Supporting Information
for
Investigation and Development of Novel Synthetic Approaches to Syntheses of Euxanthone and Derived Dyes

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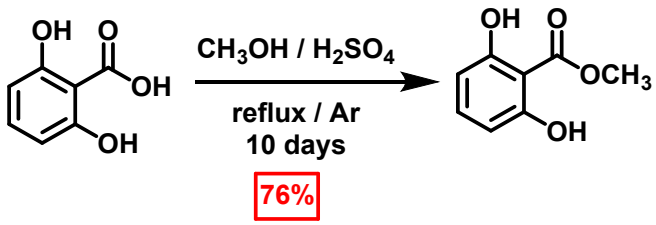
Section A. Materials / General Methods / Instrumentation

All chemicals and reagents were purchased from commercial suppliers (Aldrich, Alfa Aesar, or Fisher) and used without further purification. All reactions were carried out in dried glassware under an argon and/or a nitrogen atmosphere. Dichloromethane (CH_2Cl_2) and methanol (CH_3OH) were either freshly distilled over CaH_2 under nitrogen or dried over molecular sieves. Thin-layer chromatography (TLC) was performed on silica gel 60 F254 (E. Merck). Reactions were monitored by TLC using UV light as visualizing agent. Column (Flash) chromatography was carried out on silica gel 60F (Merck 9385, 0.040–0.063 mm). Yield refers to chromatography and spectroscopically pure compounds, unless otherwise noted. All NMR spectra (^1H and ^{13}C) were recorded on a Bruker Avance 300 or 400 with a working frequency of 300/400 MHz, respectively. Chemical shifts were reported in ppm relative to the signals corresponding to the residual non-deuterated solvents ($CDCl_3$: δ 7.26 ppm; DMSO-d_6 δ 2.50 ppm; acetone-d_6 δ 2.05 ppm for ^1H NMR, and $CDCl_3$: δ 77.06 ppm; DMSO-d_6 δ 39.53 ppm; acetone-d_6 δ 29.82 and 206.03 ppm for ^{13}C NMR) or tetramethylsilane (TMS, δ = 0.00). Coupling constants, *J*, are reported in hertz (Hz). ^1H-^1H 2D-COSY NMR spectra were recorded on the Bruker Avance 300 spectrometer. UV-Vis absorbance spectra were collected (measured in highly diluted solutions ($<10^{-5}$ M)) at RT on a UV–3600 Shimadzu spectrophotometer. Mass spectra were measured with a Finnigan Trace DSQ GC-MS mass spectrometer (ESI). Melting points were determined by the open capillary tube method using a Mel-Temp melting point device. Some reactions were run with a microwave reactor (CEM Discover BenchMate). To remove solvents and other volatile impurities under reduced pressure, a Büchi Rotavapor R-114 and an Edwards oil pump were used.

Section B. Synthetic Protocols

The detailed synthetic procedures and structural characterization data for the intermediates and desired compounds are presented below.

Synthesis of 2,6-dihyroxybenzoic acid methyl ester



Scheme S1. Synthesis of 2,6-dihyroxybenzoic acid methyl ester.

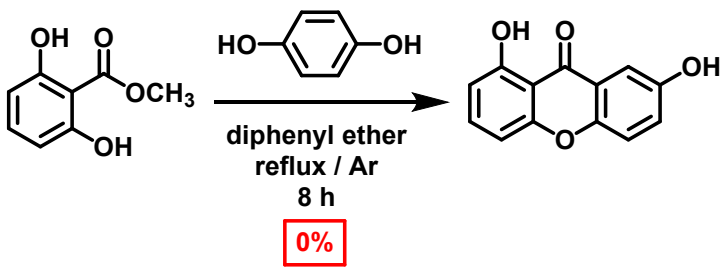
For the synthesis of Euxanthone, 2,6-dihyroxybenzoic acid methyl ester was obtained first. In a 500 mL two-neck Schlenk flask equipped with a stirring bar under an argon atmosphere, 2,6-dihyroxybenzoic acid (25.0 g, 0.150 mol) and methanol (200 mL) were charged. To this mixture, concentrated sulfuric acid (H_2SO_4, 8 mL) was cautiously added at room temperature (RT), and the mixture was stirred and refluxed for 10 days. Upon completion, the solvent was removed, and the residue was dissolved in ethyl acetate (200 mL). After addition, the reaction mixture was stirred a few minutes at RT, washed with saturated aqueous sodium chloride (NaCl) solution (2 x 100 mL) and then with saturated aqueous sodium bicarbonate ($NaHCO_3$) solution (2 x 100 mL), dried over Na_2SO_4, and concentrated on a rotary evaporator. The residue was purified by column chromatography on silica eluting with CH_2Cl_2:CH_3OH (95:5). Fractions with 2,6-dihyroxybenzoic acid methyl ester were combined and the solvent was removed using the rotary evaporator. The product was recrystallized with a hexane:ethyl acetate mixture (4:1) and isolated as white crystals with m.p. of 68 ^oC, (18.9 g, 76%); ^1H NMR (300 MHz, $CDCl_3$, 298 K) δ 9.65 (s, 2H, -OH), 7.35-

7.26 (m, 1H), 6.50 (d, *J*=aromatic, 2H), 4.09 (s, 3H). The NMR data obtained for the 2,6-dihyroxybenzoic acid methyl ester matched the literature.

For the synthesis of 1,7-dihyroxyxanthone (Euxanthone), different existing literature protocols[1-8] have been utilized and a novel way of obtaining the desired product (proposed protocol) has been reported below. The weak points (if any) of each protocol has also been provided in the synthetic procedures.

Synthesis of 1,7-dihydroxyxanthone (Euxanthone)

1st Protocol[1]

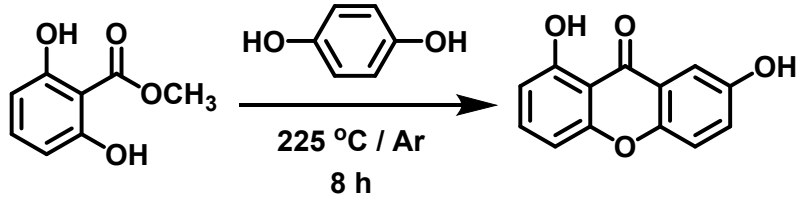


Scheme S2. Attempted synthesis of 1,7-dihyroxyxanthone (Euxanthone).

In a 50 mL two-neck Schlenk flask equipped with a stirring bar under an argon atmosphere, a mixture of hydroquinone (1.10 g; 10.0 mmol), methyl 2,6-dihyroxybenzoate (1.70 g; 10.0 mmol) and diphenyl ether (5.00 mL) were charged, and the mixture was refluxed for 8 h. Removal of solvent by steam distillation gave 1.20 grams of a black crude, which was purified by column chromatography on silica eluting with hexane:ethyl acetate (5:1) to obtain a small amount of a yellow solid, which was recrystallized from hexane and ethyl acetate. However, ^{1}H-NMR (**Figure S7**) and Gas Chromatography/Mass Spectrometry (GC/MS) results did not support the structure of the desired product. It was found by GC/MS that methyl 2,6-dihyroxybenzoate was

decomposing to 1,3-dimethoxybenzene, *m*-methoxyphenol and CO_2. Upon obtaining these results, the synthetic protocol was modified and utilized as reported below.

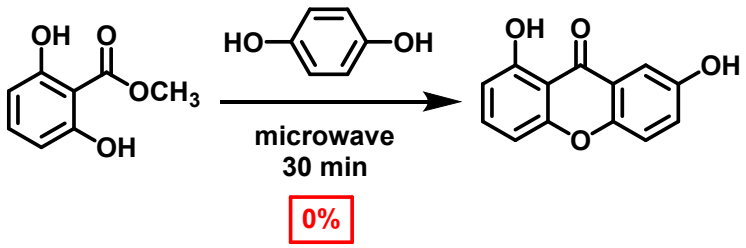
Modified 1st Protocol



Scheme S3. Synthesis of 1,7-dihyroxyxanthone (Euxanthone).

In a 50 mL two-neck Schlenk flask equipped with a stirring bar under an argon atmosphere, a mixture of hydroquinone (1.10 g; 10.0 mmol) and methyl 2,6-dihyroxybenzoate (1.70 g; 10.0 mmol) were charged, and the mixture was heated to 225 °C with a sand bath for 8 h. The reaction resulted with 1.30 grams of a black crude, which was dissolved in methanol, and purified by column chromatography on silica eluting with hexane:ethyl acetate (5:1) providing a yellow solid that was recrystallized from hexane and ethyl acetate to obtain 1,7-dihydroxyxanthone as yellow seeds with m.p. of 230-232 °C, (10.0 mg, 0.45%); ^{1}H NMR (300 MHz, DMSO-d_6, 298 K) δ 8.08 (d, *J*=9.0 Hz, 1H), 7.52 (t, *J*=9.0 Hz, 1H), 7.45 (s, 1H), 6.85-6.95 (m, 2H), 6.72 (dd, *J*=9.0 and J=2.0 Hz, 1H), 12.9 (s, 1H, -*OH exchangeable with* D_2O); MS(EI): *m/z* calcd for $C_{13}H_8O_4$ $[M]^+$ 228.04, found 227.97. The desired product, Euxanthone, was obtained only once with this method and not able to be reproduced with the same protocol (although the conditions were revised and optimized multiple times).

Modified 1st Protocol by Microwave



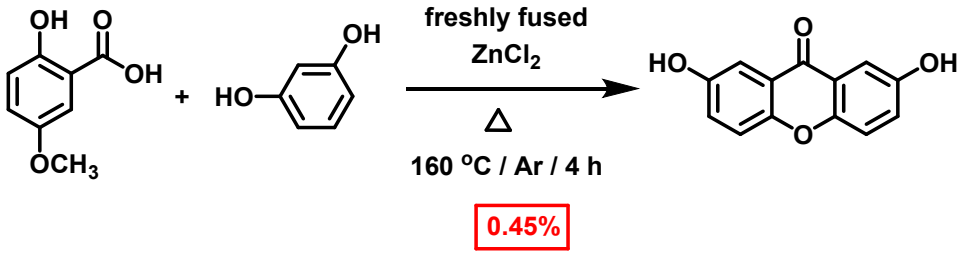
Scheme S4. Attempted synthesis of 1,7-dihyroxyxanthone (Euxanthone) by microwave.

In a small microwave flask equipped with a stirring bar and purged with argon, a mixture of hydroquinone (54 mg; 0.50 mmol) and methyl 2,6-dihyroxybenzoate (98 mg; 0.58 mmol) were charged and heated in a microwave reactor (CEM Discover BenchMate) for 30 minutes (standard mode, 300 Watt, 250 °C, 293 psi, high stirring). The reaction resulted with a black solid, which was then dissolved in methanol and a small amount of the sample was injected to GC/MS. The desired product, Euxanthone, was not obtained by this method even though conditions were revised and optimized many times (**Table S1**).

Table S1. Attempted synthetic trials of 1,7-dihyroxyxanthone (Euxanthone) by microwave.

Hydroquinone, g	Methyl Ester, g	Mode	Power, Watt	Temp., ^{0}C	Time, min	Pressure, psi
0.0054	0.098	Single	300	200	5	293
0.0054	0.098	Single	300	200	10	293
0.0054	0.098	Single	300	200	25	293
0.0054	0.098	Single	300	200	50	293
0.0054	0.098	Single	300	200	60	293
0.0054	0.098	Single	300	250	25	293
0.0054	0.098	Single	300	250	55	293

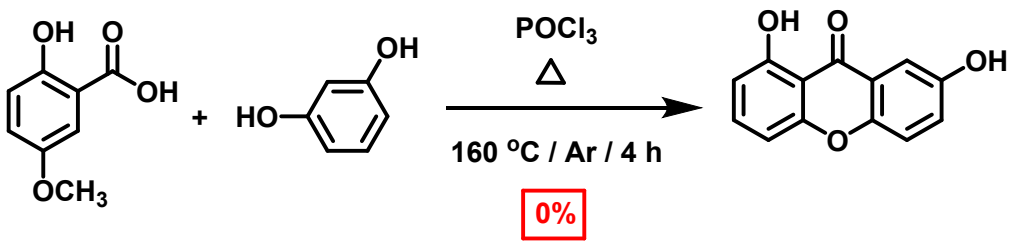
2nd Protocol[9]



Scheme S5. Attempted synthesis of 1,7-dihyroxyxanthone (Euxanthone) with $ZnCl_2$.

In a 50 mL two-neck Schlenk flask equipped with a stirring bar under an argon atmosphere, a mixture of 2-hydroxy-5-methoxybenzoic acid (1.00 g, 5.95 mmol), resorcinol (10.0 g, 90.0 mmol), and freshly fused zinc chloride (2.00 g, 15.0 mmol) were charged, and the mixture was heated to 160 °C for 4 h. The reaction was cooled to RT, washed with hot water (5 x 100 mL), and the residue was dissolved in ethyl acetate. The resulting solution was washed with aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated on a rotary evaporator. The crude was purified by column chromatography on silica eluting with CH_2Cl_2:CH_3OH (95:5). Fractions with the desired product were combined and the solvent was removed using the rotary evaporator, and the desired product was assumed to be obtained as yellow needles (6.1 mg, 0.45%); however, the ^{1}H-NMR spectrum (**Figures S8** and **S9**) provided signals for 2,7-dihydroxyxanthone (*proposed to be due by equilibration of benzophenone intermediate*) derivative although the GC/MS result provided same molecular weight; ^{1}H NMR (300 MHz, acetone-d_6, 298 K) δ 8.79 (s, 2H), 7.35 (dd, J=9.0 and J=2.9 Hz, 2H), 7.47 (d, J=9.0 Hz, 2H), 7.61 (d, J=2.9 Hz, 2H) (**Figures S8** and **S9**); MS(EI): *m/z* calcd for $C_{13}H_8O_4$ $[M]^+$ 228.04, found 228.01. As a result, Euxanthone was not able to be obtained via this protocol. Replacing zinc chloride with polyphosphoric acid (PPA) was also examined. However, there was no proof of 1,7-dihyroxyxanthone (Euxanthone). Upon obtaining this result, the synthetic protocol was modified and utilized as reported below.

Modified 2nd Protocol

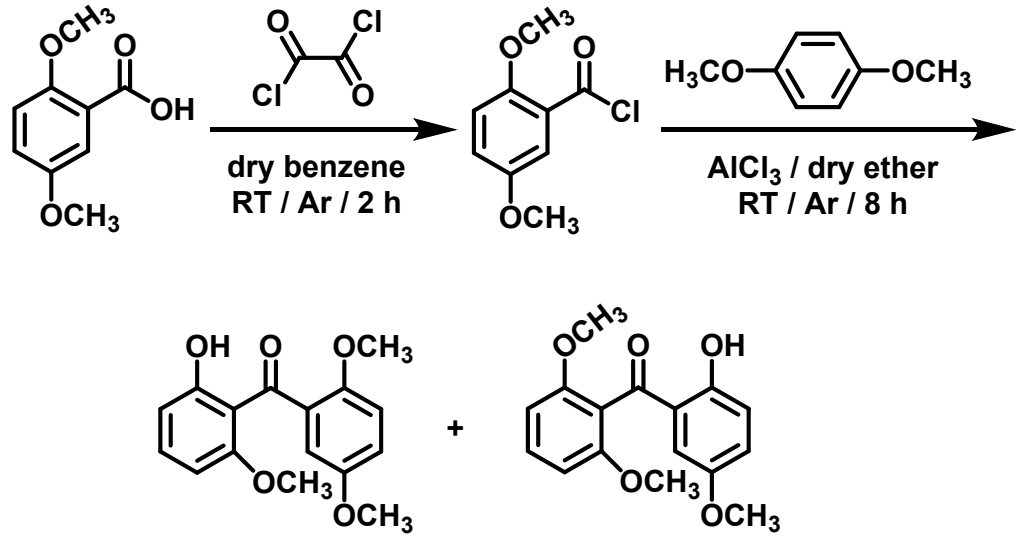


Scheme S6. Attempted synthesis of 1,7-dihyroxyxanthone (Euxanthone) with $POCl_3$.

