Understanding Anthracyclines: Synthesis of a Focused Library of Doxorubicin/Aclarubicin - Inspired Structures

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Chapter 1

Introduction and outline

In the 1950s, the anthracycline daunorubicin (**1**, Figure 1) was isolated from a soil sample found in the area of Castel del Monte, a castle in Italy.[1] Daunorubicin was produced by a strain of the actinobacterium *Streptomyces peucetius* and was initially studied for its antibiotic properties. It was soon found that daunorubicin possessed good activity against murine tumors after which it entered clinical trials as a drug for the treatment of various hematological cancers in the 1960s. In spite of the discovery of fatal cumulative cardiotoxicity as a side-effect of this drug 1967,[2] it received FDA approval in the United States of America in 1972.[3] In 1969, doxorubicin (**2**), also known as adriamycin, was isolated from a culture of *Streptomyces peucetius* var. *caesius*[4]. Doxorubicin showed to be an even more potent anti-cancer drug than daunorubicin, and also to have a broader spectrum of activity against a variety of cancers[5] and was FDA approved in the United States only 5 years later.[6]

Daunorubicin (**1**) and doxorubicin (**2**) differ only in the presence or absence of a hydroxyl group on the 14-position.[7] Both contain four fused rings, three of which comprise an anthraquinone moiety. The individual rings in these tetracyclic systems are commonly referred to as the A, B, C and D rings according to the nomenclature proposed by Brockmann (Figure 1).[8] Their 7-position is decorated with an α-L-daunosamine glycoside. The presence of this sugar moiety is essential for its anti-cancer activity, as the aglycones of daunorubicin and doxorubicin (termed daunomycinone and doxorubicinone, respectively) were shown to have no anti-tumor activity.[9] Different from daunorubicin and doxorubicin, aclarubicin (**3**) contains a trisaccharide with an α-L-rhodosamine (*N,N*-dimethyldaunosamine) at the reducing end, connected to an α-L-oliose (2-deoxy fucose) and an α-L-cinerulose.

Structural differences between anthracyclines **1**-**3** can be divided into three categories: oxidation/substitution pattern on the tetracycle portion, variation in the sugar moiety/moieties and the substitution pattern on the amine.

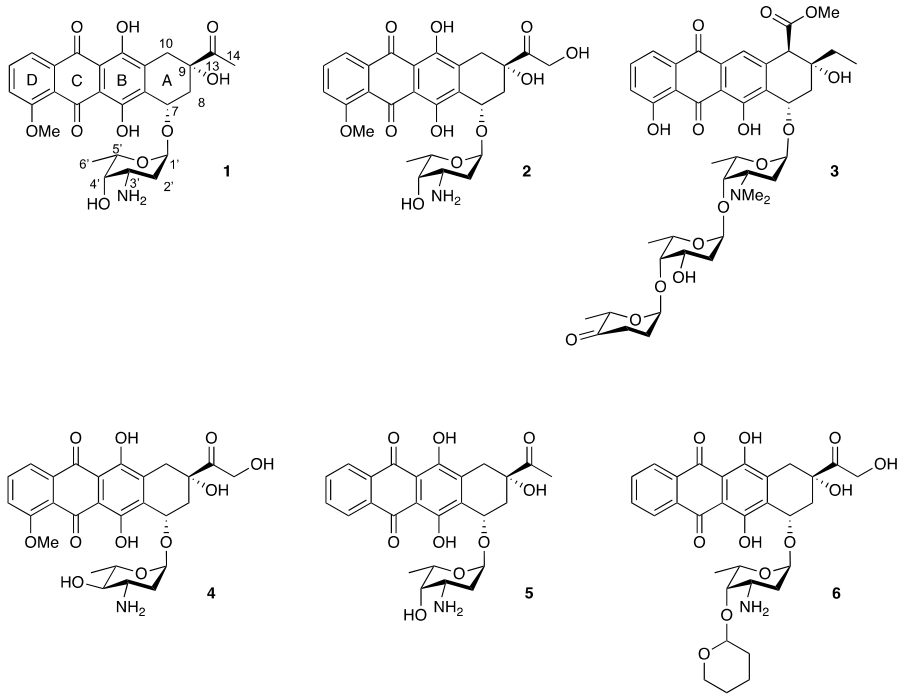


Figure 1. Structures of clinically used anthracyclines daunorubicin (**1**), doxorubicin (**2**), aclarubicin (**3**) epirubicin (**4**), idarubicin (**5**) and pirarubicin (**6**).

The most important anthracyclines currently in the clinic are doxorubicin (**2**), daunorubicin (**1**), aclarubicin (**3**), epirubicin (**4**), idarubicin (**5**) and pirarubicin (**6**). Aclarubicin (**3**, Figure 1) was isolated from the culture broth of *Streptomyces galilaeus* in 1979 by Oki *et al*.,[10] who elucidated the structure of this and related anthracyclines shortly thereafter.[11] It is commonly used for the treatment of various cancers in China and Japan, but not in the rest of the world.[12] This despite the finding that aclarubicin is about tenfold less cardiotoxic than doxorubicin.[13]

Epirubicin (**4**) is the 4'-epimer of doxorubicin, positioning the hydroxyl group on the sugar ring equatorially instead of axially. It has a comparable anti-tumor activity to doxorubicin, but causes fewer side effects in certain treatment regimens, in particular lower cardiotoxicity at comparable dose.[9] This allows for epirubicin to be used at higher doses, without increasing the incidence of heart damage. It was initially prepared through glycosylation of the appropriately protected L-acosamine donor, but a later

developed method entailed C4'-inversion and 14-hydroxylation on biosynthetic daunorubicin.[14] Idarubicin (**5**) is a semisynthetic product that represents the 4-desmethoxy analog of daunorubicin, and possesses broader-spectrum activity.[15] Epirubicin (**4**) and idarubicin (**5**) are part of a library of 200 doxorubicin variants prepared through chemical synthesis at Farmitalia, the company that discovered doxorubicin and daunorubicin.[16] Pirarubicin (**6**) was developed by Umezawa *et al.* and showed similar antitumor efficacy to doxorubicin.[17] However, it was found to be active against a number of doxorubicin-resistant cell lines. Cellular uptake for pirarubicin is faster than doxorubicin in tumor cells *in vitro* and is now used for head and neck cancer, stomach cancer, upper urinary tract cancer, uterus cancer, ovarian cancer, acute leukemia and malignant lymphoma in Japan.[18]

Through mutation of the enzymes required for anthracycline saccharide biosynthesis and feeding of non-natural glycosyl donors as well as organic synthetic efforts, thousands more such analogs have been prepared, most notably between the early 1980s and early 2000s.[19] Nevertheless, almost fifty years after its discovery, doxorubicin still remains the most used anthracycline in cancer treatments. Its global annual market had reached $800 million by 2015,[20] and is expected to increase even further. Doxorubicin induced cardiotoxicity still severely hampers treatment and an anthracycline that would display less toxicity of the heart, or is more potent and therefore could be used at a lower dose, remains much desired. The recent discovery of histone eviction as a major mechanism of action brings new information regarding the mechanisms of action of anthracyclines, consequently incentive to reinvestigate the exact molecular mechanism.

