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DISSERTATION

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„Design and evaluation of novel anthracycline derivatives“

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Contents

1 Abstract

For over four decades the anthracycline antibiotics have played a leading role in the treatment of solid and hematological malignancies and have formed the basis of most cancer treatment regimes. Although the third generation of anthracycline drugs is meanwhile under investigation and partially approved, substances available on the market have a great potential for improvements, mainly because of their dose limiting toxicities.

The ambition of this thesis was the modification of therapeutically used anthracyclines in view of their physicochemical properties, in particular their hydrophilic-lipophilic balance (HLB). Targeted structural modifications were made to modulate their pharmacokinetic properties and to increase their therapeutic efficacy.

Polyethylene glycol (PEG) is a widely used amphiphilic polymer known to improve the *in vivo* efficacy mostly of biological macromolecules. In the present work we used the new concept of a short linear methoxy oligoethylene glycol (mOEG) moiety as covalent modifier for the established anthracyclines daunorubicin and doxorubicin.

Crystallographic data of the drug-receptor complex of different anthracyclines and the relatively high tolerance for structural modification in the sugar moiety were crucial for a target based design of novel anthracycline analogues, modified at the 6'-position of their pyranose sugars. For this purpose a new and versatile synthetic route to daunosamine bearing a 6'-methoxy-oligoethylene-glycol chain has been developed, starting from commercially available (2R,3R)-tartaric acid. In this total synthesis 6'-methoxy-oligoethylene-glycol-daunosamine could be obtained in 13 steps in appropriate yield. Subsequent glycosylation with daunorubicinone and doxorubicinone followed by gradual cleavage of the protecting groups resulted in the two novel anthracycline derivatives msoep218 and msoep260 which showed remarkable amphiphilic properties.

To assess the pharmacodynamic performance of the newly synthesized anthracycline derivatives, first their cytotoxicities were tested against the human breast adenocarcinoma cell line MCF-7 by a non-radioactive cell proliferation and cytotoxicity assay. The results showed that the modified title compounds retained activity. Although having a lower in vitro activity against cancer cells (MCF-7) than doxorubicin, the compounds were still active at the therapeutically relevant low µM range of other anthracyclines. Investigation of cellular uptake behaviour of msoep218 showed a reduced cellular uptake compared to doxorubicin, which might explain the observed lower cytotoxicity. An experimental study on ABC-transporter mediated drug resistance to msoep218, comparing non-resistant epidermoid carcinoma-derived cell line KB-3-1 to its multidrug-resistant subline KBC-1, revealed that msoep218 is a substrate for ABC-transporter mediated drug efflux, such as the comparator drugs.

2 Kurzfassung

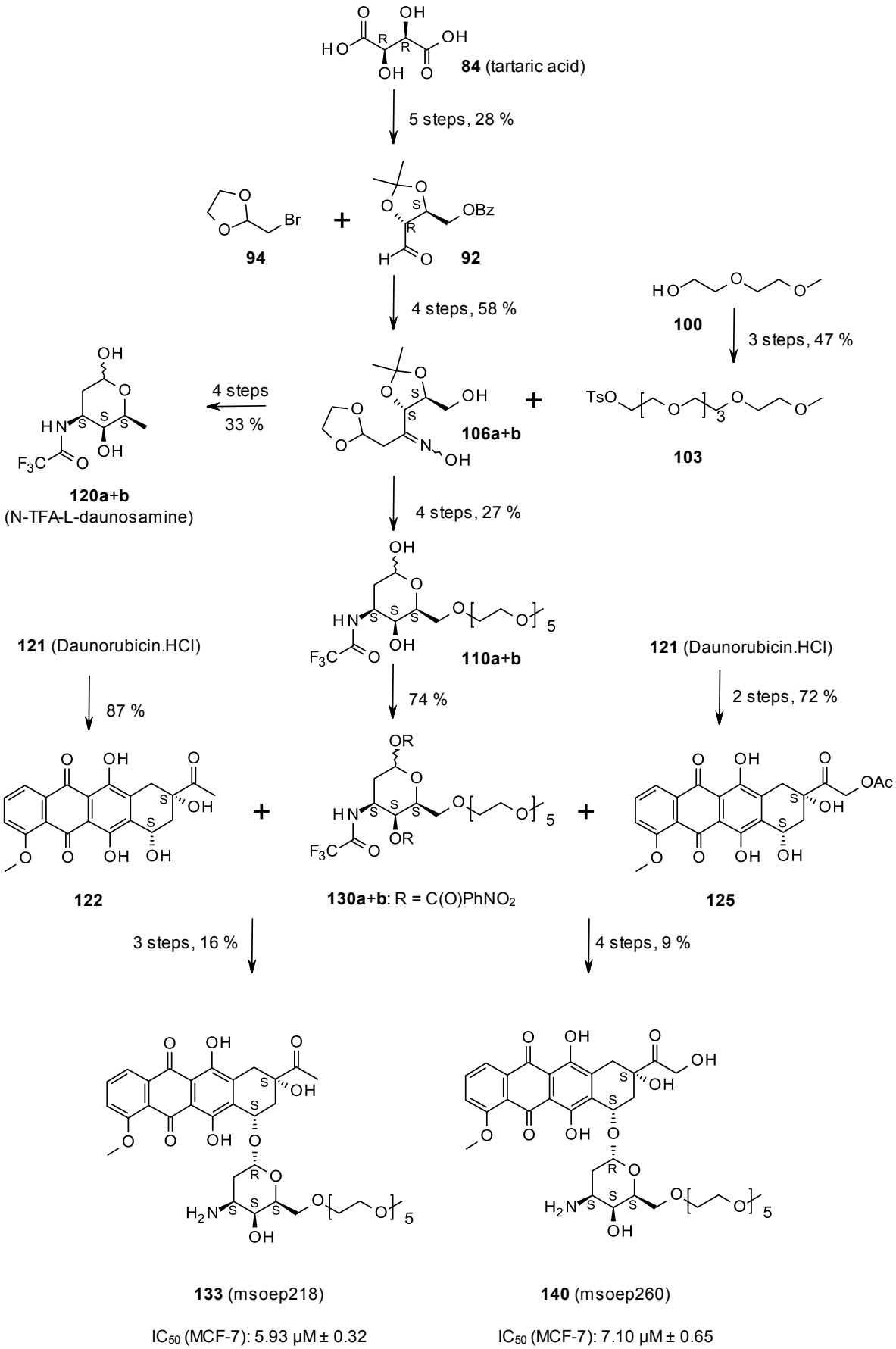
Seit über vier Jahrzenten spielen Anthracycline eine führende Rolle bei der Behandlung solider und hämatologischer Malignome und bilden nach wie vor die Basis der meisten Chemotherapie-Schemata. Obwohl mittlerweile die dritte Generation an Anthracyclinen den Markt erreicht hat, sind die verfügbaren Substanzen, vor allem wegen ihrer dosislimitierenden Nebenwirkungen, stark verbesserungswürdig.

Ziel dieser Dissertation war die Modifikation von therapeutisch relevanten Anthracyclinen im Hinblick auf ihre physikalisch-chemischen Eigenschaften, insbesondere hinsichtlich ihres hydrophilen-lipophilen Gleichgewichts (HLB). Gezielte Strukturmodifikationen wurden vorgenommen, um ihre pharmakokinetischen Eigenschaften zu modulieren und ihre therapeutische Wirksamkeit zu erhöhen.

Polyethylenglykol (PEG) ist ein weit verbreitetes amphiphiles Polymer, welches dafür bekannt ist, die *in vivo* Wirksamkeit vorwiegend von biologischen Makromolekülen zu verbessern. In der vorliegenden Arbeit verwendeten wir das neue Konzept einer kurzen linearen Methoxy-Oligoethylenglycolkette (mOEG) als kovalenten Modifikator bei den beiden etablierten Anthracyclinen Daunorubicin und Doxorubicin. Kristallographische Daten zur Arzneistoff-Rezeptor-Interaktion verschiedener Anthracycline und die Tatsache, dass die Toleranz bezüglich Strukturmodifikationen im Zuckerteil relativ hoch ist, waren die Grundlage für ein strukturbasiertes Wirkstoffdesign, bei welchem die strukturellen Veränderungen ausschließlich an der 6'-Position des Zuckerteils vorgenommen wurden. Zu diesem Zweck wurde eine neue Totalsynthese für 6'-methoxy-oligoethylenglycol-daunosamin entwickelt. Ausgehend von kommerziell erhältlicher (2R,3R)-Weinsäure konnte in 13 Stufen 6'-methoxy-oligoethylenglycol-daunosamin mit angemessener Ausbeute synthetisiert werden. Anschließende Glycosylierung mit Daunorubicinon und Doxorubicinon, gefolgt von schrittweiser Abspaltung der Schutzgruppen, resultierte in den zwei neuen Anthracyclin-Derivaten Msoep218 und Msoep260 mit bemerkenswerten amphiphilen Eigenschaften.

Zur Evaluierung der pharmakodynamischen Eigenschaften der neu synthetisierten Substanzen wurde als erster Schritt ihre Zytotoxizität gegenüber der humanen Brustkrebszelllinie MCF-7 getestet. Die mittels Zellproliferation- und Zytoxizitätstests erhobenen Daten zeigten, dass die modifizierten Anthracycline ihre Aktivität beibehielten. Obwohl eine geringere *in vitro* Aktivität als bei Doxorubicin gemessen wurde, waren Msoep218 und Msoep260 dennoch signifikant zytotoxisch, vergleichbar mit anderen therapeutisch eingesetzten Anthracyclinen. Im Vergleich zu Doxorubicin zeigten weitere Untersuchungen eine reduzierte Zellaufnahme, die für die geringere Zytotoxizität verantwortlich zu sein scheint. Eine experimentelle Studie mit Msoep218 zum Einfluss ABC-Transporter-vermittelter Resistenzen auf die Zytotoxizität zeigte, dass Msoep218 – so wie die Vergleichswirkstoffe – Substrat für ABC-Transporter vermittelten Efflux ist.

3 Graphical abstract



4 Introduction

4.1 Cancer therapeutics: General considerations

Global cancer burden rises steadily, as data recently published by the International Agency for Research on Cancer (IARC) confirmed. While in 2008, 12.7 million new cases and 7.6 million cancer-related deaths occurred, the burden rose to 14.1 million new cases and 8.2 million cancer deaths in 2012, and the trend is expected to continue upward.[1] Although, at least in the industrialized countries cancer survival is increasing as well, through inception of screening and early detection initiatives, lifestyle changes, treatment in specialist centres and improved cancer therapies[2]. Associated with these improvements are enormous costs, in 2009 only the cancer-related drug costs increased up to 343 million euro in Austria, 2705 million euro in Germany and throughout the European Union (EU) 13.6 billion euros were spent on cancer-related drugs[3].

Though numerous new cancer treatments have been approved in the last decades, the general success with cancer therapeutics has been mixed. The greatest hits of molecular biology as stem cell and gene therapy have not yet become established in the desired form and also the immunotherapy has underperformed its expectations so far. The hope was then placed again on small molecules. The increasing understanding of cancer biology resulted in numerous targets as growth factor receptors and signaling molecules which are essential for cancer cell survival. In this molecularly targeted therapy the kinase inhibitors brought promising results. Starting with Imatinip (Gleevec®) meanwhile numerous substances have reached the market.[4]

But the biomedical progress also improved the understanding of the first generation of effective anticancer drugs, made them switch from simple cytotoxics to small molecules with specific modes of action. Because many of these substances among them the anthracyclines still form the basis of most cancer treatment regimes their improvement with respect to reduce their side-effect profile is a declared goal.

4.2 Intercalation as therapeutical concept

In medicinal chemistry we understand by intercalation the interaction of a small molecule and DNA without forming covalent bonds, in particular, the insertion of a planar substituent between the DNA base pairs.

This very uncommon binding mode was first published by Leonard Lerman in 1961, who explained the high DNA affinity of "Acridine dyes"[5]. Intercalation exerts a great influence on DNA, and has therefore high biophysical and clinical significance. As double stranded DNA (dsDNA) has a coplanar arrangement of base pairs, intercalating small molecules, which usually carries a planar polycyclic aromatic unit, can interact by sandwich π– stacking with DNA nucleotides. Furthermore hydrogen bonding, van der Waals' forces and hydrophobic interactions often stabilize the intercalating compound at the binding site. The extent of these effects is essentially determined by the structure of the compound and its functional groups.

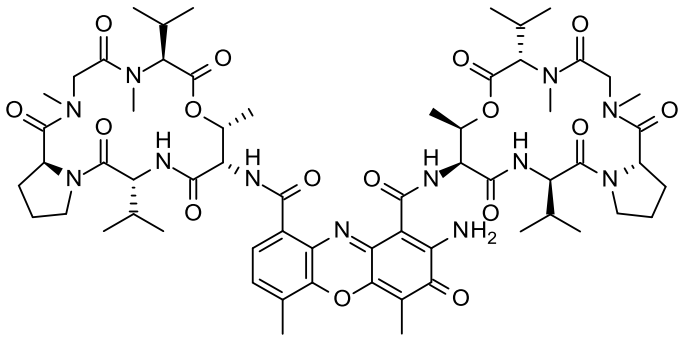
The intercalation leads to a local unwinding and lengthening of the double stranded DNA helices. The degree of unwinding is dependent on the intercalator, for example the unwinding angle of ethidium bromide is about 15 degrees, the one published for daunorubicin about 12 degrees[6]. The main effect of intercalation is the inhibition or stabilisation of DNA interacting enzymes like polymerases, which leads to a suppressed DNA replication and RNA transcription, which further results in growth arrest and/or cell killing[7].

Generally we can distinguish between two intercalation modes: The classical intercalation in which the intercalator inserts with its complete aromatic system between DNA base pairs, and the threading intercalation, whereas the molecule shows only partial insertion between DNA base pairs. Threading intercalation also shows strong molecule interactions with the DNA-grooves where the intercalating compound is stabilized by non-covalent interactions with the phosphate backbone.

To determine the exact binding mode of an intercalating compound, numerous tools (^{1}H-, ^{13}C-, ^{31}P-NMR-Spectroscopy, X-ray diffraction, linear- and circular dichroism, calorimetric methods and theoretically calculations) are available, which are often used in combination for more precisely results[8]. Although the exact DNA binding site and 3D conformation of numerous compounds is already determined, so far there is no general agreement on the kinetic of drug intercalation[9]. Most studies indicate a fast outside binding of the drug followed by a slow intercalation into the DNA, in which the drug forces the opening of a cavity and slides into the hydrophobic surrounding between the DNA base pairs, whereas "slow intercalation" has to be considered relatively, since the intercalation still happens in a millisecond time scale[10].

Despite the large number of existing DNA intercalators only a very limited number is in clinical use. The reason why they do not make it into clinical application is more often attributed to pharmacokinetics than to pharmacodynamics[8].

In clinic, DNA Intercalation is relevant for the treatment of various types of cancer. Prominent representatives in there are the class of antitumor antibiotics. Beside the anthracyclines which will be discussed in detail in the next chapter, there exist only a handful of further cytotoxic antibiotics which are used in chemotherapy. From the group of the actinomycines it is dactinomycin also called actinomycin D, first isolated from *Streptomyces* cultures, which is used against nephroplastoma and paediatric rhabdomyosarcoma[11]. It consists out of a tricyclic phenoxazone ring with two identical cyclic pentadepsipeptides (fig.1).

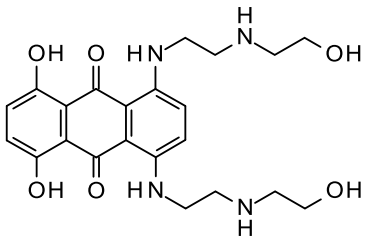


1

Figure 1 | Molecular structure of dactinomycin (1). Formula: $C_{62}H_{86}N_{12}O_{16}$, MW: 1255.42 g/mol, CAS registry number: 50-76-0, Plasma half-life: 36 hrs[12,13].

Dactinomycin intercalates with its phenoxazone ring at DNA guanine – cytosine sites and prevents thereby transcription of RNA polymerase[14].

Mitoxantrone and amsacrine (fig. 2) are chemically synthesized intercalators which also intercalate with their tricyclic ring systems into the DNA. Mitoxantrone is used against breast cancer, non-Hodgkin lymphomas, acute myeloid leukaemia and prostate cancer. Amsacrine which is particularly used against acute myeloid leukaemia, also acts on the inhibition of topoisomerase II, resulting in DNA double strand breaks, arrest of the S/G2 phase of the cell cycle and cell death[15].



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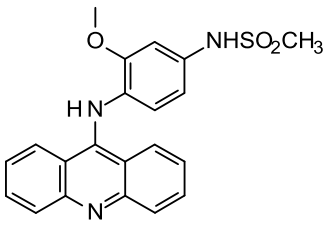

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Figure 2| **Molecular structure of mitoxantrone (2) and amsacrin (3).** Mitoxantrone / Formula: $C_{22}H_{28}N_4O_6$, MW: 444.48 g/mol, CAS registry number: 65271-80-9, Plasma half-life: 75 hrs[13]. Amsacrin / Formula: $C_{21}H_{19}N_3O_3S$, MW: 393.46 g/mol, CAS registry number: 51264-14-3, Plasma half-life: 8-9 hrs[13].

A further active substance out of the class of antitumor antibiotics is bleomycine. Bleomycine, which belongs to the glycopeptides and is isolated as dactinomycin from a *Streptomyces* strain (*streptomyces verticillus*), interacts with the DNA by intercalation and via minor groove binding. *In vivo* bleomycin forms with oxygen and iron(II)-ions an activated complex which abstracts a hydrogen from the DNA backbone. Thereby a sugar centered radical is produced, which reacts further by cleaving the DNA strand[16,17]. The ability of bleomycine to induce single- and double strand breaks lets it be very effective against Hodgkin- and non-Hodgkin lymphomas, testicular cancer and squamous-cell carcinoma[18].

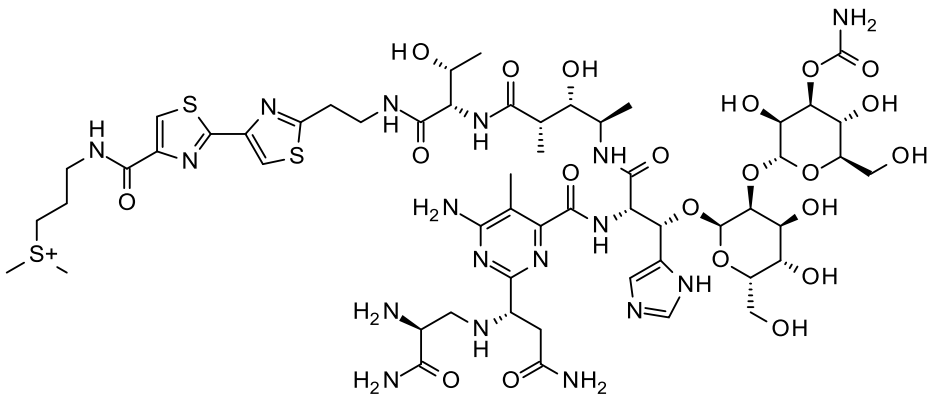

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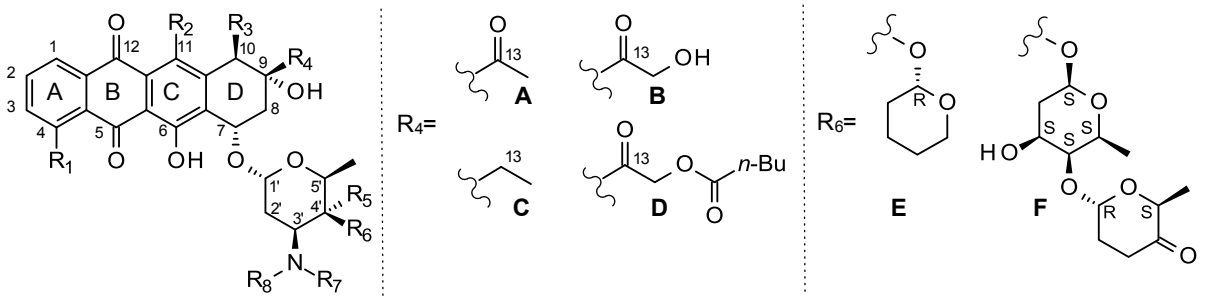
Figure 3| **Molecular structure of bleomycin (4).** Formula: $C_{55}H_{84}N_{17}O_{21}S_3$, MW: 1415.552 g/mol, CAS registry number: 11056-06-7, Plasma half-life: 3 hrs[12].

Although all these substances have an extensive side-effect profile, DNA intercalation belongs to the most important pharmaceutical concepts for cancer treatment.

4.3 Anthracyclines as anticancer agents

The first Anthracycline was detected in 1950 by Brockman and Bauer searching for new antibiotics. They isolated it from *Streptomyces purpurascens* and named it beta-rhodomycin I[19]. In the early 60s the companies "Farmitalia" and "Rhone-Poulenc" independently discovered daunorubicin, which was the first clinically effective anthracycline. In the following years numerous studies proved its cytotoxic potential against malignant cells[20,21]. Only a few years after the discovery of daunorubicin, doxorubicin was isolated in 1969, which shows a better therapeutic index than daunorubicin and is effective against a much broader spectrum of tumours[22]. The remarkable pharmaceutical properties of the detected anthracyclines lead to an enormous increase in research and development of new analogs. Nevertheless, only few of them made it into clinical use. Those in clinical use are: Daunorubicin (DNR), doxorubicin (DOX), epirubicin (EPI), idarubicin (IDA), aclarubicin, pirarubicin and valrubicin[23].



5 Daunorubicin	**6** Doxorubicin	**7** Epirubicin	**8** Idarubicin	**9** Aclarubicin	**10** Pirarubicin	**11** Valrubicin
$R_1 = OCH_3$	$R_1 = OCH_3$	$R_1 = OCH_3$	$R_1 = H$	$R_1 = OH$	$R_1 = OCH_3$	$R_1 = OCH_3$
$R_2 = OH$	$R_2 = OH$	$R_2 = OH$	$R_2 = OH$	$R_2 = H$	$R_2 = OH$	$R_2 = OH$
$R_3 = H$	$R_3 = H$	$R_3 = H$	$R_3 = H$	$R_3 = COOMe$	$R_3 = H$	$R_3 = H$
$R_4 =$ **A**	$R_4 =$ **B**	$R_4 =$ **B**	$R_4 =$ **A**	$R_4 =$ **C**	$R_4 =$ **A**	$R_4 =$ **D**
$R_5 = H$	$R_5 = H$	$R_5 = OH$	$R_5 = H$	$R_5 = H$	$R_5 = H$	$R_5 = H$
$R_6 = OH$	$R_6 = OH$	$R_6 = H$	$R_6 = OH$	$R_6 =$ **F**	$R_6 =$ **E**	$R_6 = OH$
$R_7 = H$	$R_7 = H$	$R_7 = H$	$R_7 = H$	$R_7 = Me$	$R_7 = H$	$R_7 = H$
$R_8 = H$	$R_8 = H$	$R_8 = H$	$R_8 = H$	$R_8 = Me$	$R_8 = H$	$R_8 = COCF_3$

Figure 4| **Structure of clinically used anthracyclines.** Absolute stereochemistry and formula of the anthracyclines was determined by SciFinder- structure database[24].

Anthracyclines naturally can exist as glycosides and as aglycons (anthracyclinones), those in clinical use are all glycosides, consisting out of a tetracyclic aglycon (ring A, B, C and D) derived from anthraquinone and of one or more sugar moieties, attached via a glycosidic bond to C-7 of ring D. The hydroxyanthraquinone chromophore is responsible for the intense colours of anthracyclines ranging from yellow over red to purple[25]. At C-9 anthracyclines

carry a short side chain with a carbonyl group at C-13, except aclarubicin which is carrying an ethyl group at C-9 (see fig. 4).

Although the structural differences of anthracyclines are sometimes very small, their impact on the pharmacokinetic and pharmacodynamic behaviour is significant. Doxorubicin for example, which is meanwhile produced semi-synthetically out of daunorubicin, terminates with a primary hydroxyl group at the C-9 side chain, whereas daunorubicin terminates with a methyl group[26]. This slight change results in a completely different therapeutic application. Whereas doxorubicin is also effective against several solid tumours, daunorubicin shows its activity only against haematological malignancies, such as acute lymphoblastic or acute myeloid leukaemia (ALL/AML)[27]. Another example for big change in the pharmacological profile due to minor structural modification is epirubicin, which is a semi-synthetic epimer of doxorubicin. It differs only in the configuration of the C-4 hydroxyl group in the sugar moiety (L-daunosamine), which is axial (S-configurated) in doxorubicin and equatorial (R-configurated) in epirubicin. This structural change is generally done by oxidation of the hydroxyl group to the corresponding ketone, followed by a stereoselective reduction to the desired L-acosamine. The spectrum of tumours against which epirubicin is effective is comparable with doxorubicin but epirubicin can be used in the almost doubled cumulative dose, without increasing cardiotoxicity, which is the most severe side effect in anthracycline therapy[28].

A further successful anthracycline is idarubicin, which has an unsubstituted ring- A in the aglycon part, but is apart from that structurally identical with daunorubicin. The removal of the 4-methoxy group results in an enhanced lipophilicity, better cellular uptake and idarubicin has therefore a broader cancer spectrum than daunorubicin, which is limited to haematological malignancies. Moreover idarubicin is the only anthracycline that is also orally available with around 10 to 30 % bioavailability[29,30].

Aclarubicin, pirarubicin and valrubicin emerged from the conventional anthracyclines. The modifications especially in the sugar moiety set out to be useful for specific tumour applications. Valrubicin for example, which was launched as Valstar® by "Endo Pharmaceuticals" in 2009, is used for the treatment of a distinct form of bladder cancer[31].

4.3.1 Mode of action

The cytotoxic effect of anthracyclines is multifactorial. It is based on various mechanisms, which are in total responsible for the high efficacy of this type of drugs. A major mechanism of action is the intercalation into DNA, which is the primary target for their pharmacological effect. Anthracyclines act thereby as threading intercalators, the apolar and planar tetracyclic aglycon is stabilized between the DNA base pairs by electrostatic interactions (pi – pi interactions) especially of ring B and D with the cytosine-phosphate-guanine sites of the DNA helix. The sugar moiety of anthracyclines interacts strongly with the DNA phosphate backbone by non-covalent interactions. The functional groups in the sugar moiety form direct hydrogen bonds to the minor groove of DNA[32]. The number and direction of the interactions differ in the various anthracyclines, which probably results in their diverse clinical properties. A graphical representation of the intercalation is presented in chapter 4.5.1 figure 10/C, where a doxorubicin DNA-complex is modelled out of a crystal structure from the protein data bank (PDB code 1D12). A further proposal for the mode of action implicates formaldehyde as mediator for anthracycline-DNA cross-linking via a covalent bond. Thereby a bond between the amino-group of the sugar moiety and the 2-amino group of guanine is formed by a methylene from formaldehyde. Since the origin of formaldehyde *in vivo* is unclear, this theory remains controversial. A detailed review on this topic is given by Koch et al.[33]

However, the main effect of intercalation is the inhibition or stabilisation of DNA interacting enzymes which leads to disrupted DNA- and RNA synthesis, which further results in growth arrest and/or cell killing. Two of these enzymes are topoisomerase I and II.

DNA toposiomerases are nuclear enzymes that control the topology of DNA. They unwind, untangle and unknot the DNA and play a major role in the different steps of replication. They cause temporary DNA single-strand (topoisomerase I) and double-strand (topoisomerase II) breaks. Topoisomerase II induces cleavage by a nucleophilic attack with its active site to the phosphate backbone, cleave the ester bond via transesterification and bind covalently to the newly generated 5'-terminus of DNA[34,35]. This DNA Enzyme Complex is referred to as the "cleavable complex". After successful replication the DNA strands are reconnected and the enzyme dissociates from the DNA. There exist two topoisomerase II isozymes (Top2α and Top2ß), Top2α is preferentially expressed in tumours, while Top2ß is expressed in equal levels in tumours as in quiescent tissues[36,37].

Anthracyclines, intercalated between the DNA, act as topoisomerase poison by forming a ternary complex with DNA and the bound enzyme, and stabilize the so called "cleavable complex". The ligation of the DNA strands is thereby inhibited, causing permanent strand breaks, which result in cell cycle arrest and/or cell killing.[38-40] Anthracyclines are particularly toxic during the S-phase (synthesis phase) in which the DNA is replicated. They mainly

inhibit topoisomerase II, but their cytotoxic activity is also dependent on cellular topoisomerase I content. Especially doxorubicin and idarubicin, are also known topoisomerase I poisons[41,42].

Further cytotoxic effects of anthracyclines can be attributed to the quinone moiety in ring B, which undergo a reduction to its hydroquinone form under certain conditions. That happens via an intermediate semiquinone, which can reduce oxygen into reactive oxygen species (ROS) such as hydroxyl radicals and hydrogen peroxide. ROS have damaging effects on the cell membrane, DNA and other intracellular macromolecules. This reduction-oxidation cycle is catalyzed through a number of NAD(P)H-oxidoreductases (cytochrome P450 reductase (CPR*),* cytochrome b_5 reductase*,* xanthin dehydrogenase (XDH), mitochondrial NADH dehydrogenase)[27,43].

The pharmacological input of the free radical formation of anthracyclines is still under debate, the question rises whether the concentration is high enough to contribute actively to anthracyclines antitumor efficacy[44]. The general opinion in this case is that considerable effects occur only at higher drug concentrations, whereas the effects triggered by intercalation and topoisomerase poisoning appear even at low drug concentrations[45]. Reactive oxygen species (ROS) are further known to have direct membrane effects. They induce lipid peroxidation an oxidative degradation of cell membrane lipids resulting in cell damage. A natural product in lipid peroxidation is malondialdehyde (MDA) which reacts with the amino group of the nucleic acid bases deoxyguanosine and deoxyadenosine to form alkylating adducts. Malondialdehyde is the most mutagenic product in lipid peroxidation[46].

The assumption for the cytotoxic effect of reactive oxygen species is confirmed by the fact that many scavenger molecules like N-acetylcysteine, glutathione (GSH), GSH reductase and superoxide dismutase/catalase are able to reduce the cytotoxicity of anthracyclines [47].

The response on DNA damage, triggered by anthracyclines, is the stimulation of certain signalling pathways including cell cycle arrest and apoptosis. Double strand breaks result in a complex cellular DNA-damage response (DDR) cascade to control the repair machinery. If the strand breaks persist the DDR activates apoptosis pathways or initiates cellular senescence.[48]

Summing up, DNA intercalation, inhibition of topoisomerase II and production of reactive oxygen species (ROS) are seen as the major mechanisms which are responsible for the pharmacological effects of anthracyclines. Although a recent publication in "nature communications" by Pang et al.[49] describes a novel chemotherapeutic effect of doxorubicin. Thereby DNA double-strand breaks are induced by histone eviction from open transcriptionally chromatin regions. This new effect on chromatin biology is a further confirmation for the variety in the modes of action of this drug class. The absolute relevance

of the different mechanisms to cytotoxicity, as well as their impact on the tumour selectivity of the different anthracyclines is still object of research.

4.3.2 Anthracyclines and cardiotoxicity

Beside the usual adverse side effects of chemotherapy (asthenia, fatigue, fever, anorexia, nausea, vomiting, stomatitis, diarrhea, constipation, hand and foot syndrome, rash, neutropenia, thrombocytopenia and anaemia[50]) which occur with a frequency > 20 % during anthracycline therapy, their clinical use is seriously limited by dose related cardiotoxicity. There are four different types of anthracycline cardiotoxicity indexed: (1) Acute cardiotoxicity, which occurs immediately after bolus administration and involves arrhythmias, hypotension and vasodilatation. (2) Subchronic cardiotoxicity, which occurs about 5 and 90 days after drug administration and is most associated with inflammation of the myocardium or pericardium, but is rather unusual. (3) Early-onset chronic cardiotoxicity, occurs within the first year of treatment and is characterized by dilative cardiomyopathy and congestive heart failure (CHF). (4) Late-onset chronic cardiotoxicity, occurs at least a year after completion of anthracycline therapy, but can also occur decades after treatment.

Acute cardiotoxicity is clinically relatively unproblematic since it does not infrequently occur and patients recover quickly after treatment. The different types of chronic cardiotoxicity are more serious and represent a life-threatening condition, since they are unresponsive to cardiovascular drugs like digitalis and ß-blockers[51]. A retrospective analysis of three clinical trials indicates that at a cumulative dose of 550 mg/m², 26 % of patients would experience doxorubicin-related congestive heart failure (CHF)[52]. Also very late forms of cardiomyopathy are not uncommon, a study published by Oeffinger et al.[53] in 2006 showed that 30-year-old people who have been successfully treated with doxorubicin in their childhood have a 15-fold higher rate of a heart failure, a 10-fold higher rate of cardiovascular disease and a 9-fold higher rate of stroke[54]. Currently, there is no appropriate therapy to avoid anthracycline associated cardiotoxicity. The most effective way is the reduction of single and cumulative doses. These dose limiting strategies help to control cardiotoxicity. There exist maximum doses for all anthracyclines, which may not be exceeded.

The cause and molecular basis of anthracycline induced cardiotoxicity is discussed controversially in literature. Many different pathways and hypotheses were published, which will be briefly summarized in this chapter.

One of the most frequently published mechanisms, responsible for dose dependent cardiotoxicity, is the formation of reactive oxygen species (ROS) through redox cycling. This is of particular importance since cardiomyocytes are only poorly equipped with radical detoxifying enzymes, such as catalase and superoxide dismutase[55,56]. As mentioned in chapter 4.3.1 the quinone moiety in ring B can undergo a one-electron reductive bioactivation

to a semiquinone free radical (fig. 5/A), which regenerates itself by reducing molecular oxygen to hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\bullet-}$). This reaction sequence can be highly damaging, since a small amount of anthracycline is sufficient for the formation of numerous superoxide radicals[57]. Hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\bullet-}$) can be further converted (Haber-Weiss reaction) into hydroxyl radicals ($OH^{\bullet}$) which belong to the most potent cell damaging oxidants[58].

(Haber- Weiss reaction[54]) $O_2^{\bullet-} + H_2O_2 \rightarrow O_2 + OH^- + OH^{\bullet}$

1. step $O_2^{\bullet-} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$

2. step $H_2O_2 + Fe^{2+} \rightarrow OH^- + OH^{\bullet} + Fe^{3+}$

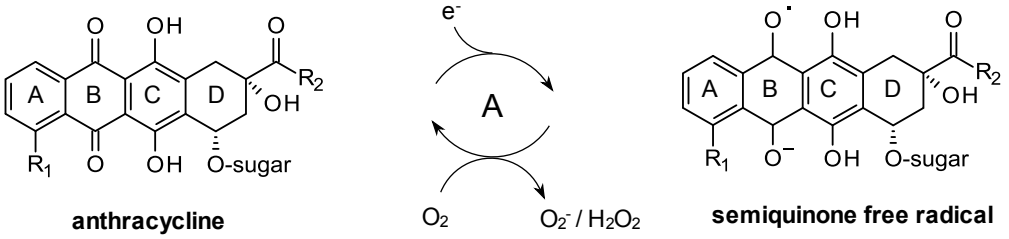
In this reaction Iron (Fe) plays a crucial role for reduction catalysis. Iron is an important element for numerous cellular processes, as it can act as electron donor and acceptor.

A further mechanism involving iron in the promotion of oxidative stress is the anthracyclines' ability of forming anthracycline-Fe complexes, which are able to generate radicals in presence as well as in absence of a reducing system[54]. The resulting myocardial oxidative stress is supposed to cause severe cell damage in cardiomyocytes, resulting in their death by different necrosis or apoptosis pathways[27].

The anthracycline induced ROS hypothesis seemed to give the perfect rationale for using antioxidants for cardioprotection. Preclinical models with variant compounds like probucol, melatonin, N-acetylcysteine and vitamin E showed promising results. But so far all these agents failed during their clinical trials due to ineffectiveness or other disqualifications[59,60].

Better results were achieved by another strategy using an iron chelator for cardioprotection. Dexrazoxane, which is structurally similar with EDTA, can prevent contractile dysfunction and histological lesions. It granted approval by the FDA for patients with advanced or metastatic breast cancer, who have already received a certain amount of the anthracyclines doxorubicin (300 mg/m^2) or epirubicin (540 mg/m^2)[61]. The fact that antioxidants showed no clinical effect, whereas dexrazoxane showed a significant cardioprotection during anthracycline therapy suggests the following: First, the role of Fe is more diverse than just catalysing hydroxyl radical formation, or second the ability of dexrazoxane to chelate Fe is not its major determination. It also must be mentioned that so far there is no further Fe chelating agent that has reached the cardioprotective efficacy of dexrazoxane, although many substances can form more stable iron complexes[62].

Beside the one-electron reduction of the quinone moiety, anthracyclines can also be substrate of a two-electron reduction of the carbonyl group at C-13, catalyzed by aldo-keto reductases in cardiomyocytes (fig. 5/B)[63].



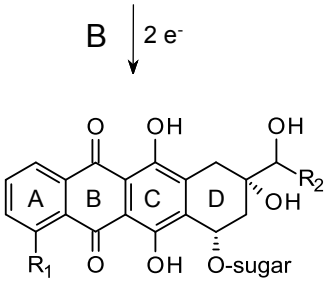


Figure 5| **One- and two- electron reductive bioactivation of anthracyclines. A)** One-electron reduction of the quinone moiety and formation of semiquinone free radical. **B)** Two-electron side chain reduction to a secondary alcohol metabolite referred to as doxorubicinol (DOXOL), daunorubiciniol (DNROL), epirubicininol (EPIOL) or idarubicinol (IDAOL), depending on the administered anthracycline.

The anthracyclines are thereby transformed to a secondary alcohol metabolite which showed to be ~ 30 - 40 times more potent in inactivation of Ca^{2+} handling proteins than the originally anthracyclines do. Secondary alcohol metabolites further exhibit high reactivity towards human cytoplasmic aconitase (iron regulatory protein 1), a major regulatory factor in iron homeostasis and in the redox balance of the cell.[58] Although it has been shown that the fractional conversion of doxorubicin and epirubicin to doxorubicinol (DOXOL) and epirubicinol (EPIOL) occurs only in a frequency ≤ 1 %, a concentration which is apparently too low to have a pharmacological effect, nevertheless they are supposed to play a major role in cellular damage due to their pharmacokinetic properties. Anthracylines are lipophilic enough to diffuse from the inner cell back to plasma and vice versa, whereas the secondary alcohol metabolites are too polar and show therefore no efflux[64]. For this reason it comes to an enrichment of secondary alcohol metabolites in cardiomyocytes with continuous therapy, reaching concentrations which will actively contribute to chronic cardiomyopathy. Numerous mice bioassays confirmed this assumption[65-67]. The ~ 30 % reduced cardiotoxicity of epirubicin in comparison to doxorubicin is supposed to be the result of an impaired catalytic specificity to those aldo-keto reductases which convert anthracyclines to their correspondent secondary alcohol metabolite in cardiomyocytes[63,64].

A different molecular basis of anthracycline induced cardiotoxicity as described above was recently published in "nature medicine" by Zhang et al.[68] As mentioned in chapter 4.3.1 anthracyclines acts as topoisomerase (Top2ß + Top2α) poisons inducing DNA double strand breaks via a ternary cleavage-complex. Cardiomyocytes only express Top2ß but not Top2α which is known to be preferentially expressed in proliferating cells. Zhang et al.[68] hypothesized that Top2ß directly contribute to anthracycline induced cardiotoxicity.

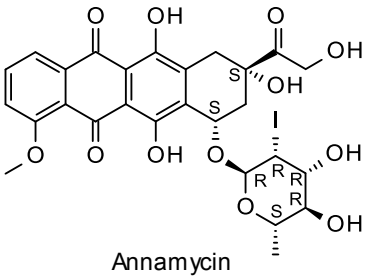
To prove this hypothesis they established a mouse model with cardiomyocyte specific deletion of the *Top2b* gene, which encodes for topoisomerase IIß (Top2ß). After treatment with doxorubicin they could show that Top2ß expression in cardiomyocytes correlates with doxorubicin effects on the transcriptome, induction of double strand breaks and apoptosis. Cardiomyocytes carrying *Top2b* showed a significant upregulation of transcripts (*Trp53inp1, Apaf1, Bax and Fas*) which are important in the apoptotic pathway, whereas *Top2b* deleted cardiomyocytes showed no expression changes. It was further shown that ROS generation in cardiomyocytes is not solely a result of redox cycling it seems to be mainly a result of Top2ß mediated changes in the transcriptome. Doxorubicin-induced ROS was reduced about 70 % in *Top2b* gene deleted mice compared to those carrying *Top2b*.[68]

To sum up it can be said that similar to the mode of action, anthracyclines cardiotoxicity cannot be linked to single mechanism. This recent publication suggests that the classical ROS hypothesis, which has gained wide acceptance over the last years, is neither the main trigger nor the key executor of anthracycline cardiotoxicity, and lets us suppose that ROS formation is more likely to be only a consequence of changes in the cellular transcriptome.

4.3.3 Anthracycline analogs

Numerous anthracycline analogs have been and are still synthesized by chemical modification of the parent anthracyclines, or by total synthesis. After the first (doxorubicin, daunorubicin) and second (epirubicin, idarubicin, pirarubicin, aclarubicin, valrubicin) generation of anthracyclines, described in chapter 4.3, meanwhile the third generation of anthracycline drugs is currently under investigation. Improved understanding of the mode of action of the naturally occurring anthracyclines lead to a preferentially derivatization at the sugar moiety (3'-amino group, 4'-hydroxyl group) and/or the aglycone side chain (hydroxyl group at C-13, keto group at C-12) since the planar quinone-hydroquinone structure must be maintained for intercalation. It further turned out that the tolerance for structural modifications in the sugar moiety is relatively high[69]. In this chapter the focus is placed on those anthracycline derivatives that have reached clinical evaluation.

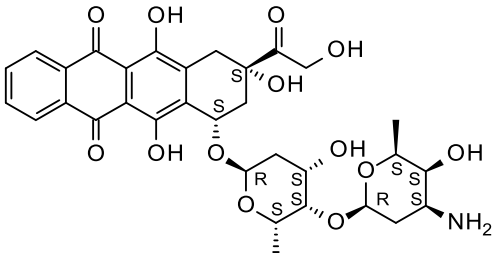
During the investigation of the pharmacological effects of the amino group in anthracyclines, it turned out that a C-3' basic center is not absolutely necessary for anticancer activity, although most of the pharmacologically active anthracyclines carry a C-3' amino group. An example for this fact is annamycin **12**, a drug candidate which is currently in clinical trials, carries a 2,6-dideoxy-2-iodo-L-*talo*-pyranose instead of L-daunosamine. Although the binding affinity to the DNA is lower than for daunorubicin, annamycin showed good results against multidrug resistant leukaemias, especially when applied in liposomal formulations[70,71].



Annamycin
12

Figure 6| **Molecular structure of annamycin.** $C_{26}H_{25}IO_{11}$, CAS registry number: 92689-49-1.

Another important discovery are disaccharide derivatives, in which an additional sugar is inserted (α 1-4) between the aglycone and daunosamine[72]. Disaccharide derivatives show a similar binding mechanism to those of doxorubicin, the second sugar moiety does not seem to disturb the intercalation process. The intercalation occurs similar to other anthracyclines at the cytosine-phosphate-guanine sites of the DNA helix. It was further discovered that 4-demethoxy derivatives in combination with an additional 4'-axial (4-S) configurated sugar moiety were the most potent substances[73]. Extended synthesis of numerous derivatives resulted in a promising candidate named sabarubicin (former name MEN 10755) which is currently undergoing clinical evaluation. Sabarubicin, which is carrying a 2,6-dideoxy-L-fucose between the aglycon and daunosamine, was successfully investigated in a phase II clinical trial and a further multinational phase III study is planned[74].



Sabarubicin
13

Figure 7| **Molecular structure of sabarubicin.** $C_{32}H_{37}NO_{13}$, CAS registry number: 211100-13-9.

Already in 2004 orphan drug designation was granted to the Menarini group for the treatment of small lung cancer with sabarubicin[75]. Sabarubicin showed remarkable antitumor activity against a broad series of solid tumours and a lower potential for cardiotoxicity[76]. The better tolerability seems to be a combination of the changed pharmacokinetic properties and the higher potency at the drug target.[77]

A further promising derivatization was the insertion of a morpholinyl group at position 3' in the sugar moiety, replacing the amino group. The most advanced representative for this

derivatization is nemorubicin, which successfully concluded a phase II clinical trial and is currently part of the pipeline of Nerviano Medical Sciences s.r.l.[78]

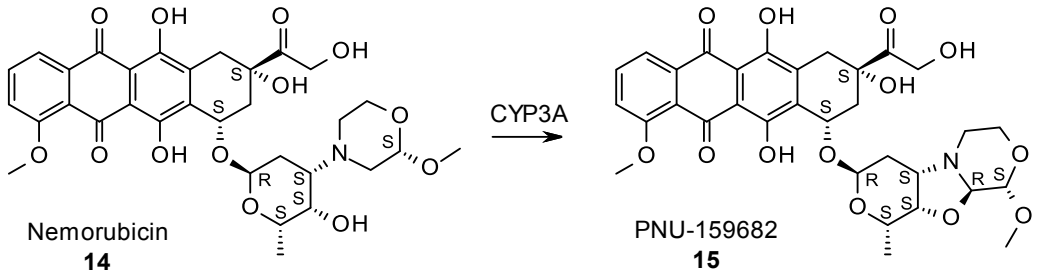


Figure 8 | Conversion of nemorubicin to its active metabolite PNU-159682 by cytochrome P450, subfamily 3A enzymes[79]. Nemorubicin: $C_{32}H_{37}NO_{13}$, CAS registry number: 108852-90-0; PNU-159682: $C_{32}H_{35}NO_{13}$, CAS registry number: 202350-68-3.

Nemorubicin is a 3'-deamino-3'[2-(*S*)-methoxy-4-morpholinyl] derivative of doxorubicin. Due to the morpholinyl group the lipophilicity of the molecule is increased, resulting in a faster cellular uptake and higher intracellular level compared to doxorubicin[80]. In human liver nemorubicin undergoes a transformation to the metabolite PNU-159682 (fig. 8) catalyzed by CYP3A enzymes (mainly by CYP3A4)[81]. Initially nemorubicin was tested for its activity against chemoresistance, especially against multi-drug resistant cells with promising results. Nemorubicin is active against a series of doxorubicin resistant tumour cell lines as well as against cell lines which show resistance to platinum derivatives and alkylating agents. The *in vitro*/*vivo* obtained data suggest a broad antitumor spectrum, both in resistant and sensitive tumours[82]. It was further shown that cells resistant to nemorubicin show collateral sensitivity to platinum derivatives and alkylating agents. This synergistic activity makes nemorubicin an appropriate drug for a combination with cisplatin (DDP). The extended effective range towards classical anthracyclines and its activity against doxorubicin resistant cell lines suggest an additional mode of action beside DNA intercalation. It was shown that nemorubicin as well as its metabolite PNU-159682 are more active in cells with an up-regulated nucleotide excision repair (NER) system, which are normally resistant to anticancer drugs. Although the exact mechanism is not yet clear, it is hypothesized that this particular DNA repair system is essential for the high activity of nemorubicin[83]. In difference to doxorubicin, which is only active in the S phase, nemorubicin is active independently of the cell cycle[82]. The results achieved in the preclinical evaluation so far could be confirmed in different clinical trials, in which nemorubicin is given alone or in combination with cisplatin to patients with hepatocellular carcinoma (HCC)[82,84].

4.4 Modulation of pharmacokinetic properties as strategy in drug design

4.4.1 General remarks

The efficacy of a drug is determined both by its pharmacodynamic and pharmacokinetic properties. Obviously drug discovery has a dominant focus on pharmacodynamic properties. Many strategies exist to optimise target affinity and other substance properties related to the pharmacodynamic effect of a drug candidate. However, the human body is a very complex system. It is well known that the efficacy of a drug strongly depends on its ADME properties. Nowadays many tools and techniques exist to improve the pharmacokinetic properties of compounds. Absorption is generally improved by means of drug formulation. Distribution of a drug has a strong focus on cell surface recognition and also depends on its hydrophilic-lipophilic balance (HLB). Metabolism may be influenced by prodrugs or metabolism preventing structural modifications. Even excretion can be influenced by specific concepts. Nevertheless, there are by far fewer projects aiming primarily at improving drug pharmacokinetics than those aiming either at new drug targets or improvement of pharmacodynamic properties.[85]

4.4.2 Carriers, prodrugs and conjugates – Modifying pharmacokinetic properties of anthracyclines

The goal to increase the tumour specificity and the safety of anthracyclines led to the development of various prodrug- and drug delivery strategies for the improvement of this drug class. Passive strategies include preferential drug distribution and retention within tumour tissue, whereas healthy tissue is not exposed to toxic drug levels. The active tumour target strategies are based on less toxic derivates of common anthracyclines, which are activated at the tumour site, or anthracyclines that are conjugated with tumour targeting ligands.[86]

An already established passive strategy is the encapsulation of anthracyclines into liposomes. Liposomes are self assembling vehicles consisting of a phospholipid bilayer with an inner aqueous compartment. They can carry hydrophilic (in the inner aqueous compartment) as well as lipophilic and amphiphilic (insertion into the bilayer) drugs. Liposome-encapsulated anthracyclines show a completely different pharmacokinetic behaviour from their parent drugs, resulting in a reduced volume of distribution (V_d), reduced clearance, limited conversion into secondary metabolites and limited accumulation in healthy tissues[87]. Liposomes are ideal vehicles for a protected drug transport, since they are non immunogenic, have a very low intrinsic toxicity and are biodegradable[88]. The ability of liposomes to accumulate in tumour tissues is based on a tumour characteristic leaky microvasculature. Over these gaps between the endothelial cells liposomes can enter the

tissue. In addition tumour tissues show an insufficient lymphatic drainage, leading to further liposome accumulation. These two effects are described in literature as EPR-effect (*enhanced permeability and retention effect*)[89,90]. So far two liposomal formulations have been developed and approved for clinical use. Myocet® (liposomal doxorubicin) is in clinical application against metastatic breast cancer and DaunoXome® (liposomal daunorubicin) has been approved for treatment of AIDS-related Kaposi's sarcoma. A progression of passive drug targeting using liposomes was their additional coating with polyethylene glycol (PEG), which prolongs the circulating half-life and increases therefore the probability to reach the tumour tissues. Pegylated-liposomal-doxorubicin is marketed under the brand names Doxil®, Lipodox® and Caelyx® in the US and Europe.[91,92]

A further promising strategy to increase the tumour specificity of anthracyclines is the design of acid-sensitive prodrugs. In living systems we can observe a significant drop of the pH-value in different intracellular compartments like in endosomes and lysosomes. Additionally the microenvironment of tumour tissues shows a slightly reduced pH in comparison to healthy tissues[93]. These two facts make it possible that acid-sensitive anthracycline prodrugs can be activated on an extracellular as well as on an intracellular level. Chemically the ketogroup at C-13 and the 3'-amino group of the sugar moiety were selected for the conjugation with acid-sensitive linkers, forming a carboxylic hydrazone bond at C-13, or attaching a cis-aconityl spacer at the 3'-amino group. These bonds are highly stable under physiological pH, but under slight acidic conditions the anthracyclines are released. The linker can be further connected with a macromolecular carrier, which is responsible for selective tumour targeting. As macromolecular carriers antibodies and serum proteins delivered the most promising results.

Bristol-Myers Squib developed a series of antibody conjugates with doxorubicin. A prominent representative is BR96-doxorubicin immunoconjugate. Thereby a thiol-bearing monoclonal antibody binds selectively to tumour selective Lewis-Y-antigen which is expressed in several human carcinomas. Doxorubicin is further released in the acidic environment of endosomes and lysosomes. After promising *in vitro* and *in vivo* results BR96-doxorubicin has been evaluated in phase I and II clinical trials. Three different phase II clinical studies were conducted, which unfortunately showed cross-reactivity of BR96-doxorubicin with normal gastrointestinal tissue, which resulted in severe toxicities[94,95]. Consequently the further development of BR96-doxorubicin was therefore not continued.

The use of serum proteins, such as albumin and transferring as macromolecular carriers was more successful. There are a number of reasons why serum proteins are very well suited as macromolecular carriers: They are biologically stable, nontoxic, nonimmunogenic, undergo a preferential uptake into tumour cells and they are biodegradable. Additionally It was shown that albumin and transferrin accumulate in tumours

by receptor-mediated endocytosis[96]. Numerous doxorubicin conjugates were synthesized by Kratz et al., which significant reduced toxicity while maintaining their anti tumour activity[97]. In further drug development the focus was mainly set on albumin-conjugates due to its 10 times lower costs in comparison to transferrin. Initially doxorubicin was directly linked to commercially available albumin, but further development resulted in a modified concept in which a doxorubicin-prodrug binds selectively to the cystein-34 position of endogenous albumin, which is the most abundant protein in the blood stream. The structure of this (6-maleimidocaproyl)-hydrazone doxorubicin derivative is presented in figure 9.

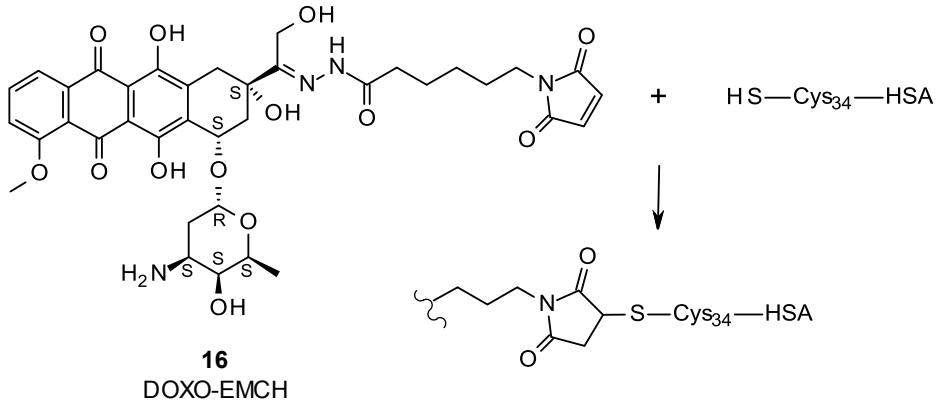


Figure 9| Conjugation of (6-maleimidocaproyl)hydrazone doxorubicin derivative 16 (DOX-EMCH) with human serum albumin. The thiol group of Cys34 of human serum albumin (HSA) reacts and couple with maleimide within a few minutes after intravenous application.

This doxorubicin-prodrug, first published as DOXO–EMCH, showed a significantly reduced side effect profile and was successfully tested in a phase I clinical trial[98]. Meanwhile it has been granted orphan drug designation by FDA and is renamed to aldoxorubicin. Aldoxorubicin which is in the pipeline of the CytRx Corporation is currently undergoing two clinical trials (phase II) in patients with soft tissue sarcomas and pancreatic ductual adenocarcinomas (PDAC).[99]

Anthracyclines carrying acid-sensitive linkers were also conjugated with different synthetic polymers. Doxorubicin directly linked via a hydrazone bond to polyethylene glycol (PEG) with a molecular weight between 5 – 70 kDa was synthesized in different versions, to improve their pharmacokinetic profile[100,101].

Although the different active or passive delivery systems described above have shown important therapeutic advantages towards the parent anthracyclines, there are still some inherent drawbacks which should not be underestimated. These include limited drug loading capacity of the different delivery systems, unpredictable aggregation, toxicities and allergic reactions induced by administration of large amounts of inactive carriers[102].

To improve the drug loading capacity of the different carriers, doxorubicin conjugations with dendrimers or dendritic polymers were especially developed in the last decade. This resulted in partially strange structures with a few promising results[103].

4.5 Aim of the thesis

Anthracyclines are used against a wide range of tumour diseases, but their clinical use is still severely limited by dose-related side effects and occurring tumour resistance. To circumvent these problems so far two basic strategies have been pursued. On one hand, an attempt is made by the techniques of "drug targeting", to increase the release and accumulation of active agents in tumour tissue. For that prodrugs are used, in which common anthracyclines are linked to peptides[96], carbohydrates[104], antibodies[94], synthetic polymers[105], or encapsulated in liposomes[106]. The second strategy is the design of new anthracycline drugs. Although various anthracycline derivatives have already been synthesized, basing on a variety of pharmacological strategies, those substances available on the market have a great potential for improvements, mainly because of their unwanted side effects. The anthracyclines therefore provide an ideal platform to discover new drug candidates, with an extended area of application and a better side-effect profile.

The aim of this thesis is to individualize therapeutically used anthracyclines according to their physicochemical properties. To improve pharmacokinetic behaviour the hydrophilic-lipophilic balance should be altered by targeted structural modifications. In a second step the newly synthesized substances should be tested for their anti-tumour effects on malignant cell systems.

4.5.1 In silico validation of the concept

In any case, modulation of pharmacokinetic properties of a drug is limited by the requirement that the pharmacodynamic properties must not be impaired or even lost. Since the pharmacodynamic effect of a drug is nowadays mostly described by its action on a receptor, a solid hypothesis on the drug-receptor interaction at the binding site is a prerequisite for pharmacokinetics driven drug discovery. As a rule, modifications have to take positions that do not interfere with the decisive drug target interaction.

Our initial hypothesis refers to various experiments described in the literature, in which the drug-receptor complex was crystallized and measured by X-ray crystallography[32,107-111]. These experiments showed that the planar aglycone part of anthracyclines intercalates between the nucleic bases of DNA, and that the sugar moiety is stabilized in the DNA backbone (fig. 10/C,B). Since conventional anthracyclines are usually obtained by fermentation, the chemical modification options remain limited to a few functional groups. Modifications of these groups are therefore not always purposeful, since the planar structure of the molecule must be maintained to achieve the effect of DNA intercalation, and furthermore they may reduce the affinity of non-bonded interactions with the receptor. For this reason, modifications of the 3'-NH_2 group and 4'-OH group in the sugar moiety were not

considered in our design because they can form direct hydrogen-bonds to the minor groove of DNA. The protonated 3'-NH_2 group is forming a hydrogen bond with the O-4' atom (ring O) of deoxycytidine and with O-2 in deoxythymidine in the doxorubicin-d(CGATCG) complex[32]. The 4'-OH group, if epimerized, can form a hydrogen bond with O-2 in deoxycytidine as shown in the epidoxorubicin-d(CGCGCGCG) complex[109].

Detailed investigation of the drug-receptor complex of different therapeutically used anthracyclines showed that the C-5' methyl group of the amino sugars extends into the "minor groove" of the DNA where is enough space for even bulky residues without impeding the intercalation (fig. 10/A). So far only hydroxy and fluoro derivatives were described at this position with some promising results[112,113].

