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Fluorescent Penta- and Hexaene Fatty Acids by a Wittig–Horner/Elimination Strategy

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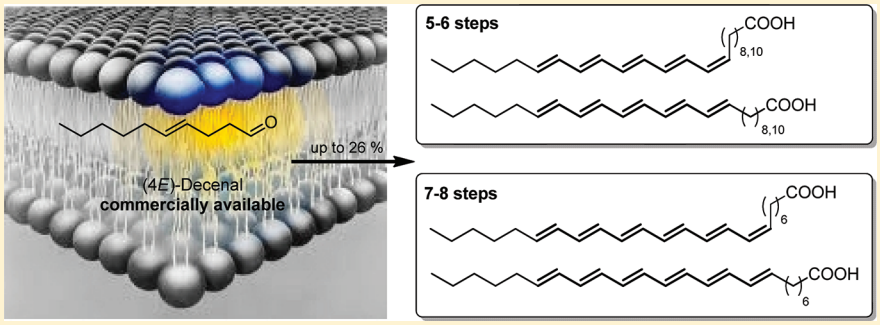
Supporting Information



ABSTRACT: Molecular fluorescent probes have revolutionized biochemical and biophysical studies in the last decades, but with regard to lipids there has been a lack of combining the slim shape of saturated acyl chains with fluorescent properties. Our strategy to pentaene and hexaene fatty acids builds upon commercially available 4-(*E*)-decenal, which is subjected to a Wittig–Horner reaction after chlorination in α-position. DBU-mediated β-elimination of HCl proceeding the olefination establishes a highly conjugated system to which a salt-free Wittig reaction adds a final double bond leading to a good (*Z*)-selectivity of 83–86%. The double bond geometry can be optionally isomerized with I_2 to furnish the all-(*E*)-species. The five conjugated alkene moieties result in a longest-wavelength absorption maximum of about 350 nm. A red-shift to 380 nm was realized by addition of another double bond employing a common Wittig–Horner prolongation sequence. Stokes shifts of about 7300 and 7800 cm^{-1}, respectively, were observed.

INTRODUCTION

Apart from carbohydrates and proteins, lipids embody another substance class of fundamental importance for metabolic processes and structural shapes of living cells. Whereas their role as an energy source as well as a constituent of lipid membranes is undisputed, modern studies embark on tracking more detailed metabolic pathways or question the extent of organization and lipid phase separation within a plasma membrane.[1] Such investigations have raised interest in visualizing fatty acid-containing cellular molecules and impelled the development of fluorescently active species. Some prominent examples are NBD-,[2] BODIPY-,[3] or pyrene-labeled[4] fatty acids (Figure 1).

Although these fluorescent species yielded a versatile bunch of meaningful and convincing new insights, other results may suffer either from the sterical perturbance or the polar properties of these fluorophores.[5] Building upon the work of Amat-Guerri,[6] Thiele and co-workers[7] introduced fluorescent pentaene fatty acids bearing five double bonds in a linear fashion at the end of an acyl chain (Figure 2). Integrated into several functional lipid species, their unique feature of mimicking natural counterparts was demonstrated with respect to microdomain formation and cellular metabolism.[7]

In this paper, we introduce an alternative, efficient, and convenient synthetic approach to a new class of pentaene and hexaene fatty acids **3**–**8** in which the fluorescent oligoene system is embedded as an integral part of the acyl chain (Figure 3). Our interest in sphingolipids guided us to provide the fatty acids with long acyl chains of 24 and 26 carbons referring to

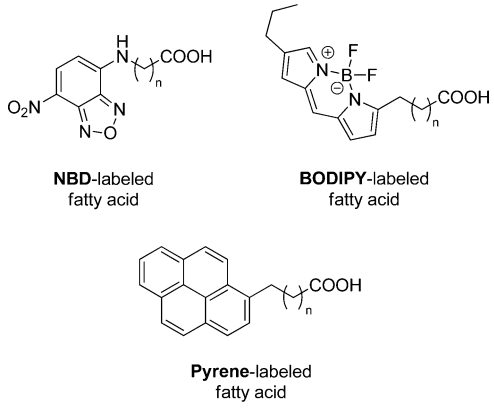


Figure 1. Examples of widely used fluorophores to derivatize fatty acids.

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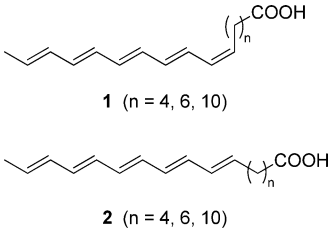


Figure 2. Pentaene fatty acids as biomimetic alternatives investigated by Thiele et al.[7]

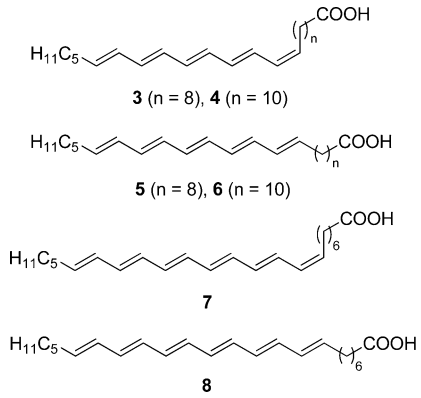


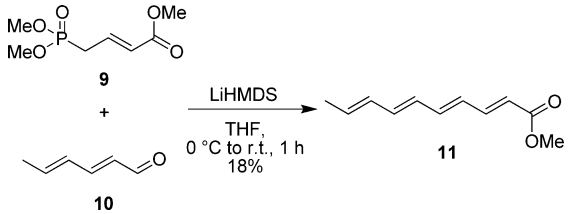
Figure 3. Pentaene and hexaene fatty acids **3**–**8** with an integrated fluorescent tag.

their widespread appearance in natural sphingolipid counterparts.

RESULTS AND DISCUSSION

A traditional approach to sensitive oligoene structures can be realized by cross-coupling reactions[8] or by a sequential strategy based on Wittig-type reactions,[9] which, as low-temperature methods, avoid harsh reaction conditions, as they are often demanded in common Pd-catalyzed coupling reactions. To shorten the synthetic route to fatty acids as investigated by Thiele,[7] we first envisioned a reaction of the unsaturated phosphonate derivative **9** with hexadienal **10** to furnish **11** (Scheme 1).

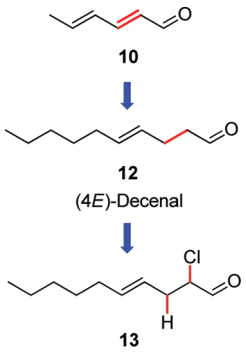
Scheme 1. Disappointing Reaction of Unsaturated Phosphonate 9 with Hexadienal 10



Several attempts to establish a satisfying reaction yield under various conditions turned out to be fruitless, possibly because of the variety of reaction pathways, which either arise from two nucleophilic centers of compound **9** and three electrophilic ones at **10** or because of the relative high basicity of deprotonated **9** in interaction with an enolizable aldehyde such as **10**.

Since the easily available phosphonate[10] **9** gave gratifying results with benzaldehyde, we decided to change the type of aldehyde used. Disguising its α-double bond with a chlorine substituent should improve its selective reactivity and provide an excellent substrate for a further elimination step (Scheme 2).

