CHARLES UNIVERSITY
FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ
DEPARTMENT OF PHARMACEUTICAL CHEMISTRY
AND PHARMACEUTICAL ANALYSIS

DIPLOMA THESIS

XANTHONE-BORONIC ACIDS: AN INSIGHT INTO THE SYNTHESIS OF BORYLATED XANTHONE DERIVATIVES

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„I declare that this thesis is my original work, which I have developed on my own under supervision. I further certify that all the literature and other sources of information used for the development of this work appear in the list of literature and are properly cited. This work was not used to obtain another or a similar title. “

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Priam-Amedeo Houngbedji
In Hradec Králové the 25.04.2021

Abstract

The development of Bortezomib **5** and discovery of its potential in the treatment of multiple myeloma has sparked hope, and a close attention is paid in medicinal chemistry to the synthesis of boronic acid derivatives as well as the evaluation of their anticancer, antimicrobial, and other activities. Parallelly, xanthones and xanthone derivatives are compounds thoroughly studied for their potential in the treatment of cancer, and their good antimicrobial, anti-inflammatory, antiviral and anticonvulsant activity. It is only natural that efforts are oriented towards the synthesis of xanthone boronic derivatives.

3,6-Dihydroxyxanthone **10** was chosen as a precursor for the development of a procedure to borylate xanthones, mainly due to its easy synthesis and availability. From **10**, 3,6-ditrifylxanthone **11** was easily prepared. The synthesis of 3,6-bis(pinacolatoboron)xanthone **8** from **11** was achieved using bis(pinacolatodiboron) B_2pin_2 as a borylation agent under $Pd(dppf)Cl_2$ catalysis with an addition of dppf complex in the presence of KOAc. A different procedure was explored by replacing B_2pin_2 with pinacolborane HBpin in the presence of Et_3N as base and higher yield was achieved. 3,6-Bis(pinacolatoboron)xanthone **8** was then deprotected into xanthone-3,6-diboronic acid (1**4**) by transformation of the pinacolboronate into a DEA-protected salt in the presence of diethanolamine, and byphasic (water/acetone) acidic hydrolysis of the obtained salt.

In an attempt to explore other borylation procedures, **10** was combined with NaH in the presence of 4,4,5,5-tetramethyl-1,3,2-dioxaborolane in order to obtain 3,6-bis((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methoxy)-9H-xanthen-9-one **12** and consequently deprotect it into (((xanthone-3,6-diyl) bis(oxy))bis(methylene))diboronic acid **13**. However, the used conditions resulted in a partial reaction and 3-hydroxy-6-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methoxy)-9H-xanthen-9-one **12b** was obtained. Keeping in mind the extension of the borylation procedure to other xanthone derivatives, the synthesis of 2,7-dibromoxanthone **22** and 3,4-ditrifyl-1-methylxanthone **17** were attempted.

Abstrakt

Vývoj léčiva bortezomibu **5** a jeho zásadní význam pro léčbu mnohočetného myelomu odstartoval zvýšený zájem o syntézu a biologické hodnocení derivátů boronových kyselin, především k jejich protirakovinným, protimikrobiálním, popř. dalším aktivitám. Zároveň jsou xanthony a jejich deriváty důkladně studovány pro potenciál v protirakovinné terapii, a pro jejich antimikrobiální, protizanětlivé, protivirové a antikonvulzivní účinky. Logickým spojením těchto dvou skutečností je orientace na syntézu boronových derivátů xanthonů.

3,6-Dihydroxyxanthon **10** byl zvolen jako prekurzor pro vývoj borylační procedury xanthonů, zejména pro svou snadnou syntézu a dostupnost. Z **10** byl snadno připraven 3,6-ditrifylxanthon **11**. Syntézy 3,6-bis(pinakolatoboron)xanthonu **8** z **11** bylo dosáhnuto použitím bis(pinakolatodiboronu) B_2pin_2 jako borylačního činidla, za katalýzy $Pd(dppf)Cl_2$ komplexem s přísadou dppf ligandu a v přítomnosti KOAc zásady. Alternativní cesta byla realizována záměnou B_2pin_2 za pinakolboran HBpin, v přítomnosti Et_3N jako báze a bylo tak dosáhnuto vyšších výtěžků. 3,6-Bis(pinakolatoboron)xanthon **8** byl následně odchráněn na přislušnou xanthon-3,6-diboronovou kyselinu **14**, a to přeměnou pinakolboronátu na DEA-chráněnou sůl v přítomnosti diethanolaminu, a dvoufázovou (voda/aceton) kyselou hydrolýzou získané soli.

Ve snaze prostudovat další borylační metody byla sloučenina **10** smíchána s NaH v přítomnosti 4,4,5,5-tetramethyl-1,3,2,-dioxaborolanu, se záměrem získat 3,6-bis((4,4,5,5,-tetramethyl-1,3,2,-dioxaborolan-2-yl)methoxy)-9*H*-xanthen-9-on **12** a odchránit jej v (((xanthon-3,6-diyl)bis(oxy))bis(methylen))diboronovou kyselinu **13**. Použité reakční podmínky ale rezultovaly pouze v částečnou reakci a byl získán 3-hydroxy-6-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methoxy)-9H-xanten-9-on **12b**. Posledním pokusem rozšířit borylace na další deriváty xanthonů byla syntéza 2,7-dibromxanthonu **22** a 3,4-ditrifyl-1-methylxanthonu **17**.

Abbreviations, acronyms, and symbols

δ – Chemical shift
DCM - Dichloromethane
DMSO – Dimethylsulfoxide
Dppf - 1,1'-Bis(diphenylphosphino)ferrocene
Et – Ethyl
FDA – Food and Drugs Administration
^{1}H NMR – Proton Nuclear Magnetic Reasonance
J – Coupling constant
Me – Methyl
Tf – Triflate
TLC – Thin Layer Chromatography

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1. Introduction

1.1. Xanthone derivatives – a promising field in medicinal chemistry

Named after the Greek word "Xanthos" meaning "yellow" for their yellow colouring, xanthones or xanthen-9*H*-ones are heterocycles with a dibenzo-gamma-pyrone structure (

Fig. 1)[1].

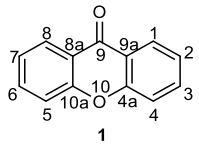

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Fig. 1 – Xanthone **1** structure and numbering

Xanthones and their various derivatives are compounds found in many natural sources such as plants, fungi, ferns, and lichens[2]. Xanthones isolated from natural sources present different structures and substituents. Based on these, they can be classified into six main groups, namely simple xanthones, xanthone glycosides, prenylated xanthones, xanthonolignoids, bisxanthones, and miscellaneous xanthones[2]. These naturally-occurring xanthones are one of many privileged structures in medicinal chemistry, and have been proved to show various properties such as cardioprotective, gastroprotective, immunosuppressive, insecticidal, antiprotozoal properties and many more[3]. However, naturally occurring xanthones do not cover all the possible structures a xanthone can have. Therefore, several libraries of xanthone derivatives have been synthesised with various groups on their structure such as -OH, -Me, -COOH and more complex groups such as epoxide, azole, amino alcohol, sulfamoyl, or pyridine [4].

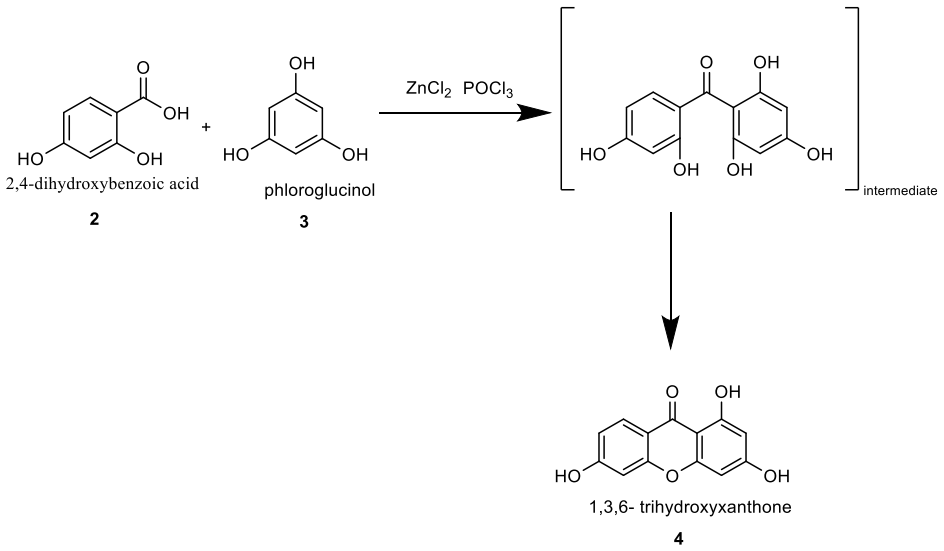
Synthetic xanthones present a wide range of activities just like natural ones [3, 5]. These compounds can modulate numerous enzymes such as the alpha glucosidase, topoisomerase, protein kinase C, aromatase, intestinal P-glycoprotein, miRNA, acyl-CoA cholesterol acyltransferase, anticholinesterase, xanthine oxidase, among others [5]. Hence, these compounds have a considerable potential as anticancer, antimalarial, antimicrobial, anticonvulsant, anti- HIV, antioxidant, and anti-inflammatory agents [5]. In general, the quality and quantity of the modulatory activity is influenced by the xanthone's structure and the type of derivative. Furthermore, the type of substituent (-OH, -COOH, -OMe, -OEt…) and its position may increase or decrease the activity [6]. Hence, it is essential to establish a structure-activity relationship when studying their activity [7].

1.2. Xanthone derivatives – Synthetic approaches

Xanthone derivatives can be obtained through different routes such as the Grover, Shah, and Shah (GSS) method, via benzophenone or via diaryl ether intermediates.

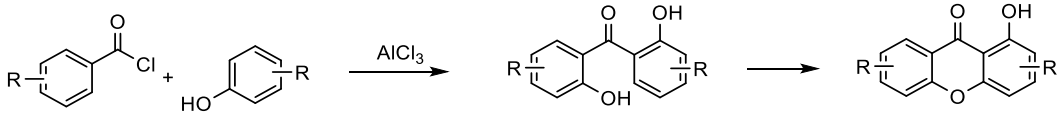
The GSS method consists of heating a salicylic acid derivative and an activated polyphenol (

Scheme 1). The reaction is catalysed by zinc chloride in phosphoryl chloride as solvent. The reaction mechanism goes through a benzophenone intermediate and provides a xanthone in one step, provided the intermediate has three -OH groups at positions 2, 2', 6 or 6'.Otherwise, an additional dehydration or appropriate reaction is required to obtain the xanthone. [8]



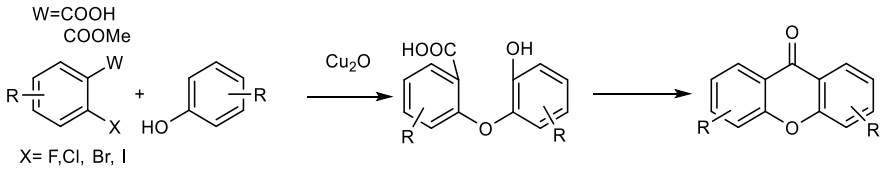
Scheme 1 - Example of xanthone synthesis by Grover, Shah and Shah method.

The synthesis of xanthones via benzophenone route (Scheme 2) requires a benzoyl chloride to react via Friedel-Crafts acylation with a phenolic derivative in an appropriate solvent with aluminium chloride as Lewis acid catalyst. This reaction lacks regioselectivity and the acylation follows the electrophilic aromatic substitution rules. The final xanthone is then obtained from the benzophenone by an appropriate reaction (dehydration, oxidation, nucleophilic substitution…). [9]



Scheme 2 – Synthesis of xanthones via benzophenone intermediate.

The third methodology that can be applied for the synthesis of xanthones is via diaryl ether intermediate route, which consists of reacting a sodium phenolate with benzoic acids halogenated in the *ortho* position, through an Ullmann condensation (Scheme 3). The ring formation is then accomplished by electrophilic cycloacylation of the 2-aryloxybenzoic acids. [4]



Scheme 3 – Synthesis of xanthones via diaryl ether intermediate.

Some less conventional methods for the synthesis of xanthones have also been reported [4] . In addition, the classical methods of synthesis have been modified and higher yields have been achieved with milder experimental conditions. [4]

1.3. Borylation and borylated xanthone derivatives

1.3.1. Boronic acids

A boronic acid (Fig. **2**) is chemically related to a boric acid in which one of the hydroxyl groups is replaced by an alkyl or an aryl. Boronic acids belong to the class of organoboranes [10] and their derivatives are common compounds in synthetic chemistry. On the other hand, their use in medicinal chemistry is fairly new, mainly due to the long-lasting belief in their toxicity. However, they have been proven non-toxic [11] and the discovery of drugs like bortezomib **5** (Fig. **2**) has sparkled new hopes and interests in boronic derivatives [12]. Boronic acids are Lewis acids with a p*K*a between 4-10 depending on the substituents. They can form complexes with Lewis bases (anions, oxygen, nitrogen, electron donating groups) and behave as electrophiles. Hence, boronic acids can interact with groups present in biological compounds such as enzyme residues, nucleic acids, or hydroxyl groups from carbohydrates. This fact helped discovering a range of boronic derivatives with antibacterial, antiviral, and anticancer activities [12].

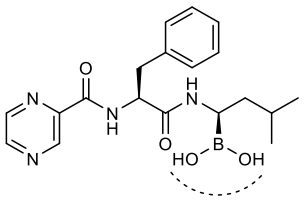


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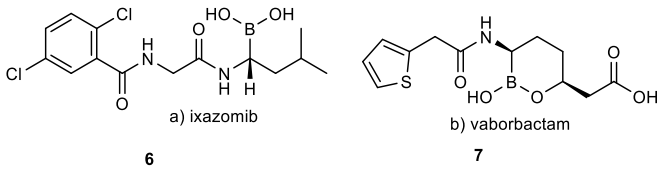
Fig. 2 – Bortezomib **5** and the boronic acid functional group

Bortezomib **5**, a drug commercially available under the name Velcade[tm], is the first proteasome inhibitor used in the treatment of multiple myeloma [13]. It is known that boronic acid groups show a high selectivity and low dissociation rate towards the active site of the proteasome [12]. Also, the replacement of the aldehyde functional group with boronic acids in previously synthesised aldehyde proteasome inhibitors solved several issues linked with their activity (rapid dissociation from proteasome, inactivation by oxidation…) as well as increased the potency of the proteasome inhibitor, leading to the discovery of later Food and Drug Administration (FDA) approved Bortezomib **5** [12].

Besides Bortezomib **5**, two more drugs containing boronic acids were approved by FDA – Ixazomib **6** (Fig. **3**a) and Vaborbactam **7** (Fig. **3**b). Ixazomib is an *N*-dipeptidyl boronic acid which follows the footsteps of Bortezomib **5** in the treatment of multiple myeloma, whereas Vaborbactam with its cyclic structure has found use in the treatment of urinary, abdominal and lung infections as a β-lactamase inhibitor [12].