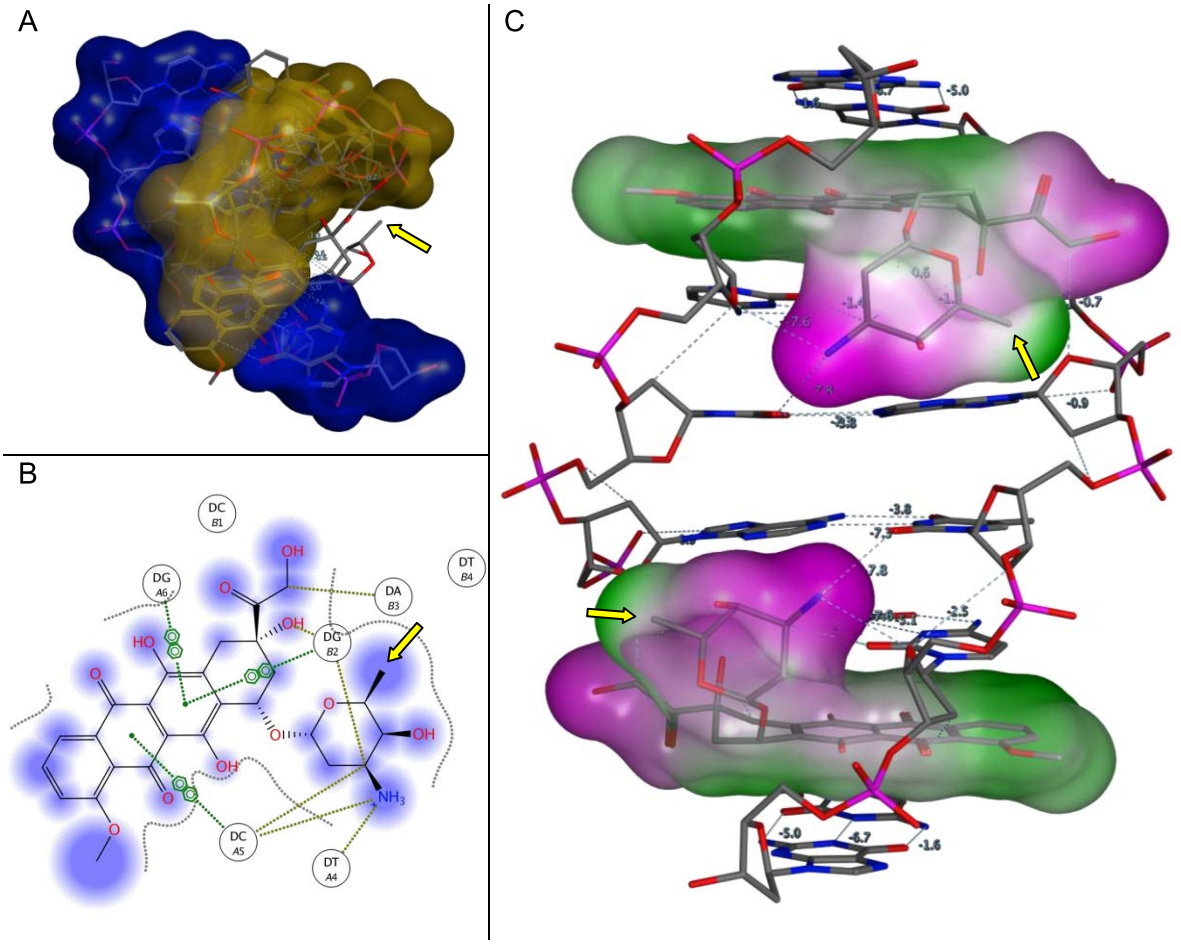


Figure 10 | Representation of DNA fragment d(CGATCG) and doxorubicin. A) Doxorubicin-d(CGATCG) crystal structure complex. The yellow arrow indicates the daunosamine–methyl group which is positioned in minor groove of DNA. **B)** 2D ligand interactions between doxorubicin and the deoxynucleotides from DNA. **C)** Doxorubicin (Molecular surface: Hydrophilic = pink, lipophilic = green) intercalated at the cytosine-phosphate-guanine sites of DNA. | Representation of the graphics and calculation of non-covalent interactions was done with Molecular Operating Environment (MOE) version 2011.10. As modelling template the crystal structure published by Frederick et al.[32] was used (PDB code 1D12).

Since a methyl group, as in the sugar part of the applied anthracyclines, cannot be functionalized easily by known synthetic methods, the sugar component has to be synthesized and modified separately, to be subsequently linked to the aglycone. An essential part of this thesis is the elaboration of an amino sugar synthesis, in which the C-5' methyl group can be derivatized.

4.5.2 Drug improvement by molecular OEGylation

In drug discovery ADME parameters were more often responsible for failure in clinical trials than efficacy or safety did. As mentioned above, nowadays many tools and techniques exist to improve the pharmacokinetic properties of compounds[114]. Polyethylene glycol (PEG) is a widely used polymer. When conjugated to a molecule, it leads to a severe change in the physicochemical properties. It exists out of polar ether and apolar alkyl groups, is amphiphilic, nontoxic and practically unlimitedly soluble in water. These specific conjugates can be synthesized in linear or branched structures and the conjugation bond to the target molecule can be stable or cleavable, to synthesize completely new chemical entities, or to pursue a prodrug strategy.

Potential advantages of PEGylation are an improved safety profile, prolonged plasma half-life, modified biodistribution and metabolism, and improved oral bioavailability. In clinic it is associated with prolonged dosage intervals and decrease of unfavourable side effects.[115,116]

Initially macromolecules in particular proteins have been modified by PEGylation, but meanwhile it is also used for low molecular weight compounds having poor aqueous solubilities.

Attaching a stable polyethylene glycol to small organic molecules is still controversial, since small molecules have only few sites where a PEG can be attached without disturbing their functionality. For permanently small molecule derivatization only the lower molecular-weight PEGs (Mw < 1000 Da) make sense because macro-molecular PEGs may block their activity via steric hindrance[115]. It is also very important that the structure activity relationship (SAR) of the compound is well understood to find an ideal attachment site for PEGgylation.[117]

In the present work we introduce a short oxyethylene chain, referred as oligoethylene glycol (OEG), for the modification of the established anthracyclines daunorubicin and doxorubicin. Parallel ongoing work with a naloxone derivative (naloxegol) carrying an oligoethylene glycol moiety, demonstrated the potential of this new concept to improve pharmacokinetics of small molecule drugs and has led to clinical development[118].

For our derivatization on the anthracycline sugar moiety a linear OEGylation design has been planned, in which the methyl group is replaced by a stable methyl ether terminated

oligoethylene glycol (mOEG) chain, with a length of 18 elements [-$CH_3(OCH_2CH_2)_5OCH_3$]. The selection of this chain length results from the fact that triethylene glycol dimethyl ether [$(CH_3(OCH_2CH_2)_3OCH_3$] is the shortest oxyethylene compound that has the same intrinsic structural properties as oxyethylene compounds with increased chain length[119]. Our design includes three more oxyethylene (-OCH_2CH_2O-)$_3$ units, to induce a significant change in the physicochemical properties of the synthesized molecule. The molecular weight ratio between the anthracycline and the oligoethylene glycol is about 2:1. A further advantage of the OEGyl moieties in comparison to long PEG-modifications is that construction is feasible in organic solvent.

The designed chain is flexible and able to change the relative position of its constituents. Due to the stereoelectronic effects in particular the gauche-effect the oxyethylene chain tends to form a linear helix pointing away from the sugar moiety, in contrast to long alkyl chains which tend to collapse through hydrophobic effects. This collapse of long alkyl chains is associated with a steep decrease in the solubility of the molecule and might be also responsible for decrease in binding affinity due to steric hindrance[120,121]. Figure 11 represents the theoretical conformation of the oxyethylene chain which was selected for derivatization of the sugar moiety.

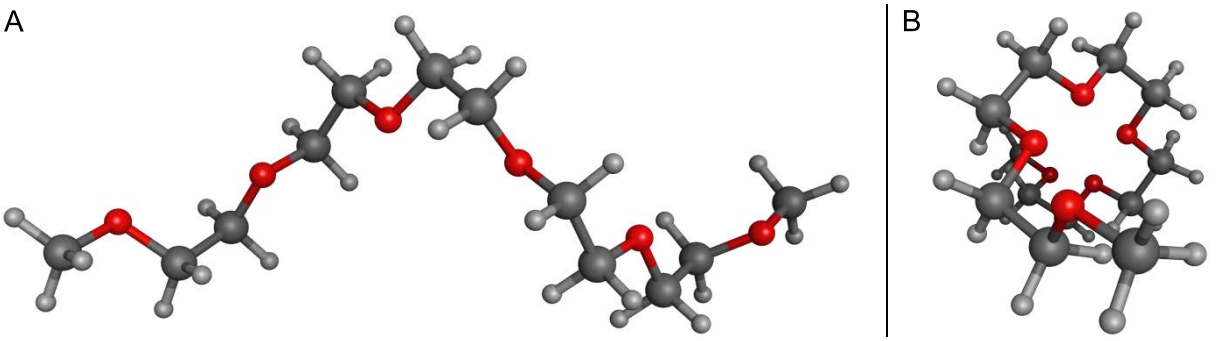


Figure 11 | Three-dimensional representation of the oxyethylene chain (-$CH_3(OCH_2CH_2)_5OCH_3$) in the trans-gauche-trans conformation (TGT). The C-O bond has a preferential trans-conformation and the C-C bond has a preferential gauche-conformation. **A)** Plan view of the helical structure **B)** Side view of the helical structure which shows that hydrogen atoms are arranged outside of the helix and the oxygen atoms are arranged in the inner. | Representation of the graphics was done with Molecular Operating Environment (MOE) version 2011.10.

The good water solubility of oxyethylene chains (-OCH_2CH_2O-)$_n$ and the fact that other polyethers like polyoxymethylene (-OCH_2-)$_n$ and polyoxytrimethylene (-$OCH_2CH_2CH_2$-)$_n$ which have only one CH_2-group less or more are insoluble in water, indicate that conformation of the oxyethylene group plays an important role. Published infrared absorption spectra of oxyethylene chains in different solvents showed, that conformation is preferable trans for the C-O bond and gauche for the C-C bond[122,123]. This

trans-gauche-trans conformation is predicted to be most favoured conformation of oxyethylene chains in water and other hydrogen-bonding solvents[124,125]. The hydrogen bonds play an essential role in the conformation stability of the oxyethylene chain. The gauche conformation of the C-C bond is stabilized by hydrogen bonding between the ether oxygens and the surrounding liquid. The distance between oxygen atoms in the gauche conformation is about 0.29 nm, which is about the same distance as oxygen atoms can have at their nearest approximation in liquid water (0.285 nm). This suggests that oxyethylene chains participate well in the hydrogen bond network of water[119,126].

4.6 Synthesis of anthracyclines

For the production of anthracyclines we generally have to distinguish between biosynthetic and semi-synthetic/synthetic methods.

Biosynthetic methods include genetic engineering, especially pathway manipulation of *Streptomyces* cultures by recombinant DNA methods, bioconversation and chemoenzymatic synthesis using purified enzymes. Since Anthracyclines are large chemically labile molecules carrying numerous chiral centres, biosynthetic methods based on enzymatic reactions have the advantage to be highly specific, building up chiral products from simple precursors. The ployketide chain of the aglycon is built by numerous biosynthetic steps starting with condensation of acyl thioesters. Starting material for the formation of the deoxysugar moieties is glucose-3-phosphate. However, biosynthetic anthracycline production is also associated with serious disadvantages: large costs due to the numerous biosynthetic steps, low productivity and only modifications for which an enzyme exists can be performed. This is also the reason why currently used second generation anthracyclines like doxorubicin, epirubicin and idarubicin are produced semi-synthetically out of daunorubicin, which is still produced by fermentation.[127,128]

Over the last decades many laboratories and research groups focused on the total synthesis of anthracyclines and its derivatives. The organic synthesis of enantiomerically pure anthracyclinones which consist of an ABCD ring system has been a challenging task since the regiochemical orientation of substituents in ring A and D, and the absolute configuration of the substituents in ring A necessary for the biological activity. Total synthesis of the aglycone was achieved by various synthetic strategies including carbanion cyclization, Friedel-Crafts, Marschalk and Diels-Alder reactions[129]. The synthesized aglycons were further glycosylated with a functionalized sugar moiety, whose syntheses will be described in the next chapter. Detailed information about the glycosylation reaction is given in chapter 5.5.

4.6.1 Synthesis of the sugar moieties

The naturally occurring sugar moieties of the anthracycline antibiotics (fig. 12) belong to the deoxyhexoses and were first isolated by hydrolysis from their parent antibiotics.

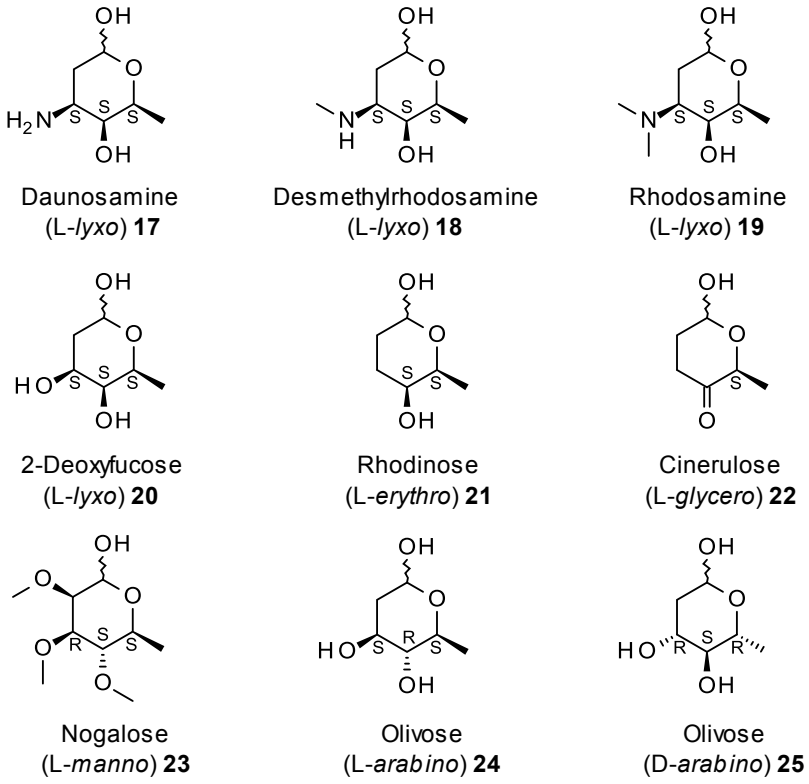


Figure 12 | Naturally occurring sugar moieties of anthracycline antibiotics[130].

The most well known deoxyhexose of the anthracycline antibiotics is L-daunosamine (3-Amino-2,3,6-trideoxy-L-*lyxo*-hexose) and was first isolated by Arcamone et al.[131], by hydrolysis of daunorubicin. With the increasing importance of daunorubicin and doxorubicin as therapeutically effective anti-tumour antibiotics and the high costs for their fermentative production, total synthesis was a declared goal.

Since the sugar moiety synthesized in this work is a derivative of L-daunosamine, in the next two chapters daunosamine syntheses of carbohydrate and non- carbohydrate precursors are briefly summarized.

4.6.1.1 Synthesis from carbohydrate precursors

Many preparations of daunosamine were base on carbohydrate precursors, with the introduction of the C-3 nitrogen functionality as a key step. The first synthesis of daunosamine, published by Marsh et al.[132] had L-rhamnose as starting material. L-rhamnose is well suited for the synthesis of 2,3,6-trideoxy-3-aminohexoses since it is L-configurated and possesses a methyl group at C-5.

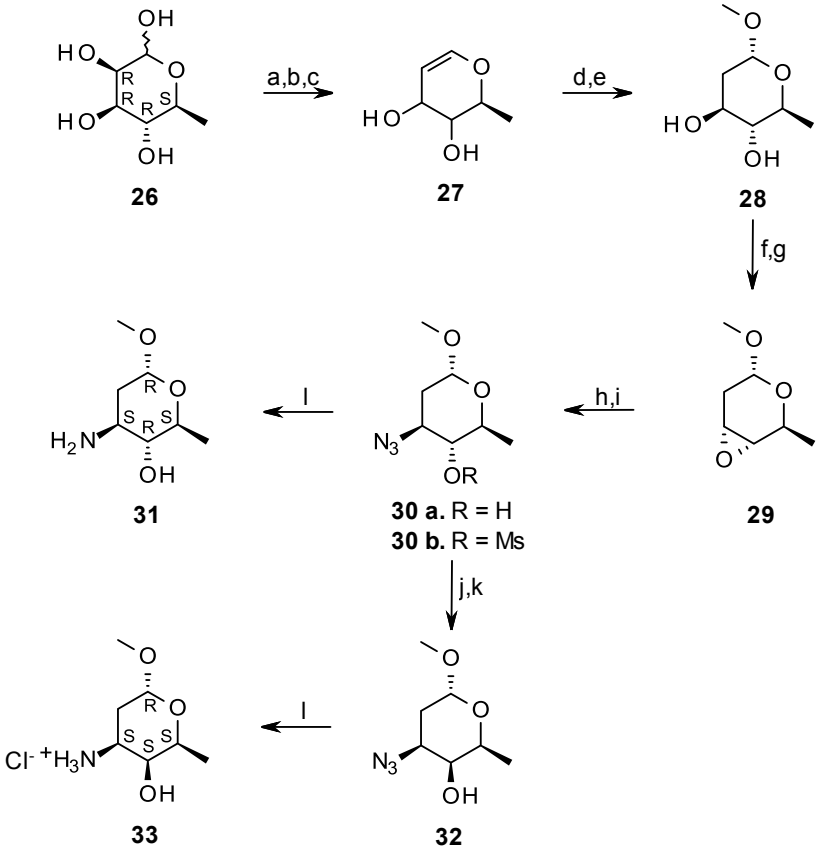


Figure 13 | Synthesis of L-daunosamine hydrochloride from L-rhamnose[132,133]. Reagents and solvents: a. Ac_2O, Py; b. HBr, HOAc, Ac_2O, Zn-Cu, HOAc; c. $Ba(OH)_2$, MeOH; d. Hg-$(OAC)_2$, CH_3OH; e. KBH_4; f. TsCl, Py; g. $NaOCH_3$; h. NaN_3; i. MsCl, Py; j. NaOBz, DMF; k. $NaOCH_3$, H_2O; l. catalytic hydrogenation.

L-rhamnose is first tetraprotected, converted to the corresponding pyranosyl bromide, which is then treated with zinc dust to give after alkaline hydrolysis glycal **27**. Methoxy-mercuration with following in situ borohydride reduction gave the dideoxysugar **28**. Sulfonylation and treatment with base gave epoxide **29**. The nitrogen functionality is incorporated via nucleophilic opening of the epoxy derivative **29**, gives azido sugar **30a**. Catalytic hydrogenation of the azido functionality results in acosamine methyl glycoside **31**. Although this preparation presents a total synthesis of L-acosamine, which is the glycone of epirubicin, this sugar was not isolated before 1973[134]. If the azido sugar **20a** is mesylated, followed by

substitution with sodium benzoate, subsequent hydrolysis gives an inversion of the 4'-hydroxyl group. Catalytic hydrogenation gives daunosamine methyl glycoside **33**.

Beside L-rhamnose also L-rhamnal and its derivatives were often used as starting materials for the synthesis of amino sugars. A synthetic approach that not looked very efficient at first sight, because of lack of stereoselectivity, was the introduction of the C-3 nitrogen functionality by Michael addition of hydrozoic acid[135]. But development of efficient separation strategies and synthesis of intermediates, which can be directly used for glycosylation, made L-rhamnal a suitable starting material[136,137]. An example for the synthesis of C-3-amiosugars by Michael addition of hydrozoic acid, including a separation strategy, is shown in figure 14.

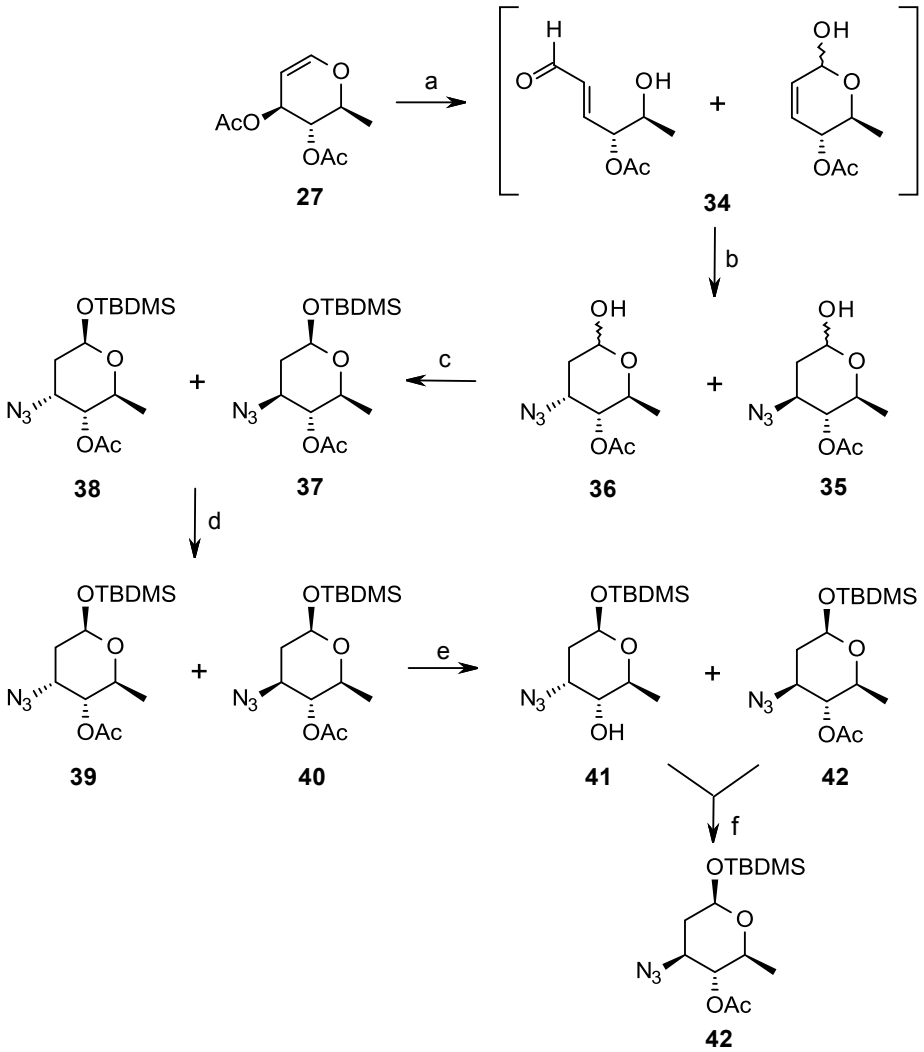


Figure 14 | Synthesis of C-3-amiosugars by Michael addition of hydrozoic acid starting from L-rhamnal[137]. Reagents and solvents: a. H_2O, 70°C; b. NaN_3, AcOH; c. TBDMSCl, Imidazole, DMF; e. 1N NaOMe, MeOH; f. silica gel filtration.

After controlled saponification of **39** + **40**, the undesired deprotected azido alcohol **41** can be separated by a simple silica gel filtration. The still acetylated acidoalcohol **42** can be further converted into appropriate glycosylating donor.

Beside L-rhamnose/L-rhamnal it seemed obvious to use natural D-hexoses as cheap and readily available starting materials for the preparation of L-daunosamine. However, they have the disadvantage that for the desired L-stereochemistry the configuration at C-5 has to be switched. The first who found a solution to this problem, based on some novel acetal transformations, were Horton et al. [138], who published a 11 step synthesis of L-daunosamine starting from α-D-mannoside with 40 % overall yield (fig. 15).

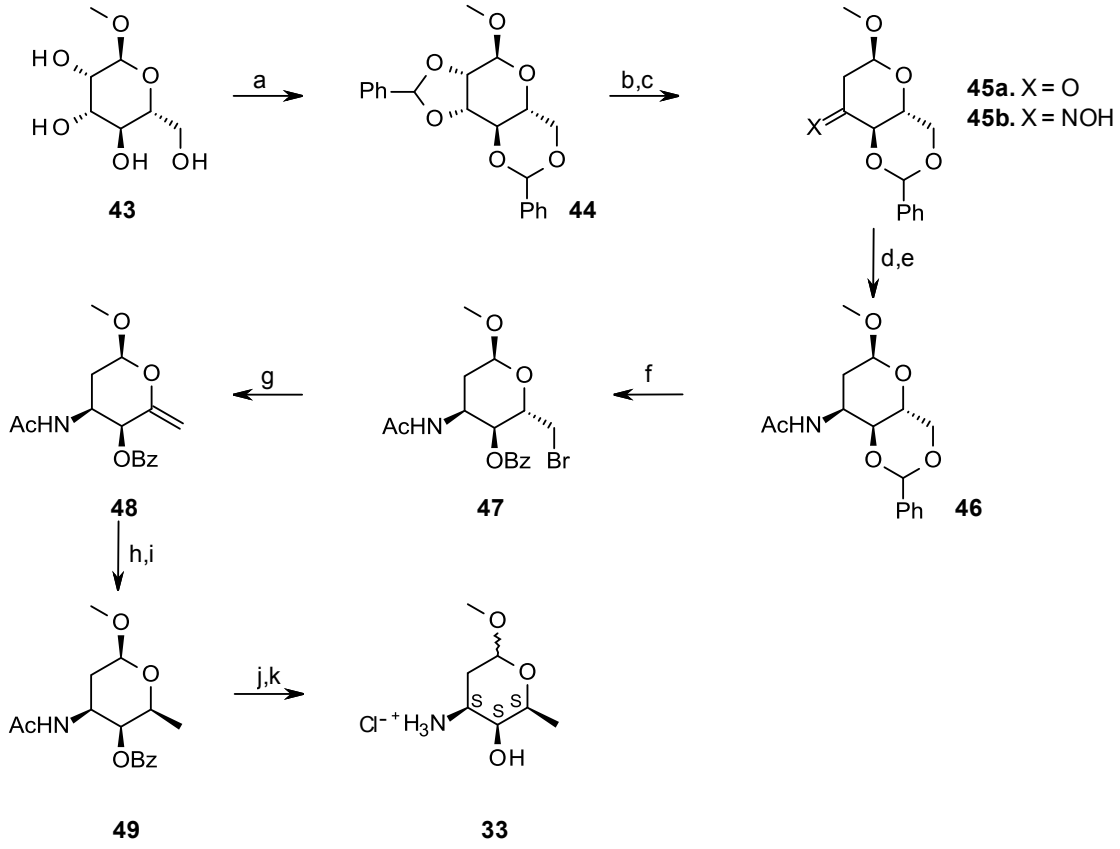


Figure 15 | Synthesis of L-daunosamine hydrochloride from α-D-mannoside[138,139]. Reagents and solvents: a. $PhCH(OCH_3)_2$, TsOH, DMF; b. BuLi, THF; c.H_2NOH, NaOH, EtOH; d. LAH, Et_2O; e. Ac_2O, Py; f. NBS, CCl4, $BaCO_3$; g. AgF, Py; h. $NaOCH_3$; i. $Pd/BaCO_3$, CH_3OH, H_2; j. $Ba(OH)_2$, H_2O; k. HCl(aq).

The highlights of this synthesis are the stereoselective reduction of the oxime with lithium aluminium hydride (LAH) and the stereospecific generation of the L-lyxo configuration.

Another group of natural starting materials are certain L-pentoses that can be converted into 2-deoxy-L-hexoses. Grethe et al[140]. published a total synthesis of L-daunosamine starting from L-arabinose (fig. 16).

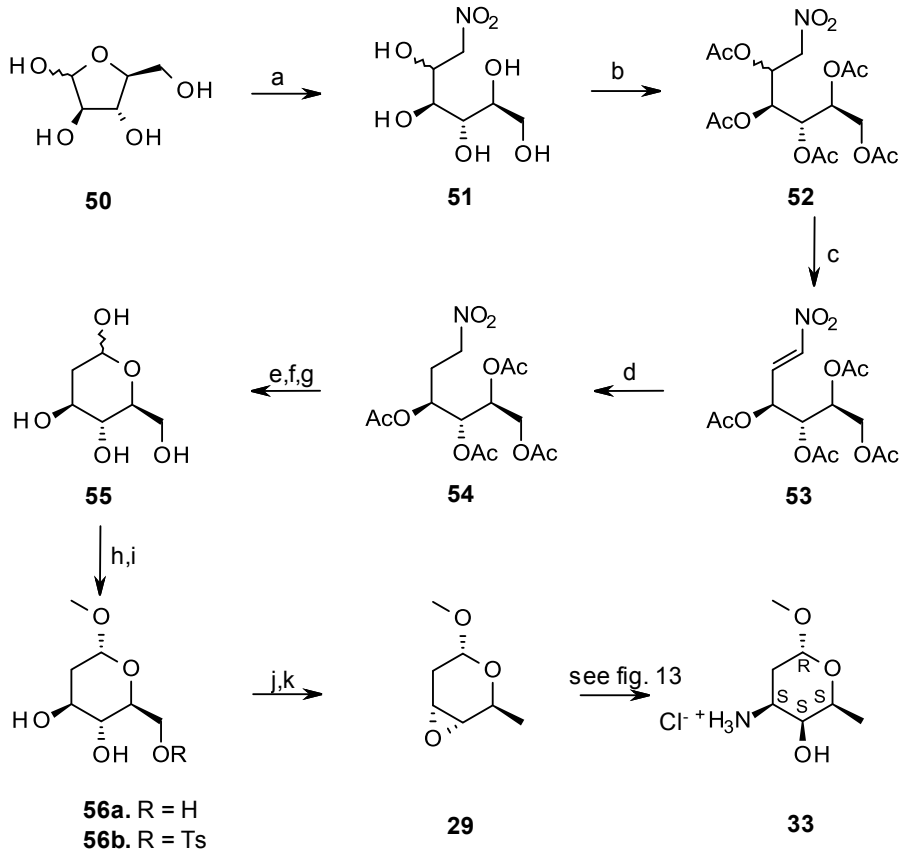
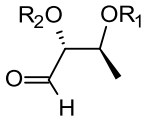


Figure 16 | Synthesis of L-daunosamine hydrochloride from L-arabinose. Reagents and solvents: a. CH_3NO_2, $NaOCH_3$; b. $BF_3 \cdot Et_2O$, Ac_2O; c. $NaHCO_3$, $PhCH_3$; d. Pd/C, H_2, EtOAc; e. $Ba(OH)_2$; f. H_2SO_4; g. $BaCO_3$; h. Amberlite-H^+, CH_3OH; i. TsCl, Py; j. $NaBH_4$, DME; k. Amberlite-OH^-, CH_3OH.

The conversion of L-arabinose **50** to 2-deoxy-L-hexose **55** runs via an (*E*)-nitro olefin **53**, which is reduced and reacts further to L-arabino-hexoses via a Nef reaction. This synthesis includes the same epoxide **29** as published before by Marsh et al.[132] Starting from the epoxide **29** L-daunosamine was synthesized via an identical pathway with 26 % overall yield[139].

4.6.1.2 Synthesis from non-carbohydrate precursors

Although deoxy-aminohexoses traditionally were synthesized by transformation of available carbohydrates, considerable efforts have been concentrated on the use of non-carbohydrate precursors. Especially hydroxyacids (L-tartaric, L-lactic) and amino acids (D-threonine, L-aspartic) were often used as chiral starting materials. The chiral aldehydes **57a**/**b** (fig. 17) prepared from (2R,3R)-tartaric acid or from D-threonine have been studied intensively[139]. Since this aldehydes have already two of the three required stereocenters of L-daunosamine only the stereocenter of the amino group has to be created.



57a: $R_1/R_2 = (CH_3)_2C$
57b. $R_1/R_2 = (CH_2)_5C$

Figure 17| (4R,5S)-2,2,5-trimethyl-1,3-dioxolane-4-carbaldehyde **57a** and (2S,3R)-2-methyl-1,4-dioxaspiro[4.5]decane-3-carbaldehyde **57b**.

Fronza et al.[141] first used these compounds as key intermediates in the synthesis of acosamine and daunosamine. The unsaturated ester **58** was synthesized via a Wittig reaction of **57a** with (Carbethoxymethylene)triphenylphosphorane. Further addition of ammonia gave stereoselectively **59** which was the hydrolyzed to the lactone **60**.

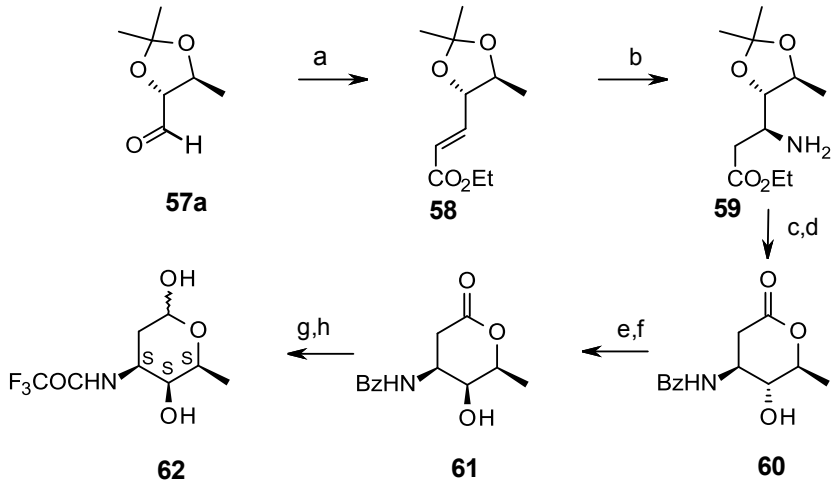


Figure 18|**Synthesis of L-daunosamine from aldehyde 57a[139,141,142].** Reagents and solvents: a. $Ph_3P{=}CHCO_2Et$; b. NH_3, CH_3OH; c. HCl, Et_2O; d. NaOH, PhCOCl; e. MsCl, Py; f. NaOAc; g. TFAA, Py; h.THF, DIBAL, 50 °C.

The epimerisation of the hydroxyl group was performed by mesylation to give lactone **61**. Trifluoroacetylation and DIBAL reduction gave N-(trifluoroacetyl)daunosamine **62**.

Further syntheses of L-daunosamine using the chiral aldehydes **57a**/**b** as key intermediates were published by Mukaiyama et al.[143], DeShong et al.[144] and Kita et al.[145]

A synthesis using lactic acid as starting material was published by Hamada et al.[146,147] They used diphenyl phosphorazidate (DPPA) for C-acylation of methyl isocyanoactetate with lactic acid to give oxazole **65**. The oxazole ring is further cleaved under acidic conditions to give lactone **66**. After protection of the amino group a stereoselective hydrogenation using rhodium on aluminia as catalyst give lactone **68** which was further reduced with DIBAL-H to give lactole **69**. A further C-1 unit was introduced via Wittig reaction with (methoxymethyl)-triphenylphosphonium chloride. The resulting enol ether **70** was further hydrolyzed to give L-daunosamine **71** as hydrochloride (fig. 19).

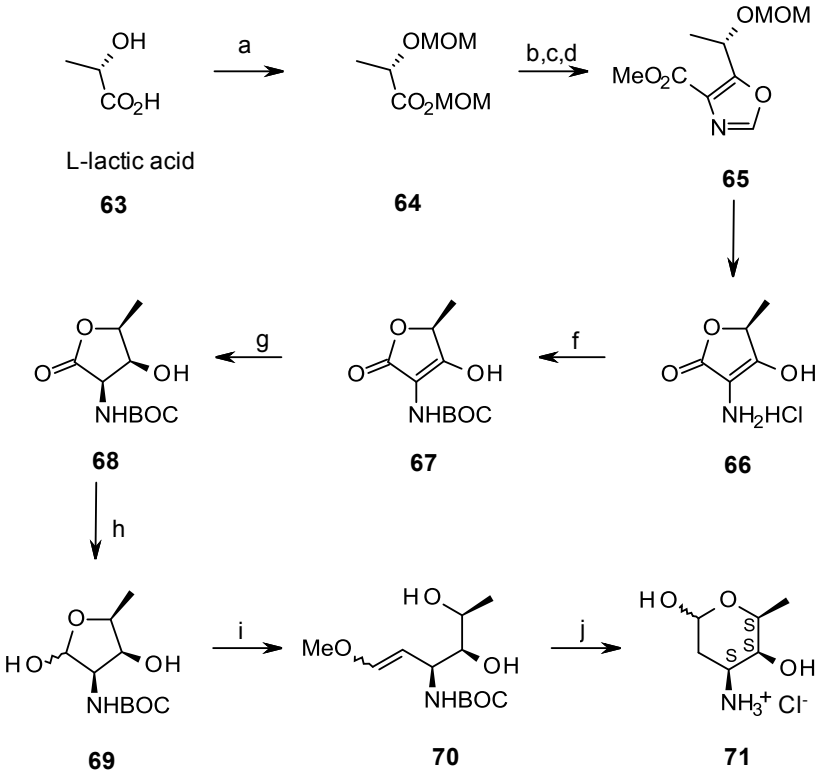


Figure 19 | Synthesis of L-daunosamine hydrochloride from L-lactic acid[146]. Reagents and solvents: a. CH_3OCH_2Cl, DIPEA, CH_2Cl_2; b. LiOH, H_2O/THF; c. DPPA, DMF; d. $CNCH_2COOMe$, NaH, DMF; e. 10 % HCl, MeOH; f. BOC_2O, $NaHCO_3$, dioxane/H_2O; g. 5 % Rh-Al_2O_3/H_2, EtOAc; h. DIBAL-H, CH_2Cl_2; i. $(PH_3P^+CH_2OCH_3)Cl^-$, KOtBu, glyme/toluene; j. 20 % HCl, THF.

Another total synthesis of L-daunosamine published by Jurczak et al.[148] uses L-aspartic acid as starting material (fig. 20).

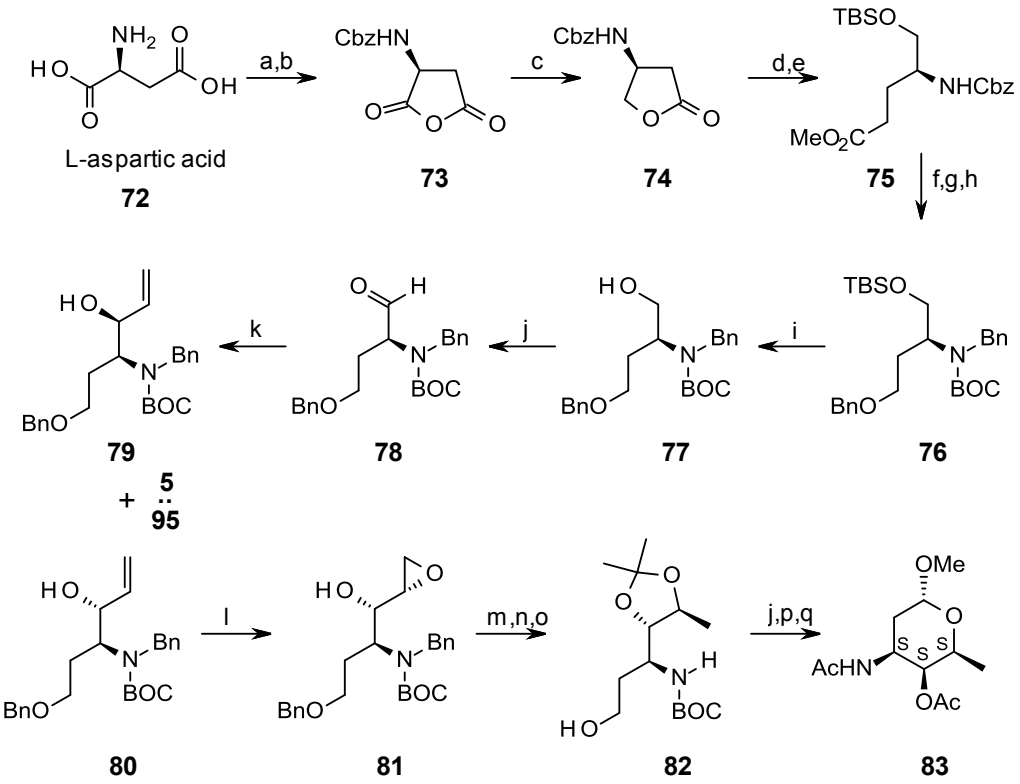


Figure 20 | Synthesis of L-daunosamine hydrochloride from L-aspartic acid[148]. Reagents and solvents: a. $ClCO_2Bn$, K_2CO_3, H_2O; b. Ac_2O, AcOH; c. $NaBH_4$, THF; d. DCC, MeOH; e. TBSCl, imidazole, DMF; f. H_2, Pd/C, BOC_2O, MeOH; g. LAH, Et_2O; h. BnBr, NaH, DMF; i. Bu_4NF, THF; j. SO_3/Py, DMSO; k. Vinyl-MgBr, Et_2O; l. mCPBA, CH_2Cl_2; m. DIBAL-H, Et_2O; n. DMP, acetone, p-TsOH; o. Na, NH_3; p. HCl/MeOH; q. Ac_2O, Py.

Lactone **74** was synthesised from L-aspartic acid via anhydride **73** in three steps. Transesterification of **74** and protection of the hydroxyl group gave compound **75**. Exchange of the N-protecting group, reduction of the ester group and benzylation gave compound **76**. Cleavage of the silyl group and oxidation to an aldehyde-functionality was followed by addition of vinylmagnesium bromide with a good diastereoselectivity (95:5). Subsequent epoxidation gave *syn*-epoxide **81** which was reductively opened and the resulting diol was protected with an ispropylidene group. Treatment with sodium in ammonia gave alcohol **82**. Oxidation of the hydroxyl group was followed by deketalisation and *in situ* cyclisation with methanolic hydrochloride. Further acetylation led to methyl *N,O*-diacetyl-L-daunosamine **83**.
In the following table 1 further syntheses of L-daunosamine and related glycosylating agents from non-carbohydrate precursors are summarized.

Table 1 | Publications of L-daunosamine syntheses and related glycosylating agents from non-carbohydrate precursors

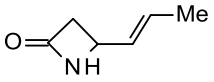
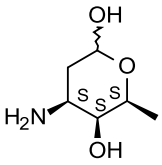
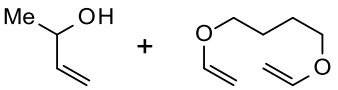
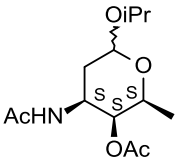
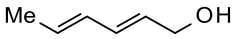
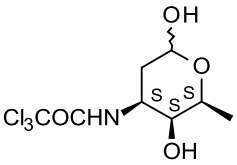
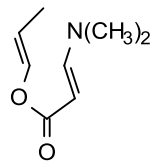
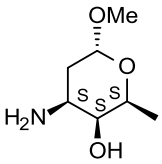
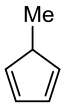
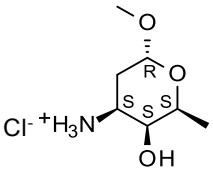
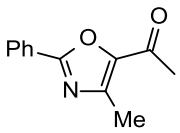
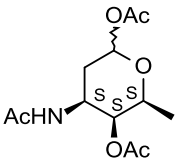
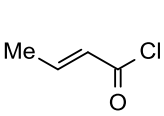
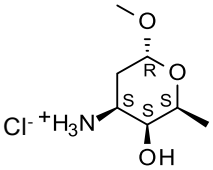
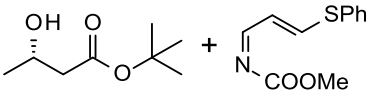
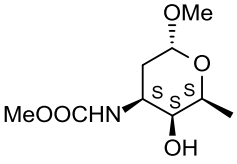
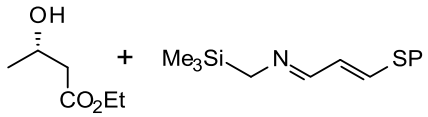
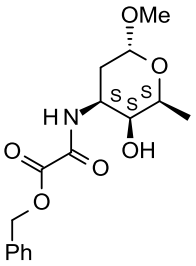
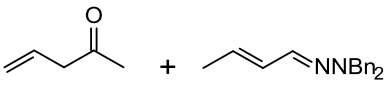
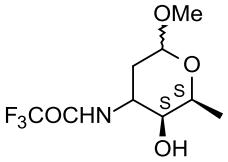
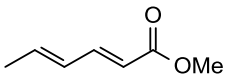
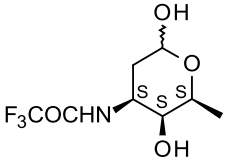
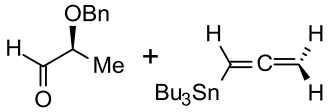
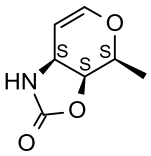
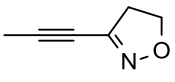
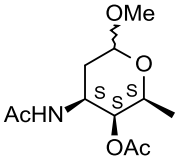
Starting material	Number of steps	Product	Overall yield	Reference
	7 steps		9 %	Hauser et al.[149]
	9 steps		17 %	Dyong et al.[150]
	7 steps		–	Hauser et al.[151]
	10 steps		–	Wovkulich et al.[152]
	12 steps		4 %	Grethe et al.[153] Marsh et al.[132]
	10 steps		–	Wong et al.[154]
	6 steps		–	Iwataki et al.[155]
	8 steps		19 %	Hatanaka et al.[156]

Table 1 (continued) | **Publications of L-daunosamine syntheses and related glycosylating agents from non-carbohydrate precursors**

Starting material	Number of steps	Product	Overall yield	Reference
	9 steps		9.8 %	Gallucci et al.[157]
	10 steps		32 %	Friestad et al.[158]
	14 steps		–	Nagumo et al.[159] Fronza et al.[142]
	5 steps		44 %	Parker et al.[160]
	10 steps		8.5 %	Wade et al.[161]

The daunosamine syntheses outlined in this chapter share the fact of relatively complex and many stage synthesis routes caused by numerous stereogenic centers with different functionalities, which makes daunosamine synthesis a really challenging task.

5 Results and discussion

5.1 Retrosynthetic considerations

For synthesis of sugar modified anthracyclines our retrosynthetic analysis led to a disconnection of the glycosidic bond between the aglycone and the sugar moiety.

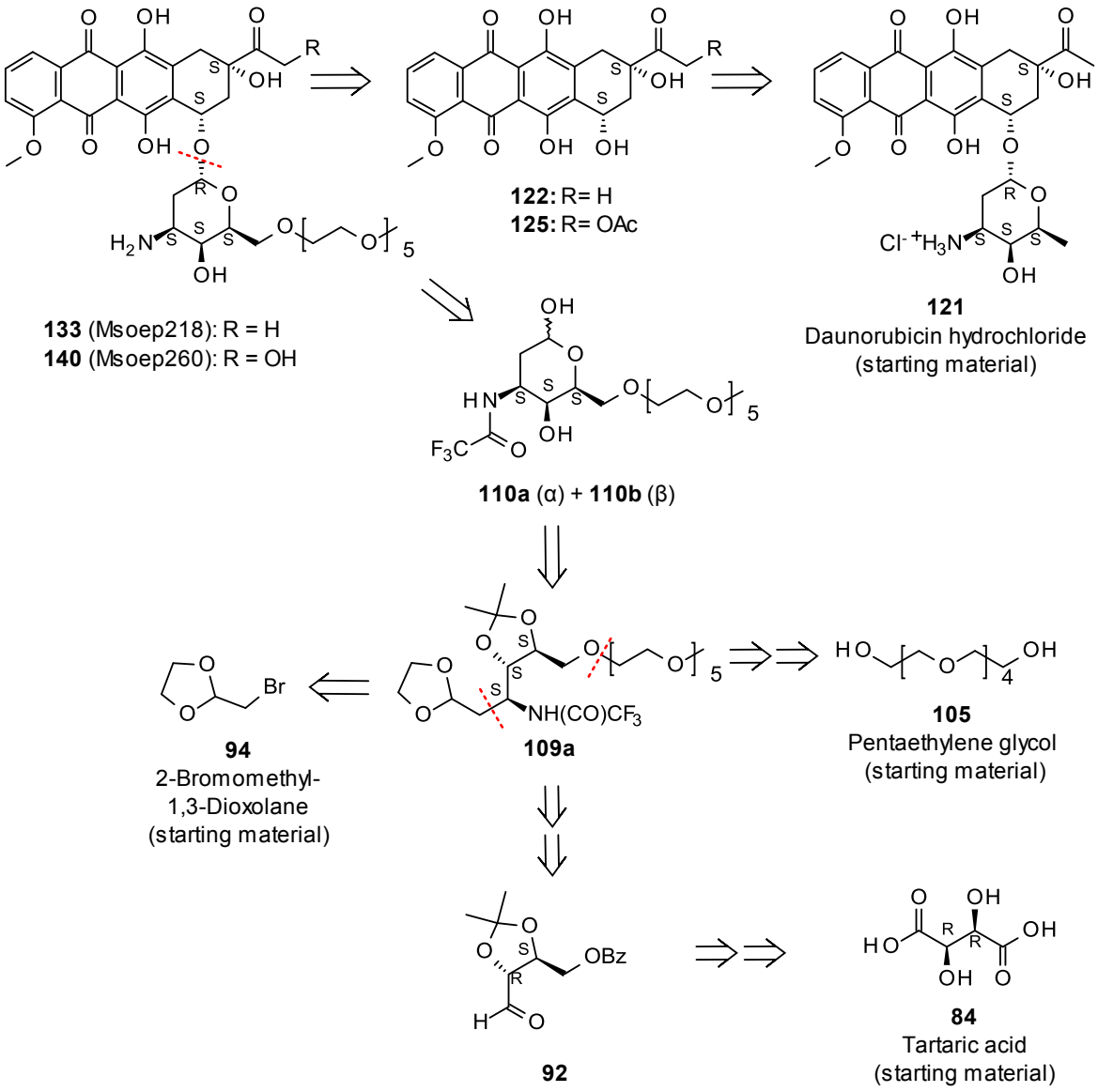


Figure 21 | Retrosynthesis analysis of the novel anthracycline derivatives **133** (msoep218) and **140** (msoep260)

Whereas the aglycons (daunorubicinone **122** and doxorubicinone **125**) can be generated out of commercially available daunorubicin hydrochloride **121**, the modification of the sugar moiety at the 6´-position requires a total synthesis, since the methyl group cannot be functionalized easily by known synthetic methods.

In search of a suitable protecting group strategy for the sugar synthesis we decided to protect the amino group with a trifluoroacetyl group, since detailed study of the glycosylation literature showed that the coupling of unprotected or protected aglycone can be efficiently done with sugar derivatives whose amino group is protected by a trifluoroacetyl group. As further protecting groups 1,3-dioxolane was selected for the formyl group and isopropylidene ketal for the hydroxyl groups.

The search for suitable precursors, for the synthesis of the protected open chain sugar moiety **109a** resulted in three disconnections, for which three building blocks were selected.

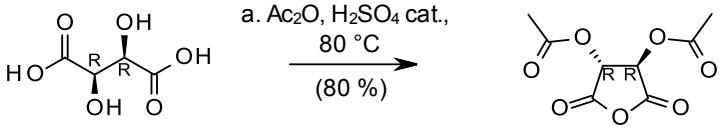
Building block 1: The chiral aldehyde **92**, which already carries two of three required stereocenters.
Building block 2: 2-Bromomethyl-1,3-Dioxolane **94**.
Building block 3: Pentaethylene glycol **105** for the construction of oxyethylene side chain.

Although the aldehyde **92** is already documented in literature[162,163], previously published syntheses provided a poor yield or lack of stereoselectiviy. Therefore it was decided to develop a new synthesis starting from (2R,3R)-tartaric acid, which was identified as the appropriate precursor. Aldehyde **92** was considered to be suitable to create a C-C connection with commercially available 2-Bromomethyl-1,3-Dioxolane **94,** completing the required six carbon atoms. Subsequently the introduction of the nitrogen functionality via an oxime reduction strategy was foreseen, a key step of the synthesis. Finally the oxyethylene building block was to be connected via an alkylation reaction.

5.2 Synthesis of 6'-derivatives of 3-amino-2,3-dideoxy-L-hexoses related to daunosamine

5.2.1 Anhydride formation of tartaric acid

For the acetylation and anhydride formation of tartaric acid **84** standard protocols are described in literature[164-166] using either acetyl chloride or acetic anhydride. Proton (H_2SO_4) catalyzed acetylation and anhydride formation of tartaric acid goes on with discharge of water and consequently acetic acid in a self-propelling exothermic reaction. The following representative procedure was taken from Dobashi et al. [166]



84 (commercial) **85**

Figure 22 | Anhydride formation of tartaric acid[166]. Reagents and conditions: a. Acetic anhydride (3.0 equiv.), sulfuric acid cat., ~ 80 °C, 10 min (80 %).

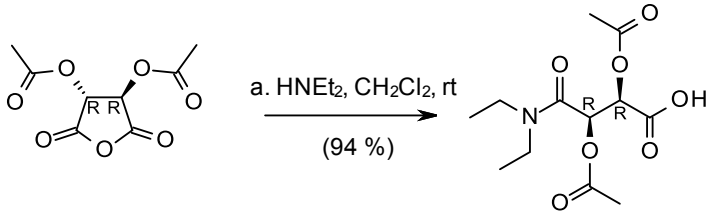
During the optimisation process further experiments were performed concerning the reaction temperature, the excess of acetic acid anhydride and the work-up procedure. The procedure from the literature was modified by avoiding benzene and using MTBE instead of diethyl ether. It could be demonstrated that the conversion was sufficiently fast at a temperature of about 80 °C. When the reaction temperature was too low (< 60 °C), the reaction did not proceed to completion. The optimum reaction temperature was investigated to be between 80 – 85 °C.

The molar ratio of acetic acid anhydride should be kept carefully. More than two equivalents of acetic acid anhydride were needed to complete the reaction. Too little acetic acid anhydride led to incomplete turnover. Excess acetic acid anhydride did not harm the reaction, but had to be removed for the next step.

By the end of the reaction, the precipitation of anhydride **85** was promoted by cooling down the reaction mixture below 5 °C and addition of MTBE. After filtration and drying a comparatively high yield (80 %) of anhydride **85** was obtained. For the reaction conversion no TLC method was applied since the substances did not move on silica gel plates. Measurement of the melting point, HPLC analysis and NMR-Spectroscopy were performed to determine the purity of the anhydride **85**.

5.2.2 Selective monoamidation and ring opening of the tartaric acid anhydride

In literature it has been shown, that the cyclic anhydride can be opened by nucleophilic attack, allowing the distinction between the both carboxylic acid moieties of tartaric acid. From Dobashi et al.[166], Bell[167], Huh et al.[168], and Gonzalez et al.[169] similar opening strategies of the cyclic anhydride with amines are reported, whereas Gawroński et al.[170] elucidated the stereochemistry of the ring-opening by nucleophilic attack. The experimental procedure applied for the first laboratory experiments followed basically the procedure as described by Dobashi et al.[166]



85 **86**

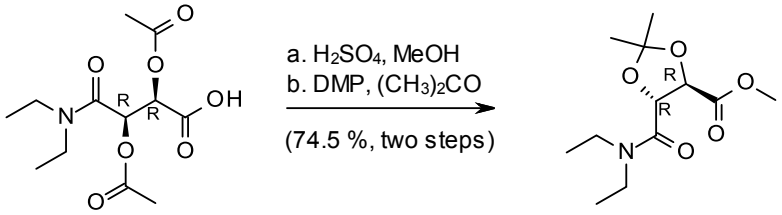
Figure 23│**Monoamidation with diethyl amine**. Reagents and conditions: a. Diethylamine (1.1 equiv.), dichloromethane, room temperature, 1 hr (94 %).

During the optimization process it could be found out that the amount of amine is critical as well in case of stoichiometric deficit as in case of stoichiometric excess. Unreacted amine disturbed the next reaction step (by forming a salt with the acid moiety). On the other hand, by subequimolar amine addition, some anhydride was left over in the reaction mixture, which led to undesired side-products, which was even more disturbing during the following steps. A washing-step using diluted acid was crucial to remove excess amine and eventually unreacted starting material.

The result, after investigating the critical parameters, was, that a low excess of diethylamine (1.08 equiv.), work up of the reaction mixture with an acidic water phase and extraction with ethyl acetate guarantee complete conversion to **86**. After removal of the volatiles the product solidifies. Recrystallization from MTBE/hexane gave an extremely hygroscopic product which liquefied when exposed to moisture but did not decompose. At the end of the development phase, yield (94 %) and purity were sufficient to go for the next step without any further purification.

5.2.3 Esterification and ketalisation of the diacetylated monoamide of tartaric acid

The third intermediate of the tartaric acid pathway resulted from a one-pot reaction of two consecutive steps using the diacetylated monoamide of tartaric acid **86** as starting material. The conversion of the diacetylated monoamide **86** into the acetonide **87** has not been previously reported, but a very similar conversion has been described[171-173]. Beside this sort of "one-pot" procedure with, simultaneous conversion of the acid functions into the ester and building up the acetal, a step-wise reaction starting from tartaric diester, has been reported by Denmark et al.[174] and Colobert et al.[175]



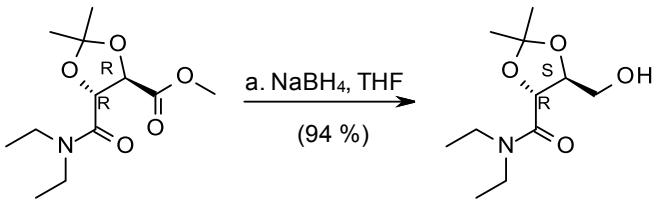
86 **87**

Figure 24 | Esterification and ketalisation of the monoamide 86. Reagents and conditions: a. Sulfuric acid (0.22 equiv.), MeOH, reflux, 10 hrs; b. 2,2-dimethoxypropane (2.81 equiv.), acetone, reflux, 2 hrs (74.5 % over two steps).

Our reaction sequence started with the mono-esterification of the carboxyl group under acidic conditions, at the same time the two acetoxy groups were cleaved and a trans-esterification of the 2,3-acetoxy groups with methanol took place, leaving two free hydroxyl groups, which were ketalised with dimethoxypropane in the second step. During the investigation of critical parameters for this reaction sequence it could be found that the more acid was used, the faster the reaction occurred. This was not a problem for the conversion of **86** into the reaction intermediate, which was very stable. However, the formation of the acetal turned out to be reversible (if the reaction time was chosen too long, a re-opening of the ring as well as hydrolysis of the ester was observed). Water was playing a crucial role in the second step of the reaction. To remove water dimethoxypropane was used in excess. The final product **87** was not sensitive against water. At the workup washing with water and aqueous saturated $NaHCO_3$ was necessary to remove the acid traces and unreacted starting material from the organic layer. The acetonide **87** was stable at room temperature for at least 2 months.

5.2.4 Reduction of the methyl ester to the correspondent primary alcohol

The following step in the development of the new procedure was a selective reduction of the ester group.



87 **88**

Figure 25│**Reduction of the methyl ester 87**. Reagents and conditions: a. Sodium borohydride (1.2 equiv.), tetrahydrofuran, room temperature, 4 hrs, (94 %).

