CHAPTER II

EXPERIMENTS

2.1 Instruments and Equipments

Melting points were determined with a Stuart Sciencetific Melting Point SMP1 (Bibby Sterlin Ltd., Staffordshire, UK). The FT-IR spectra were recorded on a Nicolet Fourier Transform Infrared Spectrophotometer: Impact 410 (Nicolet Instruments Technologies, Inc. WI, USA). Solid samples were incorporated into a pellet of potassium bromide and liquid samples were run as neat. The ^{1}H-NMR and ^{13}C-NMR spectra were obtained in deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) using Varian Mercury NMR spectrometer which operated at 400.00 MHz for ^{1}H and 100.00 MHz for ^{13}C nuclei (Varian Company, CA, USA). The mass spectra were recorded on Mass Spectrometer: Waters Micromass Quatto micro API ESCi (Waters, MA, USA.). Samples were dissolved in CH_2Cl_2 and direct injected to Mass Specrometer in 50 µL. The absorption spectra were recorded on UV-VISIBLE Spectrometer: UV-2550 (Shimadzu Corporation, Kyoto, Japan).

2.2 Chemicals

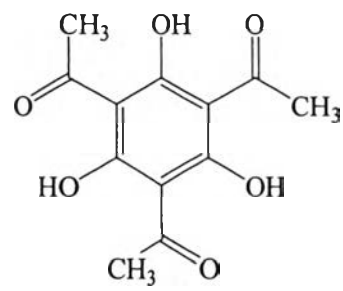
Thin layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel (Merck Kieselgel 60 F_{254}) (Merck KgaA, Darmstadt, Germany). Column chromatography was performed in silica gel (0.06-0.2 mm, 70-230 mesh ASTM), Merck Kieselgel 60 G (Merck KgaA, Darmstadt, Germany) and Scharlau Chemie S. A. (Barcelona, Spain). Solvents used in synthesis were reagent or analytical grades. Solvents used in column chromatography were distilled from commercial grade solvents prior to use. The reagents used for synthesizing were purchased from the following vendors:

- Labscan (Bangkok, Thailand): chloroform, concentrated hydrochloric acid, *n*-heptane, potassium hydroxide, toluene
- Acros Organic (New Jersey, USA): phloroglucinol dihydrate, octanoyl chloride

- Aldrich Chemical Company (Steinheim, Germany): allyl bromide, benzyl bromide, copper(II) acetate
- Carlo Erba Reagent (Milan, Italy): benzoyl chloride, benzyl chloride, anhydrous calcium chloride, iodine, anhydrous potassium carbonate
- Fluka Chemical Company (Buchs, Switzerland): *N,N*-dimethyl formamide, ethyl formate, triethylamine, anhydrous zinc(II) chloride
- Merck Co. Ltd. (Darmstadt, Germany): ethanol, anhydrous sodium hydrogencarbonate, sodium hydroxide, sodium iodide, anhydrous sodium carbonate, acetone
- Riedel-de Haën: anhydrous aluminum(III) chloride, anhydrous iron(III) chloride, anhydrous sodium sulfate
- May & Baker Co. Ltd. (Dogenham, England): chlorobenzene
- BDH Chemicals (Poole, England): potassium iodide, phenyl hydrazine
- Wilmad (New Jersey, USA): deuterated chloroform, hexadeuterated dimethylsulfoxide

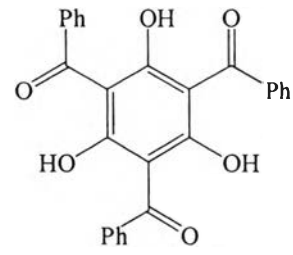
2.3 Synthesis of Hexasubstituted Benezene Derivatives

2.3.1 1,3,5-triacetyl-2,4,6-trihydroxybenzene 60



Phloroglucinol dihydrate (1,3,5-trihydroxy benzene dihydrate, 0.32 g, 2 mmol) was dissolved in excess acetyl chloride 5 mL and anh. $AlCl_3$ (1.30 g, 10 mmol) was then added. The reaction mixture was refluxed for 1 hour and then quenched with water (15 mL). The mixture was filtered and the precipitate was collected. The crude product was recrystalized in EtOH to give colorless needle crystals (0.43 g, 85% yield). m.p. 149-151 °C; IR (KBr, cm^{-1}): 3433 (O-H st), 1622 (C=O st), 1576 (C=C st) (**Figure A.3**); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.8 (s, 9H), 17.2 (s, 3H) (**Figure A.1**); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 32.9, 103.1, 175.8, 205.0 (**Figure A.2**); MS-H^+ (CH_2Cl_2): m/z = 253 (**Figure A.4**)

