The Synthesis and Evaluation of a Solution-Phase Indexed Combinatorial Library of Non-natural Polyenes for Multidrug Resistance Reversal

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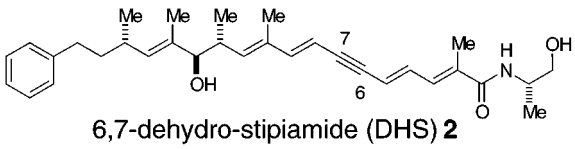
While small molecule libraries have gained great prominence as a means of identifying new inhibitors for biological targets,[2] unified approaches that allow for efficient synthesis, direct screening, and facile individual member identification are still needed. Solid-phase libraries that provide for synthetic efficiency are limited to screening with isolable receptors and are not applicable to cell assays.[3] In contrast to the split-pool peptide approach, solid-phase small molecule libraries have been made almost exclusively using spatially localized, serial methods. Solution-phase libraries offer the advantages of using standard reagents, no linker or support, and the ability to directly screen with isolated receptors or cellular assays.[4] Natural product libraries, while more focused 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 report a solution-phase library,[6] based on the multidrug resistance (MDR) reversing polyene (−)-stipiamide, that consists of mixtures indexed in two dimensions that provides for efficient combinatorial synthesis, direct screening with a cellular assay, and the isolation and testing of individual compounds.

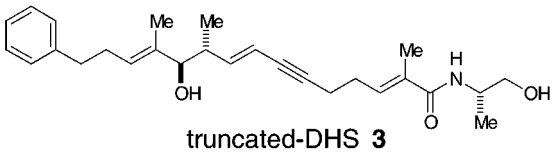
Recently, we showed that synthetic (−)-stipiamide (**1**)[7] had only moderate reversal activity with colchicine resistant cells,[8] while **2** (ED_{50} 6.5 μM) and **3** (4 μM) were potent with a variety of drugs with resistant MCF7-adrR cells that express Pgp (P-glycoprotein), the membrane-bound small-molecule MDR pump.[9] Importantly, **2** and **3** were far less toxic (4 and 14 μM) compared to stipiamide **1** (0.01 nM). Also, **3** was shown to bind Pgp by monitoring ATPase activity (10 μm) and through displacement of a known label arylazidoprazosin (12 μm).[10]

Polyene **3** was selected as the template due to its activity and convenience for varying the end groups (Ph and amide). Critical to the effort was the selection of a diversity generating reaction that would produce equal relative amounts of each compound in the pools. Amide formation was unsuitable in that primary and secondary amines would give widely varying rates of reaction. Thus, the convergent Sonogashira coupling route to **3**, involving formation of a central carbon−carbon bond remote from the two ends, was selected as the key step.[7b] This provides the indexed pools with the compounds present in equal amounts and allows for direct analysis in the cellular assay. False positive and negative results, synergistic effects of weak compounds or a potent compound being masked by weak members within a pool, were a concern. It remained to be seen if the inherent redundancy of the library, in which each individual member is present in two distinct pools, would minimize these effects. The identification and isolation of individual compounds addresses this concern verifying the results from the pools. End groups were selected to provide a range of steric, as with the adamantyl groups, polar, as with the dimethoxyphenyl and bis-hydroxyethyl amides, and nonpolar functionality with some compounds, as in **3** (**10b**−**l**), expected to be potent while others would be 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.

(-)-stipiamide **1** (*Z*-6,7-dihydro)



6,7-dehydro-stipiamide (DHS) **2**



truncated-DHS **3**

Vinyl iodides **7a**−**f** were prepared individually beginning with the enals **4a**−**f**, obtained from the corresponding propanals, following the previous route (Scheme 1).[7b] Aldehydes **4a**−**f** were reacted with crotylborane[11] derived from (−)-(α)-pinene to give the antihomoallylic alcohols (34−76% yield) with selectivities ranging from 4:1 to 8.5:1.[7b] After protection, the dienes were subjected to the AD-mix-β reagent, according to the procedure of Sharpless,[12] to selectively dihydroxylate the terminal alkene.[13] The diols were reacted with sodium periodate to afford aldehydes **6a**−**f**, which were transformed to the (*E*)-vinyl iodides (*E*:*Z* > 20:1) using the conditions of Takai.[14] Removal of the TBS group gave the desired vinyl iodides **7a**−**f**.

The amides **9g**−**m** were made in a more convergent manner (Scheme 2). Acid **8**, obtained from 4-pentyn-1-ol using PCC, Wittig coupling, and hydrolysis,[7b] was reacted with each of the amines (R′**g**−**m**, 1.2 equiv) using PyBrop.[15] Widely varying yields (63−100%) and rates confirmed the decision not to use this reaction as the key step for the library.

