Chapter 2
Recent Advances in the Chemistry and Biology of Stable Synthetic Lipoxin Analogues

2.1 Introduction

The Lipoxin metabolites, discussed in Chap. 1, dramatically reduce the bioactivity of this class of compounds and render them poor potential pharmacological agents. In light of the findings associated with the stabilisation and market value of the synthetic prostaglandin and prostacyclin analogues [1], it was thought that a similar approach could be beneficial with respect to the native Lipoxins (LX).

2.2 Design, Synthesis and Biological Evaluation of Stable Lipoxin Analogues

Recent synthetic efforts include mimicking the core structure of the native LXA₄ by replacing certain functionalities with chemically stable motifs with the aim of retaining the potent biological activity. These stable analogues will be sub-divided into three distinct categories (A, B and C), based on the target area being modified, Fig. 2.1. The strategies include (A) structural modifications of the C₁₅–₂₀ chain: [2] (B) replacement of the triene with chemically stable aromatic/heteroaromatic systems: [3, 4] and (C) modifications of the C₁–₈ unit [5]. While excellent reviews have extensively covered the synthesis and biological relevance of the native LX and their stereoisomers [6, 7], this chapter will focus on the synthesis and biological evaluation of enzymatically durable analogues.

2.3 (A) Structural Modifications of the C₁₅–₂₀ Chain

The desire to prevent oxidation at C₁₅–₂₀ led to the design of the first LXA₄ analogues which showed resistance to oxidation [8]. Replacement of this alkyl chain with several different groups furnished a number of analogues with increased pharmacokinetic
profiles. Structural adaptations incorporated 15-deoxy-LXA$_4$ 2, 15-(R/S)-methyl 3, 16-phenoxy 4, and 15-cyclohexyl 5 into the C$_{15-20}$ chain, Fig. 2.2.

The synthetic routes used for these analogues were not reported in the literature, although they were clearly constructed by using previously reported syntheses for the related native LX [9]. The authors observed that these structural modifications dramatically increased biostability compared to the native LX by preventing dehydrogenation by differential HL-cells and recombinant 15-hydroxyprosta-glandin dehydrogenase. The bioactivity was also secured in the 15-(R/S)-methyl 3, 16-phenoxy 4 and 15-cyclohexyl 5 analogues due to their ability to prevent PMN transmigration and adhesion in leukocyte migration. The 15-deoxy-LXA$_4$ 2 showed the least activity suggesting that the hydroxyl group at C$_{15}$ is essential for the preservation of bioactivity.

Alternative analogues have been developed which resulted in enhanced bioactivity compared to the native LX. These designs include the addition of a fluoro 6 and trifluoromethyl 7 group onto the 16-phenoxy analogue 4, Fig. 2.3 [7, 10].
The para-fluorophenoxy analogue 6 has proven itself to be an extremely potent derivative as it inhibited tumor necrosis factor (TNF)-α-induced leukocyte recruitment into the dorsal air pouch [10]. It was also found to suppress both LTB₄- and PMA-induced recruitment, when applied to mouse ear skin. Furthermore, this analogue has shown potential as an anti-cancer agent, as it inhibits endothelial cell proliferation leading to suppressed angiogenesis at the 1–10 nM range [11]. Realising the potential of these fluorinated analogues, a number of research groups began to develop efficient synthetic routes to these biologically important derivatives. The key synthetic transformations combine a cis-reduction of an alkyne, a palladium-catalysed Sonogashira reaction and a Wadsworth–Emmons alkene transformation, Scheme 2.1. Phillips and co-workers reported the first synthesis of the para-fluorophenoxy analogue 6 by adopting a chiral pool strategy [2], starting from 2-deoxy-D-ribose 11 [12]. This approach has the advantage of using a readily available starting material which incorporates the two stereocentres which will ultimately appear at C₅ and C₆.

