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First total synthesis of labeled EPA and DHA-derived A-type cyclopentenone isoprostanoids: [D_2]-15-A_{3t}-IsoP and [D_2]-17-A_{4t}-NeuroP

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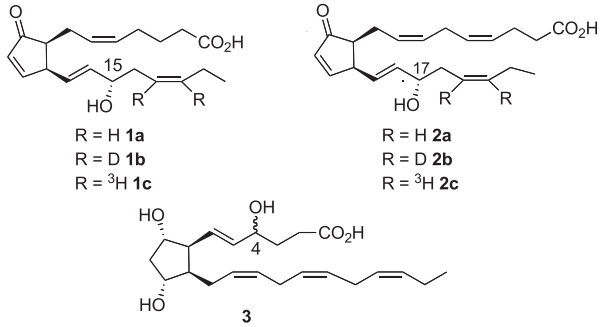
ABSTRACT

A-type cyclopentenone isoprostanoids are abundantly formed in vivo by radical peroxidation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are consumed daily for the prevention of cardiovascular and neurological pathologies. To facilitate in depth studies concerning the effects of these oxidized isoprostanoids on human health, labeled derivatives are necessary. In this paper, we have accomplished the first total synthesis of labeled A-type cyclopentenone isoprostanoids, namely 17,18-[D_2]-15-A_{3t}-IsoP and 19,20-[D_2]-17-A_{4t}-NeuroP. The two enantioselective routes are highly convergent, stemming from a common intermediate, readily available by a Julia–Kocienski reaction, and feature the semihydrogenation of an alkyne moiety for the installation of the labeled lower side chain.

1. Introduction

An appropriate dietary supply of eicosapentaenoic acid [EPA, C20:5 ω-3] and docosahexaenoic acid [DHA, C22:6 ω-3], occurring mainly in fish-oils, is essential to humans, who are unable to produce adequate levels of them. In fact, DHA, which is the most abundant polyunsaturated fatty acid esterified in neuronal plasma membrane phospholipids,[1,2] is essential for the development and normal functioning of the human central nervous system (CNS), and the susteinance of cognitive health.[1] Moreover, considerable evidence suggests that dietary consumption of these ω-3 fatty acids have beneficial effects in the primary and secondary prevention of cardiovascular pathologies, including atherosclerosis, myocardial infarction, hypertension, as well as other chronic disorders having an inflammatory or degenerative pathogenesis, such as, for example, rheumatoid arthritis, Alzheimer's disease (AD), macular degeneration, and a few types of cancer. It is noteworthy that most of these pathologies are believed to be strictly associated to the so-called oxidative stress and the consequent in vivo oxidative damage to biomolecules, including membrane lipids.

A few years ago, Nourooz-Zadeh and collaborators, and Morrow and collaborators, independently demonstrated that massive peroxidation of EPA and DHA, caused by reactive oxygen species (ROS), occurred in vitro as well in vivo in settings of oxidative stress.[2–4] A cascade of non-enzymatic free radical reactions is thus promoted, producing a large amount of different cyclopentanoid derivatives, namely isoprostanes (ISOPs) from EPA and neuroprostanes (NeuroPs) from DHA, which are structurally related to prostaglandins. However, unlike the trans-disubstituted PGs, the largest majority of IsoPs and NeuroPs shows cis-oriented side chains on cyclopentane ring, as exemplified by the structures of representative examples **1a**, **2a**, and **3** (Scheme 1).



Scheme 1. Structures of compounds 15-A_{3t}-IsoP (**1a**). 17,18-[D_2]-15-A_{3t}-IsoP (**1b**), 17,18-[$^3H_2$]-15-A_{3t}-IsoP (**1c**), 17-A_{4t}-NeuroP (**2a**), 19,20-[D_2]-17-A_{4t}-NeuroP (**2b**), 19,20-[$^3H_2$]-17-A_{4t}-NeuroP (**2c**), and 4-(*R*,*S*)-F_{4t}-NeuroP (**3**).

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These compounds have been divided in classes according to the accepted prostaglandin (PG) nomenclature.[2,5]

Optimization of therapies based on ω-3 PUFAs-enriched food supplementation would thus require the knowledge of IsoP and NeuroP biological activities in vivo; above all, their effective health benefits and absence of harmful effects to humans must be firmly established.[6] Moreover, since DHA is highly concentrated in the brain, some authors have proposed NeuroPs and, in particular, F_4-NeuroPs, as potential effective markers of oxidative stress processes in various neurodegenerative disorders, including AD.[2,7]

Among the different classes of IsoPs and NeuroPs, we have been interested for years in the synthesis of A/J cyclopentenone prostanoids.[8] Actually, in brain samples of AD patients, in which oxidized DHA levels are significantly higher than in healthy control subjects,[9] the level of A_4/J_4-cyclopentenones was determined to be at least fivefold higher than other NeuroPs, including the highly abundant cerebral F_4-NeuroPs.[2,10] It was, therefore, suggested that the study of A_4/J_4-NeuroP biology could provide valuable insights into the pathophysiology of oxidant injury in the CNS and that quantification of A_4/J_4-NeuroPs or their derivatives might be a more sensitive indicator of DHA oxidation, and neural tissue oxidative stress, than other classes of NeuroPs.[4b]

In addition, in seminal studies, Morrow et al. clearly demonstrated that A_3/J_3-IsoPs as well as A_4/J_4-NeuroPs showed potent anti-inflammatory effects in murine macrophage cells stimulated with lipopolysaccharide (LPS), exerting this bioactivity through inhibition of the NF-κB signaling pathway.[11] Moreover, the vasoprotective potential of 15-A_{3t}-IsoP was underscored by the ability of this compound to block oxidized lipid accumulation, a crucial step in foam cell transformation and atherosclerotic plaque formation.[11b] Finally, in another study, Gao and collaborators demonstrated that EPA and DHA oxidation products induced Nrf2-directed gene expression, regulating detoxification of reactive oxygen species (ROS), by destabilizing the association between Keap1 and Cullin3.[12]

To gain further insight into the biology and in vivo biological activity of oxidized EPA and DHA-derived A-type cyclopentenones, and to study their distribution in human tissues and biological fluids, the synthesis of labeled A_3-IsoP and A_4-NeuroP derivatives is, therefore, mandatory.

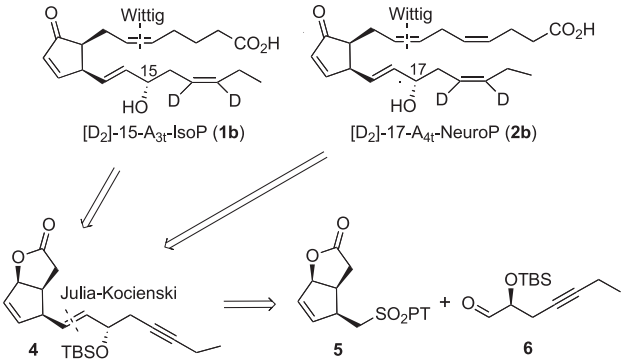
Herein, in connection with our ongoing synthetic program on IsoP and NeuroP derivatives, we report the first synthesis of a labeled A_3-IsoP, as well as of a labeled A_4-NeuroP, namely 17,18-[D_2]-15-A_{3t}-IsoP (**1b**) and 19,20-[D_2]-17-A_{4t}-IsoP (**2b**), respectively.[13,14]

2. Results and discussion

2.1. Synthesis of 17,18-[D_2]-15-A_{3t}-IsoP

The reason of choosing labeled compounds **1b** and **2b** as synthetic targets stemmed from our plan to generate both of them by a flexible convergent strategy and to introduce labeled atoms into the lower side chains of IsoPs and NeuroPs, as this would avoid the loss of the label as a result of acid β-oxidation.[14] An additional advantage in labeling the ω-3 double bond in **1b** and **2b**, was the possibility of using an alkyne derivative as the immediate precursor of the (*Z*)-double bond, thus delaying the labeling step onto an advanced synthetic intermediate. This strategy also permitted us to envisage the practical synthesis of the ditritiated analogues **1c** and **2c** by tritium hydrogenation of the triple bond.