Scheme 2. Considerations for the Choice of a New Substrate with Respect to the Wittig–Horner Reaction and the Possibility to Introduce a Disguised Conjugation at (4*E*)-Decenal (12)

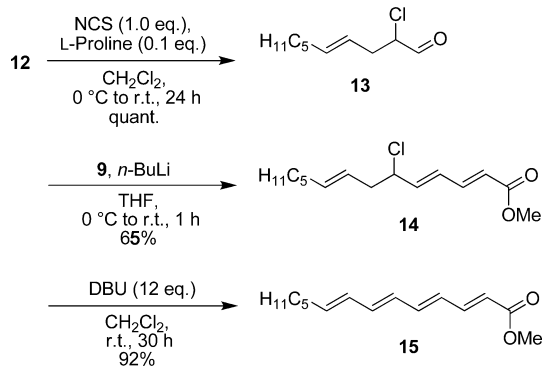


Thus, we selected commercially available (4*E*)-decenal (**12**) as starting material and were glad to see our choice appreciated with two crucial advantages. Embedding the oligoene structure not at the terminus, but further in the middle of the acyl chain the artificially incorporated rigidity of the π system should be significantly smoothened as a result of suppressed $\pi-\pi$ stacking.[11,12] An even more accurate copy of the untouched acyl chain would be the convenient consequence.

Indeed the embedded oligoene structure increased the solubility in organic solvents, preferably THF, in comparison with **1** and **2**. In contrast to a purification by means of crystallization as exemplified in the case of **1** and **2**, the fatty acids with an internal oligoene moiety were available in good yields by a convenient and reliable column chromatography employing traces of formic acid.

The synthesis commenced by chlorination of (4*E*)-decenal (**12**) using an equimolar amount of *N*-chlorosuccinimide (NCS) in almost quantitative yield (Scheme 3). L-Proline in

Scheme 3. α-Chlorination and Wittig–Horner Reaction As Preliminary Steps for the β-Elimination of HCl to Afford 15



catalytic amount was inevitable and acted as a proton shuttle.[13] A small excess of NCS enforces a double chlorination in the α-position and should be avoided. Aqueous workup at this point provides enough purity for the further steps.

Wittig–Horner reactions with α-halogenated aldehydes are literature-known[14] but might be problematic when bromine is chosen as substituent. Recently, a Polish group demonstrated

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an efficient alkylation by use of appropriate bases.[15] As anticipated, our chlorinated species **13** underwent straightforward olefination with phosphonate **9** to afford **14** in 68% yield as all-(*E*) product. For the crucial DBU-mediated[16] *β*-elimination of HCl to give the highly unsaturated ester **15**, a thorough optimization proved to be necessary (Table 1).

Table 1. Reaction Conditions for the DBU-Mediated *β*-Elimination of HCl to Afford 15

	temp	reaction time [h]	DBU [equiv]	yield [%]	note
1	0 °C, rt, reflux	12, 48, 24	3, 5[a]	12	
2	rt	48	2	28	incomplete elimination after 24 h
3	rt	32	6	41	incomplete elimination after 8 h
4	rt	30	12	92	

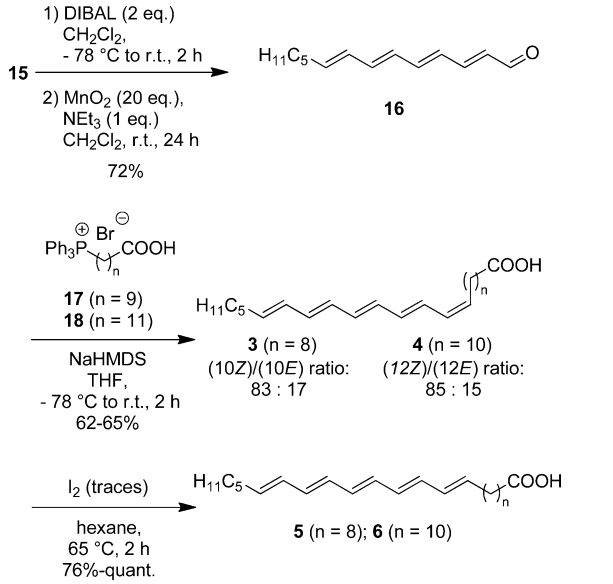
[a]Another 2 equiv were added after 12 h as well as after 48 h of reaction time.

Surprisingly, the elimination could not be established with satisfying yields by use of equimolar or small excess of base. Only 12 equiv of DBU and an accurate reaction time of 30 h furnished the highly conjugated ester in a yield of 92%. Longer reaction times seem to open a pathway for decomposition; decreasing the time leaves unconverted starting material being almost inseparable from the product by means of column chromatography.[17] We attribute the need for high amounts of DBU to the proneness of ester **14** to be enolized at its most acidic proton. Whereas this happens in equilibrium, the competitive *β*-elimination is irreversible. Both reaction pathways will benefit from higher amounts of DBU; however, the enolized species must still find a reactant, which could cause this to become a rate-determining step and handicap respective side reactions. The ratio of (6*E*)- to (6*Z*)-ester was determined to be 9:1, whereupon a final isomerization with iodine was able to turn each (*Z*)- to the respective (*E*)-geometry.

For the following transformation of the ester **15** to the homologous aldehyde **16**, the method of choice proved to be a one-pot reduction−oxidation protocol using DIBAL and MnO_2 affording the desired compound **16** in 72% yield (Scheme 4). Even at temperatures below −78 °C, 1 equiv of DIBAL led to a significant amount of the respective alcohol.[18] Triethylamine as an additive seems to play an essential role, we suppose either by weakening the Lewis acidity of aluminum fragments after the reduction or by moderating unconverted DIBAL before the addition of MnO_2. For purification of aldehyde **16**, a quick filtration over silica gel was found to be sufficient.

The concluding Wittig olefination is faced with two distinctions from its prototypical realization. First, the aldehyde is highly conjugated and thus prone to give a higher (*E*)-selectivity. Second, the anionic carboxylate group at the ylide is believed to open the initially formed *cis*-oxaphosphetanes by a "biting-back" mechanism.[19] Both aspects pronounce a stereochemical drift from the kinetically formed (*Z*)-configured double bond to the thermodynamically favored (*E*)-configured system. Maryanoff investigated such a dependence encompassing amino, carboxylate, and hydroxyl groups in different distances from the ylide center.[19] Representative examples of three different phosphonium bromides **19**−**21**, and their reaction with aldehydes are provided in Figure 4.

Scheme 4. Final Wittig Olefination under Salt-Free Conditions Yielding Predominantly Fatty Acids with a (*Z*)-Configured Double Bond and Further (*E*)/(*Z*)-Isomerization Catalyzed by I_2 to Yield 5 and 6, Respectively



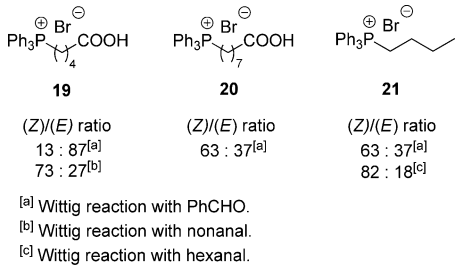


Figure 4. Illustration of the "biting-back" mechanism: (*Z*)/(*E*)-ratios using different phosphonium salts. In all cases, LiHMDS was used as base.[18]

Whereas the phosphonium salt **19** leads to a (*Z*)/(*E*) ratio of 13:87 with benzaldehyde but to 73:27 with nonanal, larger numbers of methylene units continuously attenuate the formation of the (*E*)-isomer. The phosphonium salts we employed in our fatty acid synthesis consisting of 9 or 11 methylene units were thus too hampered to utilize their carboxylate function for an equilibration mechanism. With NaHMDS as base at −78 °C, we observed similar results as in Maryanoff's study and obtained the fatty acids **3** and **4** in 62−65% yield (Scheme 4).

