Synthesis and Biological Evaluation of the Geometric Farnesylated Analogues of the a-Factor Mating Peptide of *Saccharomyces cerevisiae*

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The **a**-factor of *Saccharomyces cerevisiae* is a dodecapeptide pheromone (YIIKGVFWDPAC-(Farnesyl)-OCH_3, **1**), in which post-translational modification with a farnesyl isoprenoid and carboxymethyl group is required for full biological activity. This peptide has been used as a model system to explore the biological function of the farnesylcysteine moiety, which is found on and required for the biological activity of many key mammalian proteins. The objective of this particular study was the determination of the biological effect of double bond isomerization of the natural *E*,*E*-farnesyl moiety on the biological activity of the **a**-factor. A unified, stereoselective synthetic route to the three geometric isomers of *E*,*E*-farnesol (**12**, **13**, and **14**) has been developed. The key feature of this synthesis is the ability to control the stereochemistry of triflation of the β-ketoester **22** to give either **23** or **25**. The three farnesol isomers were converted to the corresponding isomeric **a**-factors (**9**, **10** and **11**) via a modified version of a previously utilized synthetic route. Biological evaluation of these peptides indicates that, surprisingly, all three possess nearly equivalent activity to the natural **a**-factor bearing the *E*,*E*-farnesyl moiety.

The yeast *Saccharomyces cerevisiae* exists in two haploid types, **a** and α. Both types of haploid fungi cells synthesize and secrete mating pheromones that trigger cell fusion with cells of the opposite mating type to form diploid zygotes. The α type cells secrete α-factor pheromone, which is an unmodified 13-amino acid peptide (WHWLQLKPGQPMY). The **a** type cells secrete **a**-factor pheromone, which is a farnesylated dodecapeptide mating pheromone (YIIKGVFWSPAC(S-farnesyl)-OCH_3, **1**, Figure 1). This pheromone binds to a receptor, the Ste3p protein, on the surface of α-cells,[1−3] and this binding event leads to a series of well-characterized changes in α-cell behavior, starting with cell growth arrest and ending with fusion with an **a**-cell.[4] The post-translational modification of **a**-factor by addition of a farnesyl isoprenoid and carboxymethyl ester to its C-terminal cysteine residue is critical for its export and bioactivity.[5]

Farnesylation is a very common and critically important post-translational modification, which occurs in all eucaryotic organisms.[6] In particular, the Ras protein and other key signal transduction proteins are farnesylated. Blocking the farnesylation of these proteins is thus an attractive target for drug design in the chemotherapy of cancer,[7,8] and several farnesyltransferase inhibitors are in clinical trials.[9] However, the exact role of prenylation in the cellular localization and biological function of prenylated proteins remains to be fully elucidated.[10,11] The **a**-factor pheromone provides an ideal model system to further study lipopeptide structure−function relationships.[4] The simplicity of this fungal system has allowed for biochemical and pharmacological dissection of the structural and functional requirements for the peptide prenylation process. It is particularly attractive to study **a**-factor because of its easily assayed biological activity and its relatively small size. A variety of farnesyl- and peptide-modified analogues can be prepared entirely via chemical synthesis, and the resulting compounds can be fully characterized for purity and identity by HPLC, FAB-MS, and NMR, and then through several well-established biological assay methods. Moreover, it should be noted that studies on yeast model systems have proved crucial

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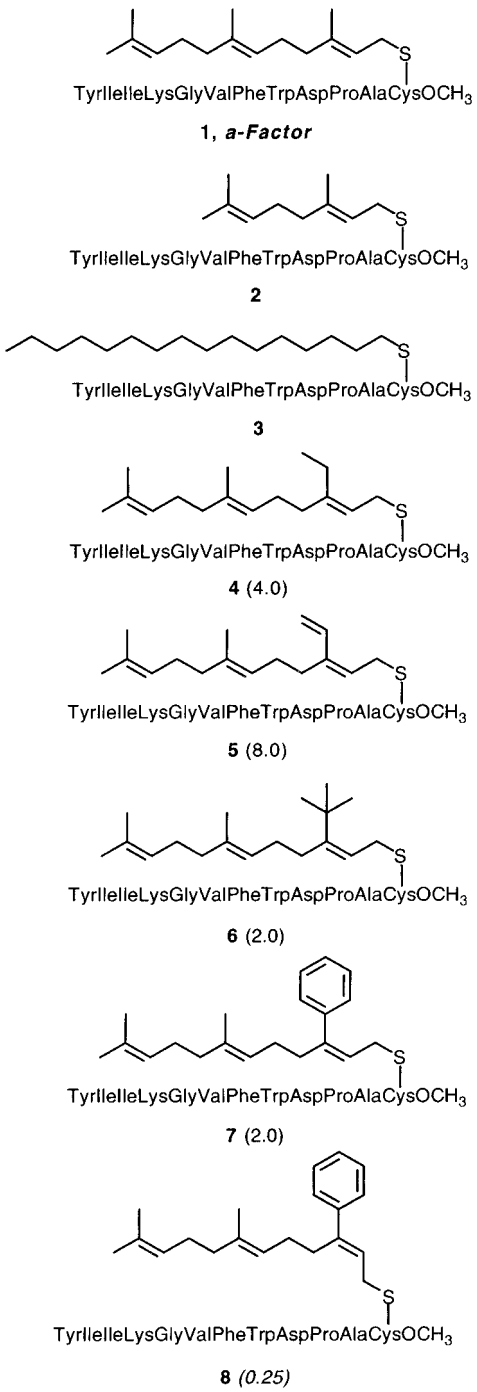

Figure 1. **a**-Factor and selected **a**-factor analogues previously synthesized. Values in parentheses are activities for the analogues **4**−**8** in the growth arrest assay (vide infra), relative to **1**.

to the elucidation of other features of signal transduction pathways important in carcinogenesis.

Several studies on lipid-modified analogues of **a**-factor have indicated that the farnesyl moiety does not simply enhance hydrophobicity, but also plays a more active role in the biological actions of this pheromone. The removal of one isoprene unit from the farnesyl group of **a**-factor, which affords the less hydrophobic geranylated **a**-factor analogue **2**, results in a 50% decrease in biological activity.[5] However, replacement of the entire farnesyl group with the simple yet equally hydrophobic hexadecyl lipid (to give **3**) leads to a much greater decrease in biological activity.[5,12] Recently, we have prepared and tested a series of novel farnesyl-modified **a**-factor analogues (**4**−**8**, Figure 1).[13] In these analogues, replacement of the 3-methyl group with ethyl, vinyl, *tert*-butyl, or phenyl groups at 3-position of the farnesyl chain of **a**-factor leads in general to a more active pheromone. However, note that the bulkier and more hydrophobic groups (*tert*-butyl and phenyl, **6** and **7**) exhibit lower biological activity than the smaller moieties (ethyl and vinyl, **4** and **5**). In the course of preparing the phenyl analogue, two pheromones were produced that differ only in the geometry of the allylic double bond (**7** and **8**). Depending on the assay used, these analogues show up to a 32-fold difference in their biological function. More recently, we have determined the membrane partitioning coefficients for the **a**-factor analogues **4**−**6** and have demonstrated that their biological activities do not correlate with simple membrane affinity.[14] These observations indicate that even modest structural modifications of the farnesyl moiety can lead to significant changes in its bioactivity, and emphasize that hydrophobicity differences alone cannot explain the differences in pheromone activity.

The results observed with the two phenyl isomers, **7** and **8**, provided the impetus to further explore the effects of double bond isomerization of the farnesyl moiety on **a**-factor biological activity. Therefore, we wished to prepare **9**, **10**, and **11**, the three geometric farnesylated stereoisomers of **a**-factor (Figure 2). Recently, we have developed a novel, unified synthetic route to the three isomeric derivatives of natural *E*,*E*-farnesol (**12**, **13**, and **14**), employing the vinyl triflate chemistry previously developed in this laboratory.[15] These farnesol isomers were used to synthesize the three unnatural geometric isomers of FPP, which were then assayed as potential protein-farnesyltransferase inhibitors. This synthetic route has now been adapted for the preparation of the three geometric isomers of **a**-factor. Note that these are the only geometric isomers possible (that is, the only ones where the only change is in double bond stereochemistry). This paper presents the full experimental details of our synthesis of **12**−**14**, their conversion to the corresponding **a**-factors **9**−**11**, and the biological evaluation of these novel lipopeptide analogues.

Results

Synthesis of Farnesol Isomers. There are several methods that have been developed to stereoselectively synthesize *trans*-isoprenoids. However, much less work has been done on preparation of *cis*-isoprenoids.[16,17]

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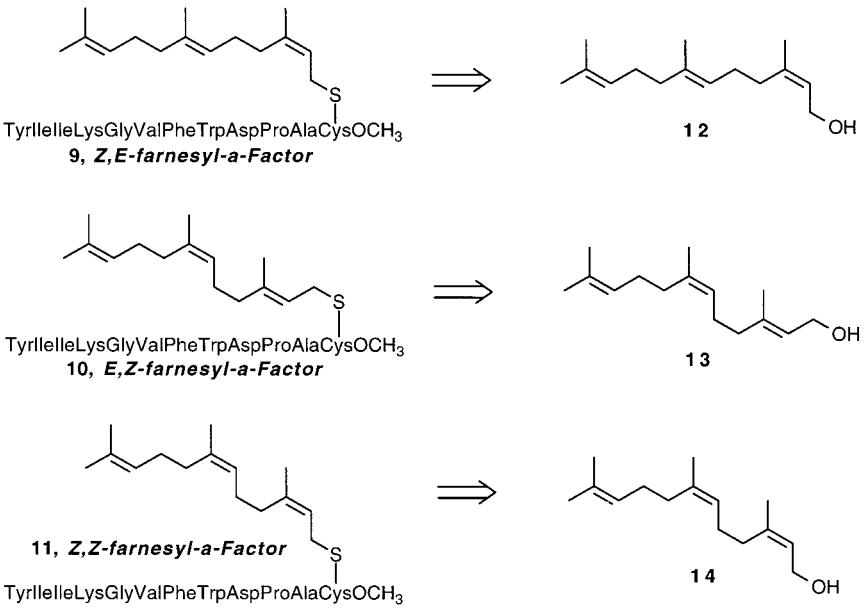

Figure 2. **a**-Factor analogues synthesized in this study.

