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Approaches to polyunsaturated sphingolipids: new conformationally restrained analogs with minimal structural modifications

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ABSTRACT

Conformationally restrained sphingolipids **1**–**4**, arising from the introduction of a polyene or a polyenyne moiety as part of the sphingoid backbone, have been synthesized. While addition of polyene acetylides **6** and **7** to Garner's aldehyde afforded the corresponding Felkin-Anh *anti*-addition adducts, addition of alkenylzirconocenes showed no diastereoselectivity. This behaviour was also observed from protected serinal **14**, which allowed the synthesis of the acid-sensitive pentaene sphingosine backbone present in ceramide **4**. Despite the inherent limitations of the synthetic approaches here reported, their application to such highly conjugated polyene sphingoid analogs is unprecedented.

1. Introduction

One of the ultimate goals in biology is to understand the relationship between structure, function, and dynamics of biomolecules in their natural environment, namely the living cell. In this context, the use of conformationally restrained analogs has become a common strategy in medicinal chemistry programs to delineate the active conformation of flexible molecules and, indirectly, to gain insight into the three-dimensional requirements of their receptors.[1] This approach has also been applied in the sphingolipid arena by incorporating part of the aminodiol moiety of the sphingoid backbone into cyclic structures[2–4] or, less frequently, by introduction of unsaturations in the sphingoid chain.[5–10] However, the full understanding of the roles of sphingolipids (SLs) in the cell environment is far for complete due to a lack of adequate experiments able to directly visualize molecular interactions under physiological conditions. In this context, fluorescent lipid analogs become useful biophysical tools. In particular, polyene systems[11–14] are suitable fluorescent tags that introduce minimize structural alterations in the resulting probe,[15] in comparison with commercially available bulkier chromophores, such as BODIPY or NBD.[16,17] Most importantly, the pentaene moiety has been reported to behave like its endogenous saturated counterparts in vivo, with respect to uptake, metabolism, transport and localization in membrane microdomains.[12,18,19] However, when these labeled lipids are part of the *N*-acyl or *O*-acyl SLs chains,[11,12,15,20,21] the fluorophore can be released by hydrolysis of the amide or the ester bonds by specific enzymes, and the resulting sphingoid base is no longer traceable. This is not the case when the polyene system is part of the sphingoid backbone. On the other hand, the chemical modification of the sphingoid backbone by incorporation of an acetylene group has received considerable attention.[8,22–24] The triple bond increases the rigidity of the molecule, affecting its physicochemical and biological properties.

Based on the above considerations and in our interested in the development of new sphingolipid probes with potential biophysical and biochemical applications, we wish to report on the development of synthetic protocols for the elaboration of new conformationally constrained sphingosines and ceramide analogs resulting from the incorporation of a polyunsaturated system as part of the sphingoid backbone. In this account, the synthesis of

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polyenyne sphingosines **1** and **2**, polyene sphingosine **3** and polyene ceramide **4** are described as representative examples of the above general approach (Fig. 1).

2. Results and discussion

Access to the alkyne polyene framework present in probes **1** and **2** was planned by nucleophilic addition of the corresponding lithium acetylides of **6** or **7** (Scheme 1) to Garner's aldehyde under conditions similar to those described for simpler acetylenes.[23,25–27] The synthesis of the required acetylenes started from the phosphonium salt **5**, easily obtained in two steps (85% overall yield) from commercially available 4-pentyn-1-ol.[28] Salt **5** was submitted to (*E*)-selective Wittig olefination[29] with the required polyene aldehyde (2,4-hexadienal for **6** and 2,4,6,8-decatetraenal[30] for **7**), followed by iodine-promoted isomerization[31–34] of the resulting polyene system. The initial condensation attempts of **6** with Garner's aldehyde, using BuLi/HMPA or LiHMDS in THF at low temperature for acetylide generation, were unsuccessful. Only the use of LDA in THF at −78 °C afforded acceptable yields of the condensation adduct **8**. Application of the above conditions to acetylene **7** led to the corresponding adduct **9**, albeit in lower yields. Gratifyingly, only the corresponding C3-*erythro* isomers of **8** and **9** were obtained, in agreement with the operation of a Felkin-Anh transition state model. The low temperature used in this condensation step becomes crucial for the exclusive formation of the *erythro* isomers and avoids the use of HMPA or HMPT as co-solvents.[23] In our case, the *erythro* configuration of **8** and **9** was confirmed by comparison with the reported ^{1}H NMR data for the corresponding saturated alkyne in $CDCl_3$.[35] The simultaneous removal of the isopropylidene and Boc groups of the above adducts under acidic conditions (HCl in MeOH)[36] led to the target acetylene polyene probes **1** and **2** in acceptable yields (Scheme 1). Contrary to our initial expectations, the acidic conditions used in this deprotection step were compatible with the presumably acid-sensitive polyene framework of the sphingoid backbone.

For the synthesis of the polyene probes **3** and **4** (Fig. 1), initial attempts relied on the stereoselective Red-Al® reduction of the propargylic alcohols **8** and **9** to the corresponding *E*-allylic alcohols, following the reported methodology of Herold et al.[37] However, complex reaction mixtures were obtained in both cases, as well as from the fully deprotected amino diol **1**. In light of these results, hydrozirconation of the alkyne polyenes **6** and **7** with Schwartz reagent ($Cp_2Zr(H)Cl$),[38] followed by addition of the intermediate alkenyl zirconocenes to Garner's aldehyde, was considered (Scheme 2). According to the literature, this sequence has been successfully applied to the elaboration of the allylic alcohol framework present in simple sphingolipids and related compounds.[39–44] In particular, the addition of alkenyl zirconocenes to Garner's aldehyde has been reported to lead to *anti* adducts by the use of substoichiometric $ZnBr_2$ as carbonyl-activating Lewis acid. On the other hand, by addition of a transient alkenylzinc intermediate, arising from transmetallation of the above alkenyl zirconocene, the opposite *syn* stereoselectivity is obtained.[41,42] In our case, initial hydrozirconation attempts of **6** using $ZnBr_2$ as Lewis acid were unsuccessful, since no addition products were obtained. After much experimentation (variation of $ZnBr_2$ stoichiometry, solvent systems,[45] and even the in situ generation of Schwartz reagent[46]) we turned our attention to the use of silver salts, which have also been reported as suitable catalysts for the addition of alkenyl zirconocenes to aldehydes.[47–49] Thus, coupling of Garner's aldehyde, in the presence of $AgClO_4$ (20% mol), with the alkenyl zirconocene arising from alkyne **6** (5*E*/*Z*: 4/1) afforded the desired addition product **10** in 50% yield in a roughly 1:1 *syn*/*anti* ratio, as expected from the reported drop of diastereoselectivity under silver promoted additions.[41,47] Similarly, the hydrozirconation-coupling of alkyne (all-*E*)-**7** with Garner's aldehyde under the above conditions led to the expected adduct **11** (*syn*/*anti* 1:1), albeit in lower yield (Scheme 2). Despite the simultaneous deprotection of the isopropylidene and *N*-Boc groups of **10** in HCl–MeOH afforded the expected polyene sphingosine **3**, these conditions were not suitable for the deprotection of pentaene **11**, since a complex mixture of products were obtained in all cases.

