Linotrins: Omega-3 oxylipins featuring an E,Z,E conjugated triene motif are present in the plant kingdom and alleviate inflammation in LPS-challenged microglial cells

Laurence Balas, Sujit Kumar Dey, Sophie Béraud-Dufour, Dean Edward Riechers, Olivia Augusta Landau, Justine Bertrand-Michel, Thierry Durand, Nicolas Blondeau

Linotrins: Omega-3 Oxylipins Featuring an E,Z,E Conjugated Triene Motif are Present in the Plant Kingdom and Alleviate Inflammation in LPS-challenged Microglial Cells

Laurence Balas*[a], Sujit Kumar Dey[a], Sophie Béraud-Dufour[b], Dean Edward Riechers[c], Olivia Augusta Landau[c], Justine Bertrand-Michel[d], Thierry Durand[a] and Nicolas Blondeau[b]

[a] IBMM, Univ Montpellier, CNRS, ENSCM, Montpellier, France.

[b] Université Côte d'Azur, CNRS, IPMC, UMR7275, Sophia Antipolis, F-06560, France.

[c] Department of Crop Sciences, University of Illinois, Urbana, Illinois U.S.A. 61801.

[d] Plateau Lipidomique – MetaToul, Institut des Maladies Métaboliques et Cardiovasculaires (I2MC), Inserm/Université Paul Sabatier UMR1048, 1 Av. Jean Poulhes, BP 84225, 31432 Toulouse Cedex 4, France.

ABSTRACT

Alpha-linolenic acid (ALA), an essential omega-3 polyunsaturated fatty acid found in plants, exerts neuroprotection and anti-inflammatory effects in chronic and acute CNS disease models. However, the underlying mechanisms are not yet understood. Since ALA is not incorporated into the brain, the observed health benefits may result from some of its metabolites. The putative formation of dihydroxylated ALA derivatives (called linotrins) was recently shown *in vitro* in the presence of lipoxygenases. However, the *in vitro* biosynthesis of linotrins was neither stereoselective nor quantitatively efficient for studying their physiological roles as enantiomeric pure forms. Herein, we report the first stereo-controlled synthesis that features regio- and stereoselective hydrometalations of alkynes for assembling the sensitive *E*,*Z*,*E*-conjugated trienes, as well as LC-MS investigations that provide evidence of linotrins occurrence in plants. Moreover, strong anti-inflammatory effects on microglia highlight the potential physiological importance of linotrins and open new perspectives in search of CNS therapeutics.

KEYWORDS

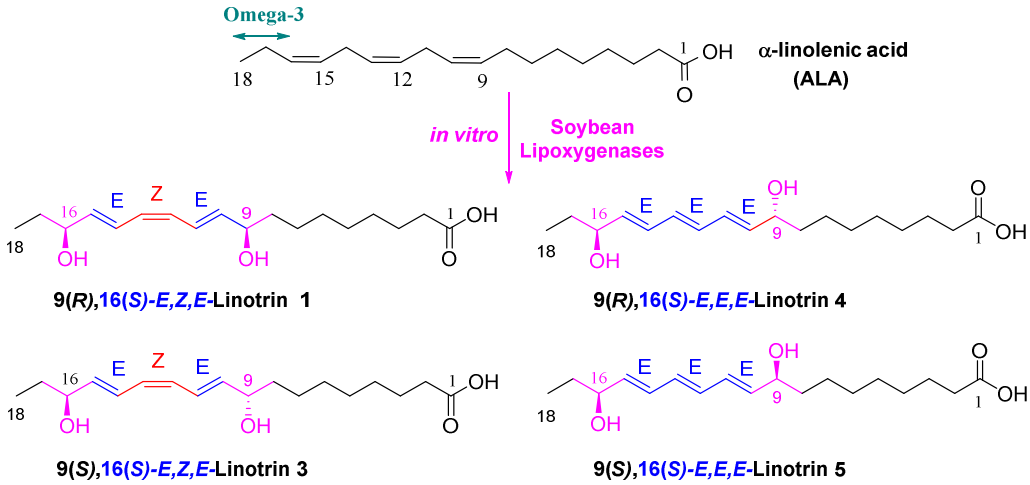
cytokine, conjugated triène, inflammation, linolenic acid, microglial cell, total synthesis.

INTRODUCTION

The omega-3 polyunsaturated fatty acids (n-3 PUFAs) present in our daily diet play important roles in various cardiovascular and cerebral diseases, including stroke.[1, 2] Within the n-3 family, only alpha-linolenic acid (ALA) cannot be synthesized by the human body. Mainly found in edible plants, ALA is the precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are the major lipid components of the brain. While ongoing confusion persists that equates the term 'omega-3' almost exclusively with longer-chain PUFAs for health improvement, it is now accepted that ALA supplementation exerts similar beneficial effects as EPA and DHA[3], including prevention of neuronal death in several *in vivo* neuropathological models[2]. Interestingly, elevated ALA intake is associated with a reduced risk of stroke in humans[4]; however, paradoxically ALA is not incorporated into the brain (unlike DHA, whose oxygenated metabolites were proposed to exert direct protection in neurons[5, 6]) and does not increase DHA concentration in the brain, modify the n-6/n-3 PUFA ratio, or the proportion of saturated, mono- and polyunsaturated fatty acids in the brain.[7] Thus, further research is needed to explore the pathways of ALA metabolism (in humans, mammals), other than its conversion to EPA/DHA, to help explain its protective effects. Like DHA, ALA is a peroxidation target that generates n-3-oxygenated compounds, which could regulate chronic or acute inflammatory state.[8] Although non-stereoselective[9], the *in vitro* double oxygenation of ALA by 15-lipoxygenase produced (scheme 1) oxylipins, called linotrins, which inhibit the *in vitro* activity of human recombinant cyclooxygenase (COX-2). Since COX-2 is a key mediator of the inflammatory storm, linotrins may have a potential anti-inflammatory effect.

This context prompted us to investigate the relevance of linotrins in neuroinflammation and their potential availability in animal and human diets by ingesting edible plants containing ALA. For this purpose, we report herein the first total synthesis of linotrins, namely the stereoisomer (9*R*),16(*S*)-

linotrin **1** and its epimer at carbon C16 (linotrin **2,** i.e. 9(*R*),16(*R*)-enantiomer of linotrin **3**), their biological evaluation in microglial cells and detection *in vivo*.



Scheme 1. *In vitro* enzymatic synthesis of linotrins from ALA.[9]

RESULTS AND DISCUSSION

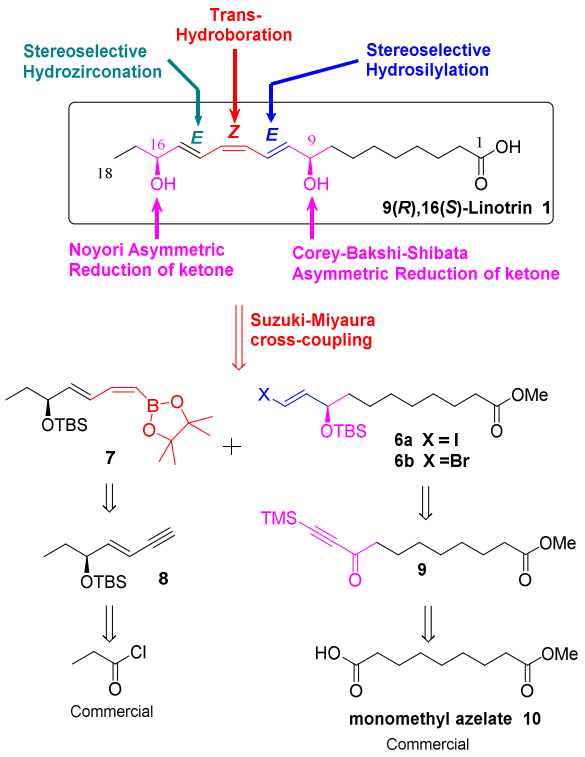
Chemical synthesis of linotrins.

Retrosynthetic analysis.

Linotrins are challenging synthetic targets displaying a *E,Z,E*-conjugated triene flanked by two optically active allylic alcohols. Due to the expected poor stability of the conjugated triene moiety, it must be elaborated at a late stage in the synthesis to facilitate the handling of the key precursors. In addition, regarding the preparation of the (Z)-double bond, our preference was to avoid a chemoselective semi-reduction of an internal alkyne (e.g. Lindlar, Brown, and Boland reductions). Based on our own experience and published literature, handling of such a step occurred with modest yield and was not convenient, albeit often employed in the synthesis of *E,E,Z*-conjugated trienes.[10]

The present synthetic approach to linotrins relied on the disconnection of the central triene at the C11-C12 single bond (scheme 2), requiring the elaboration of a (*E*)-vinyl halide **6,** and a (1*Z*,3*E*)-dienyl organometallic precursor **7**. The retrosynthetic analysis of linotrin **1** is outlined in Scheme 2. The desired (*Z*)-geometry for the vinylmetal **7** could arise from a rhodium-catalyzed *trans*-hydroboration of a terminal alkyne (**8**), suggesting an assembly of the conjugated triene through a Suzuki cross-coupling reaction. By choosing the suitable organometallic reagents, the two (*E*)-double bonds may also result from stereoselective hydrometalations of terminal alkynes.

Scheme 2. Retrosynthetic analysis of 9(*R*),16(*S*)-linotrin 1.

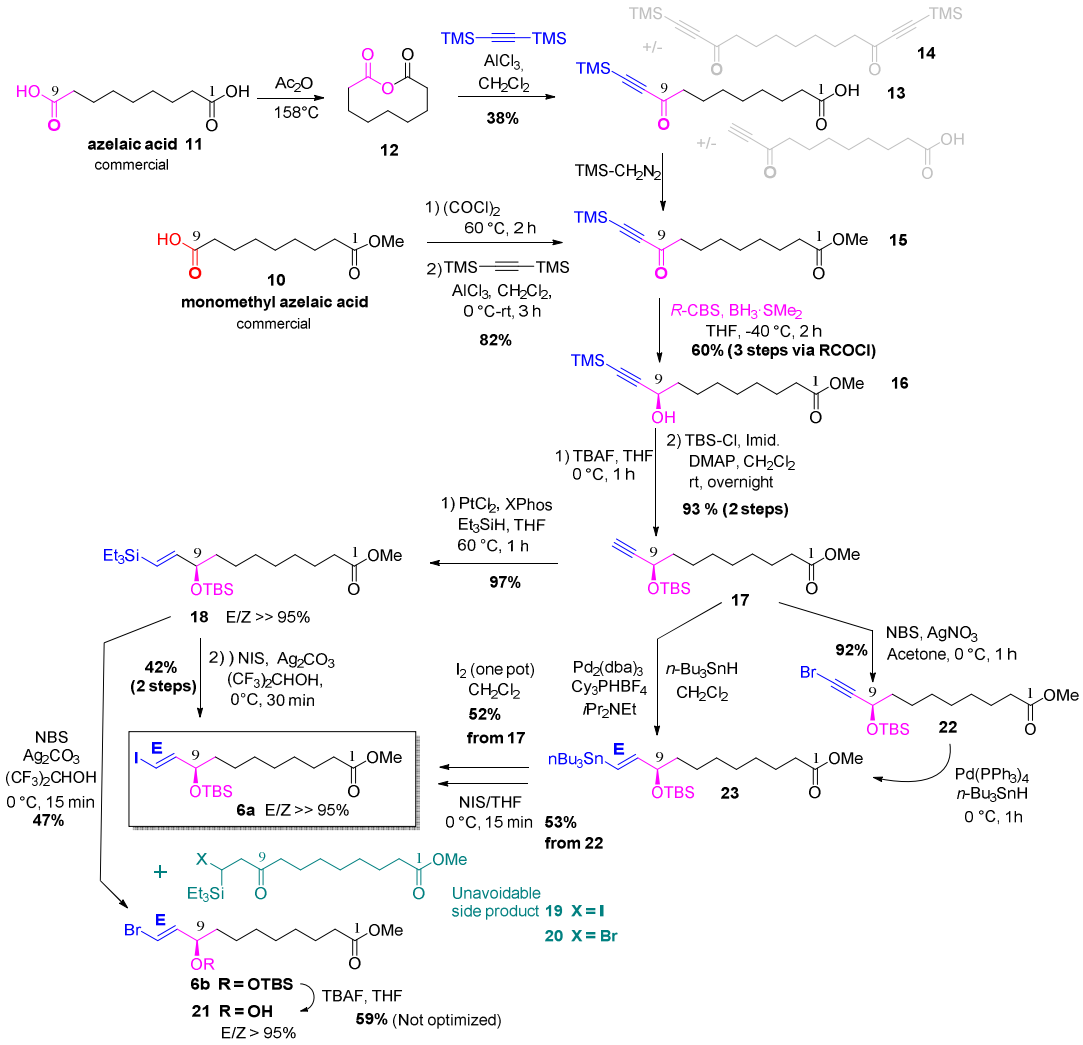


Both optically active allylic alcohols may be obtained by an enantioselective reduction of ketones, which, in turn, may derive from alkynylation of acid chlorides, namely the propionyl chloride (commercially available) for the tail chain, or readily accessible from the monomethyl azelate **10** for the polar head chain.

Synthesis of the two key intermediates 6 and 7.

The synthesis of (*E*)-vinyliodide **6a** commenced (scheme 3) by conversion of the monomethyl azelaic acid **10** into its corresponding acid chloride and subsequent $AlCl_3$-mediated alkynylation with bis(trimethylsilyl)acetylene results in excellent yields. In our hands, alkynylation[11, 12] of the nonanedioic anhydride **12** (which is readily obtained[13] from azelaic acid **11**) was not efficient enough since the starting commercially available azelaic acid **11** contained an impurity (barely visible by NMR due to overlapping), generating bis-trimethylsilyl azelate **14** that disturbed the purification steps and dramatically lowered yields of the desired pure ynones **13** or **15**. An enantioselective reduction of the resulting ynone **15** using the Corey-Bakshi-Shibata (CBS) oxazaborolidine reagent[14] afforded the desired optically active propargylic alcohol **16** in good yield (60 % over three steps) and enantiomeric excess (> 91%, calculated[15] by comparison of ^1H NMR spectra of the corresponding α-hydroxylated mandalate esters). Even if several equivalents of both reagents could be saved compared to previous[11] reports, large amounts (1.2 equivalents of the asymmetric CBS-ligand instead of two equivalents; and three equivalents of reducing BH_3.DMS agent instead of five equivalents) were required for completion of the reaction. Upon TMS-deprotection with fluoride anions in quantitative yield, the secondary hydroxyl was subsequently protected as a TBS ether to obtain the terminal alkyne **17.**

Scheme 3. Synthesis of the polar head chain.



Although it is well known that the Schwartz's reagent may reduce methyl ester[16, 17] groups, an efficient chemoselective hydrozirconation of the terminal alkyne bearing a α,β-unsaturated ester reported by Fûrstner[18] encouraged us to subject the terminal alkyne 17 to stoichiometric hydrozirconation conditions[19]. Unfortunately, but unsurprisingly, the formation of the (*E*)-vinylzirconocene was hampered by the competitive production of alcohols. The concomitant reduction of the ester group occurred within the first minutes of the reaction (even with a reduced quantity of hydride, dilution effect and reverse slow dropwise addition), suggesting a similar reactivity at this temperature (18-25°C) for both functionalities.

However, platinum-catalyzed *trans*-hydrosilylation[20] reaction of the terminal alkyne **17** provided the expected (*E*)-vinylsilane **18** in 97% yield. The ^{1}H NMR spectrum showed a large coupling constant (J = 18.8 Hz) for the two olefinic protons. There was no detectable regio-isomer or (*Z*)-isomer. Subsequent iododesilylation in the presence of N-iodosuccinimide (NIS) afforded the required key intermediate (*E*)-vinyliodide **6a** with excellent retention of the (*E*)-double bond configuration (J =14.4 Hz, no Z-isomer detected). However, this step turned out to be more difficult than expected due to the formation of a side-product, identified by 2D-NMR as the C-silylated iodo-ketone **19**. Several conditions were tested, including the mild conditions (NIS in acetonitrine/chloroacetonitrile: 9/1) advised by Stamos et al.[21] to avoid epimerization at the OTBS-allylic chiral center, the use of hexafluoroisopropanol (HFIP) as a solvent with strong hydrogen bonding suggested by Ilardi et al.[22], addition of silver salts (Ag_2CO_3 or AgOAc/2,6-lutidine) in HFIP reported by Sidera et al.[23], and including modification of the number of equivalents and reaction temperatures. However, we could not avoid the side reaction, which certainly arose from rapid rearrangement of the OTBS-protected enol intermediate[21] and dampened the yield (40-61%) of the desired (*E*)-vinyliodide **6**a.

