The Synthesis and Evaluation of a Solution Phase Indexed Combinatorial Library of Non-Natural Polyenes for Reversal of P-Glycoprotein Mediated Multidrug Resistance

Merritt B. Andrus,*,† Timothy M. Turner,† Zuben E. Sauna,‡ and Suresh V. Ambudkar‡

Brigham Young University, Department of Chemistry and Biochemistry, C100 BNSN, Provo, Utah 84602-5700, and National Cancer Institute, NIH, Laboratory of Cell Biology, Building 37, Room 1B-17 37 Convent Drive, MSC4255, Bethesda, Maryland 20878-4255

mbandrus@chemdept.byu.edu

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A combinatorial library of polyenes, based on (−)-stipiamide, has been constructed and evaluated for the discovery of new multidrug resistance reversal agents. A palladium coupling was used to react each individual vinyl iodide with a mixture of the seven acetylenes at near 1:1 stoichiometry. The coupling was also used to react each individual acetylene with the mixture of six vinyl iodides to create 13 pools indexed in two dimensions for a total of 42 compounds. Individual compounds were detected at equimolar concentration. The vinyl iodides, made initially using a crotylborane addition to generate the anti1,2-hydroxylmethyl products, were now made using a more efficient norephedrine propionate boron enolate aldol reaction. The indexed approach, ideally suited for cellular assays that involve membrane-bound targets, allowed for the rapid identification of reversal agents using assays with drug-resistant human breast cancer MCF7-adrR cells. Intersections of potent pools identified new compounds with promising activity. Aryl dimension pools showed R = ph and naphthyl as the most potent. The acetylene dimension had R′ = phenylalaninol and alaninol as the most potent. Isolated individual compounds, both active and nonpotent, were assayed to confirm the library results. The most potent new compound was **4ek** (R = naphthyl, R′ = phenylaninol) at 1.45 μM. Other nonnatural individual naphthyl-amide compounds showed potent MDR reversal including the morpholino-amide **4ej** (1.69 μM). Synergistic activities attributed to the two ends of the molecule were also identified. Direct interaction with Pgp was established by ATPase and photoaffinity displacement assays. The results indicate that both ends of the polyene reversal agent are involved in Pgp interaction and can be further modified for increased potency.

While small molecule libraries have gained great prominence as a means of identifying new inhibitors for biological targets,[1] unified approaches that allow for efficient synthesis, direct screening, and facile individual member identification are still needed. Solid-phase libraries that provide for synthetic efficiency are more amenable to screening with isolable, gobular receptors and are generally not applicable to cell assays where membrane-bound targets are involved.[2] In contrast to the split-pool peptide approach, solid-phase small molecule libraries have been more focused on spatially addressed, serial methods. Solution-phase libraries[3] offer the advantages of being able to use standard reagents, no linker or support, and the ability to directly screen with isolated receptors or cellular assays.[4] Natural product-like libraries, while more constrained with less total numbers compared to peptides and nucleotides, are inherently more diverse in that a great variety of functionality can be explored within a core template.[5] We now provide a full report of a solution-phase library,[6] based on the multidrug resistance (MDR) reversing polyene (−)-stipiamide, that consists of mixtures indexed in two dimensions that allows for efficient combinatorial synthesis, direct screening with a cellular assay, together with the isolation and testing of individual compounds.

Recently we showed that synthetic (−)-stipiamide **1**[7] had only moderate reversal activity with colchicine resistant cells,[8] while 6,7-dehydrostipiamide (DHS) **2** (ED_{50} 6.5 μM) and the truncated compound **3** (4 μM) were potent using a variety of drugs with resistant MCF7-adrR (human breast cancer) cells that express Pgp (P-glycoprotein), the membrane-bound small molecule MDR pump (Chart 1).[9] Importantly, **2** and **3** were far less toxic (4 and 14μM) compared to stipiamide **1** (0.01nM). Importantly, **3** was further shown to directly bind Pgp by monitoring ATPase activity (10 μm max. stimulation)[10] and through direct displacement of a known photoaffinity label iodoarylazidoprazosin (12 μm).[10] Having explored

† Brigham Young University.
‡ NIH.

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Chart 1

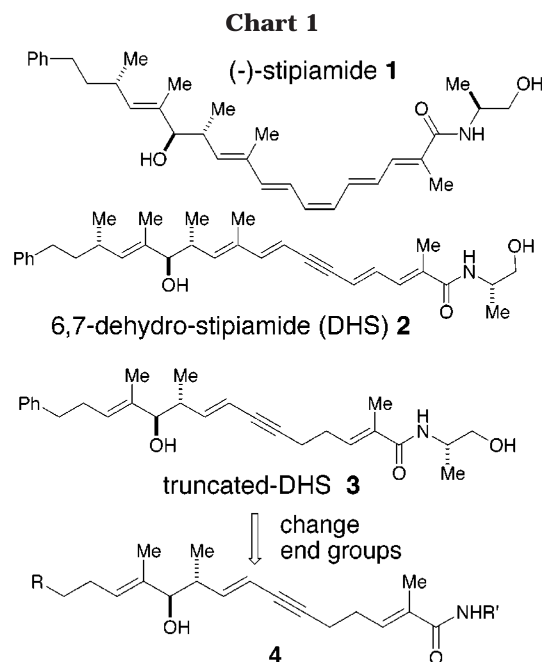

Chart 2

the length and conjugation as functions of MDR reversal efficacy, our attention next turned to perturbing the end groups of DHS **3** to improve binding to Pgp. Selection of end groups for Pgp mediated MDR modulation is difficult for several reasons. The structural features for small molecule binding to Pgp are poorly understood since no crystal structure for the protein has yet been reported. Indirect methods, such as site directed mutagenesis studies, have indicated that small molecule binding may occur at several domains on along the protein. In particular, the regions of transmembrane 5–6 and 11–12 have been implicated in the binding of several modulators.[11] Additionally, it is believed that Pgp may contain additional binding sites, thus complicating the situation.[12] Since highly refined three dimension data regarding the structure of Pgp is not available, it is not entirely clear at this time how these binding regions interact with one another.[13]

While solution phase libraries have centered on high-throughput parallel synthesis and evaluation, there are a few reports of solution phase libraries generated as mixtures. Rebek generated amide diversity around the periphery of a core dibenzopyran template to screen for trypsin inhibitors.[14] Smith and Pirrung utilized coupling reactions to generate linear amides with diversity present at the terminal positions to evaluate binding with the NK3 receptor and acetylcholinesterase inhibition.[15] Both approaches used a two-dimensional indexing strategy to evaluate the libraries with the receptor. Standard heteroatom coupling procedures formed the mixtures in reasonable yield and purity. Prior to our work a carbon–carbon bond coupling had not been explored as the diversity-generating step of a solution-phase library.

Exploration of the effect of the two end groups R and R′ of **4** was thought to be ideally suited to this 2D indexing approach (Chart 2). By reacting vinyliodides **5** with alkynyl amides **6** the library was to be constructed. The new compounds formed would contain both elements derived from the reacting ligands. Both R and R′ are basis sets which possess the various structural features to be explored. The reacting ligands were coupled in such a way to produce mixtures of compounds having a specific group at one terminus while being diversified at the other. These pools of compounds were assayed to give an average potency on either the *x*- or *y*-axis at the position pertaining to the end group that was held constant. Once evaluated, the intersection points of the most potent pools determined the optimal end groups for potent activity. It was anticipated that synergistic or antagonistic effects involving a particular combination of compounds might make it difficult to effectively determine optimal compounds. However, the chance of finding potent new compounds is significantly increased in that each compound is represented twice in two entirely different

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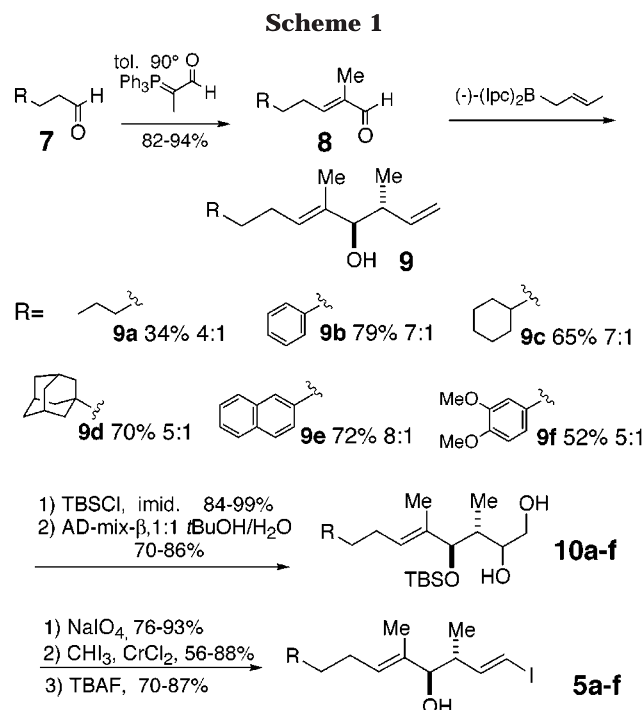
Scheme 1



mixtures in opposite dimensions. An additional concern was the requirement to achieve equimolar representation of each member of a pool. The centrally located coupling site remote from the ends was thought to ensure comparable reaction rates to provide adequate representation of each compound. The ready identification, isolation, and assay results of numerous individual compounds now addresses this concern verifying the results from the pools. The efficiency of this library is realized in that only 13 reactions and assays are required to screen 42 compounds. This represents an efficiency of 3.2 over a serial approach. End groups were selected to provide a range of steric and electronic variation. The large nonpolar adamantyl groups as well as the polar dimethoxyphenyl and bis-hydroxyethyl amides were convenient and typical. Nonpolar aromatic functionality on the left side was expected to be potent while other substituents, as with removal of the amide hydroxyl group, were expected to be ineffective. The library was restricted to 6 × 7 with 42 compounds to allow for rapid characterization of the mixtures and easy separation of individual members.