Although the stereoselectivity of the reported procedures is generally high, they are lengthy and relatively inflexible, not allowing for the ready preparation of analogues. Thus, it would be desirable to develop a general, short, flexible stereoselective method that would allow for the preparation of analogues of all geometric farnesyl isomers from a common starting material. Our vinyl triflate route to *Z*-isoprenoids is based on an observation on the solvent effect on triflation of β-ketoesters (Scheme 1). Using THF as the reaction solvent, the β-ketoester **15** was converted to the triflate **16** with high stereoselectivity (**16**/**17** 95:5) and modest yield. In an attempt to optimize the yields of **16**, the more polar solvent 1,2-dimethoxyethane (DME) was used instead of THF in the reaction. However, this increase in solvent polarity resulted in a loss of stereocontrol, which lead to a mixture (~50:50) of triflate stereoisomers **16** and **17**, with a low overall yield. In contrast, the less polar solvent diethyl ether afforded only the stereoisomer **16**, in modest yield. When the polar aprotic solvent DMF was used in the triflation reaction, only the stereoisomer **17** was obtained, in good to excellent yield. Triflation of compound **15** in DMF thus could be used as the key step in the preparation of the *cis*-isoprenoid double bond in a stereocontrolled fashion.[18]

The three unnatural isomers of farnesol (**12**, **13**, and **14**) were thus synthesized using the stereoselective triflation procedures described above. The synthesis of *Z*,*E*-farnesol **12** is illustrated in Scheme 1. Using potassium bis(trimethylsilyl)amide (KHMDS) as base and 2-[*N*,*N*-bis(trifluoromethylsulfonyl)amino]-5-chloropyridine as triflating reagent, the β-ketoester **15** was exclusively converted to the *E*-triflate **17** in DMF solvent with 70% yield. Note that **17** has been previously reported and characterized.[19] As previously demonstrated, using Pd(0)/CuI as co-catalysts triflate **17** is coupled with tetramethyltin in a highly stereoselective fashion to afford the cis,trans-ester **18** in excellent yield.[19] The ester **18** is then reduced with diisobutylaluminum hydride (DIBAL-H) to afford the desired 2*Z*,6*E*-alcohol **12**.[20]

A hypothetical mechanism for stereocontrol of the triflation product, which assumes that the triflate geometry is controlled by the geometry of the potassium enolate, is shown in Scheme 1. The β-ketoester **15** is converted initially into the *Z*-potassium enolate **19** by the base KHMDS. The *Z*-potassium enolate form allows for intramolecular chelation of the potassium ion between the enolate oxyanion and the ester carbonyl group, as shown. When the polar aprotic solvent DMF is used in the triflation reaction, the carbonyl group on DMF can compete with the β-ketoester carbonyl group for chelation to the potassium enolate. Thus, the original *Z*-configuration of potassium enolate could be disrupted, and this would lead to isomerization to the more stable E-enolate **20**.[21] Trapping of the enolate with the triflating reagent would then lead to the E-triflate **17**.[18] In contrast, the much less polar solvent THF presumably does not disrupt the intramolecular chelation of the potassium enolate **19**; thus the cis-enolate configuration is retained and reflected in the geometry of the *Z*-triflate product **16**.

The syntheses of *E*,*Z*-farnesol **13** and *Z*,*Z*-farnesol **14** required the preparation of the *Z*-β-ketoester **22** (Scheme 2). This compound was generated in a straightforward manner via the coupling of neryl bromide (**21**) with the dianion of ethyl acetoacetate. The geometry of the central double bond in **22** was confirmed by NMR comparison to **15**. The ^{13}C NMR signals were assigned for both **15** and

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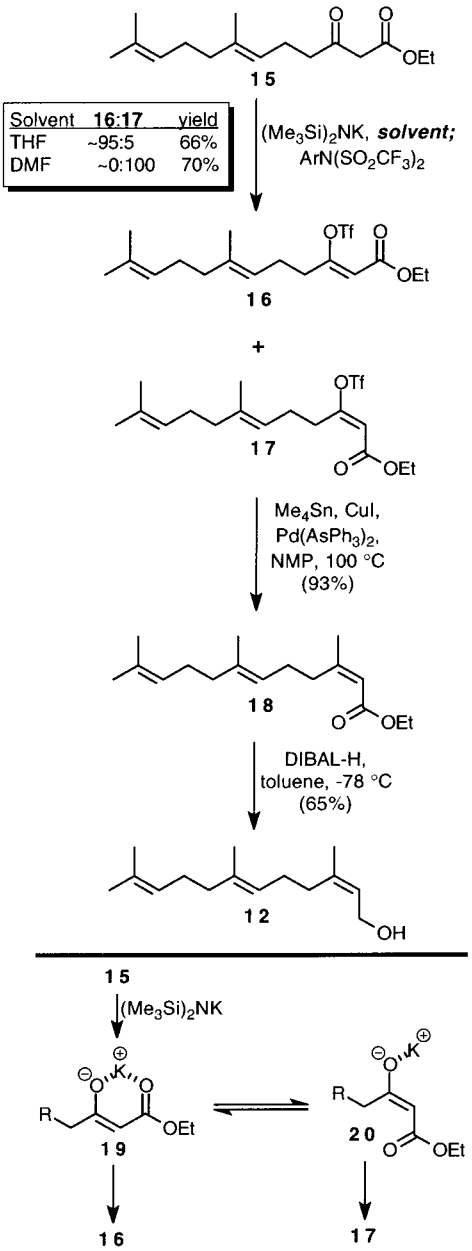
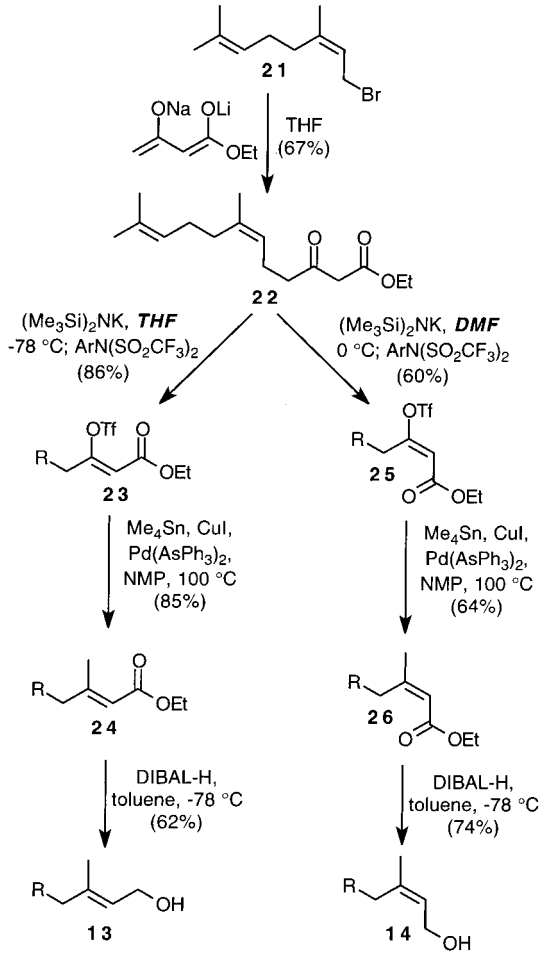
Scheme 1. Synthesis of 12



Scheme 2. Synthesis of 13 and 14



22 using 2D ^{1}H-^{13}C correlation in combination with DEPT experiments. A comparison of the C_7-Me signals for **15** and **22** indicated that the chemical shift for the former (16.7 ppm) was significantly upfield from that of the latter (24 ppm). This is consistent with the shielding expected for a methyl group cis to a methylene group versus that for a methyl group cis to a proton. A similar but less pronounced shift was observed in the proton NMR signals for the C_7-Me groups (**15**, 1.62 ppm; **22**, 1.69 ppm). As the subsequent modifications do not affect any bonds adjacent to the *cis*-C_6-C_7 double bond of **22**, these NMR results provide confirmation of the stereochemistry of this bond in alcohols **13** and **14**, and thus in **a**-factor analogues **10** and **11**.

Triflation of **22** using THF as a solvent afforded **23** in excellent yield (85%) and stereoisomeric purity after flash chromatography. Coupling of **23** with tetramethyltin generated ester **24**, also in excellent yield. Reduction of **32** gave the desired *E,Z*-farnesol isomer **13**. Note that the proton NMR spectrum of **24** was consistent with that previously reported for *trans,cis*-methyl farnesoate.[20] The *Z,Z*-farnesol isomer **14** was prepared from the *Z*-β-ketoester **22**, using the same route employed for the preparation of the *Z,E*-isomer **12**. Triflation of compound **22** in DMF affords the isomer **25** as the sole product. Methylation of **25** generated ester **26**,[22] which was reduced to the desired farnesol isomer **14** using DIBAL-H.[16,20]

Crude samples of the three farnesol isomers were subjected to GC−MS evaluation, to define the stereochemical control in our synthetic route. The analysis of the three alcohol isomers **12**, **13** and **14**, along with commercial *E,E*-farnesol, indicated high stereochemical purity for **12** and **14** as shown in Table 1, but the presence of a significant amount of *Z,Z*-farnesol in the sample of the *E,Z*-farnesol **13**. The loss of stereocontrol may be at the stage of the Stille coupling of **23** with tetramethyltin to yield the *E,Z*-ester **24**. We[19] and others[23,24] have previously observed that Stille couplings

(22) The ^{1}H NMR and ^{13}C NMR spectra of compounds **23** and **25** were consistent with their stereochemical assignments. A comparison of the protons of C_4-methylene signals for **23** and **25** indicated that the chemical shift for **23** (2.395 ppm) was significantly upfield from that of **25** (2.931 ppm), which is consistent with the deshielding effects of carbonyl group cis to the methylene protons of **25** versus trans to those of **23**.