Replacement of NIS with NBS did not change the course of the reaction, providing the same yields and same vinyl halide to side-product ratios. In that case, as the two olefinic protons of the corresponding OTBS protected derivative **6b** overlapped, the removal of the OTBS group with TBAF was necessary to confirm the (*E*)-geometry of the double bond in the vinylbromide **21** by ^{1}H NMR (J = 13.6 Hz).

Alternatively, the synthesis of (*E*)-vinyl iodide **6a** was achieved *via* palladium-catalyzed hydrostannation[24, 25]. Thus, treatment of the bromoalkyne **22** (readily obtained *via* silver nitrate mediated-bromination[26]) with $Pd(PPh_3)_4$ or $Pd_2(dba)_3$/triphenylphosphine catalytic systems in the presence of an excess of tributyltin hydride according to Guibé procedure[27] or tricyclohexylphosphonium tetrafluoborate-$Pd_2(dba)_3$-isopropylethyl amine catalyzed[28]

hydrostannylation of the terminal alkyne **17,** and subsequent *in situ* iodination showed an excellent (*E*)-stereoselectivity and good regioselectivity (92 to 95 %), albeit moderate yields (ca. 55%). Considering the toxicity of tin derivatives and the similar efficiency of the Silane vs Tin approaches, the stereo- and regio-selective hydrosilylation/Ag_2CO_3-promoted iododesilylation route was finally preferred to scale up the synthesis of the (*E*)-vinyl iodide **6a**.

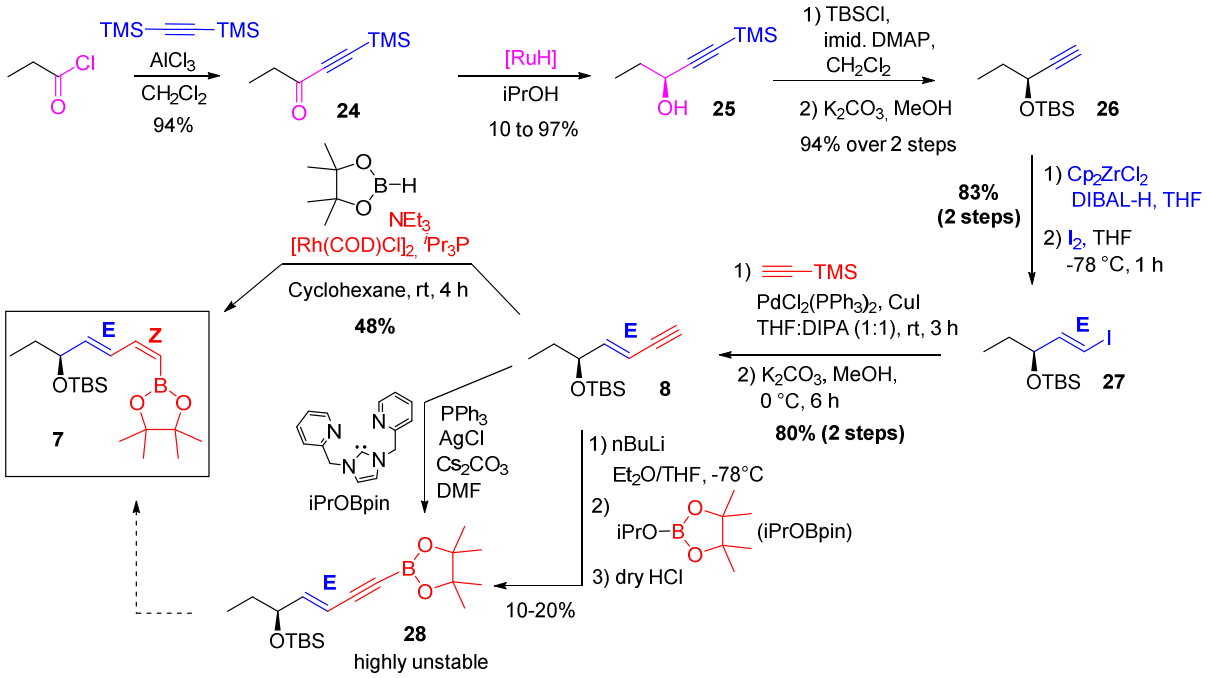
The synthesis of the (*E*)-enyne **8** (schemes 2 and 4) began[29] with an alkynylation reaction of propionyl chloride with bis(trimethylsilyl)acetylene, which resulted in excellent yields, followed by an asymmetric reduction of the resulting propargylic ketone **24** with[30, 31] the *in situ* prepared Noyori catalyst. The desired (*S*)-configuration and enantiomeric purity (ee = 96 %) were established by derivatization of the propargylic alcohol function **25** with enantiopure O-acetylmandelic acids and subsequent NMR analysis.

Upon TBS-protection of the newly formed propargylic alcohol **25**, a chemoselective TMS-deprotection in mild basic conditions afforded the expected optically active terminal alkyne **26** in excellent yields (special care is required for the low boiling point lipid precursors **24**, **25,** and **26**).

A highly regio- and stereoselective hydrozirconation-iodination sequence using *in situ* prepared Schwartz reagent[19], followed by a Sonogashira cross-coupling reaction[32-35] between the resulting (*E*)-vinyl iodide **27** (J = 14.4 Hz) and trimethylsilylacetylene and a subsequent K_2CO_3-mediated TMS-deprotection, provided the expected (*E*)-enyne **8** in 80% yield over two steps. Most metal-free and metal-catalyzed hydroborations of terminal alkynes provide *syn*-addition of a B-H moiety, leading to the more favorable anti-Markovnikov kinetic (*E*)-alkenyl products.[35-40] The choice of the metal catalyst systems dramatically influences the stereochemical outcome of the hydroboration. Of particular interest is the non-conventional *trans*-addition of pinacolborane in some specific rhodium-based catalytic conditions developed by Miyaura[41, 42], furnishing (*Z*)-alkenylboronates. Thus, treatment of the (*E*)-enyne **8** with pinacolborane in the presence of

[Rh(cod)Cl]$_2$ catalyst, a bulky phosphine and triethylamine provided the desired (1*Z*,3*E*)-dienylboronate **7** (J = 13.0 Hz for the borylated olefin), with an excellent regio-, chemo- and stereoselectivity, albeit moderate yield.

Scheme 4. Synthesis of the (1*Z*,3*E*)-dienylboronate 7.



The pinacol (*Z*)-alkenylboronic ester **7** was also prepared *via* regio- and stereoselective rhodium-hydroboration using catecholborane followed by transesterification with pinacolborane. However, this two-step sequence[43] did not improve the reactivity and yields.

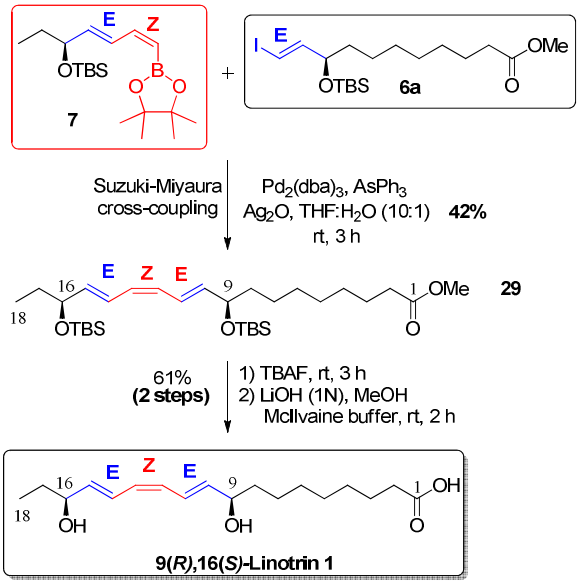
It should be noted that these *trans*-hydroborations were highly sensitive to relative reagent equivalencies and the purity and nature of the oxygen-sensitive phosphine. However, triisopropylphosphine yielded better results than tricyclohexylphosphine. In some cases, the ^{1}H NMR spectra showed some (1*E*,3*E*)-dienylboronate (5-8%) that was not separable, and thus precluded the ability to proceed in the synthesis.

A two-step reaction sequence based on a selective syn-hydrozirconation[44] or hydroboration[45] of 1-alkynyl boronate[46, 47] was also envisioned. Unfortunately, the borylation of the (*E*)-enyne **8** with isopropylpinacolborane (*via* a lithium acetylide or a Ag(I) catalyst in the presence of a 1,3-di(2-picolyl)imidazol carbene ligand) failed to give the 1-alkynyl boronate **28** in sufficient yields for total syntheses (<25%). Highly sensitive to moisture[46], the borylated enyne **28** tends to be converted back to the starting enyne **8**. In addition, although not extensively investigated, an alternative Ag(I)-catalyzed borylation of the (*E)*-enyne **8** in the presence of a 1,3-di(2-picolyl)imidazol carbene ligand, based on a C(sp)-H borylation of terminal alkyne without using any strong base[48], was also unsuccessful. Since the obtained 1-alkynylboronate **28** was highly unstable, we did not persist in this delicate pathway.

Assembling the triene subunit and completion of the linotrin synthesis.

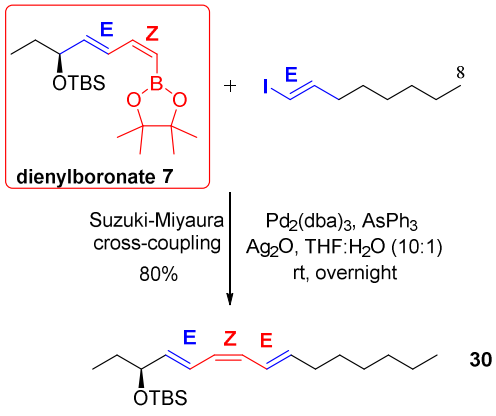
Subsequent Suzuki-Miyaura cross-coupling reaction[49-51] of the (1*Z*,3*E*)-dienylboronate **7** with the (*E)*-vinyl iodide **6a** under exceptionally mild conditions ($Pd_2(dba)_3$, Ph_3As, Ag_2O, THF/H_2O:10/1, 25°C, scheme 5) afforded a new lipid compound **29** whose U.V. spectrum showed a three-band absorption (λmax in EtOH: 260.0, 269.0, 279.0 nm), characteristic of a conjugated triene.

Scheme 5. Assembly of the *E,Z,E*-triene and completion of the linotrin synthesis.



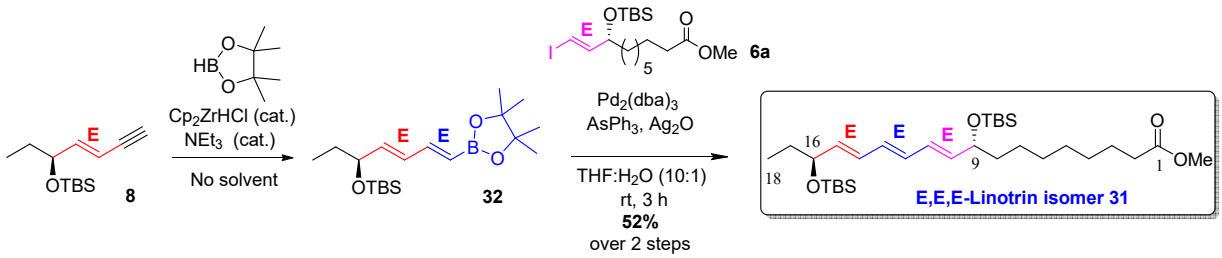
The ESI mass spectrum was in agreement with the expected molecular formula. However, no coupling constant in the ^{1}H NMR spectra confirmed the configuration of the three double bonds as several olefinic protons overlap at 300 MHz, 500 MHz, and 600 MHz in $CDCl_3$ and benzene-d6, while well-defined NMR signals (four doublets of doublet and a quintet, with J = 15.0 Hz for both (*E*)-double bonds and J = 10.7 Hz for the (*Z*)-olefin, common values in conjugated polyene structures), were observed for a pilot *E,Z,E*-triene **30** (same UV shape) derived from the same (1*Z*,3*E*)-dienylboronate **7** and (*E*)-octenyl iodide (scheme 6).

Scheme 6. Pilot Suzuki-Miyaura cross-coupling reaction with the (1*Z*,3*E*)-dienylboronate 7.



As stability may be different for both these sensitive conjugated *E,Z,E*-trienes **29** and **30**, the successful preparation of the pilot *E,Z,E*-triene **30** with retention of the starting stereochemistries cannot be a convincing argument to rule out the possibility of a *Z* to *E*-isomerisation of the central double bond in the sample **29**. Therefore, the *E,E,E*-triene isomer **31** was synthesized from the (*E*)-enyne **8** (scheme 7) using a regio- and (*E*)-stereoselective hydroboration via[52, 53] an *in situ* catalytic hydrozirconation with the pre-prepared Schwartz reagent. The resulting (1*E*,3*E*)-dienylboronate **32** (J = 17.7 Hz for the borylated olefin) was subsequently coupled with the (*E*)-vinyl iodide **6a** in the above-mentioned Suzuki-Miyaura conditions, affording the conjugated triene **31,** with three similar UV characteristic bands (λ_{max} in EtOH: 259.0, 268.0, 280.0 nm) and same ESI mass spectrum as the previous triene **29**. Due to overlapping in the olefinic region, no coupling constant could be measured. However, the chemical shifts of the olefinic protons (and the shapes of the broad multiplet signals) in the ^{1}H NMR spectra of both trienes **29** and **31** were dramatically different, allowing us to conclude that the triene **29** possesses the expected 10*E*,12*Z*,14*E*-configuration.

Scheme 7. Synthesis of the *E,E,E*-triene isomer 31.



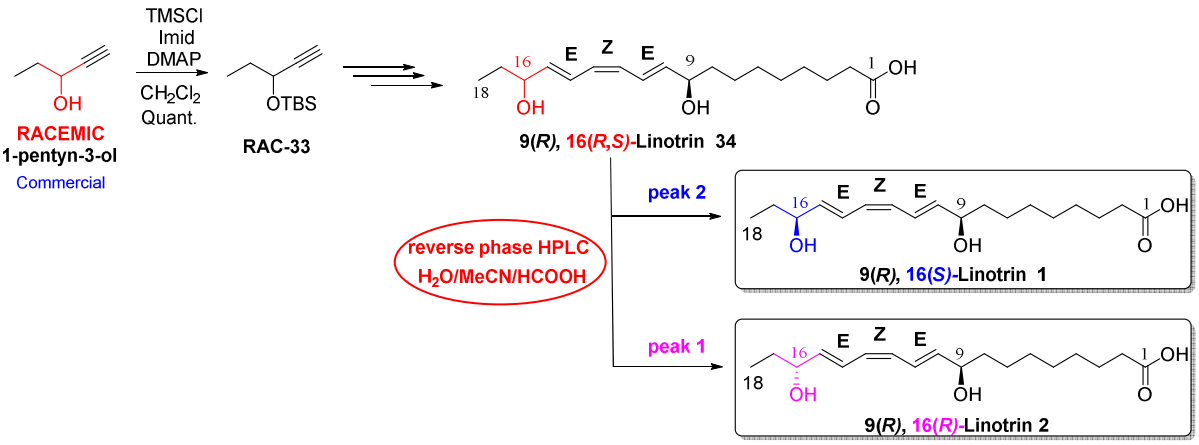
Completion of the linotrin synthesis (scheme 5) was achieved by TBAF deprotection of both hydroxyl functions, followed by the sensitive LiOH-mediated saponification of the methyl ester group. Despite the apparent simplicity of these two last steps, the reaction conditions must be carefully controlled (mainly pHs) to proceed successfully as the allylic functional groups **1** (or their

OTBS ethers **29**) tended to undergo elimination reactions, and the (*Z*)-double bond is prone to isomerization.

