CHAPTER 1 INTRODUCTION

Cordyline fruticosa Back has been traditionally used as an herbal medicine to treat various diseases in Indonesia [1]. This plant is also known as natural dyes in organic natural colorants [2]. However, there has been no scientific report of its application on biological activities. Therefore, the investigation of antioxidant and antibacterial activities from the extracts of *C. fruticosa* Back leaves is carried out in this research.

As a part of natural dyes, xanthenes are also pharmaceutically useful in medicinal and material chemistry [3]. The synthesis of various xanthene derivatives has drawn notable attention due to these extensive benefits. Recently, a number of diverse approaches to synthesize xanthene derivatives have been developed [4], however several of them suffer from one or more drawbacks. To face this challenge, hexabromoacetone ($Br_3CCOCBr_3$), an infrequent halogenated reagent in organic synthesis application, has been established to synthesize xanthene derivatives as a novel and efficient method under solvent-free conditions.

1.1 Objectives

This research was divided into two parts:

1. To investigate antioxidant and antibacterial activities from the extracts of *Cordyline fruticosa* Back leaves.
2. To synthesize xanthene derivatives from the reactions of various aromatic aldehydes with β-naphthol, 5,5-dimethyl-1,3-cyclohexanedione or 5-methyl-1,3-cyclohexanedione using $Br_3CCOCBr_3$ under solvent-free conditions.

1.2 Scopes of Research

Evaluation of antioxidant and antibacterial activities from the extracts of *Cordyline fruticosa* Back leaves and synthesis of xanthene derivatives from the reactions of various aromatic aldehydes with β-naphthol, 5,5-dimethyl-1,3-cyclohexanedione or 5-methyl-1,3-cyclohexane-dione in the presence of $Br_3CCOCBr_3$ under solvent-free conditions.

1.3 Expected Benefits

1. The first data report of antioxidant and antibacterial activities from the extracts of *Cordyline fruticosa* Back leaves.
2. The utilization of $Br_3CCOCBr_3$ as a novel and efficient method for the synthesis of xanthene derivatives from the reactions of various aromatic aldehydes with β-naphthol, 5,5-dimethyl-1,3-cyclohexanedione or 5-methyl-1,3-cyclohexanedione under solvent-free conditions.

CHAPTER 2 ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF LEAVES EXTRACTS OF *Cordyline fruticosa* Back

2.1 Introduction

The utilization of traditional herbal medicine is well recognized by local people however this behavior has not been recorded properly in scientific region. Researchers have been challenged to develop those potentials to appreciate the role of medicinal plants in health care delivery system [1]. The National Park of Kutai East Kalimantan, one of the resource of heritage traditional herbal medicine in Indonesia, screened their potential plants in scientific field. Using 5 types of traditional shrub plants, they tested the antibacterial activity against pathogenic bacteria, *Basillus cereus*, toward *Cordyline fruticosa* Back, *Asystasia indusa* Bl., *Acalypta wilkesiana*, *Prunus cistena* and *Codiaeum variegatum* leaves. The result was astonishing, among these potential shrubs utilized by local people as diarrhea treatment, only *C. fruticosa* Back showed a promising antibacterial activity (9.1 mm) than others (*A. indusa* 6.2 mm; *A. wilkesiana* 7.0 mm; *P. cistena* 6.3 mm; *C. variegatum* 8.1 mm). Therefore, further investigation of potential biological activities from *C. fruticosa* Back leaves was then established.

The genus of *Cordyline*, with about 20 species, is widely distributed in Southeast Asia, Australia and New Zealand [5]. Cordylines are known to the world by many names and extensively used in Southeast Asia for the treatment of various diseases [6] and natural dyes application [2]. In Indonesia, *C. fruticosa* Back (Liliaceae), locally called “Andong”, has been used in folklore medicine to treat various diseases utilizing the roots and leaves [1, 7]. Some scientific data reported that Cordylines had a potential activity as antioxidant [7], cytotoxic agent [8, 9], antimicrobial [9, 10] and anthelmintic [11]. However, there are no reports describing the biological activity from *C. fruticosa* Back, specifically.

Preliminary screening of ethanol (EtOH) extract of *C. fruticosa* (L.) A. Cheval showed that it had scavenging activity against 1,1-diphenyl-2-picrylhydrazil (DPPH) around 283.82 ± 1.02 μg/mL [7]. The isolated saponins from methanol (MeOH) extract were not able to inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*,

Pseudomonas aeruginosa and *Candida albicans*, nevertheless it showed a moderate antibacterial activity against the Gram-positive *Enterococcus faecalis* [9].

Due to the fact that *C. fruticosa* Back has been used as a traditional medicine and there are not any reports on its biological activities, the antioxidant and antibacterial activities are investigated and reported herein.

2.1.1 Traditional Utilization of *Cordyline fruticosa* Back

Cordyline fruticosa Back is well known by Indonesian people as "Andong" or "Hanjuang" at some area. This monocotyledon plant is widely distributed along Indonesian islands and recognized as shrub or decorative plant besides medicinal herb [1].

Clasification of the plant [12] :

Kingdom : *Plantae*
Division : *Magnoliophyta*
Class : *Liliopsida*
Orde : *Liliales*
Family : *Liliaceae*
Genus : *Cordyline*
Species : *Cordyline fruticosa* Back

Figure 2.1 *Cordyline fruticosa* Back

In Indonesia, *C. fruticosa* Back has been used as traditional medicine for long time ago. People used the roots to treat peptic and liver ulcers, meanwhile the leaves were used for headache, lung tuberculosis, asthma, skin disorder, eye inflammation, back pain, rheumatism and arthritis treatments [7]. The National Park of Kutai East Kalimantan Indonesia, a resource of heritage traditional herb, reported that *C. fruticosa* Back can be applied for cooling sensation, hemostatic, anti-swelling, bloody cough, diarrhea, dysentery, hyper menstruation, hematuria and hemorrhoids [1].

2.1.2 Research Relations of *Cordyline fruticosa* Back

There have been some previous reports of *C. fruticosa* species, including other species in the same genus, such as *C. terminalis* Kunth. However, there has not been any report of *C. fruticosa* Back, specifically.

Ahmed and co-workers [10] in 2003 screened the antibacterial activity from leaves extracts of *C. terminalis* Kunth. The MeOH extract showed a moderate antibacterial activity against *Escherichia coli, Shigella boydii, Streptococcus pyogenes* and *Staphylococcus epidermis.* The hexane fraction presented a mild antibacterial activity against *Salmonella typhi, Shigella boydii* and *Shigella dysenteriae,* whereas the acetone and chloroform fractions did not show any activity.

The leaves of *C. terminalis* Kunth was also extracted by Bogoriani, Santi and Asih [8] in 2007 using *n*-butanol. The cytotoxic activity was examined using brine shrimp lethality test (BSLT) and showed the LC_{50} (lethal dose concentration) value of 41.64 ppm.

In 2009, Atjanasuppat and colleagues [11] screened the *in vitro* anthelmintic activity against *Schistosoma mansoni* using dichloromethane (CH_2Cl_2) extract of *C. fruticosa* (L.) A. Chev. leaves and showed a low anthelmintic activity (IC_{50} 17.77 μg/mL) against this infective cercarial worm.

Marliana [7] in 2012 extracted the leaves of *C. fruticosa* (L) A. Cheval with EtOH. On the preliminary of phytochemical test, flavonoids, phenolics, saponins and steroids were found. Antioxidant activity of the EtOH extract was evaluated using scavenging DPPH radical method and gave an EC_{50} (half-maximal effective response concentration) value of 283.82±1.02 μg/mL.

Fouedjou and co-workers [9] in 2014 evaluated the *in vitro* cytotoxic and antimicrobial activities from *C. fruticosa* (L.) A. Chev. using the micro culture tetrazolium (MTT) assay. Three steroidal saponins of EtOAc extract showed a moderate antiproliferative activity against three human cancer cell lines. However, these extract compounds were not able to inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* at the highest of concentration (256 mg/L), nevertheless it could perform a moderate activity against *Enterococcus faecalis.*

2.2 Experimental

2.2.1 Instruments and Chemicals

UV spectra were recorded on a PerkinElmer Instruments Lamda 35 UV/Vis Spectrometer. 1,1-Dipheny-2-picrylhydrazyl (DPPH), 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4"-disulfonic acid mono-sodium salt (ferrozine), 2,4,6-Tris(2-pyridyl)-s-

triazine (TPTZ) and Folin-Ciocalteau reagent were from Fluka or Sigma-Aldrich. All solvents in this research were used commercial grade and have been purified prior to use by standard methodology.

2.2.2 Plant Sample

The leaves of *Cordyline fruticosa* Back were collected from East Kalimantan Province, Indonesia, in June 2013. The specimen was identified by Technology Research Institute of Natural Resources Conservation, Indonesian Forestry Ministry, and the voucher specimen was deposited at Laboratory of Forest Product Chemistry, Faculty of Forestry, Mulawarman University, Indonesia, with voucher number CF-DD-3.

2.2.3 Extract Preparations

The extraction and fractionation were done according to previous method [13] with some modification in the choice of partitioning solvents. The dry leaves (432 g) were extracted with 6 L MeOH affording the MeOH extract. The MeOH extract (49.2 g) was dissolved in water (1.5 L) and exhaustively extracted by consecutive liquid-liquid partition with hexane (0.7 L), CH_2Cl_2 (0.7 L), EtOAc (0.7 L) and n-BuOH (0.7 L). The fractions were concentrated under reduced pressure to yield hexane, CH_2Cl_2, EtOAc, n-BuOH and water extracts, respectively. Finally, all extracts were stored in dark bottle at 4 ^oC before analysis. Only MeOH, EtOAc and hexane extracts were selected to be evaluated on antioxidant and antibacterial activities.

2.2.4 Phytochemical Screening

The phytochemical screening of leaves extracts of *C. fruticosa* Back was conducted by previous methods [14-17] with minor modification.

- **Alkaloids**: the screening of alkaloids was done using Dragendorff, Mayer and Wagner tests by giving every 1 mL of sample extract with 2 mL of Dragendorff reagent, 1 mL of Mayer reagent and 3-5 drops of Wagner reagent. The appearance of red, yellow or reddish brown precipitation in the solution was the indicator of alkaloids.

- **Anthraquinones**: five milliliters of 10% NH_3 were added to 1 mL of sample in 10 mL of benzene solution. The pinkish color on the solution after shaking was the indicator of anthraquinones.

- **Antocyanins**: pink color was the indicator of antocyanins after adding 5 mL of diluted HCl to 1 mL of sample extract.

- **Saponins**: the saponins were detected by observation for 10 minutes foam persistence after shaking 1 mL of sample extract with 2 mL of water.

- **Steroids**: the Salkowski test was used to identify the steroids by mixing 1 mL of sample extract with 10 mL of $CHCl_3$ and concentrated H_2SO_4. The presence of yellow-green layer of solution was the indicator of steroids.

- **Terpenoids**: the Salkowski test was also used to identify the terpenoids by diluting 2 mL of chloroform to 0.2 g of sample extract. Then, 3 mL of concentrated H_2SO_4 were added carefully to show red brown color at the interface of solution.

- **Flavanoids**: the flavonoids were detected by alkaline reagent, $FeCl_3$, and Pb-acetate tests. The colorless of mixture solution from yellow color after adding diluted HCl to 2 mL of sample extract with few drops of 20% NaOH was the sign of flavonoids using alkaline reagent test. The blackish-red color was the indicator of flavonoids after adding few drops of $FeCl_3$ to the sample extract. Meanwhile, Pb-acetate test was conducted by adding few drops of 10% Pb-acetate to the sample extract and performing yellow precipitation in the solution.

- **Phenols**: phenol compounds were also detected by Pb-acetate and $FeCl_3$ test. The brown precipitation was indicator of phenol compound after adding 2 mL of 10% Pb-acetate to 1 mL of sample extract. While, blue-black color would appear after adding 5% $FeCl_3$ to sample solution.

- **Tannins**: tannins were indicated by $FeCl_3$, gelatin and vanilin-HCl test. Dark green color would appear after giving 2 mL of $FeCl_3$ to 1 mL of sample extract, while white precipitation would be shown after adding 1% gelatin-NaCl and the vanilin-HCl reagent would perform pink color in the mixture solution.

- **Cardiac glycosides**: the Keller Keliani and modified-Borntragers tests were used to identify the cardiac glycosides. For Keller Keliani test, the appearance of brown ring in the solution was an indicator of cardiac glycosides by carefully adding 1 mL of concentrated H_2SO_4 to the mixture of 5 mL sample, 2 mL acetic glacial and 3 drops of $FeCl_3$. The modified-Borntragers test was done by heating up the mixture of sample solution, HCl dilution and few drops of $FeCl_3$ to perform rose-pink color after adding benzene and NH_3 to the solution.

2.2.5 Antioxidant Activity

2.2.5.1 DPPH Radical Scavenging Activity

The scavenging activity of extracts for the DPPH radical was measured as described [18] with some modifications. *C. fruticosa* Back extracts were dissolved in MeOH at a concentration of 0.01-16.0 mg/mL. Various concentrations of extracts (100 μL) were mixed with 3 mL of DPPH methanol solution (60 μM). The solution was stirred and left to stand for 20 minutes in the dark and measured the absorption at 517 nm. The percentage inhibition was calculated using the equation:

$$\%\ Inhibition = \frac{(A_1 - A_2)}{A_1} \text{x } 100$$

where A_1 and A_2 are the absorbance of DPPH and extract solution, respectively. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentrations. Ascorbic acid was used as a standard. All experiments were performed in triplicate.

2.2.5.2 Ferric Reducing Antioxidant Power Assay

The ferric reducing power was determined using a modified version of the FRAP assay [19]. The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6) with 10 mM TPTZ in 40 mM hydrochloric acid and 20 mM ferric chloride (10:1:1). The freshly prepared FRAP reagent (1.5 mL) was added to 50 μL of sample along with 150 μL distilled water. The reaction mixture was incubated for 30 minutes in room temperature. Then, the absorbance of the sample was measured at 593 nm. A standard curve ($y = 0.012x + 0.448$; $r^2 = 0.985$) was prepared using various concentrations of $FeSO_4.7H_2O$ (5-30 mM). All measurements were done in triplicate. FRAP values were expressed as mmol Fe^{2+} equivalents (eq)/1 g sample.

2.2.5.3 Fe^{2+} Chelating Activity

The chelating activity on ferrous ion was adapted according to the method [20] with a minor modification. The reaction contained 1 mL of sample at various concentrations (0.01-6.5 mg/mL) with 1 mL of 0.1 mM $FeSO_4$ mixed for 30 seconds, then 1 mL of 0.25 mM ferrozine was added and the mixture was incubated for 10 minutes at room temperature. The absorbance of the Fe^{2+}-ferrozine complex was measured at 562 nm. EDTA-2Na was used as the standard. The chelating activity of the extract was calculated as:

$$\%\ Chelating\ rate = 1 - \frac{(A_1 - A_2)}{A_0} \text{x } 100$$

where A_0 is the absorbance of control (without extract), A_1 is the absorbance of sample with ferrozine and A_2 is the absorbance of sample without ferrozine.

2.2.6 Total Phenolic Contents

The total phenol content was obtained by a modification of the Folin-Ciocalteau method [21]. A 200 μL of sample was dissolved in MeOH : water (1:1) and mixed with 1 mL of 0.2 N Folin-Ciocalteau reagents and stirred for 5 minutes. Then, 80 μL of 7.5% sodium carbonate (Na_2CO_3) was added and the mixture was incubated at room temperature in the dark for 1 hour. The absorbance was measured at 760 nm. Gallic acid was used as a calibration curve ($y = -10^{-5}x^2 + 0.007x - 0.017$; $r^2 = 0.998$) by various concentrations (20-220 μg/mL). All experiments were performed in triplicate. The results are expressed as mg gallic acid equivalents (GAE)/ 1 g sample.

2.2.7 Antibacterial Activity

Antibacterial assay was conducted using the disc diffusion method as previously described [22] with minor modification. Nutrient dextrose agar was used as a media by combining 10 g agar powder, 8 g nutrient broth and 5 g dextrose in 500 mL of water. MeOH extract (500 ppm) was used to investigate the antibacterial activity against *Bacillus cereus, Salmonella thypii* and *Streptococcus sobrinus*. The bacterial were uniformly spread using sterile cotton swab on the plates of nutrient agar after turbidity test using spectroscopy visible till 75% light transmittance at 620-650 nm. The plates then were kept in an incubator at 37 ^{o}C for 24 hours. Chloramphenicol (5 mg/mL) was used as a standard drug to ensure the activity of standard antibiotic against the test organisms. The diameters of inhibition zone were measured in mm.

2.3 Results and Discussion

2.3.1 Extract Preparation

The dried leaves of *C. fruticosa* Back (432 g), collected from East Kalimantan Province, Indonesia, were pulverized to get the powder leaves. The process was continued by maceration in 6 L of MeOH for 72 hours. The filtrate was concentrated under reduced pressure using rotary evaporator to yield the MeOH extract.

After completing the partition process, 49.2 g of MeOH extract could yield 1.7 g of EtOAc extract (3.7 %) and 9.5 g of hexane extract (19.3 %), respectively. This result indicated that the leaves of *C. fruticosa* Back dominantly contained lots of polar compounds, especially in MeOH extract. Cowan *et al.* [23] reported that high polarity compounds in the MeOH extract possibly contain several kinds of major phytochemical compounds such as polyphenols, flavones, antocyanins and tannins.

The physical appearances of these extracts were semi solid with green color (MeOH extract), brown solid (EtOAc extract) and oily semi solid with dark green color (hexane extract). Afterwards, the crude MeOH extract and these two partitioned extracts were examined for their phytochemical constituents, total phenolic contents and antioxidant activity.

2.3.2 Phytochemical Screening

The phytochemical screening on this research revealed the presence of different classes of secondary metabolites, such as alkaloids, phenols, flavonoids, steroids, saponins, cardiac glycosides, anthraquinones, antocyanins and terpenoids. The results of phytochemical screening test of *C. fruticosa* Back leaves extracts are presented in Table 2.1.

Table 2.1 Phytochemical screening test of *Cordyline fruticosa* Back leaves extracts

Compounds	MeOH Extract	EtOAc Extract	Hexane Extract
Alkaloids	+	+	+
Phenols	+	+	+
Tannins	+	+	+
Flavonoids	+	+	-
Steroids	-	+	+
Saponins	-	+	+
Cardiac glycosides	-	-	+
Anthraquinones	-	-	-
Antocyanins	-	-	-
Terpenoids	-	-	-

+ : present; - : absent

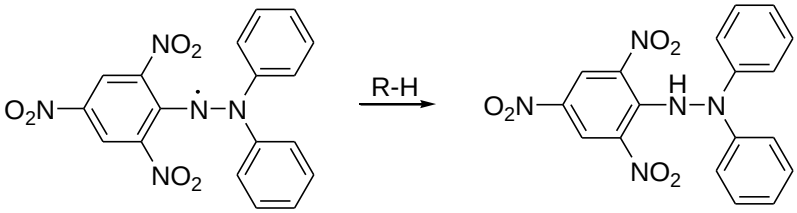
Based on the result, MeOH extract contained alkaloids and phenols, including flavonoids and tannins. This result indicated that MeOH extract consisted of high polarity compounds. EtOAc extract showed alkaloids, phenols, flavonoids, tannins, saponins and steroids. Saponins was the striking result on this test, practically, it showed persistent foam for more than 10 minutes. The latest scientific data [7, 8, 24] also discovered that saponins were exactly found on *Cordyline* genus and some of them had been identified as new saponin compounds. Furthermore, the phytochemical screening on EtOAc extract also found the flavonoids and other phenolic compounds. This finding was not astonishing because the previous reviews from another *Cordyline* species had already found new flavone in EtOAc extract [6]. Other phytochemical groups like alkaloids and steroids were also contained in EtOAc extract. This could be indicated that EtOAc extract consists of diverse varieties of phytochemical contents. Interestingly, EtOAc extract was the lowest amount on *C. fruticosa* Back leaves extract (3.7%).

Hexane extract contained alkaloids, phenols, tannins, saponins, steroids and cardiac glycosides. The lipophilic parts of these compounds should be dominantly attracted by this less polar solvent (hexane) during extraction process. Bazzaz et al. [25] reported that the less polar extract should contain lipophilic compounds. However, the odd result was showed by this extract containing higher polarity substituents (phenols, tannins and cardiac glycosides) which cannot be explained clearly. Normally, secondary metabolites are likely isolated in polar extract [26], however the exception result by this process might possibly due to the different method of extractions and the process of sample preparation that might destroy or evaporate some crucial compounds [27].

2.3.3 Antioxidant Activity

2.3.3.1 DPPH Radical Scavenging Activity

One of the simple methods to evaluate antioxidant activity is the scavenging activity on DPPH radical. DPPH is known as a stable free radical that becomes paired off in the presence of hydrogen donor from antioxidant compound. When DPPH accepts the donated electron, it will be decolorized from deep violet into colorless (pale yellow color). Then, the transformation can be measured quantitatively by absorbance measurement at 517 nm [28, 29].



Scheme 2.1 DPPH reaction mechanism

The DPPH radical scavenging test on this research was preceded by dissolving various concentrations of each extracts in MeOH. The IC_{50} value was measured by calculation of 50% scavenging activity in the equation curve. The equation curve of MeOH extract was $y = -0.778x^2 + 15.61x + 3.428$ with $r^2 = 0.997$ by diluting the concentration between 1.0-7.0 mg/mL. Meanwhile, the equation curve of EtOAc extract was $y = 20.94\ln(x) + 51.21$ with $r^2 = 0{,}948$ by diluting the concentration from 0.5 to 8.0 mg/mL. Then, the equation curve of hexane extract was $y = -0.264x^2 + 8.518x - 2.053$ with $r^2 = 0.998$ by diluting the concentration at 2.0-16.0 mg/mL. Ascorbic acid was selected to be standard for this assay. The equation curve of ascorbic acid ($y = -3493x^2 + 1205x - 6.287$; $r^2 = 0.992$) was obtained by diluting various concentration between 0.01-0.2 mg/mL.

Table 2.2 DPPH scavenging activity of leaves extract of *Cordyline fruticosa* Back

Sample Extracts	IC_{50} (mg/mL)
MeOH	3.65 ± 0.04
EtOAc	0.94 ± 0.01
Hexane	8.19 ± 0.34
Ascorbic acid[a]	0.06 ± 0.00

[a]Standard

Based on the Table 2.2, IC_{50} of DPPH radical scavenging among MeOH, EtOAc and hexane extracts were 3.65 ± 0.04 mg/mL, 0.94 ± 0.01 mg/mL and 8.19 ± 0.34 mg/mL, respectively. These results were still lower than the scavenging activity of standard (0.06 ± 0.00 mg/mL). Nevertheless, among these leaves extracts, the EtOAc extract presented the strongest activity on DPPH radical scavenging than the others.

Particularly, the EtOAc extract contained phenols (tannins and flavonoids), alkaloids, steroids and saponins. The scavenging of DPPH radical by phenols and

alkaloids has been clearly understood [30]. The hydrogen donor from phenolic compounds were able to scavenge DPPH radicals very well [31], while alkaloids such as discretamine had a strong hydrogen-donating capacity which could efficiently scavenge DPPH radicals [32]. Recent studies have shown that steroids also played important pharmacological roles against oxidative stress, nevertheless the mechanism of this modulation have not been elucidated yet [33, 34]. However, it was suggested that the antioxidant effect of steroids might be due to its benzenic ring which inherited from the antioxidant potency. Moreover the high antiradical power might possibly attribute to the acetoxyl groups in the benzenic ring by increasing productivity of a stable radical via an inductive effect [34, 35]. Meanwhile, saponins have also been reported for its potent antioxidant activity on DPPH radical scavenging. Although it was difficult to explain clearly a scavenging effect of saponins, however this activity was likely due to the number of hydroxy groups presented on its structure [36].

2.3.3.2 Ferric Reducing Antioxidant Power Assay

FRAP assay uses antioxidant as reductant to reduce of ferric tripyridyl triazine complex (Fe III-TPTZ) to ferrous form at low pH. This process can be monitored by measuring the color change (intense blue color) in spectrophotometry absorption at 593 nm. The ferric reducing activity is mainly influenced by the size of the conjugated double bond system [19, 29, 37]. On this research, the reduction of ferric complex was expressed as mmol Fe^{2+} equivalents/1 g sample obtained by calibration curve of $FeSO_4.7H_2O$ using concentration range at 5-30 mM.

Table 2.3 FRAP assay of leaves extract of *Cordyline fruticosa* Back

Sample Extracts	Reducing Power (mmol Fe^{2+} eq/g sample)
MeOH	15.83 ± 1.96
EtOAc	31.36 ± 0.21
Hexane	9.58 ± 0.38

As shown in Table 2.3, this assay indicated that the EtOAc extract exhibited the strongest ferric reducing power activity (31.36 ± 0.21 mmol Fe^{2+} equivalents (eq)/1 g sample) followed by MeOH extract (15.83 ± 1.96 mmol Fe^{2+} eq/g) and hexane extract

(9.58 ± 0.38 mmol Fe^{2+} eq/g). The strongest activity of EtOAc extract ensured that this extract had a potential as antioxidant agent.

The phytochemical screening test of EtOAc extract presented that this extract consisted of phenols (tannins and flavonoids), alkaloids, steroids and saponins. The concept behind the reducing power assay is reduction mechanism itself. The effective of reduction activity by phenols, alkaloids, saponins and steroids was presented by reducing the oxidized form of iron (Fe^{3+}) to its reduced form (Fe^{2+}) by donating an electron [38]. Thus, it can be assumed that the presence of reductants in EtOAc extract causes the reduction of the Fe^{3+} complex to the ferrous form, however the mechanism of each constituent has not been explained specifically [30].

2.3.3.3 Fe^{2+} Chelating Activity

The Fe^{2+} chelating activity can be determined by ferrozine which can quantitatively form complexes with Fe^{2+}. Iron and other transition metals (copper, chromium, cobalt, vanadium, cadmium, arsenic and nickel) promote oxidation by acting as catalysts of free radical reactions. In the presence of chelating agents, the complex formation is disrupted and the result is revealed by decreasing the red color of the complex formation. The main strategy to avoid radical-oxidative in Fe^{2+} chelating assay is by capturing ferrous ion faster than ferrozine. The chelating agents will form sigma bond with a metal, decrease the redox potential and steady the oxidized form of the metal ion [20, 39, 40].

The IC_{50} of chelating activity was measured by plotting the 50% of inhibition value to the equation curve of each extract after absorbance measurement at 562 nm. The MeOH extract was diluted from 0.5 to 6.5 mg/mL to get $y = 14.00x - 3.996$ with $r^2 = 0.995$. The equation curve of EtOAc extract was $y = -5.056x^2 + 34.92x - 5.114$, with $r^2 = 0.993$, by diluting the concentrations at 0.25-4.0 mg/mL. Meanwhile, hexane extract was diluted between 0.24 to 4.0 mg/mL to obtain the equation at $y = -11.48x^2 + 72.70x - 6.509$ with $r^2 = 0.999$. EDTA-2Na was used as standard and had been diluted by various concentrations at 0.01-0.05 mg/mL to get the equation curve at $y = -11736x^2 + 3285x - 30.60$ with $r^2 = 0.971$.

Table 2.4 Fe^{2+} chelating activity of leaves extract of *Cordyline fruticosa* Back

Sample Extracts	IC_{50} (mg/mL)
MeOH	3.86 ± 0.04
EtOAc	2.44 ± 0.06
Hexane	0.91 ± 0.01
EDTA-2Na[a]	0.03 ± 0.00

[a]Standard

The result of Fe^{2+} chelating assay, presented in Table 2.4, identified that all extracts had potent antioxidant activity on chelating metal performance. The hexane extract showed the highest antioxidant activity (0.91 ± 0.01 mg/mL) followed by EtOAc extract (2.44 ± 0.06 mg/mL) and MeOH extract (3.86 ± 0.04 mg/mL). These activities were still lower than IC_{50} of standard which could perform chelating activity by 0.03 ± 0.00 mg/mL. In spite of this difference, hexane extract could perform higher chelating activity than other extracts. The strong activity of hexane extract indicated that the active compounds which have contribution on antioxidant activity in *C. fruticosa* Back might come from non-polar compounds [41]. Steroids or saponins allegedly contributed for antioxidant activity of hexane extract. Since saponins were known as potent chelators of free iron or copper, their activity on antioxidant have been more observed as chelating factor in antioxidant therapy [42-44].

2.3.4 Total Phenolic Contents

The reaction of Folin-Ciocalteau reagent is based on colorimetric method which is widely used for determination of total phenolic contents [45]. The reagent consists of tungstates and molybdates which work on reduction-oxidation mechanism. The phenolic compound undergoes dissociation to form phenolate anions which reduce the reagent under base condition giving blue colored chromogen [21, 46]. This method consists of calibration by a pure phenolic compound and the absorbance measurement is examined after the coloring reaction using spectrophotometer at 760 nm of wavelength [45].

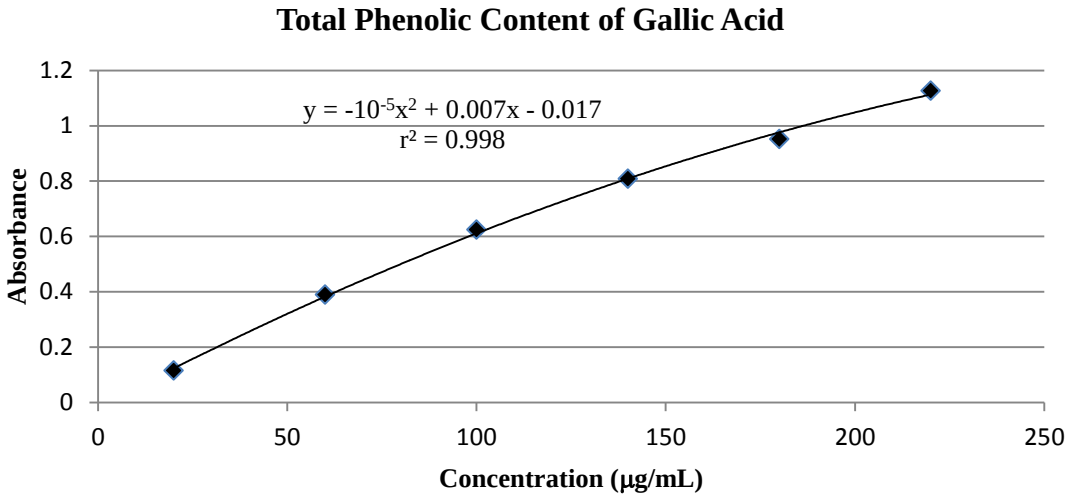


Figure 2.2 Calibration curve of gallic acid

All extracts were measured their absorbance at 760 nm after incubation for 1 hour. Then, the calculation was following the equation curve of gallic acid:

$$y = -10^{-5}x^2 + 0.007x - 0.017$$

where y is absorbance and x is amount of gallic acid in µg/mL (Figure 3.2). The total phenolic contents of leaves extract of *C. fruticosa* Back were reported as gallic acid equivalents/1 g sample (GAE/g) and shown at Table 2.5.