This could be achieved following standard protocols[176,177] for the selective reduction of an ester group with sodium borohydride or lithium aluminium hydride. Sodium borohydride was chosen as reducing agent due to its easier handling compared to lithium aluminium hydride. The reaction itself was very exothermic, ice-water cooling was required to maintain the reaction temperature below 30 °C. The optimum temperature for the reaction was between 10 - 25 °C, reaction time 4 hours, lower temperature led to long reaction times, at higher temperatures the formation of the diol derivative (ester and amide are reduced) was observed. The applied reduction procedure resulted in the novel hydroxyamide **88** in sufficient quality to go for the next step without any further purification. If required solid hydroxyamide **88** can be precipitated from a mixture of toluene and petroleum ether.

5.2.5 Reduction of the hydroxyamide 88 to the protected L-threoses 90 and 92

C_4 chiral aldehydes like the threoses **90** and **92** are well reported substances in literature, synthesized from carbohydrate and non-carbohydrate precursors[139,162,163,178-182].

In our procedure the first strategy for the reduction of the hydroxyamide **88** to an aldehyde was an interim protection of the free hydroxyl group followed by reduction of the amide to the aldehyde. The trityl group was chosen according to the requirements of the following steps. The reduction of the tritylated amide **89** to the aldehyde **90** could be achieved by use of Red-Al® under mild reaction conditions.

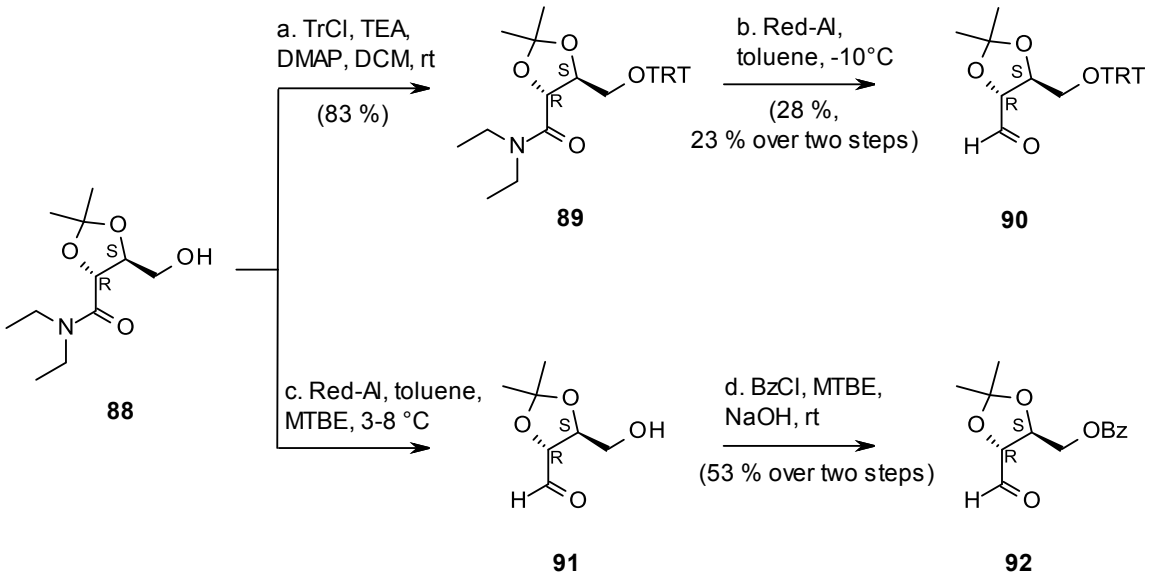


Figure 26 | Synthesis of the aldehydes 90 and 92. Reagents and conditions: a. Triphenylmethyl chloride (1.4 equiv.), triethylamine (1.2 equiv.), 4-dimethylaminopyridine, dichloromethane, room temperature, 4 hrs, (83 %); b. sodium bis(2-methoxyethoxy)aluminumhydride (1.0 equiv.), toluene, -10 °C, 60 min, (94 %); c. sodium bis(2-methoxyethoxy)aluminumhydride (1.4 equiv.), toluene, methyl tert-butyl ether, 3-8 °C, 70 min; d. Benzoyl chloride (10.0 equiv.), sodium hydroxide (10.0 equiv.), methyl tert-butyl ether, room temperature, 4 hrs, (53 % over two steps).

Reduction of dialkylamides to aldehydes is described in literature using a large variety of reducing agents, also Red-Al® has been used for this purpose[183-185]. By replacing of tetrahydrofuran by toluene as solvent the reduction slowed down significantly at the aldehyde oxidation stage, so that the isolation of the product, even with slight excess of reducing agent was possible. The following table shows a compilation of all trials to reduce the tritylated amide **89** to the aldehyde **90** using Red-Al®.

Table 2 | Compilation of trials to reduce amide 89 to aldehyde 90

Amount of starting material	Purity of the starting material	Eq. Red-Al®	Solvent [f]	Reaction Time	Reaction Temp.	Yield	Purity [a]
9.630 g	~ 40 %	1.5	THF	2 hrs	-10 °C	[b]	
0.585 g	> 90 %	1.0	THF	5 hrs	-10 °C	19 % [c]	~20 %
0.509 g	> 90 %	1.5	THF	30 min	-10 °C (15 min) 0 °C (15 min)	86 %	~30 %
0.500 g	> 90 %	1.0	THF	6 hrs	-10 °C	20 % [e]	~65 %
1.150 g	> 90 %	1.0	THF	2 h 30 min	-10 °C (30 min) 0 °C (2 hrs)	87 %	~10 %
7.080 g	> 90 %	1.5	THF	45 min	-10 °C (15 min) 0 °C (30 min)	100 %	~17 %
0.829 g	> 90 %	1.0	Toluene	2 h 30 min	-10 °C	85 %	[d]
0.512 g	> 90 %	1.5	Toluene	30 min	-10 °C	80 %	~23 %
0.793 g	> 90 %	1.2	Toluene	60 min	-20 °C	96 %	~22 %
0.324 g	> 90 %	1.0	Toluene	60 min	-10 °C	94 %	~30 %
1.304 g	~ 68 %	1.8	Toluene	2 hrs	-10 °C	92 %	~13 %

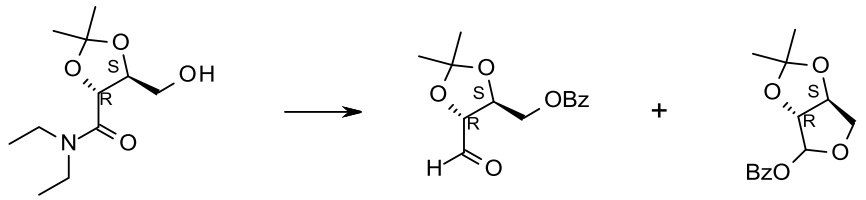
[a](The Purity was detected according to the 1H aldehyde signal relative to the trityl-signals in ^{1}H-NMR spectrometry); [b](No reaction proceeded, the reaction was quenched and discarded); [c](34 % of the starting material , and 20 % of over reduced alcohol); [d](25 % purity directly after work up, after 24 hrs in the NMR-tube the purity was 43 %); [e](after flash chromatography); [f](All reactions were performed in 10 % (w/v) solution)

In THF as solvent the complete reduction to the amine took place much more easily and led to a significant formation of the undesired amine, if reaction conditions were not observed extremely carefully. Reduction with DIBAL also led to a higher tendency towards complete reduction of the amide to the amine.

Due to the relatively low yield of aldehyde **90** after carrying out several different reaction procedures, it was decided to change the strategy (fig. 26). The new strategy included a direct reduction of hydroxyamide **88** with Red-Al® in toluene, followed by benzoylation of the hydroxyaldehyde **91** in a biphasic organic aqueous system using benzoylchloride and NaOH. While in the beginning of this development the intermediate hydroxy aldehyde **91** was isolated. During the laboratory development of this sequence it could be shown that no advantage with respect to yield or purity could be achieved by the isolation of the free hydroxy aldehyde **91**. Therefore the sequence as described in figure 26 was further developed to a one-pot procedure, which then actually consisted of two consecutive reaction steps. The reaction was followed by TLC/HPLC, but it was performed without isolation of the intermediate product. The following table 3 summarizes experiments for the synthesis of aldehyde **92** starting from hydroxyamide **88**, which were performed for the one-pot reaction using 1 -1.7 equivalents of Red-Al® under various conditions.

Table 3 | Experiments for the synthesis of aldehyde 92 starting from hydroxyamide 88

Amount of starting material	Eq. RedAl, Eq. BzCl Eq. Base	Solvent [a](	Reaction Temp.	Reaction Time	Results
0.575 g	1.0 eq. RedAl 1.7 eq. BzCl 14.0 eq. NaOH	THF	1. -78 °C -RT 2. RT 3. RT	1. 20 hrs 2. 5 min 3. 5 min	No yield of **92** or **92a**
0.556 g	2.0 eq. RedAl, 4.0 eq. BzCl 10.0 eq. Py.	Toluene	1. -78 °C 2. 0 °C 3. 0 °C	1. 20 min 2. 1 min 3. 1 hrs	Only traces of **92** + benzaldehyde
0.515 g	1.7 eq. RedAl, 5.0 eq. Bz_2O 10.0 eq. $NaHCO_3$	Toluene	1. -10 °C 2. -10 °C 3. RT	1. 3 hrs 2. 1 min 3. 20 hrs	Only traces of **92** + benzaldehyde
0.534 g	1.3 eq. RedAl 5.0 eq. Bz_2O 10.0 eq. K_2CO_3	Toluene	1. -10 °C 2. -10 °C 3. RT	1. 3.2 hrs 2. 1 min 3. 23 hrs	~ 5 % of **92** + benzaldehyde
0.653 g	1.5 eq. RedAl 5.0 eq. BzCl 10 eq. K_2CO_3	Toluene	1. -10 °C 2. -10 °C 3. RT	1. 1.5 hrs 2. 5 min 3. 24 hrs	~ 5 % of **92** + benzaldehyde
1.070 g	1.7 eq. RedAl 6.0 eq. BzCl 5.0 eq. NaOH	Toluene	1. -10 °C 2. -10 °C 3. RT	1. 30 min 2. 1 min 3. 3.17 hrs	~ 28 % of **92** and ~ 20 % of **92a**
11.60 g	1.4 eq. RedAl 4.0 eq. NaH_2PO_4 4.0 eq. BzCl 4.0 eq. NaOH	Toluene	1. 4-15 °C 2. 0 °C 3. 0 °C 4. RT	1. 60 min 2. 30 min 3. 2 min 4. 1.5 hrs	~ 36 % of **92** [b](
5.00 g	1.4 eq. RedAl 4.0 eq. NaH_2PO_4 10.0 eq. BzCl 10.0 eq. NaOH	Toluene	1. 3-8 °C 2. 0 °C 3. 0 °C 4. RT	1. 60 min 2. 2 min 3. 2 min 4. 4 hrs	~53 % of **92** [b](



88 **92** **92a**

[a](All reactions were performed in 10 % (w/v) solution); [b](After purification by extraction with sodium hydrogensulphite solution)

During the investigation of the critical parameters it was shown that only the first hydride atom of Red-Al® was reactive enough to achieve the amide reduction to the aldehyde. In principle the hydroxy aldehyde **91** was not the final product in the reduction step, this would be in theory the amine by further reduction of the aminal, or the diol by reduction of the aldehyde set free from the aminal in the reduction mixture. Temperature dependence of the reduction steps was examined. At low temperatures of around -70 °C conversion was very slow. At temperatures between 0 °C and 10 °C the best results were obtained. No further

reduction products were observed, when the reaction was kept within this temperature range. At room temperature side product formation became significant.

Concerning the solvents the reaction in THF showed formation of more side-products, derived from further reduction in comparison to toluene. The same observation had already been made in the experiments with the tritylated amide **89**. Therefore toluene was the solvent of choice.

Since the reaction proceeded in a straightforward manner, Red-Al® was added as quickly as possible. The speed of addition was limited by the exothermic nature of the reaction. Therefore efficient stirring supported quick conversion of the reduction. After addition of the full amount of Red-Al® the reaction should not be kept for more than 1 hour to complete the reduction. Stirring for much longer periods reduced the yield due to further reduction.

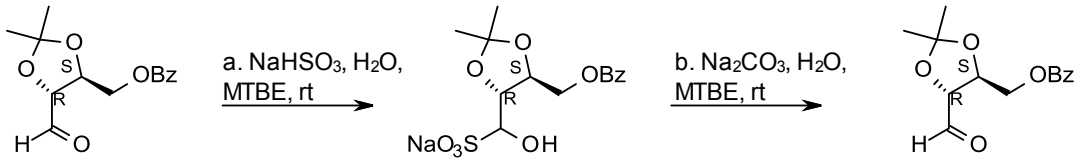
Hydrolysis of the intermediate aluminium complex under basic pH-values led to the formation of a cyclic lactol as main side product, reducing the yield significantly. To avoid unwanted basicity, hydrolysis was carried out in a buffered solution. A phosphate buffer showed to be best suited for this purpose. No cyclic lactol formation was observed with this system. A particular advantage of the phosphate buffer system was given by the fact, that the aluminium salts formed from the Red-Al® became very well stirrable and could be easily separated from the reaction mixture.

The hydroxyaldehyde **91** could be isolated from the reaction mixture by extraction with ethyl acetate. Experiments to apply this work up step did not seem to be superior to a procedure, by which the hydroxyaldehyde **91** was left in the organic phase to be subsequently benzoylated in a two phase benzoylation reaction.

The benzoylation was carried out at 0 °C and was not allowed to exceed a temperature of 5 °C during addition, since benzoyl chloride will underwent hydrolysis to benzoic acid at elevated temperatures. As each mol of Red-Al® contains 2 mols of methoxyethanol, this alcohol was benzoylated as well. A five molar excess of benzoyl chloride (4 equivalents for methoxyethylesters and one for aldehyde **92**) was required, if there was no aldehyde work up before the acylation step. The use of benzoic anhydride was feasible in principle. However it generated additional benzoic acid. Ten molar equivalents of sodium hydroxide were required to correspond to the amount of benzoyl chloride. This ratio had to be kept strictly because of potential benzoyl chloride hydrolysis and side reactions of the aldehyde under basic conditions. It was however necessary to observe the pH of the reaction mixture carefully.

The benzoylated aldehyde **92** was then isolated by extraction of the sodium bisulfite-adduct into the aqueous phase, which allowed its separation from all other components in the work up mixture and was then re-extracted into the organic phase after decomposition of

the bisulfite adduct under basic conditions (fig. 27). This step provided an excellent purification of the product for the subsequent Grignard reaction.



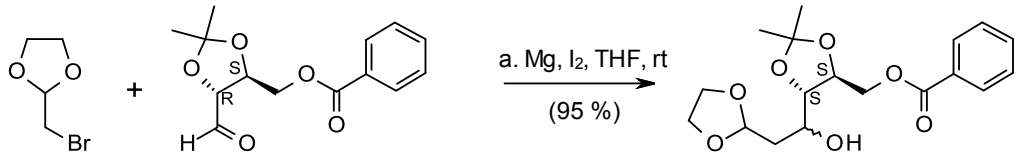
92 **93** **92**

Figure 27 | Bisulfite addition. Reagents and conditions: a. Sodium bisulfite, H_2O, methyl tert-butyl ether, room temperature, 3 hrs; b. Sodium carbonate, H_2O, methyl tert-butyl ether, room temperature, 18 hrs.

Since the overall yield of the one-pot reduction of the hydroxyamide **88** to the threo-aldehyde **92** was higher than in the two step procedure tried first to synthesize threo-aldehyde **90** (fig. 26), aldehyde **92** was chosen to continue synthesis.

5.2.6 Nucleophilic C-2-addition to the L-threose derivative 92

The addition of the C_2-unit was achieved successfully by an in situ Grignard reaction in an aprotic solvent.



94 commercial **92** **95a+b**

Figure 28 | Grignard reaction. Reagents and conditions: a. Magnesium (2.2 equiv.), Iodine (catalytic), 2-Bromomethyl-1,3-Dioxolane (1.6 equiv.), tetrahydrofuran, room temperature, 5 hrs (95 %).

Grignard reactions of (2-Bromomethyl-1,3-Dioxolane) with similar aldehydes have already been described[186-188]. In consideration of previous work in our group, the nucleophilic addition of 2-Bromomethyl-1,3-Dioxolane **94** to the threo-aldehyde **92** succeeded for the first time in context with the total synthesis of N-benzyl daunosamine and N-benzyl acosamine [189]. The (1,3-dioxolane-2ylmethyl) magnesium bromide was freshly prepared with a catalytic amount of Iodine in THF before the threo-aldehyde **92** was added. During the optimization process of this reaction the amount of (1,3-dioxolane-2ylmethyl) magnesium bromide could be reduced without any influence on the yield of the alcohols **95a+b**. The risk that the still existing excess

of Grignard reagent also reacts with the benzoyl ester, leading to a tertiary alcohol as side product, could not be observed. After hydrolysis under weakly acidic conditions the resulting secondary alcohols **95a**+**b** were obtained in a 1:1 ratio. A diastereoselectivity for the C-C coupling was not observed in this reaction. However, the stereoconfiguration of the hydroxyl group was not crucial for the further synthesis because the alcohols **95a**+**b** were oxidized to the correspondent ketone in the next step.

5.2.7 Introduction of the nitrogen functionality

To introduce the characteristic daunosamine amino functionality into the open-chain sugar moiety, the secondary hydroxyl groups of **95a**+**b** had to be oxidized first to the corresponding carbonyl functions. For this purpose the Swern oxidation, with dimethyl sulfoxide and oxalyl chloride as oxidants, was selected as the appropriate method due to its mild reaction conditions.

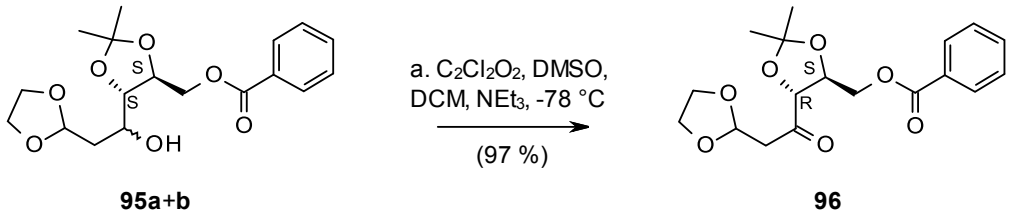


Figure 29│**Swern oxidation**. Reagents and conditions: a. Oxalyl chloride (1.5 equiv.), dimethyl sulfoxide (3.0 equiv.), dichloromethane, triethylamine - 78 °C, 2 hrs (97 %).

In this oxidation, dimethyl sulfoxide reacted first with oxalyl chloride to a sulfonium salt which subsequently reacted with the secondary alcohols **95a**+**b**. This alkoxysulfonium ion intermediate reacted under addition of a base (NEt_3) to a sulphur ylide which then underwent a transformation to the desired product **96** and Dimethyl sulfide (DMS)[190]. Low temperatures around -70 °C were necessary, otherwise thioacetals arose as side products. The applied method was consistent with standard oxidation protocols from literature[187,191], and gave ketone **96** in excellent yield, which could be used for the next step without any further purification.

After investigation of available methods for the introduction of the amino functionality we decided to transform the keto group to the corresponding ketoxime and reduce it further to the free amine. Methods for the condensation of a keto group with hydroxylamine are numerously published[155,192,193]. In our case oximes **97a**+**b** were successfully synthesized with an excess of hydroxylamine hydrochloride in pyridine as solvent (fig. 30).

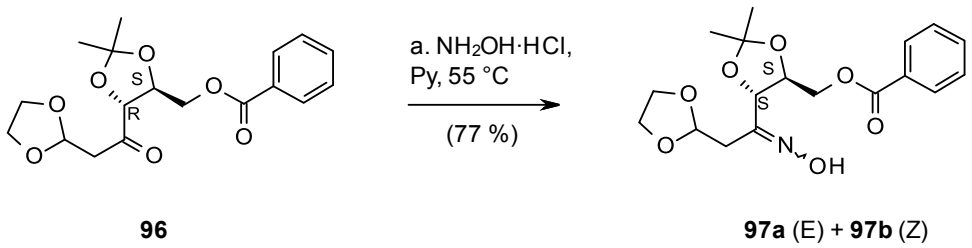


Figure 30 | Transformation to the oxime. Reagents and conditions: a. Hydroxylamine hydrochloride (6.5 equiv.), pyridine, 55 °C, 17 hrs (77 %).

The isomeric ratio of *E*-isomer and *Z*-isomer formed in the reaction was determined by quantitative ^{1}H-NMR spectroscopy gave a 75:25 (*E*:*Z*) ratio for the two isomers (**97a** and **97b**). Since the oximes **97a**+**b** were reduced in the later synthesis both isomers were used for the further steps.

5.2.8 Synthesis of the oxyethylene side chain

Our first strategy for the synthesis of the oxyethylene side chain, using commercial triethylene glycol as starting material, began with the ditosylation of the primary hydroxyl groups. This transformation to the toluenesulfonate ester was done by reaction with *p*-toluenesulfonyl chloride. After investigation of several standard tosylation-methods[192,194,195] those with sodium hydroxide as base and THF/H_2O as solvent delivered the best results.

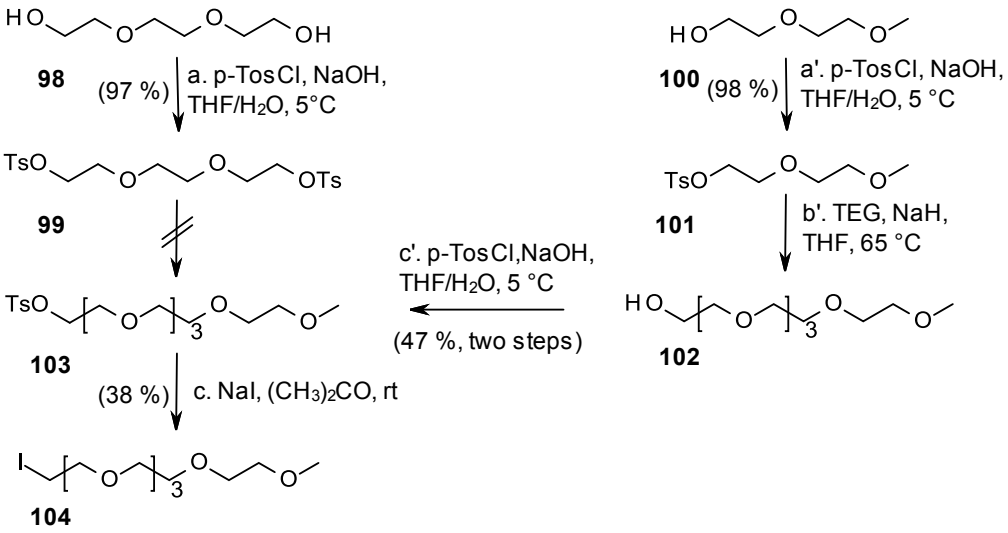


Figure 31 | Synthesis of the oxyethylene side chain (procedure 1). Reagents and conditions: a./a'. p-Toluenesulfonyl chloride, sodium hydroxide, tetrahydrofuran/water (1:1/1:1.5, v/v), 5 °C, 2/3.5 hrs (97 %/98 %); b'. Triethylene glycol (1.2 equiv.), sodium hydride (60 %, 1.2 equiv.), tetrahydrofuran, 65 °C, 14 hrs; c'. p-Toluenesulfonyl chloride (1.2 equiv.), sodium hydroxide (1.6 equiv), tetrahydrofuran/water (1:1, v/v), 5 °C, 2.5 hrs (47 % over two steps)[195]; c. Sodium iodine (1.5 equiv.), acetone, room temperature, 48 hrs, (38 %).

To achieve the required chain length of five ethylene oxide units, the ditoslyate **99** was to be connected with commercial methyl diglycol, which was first converted to the corresponding alkoxide using a strong base such as sodium hydride, potassium hydride or potassium tert-butoxide in THF or DMF as solvents, under various conditions.

Since this reaction did not lead to the desired tosylate **103** the strategy was changed. Methyl diglycol **100** was used as starting material, which was monotosylated under the same conditions as before. Monotosylate **101** was further connected with triethylene glycol in THF and sodium hydride as base to give the alcohol **102**, which was again tosylated to give the desired tosylate **103** (fig. 31). The last step in the synthesis of this alkylating agent was the formation of the halide out of tosylate **103,** which was achieved in acetone using sodium iodine. This formation led to a replacement of the tosyl group via an S_N2 reaction[196]. Since the iodide compound **104** was not detectable via common UV_{254}-TLC, the reaction was monitored by disappearance of the starting material. The chemical structure of the desired product **104** was proved by ^{1}H -^{13}C-NMR-spectroscopy.

The synthesis described above could be shortened by using commercial pentaethylene glycol as starting material (fig. 32)[121].

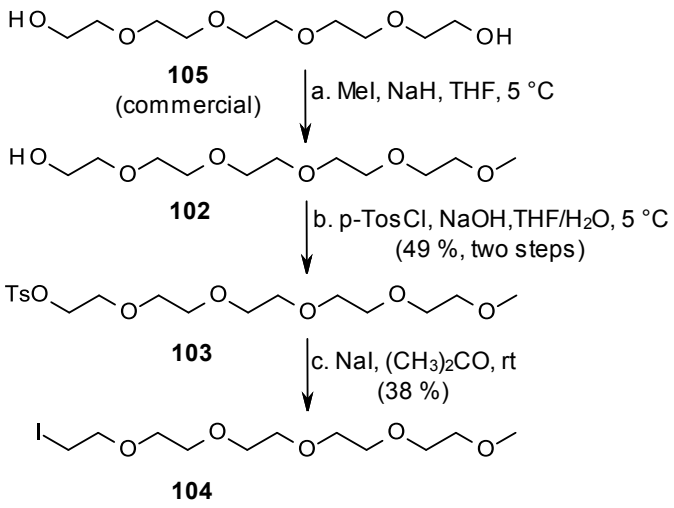
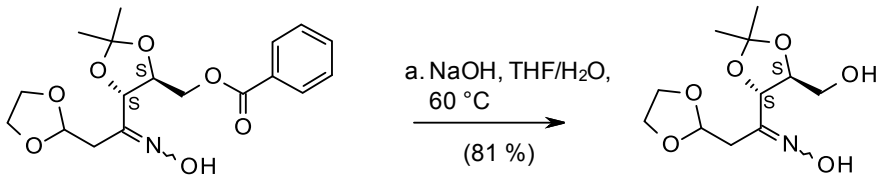


Figure 32 | Synthesis of the oxyethylene side chain (procedure 2). Reagents and conditions: a. Methyl iodine, sodium hydride (60 %, 1.1 equiv.), tetrahydrofuran, 5 °C, 2 hrs; b. *p*-Toluenesulfonyl chloride (1.2 equiv.), sodium hydroxide (1.6 equiv), tetrahydrofuran/water (1:1, v/v), 5 °C, 2.5 hrs (49 % over two steps)[195]; c. Sodium iodine (1.5 equiv.), acetone, room temperature, 48 hrs, (38 %).

Thereby the first reaction was monomethylation of pentaethylene glycol using sodium hydride (1.1 equiv.) and methyl iodine (1.0 equiv.) in THF. This S_N2 reaction led to the alcohol **102,** which was further reacted to the iodine compound **104** via two steps by the methods described above.

5.2.9 Alkylation of the open-chain sugar moiety

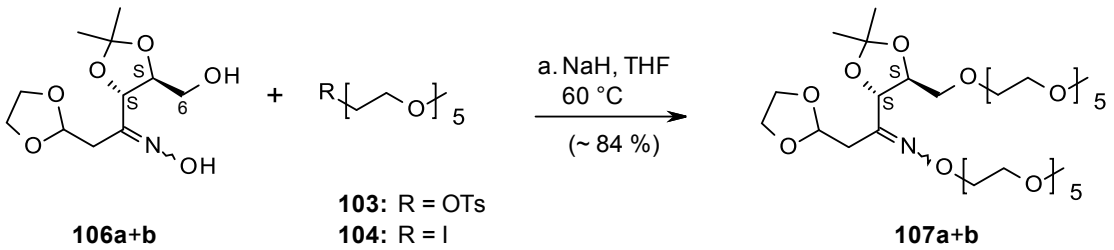
Before the alkylation could be carried out, the hydroxyl group had to be deprotected. This was done by base catalysed ester hydrolysis of **97a**+**b** and was first achieved with potassium carbonate (K_2CO_3) in MeOH, using known standard protocols for the hydrolysis of a benzoyl-group[197-200]. The deprotection was also accomplished with sodium hydroxide in THF/H_2O, this method was preferred later as standard method for the synthesis of **106a**+**b**.



97a+**b** **106a**+**b**

Figure 33│**Hydrolysis of the benzoyl group**. Reagents and conditions: a. Aqueous NaOH (2 N, 1.5 equiv.), tetrahydrofuran/water (1:1, v/v), 60 °C, 18 hrs (81 %)

In the next step the oxyethylene ether was obtained by reacting oxyethylene-halide **104** or oxyethylene-tosylate **103** via a nucleophilic substitution with the oximes **106a**+**b**. Thereby the alkoxide (RO^-) was prepared in situ from ROH with sodium hydride (95 %) in an anhydrous aprotic solvent such as tetrahydrofuran or dimethylformamide. Due to the two hydroxyl groups of the molecule at least two equivalents of sodium hydride were necessary for deprotonation, in practice three equivalents of sodium hydride yielded the best results.



106a+**b** **103:** R = OTs **104:** R = I **107a**+**b**

Figure 34│**Alkylation of the open-chain sugar moiety**. Reagents and conditions: a. Sodium hydride (95 %, 3.0 equiv.), tetrahydrofuran, 60 °C, 5 hrs, (~ 84 %).

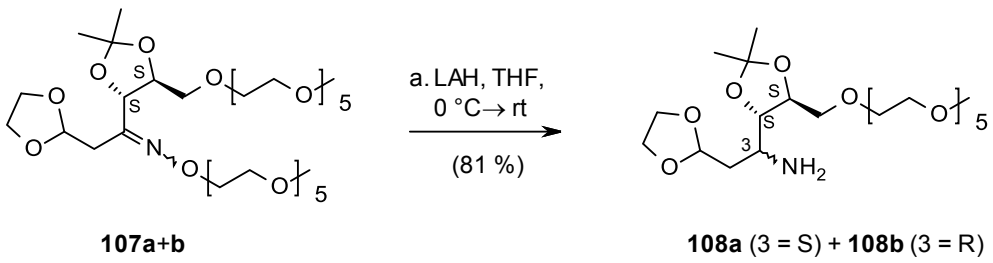
A selective alkylation of the primary hydroxyl group at C-6 of the oximes **106a**+**b** could not be accomplished, since the oxime group showed a higher reactivity towards the alkylating reagent, than the hydroxyl group. For this reason only the double alkylated products **107a**+**b**

were isolated in this reaction step (fig. 34). However, the double alkylation had no impact on the further synthesis since the oxime group was reduced in the subsequent step.

During the investigation of this reaction both alkylation agents (R-I and R–OTs) were used under different reaction conditions, but no difference with respect to the yield could be observed. Concerning the solvents, the reactions in THF showed comparative results to those made in DMF, due to the ease of handling THF was the solvent of choice for this alkylation step.

5.2.10 Reduction and protection of the nitrogen functionality

In this reduction step a new stereocenter at C-3 was created. To obtain the daunosamine characteristic (S)-configuration, the reduction of the oxime was tried with various complex metal hydrides (LAH, Red-Al®, DIBAL, $NaBH_4$, Borane tetrahydrofuran complex, L-selectride®). Our consideration that the steric demand of the hydride reagent influences the diastereoselectivity of the reaction could not be confirmed. Only with LAH and Red-Al® a reduction could be achieved, but a complete diastereoselectivity in the desired sense could not be obtained. The best result concerning yield and stereoselectivity was achieved with Lithium aluminium hydride (LAH) in tetrahydrofuran giving 81 % of **108a**+**b** in a 58:42 diastereomeric ratio at C-3 (fig. 35).

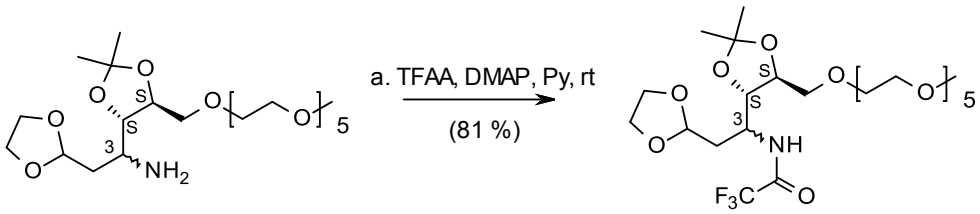


107a+b **108a** (3 = S) + **108b** (3 = R)

Figure 35│**Reduction of the oxime group**. Reagents and conditions: a. Lithium aluminium hydride (3.3 equiv.), tetrahydrofuran, room temperature, 12 hrs (81 % of **108a**+**b**, 58**:**42 diastereomeric ratio at C-3 as inseparable mixture)

More than three equivalents of lithium aluminium hydride were necessary to obtain a full reduction to the amines **108a**+**b**, which suggested that only the first hydride atom of LAH was reactive enough to achieve an oxime reduction in this case. The amount of LAH could be reduced by heating the reaction. However, this led to the loss of the anyway low stereoselectivity. The ratio of the two isomers formed in the reaction was determined by quantitative ^{1}H-NMR spectroscopy. As chromatographic separation was not possible at this stage, the diastereomeric mixture was used for further synthesis.

In view of the applied protection step, a detailed study of the glycosylation literature[201-206] showed that the coupling of unprotected or protected aglycons can efficiently be done with sugar derivatives, the amino and hydroxyl groups of which are protected with a trifluoroacetyl group. This protective group has the advantage that it can be cleaved after glycosylation under alkaline conditions without cleavage of the glycoside bond. For this reason we decided to use trifluoroacetyl as protecting group.



108a (3 = S) + **108b** (3 = R) **109a** (3 = S) + **109b** (3 = R)

Figure 36│**Trifluoroacetylation of the amino group**. Reagents and conditions: a. Trifluoroacetic anhydride (1.5 equiv.), 4-dimethylaminopyridine (0.02 equiv.), pyridine, room temperature, 4 hrs (81 % of **109a**+**b**, 58**:**42 diastereomeric ratio at C-3 as inseparable mixture).

The protection of the amino group was achieved with trifluoroacetic anhydride in pyridine, using standard protocols[207,208], to give the trifluoroacetamides **109a**+**b** in appropriate yield (fig. 36). Again no satisfactory separation of the two diastereomers was possible at this reaction stage, therefore the diastereomeric mixture of **109a**+**b** was used for the subsequent ring closure reaction.

5.2.11 Ring closure reaction

For the ring closure the aldehyde protecting group (1,3-Dioxolane) and the diol protecting group (Isopropylidene Acetal) had to be cleaved. Various attempts using aqueous acetic acid[209,210], hydrochloric acid[188,211-213], *p*-Toluenesulfonic acid[214,215] and ion-exchange resins (AMBERLITE® and DOWEX®)[216,217] failed and led to decomposition or only partial deprotection of the starting materials **109a**+**b**. A complete deprotection was first achieved by acid-catalyzed hydrolysis with trifluoroacetic acid in a mixture tetrahydrofuran/water, forming ethylene glycol and acetone as by-products[186].

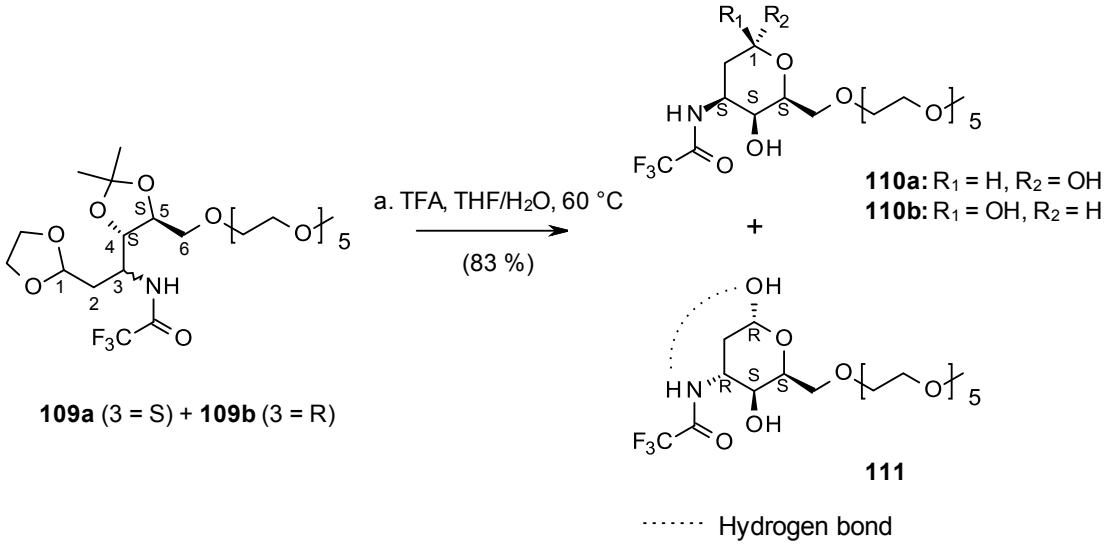


Figure 37│**Ring closure reaction**. Reagents and conditions: a. Trifluoroacetic acid, tetrahydrofuran/water (4:1, v/v), 60 °C, 30 min (48 % of **110a**+**b** in a 76:24 diastereomeric ratio at C-1 as inseparable mixture, 35 % of **111**).

During the ring closure reaction of **109a**+**b** a new asymmetric carbon atom was formed, that resulted in three different products, **110a**, **110b** and **111**. All three amino sugars formed an intramolecular hemiacetal by reaction of the C-5 hydroxyl group with the C-1 aldehyde group, which led to a six-membered pyranose ring, which is thermodynamically favoured. A five-membered furanose ring caused by the reaction of the C-4 hydroxyl group with the C-1 aldehyde group was not observed. The C-3 diasteriomeric mixture of the amino group could be first separated at this reaction step, done by column chromatography on silica gel, with DCM/MeOH (100:3→100:5→10:1, v/v) to elute first **111** (35 % yield) and then a mixture of the two anomers **110a**+**b** in a 76:24 (48 % yield), all substances as clear oils. Amino sugar **111** was purely isolated in the alpha configuration, which most likely was formed due to a hydrogen bond stabilization between the C-1 hydroxyl group and the C-3 amino group. The determination of the relative configuration of the amino group was done by nuclear Overhauser effect spectroscopy (NOESY) of each isolated isomer (fig. 38).

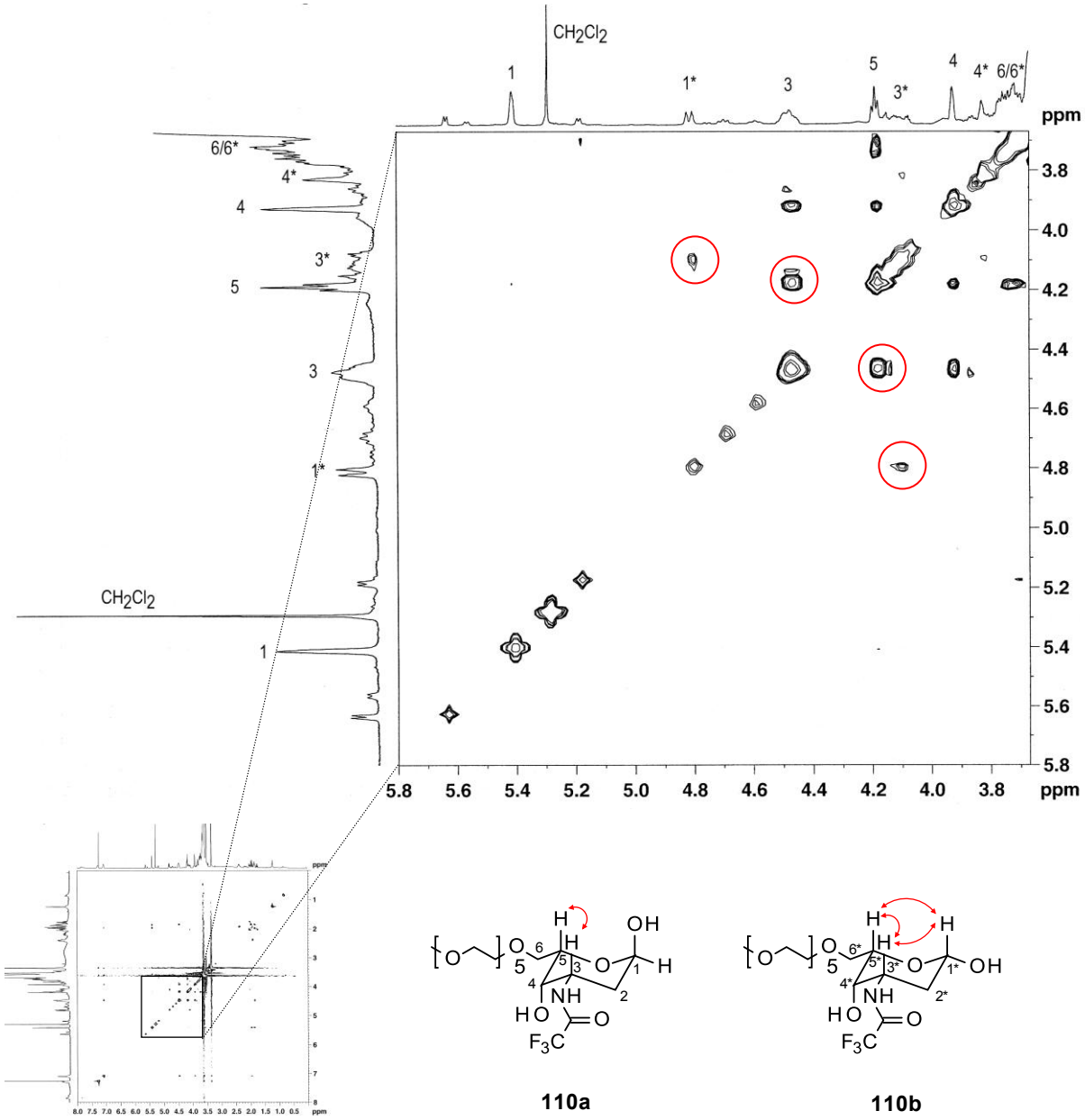


Figure 38│**NOESY of 110a+b**. Reagents and conditions: Solvent = deuterated chloroform ($CDCl_3$), mixing time = 2.0 seconds

Amino sugar **110a** showed a NOE-effect between the protons on C-3 and C-5. Under this stereochemical configuration (C-3 = S and C-5 = S) the hydrogen atoms were close enough to show cross peaks. Amino sugar **110b** showed a NOE-effect between the protons on C-3* and C-1*, cross peaks between Protons on C-3* and C-5* as well as C-1* and C-5* are not visible in figure 38 since the proton at C-5* has the same chemical shift as the oxyethylene side chain which led to a signal overlap (see fig. 39).

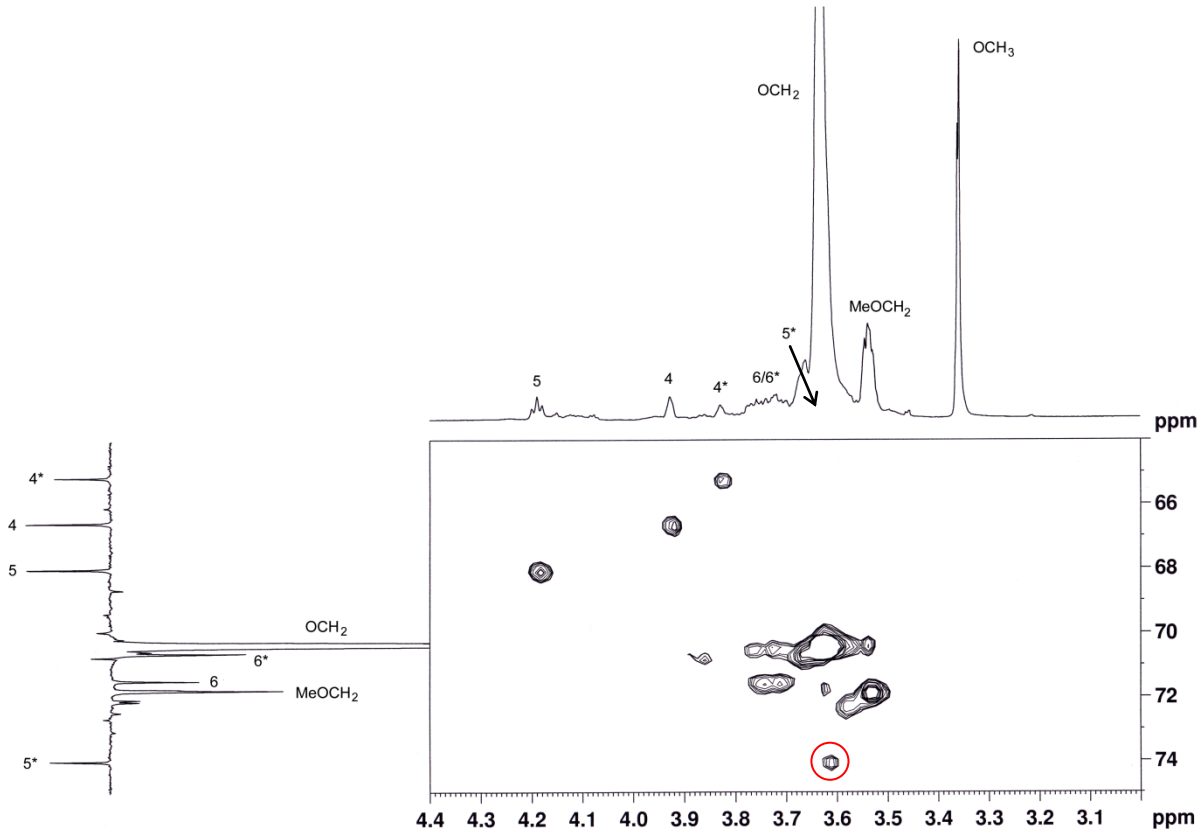


Figure 39 | 2D ^{1}H,-^{13}C heteronuclear singlequantum correlation (HSQC) spectrum of 110a+b. Reagents and conditions: Solvent = Deuterated chloroform ($CDCl_3$)

5.3 Alternative synthetic approaches towards the synthesis of daunosamine and its 6'-derivatives

During the optimisation process it was tried to reduce the number of synthesis steps by changeovers in the synthesis sequence. The first alternative sequence is shown in fig. 40 (blue). Instead of the reduction and benzolyation of the hydroxyamide **88**, the alkylation with the oxyethylene-tosylate was already carried out at this stage to evade the otherwise necessary transient protection of the hydroxyl group of hydroxyamide **88**. In the subsequent reduction of the diethylamino group the yield was equal to the reduction in the original synthesis sequence. The yield of the following Grignard reaction, however, was with 43 % far less than the equivalent reaction in the original synthesis. For this reason this synthetic approach was not continued.

Another synthetic approach with the potential to shorten the original synthesis by 3 steps to give an N-benzyl-L-amino sugar as final product was not successful (fig. 40, red). Addition of benzylamine to the aldehyde **92** in presence of anhydrous Na_2SO_4 gave the secondary aldimine **115**. But we did not succeed in the subsequent Grignard reaction with 2-Bromomethyl-1,3-dioxolane, although literature[218,219] describes highly stereoselective results for a similar reaction, using an aldimine (derived from glyceraldehyde) and vinylmagensium bromide.

In addition to the synthesis of 6'-oxyethylene-daunosamine **110a**+**b**, also a novel synthesis for conventional daunosamine was developed, starting from oxime **106a**+**b** (fig. 40, green). O-tosylation with *p*-toluenesulfonyl chloride and sodium hydroxide in THF/H_2O gave the ditoslyates **117a**+**b**. In the subsequent reduction step with LAH in THF, equivalent to the synthesis of 6'-oxyethylene-daunosamine a new stereocenter was created in a 60:40 (S:R) diastereomeric ratio at C-3, additionally the daunosamine characteristic 6-deoxy functionality is formed by transfer of a hydride ion. After chromatographic separation of the diasteriomeric mixture **118a**+**b**, the S-configurated compound **118a** was used for further synthesis. Protection of the amino group with trifluoroacetic anhydride in pyridine gave **119**. Subsequent deprotection and ring closure under the same conditions as described in chapter 5.2.11 gave N-trifluoroacetyl-α/β-L-daunosamine **120a**+**b** in 13 steps in a 5.3 % overall yield starting from (2R,3R)-tartaric acid. By combining different NMR techniques, such as APT, COSY, HSQC, HMBC and NOE difference spectroscopy, the target compounds and their precursors could be unambiguously characterized.

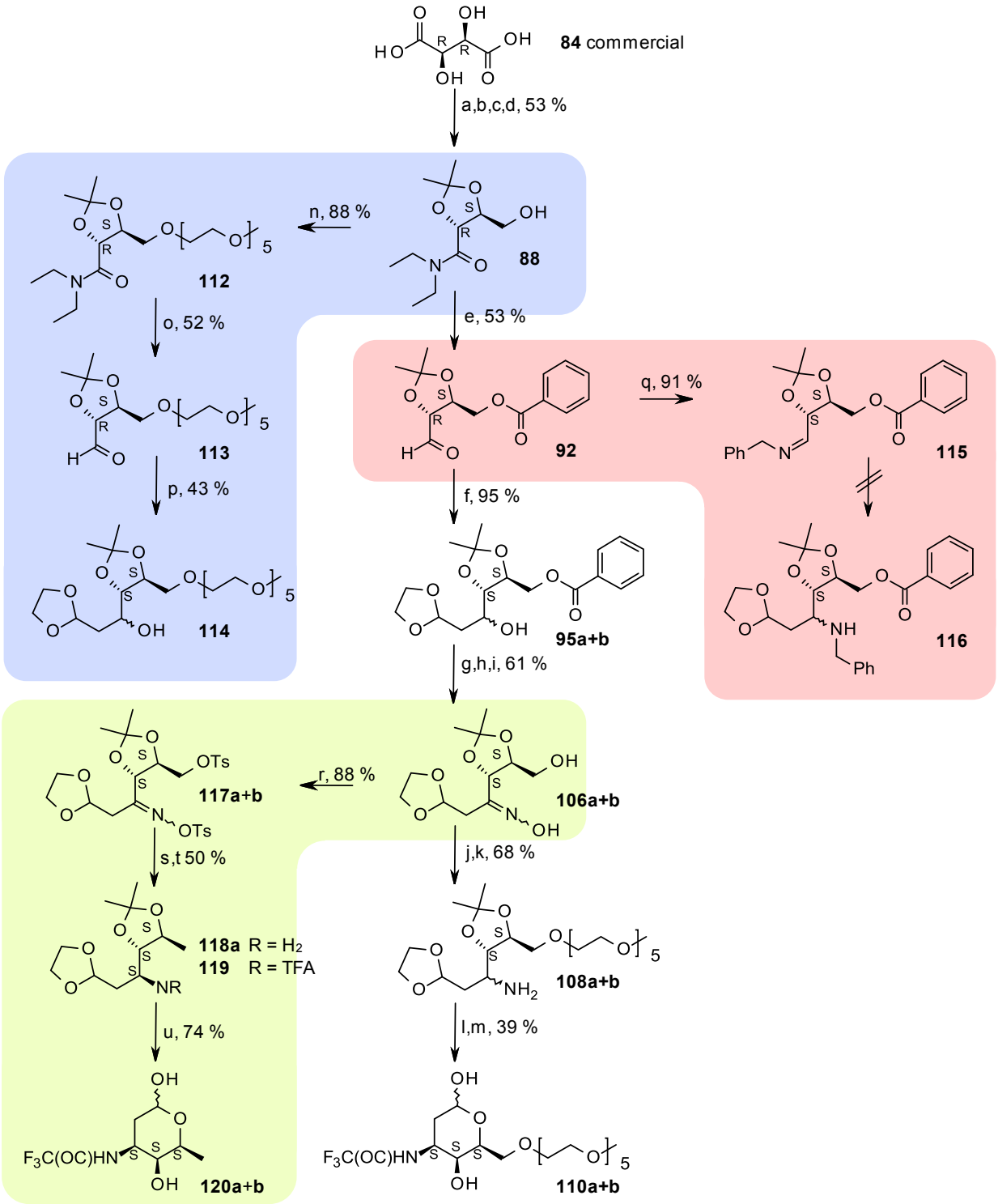


Figure 40 | Synthesis strategies towards N-trifluoroacetyl-L-daunosamine and its 6'-derivatives. Reagents and solvents: a. Ac_2O, H_2SO_4; b. $HNEt_2$, $CHCl_2$; c. H_2SO_4, MeOH, DMP, $(CH_3)_2CO$; d. $NaBH_4$, THF; e. Red-Al, toluene/MTBE, BzCl, MTBE, NaOH; f. 2-Bromomethyl-1,3-Dioxolane, Mg, I_2, THF; g. $C_2Cl_2O_2$, DMSO, DCM, Net_3; h. NH_2OH, Py; i. NaOH, THF/H_2O; j. NaH, Methoxy-OEG-Ts (**103**), THF; k. LAH, THF; l. TFAA, DMAP, Py; m. TFA, H_2O/THF (incl. diasteriomeric separation); n. NaH, Methoxy-OEG-Ts (**103**), THF; o. Red-Al, toluene; p. 2-Bromomethyl-1,3-Dioxolane, Mg, I_2, THF; q. $BnNH_2$, Na_2SO_4, Et_2O; r. TsCl, NaOH, THF/H_2O; s. LAH, THF (incl. diasteriomeric separation); t. TFAA, DMAP, Py; u. TFA, H_2O/THF

5.4 Synthesis of dauno- and doxorubicinone

5.4.1 Methanolysis of daunorubicin hydrochloride

A short and effective way to generate daunorubicinone **122** in good yield was the cleavage of the α-glycosidic bond of commercially available daunorubicin hydrochloride **121**. Several procedures have been described for the hydrolysis of the glycosidic bond between the amino sugar and the aglycon using hydrochloric acid[220-222]. We selected another effective procedure first published in 1998[203]. In this synthesis hydrochloric acid was produced in situ out of acetyl chloride and methanol. The resulting daunosamine methyl ether **123** precipitated in methanol and could be filtered off easily. After evaporation of all solvents, daunorubicinone **122** could be recrystallized from diisopropylether in 87 % yield. The purity was sufficient to go for the next steps without any further purification.

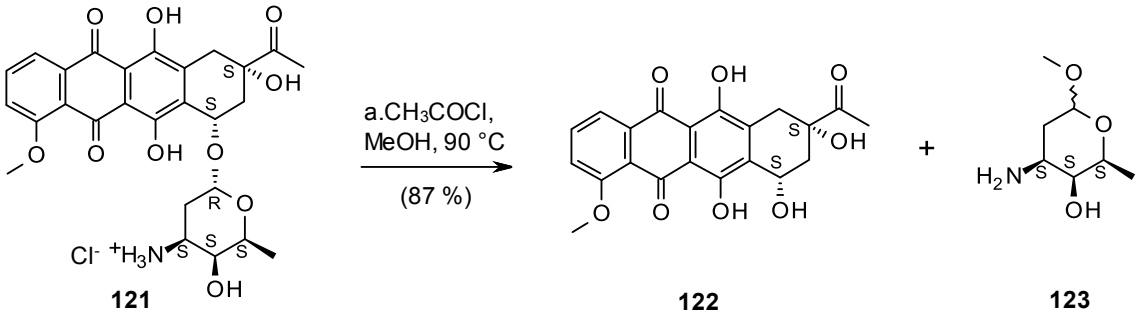


Figure 41 | Methanolysis of daunorubicin hydrochloride. Reagents and conditions: a. Acetyl chloride (5.6 equiv., methanol, 90 °C, 1.5 hrs (87 %).

5.4.2 Conversion of dauno- to doxorubicinone

For the conversion of daunorubicinone **122** to doxorubicinone a hydroxyl group had to be added at C-14. This hydroxyl group addition was first performed by Arcamone et al.[223,224] The synthesis included a bromination of daunorubicin followed by direct hydrolysis or via a carboxylate. A further development of the synthesis was successive acetalization and bromination of the aglycon, published by Kimura et al.[201] All these syntheses on aglycons, in which the sugar is already attached, show a relatively low yield mainly caused due to side reactions, such as partial cleavage of the sugar moiety during synthesis[203].

For this reason we decided to attach the hydroxyl group before the glycosylation reaction. Bromination and subsequent acetoxylation of an anthracyclin aglycon (4-Demethoxydaunomycinone) was first described by Tomato et al.[225] using pyridinium tribromide in THF followed by substitution with potassium acetate in acetone. Other methods for aglycone modification make use of elemental bromine for halogenation and sodium

acetate or potassium acetate for acetoxylation[202,203,222,226-229]. Figure 42 shows conditions and results of our synthesis which were mainly based on the publication of van der Rijst et al.[203]

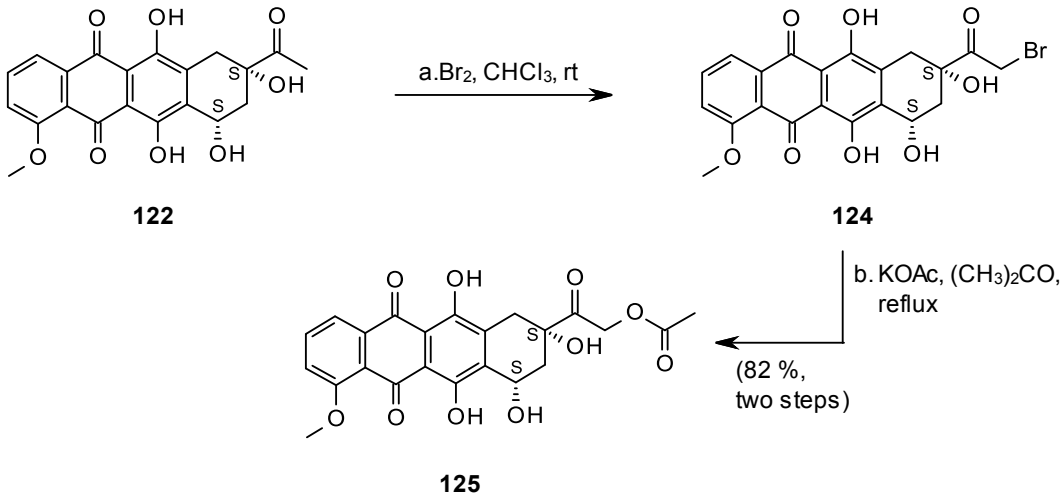


Figure 42 | Aglycone modification. Reagents and conditions: a. Bromine (2.5 equiv.), chloroform, room temperature, 18 hrs; b. Potassium acetate (22.0 equiv.), acetone chloride, reflux, 5 min (82 %, over two steps).