This Chapter first outlines the mechanisms of action of the anthracycline class of drugs, in addition to side effects that accompany their use. Then, it gives information on the biosynthesis of anthracyclines as well as the chemical synthesis of analogs. Finally, it will provide an overview of the work described in this Thesis.

1.1 Mechanisms of action and side effects of anthracyclines

Since the discovery of daunorubicin (**1**), multiple mechanisms of action for the anthracycline class of anti-cancer drugs have been reported. The most commonly accepted mode of action is the ability of anthraquinone glycosides to inhibit the catalytic cycle of the DNA (un)winding enzyme Topoisomerase II (Topo II). This enzyme plays an important role in the decatenation of intertwined DNA strands, and the relaxation of tension in the DNA strand in front of the replication fork. Topo II does this by creating a break in one of the strands of double stranded DNA, hereby allowing the

second strand to pass and subsequently closing the initial break by religation of the two DNA strand ends. Doxorubicin and daunorubicin bind and inhibit Topo-II in its catalytic steps following initial DNA double-strand break (DSB) by forming a stable DNA-drug complex, preventing religation of the broken strands, ending up with DNA damage.[21] As a result, the cell cycle is arrested, DNA repair processes are activated and p53-mediated apoptosis is induced.[22] At high concentrations, anthracyclines are also able to inhibit Topo-II activity by intercalation into DNA without inducing DNA damage.[23] This intercalation leads to an inability of DNA transcription enzymes to perform their function.[24,25] As demonstrated by Pang *et al*.,[26] aclarubicin (**3**) does not induce DNA DSBs, because it inhibits loading of the DNA strands into the enzyme before it makes a DSB.[27]

Owing to the anthraquinone function present in all anthracyclines, their anti-tumor activity has also been ascribed to the formation of Reactive Oxygen Species (ROS) through this moiety.[28] As depicted in Figure 2, addition of a free electron to this moiety by oxidative enzymes such as NADH dehydrogenase, cytochrome P450 reductase and xanthine oxidase yields a stable semi-quinone radical anion of doxorubicin. This radical can react with molecular oxygen to form superoxides and hydrogen peroxide. Under the agency of iron(II) and iron(III), this will generate hydroxyl radicals through the Fenton reaction.[29] These radicals can cause DNA damage, protein modification and lipid peroxidation.[30,31] However, this effect has only been shown at higher than clinical doxorubicin/daunorubicin concentrations, so its actual *in vivo* effect is still under debate.

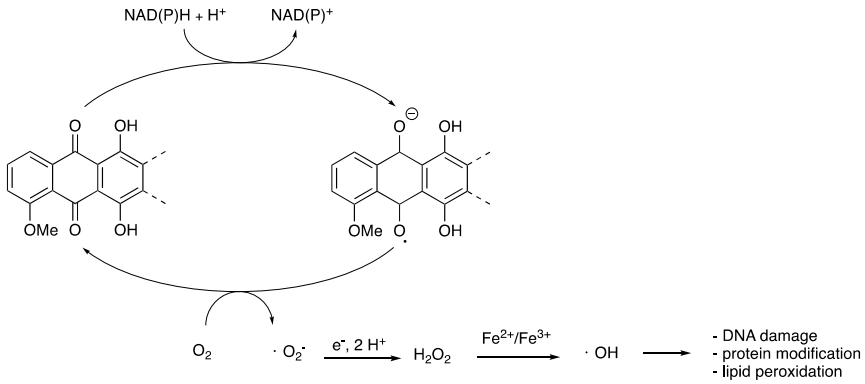


Figure 2. Generation of reactive oxygen species (ROS) as induced by anthracyclines.

A recent work by Pang *et al.*[26] showed that certain anthracyclines are able to evict histones from different areas of chromatin, an effect also known as chromatin damage. Histones are responsible for chromosome organisation at the most basic level and act

as spools around which DNA is wound in compact structures. Chromatin exists either in a loosely packed, also known as euchromatin form, or in a compacted form known as heterochromatin. The open chromatin is in general more transcriptionally available compared to the heterochromatin, which is usually associated with transcriptionally repressed genomic regions. Upon intercalation of the aglycone moiety of doxorubicin into the DNA major groove, the aminosugar sticks out into the minor groove. There, it competes for space with the histone H4 tail, causing destabilization of the complex resulting in its collapse. This results in release of histones from chromatin, chromatin damage and finally cytotoxicity.[32] A model of doxorubicin (**2**) in a histone-wound DNA duplex is depicted in Figure 3. Notable is the finding by Pang *et al.* that doxorubicin possesses both the Topo-II mediated DNA damage activity, as well as the ability to induce histone eviction. The non-anthracycline Topo-II inhibitor etoposide is able to induce DNA damage only, but not histone eviction. Aclarubicin (**3**) was found to be able to evict histones without causing DNA damage. This prompts the notion that the DNA damage ability is not crucial for the anti-tumor activity of anthracyclines and that histone eviction alone might suffice for cytotoxicity. Furthermore, this chromatin damage was found to occur with a certain regiospecificity, that is different for doxorubicin and aclarubicin. Whether this divergence in binding to specific regions within the genome is therapeutically relevant remains to be investigated.[33,34]

