CHAPTER 2
A novel approach towards syntheses of Levuglandins
E₂ and D₂ analogues
2.1 Abstract

Design and development of a novel approach for the synthesis of Levuglandins LGE₂ analogues 70 using the cheap and readily available starting materials isopropenyl acetate 61 and methyl oleate 62 has been reported. The reaction sequence involves photochemical [2+2] cycloaddition, hydrolysis, esterification, elimination and oxidative cleavage. All the intermediates were isolated using chromatographic techniques and characterized by their spectral and analytical analysis.
2.2 Introduction and objective

Lipids are essential components of cell membranes. They incorporate numerous polyunsaturated fatty acids (PUFAs) and are extremely important compounds in all organisms. PUFAs are the substrates for the various enzymatic and non-enzymatic transformations that provide a variety of important signalling molecules, mediators, and other biologically active metabolites.\(^1\) The main PUFAs in living organisms are \(\alpha\)-linolenic acid (LA) \(^1\), arachidonic acid (AA) \(^2\), eicosapentaenoic acid (EPA) \(^3\) and docosahexaenoic acid (DHA) \(^4\). (Figure 2.1)

\[
\begin{align*}
&\text{\(\alpha\)-Linolenic acid 1} \\
&\text{Arachidonic acid 2} \\
&\text{Eicosapentaenoic acid 3} \\
&\text{Docosahexaenoic acid 4}
\end{align*}
\]

**Figure 2.1:** Structures of polyunsaturated fatty acids

Lipid oxidation in various biological systems contributes in normal physiological processes and is involved in the development of many chronic and inflammatory diseases such as antiphospholipid antibody syndrome, rheumatoid arthritis, multiple sclerosis and bowel diseases.\(^2\) The presence of aerobic environment and readily oxidizable nature of polyunsaturated fatty acids plays a very important role in human health. The first observation of lipid oxidation problems was observed by the Swiss chemist Nicholas-Theodoro who noticed around 1800 that oil became viscous and had a bad smell when exposed to air. A systematic study on lipid auto oxidation was initiated around 1940 by Criegee *et al.* They proposed that hydroperoxides are the primary products of hydrocarbon oxidation.\(^3\) The detailed study of auto oxidation of polyunsaturated fatty acid was initiated in 1970 by several research groups disclosing complex mixtures than those previously proposed.\(^4\)
The most thoroughly studied enzymatic oxidation of a polyunsaturated fatty acid (PUFA) is the conversion of arachidonic acid into prostaglandins (PGs). Prostaglandins were first detected in 1930 by Kurzrok and Lieb due to their biological activity. They showed that the human semen could induce strong contractions or relaxations when applied to a human uterus. A few years later, Von Euler and Goldblatt demonstrated independently the presence of a vasodepressor agent and a stimulating factor of muscles in human seminal plasma and sheep vesicular glands. Von Euler indicated that the biological activity was due to a lipid-soluble material with acidic properties and named it as prostaglandin. About 30 years after the discovery of the biological activity of prostaglandins, Bergstrom, Sjovall, and Samuelsson isolated the first two prostaglandins PGE \textsubscript{1} 5, PGF \textsubscript{2\alpha} 6 and elucidated their structures in 1960. (Figure 2.2)

![Diagram of PGF \textsubscript{1} 5 and PGF \textsubscript{2\alpha} 6]

(Figure 2.2: The structures of PGE \textsubscript{1}, PGF \textsubscript{2\alpha})

Levuglandins (LGs) and isolevuglandins (isoLGs) are the products of lipid peroxidation that are biologically active. The discovery of LGs was the result of an effort to elucidate the chemistry of prostaglandin endoperoxide PGH \textsubscript{2} 7. PGH \textsubscript{2} 7 is the cyclooxygenase metabolite of the arachidonic acid (AA) 2. It is a key intermediate in regulating the wide variety of cellular activities and undergoes various enzymatic and non-enzymatic rearrangements to provide physiologically active molecules. The spontaneous rearrangement of PGH \textsubscript{2} produces prostaglandins E \textsubscript{2} (PGE \textsubscript{2}) 8 and prostaglandins D \textsubscript{2} (PGD \textsubscript{2}) 9.\textsuperscript{10}

Salomon et al proposed that, rearrangement of PGH \textsubscript{2} 7 generates two levulinaldehyde derivatives termed as Levuglandins, LGE \textsubscript{2} 10 and LGD \textsubscript{2} 11, because of their structural similarities with levulinaldehyde and their relation to Prostaglandins.
E₂ and D₂.¹¹ (Scheme 2.1) Both the levuglandins 10 and 11 are chemically sensitive compounds and are converted into anhydrolevuglandins, AnLGE₂ 12 and AnLGD₂ 13, after losing a water molecule. They also produce Δ⁹-LGD₂ 14 and Δ⁹-LGD₂ 15 isomers by allylic rearrangement.¹¹,¹²

Scheme 2.1: Rearrangement of PGH₂ generation of PGs and LGs

In addition to the rearrangement of AA 2 to PGH₂ 7 via the cyclooxygenase pathway Saloman et al reported the formation of other structurally different prostaglandin endoperoxide isomers by non-enzymatic free radical induced oxidation of arachidonic acid phospholipid (AA-PL) 16.¹³ They have discovered that the non-enzymatic free radical-induced oxidation of arachidonic acid phospholipid (AA-PL) gave four structurally different phospholipid endoperoxide stereoisomers that are named as isoprostanes (isoPS) 17-20. The rearrangement of these endoperoxide

