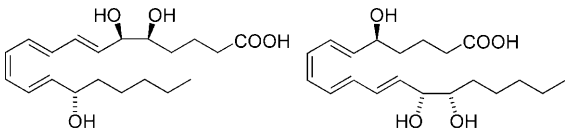
DOI: 10.1002/cmdc.200900533

Synthesis and Biological Evaluation of Pyridine-Containing Lipoxin A_4 Analogues

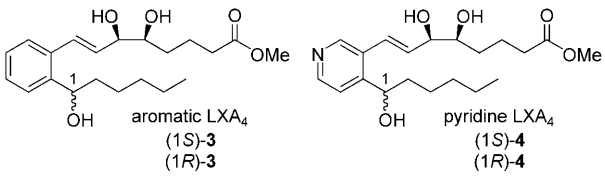
Colm D. Duffy,[a] Paola Maderna,[b] Ciara McCarthy,[c] Christine E. Loscher,[c] Catherine Godson,[b] and Patrick J. Guiry*[a]

The natural eicosanoids lipoxin A_4 and lipoxin B_4 have been shown to possess multiple biological activities and are fundamental in resolving inflammation.[1] A growing interest in this new class of compounds has emerged over the last 20 years ever since their role in inflammatory diseases became apparent.[2,3] Discovered and isolated from human leukocytes in the early 1980s by Serhan and Samuelsson,[4,5] they are a product of the lipoxygenase pathway produced from arachidonic acid. These secondary metabolites contain attractive biochemical



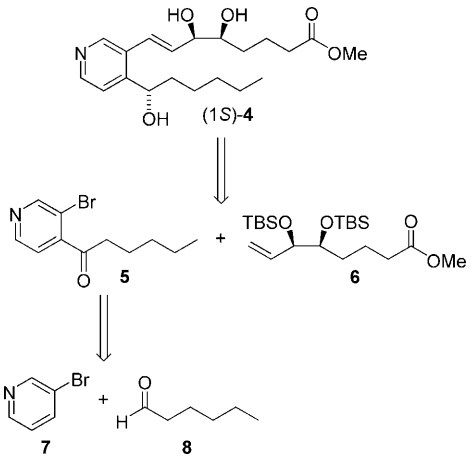
properties and have the potential to be used as pharmacological agents.[6] The first efficient synthetic route to lipoxins (LX) was described in the 1980s, and soon after their isolation, many excellent synthetic pathways have emerged.[7,8]

The rapid metabolism of LX in vivo hinders the use of these compounds as effective pharmaceutical agents.[9,10] LX are rapidly metabolized either by oxidation at C15 or reduction at the C13=C14 double bond. This problem has been recently overcome by the stereoselective synthesis of aromatic LXA_4 of type **3**, and LXB_4 analogues by our research group.[11] The synthetic route developed establishes the required stereochemistry by way of Sharpless epoxidation, palladium-mediated Heck coupling, and diastereoselective reduction reactions. The synthetic stable LXA_4 analogues caused a significant increase in the phagocytosis of apoptotic human polymorphonuclear neutrophils (PMNs) relative to the effect of native LXA_4, while the LXB_4 analogue also stimulated phagocytosis. In addition, stimu-



lation of differentiated THP-1 cells with all of the compounds tested caused F-actin rearrangement similar to that described for native lipoxins. These exciting results, in addition to the potential for an enhanced pharmacological profile, prompted us to investigate the synthesis of epimeric heteroaromatic LXA_4 analogues of type **4** for biological evaluation, as replacing benzene by heteroaromatic bioisosteres is a well-known and successful strategy in medicinal chemistry.[12] This would allow a structure–activity relationship study whereby we could determine the effect of the decreased electron density of the heteroaromatic ring and how the extra heteroatom may alter its ability to accept hydrogen bonds from the receptor in comparison with analogue **3**.

The retrosynthetic analysis of the pyridine-containing LXA_4 analogue (Scheme 1) includes an asymmetric reduction of a ketone, a palladium-catalyzed Heck reaction, a Sharpless asym-



Scheme 1. Retrosynthetic analysis of pyridine LXA_4 (1*S*)-**4**.

metric epoxidation, and a regiospecific pyridine lithiation. The procedure reported by Gribble and Saulnier[13] allows the formation of 3,4-disubstituted pyridines in excellent yields and without the formation of unwanted side products. The key to the success of this reaction is the stability of the lithiated intermediate, which is only stable for 10 min at −78 °C. The internal

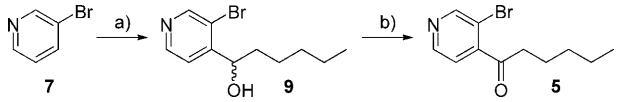
[a] *C. D. Duffy, Prof. P. J. Guiry*
Centre for Synthesis and Chemical Biology
UCD School of Chemistry and Chemical Biology
University College Dublin, Belfield, Dublin 4 (Ireland)
Fax: (+353) 1-7162501
E-mail: patrick.guiry@ucd.ie

[b] *Dr. P. Maderna, Prof. C. Godson*
UCD Diabetes Research Centre
Conway Institute of Biomolecular and Biomedical Research
UCD School of Medicine and Medical Science
University College Dublin, Belfield, Dublin 4 (Ireland)

[c] *C. McCarthy, Dr. C. E. Loscher*
School of Biotechnology
Dublin City University, Dublin 9 (Ireland)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.200900533.