**Fig. 2.3** Fluoro and trifluoromethyl stable analogues [7, 10]

Protection of 2-deoxy-D-ribose 11 was achieved through its propylidene acetal 12 using 2-methoxypropene and pyridinium p-toluenesulfonate (PPTS) in ethyl acetate at room temperature, giving a 43% yield, Scheme 2.2. A Wittig reaction of
methyl(triphenylphosphoranylidine)acetate and the aldehyde form of 12, followed by a catalytic hydrogenation using 10% Pd/C furnished alcohol 13 in high yields of 81 and 87%, respectively. Oxidation of 13 using Swern conditions afforded aldehyde 9 in 86% yield. This was subjected to a Wadsworth–Emmons transformation with phosphonate 8 and deprotected using KF and 18-crown-6 to form the key intermediate 14 in 99% yield.

\[ \text{Scheme 2.2 Formation of key intermediate 14 [2]} \]

Phosphonate 8 was itself assembled by the treatment of alkyne 15 with ethylmagnesium chloride and quenching with chlorotrimethylsilane followed by an Appel-type reaction gave the corresponding bromide in 90 and 74% yields,
respectively, Scheme 2.3. This bromide was subjected to Arbusov reaction conditions to afford 8 in 90% yield.

Scheme 2.3 Formation of phosphonate 8 [2]

The synthesis of the Sonogashira coupling partner 10 was accomplished in five steps, beginning with the alkylation of p-fluorophenol with 3-chloropropane-1,2-diol 16 in 56% yield, Scheme 2.4. Cleavage of the diol with silica-supported sodium periodate in dichloromethane afforded aldehyde 17 in 98% yield. Addition of lithium 2-trimethylsilylacetylide to 17, followed by treatment with NaOH to

Scheme 2.4 Synthesis of Sonogashira coupling partner 10 [2]
remove the TMS group, gave alkyne 18 in 76% yield. Vinylstannane 19 was constructed by treating 18 with tri-n-butyltin hydride. Addition of NBS in dichloromethane to 19 gave the vinylbromide 20 in 95% yield. An attempted kinetic resolution of vinylstannane 19 using Sharpless epoxidation, followed by treatment of the unreacted alcohol with NBS to give 10, proceeded with poor ee. Subsequently racemic 20 was resolved with chiral supercritical fluid chromatography to give vinylbromide 10 in 42% yield and 99% ee.

The Sonogashira reaction, employing Pd(PPh₃)₄ and CuI in the presence of n-propylamine at room temperature, was used to cross-couple vinylbromide 10 and the terminal alkyne 14, resulting in the formation of 21 in 75% yield, Scheme 2.5. The catalyst loading was not given for this Sonogashira coupling. The acid sensitive acetal group was cleaved by the addition of methanolic HCl to give the corresponding diol. At this stage Lindlar’s catalyst can be employed to access the C₁₁–₁₂ cis-double bond. However, problems have arisen with this method including over-reduction and isomerisation of the C₁₁–₁₂ trans-double bond isomer during the synthesis of other Lipoxin analogues [13]. Selective cis-reduction with an activated zinc alloy has previously been described by Boland [14], and this protocol afforded the para-fluorophenoxy Lipoxin analogue 6 in 80% yield. Activation of the zinc requires the addition of 2N HCl for 1–2 min for a clean reaction to take place.

In a similar synthetic approach, starting from 2-deoxy-D-ribose 11, Petasis and co-workers synthesised stable Lipoxin analogues varying at the C₁₅–₂₀ chain, via the introduction of aliphatic, aromatic and fluoroaromatic groups, Scheme 2.6 [7]. The synthetic strategy incorporates a Wittig reaction for the construction of the C₇–₈ double bond, a Sonogashira reaction followed by a cis-reduction of the alkyne to establish the C₁₁–₁₂ double bond. Simple structural variations of the Sonogashira coupling partners gave rise to many synthetic analogues.

The precise details of the synthesis, including % yields and mol% of catalysts, were not reported as this was part of a review article. The tert-butyldimethylsilyl-protected aldehyde 22 was accessed through the chiral pool strategy using 2-deoxy-D-ribose 11. Compound 23, previously prepared [13], was reacted with 22 in a Wittig reaction. Double bond isomerisation with I₂ in dichloromethane followed by removal of the trimethylsilyl group by AgNO₃ and KCN in EtOH, THF and H₂O gave the alkyne coupling partner 24. Reaction conditions employed for the Sonogashira reaction included Pd(PPh₃)₄, CuI in n-propylamine followed by the addition of the corresponding vinyl bromide or iodide. The tert-butyldimethylsilyl protecting groups were cleaved using TBAF in THF, followed by reduction of the alkyne, by either H₂ in the presence of Lindlar’s catalyst, or by selective cis-reduction with an activated zinc alloy, to afford the series of analogues 26. The 15-cyclohexyl, 15-cyclooctyl and the 16-phenoxy analogues were all found to retain the native Lipoxin bioactions. The inactivation by 15-PDGH and P-450-mediated ω-oxidation were hindered due to the absence of the free ω-alkyl chain. These analogues, of type 26, were also found to be extremely useful in studying the exact binding site in vivo [15]. The fluorinated analogues were found to be the most stable and active in vivo [10].
In recent years, researchers have focused their attention on modifying the triene structure of the Lipoxin A\(_4\) and B\(_4\) framework. Derivitisation of this part of the molecule has major advantages in terms of (i) considerably increasing the stability of the molecule towards enzymatic decomposition (ii) development of a short and economical synthesis in an effort to access and screen numerous analogues to further tune the pharmacological profile and (iii) prevention of the double bond isomerisation as described above. Significant advances in the area include the substitution of the triene with aromatic [3, 4] and heteroaromatic rings [16], Fig. 2.4.