**Scheme 2.2:** Free radical-induced oxidation of arachidonic acid phospholipid (AA-PC) generation of isoLGs

LGs and isoLGs are highly cytotoxic, and bind covalently with the amino group in proteins to produce covalent adducts with greater avidity than the other lipid oxidation products.¹⁴,¹⁵

Salomon et al have proposed that the electrophilic γ-ketoaldehyde functionality in LGs has an extraordinary proclivity towards rapid covalent adduction with biomolecules.¹⁶ The LGs 29 initially bind covalently with the amino group in proteins 30 via Paal-Knorr condensation to produce Schiff base adducts 31 which are transformed into pyrrole derivatives 33 by rapid cyclization and dehydration.¹⁷,¹⁸ These highly alkylated pyroles 33 are chemically sensitive compounds. In the
presence of oxygen they get oxidized to stable end products such as lactams 34 and hydroxy lactams 35. The LG-protein Schiff base adducts 31 also bind with primary amino group of other proteins leading to the formation of protein-protein crosslinks 36 and 37. LGs also cause DNA-protein cross-links (DPCs) 38, 39 which are responsible for cell killing.\textsuperscript{24} (Scheme 2.3)

*Scheme 2.3: Covalent adduction of LGs with protein*
The protein-protein crosslink and protein polymerisation also occur in conjunction with the binding and are associated with various diseases such as alzheimer’s diseases (AD), atherosclerosis, renal diseases etc.\textsuperscript{21,22}

Levuglandin-protein adducts are detected \textit{in vivo} by using polyclonal rabbit antibodies and are used as markers of oxidative injury.\textsuperscript{19} The mean levels of isoLGs-protein adducts, as well as LGE\textsubscript{2}-protein adducts, are elevated in the plasma of individuals with atherosclerosis and renal disease as compared to normal individuals.\textsuperscript{22,23} Hence, the LG-protein adducts provide a quantitative assessment of oxidative stress.\textsuperscript{24}

The synthesis of LGs constitutes a challenge for organic chemists due to their complicated structures. Only few methods for the syntheses of LGs have been developed by Salomon and co workers.\textsuperscript{25} LGE\textsubscript{2} was the first member of levuglandin family synthesised by a chemist. Salomon \textit{et al} has reported the synthesis of LGE\textsubscript{2} form ketophosphonoacetone \textbf{41} outlined in \textbf{scheme 2.4}. The alkylation of \textbf{41} with alkyl bromide \textbf{42} in the presence of NaH gave the corresponding ketophosphonate \textbf{43}. The Horner-Emmons condensation of sodium salt of \textbf{43} with isopropylidene glyceraldehydes \textbf{44} furnished nonracemic chiral enones \textbf{45E} and \textbf{45Z}. The magnesium bromide catalysed 1,4-addition of cuprate \textbf{46} with isomeric enone either \textbf{45E} or \textbf{45Z} afforded an identical mixture \textbf{47SR} and \textbf{47RR}. Deprotection of three hydroxy groups of \textbf{47SR} and \textbf{47RR} with acetic acid water followed by oxidative cleavage of triol \textbf{48} using NaIO\textsubscript{4} furnished the LGE\textsubscript{2} methyl ester \textbf{49RR} and \textbf{49SR}.\textsuperscript{25a} (Scheme 2.4) Following similar procedure Salomon \textit{et al} has also reported the synthesis of iso[4]LGE\textsubscript{2}, iso[7]LGD\textsubscript{2} and 17-isoLGE\textsubscript{4} natural products.\textsuperscript{25b-d}
Scheme 2.4: synthesis of LGE₂

In the area of synthesis of LGs natural products the Amarnath et al reported the synthesis of LGE₂ from methyl-7-bromoheptanoate 50 and oct-1-yne-3-one 54. The condensation of 50 with ketophosphonoacetone 41 using sodiumhydride in THF
furnished 51. The compound 51 gave the precursor 53 by condensation with 52 in presence sodium hydride. Similarly the stannane 57 was prepared from 54 in three steps. The reduction of 54 with sodium borohydride gave the hydroxy alkyne 55 which on treatment tert-butylidimethylsilyl chloride afforded corresponding silyl derivative 56. The silyl derivative 56 converted to stannane 57 on heating in presence of tributyltin hydride. The treatment of enone 53 and stannane 57 with CuCN in presence of methyllethium in THF afforded the protected LGE₂ 58. The removal of the silyl group using bromine walter followed by hydrolysis of ester group using lithium hydroxide and acetal by K-10 gave the LGE₂ 60. (Scheme 2.5)

**Scheme 2.5: Synthesis of Levuglandin E₂**
In this chapter the design and development of a novel and general method for the construction of LGs analogues via a sequence [2+2] cycloaddition, hydrolysis, elimination and oxidative cleavage using cheap and readily available starting materials like isopropenyl acetate 61 and methyl oleate 62 has been reported. (Scheme 2.6)

Scheme 2.6: Synthesis of LGE$_2$ analogue 70
2.3 Results and discussion

Photochemical reactions are most versatile tools in synthetic organic chemistry towards target oriented synthesis of the natural products and complex molecular architectures. In photochemical reactions, the activation of substrate molecules and their transformation to the desired products takes place by absorption of electromagnetic radiation. The reactant molecule absorbs energy in the form of radiation and goes to an excited state. The excited states of molecules are rich in energy therefore reactions occurred in these states may be highly endothermic in the ground state. Numerous organic transformations can be achieved in sunlight or visible light with renewable energy sources. Thus a photochemical reaction provides the most significant way to access the exceptional molecular structures that cannot be achieved by other conventional methods. Photochemical transformation occurs without the use of any chemical reagents, thereby providing a greener pathway to the reactions.