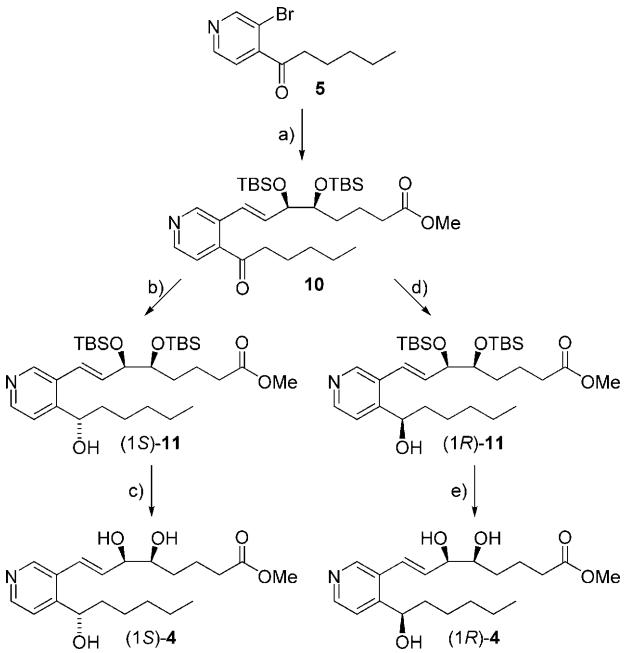
reaction vessel temperature must be monitored continuously, as an increase in temperature above −75 °C gives rise to lithium/halogen exchange and the formation of 3,4-pyridyne. Decreasing the temperature to −100 °C prevented the formation of these unwanted by-products and gave the required intermediate **9** in 75% yield (Scheme 2). We had also attempted a regioselective *ortho*-lithiation of 3-bromopyridine in order to



Scheme 2. Synthesis of 1-(3-bromopyridin-4-yl)hexan-1-one (**5**). Reagents and conditions: a) 1. LDA, −78 °C, 10 min, 2. **8**, −100 °C, 2 h, 75%; b) PCC, AcOH, CH_2Cl_2, room temperature, 5 h, 70%.

prepare the 2,3-disubstituted pyridine LXA_4 analogue. However, all reactions carried out at −78 °C failed to produce the desired product, and instead these conditions led to the formation of the 3-substituted product obtained from the quenching of 3-lithiopyridine with hexanal, due to the extremely fast lithium/halogen exchange[14] in this system. The oxidation method of choice for the preparation of 1-(3-bromopyridin-4-yl)hexan-1-one (**5**) was the use of pyridinium chlorochromate (PCC) in the presence of glacial acetic acid (Scheme 2)[15,16] which, after 5 h at room temperature, gave **5** in 70% yield. Direct preparation of 1-(3-bromopyridin-4-yl)hexan-1-one (**5**) was attempted by quenching the lithiated intermediate of 3-bromopyridine with the corresponding ester and acid chloride as reported for related compounds,[17] but the yields obtained for this reaction were between 5 and 10%.

The palladium-catalyzed Heck reaction is an excellent method for the formation of *trans*-alkenes[18] and has been exploited in many total syntheses because of its high yield and excellent stereochemical control.[19,20] This reaction has also been applied to many substrates on an industrial scale in the synthesis of important pharmaceutical agents.[21] Olefin **6** (Scheme 1) was a substrate for the development of a zirconium-catalyzed one-pot protection/deprotection synthetic methodology[22] and is a key intermediate in the synthesis of aromatic lipoxins.[11] However, attempts to apply the conditions used in the latter synthesis, using palladium acetate and tri-*o*-tolylphosphine with tributylamine as the solvent and the base, only resulted in the isolation of trace amounts of product **10**. Reaction conditions employed by Zhang et al.[23] in the synthesis of nicotinic acetylcholine receptors gave 40% yield, but required extremely long reaction times (7 days). Robert and coworkers also reported alternative conditions for the Heck reaction of bromopyridines,[24] and employing these reaction conditions gave product **10** in 82% yield after a relatively short reaction time (Scheme 3). Sodium borohydride was used to reduce ketone **10** to give a mixture of epimeric alcohols, but unfortunately these compounds were inseparable by column chromatography. Hence, reduction with Brown's (−)- and (+)-chlorodiisopinocampheylborane[25] was carried out, and afforded the desired alcohols (1*S*)-**11** and (1*R*)-**11** in 69 and 65% yields, respectively. Removal of the silyl ether protecting groups under the mild conditions of *para*-toluenesulfonic acid in methanol provided pyridine LXA_4 (1*R*)-**4** and (1*S*)-**4** in 62 and 52% yields, respectively (Scheme 3). With these epimeric molecules in hand, we evaluated their biological activity and compared the results obtained with the parent aromatic analogues **3**.



Scheme 3. Synthesis of pyridine LXA_4 (1*R*)-**4** and (1*S*)-**4**. Reagents and conditions: a) $[\eta^3\text{-}(C_3H_4)Pd(\mu\text{-}Cl)_2]_2$, P(*o*-tolyl)$_3$, **6**, NaOAc, toluene/DMA (3:1), 115 °C, 12 h, 82%; b) (−)-DIPCl, Et_2O, −25 °C, 48 h, 69%; c) *p*-TSA (1.5 equiv), MeOH, 30 °C, 48 h, 52%; d) (+)-DIPCl, Et_2O, −25 °C, 48 h, 65%; e) *p*-TSA (1.5 equiv), MeOH, 30 °C, 48 h, 62%.