The LXA\(_4\) and LXB\(_4\) analogues reported by Guiry and co-workers, 27 and 28 respectively, were constructed using Sharpless asymmetric epoxidation,
Pd-mediated Heck coupling and diastereoselective reduction reactions as the key synthetic transformations [3]. These reactions provided enantio- and diastereoselective generation of each stereocentre and complete control for the formation of the trans olefin. In a similar synthetic route Guiry and co-authors synthesised a novel pyridine-containing LXA₄ 29 that was also found to possess important biological properties. The synthesis and biological evaluation of this pyridine-containing LXA₄ 29 will be discussed in detail in Chap. 4.

Scheme 2.6 Synthesis of aliphatic, aromatic and fluoroaromatic LXA₄ analogues [7]
The first stereoselective route to the novel aromatic analogue 27 described by Guiry and co-workers employed the commercially available divinylcarbinol 30 as the starting material, Scheme 2.7 [3].

This allylic alcohol 30 was subjected to Sharpless asymmetric epoxidation reaction conditions to give the chiral epoxide 31 in 85% yield and with an enantio-meric excess of greater than 99%. Ring opening of 31 with the Grignard derivative of 32 in the presence of a catalytic amount of CuI afforded the desired diol 33 in 82% isolated yield. This diol required an acid stable protecting group as the acidic Jones’ reagent was applied to cleave the dioxane in the following transformation. The diol protection was successfully achieved by the addition of acetyl chloride and pyridine in THF at 0°C to give the bisacetate in 97% yield. The addition of Jones’ reagent in acetone for 2 h yielded the corresponding acid 34, which was esterified using diazomethane in diethyl ether. A change of protecting group strategy was employed at this stage as the bis-acetate methyl ester was an unsuitable coupling partner for the Heck reaction. For this reason, deprotection with NaOMe in MeOH followed by reprotection with a tert-butylidimethylsilyl group was necessary in order to afford the bis-silyl ether 35 in high yield. This protected olefin was then successfully applied in a palladium-mediated Heck reaction in both the benzene- and pyridine-containing LXA₄ analogues, 27 and 29, respectively. The authors also found that zirconium tetrachloride was an efficient catalyst for a one-pot protection/deprotection synthetic methodology and used this for the synthesis of 35 [17]. This protocol also led to the synthesis of 6-acetoxy-5-hexadecanolide, a component of mosquito oviposition.
attractant pheromones [18], and also a microwave-assisted asymmetric synthesis of exo- and endo-brevicomin [19].

Scheme 2.7 Synthesis of key intermediate 35 [3, 16]

The preparation of aryl bromide 38 required as the other Heck coupling partner was achieved through the addition of the Grignard derivative of 1-bromopentane 37 to acid chloride 36, Scheme 2.8. The reaction was performed at $-78^\circ C$ to prevent any of the double addition product forming. An initial screening of Heck reaction conditions revealed that tributylamine, with its high boiling point, afforded the coupled product 39 in a very high yield (88%). Reduction of this ketone was achieved using sodium borohydride giving rise to a mixture of epimeric alcohols which were easily separated by column chromatography. The authors also employed (–)-β-chlorodiisopinocampheylborane to give alcohol 40 in 67% yield and with a 92% diastereomeric excess. Finally this alcohol was deprotected using $p$-toluenesulfonic acid in MeOH giving the triol (1S)-27 in 84% yield. This triol and the (1R)-27 analogue were both converted to their corresponding acids by LiOH in a mixture of methanol and water and were also investigated for their ability to aid in the resolution of inflammation.
The stereoselective synthesis of the aromatic LXB₄ analogue (5S)-28 exploited a similar synthetic route, assembling the trans double via a palladium-catalysed Heck reaction with aryl bromide 43, Scheme 2.9. The aryl bromide 43, required for the Heck reaction, was formed through a Sonogashira coupling of 1-bromo-2-iodobenzene 41 and the commercially available terminal alkyne 42, followed by oxidation with sulfonic acid and esterification.