In light of the above results and taking into account the apparent instability of the conjugated pentaene system under the acidic conditions required for the deprotection step, we considered the use of an alternative protection strategy in the starting aldehyde. Thus, the previously unreported protected serinal **14** was considered for our purposes. In this case, the Fmoc and TBDMS protecting groups would enable the use of non-acidic deprotection conditions in the final step. The protected serinal **14** was obtained from Fmoc serine methyl ester, after hydroxyl protection (as the TBDMS ether **12**) and reduction of the intermediate Weinreb amide **13** (Scheme 3). Alkenylation of aldehyde **14** was optimized using 1-tetradecenylzirconocene as a model, obtained from treatment of 1-tetradecyne with Schwartz reagent. Under the above silver promoted alkenylation conditions, a marked stereoselectivity leading to the unnatural *syn* adduct (*anti*/*syn* 1:4) was observed. The configurational assignment at C3 in this mixture was unambiguously confirmed by its derivatization to the corresponding (*R*) and (*S*)-MPA esters, following the standard methodology of Riguera et al.,[50] as illustrated in Fig. 2.

Attempts to revert this stereoselectivity were next undertaken. After much experimentation, the use of $ZnCl_2$ (50 mol %) in DCM allowed the alkenylation of **14** with polyene (all-*E*)-**7** to afford the adduct **15** as an *anti*/*syn* 1:1.5 mixture, albeit in modest yield (Scheme 3). The diastereoisomeric composition of **15** was inferred from that of the above model reaction of aldehyde **14** with 1-tetradecyne.

In order to avoid an excessive manipulation of the sensitive polyene sphingosine system, we decided to carry out an one-pot deprotection-acylation of **15**. Thus, the simultaneous removal of

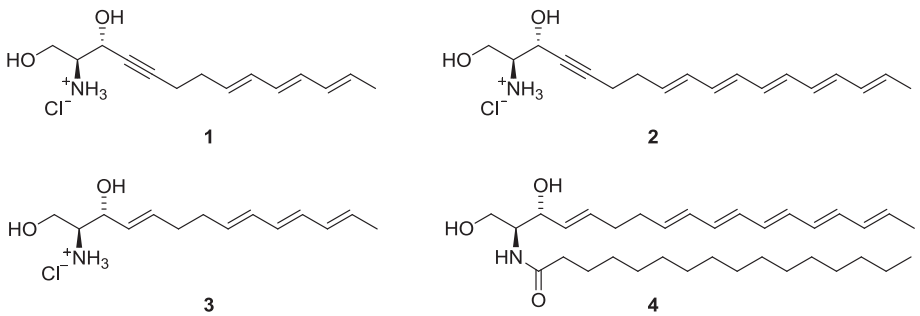
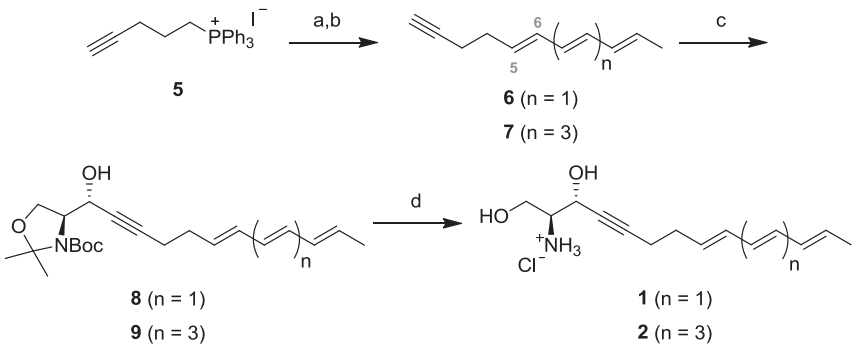


Fig. 1. Unsaturated probes described in this work.



Scheme 1. (a) BuLi (2.5 M hexanes), HMPA, THF, 2,4-hexadienal (for **6**) or 2,4,6,8-decatetraenal (for **7**), −78 °C, 4 h; (b) I_2 in hexanes, reflux (for **6,** 5*E*/*Z* 4:1, 80% combined; for 'all *E*'-**7**, 25% yield after preparative HPLC, see Experimental); (c) 1) LDA, −78 °C, 30 min; 2) Garner's aldehyde, THF, 3 h from −78 °C to rt (**8**, 45% or **9**, 25%); (d) CH_3COCl (1.5% vol), MeOH, 0 °C to rt, 30 min (**1**, 60% or **2**, 40%).

the Fmoc[51] and TBDMS groups[52–54] with TBAF in THF, followed by N-acylation of the crude reaction mixture with palmitic acid and EDC-HOBt, afforded the expected polyene ceramide **4** after chromatographic purification, albeit as an inseparable mixture of *syn*/*anti* diastereomers.

3. Conclusion

Synthetic approaches to polyene sphingosine analogs, based on the alkynylation or hydrozirconation of Garner's aldehyde with the unprecedented polyene alkynes **6** and **7** are reported. While addition of lithium acetylides afforded the natural *anti* adducts, addition of alkenylzirconocenes showed no diastereoselectivity. This trend was also observed from aldehyde **14**, which allowed the synthesis of the acid-sensitive pentaene sphingosine backbone present in ceramide **4**. Despite the inherent limitations of the synthetic approaches here reported, their application to such highly conjugated polyene sphingoid analogs is unprecedented. Experiments addressed at illustrating the applicability of these fluorescent probes in a variety of biophysical studies will be reported in due course.

4. Experimental

General: Commercial grade reagents and solvents were directly used as supplied with the exception of THF that was distilled over Na/benzophenone under Ar. Reactions sensitive to moisture and oxygen were carried out under argon or nitrogen atmosphere and were monitored by thin layer chromatography (TLC) on aluminum foil pre-coated silica gel plates. Visualization of UV-inactive materials was accomplished by soaking the TLC plates in an ethanol solution of phosphomolybdic acid (5%) and developing of the stained material with a heating gun. Organic solutions obtained from workup of crude reaction mixtures were dried over anhydrous $MgSO_4$. Flash column chromatography was performed with the indicated solvents using silica gel 60 (230–400 mesh). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. ^1H and ^{13}C NMR spectra were obtained in $CDCl_3$ solutions at 400 MHz (for ^1H) and 101 MHz (for ^{13}C), respectively, unless otherwise indicated. Chemical shifts (δ) are reported in ppm relative to the solvent ($CDCl_3$) signal. The polyenes described in this work, due to their labile nature, were stored at −80 °C as THF solutions (1 mg/mL) solutions stabilized with BHT (butylated hydroxytoluene).

