Studies toward synthesis of mono-glycosylated dipyrromethanes and analogues of the anti-cancer natural product tolyporphin

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Abstract


The tolyporphin A structure contains a tetrapyrrolic bacteriochlorin macrocycle and also consists of two glycosyl groups directly connected to the pyrroles. In this thesis, 3-glycosylated pyrrole was reacted with Eschenmoser's salt to produce $N,N$-dimethylamino methylated derivative in 95 % yield. Then, the product was reacted with pyrrole under microwave irradiation to produce glycosylated dipyrromethane in 44 % yield. Mono-glycosylated porphyrin was formed by reacting glycosylated dipyrromethane with 1,9-bis (imino)-5-phenyl dipyrromethane under the standard procedure, $^1$H NMR was used to confirm the new products.

Keywords: toloporphins, Eschenmoser salt, pyrrole, 3-glycosylated pyrrole, glycosylated dipyrromethane, microwave reaction, mono-glycosylated porphyrin.

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To my wife, my daughter, and my parents.
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Abbreviations

1- Ac: Acetate.
2- BGl$_{c4}$: Thioglycosylated bacteriochlorin.
3- Bn: Benzyl.
4- CGI$_{c4}$: Thioglycosylated chlorin.
5- DBU: 1,6-diaza-bicyclo[5,4,0]undec-6-ene.
6- DCM: Dichloromethane.
7- DDQ: 2,3-dichloro 5,6-dicyano 1,4-benzoquinone.
8- DEA: Diethylamine
9- DIPEA: Diisopropylethylamine.
10- Gal: Galactose.
11- Glc: Glucose.
12- HIV: Human immunodeficiency virus.
13- IGI$_{c4}$: Thioglycosylated isobacteriochlorin.
14- Lac: Lactose.
15- LG: Leaving group.
16- Man: Mannose.
17- MDR: Multidrug resistance.
18- MDRR: Multidrug resistance reversing
19- MesMgBr: Mesitylmagnesium bromide.
20- NBS: N-bromosucinimide.
21- NIS: N-Iodosucinimide.
22- PDT: Photodynamic therapy.
23- rt: Room temperature.
24- PDT: photodynamic therapy.
25- PTFAI: N-phenyl trifluoromethylacetimidate
26- TBAF: tetrabutylammoniumfluoride
27- TCAI: trichloroacetimidate.
28- TFA: Trifluoroacetyl.
29- THF: Tetrahydrofuran.
30- TIPS: Triisopropylsilyl.
31- TPP: Tetrphenylporphyrin.
32- TPPF: Tetra(pentafluoro)phenylporphyrin.
1- Introduction

Tetrapyrrole macrocycle compounds.

Tetapyrrolic macrocycles such as chlorins and porphyrins are heterocyclic aromatics containing $18-\pi$ aromaticity of the ring system that is responsible for the high stability of these compounds. In addition, the presence of four isoindole or pyrrole nitrogen atoms allows tetapyrrolic macromolecules to coordinate with several metal ions. Chlorin and bacteriochlorin are partially hydrated, 2,3-dihydrochlorins and 7,8,17,18-tetra-hydrochlorin. These macrocycles are strongly coloured and fluorescent, but chlorins show stronger absorption at lower wavelengths compared to the analogous porphyrins.

1.1- Porphyrins.

The porphyrin ring contains a $22-\pi$ electron system of fully unsaturated carbon atoms. It is a biologically active unit found in some natural species like heme that works as the oxygen-carrying cofactor of hemoglobin and in cytochrome P_{450} enzymes enabling catalysis of oxidation processes.

*Figure 1. Structure of porphyrin, heme and TPP*
The porphyrin ring system is built up of four pyrrole rings that are joined by –CH groups to form the macrocycle. Metalated porphyrins are formed by replacing the pyrrole hydrogens in the pyrrole core. The diversity of porphyrin functions in nature and biological systems is caused by the variety of metals that can bind to the pyrrole core. Because of this, and because of their characteristic electronic and photo-physical properties, the metal derivatives have gained great attention for their roles in biological systems.

The photosensitizing properties of porphyrin derivatives has led to studies of their use as sensitizers in certain medical applications such as photodynamic therapy (PDT) \(^1\). Also, in recent years, the advantages of the high stability of porphyrins has opened new avenues of applications in molecular electronics, information storage \(^2\), solar cells \(^3\), and opto-electronics \(^4\).

