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6-1-2005

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Brief Communication

Biologically Active Fluorescent Farnesol Analogs

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Summary

We describe ten polyene analogs of farnesol, typified by 3,7,11-trimethyl-2,4,6,8,10-dodecapentenaldehyde oxime, which preserve the length, cross-section, and approximate hydrophobicity of farnesol. Four of the ten display strong quorum-sensing activity in the human pathogen *Candida albicans*, with IC_{50} values for inhibition of germ-tube formation as low as 10 µM. The polyenes display absorption maxima between 320 and 380 nm, with the extinction coefficients for the oximes approaching 100,000. All but two of the analogs are fluorescent, with excitation maxima varying over the range of 320–370 nm. Oxime *anti*-4, which can undergo fluorescence excitation at wavelengths beyond 400 nm, is demonstrated to be useful for confocal fluorescence microscopic imaging of fungal cells. The farnesol analogs are also expected to be useful for detection of farnesol binding proteins and in determination of farnesol pharmacokinetics.

Introduction

We have been investigating the basis for quorum sensing in *Candida albicans*, an important human pathogen. Quorum sensing involves accumulation of a secreted quorum-sensing molecule (QSM) to a concentration sufficient to elicit a response from the producing cells. In the case of *C. albicans*, the first eukaryotic organism shown to engage in quorum sensing, the accumulation of the sesquiterpene farnesol blocks the morphological shift from yeast to mycelia, eliminating germ-tube formation [1]. We became interested in following the process of QSM uptake and localization with fluorescence spectroscopy and microscopy. Fluorophore-containing analogs have been used to study ligand interactions for undecaprenyl pyrophosphate [2], and Kim et al. demonstrated uptake of farnesyl pyrophosphate analogs incorporating anthranylate or dansyl fluorophores by leukemia cells [3]. However, the results of our investigations of the relationship between structure and QSM activity in forty synthetic and natural farnesol analogs suggested that any attempt to incorporate a typical fluorescent reporter would preclude binding to a farnesol receptor or binding protein [4]. In approaching this problem, we were inspired by the example of the parinaric acids. These fluorescent

fatty acid natural products contain a conjugated tetraene subunit with absorption and emission maxima near 300 and 410 nm, respectively, and have been applied as fluorescent membrane probes [5]. Prestwich and coworkers reported a conjugated pentaene based upon geranylgeranyl pyrophosphate that exhibited absorption and emission near 360 and 450 nm, respectively [6]. During the course of our studies, the same group reported a pentaene analog (*E*-3) of farnesol featuring similar photophysical properties [7]; a separate group has recently reported pentaene analogs of lipids [8]. However, fungal cells often display significant emission upon excitation in the 320–380 nm range; this autofluorescence interferes with the use of fluorescent probes [9 and 10]. Accordingly, we set out to develop a series of farnesol analogs that would preserve the approximate length, cross-section, and hydrophobicity of farnesol while extending fluorescence excitation to wavelengths beyond 380 nm. We now report the synthesis and photophysical properties of a series of fluorescent farnesol analogs that combine a polar head group with a pentaene backbone and that undergo fluorescent excitation at wavelengths beyond 400 nm (Figure 1).

Results and Discussion

Fluorescent Farnesol Analogs

The head groups include an ester, aldehyde, alcohol, oxime, and carboxylic acid (Figure 1); synthetic procedures and spectral characterization are included in the Supplemental Data available with this article online and presented here in an Appendix. The photophysical and biological properties of selected probes are summarized in Table 1. With the exception of the two esters (E-1 and Z-1), the probes were fluorescent.

Most of the probes have excitation maxima at or below 360 nm. However, oxime *anti*-4 features maxima at 362 and 382 nm with excitation extending beyond 400 nm, an important spectral range for confocal fluorescence microscopy; fluorescence spectra for *anti*-4 and most of the other new compounds are provided in the Supplemental Data available with this article online, and presented here as an Appendix.

Biological Activity

Our report of quorum sensing in *C. albicans* was the first such example reported for fungi [1]. *C. albicans* cells excrete farnesol continuously. When the accumulated farnesol exceeds a threshold value, it causes the cells to grow in the yeast rather than the mycelia morphology, as evidenced by a lack of germ-tube formation. For E,E-farnesol, a concentration of 1.2 µM results in a 50% reduction in the fraction of cells exhibiting a mycelial morphology. At levels up to 300 µM, accumulated or added farnesol alters only cell morphology not growth rate [1]. Thus, active farnesol analogs prevent mycelia growth, causing the cells to instead grow as yeasts. All ten pentaenes from the current study were compared with farnesol for the ability to inhibit germ-tube formation in *C. albicans*; val-
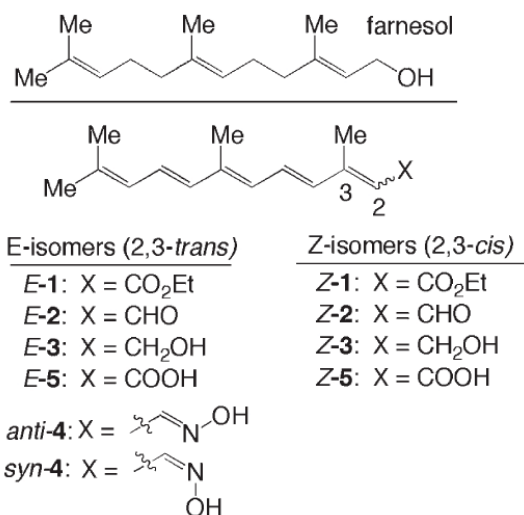
Figure 1. Farnesol and Fluorescent Analogs

Figure 2. *C. albicans* Stained with Fluorescent Oxime
Left: fluorescence microscopy (405 nm excitation) of a *C. albicans* cell (approximately 4.2 × 3.2 μm in size) stained with *anti*-**4**. Right: differential interference contrast image of the same cell.