First step in the procedure was the halogenation of the methyl group with Br_2 in chloroform at room temperature, the resulting bromide **124** which was insoluble in chloroform, precipitated and could be filtered off. The bromide was then dissolved in acetone, and an excess of potassium acetate was added, which led under reflux to a nucleophile substitution through a carboxylate anion gave doxorubicinone acetate **125**. The crude product was then filtered through a short silica-gel column, eluting with DCM/MeOH to give doxorubicinone acetate **123** over two steps as a red solid in 82 % yield. Since the hydroxyl group of **125** was protected as acetate, it could be used directly for the following glycosylation.

5.5 Glycosylation

In the glycosylation reaction of anthracyclines a functionalized carbohydrate component (glycosyl donor) is attached to an anthracyclinone (glycosyl acceptor). Several methods have been reported in recent decades. At the beginning of anthracycline synthesis in the 1970s the glycoside formation has been carried out by the reaction of the C-7 hydroxyl group of an anthracyclinone with 1'- halogenated amino sugar derivatives (glycosyl chloride or bromide) using mercury salts (Koenigs – Knorr methodology)[230,231] or silver trifluoromethanesulfonate[232]. The main drawbacks of these methodologies are the use of toxic reagents (Hg(II) salts) and the instability of 1'- halogenated amino sugars which have to be used for glycosylation immediately after the synthesis[233]. A further method for the glycosylation reaction is the coupling of a glycal, derived from an amino sugar derivative, with

an anthracyclinone under acidic catalysis with *p*-toluenesulfonic acid[234]. In this method, it is reported that glycosylation gives anomeric mixtures of α- and β-glycosides. This lack of stereoselectivity is important in the context, as β-glycosides have a much lower biological activity than α-glycosides[223,230] and are therefore undesirable byproducts, which must be separated by chromatography. A solution of these problems was published by Kimura et al.[205] using 1-O-acyl-L-daunosamine derivatives as glycosyl donors which were attached to optically pure 4-demethoxyanthracyclinones in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as promoter for the glycosylation reaction. This novel method showed the synthesis of α-glycosides in almost quantitative yield.

Investigations of the stereochemistry in the glycosylation reaction were published by Dejter-Juszynski and Flowers et al.[235-238] They described the steric control of 4-O-acyl groups of related sugars, which exclusively gave trans C-4, C-1 products. The reaction mechanism in the glycosylation of anthracyclines was first described by Smith et al.[239] Referring to the work of Dejter-Juszynski and Flowers, Smith et al. explained the glycosylation reaction through an intermediate cationic species. The same cationic species was mentioned by Kimura et al.[201,205], established by treatment of acylated ($COCF_3$, $COPhNO_2$) daunosamine derivatives with trimethylsilyl trifluoromethanesulfonate (TMSOTf) in a mixture of dichloromethane and diethyl ether (fig. 43). The C-7 hydroxyl group of an anthracyclinone can only attack from top face to the cationic species **127a**+**b**, which will lead exclusively to the desired α-glycosides.

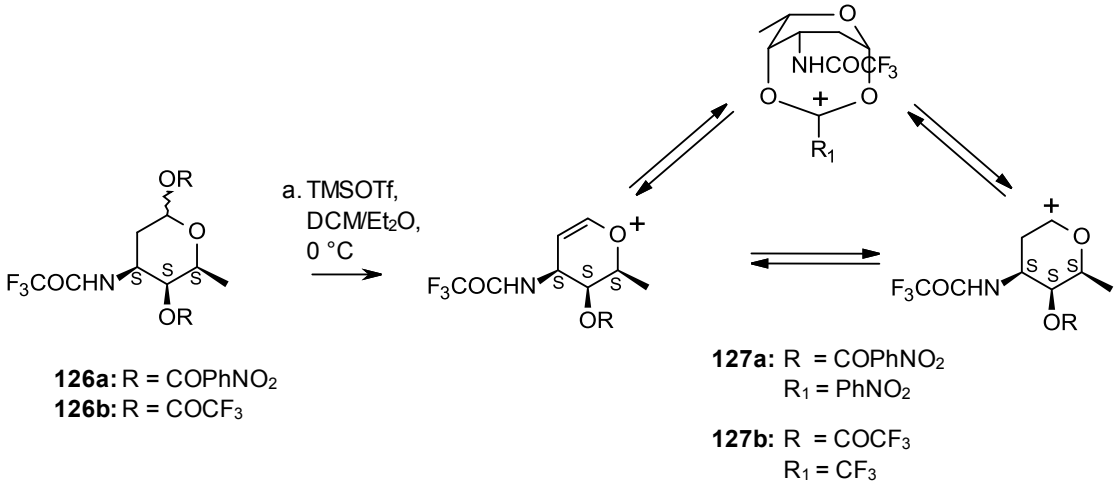


Figure 43 | Formation of the cationic species 127a+b out of the 1,4-di-O-acyl daunosamine derivatives 126a+b[201]. Reagents and conditions: a. Trimethylsilyl trifluoromethanesulfonate, 4Å molsieve, dichloromethane/diethylether (1.2:1, v/v), 0 °C, 1 hr.

The presence of ether in the glycosylation reaction is quite important, it might be explained that ether can effectively stabilize cationic species by chelation. Investigations of the

glycosylation reactions under exclusion of ether resulted in a dramatic decrease of the yield[201].

A further effect which supports the formation of α-glycosides is the anomeric effect that exhibits a greater preference for the axial orientation of the glycosidic bond. It is explained by a binding interaction of the unshared electron pair on the endocyclic oxygen with the δ* - orbital of the axial C–O bond[196].

Based on the promising results of Kimura et al.[201,205], we chose this methodology as basis for the glycosylation of the amino sugar derivatives **110a**+**b** with the anthracyclinones **122** and **125**. Therefore the amino sugars **110a**+**b** were functionalized with a suitable leaving group at the anomeric position. Treatment of **110a**+**b** with trifluoroacetic anhydride (TFAA) in diethylether under standard conditions[230] led to the *N,O*-trifluoroacetyl derivatives **128a**+**b** as a mixture of both anomers. However, the two anomers showed low stability, partially elimination of trifluoroacetic acid at the anomeric center resulted in correspondent glycal **129** (fig. 44).

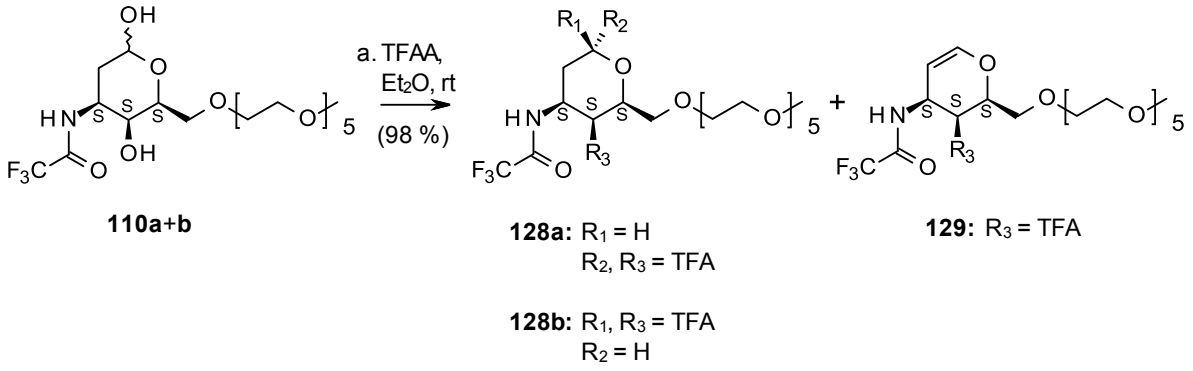


Figure 44│**Trifluoroacetylation of the amino sugars 110a+b.** Reagents and conditions: a. Trifluoroacetic anhydride (excess), diethylether, room temperature, 3 hrs (98 % of **128a**+**b** and **129**)

The attempt to glycosylate the inseparable mixture **128a**+**b**/**129** with daunorubicinone **122** failed under the standard conditions described by Kimura et al.[201]

So we changed strategy and decided to use the *p*-nitrobenzoyl group for the functionalization of the amino sugar derivative. Treatment of **110a**+**b** with 4-Nitrobenzoyl chloride in pyridine under the conditions described by Smith et al.[239] led to the desired 1,4-di-O-acyl derivatives **130a**+**b** (fig. 45) in a 76:24 anomeric ratio (determined by ^{1}H-NMR spectroscopy). Separation of the two anomers **130a**+**b** was possible using column chromatography on silica gel, with EtOAc/PE (1:1→3:2→2:1, v/v) to give in order of eluting the α-O-acyl-derivative **130a** and the β-O-acyl-derivative **130b** as white resins.

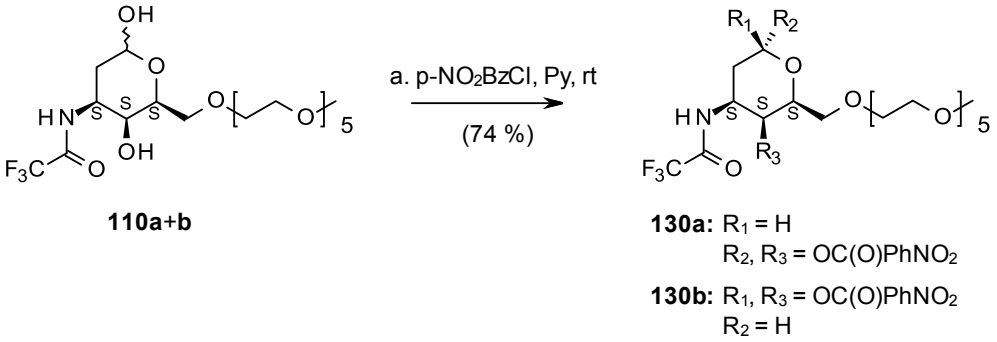


Figure 45│**Functionalization of 110a+b with 4-Nitrobenzoyl chloride**. Reagents and conditions: a. 4-Nitrobenzoyl chloride (2.8 equiv.), pyridine, room temperature, 14 hrs (56 % of **130a**, 18 % of **130b**)

For the following glycosylation reaction both anomers were used, as mixture or as optically pure substances, showing no differences in reactivity or yield. The claim that β-O-acyl amino sugar derivatives react preferentially with the anthracyclinones in the glycosylation reaction published by Kolar et al.[240] could not be confirmed with our substances **130a+b**.

5.5.1 Coupling of the sugar moiety with daunorubicinone

The glycosylation of sugars **130a+b** with daunorubicinone **122** was following in principle those procedures published by Kimura et al.[201] and Matsumoto et al.[202] TMSOTf was added to a mixture of the functionalized anomers **130a+b** and molecular sieve in a mixture of dichloromethane/diethylether in an ice bath.

After one hour a solution of **122** in tetrahydrofuran was added and the resulting solution was then stirred between -10 °C and -15 °C. Aglycone **122** was poorly soluble in dichloromethane for this reason dichloromethane was displaced by tetrahydrofuran in which aglycone **122** showed good solubility. After about 6 hrs, TLC analysis (DCM/MeOH, 100:5, v/v) showed a nearly complete consumption of **122** and the reaction was quenched by addition of aqueous saturated $NaHCO_3$. After work-up the purification by column chromatography on silica gel with DCM/MeOH gave in order of eluting unreacted aglycone **122** (19 %) and the desired α-glycoside **131** (68.8 %) as a red solid (fig. 46).

The preparative separation on silica gel with a DCM/MeOH gradient of increasing polarity turned out to be the best method. Chloroform which is often mentioned in older lab procedures should be avoided since hydrogen chloride is formed easily under the influence of light, which leads together with MeOH to cleavage of the glycosidic bond. The α-configuration of the glycosidic bond was determined by ^{1}H-NMR-spectroscopy, which showed a narrow doublet (2.9 Hz) at δ 5.69 ppm ($CDCl_3$) for the anomeric $C_{1'}$-proton. This result was consistent with the literature data for α-glycosidic linkage[221,241]. No undesired β-glycoside, which exhibits a double doublet was detected.

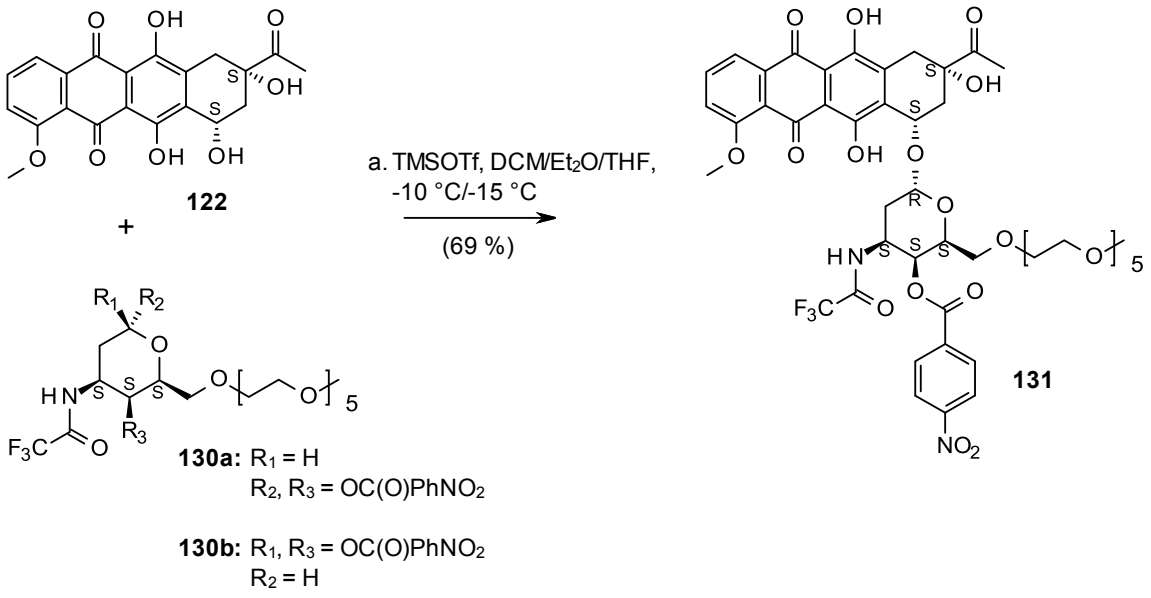


Figure 46│**Glycosylation of 130a+b with daunorubicinone 122**. Reagents and conditions: a. Trimethylsilyl trifluoromethanesulfonate (2.05 equiv.), 4Å molsieve, dichloromethane/diethylether/tetrahydrofuran (5:4:3, v/v), -10 °C/-15 °C, 6 hrs (69 %).

Prolongation of the reaction time did not lead to a complete conversion to the desired product. Also an increase of the sugar to aglycone ratio, changes in the reaction temperature and in the composition of the different solvents gave no better results than those described. The molecular sieve seemed to play an important role in the glycosylation reaction. Reactions in which molecular sieve was omitted showed no, or only poor, formation of the desired product. It has been also reported that the physical state of the molecular sieve exerts an influence on the yield in the glycosylation reaction[204].

5.5.2 Cleavage of the protective groups

The cleavage of the 4-O-*p*-Nitrobenzoyl group and the N-trifluoroacetyl group was done under basic conditions following the procedure of Broadhurst et al.[241] with the difference that the concentration of the aqueous sodium hydroxide was 1.0 N instead of 0.1 N. Deprotection with 0.1 N aqueous sodium hydroxide did not lead to full conversion of the starting material. Glycoside **131** was solved in a mixture of dichloromethane and methanol (100:1). Subsequently an aqueous solution of sodium hydroxide (1.0 N) was added at 0 °C. Under basic conditions the colour of the reaction mixture changed from light red to deep purple and the solution was then stirred for 30 min. The reaction was neutralized with pure acetic acid until the colour of the reaction mixture became light red again. After work-up the crude product was purified by column chromatography on silica gel with DCM/MeOH (100:4, v/v) to give **132** in 90 % yield as orange-red solid (fig. 47).

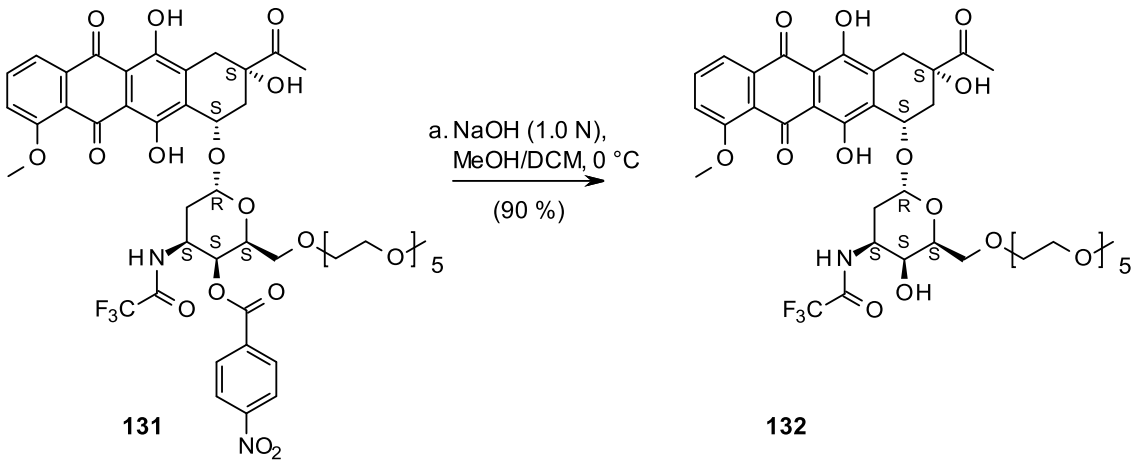


Figure 47 | Cleavage of the 4-O-p-Nitrobenzoyl group. Reagents and conditions: a. aqueous solution of sodium hydroxide (1.0 N), dichloromethane/methanol (100:1, v/v), 0 °C, 30 min (90 %).

The cleavage of the N-trifluoroacetyl group required stronger basic conditions. It was done in pure aqueous sodium hydroxide (1.0 N) stirred at room temperature for 30 min.

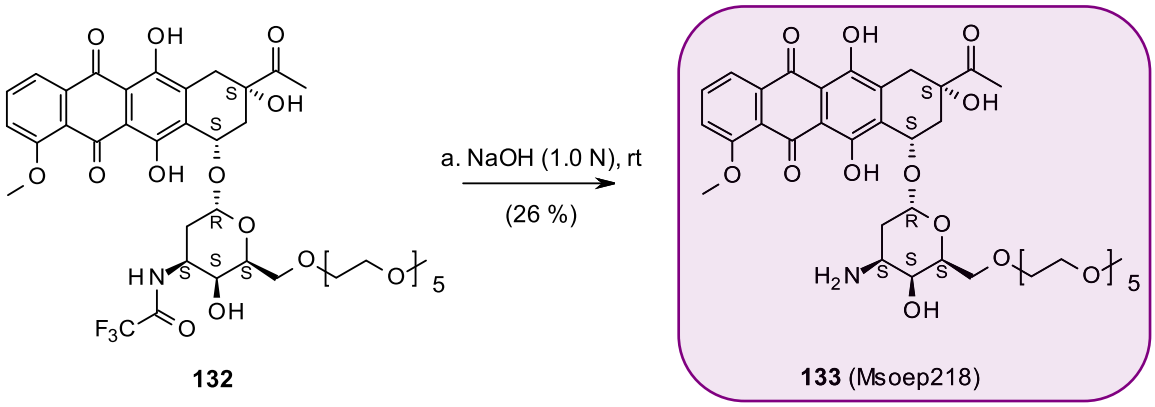


Figure 48 | Cleavage of the N-trifluoroacetyl group. Reagents and conditions: a. aqueous solution of sodium hydroxide (1.0 N), room temperature, 30 min (26 %).

For neutralisation hydrochloric acid (1.0 N) was added to the deep purple solution until pH 8 was reached, to accomplish that the C-3 amino group is not protonated anymore. The extraction from the aqueous phase was done with $CHCl_3$ in which glycoside **133** showed a good solubility caused by the oxyethylene side chain. Conventional daunorubicin **5** is hardly soluble in $CHCl_3$. The crude glycoside **133** was then purified by column chromatography on silica gel eluting with DCM/MeOH (10:1→10:2, v/v) to give **133** (msoep218 = protocol name) in 26 % yield as orange-red solid (fig. 48). The relatively low yield of 26 % was caused by column chromatography, because glycoside **133** showed a strong interaction with silica gel, which made it difficult to achieve a complete elution without destruction of the product.

To determine the purity of the novel anthracycline glycoside **133** in addition to NMR spectroscopy a reversed-phase HPLC method was established in parallel to the synthesis, based on a method published by Badea et al.[242] for conventional anthracyclines. An aqueous solution (pH 2.00) containing 0.4 % sodium dodecylsulfate mixed with methanol/acetonitrile (1:1) in a 40/60 ratio was used as mobile phase. The detection was done at λ = 254 nm, reference λ = 360 nm. The exact method is described in the experimental part. Figure 49 shows the reversed-phase HPLC-chromatogram of the novel anthracycline derivative **133** (msoep218 = protocol name) and figure 50 those of daunorubicin. Anthracycline derivative **133** showed an about two minutes longer retention time, in comparison to daunorubicin. Regarding the elution order of daunorubicin and the novel anthracycline derivative **133** (msoep218), under the conditions of a reversed-phase HPLC, an increased lipophilicity can be assumed. In addition a better overall solubility of **133** (msoep218) in aqueous media was achieved by OEGylation.

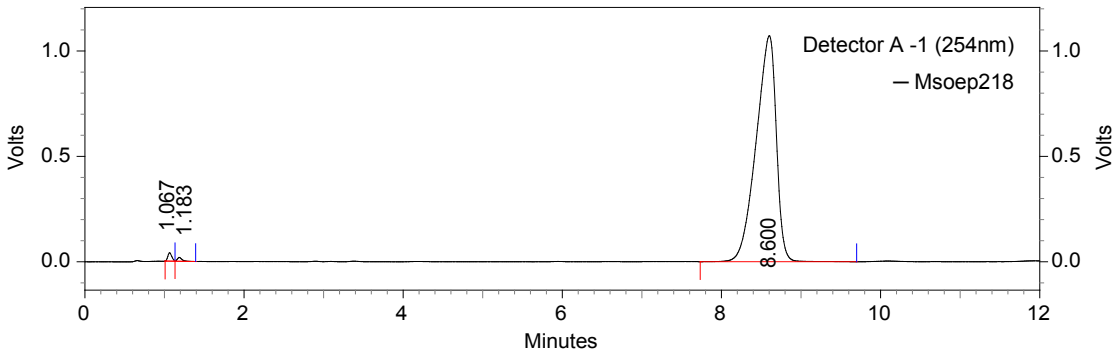


Figure 49 | HPLC-chromatogramm of anthracycline 133 (msoep218). Pk 1: Retention time = 1.067 min, area % = 0.731; Pk 2: Retention time = 1.183 min, area % = 0.392; Pk 3: Retention time = 8.600 min, area % = 98.878.

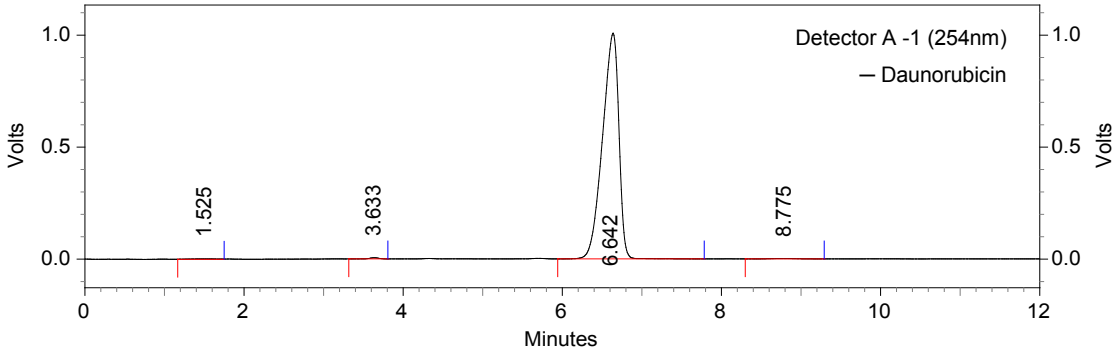


Figure 50 | HPLC-chromatogramm of daunorubicin. Pk 1: Retention time = 1.525 min, area % = 0.270; Pk 2: Retention time = 3.633 min, area % = 0.271; Pk 3: Retention time = 6.642 min, area % = 99.168; Pk 4: Retention time = 8.775 min, area % = 0.290.

The High-Resolution Mass-Spectrum of anthracycline **133** showed the [M+Na]+ ions as the most prominent species, followed by the $[M+H]^+$ and $[M+K]^+$ ions (fig. 51).

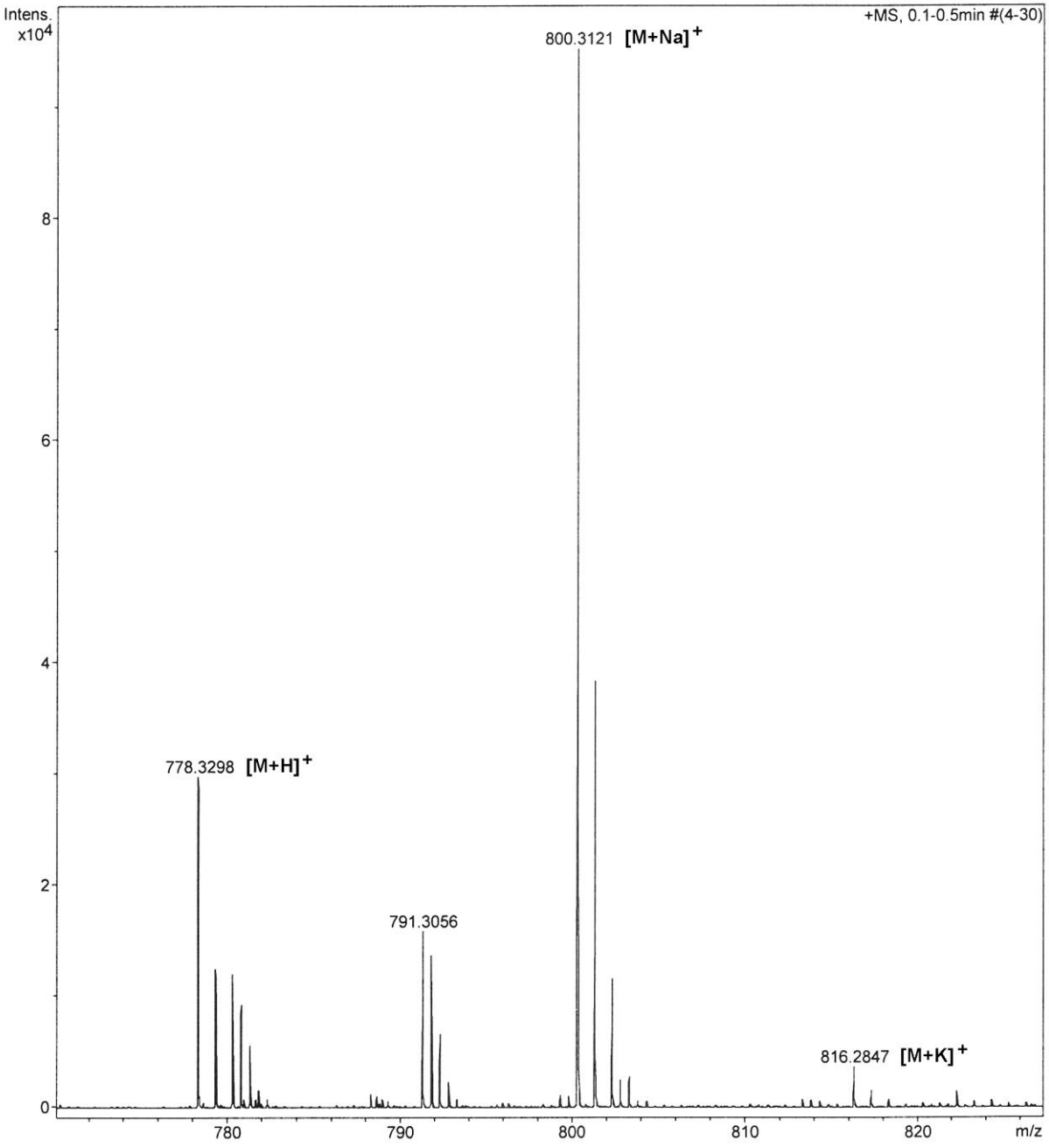


Figure 51 | HRMS spectrum of anthracycline 133 (msoep 218). Measured with MaXis, ESI-Qq-TOF-MS system with an accuracy of +/- 5ppm. Calculated for $[C_{38}H_{51}NO_{16}+H]^+$ 778.3286, found 778.3298; calculated for $[C_{38}H_{51}NO_{16}+Na]^+$ 800.3105, found 800.3121; calculated for $[C_{38}H_{51}NO_{16}+K]^+$ 816.2844, found 816.2847

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5.5.3 Coupling of the sugar moiety with doxorubicinone

The procedure for the glycosylation of the sugars **130a**+**b** with doxorubicinone acetate **125** was comparable to the glycosylation with daunorubicinone, with the difference that tetrahydrofuran was not necessary for dissolution of the aglycone and could be omitted (fig. 52).

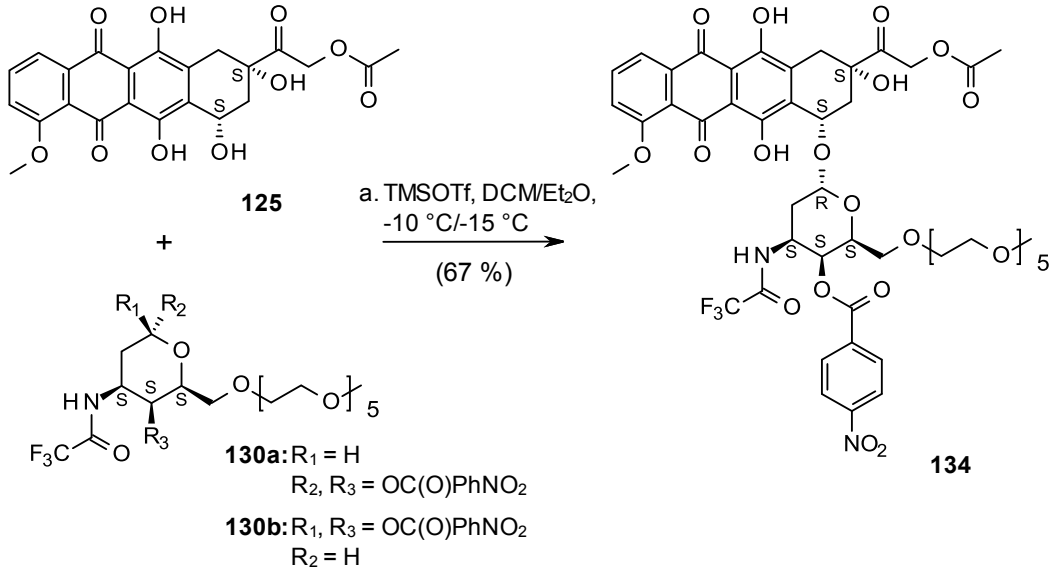
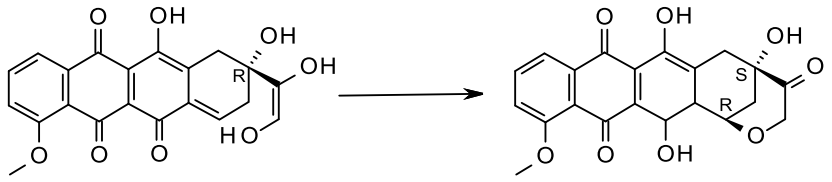


Figure 52│**Glycosylation of 130a+b with doxorubicinone acetate 125**. Reagents and conditions: a. Trimethylsilyl trifluoromethanesulfonate (2.3 equiv.), 4Å molsieve, dichloromethane/diethylether (2.8:1, v/v), -10 °C/-15 °C, 6 hrs (67 %).

After purification by column chromatography on silica gel, eluting with DCM/MeOH (1000:12, v/v) the desired α-glycoside **134** was obtained as a red solid in 66.6 % yield. However, again no complete conversion to the desired product was achieved. Unreacted starting material **125** was recovered in 14.6 % yield.

5.5.4 Cleavage of the protective groups

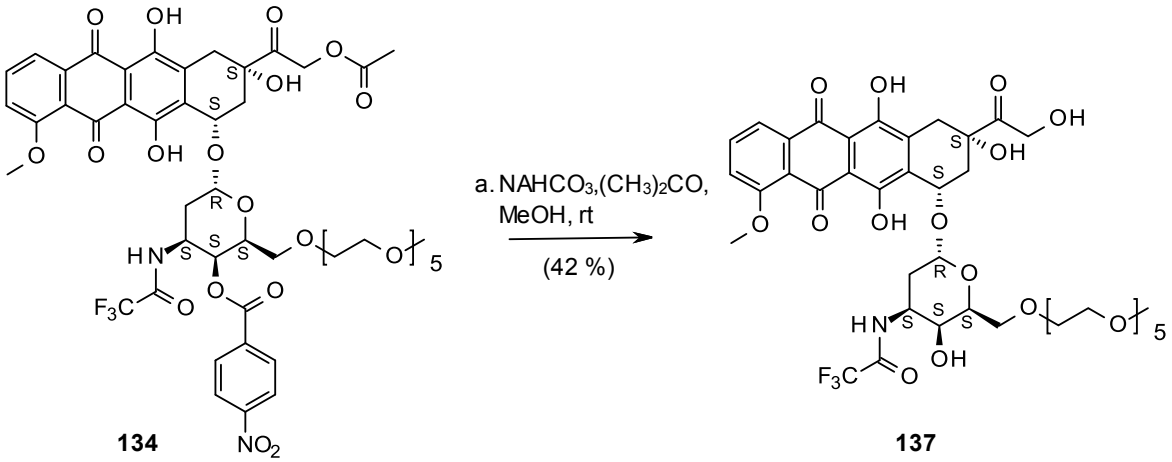
The cleavage of the protecting groups of α-glycoside **134** under the same basic conditions (aqueous sodium hydroxide in MeOH/DCM) as applied for glycoside **131** failed with glycoside **134**, giving a complex mixture of products with only a very small quantity of the desired product. The instability of doxorubicin in an aqueous solution of sodium hydroxide has already been described by Abdeen et al.[243] Also Kimura et al.[201] described this instability of compounds having a free hydroxyl group at C-14. During the degradation in aqueous sodium hydroxide first an enolate **135** was formed by elimination of the sugar moiety, which subsequently cyclised to a ring-A oxabicyclononanone **136** (fig. 53).



135 **136**

Figure 53｜ **Formation of the ring-A oxabicyclononanone 136.** Reagents and conditions: Aqueous solution of sodium hydroxide, room temperature.

Better results were achieved using aqueous $NaHCO_3$ in a mixture of acetone and methanol, simultaneous cleavage of the p-nitrobenzoyl group and the acetoxy group gave glycoside **137**. After purification by column chromatography on silica gel, eluting with DCM/MeOH (100:3, v/v) glycoside **137** was isolated in 41.5 % yield as a red solid. In comparison to the deprotection using sodium hydroxide, the use of $NaHCO_3$ proved to be much gentler and therefore did not lead to decomposition.



134 **137**

Figure 54｜**Cleavage of the 4-O-p-Nitrobenzoyl group and the acetoxy group.** Reagents and conditions: a. Aqueous saturated $NaHCO_3$, acetone/methanol (2:1, v/v), room temperature, 3 hrs (42 %).

Since the cleavage of the N-trifluoroacetyl group required even stronger basic conditions the base labile hydroxyl group at C-14 had to be protected first. Protection method via an acetonide as published by Van der Rijst et al.[203] was chosen for this purpose (fig. 55).

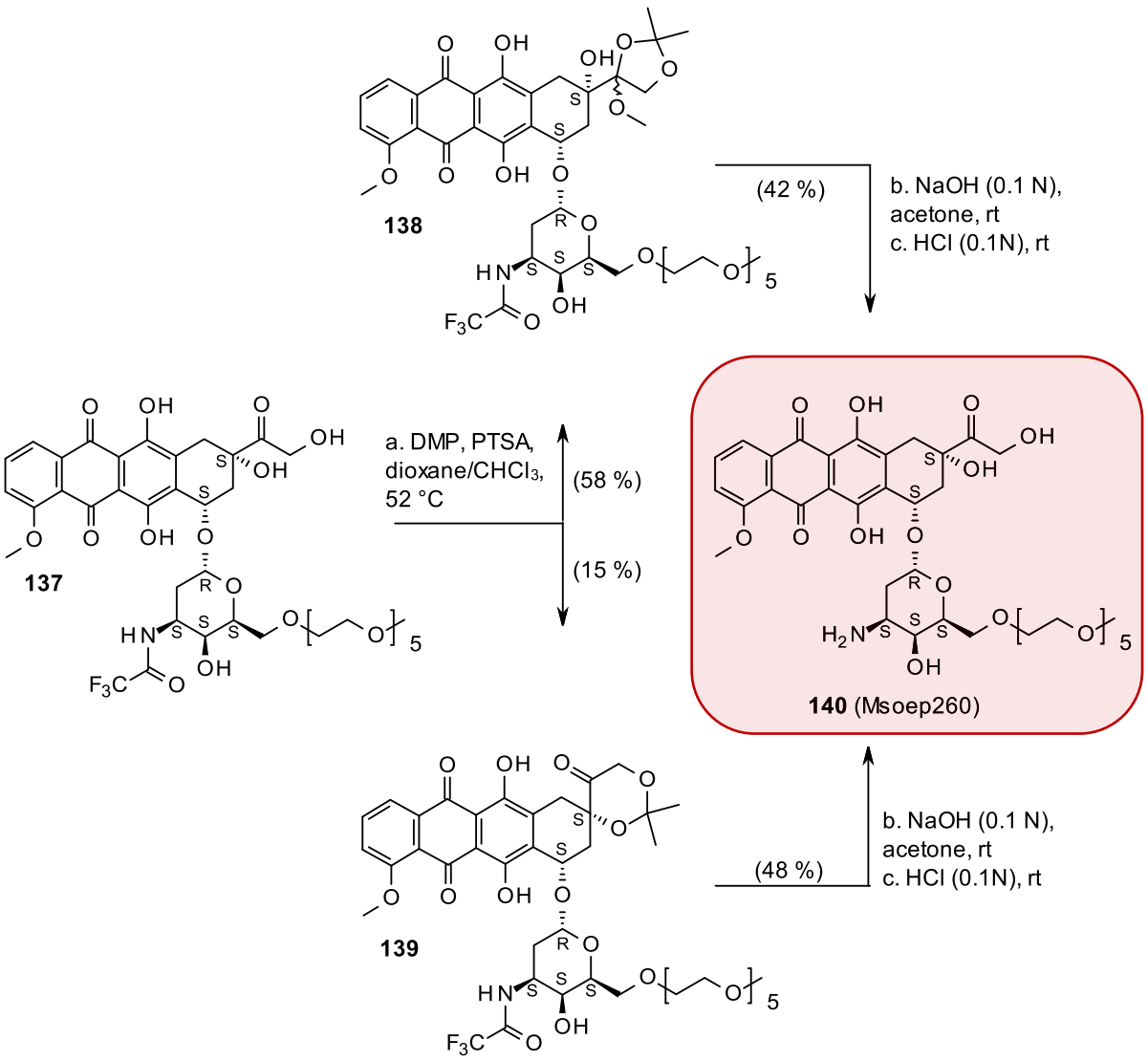


Figure 55 | Temporary acetonide formation for the cleavage of N-trifluoroacetyl group. Reagents and conditions: a. 2,2-Dimethoxypropane (excess), p-Toluenesulfonic acid, 1,4-Dioxane/chloroform (1:20, v/v), 52 °C, 22 hrs (58 % of **138**, 15 % of **139**); b. Aqueous solution of sodium hydroxide (0.1 N), acetone, room temperature, 30 min; c. Aqueous solution of HCl (0.1 N), room temperature 42 hrs (42 %, over two steps out of **138**, 48 %, over two steps out of **139**)

The glycoside **137** was treated with an excess of 2,2-Dimethoxypropane in a mixture of dioxane and chloroform with *p*-Toluenesulfonic acid as catalytic acid. The formation of the acetonide was a very slow reaction, a complete conversion was achieved stirring at 52 °C for 22 hrs. Higher reaction temperatures resulted in a complex mixture of products, which was not further investigated. In contrast to the results of Van der Rijst et al.[203], who described the acetonide formation in N-protected doxorubicin between the hydroxyl group at C-14 and the carbonyl oxygen at C-13, forming a cyclic orthoester, we observed a further product resulting in acetonide formation between the hydroxyl groups at C-14 and C-9, forming a six membered ring (fig. 55).

The ratio of formation is approximately 4:1 in favour of the orthoester. The mixture of the acetonides was purified by column chromatography on silica gel with DCM/MeOH (100:2, v/v) eluting first acetonide **139** (14.9 % yield) and then acetonide **138** (57.5 % yield, as mixture of two diastereomers) as a red solids.

Both acetonides were used for the further synthesis. Since the unstable hydroxyl group was protected the N-trifluoroacetyl group could be cleaved. For this purpose the acetonides **138** and **139** were stirred in a mixture of aqueous sodium hydroxide (0.1 N) and acetone (5:1, v/v) for 30 min at room temperature. The deep purple solution was neutralized with hydrochloric acid (0.1 N) until pH 8 was reached. After extraction with chloroform all solvents were removed and the residue was dissolved in hydrochloric acid (0.1 N) for the cleavage of the acetonide group. After stirring for about 40 hrs at room temperature the aqueous reaction mixture was extracted with chloroform to remove impurities and eventually hydrolysed aglycone. The solution was then adjusted to pH 8 with aqueous sodium hydroxide, of which the product **140** (msoep260 = protocol name) was extracted with chloroform. The extracts were dried and concentrated under reduced pressure to give glycoside **140** in 42 - 48.3 % yield (depending on starting material) without any further purification as orange-red solid.

The following figures 56/57/58 show the reversed-phase HPLC-chromatograms of glycoside **140** and doxorubicin. Glycoside **140** (msoep260) showed a longer retention time compared with its parent compound doxorubicin, which indicates an increased lipophilicity. As with msoep218 a better overall solubility of msoep260 in aqueous media was achieved by OEGylation.

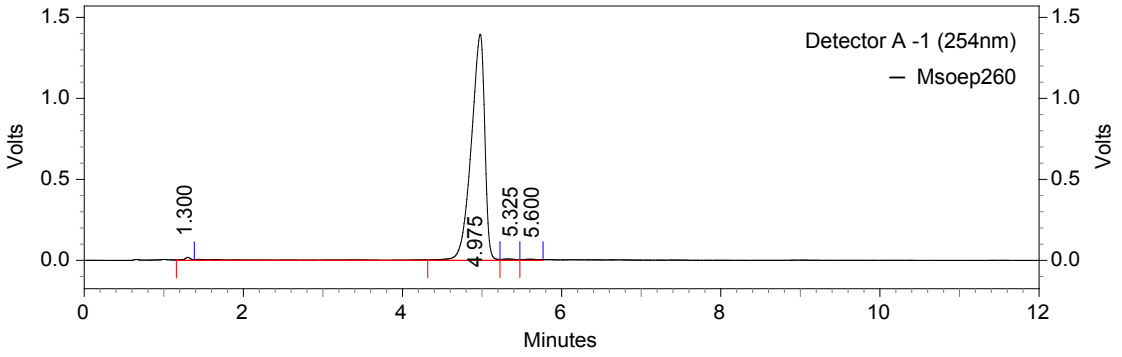


Figure 56 | HPLC-chromatogramm of glycoside 140 (msoep260). Pk 1: Retention time = 1.300 min, area % = 0.633; Pk 2: Retention time = 4.975 min, area % = 98.153; Pk 3: Retention time = 5.325 min, area % = 0.603; Pk 4: Retention time = 5.600 min, area % = 0.612.

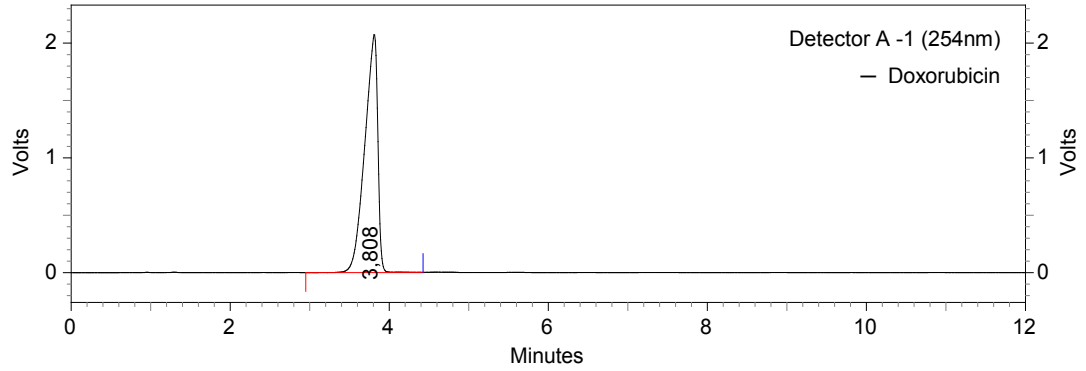


Figure 57 | HPLC-chromatogramm of doxorubicin. Pk 1: Retention time = 3.808 min, area % = 100.

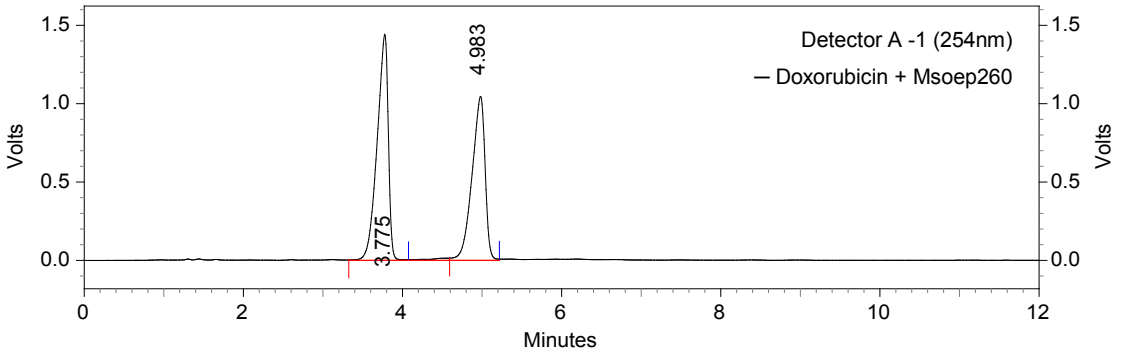


Figure 58 | HPLC-chromatogramm of glycoside 140 (msoep260) and doxorubicin. Pk 1: Retention time = 3.775 min, area % = 54.130; Pk 2: Retention time = 4.983, area % = 45.870.

The integrity of the synthesized anthracycline **140** was verified by High-Resolution Mass-Spectrometry. Anthracycline **140** shows the $[M+Na]^+$ ions as the most prominent species followed by the $[M+H]^+$ and $[M+K]^+$ ions (fig. 59).

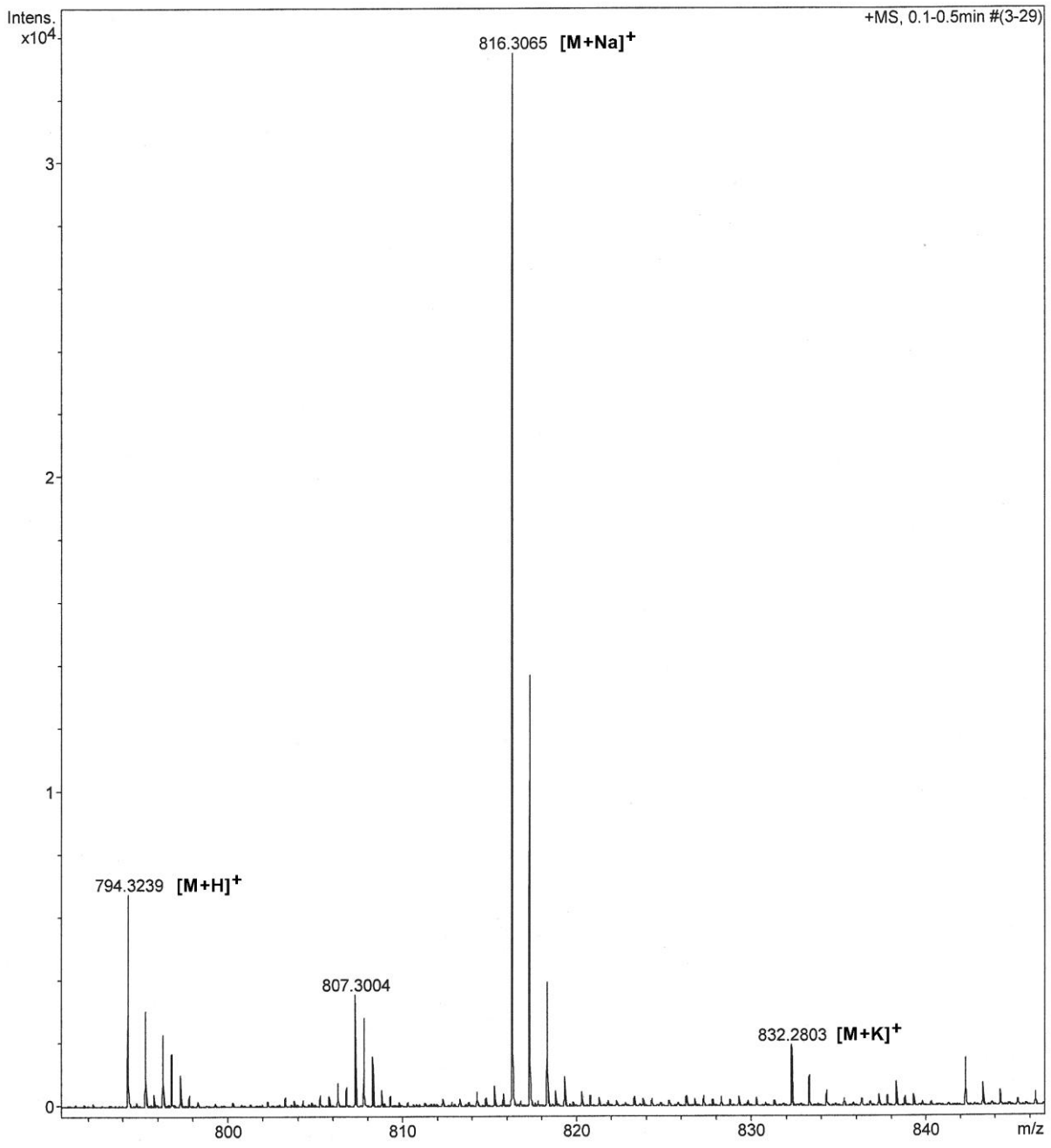


Figure 59 | HRMS spectrum of anthracycline 140 (msoep 260). Measured with MaXis, ESI-Qq-TOF-MS System with an accuracy of +/- 5ppm. Calculated for $[C_{38}H_{51}NO_{17}+H]^+$ 794.3235, found 794.3239; calculated for $[C_{38}H_{51}NO_{17}+Na]^+$ 816.3054, found 816.3065; calculated for $[C_{38}H_{51}NO_{17}+K]^+$ 832.2794, found 832.2803

5.6 In vitro antitumor activity

For the pharmacological profiling of the newly synthesized anthracyclines derivatives **133** (msoep218) and **140** (msoep260), an *in vitro* proliferation assay is a suitable method for the determination of the antiproliferative and cytotoxic effects. That for the non-radioactive cell proliferation and cytotoxicity assay (*EZ4U*, Biomedica GmbH, Vienna) was used. The method is based on the fact that living cells reduce colourless or only slightly coloured tetrazolium salts to intensively coloured formazan derivatives.

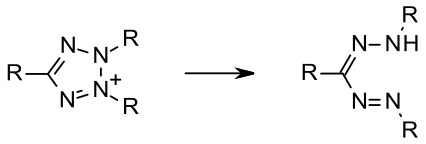


Figure 60 | Enzymatic reduction of a tetrazolium salt to a formazan derivative. Catalyzed by cellular reductase[244,245]

This reduction presupposes functioning mitochondria, which are inactivated several minutes after cell death. For this reason this test is well-suited to distinguish between living metabolic active cells and dead cells. The incubation time of the dye solution is dependent on the metabolic capacity of the cell line and varies between 2 and 5 hours. The amount of the formazan dye can be quantified through its absorption, which has its maximum at 492 nm.[246]

5.6.1 Cytotoxicity of the anthracycline drugs towards MCF-7 cell line

During evaluation of the synthesized anthracyclines **133** (msoep218) and **140** (msoep260) the cytotoxicity was determined against the breast adenocarcinoma cell line MCF-7 (Michigan Cancer Foundation – 7). As reference substance doxorubicin was chosen, a common cytostatic against breast cancer. MCF-7 cells were incubated with 0.001-100 µM of anthracycline to measure the time dependent *in vitro* cytotoxicity. Incubation was stopped after 48 hours continuous drug exposure and the cell viability was assessed, using the above described dye reduction assay.

Figure 61 shows the dose response curves of the examined anthracyclines after 48 hours. For both derivatives, **133** (msoep218) and **140** (msoep260), a significant decrease in cell viability was observed at a concentration ≥ 10 µM (fig. 61 A/B) under these assay conditions. In comparison, doxorubicin significantly decreased cell viability already at a concentration ≥ 1 µM (fig. 61 C). All data points are presented as the mean ± SD, the cell viability was calculated as a percentage of the untreated control group.

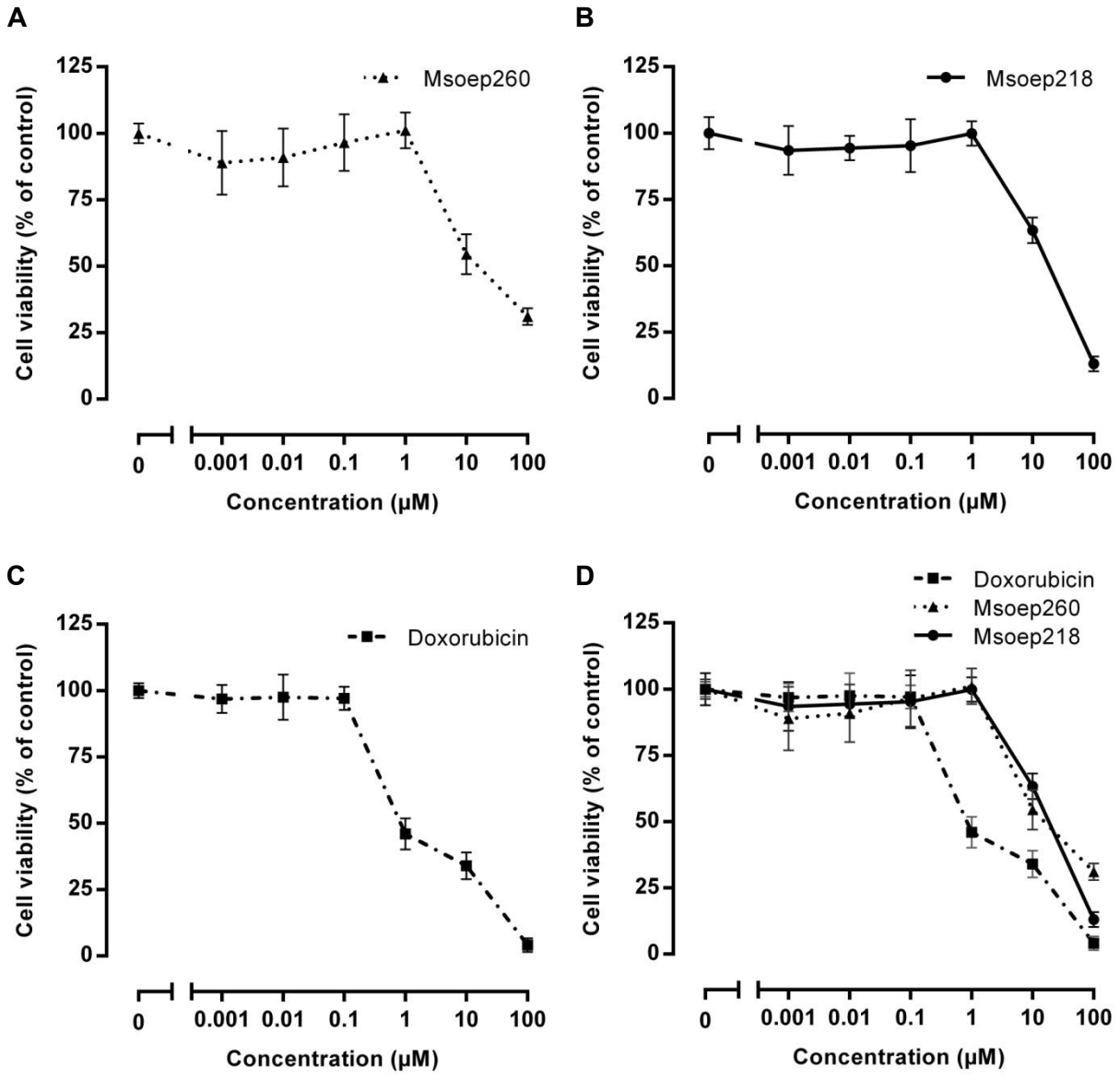


Figure 61 | Concentration response curves of synthesized anthracyclines 133 (msoep218), 140 (msoep260) and doxorubicin against MCF-7 cells after 48 h incubation time. 2500 cells were incubated with 0.001-100 µM test compound for 48 h. Data are presented as mean ± SD (n = 12 to 24) from two independent experiments, vehicle treated control was set to 100 %.

Expansion of the incubation time to 72 hours led to a further reduction of cell viability in all three substances. Figure 62 the concentration-response curves after 72 incubation time are shown.