Table 2.5 Total phenolic contents of leaves extracts of *Cordyline fruticosa* Back

Sample Extract	Total Phenolic Contents (mg GAE/g sample)
MeOH	41.91 ± 1.32
EtOAc	23.16 ± 0.94
Hexane	24.62 ± 0.84

Based on the Table 2.5, among these three extracts, MeOH extract showed the highest total phenolic content value (41.91 ± 1.32 mg GAE/g) followed by hexane extract (24.62 ± 0.84 mg GAE/g) and EtOAc extract (23.16 ± 0.94 mg GAE/g). This result showed that methanol extract consisted lots of phenolic compounds. The phytochemical screening test of MeOH extract also revealed that this extract abundantly contained phenolic compounds. Phenolic acids should be considered as hydrogen donors due to the characteristic of carboxylic group which is easily ionized and will perform good activity on antioxidant [31, 47]. However, the results of antioxidant

activity on this research revealed that MeOH extract did not perform a strong activity of antioxidant. This finding indicated that phenolic compounds might not take a role as antioxidant in *C. fruticosa* Back, however the less polar compound such as chlorophyll might contribute on the antioxidant activity rather than phenolic compounds [41, 48]. Zulkifli et al. [49] also described that the correlation of antioxidant effect might come from other secondary metabolites, such as alkaloids, steroids or saponins. Another reasonable argument about this case was also supported by Palavox et al. [50] who proved that the accumulative composition of various phenol contents may induce antagonistic interactions, giving low antioxidant capacity on its performance.

2.3.5 Antibacterial Activity

The MeOH extract of *C. fruticosa* Back leaves was screened for its antibacterial activity against *Bacillus cereus*, *Salmonella thypii* and *Streptococcus sobrinus* at a concentration of 500 ppm. Chloramphenicol was used as standard drug because of its broad-spectrum that can terminate polypeptide synthesis of *Bacillus spp.*, *Salmonella spp.* and even *Streptococcus spp.*[51]. Using the disc diffusion method, the diameter of inhibition zone of bacterial growth was measured and compared with standard value. The results are presented in Table 2.6.

Table 2.6 Antibacterial activity of MeOH extract of *Cordyline fruticosa* Back

Sample Extracts	Inhibition Zone (mm)		
	B. cereus	*S. thypii*	*S. sobrinus*
MeOH	9.1 ± 1.3	8.1 ± 0.2	8.1 ± 0.2
Chloramphenicol[a]	18.6 ± 0.3	20.0 ± 0.6	18.1 ± 0.2

[a]Standard drug

As presented in Table 2.6, the diameter of inhibition showed that *B. cereus*, *S. thypii* and *S. sobrinus* had a diameter of inhibition zone of 9.1, 8.1 and 8.1 mm, respectively. This result indicated that MeOH extract showed low activity against these three bacteria strains. The standard of zone size from National Committee of Clinical Laboratory Standard [52] conveyed that the diameter of 12 mm or less using chloramphenicol against these three bacteria strains was categorized as low antibacterial activity.

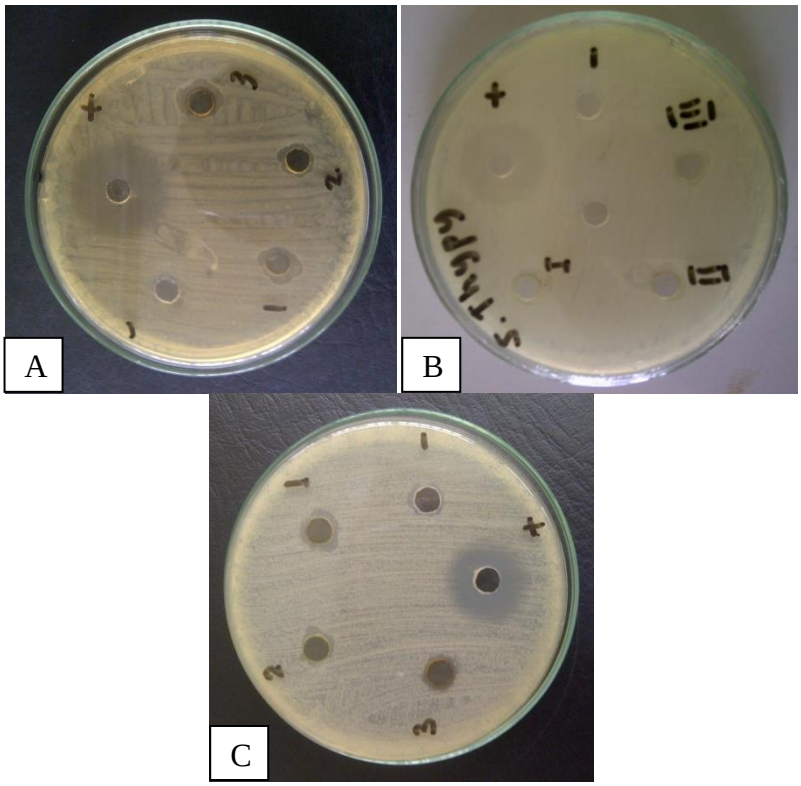


Figure 2.3 Antibacterial plates of *Bacillus cereus* (A), *Salmonella thypii* (B) and *Streptococcus sobrinus* (C)

This result revealed that MeOH extract of *C. fruticosa* Back containing high phenolic contents displayed low activity against these bacterial growths. This indicated that phenol compounds in MeOH extract might not capable enough as antibacterial agent.

CHAPTER 3 SYNTHESIS OF XANTHENE DERIVATIVES USING HEXABROMOACETONE UNDER SOLVENT-FREE CONDITIONS

3.1 Introduction

Xanthene is a tricyclic dibenzopyran (Figure 3.1) which represents as the central core of many naturally occurring compounds, for instances, xanthone, fluorescein, eosine, rhodamine, rosamines, tokyo green and other pigment compounds [53-55].

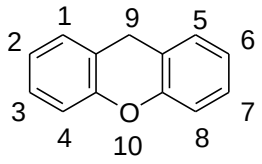


Figure 3.1 Core structure of xanthenes: tricyclic dibenzopyran

Xanthene derivatives have become considerable attention due to their extensive benefits on pharmaceutical fields, such as antibacterial agent against *Escherichia coli*, *Staphylococcus aureus* and *Saccharomyces cerevisiae* [56], analgesic and anti-inflammatory agents [57, 58], anti-proliferative substances to prevent the spread of malignant cells [59], potential cytotoxic activities in cancer therapy [60] and application on photodynamic treatment [61, 62]. In material science, xanthene derivatives have been used as fluorescent dyes in photo physic of surfactant solutions [63], bio molecular visualization or labeling support in pH-sensitive fluorescent materials [64-66] and photo stable performance at laser technology application [67, 68].

Many methods have been reported on the preparation of xanthene derivatives. In 1990s, the classical methods [69-78] have preceded as a pioneer to synthesize xanthene derivatives. However, these old-fangled methods suffered of many disadvantages. Recently, the developed methods for the synthesis of xanthenes have been reported by using halogenating reagents [79-89], metal halides [90-93], ionic liquids [94-96], organosulfurs [80, 97-106], inorganic acid salts [107], heteropolyacids (HPAs) [108, 109], polymeric acids [82, 110-112] and solid acid catalysts [91, 113-121]. Nevertheless, most of these existing methodologies endure one or more disadvantages,

for instances, low yields, long reaction times, harmful solvent usages, severe reaction conditions and excess catalyst or reagent requirements.

More recently, the organic reactions under solvent-free conditions have received extensive interest by many researchers because of their high efficiency, good separation and purification and other benefits for industry and environment. Hexabromoacetone ($Br_3CCOCBr_3$) is an organic reagent synthesized and used inorganic reactions since 1969 [122]. However, there have been a few reports on its application as a reagent or catalyst in organic synthesis. Based on these circumstances, this research has been encouraged to explore a novel and efficient method for the synthesis of xanthene derivatives in the presence of hexabromoacetone under solvent-free conditions.

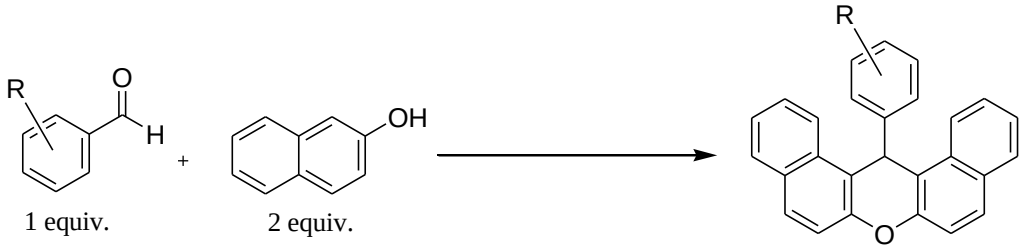
3.1.1 Classical Methods for the Synthesis of Xanthenes

Due to the extensive benefits and wide chances for industrial applications, the synthesis of xanthenes was initially conducted in 1990s by regiospecific reaction of alkylphenoxy-magnesiumhalides with triethylorthoformate [69, 70], γ-alkylation of heteroatoms [71, 77], intramolecular trapping of benzynes using phenolic nucleophiles [73-75] and cyclization of polycyclic aryltriflate esters using palladium [78]. In particular, dibenzoxanthenes were synthesized by cyclocondensation of 2-tetralone with substituted salicylaldehydes [72] as well as the condensation of primary alcohols with resorcinol and other hydroxy aromatic compounds [76]. However, these methods had been disposed because of the worst advantages on long time reactions, side reactions, low efficiency of procedures and selectivity of target molecules.

3.1.2 Literature Reviews on the Synthesis of 14-(Aryl)-14*H*-dibenzo[*a,j*]-xanthenes

Recently, many researchers have discovered the synthetic approaches of xanthenes using aldehydes and β-naphthol as starting materials in the presence of various types of catalyst in different conditions. Some developed methods for the synthesis of 14-(aryl)-14*H*-dibenzo[*a,j*]xanthenes using aromatic aldehydes and β-naphthol are described in Table 3.1.

Table 3.1 Developed methods for the synthesis of 14-(aryl)-14*H*-dibenzo[*a*,*j*]-xanthenes in the presence of various catalysts



1 equiv. 2 equiv.

Catalyst	Amount	Condition	Time	Yield (%)	Ref.
Iodine	10% mol	90 ^{o}C/neat	2-5 h	85-95	[79]
Selectfluor^{TM}	10% mol	125 ^{o}C/neat	6-12 h	90-95	[84]
BF_3-SiO_2	0.08 g	60 ^{o}C/neat	**nd*	82-97	[86]
BF_3-SiO_2	0.08 g	$CHCl_3$	6 min	85-96	[86]
TPPMS/CBr_4	10% mol	100 ^{o}C/neat	15-60 min	86-97	[89]
$NbCl_5$	25% mol	a.t./CH_2Cl_2/N_2	24-48 h	70-98	[90]
LiBr	**nd*	130 ^{o}C/neat	1 h	82	[92]
$BiCl_3$	20% mol	110 ^{o}C/neat	3-180 min	74-98	[93]
*p*TSA	**nd*	125 ^{o}C/neat	2.5-6 h	80-96	[95]
*p*TSA	**nd*	refluxing DCE	15-24 h	85-95	[95]
$KAl(SO_4)_2$-$12H_2O$	5% mol	100 ^{o}C/water	3-4 h	82-91	[97]
H_2SO_4	10 mL	80 ^{o}C/AcOH	72 h	60-90	[100]
Silica-SO_3H	13% mol	125 ^{o}C/neat	13-80 min	81-91	[101]
SuSA	5.6% mol	80 ^{o}C/neat	15-35 min	90-94	[102]
Cellulose-SO_3H	0.08 g	110 ^{o}C/neat	1.5 h	95	[103]
SaSA	15 % mol	120 ^{o}C/neat	50-100 min	87-96	[104]
Sulfamic acid	10% mol	125 ^{o}C/neat	6-12 h	90-95	[105]
Sulfamic acid	4% mol	microwave	2-4 min	92-96	[105]
(Et_3N-SO_3H)Cl	15% mol	120 ^{o}C/neat	15-40 min	92-97	[106]
Poly(AMPS-co-AA)	0.04 g	110 ^{o}C/neat	20-30 min	92-75	[110]
PVSA	20 % mol	90 ^{o}C/water	1-4.5 h	78-93	[111]
NSPVPC	0.02 g	100 ^{o}C/neat	20-45 min	80-98	[112]
Amberlyst-15	**nd*	125 ^{o}C/neat	0.5-2 h	80-94	[114]
Nano TiO_2	15% mol	90 ^{o}C/neat	12 min	82-96	[116]

Table 3.1 (cont.)

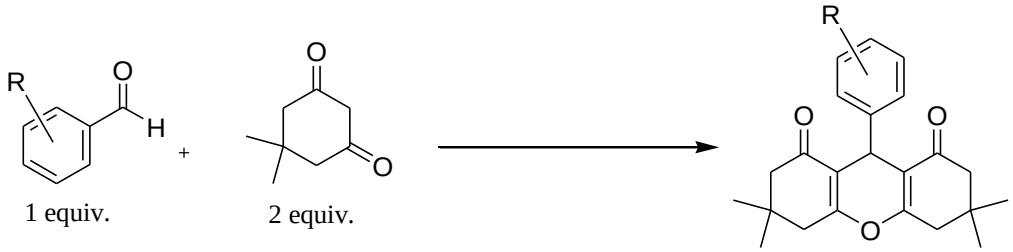
Catalyst	Amount	Condition	Time	Yield (%)	Ref.
Nano $SnCl_4$-SiO_2	0.015 g	90 ^oC/neat	40 min	73-95	[117]
Nano ZnO	10% mol	80 ^oC/neat	20-40 min	78-95	[118]
Dowex-50W	0.1 g	100 ^oC/neat	1-2 h	78-91	[119]

**nd = not described*

3.1.3 Literature Reviews on the Synthesis of 9-(Aryl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthenes

The developed methods for the synthesis of 9-(aryl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthenes using various aromatic aldehydes and 5,5-dimethyl-1,3-cyclohexanedione in the presence of various catalysts are reported in Table 3.2.

Table 3.2 Developed methods for the synthesis of 9-(aryl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthenes in the presence of various catalysts



1 equiv. 2 equiv.

Catalyst	Amount	Condition	Time	Yield (%)	Ref.
Silica chloride	**nd*	refluxing CH_3CN	6 h	90-95	[80]
$NaHSO_4$-SiO_2	**nd*	refluxing CH_3CN	6.5 h	90-98	[80]
NBS	20% mol	refluxing EtOH	10-12 h	82-96	[81]
NBS	20% mol	DMAC/microwave	4-8 min	75-93	[81]
$HClO_4$-SiO_2	**nd*	140 ^oC/neat	3 h	22-32	[82]
PPA-SiO_2	10% mol	140 ^oC/neat	30 min	66-92	[82]
ZnO-CH_3COCl	2% mol	80 ^oC/CH_3CN	2-6 h	82-96	[85]
$SbCl_3$-SiO_2	0.3 g	120 ^oC/neat	40-80 min	85-95	[88]
(Hmim)TFA	0.1 g	80 ^oC/neat	2.5-4 h	80-94	[94]
DBSA	10% mol	25-30 ^oC/ultrasonic	1 h	89-94	[98]
SiO_2-R-SO_3H	5-20% mol	80 ^oC/neat	3.5-5 h	35-93	[99]
SuSA	5.6% mol	80 ^oC/neat	15-27 min	88-96	[102]

Table 3.2 (cont.)

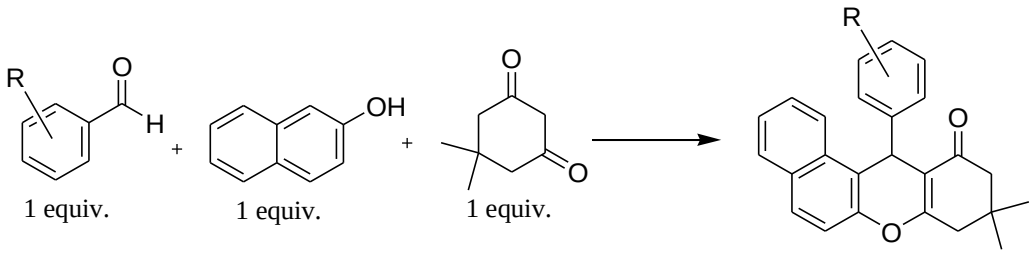
Catalyst	Amount	Condition	Time	Yield (%)	Ref.
SaSA	15% mol	90 ^{o}C/neat	10-45 min	90-97	[104]
$(Et_3N\text{-}SO_3H)Cl$	25% mol	80 ^{o}C/neat	0.5-1 h	85-97	[106]
SPNP	5% mol	100 ^{o}C/water	3 h	82-96	[108]
Poly(AMPS-co-AA)	0.04 g	110 ^{o}C/neat	25 min	70-86	[110]
NSPVPC	0.02 g	100 ^{o}C/neat	2-13 min	95-98	[112]
Amberlyst-15	0.2 g	refluxing CH_3CN	5 h	90-96	[113]
Nano ZnO	10% mol	80 ^{o}C/neat	15-35 min	82-97	[118]
Dowex-50W	0.4 g	100 ^{o}C/neat	2-5 h	78-91	[119]
PMA-SiO_2	0.1% mol	refluxing CH_3CN	4-5 h	15-95	[120]

**nd = not described*

3.1.4 Literature Reviews on the Synthesis of 12-(Aryl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-ones

The three-component reaction for the synthesis of 12-(aryl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-ones using different aromatic aldehydes, β-naphthol and 5,5-dimethyl-1,3-cyclohexanedione as starting materials has been reported. The developed methods of this reaction are presented in Table 3.3.

Table 3.3 Developed methods for the synthesis of 12-(aryl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-ones in the presence of various catalysts



Catalyst	Amount	Condition	Time	Yield (%)	Ref.
TrCl	7% mol	110 ^{o}C/neat	40-70 min	82-94	[83]
Iodine	10% mol	60 ^{o}C/neat	45-90 min	82-95	[87]
TPPMS/CBr_4	5% mol	110 ^{o}C/neat	30-90 min	78-90	[89]
$InCl_3$	30% mol	120 ^{o}C/neat	25-75 min	68-88	[91]
P_2O_5	20% mol	120 ^{o}C/neat	30-80 min	62-80	[91]

Table 3.3 (cont.)

Catalyst	Amount	Condition	Time	Yield (%)	Ref.
*p*TSA	10% mol	80 ^{o}C/(bmim)BF_4	2-3.5 h	83-95	[96]
*p*TSA	2% mol	120 ^{o}C/neat	45 min	88	[96]
SuSA	5.6% mol	80 ^{o}C/neat	30-48 min	78-96	[102]
$Zr(HSO_4)_4$	2% mol	125 ^{o}C/neat	20-60 min	15-90	[107]
$H_3PW_{12}O_{40}$	5% mol	60 ^{o}C/neat	40-90 min	81-94	[109]
Poly(AMPS-co-AA)	0.06 g	110 ^{o}C/neat	30-50 min	74-88	[110]
NSPVPC	0.02 g	120-130 ^{o}C/neat	5-30 min	60-90	[112]
Proline triflate	10% mol	water	2.5-4 h	79-88	[115]
$Sr(OTf)_2$	10% mol	80 ^{o}C/DCE	5-7 h	70-88	[121]

**nd = not described*

Overall, most of these methods suffered from the drawback conditions, such as toxic metal catalysts, volatile or harmful organic solvents, high cost operations, low yield of products, long reaction times, harsh conditions, excess catalyst requirements and exhausting laboratory works. To avoid these limitations, new system for the synthesis of xanthene derivatives with a good catalytic performance and simple work-up procedure in short reaction time has been addressed in this research.

3.1.5 Literature Reviews on Halogenating Agents for the Synthesis of Xanthenes

The methodologies for the synthesis of xanthene derivatives using halogenating agents have been studied by many research groups since the 20th centuries. The first report was published by Das and co-workers [79] in 2006 using iodine in a mixture of β-naphthol and aromatic aldehydes to synthesize a series of 14*H*-dibenzo[*a*,*j*]xanthenes under solvent-free conditions. Afterwards, Wang and colleagues [87] developed the reaction conditions for the multi-component reaction of β-naphthol, aromatic aldehydes and 5,5-dimethyl-1,3-cyclohexanedione to prepare tetrahydrobenzo[*a*]xanthene-11-ones.

In 2006, Kumar and co-workers [84] addressed the application of selectfluor^{TM} [1-(chloromethyl)-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane-bis(tetrafluoroborate)] as a catalyst in the electrophilic fluorination of β-naphthol and diverse aromatic aldehydes to

synthesize 14*H*-dibenzo[*a*,*j*]xanthenes at 125 ^{o}C without using any solvent, providing the high yield of desired products.

The use of silica chloride was demonstrated by Das and co-workers [80] in 2007 for the synthesis of 1,8-dioxo-octahydroxanthenes using 5,5-dimethyl-1,3-cyclohexanedione and aromatic aldehydes in refluxing acetonitrile. The silica-supported acid catalyst was also implicated by other halogenated reagents, such as BF_3-SiO_2 [86], $HClO_4$-SiO_2 [82], $SbCl_3$-SiO_2 [88] and nano $SnCl_4$-SiO_2 [117]. The reaction gave poor to high yield of xanthene products under solvent-free conditions.

In 2010, Mahdavinia, Bigdeli and Nemati [81] discovered that *N*-bromosuccinimide (NBS) could promote the synthesis of 9-(aryl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthenes under reflux and microwave conditions, proceeding the high yield of corresponding products within 10-12 hours and 4-8 minutes, respectively.

In 2012, Khazaei and colleagues [83] described that triarylmethyl chloride (TrCl) could lead the one-pot synthesis of tetrahydrobenzo[*a*]xanthene-11-ones derivatives using β-naphthol, aromatic aldehydes and 5,5-dimethyl-1,3-cyclohexanedione under solvent-free conditions.

In 2014, nobium pentachloride ($NbCl_5$), a metal halogenated reagent, was demonstrated by de Andrade Bartolomeu and co-workers [90] to synthesize 14*H*-dibenzo[*a*,*j*]xanthene derivatives at ambient temperature under N_2 atmosphere in anhydrous CH_2Cl_2. The reaction proceeded high yield of desired products for long time period of 1-2 days. The other metal halide catalysts, for example, $InCl_3$ [91], $BiCl_3$ [93], $TaCl_5$ [60], $GdCl_3$, $TiCl_4$, $SbCl_3$ [88] and LiBr [102], had also been applicated to promote various type of xanthene derivatives.

A solid complex halogenated reagent, sodium triphenylphosphine-*m*-sulfonate and carbon tetrabromide (TPPMS/CBr_4), was developed by Huo and friends [89] in 2014 as a recoverable catalyst for the synthesis of 14-(aryl)-14*H*-dibenzo[*a*,*j*]xanthenes and 12-(aryl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]- xanthene-11-ones derivatives under solvent-free conditions.

Despite of there are many halogenated reagents for the synthesis of xanthene derivatives, this kind of methods still have some disadvantages, such as long time period of reaction, complicated preparation of catalyst and low yield of product.

3.1.6 Literature Reviews on Hexabromoacetone ($Br_3CCOCBr_3$) in the Organic Synthesis

Hexabromoacetone ($Br_3CCOCBr_3$) (Figure 3.2) has been found since 1969 and could be prepared by bromination of acetone in acetic acid and sodium acetate [122]. Nowadays, $Br_3CCOCBr_3$ has been reported as an efficient reagent for bromination of alcohols and amines [123, 124], conversion of carboxylic acid to acid bromide [124], drug intermediate preparations [125] and bromosilane synthesis [126].

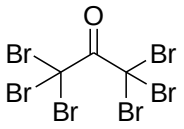


Figure 3.2 Chemical structure of hexabromoacetone

Based on the literature reviews, there has no report on the synthesis of xanthene derivatives utilizing $Br_3CCOCBr_3$. Therefore, the reaction of various aromatic aldehydes and β-naphthol, 5,5-dimethyl-1,3-cyclohexanedione or 5-methyl-1,3-cyclohexanedione to produce xanthene derivatives in the presence of $Br_3CCOCBr_3$ will be established.

3.2 Experimental

3.2.1 Instruments and Equipment

Thin layer chromatography (TLC) was performed on aluminium sheets pre-coated with silica gel (Macherey-Nagel, Alugram 60 SIL G/UV_{254}). Melting points were determined with a Mel-Temp melting point apparatus. The ^{1}HNMR spectra was performed in deuterated chloroform ($CDCl_3$) with tetramethylsilane (TMS) as an internal reference on Varian nuclear magnetic resonance spectrometer, model Mercury plus 400 NMR spectrometer which was operated at 400 MHz. The chemical shifts (δ) are assigned by comparison with residue solvent protons.

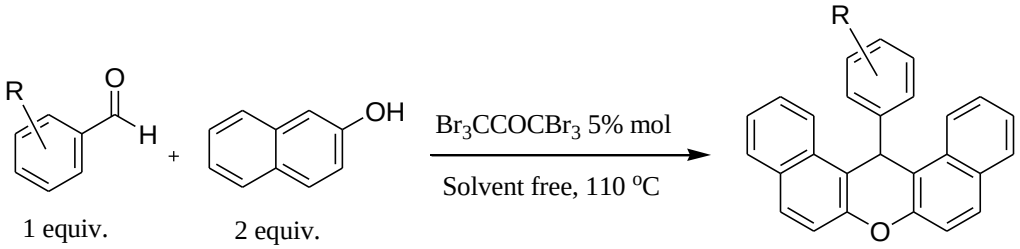
3.2.2 Chemicals

All solvents on this research were used commercial grade and have been purified prior to use by standard methodology. The reagents used for synthesis were purchased

from Sigma-Aldrich and Fluka chemical company or otherwise stated and were used without further purification.

3.2.3 General Procedure for the Synthesis of Xanthenes

3.2.3.1 General Procedure for the Synthesis of 14-(Aryl)-14*H*-dibenzo[*a,j*]-xanthenes



A mixture of aldehyde (1 mmol, 1 equiv.), β-napthol (2 mmol, 2 equiv.) and $Br_3CCOCBr_3$ (0.05 mmol) was heated and stirred at 110 °C for the appropriate time. The completion of reaction was monitored by TLC. Then, the reaction mixture was cooled down to room temperature. Water (3 mL) was added and then stirred for 5 minutes. The solid product was filtered and recrystallized from ethanol. The identity of products was characterized by using ^{1}HNMR spectroscopy. The known products were characterized by physical data comparison with reported literature. All new compounds were fully characterized with relevant spectroscopic data and their experimental data are presented below.

14-(4-*tert*-Butylphenyl)-14*H*-dibenzo[*a,j*]xanthene (4d): Red solid (415.2 mg, 100%); m.p. 296-298 °C; R_f 0.69 (14% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.42 (d, *J* = 8.48 Hz, 2H, 2xAr<u>H</u>), 7.83 (d, *J* = 8.08 Hz, 2H, 2xAr<u>H</u>), 7.79 (d, *J* = 8.87 Hz, 2H, 2xAr<u>H</u>), 7.59 (t, *J* = 8.27 Hz, 2H, 2xAr<u>H</u>), 7.49 (d, *J* = 8.88 Hz, 2H, 2xAr<u>H</u>), 7.43-7.40 (m, 4H, 4xAr<u>H</u>), 7.14 (d, *J* = 8.44 Hz, 2H, 2xAr<u>H</u>), 6.48 (s, 1H, ArC<u>H</u>), 1.14 (s, 9H, (C<u>H</u>$_3$)$_3$).

14-Phenyl-14*H*-dibenzo[*a,j*]xanthene (4a): [97, 112] Red solid (318.1 mg, 90%); m.p. 186-188 °C; R_f 0.77 (14% EtOAc/hexane).

14-(4-Methoxyphenyl)-14*H*-dibenzo[*a,j*]xanthene (4b): [95] Red semi solid (410.3 mg, 100%); R_f 0.53 (14% EtOAc/hexane).

14-(4-Methylphenyl)-14*H*-dibenzo[*a,j*]xanthene (4c): [97] Red solid (392.2 mg, 100%); m.p. 226-228 °C; R_f 0.69 (14% EtOAc/hexane).

14-(4-Chlorophenyl)-14*H*-dibenzo[*a,j*]xanthene (4e): [112] Orange solid (573.3 mg, 100%); m.p. 280-282 ^{o}C; R_f 0.62 (14% EtOAc/hexane).

14-(4-Bromophenyl)-14*H*-dibenzo[*a,j*]xanthene (4f): [112] Red solid (406.1 mg, 93%); m.p. 293-295 ^{o}C; R_f 0.63 (14% EtOAc/hexane).

14-(3-Hydroxyphenyl)-14*H*-dibenzo[*a,j*]xanthene (4g): [116] Black solid (348.2 mg, 93%); m.p. 188-190 ^{o}C; R_f 0.17 (14% EtOAc/hexane).

14-(3-Chlorophenyl)-14*H*-dibenzo[*a,j*]xanthene (4h): [112] Red solid (390.4 mg, 99%); m.p. 204-206 ^{o}C; R_f 0.15 (14% EtOAc/hexane).

14-(3-Nitrophenyl)-14*H*-dibenzo[*a,j*]xanthene (4i): [112] Orange solid (382.2 mg, 95%); m.p. 214-216 ^{o}C; R_f 0.46 (14% EtOAc/hexane).

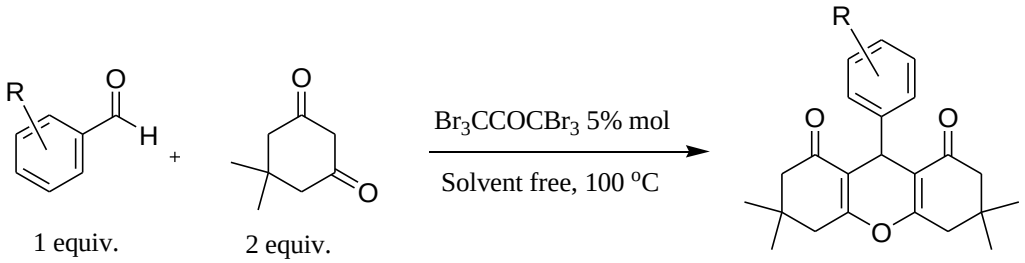
14-(2-Hydroxyphenyl)-14*H*-dibenzo[*a,j*]xanthene (4j): [116] Brown solid (331.1 mg, 88%); m.p. 187-189 ^{o}C; R_f 0.29 (14% EtOAc/hexane).

14-(2-Methoxyphenyl)-14*H*-dibenzo[*a,j*]xanthene (4k): [95] Brown semi solid (383.4 mg, 99%); R_f 0.29 (14% EtOAc/hexane).

14-(2-Chlorophenyl)-14*H*-dibenzo[*a,j*]xanthene (4l): [112] Red solid (393.3 mg, 100%); m.p. 207-209 ^{o}C; R_f 0.13 (14% EtOAc/hexane).

14-(2-Nitrophenyl)-14*H*-dibenzo[*a,j*]xanthenes (4m): [127] Black solid (386.3 mg, 96%); m.p. 215-217 ^{o}C; R_f 0.47 (14% EtOAc/hexane).

3.2.3.2 General Procedure for the Synthesis of 9-(Aryl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthenes



1 equiv. 2 equiv.

A mixture of aldehyde (1 mmol, 1 equiv.), 5,5-dimethyl-1,3-cyclohexanedione (2 mmol, 2 equiv.) and $Br_3CCOCBr_3$ (0.05 mmol) was heated and stirred at 100 ^{o}C for the appropriate time. The completion of reaction was monitored by TLC. Then, the reaction mixture was cooled down to room temperature. Water (3 mL) was added and then stirred for 5 minutes. The solid product was filtered and recrystallized from ethanol. The identity of products was characterized by using ^{1}H NMR spectroscopy. The known products were characterized by physical data comparison with reported

literature. All new compounds were fully characterized with relevant spectroscopic data and their experimental data are presented below.