2.3.2 1,3,5-tribenzoyl-2,4,6-trihydroxybenzene 61



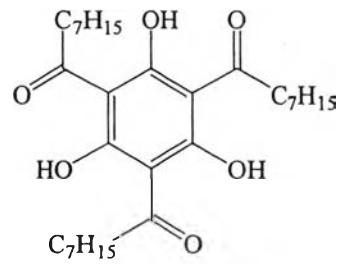
Method I The similar procedure as the synthesis of compound **60** was followed using benzoyl chloride as the electrophile and the medium in place of acetyl chloride but the reaction times were 2 hours. The soild crude was washed with 1M $NaHCO_3$ and then purified by column chromatography (80:20 Hexane/EtOAc) to give 1,3,5-tribenzoyloxybenzene (compound **63**, 0.68 g, 79% yield) as colorless needle crystals. m.p. 174-176 °C; IR (KBr, cm^{-1}): 3436 (O-H st), 1692, (C=O st), 1616 (C=C st) (**Figure A.15**); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.2 (s, 3H), 7.5 (t, 6H), 7.6 (t, 3H), 8.2 (d, 6H) (**Figure A.13**); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 113.3, 128.7, 129.0, 130.2, 133.9, 151.5, 164.4 (**Figure A.14**); MS-H^+ (CH_2Cl_2): m/z: 439 (**Figure A.16**)

The triester intermediate (0.44 g, 1 mmol) was rearranged to compound **61** by heating with anhydrous $AlCl_3$ (0.44 g, 3.3 mmol) at 150 °C for 3 hours. After cooling, 15 mL of 10% HCl was added to the mixture. The obtained precipitate was filtered and redissolved in 1 M NaOH. The insoluble solid impurities were removed by filtration. The mother liquor was reacidified with 10% HCl. The solid was collected and purified by recrystalization in EtOH (0.06 g, 13% yield). m.p. 168-170 °C; IR (KBr, cm^{-1}): 1744, (C=O st), 1604 (C=C st) (**Figure A.7**); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.4 (t, 6H), 7.5 (t, 3H), 7.6 (d, 6H), 14.6 (s, 3H) (**Figure A.5**); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 102.9, 128.0, 132.0, 140.3, 172.4, 199.9 (**Figure A.6**); MS-H^+ (CH_2Cl_2): m/z = 439 (**Figure A.8**)

Method II (one-pot) Phloroglucinol (0.16 g, 1 mmol) was dissolved in benzoyl chloride (0.7 mL, 6 mmol) and chlorobenzene (2 mL) was used as a solvent then $AlCl_3$ (1.33 g, 10 mmol) was added. The reaction mixture was stired at 150 °C under N_2 atmosphere for 5 hours and then quenched with 10% HCl (15 mL). The precipitate was filtered and collected. It was then redissolved in 1 M NaOH and the insoluble solid impurities were removed by filtration. The filtrate was reacidified with

10% HCl. The obtain precipitate was collected and purified by recrystalization in EtOH (0.16 g, 37% yield).

2.3.3 1,3,5-trihydroxy-2,4,6-trioctanoylbenzene 62

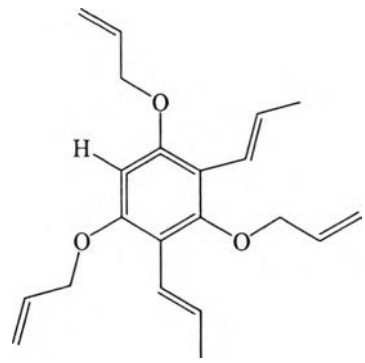


The similar procedure as the synthesis of compound **60** was followed using benzoyl chloride as the electrophile and the medium in place of acetyl chloride but the reaction times were 2 hours. When the reaction completed, it was quenched with 10% HCl (15 mL). The crude mixtured was extracted with hexane and the organic layer was dried with Na_2SO_4 anhydrous. The product was purified by column chromatography eluted with pure hexane, yielding the desired product as a yellow oil (0.14 g, 14% yield). m.p. 28-30 °C; IR (KBr, cm^{-1}): 3427 (O-H st), 2924 and 2851 (=C-H st), 1698, (C=O st), 1619, 1573 (C=C st) (**Figure A.11**); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.8-1.6 (m, 39H), 3.0 (t, 6H, J = 7.2 Hz), 17.2 (s, 3H) (**Figure A.9**); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 14.0, 22.6, 24.6, 29.1, 29.3, 31.7, 44.2, 102.9, 175.7, 208.0 (**Figure A.10**); MS-H^+ (CH_2Cl_2): m/z = 505 (**Figure A.12**)