In a 50 mL two-neck Schlenk flask equipped with a stirring bar under an argon atmosphere, a mixture of 2-hydroxy-5-methoxybenzoic acid (1.00 g, 5.95 mmol), resorcinol (4.00 g, 36 mmol), and $POCl_3$ (12.0 mL) were charged, and the mixture was heated to 160 °C for 4 h. The reaction was cooled to RT, poured into ice-cold water (300 mL), and extracted with ethyl acetate (2 x 100 mL). The organic layer was collected, and the resulting solution washed with aqueous $NaHCO_3$ (2 x 100 mL), dried over Na_2SO_4, and concentrated on a rotary evaporator. The sample was analyzed by GC/MS chromatogram showed two peaks at 12.06 and 12.42 min (at RT) with *m/z* of 302. As a result, Euxanthone was not able to be obtained by this protocol.

From all of the above synthetic protocols and observations, it was concluded that such synthetic methods were not viable to synthesize 1,7-dihyroxyxanthone (Euxanthone) in sufficient quantities.

3rd Protocol[10,11]

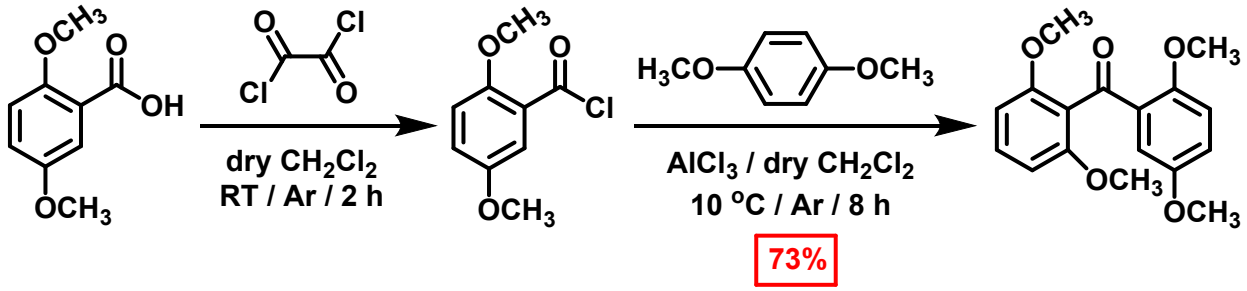


Scheme S7. Attempted synthesis of 2-hydroxy-6-methoxy-2',5'-dimethoxybenzophenone and 2,6-dimethoxy-2'-hydroxy-5'-methoxybenzophenone.

In the first step, the reaction was started with the synthesis of 2-hydroxy-6-methoxy-2',5'-dimethoxybenzophenone and 2,6-dimethoxy-2'-hydroxy-5'-methoxybenzophenone. In a 250 mL two-neck Schlenk flask equipped with a stirring bar under an argon atmosphere, a mixture of 2,6-dimethoxybenzoic acid (3.00 g, 16.5 mmol) in anhydrous (dry) benzene (60 mL) was treated with oxalyl chloride (5.0 mL), and thoroughly stirred at RT. After 2h-stirring, the excess reagent and solvent were removed under reduced pressure. The residue, 2,6-dimethoxybenzoyl chloride, was dissolved in anhydrous ether (80 mL) and 1,4-dimethoxybenzene (2.20 g, 16.0 mmol) and $AlCl_3$ (5.00 g, 36.7 mmol) were added. After stirring for 8 h at RT, the mixture was hydrolyzed with ice-cold deionized water (500 mL) containing concentrated HCl (45 mL) and extracted with chloroform ($CHCl_3$). Removal of solvent resulted with a crude that was purified by column chromatography on silica eluting with $CHCl_3$ yielding with yellow oil (3.28 g). The ^{1}H-NMR spectrum of the sample proved that the mixture of both benzophenone derivatives were not

obtained. However, after leaving the yellow oil overnight, white needle formation was observed. Once the sample was run on both ^{1}H-NMR and GC/MS instruments, the results provided proof for the colorless crystals of 2,6,2',5'-tetramethoxybenzophenone. This result was examined in the revised and enhanced new synthetic procedure described in the next protocol.

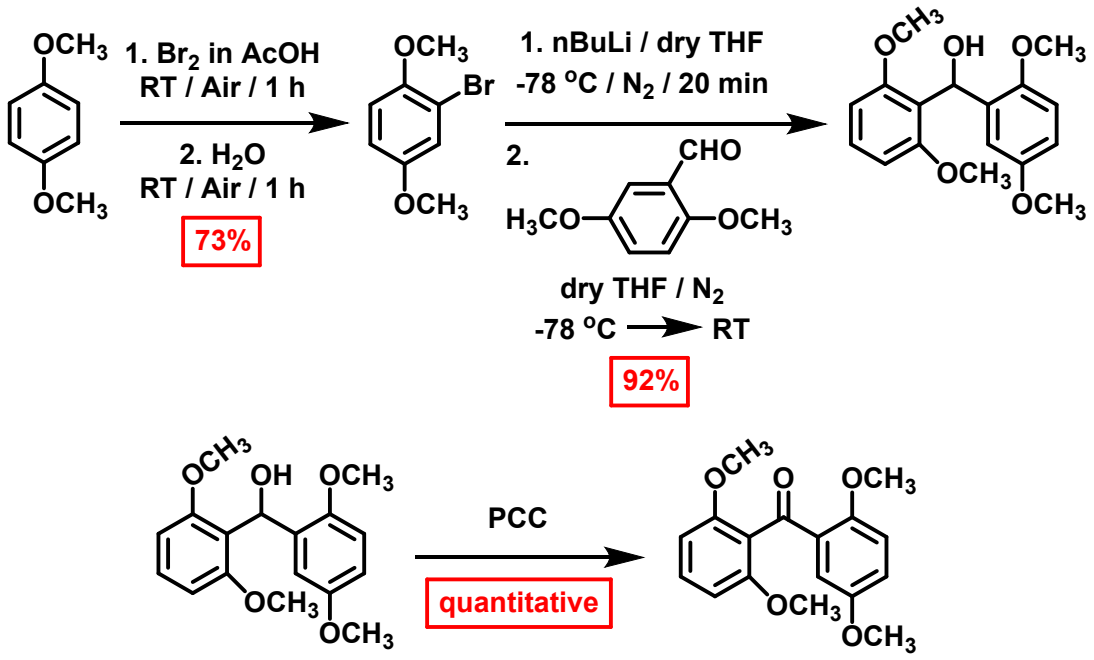
4th Protocol, a New Synthetic Procedure Revised and Enhanced Utilizing Existing Literature Protocol[10]



Scheme S8. Synthesis of 2,6,2',5'-tetramethoxybenzophenone.

After obtaining some stimulating results in the 3rd protocol,[10,11] it was decided to utilize an existing literature protocol[10] by revising and enhancing details for the synthesis of 2,6,2',5'-tetramethoxybenzophenone. In a 250 mL two-neck Schlenk flask equipped with a stirring bar under an argon atmosphere, a mixture of 2,6-dimethoxybenzoic acid (3.00 g, 16.5 mmol) in anhydrous (dry) dichloromethane (100 mL) was treated with oxalyl chloride (5.0 mL), and stirred at RT. After 2h-stirring, the excess reagent and solvent were removed under reduced pressure. The residue, 2,6-dimethoxybenzoyl chloride was dissolved in dry dichloromethane (100 mL) and added 1,4-dimethoxybenzene (2.23 g, 16.1 mmol) to the mixture. After an hour, the first aliquot of $AlCl_3$ (2.50 g, 18.0 mmol) was added and the mixture was stirred while also being cooled with ice (~10 °C) under the argon atmosphere. After stirring 30 min, second aliquot of $AlCl_3$ (2.50 g, 18.0 mmol) was added to the mixture and the mixture was stirred for 7 h. The mixture was then

hydrolyzed with ice-cold deionized water (500 mL) containing concentrated HCl (70 mL) and extracted with chloroform ($CHCl_3$). Removal of solvent resulted with a yellow crude oil that was purified by column chromatography on silica eluting with hexane:ethyl acetate (1:3) yielding with colorless crystals of 2,6,2',5'-tetramethoxybenzophenone with m.p. of 98 °C, (3.49 g, 73%); IR (KBr): υ (cm^{-1}) 1675, 1600; UV/Vis: λ_{max}: 302 nm; ^1H NMR (400 MHz, $CDCl_3$, 298 K) δ 7.33 (s, 1H), 7.25 (t, *J*=8.4 Hz, 1H), 7.02 (dd, *J*=9.2 Hz, *J*=3.2 Hz, 1H), 6.87 (d, *J*=8.8 Hz, 1H), 6.57 (d, *J*=8.4 Hz, 1H), 3.78 (s, 3H), 3.70 (s, 6H), 3.60 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, 298 K) δ 193.5, 157.3, 154.4, 153.4, 130.1, 128.9, 121.7, 120.3, 115.6, 114.7, 104.2, 57.0, 56.0, 55.8 (**Figures S13** and **S14**); MS(EI): *m/z* calcd for $C_{17}H_{18}O_5$ $[M]^+$ 302.12, found 302.25 with a retention time of 13.60 min (**Figure S16**).



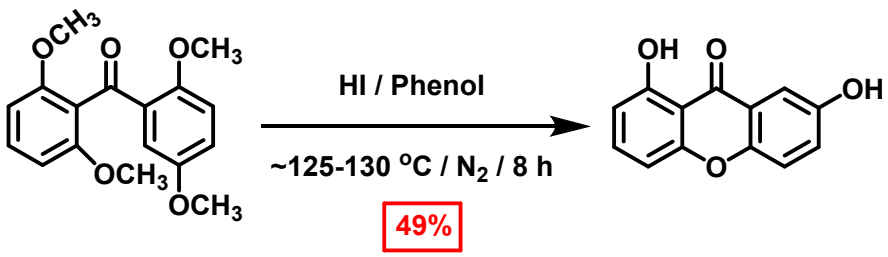
Scheme S9. Alternative synthesis of 2,6,2',5'-tetramethoxybenzophenone.

Alternatively (1st step in **Scheme S9**), in a 250 mL single-neck Schlenk flask equipped with a stirring bar under open air atmosphere, to a solution of 1,4-dimethoxybenzene (16.8 g, 121 mmol)

in glacial acetic acid (AcOH, 170 mL) was added a solution of bromine (6.70 mL, 130 mmol) in AcOH (90 mL) dropwise at RT. After stirring for an hour, deionized water (100 mL) was added to the reaction mixture. Upon stirring for another hour, the organic layer was extracted with $CHCl_3$ (2 x 100 mL). The extracts were combined and dried over anhydrous $MgSO_4$, and then the solvent was removed under reduced pressure. The residue was distilled in vacuum (78-80 ^oC at 2 mmHg) to afford 2-bromo-1,4-dimethoxybenzene as brown liquid (19.3 g, 73%); ^1H NMR (300 MHz, $CDCl_3$, 298 K) δ 7.12 (d, *J*=2.4 Hz, 1H), 6.81-6.83 (m, 2H), 3.84 (s, 3H), 3.78 (s, 3H).

In a 250 mL two-neck Schlenk flask equipped with a stirring bar under a nitrogen atmosphere, n-butyllithium (2.5 M in hexane, 4.10 mL, 10.0 mmol) was added over a minute to the solution of 2-bromo-1,4-dimethoxybenzene (2.17 g, 10.0 mmol) in dry THF (~20 mL) under a nitrogen atmosphere at –78 ^oC. After stirring the mixture for 20 min, 2,6-dimethoxybenzaldehyde (1.70 g, 10.0 mmol) in dry THF (5 mL) was added in over a min. Upon allowing the mixture to warm to RT (2^{nd} step in **Scheme S9**), the solution was quenched with deionized water (100 mL) and neutralized with 1 M HCl. The mixture was extracted with CH_2Cl_2 and dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure yielding a waxy solid. The desired product, 2,6,2',5'-tetramethoxybenzophenone was obtained as colorless crystals after oxidizing the reaction mixture with pyridinium chlorocromate (PCC) (3^{rd} step in **Scheme S9**) in 20 mL of CH_2Cl_2, with m.p. of 98 ^oC (2.80 g, 92%); collected data matched with the previous results as show in **Figures S13**, **S14** and **Figure S16**.

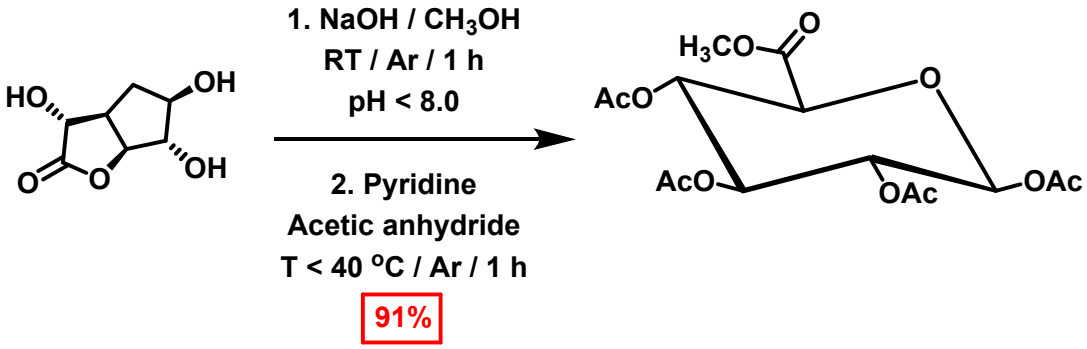
The yield of the desired product, 2,6,2',5'-tetramethoxybenzophenone, was improved with this alternative synthetic protocol.



Scheme S10. Synthesis of 1,7-dihydroxyxanthone (Euxanthone).

Upon synthesis of 2,6,2',5'-tetramethoxybenzophenone, this intermediate was used to synthesize 1,7-dihydroxyxanthone (Euxanthone) as shown in **Scheme S10**. In a 50 mL two-neck Schlenk flask equipped with a stirring bar under a nitrogen atmosphere, 2,6,2',5'-tetramethoxybenzophenone (1.30 g, 4.20 mmol) was dissolved in phenol (~15 mL) and hydrogen iodide (10 mL) was added to the mixture, and the solution was heated to 125-130 °C for 8 h. Upon completion, the reaction mixture was poured into ice-cold deionized water (100 mL). The resulting black-dark green precipitate was collected and dissolved in hexane-ethyl acetate (2:1), and then purified by column chromatography on silica eluting with hexane:ethyl acetate (2:1). The fractions were partially evaporated in open air, and then stored in a refrigerator for overnight. The yellow needles were collected on the next day with m.p. of 194-195 °C, (0.88 g, 49%); IR (KBr): υ (cm^{-1}) 3330, 1640; ^{1}H NMR (300 MHz, DMSO-d_6, 298 K) δ 12.9 (s, 1H, -*OH exchangeable with* D_2O), 10.1 (s, 1H, -*OH exchangeable with* D_2O), 7.71 (t, *J*=8.4 Hz, 1H), 7.57 (d, *J*=1.1 Hz, 1H), 7.46 (d, *J*=8.4 Hz, 1H), 7.39 (dd, *J*=1.1 and *J*=8.4 Hz, 1H), 7.06 (dd, *J*=1.1 and *J*=8.4 Hz, 1H), 6.80 (dd, *J*=1.1 and 8.4 Hz, 1H) (**Figures S1**, **S2** and **S3**); ^{13}C NMR (100 MHz, DMSO-d_6, 298 K) δ 179.8, 161.9, 157.1, 153.9, 148.2, 136.7, 124.6, 121.3, 118.7, 110.3, 109.9, 109.8, 108.1 (**Figure S4**); MS(EI): *m/z* calcd for $C_{13}H_8O_4$ $[M]^+$ 228.04, found 227.97 with a retention time of 13.95 min (**Figure S15**).

