.9 (s, C12), 171.1 (s, C6#), 173.6 (s, C13), 183.0 (s, C30), 202.2 (s, C11)

3.4.51 (*3S*, *18R*, *20S*)-3-[2-(1-Thio-β-D-glucopyranosyl) uronic acid)-ethylamino]-11-oxo-olean-12-en-30-oic acid [57]



Procedure: Ester hydrolysis

Methyl ester **56** (0.200g, 0.2768mmol, 1.00equiv.) was dissolved in dry MeOH and 0.2N NaOH in MeOH (6mL) was added at 0°C. The reaction mixture was stirred at rt under TLC-monitoring. For proper results TLC samples have to be acidified with little AcOH and be developed in $CHCl_3$:MeOH 10:3 really long-time. Upon complete conversion (3h) the reaction mixture was neutralized with ion exchange resin to pH=6-7, filtered, washed with MeOH and evaporated to give the target compound with uncertain content of sodium.

Yield: 200mg (>91%) as white solid.

Physical properties

R_f-value = 0.12 (EtOAc/MeOH 1:1 + 0.5% AcOH

Retention time = 4.02min (HPLC-method B)

Optical rotation: $[\alpha]_D^{20}$ = +119.5° (c=1.0, MeOH)

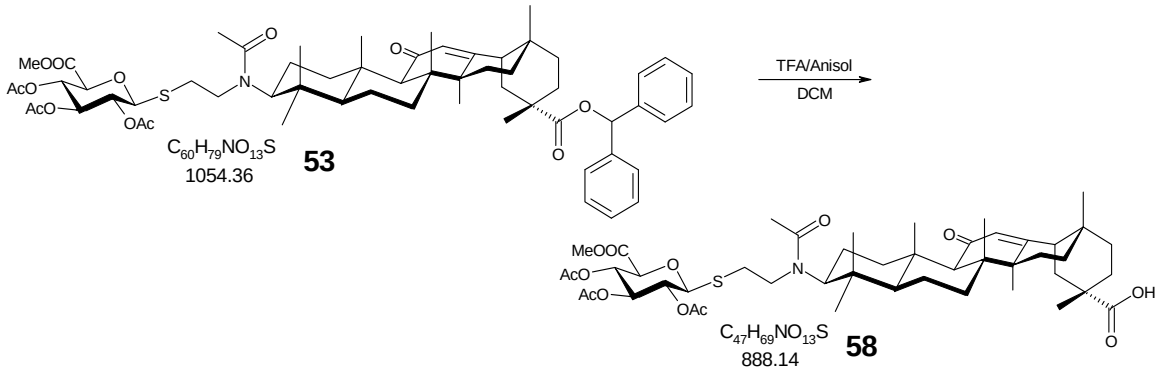
HRMS: calcd. [M+1]: 706.3983;
found [M+1]: 706.3984; dev.: -0.04ppm

^{1}H-NMR (MeOD): δ 0.74 (s, 3H, H28), 0.85-0.95 (m, 2H, H5, H16b), 0.91 (s, 3H, H24), 1.01 (m, 1H, H1b), 1.02 (s, 3H, H29), 1.06 (s, 3H, H26), 1.09 (s, 3H, H25), 1.10 (s, 3H, H23), 1.10-1.30 (m, 3H, H15b, H21b, H22b), 1.34 (s, 3H, H27), 1.34-1.47 (m, 3H, H6b, H7b, H22a), 1.50-1.65 (m, 2H, H6a, H19b), 1.66-1.90 (m, 6H, H2a, H2b, H7a, H15a, H19a, H21a), 2.07 (td, *J*=13.5Hz, *J*=4.3Hz, 1H, H16a), 2.20 (dd, *J*=13.1Hz, *J*=4.7Hz, 1H, H18),

2.42 (s, 1H, H9), 2.78 (m, 1H, H1a), 2.90 (dd, *J*=11.8Hz, *J*=4.7Hz, 1H, H3), 2.95-3.10 (m, 2H, SCH_2), 3.12-3.17 (m, 1H, H2#), 3.28-3.40 (m, 2H, H3#, H4, NCH_2), 3.53-3.60 (m, 1H, H5#), 4.50 (d, *J*=9.8Hz, 1H, H1#), 5.59 (s, 1H, H12)

^{13}C-NMR (MeOD): δ 16.7 (q, C24, C25), 18.5 (t, C6), 19.3 (q, C26), 20.9 (t, C2), 23.8 (q, C27), 27.2 (t, SCH_2), 27.5, 27.7 (2×t, C15, C16), 28.2 (q, C23), 29.3 (q, C28, C29), 32.7 (t, C7), 33.0 (s, C17), 33.6 (t, C21), 38.1 (s, C10), 38.8 (s, C4), 39.36 (t, C22), 39.44 (t, C1), 43.3 (t, C19), 44.7 (s, C20), 45.3 (t, NCH_2), 45.8, 46.6 (2×s, C14, C8), 50.0 (d, C18), 56.3 (d, C5), 62.6 (d, C9), 65.8 (d, C3), 73.4 (d, C4#), 74.2 (d, C2#), 78.8 (d, C5#), 79.2 (d, C3#), 86.8 (d, C1#), 128.8 (d, C12), 173.7 (s, C13), 176.2 (s, C6#), 183.1 (s, C30), 202.2 (s, C11)

3.4.52 (*3S*, *18R*, *20S*)-3-[*N*-[2-[Methyl (2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl) uronate]-ethyl]-(acetylamino)]-11-oxo-olean-12-en-30-oic acid [58]



Procedure: Acidic diphenylmethyl ester cleavage

To a cooled solution of ester **53** (2.00g, 1.90mmol, 1.00equiv.) in dry DCM (190mL) first anisol (2.07mL, 18.9mmol, 10.0equiv.) and then slowly TFA (6.3mL, 85.4mmol, 45.0equiv.) was added. The reaction mixture was stirred at cooling bath temperature of 0°C for 1h and then at rt monitored by TLC ($CHCl_3$:MeOH 15:1). Upon complete conversion (3h) the reaction mixture was neutralized with $NaHCO_3$ (70mL) (pH=8) before pH was adjusted with AcOH to 4-5. The phases were separated, the aqueous layer was extracted with DCM, and the combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated. The crude material was purified by MPLC (SiO_2: 60g, $CHCl_3$ to $CHCl_3$/MeOH 30:1) to give pure target compound **58** as white solid.

Yield: 1.50g (89.0%) as white solid

Physical properties:

R_f-value = 0.24 ($CHCl_3$:MeOH 9:1 + 0.1% AcOH)

Optical rotation: $[\alpha]_D^{20}$ = +47.7 (c=0.9 in $CHCl_3$)

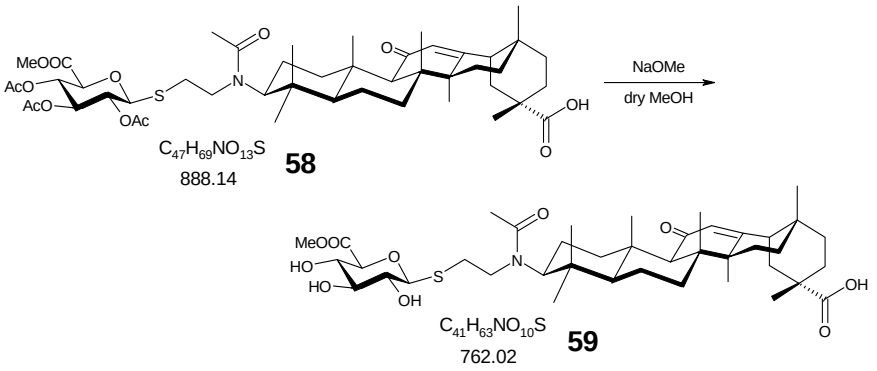
HRMS: calcd. [M+1]: 888.4562;
found [M+1]: 888.4552; dev.: 1.23ppm

^{1}H-NMR ($CDCl_3$): δ 0.76-1.10 (m, 12H, H1b, H5, H16b, 2×H23/24, H28), 1.10-1.22 (m, 7H, H15b, H25, H26), 1.23 (s, 3H, H29), 1.25-1.73 (m, 12H, H2b, H6a, H6b, H7a, H7b, H19b, H21b, H22a, H22b, H27), 1.75-2.10 (14H, H2a, H15a, H19a, H21a, H16a, 3×OAc), 2.12-2.24 (m, 1H, 18H), 2.15 (s, 1.8H, NAc), 2.20 (s, 1.2H, NAc, 2.38 (s, 0.6H, H9), 2.40 (s, 0.4H, H9), 2.53-2.76 (m, 1.2H, SCH_2), 2.80-3.10 (m, ~2H, H1a, SCH_2), 3.22-3.32 (m, 0.6H, NC**H**H), 3.37 (dd, *J*=12.3Hz, *J*=3.0Hz, 0.6H, H3), 3.42-3.76 (m, 1.4H, NC**H**H, NCH**H**), 3.73 (s, 1.8H, OCH_3), 3.74 (s, 1.2H, OCH_3), 4.07 (d, *J*=9.9Hz, 0.4H, H5#), 4.14 (d, *J*=10.0Hz, 0.6H, H5#), 4.46 (dd, *J*=13.0Hz, 3.2Hz, 0.4H, H3), 4.55 (d, *J*=10.0Hz, 0.4H, H1#), 4.81 (d, *J*=10.2Hz, 0.6H, H1#), 5.00-5.07 (m, 1H, H2#), 5.17 (app. t, *J*=9.7Hz, 0.4H, H4#), 5.20 (app. t, *J*=9.7Hz, 0.6H, H4#), 5.308 (app. t, *J*=9.4Hz, 0.4H, H3#), 5.313 (app. t, *J*=9.3Hz, 0.6H, H3#), 5.70 (s, 0.4H, H12), 5.73 (s, 0.6H, H12)

^{13}C-NMR ($CDCl_3$): δ 16.5 (q, C25), 17.5 (t, C6), 17.7 (t, C6), 18.6 (q, C26), 18.8 (q, C23/24), 20.4 (q, OCO**C**H_3), 20.5 (q, OCO**C**H_3), 20.6 (q, OCO**C**H_3), 20.6 (q, OCO**C**H_3), 20.7 (q, OCO**C**H_3), 22.0 (q, NCO**C**H_3), 23.0 (q, NCO**C**H_3), 23.3 (t, C2), 23.4 (q, C27), 24.0 (t, C2), 26.4 (t, C15), 26.5 (t, C16), 28.4 (q, C23/24), 28.5 (q, C29), 28.7 (q, C28), 29.0 (t, SCH_2), 30.4 (t, SCH_2), 30.9 (t, C21), 31.9 (s, C17), 32.6 (t, C7), 37.1 (s, C10), 37.3 (s, C10), 37.7 (t, C22), 40.3, 40.8, 40.86, 40.92 (4×t, 2×C1, 2×C19), 41.2 (s, C4), 43.2 (s, C14), 43.3 (s, C14), 43.7 (s, C20), 45.3 (s, C8), 45.4 (s, C8), 45.6 (t, NCH_2), 47.4 (t, NCH_2), 48.2 (t, C18), 48.3 (t, C18), 52.8 (q, OCH_3), 53.0 (q, OCH_3), 55.8 (t, C5), 57.1 (t, C5), 60.1 (t, C3), 61.7 (t, C9), 66.8 (t, C3), 69.2, 69.3, 69.4, 69.8 (4×t, 2×C4#, 2×C2#), 72.7 (t, C3#), 73.1 (t, C3#), 76.0 (t, C5#), 76.3 (t, C5#), 83.6 (t, C1#), 84.3 (t, C1#), 128.5 (t, C12), 166.6 (s, C6#), 167.0 (s, C6#), 169.26, 169.31, 169.4, 169.6 (4×s, 2×C13,

2×OCO**C**H$_3$), 169.9 (s, OCO**C**H$_3$), 170.0 (s, OCO**C**H$_3$), 171.8 (s, NCO**C**H$_3$), 171.9 (s, NCO**C**H$_3$), 180.7 (s, C30), 180.9 (s, C30), 200.0 (s, C11), 200.3 (s, C11)

3.4.53 (*3S*, *18R*, *20S*)-3-[*N*-[2-[Methyl (1-thio-β-D-glucopyranosyl) uronate]-ethyl]-(acetylamino)]-11-oxo-olean-12-en-30-oic acid [59]



Procedure: Zemplén deacetylation

Peracetate **58** (1.57g, 1.76mmol, 1.00equiv.) was dissolved in dry MeOH and 1M NaOMe in MeOH (2.3mL) was added at 0°C. The suspension turned to a clear solution and the pH was adjusted to ~10, the reaction mixture was allowed to reach rt and was stirred under TLC (EtOAc:MeOH 3:2 + 0.5% AcOH) monitoring. Upon complete conversion the reaction mixture was neutralized with freshly washed ion exchange resin to pH 4-5 and filtered. Evaporation of the filtrate gave a white solid, pure target compound **59** according to ^{1}H-NMR, HPLC and MS.

Yield: 1.31g (97.4%) as white solid

Physical properties:

R_f-value = 0.22 (EtOAc:MeOH 5:1 + 0.5% AcOH)

Retention time = 5.03min (HPLC-method B)

Optical rotation: $[\alpha]_D^{20}$ = +39.9° (c=1.1, $CHCl_3$:MeOH 3:1)

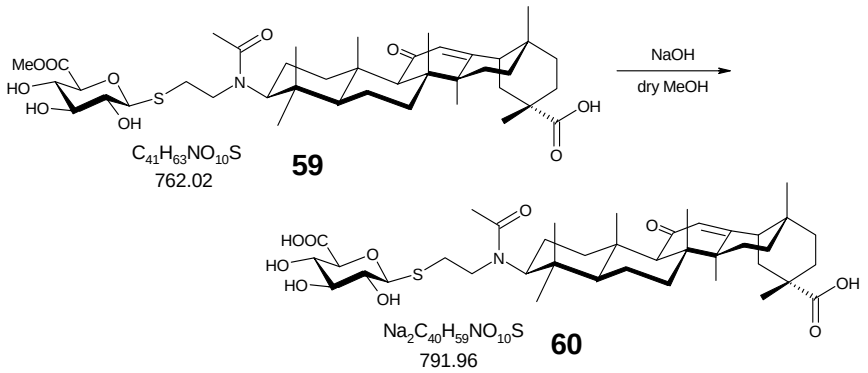
HRMS: calcd. [M+1]: 762.4245;
found [M+1]: 762.4256; dev.: -1.45ppm

NMR-analysis: Two rotamers in a ratio of 6:4 are observed.

^{1}H-NMR ($CDCl_3$:MeOD 5:1): δ 0.78-1.12 (m, 12H, H1b, H5, H16b, 2xH23/24, H28), 1.13-1.33 (11H, H15b, H21b, H25, H26, H29), 1.33-1.77 (m, 11H, H2b, H6a, H6b, H7a, H7b, 19b, H22a, H22b, H27), 1.79-2.28 (m, 9H, H2a, H15a, H16a, H18, H19a, H21a, NAc), 2.43 (s, 1H, H9), 2.44 (s, 1H, H9), 2.55-3.14 (m, 3H, H1a, SCH_2), 3.27-3.53 (m, ~3H, H3*, H2#, H3#, NCHH*), 3.53-3.77 (m, ~2.5H, NCHH*, H4#), 3.80 (s, 1.8H, OCH_3), 3.81 (s, 1.2H, OCH_3), 3.88 (d, *J*=6.6Hz, 0.4H, H#5), 3.91 (d, *J*=6.7Hz, 0.6H, H#5), 4.42 (m, ~0.5H, H3), 4.48 (d, *J*=9.9Hz, ~0.5H, H1#), 4.51 (d, *J*=9.6Hz, ~0.5H, H1#), 5.66 (s, 0.4H, H12), 5.68 (s, 0.6H, H12)

^{13}C-NMR ($CDCl_3$:MeOD 5:1) δ 16.1, 17.1, 17.3, 18.2, 18.4, 21.2, 22.5, 22.9, 23.0 (4×q, 2×NCO**C**H_3, 2×C27), 23.5, 26.0, 28.0, 28.1, 28.2, 28.4, 30.1, 30.6, 31.5, 32.2, 36.8, 36.9, 37.3, 40.0, 40.3, 40.5, 40.8, 43.0, 43.4, 45.1, 45.2, 45.6 (t, NCH_2), 47.2 (t, NCH_2), 47.9 (d, C18), 52.05 (q, OCH_3), 52.13 (q, OCH_3), 55.6 (d, C5), 56.6 (d, C5), 60.1 (d, C3), 61.4 (d, C9), 66.6 (d, C3), 70.9 (d, C4#), 71.1 (d, C4#), 71.8 (d, C2#), 71.9 (d, C2#), 77.2 (d, C3#), 78.3 (d, C#5), 85.8 (d, C1#), 86.7 (d, C1#), 127.6 (d, C12), 168.9 (s, C6#), 169.2 (s, C6#), 170.9 (s, C13), 172.34 (s, N**C**OCH_3), 172.39 (s, N**C**OCH_3), 178.96 (s, C30), 179.01 (s, C30), 200.6 (s, C11), 201.0 (s, C11)

3.4.54 (*3S*, *18R*, *20S*)-3-[*N*-[2-(1-Thio-β-D-glucopyranosyluronic acid)-ethyl]-(acetylamino)]-11-oxo-olean-12-en-30-oic acid [60]



Procedure: Ester Hydrolysis

Methylester **59** (1.31g, 1.72mmol, 1.00equiv.) was dissolved in 0.2N NaOH in dry MeOH (91mL) and stirred under TLC-monitoring (EtOAc:MeOH 3:2 + 0.5% AcOH). Upon complete conversion the reaction mixture was neutralized with freshly washed ion exchange

resin to pH=4-5, filtered and evaporated to give pure target compound. The residue was lyophilized from water dioxane to give pure target compound **60** as colorless lyophilisate.

Yield: 1.34g (quantitative) as colorless lyophilisate

Physical properties:

R_f-value = 0.07 (EtOAc:MeOH 3:2 + 0.5% AcOH)

Retention time = 4.82min (HPLC-method B)

Optical rotation: $[\alpha]_D^{20}$ = +38.0° (c=9.7, MeOH:$CHCl_3$ 5:1)

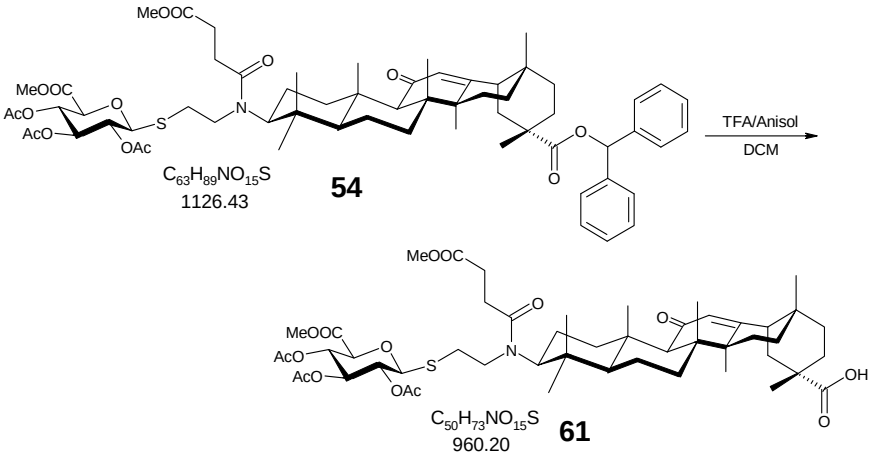
HRMS: calcd. [M+1]: 748.4089;
found [M+1]: 748.4097; dev.: -0.89ppm

NMR-analysis: Two rotamers in a ratio of 6:4 are observed.