The first objective was to synthesize the coupling precursors **5** and **6**. Six vinyl iodides **5a**−**f** were prepared, each containing a different end group remote from the coupling site (Scheme 1). The starting materials were the aldehydes **7a**−**f** obtained commercially or formed using standard protocols.[16] The aldehydes **7a**−**f** were subjected to Wittig olefination to form the *E*- α,β-unsaturated aldehydes **8a**−**f**.[17] *E*-Crotylborane addition to the aldehydes using Brown's reagent derived from (−)-pinene gave the *homo*-allylic alcohols **9a**−**f** in modest yields (34−76%).[18] Low yields in some cases were attributed to the difficulty in separating the desired alcohols from the pinenol byproduct. Although the *anti: syn* selectivity was low in some cases (4:1), the unwanted syn isomer could be removed at a later stage of the synthetic sequence. Protection of alcohols **9a**−**f** followed by selective dihydroxylation of the terminal olefin gave the 1,2,-diols **10a**−**f**.[19] Analogous with the synthesis of 4,5-dehydrostipiamide, the TBS ether blocks dihydroxylation from occurring at the internal olefin.[20] The diols were cleaved with sodium periodate to afford aldehydes which were converted to the *E*-vinyl iodides using Takai reaction conditions.[21] Finally, removal of the silyl protecting group gave the desired vinyl iodides **5a**−**f**.

Although this route proved quite useful, a plan was underway to generate the *anti*-hydroxy-methyl stereocenters in a more stereoselective manner. While numerous alternatives abound for syn stereocontrol, only a limited number of reliable methods for anti selective additions to enals are known.[22] Previous attempts to generate this anti intermediate in the route to stipiamide using Heathcock's modification[23] of the Evan's aldol[24] procedure resulted in poor yield and selectivity. Recently Masamune has developed a norephedrine based chiral propionate that generates the *anti*-aldol product in high selectivity with a variety aldehydes including enals.[25] Following this protocol addition of α,β-unsaturated aldehyde **11e** to the boron enolate of the propionate auxiliary afforded the *anti*-aldol product with 14:1 diastereoselectivity (Scheme 2).[26] No *syn*-aldol products were observed. Protection with TBS-OTf gave **13** in 77% overall yield for the aldol and protection steps. At this point, concern was focused on removal of the bulky ester auxiliary. $LiEt_3BH$, LAH and DIBAL afforded alcohol in low yield. Prolonged reaction times using these reagents

(16) Aldehyde **7a** was commercially available. **7b** and **7c** were prepared via PCC oxidation of 3-phenylpropanol and 3-cyclohexylpropanol, respectively. **7d** and **7e** originated from 1-adamantylaldehyde and 2-naphthaldehyde. Both of these aldehydes were treated with triethylphosphonoacetate to form the *E*-α,β unsaturated esters. Subsequent hydrogenation (Pd/C) followed by DIBAL reduction afforded aldehydes **7d** and **7e**. **7f** was made from 3,4-dimethoxypropanoic acid. Methylation (MeI, K_2CO_3) followed by DIBAL reduction afforded the desired aldehyde.

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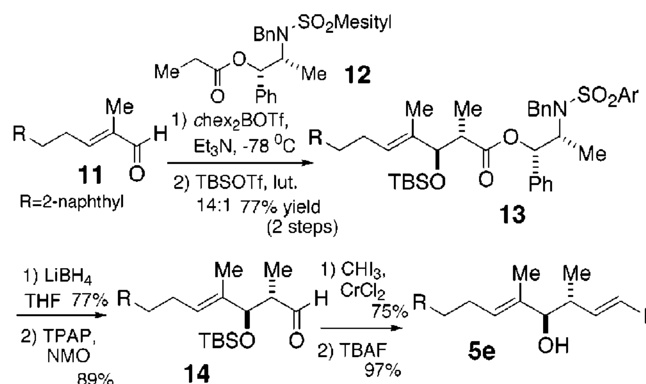
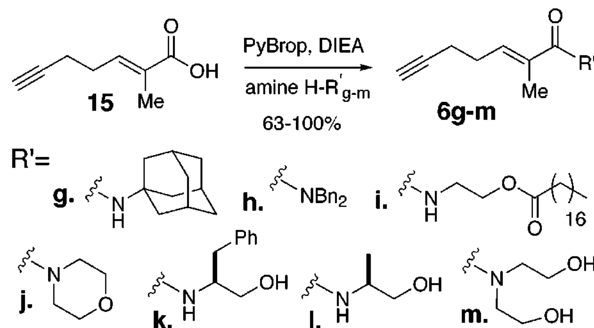
(26) Aldehydes $\mathbf{11_a}$ and $\mathbf{11_b}$ gave similar diastereoselectivities (10−13:1) for the *anti*-aldol product as determined by ^{1}H NMR and HPLC analysis. No *syn*-aldol products were observed. The diastereomerics were readily separated by flash chromatography.

Scheme 2





Scheme 3

Table 1. 6 × 7 Indexed Library Synthesis and MDR Activity

	R	R′	yield[a] (%)	ED50[b] (μM)	STD[c]
left side constant	a	g−m	90	6.6	0.9
	b	g−m	100	3.5	0.3
	c	g−m	100	9.7	1.5
	d	g−m	100	16	5
	e	g−m	89	1.3	0.1
	f	g−m	100	9.4	1.2
right side constant	a−f	g	99	23	7
	a−f	h	64	12	2
	a−f	i	90	>70	
	a−f	j	71	2.3	0.9
	a−f	k	60	1.6	0.1
	a−f	l	87	0.9	0.1
	a−f	m	42	1.8	0.2

[a] Following silica gel filtration, TLC, NMR, MS, and HPLC identified individual compounds. [b] MCF7-adrR cells and 37 nM adriamycin. [c] Standard deviation.

often lead to removal of the TBS ether. Gratifyingly, use of $LiBH_4$ in THF at 90 °C for 9 h gave the desired product in 77% yield along with the recovered auxiliary (92%). The stereocenters were maintained in both the product and recovered auxiliary as determined by [1]NMR and optical rotation analysis. Oxidation to the aldehyde **14** and ensuing Takai reaction and removal of the TBS group afforded the desired iodide **5e**. Although application of the improved synthetic route shown concerns the naphthyl vinyl iodide, the other aldehydes, **11a**−**d**,**f** work equally well.

The synthesis of the alkynyl amides started with α,β-unsaturated acid **15** derived from 4-pentyn-1-ol (Scheme 3). Coupling separately to the seven amines using PyBrop afforded **6g**−**m**.[27] Primary and secondary amines varying in ring structure as well as degree of polarity were selected. Lipophilic moieties such as the dibenzyl **6h** and ethyl-stearate **6i** were expected to partition into the lipid-bilayer region adjacent to Pgp. Other more polar amides were anticipated to bind more tightly to Pgp. The widely varying yields (63−100%) with the various amines and rates confirmed the decision not to use this reaction as the key step for the library.

Iodides **5a**−**f** were coupled with the acetylenes **6g**−**m** to give enynes **4** in an indexed manner using the optimized Sonogashira coupling conditions (Table 1).[28] This reaction proved ideal with short reaction times and tolerance of the wide array of unprotected functionality on the two ends. Each of the six left-hand iodides **5a**−**f** were reacted individually with a mixture of the seven acetylenes **6g**−**m** with $(Ph_3P)_2PdCl_2$ (5 mol %), CuI (17 mol %) at low temperature (−20 °C) in ethyl acetate at 1:1.4 stoichiometry with 0.2 equiv of each acetylene present in the mixture. This new set of conditions for this reaction proved critical to the success of the library.[7b] High yields were only obtained using ethyl acetate as solvent and starting the reaction at −20 °C. Use of benzene or THF as solvents resulted in low yields. The scale (~100 mg) allowed for multiple MDR assays (1−2 mg/run) and for subsequent isolation and testing of selected individual compounds. The yields, following filtration through silica gel to remove impurities, were quantitative in many cases. The pools were purified using radial chromatography with aggregate collection of the UV-active enynes discarding higher and lower moving impurities. Yields for the mixtures ranged from 42 to 100% and were based on an average molecular weight for the set of compounds within the respective pools. Individual compounds, in both high and lower yielding reactions, were shown by TLC, NMR, and HPLC to be present in approximately equal amounts in the pools. Uniform coupling rates were essential to ensure that each compound would have an effect in the assay. The reactions resulted in six pools, constituting the left-hand dimension of the library, where (R) was homogeneous within each pool. Conversely, seven pools homogeneous for the amide end group were made by reacting each of the individual acetylenes **6g**−**m** with a mixture of the six vinyliodides to generate the right-hand dimension of the library. In total, 13 couplings resulted in the formation of 42 compounds indexed in two dimensions. It is important to note that each individual compound appears in two distinct pools and is never found with another compound twice.

Characterization of the library mixtures for content and purity included TLC, FAB-MS, HPLC, and ^{1}H and ^{13}C NMR. Not all tools gave full characterization for all the library mixtures. Reverse phase HPLC worked nicely to resolve all six compounds for the morpholino pool **4a**−**f**,**j**.[29] This trace indicated that the desired enynes were present at approximately 97% purity. Aside from the naphthyl peak due to a greater response factor, the

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(29) See the Supporting Information.