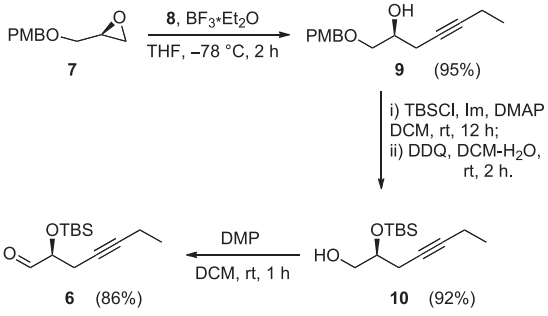
From a retrosynthetic point of view, we planned to introduce the labeling of both **1b** and **2b** by the regio- and stereoselective cis-deuteration reaction of alkyne **4** (Scheme 2), from which the upper chains of target compounds could subsequently be installed through two different prostaglandin-like Wittig olefination reactions.[8] In accordance with our previous work in the field,



Scheme 2. Retrosynthetic analysis of **1b** and **2b**. TBS=*tert*-butyldimethylsilyl; PT=phenyltetrazolyl.

protected allylic alcohol **4** could arise from Julia–Kocienski condensation between known enantiopure sulphone **5** and aldehyde **6** (Scheme 2).[8f]

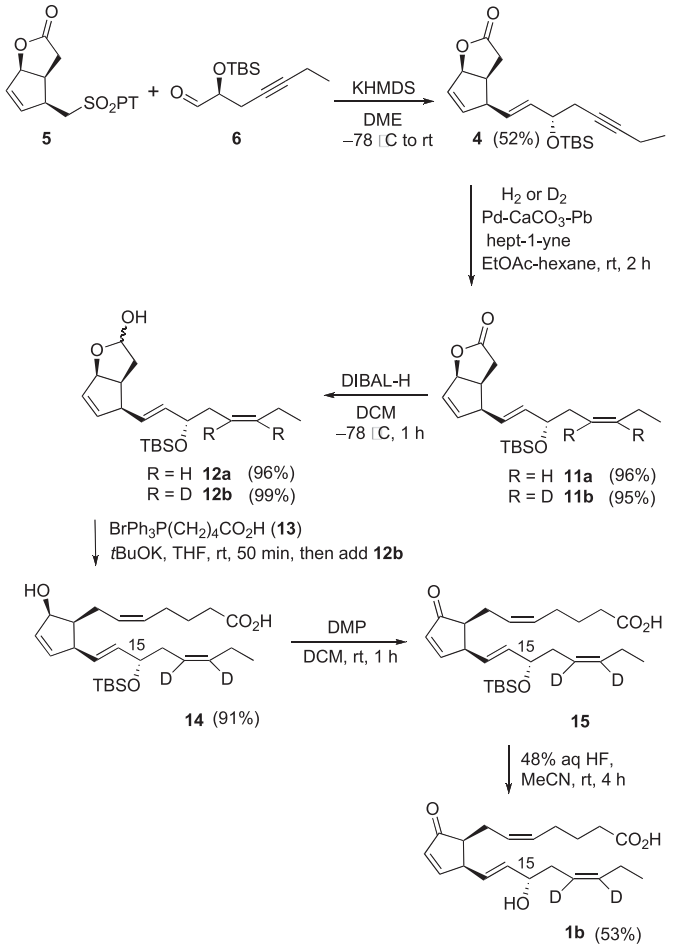
The synthesis of the required aldehyde **6** started from known (*S*)-glycidyl *p*-methoxybenzyl ether **7**,[15] which, upon exposure to the complex formed between lithium butylide **8** and $BF_3 \cdot Et_2O$, under the Yamaguchi conditions,[16] afforded the secondary alcohol **9** with complete regioselectivity in 95% yield (Scheme 3). Subsequent standard group manipulation in **9** readily gave primary alcohol **10** that was smoothly oxidized by the Dess–Martin periodinane (DMP) reagent[17] to aldehyde **6** in 86% yield. The subsequent Julia–Kocienski condensation between sulphone **5** and aldehyde **6** was performed according to the original conditions described by Kocienski for achieving *E*-olefins with high stereoselectivity.[18] As to the competitive enolization of the sulfone relative to the γ-lactone group, we have previously shown that kinetic factors govern the desired regioselective α-deprotonation of the former group, thus making the protection of the carbonyl moiety unnecessary.[8f] Under optimized conditions, sulfone **5** immediately delivered a yellow anion upon exposure to a slight excess of KHMDS in DME at −78 °C, and smoothly reacted with aldehyde **6**, affording the expected enyne **4** in 52% isolated yield (Scheme 4).



Scheme 3. Synthesis of aldehyde **6**. PMB=4-methoxybenzyl; Im=imidazole; DMAP=4-*N*,*N*-dimethylaminopyridine; DCM=dichloromethane; DDQ=2,3-dichloro-5,6-dicyano-*p*-benzoquinone; DMP=Dess–Martin periodinane.

After chromatographic purification, the ^{13}C NMR spectrum indicated compound **4** to be diastereomerically pure, while the vicinal coupling constant of 15.5 Hz between 13-H and 14-H in the corresponding ^1H NMR spectrum established the (*E*)-stereochemistry for the Δ^{13}-olefin, proving the excellent stereoselectivity of the Julia–Kocienski reaction.

Having secured a reliable and scalable access to the key intermediate **4**, the synthesis proceeded with the regio- and stereoselective cis-reduction of the alkyne functionality in **4** to give the desired diene **11a** with *E*,*Z*-configured double bonds in the side chain. After some experiments we found that hydrogenation of **4** in



Scheme 4. Synthesis of 17,18-[D_2]-15-A_{3t}-IsoP (**1b**). KHMDS=potassium hexamethyldisilyl amide; DME=dimethoxyethane; DIBAL-H=diisobutylaluminium hydride.

EtOAc/hexane, using Lindlar catalyst[19] (5% Pd/$CaCO_3$ poisoned with lead), in the presence of excess hex-1-ene, added as a hydrogenation competitive olefin, provided **11a** in quantitative yield. NMR spectroscopy analysis, as well as GC/MS (EI) analysis revealed that a chromatographically isolated sample was >98% pure, containing insignificant amounts of over-reduced products.

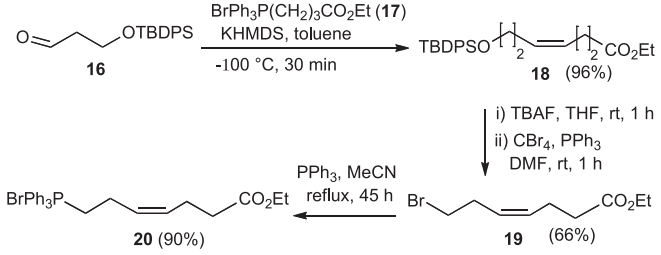
With this protocol in hand, switching H_2 gas for D_2 gas (≥99.9% pure), we then submitted alkyne **4** to the dideuteration reaction; however, the formed reduction product **11b** contained significant amounts of the dihydro derivative **11a**, possibly resulting from hydrogen-transfer from the added olefin. In fact, by substituting hex-1-ene with a large excess of hept-1-yne as a deuteration competitive substrate, uncontaminated dideuterated (*E*,*Z*)-diene **11b** was obtained in 95% yield after flash column chromatography purification. Comparison of the ^1H broad-band decoupled ^{13}C NMR spectra of **11a** and **11b** clearly showed the absence of protonated carbons C-17 and C-18 in the spectrum of **11b**, while the corresponding deuterium bonded olefinic carbons were shifted upfield of about 0.5 ppm (see the Experimental section), as expected.[20] On the basis of these results, deuterium-labeling of **11b** was estimated to be at least 95%.

Subsequent conversion of lactone **11b** to neuroprostane **1b**, was accomplished uneventfully by using standard prostaglandin chemistry,[8] submitting [D_2]-derivatives to reactions, which were previously optimized on unlabeled compounds. The absence of the signals for protonated carbons C-17 and C-18 in the ^{13}C NMR spectra of different deuterated intermediates indicated that the high percentage of D-labeling in **11b** remained unaffected until the end of the synthesis.