4.1. Pent-4-yn-1-yltriphenylphosphonium iodide (5)

a) 5-Iodo-1-pentyne: A solution of 4-pentyn-1-ol (2.5 g, 29.7 mmol) in DCM (30 mL) was added to a rapidly stirred suspension of triphenylphosphine (10.1 g, 38.9 mmol), imidazole (2.6 g, 38.6 mmol) and iodine (9.8 g, 38.6 mmol) in DCM (120 mL). After stirring for 1 h, the solvent was evaporated and the residue purified by flash column chromatography (hexane:EtOAc; 95:5) to give the 5-iodo-1-pentyne as an orange oil (60%).

Rf: 0.86 (hexane:EtOAc; 9:1).

^1H NMR ($CDCl_3$): δ 3.44–3.11 (m, 2H), 2.33 (qt, *J*=7.0, 3.0 Hz, 3H), 2.09–1.92 (m, 3H).

^{13}C NMR ($CDCl_3$): δ 82.2 (C), 69.5 (CH), 31.8 (CH_2), 19.4 (CH_2), 5.1 (CH_2).

b) A mixture of 5-iodo-1-pentyne (2.7 g, 14.0 mmol) and triphenylphosphine (11 g, 42.0 mmol) was heated at 86 °C for 16 h under Ar. The wax mixture was cooled down to rt and then was added hexane (50 mL) affording a precipitate. The supernatant was discarded and the crude was concentrated under reduced pressure. The residue was purified by flash column chromatography (DCM:MeOH; 96:4) to give a yellowish solid (85%).

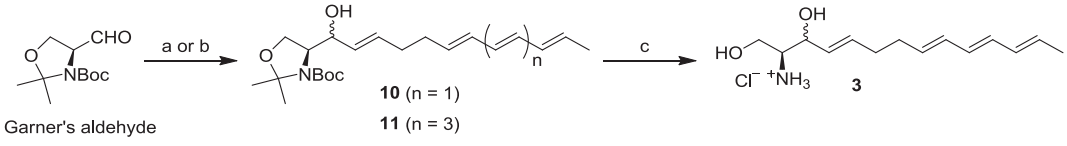
Rf: 0.72 (DCM:MeOH; 9:1).

^1H NMR ($CDCl_3$) δ 7.87–7.78 (m, 9H), 7.74–7.68 (m, 6H), 3.96–3.87 (m, 2H), 2.66 (ddd, *J*=12.0, 6.5, 1.0 Hz, 2H), 2.01 (t, *J*=3.0 Hz, 1H), 1.96–1.84 (m, 2H).

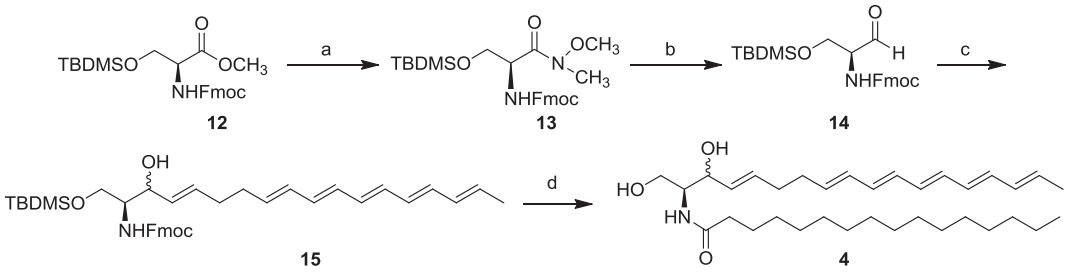
^{13}C NMR ($CDCl_3$) δ 135.2 (J_{C-P}=2.2 Hz, CH), 133.5 (J_{C-P}=9.8 Hz, CH), 130.5 (J_{C-P}=12 Hz, CH), 118.2 (C), 117.1 (C), 82.3 (C), 70.6 (CH), 21.8 (J_{C-P}=51.8 Hz, CH_2), 21.7 (J_{C-P}=2.3 Hz, CH_2), 19.2 (J_{C-P}=18.8 Hz, CH_2).

4.2. (5*E*,7*E*,9*E*)-Undeca-5,7,9-trien-1-yne (6)

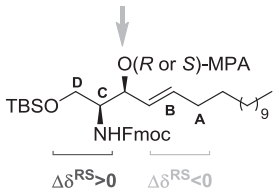
To a suspension of phosphonium iodide **5** (6.3 g, 13.8 mmol) in anhydrous THF (150 mL) under Ar was added HMPA (7.5 mL, 5% vol) at rt and then cooled to −78 °C. To the reaction mixture was added



Scheme 2. (a) **6** (5*E*/*Z*: 4/1), $Cp_2Zr(H)Cl$, $AgClO_4$, DCM, 0 °C to rt, 40 min, (*anti*/*syn* 1:1) (50%); (b) idem from (all-*E*)-**7**, (*anti*/*syn* 1:1) (20%); (c) CH_3COCl (3% vol), MeOH, rt, 1 h, quantitative (*anti*/*syn* 1:1 from **10**).



Scheme 3. (a) *N,O*-dimethylhydroxylamine·(HCl), DCM, Me_3Al (2 M in toluene) (93%); (b) $LiAlH_4$. THF, (90%); (c) (all-*E*)-**7**, $Cp_2Zr(H)Cl$, $ZnCl_2$, DCM, 0 °C to rt, 40 min, (*anti*/*syn*=1:1.5) (20%); (d) 1) TBAF (1 M in THF), THF, rt, 90 min; 2) Palmitic acid, HOBt, EDC, Et_3N, DCM, rt, 90 min, (*anti*/*syn*: 1:1.5), (20% combined).



$\Delta\delta^{RS}$ values (*syn* diastereomer, ^{1}H-NMR spectra)

	δ H_A	δ H_B	δ H_C	δ H_D
R-MPA	1.88	5.54	3.85	3.52
S-MPA	2.01	5.80	3.79	3.22
$\Delta\delta^{RS}$	-0.13	-0.26	0.06	0.30

Fig. 2. (*R*)- and (*S*)-MPA derivatives of the major *syn* diastereomer from the alkenylation of aldehyde **14** with 1-tetradecenylzirconocene.

dropwise a solution of *n*-butyllithium (2.5 M in hexanes, 5.8 mL, 14.6 mmol) and then was warmed to 0 °C with stirring for 1 h. After cooling the reddish orange solution to −78 °C, commercial *trans,-trans*-2,4-hexadienal (1 mL, 8.6 mmol) was added dropwise under Ar and protected from light. After 4 h the conversion was complete. Then, MeOH (60 mL) was added at −78 °C and the resulting mixture was gradually warmed overnight to rt. The mixture was concentrated under reduced pressure and then extracted with Et_2O (3×50 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (hexane) with silica gel neutralized with Et_3N (1% vol, using hexane as solvent) to afford **6** as a pale yellow oil in 80% yield, (5*E*:*Z*): (1:1).