1.1.1 Existing Methods of preparation porphyrins.

The most commonly used synthesis of porphyrins was presented by Poul Rothemund. It uses an condensation and oxidation of pyrrole in the presence of aldehyde in acidic medium \(^5\). After that, Alder and Longo refined the synthesis and also other groups around the world found various ways to make porphyrins and porphyrin analogues \(^6\). Figure 2 shows the synthetic routes of trans-substituted porphyrins with two different substituents \(^7\).

\[ \text{Figure 2. Routes to trans-substituted porphyrins.} \]
1.2 Chlorins.

Chlorins are important cofactors in Nature. In plants, chlorophyll \( a \) and \( b \) and other analogues are providing the basis of one of the most important vital processes for life \(^8\), the photosynthesis. Chlorins also play a role as a medium in the redox processes catalyzed by some enzymes \(^9\).

![Figure 3. Structure of chlorin.](image)

Chlorins are also called hydroporphyrins as they have one pyrrole ring saturated at the \( \beta \)-position. Although porphyrins and chlorins have many similarities, changing the symmetry and conjugation pattern as a result of reduction of one pyrrole ring gives rise to important differences in their photochemical properties and applications.

![Figure 4. Chlorophyll a and b](image)

Because of the instability of chlorophyll \( a \) and \( b \), several modifying studies have been performed aiming to stabilize there naturally occurring chlorophylls. Relatively few functionalities available for modification, however, made the researchers focus on the synthesis of stable chlorin \(^10\).
Selective methods for the total synthesis of some chlorin model system were developed by Montforts, Battersby and their co-workers \[^{11}\]. Later on, the synthesis of naturally occurring chlorin and other dialkylated chlorins was investigated from the information gathered in these studies.

### 1.3 Bacteriochlorins.

Bacteriochlorins are tetrapyrrolic macromolecules in which two pyrrole rings are saturated at the $\beta$-position. There are two types of bacteriochlorins, one with the saturated carbons at opposite sites and the other with the saturated carbons at neighboring sites, called isobacteriochlorin. Bacteriochlorins are derived from bacteriochlorophylls prevent in photosynthetic bacteria (Figure 5).

![Figure 5. Structures of bacteriochlorin, isobacteriochlorin and bacteriochlorophyll a.](image)

There are some differences between bacteriochlorophyll derivatives. For instance, bacteriochlorophyll $g$ differ from bacteriochlorophyll $b$ by containing a vinyl group at the 3-position \[^{12}\], while bacteriochlorophyll $b$ contains an acetyl group at the same position. In addition, bacteriochlorophyll $b$ is similar
to bacteriochlorophyll \( \alpha \), but contains an exocyclic ethylidene group at the 8-position. While studying the stability of bacteriochlorophylls, bacteriochlorophyll \( \beta \) and \( \gamma \) were found to be particularly unstable and are still under research.

1.4 Tolyporphins.

The emergence of broad-spectrum multi-drug resistance (MDR) is a major problem in cancer chemotherapies \(^{13}\). Therefore, compounds that are able to reverse MDR have high potential to improve the prognosis of the disease.

Moore and co-workers isolated tolyporphin A from the lipophilic extract of the cyanophyte microalgae *Tolypothrix nodosa*. After that, tolyporphins (B-K) have been isolated. Tolyporphin analogues are investigated as MDR-reversing agents with the development of new routes to functionalize heterocycles.

![Figure 6. Building blocks for tolyporphin A.](image)

Tolyporphins make up a series of bacteriochlorin natural products that display varying levels of MDR-reversing activity (MDRR) \(^{14}\). The total synthesis of tolyporphin A was carried out on a 0.38 mg scale and in \( \sim 25 \) steps (longest linear sequence) \(^{15}\). A set of key precursors are shown in Figure 6. These precursors are expected to be available via modifications of existing procedures \(^{16}\). A variety of substituents can be introduced, enabling the identification of the substitution pattern necessary for biological activity. However, a problem associated with tolyporphin is its phototoxicity \(^{14}\).
1.5 Glycosylation reactions.