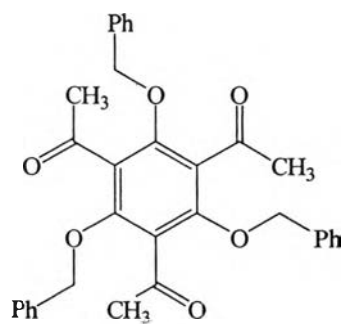
2.3.4 Allylation of phloroglucinol

The phloroglucinol (0.32 g, 2 mmol) was dissolved in DMF 15 mL and stirred with K_2CO_3 (4.14 g, 30 mmol) for 1 hour at refluxed temperature then added allyl bromide (1 mL, 12 mmol). After 3.5 hours and reaction was cooled down, 15 mL of water was added and the reaction mixture was neutralized with 10% HCl. The solution was extracted with CH_2Cl_2 (20 mL, 3 times) and the organic layer was separated and dried with Na_2SO_4 anhydrous. The concentrated crude product was purified by column chromatography (90:10 Hexane/EtOAc) to obtain a yellow oil product that has been indentified to be 1,3,5-triallyloxy-2,4-di(1-propenyl)benzene **67** (0.17 g, 35% yield). IR (neat, cm^{-1}): 3078 (=C-H st), 2980 and 2925 (C-H st), 1709 (C=O st), 1690-1610 (C=C st), 1216 (C-O st) (**Figure A.19**); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.5 (m, 6H), 2.66 (d, 1H, J = 8 Hz), 2.69 (d, 1H, J = 6.8 Hz), 4.43 (d, 2H, J =

5.5 Hz), 5.00 (d, 6H, J = 10.2 Hz), 5.02 (d, 2H, J = 4.5 Hz), 5.34 (d, 1H, J = 10.2 Hz), 5.41 (d, 1H, J = 16.5 Hz), 5.54 (m, 4H), 5.67 (s,1H), 5.98 (octet, 1H, J = 5.5 Hz) (**Figure A.17**); ^{13}C-NMR ($CDCl_3$): δ (ppm); 40.1, 41.8, 56.5, 62.8, 69.5, 104.4, 118.9, 119.0, 130.9, 132.5, 132.7, 174.2, 197.1, 209.2 (**Figure A.18**); MS-H^+ (CH_2Cl_2): m/z = 327 (**Figure A.20**)



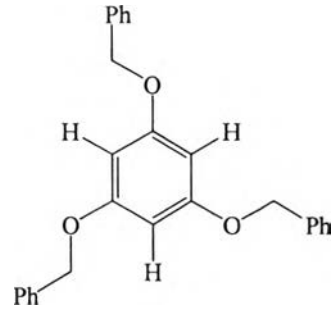
2.3.5 1,3,5-triacetyl-2,4,6-tribenzyloxybenzene 69



Compound **60** (0.25 g, 1 mmol) was dissolved in 20 mL MeCN and K_2CO_3 (2.07 g, 15 mmol) was added. The reaction mixture was stirred at reflux temperature for 1 hour and benzyl bromide (0.7 mL, 6 mmol) was added. Stirring was continued until no more substrate was found by TLC monitoring (about 8 hours). The reaction was then cooled down, added 15 mL of water and neutralized with 10% HCl. It was then extracted with CH_2Cl_2 (20 mL, 3 times) and the organic layer was separated and dried with Na_2SO_4 anhydrous. The concentrated crude product was recrystalized by 3:7 CH_2Cl_2/EtOH to give colorless needle crystals of the desired product (0.52 g, 65% yield). m.p. 189-191 °C; IR (KBr, cm^{-1}): 3027 (=C-H st), 2920 (C-H st), 1706 (C=O st), 1578 (C=C st), 1417 and 1361 (C-H bend), 1204 and 1085 (C-O st) (**Figure A.23**); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.5 (s, 9H), 4.9 (s, 6H), 7.4 (m, 15H) (**Figure**