Differentiated THP-1 cells were exposed to the pyridine LXA_4 analogues (1*R*)-**4** and (1*S*)-**4** at concentrations ranging from 0.1 to 10 nM for 15 min at 37 °C before the addition of apoptotic human PMNs. 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. Pretreatment of differentiated THP-1 cells with compound (1*S*)-**4** at 1 and 10 nM resulted in a significant increase in phagocytosis of apoptotic PMNs, similar to the effect of native LXA_4 (Figure 1). No effect was observed at a concentration of 0.1 nM (Figure 1 A). Compound (1*R*)-**4** significantly stimulated phagocytosis only at 1 nM, although there is no statistical difference between the results determined for (1*R*)-**4** and (1*S*)-**4** compared with those obtained with (1*R*)-**3** and (1*S*)-**3** (Figure 1 B).

Given that lipoxins have been reported to affect the production of inflammatory cytokines,[26] we assessed the ability of our pyridine-containing LXA_4 analogues (1*R*)-**4** and (1*S*)-**4** to modulate the production of interleukin-12p40 (IL-12p40), IL-1β, and monocyte chemoattractant protein-1 (MCP-1) using a J774 murine macrophage cell line. Lipopolysaccharide (LPS; 100 ng mL^{-1}) was used to induce cytokine production in the cells over 24 h. Addition of (1*R*)-**4** at a concentration of 10 μM 1 h before LPS stimulation resulted in the suppression of IL-12p40 (Figure 2). Exposure of cells to (1*S*)-**4** had a more potent

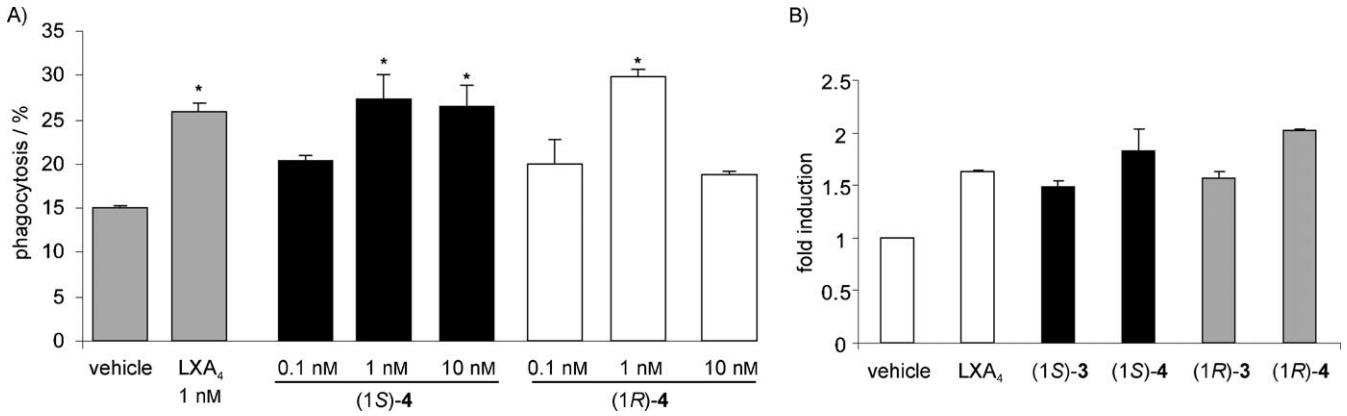


Figure 1. LXA_4 analogues promote phagocytosis of apoptotic PMNs by differentiated THP-1 cells: A) Differentiated THP-1 cells (5×10^5) were treated with vehicle (control), LXA_4 (1 nM), or LXA_4 analogues at the concentrations indicated for 15 min at 37 °C prior to co-incubation with apoptotic PMNs (1×10^6) for 2 h at 37 °C. Phagocytosis was detected by staining of PMNs and quantified by light microscopy. Data are expressed as percent phagocytosis and represent mean values ± SEM ($n=3$): *$p<0.05$ vs. vehicle (control). B) THP-1 cells (5×10^5) were treated with vehicle (control), LXA_4, benzo analogues or pyridine analogues at 1 nM for 15 min at 37 °C prior to co-incubation with apoptotic PMNs (1×10^6) for 2 h at 37 °C. Data are expressed as fold induction over basal and represent mean values ± SEM ($n=3$).

effect on IL-12p40 production, with significant suppression of this cytokine at 10 μM, 1 μM, and 1 nM. Both (1*R*)-**4** and (1*S*)-**4** suppressed LPS-induced production of IL-1β at both 1 μM and 1 nM (Figure 2). There was no effect on MCP-1 production, demonstrating that the effects of the analogues are specific.

In summary, we report herein a short and efficient synthesis of two diastereomers of a novel pyridine-containing LXA_4 analogue by means of an asymmetric reduction of a ketone, a palladium-catalyzed Heck reaction, a Sharpless asymmetric epoxidation, and a regiospecific pyridine lithiation. Both compounds (1*R*)-**4** and (1*S*)-**4** significantly enhanced phagocytosis of PMNs which is a key requirement for the resolution of inflammation. Furthermore, they suppressed the production of the pro-inflammatory cytokines IL-12p40 and IL-1β, demonstrating their anti-inflammatory potential. With these preliminary results in hand for epimeric 3,4-pyridine-containing analogues, future efforts will focus on tuning the pharmacological profile of these compounds through structural modification, and the results of these investigations will be reported in due course from these laboratories.