Different types of photochemical transformations such as photo cycloaddition, photo rearrangement, photo electron-transfer, photo Friedel-Crafts, photo oxygenation and their use in assembling of highly functionalised structures and polycycles have been established from very simple and readily available materials. Among all the photochemical reactions, the photo cycloaddition reaction in alkenes leading to cyclobutane is a valuable organic transformation to achieve the total syntheses of various natural products.

Towards the synthesis of the LG's analogue 70, a $\pi^2 + \pi^2$ photocycloaddition reaction of 61 and methyl oleate 62 was performed under UV radiation in a quartz immersion well using a low pressure mercury lamp. Thus, a solution of 61 and 62 in acetone was irradiated with UV irradiation for 3.5h while maintaining temperature between 10-15 °C. After completion of the reaction the solvent was removed in a rotary evaporator under reduced pressure which gave a thick yellow liquid. This liquid was then chromatographed over a column of silica gel. The elution of column with a mixture of light petroleum and ethyl acetate furnished products 63 at $R_f$ 7.5 and 64 at $R_f$ 6.0 in almost equal amounts. (Scheme 2.6)

The structures of both the products 63 and 64 were readily discernible through their FTIR, $^1$H and $^{13}$C NMR and mass analysis data. FTIR spectrum of the
cycloadduct 63 showed a band at 1240 cm\(^{-1}\) for the C-O-C stretching of the acetate group along with bands at 1723, 1735 cm\(^{-1}\) for the characteristic carbonyl groups. Its \(^1\)H NMR spectrum gave a triplet at δ 0.87, singlets at δ 2.05, 2.38, 3.66 for the protons of four methyl groups along with signals between δ 0.90-1.56 for the protons on methylene groups. It also displayed the multiplet between δ 2.10-2.12 for the methine protons. The \(^{13}\)C NMR spectrum of 63 was also consistent with the proposed structure. It gave resonances at δ 13.52, 24.32, 27.60, 51.39 for the four methyl carbons and peaks at δ 28.98, 29.01, 29.04, 29.07, 29.25, 29.38, 29.50, 29.58, 29.73, 31.82, 31.85, 33.91, 33.98, 34.01, 38.30 (15C, CH\(_2\)) for the methylene carbons. Similarly, the signals at δ 49.01, 50.01 for methine carbons, a signal at δ 81.68 for the carbon attached with acetate group and signals at δ 177.41, 177.45 for the two carbonyl carbons. The structure was further confirmed by its mass spectrum which gave a molecular ion peak at 396.21.

The FTIR spectrum of 64 showed a band at 1166 cm\(^{-1}\) for the C-O stretching of the acetate group along with 1730 and 1750 cm\(^{-1}\) for the characteristic carbonyl groups. Its \(^1\)H NMR spectrum exhibited a triplet at δ 0.87, singlets at δ 1.61, 2.31 and 3.59 for the protons of four methyl groups. It also displayed multiplets between δ 0.97-1.62, 2.01-2.18 for the protons of the methylene groups along with multiplets between δ 1.86-1.98, 2.20-2.28 for the protons of methine groups. The \(^{13}\)C NMR of compound 64 displayed signals at δ 14.23, 20.64, 22.27, 51.37 for the four methyl carbons and signals at δ 22.59, 24.65, 24.85, 26.39, 26.95, 27.83, 28.06, 28.86, 29.03, 29.16, 29.90, 31.63, 31.81, 32.51, 32.61, 33.98 for the carbons of methylene group. Similarly, the signals at δ 33.98, 48.56 for two methine carbons along with a signal at δ 80.09 for the quaternary carbon attached to the acetate group. It also showed resonances at 174.24, 174.28 for the carbonyl carbons of acetate groups.

The presence of acetate groups in the cyclobutane ring in photocycloadduct 63 and 64 provide unique opportunities for their elaboration to the desired product 70. Hence we attempted the next step of our strategy, which involved the hydrolysis of photo cycloadducts 63 and 64. The photo cycloadduct 63 in methanol was treated with 10% aqueous solution of potassium hydroxide at room temperature for 2.5h. After usual work up followed by column chromatography, the crude product furnished the hydroxy acid 65 as a colourless liquid. (Scheme 2.6)
The structure of compound 65 was confirmed by its spectral analysis. Its FTIR spectrum showed a very strong broad band at 2450-3450 cm\(^{-1}\) which confirms the presence of carboxylic acid group. The \(^1\)H NMR spectrum of compound 65 exhibited a broad singlet at \(\delta\) 11.85 for the proton of acid group. The product has one OH group whose signal merged with the signals of aliphatic protons between \(\delta\) 1.97 and 2.59. The presence of OH group was confirmed by D\(_2\)O exchange proton NMR spectrum, which showed the decrease in the peak area between \(\delta\) 1.97 and 2.29. The \(^{13}\)C NMR spectrum of compound 65 exhibited signals at \(\delta\) 14.15, 23.04 for the carbons of two methyl groups and signals at \(\delta\) 24.11, 27.08, 27.59, 28.46, 29.01, 29.23, 29.55, 30.56, 31.17, 31.86, 32.44, 32.71, 34.06, 35.59 represent the carbons of methylene groups. It also gave absorptions at \(\delta\) 38.86, 50.08 for two methine carbons along with signals at \(\delta\) 81.77, 179.50 for the carbon attached to OH group and one carbonyl carbon respectively.