9-(4-*tert*-Butylphenyl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthene (5d): White solid (394.4 mg, 97%); m.p. 226-228 ^{o}C; R_f 0.66 (33% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.18 (d, *J* = 4.05 Hz, 4H, 4xAr<u>H</u>), 4.73 (s, 1H, ArC<u>H</u>), 2.45 (s, 4H, 2xC<u>H</u>$_2$), 2.21 (s, 2H, C<u>H</u>$_2$), 2.19 (s, 2H, C<u>H</u>$_2$), 1.23 (s, 9H, (C<u>H</u>$_3$)$_3$), 1.09 (s, 6H, 2xC<u>H</u>$_3$), 1.00 (s, 6H, 2xC<u>H</u>$_3$).

9-Phenyl-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthene (5a): [112, 128] White solid (298.4 mg, 85%); m.p. 197-199 ^{o}C; R_f 0.14 (14% EtOAc/hexane).

9-(4-Methoxyphenyl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthene (5b): [128] Yellow solid (387.3 mg, 100%); m.p. 242-244 ^{o}C; R_f 0.37 (33% EtOAc/hexane).

9-(4-Methylphenyl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthene (5c): [128] White solid (374.6 mg, 100%); m.p. 218-220 ^{o}C; R_f 0.54 (33% EtOAc/hexane).

9-(4-Chlorophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthene (5e): [112] White solid (366.2 mg, 95%); m.p. 228-230 ^{o}C; R_f 0.27 (33% EtOAc/hexane).

9-(4-Bromophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthene (5f): [104] Yellow solid (489.3 mg, 100%); m.p. 239-241 ^{o}C; R_f 0.43 (33% EtOAc/hexane).

9-(3-Hydroxyphenyl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthene (5g): [102] Grey solid (352.3 mg, 96%); m.p. 218-220 ^{o}C; R_f 0.11 (33% EtOAc/hexane).

9-(3-Chlorophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthene (5h): [112] White solid (351.9 mg, 91%); m.p. 187-189 ^{o}C; R_f 0.54 (33% EtOAc/hexane).

9-(3-Nitrophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthene (5i): [112] Yellow solid (388.1 mg, 98%); m.p. 166-168 ^{o}C; R_f 0.29 (33% EtOAc/hexane).

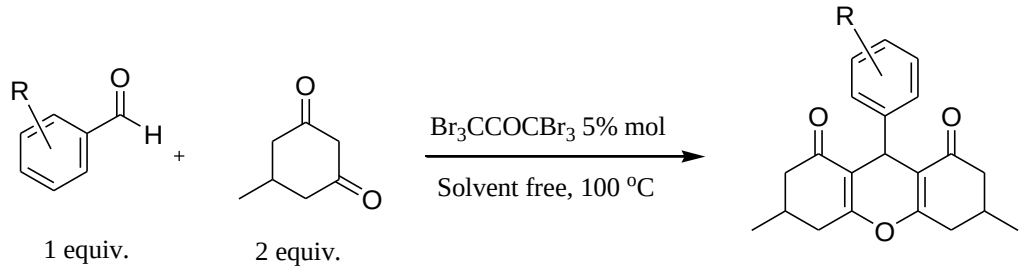
9-(2-Hydroxyphenyl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthene (5j): [128] Pink solid (346.8 mg, 95%); m.p. 226-228 ^{o}C; R_f 0.31 (33% EtOAc/hexane).

9-(2-Methoxyphenyl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthene (5k): [112] Orange solid (367.6 mg, 97%); m.p. 212-214 ^{o}C; R_f 0.37 (33% EtOAc/hexane).

9-(2-Chlorophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthene (5l): [112] Yellow solid (338.6 mg, 88%); m.p. 223-225 ^{o}C; R_f 0.37 (33% EtOAc/hexane).

9-(2-Nitrophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthene (5m): [104] Yellow solid (388.8 mg, 98%); m.p. 250-252 ^{o}C; R_f 0.37 (33% EtOAc/hexane).

3.2.3.3 General Procedure for the Synthesis of 9-(Aryl)-3,6-dimethyl-1,8-dioxo-octahydroxanthenes



1 equiv. 2 equiv.

A mixture of aldehyde (1 mmol, 1 equiv.), 5-methyl-1,3-cyclohexanedione (2 mmol, 2 equiv.) and $Br_3CCOCBr_3$ (0.05 mmol) was heated and stirred at 100 ^oC for the appropriate time. The completion of reaction was monitored by TLC. Then, the reaction mixture was cooled down to room temperature. Water (3 mL) was added and then stirred for 5 minutes. The solid product was filtered and recrystallized from ethanol. The identity of products was characterized by using ^1H NMR spectroscopy. All new compounds were fully characterized with relevant spectroscopic data and their experimental data are presented below.

9-Phenyl-3,6-dimethyl-1,8-dioxo-octahydroxanthene (6a): Yellow solid (354.3 mg, 100%); m.p. 201-203 ^oC; R_f 0.51 (50% EtOAc/hexane).

9-(4-Methoxyphenyl)-3,6-dimethyl-1,8-dioxo-octahydroxanthene (6b): Yellow solid (349.2 mg, 99%); m.p. 199-201 ^oC; R_f 0.29 (33% EtOAc/hexane); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.19 (d, *J* = 8.65 Hz, 2H, 2xAr<u>H</u>), 6.74 (d, *J* = 8.58 Hz, 2H, 2xAr<u>H</u>), 4.73 (s, 1H, ArC<u>H</u>), 3.72 (s, 3H, OC<u>H</u>$_3$), 2.65-2.01 (m, 10H, 4xC<u>H</u>$_2$ and 2xC<u>H</u>CH$_3$), 1.08 (d, *J* = 6.22 Hz, 6H, 2xC<u>H</u>$_3$).

9-(4-Methylphenyl)-3,6-dimethyl-1,8-dioxo-octahydroxanthene (6c): White solid (336.4 mg, 100%); m.p. 218-220 ^oC; R_f 0.40 (33% EtOAc/hexane); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.17 (d, *J* = 7.80 Hz, 2H, 2xAr<u>H</u>), 7.01 (d, *J* = 7.82 Hz, 2H, 2xAr<u>H</u>), 4.75 (s, 1H, ArC<u>H</u>), 2.66-1.98 (m, 10H, 4xC<u>H</u>$_2$ and 2xC<u>H</u>CH$_3$), 2.24 (s, 3H, ArC<u>H</u>$_3$), 1.08 (d, *J* = 6.31 Hz, 6H, 2xC<u>H</u>$_3$).

9-(4-Chlorophenyl)-3,6-dimethyl-1,8-dioxo-octahydroxanthene (6d): White solid (321.4 mg, 90%); m.p. 239-241 ^oC; R_f 0.43 (33% EtOAc/hexane); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.22 (d, *J* = 8.51 Hz, 2H, 2xAr<u>H</u>), 7.16 (d, *J* = 8.44 Hz, 2H, 2xAr<u>H</u>), 4.74 (s, 1H, ArC<u>H</u>), 2.67-2.03 (m, 10H, 4xC<u>H</u>$_2$ and 2xC<u>H</u>CH$_3$), 1.09 (d, *J* = 6.24 Hz, 6H, 2xC<u>H</u>$_3$).

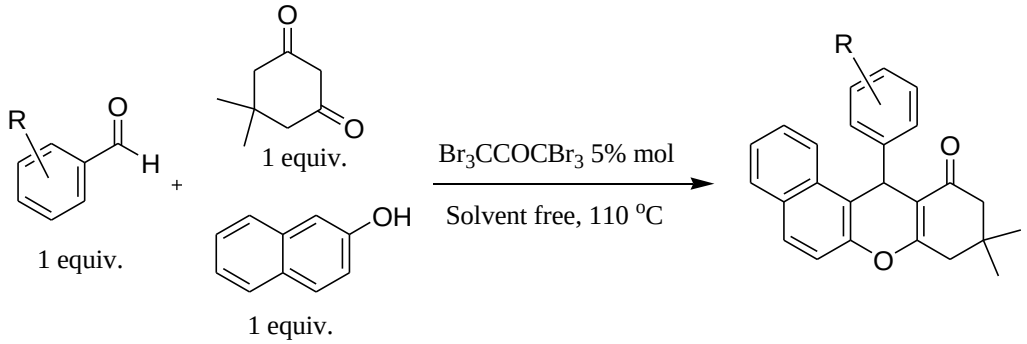
9-(3-Hydroxyphenyl)-3,6-dimethyl-1,8-dioxo-octahydroxanthene (6e): White solid (336.4 mg, 99%); m.p. 217-219 ^{o}C; R_f 0.20 (50% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.05 (t, *J* = 7.84 Hz, 1H, ArH), 6.93 (s, 1H, ArH), 6.73 (d, *J* = 7.43 Hz, 1H, ArH), 6.58 (d, *J* = 7.92 Hz, 1H, ArH), 4.76 (s, 1H, ArCH), 2.66-2.03 (m, 10H, 4xCH_2 and 2xCHCH_3), 1.08 (d, *J* = 5.76 Hz, 6H, 2xCH_3).

9-(3-Nitrophenyl)-3,6-dimethyl-1,8-dioxo-octahydroxanthene (6f): White solid (365.0 mg, 99%); m.p. 210-212 ^{o}C; R_f 0.26 (33% EtOAc/hexane).

9-(2-Methoxyphenyl)-3,6-dimethyl-1,8-dioxo-octahydroxanthene (6g): Black solid (346.6 mg, 98%); m.p. 164-166 ^{o}C; R_f 0.20 (33% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.37 (d, *J* = 5.98 Hz, 1H, ArH), 7.09 (t, *J* = 7.71 Hz, 1H, ArH), 6.85 (t, *J* = 7.38 Hz, 1H, ArH), 6.77 (d, *J* = 8.12 Hz, 1H, ArH), 4.87 (s, 1H, ArCH), 3.79 (s, 3H, OCH_3), 2.61-1.99 (m, 10H, 4xCH_2 and 2xCHCH_3), 1.06 (d, *J* = 6.35 Hz, 3H, CH_3), 1.03 (d, *J* = 6.33 Hz, 3H, CH_3).

9-(2-Chlorophenyl)-3,6-dimethyl-1,8-dioxo-octahydroxanthene (6h): White solid (314.1 mg, 88%); m.p. 188-190 ^{o}C; R_f 0.33 (33% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.45 (d, *J* = 7.89 Hz, 1H, ArH), 7.22 (d, *J* = 7.88 Hz, 1H, ArH), 7.17 (t, *J* = 7.44 Hz, 1H, ArH), 7.07 (t, *J* = 7.55 Hz, 1H, ArH), 5.01 (s, 1H, ArCH), 2.66-2.04 (m, 10H, 4xCH_2 and 2xCHCH_3), 1.10 (d, *J* = 6.12 Hz, 3H, CH_3), 1.08 (d, *J* = 4.99 Hz, 3H, CH_3).

3.2.3.4 General Procedure for the Synthesis of 12-(Aryl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-ones



A mixture of aldehyde (1 mmol, 1 equiv.), β-naphthol (1 mmol, 1 equiv.) 5,5-dimethyl-1,3-cyclohexanedione (1 mmol, 1 equiv.) and $Br_3CCOCBr_3$ (0.05 mmol) was heated and stirred at 110 ^{o}C for the appropriate time. The completion of reaction was monitored by TLC. Then, the reaction mixture was cooled down to room temperature. Water (3 mL) was added and then stirred for 5 minutes. The solid product was filtered

and recrystallized from ethanol. The identity of products was characterized by using ^{1}H NMR spectroscopy. The known products were characterized by physical data comparison with reported literature. All new compounds were fully characterized with relevant spectroscopic data and their experimental data are presented below.

12-(4-*tert*-Butylphenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (7d): White solid (452.5 mg, 100%); m.p. 196-198 ^oC; R_f 0.73 (33% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.06 (d, *J* = 8.41 Hz, 1H, Ar<u>H</u>), 7.81-7.76 (m, 2H, 2xAr<u>H</u>), 7.47 (t, *J* = 8.01 Hz, 1H, Ar<u>H</u>), 7.40 (t, *J* = 7.16 Hz, 1H, Ar<u>H</u>), 7.35 (d, *J* = 8.90 Hz, 1H, Ar<u>H</u>), 7.28-7.18 (m, 4H, 4xAr<u>H</u>), 5.70 (s, 1H, ArC<u>H</u>), 2.60 (d, *J* = 4.60 Hz, 2H, C<u>H</u>$_2$), 2.31 (d, *J* = 4.79 Hz, 2H, C<u>H</u>$_2$), 1.22 (s, 9H, (C<u>H</u>$_3$)$_3$), 1.14 (s, 3H, C<u>H</u>$_3$), 1.01 (s, 3H, C<u>H</u>$_3$).

12-Phenyl-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (7a): [91] Brown semi solid (403.8 mg, 100%); R_f 0.69 (33% EtOAc/hexane).

12-(4-Methoxyphenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (7b): [91] Orange semi solid (440.9 mg, 100%); R_f 0.31 (14% EtOAc/hexane).

12-(4-Methylphenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (7c): [91] Orange semi solid (403.3 mg, 100%); R_f 0.74 (33% EtOAc/hexane).

12-(4-Chlorophenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (7e): [112] Yellow solid (434.9 mg, 100%); m.p. 176-178 ^oC; R_f 0.60 (33% EtOAc/hexane).

12-(4-Bromophenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (7f): [112] White solid (440.3 mg, 100%); m.p. 185-187 ^oC; R_f 0.74 (33% EtOAc/hexane).

12-(3-Hydroxyphenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (7g): [91] Brown solid (381.6 mg, 100%); m.p. 242-244 ^oC; R_f 0.33 (33% EtOAc/hexane).

12-(3-Chlorophenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (7h): [96] Orange semi solid (450.3 mg, 100%); R_f 0.69 (33% EtOAc/hexane).

12-(3-Nitrophenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (7i): [91] Yellow solid (223.4 mg, 56%); m.p. 165-167 ^oC; R_f 0.60 (33% EtOAc/hexane).

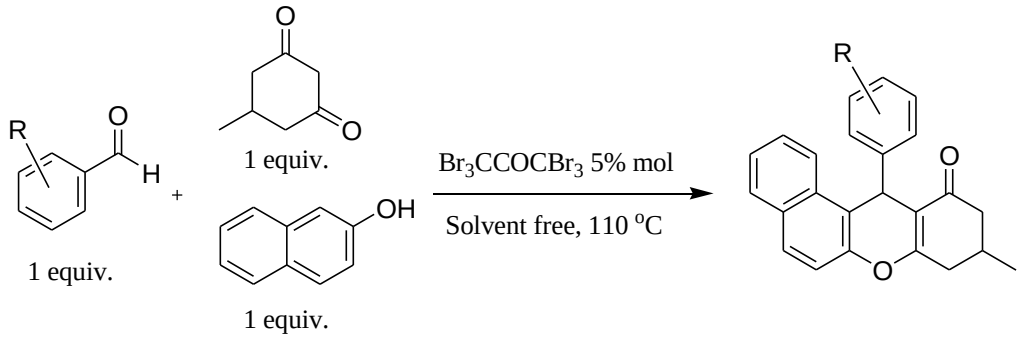
12-(2-Hydroxyphenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (7j): [87] Brown solid (322.7 mg, 90%); m.p. 202-204 ^oC; R_f 0.68 (33% EtOAc/hexane).

12-(2-Methoxyphenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (7k): [91] Brown semi solid (423.2 mg, 100%); R_f 0.57 (33% EtOAc/hexane).

12-(2-Chlorophenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (7l): [91] Orange semi solid(473.8 mg, 100%); R_f 0.64 (33% EtOAc/hexane).

12-(2-Nitrophenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (7m): [112] Black solid (254.4 mg, 64%); m.p. 221-223 ^oC; R_f 0.26 (20% EtOAc/hexane).

3.2.3.5 General Procedure for the Synthesis of 12-(Aryl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-ones



A mixture of aldehyde (1 mmol, 1 equiv.), β-naphthol (1 mmol, 1 equiv.), 5-methyl-1,3-cyclohexanedione (1 mmol, 1 equiv.) and $Br_3CCOCBr_3$ (0.05 mmol) was heated and stirred at 110 ^oC for the appropriate time. The completion of reaction was monitored by TLC. Then, the reaction mixture was cooled down to room temperature. Water (3 mL) was added and then stirred for 5 minutes. The solid product was filtered and recrystallized from ethanol. The identity of products was characterized by using ^1H NMR spectroscopy. All new compounds were fully characterized with relevant spectroscopic data and their experimental data are presented below.

12-Phenyl-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (8a): Brown solid (402.8 mg, 100%); m.p. 144-146 ^oC; R_f 0.63 (33% EtOAc/hexane); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.97 (t, *J* = 8.30 Hz, 1H, Ar<u>H</u>), 7.77 (t, *J* = 6.20 Hz, 2H, Ar<u>H</u>), 7.44-7.33 (m, 5H, 5xAr<u>H</u>), 7.17 (t, *J* = 7.50 Hz, 2H, 2xAr<u>H</u>), 7.06 (t, *J* = 7.30 Hz, 1H, Ar<u>H</u>), 5.73 (d, *J* = 8.5 Hz, 1H, ArC<u>H</u>), 2.78-2.33 (m, 4H, 2xC<u>H_2</u>), 2.17-2.07 (m, 1H, C<u>H</u>CH_3), 1.11-1.04 (dd, *J* = 6.40, 18.00 Hz, 3H, C<u>H_3</u>).

12-(4-Methoxyphenyl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (8b): Brown solid (427.0 mg, 100%); m.p. 192-194 ^oC; R_f 0.63 (33% EtOAc/hexane);

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.01 (d, *J* = 3.55 Hz, 1H, ArH), 7.79 (t, *J* = 7.92 Hz, 2H, 2xArH), 7.35-7.26 (m, 5H, 5xArH), 6.73 (d, *J* = 7.40 Hz, 2H, 2xArH), 5.69 (s, 1H, ArCH), 3.71 (s, 3H, OCH_3), 2.79-2.12 (m, 5H, 2xCH_2 and $CHCH_3$), 1.08 (d, *J* = 6.60 H, 3H, CH_3).

12-(4-*tert*-Butylphenyl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (8c): Brown solid (451.1 mg, 100%); m.p. 197-199 ^oC; R_f 0.74 (33% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.05-7.18 (m, 10H, 10xArH), 5.73 (s, 1H, ArCH), 2.80-2.09 (m, 5H, 2xCH_2 and $CHCH_3$), 1.23 (s, 9H, $(CH_3)_3$), 1.09 (d, *J* = 6.61 Hz, 3H, CH_3).

12-(4-Chlorophenyl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (8d): Brown solid (430.1 mg, 100%); m.p. 191-193 ^oC; R_f 0.66 (33% EtOAc/hexane).

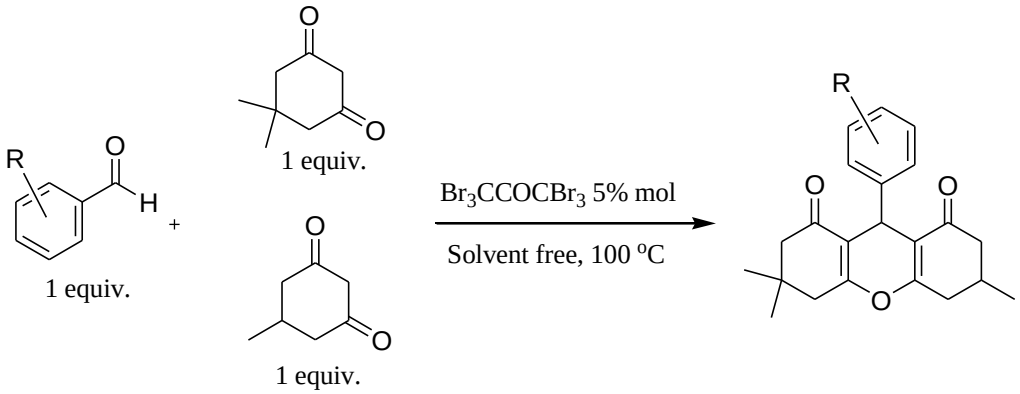
12-(3-Chlorophenyl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (8e): White solid (416.7 mg, 100%); m.p. 209-211 ^oC; R_f 0.73 (33% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.93 (d, *J* = 8.33 Hz, 1H, ArH), 7.82-7.27 (m, 7H, 7xArH), 7.13 (t, *J* = 7.84 Hz, 1H, ArH), 7.06 (d, *J* = 7.97 Hz, 1H, ArH), 5.71 (s, 1H, ArCH), 2.78-2.14 (m, 5H, 2xCH_2 and $CHCH_3$), 1.09 (d, *J* = 6.62 Hz, 3H, CH_3).

12-(2-Hydroxyphenyl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (8f): Yellow solid (323.0 mg, 91%); m.p. 224-226 ^oC; R_f 0.67 (33% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.43 (s, 1H, ArOH), 7.77 (t, *J* = 8.52 Hz, 2H, 2xArH), 7.67-6.58 (m, 8H, 8xArH), 5.76 (s, 1H, ArCH), 2.82-2.19 (m, 5H, 2xCH_2 and $CHCH_3$), 1.13 (d, *J* = 6.11 Hz, 3H, CH_3).

12-(2-Methoxyphenyl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (8g): Brown solid (403.6 mg, 100%); m.p. 209-211 ^oC; R_f 0.56 (33% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.31 (d, *J* = 8.47 Hz, 1H, ArH), 7.76 (d, *J* = 8.02 Hz, 1H, ArH), 7.72 (d, *J* = 8.86 Hz, 1H, ArH), 7.45 (t, *J* = 8.21 Hz, 1H, ArH), 7.38 (t, *J* = 7.15 Hz, 1H, ArH), 7.31-6.81 (m, 5H, 5xArH), 6.00 (s, 1H, ArCH), 3.98 (s, 3H, OCH_3), 2.79-2.10 (m, 5H, 2xCH_2 and $CHCH_3$), 1.10 (d, *J* = 6.64 Hz, 3H, CH_3).

12-(2-Chlorophenyl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (8h): White solid (419.1 mg, 100%); m.p. 197-199 ^oC; R_f 0.66 (33% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.23 (d, *J* = 8.44 Hz, 1H, ArH), 7.78 (t, *J* = 6.81 Hz, 2H, 2xArH), 7.50 (t, *J* = 7.24 Hz, 1H, ArH), 7.41 (t, *J* = 7.67 Hz, 1H, ArH), 7.33-7.28 (m, 3H, 3xArH), 7.08-7.01 (m, 2H, 2xArH), 6.03 (s, 1H, ArCH), 2.85-2.10 (m, 5H, 2xCH_2 and $CHCH_3$), 1.10 (d, *J* = 6.63, 3H, CH_3).

3.2.3.6 General Procedure for the Synthesis of 9-(Aryl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthenes



A mixture of aldehyde (1 mmol, 1 equiv.), 5,5-dimethyl-1,3-cyclohexandione (1 mmol, 1 equiv.), 5-methyl-1,3-cyclohexanedione (1 mmol, 1 equiv.) and $Br_3CCOCBr_3$ (0.05 mmol) was heated and stirred at 100 ^oC for the appropriate time. The completion of reaction was monitored by TLC. Then, the reaction mixture was cooled down to room temperature. Water (3 mL) was added and then stirred for 5 minutes. The solid product was filtered and recrystallized from ethanol. The identity of products was characterized by using ^1H NMR spectroscopy. All new compounds were fully characterized with relevant spectroscopic data and their experimental data are presented below.

9-Phenyl-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (9a): White solid (305.7 mg, 91%); m.p. 174-176 ^oC; R_f 0.30 (33% EtOAc/hexane); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.32-7.10 (m, 5H, 5xAr<u>H</u>), 4.77 (s, 1H, ArC<u>H</u>), 2.69-2.02 (m, 9H, 4xC<u>H</u>$_2$ and C<u>H</u>CH$_3$), 1.12 (s, 6H, 2xC<u>H</u>$_3$), 1.02 (d, *J* = 4.96 Hz, 3H, C<u>H</u>$_3$).

9-(4-Methoxyphenyl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (9b): Yellow solid (357.5 mg, 98%); m.p. 208-210 ^oC; R_f 0.65 (33% EtOAc/hexane); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.19 (d, *J* = 8.53 Hz, 2H, 2xAr<u>H</u>), 6.74 (d, *J* = 8.54 Hz, 2H, 2xAr<u>H</u>), 4.69 (s, 1H, ArC<u>H</u>), 3.71 (s, 3H, OC<u>H</u>$_3$), 2.65-1.98 (m, 9H, 4xC<u>H</u>$_2$ and C<u>H</u>CH$_3$), 1.08 (s, 6H, 2xC<u>H</u>$_3$), 0.99 (d, *J* = 4.44 Hz, 3H, C<u>H</u>$_3$).

9-(4-Methylphenyl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (9c): White solid (324.1 mg, 92%); m.p. 198-200 ^oC; R_f 0.74 (100% EtOAc/hexane); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.17 (d, *J* = 8.04 Hz, 2H, 2xAr<u>H</u>), 7.01 (d, *J* = 7.90 Hz, 2H, 2xAr<u>H</u>), 4.73 (s, 1H, ArC<u>H</u>), 2.65-2.02 (m, 9H, 4xC<u>H</u>$_2$ and C<u>H</u>CH$_3$), 2.24 (s, 3H, ArC<u>H</u>$_3$), 1.09 (s, 6H, 2xC<u>H</u>$_3$), 1.00 (d, *J* = 5.54 Hz, 3H, C<u>H</u>$_3$).

9-(4-Chlorophenyl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (9d): White solid (341.0 mg, 92%); m.p. 199-201 ^{o}C; R_f 0.31 (33% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.24-7.20 (m, 4H, 4xAr<u>H</u>), 4.75 (s, 1H, ArC<u>H</u>), 2.64-2.02 (m, 9H, 4xC<u>H</u>$_2$ and C<u>H</u>CH$_3$), 1.12 (s, 6H, 2xC<u>H</u>$_3$), 1.02 (d, *J* = 4.12 Hz, 3H, C<u>H</u>$_3$).

9-(3-Hydroxyphenyl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (9e): White solid (366.6 mg, 100%); m.p. 214-216 ^{o}C; R_f 0.46 (100% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.04 (t, *J* = 7.81 Hz, 1H, Ar<u>H</u>), 6.95 (s, 1H, Ar<u>H</u>), 6.71 (d, *J* = 7.21 Hz, 1H, Ar<u>H</u>), 6.58 (d, *J* = 7.96 Hz, 1H, Ar<u>H</u>), 6.51 (s, 1H, ArO<u>H</u>), 4.76 (s, 1H, ArC<u>H</u>), 2.65-2.03 (m, 9H, 4xC<u>H</u>$_2$ and C<u>H</u>CH$_3$), 1.88 (s, 3H, C<u>H</u>$_3$), 1.08 (d, *J* = 5.86 Hz, 6H, 2xC<u>H</u>$_3$).

9-(3-Chlorophenyl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (9f): White solid (333.5 mg, 90%); m.p. 178-180 ^{o}C; R_f 0.82 (100% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):7.24-7.08 (m, 4H, 4xAr<u>H</u>), 4.74 (s, 1H, ArC<u>H</u>), 2.68-2.03 (m, 9H, 4xC<u>H</u>$_2$ and C<u>H</u>CH$_3$), 1.10 (s, 6H, 2xC<u>H</u>$_3$), 1.01 (d, *J* = 4.77 Hz, 3H, C<u>H</u>$_3$).

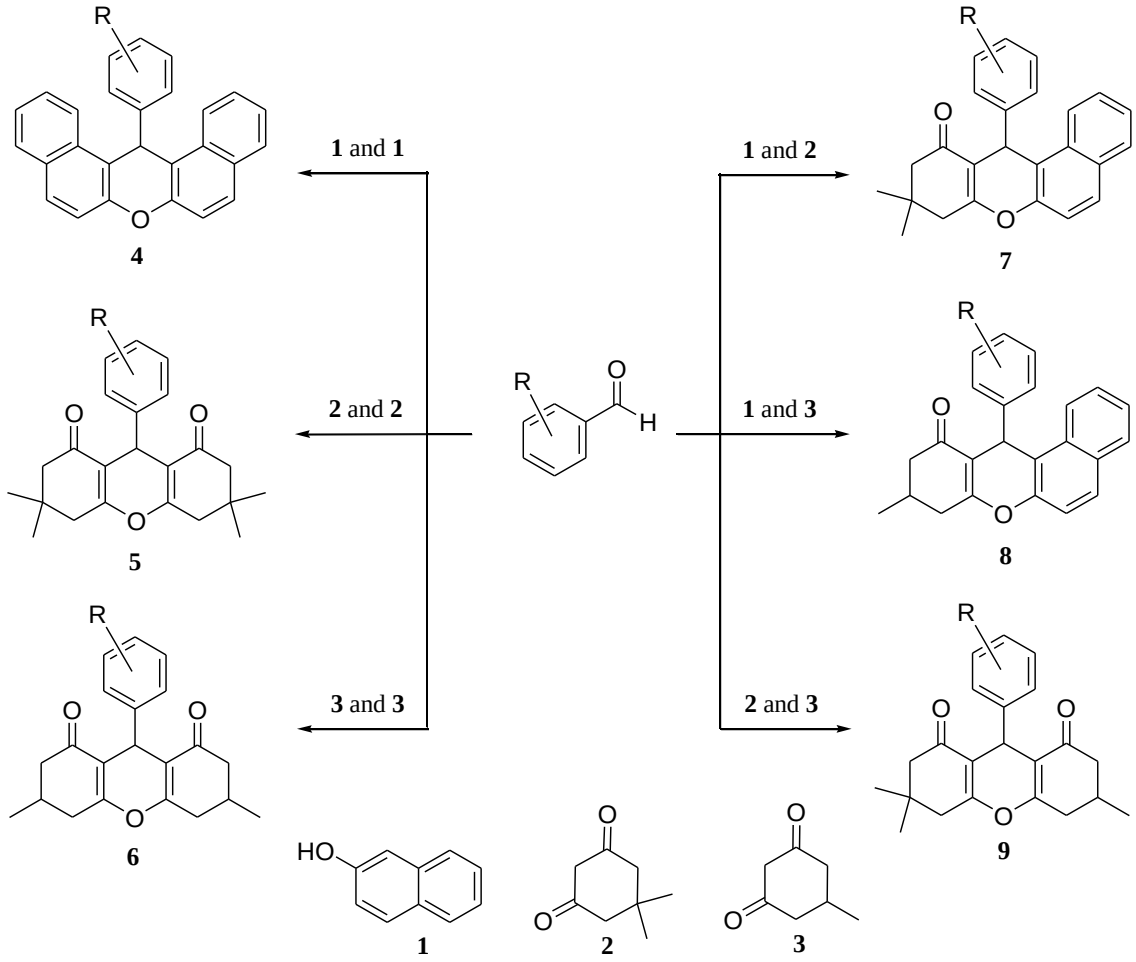
9-(3-Nitrophenyl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (9g): White solid (382.8 mg, 100%); m.p. 172-174 ^{o}C; R_f 0.72 (100% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.99-7.82 (m, 3H. 3xAr<u>H</u>), 7.39 (t, *J* = 6.54 Hz, 1H, Ar<u>H</u>), 4.85 (s, 1H, ArC<u>H</u>), 2.72-2.01 (m, 9H, 4xC<u>H</u>$_2$ and C<u>H</u>CH$_3$), 1.11 (s, 6H, 2xC<u>H</u>$_3$), 1.01 (d, *J* = 5.61 Hz, 3H, C<u>H</u>$_3$).