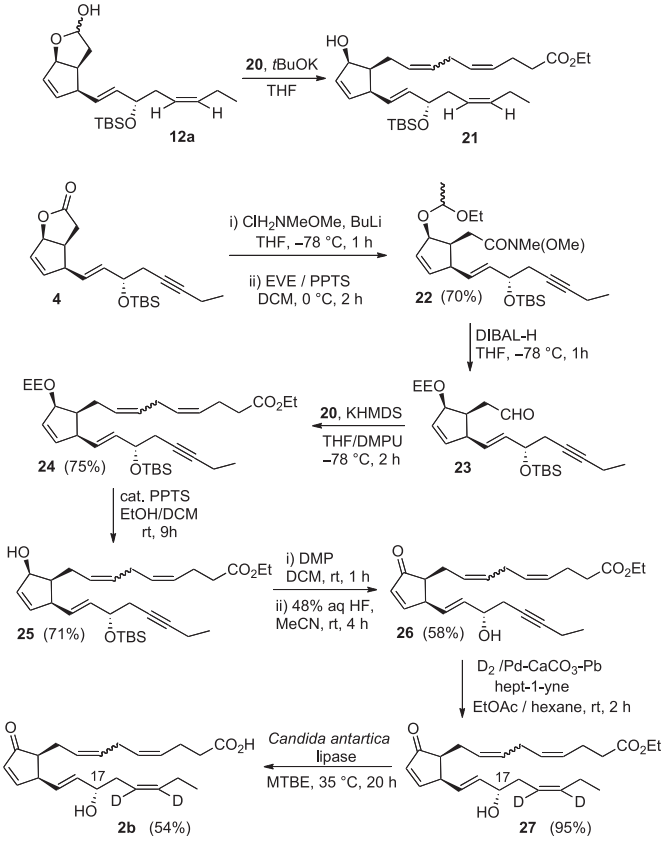
DIBAL-H reduction of **11b** provided lactol **12b**, as a mixture of anomers, in quantitative yield. Subsequent Wittig condensation with commercially available phosphonium salt **13** delivered the desired olefin **14** as a single *Z*-stereomer (^{13}C NMR) in 90% isolated yield. Finally, cyclopentenol Dess–Martin periodinane (DMP) oxidation,[17] followed by 48% aqueous HF cleavage of the *O*-TBS ether group, smoothly gave 15-A_{3t}-IsoP (**1b**) in 53% overall isolated yield, with complete conservation of the cis-stereochemistry at the labile stereocenters C-8 and C-12 (NOE experiments).

2.2. Synthesis of 19,20-[D_2]-17-A_{4t}-NeuroP

Contrary to our expectations, the preparation of 19,20-[D_2]-17-A_{4t}-NeuroP (**2b**) required a modification of the synthetic strategy developed for achieving **1b**, namely, through Wittig olefination of lactol **12b**. Actually, in an exploratory experiment, Wittig condensation of lactol **12a** with the ylide generated by deprotonation of phosphonium salt **20**,[21] readily prepared from known aldehyde **16**[22] (Scheme 5), afforded diene **21** with low (7*Z*)-stereoselectivity (7*Z*/7*E*, 4:1) (Scheme 6).



Scheme 5. Synthesis of phosphonium salt **20**. TBDPS=*tert*-butyldiphenylsilyl; TBAF=tetrabutylammonium fluoride; DMF=*N*,*N*-dimethylformamide.



Scheme 6. Synthesis of 19,20-[D_2]-15-A_{3t}-IsoP (**2b**). EE=1-ethoxyethyl; EVE=ethyl vinyl ether; PPTS=pyridinium *p*-toluenesulfonate; DMPU=1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone; MTBE=methyl *tert*-butyl ether.

To surmise this difficulty, we decided to construct the upper side chain of **2b** via Wittig olefination of *O*-EE-protected cyclopentenol-aldehyde **23**, which was smoothly achieved from lactone **4** via Weinreb amide **22** (Scheme 6). To maximize (*Z*)-stereoselectivity in the subsequent Wittig reaction, aldehyde **23** was then exposed to the ylide generated from salt **20**, in THF–DMPU at −78 °C.[23] Under this condition, tetraenyne **24** was produced in 56% overall yield from **4**, as a mixture 9:1 of 7*Z*/7*E* olefins.

Having assembled the entire carbon backbone of the target product, conversion of protected cyclopentenol **24** to neuroprostane **2b** required at first selective *O*-EE acetal deprotection, which was executed on treatment **24** with PPTS in CH_2Cl_2/EtOH to deliver allylic alcohol **25**. Subsequently, cyclopentenol DMP oxidation,[17] followed by *O*-TBS ether cleavage, readily afforded key enone **26** in 41% overall yield from **24**, with complete conservation of the cis-stereochemistry at the labile stereocenters C-10 and C-14 (NOE experiments).

Regioselective deuterium cis-addition to the alkyne moiety of tetraenyne **26** to afford 19,20-dideuterated ester **27** then proceeded uneventfully by using the same procedure previously optimized in the deuteration of lactone **4**. The ≥95% D-labeling of **27** was established by the procedure described for olefin **11b**. Finally, smooth ethyl ester cleavage catalyzed by polymer supported lipase from *Candida antartica*,[24] afforded [D_2]-17-A_{4t}-NeuroP (**2b**) in 54% yield. The spectra of **2b** were consistent with the structure.

3. Conclusion

In conclusion, we have accomplished the synthesis of 17,18-[D_2]-15-A_{3t}-IsoP (**1b**) and 19,20-[D_2]-17-A_{4t}-NeuroP (**2b**), thus achieving the first total synthesis of labeled A-type cyclopentenone derivatives formed by in vitro as well as in vivo radical-catalyzed peroxidation of the physiologically important ω-3 PUFAs EPA and DHA.

Our synthetic strategy features a stereoselective Julia–Kocienski olefination between enantiopure sulphone **5** and aldehyde **6** for the preparation of the key intermediate enyne **4**, and two highly regio- and stereoselective cis-deuteration reactions of alkynes **4** and **26**, by using Lindlar Pd-catalyst, to deliver the desired labeled compounds. We envision that the synthesis of the ditritiated analogues **1c** and **2c** could also be achieved by this standardized protocol.

The availability of labeled compounds will facilitate further in depth studies on the biology and in vivo biological activity of EPA and DHA-derived A-type cyclopentenones, as well as their distribution in human tissues and biological fluids. In particular, the role played by these molecules in the multi-faceted relationship between ROS oxidation of membrane lipids and oxidative stress-related diseases, is far for having been fully clarified. Indeed, further shedding light on the etiology of severe diseases, a more thorough comprehension of the mechanisms of action of DHA and EPA, as well as the oxidized lipids formed in vivo may lead to the development of targeted strategies that afford greater therapeutic potential for diets based on ω-3 fatty acids.

4. Experimental section

4.1. Instrumentation and chemicals

All reactions requiring anhydrous conditions were conducted in flame-dried glassware with magnetic stirring under a positive static atmosphere of argon. Syringes and needles for the transfer of reagents were dried at 140 °C and allowed to cool in a dessicator over $CaCl_2$ before use. THF, Et_2O were redistilled from sodium diphenyl ketyl; CH_2Cl_2 from CaH_2, toluene from Na/K. Other solvents and reagents were of commercial quality and used as obtained from supplier. Organic layers were dried using $MgSO_4$. Reactions were monitored by TLC, using pre-coated silica gel 60 (0.25 mm), aluminum-supported TLC plates. Visualization of reaction components was achieved by UV irradiation at a wavelength of 254 nm, or stained by exposure to a 0.5% solution of vanillin in H_2SO_4/EtOH, followed by gentle heating. Column chromatography was carried using silica gel 40–63 μm. Infrared spectra were reported as wavenumber (cm^{-1}) of significant peaks. Electrospray mass spectra (MS) were obtained by ionization methods. Melting points are uncorrected. ^1H NMR spectra were obtained at 300 MHz. The ^1H NMR spectra are reported as follows: chemical shift in parts per million (ppm) (multiplicity, coupling constant(s) *J* (Hz), relative integral) where multiplicity is defined as: br=broad, m=multiplet, s=singlet, d=doublet, t=triplet, q=quartet, qu=quintuplet, or combination thereof. Selected ^{13}C NMR spectra were determined at 75 MHz using a *J* modulated sequence and, where appropriate, the central peak of the $CDCl_3$ triplet (77.00 ppm), or the CD_2Cl_2 quintuplet (54.22 ppm), or the MeOH-d_4 septuplet (49.86 ppm), or the MeCN-d_3 singlet (117.3 ppm), was used as an internal reference. The assignments of NMR spectra were determined by DEPT experiments and are reported as follows: CH_3=q, CH_2=t, CH=d, CD=t, and C=s.