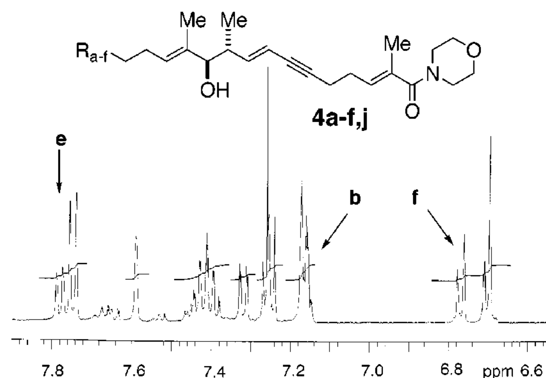


Figure 1. Selected aromatic portion of ^{1}H NMR (300 MHz) of morpholino pool **4a**−**f**,**j** shows compounds present in equimolar ratio as seen by integration of the ortho hydrogens.

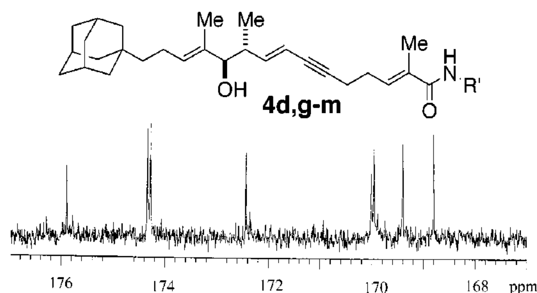


Figure 2. Carbonyl region of the ^{13}C NMR spectrum for the adamantyl pool **4d**,**g**−**m** shows seven compounds present. The eighth signal is due to the sterate ester in **4d**,**i**.

remaining compounds, are clearly present in roughly equimolar quantities. FAB-MS also allowed for identification of the compounds present within the library pools.[29] Direct injection of the library mixture and subsequent high-resolution mass spectrometry (HRMS) performed on the identified parent ion peaks allowed for determination of the mass of the enynes **4a**−**f**,**j**. This method was particularly advantageous in that it was applicable for most of the library pools.

Since the mixtures contained a relatively small number of molecules (6 or 7), ^{1}H and ^{13}C was useful not only for identifying the compounds present but also for determining their relative molar ratio. For example, ^{1}H NMR (6.7−7.8 ppm) of mixture **4a**−**f**,**j** identified three resolvable splitting patterns for the compounds R_e−R'_j, R_b−R'_j, and R_f−R'_j (Figure 1). Integration of the three sets of peaks revealed a molar ratio of 0.99:1.0:0.97. Again it was found that the coupling reaction occurred at equal rates among the various coupling partners. ^{13}C NMR was most useful when examining the carbonyl region. Using the same morpholino pool, **4a**−**f**,**j**, one notices that the diversity is present at a remote site from the carbonyl. Thus, only one peak was observed in the carbonyl region (172.5 ppm). However, in the case of the left-side adamantyl pool **4d**,**g**−**m**, the diversity is now adjacent to the carbonyl region (Figure 2). The resulting spectrum displayed seven different carbonyl peaks. An additional carbonyl peak was also observed which was attributed to the ester present in the stearate compound **4di**.

The 13 pools were assayed for MDR reversal efficacy using adriamycin resistant MCF7-adrR human breast cancer cells with 37 nM adriamycin. ED_{50} MDR reversal concentrations were determined for the mixtures, the values were then placed on the respective R and R′ dimensions of a 3D graph (Table 1 and Figure 3). As illustrated, pools on the R dimension, blue bars, having a naphthyl (**4e**) or phenyl (**4b**) group in common demonstrated the most potent MDR reversal (3.5 and 1.3 μM). In contrast, mixture having adamantyl (**4d**) group held constant showed the least potent ED_{50} value (16 μM). Along the R′ axis, colored bars to the rear, pools with hydroxylamine (**4k**−**m**) groups along with the morpholine (**4j**) group proved to be the most effective modulators (2.3−0.9 μM). The nonpotent mixtures of the series included those containing the adamantyl (**4g**) and ethyl stearate (**4i**) groups at 23 and >70 μM, respectively.

Table 2. MDR Activity of Individual Isolated Compounds 4

R(left)	R′(right)	ED_{50}[a] (μM)	STD[b]
b, Ph	**l**, ala-OH	1.85	0.04
b	**m**, bis-EtOH	8.8	0.1
c, *c*-hexyl	**g**, adamantyl	12	2
c	**h**, Bn_2	12	3
c	**l**, ala-OH	3.1	0.6
d, adamantyl	**h**, Bn_2	>70	
d	**i**, ethyl stearate	>70	
d	**l**, ala-OH	2.6	0.5
e, 2-naphthyl	**h**, Bn_2	6.6	1.1
e	**i**, ethyl stearate	46	11
e	**j**, morpholino	1.69	0.02
e	**k**, phe-OH	1.45	0.08
e	**l**, ala-OH	1.73	0.05
e	**m**, bis-EtOH	1.48	0.02

[a] MCF7-adrR cells and 37 nM adriamycin. [b] Standard deviation.

From the ED_{50} values obtained from the pools, one can then predict which individual compounds from the library are most potent. The individual compound most likely to be the most potent is the enyne **4e**,**l** (naphthyl-alaninol), which has the most potent groups in both dimensions. Similarly, the most nonpotent of the compounds should be **4d**,**i** (adamantyl-ethyl stearate). To validate the viability of the approach, a number of individual compounds were separated from the library mixtures and tested individually for reversal efficacy in the same MCF7adrR assay. Although HPLC was considered, it was found that radial chromatography was most convenient for compound isolation. Based on the observation of the pool potencies, selected compounds were isolated and evaluated that were predicted to give a wide range of potencies. The ED_{50} of each compound was plotted on the 3D graph at the intersections of the R and R′ groups which reflect the compound (Table 2, Figure 3). The interior portion of the graph, colored bars, now shows the ED_{50} values for selected individual compounds.

The **4e**,**k**-naphthyl-phenylalaninol compound was found to be the most potent (1.45 μM), while **4d**,**h**-adamantyl-dibenzyl and **4d**,**i**-adamantyl-ethyl stearate were least active (>70 μM). The discrepancy for predicting the most potent group on the R′ dimension can be explained on the basis that the four most potent pools (**4e**,**j**−**m**) of the library have very similar potencies. As shown (Table 1), within two standard deviations, these four potencies overlap one another. Thus, the library revealed a number of potent compounds of similar MDR modulating activity. This finding is reasonable when considering that several

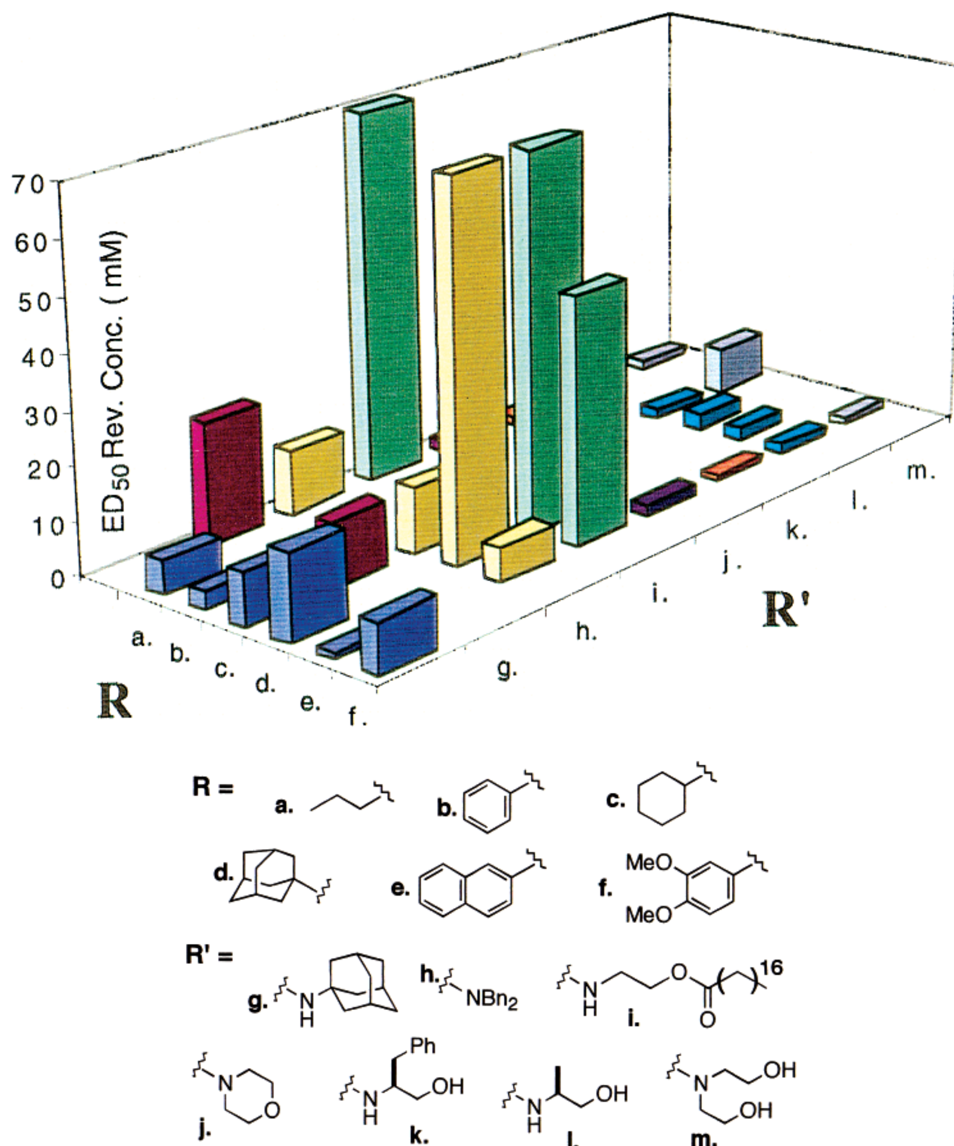
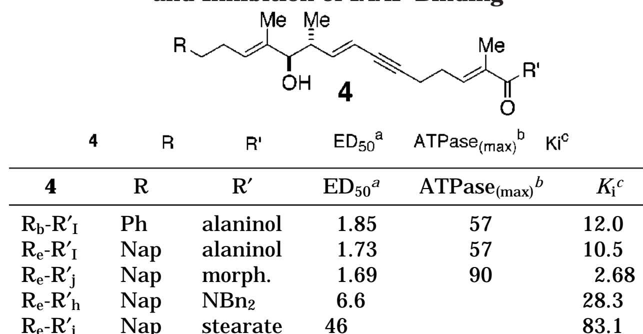