Scheme 2.8 Synthesis of aromatic LXA₄ (1S)-27 [3]

Scheme 2.9 Synthesis of Heck coupling partner 43 [3]
Another epoxide ring opening reaction via Grignard chemistry produced the olefin Heck coupling partner 44, Scheme 2.10.

\[
\begin{align*}
\text{Br} & \quad + \\
37 & \quad \text{Mg, THF, } 30 \, ^\circ\text{C} \quad \text{CuI (20 mol\%)} \\
& \quad \text{THF, } -30 \, ^\circ\text{C}, 3 \, \text{h, 63}\% \\
\text{O} & \quad \text{Me}_2C(\text{OMe})_2, \, \text{p-TSA} \\
31 & \quad \text{r.t., 18 h, 76}\% \\
\end{align*}
\]

1. (-)-DIP chloride, Et\(_2\)O
-20 °C, 24 h, 67%
2. 2N HCl, THF, r.t.
20 h, 59%

The Heck reaction proceeded under similar reaction conditions to those employed for the synthesis of aromatic LXA\(_4\) (1S)-27, furnishing 45 in 41% yield. Asymmetric reduction of ketone 45 was again accomplished by way of Brown’s (-)-\(\beta\)-chlorodiisopinocampherylborane to give the alcohol in 67% yield with a de value of 97%. The final step was acetal cleavage using 2N HCl in THF at room temperature to furnish triol (5S)-28 in 59% yield. These new aromatic analogues possess great potential as therapeutic agents as the modular synthetic approach to these compounds renders them extremely accessible and their pharmacodynamics can be further tuned by the addition of known classical bioisosteres.

The novel aromatic LXA\(_4\) analogues (1S)-27 and (1R)-27 promoted increased clearance of apoptotic PMNs when compared to the effect of the native LXA\(_4\), Fig. 2.5.

The aromatic LXB\(_4\) (5S)-28 analogue also stimulated phagocytosis of apoptotic PMNs with a maximum effect observed at 10^{-11} M, Fig. 2.6.
In addition to this, both analogues (27 and 28) caused F-actin rearrangement which has also been observed with the native compounds, Fig. 2.7 [20].

Phagocytosis of PMNs was inhibited by pre-treatment with the pan-FPR inhibitor Boc2. This strongly suggests that the effect of these analogues is mediated by the activation of the LX receptor, Fig. 2.8.

These analogues were also screened for their ability to stimulate adherence of monocytes to a matrix such as laminin, Fig. 2.9, which is a previously known property of the native LX and also some of the synthetically stable analogues [21, 22]. In the experiments the acids did not exhibit an increase in phagocytosis over the same concentration range as the methyl esters [3]. This lack of activity...
was attributed to the fact that the esters act as prodrugs, converting in vivo to the free acid and evoking LX-mediated biological actions [23].

Bannenberg and co-workers also showed that oral administration of LXA₄ has the ability to inhibit leukocyte infiltration in zymosan A-induced peritonitis [24]. Guiry and co-workers found that their (1R)-27 analogue caused a significant decrease in neutrophil accumulation at 50 µg/kg while the (1S)-27 analogue also showed a decrease at the highest dose tested, Fig. 2.10.

**Fig. 2.7** Effect of LX analogues on actin rearrangement in THP-1 cells [3]

**Fig. 2.8** LXA₄ analogues-stimulated phagocytosis of apoptotic PMNs is blocked by the receptor antagonist BOC2 [3]
Petasis and colleagues have also successfully managed to stabilise the native LXA$_4$ with the same approach, replacement of the triene with a more durable benzene ring [4, 25]. Their synthetic route allowed for the synthesis of an array of analogues (27, 46–49) Fig. 2.11. Compounds 46–49 were designed from a strategy combining domain modifications (A) and (B), Fig. 2.1.