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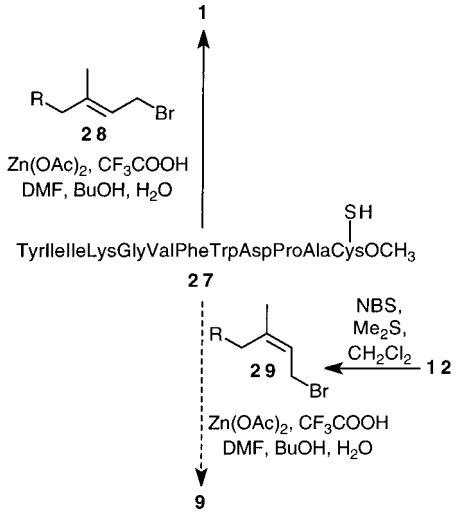
Table 1. GC−MS Analysis (%) of Farnesol Isomers[a]

t_R (min)	*E,E*-farnesol[b]	*Z,E*-farnesol (**12**)	*E,Z*-farnesol (**13**)	*Z,Z*-farnesol (**14**)
7.31	nd[c]	2.6	9.0	97.9
7.69	1.4	nd[c]	87.3	2.1
7.70	nd[c]	93.6	nd[c]	nd[c]
7.90	94.0	3.7	3.8	nd[c]

[a] GC−MS conditions: HP5988A GC/MS, DB5ms column, 100−250 °C at 10°/min. The retention times for the isomers are as follows: 7.31 min, *E,E*-farnesol; 7.69 min, *E,Z*-farnesol; 7.70 min, *Z,E*-farnesol; 7.90 min, *Z,Z*-farnesol. These retention times are assigned on the basis of the major component expected for each sample based on its method of synthesis or origin and are essentially consistent with the original GLC results of Burrell and co-workers, although they observed an inversion in the relative mobilities of *E,Z*- and *Z,E*-farnesol.[20] [b] Commercial sample of *E,E*-farnesol. Other impurities account for the other 4.6%. [c] nd = not detected in this sample.

Scheme 3. Attempted Synthesis of 9



with *Z*-isoprenoid triflates can lead, in some instances, to loss of stereocontrol and production of the more stable isomeric product. The isomeric farnesol **14** can be removed from **13** by careful flash column chromatography.

Synthesis of the Isomeric *a*-Factor Analogues. As discussed in the Introduction, two isomeric, phenyl-substituted **a**-factor analogues (**7** and **8**, Figure 1) exhibit up to a 32-fold difference in their biological function.[13] Therefore we proposed to stereospecifically synthesize the three geometric isomers of **a**-factor (**9**−**11**, Figure 2), which could then be used as probes to explore the effects of double bond isomerization on the biological activity of a farnesylated lipopeptide. Various methods for the synthesis of **a**-factor and its analogues have been previously reported. The methods developed in our laboratory involve solution-phase synthesis, solid-phase synthesis and a combination of both techniques.[25] The synthetic method initially chosen for this project involves coupling of farnesyl bromide (**28**) with the unfarnesylated dodecapeptide **27** to generate the **a**-factor, using zinc acetate and trifluoroacetic acid as catalysts (Scheme 3).[26] This procedure was previously used for the preparation of analogues **4**−**8** (Figure 1).[13]

The *Z,E*-farnesyl bromide **29** was synthesized from farnesol isomer **12** using CBr_4/PPh_3 as previously described.[15] Unfortunately, the conjugation of **29** with the dodecapeptide **27** afforded the desired **a**-factor analogue **9** in very low yield. Note that in a parallel control reaction the same sample of **27** coupled in an efficient manner with commercial **28** to give **a**-factor itself. We initially supposed that the lack of coupling was due to the impure nature of the bromide **29**. However, RP-HPLC purification of **29** did not significantly improve the yield of **9**. Thus the poor yield may be due to a higher degree of instability of the *Z,E*-farnesyl bromide under the peptide farnesylation conditions, although the stability of **29** under the reaction conditions was not addressed directly in a control experiment. Since isoprenoid chlorides are more stable than the corresponding bromides, it would be desirable to use *Z,E*-farnesyl chloride as the farnesyl source. Unfortunately, we have previously established that *E,E*-farnesyl chloride does not undergo efficient coupling with the dodecapeptide **27**.[13] However, Poulter and co-workers have demonstrated that *E,E*-farnesyl chloride couples efficiently and in high yield with cysteine methyl ester, using ammonia in methanol as the reaction medium.[27] Thus, we planned to use *Z,E*-farnesyl chloride (**30**) as the farnesyl source, couple it with cysteine methyl ester, and then prepare the farnesylated dipeptide Fmoc-NH-Ala-Cys[S-(*Z,E*-farnesyl)]-OCH_3 (**32**, Scheme 4). This dipeptide can be purified to homogeneity by RP-HPLC and characterized, prior to coupling with the decapeptide portion of **a**-factor. This strategy has been employed previously to synthesize **a**-factor itself from the dipeptide FmocNH-Ala-Cys[S-(*E,E*-farnesyl)]-OCH_3 and the protected decapeptide Fmoc-Tyr-Ile-Ile-Lys(Fmoc)-Gly-Val-Phe-Trp(Fmoc)-Asp(OFm)-ProOH.[28]

The stepwise route to the *Z,E*-**a**-factor analogue **9** is illustrated in Scheme 4. The synthesis started from *Z,E*-farnesol **12**, which was chlorinated using *N*-chlorosuccinimide (NCS) and dimethyl sulfide.[29] The resulting farnesyl chloride **30** was coupled with cysteine methyl ester, using 6M ammonia in methanol at room temperature for 3 h.[27] Following solvent evaporation, silica gel flash column purification afforded farnesylated cysteine methyl ester **31** as the sole farnesylated product. Its ^1H NMR spectrum confirmed that the alkylation occurred at the sulfhydryl moiety of cysteine methyl ester. The farnesylated cysteine methyl ester is then coupled with Fmoc-alanine to give the farnesylated dipeptide Fmoc-Ala-Cys(*Z,E*-farnesyl)-OCH_3 (**32**). The observed proton NMR and FAB mass spectra are consistent with those expected for the desired product (vide infra). The desired **a**-factor analogue **9** was synthesized by condensing the protected decapeptide **34** with the farnesylated dipeptide as previously performed in this laboratory.[28] The Fmoc-protected dipeptide **32** was deprotected to give **33**, which was then coupled with **34** using the BOP reagent. Global deprotection of the Fmoc and Fm groups afforded the desired *Z,E*-**a**-factor analogue **9**.

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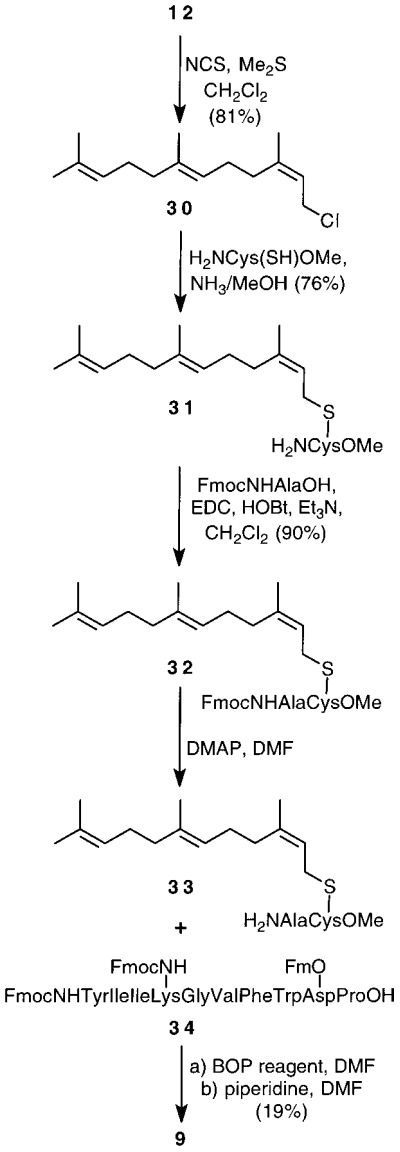
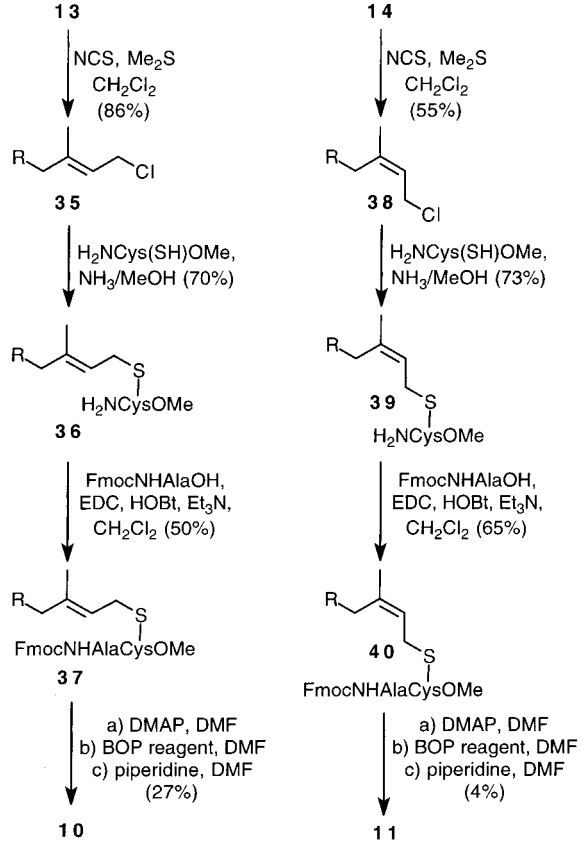
Scheme 4. Stepwise Synthesis of 9



Scheme 5. Stepwise Syntheses of 10 and 11



The syntheses of the *E*,*Z*- and *Z*,*Z*-farnesylated dipeptides **37** and **40** followed the same synthetic pathway as for the *Z*,*E*-farnesylated dipeptide (Scheme 5). The farnesylated dipeptide **37**, following deprotection of the Fmoc group on the Ala, was coupled with protected decapeptide **34**. Deprotection of the Fmoc and OFm groups afforded the desired **a**-factor analogue **10** (Scheme 5). In the same manner, the farnesylated dipeptide **40**, was deprotected and then coupled with protected decapeptide **34**. Deprotection of the Fmoc and OFm groups afforded the final desired isomeric **a**-factor analogue **11** (Scheme 5). The isolated yields for **9**−**11** appear modest (see Table 2), but they represent the combined yields for deprotection of the dipeptides (**32**, **37**, and **40**), coupling with **34**, global deprotection, and careful RP-HPLC purification.