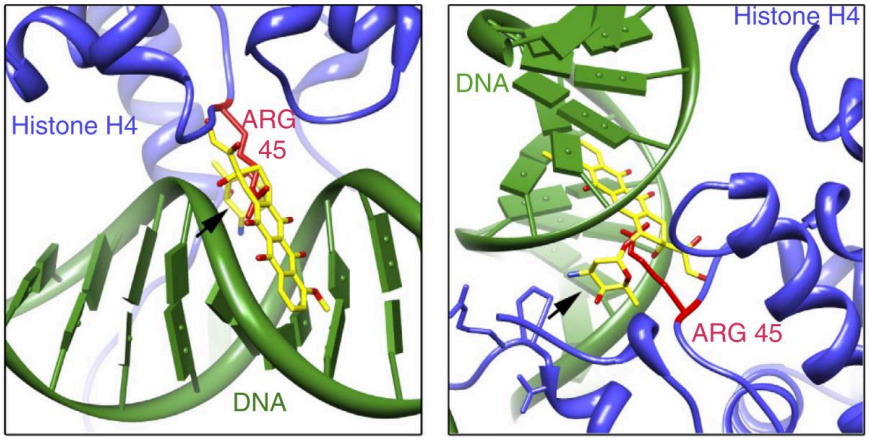


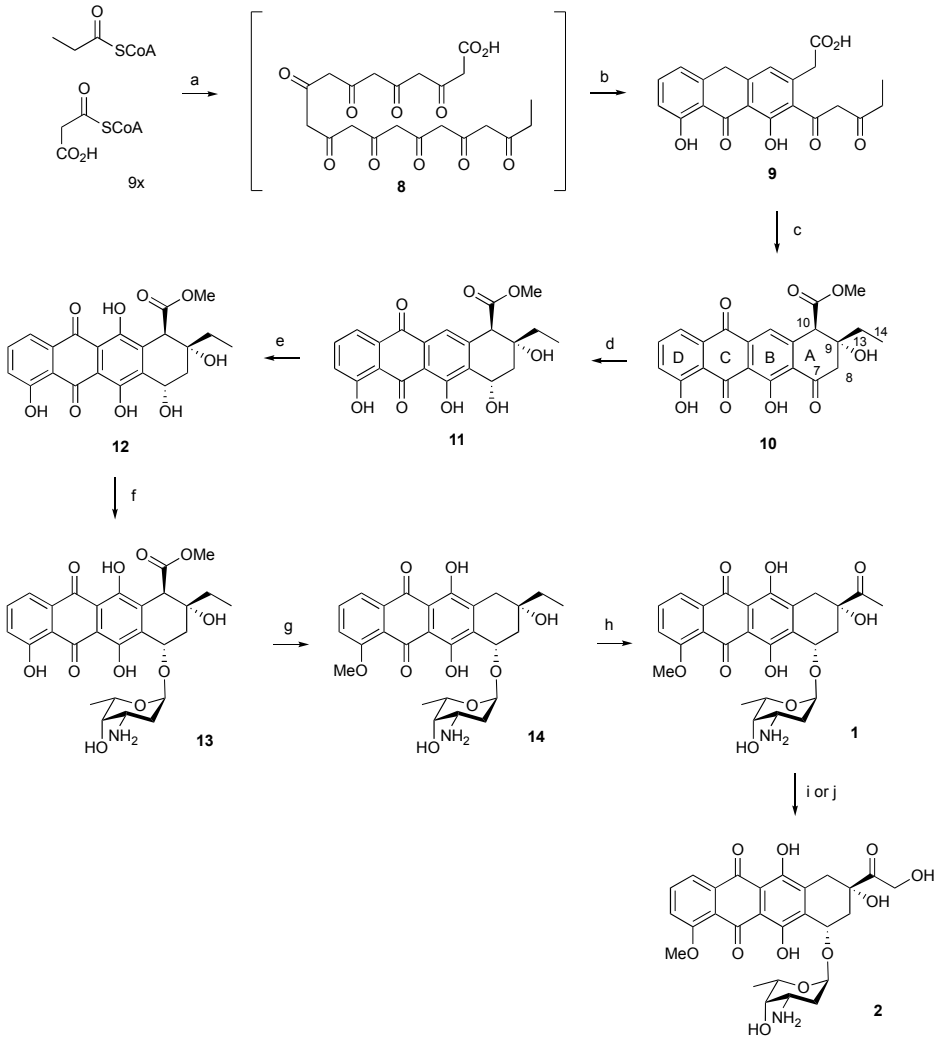
Figure 3. A model of doxorubicin (**2**) intercalation in chromatin, with the aminosugar moiety of doxorubicin competing with histone tail H4 for access to space in the DNA minor groove. Shown is a snapshot of the relevant area of the model under two angles. DNA is visualized in green, doxorubicin in yellow, histone H4 in blue and the H4-arginine residue (at position 45) that enters the DNA minor groove is shown in red.[26]

As with most anti-cancer drugs, the use of anthracyclines comes with a range of adverse effects. Most notable amongst these is the incidence of dose-related cardiotoxicity, an effect that was already uncovered for daunorubicin in 1967.[2] This effect, which is much

more prominent for doxorubicin than aclarubicin, is irreversible and can be lethal, leading patients to require alternative treatment strategies to avoid heart failure.[22,35] ROS formation has been studied as a cause for anthracycline induced cardiotoxicity, but as the co-administration of anti-oxidants failed to ameliorate it, this mechanism appears unlikely.[36] A second well known side effect for most of the anthracyclines is the formation of therapy related tumor formation, often in the form of acute myeloid leukemia.[37] Infertility is another side-effect of this class of drugs.[38] Although the anthracycline drugs are widely used anti-cancer drugs, a clear relation between their biological activities and the side effects is still poorly understood. Therefore, the availability of coherent sets of anthracyclines is of great value to study this. The biosynthetic and synthetic organic preparation of such compound collections is discussed in the upcoming paragraphs.