Isomerization of the C5–C6 double bond: To a mixture of the above isomers **6** (50 mg, 0.3 mmol) in hexane (125 mL) under Ar, was added a saturated solution of iodine in hexane (5 μL). The resulting solution was heated at 69 °C for 20 min, after which the solution was cooled to rt. The mixture was washed thoroughly with saturated aqueous sodium metabisulfite ($Na_2S_2O_5$) and the yellow solution was washed with water, dried, and concentrated under reduced pressure to furnish **6** (quantitative) as a pale yellow oil of a mixture of diastereomers (5*E*:*Z*, 4:1).

Rf: 0.36 (hexane).

^{1}H NMR ($CDCl_3$): (5*E*,7*E*,9*E*) δ 6.23–6.00 (m, 4H), 5.78–5.62 (m, 2H), 2.37–2.22 (m, 2H), 1.97 (t, *J*=2.5 Hz, 1H), 1.77 (dd, *J*=7.0, 1.5 Hz, 3H).

(5*Z*,7*E*,9*E*) δ 6.42–6.29 (m, 1H), 6.23–6.02 (m, 3H), 5.81–5.66 (m, 1H), 5.49–5.38 (m, 1H), 2.47–2.39 (m, 2H), 2.29–2.22 (m, 2H), 1.97 (t, *J*=3.0 Hz, 1H), 1.79 (d, *J*=7.0 Hz, 3H).

^{13}C NMR ($CDCl_3$): (5*E*,7*E*,9*E*) δ 131.9 (CH), 131.8 (CH), 131.5 (CH), 130.2 (CH), 130.1 (CH), 129.6 (CH), 84.0 (C), 68.8 (CH), 31.9 (CH_2), 18.8 (CH_2), 18.4 (CH_3).

(5*Z*,7*E*,9*E*) δ 133.8 (CH), 131.8 (CH), 130.3 (CH), 130.2 (CH), 129.0 (CH), 125.4 (CH), 84.0 (C), 68.8 (CH), 27.1 (CH_2), 19.0 (CH_2), 18.5 (CH_3).

Analytical HPLC for **6**. Column (Kromasil 100, C18, 5 μm, 15×0.4 cm). Isocratic method (30:70, H_2O:ACN). Sample volume: 25 μL; **6** (1 mg/mL DMSO); Rt: 6.6 min (*Z*-isomer); Rt: 7.1 min (*E*-isomer) (λ: 254 nm).

4.3. (5*E*,7*E*,9*E*,11*E*,13*E*)-Pentadeca-5,7,9,11,13-pentaen-1-yne (7)

Using the protocol described above for **6**, pentaenyne **7** (650 mg, 55% yield) was obtained from phosphonium salt **5** (4.5 g, 9.8 mmol) and *trans*-2,4,6,8-hexatetraenal[30] (0.70 mL, 6.1 mmol) as a mixture of isomers 5*E*:5*Z*: 2:1. Isomerization of the C5–C6 double bond led to a 5*E*:5*Z*: 6:1 mixture of isomers. Purification by preparative HPLC afforded pure **7** (see conditions below).

Rf: 0.30 (hexane).

^{1}H NMR ($CDCl_3$): (5*E*,7*E*,9*E*,11*E*,13*E*) δ 6.37–6.03 (m, 8H), 5.73 (dq, *J*=14.5, 7.0 Hz, 2H), 2.40–2.24 (m, 4H), 1.97 (t, *J*=2.5 Hz, 1H), 1.78 (dd, *J*=7.0, 1.4 Hz, 3H).

(5*Z*,7*E*,9*E*,11*E*,13*E*) δ 6.75–6.35 (m, 2H), 6.33–5.86 (m, 7H), 5.49 (ddd, *J*=18.0, 9.0, 7.0 Hz, 1H), 2.50–2.40 (m, 2H), 2.39–2.31 (m, 2H), 1.97 (t, *J*=2.5 Hz, 1H), 1.82 (d, *J*=7.0 Hz, 3H).

^{13}C NMR ($CDCl_3$): (5*E*,7*E*,9*E*,11*E*,13*E*) δ 133.3 (CH), 133.1 (CH), 132.5 (CH), 132.4 (CH), 132.3 (CH), 132.1 (CH), 132.0 (CH), 131.9 (CH), 130.7 (CH), 130.3 (CH), 83.92 (C), 68.9 (CH), 32.0 (CH_2), 18.8 (CH_2), 18.5 (CH_3).

(5*Z*,7*E*,9*E*,11*E*,13*E*) δ 133.8 (CH), 133.7 (CH), 133.6 (CH), 132.1 (CH), 132.0 (CH), 130.6 (CH), 130.5 (CH), 130.3 (CH), 129.8 (CH), 127.6 (CH), 83.9 (C), 68.8 (CH), 27.2 (CH_2), 18.9 (CH_2), 18.6 (CH_3).

Preparative-HPLC for (all-*E*)-**7**: Column (X-Bridge C18, 5 μm OBD, 19×250 mm). Isocratic method (35:65, H_2O:ACN, 10 mL/min); Rt: 35.0 min; λ: 330 nm.

4.4. (*S*)-*tert*-Butyl 4-((*R*,6*E*,8*E*,10*E*)-1-hydroxydodeca-6,8,10-trien-2-yn-1-yl)-2,2-dimethyloxazolidine-3-carboxylate (8)

To a stirred solution of alkyne **6** (5*E*/*Z* 4/1) (400 mg, 2.4 mmol) in anhydrous THF (40 mL), was added dropwise a solution of LDA (3.3 mmol, 1.8 M in THF/heptane/ethylbenzene) at −78 °C under Ar. The mixture was stirred at −78 °C for 30 min, and then was added a solution of Garner's aldehyde (815 mg, 3.6 mmol) in THF (20 mL). After 3 h, a saturated aqueous NH_4Cl (5 mL) solution was added to the reaction mixture at −78 °C and was warmed to rt. The resulting white suspension was diluted with H_2O, and extracted with Et_2O (3×50 mL). The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane:EtOAc, 90:10) with silica-gel neutralized with Et_3N (1% vol, using hexane as solvent) to afford *erythro*-**8** in 45% yield (enriched in the (*all-E*) isomer).

Rf: 0.34 (hexane:EtOAc; 8:2); **$[\alpha]_D$**: −22.6 (*c* 1.3, $CHCl_3$)

^{1}H NMR ($CDCl_3$): Data for (*all-E*)-isomer. δ 6.25–5.95 (m, 4H), 5.77–5.60 (m, 2H), 4.62–4.40 (m, 1H), 4.21–3.82 (m, 3H), 2.33–2.25 (m, 4H), 1.76 (d, *J*=7.0 Hz, 3H), 1.51 (s, 6H), 1.49 (s, 9H).

^{13}C NMR ($CDCl_3$): Some splitting observed due to rotamers. δ 154.2 (C), 133.6 (CH), 131.8 (CH), 131.7 (CH), 130.1 (CH), 129.4 (CH), 125.4 (CH), 95.0 (C), 85.8 (C), 81.3 (C), 78.7 (C), 64.8 (CH_2), 64.0 (CH), 62.8 (CH), 32.0 (CH_2), 28.5 (CH_3), 28.3 (CH_3), 19.1(CH_2), 18.3 (CH_3).