The 16(R)-epimer 2.

Additionally, the above-mentioned strategy was reproduced with precursors containing a racemic center at the C16 carbon atom (scheme 8). Thus, the inexpensive, commercially available racemic 1-pentyn-3-ol was readily converted to its TBS-ether **rac-33** (racemic form of the terminal alkyne **26,** scheme 4). Although an HPLC separation of the diastereomeric mixture **34** was required, this alternative strategy is justified because the total synthesis is three steps shorter, avoiding highly volatile precursors **24**, **25,** and **26,** and the delicate chemoselective TMS-deprotection. Another advantage is the concomitant synthesis of the 9(*R*),16(*R*)-linotrin epimer **2**, which has never been reported to date.

Scheme 8. linotrin epimers from a 9(*R*)-16(*R,S*)-diastereomeric mixture.



Biological evaluation in microglia cells.

The success of these syntheses offered new perspectives to study the bioactivities of linotrins. The protective action of ALA has been frequently described in numerous central nervous system (CNS)

diseases[2, 54] displaying chronic or acute neuroinflammation. Both types of neuroinflammation are primarily mediated by microglial cells, whose response is stereotypic and includes the release of major pro-inflammatory cytokines capable of compromising neuronal viability[55, 56]. Lipopolysaccharide (LPS) is a major component of the cell wall of gram-negative bacteria, which is considered the gold-standard stimulus for microglial activation in culture. Therefore, we investigated the effect of 9(*R*),16(*R*,*S*)-linotrins against LPS-induced pro-inflammatory response in microglial cells. Among the major pro-inflammatory cytokines, tumor necrosis factors (TNF-α) and interleukines (IL-6 and IL-1β) are associated with the pro-inflammatory microglial M1 phenotype[57] and induction of both direct neurotoxicity and indirect neurodegeneration.[58] To exert their effect, cytokines must be released from cells and the time course of LPS-induced release is specific to each cytokine. The most characteristic example is the difference between LPS-induced TNF-α and IL-6 release: the peak level of LPS-induced TNF release occurs 6 hours after LPS stimulation, whereas IL-6 release is usually observed at 24 hours. Therefore, we first evaluated the anti-inflammatory capacity of 9(*R*),16(*R*,*S*)-linotrins in reducing the quantity of cytokines released in the medium by microglial cells, during 6 and 24 hours of LPS-exposure. Our results revealed a substantial reduction by 14.1±4.3% and 20.79±4.3%, respectively of TNF-α and IL-6 proteins released from linotrins-pretreated microglial cells after 6h of LPS exposure (Figure 1 A). Interestingly, this protection was still observed after 24 hours of LPS exposure as shown by the reduction by 22.7±3.0% of TNF-α and by 30.5±5.7% of IL-6 protein released (Figure 1A). This anti-inflammatory effect of linotrins on the cytokine release was correlated with a reduction in the protein level of both IL-6 and TNF-α in the cellular content of microglial cells after 6 hours (data not shown) and 24 hours (Figure 1B) of LPS exposure. While AlphaLISA technology does not detect levels of IL-1β released into the medium, it revealed that pretreatment with linotrins induced a marked reduction of these pro-inflammatory cytokines in the cellular content of microglial cells exposed to LPS (Figure 1B).

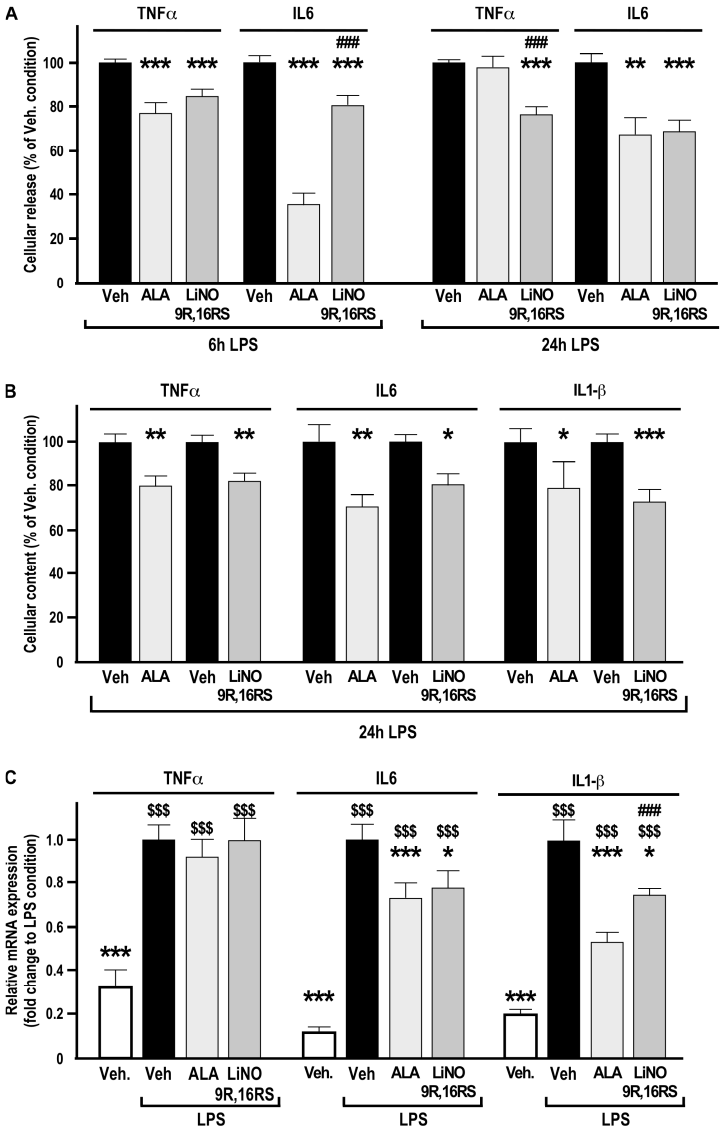


Figure 1: Anti-inflammatory effects of pretreatment with 9(*R*),16(*R*,*S*)-linotrins on LPS-challenge BV2 microglial cells. A, linotrins pretreatment reduces the quantity of TNF-α and IL-6 proteins released after 6 and 24h of LPS exposure.; B, linotrins and ALA reduced TNF-α, IL-6 and IL-1β protein content after 24 hours of LPS-challenge. C, linotrins and ALA pretreatment reduced mRNA expression of IL-6 and IL-1β but not of TNF-α 24h after LPS-challenge. Data are expressed as the mean±SEM of at least four experiments consisting of four replicates per condition. $^{\$\$\$}$P<0.001 *vs*. Veh. Group. *P<0.05, **P<0.01, ***P<0.001 *vs*. Veh+LPS condition. $^{\#\#}$P<0.01 *vs*. ALA condition.

Since the reduction in protein content could result from an effect of linotrins on transcription and/or translation[56], we then evaluated the anti-inflammatory capacity of 9(*R*),16(*R*,*S*)-linotrins in reducing the LPS-induced mRNA expression of TNF-α, IL-6 and IL-1β. LPS-induced mRNA expression of both IL-6 and IL-1β cytokines, but not of TNF-α, were substantially reduced in BV2 microglial cells by linotrins or ALA pre-treatment (Fig. 1B). The absence of effect, observed 24 hours after LPS exposure, on TNF-α mRNA expression by ALA and its oxygenated metabolite (Figure 1C) is probably due to its rapid and transient expression in LPS-stimulated microglia[59]. This result is probably due to the late timing of the measurement (24 hours after LPS stimulation), rather than to the lack of anti-inflammatory effect of linotrins due to the overall cellular content and release in TNF-α protein being significantly reduced after 6h and 24h of LPS challenge.

Altogether, our results indicate that 9(*R*),16(*R*,*S*)-linotrins possess similar biological anti-inflammatory activity to ALA. Moreover, like ALA, it limits the LPS-induced pro-inflammatory response of BV2 microglial cells by reducing expression and release of TNF-α, IL-1β and IL-6 cytokines. However, this protective effect was achieved at an effective concentration 3-fold lower than that of ALA.

Interestingly, Protectin D1 (PD1, NPD1, an oxygenated DHA-derivative containing a conjugated *E*,*E*,*Z*-triene) that was detected in the brain also demonstrates a similar protective anti-inflammatory effect against LPS-induced pro-inflammatory cytokines expression in microglial cells[60]. This neuroprotectin was proposed to mediate DHA-induced neuroprotection and anti-inflammatory effects in several experimental models of chronic and acute CNS diseases, such as Alzheimer's disease and stroke, respectively[60]. While it is therefore tempting to speculate that linotrins may contribute to the previously reported therapeutic effects of ALA supplementation [2, 54]), further research is needed to address this hypothesis.

Detection of linotrins in wheatgrass.

Given the beneficial bioactivity of linotrins, detection of these mediators in edible portions of plants, such as leaves, seeds, roots, tubers, etc., that provide ALA in livestock and the human diet represents a crucial step in evaluating the potential health benefits of linotrins. Our initial interest focused on green leaves in which ALA is a predominant component of the membrane[61, 62] of chloroplasts. As consumption of wheatgrass is becoming increasingly popular as food, drink, or dietary supplements, wheatgrass (*Triticum aestivum*) was selected for its potential health benefits.[63] Interestingly, several natural linotrin diastereomers were detected (Figure 2, panel A) in 'Kaskaskia' wheatgrass leaves. Comparison of the plant extracts with the profile of the synthetic sample (panel B) identified not only the two main peaks, i.e. two 9(*R*),16(*R*,*S*)-diastereomers, but also two smaller peaks (9(*S*),16(*R*,*S*)-diastereomers) were detected in all the analyzed extracts (Figures S1-2). Their heterogeneous concentrations may be due to the growing conditions experienced by the plants that could affect ALA content. However, the relative production of the two lipoxygenase-derived monohydroxylated ALA metabolites i.e. 9-hydroxy-10(*E*),12(*Z*),15(*Z*)-octadecatrienoic acid (9-HOTrE, precursor of linotrins) and 13-hydroxy-9(*Z*),11(*E*),15(*Z*)-octadecatrienoic acid (13-HOTrE, precursor in jasmonic acid biosynthesis) appears less sensitive to the experimental growth conditions (Figure S3), indicating that linotrin production may be controlled by a finer signaling pathway or biochemical regulation that requires further investigations.

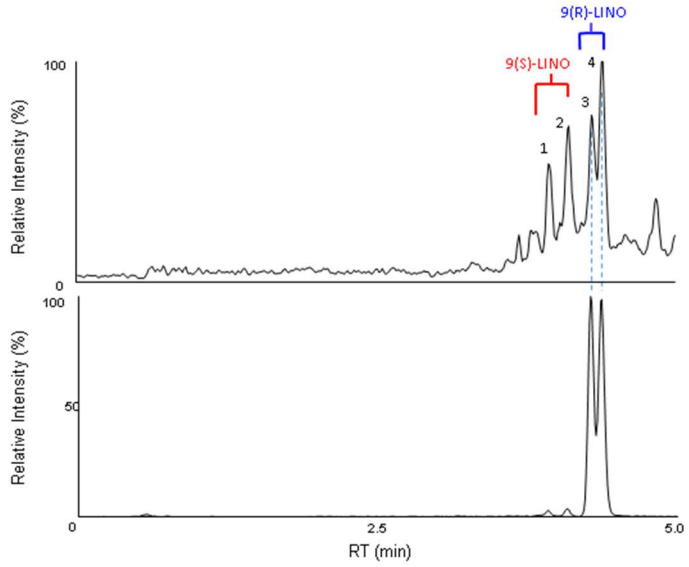


Figure 2. Evidence of linotrins in plants by LC-MS/MS with selected reaction monitoring of 'Kaskaskia' freeze-dried wheatgrass leaves.

Selected reaction monitoring (SRM) tandem mass spectrometry using a triple quadrupole mass spectrometer. Ion chromatograms relative to specific fragmentations of linotrins (m/z 309.2 to 291.1) are presented for a wheatgrass leaf extract (Panel A) and the synthetic linotrin diastereomeric mixture **34** (Panel B).

In Panel B, the four peaks detected displayed exactly the same retention times as the peaks 1,2,3, and 4 of the wheatgrass extract. The two main peaks (peaks 3 and 4) co-chromatograph with the two 9(*R*),16(*R*,*S*)-diastereomers while the two smaller peaks (peaks 1 and 2) are 9(*S*),16(*R*,*S*)-diastereomers (respectively 3.22 and 3.55% of the peak 2 of mixture **34**), stemming from the formation of a small amount of the (*S*)-propargylic alcohol enantiomer during the asymmetric reduction of the propargylic ketone **15** with the (*R*)-CBS-oxazaborolidine (enantiomeric excess ee > ca.91% i.e. formation of ca. 4.5% of the *(S)*-enantiomer).

CONCLUSION

The first total syntheses of linotrins, containing sensitive conjugated *E*,*Z*,*E*-trienes or *E*,*E*,*E*-trienes, were achieved using stereoselective (*E*) or (*Z*)-hydrometalation of (*E*)-enynes. Our strategy utilized a trans-hydroboration for the preparation of the (Z)-olefins as an alternative to the commonly used hydrogenation or zinc-mediated semi-reduction in lipid metabolite syntheses. In addition, we provide evidence of the presence of linotrins in wheatgrass leaves, whose health benefits are currently attracting great levels of interest for human consumption and could potentially represent an alternative way to feed animals in an effort to increase the ALA levels in the human diet.

These lipoxygenase-derived ALA metabolites may contribute to health benefits in humans and/or mammals through anti-inflammatory effects on inflamed microglia, the hallmark of neuroinflammation in the CNS. Linotrins reduce microglial-induced production and secretion of toxic cytokines. Cytokines negatively influence outcomes related to acute and chronic neurodegenerative conditions by direct lethal actions on neurons and indirect actions on other cell types of the neurovascular unit.

Inflammation is associated with many chronic and lethal diseases, correlating with the fact that American and Western diets are deficient in omega-3 lipids. Our findings may support a potential change in the current paradigm that individuals should increase their consumption of highly unsaturated fatty acids (particularly EPA and DHA) by eating high doses of marine-derived food to provide health benefits. Effectively, our work highlights the notion that ALA, the main omega-3 lipid in the plant kingdom that is poorly converted to EPA and DHA *in vivo*, may be an efficient alternative owing to the beneficial bioactive properties of its di-hydroxylated linotrin metabolite.

Further investigations are needed, notably to decipher whether linotrins are present in other plants and in humans under normal physiological conditions, whether their concentration is affected in pathological or stressful conditions, and whether ALA supplementation by diet modification could be an effective option to increase the content of natural anti-inflammatory linotrins.

EXPERIMENTAL SECTION

General Procedures. All reactions were performed in oven-dried glassware (120 °C, minimum 12 hours) under an atmosphere of argon. Inert gas was dried by passing it through solid anhydrous calcium sulfate (Drierite). Anhydrous diethyl ether, tetrahydrofuran (THF), dichloromethane, methanol, acetone, cyclohexane, and acetonitrile were purchased from Acros organics, and chloroacetonitrile was purchased from Sigma Aldrich (Merck). All previous listed reagents wereused without further purification. Isopropanol was freshly distilled over calcium hydride under vacuum (membrane pump) and maintained under argon atmosphere before use. Fluorinated solvent 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), purchased from Sigma Aldrich (Merck), was used without further purification. All bases like triethylamine, diisopropylethylamine (DIPA), and 2,6-lutidine were distilled over calcium hydride under an argon atmosphere and stored over KOH pellets in glass bottles protected from light. Commercially available oxalyl chloride was freshly distilled before use. Potassium carbonate and silver carbonate (protected from light) were dried at 120 ºC in an oven for two days minimum and cooled down to rt under vacuum. Copper iodide was dried overnight at 80 ºC under high vacuum pump before each use. Pinacolborane and catecholborane were purchased from Sigma Aldrich (Merck) and used as received. Highly oxygen-sensitive and moisture-sensitive tricyclohexyl phosphine (Cy_3P), triisopropyl phosphine, catalysts $Pd(PPh_3)_4$ and $Pd_2(dba)_3$, were purchased from Sigma Aldrich (Merck) or StremChemicals and stored in Schlenk tubes under inert atmosphere.