ues for the aldehydes (*E*-**2**, *Z*-**2**), alcohols (*E*-**3**, *Z*-**3**), and E-oximes (*syn*-**4**, *anti*-**4**) are illustrated in Table 1 (the IC_{50} values for *E*-**1**, *Z*-**1**, *E*-**5**, and *Z*-**5** are not shown but were found to be >100 μM). Four of the fluorescent probes are active in quorum-sensing assays (Table 1) with two, alcohol *E*-**3** and oxime *anti*-**4**, possessing greater activity than 4-thia-2,3-dihydrofarnesol, the most potent synthetic lead identified in our earlier study of forty farnesol analogs [4]. These results demonstrate that the fluorescent probes possess sufficient solubility and stability to be accessible to *C. albicans* cells and, once there, interact strongly with the receptors or binding proteins responsible for fungal quorum sensing. The relative activity of the probes is in agreement with trends observed in the earlier studies in which the most potent quorum-sensing activity was displayed by primary alcohols possessing similar length and cross-section as farnesol. In the current study, the active analogs feature a weakly acidic head group (alcohols *E*-**3** or *Z*-**3**; oximes *syn*-**4** and *anti*-**4**). The earlier studies demonstrated that significant modifications of the farnesyl backbone led to dramatic reductions in quorum-sensing activity, and the biological activity of the alcohols and oximes validates the decision to embed the fluorophore within the hydrophobic backbone [4]. In the earlier work, farnesoic acid, farnesaldehyde, and methyl farnesoate possess only 3.3%,

0.4%, and 0.1%, respectively, of the quorum-sensing activity [4] of farnesol. It was, therefore, not entirely surprising to find minimal activity (IC_{50} > 100 μM) for the pentaene acids (**5**), esters (**1**), or aldehydes (**2**). Although it is natural to assume a correlation between reduced QSM and weaker interactions with a receptor or binding protein, it is important to note that the lower activity could also reflect reduced analog availability resulting from inability to enter cells, sequestration by another protein or binding agent, or enzymatic modification.

To demonstrate the effectiveness of these fluorescent analogs in fungal cell biology, we observed *C. albicans* A72 cells that had been stained with *anti*-oxime **4** by fluorescence microscopy (405 nm excitation) and differential-interference-contrast microscopy (Figure 2). Both the cytoplasmic and nuclear membranes are stained, and there appears to be a significant nuclear localization of the fluorescent analog. The latter observation may be significant; in mammalian cells, the Farnesol X receptor (FXR) is a nuclear receptor involved in the regulation of sterol and bile acid metabolism [11].

Significance

Farnesol is a recently discovered regulatory molecule in fungal biology. In addition to acting as a QSM in *C. albicans* [1], farnesol alters circadian rhythms in *Neurospora crassa* [12] and triggers apoptosis in *Aspergillus nidulans* (S. Harris, personal communication). In none of these cases is there an understanding of the molecular

Table 1. Photophysical and Biological Properties of Selected Analogs

Compound	UV[a] λ (ϵ)	Fluorescence[a] λ (ϕ)	Bioactivity IC_{50} (μM)[b]
E-2	384 (2.4×10^4)	321 (6×10^{-4}); 336 (4×10^{-4}); 353 (3×10^{-4})	>100
Z-2	392 (2.9×10^4)	321 (4×10^{-4}); 336 (4×10^{-4}); 353 (2×10^{-4})	>100
E-3 [7]	321 (2.0×10^4), 336 (2.8×10^4), 353 (2.6×10^4)	321, 336, 353 (1×10^{-2})	10
Z-3	323 (4.5×10^4), 338 (6.8×10^4), 356 (6.3×10^4)	323 (4×10^{-3}); 338 (3×10^{-3}); 356 (4×10^{-3})	50
syn-4	368 (6.0×10^4), 389 (5.2×10^4)	368, 389 (2×10^{-3})	25
anti-4	345 (6.4×10^4), 363 (9.6×10^4), 382 (9.1×10^4)	345, 363, 382 (1×10^{-3})	10
Farnesol [4]	—	—	1.2
4-thia-2,3-dihydrofarnesol [4]			16

[a] Wavelengths in nm.
[b] Concentration resulting in 50% reduction of germ-tube formation in *C. albicans*.

basis for signaling/regulation, and the availability of fluorescent analogs may help to elucidate farnesol's mode of action. The incorporation of a conjugated system containing both a pentaene and a carbonyl or oxime head group extends the absorption spectra to beyond 400 nm, minimizing autofluorescence of the fungal cells and facilitating applications in confocal microscopy. The analogs, which achieve useful fluorescence while preserving much of the length, shape, and hydrophobicity of farnesol, are expected to effectively mimic interactions of farnesol with binding or transport proteins. For *C. albicans*, in which the mode of farnesol's activity as a QSM remains unknown, the biologically active fluorescent analogs provide a means of identifying presumptive farnesol targets or farnesol binding proteins as well as any subcellular or organellar localization of exogenous farnesol. *C. albicans* is the most important fungal pathogen of humans. Studies of the ability of farnesol to control fungal morphology have until now been conducted in vitro. However, in mammalian systems, the secretion of farnesol by *C. albicans* may contribute to pathogenesis by acting as a virulence factor or virulence determinant. The fluorescent farnesol analogs described above may help identify mammalian targets of secreted farnesol and, if the metabolic fates of farnesol and the fluorescent farnesol analogs are at all comparable, may be useful in monitoring the pharmacokinetics of exogenous farnesol. In conclusion, we have developed a class of farnesol analogs that achieve useful fluorescence properties while maintaining the core sesquiterpene structure. Application of these analogs to investigation of quorum sensing is currently in progress and will be reported separately.

Experimental Procedures

Standard procedures and methods for bioassay of QSM activity have been described elsewhere [1 and 4]. Briefly, bioassays were performed in 25 ml flasks in a pH 6.5 medium consisting of 11 mM imidazole, 3 mM $MgSO_4$, and 2.6 mM *N*-acetyl-*D*-glucosamine.

For microscopy, *C. albicans* A72 was inoculated into the same medium in the presence of 50 µM of the *anti*-oxime farnesol analog (*anti*-**4**) taken from a freshly prepared stock (4.3 mM in methanol). The cells were shaken at 250 rpm in a New Brunswick Scientific G2 shaker for 4 hr at 37°C. For fluorescence microscopy, the cells were washed three times in 50 mM potassium phosphate buffer (pH 6.5) and observed with an Olympus FluorView FV500 microscope with the 100× lens with oil immersion. Synthetic procedures and characterization data for new compounds are included in the Supplemental Data, presented here as an Appendix.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, spectral characterization for new compounds, and references for preparation of known compounds. These can be found with the online version of this article at http://www.chembiol.com/cgi/content/full/12/6/639/DC1/ or appended to this edition of this document.