Similarly the hydrolysis of photocycloadduct 64 in methanol using an aqueous solution of KOH 10% under similar reaction conditions afforded hydroxy acid 66. The structure of compound 66 was confirmed by its FTIR, \(^1\)H, \(^{13}\)C NMR and mass spectral analysis that were consistent with its structure.

To protect the acid functionality the hydroxy acid 65 converted into its corresponding methyl ester 67 by treatment with catalytic amount sulphuric acid in methanol. (Scheme 2.6)

The structure of the product 67 was confirmed by their spectral analysis. Its IR spectrum of showed an absorption band at 3380 cm\(^{-1}\) due to the presence of phenolic OH group, and strong bands at 1740 cm\(^{-1}\) for the presence of carbonyl groups. The \(^1\)H NMR spectrum of compound 67 exhibited a sharp singlet at \(\delta\) 3.66 which indicated the presence of methyl ester group along with signals at \(\delta\) 0.95, 1.61 for two methyl groups. It also showed multiplets at \(\delta\) 1.25-1.50, 2.19, 2.30 for the protons of methylene groups and a signal at \(\delta\) 1.88 for the proton of OH group. Its \(^{13}\)C NMR spectrum displayed signals at \(\delta\) 13.92, 21.23 and 54.98 for carbons of methyl groups. It also displayed a signal at \(\delta\) 71.94 for the carbon attached with OH group, along with a signal at \(\delta\) 169.29 for the carbonyl carbon of acetate group.
We then attempted the next step involving the elimination of the hydroxy group of compound 67 to its corresponding cyclobutene derivative 68, which is the key intermediate for the synthesis of the LG analogues 70. However this process is tedious since there is a possibility for the formation of three different products 68, 71 and 72 by dehydration of compound 67. (Scheme 2.7)

We attempted the catalytic dehydration of compound 67 in toluene using two different reagents; p-toluene sulphonic acid (p-TSA) and sulfuric acid (H₂SO₄). Thus a solution of 67 in toluene was heated in the presence of p-TSA at reflux temperature but the reaction did not show the formation of any product on TLC. So we attempted the sulphuric acid catalysed dehydration of 67.

![Scheme 2.7: Dehydration of hydroxy ester 67](image)

A mixture of H₂SO₄ and toluene was added to a stirred solution of hydroxy ester 67 in dry toluene at 0 °C. The stirring was further continued for 45 min with maintained temperature. The reaction mixture was then allowed to warm up to room temperature ~ 27 °C. The product was extracted in ethyl acetate and the solvent was removed in a rotatory evaporator under reduced pressure which gave a thick yellow liquid as a crude product. The column chromatography of the product over a column of silica gel using a mixture of light petroleum/ethyl acetate as eluents furnished an
inseparable mixture of two *regio* isomers 68 and 72 in the ratio 92.5:7.5. However the product 73 was not obtained at all. (Scheme 2.7, Figure 2.3)

The ratio of both the products 68, 72 was calculated by the ratio of peak area of acetate methyl group in their $^1$H NMR spectrum which gave singlets at δ 3.43, 3.67. (Figure 2.3) Based on the ratio of peak area it was found that the desired product 68 was formed in major amount.

![Diagram](image)

**Figure 2.3:** $^1$H NMR of inseparable mixture of compound 68 and 72

Towards the synthesis of LG analogue 70 it was necessary to cleave the alkene moiety of precursor 68 to corresponding γ-ketoaldehyde. Numerous methods have been reported in the literature for the cleavage of alkenes using various reagents such as Pb(CH$_3$COO)$_4$, HIO$_4$, O$_3$O$_4$-KMnO$_4$, O$_3$, MnO$_2$, PCC etc. Use of most of these reagents converts alkene to diol and further diol to aldehyde or acid. Simandi *et al* in 1986 reported the selective cleavage of alkene to aldehyde using KMnO$_4$ in THF-water. This method was applied to cleave the double bond in cyclobutene ring of precursor 68 for the synthesis of the desired product 70.
Thus, to a stirred mixture of alkene \( \text{68, 72} \) in THF, a solution of potassium permanganate in water was added over a period of 1h. Stirring was further continued for 3.5 h after which the reaction mixture was filtered on celite pad. THF was removed under reduced pressure and product was extracted with ethyl acetate (25 ml x 3). The combined extracts were washed with water, brine and dried over anhydrous sodium sulphate. Removal of solvent under reduced pressure followed by column chromatography furnished diol \( \text{69 (48%)} \) as a colourless liquid. However, the diol \( \text{74} \) and desired \( \gamma \)-keto aldehyde \( \text{70} \) was not obtained. (Scheme 2.8)