Glycosylation reactions attach a carbohydrate from a glycosyl donor to functional groups like hydroxyl in an acceptor molecule (Figure 7). In biology, glycosylation refers specially to the enzymatic process which attaches glycans to lipids, proteins, or other organic compounds. Glycans serve a variety of structural and functional roles in membrane and excreted proteins.

\[ \text{Figure 7. General glycosylation reaction}^{[17]} \]

1.6 The anomeric selectivity

The neighbouring carbon atom adjacent to the anomeric carbon can become involved in the reaction, a situation known as "neighbouring group participation". This is related to that the interaction of the $\beta$-anomer is highly favoured rendering it more reactive than other atoms. The Figure below shows the mechanism.

\[ \text{Figure 8. Anomeric selectivity mechanism.} \]

1.7 Pyrroles.

Pyrrole is an aromatic five-membered heterocyclic compound. Pyrrole reactions are controlled by electrophilic aromatic substitution, where the pyrrole reacts as a nucleophile due to its electron rich structure. The stability of the pyrrole molecule comes from the high number of resonance structures, (Figure 9).
Pyrrole is one of the more important heterocycles, and is found in a wide range of natural products and drug compounds. There are several examples of natural product molecules containing pyrrole ring. In (Figure 10), compounds 1 and 2 are antibacterial and both isolated from bacterial sources. Nakamunic acid (compound 3) which has a dimeric structure, is isolated from marine sources \[^{19}\].

**Figure 9.** Resonance of pyrrole \[^{18}\].

Furthermore, the pyrrole substructure is also present in a large number of bioactive compounds like HIV fusion inhibitors \[^{20}\]. (Figure 11) shows examples of some drugs containing pyrrole substructures.
Figure 11. Drugs containing pyrrole substructures.

1.8 Glycosylation of pyrrole.

The leaving group connected to the anomeric carbon atom of a glycone (glycosyl donor) in glycosylation reactions can be substituted by a nucleophilic acceptor in the presence of a promoter like a Lewis acid (Figure 7). During the reaction, an oxonium ion is formed as an intermediate due to the activation of the glycosyl donor; this oxonium ion is usually considered as the active species and the key intermediate in a glycosylation reaction. Stereoselectivity, yield, and by-products are determined by the mixture of oxonium-ion and its protecting groups, the promoter, the solvent, the leaving group, and the nucleophile [17]. Armitt and co-workers reported direct formation of a C-glycosidic bond to pyrrole. They reported the selective glycosylation of pyrrole using trichloroacetimidate (TCAI) donors and BF$_3$·Et$_2$O as the promoter. (Figure 12) shows the glycosylation of pyrrole and N-TIPS-pyrrole with the maltosyl pyrrole TCAI donor to give the 2-substituted glycosyl pyrrole and the 3-substituted glycosyl pyrrole.
Figure 12. Glycosylation of pyrrole using the TCAI method, (a) pyrrole (5.0 eq), BF$_3$.Et$_2$O (2.5 eq), DCM. 4Å powdered molecular sieves, -50 °C, 20 min, 84% yield (b), same conditions as in a, but with N-TIPS-pyrrole, 31% yield (1:1.25 mix of α- and β- anomers).

In 2005 another method for selective C-glycosylation of pyrrole was published. 2,3,4,6-tetra-acetyl bromoglucose was used with InCl$_3$ as a promoter. The product was a mixture of 2-, and 3-substituted pyroles in 3:1 ratio respectively.

Figure 13. Glycosylation using acetobromoglucose and InCl$_3$, the reaction conditions were, pyrrole, InCl$_3$, DCM, at 0 °C, 84% yield.

The same method was used in a reaction with indole to form the glycosylated indole (Figure 14).

Figure 14. The product form from the same reaction conditions as given in Figure 12.
2 Aim of the project

Many researches have worked on the chemistry of porphyrins and chlorins, and a wide range of methods have been applied, in which pyrroles and dipyromethane fulfill key roles in the synthesis. One of our goals is the synthesis of multi-drug resistance-reversing natural products and synthetic analogues thereof. A second objective is to apply new strategies for the production of functionalized hetero-cycle compounds using glycosylated donors, and the development of new synthesis routes resulting in selectivity and better yields (Figure 15). Comparisons between this work and other strategies regarding selectivity and the yields is described.

Figure 15. Overview of reaction steps of the project.
3 Methodology

Bacteriochlorins can be prepared by known methods, and here follows a summary of methods that can be used to prepare and modify bacteriochlorin and tolyporphin derivatives for use as synthetic building blocks. The advantages as well as disadvantages of these methods are also discussed.

3.1 Existing methods for bacteriochlorins and tolyporphins.

In 1952 Dorough and Miller reduced meso-tetraphenylchlorin (TPC) to meso-tetraphenylbacteriochlorin (TPBC) by using catalytic hydrogenation shown in (Figure 16) [22], the absorption spectrum of the product was similar to bacteriochlorophyll a. On the other hand, total synthesis process can be used to produce other series of bacteriochlorin such as tolyporphins (Figure 6).

![Figure 16. Reduction of TPC to TPBC](image)

Later on, more efficient methods were presented, which studied the selectivity of preparation of bacteriochlorin from porphyrin [22-23].

