Total Synthesis of Stipiamide and Designed Polyenes as New Agents for the Reversal of Multidrug Resistance

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Abstract: The synthesis of (−)-stipiamide (**1**) is reported together with the designed enynes **2** (6,7-dehydrostipiamide) and **3** that are now shown to reverse the multidrug resistance (MDR) of human breast cancer cells (MCF-7adrR). Stipiamide was assembled using a Stille coupling with (*E*)-vinyl iodide **17** and (*Z*)-stannyl amide **16** in 78% yield. (*E*)-Vinyl iodide **17** was made using a Takai reaction and a selective dihydroxylation of the terminal olefin of nonconjugated diene **7** using the Sharpless AD-mix reagent. The precursor to **16**, (*E,Z*)-stannyl diene ester **13**, was assembled with high selectivity in a single operation using a tandem *syn*-addition of tributyltin cuprate to acetylene followed by conjugate addition to ethyl propiolate. Structural variants **2** and **3** were assembled using palladium-catalyzed Sonogashira couplings with vinyl iodides **17** and **35** and acetylenes **22** and **26** in high yield at near 1:1 stoichiometry. Compound **2** was found to be far less toxic than stipiamide and performed much better as an MDR reversal agent. Compound **3** was better still due to even lower toxicity.

Introduction

(−)-Stipiamide (**1**) (Chart 1) was discovered by Seto from the soil bacterium *Myxocaccus stipiatus* using a colchicine-resistant screen.[2] This resistant KB cell line is known to express Pgp (P-glycoprotein),[3] the well-known membrane-bound multidrug transporter, a critical factor in the development of multidrug resistance displayed by transformed cells.[4] Höfle also independently reported the isolation of this compound, calling it phenalamide A1 using an anti-HIV screen.[5] While the related polyenes, the myxalamides differing only slightly at the left-hand terminus, are potent antifungal and antibacterial agents,[6] stipiamide was found to be only slightly effective as an antimicrobial agent. Further experiments showed that myxalamide B (Chart 2) potently inhibits NADH oxidation by complex I in isolated beef heart submitochondrial particles (IC_{50} 170 pm/mg of protein)[7] with the same level and manner of electron transfer inhibition exhibited by the powerful insecticides piericidin A and rotenone.[8]

Pgp is a heavily glycosylated membrane bound small-molecule efflux pump that is greatly overexpressed in most cancer cells found to be cross resistant to therapeutic agents.[4]

Chart 1

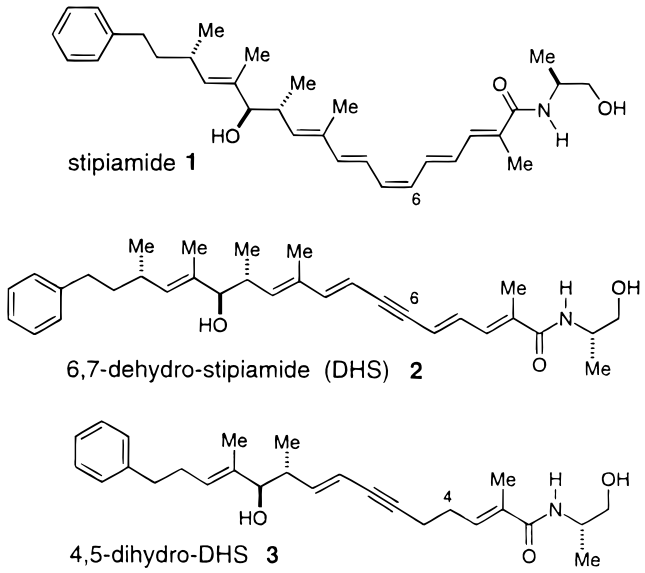
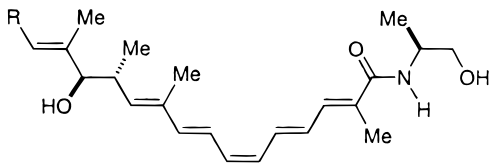


Chart 2



This condition is commonly developed following exposure to a single anticancer agent. Its action, the transport of multiple classes of compounds, is believed to be responsible in most cases for the failure of therapy, the clinical expression of multidrug resistance (MDR).[9] The ability to bind and transport

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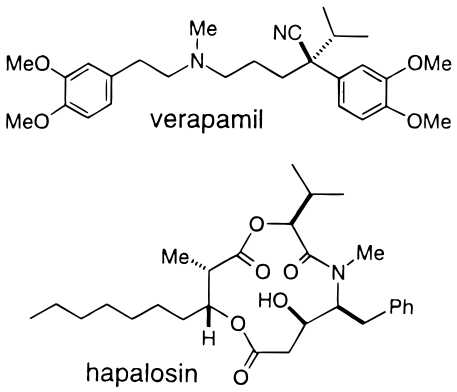
Chart 3



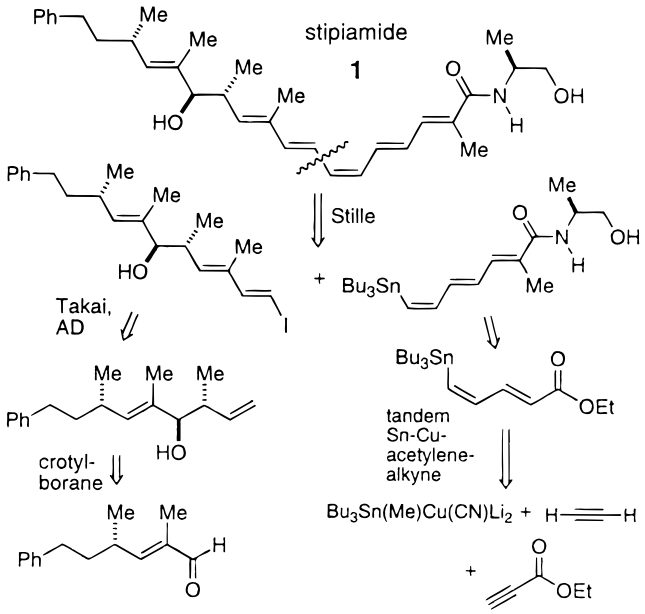
a range of structurally dissimilar compounds is truly remarkable and without precedent. These include the tetracycline antibiotics daunorubricin and adriamycin and the vinca alkaloids vinblastine and vincristine, along with others such as colchicine, mitomycin C, actinomycin D, topotecan, mitoxanantrone, etoposide, teniposide, paclitaxel, emetine, ethidium bromide, puromycin, and mithramycin, to name a few. Bleomycin, methotrexate, and cisplatinum are known not to be transported.

Of the numerous compounds that have been found to reverse MDR through inhibition of Pgp, the most studied compound, verapamil (Chart 3), a well-known calcium ion channel blocker used to treat hypertension, is still the standard by which all others are compared.[10] It has been shown to interact directly with Pgp using photolabeling and competitive displacement studies (600 nM).[11] Clinical trials, however, have proven disappointing in that the concentration required to reverse MDR (10 μM) leads to hypotension and heart failure.[9] Other compounds, also based on known channel blockers, dexniguldipine,[12] S9788,[13] and GF 120918,[14] have also suffered from hypotension problems. Hapalosin, a cyclic depsipeptide discovered recently, has demonstrated MDR reversal at 2.5 μM with MCF-7adrR cells with added adriamycin (15 μM).[15]

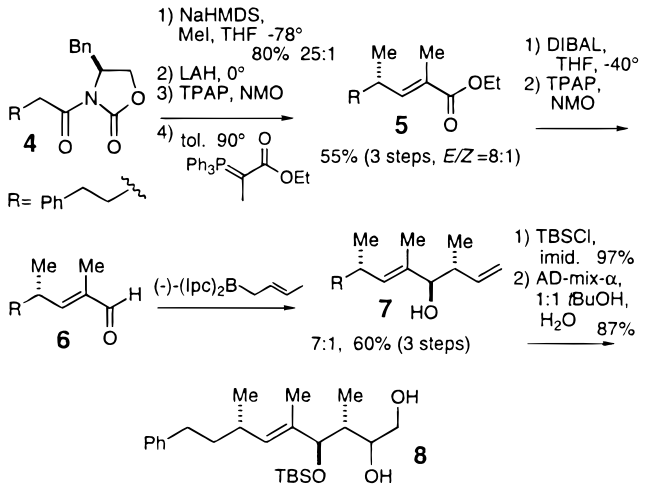
We now report the full details of our recent synthesis of (−)-stipiamide (**1**),[16] together with the design and synthesis of new less toxic polyenes for the reversal of MDR. 6,7-Dehydrostipiamide (**2**), the result of the transposition of the C-6,7 (*Z*)-alkene of stipiamide to an alkyne, was made and is shown to be far less toxic and more potent as an MDR reversal agent. In addition a less toxic and further simplified enyne (**3**) designed lacking the extended π conjugation was produced.

The final step for the synthesis of stipiamide employs a Stille coupling with an (*E*)-vinyl iodide and a (*Z*)-stannyl triene amide (Chart 4). The vinyl iodide was produced from an enal using a highly selective Takai reaction. The enal precursor was generated by oxidative cleavage of a diol that was produced using a new application of the Sharpless osmium tetroxide AD-mix reaction that is selective for the terminal olefin of the nonconjugated diene substrate.[17] A Brown crotylborane addition was used to create the vicinal 1-hydroxy-2-methyl functionality.

Chart 4



Scheme 1



The stannyl triene coupling partner was derived from the product of a new single-flask stannyl cuprate−acetylene−alkyne ester tandem addition sequence to give the key (*E,Z*)-stannyl diene ester in high yield and selectivity. Structural variants **2** and **3** were assembled using improved conditions for the palladium-catalyzed Sonogashira coupling with (*E*)-vinyl iodides and terminal acetylenes in high yield at near 1:1 stoichiometry.

Results and Discussion

The synthesis of the left-hand portion of stipiamide (Scheme 1) began with acyloxazolidinone **4** methylated with greater than 25:1 selectivity according to the procedure of Evans.[18] The product was reacted with LAH in 60% yield and the resultant alcohol was oxidized to the aldehyde with tetrapropylammonium perruthenate (TPAP) and *N*-methylmorpholine *N*-oxide (NMP).[19] The crude aldehyde was treated with (carbethoxyethylidene)-triphenylphosphorane to give **5** as an 8:1 diastereomeric mixture of unsaturated esters which were not separated.

Fragment **5** was reduced to the alcohol using 2.5 equiv of DIBAL in THF at −40 °C. This intermediate was oxidized

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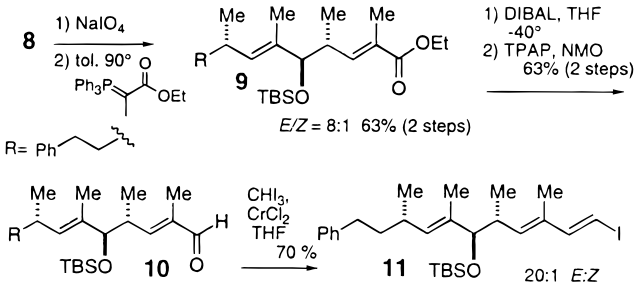
(19) Griffith, W. P; Ley, S. V. *Aldrichim. Acta* **1990**, *23*, 13.