9-(2-Methoxyphenyl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (9h): Brown solid (378.2 mg, 100%); m.p. 161-163 ^{o}C; R_f 0.71 (100% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.40 (d, *J* = 7.50 Hz, 1H, Ar<u>H</u>), 7.09 (t, *J* = 7.52 Hz, 1H, Ar<u>H</u>), 6.86 (t, *J* = 7.38 Hz, 1H, Ar<u>H</u>), 6.75 (d, *J* = 8.05 Hz, 1H, Ar<u>H</u>), 4.85 (s, 1H, ArC<u>H</u>), 3.76 (s, 3H, OC<u>H</u>$_3$), 2.61-1.96 (m, 9H, 4xC<u>H</u>$_2$ and C<u>H</u>CH$_3$), 1.08 (s, 6H, 2xC<u>H</u>$_3$), 0.95 (d, *J* = 3.93 Hz, 3H, C<u>H</u>$_3$).

9-(2-Chlorophenyl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (9i): White solid (337.2 mg, 91%); m.p. 196-198 ^{o}C; R_f 0.77 (100% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.45-7.05 (m, 4H, 4xAr<u>H</u>), 5.01 (s, 1H, ArC<u>H</u>), 2.66-2.04 (m, 9H, 4xC<u>H</u>$_2$ and C<u>H</u>CH$_3$), 1.11 (s, 6H, 2xC<u>H</u>$_3$), 1.03 (s, 3H, C<u>H</u>$_3$).

3.3 Results and Discussion

A novel and simple method for the synthesis of xanthene derivatives was carried out using hexabromoacetone ($Br_3CCOCBr_3$) as a halogenating agent under solvent-free conditions. In this research, three types of nucleophile: β-naphthol (**1**), 5,5-dimethyl-1,3-cyclohexanedione (**2**) and 5-methyl-1,3-cyclohexanedione (**3**) were used to demonstrate the effectiveness of reactions toward various aromatic aldehydes. The general equation for the synthesis of symmetrical (**4-6**) and unsymmetrical xanthenes (**7-9**) is overviewed in Scheme 3.1.



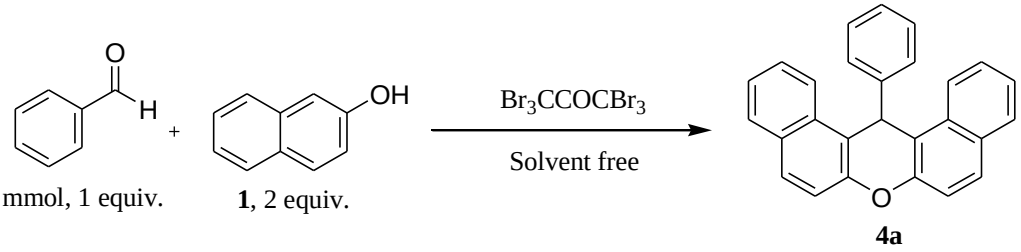
Scheme 3.1 General equation for the synthesis of xanthene derivatives

3.3.1 Condition Optimization for the Synthesis of Xanthenes

To obtain the efficient method for the synthesis of xanthene derivatives, the optimized condition was investigated by using β-naphthol (**1**, 2 mmol) and benzaldehyde (1 mmol) as a model compound to synthesize 14-phenyl-14*H*-

dibenzo[*a,j*]xanthene (**4a**). The effect of temperature and amount of $Br_3CCOCBr_3$ was studied under solvent-free conditions and the results are displayed in Table 3.4.

Table 3.4 Effect of the amount of $Br_3CCOCBr_3$ and temperature on the synthesis of 14-phenyl-14*H*-dibenzo[*a,j*]xanthene



1 mmol, 1 equiv. **1**, 2 equiv. **4a**

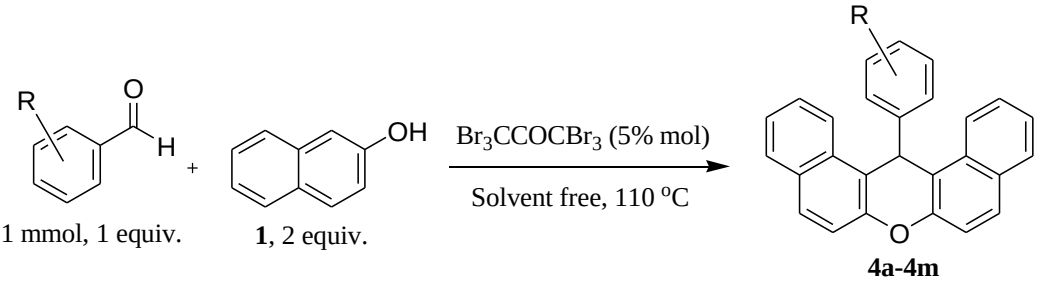
Entry	$Br_3CCOCBr_3$ (% mol)	Temperature (^oC)	Time (min)	Isolated Yield **4a** (%)
1	-	110	24 h	-
2	2	110	5	22
3	5	110	5	90
4	10	110	5	61
5	5	90	40	36

Initially, benzaldehyde was refluxed with β-naphthol at 110 ^oC in the absence of $Br_3CCOCBr_3$, no product **4a** was obtained even after 24 hours (entry 1). In order to demonstrate the need of catalyst, the reaction was then studied in the presence of different amount of $Br_3CCOCBr_3$ (2%, 5% and 10% mol) at 110 ^oC for 5 minutes under solvent-free conditions (entries 2-4). It was observed that 5% mol of $Br_3CCOCBr_3$ was an optimum amount for this model reaction to furnish the product **4a** in high yield (90%, entry 3). Increasing the amount of $Br_3CCOCBr_3$ to 10% mol did not improve the reaction yield, providing the product **4a** in moderate yield (61%, entry 4). These results indicated that $Br_3CCOCBr_3$ plays an important role and can be used to accelerate the reaction. In order to evaluate the effect of temperature, 5% mol of $Br_3CCOCBr_3$ was used to carry out the reaction at 90 ^oC. The result showed that the reaction needed a longer time period of 40 minutes affording the desired product in poor yield (36%, entry 5). According to these results, the optimized condition for the synthesis of xanthene derivatives was accomplished using 5% mol of $Br_3CCOCBr_3$ at 110 ^oC under solvent-free conditions.

3.3.2 Synthesis of 14-(Aryl)-14*H*-dibenzo[*a,j*]xanthenes

Xanthene derivatives have showed wide advantages in many aspects, specifically, 14*H*-dibenzo[*a,j*]xanthenes have proved their activities as positive allosteric modulators of metabotropic (mGlu) receptors [129] and nonpeptidic inhibitors of recombinant human Caplain I in medicinal utilization area [130]. The synthesis of 14-(aryl)-14*H*-dibenzo[*a,j*]xanthenes (**4**) from the reaction of β-naphthol (**1**, 2 mmol) and different aromatic aldehydes (1 mmol) under solvent-free conditions was then explored to test the scope and limitation of optimized condition. The results are shown in Table 3.5.

Table 3.5 Synthesis of 14-(aryl)-14*H*-dibenzo[*a,j*]xanthenes in the presence of $Br_3CCOCBr_3$ at 110 ^oC



1 mmol, 1 equiv. **1**, 2 equiv. **4a-4m**

Entry	R	Time (min)	Isolated Yield (%)
1	H	5	**4a**,90
2	4-OCH_3	30	**4b**, Quant.
3	4-CH_3	5	**4c**, Quant.
4	4-$C(CH_3)_3$	20	**4d**,Quant.
5[a]	4-Cl	1	**4e**,Quant.
6	4-Br	5	**4f**,93
7	3-OH	15	**4g**,93
8	3-Cl	5	**4h**,99
9	3-NO_2	1	**4i**,95
10	2-OH	35	**4j**,88
11	2-OCH_3	30	**4k**,99
12	2-Cl	5	**4l**,Quant.
13	2-NO_2	25	**4m**,96

[a]100 ^oC was used.

In the reaction of benzaldehyde containing no substituent on aromatic ring, the reaction was completed within 5 minutes to furnish the product **4a** in 90% yield (entry 1). The different types of aromatic aldehyde containing electron-withdrawing and electron-donating substituents at *ortho*, *meta* or *para* positions were also selected to test the reaction. At *para* position, the reactions proceeded well and gave the corresponding products **4b**-**4f** in excellent yields (93-100%, entries 2-6). Specially, in the case of 4-chlorobenzaldehyde, the reaction temperature could be reduced to 100 ^{o}C, providing the corresponding product **4e** in quantitative yield within 1 minute (entry 5). The similar excellent yields were also obtained from aromatic aldehydes bearing a substituent at the *meta* position (93-99%, entries 7-9). However, the use of 3-nitrobenzaldehyde containing a strong electron-withdrawing substituent, the reaction performed very fast and gave the desired product **4i** in 95% yield over 1 minute (entry 9). The reaction of 2-hydroxy and 2-methoxybenzaldehydes bearing a strong electron-donating substituent exhibited the corresponding products **4j** and **4k** in excellent yields within 30-35 minutes (88-99%, entries 10 and 11). While, the electron-withdrawing substituents at the *ortho* position of aromatic ring could also afford the excellent yield of corresponding products **4l** and **4m** within 5-25 minutes (96-100%, entries 12 and 13). For these aforementioned results, the aromatic aldehydes containing electron-withdrawing substituents could present the higher yields and faster reaction times than another substituent's nature on the aromatic aldehydes. The increase of electrophilicity on carbonyl carbon at aromatic aldehydes by electron-withdrawing groups could be the reason for this phenomenon.

14-(4-*tert*-Butylphenyl)-14*H*-dibenzo[*a*,*j*]xanthene (**4d**) was a new compound and its identity was characterized by ^{1}H NMR. The spectrum (Fig 3.3) displayed that five doublet signals around δ_H 8.42 (J = 8.48 Hz), 7.83 (J = 8.08 Hz), 7.79 (J = 8.87 Hz), 7.49 (J = 8.88 Hz) and 7.14 (J = 8.44 Hz), one triplet signal at δ_H 7.59 (J = 8.27 Hz) and one multiplet signal at δ_H7.43-7.40 were ascribed for sixteen aromatic protons. Methine proton (CH) was observed from the presence of one singlet signal at δ_H 6.48. The singlet signal at δ_H 1.14 was assigned to nine protons of *tert*-butyl group.

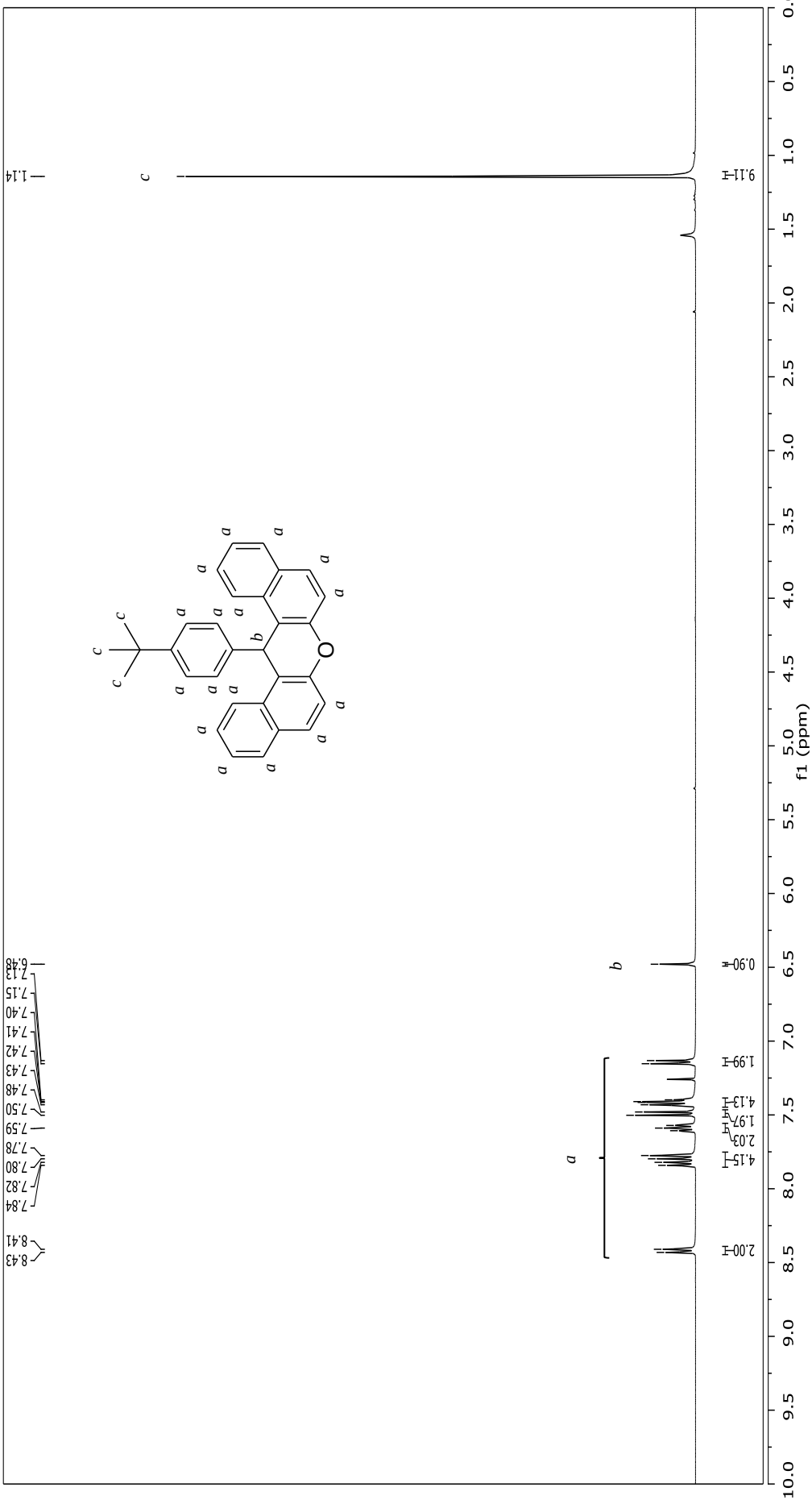
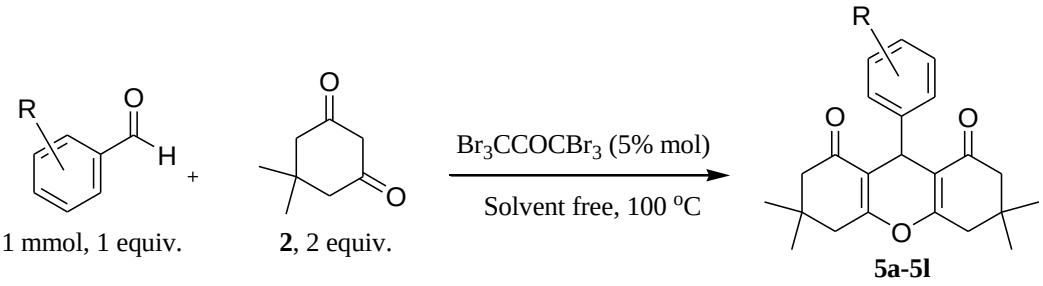


Figure 3.3 The ^{1}H NMR spectrum of 14-(*4-tert*-butylphenyl)-14*H*-dibenzo[*a,j*]xanthene (**4d**)

3.3.3 Synthesis of 9-(Aryl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydro-xanthenes

Many natural products containing a xanthenedione structure [131] have been reported to have promising biological activities such as antioxidant and acetylcholinesterase inhibitory activities [132]. To extend the utilization of $Br_3CCOCBr_3$, the method for the synthesis of 9-(aryl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthenes (**5**) has been developed by using diverse aromatic aldehydes (1 mmol) reacted with 5,5-dimethyl-1,3-cyclohexanedione (**2**, 2 mmol) in the presence of 5% mol $Br_3CCOCBr_3$ under solvent-free conditions. The results are presented in Table 3.6.

Table 3.6 Synthesis of 9-(aryl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthenes in the presence of $Br_3CCOCBr_3$ at 100 ^oC



1 mmol, 1 equiv. **2**, 2 equiv. **5a-5l**

Entry	R	Time (min)	Isolated Yield (%)
1	H	5	**5a**, 85
2	4-OCH_3	5	**5b**, Quant.
3	4-CH_3	1	**5c**, Quant.
4	4-$C(CH_3)_3$	5	**5d**, 97
5	4-Cl	10	**5e**, 95
6	4-Br	5	**5f**, Quant.
7	3-OH	10	**5g**, 96
8	3-Cl	1	**5h**, 91
9	3-NO_2	10	**5i**, 98
10	2-OH	1	**5j**, 95
11	2-OCH_3	1	**5k**, 97
12	2-Cl	5	**5l**, 88
13	2-NO_2	1	**5m**, 98

In the presence of 5% mol of $Br_3CCOCBr_3$ at 100 ^oC under solvent-free conditions, the reaction of benzaldehyde afforded well the product **5a** in good yield within 5 minutes (85%, entry 1). In order to investigate the effect of the substituent's nature on the aromatic ring, the synthesis of compounds **5b**-**5m** was carried out. At the *para* position of aromatic aldehydes, the reaction gave the corresponding products **5b**-**5f** in excellent yields (95-100%, entries 2-6). Particularly, the reaction of 4-methylbenzaldehyde performed very fast to give the quantitative yield of product **5c** within 1 minute (entry 3). In the case of aromatic aldehydes containing substituents at the *meta* position, the corresponding products **5g**-**5i** were obtained in excellent yields (91-98%, entries 7-9). The reaction of aromatic aldehyde containing electron-withdrawing substituents could give higher yield of product (98%, entry 9) than aromatic aldehyde with electron-donating substituents (96%, entry 7). For 3-chloro-benzaldehyde, halogenating aromatic aldehyde, the corresponding product **5h** was obtained in excellent yield with a short reaction time (91%, entry 8). The excellent yield of products was also considerably obtained from the reaction of aromatic aldehydes bearing a substituent at the *ortho* position (88-98%, entries 10-13). The reaction of 2-hydroxy and 2-methoxybenzaldehydes, bearing a strong electron-donating substituent on aromatic ring, could produce the corresponding products **5j** and **5k** in excellent yields within 1 minute (95-97%, entries 10 and 11). However, the reaction of 2-nitrobenzaldehyde, the electron-withdrawing substituents on aromatic ring, provided higher yield of corresponding product **5m** within 1 minute (98%, entry 13). Overall, these results clearly described that the synthesis of 9-(aryl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydro-xanthenes towards aromatic aldehydes containing electron-withdrawing substituents presented higher yields of desired products than electron-donating substituents. The possible reason of this occurrence revealed that the electron-withdrawing groups could increase the electrophilicity of carbonyl carbon at aromatic aldehydes giving higher yields of desired product within short reaction times.

9-(4-*tert*-Butylphenyl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthene (**5d**) was a new compound and its identity was characterized by ^1H NMR. The spectrum (Fig 3.4) displayed that one doublet signal at δ_H 7.18 (J = 4.05 Hz) was belonged to four aromatic protons. A singlet signal at δ_H 4.73 indicated the presence of methine proton (CH). Three singlet signals around δ_H 2.45, 2.21 and 2.19 were assigned to eight

protons of methylene group (CH_2). Nine protons of *tert*-butyl group were revealed from singlet signal at δ_H 1.23. Four methyl groups (CH_3) was observed from the presence of two singlet signals at δ_H 1.09 and 1.00.

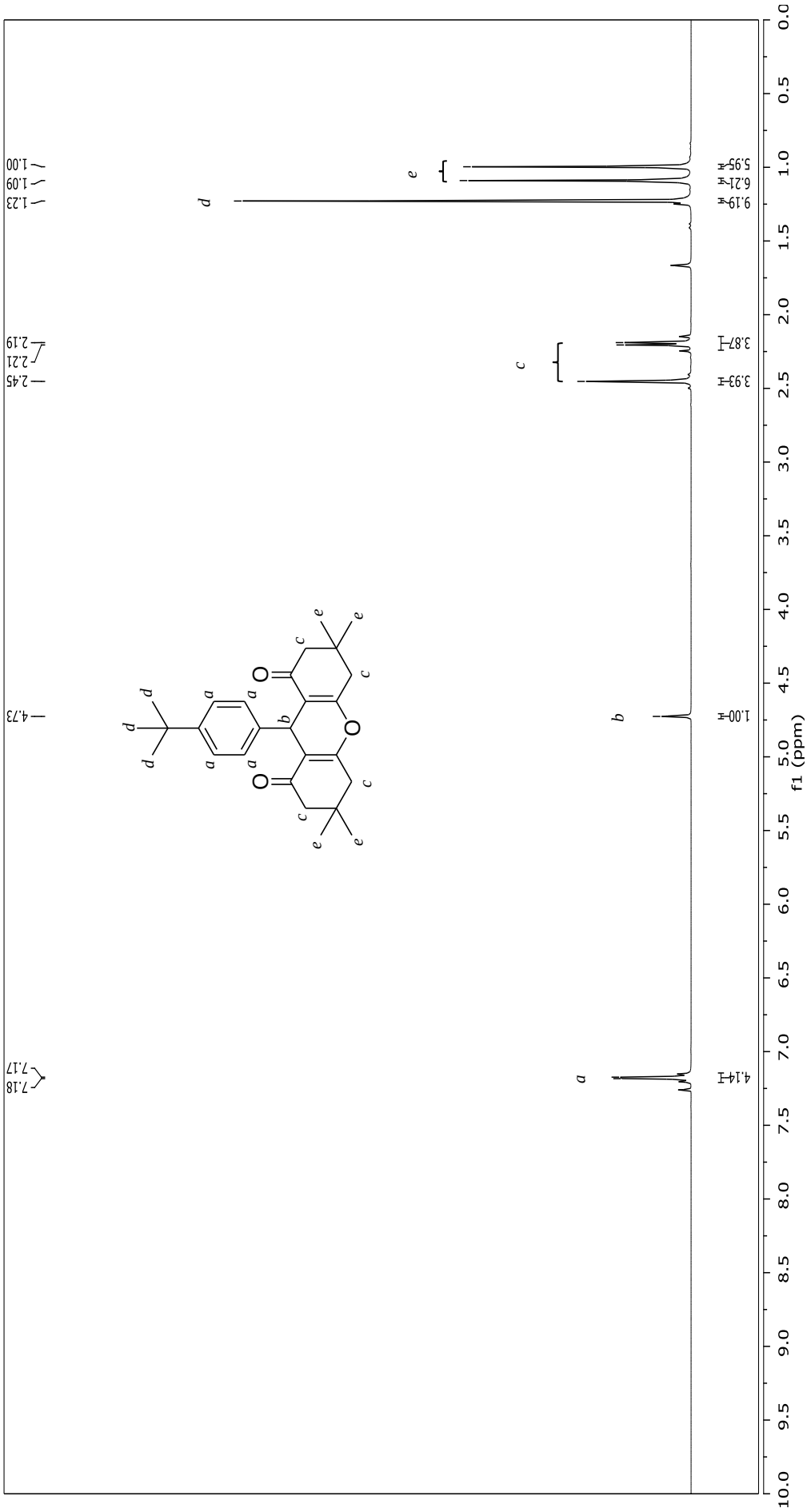
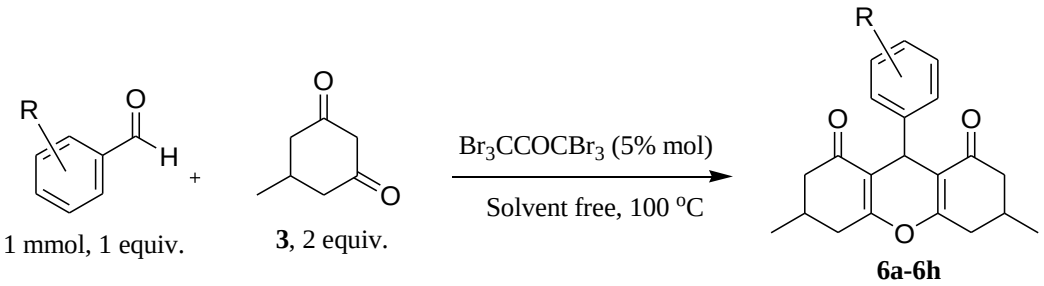


Figure 3.4 The ^{1}H NMR spectrum of 9-(*4-tert*-butylphenyl)-3,3,6,6-tetramethyl-1,8-dioxo-octahydroxanthene (**5d**)

3.3.4 Synthesis of 9-(Aryl)-3,6-dimethyl-1,8-dioxo-octahydroxanthenes

The synthesis of 9-(aryl)-3,6-dimethyl-1,8-dioxo-octahydroxanthenes (**6**) which are a new xanthene derivative has been established. They could be prepared from the reaction of 5-methyl-1,3-cyclohexanedione (**3**, 2 mmol) and various aromatic aldehydes (1 mmol) in the presence of 5% mol $Br_3CCOCBr_3$ under solvent-free conditions and the results are shown in Table 3.7.

Table 3.7 Synthesis of 9-(aryl)-3,6-dimethyl-1,8-dioxo-octahydroxanthenes in the presence of $Br_3CCOCBr_3$ at 100 ^oC



1 mmol, 1 equiv. **3**, 2 equiv. **6a-6h**

Entry	R	Time (min)	Isolated Yield (%)
1[a]	H	30	**6a**, Quant.
2	4-OCH_3	5	**6b**, 99
3[a]	4-CH_3	10	**6c**, Quant.
4	4-Cl	5	**6d**, 90
5	3-OH	10	**6e**, 99
6	3-NO_2	1	**6f**, 99
7	2-OCH_3	1	**6g**, 98
8[a]	2-Cl	10	**6h**, 88

[a]110 ^oC was used.

As shown in Table 3.7, the aromatic aldehydes containing electron-donating and electron-withdrawing groups were used and 9-(aryl)-3,6-dimethyl-1,8-dioxo-octahydroxanthenes were obtained in excellent yields at 100 ^oC. Interestingly, for the reaction of benzaldehyde, no substituent on aromatic ring, the product **6a** was isolated in quantitative yield by increasing the reaction temperature to 110 ^oC (entry 1). The reaction temperature at 110 ^oC was also carried out on 4-methylbenzaldehyde and 2-chlorobenzaldehyde to give the excellent yields of products **6c** and **6h** within 10 minutes (88-100%, entries 3 and 8). The reaction of aromatic aldehydes with a *para*

substituted group could give the corresponding products **6b**-**6d** in excellent yields (90-100%, entries 2-4). Moreover, the strong electron-donating and electron-withdrawing substituents on the *meta* position also could afford the desired products **6e** and **6f** in almost quantitative yield in short reaction times (99%, entries 5 and 6). Especially, in the case of 3-nitrobenzaldehyde, bearing electron-withdrawing substituent, the reaction could be completed very fast by 1 minute (entry 6). The fast reaction time was also occurred on 2-methoxybenzaldehyde providing the corresponding product **6g** in 98% yield within 1 minute (entry 7). These results obviously indicated that electron-withdrawing groups on an aromatic aldehyde affected on the yield of 9-(aryl)-3,6-dimethyl-1,8-dioxo-octahydroxanthenes. The increase of electrophilicity effect on carbonyl carbon at aromatic aldehydes might possibly due to the natural substituents from electron-withdrawing groups, giving the reaction in high yields within short reaction times.

All products **6a**-**6h** were a new compound and their identities were characterized by ^{1}H NMR. As a representative, the spectrum of 9-(4-methoxyphenyl-3,6-dimethyl-1,8-dioxo-octahydroxanthene (**6b**) (Fig 3.5) displayed two doublet signals of four aromatic protons around δ_H 7.19 (J = 8.65 Hz) and 6.74 (J = 8.58 Hz). One singlet signal at δ_H 4.73 was assigned to methine proton (CH) connecting to aromatic group. Methoxy proton (OCH_3) was observed from singlet signal at δ_H 3.72. Four methylene protons (CH_2) and two methine protons (CH) were identified from the presence of multiplet signal at δ_H 2.65-2.01. The doublet signal at δ_H 1.08 (J = 6.22 Hz) was ascribed for six protons of two methyl groups (CH_3).

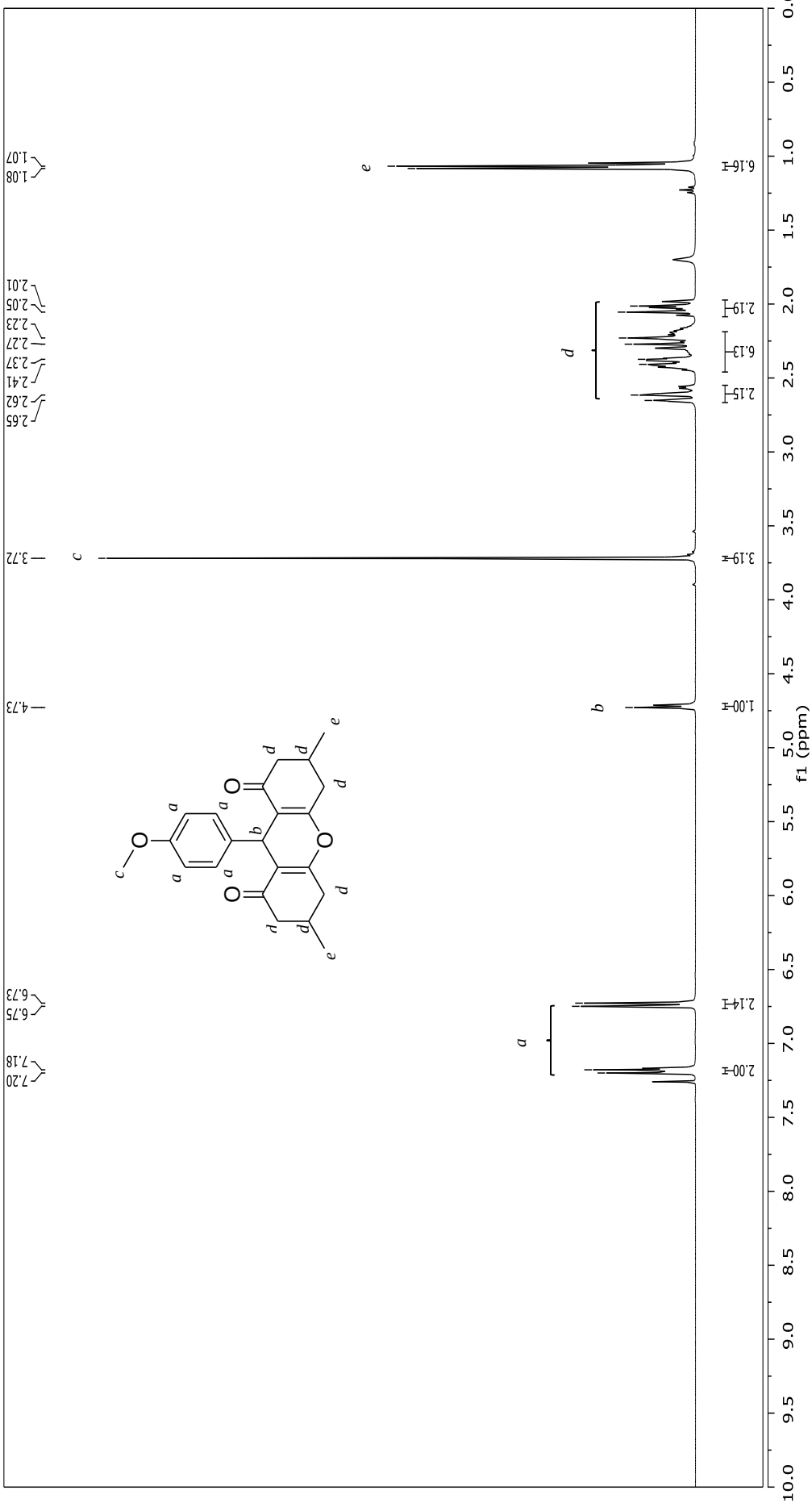
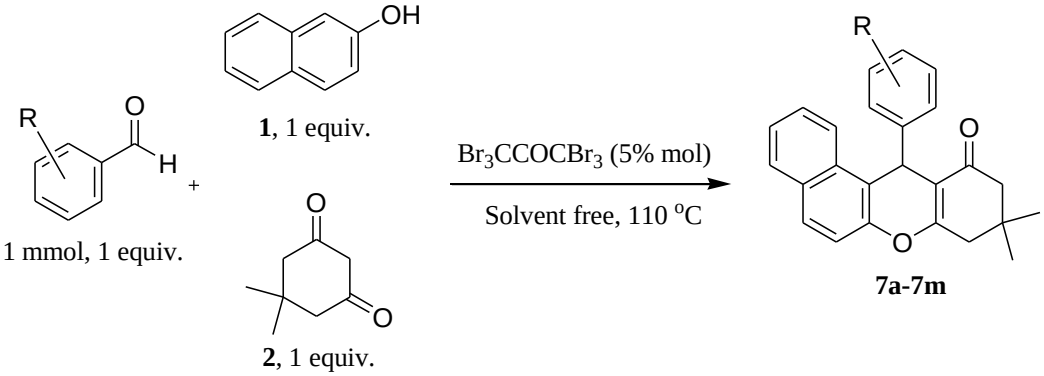


Figure 3.5 The ^{1}H NMR spectrum of 9-(4-methoxyphenyl)-3,6-dimethyl-1,8-dioxo-octahydroxanthene (**6b**)

3.3.5 Synthesis of 12-(Aryl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]-xanthene-11-ones

Tetrahydroxanthenones have a great potential for further development of synthetic method due to their distinctive structures and biological activities [133]. The three-component reaction of diverse aromatic aldehydes (1 mmol), β-naphthol (**1**, 1 mmol) and 5,5-dimethyl-1,3-cyclohexanedione (**2**, 1 mmol) was subjected to synthesize 12-(aryl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo-[*a*]xanthene-11-ones (**7**) in the presence of 5% mol $Br_3CCOCBr_3$ under solvent-free conditions. The results of these reactions are displayed in Table 3.8.