6 **7**

Fig. 3 – Chemical structure of ixazomib **6** and vaborbactam **7**

Another class of compounds whose antiviral activity was reinforced with the introduction of a boronic acid group are chalcones [12]. The replacement of the carboxylic group with the boronic acid has considerably increased the chalcones selectivity and toxicity towards breast cancer cells [12]. Figure 4 presents the general structure of chalcone-boronic acids.

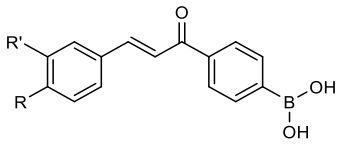


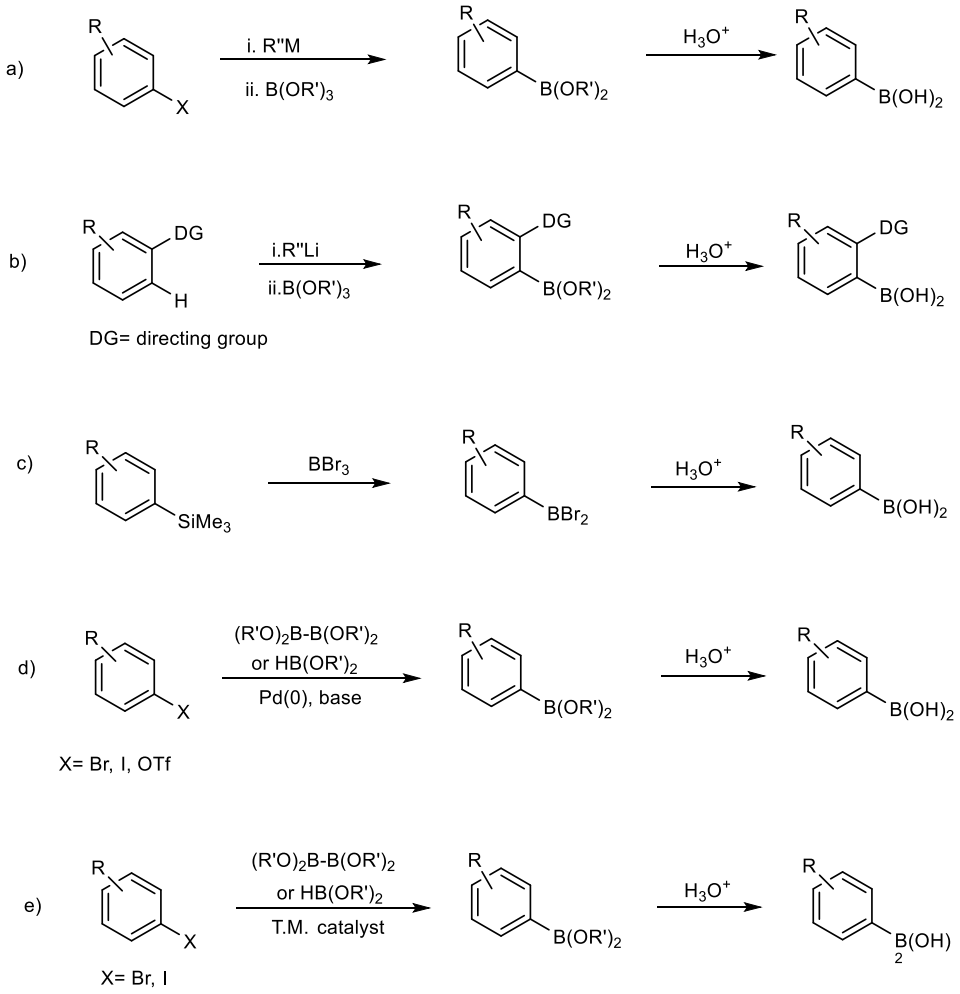
Figure 4 – General structure of chalcone boronic acids.

Given this reported boost of anticancer and antibacterial activities by boronic acid groups, it would be interesting to explore the borylation of xanthones and the biological activities of xanthone-boronic acids.

1.3.2. Synthesis of arylboronic acids and their derivatives

1.3.2.1. General synthetic approaches

The synthesis of arylboronic acids and their derivatives is well-mapped and can be summed up in Scheme 4 [10, 12]:



Scheme 4 – Methods of synthesis of arylboronic acids and their derivatives

a) Electrophilic borate trapping of arylmetal intermediates from aryl halides:

Electrophilic trapping of arylmetal intermediates with borate esters at low temperature is one of the first and most common methods to synthesise arylboronic acids. This reaction consists of adding a methylborate to a solution of phenylmagnesium bromide but results in low yields [12].

b) Electrophilic borate trapping of arylmetal intermediates from directed *ortho*-metalation

The preparation of arylboronic acids through lithium-halogen exchange is an alternative to the use of aryl halides [12].

c) Transmetallation of arylsilanes and arylstannanes

The use of silanes and stannanes is a suitable alternative to one of the earliest methods of boronic acids synthesis involving diaryl mercury compounds and boron trichloride. Trialkylaryl silanes and stannanes can both be transmetallated efficienty with a hard boron halide such as boron tribromide. This step is then followed by an aqueous acidic workup to hydrolyze the arylboron dibromide product [12].

d) Transition metal-catalysed coupling between aryl halides/triflates and diboronyl reagents

The coupling between aryl halides/triflates and diboronyl reagents was discovered by Miyaura *et al.* [14] These researchers found that diboronyl reagents such as B_2pin_2 undergo a smooth cross-coupling reaction with aryl bromides, iodides, and triflates under palladium catalysis [10]. The challenge during this method comes with the deprotection of the obtained pinacolborane esters [15].

e) Direct boronylation by transition metal catalyzed aromatic C-H functionalization

Several research groups have reported efficient procedures of direct boronylation using iridium and rhodium as catalysts. This reaction process also generated much interest for its mechanism. [10]. These methods can be used in the synthesis or arylboronic derivatives and by extention, borylated xanthone derivatives.

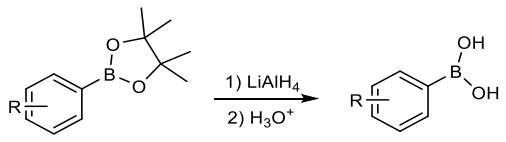
1.3.2.2. Pinacol arylboronate esters – hydrolysis and deprotection methods

As opposed to what could be believed, the deprotection of pinacol arylboronate esters can prove challenging [15]. However, several methods and approaches have been reported in the literature:

A – Hydrolysis with lithium aluminium hydride $LiAlH_4$

Lithium aluminium hydride presents itself as a white powder susceptible to combust and explode if in contact with water or exposed to considerable friction[16]. It is widely used in organic chemistry as a source of hydrogen, for the synthesis of other hydrides or as a reducing agent [16].

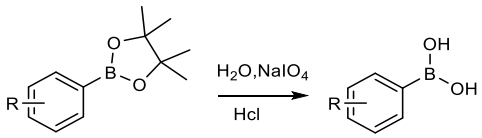
As represented in (Scheme 5), $LiAlH_4$ can be used for the hydrolysis of aryl boronates [17, 18]. This reaction has been reported to work under argon atmosphere at 0 °C with following heat-up to room temperature after 2 h and the addition of water in form of hydrates ($Na_2SO_4.10H_2O$, $MgSO_4.7H_2O$) [17]. The then formed aluminium salts are dissolved by addition of H_2SO_4 and the reaction mixture is extracted with the appropriate organic phase, dried over Na_2SO_4 and the residue obtained after evaporation of organic solvents is purified to give the desired product [17].



Scheme 5 – Arylboronate ester hydrolysis with $LiAlH_4$

B – Oxidative hydrolysis by $NaIO_4$

A white efflorescent salt, sodium periodate ($NaIO_4$) is a powerful oxidising agent in acidified solutions [19]. It is widely used in analytical chemistry for the oxidation of manganese into permanganate [19]. $NaIO_4$ has also found its use in organic chemistry for the oxidation of various compounds [19]. As Scheme 6 depicts, some of these compounds are boronate esters. These can be subjected to oxidative hydrolysis by $NaIO_4$, hence yielding the corresponding boronic acids [20, 21].



Scheme 6 – Arylboronate ester oxidative hydrolysis by $NaIO_4$

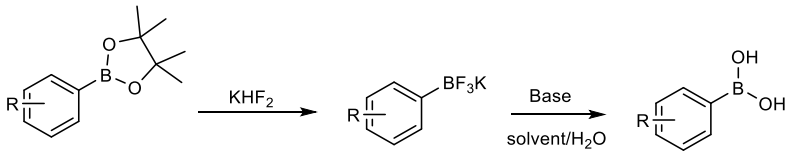
The beforehand dried boronate esters are dissolved in a 4:1 mixture of THF/H_2O and $NaIO_4$ is added. After the reaction is completed, an aqueous solution of HCl is added and the mixture extracted with ethyl acetate. The desired boronic acids are then isolated from the obtained organic phase [21].

C – Preparation of aryltrifluoroborates and their hydrolysis

As presented on Scheme 7, one possible pathway for the hydrolysis of boronate esters leads through the use of potassium bifluoride (KHF_2). This reagent is commercially available either as a liquid or a dry powder of colourless crystals, which are corrosive to tissue [22].

In a two-step procedure, the boronate esters are first converted into aryltrifluoroborates by exposition to KHF_2 as reported in the work of Vedejs *et al* [23], who investigated the conversion of arylboronic acids into aryltrifluoroborates by reacting their esters with KHF_2 in a 5:1 mixture of MeOH:H_2O [23]. This work was further modified by Genet et Darses [24] who explored the use of acids and esters without prior purification and the use of KHF_2 *in situ* in combination with classical methods of organic synthesis of arylboronic acids [24]. The herein obtained aryltrifluoroborate salts are crystalline [25] and in many cases precipitate from the reaction mixture, which makes them easy to filtrate [24]. In addition, recrystallization from acetonitrile or acetone/ethyl acetate allows the isolation of analytically pure compounds, for KHF_2 is insoluble in acetonitrile or acetone [24]. However traces of KHF_2 might be observed as contaminant when acetonitrile is used as a recrystallization solvent [24].

The second step consists of hydrolysing the obtained and isolated aryltrifluoroborate salts. This can be achieved either through aqueous basic hydrolysis or using trimethylsilylchloride $(CH_3)_3SiCl$ as a fluorophile [25]. An investigation conducted by Yuen and Hutton [25] revealed that LiOH is more effective than KOH or NaOH although all free bases were effective in converting the salts into corresponding boronic acids. Additionally, Na_2CO_3, Li_2CO_3 and K_2CO_3 were found as fairly effective bases with K_2CO_3 being less effective [25].

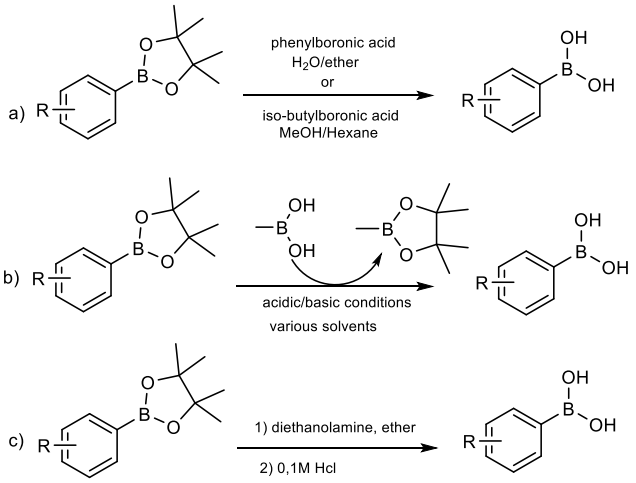


Scheme 7 – preparation of aryltrifluoroborates and their hydrolysis

D – Transesterification

The deprotection of boronic acid esters through hydrolysis does not always succeed. Also, when reagents such as $LiAlH_4$ or $NaIO_4$ prove too hazardous, and aryltrifluoroborate salts too unstable, an easier option is transesterification. As presented on Scheme 8, several transesterification methods have been reported in the literature such as byphasic transesterifications involving phenylboronic acid or iso-butylboronic acid [20, 26], a monophasic

transesterification involving methylboronic acid [15] and a two-step procedure introducing diethanolamine as a transesterification agent [27].



Scheme 8 - Transesterification methods – a) byphasic transesterification – phenylboronic or iso-butylboronic acid b) monophasic transesterification – methylboronic acid c) transesterification with diethanolamine and hydrolysis of intermediate DEA-protected product

One of the most important drugs synthesized by transesterification with another boronic acid is Bortezomib **5** (Fig. **2**) [28]. In general, the success of transesterification methods depends on the different solubility of boronate esters and the corresponding acids in two different solvents(ex. H_2O/ethyl ether , hence the term "byphasic") [15]. A possible solution to this challenge comes in the use of methylboronic acid, a semi volatile solid whose pinacol ester is an easily evaporated liquid with a boiling point of 120-122 °C under atmospheric pressure [15].

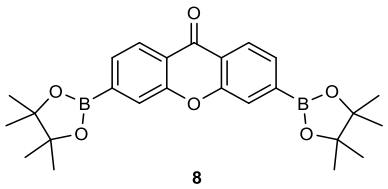
Another interesting method is the transformation of pinacol esters into DEA-protected acids by their exposition to diethanolamine at room temperature [27]. The formed salts are easily hydrolysed with an addition of aqueous HCl and the desired boronic acids are obtained in high yields [27].

1.3.3. Borylated xanthone derivatives

Xanthone derivatives are compounds that, besides their biological activities, exhibit fluorescence properties. These have made them potential compounds in the synthesis and study of fluorescent probes [29-31]. Among those derivatives figure also borylated xanthone derivatives or xanthone-boronic acids, synthesized as bis(pinacolatoboron) esters. One of these compounds, 3,6-bis(pinacolatoboron)xanthone (**3**, Figure 3) was studied as a potential probe for the detection of micromolecular changes in H_2O_2 in living cells and the further questioning of the physiology and pathology of cellular H_2O_2 [32]. The synthesis of 3,6-bis(pinacolatoboron)xanthone (**3**) was performed by palladium-catalysed transmetalation of 3,6-bis-triflate (**2**) with bis(pinacolato)diboron under Miyaura conditions [14] and delivered the product in 51% yield [32].

Although xanthone-boronic acids with biological applications have not been described to the best of our knowledge, several other arylboronic acid derivatives with promising biological

activities have been reported [12] . This is the case of chalcone-boronic acids, synthesized and studied for their toxicity towards breast cancer cells [12].

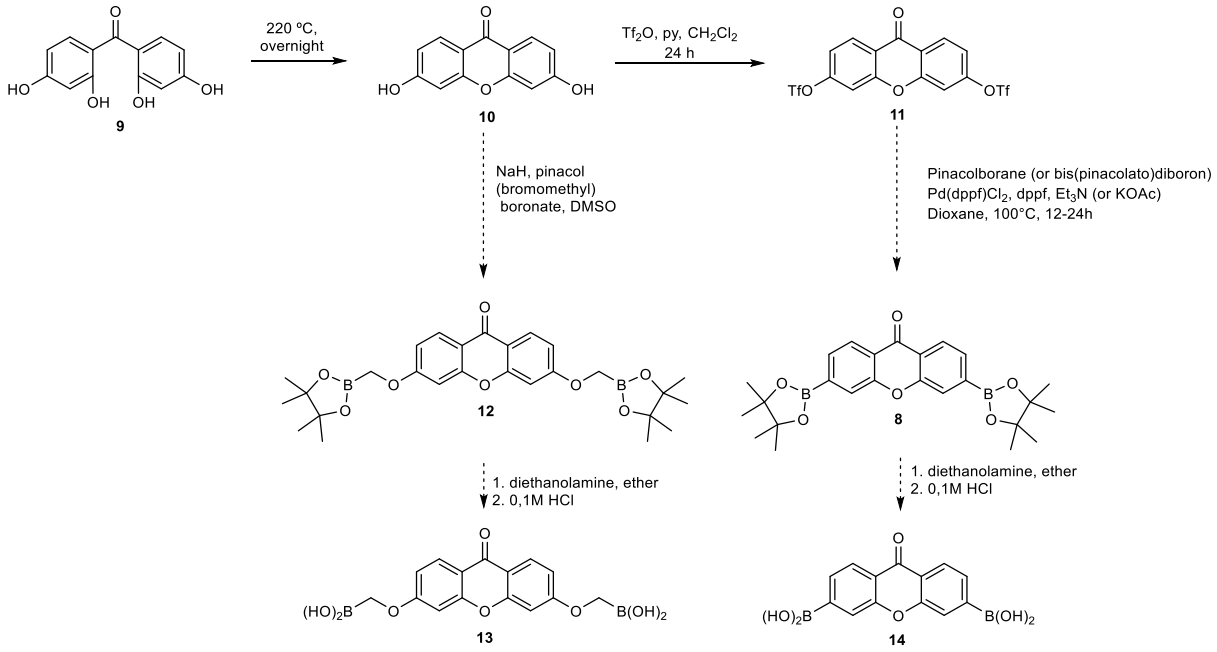


8

Figure 5 - 3,6-Bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9*H*-xanthen-9-one (**8**).

2. Aims

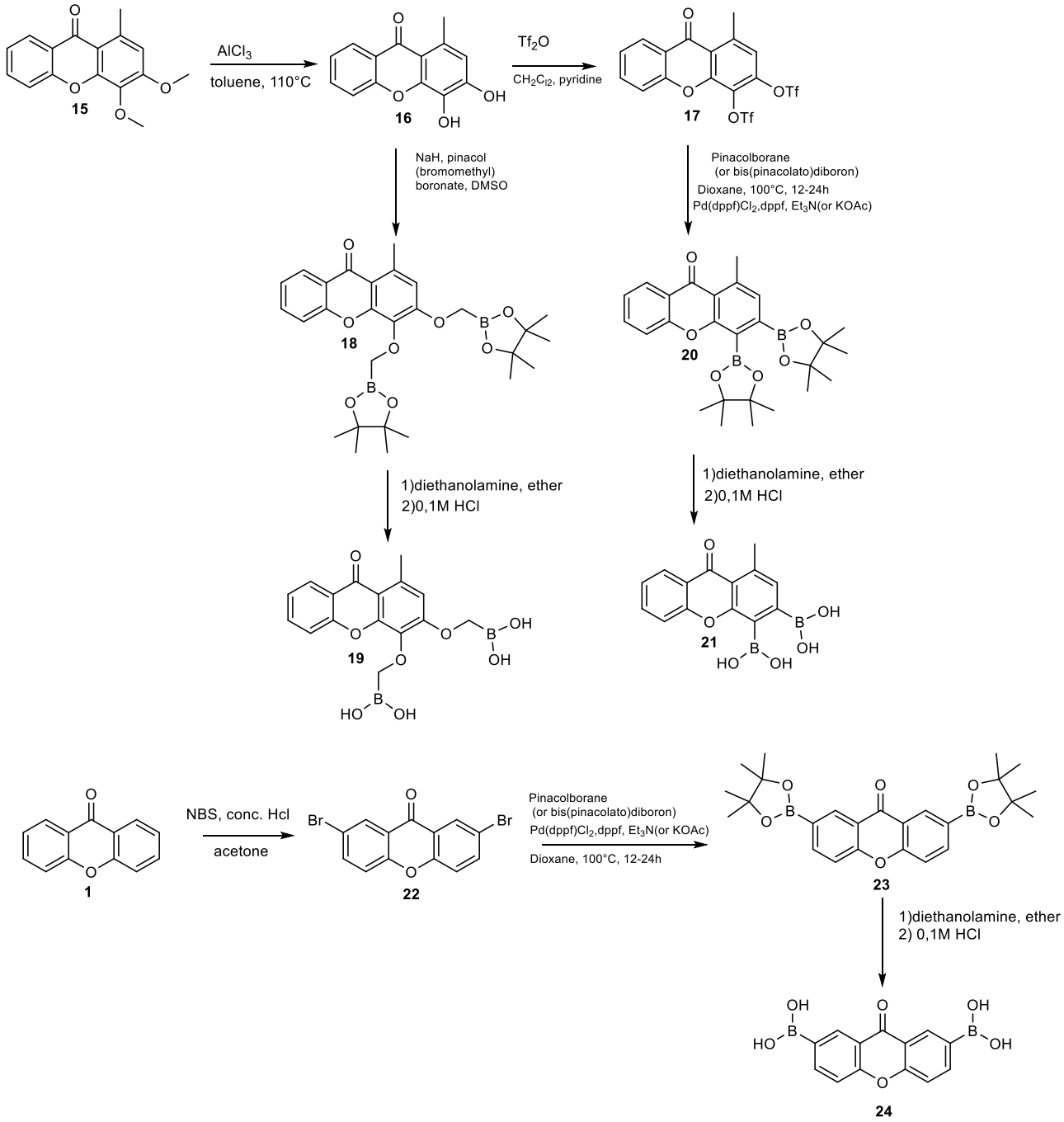
The main aim of this project was to synthesise two types of xanthone-diboronic acids, the xanthone-3,6-diboronic acid **14** and the (((9-oxo-9H-xanthene-3,6 diyl)bis(oxy))bis(methylene))diboronic acid **13** (Scheme 9).



Scheme 9 – Main objective – synthesis of compounds **13** and **14**.

The desired compounds were synthesised according to methods previously reported in the literature [32] or by adapting methods used for the borylation of similar aromatic compounds [14, 33, 34] to the borylation of xanthones.

Other objectives (Scheme 10) consisted in obtaining various precursors of xanthone-boronic acids for the study of structure activity relationship, such as 1-methyl-3,4-ditrifylxanthone **17**. In addition, the synthesis 2,7-dibromoxanthone **22** was attempted in order to compare the borylation on positions 2,7 to positions 3,6 and its influence on activity. Another objective was the structure elucidation of the synthesized compounds. Additional attention has been paid to the proposal of alternative routes of borylation and boronate ester deprotection.



Scheme 10 – Other objectives – synthesis of compounds **17** and **22** and borylation procedure proposal

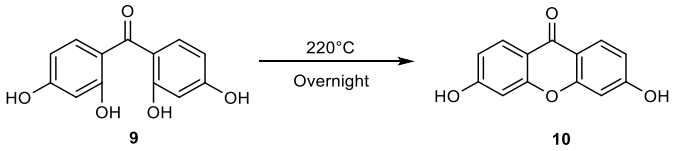
3. Results and Discussion

3.1. Reactions and reaction mechanisms

3.1.1. Synthesis of xanthone-3,6-diboronic acid **14**

3.1.1.1. Synthesis of 3,6-dihydroxyxanthone **10**

Scheme 11 represents the synthesis of 3,6-dihydroxyxanthone **10**

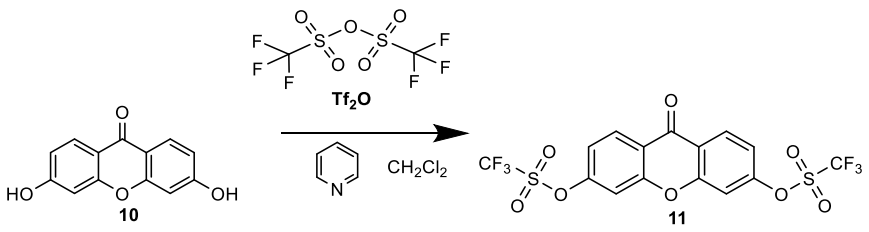


Scheme 11 – Synthesis of 3,6-dihydroxyxanthone **10**

The reaction procedure was adopted from the literature [35]. The synthesis of 3,6-dihydroxyxanthone **10** was possible by heating 2,2',4,4'-tretrahydrobenzophenone **9** in a furnace at 220 °C overnight and hence achieving a cyclodehydration in yields as high as 93 % [36]. The initially beige-coloured benzophenone turned into a light-brown compound, signalling the occurrence of a reaction. The Thin Layer Chromatography (TLC) plate showed no starting material or impurity, and a spot identical to that of a previously synthesised 3,6-dihydroxyxanthone **10**. The structure of the product and its identity was further confirmed by ^{1}H NMR (see Figure 7).

3.1.1.2. Synthesis of 3,6-ditrifylxanthone **11**

The synthesis of 3,6-ditrifylxanthone **11** was achieved following a procedure previously reported in the literature [32]. First, 3,6-dihydroxyxanthone **10** was dissolved in dichloromethane (DCM) and pyridine was added slowly at 0 °C. The reaction mixture was stirred for 5 min and then trifluoromethanesulfonic anhydride (Tf_2O) was added dropwise over 10 min. The addition of Tf_2O is followed by the formation of fumes and possibly a green-coloured precipitate. The reaction was then warmed to room temperature and left to react for 24 hours (Scheme 12)

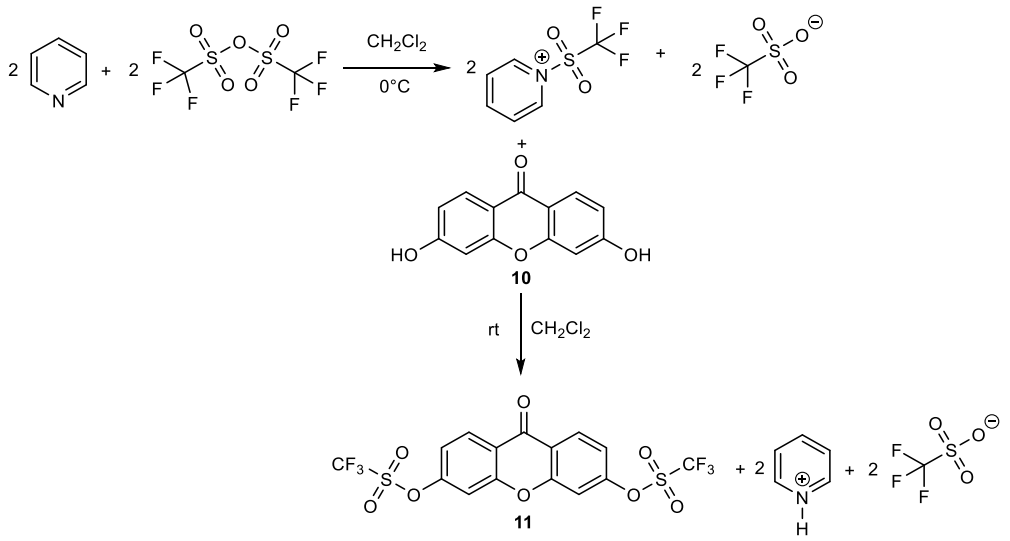


Scheme 12 - Synthesis of 3,6-ditrifylxanthone **11**

Trifluoromethansulfonic anhydride (Tf_2O) or triflic anhydride is a colourless liquid stored and manipulated preferably under N_2, widely used for the preparation of triflates [37]. At 0 °C, in the presence of a base (mostly pyridine), in an inert solvent (DCM), this reagent reacts with alcohols and phenols to produce the corresponding trifluoromethanesulfonate esters (triflates) [37]. The

reaction begins with the combination of Tf_2O with pyridine to form a pyridinium salt, which then reacts with alcohols to give the appropriate triflates (Scheme 13). Although this salt precipitates from the reaction mixture, it is reactive enough to be an effective esterifying agent [37].

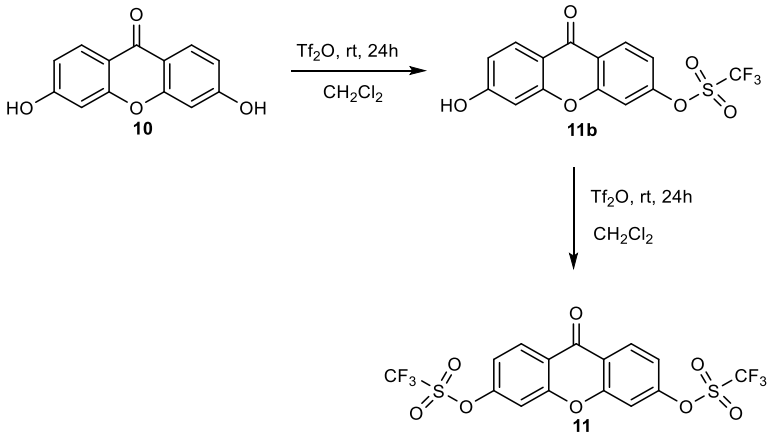
This general mechanism can be applied to the synthesis of 3,6-ditrifylxanthone **11** from 3,6-dihydroxyxanthone **10** and summed up in Scheme 13:



Scheme 13 – Synthesis of 3,6-ditrifylxanthone **11** reaction mechanism- Tf_2O combines with pyridine to form a pyridinium salt, which reacts with **10** to give **11** in high yields.

The reaction progress and completion were monitored by TLC using a mobile phase of acetone:$CHCl_3$ 2:8. The identity of the desired product **11** on the plate was confirmed by using a sample of a previously synthesised 3,6-ditrifylxanthone **11**. Upon completion, the reaction mixture is quenched with water and the organic layer is washed with water, acidified with 1N HCl, washed with brine and dried over Na_2SO_4. The solvent (DCM) is removed under reduced pressure to give a brown solid. The obtained solid was purified by column chromatography (acetone:chloroform 1:9) to give a pure white product (**11**, 81% yield). It has been further observed that this mobile phase is too polar, and a less polar phase (CH_2Cl_2 7:3 *n*-hexane) is preferable for the purification of this product.

Given its mechanism and the presence of two phenol groups on the 3,6-dihydroxyxanthone (**10**), the reaction could possibly result in the formation of 3-trifyl-6-hydroxyxanthone **11b** (Scheme 14). This was avoided using 3 equivalents of Tf_2O. Hence, no side product has been observed nor isolated.



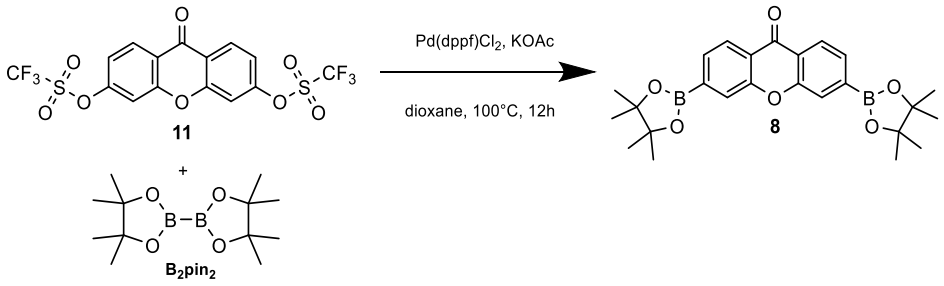
Scheme 14 – Synthesis of 3,6-ditrifylxanthone possible side product **11b**

In order to verify the absence of side products or impurities, a sample of **11** was taken and its spot was concentrated on a TLC plate. The plate was eluted (CH_2Cl_2:*n*-hexane 7:3) and no spots other than those of starting material and product were observed. Finally, the plate was sprayed with a MeOH solution of $FeCl_3$ (see 3.2.1.1). No coloration disclosing a phenol (Ar-OH) group was observed [38]. The identity of the desired product 3,6-ditrifylxanthone (**11**) was further assessed by ^{1}H NMR (see Figure 9)

3.1.1.3. Synthesis of 3,6-bis(pinacolatoboron)xanthone **8**

Method A - bis(pinacolato)diboron B_2pin_2

Scheme 15 represents the synthesis of 3,6-bis(pinacolatoboron)xanthone **8** with the use of bis(pinacolato)diboron B_2pin_2 as a borylation agent.

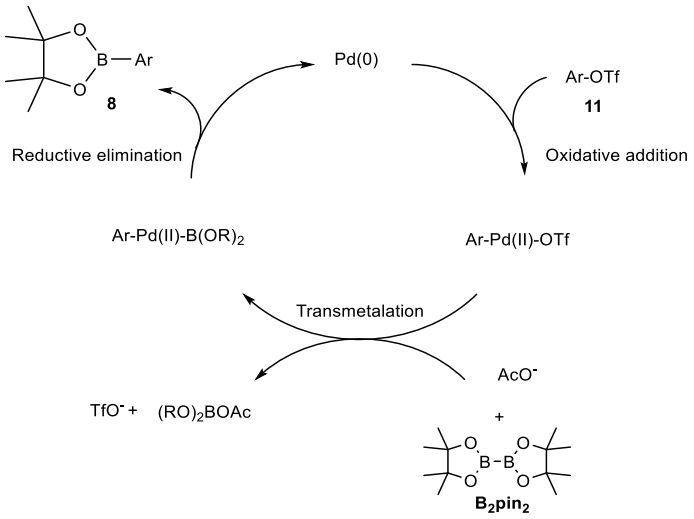


Scheme 15 – Synthesis of 3,6-bis(pinacolatoboron)xanthone **8** using bis(pinacolato)diboron B_2pin_2

Bis(pinacolato)diboron B_2pin_2 is a white/colourless powder soluble in organic solvents. B_2pin_2 is thermally stable and is not moisture sensitive, which makes it a reagent easily handled in air and suitable for palladium catalysed cross-coupling reactions [14]. The reaction involving B_2pin_2 as a borylation agent, $PdCl_2$(dppf) as a catalyst and KOAc as a base has been reported in the literature for the borylation of aryl halides or triflates [14] as well as for the synthesis of 3,6-bis(pinacolatoboron)xanthone **8** [32]. This reaction requires strictly anhydrous conditions, also all the glass (25 ml or 50 ml Schlenk flask, beakers, funnels...) and used material has been washed and dried in an oven overnight and left to cool down in a desiccator. The starting material **11** was dried overnight under vacuum at 40 °C. Before the reaction, the Schlenk flask was reassembled inside a

fume hood, all air was removed using a Buchi type vacuum pump and the flask was filled with nitrogen (x3 times). This step was repeated every time the anhydrous area was broken. Inside the flask were combined bis-triflate **11**, bis(pinacolato)diboron (B_2pin_2), $Pd(dppf)Cl_2.CH_2Cl_2$, dppf, KOAc and anhydrous dioxane. The reaction mixture was stirred for 5 min at room temperature, slowly heated to 100 °C and left to react for 12 h.

The reaction mechanism may comprise a catalytic cycle [39] as displayed in the following Scheme 16:



Scheme 16 – Proposed mechanism for the synthesis of 3,6-bis(pinacolatoboron)xanthone **8**, method A (B_2pin_2)

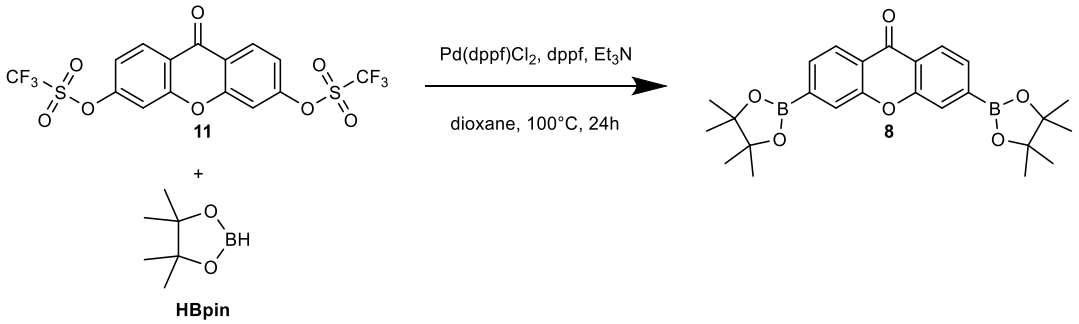
The catalytic cycle begins with the oxidative addition of **11** to the palladium(0) complex and the formation of a ArPd(II)OTf intermediate [14]. This intermediate proceeds to the transmetalation with B_2pin_2 in the presence of KOAc to produce a second intermediate, Ar-Pd(II)$B(OR)_2$ which finally provides **8** by reductive elimination and thus regenerates the palladium(0) complex [14]. The base KOAc accelerates the reaction and prevents the formation of biaryl byproducts [14]. The addition of the ligand (dppf) has been reported to increase the reaction yields [40]. It is worth noting that the reaction is accelerated in polar solvents (DMSO>dioxane>toluene) [14]. The reaction progress and completion were monitored by TLC (2% $MeOH/CH_2Cl_2$). 3 drops of HCOOH can be added to the mobile phase for a far better division. The presence of a product was after observation under UV light (245 nm and 365 nm) further proved with the use of a curcumin dip (3.2.1.2).

After completion, the reaction was cooled to room temperature, diluted with toluene, and washed three times with brine. The organic layer was then dried over Na_2SO_4 and the solvent was removed under vacuum by rotary evaporation to leave a brown residue. The brown residue may be washed with cold MeOH to yield pure boronate as a white powder. However, the efficacy of this step is debatable, as the ability of MeOH to wash off the brown impurities depends on the success of the workup. In addition, it has been observed that the boronate tends to dissolve in MeOH at room temperature. Recrystallization of the brown residue from MeOH provides white crystals of the desired product **8**. But a longer period (days) is required for the crystals to fully appear. Hence, preparative chromatography might be preferable for a more effective purification. The product **8** was however isolated in 11% yield.

The desired product was further characterised by ^{1}H NMR (see 3.2. structure characterisation)

Method B – pinacolborane (HBpin)

Scheme 17 represents the synthesis of 3,6-bis(pinacolatoboron)xanthone **8** with the use of pinacolborane (HBpin) as a borylation agent.



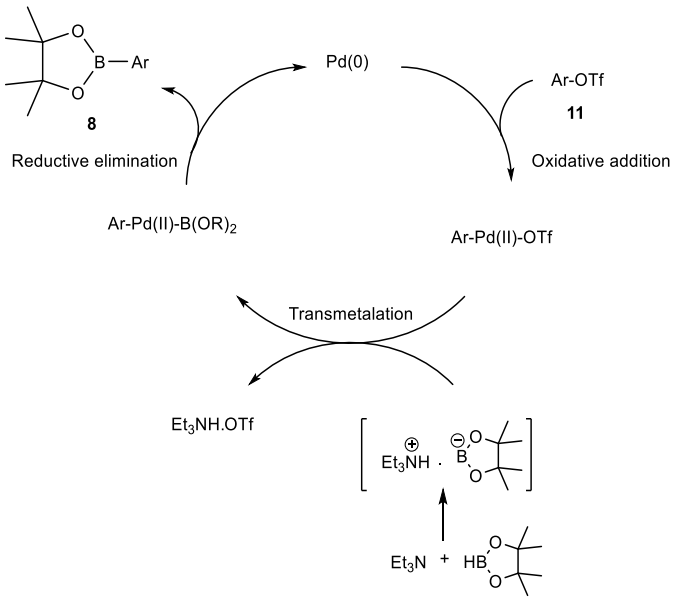
Scheme 17 - Synthesis of 3,6-bis(pinacolatoboron)xanthone **8** with the use of pinacolborane (HBpin)

Pinacolborane (HBpin) is a colourless and odourless liquid stored at lower temperatures (in the fridge). The borylation procedure is obtained by adapting procedures previously reported in the literature for the borylation of aryl halides with the use of pinacolborane [33, 34].

The overall approach of synthesis is parallel to the one adapted for the use of B_2pin_2 (anhydrous conditions, nitrogen atmosphere, dry material…). In a 25 ml Schlenk flask in a nitrogen atmosphere were combined bis-triflate **11**, Pd(dppf)Cl_2, dppf and dioxane and the mixture was stirred for 5 minutes at room temperature. Then were added dropwise HBpin and Et_3N, and the mixture was heated to 100 °C and left to react for 24 h.

The reaction progress was monitored by TLC and the reaction was stopped after no starting material was detected. The reaction was then cooled to room temperature, quenched with water, extracted with CH_2Cl_2, washed with brine, and dried over Na_2SO_4. The organic solvent was evaporated, and a brown residue was obtained. The residue was adsorbed on silica and passed through a small column (2% MeOH/CH_2Cl_2 + HCOOH) to separate the more polar compounds from the less polar ones. After checking with a curcumin dip, the presumed fractions containing the product were gathered and purified by preparative chromatography (100% CH_2Cl_2) and five bands were obtained, of which two are isolated: one band that is theorised as a complex formed during the reaction (this complex shows a stain with curcumin only after elution with a mobile phase acidified by HCOOH) and one band theorised as the desired product (shows a stain with and without HCOOH). The two isolated bands were extracted from silica and compounds (yields, mg) are further characterised by ^{1}H NMR (see 3.2.).

The proposed reaction mechanism can be summed in the following Scheme 18 [34]:

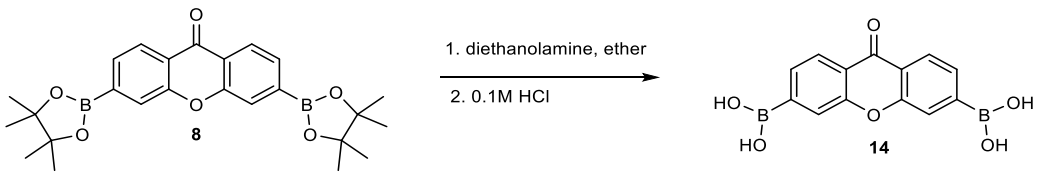


Scheme 18 – Proposed mechanism for the synthesis of 3,6-bis(pinacolatoboron)xanthone **8**, method B (HBpin)

The catalytic cycle begins with the oxidative addition of **11** to the palladium(0) complex and the formation of a ArPd(II)OTf intermediate [34]. This intermediate proceeds to the transmetalation with HBpin in presence of Et_3N to produce a second intermediate, Ar-Pd(II)B$(OR)_2$ which finally provides **8** by reductive elimination and thus regenerates the palladium(0) complex [34]. An attempt at this reaction with KOAc instead of Et_3N resulted mostly in the formation of xanthone (**1**). The success of the reaction highly depends on the maintained temperature (100 °C), solvent polarity (DMSO>dioxane>toluene), reaction time (24-48 h or even more) and the ratio base: borylation agent. Although the reaction was started with a ratio Et_3N/HBpin 1.2: 1, the reaction was not complete until 1eq of HBpin and 2eq of Et_3N were added. In addition, the presence of the complex intermediate and lower reaction yield after the reaction was stopped lead to believe a longer reaction time is necessary.

3.1.1.4. Deprotection of 3,6-bis(pinacolatoboron)xanthone **8** to xanthone-3,6-diboronic acid **14**

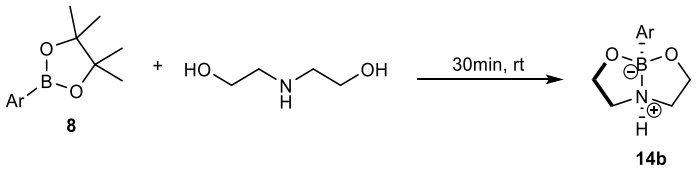
Scheme 19 represents the deprotection of 3,6-bis(pinacolatoboron)xanthone **8** to xanthone-3,6-diboronic acid **14**:



Scheme 19 – Deprotection of 3,6-bis(pinacolatoboron)xanthone **8**

The deprotection method is adapted from a procedure previously reported in the literature for the deprotection of alkylpinacolyl boronate esters [27].

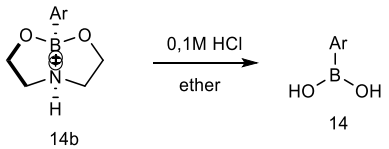
During the first step, 3,6-bis(pinacolatoboron)xanthone **8** was combined with 2.5 eq of ethanolamine in ether and stirred for 30 min at room temperature. After a few minutes, a white precipitate formed. The reaction was monitored by TLC after 30 minutes. The reaction progress and completion were marked by the progressive disappearing of **8** from the plate. If the reaction does not complete, it is possible to add 1eq of diethanolamine and keep stirring until no **8** is detected. The disappearance of **8** and apparition of a white precipitate marks the transesterification of **8** with diethanolamine to form a sp^3-hybridized boron.DEA adduct **14b** [27], as showed in the following Scheme 20:



Scheme 20 – Boronic ester transesterification with diethanolamine

After the reaction is complete, the white precipitate should be, according to the literature [27], filtered and washed with ether to yield pure **14b**. However, in the case of **8**, if diethanolamine was added in excess, the precipitate resulted in a wet compact mass. It was nevertheless possible to wash this mass with ether which was then removed carefully. The obtained mass can either be purified, isolated and dried, or used for the next step. In this work, it was directly used for the second step without purification.

During the second step, DEA-boronate **14b** was stirred in a byphasic solution of 0.1 M HCl in ether. The reaction was monitored by TLC and its progress marked by the apparition of a spot detectable by curcumin dip. After 30 min -1h the reaction was completed as judged by TLC, and the crude product extracted with ether (3×), washed with brine (1×) and dried with Na_2SO_4. The organic solvent was then evaporated under reduced pressure to provide the analytically pure product **8** as a white solid. The identity of the product was further assessed by ^{1}H NMR. (Figure 13). This step can be summed up in the following Scheme 21:



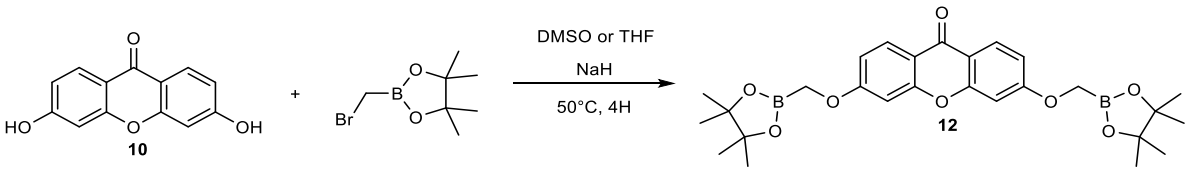
Scheme 21 – DEA-boronate **14b** hydrolysis

Due to the presence of two -$B(OH)_2$ groups, 1**4** dissolves poorly in ether. This issue was solved by replacing ether with acetone, a more polar solvent, and by increasing the aqueous acidic phase during the reaction. The compound **14** was isolated in 54% yield.

3.1.2. Synthesis of (((9-oxo-9*H*-xanthene-3,6-diyl)bis(oxy))bis(methylene))diboronic acid **13**

3.1.2.1. Synthesis of 3,6-bis((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methoxy)-9H-xanthen-9-one **12**

Scheme 22 represents the synthesis of **12** starting from **10** using pinacol(bromomethyl)boronate:



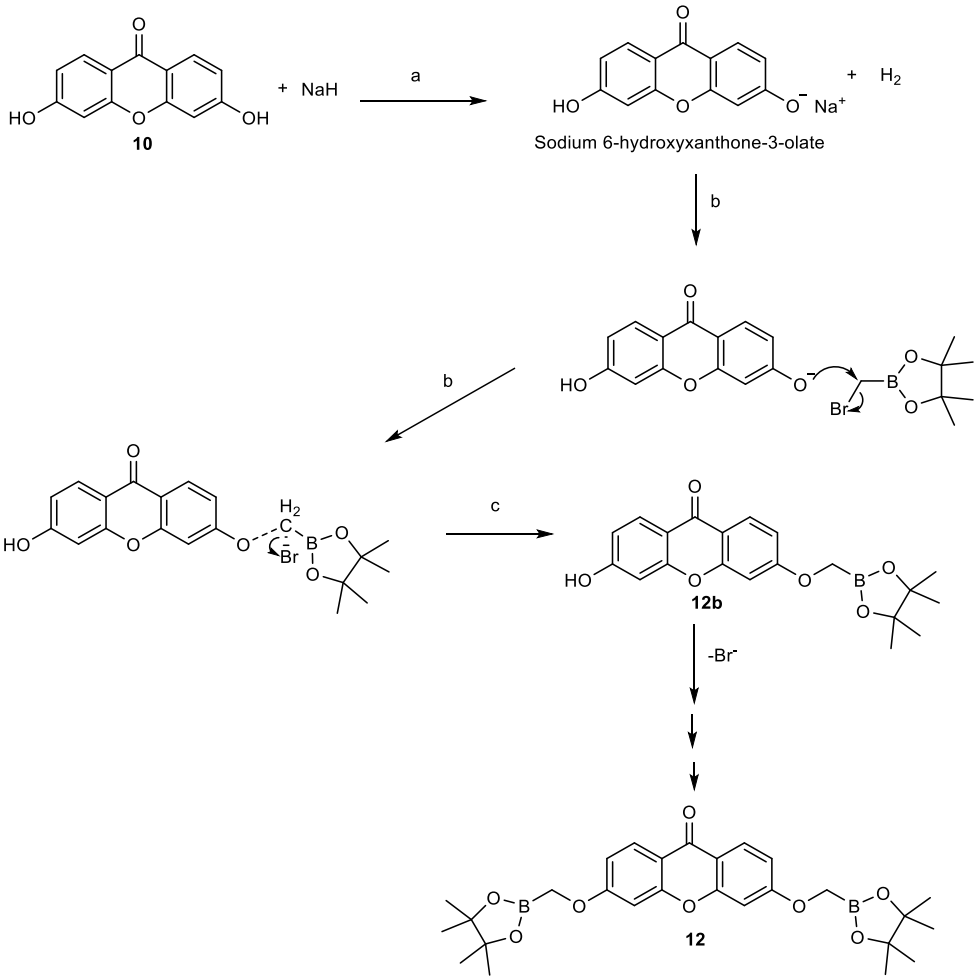
Scheme 22 – Synthesis of 3,6-bis((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methoxy)-9H-xanthen-9-one **12**

Pinacol(bromomethyl)boronate is a colourless liquid stored at lower temperatures(fridge) [41]. Although stable and relatively safe, this reagent is a lacrymator and should be manipulated in a fume hood [42]. Skin contact should be avoided [42].

Sodium hydride (NaH) is a grey powder available commercially in its dry state or dispersed in mineral oil [43]. Although stable in dry air, NaH is susceptible to decompose violently in contact with air moisture, with risks of ignition. Any manipulation should be done carefully in the fume hood using dry material and all weighing material (spoons, pipettes..) should be quenched in MeOH or another appropriate alcohol before disposal [43]. NaH is a strong base widely used in organic chemistry to deprotonate weak Bronsted acids (Ar-OH, -SH) or as a reducing agent [43].

All the glass and used material was washed with acetone, dried in an oven overnight, and left to cool down in a desiccator prior to reaction. The starting material **10** was left to dry at 40 °C under reduced pressure. The base and the reagent were used without additional treatment. In a 25 ml Schlenk flask, after it was vacuumed and filled with nitrogen (strictly anhydrous conditions) were combined 3,6-dihydroxyxanthone **10,** DMSO (or THF in a different attempt) and NaH at room temperature. After stirring for 15 min, pinacol(bromomethyl)boronate was added, the mixture was slowly heated to 50 °C and left to react for 4 h. The reaction progress was monitored by TLC (2%MeOH/CH_2Cl_2, EtOAc:*n*-hex 9:1 or MeOH:CH_2Cl_2 9:1) and the presence of a borylated product was verified using a curcumin dip. After 4 h no further evolution was observed despite the presence of starting material, hence 1eq of pinacol reagent was added and the reaction was stirred for another 4 h.

Given the properties of the used reagents and solvents, the proposed reaction mechanism is a S_N2 nucleophilic substitution [44], as displayed in the following Scheme 23:

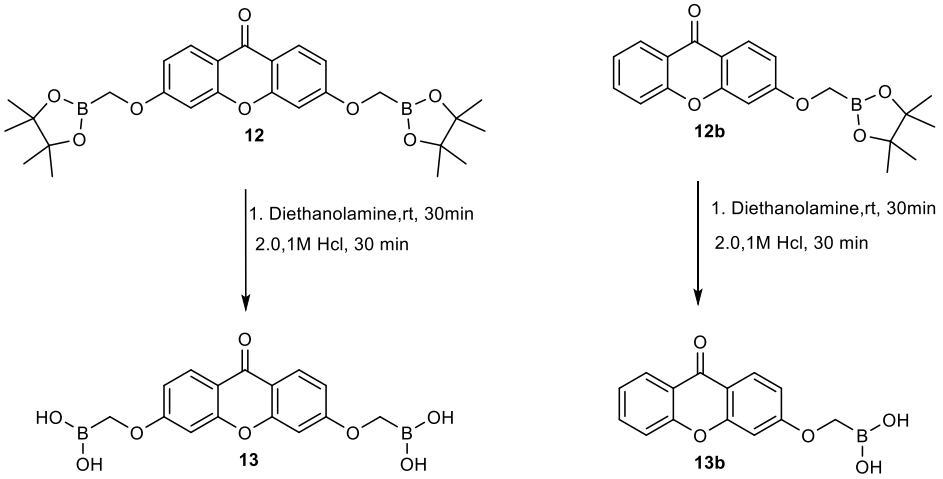


Scheme 23 - Synthesis of 3,6-bis((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methoxy)-9H-xanthen-9-one **12** – proposed reaction mechanism

First, NaH attacks the phenol group of **10**, deprotonating it into the analogous phenolate sodium-6-hydroxyxanthone-3-olate. This phenolate then attacks the pinacol(bromomethyl)boronate reagent, binding onto the methylene group just as the Br^- group leaves. This results into the formation of the intermediate **12b**, which further reacts to provide the desired product **12**.

After completion, the reaction was quenched with water, extracted with ethyl acetate and the organic layers were washed with brine and dried over Na_2SO_4. The organic layer was concentrated under reduced pressure and the concentrate was purified by preparative chromatography (10 plates, MeOH:*n*-hexane 7:3 + HCOOH). 3 fractions were obtained, of which two were sent for NMR and only one revealed a borylated product, this being the **12b** intermediate (3.2.).

The attempt to replace DMSO with THF led to difficulties to dissolve starting material and reagents in the mixture, as well as a harder workup. The compound **12b** was isolated in 35% yield. This compound, as well as the originally intended compound **12**, could be subjected to the transesterification method involving diethanolamine used to deprotect **8** into **14**, in an attempt to obtain **6** as portrayed by Scheme 24. In case of difficulties or failure, it is possible to try the transesterification with methylboronic acid [15].

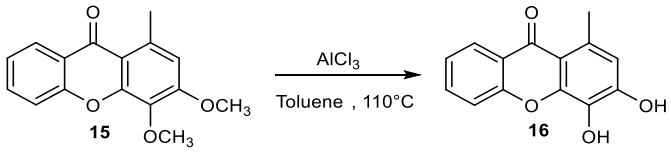


Scheme 24 – Proposal for the deprotection of **12** (**12b**) into **13** (**13b**)

3.1.3. Synthesis of precursors of 1-methylxanthone-3,4-diboronic acid **21**

3.1.3.1. Synthesis of 3,4-dihydroxy-1-methylxanthone **16**

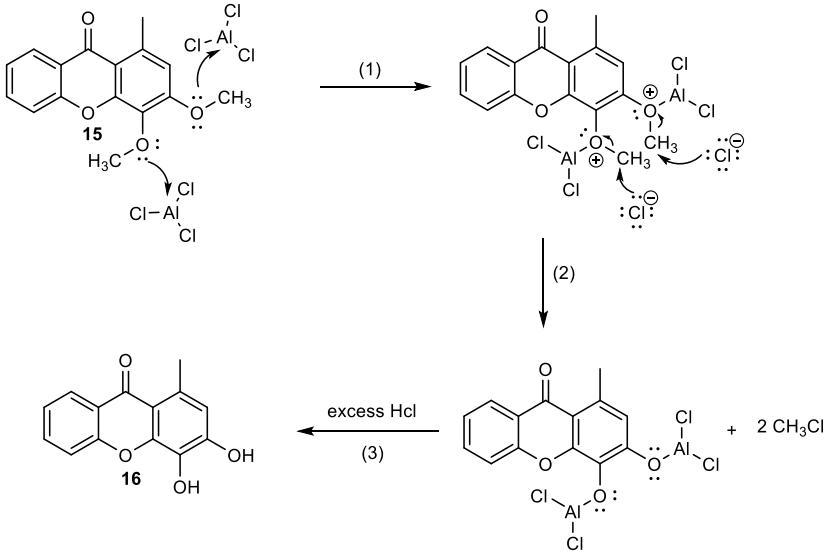
Scheme 25 represents the synthesis of 3,4-dihydroxy-1-methylxanthone **16** starting from 3,4-dimethoxy-1-methylxanthone **15**, previously synthesised in the laboratory.



Scheme 25 – Synthesis of 3,4-dihydroxy-1-methylxanthone **16**

The deprotection of 3,4-dimethoxy-1-methylxanthone **15** into 3,4-dihydroxy-1-methylxanthone **16** was achieved following a procedure previously reported in the literature [45]. The starting material was dissolved in dry toluene and aluminium chloride $AlCl_3$ was carefully added. The reaction mixture was stirred and heated to 110 °C under reflux. The evolution of the reaction was monitored by TLC ($CHCl_3$:MeOH 9:1). After completion, the reaction was cooled to room temperature, excess conc. HCl was added, and the mixture was extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to provide a dark-brown residue. The acquired residue was purified by preparative chromatography ($CHCl_3$: MeOH 9:1). The obtained product's identity was verified by comparison to a previously synthesised compound and by ^{1}H NMR. The reaction yield was 54%.

The reaction mechanism can be summed up in the following Scheme 26 [46]:

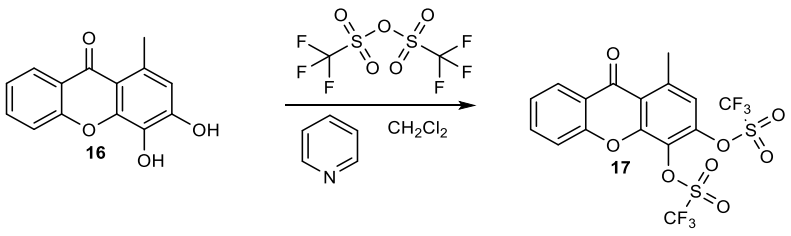


Scheme 26 – Proposed reaction mechanism of 3,4-dimethoxy-1-methylxanthone **15** deprotection

As the scheme shows, the presence of $AlCl_3$ is essential, for the free electron pair of the methoxy group to bind to the central Al atom, leading to the departure of Cl^- [46]. Due to its nucleophilic nature, the chlorine ion binds to the methyl group and hence produces methylchloride as a side product, next to the of Ar-O-$AlCl_2$ complex. While being detectable by TLC (top stain), this complex disappears after reaction workup, for excess HCl decomposes it into 3,4-dihydroxy-1-methylxanthone **16**. The anhydrous atmosphere achieved by presence of N_2 is important, as it reduces the contact of $AlCl_3$ with air moisture and its transformation into unreactive $Al(OH)_3$ [46]. The presence of the desired product after workup can besides the absence of complexes be verified by staining the TLC plate with a 10% MeOH solution of $FeCl_3$.

3.1.3.2. Synthesis of 3,4-trifyl-1-methylxanthone **17**

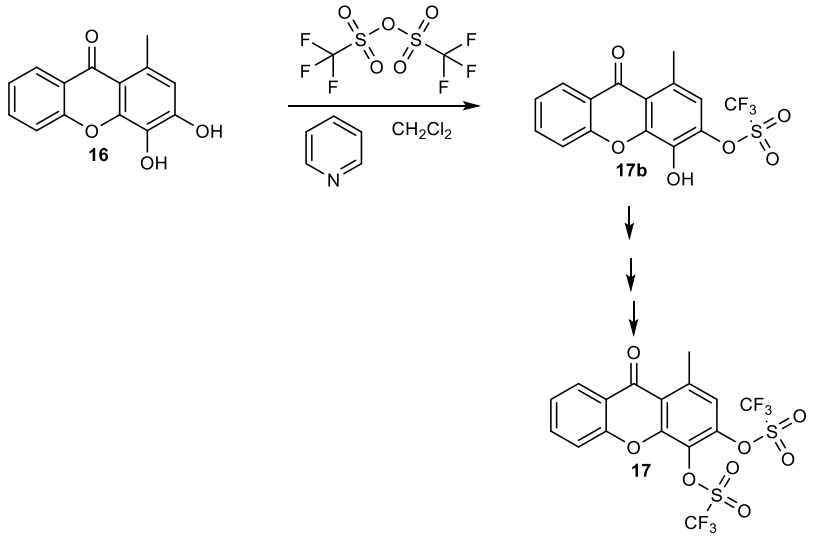
The synthesis of 3,4-trifyl-1-methylxanthone **17** was attempted by reproducing the procedure used for the synthesis of 3,6-ditrifylxanthone **11** (see 4-1-2).



Scheme 27 – Synthesis of 3,4-trifyl-1-methylxanthone **17**

3,4-Dihydroxy-1-methylxanthone **16** was dissolved in DCM and pyridine was slowly added over 5 min at 0 °C. The mixture was stirred for 10 min and Tf_2O was added. The reaction was stirred for 24 h at room temperature and monitored by TLC. After no evolution was detected, the reaction was quenched with water, and the organic phase was washed with water (1x15ml), 1N HCl (3x15ml), brine(1x15ml) and dried over Na_2SO_4. The organic solvent was removed under reduced pressure to provide a brown residue. A TLC plate sprayed with a 10% MeOH solution of $FeCl_3$

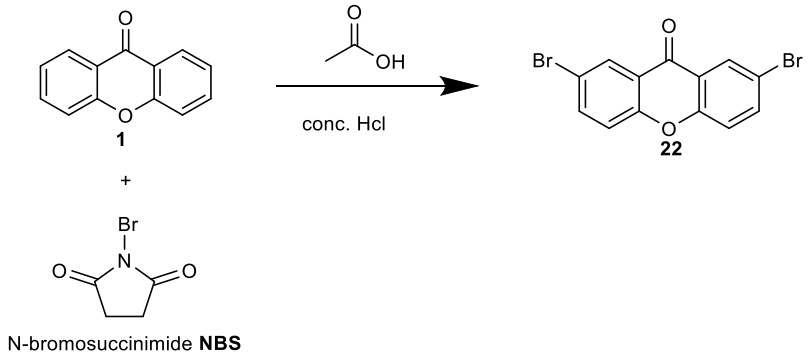
revealed that out of two spots theorised as products, only one contains a free phenol group. Considering this information, the residue was purified by preparative chromatography (7 plates, CH_2Cl_2: *n*-hexane 7:3) and two fractions were obtained. The main fraction (34% yield) was identified by ^{1}H NMR as a side product, 3-trifyl-4-hydroxy-1-methylxanthone (**17b**, Scheme 28). The second fraction (10% yield) showed no stain with $FeCl_3$, and although the obtained ^{1}H NMR was impure, this fraction could represent the desired product. From this information it was concluded that the reaction was incomplete, and it required either more reaction time as 3 equivalents of Tf_2O were used to avoid the occurrence of side products. The higher fraction of side product **17b** could also be due to the size of the triflate substituent and its steric hindrance to further substitution in *ortho* position.



Scheme 28 – Synthesis of 3,4-trifyl-1-methyl-xanthone **17** and possible side product **17b**

3.1.4. Synthesis of 2,7-dibromoxanthone **22**

The synthesis of 2,7-dibromoxanthone **22** as attempted by adapting methods previously reported in the literature for aromatic halogenation [47, 48].

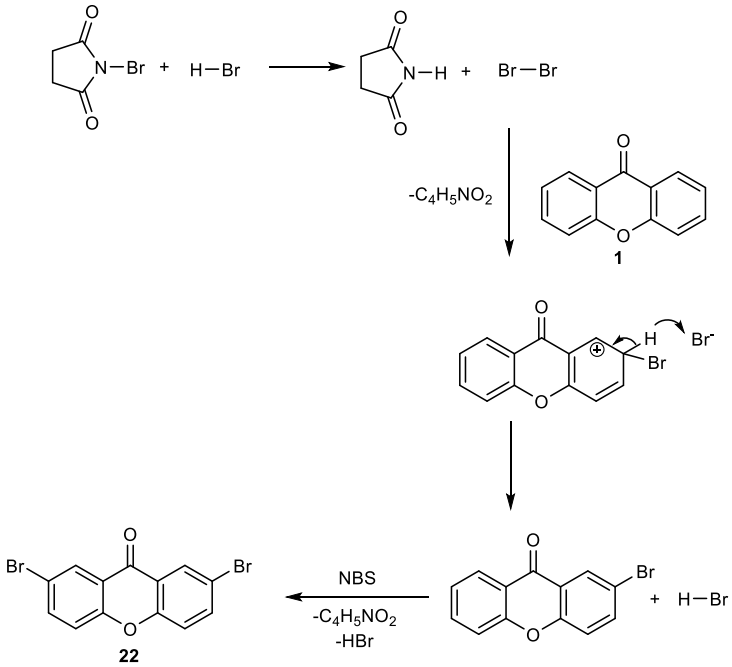


Scheme 29 – Synthesis of 2,7-dibromoxanthone **22**

A suspension of xanthone **1** and NBS was prepared in acetic acid, to which HCl was added dropwise after 5 min. The reaction was stirred at room temperature for 12 hours, a period during which the orange coloration provided by the addition of HCl disappeared, and a white precipitate

was formed. After this period, the TLC plates (CH_2Cl_2:*n*-hexanes 5:5) revealed a certain product, but no more progress and the presence of a considerable amount of starting material. Hence, 1 more equivalent of NBS, HCl and acetic acid was added, and the reaction was stirred for another 12 h.

N-Bromosuccinimide or 1-bromopyrrolidine-2,5-dione is a white solid with a slight odour of bromine, used in organic chemistry for various reactions such as allylic bromination (Wohl-Ziegler reaction), aromatic ring and side-chain bromination or oxidation [49]. During these reactions, NBS provides a constant, low concentration of Br_2, this being catalysed by HBr which is usually present in NBS in small amounts [50]. This release as well as the following reaction between Br_2 and xanthone can be summed up in the following Scheme 30:



Scheme 30 – Proposed reaction mechanism for the synthesis of 2,7-dibromoxanthone **22**

After its release from NBS, Br_2 reacts with xanthone by aromatic electrophilic substitution [51]. In a first step, the Br^+ electrophile part of Br_2 attacks the aromatic ring, forming a carbocation intermediate. Next, this intermediate deprotonates under the influence of the Br^- basic part of Br_2, forming HBr as a side product and the brominated xanthone. It is worth noting that the addition of HCl decreases the reaction time by promoting the release of Br_2 [47]. After the reaction showed no more progress, it was quenched with water and the precipitated solid was filtered. The obtained white solid was then dissolved in CH_2Cl_2, washed with water, and dried with Na_2SO_4. The solid was next purified by preparative chromatography (22 plates, CH_2Cl_2:*n*-hexanes 1:1). This provided 3 fractions that were sent for ^{1}H NMR. Fraction 1 (revealed to most likely 2-chloro-7-bromoxanthone or a similarly halogenated side product. Fraction 2 revealed to be a mixture of compounds, whereas fraction 3 was starting material.

Fraction 2 was further purified by preparative chromatography, which revealed a mixture of around 5 different fractions, of which the main was isolated and sent for ^{1}H NMR.

3.2. Structure characterisation

3.2.1. TLC stain tests

3.2.1.1. 10% MeOH solution of $FeCl_3$

Phenols react with $FeCl_3$ to form complexes of various colours (purple, blue, red-brown…) [38]. The structure of the formed complex has long been debated [52]. However, the reaction can be represented by the following equation [52]:

$$C_6H_5OH + FeCl_3 \text{ ----------> } (Fe\ (OC_6H_5)_6)^{3-} + 3H^+ + 3HCl$$

This allows a 10%MeOH solution of $FeCl_3$ to be used as a stain test for the detection of phenol groups on TLC. The method consists of spraying the TLC plate inside a fumehood with the prepared solution. The stains appear 3-5 seconds after spraying. The apparition of a stain proves the presence of phenol groups, whereas various colours of the stain may indicate differently substituted compounds.

3.2.1.2. Curcumin dip

The curcumin dip is a solution prepared by dissolving 100 mg of curcumin in a 100 mL solution of ethanol with 2 N HCl (99:1 v/v) [53].

Curcumin is a chemical produced by *Curcuma longa*, a plant from the family *Zingiberaceae*. Also called "Indian solid gold", it is the principal chemical component of turmeric (a spice widely used in the Indian subcontinent) to which it gives its yellow colour [54]. In terms of chemistry, curcumin is a diarylheptanoid from the group of curcuminoids existing in solutions as keto-enol tautomers (Figure 6) [55]. Curcumin combines with boric acid $B(OH)_3$ to form rosocyanine, a 2:1 complex of bright red colour in solutions (Figure 6) [53]. This provides a reliable colourimetric method of detection of boron in waters and solutions [53]. A similar complex is formed when curcumin combines with boronic acids and their derivatives, allowing an acidified ethanol solution of curcumin to be used as a TLC-staining dip [53].

In order to use the dip, the sample containing the desired boronic acid or its derivative (in our case xanthone-boronic acid or its ester) was placed on a TLC plate using a glass spotter. The plate was eluted in a suitable mobile phase, submerged in the curcumin dip for 5-10 seconds and left to dry in an oven for another 10 seconds. An observation of the spots under UV light at 245 nm and 365 nm is recommended prior to the use of the dip. The orange-coloured spots of various intensity appeared progressively as the plate dried. The curcumin dip was successfully used to monitor the progress of the borylation reactions and ester deprotections. Another variant was the use of the curcumin solution as a spray for the detection of borylated compounds during preparative chromatography.

To reduce the consumption of curcumin for the preparation of the curcumin dip, the initial solution (100 mg/100 ml) was diluted. Concentrations as low as 6.25 mg/ml still provided orange-coloured stains. However, a more concentrated solution (25-50 mg/100 ml) might be preferable for the detection of borylated compounds in lower concentrations. After preparation, the curcumin dip was simply replenished with ethanol and drops of HCl as the solution diminished or the ethanol

solvent evaporated. This option represents another reason for initially preparing a more concentrated dip.

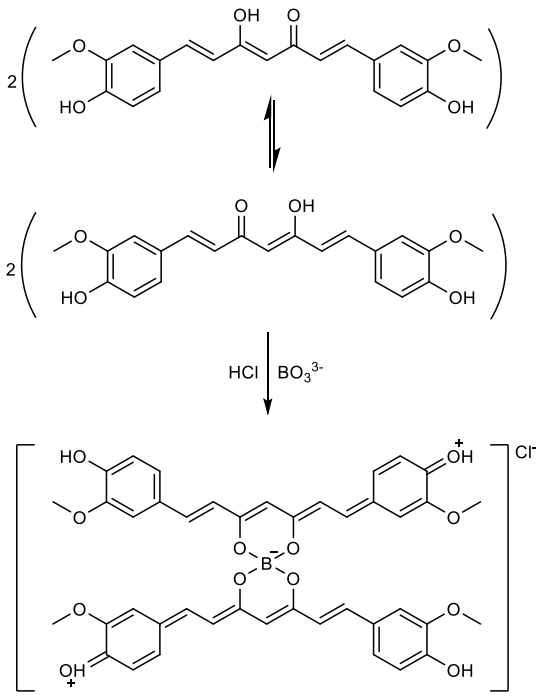


Figure 6 – Curcumin tautomers and rosocyanine complex formation

3.2.2. ^{1}H NMR

3.2.2.1. 3,6-dihydroxyxanthone **10**

The ^{1}H NMR of **10** dissolved in DMSO-d_6 without purification shows four signals in the aromatic area corresponding to the resonance of the protons of 3,6-dihydroxyxanthone **10**. The integral of each signal corresponds to two protons. The signals are:

- δ = 10.83 ppm – the higher shift in comparison to other signals, the absence of a coupling constant, the integral size and smaller shape of the signal all hint to the presence of protons from groups 3-OH and 6-OH. The broader shape of the signal is due to proton exchange at room temperature between the -OH groups and the solvent[56]. With the use of DMSO-d_6, the signals appear sharper[56]. In addition, with the use of DMSO-d_6 the -OH group peaks appear highly deprotected, well outside the aromatic region[56].
- δ = 7.98 ppm – this signal appears as a doublet. Signals in ^{1}H NMR are split by the influence of protons present on neighbouring carbons. The resulting multiplicity can be expressed by the formula n+1, where n represents the number of protons. This signal being a doublet (multiplicity 2, or n+1=1+1) hints at the presence of protons on the neighbour carbon. The coupling constant (J = 8.7 Hz) discloses an interaction with an *ortho* proton. The signal with a high chemical shift due to the aromatic ring is further deprotected by the proximity of the xanthone carbonyl group. All being taken into consideration, this signal represents the protons H_1 and H_8.

- δ = 6.86 ppm – a doublet of doublets with coupling constants J = 8.7, 2.5 Hz, this signal presents protons interacting with protons in position *ortho* and *meta*. Such protons can be none other than H_2 and H_7
- δ = 6.82 ppm – The protons represented by a doublet with a coupling constant J = 2.5 Hz are positioned in *meta* position to protons H_2 and H_7 respectively and hence are protons H_4 and H_5.

Table 1 – ^{1}H NMR data analysis for 3,6-dihydroxyxanthone **10**

Chemical shift (δ, ppm)	**Multiplicity**	**Coupling constant *J* (Hz)**	**Number of protons**	**Corresponding proton**
10.83	bs	-	2	3-OH, 6-OH
7.98	d	8.7	2	H_1, H_8
6.86	dd	8.7, 2.5	2	H_2, H_7
6.82	d	2.5	2	H_4, H_5

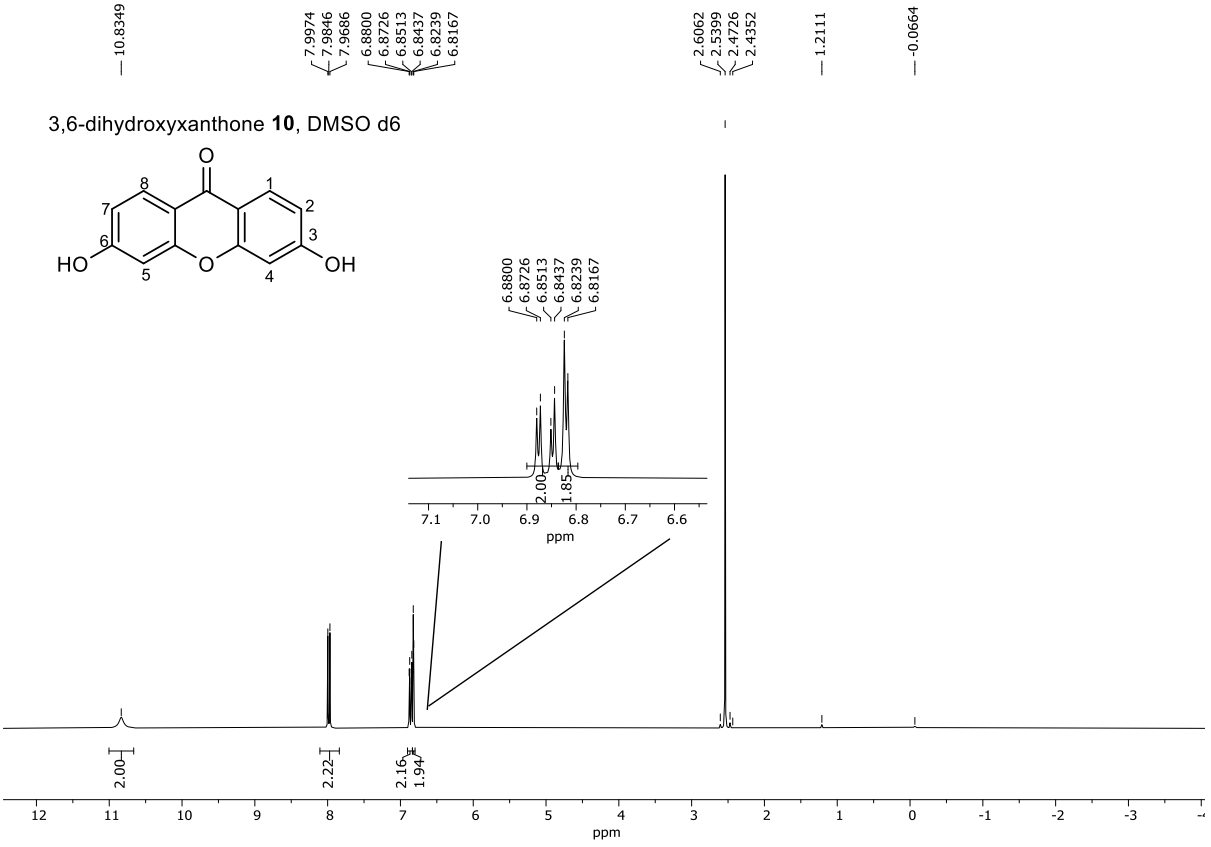


Figure 7 – ^{1}H NMR spectra of 3,6-Dihydroxyxanthone **10**

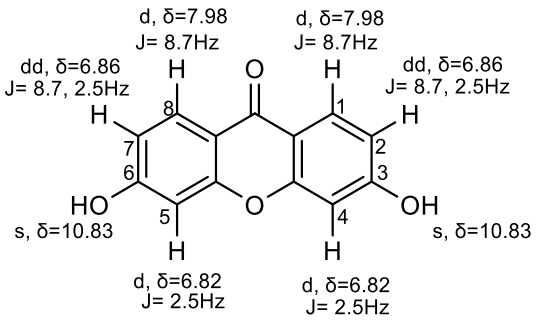


Figure 8 – ^{1}H NMR data of compound **10** (chemical shifts δ in ppm, J-coupling in Hz)

