Articles

Aromatic Lipoxin A_4 and Lipoxin B_4 Analogues Display Potent Biological Activities

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Lipoxins are a group of biologically active eicosanoids typically formed by transcellular lipoxygenase activity. Lipoxin A_4 (LXA_4) and Lipoxin B_4 (LXB_4) biosynthesis has been detected in a variety of inflammatory conditions. The native lipoxins LXA_4 and LXB_4 demonstrate potent antiinflammatory and proresolution bioactions. However, their therapeutic potential is compromised by rapid metabolic inactivation by PG dehydrogenase-mediated oxidation and reduction. Here we report on the stereoselective synthesis of aromatic LXA_4 and LXB_4 analogues by employing Sharpless epoxidation, Pd-mediated Heck coupling, and diastereoselective reduction as the key transformations. Subsequent biological testing has shown that these analogues display potent biological activities. Phagocytic clearance of apoptotic leukocytes plays a critical role in the resolution of inflammation. Both LXA_4 analogues (1*R*)-**3a** and (1*S*)-**3a** were found to stimulate a significant increase in phagocytosis of apoptotic polymorphonuclear leukocytes (PMN) by macrophages, with comparable efficacy to the effect of native LXA_4, albeit greater potency, while the LXB_4 analogue also stimulated phagocytosis with a maximum effect observed at 10^{-11} M. LX-stimulated phagocytosis was associated with rearrangement of the actin cytoskeleton consistent with that reported for native lipoxins. Using zymosan-induced peritonitis as a murine model of acute inflammation (1*R*)-**3a** significantly reduced PMN accumulation.

Introduction

In 1984, a new class of arachidonic acid metabolites was isolated by Samuelsson and Serhan.[1] These molecular agents, named Lipoxin A_4 (**1**) and B_4 (**2**), are produced by transcellular metabolism of eicosanoid derivatives (Figure 1). Lipoxins (LX) are typically formed by the sequential actions of 15- and 5-, or 5- and 12-lipoxygenases, on arachidonic acid, depending on the cellular context.[2] These compounds are biologically important with LX deficiency being observed in many human diseases, including asthma, glomerulonephritis, and rheumatoid arthritis.[3−6] Both *in vivo* and *in vitro* studies show that LX regulate polymorphonuclear leukocytes (PMN), chemotaxis, adhesion and transmigration.[7−10] LXA_4 appears to block some leukocyte responses to leukotrienes including PMN adhesion mediated by CD11/CD18 expression and endothelial-neutrophil adhesion dependent on endothelial P-selection, and PMN-mesangial cell adhesion.[10,11] We have previously demonstrated that LX are involved in the resolution of inflammation by promoting nonphlogistic phagocytosis of apoptotic PMN by macrophages *in vitro* and *in vivo*.[12,13] Consistent with a biological role for LX in the resolution of inflammation is evidence that their production at an inflammatory locus is co-incident with the resolution of inflammation.[14,15]

LX are rapidly metabolized either by oxidation at C-15 or reduction of the C13−C14 double bond.[16] 15-Hydroxyprostaglandin dehydrogenase (15-PGDH) catalyzes the dehydrogenation of the C-15 hydroxyl to afford the corresponding ketone 15-oxo-LXA_4. Alternatively, leukotriene B_4 12-hydroxydehydrogenase (PGR/LTB_4DH) may catalyze the reduction of the C13−C14 double bond of LXA_4 or 15-oxo-LXA_4 to give 13,14-dihydro-LXA_4 or 13,14-dihydro-15-oxo-LXA_4, respectively. LX are also subject to ω oxidation at C20.[17] This represents a major obstacle to the application of these compounds as pharmacological agents although chemically and metabolically stable LXA_4 analogues which are equipotent in animal models of inflammation have been reported.[18,19] We propose that the active region of these molecules encompasses the alcohol and carboxylic acid functionalities as outlined in Figure 1. Therefore, we have

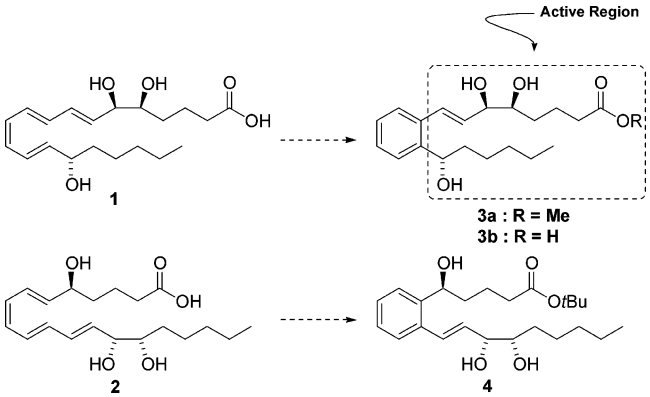

Figure 1. Lipoxin A_4 (**1**) and Lipoxin B_4 (**2**) and their corresponding analogues.

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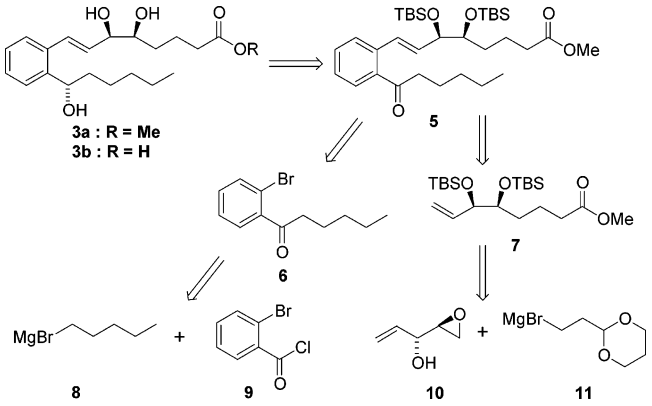
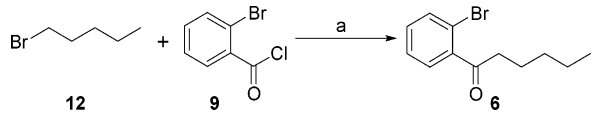
Scheme 1. Retrosynthetic Analysis of Lipoxin Analogue **3**



Scheme 2. Preparation of Aryl Bromide **6**[a]



[a] (a) **12**, Mg, diethyl ether, reflux then **9**, THF, −78 °C, 2.5 h, 87%.

designed compounds **3** and **4** as analogues of LXA_4 and LXB_4 which, while retaining the active region associated with *in vivo* cellular interactions, possess an aromatic ring in place of the reactive hexatriene system.[20] Additionally, the pharmacological profile of **3** and **4** can be further optimized by the introduction of substituents onto the aromatic ring.

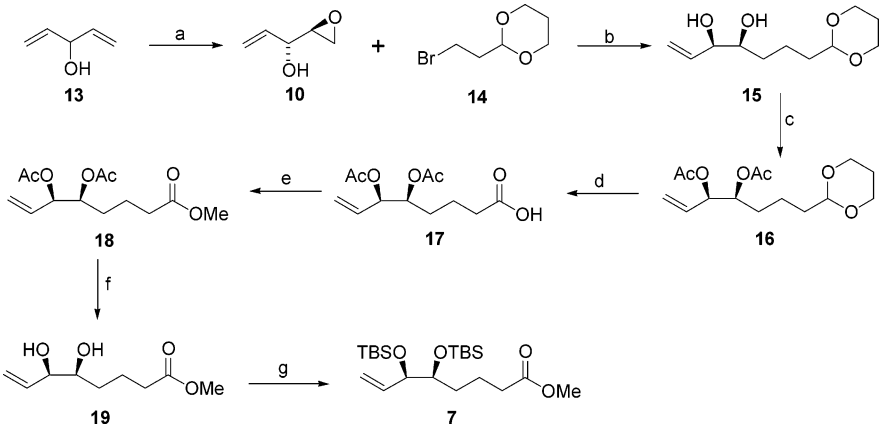
Synthesis of Lipoxin A_4 Analogue The retrosynthetic analysis for the LXA_4 analogue **3** relies upon advanced intermediate **5** being formed by the coupling of aryl bromide **6** with alkene **7** *via* a palladium-mediated Heck reaction (Scheme 1). It is proposed that ketone **6** be synthesized by the reaction of Grignard reagent **8** with acid chloride **9**. Intermediate **7** can be obtained by the regioselective ring-opening of epoxy alcohol **10** by Grignard reagent **11**.