4.2. Synthetic procedures

*4.2.1. (S)-1-(4-Methoxybenzyloxy)hept-4-yn-2-ol (**9**).* 1-Butyne gas was condensed in a flask cooled at −30 °C under an Ar atmosphere and dry THF was added to give a 0.25 M solution, 4.0 mL of which (1.0 mmol) were then transferred to another flask cooled to −78 °C. 1.6 M BuLi in hexane (1.25 mL, 2.0 mmol, 2 equiv) was added and the solution was stirred at −30 °C for 30 min. Subsequently, $BF_3{\cdot}Et_2O$ (2.0 mmol, 2 equiv) was added and, after 10 min, the solution was cooled to −78 °C and epoxide **7** (100 mg, 0.515 mmol) in dry THF (0.75 mL) was added. After 2 h of stirring, Et_2O (20 mL) and saturated aqueous NH_4Cl (20 mL) were added, the two layers were separated, and the aqueous phase was extracted with Et_2O (3×25 mL). The combined organic phases were dried ($MgSO_4$) and evaporated in vacuo. The resulting residue was purified by flash column chromatography on silica gel. Elution with hexane/EtOAc, 4:1, gave alcohol **9** (122 mg, 95%) as a colorless oil, TLC R_f (EtOAc/hexane 15:85) 0.35; $[\alpha]_D^{20}$ +9.3 (*c* 1.3, CH_2Cl_2); ν_{max} (liquid film) 3430 (br), 2975, 1613, 1514, 1248, 1103, 1035 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.24 (2H, d, *J* 7.9 Hz), 6.80 (2H, d, *J* 7.9 Hz), 4.53 (2H, s), 3.96–3.82 (1H, m), 3.79 (3H, s), 3.55 (1H, dd, *J* 9.5, 3.5 Hz), 3.47 (1H, dd, *J* 9.5, 6.7 Hz), 2.72 (1H, br, O*H*), 2.44–2.32 (2H, m), 2.20–2.10 (2H, m), 1.09 (3H, t, *J* 7.1 Hz); δ_C (75 MHz, $CDCl_3$) 159.2 (s), 130.0 (s), 129.3 (2×d), 113.7 (2×d), 84.0 (s), 74.8 (s), 72.9 (t), 72.6 (t), 69.0 (d), 55.1 (q), 23.7 (t), 14.0 (q), 12.3 (t); HRMS (ESI^+): MH^+, found 249.1492. $C_{15}H_{20}O_3$ requires 249.1491.

*4.2.2. (S)-2-(tert-Butyldimethylsilyloxy)hept-4-ynal (**6**).* DMP (187 mg, 1.2 equiv) was added to alcohol **10** (90.2 mg, 0.369 mmol) in CH_2Cl_2 (4 mL), obtained from **9** by a standard protocol. After stirring the solution at room temperature for 1 h, a saturated aqueous solution of $Na_2S_2O_3$ (5 mL) was added, the two layers were separated, and the aqueous phase was extracted with CH_2Cl_2 (3×5 mL). The combined organic phases were dried ($MgSO_4$) and evaporated in vacuo. The resulting residue was purified by flash column chromatography on silica gel. Elution with hexane/EtOAc, 9:1, gave aldehyde **6** (77.8 mg, 86%) as a colorless oil, TLC R_f (EtOAc/hexane 10:90) 0.31; $[\alpha]_D^{20}$ −14.2 (*c* 0.8, CH_2Cl_2); ν_{max} (liquid film) 2955, 2930, 1741, 1254, 839, 780 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 9.61 (1H, d, *J* 1.4 Hz), 4.08 (1H, td, *J* 6.0, 1.4 Hz), 2.65–2.37 (2H, m), 2.25–2.08 (2H, m), 1.10 (3H, t, *J* 7.2 Hz), 0.98 (3×3H, s), 0.09 (2×3H, s); δ_C (75 MHz, $CDCl_3$) 202.2 (d), 84.2 (s), 76.3 (d), 74.0 (s), 25.4

(3×q), 23.3 (t), 18.1 (s), 13.8 (q), 12.3 (t), −4.9 (q), −5.0 (q); HRMS (ESI$^+$): MH$^+$, found 241.1620. $C_{13}H_{25}O_2Si$ requires 241.1624.

*4.2.3. (3aS,4R,6aR)-4-[(S,E)-3-(tert-Butyldimethylsilyloxy)oct-1-en-5-ynyl]-3,3a,4,6a-tetrahydro-2H-cyclopenta[b]furan-2-one (**4**).* KHMDS (0.66 M in toluene, 1.58 mL, 1.04 mmol, 1.14 equiv) was added to a stirred solution of sulfone **5**[8f] (315 mg, 0.91 mmol, 1 equiv) in dry DME (10 mL) under Ar at −78 °C. After 50 min a solution of aldehyde **6** (262 mg, 1.09 mmol, 1.2 equiv) in dry DME (5 mL) was added and the reaction mixture was allowed to warm to room temperature for 2 h, then a saturated solution of NH_4Cl (30 mL) and Et_2O (10 mL) was added. The layers were separated and the aqueous phase was extracted with Et_2O (3×50 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The resulting residue was purified by flash chromatography on silica gel. Elution with hexane/EtOAc (92:8) gave compound **4** (171 mg, 52%) as a colorless oil, TLC R_f (EtOAc/hexane 15:85) 0.38; $[\alpha]_D^{20}$ +21.2 (*c* 2, CH_2Cl_2); ν_{max} (liquid film) 3057, 2956, 2930, 2857, 1775, 1266, 1170, 739 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.02 (1H, d, *J* 5.8 Hz), 5.97 (1H, d, *J* 5.8 Hz), 5.67 (1H, dd, *J* 15.5, 5.6 Hz), 5.51 (1H, dd, *J* 15.5, 7.8 Hz), 5.48 (1H, d, *J* 7.5 Hz), 4.24 (1H, q, *J* 6.5 Hz), 3.58 (1H, t, *J* 7.2 Hz), 3.26 (1H, qu, *J* 7.5 Hz), 2.50–2.12 (6H, m), 1.10 (3H, t, *J* 7.5 Hz), 0.88 (3×3H, s), 0.08 (3H, s), 0.06 (3H, s); δ_C (75 MHz, $CDCl_3$) 176.8 (s), 139.3 (d), 136.1 (d), 129.2 (d), 128.2 (d), 88.6 (d), 83.7 (s), 75.9 (s), 72.0 (d), 49.1 (d), 40.1 (d), 30.5 (t), 28.8 (t), 25.8 (3×q), 18.2 (s), 14.1 (q), 12.4 (t), −4.6 (q), −4.8 (q); HRMS (ESI$^+$): MH$^+$, found 361.2198. $C_{21}H_{33}O_3Si$ requires 361.2199.

*4.2.4. Hydrogenation and deuteration protocol: (3aS,4R,6aR)-4-[(S,1E,5Z)-3-(tert-butyldimethylsilyloxy)octa-1,5-dienyl]-3,3a,4,6a-tetrahydro-2H-cyclopenta[b]furan-2-one (**11a**) and (3aS,4R,6aR)-4-{5,6-[D$_2$]-(S,1E,5Z)-3-(tert-butyldimethylsilyloxy)octa-1,5-dienyl}-3,3a,4,6a-tetrahydro-2H-cyclopenta[b]furan-2-one (**11b**).* Palladium on calcium carbonate poisoned with lead (Lindlar catalyst, 4.2 mg) was added to a stirred solution of hept-1-yne (212.5 μL, 1.625 mmol, 25 equiv) in 1:1 EtOAc/hexane (1.4 mL). A static atmosphere of H_2 was created, and the mixture was stirred for 40 min. A solution of alkyne **4** (25 mg, 0.065 mmol) in EtOAc (0.5 mL) was then added and the resulting mixture was stirred under H_2 atmosphere for 1 h. The mixture was then filtered through a small pad of Celite® and concentrated in vacuo at room temperature. The resulting residue was purified by flash column chromatography on silica gel. Elution with hexane/EtOAc, 7:3, gave diene **11a** (24 mg, 96%) as a colorless oil, TLC R_f (EtOAc/hexane 10:90) 0.35; $[\alpha]_D^{20}$ +29.9 (*c* 1.1, CH_2Cl_2); ν_{max} (liquid film) 2958, 2930, 2857, 1780, 1166, 835, 778 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.02 (1H, d, *J* 6.0 Hz), 5.97 (1H, d, *J* 6.0 Hz), 5.66 (1H, dd, *J* 15.5, 5.5 Hz), 5.50–5.25 (4H, m), 5.48 (1H, d, *J* 8.5 Hz), 4.15 (1H, q, *J* 6.1 Hz), 3.55 (1H, t, *J* 7.2 Hz), 3.25 (1H, qu, *J* 7.5 Hz), 2.50 (2H, d, *J* 8.5 Hz), 2.40–2.13 (2H, m), 2.12–1.99 (2H, distorted qu, *J* 7.4 Hz), 0.96 (3H, t, *J* 7.5 Hz), 0.90 (3×3H, s), 0.06 (3H, s), 0.04 (3H, s); δ_C (75 MHz, $CDCl_3$) 176.8 (s), 139.2 (d), 136.9 (d), 133.7 (d), 129.2 (d), 127.4 (d), 124.3 (d), 88.7 (d), 72.7 (d), 48.9 (d), 40.1 (d), 36.2 (t), 30.5 (t), 25.8 (3×q), 20.7 (t), 18.2 (s), 14.2 (q), −4.5 (q), −4.7 (q); HRMS (ESI$^+$): MH$^+$, found 363.2358. $C_{21}H_{35}O_3Si$ requires 363.2355.