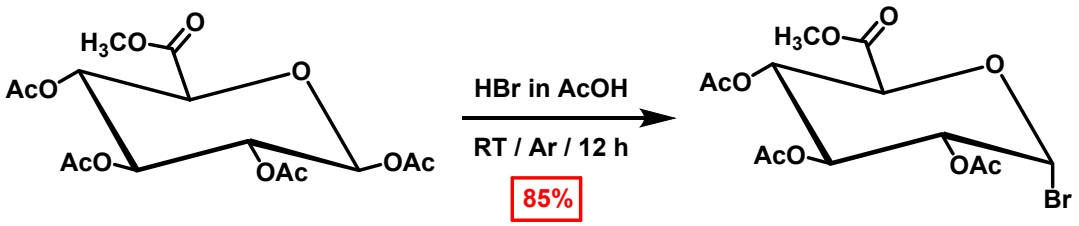
***Synthesis of Methyl tetra-O-acetyl-β-D-glucopyranuronate by Pyridine Catalyzed Acetylation*[12]**



Scheme S11. Synthesis of methyl tetra-*O*-acetyl-β-D-glucopyranuronate.

In a 250 mL two-neck Schlenk flask equipped with a stirring bar under an argon atmosphere, sodium hydroxide (70.0 mg, 1.75 mmol) was dissolved in methanol (150 mL) and glucuronolactone (20.0 g, 0.110 mmol) was added (the lactone should dissolve almost immediately; additional base should be added if pH drops below 8.0). The pH of mixture was confirmed with litmus paper (between 8.5 and 9.5). The reaction mixture was stirred at RT for an hour, and after then methanol was removed under reduced pressure (bath temperature below 50 °C). Upon removal of methanol, the sample was kept under vacuum with a vacuum pump to remove the remaining trace amount of solvent. The obtained syrup like sample was dissolved in acetic anhydride (50 mL), and a solution of pyridine (50 mL) in acetic anhydride (25 mL) was added dropwise at a rate such that the reaction mixture never exceeded 40 °C (temperature was monitored and kept below 40 °C with an ice-cold water bath). On standing in the refrigerator overnight, the desired product, methyl tetra-*O*-acetyl-β-D-glucopyranuronate, was crystallized from the reaction mixture with m.p. of 155-158 °C, (38.9 g, 91%); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K) δ 5.30 (t, *J*=9.0 Hz, 1H); 5.24 (d, *J*=9.6 Hz, 1H); 5.15 (t, *J*=8.4 Hz, 1H); 4.19 (d, *J*=9.4 Hz, 1H); 3.75 (s, 3H), 2.12 (s, 3H); 2.03 (s, 6H); 1.56 (s, 3H) (**Figure S5**); MS(EI): *m/z* calcd for $C_{15}H_{20}O_{11}$ $[M]^+$ 376.10, found 377.00.

***Synthesis of Methyl (tri-O-acetyl-α-D-glucopyranosyl bromide) uronate*[10]**



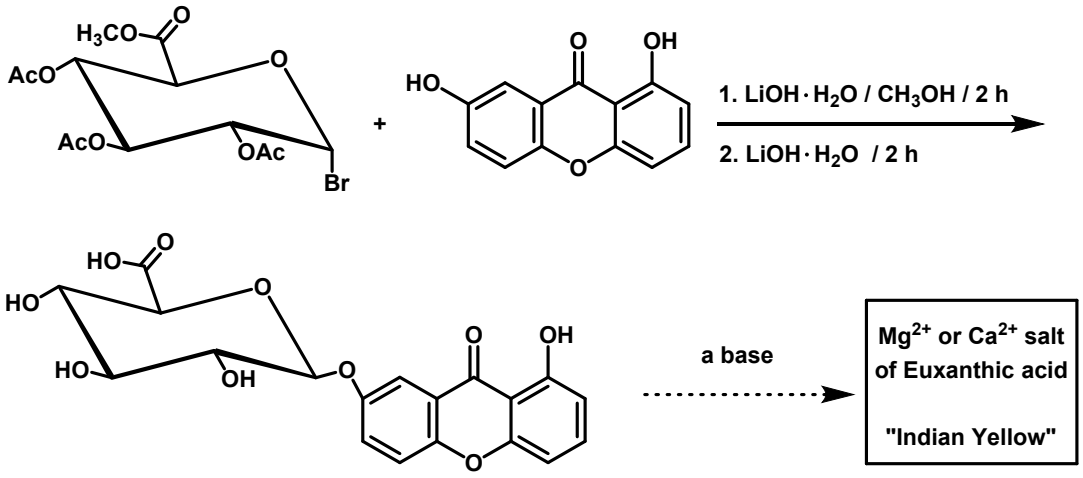
Scheme S12. Synthesis of Methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide) uronate.

In a 100 mL two-neck Schlenk flask equipped with a stirring bar under an argon atmosphere, a solution of methyl tetra-*O*-acetyl-β-D-glucopyranuronate (1.00 g, 2.65 mmol) in 27% hydrobromic acid in acetic acid (20 mL) was stirred at RT for 12 hours (see the ***NOTE*** for preparation of 27% hydrobromic acid solution). The solution was kept in the refrigerator overnight, and then the reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in $CHCl_3$ (50 mL), washed with cold saturated aqueous $NaHCO_3$ (2 x 30 mL) and then with deionized water (2 x 25 mL), dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residual syrup was recrystallized from ethanol to give yellow crystals of methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide) uronate with m.p. of 74-76 ^{o}C (1.06 g, 85%); ^{1}H NMR (300 MHz, DMSO-d_6, 298 K) δ 6.94 (d, *J*=4.0 Hz, 1H); 5.42 (t, *J*=9.4 Hz, 1H); 5.28 (t, *J*=9.4 Hz, 1H); 5.14 (dd, *J*=4.0 and *J*=9.2 Hz, 1H); 4.47 (d, *J*=9.4 Hz, 1H); 3.67 (s, 3H), 2.02 (s, 6H) (**Figure S6**); MS(EI): *m/z* calcd for $C_{13}H_{17}BrO_9$ $[M]^+$ 396.01, found 396.78.

NOTE: To 50 mL of 48% hydrobromic acid, deionized water (39 mL) was added to result in preparation of 27% hydrobromic acid. And then acetic anhydride (17.8 g, 19.5 mL, d=1.08 g/mL) was added to 10 mL of the prepared solution (including 15.9 g HBr and 3.53 g deionized water) to *in situ* produce acetic acid. The final solution was 27% HBr in acetic acid (20 mL, d=1.049 g/mL).

In order to validate the synthetic route and improve the isolated yield, methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide) uronate was resynthesized with the scaled-up reaction (3x) using 33% commercially available HBr. In a 100 mL two-neck Schlenk flask equipped with a stirring bar under an argon atmosphere, a solution of methyl tetra-*O*-acetyl-β-D-glucopyranuronate (3.00 g, 7.96 mmol) in 33% hydrobromic acid in acetic acid (20 mL) was stirred at RT for 12 hours (see the ***NOTE*** above for preparation of 27% hydrobromic acid solution). The solution was kept in the refrigerator overnight, and then the reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in $CHCl_3$ (50 mL), washed with cold saturated aqueous $NaHCO_3$ (2 x 30 mL) and then with deionized water (2 x 25 mL), dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residual syrup was recrystallized from ethanol to give yellow crystals of methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide) uronate with m.p. of 74-76 ^{o}C (2.92 g, 92%); ^{1}H NMR (300 MHz, DMSO-d_6, 298 K) δ 6.94 (d, *J*=4.0 Hz, 1H); 5.42 (t, *J*=9.4 Hz, 1H); 5.28 (t, *J*=9.4 Hz, 1H); 5.14 (dd, *J*=4.0 and *J*=9.2 Hz, 1H); 4.47 (d, *J*=9.4 Hz, 1H); 3.67 (s, 3H), 2.02 (s, 6H); MS(EI): *m/z* calcd for $C_{13}H_{17}BrO_9$ $[M]^+$ 396.01, found 396.38.

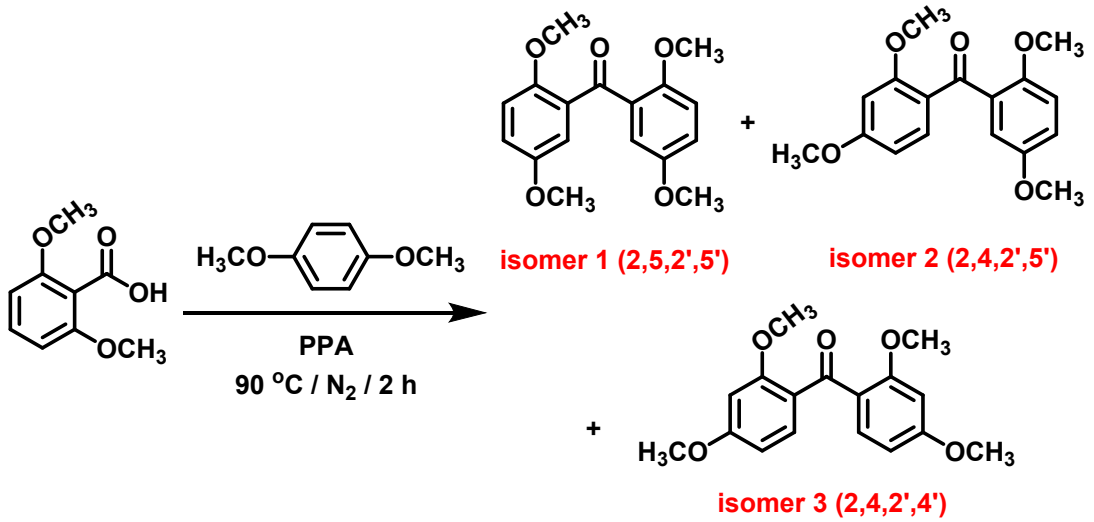
Attempted Synthesis of Indian Yellow



Scheme S13. Attempted synthesis of Indian yellow.

In a 50 mL two-neck Schlenk flask equipped with a stirring bar under an argon atmosphere, a solution of 1,7-dihydroxyxanthone (52.0 mg, 0.230 mmol) and $LiOH{\cdot}H_2O$ (9.65 mg, 0.230 mmol) in methanol (5 mL) were charged with methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide) uronate (90.0 mg, 0.230 mmol), and the mixture was allowed to stand and stir at RT for 2 h. After then additional amount of $LiOH{\cdot}H_2O$ (28.9 mg, 0.690 mmol) was added and stirred for 2 additional hours. Upon completion, the mixture was evaporated to dryness, the residue was dissolved in CH_2Cl_2, and purified by column chromatography on silica eluting with CH_2Cl_2:CH_3OH (4:1) to give a yellow solid, which was one of starting materials, Euxanthone, according to the results of GC/MS and NMR. Although the reaction was repeated many times with different reaction conditions, the desired product, Indian yellow, was not observed even in trace amounts. However, this reaction has led to a new discovery of equilibration reactions of 2,6,2′,5′-tetramethoxybenzophenone.

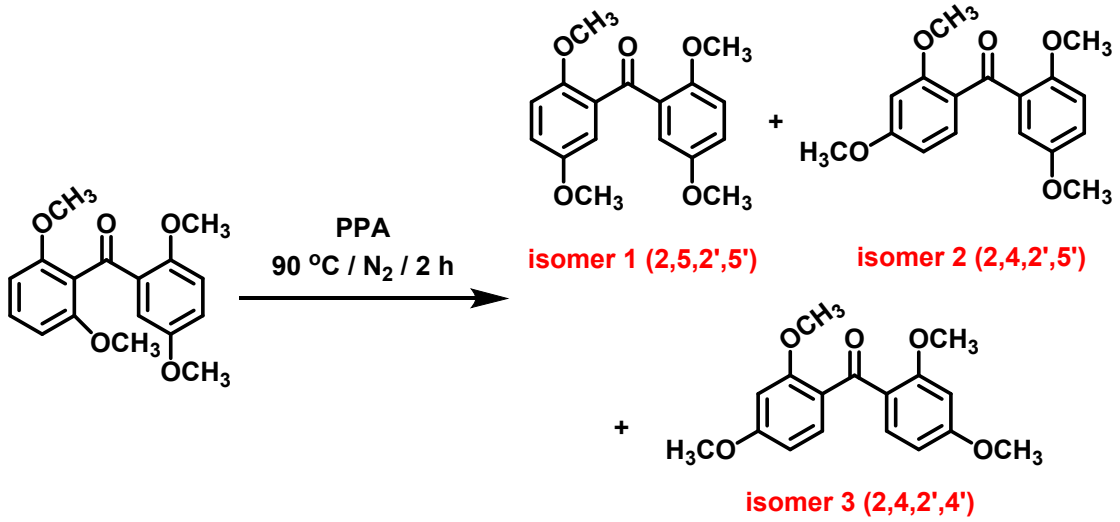
Discovery of an Equilibration Reaction: Synthesis of 2,6,2',5'-Tetramethoxybenzophenone Derivatives



Scheme S14. A new discovery of an equilibration reaction while obtaining 2,6,2′,5′-tetramethoxybenzophenone.

In a 50 mL two-neck Schlenk flask equipped with a stirring bar under a nitrogen atmosphere, a mixture of 2,6-methoxybenzoic acid, 1,4-dimethoxybenzene, and polyphosphoric acid (PPA) were heated to 90 °C for 1-2 hours. Upon completion, the reaction mixture was cooled down to RT, washed with saturated aqueous $NaHCO_3$ and deionized water, and then dried over $MgSO_4$, and purified by column chromatography on silica eluting with hexane:ethyl acetate (3:1). Three different fractions were collected and subjected to GC/MS and NMR analyses for determination and identification of each component, which were found to be three structural isomers (**Figures S10**, **S11**, **S12**, **S17**, **S18** and **S19**). ***Isomer 1*****:** ^{1}H NMR (300 MHz, $CDCl_3$, 298 K) δ 7.09 (d, *J*=3.0 Hz, 2H), 6.99 (dd, *J*=3.0 and *J*=8.8 Hz, 2H), 6.85 (d, *J*=8.4 Hz, 2H), 3.79 (s, 6H), 3.61 (s, 6H) (**Figure S10**); MS(EI): *m/z* calcd for $C_{17}H_{18}O_5$ $[M]^+$ 302.12, found 302.11 with a retention time of 14.12 min (**Figure S17**); ***Isomer 2*****:** ^{1}H NMR (300 MHz, $CDCl_3$, 298 K) δ 7.54 (d, *J*=8.6 Hz, 1H), 6.94 (d, *J*=2.8 Hz, 1H), 6.89 (dd, *J*=3.1 and *J*=5.6 Hz, 1H), 6.80 (d, *J*=8.8 Hz,

1H), 6.44 (dd, *J*=2.3 and *J*=6.3 Hz, 1H), 6.40 (s, 1H), 3.82 (s, 3H), 3.74 (s, 3H), 3.65 (s, 3H), 3.59 (s, 3H) (**Figure S11**); MS(EI): *m/z* calcd for $C_{17}H_{18}O_5$ $[M]^+$ 302.12, found 302.02 with a retention time of 15.70 min (**Figure S18**) ; ***Isomer 3*:** ^{1}H NMR (300 MHz, $CDCl_3$, 298 K) δ 7.49 (d, *J*=8.7 Hz, 2H), 6.49 (dd, *J*=2.3 and *J*=8.6 Hz, 2H), 6.40 (s, 2H), 3.84 (s, 6H), 3.66 (s, 6H) (**Figure S12**); MS(EI): *m/z* calcd for $C_{17}H_{18}O_5$ $[M]^+$ 302.12, found 302.14 with a retention time of 16.52 min (**Figure S19**).