Thin-layer chromatography (TLC) was performed on aluminum pre-coated silica gel plates from Merck, and developed plates were visualized by UV light (254 nm), p-anisaldehyde spray, or potassium permanganate stain. Purification was performed using Reveleris® automated flash chromatographic technique or manual column chromatography with the indicated eluent on Davisil

40-63 µm, 100-200 mesh silica. In some cases, for flash column chromatography, deactivated silica (prepared by addition of 46 mL of water to 100 g of silica then stirring on rotavapor for 2 h at rt without applying vacuum) was used.

^{1}H NMR spectra (300 MHz, 500 MHz, and 600 MHz) and ^{13}C NMR spectra (75 MHz, 125 MHz) were recorded on Bruker spectrometers. According to Gottlieb et al. [64], chemical shifts are reported relative to residual chloroform (δ 7.24 ppm) or methanol quintet (δ 3.31 ppm) for ^{1}H NMR spectra. ^{13}C NMR spectra were calibrated at the central pic of deuterated chloroform (δ 77.16 ppm) or methanol (δ 49.00 ppm). The ^{1}H NMR spectra data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, br. = broad), coupling constant(s) in Hertz (Hz), integration and assignments. All the NMR spectra were assigned with the help of 2D NMR techniques (COSY ^{1}H-^{1}H, HMBC, and HSQC).

Infrared spectra were obtained using a Perkin-Elmer Spectrum One spectrophotometer and were reported as wavenumbers (cm^{-1}) of significant peaks. Mass spectra (negative or positive ESI) and high-resolution mass spectra (HRMS) were measured at the Faculty of Sciences (University of Montpellier, France) on Q-Tof micro (resolution 100000, Waters) or Synapt G2-S mass spectrometers (Waters). UV-Vis spectra were performed on a double beam Cary 100 spectrophotometer (version 9.00).

(9*R*,10*E*,12*Z*,14*E*,16*S*)-9,16-dihydroxyoctadeca-10,12,14-trienoic acid (Linotrin 1).

Step 1. TBS-deprotection. **(9*R*,10*E*,12*Z*,14*E*,16*S*)-methyl 9,16-bis((*tert*-butyldimethylsilyl)oxy) octadeca-10,12,14-trienoate (diol).** TBAF (117 µL, 1.0 M in THF, 0.115 mmol, 5 equiv) was added slowly at 0 °C to a stirred solution of the starting di-OTBS compound **29** (13.0 mg, 0.023 mmol, 1.0 equiv) in degassed THF (0.6 mL). The reaction mixture was slowly allowed to come to rt and stirred at same temperature for 10 h. After completion (monitored by TLC), the reaction

mixture was diluted with diethyl ether (5 mL) prior addition of a saturated ammonium chloride solution (2 mL). The aqueous phase was extracted with ethyl acetate (2 x 8 mL previously passed through basic alumina). The combined organic phases were washed with water (2 mL), then brine (2.0 mL), dried over $MgSO_4$, filtered and evaporated. Purification on column chromatography (deactivated silica, diethyl ether/pentane: 75/25 to 70/30) provided the desired diol (7.1 mg, 93% yield). $\mathbf{R}_f$ = 0.31 (pentane/diethyl ether: 60/40). **U.V.** (EtOH) λmax 260.0, 269.0, 279.0 nm. **^{1}H NMR (300 MHz, $CDCl_3$):** 6.75-6.59 (m, 2H, H11 and H14), 6.02-5.90 (m, 2H, H12 and H13), 5.72 (dd, *J* = 15.0 Hz and *J* = 6.6 Hz, 1H, H10 or H15), 5.71 (dd, *J* = 15.0 Hz and *J* = 6.6 Hz, 1, H10 or H15), 4.22 (m, 2H, H16 and H9), 3.64 (s, 3H, OMe), 2.28 (t, *J* = 7.5 Hz, 2H, H2), 1.68 (br s, 2H, OH), 1.65-1.47 (m, 6H, H8, H17 and CH2), 1.40-1.20 (m, 8H, CH2), 0.92 (t, *J* = 7.4, 3H, H18) ppm. **^{13}C NMR (75 MHz, $CDCl_3$):** δ 174.51 (CO_2R), 137.66 (C10), 137.40 (C15), 129.14 (C12 or C13), 129.06 (C13 or C12), 125.74 (C11 or C14), 125.54 (C14 or C11), 74.17 (C16), 72.84 (C9), 51.63 (OMe), 37.48 (C8), 34.26 (C2), 30.38 (C17), 29.47 (C3 or C4, or C6 or C5), 29.30 (C3 or C4, or C6 or C5), 29.19 (C3 or C4, or C6 or C5), 25.46 (C7), 25.06 (C4 or C3), 9.87 (C18) ppm. **ESI-MS:** 347.22 (M+Na). **HMRS (ESI, positive mode) m/z:** calculated for $C_{19}H_{32}O_4Na$ [M +Na]$^+$347.2198, found 347.2202.

Step2. Ester saponification. At 0 °C, a LiOH solution (1N, 16.8 mg, 0.4 mL H_2O, 50 equiv) was added dropwise to the methyl ester (2.6 mg, 0.008 mmol, 1.0 equiv) in degassed MeOH (0.6 mL) and the reaction mixture was covered with aluminum foil and stirred at rt for 3 h. Upon completion, the reaction mixture was diluted with McIlvaine buffer[65] (ca.12 mL) to adjust the pH at 5. The aqueous phase was extracted with EtOAc (2 x 15 mL). The organic phases were combined and washed with water (3 x 3 mL), brine (3 mL), and water (5 mL). The organic phase was dried over $MgSO_4$, filtered, and evaporated to dryness to afford the desired target **Linotrin 1** (1.73 mg, 68%, NMR purity >95%, 5% *E*, *E*, *E*) as a colorless liquid.

Application of this procedure to the diastereomeric diol mixture provided the two corresponding acids (Linotrin **34)**, which were separated by HPLC (conditions and chromatograms, see the supporting information file). Assignment of the configurations at C9 in the molecules isolated from peaks 1 and 2 were deduced from co-injections in HPLC with the target **1** obtained from the enantioselective synthesis.

Note: *We recorded the ^{1}H NMR spectrum in $CDCl_3$ to compare with literature data. However, as this diol-acid compound **1** was poorly soluble in $CDCl_3$, we also recorded the NMR spectra in CD_3OD.*

Linotrin 1 and linotrin 34. R_f= 0.34 (acetone/hexane: 4/6). **U.V.** (EtOH) λmax 259.0, 268.0, 279.0 nm. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 6.78–6.61 (m, 2H, H11 and H14), 6.01-5.85 (m, 2H, H12 and H13), 5.67 (dd, *J* = 6.6 and *J* = 15.0 Hz, 2H, H10 and H15), 4.15-4.02 (m, 1H, H9), 4.08-3.96 (m, 1H, H16), 2.24 (t, *J* =7.3 Hz, 2H, $CH2_{alphaCO}$),1.67-1.18 (m, 14H, CH2), 0.90 (t, *J* = 7.3 Hz, 3H, H18) ppm. **^{13}CNMR (300MHz, $CDCl_3$, Linotrin 34** (racemic C16 carbon atom): δ (two diastereomers) 179.06 (COOR), 178.96 (COOR), 137.46 (C10 or C15), 137.41 (C10 or C15), 137.17 (C10 or C15), 137.09 (C10 or C15), 129.11 (C12 or C13), 129.04 (C12 or C13), 125.80 (C11 or C15), 125.71 (C11 or C15), 125.63 (C11 or C15), 125.59 (C11 or C15), 74.20 (C16), 74.08 (C16), 72.91 (C9), 72.82 (C9), 37.23 (CH2), 37.19 (CH2), 34.04 (C2), 30.25 (C17), 29.83 (CH2), 29.29 (CH2), 29.17 (CH2), 28.96 (CH2), 28.91(CH2), 25.31(C3 or C4), 24.72 (C3 or C4), 9.85 (C18). **ESI-MS (negative mode, Linotrin 34):** 309.21 (M-H). **HMRS (ESI, negative mode) m/z:** calculated for $C_{18}H_{29}O_4$ $[M-H]^-$ 309.2066, found 309.2069.

^{1}H NMR (600 MHz, MeOD-*d4*, Linotrin 1 = Peak2): δ 6.76–6.67 (m, 2H, H11 and H14), 6.01-5.94 (m, 2H, H12 and H13), 5.71 (dd, *J* = 6.6 and *J* = 15.1 Hz, 1H, H10), 5.70 (dd, *J* = 6.6 and *J* = 15.1 Hz, 1H, H15), 4.14-4.09 (m, 1H, H9), 4.05 (dq, 1H, *J* = 6.5 Hz and *J* = 1.1 Hz, H16), 2.24 (t, *J* =7.4 Hz, 2H, H2), 1.64-1.46 (m, 6H, CH2), 1.44-1.30 (m, 8H, CH2), 0.92 (t, *J* = 7.4 Hz, 3H, H18) ppm. **^{13}CNMR (125 MHz, MeOD-*d4*, Linotrin 1 = Peak2)**: δ 179.18 (CO_2H), 138.75 (C10),

138.40 (C15), 129.93 (C12 or C13), 129.90 (C13 or C12), 126.51 (C11), 126.29 (C14), 74.62 (C16), 73.21 (C9), 38.41 (C8 or C7), 36.04 (C2), 31.18 (C17), 30.59 (CH2), 30.47 (CH2), 30.36 (CH2), 26.57 (C7 or C8, and C3 or C4), 26.52 (C4 or C3), 10.19 (C18) ppm. **ESI-MS (negative mode):** 309.21 (M-H). **HMRS (ESI, negative mode) m/z:** calculated for $C_{18}H_{29}O_4$ $[M-H]^-$ 309.2066, found 309.2067.

(9*R*,10*E*,12*Z*,14*E*,16R)-9,16-dihydroxyoctadeca-10,12,14-trienoic acid (diastereoisomer 2, peak 1). U.V. (EtOH) λmax 259.1, 268.0, 279.0 nm. **^{1}H NMR (600 MHz, MeOD-*d4*)**: δ 6.76–6.67 (m, 2H, H11 and H14), 6.01-5.94 (m, 2H, H12 and H13), 5.70 (dd, *J* = 6.6 and *J* = 15.1 Hz, 1H, H10), 5.69 (dd, *J* = 6.6 and *J* = 15.0 Hz, 1H, H15), 4.14-4.08 (m, 1H, H9), 4.05 (dq, 1H, *J* = 6.4 Hz and *J* = 1.0 Hz, H16), 2.24 (t, *J* =7.4 Hz, 2H, H2), 1.64-1.46 (m, 6H, CH2), 1.44-1.30 (m, 8H, CH2), 0.92 (t, *J* = 7.4 Hz, 3H, H18) ppm. **^{13}CNMR (125 MHz, MeOD-*d4*)**: δ 179.18 (CO_2H), 138.75 (C10), 138.38 (C15), 129.94 (C12 or C13) 129.89 (C13 or C12), 126.55 (C11), 126.31 (C14), 74.65 (C16), 73.22 (C9), 38.42 (C8 or C7), 36.57 (C2), 31.17 (C17), 30.60 (CH2), 30.50 (CH2), 30.43 (CH2), 26.73 (C7 or C8, and C3 or C4), 26.58 (C4 or C3), 10.19 (C18) ppm. **ESI-MS (negative mode):** 309.21 (M-H). **HMRS (ESI, negative mode) m/z:** calculated for $C_{18}H_{29}O_4$ $[M-H]^-$ 309.2066, found 309.2068.

(*R*,*E*)-methyl 9-((*tert*-butyldimethylsilyl)oxy)-11-iodoundec-10-enoate (6a) via iododesilylation of (*E*)-vinylsilane 18.

Method 1 *(based on procedure reported in Reference[23])*: Silver carbonate (60 mg, 0.20 mmol, 0.3 equiv) was added to a stirred solution of (*E*)-vinylsilane **18** (300 mg, 0.677 mmol, 1.0 equiv) in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (3.4 mL) at 0 ºC. The flask was protected from light with aluminum foil before the addition of *N*-iodosuccinimide (182.9 mg, 0.813 mmol, 1.2 equiv) in one portion at 0 °C. The reaction mixture was stirred for 30 min. When no starting material was observed

by TLC, the reaction mixture was quenched at 0 °C with the addition of a saturated $Na_2S_2O_3$ solution (0.8 mL), followed by water (0.8 mL). The two layers were separated and the aqueous phase was extracted with CH_2Cl_2 (5 × 5 mL). The combined organic phases were washed with brine (1 mL), dried over $MgSO_4$, filtered, and evaporated to dryness under reduced pressure. Purification of the crude by flash column chromatography (silica, pentane/diethyl ether: 99/1) afforded the desired (*E*)-vinyl iodide **6a** (134 mg, 43%) as a colorless oil.

Method 2 *(based on procedure reported in Reference[21])***:** To a solution of the (*E)*-vinylsilane **18** (330 mg, 0.745 mmol, 1.0 equiv) in a mixture of CH_3CN and $ClCH_2CN$ (9:1 v/v, 9 mL), was added *N*-iodosuccinimide (335 mg, 1.49 mmol, 2.0 equiv) at 0°C in one portion. Then, the water bath was removed and the reaction mixture was stirred for 2 h at rt. Upon completion, the reaction mixture was quenched with water (5 mL) and extracted with diethyl ether (3 x 12 mL). The combined organic layers were washed with sodium thiosulfate (2 mL), water (1 mL), and brine (2 mL), then dried over $MgSO_4$, filtered, and evaporated. Purification by flash column chromatography (silica, pentane/diethyl ether: 99/1) provided the desired (*E*)-vinyl iodide **6a** (134 mg, 40%) as a colorless oil.

Method 3 *(based on procedure reported in Reference[22])***:** *N*-iodosuccinimide (53.3 mg, 0.237 mmol, 1.5 equiv) was added to a mixture of (*E)*-vinylsilane **18** (70 mg, 0.158 mmol, 1.0 equiv) and 2,6-lutidine (26 µL, 0.221 mmol, 1.4 equiv) in a mixture of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and CH_2Cl_2 (2 mL, 1:1 v/v, CH_2Cl_2 used to solubilize the silane) at 0 °C. Upon completion in ca. 15 min, the reaction mixture was quenched by the addition of water (1 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 5 mL). The organic layers were combined, washed with saturated aqueous sodium thiosulfate (0.5 mL), 1 M HCl (0.5 mL), water (1 mL), saturated aqueous sodium bicarbonate (1 mL), dried over $MgSO_4$, filtered, and concentrated. The resulting residue was purified by flash column chromatography (Silica, pentane/diethyl ether: 99.5/0.5), yielding the desired (*E*)-vinyl iodide **6a** (30 mg, 42%) as a colorless liquid.

(*R*, *E*)-methyl 9-((*tert*-butyldimethylsilyl)oxy)-11-iodoundec-10-enoate (6a) via the (*E*)-vinylstannane 23.

Method 1: via bromoalkyne 22. At 0°C, a solution of bromoalkyne **22** (80 mg, 0.197 mmol, 1.0 equiv) in anhydrous THF (3 mL) was cannulated to a mixture of $Pd(PPh_3)_4$ (11.4 mg, 9.9 µmol, 5 mol%) in THF (1 mL). The resulting dark brown solution was stirred for 2 min. Then, n-tributyltin hydride (160 µL, 592 µmol, 3.0 equiv) was added at 0 ºC. The reaction mixture was stirred for 1 h. Upon completion, a few drops of water were added and the mixture subsequently dried over Na_2SO_4, filtered, and evaporated to dryness. The resulting crude (*E)*-vinyl stannane **23** was immediately used for the following ioodestannylation step without further purification.

NIS (222 mg, 1.00 mmol, 5.0 equiv) was added at 0 ºC in one portion to the crude vinylstannane **23** (dissolved in 3.0 mL of anhydrous THF) and the reaction mixture was stirred at the same temperature for 15 min. Upon completion (monitored by TLC), the reaction was quenched by the addition of a saturated $Na_2S_2O_3$ solution (1 mL) and diluted with ethyl ether (5 mL). The organic phase was washed with water (2 mL), then HCl (1N, 1 mL) and water (2 mL, until neutral pH). dried over $MgSO_4$, filtered, and evaporated. Purification by column chromatography (pentane/diethyl ether: 100/0 to 98/2) provided the desired (*E)*-vinyl iodide **6a** (48 mg, 53% yield). Only traces of regioisomers were detected. Replacement of the $Pd(PPh_3)_4$ catalyst with $Pd_2(dba)_3$ and triphenylphosphine furnished a much lower yield, due to poor conversion.