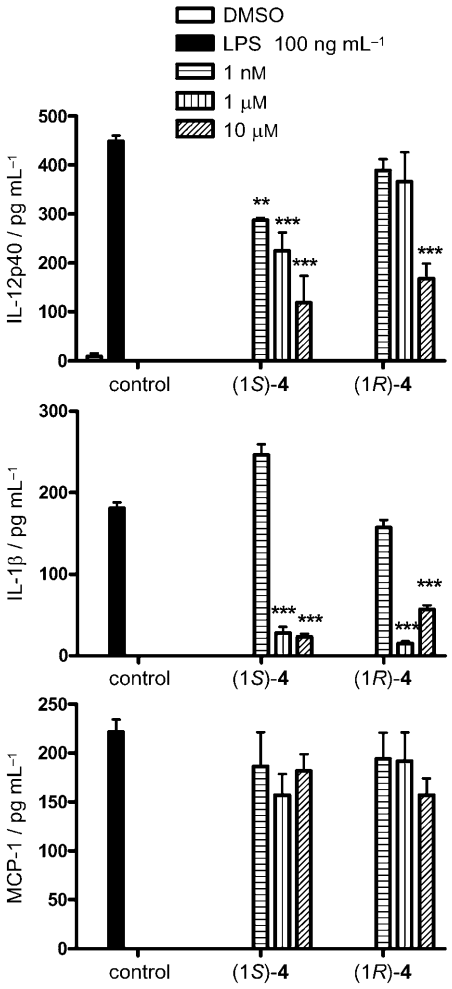


Figure 2. LXA_4 analogues suppress pro-inflammatory cytokine production by J774 macrophages: J774 macrophages (1×10^6) were treated with vehicle (control), (1*R*)-**4**, or (1*S*)-**4** at the concentrations indicated for 1 h prior to stimulation with LPS (100 ng mL^{-1}). Cytokine concentrations were determined by ELISA; data represent mean values ± SEM ($n=4$): **$p<0.01$, ***$p<0.001$, determined by one-way ANOVA comparing all groups.

Experimental Section

Chemistry

General experimental conditions: All reactions were carried out in an inert atmosphere of N_2 using oven-dried glassware, and reagents were purchased from Sigma–Aldrich. Et_2O, THF, and CH_2Cl_2 were obtained from a PureSolv-300-3-MD dry solvent dispenser and were used without further purification. *N*,*N*-Dimethylacetamide was purchased from Sigma–Aldrich and was used without further purification; toluene was dried over sodium. ^1H and ^{13}C NMR spectra were recorded on Varian Oxford 500 spectrometer at room temperature using $(CH_3)_4Si$ as an internal standard. The reference values used for $CDCl_3$ were 7.26 and 77.02 ppm for ^1H and ^{13}C NMR spectra, respectively. Chemical shift (δ) values are given in ppm, and coupling constants are given as absolute values expressed in Hz. HRMS data were obtained using a Micromass/Waters LCT instrument. IR spectra were recorded on a Varian 3100 FTIR Excalibur

Series spectrometer. Optical rotation values were measured on a PerkinElmer 241 polarimeter; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. HPLC analysis was carried out using a Supelco 2-4304 beta-Dex® 120 (30 m × 0.25 mm, 0.25 mm film) and a Chiralcel OD column (0.46 cm i.d. × 25 cm). Flash chromatography was carried out using Merck Kiesegel 60 F_{254} (230–400 mesh) silica gel. Evaporation in vacuo refers to the removal of volatiles on a Büchi rotary evaporator with an integrated vacuum pump. Thin-layer chromatography (TLC) was performed on Merck DC-Alufolien plates precoated with silica gel 60 F_{254}. They were visualized either by quenching of UV fluorescence or by charring with an acidic vanillin solution (vanillin, H_2SO_4, and AcOH in MeOH).

1-(3-Bromopyridin-4-yl)hexan-1-ol (9). *n*BuLi (*c* = 2.5 M, 2.8 mL, 6.9 mmol) was added to a solution of diisopropylamine (0.88 mL, 6.3 mmol) in THF (20 mL) at −78 °C under an atmosphere of N_2, and stirring was continued for 15 min. 3-Bromopyridine **7** (0.62 mL, 6.3 mmol) in THF (1 mL) was added over 10 min (maintaining the internal temperature < −75 °C). The reaction was brought to −100 °C for 10 min, and hexanal **8** (1.26 g, 12.6 mmol) in THF (3 mL) was added over 10 min (again maintaining the internal temperature < −75 °C). The reaction mixture was stirred at −100 °C for 1 h and then warmed to −20 °C over 20 min. The mixture was quenched with a saturated solution of NH_4Cl (3 mL), extracted with Et_2O (3 × 25 mL), washed with water (25 mL), brine (25 mL), and dried over Na_2SO_4. The solvent was removed in vacuo, and the residue was purified using silica gel chromatography (pentane/EtOAc 9:1, then 4:1) to afford **9** (737 mg, 74% yield) as a viscous yellow oil. TLC: R_f = 0.21 (pentane/EtOAc 4:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.57 (s, 1H), 8.43 (d, *J* = 4.9 Hz, 1H), 7.51 (d, *J* = 4.9 Hz, 1H), 4.98 (m, 1H), 3.22 (br s, 1H, exchanges with D_2O), 1.73–1.79 (m, 1H), 1.49–1.65 (m, 1H), 1.40–1.49 (m, 2H), 1.26–1.39 (m, 4H), 0.9 ppm (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 153.3, 151.5, 148.3, 122.2, 120.0, 71.9, 37.1, 31.5, 25.3, 22.5, 14.0 ppm; IR (neat): $\tilde{\nu}_{max}$ = 3583, 3296, 2929, 2361, 1588, 1466, 1401, 1343, 1217, 1162, 1084, 756 cm^{-1} cm^{-1}; HRMS (EIMS) *m/z*: found: 258.0486 $[M+H]^+$, $C_{11}H_{16}BrNO$ requires 258.0494.