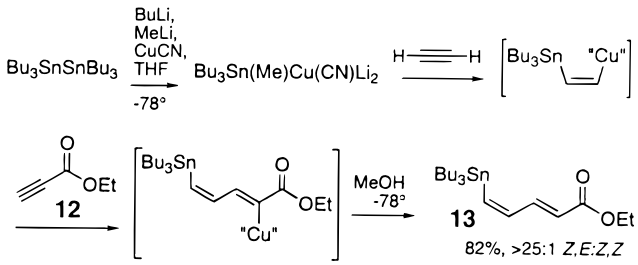
Scheme 2



using TPAP to give aldehyde **6** which was subsequently reacted with diisopinocampheyl-(*E*)-crotylborane derived from (−)-α-pinene according to the procedure of Brown.[20] This gave the key *anti*-homoallylic alcohol **7** in 60% yield for the three combined steps. In order to continue the synthetic sequence, oxidative cleavage of the terminal olefin in the presence of the internal trisubstituted olefin was required. Initially it was considered that this would be a limitation of the crotylboration method. Initial attempts to selectively oxidize the terminal olefin of TBS-protected diene **7** were made using standard osmium tetroxide−NMO conditions with added amines in various solvents.[21] These reactions were found to be sluggish, giving mixtures of internal and terminal diol products. The attempt was then made using the commercially available asymmetric osmium tetroxide dihydroxylation reagent, AD-mix of Sharpless.[22] The terminal olefin of **7** was smoothly converted to the 1,2-diol **8** in 87% yield with no internal dihydroxylation or tetrahydroxylation products observed. Many of the factors contributing to the selectivity of this useful transformation, in particular the effect of added base, along with various substrates are included in a recent report.[17]

With the nonconjugated diene problem solved, diol **8** was treated with sodium periodate, producing the corresponding aldehyde which was reacted with (carbethoxyethylidene)-triphenylphosphorane to give ester **9** in 75% yield as an 8:1 *E/Z* mixture (Scheme 2). Conversion of the ester to the alcohol was achieved using DIBAL followed by TPAP and NMO to provide aldehyde **10**. Treatment of enal **10** with iodoform and chromous chloride in THF gave vinyl iodide **11** in 70% yield with 20:1 *E/Z* selectivity.[23] Several features of the Takai reaction deserve some comment in that the initial attempts following reported procedures gave a wide range of yields (10−50%) and low selectivity. A recent investigation concerning the effect of solvent found an optimal solvent system of 6:1 dioxane/THF that gave a 69% yield after 8 h with good selectivity (13:1 *E/Z*).[24] With enal **10**, dioxane as cosolvent produced an extremely sluggish reaction and provided only slightly enhanced selectivity at the expense of yield compared to THF alone. After exploring numerous variations, the remarkably high 20:1 *E/Z* selectivity was finally achieved only after the newly purchased chromous chloride was gently flame dried under vacuum immediately prior to use. Following addition of THF to the cooled material, care was taken to mechanically agitate the chromous chloride to ensure that it had not fused. This precaution greatly decreased the reaction time to 3 h at 0 °C, at the same time increasing the yield. In addition,

Scheme 3



as noted by others, we have found that the yield is lowered upon scale up above 1 g.[25]

Inspiration for the new tandem acetylene−propiolate−cuprate addition reaction developed to construct the right-hand portion came from the work of Corey and others who found that alkylcopper reagents add to acetylenic esters to form α,*β*-unsaturated esters in high yield and selectivity.[26] This useful *cis*-stannylvinyl cuprate intermediate, first made by Westmijze, is the product of a stannylcopper(I) bimetallic reagent reacted with acetylene in *syn*-fashion.[27] Marino reported that 5 equiv of *cis*-(tributylstannyl)vinyl cuprate added in a 1,4-fashion to cyclohexenonegave the *cis*-vinylstannane ketone adduct in 80% yield.[28] Subsequent studies by Marino and others have shown that the reactivity of the (stannylvinyl)copper(I) intermediate can be increased by starting with copper(I) cyanide.[29] Following this precedence, ethyl propiolate (**12**) was developed as a substrate for a new tandem stannylcopper−acetylene−alkyne ester addition reaction (Scheme 3).

Stannyl cuprate was first generated from hexabutylditin, butyllithium, methyllithium, and copper(I) cyanide added sequentially in THF at −78 °C.[29] Excess acetylene was then added directly to the cold solution followed by ethyl propiolate (**12**). After quenching with methanol, diene ester **13** was obtained in 82% yield, based on ethyl propiolate using 6 equiv of the cuprate, with greater than 25:1 *Z,E/Z,Z* selectivity. Use of only 1 equiv of cuprate gave **13** in only 17% yield, and 3 equiv improved the yield to 53%. The kinetic quench conditions of Piers, with **12** added together with methanol to the cuprate reagent, also gave **13** with high selectivity.[30] As found previously with the alkylcopper reagents, the stereochemistry of intermediate copper−enoate adduct was maintained upon protonation at low temperature.

To continue, DIBAL reduction of ester **13** gave the corresponding allyl alcohol which was oxidized using TPAP and NMO to give aldehyde **14** (Scheme 4). Treatment with the Horner−Emmons reagent then gave ester **15** in 73% overall yield. Only the (*E,E,Z*)-product shown was detected for this step. For the hydrolysis of ester **15** to the corresponding acid, several conditions were explored using sodium and lithium hydroxide in various concentrations and combinations of water, methanol, and THF. All gave extensive destannylation products with yields ranging from only 25% to 35%. Finally it was found

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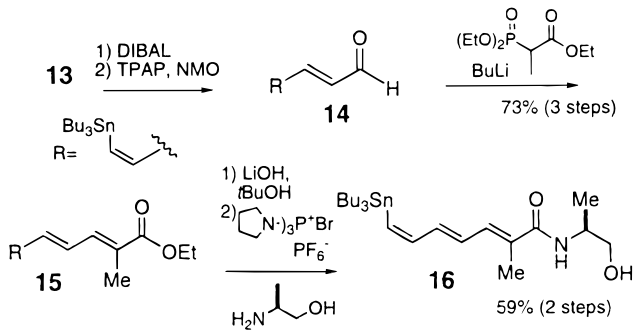
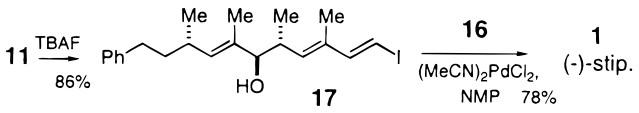
Scheme 4



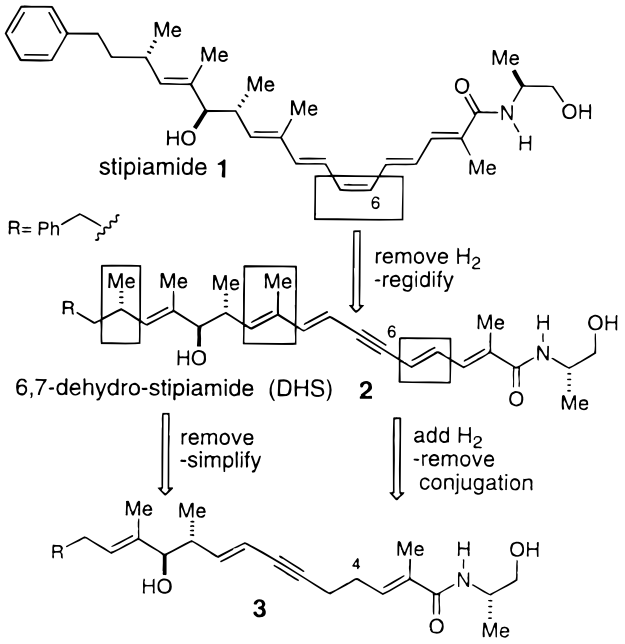
Scheme 5



that slow addition of saturated aqueous lithium hydroxide to a *tert*-butyl alcohol solution of ester **15** at 0 °C produced the desired acid with no destannylation.[24] The crude acid was then coupled with (*S*)-alaninol using bromotris(pyrrolidino)phosphonium hexafluorophosphate (PyBroP) to give amide **16** in 59% yield for the two combined steps.[31] With routes to the right- and left-hand fragments, the key coupling to form stipiamide was undertaken.

Vinyl iodide **11** was deprotected in 86% yield using tetrabutylammonium fluoride (TBAF) to give **17** (Scheme). In a recent synthesis, a key Stille coupling between vinyltin and vinyl iodide fragments of similar size and comparable functionality to this system using $Pd_2(dba)_3CHCl_3$ as catalyst was reported where a number of problems with low yields and byproduct formation were encountered.[32] Further review of the literature provided a number of additional cases where Pd(II) catalysts worked well, prompting us to investigate the more active $(MeCN)_2PdCl_2$ catalyst.[33] It was found that at room temperature in 15 min the reaction of **16** and **17**, at 1:1.1 stoichiometry, gave the desired product in 78% yield. NMR analysis of this material showed a 4:2:1 mixture of (−)-stipiamide and the all-*trans*- and the (4*Z*)-isomers, respectively. The coupling experiment was repeated with the exclusion of light followed by rapid filtration through neutral alumina. The same isomeric ratio was observed. Due to the stereochemical fidelity of the Stille reaction, it can be reasoned that the isomerization did not occur during the brief palladium coupling conditions, but rather during the workup and isolation steps. Indeed, a similar ratio of isomers was also observed in a sample of authentic material. In the case of the heptaene dimethylcrocetin visible light readily converts the *cis*-form to the all-*trans*-form in the time needed to perform the spectroscopy.[34] The process is also reminiscent of the conversion of all-*trans*-β-carotene to the mono-*cis*-isomers.[35] In similar fashion, the pentaene−amide functionality of stipiamide is readily isomerized. Attempts were made to separate the stipiamide isomers using reversed-phase HPLC following the report of Höfle.[5] Although complete separation was not achieved, direct comparison by ^{1}H NMR to the authentic

Chart 5



material clearly revealed the presence of stipiamide, the all-*trans*-isomer, and the 4-*cis*-isomer in a similar ratio. In addition, the optical rotation of the synthetic material ($[\alpha]_D$ −100°) approximated the weighted average of the rotations reported for the three natural isomers (−112°).

Design and Synthesis of Simplified Variants. Due to the structural similarity with the myxalamides, it was reasoned that the toxicity of stipiamide was due to its ability to function as an electron acceptor inhibiting mitochondrial NADH oxidation.[7] In the design of DHS (**2**), the goal was to create a molecule of similar geometry but with different electronic characteristics. Thus, the conversion of the C-6,7 *cis*-alkene to a triple bond served two purposes, a significant synthetic simplification eliminating the isomerization problem and an electronic variation. The next goal was to further simplify **2** by interrupting the extended polyene conjugation in an effort to probe the connection between toxicity and electron transport inhibition. This was accomplished by saturating the C-4,5 alkene and removing the C-16 carbon with its attendant stereocenter along with the C-10,11 ethylene methyl functionality to give compound **3** (Chart 5).