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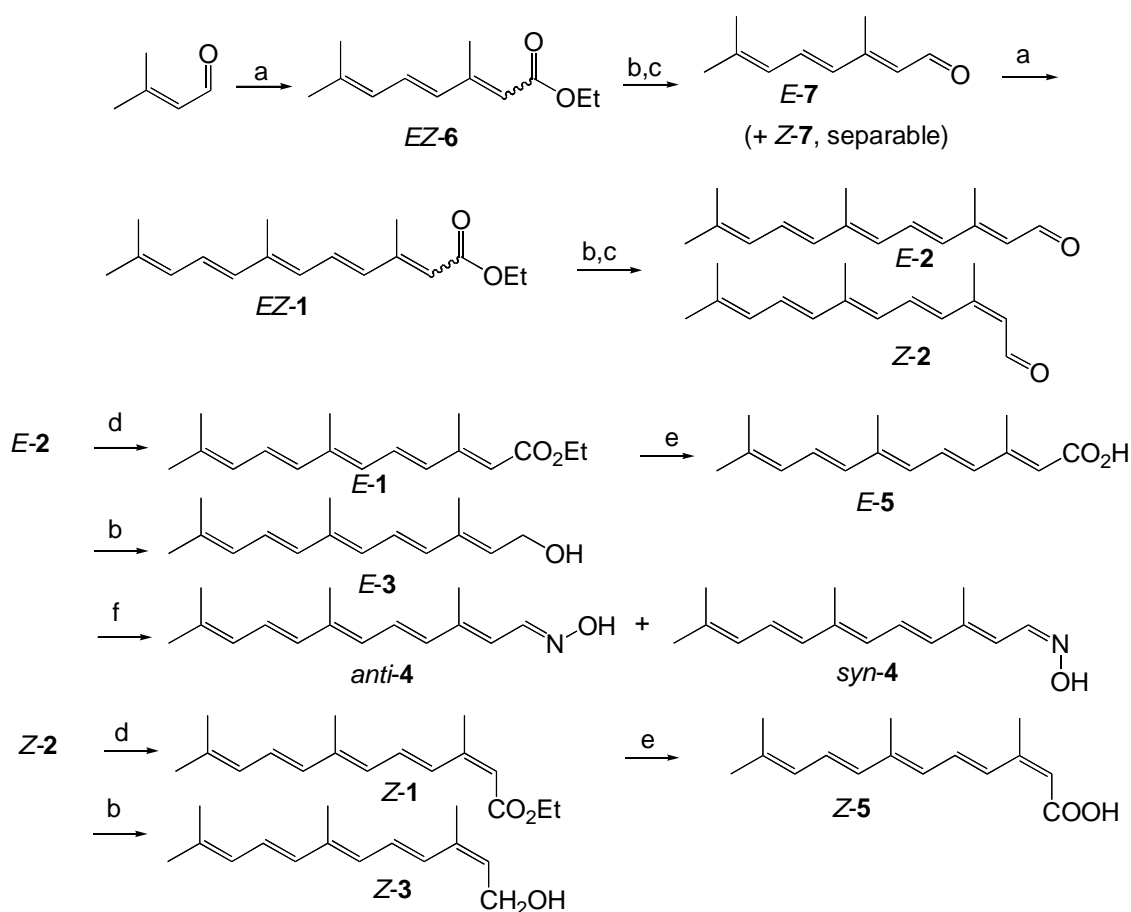
General experimental conditions have been described [1]. All reactions were performed in the dark, under N_2 and in flame-dried glassware. Polyenes were protected from light whenever possible. UV and fluorescence spectra were recorded as dilute methanol solutions on Shimadzu 2401PC (UV) or RF-5301PC (fluorescence) spectrometers. Melting points are uncorrected. All new compounds were purified by silica gel chromatography and were characterized by ^{1}H, ^{13}C, IR, UV fluorescence spectroscopy, and HRMS. Quantum yields were calculated using commercially available quinine sulfate dihydrate (Alfa Aesar, FW = 782.95, ϕ = 0.55; ε = 10,600 at 346 nm) as a standard [2].

Scheme Supplemental-1: Synthesis of Fluorophores

a. triethyl-3-methyl-4-phosphonobutanoate, *n*-BuLi; b. DIBAL;
c. MnO_2; d. MnO_2,EtOH NaCN; e. KOH; f. NH_2OH.

<u>Synthetic overview:</u> The ten analogs were derived from (*E,E*)-3,7-dimethyl-2,4,6-octatrienal (*E*-**7**) [3], prepared from 3-methyl-2-butenal (see Scheme Supplemental-1 on previous page). Homologation of the octatrienal with triethyl-3-methyl-4-phosphono-2-butenoate produced the

pentaenoate ethyl ester **1** as an inseparable mixture of E and Z-isomers at the 2,3-alkene. Reduction furnished an isomeric mixture of pentaene alcohols (Z-**3** and E-**3**) which tended to be difficult to separate and to undergo decomposition in the presence of trace acid to furnish complex mixtures of very nonpolar and strongly colored compounds. Oxidation of the alcohols furnished intensely colored aldehydes, E-**2** and Z-**2**, which were more stable than the alcohols and which could be separated by silica gel chromatography. The stereochemical identity of the aldehydes was determined by nOe and COSY pulse sequences, illustrated below for the individual isomers. Aldehyde *E*-**2** was used to prepare oximes *syn*-**4** and *anti*-**4**, ester *E*-**1**, and acid *E*-**5**. Aldehyde *Z*-**2** was similarly converted to ester *Z*-**1**, alcohol *Z*-**3**, and acid *Z*-**5**.