^{1}H-NMR (MeOD): δ 0.78-1.10 (m, 11H, H5, H16b, 2xH23/24, H28), 1.10-1.33 (11H, H1b, H15b, H25, H26, H29), 1.33-1.66 (m, 10H, H2b, H6a, H6b, H7b, H21b, H22a, H22b, H27), 1.66-2.05 (m, 5H, H7a, H15a, H19a, 19b, H21a), 2.06-2.27 (m, 6H, H2a, H16a, H18, NAc), 2.51 (s, 0.4H, H9), 2.53 (s, 0.6H, H9), 2.60-3.09 (m, 3H, H1a, SCH_2), 3.24-3.29 (m, 1H, H2#), 3.35-3.46 (m, ~1.5H, H3#, NCHH*), 3.48-3.78 (m, ~3H, H3,H4#, NCHH*), 3.83 (d, *J*=9.7Hz, 1H, H5#), 3.84 (d, *J*=9.8Hz, 1H, H5#), 4.43 (dd, J=13.0Hz, 3.1Hz, 0.4H, H3), 4.53 (d, *J*=9.8Hz, 1H, H1#), 4.54 (d, *J*=9.6Hz, 1H, H1#), 5.59 (s, 0.6H, H12), 5.60 (s, 0.4H, H12)

^{13}C-NMR (MeOD): δ 17.19, 17.2, 18.7, 18.8, 19.2, 19.5, 19.6, 22.1 (q, NCO**C**H_3), 23.1 (q, NCO**C**H_3), 23.8 (q, C27), 23.9, 24.4, 24.8, 27.4, 27.6, 28.8, 29.0, 29.2, 29.8 (t, SCH_2), 31.3 (t, SCH_2), 32.0, 33.0, 33.6, 33.7, 38.4, 38.5, 39.0, 41.5, 41.7, 41.9, 42.2, 42.4, 44.6, 44.9, 46.65, 46.69, 47.5 (t, NCH_2), 49.9 (d, C18), 57.5 (d, C5), 57.7 (d, C5), 61.9 (d, C3), 63.0 (d, C9), 63.1 (d, C9), 68.0 (d, C3), 73.0 (d, C4#), 73.9 (d, C2#), 74.0 (d, C2#), 78.85 (d, C3#), 78.94 (d, C3#), 80.1 (d, C5#), 87.2 (d, C1#), 88.4 (d, C1#), 128.9 (d, C12), 172.5 (s, C13), 172.8 (s, C13), 174.47 (s, N**C**OCH_3), 174.52 (s, N**C**OCH_3), 180.3 (s, C30), 202.4 (s, C11), 202.5 (s, C11)

3.4.55 (*3S*, *18R*, *20S*)-3-[*N*-[2-[Methyl (2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl) uronate]-ethyl]-[(methylsuccinyl)amino]]-11-oxo-olean-12-en-30-oic acid [61]



Procedure: Acidic diphenylmethyl ester cleavage

To a chilled solution of ester **54** (0.350g, 0.311mmol, 1.00equiv.) in dry DCM (15mL) first anisole (0.34mL, 3.1mmol, 10.0equiv.) and then TFA (1.04mL, 14.0mmol, 45equiv.) was added gradually at 0°C. The reaction mixture was stirred under TLC-monitoring (Hex:EtOAc 1:2 + 0.5% AcOH) at 0°C. Upon complete conversion (3h) the reaction mixture was allowed to reach rt and was diluted with EtOAc. The organic layer was washed with water and brine, the aqueous layers were once more extracted with EtOAc (TLC-check) and again washed with brine. The combined organic layers were dried over Na_2SO_4 and evaporated to give a crude material (800mg) which was purified by column chromatography (SiO_2: 45g, Hex:EtOAc 2:1 to Hex:EtOAc 1:2 + 0.1% AcOH) to pure target compound **61** as white solid after co-evaporation from toluene.

Yield: 248mg (83.1%) as white solid

Physical properties:

R_f-value = 0.46 ($CHCl_3$:MeOH 9:1 + 0.1% AcOH)

Optical rotation: $[\alpha]_D^{20}$ = +38.3° (c=0.3, $CHCl_3$)

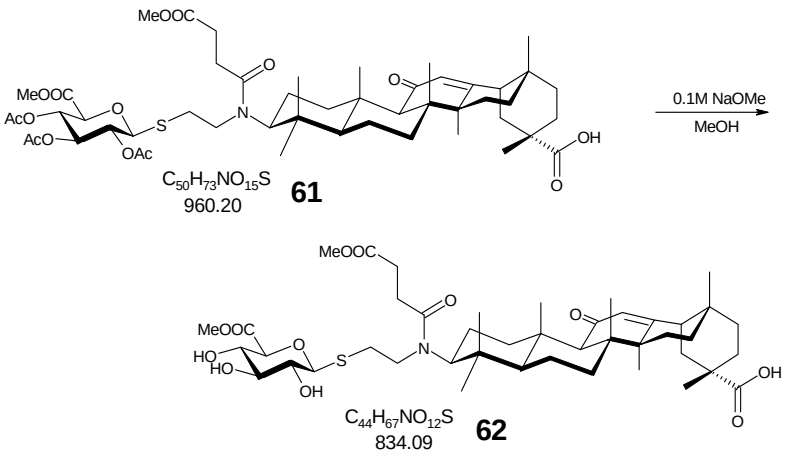
HRMS: calcd. [M+1]: 960.4774
found [M+1]: 960.4776

NMR-analysis: Two rotamers in a ratio of 6:4 are observed.

^{1}H-NMR ($CDCl_3$): δ 0.71-1.09 (m, 12H, H1b, H5, H16b, 2×H23/24, H28), 1.10-2.30 (m, 37H, H2a, H2b, H6a, H6b, H7a, H7b, H15a, H15b, H16a, 18H, H19a, H19b, H21a, H21b, H22a, H22b, H25, H26, H27, H29, 3×OAc), 2.36 (s, 0.4H, H9), 2.40 (s, 0.6H, H9), 2.40-3.00 (m, 7H, SCH2, 2×$COCH_2$, H1a), 3.21-3.74 (m, 2.6H, NCH_2, H3), 3.69, 3.70 (2×s, 3H, OCH_3), 3.74 (bs, 3H, OCH_3), 4.10 (d, *J*=9.8Hz, 0.4H, H5#), 4.16 (d, *J*=9.8Hz, 0.6H, H5#), 4.45 (dd, *J*=13.0Hz, 3.0Hz, 0.4H, H3), 4.60 (d, *J*=9.9Hz, 0.4H, H1#), 4.81 (d, *J*=10.2Hz, 0.6H, H1#), 5.01 (app. t, *J*=9.6Hz, 0.6H, H2#), 5.11 (app. t, *J*=9.6Hz, 0.6H, H2#), 5.18 (app. t, *J*=9.7Hz, 0.4H, H4#), 5.19 (app. t, *J*=9.6Hz, 0.4H, H4#), 5.31 (app. t, *J*=9.3Hz, 1H, H3#), 5.31 (app. t, *J*=9.3Hz, 1H, H3#), 5.67 (s, 0.4H, H12), 5.70 (s, 0.6H, H12)

^{13}C-NMR ($CDCl_3$): δ: 16.4, 17.45, 17.7, 18.5, 18.8, 20.3 (q, CO**C**H_3), 20.4 (q, CO**C**H_3), 20.5 (q, CO**C**H_3), 20.6 (q, CO**C**H_3), 23.2, 23.3, 23.8, 26.3, 28.34, 28.4, 28.9, 29.2, 29.45, 31.0, 31.8, 32.5, 37, 37.2, 37.7, 40.3, 40.6, 40.8, 41.0 (t, C1), 43.2, 43.6, 45.3, 45.4, 45.7 (t, NCH_2), 46.6 (t, NCH_2), 48.2 (d, C18), 51.7 (q, OCH_3), 52.8 (q, OCH_3), 52.9 (q, OCH_3), 55.9 (d, C5), 57.0 (d, C5), 60.4 (d, C3), 61.7 (d, C9), 65.4 (d, C3), 69.2 (d, C2#), 69.4 (d, C4#), 69.8 (d, C2#), 72.7 (d, C3#), 73.1 (d, C3#), 75.9 (d, C5#), 76.1 (d, C5#), 83.5 (d, C1#), 84.0 (d, C1#), 128.3 (d, C12), 166.7 (s, C6#), 167.1 (s, C6#), 169.3, 169.4, 169.5, 169.9 (4×s, 2×C13, 2×CO), 169.9 (s, CO), 170.0 (s, CO), 170.1 (s, CO), 172.4 (s, CO), 172.7 (s, CO), 173.6 (s, CO), 173.8 (s, CO), 179.3 (s, C30), 200.2 (s, C11), 200.5 (s, C11)

3.4.56 (*3S*, *18R*, *20S*)-3-[*N*-[2-[Methyl (1-thio-β-D-glucopyranosyl) uronate]-ethyl]-[succinylamino]]-11-oxo-olean-12-en-30-oic acid [62]



Procedure: Zemplén deacetylation

Peracetate **61** (70mg, 0.073mmol, 1.00equiv.) was dissolved in dry MeOH (2.5mL) and 0.1M NaOMe was added at 0°C. The pH was adjusted to 10.0 and the reaction mixture was stirred at 0°C under TLC- (EtOAc:Hex 2:1, DCM:MeOH10:1 + 0.5% AcOH) and HPLC-monitoring for several hours. Upon complete conversion (2.5h), the reaction mixture was neutralized with ion exchange resin (pH 4-5) filtered, evaporated onto SiO_2 (200mg) and purified by column chromatography (SiO_2: 8g, DCM:MeOH 20:1 + 0.1% AcOH to DCM:MeOH 15:1 + 0.1% AcOH) to give pure target compound after co-evaporation from toluene.

Yield: 60mg (98.7%) as white solid

Physical properties:

R_f-value = 0.44 (EtOAc:MeOH 5:1 + 0.5% AcOH)

Retention time = 5.19min (HPLC-method B)

Optical rotation: $[\alpha]_D^{20}$ = +37.4° (c=0.5, $CHCl_3$:MeOH 5:1)

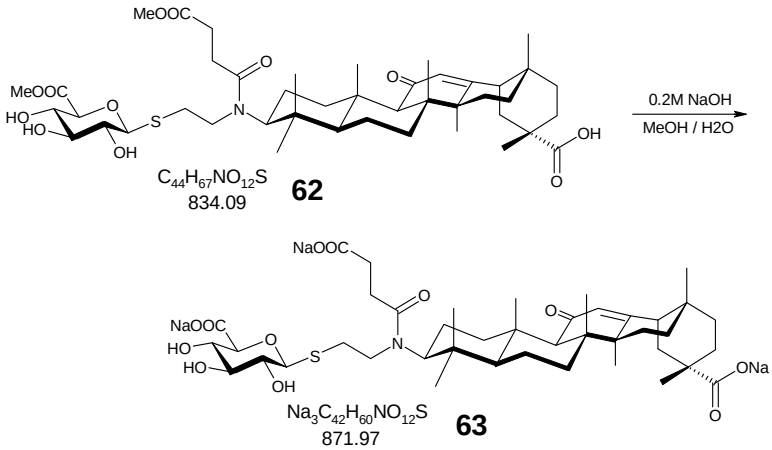
HRMS: calcd. [M+1]: 834.4457;
found [M+1]: 834.4459; dev.: -0.22ppm

NMR-analysis: Two rotamers in a ratio of 6:4 are observed.

^{1}H-NMR ($CDCl_3$:MeOD 5:1): δ 0.73-0.99 (m, 10H, H5, 2xH23/24, H28), 0.98-1.24 (12H H1b, H15b, H16b, H25, H26, H29), 1.25-1.76 (m, 12H, H2b, H6a, H6b, H7a, H7b, 19b, H21b, H22a, H22b, H27), 1.77-2.28 (m, 6H, H2a, H15a, H16a, H18, H19a, H21a), 2.42 (s, 1H, H9), 2.55-3.08 (m, 7H, H1a, 2x$COCH_2$, SCH_2), 3.27-3.61 (m, ~3H, H3, H2#, H3#, NCHH*), 3.62-3.72 (m, ~5H, NCHH*, H4#), 3.68 (s, 1.2H, OCH_3), 3.69 (s, 1.8H, OCH_3), 3.79 (s, 3H, OCH_3), 3.89 (d, *J*=9.7Hz, 0.4H, H5#), 3.91 (d, *J*=9.8Hz, 0.6H, H5#), 4.42 (dd, *J*=13.0Hz, *J*=3.2Hz, 0.4H, H3), 4.48 (d, *J*=9.4Hz, 0.4H, H1#), 4.50 (d, *J*=9.5Hz, 0.6H, H1#), 5.66 (s, 0.4H, H12), 5.68 (s, 0.6H, H1)

^{13}C-NMR ($CHCl_3$:MeOD 5:1): δ 16.20, 16.24, 17.3, 17.5, 18.37, 18.40, 18.6, 23.0, 23.1, 23.6, 26.2, 26.3, 28.2, 28.3, 28.4, 29.1, 29.3, 29.9, 30.9, 31.7, 32.4, 36.9, 37.1, 37.5, 40.2, 40.5, 40.7, 40.9, 41.0, 43.2, 43.6, 45.2, 45.3, 45.9 (t, NCH_2), 46.5 (t, NCH_2), 48.2 (d, 18), 51.6 (q, OCH_3), 52.26 (q, C6#), 52.32 (q, C6#), 55.8 (d, C5), 56.8 (d, C5), 60.4 (d, C3), 61.6 (d, C9), 65.4 (d, C3), 71.0 (d, C4#), 71.2 (d, C4#), 71.85 (d, C2#), 71.94 (d, C2#), 77.2 (d, C#3), 77.3 (d, C#3), 78.3 (d, C5#), 78.4 (d, C5#), 85.7 (d, C1#), 86.7 (d, C1#), 127.9 (d, C12), 169.1 (s, C6#), 169.3 (s, C6#), 170.8 (s, C13), 172.7 (s, **C**OCH_2), 172.8 (s, **C**OCH_2), 173.7 (s, **C**OCH_2), 173.8 (s, **C**OCH_2), 179.6 (s, C30), 200.6 (s, C11), 200.9 (s, C11)

3.4.57 (*3S*, *18R*, *20S*)-3-[*N*-[2-(1-Thio-β-D-glucopyranosyluronic acid)-ethyl]-[(methylsuccinyl)amino]]-11-oxo-olean-12-en-30-oic acid [63]



Procedure: Ester hydrolysis:

Dimethyl ester **62** (60mg, 0.072mmol, 1.00equiv.) was dissolved in 0.2N NaOH at rt, water (0.4mL) was added and the reaction mixture was allowed to stir at rt overnight. According to HPLC complete conversion from starting material to target compound was achieved and the reaction mixture was neutralized with freshly washed (MeOH, H_2O) ion exchange resin and pH was adjusted to ~8. The mixture was filtered, washed and evaporated, taken up in a mixture of dioxane:water 1:1 (3mL) and lyophilized to yield 62mg of white lyophilisate.

Yield: 62mg (98.8%) as white lyophilisate

Physical properties:

R_f-value = 0.04 (EtOAc:MeOH 3:2 + 0.5% AcOH)

Retention time = 4.70min (HPLC-method B)

Optical rotation: $[\alpha]_D^{20}$ = +33.9° (c=0.6, MeOH)

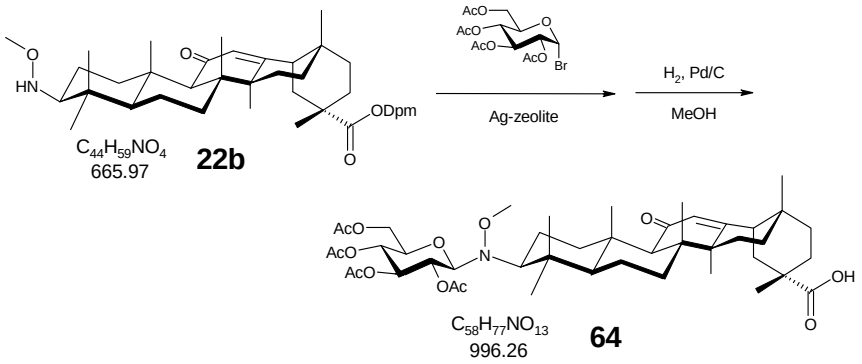
HRMS: calcd. [M+1]: 806.4144;
found [M+1]: 806.4151; dev.: -0.95ppm

NMR-analysis: Two rotamers in a ratio of 6:4 are observed.

^{1}H-NMR (MeOD): δ 0.75-1.36 (m, 24H, H1b, H5, H15b, H16b, H22a, H22b, 2×H23/H24, H25, H26, H28, H29), 1.37-1.76 (m, 16H, H2a, H2b, H6a, H6b, H7a, H7b, H15a, H16a, H18, 19b, H19a, H21a, H21b, H27), 2.26-3.22 (m, 9H, H1a, H9, H18, 2×COCH2, SCH2), 3.20-3.53 (m, 4H, H2#, H3#, H4#, NC**H**H), 3.55-3.75 (m, 2.6H, H5#, NCH**H**, H3), 4.37-4.47 (m, 0.4H, H3), 4.45 (d, *J*=9.7Hz, 0.6H, H1#), 4.50 (d, *J*=9.6Hz, 0.4H, H1#), 5.73 (s, 0.6H, H12), 5.74 (s, 0.4H, H12)

^{13}C-NMR (MeOD): δ 17.1, 17.2, 18.7, 18.8, 19.2, 19.5, 19.7, 23.7, 23.8, 24.4, 24.8, 27.6, 29.0, 29.3, 29.36, 29.43, 29.6, 31.5, 31.7, 32.4, 33.0, 33.1, 33.7, 33.8, 34.7, 38.4, 38.5, 39.6, 41.5, 41.95, 42.00, 43.9, 44.6, 44.7, 46.4, 46.7, 46.9 (t, NCH_2), 47.9 (t, NCH_2), 50.0 (d, C18), 57.6 (d, C5), 57.8 (d, C5), 61.9 (d, C3), 62.9 (d, C9), 63.0 (d, C9), 66.9 (d, C3), 73.4 (d, C4#), 73.8 (d, C2#), 74.0 (d, C2#), 79.2 (d, C3#), 80.0 (d, C5#), 87.0 (d, C1#), 87.6 (d, C1#), 128.7 (d, C12), 174.4 (s, C13), 174.4 (s, C13), 176.4 (s, C6#), 176.5 (s, C6#), 176.7 (s, **C**OCH_2), 181.2 (s, **C**OCH_2), 181.2 (s, **C**OCH_2), 185.0 (s, C30), 202.9 (s, C11), 203.0 (s, C11)

3.4.58 (*3R*, *18R*, *20S*)-3-[*N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-methoxyamino]-11-oxo-olean-12-en-30-oic acid [65]



Step 1: *N*-Glycosylation with silver-zeolite

O-Methyl-hydroxylamine **22b** (0.300g, 0.45mmol) and tetraacetyl-D-glucopyranosyl bromide (0.556g, 1.35mmol, 3.00equiv.) were dissolved in dry DCM in oven-dried glassware and molecular sieve 4Å (powdered and activated) and silver-zeolite[77] (900mg, 1h dried *in vacuo*) were added at rt. The reaction mixture was stirred at rt under TLC and ^{1}H-NMR-monitoring. After 10 days of stirring and another addition of fresh silver-zeolite (900mg) after 3 days the reaction mixture was filtered over a bed of Celite®, washed with DCM and evaporated. The residue was dissolved in acetone (2.4mL) and this solution was added dropwise to a stirred suspension of Ag_2CO_3 (400mg) in acetone:water (5.2mL) and was stirred for 2h. After 3h the reaction mixture was filtered over a bed of Celite® and the filtrate was evaporated. According to TLC (Hex:EtOAc 3:1) the excess of bromide was hydrolyzed to a mixture of two more polar compounds.