Scheme S15. The control reaction for the new discovery of an equilibration reaction.

Similarly, in a 100 mL two-neck Schlenk flask equipped with a stirring bar under a nitrogen atmosphere, 2,6,2′,5′-tetramethoxybenzophenone (1.00 g, 3.31 mmol) was charged with polyphosphoric acid (PPA, 20 mL), heated to 90 °C for 2 hours, and similar steps of the abovementioned protocol were conducted. Three different fractions were collected and subjected to GC/MS and NMR analyses for determination and identification of each component, which were confirmed to be the same structural isomers as in the above analyses.

Section C. NMR Spectroscopy

[1]H NMR Spectrum of 1,7-dihydroxyxanthone (Euxanthone) in DMSO-d_6

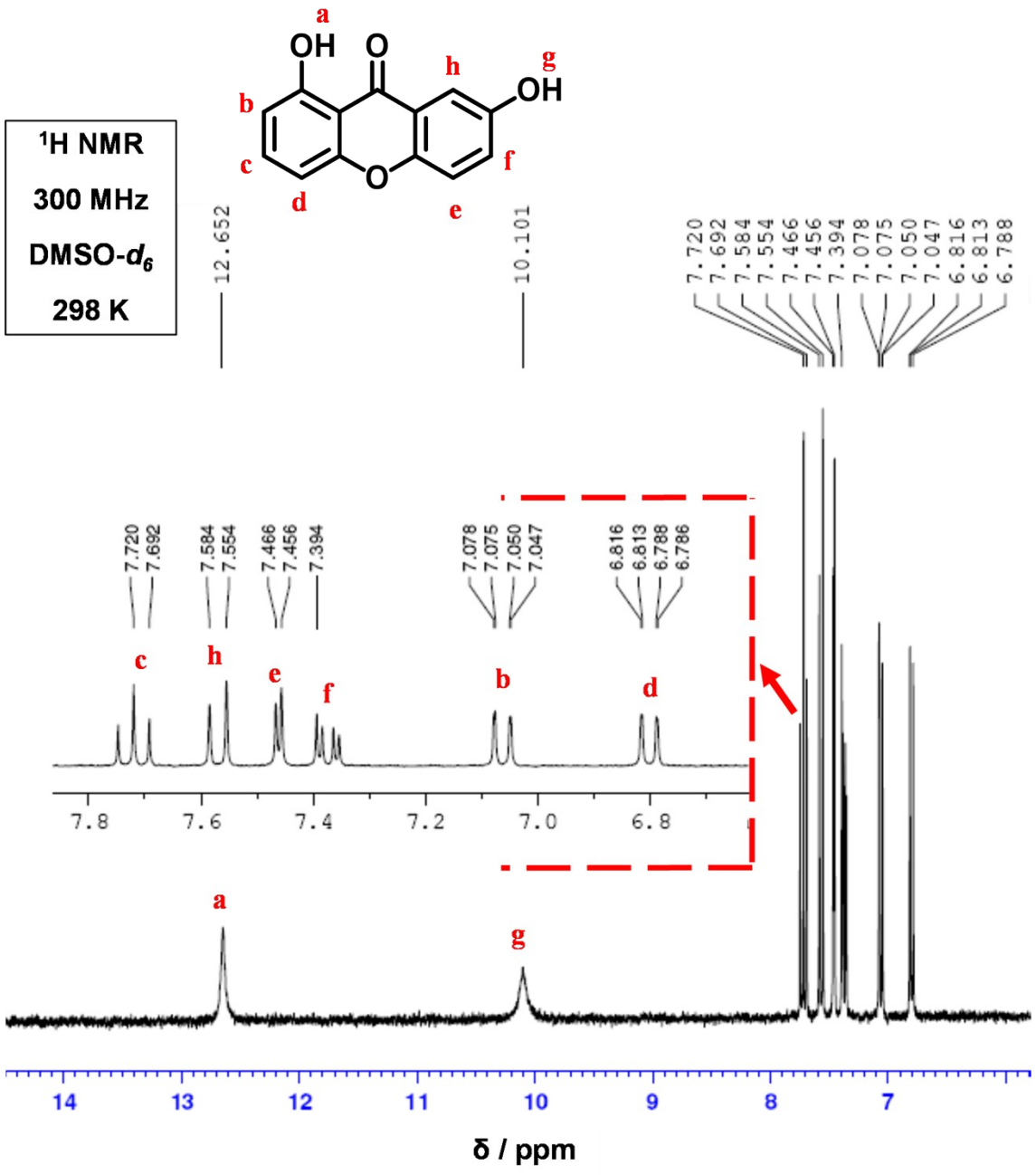


Figure S1. Annotated [1]H NMR spectrum (300 MHz, DMSO-d_6, 25 ºC) of 1,7-dihydroxyxanthone (Euxanthone).

^{1}H-^{1}H 2D-COSY NMR Spectrum of 1,7-dihydroxyxanthone (Euxanthone) in DMSO-d_6

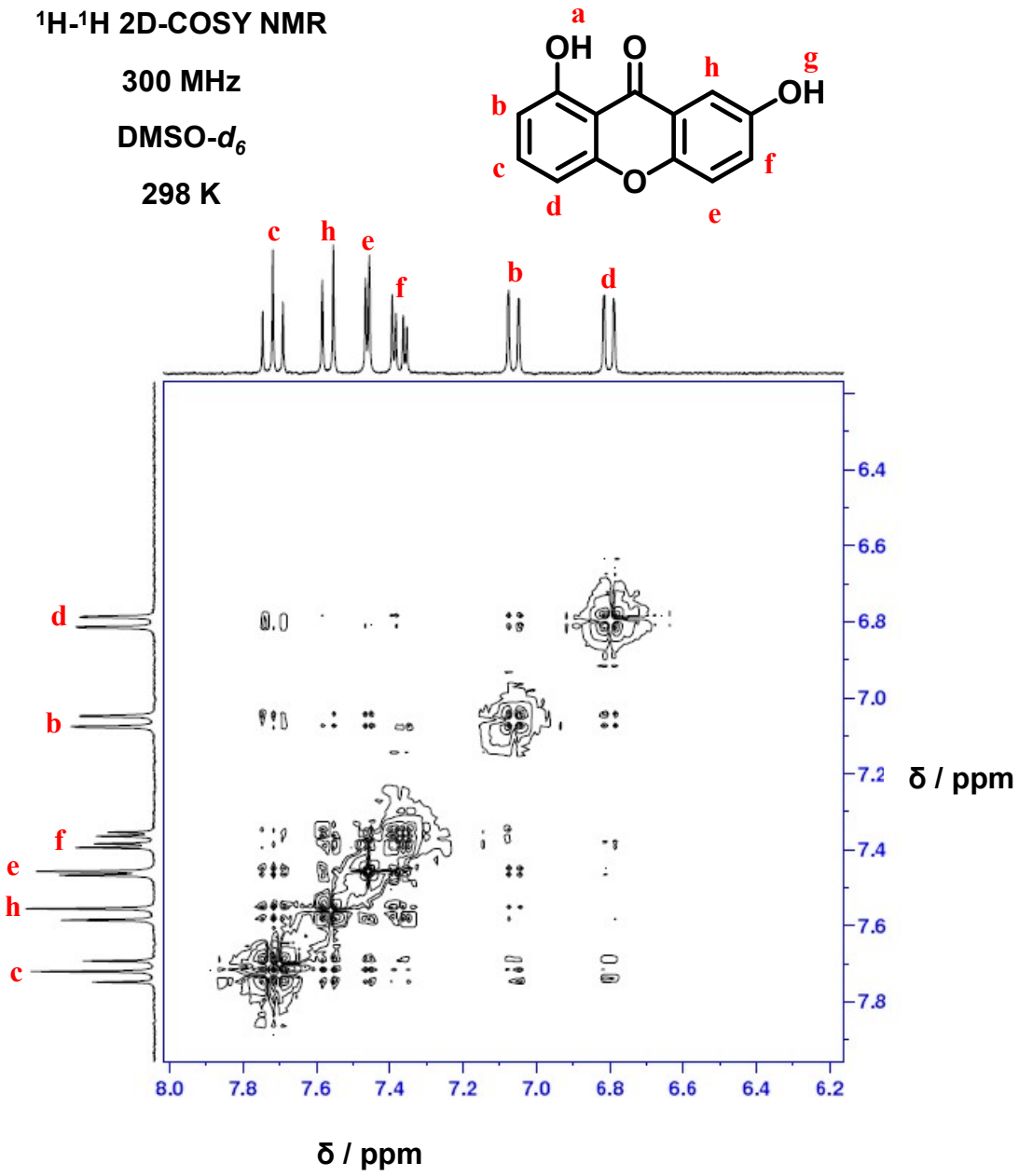


Figure S2. Annotated ^{1}H-^{1}H 2D-COSY NMR spectrum (300 MHz, DMSO-d_6, 25 ºC) of 1,7-dihydroxyxanthone (Euxanthone).

Simplified ^{1}H-^{1}H 2D-COSY NMR Spectrum of 1,7-dihydroxyxanthone (Euxanthone) in DMSO-d_6

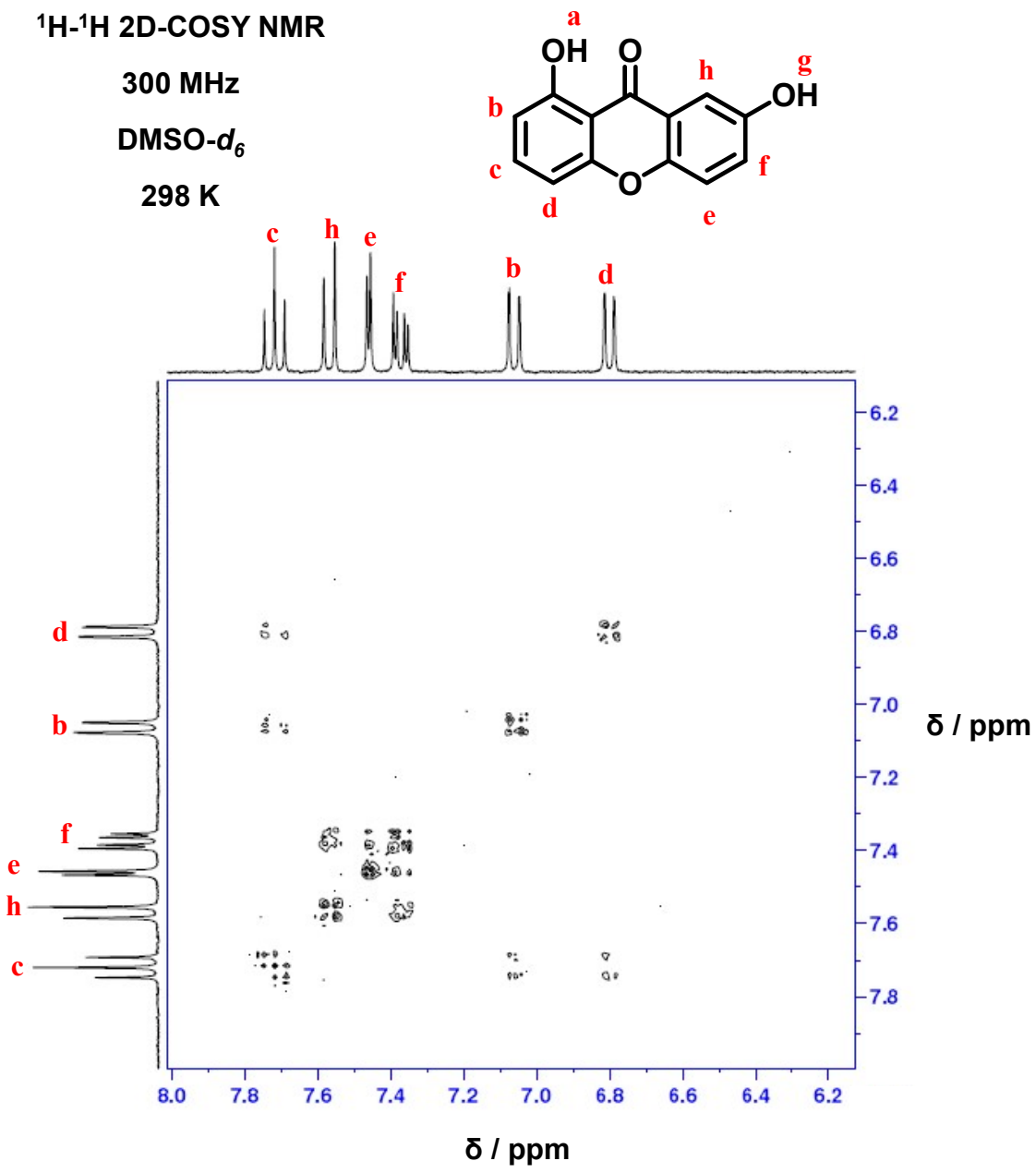


Figure S3. Annotated and simplified ^{1}H-^{1}H 2D-COSY NMR spectrum (300 MHz, DMSO-d_6, 25 ºC) of 1,7-dihydroxyxanthone (Euxanthone).

^{13}C NMR Spectrum of 1,7-dihydroxyxanthone (Euxanthone) in DMSO-d_6

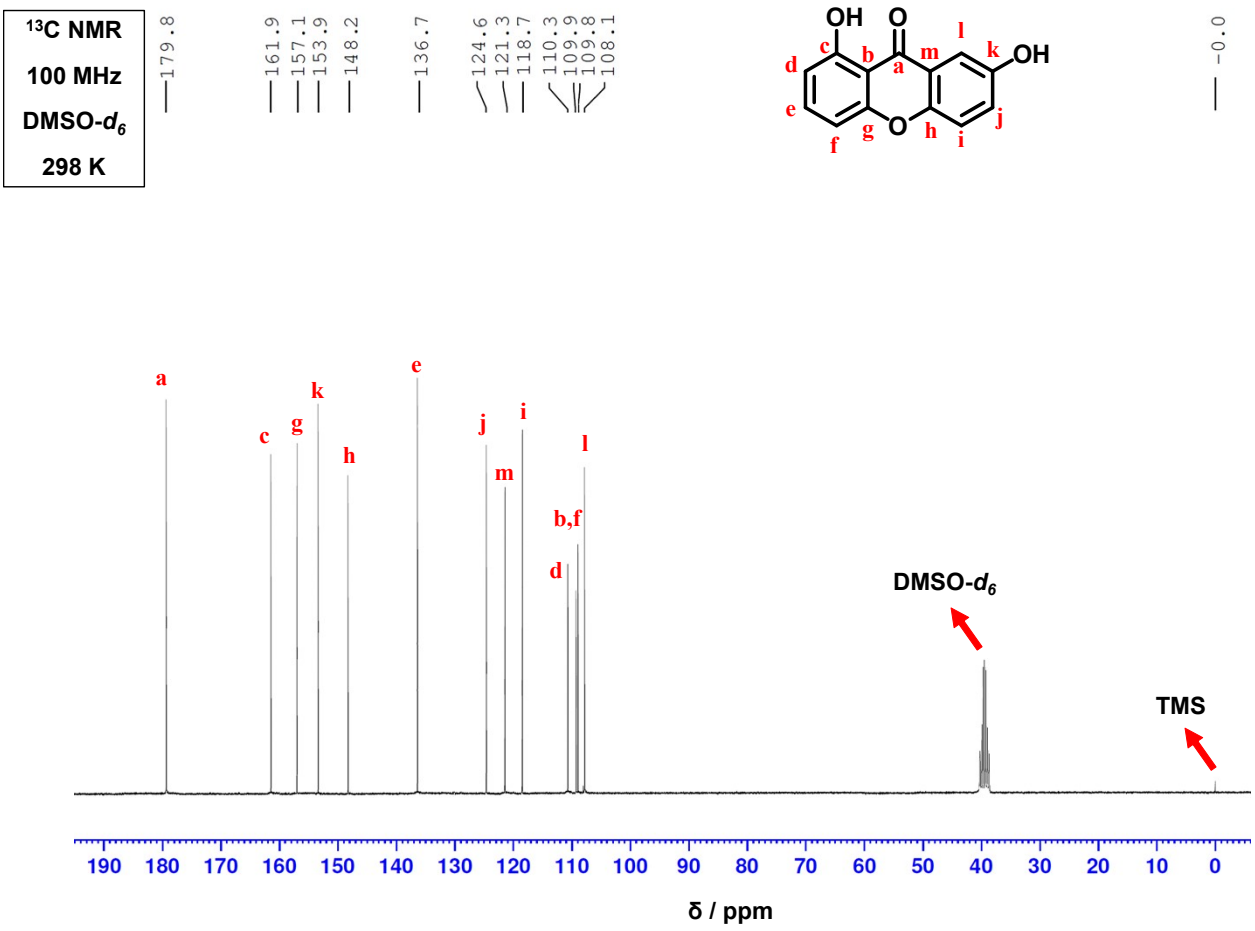


Figure S4. Annotated ^{13}C NMR spectrum (100 MHz, DMSO-d_6, 25 ºC) of 1,7-dihydroxyxanthone (Euxanthone).