The Grignard derivative of 1-bromopentane (**12**) was prepared *in situ* and slowly added to a solution of the acid chloride **9** at −78 °C, affording aryl bromide **6** in 87% yield (Scheme 2). Attempts at synthesizing the iodo analogue of **6** utilizing similar chemistry proved unsuccessful. Addition of iron(III) chloride, a reagent known to increase the reactivity of acid chlorides,[21] also failed and while the reaction between the cuprate derivative of **8** and acid chloride **9** did proceed, the final yield was poor.

Sharpless asymmetric epoxidation of divinylcarbinol (**13**) was carried out using the procedure of Wong and Romero, furnishing epoxide **10** in 85% yield and with excellent enantioselectivity (>99%) (Scheme 3).[22] Addition of the Grignard derivative of **14** to epoxide **10** in the presence of a catalytic amount of copper iodide furnished *syn*-diol **15** in 82% yield. Surprisingly, yields for this reaction were consistently lower when epoxide **10** was protected as the silyl ether despite some literature evidence to the contrary.[23−25] Diol **15** was protected in near quantitative yield as the acid-stable diacetate **16**. Addition of an excess of Jones' reagent to a concentrated solution of **16** in acetone led to both the cleavage of the acetal group and oxidation of the resultant aldehyde to the carboxylic acid **17**, which was subsequently converted in 97% yield to methyl ester **18** after reaction with diazomethane. At this point, a change in protecting groups for the hydroxyls was required. In the course of our studies, we discovered that **18** was an unsuitable candidate for the Heck reaction, with coupling to the aryl bromide being accompanied by elimination of the acetate groups, in line with results obtained by Lautens and co-workers.[26] Therefore, hydrolysis of the acetate groups, followed by reprotection of the diol **19** using standard conditions of *tert*-butyldimethylsilyl chloride and imidazole in DMF, afforded advanced intermediate **7** in 87% yield.

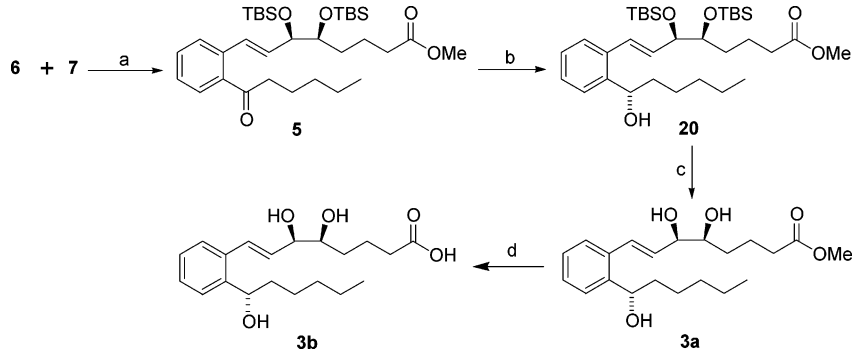
A range of conditions were screened for the Heck reaction of aryl bromide **6** and alkene **7**, including those employed by Nokami et al. in their synthesis of LXA_4 analogues.[24] However, only the use of palladium acetate and tri-*o*-tolylphosphine with triethylamine as the solvent provided **5**, albeit in poor yield and requiring very long reaction times. We reasoned that a higher boiling-point amine might furnish the desired compound in higher yield and in a shorter period of time and were gratified to find that the substitution of triethylamine with tributylamine afforded **5** in 88% yield after 12 h (Scheme 4). No decomposition of the methyl ester was observed under these conditions. The presence of a doublet at 6.85 ppm with a large coupling constant of 15.8 Hz (indicating a trans relationship) in the ^{1}H NMR spectrum confirmed that the required *E*-stereochemistry was in place. While **5** could be reduced with sodium borohydride to produce a mixture of epimeric alcohols which were separable by column chromatography, reduction with Brown's (−)-β-chlorodiisopinocampheylborane afforded stereoisomer **20** in 67% yield and 92% de as determined by ^{1}H NMR analysis. The configuration of the newly formed stereocenter is (1*S*)-OH as expected from the use of this chiral reducing agent.[27] Removal

Scheme 3. Synthesis of Alkene **7**[a]



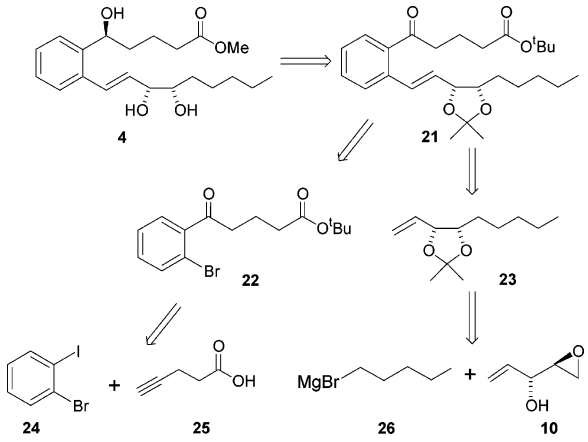
[a] (a) (−)-DIPT, cumene hydroperoxide, CH_2Cl_2, −35 °C, 36 h, 85%, >99% ee; (b) **14**, Mg, THF, reflux then CuI (20 mol %), **10**, THF, −35 °C, 3 h, 82%; (c) AcCl, pyridine, THF, 0 °C, 17 h, 97%; (d) Jones' reagent, acetone, 2 h, 83%; (e) CH_2N_2, diethyl ether, 0 °C, 16 h, 97%; (f) NaOMe, MeOH, −40 °C to −10 °C, 14.5 h, 87%; (g) TBDMSCl, imidazole, DMF, 24 h, 99%.

Scheme 4. Synthesis of LXA_4 Analogue **3**[a]



[a] (a) $Pd(OAc)_2$, (*o*-tolyl)$_3$P, Bu_3N, 120 °C, 12 h, 88%; (b) (−)-DIP chloride, diethyl ether, −25 °C, 48 h, 67%; (c) *p*-TsOH, methanol, room temp, 5 h, 84%; (d) LiOH, methanol/water, 1 h, 89%.

Scheme 5. Retrosynthetic Analysis of Lipoxin Analogue **4**



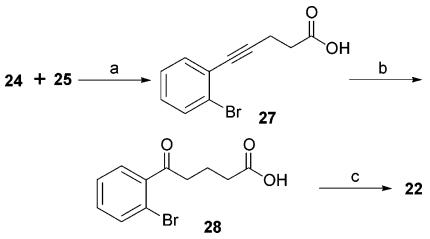
of the silyl ether protecting groups under the mild conditions of *p*-toluenesulfonic acid in methanol furnished the target compound **3a** in 84% yield. Subsequent hydrolysis afforded the corresponding carboxylic acid **3b** although the acid was found to be prone to lactonization. The (1*R*)-OH epimers of **3a** and **3b** were also prepared as there is considerable literature evidence to suggest that the epimeric compounds would be biologically active.[28,29]

Synthesis of Lipoxin B_4 Analogue. The retrosynthetic analysis for the LXB_4 analogue **4** divides the molecule into two main fragments, a bromo keto-ester **22** and an olefin **23** (Scheme 5). The *E*-configuration of the double bond in **21** is to be established by carrying out a Heck reaction between fragments **22** and **23**. Ketone **22** may be obtained by way of Sonagashira coupling of aryl iodide **24** and alkyne **25**, followed by oxidation. The stereochemistry in **23** is to be established *via* Sharpless asymmetric epoxidation on divinyl carbinol followed by a Grignard reaction.