1-(3-Bromopyridin-4-yl)hexan-1-one (5). Glacial acetic acid (0.21 mL) was added to a well-stirred solution of PCC (821 mg, 3.81 mmol) in dry CH_2Cl_2 (20 mL). After 5 min at room temperature, alcohol **9** (655 mg, 2.54 mmol) in CH_2Cl_2 (5 mL) was added, and the mixture was stirred at room temperature for 5 h. Et_2O (40 mL) was added, and the mixture was filtered twice. The solvent was removed in vacuo, and the residue was purified by silica gel chromatography (pentane/EtOAc 4:1) to afford ketone **5** (458 mg, 70% yield) as an orange oil. TLC: R_f = 0.67 (pentane/EtOAc 4:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.79 (s, 1H), 8.60 (d, *J* = 4.9 Hz, 1H), 7.22 (d, *J* = 4.9 Hz, 1H), 2.88 (t, *J* = 7.4 Hz, 2H), 1.74–1.69 (m, 2H), 1.37–1.33 (m, 4H), 0.91 ppm (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 202.6, 152.8, 148.5, 148.5, 121.6, 116.0, 42.5, 31.2, 23.2, 22.3, 13.8 ppm; IR (neat):: $\tilde{\nu}_{max}$ = 2958, 2931, 1710, 1578, 1466, 1396, 1378, 1276, 1250, 1089, 1022 cm^{-1}; HRMS (EIMS) *m/z*: found: 256.0336 $[M+H]^+$, $C_{11}H_{14}BrNO$ requires 256.0337.

(5*S*,6*R*,*E*)-Methyl-5,6-bis(*tert*-butyldimethylsilyloxy)-8-(4-hexanoylpyridin-3-yl)oct-7-enoate (10). $[\eta^3$-$(C_3H_4)Pd(\mu$-$Cl)_2]_2$ (17 mg, 0.048 mmol), P(*o*-tolyl)$_3$ (34 mg, 0.096 mmol), and NaOAc (234 mg, 2.88 mmol) were dissolved in dry freshly distilled toluene (2 mL) to which ketone **5** (250 mg, 0.96 mmol) in toluene (1 mL) and olefin **6** (406 mg, 0.96 mmol) in toluene (1 mL) were added. *N*,*N*-Dimethylacetamide (DMA; 1.3 mL) was added, and the reaction mixture was sealed under N_2; stirring was continued for 12 h at 115 °C followed by filtration through a pad of Celite®. The solvent was removed in vacuo, and the residue was purified by silica gel chromatography (neat pentane, then pentane/Et_2O 9:1, then 4:1, then 3:2) to afford **10** (486 mg, 82% yield) as a viscous yellow oil. TLC: R_f = 0.23 (pentane/Et_2O 3:2); $[\alpha]_D^{20}$ = −12.9 10^{-1} deg cm^2 g^{-1} (*c* = 0.84, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.79 (s, 1H), 8.56 (d, *J* = 5.0 Hz, 1H), 7.27 (d, *J* = 5.0 Hz, 1H), 6.75 (d, *J* = 16.0 Hz, 1H), 6.22 (dd, *J* = 16.0, 6.5 Hz, 1H), 4.16 (dd, *J* = 6.5, 4.8 Hz, 1H), 3.70 (m, 1H), 3.66 (s, 3H), 2.81 (dt, *J* = 7.3, 9.5 Hz, 2H), 2.31 (t, *J* = 7.4, 2H), 1.31–1.79 (m, 10H), 0.91 (s, 9H), 0.87 (s, 12H), 0.05–0.10 ppm (3 × s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 204.1, 173.9, 149.0, 148.4, 144.2, 135.7, 129.9, 125.4, 120.4, 77.0, 76.2, 51.5, 42.4, 34.2, 33.0, 31.3, 26.0, 23.6, 22.5, 20.7, 18.3, 18.2, 13.9, −4.0, −4.2, −4.6, −4.7 ppm; IR (neat): $\tilde{\nu}_{max}$ = 2995, 2929, 2857, 1740, 1701 cm^{-1}; HRMS (EIMS) *m/z*: found: 592.3870 $[M+H]^+$, $C_{32}H_{57}NO_5Si_2$ requires 592.3881.