Figure 3. Bargraph of 6 × 7 pool MDR reversal results, R = a−f left-side axis (blue bars) and R′ = g−m right-side axis (colored bars to the rear). Individual isolated compounds are also shown in the off-axis interior region (colored bars).

hydroxy amines were chosen to comprise part of the right-side library R′ basis set. It is expected that a more functionally diverse set would lead to larger difference in potencies between the chosen groups. It is interesting to note that the most active pool, **4a**−**f**,**l** (0.9 μM) does not contain the most active compound **4e**,**k** (1.45 μM). The alaninol pool is five standard deviations removed from the phenylalaninol pool. Drug−drug interactions may be the cause of the discrepancy, especially with compounds of similar potency and a receptor with multiple binding sites.

Upon close examination of the 3D ED_{50} profile, a distinct division between potent and nonpotent individual compounds is seen. The left half of the graph containing R'_{g-i} possesses the less potent enynes while the right half R'_{j-m} composes the potent region. Most interesting, the presence of a potent group on either the left or right end of the molecule can dramatically affect the activity regardless of the functional group placed at the other terminal site. For example, **4d**−**h**,**i** (adamantyl- dibenzyl, ethyl stearate) were both found inactive (>70 μM) while **4d**,**l** (adamantyl-alaninol) gave a potent activity of 2.6 μM. Thus, even though the adamantyl group in the R dimension is the least potent of the series, the addition of the alaninol amine to the right-hand part of the molecule produced a potent modulator. Alternatively, **4e**,**h** (naphthyl- dibenzyl) with a poor group on the right was moderately active at 6.6 uM while the above-mentioned **4d**,**h** (adamantyl-dibenzyl), with both groups poor, was not. It is interesting to note that from the left-hand pool results for **4b**, **4c**, and **4d**, it is difficult to predict the relative high potency of the individual compound with alaninol on the right, **4b**,**l**, **4c**,**l**, and **4d**,**l**. Without isolating these compounds, the library results may have missed these compounds. Yet the primary goals of the approach of finding and varifying potent compounds have been met, even while some hits may have been missed. The most potent compound **4e**,**k** was identified as being present in the most potent left-hand pool and the phenylalaninol component was shown to be very similar to the most potent right-hand alaninol pool. A somewhat surprising result is the potency of the morpholino pool (2.3 μM) and the individual compound **4e**,**j**. All others that lack a hydrogen bond donor were

Table 3. Comparison of MDR Reversal, ATPase Activity, and Inhibition of IAAP Binding



4	R	R′	ED_{50}[a]	$ATPase_{(max)}$[b]	K_i[c]
R_b-R'_I	Ph	alaninol	1.85	57	12.0
R_e-R'_I	Nap	alaninol	1.73	57	10.5
R_e-R'_j	Nap	morph.	1.69	90	2.68
R_e-R'_h	Nap	NBn_2	6.6		28.3
R_e-R'_i	Nap	stearate	46		83.1

[a] MDR reversal concentration (μM), adriamycin present at 37 nm. [b] Maximum stimulation of ATPasa (nmol Pi/min/mg protein). Values are not normalized for Pgp concentration in membrane. [c] K_i (μM) for the competition of ^{125}IAAP binding to Pgp by the test compound.

far less potent. While the approach does not unambiguously identify the least potent compound, a wide range of compounds, **4d** (adamantyl) and **4h** (dibenzyl) in particular, can be safely disregarded as being nonpotent.

If the end groups were truly independent from each other, we would expect to see a close correlation between the potencies of the pools and the individual compounds. As discussed, this seems to be the case. However, there are some notable exceptions. For example, **4d**,**h** (adamantyl, dibenzyl) is inactive (>70 μM) while the pools corresponding to both of the subgroups R_d−R'_{g-m} and R_{a-f}−R'_h gave activities of 23 and 12 μM, respectively. The addition of two nonpolar groups to the enyne dramatically lowered reversal ability. Synergistic activities attributed by the two ends are also seen in the alaninol amides **4d**,**l** (2.6 μM) and naphthyl-stearate **4e**,**i** (46 μM). The more potent alaninol on the right can override the poor binding of the left-side adamantyl group pool (16 μM) and the naphthyl group can overcome to some extent the poor stearate amide activity (>70 μM). These results indicate that both ends of the polyene reversal agent are involved in Pgp interaction. The results indicate that polar endgroups on the right end of the template combined with aromatic left end groups leads to enhanced potency. Conversely, highly nonpolar species on either end will reduce potency.

Compounds isolated from the library were tested for their ability to stimulate ATP hydrolysis[10] as well as displace ^{125}I[IAAP], a photoaffinity analogue of prazosin, a substrate of Pgp (Table 3).[10] The new naphthyl compounds **4**-naphthyl-alaninol and **4**-naphthyl-morpholino had MDR reversal, ATPase stimulation and K_i values which were similar or better than that for truncated-DHS **3**, the original compound (**4b**,**l**). In particular, **4**-naphthyl-morpholino displayed significantly higher ATPase activity above any tested (90 nmolesPi/min/mg protein) and a K_i for inhibition of IAAP binding at a low level of 2.7 μM. In contrast, the dibenzyl or stearate ester left end groups had poor binding characteristics (K_i > 28 μM). The comparison between MDR reversal efficacy and K_i binding show a good linear correlation. When compounds of the naphthyl series were compared (R = naphthyl, R′ = varied), the correlation coefficient was 0.96.[29] The correlation dropped when all isolated compounds from the library had their ED_{50} and K_i values correlated (r^2 = 0.76). Both **4**-naphthyl, dibenzyl and **4**-naphthyl-stearate displayed distinct ATPase profiles which were different from that of the potent modulators. Compounds **4**-naphthyl-alaninol, and several other potent modulators gave sharp $ATPase_{(max)}$ peaks while **4**-naphthyl, dibenzyl and **4**-naphthyl-stearate did not.

It is also interesting to determine what affect might occur by using the enantiomeric form of the reversal agent. (11*S*, 10*R*)-**4**-naphthyl-morpholino (18.2 ED_{50})[30] was an order of magnitude less active than (11*R*, 10*S*)-**4**-naphthyl-morpholino (1.69 ED_{50}) with stereochemistry that corresponds to the natural product (−)-stipiamide. The K_i for inhibition of IAAP binding was also weaker but not to the same degree. This finding is parallel with that of *S*-verapamil that was proven to be seven times more potent than its *R*-counterpart.[31]

The approach has allowed for discovery of new potent MDR modulators in a quick and resourceful manner. It represents a unique example of a solution phase carbon−carbon bond forming library targeted to a challenging membrane bound receptor. The library has provided valuable information regarding structure activity relationships of the truncated stipiamide structural variants. Future libraries and SAR studies involving the template can now be performed and characterized using the information gathered herein to further increase potency.

Experimental Section

General Methods. Air-sensitive reactions were performed under a positive pressure of nitrogen. Air and moisture-sensitive reagents were introduced by syringe or cannula through rubber septa. All reaction solvents were of HPLC grade quality or 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. Starting materials and reagents were purchased from Aldrich, Sigma, or Novabiochem and used without further purification. [^{125}I]-Iodoarylazidoprazosin (2200 Ci/mmol) was obtained from NEN. Purification by flash 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. When filtering through a silica plug, a buchner funnel was loaded with silica gel (diameter x height) topped off with an Ottawa sand layer. TLC analysis was conducted on silica gel 60 F_{254}, 0.25 mm precoated glass plates. All ^{1}H NMR spectra were obtained using either a 200, 300, or 500 MHz spectrometer employing chloroform (7.26 ppm) or TMS (0.0 ppm) as an internal reference. Signals are reported as: m (multiplet), s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet), bd (broad doublet), bt (broad triplet), ABq (AB quartet); coupling constants (*J*) are reported in hertz (Hz). Carbon spectra were obtained at 50, 75, or 125 MHz and referenced against deuterated $CDCl_3$. Infrared spectra were obtained using a Varian FTIR spectrometer. Mass spectra were run by the Brigham Young mass spectrometry facility. Generally, FAB or CI analysis was performed. Optical rotations were obtained using a polarimeter at rt employing the sodium D line. Concentrations are reported in g/100 mL. Combustion analysis was run by M-H-W Laboratories (Pheonix, AZ). Bicinchoninic acid MDR cytotoxicity assays were performed by the Purdue Cancer Research Center employing MCF-7-adrR human breast cancer cells.

(2*E*)-2-Methyl-5-phenyl-2-pentenal. To a stirring solution of 3-phenylpropanal (4.3 g, 32 mmol) in toluene (160 mL, 0.2M) was added 2-(triphenylphosphoranylidene)propionaldehyde (13.7 g, 43 mmol). The solution was then heated at 90 °C for 24 h. After cooling, the toluene solvent was removed *in vacuo*

(30) *ent*-**4ej** was produced in analogous fashion using the enantiomeric form of the norephedrine ester. See supplementary data.