The preparation of δ-keto acid **28**, the precursor to ester **22**, is a known synthetic route.[30] Sonogashira coupling of 1-bromo-2-iodobenzene (**24**) and 4-pentynoic acid (**25**) gave acetylenic acid **27** in 69% yield which was treated with concentrated sulfuric acid to afford δ-keto acid **28** (Scheme 6). **28** was subsequently converted to *tert*-butyl ester **22** in 60% yield following a procedure described by Wright.[31]

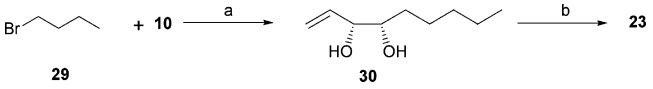
The chiral epoxide **10**, which was previously prepared during the synthesis of the LXA_4 analogues, underwent ring-opening on addition of butylmagnesium bromide to produce diol **30** in 63% yield (Scheme 7). Diol **30** was protected as the corresponding acetal **23** in 76% yield using 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid.

Scheme 6. Preparation of Aryl Bromide **22**[a]



[a] (a) $Pd(PPh_3)_4$, CuI (20 mol %), Et_2NH, room temp, 20 h, 69%; (b) H_2SO_4, room temp, 15 min, 74%; (c) *t*-BuOH, H_2SO_4, magnesium sulfate, CH_2Cl_2, room temp, 48 h, 60%.

Scheme 7. Synthesis of Olefin **23**[a]

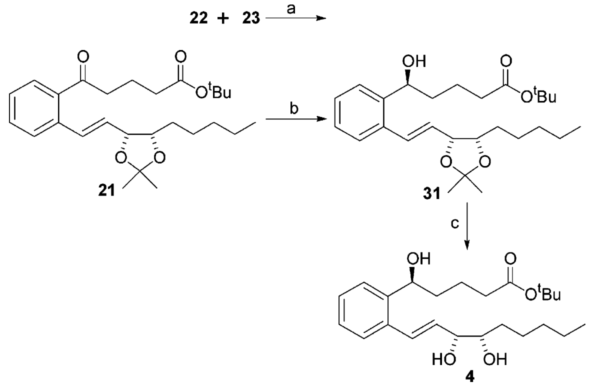


[a] (a) **29**, Mg, THF, 30 °C then CuI (20 mol %), **10**, THF, −30 °C, 3 h, 63%; (b) $Me_2C(OMe)_2$, *p*-TSA, room temp, 18 h, 76%.

A palladium-catalyzed Heck reaction between aryl bromide **22** and olefin **23** provided keto-ester **21** in 41% yield (Scheme 8). In contrast to the synthesis of intermediate **5**, higher yields of **21** were obtained when triethylamine was employed as the base rather than tributylamine. As before, the ketone could be asymmetrically reduced at C-5 using (−)-β-chlorodiisopinocampheylborane to afford (5*S*)-hydroxy ester **31** in 61% yield and 97% de. Cleavage of the acetal **31** under acidic conditions furnished the target compound, *tert*-butyl ester **4**, in 59% yield. The corresponding carboxylic acid was found on formation to undergo spontaneous lactonization and was not subjected to biological evaluation.

Biological Evaluation. Differentiated THP-1 cells were exposed to the LXA_4 analogues (1*R*)-**3a** and (1*S*)-**3a** or LXB_4 analogue **4** at different concentrations for 15 min at 37 °C before addition of apoptotic human PMNs, and the extent of phagocytosis was compared with that obtained with native LXA_4 (1 nM; 15 min at 37 °C), previously shown to significantly enhance phagocytosis.[12,13] Pretreatment of differentiated THP-1 cells with (1*R*)-**3a** over a range of concentrations between 10^{-13} and 10^{-9} M resulted in a significant increase in phagocytosis of apoptotic PMNs, comparable to the effect of the native LXA_4 (Figure 2). No effect was observed when a concentration of 10^{-14} M was used (Figure 2). The bell-shaped dose−response is characteristic of chemokine-type receptor mediated events such as the receptor for formylated peptides (FPR) family and has already been described for native LX-stimulated phagocytosis.[12] A significant increase in phagocytosis of apoptotic PMNs was observed only

Scheme 8. Synthesis of LXB_4 Analogue **4**[a]



[a] (a) $Pd(OAc)_2$, (*o*-tolyl)$_3$P, Et_3N, 80 °C, 48 h, 41%; (b) (−)-DIP chloride, diethyl ether, −20 °C, 24 h, 67%; (c) 2 N HCl, THF, room temp, 20 h, 59%.

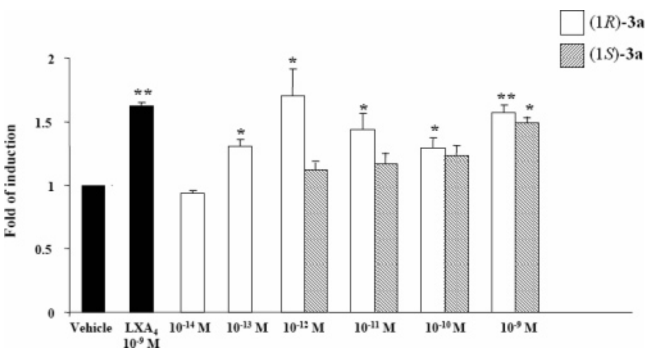


Figure 2. LXA_4 analogues promote phagocytosis of apoptotic PMNs by differentiated THP-1 cells. Differentiated THP-1 cells were treated with vehicle or LXA_4 (1 nM), or with the indicated concentrations of (1*R*)-**3a** and (1*S*)-**3a**, before coincubation with apoptotic PMNs for 2 h. Data are expressed as fold of induction over basal ± SEM ($n = 3$); *$p < 0.05$; **$p < 0.01$.

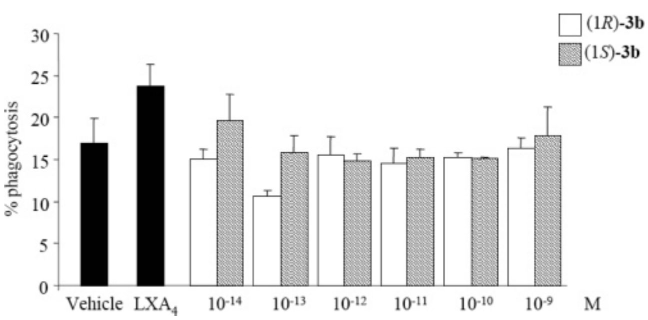


Figure 3. The acid form of LXA_4 analogues did not increase phagocytosis. Differentiated THP-1 cells were treated with vehicle or LXA_4 (1 nM), or with the indicated concentrations of (1*R*)-**3b** and (1*S*)-**3b**, before coincubation with apoptotic PMNs for 2 h. Data are expressed as percentage of phagocytosis ± SEM ($n = 3$).

in the presence of 10^{-9} M (1*S*)-**3a** but not at lower concentrations (Figure 2). By contrast, the corresponding acids (1*S*)-**3b** and (1*R*)-**3b** did not increase phagocytosis over a concentration range of 10^{-14} to 10^{-9} M (Figure 3). A recent review by Brink and co-authors suggests that the pharmacological addition of LXA_4 analogues as esters are prodrugs that require conversion to their corresponding free acids to evoke LX-mediated biological actions.[32] In our case it appears that addition as the prodrug ester is necessary, and this may be due to differences in the rates of their transfer across cell membranes.