ESI-MS *m*/*z* $C_{22}H_{33}NO_4$ $[M+Na]^+$ Found:398.2315; Calculated: 398.2307.

4.5. (*S*)-*tert*-Butyl 4-((*R*,6*E*,8*E*,10*E*,12*E*,14*E*)-1-hydroxyhexadeca-6,8,10,12,14-pentaen-2-yn-1-yl)-2,2-dimethyloxazolidine-3-carboxylate (9)

Following the method described for **8**, condensation of Garner's aldehyde with (all-*E*)-**7** (500 mg, 2.5 mmol) afforded *erytrho*-**9** in 25% yield.

Rf: 0.24 (hexane:EtOAc; 8:2); **$[\alpha]_D$**: −28.2 (*c* 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$): Data described for (*all-E*)-isomer. **(6*E*,8*E*,10*E*,12*E*,14*E*)** δ 6.34–6.00 (m, 8H), 5.83–5.62 (m, 2H), 4.74–4.58 (m, 1H), 4.59–4.45 (m, 1H), 4.24–3.81 (m, 3H), 2.35–2.24 (m, 4H), 1.78 (dt, *J*=7.0, 2.0 Hz, 3H), 1.63–1.53 (m, 6H), 1.56–1.39 (m, 9H).

^{13}C NMR ($CDCl_3$): (6*E*,8*E*,10*E*,12*E*,14*E*). δ 154.3(C), 133.8 (CH), 133.7 (CH), 132.3 (CH), 132.1 (CH), 132.0 (CH), 131.9 (CH), 130.7 (CH), 130.6 (CH), 130.5 (CH), 130.3 (CH), 95.1 (C), 86.0 (C), 81.4 (C), 79.3 (C), 65.2 (CH_2), 64.4 (CH), 62.9 (CH), 53.6 (C), 32.1 (CH_2), 29.8 (CH_3), 26.0 (CH_3), 19.3 (CH_2), 18.5 (CH_3).

ESI-MS *m*/*z* $C_{26}H_{37}NO_4$ $[M+Na]^+$ Found:450.2610; Calculated: 450.2620.

Analytical HPLC conditions for **9**: Column (Kromasil 100, C18, 5 μm, 15×0.4 cm). Isocratic method (30:70, H_2O:ACN). Sample (v:25 μL, sample 1 mg/mL DMSO); (λ: 331 nm). Rt: 23.6 min.

4.6. (*S*)-*tert*-Butyl 4-((*R*:*S*,2*E*,6*E*,8*E*,10*E*)-1-hydroxydodeca-2,6,8,10-tetraen-1-yl)-2,2-dimethyloxazolidine-3-carboxylate (10)

To a flame-dried Schlenk with $Cp_2Zr(H)Cl$ (350 mg, 1.4 mmol), was added a solution of alkyne **6** (5*E*/*Z*: 4/1; 200 mg, 1.4 mmol) in anhydrous DCM (1.7 mL) at 0 °C under Ar and protected from light. During warming to room temperature (20 min), the zirconocene complex gradually dissolved to give a clear orange solution. A solution of Garner's aldehyde (224 mg, 1.0 mmol) in DCM (1.2 mL) was added followed by $AgClO_4$ (20% mol). After 20 min, the reaction mixture turned dark red and was diluted with Et_2O and quenched by addition of 3 mL of saturated $NaHCO_3$. The mixture was filtered through a pad of Celite® and extracted with Et_2O (3×10 mL). The combined ethereal solution were washed with brine, dried and concentrated under reduced pressure. Flash chromatography with silica-gel neutralized with Et_3N (1% vol, using hexane as solvent) in hexane:EtOAc (stepwise gradient from 0 to 12% of EtOAc) afforded **10** (50%) as an *anti*:*syn* 1:1mixture of diastereomers.

Rf: (*anti*) 0.38; (*syn*) 0.32 (hexane:EtOAc; 8:2).

^{1}H NMR ($CDCl_3$): Mixture of diastereomers δ 6.19–5.93 (m, 4H), 5.77–5.57 (m, 3H), 5.51–5.38 (m, 1H), 4.45–3.73 (m, 5H), 2.24–1.96 (m, 4H), 1.74 (d, *J*=6.5 Hz, 3H), 1.47 (s, 6H), 1.46 (s, 9H).

^{13}C NMR ($CDCl_3$): δ 152.8 (C), 133.0 (CH), 132.4 (CH), 131.9 (CH), 131.3 (CH), 131.2 (CH), 130.5 (CH), 130.4 (CH), 129. (CH), 94.6 (C), 81.2 (C), 74.1 (CH), 65.0 (CH_2), 62.4 (CH), 32.5 (CH_2), 32.3 (CH_2), 28.5 (2 CH_3), 18.4 (CH_3).

ESI-MS *m*/*z* $C_{22}H_{35}NO_4$ $[M+Na]^+$ Found: 400.2379; Calculated: 400.2464.

Analytical HPLC for **10** (*anti*:*syn* 1:1): Column (Kromasil 100, C18, 5 μm, 15×0.4 cm). Isocratic method (30:70, H_2O:ACN). Sample (v:25 μL, sample 1 mg/mL DMSO); (λ: 254 nm). Rt: 11.8 and 12.3 min.

4.7. (*S*) *tert*-Butyl 4-(*R*:*S*(2*E*,6*E*,8*E*,10*E*,12*E*,14*E*)-1-hydroxyhexadeca-2,6,8,10,12,14-hexaen-1-yl)-2,2-dimethyloxazolidine-3-carboxylate (11)

Following the same protocol used for **10**, condensation of alkenylzirconocene, obtained from alkyne **7** (200 mg, 1.0 mmol), with Garner's aldehyde afforded **11** in 20% yield as a 1:1 *syn*:*anti* mixture after flash chromatography with silica-gel neutralized with Et_3N (1% vol, using hexane as solvent) (hexane:EtOAc; stepwise gradient from 0 to 18% of EtOAc).

Rf: 0.30 (hexane:EtOAc; 8:2) (mixture *syn*:*anti* 1:1).

^{1}H NMR ($CDCl_3$): (*syn:anti:* 1:1). δ 6.28–6.00 (m, 7H), 5.80–5.58 (m, 3H), 5.56–5.37 (m, 2H), 4.44–4.30 (m, 1H), 4.26–4.06 (m, 2H), 4.06–3.77 (m, 3H), 2.25–2.11 (m, 4H), 1.78 (dd, *J*=7.5, 1.5 Hz, 3H), 1.50 (s, 6H), 1.48 (s, 9H).