^{1}H NMR Spectrum of methyl tetra-O-acetyl-β-D-glucopyranuronate in $CDCl_3$

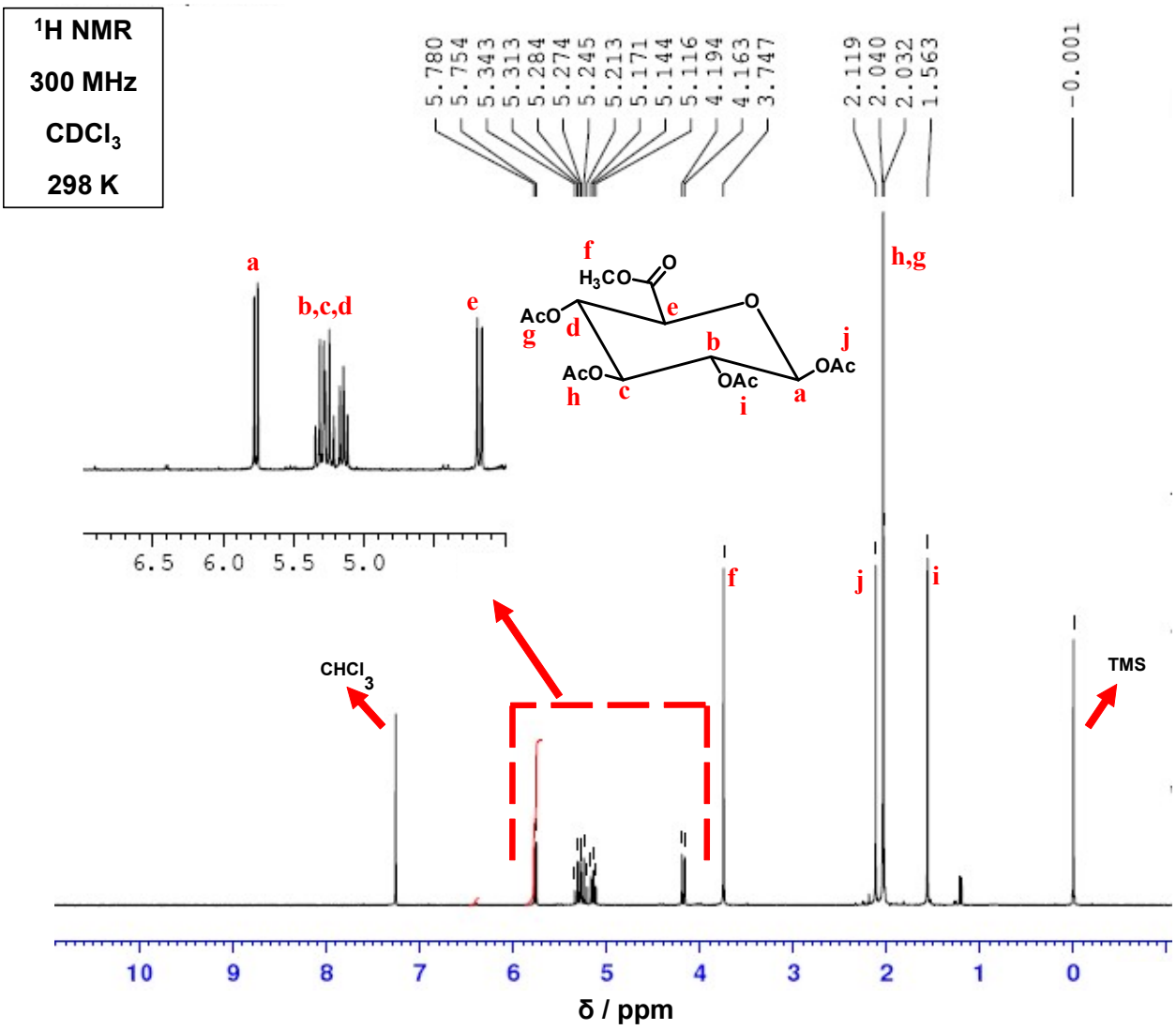


Figure S5. Annotated ^{1}H NMR spectrum (300 MHz, $CDCl_3$, 25 ºC) of methyl tetra-*O*-acetyl-β-D-glucopyranuronate.

[1]H NMR Spectrum of methyl (tri-O-acetyl-α-D-glucopyranosyl bromide) uronate in DMSO-d_6

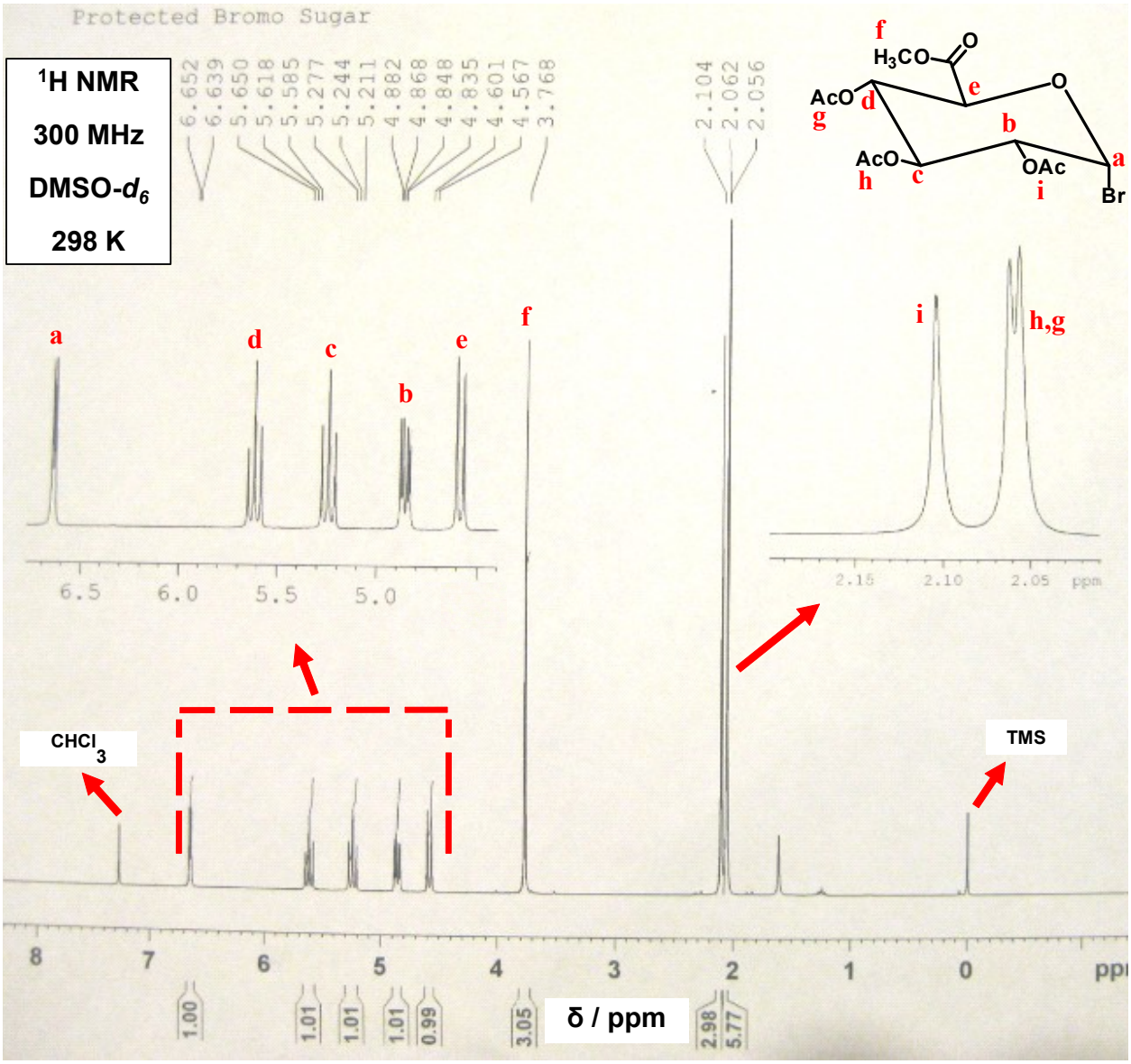


Figure S6. Annotated [1]H NMR spectrum (300 MHz, DMSO-d_6, 25 ºC) of methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide) uronate.

[1]H NMR Spectrum of 1,7-dihydroxyxanthone (synthesized by the Indian Method)[1] in $CDCl_3$

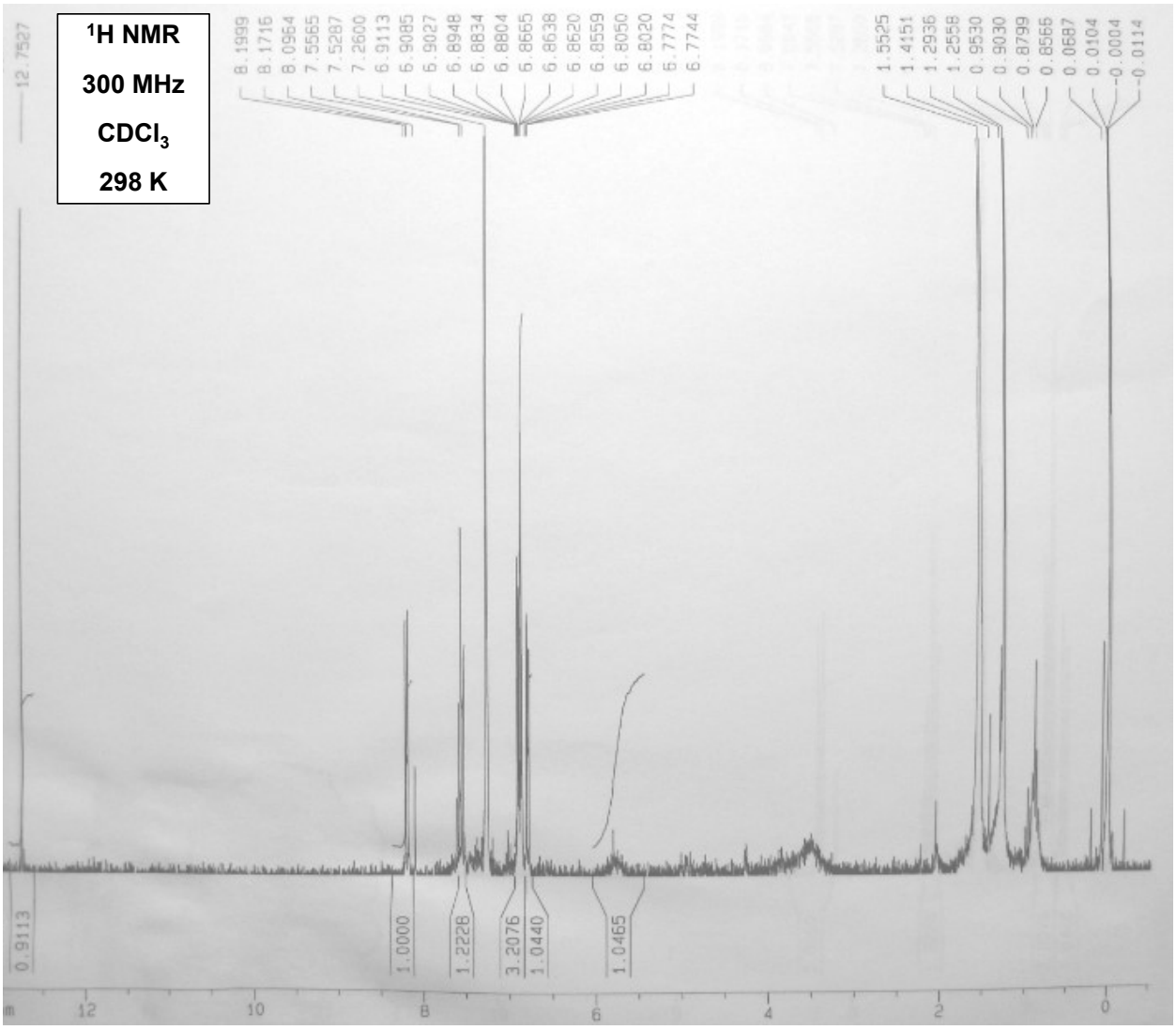


Figure S7. Annotated ^{1}H NMR spectrum (300 MHz, $CDCl_3$, 25 ºC) of 1,7-dihydroxyxanthone (Euxanthone) (synthesized by the Indian Method[1]).