The LXB_4 analogue also stimulated phagocytosis of apoptotic PMNs with a maximum effect observed at 10^{-11} M (Figure 4).

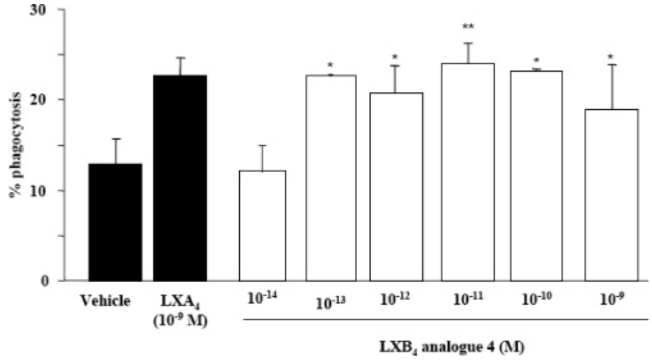


Figure 4. LXB_4 analogue **4** stimulates phagocytosis of apoptotic PMNs by differentiated THP-1 cells. Differentiated THP-1 cells were treated with vehicle or LXA_4 (1 nM), or with the indicated concentrations of **4** before coincubation with apoptotic PMNs for 2 h. Data are expressed as percentage of phagocytosis ± SEM ($n = 3$); *$p < 0.05$; **$p < 0.01$.

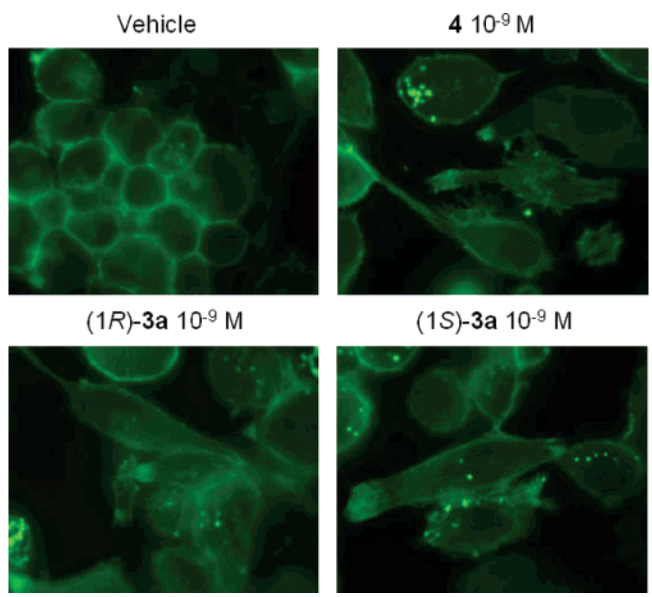


Figure 5. Effect of LX analogues on actin rearrangement in THP-1 cells. Differentiated THP-1 cells were exposed to vehicle or to the LX analogues (1 nM for 15 min). Cells were fixed with paraformaldehyde, and localization of actin was determined using Oregon Green phalloidin and visualized by fluorescence microscopy using a ×100 oil objective. Images are representative of one of three independent experiments.

In addition, stimulation of differentiated THP-1 cells with all of compounds tested caused F-actin rearrangement similar to the described effect of native LX (Figure 5).[33]

The effect of LX analogues on monocyte adhesion was also investigated. We examined their ability to stimulate adherence of monocytes to a matrix such as laminin, previously shown to be significantly enhanced by LXA_4 and LXB_4 as well as LXA_4 stable analogues.[34,35] In these experiments, the LXA_4 methyl ester analogues significantly increased adherence while a modest effect was observed when the corresponding acids were used (Figure 6).

LXA_4 and aspirin-triggered LX exert their bioactions through a high-affinity, seven-transmembrane G protein-coupled receptor named ALXR. Boc2 (*N*-*tert*-butyloxycarbonyl-Phe-D-Leu-Phe-D-Leu-Phe) is a nonselective formyl peptide receptor antagonist described in several studies as a nonspecific antagonist of the FPRL1/ALXR.[36−40] Differentiated THP-1 cells were treated with the pan-FPR/ALXR antagonist, Boc2, for 15 min in the presence of vehicle, LXA_4 (1 nM), (1*R*)-**3a** (10^{-12} M), or (1*S*)-**3a** (1 nM)

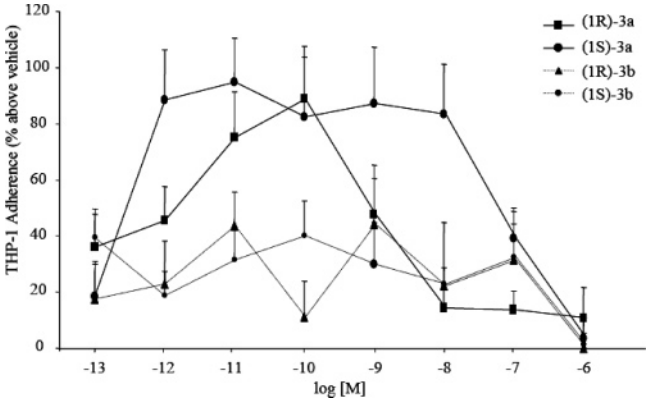


Figure 6. Effect of stable analogues on THP-1 cell adherence to laminin. THP-1 (3×10^6/well) labeled with BCECF-AM were incubated with vehicle or LX analogues for 30 min at 37 °C. Values represent means ± SE for three separate experiments performed in triplicate and are expressed as the percent adherence above that with cells exposed to vehicle alone.

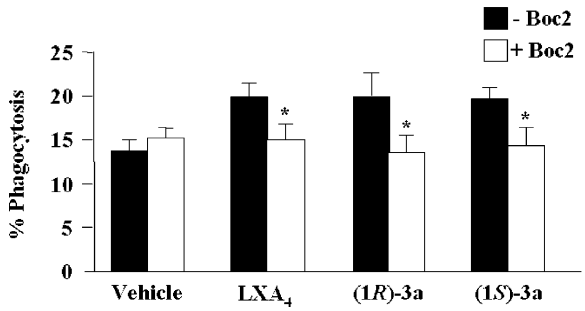


Figure 7. LXA_4 analogues-stimulated phagocytosis of apoptotic PMNs is blocked by the receptor antagonist Boc2. Differentiated THP-1 cells were treated with Boc2 compound (100 μM) in the absence or in the presence of LXA_4 (1 nM) or (1*R*)-**3a** (10^{-12} M) or (1*S*)-**3a** (1 nM) for 15 min at 37 °C and then coincubated with apoptotic PMNs for 2 h. Data are mean percentage of phagocytosis ± SEM ($n = 3$). *$p < 0.05$ vs − Boc2.

for 15 min before coincubation with apoptotic PMNs for 2 h. Phagocytosis of PMNs induced by LXA_4 and LXA_4 analogues was significantly inhibited by pretreatment with the pan-FPR inhibitor Boc2, suggesting that the effect of these LX analogues may be mediated by the activation of the LX receptor.