We could induce a good reliability of the stereochemical outcome when 1.3 equiv of the respective phosphonium salt were applied with a small shortfall of NaHMDS (2.5 equiv). The deprotonation time must not be too short; otherwise, the rest of the base could deprotonate the oxaphosphetanes and trigger a Schlosser-like equilibration leading to a higher amount of all-(*E*) product, especially in highly concentrated reaction mixtures.[20]

All products onward from the conjugated ester **15** are highly capricious compounds that tend to polymerization, even if stored at −32 °C under argon atmosphere. Consequently they should quickly be utilized in further transformations. However, as soon as the final fatty acid is coupled, an example of amide

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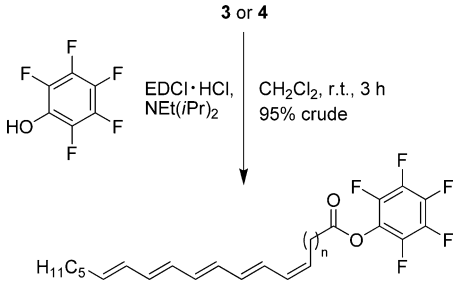
bond formation was investigated, the stability of the oligoene system is significantly enhanced.

An optional isomerization to all-(*E*) congeners was a risky endeavor in our first approaches. To decrease the propensity for polymerization, the substrate is recommended to be used in very small concentrations down to 0.001 M. Since this is still too much substance for establishing a solution in the inert solvent hexane, small amounts of THF might help here to dissolve the suspension upon heating. A thorough exclusion of oxygen and a reaction time of 2 h at 65 °C combined with catalytic amounts of iodine provided the isomerized products **5** and **6**, respectively, in good to excellent yields (Scheme 4). Polymerized particles, if observed, could easily be filtrated off.

It seems probable that the fatty acid suffers from its own acidity, especially when submitted to higher temperatures. An intermediate protonation of the oligoene system is liable to be quenched by a carboxylate function either in an intermolecular or intramolecular way. Our concerns were somehow confirmed as we found that a preceding activation of the acid with pentafluorophenol[21] showed virtually no decomposition even at raised concentrations during isomerization. Accordingly, we suppose that an induced polymerization is predominantly acid-mediated. Our activation procedure gives the pentafluorophenol-derivatized fatty acids **22** and **23**, respectively, in 95% yield as crude product, whereas even a quick column chromatography on silica gel led to obvious decomposition and decreased the yield to 40−60% (Scheme 5).

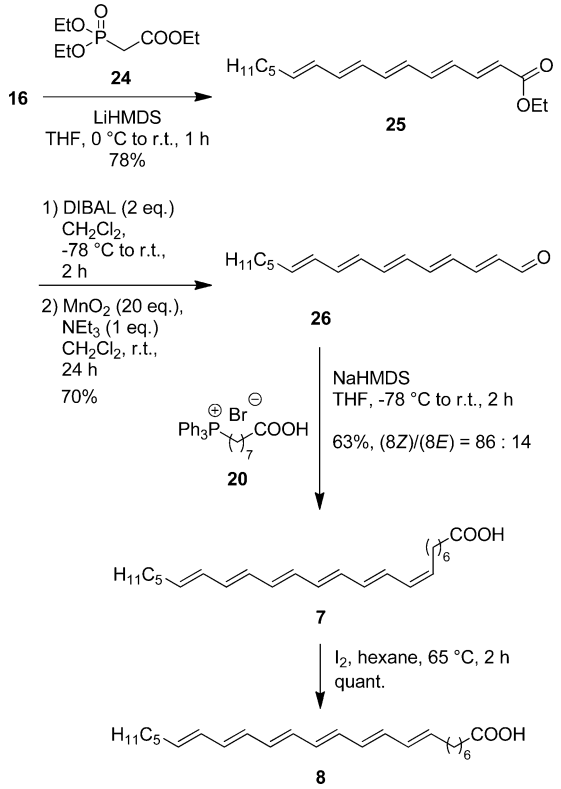
Scheme 5. Activation of Fatty Acids with Pentafluorophenol



22 (n = 8); **23** (n = 10)

Our synthesized fatty acids exhibit beneficial optical properties. UV experiments reveal a strong absorption at 350 nm (all-(*E*)) and at 352 nm ((10*Z*) and (12*Z*), respectively), considering the required wavelengths, a region that might still be troublesome for standard laser equipment. Hence we were interested in shifting the absorption to longer wavelengths by incorporation of a sixth double bond. Indeed, our procedure proved to be compatible even with these higher conjugated species (Scheme 6). Thus, aldehyde **16** was easily converted into the homologous ester **25** in 78% yield. The subsequent one-pot reduction−oxidation procedure was equally successful providing the blood-red aldehyde **26** in 70% yield. Also the Wittig olefination to afford **7** did not sustain a loss in efficiency. The configurational outcome ((*Z*)/(*E*) = 86:14) was gratifying despite the shorter alkyl chain of the involved phosphonium salt **20**. Freshly prepared fatty acid **7** turned out to be an ideal substrate for isomerization furnishing the all-(*E*) hexaene fatty acid **8** in quantitative yield. Although the solubility of the all-(*E*) isomer is noticeably decreased in THF, a column chromatography with 0.1% of formic acid can maintain the

Scheme 6. Synthetic Route to Fatty Acids 7 and 8 with Six Embedded Double Bonds



results from former purifications with the related pentaene species.

The shelf life of all presented fatty acids is restricted. Even storage at −32 °C under an argon atmosphere can not prevent a gradual, likely acid-induced, decomposition. Therefore, we recommend a storage of dry substance at −78 °C, still giving preference to a subsequent coupling, immediately after preparation. The promising option to store the fatty acids as ammonium salts is currently under investigation.

During isomerization, the electronic shape of the π system is dramatically changed, which becomes strikingly visible in the ^{13}C NMR spectra. The transformation to an almost C_2-symmetrical all-(*E*) geometry renders the termini of the oligoene system hardly distinguishable, leading to strongly overlapping signals (Figure 5).

In comparison to tail-positioned pentaene moieties in Amat-Guerri's or Thiele's fatty acids,[6,7] the embeddedness of the conjugation in our case leads to a red-shift of approximately 2 nm in THF, whereas the shapes of the spectra appear very similar. In changing the proximal double bond from (*E*)- to (*Z*)-configuration, another red-shift is observed from 350 nm to approximately 352 nm, having regard to an isomeric purity of 83−85%. The absorption and emission maxima for all fatty acids we synthesized are compiled in Table 2.