The synthesis of DHS (**2**) was accomplished by using a Sonogashira reaction with the same vinyl iodide **17** used for the synthesis of stipiamide and a new terminal alkyne amide. Alcohol **18**[36] was oxidized to the corresponding aldehyde which was treated with the phosphonate ester anion to give **19** in 55% yield for the two steps (Scheme 6).[32] Stereospecific bromine−tin exchange was brought about using bromine in methylene chloride to provide the vinyl bromide in 92% yield.[37]

The terminal alkyne was installed by coupling with TMS-acetylene to give **20** in 91% yield. Removal of the TMS group was accomplished using potassium carbonate in ethanol. Treatment with lithium hydroxide then provided **21** in 76% yield. This yellow-orange acid was taken on, without purification, to the amide using (*S*)-alaninol and PyBrop to give **22**. The purification of fragment **22** proved difficult due to stability problems. Thus, it was carried on without delay to the next reaction.

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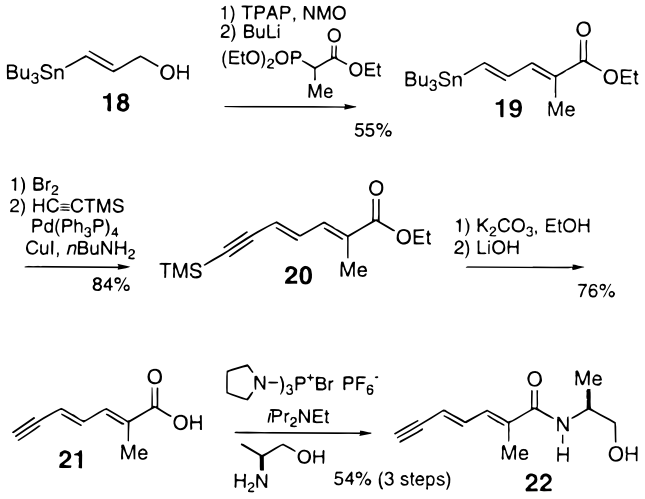
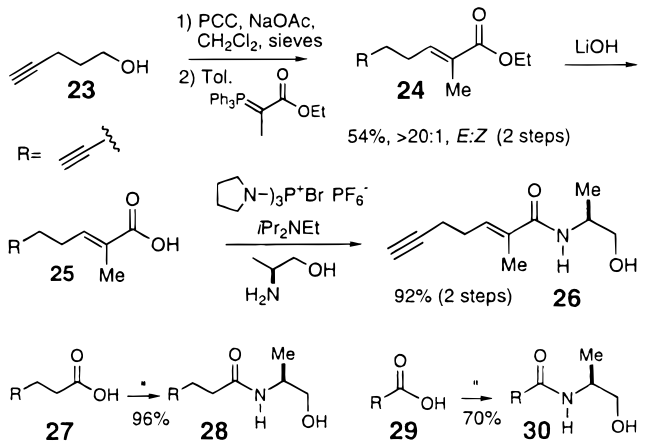
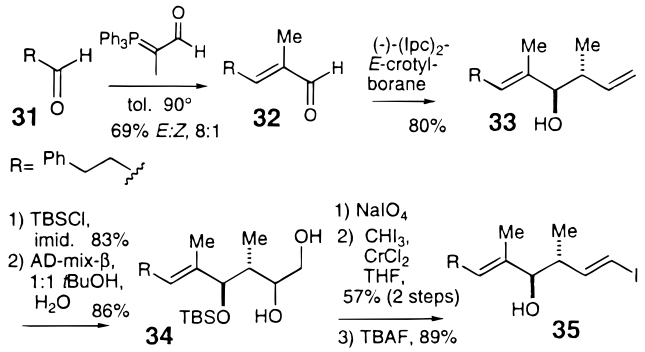
Scheme 6



Scheme 7



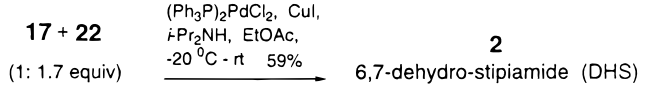
Scheme 8



The terminal alkynes used to form the simplified stipiamide variants were prepared as shown in Scheme 7. 4-Butyn-1-ol (**23**) was oxidized with PCC, providing the volatile aldehyde which was carefully concentrated and treated with (carbethoxyethylidene)triphenylphosphorane to give ester **24** in 54% yield. Hydrolysis of ester **24** with lithium hydroxide gave acid **25** which was coupled with (*S*)-alaninol in 92% overall yield. Similar amidation reactions were used with **27** and **29** to access the corresponding amides **28** and **30**.

The synthesis of a simplified left-hand fragment began with 3-phenylpropanal (**31**) olefinated using 2-(triphenylphosphoranylidene) propionaldehyde in 69% yield (Scheme 8). The resultant enal **32** was treated with diisopinocampheyl (*E*)-crotylborane derived from (−)-α-pinene to give *anti*-homoallylic alcohol **33** in 80% yield followed by TBS protection. In contrast

Scheme 9



to diene **7** in the synthesis of stipiamide, it was found the protected diene **33** gave poor yields (35−45%) when reacted with AD-mix-α. Interestingly, use of the opposite enantiomeric antipode AD-mix-*β* gave the desired diol **34** in 86% yield with no internal dihydroxylation.[17] Diol **34** was then reacted with sodium periodate, and the resulting crude aldehyde was treated under the optimized Takai reaction conditions to give the vinyl iodide in 57% overall yield for the two steps. TBAF deprotection provided the simplified left-hand fragment **35** in 89% yield.

DHS (**2**) was formed by a Sonogashira coupling of vinyl iodide **17** and alkyne **22** (Scheme 9).[38] The optimized conditions included $(PPh_3)_2PdCl_2$ as catalyst with CuI as cocatalyst with addition of diisopropylamine to an ethyl acetate solution at −20 °C of the coupling partners at 1:1.7 stoichiometry to give DHS (**2**) in 59% yield. Acetylenes **26**, **28**, and **30** were coupled to vinyl iodide **35** to give the desired enyne products **3**, **36**, and **37**, as shown in Chart 6. Selected conditions and reagents explored are shown in Table 1.

While there are many synthetic applications of the coupling of a vinyl iodide with a terminal alkyne under palladium−copper catalysis,[39] there have been few systematic studies reported that investigate each feature of the reaction. Initially (entries 1−3) the widely used protocol was employed using catalytic $Pd(PPh_3)_4$ with stoichiometric *n*-butylamine in benzene.[40] The use of 1 equiv of acetylene **22** gave a low yield of 15% for **2**. Even with 3 equiv of alkyne, **2** was obtained in only 50% yield. Numerous variations were performed to determine the effect of the hydroxyl and amide functionalities on the yield (not shown). These findings suggested that an alkynyl amide is inherently a poorer coupling partner compared to an analogous alkynyl ester, giving yields 15−20% lower. The few reported couplings with amide substrates also occur with low yields in parallel with our findings.[41] Other attempts to remedy the low yield problem that also proved unsuccessful included slow addition, changing the mole percent of the copper cocatalyst, and colder initial temperatures.[42] Only with **22** added rapidly was **2** obtained in 70% yield (entry 3) using this catalyst system.

Alternative forms of palladium were explored in an effort to increase the yield closer to the ideal 1:1 substrate stoichiometry. With enyne **3**, $Pd(PPh_3)_4$ gave yields of 10−12% (not shown) whereas use of $PdCl_2(MeCN)_2$ significantly improved the yield to 31% (entry 4). The optimal results were finally achieved using $PdCl_2(PPh_3)_2$ as catalyst (entries 6−9). Indeed, in the case of DHS (**2**) $PdCl_2(PPh_3)_2$ (entry 5) gave a much improved 59% yield. Most importantly, in this example only 1.7 equiv of **22** was used.

Following the lead of Johnson, further improvement could be realized by starting the reaction at −20 °C with warming to

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Chart 6

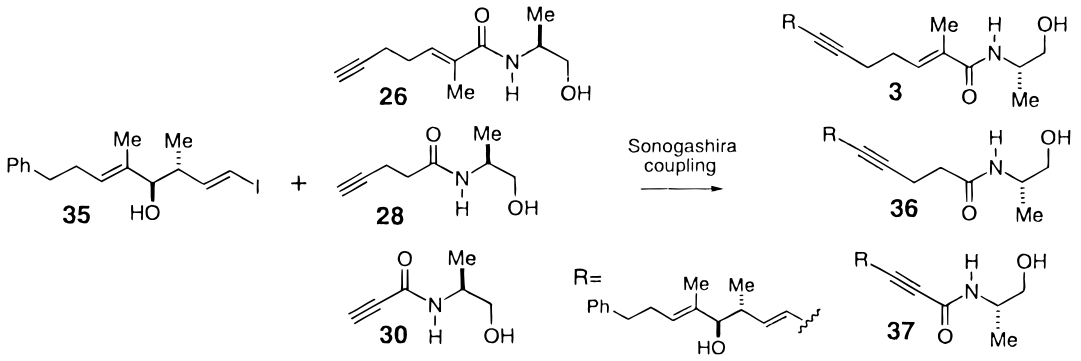

Table 1. Sonogashira Coupling for the Formation of Enynes **2**, **3**, **36**, and **37**

entry	vinyl iodide	alkyne	equiv of alkyne	product	palladium catalyst (%)[a]	CuI (%)[a]	solvent	temp (°C)	base[b]	% yield
1	**17**	**22**	1	**2**	$Pd(PPh_3)_4$ (10)	5	benzene	rt	*n*-$BuNH_2$	15
2	**17**	**22**	3	**2**	$Pd(PPh_3)_4$ (10)	5	benzene	rt	*n*-$BuNH_2$	50
3	**17**	**22**	10	**2**	$Pd(PPh_3)_4$ (10)	5	benzene	rt	*n*-$BuNH_2$	**70**
4	**35**	**26**	1.5	**3**	$(MeCN)_2PdCl_2$ (5)	10	DMF	rt	Et_3N	31
5	**17**	**22**	1.7	**2**	$(PPh_3)_2PdCl_2$ (5)	35	EtOAc	−20 to rt	*i*-Pr_2NH	**59**
6	**35**	**26**	1.5	**3**	$(PPh_3)_2PdCl_2$ (10)	10	EtOAc	rt	Et_3N	54
7	**35**	**26**	1.3	**3**	$(PPh_3)_2PdCl_2$ (5)	7	EtOAc	0	*i*-Pr_2NH	61
8	**35**	**26**	1.5	**3**	$(PPh_3)_2PdCl_2$ (5)	30	EtOAc	0	*i*-Pr_2NH	72
9	**35**	**26**	1.5	**3**	$(PPh_3)_2PdCl_2$ (8)	10	EtOAc	−20 to rt	*i*-Pr_2NH	**87**
10	**35**	**28**	1.5	**36**	$(PPh_3)_2PdCl_2$ (5)	10	EtOAc	rt	Et_2NH	61[c]
11	**35**	**28**	1.5	**36**	$(PPh_3)_2PdCl_2$ (5)	15	EtOAc	0	*i*-Pr_2NH	70
12	**35**	**28**	1.5	**36**	$(PPh_3)_2PdCl_2$ (6)	32	EtOAc	−20 to rt	*i*-Pr_2NH	**85**
13	**35**	**30**	1.5	**37**	$(PPh_3)_2PdCl_2$ (5)	10	EtOAc	rt	Et_2NH	31
14	**35**	**30**	1.5	**37**	$(PPh_3)_2PdCl_2$ (5)	33	EtOAc	0 to rt	*i*-Pr_2NH	54
15	**35**	**30**	1.5	**37**	$(PPh_3)_2PdCl_2$ (5)	18	EtOAc	−20 to rt	*i*-Pr_2NH	**95**