![Chemical Structure](image)

**Scheme 2.8:** Synthesis of \( \gamma \)-keto aldehyde \( \text{70} \)

The structure of the diol \( \text{69} \) was fully discernible through its spectral analysis. Its IR spectrum showed a strong absorption band at 3284 cm\(^{-1}\) due to the presence of OH group, and a strong band at 1739 cm\(^{-1}\) for the carbonyl of acetate group. The \(^1\)H NMR spectrum of diol \( \text{69} \) exhibited signals at \( \delta \) 0.89, 1.54, 3.68 for the protons on methyl groups and signals at \( \delta \) 1.21, 2.29 for protons of methylene groups. It also showed a singlet at \( \delta \) 2.19 for the protons of two hydroxy group along with a signals at \( \delta \) 2.45, 4.18 for the two methine protons. The \(^{13}\)C NMR spectrum of \( \text{69} \) displayed signals at \( \delta \) 14.12, 22.67 and 51.52 for carbons of three methyl groups and signals at \( \delta \) 23.65, 24.78, 28.90, 29.01, 29.11, 29.32, 29.47, 30.98, 31.98, 31.85, 33.72, 33.77, 34.02, 34.05 for the methylene carbons. Similarly the signals at \( \delta \) 74.33, 75.53 for
methine and quaternary carbons attached with OH group along with a signal at δ 174.33 for the carbonyl carbon of acetate group were obtained.

In order to synthesize the γ-keto aldehyde 70, the diol 69 was subjected to oxidative cleavage. Thus, a solution of diol 69 in acetone-water (1:1) was added to a stirred solution of NaIO₄ in water-acetone (1:1) at 10 °C over a period of 15 min. The resulting mixture was stirred further for 1.5h, and then reaction was quenched by addition of ethylene glycol. The reaction mixture was extracted with ethyl acetate (25 ml x 3), washed with water, brine and dried over anhydrous sodium sulphate. Removal of solvent under reduced pressure gave a thick yellow liquid which was chromatographed over a column of silica gel. The elution of the column using a mixture of light petroleum/ethyl acetate afforded the keto aldehyde 70 as a colourless liquid (Scheme 2.8)

The structure of 70 was confirmed by their spectral analysis. Its IR spectrum showed absorption bands at 1710, 1730 cm⁻¹ due to the presence of the carbonyl groups. The ¹H NMR spectrum of compound 70 exhibited a sharp singlet at δ 9.37 which indicated the presence of aldehyde group along with signals at δ 0.89, 2.19 and 3.68 for three methyl groups. It also displayed cluster of multiplets between δ 0.96-1.71, 2.34 for the protons of methylene groups and a signal at δ 2.45 for the protons on methinc groups. Its ¹³C NMR spectrum gave resonances at δ 15.12, 19.24 and 51.52 for the carbons of three methyl groups. It also displayed signals at δ 35.75, 37.87 for the two methine carbons, along with signals at δ 174.33, 196.29, 198.93 for the three carbonyl carbons.

2.4 Experimental section

Ultraviolet spectra were recorded on a Perkin-Elmer Lambda-19 Spectrometer. Infrared spectra were recorded on a Perkin-Elmer PC-16 FTIR Spectrophotometer. ¹H and ¹³C NMR spectra were recorded on Bruker 500/400 MHz NMR spectrometer (125/100 MHz for ¹³C respectively) using CDCl₃ or DMSO-δ₆ (TMS as an internal standard). Mass spectra were obtained on Thermo-Fisher DSQ II GCMS instrument.

Column chromatography was performed using Acme’s silica gel (60–120# mesh) and the elution was done using mixtures of light petroleum and ethyl acetate.
Yields (%) were reported based on the isolated material after column chromatography. Thin layer chromatography was performed using Acme’s silica gel for TLC and the spots were visualized in iodine vapor.

Experimental procedures:

Synthesis of adducts (63) and (64):

A solution of methyl oleate (62) (1.00 g, 0.0034 mole) and isopropenyl acetate (61) (0.34 gm, 0.0034 mole) in acetone (~ 600 ml) was placed in an immersion well-type photoreactor and was irradiated for 6 h at 10-15 °C, with a 250 W low pressure mercury vapour lamp. After near completion of reaction (TLC), the solvent was removed under reduced pressure which gave a thick yellow liquid as a crude product. This product was then chromatographed over a column of silica gel. Elution of the column using a mixture of light petroleum/ethyl acetate (90:7) afforded the adduct (64) as a colourless liquid (0.23 gm, 17 % yield).

\[
\text{IR (KBr): } 1160, 1730, 1750, 2838, 2875 \text{ cm}^{-1}.
\]

\[
\text{\textsuperscript{1}H NMR (500 MHz, CDCl}_3\text{): } \delta 0.87 (3H, t, J = 6.75 Hz, CH}_3, 0.97-1.62 (28H, m, 14CH}_2, 1.61 (3H, s, CH}_3, 1.86-1.98 (1H, m, CH), 2.01-2.18 (2H, m, CH}_2, 2.20-2.28 (1H, m, CH), 2.31 (3H, s, CH}_3, 3.59 (3H, s, CH}_3).
\]

\[
\text{\textsuperscript{13}C NMR (100 MHz, CDCl}_3\text{): } \delta 14.03, 20.64, 22.27 (3C, CH}_3, 22.59, 24.65, 24.85, 26.39, 26.95, 27.83, 28.06, 28.86, 29.03, 29.16, 29.90, 31.63, 31.81, 32.51, 32.61 (15C, CH}_2), 33.98, 48.56 (2C, CH), 51.37 (1C, CH}_3), 80.09 (1C, Cq), 174.24, 174.28 (2C, CO).
\]

\[
\text{MS (EI): } m/z \text{ Calculated for C}_{24}H_{44}O_4: 396.32; \text{ found } 396.20 (M}^+.\]