Ethyl (2*E/Z*, 4*E*)-3,7-dimethyl-2,4,6-octatrienoate (*EZ*-**6**): [2]

Into a 0 °C solution of triethyl 3-methyl-4-phosphonocrotonate (14.8 ml, 60 mmol) in dry THF (50 ml) was added dropwise n-BuLi (60 mmol, 2.5 M hexane solution). After 15 min, 3-methyl-2-butenal (4.8 ml, 50 mmol) was added as a solution in 10ml of THF and the reaction was stirred for 1h. The reaction was then warmed to room temperature and stirred for 20 min before being quenched with water (30 ml). The residue obtained after concentration in vacuo was diluted with water (200 ml) and extracted with 10% ethyl acetate/hexane (3 x 200 ml). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash chromatography (3% EA/Hex) to afford 6.08 g (81%) of ester **6** as an inseparable mixture of E and Z isomers (2:1).

(2*E/Z*,4*E*)-3,7-Dimethyl-2,4,6-octatrienal (*E*-**7** and *Z*-**7**):

Into a -78°C solution of esters **6** (30 mmol) in THF (70 ml) was added DIBAL (90 mmol, nominally 1M in hexane). After 3h, the reaction was quenched with sat, aq, potassium sodium tartrate (150 ml) and the resulting mixture was allowed to warm to room temperature. After stirring vigorously for 3h, the mixture was extracted with 20% ethyl acetate/hexane (EA/Hex, 3 x150 ml). The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude allylic alcohol, a 2:1 mixture of 2E- and 2Z-isomers, was directly applied to the next step.

Into a solution of the crude 3,7-dimethyl-2,4,6-octatrienol in THF (50 ml) was added MnO_2 (52.16 g, 600 mmol). After 12h, the reaction was diluted with ethyl ether (200 ml) and filtered (paper). The residue was washed with two more portions of ether (200 ml). The combined organic washes were concentrated *in vacuo* and the residue was purified by flash chromatography (5% ether/hexane) to afford individual samples of *E*-**7** (42%, 1.892g, two steps) and *Z*-**7** (23%, 1.036 g, two steps). The spectral properties of the aldehydes have been previously reported [2].

Ethyl (2*E/Z*, 4*E*,6*E*,8*E*)-3,7,11-trimethyl dodecapentenoate (*EZ*-**1**)

Using a similar procedure as described above for synthesis of ester **6**, Horner-Emmons reaction of E-**7** furnished ester **1** in 78% yield (10.15g) as an inseparable 2:1 mixture of E- and Z-isomers at the 2,3-alkene. The spectra for the individual isomers of **2** are described later.
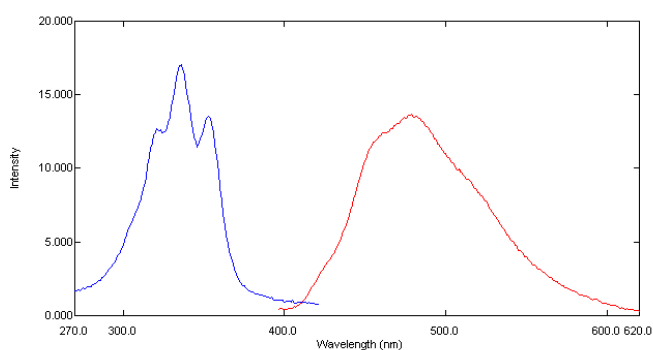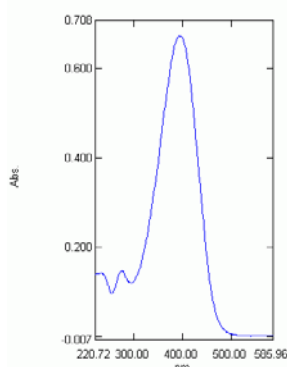
(2E,4E,6E,8E)-3,7,11-Trimethyldodecapentenal (*E*-2**):**

2
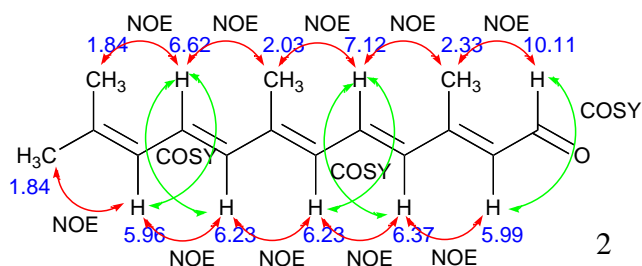
DIBAL reduction of the mixture of *EZ*-**1** under similar conditions as described above furnished the pentaene alcohol (*EZ*-**3**) which was used without purification (spectra of the individual isomers of **3** are reported later).

Into a solution of 2E/Z ,4E,6E,8E)-3,7,11-trimethyldodecapentenol in THF (50 ml) was added MnO_2 (52.16 g, 600 mmol). After 12h, the reaction was diluted with benzene/ethyl ether (1:50, 200 ml) and filtered (paper). The residue was washed with two more portions of 2% benzene/ether (200 ml). The combined organic washes were concentrated *in vacuo* and the residue was separated by flash chromatography (2.5% EA/Hex) to afford individual samples of *E*-**2** (54%, 3.504g, two steps) and Z-**2** (26%, 1.687g).

(*E*-**2**): R_f=0.3 (10% EA/Hex); mp = 92-94 ^{0}C; [1]H (CDCl$_3$, 500 MHz) δ 10.11 (d, 1H, *J*=8), 7.12 (dd, 1H, *J*=15 and 11.5), 6.62 (dd, 1H, *J*=15 and 11), 6.37 (d, *J*=15), 6.2 [m, 2H; peak at 6.23 (d, *J*=12) visible upon NOE irradiation at 6.37; 6.23 (d, *J*=15) visible upon NOE irradiation at 6.0], 6.0 [m, 2H; peak 5.99 (*J*=8.5) visible upon NOE irradiation at 6.37; peak 5.96 (*J*=11) visible upon NOE irradiation at 1.84], 2.33(s, 3H), 2.03(s, 3H), 1.84(s, 6H); [13]C (CDCl$_3$) (125MHz) δ 191.0, 154.7, 141.4, 138.2, 134.5, 134.2, 132.5, 129.8, 129.0, 127.5, 125.9, 26.4, 18.7, 13.13, 13.08; IR 2926, 1802, 1648, 1564, 1442, 1217, 966; UV λ 384 (ε = 2.5 x 10^4), 353 (ε = 1.6 x 10^4), 336 (ε = 1.1 x 10^4) 321 (ε = 7.4 x 10^4); Fluorescence $E_{emission}$ =479 nm; E_{ex}= 321, 336, 353. MS (HRFAB, 3-NBA): calc. for $C_{15}H_{20}O$: 216.1514; found: 216.1509 (2.4 ppm).