1.2 On the biosynthesis of anthracyclines

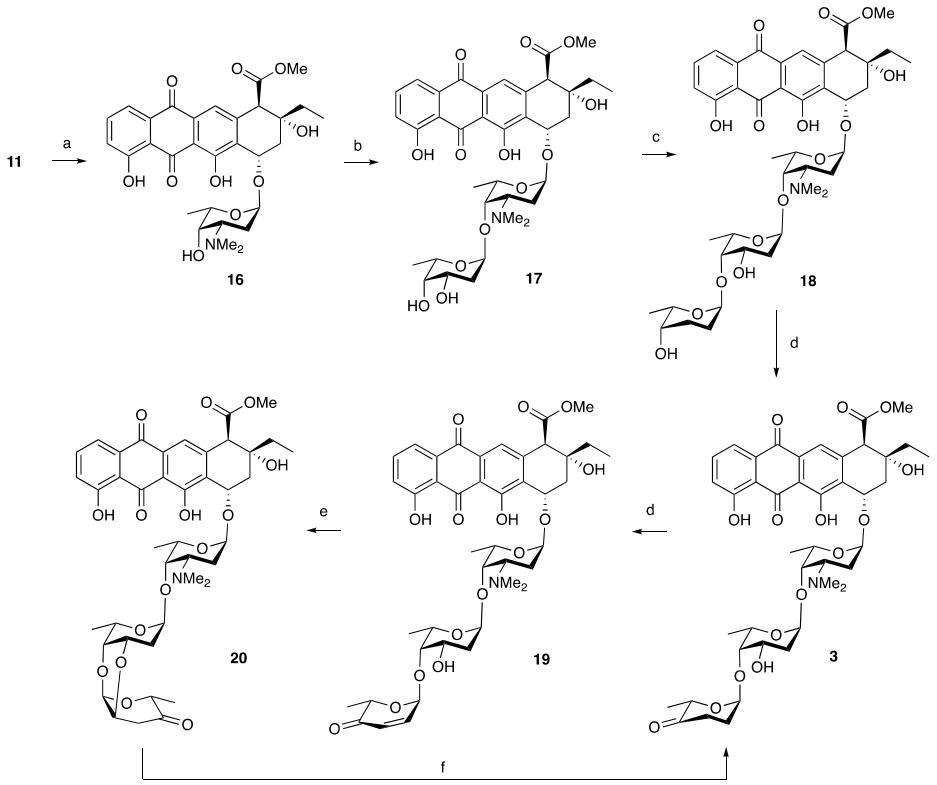
The biosynthesis of daunorubicin and doxorubicin has been extensively optimized to allow for the large-scale production of these drugs by fermentation. Scheme 1 outlines the biosynthetic steps in the production of daunorubicin and doxorubicin. The initial A,B,C,D ring system is produced by a type-II polyketide synthase. Propionyl-CoA is elongated by the sequential decarboxylative addition of nine units of malonyl-CoA to yield 21-carbon polyketide **8**. A sequence of cyclization reactions and other modifications then gives the aglycone found in aclarubicin, aklavinone **11**. This compound is hydroxylated on the C-11 position to give ε-rhodomycinone **12**. Appendage of TDP-daunosamine then delivers rhodomycin D (**13**) as the first anthraquinone glycoside in this biosynthesis. With this sugar in place, several steps take place to transform the aglycone. The C-9 methyl ester is enzymatically demethylated, and decarboxylated by *DnrK* to provide **14**.[39] Oxidation of the C-13 methylene leads to the corresponding ketone, with a final C-4 phenol methylation completing daunorubicin (**1**). Initially, the mutant *Streptomyces peucetius* ATCC 27952[40] was found to produce doxorubicin in small quantities and the enzyme responsible for hydroxylation of C-14 turned out to be the same as the one that is responsible for the C-13 oxidation (*DoxA*). However, the enzyme has a low turnover for this hydroxylation reaction in the wild-type strain. Although some mutants that overexpress *DoxA* were able to double the yield of doxorubicin, complete hydroxylation has not been achieved yet, yielding mixtures of doxorubicin and daunorubicin.[41] Daunorubicin then still needs to be converted to doxorubicin by means of chemical synthesis (bromination of C-14 followed by hydrolysis).[42,43] This complicates the production of doxorubicin and as a result there is still significant attention to the improvement of its production.[44]



Scheme 1. Biosynthesis of daunorubicin and doxorubicin by *S. peucetius*.[45] (a) Assembly of the polyketide by sequential decarboxylative addition of 9 units of malonyl-CoA to propionyl-CoA; (b) Reduction at C-4 and cyclization to form the D, C and B rings; (c) Oxidation at C-12, methylation of the carboxylic acid and Claisen condensation to cyclize the A-ring; (d) Reduction of the C-7 ketone to give aklavinone; (e) Hydroxylation at C-11 to yield ε-rhodomycinone (**13**); (f) Glycosylation on C-7 with TDP-daunosamine; (g) Demethylation of the ester at C-10 by *DnrP*, followed by decarboxylation and C-4 phenol methylation by *DnrK*; (h) Oxidation at C-13 by *DoxA*; (i) Chemical hydroxylation of C-14 by *i.* Bromination of C-14; *ii.* Hydrolysis; (j) Enzymatic hydroxylation at C-14 by enzymatic overexpression of *DoxA*, as in mutant ATCC 27952.[40]

Aclarubicin (**3**) is also produced by means of fermentation, and the biosynthetic steps as performed by *Streptomyces galilaeus* are shown in Scheme 2. The biosynthesis commences with the assembly of aklavinone (**11**), as described before for *Streptomyces*

peucetius.[46] Appendage of the rhodosamine moiety on C-7 gives aclacinomycin T (**16**), which is transformed into aclacinomycin S (**17**) by appending an oliose moiety. Next a rhodinose is attached to the terminal sugar, giving aclacinomycin N (**18**). Oxidation of the terminal 4'''-alcohol to furnish the cinerulose moiety is performed extracellularly by the enzyme *AknOx* and yields aclarubicin (**3**). However, the same enzyme is also able to oxidize the cinerulose C2,C3-bond to yield the corresponding enone aclacinomycin Y (**19**).[47] Cyclization by the addition of the 3"-OH onto the enone functionality of the resulting sugar moiety (which is named aculose) delivers aclacinomycin B (**20**). Upon reuptake into the cell, this compound can be converted back into aclarubicin. In practice, mixtures of aclarubicin, aclacinomycin B and Y are obtained by fermentation, making the purification process of aclarubicin difficult.



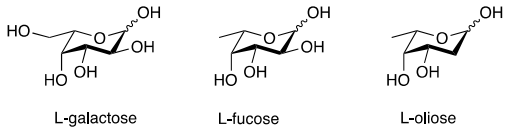
Scheme 2. Biosynthesis of aclarubicin (**3**) by *S. galilaeus*.[46] (a) Glycosylation on C-7 with TDP-L-rhodosamine; (b) Glycosylation on 4'-OH with TDP-L-oliose; (c) Glycosylation on 4''-OH with TDP-L-rhodinose; (d) Oxidation of 4'''-OH by *AknOx*, or oxidation of 2'''-3''' by the same enzyme; (e) Cyclisation between the enone and C3"-OH; (f) Cleavage of the bond between 3"-OH and C2'''.

The synthesis of coherent sets of anthracyclines to facilitate the elucidation of the structure-activity relationships of doxorubicin (**2**) and aclarubicin (**3**) would require careful tailoring of mutant enzymes for each of the individual desired analogs. Therefore, a divergent synthetic strategy was instead chosen to obtain such sets of compounds. General considerations in the organic synthesis of anthracyclines, as well as examples of their (semi)synthesis are discussed in the upcoming paragraphs.