One particular concern in the preparation of the isomeric farnesylated peptides was to ensure that the double bond geometry had been maintained at the C_2−C_3 double bond. It was assumed that the geometry of the isolated C_6−C_7 double bond would not be affected by any of the manipulations after its formation. We had thought that simple 1D NOE experiments would provide evidence supporting the geometry of these isomers. However, these experiments were inconclusive due to the fact that H_6 and H_{10} vinylic proton signals overlap and both appear at 5.15 ppm. Thus we tried to use the chemical shift values of the four methyl groups in these isomers to make the stereochemical assignments. Considering the structural similarity of these isomers, we assumed that all vinylic protons (H_2, H_6, and H_{10}) should show a similar shielding effect on chemical shift values of the methyl groups at position 3, 7, and 11 (C_3 Me, C_7 Me, and C_{11} Me), respectively.[20] Therefore the chemical shift values of C_3 Me should be the same in the natural *E*,*E*-dipeptide and *E*,*Z*-isomer **37**, and the *Z*,*E*-isomer **32** and the *Z*,*Z*-isomer **40**. However, this chemical shift should differ between the *E*,*Z*-isomer **37** and the *Z*,*Z*-isomer **40**, thus allowing one to determine if isomerization of the C_2−C_3 double bond had occurred during the synthesis of either isomer. Similarly, the chemical shift values of C_7 Me should be the same between the natural *E*,*E*-dipeptide and *E*,*Z*-isomer **37**, and the *Z*,*E*-isomer **32**, and in *E*,*Z*-isomer **37** and the *Z*,*Z*-isomer **40**. Assuming that the shielding effect is about 0.07−0.09 ppm, we assigned chemical shift values for the 4 methyl groups of 4 dipeptide stereoisomers as shown in Figure 3. Note that the signal of C_7 Me always overlaps one of C_{11} Me signals. This is due to the H_6 and H_{10} vinylic protons, which possess the same chemical shift values, shielding the C_7 Me and one of C_{11} Me groups (the one with the same configuration as the C_7 Me) to the same extent. Note that the same proton chemical shift patterns seen with the dipeptides are also seen with the **a**-factor isomers **9**−**11** (Table 2).

Biological Assay of the Isomeric *a*-Factor Analogues. To assess the effects of various modifications of the **a**-factor, a number of biological assays have been employed.[4] These utilized different characteristics of the

Table 2. Characterization of the Isomeric a-Factor Peptides

	E,E-**a**-factor (**1**)	*Z,E*-**a**-factor (**9**)	*E,Z*-**a**-factor (**10**)	*Z,Z*-**a**-factor (**11**)
yield[a]		19	27	4
MS (MH+)[b]		1629.1	1626.3	1629.4
K′[c]		7.9	7.9	7.9
C3 Me[d]	1.61 ppm	1.68 ppm	1.61 ppm	1.69 ppm
C7 Me[d]	1.54 ppm	1.56 ppm	1.63 ppm	1.63 ppm
C11 (2Me)[d]	1.54/1.63 ppm	1.54/1.62 ppm	1.54/1.63 ppm	1.55/1.63 ppm
halo assay endpoint[e]	50 pg	50 pg	100 pg	50 pg

[a] Yield is for dipeptide deprotection, 10 + 2 coupling, final deprotection, and purification steps. [b] Calculated value is 1628.9. Experimental values shown are from electrospray mass spectroscopy. [c] *K′* is defined as $(V_p - V_f)/V_f$, where V_p is the elution volume for the peptide and V_f is the breakthrough volume. HPLC was performed on a Waters μ-Bondapak-C_{18} column (3.9 × 300 mm) using a linear gradient of water (0.025%TFA) and MeCN (0.025%TFA) with water from 20% to 80% over 30 min. [d] Chemical shift of the 600 MHz proton NMR signals assigned to the indicated methyl group. [e] Lawns of *Saccharomyces cerevisiae* cells of the supersensitive strain RC757 were subjected to a dilution series containing identical amounts of each **a**-factor peptide. Endpoints were defined as the least amount of pheromone that was capable of inducing growth arrest resulting in a region of significantly reduced lawn density.

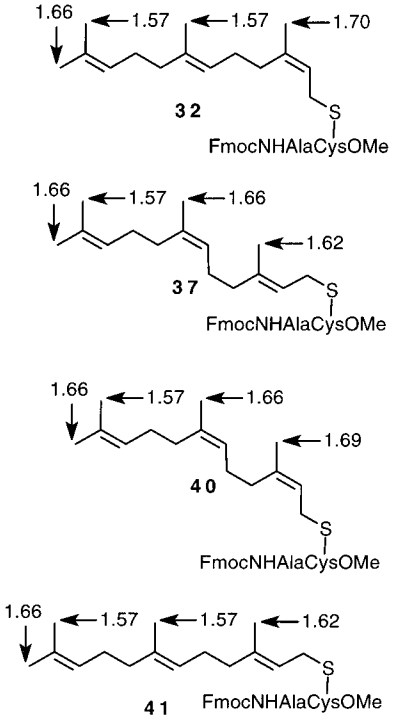


Figure 3. 600 MHz proton NMR chemical shifts for the farnesyl methyl groups of the isomeric dipeptides **32**, **37**, **40**, and **41**.

pheromone response pathway, including growth arrest of α-cells in response to **a**-factor, induction of specific genes in response to **a**-factor, and mating between two strains that will not mate in the absence of exogenous pheromone. The growth arrest assay was chosen for the biological evaluation of isomeric **a**-factors **9**−**11**. It has been previously established[5,28,30] that this assay is able to reflect the biological activity of a given analogue. This assay is readily performed and gives a straightforward readout of **a**-factor biological activity.

In the growth arrest (halo) assay, lawns of cells of the supersensitive strain RC757 were subjected to a dilution series containing identical amounts of each analogue. Endpoints were defined as the least amount of pheromone that was capable of inducing growth arrest resulting in a region of significantly reduced lawn density. Farnesylated synthetic **a**-factor (compound **1**) was used for reference as described in the Experimental Procedures, and shows an end point of 50 pg. Each of the modified farnesyl additions shows an end point that indicates a biological potency remarkably similar to that of **a**-factor. Activities for each pheromone analogue were determined from at least three experiments, and are as follows: **9**, 50 pg; **10**, 100 pg; **11**, 50 pg. Thus, only the biological activity of the *E,Z*-farnesyl isomer **10** varies from that of **a**-factor, and even in this case it exhibits a 2-fold decrease, which is close to the reproducibility of the assay.

Discussion

The development of general and efficient chemical methods for the synthesis of isoprenoids has been a subject of continuing interest.[31] For example, at least four improved methods for the synthesis of *all-trans*-geranylgeraniol have been developed over the past decade.[32−35] These methods allow for the rapid and ready assembly of this natural product in stereochemically enriched or pure form. However, they are specialized for the synthesis of the natural *trans*-isoprenoid unit, and thus lack flexibility for the synthesis of isoprenoid analogues, particularly geometric isomers. The synthesis of isoprenoid analogues is important not only for the study of protein prenylation,[36−38] but it is also crucial for the development of probes for squalene synthase and other key FPP-utilizing enzymes.[39] The synthetic strategy chosen for the stereoselective preparation of the isoprenyl backbone was based on a route previously developed in

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our laboratory.[19] The key step involves a Stille cross-coupling reaction[40] with organostannanes using Pd-$(AsPh_3)_2$ and CuI as catalysts. The presence of CuI as cocatalyst significantly increases the stereoselectivity of the coupling reaction and increased the yield as well. Liebeskind and co-workers were the first to document the beneficial effect of CuI on the Stille reaction.[41] Johnson and co-workers have also described the combined use of CuI and $Pd(AsPh)_2$ to effect some difficult Stille reactions.[42] The palladium-catalyzed cross-coupling between organic triflates and organostannanes is a very popular synthetic tool for forming the carbon−carbon bond.[40] It has been demonstrated that vinyl triflates can also undergo palladium-catalyzed cross-coupling reactions with a variety of other nucleophiles, including in particular organoboron reagents.[35,43] One of the important advantages of the Stille reaction is that the organostannane reagents display very restricted reactivity with common functional groups, thus allowing carbon−carbon bond formation to occur in the presence of unprotected functional groups, such as alcohols, aldehydes, ketones, esters, nitriles, azides, or epoxides. Another attractive feature of the Stille coupling is that the reaction occurs under neutral conditions.

The chemical results described herein provide a general method for the synthesis of isoprenoid analogues with either double bond geometry. This synthetic pathway is simple, efficient, provides high stereoisomeric purity of products, and does not involve protection of functional groups. It has been previously demonstrated that a wide variety of β-ketoesters can be prepared via alkylation of acetoacetate dianions,[44] and isoprenoid triflates can be coupled with a wide variety of organotin,[19] organoboron,[35] organozinc,[45] and organocopper reagents.[46] Thus this route may also be of more general interest, in that it should be applicable to the stereoselective synthesis of a broad range of trisubstituted allylic alcohols.