The effects of the LXA_4 analogues (1*R*)-**3a** and (1*S*)-**3a** (0.5 to 50 μg/kg) on the peritoneal neutrophil accumulation (GR-1+ cells) triggered by zymosan were also studied. Treatment with (1*R*)-**3a** (50 μg/kg) caused a statistically significant ($p < 0.05$) decrease in neutrophil accumulation (Figure 8), with no effect at lower doses. Treatment with (1*S*)-**3a** evoked a 31.46% decrease in the GR-1+ cell accumulation at the highest dose tested (50 μg/kg); however, this decrease was not statistically significant.

Conclusions

A stereoselective synthesis of aromatic LXA_4 and LXB_4 analogues has been reported. The synthetic route developed establishes the required stereochemistry by way of Sharpless epoxidation, Pd-mediated Heck coupling and diastereoselective reduction reactions. Both LXA_4 analogues (1*R*)-**3a** and (1*S*)-**3a** were found to result in a significant increase of phagocytosis of apoptotic PMNs, comparable to the effect of native LXA_4, while the LXB_4 analogue **4** also stimulated phagocytosis with a maximum effect observed at 10^{-11} M. In addition, stimulation of differentiated THP-1 cells with all of the compounds tested caused F-actin rearrangement similar to the described effect of

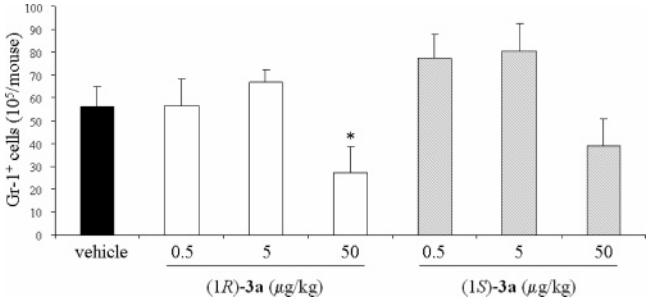


Figure 8. Effect of LXA_4 analogues (1*R*)-**3a** and (1*S*)-**3a**, on zymosan-induced peritonitis. Mice were pretreated with (1*R*)-**3a** and (1*S*)-**3a** (0.5, 5, 50 μg/kg) 5 min prior to intraperitoneal injection of zymosan A (1 mg in 0.5 mL of sterile PBS). Neutrophil (Gr-1+ cells) accumulation was measured 4 h later. Data are means ± SEM of $n = 6$ mice per group (vehicle group = 15 mice), *$p < 0.05$ vs vehicle.

native lipoxins. Furthermore, a significant increase of phagocytosis of apoptotic PMNs was observed only in the presence of a 10^{-9} M concentration of (1*S*)-**3a** but not at lower concentrations. The LXB_4 analogue **4** was likewise observed to stimulate phagocytosis of apoptotic PMNs. Phagocytosis of PMNs induced by (1*R*)-**3a**, (1*S*)-**3a**, and **4** was significantly inhibited by pretreatment with the pan-FPR inhibitor Boc2. This strongly suggests that the effect of these LX analogues is mediated by the activation of the LX receptor.

Oral administration of LXA_4 (~5 μg/kg) has previously been reported by Bannenberg and colleagues to inhibit leukocyte infiltration in zymosan A-induced peritonitis.[41] Here, using the same experimental model, we investigated the possible antiinflammatory effects of the lipoxin analogues (1*R*)-**3a** and (1*S*)-**3a** (0.5 to 50 μg/kg) on the acute neutrophil accumulation (GR-1+ cells) at 4 h. The zymosan-induced neutrophil migration was attenuated by the treatment with (1*R*)-**3a** at the highest dose examined (50 μg/kg). A similar trend was observed for (1*S*)-**3a**, however, the degree of attenuation was not significant. This difference in neutrophil accumulation may be due to potency differences of these analogues and to assess this possibility, further animal studies will be carried out using higher doses of each compound as well with LXB_4 analogue **4**. The difference observed in the biological activities of the epimeric alcohols (1*R*)-**3a** and (1*S*)-**3a** correlate well with other epimeric LXA_4 analogues and is presumably due to diastereomeric interactions between the epimeric compounds and their biologically active sites.[41]

There is an increasing appreciation that the resolution of inflammation is a dynamic process that may be regulated by lipid and peptide mediators.[12,13,40] Phagocytosis of apoptotic cells is a key event in promoting the resolution of inflammation. Macrophage phagocytosis of leukocytes at a site of inflammation spares tissue from exposure to the noxious and immunogenic contents of necrotic cells and is associated with the release of antiinflammatory mediators.[42−44] Defects in clearance of apoptotic cells have been closely associated with several chronic inflammatory conditions including systemic lupus erythematosus,[45] cystic fibrosis,[46] and immune complex-mediated arthritis.[47] Such conditions may be amenable to therapeutic intervention by agents such as stable synthetic lipoxin analogues.

In view of the findings reported here, further work is currently underway in the synthesis of heteroaromatic LXA_4 and LXB_4 analogues and their subsequent biological evaluation, the results of which will be presented shortly. In the course of preparing this article, we became aware of a nonstereoselective synthesis of (1*S*)-**3a** and (1*R*)-**3a** employing a related, but different, synthetic strategy to the one described herein.[48]

Experimental Section

^{1}H NMR (300 or 500 MHz) and ^{13}C NMR (75 or 126 MHz) were recorded at room temperature in $CDCl_3$ with Varian-Unity spectrometers. Chemical shifts (δ) are in parts per million relative to $CHCl_3$ (7.26, ^{1}H), $CDCl_3$ (77.0, ^{13}C). Coupling constants are given as absolute values expressed in hertz. High-resolution mass spectra were measured on a Waters/Micromass instrument. Elemental analyses were performed by Ms Anne Connolly, School of Chemistry and Chemical Biology, University College Dublin. Infrared spectra were recorded on a Perkin-Elmer infrared FT spectrometer. Optical rotation values were measured on a Perkin-Elmer polarimeter. Thin layer chromatography was carried out using Merck Kieselgel 60 F254 silica gel plates. Column chromatography separations were performed using Merck Kieselgel 60 (Art. 7734). Solvents were dried immediately before use by distillation from standard drying agents. (−)-DIP-Cl was used as received from commercial sources. HPLC analyses were performed using a Schimadzu LC-2010A machine.

1-(2′-Bromo-phenyl)-hexan-1-one (6). The Grignard derivative of 1-bromopentane was prepared by addition of the bromide (3.79 g, 3.11 mL, 25.10 mmol) to magnesium turnings (603 mg, 25.13 mmol) in diethyl ether (40 mL) followed by heating to reflux for 1 h. The Grignard solution was then transferred over 30 min to a two-necked flask containing 2-bromobenzoyl chloride (5 g, 22.84 mmol) dissolved in THF (40 mL) and cooled to −78 °C. The solution was stirred at −78 °C for 30 min and then allowed to warm to room temperature over 2 h. Water (40 mL) and 1 M HCl (10 mL) were added, and the mixture was extracted with diethyl ether (3 × 50 mL). The combined organic layer was washed with water (50 mL) and brine (50 mL) and dried over magnesium sulfate. After removal of the solvent *in vacuo*, the residue was purified by column chromatography using silica gel (pentane:ethyl acetate = 10:1) to afford the ketone (5.08 g, 87%) as a pale yellow oil.