The synthesis of 46 and 47 relied on two sequential Suzuki–Miyaura coupling reactions, Scheme 2.11. The first combines 2-bromophenylboronic acid 51 and vinyl iodide 50, which was constructed by a Takai olefination of 22 [13]. Suzuki–Miyaura reaction conditions incorporated Pd(PPh$_3$)$_4$ and K$_2$CO$_3$ using dioxane as the solvent at 60°C furnished 52 in 70% yield. The catalyst loading was not reported in this coupling reaction.
Boronic esters 55 and 56 were both synthesised from the corresponding alkynes 53 and 54, respectively, Scheme 2.12. Compound 54 was synthesised by the protection of the corresponding alcohol [26, 27].

The second Suzuki–Miyaura coupling combined aryl bromide 52 and boronic esters 55 and 56 in the presence of Pd(PPh₃)₄ and K₂CO₃ using a mixture of dioxane and water as the solvent at 80°C, giving 57 and 58 in moderate yields, Scheme 2.13. Deprotection followed with the use of TBAF in THF affording triol 47 and diol 46 in excellent yields.

**Fig. 2.11** Analogues designed and synthesised by Petasis and co-workers [4]

**Scheme 2.11** Synthesis of key intermediate 52
The same authors also described an interesting and alternative generation of 47 involving a novel and time-conserving one pot boronic acid Heck-type coupling, Scheme 2.14. Both alkenes 35 and 59 were prepared from their corresponding aldehyde precursors, by way of an extremely useful titanium-mediated methylenation developed by Petasis and Bzowej [28]. Firstly, boronic acid 51 reacts with olefin 35 and reactivity is observed solely at the boronic acid position. In the same reaction vessel, a second Heck reaction occurs under reaction conditions reported by Jeffery [29], using Pd(OAc)$_2$, NaHCO$_3$, Bu$_4$NCl, PPh$_3$ in acetonitrile at 60°C, giving 57 in 47% yield.

The authors also described the first reported synthesis of a novel meta-LXA$_4$ analogue 48 using a related synthetic pathway starting from 3-bromophenylboronic acid 60, Scheme 2.15. The vinyl iodide derivative 50 was coupled to 60 by way of a palladium-catalysed Suzuki–Miyaura reaction affording 61 in 70% yield. This aryl bromide 61 was further reacted in a consecutive Suzuki–Miyaura reaction with boronic ester 56, followed by deprotection with TBAF to give the meta-LXA$_4$ analogue 48 in 42% yield over the final two steps.
Scheme 2.14 Alternative synthesis of 47 [4]

Scheme 2.15 Synthesis of a meta-LXA4 analogue 48 [4]
The LXA₄ analogue 49 was prepared in order to determine the impact of increasing the chain length of the analogues on its ability to act as an agonist in the known receptor site of ALXR. The vinylboronic acid 63 was synthesised by hydroboration of the available 2-bromophenyl alkyne 62, using the reaction conditions reported by Matteson and co-workers, Scheme 2.16 [30]. The Suzuki–Miyaura reaction of 63 with vinyl bromide 64, prepared previously [31], gave the aryl bromide 65 in 65% yield. Conversion of this aryl bromide 65 to its pinacol boronate 66 using bis-pinacolato diboron, PdCl₂ (dppf) and AcOK in dimethylsulfoxide at 80°C proceeded in 40% yield. Boronate 66 was coupled with vinyl iodide 50 by a Suzuki–Miyaura reaction to give the silyl-protected intermediate which was then deprotected to furnish the novel analogue 49 in 43% over the final two steps.

The same authors also outline a non-stereoselective (at the benzylic position) synthesis of the same benzene-containing LXA₄ 27, Scheme 2.17, [4] prepared in an asymmetric manner by Guiry and co-workers [3]. The Grignard derivative of bromopentane was prepared and reacted with the Weinreb amide derived from acid chloride 36 to give the aryl ketone in 70% yield. This ketone was then reduced using NaBH₄ in MeOH, followed by silyl protection to furnish 67 in high yield. Aryl bromide 67 was converted to its corresponding boronate 68 in a modest 40% yield. The trans olefin was constructed by the Suzuki–Miyaura coupling of boronate 68 and vinyl iodide 50 and the epimeric triol 27 was produced in 95% yield after removal of the silyl ethers.