Further elution of the column with light petroleum / ethyl acetate (90:10) furnished adduct (63) (0.24 gm, 18 % yield).
IR (KBr): 1240, 1723, 1735, 2838, 2875 cm⁻¹.

¹H NMR (500 MHz, CDCl₃): δ 0.87 (3H, t, J = 6.75 Hz, CH₃), 0.90-1.56 (28H, m, 14CH₂), 1.62 (3H, s, CH₃), 2.05 (2H, m, CH₂), 2.10-2.32 (2H, m, 2CH), 2.38 (3H, s, CH₃), 3.66 (3H, s, CH₃).

¹³C NMR (100 MHz, CDCl₃): 13.52, 22.78, 24.32 (3C, CH₃), 27.60, 28.83, 29.01, 29.07, 29.25, 29.38, 29.50, 29.58, 29.73, 31.82, 31.85, 33.91, 33.98, 34.01, 38.30 (15C, CH₂), 39.84, 50.01 (2C, CH), 51.39 (1C, CH₃), 81.68 (1C, Cq) 177.41, 177.45 (2C, CO)

MS (EI): m/z calculated for C₂₄H₄₄O₄ 396.32; found 396.21 (M⁺).

Synthesis of hydroxy acid (65):
To a stirred solution of cycloadduct (63) (4.00 gm, 0.012 mol) in methanol (50 ml) an aqueous solution of KOH (10%, 3.5 ml) was added over a period of 15 min at room temperature ~ 27 °C. The stirring was further continued for 2.5h. After completion of the reaction the reaction mixture was neutralised with aqueous HCl (1:4). The product was extracted in ethyl acetate (25 ml x 3). The combined organic extracts were washed with water, brine solution and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure furnishing a thick liquid which was then chromatographed over a column of silica gel. Elution of the column using a mixture of light petroleum ethyl acetate (90:30) furnished the hydroxy acid (65) a colourless liquid. (3.12 gm, 91 % yield)

IR (KBr): 1163, 1712, 2561-3255 cm⁻¹

¹H NMR (400 MHz, CDCl₃): δ 0.86 (3H, t, J = 6.00 Hz, CH₃), 1.27-1.97 (28H, m, 14CH₂), 2.17 (1H, s, OH superimposed with CH₂), 2.34 (2H, m, CH₂), 2.43 (2H, m, 2CH), 2.59 (3H, s, CH₃), 11.85 (1H, bs, COOH).

¹³C NMR (100 MHz, CDCl₃): 14.15, 23.04 (2C, CH₃), 24.11, 27.08, 27.59, 28.46, 29.01, 29.23, 29.55, 30.56, 31.17, 31.86, 32.44, 32.71, 34.06, 35.59, 38.33, 38.86 (15C, CH₂), 45.93, 50.08 (2C, CH), 81.77 (1C, Cq), 179.50 (1C, CO).
MS (ESI): m/z calculated for C\textsubscript{21}H\textsubscript{40}O\textsubscript{5}: 340.54; found 340.8 (M\textsuperscript+).

Synthesis of hydroxy acid (66):
To a stirred solution of cycloadduct (64) (4.00 g, 0.012 mol) in methanol, an aqueous solution of NaOH (10%, 1.5 ml) was added over a period of 15 min at room temperature ~27 °C. The stirring was further continued for 2.5h. After completion of the reaction, the reaction mixture was neutralised with aqueous HCl (1:4). The product was extracted in ethyl acetate (25 ml x 3), the organic extracts were combined and washed with water, brine solution and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure furnishing a thick liquid which was chromatographed over a column of silica gel. Elution of column using a mixture of light petroleum ethyl acetate (90:30) furnished the hydroxy acid (66) a colourless liquid. (3.00 gm, 90 % yield)

IR (KBr): 1136, 1369, 1710, 3279 cm\textsuperscript{-1}.

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta\) 0.85 (3H, t, \(J = 6.00\) Hz, CH\textsubscript{3}), 1.14-1.62 (28H, m, 14CH\textsubscript{2}), 1.86-1.92 (2H, m, 2CH), 2.01 (1H, s, OH superimposed with CH\textsubscript{2}), 2.03 (2H, m, CH\textsubscript{2}), 2.30-2.35 (3H, t, CH\textsubscript{3}), 10.86 (1H, bs, COOH).

\textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): \(\delta\) 14.01, 22.57 (2C, CH\textsubscript{3}), 24.61, 25.38, 26.34, 26.81, 27.76, 27.94, 28.02, 28.97, 29.00, 29.64, 31.79, 32.48, 32.59, 33.97, 37.13 (15C, CH\textsubscript{2}), 48.49, 54.82 (2C, CH), 73.21 (1C, Cq), 179.05 (1C, CO).