Method 2: (*E)*-vinyl iodide **6a** from the **terminal alkyne 17 via the *E*)-vinylstannane 23.** Distilled diisopropylethylamine (533 µL, 3.1 µmol, 0.01 equiv) was added to a solution of $Pd_2(dba)_3$ (0.7 mg, 0.76 µmol, 0.25 mol%) and tricyclohexylphosphonium tetrafluoroborate (0.6 mg, 1.5 µmol, 0.5 mol%) in CH_2Cl_2 (1.5 mL) and the resulting mixture was stirred at rt for 15 min. The terminal alkyne **17** diluted in CH_2Cl_2 (1.5 mL) was cannulated (100 mg, 0.307 mmol, 1.0 equiv). The reaction was cooled to 0°C prior dropwise addition of n-Bu_3SnH (100 µL, 0.368 mmol, 1.2 equiv) diluted in

CH_2Cl_2 (0.5 mL) over 20 min. The reaction was stirred at 0°C for 30min. Upon completion, iodine (101 mg, 3.9 µmol, 1.3 equiv) dissolved in CH_2Cl_2 (1.3 mL) was cannulated dropwise within 10 min at 0°C and the mixture was stirred at 0 °C for a further 15 min. The reaction mixture was washed with saturated $Na_2S_2O_3$ solution (1.2 mL), then water (1 mL), brine (2 mL) then extracted with ethyl ether (3 x 5 mL), dried over $MgSO_4$, filtered, and evaporated. The resulting oil was purified by silica gel chromatography (pentane/ether) to afford the corresponding (*E*)-vinyl iodide **6a** (72.5 mg, 52%).

R*f* = 0.45 (pentane/diethyl ether: 98/2). **IR (cm^{-1})**: 2929, 2856, 1739, 1462, 1435, 1361, 1251, 1194, 1165,1102, 1082, 1005, 941, 834, 774. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 6.48 (dd, *J* = 5.9 Hz and *J* = 14.4 Hz, 1H, H10), 6.16 (dd, *J* = 1.0 Hz and *J* = 14.4 Hz, 1H, H11), 4.04 (br q, *J* = 5.9 Hz, 1H, H9), 3.64 (s, 3H, OMe), 2.28 (t, *J* = 7.5 Hz, 2H, H2), 1.68-1.52 (m, 2H, H3), 1.52-1.34 (m, 2H, H8), 1.34-1.17 (m, 8H, CH2), 0.86 (s, 9H, tBu), 0.02 (s, 3H, CH3Si), 0.00 (s, 3H, CH3Si) ppm. **^{13}C NMR (75 MHz, CDCl3)**: δ 174.46 (CO_2R), 149.46 (C10), 75.64 (C11), 75.28 (C9), 51.62 (OMe), 37.62 (C8), 34.22 (C2), 29.47 (CH2), 29.30 (CH2), 29.21 (CH2), 25.96 (tBu), 25.05 (C3), 24.91 (CH2), 18.35 ($C_{quat\ TBS}$), -4.39 (CH3Si), -4.74 (CH3Si) ppm. **ESI-MS**: 327.24 (M-I), 477.13 (M+Na). **HRMS (ESI, positive mode) m/z**: calculated for $C_{18}H_{35}O_3Si$ $C_{18}H_{35}O_3Si$ $[M - I]^+$ 327.2355, found 327.2360.

(*R*,*E*)-methyl 11-bromo-9-(*tert*-butyldimethylsilyloxy)undec-10-enoate (6b) via bromodesilylation of (*E*)-vinylsilane 18. Silver carbonate (9.3 mg, 0.04 mmol, 0.3 equiv) was added to a stirring solution of the (*E*)-vinylsilane **18** (50 mg, 0.113 mmol, 1.0 equiv) in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, 0.8 mL) at 0 ºC. The flask was protected from light before the addition of *N*-bromosuccinimide (NBS) (30 mg, 0.169 mmol, 1.5 equiv) in one portion at 0 °C. The reaction mixture was stirred for 30 min. When no starting material was observed by TLC, the reaction mixture was quenched at 0 °C by addition of water (2 mL). After separation of the two

layers, the aqueous phase was extracted with CH_2Cl_2 (2 × 5 mL). The combined organic phases were washed with brine (2 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (silica, pentane/diethyl ether: 99/1) afforded the desired (*E*)-vinyl bromide **6b** (21.7 mg, 47%) as a colorless oil along with the ketone side product **20**. *At that stage, the coupling constants do not allow the assignment of the (E)-stereoselectivity. It was confirmed after deprotection of the silyl group (compound **21**, see below).* **R_f** = 0.44 (pentane/diethyl ether: 98/2). **IR (cm^{-1})**: 2931, 2857, 1741 (COOR), 1621, 1463, 1361, 1252, 1171, 1086, 937, 836, 776. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 6.19-6.16 (m, 2H, H10 and H11), 4.12-4.04 (m, 1H, H9), 3.66 (s, 3H, OMe), 2.30 (t, *J* = 7.6 Hz, 2H, H2), 1.65-1.51 (m, 2H, H3), 1.51-1.42 (m, 2H, H8), 1.37-1.20 (m, 8H, CH2), 0.88 (s, 9H, tBu), 0.04 (s, 3H, CH3Si), 0.03 (s, 3H, CH3Si) ppm. **ESI-MS**: 327.24 (M-Br), 429.14 (M+Na) for isotope ^{79}Br, 431.14 (M+Na) for isotope ^{81}Br. **HRMS (ESI, positive mode) m/z**: calculated for $C_{18}H_{35}O_3NaSiBr$ $[M + Na]^+$ with isotope ^{79}Br 429.1437, found 429.1434; calculated for $C_{18}H_{35}O_3Si$ $[M - Br]^+$ 327.2355, found 327.2357.

***tert*-butyldimethyl(((S,4E,6Z)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)hepta-4,6-dien-3-yl)oxy)silane (7).** *Based on procedures reported in reference[41].* At rt, pinacolborane (145 µL, 1.0 mmol, 1.0 equiv) was added to a degassed solution of $[Rh(COD)Cl]_2$ (7.4 mg, 0.015 mmol, 0.15 equiv) and $^iPPr_3$ (11.5 µL, 0.06 mmol, 0.06 equiv) in degassed and freshly distilled cyclohexane (3.0 mL) and Et_3N (697 µL, 5.0 mmol). After stirring for 30 min, (*E*)-enyne **8** (269.3 mg, 1.2 mmol, 1.2 equiv), diluted in degassed cyclohexane (1.2 mL), was added dropwise via cannula and stirred for 4 h at rt. (Prolonged reaction time led to more by-products). After 4 h, the reaction mixture was carefully evaporated under vacuum (bath temperature below 30°C, rotary evaporator backfilled with argon at rt when evaporation is complete). The resulting residue was purified by column chromatography (pentane/diethyl ether: 96/4) using NEt_3-desactivated silica (silica was pre-treated with 2% Et_3N in pentane, then stirred for 2 h in a rotary evaporator without vacuum prior

evaporation of pentane, process repeated twice). Noteworthy, on normal silica the desired dienylboronate **7** suffers from decomposition. In addition, deactivated silica with water (46 mL of water poured to 100 g of silica, and then stirring for at least 2h) induced the formation of several by-products during the column chromatography, seriously lowering the yield). The expected (*E*,*Z)*-boronate **7** was obtained as a pale yellow oil in 48% yield (140.1 mg). Comparison with the corresponding (*E*, *E)*-boronate **32** confirmed the absence of the (*E*, *E)*-isomer, suggesting that this rhodium mediated-hydroboration procedure was highly stereoselective [(*Z*)-selectivity over 95 %]. **R*f*** = 0.47 (pentane/diethyl ether: 96/4). **IR (cm^{-1})**: 2957, 2929, 2857, 1698, 1643, 1592, 1471, 1437, 1371, 1330, 1299, 1255, 1144, 1111, 1085, 1005, 967, 833, 774. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 7.10–6.85 (m, 1H, H5), 6.81 (t1, *J* = 3.0 Hz, 1H, H6), 5.76 (dd, *J* = 5.7 Hz and *J* = 14.9 Hz, 1H, H4), 5.31 (d, *J* = 13.0 Hz, 1H, H7), 4.20-4.08 (m, 1H, H3), 1.59-1.40 (m, 2H, H2), 1.25 (s, 12H, Me $_{pinacol}$),0.92 (s, 9H, tBu),0.87 (t, *J* = 7.5 Hz, 3H, H1), 0.05 (s, 3H, CH_3Si), 0.04 (s, 3H, CH_3Si) ppm. **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 150.56 (C6), 141.79(C4), 129.72 (C5),118.27 (C7, very broad, confirmed by HMBC spectrum), 83.14 (C_{quat}-O),74.0 (C3), 31.06 (C2), 26.16 (tBu), 25.02 ($CH_{3pinacol}$), 24.95 ($CH_{3pinacol}$), 18.46 ($C_{quat\ TBS}$), 9.76 (C1), -4.14 (CH_3Si), -4.73 (CH_3Si) ppm. **ESI-MS**: Too unstable - Failed with both positive and negative mode.

(*S*,*E*)-*tert*-butyl(hept-4-en-6-yn-3-yloxy)dimethylsilane (8).

Step 1: (*S*,*E*)-t*ert*-butyldimethyl((7-(trimethylsilyl)hept-4-en-6-yn-3-yl)oxy)silane (TMS-enyne). Anhydrous CuI (129 mg, 0.677 mmol, 0.1 equiv) and bis(triphenylphosphine)palladium(II) dichloride (237 mg, 0.338 mmol, 0.05 equiv) were loaded into a two-neck flask which was subsequently evacuated and backfilled with argon, three times. A solution of (*E*)-vinyl iodide **27** (2.21 g, 6.77 mmol, 1.0 equiv) in THF (34 mL, degassed by argon bubbling for 30 min prior to use) was added via cannula. After addition of degassed piperidine (34 mL), the flask was evacuated and backfilled with argon (3 cycles). The flask was protected from light with aluminum foil. A solution

of trimethylsilylacetylene (1.1 mL, 8.12 mmol, 1.2 equiv) in degassed THF (10.0 mL) was added over a period of 30 min. After completion, the reaction mixture was filtered through a pad of Celite® (washed with diethyl ether, 2 x 50 mL). Evaporation of the volatiles under vacuum followed by flash chromatography purification (normal silica gel, pentane/diethylether: 98/2 to 97/3) afforded the expected (*E*)-enyne **8** (1.83 g, 91%) as a colorless oil. $\mathbf{R}_f$ = 0.42 (100% pentane). **IR (cm^{-1})**: 2958, 2931, 2904, 2858, 2169 (alkyne), 2133 (alkyne), 1472, 1460, 1361, 1250, 1445, 1087, 1042, 1005, 955, 838, 775, 759. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 6.16 (dd, *J* = 5.0 Hz and *J* = 15.9 Hz, 1H, H4), 5.66 (dd, *J* = 1.7, 15.9 Hz, 1H, H5), 4.09 (dq, *J* = 1.6 Hz and *J* = 5.9 Hz, 1H, H3), 1.55-1.37 (m, 2H, H2), 0.88 (s, 9H, tBu), 0.85 (t, *J* = 7.5 Hz, 3H, H1), 0.17 (s, 9H, TMS) 0.02 (s, 3H, CH_3Si), 0.01 (s, 3H, CH_3Si) ppm. **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 147.65 (C4), 108.75 (C5), 103.90 (C6), 94.35 (C7), 73.48 (C3),30.70 (C2), 25.99 (tBu), 18.36 ($C_{quat\ TBS}$), 9.36 (C1), 0.12 (TMS), -4.42 (CH_3Si), -4.74 (CH3Si) ppm.

Step 2: TMS-deprotection. At 0 ºC, the trimethylsilyl-protected (*E*)-enyne (2.17 g, 7.31 mmol, 1.0 equiv) diluted in CH_2Cl_2 (35 mL) was cannulated to a stirred suspension of dry K_2CO_3 (3.04 g, 21.9 mmol, 3.0 equiv) in anhydrous MeOH (35 mL). The reaction mixture was stirred at 0 °C for 6 h. The reaction was quenched with 1N HCl (10 mL) and the two phases were separated. The aqueous phase was extracted with diethyl ether (2 x 50 mL). The organic layers were combined and washed with water (2 x 25 mL) and brine (20 mL), dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The crude product was purified by column chromatography (pentane 100%, deactivated silica which was prepared as follows: 46 mL water was added to 100 g of silica, then stirred for at least 2h), affording the expected (*E*)-enyne **8** (1.33 g, 81 %) as a light-yellow oil. $\mathbf{R}_f$ = 0.71 (100% pentane). **IR (cm^{-1})**: 3316 (alkyne), 2957, 2930, 2904, 2889, 2857, 1773, 1463, 1255, 1146, 1089, 1032, 1006, 957, 836, 776. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 6.21 (ddd, *J* = 15.9 Hz, *J* =5.1 Hz and *J* = 0.6 Hz, 1H, H4), 5.63 (ddd, *J* = 15.9 Hz, *J* =2.3 Hz and *J* = 1.7 Hz, 1H, H5), 4.10 (qd, *J* = 5.8 Hz

and *J* =1.7 Hz, 1H, H3), 2.84 (dt, *J* = 2.3 Hz and *J* = 0.6 Hz, 1H, H7), 1.53 – 1.44 (m, 2H, H2), 0.88 (s, 9H, tBu), 0.85 (t, *J* = 7.4 Hz, 3H, CH3Si), 0.03 (s, 3H), 0.01 (s, 3H, CH3Si) ppm. **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 148.49 (C4), 107.69 (C5), 82.33 (C6), 77.33 (C7), 73.45 (C3), 30.63 (C2), 25.97 (tBu), 18.37 ($C_{quat\ TBS}$), 9.36 (C1), -4.45 (CH3Si), -4.74 (CH3Si) ppm. **ESI-MS**: 247.15 (M+Na). **HRMS (ESI, positive mode) m/z**: calculated for $C_{13}H_{24}OSiNa$ $[M+Na]^+$ 247.1497, found 247.1495.

(*R*,*E*)-methyl 9-((*tert*-butyldimethylsilyl)oxy)-11-(triethylsilyl)undec-10-enoate (18). *(based on procedure reported in Reference[20])* Under an argon atmosphere, platinum dichloride (12.2 mg; 0.046 mmol, 0.05 equiv) and Xphos (43.79 mg, 0.092 mmol, 0.1 equiv) in anhydrous THF (1 mL) were heated at 60°C for 15 min. Then, terminal alkyne **17** (300 mg, 0.920 mmol, 1.0 equiv) in anhydrous THF and triethylsilane (220 µL, 1.38 mmol, 1.5 equiv) were added via syringe. The mixture was stirred at 60°C for 1 h. After evaporation of the solvent, column chromatography (silica, pentane/diethyl ether: 99/1) afforded the desired (*E*)-vinylsilane **18** (395 mg, 97%) as a light-yellow oil. **R_f** = 0.82 (pentane/diethyl ether: 90/10). **IR (cm^{-1})**: 2951, 2931, 2856, 2869, 1743 (COOR), 1621, 1462, 1437, 1361, 1251, 1198, 1170, 1079, 992, 835, 775, 720. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 5.95 (dd, *J* = 5.7 Hz and *J* = 18.8 Hz, 1H, H10), 5.64 (dd, *J* = 1.2 Hz and *J* = 18.8 Hz, 1H, H11), 4.01 (quint, *J* = 5.6 Hz, 1H, H9), 3.64 (s, 3H, OMe), 2.27 (t, *J* = 7.4 Hz, 2H, H2), 1.66-1.52 (m, 2H, H3), 1.52-1.34 (m, 2H, H8),1.34-1.11 (m, 6H, CH2), 0.90 (t, *J* = 7.7 Hz, 9H, CH3), 0.87 (s, 9H, tBu), 0.53(br q, *J* = 7.7 Hz, 6H, CH2Si), 0.01 (s, 3H, CH3Si), -0.01 (s, 3H, CH3Si) ppm. **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 174.50 (CO_2R), 151.04 (C10), 124.43 (C11), 76.19 (C9), 51.60 (OMe), 38.06 (C8), 34.26 (C2), 29.55 (CH2), 29.36 (CH2), 29.24 (CH2), 26.05 (tBu), 25.42 (CH2), 25.09(C3), 18.48 ($C_{quat\ TBS}$), 7.49 (CH3), 3.58 (CH2Si), -4.17 (CH3Si), -4.67 (CH3Si) ppm. **ESI-MS**: 311.24 (M-OTBS), 465.32 (M+Na). **HRMS (ESI, positive mode) m/z**: calculated for $C_{24}H_{50}O_3NaSi_2$ $[M+Na]^+$ 465.3196, found465.3201; calculated for $C_{18}H_{35}O_2NaSi$ $[M - OTBS]^+$ 311.2406, found 311.2412.