(5*S*,6*R*,*E*)-Methyl-5,6-bis(*tert*-butyldimethylsilyloxy)-8-{4-[(*R*)-1-hydroxyhexyl]pyridin-3-yl}oct-7-enoate ((1*R*)-11). Ketone **10** (190 mg, 0.32 mmol) in Et_2O (2 mL) was added to a solution of (+)-chlorodiisopinocampheylborane [(+-DIPCl; 0.41 g, 1.28 mmol)] in Et_2O (2 mL) at −25 °C, and stirring was continued for 48 h. The reaction mixture was diluted with pentane and Et_2O (2 mL each), and diethanolamine (64.3 mg, 0.64 mmol) was added. Stirring was continued for 4 h at room temperature, followed by filtration and removal of the solvent in vacuo. The residue was purified by silica gel chromatography (neat pentane, then pentane/Et_2O 1:1, then 1:2) to afford (1*R*)-**11** (125 mg, 53% yield) as a viscous yellow oil at 92.3% *de* as determined by chiral HPLC using an OD column (hexanes/*i*PrOH 99:1) at a flow rate of 1 mL min^{-1}: t_R = 24.0 min for the *R* isomer, t_R = 30.3 min for *S*. TLC: R_f = 0.15 (Et_2O/pentane 2:1); $[\alpha]_D^{20}$ = + 29.4 10^{-1} deg cm^2 g^{-1} (*c* = 0.36, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.57 (s, 1H), 8.45 (d, *J* = 5.0 Hz, 1H), 7.40 (d, *J* = 5.0 Hz, 1H), 6.72 (d, *J* = 15.8 Hz, 1H), 6.15 (dd, *J* = 15.8, 6.0 Hz, 1H), 4.94 (t, *J* = 6.7 Hz, 1H) 4.22 (m, 1H), 3.71 (m, 1H), 3.64 (s, 3H), 2.30 (t, *J* = 7.5 Hz, 2H), 1.27–1.79 (m, 10H), 0.93 (s, 9H), 0.88 (s, 12H), 0.06–0.11 ppm (3 × s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 174.0, 151.0, 147.9, 147.0, 135.2, 130.7, 124.3, 120.2, 77.0, 76.2, 70.2, 51.5, 37.9, 34.2, 32.8, 31.7, 25.9, 25.4 22.6, 20.9, 18.3, 18.2, 13.9, −4.0, −4.3, −4.6, −4.7 ppm; IR (neat): $\tilde{\nu}_{max}$ = 3365, 2954, 2930, 2857, 1741, 1252 cm^{-1}; HRMS (EIMS) *m/z*: found: 594.4033 $[M+H]^+$, $C_{32}H_{59}NO_5Si_2$ requires 594.4010.

(5*S*,6*R*,*E*)-Methyl-5,6-bis(*tert*-butyldimethylsilyloxy)-8-{4-[(*S*)-1-hydroxyhexyl]pyridin-3-yl}oct-7-enoate ((1*S*)-11). Ketone **10** (97 mg, 0.163 mmol) in Et_2O (1 mL) was added to a solution of (−)-DIPCl (210 mg, 0.64 mmol) in Et_2O (1 mL) at −25 °C, and stirring was continued for 48 h. The reaction mixture was diluted with pentane and Et_2O (1 mL each), and diethanolamine (34 mg, 0.326 mmol) was added. Stirring was continued for 4 h at room temperature, followed by filtration and removal of the solvent in vacuo. The residue was purified by silica gel chromatography (neat pentane, then pentane/Et_2O 1:1, then 1:2) to afford (1*S*)-**11** (66 mg, 67% yield) as a clear oil at 94.9% *de*. TLC: R_f = 0.15 (Et_2O/pentane 2:1); $[\alpha]_D^{20}$ = −31.9 10^{-1} deg cm^2 g^{-1} (*c* = 0.56, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.60 (s, 1H), 8.45 (d, *J* = 5.0 Hz, 1H), 7.41 (d, *J* = 5.0 Hz, 1H), 6.65 (d, *J* = 15.8 Hz, 1H), 6.18 (dd, *J* = 15.8, 6.5 Hz, 1H), 4.98 (t, *J* = 6.2 Hz, 1H) 4.18 (dd, *J* = 6.5, 4.9 Hz, 1H), 3.70 (m, 1H), 3.65 (s, 3H), 2.31 (t, *J* = 7.3, 2H), 1.27–1.80 (m, 10H), 0.92 (s, 9H), 0.87 (s, 12H), 0.05–0.10 ppm (3 × s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 174.0, 150.3, 148.7, 147.6, 134.8, 130.5, 124.5, 119.8, 77.2, 76.2, 69.8, 51.5, 38.1, 34.3, 33.0, 31.7, 26.0, 26.0, 25.4, 22.6, 20.7, 18.3, 18.2, 14.0, −4.0, −4.1, −4.6, −4.6 ppm; IR (neat): $\tilde{\nu}_{max}$ = 3350, 2954, 2930, 2857, 1742, 1252 cm^{-1}; HRMS (EIMS) *m/z*: found: 594.3992 $[M+H]^+$, $C_{32}H_{57}NO_5Si_2$ requires 594.4010.

(5*S*,6*R*,*E*)-Methyl-5,6-dihydroxy-8-{4-[(*R*)-1-hydroxyhexyl]pyridin-3-yl}oct-7-enoate ((1*R*)-4). Alcohol (1*R*)-**11** (160 mg, 0.266 mmol)

was dissolved in dry MeOH (1 mL) to which *p*-toluenesulfonic acid (*p*-TSA; 77 mg, 0.4 mmol) was added, and the resultant mixture was stirred at 30 °C for 48 h. The solvent was removed in vacuo at 30 °C to prevent formation of a lactone by-product, and the residue was purified by silica gel chromatography (pentane/EtOAc 1:1, then neat EtOAc, then EtOAc/MeOH (1%)) to afford (1*R*)-**4** (60.8 mg, 62% yield) as a clear viscous oil. TLC: $R_f = 0.26$ (CH_2Cl_2/MeOH 9.5:0.5); $[\alpha]_D^{20} = +12.2\ 10^{-1}\,deg\,cm^2\,g^{-1}$ ($c = 2.3$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 8.39$ (s, 1H), 8.35 (app d, $J = 4.8$ Hz 1H) 7.38 (d, $J = 4.8$ Hz, 1H), 6.76 (d, $J = 15.8$ Hz, 1H), 6.15 (dd, $J = 15.8$, 6.1 Hz, 1H), 4.88 (br s, 1H) 4.22 (br s, 1H), 3.76 (br s, 1H), 3.65 (s, 3H), 3.01 (br s, 3H), 2.35 (t, $J = 7.27$, 2H), 1.26–1.89 (m, 13H), 0.86 ppm (t, $J = 6.58$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 174.3$, 152.2, 147.4, 146.4, 132.9, 130.8, 125.9, 120.7, 75.6, 74.0, 69.3, 51.6, 37.5, 33.7, 31.9, 31.6, 25.3, 22.5, 21.2, 14.0 ppm; IR (neat): $\tilde{\nu}_{max} =$ 3389, 2953, 2928, 2856, 1737, 1457, 1235 cm^{-1}; HRMS (EIMS) *m/z*: found: 366.2278 $[M+H]^+$, $C_{20}H_{31}NO_5$ requires 366.2280.