The biological objective of these studies was to further define the role of the farnesyl moiety of the *Saccharomyces cerevisiae* **a**-factor. To this end, we have synthesized the three geometric isomers of the farnesyl moiety and examined the biological activities of the corresponding **a**-factor analogues in a well-characterized assay system. The purpose of isoprenoid modifications is a basic question in the field of protein prenylation. Whether these additions serve to locate an attached peptide to membranes via lipid−lipid interactions, or bind to proteins through lipid−protein interactions has been a contentious issue,[6,10,11,47] and evidence to support several models can be found in the literature. Investigation of the mammalian Ras protein in soluble assay systems,[48−50] and the *S. cerevisiae* Ras protein in vivo[51] have demonstrated that the farnesyl group is required for biological activity in the absence of membrane binding, implying the involvement of prenyl-protein binding sites. In addition, the fact that prenyl cysteine analogues can block certain signal transduction pathways has been taken as evidence for prenyl binding sites on intracellular proteins.[52−54] Moreover, the recently determined structure of the Cdc42-rhoGDI heterodimer clearly indicates a deep hydrophobic pocket on the rhoGDI protein that binds to the prenyl group of Cdc42.[55] However, studies in biological systems[56,57] and model membrane/prenylated peptide systems[58,59] suggest a purely hydrophobic, lipid−lipid based, interaction between membranes and certain farnesylated peptides. Furthermore, Parish and Rando have summarized the evidence concerning the γ subunit of heterotrimeric G proteins and concluded that the isoprenoid moiety in this case also facilitates membrane attachment strictly through lipid−lipid interactions.[11,60]

Previous studies from our laboratories, however, have indicated that hydrophobic factors alone do not predict the behavior of the **a**-factor. Replacement of the farnesyl group with the more hydrophobic hexadecanyl moiety leads to a drastic reduction in biological activity,[61] and the more hydrophobic S-geranylgeranyl **a**-factor is also significantly less active than the natural compound.[62] More recently, in the work that led to the present study,

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analysis of the activities of the 3-substituted analogues **4**−**8** shown in Figure 1 demonstrated that all were capable of initiating a biological response, but to varying degrees depending on the nature of the modification.[13] The more hydrophobic 3-phenyl and 3-*tert*-butyl derivatives were clearly less active than the 3-vinyl and 3-ethyl compounds in all assays. Large differences in biological activity between the two 3-phenyl isomers and the significantly higher biological activity of the 3-vinyl isomer than the most active of the 3-phenyl isomers (compound **7**) are also in direct conflict with the simple hydrophobic model. Moreover, we have recently determined the affinities of **4**−**6** for model lipid membranes, and found that the change in biological activities observed with these compounds does not correlate with simple membrane affinity.[14]

The lack of variation in biological activity observed with the isomeric **a**-factor analogues **1**, **9**, **10**, and **11** stands in sharp contrast to the dramatic variation in activities associated with compounds **7** and **8**, the different isomers of the 3-phenyl-modified farnesyl group. Previous biophysical studies by Epand and co-workers on lipid-modified **a**-factor analogues demonstrated that the farnesyl group results in the attached peptide being anchored more deeply in the membrane than a simple *n*-alkyl chain,[12] and this correlated with the lower biological activity of the *n*-alkyl **a**-factor analogue. The different spatial relationship of the peptide relative to the farnesyl moiety that is imparted by the isomerization of the 2*Z*-double bond (**7**) to the 2E-conformation (**8**) could affect the ability of the lipopeptide to interact with the Ste3p receptor in the same manner. However, it may be that without the bulky phenyl group at the 3-position, the isomeric farnesyl chains of **9**−**11** may be able to adopt conformations similar to that of the *E*,*E*-farnesyl moiety of the natural **a**-factor **1**. In general, it would appear that the relatively small differences in biological activity observed in the present study and a previous study, which examined the effect of stereochemical variation on the point of attachment of the farnesyl group to the **a**-factor peptide moiety,[30] are not consistent with a traditional tight ligand−receptor interaction. This could result from a simple hydrophobic, membrane attachment role for the isoprenoid in **a**-factor. However, we have demonstrated that the three geometric isomers of farnesyl diphosphate (derived from **12**−**14**) surprisingly all exhibit nanomolar binding to the high affinity isoprenoid binding site of mammalian protein-farnesyltransferase.[15] This demonstrates that the isomeric farnesyl moieties can adopt conformations that allow them to mimic the natural *E*,*E*-farnesyl moiety in protein binding sites, as we have proposed above for the **a**-factor geometric isomers **9**−**11**.

Experimental Procedures

General Synthetic Procedures. Reactions that were sensitive to air and/or water were conducted in flame dried glassware under an argon atmosphere. Diisopropylethylamine (DIEA), benzotriazol-1-yloxy-tris(dimethylamino) phosphonium hexafluorophosphate (BOP), trifluoroacetic acid (TFA), *N*,*N*-dimethylaminopyridine (DMAP), and all other reagents were purchased from Aldrich (Milwaukee, WI), unless otherwise noted. Solvents were obtained from Fisher Scientific or VWR Scientific unless otherwise noted. All reagents and solvents were used without further purification unless otherwise noted. To prepare anhydrous solvents used in reaction mixtures, the following procedures were used: THF was distilled from Na/benzophenone; dichloromethane was distilled from CaH_2; toluene (HPLC grade) was dried and stored over activated 4 Å molecular sieves. Flash column chromatography was performed with silica gel 60 (230−440 mesh). Thin-layer chromatography was conducted on silica gel-precoated aluminum plates or glass plates, which were visualized with UV and/or a 10% phosphomolybdic acid/ethanol solution. HPLC was performed on one of two different Waters systems, with column, solvents, and gradient programs as indicated.

Ethyl 3-((Trifluoromethylsulfonyl)oxy)-7,11-dimethyldodeca-2(*E*),6(*E*),10-trienoate (17). A solution of the β-ketoester **15** (4.0 mmol, 1.072 g)[19] in DMF (10.0 mL; 99.8% anhydrous) was added to potassium bis(trimethylsilyl)amide (0.5 M in toluene, 4.8 mmol, 9.6 mL) at 0 °C. While at 0 °C, 2-[*N*,*N*-bis(trifluoromethylsulfonyl)amino]-5-chloropyridine (4.8 mmol, 1.88 g) was added and the mixture was allowed to warm to room temperature overnight. The mixture was taken up in 30 mL of ether and washed with a 10% citric acid solution (2 × 20 mL) and water (20 mL). The ether layer was dried over $MgSO_4$ and the solvent removed in a vacuum. Purification by flash chromatography (19:1 hexane/ethyl acetate) gave 927 mg (58%) of compound **17**, identical to the material previously obtained as a byproduct of the synthesis of **16**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (t, 3H), 1.60 (two, s, 6H), 1.67 (s, 3H), 2.00 (m, 4H), 2.33 (q, 4H), 2.95 (t, 2H), 4.22 (q, 2H), 5.09 (m, 2H), 5.93 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.21, 14.88, 24.81, 26.54, 61.13, 111.68, 113.86, 116.22, 120.47, 124.72, 163.98, 165.28.

Ethyl 3,7,11-Trimethyldodeca-2(*Z*),6(*E*),10-trienoate (18). Triflate **17** (0.98 mmol, 392 mg), CuI (0.099 mmol, 18.9 mg), Ph_3As (0.099 mmol, 30.6 mg), and bis(benzonitrile)-palladium(II) chloride (0.0495 mmol, 18.9 g) were placed in an argon-flushed flask and dissolved in NMP (*N*-methylpyrrolidone; anhydrous grade, used as obtained; 1.2 mL). The mixture was immersed in an oil bath maintained at a temperature of 100 °C, tetramethyltin (1.98 mmol, 0.27 mL; **CAUTION:** poisonous and volatile; handle only in hood) was added, and the reaction mixture was stirred for 18 h. It was then cooled, taken up in ethyl acetate (25 mL), and washed with saturated aqueous KF (2 × 20 mL) and water (2 × 20 mL). The aqueous layers were back extracted with ethyl acetate (30 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (hexane/ethyl acetate 19:1) afforded 241 mg (93%) of **18**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (t, 3H), 1.60 (two, s, 6H), 1.68 (s, 3H), 1.97 (m, 4H), 2.16 (q, 2H), 2.62 (t, 2H), 4.13 (q, 2H), 5.09 (t, 1H), 5.17 (t, 1H), 5.65 (s, 1H).

3,7,11-Trimethyldodeca-2(*Z*),6(*E*),10-trien-1-ol (12). A solution of the ester **18** (1.50 mmol, 400 mg) in toluene (7.5 mL) was treated at −78 °C under argon with diisobutylaluminum hydride (1.0 M in toluene; 4.28 mmol, 4.28 mL). After the addition, the mixture was stirred for 1 h at −78 °C. The reaction was quenched by addition to saturated aqueous potassium sodium tartrate (40 mL), the organic phase was separated, and the aqueous phase was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL) and dried by $MgSO_4$. Filtration and concentration followed by flash chromatography (hexane/ethyl acetate 9:1) gave 216 mg (65%) of alcohol **12**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60 (s, 6H), 1.68 (s, 3H), 1.76 (s, 3H), 2.01 (m, 4H), 2.11 (m, 4H), 4.12 (d, 2H), 5.11 (app t, 2H), 5.45 (t, 1H). The proton spectrum obtained for **13** is essentially identical to that reported previously for this compound.[20]

Neryl Bromide (21). NBS (*N*-bromosuccinimide; 12 mmol, 3.2 g) was dissolved in 60 mL of CH_2Cl_2 (distilled from CaH_2), and the resulting solution was cooled to −30 °C with a dry

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ice/acetonitrile bath. Dimethyl sulfide (8 mmol, 1.77 mL) was added dropwise by a syringe, and the mixture was warmed to 0 °C, maintained at that temperature for 5 min, and then recooled to −40 °C. To the resulting milky white suspension was added dropwise nerol (3,7-dimethylocta-2*Z*,6-dien-1-ol; 24 mmol, 2.11 mL). The suspension was warmed to 0 °C and stirred for 2 h. The ice bath was removed, and reaction mixture was warmed to room temperature and stirred for an additional 30 min. The resulting solution was washed with hexane (2 × 20 mL). The hexane layers were then washed with brine (2 × 20 mL) and dried over $MgSO_4$. Concentration afforded 2.305 g (89%) of neryl bromide **21** as an oil which was used directly in the next step without purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.54 (s, 3H), 1.62 (s, 3H), 1.69 (s, 3H), 2.03 (m, 4H), 4.02 (d, 2H), 5.03 (t, 1H), 5.38 (t, 1H).