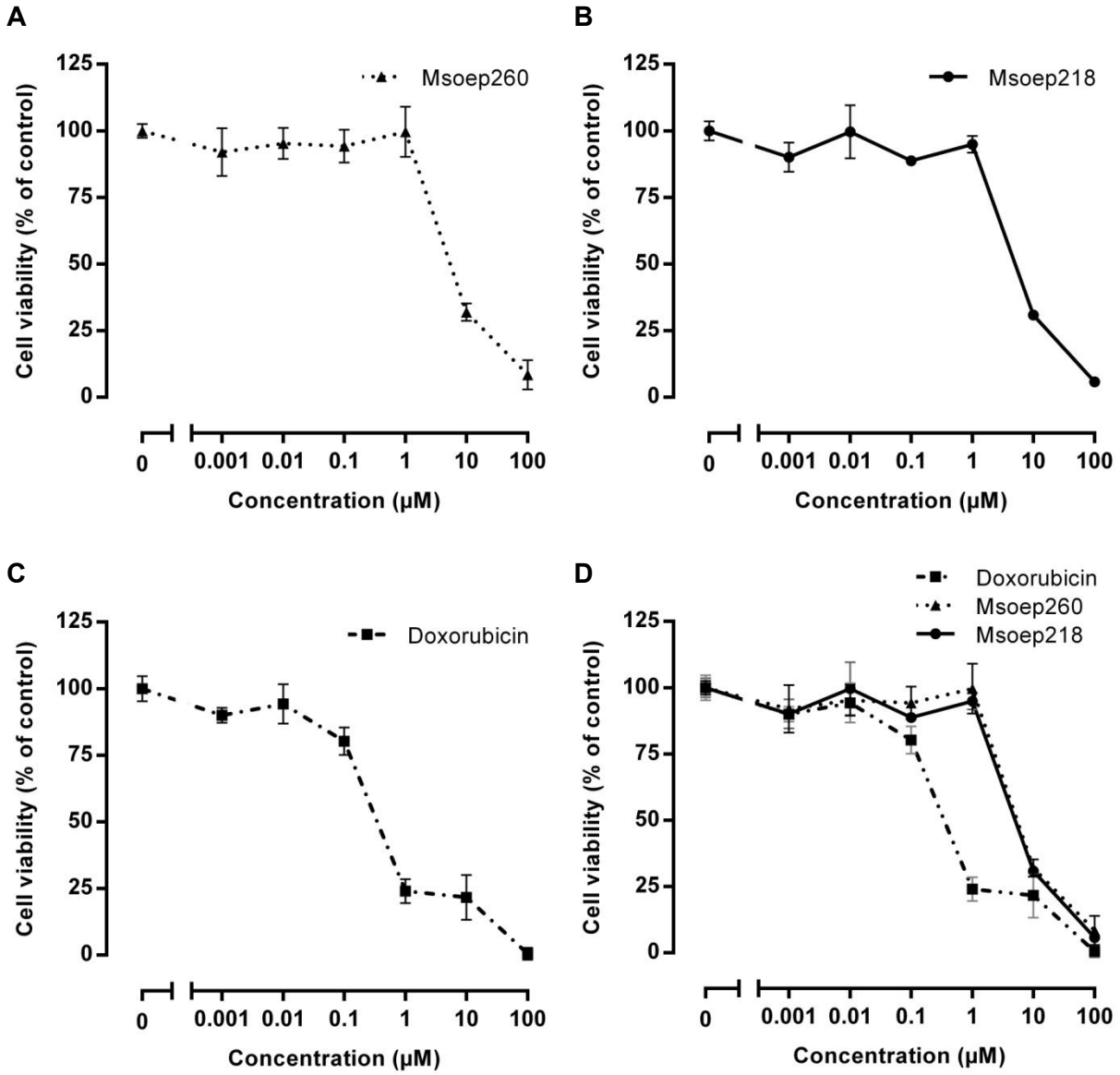


Figure 62 | Concentration response curves of synthesized anthracyclines 133 (msoep218), 140 (msoep260) and doxorubicin against MCF-7 cells after 72 h incubation time. 2500 cells were incubated with 0.001-100 µM test compound for 72 h. Data are presented as mean ± SD (n = 12 to 24) from two independent experiments, vehicle treated control was set to 100 %.

The following bar chart (fig. 63) shows the relative reduction of the cell viability of MCF-7 cells by **133** (msoep218), **140** (msoep260) and doxorubicin after 48 hours, equivalent to the dose response curve in fig. 61/D, giving additionally the level of significance calculated by a two-sided t-test with same variances. As before the untreated control group was set to 100 % viability.

Msoep218 highly significantly decreased ($p < 0.001$) cell viability at concentrations of 100 and 10 µM (13.02 % ± 2.79 %, 63.33 % ± 4.79 %, mean ± SD, n = 12). A significant decrease ($p < 0.05$) of cell viability was further observed at 0.01 µM (94.40 % ± 4.61%, mean

± SD, n = 12), although this value must be interpreted cautiously, because in the two intervening concentrations (0.1 µM and 1 µM) no significant decrease of cell viability was observed.

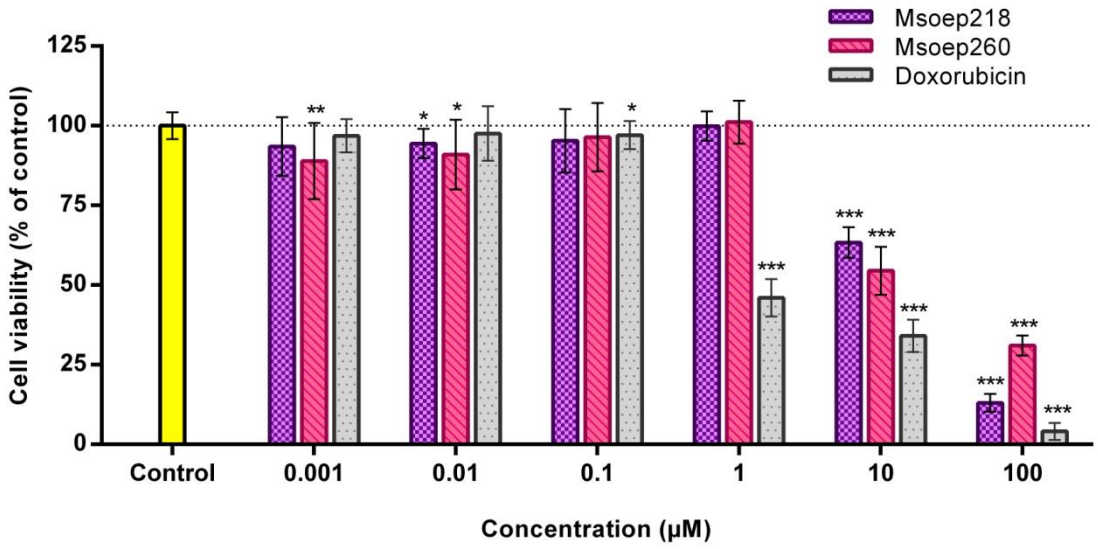


Figure 63 | Reduction of cell viability of MCF-7 cells by the novel anthracyclines msoep218 and msoep260 in comparison with doxorubicin after 48 h incubation time. Data are presented as mean ± SD (n = 12 to 24) from two independent experiments normalized to control. Statistical significance of individual concentrations versus the untreated control group was calculated by a two-sided t-test with same variances (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Msoep260 showed similar to msoep218 a highly significant decrease ($p < 0.001$) of cell viability at concentrations of 100 and 10 µM (31.05 % ± 3.14 %, 54.48 % ± 7.52 %, mean ± SD, n = 12 - 24). In comparison to msoep218, msoep260 showed a higher cytotoxicity at 10 µM but a lower cytotoxicity at 100 µM than msoep218. At concentrations of 0.1 µM and 1 µM, msoep260 showed no significant decrease of cell viability. Surprisingly, with the two lowest concentrations, 0.01 and 0.001 µM, a significant decrease ($p < 0.05$) in cell viability (90.92 % ± 10.90 %, 88.89 % ± 11.96 %, mean ± SD, n = 12), was observed.

Doxorubicin showed the strongest effects in this 48-hour-proliferation assay. The cell viability was highly significantly decreased ($p < 0.001$) at 100, 10 and 1 µM (4.04 % ± 2.67 %, 34.03 % ± 5.10 %, 46.04 % ± 5.90 %, mean ± SD, n = 12 - 24). Even at the concentration of 0.1 µM a small but significant decrease ($p < 0.05$) was observed (97.05 % ± 4.38 %, mean ± SD, n = 12). No significant decrease was observed at 0.01 and 0.001 µM.

After 72 hours incubation time using the identical concentrations as in the 48-hour-assay, further reduction of the cell viability was observed with at all three substances (fig. 64).

Msoep218 highly significantly decreased ($p < 0.001$) cell viability at 100 and 10 µM (5.72 % ± 0.87 %, 30.89 % ± 1.74 %, mean ± SD, n = 12-24). Also at lower concentrations 1, 0.1 and 0.001 µM (94.89 % ± 3.19 %, 88.72 % ± 1.27 %, 90.11 % ± 5.48 %m mean ± SD, n = 12 - 24) a small, but significant decrease ($p < 0.005$) of cell viability was observed.

Msoep260 highly significantly decreased ($p < 0.001$) cell viability at 100 and 10 µM (8.43 % ± 5.5 %, 31.96 % ± 3.28 %, mean ± SD, n = 12-24). No significant decrease was observed at 1 µM, whereas the further concentrations 0.1, 0.01 and 0.001 µM (94.24 % ± 6.17 %, 95 % ± 5.85 %, 92.05 % ± 8.98 %, mean ± SD, n = 12-24) showed a small but significant decrease ($p < 0.05$) of cell viability.

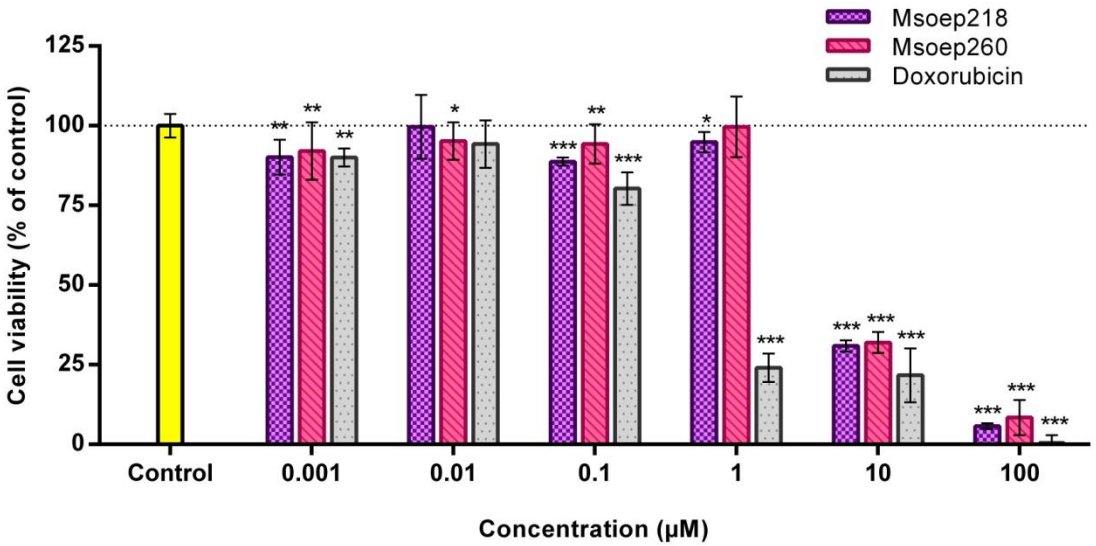


Figure 64 | Reduction of cell viability of MCF-7 cells by the novel anthracyclines msoep218 and msoep260 in comparison with doxorubicin after 72 h incubation time. Data are presented as mean ± SD (n = 12 to 24) from two independent experiments normalized to control. Statistical significance of individual concentrations versus the untreated control group was calculated by a two-sided t-test with same variances (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Doxorubicin highly significantly decreased ($p < 0.001$) the cell viability at 100, 10, 1 and 0.1 µM (0.50 % ± 2.35 %, 21.67 % ± 8.41 %, 24.02 % ± 4.43 %, 80.27 % ± 5.14 %, mean ± SD, n = 12-24). At 0.01 µM no significant decrease was observed, however at 0.001 µM (90.01% ± 2.86% mean ± SD, n = 12) again a significant decrease ($p < 0.001$) was observed.

Ending up the inhibitory concentration (IC_{50}), which is the median concentration that causes 50 % inhibition[247], was calculated for each substance after 48 and 72 hours

incubation time. Basis for the calculation were the dose response curves (fig. 61/62) using nonlinear least-squares regression analysis of GraphPad Prism® 6. The IC_{50} values of all three compounds against MCF-7 cells are summarized in Table 4.

Table 4 | Cytotoxicity (IC_{50}) against MCF-7 breast cancer cells determined after 48 h and 72 h incubation time.

	Cell line [MCF-7]	
Compounds	**IC_{50} [µM] / 48 h**	**IC_{50} [µM] / 72 h**
Msoep218	16.55 ± 1.03	5.93 ± 0.32
Msoep260	18.48 ± 2.25	7.10 ± 0.65
Doxorubicin	1.73 ± 0.18	0.40 ± 0.04

Data are presented as mean ± SEM (n = 12 to 24) from two independent experiments.

In general, the sensitivity of MCF-7 cells against the synthesized anthracycline derivatives was at a therapeutically relevant low µM range. The results showed that the anthracycline derivative mseop218, which lacks a hydroxyl group at C-14, has a slightly higher cytotoxicity than msoep260, both after 48 and 72 hours incubation time. However, msoep218 and msoep260 both carrying an oxyethylene side chain on their sugar moiety, exhibit a 10 to 11 fold lower cytotoxicity than doxorubicin after 48 hours and a 15 to 18 fold lower cytotoxicity after 72 hours incubation time. The *in vitro* potency of the novel anthracyclines is approximately comparable with those of annamycin **12**, an anthracycline drug candidate, currently in clinical phase II development, which also exhibits a 10 to 12 fold lower *in vitro* cytotoxicity against MCF-7 cells than doxorubicin[248,249].

5.7 Cell uptake studies

To investigate the cell uptake kinetics of the synthesized anthracyclines a time-dependent *in vitro* cellular uptake of **133** (msoep218), which showed slightly higher *in vitro* cytotoxicity than **140** (msoep260), was measured in MCF-7 cells. As reference substance doxorubicin was used. Both doxorubicin and msoep218 contain a hydroxy substituted anthraquinone chromophore thereby the cellular drug uptake can be measured by flow cytometry, without any further derivatization.

First, the fluorescence intensity of doxorubicin and msoep218 was determined in a concentration range of 0.1 to 10 µM. The fluorescence intensity followed an almost linear increase in relation to the drug concentration for both substances, whereas doxorubicin showed higher fluorescence intensity at equimolar concentrations in comparison to msoep218. The difference in the fluorescence/mole ratio was determined by the slopes of the regression lines. The calculated slope factor (4398.8/2152.6 = 2.04) indicates that doxorubicin fluorescence intensity is twofold higher than that of msoep218 (fig. 65).

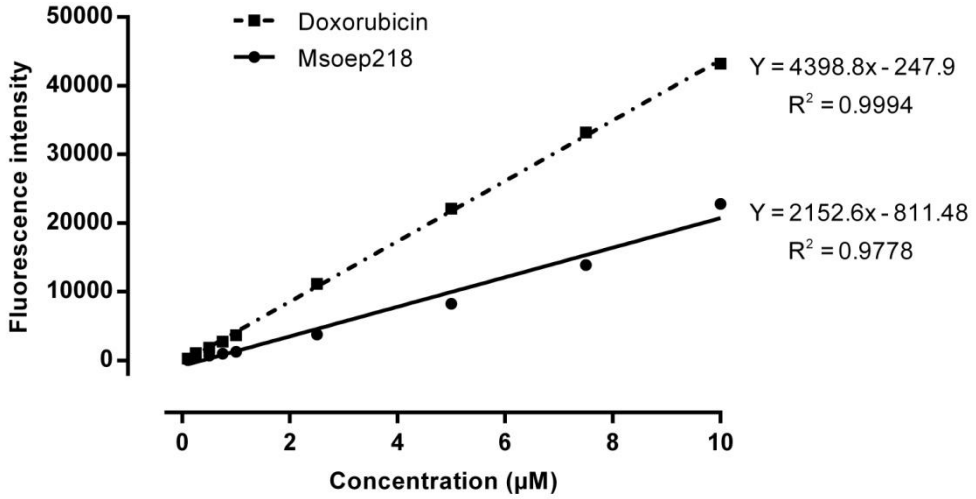
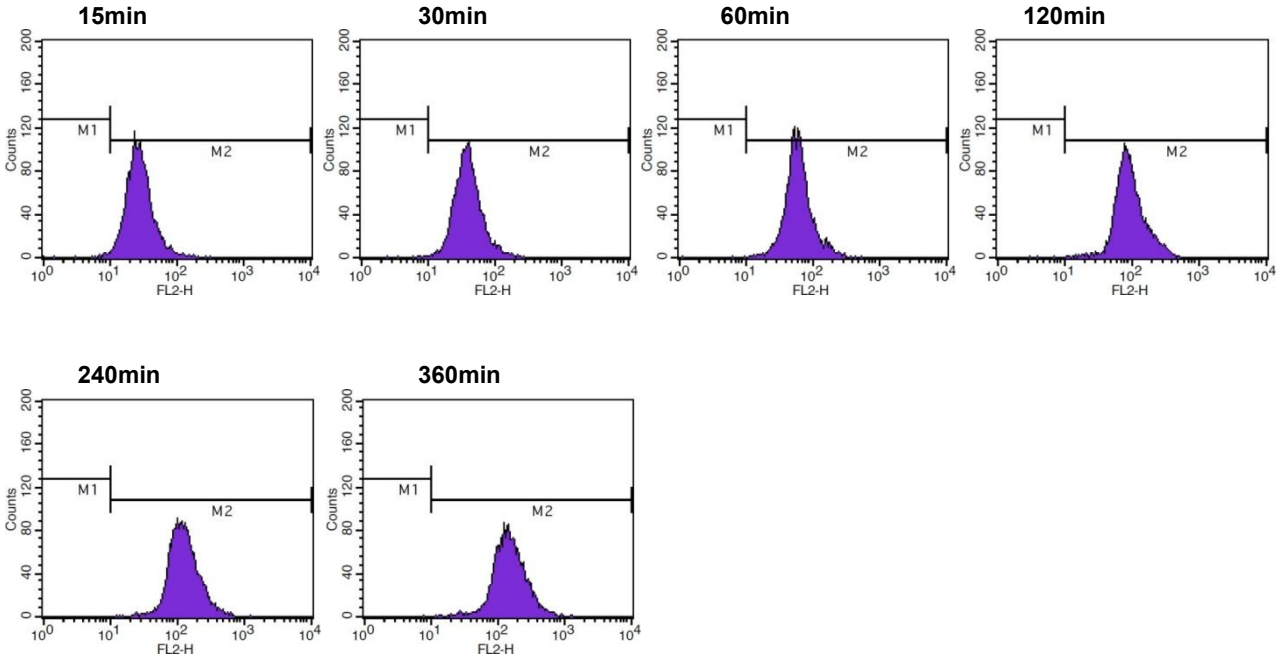


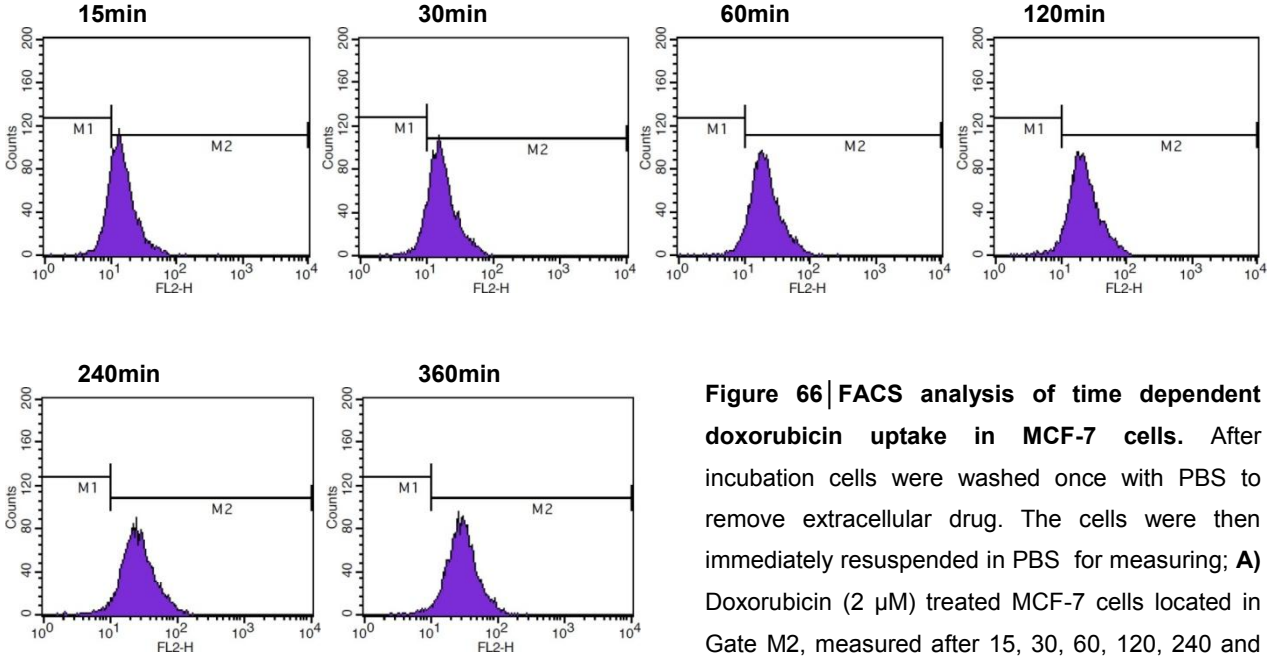
Figure 65 | Linearity of msoep218 and doxorubicin. Measured drug concentrations: 0.1 µM, 0.25 µM, 0.5 µM, 0.75 µM, 1 µM, 2.5 µM, 5 µM, 7.5 µM, 10 µM in PBS 1x. Device: Microplate reader, Infinite 200 Pro, Tecan (excitation at λ = 488 nm, emission at λ = 575 nm).

The uptake measurement was performed over a period of 360 min. MCF-7 cells were incubated at 37 °C with msoep218 and doxorubicin at a concentration of 2 µM in fresh cell media. The intracellular anthracycline content was determined after 15, 30, 60, 120, 240 and 360 min by FACS analysis.

A (Doxorubicin)



B (Msoep218)



C (Untreated Cells)

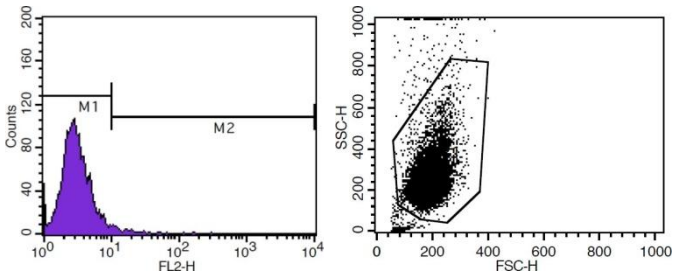


Figure 66 | FACS analysis of time dependent doxorubicin uptake in MCF-7 cells. After incubation cells were washed once with PBS to remove extracellular drug. The cells were then immediately resuspended in PBS for measuring; **A)** Doxorubicin (2 µM) treated MCF-7 cells located in Gate M2, measured after 15, 30, 60, 120, 240 and 360 minutes incubation time; Device: BD (Becton-Dickinson) FACSCalibur flow cytometer (λ_{ex} = 488nm, λ_{em} = 575 ± 30nm); **B)** Msoep218 (2 µM) treated MCF-7 cells located in Gate M2, measured after 15, 30, 60, 120, 240 and 360 minutes incubation time; Device: BD (Becton-Dickinson) FACSCalibur flow cytometer (λ_{ex} = 488nm, λ_{em} = 575 ± 30nm); **C)** Untreated MCF-7 cells located in Gate M1;

All values measured for msoep218 were multiplied with 2.04 (slope factor, calculated from the linear equations) to compensate the difference in the fluorescence intensity of doxorubicin and msoep218. The results obtained are presented in figure 67, in which the fluorescence intensity is plotted versus incubation time.

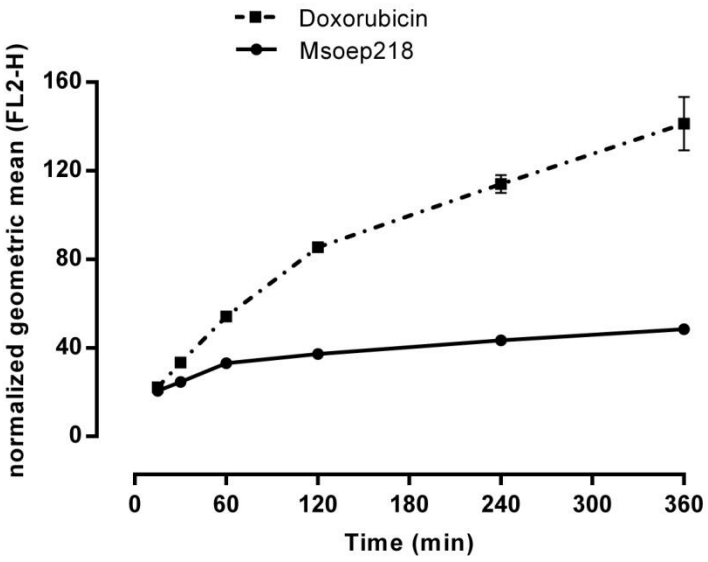


Figure 67 | Time dependent cell uptake of doxorubicin and msoep218 (2 µM) in MCF-7 cells. Data are presented as mean ± SD (n = 3 to 6); Data (doxorubicin): 15 min / 22.14 ± 0.69, 30 min / 33.43 ± 1.45, 60 min / 54.12 ± 2.11, 120 min / 85.48 ± 1.95, 240 min / 113.96 ± 4.11, 360 min / 141.34 ± 12.16; Data (msoep218): 15min / 20.57 ± 0.69, 30 min / 24.61 ± 0.24, 60 min / 33.04 ± 0.63, 120 min / 37.21 ± 0.80, 240 min / 43.37 ± 1.10, 360 min / 48.39 ± 1.23

After 15 min incubation time a significant drug accumulation in MCF-7 cells was observed for both substances (msoep218 and doxorubicin). With continuous incubation time doxorubicin showed significantly higher cell accumulation compared to msoep218, whose uptake slowed down after 60 min. The data showed that the amount of doxorubicin uptake was about four times higher than that of msoep218 in the measured time slot (15 - 360 min), calculated by dividing the change in fluorescence of doxorubicin by the change in fluorescence of msoep218.

The lower cellular uptake of msoep218 compared to doxorubicin, probably caused by changes in the physicochemical properties, might be a reason for the lower cytotoxicity of the newly designed anthracycline derivatives in comparison with doxorubicin.

5.8 The influence of MDR modulators on the cytotoxicity

The aim of this experiment was to investigate the influence of ABC-transporter mediated drug resistance on the cytotoxicity of the novel anthracycline derivative msoep218. For the experiment two human cell lines were used: The epidermoid carcinoma-derived cell line KB-3-1, and its multidrug-resistant subline KBC-1. Both cell lines were donated by Dr. D.W. Shen, Bethesda, USA[250]. For the cytotoxicity assay the cells were plated (2 x 10^4 cells/ml) in 100 µL per well in 96 well microplates. The cells were incubated for 24 hours for culture plate surfaces adhesion. The various drug concentrations were dissolved in another 100 µl of growth medium and were added to the cells. The cells were incubated with msoep218 (5, 10, 20, 40, 80 µM), with and without co-administration of the multidrug resistance protein 1 (ABCB1) inhibitors, verapamil (10 µM) or cyclosporin-A (1 µM), for 72 hours at 37 °C. For the determination of cytotoxic effects a non-radioactive cell proliferation and cytotoxicity assay (*EZ4U*, Biomedica GmbH, Vienna, Austria) was used following the manufacturer's instructions.

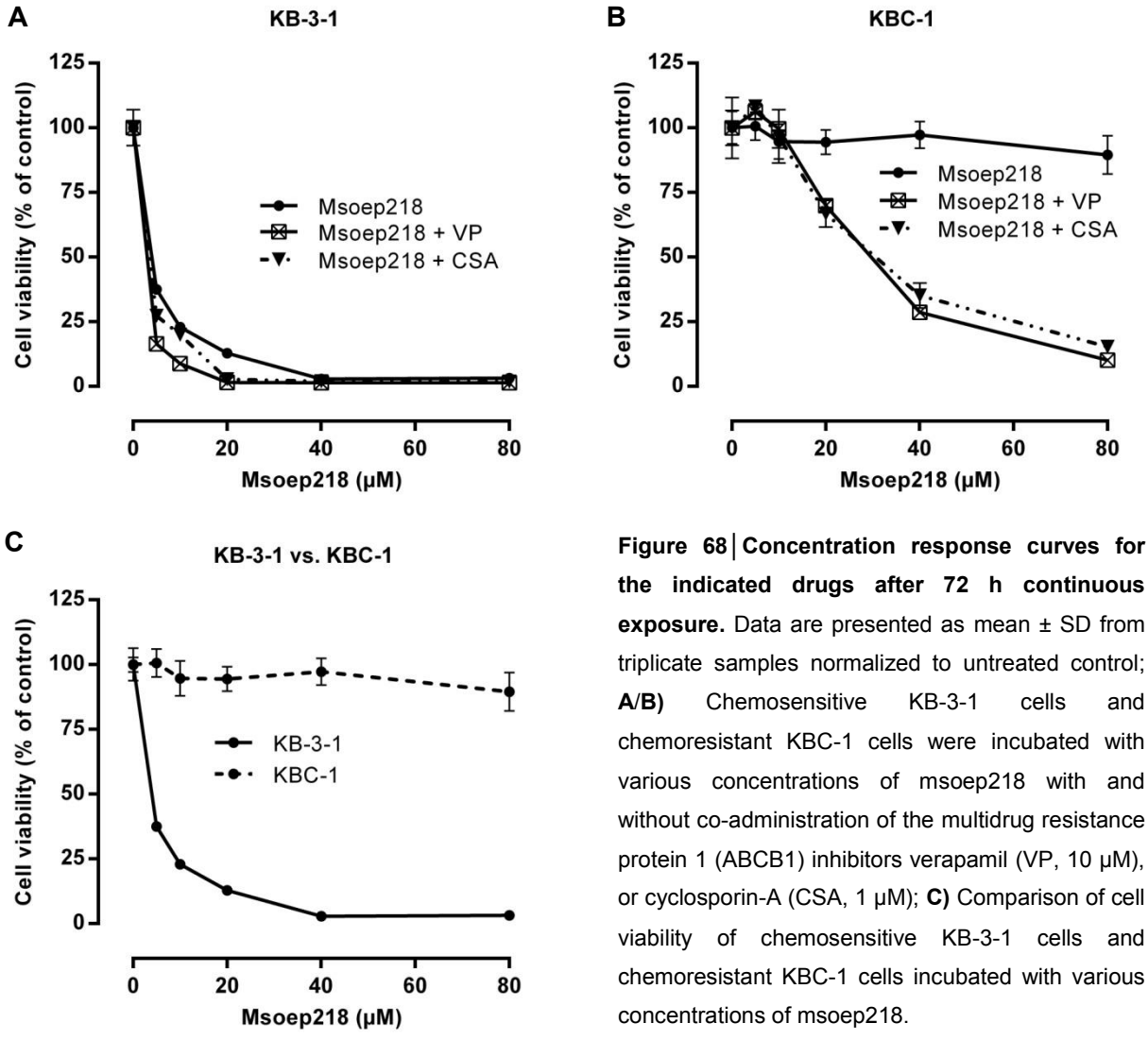


Figure 68 | Concentration response curves for the indicated drugs after 72 h continuous exposure. Data are presented as mean ± SD from triplicate samples normalized to untreated control; **A/B)** Chemosensitive KB-3-1 cells and chemoresistant KBC-1 cells were incubated with various concentrations of msoep218 with and without co-administration of the multidrug resistance protein 1 (ABCB1) inhibitors verapamil (VP, 10 µM), or cyclosporin-A (CSA, 1 µM); **C)** Comparison of cell viability of chemosensitive KB-3-1 cells and chemoresistant KBC-1 cells incubated with various concentrations of msoep218.

The sensitivity of cell line KB-3-1 against msoep218 was in a very low µM range (fig. 68/A & C), 5 µM of msoep218 decreased the cell viability to 37.53 % ± 1.19 %, 10 µM to 22.94 % ± 1.11 %, 20 µM to 12.82 % ± 1.25 %, 40 µM to 2.84 % ± 1.31 % and 80 µM to 3.17 % ± 1.54 %.

Different results were obtained for its multidrug-resistant subline KBC-1, which expresses high levels of the multidrug-resistance protein ABCB1[251]. The KBC-1 cells did not respond to the treatment with msoep218 alone, no significant decrease of cell viability was observed (fig. 68/B & C). Verapamil (VP) and cyclosporine A (CSA) are known inhibitors of multidrug resistance protein 1 (gene: ABCB1) and multidrug resistance-associated protein (gene: ABCC1), which substantially contribute to drug efflux in multidrug resistance cell lines[252]. Both verapamil (VP) and cyclosporine A (CSA) are known to restore *in vitro* cell sensitivity against anti-tumour agents by inhibition of these efflux pumps. Doxorubicin and daunorubicin, which are known substrates for ABCB1 and ABCC1, give a good example for this effect, their cytotoxicity against multidrug-resistant KBC-1 cells could be restored by verapamil and cyclosporine[251,253,254]. Similar results were obtained in our experiment with KBC-1 cells. Co-administration of msoep218 and verapamil (10 µM) significantly reduced cell viability of chemoresistant KBC-1 cells. Comparable results were achieved with cyclosporine A (1 µM), results see fig. 68/B.

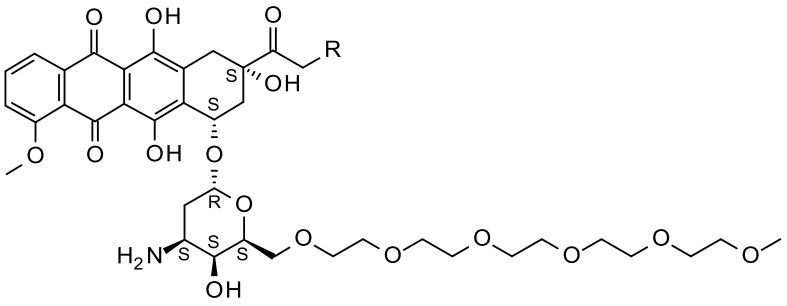
These results allow the conclusion that msoep218 is a substrate for ABC transporter-mediated drug efflux in particular msoep218 is supposed to be transported by multidrug resistance protein 1 (ABCB1) and/or multidrug resistance-associated protein (ABCC1). For this reason msoep218 showed no significant decrease of cell viability in ABCB1-overexpressing KBC-1 cells.

Chemosensitive KB-3-1 cells showed significant sensibility against msoep218. This effect was further increased by co-administration of msoep218 with verapamil (10 µM) or cyclosporine A (1 µM), results see fig. 68/A. A possible reason for this effect might be moderate ABCB1/ ABCC1 expression in KB-3-1 cells and additionally a slightly toxic effect on KB-3-1 cells by verapamil and cyclosporine-A itself.

The practical implementation of this experiment was done by Volker Baumann, M.Sc. in collaboration with the institute of Cancer Research, Department of Medicine I, Medical University Vienna, Borschkegasse 8a, 1090 Vienna, Austria.

6 Conclusion

The main goal of this thesis was synthesizing novel anthracycline derivatives with altered physicochemical properties, by targeted structural modifications, to improve the pharmacokinetic profile. This goal was fully achieved.



133 (Msoep218): R = H
140 (Msoep260): R = OH

Figure 69| **Oligoethylene glycol (OEG) modified daunorubicin 133** (msoep218) **and doxorubicin 140** (msoep260)

Detailed investigation of the drug-receptor complex of different therapeutically used anthracyclines was crucial for a target based design of novel analogs modified at the 6'-position of their sugar moiety. The 6'-position of anthracycline monosaccharides, which is occupied by a methyl group at therapeutically used anthracyclines, is located in the "minor groove" of DNA where is enough space to introduce a covalent modifier without restricting the intercalation. The replacement of the methyl group with an oligoethylene glycol chain is to our knowledge the first attempt to improve the physicochemical properties of anthracyclines by small molecular-weight OEGgylation, which is not cleavable under physiological conditions.

The synthetic route described here in detail represents an easy access to new and established glycosyl donors which can be used for total and semi-synthesis of anthracyclines. For the introduction of a linear oxyethylene chain instead of the methyl group in the sugar moiety of anthracyclines, first a synthetic route to daunosamine bearing a 6'-methoxy-oligoethylene-glycol chain has been developed, starting from commercially available (2R,3R)-tartaric acid. Subsequent glycosylation with daunorubicinone and doxorubicinone, followed by gradual cleavage of the protecting groups resulted in the two novel anthracycline derivatives msoep218 and msoep260.

The newly synthesized title compounds showed a severe change in their physicochemical properties. Both lipophilicity and hydrophilicity were increased compared to their parent drugs (daunorubicin and doxorubicin). The change in the physicochemical

properties may lead to substantially altered organ distribution, which will shift or expand the spectrum of tumours to be treated.

A set of pharmacological *in vitro* tests was carried out for a preliminary assessment of the newly synthesised compounds. These experiments included cytotoxicity as pharmacodynamic property and drug uptake as experiment towards evaluation of pharmacokinetic properties. The first pharmacological tests showed, regarding *in vitro* activity, promising results. The novel anthracyclines were active at a therapeutically relevant concentration range, comparable with the anthracycline drug candidate, annamycin. Although having a lower *in vitro* activity against cancer cells than doxorubicin, the experiments carried out suggest that the decreased activity of msoep218 and msoep260 might be caused by reduced cellular uptake, and not by a reduced affinity to the target. The lower *in vitro* potency of the novel anthracyclines compared to doxorubicin should not be seen as a negative result, because the extremely high potency of doxorubicin has been considered as a source of side effects. The lower potency indicates at the same time a reduced *in vivo* toxicity, which is of relevance in prolonged treatment with high cumulative doses of anthracyclines.

In vivo studies will give detailed information on the impact of the amphiphilic OEGyl-modification on efficacy and safety of a novel class of anthracyclines.

7 Experimental part

7.1 Material and methods

7.1.1 Analytical methods

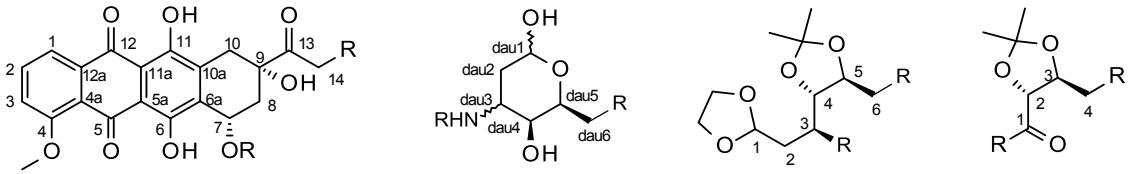
Nuclear magnetic resonance spectroscopy (NMR)

The NMR spectra were measured in the indicated solvent using a Bruker Avance DPX-200 MHz spectrometer, measuring at 27 °C (200.13 MHz for ^{1}H-NMR and 50.32 MHz for ^{13}C-NMR), or a Brucker Avance 500 MHz spectrometer, measuring at 20 °C (500.13 MHz for ^{1}H-NMR and 125.77 MHz for ^{13}C-NMR). The chemical shifts (δ) are specified in ppm and the coupling constants (*J*) in Hertz (*Hz*). The solvent signal is utilized as internal standard and the spectra were calibrated as follows:

- $CDCl_3$: 7.26 ppm for ^{1}H and 77.00 ppm for ^{13}C-spectra
- d_6-DMSO: 2.50 ppm for ^{1}H and 39.50 ppm for ^{13}C-spectra
- d_4-methanol: 3.31 ppm for ^{1}H and 49.00 ppm for ^{13}C-spectra

For the differentiation of the proton signals the multiplicities of the signals are mentioned as: "s" = singlet, "brs" = broad singlet, "d" = doublet, "dd" = doublet of doublets, "t" = triplet, "q" = quartet, "m" = multiplet.

For the numbering of the anthraquinone, the sugar moiety and its precursors templates are illustrated below.



Mass Spectrometry and High-Resolution Mass Spectrometry (MS/HRMS)

ESI-MS was done with a Finnigan Thermo Quest LCQ Deca Mass Spectrometer. The High-resolution Mass Spectrograms were measured with a MaXis, ESI-Qq-TOF-MS System, (Bruker Daltonics) with a resolution of 40 000 for m/z = 922Da. The exact molecular masses were determined with accuracy of +/- 5ppm. All sample preparations were done in ACN/MeOH.

Optical rotation

The optical rotation was measured on a Perkin Elmer, Model 341 LC polarimeter at the wavelength of the D-line of sodium (λ = 589 nm), at 20 °C. To calculate the specific optical rotation the following formula was used: $[\alpha_D^{20}] = \alpha * 1000/l * c$; $\alpha =$ angle of rotation in degrees, read at 20 °C; $l =$ length in decimeters of the polarimeter tube; $c =$ concentration of the solution in grams per liter. The specification of the rotation values was done as follows: $[\alpha_D^{20}] =$ -11.2° (c = 1.16, $CHCl_3$) in which c = concentration is specified in g/100 ml.

Melting points

The instantaneous melting points were determined on a Leica Galen III melting point apparatus (Ser. No. 1413 WT) and are uncorrected.

Thin layer chromatography (TLC)

The analytical TLC was carried out using Merck pre-coated aluminium sheets (Silicagel 60 F_{254}), layer thickness 0.25 mm; Machery-Nagel Pre-coated TLC-sheets ALUGRAM® Xtra SIL G/UV_{254}, layer thickness 0.20 mm; Machery-Nagel HPTLC-plates Nano-SIL C18-100 with fluorescent indicator UV_{254}, layer thickness 0.20 mm. For the application capillaries (ringcaps®) 5/10 µL were used. Visualization of the substances was done with ultraviolet light at 254 nm. Non UV-active substances were visualized by spraying with following visualisation reagents:

Seebach reagent (12.5 g of ammonium molybdate tetrahydrate $[(NH_4)_6Mo_7O_{24}\cdot 4H_2O]$, 5 g of cerium (IV) sulfate tetrahydrate $[Ce(SO_4)_2\ 4H_2O]$, 470 ml H_2O and 30 ml Sulfuric acid) and

Anisaldehyde / sulfuric acid (0.5 ml 4-Methoxybenzaldehyde, 50 ml glacial acetic acid and 1.0 ml sulphuric acid). The TLC-sheets were then heated with a heat gun until the spots appeared.

High-performance liquid chromatography (HPLC)

As chromatographic system a Shimadzu High-performance Liquid-Chromatograph (LC-2010A) was used. The following columns were used: Macherey- Nagel Nucleosil C18, 5 µm, l = 125 mm, Ø = 4mm and Zorbax Eclipse XDB-C8, 5 µm, l = 150 mm, Ø = 4.6 mm.

7.1.2 Preparative methods

Preparative layer chromatography (PLC)

The PLC was done with Merck Pre-coated glass plates (Silicagel 60 F_{254}), layer thickness 2.00 mm. The plates were pre-treated, done by migration of MeOH followed by drying at 80 °C for 20 min.

Column chromatography

Normal-phase chromatography was conducted with Acros Organics unmodified Silica gel, for column chromatography, 0.035 - 0.070mm, 60 Å.
The column was equilibrated with the prescribed mobile phase. The substance was dissolved in the eluent until it was free from solid particles and was then pipetted on top of the stationary phase covered with a 0.5 cm layer of sea-sand. The chromatography was done with a pressure between 0.2 - 0.5 bar N_2.

7.1.3 Solvents

The solvents were purchased from commercial chemical suppliers (Acros Organics, Brenntag CEE GmbH, Fisher Scientific GmbH, Lab Scan, Sigma Aldrich Corporation, VWR International GmbH) and were used in the offered quality without any further purification.

Anhydrous solvents were dried via distillation over a drying agent or storage over molecular sieves and were then directly used for synthesis.

Tetrahydrofuran (THF)	distillation from sodium metal (Na)
Chloroform	distillation from Potassium carbonate (K_2CO_3)
Dichloromethane (DCM)	distillation from phosphorus pentoxide (P_2O_5)
Pyridine	distillation from Calcium hydride (CaH_2)
Toluene	distillation from sodium metal (Na)
Dimethylsulfoxide	molecular sieves 4Å
Diethylether	distillation from sodium metal (Na)

7.1.4 Reagents

Acetic acid (AcOH)	Sigma-Aldrich, ACS reagent, ≥ 99.7 %
Acetyl chloride (CH_3COCl)	Acros Organics, 98 %
Acetic anhydride	Sigma-Aldrich, ReagentPlus®, ≥ 99 %
Ammonium chloride (NH_4Cl)	J.T. Baker, ACS Grade, 99.5 % min.

Benzoyl chloride (C_6H_5COCl)	Acros Organics, 99 %, pure
Bromine (Br_2)	Acros Organics 99.8 %, for analysis
2-Bromomethyl-1,3-Dioxolane	Acros Organics, 97 %
Daunorubicin-HCl	Iffect Chemphar Co., LTD.
4-(Dimethylamino)-pyridin (DMAP)	Fluka, purum, ≥ 98.0 %
2,2-dimethoxypropane	Sigma-Aldrich, reagent grade, 98 %
Hydrochloric acid (HCl)	Sigma-Aldrich, pure, fuming, 37 % solution in water
Hydroxylamine hydrochl. ($NH_2OH \cdot HCl$)	Acros Organics, 97 %
Iodine (I_2)	Riedel-de haen, puriss.
Lithium aluminium hydride ($LiAlH_4$)	Acros Organics, 95 %, powder
Magnesium (Mg)	Sigma-Aldrich, chips, 4-30 mesh, 99.98 % trace metals basis
Methyldiglycol	Fluka, purum, ≥ 99.0 % (GC)
4-Nitrobenzoyl chloride	Fluka, ≥ 99.0 % (GC)
Oxalyl chloride	Acros Organics, 98 %
Pentaethylene glycol	Sigma-Aldrich, 98 %
Potassium acetate	Sigma-Aldrich, ACS reagent, ≥ 99.0 %
Potassium carbonate (K_2CO_3)	Sigma-Aldrich, ACS reagent, ≥ 99.0 %
Sodium borohydride ($NaBH_4$)	Acros Organics, 98+ %, powder
Sodium bis(2-methoxyethoxy)aluminum hydride solution	Sigma-Aldrich, ≥ 60 wt. % in toluene
Sodium carbonate (Na_2CO_3)	Carl Roth GmbH + Co. KG, ≥ 99.5 %, p.a., ACS, anhydrous
Sodium hydride (NaH)	Sigma-Aldrich, dry, 95 %
Sodium iodide (NaI)	Sigma-Aldrich, ACS reagent, ≥ 99.5 %
Sodium sulfate (Na_2SO_4)	Carl Roth GmbH+Co. KG, ≥ 99 %, p.a., ACS, ISO, gepulvert
Sodium bicarbonate ($NaHCO_3$)	Carl Roth GmbH + Co. KG, ≥ 99 %,
Sodium bisulfite ($NaHSO_3$)	Acros Organics
Sodium dihydrogen phosphate (NaH_2PO_4)	Acros Organics, 99 %, for analysis, anhydrous
Sodium hydroxide solution (1M)	Gatt - Koller GmbH
Sulfuric acid (H_2SO_4)	Sigma-Aldrich, ACS reagent, 95.0-98.0 %
L(+)-tartaric acid	Merck
p-Toluenesulfonyl chloride	Sigma-Aldrich, puriss., ≥ 99.0 %

Triethylene glycol	Acros Organics, 99+ %
Triphenylmethyl chloride	Acros Organics, 98 %
Trimethylsilyl trifluoromethanesulfonate	Acros Organics, 99 %
Trifluoroacetic anhydride (TFAA)	Acros Organics, 99+ %
Trifluoroacetic acid (TFA)	Merck. > 99.8 %
p-Toluenesulfonic acid monohydrate	Sigma-Aldrich, ACS reagent, ≥ 98.5 %

7.1.5 Abbreviations

Ar	aryl group
Ac_2O	acetic anhydride
Aq.	aqueous
Bz	benzoyl
BzCl	benzoyl chloride
CAS	Chemical Abstracts Service
CHF	congestive heart failure
DCC	N,N'-Dicyclohexylcarbodiimide
DCM	dichloromethane
DIBAL	diisobutylaluminiumhydride
DIPEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DME	dimethyl ether
DMF	dimethylformamide
DMP	dimethoxypropane
DMS	dimethyl sulfide
DMSO	dimethylsulfoxide
DNR	daunorubicin
DOX	doxorubicin
DOXOL	doxorubicinol
DPPA	diphenoxyphosphoryl azide
EPI	epirubicin
EPIOL	epirubicinol
EtOAc	ethyl acetate
EtOH	ethanol
Et_2O	diethyl ether
FACS	fluorescence Activated Cell Sorting
FBS	fetal bovine serum
FDA	Food and Drug Administration

HRMS	high-resolution mass spectrometry
HPLC	high-performance liquid chromatography
Hz	hertz
IDA	idarubicin
IDAOL	idarubicinol
MCF-7	Michigan Cancer Foundation - 7
MS	mass spectrometry
MTBE	methyl tert-butyl ether
MW	molecular weight
NaH	Sodium hydride
NBS	N-Bromosuccinimide
NMR	nuclear magnetic resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
OEG	oligoethylene glycol
PBS	phosphate buffered saline
PE	petroleum ether
PEG	polyethylene glycol
PLC	preparative layer chromatography
PTSA	*p*-Toluenesulfonic acid
Py	pyridine
pNO_2Bz	*p*-nitrobenzoyl
Red-Al	sodium bis(2-methoxyethoxy)aluminumhydride
RID	refractometric index detector
ROS	reactive oxygen species
TBDMSCl	tert-Butyldimethylsilyl chloride
TEA	triethylamine
TEG	triethylene glycol
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TOF	time-of-flight
TrCl	triphenylmethyl chloride
TRT	triphenylmethyl
Ts	tosyl group
TsCl	tosyl chloride

7.1.6 Cell biology

7.1.6.1 Cell cultivation

Cytotoxicity and cellular uptake studies were done with the breast adenocarcinoma cell line MCF-7 (Michigan Cancer Foundation – 7). The cells were maintained in 25 cm² or 75 cm² tissue culture flasks, each of which delivered at confluency about 5 millon (25 cm²) or 15 million (75 cm²) cells. As culture medium DMEM (Dulbecco's Modified Eagle Medium 1x, [+] 4.5 g/l glucose, [-] pyruvate + 10 % FBS) was used. The cells were incubated at 37 °C, 5 % CO_2, 95 % air atmosphere and 95 % humidity.

Cells were passaged twice a week depending on their confluency status. For this, the culture medium was removed with a pipette and the cells were washed with 5 ml (25 cm²) or 15 ml (75 cm²) sterile prewarmed PBS (Dulbecco's Phosphate buffered without Ca^{2+} and Mg^{2+}). PBS was then removed and 0.5 ml (25 cm²) or 1.0 ml (75 cm²) trypsine solution (0.5 % Trypsine EDTA 1x) was added to cover the whole bottom of the flask. Subsequently the flask was incubated for 5 min in the incubator at 37 °C. The trypsinized detached cells were diluted in an appropriate amount of culture medium (1:8, 1:4), which stops the trypsin reaction immediately. Following 1 ml (25 cm²) or 1.5 ml (75 cm²) of the cell suspension was transferred into a new tissue culture flask, in which additional 6 ml (25 cm²) or 15 ml (75 cm²) of culture medium was added. As rule of thumb, a minimum of 500,000 cells (25 cm²) or 1 million cells (75 cm²) should be transferred in every passage to provide exponential cell growth.

7.1.6.2 Cell viability assay

The cell viability was determined by a nonradioactive cell proliferation and cytotoxicity assay (EZ4U, Biomedica GmbH, Vienna). Exponentially growing MCF-7 cells suspended in fresh cell media (DMEM, [+] 4.5 g/l glucose, [-] pyruvate + 10 % FBS) were plated (1.5 x 10^4 cells/ml) in 180 µL media per well (~ 2700 cells) using 96 well microplates. After 24 h incubation at 37 °C, 5 % CO_2, 95 % air atmosphere and 95 % humidity the cells were resettled on the surface and 20 µL various concentrations of anthracyclines in PBS (Dulbecco's Phosphate buffered without Ca^{2+} and Mg^{2+}) were added, giving a final volume of 200 µL/well. At least 6 wells were used for each concentration per experiment. The cell viability was determined after 48 and 72 h incubation (37 °C, 5 % CO_2, 95 % air atmosphere and 95 % humidity). For this the culture media with the drug was replaced with 200 µL fresh media (DMEM), then 20 µL of dye solution was added to each well and the microplates were incubated at 37 °C, 5 % CO_2, 95 % air atmosphere and 95 % humidity. Absorption was measured every 60 min until untreated cells yielded an absorbance value of 1.8 to 2.0. Absorption measurements and addition of the dye solution were done using a microplate

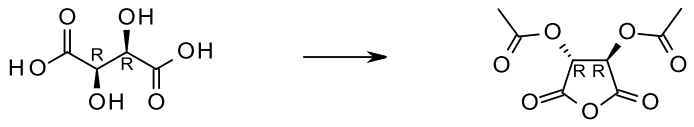
reader (Infinite 200 Pro, Tecan) measuring at λ = 492 nm and λ = 690 nm reference wavelength which was subtracted from the measured value. The cell viability of each fraction was calculated relatively to the untreated control, which was set to 100 % viability (cell viability % = absorbance treated cells/absorbance untreated cell). The IC_{50} values were calculated from the dose response curves using nonlinear least-squares regression analysis of GraphPad Prism® 6.

7.1.6.3 Cellular uptake / Flow Cytometry (FACS)

The flow cytometry assay was done with a BD (Becton-Dickinson) FACSCalibur system. The measurement to detect the anthracycline fluorescence was performed using a FL2-filter (excitation at λ = 488 nm, emission at λ = 575/30 nm). 490 µL fresh cell media (DMEM, [+] 4.5 g/l glucose, [-] pyruvate + 10 % FBS) containing 210,000 MCF-7 cells was placed into an Eppendorf® tube. Then 10 µL of the anthracycline of interest (100 µM in Dulbecco's PBS) was added to a final concentration of 2 µM. Three untreated tubes were used as control. The cells were then incubated at 37 °C, shaking at 450 rpm, for 15, 30, 120, 240 and 360 min for each time in triplicate. During the following steps the Eppendorf® tubes were kept on ice. After centrifugation (500 g's/2min/4 °C) the supernatant was removed and the cells were washed once with 500 µL cold PBS to remove extracellular drug excess. The cells were then immediately resuspended in 750 µL PBS, and the suspension was transferred to FACS tubes for the measurement. For data collection 10,000 events were counted, the fluorescence was analyzed by using the geometric mean of the measured values.

7.2 Synthesis

[(3R,4R)-4-acetoxy-2,5-dioxo-tetrahydrofuran-3-yl] acetate **85**



84 **85**

To a stirred solution of L(+)-tartaric acid **84** (10.0 g, 66.63 mmol, 1.0 equiv.) in acetic anhydride (21.8 ml, 197.3 mmol, 3.0 equiv.), conc. H_2SO_4 (0.36 ml) was added at room temperature under an atmosphere of argon. The resulting mixture was stirred at 85 °C for 10 min and then cooled down to 0 °C. The reaction mixture was filtered and washed with MTBE. The crude product was triturated in MTBE, filtrated, washed with MTBE and dried under high vacuum to give **85** (11.6 g, 53.58 mmol, 80.04 % yield) as a white solid.

^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 5.68 (s, 2H, $(CH)_2$); 2.23 (s, 6H, $(CH_3)_2$)

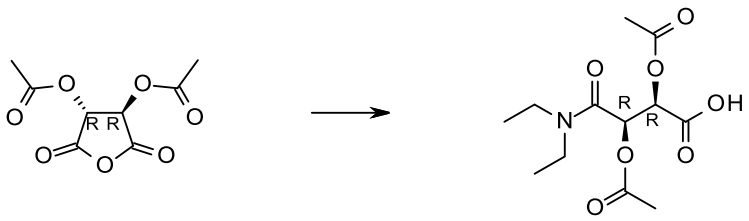
^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 169.86 (Cq); 163.46 (Cq); 72.17 ($(CH)_2$); 20.20 ($(CH_3)_2$)

m.p.: 134-135°C

Analytical data were in agreement with those reported in literature[166].

HPLC: Retention time: 1.5 min; Column: Macherey- Nagel Nucleosil C18, 5 µm, l = 125 mm, Ø = 4 mm; Mobile Phase: Acetic acid (70 %) 1 % in H_2O/30 % MeOH (HPLC grade); Detection: RID; Injection volume: 20 µL; Temperature: 40 °C; Flow rate: 1 ml/min; Run time: 15 min; Standard prep.: 20 mg/ml mobile phase

(2R,3R)-2,3-diacetoxy-4-(diethylamino)-4-oxo-butanoic acid **86**



85 **86**

To a stirred solution of **85** (100 g, 0.46 mol, 1.0 equiv.) in DCM (180 ml), diethylamine (52 ml, 0.498 mmol, 1.08 equiv.) was added at 10 °C under an atmosphere of argon. The white suspension is transformed into a clear yellow solution. The resulting solution was allowed to stir at room temperature for one hour. After this time TLC analysis (DCM/MeOH, 2:1, v/v) showed complete consumption of **85** and all solvents were removed under reduced pressure. The residue was dissolved in EtOAc (200 ml) and was washed with 5N HCl and then with brine. The layers were separated and the aqueous phase was extracted twice with EtOAc. The combined organic extracts were washed then with brine, dried over $NaSO_4$, filtered and concentrated under reduced pressure to give a yellow gum. The crude product was recrystallized from MTBE/hexane (1:1, v/v) to give 125.83 g of **86** (0.4349 mol, 94.0 %) as colourless solid.

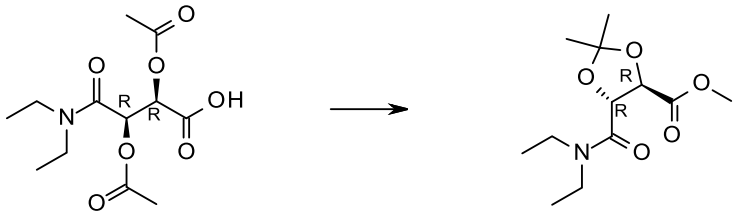
^{1}H NMR (200 MHz; $CDCl_3$): δ (ppm) = 9.21 (brs, 1H, COOH); 5.78 (d, *J* = 4.16, 1H; H-2); 5.58 (d, *J* = 4.04, 1H, H-3); 3.62-3.37 (m, 4H, $(CH_2)_2$); 2.15 (s, 6H, $(CH_3)_2$); 1.23 (t, *J* = 7.06, 3H, CH_3); 1.06 (t, *J* = 7.00, 3H, CH_3)

^{13}C NMR (50 MHz; $CDCl_3$): δ (ppm) = 170.2 (Cq, CO); 169.9 (Cq, CO); 169.0 (Cq, C-1); 165.2 (Cq, C-4); 69.9 (CH, C-2); 68.9 (CH, C-3); 42.2 (-N-$\underline{C}H_2$-CH_3); 41.2 (-N$\underline{C}H_2$-CH_3); 20.4 (CH_3); 20.3 (CH_3); 13.8 (-N-CH_2-$\underline{C}H_3$); 12.3 (N-CH_2-$\underline{C}H_3$)

TLC: R_f = 0.5 (DCM/MeOH, 2:1, v/v)

HPLC: Retention time: 2.5 min; Column: Macherey- Nagel Nucleosil C18, 5 µm, l = 125 mm, Ø = 4 mm; Mobile Phase: Acetic acid (70 %) 1 % in H_2O/30 % MeOH; Detection: RID and UV [λ = 230 nm]; Injection volume: 20 µL; Temperature: 40 °C; Flow rate: 1 ml/min; Run time: 15 min; Standard prep.: 10 mg/ml mobile phase

Methyl (4R,5R)-5-(diethylcarbamoyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylate **87**



86 **87**

To a stirred solution of **86** (249.2 g, 0.86 mol, 1.0 equiv.) in MeOH (400 ml), conc. sulphuric acid (10 ml, 0.19 mol 0.22 equiv.) was added at room temperature under an atmosphere of argon. The resulting mixture was heated under reflux for 10 hrs. After this time the reaction mixture was concentrated under reduced pressure. The residue was dissolved in acetone (300 ml) and 2,2-dimethoxypropane (300 ml, 2.42 mol, 2.81 equiv.) and was heated under reflux for 2 hours. After this time, TLC analysis (MTBE/PE, 1:1, v/v) showed complete consumption of **86** and the reaction mixture was evaporated under reduced pressure. The crude product was dissolved in 2 l EtOAc and was washed with 200 ml water. The layers were separated and the aqueous phase was extracted twice with EtOAc. The combined organic extracts were washed with aqueous saturated $NaHCO_3$ and brine, dried over $NaSO_4$, filtered and concentrated under reduced pressure to give 233.2 g of **87** (0.9 mol, 74.5 %) as yellow oil.