(5*S*,6*R*,*E*)-Methyl-5,6-dihydroxy-8-{4-[(*S*)-1-hydroxyhexyl]pyridin-3-yl}oct-7-enoate ((1*S*)-4). Alcohol (1*S*)-**11** (50 mg, 0.084 mmol) was dissolved in dry MeOH (1 mL) to which *p*-TSA (24.1 mg, 0.126 mmol) and the resultant mixture was stirred at 30 °C for 48 h. The solvent was removed in vacuo at 30 °C to prevent formation of a lactone by-product, and the residue was purified by silica gel chromatography (pentane/EtOAc 1:1, then neat EtOAc, then EtOAc/MeOH (1%)) to afford (1*S*)-**4** (16.4 mg, 52% yield) as a clear viscous oil. TLC: $R_f = 0.26$ (CH_2Cl_2/MeOH 9.5:0.5); $[\alpha]_D^{20} = -8.8\ 10^{-1}\,deg\,cm^2\,g^{-1}$ ($c = 0.34$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 8.36$ (app br s, 2H), 7.36 (d, $J = 5.0$ Hz, 1H), 6.77 (d, $J = 15.9$ Hz, 1H), 6.07 (dd, $J = 15.9$, 6.4 Hz, 1H), 4.85 (dd, $J = 7.3$, 5.2 Hz, 1H) 4.21 (m, 1H), 3.72 (m, 1H), 3.65 (s, 3H), 2.34 (t, $J = 7.3$, 2H), 1.26–1.89 (m, 13H), 0.86 ppm (t, $J = 6.7$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 174.3$, 151.3, 148.2, 147.2, 132.8, 130.7, 126.1, 120.6, 75.4, 73.9, 70.1, 51.6, 37.6, 33.7, 31.7, 31.6, 25.4, 22.5, 21.1, 14.0 ppm; IR (neat): $\tilde{\nu}_{max} =$ 3383, 2954, 2857, 1733, 1460, 1259 cm^{-1}; HRMS (EIMS) *m/z*: found: 366.2291 $[M+H]^+$, $C_{20}H_{31}NO_5$ requires 366.2280.

Phagocytosis of apoptotic PMNs by THP-1 cells

The human myelomonocytic cell line THP-1 (European Collection of Cell Cultures, Salisbury, UK) was maintained as a suspension in RPMI 1640 supplemented with glutamine (2 mmol L^{-1}), penicillin (100 IU mL^{-1}), streptomycin (100 μg mL^{-1}), and 10% fetal calf serum (FCS; Life Technologies Inc., Grand Island, NY, USA). THP-1 cells at 5×10^5 mL^{-1} 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. 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^{-1}, and spontaneous apoptosis was achieved by culturing PMNs in RPMI 1640 supplemented with 10% autologous serum, glutamine (2 mmol L^{-1}), penicillin (100 IU mL^{-1}), and streptomycin (100 μg mL^{-1}) for 20 h at 37 °C under a 5% CO_2 atmosphere. Cells were 25–50% apoptotic on average, with ~3% necrosis as assessed by light microscopy on stained cytocentrifuged preparations. Differentiated THP-1 cells (5×10^5 cells per 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 per well) at 37 °C for 2 h. Non-ingested cells were removed by three washes with cold phosphate-buffered saline. 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 PMN 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.

Cytokine production by J774 macrophages

Murine J774 macrophages (European Collection of Cell Cultures, UK) were maintained in suspension of RPMI 1640 supplemented with glutamine (2 mmol L^{-1}), penicillin (100 IU mL^{-1}), streptomycin (100 μg mL^{-1}), and 10% FCS. Cells were seeded at 1×10^6 cells mL^{-1} for experiments and exposed to LPS at a concentration of 100 ng mL^{-1} for 24 h at 37 °C under 5% CO_2. (1*R*)-**4** and (1*S*)-**4** were added to the cells 1 h before addition of LPS, at a concentrations of 1 nM, 1 μM, and 10 μM. After 24 h the supernatants were collected for cytokine analysis. IL-1β, MCP-1, and IL-12p40 concentrations in cell culture supernatants were quantified by commercial DuoSet ELISA kits (R&D Systems), according to the manufacturer's instructions.

Acknowledgements

C.D.D. thanks University College Dublin for the award of an Ad Astra Scholarship. We thank University College Dublin for providing financial support for this project. We also acknowledge the facilities provided by the Centre for Synthesis and Chemical Biology (CSCB), funded by the Higher Education Authority's Programme for Research in Third-Level Institutions (PRTLI). We are grateful to Dr. Jimmy Muldoon and Dr. Dilip Rai of the CSCB for NMR and mass spectra, respectively. C.McC. is funded by an IRCSET PhD scholarship. C.G. and P.M. are funded by Science Foundation Ireland and by the EU FP6 EICOSANOX programme LSHM-CT-2004-00503.