![Figure 17. Reduction of porphyrin to bacteriochlorin.](image)

Note that most bacteriochlorins prepared by reductive hydrogenation are not stable, because of their high sensitivity towards oxygen, resulting in conversion back to chlorin and ultimately to porphyrin (Figure 17) [24].
Hydroxylation of substituted porphyrins was achieved for the first time in 1930 by Hans Fischer, with the expected product being a chlorin-epoxide, in which the double bond in pyrrole had undergone epoxidation \[^{25}\]. Later on, other strategies of oxidation reactions using symmetrical \(\beta\)-substituted porphyrins were performed by other researchers, and it was determined that the structure of the major product was a keto-chlorin that had arisen from hydrogen peroxide/sulfuric acid oxidation, and formed by rearrangement of a diol or epoxide \[^{26}\]. After that, more selective reactions were investigated. Chang et al. reported that free base octaethylporphyrin converted into tetrahydroxybacteriochlorin by a selective reaction that included OsO\(_4\) in CH\(_2\)Cl\(_2\) and zinc oxochlorin, produced the isomeric tetrahydroxisobacteriochlorin as a major product \[^{27}\].

Later on, more reactions were performed using OsO\(_4\)-mediated hydroxylation as a regioselective reaction. Pandy et al. studied the effect of electron-withdrawing groups on the OsO\(_4\) mediated hydroxylation and the rearrangement of pinacol-pinacolone, and the two pairs of vicinal hydroxy groups were produced, and can exist in a syn or anti relationship (Figure 18) \[^{28}\].

![Figure 18. Rearrangement of pinacol-pinacolone.](image)

The migration efficiency of electron-withdrawing groups in the pinacol-pinacolone rearrangement can be controlled by the substitution pattern of electron-withdrawing groups at the peripheral positions of the macrocycles \[^{28}\].
One of the most effective methods for the preparation bacteriochlorins is the hydroxylation of porphyrin since it provides high selectivity and good yields. In the hydroxylation method, free base porphyrin controls the selective formation of bacteriochlorin more efficiently as compared to metalated porphyrins [27]. Another advantage of hydroxylation reactions of porphyrins is that bacteriochlorins showed higher stability compared to those that were prepared by hydrogenation reactions. Still, some disadvantages of hydroxylation reactions methods exist. Firstly, no other β-alkylated porphyrin can be converted to dioxobacteriochlorins. Secondly, the starting material should display high symmetry to minimize the number of isomers products, (Figure 19) shows a number of products produced from non-symmetry starting material. Also, other by-products can be formed such as chlorins and isobacteriochlorins.

![Figure 19](image.png)

*Figure 19.* The number of products from hydroxylation non-symmetrical porphyrins.

### 3.2 Other methods for producing porphyrin and chlorin derivatives.

Until now, many reactions have been discovered for addition of functional groups to porphyrins and chlorins to increase their scope of use such as PDT, solar cells, opto-electronics and other applications. One of these methods include a double Diels-Alder reaction involving di-vinylporphyrins [29], 1,3 dipolar addition and others, but all of these methods include isomers and some result in unstable products.
3.3 Glycosylated porphyrins, chlorins and bacteriochlorins.

In this section, methods used to prepare meso-substituted and β-substituted porphyrins, chlorins, and bacteriochlorins will be described. In 2010, Drain and co-workers reported the facile synthesis and analysis of the photophysical properties, and in-vitro studies of tetrathioglycosylated porphyrinoids: chlorin CGlc₄, bacteriochlorin BGLc₄, and isobacteriochlorin IGLc₄, by adjusting the photophysical properties relative to TPPF₂₀[30] (Figure 20).

![Synthesis of CGlc₄, IGlc₄ and BGLc₄](image)

*Figure 20. Synthesis of CGlc₄, IGlc₄ and BGLc₄.*
In two- or three-steps procedures, they prepared CGLc₄, BGLc₄, and IGLc₄ as a suitable platform for developed biochemical tag, diagnostics and photodynamic therapeutic agents related to the photophysical properties of TPPF₂₀ and glycosylated conjugates PGLcAc₄ and PGLc₄[^31] (Figure 21).