1.3 Organic synthesis of anthracyclines

1.3.1 Challenges in the preparation and glycosylation of deoxy glycosides

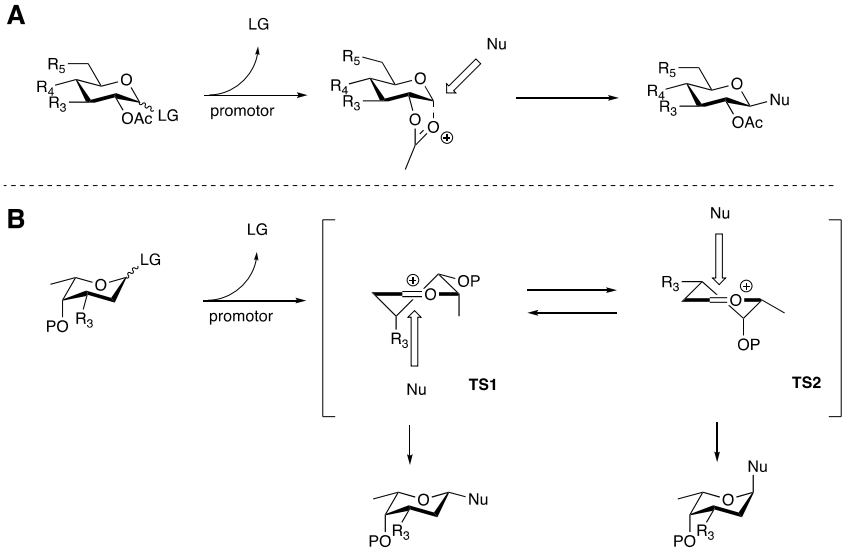
As shown in Figure 1, the sugars present in anthracyclines can all be characterized as 'deoxy' sugars: they lack one or more hydroxyls when compared to the more common sugars (such as glucose, mannose, galactose). Deoxy glycosides are widely found as components in antibiotics and anti-cancer agents, originating from bacterial sources. These compounds show immense variation as well as structural complexity and have therefore been subject of many total synthesis efforts.[48] The lacking hydroxyl group(s) are substituted for nitrogen substituents (primary/secondary/tertiary amines, acetamides, nitro groups) or by hydrogen. Quaternary stereocenters featuring amine or hydroxyl groups may also be present. These differences when compared to fully oxygenated saccharides have a tremendous effect on their synthetic preparation and glycosylating properties of their corresponding glycosyl donors.



L-galactose L-fucose L-oliose

Figure 4. Structures of L-galactose, L-fucose and L-oliose.

The structure of L-galactose, its 6-deoxy variant L-fucose and 2,6-dideoxygenated L-galactose (L-oliose) are depicted in Figure 4. Because fewer electron-withdrawing oxygen substituents are present on the pyranose ring going from L-galactose to L-fucose and finally L-oliose, the electron density on the ring increases, which leads to higher reactivity of the corresponding glycosyl donors. For the same reason, the glycosidic linkages of deoxy sugars are more labile towards (Lewis-)acidic conditions than their fully oxygenated counterparts. These effects are especially pronounced when the deoxygenated positions are close to the anomeric center (*i.e.* at C2 and C6).



Scheme 3. A) Formation of a β-glycosidic bond through neighboring group participation for glucose donors. B) Rationale of the stereochemical outcome in the glycosylation of 2-deoxy fucosyl donors.

The lack of a 2-substituent strongly impacts the stereoselectivity of the glycosylation of 2-deoxy glycosyl donors. While anchimeric assistance of a C2 participating group can be called upon for the stereoselective construction of 1,2-*trans* glycosidic bonds in carbohydrates with a C2-oxygen or nitrogen substituent (Scheme 3A), this type of stereocontrol cannot be applied to C2-deoxy glycosides, present in the anthracyclines. Because of the higher reactivity of 2-deoxy glycosyl donor, glycosylations take place through the intermediacy of species bearing more carbocation character, *i.e.* oxocarbenium ion like species. The reactivity of these latter species is governed by their overall shape, which is dictated by the nature of the groups on the 3-, 4- and 6-position (See Scheme 3). Factors such as the solvent, temperature and the nature of the nucleophile strongly influence the outcome of the glycosylation reactions.

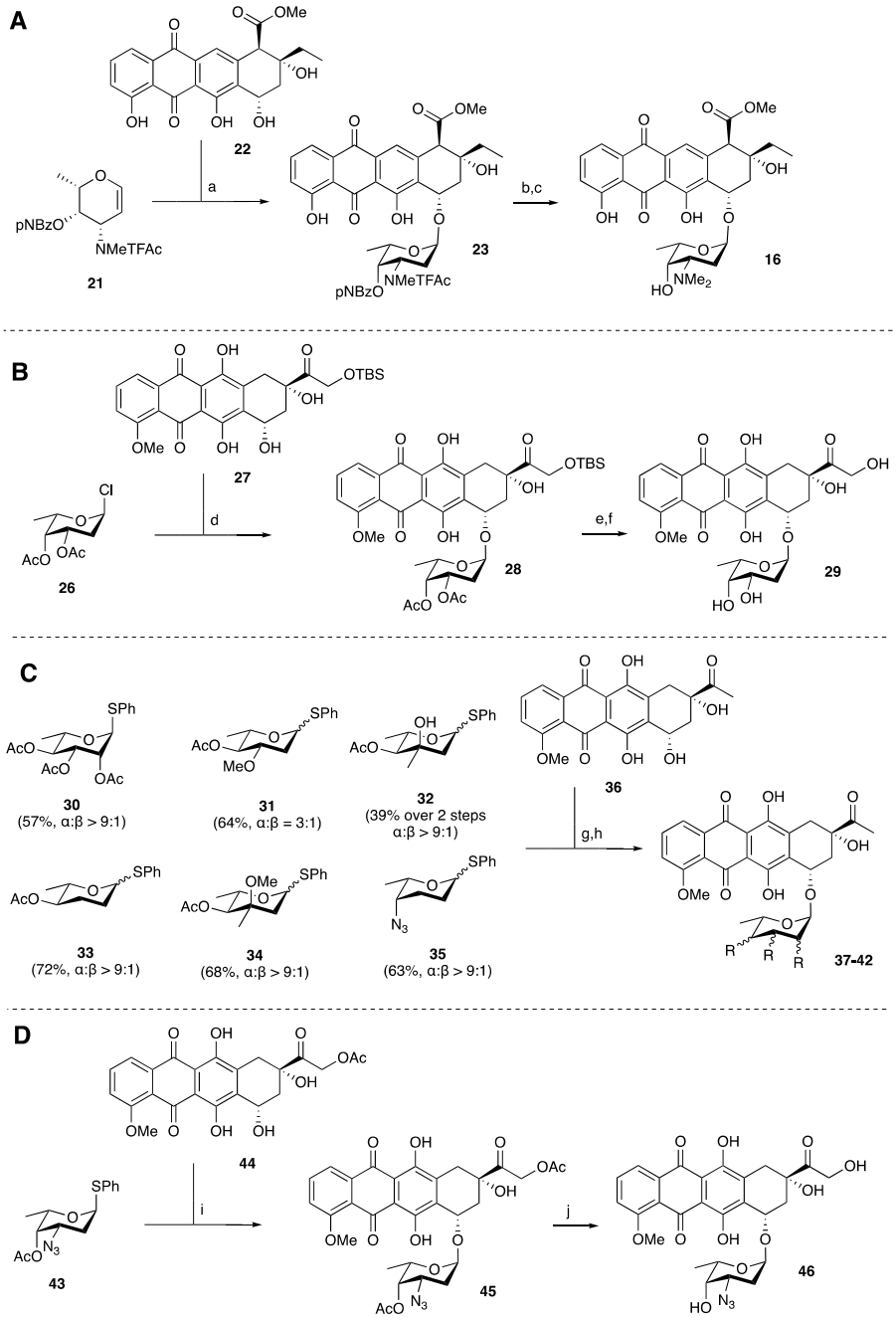
The scarcity of many of the deoxygenated monosaccharides in nature is reflected in the costs and efforts required for their preparation. Only a few are commercially available, and they become significantly more expensive as their natural abundance diminishes. Although they can be synthesized from more abundant sugars, these routes of synthesis are normally quite lengthy. Depending on the deoxygenation site, a multitude of methods has been developed to prepare the desired ring-substitution motif. Examples include tin-mediated radical deoxygenation of halides,[49] formation of glycals from glycosyl bromides,[50] Ferrier-rearrangement of glycals to obtain 2,3-dideoxyglycosides,[51] Barton-McCombie deoxygenation,[52] hydrogenation of ring-