A.21); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 32.7, 79.9, 128.6, 128.7, 128.9, 135.7, 153.8, 200.9 (**Figure A.22**); MS-H^+ (CH_2Cl_2): m/z = 523 (**Figure A.24**)

2.3.6 1,3,5-tribenzyloxybenzene 70



The phloroglucinol (0.32 g, 2 mmol) was dissolved in MeCN 15 mL and stirred with K_2CO_3 (4.14 g, 30 mmol) for 1 hour at refluxed temperature then added benzyl bromide (1.4 mL, 12 mmol). After 1.5 hours and reaction was cooled down, 15 mL of water was added and the reaction mixture was neutralized with 10% HCl. The solution was extracted with CH_2Cl_2 (20 mL, 3 times) and the organic layer was separated and dried with Na_2SO_4 anhydrous. The concentrated crude product was purified by column chromatography (80:20 Hexane/EtOAc) to give colorless needle crystals (0.54 g, 69% yield). m.p. 80-83 °C; IR (KBr, cm^{-1}): 3030 (=C-H st), 2863 (C-H st), 1601 (C=C st), 1451 and 1378 (C-H bend), 1159 (C-O st) (**Figure A.27**); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 5.03 (s, 2H), 5.05 (s, 1H), 6.32 (s, 2H), 6.34 (s, 1H), 7.4 (m, 15H) (**Figure A.25**); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 70.1, 70.3, 127, 128, 157.9, 158.7, 160.7 (**Figure A.26**); MS-H^+ (CH_2Cl_2): m/z = 397 (**Figure A.28**)

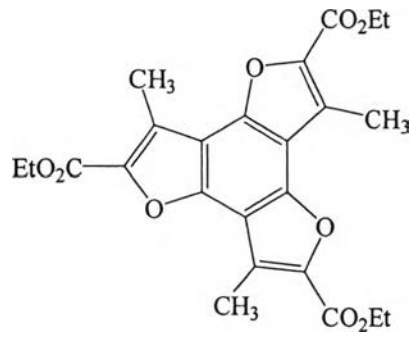
2.3.7 Trifuranyl triester derivative 71

Ethyl iodoacetate

Chloroacetyl chloride (5 mL, 63 mmol) was stirred in ice-bath and added ethanol (5 mL) dropwise in 10 minutes. The mixture was stirred at room temperature for 2 hours then the excess ethanol was removed to give the colorless oil of ethyl chloroacetate as the intermediate. After that, the intermediate was dissolved in acetone (20 mL), added NaI (10.4 g, 69 mmol) and refluxed for 3 hours. The reaction was filtered and the filtrate was collected and removed the solvent, yielding the ethyl iodoacetate as yellow oil in 89% overall from chloroacetyl chloride yield. IR (neat, cm^{-1}): 2981 (C-H st), 1730 (C=O st), 1092 (C-O st) (**Figure A.31**); ^{1}H-NMR ($CDCl_3$):

δ (ppm) = 1.2 (t, 3H, J = 7.2 Hz), 3.6 (s,2H), 4.1 (q, 2H, J = 7.2 Hz) (**Figure A.29**); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 0, 18.9, 67.1, 173.9 (**Figure A.30**)

Trifuranyl triester derivative 71



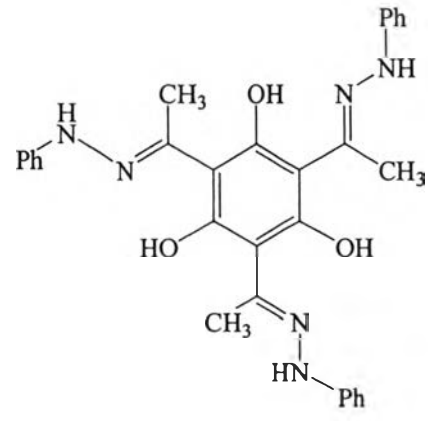
Compound **60** (0.25 g, 1 mmol) was dissolved in 20 mL MeCN and K_2CO_3 (2.07 g, 15 mmol) was added. The reaction mixture was stirred at reflux temperature for 1 hour and ethyl iodoacetate (1.28 g, 6 mmol) was added. Stirring was continued until no more substrate was found by TLC monitoring (about 23 hours). The reaction was then cooled down, added 15 mL of water and neutralized 10% HCl. It was then extracted with CH_2Cl_2 (20 mL, 3 times) and the organic layer was separated and dried with Na_2SO_4 anhydrous. Then MeCN was added in the concentrated crude product and the precipitate was filtered and collected. It was recrystalized by EtOAc to give colorless needle crystals of the desired product (0.13 g, 18% yield). m.p. 269-273 °C; IR (KBr, cm^{-1}): 2972 (C-H st), 1714 (C=O st), 1581 (C=C st), 1267-1075 (C-O st) (**Figure A.34**); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.4 (t, 9H, J = 7.2 Hz), 2.8 (s,9H), 4.4 (q, 6H, J = 7.2 Hz) (**Figure A.32**); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 9.0, 13.3, 60.0, 110.7, 123.9, 139.8, 147.4 (**Figure A.33**); MS-H^+ (CH_2Cl_2): m/z = 457 (**Figure A.35**)