Table 3.8 Synthesis of 12-(aryl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-ones in the presence of $Br_3CCOCBr_3$ at 110 ^oC



Entry	R	Time (min)	Isolated Yield (%)
1	H	45	**7a**, Quant.
2	4-OCH_3	25	**7b**, Quant.
3	4-CH_3	40	**7c**, Quant.
4	4-$C(CH_3)_3$	30	**7d**, Quant.
5	4-Cl	25	**7e**, Quant.
6	4-Br	25	**7f**, Quant.
7	3-OH	20	**7g**, Quant.
8	3-Cl	20	**7h**, Quant.
9	3-NO_2	20	**7i**, 56
10	2-OH	35	**7j**, 90
11	2-OCH_3	45	**7k**, Quant.
12	2-Cl	35	**7l**, Quant.
13	2-NO_2	35	**7m**, 64

From the results in Table 3.8, it was observed that the reaction of benzaldehyde and all aromatic aldehydes containing electron-donating and electron-withdrawing substituents on aromatic rings could give the unsymmetrical xanthenes **7a**-**7m** in good to excellent yields (56-100%, entries 1-13). All substituents at the *para*, *meta* or *ortho* position promoted the yields of unsymmetrical xanthenes**7** in 20-45 minutes. However, 3-nitro and 2-nitrobenzaldehydes, the aromatic aldehydes containing electron-withdrawing substituent at the *meta* and *ortho* position, afforded the corresponding product **7i** and **7m** in less yield (56-64 %, entry 9 and 13). Based on these results, there was an effect of substituent's nature at the aromatic rings on these reactions. The aromatic aldehydes containing electron-donating substituents could stabilize the activated carbonyl of aromatic aldehydes performing the mild reaction and yielded the higher amount of products.

12-(4-*tert*-Butylphenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (**7d**) was a new compound and its identity was characterized by ^{1}H NMR. The spectrum (Fig 3.6) showed that two doublet signals at δ_H 8.06 (J = 8.41 Hz) and 7.35 (J = 8.90 Hz), two triplet signals at δ_H 7.47 (J = 8.01 Hz) and 7.40 (J = 7.16 Hz) and two multiplet signals at δ_H 7.81-7.76 and 7.28-7.18 were due to ten aromatic protons. Methine proton (CH) was identified from the presence of singlet signal at δ_H 5.70. Two doublet signals at δ_H 2.60 (J = 4.60 Hz) and 2.31 (J = 4.79 Hz) were assigned to four methylene protons (CH_2). A singlet signal at δ_H 1.22 was ascribed for nine protons of *tert*-butyl group. Two methyl protons (CH_3) were identified from the presence of two singlet signals at δ_H 1.14 and 1.01.

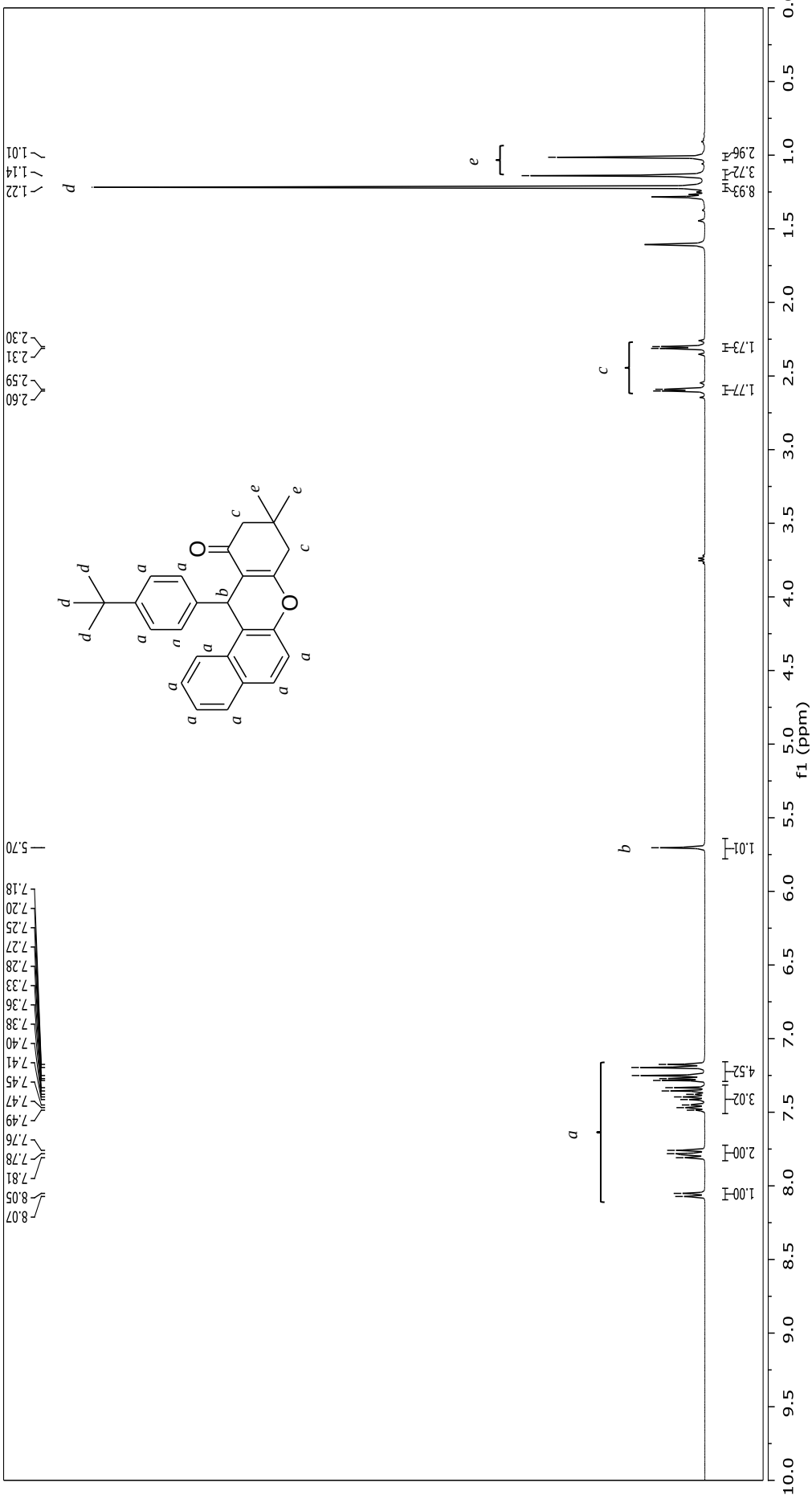
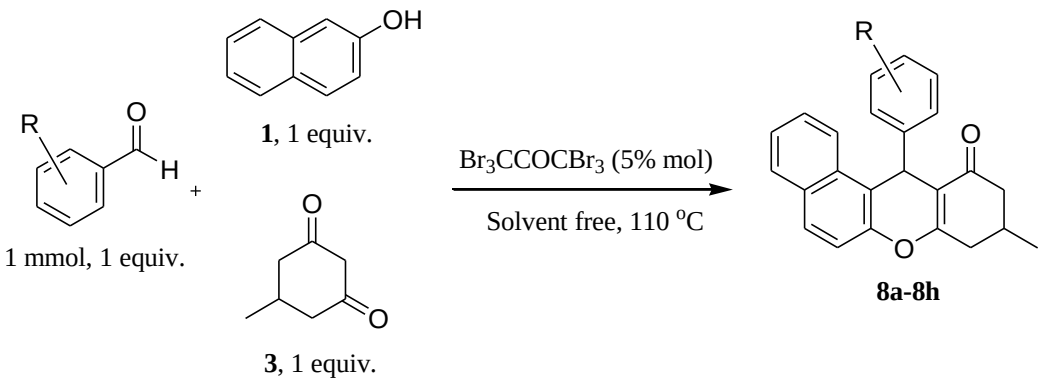


Figure 3.6 The ^{1}H NMR spectrum of 12-(4-*tert*-butylphenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (**7d**)

3.3.6 Synthesis of 12-(Aryl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]-xanthene-11-ones

Encouraged by previous results, the three-component reaction of β-naphthol (**1**, 1 mmol), 5-methyl-1,3-cyclohexanedione (**3**, 1 mmol) and different aromatic aldehydes (1 mmol) was then developed in the presence of 5% mol $Br_3CCOCBr_3$ under solvent-free conditions to furnish 12-(aryl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-ones (**8**) which are a new xanthene derivative. The results are presented in Table 3.9.

Table 3.9 Synthesis of 12-(aryl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-ones in the presence of $Br_3CCOCBr_3$ at 110 ^oC



Entry	R	Time (min)	Isolated Yield (%)
1	H	25	**8a**, Quant.
2	4-OCH_3	35	**8b**, Quant.
3	4-$C(CH_3)_3$	40	**8c**, Quant.
4	4-Cl	50	**8d**, Quant.
5	3-Cl	30	**8e**, Quant.
6	2-OH	15	**8f**, 91
7	2-OCH_3	60	**8g**, Quant.
8	2-Cl	50	**8h**, Quant.

It was clearly seen in Table 3.9 that the reaction of benzaldehyde, containing no substituent on aromatic ring, regioselectively proceeded to give the unsymmetrical xanthenes **8a** in quantitative yield (100%, entry 1). The quantitative yield and high regioselectivity were also afforded when aromatic aldehydes containing the substituents

at the *para* or *meta* position were used (100%, entries 2-5). At *ortho* position, the reactions also generated the desired products **8f**-**8h** in excellent yields (91-100%, entries 6-8). Particularly, the reaction of 2-hydroxybenzaldehyde, bearing a strong electron-donating substituent, provided the corresponding product **8f** in 91% within 15 minutes (entry 6). For these aforementioned results, the synthesis of 12-(aryl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-ones with exclusive regioselectivity was performed very well by substituent's nature on the aromatic rings.

All products **8a**-**8h** were a new compound and their identities were characterized by ^{1}H NMR. As a representative, the spectrum of 12-phenyl-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (**8a**) (Fig 3.7) showed that four triplet signals around δ_H 7.97 (J = 8.30 Hz), 7.77 (J = 6.20 Hz), 7.17 (J = 7.50 Hz) and 7.06 (J = 7.30 Hz) and multiplet signal at δ_H 7.44-7.33 were assigned for eleven aromatic protons. The doublet signals at δ_H 5.73 were ascribed for methine proton (CH) connecting to aromatic group. Two methylene protons (CH_2) were revealed from the presence of two multiplet signals at δ_H 2.78-2.70 and 2.54-2.33. Methine proton (CH) was observed from multiplet signal at δ_H 2.17-2.07. A methyl proton (CH_3) was identified from double doublet signal at δ_H 1.11-1.04 (J = 6.40 and 18.00 Hz).

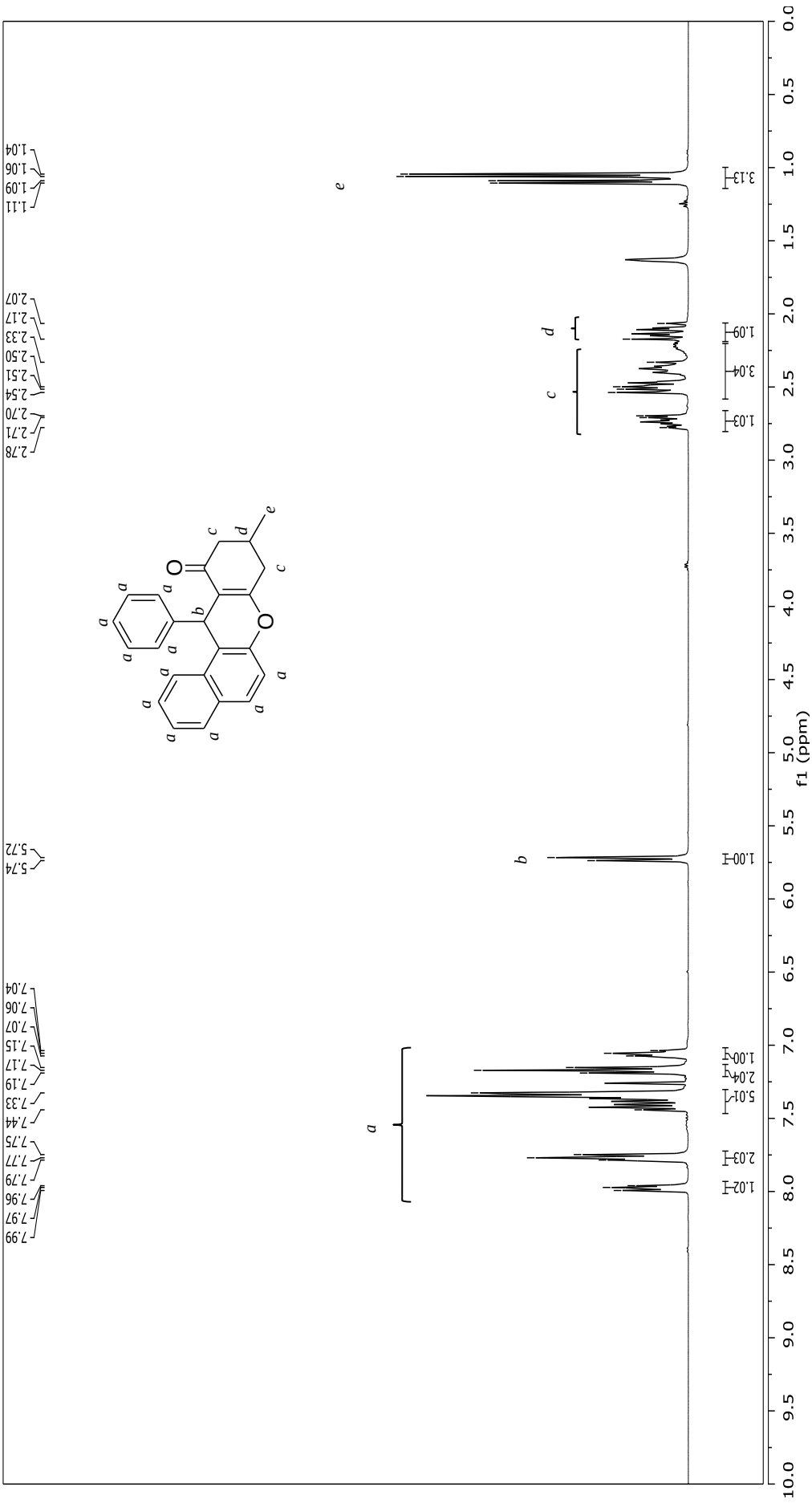
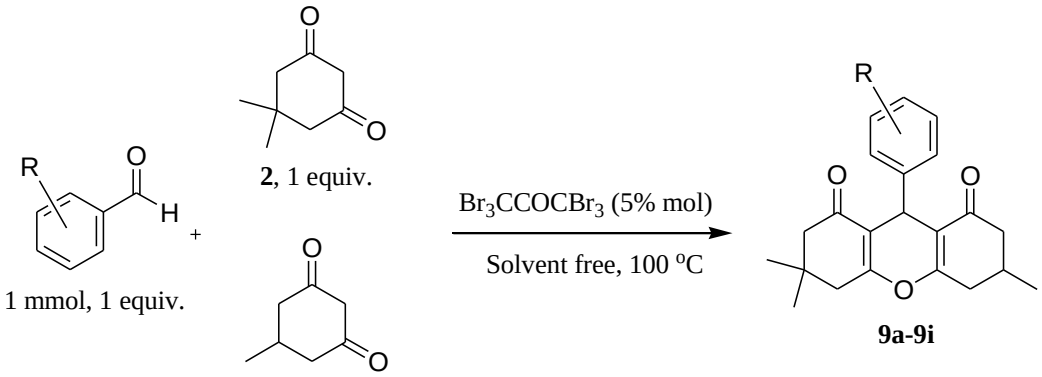


Figure 3.7 The ^{1}H NMR spectrum of 12-phenyl-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (**8a**)

3.3.7 Synthesis of 9-(Aryl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthenes

Another three-component reaction of new xanthene derivatives has also been established in this research. In the presence of 5% mol $Br_3CCOCBr_3$ under solvent-free conditions, the synthesis of 9-(aryl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthenes (**9**) was conducted using various aromatic aldehydes (1 mmol), 5,5-dimethyl-1,3-cyclohexanedione (**2**, 1 mmol) and 5-methyl-1,3-cyclohexanedione (**3**, 1 mmol). The results are shown in Table 3.10.

Table 3.10 Synthesis of 9-(aryl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthenes in the presence of $Br_3CCOCBr_3$ at 100 ^oC



Entry	R	Time (min)	Isolated Yield (%)
1[a]	H	15	**9a**, 91
2	4-OCH_3	1	**9b**, 98
3	4-CH_3	1	**9c**, 92
4	4-Cl	25	**9d**, 92
5[a]	3-OH	25	**9e**, Quant.
6[a]	3-Cl	10	**9f**, 90
7[a]	3-NO_2	15	**9g**, Quant.
8[a]	2-OCH_3	15	**9h**, Quant.
9[a]	2-Cl	25	**9i**, 91

[a]110 ^oC was used.

From the Table 3.10, at 100 ^oC, the excellent regioselectivity and yield from the synthesis of unsymmetrical xanthenes **9** using aromatic aldehydes bearing electron-

donating and electron-withdrawing substituents were observed. In the reaction of aromatic aldehydes containing substituents at *para* position also provided the corresponding products **9b**-**9d** in excellent yields (92-98%, entries 2-4). Specially, for 4-methoxy and 4-methylbenzaldehydes, the reactions delivered the excellent yield of products **9b** and **9c** very fast within 1 minute (92-98%, entries 2 and 3). However, the reaction of benzaldehyde and aromatic aldehydes containing substituents at the *meta* or *ortho* position required the higher reaction temperature up to 110 ^{o}C to give the products **9a** and **9e**-**9i** in excellent yields (90-100%, entries 1, 5-9). Particularly for natural substituents on aromatic aldehydes at *meta* positions, 3-hydroxy and 3-nitrobenzaldehydes, the electron-withdrawing group could perform faster reaction time to give the quantitative yield within 15 minutes (100%, entry 7) than electron-donating group that needed 25 minutes to yield the same amount (100%, entry 5). According to these results, the synthesis of 9-(aryl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthenes was influenced by substituent's nature on aromatic aldehydes specially from the electron-withdrawing substituents. The electron-withdrawing group could increase the electrophilicity of carbonyl carbon at aromatic aldehydes giving high yields of product within short reaction time.

All products **9a**-**9i** were a new compound and their identities were characterized by ^{1}H NMR. As a representative, the spectrum of 9-phenyl-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (**9a**) (Fig 3.8) displayed a multiplet signal of five aromatic protons at δ_H 7.32-7.10. Methine proton (CH) connecting to aromatic group was observed from singlet signal at δ_H 4.77. Four methylene protons (CH_2) and one methine proton (CH) were revealed from the presence of multiplet signal at δ_H 2.69-2.09. The singlet signal at δ_H 1.12 was assigned to six protons of two methyl groups (CH_3). Another methyl proton (CH_3) was identifed from the presence of doublet signal at δ_H 1.02 (J = 4.96 Hz).

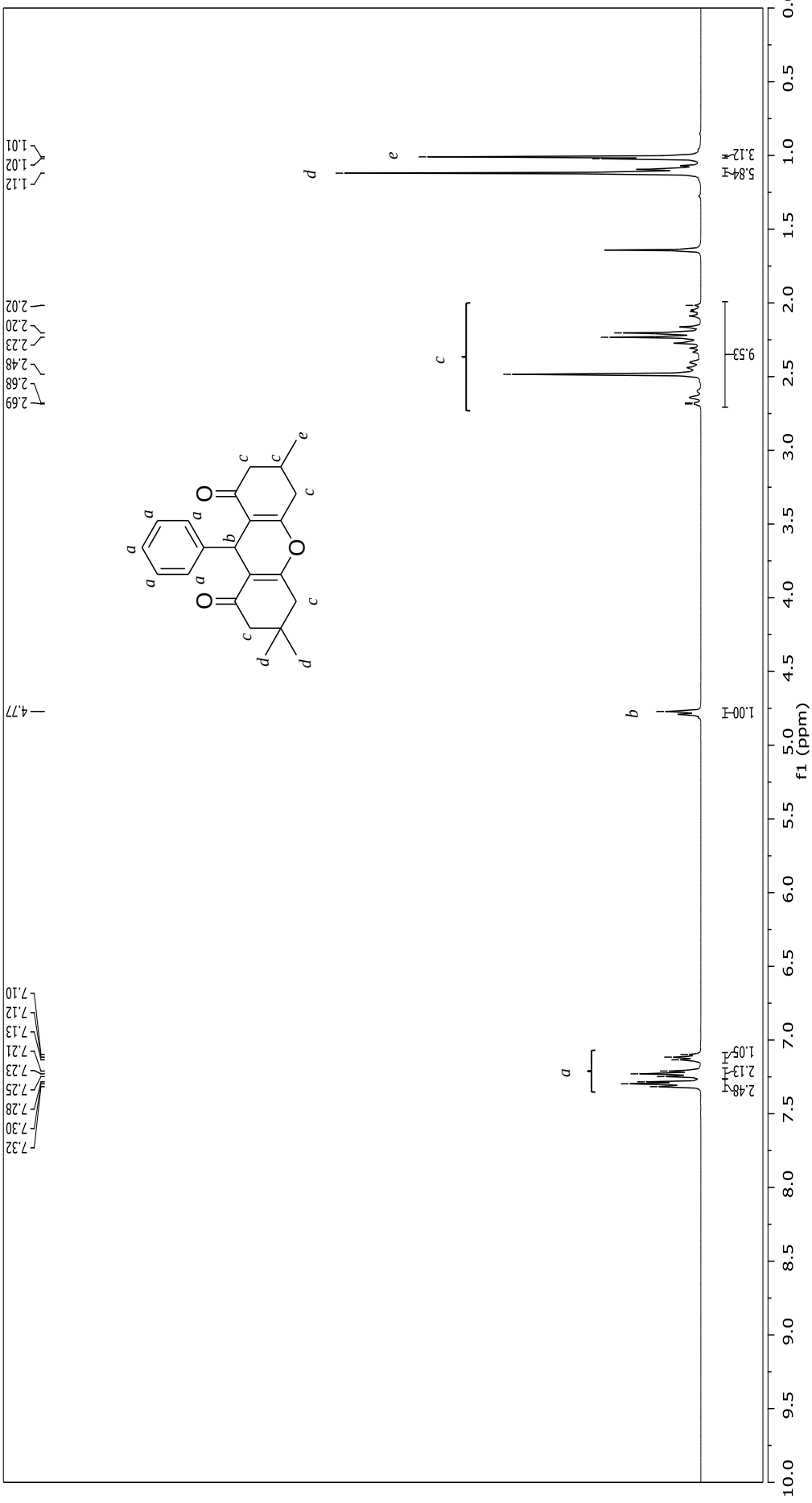
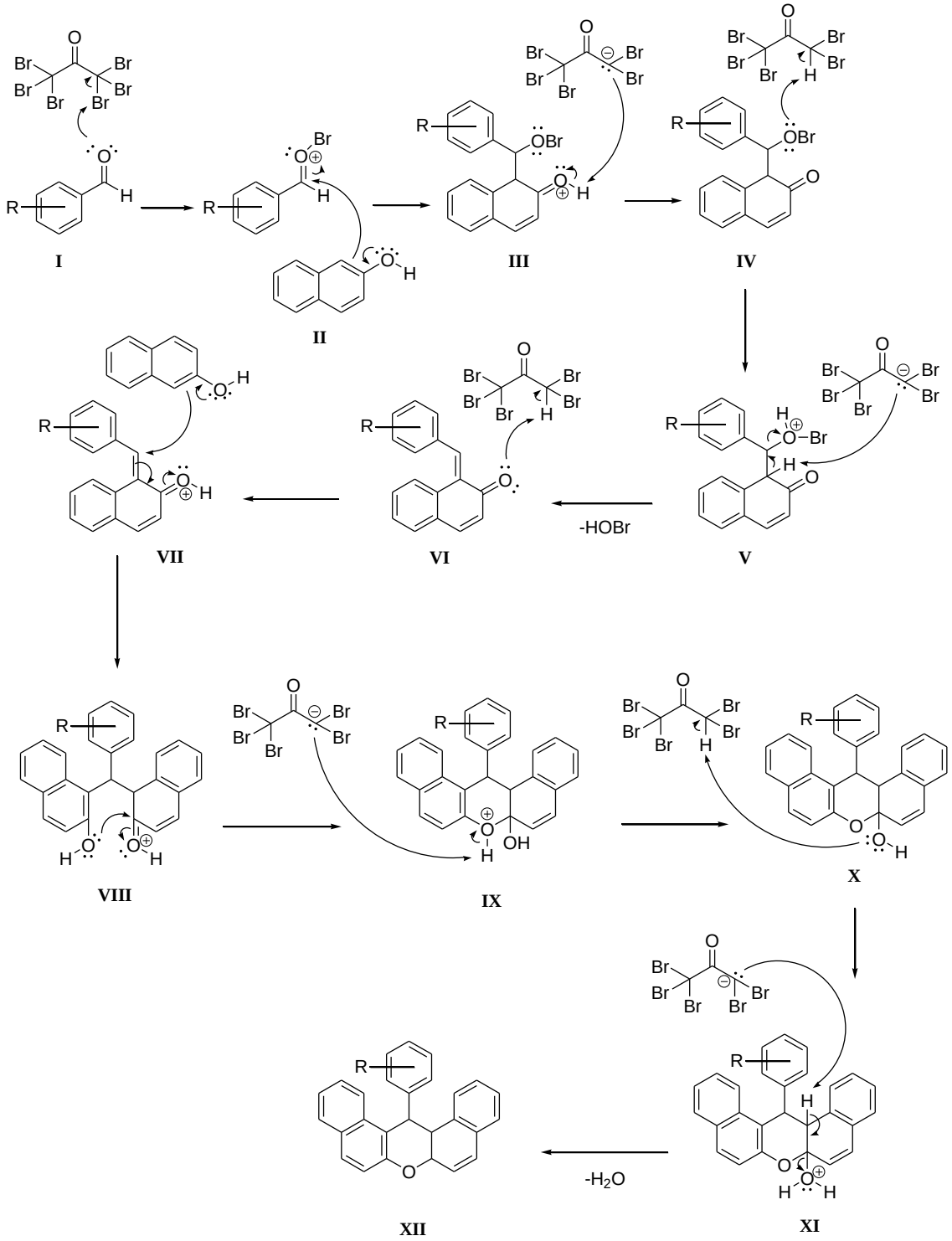


Figure 3.8 The ^{1}H NMR spectrum of 9-phenyl-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (**9a**)

3.3.8 Proposed Reaction Mechanism for the Synthesis of Xanthene Derivatives

A plausible reaction mechanism for the synthesis of 14-(aryl)-14*H*-dibenzo[*a*,*j*]-xanthenes, as a representative case, is shown in Scheme 3.2. The $Br_3CCOCBr_3$ can induce the initiation of nucleophilic attack by β-naphthol (**II**) toward activated carbonyl carbon of aromatic aldehyde providing the intermediate **III**. The process will then release hydroxidobromine (HOBr) to lead the formation of intermediate **VI** and subsequently induce nucleophilic attack of **VII** by another β-naphthol to give **VIII**. Afterward, **IX** is formed by ring closure of **VIII** and dehydration of this intermediate gives the desired product **XII**. A similar mechanism can be expected by replacing β-naphthol with 5,5-dimethyl-1,3-cyclohexanedione or 5-methyl-1,3-cyclohexanedione.



Scheme 3.2 Proposed reaction mechanism for the synthesis of 14-(aryl)-14*H*-dibenzo[*a*,*j*]-xanthenes

CHAPTER 4 CONCLUSION

4.1 Conclusion

1. This research found that the leaves extracts of *C. fruticosa* Back, collected from East Kalimantan Province, Indonesia, exhibited the potent antioxidant activity on EtOAc and hexane extracts, but were low antibacterial activity using MeOH extract.
2. This synthesis of xanthene derivatives using hexabromoacetone demonstrated a novel and efficient method for the synthesis of xanthene derivatives under solvent-free conditions. A variety of aromatic aldehydes reacted successfully with β-naphthol, 5,5-dimethyl-1,3-cyclohexanedione or 5-methyl-1,3-cyclohexanedione to afford the desired products in excellent yields at short reaction times.

4.2 Suggestion of Further Work

1. The further study about chemical constituents of leaves extracts of *C. fruticosa* Back is highly recommended to identify the specific compound which responsible to biological activities of *C. fruticosa* Back.
2. The utilization of $Br_3CCOCBr_3$, as a novel synthesis method, would open the new opportunity to mediate the other organic syntheses under solvent-free conditions. The other developed methods using halogenated reagents for the synthesis of xanthene derivatives would be interesting to be established.