[a] mol %. [b] The reactions were 0.2 M in base which was added last. [c] The yield was obtained with Et_2NH added last. Slow addition of alkyne **28** gave **36** in 30% yield.

room temperature.[43] Ethyl acetate was used for these reactions since the polar alkynyl amides were found to be poorly solvated by benzene at lower temperatures. The palladium(II) chloride catalyst again proved optimal in the synthesis of the simplified variants, giving excellent yields of 85−95% (entries 9, 12, and 15). Diminished yields of 10−15% were obtained using the less active catalyst $Pd(PPh_3)_4$ (not shown). Temperature clearly plays an important role in the formation of the enynes especially in the case of **37**. This coupling reaction when performed at 0 °C gave only a 54% yield (entry 14), but when started at −20 °C (entry 15) with warming to room temperature, the product was obtained in 95% yield. The fact that coupling occurs with high efficiency is significant in this case in that others have recently reported that alkynes conjugated to electron-withdrawing groups react with very poor yields.[44]

Biological Evaluation. Seto found that stipiamide, in the MDR assay with colchicine resistant KB cells, was able to restore cytotoxicity to within a factor of six to the wild type response.[2] However, with other anticancer agents, MDR reversal was not observed using stipiamide. Now with synthetic stipiamide, MDR reversal using human breast cancer cells (MCF-7adrR) resistant to multiple anticancer compounds was investigated.[45] Again with several added agents, exclusive MDR reversal activity for colchicine was observed (Table 2). Only in the case of colchicine (entry 5) was stipiamide shown to reduce resistance to within a factor of 100 of the wild type response, whereas in all other cases the difference between transformed and wild type response was over 5 orders of magnitude. Besides the exclusive MDR effect, stipiamide was also found to be incredibly toxic (ED_{50}, 0.01 nM). This activity can be attributed to inhibition of mitochondrial ATP synthesis as exhibited by other members of the polyene class.[6c]

Table 2. MDR Reversal Activity of Stipiamide with Chemotherapeutic Drugs Using MDR-7adrR (Adriamycin Resistant) Breast Cancer Cells

entry	anticancer drug	ED_{50} of anticancer drug in wild type cell (nM)	ED_{50} of anticancer drug in MCF-7adrR cell + stipiamide[a] (nM)
1	adriamycin	0.5	2500
2	vinblastine	4.4×10^{-6}	12
3	vincristine	0.01	400
4	colchicine	0.02	3

[a] Stipiamide was present at 50% of its ED_{50} (4×10^{-12} M).

In contrast, the same assay found that the ED_{50} of DHS (**2**) was 4.4 μM, 10 000 times less toxic than stipiamide. Remarkably, the simple modification of the C-6,7 (*Z*)-alkene to alkyne greatly lowers the toxicity displayed in the normal, wild type cells. In order to then determine the concentration for MDR reversal, the standard protocol developed by Chang was used with **2**.[46] MCF-7adrR cells were treated with **2** followed by the addition of adriamycin at its ED_{50} concentration for the wild type cells (4 nM). The percentage of cells remaining viable at a given concentration of **2** was then determined for each assay performed in quadruplicate at each concentration. The assays were performed using consistent concentrations both above and below the ED_{50} of the compound, allowing for comparisons of **2** and the other simplified variants. The average of the percent viable cells for the assays was then plotted as a function of DHS (**2**) concentration (Figure 1).

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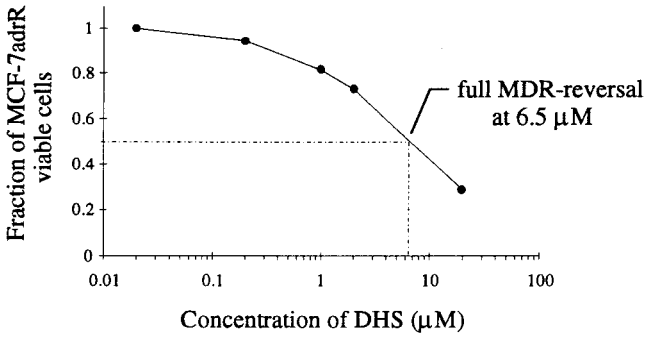

Figure 1. Fraction of viable MCF-7adrR cells versus the concentration of DHS with adriamycin present at 4 nM.

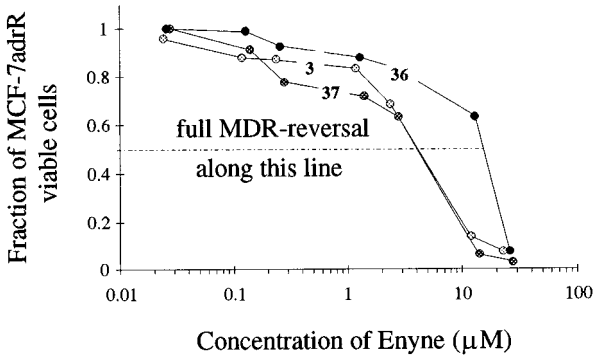

Figure 2. Fraction of viable MCF-7adrR cells versus the concentration of enynes **3**, **36**, and **37** with adriamycin present at 4 nM.

Table 3. Comparison of the ED_{50} and TIs for Enynes **2**, **3**, **36**, and **37** in MDR-7adrR Adriamycin Resistant Breast Cancer Cells

entry	compd	ED_{50} (μM)	reversal concn (μM)	TI[a]
1	**2**	4.4	6.5	1.48
2	**3**	14	4	0.29
3	**36**	18	14	0.78
4	**37**	5	4	0.8

[a] TI = concentration to completely reverse MDR/the ED_{50}.

From the graph it was determined that the concentration for MDR reversal by **2** required for adriamycin to kill half of the resistant cells was 6.5 μM. This value is slightly greater than its own ED_{50} suggesting that **2**, at the MDR reversal concentration, may not be acting exclusively as a reversal agent but also may be functioning as a cytotoxin. More importantly **2**, in contrast to stipiamide, now shows MDR reversal activity with a broad range of cancer agents, not being limited to colchicine.

MDR reversal analysis was then performed with the simplified enynes **3**, **36**, and **37**. It was found that MDR reversal now occurred at concentrations lower than the toxicity ED_{50} (Figure 2). The therapeutic index (TI) was then used as a helpful convention to rank the success of the new reversal compounds, being defined as the ratio of the reversal concentration to the toxicity ED_{50} as shown in Table 3.

By this definition, smaller TI values (<1) correspond to better reversal agents. This range indicates that the compounds are able to reverse MDR at concentrations below their toxicity level (ED_{50}). DHS (**2**), with a TI of 1.48 (entry 1), demonstrates very good MDR reversal when directly compared to verapamil (TI $>$ 5), the clinical standard. Clearly the lower toxicity generated by the change to the 6,7-acetylene is a step in the right direction. More remarkable is the low TI of 0.29 observed for **3** (entry 2). Although enynes **2**, **3**, and **37** have similar MDR reversal concentrations, **3** is less toxic than the other two (14 vs 4.4 and 5 μM). This finding for **3** supports the reasoning of the design criteria where the interruption of the polyene conjugation impairs the compound's ability to function as a one-electron acceptor, leading to lower toxicity. Continuing the trend, **36** (entry 3) further lacking the α,β-unsaturation of **3** is the least toxic of the simplified variants. Again the loss of conjugation appears to play a role in lowering the toxicity. However, the lack of rigidity and reduced size relative to **3** diminished the ability of **36** to reverse MDR. These findings together with the optimized coupling conditions will allow for the design and synthesis of new compounds that will be used to further define the structural requirements for effective Pgp transport inhibition and improved MDR reversal.

Experimental Section

General Information. Air-sensitive reactions were performed under a positive pressure of argon unless otherwise indicated. Air- and moisture-sensitive reagents were introduced *via* syringe or cannula through rubber septa. All solvents were distilled prior to use from an appropriate drying agent. Methylene chloride was distilled from CaH_2. THF and diethyl ether were distilled from sodium−benzophenone ketyl. Reagents were purchased and used without further purification. Purification by flash column chromatography was carried out in the indicated solvent system using 70−230 mesh silica gel. Purification by radial chromatography was performed using 1, 2, and 4 mm plates loaded with 230−400 mesh PF-254 gypsum-bound silica. Chromophore-containing compounds were visualized during the radial chromatography using 254 nm UV light. TLC analysis was conducted on silica gel F_{254}, 0.25 mm precoated glass plates. The developed plates were visualized by UV light (254 nm) and/or by dipping into staining solutions followed by charring. All ^{1}H NMR spectra were obtained using either a 500 or 200 MHz spectrometer employing chloroform (7.26 ppm) as an internal reference. Signals are reported as m (multiplet), s (singlet), d (doublet), t (triplet), q (quartet), etc.; coupling constants (J) are reported in hertz (Hz). Carbon spectra were obtained at 50 MHz. Infrared spectra were obtained using an FTIR spectrometer. Only several characteristic absorbencies are reported (in units of wavenumbers, cm^{-1}). Mass spectra were run by the Purdue campus-wide mass spectrometry facility. Low-resolution electron impact (EI) or chemical ionization (CI) spectra were obtained using a mass spectrometer. Prominent mass fragments are reported along with their relative intensities (in parentheses). Optical rotations were obtained using a polarimeter at room temperature (rt) employing the sodium D line. In all cases, the length of the rotation cell was 10 cm (1 dm), and solvents employed were of spectroscopic grade. Optical rotation concentrations are given in grams per milliliter. Microanalyses were performed by the Purdue Chemistry Department Microanalytical Laboratory. Finally, bicinchoninic acid MDR assays were performed by the Purdue Cancer Research Center.

Preparation of Ethyl (2*E*,4*S*)-2,4-Dimethyl-6-phenyl-2-hexenoate (5). To a stirring solution of (4*S*)-3-[(2*S*)-2-methyl-4-phenyl-1-oxobutyl]-4-(phenylmethyl)-2-oxazolidinone (10.41 g, 30.9 mmol) in ether (0.15 M, 200 mL) at 0 °C was added lithium aluminum hydride (1 M, 46.3 mL, 46.3 mmol) as a THF solution via syringe pump over 1 h. The mixture was allowed to stir for 2 h and was quenched by the slow addition of 9:1 THF/water (20 mL) followed by aqueous NaOH (2 N, 3 mL).

The THF and water layers were separated, and the aqueous layer was extracted with methlyene chloride (2 × 100 mL). The organic phases were combined, dried over $MgSO_4$, and distilled down to a crude oil which was passed through a plug of silica gel (7 × 4 cm) and distilled down to give 4.3 g of essentially pure alcohol (by TLC).