substituted thiolates or selenides[53] and these methods will be elaborated upon in Chapter 2-5 of this Thesis.

1.3.2 Glycosylation strategies in the synthesis of anthracyclines

Since the discovery of doxorubicin and aclarubicin, the synthesis of anthracyclines has gathered considerable attention. The formation of the α-glycosidic linkages in anthracyclines is no trivial matter but has nevertheless been accomplished by the use of several donor-promoter systems, some of which are highlighted in this paragraph. Pearlman *et al*. reported the synthesis of aklavin (**16**) and *N*-demethylaklavin (**24**) in 1981 (Scheme 4A).[54] They prepared glycal donor **21** from daunosamine and were able to stereoselectively glycosylate this to aklavinone (**22**) using a catalytic amount of *p*-toluenesulfonic acid to yield **23**. Treatment with excess sodium methoxide removed the *N*-trifluoroacetyl and *p*-nitrobenzoate groups, which was followed by Borch conditions (aq. CH_2O, $NaBH_3CN$, AcOH) to yield aklavin (**16**). A few years later, Horton *et al.* prepared 3'-desamino-3'-hydroxydoxorubicin **29** (Scheme 4B).[55] Activation of L-oliosyl chloride **26** under Koenigs-Knorr conditions ($HgBr_2$, HgO) in the presence of 14-TBS-doxorubicinone **27** afforded protected anthracycline **28**. Zemplén deacylation followed by treatment with tetra-*n*-butylammonium fluoride (TBAF) afforded hydroxyrubicin **29**. Wang *et al*. prepared the set of daunorubicin analogs **37**-**42** with uncommon deoxysugars in 2005 (Scheme 4C).[56] Thioglycosides **30**-**35** were activated by $AgPF_6$ in the presence of TTBP (2,4,6-tri-*tert*-butylpyrimidine) and daunorubicinone **36** to generate the daunorubicin glycosides, which were deacylated to yield the corresponding daunorubicin analogs in varying yield and stereoselectivity. The same group prepared 3'-azidodoxorubicin (**46**) (Scheme 4D).[57] In this case, thioglycoside **43** was activated by *N*-iodosuccinimide (NIS) and a catalytic amount of triflic acid in the presence of **44** to yield the target compound **46** after deacylation. Weil *et al.* realized the preparation of the same compound by one-step diazotransfer on doxorubicin.[58]

In 1990, the group of Danishefsky reported the synthesis of the natural product anthracycline "ciclamycin 0" (**56**) shown in Scheme 5A.[59] Their synthesis features the first fully synthetic oligosaccharide to be glycosylated to an anthracycline aglycone, using a strategy relying on the activation of glycals by means of iodonium dicollidinium perchlorate (IDCP).