Methyl 11-iodo-9-oxo-11-(triethylsilyl)undecanoate (19). Obtained as a side-product during the iododesilylation of (*E*)-vinylsilane **18** (see the above-mentioned synthesis of the (*E*)-vinyliodide **6a**). $\mathbf{R_f}$ = 0.23 (pentane/diethyl ether: 95/5). **IR (cm^{-1}):** 2940, 2933, 2875, 2853, 1737 (C=O), 1718 (COOR), 1457, 1435,1414, 1362, 1237, 1195, 1169, 1105, 1067, 1006, 766, 719. **^{1}H NMR (300 MHz, $CDCl_3$):** δ 3.64. (s, 3H, OMe), 3.59 (dd, *J* = 3.0 Hz and *J* = 12.0 Hz, 1H, H11), 3.04 (dd, *J* = 12.0 and *J* = 17.4 Hz, 1H, H10'), 2.86 (dd, *J* = 3.0 Hz and *J* = 17.4 Hz, 1H, H10), 2.40 (t, *J* = 7.5 Hz, 2H, H8), 2.28 (t, *J* = 7.5 Hz, 2H, H2), 1.67-1.49 (m, 4H, H3 and H7), 1.35-1.20 (m, 6H, H4, H5, H6), 0.98 (t, *J* = 7.5 Hz, 9H, CH_3), 0.67 (q, *J* = 7.8 Hz, 6H, CH_2Si) ppm. **^{13}C NMR (75 MHz, $CDCl_3$):** δ 208.55 (C=O), 174.46 (COOR), 51.63 (OMe), 46.86 (C10), 43.47 (C8), 34.19 (C2), 29.14 (CH2), 29.10 (CH2), 25.00 (C3 or C4), 23.49 (C7 or C6), 7.57 (CH3), 7.10 (C11), 3.22 (CH2Si), 3.13 (CH2Si) ppm. **ESI-MS:** 279.19 (M-I-Et), 327.24 (M-I), 455.15 (M+H), 472.17 ($M+NH_4^+$). **HRMS (ESI, positive mode) m/z:** calculated for $C_{18}H_{36}O_3SiI$ $[M + H]^+$ 455.1478, found 455.1484; calculated for $C_{18}H_{39}NO_3SiI$ $[M + NH_4]^+$472.1744, found 472.1749;calculated for $C_{18}H_{35}O_3Si$ $[M-I]^+$ 327.2355, found 327.2361; calculated for $C_{16}H_{29}O_3Si$ $[M-I-Et]^+$ 297.1886, found 297.1892.

Methyl 11-bromo-9-oxo-11-(triethylsilyl)undecanoate (20). Obtained as a side-product during the bromodesilylation of (*E*)-vinylsilane **18** (see the above-mentioned synthesis of the (E)-vinyliodide **6b**). $\mathbf{R_f}$ = 0.21 (pentane/diethyl ether: 95/5). **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 3.80 (dd, *J* = 2.7, 11.9 Hz, 1H, H11), 3.64 (s, 3H, OMe), 2.98 (dd, *J* = 11.9 and *J* = 17.0 Hz, 1H, H10), 2.72 (dd, *J* = 2.7 Hz and *J* = 17.0 Hz, 1H, H10'), 2.42 (t, *J* = 7.6 Hz, 2H, H8), 2.27 (t, *J* = 7.5 Hz, 2H, H2), 1.66-1.49 (m, 4H, CH2), 1.35-1.23 (m, 6H, CH2), 0.97 (t, *J* =7.9 Hz, 9H, CH3), 0.67 (m, 6H, CH2Si) ppm. **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 208.35 (C=O), 174.70 (COOR), 51.62 (OMe), 46.22 (C10), 43.67 (C8), 34.19 (C2), 32.76 (C11), 29.14 (CH2), 29.07 (CH2), 25.00 (C3 or C4), 23.49

(C7 or C6), 7.53 (CH3), 2.52 (CH2Si) ppm. **ESI-MS:** 327.24 (M-Br), 407.16 (M+H) for isotope ^{79}Br, 409.16 (M+H) for isotope ^{79}Br. **HRMS (ESI, positive mode) m/z:** calculated for $C_{18}H_{36}O_3SiBr$ $[M + H]^+$ with isotope ^{79}Br 406.1539, found 406.1542 ; calculated for $C_{18}H_{35}O_3Si$ $[M-Br]^+$ 327.2355, found 327.2360.

(*R*,*E*)-methyl 11-bromo-9-hydroxyundec-10-enoate (21). TBAF (32 µL, 1 M solution in THF, 0.032 mmol, 1.1 equiv) was added dropwise to a solution of (*E)*-vinyl bromide- **6b** (12 mg, 0.029 mmol, 1.0 equiv) in anhydrous THF (1 mL) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C. Upon completion (monitoring by TLC), the reaction mixture was diluted with EtOAc (2.0 mL), washed with water (1.0 mL), extracted with EtOAc (2 x 40 mL) and dried over $MgSO_4$. The solvent was removed by rotary evaporation to obtain a crude residue, which was purified by normal silica gel chromatography to afford the desired deprotected (*E)*-vinyl bromide **21** (5.1 mg, 59%, not optimized), as a light-yellow oil. $\mathbf{R}_f$ = 0.23 (pentane/EtOAc: 90/10). **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 6.31 (dd, *J* = 0.7 Hz and *J* = 13.6 Hz, 1H, H11), 6.20 (dd, *J* = 6.3 Hz and J = 13.6 Hz, 1H, H10), 4.15-4.05 (m, 1H, H9), 3.64 (s, 3H, OMe), 2.28 (t, *J* = 7.5 Hz, 2H, H2), 1.67-1.44 (m, 6H, CH2), 1.41-1.19 (m, 6H, CH2) ppm. **ESI-MS**: 213.07 (M-Br), 315.07 (M+Na) for isotope ^{79}Br, 317.07 (M+Na) for isotope ^{81}Br. **HRMS (ESI, positive mode) m/z**: calculated for $C_{12}H_{21}O_3NaBr$ $[M+Na]^+$ with isotope ^{79}Br 315.0674, found 315.0570.

(*R*)-methyl 11-bromo-9-((*tert*-butyldimethylsilyl)oxy)undec-10-ynoate (22). *N*-bromosuccinimide (218.0 mg, 1.22 mmol, 2.0 equiv) and silver nitrate (10.4 mg, 0.061 mmol, 0.1 equiv, dried overnight at 120 ºC in absence of light) were added sequentially in absence of light to a solution of terminal alkyne **17** (200.0 mg, 0.612 mmol, 1.0 equiv) in acetone (10 mL). The mixture was stirred for 30 min at rt in the dark before it was diluted with CH_2Cl_2 (5 mL) and water (5 mL). The two layers were separated and the aqueous phase was extracted with CH_2Cl_2 (2 x 10 mL) and

brine (5 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (silica gel, 100% pentane to pentane/diethyl ether: 99/1) to produce the desired bromoalkyne **22** (206.2 mg, 83%) as a colorless oil. **R*f*** = 0.54 (pentane/diethyl ether: 95/5). **IR (cm^{-1})**: 2929, 2856, 2209 (alkyne), 1739 (COOR), 1462, 1435, 1361, 1250, 1196, 1170, 1101, 1082, 1005, 939, 834, 776. **^{1}H NMR (300 MHz, $CDCl_3$)** : δ 4.32 (t, *J* = 6.4 Hz, 1H, H9), 3.64 (s, 3H, OMe), 2.28 (t, *J* = 7.5 Hz, 2H, H2), 1.71-1.51 (m, 4H, H8 and H3), 1.46-1.18 (m,8H, CH2), 0.87 (s, 9H, tBu), 0.10 (s, 3H, CH3Si), 0.07 (s, 3H, CH3Si) ppm. **^{13}C NMR (75 MHz, $CDCl_3$)** : δ 174.46 (COOR), 81.98 (C10), 63.96 (C9), 51.61 (OMe), 43.80 (C11), 38.55 (C8), 34.23 (C2), 29.27 (CH2), 29.19 (CH2), 29.14 (CH2), 25.91 (tBu), 25.14 (C7), 25.06 (C3 or C4), 18.37 ($C_{quat\ TBS}$), -4.44 (CH_3Si), -4.94 (CH_3Si) ppm. **ESI-MS**: 405.15 (M+H) for isotope ^{79}Br, 407.14 (M+H) for isotope ^{81}Br. **HRMS (ESI, positive mode) m/z**: calculated for $C_{18}H_{34}O_3SiBr$ $[M+H]^+$with isotope ^{79}Br 405.1461, found 405.1463.

(*S*,*E*)-*tert*-butyl((1-iodopent-1-en-3-yl)oxy)dimethylsilane 27. To a suspension of Cp_2ZrCl_2 (4.42 g, 15.1mmol, 1.5 equiv) in anhydrous THF (34 mL) was added dropwise a solution of *i*Bu_2AlH (15.1 mL, 1 M in hexane, 15.1 mmol, 1.5 equiv) at 0 °C under argon. The resulting suspension was protected from light (with aluminum foil) and stirred for 30 min at 0 °C followed by cannulation of terminal alkyne **26** (2.0 g, 10.1 mmol, 1.0 equiv) in anhydrous THF (16 mL, 8mL+2x 4mL rinse). The mixture was warmed to rt and stirred until it turned to a homogeneous solution (around 1 h). After cooling to -78°C, iodine (3.84 g, 15.1 mmol, 1.5 equiv, until brown-red color persistence) diluted in anhydrous THF (15.1 mL) was cannulated. After 30 min at -78°C, the reaction mixture was quenched with 1N HCl (16 mL), followed by water (10 mL). The aqueous phase was extracted with diethyl ether (2 x 50 mL). The combined organic phases were washed successively with saturated $Na_2S_2O_3$ aqueous solution (5 mL), and brine (15 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. The crude product was purified by column chromatography, providing the

expected (*E)*-vinyl iodide **27** (1.87 g, 57% yield) as a pale-yellow oil. $\mathbf{R}_f$ = 0.68 (100% pentane). **IR (cm^{-1})**: 2956, 2929, 2893, 2857, 1607, 1471, 1462, 1361, 1253, 1113, 1077, 1005, 944, 895, 854, 834, 775. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 6.49 (dd, *J* = 5.8 Hz and J = 14.4 Hz, 1H, H2), 6.17 (dd, *J* = 1.3, 14.4 Hz, 1H, H1), 4.00 (dq, *J* = 1.3 Hz and J = 6.0 Hz,1H, H3), 1.48 (m, 2H, H4), 0.87 (s, 9H, tBu), 0.85 (t, *J* = 7.5 Hz, 3H, H5), 0.02 (s, 3H, CH3Si), 0.02 (s, 3H, CH3Si) ppm. **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 149.21 (C2), 76.37 (C3), 75.70 (C1), 30.49 (C4), 25.96 (tBu), 18.38 ($C_{quat\ TBS}$), 9.35 (C5), -4.43 (CH_3Si), -4.74 (CH_3Si) ppm.

(9*R*,10*E*,12*Z*,14*E*,16*S*)-methyl-9,16-bis((*tert*-butyldimethylsilyl)oxy)octadeca-10,12,14-trienoate (29). *Based on procedures reported in references[66, 67].*At rt, (*E*)-vinyl iodide **6a** (50 mg, 0.110 mmol, 1.0 equiv) and (1*Z,* 3*E*)-dienylboronate **7** (58.1 mg, 0.165 mmol, 1.5 equiv), both dissolved in degassed THF (each in 1.5 mL), were cannulated to a degassed solution of PPh_3As (20.2 mg, 0.066 mmol, 0.6 equiv) and Ag_2O (127.4 mg, 0.55 mmol, 5.0 equiv) in anhydrous THF (3 mL). The required quantity of degassed water (600 μL) was then added to adjust the ratio of THF/H_2O at 10/1 (v/v). The mixture was degassed for additional 10 min. Then, $Pd_2(dba)_3$ (15.1 mg, 0.016 mmol, 0.15 equiv) was added in one portion under an argon atmosphere. The flask was protected from light with some aluminum foil and the reaction mixture was stirred 3 h at rt. After completion, THF was evaporated and the resulting crude residue was purified by column chromatography (silica, pentane/diethyl ether: 99/1), yielding the desired triene **29** (50.1 mg, 82%) as a light-yellow oil. $\mathbf{R}_f$= 0.49 (pentane/diethyl ether: 96/4). **U.V.** (EtOH) λmax 260.0, 269.0, 279.0 nm. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 6.65–6.47 (m, 2H, H11 and H14), 6.00-5.83 (m,2H, H12 and H13), 5.65 (dd, *J* =6.2 Hz and *J* =15.1 Hz, 1H, H10 or H15), 5.64 (dd, *J* =6.4 Hz and *J* =15.0 Hz, 1H, H10 or H15), 4.17-4.00 (m, 2H, H9 and H16), 3.64 (s, 3H, OMe), 2.28 (t, *J* =7.5 Hz, 2H, H2), 1.68-1.38 (m, 6H, CH2), 1.36-1.17 (m, 8H, CH2), 0.89 (s, 9H, tBu), 0.88 (s, 9H, tBu), 0.87 (t, *J* =7.4 Hz, 3H, H18), 0.04 (s, 3H, CH_3Si), 0.03 (s, 3H, CH_3Si), 0.02 (s, 3H, CH_3Si), 0.01 (s, 3H,

CH_3Si) ppm. **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 174.49 (CO_2R), 138.21 (C10 or C15), 137.97 (C15 or C10), 128.69 (C13 or C12), 128.62 (C12 or C13), 124.70 (C11 or C14), 124.59 (C14 or C11), 74.66 (C16), 73.46 (C9), 51.60 (OMe), 38.45 (C8), 34.25 (C2), 31.26 (C17), 29.86 (CH2), 29.57(CH2),29.36 (CH2), 29.26 (CH2), 26.06 (tBu), 25.37 (CH2), 25.09 (CH2), 18.44($C_{quatTBS}$), 9.84 (C18), -4.13 (CH_3Si), -4.19 (CH_3Si), -4.60 (CH3Si) ppm. **ESI-MS:** 421.31(M-OTBS), 575.39 (M+Na). **HRMS (ESI, positive mode) m/z:** calculated for $C_{31}H_{60}O_4Si_2Na$ $[M+Na]^+$575.3928, found 575.3924.