[1]H NMR Spectrum of 2,7-dihydroxyxanthone (synthesized by Nencki Method)[2] in DMSO-d_6

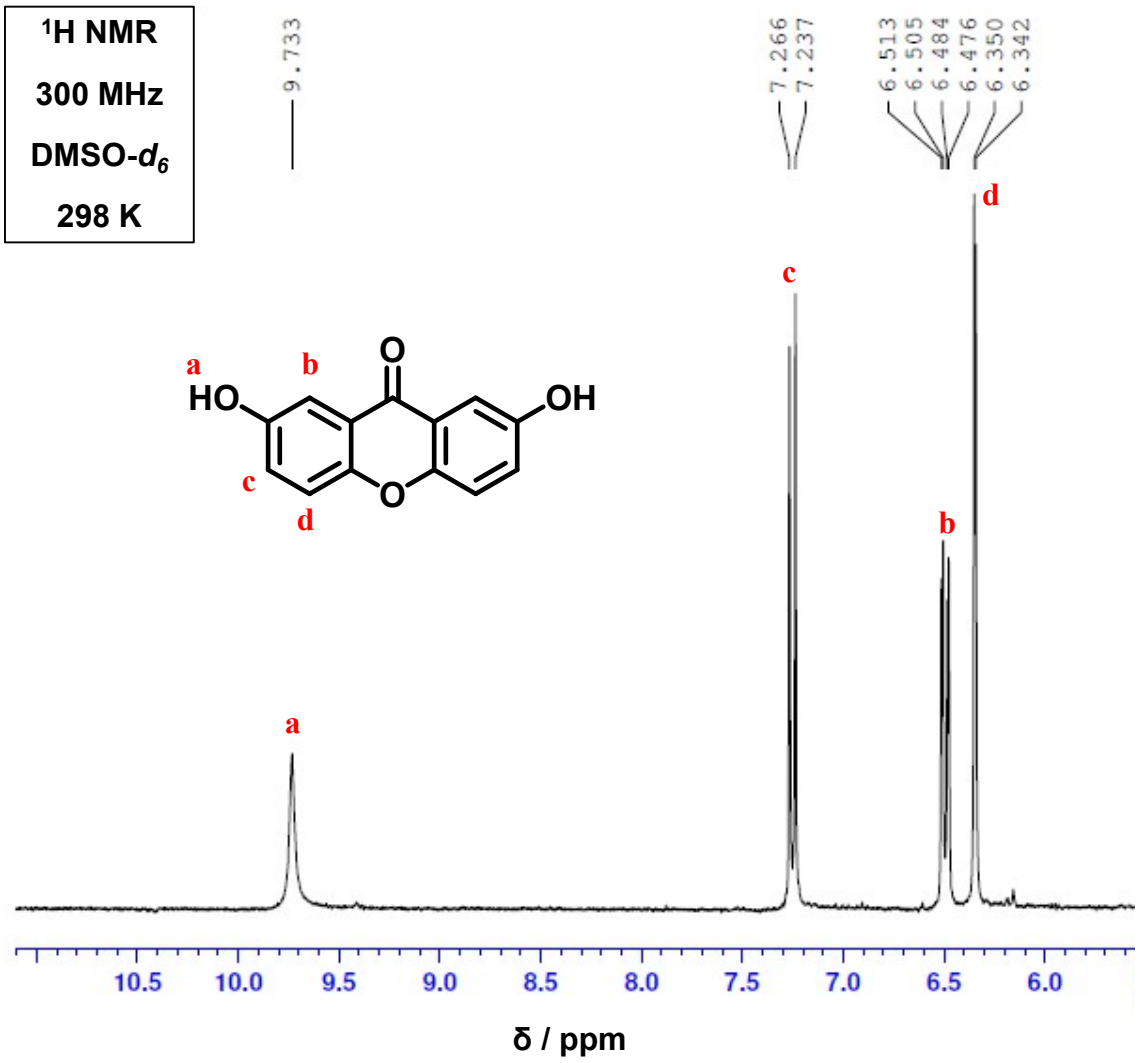


Figure S8. Annotated [1]H NMR spectrum (300 MHz, DMSO-d_6, 25 ºC) of 2,7-dihydroxyxanthone (synthesized by the Nencki Method[2]).

^{1}H-^{1}H 2D-COSY NMR of 2,7-dihydroxyxanthone in DMSO-d_6

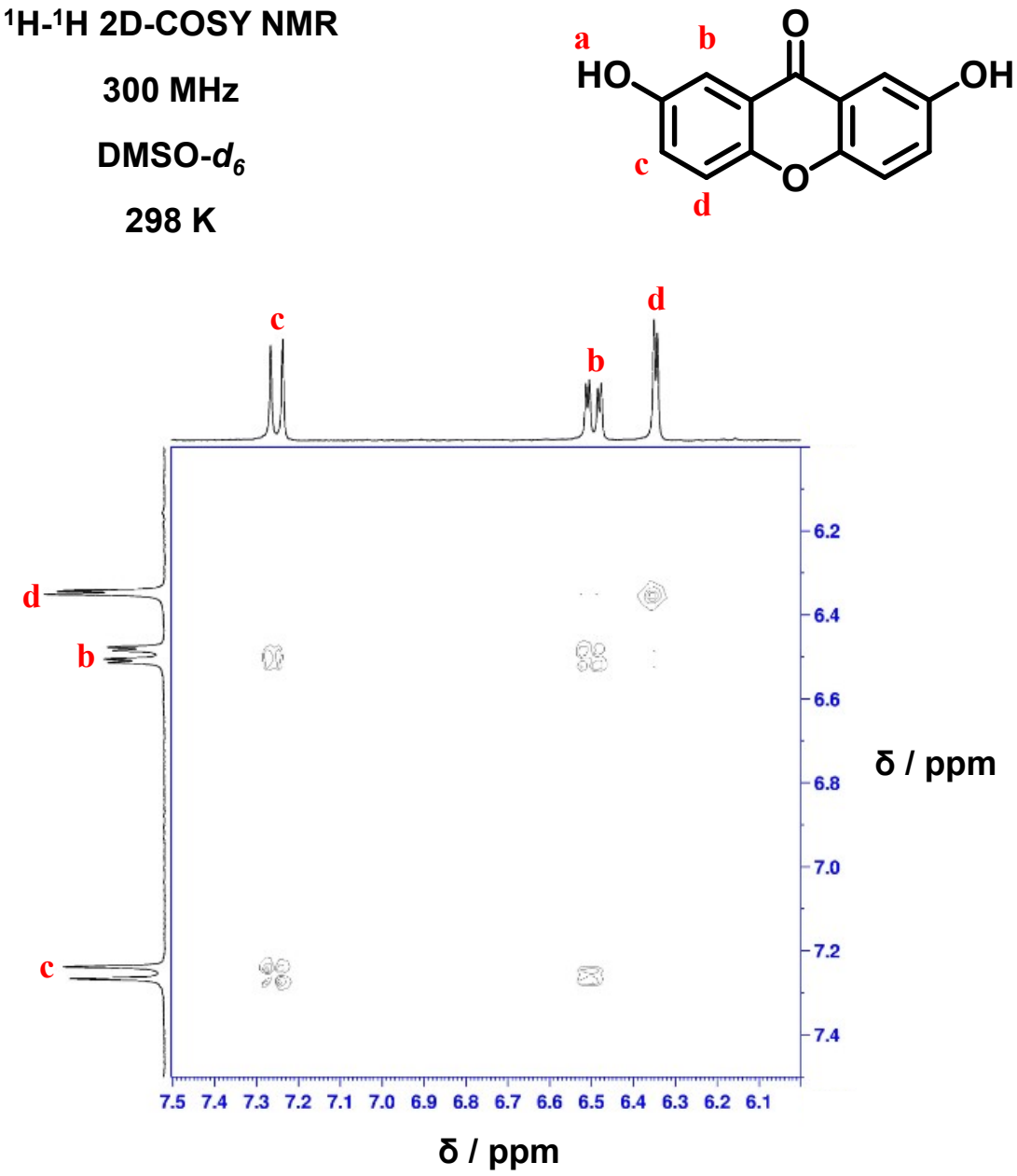


Figure S9. Annotated ^{1}H-^{1}H 2D-COSY NMR spectrum (300 MHz, DMSO-d_6, 25 ºC) of 2,7-dihydroxyxanthone.

^{1}H NMR Spectrum of 2,5,2',5'-tetramethoxybenzophenone (isomer 1) leading to 2,7-dihydroxyxanthone in $CDCl_3$

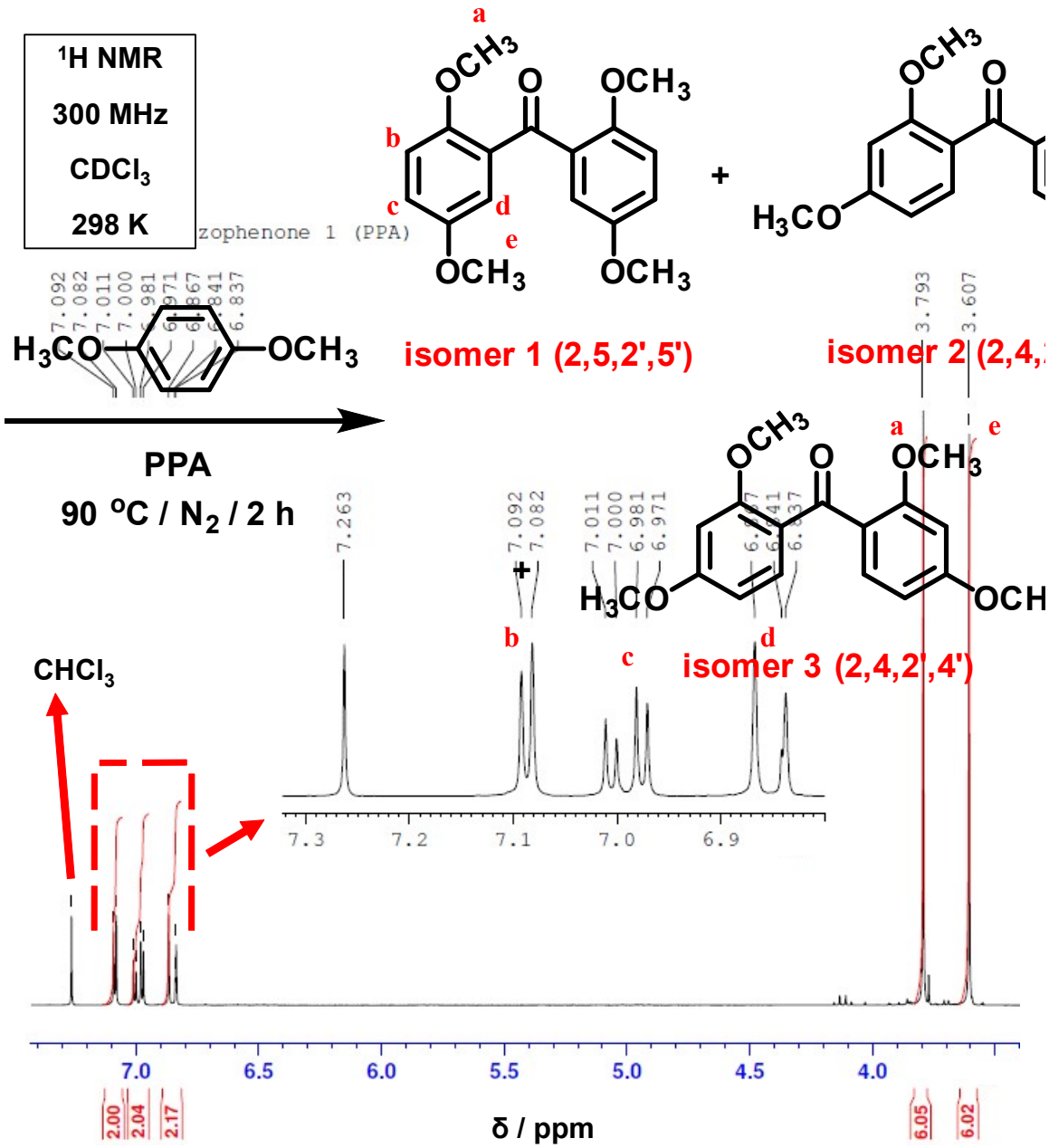


Figure S10. Annotated ^{1}H NMR spectrum (300 MHz, in $CDCl_3$, 25 ºC) of 2,5,2',5'-tetramethoxybenzophenone (***isomer* 1**) leading to 2,7-dihydroxyxanthone.

^{1}H NMR Spectrum of 2,4,2',5'-tetramethoxybenzophenone (isomer 2) leading to 2,6-dihydroxyxanthone in $CDCl_3$

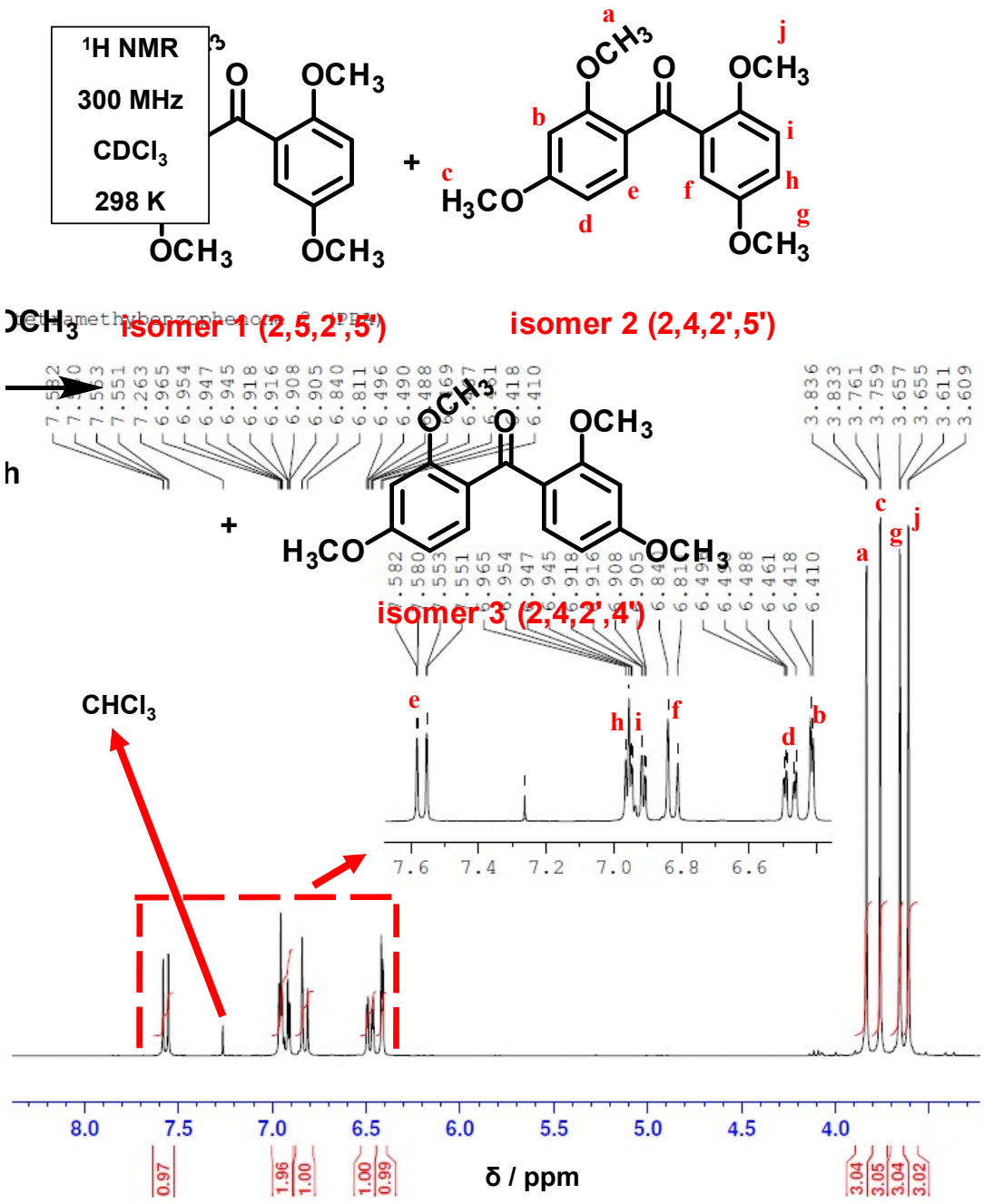


Figure S11. Annotated ^{1}H NMR spectrum (300 MHz, in $CDCl_3$, 25 ºC) of 2,4,2',5'-tetramethoxybenzophenone (***isomer* 2**) leading to 2,6-dihydroxyxanthone.

