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Small Molecule Macroarray Construction via Palladium-Mediated Carbon–Carbon Bond-Forming Reactions: Highly Efficient Synthesis and Screening of Stilbene Arrays

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The use of small molecule probes to interrogate biological phenomena has become a prominent research approach in chemical biology.[1,2] As the demand for probe molecules continues to grow, the need to develop new methodology for the rapid generation and evaluation of small molecules has also become more urgent.[3,4] Our laboratory is engaged in the development of the small molecule macroarray as tool for the rapid parallel synthesis and biological screening of drug-like molecules and peptidomimetics.[5–8] Macroarrays are readily constructed via the SPOT-synthesis technique on planar cellulose supports (i.e., laboratory filter paper)[9] yielding spatially addressed libraries of 10–1000 unique compounds (≈50–200 nmol of compound/spot; Figure 1A). The simplicity of macroarray construction, coupled to its compatibility with a range of biological assay formats performed either on or off of the planar support, renders this library synthesis technique attractive for a range of research applications. To find wider usage, however, the scope of chemical reactions that are compatible with the macroarray platform must be extended, and this has motivated considerable recent research in this area.[10]

To date, the majority of reactions performed on planar cellulose supports have been simple, acylation-type reactions.[11–13] In view of the prevalence of metal-mediated, carbon–carbon bond forming reactions in both chemical probe and pharmaceutical discovery,[14] we sought to explore the feasibility of these reactions on the small molecule macroarray. Herein, we report the application of palladium-mediated Heck reactions for the efficient construction of stilbene macroarrays. Preliminary bacteriological screening of these arrays post-synthesis revealed several stilbenes with novel activities. This work represents both a chemical and biological advance for the small molecule macroarray technique, and further underscores the utility of this approach for chemical probe discovery.

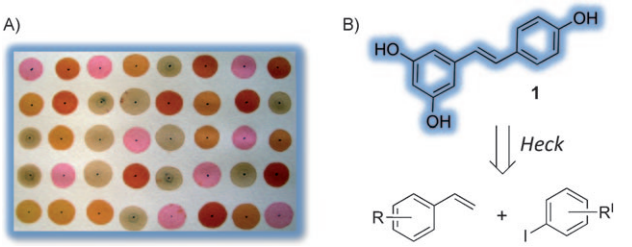

Figure 1. A) Image of a representative small molecule macroarray (scale = 12 × 8 cm). B) The natural product resveratrol (**1**) and a general retrosynthesis of stilbenes *via* the Heck reaction.

We selected the Heck reaction for examination on planar cellulose as this transformation has been well-studied in solution[15,16] and, to a lesser extent, on traditional solid supports.[17] As our laboratory has an on-going interest in the development of chemical probes to study virulence pathways in bacteria (e.g., quorum sensing (QS)[18–20]), we chose a chemical scaffold for macroarray design that i) was readily accessible *via* the Heck reaction and ii) has been previously shown to modulate QS pathways. The phytoalexin resveratrol (**1**; Figure 1B) was recently shown to inhibit QS receptors in Gram-negative bacteria (i.e., *Proteus mirabilis*, *Vibrio fischeri*, and *Pseudomonas aeruginosa*); however, its mechanisms of action remain unknown.[21–23] Resveratrol (**1**), along with other naturally occurring stilbenes, exhibits a rich range of additional biological activities, spanning from anticancer and cardioprotective effects to life prolongation effects.[24–26] As such, the synthesis and screening of stilbenes could provide valuable new chemical tools to examine not only QS and virulence, but also a range of other pertinent biological pathways. These attributes, coupled with the ready applicability of Heck chemistry to stilbene synthesis