***tert*-butyldimethyl((*S*,4*E*,6*Z*,8*E*)-pentadeca-4,6,8-trien-3-yloxy)silane (30).** At rt, (*E*)-1-iodooctene (10 mg, 0.042 mmol, 1.0 equiv) and (1*Z,* 3*E*)-dienylboronate **7** (22.2 mg, 0.063 mmol, 1.5 equiv), both dissolved in degassed THF (0.5 mL), were cannulated to a degassed solution of $AsPh_3$ (7.7 mg, 0.025 mmol, 0.6 equiv) and Ag_2O (46.3 mg, 0.21 mmol, 5.0 equiv) in THF (1.1 mL THF). The required quantity of degassed water (200 µL) was then added to adjust the ratio of THF/H_2O (10/1 v/v) and the mixture was degassed for additional 10 min. Subsequently, $Pd(dba)_3$ (5.8 mg, 0.006 mmol, 0.015 equiv) was added in one portion under an argon atmosphere. The flask was protected from light with aluminum foil and the reaction was stirred overnight at rt. After completion, THF was evaporated and the resulting crude residue was purified by column chromatography (normal silica gel, pentane/diethyl ether: 100/0 to 99/1), yielding the desired triene **30** (11.5 mg, 80 %) as a light-yellow oil. **R_f** = 0.49 (100% pentane). **IR (cm^{-1}):** 2956, 2927, 2855, 1470, 1462, 1434, 1360, 1254, 1075, 1044, 1004, 963, 835, 774, 735, 695. **U.V.** (EtOH) λmax 259.0, 268.0, 278.0 nm. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 6.60 (dd, *J* = 10.7 Hz and *J* = 15.0 Hz, 1H, H5), 6.46 (br dd, *J* = 10.3 Hz and 15.0 Hz, 1H, H8), 5.88 (quint, *J* = 10.7 Hz, 2H, H6 and H7), 5.71 (td, *J* = 7.0 Hz and *J* = 15.0 Hz, 1H, H9), 5.63 (dd, *J* = 6.0 Hz and *J* = 15.0 Hz, 1H, H4), 4.10 (quint, *J* = 6.0 Hz, 1H, H3), 2.10 (quint, *J* = 7.0 Hz, 2H, H10), 1.58-1.47 (m, 2H, H2), 1.58-1.45 (m, 2H, H2), 1.45-1.17 (m, 6H, CH2), 0.95 (s, 9H, tBu), 0.95-0.81 (m, 6H, H1 and H15), 0.05 (s, 3H,

CH3Si), 0.03 (s, 3H, CH3Si) ppm. **^{13}C NMR (75 MHz, $CDCl_3$):** δ 137.26 (C4), 136.31 (C9), 129.31 (C7 or C6), 127.02 (C6 or C7), 125.70 (C8), 124.67 (C5), 74.48 (C3), 33.11 (C10), 31.88 (CH2), 31.33 (C2), 29.30 (CH2), 29.06 (CH2), 26.05 (tBu), 22.76 (CH2), 18.44 ($C_{quatTBS}$), 14.27 (C15), 9.86 (C1), -4.23 (CH3Si), -4.61 (CH3Si) ppm. **ESI-MS:** 205.20 (M-OTBS). **HRMS (ESI, positive mode) m/z**: calculated for $C_{15}H_{25}$ $[M\text{ -OTBS}]^+$ 205.1956, found 205.1958.

(7*R*,9*E*,11*E*,13*E*,15*S*)-methyl-7,15-bis((tert-butyldimethylsilyl)oxy)heptadeca-9,11,13-trienoate (31). Compound **31** was synthesized according to the above-mentioned Suzuki procedure, replacing the *E,Z*-diene-borane **7** with the *E,E*-diene-borane **32** (stoichiometric conditions, 3h at 23°C, 20 mg scale, 52% over 2 steps, not optimized). **R*f*** = 0.39 (pentane/diethyl ether: 99/1). **U.V.** (EtOH) λmax 259.0, 268.0, 280.0 nm. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 6.22-6.03 (m,4H, H_{olefin}), 5.71-5.55 (m, 2H, H10 and H15), 4.16-3.99 (m, 2H, H9 and H16), 3.64 (s, 3H, OMe), 2.27 (t, *J* = 7.5 Hz, 2H, H2), 1.71-1.52 (m, 4H, CH2),1.52-1.34 (m, 2H, CH2), 1.34-1.12 (m, 8H, CH2), 0.87 (s, 9H, tBu), 0.86 (s, 9H, tBu),0.84 (t, *J* = 7.5 Hz, 3H,H18), 0.02 (s, 3H, CH3Si), 0.01(s, 3H, CH3Si), 0.00 (s, 3H, CH3Si), -0.01 (s,3H, CH3Si) ppm. **^{13}C NMR (75 MHz, $CDCl_3$):** δ 174.50 (CO_2Me), 137.43 (C15 or C10), 137.21 (C10 or C15), 131.94 (CH_{olefin}), 131.87 (CH_{olefin}), 129.46 (CH_{olefin}), 129.35 (CH_{olefin}), 74.56 (C16or C9), 73.36 (C9 or C16), 51.61 (OMe), 38.51(CH2), 34.24 (C2), 31.32 (CH2), 29.56 (CH2), 29.35 (CH2), 29.24 (CH2), 26.05 (^{t}Bu), 25.30 (CH2), 25.08 (CH2), 18.43 (C_{quat}TBS), 9.78 (C18), -4.14 (CH_3Si), -4.20 (CH_3Si), -4.65 (2 CH_3Si) ppm. **ESI-MS:** 421.31 (M-OTBS), 575.39 (M+Na). **HMRS** (ESI, positive mode) m/z: calculated for $C_{31}H_{60}O_4Si_2Na$ $[M+Na]^+$575.3928, found 575.3926; calculated for $C_{25}H_{45}O_3Si$ $[M\text{ -OTBS}]^+$ 421.3138, found 421.3134.

***tert*-butyldimethyl(((4E,6*E*)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)hepta-4,6-dien-3-yl)oxy)silane 32 (*E*,*E*-boronate).** To a mixture of (*E*)-enyne **8** (21.9 mg, 1.0 equiv, 0.097mmol)

and freshly distilled triethylamine (3 µL, 0.2 equiv, 0.019 mmol), was added 4,4,5,5-tetramethyl[1,3,2]dioxaborolane (141 µL, 10 equiv, 0.97 mmol) and bis(cyclopentadienyl)zirconium chloride hydride (5.1 mg, 0.2 equiv, 0.01 mmol, Strem Chemicals). The resulting mixture was protected from light with aluminum foil and heated at 60°C overnight. Dilution with n-hexane and filtration of the white precipitate over a short pad of Florisil® (rinsed with pentane then pentane/diethyl ether: 98/2), evaporation to dryness under vacuum afforded the desired (*E*)-vinyl-boronate **32**. The resulting crude residue was used for the next step without further purification. $\mathbf{R}_f$ = 0.32 (pentane/diethyl ether: 98/2). **^{1}H NMR (300 MHz, $CDCl_3$):** δ 6.97 (dd, *J* = 17.7 Hz and *J* = 10.5 Hz, 1H, H6), 6.18 (br ddt, *J* = 15.3 Hz, *J* = 10.5 Hz and *J* = 1.0 Hz, 1H, H5), 5.81 (br ddt, *J* = 15.3 Hz, *J* = 6.2 Hz and *J* = 0.5 Hz, 1H, H4), 5.49 (d, *J* = 17.7 Hz, 1H, H7), 4.09-4.00 (m, 1H CHOSi), 1.60-1.45 (m, 2H, CH2), 1.25 (s, 12H, 4 x Me in pinacol), 0.88 (s, 9H, tBu), 0.83 (t, *J* = 7.80 Hz, 3H, $CH3_{alphaCH2}$), 0.01 (s, 3H, CH3Si), -0.03 (s, 3H, CH3Si) ppm. **ESI-MS:** Too unstable - Not succeeded with both positive and negative mode.

In vitro assessment of linotrins anti-inflammatory activity in LPS-challenged microglial cells.

Cell Culture and Treatments. The commonly used BV-2 microglia cell lines are C57/BL6 murine microglial cells immortalized by the v-raf/v-myc oncogene carrying J2 retrovirus[68]. These lines were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) (4.5 g/L glucose, 4 mmol/L L-alanyl-glutamine, supplemented with 5 % fetal bovine serum (FBS, Sigma-Aldrich) and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin, Gibco) as previously described[69]. Briefly, BV-2 cells were seeded in a complete DMEM medium in 12-well plates at a density $2x10^5$ cells/well, and the following day the medium was replaced by a serum-free medium to synchronize/stop their proliferation during 3 hours. BV2 cells were then pretreated for 3 h with 30 µmol/L 9(*R*),16(*RS*)-linotrin (LiNO 9*R*,16*RS*) or 100 µmol/L ALA dispersed in 0.5 % EtOH or

with a 0.5% EtOH solution alone (vehicle) before the application of 1 µg/mL LPS from *E. coli* 0111:B4 (Sigma-Aldrich) freshly dissolved in PBS for 16 hours[69]. The supernatants and cell contents were then collected and used subsequently to assess the levels of cytokines.

Determination of Proinflammatory Cytokine Expression.

***At the transcription level: isolation of RNA and qRT-PCR.** Total RNA was extracted using RNAzol-RT (Euromedex) and TURBO DNAse (Invitrogen)-treated RNA was used for reverse transcription using 200U/µL Superscript™ IV Reverse transcriptase (Invitrogen) in a final reaction volume of 20 µL containing 2 µg of total RNA, 2.5 µmol/L oligo d(T)20 primers (Eurogentec), 0.5 mmol/L desoxyribonucleotide triphosphate (dNTP) mix (Promega), and 5 mmol/L dithiothreitol (DTT) (Invitrogen). The reaction was incubated for 5 min at 65°C, then for 10 min at 50°C, and was finally inactivated by heating at 80°C for 10 min.

The synthesized first-strand cDNA was amplified by qRT-PCR using SYBR Green I Master Mix (Roche Diagnostics) and detected with a LightCycler(R) 480 sequence detector (Roche Diagnostics) in a final reaction volume of 20 µL, containing 7 µL ddH_2O, 1 µL of each primer (0,5 uM), 2 µL of cDNA template and 10 µL of 2 × SYBRgreen I Master Mix (Roche Diagnostics). The qRT-PCR was performed as described previously[69]: pre-incubation at 95◦C for 5 min followed by 45 cycles of denaturation at 95°C for 10 s and annealing at 60°C for 10 s and elongation at 72°C for 10 s. *GAPDH* was used as a reference gene for normalization. The following sequences of primers (Eurogentec) were used: mIL-1β (forward 5´-TGGTGTGTGACGTTCCCATT-3´, reverse 5´-CAGCACGAGGCTTTTTTGTTG-3´), mTNF-α (forward 5´-CATCTTCTCAAAATTCGAGTGACAA-3´, reverse 5´-TGGGAGTAGACAAGGTACAACCC-3´), and mGAPDH (forward 5´-CCAGTGAGCTTCCCGTTCA-3´, reverse GAACATCATCCCTGCATCC-3´).

***At the expression and secretion level.** The expression of TNF-α, IL6, and IL-1β was determined by testing cell contents and supernatants, using AlphaLISA technology and mTNF-α (AL505C), mIL6 (AL503F), and mIL1β (AL503C) kits (Perkin-Elmer). The total quantities of cytokines produced were calculated and normalized to the amounts of total proteins determined by Bradford protein assay (Bio-Rad).

Statistical Analysis. Statistical analysis was carried out using GraphPad Prism 6.00 software. Data were expressed as the mean±SEM. Statistical analyses of differences between groups were performed using the non-parametric ($n < 30$) Mann-Whitney (for comparisons of 2 groups), and Kruskal-Wallis analysis, together with Dunn's post-hoc test (for more than 2 groups). Values of p less than 0.05 were considered statistically significant.

Analysis of Wheatgrass Leaves (*Triticum aestivum*).

Plant Growth Methods. Wheatgrass variety 'Kaskaskia' seeds were planted 4 cm deep in 380 cm^3 pots containing a 1:1:1 soil mixture of soil, peat, and sand. Pots were placed in a growth chamber with a light intensity of 500 µmol/m^2/sec and day/night periods of 16 h light:8 h dark at temperatures of 28ºC day/22ºC night, and plants were watered daily. Ten days after planting, the first leaf (approximately 200 mg tissue) of each wheat plant was collected, frozen in liquid nitrogen, and tissue was stored at -80°C. Frozen tissue was lyophilized before extraction and further LC-MS analysis as described below.

Lipidomic Analysis and Quantification. LC-MS/MS analyses of ALA metabolites were performed as described[70] for 9(*S*)-hydroxy-10(*E*),12(*Z*),15(*Z*)-octadecatrienoic acid (9-HOTE) and 13(*S*)-hydroxy-9(*Z*),11(*E*),15(*Z*)-octadecatrienoic acid (13(*S*)-HOTE) (purchased from CAYMAN). Briefly, lipid mediators were separated on a ZorBAX SB-C18 column (2.1 mm, 50

mm, 1.8 µm) (Agilent Technologies) maintained at 40 ºC. The mobile phases consisted of water, acetonitrile, and FA (75/25/0.1, v/v/v) (A) and acetonitrile, FA (100/0.1, v/v) (B). The linear gradient was as follows: 0% B at 0 min, 85% B at 8.5 min, 100% B at 9.5 min, 100% B at 10.5 min, and 0% B at 12 min. The flow rate was 0.35 mL/min. The autosampler was set at 5 ºC and the injection volume was 5 µL. The HPLC system was coupled online to an Agilent 6460 triple quadrupole MS (Agilent Technologies) equipped with an electrospray ionization source. Electrospray ionization (ESI) was performed in negative ion mode. After optimization, the source parameters used were as follows: source temperature was set at 325 °C, nebulizer gas (nitrogen) flow rate was 10 L/min, sheath gas temperature was 400 ºC, sheath gas (nitrogen) flow rate was 12 L/min and the spray voltage was adjusted to −3500 V.

Data were acquired in Multiple Reaction Monitoring (MRM) mode with optimized conditions (ion optics and collision energy). Peak detection, integration, and quantitative analysis were done using Mass Hunter Quantitative analysis software (Agilent Technologies), according to our previous[70] procedure.

The parameters for the detection of Linotrins were determined using Optimizer software (Agilent Technologies). Linotrins are detected in a double peak (with a retention time at 4.29 and 4.37min) for the 2 diastereoisomer *9(R), 16(S) and 9(R),16(R)* form with MRM transition: parent ion m/z = 309.2 ; product ion m/z = 291.1 conditioned by a fragmentor energy = 86 V and a collision energy = 10 eV. Linotrin quantification was obtained through external calibration curves in pg/µL and normalized (divided) by the weight of tissue (in mg) to obtain a quantity in pg/µg of tissue. The calibration curve was established with 10 successive dilutions by half in MeOH with concentrations of 1.95, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, and 500 ng/mL used to quantify linotrins with linear equation y= 1218.7 * x -1423.9 and a coefficient of determination r^2 =0.9997. The limit of detection (LOD) was 1.95 pg/µL injected, and the limit of quantification (LOQ) was 3.9 pg /µL injected.

ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via internet and shows:

- Chemical procedures for the synthesis of *E*,*Z*,*E*-linotrin targets (**1**, **2** and **34**), the *E*,*Z*,*E*-triene **30** and the *E*,*E*,*E*-triene **31**, and all their precursors.
- ^{1}H and ^{13}C NMR spectra.
- UV spectra for the trienes.
- Purity: HPLC chromatograms for linotrins **1** and **2**.
- Determination of the 9(*R*)- and 16(*S*)-configurations and measurement of the enantiomeric excess (ee).
- Procedure of extraction of wheatgrass leaves.
- Figure S1. Absolute quantification of 9(R)-linotrin diastereomers.
- Figure S2. Relative quantification of four linotrin isomers in area/mg of leaf.
- Figure S3. Relative quantification of mono-hydroxylated ALA metabolites 9-HOTrE and 13-HOTrE.

AUTHOR INFORMATION

Corresponding Author

*E-mail: laurence.balas@umontpellier.fr

Notes

The authors declare no competing financial interest. All authors have approved the final article.

ACKNOWLEDGMENTS

This work was supported by the Centre National de la Recherche Scientifique (CNRS) and the French Government for the "Investments for the Future LabEx ICST #ANR-11 LabEx 0015". S.K.D. thanks the Fondation Infectiopole Sud for his post-doctoral fellowship. We are greatly indebted to Luc Brunel (SynBio3 platform, Montpellier) for technical assistance regarding the demanding purification of Linotrins.

ABBREVIATIONS

ALA, alpha-linolenic acid (C18:3, n-3); br., broad signal; Cat., catalytic amount; CBS, Corey-Bakshi-Shibata oxazaborolidine; dba, dibenzylideneacetone; ddH_2O, double distilled water; DIPA, diisopropylamine; IL, Interleukin; imid., imidazole; LINO, Linotrin; LPS, lipopolysaccharide; MRM, multiple reaction monitoring; NIS, *N*-iodosuccinimide; NBS, *N*-bromosuccinimide; pin, pinacol group; quat, quaternary; THF, tetrahydrofuran; veh., vehicle; Xphos, 2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl – CAS number: 564483-18-7.