^{13}C NMR ($CDCl_3$): (*syn:anti:* 1:1). δ 159.3 (C), 133.1 (CH), 132.9 (CH), 132.8 (CH), 132.8 (CH), 132.8 (CH), 132.7 (CH), 132.5 (CH), 132.1 (CH), 131.4 (CH), 131.2 (CH), 130.8 (CH), 130.1 (CH), 118.0 (C), 94.6 (C), 74.0 (CH), 65.0 (CH_2), 62.4 (CH), 32.6 (CH_2), 32.3 (CH_2), 28.6 (CH_3), 26.5 (CH_3), 18.6 (CH_3).

ESI-MS *m*/*z* $C_{26}H_{39}NO_4$ $[M+Na]^+$ Found: 452.2776; Calculated: 452.2777; $[M+H]^+$ Found:430.2995; Calculated: 430.2957.

4.8. Methyl *N*-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-*O*-(*tert*-butyldimethylsilyl)-*L*-serinate (12)

To a solution of Fmoc serine methyl ester[55] (8.1 g, 23.7 mmol) in anhydrous DCM (180 mL) was added TBDMSCl (7.1 g, 47.1 mmol) followed by imidazole (4.8 g, 71.2 mmol) at 0 °C. The reaction mixture was warmed to rt and stirred for 3 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl solution and then was extracted with DCM (3×150 mL). The combined organic extracts were washed with brine, dried with Mg_2SO_4 and concentrated. The residue was purified by flash chromatography (hexane:EtOAc; 95:5) to afford **12** (10.3 g, 94%) as a white solid.

Rf: 0.81 (hexane:EtOAc; 7:3); **$[\alpha]_D$** +19.6 (*c* 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ 7.77 (d, *J*=7.5 Hz, 2H), 7.62 (t, *J*=8.0 Hz, 2H), 7.40 (t, *J*=7.5 Hz, 2H), 7.32 (td, *J*=7.5, 1.0 Hz, 2H), 5.64 (d, *J*=9.0 Hz, 1H), 4.48–4.43 (m, 1H), 4.42–4.33 (m, 2H), 4.26 (t, *J*=7.5 Hz, 1H), 4.08 (dd, *J*=10.0, 3.0 Hz, 1H), 3.87 (dd, *J*=10.0, 3.0 Hz, 1H), 3.77 (s, 3H), 0.89 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H).

^{13}C NMR ($CDCl_3$): δ 171.1 (C), 156.1 (C), 144.1 (C), 143.9 (C), 141.4 (C), 127.9 (CH), 127.2 (CH), 125.3 (CH), 125.3(CH), 120.1 (CH), 67.4 (CH_2), 63.8 (CH_2), 56.1 (CH), 52.6 (CH_3), 47.3 (CH), 25.9 (CH_3), 18.4 (C), −5.4 (CH_3), −5.5 (CH_3).

ESI-MS *m*/*z* $C_{25}H_{33}NO_5Si$ $[M+Na]^+$ Found: 478.2008; Calculated: 478.2026; $[M+H]^+$ Found: 456.2193; Calculated: 456.2206.

4.9. (9*H*-Fluoren-9-yl)methyl (*S*)-(3,8,8,9,9-pentamethyl-4-oxo-2,7-dioxa-3-aza-8-siladecan-5-yl)carbamate (13)

A solution of *N*,*O*-dimethylhydroxylamine·(HCl) (13.5 g, 138.3 mmol) in DCM (50 mL) under Ar, was treated at 0 °C with 69.1 mL Me_3Al (2 M in toluene, 138.3 mmol). The reaction mixture was stirred at rt for 30 min, treated with a solution of **12** (21 g, 46.1 mmol) in DCM (150 mL) and heated to reflux for 16 h. The reaction mixture was next cooled to 0 °C and carefully quenched with aqueous sodium potassium tartrate (10%, 75 mL). After stirring at rt for 1 h, the resulting suspension was filtered through a pad of Celite®, which was then washed with DCM. The filtrates were concentrated under reduced pressure to give the crude product, which upon purification by flash chromatography (hexane:EtOAc; 85:15) afforded the Weinreb amide **13** (20.8 g, 93%) as a colorless oil.

Rf: 0.35 (hexane:EtOAc; 7:3); **[α]$_D$** +13.9 (*c* 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ 7.76 (d, *J*=7.5 Hz, 2H), 7.61 (t, *J*=8.0 Hz, 2H), 7.40 (tq, *J*=7.5, 1.0 Hz, 2H), 7.31 (tt, *J*=7.5, 1.0 Hz, 2H), 5.69 (d, *J*=9.0 Hz, 1H), 4.89–4.79 (m, 1H), 4.36 (d, *J*=7.5 Hz, 2H), 4.24 (t, *J*=7.0 Hz, 1H), 3.88 (qd, *J*=10.0, 5.0 Hz, 2H), 3.77 (s, 3H), 3.24 (s, 3H), 0.89 (s, 9H), 0.05 (d, *J*=2.0 Hz, 6H).

^{13}C NMR ($CDCl_3$): The methyl group bonded to the Weinreb amide is not observed in the ^{13}C spectra. δ 156.1 (C), 156.1 (C), 144.1 (C), 144.0 (C), 141.4 (C), 127.8 (CH), 127.2 (CH), 125.4 (CH), 125.3 (CH), 120.1 (CH), 67.3 (CH_2), 63.5 (CH_2), 61.7 (CH_3), 53.2 (CH), 47.3 (CH), 25.9 (CH_3), −5.4 (CH_3).

ESI-MS *m*/*z* $C_{26}H_{36}N_2O_5Si$ $[M+Na]^+$ Found: 507.2280; Calculated: 507.2291; $[M+H]^+$ Found: 485.2451; Calculated: 485.2472.

4.10. (*S*)-(9*H*-Fluoren-9-yl)methyl (1-((*tert*-butyldimethyl silyl)oxy)-3-oxopropan-2-yl)-carbamate (14)

To a solution of Weinreb amide **13** (5 g, 10.3 mmol) in anhydrous THF (100 mL) at −40 °C, was added dropwise a solution of $LiAlH_4$ (0.7 g, 19.6 mmol) in 20 mL of THF. After stirring for 2.5 h at −40 °C, the reaction was quenched by addition of saturated aqueous Na_2SO_4 solution. The resulting white solid was filtered through a pad of Celite®, which was then washed with EtOAc. The filtrates were concentrated under reduced pressure to give the crude product, which was flash chromatographed (hexane:EtOAC; 98:12) to afford the aldehyde **14** (3.9 g, 90%) as a colorless oil.

Rf: 0.44 (hexane:EtOAc; 8:2); **[α]$_D$** +19.3 (*c* 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ 9.67 (s, 1H), 7.77 (d, *J*=8.0 Hz, 2H), 7.65–7.57 (m, 2H), 7.41 (t, *J*=7.5 Hz, 2H), 7.37–7.28 (m, 2H), 5.65 (d, *J*=7.0 Hz, 1H), 4.42 (d, *J*=7.0 Hz, 2H), 4.35 (dt, *J*=7.0, 4.0 Hz, 1H), 4.29–4.18 (m, 3H), 3.90 (dd, *J*=10.5, 4.0 Hz, 1H), 0.88 (s, 9H), 0.06 (s, 6H).