To a stirring solution of the crude alcohol (4.3 g, 26 mmol) in 50 mL of methylene chloride were added oven-dried, crushed 3 Å molecular sieves (13 g), NMO (4.6 g, 39 mmol), and TPAP (350 mg, 1 mmol). The solution was stirred for 15 min and passed through a 7 cm diameter course fritted funnel containing sand (0.5 cm) on top, filter paper in between, and silica gel (3 cm) on the bottom. The material was rinsed through with ether. The filtered crude was concentrated, giving 4.18 g of crude aldehyde (one spot by TLC) which was dissolved in 125 mL of toluene (0.2 M). To this was added (carbethoxyethylidene)triphenylphosphorane (14 g, 38 mmol), and the solution was

warmed to 90 °C for 15 h. After cooling, the toluene was removed, and the oil was dissolved in hexanes, causing triphenylphosphine oxide and excess ylide to precipitate. This suspension was passed through a plug of silica gel (7 × 3 cm) and divided into three equal portions (^{1}H NMR showed *E*:Z = 8:1). Each portion was radial chromatographed on a 4 mm plate with 5% EtAOc/Hex as the eluent, giving all together 4.2 g (55%, three steps) of the pure *E* isomer. Close eluting higher fractions (the (*Z*)-isomer) were discarded along with some overlapping fractions: R_f = 0.56 in 35% EtOAc/Hex; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.36−7.12 (m, 5H), 6.62 (d, J = 11.6 Hz, 1H), 4.22 (q, *J* = 7.0 Hz, 2H), 2.73−2.46 (m, 3H), 1.82 (s, 3H), 1.82−1.58 (m, 2H), 1.32 (t, *J* = 7.0 Hz, 3H), 1.05 (d, *J* = 7.4 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.1, 14.8, 20.5, 22.2, 33.3, 34.2, 39.0, 61.0, 126.3, 127.4, 128.8, 142.6, 147.9, 168.9; IR (film) 1712, 1256, 1086. Anal. Calcd for $C_{16}H_{22}O_2$: C,78.01; H, 9.00. Found: C, 77.76; H, 9.29.

Preparation of (3*S*,4*R*,5*E*,7*S*)-4-Hydroxy-9-phenyl-3,5,7-trimethylnona-1,5-diene (7). To a stirring solution of ethyl (2*E*,4*S*)-2,4-dimethyl-6-phenyl-2-hexenoate (**5**) (6.0 g, 24.4 mmol) in THF (0.2 M, 120 mL) at −40 °C was added DIBAL (1.5 M, 49 mL, 73.1 mmol) rapidly as a THF solution. The mixture was allowed to stir for 1 h and was quenched by the slow addition of methanol (2 mL) followed by saturated aqueous Rochelle salts (15 mL). The solution was allowed to stir until the aqueous and organic layers separated into distinct phases (approximately 3 h). The THF and water layers were separated, and the aqueous layer was extracted with methlyene chloride (2 × 50 mL). The organic phases were combined, dried over $MgSO_4$, and distilled down to a crude oil which was passed through a plug of silica gel (7 × 4 cm) and distilled down to give 4.38 g of essentially pure alcohol (by TLC).

To a stirring solution of the crude alcohol (4.38 g, 21.4 mmol) in 45 mL of methylene chloride were added oven-dried, crushed 3 Å molecular sieves (10 g), NMO (3.8 g, 32 mmol), and TPAP (300 mg, 0.8 mmol). The solution was stirred for 15 min and passed through an 8 cm diameter course fritted funnel containing sand (0.5 cm) on top, filter paper in between, and silica gel (3 cm) on the bottom. The material was rinsed through with ether. The filtered crude was concentrated, giving 3.32 g of crude aldehyde (one spot by TLC).

To a stirring −78 °C solution of potassium *tert*-butoxide (3.13 g, 27.9 mmol) in 15 mL of THF was added via cannula *trans*-2-butene (6 mL, ∼54 mmol, condensed into a graduated column at −78 °C). To the solution was added via syringe *n*-butyllithium (2.5 M, 11.2 mL, 27.9 mmol) as a hexane solution. The mixture was allowed to stir for 15 min, and ^{l}Ipc$_2$BOMe (diisopinocampheylmethoxyborane derived from (−)-α-pinene) was slowly added by syringe (2 M, 16.4 mL, 32.8 mmol) as a solution in ethyl ether. The solution was stirred for 30 min, and boron trifluoride etherate was slowly added (4.64 mL, 37.7 mmol) by syringe. The mixture was stirred for 5 min followed by the addition of the above formed crude aldehyde as an ether solution (5 mL) with two 1 mL ether washings. After 15 h at −78 °C, 10 mL of saturated NaOAc was added followed by 10 mL of 30% H_2O_2. The biphasic mixture was then allowed to warm to rt and stir for 8 h. The mixture was diluted with ether (10 mL) and washed with brine. The separated aqueous layer was then washed twice with methylene chloride (2 × 25 mL), and the combined organic layers were dried over $MgSO_4$. The solution was concentrated and flashed chromatographed through silica gel (4 × 10 cm) eluting with 250 mL of 7%, 9%, 11%, and 15% EtOAc/hexanes. The product-containing fractions were concentrated to give 3.71 g (60% from the ester) of product as clear oil. ^{1}H NMR showed the product to be an approximately 7:1 inseparable mixture of diastereomers: R_f = 0.43 in 20% EtOAc/Hex; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.33−7.12 (m, 5H), 5.87−5.66 (m, 1H), 5.26−5.09 (m, 3H), 3.65 (d, *J* = 8 Hz, 1H), 2.66−2.22 (m, 4H), 1.73−1.51 (m, 6H), 0.96 (d, *J* = 7.2 Hz, 3H), 0.89 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 143.2, 141.8, 135.4, 134.3, 128.9, 128.7, 126.1, 116.9, 81.8, 42.7, 39.7, 34.4, 32.3, 21.5, 17.4, 11.8; MS (CI) *m/z* 241 (1.0, M + H (−H_2O)). Anal. Calcd for $C_{18}H_{26}O$: C, 83.67; H, 10.14. Found: C, 83.35; H, 10.08.

Preparation of (3*S*,4*R*,5*E*,7*S*)-4-[(Dimethyl-*tert*-butylsilyly)oxy]-9-phenyl-3,5,7-trimethylnona-1,5-diene. To a stirring solution of (3*S*,5*E*,4*R*,7*S*)-4-hydroxy-9-phenyl-3,5,7-trimethylnona-1,5-diene (**7**) (3.37 g, 13 mmol) in DMF (0.6 M, 27 mL) were added imidazole (2.6 g, 39.1 mmol) and *tert*-butyldimethylsilyl chloride (3.92 g, 26.1 mmol) at rt. The reaction was stirred overnight, diluted with 50 mL of methylene chloride, and extracted with water (50 mL). The separated water layer was washed with methylene chloride (2 × 50 mL). Combined extracts were dried over $MgSO_4$. The solution was concentrated and flashed chromatographed through silica gel (4 × 10 cm) eluting with 250 mL of 1%, 2%, 5% EtOAc/hexanes. Product-containing fractions were collected and concentrated to give 4.64 g (95%) of a clear oil as an inseparable mixture of diastereomers (∼7:1 determined by ^{1}H and ^{13}C NMR): $[\alpha]_D$ + 2.72° (*c* = 0.054, CH_2Cl_2); R_f = 0.68 in 5% EtOAc/Hex; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.33−7.13 (m, 5H), 5.97−5.77 (m, 1H), 5.16−4.93 (m, 3H), 3.68 (d, *J* = 8 Hz, 1H), 2.72−2.21 (m, 4H), 1.73−1.47 (m, 5H), 0.98−0.83 (m, 15H), 0.04 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 143.4, 142.7, 135.8, 133.6, 128.8, 128.7, 126.0, 114.2, 83.3, 42.7, 40.0, 34.5, 32.3, 26.4, 21.6, 18.8, 17.3, 12.3, −3.8, −4.4. Anal. Calcd for $C_{24}H_{40}OSi$: C, 77.35; H, 10.82. Found: C, 77.19; H, 10.86.

Preparation of Ethyl (2*E*,4*S*,5*R*,6*E*,8*S*)-5-[(*tert*-Butylsilyl)oxy]-10-phenyl-2,4,6,8-tetramethyldodeca-2,6-dienoate (9). To a stirring solution of (3*S*,4*R*,5*E*,7*S*)-4-[(dimethyl-*tert*-butylsilyl)oxy]-9-phenyl-3,5,7-trimethylnona-1,5-diene (1.25 g, 3.35 mmol) in a mixture of *tert*-butyl alcohol (15 mL) and water (15 mL) was added AD-mix-α (Aldrich, 5 g, 1.5 g/mmol) at rt. The reaction was allowed to proceed for 30 h, giving exclusively terminal alkene dihydroxylation (R_f = 0.41 in 35% EtOAc/Hex) and a faint starting material spot. The reaction mixture was then passed through an 8 cm diameter course fritted funnel containing sand (0.5 cm) on top, filter paper in between, and silica gel (4 cm) on the bottom. The material was rinsed through with ether. The filtered crude was concentrated, giving 1.40 g of crude diol which was dissolved in a mixture of THF (10 mL) and water (10 mL). To this solution was added sodium periodate (1.1 g, 5.2 mmol), and the mixture was allowed to stir for 30 min, giving aldehyde (R_f = 0.38 in 10% EtOAc/Hex). The reaction mixture was then passed through an 8 cm diameter course fritted funnel containing sand (0.5 cm) on top, filter paper in between, and silica gel (4 cm) on the bottom. The material was rinsed through with ether and concentrated, giving 1.13 g of crude aldehyde (one spot by TLC) which was dissolved in 30 mL of toluene (0.1 M). To this was added (carbethoxyethylidene)-triphenylphosphorane (2.1 g, 6.0 mmol), and the solution was warmed to 90 °C for 12 h. After cooling, the toluene was removed, and the oil was dissolved in hexanes, causing triphenylphosphine oxide and excess ylide to precipitate. This suspension was passed through a plug of silica gel (7 × 3 cm) rinsing with 1:1 EtAOc/hexanes. The solution was concentrated and radial chromatographed on a 4 mm plate using 10% EtAOc/hexanes to give 0.96 g (62% from terminal alkene) of product (a clear oil) as a mixture of diastereomers (∼7:1 by NMR). Close eluting higher fractions (the (*Z*)-isomer) was discarded along with some overlapping fractions: $[\alpha]_D$ + 6.08° (*c* = 0.36, CH_2Cl_2); R_f = 0.56 in 35% EtOAc/Hex; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30−7.24 (m, 2H), 7.21−7.13 (m, 3H), 6.64 (d, *J* = 11.6 Hz, 1H), 5.35 (d, *J* = 11.1 Hz, 1H), 4.22−4.12 (m, 2H), 3.76 (d, *J* = 8.9 Hz, 1H), 2.70−2.47 (m, 3H), 2.46−2.36 (m, 1H), 1.85 (s, 3H), 1.68−1.60 (m, 1H), 1.6−1.52 (m, 1H), 1.57 (s, 3H), 1.26 (t, *J* = 8.3 Hz, 3H), 0.95 (d, *J* = 7.4 Hz, 3H), 0.87 (s, 3H), 0.84 (s, 9H), 0.01 (s, 3H), −0.01 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.8, 146.8, 143.3, 135.8, 135.4, 128.6, 127.8, 126.1, 83.4, 60.7, 39.9, 38.7, 38.6, 34.4, 32.3, 26.3, 21.7, 18.6, 14.8, 13.2, 12.0; IR (film) 1712, 1184, 1086. Anal. Calcd for $C_{28}H_{46}O_3$: C,73.31; H, 10.11. Found: C, 73.06; H, 10.39.