REFERENCES

[1] J.M. Bourre, O. Dumont, M. Piciotti, M. Clement, J. Chaudiere, M. Bonneil, G. Nalbone, H. Lafont, G. Pascal, G. Durand, Essentiality of omega 3 fatty acids for brain structure and function, World Rev. Nutr. Diet, 66 (1991) 103-117.
[2] N. Blondeau, The nutraceutical potential of omega-3 alpha-linolenic acid in reducing the consequences of stroke, Biochimie, 120 (2016) 49-55.
[3] G. Barcelo-Coblijn, E.J. Murphy, Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: benefits for human health and a role in maintaining tissue n-3 fatty acid levels, Prog. Lipid Res., 48 (2009) 355-374.
[4] J. de Goede, W.M.M. Verschuren, J.M.A. Boer, D. Kromhout, J.M. Geleijnse, Alpha-linolenic acid intake and 10-year incidence of coronary heart disease and stroke in 20,000 middle-aged men and women in the Netherlands, PLOS ONE, 6 (2011) e17967.
[5] C.N. Serhan, Pro-resolving lipid mediators are leads for resolution physiology, Nature, 510 (2014) 92-101.
[6] C.N. Serhan, N. Chiang, T.E. Van Dyke, Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators, Nat. Rev. Immunol., 8 (2008) 349-361.
[7] M. Bourourou, E. Gouix, N. Melis, J. Friard, C. Heurteaux, M. Tauc, N. Blondeau, Inhibition of eIF5A hypusination pathway as a new pharmacological target for stroke therapy, J. Cereb. Blood Flow Metab., 0 (2020) 0271678X20928882.
[8] A.D. Dobrian, D.C. Lieb, B.K. Cole, D.A. Taylor-Fishwick, S.K. Chakrabarti, J.L. Nadler, Functional and pathological roles of the 12- and 15-lipoxygenases, Prog. Lipid Res., 50 (2011) 115-131.
[9] M. Liu, P. Chen, E. Vericel, M. Lelli, L. Beguin, M. Lagarde, M. Guichardant, Characterization and biological effects of di-hydroxylated compounds deriving from the lipoxygenation of ALA, J. Lipid Res., 54 (2013) 2083-2094.
[10] L. Balas, T. Durand, Dihydroxylated E,E,Z-docosatrienes. An overview of their synthesis and biological significance, Prog. Lipid Res., 61 (2016) 1-18.
[11] Y.-J. Jian, C.-J. Tang, Y. Wu, Enantioselective total synthesis of Phomallenic acid C, J. Org. Chem., 72 (2007) 4851-4855.
[12] M. Yoshida, M. Al-Amin, K. Shishido, Total syntheses of phomallenic Acids B and C utilizing palladium-catalyzed coupling of propargylic tosylates with terminal alkynes, Synthesis, DOI 10.1055/s-2008-1032116(2008) 1099-1105.
[13] S. Zhu, Y. Wu, Synthesis of the anti-melanogenic glycerol fatty acid ester isolated from the tuber-barks of Colocasia antiquorum var. esculenta, Synlett, 25 (2014) 261-264.
[14] E.J. Corey, C.J. Helal, Reduction of carbonyl compounds with chiral oxazaborolidine catalysts: A new paradigm for enantioselective catalysis and a powerful new synthetic method, Angew. Chem. Int. Ed., 37 (1998) 1986-2012.
[15] I. Chataigner, J. Lebreton, D. Durand, A. Guingant, J. Villiéras, A new approach for the determination of the absolute configuration of secondary alcohols by 1H NMR with O-substituted mandelate derivatives, Tetrahedron Lett., 39 (1998) 1759-1762.
[16] J. Schwartz, J.A. Labinger, Hydrozirconation: A new transition metal reagent for organic synthesis, Angew. Chem. Int. Ed., 15 (1976) 333-340.
[17] P. Wipf, H. Jahn, Synthetic applications of organochlorozirconocene complexes, Tetrahedron, 52 (1996) 12853-12910.
[18] A. Fürstner, D. De Souza, L. Turet, M.D.B. Fenster, L. Parra-Rapado, C. Wirtz, R. Mynott, C.W. Lehmann, Total syntheses of the actin-binding macrolides Latrunculin A, B, C, M, S and 16-epi-Latrunculin B, Chem. Eur. J. , 13 (2007) 115-134.
[19] Z. Huang, E.-i. Negishi, A convenient and genuine equivalent to HZrCp2Cl generated in situ from ZrCp2Cl2−DIBAL-H, Org. Lett., 8 (2006) 3675-3678.
[20] A. Hamze, O. Provot, J.-D. Brion, M. Alami, Xphos ligand and platinum catalysts: A versatile catalyst for the synthesis of functionalized β-(E)-vinylsilanes from terminal alkynes, J. Organomet. Chem., 693 (2008) 2789-2797.
[21] D.P. Stamos, A.G. Taylor, Y. Kishi, A mild preparation of vinyliodides from vinylsilanes, Tetrahedron Lett., 37 (1996) 8647-8650.

[22] E.A. Ilardi, C.E. Stivala, A. Zakarian, Hexafluoroisopropanol as a unique solvent for stereoselective iododesilylation of vinylsilanes, Org. Lett., 10 (2008) 1727-1730.
[23] M. Sidera, A.M. Costa, J. Vilarrasa, Iododesilylation of TIPS-, TBDPS-, and TBS-substituted alkenes in connection with the synthesis of Amphidinolides B/D, Org. Lett., 13 (2011) 4934-4937.
[24] N.D. Smith, J. Mancuso, M. Lautens, Metal-catalyzed hydrostannations, Chem. Rev., 100 (2000) 3257-3282.
[25] J.K. Stille, The Palladium-catalyzed cross-coupling reactions of organotin reagents with organic electrophiles [new synthetic methods (58)], Angew. Chem. Int. Ed., 25 (1986) 508-524.
[26] H. Hofmeister, K. Annen, H. Laurent, R. Wiechert, A novel entry to 17α-bromo- and 17α-iodoethynyl steroids, Angew. Chem. Int. Ed., 23 (1984) 727-729.
[27] H.X. Zhang, F. Guibe, G. Balavoine, Palladium- and molybdenum-catalyzed hydrostannation of alkynes. A novel access to regio- and stereodefined vinylstannanes, J. Org. Chem., 55 (1990) 1857-1867.
[28] A. Darwish, J.M. Chong, Synthesis of E-vinyl iodides via Pd-catalyzed hydrostannation of terminal alkynes, Tetrahedron, 68 (2012) 654-658.
[29] P. Ghosh, S.D. Lotesta, L.J. Williams, Spirodiepoxide reaction with cuprates, J. Am. Chem. Soc., 129 (2007) 2438-2439.
[30] V.R. Krishnamurthy, A. Dougherty, C.A. Haller, E.L. Chaikof, Total synthesis and bioactivity of 18(R)-hydroxyeicosapentaenoic acid, J. Org. Chem., 76 (2011) 5433-5437.
[31] F. Ungeheuer, A. Fürstner, Concise total synthesis of Ivorenolide B, Chem. Eur. J. , 21 (2015) 11387-11392.
[32] K. Sonogashira, Y. Tohda, N. Hagihara, A convenient synthesis of acetylenes: catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes and bromopyridines, Tetrahedron Lett., 16 (1975) 4467-4470.
[33] R. Chinchilla, C. Najera, The Sonogashira reaction: a booming methodology in synthetic organic chemistry, Chem. Rev., 107 (2007) 874-922.
[34] R. Chinchilla, C. Nájera, Recent advances in Sonogashira reactions, Chem. Soc. Rev., 40 (2011) 5084-5121.
[35] J. Bhattacharjee, A. Harinath, K. Bano, T.K. Panda, Highly chemoselective hydroboration of alkynes and nitriles catalyzed by group 4 metal amidophosphine–borane complexes, ACS Omega, 5 (2020) 1595-1606; and herein references.
[36] S. Pereira, M. Srebnik, A study of hydroboration of alkenes and alkynes with pinacolborane catalyzed by transition metals, Tetrahedron Lett., 37 (1996) 3283-3286.
[37] B.M. Trost, Z.T. Ball, Addition of metalloid hydrides to alkynes: hydrometallation with boron, silicon, and tin, Synthesis, 36 (2005) 853–887.
[38] N.W.J. Ang, C.S. Buettner, S. Docherty, A. Bismuto, J.R. Carney, J.H. Docherty, M.J. Cowley, S.P. Thomas, Borane-catalysed hydroboration of alkynes and alkenes, Synthesis, 50 (2018) 803-808; and herein references.
[39] S. Mandal, S. Mandal, K. Geetharani, Zinc-catalysed cydroboration of terminal and internal alkynes, Chem. Asian J. , 14 (2019) 4553-4556.
[40] Y. Wang, R. Guan, P. Sivaguru, X. Cong, X. Bi, Silver-catalyzed anti-Markovnikov hydroboration of C–C multiple bonds, Org. Lett., 21 (2019) 4035-4038.
[41] T. Ohmura, Y. Yamamoto, N. Miyaura, Rhodium- or Iridium-catalyzed trans-hydroboration of terminal alkynes, giving (Z)-1-alkenylboron compounds, J. Am. Chem. Soc., 122 (2000) 4990-4991.
[42] J. Cid, J.J. Carbó, E. Fernández, Catalytic non-conventional trans-hydroboration: A theoretical and experimental perspective, Chem. Eur. J. , 18 (2012) 1512-1521.
[43] K. Shirakawa, A. Arase, M. Hoshi, Preparation of (E)-1-alkenylboronic acid pinacol esters via transfer of alkenyl group from boron to boron, Synthesis, 36 (2005) 1814–1820.
[44] L. Deloux, M. Srebnik, Preparation of (Z)-1-alkenyl dioxaborolanes by hydrolysis of boryl zirconocene 1,1-dimetalloalkenes, J. Org. Chem., 59 (1994) 6871-6873.
[45] G.A. Molander, N.M. Ellis, Highly stereoselective synthesis of cis-alkenyl pinacolboronates and potassium cis-alkenyltrifluoroborates via a hydroboration/ protodeboronation approach, J. Org. Chem., 73 (2008) 6841-6844.
[46] H.C. Brown, N.G. Bhat, M. Srebnik, A simple, general synthesis of 1-alkynyldiisopropoxyboranes, Tetrahedron Lett., 29 (1988) 2631-2634.

[47] T. León, E. Fernández, The Pauson–Khand reaction using alkynylboronic esters: solving a long-standing regioselectivity issue, Chem. Commun., 52 (2016) 9363-9366.
[48] J.-R. Hu, L.-H. Liu, X. Hu, H.-D. Ye, Ag(I)-catalyzed C–H borylation of terminal alkynes, Tetrahedron, 70 (2014) 5815-5819.
[49] S. Kotha, K. Lahiri, D. Kashinath, Recent applications of the Suzuki–Miyaura cross-coupling reaction in organic synthesis, Tetrahedron, 58 (2002) 9633-9695.
[50] A. Suzuki, Cross-coupling reactions of organoboranes: An easy way To construct C□C bonds (Nobel Lecture), Angew. Chem. Int. Ed., 50 (2011) 6722-6737.
[51] C.C.C. Johansson Seechurn, M.O. Kitching, T.J. Colacot, V. Snieckus, Palladium-catalyzed cross-coupling: A historical contextual perspective to the 2010 Nobel Prize, Angew. Chem. Int. Ed., 51 (2012) 5062-5085.
[52] S. Pereira, M. Srebnik, Hydroboration of alkynes with pinacolborane catalyzed by HZrCp2Cl, Organometallics, 14 (1995) 3127-3128.
[53] Y.D. Wang, G. Kimball, A.S. Prashad, Y. Wang, Zr-Mediated hydroboration: stereoselective synthesis of vinyl boronic esters, Tetrahedron Lett., 46 (2005) 8777-8780.
[54] C. Nguemeni, E. Gouix, M. Bourourou, C. Heurteaux, N. Blondeau, Alpha-linolenic acid: A promising nutraceutical for the prevention of stroke, PharmaNutrition, 1 (2013) 1-8.
[55] S. Hickman, S. Izzy, P. Sen, L. Morsett, J. El Khoury, Microglia in neurodegeneration, Nat. Neurosci., 21 (2018) 1359-1369.
[56] S.M. Allan, N.J. Rothwell, Cytokines and acute neurodegeneration, Nat. Rev. Neurosci., 2 (2001) 734-744.
[57] X. Hu, R.K. Leak, Y. Shi, J. Suenaga, Y. Gao, P. Zheng, J. Chen, Microglial and macrophage polarization-new prospects for brain repair, Nature reviews. Neurology, 11 (2015) 56-64.
[58] C.C. Chao, S.X. Hu, L. Ehrlich, P.K. Peterson, Interleukin-1 and tumor necrosis factor-α synergistically mediate neurotoxicity: Involvement of nitric oxide and of N-methyl-D-aspartate receptors, Brain Behav. Immun., 9 (1995) 355-365.
[59] Y. Nakamura, Q.S. Si, K. Kataoka, Lipopolysaccharide-induced microglial activation in culture: temporal profiles of morphological change and release of cytokines and nitric oxide, Neurosci. Res., 35 (1999) 95-100.
[60] N.G. Bazan, Neuroprotectin D1-mediated anti-inflammatory and survival signaling in stroke, retinal degenerations, and Alzheimer's disease, J. Lipid Res., 50 (2009) S400-S405.
[61] A.P. Simopoulos, Omega-3 fatty acids in wild plants, nuts and seeds, Asia Pac. J. Clin. Nutr., 11 (2002) S163-S173.
[62] R.P. Poincelot, Lipid and Fatty Acid Composition of Chloroplast Envelope Membranes from Species with Differing Net Photosynthesis, Plant Physiol., 58 (1976) 595-598.
[63] G. Bar-Sela, M. Cohen, E. Ben-Arye, R. Epelbaum, The medical use of wheatgrass: review of the gap between basic and clinical applications, Mini Rev. Med. Chem., 15 (2015) 1002-1010.
[64] H.E. Gottlieb, V. Kotlyar, A. Nudelman, NMR chemical shifts of common laboratory solvents as trace impurities, J. Org. Chem., 62 (1997) 7512-7515.
[65] T.C. McIlvaine, A buffer solution for colorimetric comparison, J. Biol. Chem., 4 (1921) 183-186.
[66] H. Fuwa, T. Suzuki, H. Kubo, T. Yamori, M. Sasaki, Total synthesis and biological assessment of (−)-Exiguolide and analogues, Chem. Eur. J. , 17 (2011) 2678-2688.
[67] H. Fuwa, K. Mizunuma, M. Sasaki, T. Suzuki, H. Kubo, Total synthesis and biological evaluation of (−)-exiguolide analogues: importance of the macrocyclic backbone, Org. Biomol. Chem., 11 (2013) 3442-3450.
[68] E. Blasi, R. Barluzzi, V. Bocchini, R. Mazzolla, F. Bistoni, Immortalization of murine microglial cells by a v-raf / v-myc carrying retrovirus, J. Neuroimmun., 27 (1990) 229-237.
[69] V. Leláková, S. Béraud-Dufour, J. Hošek, K. Šmejkal, V. Prachyawarakorn, P. Pailee, C. Widmann, J. Václavík, T. Coppola, J. Mazella, N. Blondeau, C. Heurteaux, Therapeutic potential of prenylated stilbenoid macasiamenene F through its anti-inflammatory and cytoprotective effects on LPS-challenged monocytes and microglia, J. Ethnopharmacol., 263 (2020) 113147.
[70] P. Le Faouder, V. Baillif, I. Spreadbury, J.-P. Motta, P. Rousset, G. Chêne, C. Guigné, F. Tercé, S. Vanner, N. Vergnolle, J. Bertrand-Michel, M. Dubourdeau, N. Cenac, LC–MS/MS method for rapid and concomitant quantification of pro-inflammatory and pro-resolving polyunsaturated fatty acid metabolites, J. Chrom. B, 932 (2013) 123-133.