(31) Wilson, W. H.; Jamis-Dow, C.; Bryant G. *J. Clin. Oncol.* **1995**, *13*, 1985 (b) Kayes, S. B. *Br. Cancer* **1993**, *67*, 641.

and the remaining oil was triturated with cold hexanes causing a large fraction of the insoluble triphenylphosphine oxide to precipitate. Following filtration (rinsing with hexanes), the eluent was concentrated to give an oil. Purification was performed using flash chromatography eluting with 250 mL of 5, 10, and 15% EtOAc/hexanes. The product containing fractions were concentrated to give 3.97g (71% yield) of the title compound. ^{1}H NMR shows >20:1 *E*/*Z* selectivity: R_f = 0.45 in 10% EtOAc/hexanes; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 − 7.16 (m, 5 H), 6.51 (t, *J* = 7.3 Hz, 1 H), 2.81 (d, *J* = 7.5 Hz, 2 H), 2.69 (t, *J* = 7.5 Hz, 2 H), 1.68 (s, 3H); ^{13}C (75 MHz, $CDCl_3$) δ 195.5, 153.5, 140.8, 128.8, 128.7, 128.5, 126.5, 34.6, 30.9, 9.4; HRMS CI (M + H) calcd for $C_{12}H_{15}O$ 175.1112, found 175.1101.

(3*R*,4*R*,5*E*)-4-Hydroxy-8-phenyl-3,5-dimethyl-1,5-octadiene 9b. To a stirring −78 °C solution of potassium *tert*-butoxide (5.8 g, 51.7 mmol) in 150 mL of THF was added via cannula *trans*-2-butene (10 mL, ~91 mmol, condensed into a graduated column at −78 °C). To the solution was added via syringe n-butyllithium (2.5 M, 20.7 mL, 51.7 mmol) as a hexane solution. The mixture was allowed to stir for 15 min and (-)-Ipc_2BOMe (diisopinylcampheylmethoxyborane derived from (-)-α-pinene) was slowly added by syringe (2 M, 25.9 mL, 51.7 mmol) as a solution in ethyl ether. The solution was stirred for 30 min and borontrifluoride etherate was slowly added (8.6 mL, 70.0 mmol) by syringe. The mixture was stirred for 5 min followed by the addition of (*2E*)-2-methyl-5-phenyl-2-pentenal (4.24 g, 24.3 mmol) as an ether solution (5 mL) with two 1 mL ether washings. After 15 h at −78 °C, 50 mL of 3 N NaOH was added followed by 20 mL of 30% H_2O_2. The biphasic mixture was then allowed to warm to rt and stir for 3 h. The mixture was diluted with ether (15 mL) and washed with brine. The separated aqueous layer was then washed twice with methylene chloride (2 × 30 mL) and the combined organic layers were dried over $MgSO_4$. The solution was then concentrated and flash chromatographed eluting with 250 mL of 7, 9, 11, and 15% EtOAc/hexanes. The product containing fractions were concentrated to give 4.4 g (79% yield) of the title product as a clear oil: ^{1}H NMR showed the product to be an approximate 7:1 inseparable mixture of diastereomers: $[\alpha]_D$ +21.2 (c 2.28, $CHCl_3$); R_f = 0.43 in 20% EtOAc/hexanes; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.36−7.15 (m, 5 H), 5.85−5.64 (m, 1 H), 5.45 (t, *J* = 7.0 Hz, 1 H), 5.24−5.07 (m, 2 H), 3.65 (d, *J* = 8.9 Hz, 1 H), 2.77−2.64 (m, 2 H), 2.48 - 2.23 (m, 3 H), 1.82 (s, 1 H), 1.60 (s, 3 H), 0.85 (d, *J* = 7.4 Hz, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 142.5, 141.8, 136.1, 128.9, 128.8, 128.5, 126.3, 116.8, 81.8, 42.6, 36.2, 30.0, 17.2, 11.5. Anal. Calcd for $C_{16}H_{22}O$; C, 83.40; H, 9.62. Found: C, 83.04, H, 9.92.

(3*R*,4*R*,5*E*)-4-Dimethyl-*tert*-butylsilyloxy-8-phenyl-3,5-dimethyl-1,5-octadiene. To a stirring solution of (3*S*,4*R*,5*E*)-4-hydroxyl-8-phenyl-3,5-dimethyl-1,5-octadiene **9b** (4.05 g, 17.6 mmol) in DMF (0.6 M, 30 mL) was added imidazole (3.6 g, 52.8 mmol) and *tert*-butyldimethylsilylchloride (5.3 g, 35.2 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 and the organic layers were combined. The solution was dried, concentrated and flash chromatographed eluting with 250 mL of 1, 2, 5% EtOAc/hexanes. Product containing fractions were collected and concentrated to give 5.02 g (83%) of a clear oil as an inseparable mixture of diastereomers (7:1 determined by ^{1}H-formed during the previous reaction): R_f = 0.68 in 5% EtOAc/hexanes; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.38−7.17 (m, 5 H), 5.99−5.81 (m, 1 H), 5.36 (t, *J* = 7.5 Hz, 1 H), 5.13−4.97 (m, 2 H), 3.68 (d, *J* = 8.7, 1 H), 2.77−2.66 (m, 2 H), 2.48−2.24 (m, 3 H), 1.60 (s, 3 H), 0.93 (s, 9 H), 0.84 (d, *J* = 7.9 Hz, 3 H), 0.06 (s, 3 H), −0.01 (s, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 142.8, 137.6, 128.9, 128.8, 126.8, 126.3, 114.1, 83.6, 43.7, 42.6, 36.2, 29.8, 26.4, 18.9, 17.0, 11.8, −4.0, −4.5. Anal. Calcd for $C_{22}H_{36}OSi$; C, 76.68; H, 10.53. Found: C, 76.32; H, 10.88.

(1*E*,3*R*,4*R*,5*E*)-4-*tert*-Butylsilyloxy-1-iodo-8-phenyl-3,5-dimethyl-1,5-octadiene. To a stirring solution of (3*R*,4*R*,5*E*)-4-dimethyl-*tert*-butylsilyloxy-8-phenyl-3,5-dimethyl-1,5-octadiene (90 mg g, 0.26 mmol) in a mixture of *tert*-butanol (1 mL) and water (1 mL) was added AD-mix-β (390 mg, 1.5 g/mmol) at rt. The reaction was allowed to proceed for 24 h giving predominantly terminal alkene dihydroxylation (R_f = 0.41 in 35% EtOAc/hexanes). Occasionally, the purchased AD mix reagent contained variable reactivity. It was often necessary to add an additional portion of AD-mix-β (390 mg, 1.5 g/mmol) following the initial 24 h. After another 24 h period of stirring, the reaction mixture was then passed through a silica gel plug (4 × 3 cm). The material was rinsed through with ether and concentrated. Flash chromatography (10% EtOAc/hexanes) afforded a small amount of the starting diene. Flash chromatography with 35% EtOAc/hexanes gave 0.085 mg (86% yield) of the desired diol product **10b**.

The crude diol (2.14 g, 5.6 mmol) was dissolved in a mixture of THF (11 mL) and water (11 mL). To this solution was added sodium periodate (1.8 g, 8.5 mmol) and the mixture was allowed to stir for 30 min giving aldehyde (R_f = 0.38 in 10% EtOAc/hexanes) exclusively. The reaction mixture was then passed through a silica plug (8 × 4 cm). The material was rinsed through with ether and concentrated giving 1.95 g of crude aldehyde (one spot by TLC).

$CrCl_2$ (660 mg, 5.1 mmol) was added to a 25 mL round bottom flask and gently flame dried under high vacuum. Upon cooling, the flask was released under nitrogen and charged with 1.5 mL of THF and allowed to stir for 10 min at rt. The slurry was cooled to 0 °C and allowed to stir for 10 min. To another flask containing the crude aldehyde (252 mg, 0.73 mmol) was added 1 mL of THF followed by iodoform (660 mg, 1.68 mmol). This solution was then added dropwise by syringe to the $CrCl_2$ slurry which turned a brownish-red color. The reaction was allowed to proceed for 4 h at 0 °C after which, was poured in ice water (10 mL). The solution was extracted with ether (3×), dried ($MgSO_4$) and concentrated. Purification via radial chromatography on a 1 mm plate using 2% EtOAc/hexanes gave 194 mg (57%) of product (a clear oil) as an mixture of diastereomers (E/Z, 20:1 by 200 MHz ^{1}H NMR): R_f = 0.56 in 35% EtOAc/hexanes; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.34−7.14 (m, 2 H), 6.47 (dd, *J* = 14.8, 8.0 Hz, 1 H), 5.94 (d, *J* = 15.7 Hz, 1 H), 5.32 (t, *J* = 14.4 Hz, 1 H), 3.61 (d, *J* = 8.9 Hz, 1 H), 2.73−2.62 (m, 2 H), 2.44−2.19 (m, 3 H), 1.53 (s, 3 H), 0.87 (s, 9 H), 0.77 (d, *J* = 7.4 Hz, 3 H), 0.02 (s, 3 H), −0.07 (s, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 150.4, 142.4, 136.8, 128.9, 128.8, 127.5, 126.3, 82.8, 75.0, 45.5, 36.1, 29.8, 26.3, 19.3, 18.6, 11.6, −4.1, −4.4. Anal. Calcd for $C_{22}H_{35}IOSi$: C, 56.16; H, 7.50. Found: C, 52.49; H, 7.49.