MS (ESI): m/z calculated for C\textsubscript{21}H\textsubscript{40}O\textsubscript{5}: 340.54; found 340.3.

Synthesis of hydroxy ester (67):
To a stirred solution of hydroxy acid (65) (3 gm, 0.008 mole) in dry methanol (20 ml), concentrated sulphuric acid (0.01 ml) was added at room temperature ~27°C. The resulting mixture was further stirred for 1h. The reaction mixture was neutralised with a saturated solution of sodium bicarbonate and the product was extracted with ethyl acetate (25 ml x 3). The organic layers were combined, washed thoroughly with water, brine solution and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure which gave the crude product as a pale yellow liquid. This product was then chromatographed over a column of silica gel using light
petroleum and ethyl acetate afforded the hydroxy ester (67) as a colour less liquid. (2.63 gm, 88 % yield)

![Chemical Structure](image)

IR (KBr): 1150, 1742, 2891, 3380 cm⁻¹

$^1$H NMR (400 MHz, CDCl₃): $\delta$

0.95 (3H, t, $J = 7.00$ Hz, CH₃), 1.10-1.50 (26H, s, 13CH₂), 1.61 (3H, s, CH₃), 1.73 (2H, m, 2CH), 1.88 (1H, s, OH), 2.19 (2H, m, CH₂), 2.30 (2H, t, $J = 10$ Hz, CH₂), 3.66 (3H, s, CH₃).

$^{13}$C NMR (100 MHz, CDCl₃): $\delta$ 13.92, 21.23 (2C, CH₃), 24.21, 25.47, 27.50, 27.87, 28.08, 28.67, 28.87, 29.16, 29.35, 29.91, 30.01, 31.32, 32.62, 37.45, 48.65 (15C, CH₂), 51.34, 52.49 (2C, CH), 54.98 (1C, CH₃), 71.94 (1C, Cq), 174.19 (1C, CO)

MS (ESI): m/z calculated for C₂₂H₄₂O₃: 354.31; found 354.0 (M⁺).

**Synthesis of alkene (68):**

A solution of H₂SO₄ in toluene (1 %) (5 ml) was added to a stirred solution of hydroxy ester (67) (2.50 g, 0.007 mol) in dry toluene (20 ml) at 0 °C. The stirring was further continued for 45 min with the maintained temperature 0 °C. The reaction mixture was allowed to warm up to room temperature and poured into a saturated solution of sodium bicarbonate to neutralise the excess acid. The products were extracted in ethyl acetate (25 ml x 3), washed successively with water, brine and dried over anhydrous sodium sulphate and concentrated in rotator evaporator under reduced pressure to give the crude product as a thick liquid. Column chromatography of the crude product over a column of silica gel using light petroleum ethyl acetate as eluents furnished an inseparable mixture of colourless liquid (68) (1.60 gm, 67 % yield) and (72) (0.12 gm, 5.00 % yield).

![Chemical Structure](image)

IR (KBr): 1435, 1458, 1743, 2924, 3005 cm⁻¹

$^1$H NMR (400 MHz CDCl₃): $\delta$

0.89 (3H, t, $J = 7.2$ Hz, CH₃), 1.20-1.4 (20H, m, 10CH₂), 1.50-1.68 (8H, m, 4CH₂), 1.97-2.06 (2H, m, 2CH), 2.31 (3H, s, CH₃), 3.67 (3H, s, CH₃), 4.69 (IH, d, $J = 2.6$ Hz, olefinic).
\(^{13}\)C NMR (100 MHz): \(\delta\) 14.00, 19.37 (2C, CH\(_3\)), 20.97-34.42 (14C, CH\(_2\)), 33.63, 34.12, (2C, CH), 50.30 (1C, CH\(_3\)), 130.56, 132.48 (2C, olefinic), 174.74 (1C, CO).

**MS (EI):** m/z calculated for C\(_{22}\)H\(_{40}\)O\(_2\) 336.31; found 335.81 (M\(^+\)).

\[\text{IR (KBr): } 1435, 1458, 1743, 2924, 3005 \text{ cm}^{-1}.\]

\(^1\)H NMR (400 MHz CDCl\(_3\)): \(\delta\) 0.89 (3H, t, \(J = 7.2 \text{ Hz}, \text{CH}_3\)), 1.20-1.4 (20H, m, 10CH\(_2\)), 1.50-1.68 (8H, m, 4CH\(_2\)), 1.97-2.06 (1H, m, CH), 2.31 (3H, s, CH\(_3\)), 2.58 (2H, m, CH\(_2\)), 3.43 (3H, s, CH\(_3\)).

\(^{13}\)C NMR (100 MHz): \(\delta\) 14.00, 19.37 (2C, CH\(_3\)), 20.97-32.83 (15C, CH\(_2\)), 44.84 (1C, CH), 51.50 (1C, CH\(_3\)), 130.56, 132.48 (2C, olefinic), 174.74 (1C, CO).