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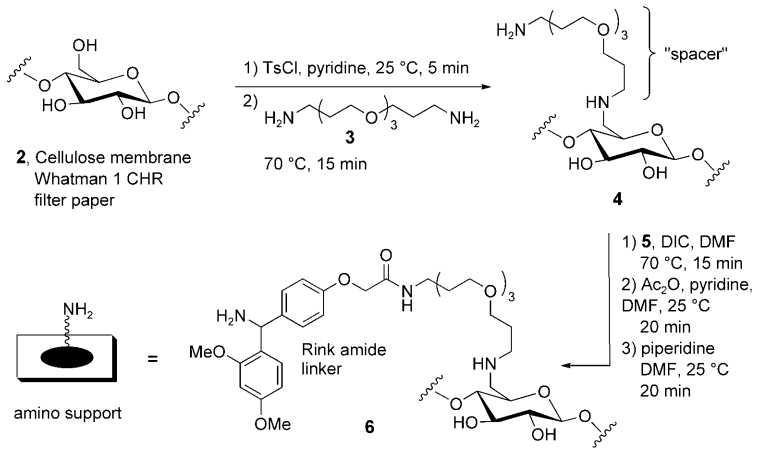
(Figure 1 B), caused us to select stilbenes as our scaffold for this study.

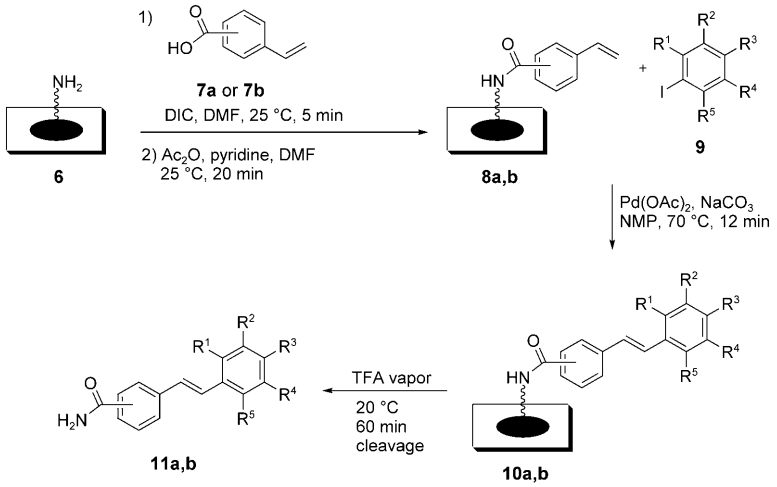
Macroarray construction commenced with the selection of a robust linker system (Scheme 1). We chose the Rink-amide linker, as this linker has been shown to be compatible with a range of solid-phase syntheses.[10,27] Prior to the attachment of the linker to planar cellulose, we derivatized the cellulose (Whatman 1 CHR filter paper, **2**) with a flexible diamine spacer unit **3** to yield support **4**. The spacer was installed by first converting the primary hydroxyl groups of cellulose membrane **2** to tosyl groups at 25 °C, then reacting this tosylated support with the neat diamine **3** at 70 °C. Next, the Rink-amide linker (**5**, 4-[(2,4-dimethoxyphenyl)-(Fmoc-amino)methyl]phenoxyacetic acid) was attached to support **4** via a standard *N*,*N*′-diisopropylcarbodiimide (DIC) coupling reaction at 70 °C. Notably, we applied linker **5** to support **4** in an arrayed "spot" format using a micropipette (0.3 cm^2 per spot) to minimize the use of this relatively expensive reagent. Following linker loading, the membrane was acetylated (to cap any unreacted spacer amines), and subjected to basic Fmoc deprotection to generate amine support **6**. Using this method, Rink linker loadings of $\approx$550 nmol cm^{-2} were routinely generated (as determined by UV Fmoc quantification) in 75 min reaction times (**2**→**6**).



Scheme 1. Initial planar cellulose support modification and Rink linker installation to generate support **6**.