![Figure 21](image)

**Figure 21.** Synthesis of PGLcAc₄ and PGLc₄ from TPPF₂₀.

### 3.4 Synthesis of tolyporphin A derivatives.

In 1999 Kishi and his group synthesized O,O-diacetatetolyporphin A by using iminoester cyclization with extension of the Eschenmoser Sulfide contraction[^32]. The spectroscopic and chromatographic analysis showed that the synthetic O,O-diacetatetolyporphin A was identical to the natural tolyporphin A[^32], unambiguously establishing the stereochemistry of tolyporphin A, the figure below shows the O, O-diacetatetolyporphin A building blocks.

![Figure 22](image)

**Figure 22.** Tolyporphin A O,O-diacetate with building blocks.
The stepwise synthesis of tolyporphin A is too complex for practical use (more than 20 steps) and with low yield (< 5 mg) to serve as a general method for the preparation of bacteriochlorin. Therefore, for fundamental studies or other applications in materials chemistry, in naturally occurring bacteriochlorin, de novo synthesis is the only way for tolyporphin synthesis.

3.5 Glycosylated pyrroles.

Of the two positions in the pyrrole ring that can react with electrophiles, position, 2 is more reactive than position 3. In 2002, Armit et al. prepared C-glycosides by using O-glycosylimidates and pyrrole in presence of BF$_3$.Et$_2$O. The reaction produced 2-galacto, manno- and lactopyrroles as well as the benzyl protected 2-glycosyl pyrrole which were acquired in good yields.

![Figure 23. Preparation of pyrrole-C-glycosylated using the glycosyl donors under BF$_3$.Et$_2$O.](image)

3.6 Glycosylation of pyrrole at position 3.

The group of Borbas tried to use the same procedure but with $N$-TIPS-pyrrole to block position 2 and steer the reaction to position 3 \cite{33}. In this work $N$-TIPS-pyrrole with a trichloroacetimidate donor was used under the same conditions to produce 3-glycosylated pyrroles as building blocks for glycosylated tetrapyrrrole macrocycles. The reaction of the $O$-benzyl protected trichloroacetimidate was, however, poorly selective.
The same group also attempted a potentially more selective pathway to prepare 3-glycosylated pyroles, (Figure 25 and 26) \(^{[34]}\).

This glycosyl donor was reacted with \(N\)-TIPS pyrrole under TMSOTf promotion in dry DCM at -20 °C. The reaction was quenched by addition of TBAF·3H\(_2\)O to get 3-glycosylpyrrole (Figure 26).

\[\text{NH}_2 + \text{Cl}_{2}\text{CF}_3 \xrightarrow{\text{Ph}, \text{Et}_3\text{N, CCl}_4} \text{Cl}_{2}\text{CF}_3 \xrightarrow{0 \text{ C to reflux 4 h}} \text{Cl}_{2}\text{CF}_3 \]

Figure 25. Preparation of glycosyl donor with new substrate.

Figure 26. Preparation of 3-glycosylated pyrrole \(^{[33]}\).
3.7 Conversion of glycosylated pyrrole to dipyrromethane.

Dipyrromethanes can be synthesized from aldehydes and pyrrole which are reacted in the presence of acid \[^{35}\]. 3-Glycosylated pyrroles prepared by the described method, synthesis of mono- and diglycosylated dipyrromethane can be used by different synthetic routes (Figure 27).

![Diagram of pathways of preparation of glycosylated dipyrromethanes](image)

*Figure 27. Pathways of preparation of glycosylated dipyrromethanes \[^{33}\].*

3.8 Diglycosylated dipyrromethanes.

Earlier studies have demonstrated the possibility of preparing diglycosylated dipyrromethanes from 2-glycosyl-pyrrole which had reacted with benzaldehyde in the presence of TFA \[^{35}\]. The same protocol gave traces for 3-glycosylated pyrroles. The reasons will be discussed later, (Figure 28) shows the reactions and the reaction conditions.

![Reaction diagram for preparation of diglycosylated dipyrromethane](image)

*Figure 28. Preparation of diglycosylated dipyrromethane \[^{33}\].*
3.9 Preparing porphyrin.

Lindsey et al. developed a rational route to \(\text{trans-AB-porphyrins}\) \[^{[36]}\]. They chose Western half dipyrrromethane (5-phenyl dipyrrromethane) to interact with mono-glycosylated dipyrrromethane. The Western half can be prepared by reacting pyrrole with benzaldehyde in presence of \(\text{InCl}_3\) as a catalyst \[^{[37]}\]. Following this step, dipyrrromethane was diformylated with \(\text{POCl}_3\) and DMF (Vilsmeier formylation) \[^{[38]}\], then reacted with propylamine in THF for 1 h to give \(1,9\)-bis(imino)-5-phenyl dipyrrromethane.

\[
\begin{align*}
\text{Ph} & \quad \text{Ph} \\
\text{NH} & \quad \text{NH} \\
\text{HN} & \quad \text{HN} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\end{align*}
\]

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\end{align*}
\]

\textit{Figure 29}. Preparation of Eastern half of dipyrrromethane.