^{13}C NMR ($CDCl_3$): δ 198.9 (CH), 156.2 (C), 144.0 (C), 143.9 (C), 141.5 (C), 127.9 (CH), 127.2 (CH), 125.3 (CH), 125.2 (CH), 120.2 (CH), 67.4 (CH_2), 62.0 (CH), 61.4 (CH_2), 47.3 (CH), 25.9 (CH_3), −5.4 (CH_3), −5.4 (CH_3).

ESI-MS *m*/*z* $C_{24}H_{31}NO_4Si$ $[M+H]^+$ Found: 426.2087; Calculated: 426.2101.

4.11. (9*H*-Fluoren-9-yl)methyl ((2*S*,3*R*:*S*,4*E*,8*E*,10*E*, 12*E*,14*E*,16*E*)-1-((*tert*-butyldimethyl-silyl)oxy)-3-hydroxy-octadeca-4,8,10,12,14,16-hexaen-2-yl)carbamate (15)

To a flame-dried Schlenk with a suspension of $Cp_2Zr(H)Cl$ (100 mg, 0.4 mmol) in DCM (0.4 mL), was added a solution of the alkyne (all-*E*)-**7** (55 mg, 0.3 mmol) in anhydrous DCM (0.4 mL), at 0 °C under Ar and protected from light. During warming to room temperature, the zirconocene complex gradually dissolved to give a clear red solution (20 min). A solution of the aldehyde **14** (91 mg, 0.2 mmol) in DCM (0.6 mL), previously activated for 10 min with $ZnCl_2$ (15 mg, 0.1 mmol, dried under vacuum for 1 h before use), was added to the reaction mixture. The solution was stirred for 30 min at rt and turned clear orange. Next, it was diluted with DCM (2 mL) and stirred with a 10% aqueous potassium sodium tartrate solution (2 mL) for 10 min. The resulting suspension was filtered through a pad of Celite® and washed thoroughly with DCM (5 mL). The combined filtrates were successively washed with H_2O and brine. The aqueous phase was extracted with DCM (3×10 mL), and the combined organic layers were dried over Mg_2SO_4. Purification by flash chromatography with silica-gel neutralized with Et_3N (1% vol, using hexane as solvent) (hexane:EtOAc; stepwise gradient from 0 to 10% of EtOAc) afforded **15** in 20% yield as a 1:1.5 *anti*:*syn* mixture.

Rf: 0.43 (*syn*); 0.37 (*anti*) (hexane:EtOAc; 8:2).

^{1}H NMR ($CDCl_3$): From a mixture of diastereomers.

(*anti*): δ 7.77 (d, *J*=8.0 Hz, 2H), 7.60 (d, *J*=7.5 Hz, 2H), 7.40 (ddt, *J*=8.5, 7.5, 1.5 Hz, 2H), 7.31 (tt, *J*=7.5, 2.0 Hz, 2H), 6.30–5.88 (m, 8H), 5.91–5.21 (m, 4H), 5.05 (d, *J*=13.0 Hz, 1H), 4.54–4.30 (m, 3H), 4.23 (t, *J*=7.0 Hz, 1H), 3.96 (d, *J*=10.0 Hz, 1H), 3.76 (d, *J*=10.0 Hz, 1H), 3.71–3.62 (m, 1H), 3.26 (d, *J*=5.0 Hz, 1H), 2.24–1.94 (m, 3H), 1.78 (dd, *J*=7.0, 1.5 Hz, 3H),0.91 (s, 9H), 0.08 (s, 6H).

(*syn*): δ 7.77 (d, *J*=8.0 Hz, 2H), 7.60 (d, *J*=7.5 Hz, 2H), 7.40 (ddt, *J*=8.5, 7.5, 1.5 Hz, 2H), 7.31 (tt, *J*=7.5, 2.0 Hz, 2H), 6.30–5.88 (m, 8H), 5.91–5.21 (m, 4H), 4.97 (d, *J*=12.0 Hz, 2H), 4.54–4.30 (m, 3H), 4.23 (t, *J*=7.0 Hz, 1H), 3.89–3.81 (m, 2H), 3.70–3.61 (m, 1H), 3.17 (d, *J*=12.5 Hz, 1H), 2.24–1.94 (m, 4H), 1.78 (dd, *J*=7.0, 1.4 Hz, 3H), 0.91 (s, 9H), 0.08 (s, 6H).

^{13}C NMR ($CDCl_3$): (mixture *syn*:*anti*): δ 156.3 (C), 144.1 (C), 141.5 (C), 133.1 (CH), 133.1 (CH), 132.8 (CH), 132.5 (CH), 132.4 (CH), 132.1 (CH), 132.1 (CH), 131.0 (CH), 130.8 (CH), 130.2 (CH), 129.9 (CH), 129.6 (CH), 127.85 (CH), 127.2 (CH), 125.23 (CH), 120.1 (CH), 73.4 (CH), 67.1 (CH_2), 65.5 (CH_2), 55.2 (CH), 47.4 (CH), 32.2 (CH_2), 32.1 (CH), 26.0 (CH_3), 18.3 (CH_3), −5.4 (CH_3).

ESI-MS *m*/*z* $C_{39}H_{51}NO_4Si$ $[M+Na]^+$ Found: 648.3478; Calculated: 648.3485; $[M+H]^+$ Found: 626.3668; Calculated: 626.3666.

4.12. (2*S*,3*R*,8*E*,10*E*,12*E*)-1,3-Dihydroxytetradeca-8,10,12-trien-4-yn-2-amonium chloride (1)

To a solution of **8** (200 mg, 0.5 mmol) in MeOH (35 mL) was added dropwise acetyl chloride (540 μL, 1.5% vol) at −30 °C. The resulting mixture was vigorously stirred and allowed to warm to rt for 30 min. Next, addition of a saturated aqueous $NaHCO_3$ solution and concentration under reduce pressure, afforded a dark green residue, which was taken up in DCM, dried over $MgSO_4$, filtered through a cotton pad and concentrated under reduced pressure to give 80 mg (60% yield) of amino diol **1**·**HCl**.

Rf: 0.60 (DCM:MeOH; 8:2); **[α]$_D$**: −3.1 (*c* 1.2, CH_3OH).

^{1}H NMR (CD_3OD): δ 6.46–5.93 (m, 4H), 5.86–5.60 (m, 2H), 4.71–4.60 (m, 1H), 3.99–3.74 (m, 2H), 3.35–3.27 (m, 1H), 2.46–2.23 (m, 4H), 1.77 (d, *J*=7.0 Hz, 3H).

^{13}C NMR (CD_3OD): δ 131.7 (CH), 131.7 (CH), 131.6 (CH), 131.1 (CH), 129.9 (CH), 128.6 (CH), 87.4 (C), 76.4 (C), 59.3 (CH), 58.4 (CH_2), 57.2 (CH), 31.3 (CH_2), 18.3 (CH_2), 17.0 (CH_3).