(2*Z*,4*E*,8*E*)-3,7,11-Trimethyldodecapentenal (Z-2):

R_f=0.4 (10% EA/Hex); Melting point = 80-82 ^{0}C; [1]H (CDCl$_3$, 500 MHz) δ 10.20 (d, 1H, *J*=8), 7.28(d, 1H, *J*=15), 7.01 (dd, 1H, *J*= 11.5 and 15), 6.62 (dd, 1H, *J*=11 and 15), 6.24 [(d, 2H, *J*=14.5), peak at 6.24 (d, *J*=12) visible upon NOE irradiation at 7.28; 6.24 (d, *J*=15) visible upon NOE irradiation at 5.96], 5.96 (d,

1H, *J*=11), 5.84 (d, 1H, *J*=8), 2.14 (s, 3H), 2.02 (s, 3H), 1.84 (s, 6H); [13]C (CDCl$_3$, 125MHz) δ 189.8, 154.6, 141.6, 138.1, 134.1, 133.4, 129.8, 127.6, 127.5, 126.3, 125.8, 26.3, 21.1, 18.6, 13.1; IR 2910, 1655, 1564, 1439, 1382, 1115, 959, 912, 733; UV λ 392 (ε = 2.9 x 10^4), 386 (ε =
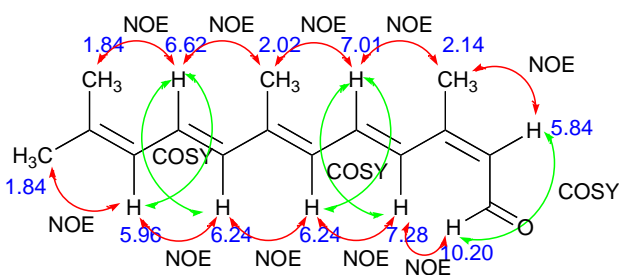
2.8 x 10^4), 354 (ε = 1.9 x 10^4), 336 (ε = 1.2 x 10^4), 321 (ε = 8.0 x 10^4); Fluorescence E$_{em}$=486; E$_{ex}$= 321, 336, 354; MS (HRFAB, 3-NBA) calc. for C$_{15}$H$_{20}$O: 216.1514; found: 216.1511(1.4ppm).
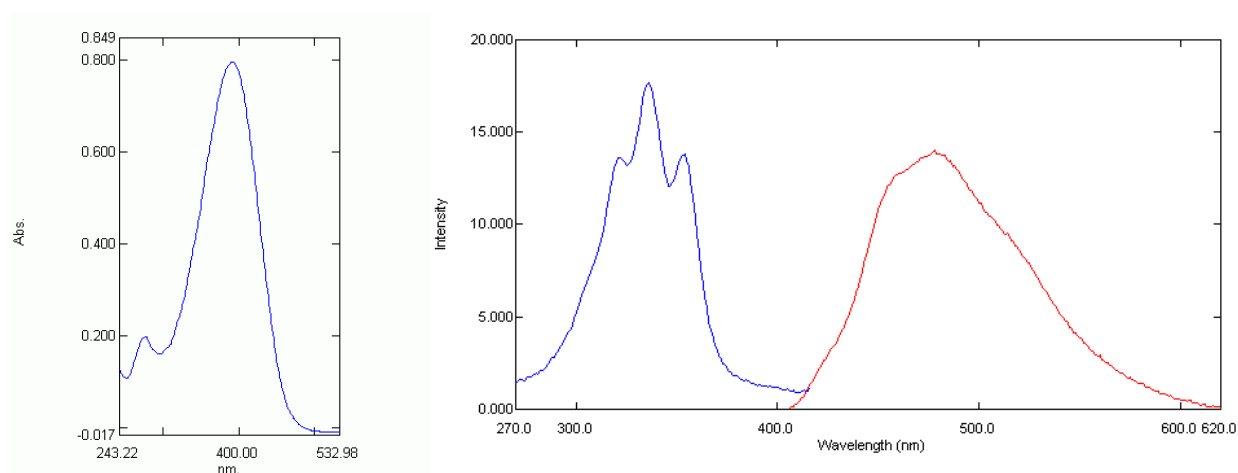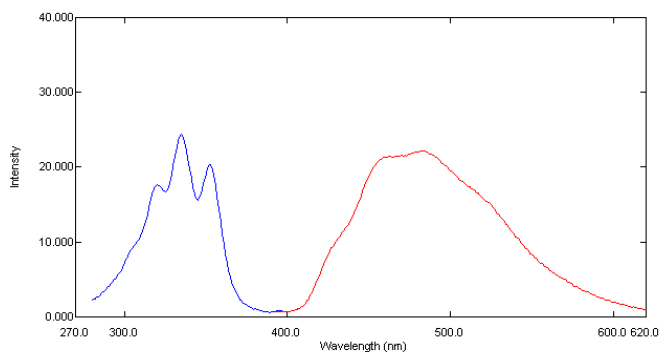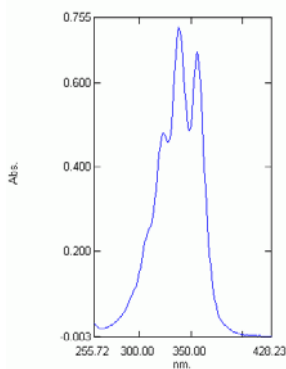