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APPENDIX A

Experimental Data of Antioxidant Activity of Leaves Extracts from *Cordyline fruticosa* Back

Table A.1 Inhibition activity of DPPH scavenging assay of MeOH extract

		Absorbance of Sample (mg/mL)							
	Control	1	1.5	2	3	4	5	6	7
Rep. I	0.3997	0.3306	0.2906	0.2651	0.2171	0.1826	0.1525	0.1215	0.0999
Rep II	0.3898	0.3200	0.2968	0.2610	0.2314	0.1903	0.1511	0.1154	0.1032
Rep III	0.3961	0.3332	0.2935	0.2656	0.2306	0.1866	0.1550	0.1174	-
Average	0.3952	0.3279	0.2936	0.2639	0.2264	0.1865	0.1529	0.1181	0.1016
% inhibition		17.02	25.70	33.22	42.72	52.81	61.32	70.12	74.30

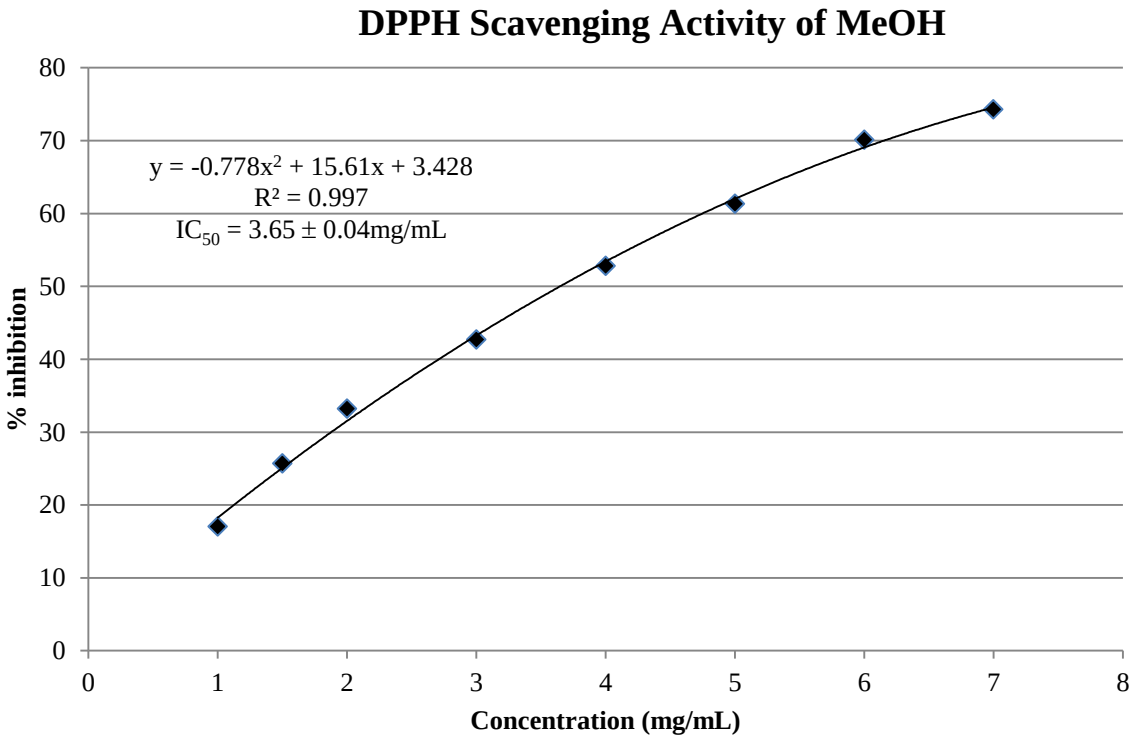

Figure A.1 DPPH scavenging activity graph of MeOH extract

Table A.2 Inhibition activity of DPPH scavenging assay of EtOAc extract

		Absorbance of Sample (mg/mL)				
	Control	0.5	1	2	4	8
Rep. I	0.4664	0.3103	0.2213	0.1256	0.0865	0.0517
Rep II	0.4591	0.3097	0.2264	0.1344	0.0700	0.0528
Rep III	0.4544	0.3117	0.2275	0.1191	0.0645	0.0526
Average	0.4600	0.3106	0.2251	0.1264	0.0737	0.0524
% inhibition		32.48 %	51.07 %	72.53 %	83.98 %	88.62 %

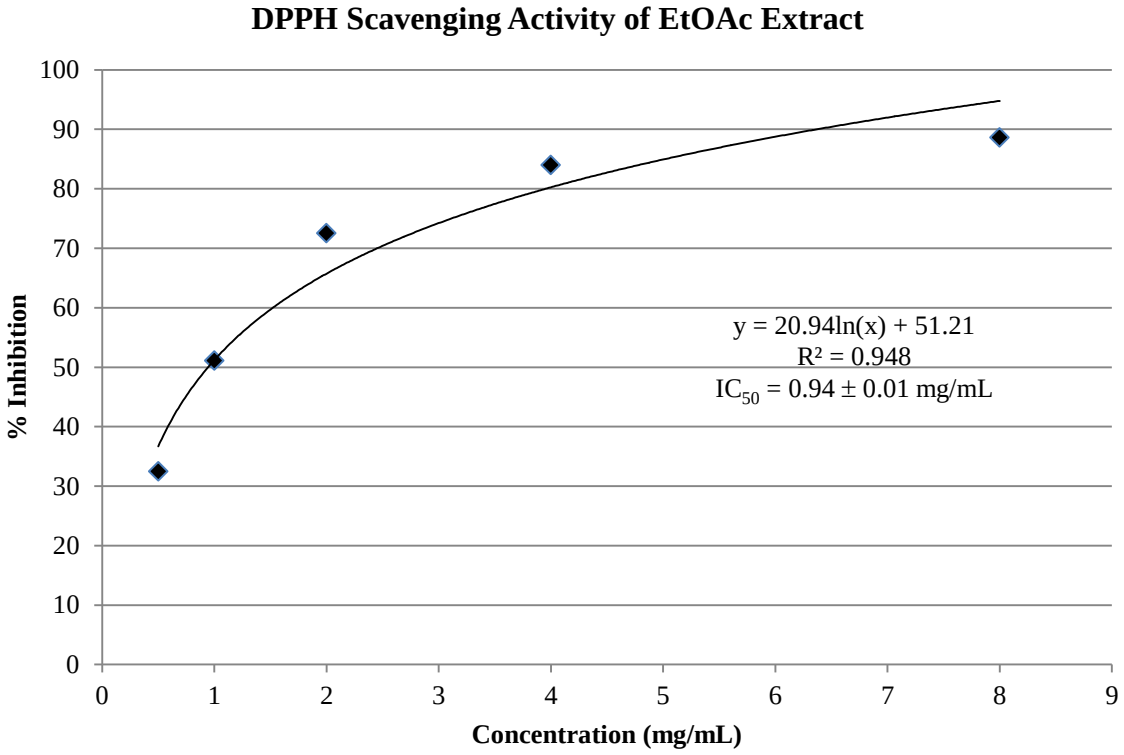

Figure A.2 DPPH scavenging activity graph of EtOAc extract

Table A.3 Inhibition activity of DPPH scavenging assay of hexane extract

		Absorbance of Sample (mg/mL)				
	Control	2	4	8	12	16
Rep. I	0.3705	0.3142	0.2677	0.1994	0.1354	0.1275
Rep II	0.3675	0.3184	0.2724	0.1843	0.1408	0.1250
Rep III	0.3765	0.3165	0.2801	0.1809	0.1387	0.1233
Average	0.3715	0.3164	0.2734	0.1882	0.1383	0.1253
% inhibition		14.83 %	26.41 %	49.34 %	62.77 %	66.27 %

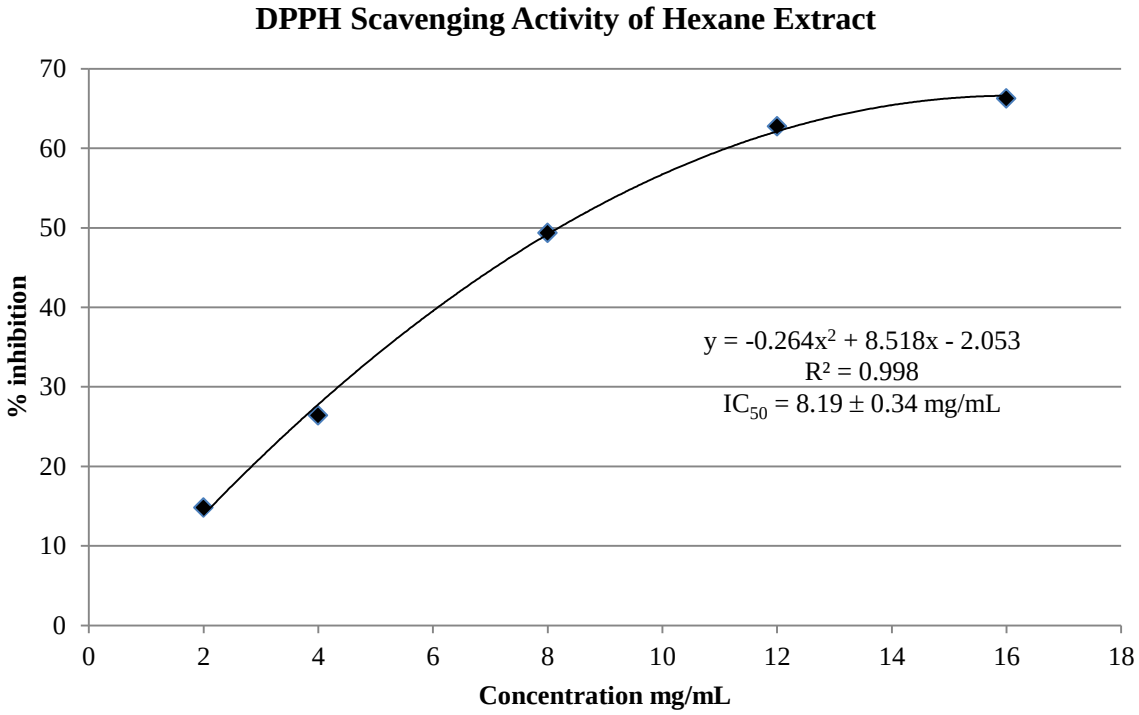

Figure A.3 DPPH scavenging activity graph of hexane extract

Table A.4 Inhibition activity of DPPH scavenging assay of ascorbic acid

		Absorbance of Sample (mg/mL)				
	Control	0.01	0.05	0.1	0.15	0.2
Rep. I	0.3921	0.3647	0.2365	0.0645	0.0189	0.0218
Rep II	0.3946	0.3591	0.2313	0.0651	0.0208	0.0178
Rep III	0.3874	0.3644	0.2309	0.0650	0.0230	0.0194
Average	0.3914	0.3627	0.2329	0.0649	0.0209	0.0197
% inhibition		7.32 %	40.49 %	83.43 %	94.66 %	94.97 %

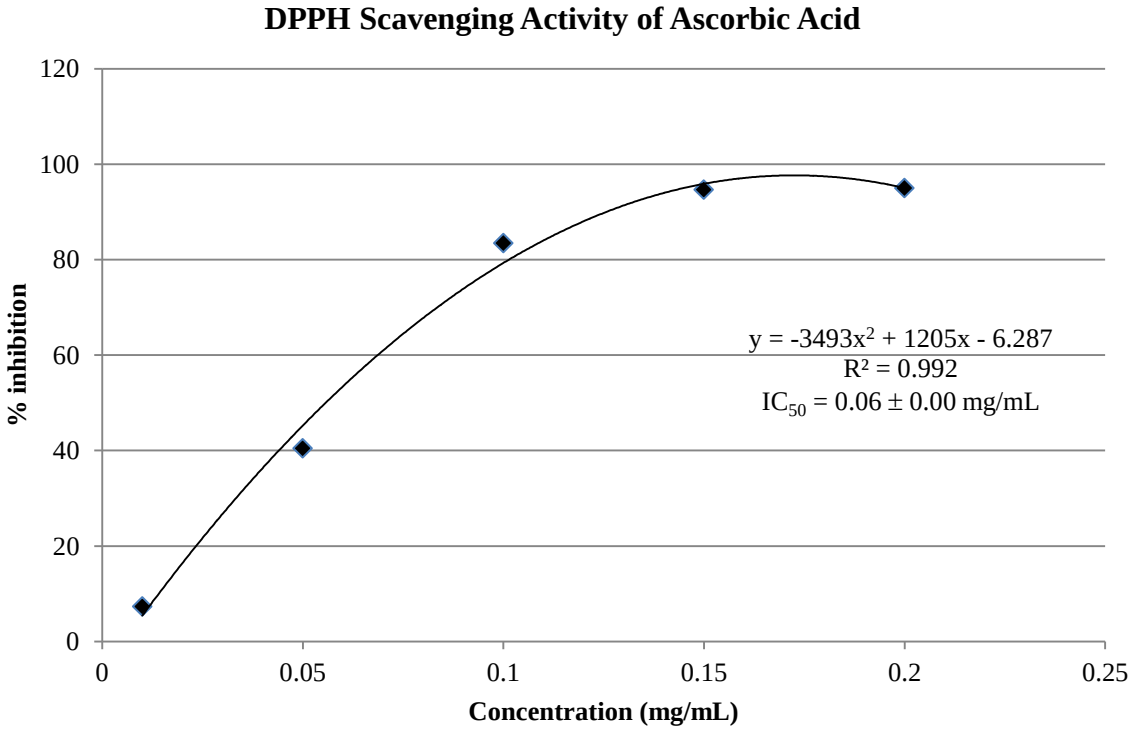

Figure A.4 DPPH scavenging activity graph of ascorbic acid

Table A.5 Reducing activity of leaves extracts of *Cordyline fruticosa* Back

	Absorbance of Sample (mg/mL)		
	MetOH 1.0	Hexane 2.0	EtOAc 1.0
Rep I	0.6257	0.6698	0.8270
Rep II	0.6650	0.6763	0.8239
Rep III	0.6231	0.6880	0.8220
Average	0.6379	0.6780	0.8243
mmol Fe^{2+} eq/g	15.83 ± 1.96	9.58 ± 0.38	31.36 ± 0.21

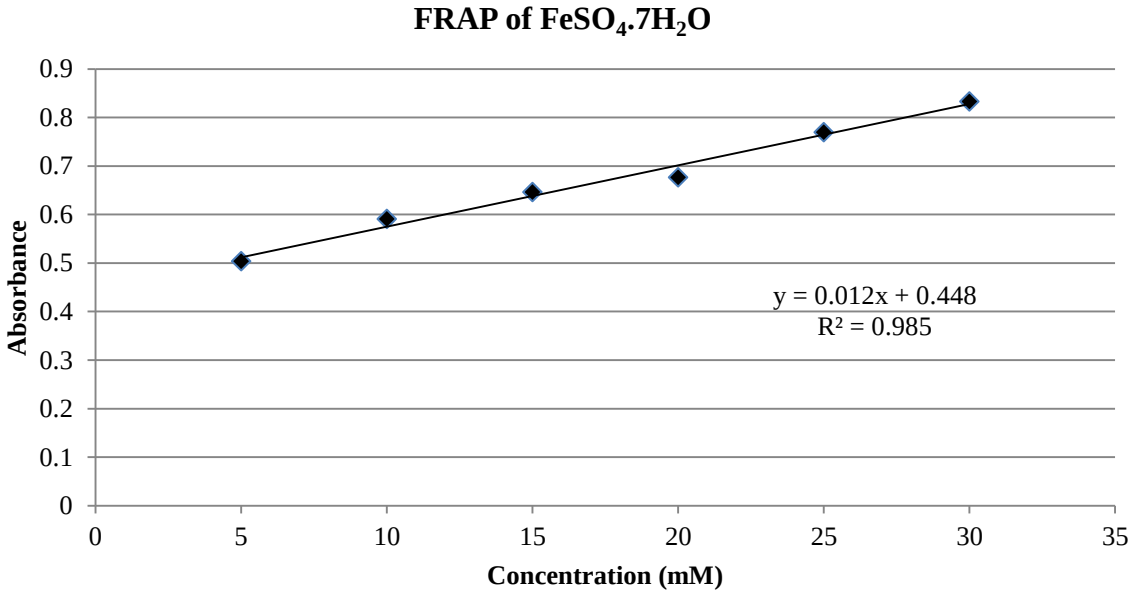

Figure A.5 Ferric reducing activity graph of $FeSO_4.7H_2O$

Table A.10 Total phenolic content of leaves extracts of *Cordyline fruticosa* Back

	Absorbance of Sample (g/mL)		
	MetOH 0.0025	Hexane 0.01	EtOAc 0.01
Rep I	0.5961	1.1174	1.0544
Rep II	0.5986	1.1003	1.0559
Rep III	0.6251	1.0828	1.0928
Average	0.6066	1.1002	1.0677
mg GAE/g	41.91 ± 1.32	24.62 ± 0.84	23.16 ± 0.94

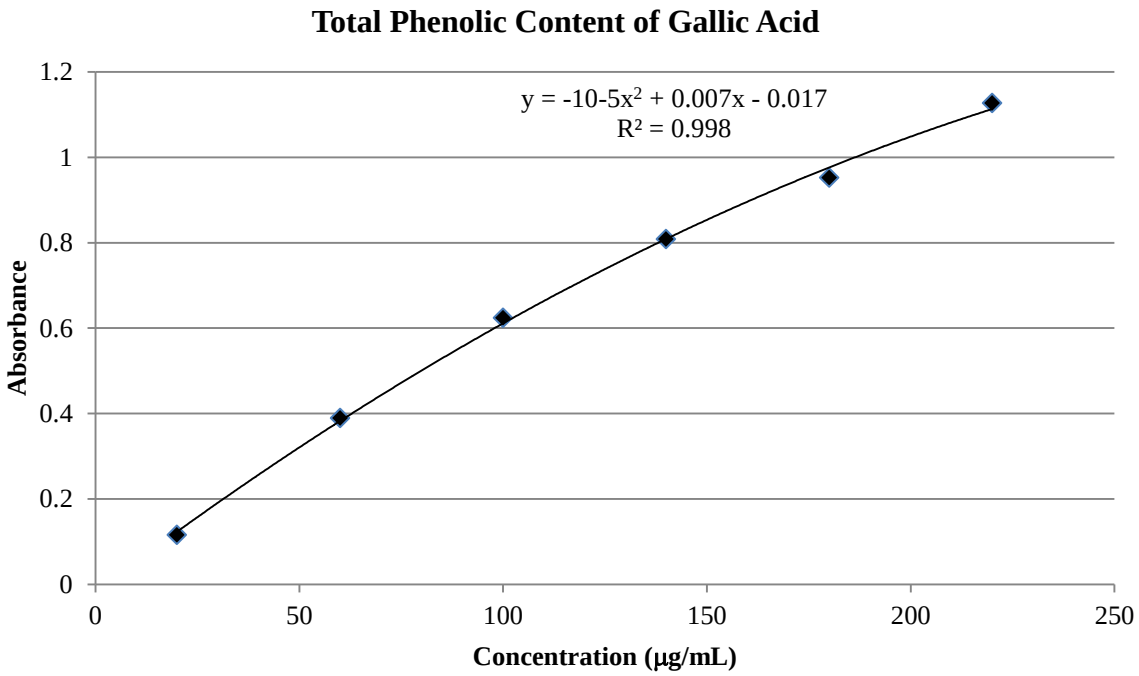

Figure A.10 Total phenolic content graph of gallic acid

APPENDIX B

The ^{1}H NMR Spectrum of New Compounds of Xanthene Derivatives

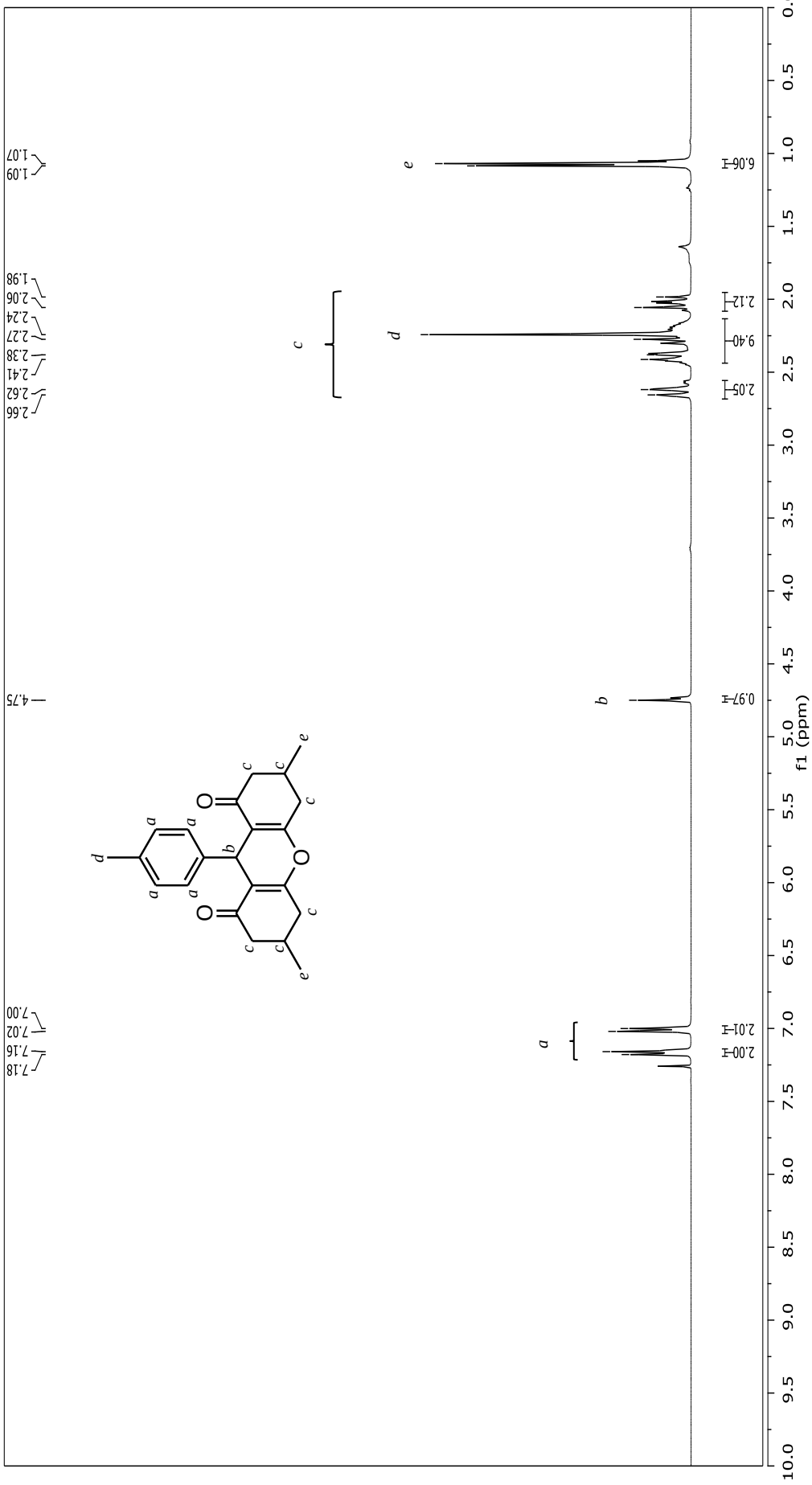

Figure B.1 The ^{1}H NMR spectrum of 9-(4-methylphenyl)-3,6-dimethyl-1,8-dioxo-octahydroxanthene (**6c**)

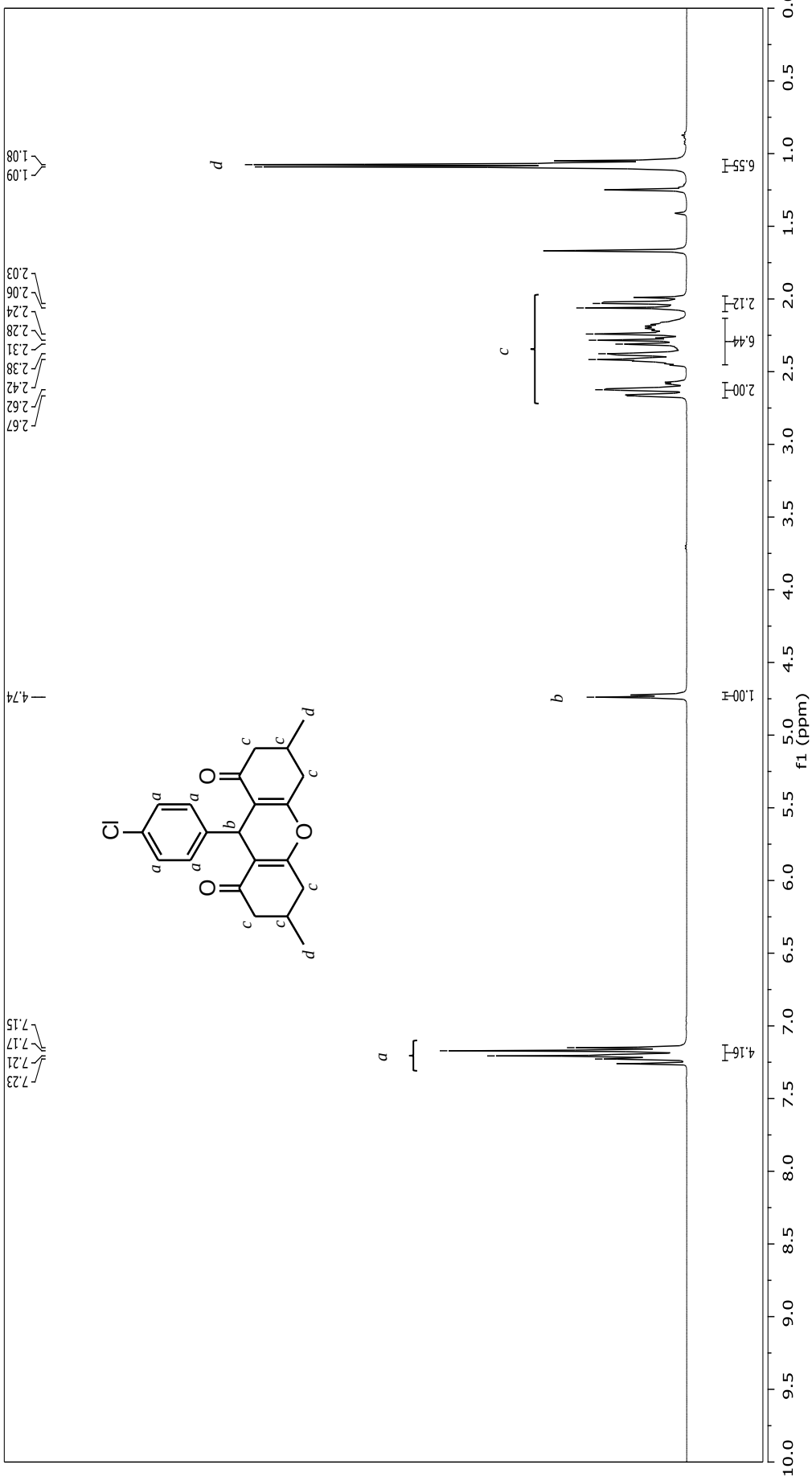

Figure B.2 The ^{1}H NMR spectrum of 9-(4-chlorophenyl)-3,6-dimethyl-1,8-dioxo-octahydroxanthene (**6d**)

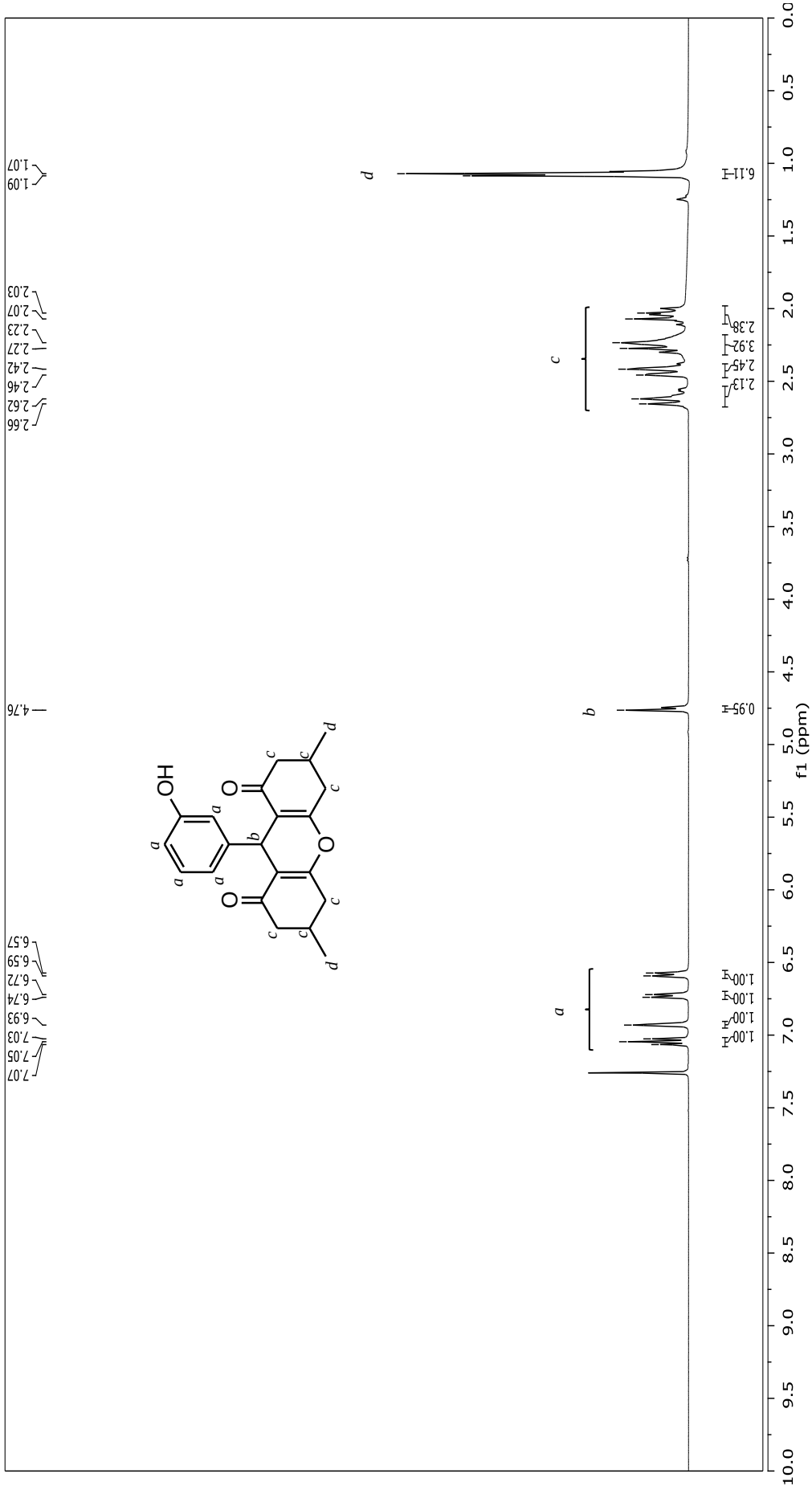

Figure B.3 The ^{1}H NMR spectrum of 9-(3-hydroxyphenyl)-3,6-dimethyl-1,8-dioxo-octahydroxanthene (**6e**)