Preparation of (1*E*,3*E*,4*S*,5*R*,7*E*,8*S*)-5-[(*tert*-Butylsilyl)oxy]-1-iodo-10-phenyl-3,5,7,9-tetramethylundeca-1,3,7-triene (11). To a stirring solution of ethyl (2*E*,4*S*,5*R*,6*E*,8*S*)-5-[(*tert*-butylsilyl)oxy]-10-phenyl-2,4,6,8-tetramethyldodeca-2,6-dienoate (**9**) (2.11 g, 4.6 mmol) in THF (0.2 M, 25 mL) at −40 °C was added DIBAL (1.5 M, 9.2 mL, 13.8 mmol) rapidly as a THF solution. The mixture was allowed to stir for 1 h and was quenched by the slow addition of methanol (1 mL) followed by saturated aqueous Rochelle salts (5 mL). The solution was allowed to stir until aqueous and organic layers separated into distinct phases (approximately 3 h). The THF and water layers were separated, and the aqueous layer was extracted with methlyene chloride (2 × 15 mL). The organic phases were combined, dried over $MgSO_4$, and distilled down to a crude oil which was passed through a plug of silica gel (8 × 4 cm) and distilled down to give 1.56 g of alcohol (81%).

This was divided into halves and radial chromatographed on a 4 mm plate using gradient elutions (5%, 7%, and 10% EtOAc/hexanes). The overlapping fractions from the two separations were combined and rechromatographed. In this manner, the minor isomers from the Wittig−crotylboration−Wittig reactions were eliminated to give 1.16 g of pure alcohol (61%, ^{1}H NMR). To a stirring solution of the alcohol (338 mg, 0.94 mmol) in 2 mL of methylene chloride were added oven-dried, crushed 3 Å molecular sieves (470 mg), NMO (166 mg, 1.41 mmol) and TPAP (33 mg, 0.094 mmol). The solution was stirred for 10 min and passed through a 4 cm diameter course fritted funnel containing sand (0.5 cm) on top, filter paper in between, and silica gel (2.5 cm) on the bottom. The material was rinsed through with ether. The filtered crude was concentrated, giving 338 mg of crude aldehyde (87%, one spot by TLC).

$CrCl_2$ (220 mg, 1.79 mmol) was added to a 25 mL round bottom flask and gently flame dried under high vacuum. Upon cooling, the flask was released under argon and charged with 2 mL of THF. The slurry was cooled to 0 °C and allowed to stir for 10 min. To another flask containing aldehyde (119 mg, 0.29 mmol) was added 1 mL of THF followed by iodoform (235 mg, 0.60 mmol). These were then added dropwise by syringe to the $CrCl_2$ slurry which turned a brown-red color. The reaction was allowed to proceed for 3 h at 0 °C and then filtered through a plug of silica gel (4 × 3 cm) using ether. The filtrate was then concentrated, dissolved in 1 mL of ether, and treated with 1 mL of TBAF (1 M in THF) with stirring to remove the excess iodoform. This solution was allowed to stir for 10 min and filtered through a plug of silica gel (4 × 3 cm) using ether. The filtered solution was concentrated and radial chromatographed on a 1 mm plate using 2% EtOAc/hexanes to give 109 mg (70%) of product (a clear oil) as a mixture of diastereomers (*E*/*Z*, 20:1, by 500 MHz ^{1}H NMR). The overall yield for the three steps was 37% (50% including the minor isomers): $[\alpha]_D$ + 7.68° (*c* 0.24, CH_2Cl_2); R_f = 0.5 in 0.5% EtOAc/Hex; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32−7.26 (m, 2H), 7.21−7.17 (m, 3H), 7.06 (d, J = 15.1 Hz, 1H), 6.09 (d, J = 15.1 Hz, 1H), 5.36 (d, J = 10.1 Hz, 1H), 5.14 (d, J = 10.1 Hz, 1H), 3.72 (d, J = 7.8 Hz, 1H), 2.68−2.56 (m, 2H), 2.54−2.46 (m, 1H), 2.46−2.36 (m, 1H), 1.71 (s, 3H), 1.69−1.59 (m, 1H), 1.59−1.48 (m, 1H), 1.56 (s, 3H), 0.95 (d, J = 7.2 Hz, 3H), 0.86 (s, 9H), 0.84 (d, J = 0.72 Hz, 3H), −0.01 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 150.5, 143.4, 139.0, 135.7, 134.4, 133.7, 128.84, 128.78, 126.1, 83.0, 73.2, 40.0, 37.9, 34.4, 32.3, 26.3, 21.7, 18.6, 18.2, 12.8, 12.4, −3.9, −4.4; HRMS (CI) calcd for $C_{27}H_{43}IOSi$ 539.2206, found 539.2222.

Preparation of Ethyl (2*E*,4*Z*)-5-(Tri-*n*-butylstannyl)penta-2,4-dienoate (13). To a solution of hexabutylditin (7.6 mL, 15 mmol) in 50 mL of THF at −20 °C was added *n*-butyllithium (6 mL, 15 mmol, 2.5 M) via syringe. After 15 min, the reaction was further cooled to −78 °C, and methyllithium was added (10.7 mL, 15 mmol, 1.4 M) and allowed to stir for an additional 10 min. Copper(I) cyanide was then added (1.34 g, 15 mmol), giving an orange-red solution. After the solution was stirred for an additional 30 min, a balloon of acetylene (∼1 L) was placed upon the flask. The flask was placed under a mild vacuum (by means of a three-way valve in the balloon apparatus), and the contents of the flask were exposed to the acetylene gas. Over the course of 30 min, the reaction mixture turned to a dark green color. After 30 min at −78 °C, the balloon was removed and ethyl propiolate (0.25 mL, 2.5 mmol) and methanol (0.2 mL, 5 mmol) were added as a THF solution (1 mL) dropwise to the flask. After 10 min, 0.5 mL of $NaHCO_3$ was added. The reaction mixture was warmed to rt and diluted with 10 mL of ether. The reaction mixture was then passed through an 8 cm diameter course fritted funnel containing sand (0.5 cm) on top, filter paper in between, and silica gel (4 cm) on the bottom. The material was rinsed through with ether. The filtered crude was concentrated and further purified by column chromatography (4 × 17 cm) using 250 mL of 0%, 1%, 3%, and 5% EtOAc/hexanes and concentrated to give 854 mg (82%) of product oil as one diastereomer by proton NMR (S/N > 25:1). Data for the major diastereomer: R_f = 0.44 in 2% EtOAc/Hex; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23−7.10 (m, 2H), 6.71 (d, J = 12.2 Hz, 1H), 5.88 (d, J = 14.8 Hz, 1H), 4.21 (q, J = 7.4 Hz, 2H), 1.56−1.47 (m, 9H), 1.35−1.27 (m, 9H), 1.01 (t, J = 7.4 Hz, 3H), 0.88 (t, J = 6.6 Hz, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 10.6, 13.8, 14.1, 27.8, 29.8, 60.7, 122.2, 143.5, 146.1, 147.3, 165.9, IR (film) 2926, 1718, 1154. Anal. Calcd for $C_{19}H_{36}O_2Sn$; C, 54.96; H, 8.74. Found: C, 54.24; H, 9.00.

Preparation of Ethyl (2*E*,4*E*,6*Z*)-2-Methyl-7-(tri-*n*-butylstannyl)-hepta-2,4,6-trienoate (15). To a stirring solution of ethyl (*2E,4Z*)-5-(tri-*n*-butylstannyl)penta-2,4-dienoate (**13**) (1.09 g, 2.6 mmol, (*ZE*:*ZZ* ≈ 4:1) in THF (0.1 M, 25 mL) at −40 °C was added DIBAL (1.5 M, 5.3 mL, 7.9 mmol) rapidly as a THF solution. The mixture was allowed to stir for 1 h and was quenched by the slow addition of methanol (1 mL) followed by saturated aqueous Rochelle salts (3 mL). The solution was allowed to stir until aqueous and organic layers separated into distinct phases (approximately 3 h). The THF and water layers were separated, and the aqueous layer was extracted with methlyene chloride (2 × 15 mL). The organic phases were combined, dried over $MgSO_4$, and distilled down to a crude oil which was passed through a plug of silica gel (8 × 4 cm) and distilled down to give the alcohol. This alcohol was radial chromatographed (4 mm plate) using 5% EtOAc/hexanes, allowing the separation of the minor diastereomer to give 580 mg of pure (*ZE*)-product (60% or 79% if only the (*ZE*)-isomer of the starting material is counted). The overlapping fractions were discarded. To a stirring solution of alcohol (792 mg, 2.12 mmol) in 5 mL of methylene chloride were added oven-dried, crushed 3 Å molecular sieves (1 g), NMO (375 mg, 3.19 mmol), and TPAP (30 mg, 0.08 mmol). The solution was stirred for 15 min and passed through a 5 cm diameter course fritted funnel containing sand (0.5 cm) on top, filter paper in between, and silica gel (3 cm) on the bottom. The material was rinsed through with ether. The filtered crude was concentrated, giving 720 mg of crude aldehyde (one spot by TLC). Butyllithium (0.85 mL, 2.13 mmol) was added to a solution of ethyl 2-(diethylphosphoryl)propionate (0.48 mL, 2.23 mmol) in THF (11 mL, 0.2 M) at 0 °C. To this was added the crude aldehyde, and the mixture was allowed to warm to rt. After 5 min, the reaction mixture was filtered through a plug of silica gel (4 × 3 cm) using ether. The filtrate was then concentrated and radial chromatographed (4 mm plate) using 5% EtOAc/hexanes to give 867 mg (71% from ester) of product (a clear oil) as one diastereomer (S/N > 20:1 in ^{13}C NMR): R_f = 0.4 in 10% EtOAc/Hex; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.28−7.12 (m, 2H), 6.60−6.38 (m, 3H), 4.24 (q, J = 8.4 Hz, 2H), 1.97 (s, 3H), 1.61−1.23 (m, 18H), 0.97 (t, J = 8.4 Hz, 3H), 0.89 (t, J = 7.8 Hz, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.1, 13.2, 14.2, 14.8, 27.7, 29.6, 61.1, 76.9, 77.5, 78.1, 128.0, 129.6, 138.4, 141.3, 142.1, 146.5, 168.9; IR (film) 2958, 1706, 1102. Anal. Calcd for $C_{12}H_{40}O_2Sn$; C, 58.04; H, 8.86. Found: C, 58.15; H, 9.08.

Preparation of *N*-[(2*E*,4*E*,6*Z*)-2-Methyl-7-(tri-*n*-butylstannyl)-hepta-2,4,6-trienoyl]-(*S*)-alaninol (16). To a stirring solution of ethyl (2*E*,4*E*,6*Z*)-2-methyl-7-(tri-*n*-butylstannyl)hepta-2,4,6-trienoate (**15**) in *tert*-butyl alcohol (11 mL, 0.03 M) was added aqueous LiOH (2.3 mL, 2 M). After 2 d, the mixture was filtered through a plug of silica gel (4 × 3 cm) with EtOAc and concentrated to give 137 mg of the crude acid. This acid was then dissolved in methylene chloride (3 mL, 0.1 M) and cooled to 0 °C. To this solution were added diisopropylethylamine (0.17 mL, 0.97 mmol), (*S*)-alaninol (72 mg, 0.97 mmol), and PyBrOP (180 mg, 0.39 mmol). After 12 h, the reaction was filtered through a plug of silica gel (4 × 3 cm) using EtOAc and radial chromatographed on a 1 mm plate to give 94 mg (59% from the ester) of a yellow oil. This material was quite unstable, forming a yellow solid even after −20 °C storage for one week. Hence, the material had to be synthesized just prior to subsequent use: R_f = 0.25 in 50% EtOAc/Hex; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (dd, J = 11.7, 10.8 Hz, 1H), 7.03 (d, J = 10.8 Hz, 1H), 6.49 (dd, J = 11.7, 10.8 Hz, 1H), 6.43−6.36 (m, 2H), 5.89 (s, 1H), 4.22−4.14 (m, 1H), 3.78−3.70 (m, 1H), 3.64−3.55 (m, 1H), 2.93 (s, 1H), 1.98 (s, 3H), 1.57−1.18 (m, 18H), 0.96 (d, J = 8.4 Hz, 3H), 0.87 (t, J = 7.8 Hz, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.0, 13.5, 14.2, 17.6, 27.7, 29.6, 48.6, 67.7, 129.4, 129.9, 134.9, 140.6, 141.3, 146.5, 169.9; IR (film) 3336, 1636, 1458, 990; HRMS (CI) calcd for $C_{23}H_{43}NO_2Sn$ 482.2389, found 482.2379.