ESI-MS *m*/*z* $C_{14}H_{21}NO_2$ $[M+H]^+$ Found:236.1655; Calculated: 236.1651.

4.13. (2*S*,3*R*,8*E*,10*E*,12*E*,14*E*,16*E*)-1,3-Dihydroxyoctadeca-8,10,12,14,16-pentaen-4-yn-2-amonium chloride (2)

Following the same protocol described for **1**, the protected amino diol **9** (20 mg, 0.04 mmol) afforded amino diol **2**·**HCl** 6 mg in 40% yield.

Rf: 0.46 (DCM:MeOH; 8:2); **[α]$_D$**: −4.2 (*c* 1, CH_3OH).

^{1}H NMR (CD_3OD): δ 6.38–5.94 (m, 8H), 5.84–5.63 (m, 2H), 4.71–4.62 (m, 1H), 4.02–3.87 (m, 1H), 3.86–3.71 (m, 2H), 2.45–2.13 (m, 4H), 1.77 (d, *J*=6.5 Hz, 3H).

ESI-MS *m*/*z* $C_{18}H_{25}NO_2$ $[M+Na]^+$ Found: 310.1779; Calculated: 310.1783; $[M+H]^+$ Found:288.1954; Calculated: 288.1964.

Analytical HPLC conditions for **2**·**HCl**: Column (Kromasil 100, C18, 5 μm, 15×0.4 cm). Isocratic method (60:40, Buffer sodium acetate (pH=4.8, 140 mM):ACN). Sample (v:50 μL, **2** at 1 mg/mL DMSO); (λ: 331 nm). Rt: 8.8 min; λ_{abs} (nm) 275, 315, 346; λ_{em} (nm) 440, 456, 470.

Φ_F (λ_{ex}: 346 nm, ethanol, 9,10-DPA as reference): 0.065; ε (λ_{ex} max=325 nm): 12,790 M^{-1} cm^{-1}.

4.14. (2*S*,3(*R*:*S*),4*E*,8*Z*:*E*,10*E*,12*E*)-1,3-Dihydroxytetradeca-4,8,10,12-tetraen-2-aminium chloride (3)

Following the method described for amino diol **1**, protected **10** (*syn*:*anti* 1:1) (185 mg, 0.5 mmol) afforded aminodiol **3**·**HCl** (160 mg) in quantitative yield as a 1:1 *syn*:*anti* mixture.

Rf: (*syn*:*anti* 1:1) 0.40 (DCM:MeOH; 85:15).

^{1}H NMR ($CDCl_3$): (2*S*,3*R*:*S*) (4*E*,8*E*,10*E*,12*E*) δ 6.26–5.80 (m, 5H), 5.76–5.42 (m, 3H), 4.47–4.26 (m, 1H), 3.92 (s, 1H), 3.84–3.42 (m, 2H), 3.31–3.20 (m, 1H), 2.27–2.04 (m, 4H), 1.76 (d, *J*=7.0 Hz, 3H).

^{13}C NMR ($CDCl_3$): (2*S*,3*R*:*S*) (4*E*,8*E*,10*E*,12*E*) δ 133.5 (CH), 133.0 (CH), 131.9 (CH), 131.3 (CH), 130.5 (CH), 130.4 (CH), 129.9 (CH), 129.5 (CH), 73.2 (CH), 63.8 (CH_2), 56.8 (CH), 32.4 (CH_2), 32.2 (CH_2), 18.4 (CH_3).

ESI-MS *m*/*z* $C_{14}H_{23}NO_2$ $[M+Na]^+$ Found: 260.1610; Calculated: 260.1626.

Analytical HPLC conditions: Column (Kromasil 100, C18, 5 μm, 15×0.4 cm). Isocratic method (25:75, buffer sodium acetate (pH:4.8, 140 mM): ACN). Sample (v:25 μL, **3** at 1 mg/mL DMSO); (λ: 254 nm). Rt: 17.6 min and 19.7 min.

4.15. *N*-((2*S*,3*R*:*S*,4*E*,8*E*,10*E*,12*E*,14*E*,16*E*)-1,3-Dihydroxyoctadeca-4,8,10,12,14,16-hexaen-2-yl) palmitamide (4)

Compound **15** (20 mg, 0.03 mmol) compound was dissolved in anhydrous THF (0.5 mL) under Ar. Next, 35 μL of TBAF solution was added via syringe (0.04 mmol, 1 M in THF). The solution turned orange immediately. After being stirred for 1 h at rt, it was observed by TLC part of the starting material, then to the reaction mixture was added an additional 15 μL of TBAF solution (1 M in THF) to fulfil the conversion. After 30 min, a saturated aqueous NH_4Cl (0.5 mL) solution was added, and the mixture was dried with $MgSO_4$ and the resulting amino diol was eluted to another flask with DCM. The solvent was concentrated under reduced pressure and the crude was used in the next reaction without further purification.

To a solution of EDC (10 mg, 0.05 mmol) and HOBt (5 mg, 0.04 mmol) in anhydrous DCM (200 μL) was added palmitic acid (9 mg, 0.04 mmol) in DCM (300 μL) under Ar. The resulting mixture was vigorously stirred at rt for 15 min, and next added dropwise to a solution of the amino diol intermediate (0.03 mmol) and Et_3N (9 μL, 0.06 mmol) in anhydrous DCM (500 μL). The reaction mixture was stirred at rt for 1.5 h and then was diluted by addition of DCM (2 mL), and washed successively with water (5 mL) and brine (5 mL). The organic layer was dried over $MgSO_4$, and filtered and concentrated under reduced pressure. The crude was purified by flash column chromatography with silica-gel neutralized with Et_3N (1% vol, using hexane as solvent) (hexane:EtOAc; stepwise gradient from 0 to 5% of AcOEt, followed by stepwise gradient from 0 to 4% of MeOH) affording a low yield of **4** (4 mg, 20% combined yield).

Rf: (mixture of diastereomers) 0.58 (DCM:MeOH; 9:1).

^{1}H NMR ($CDCl_3$): δ 6.27–5.80 (m, 5H), 5.86–5.52 (m, 3H), 5.53–5.30 (m, 2H), 5.13–5.03 (m, 1H), 5.03–4.83 (m, 2H), 4.42–4.26 (m, 1H), 4.22–3.99 (m, 3H), 2.32–2.19 (m, 1H), 2.18–2.05 (m, 3H), 2.05–1.92 (m, 4H), 1.73 (d, *J*=7.0 Hz, 3H), 1.60–1.46 (m, 2H), 1.20 (s, 22H), 0.83 (t, *J*=7.0 Hz, 3H).

ESI-MS *m*/*z* $C_{34}H_{57}NO_3$ $[M+H]^+$ Found: 528.4432; Calculated: 528.4417.

λ_{abs} (nm) 315, 325, 345; λ_{em} (nm) 440, 465, 500. Φ_F (λ_{ex}: 346 nm, ethanol, 9,10-DPA as reference): 0.070; ε (λ_{ex} max=325 nm): 10,781 M^{-1} cm^{-1}.

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