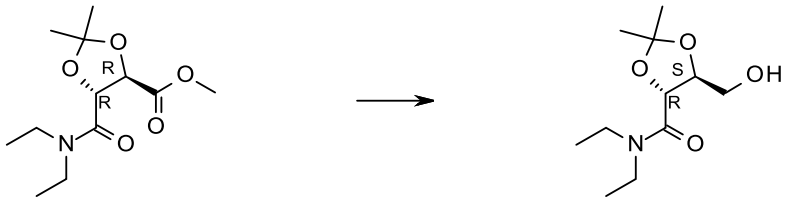
^{1}H NMR (200 MHz; $CDCl_3$): δ (ppm) = 5.26 (d, *J* = 5.5, 1H, H-3); 4.79 (d, *J* = 5.4, 1H, H-2); 3.73 (s, 3H, OCH_3); 3.49-3.23 (m, 4H, $(CH_2)_2$); 1.43 (s, 3H, CH_3, acetonide); 1.37 (s, 3H, CH_3, acetonide); 1.16 (t, *J* = 7.06, 3H, CH_3); 1.09 (t, *J* = 7.06, 3H, CH_3)

^{13}C NMR (50 MHz; $CDCl_3$): δ (ppm) = 171.1 (Cq, COO); 167.0 (Cq, CON); 112.8 (Cq, acetonide); 76.6 (CH, C-3); 76.1 (CH, C-2); 52.4 (OCH_3); 41.7 (-N-$\underline{C}H_2$-CH_3); 40.6 (-N-$\underline{C}H_2$-CH_3); 26.3 (CH_3, acetonide); 26.2 (CH_3, acetonide); 14.3 (-N-CH_2-$\underline{C}H_3$); 12.6 (-N-CH_2-$\underline{C}H_3$)

TLC: R_f = 0.53 (MTBE/PE, 1:1, v/v)

HPLC: Retention time: 15.2 min; Column: Macherey- Nagel Nucleosil C18, 5 µm, l = 125 mm, Ø = 4 mm; Mobile Phase: 70 % H_2O/30 % MeOH; Detection: RID and UV [λ = 230nm]; Injection volume: 20 µL; Temperature: 40 °C; Flow rate: 1 ml/min; Run time: 30 min; Standard prep.: 10 mg/ml mobile phase

(4R,5S)-N,N-diethyl-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolane-4-carboxamide **88**



87 **88**

Sodium borohydride (12 g, 0.317 mol 1.2 equiv.) was added in small portions to a stirred solution of **87** (100 g. 0.386 mol, 1.0 equiv.) in 150 ml dry THF at 10 - 25 °C under an atmosphere of argon. The resulting mixture was allowed to stir at room temperature for 4 hrs. The reaction was quenched by addition of water (60 ml). Then EtOAc (300 ml) was added, the layers were separated and the aqueous phase was extracted twice with EtOAc. The combined organic extracts were washed with brine, dried over $NaSO_4$ filtered and concentrated under reduced pressure to give 81.1 g of **88** (0.36 mol, 94.0 %) as yellow oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 4.63-4.60 (m, 1H, H-3); 4.46 (d, *J* = 7.6 Hz, 1H, H-2); 3.84 (dd, J^1 = 12.0 Hz, J^2 = 4.4 Hz, 1H, H-4a), 3.70 (dd, J^1 = 12.0 Hz, J^2 = 3.1 Hz, 1H, H-4b) 3.53-3.28 (m, 4H, $(CH_2)_2$); 2.67 (brs, 1H, OH); 1.42 (s, 3H, CH_3, acetonide); 1.38 (s, 3H, CH_3, acetonide); 1.18 (t, *J* = 7.1, 3H, CH_3); 1.10 (t, *J* = 7.1, 3H, CH_3)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 168.4 (Cq, CO); 110.0 (Cq, acetonide); 78.2 (CH, C-3); 75.0 (CH, C-2); 61.6 (CH_2, C-4); 41.7 (-N-$\underline{C}H_2$-CH_3); 40.4 (-N-$\underline{C}H_2$-CH_3); 26.8 (CH_3, acetonide); 26.0 (CH_3, acetonide); 14.3 (-N-CH_2-$\underline{C}H_3$); 12.5 (-N-CH_2-$\underline{C}H_3$)

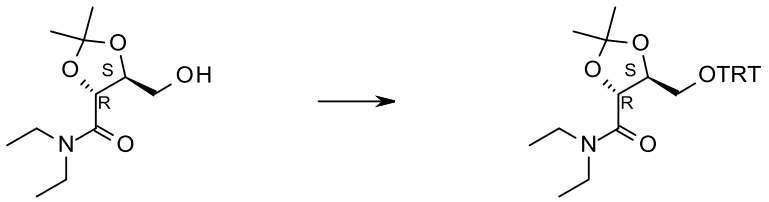
HRMS (ESI-TOF): calcd. for $[C_{11}H_{21}NO_4+H]^+$ 232.1549, found 232.1537; calcd. for $[C_{11}H_{21}NO_4+Na]^+$ 254.1368, found 254.1358

Optical Rotation $[\alpha_D^{20}]$: -9.9° (c = 2.02, $CHCl_3$)

TLC: R_f = 0.4 (MTBE)

HPLC: Retention time: 7.0 min; Column: Macherey- Nagel Nucleosil C18, 5 µm, l = 125 mm, Ø = 4 mm; Mobile Phase: 70 % H_2O/30 % MeOH; Detection: RID and UV [λ = 230nm]; Injection volume: 20 µL; Temperature: 40 °C; Flow rate: 1 ml/min; Run time: 15 min; Standard prep.: 10 mg/ml mobile phase

(4R,5S)-N,N-diethyl-2,2-dimethyl-5-(trityloxymethyl)-1,3-dioxolane-4-carboxamide **89**



88 **89**

Triethylamine (7.2 ml, 51.91 mmol, 1.2 equiv.) and DMAP (0.2g, 1.73 mmol) were added to a stirred solution of **88** (10 g, 43.26 mmol, 1.0 equiv.) in DCM (40 ml) at room temperature under an atmosphere of argon. The resulting mixture was allowed to stir for 5 min. After this time trityl chloride (16.9 g, 60.62 mmol, 1.4 equiv.) was added and the solution was allowed to stir for 4 hrs.

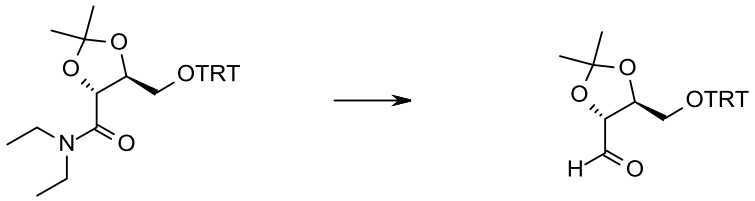
The reaction mixture was evaporated under reduced pressure and the crude product was dissolved in petroleum ether (40 ml) and washed with water (50 ml). The layers were separated and the aqueous phase was extracted with petroleum ether/MTBE (1:1, v/v). The combined organic extracts were washed with brine dried over $NaSO_4$, filtered and concentrated under reduced pressure to give 17.0 g of **89** (35.9 mmol, 83.0 % yield) as yellow oil.

^{1}H NMR (200 MHz; $CDCl_3$): δ (ppm) = 7.51-7.29 (m, 15H, Ar); 4.92-4.84 (m, 1H, H-3); 4.60 (d, *J* = 6.8, 1H, H-2); 3.49-3.31 (m, 6H, H-4a/b, $(CH_2)_2$); 1.52 (s, 3H, CH_3, acetonide); 1.50 (s, 3H, CH_3, acetonide); 1.17 (t, *J* = 7.1, 6H, $(CH_3)_2$)

^{13}C NMR (50 MHz; $CDCl_3$): δ (ppm) = 168.6 (Cq, CO); 143.7 (Cq, Ar); 128.6 (CH, Ar); 127.7 (CH, Ar); 126.8 (CH, Ar); 110.5 (Cq, acetonide); 86.9 (Cq, TRT); 77.2 (CH, C-2); 74.9 (CH, C-3); 63.5 (CH_2, C-4); 41.6 (-N-$\underline{C}H_2$-CH_3); 40.5 (-N-$\underline{C}H_2$-CH_3); 26.8 (CH_3, acetonide); 26.1(CH_3, acetonide); 14.5 (-N-CH_2-$\underline{C}H_3$); 12.8 (-N-CH_2-$\underline{C}H_3$)

TLC: Rf= 0.54 (MTBE/PE, 1:1, v/v)

(4R,5S)-2,2-dimethyl-5-(trityloxymethyl)-1,3-dioxolane-4-carbaldehyde **90**



89 **90**

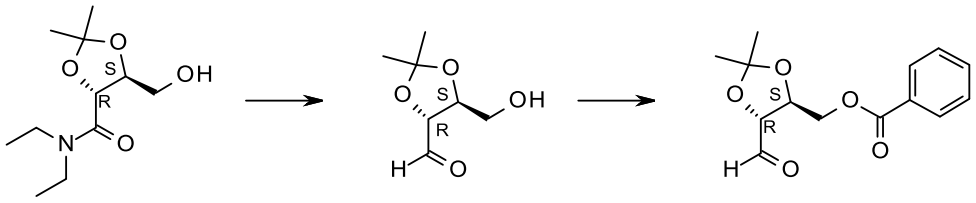
Sodium bis(2-methoxyethoxy)aluminumhydride, 65 wt % sol. in toluene (0.2 ml, 0.68 mmol, 1.0 equiv.) was added dropwise to a stirred solution of **89** (0.32 g, 0.68 mmol, 1.0 equiv.) in toluene (4 ml) at -10 °C under an atmosphere of argon. The resulting mixture was allowed to stir at -10 °C for 60 min. After this time, TLC analysis (MTBE/PE, 1:1, v/v) showed complete consumption of **89** and the reaction was quenched by addition of acetone (0.2 ml). Then MTBE (5 ml) and acetic acid (2.62 N, 5 ml) were added and the mixture was stirred at 10 °C for 10 min. The layers were separated and the aqueous phase was extracted with MTBE (5 ml). The combined organic extracts were washed with sat. $NaHCO_3$ (20 ml) and brine, dried over $NaSO_4$, filtered and concentrated under reduced pressure to give 0.256 g of **90** (0.64 mmol, 94.0 % yield, purity ~ 30 %) as cloudy oil.

^{1}H NMR (200 MHz; $CDCl_3$): δ (ppm) = 9.77 (s, 1H, H-1); 7.48-7.24 (m, 15H, Ar); 4.31-4.23 (m, 2H, H-4a/b); 3.42-3,21 (m, 2H, H-3, H-2); 1.51 (s, 3H, CH_3, acetonide); 1.45 (s, 3H, CH_3, acetonide)

^{13}C NMR (50 MHz; $CDCl_3$): δ (ppm) = 200.4 (CH, C-1); 143.6 (Cq, Ar); 128.6 (CH, Ar); 127.9 (CH, Ar); 127.1 (CH, Ar); 111.6 (Cq, acetonide); 87.0 (Cq, TRT); 82.5 (CH, C-2); 76.2 (CH, C-3); 64.0 (CH_2, C-4); 26.9 (CH_3, acetonide); 26.3 (CH_3, acetonide)

TLC: R_f = 0.22 (MTBE/PE, 1:1, v/v)

[(4S,5R)-5-formyl-2,2-dimethyl-1,3-dioxolan-4-yl]methyl benzoate **92**



88 **91** **92**

Sodium bis(2-methoxyethoxy)aluminumhydride, 70 wt % sol. in toluene (8.40 g , 30.1 mmol, 1.4 equiv.) was added dropwise to a stirred solution of **88** (5.0 g, 21.6 mmol, 1.0 equiv.) in toluene (30 ml) between 3 - 8°C under an atmosphere of argon. The resulting mixture was allowed to stir for 10 min. Then MTBE (30 ml) was added and the mixture was stirred for 1 hr. After this time TLC analysis (DCM/MeOH, 19:1, v/v) showed complete consumption of **88** and the reaction was quenched by addition of NaH_2PO_4 (10.37, 30.2 mmol, 4.0 equiv.) in water (83 ml). MTBE was added to the heterogenic mixture and then benzoylchloride (24.92 ml, 216.3 mmol, 10.0 equiv.) was added dropwise at 0 °C. To the resulting mixture NaOH (8.65 g, 0.216 mol, 10.0 equiv.) was added dropwise and the mixture was stirred at room temperature for 4 hrs.

After this time the layers were separated and the aqueous phase was extracted twice with MTBE. The combined organic extracts were washed with NH_4Cl and brine, dried over $NaSO_4$, filtered and concentrated under reduced pressure to the half of its volume.

Then a saturated solution of sodium hydrogensulphite in water (50 ml) was added and the resulting mixture was stirred at room temperature. After 3 hrs TLC analysis (MTBE) showed no more aldehyde in the organic phase. The layers were separated and the organic phase was extracted with water. The combined aqueous extracts were washed with MTBE several times. The pH of the combined aqueous extracts was adjusted to 9.0 with Na_2CO_3. Then MTBE (100 ml) was added and the mixture was stirred at room temperature for 18 hrs. The layers were separated and the aqueous phase was extracted three times with MTBE/EtOAc (1:1, v/v). The combined organic extracts were dried over $NaSO_4$, filtrated and concentrated under reduced pressure give 3.02 g of **92** (11.4 mmol, 52.9 % yield) as colourless oil.

Preparation of the sodium hydrogensulphite solution:

900.8 g (857.9 ml, 15.0 mol) acidic acid (glacial, 99-100 %) was dissolved in H_2O (2440 ml). To this solution Na_2SO_3 (1890 g) was added in 10 portion. The resulting solution

was allowed to stir at room temperature for 1hr. The precipitate was filtrated off to give a sodium hydrogensulphite solution (pH 5.29).

^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 9.83 (d, *J* = 1.2 Hz, 1H, H-1); 8.05 (m, 2H, Ar); 7.58 (m, 1H, Ar); 7.45 (m, 2H, Ar); 4.60-4.56 (m, 1H, H-4a); 4.47-4.44 (m, 2H, H-3, H-4b); 3.35-4.33 (m, 1H, H-2); 1.51 (s, 3H, CH_3, acetonide); 1.45 (s, 3H, CH_3, acetonide)

^{1}H-NMR spectroscopic data were in agreement with those reported in literature[179].

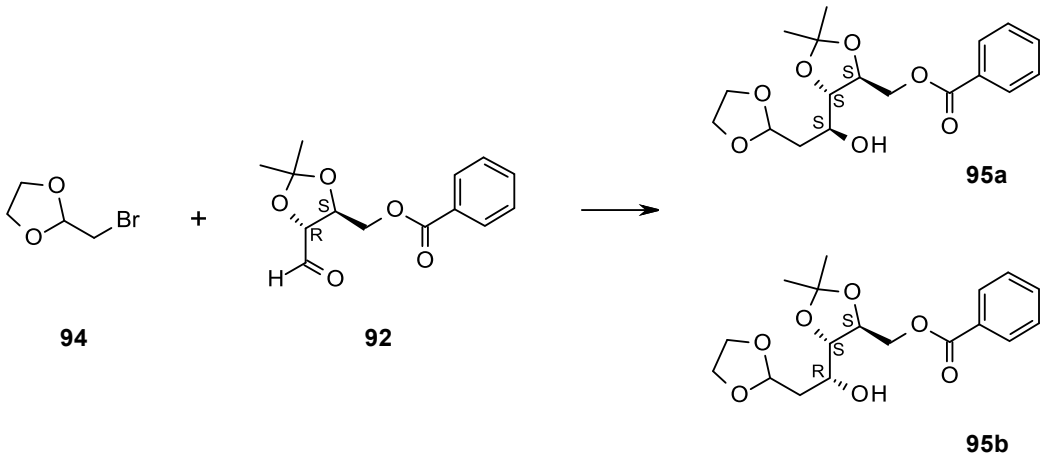
^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 200.6 (CH, C-1); 166.1 (Cq, CO); 133.9 (CH, Ar); 129.7 (CH, Ar); 129.5 (Cq, Ar); 128.4 (CH, Ar); 112.0 (Cq, acetonide); 82.0 (CH, C-2); 75.0 (CH, C-3); 64.0 (CH_2, C-4); 26.8 (CH_3, acetonide); 26.2 (CH_3, acetonide)

TLC: R_f = 0.29 (MTBE/PE, 2:1, v/v)

HRMS (ESI-TOF): calcd. for $[C_{14}H_{16}O_5+H]^+$ 265.1076, found 265.1060; calcd. for $[C_{14}H_{16}O_5+NH_4]^+$ 282.1341, found 282.1326; calcd. for $[C_{14}H_{16}O_5+Na]^+$ 287.0895, found 287.0847

HPLC: Retention time: 4.5 min; Column: Macherey- Nagel Nucleosil C18, 5 µm, l = 125 mm, Ø = 4 mm; Mobile Phase: A: H_2O, B: ACN; Gradient: 0 min 20 % B, 0-6 min 30 % B, 10-13 min 70 % B, 18-30 min 20 % B; Detection: UV [λ = 254 nm]; Injection volume: 20 µL; Temperature: 40 °C; Flow rate: 1 ml/min; Run time: 30 min; Standard prep.: 20 mg/100 ml mobile phase

[(4S,5S)-5-[(1S)-2-(1,3-dioxolan-2-yl)-1-hydroxy-ethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methyl benzoate **95a** and [(4S,5S)-5-[(1R)-2-(1,3-dioxolan-2-yl)-1-hydroxy-ethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methyl benzoate **95b**



94 **92** **95a** **95b**

A solution of magnesium (7.17 g, 0.295 mol, 2.2 equiv.) and a catalytic amount of iodine in dry THF (400 ml) was heated under reflux for 10 min under an atmosphere of argon. After this time 2-Bromomethyl-1,3-Dioxolane **94** (35.8 g, 0.214 mol, 1.6 equiv) was added dropwise and the solution was heated under reflux until the colour changed to a blur solution and the magnesium started reacting. After the complete addition, the mixture was allowed to stir at 90 °C for 2 hrs. At this stage, the reaction was cooled to room temperature and the aldehyde **92** (35.5g, 0.134 mol, 1.0 equiv.) solved in dry THF (150 ml) was added dropwise. The resulting mixture was allowed to stir at room temperature for 5 hrs. After this time, TLC analysis (PE/acetone, 1:1, v/v) showed complete consumption of **92**. The reaction was quenched by addition of aqueous saturated NH_4Cl (400 ml) and crushed ice (400 ml) and the mixture was stirred for 5 min. The layers were separated and the aqueous phase was extracted with EtOAc (2 x 200 ml). The combined organic extracts were washed with brine, dried over $NaSO_4$, filtered and concentrated under reduced pressure to give a mixture of two stereoisomers (**95a** and **95b**) in a 49:51 ratio as yellow viscous oil (44.65 g, 0.127 mol, 94.6 % yield).

[(4S,5S)-5-[(1S)-2-(1,3-dioxolan-2-yl)-1-hydroxy-ethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methyl benzoate **95a**

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 8.07-8.05 (m, 2H, Ar); 7.57-7.53 (m, 1H, Ar); 7.45-7.41 (m, 2H, Ar); 5.10-5.08 (m, 1H, H-1); 4.66 (dd, J^1 = 11.6 Hz, J^2 = 2.5 Hz, 1H, H-6a); 4.44-4.32 (m, 2H, H-6b, H-5); 4.09-3.77 (m, 6H, H-4, H-3, $(OCH_2)_2$); 2.18-2.14 (m, 1H, H-2a); 1.90-1.84 (m, 1H, H-2b); 1.42 (s, 3H, CH_3, acetonide); 1.41 (s, 3H, CH_3, acetonide)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 166.44 (Cq, CO); 132.10 (CH, Ar); 129.93 (Cq, Ar); 129.71 (CH, Ar); 128.30 (CH, Ar); 109.79 (Cq, acetonide) 103.31 (CH, C-1); 79.28 (CH, C-4); 77.76 (CH, C-5); 69.40 (CH, C-3); 65.48 (CH_2, C-6); 64.95 (OCH_2); 64.76 (OCH_2); 36.76 (CH_2, C-2); 27.13 (CH_3, acetonide); 27.09 (CH_3, acetonide)

TLC: R_f = 0.36 (MTBE/PE, 1:1, v/v)

HRMS (ESI-TOF): calcd. for $[C_{18}H_{24}O_7+H]^+$ 353.1600, found 353.1589; calcd. for $[C_{18}H_{24}O_7+NH_4]^+$ 370.1865, found 370.1855; calcd. for $[C_{18}H_{24}O_7+Na]^+$ 375.1419, found 375.1480; calcd. for $[C_{18}H_{24}O_7+K]^+$ 391.1159, found 391.1154

[(4S,5S)-5-[(1R)-2-(1,3-dioxolan-2-yl)-1-hydroxy-ethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methyl benzoate **95b**

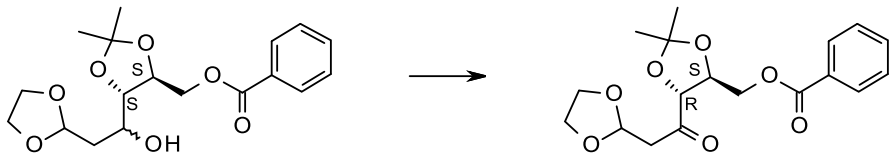
^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 8.07-8.05 (m, 2H, Ar); 7.57-7.53 (m, 1H, Ar); 7.45-7.41 (m, 2H, Ar); 5.10-5.08 (m, 1H, H-1); 4.56-4.52 (m, 1H, H-6a); 4.44-4.32 (m, 2H, H-6b, H-5); 4.09-3.77 (m, 6H, H-4, H-3, $(OCH_2)_2$); 2.03-1.92 (m, 2H, H-2a/b); 1.45 (s, 3H, CH_3, acetonide); 1.44 (s, 3H, CH_3, acetonide)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 166.33 (Cq, CO); 133.12 (CH, Ar); 129.93 (Cq, Ar); 129.71 (CH, Ar); 128.36 (CH, Ar); 109.91 (C_q, acetonide) 102.77 (CH, C-1); 80.27 (CH, C-4); 75.03 (CH, C-5); 66.78 (CH, C-3); 64.95 (OCH_2); 64.76 (OCH_2); 64.68 (CH_2, C-6); 36.38 (CH_2, C-2); 27.06 (CH_3, acetonide); 27.02 (CH_3, acetonide)

TLC: R_f = 0.51 (MTBE/PE, 1:1, v/v)

HRMS (ESI-TOF): calcd. for $[C_{18}H_{24}O_7+H]^+$ 353.1600, found 353.1589; calcd. for $[C_{18}H_{24}O_7+NH_4]^+$ 370.1865, found 370.1855; calcd. for $[C_{18}H_{24}O_7+Na]^+$ 375.1419, found 375.1480; calcd. for $[C_{18}H_{24}O_7+K]^+$ 391.1159, found 391.1154

[(4S,5R)-5-[2-(1,3-dioxolan-2-yl)acetyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methyl benzoate **96**



95a+b **96**

Oxalyl chloride (5.48 g, 43.17 mmol, 1.5 equiv.) was added dropwise to a solution of dry DMSO (6.74 g, 86.27 mmol, 3.0 equiv.) and dry DCM (40 ml) at -60 °C under an atmosphere of argon. The resulting solution was then stirred for 40 min at -60 °C. After this time a solution of **95a**+**b** (10.14 g, 28.77 mmol, 1.0 equiv.) in dry DCM (50 ml) was added dropwise to the mixture. During the addition the temperature of the mixture should not exceed -60 °C. The solution was then stirred for 50 min at -70 °C. Afterwards triethylamine (21.82 g 215.6 mmol, 7.5 equiv.) was added dropwise and the solution was stirred for further 30 min at -70 °C and was then allowed to warm to room temperature. Then water (100 ml) and DCM (20 ml) were added and the aqueous layer was separated and extracted with 100 ml DCM. The combined extracts were washed with sulphuric acid (100 ml, 0.1 %), sat. $NaHCO_3$ (100 ml) and brine, dried over $NaSO_4$, filtered and concentrated under reduced pressure to give 9.8 g of **96** (27.97 mmol, 97.2 % yield) as cloudy oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 8.02 (d, *J* = 7.2 Hz, 2H, Ar); 7.53 (t, *J* = 7.2 Hz, 1H, Ar); 7.41 (t, *J* = 7.6 Hz, 2H, Ar); 5.30 (t, *J* = 5.2 Hz, 1H, H-1); 4.62 (dd, J^1 = 11.6 Hz, J^2 = 3.1 Hz, 1H, H-6a); 4.37 (m, 3H, H-4, H-5, H-6b); 3.94 (m, 2H, OCH_2); 3.83 (m, 2H, OCH_2); 3.04 (d, *J* = 5.3, 2H, H-2a/b); 1.44 (s, 3H, CH_3, acetonide); 1.41 (s, 3H, CH_3, acetonide)

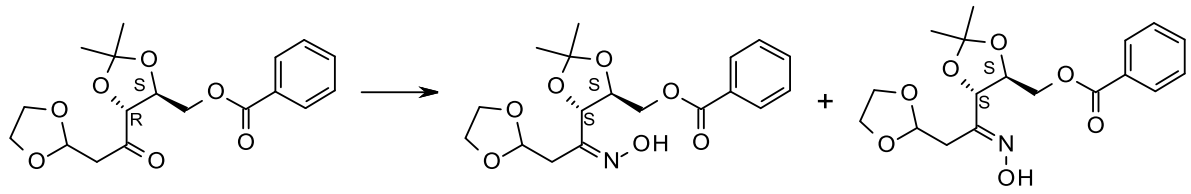
^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 206.09 (Cq, C-3); 166.03 (Cq, COO); 133.03 (CH, Ar); 129.60 (CH, Ar); 129.48 (CH, Ar); 128.34 (Cq, Ar); 128.27 (CH, Ar); 111.35 (Cq, acetonide); 100.42 (CH, C-1); 81.74 (CH, C-4); 75.52 (CH, C-5); 64.85 (OCH_2); 64.83 (OCH_2); 64.07 (CH_2, C-6); 43.53 (CH_2, C-2); 26.77 (CH_3, acetonide); 26.15 (CH_3, acetonide)

Optical rotation $[\alpha_D^{20}]$: + 9.7° (c = 2.00, $CHCl_3$)

TLC: R_f = 0.86 (MTBE) and R_f = 0.67 (MTBE/PE, 2:1, v/v)

HRMS (ESI-TOF): calcd. for $[C_{18}H_{22}O_7+NH_4]^+$ 368.1709, found 368.1702; calcd. for $[C_{18}H_{22}O_7+Na]^+$ 373.1263, found 373.1267; calcd. for $[C_{18}H_{22}O_7+K]^+$ 389.1002, found 389.1000

[(4S,5S)-5-[(E)-C-(1,3-dioxolan-2-ylmethyl)-N-hydroxy-carbonimidoyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methyl benzoate **97a** and
[(4S,5S)-5-[(Z)-C-(1,3-dioxolan-2-ylmethyl)-N-hydroxy-carbonimidoyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methyl benzoate **97b**



96 **97a** (*E*) **97b** (*Z*)

Hydroxylamine hydrochloride (18.1 g, 260.4 mmol, 6.5 equiv.) was added in 5 portions to a solution of **96** (14.04 g, 40.07 mmol, 1.0 equiv.) in dry pyridine (135 ml) under an atmosphere of argon. The resulting mixture was allowed to stir at 55 °C for 17 hrs. After this time, TLC analysis (acetone/petroleum ether, 1:3, v/v) showed complete consumption of **96** and the mixture was evaporated under reduced pressure. Then water (200 ml) and EtOAc (150 ml) were added and the mixture was stirred for 5 min. The layers were separated and the aqueous phase was extracted with EtOAc (4x 100 ml). The combined organic extracts were washed with brine, dried over $NaSO_4$, filtered and concentrated under reduced pressure give a mixture of two *isomers* (**97a** and **97b**) in a 75:25 (*E*:*Z*) ratio as yellow oil (14.6 g, 39.96 mmol 77.4 % yield).

[(4S,5S)-5-[(E)-C-(1,3-dioxolan-2-ylmethyl)-N-hydroxy-carbonimidoyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methyl benzoate **97a**

^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 8.05 (d, *J* = 7.2 Hz, 2H, Ar); 7.56 (t, *J* = 7.2 Hz, 1H, Ar); 7.43 (t, *J* = 7.7 Hz, 2H, Ar); 5.31 (t, *J* = 4.8 Hz, 1H, H-1); 4.61-4.55 (m, 3H, H-4, H-5, H-6a); 4.42-4.39 (m, 1H, H-6b); 4.03-3.76 (m, 4H, $(OCH_2)_2$); 2.90-2.66 (m, 2H, H-2a/b); 1.46 (s, 6H, $(CH_3)_2$, acetonide)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 166.29 (Cq, COO); 154.04 (Cq, CNO); 133.05 (CH, Ar); 129.90 (Cq, Ar); 129.73 (CH, Ar); 128.33 (CH, Ar); 110.14 (Cq, acetonide); 100.65 (CH, C-1); 78.05 (CH, C-4); 75.64 (CH, C-5); 64.99 (OCH_2); 64.77 (OCH_2); 63.70 (CH_2, C-6); 30.55 (CH_2, C-2); 26.00 (CH_3, acetonide); 26.83 (CH_3, acetonide)

Optical rotation $[\alpha_D^{20}]$: + 15.0° (c = 2.2, $CHCl_3$)

TLC: R_f = 0.53 (MTBE/PE, 1:1, v/v)

HRMS (ESI-TOF): calcd. for $[C_{18}H_{23}NO_7+H]^+$ 366.1553, found 366.1555; calcd. for $[C_{18}H_{23}O_7+Na]^+$ 388.1372, found 388.1374; calcd. for $[C_{18}H_{23}O_7+K]^+$ 404.1111, found 404.1107

[(4S,5S)-5-[(Z)-C-(1,3-dioxolan-2-ylmethyl)-N-hydroxy-carbonimidoyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methyl benzoate **97b**

^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 8.05 (d, *J* = 7.2 Hz, 2H, Ar); 7.56 (t, *J* = 7.2 Hz, 1H, Ar); 7.43 (t, *J* = 7.7 Hz, 2H, Ar); 5.39 (d, *J* = 8.2 Hz 1H, H-4); 5.24 (t, *J* = 5.2 Hz, 1H, H-1); 4.76-4.74 (m, 1H, H-6a); 4.52-4.47 (m, 1H, H-6b); 4.25-4.21 (m, 1H, H-5); 4.03-3.76 (m, 4H, $(OCH_2)_2$); 2.90-2.66 (m, 2H, H-2a/b); 1.46 (s, 6H, $(CH_3)_2$, acetonide)

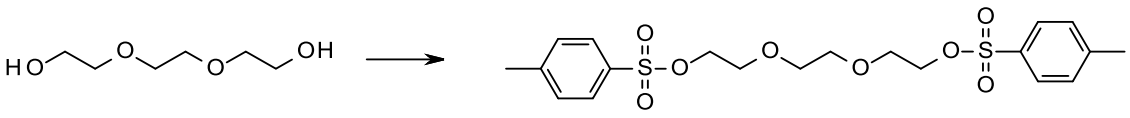
^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 166.36 (Cq, COO); 155.33 (Cq, CNO); 133.01 (CH, Ar); 129.90 (Cq, Ar); 129.69 (CH, Ar); 128.40 (CH, Ar); 110.56 (Cq, acetonide); 101.89 (CH, C-1); 77.43 (CH, C-5); 71.83 (CH, C-4); 64.93 (OCH_2); 64.79 (OCH_2); 64.22 (CH_2, C-6); 34.59 (CH2, C-2); 26.00 (CH_3, acetonide); 26.83 (CH_3, acetonide)

Optical rotation $[\alpha_D^{20}]$: - 21.6° (c = 2.6, $CHCl_3$)

TLC: R_f = 0.36 (MTBE/PE, 1:1, v/v)

HRMS (ESI-TOF): calcd. for $[C_{18}H_{23}NO_7+H]^+$ 366.1553, found 366.1555; calcd. for $[C_{18}H_{23}O_7+Na]^+$ 388.1372, found 388.1374; calcd. for $[C_{18}H_{23}O_7+K]^+$ 404.1111, found 404.1107

2-[2-[2-(p-tolylsulfonyloxy)ethoxy]ethoxy]ethyl 4-methylbenzenesulfonate **99**



98 (commercial) **99**

A mixture of triethylene glycol **98** (2.01 g, 13.38 mmol, 1.0 equiv.) in THF (8 ml) was added to a solution of sodium hydroxide (1.62 g, 40.51 mol, 3.03 equiv.) in water (8 ml) at 5 °C. Then p-toluenesulfonyl chloride (5.71 g, 29.95 mmol, 2.24 equiv.) in THF (10 ml) was added dropwise over 5 min. The resulting mixture was allowed to stir at 5 °C for 2 hrs. The reaction mixture was then poured into ice-water (100 ml). After the addition of DCM (450 ml) the layers were separated and the aqueous phase was extracted twice with DCM. The combined organic extracts were washed with H_2O and brine, dried over $NaSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with MTBE/PE (1:1, v/v) to give **99** (5.95 g, 12.98 mmol, 96.9 % yield) as a white solid.

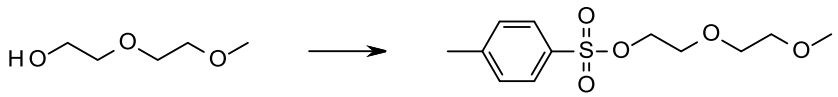
^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 7.79 (d, *J* = 8.3 Hz, 2H, Ar); 7.34 (d, *J* = 8.1 Hz, 2H, Ar); 4.13 (t, *J* = 4.8 Hz, 4H, $(CH_2)_2$); 3.65 (t, *J* = 4.8 Hz, 4H, $(CH_2)_2$); 3.52 (s, 4H, $(CH_2)_2$); 2.44 (s, 3H, CH_3)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 144.85 (Cq, Ar); 132.90 (Cq, Ar); 129.83 (CH, Ar); 127.94 (CH, Ar); 70.66 (CH_2); 69.18 (CH_2); 68.72 (CH_2); 21.62 (CH_3)

^{1}H-NMR spectroscopic data were in agreement with those reported in literature[255,256].

TLC: R_f = 0.1 (MTBE/PE, 1:1, v/v)

2-(2-methoxyethoxy)ethyl 4-methylbenzenesulfonate **101**



100 commercial **101**

Aqueous NaOH (6 N, 200 ml, 1.18 mol, 1.39 equiv.) was added to a stirred solution of 2-(2-Methoxyethoxy)ethanol (102.3 g, 0.851 mol, 1.0 equiv.) in dry THF (150 ml) at 5 °C. A solution of p-toluenesulfonyl chloride (172.9 g, 0.907 mol, 1.06 equiv.) in THF (350 ml) was added dropwise over 30 min. The resulting mixture was allowed to stir at 5 °C for 3.5 hrs. After this time, TLC analysis (DCM/MeOH, 100:3, v/v) showed complete consumption of 2-(2-methoxyethoxy)ethyl 4-methylbenzenesulfonate and the reaction was quenched by addition of crushed ice (400 ml). After the addition of DCM (450 ml) the layers were separated and the aqueous phase was once extracted with DCM. The combined organic extracts were washed with H_2O (400 ml) and brine (600 ml), dried over $NaSO_4$, filtered and concentrated under reduced pressure to give 230.1 g of **101** (0.839 mol, 98.05 %) as clear oil.

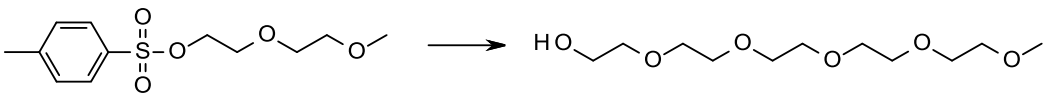
^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 7.79 (d, *J* = 8.3 Hz, 2H, Ar); 7.32 (d, *J* = 8.3 Hz, 2H, Ar); 4.16 (t, *J* = 5.1 Hz, 2H, CH_2); 3.68 (t, *J* = 5.1 Hz, 2H, CH_2); 3.60-3.44 (m, 4H, $(CH_2)_2$); 3.34 (s, 3H, OCH_3); 2.44 (s, 3H, CH_3)

^{1}H-NMR spectroscopic data were in agreement with those reported in literature[257].

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 144.93 (Cq, Ar); 133.15 (Cq, Ar); 129.94 (CH, Ar); 128.13 (CH, Ar); 71.95 (CH_2); 70.81 (CH_2); 69.35 (CH_2); 68.85 (CH_2); 59.17 (OCH_3); 21.76 (CH_3)

TLC: R_f = 0.44 (DCM/MeOH, 100:3, v/v)

2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethanol **102**



101 **102**

A 60 % dispersion of sodium hydride in mineral oil (1.76 g, 44.01 mmol, 1.2 equiv.) was washed three times with petroleum ether (3 x 10 ml) and then added in portions to a stirred solution of triethylene glycol (6.6 g, 43.95 mmol, 1.2 equiv.) in dry THF (40 ml) under an atmosphere of argon. The resulting grey slurry was allowed to stir for 10 min. After this time a solution of **101** (6.44 g, 25.52 mmol, 1.0 equiv.) in dry THF (20 ml) was added dropwise and the reaction mixture was heated at 65 °C for 14 hrs. The mixture was cooled to room temperature and the reaction was quenched by addition of saturated aqueous ammonium chloride solution (100 ml).

The layers were separated and the aqueous phase was extracted three times with DCM (3 x 80 ml). The combined organic extracts were dried over $NaSO_4$, filtered and concentrated under reduced pressure to give 9.35 g of **102** (23.0 mmol, 90.1 % yield) as yellow oil.

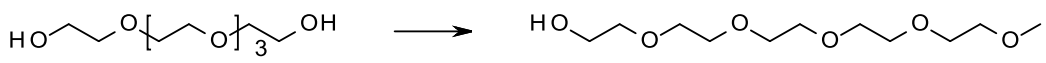
^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 3.79-3.53 (m, 20H, $(CH_2)_{10}$); 3.38 (s, 3H, OCH_3)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 72.02 ($MeO\underline{C}H_2$); 69.81, 69.65, 69.45, 68.97 ($-\underline{C}H_2-(O-\underline{C}H_2-\underline{C}H_2)_4-OH$); 58.98 ($O\underline{C}H_3$)

NMR spectroscopic data were in agreement with those reported in literature[258].

TLC: R_f = 0.14 (DCM/MeOH, 100:3, v/v)

2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethanol **102**



105 **102**

A 60 % dispersion of sodium hydride in mineral oil (0.935 g, 23.38 mmol, 1.1 equiv.) was washed three times with petroleum ether (3 x 10 ml) and then added in portions to a stirred solution of pentaethylene glycol **105** (5.04 g, 21.15 mmol, 1.0 equiv.) in dry THF (50 ml) under an atmosphere of argon. The resulting grey slurry was allowed to stir for 30 min. After this time methyl iodide (3.0 g, 21.13 mmol, 1.0 equiv.) was added and the reaction mixture was stirred at 5 °C for 2 hrs. The mixture was then warmed to room temperature and was stirred for another 2 hrs. The mixture was then poured into water and the aqueous phase was extracted with a mixture of MTBE (20 ml) and EtOAc (35 ml). The combined organic extracts were dried over $NaSO_4$, filtered and concentrated under reduced pressure to give 4.94 g of **102** (19.58 mmol, 92.6 % yield) as brown-yellow oil.

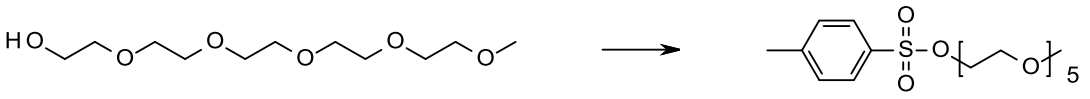
^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 3.79-3.53 (m, 20H, $(CH_2)_{10}$); 3.38 (s, 3H, OCH_3)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 72.02 ($MeO\underline{C}H_2$); 69.81, 69.65, 69.45, 68.97 ($-\underline{C}H_2-(O-\underline{C}H_2-\underline{C}H_2)_4-OH$); 58.98 ($O\underline{C}H_3$)

NMR spectroscopic data were in agreement with those reported in literature[258].

TLC: R_f = 0.14 (DCM/MeOH, 100:3, v/v)

2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethyl 4-methylbenzene-sulfonate **103**



102 **103**

Aqueous NaOH (2 N, 20 ml, 40.0 mmol, 1.6 equiv) was added to a stirred solution of **102** (6.44 g, 25.52 mol, 1.0 equiv) in dry THF (20 ml) at 5 °C. A solution of p-toluenesulfonyl chloride (5.85 g, 30.68 mol, 1.2 equiv.) in THF (20 ml) was added dropwise over 5 min. The resulting mixture was allowed to stir at 5 °C for 2.5 hrs. After this time, TLC analysis (EtOAc), showed complete consumption of **102** and the reaction was quenched by addition of crushed ice (50 ml).

After the addition of DCM (80 ml) the layers were separated and the aqueous phase was once extracted with DCM. The combined organic extracts were washed with H_2O (100 ml) and brine (100 ml), dried over $NaSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with EtOAc to give 5.52 g of **103** (13.58 mmol, 53.2 % yield) as clear yellow oil.

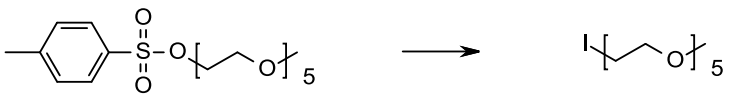
^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 7.78 (d, *J* = 8.0 Hz, 2H, Ar); 7.33 (d, *J* = 7.9 Hz, 2H, Ar); 4.14 (t, *J* = 4.7 Hz, 2H, CH_2); 3.67 (t, *J* = 4.7 Hz, 2H, CH_2); 3.68-3.57 (m, 18H, (-CH_2-CH_2-O)$_4$-CH_2); 3.54-3.52 (m, 2H, MeOCH_2); 3.36 (s, 3H, OCH_3); 2.43 (s, 3H, CH_3)

^{1}H-NMR spectroscopic data were in agreement with those reported in literature[259].

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 144.75 (Cq, Ar); 132.90 (Cq, Ar); 129.77 (CH, Ar); 127.92 (CH, Ar); 71.85 (CH_2); 70.68 (CH_2); 70.53 (CH_2); 70.49 (CH_2); 70.45 (CH_2); 69.19 (CH_2); 68.61 (CH_2); 58.98 (OCH_3); 21.59 (CH_3)

TLC: R_f = 0.43 (EtOAc)

1-[2-[2-[2-(2-iodoethoxy)ethoxy]ethoxy]ethoxy]-2-methoxy-ethane **104**



103 **104**

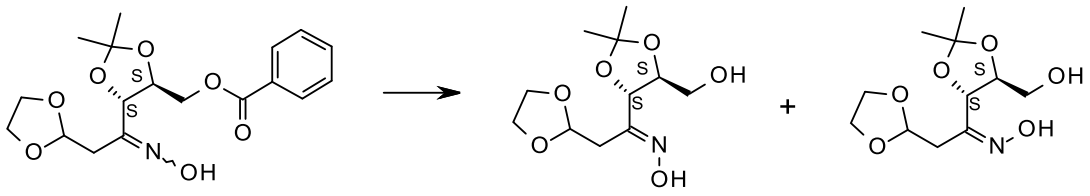
Sodium iodide (1.7 g, 11.34 mmol, 1.5 equiv.) was added to a solution of **103** (3.01 g, 7.404 mmol, 1.0 equiv.) in anhydrous acetone (25 ml) under an atmosphere of argon. The resulting mixture was allowed to stir under the exclusion of light at room temperature for 48 hrs. After this time TLC analysis (hexane/EtOAc, 10:3, v/v) showed complete consumption of **103**. The solution was then filtered and concentrated under reduced pressure. The residue was dissolved in EtOAc (30 ml) and was washed twice with a aqueous solution of sodium thiosulfate (0.1 M), dried over $NaSO_4$, filtered and concentrated under reduced pressure give **104** as brown-yellow oil (1.01 g, 2.789 mmol 37.7 % yield).

^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 3.75 (2H, CH_2), 3.63-3.55 (m, 16H, $(CH_2)_8$); 3.37 (s, 3H, OCH_3); 3.25 (d, *J* = 6.9 Hz, 2H, I-CH_2)

^{1}H-NMR spectroscopic data were in agreement with those reported in literature[260].

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 71.8 (CH_3); 70.2, 70.1 (CH_2); 61.2 (OCH_3)

(E)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl]ethanone oxime **106a** and
(Z)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl]ethanone oxime **106b**



97a+b **106a** (*E*) **106b** (*Z*)

Aqueous NaOH (2 N, 32 ml, 63.96 mmol, 1.5 equiv.) was added to a stirred solution of **97a+b** (16.0 g, 43.79 mmol, 1.0 equiv.) in THF/H_2O (340 ml, 1:1) at room temperature . The resulting solution was allowed to stir at 60 °C for 18 hrs. After this time TLC analysis (MTBE/PE, 2:1, v/v) showed complete consumption of **97a+b** and the reaction was cooled to room temperature. Afterwards MTBE (200 ml) was added, the layers were separated and the aqueous phase was extracted with EtOAc (4 x 200 ml). The combined organic extracts were washed with brine, dried over $NaSO_4$, filtered and concentrated under reduced pressure to give a mixture of two isomers (**106a** and **106b**) correspondent to the starting material in a 75:25 ratio as yellow *oil* (9.31 g, 35.63 mmol, 81.3% yield).

(E)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl]ethanone oxime **106a**

^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 9.07 (brs, 1H, OH); 5.35 (t, *J* = 5.3 Hz, 1H, H-1); 4.45 (d, *J* = 8.5 Hz, 1H, H-4,); 4.29-4.26 (m, 1H, H-5); 4.01-3.93 (m, 2H, OCH_2); 3.88-3.80 (m, 3H, OCH_2, H-6a); 3.70 (dd, J^1 = 12.0, J^2 =3.83, H-6b); 3.09 (brs, 1H, OH); 2.85-2.79 (m, 2H, H-2a/b); 1.43 (s, 6H, $(CH_3)_2$)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 154.07 (Cq, CNO); 109.66 (Cq, acetonide); 100.69 (CH, C-1); 78.23 (CH, C-5); 72.44 (CH, C-4); 64.75 (OCH_2); 64.72(OCH_2); 61.37 (CH_2, C-6); 30.60 (CH_2, C-2); 26.90 (CH_3, acetonide); 26.75 (CH_3, acetonide)

Optical rotation $[\alpha_D^{20}]$: - 1.7° (c = 2.0, $CHCl_3$)

TLC: R_f = 0.33 (MTBE/PE, 3:1, v/v)

HRMS (ESI-TOF): calcd. for $[C_{11}H_{19}NO_6+Na]^+$ 284.1110, found 284.1112

(Z)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl]ethanone oxime **106b**

^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 9.07 (brs, 1H, OH); 5.23-5.21 (m, 2H, H-1, H-4); 4.01-3.85 (m, 6H, H-5, H-6a/b, $(OCH_2)_2$); 3.09 (brs, 1H, OH); 2.79-2.63 (m, 2H, H-2a/b); 1.43 (s, 6H, $(CH_3)_2$)

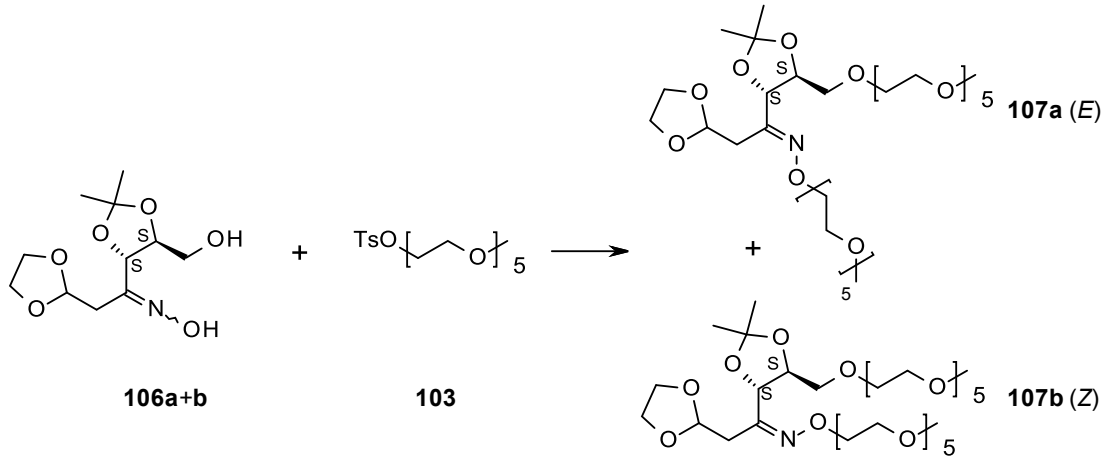
^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 155.50 (Cq, CNO); 109.88 (Cq, acetonide); 101.84 (CH, C-1); 79.56 (CH, C-5); 77.71 (CH, C-4); 64.88 (OCH_2); 64.84 (OCH_2); 62.15 (CH_2, C-6); 34.53 (CH_2, C-2); 26.90 (CH_3, acetonide); 26.75 (CH_3, acetonide)

Optical rotation $[\alpha_D^{20}]$: + 52.0° (c = 2.0, $CHCl_3$)

TLC: R_f = 0.21 (MTBE/PE, 3:1, v/v)

HRMS (ESI-TOF): calcd. for $[C_{11}H_{19}NO_6+Na]^+$ 284.1110, found 284.1112

(E)-2-(1,3-dioxolan-2-yl)-N-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]-ethoxy]-1-[(4S,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy-methyl]-2,2-dimethyl-1,3-dioxolan-4-yl]ethanimine **107a** and
(Z)-2-(1,3-dioxolan-2-yl)-N-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]-ethoxy]-1-[(4S,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy-methyl]-2,2-dimethyl-1,3-dioxolan-4-yl]ethanimine **107b**



106a+b **103**

Sodium hydride 95 % in mineral oil (2.26 g, 89.49 mmol, 3.0 equiv.) was added in portions to a stirred solution of **106a+b** (7.8 g, 29.37 mmol, 1.0 equiv.) in dry THF (150 ml) under an atmosphere of argon. The resulting grey slurry was allowed to stir for 1.5 hrs at room temperature. After this time a solution of **103** (24.3 g, 59.87mmol, 2.0 equiv.) in dry THF (50 ml) was added dropwise and the reaction mixture was heated at 60 °C for 5 hrs. After this time TLC analysis (acetone/toluene, 2:1, v/v) showed complete consumption of **106a+b**. The mixture was cooled to room temperature and quenched by addition of saturated aqueous ammonium chloride solution (100 ml). Then DCM (300 ml) was added, the layers were separated and the aqueous phase was extracted four times with DCM (4x 100 ml). The combined organic extracts were dried over $NaSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with toluene/acetone (2:1, v/v) to give a mixture of two isomers (**107a** and **107b**) correspondent to the starting material in a 89:11 ratio as yellow oil (12.4 g, 25.02 mmol, 83.8 % yield).

(E)-2-(1,3-dioxolan-2-yl)-N-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]-ethoxy]-1-[(4S,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy-methyl]-2,2-dimethyl-1,3-dioxolan-4-yl]ethanimine **107a**

^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 5.32 (t, *J* = 5.3 Hz, 1H, H-1); 4.40-37 (m, 1H, H-5); 4.28 (d, *J* = 8.2 Hz, 1H, H-4,); 4.20-4.19 (m, 2H, OCH_2); 3.99-3.91 (m, 2H, OCH_2); 3.87-3.77

(m, 2H, OCH_2); 3.71-3.57 (m, 38H, H-6, (-CH_2-(O-CH_2-CH_2)$_4$-O-)$_2$); 3.54-3.52 (m, 4H, ($MeOCH_2$)$_2$); 3.35 (s, 6H, (OCH_3)$_2$); 2.73 (d, *J* = 5.7 Hz, 2H, H-2a/b); 1.42 (s, 3H, CH_3); 1.40 (s, 3H, CH_3)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 153.37 (Cq, CNO); 109.90 (Cq, acetonide); 100.64 (CH, C-1); 77.71 (CH, C-4); 77.20 (CH, C-5); 73.37, 71.86, 71.04, 70.91, 70.53, 70.50, 70.44, 70.36 69.42 64.63 (OCH_2); 58.98 (OCH_3); 31.31 (CH_2, C-2); 27.04 (CH_3, acetonide); 26.80 (CH_3, acetonide)

Optical rotation $[\alpha_D^{20}]$: - 7.6° (c = 3.8, $CHCl_3$)

TLC: R_f = 0.56 (toluene/acetone 2:1, v/v)

HRMS (ESI-TOF): calcd. for $[C_{33}H_{63}NO_{16}+H]^+$ 730.4225, found 730.4221; calcd. for $[C_{33}H_{63}NO_{16}+NH_4]^+$ 747.4491, found 747.4498; calcd. for $[C_{33}H_{63}NO_{16}+Na]^+$ 752.4044, found 752.4059; calcd. for $[C_{33}H_{63}NO_{16}+K]^+$ 768.3784, found 768.3783

(Z)-2-(1,3-dioxolan-2-yl)-N-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]-ethoxy]-1-[(4S,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy-methyl]-2,2-dimethyl-1,3-dioxolan-4-yl]ethanimine **107b**

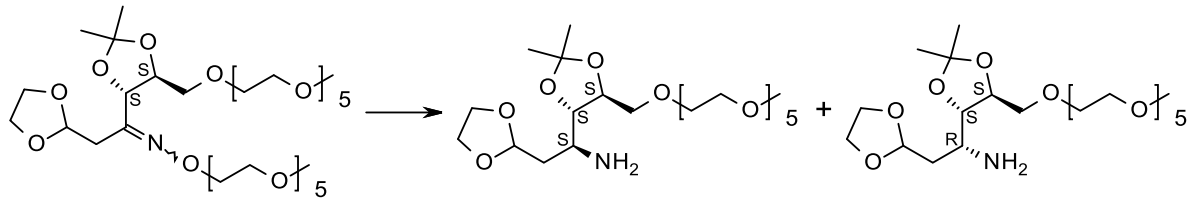
^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 5.22 (t, *J* = 5.2 Hz, 1H, H-1); 5.08 (d, *J* = 8.5 Hz, 1H, H-4,); 4.40-37 (m, 1H, H-5); 4.20-4.19 (m, 2H, OCH_2); 3.99-3.91 (m, 2H, OCH_2); 3.87-3.77 (m, 2H, OCH_2); 3.71-3.57 (m, 38H, H-6, (-CH_2-(O-CH_2-CH_2)$_4$-O-)$_2$); 3.54-3.52 (m, 4H, ($MeOCH_2$)$_2$); 3.35 (s, 6H, (OCH_3)$_2$); 2.73 (d, *J* = 5.7 Hz, 2H, H-2a/b); 1.42 (s, 3H, CH_3); 1.40 (s, 3H, CH_3)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 154.78 (Cq, CNO); 110.31 (Cq, acetonide); 100.64 (CH, C-1); 77.71 (CH, C-4); 77.20 (CH, C-5); 73.37, 71.86, 71.04, 70.91, 70.53, 70.50, 70.44, 70.36, 69.42, 64.63 (OCH_2); 58.98 (OCH_3); 34.92 (CH_2, C-2); 27.04 (CH_3, acetonide); 26.80 (CH_3, acetonide)

TLC: R_f = 0.56 (toluene/acetone 2:1, v/v)

HRMS (ESI-TOF): calcd. for $[C_{33}H_{63}NO_{16}+H]^+$ 730.4225, found 730.4221; calcd. for $[C_{33}H_{63}NO_{16}+NH_4]^+$ 747.4491, found 747.4498; calcd. for $[C_{33}H_{63}NO_{16}+Na]^+$ 752.4044, found 752.4059; calcd. for $[C_{33}H_{63}NO_{16}+K]^+$ 768.3784, found 768.3783

(1S)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]ethanamine **108a** and
(1R)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]ethanamine **108b**



107a+b **108a** **108b**

Lithium aluminium hydride (3.1 g, 81.69 mmol, 3.3 equiv.) was slowly added to a stirred solution of **107a+b** (12.4 g, 25.02mmol, 1.0 equiv.) in dry THF (125 ml) at 0 °C under an atmosphere of argon. The resulting mixture was allowed to stir for 12 hrs at room temperature. After this time TLC analysis (DCM/MeOH, 10:1, v/v) showed complete consumption of **107a+b** and the reaction was quenched by addition of aqueous NaOH (5 N, 30 ml) and EtOAc (200 ml). The resulting mixture was stirred at room temperature for 10 min and was then filtered. The organic layer was washed twice with brine, dried over $NaSO_4$, filtered and concentrated under reduced pressure to give a mixture of two diastereomers (**108a** and **108b**) in a 57:43 ratio as yellow oil (9.8 g, 20.35 mmol 81.3 % yield).