The crude mixture was attempted to be purified by two subsequent column chromatography steps (SiO_2: 40g Hex:EtOAc 3.2:1 to 2.5:1) - (SiO_2: 40g, Tol:EtOAc 7:1 to Tol:EtOAc 5:1) but only parts of the material could be isolated in pure form. Purification was easier accomplished after the oncoming deprotection step.

Yield: 125mg (27.9%) as white solid foam

Step 2: Hydrogenolysis

Ester **64** (100mg, 0.100mmol, 1.00equiv.) was dissolved in MeOH and the reaction mixture was evacuated and purged three times with argon, before Pd/C (30mg) was added and the atmosphere was exchanged to H_2 three times before it was stirred at rt for 3h. According to TLC (Hex:EtOAc 3:1) all starting material was converted to one more polar compound. The reaction mixture was filtered over a short bed of Celite®, which was washed with MeOH. After evaporation the crude product was purified by column chromatography (SiO_2: 7g, $CHCl_3$ to $CHCl_3$:MeOH 30:1) to give pure target compound **65** as white solid.

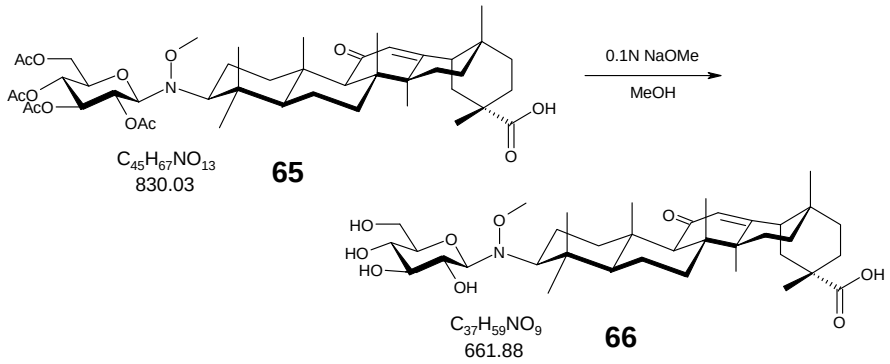
Yield: 75mg (90.0%) as white solid

Physical properties:

^{1}H-NMR ($CDCl_3$): δ 0.70-0.78 (m, 1H, H5), 0.85 (s, 3H, H28), 0.89 (s, 3H, H23/24), 0.90 (s, 3H, H23/24), 0.91-0.99 (m, 1H, H1b), 0.99-1.07 (m, 1H, H16b), 1.14-1.83 (m, 1H, H15b), 1.14 (s, 3H, H26), 1.16 (s, 3H, H25), 1.23 (s, 3H, H29), 1.29-1.50 (m, 5H, H6b, H7b, H21b, H22a, H22b), 1.37 (s, 3H, H27), 1.56-1.70 (m, 3H, H6a, H7a, H19b), 1.73-1.89 (m, 3H, H2a, H2b, H15a), 1.89-2.09 (m, 3H, H16a, H19a, H21a), 2.02 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.15-2.24 (m, 1H, H18), 2.35 (s, 1H, 9), 2.57-2.68 (m, 1H, H3), 2.84-2.93 (m, 1H, H1a), 3.48 (s, 3H, OCH_3), 3.65 (ddd, *J*=9.9Hz, *J*=6.8Hz, *J*=3.0Hz, 1H, H5#), 4.10-4.17 (m, 1H, H1#), 4.10 (dd, *J*=12.1Hz, 2.9Hz, 1H, H6b#), 4.26 (dd, *J*=12.1Hz, *J*=6.7Hz, 1H, H6a#), 4.99-5.11 (m, 1H, H4#), 5.20-5.27 (m, 2H, H2#, H3#), 5.73 (s, 1H, H12)

^{13}C-NMR ($CDCl_3$): δ 16.4 (q, C25), 17.7 (t, C6), 18.2 (q, C23/24), 18.6 (q, C26), 20.5 (t, C2), 20.6 (q, CO**C**H_3), 20.7 (q, CO**C**H_3), 20.8 (q, 2×CO**C**H_3), 23.3 (q, C27), 26.34, 26.43 (2×t, C15, C16), 28.42, 28.48 (2×q, C28, C29, C23/24) 30.8 (t, C21), 31.8 (s, C17), 32.7 (t, C7), 37.0 (s, C10), 37.7 (t, C22), 38.7 (s, C4), 40.61, 40.80 (2×t, C1, C19), 43.1 (s, C8/14), 43.8 (s, C20), 45.5 (s, C8/14), 48.2 (d, C18), 55.9 (d, C5), 61.8 (d, C9), 63.0 (t, C6#), 63.6 (q, OMe), 69.0 (d, C4#), 69.7 (d, C2#), 73.5 (d, C5#), 74.44, 74.50 (2×d, C3, C3#), 96.1 (d, C1#), 128.5 (d, C12), 169.3 (s, C13), 169.4 (s, **C**OCH_3), 169.5 (s, **C**OCH_3), 170.5 (s, **C**OCH_3), 170.7 (s, **C**OCH_3), 181.7 (s, C30), 200.4 (s, C11)

3.4.59 (*3S*, *18R*, *20S*)-3-[*N*-(β-D-Glucopyranosyl)-methoxyamino]-11-oxo-olean-12-en-30-oic acid [66]



$C_{45}H_{67}NO_{13}$
830.03

65

$C_{37}H_{59}NO_9$
661.88

66

Procedure: Zemplén deacetylation

Peracetate **65** (70mg, 0.084mmol, 1.00equiv.) was dissolved in dry MeOH and 0.1M NaOMe in MeOH (0.9mL) was added at 0°C. The reaction mixture was stirred at 0°C under TLC monitoring for 2h. Then the solution was neutralized with freshly washed anion exchange resin. Due to minor impurities the the crude product had to be purified by column chromatography (SiO_2: 11g, $CHCl_3$:MeOH 20:1 to $CHCl_3$:MeOH 10:1) to give pure target compound **66** after lyophilisation from freshly distilled dioxane.

Yield: 40mg (71.7%) as white lyophilisate

Physical properties:

R_f-value = 0.08 ($CHCl_3$: MeOH 9:1)

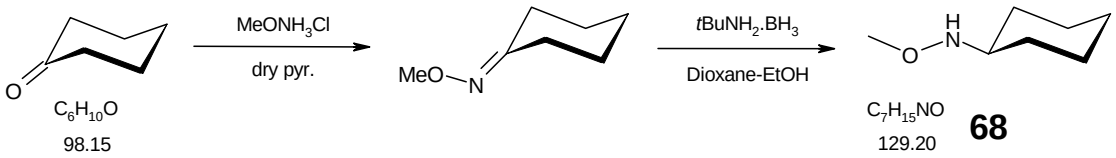
Optical rotation: $[\alpha]_D^{20}$ = +86.7° (c=0.6, MeOH)

HRMS: calcd. [M+1]: 662.4262;
found [M+1]: 662.4263; dev.: 0.11ppm

^{1}H-NMR (MeOD): δ 0.77-0.83 (m, 1H, H5), 0.83 (s, 3H, H28), 0.96 (s, 3H, H23/24), 0.98 (s, 3H, H23/24), 0.99-1.09 (m, 2H, H15/16b, H1b), 1.149 (s, 3H, H26), 1.152 (s, 3H, H29), 1.160 (s, 3H, H25), 1.20-1.27 (m, 1H, H15/16b), 1.36-1.51 (m, 5H, H6b, H7b, H21b, H22a, H22b), 1.42 (s, 3H, H27), 1.62-1.78 (m, 3H, H6a, H7a, H19b), 1.77-1.98 (m, 5H, H2a, H2b, H15/16a, H19a, H21a), 2.11-2.19 (m, 1H, H15/16a), 2.20-2.26 (m, 1H, H18), 2.47 (s, 1H, H9), 2.68-2.74 (m, *J*=13.2Hz, 1H, H3), 2.72-2.79 (m, 1H, H1a), 3.15-3.21 (m, 1H, H5#), 3.33-3.39 (m, 1H, H3#), 3.34-3.42 (m, 1H, H4#), 3.54 (t, *J*=8.9Hz, 1H, H2#), 3.67 (s, 3H, OCH_3), 3.70 (dd, *J*=12.0Hz, *J*=4.6Hz, 1H, H6b#), 3.81 (dd, *J*=12.0Hz, *J*=2.2Hz, 1H, H6a#), 3.86 (d, *J*=8.9Hz, 1H, H1#), 5.61 (s, 1H, H12)

^{13}C-NMR (MeOD): δ 17.0 (q, C25), 18.8 (q, C23/24), 18.8 (t, C6), 19.3 (q, C26), 21.8 (t, C2), 23.8 (q, C27), 27.4, 27.6 (2×t, C15, C16), 28.9 (q, C29), 29.2 (q, C23/24), 29.2 (q, C28), 32.2 (t, C21), 33.0 (s, C17), 33.9 (t, C7), 38.2 (s, C10), 39.1 (t, C22), 39.8 (s, C4), 41.6 (t, C1), 42.7 (t, C19), 44.6 (s, C20), 45.2 (s, C14), 46.8 (s, C8), 49.9 (d, C18), 57.1 (d, C5), 62.7 (t, C6#), 63.2 (d, C9), 63.8 (q, OCH_3), 71.0 (d, C4#), 72.0 (d, C2#), 74.9 (d, C3), 79.5 (d, C3#), 79.7 (d, C5#), 100.1 (d, C1#), 128.9 (d, C12), 173.0 (s, C13), 181.3 (s, C30), 202.8 (s, C11)

3.4.59.1 *N*-Methoxy-cyclohexanamine [68]



Step 1: Oxime formation in pyridine

A suspension of cyclohexanone (1.00g, 10.2mmol, 1.00equiv.) and methoxylamine hydrochloride (2.55g, 30.6mmol, 3.00equiv.) in dry pyridine (30mL) was stirred at rt. After 3h the reaction was diluted with Et_2O (150mL) resulting in precipitation of a white solid. Treatment with 2N HCl (3x150mL) and brine, drying over Na_2SO_4, and evaporation gave crude material (1.17g), which was applied to the subsequent reduction without further purification.

Step 2: Oxime reduction with BH_3.*t*BuNH_2

Borane tert-butylamine complex (2.40g, 27.6mmol, 3.00equiv.) was added to a solution of cyclohexanone, *O*-methyl-oxime (1.17g, 9.20mmol, 1.00equiv.) in dry EtOH/dioxane (1:1, 150mL) at 0°C. The solution turned white and cloudy. After 5min of stirring 2N HCl was added slowly and dropwise while keeping the temperature below 5°C. The reaction mixture turned to a clear solution and was stirred at 0°C under TLC monitoring (Hex:Et_2O 3:1, iodine chamber). Upon complete conversion, Et_2O was added, the organic phase was washed with satd. $NaHCO_3$, water and brine, dried over Na_2SO_4 and evaporated to give a crude material, which was purified by column chromatography (MPLC, SiO_2: 80g Hex:Et_2O 4:1) to give the target compound **68**[102].

Yield: 0.89g (74.9% over 2 steps)

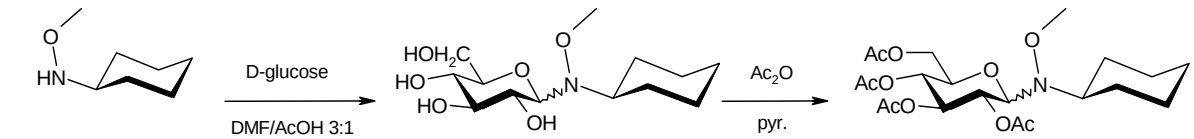
Physical properties:

[CAS-73919-25-2]

^{1}H-NMR ($CDCl_3$): δ 1.04-1.35 (m, 5H, 2×H2$_{ax}$, 2×H3$_{ax}$, H4$_{ax}$), 1.60-1.65 (m, 1H, 2×H4$_{eq}$), 1.70-1.80 (m, 2H, 2×H3$_{eq}$), 1.81-1.91 (m, 2H, 2×H2$_{eq}$), 2.79-2.89 (m, 1H, H1), 3.54 (s, 3H, OCH_3)

^{13}C-NMR ($CDCl_3$): d 24.6 (t, C3), 26.1 (t, C4), 30.6 (t, C2), 59.3 (d, C1), 62.6 (q, OCH_3)

3.4.60 *N*-Cyclohexyl *N*-methoxy-2,3,4,6-tetra-*O*-acetyl-D-glucopyranoside



$C_7H_{15}NO$ 129.20 **68** → $C_{13}H_{25}NO_5$ 275.35 → $C_{21}H_{33}NO_{10}$ 459.50 **69**

Procedure: Direct glycosylation and subsequent peracetylation

A solution of *N*-methoxy-cyclohexylamine **68** (90mg, 0.697mmol, 1.00equiv.) and D-glucose (0.351mg, 2.09mmol, 3.00equiv.) in DMF-AcOH (3:1, 6mL) was stirred in a closed vessel for 5 days before the mixture was co-evaporated from toluene twice and dissolved in pyridine (15mL). The solution was cooled to 0°C and first a mixture of cold pyridine/Ac_2O (3mL/3mL) and then a small amount (<10mg) of DMAP was added and the reaction mixture

was stirred at rt overnight. The reaction mixture was co-evaporated from toluene (twice 100mL) and submitted to column chromatography (SiO_2: 350g, Tol:EtOAc 4.5:1) to afford pure target compound **69** as an anomeric mixture with a β:α ratio of ~8:1.

Yield: 210mg (65.6%) as colorless oil

Physical properties:

R_f-value = 0.44 (Tol:EtOAc 4.5:1)

HRMS: calcd. [M+1]: 460.2177;
found [M+1]: 460.2174; dev.: 0.82ppm

NMR (α-anomer, minor product)

^{1}H-NMR ($CDCl_3$): δ 1.07-1.42 (m, 5H, H2'$_{ax}$, H3'$_{ax}$, H4'$_{ax}$, H5'$_{ax}$, H6'$_{ax}$,), 1.57-2.00 (m, 5H, H2'$_{eq}$, H3'$_{eq}$, H4'$_{eq}$, H5'$_{eq}$, H6'$_{eq}$,), 2.03 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.09 (s, 3H, OAc) 2.96-3.07 (m, 1H, H1'), 3.67 (s, 3H, OCH_3), 4.04 (dd, $J_{5,6b}$=2.3Hz, $J_{6a,6b}$=12.2Hz, 1H, H6b), 4.15 (dd, $J_{5,6a}$=4.9Hz, $J_{6a,6b}$=12.3 Hz, 1H, H6a), 4.45 (ddd, $J_{5,6b}$=2.3Hz, $J_{5,6a}$=4.9Hz, $J_{4,5}$=10.2 Hz, 1H, H5), 4.94 (dd, $J_{1,2}$=6.6Hz, $J_{2,3}$=10.3Hz, 1H, H2), 5.06 (dd, $J_{4,5}$=9.2Hz, $J_{3,4}$=10.3Hz, 1H, H4), 5.13 (d, $J_{1,2}$=6.6Hz, 1H, H1), 5.82 (dd, $J_{3,4}$=9.2Hz, $J_{2,3}$=10.3Hz, 1H, H3)

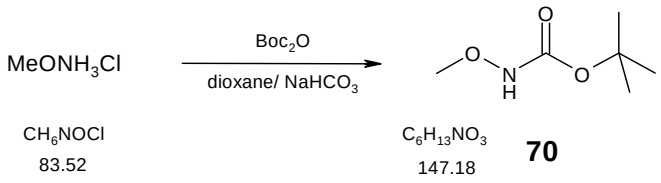
^{13}C-NMR ($CDCl_3$): d 20.7 (q, CO**C**H_3), 20.8 (q, CO**C**H_3), 20.9 (q, CO**C**H_3), 25.30 (t, C3'/C5'), 25.32 (t, C3'/C5'), 25.9 (t, C4'), 30.3 (t, C2'/C6'), 30.4 (t, C2'/C6'), 61.6 (d, C1'), 62.7 (q, OCH_3), 62.7 (d, C6), 69.1 (d, C4), 70.7 (d, C2), 71.3 (d, C3), 72.4 (d, C5), 83.0 (d, C1), 169.8 (s, **C**OCH_3), 170.0 (s, **C**OCH_3), 170.5 (s, **C**OCH_3), 170.8 (s, **C**OCH_3)

NMR (β anomer, main product, out of mixture):

^{1}H-NMR ($CDCl_3$): δ 1.06-1.40 (m, 5H, H2'$_{ax}$, H3'$_{ax}$, H4'$_{ax}$, H5'$_{ax}$, H6'$_{ax}$), 1.58-1.65 (m, 1H, H4'$_{eq}$), 1.71-1.90 (m, 3H, H2'$_{eq}$/H6'$_{eq}$, H3'$_{eq}$, H5'$_{eq}$,), 1.96-2.07 (m, 1H, H2'$_{eq}$ /H6'$_{eq}$), 2.016 (s, 3H, OAc), 2.021 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.98-3.08 (m, 1H, H1'), 3.50 (s, 3H, OCH_3), 3.60 (app. ddd, *J*=3.0Hz, *J*=6.3Hz, *J*=9.6Hz, 1H, H5), 4.13 (dd, $J_{5,6b}$=2.6Hz, $J_{6a,6b}$=12.1Hz, 1H, H6b), 4.22 (dd, $J_{5,6a}$ =5.9Hz, $J_{6a,6b}$=12.1Hz, 1H, H6a), 4.48 (d, $J_{1,2}$=8.6Hz, 1H, H1), 5.05 (app. t, *J*=9.5Hz, 1H, H4), 5.17-5.29 (m, 2H, H2, H3),

^{13}C-NMR ($CDCl_3$): 20.6 (q, CO**C**H_3), 20.68 (q, CO**C**H_3), 20.69 (q, CO**C**H_3), 20.9 (q, CO**C**H_3), 25.2 (t, C3'/C5'), 25.3 (t, C3'/C5'), 25.9 (t, C4'), 30.2 (t, C2'/C6'), 31.2 (t, C2'/C6'), 61.4 (d, C1'), 62.6 (t, C6), 63.7 (q, OCH_3), 68.6 (d, C4), 69.1 (d, C2), 73.4 (d, C5), 74.5 (d, C3), 88.7 (d, C1), 169.5 (s, **C**OCH_3), 169.7 (s, **C**OCH_3), 170.5 (s, **C**OCH_3), 170.6 (s, **C**OCH_3)

3.4.61 *t*-Butyl *N*-methoxy-carbamate [70]



Procedure: *N*-Boc-Protection

To a stirred solution of Boc_2O (25.04g, 110.2mmol, 1.15equiv.) in distilled dioxane a solution of methoxylamine hydrochloride (8.00g, 95.8mmol, 1.00equiv.) in 1M $NaHCO_3$ (172mL, 1.8equiv., strong and immediate gas evolution when dissolved) was added at rt. The resulting solution was stirred overnight (20h) at rt. The reaction mixture was concentrated and acidified to pH=4 with citric acid. The mixture was extracted three times with DCM. The combined organic layers were washed with satd. $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated to leave a crude product which was purified by vacuum distillation (b.p.: 53°C-55°C / 0.5mbar) to yield a colorless liquid[105].