(3*R*,4*S*)-7-[1′,3′]Dioxan-2′-yl-hept-1-ene-3,4-diol (15). The Grignard derivative of 2-(2-bromoethyl)-1,3-dioxane was prepared by addition of the bromide (21 mL, 0.16 mol) to preactivated magnesium turnings (3.6 g, 0.15 mol) in THF (250 mL) followed by heating to reflux for 45 min. The solution was then transferred to a two-necked flask containing copper(II) iodide (1.90 g, 0.01 mol) at −35 °C and stirred for 5 min. The epoxide **10** (5 g, 0.05 mol) in THF (50 mL) was added dropwise over 20 min, and stirring was continued for a further 3 h at −35 °C. Solid ammonium chloride (2.5 g) was added, and the solution was stirred at room temperature for 10 min. The solvent was removed *in vacuo*, and saturated ammonium chloride solution (250 mL) was added. The solution was extracted with ethyl acetate (6 × 150 mL), and the combined organic layers were washed with water (250 mL) and brine (250 mL) and dried over magnesium sulfate. After removal of the solvent *in vacuo*, the residue was purified by column chromatography using silica gel (pentane:ethyl acetate = 4:1 → 1:1 and then ethyl acetate) to afford the diol **15** (8.8 g, 82%) as a pale yellow oil.

Acetic Acid 1-(1-Acetoxy-4-[1′,3′]dioxan-2′-yl-butyl)-allyl Ester (16). Diol **15** (2.28 g, 10.54 mmol) was dissolved in THF (250 mL) to which pyridine (1.880 mL, 23.2 mmol) was added. Acetyl chloride (1.586 mL, 22.3 mmol) was added dropwise over 1 h at 0 °C, and stirring was continued for an additional 16 h. The solution was neutralized with 5% HCl solution (150 mL) and extracted with ethyl acetate (4 × 200 mL). The combined organic layers were washed with water (150 mL) and brine (150 mL) and dried over magnesium sulfate. The solvent was removed *in vacuo*, and the residue was purified by column chromatography using silica gel (pentane:ethyl acetate = 4:1) to afford the diacetate **16** (3.07 g, 97%) as a pale yellow oil.

(5*S*,6*R*)-5,6-Diacetoxy-oct-7-enoic Acid (17). Acetal **16** (1.0 g, 3.3 mmol) was dissolved in acetone (1 mL) to which Jones' reagent (5 mL) was added over 5 min. The solution was stirred at room temperature for 1.5 h. Isopropanol (10 mL) was added and stirring was continued for a further 15 min. The mixture was filtered through a pad of silica gel and washed with ethyl acetate (100 mL). The solvent was removed *in vacuo* and the residue was purified by column chromatography using silica gel (pentane:ethyl acetate = 2:1) to afford the acid **17** (711 mg, 83%) as a brown oil.

(5*S*,6*R*)-5,6-Diacetoxy-oct-7-enoic Acid Methyl Ester (18). Acid **17** (2.0 g, 7.75 mmol) was dissolved in THF (5 mL) and added dropwise over 5 min to a cooled ethereal solution of diazomethane prepared from Diazald (5 g, 3 equiv). Stirring was continued for 16 h at 0 °C. The solvent was removed *in vacuo* and the residue was purified by column chromatography using silica gel (pentane:ethyl acetate = 4:1) to afford the ester **18** (1.98 g, 97%) as a colorless oil.

(5*S*,6*R*)-5,6-Dihydroxy-oct-7-enoic Acid Methyl Ester (19). Diacetate **18** (2.5 g, 9.19 mmol) (predried under high vacuum for 2 h) was dissolved in anhydrous MeOH (42 mL). NaOMe (0.347 g, 6.42 mmol) in MeOH (8 mL) was added dropwise at −40 °C over 20 min, and stirring was continued for a further 14 h at −10 °C. The solution was neutralized with AcOH and extracted with ethyl acetate (4 × 100 mL). The combined organic layers were washed with water (100 mL) and brine (100 mL) and dried over magnesium sulfate. The solvent was removed *in vacuo*, and the residue was purified by column chromatography using silica gel (pentane:ethyl acetate = 2:1) to afford the diol **19** (1.50 g, 87%) as a colorless oil.

(5*S*,6*R*)-Methyl 5,6-Bis(*tert*-butyldimethylsilyloxy)oct-7-enoate (7). Diol **19** (1.48 g, 7.86 mmol) and imidazole (1.71 g, 25 mmol) were dissolved in DMF (40 mL) to which TBDMSCl (3.72 g, 24.55 mmol) was added. The solution was stirred at room temperature for 24 h. Following removal of the solvent *in vacuo*, the residue was purified by column chromatography using silica gel (pentane → pentane:diethyl ether = 9.5:0.5) to afford the bis-silyl ether **7** (3.25 g, 99%) as a colorless oil.

Methyl (5*R*,6*R*,7*E*)-5,6-Bis{[*tert*-butyl(dimethyl)silyl]oxy}-8-(2-hexanoylphenyl)-oct-7-enoate (5). $Pd(OAc)_2$ (14 mg, 0.062 mmol) and P(*o*-tolyl)$_3$ (38 mg, 0.125 mmol) were dissolved in Bu_3N (2 mL) and stirred at room temperature for 10 min under N_2. The bromide **6** (212 mg, 0.813 mmol) was added to the reaction vessel followed by the alkene **7** (262 mg, 0.626 mmol). The reaction mixture was stirred at 120 °C for 12 h. The reaction mixture was filtered through a pad of silica gel and washed with ethyl acetate (20 mL), and the solvent was recovered *in vacuo*. The remaining Bu_3N was removed by Kugelrohr distillation at 100 °C under high vaccum. The residue was purified by column chromatography using silica gel (pentane:diethyl ether = 9.5:0.5) to afford intermediate **5** (320 mg, 88%) as a pale yellow oil.

Methyl (5*R*,6*R*,7*E*)-5,6-Bis{[*tert*-butyl(dimethyl)silyl]oxy}-8-{2-[(1*S*)-1-hydroxy-hexyl]phenyl}oct-7-enoate ((1*S*)-20). Ketone **5** (332 mg, 0.562 mmol) was dissolved in diethyl ether (10 mL). The solution was then transferred to a dry flask under a nitrogen atmosphere. The solution was cooled to −20 °C, and (−)-DIP-chloride (288 mg, 0.92 mmol) was added. The reaction mixture was stirred at −20 °C for 48 h. The reaction was then allowed to warm to room temperature and diluted with diethyl ether (10 mL). Diethanolamine (280 mg) was added, and the reaction mixture was vigorously stirred at ambient temperature for 4 h. The mixture was then poured into pentane (10 mL), filtered, and washed with diethyl ether (10 mL) and ethyl acetate (10 mL). The solvent was removed *in vacuo*, and the residue was purified by column chromatography using silica gel (pentane:diethyl ether = 8:1) to afford the benzylic alcohol (1*S*)-**20** (225 mg, 67%) as a colorless oil. The diastereomeric excess (de) was determined by ^{1}H NMR analysis to be greater than 92% prior to purification by column chromatography. This compound was determined to be 98.1% pure by two diverse methods of HPLC analysis.

Methyl (5*R*,6*R*,7*E*)-5,6-bis{[*tert*-butyl(dimethyl)silyl]oxy}-8-{2-[(1*R*)-1-hydroxy-hexyl]phenyl}oct-7-enoate ((1*R*)-20). Ketone **5** (312 mg, 0.528 mmol) was dissolved in THF (10 mL) to which a few drops of MeOH were added. The reaction mixture was cooled to 0 °C, and sodium borohydride (24 mg, 0.633 mmol) was added. The solution was stirred at room temperature for 2.5 h. Acetone (10 mL) was added to quench the remaining sodium borohydride. The solvent was removed *in vacuo*, and saturated ammonium

chloride solution (50 mL) was added. The solution was extracted with ethyl acetate (4 × 50 mL), and the combined organic layers were washed with water (50 mL) and brine (50 mL) and dried over magnesium sulfate. After removal of the solvent *in vacuo*, the residue was purified by column chromatography using silica gel (pentane:dichloromethane = 3:1 → 1:2) to afford (1*S*)-**20** (90 mg, 28%) which was identical in all respects to the previously prepared sample and (1*R*)-**20** (65 mg, 21%) as a colorless oil.