(1*E*,3*R*,4*R*,5*E*)-4-Hydroxy-1-iodo-8-phenyl-3,5-dimethyl-1,5-octadiene 5b. To a flask containing neat (1*E*,3*R*,4*R*,5*E*)-4-*tert*-butylsilyloxy-1-iodo-8-phenyl-3,5-dimethyl-1,5-octadiene (310 mg, 0.66 mmol) was added a THF solution of tetrabutylammonium fluoride (5 mL, 9.9 mmol, 2 M) at 0 °C. The reaction was allowed to warm to rt and to stir for 6 h. Filtration of the reaction mixture through a plug of silica gel (4 × 3 cm) with ether followed by concentration gave the crude product. At this point the minor diastereomers from the olefination and the crotyl boration reactions were separated using radial chromatography (3−5% EtOAc/hexanes). Isolation of the major diastereomer gave 172 mg (73%) of product. The product was 70% enantiopure as determined by ^{1}H NMR analysis of the derivatized (*R*)-(+)-Mosher ester: $[\alpha]_D$ +1.5 (c 1.22, $CHCl_3$); R_f = 0.20 in 10% EtOAc/hexanes; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.37−7.16 (m, 5 H), 6.52 (dd, *J* = 15.0, 8.0 Hz, 1 H), 6.13 (d, *J* = 15.0 Hz, 1 H), 5.43 (t, *J* = 7.3 Hz, 1 H), 3.69 (d, *J* = 8.5 Hz, 1 H), 2.77−2.64 (m, 2 H), 2.47−2.28 (m, 3 H), 1.67 (s, 1 H), 1.06 (s, 3 H), 0.95 (d, *J* = 7.7 Hz, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 149.2, 142.3, 136.0, 128.9, 128.8, 128.6, 126.4, 81.5, 76.4, 44.9, 36.1, 29.9, 16.7, 11.6; HRMS (CI) calcd for M + H − H_2O for $C_{16}H_{21}IO$ 339.0610, found 339.0596.

Norephedrine Ester Based Route to Vinyl Iodide 9e. (1′*S*,2′*R*,2*S*,3*R*,4*E*)-2′-(*N*-Benzyl-*N*-mesitylenesulfonyl)-amino-1′-phenyl-1′-propyl-3-hydroxy-2,4-dimethyl-7-(2″-naphthyl)-4-heptenoate. To a stirred solution of (1*S*,2*R*)-2-(*N*-benzyl-*N*-mesitylenesulfonyl)amino-1-phenyl-1-propyl propionate (0.111g, 0.23 mmol) in 2 mL of CH_2Cl_2 was added triethylamine (0.08 mL, 0.56 mmol). The reaction flask was then cooled to −78 °C. A solution of 1.0 M dicyclohexylboron-triflate (0.7 mL, 0.70 mmol) in 2 mL CH_2Cl_2, precooled to −78

°C, was subsequently cannulated over to the reaction flask and stirring continued for two and a half h at −78 °C. (2*E*)-2-Methyl-5-(2′-naphthyl)-2-pentenal (0.065 g, 0.29 mmol) in 0.5 mL of CH_2Cl_2 was added dropwise to the reaction flask (with 0.5 mL CH_2Cl_2 rinse) and the reaction continued to stir for an additional h at the same temperature. The mixture was warmed to 0 °C and stirred for one h. The reaction was then charged with pH 7 buffer solution (2 mL), MeOH (4 mL), and 30% H_2O_2 (0.5 mL). The mixture stirred vigorously overnight at rt and was worked up with CH_2Cl_2 (3 extractions), dried ($MgSO_4$), and concentrated to give the crude oil. Purification of the desired product with its accompanying minor diastereomer was accomplished using radial chromatography (2 mm plate, 7% EtOAc/hexanes) affording 0.175g of material. The ratio of the two *anti* diastereomers (2′S, 3′R) and (2′R, 3′S) was determined to be 14:1 using HPLC analysis. No *syn* diastereomers were observed. Additional chromatography allowed for separation and characterization of the two diastereomers. Major isomer: $[\alpha]_D$ −31.2 (*c* 2.25, $CHCl_3$); R_f = 0.29 (20% EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) 7.79−7.72 (m, 3 H), 7.58 (s, 1 H), 7.45−7.12 (m, 11 H), 6.88 (s, 2 H), 7.82−6.79 (m, 2 H), 5.80 (d, *J* = 3.6 Hz, 1 H), 5.46 (t, *J* = 6.9 Hz, 1 H), 4.81 (A of ABq, J_{AB} = 16.8 Hz, 1 H), 4.60 (B of ABq, J_{AB} = 16.8 Hz), 4.06−4.02 (m, 2 H), 2.85−2.67 (m, 2 H), 2.59−2.36 (m, 4 H), 2.50 (s, 6 H), 2.27 (s, 3 H), 1.56 (s, 3 H), 1.12 (d, *J* = 7.5 Hz, 3 H), 0.80 (d, *J* = 7.2 Hz, 3 H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 174.9, 172.7, 140.4, 139.4, 139.1, 138.5, 134.8, 133.7, 133.7, 132.3, 132.1, 129.4, 128.5, 128.4, 128.0, 127.9, 127.8, 127.5, 127.4, 127.2, 126.6, 126.1, 125.9, 125.3, 80.3, 78.3, 57.0, 48.4, 43.4, 35.8, 29.5, 23.1, 21.0, 14.3, 14.2, 13.4, 10.7; HRMS FAB (M + Na) calcd for $C_{44}H_{49}O_5NSNa$ 726.3223, found 726.3217.

(1′*S*,2′*R*,2*S*,3*R*,4*E*)-2′-(*N*-Benzyl-*N*-mesitylenesulfonyl)-amino-1′-phenyl-1′-propyl 3-*tert*-butyldimethylsilyloxy-2,4-dimethyl-7-(2″-naphthyl)-4-heptenoate 11. 0.175 g (0.23 mmol assuming 100% theoretical yield) of the crude diastereomeric mixture isolated from the previous reaction was dissolved in 2 mL CH_2Cl_2 and cooled to 0 °C. This solution was charged with 2,6-lutidine (0.12 mL, 1.0 mmol) and dropwise addition of TBSOTf (0.17 mL, 0.74 mmol). The reaction was complete after 20 min of stirring at this temperature. The solution was diluted with brine and extracted with CH_2Cl_2 (3×), dried ($MgSO_4$), filtered, and concentrated. Purification by radial chromatography (3% EtOAc/hexanes) gave a white solid (mp 63 °C) 0.146 g (77% yield for two steps overall): $[\alpha]_D$ −33.8 (c 1.85, $CHCl_3$); R_f = 0.63 (20% EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) 7.82−7.73 (m, 3 H), 7.59 (s, 1 H), 7.47−7.06 (m, 11 H), 6.90 (s, 2 H), 6.67 (d, *J* = 7.2 Hz, 2 H), 5.66 (d, *J* = 5.7 Hz, 1 H), 5.40 (t, *J* = 6.3 Hz, 1 H), 4.95 (A of ABq, J_{AB} = 15.9 Hz, 1 H), 4.41 (B of ABq, J_{AB} = 15.9 Hz), 4.09 (d, *J* = 9.6 Hz, 1 H), 4.02−3.97 (m, 1 H), 2.85−2.78 (m, 2 H), 2.64−2.58 (m, 1 H), 2.48−2.38 (m, 1 H), 2.43 (s, 6 H), 2.33 (s, 3 H), 1.57 (s, 3 H), 1.14 (d, *J* = 6.9 Hz, 3 H), 0.80 (s, 9 H), 0.66 (d, *J* = 7.2 Hz, 3 H), −0.05 (d, *J* = 9.9 Hz, 6 H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 174.0, 142.5, 140.6, 139.5, 138.9, 138.5, 135.3, 133.8, 133.3, 132.3, 128.9, 128.7, 128.5, 128.4, 128.1, 127.9, 127.8, 127.6, 127.4, 126.6, 126.5, 126.1, 125.3, 80.9, 77.7, 56.9, 48.5, 44.8, 35.8, 29.5, 26.1, 23.1, 21.1, 18.4, 15.0, 14.5, 10.7, −4.6, −4.7; HRMS FAB (M + Na) calcd for $C_{50}H_{63}O_5NSiSNa$ 840.4108, found 840.4122. Anal. Calcd for $C_{50}H_{63}O_5NSiS$: C, 73.40; H, 7.76. Found: C, 73.14; H, 7.65.

(2*R*,3*R*,4*E*)-3-*tert*-Butyl-dimethylsilyloxy-2,4-dimethyl-7-(2″-naphthyl)-4-heptenol. (1′*S*,2′*R*,2*S*,3*R*,4*E*)-2′-(*N*-Benzyl-*N*-mesitylenesulfonyl)amino-1′-phenyl-1′-propyl 3-*tert*-butyldimethylsilyloxy-2,4-dimethyl-7-(2″-naphthyl)-4-hepten-oate (2.5 g, 3.1 mmol), was dissolved in 22 mL of THF and charged with solid $LiBH_4$ (0.20 g, 9.2 mmol) at 0 °C. The stirred solution was removed from the ice bath and heated under reflux for 9 h at 90 °C. The mixture was then cooled and extract with CH_2Cl_2 (3×), dried, and concentrated. Purification via radial chromatography (4−10% EtOAc/hexanes) gave the recovered hydroxy auxiliary (1*S*,2*R*)-2-(*N*-benzyl-*N*-mesitylenesulfonyl)-amino-1-phenyl-1-propanol (0.94 g, 92%), along with the title compound (0.941 g, 77% yield): $[\alpha]_D$ +9.23 (*c* 2.17, $CHCl_3$); R_f = 0.28 (10% EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) 7.83−7.76 (m, 3 H), 7.63 (s, 1 H), 7.48−7.40 (m, 2 H), 7.36 (d, *J* = 8.1 Hz, 1 H), 5.40 (t, *J* = 6.9 Hz, 1 H), 3.81 (d, *J* = 8.1 Hz, 1 H), 3.59 (d, *J* = 5.7 Hz, 2 H), 2.85 (dd, *J* = 7.5, 5.1 Hz, 2 H), 2.51−2.43 (m, 2 H), 1.89−1.75 (m, 1 H), 1.58 (s, 1 H), 0.89 (s, 9 H), 0.70 (d, *J* = 7.2 Hz, 3 H), 0.06 (s, 3 H), −0.04 (s, 3 H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 139.6, 136.7, 133.8, 132.2, 128.1, 127.8, 127.6, 127.4, 127.2, 126.6, 126.1, 125.3, 85.2, 67.3, 38.4, 35.8, 29.4, 26.0, 18.2, 14.4, 11.6, −4.2, −5.0; HRMS FAB (M + Na) calcd for $C_{25}H_{38}O_2SiNa$ 421.2535, found 421.2531.