An identical procedure was followed for the deuteration of alkyne **4**, by substituting a static atmosphere of H_2 with ≥99.9% pure D_2. After chromatographic purification on silica gel, diene **11b** was obtained in 95% yield as a colorless oil, TLC R_f (EtOAc/hexane 10:90) 0.35; $[\alpha]_D^{20}$ +30.2 (*c* 1.2, CH_2Cl_2); δ_H (300 MHz, $CDCl_3$) 6.00 (1H, d, *J* 5.7 Hz), 5.95 (1H, d, *J* 5.7 Hz), 5.48 (1H, d, *J* 8.0 Hz), 5.44 (1H, dd, *J* 15.5, 7.5 Hz), 4.15 (1H, q, *J* 6.0 Hz), 3.55 (1H, t, *J* 7.4 Hz), 3.25 (1H, qu, *J* 8.0 Hz), 2.47 (2H, d, *J* 8.5 Hz), 2.35–2.12 (2H, m), 2.05 (2H, distorted q, *J* 7.5 Hz), 0.97 (3H, t, *J* 7.5 Hz), 0.90 (3×3H, s), 0.07 (3H, s), 0.05 (3H, s); δ_C (75 MHz, $CDCl_3$) 176.8 (s), 139.2 (d), 136.9 (d), 133.2 (t, J^1_{c-d} 23.2 Hz), 129.2 (d), 127.4 (d), 123.8 (t, J^1_{c-d} 23.2 Hz), 88.7 (d), 72.7 (d), 48.9 (d), 40.1 (d), 36.1 (t), 30.5 (t), 25.8 (3×q), 20.6 (t), 18.2 (s), 14.1 (q), −4.5 (q), −4.7 (q); HRMS (ESI$^+$): MH$^+$, found 365.2482. $C_{21}H_{33}D_2O_3Si$ requires 365.2481.

*4.2.5. (3aS,4R,6aR)-4-{5,6-[D$_2$]-(S,1E,5Z)-3-(tert-Butyldimethylsilyloxy)octa-1,5-dienyl}-3,3a,4,6a-tetrahydro-2H-cyclopenta[b]furan-2-ol (**12b**).* A stirred solution of lactone **11b** (453 mg, 1.24 mmol) in DCM (3 mL) was cooled to −78 °C, and DIBAL-H (1.87 mL, 1 M in hexane, 1.51 equiv) was added dropwise. Stirring was continued for an additional 1 h, and then a saturated solution of NH_4Cl (5 mL) was added at −78 °C. The mixture was gradually warmed to rt, diluted with DCM (30 mL), and a saturated aqueous Rochelle salt solution (30 mL) was added. The aqueous layer was extracted with DCM (3×25 mL), and the combined organic phases were washed with H_2O and brine and dried on $MgSO_4$. Evaporation in vacuo of the volatiles gave lactol **12b** (493 mg, 98%), mixture about 9:1 of hemiacetals, as a colorless oil; TLC R_f (EtOAc/hexane 15:−85) 0.19; ν_{max} (liquid film) 3406, 3010, 2963, 2857, 1471, 1463, 1253, 1107, 1071, 1032, 834, 776; δ_H (300 MHz, $CDCl_3$) 5.82 (1H, br d, *J* 5.7 Hz), 5.68 (1H, br d, *J* 5.7 Hz), 5.55–5.40 (2H, m), 5.25 (1H, d, *J* 7.4 Hz), 4.19–4.03 (1H, m), 3.49–3.41 (1H, m), 3.24 (1H, qu, *J* 7.5 Hz), 2.85 (1H, br, OH), 2.30–2.11 (2H, m), 2.05 (2H, distorted q, *J* 7.5 Hz), 1.85–1.65 (2H, m), 0.96 (3H, t, *J* 7.6 Hz), 0.91 (3×3H, s), 0.07 (3H, s), 0.04 (3H, s); main hemiacetal δ_C (75 MHz, $CDCl_3$) 135.2 (d), 134.8 (d), 132.8 (t, J^1_{c-d} 23.2 Hz), 131.2 (d), 128.3 (d), 124.0 (t, J^1_{c-d} 23.2 Hz), 99.1 (d), 88.4 (d), 73.0 (d), 47.6 (d), 42.4 (d), 36.2 (t), 35.5 (t), 25.8 (3×q), 20.6 (t), 18.2 (s), 14.1 (q), −4.6 (q), −4.8 (q). HRMS (ESI$^+$): MH$^+$, found 367.2641. $C_{21}H_{35}D_2O_3Si$ requires 367.2638.

*4.2.6. (Z)-7-[(1S,2R,5R)-2-{5,6-[D$_2$]-(S,1E,5Z)-3-(tert-Butyldimethylsilyloxy)octa-1,5-dienyl}-5-hydroxycyclopent-3-enyl]hept-5-enoic acid (**14**).* Solid phosphonium salt **13** (2.24 g, 4.94 mmol, 4 equiv) was suspended in dry THF (15 mL) in a two-neck round-bottom flask under an argon atmosphere. To the suspension was added freshly sublimed potassium *tert*-butoxide (1.13 g, 9.88 mmol, 8 equiv) portionwise at rt. After being stirred for 20 min, the solution became deeply orange and a THF solution (8.1 mL) of lactol **12b** (461 mg, 1.23 mmol) was added via cannula dropwise at rt. Stirring was continued for an additional 2 h, and the reaction was quenched by adding a saturated aqueous solution of NH_4Cl (15 mL) and acetic acid (0.593 mL, 1.05 equiv vs *tert*-butoxide); Et_2O (60 mL) was added to the mixture and the organic layer was separated, whereas the aqueous phase was extracted with an additional Et_2O (3×50 mL). The organic phases were combined, dried over $MgSO_4$, filtered, and concentrated at reduced pressure. The residue was purified on silica gel, using hexane/EtOAc (8:2) as eluent, to give acid **14** (515 mg, 91%) as a pale yellow oil, TLC R_f (EtOAc/hexane 25:75) 0.32; ν_{max} (liquid film) 3310–2590 (OH and CO*OH*), 2931, 2854, 1714, 1463, 1250, 1066, 838, 776; δ_H (300 MHz, $CDCl_3$) 6.11–6.06 (1H, m), 6.01 (1H, dd, *J* 5.7, 2.6 Hz), 5.65–5.30 (4H, m), 4.51 (1H, dd, *J* 5.4, 2.5 Hz), 4.08 (1H, q, *J* 6.3 Hz), 3.22–3.12 (1H, m), 2.45–1.98 (11H, m), 1.74 (2H, qu, *J* 7.4 Hz), 0.97 (3H, t, *J* 7.4 Hz), 0.91 (3×3H, s), 0.06 (3H, s), 0.05 (3H, s); δ_C (75 MHz, $CDCl_3$) 178.1 (s), 139.1 (d), 134.7 (d), 133.1 (d), 132.8 (t, J^1_{c-d} 23.3 Hz), 131.8 (d), 129.5 (d), 129.2 (d), 124.1 (t, J^1_{c-d} 23.3 Hz), 76.3 (d), 73.3 (d), 49.2 (d), 46.8 (d), 36.2 (t), 33.3 (t), 26.5 (t), 25.8 (3×q), 24.4 (t), 23.9 (t), 20.6 (t), 18.1 (s), 14.1 (q), −4.5 (q), −4.8 (q). HRMS (ESI$^+$): MH$^+$, found 451.3216. $C_{26}H_{43}D_2O_4Si$ requires 451.3213.

*4.2.7. (Z)-7-[(1S,2S)-2-{5,6-[D$_2$]-(S,1E,5Z)-3-(tert-Butyldimethylsilyloxy)octa-1,5-dienyl}-5-oxocyclopent-3-enyl]hept-5-enoic acid (**15**).* To a solution of allylic alcohol **14** (70 mg, 0.156 mmol) in DCM (3.4 mL) at rt was added solid Dess–Martin periodinane (79.1 mg, 0.187 mmol, 1.2 equiv) in one portion. After the homogeneous solution was stirred for 3 h, the reaction was quenched by adding dry Et_2O (15 mL) and the suspension was quickly filtered on a short pad of Celite®. The Celite® layer was washed with Et_2O (3×30 mL), and

the organic phases were combined and concentrated at reduced pressure WITHOUT HEATING. The residue was purified by flash chromatography on silica gel (SiO_2, 4.5 g); elution with hexane/EtOAc (7:3) gave enone **15** (49 mg, 70%) as a pale yellow oil, TLC R_f (EtOAc/hexane 30:70) 0.33; ν_{max} (liquid film) 3210–2600 (COOH), 2932, 2855, 1712, 1586, 1460, 1251, 1082, 973, 836, 776; δ_H (300 MHz, CD_2Cl_2) 7.55 (1H, dd *J* 5.7, 2.8 Hz), 6.19 (1H, dd, *J* 5.7, 1.7 Hz), 5.65–5.34 (4H, m), 4.18 (1H, q, *J* 6.1 Hz), 3.79–3.69 (1H, m), 2.58–2.00 (11H, m), 1.72 (2H, qu, *J* 7.4 Hz), 0.98 (3H, t, *J* 7.4 Hz), 0.91 (3×3H, s), 0.08 (3H, s), 0.06 (3H, s); δ_C (75 MHz, CD_2Cl_2) 211.0 (s), 179.1 (s), 165.8 (d), 138.1 (d), 134.3 (d), 132.8 (t, J^1_{c-d} 23.2 Hz), 130.4 (d), 129.2 (d), 127.6 (d), 124.7 (t, J^1_{c-d} 23.2 Hz), 73.7 (d), 50.5 (d), 48.3 (d), 36.8 (t), 33.9 (t), 27.4 (t), 26.4 (3×q), 25.4 (t), 25.2 (t), 21.5 (t), 18.8 (s), 14.8 (t), −3.9 (q), −4.2 (q). HRMS (ESI$^+$): MH^+, found 449.3061. $C_{26}H_{41}D_2O_4Si$ requires 449.3056.