By refluxing the mixture of Western half and Eastern half for 15 h in presence of \(\text{Zn(OAc)}_2\), followed by purification by column chromatography gave 2 mg of the mixture of products (Figure 30).

\[
\begin{align*}
\text{AcO} & \quad \text{AcO} & \quad \text{AcO} & \quad \text{AcO} \\
\text{AcO} & \quad \text{AcO} & \quad \text{AcO} & \quad \text{AcO} \\
\text{NH} & \quad \text{HN} & \quad \text{HN} & \quad \text{HN} \\
\text{NH} & \quad \text{HN} & \quad \text{HN} & \quad \text{HN} \\
\text{Ph} & \quad \text{Ph} & \quad \text{Ph} & \quad \text{Ph} \\
\end{align*}
\]

\[
\begin{align*}
\text{Zn(OAc)}_2 & \quad \text{Zn(OAc)}_2 \\
\end{align*}
\]

\textit{Figure 30}. Preparation of monoglycosylated porphyrin.

3.10 Mono glycosylation of dipyrrromethane in regioselective reaction

Other productive pathways of more selective reactions were also worked out. 3-Glycosyl pyrrole was reacted with pyrrole to get monoglycosyl dipyrrromethane. 2 Eq of Eschenmoser salt in DCM was reacted with the substrate to produce 3-glycosylated pyrrole (Figure 31), the reaction was highly regioselective and reproducible \[^{[39]}\].
After that, the product was reacted with neat distilled pyrrole in the microwave for 30 min at 150 °C and the product was purified by column chromatography to get monoglycosyl dipyrromethane (Figure 32).

The same route was followed with the other half of 5-phenyldipyrromethane to get monoglycosylated porphyrin without isomers (Figure 46).
4 Results and discussion.

From the previous discussed results it can be concluded that the Armit et al. reaction was useful for 2-glycosylated pyrroles, but when the same procedure was applied to make N-TIPS-pyrrole to produce 3-glycosylated products, it was irreproducible.

Because the C-glycosylation of pyrrole at 3-position is challenging, strategies using a new leaving group was chosen. N-phenyl-trifluoroacetimidate was the optimal solution to achieve a selective reaction [40]. N-2,2,2-trifluoroacetimidoyl chloride was prepared from standard procedure [34]. Trifluoroacetimidate glycosyl donor was produced by using either of these bases, (DIPEA [41], NaH [42], CsCO₃ [43], DBU [44], or K₂CO₃ [45]), and was obtained in 97% yield using K₂CO₃. The compound is temperature sensitive and decomposes at room temperature after two weeks, but is stable for several months at 4 °C.

![Figure 33. Preparation of glycosyl donor.](image)

4.1 Selectivity of glycosylation of N-TIPS-pyrrole

When N-phenyl-trifluoroacetimidate glycosyl donor was applied high selectivity for the β-anomer was observed, and 40% yield after two reaction steps was achieved (Figure 33).

4.2 Glycosylation of N-TIPS-pyrrole

Based on previous results, PTFAI was chosen as donor for the reaction with N-TIPS-pyrrole by following a literature procedure [46]. A glycosidic coupling was used between N-pentenoyl hydroxamic acid and a PTFAI donor after TMSOTf activation according to published procedures [47]. Optimization of condition resulted in good yield.

Purification of 3-glycosylated TIPS pyrrole was problematic, but after the temperature changing had been studied, the optimum reaction temperature was -20 °C with 20 min waiting time.
Temperature, time, and quenching were optimized for best yield: -20 °C at 35 min with quenching with 5 eq of TBAF proved optimal condition and produced 41% yield.

4.3 Mono-glycosylated dipyrrromethanes.

Based on previous results with dipyrrromethanes, two different strategies were studied. In the first strategy, commercially available 2-formylpyrrole was used as starting material to react with benzylpyrrole which was prepared from benzylmorpholine and POCl₃ forming Vilsmeier reagent \[^{[48]}\]. (Figure 34) shows the results of previous work \[^{[33]}\].

\[ \text{Figure 34. Previous reaction steps to produce mono glycosylated porphyrin.} \]

This strategy failed as judged by the lack of product dipyrrromethane. Also, the reduction of the carbonyl function did not give any dipyrrromethane as shown in (Figure 35).
Figure 35. Failed strategies to produce glycosylated dipyrrromethanes.