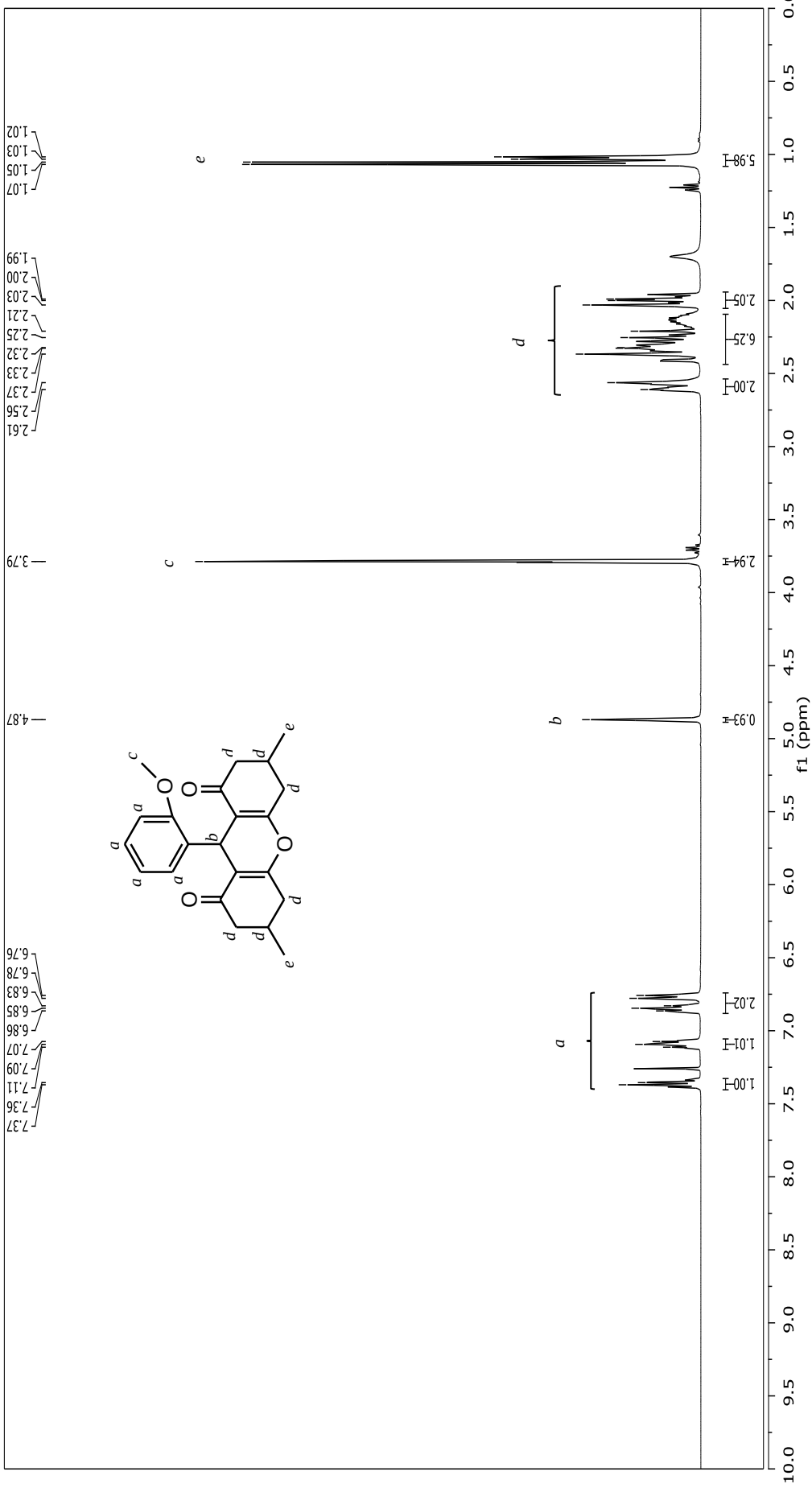

Figure B.4 The ^{1}H NMR spectrum of 9-(2-methoxyphenyl)-3,6-dimethyl-1,8-dioxo-octahydroxanthene (**6g**)

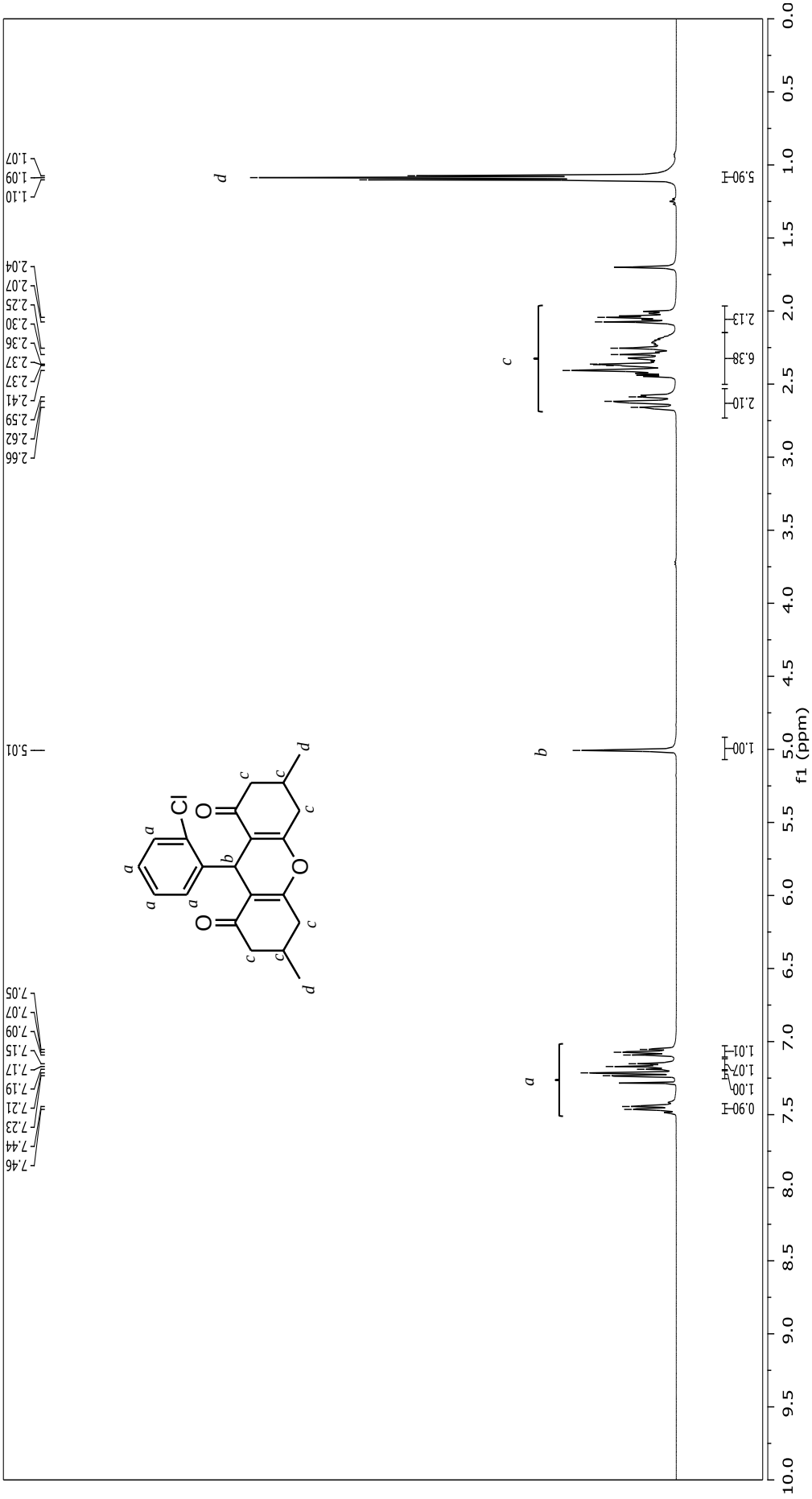

Figure B.5 The ^{1}H NMR spectrum of 9-(2-chlorophenyl)-3,6-dimethyl-1,8-dioxo-octahydroxanthene (**6h**)

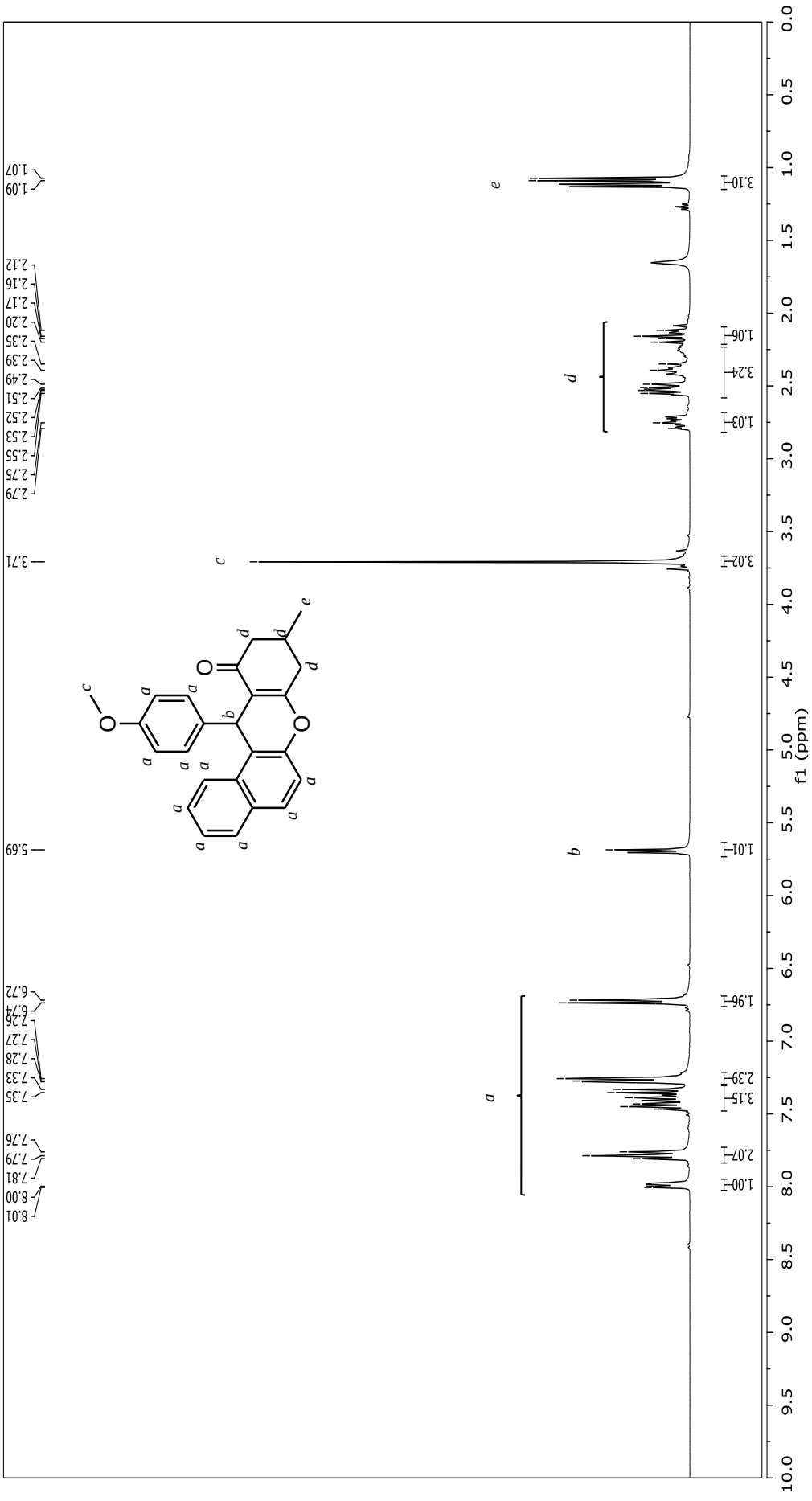

Figure B.6 The ^{1}H NMR spectrum of 12-(4-methoxyphenyl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (**8b**)

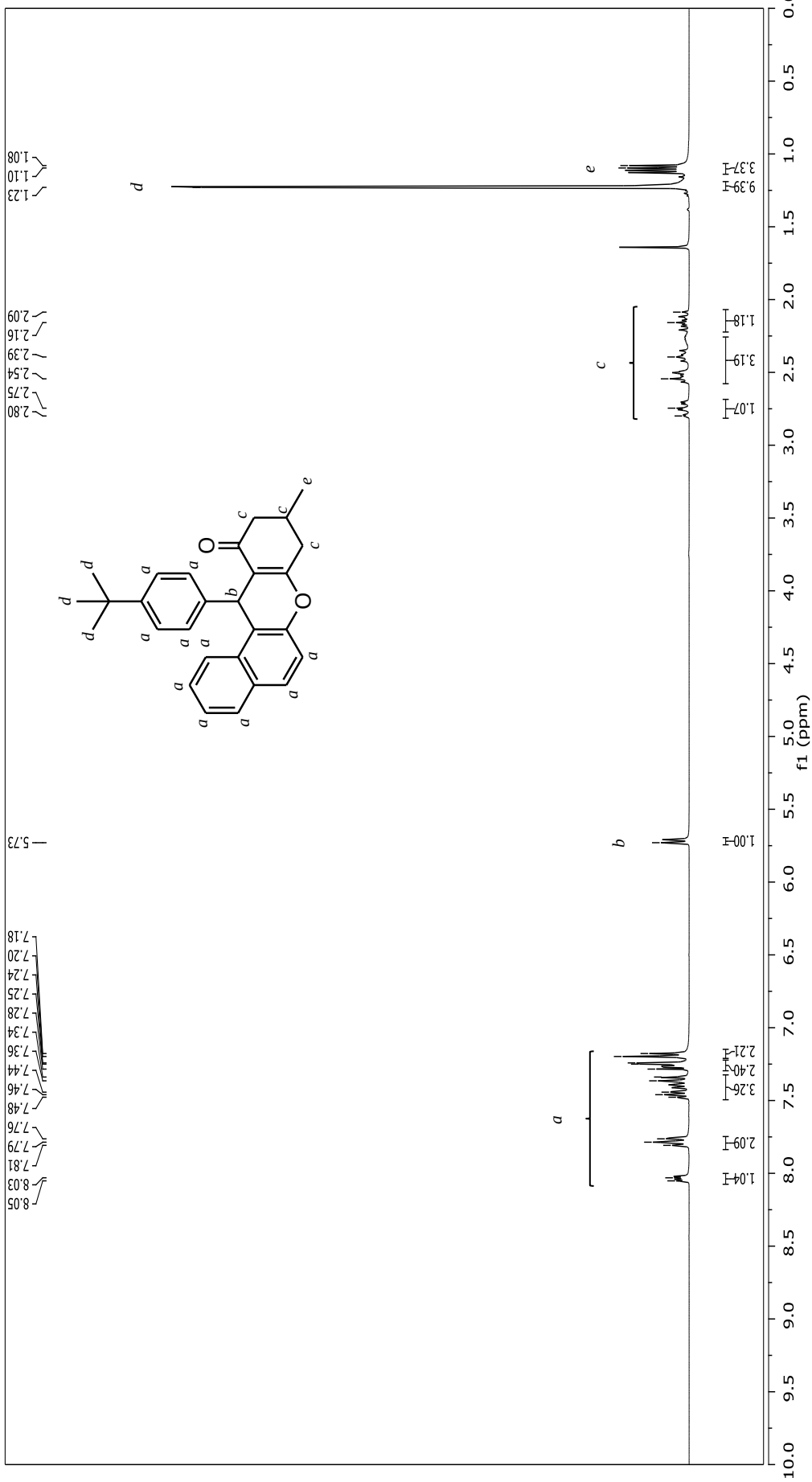

Figure B.7 The ^{1}H NMR spectrum of 12-(4-*tert*-butylphenyl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (**8c**)

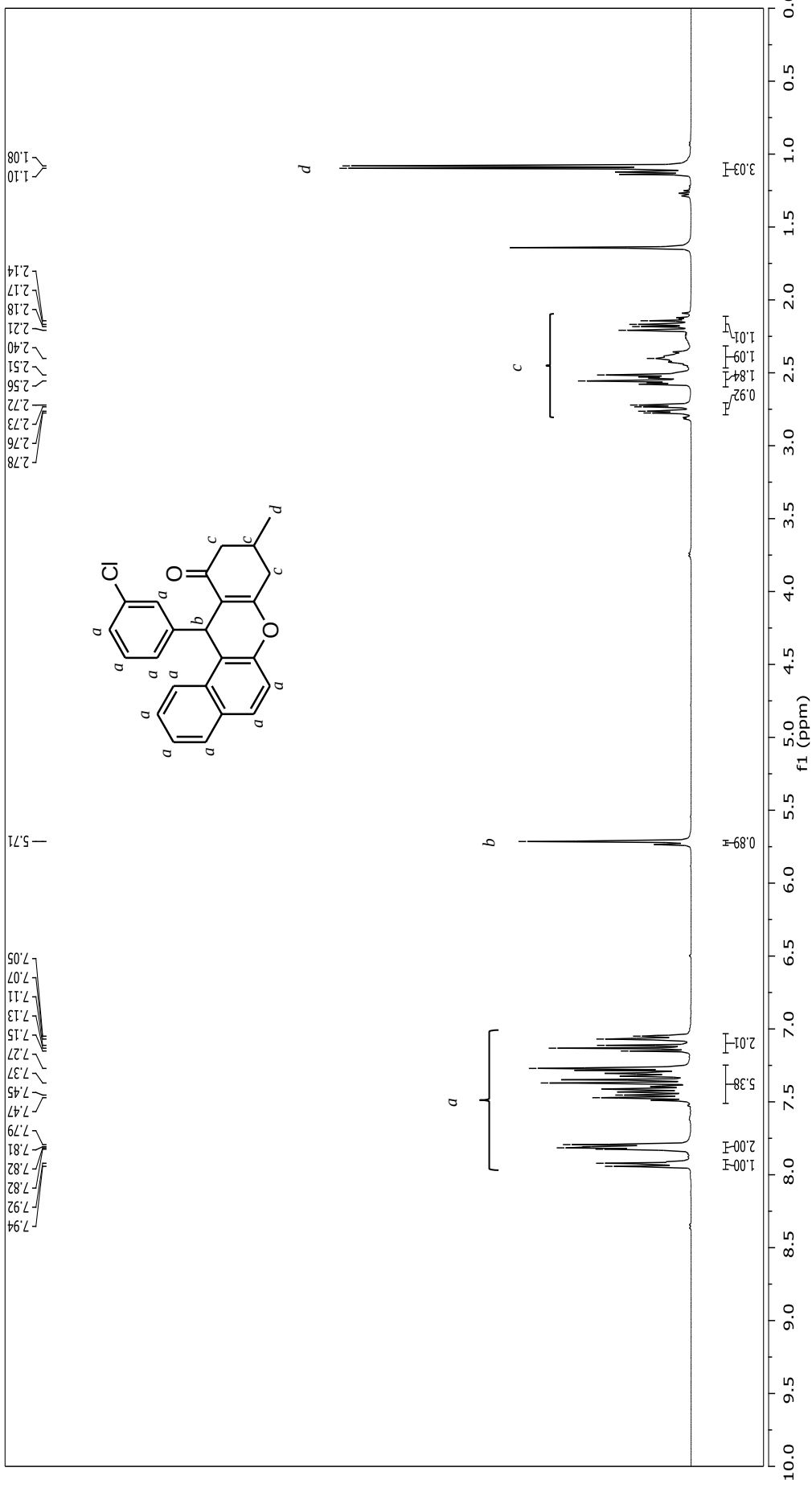

Figure B.8 The ^{1}H NMR spectrum of 12-(3-chlorophenyl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (**8e**)

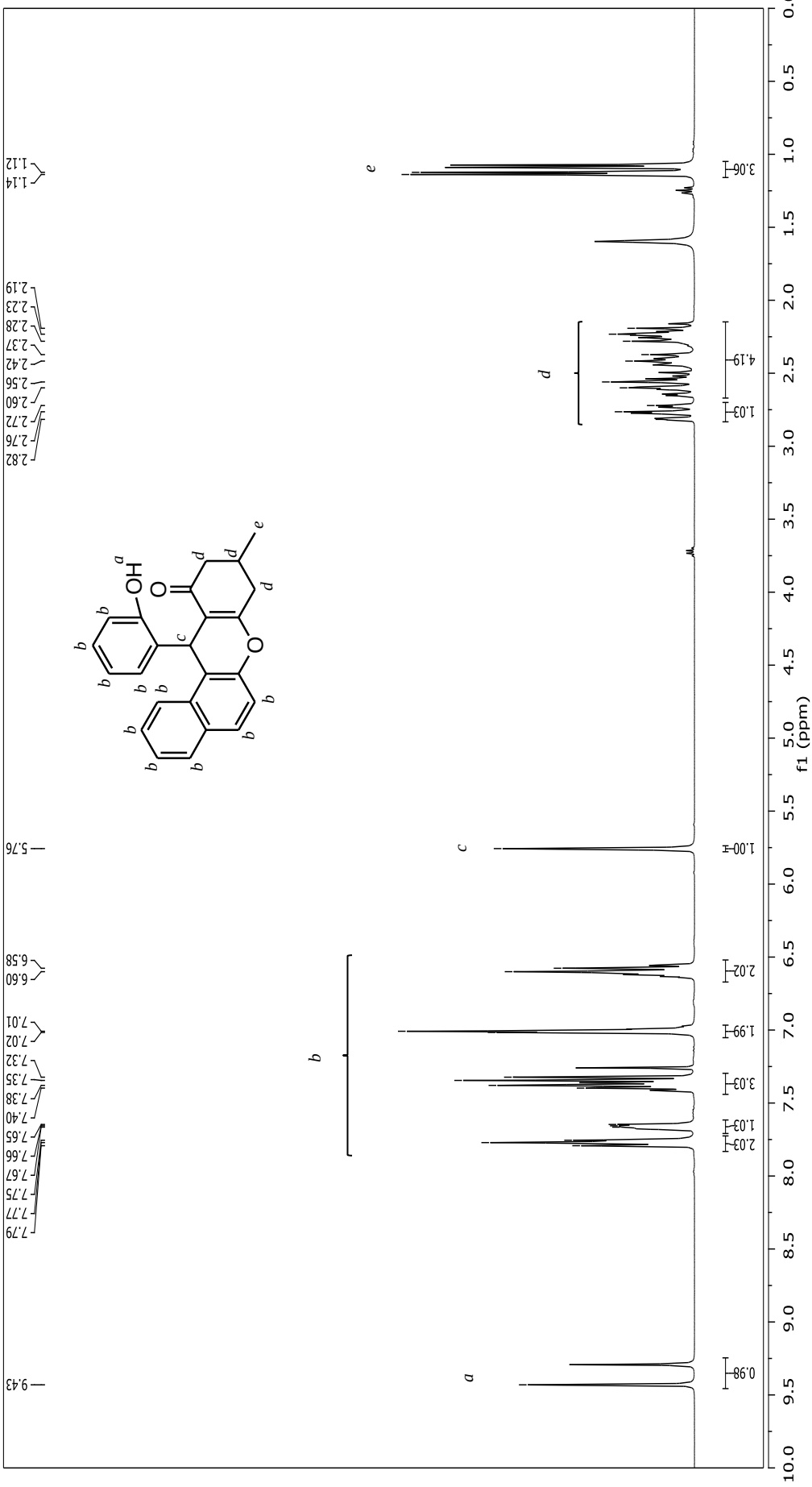

Figure B.9 The ^{1}H NMR spectrum of 12-(2-hydroxyphenyl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (**8f**)

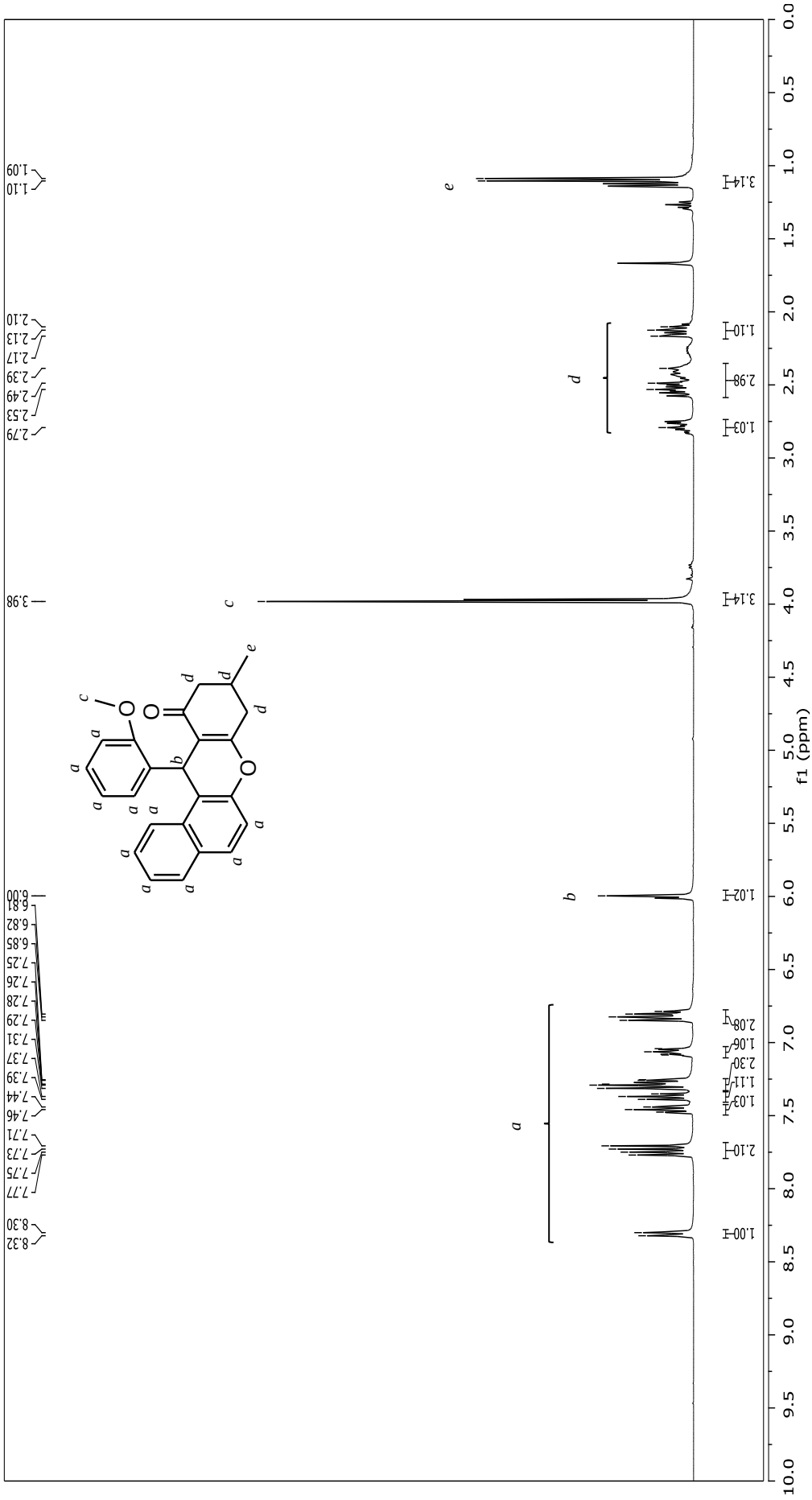

Figure B.10 The ^{1}H NMR spectrum of 12-(2-methoxyphenyl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (**8g**)

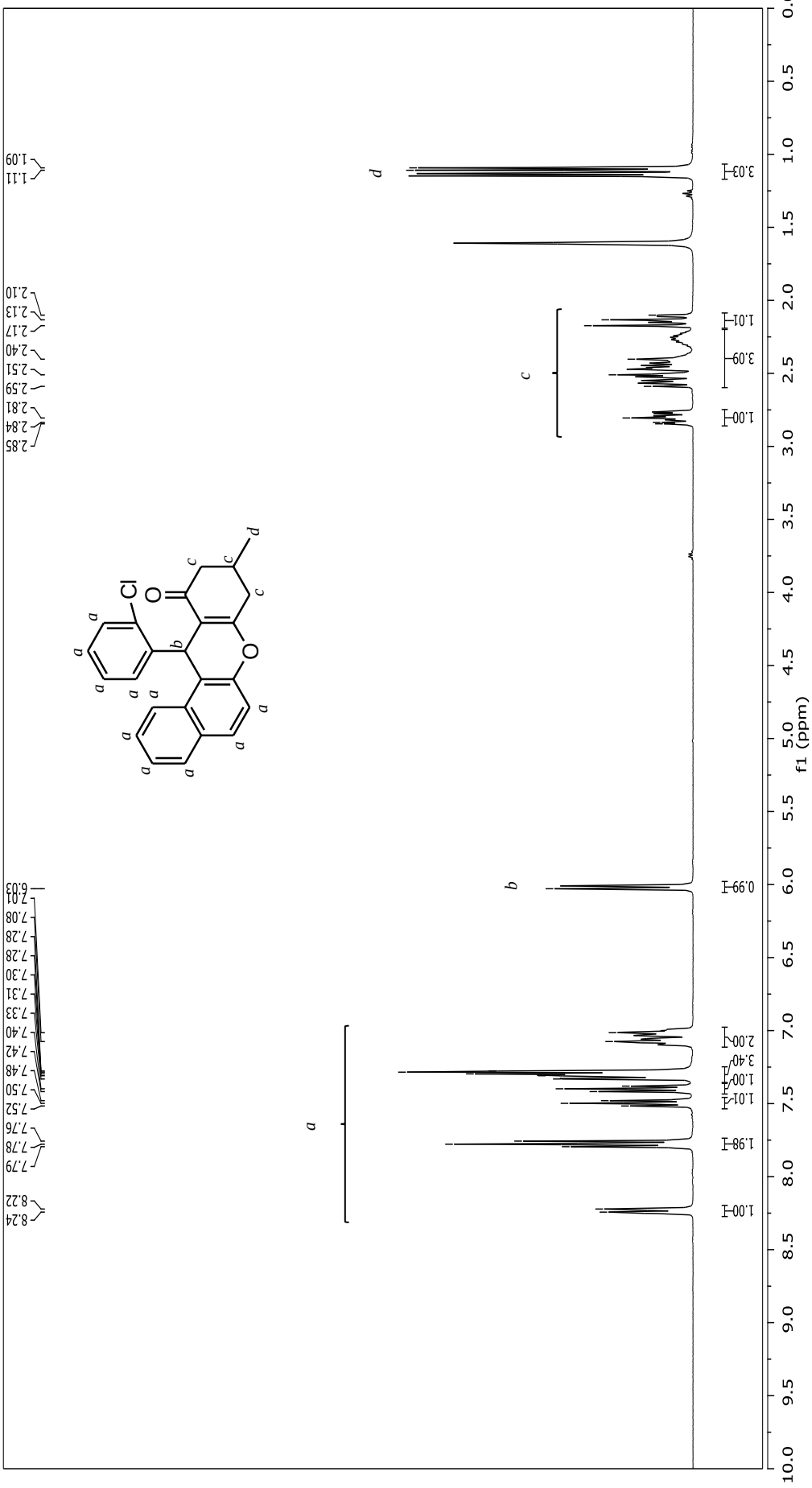

Figure B.11 The ^{1}H NMR spectrum of 12-(2-chlorophenyl)-9-methyl-8,9,10,12-tetrahydrobenzo[*a*]xanthene-11-one (**8h**)