Preparation of (1*E*,3*E*,4*S*,5*R*,7*E*,8*S*)-5-Hydroxy-1-iodo-10-phenyl-3,5,7,9-tetramethylundeca-1,3,7-triene (17). To a flask containing neat (1*E*,3*E*,4*S*,5*R*,7*E*,8*S*)-5-[(*tert*-butylsilyl)oxy]-1-iodo-10-phenyl-3,5,7,9-tetramethylundeca-1,3,7-triene (**11**) (109 mg, 0.202) was added a THF solution of tetrabutylammonium fluoride (3 mL, 3.04 mmol, 1 M) at 0 °C. The reaction was allowed to warm to rt and to stir for 30 h. Filtration of the reaction mixture through a plug of silica gel (4 ×

3 cm) with ether followed by concentration and radial chromatography on a 1 mm plate using 5% EtOAc/hexanes gave 74 mg (86%) of product: $[\alpha]_D$ +29.4° (c = 0.0125, CH_2Cl_2); R_f = 0.20 in 10% EtOAc/Hex; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31−7.25 (m, 2H), 7.21−7.15 (m, 3H), 7.10 (d, J = 15.0 Hz, 1H), 6.21 (d, J = 15.0 Hz, 1H), 5.39 (d, J = 9.6 Hz, 1H), 5.21 (d, J = 9.6 Hz, 1H), 3.69 (d, J = 9.0 Hz, 1H), 2.72−2.52 (m, 3H), 2.48−2.39 (m, 1H), 1.79 (s, 3H) 1.70−1.57 (m, 2H), 1.60 (s, 3H), 1.62−1.55 (m, 1H), 0.97 (d, J = 7.2 Hz, 3H), 0.86 (d, J = 7.2 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 150.0, 143.2, 137.3, 136.4, 135.6, 134.4, 128.9, 128.7, 126.0, 82.7, 74.7, 39.6, 37.2, 34.4, 32.4, 21.5, 17.8, 12.9, 11.9; HRMS (CI) calcd for $C_{21}H_{29}$ IO 425.1341, found 425.1349.

Preparation of (1′*S*,2*E*,4*E*,6*Z*,8*E*,10*E*,12*R*,13*R*,14*E*,16*S*)-(−)-13-Hydroxy-*N*-(2′-hydroxy-1′-methylethyl)-2,10,12,14,16-pentamethyl-18-phenyl-2,4,6,8,10,14-octadecahexaenamide ((−)-Stipiamide, 1). To a flask containing neat (1*E*,3*E*,4*S*,5*R*,7*E*,8*S*)-5-hydroxy-1-iodo-10-phenyl-3,5,7,9-tetramethylundeca-1,3,7-triene (**17**) (5.8 mg, 0.0136 mmol) and neat *N*-[(2*E*,4*E*,6*Z*)-2-methyl-7-(tri-*n*-butylstannyl)hepta-2,4,6-trienoyl]-(*S*)-alaninol (**16**) (6.6 mg, 0.0136 mmol) at 0 °C were added 1-methyl-2-pyrrolidinone (0.2 mL) and bis(acetonitrile)palladium dichloride (0.4 mg, 0.0014 mmol). After being stirred for 15 min, the reaction mixture was filtered through a plug of alumina (3 × 3 cm), rinsing with 5% MeOH/CH_2Cl_2. The crude was then concentrated and placed under a 200 μm Hg vacuum for 12 h to remove the pyrrolidinone.

The crude was then further purified on a minicolumn (0.5 × 6 cm) of alumina using 5 mL of 2%, 5%, and 7% MeOH/CH_2Cl_2. Product-containing fractions were concentrated on a rotary evaporator, keeping the water bath at rt to give 5.3 mg (80%) of a bright yellow oil. ^{1}H NMR (500 MHz) shows the product to be a ∼2:1 mixture of stipiamide/(1′*S*,2*E*,4*Z*,6*E*,8*E*,10*E*,12*R*,13*R*,14*E*,16*S*)-13-hydroxy-*N*-(2′-hydroxy-1′-methylethyl)-2,10,12,14,16-pentamethyl-18-phenyl-2,4,6,8,10,14-octadecahexaenamide by comparison with the authentic spectra: $[\alpha]_D$ −100° (c = 0.0015, MeOH). The literature value for pure stipiamide is $[\alpha]_D$ −189° (c = 1, MeOH), and that for the (*E*,*Z*,*E*,*E*,*E*)-isomer is $[\alpha]_D$ −40.3° (c = 0.3, MeOH). For a 2:1 mixture the expected rotation should be $[\alpha]_D$ 0.66° (−189°) + 0.33° (−40.3°) = −138°: R_f = 0.32 in EtOAc; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29−7.23 (m, 2H), 7.19−7.13 (m, 3H), 7.05 (d, J = 11.5 Hz, 1H), 6.98 (d, J = 11.5 Hz, 1H), 6.67 (dd, J = 15, 11.5 Hz, 1H), 6.47 (dd, J = 15, 11.5 Hz, 1H), 6.37 (d, J = 15 Hz, 1H), 6.17 (t, J = 11.5 Hz, 1H), 6.09 (t, J = 11.5 Hz, 1H), 5.88 (d, J = 7 Hz, 1H), 5.49 (d, J = 9.5 Hz, 1H), 5.22 (d, J = 9.5 Hz, 1H), 4.22−4.13 (m, 1H), 3.75−3.63 (m, 3H), 3.62−3.56 (m, 1H), 3.07−3.01 (m, 1H), 2.78−2.70 (m, 1H), 2.64−2.51 (m, 2H), 2.47−2.39 (m, 1H), 2.00 (s, 3H), 1.90 (s, 3H), 1.68−1.49 (m, 2H), 1.60 (s, 3H), 1.24 (d, J = 6.5 Hz, 3H), 0.96 (d, J = 6.5 Hz, 3H), 0.87 (d, J = 6.5 Hz, 3H); HRMS (CI) calcd for $C_{32}H_{45}NO_3$ 492.3478, found 492.3492.

Preparation of Ethyl (2*E*,4*E*)-5-Bromo-2-methyl-2,4-pentadienoate. To a stirring solution of ethyl (2*E*,4*E*)-5-(tributylstannyl)-2-methyl-2,4-pentadienoate (**19**) (367 mg, 0.86 mmol) in CCl_4 (5 mL) at −20 °C was added bromine (138 mg, 0.86 mmol) as a CCl_4 solution (2 mL) dropwise until a faint yellow color persisted. The mixture was concentrated, filtered though a plug of silica gel (3.5 × 3 cm) with ether, and concentrated. The oil was then radial chromatographed, giving 172 mg (92%) of the title compound: R_f = 0.39 in 10% EtOAc/Hex; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.13−6.94 (m, 2H), 6.79−6.63 (m, 1H), 4.21 (q, J = 8.0 Hz, 2H), 1.92 (s, 3H), 1.29 (t, J = 8.0, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.3, 135.1, 133.8, 128.8, 116.6, 61.3, 14.7, 13.3; HRMS (EI) calcd for $C_8H_{11}BrO_2$ 217.9942, found 217.9938.

Preparation of Ethyl (2*E*,4*E*)-5-Bromo-2-methyl-7-(trimethylsilyl)-2,4-hept-6-ynedienoate (20). To a stirring solution of ethyl (2*E*,4*E*)-5-bromo-2-methyl-2,4-pentadienoate (159 mg, 0.73 mmol) in benzene (0.15 M, 6 mL) at room temperature were added *n*-butylamine (100 μL, 1.02 mmol) and $Pd(Ph_3P)_4$ (8 mg, 0.01 mmol). The resulting solution was protected from light by wrapping the flask with foil. The solution was allowed to stir for 45 min, after which (trimethylsilyl)-acetylene (216 μL, 1.52 mmol) was added followed by copper(I) iodide (22 mg, 0.12 mmol). After 5 h the mixture was filtered through a plug of silica gel (3.5 × 4 cm) and concentrated. The resulting oil was radial chromatographed to give 157 mg (91%) of the title compound: R_f = 0.40 in 10% EtOAc/Hex; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.16 (d, J = 12 Hz, 1H), 7.99−6.84 (m, 1H), 5.93 (d, J = 15.2 Hz, 1H), 4.23 (q, J = 8 Hz, 2H), 1.97 (s, 3H), 1.32 (t, J = 7.2 Hz, 3H), 0.22 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.3, 137.9, 136.9, 130.4, 117.8, 104.4, 101.3, 61.2, 14.7, 13.4, 0.2; HRMS (EI) calcd for $C_{13}H_{20}O_2Si$ 236.1233, found 236.1223.

Preparation of (1′*S*,2*E*,4*E*)-*N*-(2′-Hydroxy-1′-methylethyl)-2-methyl-2,4-hept-6-ynedienamide (22). To a stirring solution of ethyl (2*E*,4*E*]-5-bromo-2-methyl-7-(trimethylsilyl)-2,4-hept-6-ynedienoate (**20**) (226 mg, 0.96 mmol) in ethanol (10 mL, 0.1 M) at rt was added all at once potassium carbonate (159 mg, 1.15 mmol). After 2 h the reaction mixture was filtered through a plug of silica gel (3.5 × 4 cm) and concentrated to give 140 mg of the desilylated ester. The crude ester was then dissolved in THF/MeOH/H_2O (3, 1, and 1 mL, respectively). To this was added LiOH (61 mg, 2.56 mmol) at rt, and the reaction was stirred vigorously. After several hours, the reaction mixture was filtered through a plug of silica gel (3.5 × 4 cm) with methanol and concentrated to give the acid as an orange solid. To this solid was added methylene chloride (9 mL, ∼0.1 M) followed by diisopropylethylamine (440 μL, 2.55 mmol) and (*S*)-alaninol (128 mg, 1.7 mmol). The acid was only slightly soluble in this mixture. Finally, the PyBrOP reagent was added (475 mg, 1.02 mmol), and the reaction was allowed to stir for 24 h in the absence of light. The reaction was worked up by filtration through a plug of silica gel (3.5 × 4 cm) with methanol followed by concentration. This orange material was further purified by radial chromatography on a 1 mm plate using ethyl acetate as the eluent. Concentration of the product-containing fractions gave 101 mg (54%, three steps) of the title compound as a clear oil that readily turned orange in less than 15 min: R_f = 0.29 in 100% EtOAc; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.01−6.83 (m, 2H), 6.13 (d, 1H), 5.91−4.73 (m, 1H), 4.18−4.02 (m, 1H), 3.68 (dd, J = 11.4, 4.6 Hz, 1H), 3.54 (dd, J = 8.4, 6.1 Hz, 1H), 3.37 (s, 1H), 3.18 (s, 1H), 2.03 (s, 1H), 1.97 (s, 3H), 1.28−1.16 (m, 1H), 1.21 (d, J = 7.2 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 169.5, 138.5, 133.3, 132.8, 115.9, 83.1, 82.7, 67.3, 48.5, 17.5, 13.8; HRMS (EI) calcd for $C_{11}H_{15}NO_2$ 194.1181, found 194.1178.