*4.2.8. 17,18-[D$_2$]-15-A$_{3t}$-IsoP (**1b**).* Excess 48% aqueous HF (0.098 mL) was added to silyl ether **15** (30 mg, 0.067 mmol) dissolved in MeCN (3.25 mL) in a polyethylene test tube. After 4 h at room temperature, a pH 6.8 phosphate buffer (5 mL) and EtOAc (10 mL) were added, and the two layers were separated. The aqueous phase was extracted with EtOAc (4×5 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by a short flash column chromatography on silica gel. Elution with hexane/EtOAc (1:1) gave [D$_2$]-15-A$_{3t}$-IsoP (**1b**) (16 mg, 76%) as a colorless oil, TLC R_f (EtOAc/hexane 50:50) 0.25; $[\alpha]_D^{20}$ +136.2 (*c* 1.0, EtOAc); ν_{max} (liquid film) 3320 (br), 2930, 1710, 1580, 1240, 970 cm^{-1}; δ_H (300 MHz, CD_2Cl_2) 7.60 (1H, dd, *J* 5.8, 2.8 Hz), 6.25 (1H, dd, *J* 5.8, 1.8 Hz), 5.65–5.34 (4H, m), 4.20 (1H, q, *J* 6.3 Hz), 3.77–3.74 (1H, m), 2.60–2.48 (1H, m), 2.45–2.25 (5H, m), 2.20–2.05 (5H, m), 1.70 (2H, qu, *J* 7.4 Hz), 0.99 (3H, t, *J* 7.4 Hz); δ_C (75 MHz, CD_2Cl_2) 211.0 (s), 178.3 (s), 165.6 (d), 136.8 (d), 135.8 (d), 133.1 (t, J^1_{c-d} 23.3 Hz), 130.3 (d), 129.5 (d), 128.8 (d), 123.9 (t, J^1_{c-d} 23.3 Hz), 72.6 (d), 50.3 (d), 48.0 (d), 35.8 (t), 33.7 (t), 27.3 (t), 25.7 (t), 25.1 (t), 21.5 (t), 14.7 (q); HRMS (ESI$^+$): MH^+, found 335.2192. $C_{20}H_{27}D_2O_4$ requires 335.2191.

*4.2.9. (Z)-Ethyl 7-(tert-butyldimethylsilyloxy)hept-4-enoate (**18**).* Commercially available phosphonium salt **17** (6.41 g, 14 mmol, 4.3 equiv) was dried under high vacuum at rt for 1 h and then suspended in dry PhMe (93 mL). KHMDS (0.5 M solution in PhMe, 26.8 mL, 13.4 mmol, 4 equiv) was added slowly, under vigorous stirring, at room temperature. The orange suspension was stirred at the same temperature for 1 h, then stirring was stopped for 1 h. The resulting clear ylide solution (82 mL, ca. 3 equiv) was transferred into a two-neck round-bottom flask and was cooled to −100 °C; a solution of known aldehyde **16**[22] (1.00 g, 3.20 mmol, 1 equiv) in dry PhMe (30 mL) was added dropwise via cannula. The resulting reaction mixture was vigorously stirred for 30 min, then quenched with a saturated solution of NH_4Cl (20 mL), and diluted with Et_2O (50 mL). The layers were separated and the aqueous phase was extracted with Et_2O (3×30 mL). The combined organic phases were then dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel. Elution with hexane/EtOAc (99:1) gave known ester **18**[21] (1.26 g, 96%) as a colorless oil, TLC R_f (EtOAc/hexane 2:98) 0.28; δ_H (300 MHz, $CDCl_3$) 7.75–7.60 (4H, m), 7.45–7.35 (6H, m), 5.52–5.35 (2H, m), 4.12 (2H, q, *J* 7.1 Hz), 3.70 (2H, t, *J* 6.8 Hz), 2.50–2.25 (6H, m), 1.28 (3H, t, *J* 7.1 Hz), 1.12 (3×3H, s); δ_C (75 MHz, $CDCl_3$) 173.0 (s), 135.5 (d), 133.8 (s), 129.5 (d), 129.2 (d), 127.5 (d), 127.2 (d), 63.4 (t), 60.2 (t), 34.2 (t), 30.7 (t), 26.7 (3×q), 22.8 (t), 19.1 (s), 14.2 (q). HRMS (ESI$^+$): MH^+, found 411.2354. $C_{25}H_{35}O_3Si$ requires 411.2355.

*4.2.10. (Z)-Ethyl 7-bromohept-4-enoate (**19**).* Silyl ether **18** (192 mg, 0.46 mmol) was dissolved in dry THF (5.0 mL) under an argon atmosphere; TBAF (1 M solution in THF, 0.54 mL, 0.54 mmol, 1.17 equiv) was added at 22 °C and the reaction mixture was stirred for 1 h. Volatiles were removed under reduced pressure and the residue was purified by flash chromatography on silica gel. Elution with hexane/EtOAc (80:20) gave the expected alcohol (*Z*)-ethyl 7-hydroxyhept-4-enoate (73 mg, 92%) as a colorless oil.[21] Subsequently, this ester (207 mg, 1.20 mmol) was dissolved in dry DMF (12 mL) under an argon atmosphere. The resulting solution was cooled to 0 °C and CBr_4 (474 mg, 1.43 mmol, 1.19 equiv) was added under vigorous stirring, followed by PPh_3 (435 mg, 1.66 mmol, 1.38 equiv). The ice bath was removed and the reaction mixture was stirred for 1 h at 22 °C, then quenched with water (25 mL) and diluted with Et_2O (40 mL); the aqueous phase was extracted with Et_2O (3×25 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude bromide was purified by flash chromatography on silica gel. Elution with hexane/EtOAc (95:5) gave known bromide **19**[21] (203 mg, 72%) as a pale yellow oil, TLC R_f (EtOAc/hexane 2:98) 0.22; δ_H (300 MHz, $CDCl_3$) 5.65–5.32 (m, 2H), 4.13 (q, *J* 7.1, 2H), 3.42 (t, *J* 7.1, 2H), 2.70 (q, *J* 7.0, 2H), 2.50–2.40 (m, 4H), 1.21 (t, *J* 7.1, 3H); δ_C (75 MHz, $CDCl_3$) 172.8 (s), 130.5 (d), 127.3 (d), 60.3 (t), 33.9 (t), 32.3 (t), 30.6 (t), 22.8 (t), 14.1 (q).

*4.2.11. (Z)-[6-(Ethoxycarbonyl)hex-3-enyl]triphenylphosphonium bromide (**20**).* Bromide **19** (212 mg, 0.90 mmol) was dissolved in dry MeCN (2 mL) under an argon atmosphere and PPh_3 (284 mg, 1.08 mmol, 1.2 equiv) was added. The reaction mixture was heated to reflux for 45 h, then MeCN was removed in vacuo. The residue was purified by flash chromatography on silica gel. Elution with DCM/MeOH (95:5) gave known phosphonium salt **20**[21] (403 mg, 90%) as a pale yellow oil, TLC R_f (DCM/MeOH 90:10) 0.25; δ_H (300 MHz, $CDCl_3$) 7.9–7.5 (m, 15H), 5.60–5.25 (m, 2H), 3.91 (q, *J* 7.1, 2H), 3.88–3.72 (m, 2H), 2.33–2.28 (m, 2H), 2.23–2.18 (m, 2H), 2.14–2.09 (m, 2H), 1.1 (t, *J* 7.1, 3H). δ_C (75 MHz, $CDCl_3$) 172.7 (s), 135.0 (d), 133.4 (d), 130.3 (d), 127.5 (d), 127.3 (d), 118.5 (s), 117.4 (s), 60.2 (t), 33.4 (t), 23.0 (t), 22.4 (t), 20.3 (t), 14.1 (q).