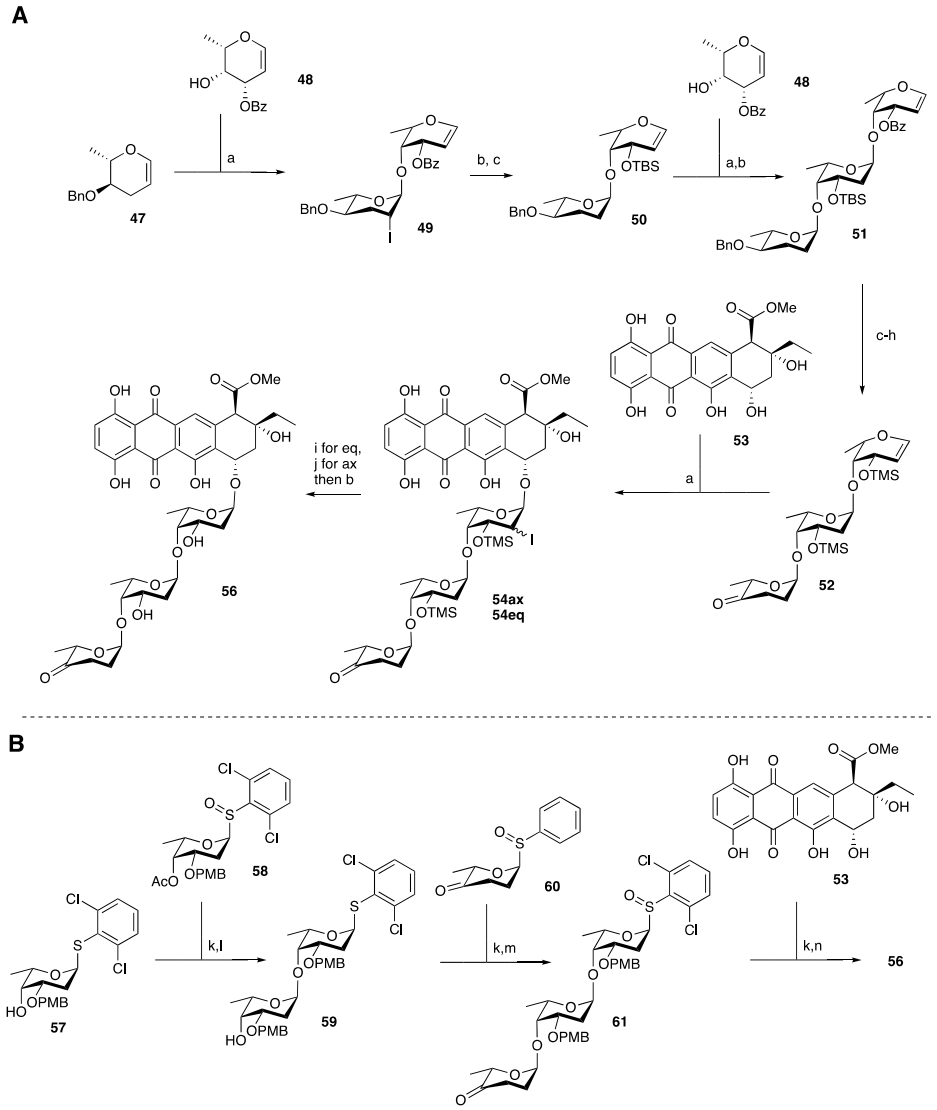


Scheme 4. Selected syntheses of monosaccharidic anthracyclines. *Reagents and conditions:* (a) *p*-toluenesulfonic acid, benzene, 50 °C, 80%; (b) NaOMe, MeOH, -20 °C; (c) aq. CH_2O, $NaBH_3CN$, AcOH; (d) HgO, $HgBr_2$, DCM, 83%; (e) NaOMe, MeOH, 88%; (f) TBAF, pyr., THF, DCM, 83%; (g) $AgPF_6$, TTBP, DCM, 0 °C, 57-72% (>9:1 – 3:1 α:β); (h) NaOH, THF, H_2O, 39-75%; (i) NIS, TfOH, DCM, 0 °C, 64%; (j) NaOH, THF, H_2O, 70%.

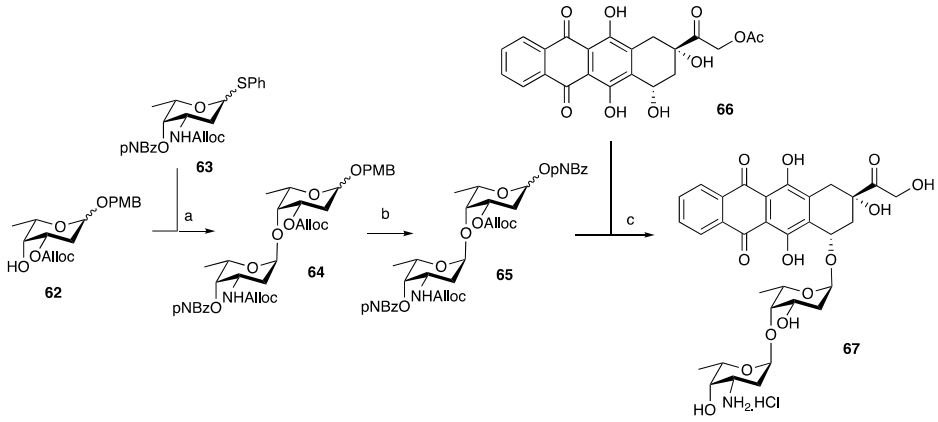
In this vein, glycal **47** was chemoselectively coupled to glycal **48** to afford the corresponding 2'-iodo disaccharide glycal **49**. The iodide was then reductively removed using Ph_3SnH. Switching the benzoyl group for a TBS group gave disaccharide **50**, which could now be activated by IDCP. Doing so in the presence of acceptor **48** gave the corresponding trisaccharide **51** after reductive removal of the 2'-iodide. Protecting group manipulation and oxidation of the terminal 4-hydroxyl gave trisaccharide donor **52**. Treatment of a mixture of this donor and ε-pyrromycinone **53** with IDCP gave 60% of the α,α,α-linked trisaccharide anthraquinone as a mixture of the axial and equatorial 2'-iodide. Removal of the TMS ethers and final reductive removal of the iodide afforded ciclamyin 0 (**56**).

In 1999, Kahne's group also prepared ciclamycin 0, by means of glycosylations using glycosyl sulfoxide donors, as depicted in Scheme 5B.[60] Initially the authors tried to use benzyl ethers in the synthesis of this compound, but noted that the hydrogenolytic conditions used for the deprotection also cleaved the glycosidic linkage to the benzylic aglycon. To synthesize the trisaccharide, sulfoxide **58** was activated with triflic anhydride in the presence of oliosyl acceptor **57**, DTBMP and 4-allyl-3,4-dimethoxybenzene as sulfenyl scavenger to afford disaccharide acceptor **59** after reductive deacylation. In the same manner, cineruloside **60** was appended to finish the trisaccharide motif. The 'latent' reducing end thioglycoside was then oxidized by means of dimethyldioxirane to afford the corresponding sulfoxide donor **61**. Adding this donor to a mixture of triflic anhydride, DTBMP and 4-allyl-3,4-dimethoxybenzene as well as aglycone **53** afforded the protected anthracycline stereoselectively in good yield. Final oxidative removal of the PMB groups by action of excess DDQ furnished ciclamycin 0.

The pharmaceutical company Menarini Richerche reported the synthesis of a series of doxorubicin-inspired di- and trisaccharides.[61,62] Representative of these is the synthesis of MEN10755, or sabarubicin **67**, which is shown in Scheme 6. Subjection of a mixture of olioside **62** and daunosaminide **63** to excess IDCP in Et_2O/DCE afforded disaccharide **64**. The reducing end PMB group was then removed oxidatively (ceric ammonium nitrate), after which the resulting lactol was acylated to obtain *p*-nitrobenzoate disaccharide **65**. Activation of this donor with TMSOTf in the presence of 14-acetoxy-4-demethoxydoxorubicinone (**66**) afforded sabarubicin (**67**), after deprotection of the acetate and Alloc groups.