Iodides **7a**−**f** were coupled with the acetylenes **9g**−**m** to give enynes **10** in an indexed manner using the optimized

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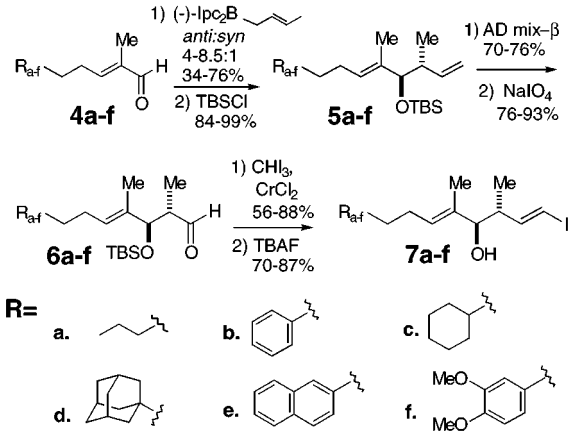
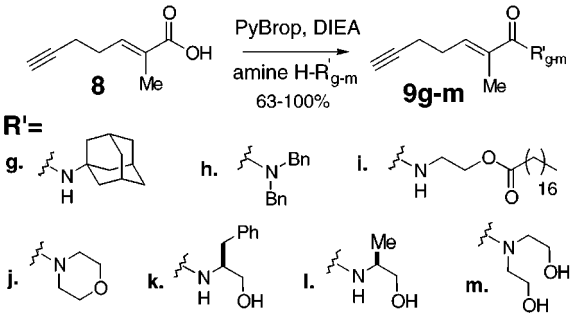
Scheme 1



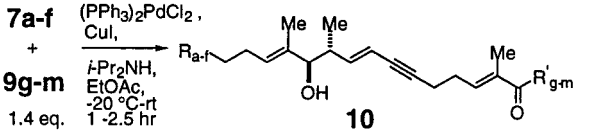
Scheme 2



Sonogashira coupling conditions (Table 1).[16] This reaction proved ideal with short reaction times and tolerance of the wide array of unprotected functionality on the two ends. The six left-hand iodides **7a**−**f** were reacted individually with a mixture of the seven acetylenes **9g**−**m** with $(Ph_3P)_2PdCl_2$ (5 mol %) and 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. The scale (∼100 mg) allowed for multiple MDR assays (1−2 mg per 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. Individual compounds, in both high and lower yielding reactions, were shown by TLC, NMR, MS, 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 **9g**−**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. The synthesis is thus 3.2 times more efficient than the serial approach. It is important to note that each individual compound appears in two distinct pools and is never found with another compound twice.

MCF-7adrR cell assays with added adriamycin were performed with the pools in quadruplicate over a range of concentrations using an average molecular weight (Table 1).[17] The R = Ph(**b**) pool ED_{50} was 3.5 μM, while the cyclohexyl pool at 10 μM was three times less potent. The dimethoxyphenyl and adamantyl pools were far less active. At least two times more potent was the naphthyl pool at 1.3 μM. Trends evident in the right-hand amide dimension include the far less potent adamantyl (**g**) and the sterate ester group (**i**). The hydroxy amide (R'_1 = (*S*)-alaninol) and morpholine (**j**) pools were active. Potent individual agents are readily identified where active pools overlap. Selected compounds were easily isolated by radial chromatography from reserved material and additional reversal assays were performed (Table 1). Isolated compound **3** (**10b,l,** R = Ph and R′ = alaninol) showed an ED_{50} of 1.9 μM and **10e,l** (2-naphthyl, alaninol) was 1.7 μM in accord with the pool assays.[18] The most potent new compound was **10e,k** (naphthyl, phenylaninol) at 1.45 μM. Compound **10e,m** (2-naphthyl, bishydroxyethylene) was also very active. Less potent combinations **10c,g** (cyclohexyl, adamantyl) at 12 μM and **10d,h** and **10d,i** (adamantyl, dibenzylamine and ethyl sterate amine) again >70 μM were also in accord with the pool results. Correlation of these and other individual activities with the library assays confirms the viability of the indexed approach using a complex natural product template. Extended solution-phase polyene libraries and applications to other natural product templates are now under investigation.

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



	R	R′	yield[a] (%)	ED_{50}[b] (μM)	STD[c]
left side	**a**	**g**−**m**	90	6.6	0.9
constant	**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	**a**−**f**	**g**	99	23	7
constant	**a**−**f**	**h**	100	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
isolated	**b**	**l**		1.85	0.04
compounds	**b**	**m**		8.8	0.1
	e	**j**		1.69	0.02
	e	**k**		1.45	0.08
	e	**l**		1.73	0.05
	e	**m**		1.48	0.02
	c	**g**		12	2
	d	**h**		>70	
	d	**i**		>70	

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

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Supporting Information Available: Experimental details, analytical data, and MDR protocols for selected compounds.

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(18) ED_{50} for *cis*-flupenthixol (ref 10) was 5.2 μM performed as a control.