(1S)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]ethanamine **108a**

^{1}H NMR (200 MHz; $CDCl_3$): δ (ppm) = 5.01 (t, *J* = 4.73 Hz, 1H, H-1); 4.20-3.77 (m, 7H, H-4, H-5, H-6, $(OCH_2)_2$); 3.72-3.58 (m, 18H, $-CH_2-(O-CH_2-CH_2)_4-O-$); 3.56-3.50 (m, 2H, $MeOCH_2$); 3.36 (s, 3H, OCH_3); 3.19-3.00 (m, 1H, H-3); 2.24-1.56 (m, 2H, H-2); 1.38 (s, 3H, CH_3); 1.37 (s, 3H, CH_3)

^{13}C NMR (50 MHz; $CDCl_3$): δ (ppm) = 108.89 (Cq, acetonide); 103.08 (CH, C-1); 81.96 (CH, C-4); 76.59 (CH, C-5); 72.66, 72.38, 72.23, 71.74, 70.80, 70.71, 70.56, 70.38, 70.16, 64.72, 64.44 (OCH_2); 58.83 (OCH_3); 48.79 (CH, C-3); 38.40 (CH_2, C-2); 27.05 (CH_3, acetonide); 26.99 (CH_3, acetonide)

TLC : R_f = 0.38 (DCM/MeOH, 10:1, v/v)

HRMS (ESI-TOF): calcd. for $[C_{22}H_{43}NO_{10}+H]^+$ 482.2965, found 482.2957

(1R)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy] ethoxy]ethoxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]ethanamine **108b**

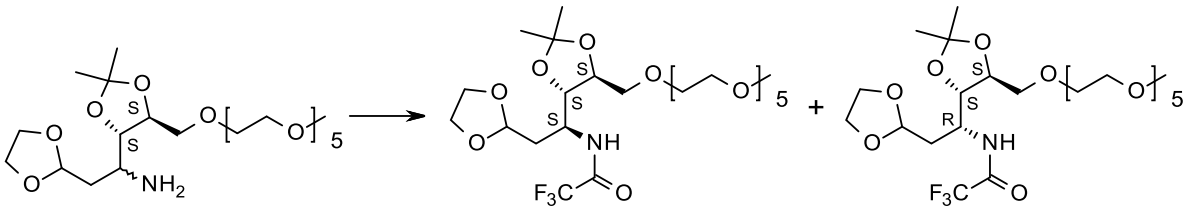
^{1}H NMR (200 MHz; $CDCl_3$): δ (ppm) = 5.01 (t, *J* = 4.73 Hz, 1H, H-1); 4.20-3.77 (m, 7H, H-4, H-5, H-6, $(OCH_2)_2$); 3.72-3.58 (m, 18H, $-CH_2-(O-CH_2-CH_2)_4-O-$); 3.56-3.50 (m, 2H, $MeOCH_2$); 3.36 (s, 3H, OCH_3); 3.19-3.00 (m, 1H, H-3); 2.24-1.56 (m, 2H, H-2); 1.40 (s, 3H, CH_3); 1.37 (s, 3H, CH_3)

^{13}C NMR (50 MHz; $CDCl_3$): δ (ppm) = 108.72 (Cq, acetonide); 103.35 (CH, C-1); 78.88 (CH, C-4); 77.46 (CH, C-5); 72.66, 72.38, 72.23, 71.74, 70.80, 70.71, 70.56, 70.38, 70.16, 64.72, 64.44 (OCH_2); 58.83 (OCH_3); 48.60 (CH, C-3); 37.15 (CH_2, C-2); 26.94 (CH_3, acetonide); 26.70 (CH_3, acetonide)

TLC : R_f = 0.38 (DCM/MeOH, 10:1, v/v)

HRMS (ESI-TOF): calcd. for $[C_{22}H_{43}NO_{10}+H]^+$ 482.2965, found 482.2957

N-[(1S)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]ethyl]-2,2,2-trifluoro-acetamide **109a** and N-[(1R)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]ethyl]-2,2,2-trifluoro-acetamide **109b**



108a+b **109a** **109b**

4-(Dimethylamino)-pyridine (50 mg, 0.409 mmol, 0.02 equiv.) was added to stirred solution of **108a+b** (9.8 g, 20.35 mmol, 1.0 equiv.) in DCM (196 ml) and dry pyridine (9.6 ml). The resulting solution was cooled to -17 °C in an ice-acetone bath. Trifluoroacetic anhydride (6.41g, 30.52 mmol, 1.5 equiv.) was added dropwise to the stirred solution. The resulting solution was allowed to warm to room temperature and was then stirred for 4 hrs. After this time TLC analysis (DCM/MeOH, 10:1, v/v) showed complete consumption of **108a+b** and the mixture was evaporated under reduced pressure.

The residue was resuspended in DCM and was washed with aqueous saturated $NaHCO_3$ and brine. The organic layer was then dried over $NaSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with petroleum ether/acetone (2:1, v/v) to give a mixture of two diastereomers (**109a** and **109b**) correspondent to the starting material in a 58:42 ratio as yellow oil (9.5 g, 16.45 mmol, 80.8 % yield).

N-[(1S)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]ethyl]-2,2,2-trifluoro-acetamide **109a**

^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 7.65 (d, *J* = 8.85 Hz, 1H, NH); 4.99 (t, *J* = 4.6 Hz, 1H, H-1); 4.33-4.27 (m, 1H, H-3); 4.04-3.81 (m, 7H, H-4, H-5, H-6, $(OCH_2)_2$); 3.63-3.61 (m, 18H, -CH_2-(O-CH_2-CH_2)$_4$-O-); 3.53-3.52 (m, 2H, $MeOCH_2$); 3.35 (s, 3H, OCH_3); 2.07-2.00 (m, 2H, H-2a/b); 1.38 (s, 3H, CH_3); 1.37 (s, 3H, CH_3)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 156.93 (Cq, *J* = 36.8 Hz, $\underline{C}OCF_3$); 115.86 (Cq, *J* = 286.4 Hz, CF_3); 109.71 (Cq, acetonide); 102.10 (CH, C-1); 79.04 (CH, C-4); 77.72 (CH, C-5); 71.83, 71.66, 71.10, 70.85, 70.73, 70.46, 70.43, 70.24, 64.89, 64.77, 64.68 (OCH_2); 58.94 (OCH_3); 48.84 (CH, C-3); 34.30 (CH_2, C-2); 27.00 (CH_3, acetonide); 26.90 (CH_3, acetonide)

TLC: R_f = 0.45 (DCM/MeOH, 10:1, v/v)

HRMS (ESI-TOF): calcd. for $[C_{24}H_{42}F_3NO_{11}+NH_4]^+$ 595.3054, found 595.3043; calcd. for $[C_{24}H_{42}F_3NO_{11}+Na]^+$ 600.2608, found 600.2596; calcd. for $[C_{24}H_{42}F_3NO_{11}+K]^+$ 616.2347, found 616.2333

N-[(1R)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy] ethoxy]ethoxy]ethoxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]ethyl]-2,2,2-trifluoro-acetamide **109b**

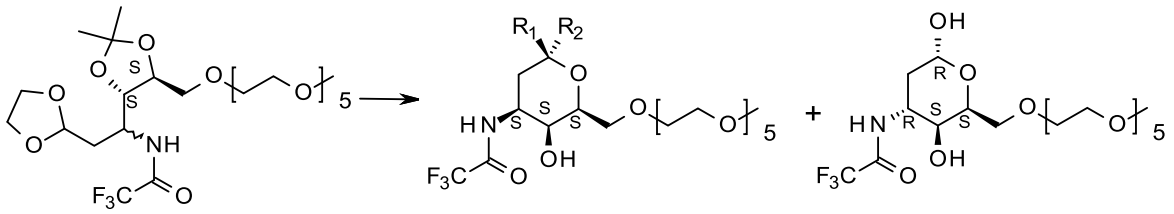
^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 6.95 (d, *J* = 9.15 Hz, 1H, NH); 4.96 (t, *J* = 4.4 Hz, 1H, H-1); 4.42-4.38 (m, 1H, H-3); 4.04-3.81 (m, 7H, H-4, H-5, H-6, $(OCH_2)_2$); 3.63-3.61 (m, 18H, -CH_2-(O-CH_2-CH_2)$_4$-O-); 3.53-3.52 (m, 2H, $MeOCH_2$); 3.35 (s, 3H, OCH_3); 2.07-2.00 (m, 2H, H-2a/b); 1.38 (s, 3H, CH_3); 1.37 (s, 3H, CH_3)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 156.86 (Cq, *J* = 36.8 Hz, $\underline{C}OCF_3$); 115.86 (Cq, *J* = 286.4 Hz, CF_3); 109.41 (Cq, acetonide); 101.95 (CH, C-1); 79.73 (CH, C-4); 75.74 (CH, C-5); 71.83, 71.66, 71.10, 70.85, 70.73, 70.46, 70.43, 70.24, 64.89, 64.77, 64.68 (OCH_2); 58.94 (OCH_3); 45.20 (CH, C-3); 36.30 (CH_2, C-2); 26.85 (CH_3, acetonide); 26.74 (CH_3, acetonide)

TLC: R_f = 0.45 (DCM/MeOH, 10:1, v/v)

HRMS (ESI-TOF): calcd. for $[C_{24}H_{42}F_3NO_{11}+NH_4]^+$ 595.3054, found 595.3043; calcd. for $[C_{24}H_{42}F_3NO_{11}+Na]^+$ 600.2608, found 600.2596; calcd. for $[C_{24}H_{42}F_3NO_{11}+K]^+$ 616.2347, found 616.2333

N-[(2S,3S,4S,6S)-3,6-dihydroxy-2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxymethyl]tetrahydropyran-4-yl]-2,2,2-trifluoro-acetamide **110a**,
N-[(2S,3S,4S,-6R)-3,6-dihydroxy-2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]-ethoxy]ethoxy-methyl]tetrahydropyran-4-yl]-2,2,2-trifluoro-acetamide **110b**,
N-[(2S,3S,4R,6R)-3,6-dihydroxy-2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]-ethoxy]ethoxymethyl] tetrahydropyran-4-yl]-2,2,2-trifluoro-acetamide **111**



109a+b

110a: R_1 = H, R_2 = OH
110b: R_1 = OH, R_2 = H

111

Trifluoroacetic acid (43.0 ml, 0.558 mol) was added dropwise to a stirred solution of **109a+b** (8.9 g, 0.015 mol) in THF/H_2O (4:1, v/v, 43 ml). The resulting solution was allowed to stir at 60 °C for 30 min. The reaction mixture was poured into ice-water (20 ml) and slowly neutralized with solid $NaHCO_3$ (87.0 g, 1.03 mol) until pH 6-7 was reached. The mixture was then filtered and extracted with DCM (3 x 30 ml). The organic layer was then dried over $NaSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, with DCM/MeOH (100:3→100:5→10:1, v/v) to give in order of eluting **111** (2.70 g, 5.46 mmol, 35.4 % yield) and a mixture of the two anomers **110a** and **110b** in a 76:24 ratio (3.66 g, 7.40 mmol, 48.3 % yield) both as clear oil.

N-[(2S,3S,4S,6S)-3,6-dihydroxy-2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy] ethoxy]ethoxymethyl]tetrahydropyran-4-yl]-2,2,2-trifluoro-acetamide **110a**,

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 7.09 (m, 1H, NH); 5.41 (brs, 1H, H-dau1); 4.52-4.43 (m, 1H, H-dau3); 4.18 (m, 1H, H-dau5); 3.92 (m, 1H, H-dau4); 3.79-3.69 (m, 2H, H-dau6a/b) 3.62 (m, 18H, -CH_2-(O-CH_2-CH_2)$_4$-O-); 3.55 (m, 2H, MeOCH_2); 3.36 (s, 3H, OCH_3); 2.00-1.91 (m, 1H, H-dau2a); 1.87-1.84 (m, 1H, H-dau2b)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 156.45 (Cq, *J* = 36.8 Hz, $\underline{C}OCF_3$); 115.83 (Cq, *J* = 286.5 Hz, CF_3); 91.49 (CH, C-dau1); 71.82 (MeO$\underline{C}H_2$), 71.52 (CH_2, C-dau6); 70.42, 70.39 (OCH_2) 68.05 (CH, C-dau5); 65.21 (CH, C-dau4); 58.90 (OCH_3); 45.48 (CH, C-dau3); 30.00 (CH_2, C-dau2)

TLC: R_f = 0.48 (DCM/MeOH 10:1, v/v)

HRMS (ESI-TOF): calcd. for $[C_{19}H_{34}F_3NO_{10}+NH_4]^+$ 511.2478, found 511.2467; calcd. for $[C_{19}H_{34}F_3NO_{10}+Na]^+$ 516.2032, found 516.2017; calcd. for $[C_{19}H_{34}F_3NO_{10}+K]^+$ 532.1771, found 532.1756

N-[(2S,3S,4S,6R)-3,6-dihydroxy-2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy] ethoxy]ethoxymethyl]tetrahydropyran-4-yl]-2,2,2-trifluoro-acetamide **110b**,

^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 7.29 (m, 1H, NH); 4.82-4.80 (m, 1H, H-dau1); 4.16-4.06 (m, 1H, H-dau3); 3.82 (m, 1H, H-dau4); 3.79-3.69 (m, 2H, H-dau6a/b); 3.62 (m, 19H, H-5, $-CH_2-(O-CH_2-CH_2)_4-O-$); 3.55 (m, 2H, $MeOCH_2$); 3.36 (s, 3H, OMe); 2.04-2.00 (m, 1H, H-dau2a); 1.80-1.74 (m, 1H, H-dau2b)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 156.54 (Cq, *J* = 36.8 Hz, $\underline{C}OCF_3$); 115.82 (Cq, *J* = 286.5 Hz, CF_3); 94.58 (CH, C-dau1); 74.05 (CH, C-dau5); 71.82 ($MeO\underline{C}H_2$), 70.60 (CH_2, C-dau6); 70.42, 70.39 (OCH_2); 66.63 (CH, C-dau4); 58.90 (OCH_3); 48.95 (CH, C-dau3); 33.18 (CH_2, C-dau2)

TLC: R_f = 0.48 (DCM/MeOH 10:1, v/v)

HRMS (ESI-TOF): calcd. for $[C_{19}H_{34}F_3NO_{10}+NH_4]^+$ 511.2478, found 511.2467; calcd. for $[C_{19}H_{34}F_3NO_{10}+Na]^+$ 516.2032, found 516.2017; calcd. for $[C_{19}H_{34}F_3NO_{10}+K]^+$ 532.1771, found 532.1756

N-[(2S,3S,4R,6R)-3,6-dihydroxy-2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy] ethoxy]ethoxymethyl]tetrahydropyran-4-yl]-2,2,2-trifluoro-acetamide **111**

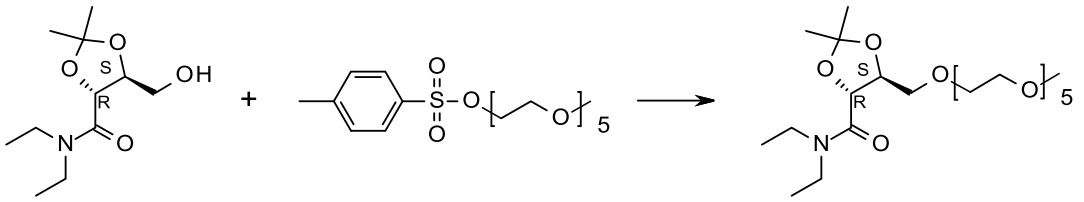
^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 8.30 (d, *J* = 7.6, 1H, NH); 5.42 (brs, 1H, H-dau1); 4.25 (m, 1H, H-dau5); 4.16 (m, 1H, H-dau3); 3.72-3-53 (m, 23H, H-4, H-dau6a/b, $-(O-CH_2-CH_2)_5-O-$); 3.35 (s, 3H, OCH_3); 2.35-2.30 (m, 1H, H-dau2a); 1.67-1.64 (m, 1H, H-dau2b)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 156.50 (Cq, *J* = 36.8 Hz, $\underline{C}OCF_3$); 115.74 (Cq, *J* = 286.5 Hz, CF_3); 91.81 (CH, C-dau1); 71.73 (CH_2, C-dau6); 71.72 ($MeO\underline{C}H_2$); 70.32 (OCH_2); 65.98 (CH, C-dau4); 65.10 (CH, C-dau5); 58.87 (OCH_3); 47.66 (CH, C-dau3); 27.09 (CH_2, C-dau2)

Optical rotation: $[\alpha_D^{20}]$: + 1.8° (c = 1.4, $CHCl_3$)

TLC: R_f = 0.56 (DCM/MeOH 10:1, v/v)

(4R,5S)-N,N-diethyl-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy] ethoxymethyl]-2,2-dimethyl-1,3-dioxolane-4-carboxamide **112**



88 **103** **112**

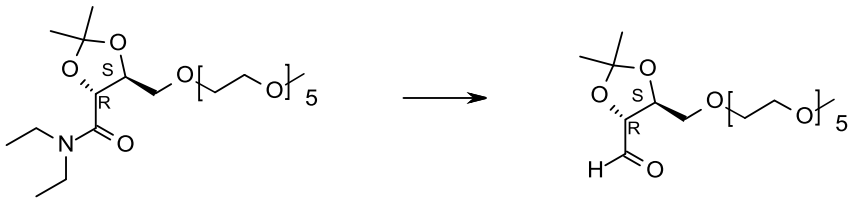
Sodium hydride, 95 % (0.84 g, 3.5 mmol, 1.6 equiv.) was added in portions to a stirred solution of **88** (0.5 g, 2.16 mmol, 1.0 equiv.) in THF (5 ml) at room temperature under an atmosphere of argon. The resulting suspension was allowed to stir at room temperature for 10 minutes. A solution of **103** (0.92 g, 3.6 mmol, 1.7 equiv.) in THF (8 ml) was added dropwise. This mixture was then warmed to 60 °C and stirred for 4.5 hrs. After this time, TLC analysis (toluene/acetone, 4:3, v/v) showed complete consumption of **88** and the reaction was quenched by addition of sat. NH_4Cl (11 ml). The layers were separated and the aqueous phase was extracted with DCM (3 x 15 ml). The combined organic extracts were dried over $NaSO_4$ filtered and concentrated under reduced pressure to give 0.80 g of **112** (1.9 mmol, 88.0 %) as yellow oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 4.75-4.67 (m, 1H, H-3); 4.43 (d, *J* = 7.2, 1H, H-2); 3.68-3,42 (m, 26H, (-CH_2-CH_2-O-)$_5$-CH_2, (CH_2)$_2$), 3.35 (s, 3H,OCH_3); 1.42 (s, 3H, CH_3); 1.38 (s, 3H, CH_3); 1.17 (t, *J* = 7.1, 3H, CH_3); 1.10 (t, *J* = 7.1, 3H, CH_3)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 168.4 (Cq, CO); 110.0 (Cq, acetonide); 78.2 (CH, C-2); 75.0 (CH, C-3); 61.6 (CH_2, C-4); 71.8 (MeO$\underline{C}H_2$); 70.46, 70.40, 70.37, 70.30, 69.27 (-$\underline{C}H_2$-(O-$\underline{C}H_2$-$\underline{C}H_2$)$_4$-O-); 58.9 (OCH_3); 41.7 (-N-$\underline{C}H_2$-CH_3); 40.4 (-N-$\underline{C}H_2$-CH_3); 26.9 (CH_3, acetonide); 26.0 (CH_3, acetonide); 14.3 (-N-CH_2-$\underline{C}H_3$); 12.5 (-N-CH_2-$\underline{C}H_3$)

TLC: R_f = 0.25 (toluene/acetone)

(4R,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxymethyl]-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde **113**



112 **113**

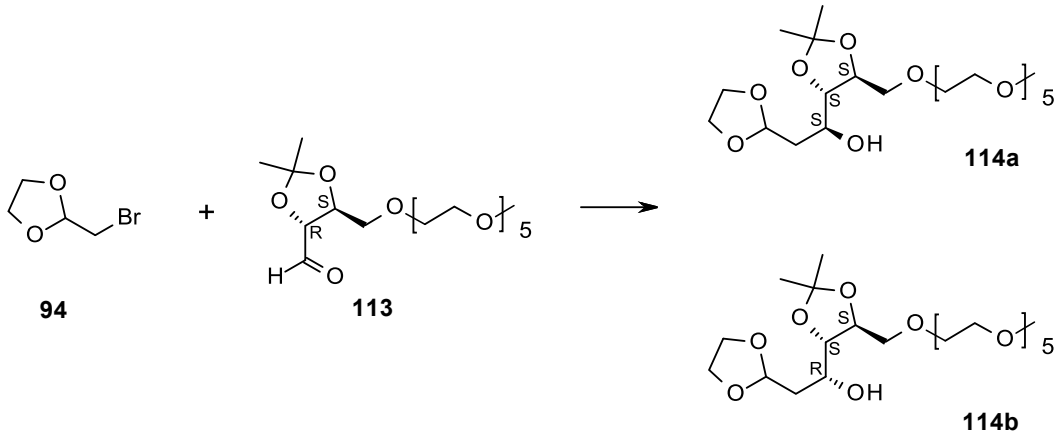
Sodium bis(2-methoxyethoxy)aluminumhydride, 70 wt % sol. in toluene (0.79 ml, 2.84 mmol, 1.5 equiv.) was added dropwise to a stirred solution of **112** (0.80 g, 1.90 mmol, 1.0 equiv.) in toluene (8 ml) at 0 °C under an atmosphere of argon. The resulting mixture was allowed to stir between 0 - 5 °C for 3 hrs. After this time, TLC analysis (toluene/acetone, 4:3, v/v) showed complete consumption of **112** and the reaction was quenched by addition of sat. NH_4Cl (15 ml). Then DCM (20 ml) was added and the mixture was filtrated. The layers were separated and the aqueous phase was extracted with DCM. The combined organic extracts were washed with sat. $NaHCO_3$ and brine, dried over NaSO4, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with toluene/acetone (2:1, v/v) to give 0.35 g of **113** (1.0 mmol, 52 % yield) as clear yellow oil.

^{1}H NMR (200 MHz; $CDCl_3$): δ (ppm) = 9.74 (s, 1H, H-1); 7.27-7.13 (m, 1H, H-2); 4.23-4.21 (m, 1H, H-3); 3.69-3.50 (m, 22H, $(-CH_2-CH_2-O)_5-CH_2$); 3.36 (s, 3H, OCH_3) 1.47 (s, 3H, CH_3); 1.40 (s, 3H, CH_3)

^{13}C NMR (50 MHz; $CDCl_3$): δ (ppm) = 200.4 (CH, C-1); 109.6 (Cq, acetonide); 82.0 (CH, C-2); 73.2 (CH, C-3); 72.3 (CH_2, C-4); 71.8 (CH_2, $MeO\underline{C}H_2$); 70.46, 70.40, 70.37, 70.30, 69.27 ($-\underline{C}H_2-(O-\underline{C}H_2-\underline{C}H_2)_4-O-$); 58.9 ($OCH_3$); 26.9 ($CH_3$, acetonide); 26.8 ($CH_3$, acetonide)

TLC: R_f = 0.22 (toluene/acetone, 4:3, v/v)

(1R)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy] ethoxy]ethoxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]ethanol **114a** and (1S)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy] ethoxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]ethanol **114b**



A suspension of magnesium (0.160 g, 6.58 mmol, 1.1 equiv.) and a catalytic amount of iodine in dry THF (8 ml) was heated under reflux for 10 min. After this time 2-Bromomethyl-1,3-Dioxolane **94** (1.0 g, 5.99 mmol, 1.0 equiv.) was added dropwise and the solution was heated under reflux until the colour changed to a blur solution and the magnesium started reacting. This mixture was allowed to stir at 90 °C under reflux for 2 hrs. After this time the mixture was cooled to room temperature. Then two ml of this mixture was added dropwise to a solution of **113** (0.348 g, 0.882 mmol) in dry THF (1.5 ml). The resulting mixture was allowed to stir at room temperature for 18 hrs. After this time TLC analysis (PE/acetone, 1:1, v/v) showed complete consumption of **113**. The reaction was quenched by addition of sat. NH_4Cl (10 ml) and crushed ice (10 ml) and stirred for 5 min. The layers were separated and the aqueous phase was extracted with DCM (3x 10 ml). The combined organic extracts were washed with brine, dried over $NaSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with petroleum ether/acetone (2:1, v/v) to give a mixture of two *stereoisomers* (**114a** and **114b**) in a 64:36 ratio as yellow viscous oil (0.184 g, 0.381 mmol 43.2 % yield).

(1R)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy] ethoxy]ethoxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]ethanol **114a:**

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 5.06 (m, 1H, H-1); 4.08 (m, 1H, H-5); 4.02-3.80 (m, 5H, H-3, $(OCH_2)_2$); 3.69-3.61 (m, 21H, H-4, H-6a/b, $-CH_2-(O-CH_2-CH_2)_4-O-$) 3.52 (m, 2H, $MeOCH_2$); 3.35 (s, 3H, OCH_3); 2.08-2.04 (m, 1H, H-2a); 1.83-1.77 (m, 1H, H-2b); 1.36 (s, 3H, CH_3); 1.35 (s, 3H, CH_3)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 109.2 (Cq, acetonide); 103.1 (CH, C-1); 80.9 (CH, C-4); 78.1 (CH, C-5); 72.3 (CH_2, C-6); 71.8 ($MeO\underline{C}H_2$); 70.9, 70.8, 70.5, 70.4, 70.3 ($-\underline{C}H_2-(O-\underline{C}H_2-\underline{C}H_2)_4-O-$); 69.3 (CH, C-3); 64.8 (OCH_2); 64.63 (OCH_2); 58.9 (OCH_3); 37.1 (CH2, C-2); 26.9 (CH_3, acetonide); 26.8 (CH_3, acetonide)

TLC: R_f = 0.25 (PE/acetone, 2:1, v/v)

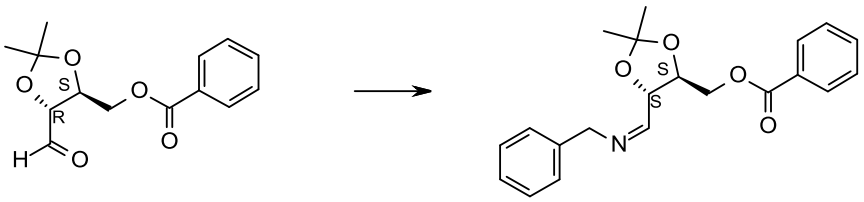
(1S)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-5-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy] ethoxy]ethoxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]ethanol **114b:**

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 5.06 (m, 1H, H-1); 4.14 (m, 1H, H-5); 4.02-3.80 (m, 5H, H-3, $(OCH_2)_2$); 3.78 (m, 1H, H-4); 3.69-3.61 (m, 20H, H-6a/b, $-CH_2-(O-CH_2-CH_2)_4-O-$); 3.52 (m, 2H, $MeOCH_2$); 3.35 (s, 3H, OCH_3); 1.95-1.86 (m, 2H, H-2a/b); 1.39 (s, 3H, CH_3); 1.38 (s, 3H, CH_3)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 109.2 (Cq, acetonide); 102.8 (CH, C-1); 80.8 (CH, C-4); 75.8 (CH, C-5); 71.8 ($MeO\underline{C}H_2$); 71.5 (CH_2, C-6); 70.9, 70.8, 70.5, 70.4, 70.3 ($-\underline{C}H_2-(O-\underline{C}H_2-\underline{C}H_2)_4-O-$); 66.9 (CH, C-3); 64.8 (OCH_2); 64.63 (OCH_2); 58.9 (OCH_3); 37.5 (CH_2, C-2); 27.1 (CH_3, acetonide); 26.9 (CH_3, acetonide)

TLC: R_f = 0.25 (PE/acetone, 2:1, v/v)

[(4S,5S)-5-[(E)-benzyliminomethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]-methyl benzoate **115**



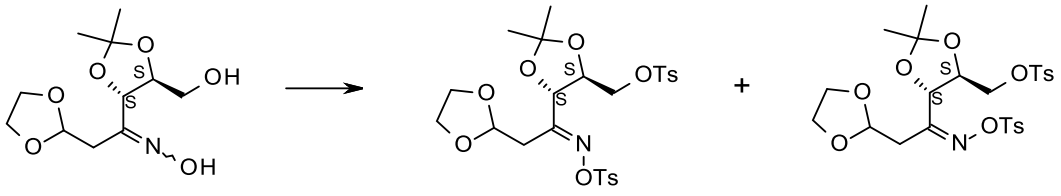
92 **115**

To a solution of **92** (0.180 g, 0.681 mmol, 1.0 equiv.) in dry Et_2O (1.0 ml) anhydrous Na_2SO_4 (0.290 g, 20.43 mmol, 3.0 equiv.) was added under an atmosphere of argon. The resulting suspension was then cooled down to 0 °C. Benzylamine (0.072 g, 0.6810 mmol, 1.0 equiv.) was added dropwise, and the mixture stirred briskly for 2 hrs. The suspension was then filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with DCM/MeOH (100:3, v/v) to give 0.22 g of **115** (0.622 mmol, 91.3 %) as yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 7.94-7.90 (m, 2H, Ar); 7.73-7.72 (m, 1H, H-1); 7.41-7.40 (m, 1H, Ar); 7.29-7.27 (m, 2H, Ar); 7.19-7.12 (m, 5H, Ar); 4.54-4.50 (m, 1H, $BnCH_2a$); 4.44-4.441 (m, 1H, H-2); 4.33-4.30 (m, 2H, $BnCH_2b$, H-3); 1.35 (s, 3H, CH_3); 1.34 (s, 3H, CH_3)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 165.98 (Cq, BzCOO); 140.34 (Cq, Bn); 132.89 (CH, Ar); 129.51 (CH, Ar); 128.34 (CH, Ar); 128.14 (CH, Ar); 127.70 (CH, Ar); 126.97 (CH, Ar); 110.56 (Cq, acetonide); 78.64 (CH, C-2); 76.47 (CH, C-3); 64.28 (CH_2, $BnCH_2$); 63.65 (CH_2, C-4); 26.65 (CH_3, acetonide); 36.61 (CH_3, acetonide)

[(4S,5S)-5-[(E)-C-(1,3-dioxolan-2-ylmethyl)-N-(p-tolylsulfonyloxy)carbonimidoyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methyl 4-methylbenzenesulfonate **117a** and
[(4S,5S)-5-[(Z)-C-(1,3-dioxolan-2-ylmethyl)-N-(p-tolylsulfonyloxy)carbonimidoyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methyl 4-methylbenzenesulfonate **117b**



106a+b **117a** **117b**

To a stirred solution of **106a**+**b** (150.0 mg, 0.57 mmol, 1.0 equiv) in THF (2.0 ml), aqueous NaOH (2.3 N, 2.0 ml, 4.55 mmol, 1.6 equiv, 8.0 equiv.) was added at 0 °C. Afterwards a solution of *p*-toluenesulfonyl chloride (0.262g, 1.37 mmol, 2.4 equiv.) in THF (1.0 ml) was added dropwise. The resulting mixture was allowed to stir at 0 °C for 60 minutes. After this time TLC analysis (MTBE/PE, 2:1, v/v), showed complete consumption of **106a**+**b** and the reaction was quenched by addition of crushed ice.
After the addition of DCM (5 ml) the mixture was stirred for further 10 minutes. The layers were separated and the aqueous phase was extracted three times with DCM (3 x 2 ml). The combined organic extracts were washed with $NaHCO_3$ (5 ml) and brine (5 ml), dried over $NaSO_4$, filtered and concentrated under reduced pressure. To give a mixture of two isomers (**117a** and **117b**) in a 85:15 (*E:Z*) ratio as white solid (0.289 g, 0.507 mmol 88.4 % yield).

[(4S,5S)-5-[(E)-C-(1,3-dioxolan-2-ylmethyl)-N-(p-tolylsulfonyloxy)carbonimidoyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methyl 4-methylbenzenesulfonate **117a**

^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 7.88 (m, 4H, Ar); 7.43-7.17 (m, 4H, Ar); 5.16 (t, *J* = 3.2 Hz, 1H, H-1); 4.40-4.34 (m, 2H, H-4, H-5); 4.11-3.94 (m, 4H, $(OCH_2)_2$); 3.79-3.78 (m, 2H, H-6a/b) 2.92-2.67 (m, 2H, H-2a/b); 2.44 (s, 3H, Ar-CH_3); 2.37 (s, 3H, Ar-CH_3) 2.371.41-1.22 (m, 6H, $(CH_3)_2$, acetonide)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 162.42 (Cq, CNO); 145.47 (Cq, Ar); 144.98 (Cq, Ar), 132.63 (Cq, Ar); 131.96 (Cq, Ar); 130.07 (CH, Ar); 129.97 (CH, Ar); 129.33 (CH, Ar); 128.95 (CH, Ar); 110.95 (Cq, acetonide); 100.16 (CH, C-1); 76.00 (CH, C-4); 75.24 (CH, C-5); 68.13

(CH_2, C-6); 65.73 (OCH_2); 64.82 (OCH_2); 32.17 (CH_2, C-2); 26.59 (CH_3, acetonide); 26.27 (CH_3, acetonide); 21.67 (CH_3, Ar); 21.61 (CH_3, Ar)

TLC: R_f = 0.5 (MTBE/PE, 2:1, v/v)

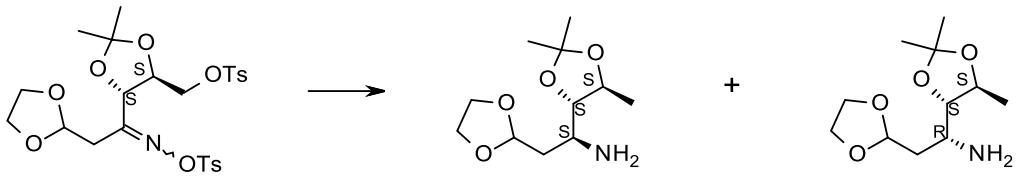
[(4S,5S)-5-[(Z)-C-(1,3-dioxolan-2-ylmethyl)-N-(p-tolylsulfonyloxy)carbonimidoyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methyl 4-methylbenzenesulfonate **117b**

^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 7.88 (m, 4H, Ar); 7.43-7.17 (m, 4H, Ar); 5.10 (t, *J* = 5.1 Hz, 1H, H-1); 4.95 (d, *J* = 7.25, 1H, H-4); (m, 5H, H-5, $(OCH_2)_2$); 3.79-3.78 (m, 2H, H-6a/b) 2.72-2.67 (m, 2H, H-2a/b); 2.44 (s, 3H, Ar-CH_3); 2.37 (s, 3H, Ar-CH_3) 1.41-1.22 (m, 6H, $(CH_3)_2$, acetonide)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 163.88 (Cq, CNO); 145.40 (Cq, Ar); 144.92 (Cq, Ar), 132.63 (Cq, Ar); 131.96 (Cq, Ar); 129.90 (CH, Ar); 129.85 (CH, Ar); 129.71 (CH, Ar); 129.64 (CH, Ar); 111.61 (Cq, acetonide); 101.32 (CH, C-1); 77.67 (CH, C-5); 72.12 (CH, C-4); 68.84 (CH_2, C-6); 65.73 (OCH_2); 64.82 (OCH_2); 34.77 (CH_2, C-2); 30.26 (CH_3, acetonide); 26.59 (CH_3, acetonide); 21.67 (CH_3, Ar); 21.61 (CH_3, Ar)

TLC : R_f = 0.5 (MTBE/PE, 2:1, v/v)

(1S)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-2,2,5-trimethyl-1,3-dioxolan-4-yl]ethanamine **118a** and (1R)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-2,2,5-trimethyl-1,3-dioxolan-4-yl]ethanamine **118b**



117a+b **118a** **118b**

Lithium aluminium hydride (50.5 mg, 1.33 mmol, 2.6 equiv.) was slowly added to a stirred solution of **117a**+**b** (0.29 g, 0.509 mmol, 1.0 equiv.) in dry THF (3.0 ml) at 0 °C under an atmosphere of argon. The resulting mixture was allowed to stir for 24 hrs at room temperature. After this time, TLC analysis (PE/acetone, 3:1, v/v) showed complete consumption of **117a**+**b** and the reaction was quenched by addition of aqueous NaOH (5 N, 1.0 ml) and EtOAc (3.0 ml). The resulting mixture was stirred at room temperature for 10 min and was then filtered. The organic layer was washed twice with brine, dried over $NaSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, with MTBE/PE (2:1, v/v) to give in order of eluting **118a** (61.2 mg, 0.265 mmol, 52.0 % yield) and **118b** (40.8 mg, 0.176 mmol, 34.7 % yield) as yellow oils.

(1S)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-2,2,5-trimethyl-1,3-dioxolan-4-yl]ethanamine **118a**

^{1}H NMR (200 MHz; $CDCl_3$): δ (ppm) = 5.00 (t, *J* = 4.7 Hz, 1H, H-1); 4.10-3.74 (m, 5H, H-5, $(OCH_2)_2$); 3.50-3.44 (m, 1H, H-4); 3.22-3.12 (m, 1H, H-3); 2.00-1.54 (m, 2H, H-2a/b); 1.37 (s, 3H, CH_3, acetonide); 1.36 (s, 3H, CH_3, acetonide); 1.31 (d, *J* = 5.9 Hz, 3H, H-6)

^{13}C NMR (50 MHz; $CDCl_3$): δ (ppm) = 107.83 (Cq, acetonide); 103.59 (CH, C-1); 85.98 (CH, C-4); 73.39 (CH, C-5); 64.91 (OCH_2); 64.59 (OCH_2); 48.82 (CH, C-3); 37.23 (CH_2, C-2); 27.33 (CH_3, acetonide); 26.96 (CH_3, acetonide) 19.56 (CH_3, C-6);

TLC: R_f = 0.29 (PE/acetone, 3:1, v/v)

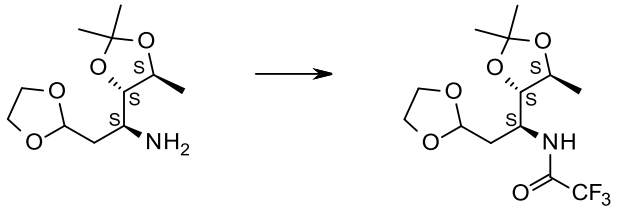
(1R)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-2,2,5-trimethyl-1,3-dioxolan-4-yl]ethanamine **118b**

^{1}H NMR (200 MHz; $CDCl_3$): δ (ppm) = 5.02 (t, *J* = 4.7, 1H, H-1); 4.08-3.78 (m, 5H, H-5, $(OCH_2)_2$); 3.46-3.40 (m, 1H, H-4); 3.05-2.96 (m, 1H, H-3); 2.24-1.73 (m, 2H, H-2a/b); 1.38 (s, 3H, CH_3, acetonide); 1.37 (s, 3H, CH_3, acetonide); 1.28 (d, *J* = 5.7 Hz, 3H, H-6)

^{13}C NMR (50 MHz; $CDCl_3$): δ (ppm) = 107.99 (Cq, acetonide); 103.17 (CH, C-1); 86.25 (CH, C-4); 73.47 (CH, C-5); 64.88 (OCH_2); 64.62 (OCH_2); 48.54 (CH, C-3); 38.94 (CH_2, C-2); 27.38 (CH_3, acetonide); 26.99 (CH_3, acetonide) 18.66 (CH_3, C-6);

TLC: R_f = 0.18 (PE/acetone, 2:1, v/v)

N-[(1S)-2-(1,3-dioxolan-2-yl)-1-[(4S,5S)-2,2,5-trimethyl-1,3-dioxolan-4-yl]ethyl]-2,2,2-trifluoro-acetamide **119**



118a **119**

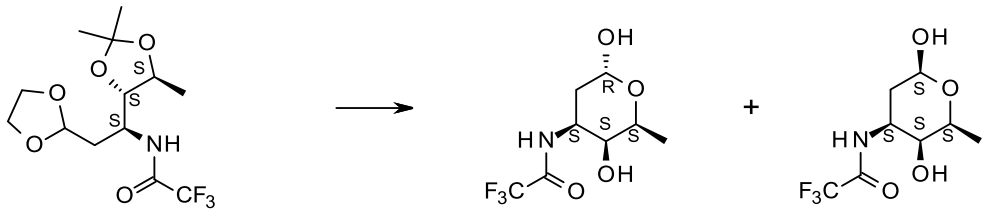
4-(Dimethylamino)-pyridine (12.9 mg, 0.106 mmol, 0.014 equiv.) was added to stirred solution of **118a** (1.754 g, 7.584 mmol, 1.0 equiv.) in DCM (50 ml) and dry pyridine (2.1 ml). The resulting solution was cooled to -10 °C in an ice-acetone bath. Trifluoroacetic anhydride (2.26, 10.77 mmol, 1.4 equiv.) was added dropwise to the stirred solution. The resulting mixture was stirred at -10 °C for 1.5 hrs. After this time, TLC analysis (petroleum ether/acetone, 1:1, v/v) showed complete consumption of **118a** and the mixture was evaporated under reduced pressure. The residue was resuspended in DCM and was washed with aqueous saturated $NaHCO_3$ and brine. The organic layer was then dried over $NaSO_4$, filtered and concentrated under reduced pressure to give 0.242 g of **119** (0.64 mmol, 97.5 % yield) as yellow oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ (ppm) = 7.18 (d, *J* = 6.95 Hz, 1H, NH); 5.05-5-04 (m, 1H, H-1); 4.30-4.25 (m, 1H, H-3); 4.02-3.83 (m, 5H, H-5, $(OCH_2)_2$); 3.59 (dd, J^1 = 8.0 Hz, J^2 = 6.5 Hz, 1H, H-4); 2.21-2.14 (m, 1H, H-2a); 1.95-1.90 (m, 1H, H-2b); 1.38 (s, 3H, CH_3, actonide); 1.37 (s, 3H, CH_3, actonide); 1.26 (d, *J* = 6.0 Hz, 3H, H-6)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 156.80 (Cq, *J* = 36.8 Hz, $\underline{C}OCF_3$); 115.86 (Cq, *J* = 286.4 Hz, CF_3); 108.62 (Cq, acetonide); 102.12 (CH, C-1); 82.65 (CH, C-4); 74.99 (CH, C-5); 64.93 (OCH_2); 64.84 (OCH_2); 58.94 (OCH_3); 48.09 (CH, C-3); 33.37 (CH_2, C-2); 27.24 (CH_3, acetonide); 26.75 (CH_3, acetonide); 18.45 (CH_3, C-6)

TLC: R_f = 0.56 (PE/acetone, 2:1, v/v)

N-[(2S,3S,4S,6S)-3,6-dihydroxy-2-methyl-tetrahydropyran-4-yl]-2,2,2-trifluoro-acetamide **120a** and N-[(2S,3S,4S,6R)-3,6-dihydroxy-2-methyl-tetrahydropyran-4-yl]-2,2,2-trifluoro-acetamide **120b**



119 **120a** **120b**

Trifluoroacetic acid (3.17 ml, 0.0188 mol) was added dropwise to a stirred solution of **119** (0.1357 g, 0.415 mmol) in THF/H_2O (4:1, v/v, 3.16 ml). The resulting solution was allowed to stir at 55 °C for 10 min. The reaction mixture was poured into ice-water (4 ml) and was slowly neutralized with solid $NaHCO_3$ (4.8 g, 0.057 mol) until pH 6-7 was reached. The mixture was then filtered and extracted several times with DCM. The organic layer was then dried over $NaSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with toluene/aceteone (3:2, v/v) to give an anomeric mixture of **120a** and **120b** in a 1:1 ratio as white solid (75 mg, 0.308 mmol, 74.4 % yield). Recrystallization from petroleum ether/ethyl acetate afforded the α-anomer **120a.**

N-[(2S,3S,4S,6R)-3,6-dihydroxy-2-methyl-tetrahydropyran-4-yl]-2,2,2-trifluoro-acetamide **120a**

^{1}H NMR (500 MHz, d_4-methanol): δ (ppm) = 5.26 (d, *J* = 3.15 Hz, 1H, H-dau1); 4.38-4.34 (m, 1H, H-dau3); 3.66-3.64 (m, 1H, H-dau5); 3.59 (d, *J* = 1.9 Hz, 1H, H-dau4); 2.08-2.03 (m, 1H, H-dau2a); 1.64-1.61 (m, 1H, H-dau2b) 1.17 (d, *J* = 5.35 Hz, 3H, H-6)

^{13}C NMR (126 MHz; d_4-methanol): δ (ppm) = 158.41 (Cq, *J* = 36.8 Hz, $\underline{C}OCF_3$); 117.45 (Cq, *J* = 285.0 Hz, CF_3); 95.53 (CH, C-1); 69.31 (CH, C-4); 67.03 (CH, C-5); 47.96 (CH, C-3); 30.47 (CH_2, C-2); 17.30 (CH_3, C-6)

TLC: R_f = 0.3 (toluene/acetone, 3:2, v/v)

m.p.: 147-149 °C (The melting point is in agreement with that reported in literature[261,262])

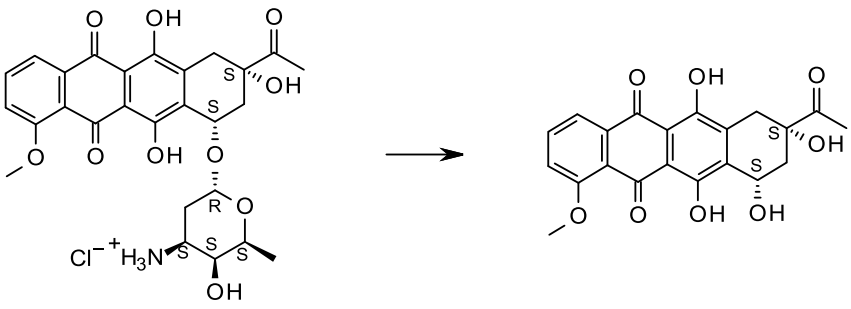
N-[(2S,3S,4S,6S)-3,6-dihydroxy-2-methyl-tetrahydropyran-4-yl]-2,2,2-trifluoro-acetamide **120b**

^{1}H NMR (500 MHz, d_4-methanol): δ (ppm) = 4.80-4.77 (m, 1H, H-dau1); 4.07-4.01 (m, 1H, H-dau3); 3.66-3.63 (m, 1H, H-dau5); 3.52 (d, *J* = 2.5 Hz, 1H, H-dau4); 1.81-1.77 (m, 2H, H-dau2a/b); 1.25 (d, *J* = 6.65 Hz, 3H, H-dau6)

^{13}C NMR (126 MHz; d_4-methanol): δ (ppm) = 158.35 (Cq, *J* = 36.8 Hz, $\underline{C}OCF_3$); 117.45 (Cq, *J* = 285.0 Hz, CF_3); 92.17 (CH, C-1); 72.88 (CH, C-5); 68.16 (CH, C-4); 51.50 (CH, C-3); 33.12 (CH_2, C-2); 17.21 (CH_3, C-6)

TLC: R_f = 0.3 (toluene/acetone, 3:2, v/v)

(7S,9S)-9-acetyl-6,7,9,11-tetrahydroxy-4-methoxy-8,10-dihydro-7H-tetracene-5,12-dione **122**



121 **122**

Acetyl chloride (0.81 g, 10.31 mmol, 5.6 equiv.) was added dropwise to a stirred solution of daunorubicin hydrochloride **121** (1.04 g, 1.844 mmol, 1.0 equiv.) in MeOH (62.5 ml) under an atmosphere of argon. The resulting solution was allowed to stir at 90 °C under reflux for 1.5 hrs. After this time TLC analysis (DCM/MeOH, 10:1, v/v) showed complete consumption of **121** and the mixture was evaporated under reduced pressure. The residue was resuspended in $CHCl_3$ causes precipitation of daunosamine methyl ether, which was filtered off. The filtrate was evaporated under reduced pressure. Afterwards diisopropyl ether (100 ml) was added and the resulting suspension was sonicated for 30 min. The precipitate was collected by filtration, washed with diisopropyl ether and dried under high vacuum, to give **122** (0.64 g, 1.607 mmol, 86.8 % yield) as an orange-red solid.

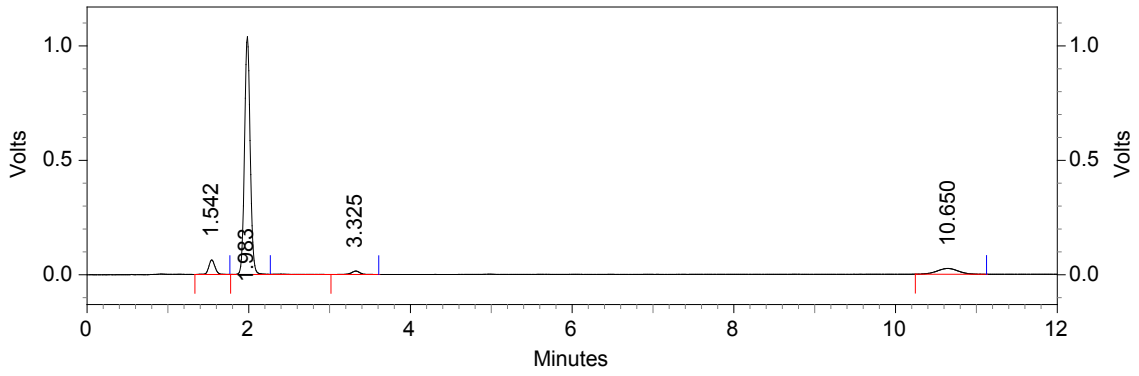
^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 13.84 (s, 1H, OH-6); 13.17 (s, 1H, OH-11); 7.96 (d, *J* = 7.55 Hz, 1H, H-1); 7.76 (t, *J* = 7.4 1H, H-2); 7.37 (d, *J* = 8.5 Hz, 1H, H-3); 5.28 (brs, 1H, H-7); 4.07 (s, 3H, $ArOCH_3$); 3.13 (d, *J* = 18.3 Hz, 1H, H-10a); 2.88 (d, *J* = 18.6 Hz, 1H, H-10b); 2.43 (s, 3H, CH_3); 2.34 (d, *J* = 14.5 Hz, 1H, H-8a); 2.13 (dd, J^1 = 14.4 Hz, J^2 = 4.3 Hz, 1H, H-8b).

^{1}H-NMR spectroscopic data were in agreement with those reported in literature[222].

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 212.04 (Cq, C-13); 186.99 (Cq, C-5); 186.51 (Cq, C-12); 160.98 (Cq, C-4); 155.92 (Cq, C-11); 155.77 (Cq, C-6); 135.93 (Cq, C-6a); 135.73 (CH, C-2); 135.38 (Cq, C-12a); 133.62 (Cq, C-10a); 120.67 (Cq, C-4a); 119.71 (CH, C-1); 118.38 (CH, C-3); 111.39 (Cq, C-5a); 111.01 (Cq, C-11a); 77.00 (Cq, C-9); 61.84 (CH, C-7); 56.66 ($ArOCH_3$); 35.27 (CH_2, C-8); 33.10 (CH_2, C-10); 24.61 (CH_3, C-14)

TLC: R_f = 0.33 (DCM/MeOH 100:5, v/v)

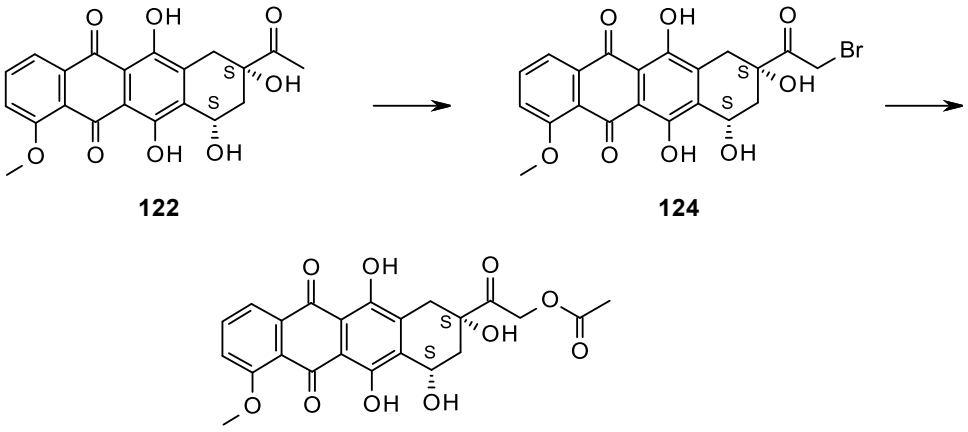
HPLC: Retention time: 1.983 min; Column: Zorbax Eclipse XDB-C8, 5 µm, l = 150 mm, Ø = 4.6 mm; Mobile Phase: 40 % H_2O containing 0.4 % sodium dodecylsulfate and 60 % MeOH/ACN, 1:1, v/v; Detection: UV [λ = 254nm, reference λ = 360nm]; Injection volume: 20 µL; Temperature: 35 °C; Flow rate: 2 ml/min; Run time: 12 min; Standard preparation: 1 mg/ml mobile phase



Detector A - 1 (254nm)

Pk #	Retention Time	Area	Area %	Height	Height %
1	1.542	341988	5.716	63606	5.566
2	1.983	5071272	84.761	1038999	90.920
3	3.325	108940	1.821	14891	1.303
4	10.650	460842	7.702	25270	2.211
Totals		5983042	100.000	1142766	100.000

[2-oxo-2-[(2S,4S)-2,4,5,12-tetrahydroxy-7-methoxy-6,11-dioxo-3,4-dihydro-1H-tetracen-2-yl]ethyl] acetate **125**



122 **124**

125

Bromine (0.234 g, 1.469 mmol, 2.5 equiv.) in $CHCl_3$ (4.4 ml) was added to a stirred solution of daunomycinone **122** (0.234 g, 0.587 mmol, 1.0 equiv.) in $CHCl_3$ (23.4 ml) under an atmosphere of argon. The resulting solution was allowed to stir at room temperature for 18 hrs. The precipitated bromide **124** was filtered off and dissolved in dry acetone (89 ml). Then KOAc (1.27 g, 12.94 mmol, 22.0 equiv.) was added in portions. After being heated under reflux for 5 min all solvent were removed under reduced pressure. The residue was dissolved in $CHCl_3$ and was washed once with H_2O and once with brine. The organic layer was dried over $NaSO_4$, filtered and concentrated under reduced pressure. Then diisopropyl ether was added and the suspension was sonicated for 20 min. The precipitate was collected by filtration, washed with diisopropyl ether and dried under high vacuum. The crude product was filtered through a short silica-gel column, eluting with DCM/MeOH (100:1→100:3→100:5, v/v) to give **125** (221 mg, 0.484 mmol, 82.4 % yield) as a red solid.

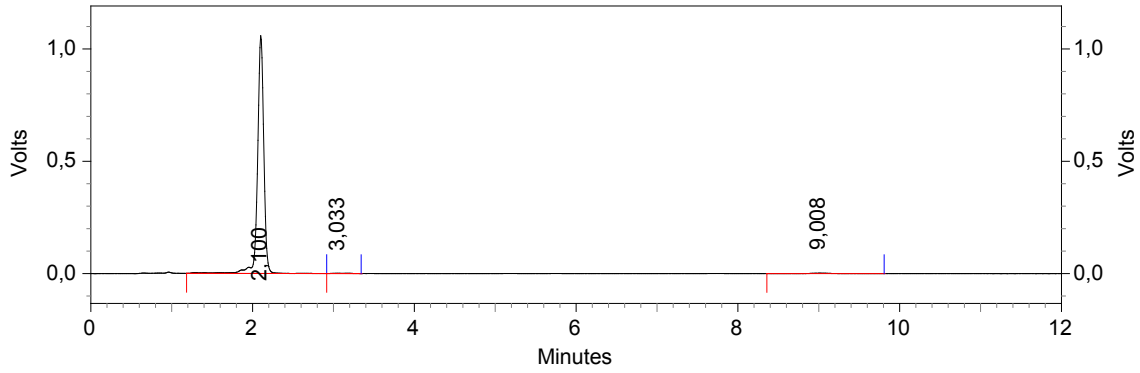
^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 13.79 (s, 1H, OH-6); 13.08 (s, 1H, OH-11); 7.96 (d, *J* = 7.55 Hz, 1H, H-1); 7.76 (t, *J* = 8.1 1H, H-2); 7.37 (d, *J* = 8.2 Hz, 1H, H-3); 5.36 (d, *J* = 18.3 Hz, H-14a); 5.30 (s, 1H, H-7); 5.16 (d, *J* = 18.3 Hz, H-14b); 4.79 (s, 1H, OH-9); 4.07 (s, 3H, $ArOCH_3$); 3.52 (brs, 1H, OH-7); 3.13 (dd, J^1 = 18.8 Hz, J^2 = 2.1 Hz, 1H, H-10a); 2.87 (d, *J* = 18.6 Hz, 1H, H-10b); 2.48 (d, *J* = 14.9 Hz, 1H, H-8a); 2.21 (s, 3H, CH_3); 2.34 (d, *J* = 14.5 Hz, 1H, H-8a); 2.07 (dd, J^1 = 14.7 Hz, J^2 = 4.6 Hz, 1H, H-8b)

^{1}H-NMR spectroscopic data were in agreement with those reported in literature[203].

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm)= 207.29 (Cq, C-13); 186.94 (Cq, C-5); 186.41 (Cq, C-12); 170.44 (Cq, COO); 160.99 (Cq, C-4); 156.03 (Cq, C-6); 155.28 (Cq, C-11); 135.93 (CH, C-2); 135.35 (Cq, C-12a); 135.04 (Cq, C-6a); 133.72 (Cq, C-10a); 120.48 (Cq, C-4a); 119.81 (CH, C-1); 118.42 (CH, C-3); 111.40 (Cq, C-5a); 111.10 (Cq, C-11a); 77.00 (Cq, C-9); 66.18 (CH2, C-14); 62.18 (CH, C-7); 56.67 ($ArOCH_3$); 35.34 (CH_2, C-8); 33.49 (CH_2, C-10); 20.57 (CH_3, Ac)

TLC: R_f = 0.41 (DCM/MeOH 100:5, v/v)

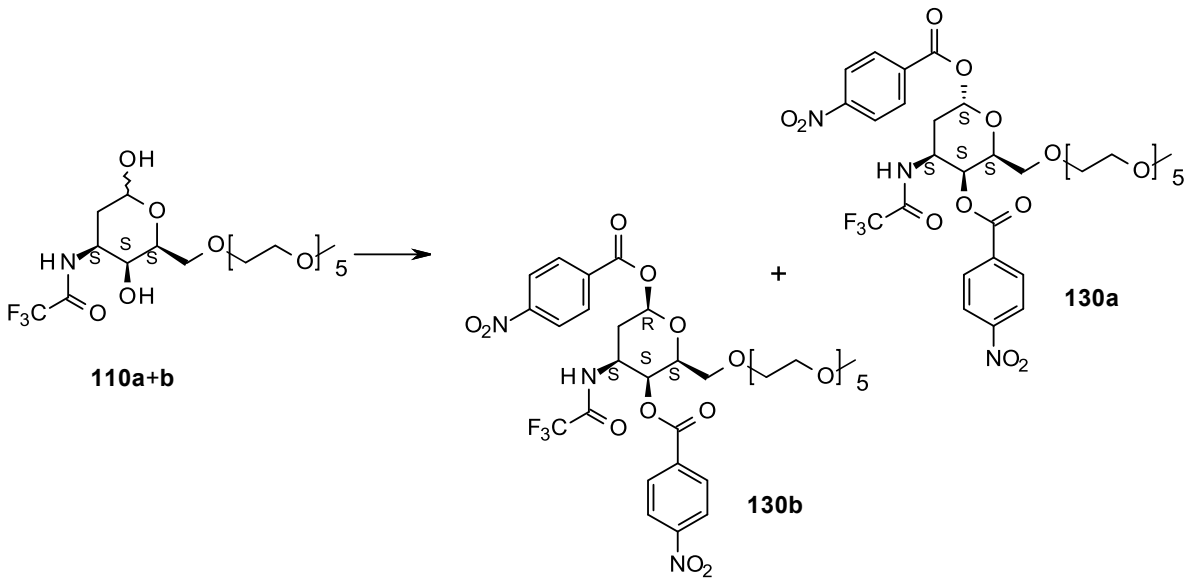
HPLC: Retention time: 2.10 min; Column: Zorbax Eclipse XDB-C8, 5 µm, l = 150 mm, Ø = 4.6 mm ; Mobile Phase: 40 % H_2O containing 0.4 % sodium dodecylsulfate (pH 2.00) and 60 % MeOH/ACN, 1:1, v/v; Detection: UV [λ= 254 nm, reference λ= 360 nm]; Injection volume: 20 µL; Temperature: 35 °C; Flow rate: 2 ml/min; Run time: 12 min; Standard preparation: 1 mg/ml mobile phase



Detector A - 1 (254nm)

Pk #	Retention Time	Area	Area %	Height	Height %
1	2.100	5699459	99.294	1058531	99.751
2	3.033	11961	0.208	894	0.084
3	9.008	28537	0.497	1752	0.165
Totals		5739957	100.000	1061177	100.000

[(2R,4S,5S,6S)-6-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxymethyl]-5-(4-nitrobenzoyl)oxy-4-[(2,2,2-trifluoroacetyl)amino]tetrahydropyran-2-yl] 4-nitro-benzoate **130a** and [(2S,4S,5S,6S)-6-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy] ethoxy]ethoxy]ethoxymethyl]-5-(4-nitrobenzoyl)oxy-4-[(2,2,2-trifluoroacetyl)amino] tetrahydropyran-2-yl] 4-nitrobenzoate **130b**



4-Nitrobenzoyl chloride (1.75 g, 9.43 mmol 2.8 equiv.) was added in small portions to a stirred solution of **110a+b** (1.67 g, 3.38 mmol, 1.0 equiv.) in dry pyridine (26 ml) at 0 °C under an atmosphere of argon. The resulting mixture was allowed to warm to room temperature and was then stirred for 14 hrs. After this time the reaction was quenched by addition of H_2O (5 ml) and all solvents were removed under reduced pressure. The resulting residue was resuspended in DCM (40 ml) and was washed three times with saturated aqueous $NaHCO_3$ and once with brine. The organic layer was then dried over $NaSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, with EtOAc/PE (1:1→3:2→2:1, v/v) to give in order of eluting **130a** (1.50 g, 1.89 mmol, 56.0 % yield) and **130b** (0.47 g, 0.594 mmol, 17.5 % yield) as white resins.