Scheme 5. Syntheses of trisaccharidic anthracycline ciclamycin 0 (**56**) by the groups of Kahne and Danishefsky. *Reagents and conditions:* (a) *i.* IDCP, DCM, 0 °C 66% for **49**, 54% for **51**, 60% for **54**; (b) Ph_3SnH, AIBN, benzene, 80 °C, 86% from **49**, 93% for **51**, 55% (77% B.R.S.M) from **54eq**, 72% (98% B.R.S.M) from **54ax**; (c) *i.* $LiAlH_4$, Et_2O, 0 °C ; *ii.* TBSCl, imidazole, DMF, 60% over 2 steps for **50**, 79% over 2 steps from **51**; (d) *i.* Li, NH_3(l), -78 °C ; *ii.* Ac_2O, Et_3N, DMAP, 87% over 2 steps; (e) TBAF, THF, quant.; (f) TMSCl, Et_3N, DMAP, DCM, 0 °C, 99%; (g) $LiAlH_4$, THF, 0 °C, 82%; (h) Dess-Martin periodinane, $NaHCO_3$, DCM, 95%; (i) *i.* AcOH, THF, MeOH, 97%; *ii.* TBAF, THF, 41% (72% B.R.S.M); (j) AcOH, THF, MeOH, 79%; (k) Tf_2O, DTBMP, 4-allyl-3,4-dimethoxybenzene, DCM, 81% from **57**, 68% (5:1 α:β) from **59**, 75% from **61**; (l) $LiAlH_4$, Et_2O, 0 °C, 92%; (m) dimethyldioxirane, -72 °C to -42 °C, 90%; (n) DDQ, DCM, H_2O, 60%.



Scheme 6. Synthesis of the anthracycline disaccharide sabarubicin (**67**) at Menarini Richerche. *Reagents and conditions:* (a) IDCP, Et_2O, DCE, 90%; (b) *i.* $(NH_4)_2Ce(NO_3)_6$, MeCN, H_2O; *ii.* pNBzCl, pyr., 0 °C, quant. over 2 steps; (d) *i.* TMSOTf, DCM, Et_2O, -10 °C; *ii.* K_2CO_3, MeOH, H_2O; *iii.* $Pd(PPh_3)_4$, Me_2NTMS, TMSOAc, DCM; *iv.* aq. HCl, 77% over 4 steps.

1.4 Outline of this Thesis

Since the discovery of daunorubicin in the 1950s, a multitude of (bio-)synthetic analogs have been prepared in an attempt to find improved, less cardiotoxic anthracyclines. Unfortunately, this has not resulted in a significantly improved anti-cancer anthracycline, and the molecular understanding of the mode of action of this class of drugs remains relatively poor. Uncovering histone eviction as an important mode of action of anthracyclines has provided a new incentive to investigate the structure-activity relationships of this class of anti-tumor drugs in detail. The research described in this Thesis focuses on the design and preparation of coherent sets of analogs that combined may inform on the molecular mechanism behind the various biological activities displayed by anthracyclines as (cardiotoxic) antitumor agents. Ultimately, the synthesized compounds, and the biological studies executed within the Chemical Immunology Department at the Leiden University Medical Center, may inform on how to 'separate' the different biological activities by the design of tailored doxorubicin/aclarubicin analogs and unveil how cardiotoxicity can be prevented while maintaining tumor cell toxicity. Possibly, new biological activities, including DNA-sequence (regio)-selective targeting, can be identified, which may lead to new therapeutic indications. The biological studies will be reported in a Thesis by Sabina Y. van der Zanden, to augment the results in anthracycline design and synthesis described in this Thesis. The Thesis comprises of the four experimental Chapters 2-5, followed by Chapter 6 that looks ahead based on the results obtained so far.

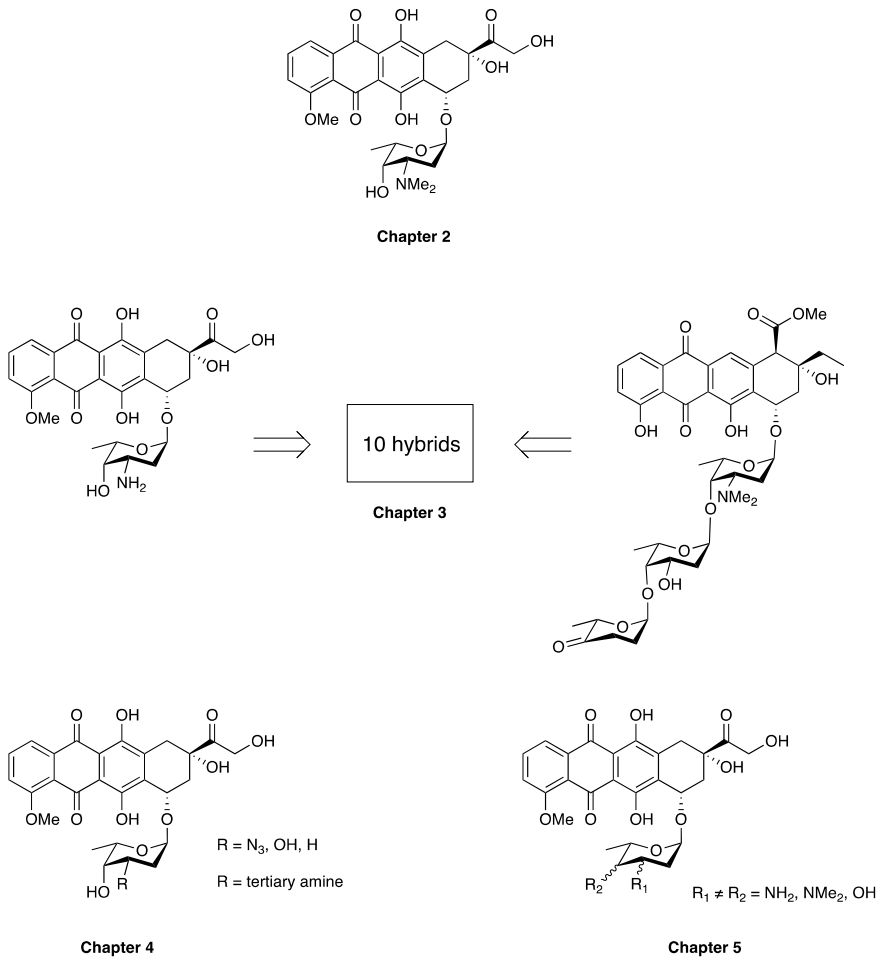


Figure 5. Global overview of the doxorubicin and aclarubicin analogs prepared and described in this Thesis.

Chapter 2 describes successful and unsuccessful (semi-)synthetic routes towards *N,N*-dimethyldoxorubicin, with the aim of developing methodology towards more complex anthracyclines. The synthesis of 10 hybrid structures, filling the chemical space between monosaccharide doxorubicin and trisaccharide aclarubicin is described in **Chapter 3**, building on the use of Yu's gold(I) catalyzed glycosylation methodology to connect the glycans and anthraquinone aglycons. **Chapter 4** describes the design and synthesis of a set of doxorubicin analogs, varying in substituent or amine functionalization on the 3'-position of its sugar moiety. A series of regio- and stereoisomers of doxorubicin is described in **Chapter 5**. Finally, **Chapter 6** provides a summary of this Thesis, and describes prospects for future research.

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