Keywords: cytokines · eicosanoids · inflammation · lipoxins · synthesis design

[1] N. Chiang, C. Serhan, S. E. Dahlan, C. Brink, *Pharmacol. Rev.* **2006**, *58*, 463 – 487.
[2] N. Kieran, P. Maderna, C. Godson, *Kidney Int.* **2004**, *65*, 1145 – 1154.
[3] C. Bonnans, I. Vachier, *Am. J. Respir. Crit. Care Med.* **2002**, *165*, 1531 – 1535.
[4] C. Serhan, M. Hamberg, B. Samuelsson, *Biochem. Biophys. Res. Commun.* **1984**, *118*, 943 – 949.
[5] C. Serhan, M. Hamberg, B. Samuelsson, *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 5335 – 5339.
[6] B. McMahon, C. Godson, *Am. J. Physiol. Renal Physiol.* **2004**, *286*, F189 – F201.
[7] a) J. Adams, B. J. Fitzsimmons, J. Rokach, *Tetrahedron Lett.* **1984**, *25*, 4713- 4716; b) E. J. Corey, W.-g. Su, *Tetrahedron Lett.* **1985**, *26*, 281 – 284; c) E. J. Corey, W.-g. Su, M. B. Cleaver, *Tetrahedron Lett.* **1989**, *30*, 4181 – 4184.
[8] a) K. C. Nicolaou, C. A. Veale, S. E. Webber, H. Katerinopoulos, *J. Am. Chem. Soc.* **1985**, *107*, 7515 – 7518; b) K. C. Nicolaou, J. Y. Ramphal, N. A. Petasis, C. N Serhan, *Angew. Chem.* **1991**, *103*, 1119 – 1136; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1100 – 1116.
[9] B. Clish, D. Levy, N. Chiang, *J. Biol. Chem.* **2000**, *275*, 25372 – 25380.
[10] C. Serhan, *Prostaglandins* **1997**, *53*, 107 – 137.
[11] a) T. P. O'Sullivan, K. S. A. Vallin, S. T. A. Shah, J. Fakhry, P. Maderna, M. Scannell, A. L. F. Sampaio, M. Perretti, C. Godson, P. J. Guiry, *J. Med. Chem.* **2007**, *50*, 5894 – 5902; b) N. A. Petasis, R. Keledjian, Y.-P. Sun, K. C. Nagulapalli, E. Tjonahen, R. Yang, C. N. Serhan, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1382 – 1387.

[12] D. A. Williams, W. O. Foye, T. L. Lemke in *Foye's Principles of Medicinal Chemistry, 5th Ed.*, Lippincott Williams & Wilkins, **2002**, 60 – 62.
[13] G. W. Gribble, M. G. Saulnier, *Heterocycles* **1993**, *35*, 151 – 169.
[14] H. Gilman, S. M. Spatz, *J. Org. Chem.* **1951**, *16*, 1485 – 1494.
[15] E. J. Corey, J. W. Suggs, *Tetrahedron Lett.* **1975**, *16*, 2647 – 2650.
[16] S. Agarwal, H. P. Tiwari, J. P. Sharma, *Tetrahedron* **1990**, *46*, 4417 – 4420.
[17] Y. Aoyagi, T. Inariyama, Y. Arai, S. Tsuchida, Y. Matuda, H. Kobayashi, A. Ohta, T. Kuribara, S. Fujihira, *Tetrahedron* **1994**, *50*, 13575 – 13582.
[18] R. F. Heck, *J. Am. Chem. Soc.* **1968**, *90*, 5518 – 5526.
[19] K. C. Nicolaou, P. G. Bulger, D. Sarlah, *Angew. Chem.* **2005**, *117*, 4516 – 4563; *Angew. Chem. Int. Ed.* **2005**, *44*, 4442 – 4489.
[20] a) A. G. Coyne, M. O. Fitzpatrick, J. G. Patrick in *The Mizoroki-Heck Reaction* (Ed.: M. Oestreich), Wiley-VCH, Chichester, **2009**, pp. 405 – 428; b) V. Coeffard, P. J. Guiry, *Curr. Org. Chem.* **2010**, *14*, 212 – 229.
[21] M. Labelle, M. Belley, Y. Gareau, J. Y. Gauthier, D. Guay, R. Gordon, S. G. Grossman, T. R. Jones, Y. Leblanc, M. McAuliffe, C. McFarlane, P. Masson, K. M. Metters, N. Ouimet, D. H. Patrick, H. Piechuta, C. Rochette, N. Sawyer, Y. B. Xiang, C. B. Pickett, A. W. Ford-Hutchinson, R. J. Zamboni, R. N. Young, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 283 – 288.
[22] S. Singh, C. D. Duffy, S. T. A. Shah, P. J. Guiry, *J. Org. Chem.* **2008**, 73, 6429 – 6432.
[23] Y. Zhang, O. A. Pavlova, S. I. Chefer, A. W. Hall, V. Kurian, L. L. Brown, A. S. Kimes, A. G. Mukhin, A. G. Horti, *J. Med. Chem.* **2004**, *47*, 2453 – 2465.
[24] N. Robert, C. Hoarau, S. Celanire, P. Ribereau, A. Godard, G. Queguiner, F. Marsais, *Tetrahedron* **2005**, *61*, 4569 – 4576.
[25] M. Srebnik, P. Ramachandran, H. Brown, *J. Org. Chem.* **1988**, *53*, 2916 – 2920.
[26] S.-H. Wu, P.-Y. Liao, L. Dong, Z.-Q. Chen, *Inflammation Res.* **2008**, *57*, 430 – 437.

Received: December 23, 2009
Revised: January 12, 2010
Published online on February 1, 2010