*4.2.12. 2-{(1S,2R,5R)-2-[(S,E)-3-(tert-Butyldimethylsilyloxy)oct-1-en-5-ynyl]-5-(1-ethoxyethoxy)cyclopent-3-enyl}-N-methoxy-N-methylethanamide (**22**).* BuLi (2.5 M in hexane, 2.39 mL, 12 equiv) was added to ClHNMeOMe (0.292 g, 3.0 mmol, 6 equiv) in dry THF (4.75 mL) under Ar at −78 °C. After 5 min the solution was stirred at room temperature for 15 min and then cooled again to −78 °C. Lactone **4** (0.180 mg, 0.5 mmol) in dry THF (5.2 mL) was then added and the mixture was stirred at −78 °C for 1 h. Saturated aqueous NH_4Cl (10 mL), followed by brine (5 mL) and Et_2O (10 mL) was added; the two layers were separated, and the aqueous phase was extracted with Et_2O (3×10 mL). The combined organic layers were then dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude residue was dissolved in 3 mL of CH_2Cl_2/EVE, 2:1, and a catalytic amount of PPTS was added. After 2 h of stirring at room temperature, the mixture was added to saturated aqueous $NaHCO_3$ (10 mL). The two layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel. Elution with CH_2Cl_2/EtOAc (98:2) gave Weinreb amide **22** (0.172 g, 70%), mixture of anomers, as a colorless oil; TLC R_f (DCM/EtOAc 80:20) 0.31; ν_{max} 2930, 1668, 1463, 1386, 1254, 1123, 1005, 964, 837 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.03–5.97 (1H, m), 5.97–5.90 (1H, m), 5.55 (1H, dd, *J* 15.5, 5.2 Hz), 5.37 (1H, dd, *J* 15.5, 5.2 Hz), 4.74 (0.5H, q, *J* 5.2 Hz), 4.67 (0.5H, q, *J* 5.2 Hz), 4.52 (0.5H, br d, *J* 3.9 Hz), 4.47 (0.5H, br d, *J* 3.9 Hz), 4.15 (1H, distorted q, *J* 6.0 Hz), 3.72 (1.5H, s), 3.71 (1.5H, s), 3.73–3.52 (1H, m), 3.51–3.31 (1H, m), 3.25–3.13 (1H, m), 3.15 (3H, s), 2.80–2.55 (2H, m), 2.40–2.12 (5H, m), 1.30–1.05 (9H, 2t+1d, 3H each), 0.90 (3×3H, s), 0.06 (3H, s), 0.04

(3H, s); δ_C (75 MHz, $CDCl_3$) 174.1 (s), 139.3 (d), 138.1 (d), 133.7 (d), 132.2 (d), 131.3 (d), 130.9 (d), 100.7 (d), 98.0 (d), 83.0 (s), 82.1 (d), 77.9 (d), 76.4 (s), 71.9 (d), 60.9 (q), 60.5 (t), 59.4 (t), 50.4 (d), 50.3 (d), 40.9 (d), 40.7 (d), 32.1 (q), 28.9 (t), 28.4 (t), 28.2 (t), 25.7 (3×q), 20.8 (q), 20.1 (q), 18.1 (s), 15.2 (q), 14.0 (q), 12.3 (t), −4.5 (q), −5.0 (q); HRMS (ESI$^+$): MH^+, found 494.3304. $C_{27}H_{48}NO_5Si$ requires 494.3302.

*4.2.13. (4Z,7Z)-Ethyl 9-{(1S,2R,5R)-2-[(S,E)-3-(tert-butyldimethylsilyloxy)oct-1-en-5-ynyl]-5-(1-ethoxyethoxy)cyclopent-3-enyl}nona-4,7-dienoate (**24**).* DIBAL-H (1 M in hexane, 0.210 mL, 1.2 equiv) was added to well-dried amide **22** (85.2 mg, 0.175 mmol) in dry THF (1.75 mL) under Ar at −78 °C. After 1 h, the reaction was quenched by adding a saturated aqueous Rochelle salt solution (10 mL) and Et_2O (10 mL). After 3 h of stirring, the two layers were separated and the aqueous phase was extracted with Et_2O (3×10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo to give crude aldehyde **23**, which was immediately submitted to the olefination reaction.

KHMDS (0.66 M in toluene, 0.70 mL, 0.46 mmol, about 2.6 equiv) was added to a suspension of phosphonium salt **21** (0.231 g, 0.46 mmol, about 2.6 equiv) in dry THF (2 mL) under Ar at −40 °C. After 30 min, DMPU (0.11 mL) was added, the mixture was cooled to −78 °C, and crude aldehyde **23** in THF (1 mL) was added via cannula. After 2 h, the reaction was quenched by adding a saturated aqueous NH_4Cl solution (10 mL) and Et_2O (10 mL). The two layers were separated and the aqueous phase was extracted with Et_2O (3×10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel. Elution with hexane/EtOAc (95:5) gave anomers **24** (65.2 mg, 75%), each constituted by a mixture 90:10 of 7*Z*/7*E* stereoisomers, as a colorless oil; ν_{max} 2929, 1730, 1465, 1372, 1251, 1163, 1124, 837, 776 cm^{-1}; δ_H (300 MHz, MeCN-d_3) 6.05–5.98 (1H, m), 6.0–5.90 (1H, m), 5.55–5.26 (6H, m), 4.80–4.71 (1H, m), 4.50–4.30 (1H, m), 4.30–4.20 (1H, m), 4.15 (2H, distorted q, *J* 6.0 Hz), 3.67–3.35 (2H, m), 3.20–3.12 (1H, m), 2.85–2.75 (2H, m), 2.40–2.0 (10H, m), 1.26–1.20 (6H, t+d, 3H each), 1.20 (3H, t, *J* 7.1 Hz), 1.09 (3H, t, *J* 7.0 Hz), 0.93 (3×3H, s), 0.05 (3H, s), 0.04 (3H, s); δ_C (75 MHz, MeCN-d_3) 172.6 (s), 139.4 (d), 138.5 (d), 133.1 (d), 132.5 (d), 131.7 (d), 131.6 (d), 129.3 (d), 129.2 (d), 127.8 (d), 127.7 (d), 100.3 (d), 97.8 (d), 83.0 (s), 81.3 (d), 77.5 (d), 76.3 (s), 72.2 (d), 60.3 (t), 59.9 (t), 59.3 (t), 50.6 (d), 50.5 (d), 46.7 (d), 33.8 (t), 28.5 (t), 25.5 (t), 25.3 (3×q), 23.9 (t), 22.5 (t), 20.3 (q), 19.8 (q), 17.8 (s), 14.7 (q), 13.1 (q), 11.9 (t), −5.2 (q), −5.6 (q); HRMS (ESI$^+$): MH^+, found 484.3368. $C_{30}H_{48}O_3Si$ requires 484.3373.

*4.2.14. (4Z,7Z)-Ethyl 9-{(1S,2R,5R)-2-[(S,E)-3-(tert-butyldimethylsilyloxy)oct-1-en-5-ynyl]-5-hydroxycyclopent-3-enyl}nona-4,7-dienoate (**25**).* A catalytic amount of PPTS was added to acetals **24** (60 mg, 0.12 mmol) in 15% CH_2Cl_2/EtOH/(7 mL) at room temperature. After 9 h, the reaction was quenched by adding excess solid $NaHCO_3$; subsequently, the mixture was filtered and evaporated in vacuo. The residue was purified by flash column chromatography on silica gel. Elution with hexane/EtOAc (90:10) gave allylic alcohol **25** (42.5 mg, 71%), as a colorless oil, TLC R_f (EtOAc/hexane 15:85) 0.31; $[\alpha]_D^{20}$ +81.3 (*c* 2.1, CH_2Cl_2); ν_{max} 3501 (br), 2930, 1720, 1470, 1360, 1255, 1076, 987, 776 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.05 (1H, br s), 6.02 (1H, br s), 5.65–5.30 (6H, m), 4.50 (1H, distorted q, *J* 6.0 Hz), 4.25–4.05 (1H+2H, m+q), 3.20–3.12 (1H, m), 2.95–2.75 (2H, m), 2.48–2.05 (11H, m), 1.26 (3H, t, *J* 7.1 Hz), 1.12 (3H, t, *J* 7.5 Hz), 0.91 (3×3H, s), 0.06 (3H, s), 0.04 (3H, s); δ_C main (7*Z*)-stereoisomer (75 MHz, $CDCl_3$) 173.2 (s), 139.1 (d), 133.9 (d), 133.3 (d), 132.4 (d), 129.4 (d), 128.9 (d), 128.4 (d), 127.9 (d), 83.4 (s), 76.3 (d), 76.2 (s), 72.2 (d), 60.3 (t), 49.5 (d), 47.0 (d), 34.3 (t), 29.0 (t), 25.8 (t), 25.8 (3×q), 24.0 (t), 22.8 (t), 18.2 (s), 14.2 (q), 14.1 (q), 12.4 (t), −4.5 (q), −4.8 (q); HRMS (ESI$^+$): MH^+, found 501.3404. $C_{30}H_{49}O_4Si$ requires 501.3400.