Although the absorption spectra were recorded shortly after the synthesis of the fatty acids, their creeping decomposition encumbers the molar absorptivities probably with a considerable error. Nevertheless, our results seem comparable with those of Amat-Guerri, referring to similar molar absorptivities in a range of 60 000−70 000 M^{-1} cm^{-1} in THF.

The fluorescence emissions are in all cases of wide and unsharp shape as expected. The spectra reveal a maximum at

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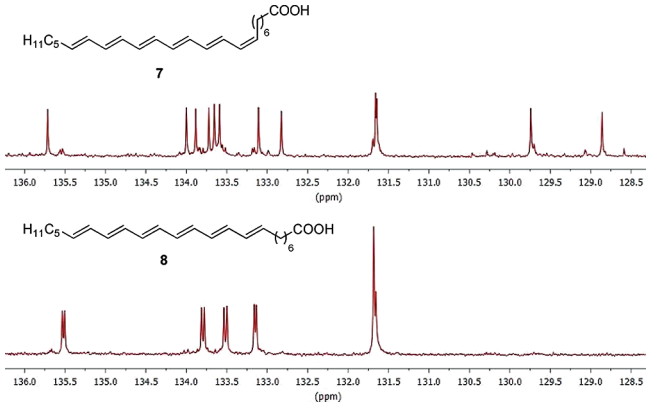

Figure 5. Contrasting juxtaposition of the ^{13}C NMR spectra (δ = 128.0−136.5, hexaene region) of **7** (top) and **8** (bottom).

Table 2. Absorption and Emission Maxima of the Electronic Excitation Spectra of Oligoene Fatty Acids 3−8[a]

compound	UV absorption maxima [nm]	fluorescence emission maxima [nm]
3 (C_{24})	**352**, 334, 319, 305	**474**, 449
5 (C_{24})	**350**, 333, 317, 304	**470**, 446
4 (C_{26})	**352**, 334, 319, 305	**474**, 450
6 (C_{26})	**350**, 332, 317, 304	**470**, 445
7	**381**, 360, 343, 327	**543**, 508, 475
8	**379**, 359, 341, 326	**538**, 504, 471

[a]Species embedding a (*Z*) double bond are **3**, **4**, and **7**. The most intense maximum is written in bold.

about 474 nm for species with a (*Z*)-configured double bond and 470 nm for the corresponding all-(*E*) isomers. Concerning the resulting Stokes shift, a value of about 7300 cm^{-1} arises from taking the major maxima at 350−352 and 474 nm, which fits very well to the data reported for the tail-positioned pentaene moieties.[6] The addition of another double bond in our hexaene fatty acids shifts all absorption maxima by about 30 nm to the red (Figure 6), whereas the difference between the two isomers stays in the same range of 1−2 nm. The molar absorptivity (measured in THF) is strongly increased up to 70 000−100 000 M^{-1} cm^{-1}.[22] The fluorescence emission spectra are less blurred and exhibit three distinct maxima, the major one at 543 nm (for **7**) and 538 nm (for **8**), respectively. If these are taken for the calculation of the Stokes shift with the absorption peaks at about 380 nm, a value of approximately 7800 cm^{-1} results, being slightly more than for their pentaene counterparts.

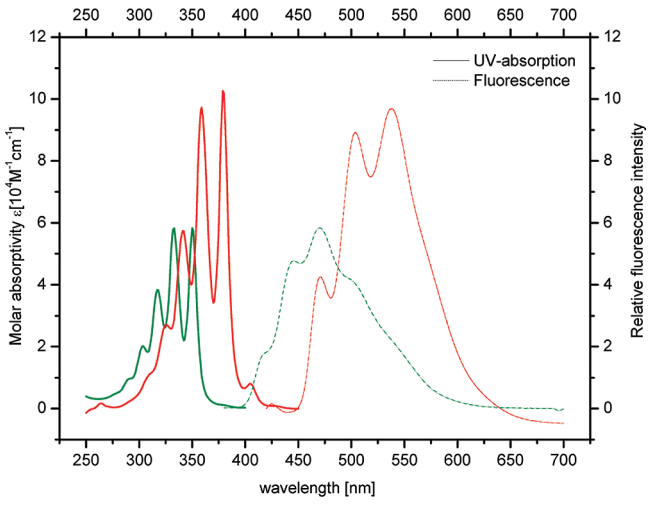

Figure 6. Electronic excitation spectra (UV absorption (solid line) and fluorescence emission (dashed line)) of the all-(*E*) pentaene fatty acid **5** (shown in green) and the respective hexaene congener **8** (shown in red).

CONCLUSION

In summary, we developed a novel synthetic strategy to fluorescent oligoene fatty acids using a DBU-mediated β-elimination of HCl as the key step of the synthetic route. Respective species containing acyl chains of 24 as well as 26 carbon atoms with an embedded pentaene moiety were synthesized in 5−6 steps, first as (*Z*)-configured isomers at the proximal double bond (revealing an isomeric purity of 83−85%) or as all-(*E*) congeners after a subsequent isomerization process induced by traces of I_2. A prolongation to hexaene C_{24}-fatty acids was found to be highly successful utilizing a repetition of Wittig−Horner reactions. The products obtained in an overall yield of up to 26% show fluorescent properties with longest-wavelength absorption maxima of about 350 and 380 nm, respectively. Large Stokes shifts of about 7300 and 7800 cm^{-1} are observed, rendering this material ideally suited for studies of lipids in model membranes. The activation with pentafluorophenol proceeded smoothly preserving their sensible oligoene structures and should act as suitable substrate for amide bond formation. The embedded oligoene moiety instead of a tail-positioned sensibly helps to improve the flexibility of the acyl chain by suppressing efficient $\pi-\pi$ stacking and probably raises the fatty acids' mimicry quality to a new extent. The incorporation of these fluorescent fatty acids into lipids is in progress, and biophysical studies with corresponding species will be reported in due course.

EXPERIMENTAL SECTION

General Methods. All solvents were distilled before use unless otherwise stated. Tetrahydrofuran (THF) was distilled from sodium/benzophenone and dichloromethane (CH_2Cl_2) from $CaCl_2$ under a nitrogen atmosphere. Air- and moisture-sensitive reactions were carried out in oven-dried or flame-dried glassware, septum-capped under atmospheric pressure of argon. Commercially available compounds were used without further purification unless otherwise stated. Proton (^1H) and carbon (^{13}C) NMR spectra were recorded on a 300, 500, or 600 MHz instrument using the residual signals from $CHCl_3$, δ 7.26 and δ 77.0 ppm, and THF, δ 1.73, 3.58 ppm and δ 25.4, 67.6 ppm, as internal references for ^1H and ^{13}C, respectively. Assignments of the respective signals were made by the combination of H,H-COSY, HSQC, HMBC, and experiments. Assignments marked with "*" remained uncertain, and "m_c" defines a symmetrical multiplet. ESI-HRMS mass spectrometry was carried out on a FTICR instrument. IR spectra were measured on a conventional FTIR spectrometer with ATR sample technique. UV and fluorescence spectra were measured with common instruments, and the fluorescence emission was induced by using the most red-shifted maximum. The ratios of (*E*)- and (*Z*)-isomers in isomeric mixtures were calculated from ^{13}C NMR spectra, taking the average signal integral of 5−6 separate peaks of the oligoene region in each case.