Yield: 11.7g (85.6%) as colorless liquid

Physical properties:

[CAS-58377-44-9]

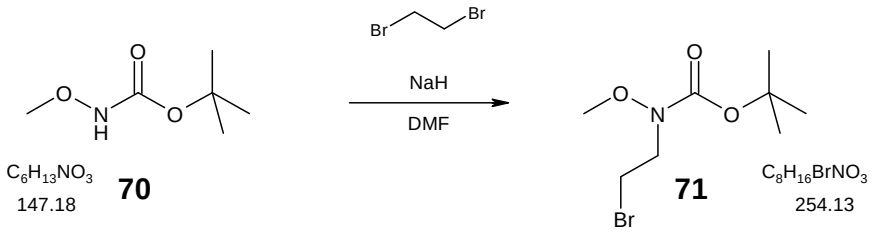
R_f-value = 0.18 (Hex:EtOAc 5:1)

b.p.: 53-55°C (0.5mbar) (Lit[109]: 83-84 °C (10 Torr))

^{1}H-NMR ($CDCl_3$) δ 1.49 (s, 9H, OC(C**H**$_3$)$_3$), 3.72 (s, 3H, OCH_3)

^{13}C-NMR ($CDCl_3$): δ 28.2 (q, OC(**C**H_3)$_3$), 64.5 (q, OCH_3), 81.8 (s, O**C**$(CH_3)_3$)

3.4.62 *t*-Butyl *N*-(2-bromoethyl)-*N*-methoxy-carbamate [71]



Procedure: Carbamate alkylation

Carbamate **70** (0.309g, 2.10mmol, 1.00equiv.) was dissolved in dry DMF (5mL) and cooled to 0°C. The solution was purged with Ar before NaH (60% suspension) (101mg, 2.52mmol, 1.2mmol) was added portionwise within a short period of time giving a foamy mixture that clarified at rt under stirring. The mixture was allowed to stir at rt for 30min before DMF (2mL) was added to support homogeneity. The reaction mixture was cooled to -10°C, dibromoethane (1.97g, 10.5mmol, 5.00equiv) was added in one portion and the reaction mixture was stirred at rt and monitored by TLC (Hex:EtOAc 5:1). Upon complete conversion the reaction mixture was worked up by dilution with satd. NH_4Cl and extraction with EtOAc. The combined organic layers were washed four times with water, once with brine, dried over Na_2SO_4 and evaporated to give a crude product (580mg) which was co-evaporated from EtOAc four times to give target compound **71** as colorless liquid, free of solvents and dibromoethane according to ^{1}H-NMR.

Yield: 490mg (91.8%) as colorless liquid

Physical Properties:

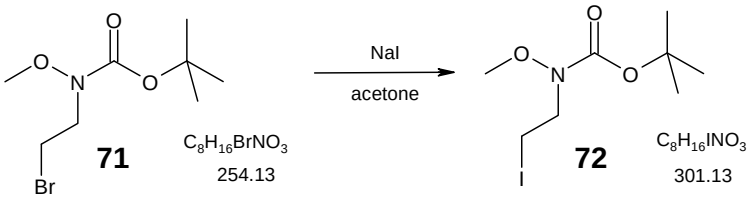
[CAS-1174534-38-3]

R_f-value = 0.33 (Hex:EtOAc 5:1)

^{1}H-NMR ($CDCl_3$) δ 1.51 (s, 9H, OC(C**H**$_3$)$_3$), 3.50 (t, *J*=7.0, 2H, $BrCH_2$), 3.73 (s, 3H, OCH_3), 3.82 (t, *J*=7.0Hz, 2H, NCH_2)

^{13}C-NMR ($CDCl_3$): δ 27.6 (t, $BrCH_2$), 28.2 (q, OC(**C**H$_3$)$_3$), 50.8 (t, NCH_2), 62.7 (q, OCH_3), 82.0 (s, O**C**(CH$_3$)$_3$), 155.8 (s, N**C**O)

3.4.63 *t*-Butyl *N*-(2-iodoethyl)-*N*-methoxy-carbamate [72]



Procedure: Finkelstein reaction

Bromide **71** (100mg, 0.393mmol, 1.00equiv.) was dissolved in acetone (1mL), NaI (177mg, 1.18mmol, 3.00equiv.) was added and the reaction mixture was heated to 45°C under TLC monitoring (Tol:EtOAc 4:1) for 6h in a closed vessel and at rt overnight. The reaction mixture was separated between Et_2O and diluted $Na_2S_2O_3$. The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated. The crude material was purified by column chromatography (SiO_2, Tol:EtOAc 98:2) to give pure target compound as clear yellowish liquid, with small amounts of starting material according to ^{1}H-NMR. The target compound should be stored in the dark and below -25°C.

Yield: 115mg (97%) as yellowish liquid

Physical properties:

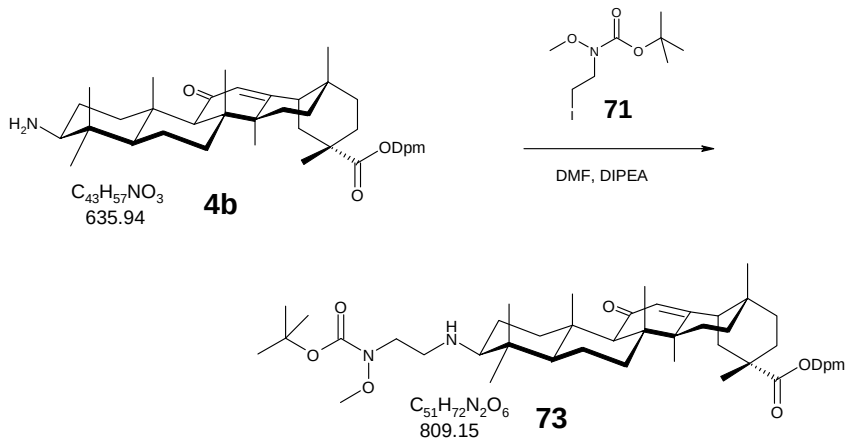
[CAS-1174537-77-9]

R_f-value = 0.63 (Tol:EtOAc 4:1)

^{1}H-NMR ($CDCl_3$) δ 1.50 (s, 9H, $OC(C\underline{\mathbf{H}}_3)_3$), 3.27 (t, *J*=7.5, 2H, ICH_2), 3.71 (s, 3H, OCH_3), 3.78 (t, *J*=7.5Hz, 2H, NCH_2)

^{13}C-NMR ($CDCl_3$): δ 0.04 (t, ICH_2), 28.2 (q, $OC(\underline{\mathbf{C}}H_3)_3$), 51.7 (t, NCH_2), 62.8 (q, OCH_3), 81.2 (s, $O\underline{\mathbf{C}}(CH_3)_3$)

3.4.64 (*3S*, *18R*, *20S*)-3-[2-(*N*-Boc-methoxyamino)-ethylamino]-11-oxo-olean-12-en-30-oic acid, diphenylmethyl ester [73]



Procedure: *N*-Alkylation

A solution of amine **4b** (1.49g, 2.50mmol, 1.00equiv.), iodide **72** (1.52g, 5.00mmol, 2.00 equiv.) and DIPEA (650mg, 5.00mmol, 2.00equiv.) in DMF (8mL) was stripped with argon for approximately 20min before it was heated to 50°C and stirred under an atmosphere of argon and with protection of light under HPLC- and TLC-monitoring. After 20h the reaction mixture was worked up by diluting with EtOAc and washing with $Na_2S_2O_3$, satd. $NaHCO_3$, satd. NH_4Cl, water and brine, drying over Na_2SO_4 and evaporation. The crude product was purified by column chromatography (SiO_2: 200g DCM:MeOH 25:1 + 0.1% AcOH to DCM:MeOH 10:1 + 0.1% AcOH). The fractions containing the target compound were treated twice with satd. $NaHCO_3$, once with brine, dried over Na_2SO_4 to give target compound as white solid foam.

Yield: 1.080g (54.5%) as white solid foam

Physical properties:

R_f-value = 0.49 (DCM : MeOH 15:1 + 0.5% AcOH)

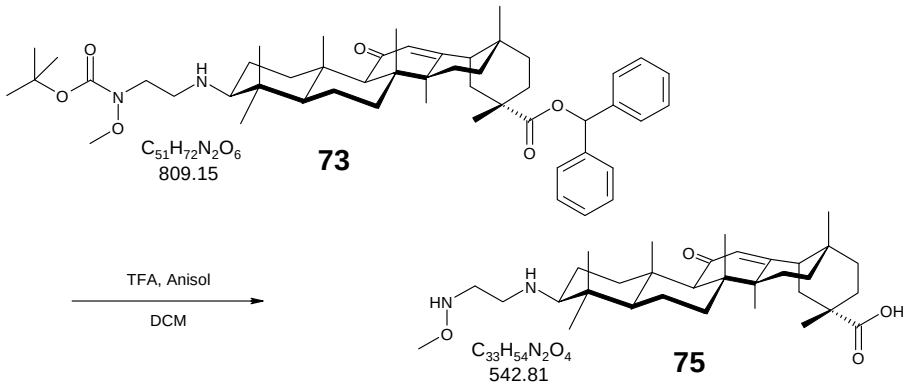
Optical rotation: $[\alpha]_D^{20}$ = +118.3°(c=0.7, CHCl3)

HRMS: calcd. [M+1]: 809.5463
found [M+1]: 809.5460, dev.: 0.51ppm

^{1}H-NMR ($CDCl_3$): δ 0.66 (s, 3H, H28), 0.65-0.75 (m, 1H, H5), 0.73 (s, 3H, H23/24), 0.87-1.05 (m, 2H, H1b, H16b), 0.98 (s, 3H, H23/24), 1.09 (s, 3H, H26), 1.13 (s, 3H, H25), 1.10-1.20 (m, 1H, H15b), 1.13 (s, 3H, H25), 1.17, (s, 3H, H29), 1.25-1.50 (m, 6H, H2b, H6b, H7b, H21b, H22a, H22b), 1.35 (s, 3H, H27), 1.50 (s, 9H, Boc-CH_3), 1.50-1.85 (m, 5H, H2a, H6a, H7a, H15a, H19b), 1.95-2.10 (m, 5H, H3, H16a, H18, H19a, H21a), 2.30 (m, 1H, H9), 2.65-2.75 (m, 1H, NHC**H**H), 2.75-2.81 (m, 1H, H1a), 2.98-3.08 (m, 1H, NHCH**H**), 3.55 (t, *J*=6.4Hz, 2H, $MeONCH_2$), 3.66 (s, 3H, OMe), 5.51 (s, 1H, H12), 6.90 (s, 1H, $OCHPh_2$), 7.25-7.41 (m, 10H, 10×PhCH)

^{13}C-NMR($CDCl_3$): δ 16.3 (q, C25), 16.5 (q, C23/24), 17.9 (t, C6), 18.7 (q, C26), 23.3 (q, C27), 24.37 (t, C2), 26.39, 26.41 (2×t, C15, C16), 28.24, 28.28, 28.32 (3×q, C23/24, C28, C29), 28.34 (q, OC(**C**H_3)$_3$) 31.2 (t, C21), 31.7 (s, C17), 32.8 (t, C7), 37.2 (s, C10), 37.5 (t, C22), 38.5 (s, C4), 39.9 (t, C1), 41.1 (t, C19), 43.1 (s, C14/C8), 44.0 (s, C20), 45.3 (s, C8/C14), 45.7 (t, C31), 48.0 (d, C18), 49.4 (t, C32), 56.1 (d, C5), 61.9 (d, C9), 62.3 (q, OMe), 66.0 (d, C3), 76.6 (d, $OCHPh_2$), 81.2 (s, O**C**(CH_3)$_3$), 127.0 (d, PhCH), 127.3 (d, PhCH), 127.8 (d, PhCH), 128.1 (d, PhCH), 128.5, 128.6, 128.6 (3×d, 2×PhCH, C12), 140.1 (s, PhC), 140.1 (s, PhC), 156.3 (s, N**C**O2*t*Bu), 168.7 (s, C13), 175.2 (s, C30), 200.3 (s, C11)

3.4.65 (*3S*, *18R*, *20S*)-3-[2-(Methoxyamino)-ethylamino]-11-oxo-olean-12-en-30-oic acid [75]



Procedure: Double deprotection

To a solution of ester **73** (0.405g, 0.501mmol, 1.00equiv.) in dry DCM (22mL) first anisole (2.71g, 25.0mmol, 50equiv.) and then TFA (7.5mL) was added gradually at -10°C and the reaction mixture was stirred at 0°C until all starting material was converted to a more polar

reaction intermediate (TLC, Hex:EtOAc 2:3). Then the reaction mixture was allowed to warm to rt and was monitored by TLC ($CHCl_3$:MeOH 5:1 + 0.5% AcOH). After 3h the reaction mixture was diluted with DCM and neutralized by treatment with water and satd. $NaHCO_3$. Then the pH was adjusted to 4-5 by addition of acetic acid, the phases were separated, the aqueous layer was extracted with DCM, the combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated. The crude material was submitted to column chromatography (SiO_2: 13g, $CHCl_3$:MeOH 30:1 + 0.1% AcOH to $CHCl_3$: MeOH 10:1 + 0.1% AcOH). All target compound containing fraction were combined, washed with water and brine, dried over Na_2SO_4 and evaporated to give a yellowish sticky foam, which was dissolved in dioxane (3mL + 2 drops of water) and lyophilized to give pure target compound.

Yield: 249mg (91.6%) as white lyophilizate

Physical properties:

R_f-value = 0.09 (DCM:MeOH 15:1 + 0.5%AcOH)

Retention time = 4.25min (HPLC-method B)

Optical rotation: $[\alpha]_D^{20}$ = +117.9° (c=0.8, MeOH)

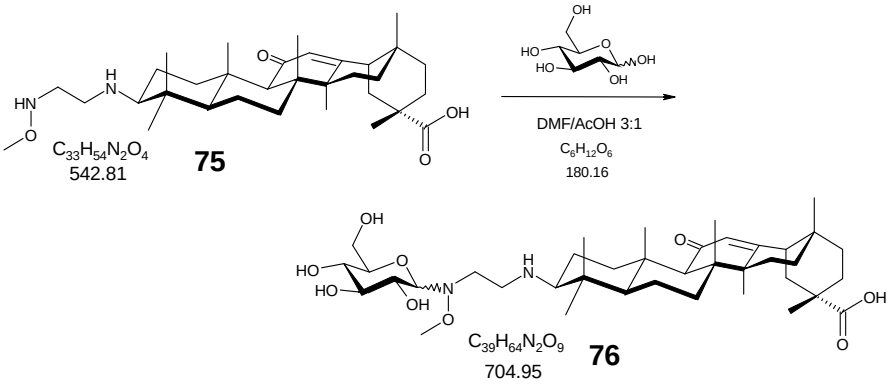
HRMS: calcd. [M+1]: 543.4156
found [M+1]: 543.4162, dev.: -1.11ppm

^{1}H-NMR (MeOD): δ 0.84 (s, 3H, H28), 0.94-0.96 (m, 1H, H5), 0.97 (s, 3H, H23/24), 1.05-1.13 (m, 2H, H1b, H16b), 1.15 (s, 3H, H23/24), 1.16 (s, 3H, H26), 1.17 (s, 3H, H29), 1.18 (s, 3H, H25), 1.25-1.40 (m, 2H, H15b, H21b), 1.41-1.43 (m, 2H, H22a, H22b), 1.43 (s, 3H, H27), 1.48-1.62 (m, 2H, H6b, H7b), 1.68-2.05 (m, 8H, H2a, H2b, H6a, H7a, H15a, H19b, H19a, H21a), 2.14-2.23 (m, 2H, H16a, H18), 2.51 (s, 1H, H9), 2.88 (dt, 13.4, 3.4, 1H, H1a), 2.97 (dd, *J*=12.3Hz, *J*=4.3Hz, 1H, H3), 3.20-3.27 (m, 4H, 2×NCH_2), 3.54 (s, 3H, OCH_3), 5.60 (s, 1H, H12)

^{13}C-NMR (MeOD): δ 16.6 (q, C25), 16.6 (q, C23/24), 18.5 (t, C6), 19.2 (q, C26), 21.3 (t, C2), 23.8 (q, C27), 27.3, 27.6 (2 × t, C16, C15), 27.9 (q, C23/24), 28.8 (q, C29), 29.2 (q, C28), 32.0 (t, C21), 33.0 (s, C17), 33.5 (t, C7), 38.1 (s, C10), 38.4 (s, C4), 39.0 (t, C22), 39.4 (t, C1), 42.4 (t, C19), 44.7 (s, C20), 44.9 (s, C14/8), 46.1 (t, NCH_2), 46.6 (s, C14/8), 47.7 (t,

N(OMe)CH_2), 49.9 (d, C18), 56.1 (d, C5), 62.1 (q, OCH_3), 62.6 (d, C9), 68.6 (d, C3), 128.8 (d, C12), 173.2 (s, C13), 180.5 (s, C30), 202.0 (s, C11)

3.4.66 (*3S*, *18R*, *20S*)-3-[2-[*N*-(β-D-Glucopyranosyl)-methoxyamino]-ethylamino]-11-oxo-olean-12-en-30-oic acid [76]



Procedure: Direct glycosylation in DMF-AcOH 3:1

Methoxyamine **75** (30mg, 0.055mmol, 1.00equiv.) and D-glucuronic acid (30mg, 0.166mmol, 3.00equiv.) were dissolved in DMF/AcOH 3:1 (2.5mL) and the reaction mixture was stirred at 40°C with TLC- and HPLC-monitoring. After 2d of stirring one dominant more polar species was formed and the reaction mixture was diluted with DCM:*i*PrOH 3:1 and washed with halve satd. NaCl solution, the aqueous layer was re-extracted with DCM:*i*PrOH 3:1 (TLC monitoring – 3-4 extractions necessary). The combined organic layer was washed with brine, dried over Na_2SO_4 and evaporated to give a crude material mainly free of glucose. It was purified by column chromatography (SiO_2: 1.5g, DCM:MeOH 98:2 + 1.0% TEA to DCM:MeOH 80:20 + 1.0% TEA) to give target compound **76**, pure according to TLC ($CHCl_3$:MeOH 3:1 +1% TEA) and NMR, only contaminated by small amounts of TEA.