Scheme 2.16 Synthesis of LXA₄ analogue 49 [4]
Each new stable LXA₄ analogue compiled by Petasis and co-workers (27, 46–49) were subjected to enzymatic stability examinations in order to accurately demonstrate their resistance to rapid metabolism by recombinant eicosanoid oxido-reductase (EOR). These compounds were compared to the native LXA₄ to determine which was metabolised the fastest, Fig. 2.12.

The deactivation was monitored by the production of the co-factor NADH. As expected, analogue 46 was the slowest to be metabolised due to the absence of a hydroxyl group on the lower chain.

These new compounds were also tested for their ability to inhibit PMN infiltration by comparison of zymosan A induced-peritonitis in mice, Fig. 2.13.

All of the above new stable analogues were found to be potentially effective anti-inflammatory agents as they increased the inhibition of PMN by up to 32% in the case of 47. This level of activity is significant as LX and their analogues possess comparable potency to current non-steroidal anti-inflammatory drugs on the market. For example, the anti-inflammatory drug indomethacin 69, Fig. 2.14, reduces PMN infiltration by 35–40% in the same model of peritonitis [32].

Further to this, the aromatic analogue 47 displayed therapeutic ability to reduce PMN infiltration in murine hind-limb ischemia-induced lung injury, comparable to synthetic analogues that lack the additional benzene ring moiety [24, 25]. Compound 47 was also shown to regulate the production of important cytokines and chemokines known to be fundamental in the inflammatory process [33, 34]. A decrease in MIP-2, TNF-α, and IFN-γ was observed and no effect was observed on the levels of RANTES or SDF-1.
2.4 (B) Structural Modifications of the Triene

Fig. 2.12  Enzymatic metabolism by eicosanoid oxido-reductase [4]
Although the Lipoxin receptor target has been sequenced [22], the tertiary structure has not been determined to date. Therefore, any extension and/or structural modifications of the upper chains could potentially lead to some attractive...
biological findings, as chemical alterations of the lower chain have proven to be extremely advantageous in the previously reported para-fluorophenoxy Lipoxin analogue 6. Structural modification of the top chain is a less researched area as the stereocentres at the hydroxyl groups are essential for bioactivity. The conversion of the stereocentre at C₆ to the corresponding (S) stereocentre results in a complete loss of activity, Fig. 2.15 [35].

The C₅ and C₆ hydroxyl groups have displayed resistance to enzymatic metabolism by EOR, therefore rendering this an undesirable part of the Lipoxin structure to alter. However, Guilford and co-workers discovered β-oxidation can occur at C₃ in the para-fluorophenoxy analogue 6, Scheme 2.18 [5].

Stability experiments carried out on plasma samples by Guilford and co-workers revealed an unexpected result. The para-fluorophenoxy analogue 6 was converted

![Chemical structure](image)

**Fig. 2.15** Inversion of stereocentre at C₆ [32]

![Chemical structure](image)

**Scheme 2.18** In vivo metabolism of para-fluorophenoxy analogue 6 [5]
into the corresponding acid 70 followed immediately by $\beta$-oxidation to furnish the 2,3-dehydro analogue 71. The assignment of this structure was aided with direct comparisons to the lipid metabolisms of the prostaglandin and the leukotriene pathways previously reported in the literature [36, 37]. With these findings in hand, Guilford designed and synthesised two new LXA$_4$ analogues (72 and 73) by directly replacing the CH$_2$ group at C3 with an oxygen to prevent this $\beta$-oxidation and hence proposed to enhance the metabolic and chemical stability, Fig. 2.16 [5]. The design of these analogues combines the useful strategy of domain modifications (C) and (A), Fig. 2.1, modifications the upper and lower chains.

The seleoselective synthesis of 72 and 73 relies upon a Wittig reaction of a known enyne reagent [38], a palladium-catalysed Sonogashira coupling reaction and an activated zinc reduction of an alkyne. A successful chiral pool strategy was utilised in order to achieve the correct stereochemistry at C5 and C6 as key intermediates for the Sonogashira coupling reaction were obtained from L-Rhamnose 74, Scheme 2.19.