[(2R,4S,5S,6S)-6-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxymethyl]-5-(4-nitrobenzoyl)oxy-4-[(2,2,2-trifluoroacetyl)amino]tetrahydropyran-2-yl]4-nitro-benzoate **130a**

^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 8.36-8.24 (m, 8H, pNO_2Bz); 6.88 (d, *J* = 6.6 Hz, 1H, NH); 6.67 (m, 1H, H-1); 5.76 (m, 1H, H-4); 4.80 (m, 1H, H-5); 4.46 (m, 1H, H-3); 3.63-3.46 (m, 22H, H-6a/b, -(O-CH_2-CH_2)$_5$-) 3.34 (s, 3H, OCH_3); 2.39-2.33 (m, 2H, H-2a/b)

^{13}C NMR (50 MHz; $CDCl_3$): δ (ppm) = 164.62 (Cq, COO); 162.86 (Cq, COO); 150.77 (Cq, pNO_2Bz); 134.55 (Cq, pNO_2Bz); 134.11 (Cq, pNO_2Bz); 131.13 (CH, pNO_2Bz); 134.03 (CH, pNO_2Bz); 123.88 (CH, pNO_2Bz); 123.80 (CH, pNO_2Bz); 92.48 (CH, C-1); 71.84 (MeO$\underline{C}H_2$), 70.99 (CH_2, C-6); 70.81 (CH, C-4); 70.56, 70.54, 70.50, 70.45, 70.35, 69.04 (OCH_2); 68.82 (CH, C-5); 58.89 (OCH_3); 45.68 (CH, C-3); 29.28 (CH_2, C-2)

TLC: R_f = 0.65 (EtOAc)

Optical rotation $[\alpha_D^{20}]$: - 57.6° (c = 3.56, $CHCl_3$)

HRMS (ESI-TOF): calcd. for $[C_{33}H_{40}F_3N_3O_{16}+NH_4]^+$ 809.2704, found 809.2694; calcd. for $[C_{33}H_{40}F_3N_3O_{16}+Na]^+$ 814.2258, found 814.2247; calcd. for $[C_{33}H_{40}F_3N_3O_{16}+K]^+$ 830.1997, found 830.1984

[(2S,4S,5S,6S)-6-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxymethyl]-5-(4-nitrobenzoyl)oxy-4-[(2,2,2-trifluoroacetyl)amino]tetrahydropyran-2-yl]4-nitro-benzoate **130b**

^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 8.33-8.21 (m, 8H, pNO_2Bz); 6.90 (m, 1H, NH); 6.15 (dd, J^1 = 9.5 Hz, J^2 = 2.2 Hz, 1H, H-1); 5.64 (m, 1H, H-4); 4.51 (m, 1H, H-5); 4.19 (m, 1H, H-3); 3.69-3.52 (m, 22H, H-6a/b, -(O-CH_2-CH_2)$_5$-) 3.35 (s, 3H, OMe); 2.46-2.17 (m, 2H, H-2a/b)

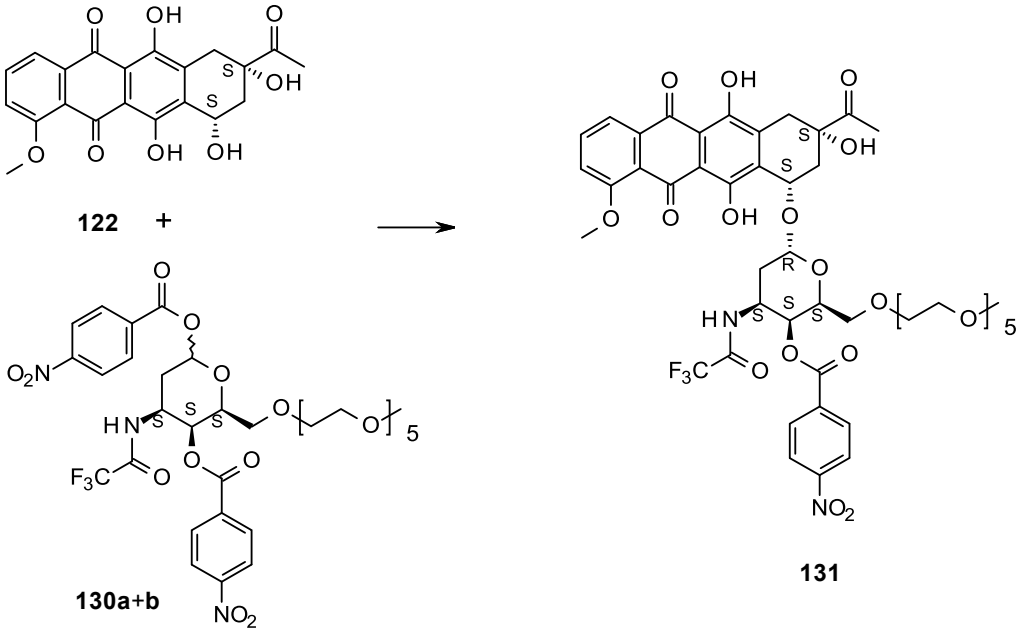
^{13}C NMR (50 MHz; $CDCl_3$): δ (ppm) = 164.75 (Cq, COO); 162.56 (Cq, COO); 150.95 (Cq, pNO_2Bz); 134.29 (Cq, pNO_2Bz); 134.05 (Cq, pNO_2Bz); 131.18 (CH, pNO_2Bz); 130.98 (CH, pNO_2Bz); 123.87 (CH, pNO_2Bz); 123.65 (CH, pNO_2Bz); 93.08 (CH, C-1); 74.27 (CH, C-4); 71.87 (MeO$\underline{C}H_2$), 71.06 (CH_2, C-6); 71.06, 70.48, 70.40, 68.96 (OCH_2); 68.19 (CH, C-5); 58.93 (OCH_3); 48.81 (CH, C-3); 30.75 (CH_2, C-2)

TLC: R_f = 0.50 (EtOAc)

Optical rotation $[\alpha_D^{20}]$: - 11.2° (c = 1.16, $CHCl_3$)

HRMS (ESI-TOF): calcd. for $[C_{33}H_{40}F_3N_3O_{16}+NH_4]^+$ 809.2704, found 809.2694; calcd. for $[C_{33}H_{40}F_3N_3O_{16}+Na]^+$ 814.2258, found 814.2247; calcd. for $[C_{33}H_{40}F_3N_3O_{16}+K]^+$ 830.1997, found 830.1984

[(2S,3S,4S,6R)-6-[[(1S,3S)-3-acetyl-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-2,4-dihydro-1H-tetracen-1-yl]oxy]-2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy] ethoxymethyl]-4-[(2,2,2-trifluoroacetyl)amino]tetrahydropyran-3-yl]4-nitro-benzoate **131**



Trimethylsilyl trifluoromethanesulfonate (105 µL, 0.581 mmol, 2.05 equiv.) was added to a stirred solution of **130a+b** (225 mg, 0.284 mmol, 1.0 equiv.) and 4Å molsieve (1.1 g) in a mixture of DCM (15 ml) and Et_2O (12 ml) at -50 °C under an atmosphere of argon. The resulting mixture was then stirred at 0 °C in an ice-bath for 1 hr. The temperature of the solution was then reduced to -30 °C and **122** (56 mg, 0.142 mmol, 0.5 equiv.) solved in THF (9 ml) was slowly added. The whole mixture was then stirred between -10 °C and -15 °C for 6 hrs. After this time TLC analysis (DCM/MeOH, 100:5, v/v) showed a nearly complete consumption of **122** and the reaction was quenched by addition of aqueous saturated $NaHCO_3$. Then DCM was added, the layers were separated and the aqueous phase was extracted several times with DCM. The combined organic extracts were washed with H_2O then brine, dried over $NaSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with DCM/MeOH (100:1→100:2→100:4, v/v) to give in order of eluting unreacted aglycone **122** (11.0 mg, 0.028 mmol, 19 %) and the desired product **131** (99.0 mg, 0.097 mmol, 68.8 % yield) as a red solid.

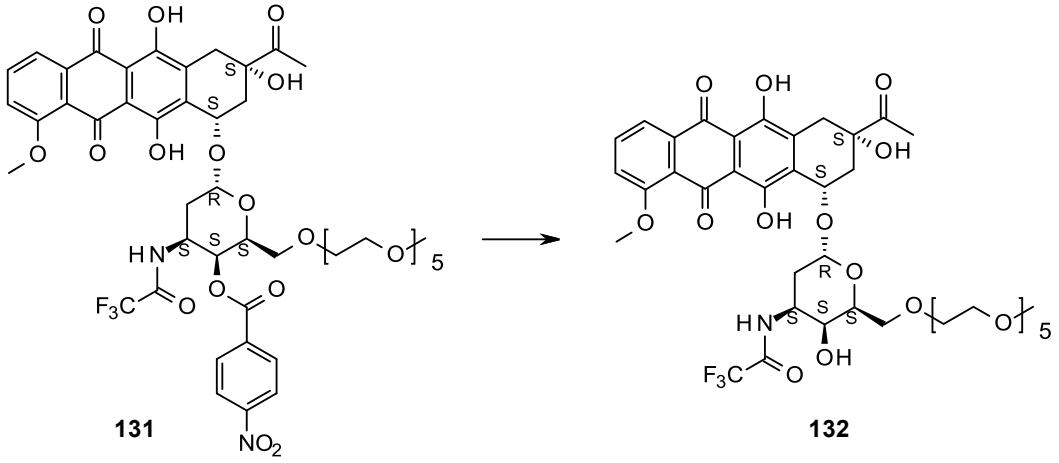
^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 13.93 (s, 1H, OH-6); 13.15 (s, 1H, OH-11); 8.30-8.23 (m, 4H, pNO_2Bz); 7.90 (d, *J* = 7.55 Hz, 1H, H-1); 7.76-7.72 (m, 1H, H-2); 7.35 (d, *J* = 8.5 Hz, 1H, H-3); 6.72 (d, *J* = 7.0 Hz, 1H, NH); 5.69 (d, *J* = 2.9 Hz, 1H, H-dau1); 5.62 (m, 1H, H-dau4); 5.20 (m, 1H, H-7); 4.56-4.53 (m, 1H, H-dau5); 4.48-4.40 (m, 1H, H-dau3); 4.04 (s, 3H, $ArOCH_3$); 3.68-3.45 (m, 22H, H-dau6, -(O-CH_2-CH_2)$_5$-); 3.33 (s, 3H, OCH_3); 3.12 (m, 1H, H-10a); 2.88 (m, 1H, H-10b); 2.57 (m, 1H, H-8a); 2.43 (s, 3H, CH_3); 2.18-2.02 (m, 3H, H-8b, H-dau2a/b)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 212.06 (Cq, C-13); 186.89 (Cq, C-5); 186.35 (Cq, C-12); 165.82 (Cq, pNO_2Bz); 160.89 (Cq, C-4); 156.77 (Cq, *J* = 36.8 Hz, $\underline{C}OCF_3$); 156.36 (Cq, C-6); 155.62 (Cq, C-11); 150.77 (Cq, pNO_2Bz); 135.69 (CH, C-2); 135.29 (Cq, C-12a); 134.52 (Cq, C-10a); 134.38 (Cq, pNO_2Bz); 133.70 (Cq, C-6a); 130.99 (CH, pNO_2Bz); 123.72 (CH, pNO_2Bz); 120.50 (Cq, C-4a); 119.63 (CH, C-1); 118.35 (CH, C-3); 115.32 (Cq, *J* = 285.0 Hz, CF_3); 111.30 (Cq, C-5a); 111.12 (Cq, C-11a); 100.31 (CH, C-dau1); 76.46 (Cq, C-9); 71.80 (MeO$\underline{C}H_2$); 70.93 (OCH_2); 70.87 (CH, C-7); 70.72, 70.43 (OCH_2); 69.28 (CH, C-dau5); 69.87 (CH-C-dau4); 58.90 (OCH_3); 56.58 (ArO$\underline{C}H_3$); 45.55 (CH, C-dau3); 34.91 (CH_2, C-8); 33.00 (CH_2, C-10); 30.10 (CH_2, C-dau2); 24.56 (CH_3, C-14)

HRMS (ESI-TOF): calcd. for $[C_{47}H_{53}F_3N_2O_{20}+Na]^+$ 1045.3041, found 1045.3059; calcd. for $[C_{47}H_{53}F_3N_2O_{20}+K]^+$ 1061.2781, found 1061.2809

TLC: R_f = 0.25 (DCM/MeOH 100:5, v/v)

N-[(2S,3S,4S,6R)-6-[[(1S,3S)-3-acetyl-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-2,4-dihydro-1H-tetracen-1-yl]oxy]-2-[2-[2-[2-[2-(2-ethoxyethoxy)ethoxy]ethoxy]ethoxy] ethoxymethyl]-3-hydroxy-tetrahydropyran-4-yl]-2,2,2-trifluoro-acetamide **132**



Aqueous sodium hydroxide (1.0 N, 1.3 ml) was added to a stirred solution of **131** (99.0 mg, 0.097 mmol) in methanol (52.1 ml) and DCM (0.5 ml) at 0 °C. The colour of the reaction mixture changed from light red to deep purple. Then the solution was stirred 0 °C in an ice-bath for 30 min. The reaction was then neutralized with pure acetic acid (0.09 ml) until the colour of the reaction mixture became light red. Then EtOAc (80 ml) and brine (80 ml) was added, the layers were separated and the organic phase was washed twice with brine, dried over NaSO4 filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with DCM/MeOH (100:4, v/v) to give 75.9 mg of **132** (0.087 mmol, 89.7 %) as orange-red solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 13.94 (s, 1H, OH-6); 13.23 (s, 1H, OH-11); 7.99 (d, *J* = 7.55 Hz, 1H, H-1); 7.77-7.74 (m, 1H, H-2); 7.36 (d, *J* = 8.5 Hz, 1H, H-3); 7.10 (m, 1H, NH); 5.57 (brs, 1H, H-dau1); 5.25 (m, 1H, H-7); 4.22-4.14 (m, 1H, H-dau3); 4.14-4.11 (m, 1H, H-dau5); 4.05 (s, 3H, $ArOCH_3$); 3.89 (m, 1H, H-dau4); 3.88-3.447 (m, 22H, H-dau6, -$(O\text{-}CH_2\text{-}CH_2)_5$-); 3.35 (s, 3H, OCH_3); 3.21-3.17 (m, 1H, H-10a); 2.88-2.84 (m, 1H, H-10b); 2.39 (s, 3H, CH_3); 2.37-2.34 (m, 1H, H-8a); 2.11-2.01(m, 2H, H-8b, H-dau2a); 1.86-183 (m, 1H, H-dau2b)

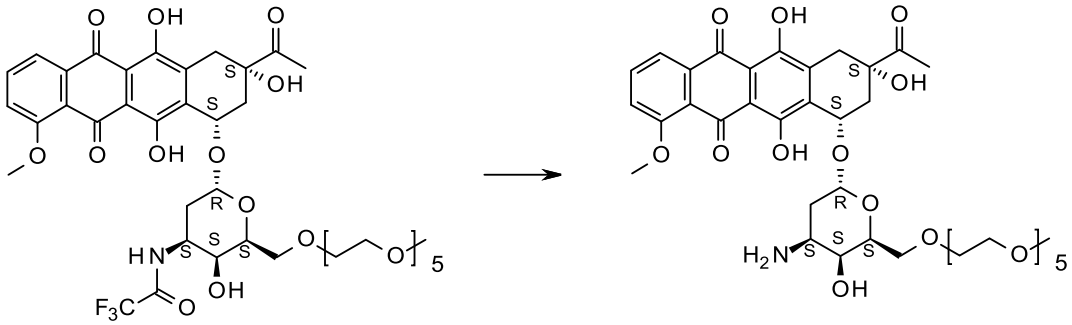
^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 212.12 (Cq, C-13); 186.96 (Cq, C-5); 186.59 (Cq, C-12); 160.92 (Cq, C-4); 156.30 (Cq, C-6); 156.27 (Cq, *J* = 36.8 Hz, $COCF_3$); 155.71 (Cq, C-11); 135.63 (CH, C-2); 135.40 (Cq, C-12a); 134.25 (Cq, C-10a); 133.83 (Cq, C-6a); 120.78

(Cq, C-4a); 119.70 (CH, C-1); 118.34 (CH, C-3); 115.70 (Cq, *J* = 286.5 Hz, CF_3); 111.39 (Cq, C-5a); 111.23 (Cq, C-11a); 100.70 (CH, C-dau1); 76.61 (Cq, C-9); 71.78 ($MeO\underline{C}H_2$); 71.26 (CH_2, C-dau6); 70.75, 70.43, 70.38, 70.36 (OCH_2); 70.20 (CH, C-7); 69.43 (CH, C-dau5); 66.15 (CH-C-dau4); 58.87 (OCH_3); 56.59 ($ArO\underline{C}H_3$); 45.62 (CH, C-dau3); 34.94 (CH_2, C-8); 33.31 (CH_2, C-10); 29.58 (CH_2, C-dau2); 24.90 (CH_3, C-14)

HRMS (ESI-TOF): calcd. for $[C_{40}H_{50}F_3NO_{17}+NH_4]^+$ 891.3374, found 891.3370; calcd. for $[C_{40}H_{50}F_3NO_{17}+Na]^+$ 896.2928, found 896.2940; calcd. for $[C_{40}H_{50}F_3NO_{17}+K]^+$ 912.26679, found 912.2655

TLC: R_f = 0.15 (DCM/MeOH 100:5, v/v)

(7S,9S)-9-acetyl-7-[(2R,4S,5S,6S)-4-amino-5-hydroxy-6-[2-[2-[2-[2-(2-methoxy-ethoxy)ethoxy]ethoxy]ethoxy]ethoxymethyl]tetrahydropyran-2-yl]oxy-6,9,11-trihydroxy-4-methoxy-8,10-dihydro-7H-tetracene-5,12-dione **133**



132 **133**

A solution of **132** (60.0 mg, 0.0687 mmol) in aqueous sodium hydroxide (1.0 N, 12.5 ml) was allowed to stir at room temperature for 30 min. The deep purple solution was the adjusted to pH 8 with 1N HCl. The neutralized solution was then extracted several times with $CHCl_3$ until the aqueous phase was colourless. The combined organic extracts were washed with brine, dried over $NaSO_4$ filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with DCM/MeOH (10:1→10:2, v/v) to give 13.9 mg of **133** (0.0179 mmol, 26.0 %) as red solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 13.81 (s, 1H, OH-6); 13.12 (s, 1H, OH-11); 7.86-7.85 (m, 1H, H-1); 7.69-7.66 (m, 1H, H-2); 7.26-7.24 (m, 1H, H-3); 5.52 (brs, 1H, H-dau1); 5.03-4.98 (m, 1H, H-7); 4.27 (m, 1H, H-dau4); 4.18 (m, 1H, H-dau5); 3.94 (s, 3H, $ArOCH_3$); 3.70-3.51(m, 23H, H-dau3, H-dau6, -$(O-CH_2-CH_2)_5$-); 3.33 (s, 3H, OCH_3); 3.13-3.10 (m, 1H, H-10a); 2.81-2.77 (m, 1H, H-10b); 2.41 (s, 3H, CH_3); 2.38-2.35 (m, 1H, H-8a); 2.18-2.00 (m, 3H, H-8b, H-dau2a/b)

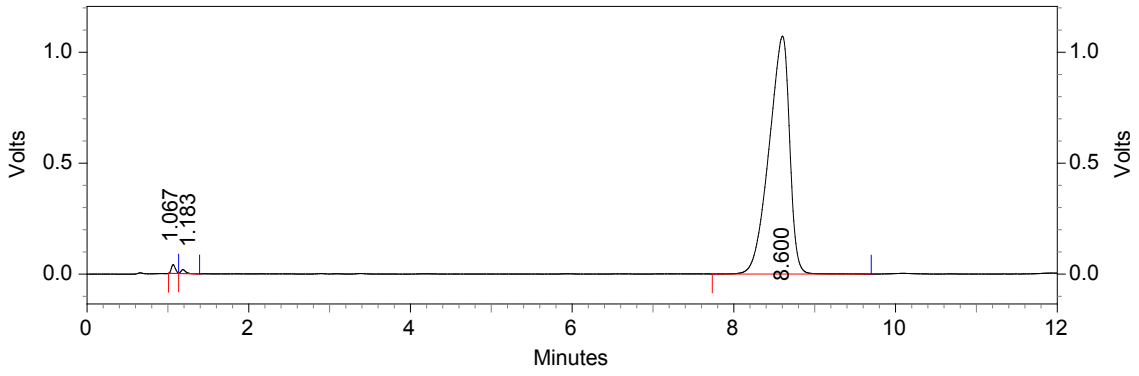
^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 212.66 (Cq, C-13); 186.45 (Cq, C-5); 186.19 (Cq, C-12); 160.72 (Cq, C-4); 156.47 (Cq, C-6); 155.54 (Cq, C-11); 135.53 (CH, C-2); 135.12 (Cq, C-12a); 134.65 (Cq, C-10a); 134.21 (Cq, C-6a); 120.50 (Cq, C-4a); 119.49 (CH, C-1); 118.29 (CH, C-3); 111.04 (Cq, C-11a); 110.86 (Cq, C-5a); 99.67 (CH, C-dau1); 76.29 (Cq, C-9); 71.52 ($MeO\underline{C}H_2$); 70.93 (CH_2, C-dau6); 70.57, 70.52, 70.39, 70.31, 70.22, 70.17, 70.12, 70.06, 69.92 (OCH_2); 69.57 (CH, C-dau5, C-7); 63.48 (CH, C-dau4); 58.91 (OCH_3); 56.57

(ArO<u>C</u>H_3); 47.23 (CH, C-dau3); 34.99 (CH_2, C-8); 33.00 (CH_2, C-10); 28.66 (CH_2, C-dau2); 25.12 (CH_3, C-14)

<u>TLC:</u> Rf= 0.56 (DCM/MeOH 10:2, v/v)

<u>HRMS</u> (ESI-TOF): calcd. for $[C_{38}H_{51}NO_{16}+H]^+$ 778.3286, found 778.3298; calcd. for $[C_{38}H_{51}NO_{16}+Na]^+$ 800.3105, found 800.3121; calcd. for $[C_{38}H_{51}NO_{16}+K]^+$ 816.2844, found 816.2847

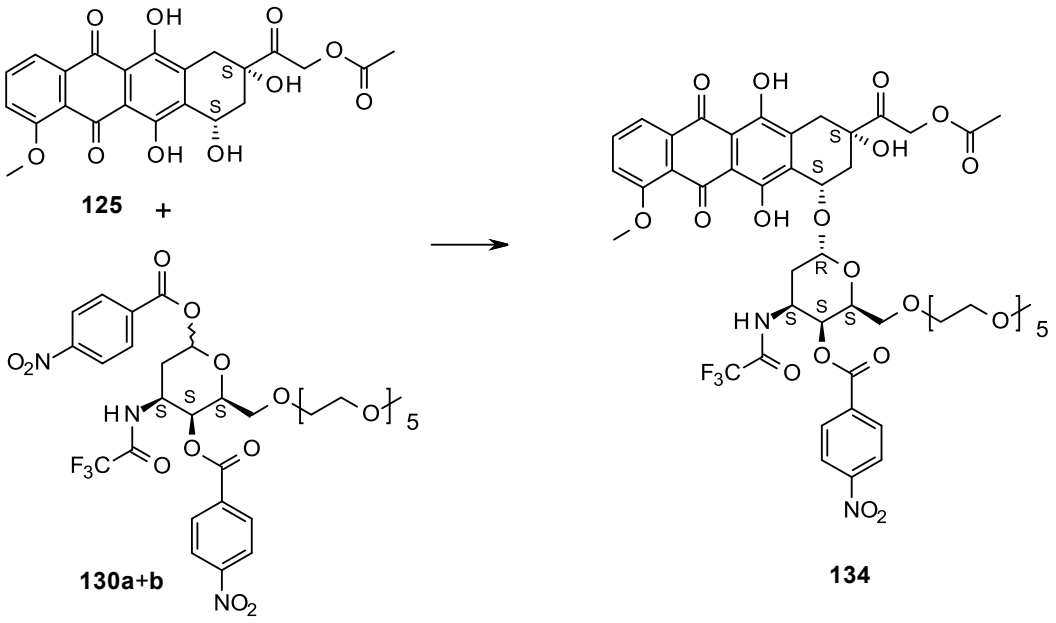
<u>HPLC:</u> Retention time: 8.6 min; Column: Zorbax Eclipse XDB-C8, 5 µm, l = 150 mm, Ø = 4.6 mm; Mobile Phase: 40 % H_2O containing 0.4 % sodium dodecylsulfate (pH 2.00) and 60 % MeOH/ACN, 1:1, v/v; Detection: UV [λ= 254 nm, reference λ= 360 nm]; Injection volume: 20 µL; Temperature: 35 °C; Flow rate: 2 ml/min; Run time: 12 min; Standard preparation: 1 mg/ml mobile phase.



Detector A - 1 (254nm)

Pk #	Retention Time	Area	Area %	Height	Height %
1	1.067	139488	0.731	39920	3.532
2	1.183	74771	0.392	18250	1.615
3	8.600	18879507	98.878	1072066	94.853
Totals		19093766	100.000	1130236	100.000

[(2S,3S,4S,6R)-6-[[(1S,3S)-3-(2-acetoxyacetyl)-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-2,4-dihydro-1H-tetracen-1-yl]oxy]-2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]-ethoxy]ethoxy]ethoxymethyl]-4-[(2,2,2-trifluoroacetyl)amino]tetrahydropyran-3-yl] 4-nitrobenzoate **134**



A solution of **130a+b** (307.4 mg, 0.388 mmol, 1.0 equiv.) and 4Å molecular sieve (1.0 g) in a mixture of DCM (16 ml) and Et_2O (13 ml) was cooled to -50 °C with carbon dioxide snow in acetone under an atmosphere of Ar. Then Trimethylsilyl trifluoromethanesulfonate (160 µL, 0.884 mmol, 2.3 equiv.) was added dropwise. The resulting mixture was then stirred at 0 °C in an ice-bath for 1 hr. The temperature of the solution was then reduced to -30 °C and **125** (85.0 mg, 0.186 mmol, 0.5 equiv.) solved in DCM (20 ml) was added dropwise. The whole mixture was then stirred between -10 °C and -15 °C for 6 hrs. After this time TLC analysis (DCM/MeOH, 100:5, v/v) showed a nearly complete consumption of **125** and the reaction was quenched by addition of aqueous saturated $NaHCO_3$. Then DCM was added, and the molecular sieve was filtered off. The layers were separated and the aqueous phase was extracted several times with DCM. The combined organic extracts were washed with H_2O then brine, dried over $NaSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with DCM/MeOH (1000:12, v/v) to give in order of eluting unreacted aglycone **125** (12.4 mg, 0.027 mmol, 14.6 %) and the desired product **134** (134.0 mg, 0.124 mmol, 66.6 % yield) as a red solid.

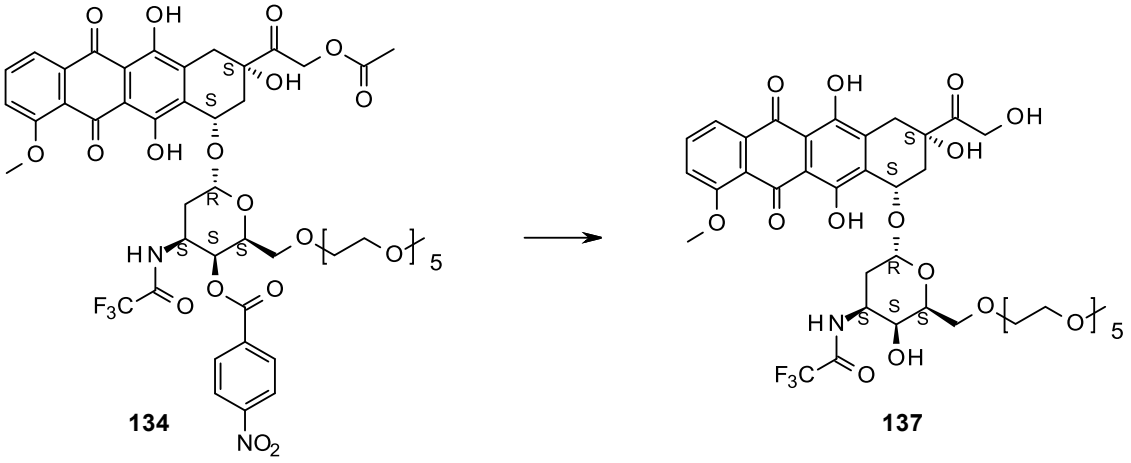
^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 13.94 (s, 1H, OH-6); 13.25 (s, 1H, OH-11); 8.37-8.18 (m, 4H, pNO_2Bz); 8.05-8.03 (m, 1H, H-1); 7.81-7.77 (m, 1H, H-2); 7.39 (d, *J* = 8.5 Hz, 1H, H-3); 6.78 (d, *J* = 6.6 Hz, 1H, NH); 5.68 (d, *J* = 4.1 Hz, 1H, H-dau1); 5.65 (s, 1H, H-dau4); 5.42 (d, *J* = 18.3 Hz, H-14a); 5.35-5.32 (m, 1H, H-7); 5.26 (d, *J* = 18.3 Hz, H-14b); 4.64 (t, *J* = 7.7 Hz, 1H, H-dau5); 4.47-4.37 (m, 1H, H-dau3); 4.07 (s, 3H, $ArOCH_3$); 3.64-3.40 (m, 22H, H-dau6, -(O-CH_2-CH_2)$_5$-); 3.35 (s, 3H, OCH_3); 3.13 (d, *J* = 18.9 Hz, 1H, H-10a); 2.90 (d, *J* = 18.9 Hz, 1H, H-10b); 2.56 (d, *J* = 14.8 Hz,1H, H-8a); 2.21 (s, 3H, $COCH_3$); 2.08-1.88 (m, 3H, H-8b, H-dau2a/b)

^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) = 207.42 (Cq, C-13); 187.09 (Cq, C-5); 187.06 (Cq, C-12); 170 (Cq, COO); 164.06 (Cq, pNO_2Bz); 161.00 (Cq, C-4); 155.67 (Cq, C-6); 155.51 (Cq, C-11); 150.64 (Cq, pNO_2Bz); 135.75 (CH, C-2); 135.52 (Cq, C-12a); 134.97 (Cq, C-10a); 134.21 (Cq, pNO_2Bz); 132.22 (Cq, C-6a); 131.14 (CH, pNO_2Bz); 123.48 (CH, pNO_2Bz); 120.86 (Cq, C-4a); 119.87 (CH, C-1); 118.39 (CH, C-3); 111.46 (Cq, C-5a); 111.38 (Cq, C-11a); 98.95 (CH, C-dau1); 76.91 (Cq, C-9); 71.82 (MeO$\underline{C}H_2$); 70.94, 70.47, 70.40, 70.23 (OCH_2); 70.02 (CH, C-7); 68.28 (CH, C-dau5); 67.60 (CH, C-dau4); 66.36 (CH2, C-14); 58.93 (OCH_3); 56.64 ($ArOCH_3$); 48.97 (CH, C-dau3); 34.41 (CH_2, C-8); 32.12 (CH_2, C-10); 29.66 (CH_2, C-dau2); 20.59 (CH_3, Ac)

TLC: R_f = 0.29 (DCM/MeOH 100:5, v/v)

HRMS (ESI-TOF): calcd. for $[C_{49}H_{55}F_3N_2O_{22}+NH_4]^+$ 1098.3542, found 1098.3527; calcd. for $[C_{49}H_{55}F_3N_2O_{22}+Na]^+$ 1103.3096, found 1103.3089; calcd. for $[C_{49}H_{55}F_3N_2O_{22}+K]^+$ 1119.2835, found 1119.2812

2,2,2-trifluoro-N-[(2S,3S,4S,6R)-3-hydroxy-2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxymethyl]-6-[[(1S,3S)-3,5,12-trihydroxy-3-(2-hydroxyacetyl)-10-methoxy-6,11-dioxo-2,4-dihydro-1H-tetracen-1-yl]oxy]tetrahydropyran-4-yl]-acetamide **137**



134 **137**

To a stirred solution of **134** (124.3 mg, 0.115 mmol,) in a mixture of acetone (26 ml) and MeOH (13 ml), aqueous saturated $NaHCO_3$ (39 ml) was added under an atmosphere of argon. The resulting purple mixture was allowed to stir at room temperature for 3 hrs. After this time TLC analysis (DCM/MeOH, 100:5, v/v) showed a complete consumption of **134** and the reaction was quenched by addition of H_2O and DCM. The layers were separated and the aqueous phase was extracted triply with DCM. The combined organic extracts were washed with brine, dried over $NaSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with DCM/MeOH (100:3, v/v) to give **137** (42.9 mg, 0.0482 mmol, 41.5 % yield) as a red solid.

^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 13.96 (s, 1H, OH-6); 13.25 (s, 1H, OH-11); 8.04 (d, *J* = 7.6 Hz, 1H, H-1); 7.78 (t, *J* = 8.2 Hz, 1H, H-2); 7.39 (d, *J* = 8.4 Hz, 1H, H-3); 7.09 (d, *J* = 8.7 Hz, 1H, NH); 5.59-5.57 (m, 1H, H-dau1); 5.29 (s, 1H, H-7); 4.77 (s, 2H, H-14a/b); 4.44 (m, 1H, H-dau3); 4.17 (m, 1H, H-dau5); 4.08 (s, 3H, $ArOCH_3$); 4.00 (m, 1H, H-dau4); 3.87-3.54 (m, 22H, H-dau6, -(O-CH_2-CH_2)$_5$-); 3.36 (s, 3H, OCH_3); 3.29 (d, *J* = 19.8, 1H, H-10a); 3.02 (d, *J* = 18.8, 1H, H-10b); 2.46 (d, *J* = 14.5, 1H, H-8a); 2.19-1.82 (m, 3H, H-8b, H-dau2a/b)

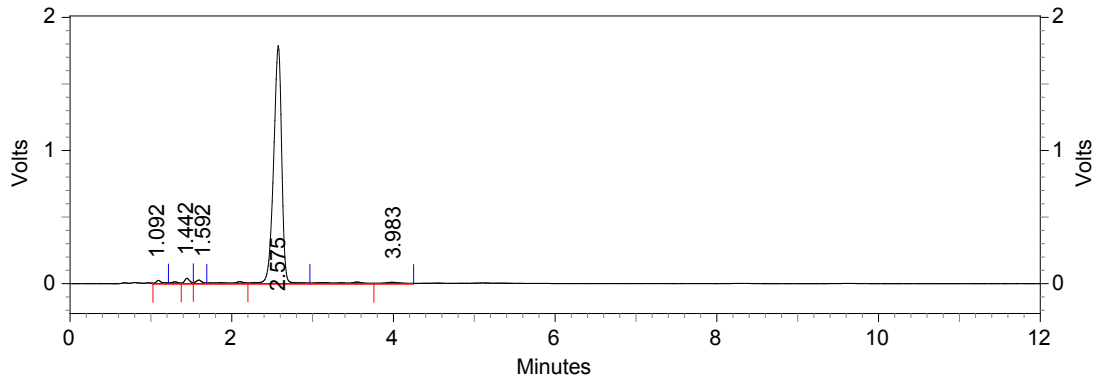
^{13}C NMR (50 MHz; $CDCl_3$): δ(ppm) = 213.85 (Cq, C-13); 187.14 (Cq, C-5); 186.76 (Cq, C-12); 161.05 (Cq, C-4); 156.19 (Cq, C-6); 155.67 (Cq, C-11); 135.75 (CH, C-2); 135.51 (Cq, C-12a); 133.56 (Cq, C-10a); 133.42 (Cq, C-6a); 121.46 (Cq, C-4a); 119.84 (CH, C-1); 118.43 (CH, C-3); 111.64 (Cq, C-5a); 111.47 (Cq, C-11a); 100.93 (CH, C-dau1); 76.64 (Cq, C-9);

71.84 (MeO<u>C</u>H_2); 70.96 (CH_2, C-dau6); 70.73, 70.45, 70.42, 70.38 (OCH_2); 70.16 (CH, C-7); 69.79 (CH, C-dau5); 65.18 (CH-C-dau4); 65.57 (CH_2, C-14); 58.91 (OCH_3); 56.68 (Ar<u>O</u>CH_3); 45.53 (CH, C-dau3); 35.61 (CH_2, C-8); 33.86 (CH_2, C-10); 29.69 (CH_2, C-dau2)

<u>TLC:</u> R_f = 0.18 (DCM/MeOH 100:5, v/v)

<u>HRMS</u> (ESI-TOF): calcd. for $[C_{40}H_{50}F_3NO_{18}+NH_4]^+$ 907.3323, found 907.3308; calcd. for $[C_{40}H_{50}F_3NO_{18}+Na]^+$ 912.2877, found 912.2880; calcd. for $[C_{40}H_{50}F_3NO_{18}+K]^+$ 928.2617, found 928.2610

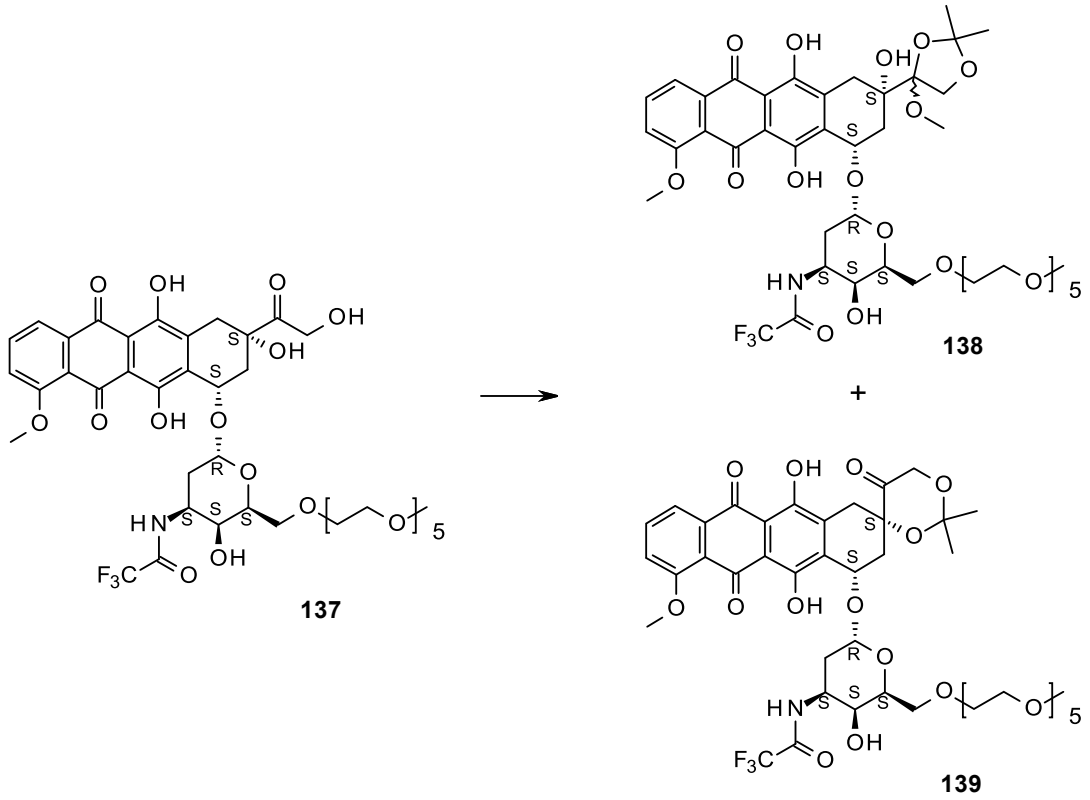
<u>HPLC:</u> Retention time: 2.575 min; Column: Zorbax Eclipse XDB-C8, 5 µm, l = 150 mm, Ø = 4.6 mm; Mobile Phase: 40 % H_2O containing 0.4 % sodium dodecylsulfate (pH 2.00) and 60 % MeOH/ACN, 1:1, v/v; Detection: UV [λ = 254 nm, reference λ = 360 nm]; Injection volume: 20 µL; Temperature: 35 °C; Flow rate: 2 ml/min; Run time: 12 min; Standard preparation: 1 mg/ml mobile phase



Detector A - 1 (254nm)

Pk #	Retention Time	Area	Area %	Height	Height %
1	1.092	128602	0.983	24432	1.293
2	1.442	180979	1.383	40240	2.130
3	1.592	146548	1.120	27020	1.430
4	2.575	12480671	95.376	1787692	94.616
5	3.983	148938	1.138	10035	0.531
Totals		13085738	100.000	1889419	100.000

2,2,2-trifluoro-N-[(2S,3S,4S,6R)-3-hydroxy-2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]-ethoxy]ethoxy]ethoxymethyl]-6-[[(1S,3S)-3,5,12-trihydroxy-10-methoxy-3-(4-methoxy-2,2-dimethyl-1,3-dioxolan-4-yl)-6,11-dioxo-2,4-dihydro-1H-tetracen-1-yl]oxy]tetra-hydropyran-4-yl]acetamide **138** and N-[(2S,3S,4S,6R)-6-[(1'S,4S)-5',12'-dihydroxy-10'-methoxy-2,2-dimethyl-5,6',11'-trioxo-spiro[1,3-dioxane-4,3'-2,4-dihydro-1H-tetracene]-1'-yl]oxy-3-hydroxy-2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]-ethoxymethyl]tetrahydropyran-4-yl]-2,2,2-trifluoro-acetamide **139**



To a stirred solution of **137** (17.0 mg, 0.0191 mmol) in a mixture of dioxane (1.0 ml) and $CHCl_3$ (20 ml), 2,2-dimethoxypropane (5.0 ml) and a catalytic amount of *p*-toluenesulfonic acid monohydrate (1.2 mg) were added under an atmosphere of argon. The resulting solution was allowed to stir at 52 °C for 22 hrs. After this time the reaction was cooled to room temperature, solid $NaHCO_3$ (18 mg) was added and the solution was stirred for further 5 min. The reaction mixture was then washed twice with H_2O and once with brine. The layers were separated and the organic layer was dried over $NaSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with DCM/MeOH (100:2, v/v) to give in order of eluting **139** (2.64 mg, 0.00284 mmol, 14.9 % yield) as a red solid and **138** (10.56 mg, 0.0110 mmol, 57.5 % yield) as a red solid.

2,2,2-trifluoro-N-[(2S,3S,4S,6R)-3-hydroxy-2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy] ethoxy]ethoxy]ethoxymethyl]-6-[[(1S,3S)-3,5,12-trihydroxy-10-methoxy-3-(4-methoxy-2,2-dimethyl-1,3-dioxolan-4-yl)-6,11-dioxo-2,4-dihydro-1H-tetracen-1-yl]oxy]tetrahydropyran-4-yl]acetamide **138**

^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 14.01 and 13.93 (2s, 1H, OH-6); 13.37 and 13.36 (2s, 1H, OH-11); 8.04 (d, *J* = 7.7 Hz, 1H, H-1); 7.77 (t, *J* = 8.1 Hz, 1H, H-2); 7.38 (d, *J* = 8.6 Hz, 1H, H-3); 6.99 (m, 1H, NH); 5.54 and 5.48 (2m, 1H, H-dau1); 5.19-5.12 (m, 1H, H-7); 4.53-4.21 (m, 3H, H-14a/b, H-dau3); 4.07-3.80 (m, 5H, $ArOCH_3$, H-dau5, H-dau4) 3.80-3.49 (m, 22H, H-dau6, -(O-CH_2-CH_2)$_5$-); 3.41 (s, 3H, OCH_3); 3.37 (s, 3H, OCH_3); 3.29 (d, *J* = 19.8 Hz, 1H, H-10a); 3.07 (d, *J* = 18.8 Hz, 1H, H-10b); 2.31-1.77 (m, 4H, H-8a/b, H-dau2a/b); 1.51 (s, 3H, CH_3, acetonide); 1.46 (s, 3H, CH_3, acetonide)

HRMS (ESI-TOF): calcd. for $[C_{44}H_{58}F_3NO_{19}+Na]^+$ 984.3453, found 984.3457; calcd. for $[C_{44}H_{58}F_3NO_{19}+K]^+$ 1000.3192, found 1000.3210

TLC: R_f = 0.21 (DCM/MeOH 100:5, v/v)

N-[(2S,3S,4S,6R)-6-[(1'S,4S)-5',12'-dihydroxy-10'-methoxy-2,2-dimethyl-5,6',11'-trioxo-spiro[1,3-dioxane-4,3'-2,4-dihydro-1H-tetracene]-1'-yl]oxy-3-hydroxy-2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxymethyl]tetrahydropyran-4-yl]-2,2,2-trifluoro-acetamide **139**

^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 13.95 (s, 1H, OH-6); 13.38 (s, 1H, OH-11); 8.04 (d, *J* = 7.3 Hz, 1H, H-1); 7.77 (t, *J* = 7.7 Hz, 1H, H-2); 7.38 (d, *J* = 8.0 Hz, 1H, H-3); 6.99 (d, *J* = 7.4 Hz, 1H, NH); 5.54 (m, 1H, H-dau1); 5.23 (s, 1H, H-7); 4.46-4.41 (m, 2H, H-14a/b); 4.18-4.14 (m, 2H, H-dau3, H-dau5); 4.07 (s, 3H, $ArOCH_3$); 3.98 (m, 1H, H-dau4); 3.88-3.55 (m, 22H, H-dau6, -(O-CH_2-CH_2)$_5$-); 3.37 (s, 3H, OCH_3); 2.94 (m, 1H, H-10a); 2.59-2.51 (m, 1H, H-10b); 2.25-1.78 (m, 4H, H-8a/b, H-dau2a/b); 1.60 (s, 3H, CH_3, acetonide); 1.43 (s, 3H, CH_3, acetonide)

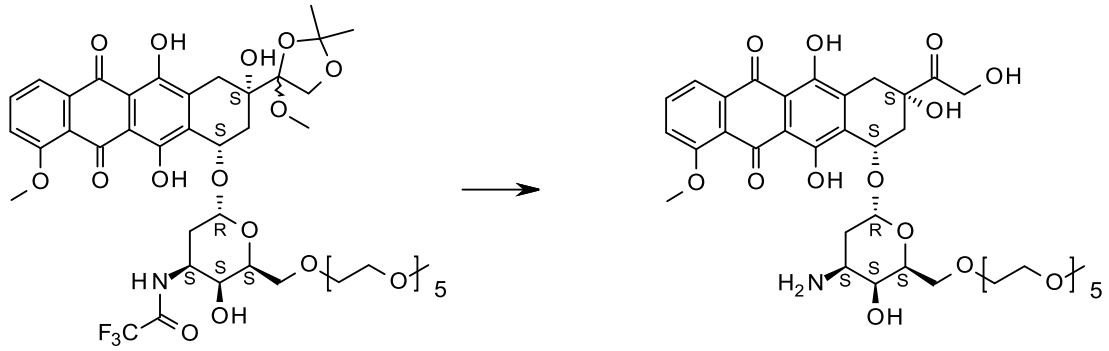
HRMS (ESI-TOF): calcd. for $[C_{43}H_{54}F_3NO_{18}+Na]^+$ 952.3191, found 952.3201; calcd. for $[C_{43}H_{54}F_3NO_{18}+K]^+$ 968.2930, found 968.2931

TLC: R_f = 0.26 (DCM/MeOH 100:5, v/v)

(7S,9S)-7-[(2R,4S,5S,6S)-4-amino-5-hydroxy-6-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxymethyl]tetrahydropyran-2-yl]oxy-6,9,11-trihydroxy-9-(2-hydroxyacetyl)-4-methoxy-8,10-dihydro-7H-tetracene-5,12-dione

140

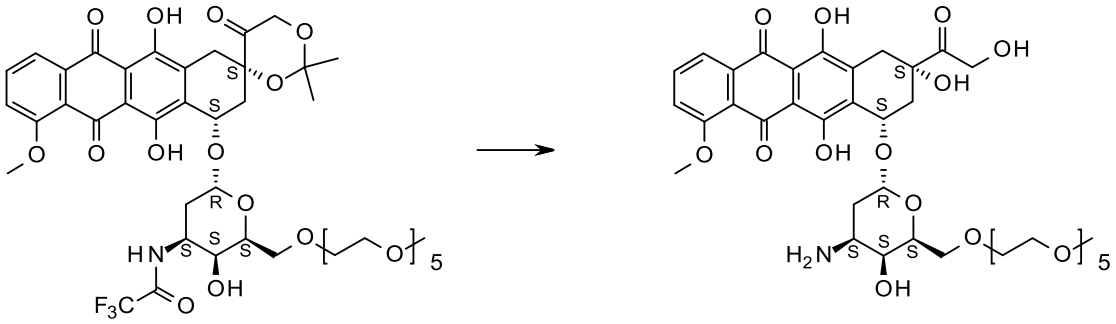
Option a:



138 **140**

Aqueous sodium hydroxide (0.1 N, 2.0 ml) was added to a stirred solution of **138** (5.5 mg, 0.0057 mmol) in acetone (0.4 ml). The deep purple solution was allowed to stir at room temperature 30 min. After this time HCl (0.1 N) was added dropwise until a pH of 8 was reached. The resulting orange-red mixture was extracted several times with $CHCl_3$ until the aqueous phase was colourless. The combined organic extracts were dried over sodium sulphate, filtered and concentrated under reduced pressure. The residue was dissolved in HCl (0.1 N, 2.0 ml) and was stirred at room temperature for 42 hrs. After this time $CHCl_3$ was added, the phases were separated and the aqueous phase was extracted twice with $CHCl_3$ to get rid of impurities and hydrolysed aglycone. The pH of the acidic aqueous phase was then adjusted to 8 with NaOH (0.1N). Afterwards $CHCl_3$ was added, the layers were separated and the aqueous phase was extracted several times with $CHCl_3$ until it was colourless. The combined organic extracts were dried over $NaSO_4$ filtered and concentrated under reduced pressure to give 1.9 mg of **140** (0.0024 mmol, 41.8 %) as orange-red solid.

Option b:



139 **140**

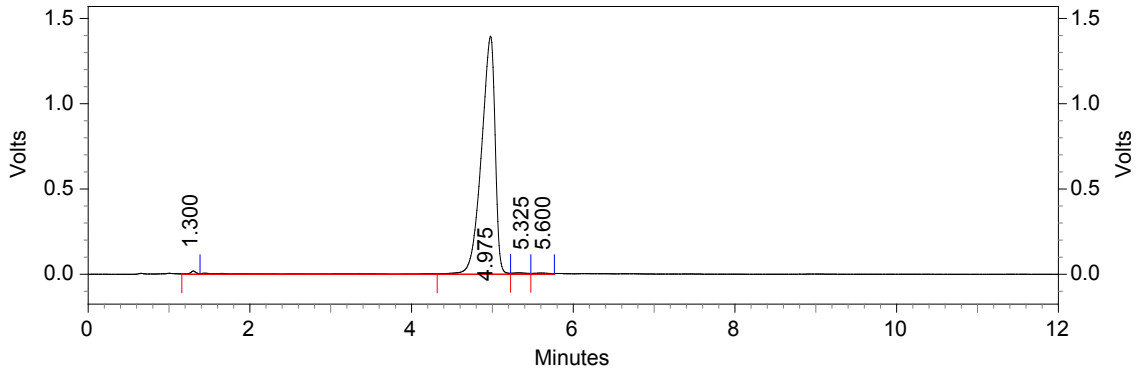
To a stirred solution of **139** (6.3 mg, 0.0065 mmol) in acetone (0.6 ml), aqueous sodium hydroxide (0.1 N, 3.0 ml) was added dropwise. The resulting deep purple solution was allowed to stir at room temperature 30 min. After this time the pH of the solution was adjusted to 8 with aqueous HCl (0.1 N). The resulting orange-red mixture was extracted several times with $CHCl_3$ until the aqueous phase was colourless. The combined organic extracts were dried over sodium sulphate, filtered and concentrated under reduced pressure. The residue was dissolved in HCl (0.1 N, 4.0 ml) and was stirred at room temperature for 39 hrs. After this time $CHCl_3$ was added, the phases were separated and the aqueous phase was extracted twice with $CHCl_3$ to get rid of impurities and hydrolysed aglycone. The pH of the aqueous phase was then adjusted to 8 with aqueous NaOH (0.1N) and $CHCl_3$ was added. The layers were separated and the aqueous phase was extracted with $CHCl_3$ until it was colourless. The combined organic extracts were dried over $NaSO_4$ filtered and concentrated under reduced pressure to give 2.6 mg of **140** (0.0030 mmol, 48.3 %) as orange-red solid.

^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 14.01 (s, 1H, OH-6); 13.125 (s, 1H, OH-11); 8.04 (d, *J* = 7.7 Hz, 1H, H-1); 7.77 (t, *J* = 8.1 Hz, 1H, H-2); 7.39 (d, *J* = 8.3 Hz, 1H, H-3); 5.54 (brs, 1H, H-dau1); 5.30 (m, 1H, H-7); 4.79 (d, *J* = 12.4 Hz, 2H, H-14a/b); 4.08 (s, 3H, $ArOCH_3$); 3.79-3.76 (m, 2H, H-dau4, H-dau5); 3.66-3.51 (m, 22H, H-dau6, -(O-CH_2-CH_2)$_5$-); 3.37 (s, 3H, OCH_3); 3.20 (m, 1H, H-dau3); 3.12-3.02 (m, 1H, H-10a); 2.73-2.65 (m, 1H, H-10b); 2.09-1.63 (m, 4H, H-8a/b, H-dau2a/b)

HRMS (ESI-TOF): calcd. for $[C_{38}H_{51}NO_{17}+H]^+$ 794.3235, found 794.3239; calcd. for $[C_{38}H_{51}NO_{17}+Na]^+$ 816.3054, found 816.3065; calcd. for $[C_{38}H_{51}NO_{17}+K]^+$ 832.2794, found 832.2803

TLC: R_f = 0.04 (DCM/MeOH 100:10, v/v)

HPLC: Retention time: 4.975 min; Column: Zorbax Eclipse XDB-C8, 5 µm, l = 150 mm, Ø = 4.6 mm; Mobile Phase: 40 % H_2O containing 0.4 % sodium dodecylsulfate (pH 2.00) and 60 % MeOH/ACN, 1:1, v/v; Detection: UV [λ= 254 nm, reference λ= 360 nm]; Injection volume: 20 µL; Temperature: 35°C; Flow rate: 2 ml/min; Run time: 12 min; Standard preparation: 1 mg/ml mobile phase



Detector A - 1 (254nm)

Pk #	Retention Time	Area	Area %	Height	Height %
1	1.300	105674	0.633	18755	1.311
2	4.975	16389410	98.153	1396455	97.621
3	5.325	100656	0.603	8070	0.564
4	5.600	102141	0.612	7208	0.504
Totals		16697881	100.000	1430488	100.000

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9 Curriculum vitae

Personal information

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Education and training

Since 12/2008	University of Vienna, Ph.D. studies in natural sciences/pharmacy, Department of Medicinal Chemistry, Faculty of Life Sciences Thesis: "Design and evaluation of novel anthracycline derivatives"
09/2004 – 11/2008	University of Applied Sciences, FH Campus Wien, Course of studies: Biotechnology Title conferred: Dipl.-Ing. (FH)
10/2003 – 09/2004	Civilian service, Austrian Red Cross, Salzburg (Paramedic certification)
09/1998 – 06/2003	Secondary Federal College for Agriculture Ursprung, Salzburg (hlfs.ursprung.at), including a 6 weeks' agricultural practical training in Canada/Ontario

Work experience

Since 12/2012	University of Vienna, Department of Medicinal Chemistry, research associate and assistant lecturer
12/2008 – 11/2012	University of Vienna, Department of Medicinal Chemistry, university assistant (prae doc) • Scientific research and development in the field of antitumor agents • Supervision of trainees and graduands • Lectureship for the courses "Drug analysis and drug development" and "Quantitative chemical analytics", holding seminars, development of new laboratory experiments

08/2007 – 06/2008	Pharmacon F&B GmbH, Vienna, Chemist, • Synthesis and process optimization of APIs including GMP/GLP-compliant documentation • Scale-up of synthesis processes for transfer to production sites • Method development and validation of analytical procedures

Personal skills and competences

Languages	German (mother tongue) English (proficient user)
Computer skills	MS Office (Access, Excel, PowerPoint, Word), SPSS Statistics, handling of several chemistry-related software packages and databases
Social skills	Graduation in various courses in communication, motivation and conflict management between 2004 and 2006
Driving licence	Category B (since September 2002)

Publications

Patent	„Neue Anthracyclinderivate“, National patent application (A114/2012)/15.10.2012 International patent application (PCT/EP2013/071520) /15.10.2013