Yield: 22mg (57%) as white solid

Physical properties:

R_f-value = 0.18 ($CHCl_3$:MeOH 3:1 + 1% TEA)

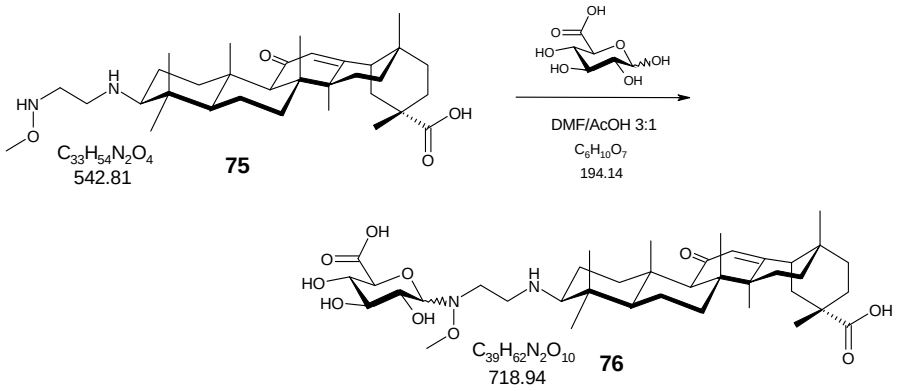
Retention time = 3.88min (HPLC-method B)

Optical rotation: $[\alpha]_D^{20}$ = +22.9° (c=0.5, MeOH:Acetone 4:1)

^{1}H-NMR (MeOD:acetone-d6 4:1): δ 0.83 (s, 3H, H28), 0.93-1.00 (m, 1H, H5), 1.02 (s, 3H, H23/24), 1.011.18 (m, 2H, H1b, H16b), 1.15 (s, 3H, H29), 1.17 (s, 3H, H26), 1.2 (s, 3H, H23/24), 1.2 (s, 3H, H25), 1.43 (s, 3H, H27), 1.22-1.65 (m, 7H, H2b, H6b, H7b, H15b, H21b, H22a, H22b), 1.65-1.75 (m, 2H, H6a, H19b), 1.75-1.99 (m, 5H, H2a, H7a, H15a, H19a, H21a), 2.11-2.31 (m, 1H, H16a, H18), 2.51 (s, 1H, H9), 2.85-2.93 (m, 1H, H1a), 3.03 (dd, *J*=4.6Hz, *J*=12.0, 1H, H3), 3.18-3.59 (m, 8H, H2#, H3#, H4#, H5#, 2×NCH_2), 3.65-3.70 (m, 1H, H6#b), 3.68 (s, 3H, OCH_3), 3.87 (dd, *J*=11.2Hz, *J*=1.9Hz, 1H, H6#a), 4.27 (d, *J*= 8.5Hz, 1H, H1#), 5.63 (s, 1H, H12)

^{13}C-NMR (MeOD:acetone-d^{6} 4:1): δ 16.7 (q, C25), 16.7 (q, C23/24), 18.5 (t, C6), 19.3 (q, C26), 21.7 (t, C2), 23.8 (q, C27), 27.4, 27.6 (2×t, C15, C16), 28.1 (q, C23/24), 29.0 (q, C29), 29.3 (q, C28), 32.2 (t, C21), 32.9 (s, C17), 33.5 (t, C7), 38.0 (s, C10), 38.6 (s, C4), 39.1 (t, C22), 39.5 (t, C1), 42.8 (t, C19), 44.6 (s, C20), 45.2 (s, C14/8), 46.5 (s, C14/8), 46.8, 47.8 (2×t, 2×NCH_2), 49.8 (d, C18), 56.1 (d, C5), 62.6 (q, OCH_3), 62.7 (t, C6#), 63.0 (d, C9), 69.3 (d, C3), 71.5 (d, C2#), 71.7 (d, C4#), 79.2 (d, C3#), 79.8 (d, C5#), 93.8 (d, C1#), 128.8 (d, C12), 172.3 (s, C13), 181.2 (s, C30), 201.5 (s, C11)

3.4.67 (*3S*, *18R*, *20S*)-3-[2-[*N*-(β-D-Glucopyranosyluronic acid)-(methoxyamino)]-ethylamino]-11-oxo-olean-12-en-30-oic acid [77]



Procedure: Direct glycosylation in DMF-AcOH 3:1

Methoxyamine **75** (30mg, 0.055mmol, 1.00equiv.) and D-glucuronic acid (32mg, 0.166mmol, 3.00equiv.) were dissolved in DMF/AcOH 3:1 (2.5mL) and the reaction mixture was stirred at 40°C with TLC- and HPLC-monitoring. After 2d of stirring one dominant more polar species was formed and the reaction mixture was diluted with DCM:*i*PrOH 3:1 and washed with

halve satd. NaCl solution, the aqueous layer was re-extracted with DCM:*i*PrOH 3:1 (TLC monitoring – 3-4 extractions necessary). The combined organic layer was washed with brine, dried over Na_2SO_4 and evaporated to give a crude material mainly free of glucuronic acid. It was purified by column chromatography (SiO_2: 4g, DCM:MeOH 9:1 to 3:1) to give target compound **77,** pure according to TLC ($CHCl_3$:MeOH 3:1 +1% TEA) and NMR..
Yield: 11.5mg (29%) as white lyophilisate

Physical properties:

R_f-value = 0.08 ($CHCl_3$:MeOH 3:1 + 1% TEA)

Retention time = 4.07min (HPLC-method B)

Optical rotation: $[\alpha]_D^{20} = 0°$ (c=0.8, MeOH:Acetone 4:1)

^{13}C-NMR (MeOD): δ 16.6 (q, C23/24), 16.7 (q, C25), 18.5 (t, C6), 19.2 (q, C26), 21.2 (t, C2), 23.8 (q, C27), 27.42, 27.5 (2×t, C16, C15), 27.8 (q, C23/24), 29.0 (q, C29), 29.2 (q, C28), 32.3 (t, C21), 32.9 (s, C17), 33.5 (t, C7), 38.1 (s, C10), 38.4 (s, C4), 39.1 (t, C22), 39.4 (t, C1), 42.9 (t, C19), 44.6 (s, C20), 45.4 (s, C8), 46.52 (t, NCH_2), 46.57 (s, C14), 49.0 (t, NCH_2), 49.9 (d, C18), 56.2 (d, C5), 62.2 (q, OCH_3), 62.6 (d, C9), 68.1 (d, C3), 71.3 (d, C2#), 73.4 (d, C4#), 77.1 (d, C5#), 78.9 (d, C3#), 93.7 (d, C1#), 128.8 (d, C12), 173.3 (s, C13), 176.8 (s, C6#), 181.9 (s, C30), 202.0 (s, C11)

^{1}H-NMR (MeOD): δ 0.83 (s, 3H, H28), 0.98 (m, 1H, H5), 0.98 (s, 3H, H23/24), 1.05-1.10 (m, 2H, H16b, H1b), 1.12 (s, 3H, H23/24), 1.14 (s, 3H, H29), 1.16 (s, 3H, H26), 1.19 (s, 3H, H25), 1.20-1.42 (m, 4H, H15b, H21b, H22a, H22b), 1.43 (s, 3H, H27), 1.48-1.99 (m, 10H, H2a, H2b, H6a, H6b, H7a, H7b, H15a, H19a, H19b, H21a), 2.11-2.20 (m, 1H, H16a), 2.23-2.30 (m, 1H, H18), 2.50 (s, 1H, H9), 2.84-2.93 (m, 1H, H1a), 2.96-3.02 (m, 1H, H3), 3.20-3.72 (m, 8H, H4#, H3#, H2#, H5#, 2×NCH_2), 3.63 (s, 3H, OCH_3), 4.18-4.22 (m, 1H, H1#), 5.66 (s, 1H, H12)

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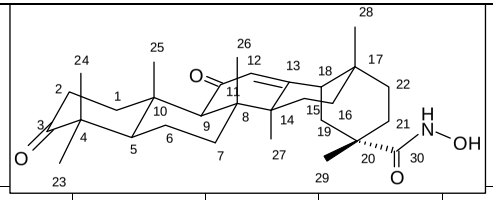
5 Appendix

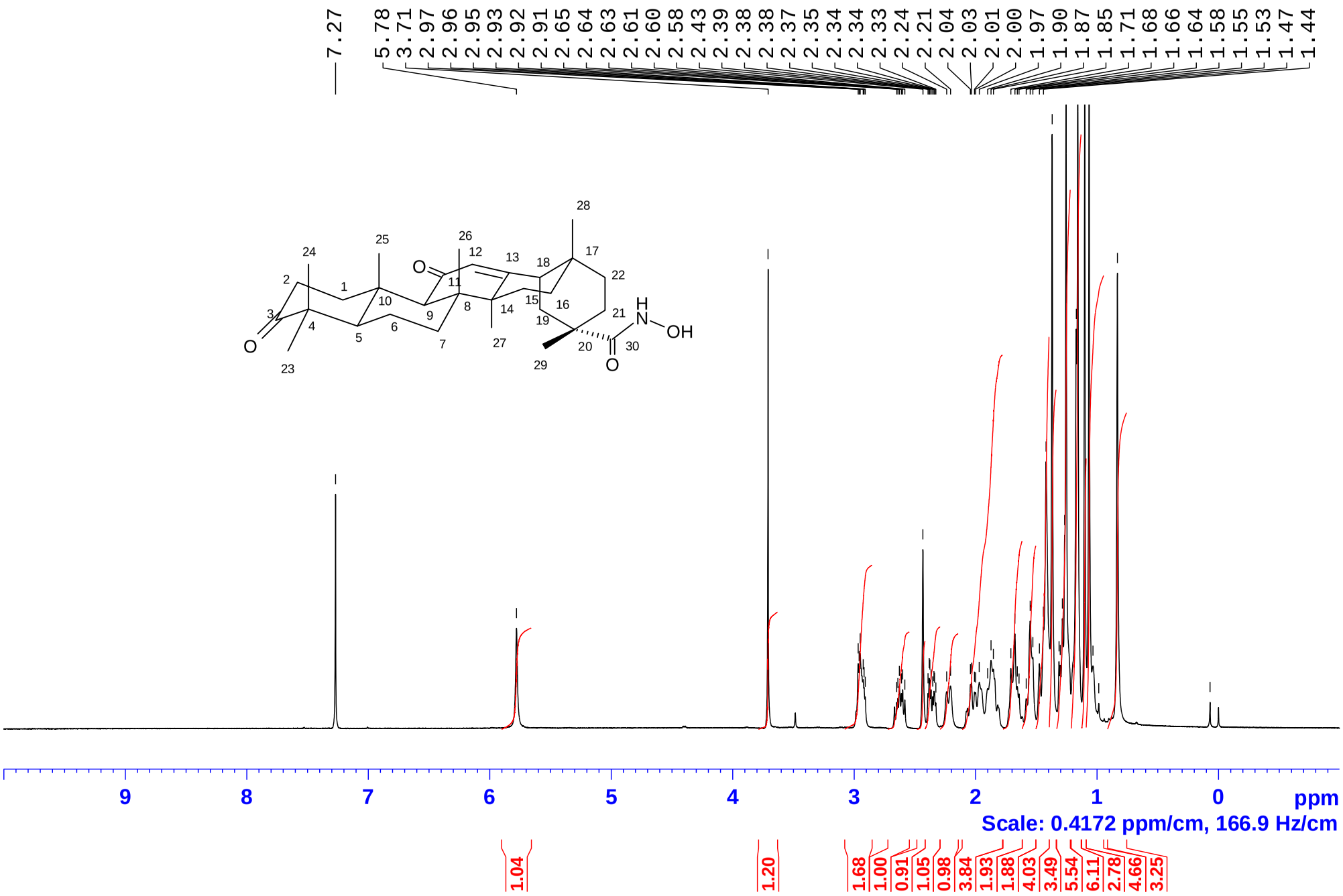
5.1 NMR-spectra and assignment table of compound [31]

CS-169-02



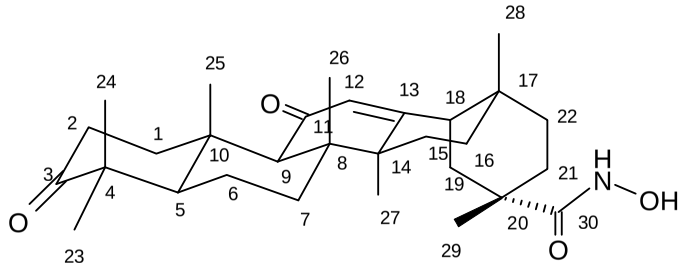
	13C-Information				1H-Information				
Peak	**Assign.**	**13C-Shift**	**Integral**	**HSQC / DEPT**	**Multiplett**	**1H-Shift (middle)**	**1H-Shift (middle)**	**Correlation peaks**	**Comment**
1	3	217.3	5.3	quaternary				shift	
2	11	199.8	7.6	quaternary				shift	
3	30	173.8	4.8	quaternary				shift and exclusion	
4	13	169.9	7.6	quaternary				shift, phase and HMBC H12	
5	12	128.5	-10.3	CH	s	5.78		shift and phase	
6	9	61.1	-12	CH	s	2.43		1H-singlet, 2xHMBC to methyl	
7	5	55.4	-12.4	CH	m	1.29		3xHMBC to methyl	
8	18	47.82	-10.9	CH	m	2.22		1xHMBC to methyl	
9	**4**	47.77	8.7	quaternary				HMBC 23, 24	
10	**8**	45.2	10	quaternary				HMBC 9	
11	**14**	43.3	11	quaternary				HMBC-H27, H26	
12	**20**	42.3	9.4	quaternary				HMBC-H26, H29	
13	19	40.9	8	CH2	2xm	1.87	1.67	COSY H18	
14	1	39.8	9.9	CH2	2xm	2.94	1.39	HSQC-split up, HMBC-C3, Spinsystem 1-2	
15	22	37.3	8.3	CH2	2xm	1.40	1.40	HMBC H28	
16	**10**	36.7	10.8	quaternary				HMBC H9, HMBC H25	
17	2	34.2	11.7	CH2	2xm	2.61	2.35	COSY H1	
18	7	32.1	8.9	CH2	2xm	1.67	1.45	Cosy 6	
19	**17**	31.8	12	quaternary				HMBC H28	
20	21	31.0	7	CH2	2xm	1.95	1.41	Cosy, HSQC Spinsystem 21- 22 no correlation to C28	
21	29	29.5	-9.2	methyl	s	1.17		HMBC C30	
22	28	28.4	-11.8	methyl	s	0.83		HMBC C18	
23	15/16	26.5	8.2	CH2	2xm	**2.03**	**1.03**	COSY + HMBCH27	
24	23/24	26.4	-5.2	methyl	s	1.10		HMBC C5	
25	15/16	26.4	overlapp	CH2	2xm	**1.85**	**1.20**	COSY + HMBCH27	
26	27	23.3	-12.8	methyl	s	1.37		HMBC C13	
27	23/24	21.4	-15	methyl	s	1.07		HMBC C5	
28	6	18.8	8.9	CH2	2xm	1.55	1.55	COSY 5	
29	26	18.5	-10.7	methyl	s	1.16		HMBC H9, HMBC C9	
30	25	15.7	-12.6	methyl	s	1.26		HMBC C9	

CS169_02 PNS am 21.10.2009 (CDCl3)



CS169_02 PNS am 21.10.2009 (CDCl3)

2.97 2.96 2.95 2.93 2.92 2.91 2.65 2.64 2.63 2.61 2.60 2.58 2.43 2.39 2.38 2.38 2.37 2.35 2.34 2.34 2.33 2.24 2.21 2.04 2.03 2.01 2.00 1.97 1.90 1.87 1.85 1.71 1.68 1.66 1.64 1.58 1.55 1.53 1.47 1.44 1.42 1.37 1.31 1.30 1.29 1.27 1.26 1.17 1.16 1.10 1.07 1.03 0.99 0.83

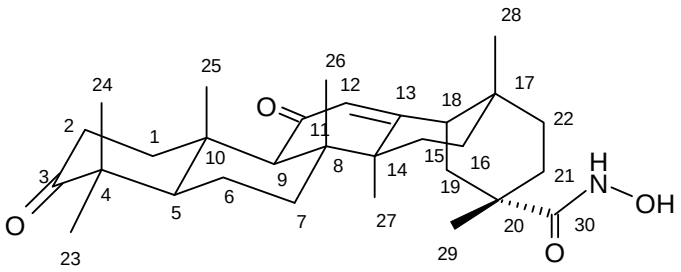


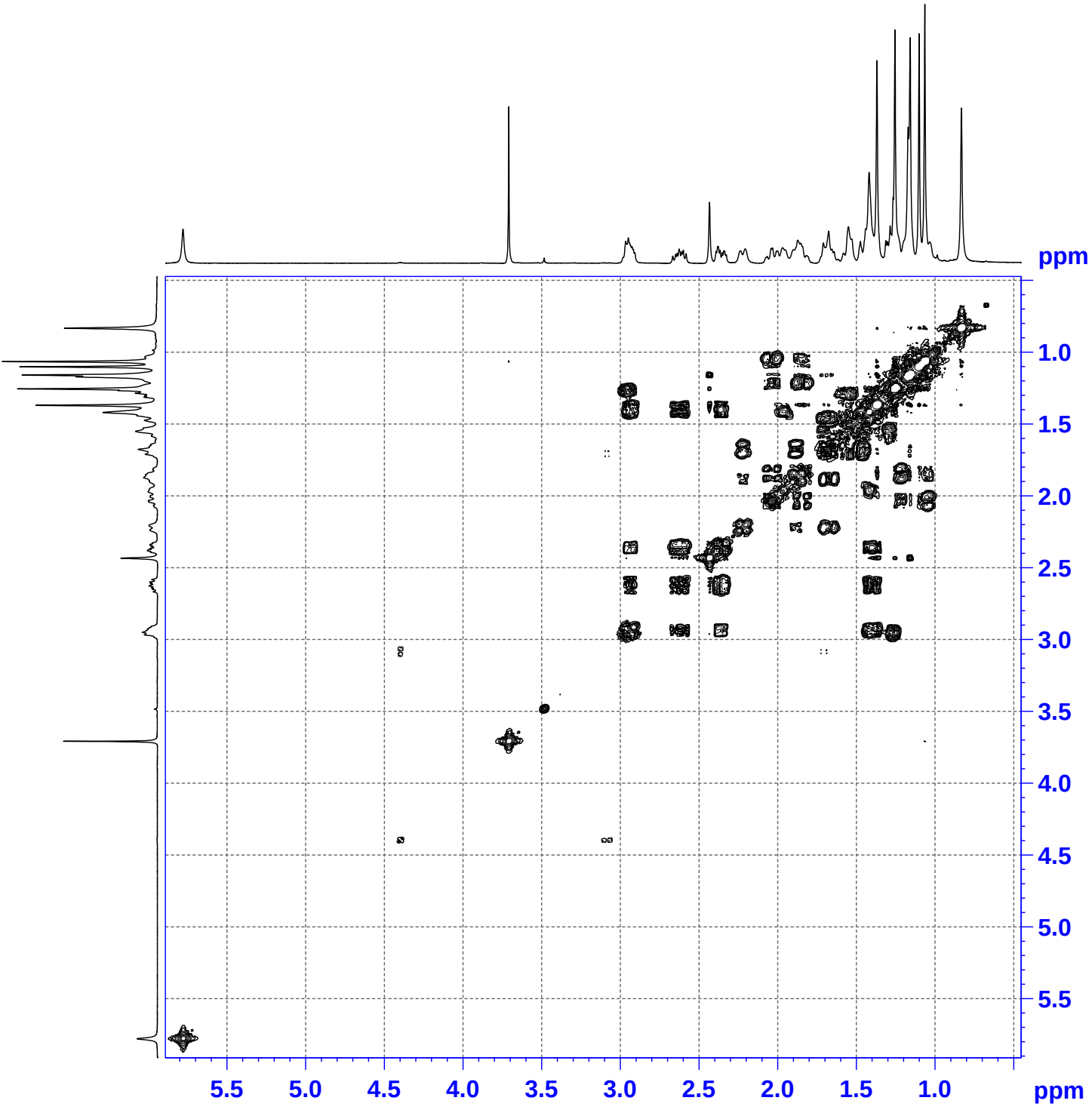
3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 ppm