*4.2.15. (4Z,7Z)-Ethyl 9-{(1S,2S)-2-[(S,E)-3-hydroxyoct-1-en-5-ynyl]-5-oxocyclopent-3-enyl}nona-4,7-dienoate (**26**).* DMP (41.9 mg, 0.098 mmol, 1.2 equiv) was added to alcohol **25** (40.2 mg, 0.082 mmol) in dry DCM (1.5 mL). After 1 h of stirring at room temperature, Et_2O (10 mL) was added and the suspension was filtered through a short pad of silica gel, which was abundantly washed with Et_2O. The filtrate was evaporated in vacuo to give a residue, which was purified by flash column chromatography on silica gel. Elution with hexane/EtOAc (90:10) gave the *O*-TBS ether of hydroxy-enone **26** (33.1 mg, 79%), as a colorless oil, TLC R_f (EtOAc/hexane 10:90) 0.33; $[\alpha]_D^{20}$ +118.5 (*c* 1.8, CH_2Cl_2); δ_H (300 MHz, MeCN-d_3) 7.61 (1H, dd, *J* 5.8, 2.8 Hz), 6.19 (1H, dd, *J* 5.8, 1.7 Hz), 5.62 (1H, dd, *J* 15.5, 5.5 Hz), 5.55–5.31 (5H, m), 4.27 (1H, q, *J* 6.0 Hz), 4.08 (2H, q, *J* 7.1 Hz), 3.80–3.72 (1H, m), 2.89–2.70 (2H, m), 2.55–2.02 (11H, m), 1.24 (3H, t, *J* 7.1 Hz), 1.10 (3H, t, *J* 7.5 Hz), 0.90 (3×3H, s), 0.10 (3H, s), 0.07 (3H, s).

Excess 48% aqueous HF (0.098 mL) was added to the *O*-TBS ether of hydroxy-enone **26** (30 mg, 0.060 mmol) dissolved in MeCN (3.25 mL). After 4 h of stirring at room temperature, a pH 6.8 phosphate buffer (5 mL) and EtOAc (10 mL) were added, and the two layers were separated. The aqueous phase was extracted with EtOAc (4×5 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel. Elution with hexane/EtOAc (1:1) gave free alcohol **26** (17.2 mg, 74%) as a colorless oil, TLC R_f (EtOAc/hexane 15:85) 0.35; $[\alpha]_D^{20}$ +96.3 (*c* 1.3, EtOAc); ν_{max} (liquid film) 3320 (br), 2930, 1710, 1580, 1240, 970 cm^{-1}; δ_H (300 MHz, MeCN-d_3) 7.61 (1H, dd, *J* 5.7, 2.7 Hz), 6.18 (1H, dd, *J* 5.7, 1.8 Hz), 5.60 (1H, dd, *J* 15.5, 5.5 Hz), 5.60–5.31 (5H, m), 4.18–4.01 (1+2H, m+q, *J* 7.0 Hz), 3.80–3.65 (1H, m), 3.10–3.0 (1H, br s), 2.89–2.70 (2H, m), 2.55–2.02 (11H, m), 1.25 (3H, t, *J* 7.1 Hz), 1.10 (3H, t, *J* 7.5 Hz); δ_C main (7*Z*)-stereoisomer (75 MHz, MeCN-d_3) 209.8 (s), 172.8 (s), 165.3 (d), 135.7 (d), 132.2 (d), 128.9 (d), 128.6 (d), 128.3 (d), 128.1 (d), 128.0 (d), 83.5 (s), 75.7 (s), 70.3 (d), 59.9 (t), 49.6 (d), 47.1 (d), 33.8 (t), 27.5 (t), 25.4 (t), 24.3 (t), 22.5 (t), 13.6 (2×q), 11.9 (t); HRMS (ESI$^+$): MH^+, found 385.2386. $C_{24}H_{33}O_4$ requires 385.2379.

*4.2.16. (4Z,7Z)-Ethyl 9-[(1S,2S)-2-{[5,6-D_2]-(S,1E,5Z)-3-hydroxyocta-1,5-dienyl}-5-oxocyclopent-3-enyl]nona-4,7-dienoate (**27**).* Tetraenyne **26** (15 mg) was submitted to the standardized deuteration protocol described in paragraph 4.2.4. After flash column chromatographic separation on silica gel, dideuterated enone **27** (14 mg, 95%) was obtained as colorless oil, TLC R_f (EtOAc/hexane 15:85) 0.38; $[\alpha]_D^{20}$ +78.3 (*c* 1.2, EtOAc); ν_{max} (liquid film) 3330 (br), 2935, 1720, 1575, 1230 cm^{-1}; δ_H (300 MHz, MeCN-d_3) 7.62 (1H, dd, *J* 5.7, 2.6 Hz), 6.18 (1H, dd, *J* 5.7, 1.8 Hz), 5.65–5.30 (6H, m), 4.17–4.01 (1+2H, m+q, *J* 7.0 Hz), 3.80–3.72 (1H, m), 2.88–2.72 (3H, m), 2.55–1.99 (11H, m), 1.23 (3H, t, *J* 7.1 Hz), 0.96 (3H, t, *J* 7.5 Hz); δ_C main (7*Z*)-stereoisomer (75 MHz, MeCN-d_3) 209.9 (s), 172.8 (s), 165.5 (d), 136.8 (d), 132.9 (t, J^1_{c-d} 23.2 Hz), 132.1 (d), 128.9 (d), 128.6 (d), 128.1 (d), 128.0 (d), 127.5 (d), 124.2 (t, J^1_{c-d} 23.2 Hz), 71.3 (d), 59.9 (t), 49.5 (d), 47.0 (d), 34.9 (t), 33.7 (t), 25.4 (t), 24.3 (t), 22.5 (t), 20.4 (t), 13.6 (q), 13.5 (q); HRMS (ESI$^+$): MH^+, found 389.2668. $C_{24}H_{33}D_2O_4$ requires 389.2661.

*4.2.17. 19,20-[D_2]-17-A_{4t}-NeuroP (**2b**).* Ethyl ester **27** (12 mg, 0.03 mmol, 1 equiv) was dissolved in HPLC grade MTBE (0.5 mL), and HPLC grade H_2O (50 μL) was added. To the resulting stirred solution was added lipase immobilized from *C. antartica* (CAL-B, 50 mg), and the suspension was gently stirred at 35 °C for 18 h. The enzyme was filtered off over a sintered glass funnel, and the solid was carefully washed with MeCN/MTBE (1:1, 25 mL). Filtrates were

collected and evaporated in vacuo. The residue was purified by flash column chromatography on silica gel. Elution with hexane/EtOAc (50:50) afforded 19,20-[D_2]-17-A_{4t}-NeuroP (**2b**) (6 mg, 54%) as a colorless oil, TLC R_f (EtOAc/hexane 50:50) 0.23; $[\alpha]_D^{20}$ +96.7 (*c* 0.2, EtOAc); ν_{max} (liquid film) 3412 (br), 2929, 1703, 1581, 1239, 969 cm^{-1}; δ_H (300 MHz, MeCN-d_3) 7.61 (1H, dd, *J* 5.7, 2.6 Hz), 6.18 (1H, dd, *J* 5.7, 1.8 Hz), 5.70–5.30 (6H, m), 4.05 (1H, distorted q, *J* 6.0 Hz), 3.80–3.72 (1H, m), 2.88–2.65 (2H, m), 2.55–2.00 (m, 11H), 0.98 (t, *J* 7.5, 3H). δ_C main (7*Z*)-stereoisomer (75 MHz, MeCN-d_3) 210.0 (s), 173.6 (s), 165.6 (d), 136.7 (d), 132.9 (t, J^1_{c-d} 23.3 Hz), 132.1 (d), 128.9 (d), 128.6 (d), 128.1 (d), 128.0 (d), 127.5 (d), 124.1 (t, J^1_{c-d} 23.3 Hz), 71.4 (d), 49.5 (d), 47.0 (d), 35.0 (t), 33.2 (t), 25.4 (t), 24.4 (t), 22.5 (t), 20.4 (t), 13.5 (q). HRMS (ESI^-): $M-H^-$, found 359.2206. $C_{22}H_{27}D_2O_4$ requires 359.2191.

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