^{1}H NMR Spectrum of 2,4,2',4'-tetramethoxybenzophenone (isomer 3) leading to 2,6-dihydroxyxanthone in $CDCl_3$

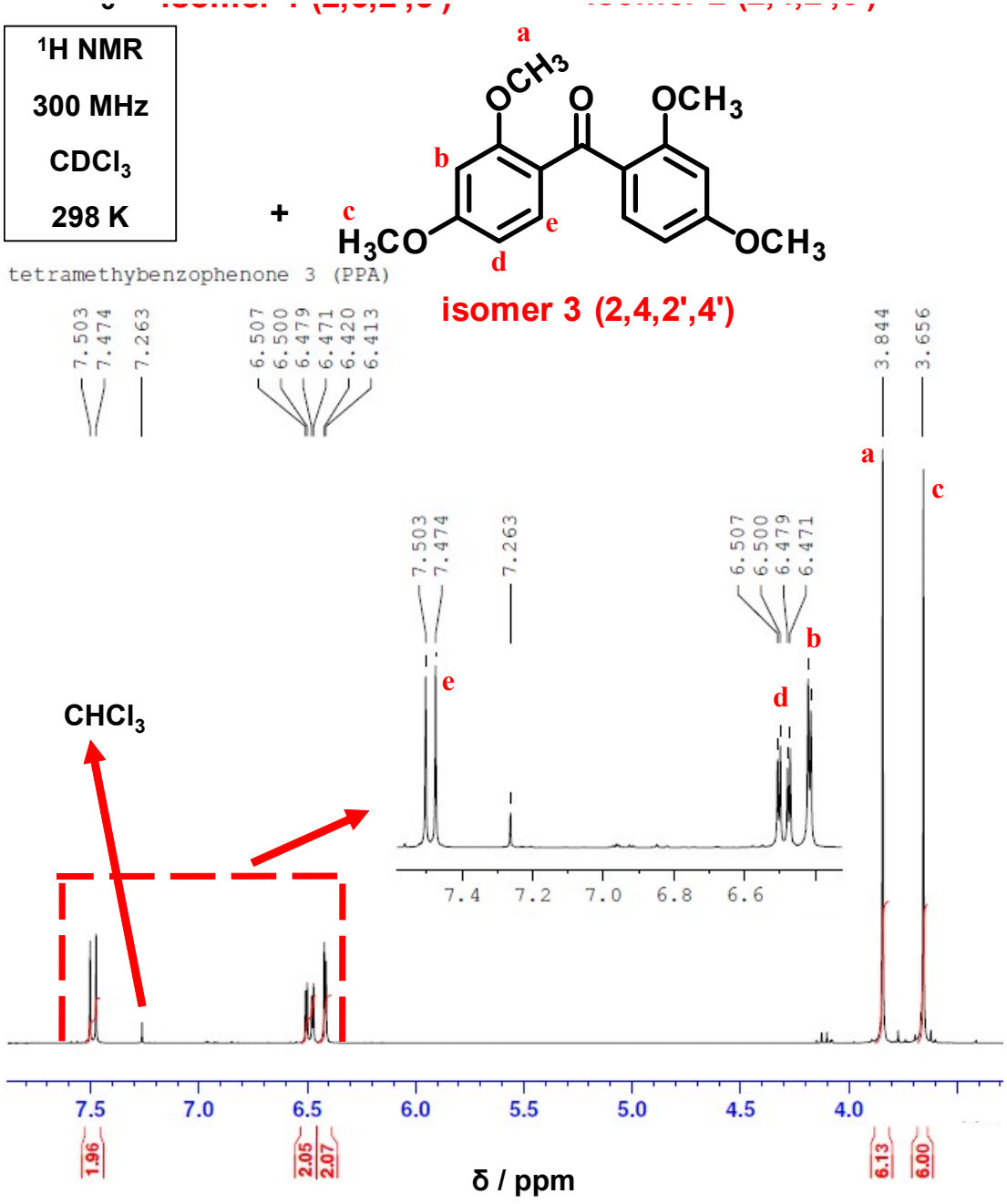


Figure S12. Annotated ^{1}H NMR spectrum (300 MHz, in $CDCl_3$, 25 ºC) of 2,4,2',4'-tetramethoxybenzophenone (***isomer* 3**) leading to 3,6-dihydroxyxanthone.

^{1}H NMR Spectrum of 2,6,2',5'-tetramethoxybenzophenone (key intermediate) leading to 1,7-dihydroxyxanthone in $CDCl_3$

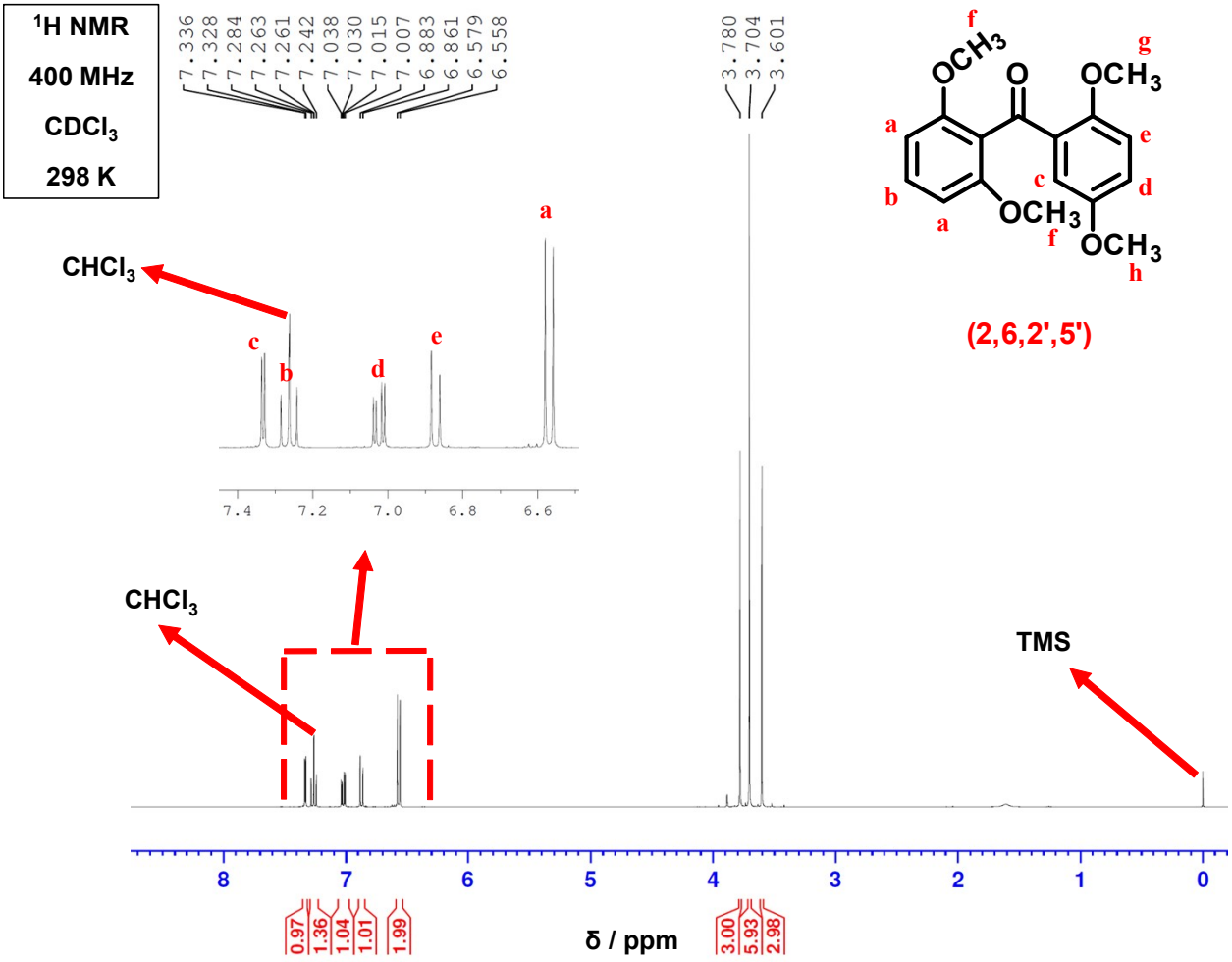


Figure S13. Annotated ^{1}H NMR spectrum (400 MHz, in $CDCl_3$, 25 ºC) of 2,6,2',5'-tetramethoxybenzophenone (***key intermediate***) leading to 1,7-dihydroxyxanthone.

^{13}C NMR Spectrum of 2,6,2',5'-tetramethoxybenzophenone (key intermediate) leading to 1,7-dihydroxyxanthone in $CDCl_3$

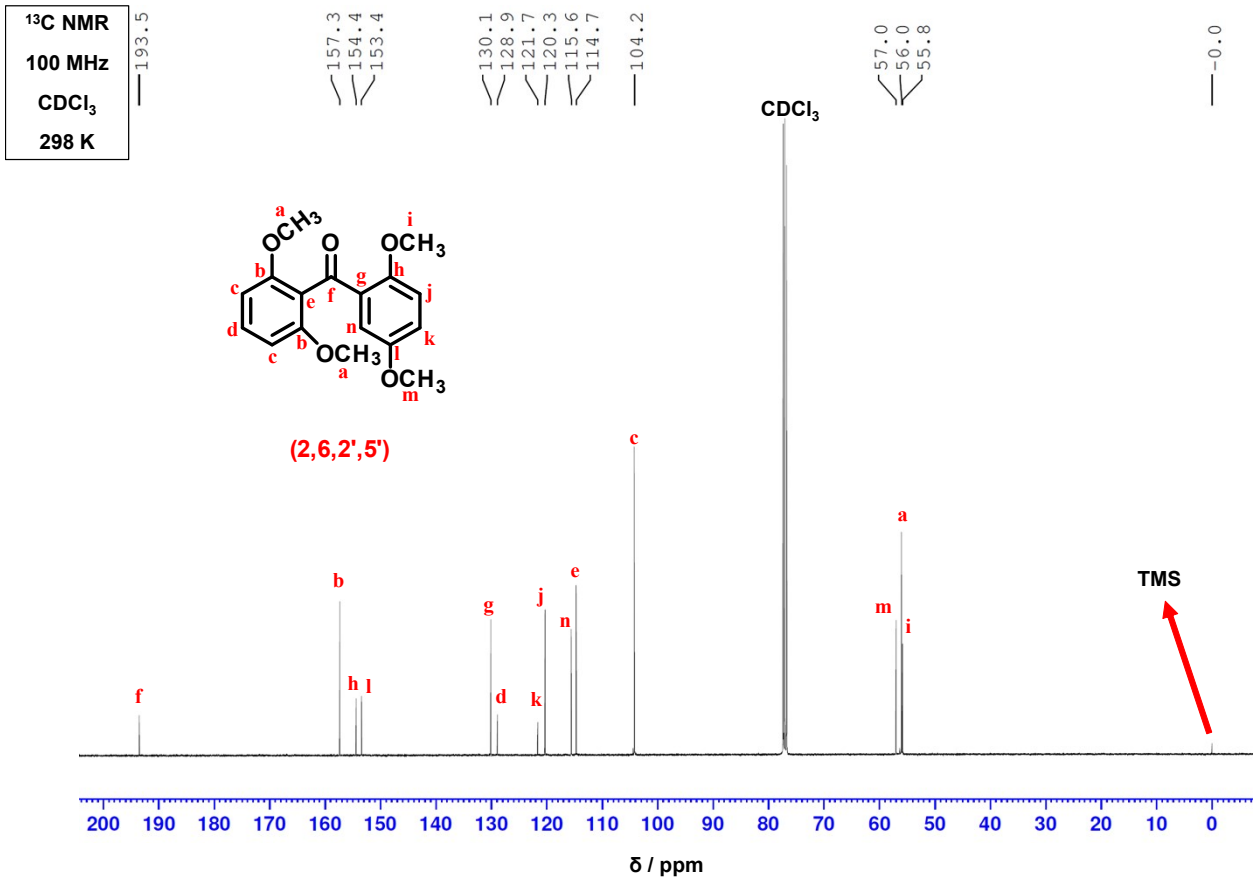


Figure S14. Annotated ^{13}C NMR spectrum (100 MHz, in $CDCl_3$, 25 ºC) of 2,6,2',5'-tetramethoxybenzophenone (***key intermediate***) leading to 1,7-dihydroxyxanthone.

Section D. Mass Spectrometry

Mass Spectrum of 1,7-dihydroxyxanthone

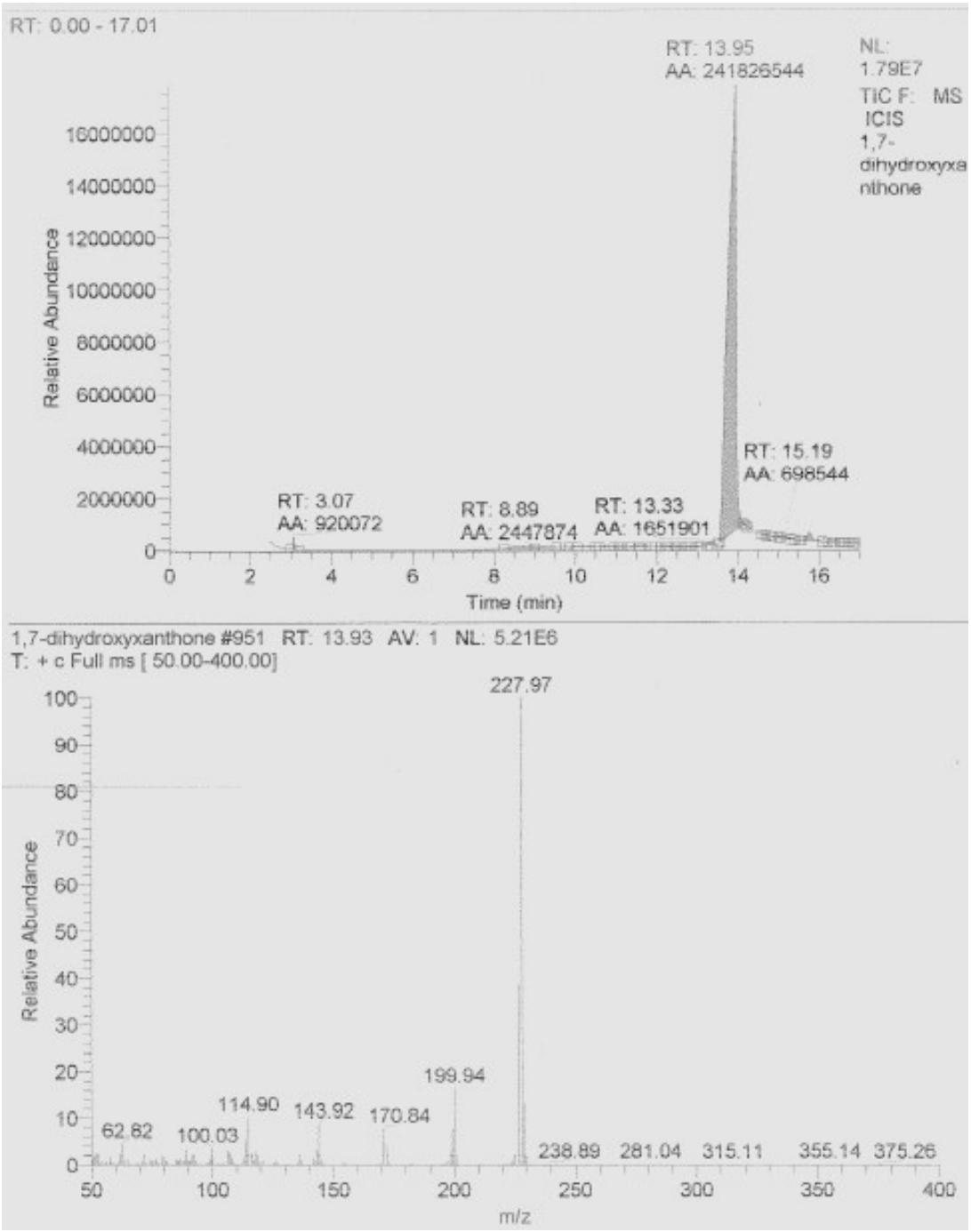


Figure S15. Mass spectrum of 1,7-dihydroxyxanthone (Euxanthone).