Methyl (5*S*,6*R*,7*E*)-5,6-Dihydroxy-8-{2-[(1*S*)-1-hydroxyhexyl]-phenyl}oct-7-enoate ((1*S*)-3a). *p*-Toluenesulfonic acid monohydrate (10 mg, 0.052 mmol) was added to a solution of bis-silyl ether (1*S*)-**18** (35 mg, 0.059 mmol) in methanol (0.5 mL). The mixture was stirred at r.t. for 5 h. The solvent was then removed *in vacuo*. The remaining residue was purified by preparative TLC (ethyl acetate:pentane = 3:2) to afford the methyl ester (1*S*)-**3a** (18 mg, 84%) as a colorless oil. This compound was determined to be 96% pure by two diverse methods of HPLC analysis.

Methyl (5*S*,6*R*,7*E*)-5,6-Dihydroxy-8-{2-[(1*S*)-1-hydroxyhexyl]-phenyl}oct-7-enoate ((1*S*)-3b). The methyl ester (1*S*)-**3a** (10 mg, 0.027 mmol) was dissolved in a mixture of methanol and water (3:1, 0.8 mL). Lithium hydroxide monohydrate (2.2 mg, 0.052 mmol) was then added, and the solution was stirred at room temperature for 1 h. Diethyl ether (1 mL) was added and then acidified to pH 5 with 1 M HCl. The solvent was removed *in vacuo*, and the resultant residue was purified by preparative TLC (ethyl acetate: methanol = 10:0.2) to afford the carboxylic acid (1*S*)-**3b** (8.6 mg, 89%) as a colorless oil. The acid was converted to its methyl ester derivative, and the purity was determined to be 96% by two diverse methods of HPLC analysis.

Methyl (5*S*,6*R*,7*E*)-5,6-Dihydroxy-8-{2-[(1*R*)-1-hydroxyhexyl]-phenyl}oct-7-enoate ((1*R*)-3a). *p*-Toluenesulfonic acid (6.5 mg, 0.034 mmol) was added to a solution of bis-silyl ether (1*R*)-**18** (20 mg, 0.033 mmol) in methanol (0.5 mL). The resultant mixture was stirred at r.t. for 5 h. The solvent then was removed *in vacuo*. The remaining residue was purified by preparative TLC (ethyl acetate: pentane = 3:2) to afford the methyl ester (1*R*)-**3a** (9 mg, 73%) as a white solid. This compound was determined to be 96.5% pure by two diverse methods of HPLC analysis.

(5*S*,6*R*,7*E*)-5,6-Dihydroxy-8-{2-[(1*R*)-1-hydroxyhexyl]phenyl}-oct-7-enoic Acid ((1*R*)-3b). The methyl ester (1*R*)-**3a** (17 mg, 0.046 mmol) was dissolved in a mixture of methanol and water (3:1, 0.8 mL). Lithium hydroxide monohydrate (4 mg, 0.093 mmol) was then added, and the solution was stirred at room temperature for 1 h. Diethyl ether (1 mL) was added and then acidified to pH 5 with 1 M HCl. The solvent was removed *in vacuo* and the residue was purified by preparative TLC (ethyl acetate: methanol = 10:0.2) to afford the carboxylic acid (1*R*)-**3b** (12 mg, 73%) as colorless oil. The acid was converted to its methyl ester derivative, and the purity was determined to be 96% by two diverse methods of HPLC analysis.

5-(2-Bromo-phenyl)-5-oxo-pentanoic Acid *tert*-Butyl Ester (22). Concentrated sulfuric acid (0.2 mL, 3.6 mmol) was added to a vigorously stirred suspension of anhydrous magnesium sulfate (1.78 g, 14.8 mmol) in dichloromethane (15 mL). The mixture was stirred at room temperature for 15 min after which acid 28 (1 g, 3.7 mmol) in dichloromethane (3 mL) was added, followed by *tert*-butanol (1.77 mL, 18.5 mmol) in dichloromethane (3 mL). The resulting mixture was stirred for 48 h then quenched with saturated aqueous sodium bicarbonate (25 mL) and stirred again until all the magnesium sulfate had dissolved. The organic layers was separated, washed with brine (10 mL), dried over magnesium sulfate, and evaporated under reduced pressure. Purification by column chromatography using silica gel (pentane:ethyl acetate = 10:1) afforded *tert*-butyl ester **22** (0.7 g, 60%) as a pale yellow oil.

(3*R*,4*S*)-Non-1-ene-3,4-diol (30). Bromobutane (6.87 mL, 64 mmol) was added dropwise to a refluxing suspension of magnesium turnings (1.44 g, 60 mmol) in dry tetrahydrofuran (20 mL) containing a catalytic amount of iodine under nitrogen. The mixture was then diluted with dry tetrahydrofuran (80 mL) and heated under reflux for 1.5 h. The resulting mixture was allowed to cool to room temperature and added *via* syringe to a solution of CuI (0.79 g, 4 mmol) in anhydrous tetrahydrofuran (5 mL) under nitrogen at −30 °C. After 30 min at −30 °C, epoxide **10** (2 g, 20 mmol) in dry tetrahydrofuran (5 mL) was added dropwise. The reaction mixture was stirred for a further 3 h at −30 °C, and solid ammonium chloride was added. Following filtration, the solution was concentrated under reduced pressure, dissolved in ethyl acetate (50 mL), and washed with saturated ammonium chloride solution (50 mL). The aqueous phase was extracted with ethyl acetate (3 × 150 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), dried over magnesium sulfate, and concentrated under reduced pressure. The crude product was purified by column chromatography using silica gel (pentane:ethyl acetate = 7:3) to afford diol **30** (2 g, 63%) as a pale yellow oil. The data obtained for diol **30** corresponded correctly with literature values.

(4*S*,5*R*)-2,2-Dimethyl-4-pentyl-5-vinyl-1,3-dioxolane (23). A mixture of diol **30** (1.7 g, 11 mmol), 2,2-dimethoxy propane (2 mL, 16.3 mmol), and *p*-toluenesulfonic acid (0.21 g, 1.1 mmol) in dry dichloromethane (40 mL) was stirred at room temperature for 18 h. The reaction mixture was concentrated under reduced pressure, and the resulting oil was purified by column chromatography using silica gel (pentane:diethyl ether = 10:1) to afford olefin **23** (1.64 g, 76%) as a clear oil.

5-{2-[(*E*)-2-((4*R*,5*S*)-2,2-Dimethyl-5-pentyl-1,3-dioxolan-4-yl)-vinyl]-phenyl}-5-oxo-pentanoic Acid *tert*-Butyl Ester (21). To a solution of *tert*-butyl ester **22** (360 mg, 1.09 mmol) and olefin **23** (290 mg, 1.48 mmol) in dry triethylamine (2 mL) were added $Pd(OAc)_2$ (14 mg, 0.06 mmol) and *o*-$(tolyl)_3P$ (32 mg; 0.10 mmol). The resulting mixture was stirred in a sealed tube at 80 °C under nitrogen for 48 h. The reaction mixture was thereafter poured into 1% NaOH (50 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with brine (40 mL), dried over magnesium sulfate, and concentrated under reduced pressure. Purification of the resulting oil by column chromatography using silica gel (pentane:ethyl acetate = 10:1) afforded intermediate **21** (200 mg, 41%) as a colorless oil.