Ethyl 7,11-Dimethyl-3-oxododeca-6(*Z*),10-dienoate (22). The monosodium salt of ethyl acetoacetate (4.26 g, 28.0 mmol) in 56.0 mL THF (distilled from Na/benzophenone) was cooled to 0 °C under argon, and treated with butyllithium (2.0 M in hexane, 14.7 mL, 29.4 mmol). After 20 min, neryl bromide **21** (3.03 g, 1.0 mmol) was added to the resulting dianion solution, and stirring was continued for additional 30 min at 0 °C. The reaction was quenched by adding ∼10 mL of 10% aqueous citric acid, and extracted with ether (3 × 50 mL). The organic layers were combined, washed with saturated NaCl (2 × 30 mL) and dried over $MgSO_4$. After purification by flash chromatography (hexanes/ethyl acetate 9:1), 2.50 g (67%) of the product **22** was obtained as a pale yellow oil. ^{1}H NMR (500 MHz) δ 1.28 (t, 3H), 1.61 (s, 3H, CH_3 at C_{11}), 1.69 (app s, 6H, CH_3 at C_7 and C_{12}-CH_3), 2.04 (narrow m, 4H), 2.30 (m, 2H), 2.56 (app t, 2H), 3.43 (s, 2H), 4.19 (q, 2H), 5.08 (m, 2H). ^{13}C NMR (125 MHz) δ 14.8 (O-CH_2*C*H_3), 18.4 (CH_3 at C_{11}), 22.6 (C_5), 24.0 (CH_3 at C_7), 26.4 (C_{12}), 27.3 (C_{10}), 32.6 (C_9), 44.0 (C_4), 50.1 (C_2), 62.1 (O-*C*H_2CH$_3$), 123.6 (C_6), 124.8 (C_{10}), 132.4, 137.5, 167.9, 203.3. The ^{13}C NMR spectrum was partially assigned as described above (see also Supporting Information).

Ethyl 3-(((Trifluoromethyl)sulfonyl)oxy)-7,11-dimethyldodeca-2(*Z*),6(*Z*),10-trienoate (23). A solution of the β-ketoester **22** (9.2 mmol, 2.45 g) in THF (23.0 mL; 99.8% anhydrous) was added to potassium bis(trimethylsilyl)amide (0.5 M in toluene, 11.0 mmol, 22.0 mL) at −78 °C. While at −78 °C, 2-[*N*,*N*-bis(trifluoromethylsulfonyl)amino]-5-chloropyridine (11.0 mmol, 4.3 g) was added and the mixture was warmed to room-temperature overnight. The mixture was taken up in 30 mL of ether and washed with a 10% citric acid solution (2 × 20 mL) and water (20 mL). The ether layer was dried over $MgSO_4$ and the solvent removed in a vacuum. Purification by flash chromatography (19:1 hexane/ethyl acetate) gave 3.17 g (86%) of compound **23** as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (t, 3H), 1.61 (s, 3H), 1.69 (s, 6H), 2.04 (s, 4H), 2.28 (t, 2H), 2.40 (t, 2H), 4.26 (q, 2H), 5.06 (t, 2H), 5.74 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.952, 17.538, 23.218, 24.324, 26.335, 31.892, 34.791, 61.167, 111.946, 121.383, 123.790, 131.902, 138.211, 158.296, 162.365.

Ethyl 3,7,11-Trimethyldodeca-2(*E*),6(*Z*),10-trienoate (24). Triflate **23** (0.84 mmol, 337 mg), CuI (0.084 mmol, 16.0 mg), Ph_3As (0.084 mmol, 25.8 mg), and bis(benzonitrile)-palladium(II) chloride (0.042 mmol, 16.0 g) were placed in an argon-flushed flask and dissolved in NMP (1.01 mL). The mixture was immersed in an oil bath maintained at a temperature of 100 °C, tetramethyltin (1.68 mmol, 0.23 mL, 397.2 mg; **CAUTION:** poisonous and volatile; handle only in hood) was added, and the reaction mixture was stirred for 18 h. It was then cooled, taken up in ethyl acetate (25 mL), and washed with saturated aqueous KF (2 × 20 mL) and water (2 × 20 mL). The aqueous layers were back extracted with ethyl acetate (30 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (hexane/ethyl acetate 19:1) gave 189 mg (85%) of **24**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (t, 3H), 1.61 (s, 3H), 1.64 (s, 6H), 2.03 (s, 4H), 2.15 (s, 4H), 4.14 (q, 2H), 5.09 (m, 2H), 5.66 (s, 1H).

3,7,11-Trimethyldodeca-2(*E*),6(*Z*),10-trien-1-ol (13). A solution of the ester **24** (0.85 mmol, 226 mg) in toluene (4.2 mL) was treated at −78 °C under argon with diisobutylaluminum hydride (1.0 M in toluene; 2.38 mmol, 2.38 mL). After the addition, the mixture was stirred for 1 h at −78 °C. The reaction was quenched with saturated aqueous potassium sodium tartrate (40 mL), the organic phase was separated, and the aqueous phase was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL) and dried with $MgSO_4$. Filtration and concentration followed by flash chromatography (hexane/ethyl acetate 9:1) gave 115 mg (62%) of alcohol **13**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.61 (s, 3H), 1.69 (9H), 2.04 (m, 4H), 2.10 (m, 4H), 4.16 (d, 2H), 5.11 (t, 2H), 5.42 (t, 1H). The proton spectrum obtained for **13** is essentially identical to that reported previously for this compound.[20]

Ethyl 3-(((Trifluoromethyl)sulfonyl)oxy)-7,11-dimethyldodeca-2(*E*),6(*Z*),10-trienoate (25). To a solution of β-ketoester **22** (867 mg, 3.26 mmol) in DMF (8.0 mL; 99.8% anhydrous) at 0 °C was added potassium bis(trimethylsilyl)-amide (0.5M in toluene, 3.85 mmol, 7.7 mL). After 2 h, 2-[*N*,*N*-bis(trifluoromethylsulfonyl)amino]-5-chloropyridine (1.39 g, 3.54 mmol) in ∼5 mL of DMF was added to the resulting enolate solution, and stirring was continued at 0 °C for 3.5 h. The reaction was then taken up in 30 mL of ether, washed with ∼5 mL of 10% aqueous citric acid (2 × 20 mL), and water (20 mL). The organic layer was dried over $MgSO_4$, and concentrated. Purification by flash chromatography (20:1 hexanes/ethyl acetate) gave 797 mg (60%) of triflate **25** as a pale yellow oil. None of the isomeric triflate **23** could be observed by proton NMR. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33 (t, 3H), 1.63 (s, 3H), 1.68 (app s, 6H), 2.11 (app s, 4H), 2.32 (m, 2H), 2.98 (t, 2H), 4.29 (q, 2H), 5.12 (m, 3H), 5.95 (s, 1H). Note the characteristic strong deshielding of the C_4-CH_2 signal, by the ester carbonyl, to 2.98 ppm; in contrast, the C_4-CH_2 signal for the isomeric triflate **23** appears at 2.39 ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 13.206, 14.883, 23.062, 24.779, 26.486, 30.084, 31.819, 33.521, 59.094, 61.067, 63.008, 111.628, 113.816, 116.215, 120.452, 122.894, 163.949, 165.196.

Ethyl 3,7,11-Trimethyldodeca-2(*Z*),6(*Z*),10-trienoate (26). In a flame-dried, argon-flushed flask were placed triflate **25** (580 mg, 1.46 mmol), $Pd(PhCN)_2Cl_2$ (28 mg, 0.073 mmol), $AsPh_3$ (45 mg, 0.146 mmol), CuI (28 mg, 0.146 mmol) and 0.80 mL of NMP (99.5%, anhydrous). This mixture was heated to ∼100 °C, tetramethyltin (0.40 mL, 1.91 mmol; **CAUTION:** poisonous and volatile; handle only in hood) was added, and the reaction was stirred for 15 h. The reaction was cooled, taken up with 100 mL ethyl acetate, and washed with saturated aqueous KF (3 × 30 mL). The aqueous layer was back-extracted with ethyl acetate (2 × 15 mL) and the combined organic layers were dried over $MgSO_4$. Concentration followed by flash chromatography (hexanes/ethyl acetate 20:1) gave **26** as a colorless oil (248 mg, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (t, 3H), 1.605 (s, 3H), 1.68 (S, 6H), 1.88 (s, 3H), 2.04 (s, 4H), 2.15 (m, 2H), 2.63 (t, 2H), 4.12 (q, 2H), 5.16 (m, 2H), 5.65 (s, 1H). The identity, and in particular the stereochemistry, of this ester was confirmed by the similarity of the methyl peaks in its ^{1}H NMR spectrum to that of the *Z*,*Z*-methyl farnesoate originally prepared by Burrell et al.[20]

3,7,11-Trimethyldodeca-2(*Z*),6(*Z*),10-trien-1-ol (14). A solution of the ester **26** (0.61 mmol, 160 mg) in toluene (3.0 mL) was treated at −78 °C under argon with diisobutylaluminum hydride (1.0 M in toluene; 1.70 mmol, 1.70 mL). After the addition the mixture was stirred for 1h at −78 °C. The reaction was quenched with saturated aqueous potassium sodium tartrate (40 mL), the organic phase was separated, and the aqueous phase was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL) and dried by $MgSO_4$. Filtration and concentration followed by flash chromatography (hexane/ethyl acetate 9:1) gave 98 mg (74%) of alcohol **14**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.61 (s, 3H), 1.69 (s, 6H), 1.75 (s, 3H), 2.10 (two, s, 8H), 4.11 (d, 2H), 5.11 (m, 2H), 5.45 (t, 1H). The proton spectrum obtained for **14** is essentially identical to that reported previously for this compound.[16,20]

1-Bromo-3,7,11-trimethyldodeca-2(*Z*),6(*E*),10-triene (29). Carbon tetrabromide (0.361 mmol, 121.4 mg), triphenylphosphine (0.293 mmol, 78.5 mg), and alcohol **12** (0.278 mmol, 78.5

mg) were dissolved in CH_2Cl_2 (distilled from CaH_2, 10.7 mL). The resulting mixture was stirred at room temperature for 3.0 h. The solvent was removed in rotary evaporator at room temperature. The residue was treated with hexane (20 mL), and the hexane solution was then centrifuged, removed from the precipitate with a pipet, transferred to a second flask, and concentrated. This procedure was repeated five to six times. The resulting bromide **29** (91 mg, 95%) could be used directly in the next step, or it could be purified by reversed-phase HPLC [linear gradient of 30% A/70% B to 100% B over 30 min (A, water; B, MeCN; column, Vydac pH-stable C_8 10 × 250 mm column; flow rate, 4 mL/min; UV monitoring at 214 and 254 nm)]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60 (s, 6H), 1.68 (s, 3H), 1.73 (s, 3H), 1.98 (m, 4H), 2.09 (m, 4H), 4.01 (d, 2H), 5.08 (t, 2H), 5.54 (t, 1H).