Preparation of Ethyl (*E*)-2-Methyl-2-hepten-6-ynoate (24). Pyridinium dichromate (0.971 g, 4.50 mmol) was added at 0 °C to a stirred solution of 4-pentyn-1-ol (0.252, 3.0 mmol), sodium acetate (0.737 g, 9.0 mmol), and 4 Å sieves (0.367 g). The mixture was warmed to room temperature and allowed to stir for 5 h. The solution was then filtered through silica gel with ether and concentrated to give crude 4-pentyn-1-al: R_f = 0.58 (EtOAc/hexane, 1:4); ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.8 (s, 1H, CHO). The crude aldehyde was dissolved in toluene (15 mL), (carbethoxyethylidene)triphenylphosphorane (1.50 g, 4.14 mmol) was added, and the reaction mixture was heated to 90 °C for 22 h. The solution was filtered through silica gel (Et_2O/pentane, 1:4) and concentrated to give the crude vinyl ester. ^{1}H NMR showed *E*/*Z* selectivity >20:1 (200 MHz, $CDCl_3$): δ 6.75 (t, J = 7.1 Hz, (*E*)-vinyl-H), 6.0 (t, J = 8.0 Hz, (*Z*)-vinyl-H). Purification of the crude mixture via radial chromatography (Et_2O/pentane, 1:19) gave the (*E*)-vinyl ester as a clear oil (0.226 g, 54% yield): R_f = 0.60 (EtOAc/hexane, 1:4); IR (neat) 3300, 1712, 1652 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.75 (t, J = 7.1 Hz, 1H), 4.15 (q, J = 7.0 Hz, 2H), 2.45−2.20 (m, 4H), 1.96 (t, J = 2.5 Hz, 1H), 1.83 (s, 3H), 1.24 (t, J = 7.0 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.3, 139.8, 129.7, 83.6, 69.5, 60.9, 28.2, 18.2, 14.7, 13.0; MS (CI) 167 (M + H, 100%). Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 71.88; H, 8.83.

Preparation of (*S*)-(−)-*N*-(2′-Hydroxy-1′-methylethyl)-(*E*)-2-methyl-2-hepten-6-ynamide (26). Ethyl (*E*)-2-methyl-2-hepten-6-ynoate (0.173 g, 1.04 mmol) was added to a 5 mL THF/MeOH/H_2O (3:1:1) solution. The mixture was cooled to 0 °C and LiOH (0.050 g, 2.08 mmol) was added followed by stirring at room temperature for 20 h. An additional amount of LiOH (0.050 g, 2.08 mmol) was added, and stirring of the reaction was continued for an additional 4 h, upon which TLC showed disappearance of the ester. The solution was filtered through silica gel (EtOAc/MeOH, 1:4) to give the yellow acid residue. The crude acid was then dissolved in CH_2Cl_2 (15 mL). PyBrop (0.826 g, 1.77 mmol) and (*S*)-(+)-2-amino-1-propanol (0.222 g, 2.95 mmol) were sequentually added followed by addition of DIEA (0.77 mL, 4.4 mmol) at 0 °C. The reaction mixture was stirred for 12 h at room temperature. The solution was filtered through silica gel (EtOAc) and purified via radial chromatography (gradient elution, EtOAc/hexane, 2:1, to 100% EtOAc), giving a yellow-white solid (0.228 g, 92%): R_f

= 0.263 (EtOAc); $[\alpha]_D^{23} = -4.08°$ (*c* 3.56 × 10^{-2}, EtOAc); IR (neat) 3297, 2877, 2117, 1661, 1617 cm^{-1}; ^1H NMR (200 MHz, $CDCL_3$) δ = 6.35 (t, *J* = 6.4 Hz, 1H), 6.0 (br d, 1H), 4.15−4.0 (m, 1H), 3.71−3.64 (dd, *J* = 11.0, 3.75 Hz, 1H), 3.59−3.50 (dd, *J* = 11.0, 6.0 Hz, 1H), 3.18 (br s, 1H), 2.41−2.26 (m, 4H), 1.97 (t, *J* = 2.4 Hz, 1H), 1.85 (s, 3H), 1.2 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.4, 134.4, 132.6, 83.8, 69.6, 67.5, 48.4, 27.9, 18.4, 17.6, 13.4; MS (CI) *m*/*z* 196 (M + H, 100%). Anal. Calcd for $C_{11}H_{17}O_2N$: C, 67.66; H, 8.78. Found: C, 67.31; H, 8.92.

Preparation of (1′*S*,2*E*,4*E*,8*E*,10*E*,12*R*,13*R*,14*E*,16*S*)-(−)-13-Hydroxy-*N*-(2′-hydroxy-1′-methylethyl)-2,10,12,14,16-pentamethyl-18-phenyl-2,4,8,10,14-octadecapentaen-6-ynamide (DHS, 2). To a flask containing (1*E*,3*E*,4*S*,5*R*,7*E*,8*S*)-5-hydroxy-1-iodo-10-phenyl-3,5,7,9-tetramethylundeca-1,3,7-triene **(17)** (5.0 mg, 0.0117 mmol) at −20 °C was added alkyne **22** (3.9 mg, 0.020 mmol) as an ethyl acetate solution (1.0 mL). $(PPh_3)PdCl_2$ (0.5 mg, 0.7 × 10^{-3} mmol), CuI (0.8 mg, 4.1 × 10^{-3} mmol), and *i*-Pr_2NH (0.06 mL, 0.2M) were subsequently added. The reaction mixture was protected from light, warmed to room temperature, and allowed to stir for 30 min, whereupon TLC showed disappearance of the starting alkyne. The reaction mixture was placed directly by pipet onto a 20 × 20 cm analytical TLC plate and eluted with 1:5 hexane/EtOAc in a TLC chamber. After eight elution/drying sequences, the appropriate band was scraped off the plate, and the scrapings were rinsed with 10 mL of ethyl acetate through a paper filter. Concentration of the ethyl acetate gave 3.4 mg (59%) of the title product as a yellow solid: $[\alpha]_D = 22.9°$ (*c* = 0.0017, $CHCl_3$); R_f = 0.32 in 100% EtOAc; IR (neat) 3377, 3029, 2955, 2924, 1638, 1605, 1524, 1451, 1382 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.30−7.23 (m, 2H), 7.21−7.11 (m, 3H), 6.97 (d, J = 11.5 Hz, 1H), 6.82 (dd, J = 15.6, 15.1 Hz, 1H), 6.70 (d, J = 17.0 Hz, 1H), 6.04 (d, J = 16.0 Hz, 1H), 5.92 (d, 1H), 5.71 (d, J = 16.0 Hz, 1H), 5.52 (d, J = 10.1 Hz, 1H), 5.23 (d, J = 9.6 Hz, 1H), 4.16 (m, 1H), 3.76−3.69 (m, 3H), 3.59 (dd, J = 11.1, 6.0 Hz, 1H), 2.78−2.70 (m, 1H), 2.63−2.41 (m, 3H), 2.00 (s, 3H), 1.82 (s, 3H), 1.71−1.48 (m, 4H), 1.62 (s, 3H), 1.23 (d, J = 6.9 Hz, 3H), 0.99 (d, J = 6.0 Hz, 3H), 0.91 (d, J = 6.9 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 169.7, 147.4, 143.2, 139.5, 136.0, 135.5, 134.4, 133.7, 131.7, 128.9, 128.7, 126.1, 117.8, 106.2, 95.9, 91.2, 82.8, 77.7, 67.9, 48.6, 39.6, 37.6, 34.4, 32.3, 21.5, 17.9, 17.6, 13.7, 12.9, 11.9; FAB HRMS (DTT) calcd for $C_{32}H_{43}NO_3$ 490.3321, found 490.3331.

Preparation of (1′*S*,2*E*,8*E*,10*R*,11*R*,12*E*)-11-Hydroxy-*N*-(2′-hydroxy-1′-methylethyl)-15-phenyl-2,11,13-trimethylpentadeca-2,8,12-trien-6-yneamide (3). To a flask containing (1*E*,3*S*,4*R*,5*E*)-4-hydroxy-1-iodo-8-phenyl-3,5-dimethylocta-1,5-diene (**35**) (11.1 mg, 0.031 mmol) at −20 °C was added alkyne **26** (9.1 mg, 0.047 mmol) as an ethyl acetate solution (1.0 mL). $(PPh_3)PdCl_2$ (1.7 mg, 2.4 × 10^{-3} mmol), CuI (0.6 mg, 3.1 × 10^{-3} mmol), and *i*-Pr_2NH (0.16 mL, 0.2M) were subsequently added. The reaction mixture was protected from light, warmed to room temperature, and allowed to stir for 1 h, whereupon TLC showed disappearance of the starting alkyne. The solution was quenched with 0.25 mL of saturated NH_4Cl, filtered through a silica plug (ca. 7 g) using 1:20 MeOH/EtOAc, and concentrated to give the crude material. Purification via radial chromatography (100%) gave 11.5 mg (87%) of the title compound as an oil: $[\alpha]_D = -10.0°$ (*c* = 0.010, $CHCl_3$); R_f = 0.32 in EtOAc; IR (neat) 3355, 3025, 2969, 2925, 1659, 1617, 1530, 1450 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.29−7.22 (m, 2H), 7.20−7.18 (m, 3H), 6.35 (t, J = 6.9 Hz, 1H), 6.00 (dd, J = 15.5, 8.5 Hz, 1H), 5.94 (d, 1H), 5.54 (d, J = 16.4 Hz, 1H), 5.42 (t, J = 7.1 Hz, 1H), 4.16−4.08 (m, 1H), 3.73−3.67 (m, 1H), 3.64 (d, J = 8.6 Hz, 1H), 3.59−3.53 (m, 1H), 3.10 (s, 1H), 2.73−2.61 (m, 2H), 2.44−2.28 (m, 8H), 1.86 (s, 3H), 1.54 (s, 3H), 1.21 (d, J = 7.3 Hz, 3H), 0.81 (d, J = 6.9 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.5, 146.3, 142.4, 136.1, 134.6, 132.6, 128.9, 128.8, 126.3, 111.5, 88.7, 82.2, 80.2, 67.7, 48.4, 41.7, 36.1, 30.0, 28.1, 19.4, 17.6, 17.2, 13.4, 11.4; HRMS (CI) calcd for $C_{27}H_{37}NO_3$ 424.2852, found 424.2834.

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Supporting Information Available: Experimental details, NMR spectra, and MDR protocols for selected compounds (26 pages). See any current masthead page for ordering and Internet access instructions.

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