(2E,4E,6E,8E)-3,7,11-Trimethyldodecapenten-1-ol (didehydrofarnesyl, E-3), which has been previously reported from a different route [4], was prepared by DIBAL reduction of E-**2** (1 mmol, 0.216g) in 71% yield (0.155g) by a similar procedure as employed for reduction of EZ-**7**. The results of UV measurements differ slightly from those reported in the previous reference: λ 353 (ε = 2.6 x 10^4), 336 (ε = 2.8 x 10^4), 321 (ε = 2.0 x 10^4).
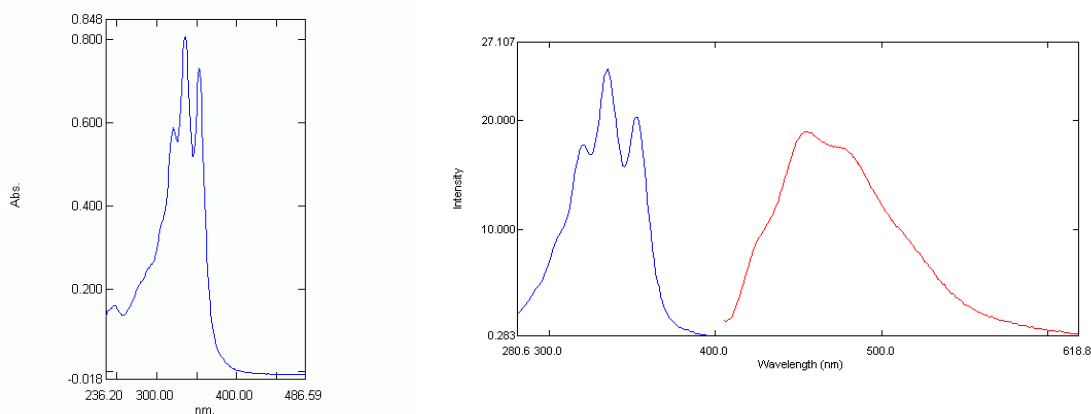
(2Z,4E,6E,8E)-3,7,11-Trimethyldodecapenten-1-ol (didehydrofarnesyl, Z-3) was prepared by DIBAL reduction of aldehyde Z-**2** (1 mmol, 0.216g) in 80% yield (0.175g): R$_f$=0.2 (20% EA/Hex); ^{1}H (CDCl$_3$, 500 MHz) δ 6.60-6.69 (m, 2H), 6.50 (dd, 1H, J=11 and 15), 6.22 (d, 1H,

J=15), 6.17 (d, 1H, *J*=10), 5.94 (d, 1H, *J*=11), 5.58 (t, 1H, *J*=7), 4.33 (d, 2H, *J*=7.5), 1.96 (s, 3H), 1.94 (s, 3H), 1.832 and 1.825 (two s, 6H); ^{13}C (CDCl$_3$, 125MHz) δ 137.2, 136.2, 136.0 130.7, 128.34, 128.30, 127.3, 125.9, 125.4, 58.5, 26.2, 20.4, 18.6, 12.9; IR 3408, 2974, 2916, 1673, 1446, 1379, 963; UV 356 (ε = 6.3 x 10^4), 338 (6.8 x 10^4), 323 (4.5 x 10^4); Fluorescence E$_{em}$=480; E$_{ex}$= 359; MS (HRFAB, 3-NBA) calc. for C$_{15}$H$_{22}$OH, MH$^+$: 219.1746; found: 219.1749 (1.2 ppm).

Ethyl (2*E*, 4*E*,6*E*,8*E*)-3,7,11-trimethyldodecapentenoate (*E*-**1**):
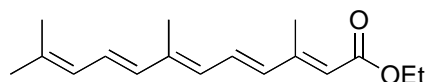
Into a 0°C solution of aldehyde *E*-**2** (1mmol, 0.216 g) in benzene/ethanol (1:5, 6 ml) was added NaCN (7 mmol) and MnO$_2$ (20 mmol). After 2h, the reaction was extracted with ethyl ether (4 x 100 ml). The filtered organic layers were concentrated under reduced pressure. The residue was purified by column chromatography (5% EA/Hex) to furnish *E*-**1** in 82% yield (0.213g): R$_f$=0.6 (10% EA/Hex); mp = 102-103^{0}C; ^{1}H (CDCl$_3$, 600 MHz) δ 6.98 (dd, 1H, *J*= 11.4 and 15), 6.57 (dd, 1H, *J*= 11.1 and 15.1), 6.29 (d, 1H, *J*=15), 6.22 (d, 1H, *J*=15.2), 6.17 (d, 1H, *J*=11.5), 5.95 (d, 1H, *J*=11), 5.78 (s, 1H), 4.18(q, 2H, *J*=7.1), 2.36 (s, 3H), 2.00 (s, 3H), 1.842 and 1.836 (two s, 6H), 1.30 (t, 3H, *J*=7.1); ^{13}C (CDCl$_3$, 150 MHz) δ 167.2, 152.6, 139.6, 137.2, 135.2, 134.4, 130.9, 130.0, 126.6, 125.9, 118.6, 59.6, 26.3, 18.6, 14.3, 13.8, 13.0; IR 2926, 1704, 1606, 1350, 1242, 1156, 963; UV λ$_{max}$ 368 nm; MS (HRFAB, 3-NBA) calc. for C$_{17}$H$_{24}$O$_2$: 260.1776; found: 260.1783 (-2.7 ppm).

Ethyl (2*Z*, 4*E*,6*E*,8*E*)-3,7,11-trimethyldodecapentenoate
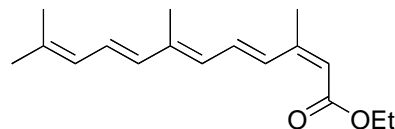
(*Z*-**1**) was prepared in a similar manner from aldehyde *Z*-**2** (1mmol, 0.216g) in 86% yield (0.224g): R$_f$= 0.6 (10% EA/Hex); mp = 76-78 ^{0}C; ^{1}H (CDCl$_3$, 400 MHz) δ 7.79 (d, 1H, *J*=15.2), 6.96 (dd, 1H, *J*= 11.4 and 15.4), 6.56(dd, 1H *J*= 10.8 and 15.2), 6.27 (d, 1H, *J*= 11.6), 6.23 (d, 1H, *J*=15.2), 5.96 (d, 1H, *J*= 11.2), 5.64 (s, 1H), 4.17 (q, 2H, *J*= 7.2), 2.07 (d, 3H, *J*= 1.2), 1.99 (s, 1H), 1.838/1.830 (two s, 6H), 1.29 (t, 3H, *J*= 7.2); ^{13}C (CDCl$_3$, 150 MHz) δ 166.4, 151.0, 139.8, 137.1, 134.6, 132.1, 130.8, 129.3, 126.4, 125.9, 116.5, 59.6, 26.3, 20.9, 18.6, 14.3, 13.0;IR 2980, 1701, 1606, 1448, 1268, 1052, 972; UV λ$_{max}$ 370 nm; Weakly fluorescent; MS (HRFAB, 3-NBA) calc. for C$_{17}$H$_{24}$O$_2$: 260.1776; found: 260.1770 (2.3 ppm).