(1*E*,3*R*,4*R*,5*E*)-4-*tert*-Butyldimethylsiloxy-1-iodo-8-(2′-naphthyl)-3,5-dimethyl-1,5-octadiene. To a stirring solution of (2*R*,3*R*,4*E*)-3-*tert*-butyldimethylsiloxy-2,4-dimethyl-7-(2″-naphthyl)-4-heptenol (1.142 g, 2.87 mmol) in 20 mL of methylene chloride was added oven-dried, crushed 4 Å molecular sieves (1.14g, 400mg/mmol), NMO (0.50g, 4.3 mmol), and TPAP (0.050g, 0.14 mmol); the latter was added at 0 °C. The solution was warmed to rt and allowed to stir for 1 h. The reaction mixture was then passed through a 4 cm diameter course fritted funnel containing silica gel (2 in). The material was rinsed through with ether. The filtered solution was concentrated giving 1.00 g of crude aldehyde (89%, one spot by TLC).

$CrCl_2$ was added to a 50 mL round bottom flask and gently flame dried under high vacuum. Upon cooling, the flask was released under nitrogen and charged with 13 mL of THF. The slurry was cooled to 0 °C and allowed to stir. To another flask containing the crude aldehyde (1.00g, 2.5mmol) stirring in 7 mL of THF was added iodoform (2.3 g, 5.9 mmol). This solution was then cooled to 0 °C and stirred for 10 min. This aldehyde/iodoform solution was then taken up in a syringe and added dropwise to the $CrCl_2$ slurry (also at 0 °C) which turned a brownish-red color. The reaction was allowed to proceed for 3 h at 0 °C and was then poured into 50 mL H_2O. The mixture was extracted with ether (3×), dried, and concentrated. The residue was purified by flash chromatography using 2% EtOAc/hexanes to give the desired product as a clear oil (0.99 g, 75% yield). Upon characterization, only the *E*-isomer was observed: $[\alpha]_D$ −1.0 (*c* 2.0, $CHCl_3$); R_f = 0.69 (10% EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82−7.76 (m, 3 H), 7.63 (s, 1 H), 7.46−7.37 (m, 2 H), 7.36 (d, *J* = 8.4 Hz, 1 H), 6.47 (dd, *J* = 14.1, 8.1 Hz, 1 H), 5.92 (d, *J* = 14.4 Hz, 1 H), 5.35 (t, *J* = 6.9 Hz, 1 H), 3.61 (d, *J* = 8.4 Hz, 1 H), 2.87−2.80 (m, 2 H), 2.50−2.40 (m, 2 H), 2.32−2.22 (m, 1 H), 1.55 (s, 3 H), 0.87 (s, 9 H), 0.76 (d, *J* = 6.9 Hz, 3 H), 0.01 (s, 3 H), −0.08 (s, 3 H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 150.1, 139.7, 136.6, 133.8, 132.2, 128.1, 127.8, 127.6, 127.4, 127.1, 126.6, 126.1, 125.3, 82.6, 74.8, 45.2, 35.9, 29.4, 26.0, 18.3, 16.2, 11.3, −4.4, −4.7; HRMS FAB (M + Na) calcd for $C_{26}H_{37}OSiNaI$ 543.1564, found 543.1572.

(1*E*,3*S*,4*R*,5*E*)-4-Hydroxy-1-iodo-8-(2′-naphthyl)-3,5-dimethyl-1,5-octadiene 5e. To a flask containing (1*E*,3*S*,4*R*,5*E*)-4-*tert*-butylsilyloxy-1-iodo-8-phenyl-3,5-dimethyl-1,5-octadiene (0.230 g, 0.44mmol) **14** was added a 1 M THF solution of *tert*-butylammonium fluoride (6.6 mL, 6.6 mmol) at 0 °C. The reaction was allowed to warm to rt and stirred for 90 min. Filtration of the reaction mixture through a plug of silica gel (2 in) with ether was followed by concentration to give a crude oil. Purification via radial chromatography (5% EtOAc/hexanes) gave the desired product (0.174 g, 97% yield) which gave a light yellow solid upon freezing. Full characterization for this compound was described previously: $[\alpha]_D$ +2.4 (*c* 1.73, $CHCl_3$). The **5e** enantiomer was also prepared (97% enantiopure-Mosher ester analysis): $[\alpha]_D$ −2.7 (*c* 1.47, $CHCl_3$).

(2*E*)-Ethyl 2-Methyl-2-hepten-6-ynoate. Pyridinium chlorochromate (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 rt and allowed to stir for 5 h. The solution was then filtered through silica gel with ether and carefully concentrated to give crude 4-pentyn-1-al as a volatile liquid: R_f = 0.58 (20% EtOAc/hexanes); ^{1}H NMR (200 MHz, $CDCl_3$) δ = 9.8 (s, 1 H, CHO). The crude aldehyde was dissolved in toluene (15 mL), and (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

(20% Et_2O/pentane) and concentrated to give the crude vinyl ester: ^{1}H NMR showed *E*/*Z* selectivity of 15:1; (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 (5% Et_2O/pentane) gave the *E* vinyl ester as a clear oil (0.226 g, 54% yield): R_f = 0.60 (20% EtOAc/hexane); IR (neat) 3300, 2983, 2916, 2119, 1712, 1652 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.75 (t, J = 7.1 Hz, 1 H), 4.15 (q, J = 2.9 Hz, 2 H), 2.45−2.20 (m, 4 H), 1.96 (t, J = 2.5Hz, 1 H), 1.83 (s, 3 H), 1.24 (t, J = 7.1Hz, 3 H); ^{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.

(2*E*)-2-Methyl-2-hepten-6-ynoic Acid 15. (2*E*)-Ethyl 2-methyl-2-hepten-6-ynoate (5.28 g, 31.8 mmol) was added to a solution containing THF (110 mL), MeOH (32 mL), and H_2O (32 mL) The mixture was cooled to 0 °C and LiOH (2.29 g, 95.4 mmol) was added followed by stirring at rt for 1 d upon which TLC showed disappearance of the ester. The solution was filtered through silica gel (25% EtOAc/MeOH) to give the yellow acid residue. The acid was then recrystallized in hexanes/EtOAc giving 3.32 g of a white solid (76% yield): mp 46 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 11.1 (bs, 1 H), 6.97 (t, J = 7.4 Hz, 1 H), 2.51−2.34 (m, 4 H), 2.02 (t, J = 2.6 Hz, 1 H), 1.90 (s, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.6, 142.4, 128.6, 83.0, 69.1, 27.8, 17.6, 12.1.