L-Rhamnose 74 was reacted with sulfuric acid, copper sulfate and cyclohexane at room temperature for 16 h to afford the corresponding protected cyclohexylidene ketal 75 in 57% yield. This was reduced using NaBH$_4$ in methanol to give the triol 76 in 88% yield. Phase transfer conditions were employed to prepare the required ester which was converted into the corresponding aldehyde 77 in 92% yield using sodium metaperiodate in a mixture of water and acetone. A Wittig coupling of aldehyde 77 and the protected alkyne 78 yielded a 2:1 of mixture of E,E and E,Z isomers as determined by $^1$H NMR spectroscopic analysis. This mixture was dissolved in dichloromethane and treated with iodine to give the required
protected E,E-dienyne in 49% yield, Scheme 2.20. This was further deprotected using TBAF in THF giving the required terminal alkyne 79 in 99% yield.

The synthesis of the Sonogashira coupling partner 83, Scheme 2.21, proceeded with the conversion of carboxylic acid 80 into its acid chloride by treatment with oxalyl chloride and a catalytic amount of DMF, followed by direct preparation of the Weinreb amide. This amide was treated with a solution of ethynylmagnesium bromide to furnish the target ketone 81 in 59% yield over three steps. Ketone 81 was reduced using R-Alpine-Borane although with a modest ee value of between 60 and 70%. This problem was overcome by the conversion of the alcohol to its dinitrobenzoyl derivative followed by a recrystallization to give ee values greater than 98%. This ester was deprotected using K₂CO₃ in MeOH, followed by bromination using NBS
and silver nitrate to form the chiral alcohol 82 in 79% yield over the final two steps. Reduction of the 82 using lithium aluminium hydride and aluminium chloride gave the vinyl bromide 83, the substrate for a subsequent Sonogashira coupling reaction, Scheme 2.21.

![Scheme 2.21 Synthesis of vinyl bromide 83 for Sonogashira coupling](image)

The Sonogashira coupling of 83 and 79 gave the required alkyne in 50% yield, Scheme 2.22. Cleavage of the acetal protecting group with AcOH gave diol 84 in 58% yield. Diol 84 was hydrolysed under basic conditions affording 72 in 58% yield. Reduction using activated zinc, followed by hydrolysis furnished 73 in a low 30% yield.

The natural LX along with stable analogues provide anti-inflammatory benefits in several models of induced skin inflammation [39]. With this information in hand, β-oxidation resistant analogues 72 and 73 were analysed in a calcium ionophore model topically applied to the mouse ear skin. This study revealed comparable potency to the native analogues, by inhibiting edema formation along with a decrease in neutrophil and granulocyte infiltration. Moreover, compounds 72 and 73 have demonstrated the ability to promote the resolution of colitis induced by the hapten trinitrobenzene sulfonic acid which is a model of Crohn’s disease [40, 41].
2.6 Conclusion

Modifications of three key target areas on the LX structure have resulted in the development of Lipoxin analogues displaying increased bioactivity and bioavailability compared to the native LX. The potential biological applications of these stable LX analogues have resulted in a number of efficient synthetic routes being developed for their preparation. Replacement of the C15–20 chain by cyclohexyl- and phenoxy-groups and later the further derivatisation of these analogues with fluoro-groups, gave rise to compounds which showed increased biostability and

Scheme 2.22  Synthesis of stable analogues 72 and 73 [5]
displayed potential anti-cancer properties. Phillips and Petasis pioneered the research involving stabilisation of this key C_{15–20} chain. Modification of the triene structure which is present in the native LX has been an active area of research. Incorporation of benzene or a heteroaromatic ring in place of this triene structure has had a number of enhanced properties, including stability towards enzymatic decomposition. Guiry and co-workers reported the first stereocontrolled synthesis of a benzene-containing analogue and found that it enhanced the phagocytosis of PMN by macrophages. Guiry and co-workers later published the synthesis of a novel analogue, where the triene had been replaced by a pyridine ring. They found that both epimers displayed potent anti-inflammatory characteristics. There have been fewer reports of structural modifications of the upper chain of the LX, mainly due to the importance of retaining the hydroxyl groups in order to maintain bioactivity. Guilford incorporated oxygen into the upper chain, replacing the β-CH₂ group. This resulted in an analogue that displayed resistance to β-oxidation, leading to heightened metabolic and chemical stability. This derivative also showed potential in the treatment of Crohn’s disease.

This chapter reports a concise review of the synthetic and biological developments of novel stable Lipoxin analogues. The major and noteworthy synthetic obstacles and achievements were outlined and discussed. There is an on-going effort to provide novel therapeutic agents to combat an array of inflammatory diseases and it is hoped that this timely review will help to stimulate the design and biological evaluation of novel Lipoxin analogues.

References

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