In the other strategy, shown in Figure 35, the Vilsmeier reagent formylation reaction was applied directly to glycosylated pyrrole. The yield after overnight reaction was satisfactory but the selectivity was not. The reaction occurred on both sides of the glycosylated pyrrole (Figure 36 and 37) and it was difficult to isolate the desired product.

Figure 36. Vilsmeier reaction with glycosylated pyrrole.

Figure 37. Other products from Vilsmeier reaction.
4.4 The new results.

At this point, new strategies producing more efficient selectivity, and better yields were tested. Reactions that described attachment of an electrophilic group at position 2 in 3-glycosylated pyrrole was tried. Other procedures such as benzoyl chloride in presence of MesMgBr was also tested.

![Figure 38. Bromination of 3- glycosylated pyrrole.](image)

Reacting the substrate with NBS in THF at -78 °C was also tested,

![Figure 39. Bromination of 3- glycosylated pyrrole with other condition.](image)

or with NIS in THF at -94 °C.

![Figure 40. Iodination of glycosylated pyrrole.](image)

After that p-bromobenzaldehyde was used in the presence of piperidine and DCM, repeating the reaction with toluene as a solvent.

![Figure 41. Preparation of diglycosylated dipyrromethane.](image)
Also morphilino(phenyl)methanone in presence of POCl₃ in THF was reacted with the substrate.

![Diagram](image1.png)

**Figure 42.** Preparation of 2-bezoyl-4-glycosylated pyrrole.

All of the described attempts were unsuccessful.

Table 1. *Unsuccessful reactions.*

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Time/ min</th>
<th>Temp</th>
<th>Solvent</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MesMgBr/benzoylchloride</td>
<td>30</td>
<td>-78 °C</td>
<td>THF</td>
<td>NR</td>
</tr>
<tr>
<td>NBS</td>
<td>30</td>
<td>-78 °C</td>
<td>THF</td>
<td>NR</td>
</tr>
<tr>
<td>NBS</td>
<td>30</td>
<td>-94 °C</td>
<td>THF</td>
<td>Mixture of products</td>
</tr>
<tr>
<td>NIS</td>
<td>30</td>
<td>-94 °C</td>
<td>THF</td>
<td>NR</td>
</tr>
<tr>
<td><em>p</em>-benzoylbromide</td>
<td>30</td>
<td></td>
<td>DCM or toluene</td>
<td>NR</td>
</tr>
</tbody>
</table>

*a* = No Reaction.

4.5 **Eschenmoser salt as a reagent.**

Finally, in a very facile reaction, including 2 eq of Eschenmoser salt in THF at room temperature for 20 min resulted in 95 % yield.

The reaction was easy to perform, not sensitive to moisture and reproducible. Figure 43 shows the reaction conditions. The reaction was highly selective and no by-product was observed.

![Diagram](image2.png)

**Figure 43.** Preparation of glycosylpyrrole donor in presence of Eschonmoser salt.

40 mg (0.094 mmol) of 3-glycosylated pyrrole was dissolved in DCM at rt, Eschonmoser salt (2 eq) was added in one portion, the mixture was stirred for 20 min, then the reaction was quenched with saturated NaHCO₃, the aqueous
layer was extracted twice. The organic layer was dried by dry Na₂SO₄, the solvent was evaporated, no more purification needed with 95% yield[39].

### 4.6 Mono-glycosylated dipyrromethanes synthesis.

The compound with a dimethyl ethylamino group was reacted with 10 % ZnCl₂ and pyrrole neat, under room temperature. This reaction was, however, unsuccessful. After this, the substrate was reacted with InCl₃ and pyrrole neat, also without success. The reaction was repeated with Zn(OAc)₂ and 5 eq of pyrrole in THF, EtOH and toluene as a solvents, again without product formation.

Finally, microwave assisted heating of the substrate and pyrrole at 150 °C for 30 min resulted in optimal results[39].

**Figure 44.** Formation of glycosylated dipyrromethane.

15 mg (0.031 mmol) of the product (Figure 43) was placed in microwave vial and dissolved in 2.5 mL pyrrole, the vial was placed in a microwave for 30 min at 150 °C. Then, the pyrrole was evaporated and the residue was purified by column chromatography with 44 % yield[39].