Scale: 0.1138 ppm/cm, 45.54 Hz/cm

1.68 1.00 0.91 1.05 0.98 3.84 1.93 1.88 4.03 3.49 5.54 6.11 2.78 4.66 3.25

CS169_02 PNS am 21.10.2009 (CDCl3) - COSY





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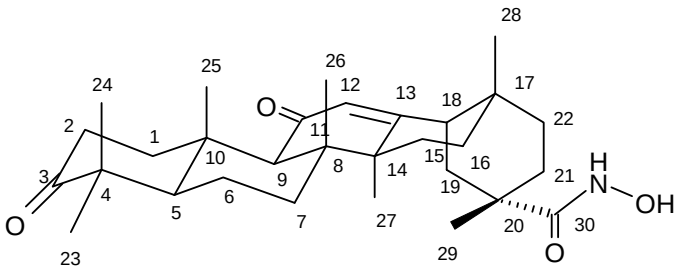

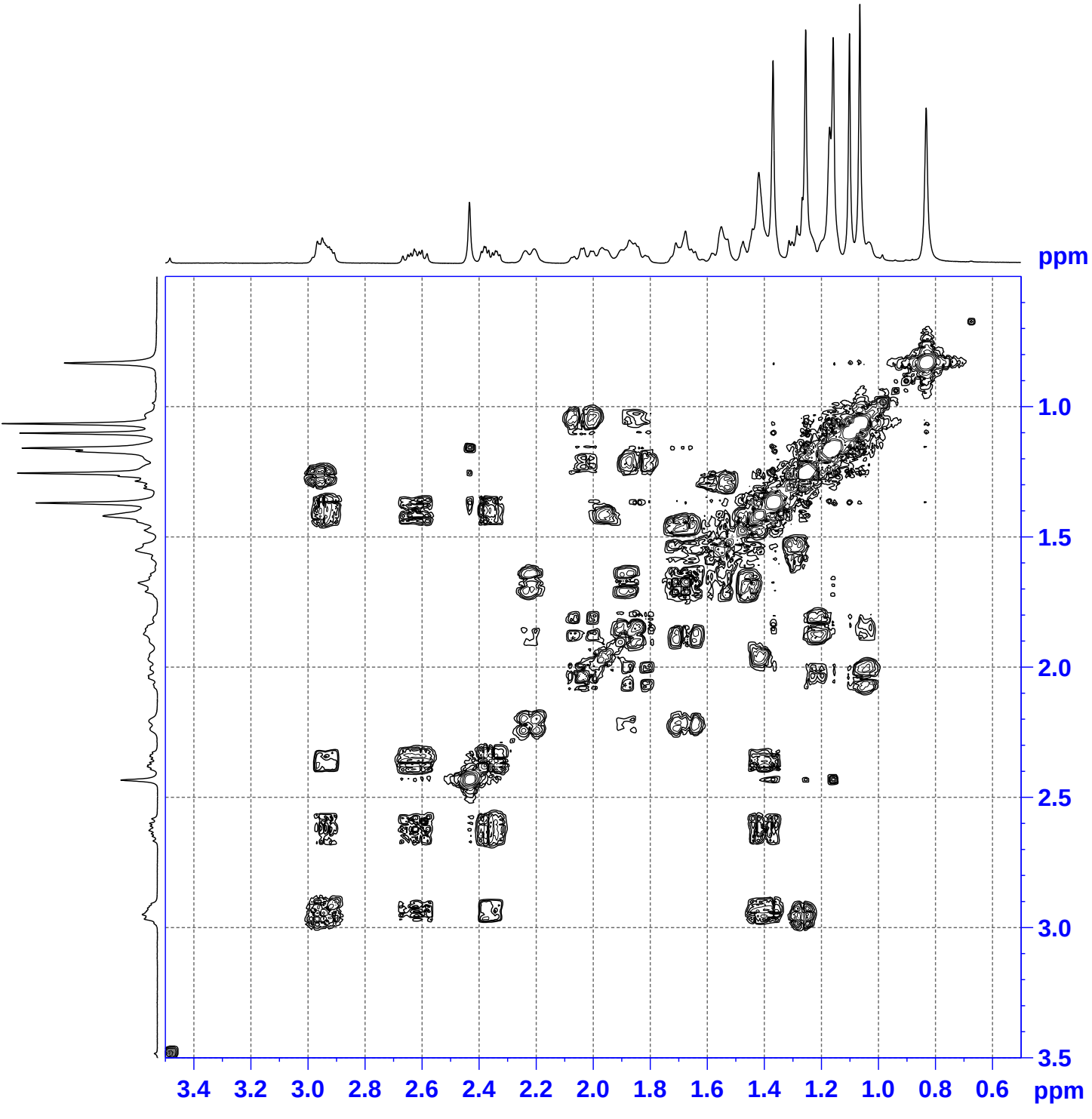

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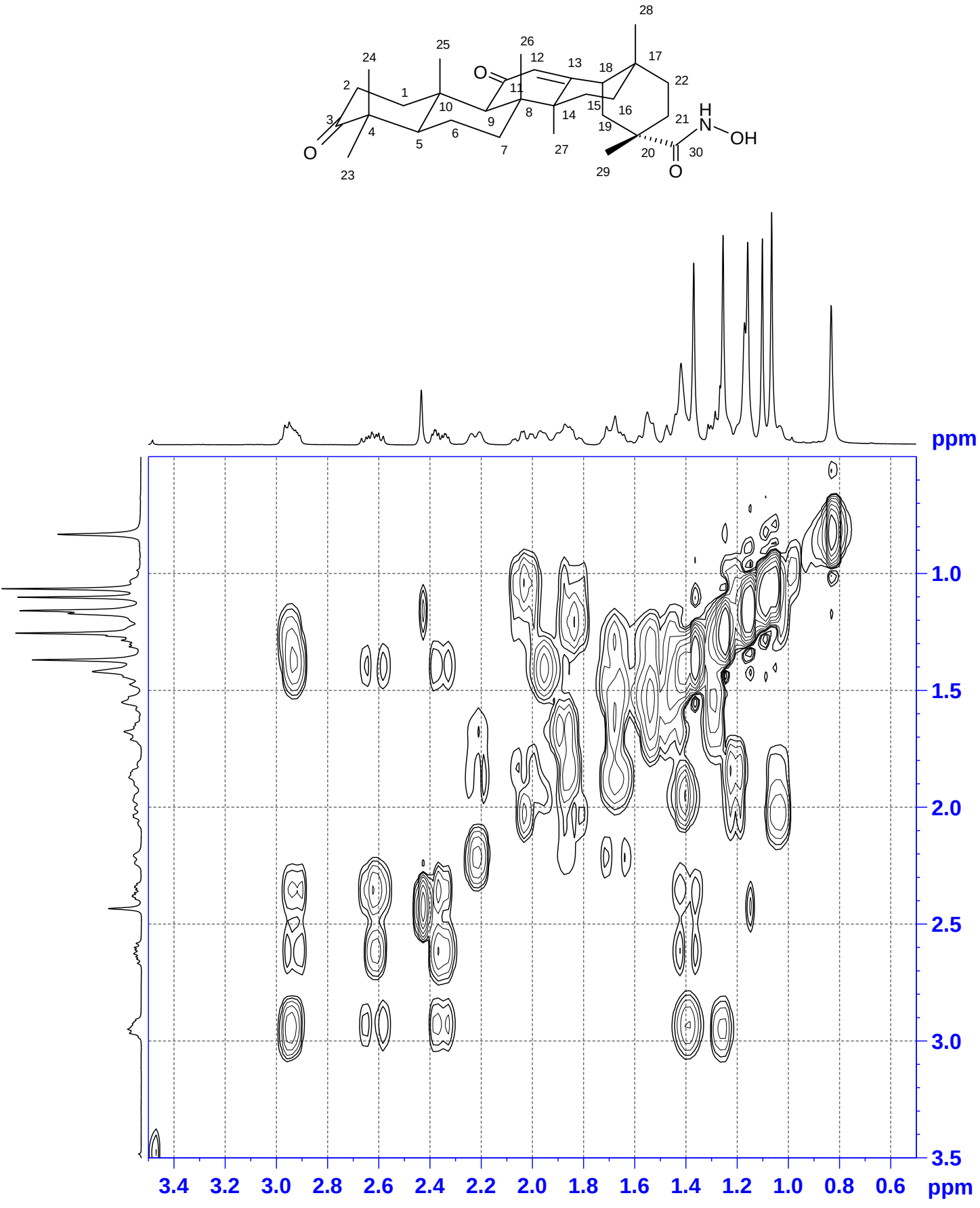

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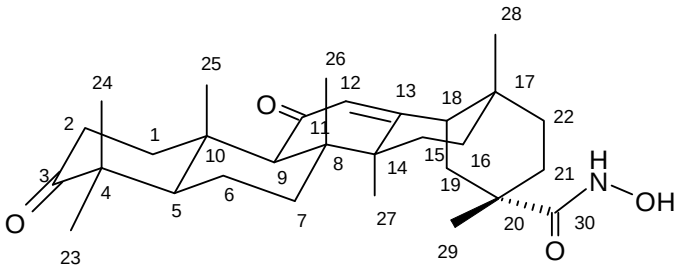

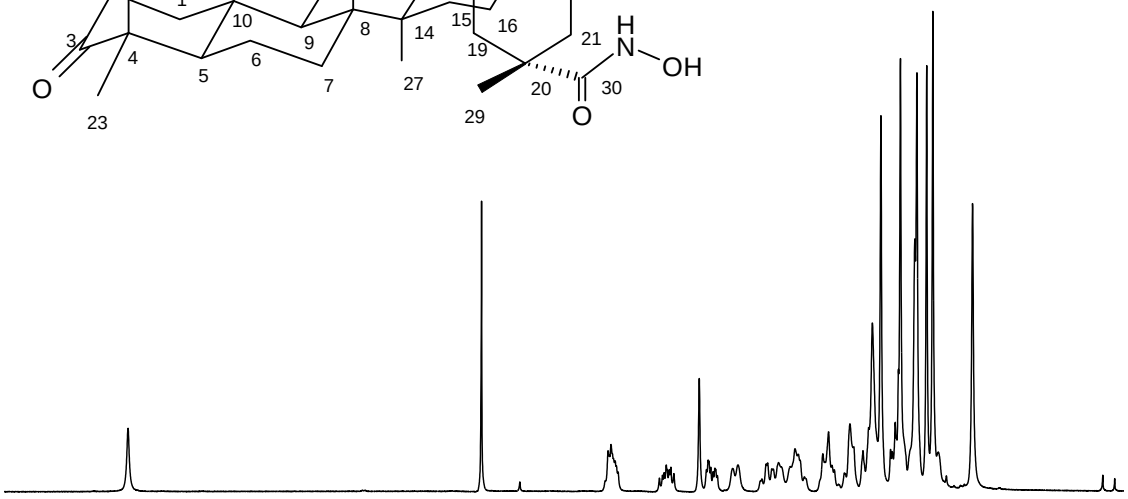

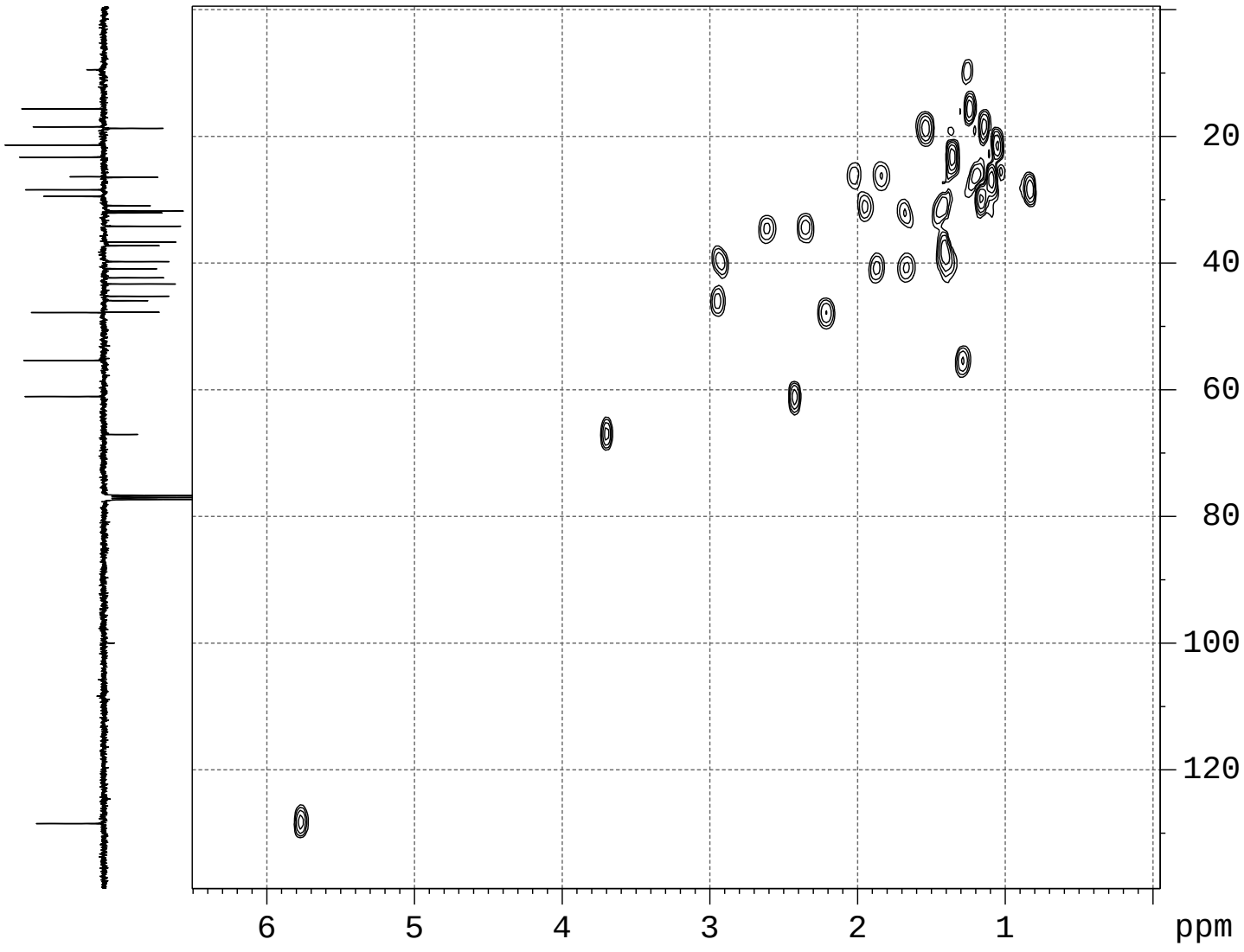

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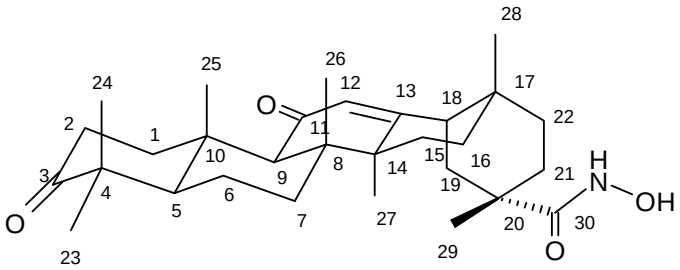

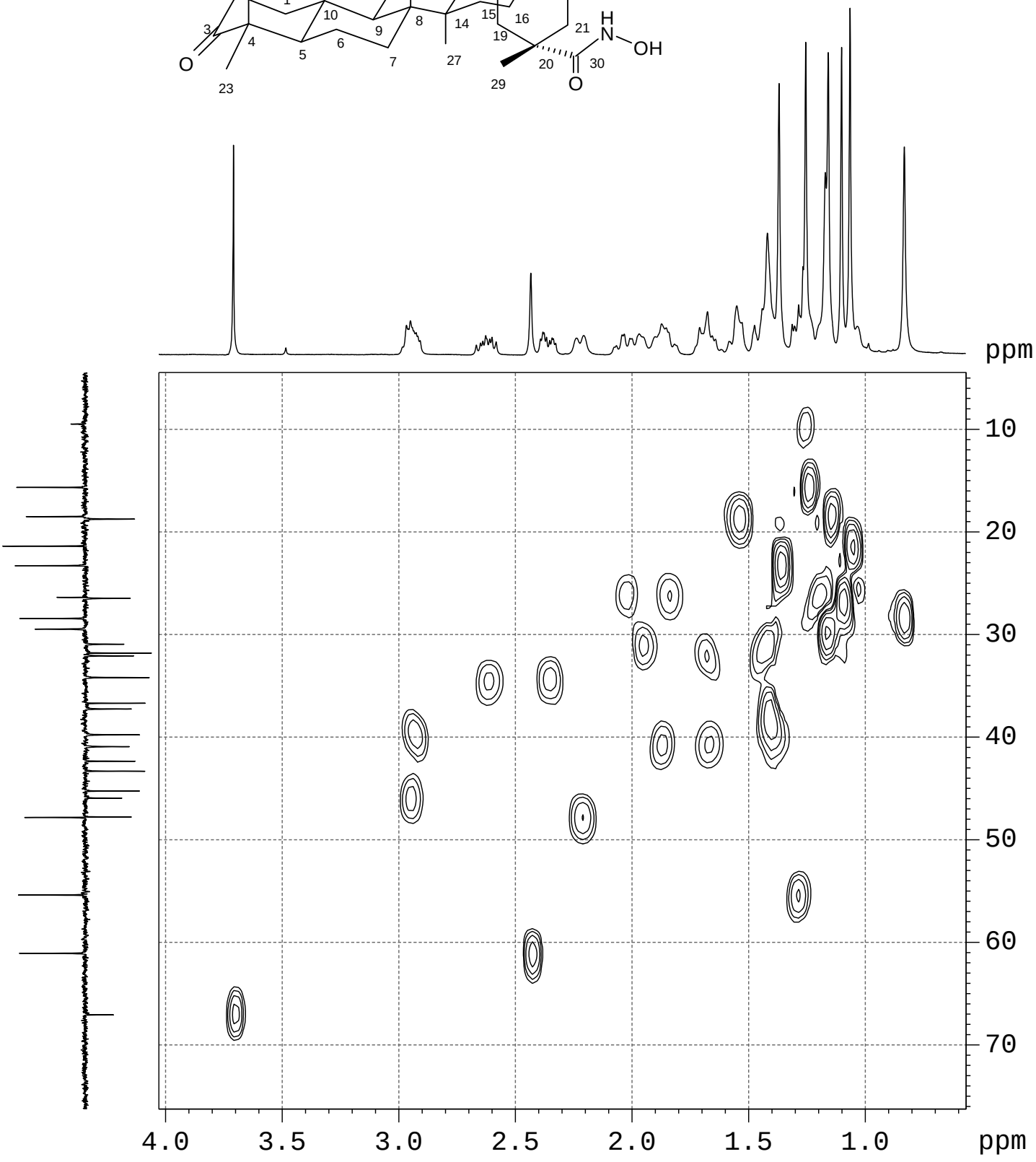

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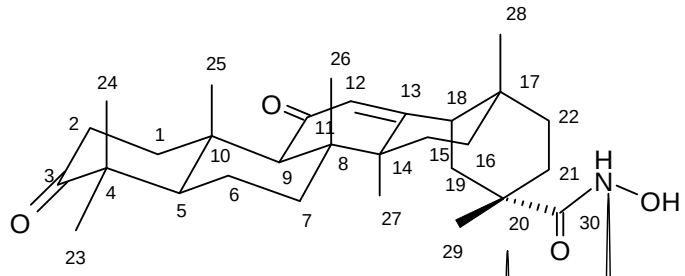