1-Chloro-3,7,11-trimethyldodeca-2(*Z*),6(*E*),10-triene (30). NCS (*N*-chlorosuccinimide; 1.08 mmol, 144 mg) was dissolved in 10 mL of CH_2Cl_2 (distilled from CaH_2), and the resulting solution was cooled to −30 °C with a dry ice/acetonitrile bath. Dimethyl sulfide (1.08 mmol, 0.08 mL) was added dropwise by a syringe, and the mixture was warmed to 0 °C, maintained at that temperature for 5 min, and cooled to −40 °C. To the resulting milky white suspension was added dropwise *Z*,*E*-farnesol **12** (0.54 mmol, 120 mg). The suspension was warmed to 0 °C and stirred to 3 h. The ice bath was removed, and the reaction mixture was warmed to room temperature and stirred for an additional 2 h. The resulting solution was washed with hexane (2 × 20 mL). The hexane layers were then washed with brine (2 × 20 mL) and dried over $MgSO_4$. Concentration afforded 107 mg (81%) of farnesyl chloride **30** as an oil, which was used directly in the next step without purification.

Cys[S-(*Z*,*E*-farnesyl)]-OCH$_3$ (31). The *Z*,*E*-farnesyl chloride **30** (0.34 mmol, 82 mg) and l-cysteine methyl ester (0.34 mmol, 58.0 mg) were dissolved in 6.0 M NH_3/MeOH (2.6 mL), stirred at 0 °C for 3 h and then at room temperature for 1 h. The resulting mixture was partitioned between ether and H_2O. The organic layer was washed with H_2O (3 × 10 mL), the combined organic layers were filtered, concentrated, and dried by $MgSO_4$. The crude compound was purified by flash column (ether/hexane 6:1) to afford 88 mg (76%) of **31**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60 (s, 6H), 1.69 (s, 6H), 2.00∼2.07 (m, 8H), 2.74, 2.88 (2q, 2H), 3.19 (d, 2H), 3.62 (q, 1H), 3.74 (s, 3H), 5.11 (t, 2H, H_6 and H_{10}), 5.24 (t, 1H, H_2).

Fmoc-Ala-Cys[S-(*Z*,*E*-farnesyl)]-OCH$_3$ (32). Cys[S-(*Z*,*E*-farnesyl)]OCH_3 (**31**; 0.22 mmol, 76 mg), Fmoc-alanine (0.22 mmol, 77.0 mg), and HOBt (0.22 mmol, 32 mg) were dissolved in CH_2Cl_2 (3.0 mL). This solution was cooled to 0 °C for 20 min, and then EDC was added (0.22 mmol, 47 mg). The mixture was warmed to 20 °C and stirred overnight. The solvent was removed, and the resulting mixture was partitioned between ether and H_2O. The organic layer was washed with H_2O (3 × 10 mL), the combined organic layer was filtered, concentrated, and dried by $MgSO_4$. The crude compound was purified by flash column (ether/hexane 6:1) to afford 125 mg (90%) of **32**. Further purification by reversed-phase HPLC, using a linear gradient from 80% A/20% B to 100% B over 25 min (A, H_2O; B, CH_3CN; Vydac pH-stable C_8 10 × 250 mm column; flow rate, 4 mL/min; UV monitoring at 214 and 254 nm), afforded pure dipeptide. The retention time of the dipeptide **32** was 24.5 min. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.416 (d, 3H), 1.571 (d, 6H), 1.657 (s, 3H), 1.709 (s, 3H), 1.92−2.09 (m, 8H), 2.81−2.99 (m, 2H), 3.06−3.20 (m, 2H), 3.758 (s, 3H), 4.219 (t, 1H), 4.316 (narrow m, 1H), 4.397 (narrow m, 2H), 4.749 (m, 1H), 5.099 (nm, 2H), 5.177 (t, 1H), 5.360 (d, Cys NH), 6.771 (d, Ala NH), 7.314 (t, 2H), 7.391 (t, 2H), 7.574 (d, 2H), 7.747 (d, 2H). FAB-MS: 633 [calcd for ($C_{37}H_{48}N_2O_5S$ + H^+) − 633.3].

1-Chloro-3,7,11-trimethyldodeca-2(*E*),6(*Z*),10-triene (35). NCS (N-chlorosuccinimide; 0.42 mmol, 60 mg) was dissolved in 1.75 mL of CH_2Cl_2 (distilled from CaH_2), and the resulting solution was cooled to −30 °C with a dry ice/acetonitrile bath. Dimethyl sulfide (0.45 mmol, 0.03 mL, 27 mg) was added dropwise by a syringe, and the mixture was warmed to 0 °C, maintained at that temperature for 15 min, and cooled to −30 °C. To the resulting milky white suspension was added dropwise *E*,*Z*-farnesol (**13**; 0.38 mmol, 84 mg). The suspension was warmed to 0 °C and stirred to 3 h. The ice bath was removed, and the reaction mixture was warmed to room temperature and stirred for an additional 20 min. The resulting solution was washed with hexane (2 × 20 mL). The hexane layers were then washed with brine (2 × 20 mL) and dried over $MgSO_4$. Concentration afforded 80 mg (86%) of farnesyl chloride **35** as an oil, which was used directly in the next step without purification. Monitoring of the reaction by TLC confirmed the disappearance of the starting material **13** and indicated the completion of the reaction.

Cys[S-(*E*,*Z*-farnesyl)]-OCH$_3$ (36). The *E*,*Z*-farnesyl chloride **35** (0.42 mmol, 100 mg) and l-cysteine methyl ester (0.42 mmol, 70.0 mg) were dissolved in 6.0 M NH_3/MeOH (3.2 mL), stirred at 0 °C for 3 h and then at 20 °C for 1 h. The resulting mixture was partitioned between ether and H_2O. The organic layer was washed with H_2O (3 × 10 mL), and then dried over $MgSO_4$. The crude compound was purified by flash column (ether/hexane 6:1) to afford 74 mg (51%) of **36**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.61 (s, 3H), 1.66 (s, 6H), 1.76 (s, 3H), 2.03 (m, 8H), 2.71, 2.87 (app dq, 2H), 3.18 (t, 2H), 3.64 (t, 1H), 3.75 (s, 3H), 5.12 (t, 2H, H_6 and H_{10}), 5.23 (t, 1H, H_2).

Fmoc-Ala-Cys[S-(*E*,*Z*-farnesyl)]-OCH$_3$ (37). Cys[S-(*E*,*Z*-farnesyl)]OCH_3 (**36**; 0.29 mmol, 100 mg), Fmoc-alanine (0.47 mmol, 146 mg) and HOBt (0.53 mmol, 71 mg), were dissolved in CH_2Cl_2 (6.5 mL). This solution was cooled to 0 °C for 20 min, then EDC was added (0.53 mmol, 102 mg). The mixture was then stirred at room temperature overnight. The solvent was removed and the resulting mixture was partitioned between ether and H_2O. The organic layer was washed with H_2O (3 × 10 mL), the combined organic layer was filtered, concentrated, and dried by $MgSO_4$. The crude compound was purified by flash column (ether/hexane 6:1) to afford 94 mg (50%) of **37**. Further purification by reversed-phase HPLC, using a linear gradient from 80% A/20% B to 100% B over 25 min (A, H_2O; B, CH_3CN; Vydac pH-stable C_8 10 × 250 mm column; flow rate, 4 mL/min; UV monitoring at 214 and 254 nm), afforded pure dipeptide. The retention time of the dipeptide **37** was 24.4 min. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.416 (d, 3H), 1.576 (s, 3H), 1.619 (s, 3H), 1.657 (s, 6H), 1.92−2.09 (m, 8H), 2.80−2.99 (m, 2H), 3.04−3.19 (m, 2H), 3.724 (s, 3H), 4.212 (t, 1H), 4.306 (narrow m, 1H), 4.372 (narrow m, 2H), 4.749 (m, 1H), 5.063 (nm, 2H), 5.151 (t, 1H), 5.340 (d, Cys NH), 6.775 (d, Ala NH), 7.295 (t, 2H), 7.380 (t, 2H), 7.574 (d, 2H), 7.743 (d, 2H). FAB-MS: 633 [calcd for ($C_{37}H_{48}N_2O_5S$ + H^+) − 633.3].

1-Chloro-3,7,11-trimethyldodeca-2(*Z*),6(*Z*),10-triene (38). NCS (N-chlorosuccinimide; 1.08 mmol, 144 mg), was dissolved in 10 mL of CH_2Cl_2 (distilled from CaH_2), and the resulting solution was cooled to −30 °C with a dry ice/acetonitrile bath. Dimethyl sulfide (1.08 mmol, 0.08 mL) was added dropwise by a syringe, and the mixture was warmed to 0 °C, maintained at that temperature for 15 min, and cooled to −30 °C. To the resulting milky white suspension was added dropwise *Z*,*Z*-farnesol **14** (0.45 mmol, 120 mg). The suspension was warmed to 0 °C and stirred to 3 h. The ice bath was removed, and the reaction mixture was stirred for an additional 2 h at room temperature. The resulting solution was washed with hexane (2 × 20 mL). The hexane layers were then washed with brine (2 × 20 mL) and dried over $MgSO_4$. Concentration afforded 72 mg (55%) of farnesyl chloride **38** as oil, which was used directly in the next step without purification.