**Table 2. Optimization reaction of glycosylated dipyrromethane.**

<table>
<thead>
<tr>
<th>Time/ min</th>
<th>Temp</th>
<th>Solvent</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>80 °C</td>
<td>Pyrrole</td>
<td>Reaction not completed</td>
</tr>
<tr>
<td>30</td>
<td>100 °C</td>
<td>Pyrrole</td>
<td>Reaction not completed</td>
</tr>
<tr>
<td>30</td>
<td>120 °C</td>
<td>Pyrrole</td>
<td>Reaction not completed</td>
</tr>
<tr>
<td>15</td>
<td>150 °C</td>
<td>Pyrrole</td>
<td>Reaction not completed</td>
</tr>
<tr>
<td>30</td>
<td>150 °C</td>
<td>Pyrrole</td>
<td>44 %</td>
</tr>
</tbody>
</table>
\[ \text{H NMR (400 MHz, Chloroform-}\text{d}) \delta 8.33 (s, 1H), 8.17 (s, 1H), 6.71 (s, 1H), 6.18 (t, J=2.6 Hz, 1H), 6.04 (d, J=2.9 Hz, 2H), 5.93-5.82 (m, 1H), 5.31 (td, J=9.5 Hz, 1H), 5.10 (dt, J=25.2, 9.7 Hz, 2H), 4.45-4.32 (m, 1H), 4.28 (dd, J=12.4, 4.8 Hz, 1H), 4.16-4.05 (m, 2H), 3.98 (d, J=2.6 Hz, 2H), 3.85-3.73 (m, 1H), 2.12-1.98 (m, 11H), 1.89 (t, J=1.3 Hz, 3H). \]

4.7 Second part of dipyrrromethane preparation.

Commercially available 5-phenyl dipyrrromethane was used to prepare di-formylated dipyrrromethane in presence of POCl\textsubscript{3} and DMF at 0 °C by standard procedure \cite{36}. The product was subsequently reacted with propylamine in THF at room temperature to give 1,9-\textit{bis}(imino)-5-phenyl-dipyrrromethane \cite{38}, (Figure 45).

\[ \text{Figure 45. Formylation of dipyrrromethane.} \]

4.8 Preparation of porphyrin.

The last step of this multi-steps synthesis was to react the two of dipyrrromethanes parts to yield the mono-glycosylated porphyrin. This was done by refluxing the reactants with Zn(OAc)\textsubscript{2} in presence of toluene, followed by column chromatography to get a redish fluorescent solid compound as shown below.

\[ \text{Figure 46. Reaction of the two parts of dipyrrromethane.} \]

A 5.5 mg (0.015 mmol) of 5-phenyl-1,9-\textit{bis}(N-propylimino) dipyrrromethane was dissolved in anhydrous toluene in dry flask, 7.2 mg (0.015 mmol) was
dissolved in 1 mL dry toluene, then the solution was added to the first reactant, 27.3 mg (0.15 mmol) of Zn(OAc)$_2$ was added. The reaction mixture was refluxed overnight, the solvent was evaporated and the residue was purified by column chromatography with 3 mg pink product

$^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 10.28 (s, 1H), 10.23 (s, 1H), 9.54 (s, 1H), 9.46 (d, 1H), 9.41 (t, $J=5.0$ Hz, 1H), 9.12 (d, $J=4.5$ Hz, 1H), 8.24 (dd, $J=19.7$, 6.8 Hz, 1H), 7.79 (dq, $J=8.7$, 5.4, 4.6 Hz, 2H), 6.35 (d, $J=9.8$ Hz, 1H), 6.06 (t, $J=9.6$ Hz, 1H), 5.88 (t, $J=9.4$ Hz, 1H), 5.70 (t, $J=9.8$ Hz, 1H), 5.30 (d, $J=1.3$ Hz, 1H), 4.60 (dd, $J=12.6$, 5.0 Hz, 1H), 4.55-4.43 (m, 1H), 2.21 (dd, $J=7.6$, 1.3 Hz, 4H), 2.09 (s, 2H), 1.26 (d, $J=2.2$ Hz, 9H).
5 Conclusion

In this work, new pathways of synthesis have been applied to produce glycosylated dipyrrromethanes and mono-glycosylated porphyrin, and the synthesis strategies have been compared with similar work.

Reaction conditions resulting in selective and high-yield production of mono glycosylated dipyrrromethane have been established.

From the results discussed here, I have some suggestions for future work. Firstly, it would be a good idea if also other mono-saccharides, such as mannose, galactose, or di-saccharides, such as sucrose could be applied in the reaction synthesis and the differences between sugar substrates could be assessed. Secondly, the physical properties of mono-glycosylated porphyrin could be studied compared to tolyporphin natural products.
Appendix
References:


