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The Synthesis of Fluorescent 3, 6-dihydroxyxanthones: A Route to Substituted Fluoresceins

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THE SYNTHESIS OF FLUORESCENT 3, 6-DIHYDROXYXANTHONES:

A ROUTE TO SUBSTITUTED FLUORESCEINS

by

Surajudeen Omolabake

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ABSTRACT

THE SYNTHESIS OF FLUORESCENT 3, 6-DIHYDROXYXANTHONES:
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Surajudeen Omolabake

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Under the Supervision of Professor Alan W Schwabacher

Xanthones belong to the family of compounds of the dibenzo-γ-pyrone framework. Naturally occurring xanthones have been reported to show a wide range of biological and medicinal activities including antifungal,[19] antimalarial,[20] antimicrobial,[21] antiparasitic,[22] anticancer,[23] and inhibition of HIV activity in cells.[24] Xanthones have also been used as a turn on fluorescent probe for metal ions,[32] including use as pH indicators, metal ion sensors, in molecular biology, medicinal chemistry and in the construction of other dyes.

Several methods have been developed for the synthesis of this important class of compounds. These methods have several limitations including commercially unavailable or very expensive starting materials, harsh reaction conditions, and multiple steps leading to low overall yields.

In this report I present a simple and efficient method to make 3,6-dihydroxyxanthones in high yields starting with cheap and commercially available starting materials. This transformation involves Friedel-Crafts acylation, Friedel-Crafts alkylation and cyclization of the resulting diarylmethyl cation in a manner mechanistically equivalent to the formation of fluorescein with trifluoroacetic anhydride playing the role of phthalic anhydride.

Fluorination of fluorophores can greatly enhance their photo-stability and improve their spectroscopic properties. 2', 7'-difluoro derivative of fluorescein has a lower pKa compared to un-substituted fluorescein thereby making it less pH sensitive. Our method offers an easier and efficient 2 steps sequence to make fluorinated xanthones in high yield compared to a 6 step sequence reported in the literature.[1]

To My Parents

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LIST OF ABBREVIATIONS

TFA	Trifluoroacetic acid
MEM-Cl	Methoxyethoxymethyl chloride
DMSO	Dimethylsulfoxide
DMF	Dimethylformamide
DDQ	Dicyanodichlorobenzoquinone
TLC	Thin layer chromatography
DCM	Dichloromethane
HIV	Human immunodeficiency virus
NMR	Nuclear magnetic resonance
MS	Mass spectrometry
MIC	Minimum inhibitory concentration
IC	Inhibitory concentration
LCMS	Liquid chromatography-mass spectrometry
ROS	Reactive oxygen species
IR	Infra-red spectrometry
UV	Ultra-violet

LIST OF SCHEMES

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1. LITERATURE REVIEW

1.1 Fluorophores

A fluorophore is the component of a compound that is mainly responsible for the absorption and emission of light.[2] After absorption of light at a specific wavelength, it re-emits at usually a longer wavelength. The wavelength of the emitted depends on the nature of the fluorophore and its chemical environment. Fluorophore usually contain either aromatic ring systems or several conjugated double bonds. Common fluorophore containing compounds are shown in figure 1 below;

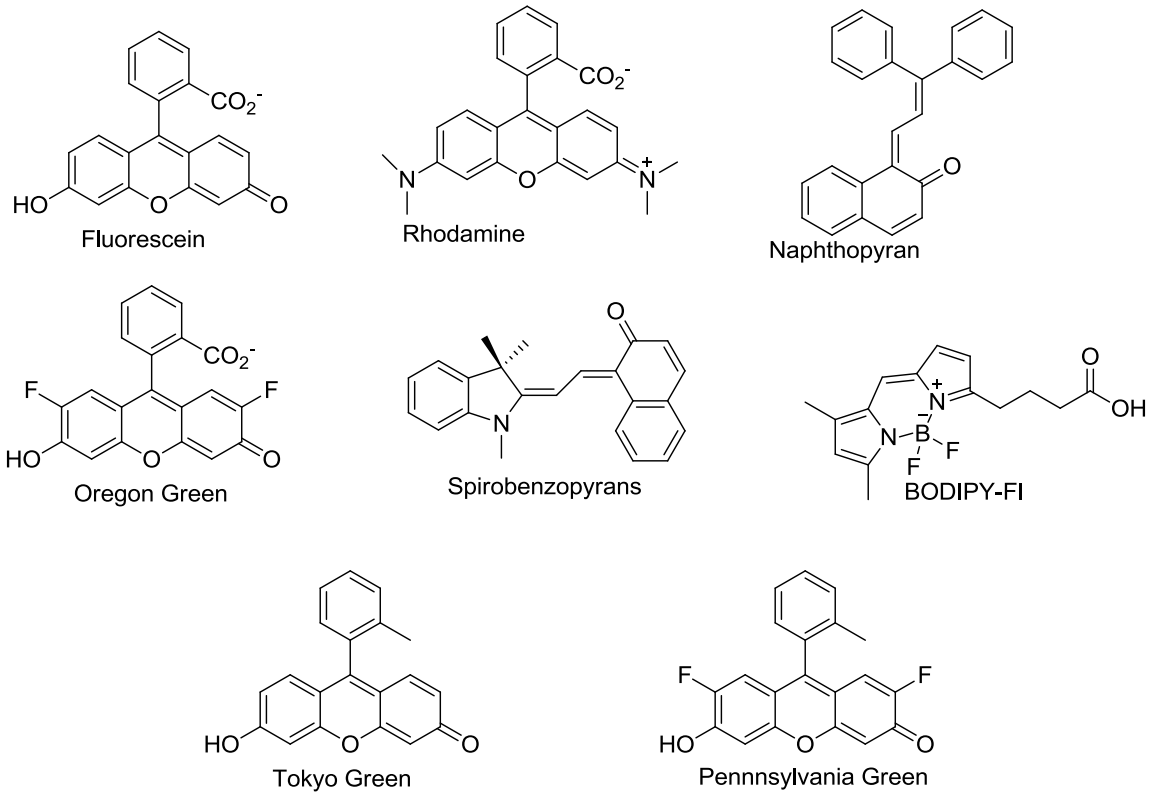


Figure 1.1 Common fluorophore containing compounds

Fluorophores have wide applications and are mostly used to stain tissues, cells, or materials in a variety of analytical methods.

1.2 The Concept of Fluorescence

Fluorescence is the emission of light from singlet excited states in which the electron in the excited orbital has opposite spin orientation as the ground-state electron.[3] Transitions to the ground state are allowed and the emission rates are very fast so that fluorescent lifetimes are typically in the nanosecond range. Measurement of the time-resolved emission involves advanced optics due of the short timescale of fluorescence, making it a sensitive process. Fluorescence data are presented as emission data which is a plot of fluorescence intensity against wave number. The Jablonski diagram is frequently used to illustrate the process that occurs when light is absorbed and re-emitted by a compound.

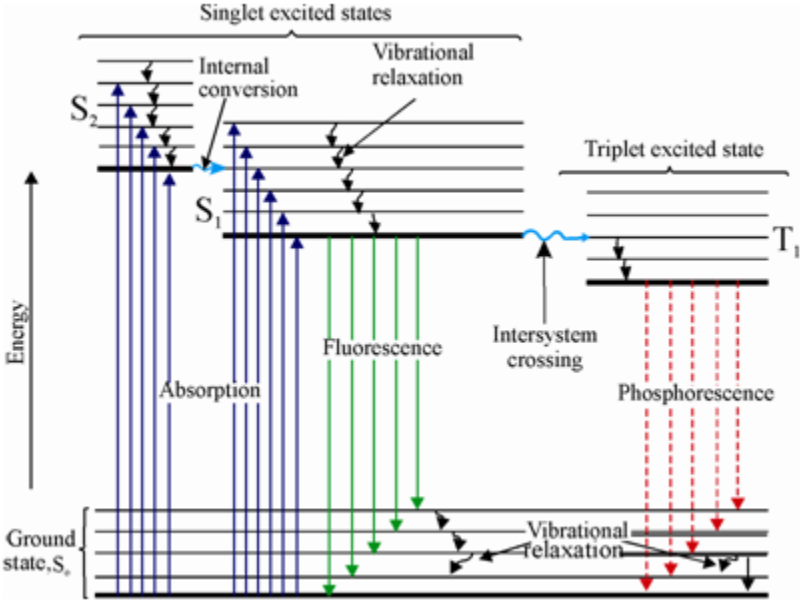


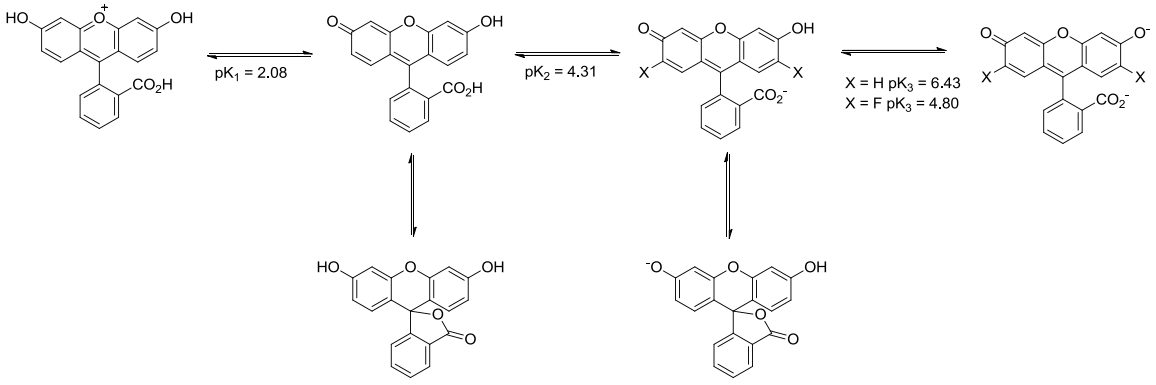
Figure 1.2 The Jablonski diagram

Light of specific wavelength interacts with an electron and causes its excitation to a higher-energy level S_2, which then undergoes internal conversion according to Kasha's rule to the first excited state S_1. Several processes compete with fluorescence. The excited electron can either relax back

to the ground state which is fluorescence or can undergo intersystem crossing to the excited triplet state and then relax to the ground state a process termed phosphorescence. Phosphorescence is a much slower process since it is spin forbidden and a result the rate constants for triplet emission are several orders of magnitude smaller than those for fluorescence. Compounds containing heavy atoms such as iodine are frequently phosphorescent. The heavy atoms promote intersystem crossing and thus enhance phosphorescence effectively reducing the efficiency of fluorescence termed quantum yield.

1.3 Fluorescein

Fluorescein is a synthetic organic fluorophore that was first reported in 1871 by Von Bayer.[4] It is a dark orange compound that is soluble in methanol and slightly soluble in water. It is a highly fluorescent compound that absorbs light at 494nm and re-emits at 517nm in water and can be excited with the readily available argon ion laser. Fluorescein has a very high quantum yield of 0.92. A problem with fluorescein is that it can exist in cationic, neutral and in anionic forms making its fluorescent properties pH dependent.[5]



Scheme 1.1 pH Dependence of Fluorescein Equilibria

Fluorine atom in certain positions in the fluorescein core reduces the pKa of the compound and is presented later in this work. A great number of fluorescein derivatives are commercially available

while the properties can be modified to tune its fluorescent properties thereby widening its range of applications

1.3.1 Applications of Fluorescein

The excitation and emission wavelength of fluorescein can be tuned by making derivatives of fluorescein. Many of such derivatives have been made and are commercially available thereby increasing the scope of fluorescein use. Fluorine substituted fluoresceins is particularly useful as tags for biomolecules. Some fluorosensor uses are highlighted below:

Metal Sensors: Metals play very important role in biological systems. An increase or decrease in their concentration can be detrimental hence the need to monitor their concentration. A derivative of fluorescein that can detect copper selectively in the presence of other divalent metal ions has been reported.[6] The fluorescence of the sensor is quenched when copper is added with a detection limit of 0.5μM

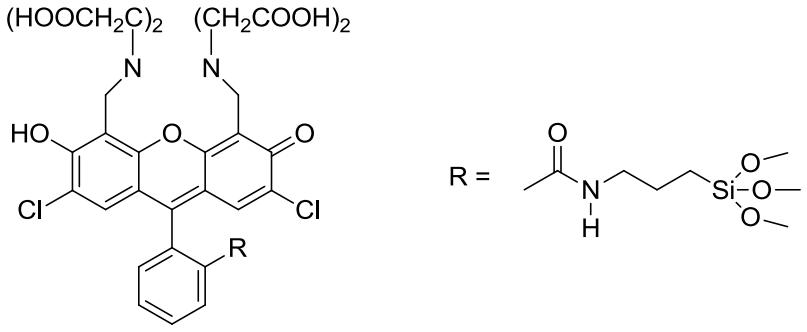


Figure 1.3 Fluorescein based copper ion sensor

pH Sensor: In some applications, it is very important to monitor the pH as a slight increase or decrease could affect the function of the system, an example is the cell. This usually would require non- invasive sensors. A fluorescein based pH sensor that can detect pH between 7 and 10 was reported.[7]

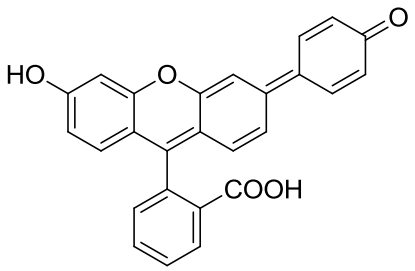
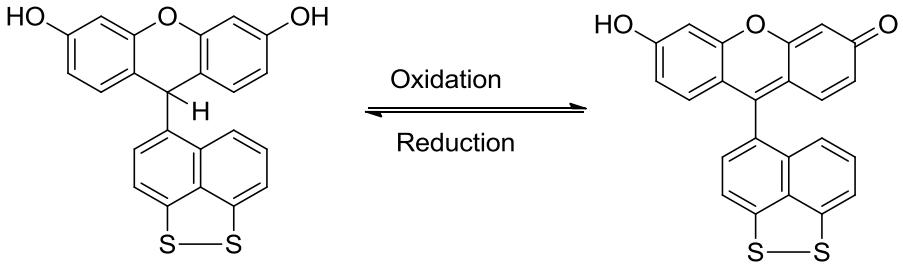


Figure 1.4 Fluorescein based pH sensor

Reactive Oxygen Species (ROS) Sensor: A fluorescein derivative incorporating a disulfide linkage that can detect reactive oxygen species has been developed.[8] The sensor turns on when oxidized and turns off in the reduced form.



Scheme 1.2 Fluorescein based ROS sensor

Enzyme activity sensors: A group prepared a fluorescein based enzyme sensor that can detect alkaline phosphatase.[9] The fluorescence of the sensor was caged because of self-quenching in a polymer structure. The enzyme breaks the phosphoramidite bonds which then releases the free fluorescein and the fluorescence of the free fluorescein is detected and used to quantify the enzyme.

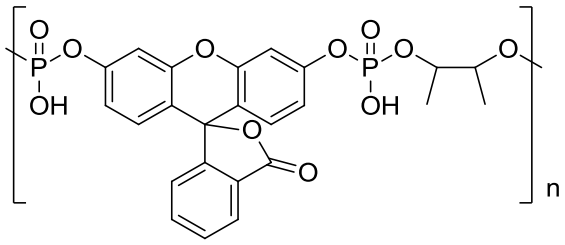


Figure 1.5 Caged fluorescein based sensor

Polymerizable Fluorescein Derivatives: Detection of nanoparticles is frequently based on fluorescent labels knowing their location and permitting quantification of cellular loading**.** A

fluorescein based sensor that can detect nanoparticles was reported.[10] In their structure, fluorescein was modified with styryl monomers and are converted into polymer particles.

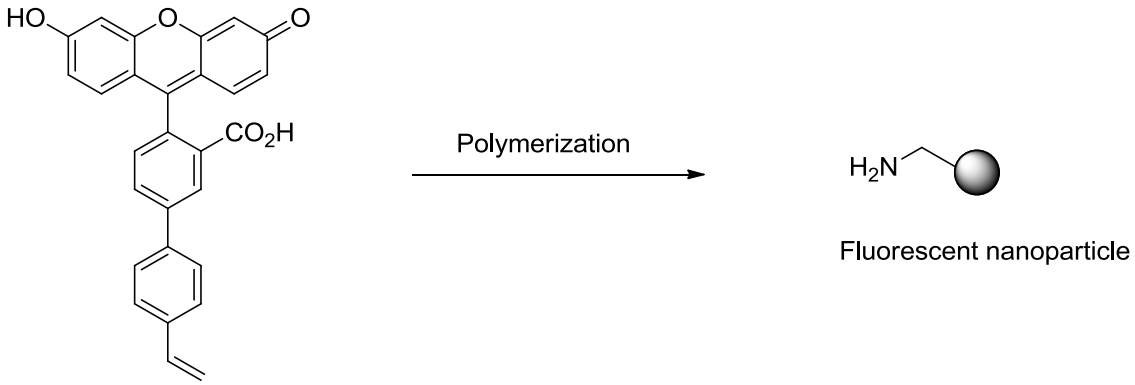
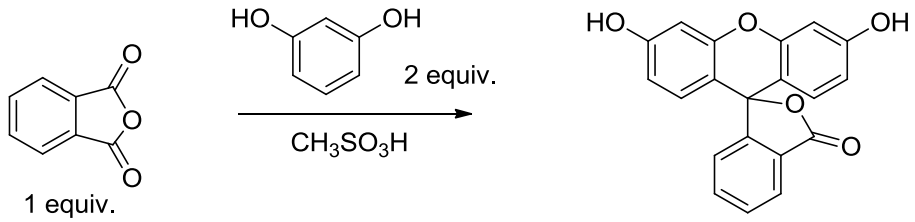


Figure 1.6 Fluorescein based nanoparticle sensor

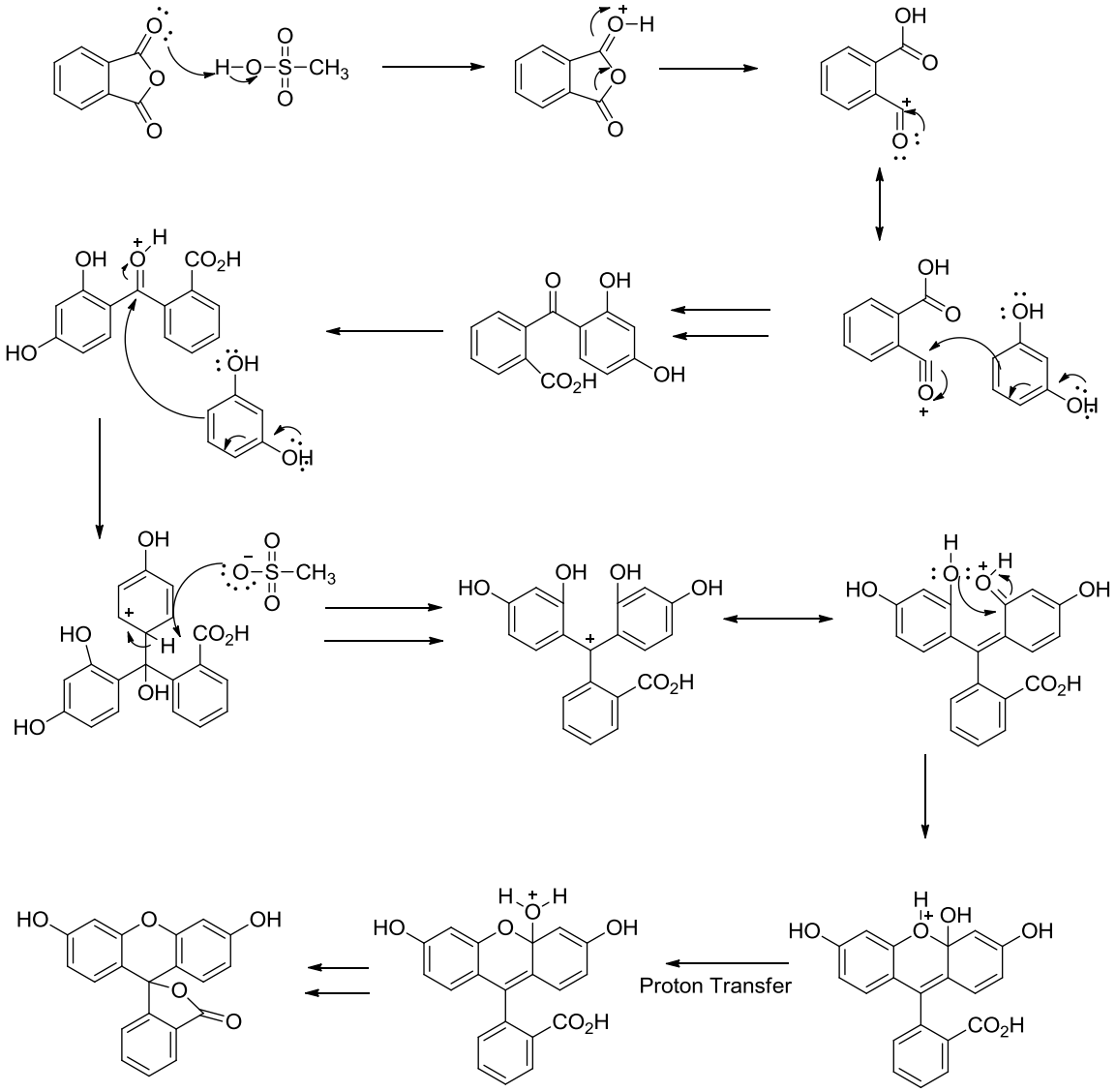
1.3.2 Synthesis of Fluorescein

Fluorescein was first prepared in the lab from the condensation of two molecules of resorcinol and one molecule of phthalic anhydride using zinc chloride as a catalyst.[4] Methanesulfonic acid is a more suitable Lewis acid and solvent for the formation of the product with improved yields.[11]



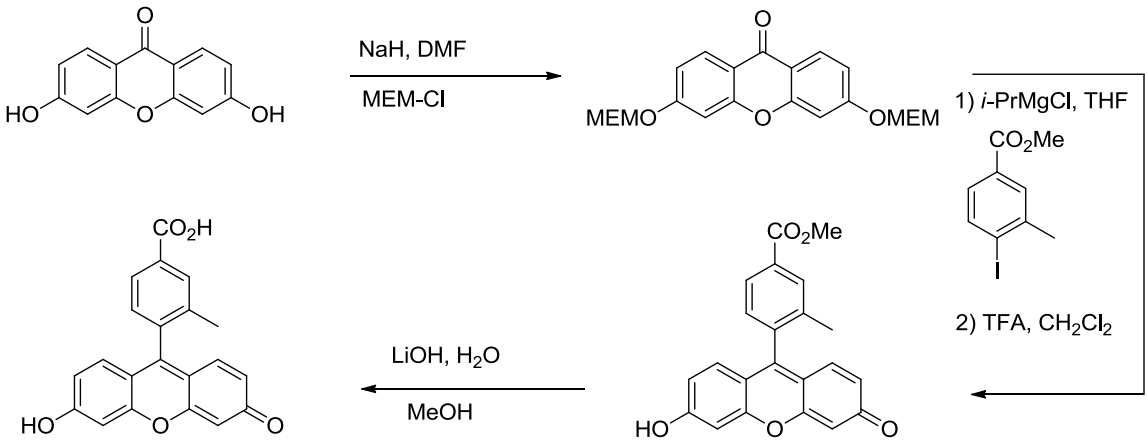
Scheme 1.3 Synthesis of Fluorescein

The mechanism of fluorescein synthesis involves the double Friedel-Craft's acylation of resorcinol using phthalic anhydride. The key step of ether bridge formation depends on conjugation.



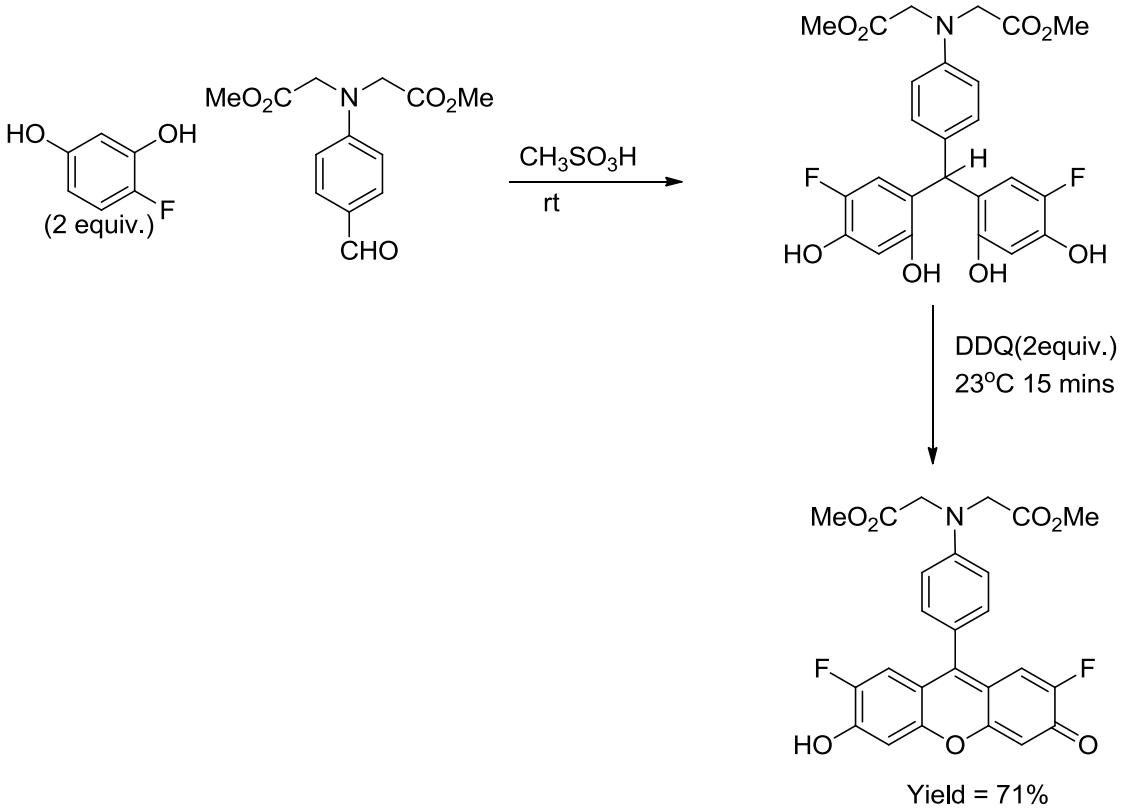
Scheme 1.4 Mechanism of Fluorescein formation

A second method to make fluorescein is the xanthone route. Several methods to convert 3,6-dihydroxyxanthones to derivatives of fluorescein have been reviewed in the literature.[12] The general method is firstly protect the oxygen of the hydroxyl groups in the 3,6-dihydroxyxanthone and add a Grignard's reagent after which an acid is applied as a dehydrating agent. In the scheme below a base lithium hydroxide is used to hydrolyze the ester to the free acid.[48]



Scheme 1.5 Formation of fluorescein derivative from 3,6-dihydroxyxanthone[48]

A third method to synthesize fluorescein derivatives is the condensation of aryl aldehydes and resorcinol using methanesulfonic acid leading to a triarylmethane intermediate which is the oxidized to the fluorescein derivative[13].

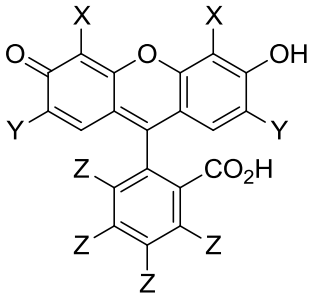


Scheme 1.6 Synthesis of fluorescein derivative using aldehyde

The conditions used here is mild and metal sensitive analogues of fluorescein can be prepared this way.

1.3.3 Effect of Fluorine on fluorescein

When fluorescein based dyes are used in assays especially as fluorescein conjugates there occur the problem of photobleaching[14] which is the loss of the fluorescent signal due to an irreversible photochemical reaction. The replacement of the hydrogen atoms in an organic molecule by fluorine results in a change in properties[15] due to the high electronegativity and small atomic radius of the atom. When the hydrogens in fluorescein was substituted with fluorine it resulted in reduction in the pKa values compared to the unsubstituted fluorescein. The lower pKa values increased to increased resistance to photo-bleaching and diminished quenching when the dye is conjugated to proteins.



1: X = Y = Z = H
2: X = H, Y = F, Z = H
3: X = Y = H, Z = F
4: X = H, Y = Z = F
5: X = Y = Z = F

Compound	Abs/Em (nm)	Quantum Yield	pKa
1	490/514	0.92	6.5
2	480/514	0.97	4.8
3	508/527	0.85	6.1
4	508/527	0.96	4.5
5	535/553	0.47	3.3

Table 1.1 Physiochemical properties of fluorinated fluoresceins

1.4 Xanthones

Xanthones are fluorescent organic compounds that are naturally occurring and whose general structure is depicted below. Over 1000 different types of xanthones have been reported in the literature.[16][17][18] The xanthone nucleus have been reported to show a wide range of biological and medicinal activities e.g. antifungal,[19] antimalarial,[20] antimicrobial,[21] antiparasitic,[22] anticancer,[23] and is able to inhibit HIV activity in cells.[24] Xanthones have also been used as a turn on fluorescent probe for metal ions.[32] The xanthone structure has also been described as a 'privileged structure' because the activity exhibited depends on the type and position of the substituents on the xanthone core.[25]

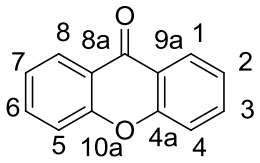


Figure 1.7 The structure of Xanthone core and numbering

Some examples of naturally occurring xanthones is shown below; these natural xanthones have been screened for drug activity.

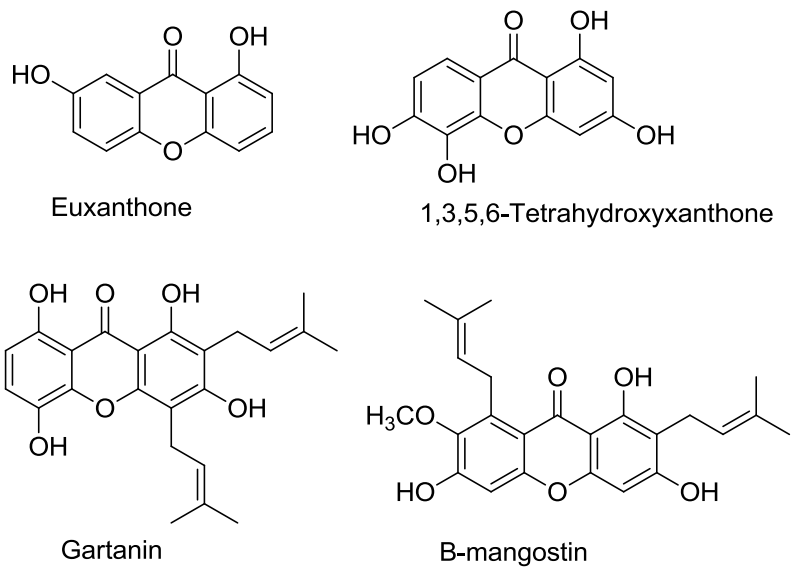


Figure 1.8 Structure of some naturally occurring xanthones

1.4.1 Classification of Xanthones

Xanthones isolated from natural products can be classified into six main groups with other subclasses. The classes includes simple xanthones which includes both oxygenated and non-oxygenated xanthones, xanthone glycosides, prenylated xanthones, xanthonolignoids, bisxanthones, and miscellaneous xanthones. Simple oxygenated xanthones is further divided into monooygenated xanthones, dioxygenated, trioxygenated xanthones, tetraoxygenated xanthones, pentaoxygenated xanthones and hexaoxygenated xanthones.[26]

Xanthone Glycosides

This group of xanthones have a glucose molecule attached to the core of the xanthone backbone and can be divided into 2 groups which includes C-glycosides and O-glycosides. In C-glycosides, C–C bond links the sugar moiety to the xanthone core and they are resistant to acidic and enzymatic breakdown due to the C-C bond strength while the O-glycosides have typical glycosidic bond linkage which are prone to hydrolysis. 2,-C-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone is

abundant in and was first isolated from *Mangifera indica.*[27] The common O-glycoside 3,7,8-trihydroxyxanthone-1-O-β-laminaribioside is gotten from the fern species.[28]

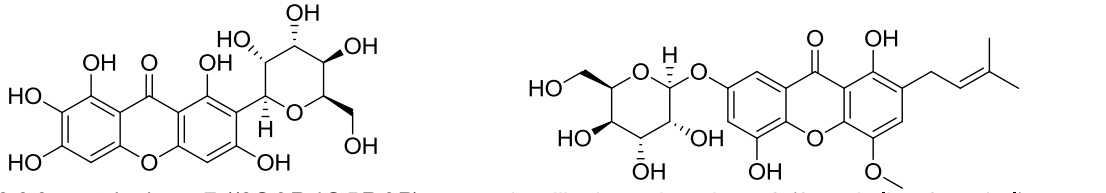


Figure 1.9 Structure of C-glycoside and O-glycoside

Prenylated Xanthones

Prenylated xanthones contains either a benzofuran or benzopyran ring fused to the xanthone core and are present in many natural products. These compounds also are bioactive. About 273 prenylated xanthones are known. caloxanthone O and caloxanthone P are two new prenylated xanthones that have been isolated from *Calophyllum inophyllum.*[29] Caloxanthone O was also reported to be cytotoxic activity against the human SGC-7901 cell line with the IC_{50} value of 22.4 μg mL^{-1}, caloxanthone P showed no activity on screening with the human gastric cancer cell line SGC-7901.[29]

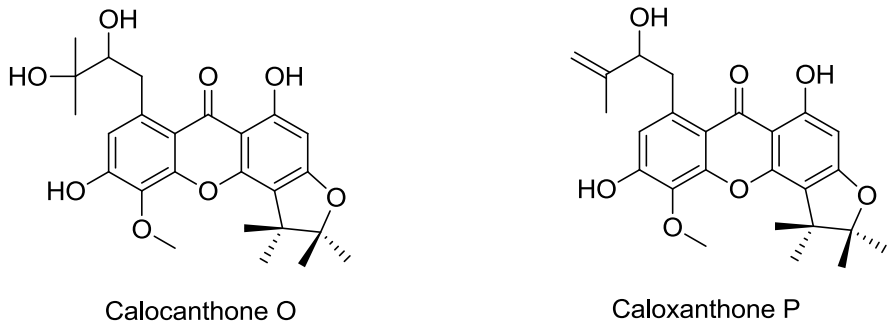
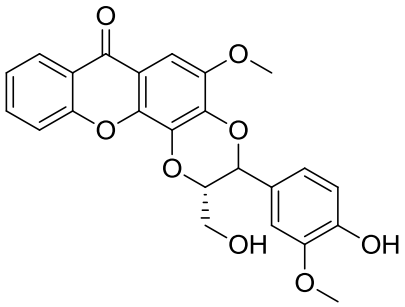


Figure 1.10 Structures of Caloxanthone O and Caloxanthone P

Xanthonolignoids

This numbers of xanthones in this class is small in numbers. The first xanthonolignoid isolated is from Kielmeyera species. The xanthonolignoid Kielcorin was isolated from *Kielmeyera variabilis.*[30]

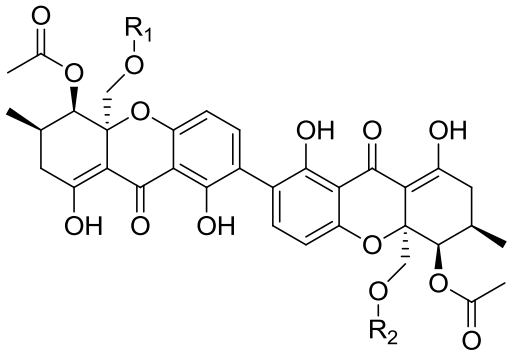


Kielcorin

Figure 1.11 Structure of Kielcorin

Bisxanthones

Twelve bisxanthones have been reportedly extracted from some higher plants, lichen and fungi. Examples include dicerandrols A, B and C were isolated from *Phomopsis longicolla.*[31]



Dicerandrol A: $R_1 = R_2 = H$
Dicerandrol A: $R_1 = Ac$ $R_2 = H$
Dicerandrol A: $R_1 = R_2 = Ac$

Figure 1.12 Structure of a bisxanthone

Other xanthones have been isolated that do not fall into any of the classes discussed and are generally classified as miscellaneous xanthones.

1.4.2 Selected Applications of Xanthones

The xanthone core have been reported to show a wide range of biological and medicinal activities e.g. antifungal,[19] antimalarial,[20] antimicrobial,[21] antiparasitic,[22] anticancer,[23] and the ability of xanthone containing compounds to inhibit HIV activity in cells have been investigated.[24] Xanthones have also been used as a turn on fluorescent probe for toxic anions and cations,[32] and has been applied as an insecticide.

1.4.2.1 Fluorescent Probe for Metal Ions

1,3,6-trihydroxyxanthone has been shown to selectively bind Pb^{2+} in the presence of other metal ions and in the process turning on the fluorescence of the compound.[32]

The emission spectra of the probe discriminating against other metal ions in the presence of Pb^{2+} is shown below

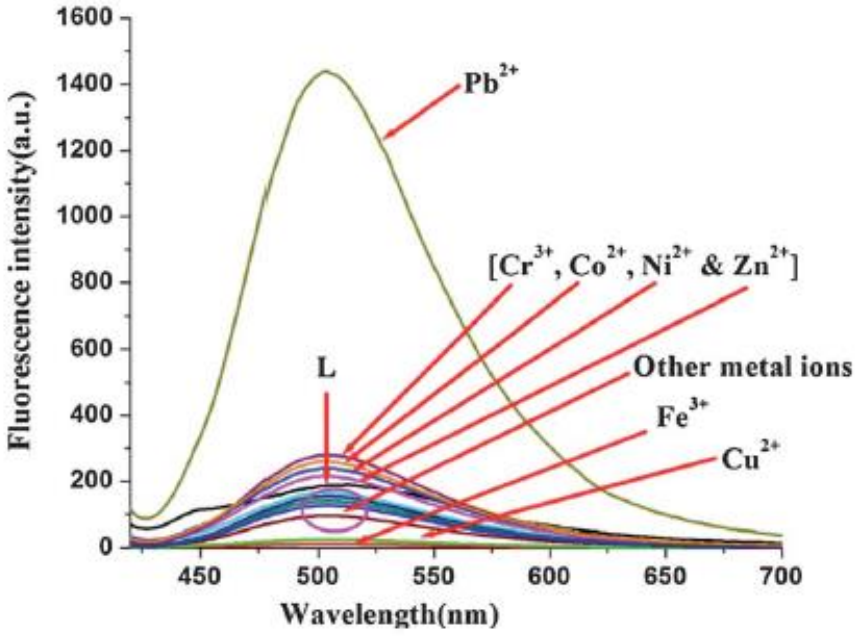
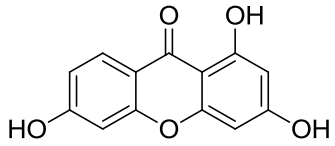


Figure 1.13 Discrimination of Pb2+ in the presence of other cations (100μM) by L (1 μM)

L = 

The proposed stoichiometry of L to Pb^{2+} was proposed to be 2:1

1.4.2.2 Antifungal Activity

Simple monooxygenated, deoxygenated and trioxygenated xanthones were screened against yeast cells (*C. albicans*, *C. glabata*, *C. neoformans*) for their inhibitory effect.[33] It was discovered that some of the xanthones tested exhibited strong inhibitory effects against those yeast cells with MIC values <10μg mL^{-1}. Some of the xanthones that were inhibited the yeast cells includes 2-hydroxyxanthone, 3-hydroxyxanthone, 3-hydroxyxanthone, 1,2-dihydroxyxanthone and 3,4-dihydroxyxanthone.

1.4.2.3 Antitumor Activity

Several natural and synthetic xanthones containing hydroxyl and or prenyl groups have been investigated for their antitumor activity against human cell lines (HepG2, HCT-116, A549, BGC823, and MDA-MB-231.[34] When the cancer cells were incubated for 48 hours with the xanthones, the compounds shown below with prenyl groups suppressed their growth with IC50 values ≤ 10μM.[34]

	IC50 (μM)				
Compound	HepG2	HCT-116	A549	BGC823	MDA-MB-231

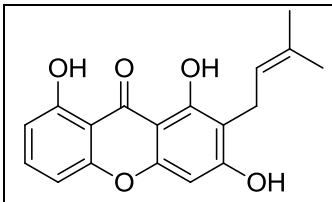
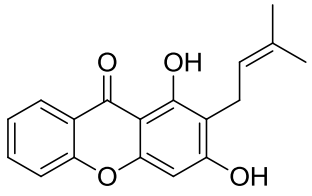
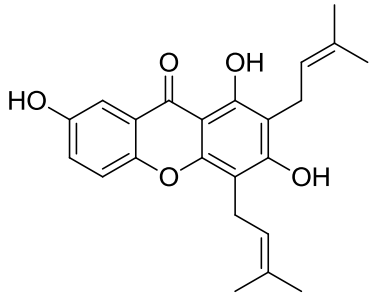
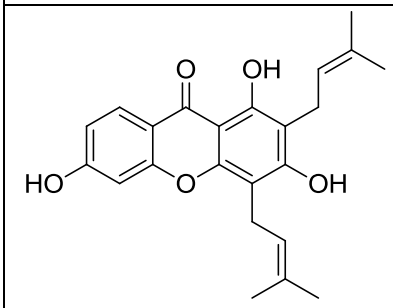
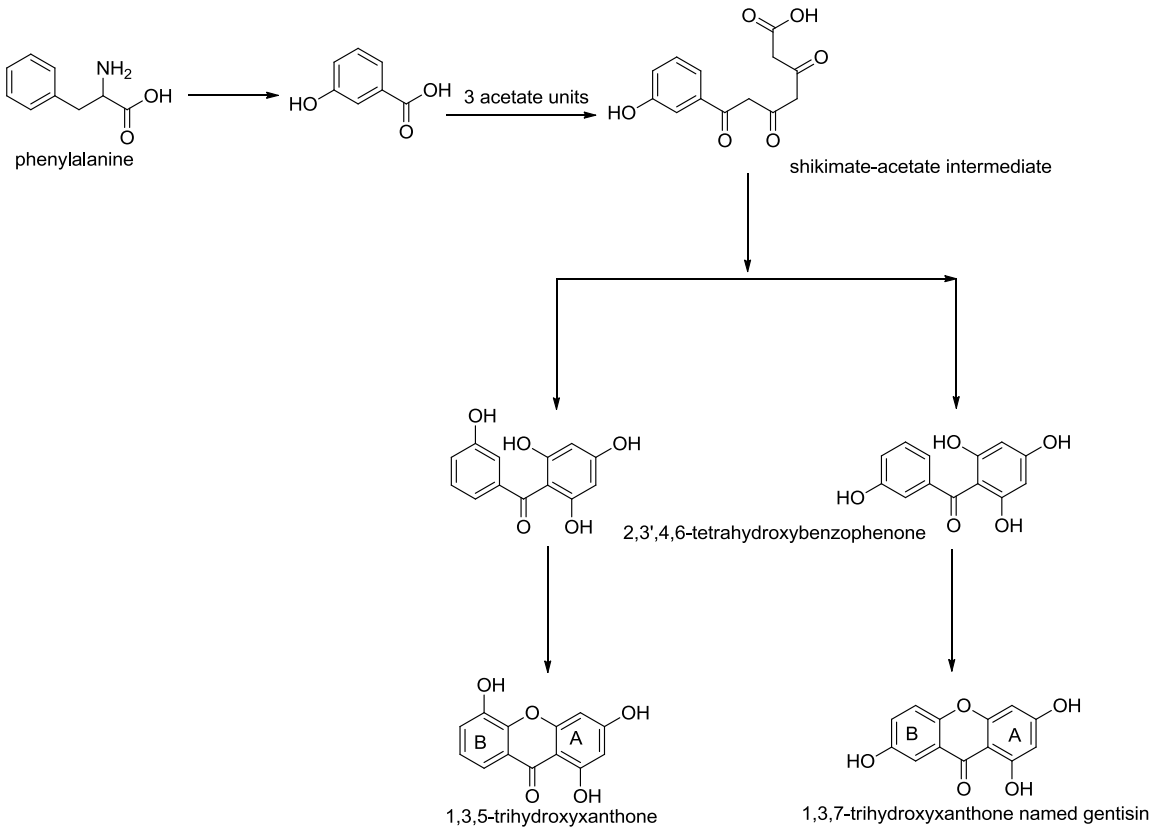
	1.49	18.5	1.96	6.72	11.1
	9.81	71.1	20.6	47.5	44.4
	16.1	8.35	8.85	73.4	>100
	18.7	6.15	9.23	10.0	25.3

Table 1.2 Antitumor activity of Xanthones

1.4.3 Biosynthesis of Xanthones

The amino acid phenylalanine derived from shikimate is the precursor in the biosynthesis of xanthones.[35] The amino acid loses two carbon atoms from the side-chain and is oxidized to form *m*-hydroxybenzoic acid. The *m*-hydroxybenzoic acid combines with three units of acetate to form the

shikimate-acetate intermediate, which then undergoes a ring closure to form the benzophenone that is further undergoes oxidative coupling catalyzed by enzymes to form the xanthone core. The benzophenone can undergo condensation to form the xanthone in two ways, it is either the attack is ortho or para to the hydroxyl group in ring B hence forming two different products. The mechanism for the pathway has been elucidated by experiments using plants fed with labelled ^{14}C phenylalanine and ^{14}C labelled acetate.[36] The final step is an oxidative coupling reaction which has also been applied to synthesize xanthones.[45]



Scheme 1.7 Biosynthesis of Xanthones from Phenylalanine

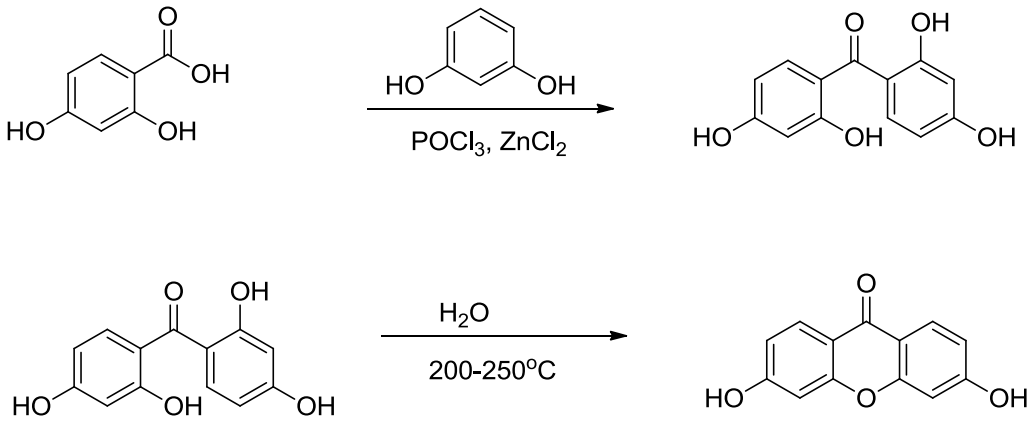
1.4.4 Synthesis of Xanthones

Naturally occurring xanthones are not readily available for drug studies due to the fact that the sources are limited, isolation and purification complexities, and substituent positions in the

xanthone core. Hence there is the dire need to synthesize these xanthones to provide a huge library of multi-substituted xanthones for structure activity relationship studies and other uses. Several methods to prepare this important class of compounds is presented in this report.

1.4.4.1 Friedel-Crafts Acylation

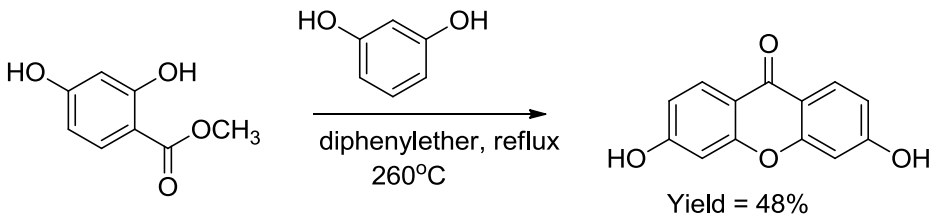
One of the earliest synthesis of xanthones is the $POCl_3$ mediated acylation of resorcinol by derivatives of benzoic acid using $ZnCl_2$ as a catalyst reported in 1955.[37] The resulting benzophenone undergoes cyclization in water at high temperature and pressure. An example of this scheme is the reaction of 2,4-dihydroxybenzoic acid and resorcinol in the presence of $POCl_3$ and $ZnCl_2$ to give 2,2',4,4'-tetrahydroxybenzophenone and the condensation of the benzophenone to give 3,6-dihydroxyxanthone.



Scheme 1.8 Condensation of Resorcinol and dihydroxybenzoic acid

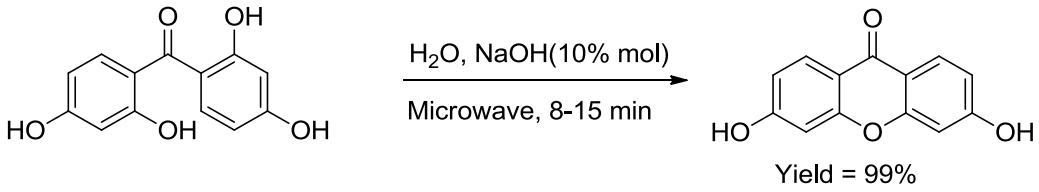
The yields using this procedure have been improved using P_4O_{10} and Eaton's reagent.[38]

This procedure does not always give the desired products and sometimes demethylation may occur in benzophenones that has methoxy groups. A drawback however is that microwave assisted synthesis cannot be applied for large scale reactions. High temperature can be applied instead of acid: hydroxylated methylethylbenzoates and resorcinols undergo thermal condensation in refluxing diethylether to give the corresponding hydroxyxanthones.[39]



Scheme 1.9 Xanthones from condensation of benzoates and resorcinol in diphenylether

Apart from the very harsh reaction conditions, the yield reported using this procedure is relatively poor. Microwaves have also been applied to cyclize the benzophenones to xanthones[40]



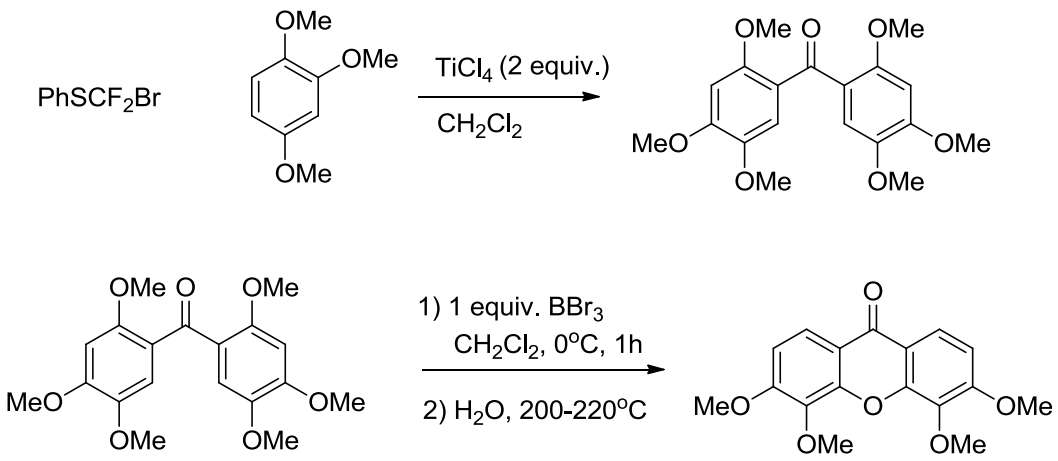
Scheme 1.10 Microwave assisted synthesis of xanthones in water

1.4.4.2 C1 Coupling Strategy

Coupling of two substituted resorcinols to a C1 electrophile can lead to xanthones. The advantage of this strategy is seen where it is difficult to prepare both precursors for the Friedel-Craft's approach.[41]

Friedel-Crafts alkylation using bromodifluoro(phenylsulfanyl)methane

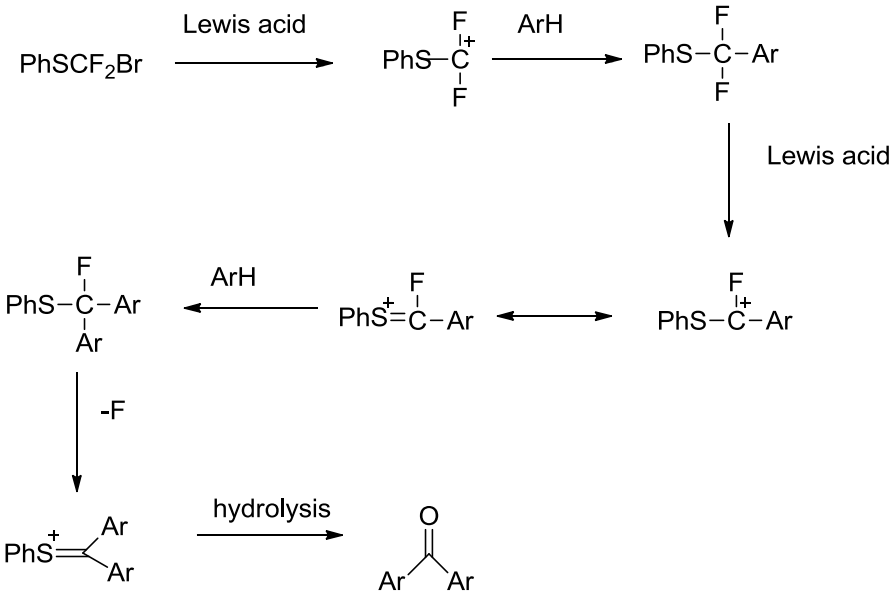
Benzophenones were synthesized by the double Friedel-Craft's acylation of various aromatics using bromodifluoro(phenylsulfanyl)methane and a lewis acid.[42] A very stable α,α-difluorocarbocation is generated by the reaction of the bromodifluoro(phenylsulfanyl)methane and the lewis acid which is then used to the cyclization reaction.[42]



Scheme1.11 Double Friedel-Craft's acylation using bromodifluoro(phenylsulfanyl)methane

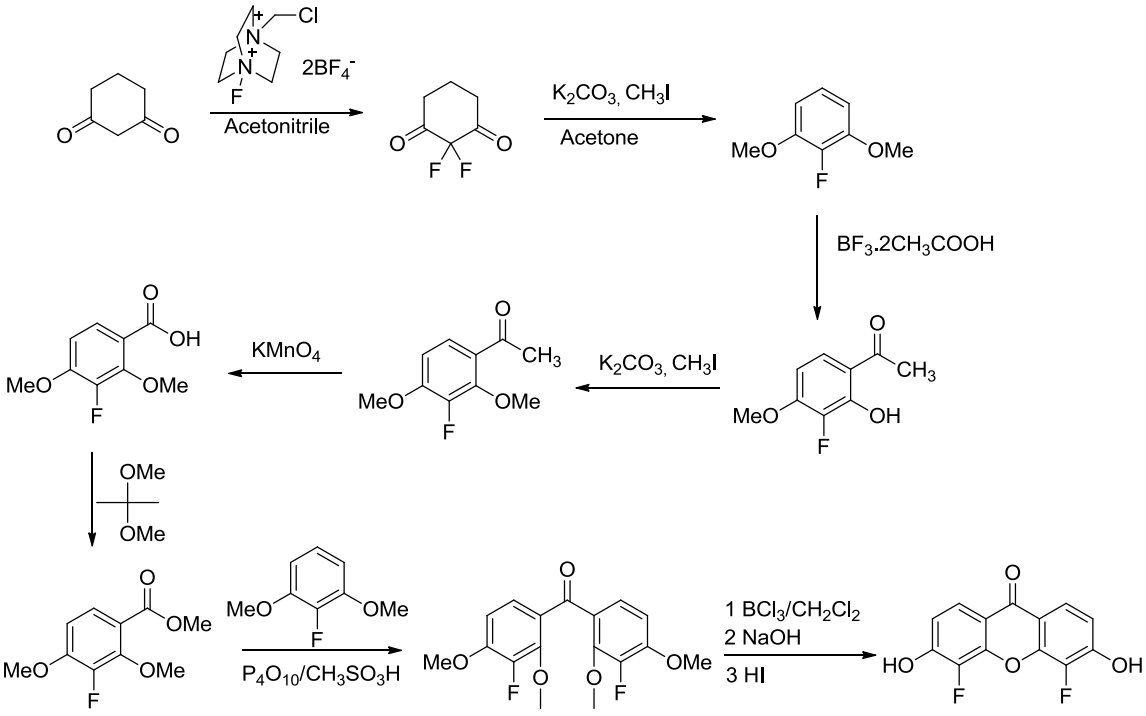
The substituted benzophenone acquired this way is then converted into the xanthone by the selective ortho-demethylation[43] followed by condensation in water in a sealed tube. A major disadvantage of this procedure is that the starting material is either expensive or are not commercially available.

The strategy adopted here is the use of difluoro(phenylsulfanyl)methane which is an excellent reagent for the generation of the α,α-difluorocarbocation which is a very stable carbocation by reacting with a lewis acid. Trapping the intermediate carbocation with an electron rich aromatic provided a succinct route for the formation of a C-C bond, and hydrolysis leads to benzophenone, convertible to xanthones.



Scheme 1.12 C-C formation using difluoro(phenylsulfanyl)methane

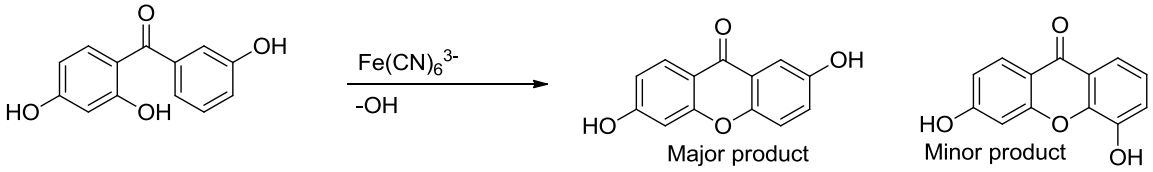
A similar C1 coupling reaction leading to xanthone formation was done using a boron trifluoride–acetic acid complex.[44] The Fluorine on the xanthone core could impart interesting properties on the compound that is why fluorinated compound is desirable[14].



Scheme 1.13 C1 Coupling Using boron trifluoride–acetic acid complex

1.4.4.3 Oxidative coupling of phenols

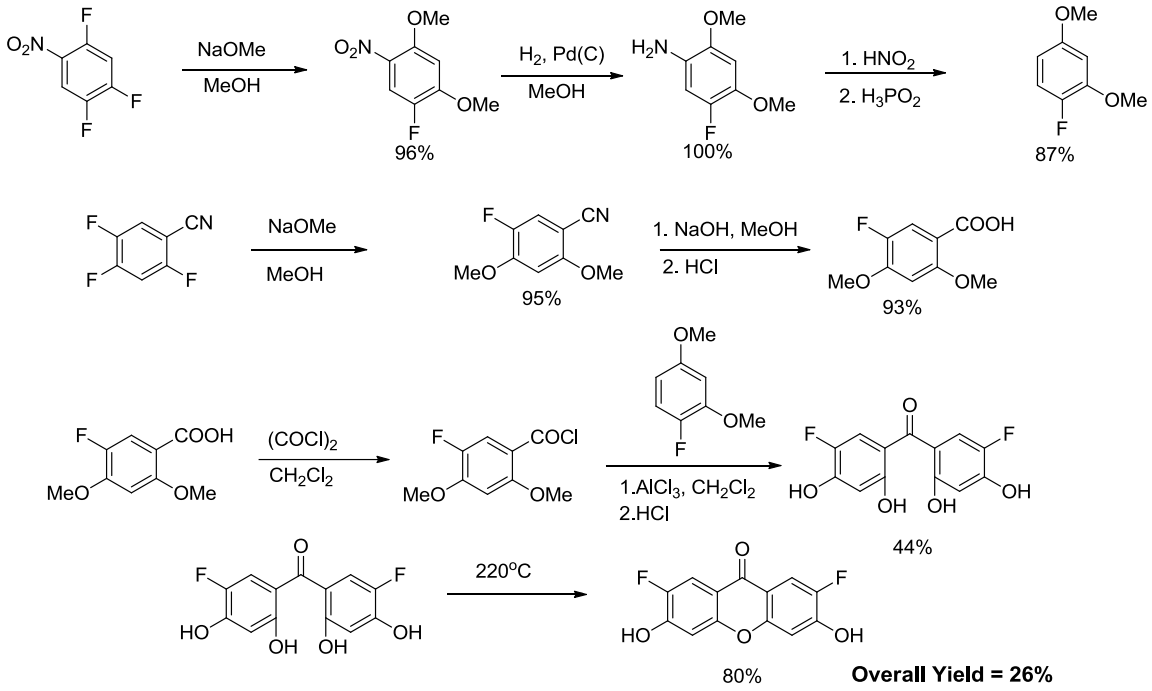
The phenolic oxidative coupling reaction is an important reaction that has been extensively studied and has been reported to be an important step in the biosynthesis of naturally occurring compounds.[45] This process is similar to the mechanism of the biosynthesis of xanthones earlier presented. Lewis and his coworkers first reported the oxidative coupling reaction of 2,3',4-trihydroxybenzophenone using alkaline ferricyanide to give 2,6-dihydroxyxanthone as the major product and 3,5-dihydroxyxanthone as a minor product.[46]



Scheme 1.14 Oxidative coupling of 2,3',4-trihydroxybenzophenone

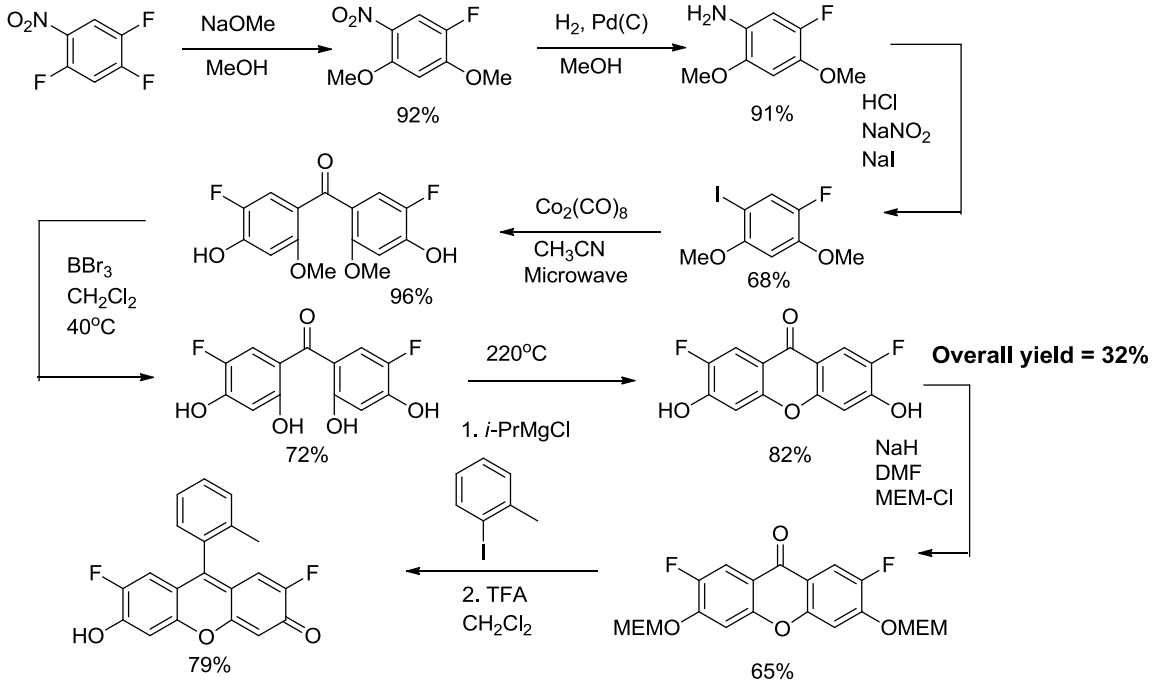
1.4.5 Fluorine Substitution

Xanthones are particularly valuable as precursors to fluorescein derivatives. The synthesis of a fluorinated xanthone starts with the synthesis of fluorinated benzophenone derivative as reported by David S. Lawrence and co-workers.[47] In the scheme shown below both fluorinated starting materials are expensive. Our method uses a relatively cheaper starting materials in less steps.



Scheme 1.15 8-steps fluorinated benzophenone synthesis

The benzophenone was synthesized in an improved 5 step sequence was done by Blake R. Peterson and co-workers[48]staring with 2,4,5-trifluoronitrobenzene.



Scheme 1.16 Improved 5 steps synthesis of benzophenone

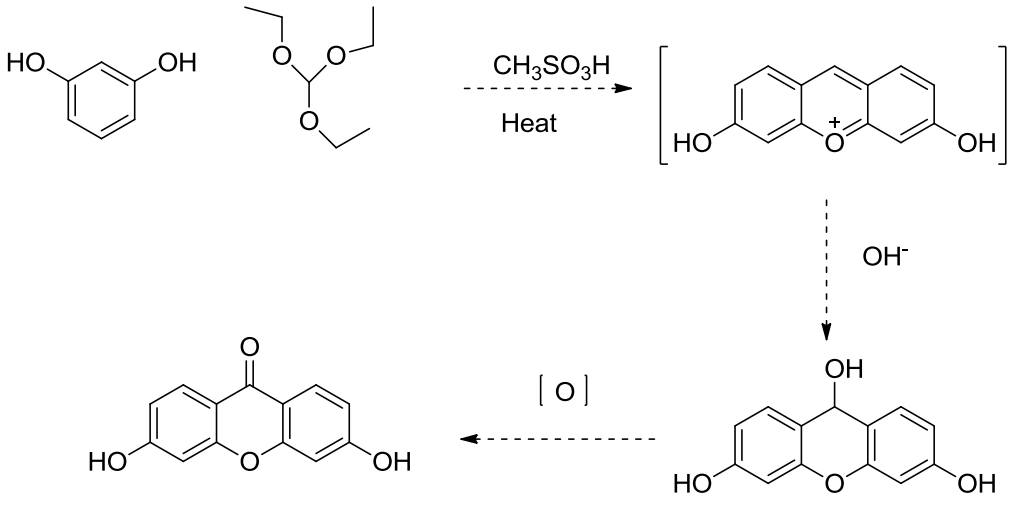
The benzophenone was then heated in a sealed tube to 200^{o}C forming the xanthone.[49]

2. RESULT AND DISCUSSION

Given the utility of fluorescein and the great versatility their precursor xanthones, we set about to devise a better preparation of xanthones. As these substance can be fluorescent in their own right, we also decided to investigate the fluorescence of various substituents.

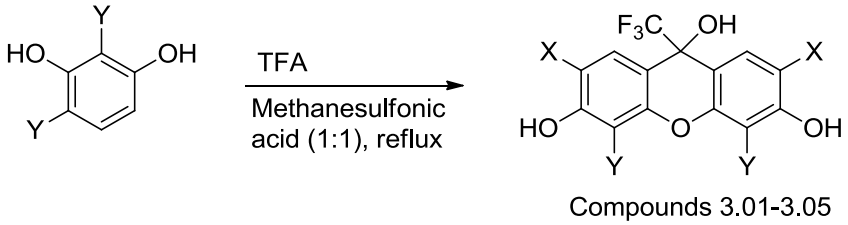
We report here a simple and efficient procedure using readily available reagents to effect acylation of resorcinol molecules by a C_1 equivalent leading to the formation of fluorescent xanthones. A key step in our procedure leading to the formation of 3,6-dihydroxyxanthones is the formation of a diarylmethyl cation as an intermediate. Trifluoroacetic acid behaves as the electrophile in this process and as the C1 equivalent. Simply heating at reflux a solution of resorcinol in 1:1 trifluoroacetic acid/methanesulfonic acid leads, after aqueous quench, to trifluoromethylcarbinols **3.01-3.05**.

Our initial strategy to make these fluorescent xanthones is using triethyl orthoformate in a fashion similar to how fluorescein is made using phthalic anhydride. We had hoped that quenching the intermediate in water would lead to the alcohol after which it can then be oxidized to the ketone.



Scheme 2.1 Initial strategy for xanthone formation

The initial reaction with resorcinol, triethyl orthoformate and with methanesulfonic acid and TFA in a 1:1 ratio did not give the product as expected. We got the trifluorocarbinol compounds **3.01** contaminated with some unidentified material which exhibits yellow fluorescence under UV. The trifluorocarbinol **3.01** looked promising so we moved forward with it as we did not investigate the yellow fluorescent contaminant. It was discovered that triethyl orthoformate was not used to form the trifluorocarbinol compound so we excluded it from the scheme going forward. The scheme below shows the transformation. We knew it was not the dehydrated form because it was not fluorescent and as confirmed by MS, IR and further alkylation reactions.



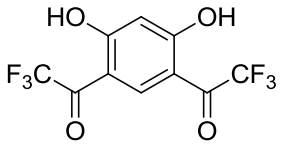
Scheme 2.2 Formation of trifluoromethylcarbinol

The transformation involves Friedel-Crafts acylation, Friedel-Crafts alkylation, and cyclization of the resulting diarylmethylcation in a manner mechanistically equivalent to the formation of fluorescein, with trifluoroacetic acid playing the role of phthalic anhydride. The trifluorocarbinols compounds **3.01**, **3.02**, **3.03, 3.04** and **3.05** on initial screening has emission max of 571nm, 620nm, 559nm, 554nm, and 590nm respectively.

Entry	X	Y	Yield (%)
1	H	H	80
2	Cl	H	98
3	H	CH_3	72
4	F	H	77
5	C_6H_{13}	H	94

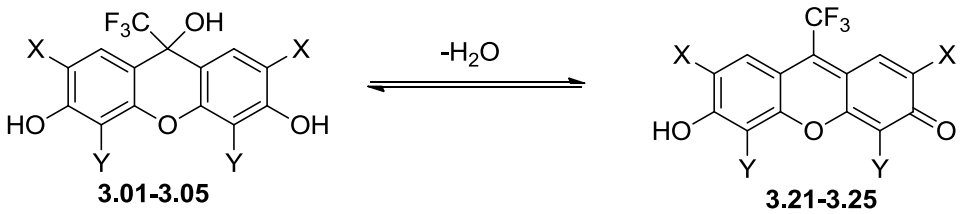
Table 2.1 Emission and yields of trifluoromethylcarbinols.

Using the same reflux conditions TFA/methanesulfonic acid, reflux) for n-hexylresorcinol did not give the desired product. Our interpretation based on the mass spectra of the compound is that the structure below was formed.



A possible explanation is the high reactivity of the phenol due to the long chain electron donating alkyl group. The reaction was done at room temperature using the same acid ratio. The desired product was isolated with a very high yield.

Table 2 shows that this procedure is effective with several substituents. Interestingly, the trifluoromethylcarbinol compounds appear primarily in the carbinol form, rather as in the dehydrated form that is fluorescent, as shown by MS, IR and alkylation reactions.



3.01-3.05 **3.21-3.25**

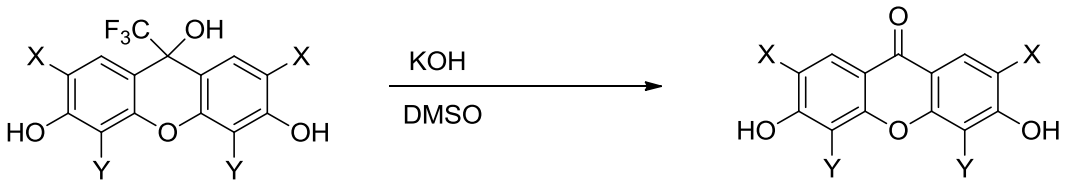
Scheme 2.3 Trimethylcarbinol and the dehydrated form

The CF_3 group favors sp3 over sp2 hybridization, encouraging formation of trifluoromethylcarbinol at the expense of the fluorescent dehydrated form. The powerfully electron withdrawing CF_3 group also lowers the pKa of these xanthines, facilitating deprotonation in neutral solution. Despite the low content of dehydrated fluorescent form, these are intensely colored compounds with significant fluorescence. Solutions of the trifluoromethylcarbinols **3.01-3.05** constitute slow-release forms of highly fluorescent form **3.21-3.25**, we speculate that these

compounds will be resistant to photobleaching since the major form is highly photostable. Fluorosensor molecules based on this chromophore may benefit from such stability.

Also noteworthy is the fact that phenols containing substituent at the 5 position (5 methyl resorcinol and 1,3,5-trihydroxyphenol) did not give the desired product. A possible explanation is the hindrance that would result between the trifluoromethyl group and the substituent on the 5 position. Friedel-Craft's acylation is rarely efficient at a site with two ortho substituents.

Conversion of substances trifluoroarylmethylcarbinols into xanthones requires removal of the CF_3 group. KOH in DMSO cleanly converts the carbinols to their corresponding xanthones, isolated by precipitation from acidic H_2O.



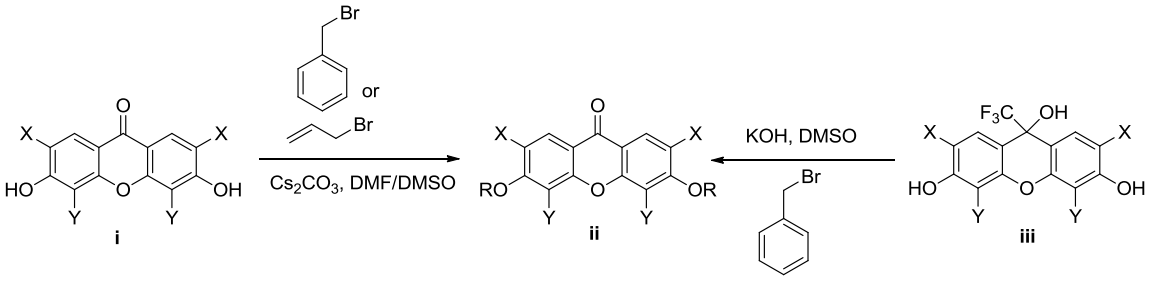
Scheme 2.4 Conversion of the trifluoromethylcarbinols to xanthones

Entry	X	Y	Yield (%)	Emλ_{max} (pH 7)
1	H	H	96	443nm
2	Cl	H	99	440nm
3	H	CH_3	90	499nm
4	F	H	94	447nm
5	C_6H_{13}	H	88	544nm

Table 2.2 Emission and yields of xanthones

In those cases where precipitation of the xanthones is not high yielding, extraction with ethylacetate gives product in very high yields. Because of the high stability of the xanthones, we have carried out the elimination at reflux in DMSO, allowing very short reaction times. However, even at 25˚C with 1M KOH, reaction was complete in approximately 1 h in most cases except in the 2,7-difluoro derivative where stirring overnight is required.

Fluorescein derivatives have been obtained by Grignard reagent addition to protected xanthones.[12] Simply adding alkylating agent to the DMSO solution after CF_3 cleavage leads in high yield to alkylated xanthones in a one-pot process from the trifluoromethylcarbinol compounds. We also alkylated the already worked up xanthones using potassium hydroxide which also lead to high yields of products. Alkylating agents used is allyl bromide and benzyl bromide. Interestingly, this sequence is more facile than the reverse order: alkylation of the xanthone with allyl bromide and Cs_2CO_3 in DMSO cleanly forms the product, which on treatment with KOH in DMSO is completely stable to 110˚C, conditions that convert the phenolic form to ketone, suggesting the intermediate that expels CF_3^- is a polyanion.

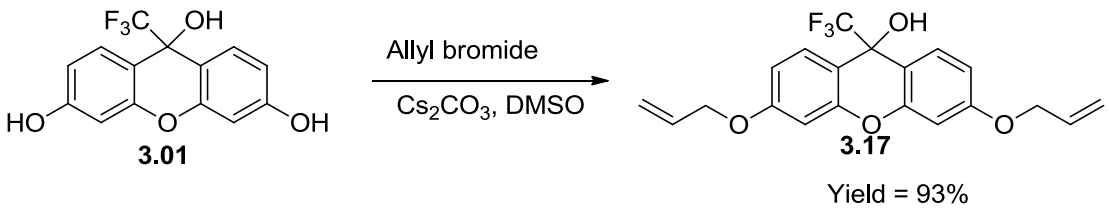


Scheme 2.5 Alkylation of Xanthones and Trifluorocarbinols

X	Y	R	Yield i (%)	Yield ii (%)
H	H	CH_2CHCH_2	91	
Cl	H	CH_2CHCH_2	86	
F	H	CH_2CHCH_2	76	
F	H	$C_6H_5CH_2$	98	91
H	H	$C_6H_5CH_2$		91
C_6H_{13}	H	$C_6H_5CH_2$	92	

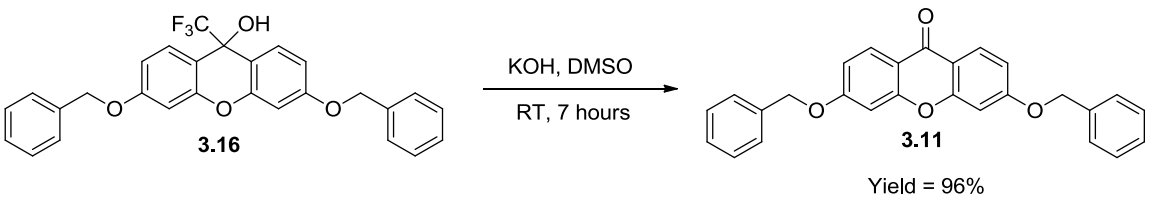
Table 2.3 Alkylation of the xanthones and yields

The trifluoromethylcarbinol obtained when X and Y = H was also alkylated using benzyl bromide and allyl bromide in excellent yields.



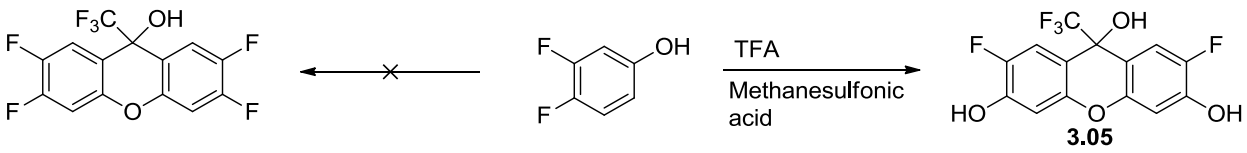
Scheme 2.6 Alkylation of trifluorocarbinol compound

We wanted to know whether the hydroxyl group of the phenol is necessary CF_3 elimination to take place. Under the same reaction condition **3.16** was converted to **3.11** after stirring at room temperature for 7 hours compared to 60 minutes it took the free phenol to completely react. The free phenol makes the reaction goes faster compared to the protected phenol.



Scheme 2.7 Conversion of protected trifluorocarbinol to ketone

One advantage of this process is the ease with which 3,6-disubstituted 2,7-dihydroxyxanthones may be prepared from a single 4-substituted resorcinol. Among related xanthine derivatives, the 3,6-difluoro derivatives are highly prized, as the fluorines lower pKa values to a convenient range without quenching of fluorescence and also diminish photobleaching. However, 4-fluororesorcinol is relatively expensive, despite several recent improvements in its preparation.[1] We wondered whether the high reactivity of carbocation intermediates caused by CF_3 substitution would allow preparation of the desired fluorinated xanthones without use of the 4-fluororesorcinol.



Scheme 2.8 Preparation of 2,7-difluoro-3,6-dihydroxyxanthone

3,4-difluorophenol was treated with trifluoroacetic acid/methanesulfonic acid in the hope of preparing 2,3,6,7-tetrafluoroxanthone, which was expected to give 2,7-difluoro-3,6-dihydroxyxanthone on treatment with KOH in DMSO by nucleophilic aromatic substitution of the fluoroxanthone In the event, treatment of 3,4-difluorophenol, with trifluoroacetic acid/methanesulfonic acid under our standard conditions led in high yield to product with a yield of **77%**, and upon KOH/DMSO treatment to the corresponding xanthone, the compound that would be formed from 4-fluororesorcinol! This is noteworthy because the price of 3,4-difluorophenol is *ca.* 4% that of 4-fluororesorcinol. On treating 3-chloro-4-fluorophenol with a 1:1 ratio of TFA and methanesulfonic acid, It was found that the rate of reaction was slow. After refluxing for 4 days and work up it was mostly starting material that was isolated even though NMR indicated the same product was formed. We speculate that the CF_3-substituted carbocation intermediate is so reactive it undergoes nucleophilic aromatic substitution with trifluoroacetic acid as the nucleophile. We believe such substitution precedes aqueous quench, since quenching into CH_3OH instead of H_2O leads to the diphenol, rather than the trimethyl ether. However, we have no data to suggest whether substitution of F precedes the similar cyclization by hydroxy substitution.

The spectrum that follows show the emission of the xanthines and xanthones followed by the absorbance of the xanthines and xanthones. **3.01, 3.02, 3.03, 3.04** and **3.05** and the xanthones **3.06, 3.07, 3.08, 3.09** and **3.10** at pH 2, 7 and 9. Samples were prepared in 1cm path length quartz cells with absorbance less than 1.5 at the excitation and all emission wavelengths to uniformly illuminate across the sample, and to avoid the inner-filter effect. As seen from the spectrum the anionic forms of the xanthones displayed the highest fluorescence as determined by the number of

counts. The neutral forms were also fluorescent and the cationic forms were the least fluorescent forms. The spectrum is diaplayed this way for easy comparison of the emission and absorbance of the xanthines and xanthones and to see the effect of substitutuin. The concentration of xanthines and xanthones used for this study is 100μM and 10μM respectively.

At pH 2, Compound **3.01** had $A_{268nm} = 4400M^{-1}cm^{-1}$

Compound **3.02** had $A_{265nm} = 3400M^{-1}cm^{-1}$

Compound **3.03** had $A_{301nm} = 8600M^{-1}cm^{-1}$ and another band at $A_{519nm} = 5200M^{-1}cm^{-1}$

Compound **3.04** had $A_{280nm} = 7700M^{-1}cm^{-1}$

Compound **3.05** had $A_{276nm} = 7300M^{-1}cm^{-1}$

At pH 7, Compound **3.01** had $A_{269nm} = 5100M^{-1}cm^{-1}$

Compound **3.02** had $A_{273nm} = 3200M^{-1}cm^{-1}$

Compound **3.03** had $A_{301nm} = 8400M^{-1}cm^{-1}$

Compound **3.04** had $A_{286nm} = 7300M^{-1}cm^{-1}$, and $A_{503nm} = 1700M^{-1}cm^{-1}$

Compound **3.05** had $A_{279nm} = 7800M^{-1}cm^{-1}$

At pH 9, Compound **3.01** had $A_{279nm} = 7800M^{-1}cm^{-1}$

Compound **3.02** had $A_{281nm} = 900M^{-1}cm^{-1}$, and $A_{569nm} = 780M^{-1}cm^{-1}$

Compound **3.03** had $A_{280nm} = 10200M^{-1}cm^{-1}$

Compound **3.04** had $A_{301nm} = 11300M^{-1}cm^{-1}$,

Compound **3.05** had $A_{294nm} = 8600M^{-1}cm^{-1}$

A methyl group at the 4 and 5 position of the xanthine core generally leads to a much lower extinction coefficient.

At pH 2, **3.06** had $A_{322nm} = 64000M^{-1}cm^{-1}$, At pH 7, **3.06** had $A_{329nm} = 50000M^{-1}cm^{-1}$ and at pH 9 3.06 had $A_{370nm} = 84000M^{-1}cm^{-1}$ and $A_{308nm} = 20000M^{-1}cm^{-1}$

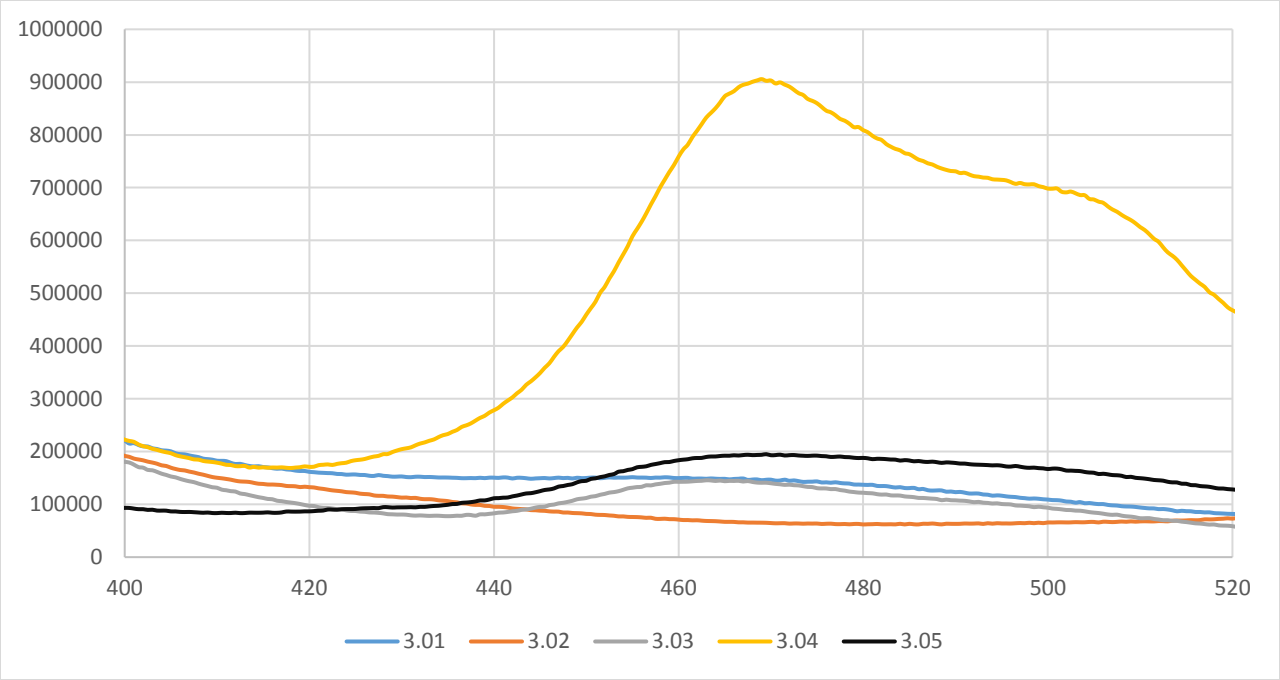


Figure 2.1 Emission spectra of xathines at pH 2

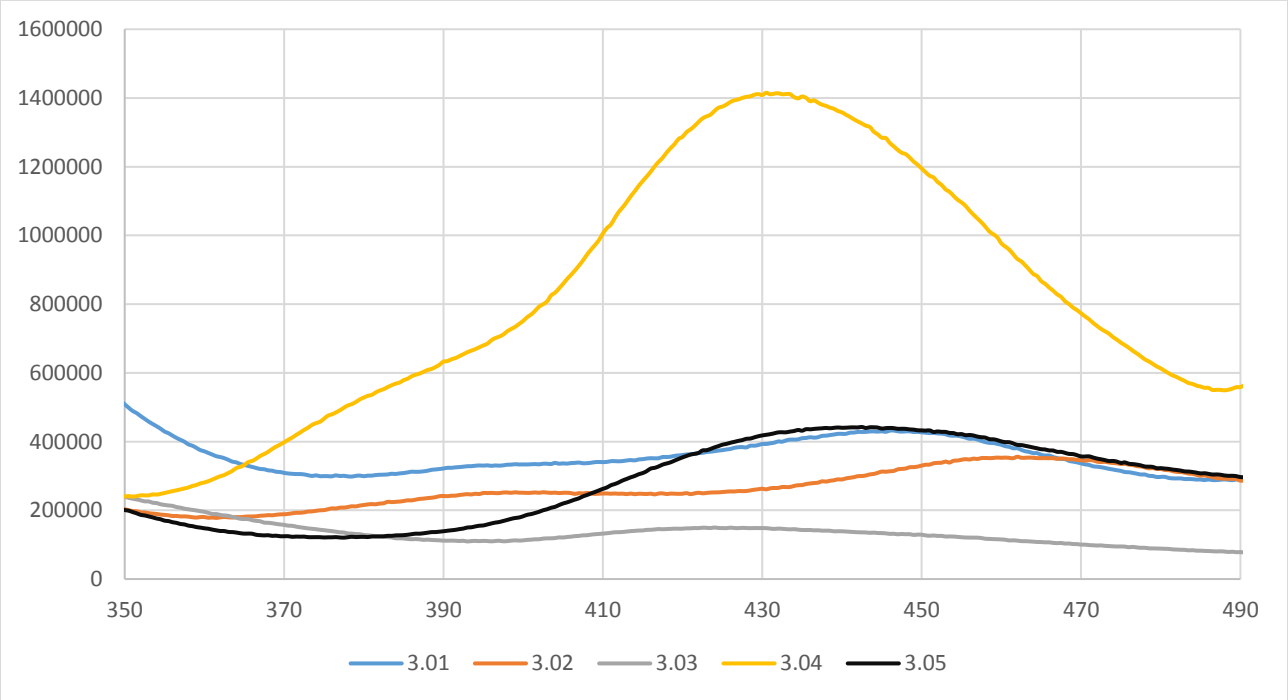


Figure 2.2 Emission spectra of the Xanthines at pH 7

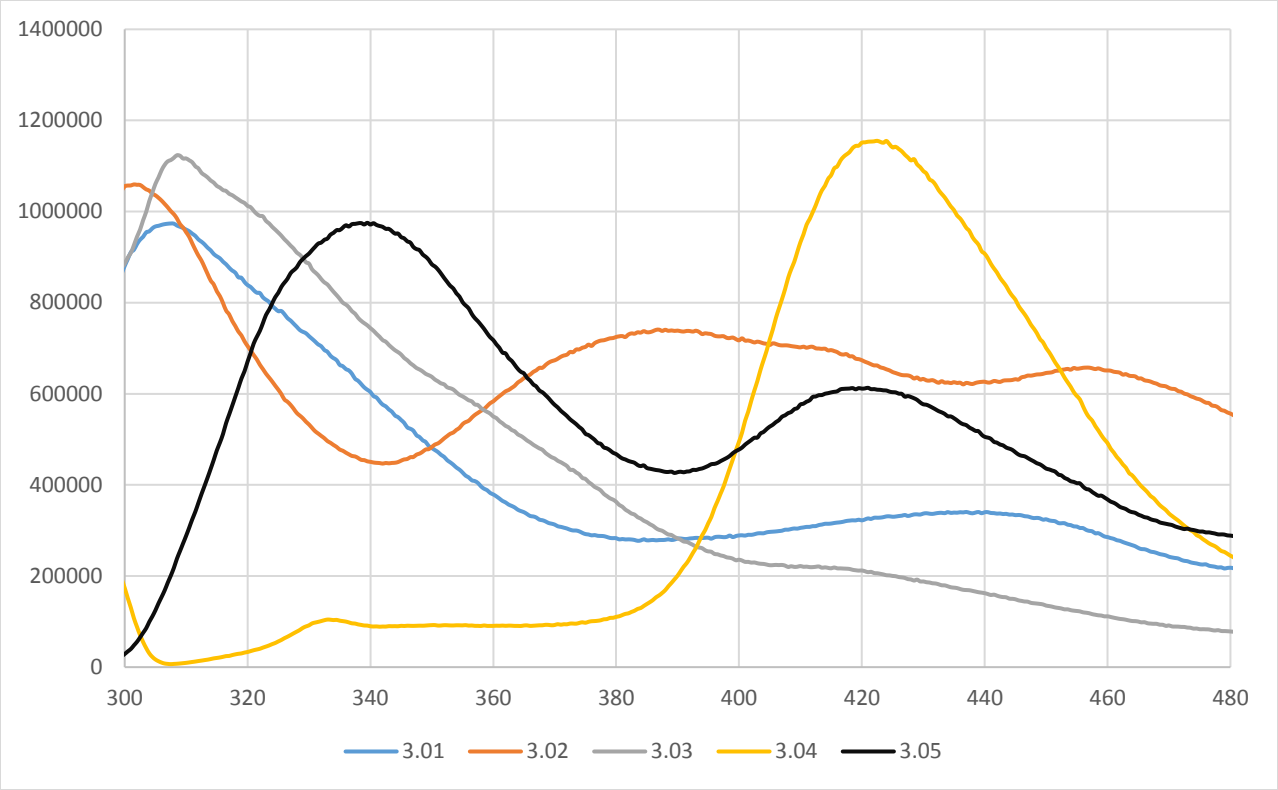


Figure 2.3 Emission spectra of the Xanthines at pH 7

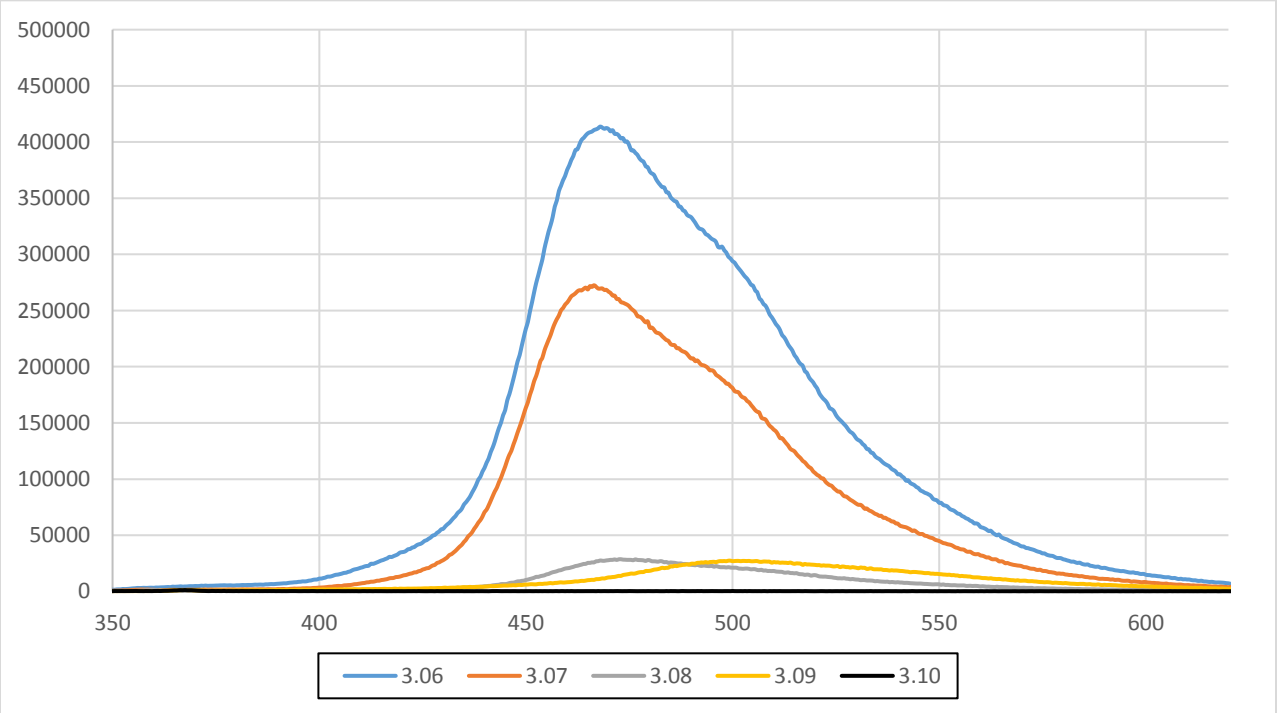


Figure 2.4 Emission spectra of the Xanthones at pH 2

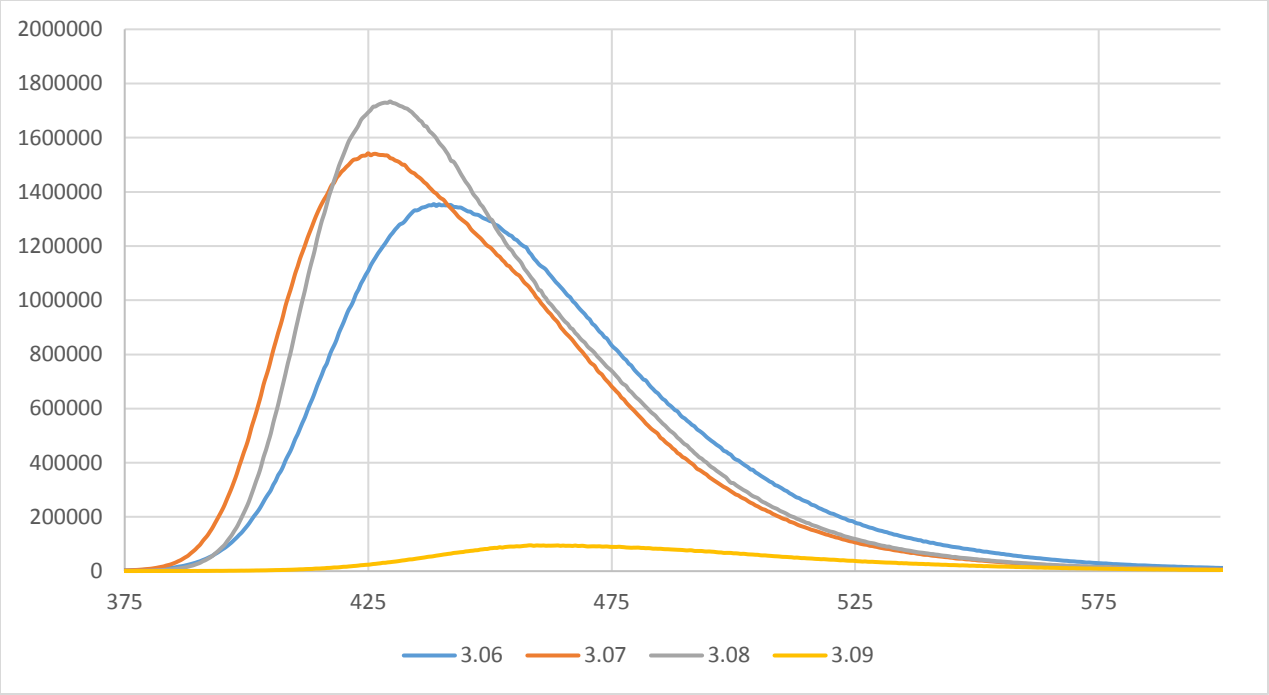


Figure 2.5 Emission spectra of the Xanthones at pH 7

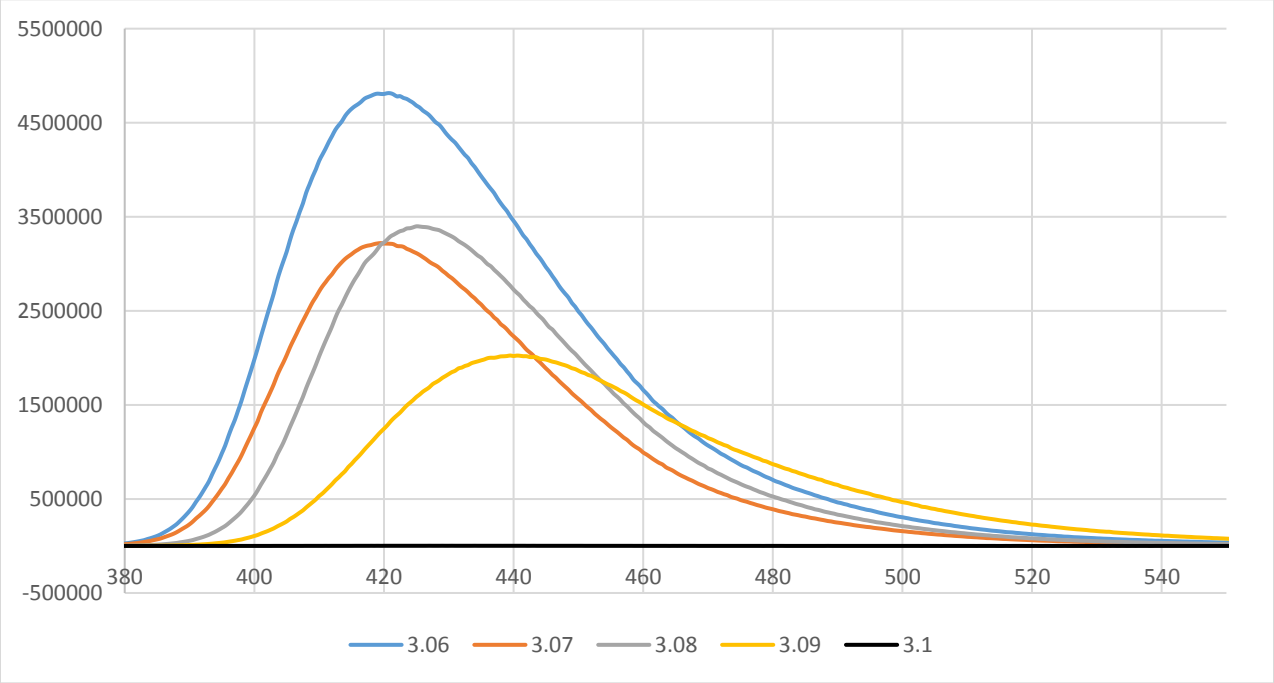


Figure 2.6 Emission spectra of the Xanthones at pH 9

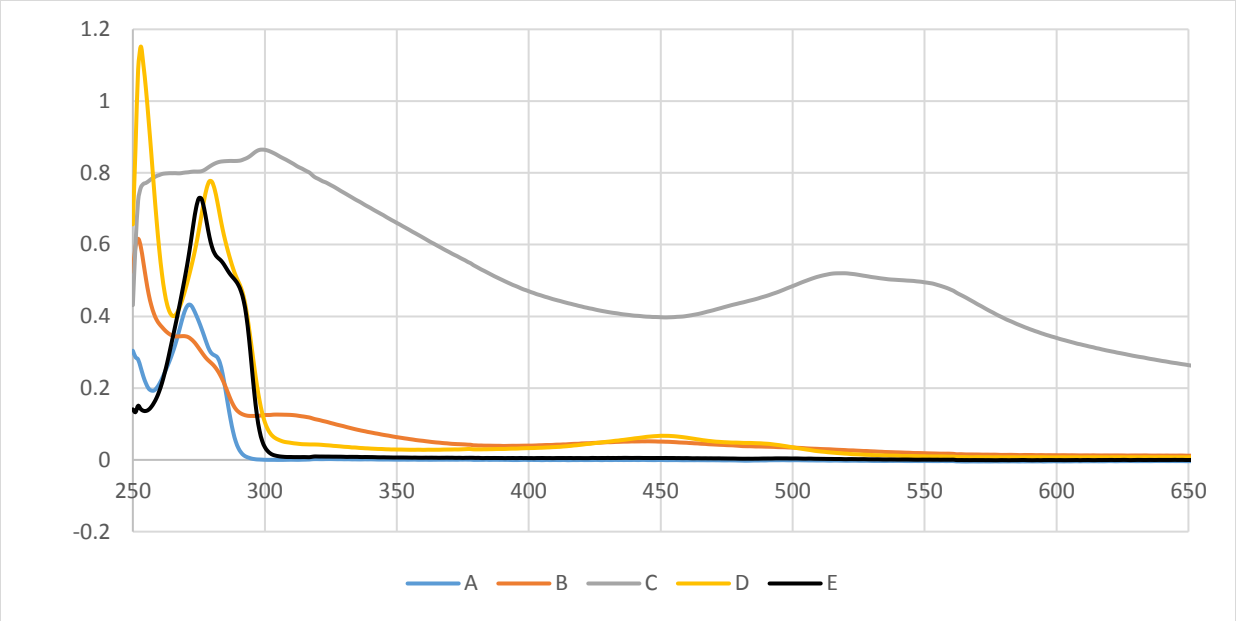


Figure 2.7 Absorbance spectra of the Xanthines at pH 2 (A= 3.01,B= 3.02,C= 3.03,D= 3.04E= 3.05)

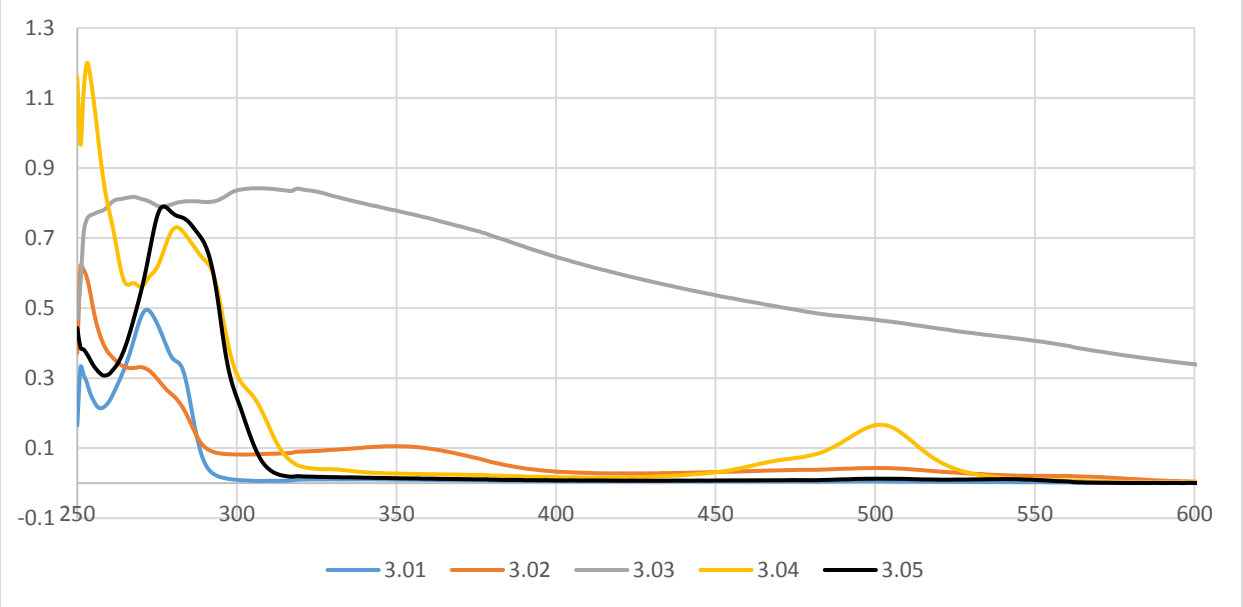


Figure 2.8 Absorbance spectra of the Xanthines at pH 7

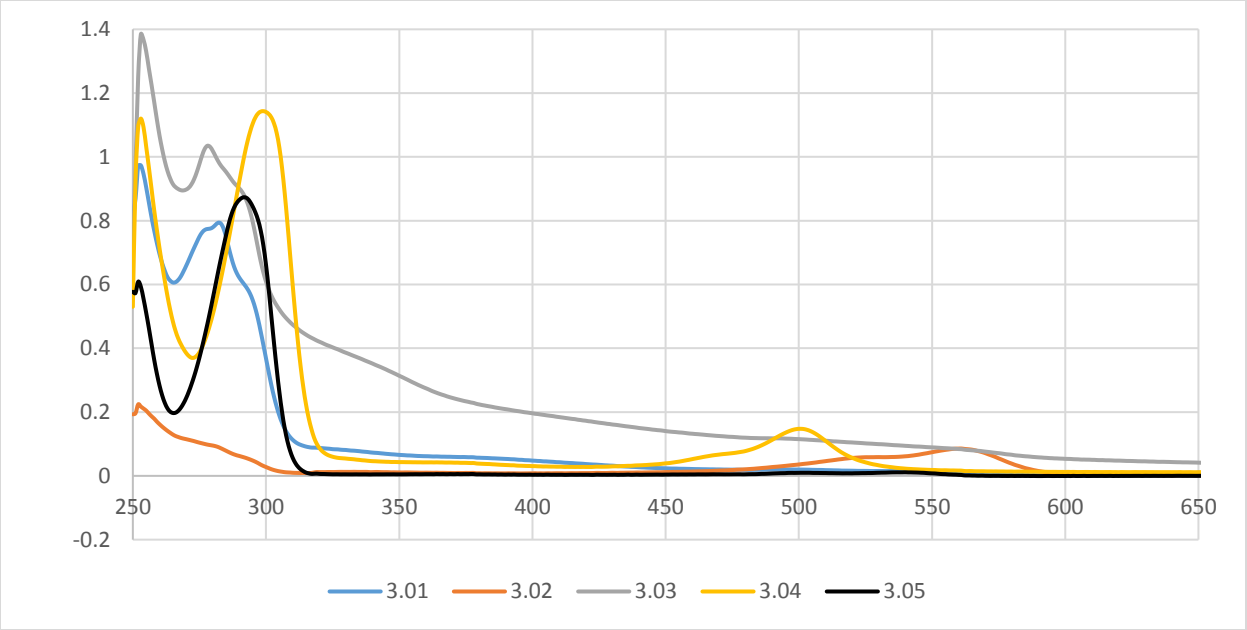


Figure 2.9 Absorbance spectra of the Xanthines at pH 9

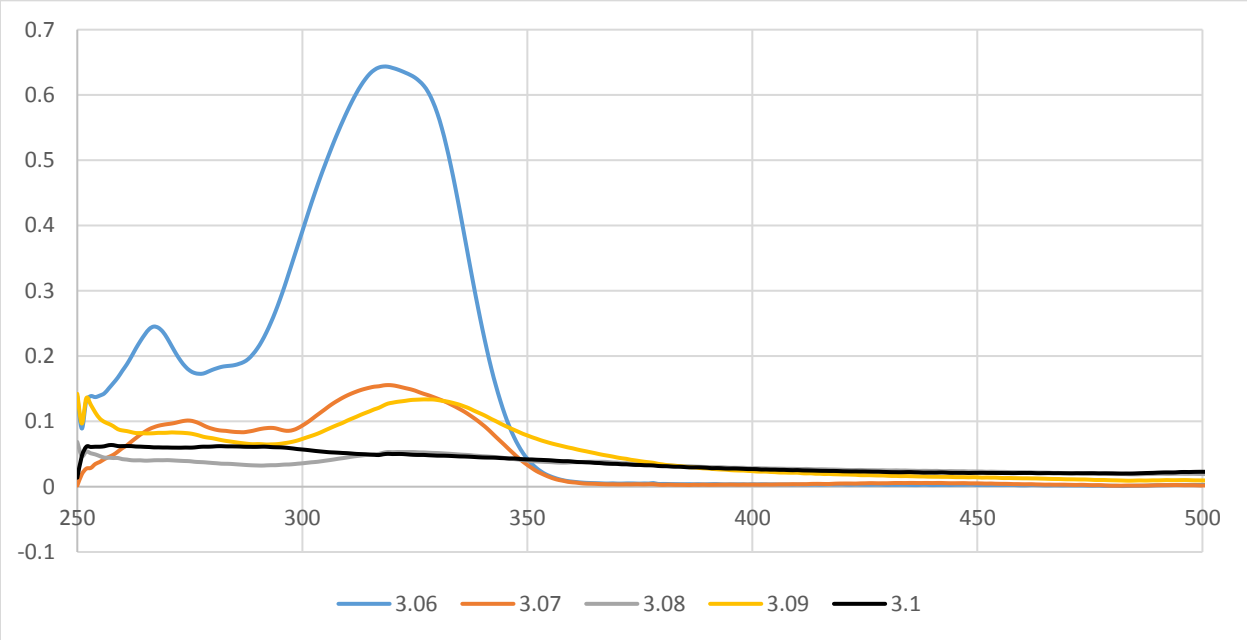


Figure 2.10 Absorbance spectra of the Xanthones at pH 2

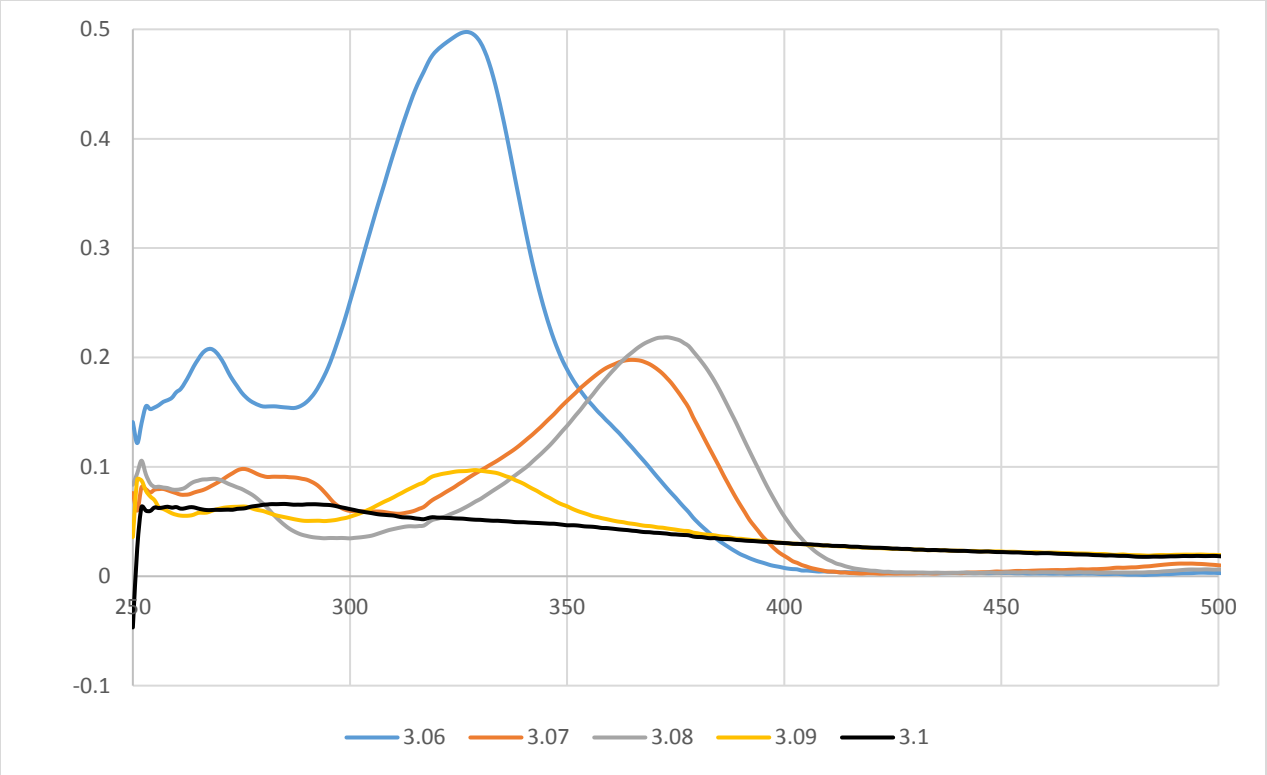


Figure 2.11 Absorbance spectra of the Xanthones at pH 7

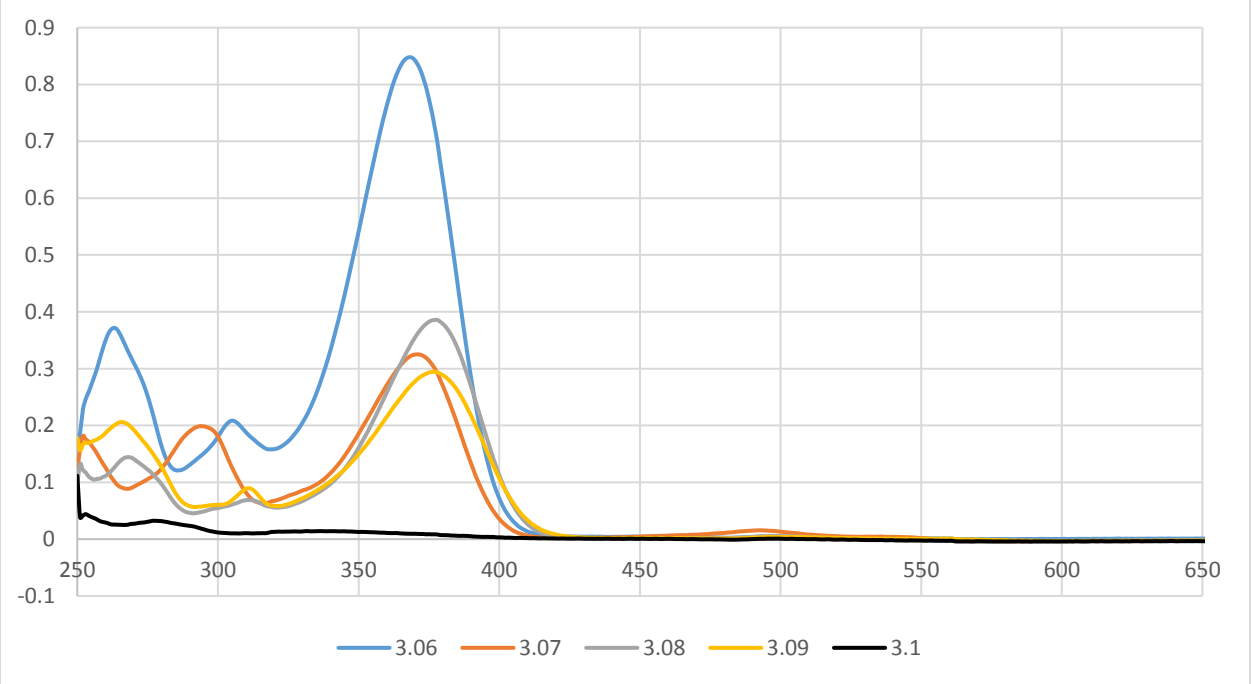
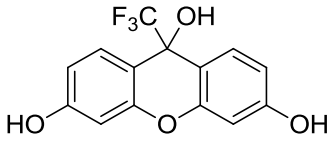


Figure 2.12 Absorbance spectra of the Xanthones at pH 9

3. EXPERIMENTAL

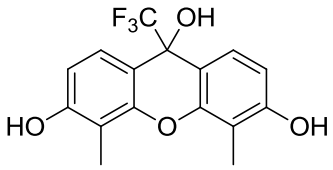
Unless otherwise noted, materials were obtained from commercial suppliers and used without purification. Tetrahydrofuran and diethyl ether were distilled from sodium and benzophenone. All experiments were performed under nitrogen atmosphere unlessotherwise noted. Organic extracts were dried over anhydrous magnesium sulfate. Nuclear magnetic resonance (NMR) experiments were performed with either a Bruker 300 MHz or Bruker 500 MHz instrument. All chemical shifts are reported relative to CDCl3 7.26 ppm for 1H. 1H and 13C NMR chemical shifts (δ) are reported in parts per million (ppm). Unless otherwise indicated, 1H and 13C NMR spectra were recorded in CDCl3 unless otherwise noted. Coupling constants (*J*) are reported in Hertz (Hz). Spectral splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Melting points were obtained using a Mel-temp II capillary apparatus and are uncorrected. MS was obtained using a Shimadzu LCMS 2020.



9-(trifluoromethyl)-9H-xanthene-3,6,9-triol (3.01)

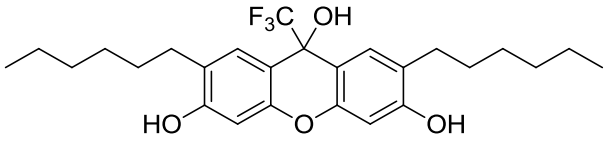
A solution of resorcinol (2 g, 18.16 mmol, 1eq.) in mixed acids of methanesulfonic acid and trifluoroacetic acid (4 mL each) was refluxed in a 25mL round bottom flask while stirring and monitored by TLC (10% methanol in dichloromethane). Reaction went to completion after 2 hours. The reaction mixture was diluted into ice cold water (50 mL) after which product precipitated out and stirred for 2 hours. It was then filtered using a Buchner funnel and left to dry overnight under reduced pressure affording 2.74g. The crude product was purified from boiling water to give 2.2g of a needle-like crystals. M.p > 350^{o}C. %. ^{1}H NMR (300MHz, Methanol) δ 7.55 (d, J= 8.7, 2H),

6.60 (d,d, J= 2.1, 8.7, 2H), 6.49 (d, J= 2.1 2H); MS (ES) Calc. for $C_{14}H_9F_3O_4$ [M-1]$^-$ 297.05; found 297.05



4,5-dimethyl-9-(trifluoromethyl)-9H-xanthene-3,6,9-triol (3.02)

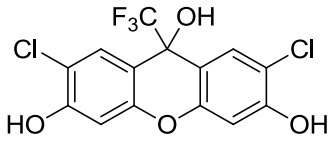
A solution of 2 methylresorcinol (442 mg, 3.56 mmol, 1eq.) in mixed acids of methanesulfonic acid and trifluoroacetic acid (2.5 mL each) was refluxed under nitrogen in a 25mL round bottom flask while stirring and monitored by TLC (10% methanol in dichloromethane). Reaction went to completion after 17 hours. The reaction mixture was diluted into ice cold water (50 mL) after which product precipitated out and was stirred for 30 mins. It was then filtered using a Buchner funnel and left to dry overnight under reduced pressure affording 420mg of the product corresponding to a yield of 72%. ^{1}H NMR (300MHz, $CDCl_3$) δ 7.54 (d, J= 8.7, 2H), 6.70 (d,d, J= 8.7, 2H), 2.35 (s, 6H); MS (ES) Calc. for $C_{16}H_{13}F_3O_4$ [M-1]$^-$ 325.08; found 325.10



2,7-dihexyl-9-(trifluoromethyl)-9H-xanthene-3,6,9-triol (3.03)

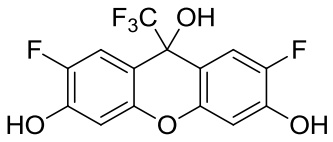
A solution of 4n-hexylresorcinol (71 mg, 0.36 mmol, 1eq.) in mixed acids of methanesulfonic acid and trifluoroacetic acid (1 mL each) was stirred under nitogen at room temperature in a 25mL round bottom flask and monitored by TLC (10% methanol in dichloromethane). Reaction went to

completion after 72 hours. The reaction mixture was then diluted into 15ml of ice cold water and. Product immediately precipitated which was stirred for 5 mins and filtered using a Buchner funnel with vacuum and dried overnight under pressure to afford 80mg of the desired compound. corresponding to a yield of 94%. No purification was attempted. ^{1}H NMR (300MHz, $CDCl_{3,}$) δ 7.89 (s, 2H), 7.42 (s, 2H), 2.73 (m, 4H), 1.63 (m, 4H), 1.25 (m, 12H), 0.89 (m, 6H); MS (ES) Calc. for $C_{26}H_{33}F_3O_4$ $[M-17]^+$ 449.53; found 449.50



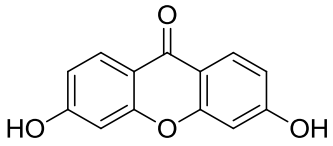
2,7-dichloro-9-(trifluoromethyl)-9H-xanthene-3,6,9-triol (3.04)

A solution of 4-chlororesorcinol (500mg, 3.5 mmol, 1eq.) in mixed acids of methanesulfonic acid and trifluoroacetic acid (2 mL each) was refluxed in a 25mL round bottom flask while stirring and monitored by TLC (10% methanol in dichloromethane). Reaction went to completion after 20 hours. The reaction mixture was diluted into ice cold water (50 mL) after which product precipitated out and stirred for 30 mins. It was then filtered using a Buchner funnel and left to dry overnight under reduced pressure to give 0.62g of the product corresponding to a yield of 98%. The crude compound was crystallized from hot toluene. ^{1}H NMR (300MHz, DMSO) δ 10.91 (s, 2H), 7.61 (s, 2H), 7.57 (s, 1H), 6.80 (s, 2H); MS (ES) Calc. for $C_{14}H_7Cl_2F_3O_4$ $[M-1]^-$ 363.97; found 364.00**.**



2,7-difluoro-9-(trifluoromethyl)-9H-xanthene-3,6,9-triol (3.05)

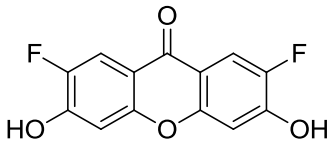
A solution of 3,4-difluorophenol (500mg, 3.8 mmol, 1eq.) in mixed acids of methanesulfonic acid and trifluoroacetic acid (2 mL each) was refluxed under nitrogen in a 25mL round bottom flask while stirring and monitored by TLC (10% methanol in dichloromethane). Reaction went to completion after 68 hours. The reaction mixture was diluted into ice cold water (50 mL) after which product precipitated out and stirred for 2 hours. It was then filtered using a Buchner funnel and left to dry overnight under reduced pressure. The filtrate was then extracted with ethyl acetate to give a combined mass of 0.50g corresponding to a yield of 77%. The crude product was crystallized using boiling dichloromethane to give a red solid. ^{1}H NMR (300MHz, CD_3OD) δ 7.24 (d, J= 11.1, 2H), 6.75 (d, J= 7.2, 2H); ^{19}F NMR (300MHz, CD_3OD) δ -81.70 (s, 3F), -143.79 (d,d J= 12, 1F); MS (ES) Calc. for $C_{14}H_7F_5O_4$ $[M-17]^+$ 317.03; found 317.00



3,6-dihydroxy-9H-xanthen-9-one (3.06)

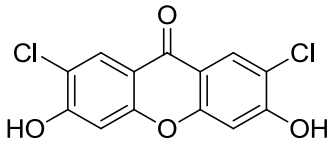
A solution of 9-(trifluoromethyl)-9H-xanthene-3,6,9-triol (322mg, 1.08 mmol, 1eq.) in dimethylsulfoxide (5mL) and potassium hydroxide (0.30g, 5.4mmol, 5eq.) was refluxed under nitrogen in a 25mL round bottom flask while stirring and monitored by TLC (10% methanol in dichloromethane). Reaction went to completion after 32 minutes. Compound is indigo fluorescence under long range ultraviolet light. The reaction mixture was diluted into 5 parts by volume of ice cold water (25 mL) and product was precipitated using 2ml of 1M hydrochloric acid

solution and stirred for 10 minutes. It was then filtered using a Buchner funnel and left to dry overnight under reduced pressure. 0.24mg was obtained after drying corresponding to a yield of 96%. The crude was purified using aqueous ethanol. M.p > 350^{o}C (corresponds to what is reported in the literature). %. ^{1}H NMR (300MHz, Methanol) δ 8.07 (d, J= 8.7, 2H), 6.86 (d, J= 8.7, 2H), 6.82 (s, 2H); MS (ES) Calc. for $C_{13}H_8O_4$ [M-1]$^{-}$ 227.04; found 227.00



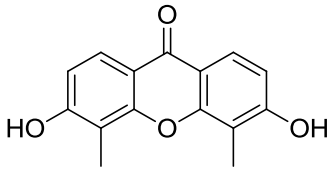
2,7-difluoro-3,6-dihydroxy-9H-xanthen-9-one (3.07)

A solution of 2,7-difluoro-9-(trifluoromethyl)-9H-xanthene-3,6,9-triol (204mg, 0.61 mmol, 1eq.) in dimethylsulfoxide (6mL) and potassium hydroxide (0.17g, 3.0mmol, 5eq.) was refluxed under nitrogen in a 25mL round bottom flask while stirring and monitored by TLC (10% methanol in dichloromethane). Reaction went to completion after 24 minutes. Compound fluorescence indigo under long range UV light. The reaction mixture was diluted into 10 parts by volume of ice cold water (56 mL) and product was precipitated using 2ml of 1M hydrochloric acid solution and stirred for 10 minutes. It was then filtered using a Buchner funnel and left to dry overnight under reduced pressure. The filtrate was then extracted with ethyl acetate to yield a combined mass of 150mg corresponding to a yield of 94%.Product is a light yellow solid which crystallized from aqueous ethanol. ^{1}H NMR (300MHz, CD_3OD) δ 7.80 (d, J= 10.5, 2H), 6.99 (d, J= 6.9, 2H); MS (ES) Calc. for $C_{13}H_6F_2O_4$ [M-1]$^{-}$ 263.02; found 263.00



2,7-dichloro-3,6-dihydroxy-9H-xanthen-9-one (3.08)

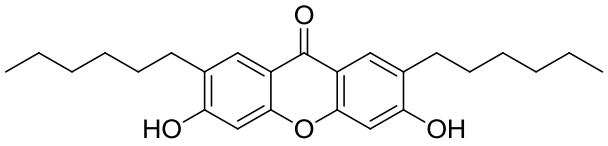
A solution of 2,7-dichloro-9-(trifluoromethyl)-9H-xanthene-3,6,9-triol (211mg, 0.57 mmol, 1eq.) in dimethylsulfoxide (5mL) and potassium hydroxide (0.16g, 2.85mmol, 5eq.) was refluxed under nitrogen in a 25mL round bottom flask while stirring and monitored by TLC (10% methanol in dichloromethane). Reaction went to completion after 25 minutes. Compound is indigo fluorescence under long range ultraviolet light. The reaction mixture was diluted into 5 parts by volume of ice cold water (25 mL) and product was precipitated using 2ml of 1M hydrochloric acid solution and stirred for 10 minutes. It was then filtered using a Buchner funnel and left to dry overnight under reduced pressure. 170mg was obtained after drying corresponding to a yield of 99%. ^{1}H NMR (300MHz, D_2O) δ 7.84 (s, 2H), 6.45 (s, 2H); MS (ES) Calc. for $C_{13}H_6Cl_2O_4$ [M-1]$^-$ 294.96; found 294.95



3,6-dihydroxy-4,5-dimethyl-9H-xanthen-9-one (3.09)

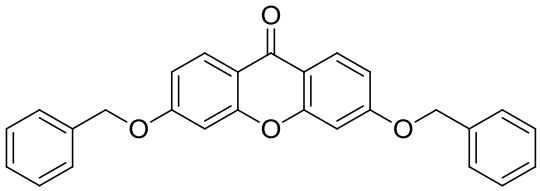
A solution of 4,5-dimethyl-9-(trifluoromethyl)-9H-xanthene-3,6,9-triol (213mg, 0.64 mmol, 1eq.) in dimethylsulfoxide (5mL) and potassium hydroxide (0.18g, 3.2mmol, 5eq.) was refluxed under nitrogen in a 25mL round bottom flask while stirring and monitored by TLC (10% methanol in dichloromethane). Reaction went to completion after 25 minutes. Compound is indigo

fluorescence under long range ultraviolet light. The reaction mixture was diluted into 5 parts by volume of ice cold water (26 mL) and product was precipitated using 2ml of 1M hydrochloric acid solution and stirred for 10 minutes. It was then filtered using a Buchner funnel and left to dry overnight under reduced pressure. 85mg was obtained after drying corresponding to a yield of 51% initial yield. The filtrate then was extracted with ethyl acetate to give a total yield of 90%. ^{1}H NMR (300MHz, D_2O) δ 7.76 (d, J= 9.0, 2H), 6.66 (d, J= 9.0, 2H), 2.24 (s, 6H); MS (ES) Calc. for $C_{15}H_{12}O_4$ $[M+1]^+$ 257.07; found 257.25



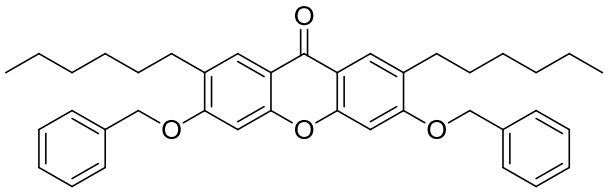
2,7-dihexyl-3,6-dihydroxy-9H-xanthen-9-one (3.10)

A solution of 2,7-dihexyl-9-(trifluoromethyl)-9H-xanthene-3,6,9-triol from (80 mg, 0.17 mmol, 1eq.) in dimethylsulfoxide (3 mL) in which potassium hydroxide (48mg, 0.85mmol, 5eq.) has been added was refluxed under nitrogen in a 25mL round bottom flask while stirring and monitored by TLC (10% methanol in dichloromethane). Reaction went to completion after 5 minutes. The reaction mixture was diluted into 25ml of ice cold water. Product immediately precipitated which was stirred for 5 mins and filtered using a Buchner funnel under vacuum and dried overnight under pressure to afford 60mg of the desired compound corresponding to a yield of 88%. ^{1}H NMR (300MHz, $CDCl_{3,}$) δ 7.98 (s, 2H), 6.80 (s, 2H), 2.99 (m, 4H), 2.67 (m, 4H), 2.60 (m, 4H), 1.25 (m, 8H), 0.83 (m, 6H), 0.87 (m, 8H); MS (ES) Calc. for $C_{39}H_{44}O_4$ $[M-H]^-$ 395.52; found 395.35



3,6-bis(benzyloxy)-9H-xanthen-9-one (3.11)

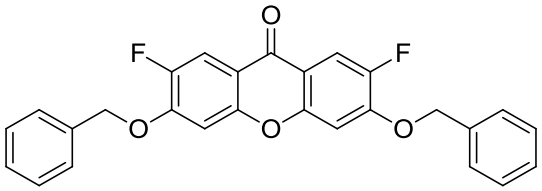
A solution of 9-(trifluoromethyl)-9H-xanthene-3,6,9-triol (300mg, 1.0mmol, 1eq.) in dimethylsulfoxide (7mL) and potassium hydroxide (282mg, 5mmol, 5eq.) was stirred under nitrogen at room temperature in a 25mL round bottom flask and monitored by TLC (10% methanol in dichloromethane). No starting material was seen on the plate after stirring for 85 minutes. Benzylbromide (430mg, 2.5mmol, 2.5eq.) was added and continued monitoring on TLC. Reaction went to completion after 10 minutes. The reaction mixture was diluted into 100ml of 1M hydrochloric acid solution. Product immediately precipitated which was stirred for 5 mins and filtered using a Buchner funnel with vacuum and dried overnight under pressure to afford 373mg of the desired compound corresponding to a yield of 91%. ^{1}H NMR (300MHz, $CDCl_{3,}$) δ 8.25 (d, J= 8.7, 2H), 7.43 (m, 10H), 7.01 (d, J= 9.0, 2H), 6.94 (s, 2H), 5.19 (s, 4H); MS (ES) Calc. for $C_{27}H_{20}O_4$ $[M+1]^+$ 409.45; found 409.60



3,6-bis(benzyloxy)-2,7-dihexyl-9H-xanthen-9-one (3.12)

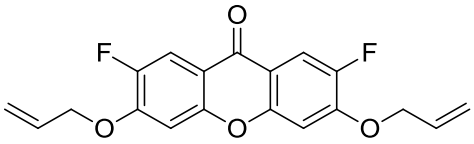
A solution of 2,7-dihexyl-3,6-dihydroxy-9H-xanthen-9-one SO-1-80b (70mg, 0.17mmol, 1eq.) in dimethylsulfoxide (7mL), benzylbromide (76mg, 0.42mmol, 2.5eq.) and cesium carbonate (172mg, 0.53mmol, 3eq.) was stirred under nitrogen at room temperature in a 25mL round bottom

flask and monitored by TLC (10% methanol in dichloromethane). No starting material was seen on the plate after stirring for 2 days. The reaction mixture was diluted into 100ml of 1M hydrochloric acid solution. Product immediately precipitated which was stirred for 5 mins and filtered using a Buchner funnel with vacuum and dried overnight under pressure to afford 94mg of the desired compound corresponding to a yield of 92%. ^{1}H NMR (300MHz, $CDCl_3$,) δ 8.08 (s, 2H), 7.45 (m, 10H), 6.85 (s, 2H), 5.19 (s, 4H), 2.74 (m, 4H), 1.66 (m, 8H), 1.29 (m, 6H), 0.87 (m, 8H); MS (ES) Calc. for $C_{39}H_{44}O_4$ $[M+H]^+$ 577.76; found 577.40.



3,6-bis(benzyloxy)-2,7-difluoro-9H-xanthen-9-one (3.13)

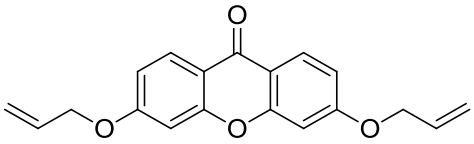
A solution of 9-(trifluoromethyl)-2, 7-difluoro-9H-xanthene-3,6,9-triol from SO-1-57b2 (72mg, 1.0mmol, 1eq.) in DMSO (2mL) and potassium hydroxide (60mg, 1mmol, 5eq.) was stirred under nitrogen at room temperature in a 25mL round bottom flask and monitored by TLC (10% methanol in dichloromethane). No starting material was seen on the plate after stirring for 22 hours. Benzylbromide (93mg, 0.54mmol, 2.5eq.) was added and continued monitoring on TLC. Reaction went to completion after 1 hour. The reaction mixture was diluted into 20ml of 1M hydrochloric acid solution. Product immediately precipitated which was stirred for 5 mins and filtered using a Buchner funnel under vacuum and dried overnight under pressure to afford 86mg of the desired compound corresponding to a yield of 91%. ^{1}H NMR (300MHz, $CDCl_3$,) δ 7.51 (d, J= 10.8, 2H), 7.43 (m, 10H), 6.97 (d, J= 6.6, 2H), 5.26 (s, 4H); MS (ES) Calc. for $C_{27}H_{18}F_2O_4$ $[M+H]^+$ 445.43; found 445.35.



3,6-bis(allyloxy)-2,7-difluoro-9H-xanthen-9-one (3.14)

A solution of 2,7-difluoro-3,6-dihydroxy-9H-xanthen-9-one (40mg, 0.15mmol, 1eq.) in dimethylsulfoxide (2mL) and allyl bromide (55mg, 0.45mmol, 3eq.) and caesium carbonate (149mg, 0.45mmol, 3eq.) was stirred under nitrogen at room temperature overnight. TLC (10% methanol in dichloromethane) the following morning showed all starting material has been used up. The reaction mixture was diluted into 20ml of 1M hydrochloric acid solution. Product immediately precipitated which was stirred for 5 mins and filtered using a Buchner funnel and dried overnight under pressure to afford 40mg of the desired compound corresponding to a yield of 75%. ^{1}H NMR (300MHz, $CDCl_{3,}$) δ 7.94

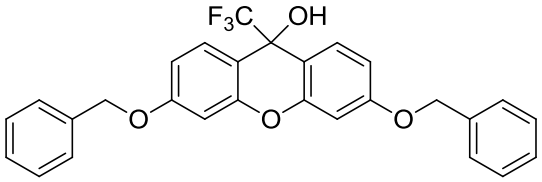
(d, J= 10.8, 2H), 6.94 (d, J=6.6, 2H), 6.10 (m, 2H), 5.52 (d, J=17.1, 2H), 5.41 (d, J=10.5, 12H), 4.73 (d, J=5.1, 4H); MS (ES) Calc. for $C_{19}H_{14}F_2O_4$ $[M+1]^+$ 345.09; found 345.25



3,6-bis(allyloxy)-9H-xanthen-9-one (3.15)

A solution of 3,6-dihydroxy-9H-xanthen-9-one (200mg, 0.87mmol, 1eq.) in DMF (6mL) and allyl bromide (212mg, 1.72mmol, 2eq.) and cesium carbonate (856mg, 2.61mmol, 3eq.) was stirred under nitrogen at room temperature overnight. TLC (10% methanol in dichloromethane) the following morning showed all starting material has been used up. The reaction mixture was diluted into 10ml of 1M hydrochloric acid solution. Product extracted with ethyl acetate (3x) and the

organic layer was washed with brine and passed through sodium sulfate and concentrated using the rotary evaporator and dried overnight under pressure to afford 254mg of the crude compound corresponding to a yield of 94%. ^{1}H NMR (300MHz, $CDCl_3$,) δ 8.25 (d, J= 8.7, 2H), 6.96 (d, J=9.0, 2H), 6.87 (s, 2H), 6.10 (3, 2H), 5.50 (d, J=17.4, 2H), 5.38 (d, J=10.8, 2H), 4.67 (d, J= 4.80 ,4H; MS (ES) Calc. for $C_{19}H_{16}O_4$ $[M+1]^+$ 309.33; found 309.25



3,6-bis(benzyloxy)-9-(trifluoromethyl)-9H-xanthen-9-ol (3.16)

To a solution of 9-(trifluoromethyl)-9H-xanthene-3,6,9-triol (106mg, 0.22mmol, 1eq.) in DMSO (2mL) was added potassium hydroxide (62mg, 1.1mmol, 5eq.) and stirred at room temperature and monitored by TLC (5% methanol in DCM). After 7 hours of stirring TLC showed that all the starting material had been used up. The reaction mixture was diluted into 20ml of 1M HCl solution. Product immediately precipitated which was stirred for 5 mins and filtered using a Buchner funnel and dried overnight under pressure to afford 80mg of the desired compound corresponding to a yield of 96%. ^{1}H NMR (300MHz, $CDCl_3$,) δ 7.94 (d, J= 10.8, 2H), 6.94 (d, J=6.6, 2H), 6.10 (m, 2H), 5.52 (d, J=17.1, 2H), 5.41 (d, J=10.5, 12H), 4.73 (d, J=5.1, 4H); MS (ES) Calc. for $C_{19}H_{14}F_2O_4$ $[M+1]^+$ 345.09; found 345.25. Mp = 176-178^{o}C

4. CONCLUSION

In conclusion, we present a simple and efficient route to xanthones, and introduce a new class of fluorescent xanthine with potentially low photo bleaching properties. We used our procedure to synthesize in very high yield various derivatives of 3,6-dihydroxyxanthones. 2,7-difluoro,3,6-dihydroxyxanthone is particularly interesting because we used a relatively cheaper starting material to synthesize 2,7-difluoro,3,6-dihydroxyxanthone in 2 steps with a 73% overall yield compared to earlier reported procedures: 8 steps process with an overall yield of 26% using a more expensive starting material and a 6 steps sequence with an overall yield of 32% again starting with a relatively more expensive starting material.

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6. APPENDIX: NMR AND MS DATA SHEET

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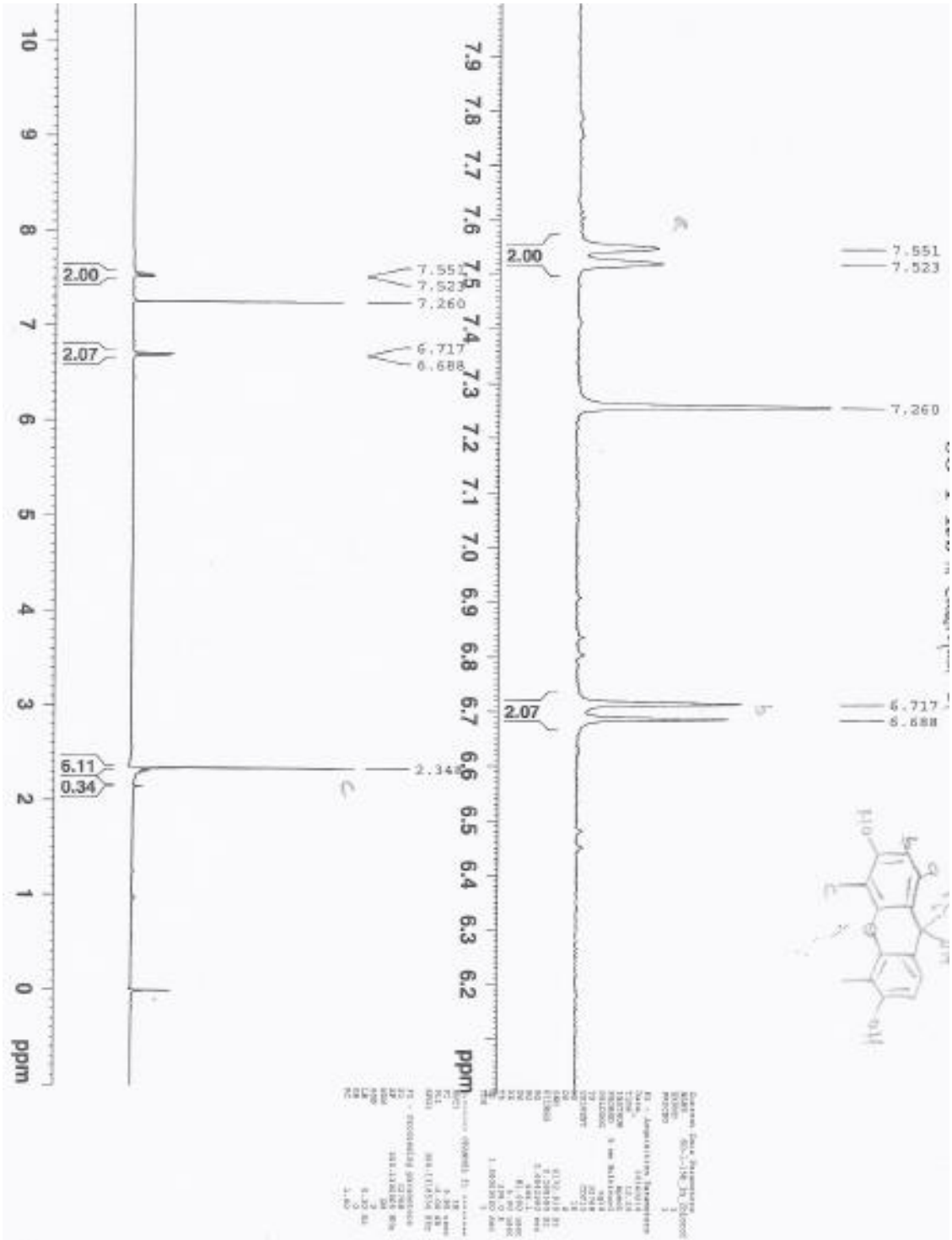

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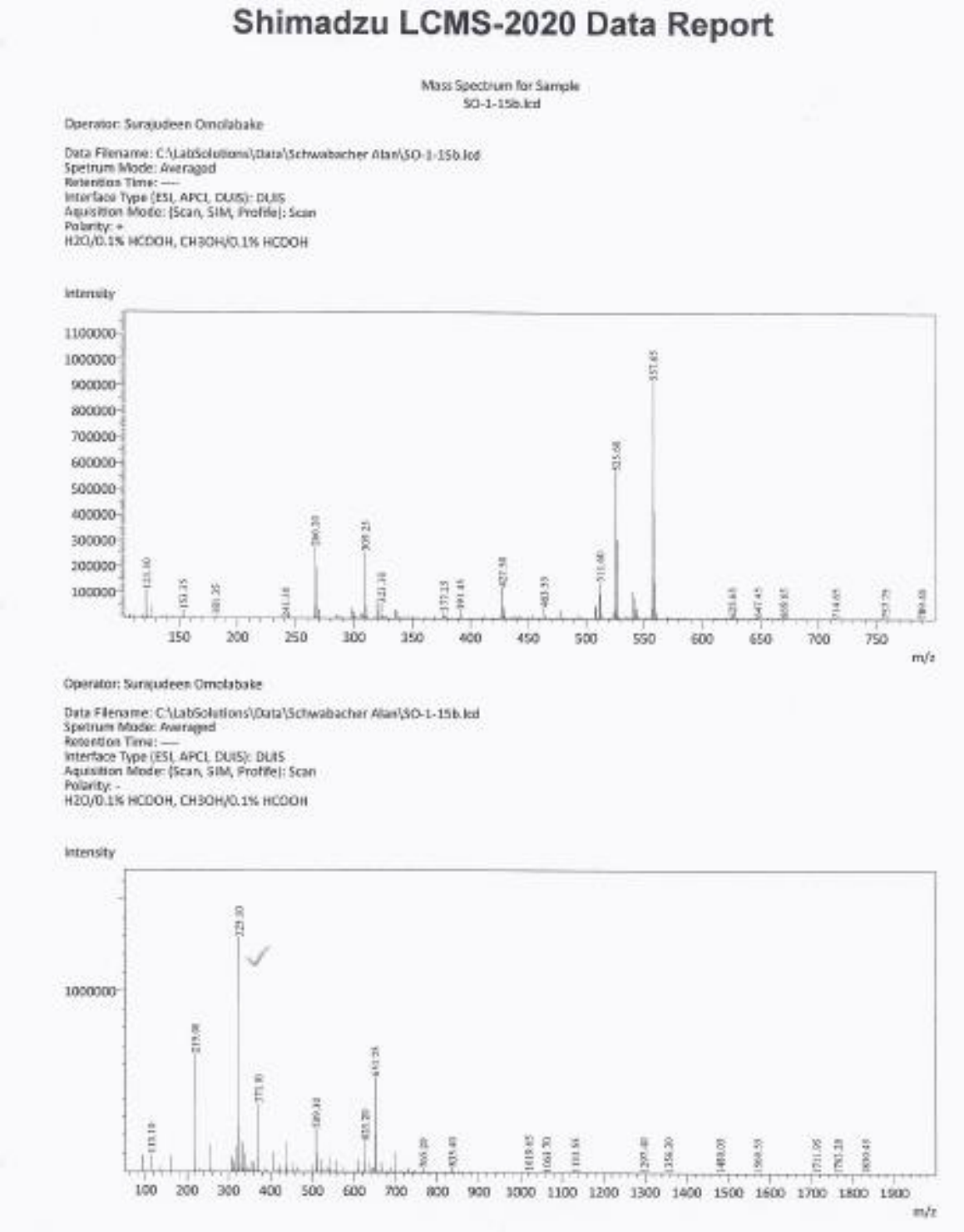

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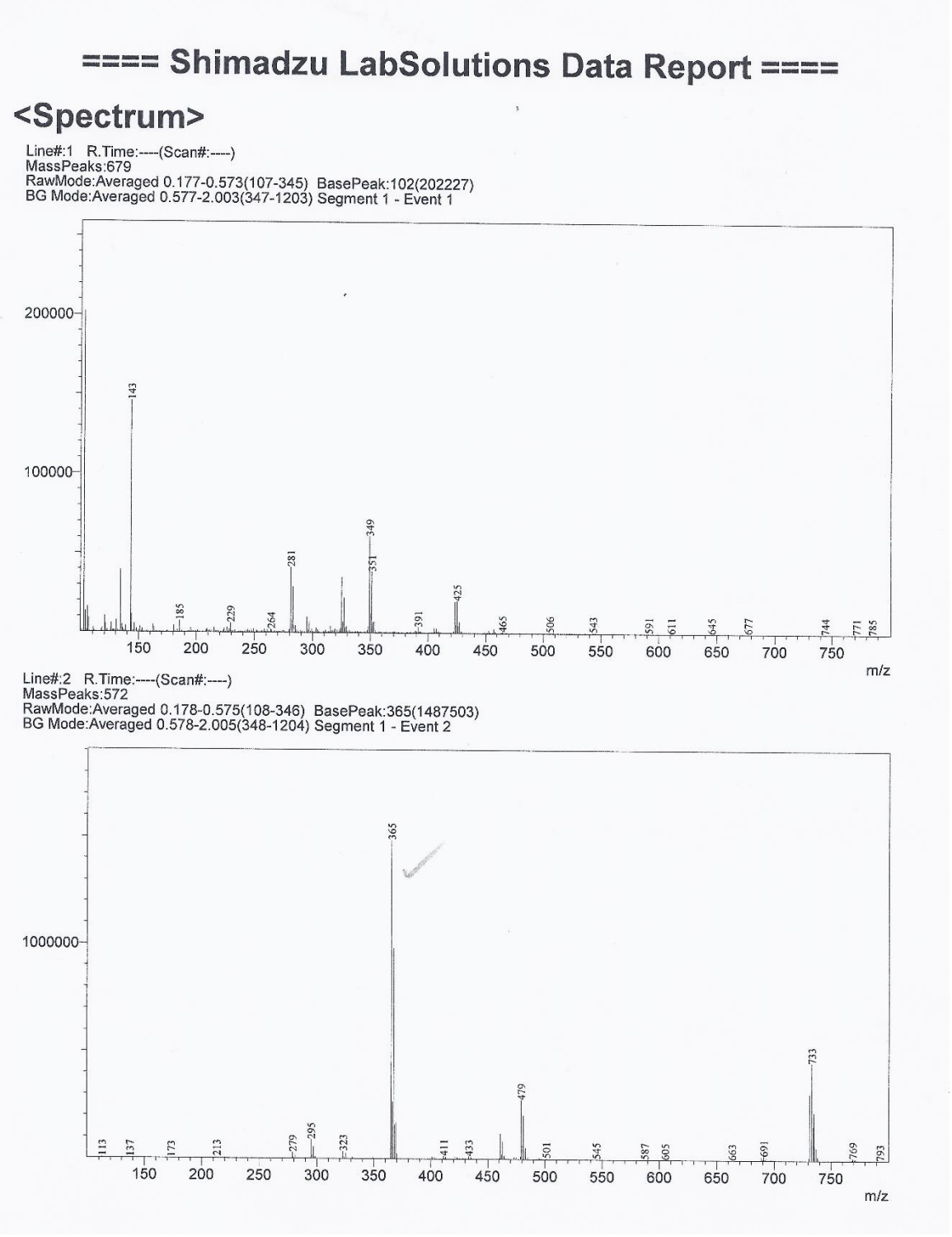

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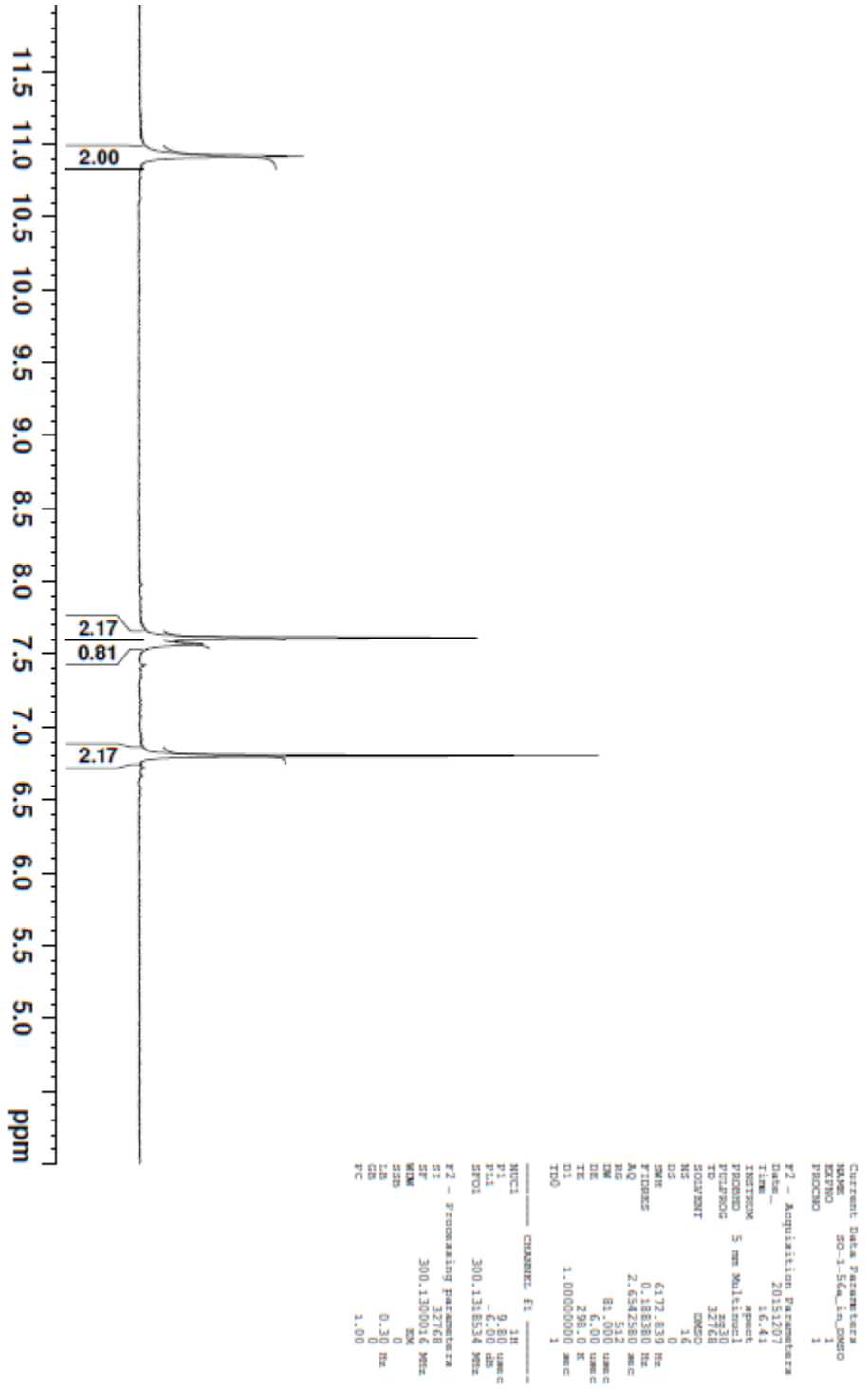

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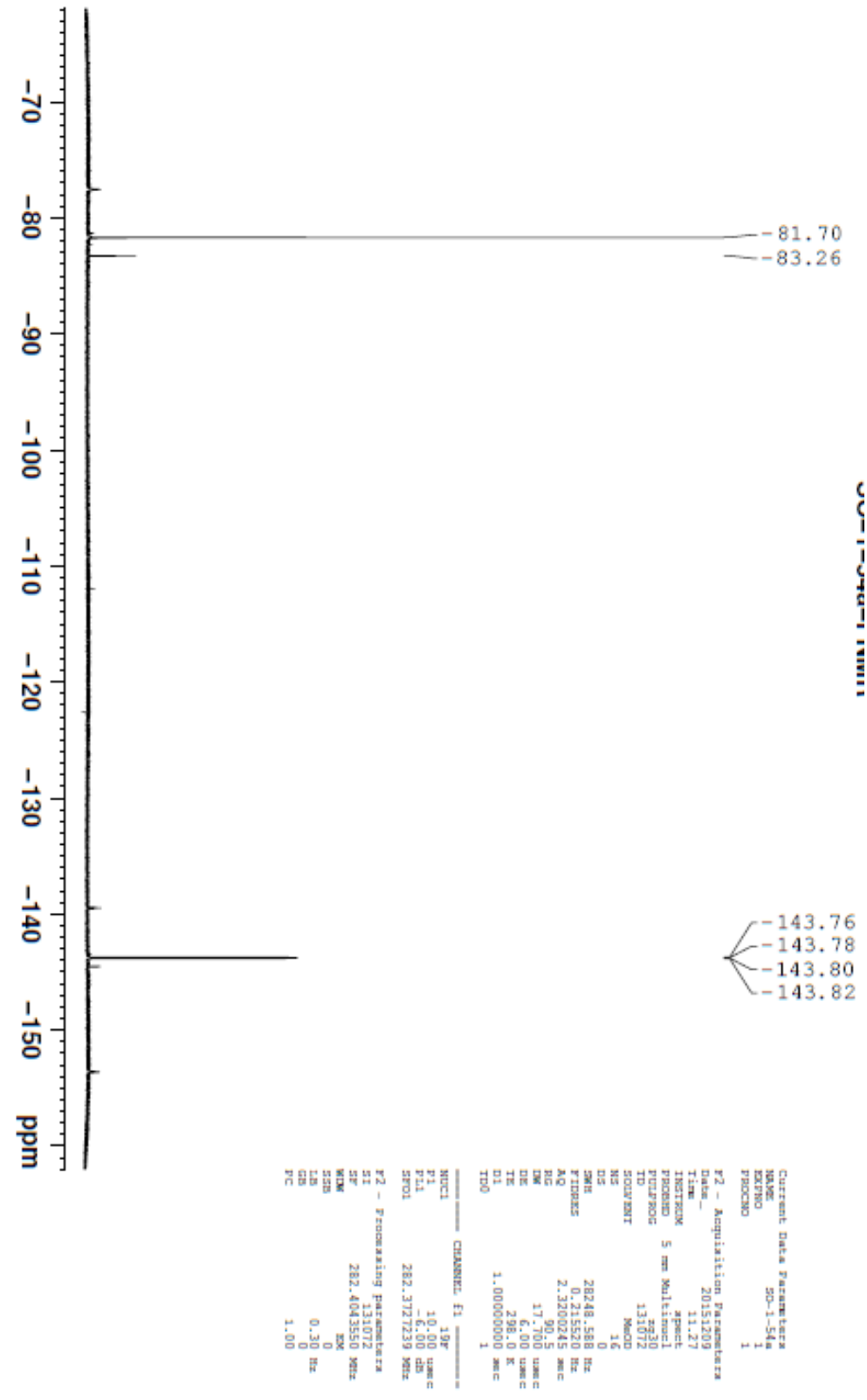

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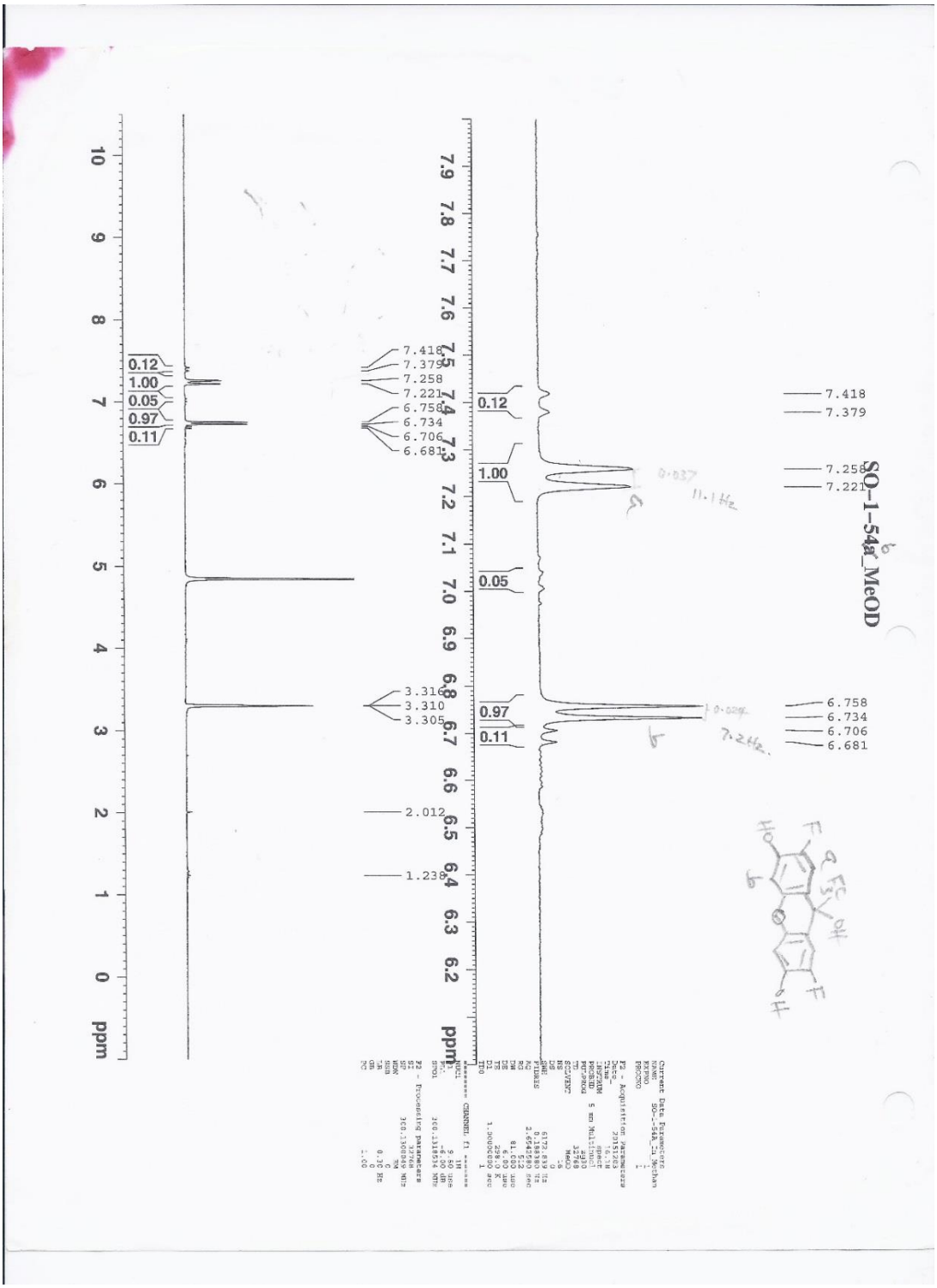

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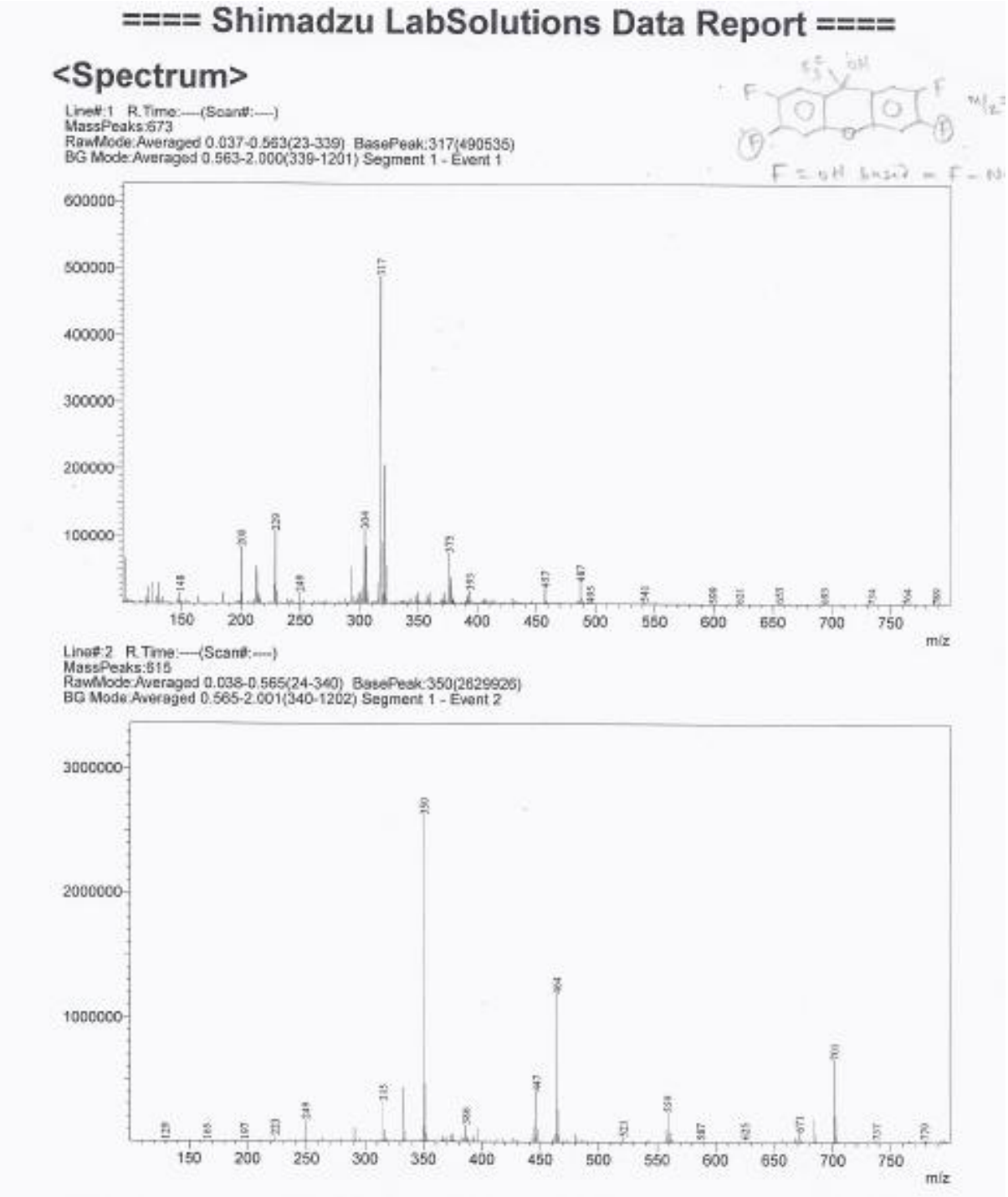

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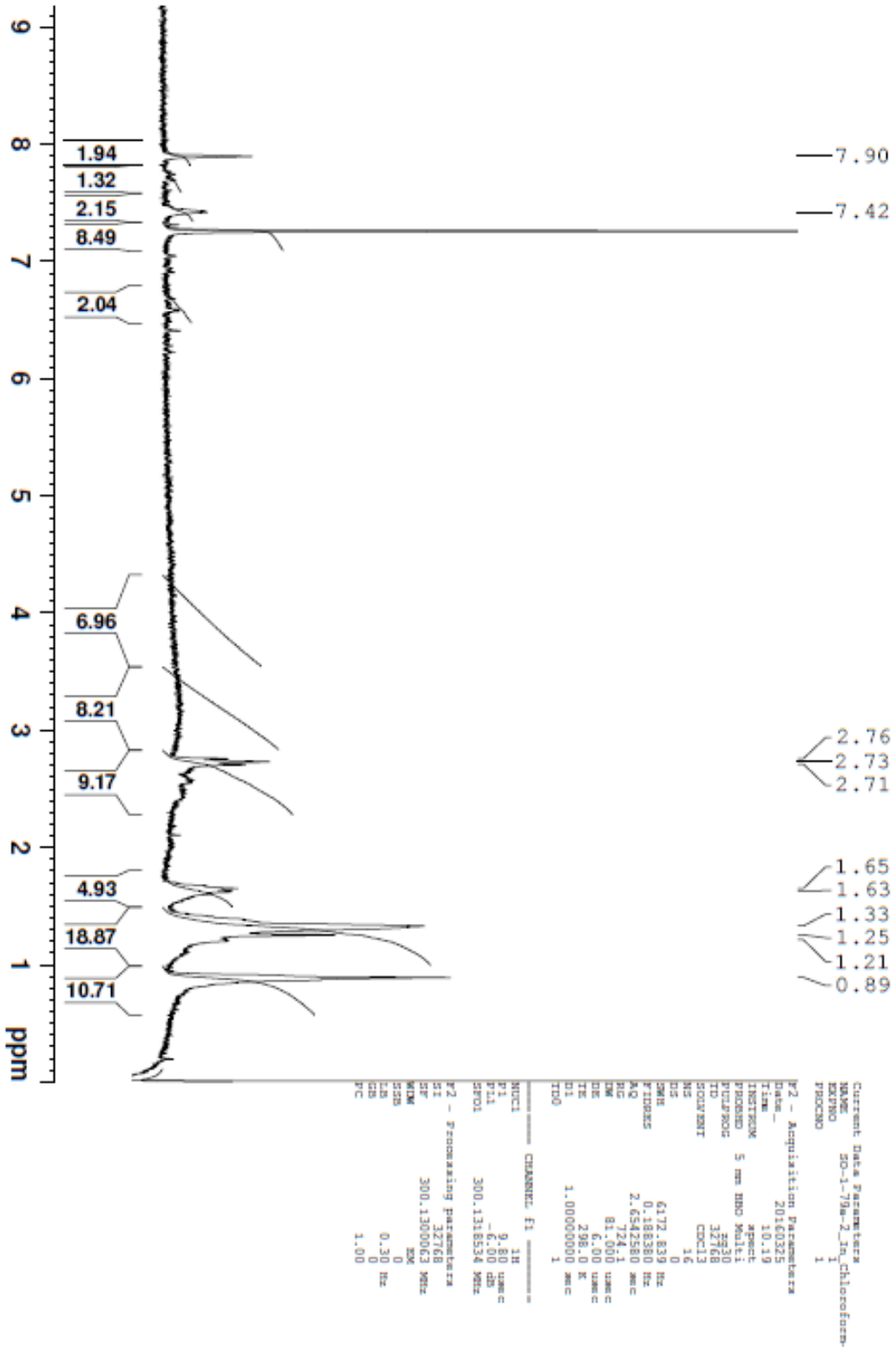

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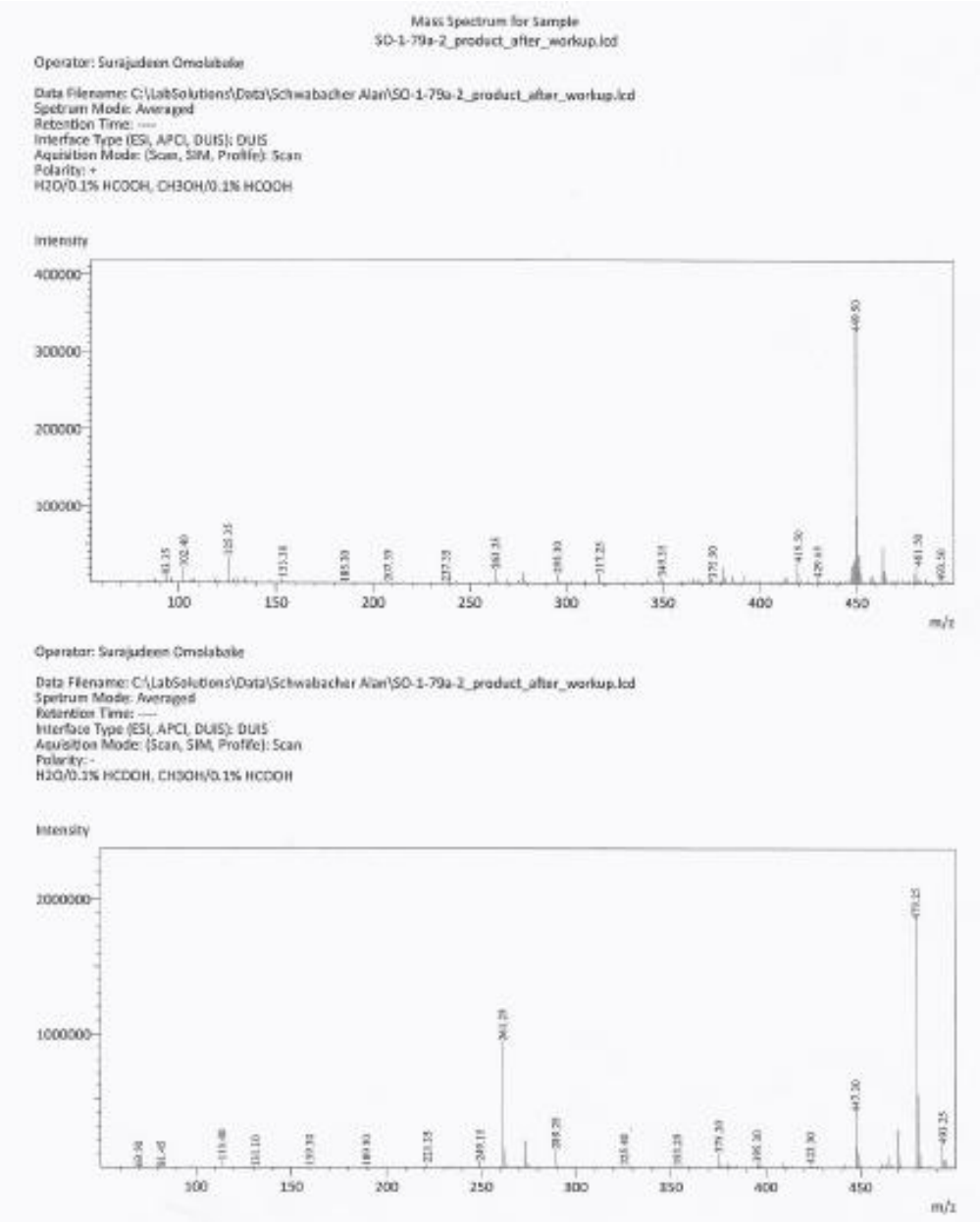

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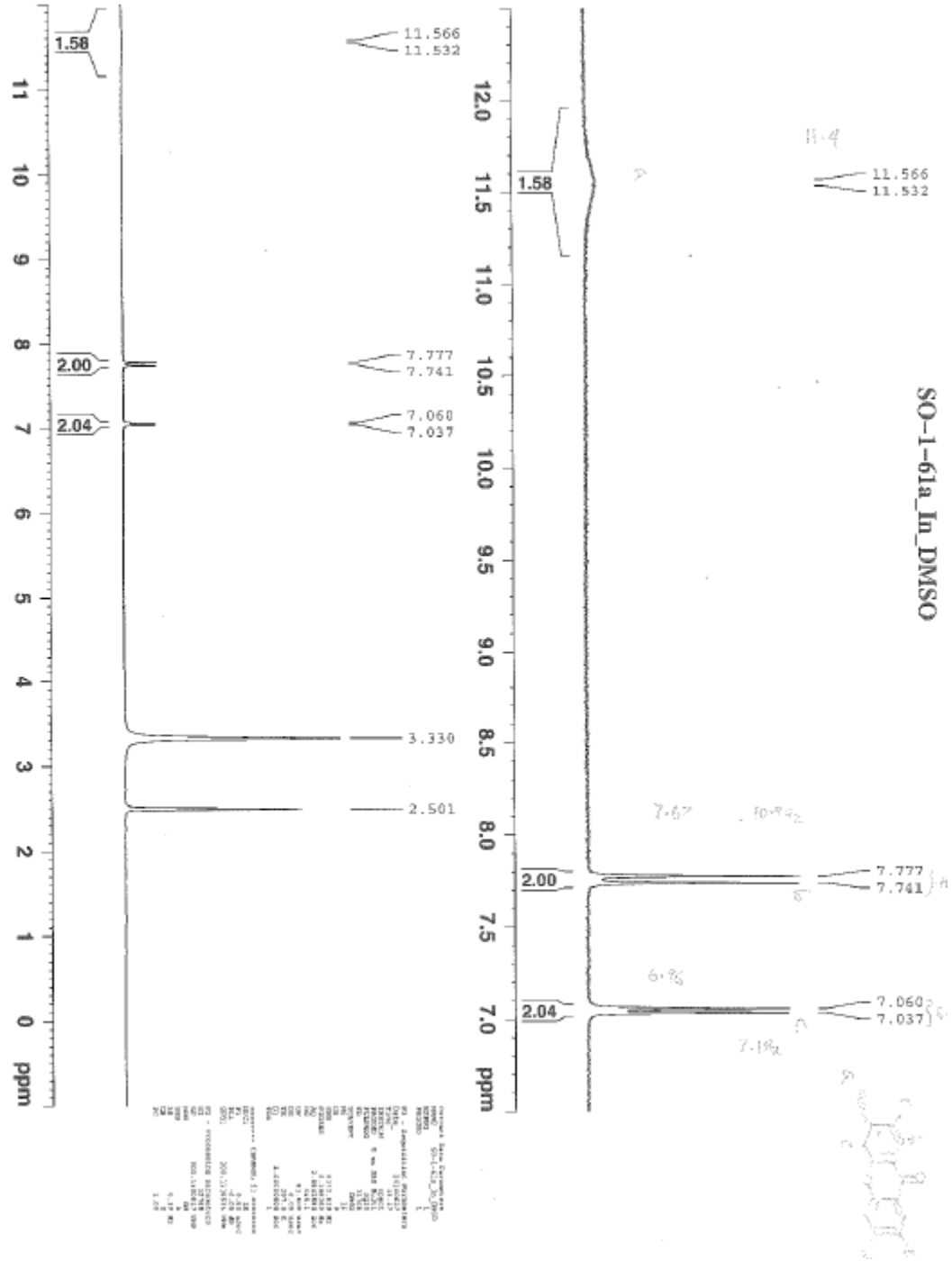

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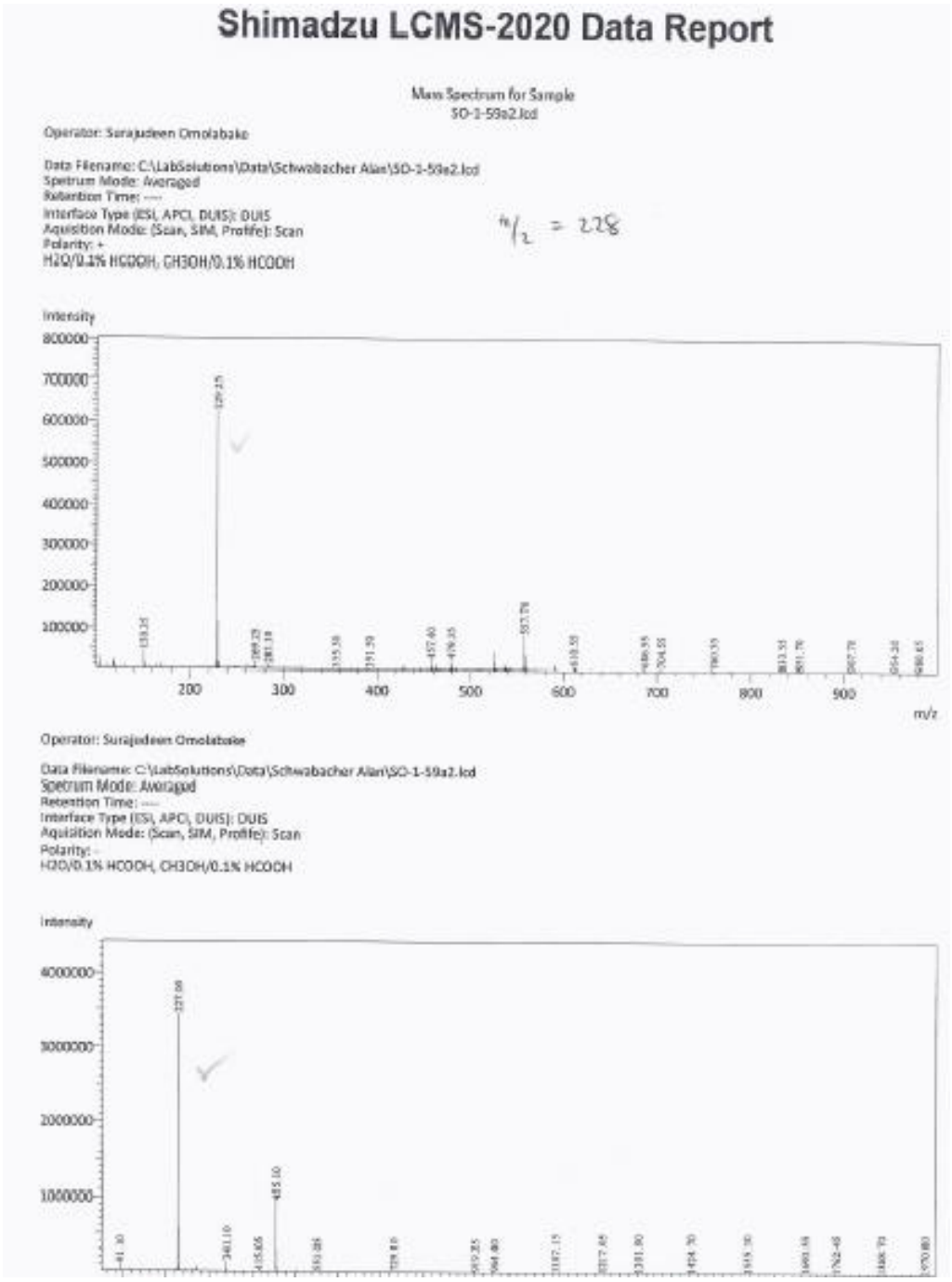

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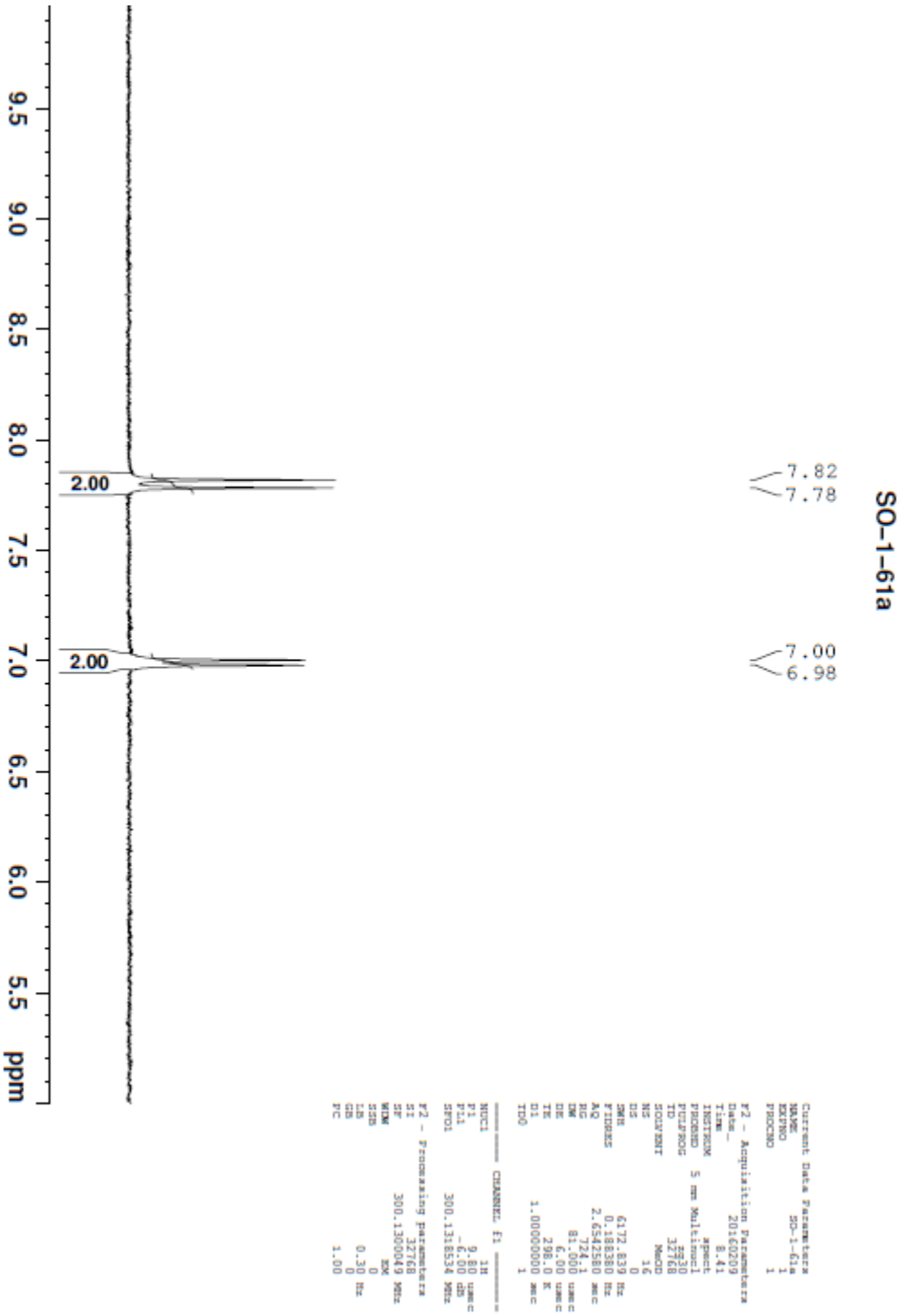

MS-2,7-difluoro-3,6-dihydroxy-9H-xanthen-9-one (3.07)

Shimadzu LCMS-2020 Data Report

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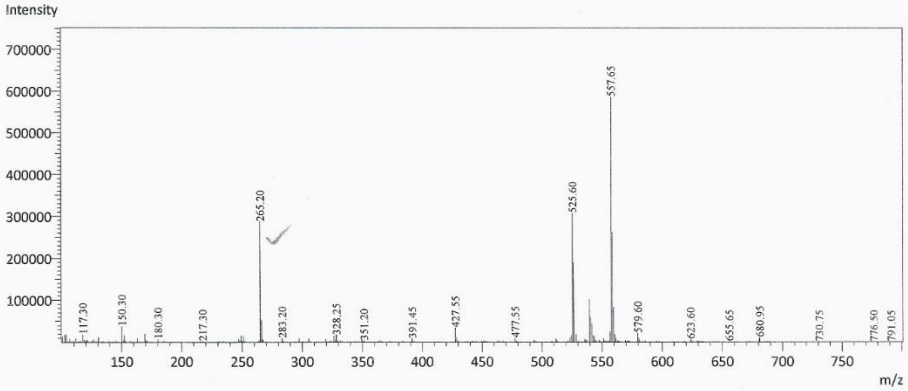

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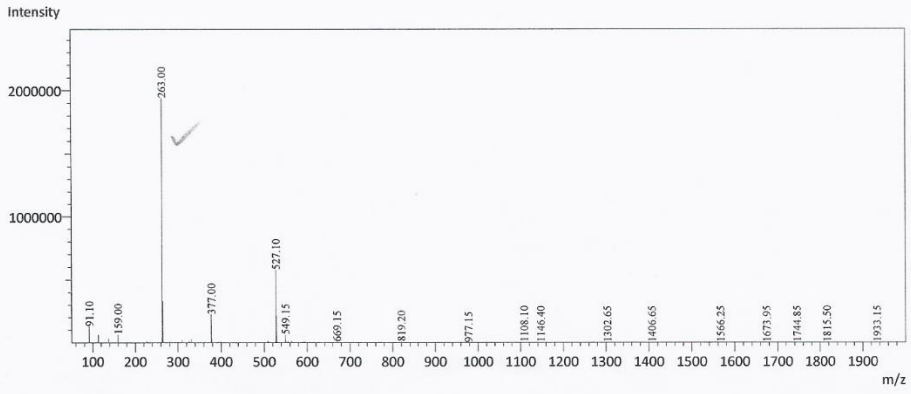

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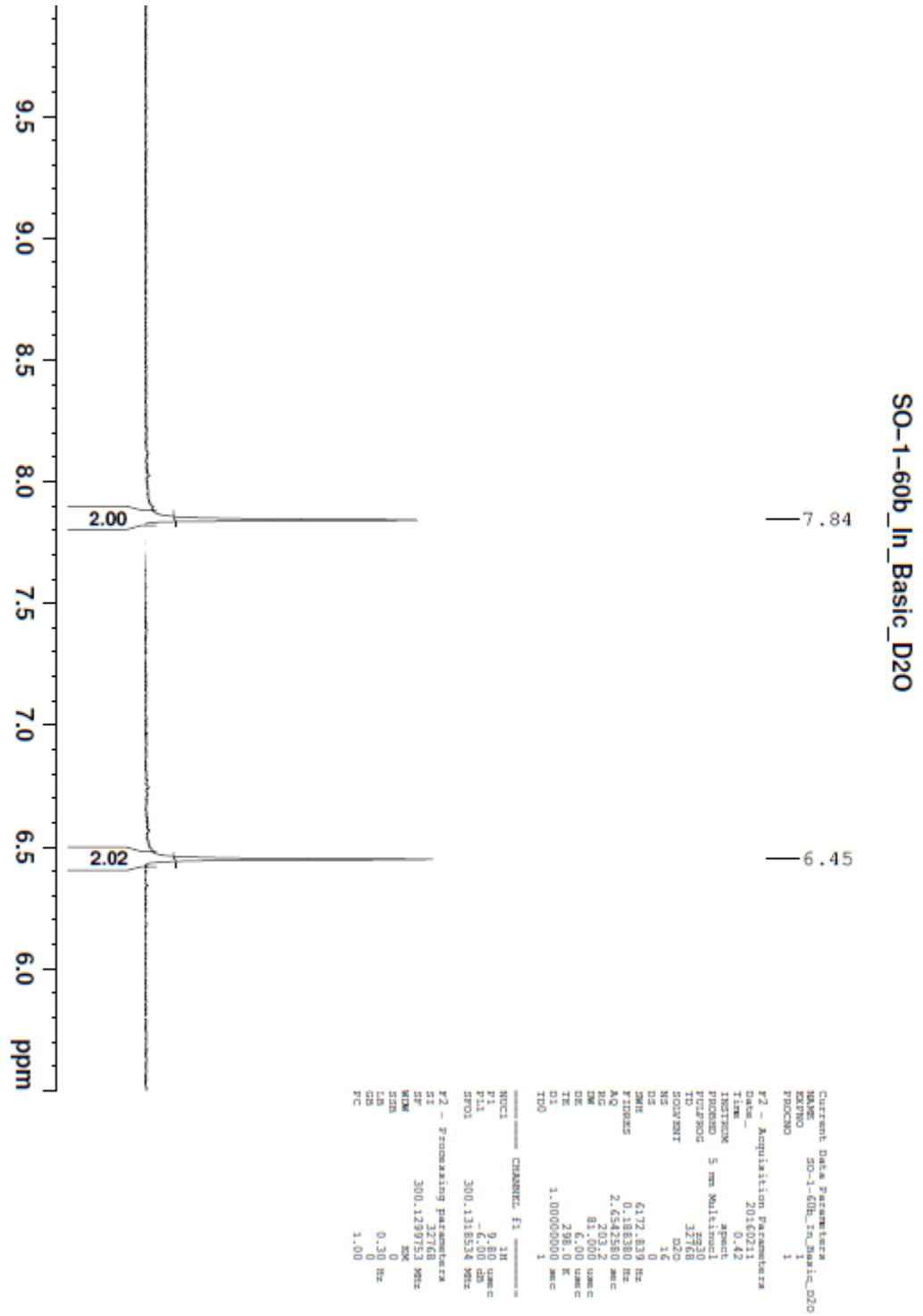

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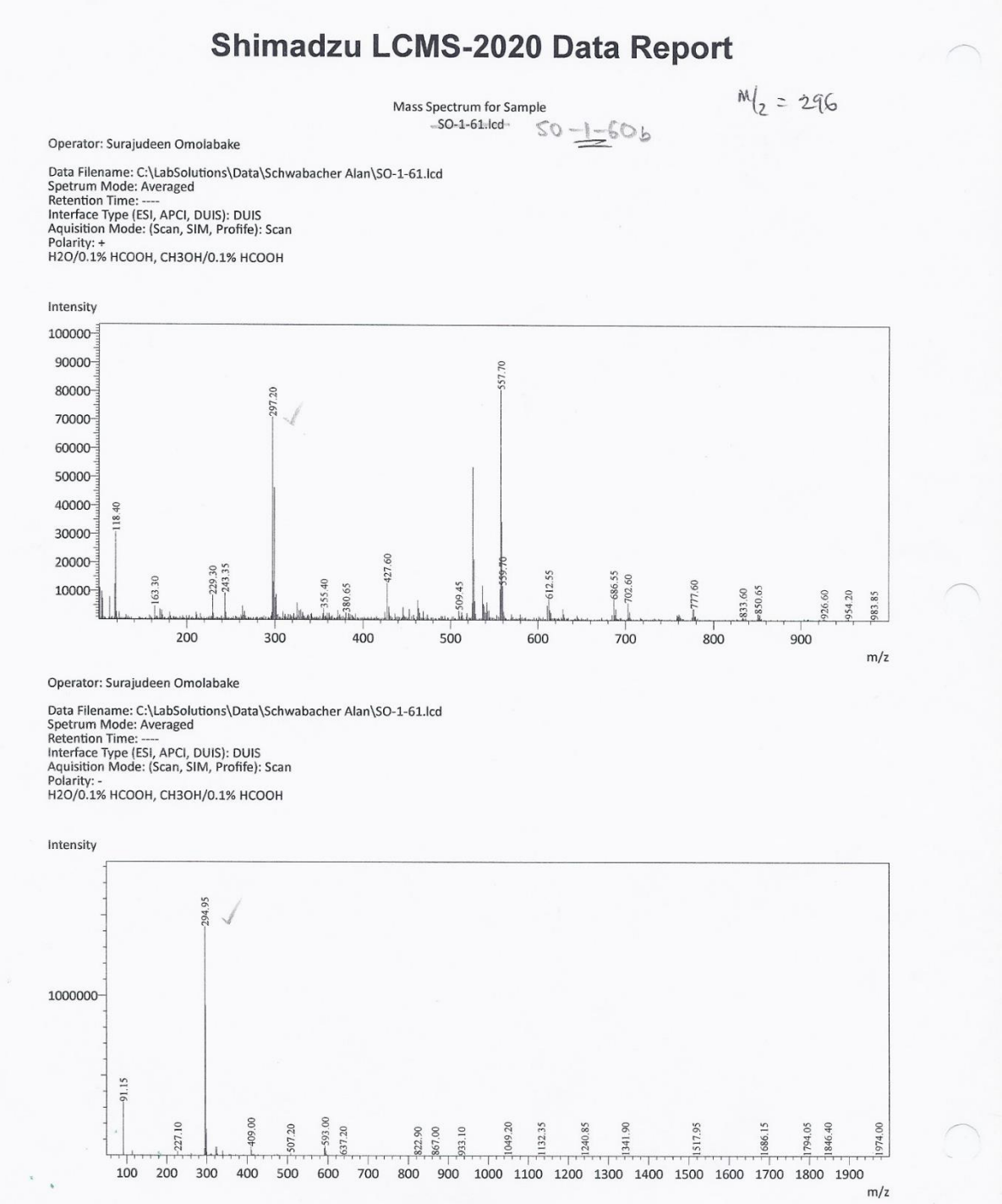

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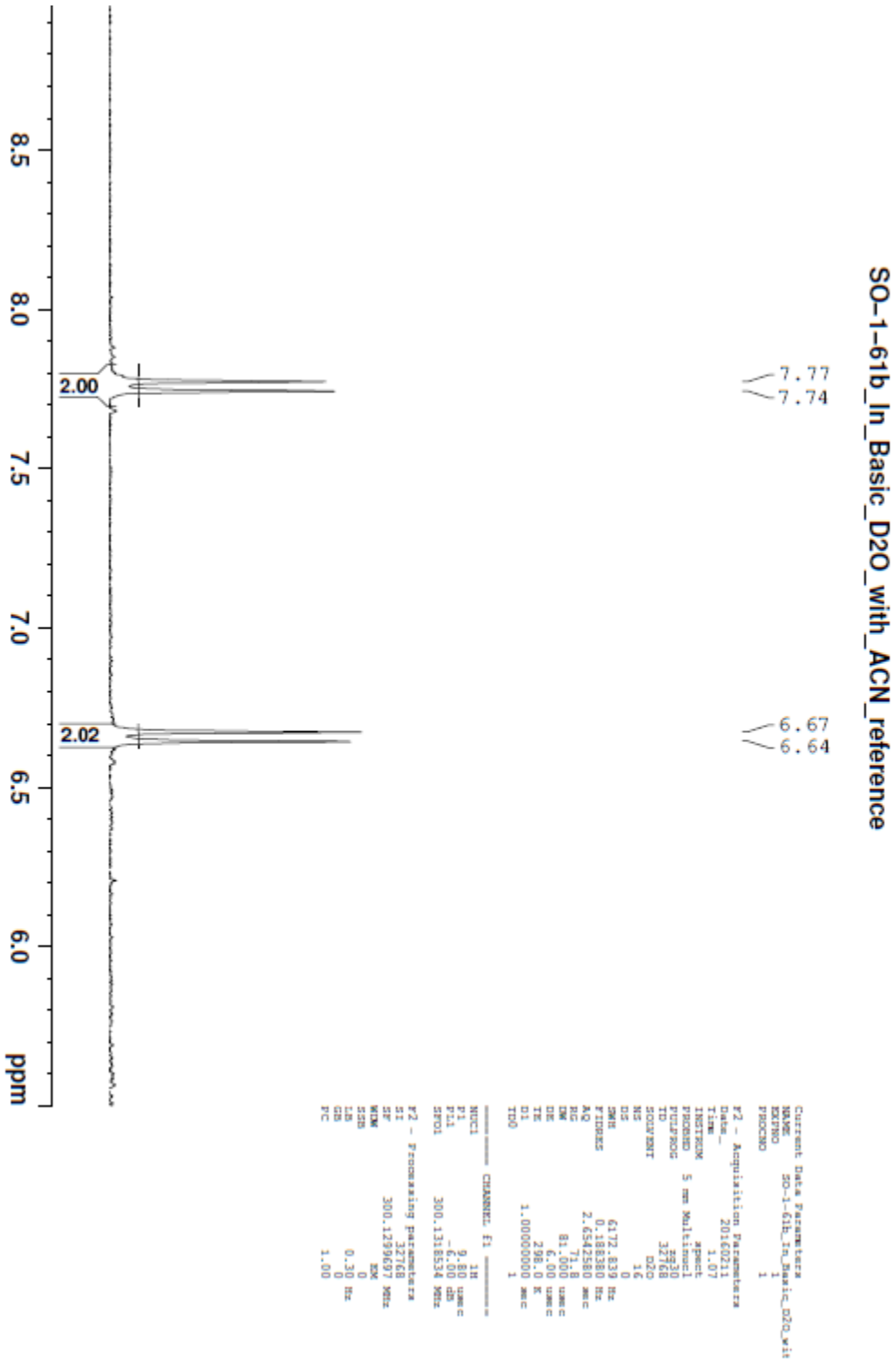

MS 3,6-dihydroxy-4,5-dimethyl-9H-xanthen-9-one (3.09)

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Shimadzu LCMS-2020 Data Report

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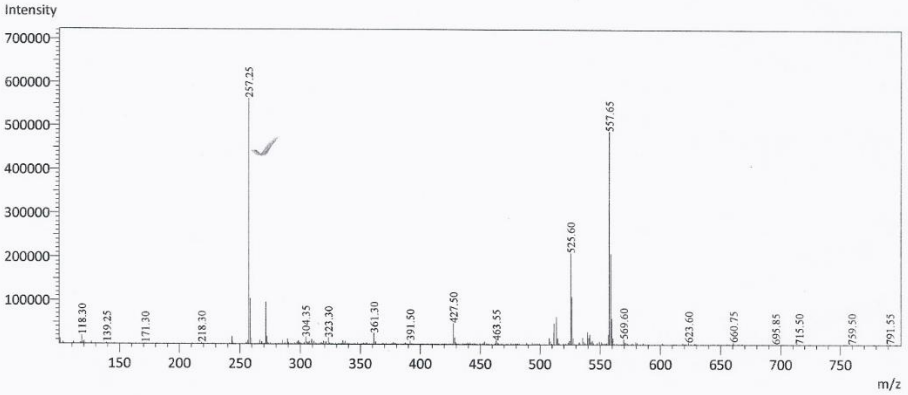

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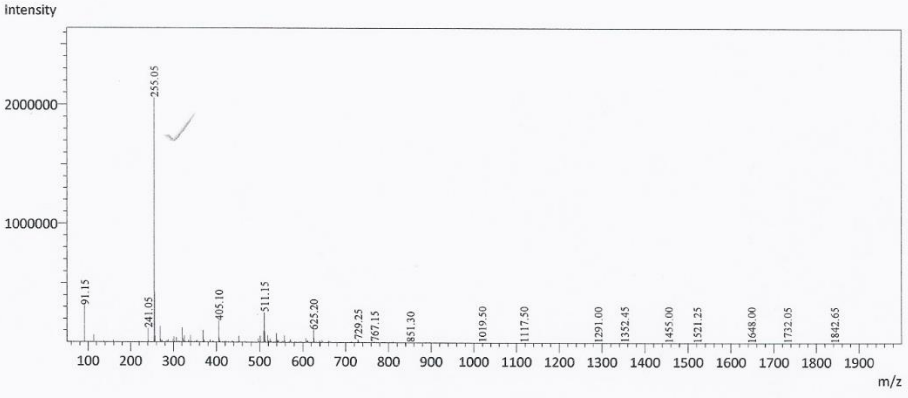

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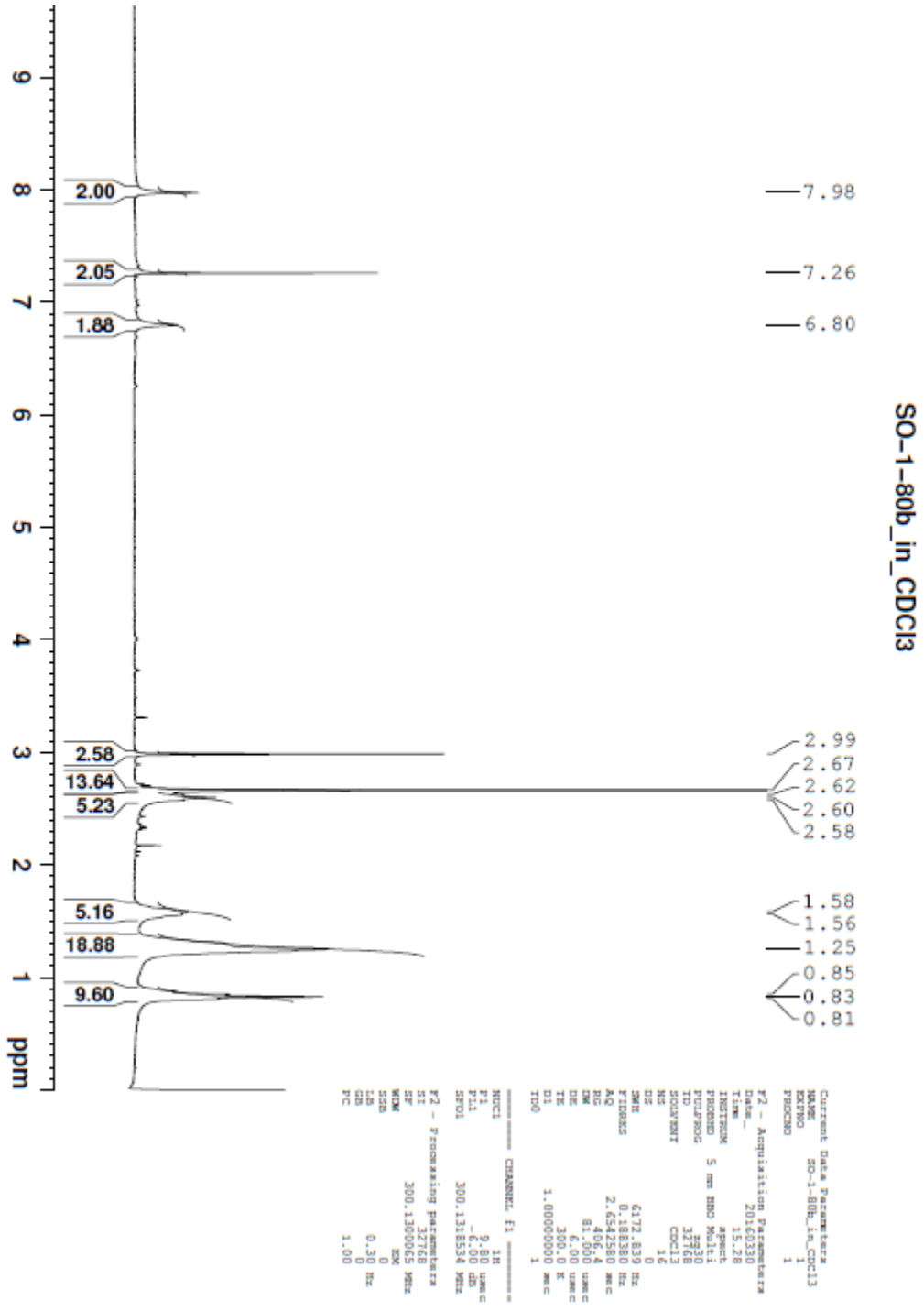

MS 2,7-dihexyl-3,6-dihydroxy-9H-xanthen-9-one (3.10)

Shimadzu LCMS-2020 Data Report

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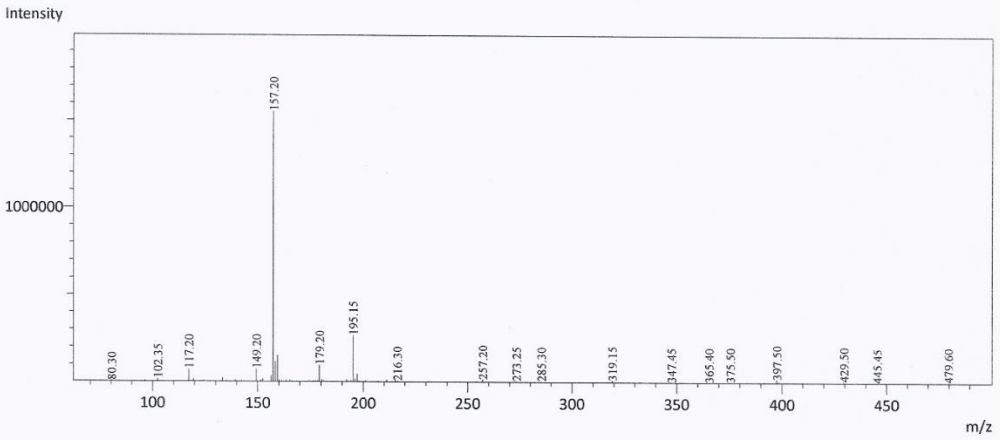

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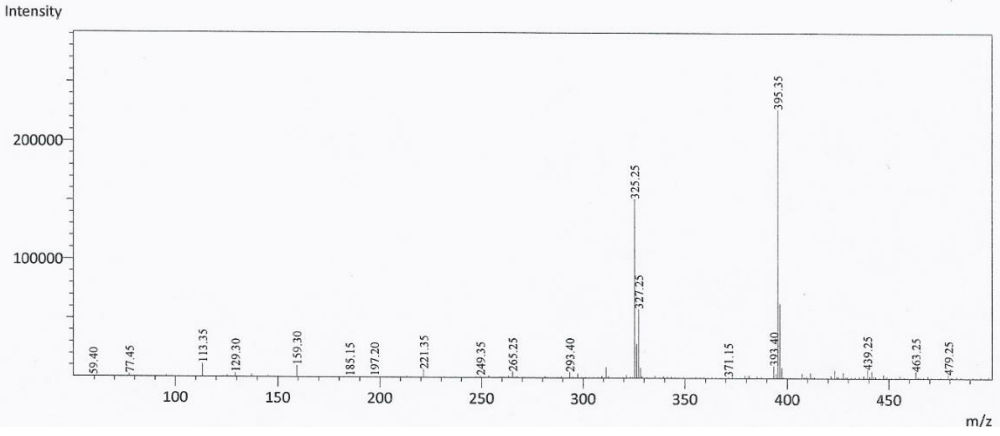
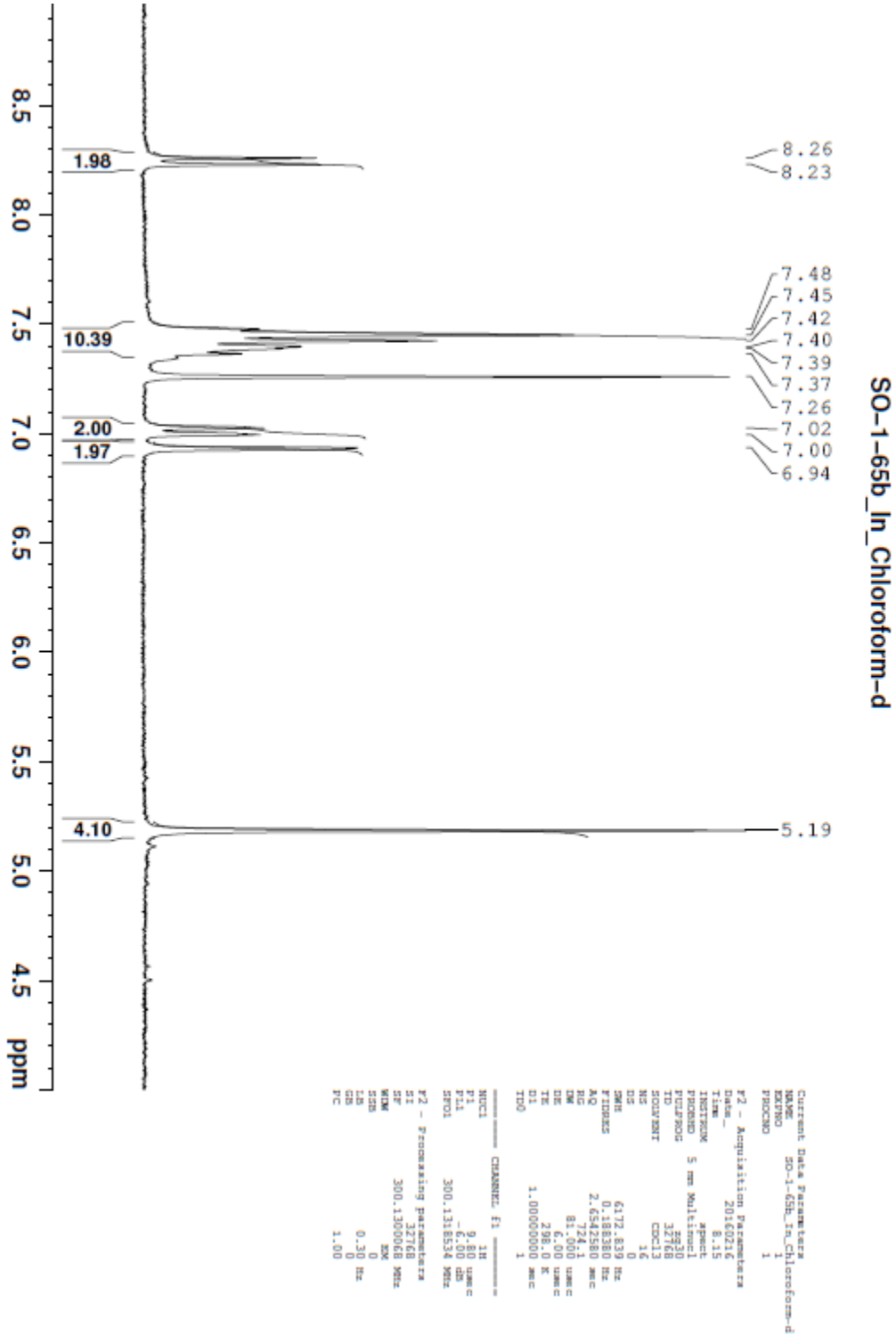


^{1}H-NMR 3,6-bis(benzyloxy)-9H-xanthen-9-one (3.11)



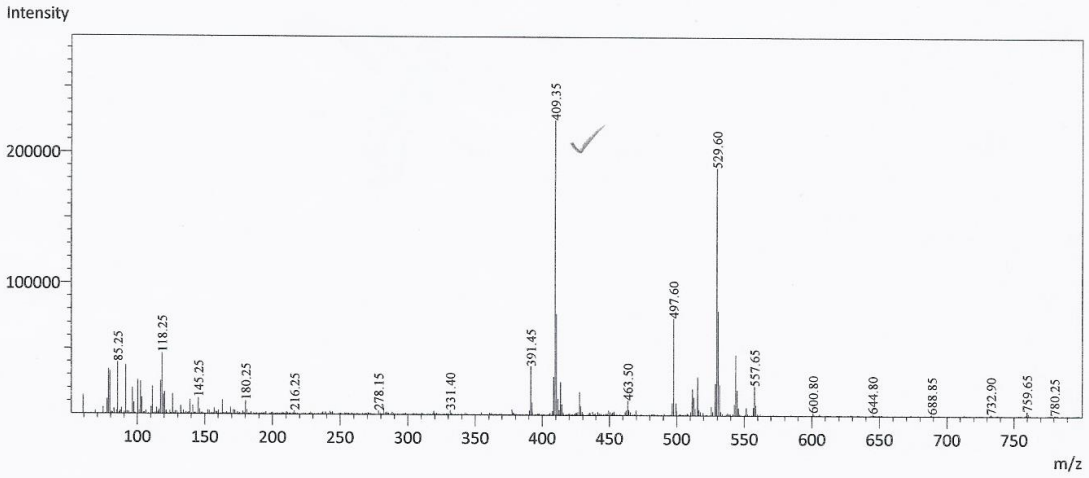
MS 3,6-bis(benzyloxy)-9H-xanthen-9-one (3.11)

Shimadzu LCMS-2020 Data Report

Mass Spectrum for Sample
SO-1-65b_2.lcd

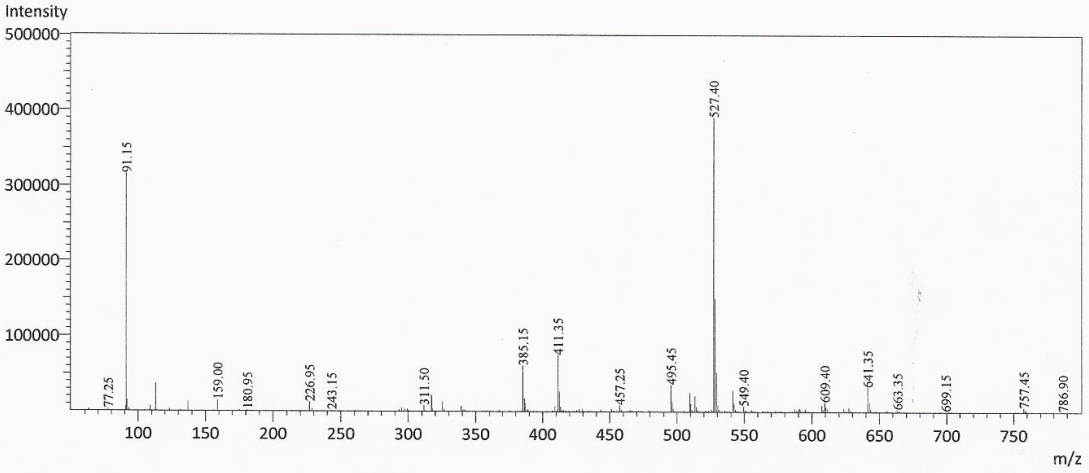
Operator: Surajudeen Omolabake

Data Filename: C:\LabSolutions\Data\Schwabacher Alan\SO-1-65b_2.lcd
Spetrum Mode: Averaged
Retention Time: ----
Interface Type (ESI, APCI, DUIS): DUIS
Aquisition Mode: (Scan, SIM, Profife): Scan
Polarity: +
H2O/0.1% HCOOH, CH3OH/0.1% HCOOH

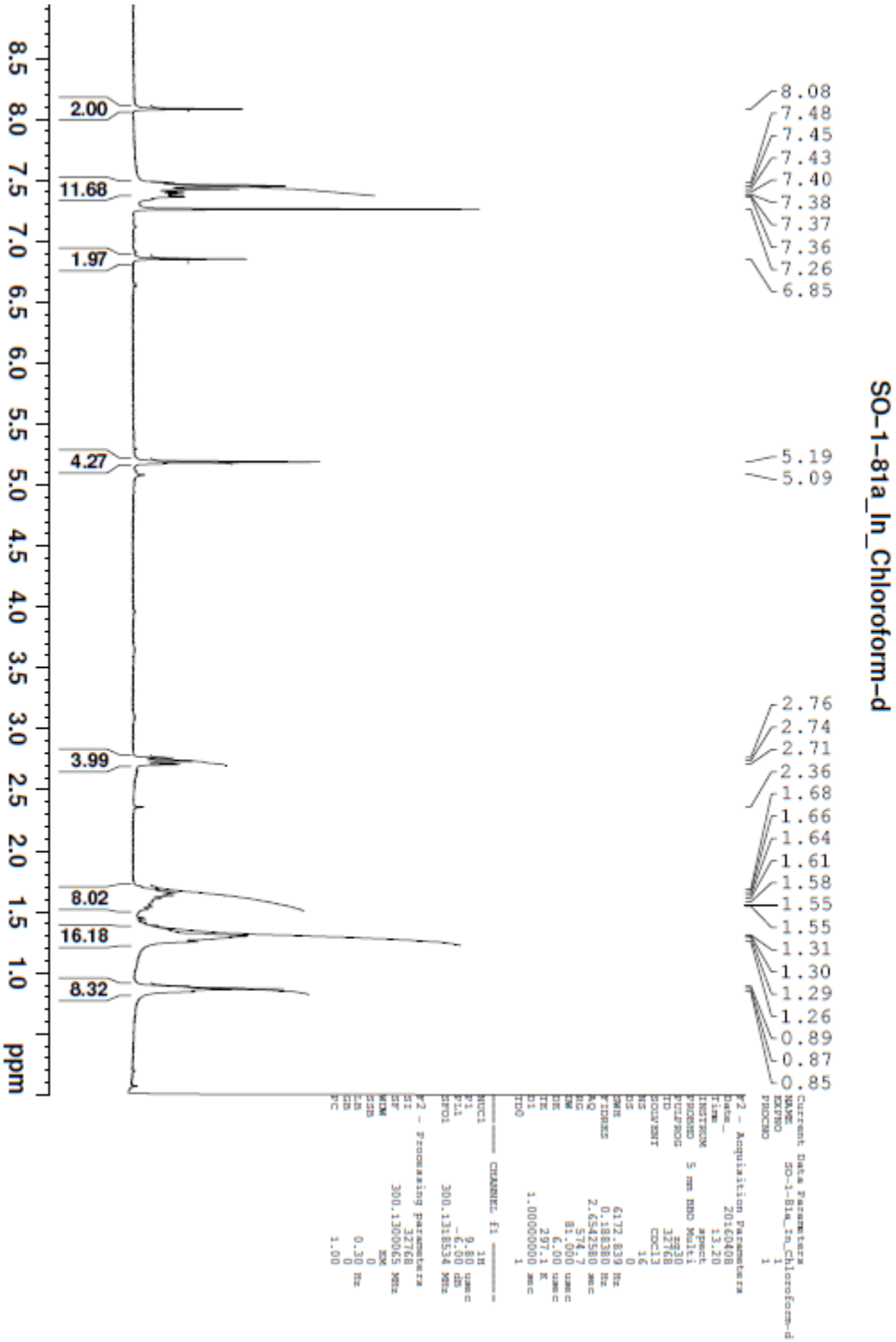


Operator: Surajudeen Omolabake

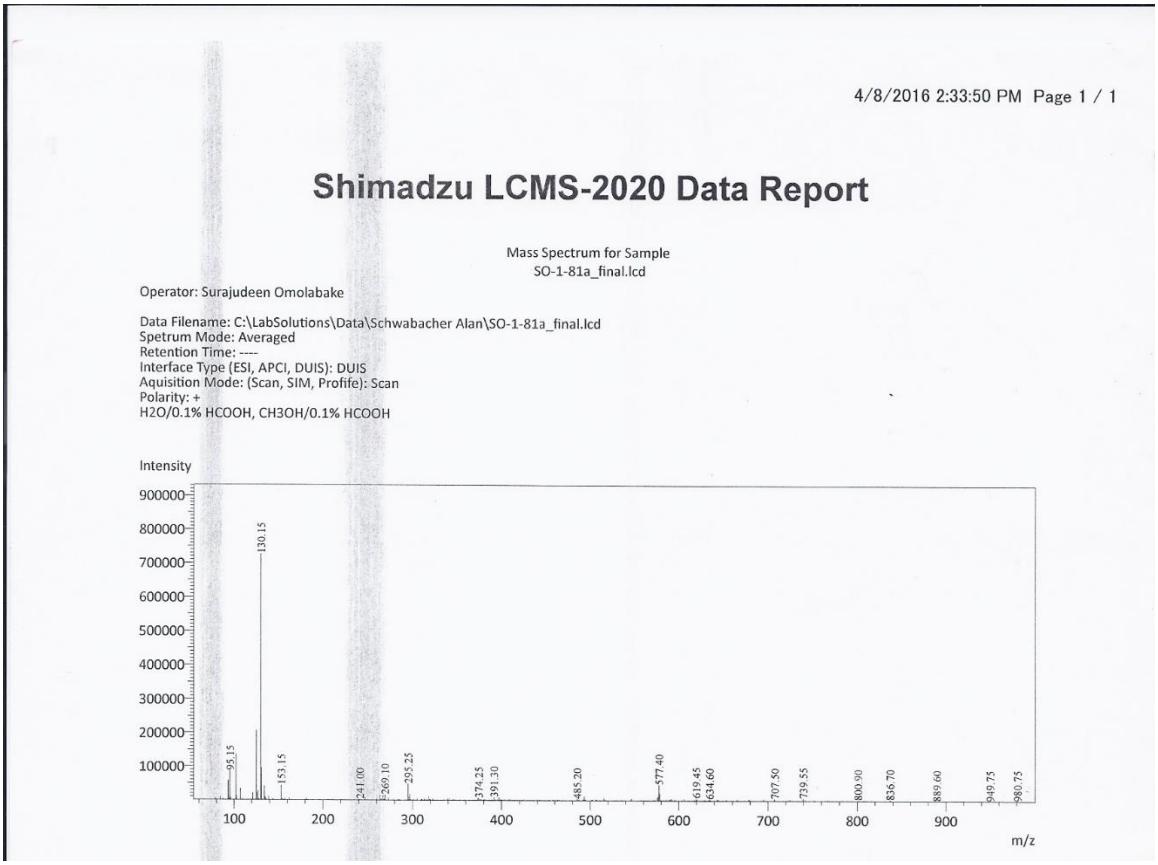
Data Filename: C:\LabSolutions\Data\Schwabacher Alan\SO-1-65b_2.lcd
Spetrum Mode: Averaged
Retention Time: ----
Interface Type (ESI, APCI, DUIS): DUIS
Aquisition Mode: (Scan, SIM, Profife): Scan
Polarity: -
H2O/0.1% HCOOH, CH3OH/0.1% HCOOH



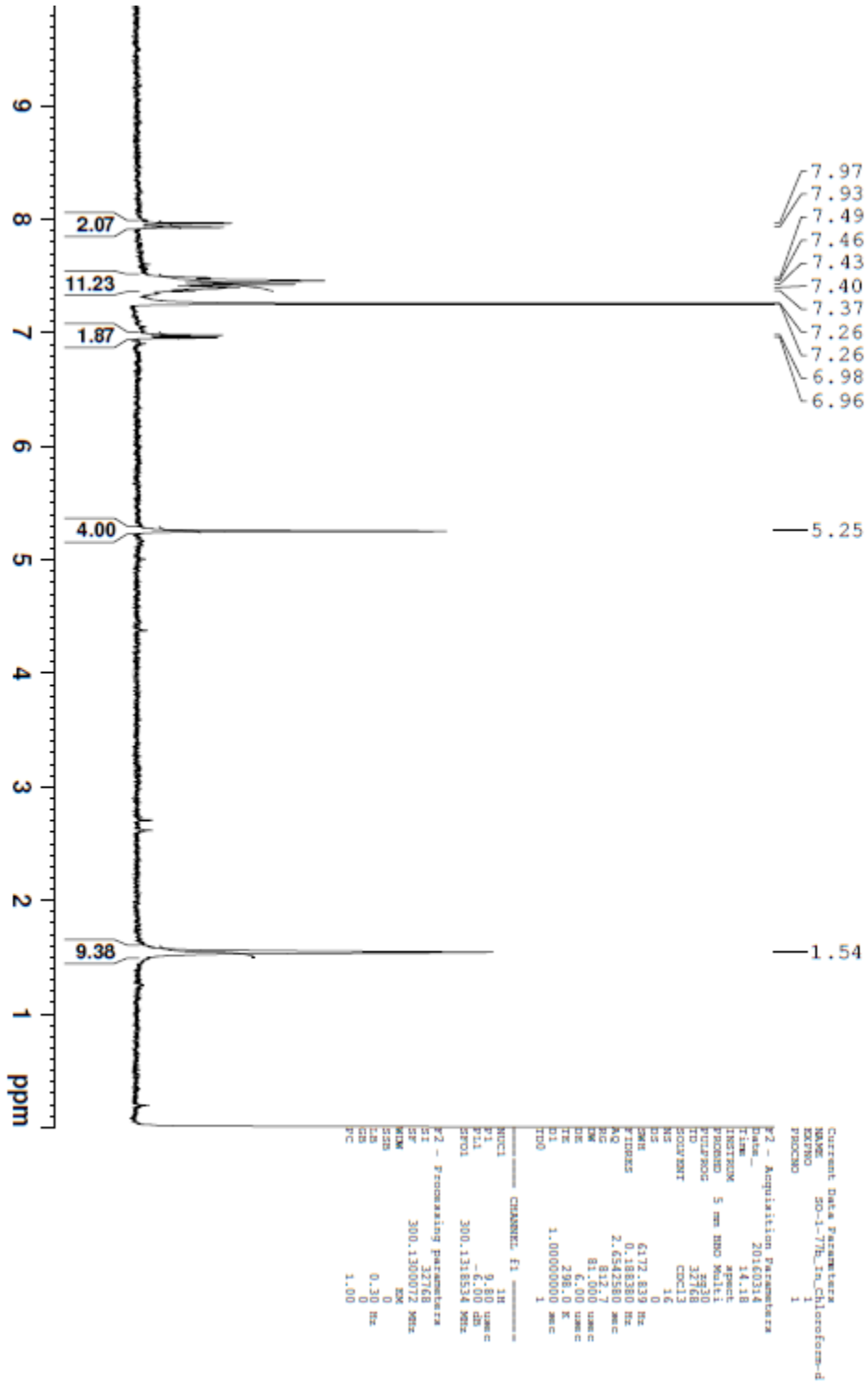
^{1}H-NMR 3,6-bis(benzyloxy)-2,7-dihexyl-9H-xanthen-9-one (3.12)



MS 3,6-bis(benzyloxy)-2,7-dihexyl-9H-xanthen-9-one (3.12)



^{1}H-NMR 3,6-bis(benzyloxy)-2,7-difluoro-9H-xanthen-9-one (3.13)



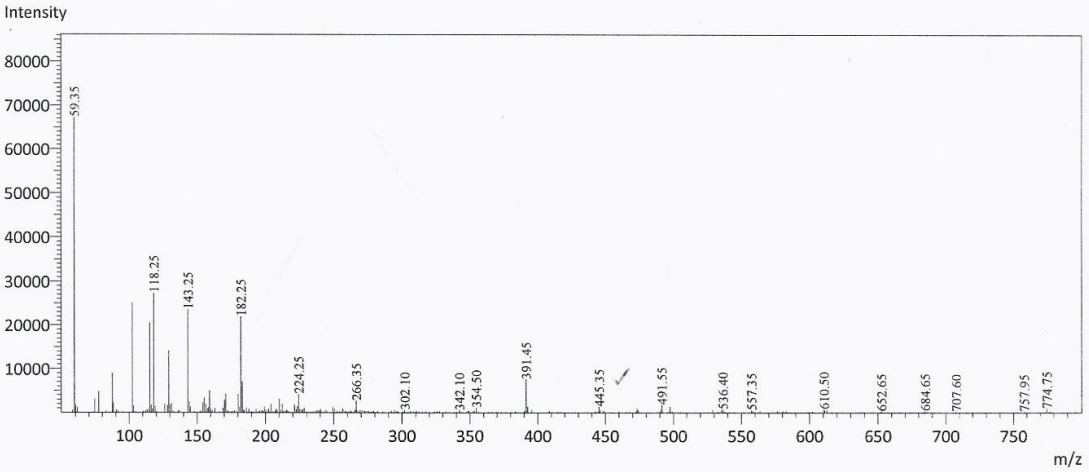
MS 3,6-bis(benzyloxy)-2,7-difluoro-9H-xanthen-9-one (3.13)

Shimadzu LCMS-2020 Data Report

Mass Spectrum for Sample
SO-1-77b.lcd

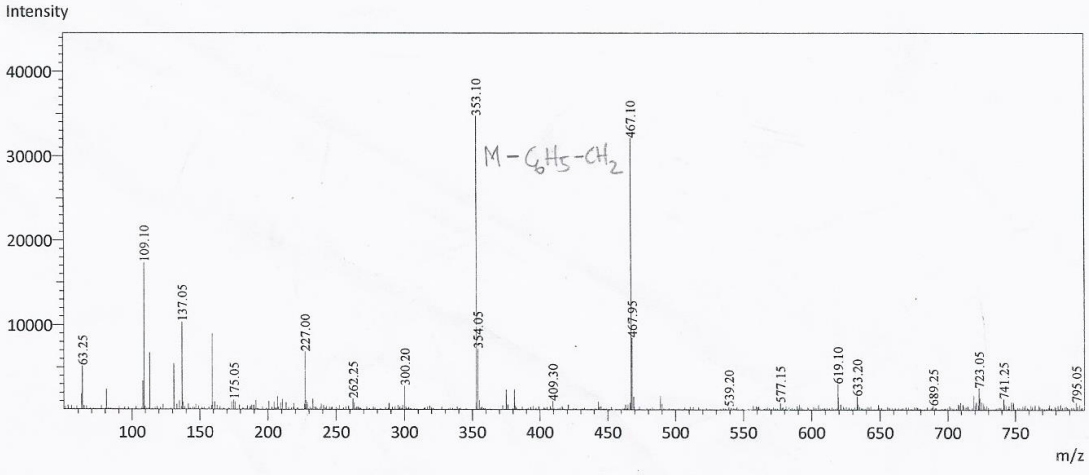
Operator: Surajudeen Omolabake

Data Filename: C:\LabSolutions\Data\Schwabacher Alan\SO-1-77b.lcd
Spetrum Mode: Averaged
Retention Time: ----
Interface Type (ESI, APCI, DUIS): DUIS
Aquisition Mode: (Scan, SIM, Profife): Scan
Polarity: +
H2O/0.1% HCOOH, CH3OH/0.1% HCOOH

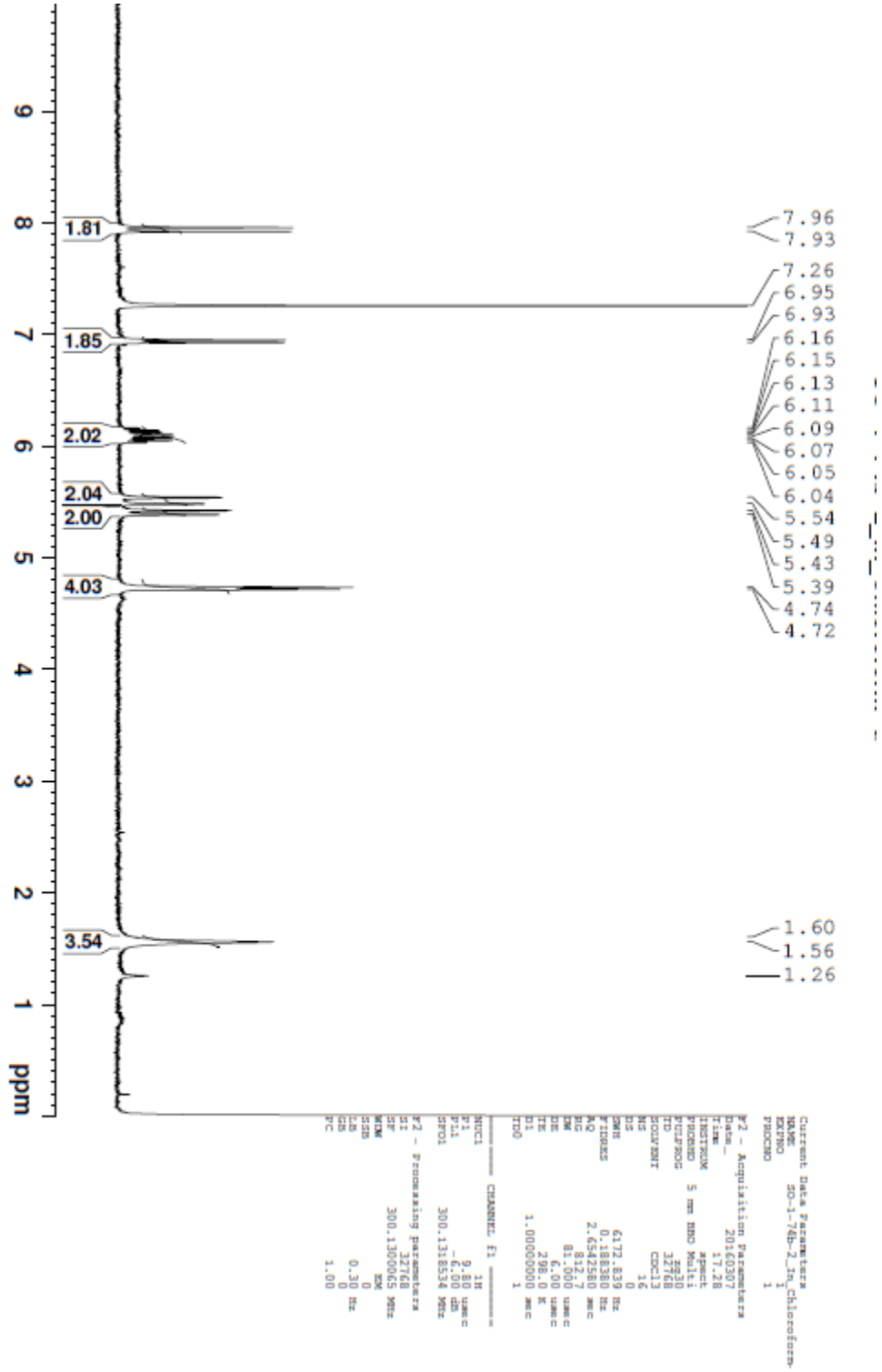


Operator: Surajudeen Omolabake

Data Filename: C:\LabSolutions\Data\Schwabacher Alan\SO-1-77b.lcd
Spetrum Mode: Averaged
Retention Time: ----
Interface Type (ESI, APCI, DUIS): DUIS
Aquisition Mode: (Scan, SIM, Profife): Scan
Polarity: -
H2O/0.1% HCOOH, CH3OH/0.1% HCOOH



^{1}H-NMR 3,6-bis(allyloxy)-2,7-difluoro-9H-xanthen-9-one (3.14)



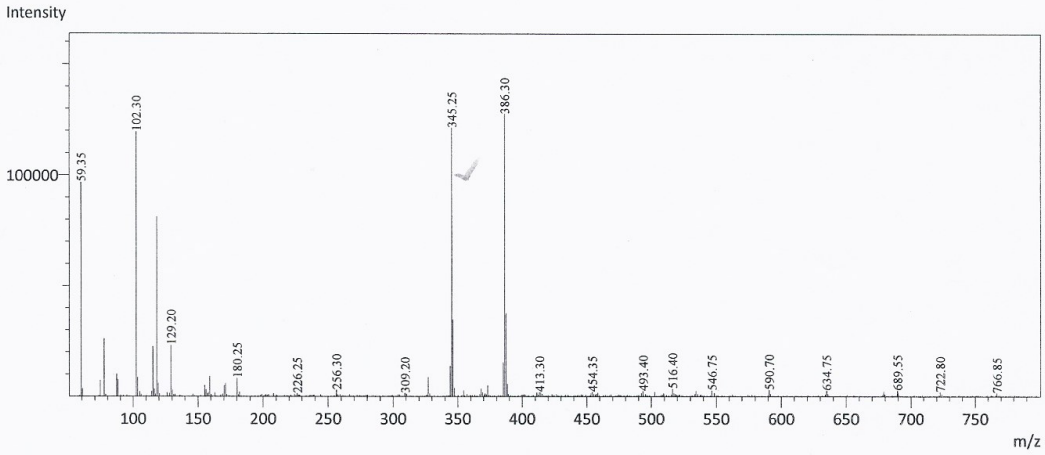
MS 3,6-bis(allyloxy)-2,7-difluoro-9H-xanthen-9-one (3.14)

Shimadzu LCMS-2020 Data Report

Mass Spectrum for Sample
SO-1-74b-2.lcd

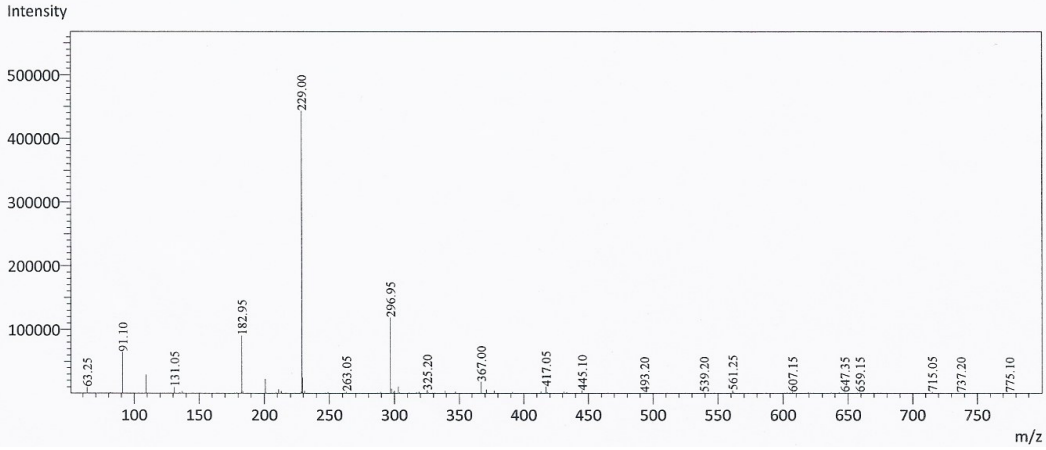
Operator: Surajudeen Omolabake

Data Filename: C:\LabSolutions\Data\Schwabacher Alan\SO-1-74b-2.lcd
Spetrum Mode: Averaged
Retention Time: ----
Interface Type (ESI, APCI, DUIS): DUIS
Aquisition Mode: (Scan, SIM, Profife): Scan
Polarity: +
H2O/0.1% HCOOH, CH3OH/0.1% HCOOH

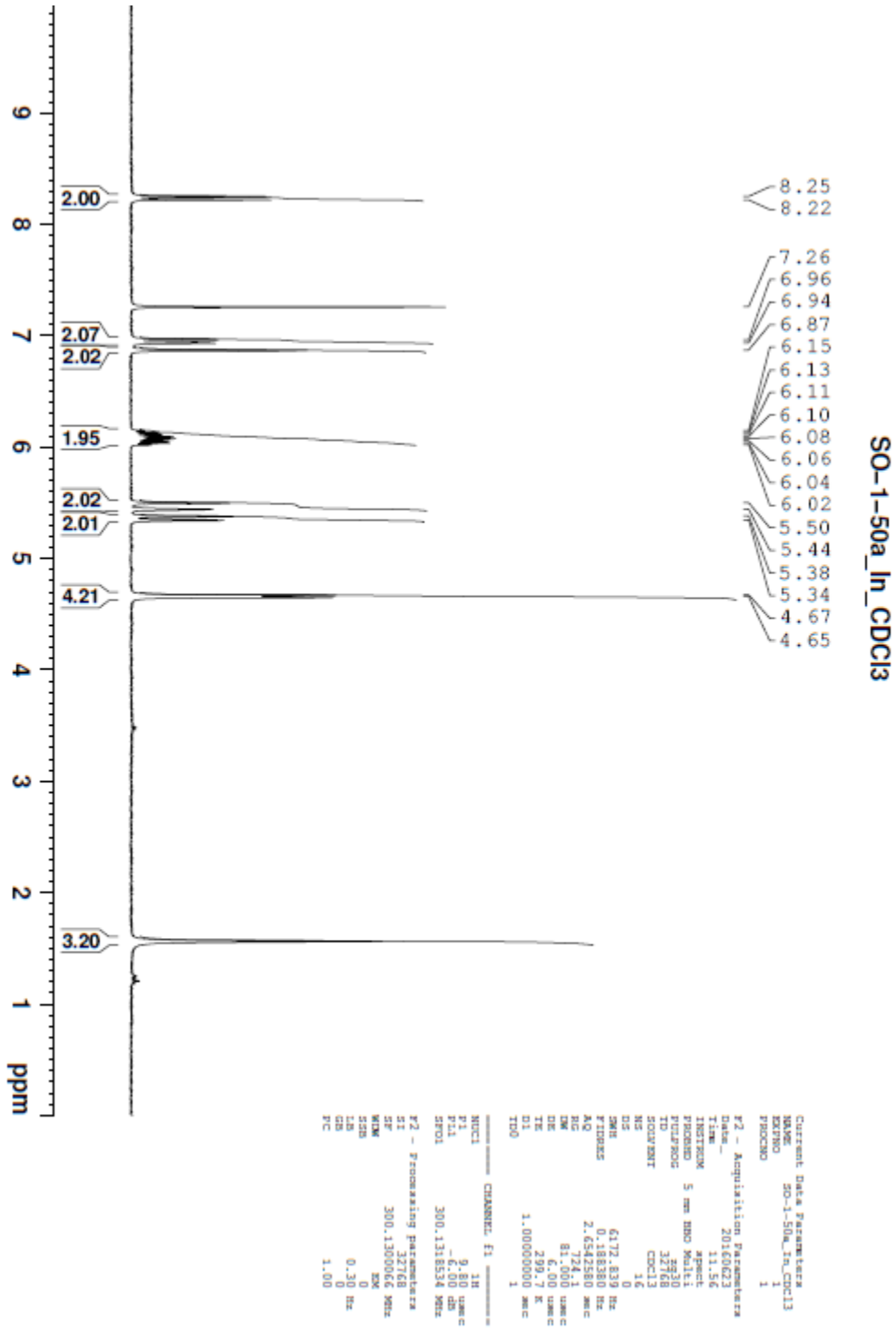


Operator: Surajudeen Omolabake

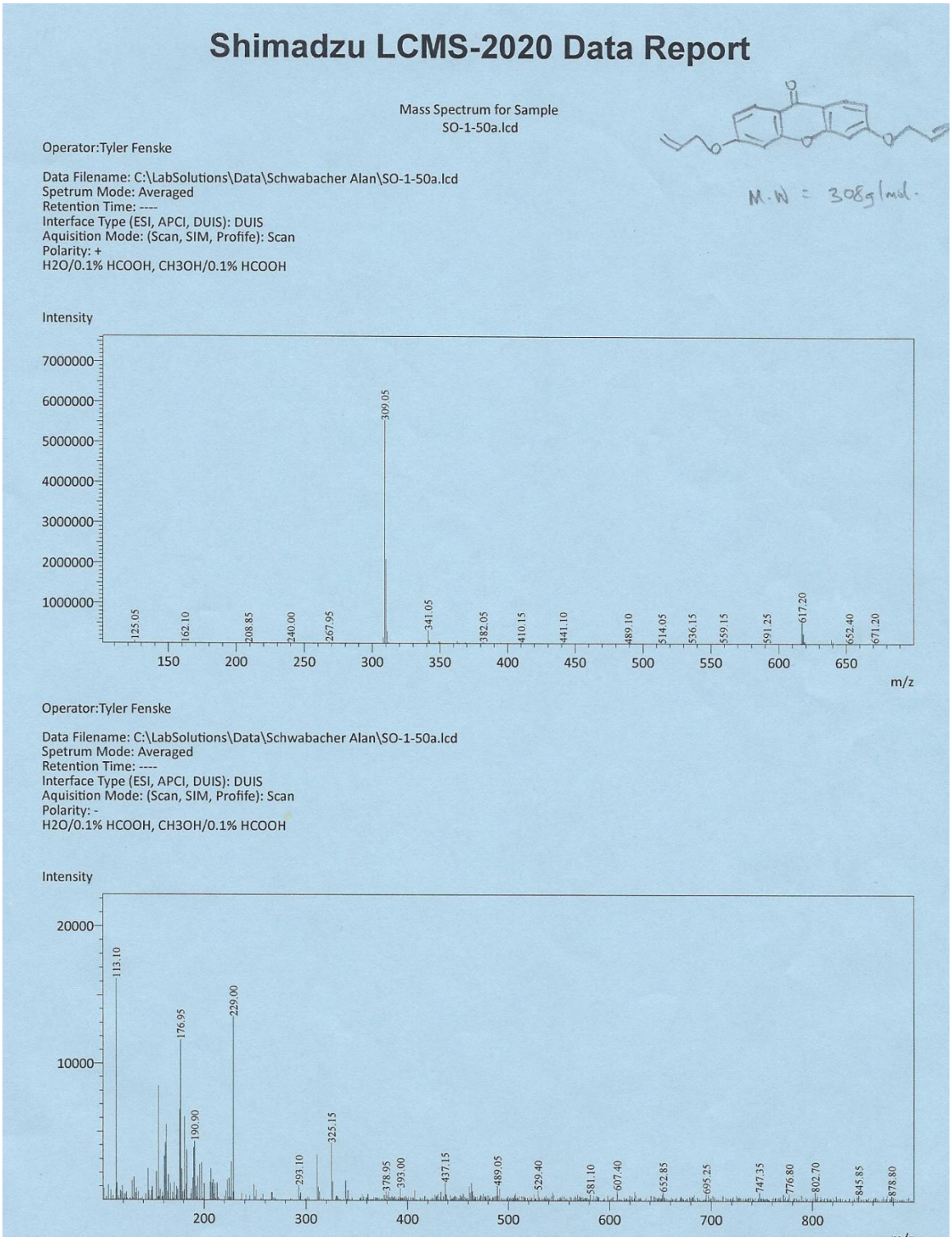
Data Filename: C:\LabSolutions\Data\Schwabacher Alan\SO-1-74b-2.lcd
Spetrum Mode: Averaged
Retention Time: ----
Interface Type (ESI, APCI, DUIS): DUIS
Aquisition Mode: (Scan, SIM, Profife): Scan
Polarity: -
H2O/0.1% HCOOH, CH3OH/0.1% HCOOH



^{1}H-NMR 3,6-bis(allyloxy)-9H-xanthen-9-one (3.15)



MS 3,6-bis(allyloxy)-9H-xanthen-9-one (3.15)



^{1}H-NMR 3,6-bis(benzyloxy)-9-(trifluoromethyl)-9H-xanthen-9-ol (3.16)