(4*E*)-2-Chlorodecenal (13). (4*E*)-Decenal (1.00 g, 6.48 mmol, 1.0 equiv) was dissolved in anhydrous dichloromethane (8 mL). *N*-Chlorosuccinimide (866 mg, 6.48 mmol, 1.0 equiv) and L-proline (74.6 mg, 0.648 mmol, 0.1 equiv) were added at 0 °C, and the stirred reaction mixture was slowly warmed up to room temperature overnight. Afterward it was diluted with dichloromethane (30 mL)

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and washed with brine (3×). Drying over Na_2SO_4 and evaporation of the solvent afforded 1.22 g (6.47 mmol, quant.) of **13** as a yellowish oil, which tends to get brownish on storage: R_f 0.4−0.7 (pentane/EtOAc = 4:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 6.8 Hz, 3 H, 10-H), 1.15−1.40 (m, 6 H, 7-H, 8-H, 9-H), 1.98 (q, J = 7.0 Hz, 2 H, 6-H), 2.61 (m, 2 H, 3-H$_a$, 3-H$_b$), 4.13 (m$_c$, 1 H, 2-H), 5.35 (m$_c$, 1 H, 4-H), 5.57 (m$_c$, 1 H, 5-H), 9.45 (d, J = 2.4 Hz, 1 H, 1-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 22.5, 28.8, 31.3, 32.4, 35.6, 63.2 (C-2), 122.6 (C-4)*, 136.2 (C-5)*, 195.0 (C-1); IR (ATR) $\tilde{\nu}$ (cm^{-1}) = 2956, 2924, 2855, 1735, 968; UV (CH_3CN) λ_{max} (lg ε) [nm] no absorption maxima detectable; MS (ESI) m/z (%) = 187.1 (30) $[M - H]^-$; HRMS (ESI) calcd for $[M - H]^-$ ($M = C_{10}H_{17}ClO$) 187.0890, found 187.0890.

Methyl (2*E*,4*E*,8*E*)-6-Chlorotetradecatrienoate (14). Methyl (2*E*)-4-(diethoxyphosphoryl)crotonate (**9**) (2.29 g, 9.71 mmol, 1.5 equiv) was dissolved in anhydrous THF (50 mL). *n*-BuLi (2.5 M, 3.88 mL, 9.71 mmol) was added dropwise at 0 °C, and stirring was continued for 30 min while warming to room temperature. (4*E*)-2-Chlorodecenal (**13**) (1.22 g, 6.47 mmol, 1.0 equiv) dissolved in 6 mL of anhydrous THF was added at room temperature, and stirring was continued for 30 min. The reaction was quenched with aq saturated NH_4Cl solution. After an aqueous workup, the crude product was purified by column chromatography (pentane/EtOAc = 20:1) to afford 1.14 g (4.21 mmol, 65%) of **14** as a pale yellow oil: R_f 0.37 (pentane/EtOAc = 20:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 6.8 Hz, 3 H, 14-H), 1.15−1.43 (m, 6 H, 11-H, 12-H, 13-H), 2.00 (q, J = 6.6 Hz, 2 H, 10-H), 2.54 (t, J = 6.8 Hz, 2 H, 7-H), 3.75 (s, 3 H, OCH_3) 4.42 (m$_c$, 1 H, 6-H), 5.36 (m$_c$, 1 H, 8-H), 5.53 (m$_c$, 1 H, 9-H), 5.92 (d, J = 15.4 Hz, 1 H, 2-H), 6.09 (dd, J = 15.2, 8.3 Hz, 1 H, 5-H), 6.34 (dd, J = 15.2, 11.0 Hz, 1 H, 4-H), 7.25 (dd, J = 15.4, 11.0 Hz, 1 H, 3-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0 (C-14), 22.5 (C-13), 28.9 (C-12), 31.3 (C-11), 32.5 (C-10), 41.4 (C-7), 51.6 (OCH_3), 61.0 (C-6), 122.4 (C-2), 124.1 (C-8), 129.5 (C-4), 135.4 (C-9), 141.0 (C-5), 143.1 (C-3), 167.0 (C-1); IR (ATR) $\tilde{\nu}$ (cm^{-1}) = 2926, 1719, 1261, 1138, 995; UV (CH_3CN) λ_{max} (lg ε) [nm] = 257 (4.49); MS (ESI) m/z (%) = 293.1 (100) $[M + Na]^+$; HRMS (ESI) calcd for $[M + Na]^+$ ($M = C_{15}H_{23}ClO_2$) 293.1284, found 293.1279.

Methyl (2*E*,4*E*,6*E*,8*E*)-Tetradecatetraenoate (15). Methyl (2*E*,4*E*,8*E*)-6-Chlorotetradecatrienoate (**14**) (1.14 g, 4.21 mmol, 1.0 equiv) was dissolved in 42 mL of anhydrous dichloromethane. DBU (7.54 mL, 50.5 mmol, 12 equiv) was added, and stirring was continued for exactly 30 h at room temperature. An aqueous workup with aq saturated NH_4Cl solution, followed by flash column chromatography (pentane/EtOAc = 20:1) afforded 908 mg (3.87 mmol, 92%) of **15** as a yellow solid: R_f 0.37 (pentane/EtOAc = 20:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 6.7 Hz, 3 H, 14-H), 1.16−1.46 (m, 6 H, 11-H, 12-H, 13-H), 2.10 (q, J = 7.2 Hz, 2 H, 10-H), 3.72 (s, 3 H, OCH_3), 5.76−5.90 (m, 2 H, 2-H, 9-H), 6.00−6.43 (m, 4 H, 4-H, 6-H, 7-H, 8-H), 6.55 (dd, J = 14.8, 10.8 Hz, 1 H, 5-H), 7.29 (dd, J = 15.2, 11.4 Hz, 1 H, 3-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0 (C-14), 22.5 (C-13)*, 28.8 (C-11), 31.4 (C-12)*, 32.9 (C-10), 51.4 (OCH_3), 119.5 (C-2), 130.0, 129.5, 130.2, 137.8 (C-7), 138.9 (C-9), 141.2 (C-5), 144.9 (C-3), 167.6 (C-1); IR (ATR) $\tilde{\nu}$ (cm^{-1}) = 2953, 2927, 2858, 1716, 1618, 1435, 1130, 1005; UV (THF) λ_{max} (lg ε) [nm] = 224 (3.46), 263 (3.31), 315 (3.34), 330 (3.35); MS (ESI) m/z (%) = 257.2 (100) $[M + Na]^+$; HRMS (ESI) calcd for $[M + Na]^+$ ($M = C_{15}H_{22}O_2$) 257.1517, found 257.1515.