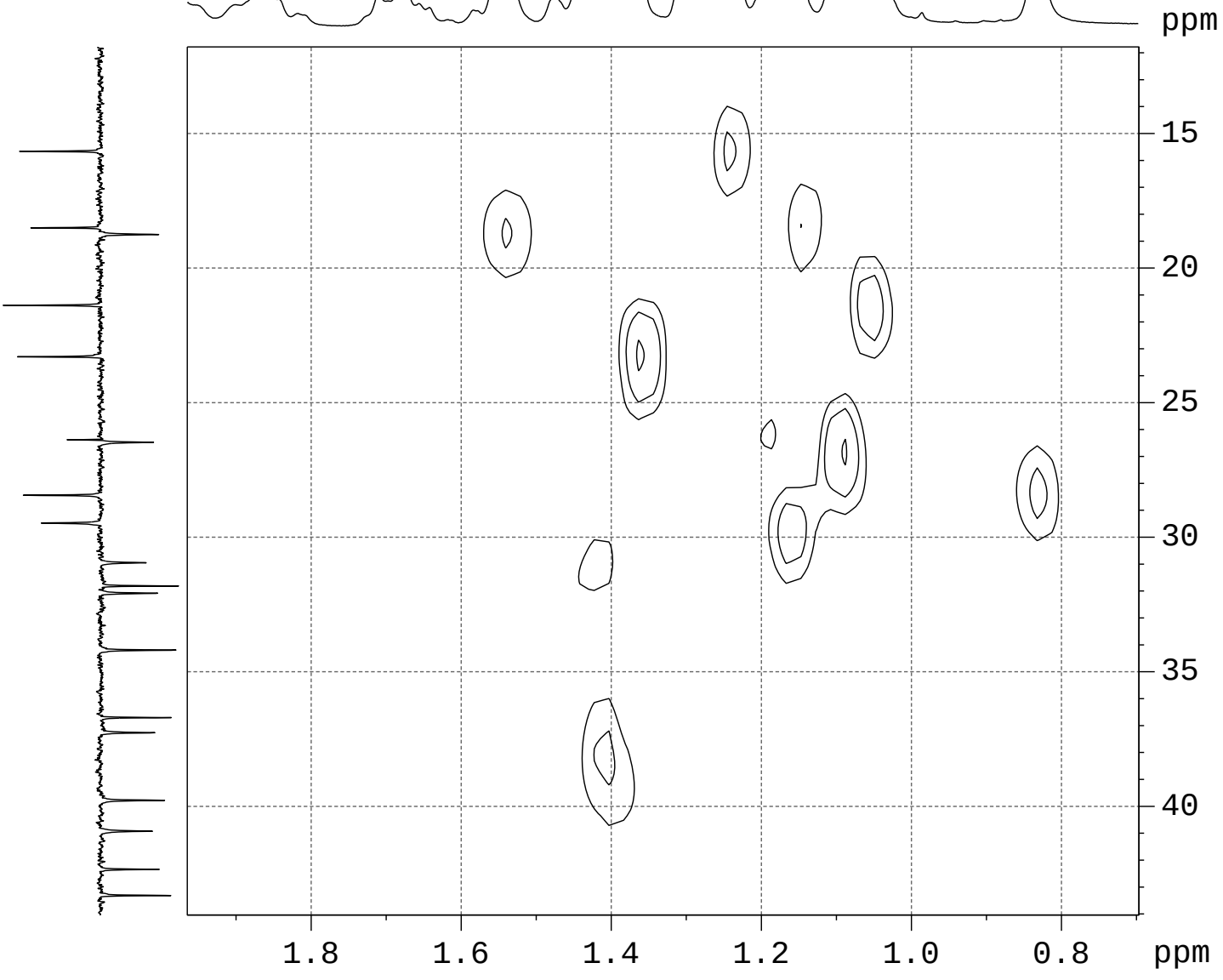

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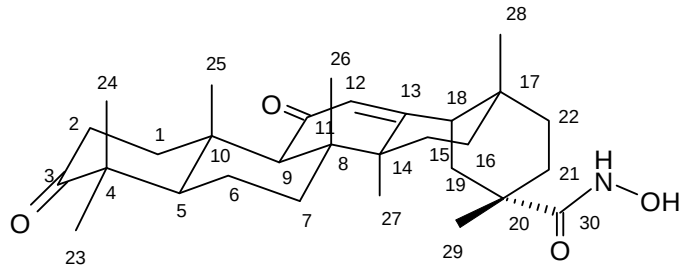

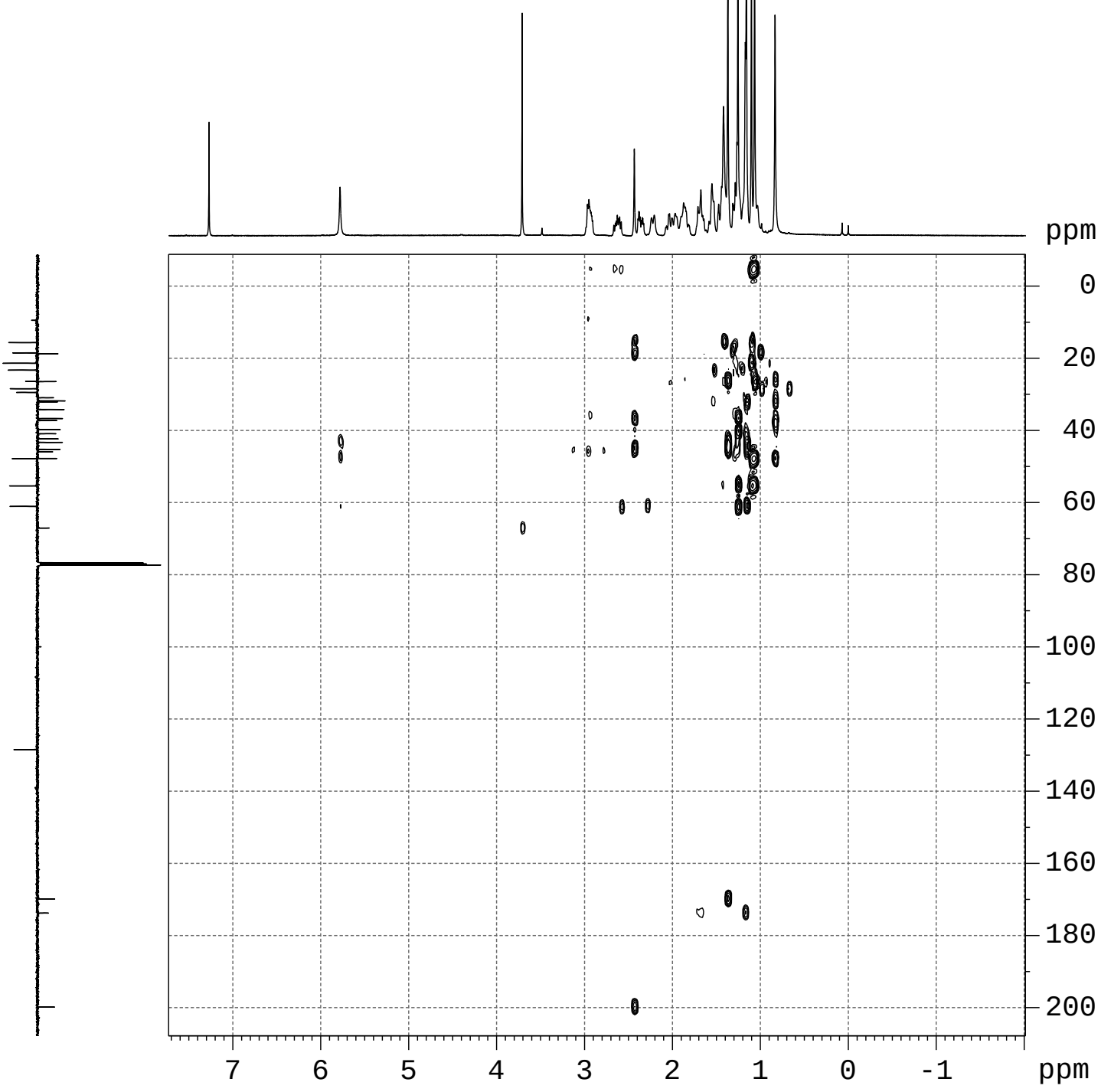

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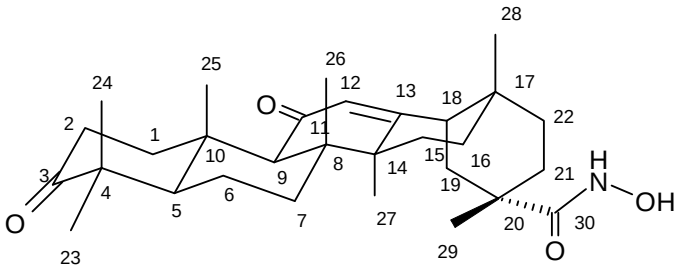

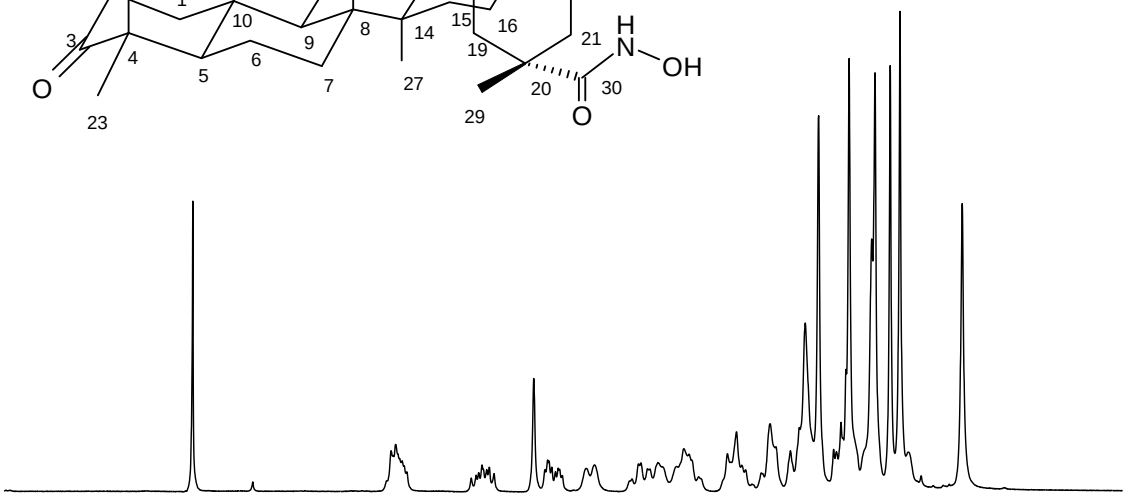

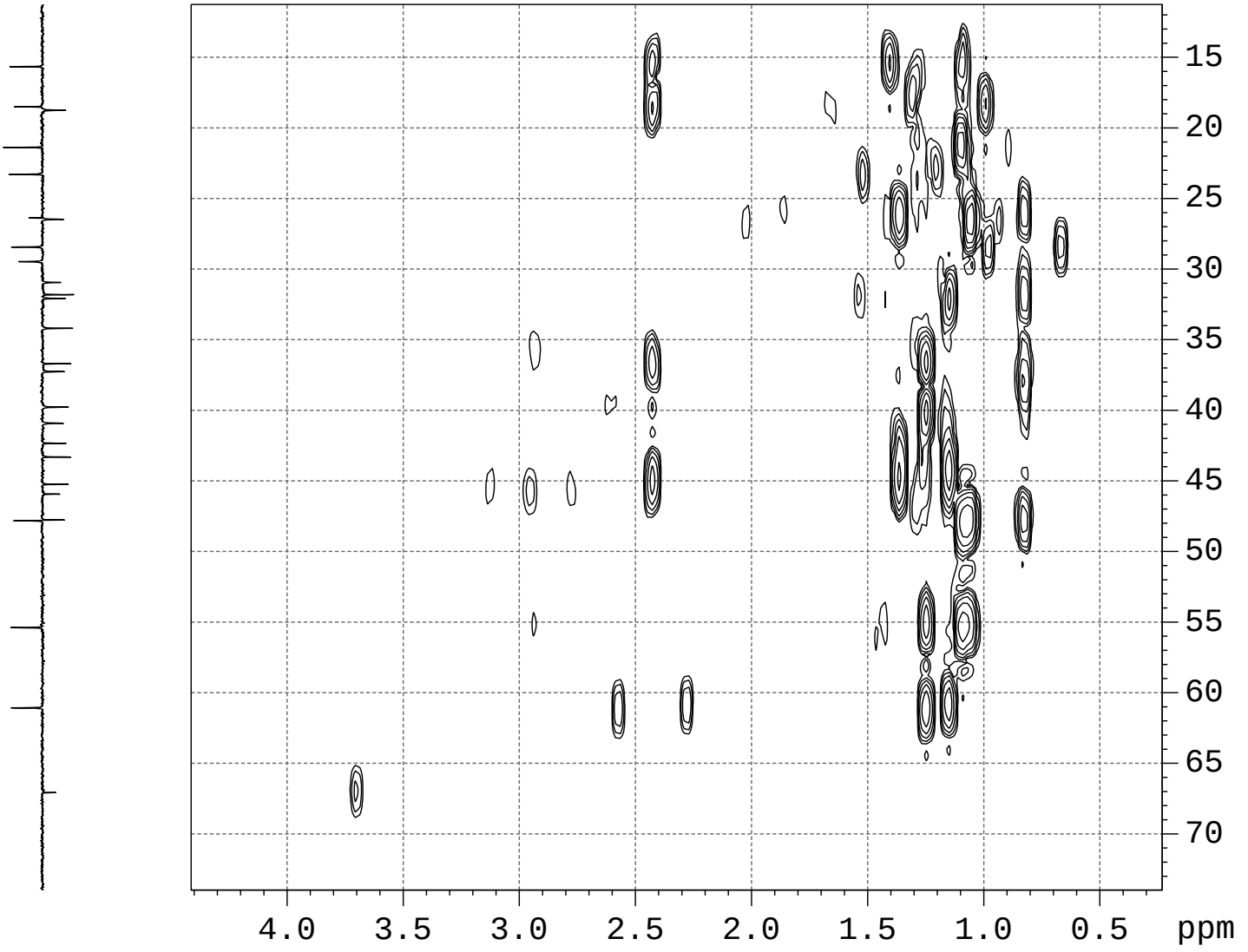

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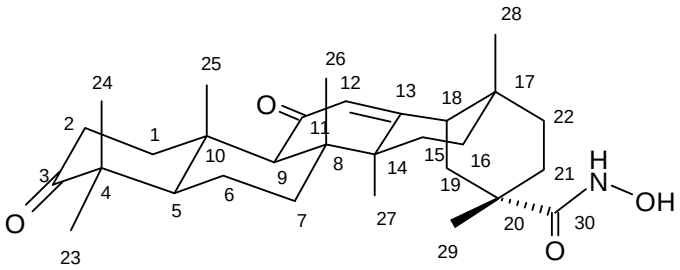

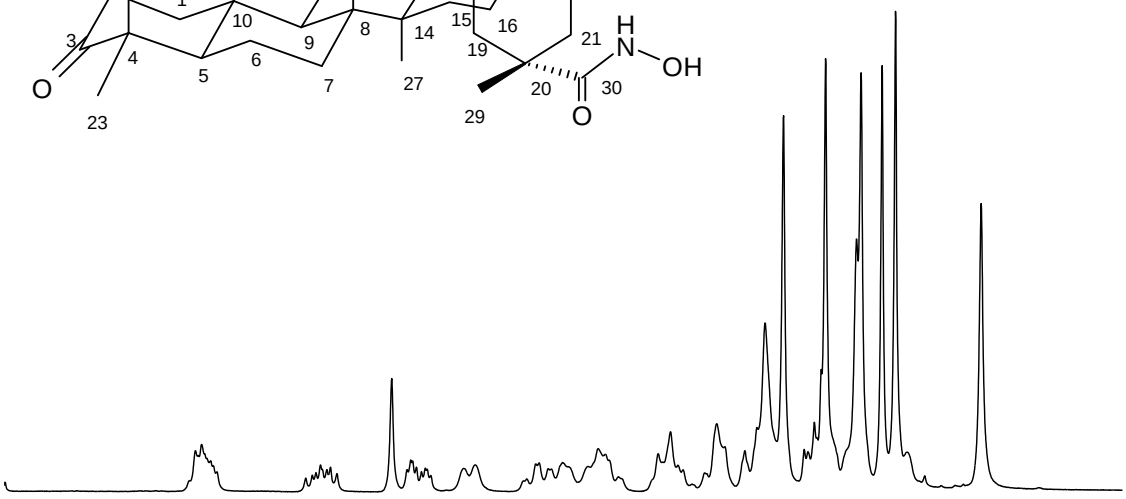

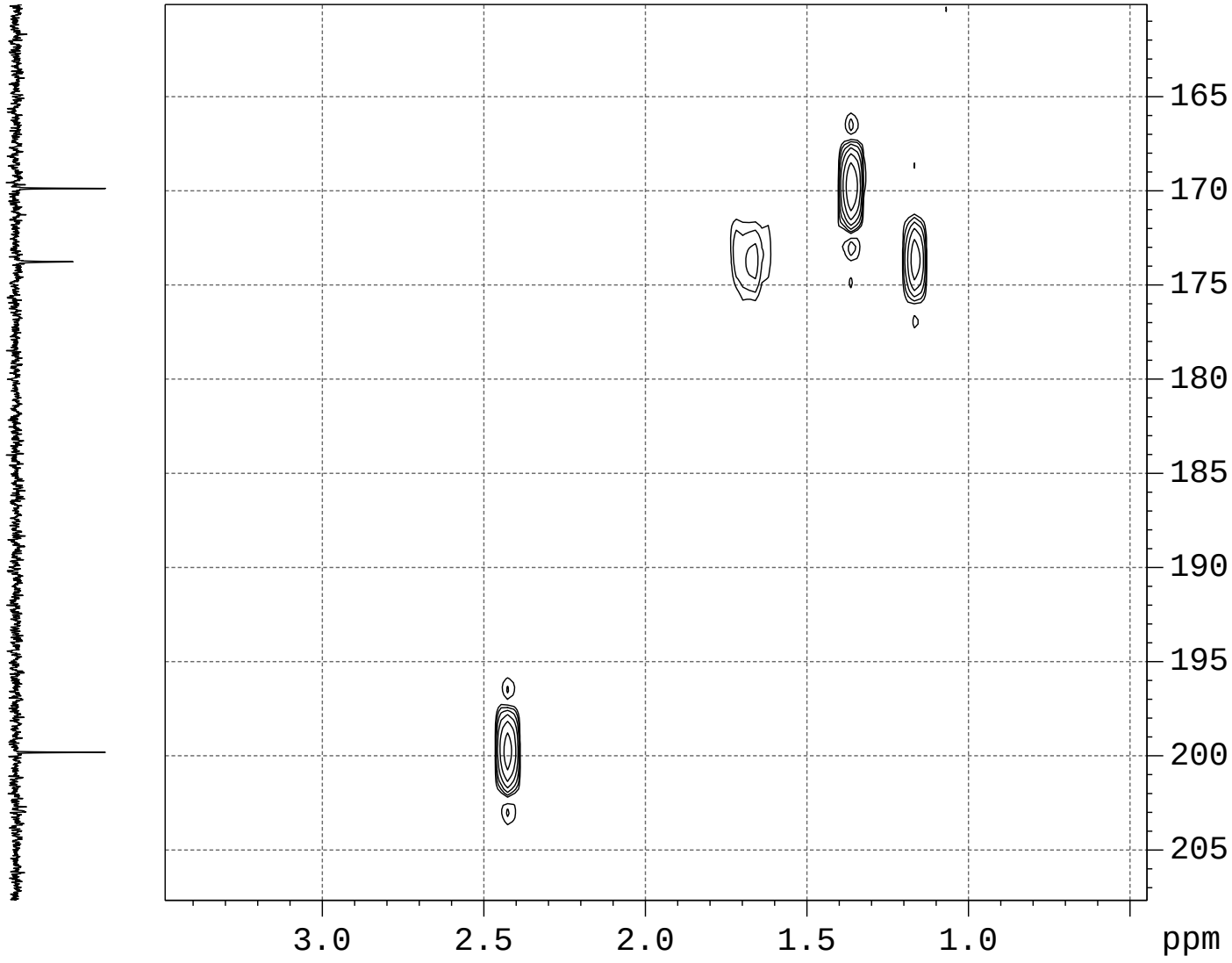

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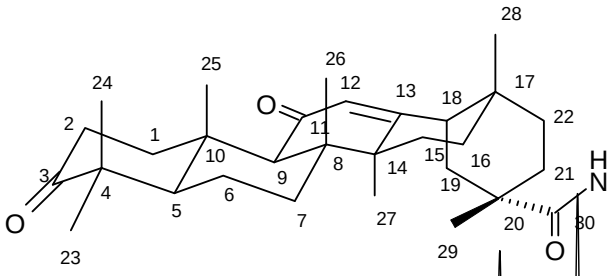