**(2*E*, 4*E*,6*E*,8*E*)-3,7,11-Trimethyl dodecapentenoic acid** (*E*-**5**):
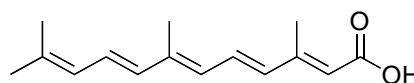
Into a solution of ester E-1 (1mmol, 0.216 g) in THF/isopropanol/benzene (6:2:1, 9 ml) was added 7N KOH (2 ml). The reaction was refluxed for 20h and then cooled to room temperature. The solution was brought to pH 2 by the successive addition of ice and 3 M HCl and then extracted with 2:10:1 EA/Hex/benzene (3 x 100 mL). The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. The resulting residue was purified by flash chromatography (EA/Hex/AcOH 100:900:5) to furnish acid *E*-**5** in 62% yield (0.144g): mp = 216-218 ^{0}C; ^{1}H (d$_6$-DMSO, 400 MHz) δ 12.10 (br. s, 1H), 7.00 (dd, 1H, *J*= 11.4 and 15), 6.58 (dd, 1H, *J*= 11.2 and 15.2), 6.40 (d, 1H, *J*=15.2), 6.21-6.27 (m, 2H), 5.95 (d, 1H, *J*= 10.8), 5.77 (s, 1H), 2.266 (d, 3H, *J*=0.8), 1.97 (s, 3H), 1.80 (s, 6H); ^{13}C (d$_6$-DMSO, 100 MHz) δ 167.9, 151.6, 139.4, 136.8, 135.4, 134.3, 130.9, 130.1, 126.6, 125.9, 119.5, 26.1, 18.5, 13.38, 12.8; IR 3454, 3055, 2988, 1673, 1265, 737; UV λ$_{max}$=357 (ε=53000); Fluorescence E$_{em}$=474; E$_{ex}$= 332, 348; MS (HRFAB, 3-NBA) calc. for C$_{15}$H$_{20}$O$_2$: 232.1463; found: 232.1458 (2.2 ppm).
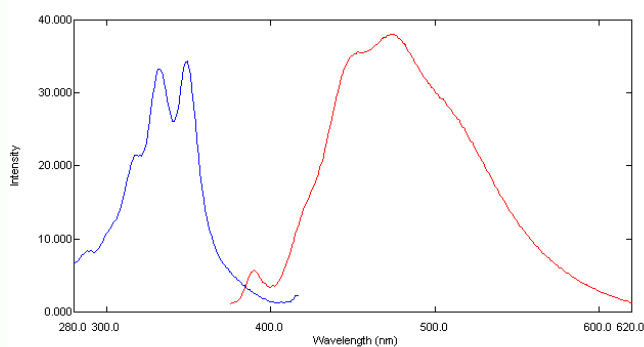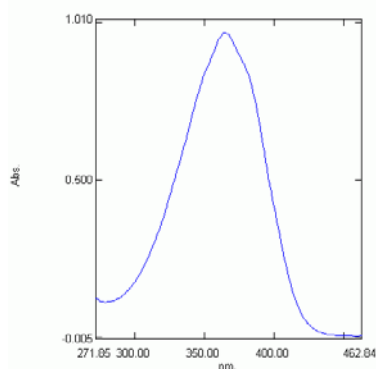
**(2*Z*, 4*E*,6*E*,8*E*)-3,7,11-Trimethyldodecapentenoic acid** (*Z*-**5**) was similarly prepared from *Z*-**1** (1.0 mmol, 0.216 g) in 77% yield (0.179 g): mp = 194-196 ^{0}C; ^{1}H (*d*^{6}-DMSO, 400 MHz) δ 7.69 (d, 1H, *J*=15.2), 6.99 (dd, 1H, *J*= 11.6 and 15.2), 6.58 (dd, 1H, *J*= 10.8 and 15.2), 6.24-6.30 (m, 2H), 5.94 (d, 1H, 10.8), 5.62 (s, 1H), 2.03 (s, 3H), 1.97 (s, 3H), 1.80 (s, 6H); ^{13}C (d-DMSO, 100 MHz) δ 167.2, 150.2, 139.9, 136.9, 134.4, 131.9, 130.5, 129.2, 126.6, 125.9, 117.5, 26.0, 20.5, 18.5, 12.8; UV λ$_{max}$=364 (ε=56000); Fluorescence E$_{em}$=475; E$_{ex}$= 331, 348; MS (HRFAB, 3-NBA) calc. for C$_{15}$H$_{20}$O$_2$, (M–H+2Li): 245.1705; found: 245.1703 (1.0 ppm).
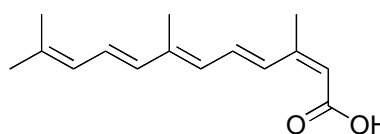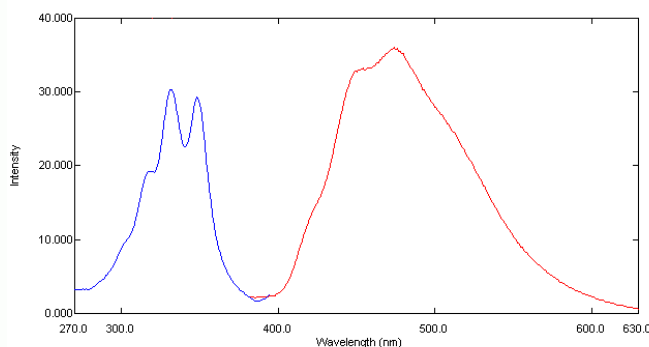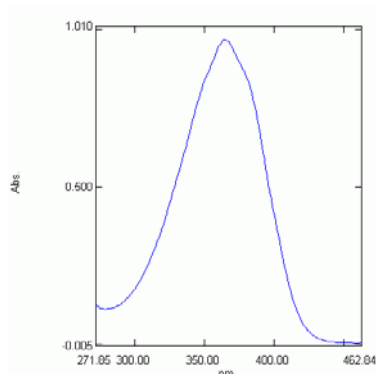
Oxime formation (***anti*-4 and *syn*-4**): To the solution of NH₂OH•HCl (6 mmol) in pyridine (10 ml) was added a solution of *E*-**2** (1 mmol, 0.216 g) in THF (4 ml). After 30min, the reaction was extracted with 20% EA/Hex (3 x 150 ml). The organic layer was washed with brine, dried over Na₂SO₄ and concentrated *in vacuo.* The residue was purified by flash chromatography (5 % EA/Hex) to afford *anti*-**4** (64%, 0.148 g) and *syn*-**4** (28%, 0.065 g).