(1′*S*,2*E*)-(−)-*N*-(2′-Hydroxy-1′-methylethyl) 2-Methyl-2-hepten-6-ynamide 6l. (2*E*)-Ethyl 2-methyl-2-hepten-6-ynoic acid **15** (0.30 g, 2.2 mmol) was then dissolved in CH_2Cl_2 (11 mL). PyBrop (1.2 g, 2.6 mmol) and (S)-(+)-2-amino-1-propanol (0.20 g, 2.66 mmol) were sequentially added followed by addition of DIEA (1.2 mL, 6.9 mmol) at 0 °C. The reaction mixture stirred for 12 h at rt. The solution was filtered through silica gel (10% MeOH/EtOAc) and purified via radial chromatography (gradient elution, 70% EtOAc/hexanes to 100% EtOAc) giving a yellow white solid (0.367g, 87%): mp = 38−40 °C; R_f = 0.263 (EtOAc); $[\alpha]_D$ −4.08 (c 3.56, EtOAc); IR (neat) 3297, 2974, 2918, 2877, 2117, 1661, 1617, 1530 cm^{-1}; ^{1}H NMR (200 MHz, $CDCL_3$) δ 6.35 (t, J = 6.4Hz, 1 H), 6.0 (bd, 1 H), 4.15−4.0 (m, 1 H), 3.71−3.64 (dd, J = 11.0, 3.75 Hz, 1 H), 3.59−3.50 (dd, J = 11.0, 6.0 Hz, 1 H), 3.18 (bs, 1 H), 2.41−2.26 (m, 4 H), 1.97 (t, J = 2.4 Hz, 1 H), 1.85 (s, 3 H), 1.2 (d, J = 6.8 Hz, 3 H); ^{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) 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 Library Mixtures. (2*E*,8*E*,10*R*,11*R*,12*E*)-N-Substituted R′g−m-11-hydroxy-2,10,12-trimethyl-2,8,12-octadecatrien-6-ynamides 4a,g−m. To a flask containing (1*E*,3*R*,4*R*,5*E*)-4-hydroxy-1-iodo-3,5-dimethyl-1,5-undecadiene **5a** (69.5 mg, 0.22 mmol) was added (2*E*)-*N*-1′-adamantyl 2-methyl-2-hepten-6-ynamide **6g** (11.8 mg, 0.044 mmol), (2*E*)-*N*,*N*-dibenzyl 2-methyl-2-hepten-6-ynamide **6h** (13.8 mg, 0.044 mmol), (2*E*)-*N*-(2′-stearoyl)ethyl 2-methyl-2-hepten-6-ynamide **6i** (19.2 mg, 0.043 mmol), (2*E*)-*N*-4′-morpholinyl 2-methyl-2-hepten-6-ynamide **6j** (8.9 mg, 0.043 mmol), (1*S*′,2*E*)-*N*-(2′-hydroxy-1′-benzyl)ethyl 2-methyl-2-hepten-6-ynamide **6k** (11.6 mg, 0.043 mmol), (1*S*′,2*E*)-(−)-*N*-(2'-hydroxy-1′-methyl)ethyl 2-methyl-2-hepten-6-ynamide **6l** (8.3 mg, 0.043 mmol), and (2*E*)-*N*,*N*-diethanol 2-methyl-2-hepten-6-ynamide **6m** (9.9 mg, 0.44 mmol) all as a solution in ethyl acetate (8 mL). The mixture was cooled to −20 °C and $(Ph_3P)_2PdCl_2$ (7.5 mg, 0.011 mmol), CuI (6.0 mg, 0.031 mmol), and *i*-Pr_2NH (1.1 mL) were sequentially added. The reaction was immediately removed from the cool bath, protected from light, and allowed to warm to rt with continued stirring for 2 h. At this point, TLC showed disappearance of the alkynyl amides and seven new UV spots appeared which stained intensely using Ce/Mo staining solution. The brown reaction mixture was filtered through a plug of silica gel (ca. 3 g) followed by purification using radial chromatography to collect the uν bands as a mixture of seven compounds (90.1 mg, 90% yield): R_f = 0.53, 0.38, 0.21 in 35% EtOAc/hexanes; R_f = 0.72, 0.59, 0.46, 0.13 in 100% EtOAc; ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.9, 174.4, 174.3, 172.4, 170.02, 169.96, 169.4, 168.8; HRMS (FAB + Na) calcd for $C_{24}H_{39}O_3NNa$ 412.2827, found 412.2808; $C_{25}H_{39}O_3NNa$ 424.2827, found 424.2817; $C_{25}H_{41}O_4NNa$ 442.2933, found 442.2918; $C_{30}H_{43}O_3NNa$ 488.3140, found 488.3141; $C_{31}H_{47}O_2NNa$ 488.3505, found 488.3490; $C_{35}H_{45}O_2NNa$ 534.3348, found 534.3334; $C_{41}H_{71}O_4NNa$ 664.5280, found 664.5276.

(2*E*,8*E*,10*R*,11*R*,12*E*)-*N*-1′-Adamantyl 11-Hydroxy-15-substituted Ra−f-2,10,12-trimethyl-2,8,12-pentadecatrien-6-ynamides 4a−f,g. To a solution of (2*E*)-*N*-1′-adamantyl 2-methyl-2-hepten-6-ynamide **6g** (67.5 mg, 0.25 mmol) was added (1*E*,3*R*,4*R*,5*E*)-4-hydroxy-1-iodo-3,5-dimethyl-1,5-undecadiene **5a** (9.7 mg, 0.030 mmol), (1*E*,3*R*,4*R*,5*E*)-4-hydroxy-1-iodo-8-phenyl-3,5-dimethyl-1,5-octadiene **5b** (10.8 mg, 0.030 mmol), (1*E*,3*R*,4*R*,5*E*)-4-hydroxy-1-iodo-8-cyclohexyl-3,5-dimethyl-1,5-octadiene **5c** (10.7 mg, 0.030 mmol), (1*E*,3*R*,4*R*,5*E*)-4-hydroxy-1-iodo-8-(1′-adamantyl)-3,5-dimethyl-1,5-octadiene **5d** (12.2 mg, 0.029 mmol), (1*E*,3*R*,4*R*,5*E*)-4-hydroxy-1-iodo-8-(2′-naphthyl)-3,5-dimethyl-1,5-octadiene **5e** (12.0 mg, 0.030 mmol), and (1*E*,3*R*,4*R*,5*E*)-4-hydroxy-1-iodo-8-(3′,4′-dimethoxyphenyl)-3,5-dimethyl-1,5-octadiene **5f** (12.3 mg, 0.030 mmol) all as a solution in ethyl acetate (12 mL). The mixture was cooled to −20 °C and $(Ph_3P)_2PdCl_2$ (6.5 mg, 0.009 mmol), CuI (5.9 mg, 0.030 mmol), and *i*-Pr_2NH (0.9 mL) were sequentially added. The reaction was immediately removed from the cool bath, protected from light, and warmed to rt with continued stirring for 3.5 h. TLC showed disappearance of the alkynyl amide and three new uν spots appeared which stained intensely using Ce/Mo staining solution. The brown reaction mixture was filtered through a plug of silica gel (ca. 3 g) followed by purification using radial chromatography to collect the uν bands as a mixture of six compounds (92.3 mg, 99% yield): R_f = 0.57, 0.46, 0.46 in 35% EtOAc/hexanes; ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.7; MS (FAB) for (M − H_2O)H$^+$ 448, 482, 488, 532, 540, 542.

Bicinchoninic Acid (BCA) Assay for the Determination of Adriamycin Cytotoxicity in the Presence of MDR Reversal Agents. MCF-7 adriamycin-resistant (MCF-7/ADR) cells were seeded into 96-well microtiter dishes (precoated with poly-D-lysine) at a concentration of 1000 cells/well (5000 cells/mL). The cells were then subsequently seeded in 200 μl of RPMI medium (containing 10% FCSH I and PS) per well and incubated for 24 h at 37 °C. The MDR reversal agent (pool or individual compound) was first dissolved in DMSO to give a concentration of less than 0.1% DMSO in the well and was then further diluted with media. Twelve aliquots of the reversal compound(s) varying in concentration ranging from 0.005 to 50 μg/mL were then added to the resistant cells followed by incubation at 37 °C for 30 min. At this point, adriamycin at a concentration of 2×10^{-2} μg/mL, was added to each well containing reversal agent. After a 6 d incubation period, the media was removed from the wells followed by washing of the wells with phosphate-buffered saline (PBS). Nonidet P-41 1% (10 μl) was added to each well of cells followed by 200 μl of a premixed bicinchoninic acid (BCA)-4% copper sulfate (50:1) solution. The 96-well plate was again incubated at 37 °C for 30 min. whereupon the purple-tinted wells indicated the presence of cellular protein and viable cells. The optical density of each well was read at 570 nm using a microplate reader. The correlation already established by Chang between cell number and optical density allowed for determination of the ED_{50} of adriamycin in the presence of reversal agent(s). The cell assays were done in quadruplicate allowing for calculation of the ED_{50} value (determined by linear interpolation) and standard deviation.

Measurement of ATPase Activity. ATPase activity of Pgp was measured by the endpoint, inorganic phosphate (P_i) assay. Pgp-specific activity was recorded as the vanadate (V_i) (0.3 mM)-sensitive ATPase activity. The compounds being tested were added from 100× stock solutions in DMSO so that the DMSO concentration was no greater than 1%; this concentration of DMSO had no effect on the activity of Pgp. The P_i assay measured the amount of inorganic phosphate released over 20 min at 37 °C (100 μL assay volume). Pgp was incubated in ATPase buffer (50 mM MES-Tris, pH 6.8, 50 mM KCl, 5 mM NaN_3, 2 mM EGTA, 1 mM ouabain, 5 mM $MgCl_2$ and 2 mM DTT) and the compounds being tested were incubated for 5

min at 37 °C, both in the presence and absence of 0.3 mM Vanadate. The ATPase reaction was initiated by the addition of ATP and quenched with SDS (2.5% final concentration); the amount of inorganic phosphate released was quantitated using colorimetric method as previously described (Ramachandra, M.; Ambudkar, S. V.; Gottesman, M. M.; Pastan, I.; Hrycyna, C. A. *Mol. Cell Biol.* **1996**, *7*, 1485−1498).

Photoaffinity Labeling with IAAP. Crude plasma membranes of High Five insect cells containing Pgp were used for this experiment. The crude plasma membrane (10−20 μg protein) was labeled in 50 mM Tris-HCl, pH 7.4 and was incubated with the MDR modulator for 3 min at rt in plastic eppendorf vials. ^{125}IAAP was added in the dark and incubated at rt for five min. Usually, between 3−6 nM of IAAP was added per sample. The membranes were then exposed to a UV lamp (G.E. no. F15T8-BLB) at 365 nm for 10 min at rt. 5× SDS PAGE sample buffer was added to each of the samples and these were subsequently incubated at rt for 30 min. Samples were then run on an 8% Tris-glycine gel at constant voltage. Gels were dried and exposed to Biomax MR (Kodak) autoradiography film.

Determination of K_i for the MDR Modulators. The crude plasma membranes (20 μg protein in 40 mL of 50 mM Tris-HCl, pH 7.4) were placed in vials containing varied concentration of the MDR modulators (0−250 μM) and incubated at rt for 3 min. IAAP (3−6 nM) was added to each test tube and incubated for another 3 min. The membranes were photocrosslinked as described in the previous section and run on an 8% Tris-glycine gel. Gels were then dried and the radioactivity of IAAP bound Pgp was estimated using the STORM 860 phospholimager system with Image Quant software. The radioactivity in the Pgp band was obtained as arbitrary units. The data was fitted to a one phase experimental decay using GraphPad Prism 2.0 software for Macintosh.

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Supporting Information Available: Experimental details, analytical data, and MDR, ATPase, and displacement protocols for selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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