(2*E*,4*E*,6*E*,8*E*)-Tetradecatetraenal (16). Methyl (2*E*,4*E*,6*E*,8*E*)-tetradecatetraenoate (**15**) (908 mg, 3.87 mmol, 1 equiv) was dissolved in dichloromethane (125 mL). DIBAL (1 M in hexane, 7.74 mL, 7.74 mmol, 2 equiv) was added slowly at −78 °C, and stirring was continued for 1 h. The reaction mixture was then warmed to room temperature for another hour. NEt_3 (0.54 mL, 3.9 mmol, 1 equiv) as well as activated MnO_2 (6.73 g, 77.4 mmol, 20 equiv) were added, and stirring was continued overnight at room temperature. If a TLC still indicated a strong alcohol spot, another 10 equiv of MnO_2 could be added, awaiting another stirring time of 2 h. Approx. 100 mL of the solvent were removed under reduced pressure, and pentane (50 mL) as well as silica gel (20 mL) were added. The dark suspension was put on a short (5 cm) plug of silica gel and rinsed with pentane/EtOAc (50:1) as eluent until the yellow aldehyde started to be eluated. The colorless fraction was rejected and the product rinsed off with pentane/EtOAc (4:1) as eluent. Removal of solvents yielded 585 mg (2.86 mmol, 74%) of aldehyde **16** as an orange oily solid, which was directly subjected to the following Wittig olefination or to a further prolongation to the ester **25**.

Ethyl (2*E*,4*E*,6*E*,8*E*,10*E*)-Hexadecapentaenoate (25). Triethyl phosphonoacetate **24** (483 mg, 2.15 mmol, 1.1 equiv) was dissolved in anhydrous THF (15 mL). LiHMDS (1 M in THF, 2.15 mL, 2.15 mmol, 1.1 equiv) was added dropwise at 0 °C, and stirring was continued for 15 min. Freshly prepared aldehyde **16** (400 mg, 1.96 mmol, 1 equiv) in 5 mL of anhydrous THF was added dropwise, and stirring was continued for 30 min at 0 °C. The reaction mixture was allowed to warm to room temperature within 30 min. Then it was quenched with aq saturated NH_4Cl solution. After an aqueous workup, the crude product was purified by column chromatography (pentane/EtOAc = 20:1) to afford 420 mg (1.53 mmol, 78%) of ester **25** as an orange solid: R_f 0.56 (pentane/EtOAc = 4:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.3 Hz, 3 H, 16-H), 1.22−1.47 (m, 9 H, OCH_2CH_3, 13-H, 14-H, 15-H), 2.11 (q, J = 7.2 Hz, 2 H, 12-H), 4.20 (q, J = 7.2 Hz, 2 H, OCH_2CH_3), 5.74−5.92 (m, 2 H, 2-H, 11-H), 6.00−6.50 (m, 6 H, 4-H, 6-H, 7-H, 8-H, 9-H, 10-H), 6.59 (dd, J = 14.8, 10.8 Hz, 1 H, 5-H), 7.29 (dd, J = 15.2, 11.4 Hz, 1 H, 3-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1 (C-16), 14.4 (OCH_2CH_3), 22.6, 28.9 (C-13), 31.5, 33.0 (C-12), 60.2 (OCH_2CH_3), 120.2 (C-2), 129.3, 130.0, 130.3, 130.9, 136.0, 137.5, 137.7 (C-11), 140.8 (C-5), 144.4 (C-3), 167.1 (C-1); IR (ATR) $\tilde{\nu}$ (cm^{-1}) = 2955, 2926, 2856, 1706, 1619, 1575, 1465, 1367, 1125, 1004; UV (THF) λ_{max} (lg ε) [nm] = 376 (4.55), 359 (4.63); MS (ESI) m/z (%) = 297.2 (50) $[M + Na]^+$; HRMS (ESI) calcd for $[M + Na]^+$ ($M = C_{18}H_{26}O_2$) 297.1830, found 297.1825.

(2*E*,4*E*,6*E*,8*E*,10*E*)-Hexadecapentaenal (26). Ethyl (2*E*,4*E*,6*E*,8*E*,10*E*)-hexadeca-pentaenoate (**25**) (400 mg, 1.46 mmol, 1 equiv) was dissolved in dichloromethane (80 mL). DIBAL (1 M in hexane, 4.38 mL, 4.38 mmol, 3 equiv) was added slowly at −78 °C, and stirring was continued for 1 h. The reaction mixture was then warmed to room temperature for another hour. NEt_3 (0.20 mL, 1.5 mmol, 1 equiv) as well as activated MnO_2 (2.53 g, 29.2 mmol, 20 equiv) were added, and stirring was continued overnight at room temperature. If a TLC still indicated an alcohol spot, another 10 equiv of MnO_2 could be added, awaiting another stirring time of 2 h. About 40 mL of solvent were removed under reduced pressure, and pentane (20 mL) as well as silica gel (15 mL) were added. The dark suspension was put on a short (5 cm) plug of silica gel and rinsed with pentane/EtOAc (50:1) as eluent until the orange aldehyde started to be eluated. The colorless fraction was rejected and the product rinsed off with pentane/EtOAc (4:1) as eluent. Removal of solvents afforded 285 mg (1.02 mmol, 70%) of aldehyde **26** as a bloody-red solid that was directly subjected to the following Wittig olefination: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 6.3 Hz, 3 H, 16-H), 1.17−1.46 (m, 6 H, 13-H, 14-H, 15-H), 2.10 (q, J = 7.2 Hz, 2 H, 12-H), 5.76−5.88 (dt, J = 15.1, 7.2 Hz, 1 H, 11-H), 6.03−6.57 (m, 7 H, 2-H, 4-H, 6-H, 7-H, 8-H, 9-H, 10-H), 6.68 (dd, J = 14.8, 11.1 Hz, 1 H, 5-H), 7.11 (dd, J = 15.1, 11.2 Hz, 1 H, 3-H), 9.53 (d, J = 8.0 Hz, 1 H, 1-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1 (C-16), 22.6, 28.9 (C-13), 31.5, 33.0 (C-12), 129.2, 129.9, 130.2, 130.5, 130.6, 137.0, 138.6 (C-11), 139.1, 142.8 (C-5), 151.8 (C-3), 193.2 (C-1); IR (ATR) $\tilde{\nu}$ (cm^{-1}) = 2955, 2926, 2857, 1672, 1571, 1153, 1102, 1001; UV (THF) λ_{max} (lg ε) [nm] = 372 (4.58); MS (ESI) m/z (%) = 253.2 (100) $[M + Na]^+$; HRMS (ESI) calcd for $[M + Na]^+$ ($M = C_{16}H_{22}O$) 253.1568, found 253.1563.

General Procedure for the Preparation of Phosphonium Salts 17, 18, and 20. The synthesis followed typical literature-known procedures.[23] Equimolar amounts of bromide and triphenylphosphine were dissolved in acetonitrile (1 M) and refluxed for 3−5 days. To avoid a gummy consistence of the products and to obtain powdered solids instead, the solvent was removed afterward, and the residue dissolved again in a mixture of dichloromethane/THF (1:1). The crystallization was usually initiated in high vacuo when the rest of the solvents was removed.

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General Procedure for the Preparation of Fatty Acids 3, 4, and 7. The corresponding phosphonium salt (0.500 mmol, 1.3 equiv) was suspended in anhydrous THF (5 mL). NaHMDS (1 M in hexane, 0.96 mmol, 2.5 equiv) was added, and stirring was continued for 15 min at room temperature. The reaction mixture was cooled to −78 °C, and the corresponding freshly prepared aldehyde (**16** or **26**, respectively) (0.384 mmol, 1 equiv), dissolved in anhydrous THF (2 mL), was added slowly. Stirring was continued for 1 h at −78 °C, and afterward the reaction mixture was allowed to warm to room temperature for another hour. The reaction was quenched with formic acid (0.96 mmol, 2.5 equiv) under vigorous stirring, and the crude product was directly adsorbed on silica gel. After a purification by means of flash column chromatography (pentane/EtOAc = 20:1, 0.1% of formic acid), the rest of the formic acid in the collected fractions was quickly removed in high vacuo. The procedure afforded the respective fatty acids with one (*Z*)-configured double bond in yields of 62−65% with a stereochemical purity of 83−86%.