Anti- (**2E,4E,6E,8E**)-3,7,11-
trimethyldodecapentenal oxime (*anti*-4):
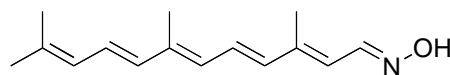
R_f=0.4 (20% EA/Hex); mp 140-160^{0}C (decomposition); ^{1}H (d₆-DMSO, 500 MHz) δ 11.1 (s, 1H), 8.10 (d, 1H, *J*=11), 6.76 (dd, *J*= 11.5 and 15), 6.51 (dd, 1H, *J*= 11.3 and 15.3), 6.43 (d, 1H, *J*= 15), 6.24-6.15 (m, 3H, appears to include three overlapping d), 5.93 (d, 1H, *J*= 11), 1.937 and 1.944 (two s, 6H), 1.79 (s, 6H); ^{13}C (d₆-DMSO, 125 MHz) δ 147.5, 140.1, 137.0, 136.3, 135.9, 134.6, 126.7, 125.9, 125.5, 124.3, 26.0, 18.4, 12.7, 12.6; IR 3445, 3057, 2917, 1644, 1265, 1265; UV λ(ε) 382 (9.1 x 10^5), 363 (9.6 x10^5), 345 (6.4 x 10^5); E_{em}=530; E_{ex}= 344, 362, 382; MS (HRFAB, 3-NBA) calc. for C₁₅H₂₁NO: 231.1623; found, 231.1622 (0.6 ppm).
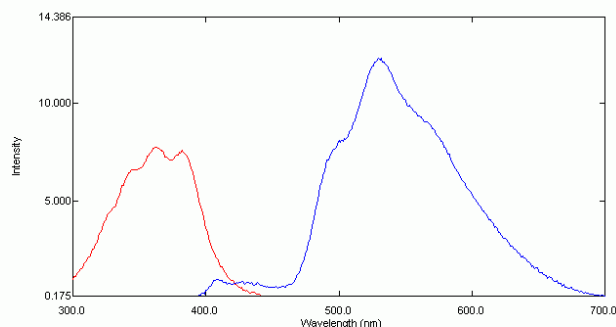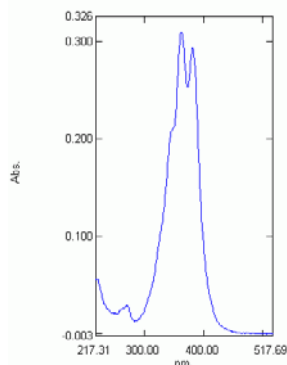
***syn*- (2*E*,4*E*,6*E*,8*E*)-3,7,11-trimethyldodecapentenal oxime (*syn*-4):**
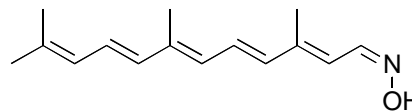
R_f=0.3 (20% EA/Hex); mp 150-170 ^{0}C (decomposition); ^{1}H (d$_6$-DMSO, 400 MHz) δ 11.18 (s, 1H), 7.55 (d, 1H, *J*= 10), 6.85 (dd, 1H, *J*= 11.6 and 15.2), 6.59 (d, 1H, *J*= 10), 6.53 (dd, 1H, *J*= 11.2 and 15.2), 6.44(d, 1H, *J*=14.8), 6.26-6.21 (m, overlapping d, 2H), 5.94 (d, 1H, *J*= 10.8), 2.00 (s, 3H), 1.95 (s, 3H), 1.79 (s, 6H); ^{13}C (d$_6$-DMSO, 100 MHz) δ 144.2, 141.5, 137.7, 136.5, 136.2, 134.5, 130.7, 128.5, 125.94, 125.87, 117.3, 26.1, 18.5, 12.8, 12.5; IR 3418, 3055, 1641, 1265, 747; UV 389 (5.2 x 10^4), 368 (6.0 x 10^4); E_{em}=565; E_{ex}= 369; MS (HRFAB, 3-NBA) calc. for $C_{15}H_{21}NO$: 231.1623; found: 231.1624 (0.2 ppm).
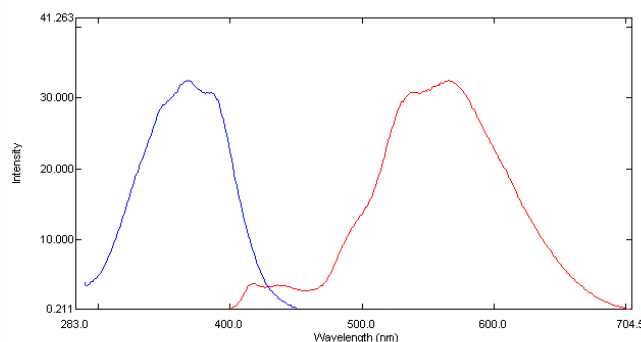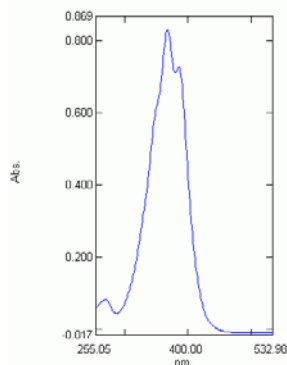
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[4] Liu, X.-h.; Prestwich, G. D. (2004) Didehydrofarnesyl diphosphate: an intrinsically fluorescent inhibitor of protein farnesyltransferase. Bioorg. & Med. Chem. Lett*. 14*, 2137-2140.