3.2.2.2. 3,6-ditrifylxanthone **11**

As expected with the symmetrical aromatic structure of **11**, the ^{1}H NMR obtained after workup, purification, and dissolution in $CDCl_3$ shows three signals in the aromatic area, each corresponding to 2 atoms after integration. The signals chemical shifts are:

- δ = 8.45 ppm This signal is a doublet closely interacting with protons in the *ortho* position (*J* = 8.8 Hz). The only other signal with a similar coupling constant is a doublet of doublets. Hence, this signal belongs to protons H_1 and H_8.
- δ = 7.50 ppm As a doublet with a lower coupling constant (*J* = 2.3 Hz) hinting at the *meta* position, this signal belongs to the only protons in a such position without any proton in the *ortho* position, protons H_4 and H_5.
- δ = 7.36 ppm A doublet of doublets and hence a signal with protons in *ortho* position (*J* = 8.8 Hz), interacting with other protons in the meta position, this signal represents protons H_2 and H_7.

Table 2 – ^{1}H NMR data analysis for 3,6-ditrifylxanthone **11**

Chemical shift (δ, ppm)	**Multiplicity**	**Coupling constant *J* (Hz)**	**Number of protons**	**Corresponding proton**
8.45	d	8.8	2	H_1, H_8
7.50	d	2.3	2	H_4, H_5
7.36	dd	8.8	2	H_2, H_7

Figure 9 – ^{1}H NMR Spectra of 3,6-ditrifylxanthone **11**.

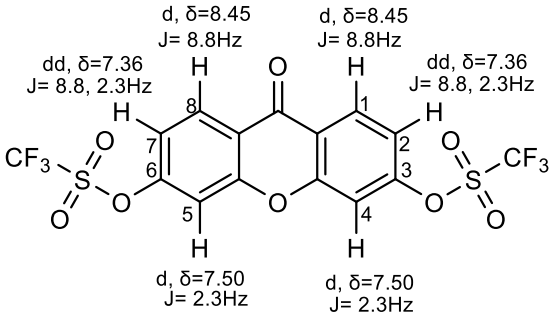


Figure 10 – ^{1}H NMR data of compound **11** (chemical shifts δ in ppm, *J*-coupling in Hz)

3.2.2.3. 3,6-bis(pinacolatoboron)xanthone **8**

In the ^{1}H NMR ($CDCl_3$) spectra, it is possible to observe the presence of four signals corresponding to the resonance of the protons of compound **8**: three signals in the aromatic area (δ = 8.32, 7.94, and 7.77 ppm) and one signal in the aliphatic area (δ = 1,39 ppm). The signals integration reveals that each signal in the aromatic area stands for two protons, whereas the signal

in the aliphatic area stands for cca 24 protons. This hints at the symmetrical structure of compound **8**. A more precise analysis of the signals reveals the following information:

- δ = 8.32 ppm: This signal is split into a doublet (d). This information is backed by the coupling J = 7.9 Hz representing an *ortho* interaction between protons. Summed up, this signal corresponds to the resonance of protons H_1 and H_8.
- δ =7.94 ppm: This signal appears as a singlet (s) and integrates to two protons (H_4 and H_5), that appear isolated on the pinacolboronate ester functional group on C_3 and C_6.
- δ =7.77 ppm: The signal corresponding to this chemical shift is split into a doublet of doublets (dd) with two coupling constants of J =7.9 Hz and 1.0 Hz. This information indicates that protons H_2 and H_7proton interact with *ortho* protons H_1 and H_8, respectively, and with *meta* protons H_4 and H_5, respectively.
- δ =1.39 ppm: A signal appearing as a singlet and integrating for 24 protons in the aliphatic area, represents the protons of CH_3 group of the pinacolboronate functional groups.

These informations can be summed up in the following table:

Table 3 – 1H NMR data analysis for 3,6-bis(pinacolboronate)xanthone **8**

Chemical shift (δ, ppm)	**Multiplicity**	**Coupling constant *J* (Hz)**	**Number of protons**	**Corresponding proton**
8.32	d	7.9	2	H_1, H_8
7.94	s	-	2	H_4, H_5
7.77	dd	7.9, 1.0	2	H_2, H_7
1.39	s	-	24	CH_3

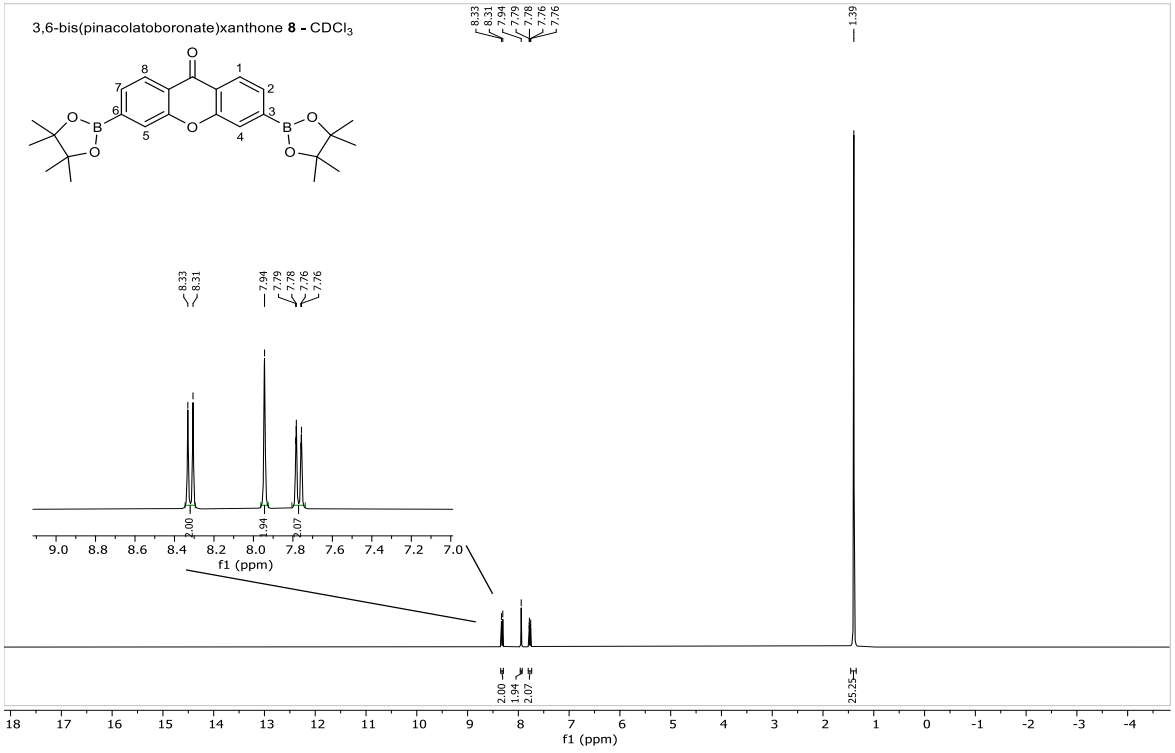


Figure 11 – 3,6-Bis(pinacolatoboron)xanthone **8** ^{1}H NMR

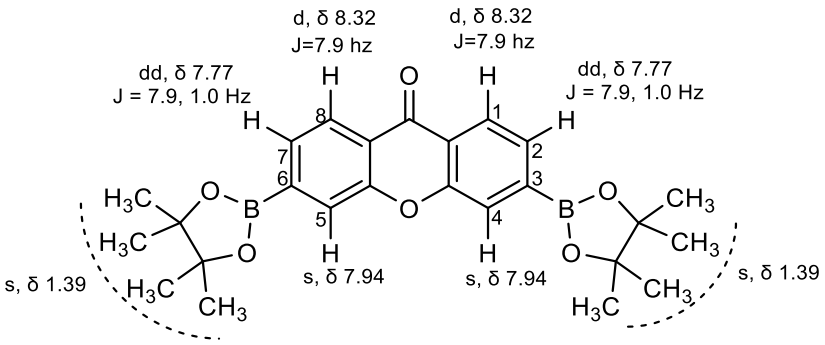


Figure 12 – ^{1}H NMR data of compound **8** (chemical shifts δ in ppm, J-coupling in Hz)

3.2.2.4. Xanthone-3,6-diboronic acid **14**

The sample was sent for NMR without purification dissolved in DMSO-d_6. The obtained ^{1}H NMR in comparison to the NMR spectrum of compound **8** allowed us to identify four signals in the aromatic area susceptible to represent the compound **14**. The forementioned signals present chemical shifts δ= 8.57, 8.14, 8.05, and 7.83 ppm with integrals of ratio 4:2:2:2. Minutious analysis of these signals revealed the following informations:

- δ= 8.57 ppm: The signal corresponding to this chemical shift is a singlet of an integral ratio size hinting at the presence of 4 protons. The comparison of this signal's chemical shift in DMSO-d_6 to that of boronic acid functional groups on compounds similar to **14** reveals that it represents the four B-OH groups present on the two -$B(OH)_2$ functional groups.
- δ= 8.14 ppm: A doublet with a coupling constant J =7.9 Hz and an integral hinting at two protons, this signal represents the atoms H_1 and H_8.
- δ= 8.05 ppm: This signal is a singlet of the size of two protons without any reported coupling constant. It represents the atoms H_4 and H_5.
- δ= 7.83 ppm: Just as in the case of compound **8**, we observed a doublet of doublets of the size of two protons and two coupling constants J =7.9, 1.0 Hz. This signal represents the atoms H_2 and H_7.

This data can be summarised in the following table:

Table 4 – ^{1}H data analysis for xanthone-3,6-diboronic acid **14**

Chemical shift (δ, ppm)	**Multiplicity**	**Coupling constant J (Hz)**	**Number of protons**	**Corresponding proton**
8.57	s	-	4	B-OH
8.14	d	7.9	2	H_1, H_8
8.05	s	-	2	H_4, H_5
7.83	dd	7.9, 1.0	2	H_2, H_7

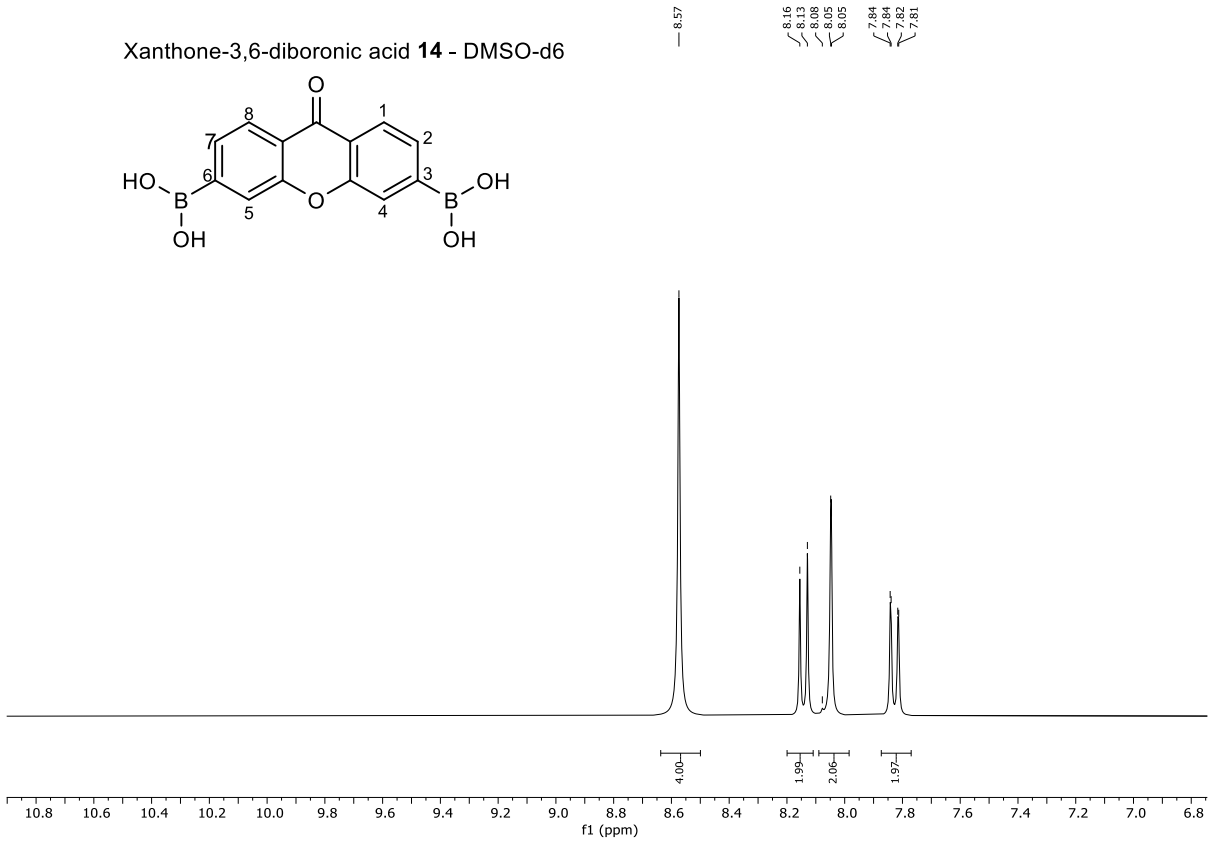


Figure 13 – ^{1}H NMR spectra of xanthone-3,6-diboronic acid **14**.