***N*-Benzyl Iodoacetamide**

Chloroacetyl chloride (5 mL, 63 mmol) was added in CH_2Cl_2 (20 mL) and stirred in ice-bath and added benzylamine (9 mL, 82 mmol) dropwise in 10 minutes. The mixture was stirred at room temperature for 1 hours then the excess CH_2Cl_2 was removed to give the white solid of *N*-benzyl chloroacetamide as the intermediate. After that, the intermediate was dissolved in acetone (20 mL), added NaI (10.4 g, 69 mmol) and refluxed for 3 hours. The reaction was filtered and the filtrate was collected and removed the solvent, yielding the *N*-benzyl iodoacetamide as white solid in 94% overall from chloroacetyl chloride yield. m.p. 103-106 °C; IR (KBr,

cm^{-1}): 3276 (N-H st), 1632 (C=O st), 1540 (C=C st), 1170 (C-N st) (**Figure A.38**); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.6 (s,2H), 4.4 (s,2H), 6.8 (s, br, 1H), 7.2-7.4 (m, 5H) (**Figure A.36**); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 0, 45.0, 128.3, 129.5, 138.1, 168.0 (**Figure A.37**)

2.3.8 Phenylhydrazone derivative 72



Compound **60** (0.25 g, 1 mmol) was dissolved in CH_2Cl_2 (20 mL) and phenylhydrazine (0.6 mL, 6mmol) was added. The reaction mixture was stirred at room temperature until no more the substrate was found by TLC monitoring (about 3 hours). The solvent was removed and the concentrated crude was purified by column chromatography (90:10 Hexane/EtOAc) to give a yellow solid (93 mg, 18% yield). m.p. 178-180 °C; IR (KBr, cm^{-1}): 3306 (N-H), 1606-1410 (C=N st and C=C st), 1263 (C-N st) (**Figure A.41**); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.5 (s, 9H), 6.9 (t, 3H), 7.0 (d, 6H), 7.3 (t, 6H), 15.4 (s, 3H) (**Figure A.39**); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 33.3, 103.7, 113.1, 120.9, 129.5, 144.2, 160.0 (**Figure A.40**); MS-H^+ (CH_2Cl_2): m/z = 523 (**Figure A.42**)

3.4 Procedures for Complexes Analysis

3.4.1 UV-VISIBLE Spectrophotometry

The 0.1 mM in EtOAc solution of compound **72** was prepared in a 10 mL volumetric flask. The resulting stock solution was then diluted to 0.01 mM in 10 mL. For the metal salts, the stock solutions were prepared 4 mM in EtOAc and then diluted to 0.2 mM in 5 mL. The mixture of 2 mL of final dilution of compound **72** and 1.5 mL of final dilution of salt (1:15 ligand/metal) were mixed and shaked together

for 2 minutes. The UV absorbance of each final dilution was recorded by using a sample in 1 cm quartz cell and scanning wavelengths between 200 and 800 nm.

3.4.2 ^{1}H-NMR Spectroscopy

Compound **71** (5 mg, 0.01 mmol) and metals (0.05 mmol), $FeCl_3$ (8.1 mg), $Cu(OAc)_2$ (7.5 mg), $Co(OAc)_2{\cdot}4H_2O$ (10.9 mg), $Zn(OAc)_2{\cdot}2H_2O$ (9.4 mg), were dissolved in $CHCl_3$ 2 mL then the mixture were stirred at room temperature for 5 days. The solvent was removed and crude mixture was redissolved in $CDCl_3$ for NMR analysis