Cys[S-(*Z*,*Z*-farnesyl)]-OCH$_3$ (39). The *Z*,*Z*-farnesyl chloride **38** (0.29 mmol, 72 mg) and l-cysteine methyl ester (0.35 mmol, 61 mg) were dissolved in 6.0 M NH_3/MeOH (2.3 mL), stirred at 0 °C for 3 h and then at room temperature for 1 h. The resulting mixture was partitioned between ether and H_2O. The organic layers were washed with H_2O (3 × 10 mL), the combined organic layers were filtered, concentrated, and dried over $MgSO_4$. The crude compound was purified by flash chromatography (ether/hexane 6:1) to afford 72 mg (73%) of **39**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.61 (s, 3H), 1.69 (s, 6H), 1.74 (s, 3H), 2.03 (m, 8H), 2.71, 2.87 (2q, 2H), 3.16 (t, 2H), 3.64 (q, 1H), 3.74 (s, 3H), 5.11 (t, 2H, H_6 and H_{10}), 5.23 (t, 1H, H_2).

Fmoc-Ala-Cys[S-(*Z,Z*-farnesyl)]-OCH_3 (40). Cys[S-(*Z,Z*-farnesyl)]-OCH_3 (**39**; 0.17 mmol, 57 mg), Fmoc-alanine (0.19 mmol, 58 mg), and HOBt (0.19 mmol, 25 mg), were dissolved in CH_2Cl_2 (2.5 mL). This solution was cooled to 0 °C for 20 min, and then EDC was added (0.19 mmol, 36 mg). The reaction was then stirred overnight at room temperature. The solvent was removed and the resulting mixture was partitioned between ether and H_2O. The organic layer was washed with H_2O (3 × 10 mL), the combined organic layer was filtered, concentrated, and dried by $MgSO_4$. The crude compound was purified by flash column (ether/hexane 6:1) to afford 70 mg (65%) of **40**. Further purification by reversed-phase HPLC, using a linear gradient from 80% A/20% B to 100% B over 25 min (A, H_2O; B, CH_3CN; Vydac pH-stable C_8 10 × 250 mm column; flow rate, 4 mL/min; UV monitoring at 214 and 254 nm), afforded pure dipeptide. The retention time of the dipeptide **40** was 25.22 min. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.416 (d, 3H), 1.570 (s, 3H), 1.657 (d, 6H), 1.69(s, 3H), 1.92−2.09 (m, 8H), 2.80−2.99 (m, 2H), 3.04−3.19 (m, 2H), 3.731 (s, 3H), 4.210 (t, 1H), 4.309 (narrow m, 1H), 4.376 (narrow m, 2H), 4.749 (m, 1H), 5.060 (nm, 2H), 5.155 (t, 1H), 5.307 (d, Cys NH), 6.605 (d, Ala NH), 7.304 (t, 2H), 7.398 (t, 2H), 7.594 (d, 2H), 7.753 (d, 2H). FAB-MS: 633 [calcd for ($C_{37}H_{48}N_2O_5S + H^+$) − 633.3].

Fmoc-Ala-Cys[S-(*E,E*-farnesyl)]-OCH_3 (41). This protected dipeptide was prepared as previously described.[28] Its proton NMR spectrum is given below for comparison to the other isomers (dipeptides **32**, **37**, and **40**). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.408 (d, 3H), 1.565 (d, 6H), 1.616 (s, 3H), 1.652 (s, 3H), 1.92−2.08 (m, 8H), 2.82−2.98 (m, 2H), 3.04−3.18 (m, 2H), 3.736 (s, 3H), 4.207 (t, 1H), 4.288 (narrow m, 1H), 4.379 (narrow, m, 2H), 4.741 (m, 1H), 5.057 (m, 2H), 5.147 (t, 1H), 5.340 (d, Cys NH), 6.646 (d, Ala NH), 7.291 (t, 2H), 7.375 (t, 2H), 7.563 (m, 2H), 7.739 (d, 2H).

Peptide Synthesis, Purification, and Characterization Methods. All stereoisomers of **a**-factor were synthesized by coupling the protected amine terminal decapeptide [Fmoc-Tyr-(tBu)-Ile-Ile-Lys(Boc)-Gly-Val-Phe-Trp(formyl)-Asp(OFm)-Pro-OH] with the appropriate AlaCys(S-Far)-OMe following the procedure originally used by Xue et al.[28] N-α-Boc-Pro-OCH_2-PAM-resin was supplied by Applied Biosystems. Protected amino acids were purchased from Bachem Inc. (Torrance, CA) and Advanced ChemTech (Louisville, KY). 1-Hydroxybenzotriazole (HOBt) was purchased from Advanced ChemTech. Reversed-phase HPLC was performed on a Waters system. Analytical HPLC was run on a Waters μ-Bondapak-C_{18} column (3.9 × 300 mm), and all peptides were eluted with either a water/acetonitrile (both containing 0.025%TFA) or a water/methanol (both containing 0.025%TFA) linear gradient. Preparative HPLC was run on a Waters μ-Bondapak-C_{18} column (19 × 300 mm or 19 × 150 mm) using a linear gradient of water (0.025%TFA) and acetonitrile (0.025%TFA) from 10% to 100% over 120 min. Detection in both cases was by UV at 220 nm. All peptides subjected to biological assays were purified to over 99% homogeneity as judged by RP-HPLC. Electron spray mass spectrometry was carried out at PeptidoGenics Inc., Livermore, CA. Amino acid analyses were performed by the Biopolymers Laboratory at the Brigham and Women's Hospital, Cambridge, MA.

Synthesis of the Protected Decapeptide [Fmoc-Tyr-Ile-Ile-Lys(Fmoc)-Gly-Val-Phe-Trp-Asp(OFm)-Pro-OH (34)]. In the synthesis of the amine terminal protected decapeptides, we utilized Boc as the temporary protecting group for all α-amines except that of Tyr. A preloaded Boc-Pro-OCH_2-PAM resin was employed as the support. The protected decapeptide was synthesized using an automated solid-phase protocol on an Applied Biosystems Model 433A peptide synthesizer. To ensure a high degree of coupling efficiency, all amino acids were double coupled using DCC/HOBt. After 9 cycles and removal of *tert*-butyl group of Tyr, the partially protected decapeptide-resin was dried completely in a vacuum and subjected to HF treatment as previously described.[28] The protected decapeptide was recovered as a white powder in about 70−80% yield (based on the starting amine content on the resin). Since the protected decapeptide is very hydrophobic, the direct evaluation of its purity by reverse- phase HPLC was complicated due to precipitation in the tubing and on the column. After removal of all protecting groups with 10% piperidine in DMF, the crude deprotected decapeptides were found to be over 85% homogeneous on HPLC. Thus the crude protected decapeptide was used for 10 + 2 couplings without purification.

Synthesis of Stereoisomeric a-Factor Analogues. The final **a**-factor analogues were synthesized by condensing the protected decapeptide with the appropriately farnesylated dipeptide as previously performed in this laboratory.[28] The procedure for the synthesis of trans, cis-farnesylated **a**-factor **10** is presented as a representative example. Fmoc-Ala-Cys-[S-(*E,Z*-farnesyl)]OCH_3 (**37**; 10 mg, 27 μmol) was dissolved in 0.4 mL DMF, 25 mg of DMAP was added, and stirring was continued at room-temperature overnight. HPLC indicated the complete deprotection of the Fmoc group, and this solution was added to a solution of protected decapeptide 24.5 mg in 1.6 mL DMF at 0 °C followed by BOP reagent (4.9 mg, 11 μmol) and HOBt (1.5 mg, 11 μmol). The reaction mixture was stirred at 0 °C for 1h, and analytical HPLC indicated that the decapeptide had been consumed. The reaction was terminated, and the Fmoc/OFm groups were removed by adding 0.5 mL piperidine. After stirring for 1 h, the solution was added to 50 mL precooled ethyl ether and the precipitate was washed with ethyl ether. The solid was dissolved in 0.5 mL methanol, acidified with 1% aqueous TFA, and applied to a Waters μ-Bondapak column (19 × 150 mm) for HPLC purification. Amino acid analyses (values were not determined for Trp and Cys): **9** − A(1)1.05; D(1)1.05; F(1)1.04; G(1)1.06; I(2)1.73 K(1)1.05; P(1) V(1)1.04; Y(1)0.98; **10** − A(1)1.05; D(1)1.04; F(1)1.04; G(1)1.06; I(2)1.73; K(1)1.03; P(1) V(1)1.05; Y(1)1.01; **11** − A(1)1.14; D(1)1.08; F(1)0.98; G(1)1.20; I(2)1.78; K(1)1.10; P(1)1.11; V(1)1.11; Y(1)0.51. Other analytical data for **9**, **10**, and **11** are presented in Table 2.

Growth Arrest Assay. *Saccharomyces cerevisiae* strain RC757 [*MAT*α *sst2-1 rme1 his6 met1 can1 cyh2*] was a gift from Russell Chan (University of Cincinnati). Biological activity of these pheromone analogues was measured by examining their ability to induce a halo of growth arrest when spotted onto a lawn of RC757 cells, a strain supersensitive to **a**-factor. The endpoint of activity is defined as the minimum quantity of peptide resulting in the formation of an observed halo, as described previously.[5] No variations in the activities of analogues from those values reported were seen across three experiments. All peptide stocks were verified to be approximately equal in concentration by absorbance at 279 nm (ϵ = 5600 cm^2 mol^{-1}).

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Supporting Information Available: 300, 500, and 600 MHz ^{1}H NMR spectra of compounds **1**, **9**−**15**, **18**, **22**−**26**, **32**, **37**, **40**, and **41**; and 500 MHz/125 MHz ^{1}H−^{13}C correlation and 125 MHz DEPT experiments for **15** and **22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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