Mass Spectrum of 2,6,2',5'-tetramethoxybenzophenone

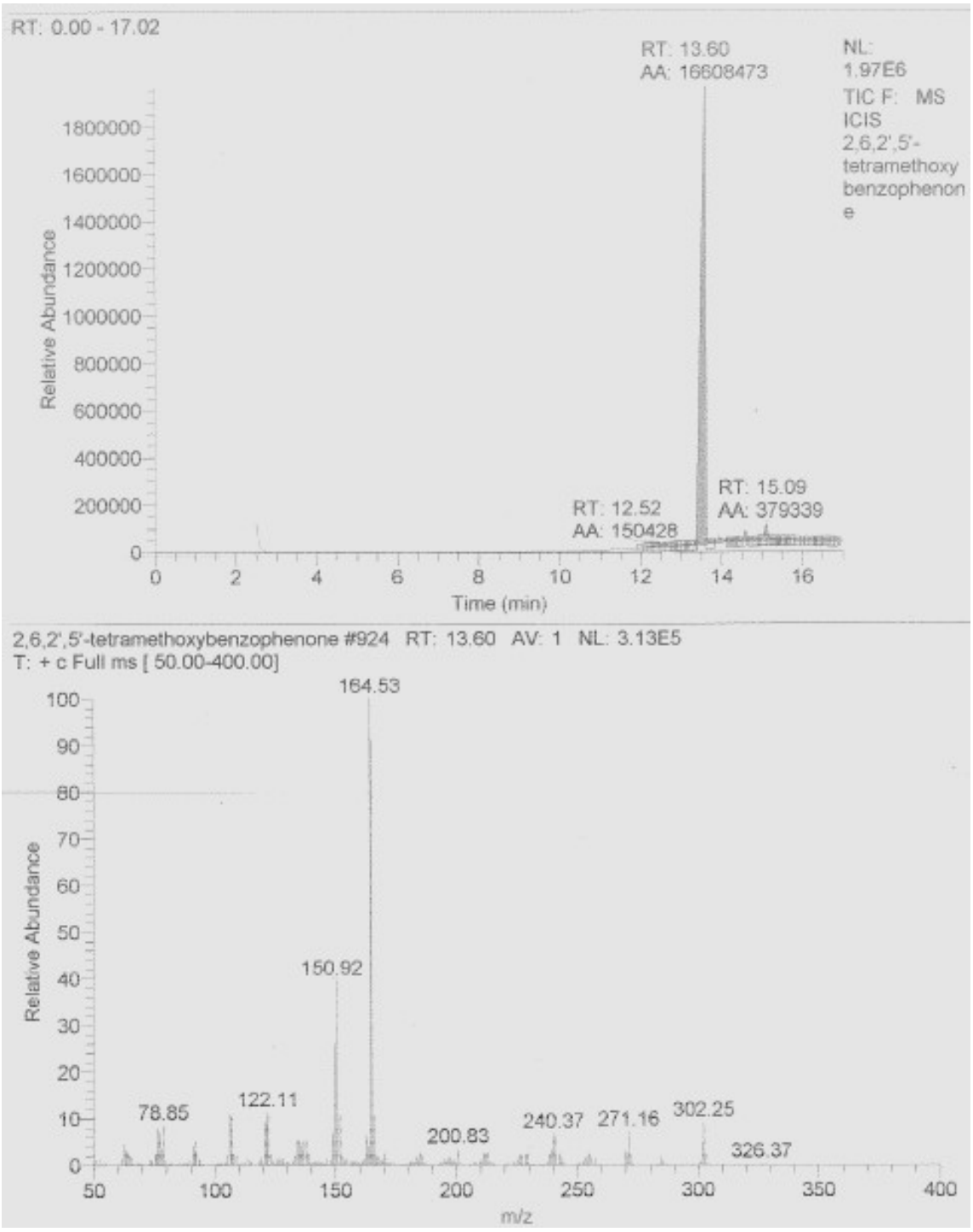


Figure S16. Mass spectrum of 2,6,2',5'-tetramethoxybenzophenone.

Mass Spectrum of 2,5,2',5'-tetramethoxybenzophenone (isomer 1)

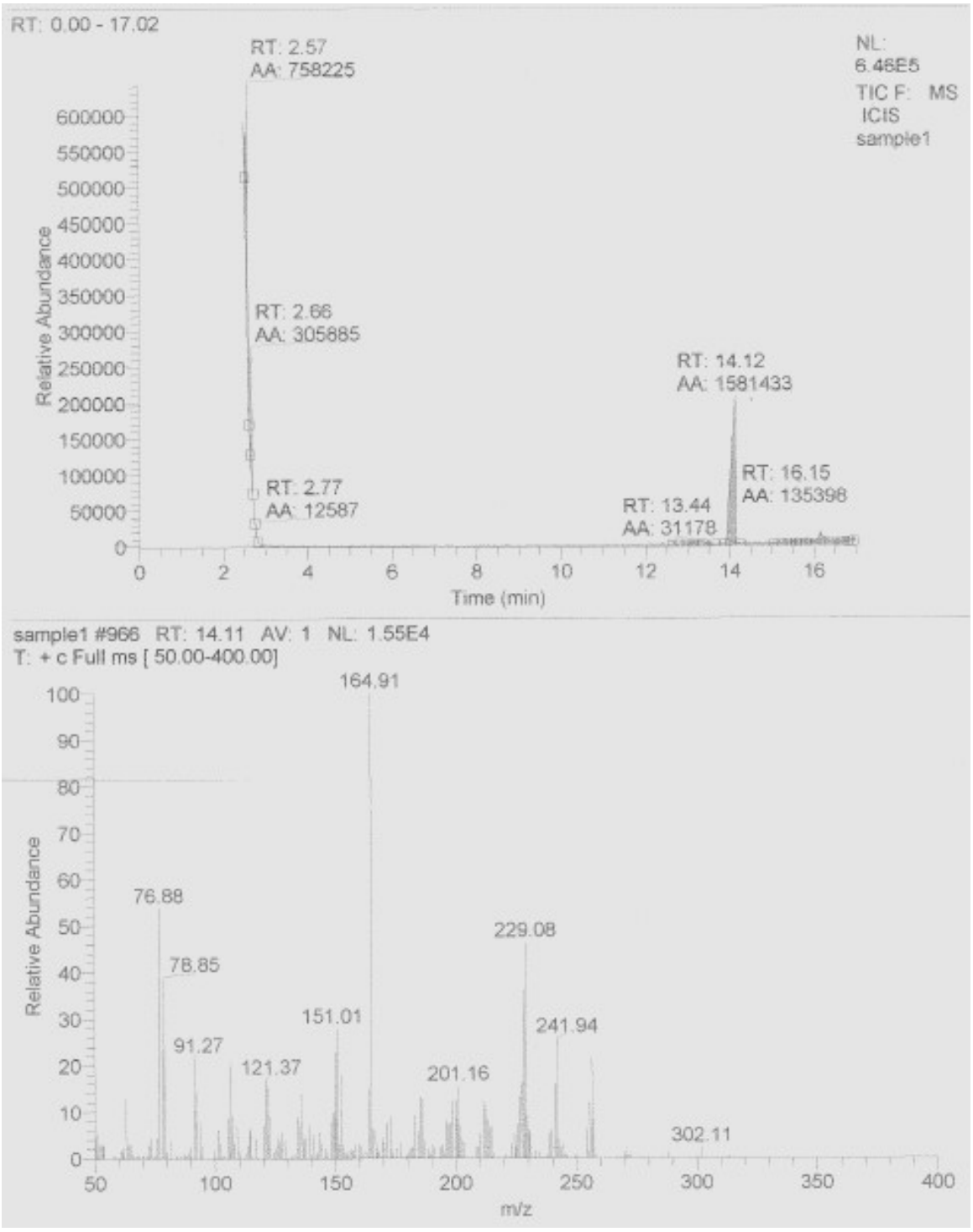


Figure S17. Mass spectrum of 2,5,2',5'-tetramethoxybenzophenone (**isomer 1**).

Mass Spectrum of 2,4,2',5'-tetramethoxybenzophenone (isomer 2)

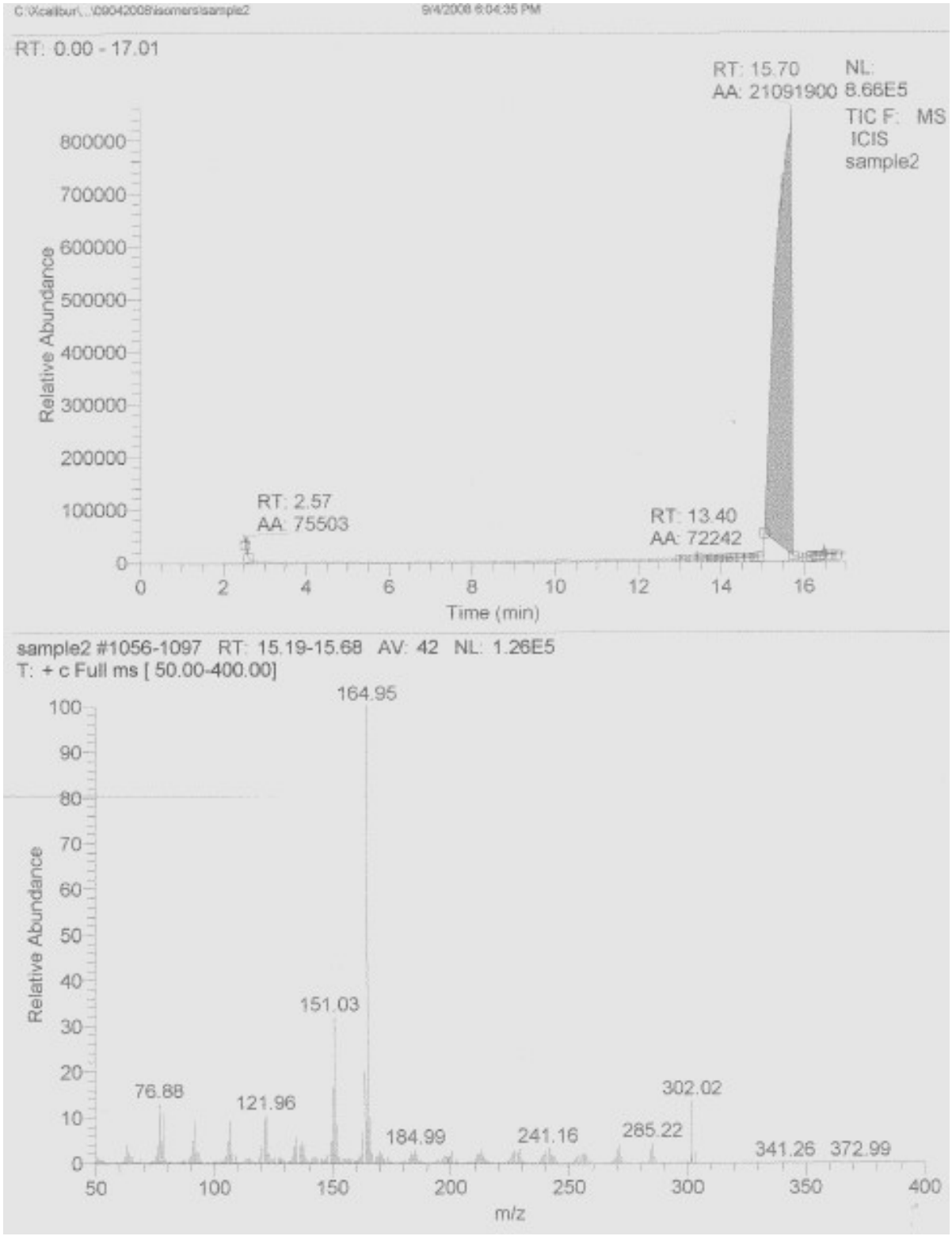


Figure S18. Mass spectrum of 2,4,2',5'-tetramethoxybenzophenone (**isomer 2**).

Mass Spectrum of 2,4,2',4'-tetramethoxybenzophenone (isomer 3)

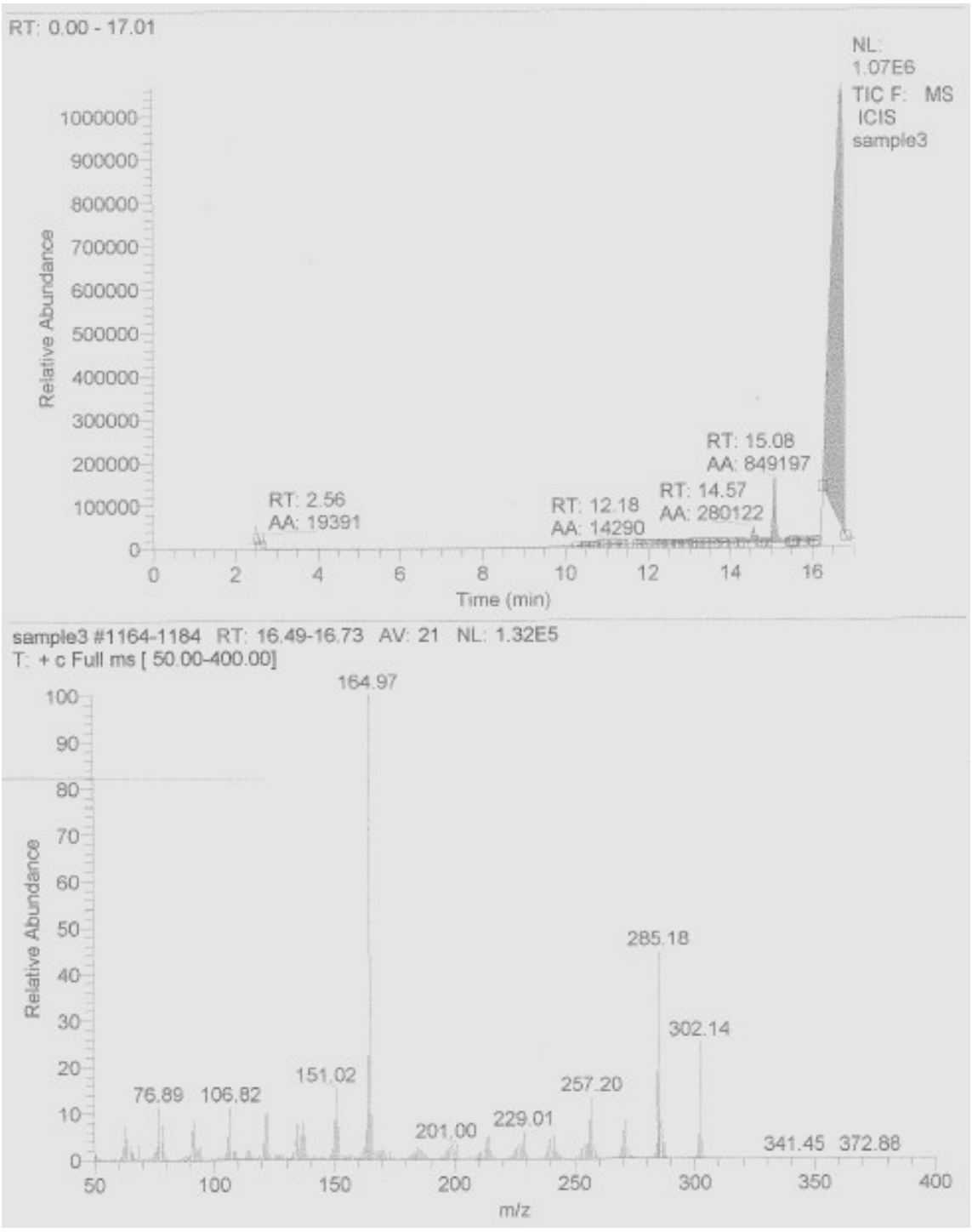


Figure S19. Mass spectrum of 2,4,2',4'-tetramethoxybenzophenone (**isomer 3**).

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