(10*Z*,12*E*,14*E*,16*E*,18*E*)-Tetracosapentaenoic Acid (3). 50.0 mg (0.245 mmol) of **16** was reacted with 163 mg (0.318 mmol) of **17** to yield 54.5 mg (0.152 mmol, 62%) of **3** in 83% stereochemical purity as a yellow solid: R_f 0.18 (pentane/EtOAc = 4:1); ^{1}H NMR (300 MHz, THF-d_8) δ 0.88 (t, J = 6.9 Hz, 3 H, 24-H), 1.19−1.47 (m, 16 H, 4-H, 5-H, 6-H, 7-H, 8-H, 21-H, 22-H, 23-H), 1.55 (m$_c$, 2 H, 3-H), 2.09 (m$_c$, 2 H, 20-H), 2.19 (m$_c$, 4 H, 2-H, 9-H), 5.39 (dt, J = 10.6, 7.8 Hz, 1 H, 10-H), 5.69 (dt, J = 14.6, 7.1 Hz, 1 H, 19-H), 5.93−6.36 (m, 7 H, 11-H, 13-H, 14-H, 15-H, 16-H, 17-H, 18-H), 6.50 (dd, J = 14.1, 11.3 Hz, 1 H, 12-H); ^{13}C NMR (125 MHz, THF-d_8) δ 14.3, 23.3, 25.7, 28.6, 29.88, 29.98, 30.04, 30.15, 30.20, 30.5, 32.2, 33.6, 34.1, 128.6, 129.7, 131.63, 131.64, 132.7, 133.2, 133.6, 133.7, 133.9, 135.6, 174.1; IR (ATR) $\tilde{\nu}$ (cm^{-1}) = 2919, 2848, 1691, 1466, 1440, 1410, 1318, 1289, 1259, 1226, 1193, 1000; UV (THF) λ_{max} (lg ε) [nm] = 381 (3.26), 352 (4.73), 334 (4.73), 319 (4.53), 305 (4.22), 292 (3.90); MS (ESI) m/z (%) = 381.3 (100) [M + Na]$^+$; HRMS (ESI) calcd for [M + Na]$^+$ (M = $C_{24}H_{38}O_2$) 381.2770, found 381.2764.

(12*Z*,14*E*,16*E*,18*E*,20*E*)-Hexacosapentaenoic Acid (4). 50.0 mg (0.245 mmol) of **16** was reacted with 172 mg (0.318 mmol) of **18** to yield 61.6 mg (0.159 mmol, 65%) of **4** in 85% stereochemical purity as a yellow solid: R_f 0.18 (pentane/EtOAc = 4:1); ^{1}H NMR (300 MHz, THF-d_8) δ 0.88 (t, J = 6.9 Hz, 3 H, 26-H), 1.20−1.47 (m, 20 H, 4-H, 5-H, 6-H, 7-H, 8-H, 9-H, 10-H, 21-H, 22-H, 23-H), 1.55 (m$_c$, 2 H, 3-H), 2.09 (m$_c$, 2 H, 22-H), 2.19 (m$_c$, 4 H, 2-H, 11-H), 5.40 (dt, J = 10.6, 7.8 Hz, 1 H, 12-H), 5.69 (dt, J = 14.6, 7.1 Hz, 1 H, 21-H), 5.93 - 6.36 (m, 7 H, 13-H, 15-H, 16-H, 17-H, 18-H, 19-H, 20-H), 6.50 (dd, J = 14.1, 11.3 Hz, 1 H, 14-H); ^{13}C NMR (125 MHz, THF-d_8) δ 14.3, 23.3, 25.7, 28.6, 29.9, 30.0, 30.1, 30.2, 30.33, 30.33, 30.4, 30.5, 32.2, 33.6, 34.1, 128.6, 129.7, 131.63, 131.63, 132.7, 133.2, 133.6, 133.7, 133.9, 135.6, 174.1; IR (ATR) $\tilde{\nu}$ (cm^{-1}) = 2918, 2848, 1692, 1466, 1437, 1410, 1296, 1273, 1244, 1216, 1189, 1001; UV (THF) λ_{max} (lg ε) [nm] = 352 (4.86), 334 (4.87), 319 (4.65), 305 (4.33); MS (ESI) m/z (%) = 409.4 (98) [M + Na]$^+$; HRMS (ESI) calcd for [M + Na]$^+$ (M = $C_{26}H_{42}O_2$) 409.3083, found 409.3077.

(8*Z*,10*E*,12*E*,14*E*,16*E*,18*E*)-Tetracosahexaenoic Acid (7). 50.0 mg (0.217 mmol) of **26** was reacted with 137.0 mg (0.282 mmol) of **20** to yield 48.7 mg (0.137 mmol, 63%) of **7** in 86% stereochemical purity as a yellow solid: R_f 0.18 (pentane/EtOAc = 4:1); ^{1}H NMR (300 MHz, THF-d_8) δ 0.89 (t, J = 6.9 Hz, 3 H, 24-H), 1.24−1.47 (m, 12 H, 4-H, 5-H, 6-H, 21-H, 22-H, 23-H), 1.57 (m$_c$, 2 H, 3-H), 2.10 (m$_c$, 2 H, 20-H), 2.20 (m$_c$, 4 H, 2-H, 7-H), 5.41 (dt, J = 10.6, 7.7 Hz, 1 H, 8-H), 5.70 (dt, J = 14.5, 7.2 Hz, 1 H, 19-H), 5.94−6.40 (m, 9 H, 9-H, 11-H, 12-H, 13-H, 14-H, 15-H, 16-H, 17-H, 18-H), 6.53 (dd, J = 13.6, 11.5 Hz, 1 H, 10-H); ^{13}C NMR (125 MHz, THF-d_8) δ 14.2, 23.3, 25.7, 28.5, 29.7, 29.8, 29.9, 30.3, 32.2, 33.6, 34.1, 128.9, 129.8, 131.66, 131.67, 132.8, 133.1, 133.6, 133.67, 133.74, 133.9, 134.0, 135.7, 174.1; IR (ATR) $\tilde{\nu}$ (cm^{-1}) = 2953, 2921, 2852, 1691, 1463, 1433, 1411, 1281, 1231, 1197, 1001; UV (THF) λ_{max} (lg ε) [nm] = 405 (3.72), 381 (4.86), 360 (4.85), 343 (4.64), 327 (4.35), 310 (4.00); MS (ESI) m/z (%) = 379.3 (96) [M + Na]$^+$; HRMS (ESI) calcd for [M − H]$^-$ (M = $C_{24}H_{36}O_2$) 355.2643, found 355.2643.