(*S*)-5-{2-[(*E*)-2-((4*R*,5*S*)-2,2-Dimethyl-5-pentyl-1,3-dioxolan-4-yl)-vinyl]-phenyl}-5-hydroxy-pentanoic Acid *tert*-Butyl Ester (31). To a solution of (−)-*β*-chlorodiisopinocampheylborane (145 mg; 0.45 mmol) in dry diethyl ether (3 mL) at −25 °C under nitrogen was added ketone **21** (180 mg; 0.41 mmol) in dry diethyl ether (1 mL). The solution was stirred at −25 °C for 24 h, allowed to warm to room temperature, and diluted with diethyl ether (3 mL). Diethanolamine (95 mg, 0.90 mmol) was then added, and the resulting mixture was stirred at room temperature for 3 h. Filtration followed by evaporation of the solvent gave an oil which was purified by column chromatography using silica gel (pentane: ethyl acetate = 5:1) to afford (5*S*)-**31** (112 mg; 61%) as a thick colorless oil. The de was 97% as determined by chiral HPLC using a OD-H column (hexane:iPrOH 99:1, flow rate: 1 mL/min), 60.2 min for (5S)-**31** and 67.6 min for (5*R*)-**31**.

(*S*)-5-[2-((*E*)-(3*R*,4*S*)-3,4-Dihydroxy-non-1-enyl)-phenyl]-5-hydroxy-pentanoic Acid *tert*-Butyl Ester (4). To a solution of alcohol **31** (80 mg; 0.18 mmol) in tetrahydrofuran (5 mL) was added dropwise 2 N HCl (1 mL), and the solution was stirred at room temperature for 20 h. The mixture was diluted with water (1 mL), extracted with diethyl ether (3 × 20 mL), washed with brine (10 mL), and dried over magnesium sulfate. The solvent was removed *in vacuo* and the residue was product was purified by column chromatography using silica gel (pentane:ethyl acetate 2:1 → 1:1) to afford target compound **4** (43 mg; 59%) as a thick colorless oil. This compound was determined to be 97% pure by two diverse methods of HPLC analysis.

Materials. LXA_4 was obtained from Biomol Research Laboratories. The antagonist Boc2 (*N*-t-Boc-Phe-D-Leu-Phe-D-Leu-Phe) was purchased from MP Biomedicals. Oregon Green phalloidin was from Molecular Probes, Invitrogen, Paisley, UK.

Reagents were dissolved in DMSO or ethanol and further diluted in medium (final concentration 0.1%). Equivalent concentrations of DMSO or ethanol were used as vehicle controls.

Methods. Phagocytosis of Apoptotic PMN by THP-1 Cells. The human myelomonocytic cell line THP-1 (European Collection

of Cell Cultures, Salisbury, UK) was maintained in suspension in RPMI 1640 supplemented with 2 mmol/L glutamine, 100 IU/mL penicillin, 100 μg /mL streptomycin, and 10% fetal calf serum (Life Technologies Inc, Grand Island, NY). THP-1 cells at 5×10^5/mL were differentiated to a macrophage-like phenotype by treatment with 100 nM phorbol 12-myristate, 13-acetate (PMA) for 48 h at 37 °C.

Human polymorphonuclear neutrophils (PMNs) were isolated from peripheral venous blood drawn from healthy volunteers, after informed written consent according to institutional ethical guidelines. Briefly, PMNs were separated by centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden) followed by dextran sedimentation (Dextran T500; Pharmacia) and hypotonic lysis of red cells. PMNs were suspended at 4×10^6 cells/mL, and spontaneous apoptosis was achieved by culturing PMNs in RPMI 1640 supplemented with 10% autologous serum, 2 mmol/L glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin for 20 h at 37 °C in 5% CO_2 atmosphere. Cells were on average 25−50% apoptotic with about 3% necrosis as assessed by light microscopy on stained cytocentrifuged preparations.

Differentiated THP-1 cells (5×10^5 cells/well) were exposed to the appropriate stimuli as indicated for 15 min at 37 °C, before co-incubation with apoptotic PMNs (1×10^6 PMNs/well) at 37 °C for 2 h. Noningested cells were removed by three washes with cold PBS. Phagocytosis was assayed by myeloperoxidase staining of co-cultures fixed with 2.5% glutaraldehyde. For each experiment, the number of THP-1 cells containing one or more PMNs in at least five fields (minimum of 400 cells) was expressed as a percentage of the total number of THP-1 cells and an average between duplicate wells was calculated.

Actin Staining. For actin studies, differentiated THP-1 cells (1×10^6 cells/well) were grown on glass coverslips and exposed to the appropriate stimuli. At the end of incubation, cells were rinsed with PBS and fixed in 3.8% paraformaldehyde−PBS for 20 min at room temperature. Cells were permeabilized with 0.1% Triton X-100 in PBS for 5 min and stained with 0.33 μmol/L Oregon Green-phalloidin for 30 min at room temperature. Coverslips were mounted on microscope glass slides with Probing Antifade medium (Molecular Probes). Cells were viewed on an Axiovert 200M fluorescent microscope (Carl Zeiss AG, Oberkochen, Germany) using Axio-vision image analysis software (Imaging Associates, UK).

Laminin Adherence. THP-1 cells were resuspended in PBS at 4×10^6/mL and incubated with 1 μM of 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM, Sigma) for 20 min at 37 °C. Cells were washed once with PBS and suspended at 3.3×10^6/mL in PBS^{2+} plus 0.1% bovine serum albumin. Aliquots (90 μL) of cells were added to wells in 96-well culture plate coated with laminin (BD Biosciences, MA) and allowed to settle for 10 min. A 10 μL aliquot of agonist or vehicle was added to each well, and plates were incubated at 37 °C for 30 min. Following incubation, wells were washed with $DPBS^{2+}$ plus 0.1% bovine serum albumin. Adherent cells were solubilized with 100 μL of 0.025 M NaOH plus 0.1% sodium dodecyl sulfate, and the plate was stirred on a rotary shaker for 20 min, followed by fluorescence quantitation.

Murine Model of Peritonitis. The peritonitis was induced by intraperitoneal injection of 1 mg of zymosan A^{49} 5 min following intravenous administration of vehicle ($n = 15$) or 0.5−50 μg/kg of the LXA_4 analogues (1*R*)-**3a** and (1*S*)-**3a** ($n = 6$ for each group). The animals were sacrificed 4 h postchallenge by CO_2 exposure. Peritoneal cavities were washed with 3 mL of PBS containing 3 mM EDTA and 25 μ/mL heparin. Cell samples were suspended and incubated in PBS containing 0.1% sodium azide, 10% rat serum (PBS-S), and FcγIIR blocking mAb (CD16/CD32; eBioscience) for 30 min at 4 °C and then labeled with the appropriate concentration of *R*-phycoerythrin-conjugated anti-GR-1 mAb (BD Biosciences) or *R*-phycoerythrin-conjugated IgG isotype control (eBioscience) diluted in PBS-S for another 30 min at 4 °C. After being labeled, cells were washed and suspended for data acquisition in a FACScalibur flow cytometer (Becton & Dickinson, San Jose, CA) using CellQuest software (Becton & Dickinson).

Statistical Analysis. Statistical analysis was conducted using one-way ANOVA or unpaired Student's *t* test with $p < 0.05$ for *n* independent samples being deemed statistically significant.

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Supporting Information Available: Spectroscopic data and HPLC purity details for all new compounds described in this article. This material is available free of charge via the Internet at http://www.pubs.acs.

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