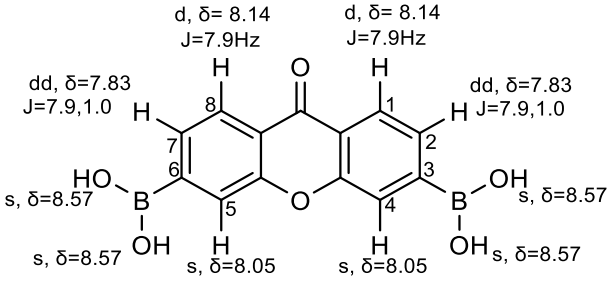


Figure 14 – ^{1}H NMR data of compound **14** (chemical shift δ in ppm, J-coupling in Hz)

4. Conclusions

The synthesis of xanthone-3,6-boronic acid **14** was achieved in a two-step procedure involving the synthesis of 3,6-bis(pinacolatoboron)xanthone **8** starting from 3,6-ditrifylxanthone **11** and further deprotection of the corresponding boronic ester. The synthesis of **8** was performed using bis(pinacolato)diboron (resp. pinacolborane) as borylation agent, in the presence of KOAc (resp. Et_3N) as base, under catalysis by $Pd(dppf)Cl_2$ with an addition of dppf. The character of the base and the borylation agent as well as their equivalents with **11** highly influenced the reaction yield and product. The reaction was done during 12h (resp. 24h). However, given the lower yields 11% (resp. 12%) as opposed to 50% reported in the literature and the presence of intermediate reaction

complexes on TLC plates, the reaction might need a longer reaction time. An optimisation regarding the equivalents of borylation agent and base is also necessary.

The deprotection of **8** into **14** was achieved by transforming **8** into a DEA-protected boronate **14b** in the presence of diethanolamine at room temperature and the acidic hydrolysis of the DEA intermediate into **4** (54% yield). The size of **14** and the presence of 2 -$B(OH)_2$ groups make this compound hard to dissolve and a more polar solvent such as acetone was preferred as organic phase instead of ether for acidic hydrolysis.

The attempt to synthesise 3,6-bis((pinacolboryl)methoxy) xanthone **12** using 2-(bromomethyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane as borylation agent in DMSO (or THF) under catalysis by NaH resulted in 3-((pinacolboryl)methoxy)-6-hydroxyxanthone (**12b**, 35% yield). The partial success of the reaction was attributed to an insufficient amount of borylation agent and catalyst. 3,4-Dimethoxy-1-methylxanthone **15** was successfully converted into 4-dihydroxy-1-methylxanthone (**16**, 54% yield) with the deprotective action of $AlCl_3$. The attempt to convert **16** into 3,4-ditrifyl-1-methyl-xanthone **17** using 3 equivalents of Tf_2O resulted mostly in 3-trifyl-4-hydroxy-1-methylxanthone (**17b**, 25% yield).

The attempt to synthesise 2,7-dibromoxanthone **22** in acetic acid using NBS as source of bromine in the presence of HCl resulted in a mixture of products which proved difficult to purify. Three fractions were obtained from initial purification, starting material, one product theorised as 2-bromo-7-chloroxanthone (18, 7% yield) and a mixture of unidentified products susceptible to contain **22** (63%).

5. Materials and methods

All reagents and solvents were purchased from Sigma Aldrich and used without prior purification. The solvents were evaporated on a rotary evaporator under reduced pressure (evaporator Buchi Waterchath B-480). The obtained compounds were purified by collumn chromatography using silica flash column using Merck silica gel 60 (0.040–0.063 mm) or preparative chromatography with silica and preparative TLC was carried out on pre-coated plates Merck Kieselgel 60 F_{254}, spots were visualized under UV light. Non – commercial starting material was dried overnight in a Buchi Glass Oven B-585 before use. The reactions and purification procedures were controlled by thin-layer chromatography(TLC) with precoated plates(0.2 mm thickness, silica gel, 60 GF254 Merck) with appropriate mobile phases, and UV detection at 245 and 365nm. ^{1}H NMR spectra were performer in the Department of Chemistry of the University of Aveiro, and were taken on BRUKER ADVANCE III 300 MHz spectrometer using $CDCl_3$ or DMSO-d_6 as solvents.

5.1. Synthesis of xanthone-3,6-diboronic acid **14**

5.1.1. Synthesis of 3,6-dihydroxyxanthone **10**

A portion of 2,2',4,4'-tretrahydrobenzophenone **9** (Aldrich) (2.00 g, 8.12 mmol) is heated at 220 °C, in a furnace, overnight. The reaction results in a nearly quantitative conversion into the pure 3,6-dihydroxyxanthone **10**.

3,6- dihydroxyxanthone 10. Brown solid. 93% yield. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.83 (2H, s, 3-OH, 6-OH), 7.98 (2H, d, *J*= 8.7 Hz, H_1, H_8), 6.86 (2H, dd, *J*= 8.7,2.5 Hz, H_2, H_7), 6.82 (2H, d, *J*= 2.5 Hz, H_4, H_5).

5.1.2. Synthesis 3,6-ditrifylxanthone **11**

3,6-Dihydroxyxanthone (**10,** 1.00 g, 4,.38 mmol) was dissolved in 40 mL of CH_2Cl_2 and pyridine (3.545 mL, 43.82 mmol) was added slowly over 5 min at 0 °C. The mixture was stirred at 0 °C for 10 min then Tf_2O (2.21 ml, 13.15 mmol) was added dropwise over 10 min. The reaction mixture was warmed to room temperature slowly and stirred for 24 h. The reaction was quenched with water and the organic layer was washed with water (1 × 30 ml, 1N HCl (3 × 30 ml), brine (1 × 30 ml) and dried over Na_2SO_4. The solvents were removed under pressure to give a brown residue. The residue was purified by column chromatography (CH_2Cl_2/*n*-hexane 7:3) to give a white product corresponding to 3,6-ditrifylxanthone **11**,

3,6-ditrifylxanthone 11, White solid. 81% yield. 1H NMR (300 MHz, Chloroform-d, δ ppm) δ 8.45 (d, *J* = 8.8 Hz, H_1, H_8), 7.50 (d, *J* = 2.3 Hz, H_4, H_5), 7.36 (dd, *J* = 8.8, 2.3 Hz, H_2, H_7).

5.1.3. Synthesis of 3,6-bis(pinacolatoboron)xanthone **8**

A - Borylation agent – bis(pinacolato)diboron(B_2pin_2)

In a 25 ml Schlenk flask, in anhydrous conditions under nitrogen, were combined 3,6-ditrifylxanthone (**11**, 100 mg, 0.203 mmol), bis(pinacolato)diboron (113 mg, 0.445 mmol), Pd(dppf)Cl_2.CH_2Cl_2(11.5mg, 0.014 mmol), dppf (8 mg, 0.0144 mmol), potassium acetate (60 mg, 0.0611 mmol) and anhydrous dioxane (3 ml). After stirring for 5 min at room temperature, the reaction was slowly heated up to 100 °C and left to react for 12 h. The reaction progress is monitored by TLC using a curcumin dip (100 mg curcumin, 100 ml EtOH, 2 N HCl 99:1 v/v). After completion, the reaction is cooled down to room temperature, diluted with toluene (15 ml) and washed with brine (3 ×10 ml). The organic layer is dried over Na_2SO_4 and the solvent was removed by rotary evaporation to leave a brown residue. The residue was recrystallised from methanol to give a white product corresponding to 3,6-bis(pinacolatoboron)xanthone **8**.

3,6-bis(pinacolatoboron)xanthone 8: White solid. 11% yield. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) δ 8.32 (2H, d, *J* = 7.9 Hz, H_1, H_8), 7.94 (2H, s, H_4, H_5), 7.77 (2H, dd, *J*= 7.9, 1.0 Hz, H_2, H_7), 1.39 (24H, s, CH_3).

B- Borylation agent – pinacolborane (HBpin)

In a 25 ml Schlenk flask, in anhydrous conditions under nitrogen, were combined 3,6-ditrifylxanthone **11** (100 mg, 0.203 mmol), $Pd(dppf)Cl_2.CH_2Cl_2$(11.5 mg, 0.014 mmol), dppf (8 mg, 0.0144 mmol), and anhydrous dioxane (3 mL) and the mixture was stirred. After 5 min, pinacolborane (64.95 mg, 0.507 mmol), and triethylamine (0.08 ml, 0.611 mmol) were respectively added dropwise over 5 min. The mixture was slowly heated to 100 °C and left to react for 24 h. The reaction progress was monitored by TLC using a curcumin dip (100 mg curcumin, 100 ml EtOH, 2N HCl 99:1 v/v). After completion, the reaction was cooled to room temperature, quenched with water, extracted with toluene (3 × 15 ml), and the organic phase was washed with brine and dried over Na_2SO_4. The organic solvent was evaporated to give a brown residue which was purified by column chromatography (100% CH_2Cl_2), and the obtained compound was washed with cold MeOH to yield pure product **8.**

3,6-bis(pinacolatoboron)xanthone 8: White solid. 12% yield. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) δ 8.32 (2H, d, *J* = 7.9 Hz, H_1, H_8), 7.94 (2H, s, H_4, H_5), 7.77 (2H, dd, *J*= 7.9, 1.0 Hz, H_2, H_7), 1.39 (24H, s, CH_3).

5.1.4. Deprotection of 3,6-bis(pinacolatoboron)xanthone **8** to xanthone-3,6-diboronic acid **14**

A- Synthesis of DEA-boronate ester **14b**

To a solution of 3,6-bis(pinacolatoboron)xanthone (25 mg, 0.056 mmol) in ether was added diethanolamine (0.14 mmol, 2.5 equiv.). After a few minutes a white precipitate formed, and the reaction was allowed to continue until the starting material was completely consumed as showed by TLC (30 min to 1 h). The precipitate was then filtered, washed with ether, and dried to afford the desired product (DEA-protected 3,6-diboronic ester).

B- Deprotection of DEA-boronate ester **14b**

To a solution of DEA-boronate in ether was added 0.1M HCl. After about 20 min as judged by TLC, the reaction was extracted with ether (3 ×), washed with brine (1 ×), dried with Na_2SO_4, and the organic solvent was removed under reduced pressure. Evaporation of residual solvent provided the analytically pure product as white solid **14**.

Xanthone 3,6-diboronic acid 14. White solid 54% yield. ^{1}H NMR (300 MHz, DMSO-d6, δ ppm) δ 8.57 (s, 4H, B-OH), 8.14 (d, *J* = 7.9 Hz, H_1, H_8), 8.05 (s, H_4, H_5), 7.83 (dd, *J* = 7.9, 1.0 Hz, H_2, H_7).

5.2. Synthesis of (((9-oxo-9*H*-xanthene-3,6-diyl)bis(oxy))bis(methylene))diboronic acid **13**

5.2.1. Synthesis of 3,6-bis((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methoxy)-9H-xanthen-9-one **12**

To a mixture of 3,6-dihydroxyxanthone (**10**, 100 mg, 0.438 mmol) in DMSO (3 mL) was added sodium hydroxide (NaH, 16 mg, 2.00 equiv.) at room temperature. After stirring for 15 min, 2-(bromomethyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (111 mg, 1.5 equiv.) was added and the resulting mixture was heated to 50 °C and stirred for 4 h. The reaction was monitored by TLC. After completion, the reaction was cooled to room temperature, quenched with water (15 mL) and extracted with ethyl acetate (2 × 30 mL). The combined organic layers were washed with brine (2 × 50 mL), dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by preparative chromatography (10 plates, MeOH:*n*-hexane 7:3 + drops of HCOOH). The product was isolated from the silica as a yellow – coloured residue. ^{1}H NMR revealed the obtained residue to be 3-hydroxy-6-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methoxy)-9*H*-xanthen-9-one **12b**.

3-hydroxy-6-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methoxy)-9H-xanthen-9-one (**12b**): yellow solid. 35% yield.

5.3. Synthesis of precursors of 1-methylxanthone-3,4-diboronic acid (**21**)

5.3.1. Synthesis of 3,4-dihydroxy-1-methylxanthone (**16**)

3,4-Dimethoxy-1-methylxanthone (**15**, 200 mg, 1 equiv., 740 μmol) was dissolved in dry toluene (20 mL) and aluminium chloride (987 mg, 10 equiv., 740 mmol) was carefully added. The reaction mixture was refluxed (110 °C) with magnetic stirring for 1.5 hours. The evolution of the reaction was followed by TLC. After completion, the reaction was cooled down to room temperature, excess conc. HCl (5 N, 10 ml) was added and the mixture was extracted with ethyl acetate (4 × 50 ml). The organic layer is dried over anhydrous Na_2SO_4 and evaporated to give a brown-coloured residue. The residue is purified by preparative chromatography (26 plates, $CHCl_3$: MeOH 9:1) and the product is isolated as a light brown coloured powder.

3,4-Dihydroxy-1-methylxanthone (16): light brown solid, 54.36% yield. 1H NMR (300 MHz, DMSO-d6) δ 10.34 (s, C_3-OH), 9.18 (s, C_4-OH), 8.11 (dd, J = 7.9, 1.7 Hz, H_8), 7.78 (ddd, J = 8.7, 7.1, 1.8 Hz, H_6), 7.57 (dd, J = 8.5, 1.0 Hz, H_5), 7.40 (ddd, J = 8.0, 7.1, 1.1 Hz, H_7), 6.68 (d, J = 1.0 Hz, H_2), 2.68 (s, 3H, -CH_3).

5.3.2. Synthesis of 3,4-ditrifyl-1-methyl-xanthone (**17**)

3,4-Dimethoxy-1-methylxanthone (**16,** 180 mg, 0.743 mmol) was dissolved in CH_2Cl_2 (10 ml) in anhydrous nitrogen atmosphere and pyridine (0.6 ml, 7.4 mmol) was added slowly over 5

min at 0 °C. The mixture was stirred for 10 min, then Tf_2O (0.373 ml, 2.22 mmol) was added dropwise over 10 min. The reaction mixture was warmed to room temperature and stirred for 24 h. The reaction was monitored by TLC. After completion, the reaction was quenched with water, and the organic layer was washed with water (1 ×15 ml), 1N HCl (3 × 15 ml), brine (1 × 15 ml) and dried over Na_2SO_4. The solvents were removed under reduced pressure to give brown residue. The obtained residue was purified by preparative chromatography (CH_2Cl_2: *n*-hexane 7:3, 7 plates) to give a white product identified by ^{1}H NMR as the intermediate 3-trifyl-4-hydroxy-1-methylxanthone **17b.**

3-Trifyl-4-hydroxy-1-methylxanthone 17b white solid, 25% yield

5.4. Synthesis of 2,7-dibromoxanthone **22**

A suspension of xanthone (**1**, 500 mg, 2.55 mmol) and *N*-bromosuccinimide (700 mg, 3.93 mmol) in acetic acid (4 mL, 69.93 mmol) was prepared in a 50 mL flask and stirred. HCl (0.1 ml, 3.2 mmol) was added to the suspension (cca 2-3 drops). The mixture was stirred at room temperature for 12h. The reaction progress was monitored by TLC (thin-layer chromatography). The product was purified by preparative chromatography (22 plates, CH_2Cl_2: *n*-hexane 1:1). 3 phases were obtained and sent for ^{1}H NMR of which one was characterised as xanthone **1**, one as 2-chloro-7-bromoxanhone and a mixture of compounds. This mixture was further purified by preparative chromatography (5 plates, CH_2Cl_2: *n*-hexane 7:3) and the most abundant phase was sent for ^{1}H NMR.

2-chloro-7-bromoxanthone – white solid, 7% yield
Unpurified mixture of products – white solid, 63% yield.

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