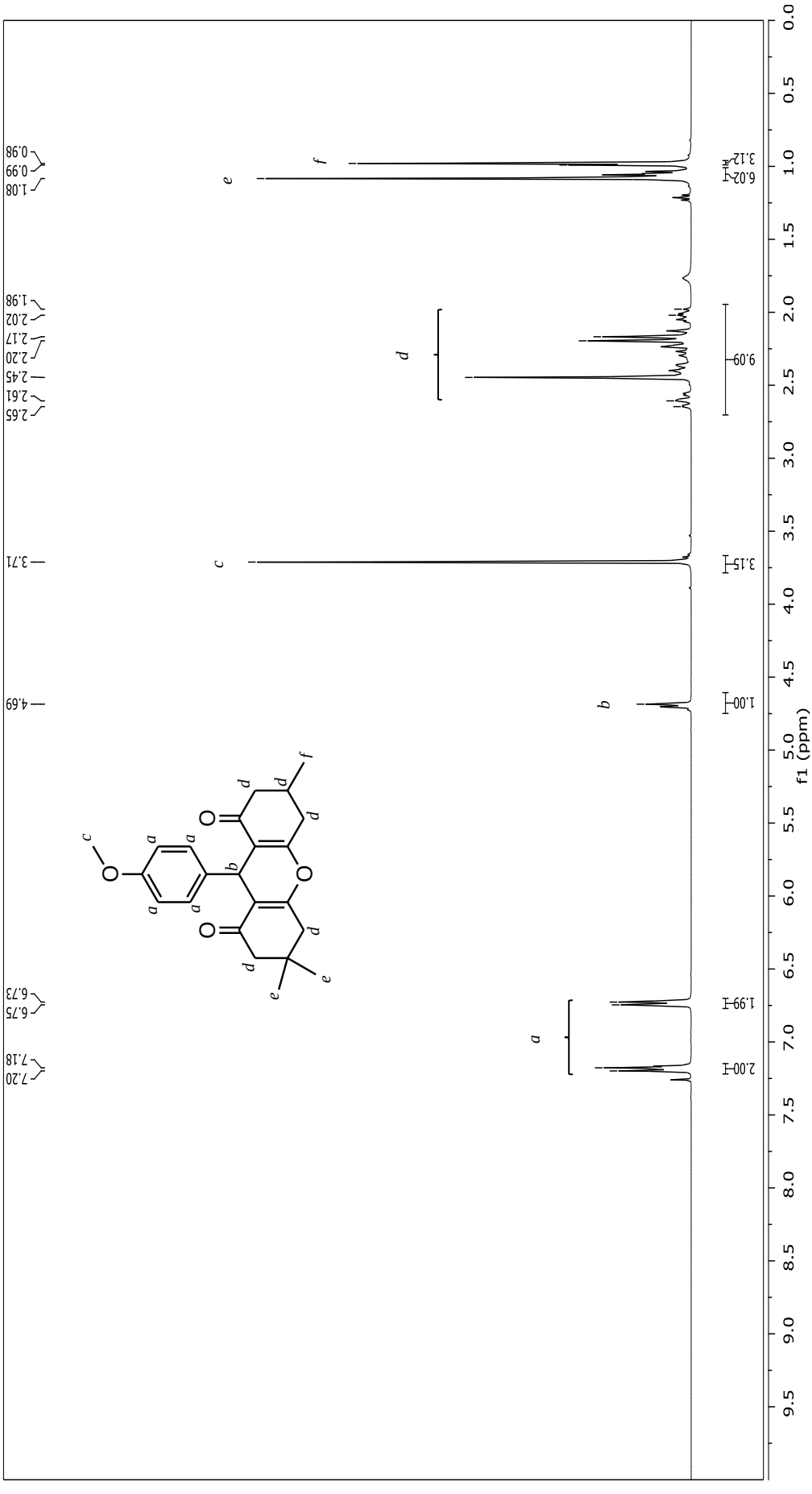

Figure B.12 The ^{1}H NMR spectrum of 9-(4-methoxyphenyl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (**9b**)

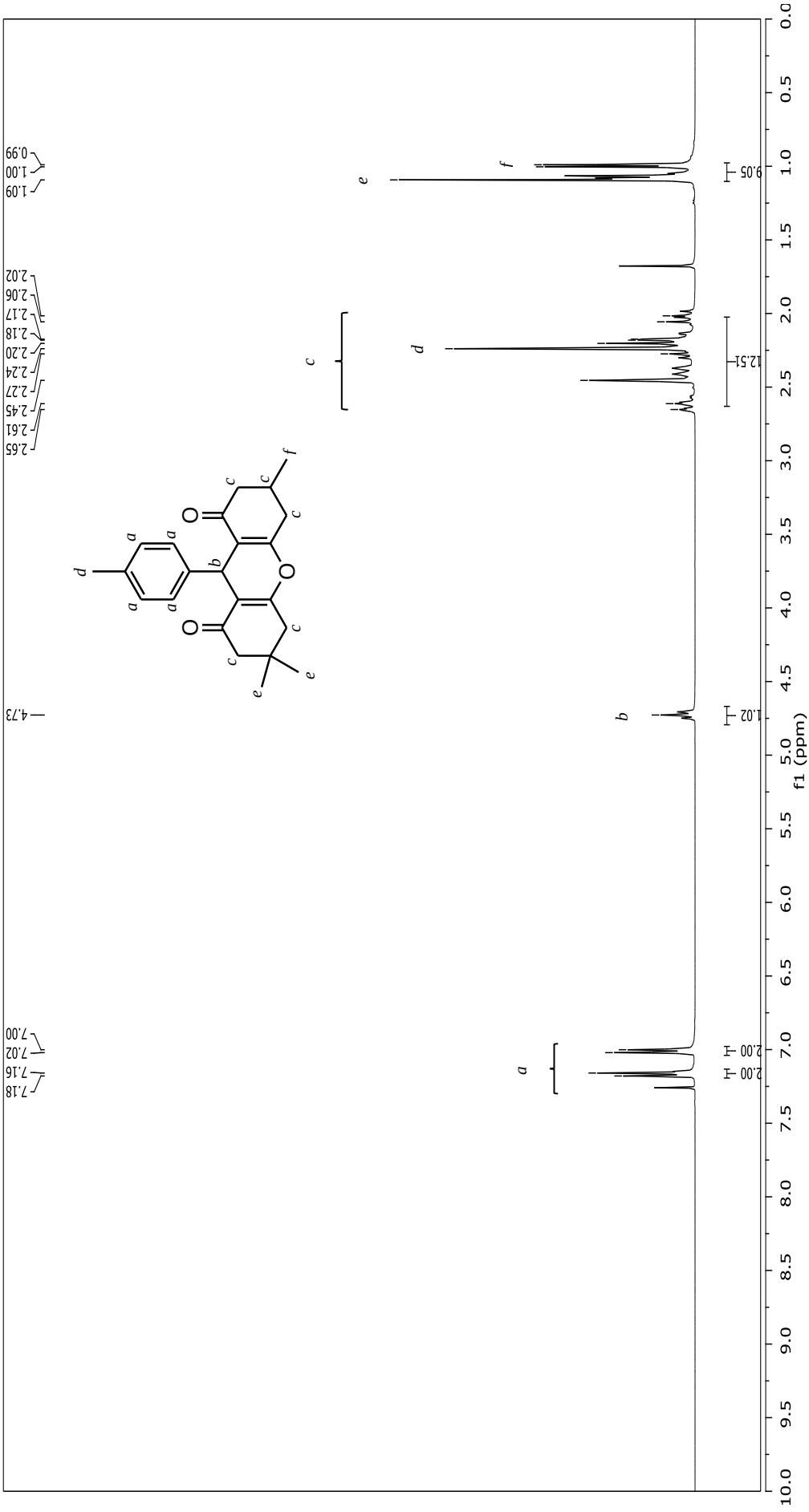

Figure B.13 The ^{1}H NMR spectrum of 9-(4-methylphenyl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (**9c**)

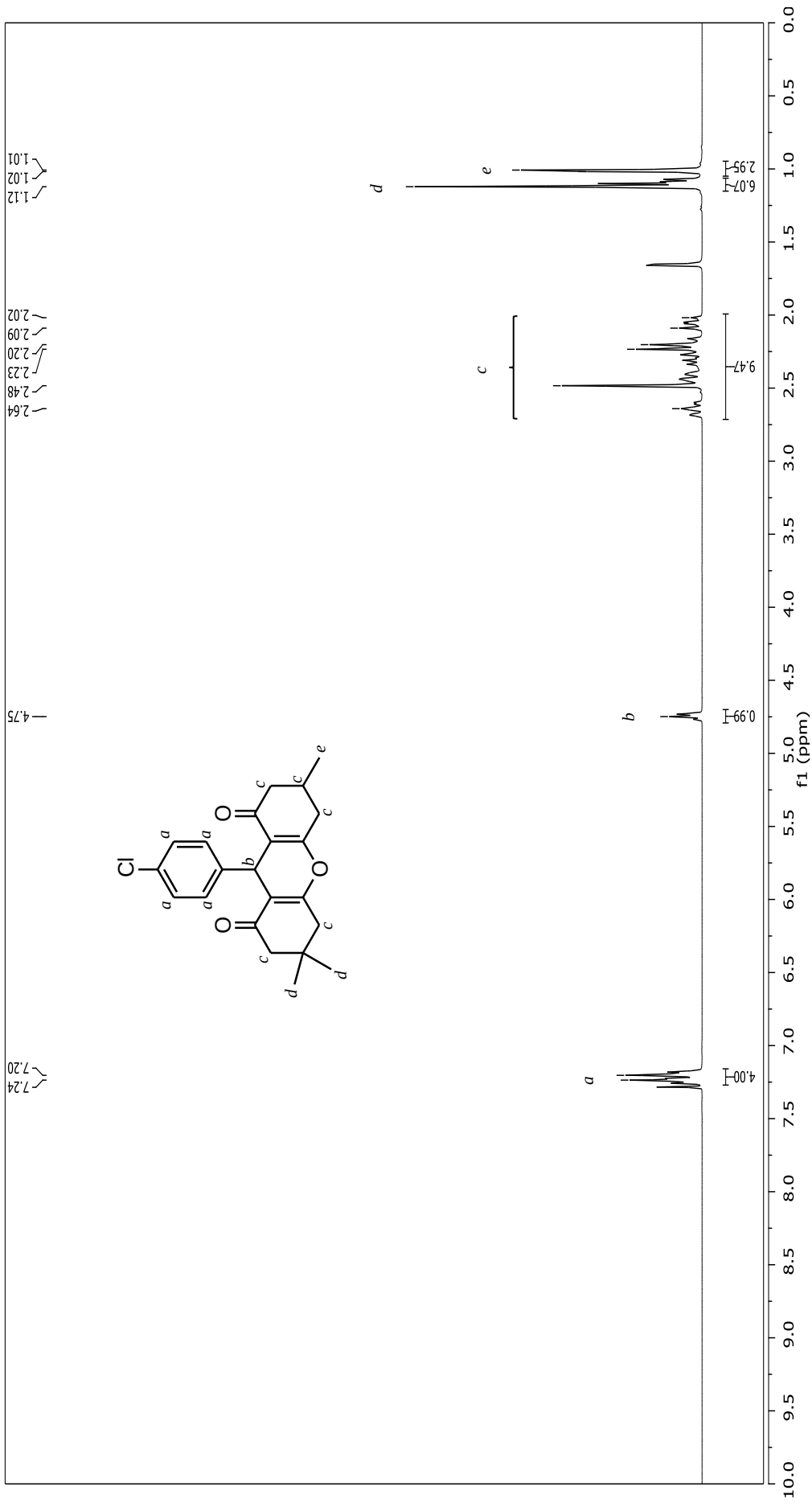

Figure B.14 The ^{1}H NMR spectrum of 9-(4-chlorophenyl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (**9d**)

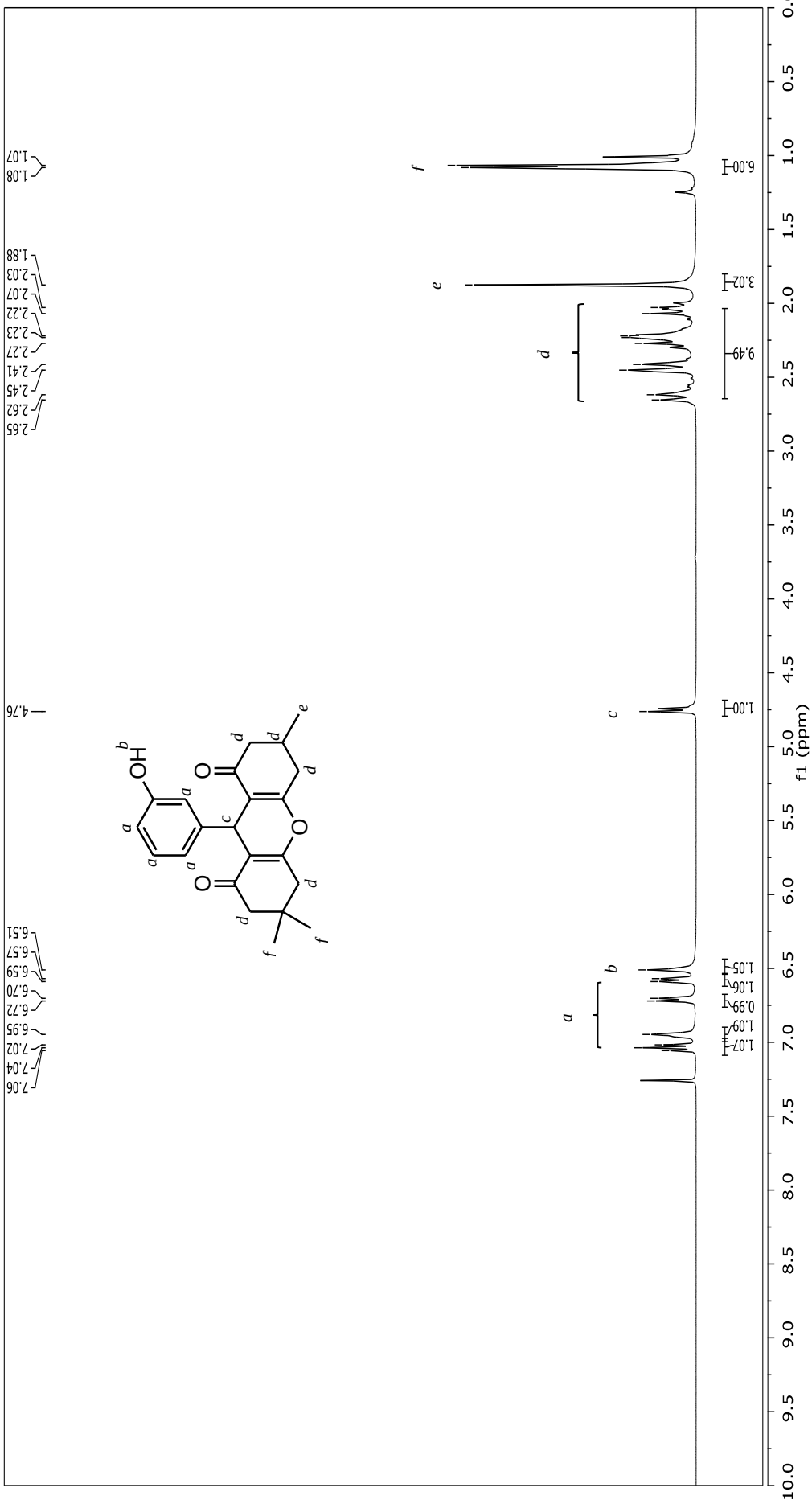

Figure B.15 The ^{1}H NMR spectrum of 9-(3-hydroxyphenyl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (**9e**)

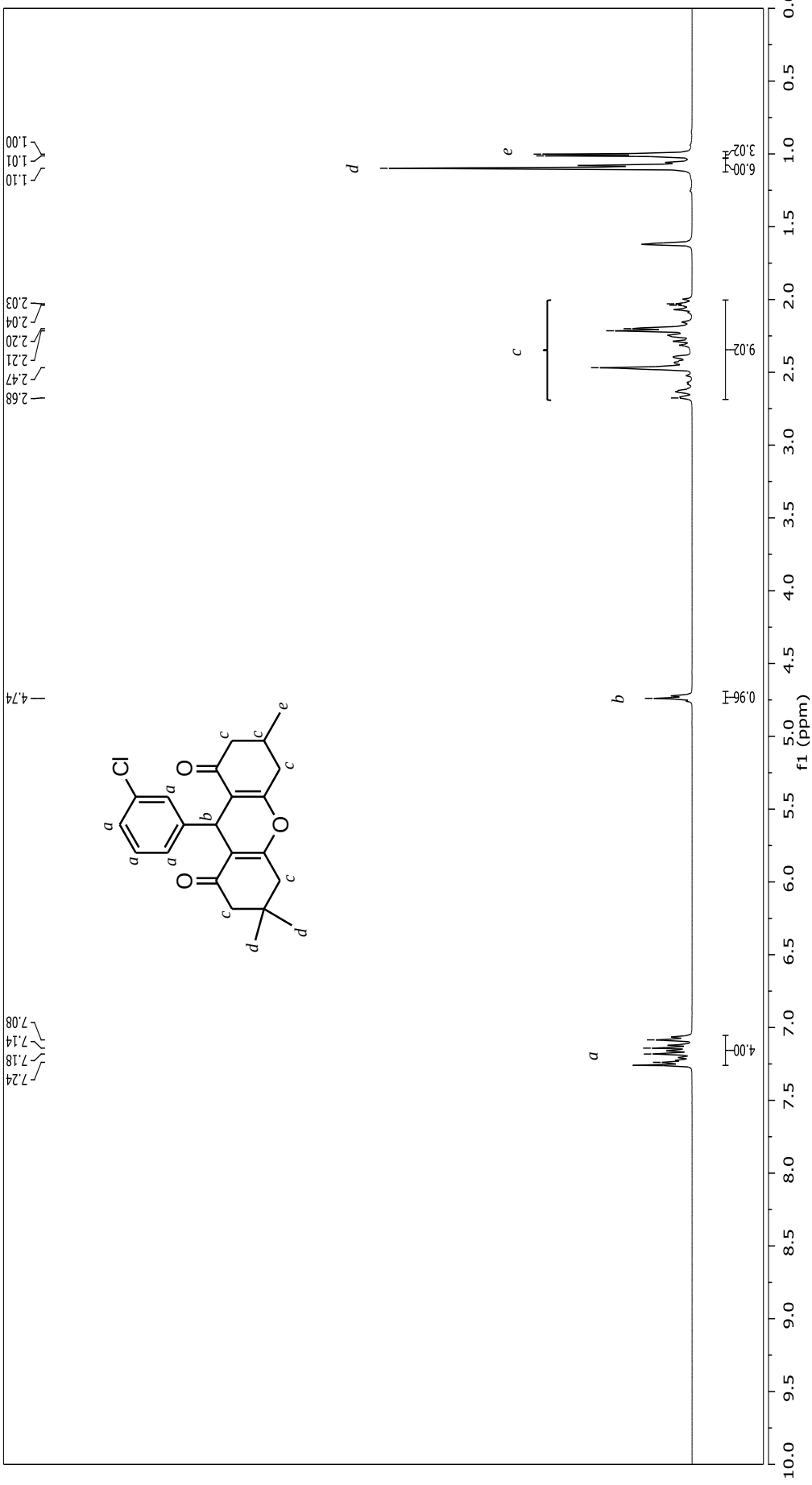

Figure B.16 The ^{1}H NMR spectrum of 9-(3-chlorophenyl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (**9f**)

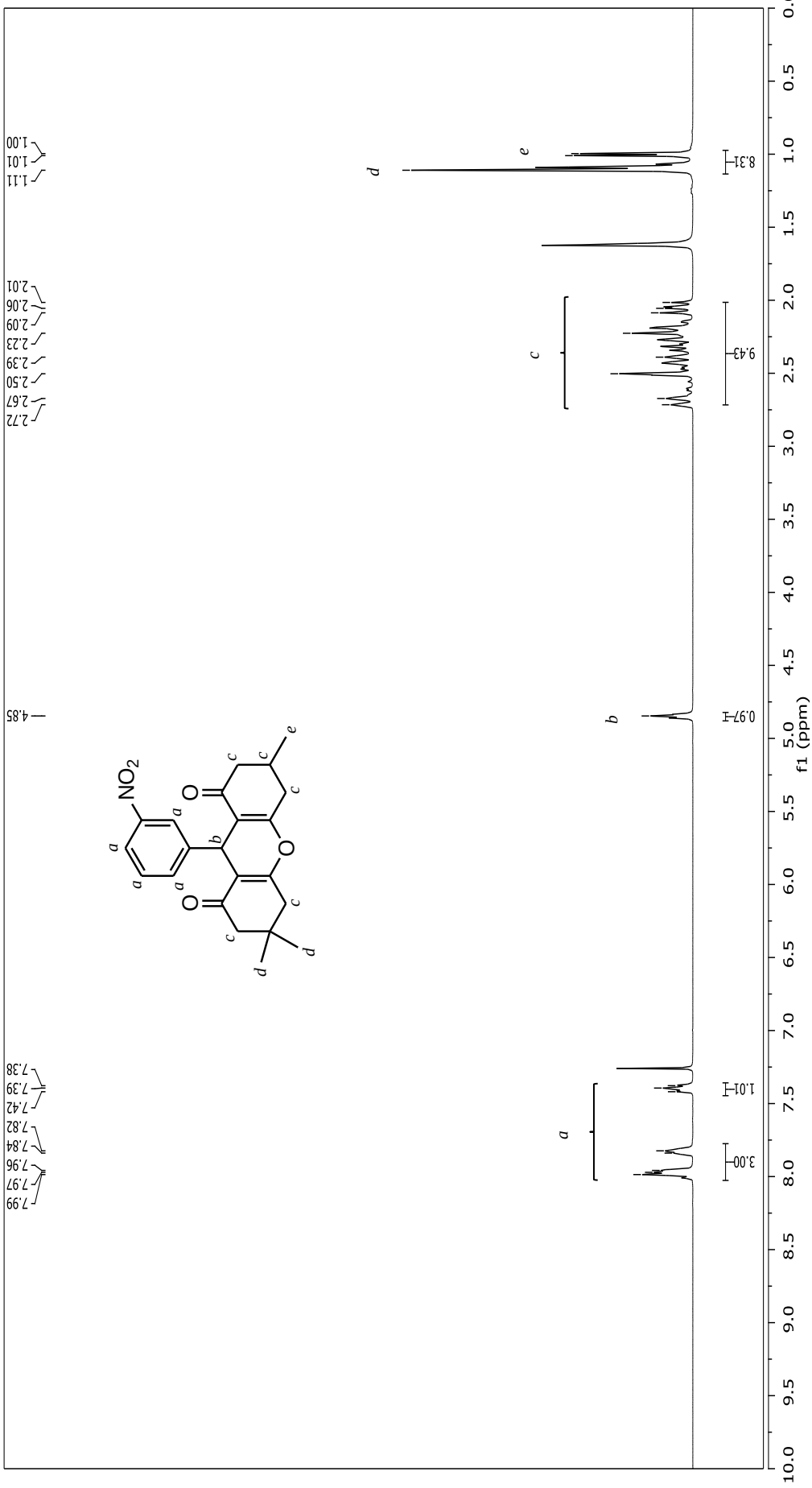

Figure B.17 The ^{1}H NMR spectrum of 9-(3-nitrophenyl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (**9g**)

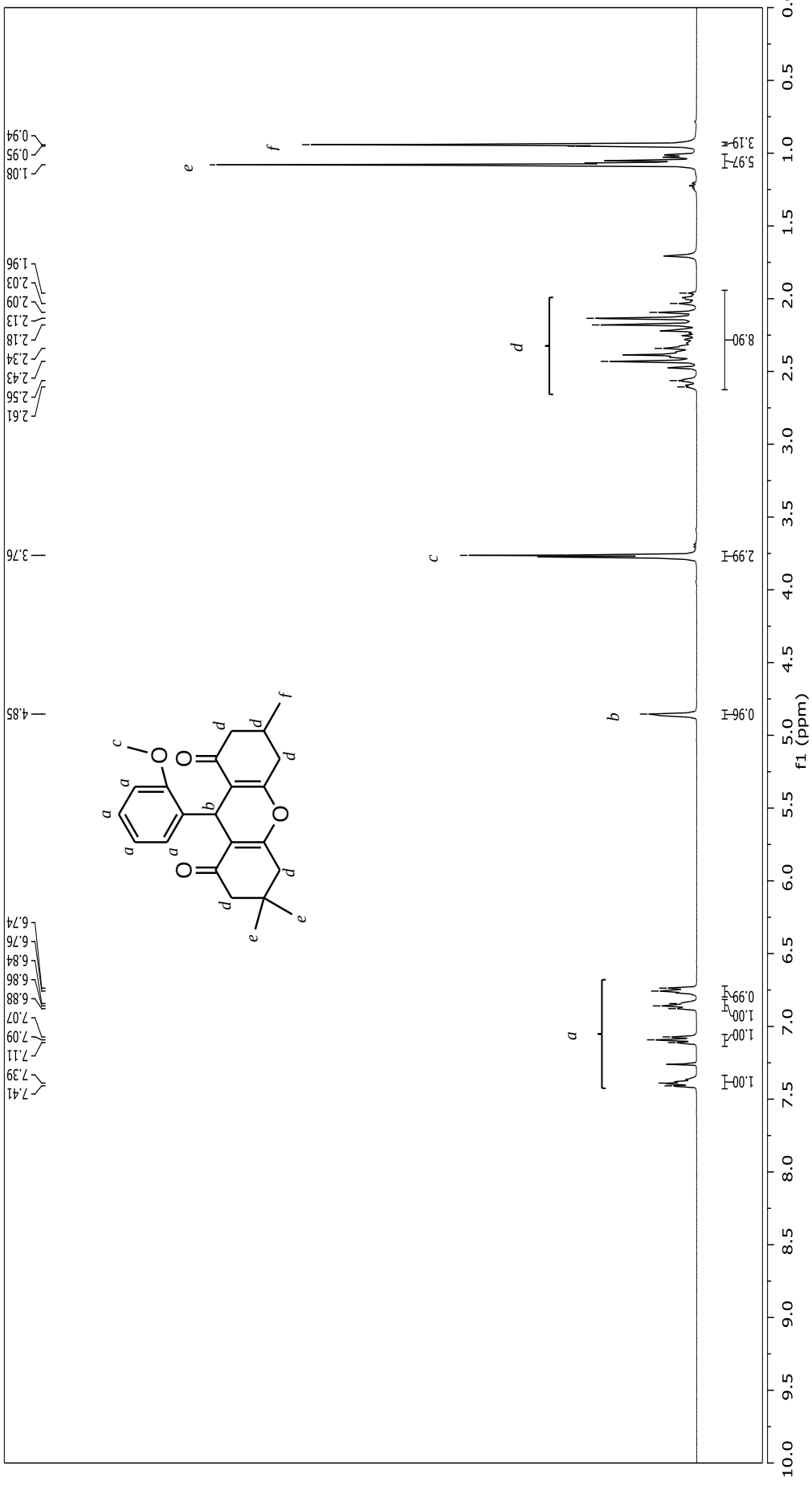

Figure B.18 The ^{1}H NMR spectrum of 9-(2-methoxyphenyl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (**9h**)

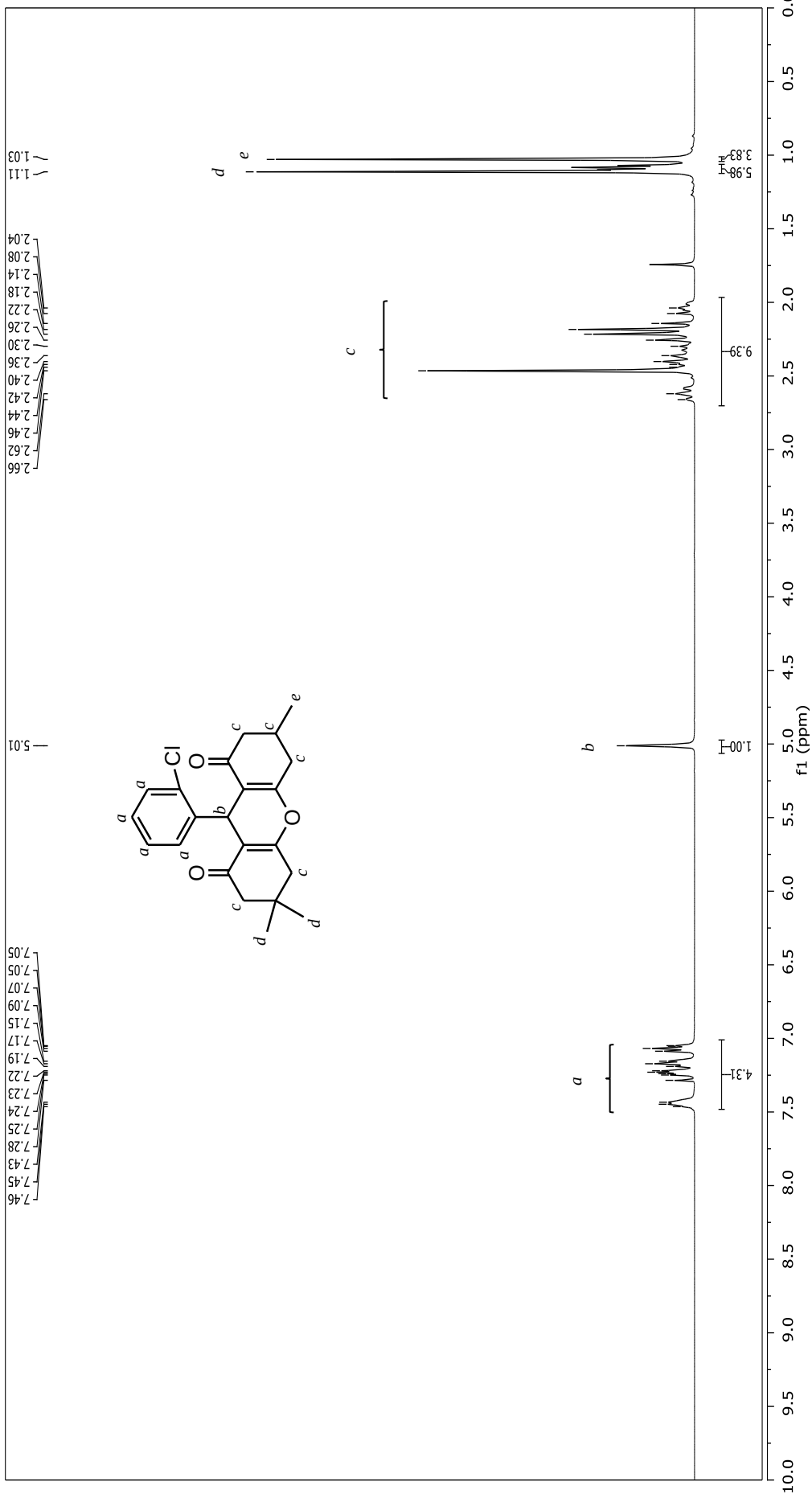

Figure B.19 The ^{1}H NMR spectrum of 9-(2-chlorophenyl)-3,3,6-trimethyl-1,8-dioxo-octahydroxanthene (**9i**)

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