General Procedure for the Isomerization of Fatty Acids 3, 4, and 7 to the Corresponding all-(*E*) Species 5, 6, and 8. The fatty acid (20 mg) was dissolved in THF (0.5 mL), optionally with slight warming. Hexane (50 mL) was added to yield a homogeneous suspension. A saturated solution of iodine (6 μL) in hexane was added, and the apparatus was thoroughly flushed with argon. The reaction mixture was heated to 60−70 °C for 2 h. Afterward, the solvent was removed under reduced pressure. Polymerized particles, if they emerged, were filtrated off. The procedure gave the respective all-*trans* fatty acid in 76% to quantitative yield.

(10*E*,12*E*,14*E*,16*E*,18*E*)-Tetracosapentaenoic Acid (5). Twenty milligrams (0.056 mmol) of **3** were subjected to isomerization to yield 15 mg (0.42 mmol, 76%) of **5** as a yellow solid: R_f 0.18 (pentane/EtOAc = 4:1); ^{1}H NMR (600 MHz, THF-d_8) δ 0.88 (t, J = 7.0 Hz, 3 H, 24-H), 1.24−1.36 (m, 12 H, 4-H, 5-H, 6-H, 7-H, 22-H, 23-H), 1.39 (m$_c$, 4 H, 8-H, 21-H), 1.56 (m$_c$, 2 H, 3-H), 2.09 (m$_c$, 4 H, 9-H, 20-H), 2.19 (t, J = 7.5 Hz, 2 H, 2-H), 5.62−5.73 (m, 2 H, 10-H, 19-H), 5.95−6.35 (m, 8 H, 11-H, 12-H, 13-H, 14-H, 15-H, 16-H, 17-H, 18-H); ^{13}C NMR (125 MHz, THF-d_8) δ 14.2, 23.3, 25.7, 29.91, 29.99, 29.99, 30.15, 30.19, 30.21, 32.24, 33.60, 33.63, 34.15, 131.80, 131.80, 131.84, 131.86, 133.28, 133.32, 133.67, 133.72, 135.55, 135.59, 174.4; IR (ATR) $\tilde{\nu}$ (cm^{-1}) = 2919, 2849, 1703, 1467, 1435, 1284, 1254, 1221, 1170, 1096; UV (THF) λ_{max} (lg ε) [nm] = 232 (3.81), 304 (4.30), 317 (4.58), 333 (4.77), 350 (4.77); MS (ESI) m/z (%) = 381.3 (100) [M + Na]$^+$; HRMS (ESI) calcd for [M + Na]$^+$ (M = $C_{24}H_{38}O_2$) 381.2770, found 381.2764.

(12*E*,14*E*,16*E*,18*E*,20*E*)-Hexacosapentaenoic Acid (6). Twenty milligrams (0.052 mmol) of **4** were subjected to isomerization to yield 15 mg (0.039 mmol, 76%) of **6** as a yellow solid: R_f 0.18 (pentane/EtOAc = 4:1); ^{1}H NMR (300 MHz, THF-d_8) δ 0.88 (t, J = 6.8 Hz, 3 H, 26-H), 1.24−1.47 (m, 18 H, 4-H, 5-H, 6-H, 7-H, 8-H, 9-H, 10-H, 23-H, 24-H, 25-H), 1.56 (m$_c$, 2 H, 3-H), 2.09 (m$_c$, 4 H, 11-H, 22-H), 2.19 (t, J = 7.5 Hz, 2 H, 2-H), 5.61−5.75 (m, 2 H, 12-H, 21-H), 5.95−6.27 (m, 8 H, 13-H, 14-H, 15-H, 16-H, 17-H, 18-H, 19-H, 20-H); ^{13}C NMR (125 MHz, THF-d_8) δ 14.2, 23.3, 25.7, 29.9, 29.98, 30.00, 30.16, 30.21, 30.29, 30.30, 30.4, 32.2, 33.6, 33.6, 34.1, 131.63, 131.65, 131.66, 131.69, 133.11, 133.13, 133.50, 133.54, 135.36, 135.41, 174.1; IR (ATR) $\tilde{\nu}$ (cm^{-1}) = 2914, 2848, 1706, 1467, 1434, 1305, 1273, 1245, 1218, 1189, 1001; UV (THF) λ_{max} (lg ε) = 350 nm (4.82), 332 (4.82), 317 (4.62), 304 (3.37); MS (ESI) m/z (%) = 409.3 (99) [M + Na]$^+$; HRMS (ESI) calcd for [M + Na]$^+$ (M = $C_{26}H_{42}O_2$) 409.3083, found 409.3077.

(8*E*,10*E*,12*E*,14*E*,16*E*,18*E*)-Tetracosahexaenoic Acid (8). Twenty milligrams (0.056 mmol) of **7** were subjected to isomerization to yield 20 mg (0.056 mmol, 100%) of **8** as a yellow solid: R_f 0.18 (pentane/EtOAc = 4:1); ^{1}H NMR (300 MHz, THF-d_8) δ 0.89 (t, J = 6.9 Hz, 3 H, 24-H), 1.24−1.49 (m, 12 H, 4-H, 5-H, 6-H, 21-H, 22-H, 23-H), 1.57 (m$_c$, 2 H, 3-H), 2.10 (m$_c$, 4 H, 7-H, 20-H), 2.19 (m$_c$, 2 H, 2-H), 5.69 (dt, J = 14.1, 7.2 Hz, 2 H, 8-H, 19-H), 5.96−6.36 (m, 10 H, 9-H, 10-H, 11-H, 12-H, 13-H, 14-H, 15-H, 16-H, 17-H, 18-H); ^{13}C NMR (125 MHz, THF-d_8) δ 14.2, 23.2, 25.7, 29.7, 29.8, 29.9, 30.0, 32.2, 33.50, 33.53, 34.1, 131.67, 131.70, 131.70, 131.70, 133.15, 133.17, 133.51, 133.55, 133.79, 133.83, 135.52, 135.55, 174.0; IR (ATR) $\tilde{\nu}$ (cm^{-1}) = 2954, 2916, 2847, 1693, 1466, 1432, 1305, 1280, 1237, 1201, 1000; UV (THF) λ_{max} (lg ε) [nm] = 405 (3.90), 379 (5.01), 359 (4.99), 341 (4.76), 326 (4.43), 310 (4.04); MS (ESI) m/z (%) = 379.3 (100) [M + Na]$^+$; HRMS (ESI) calcd for [M − H]$^-$ (M = $C_{24}H_{36}O_2$) 355.2643, found 355.2646.

General Procedure for the Activation with Pentafluorophenol. Pentafluorophenol (48 mg, 0.26 mmol, 1.3 equiv) and Hünig's base (44 μL, 0.26 mmol, 1.3 equiv) were dissolved in anhydrous dichloromethane (3 mL) and added as a solution to any fatty acid (0.20 mmol, 1 equiv). EDCI·HCl (50 mg, 0.26 mmol, 1.3 equiv) was added at once, and stirring was continued at room temperature for 2 h. The reaction mixture was diluted with diethylether (20 mL) and washed three times with brine. A separation of the organic phase, drying over Na_2SO_4, and removal of solvent under reduced pressure gave the activated fatty acid as crude product in 95% yield.

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■ ASSOCIATED CONTENT

Supporting Information

Copies of ^{1}H and ^{13}C NMR spectra for all novel compounds, as well as UV and fluorescence spectra for compounds **3**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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