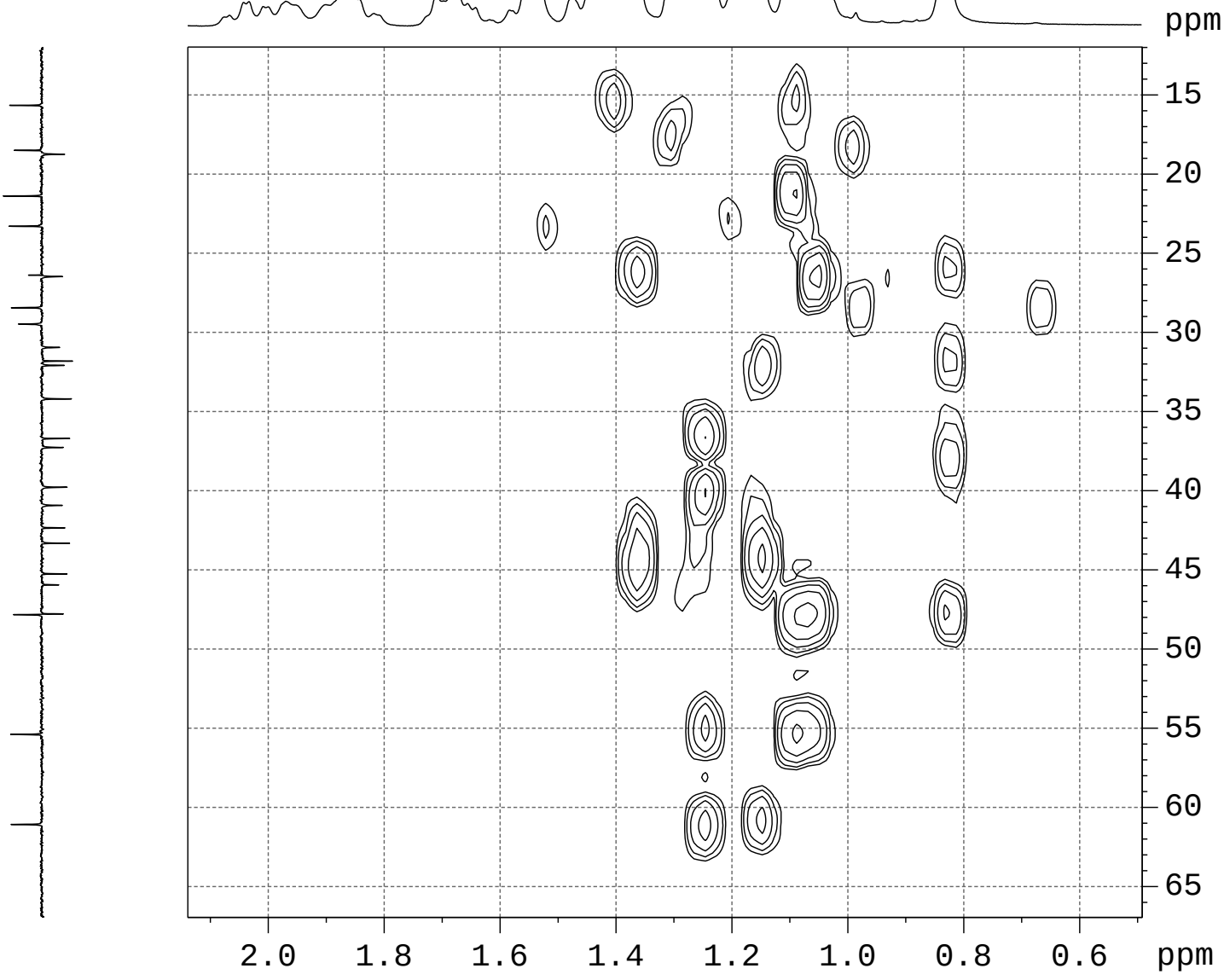

CS169_02 PNS am 21.10.2009 (CDCl3) - APT

217.3
199.8
173.8
169.9
128.5

61.1
55.4
47.8
47.8
45.2
43.3
42.3
40.9
39.8
37.3
36.7
34.2
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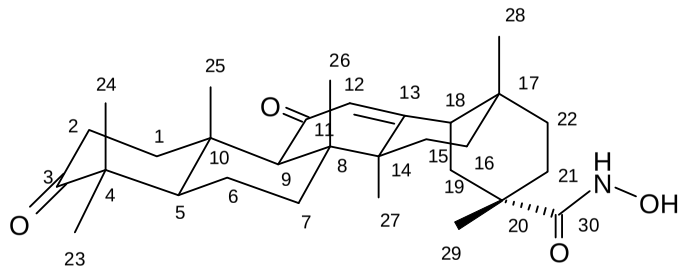
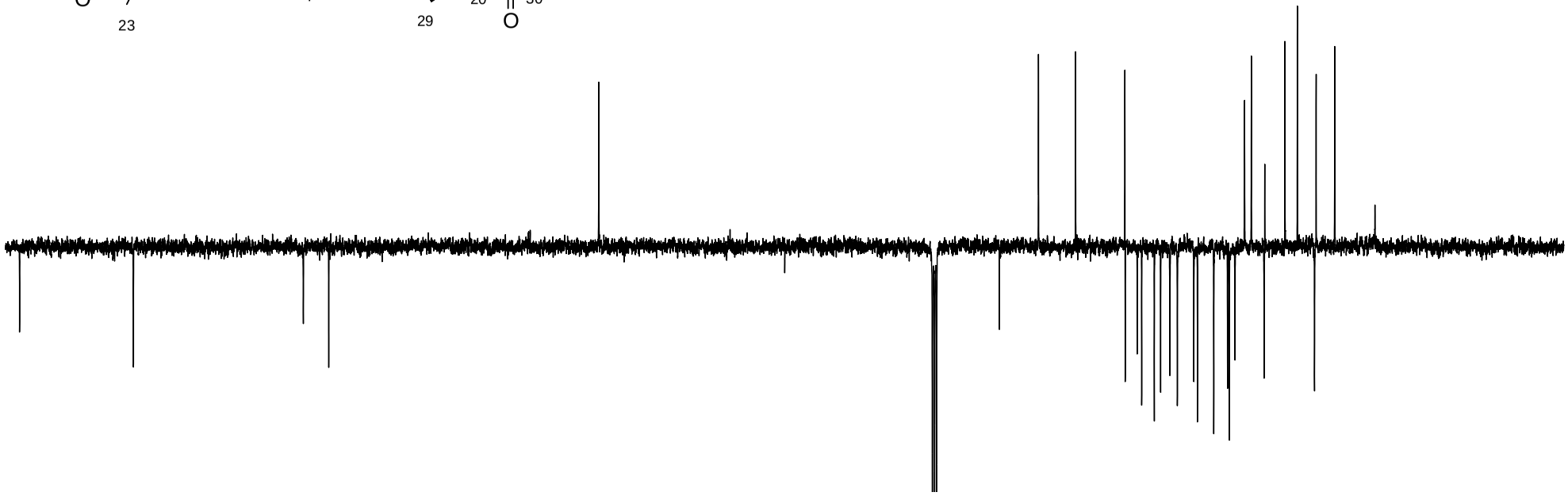
18.5
15.7





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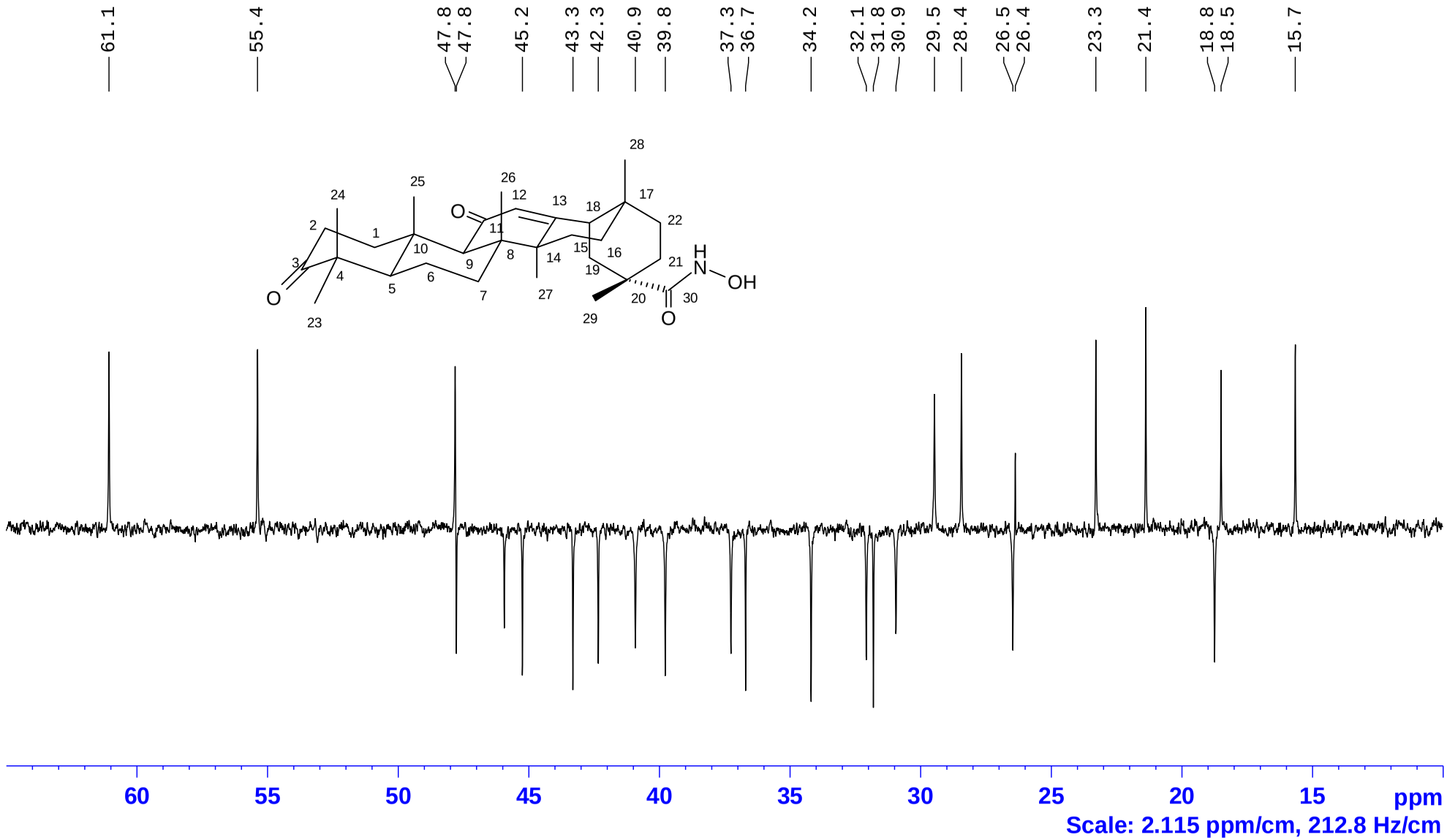
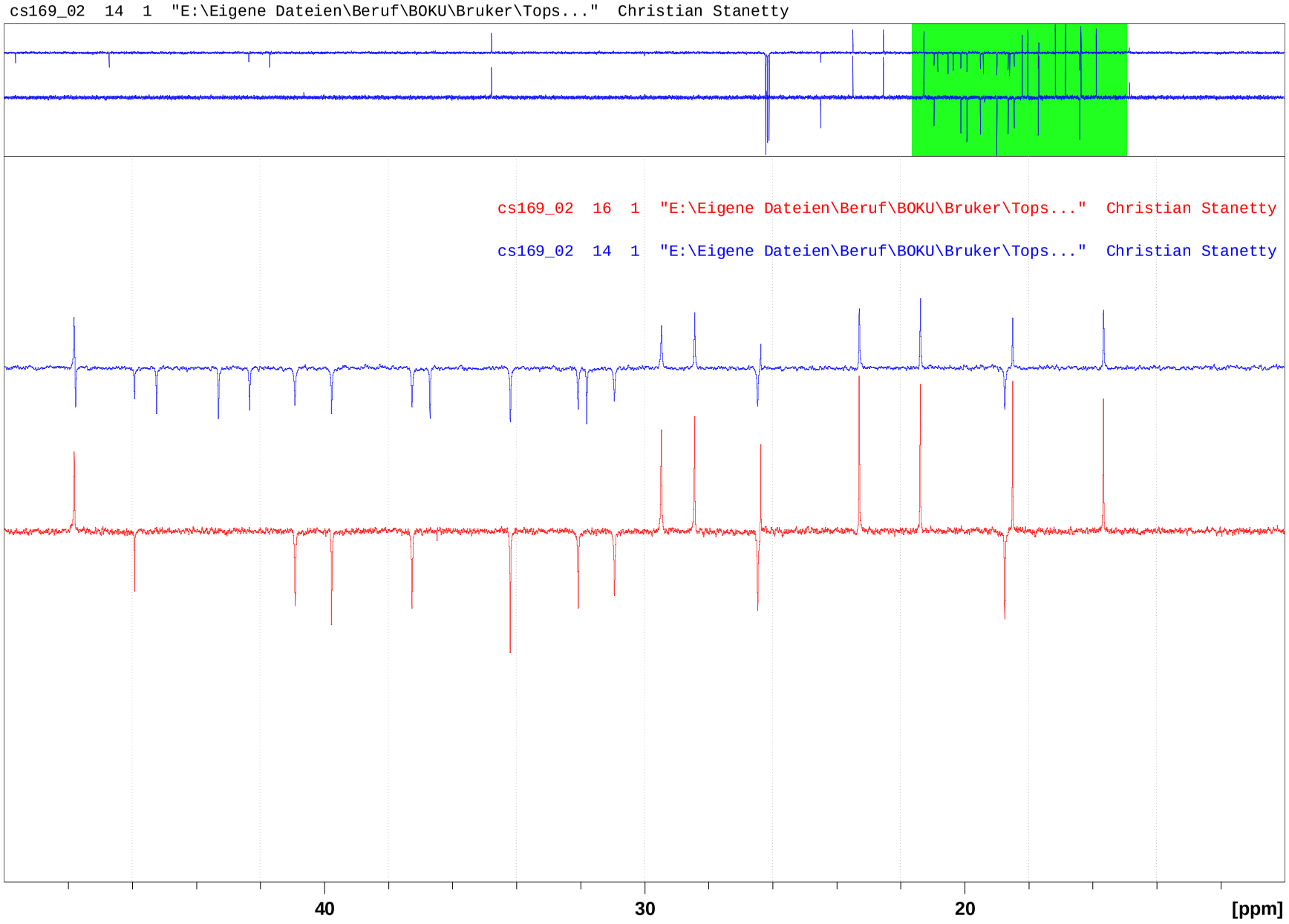
Scale: 9.189 ppm/cm, 924.6 Hz/cm

CS169_02 PNS am 21.10.2009 (CDCl3) - APT




cs169_02 14 1 "E:\Eigene Dateien\Beruf\BOKU\Bruker\Tops..." Christian Stanetty
cs169_02 16 1 "E:\Eigene Dateien\Beruf\BOKU\Bruker\Tops..." Christian Stanetty
cs169_02 14 1 "E:\Eigene Dateien\Beruf\BOKU\Bruker\Tops..." Christian Stanetty
40
30
20
[ppm]

6 Curriculum Vitae

Personal Data

Date of birth: January 5th 1980, Vienna

Adress: Schadinagasse 10/28, 1170 Vienna

Contact: christian.stanetty@boku.ac.at

School Education

1990 – 1998	Gymnasium in Stockerau, NÖ (*with distinction*)

Civil Services

October 1999 – Sept. 2000	Austrian blinds school

University Education

October 1998 to June 2005	*Technical Chemistry* at the Vienna University of Technology (TU-Wien) – *with distinction*
September 2005 – Feb. 2010	*PhD* at the Vienna University of Technology
October 2007 to date	*MBA program* (General Management) at TU-Wien / Danube University Krems

Awards and Scholarships

2002	Top-Scholarship of the county Lower Austria
2003, 2005	Twice - Excellence Scholarship (TU Wien)
2006	Price for best diploma thesis (Austrian Chemical Society)

Professional Experiences

July 2001 – Sept. 2001 & July 2002 – Sept. 2002	Two Internships – Syngenta Crop Protection, Basel

Professional Experiences

March 2004 – Nov. 2006	Student assistant and research assistant at the University of Vienna, Department for Medicinal Chemistry
December 2004 – Dec. 2008	Tutor at the FH Campus Wien – Biotechnology
December 2006 – to date	Research assistant at University of natural resources and applied life sciences, Vienna / Dep. Chemistry

Conference contributions

Posters:	Nine in total, five as *presenting author* (2005 to date)
Oral presentations:	Three in total, one as *presenting author* (2007 to date)

Scientific publications

- Berndt, U.; Stanetty, Ch.; Noe, C. R. *et al. J. Label. Compd. Radiopharm.* **2007**, *50(S1)*, 86
- Berndt, U.; Stanetty, Ch.; Noe, C. R. *et al. J. Label. Compd. Radiopharm.* **2008**, *51*, 137-145
- Del Ruiz-Ruiz, M.C.; Amer, H.; Stanetty, Ch.; Beseda, I., Czollner, L.; Shah, P.; Jordis, U.; Kueenburg, B.; Claßen-Houben, D.; Hofinger, A.; Kosma, P. *Carbohydr. Res.* **2009**, *344*, 1063-1071
- Beseda, I.M; Czollner, L.; Shah, P. S.; Khunt, R.; Gaware, R.; Kosma, P.; Stanetty, Ch.; del Ruiz-Ruiz, M.C.; Amer, H.; Mereiter, K.; Da Cunha, Th.; Odermatt, A.; Claßen-Houben, D. Jordis, U. *Bioorg.Med. Chem.* **2010** *18* 433–454
- Dell'mour, M.; Koellensperger, M.; Quirino, J.P.; Haddad, P.R.; Stanetty, C.; Oburger, E.; Puschenreiter, M.; Hann, St; *Electrophoreses*, **2010**, *in press*