**MS (EI):** m/z calculated for C\(_{22}\)H\(_{40}\)O\(_2\) 336.31; found 335.81 (M\(^+\)).

**Synthesis of diol (69):**

To a stirred mixture of (68, 72) (1.70 gm, 0.005 mol) in THF-water (1:1, 10 ml), a solution of potassium permanganate (1gm, 0.006 mol) was added over a period of 1h. The stirring was further continued for 3.5 h after which the reaction mixture was filtered on a celite pad and THF was removed under reduced pressure. The product was extracted with ethyl acetate (25 ml x 3). The combined extracts were washed with water, brine solution and dried over anhydrous sodium sulphate. Removal of the solvent under reduced pressure furnished the crude product as an oily liquid, which was purified by column chromatography over a column of silica gel using a mixture of light petroleum/ethyl acetate as eluents. This exercise afforded the diol (69) as a colourless liquid (0.84 gm, 48 % yield)

\[\text{IR (KBr): } 1739, 2851, 2918, 3284 \text{ cm}^{-1}\]

\(^1\)H NMR (400 MHz CDCl\(_3\)): \(\delta\) 0.89 (3H, t, \(J = 7.2 \text{ Hz}, \text{CH}_3\)), 1.2-1.61 (24H, m, 12CH\(_2\)), 1.64 (3H, CH\(_3\) merged with signal of methylene groups), 1.82 (2H, m, CH\(_2\)), 2.19 (2H, s,
exchangeable OH), 2.29 (2H, t, J = 7.6 Hz, CH₂), 2.45 (2H, m, 2CH), 3.68 (2H, s, CH₃), 4.18 (1H, dd, J₁ = 7.2 Hz, J₂ = 3.20 Hz, CH).

¹³C NMR (100 MHz, CDCl₃): δ 14.12, 22.67 (2C, CH₃), 23.65, 24.78, 28.90, 29.01, 29.11, 29.32, 29.47, 30.98, 31.80, 31.85, 33.72, 33.77, 34.02, 34.05 (14C, CH₂), 37.79, 37.86 (2C, CH), 51.52 (1C, CH₃), 74.33 (1C, CH), 75.53 (1C, Cq), 174.33 (1C, CO).

MS (EI): m/z calculated for C₂₂H₂₄O₄ 370.31; found 370.22 (M⁺).

**Synthesis of γ-keto aldehyde (70):**

A solution of NaIO₄ (0.43 gm, 0.002 mol) in acetone - water (10 ml, 1:1) was added to a stirred solution of diol (69) (0.80 gm, 0.004 mol) in acetone-water (15 ml, 1:3) at 10 °C over a period of 15 min. The resulting mixture was further stirred for 1.5 h and then the reaction was quenched by addition of ethyl glycol (1.00 gm, 0.016 mol). The product was then extracted with ethyl acetate (25 ml x 3), washed with water, brine and dried using anhydrous sodium sulphate. Removal of the solvent under reduced pressure gave the crude product as a thick yellow liquid which was chromatographed over a column of silica gel. Elution of the column using light petroleum ethyl acetate (90:10) afforded the γ-keto aldehyde (70) as a colourless liquid. (0.29 gm, 37 % yield).

IR (KBr): 1710, 1750, 2935, 2964 cm⁻¹.

¹H NMR (400MHz CDCl₃): δ 0.89 (3H, t, J = 6.8 Hz, CH₃), 0.96-1.71 (26H, m, 13CH₂), 2.19 (3H, s, CH₃), 2.34 (2H, m, CH₃).

2.45 (2H, m, 2CH), 3.68 (3H, s, CH₃), 9.37 (1H, s, CHO)

¹³C NMR (100 MHz, CDCl₃): δ 15.12, 19.24 (2C, CH₃), 23.65, 24.70, 24.83, 28.90, 29.01, 29.23, 29.43, 29.72, 30.70, 31.80, 33.70, 33.76, 33.92, 34.06 (14C, CH₂), 35.75, 37.87 (2C, CH), 51.52 (1C, CH₃), 174.33, 196.23, 198.93 (3C, CO).

MS (EI): m/z calculated for C₂₂H₂₄O₄ 368.29; found 368.11 (M⁺).
2.5 Conclusion

We have made an attempt to design and develop a synthetic route towards construction of Levuglandin skeleton, however no stereochemical separation is attempted at this stage. Depending upon the functionalities on two long side chains, the starting long chain alkene can be suitably modified for the synthesis of specific levuglandins.

2.6 References

8. Euler, V. Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol. 1933, 175, 78.


2.7 Spectral data of compounds
Figure 2.6: $^{13}$C NMR spectrum of compound 63.
Figure 2.8: FTIR spectrum of compound 64
Figure 2.9: $^1$H NMR spectrum of compound 64
Figure 2.10: $^{13}$C NMR spectrum of compound 64
Figure 2.13: $^1$H NMR spectrum of compound 65
Figure 2.14: $^{13}$C NMR spectrum of compound 65
Figure 2.15: ESI-MS spectrum of compound 65
Figure 2.17: ¹H NMR spectrum of compound 66
Figure 2.18: $^{13}$C NMR spectrum of compound 66
Figure 2.19: ESI-MS spectrum of compound 66
Figure 2.21: $^1$H NMR spectrum of compound 67
Figure 2.23: ESI-MS spectrum of compound 67
Figure 2.25: $^1$H NMR spectrum of compound 68 & 72
Figure 2.27: EI-MS spectrum of compound 68 & 72
Figure 2.33: $^1$H NMR spectrum of compound 70
Figure 2.35: ESI-MS spectrum of compound 70