To generate stilbenes on support **6** via the Heck reaction, we required an aryl alkene component that could be directly attached to the Rink linker. *para*-Vinyl and *meta*-vinyl benzoic acids (**7a**,**b**) met this criterion as our initial building blocks (Scheme 2), and were efficiently coupled to linker "spots" on support **6** using spatially addressed DIC reactions (loading level $\approx$350 nmol cm^{-2}). We next turned to the aryl halide component for the Heck reaction, and examined aryl iodides **9** as the second building block for stilbene macroarray synthesis. Careful optimization work with a small set of aryl iodides revealed that spotting a mixture of 3 mol% $Pd(OAc)_2$ (relative to **9**) and 1.2 equiv Na_2CO_3 (relative to **9**) in *N*-methyl-2-pyrrolidone (NMP) onto styrene support **8a**,**b** led to the productive formation of stilbene support **10a**,**b**. Spatially-addressed cleavage of **10a**,**b** using TFA vapor gave *trans*-stilbenes **11a**,**b** in excellent conversions and purities ($\approx$97 and $\approx$93%, respectively; as determined by HPLC analysis).[28] We found that the Heck reaction protocol did not require a tertiary phosphine ligand,[29,30] further streamlining this synthesis method. $Pd(OAc)_2$ was used in an excess of * 1.4 equivalents with respect to immobilized styrenes **8a**,**b**, suggesting a non-catalytic process. We note, however, that the small scale of macroarray synthesis (nanomolar) does not demand a catalytic process. Increasing the palladium loading led to lowered stilbene purities, while lowering the loading resulted in poorer stilbene conversions. Importantly, palladium contamination of the stilbenes products was found to be minimal (ppb level; see Supporting Information).

Using the optimized reaction conditions (Scheme 2), we prepared a 100-member stilbene macroarray (**10a**,**b**) on a 18×15 cm section of support **6** using the two benzoic acids



Scheme 2. Styrene loading and Heck reaction to yield stilbene macroarrays **10a**,**b** (stilbenes **11a**,**b** after TFA cleavage).

7a,**b** and 50 structurally divergent aryl-iodides (**9**; see Supporting Information for structures) as library building blocks. The stilbene products (**11a**,**b**) were isolated in ≈100 nmol per spot quantities, which was more than sufficient for analytical testing and numerous biological assays.[5] A subset of the library (20 randomly selected compounds) was evaluated by HPLC, and high product conversions and purities were obtained in almost all cases (Table 1).[31] These results are significant, as they represent to our knowledge the first demonstration of metal-mediated coupling reactions in small molecule macroarray synthesis. Notably, we were able to prepare the 100 stilbenes (**11a**,**b**) in under 12 h starting from unfunctionalized cellulose **2** (including all reactions, washing, drying, cleavage, and compound elution), further highlighting the efficiency of this synthesis method for the generation of libraries of biologically relevant molecules.

Table 1. Representative purity and conversion data for the stilbene macroarray products **11a**,**b**.

Stilbene	R^1	R^2	R^3	R^4	R^5	Conversion [%][a]	Purity [%][b]
11a-4	H	H	CH_2CN	H	H	99	92
11a-15	H	NO_2	H	NO_2	H	95	94
11a-22	H	H	CN	H	H	98	97
11a-26	H	NO_2	H	H	H	99	98
11a-27	H	CN	H	H	CH_3	96	98
11a-38	NO_2	H	CH_3	H	H	99	96
11a-41	CH_3	H	H	NO_2	H	97	93
11a-44	H	F	H	NO_2	H	95	88
11a-48	H	F	CH_3	H	H	91	87
11a-50	H	CF_3	H	H	H	88	88
11b-4	H	H	CH_2CN	H	H	>99	92
11b-15	H	NO_2	H	NO_2	H	99	94
11b-22	H	H	CN	H	H	>99	99
11b-26	H	CN	H	H	CH_3	98	97
11b-27	NO_2	H	CH_3	H	H	>99	96
11b-38	CH_3	H	H	NO_2	H	99	93
11b-41	H	F	H	NO_2	H	99	88
11b-44	H	F	CH_3	H	H	93	92
11b-48	H	NO_2	H	H	H	>99	93
11b-50	H	CF_3	H	H	H	93	94

[a] Calculated by comparing initial styrene loadings and the quantity of styrene remaining after the Heck reaction using UV calibration curves; see the Supporting Information. [b] HPLC UV detection at 278 nm (for stilbenes **11a**) and 254 nm (for stilbenes **11b**).

We next sought to evaluate the activity of the stilbene library (**11a**,**b**) as QS inhibitors, and performed a competitive antagonism screen against the LuxR receptor in the bacterial symbiont *V. fischeri*.[19] *V. fischeri* contains the canonical QS system in Gram-negative bacteria based on LuxR-type receptors, and thus represented an excellent model organism for this initial study.[18,20] We utilized a *V. fischeri* mutant strain that reported LuxR inhibition via luminescence, and examined 5 μM stilbene against 5 μM of *V. fischeri's* native QS ligand, *N*-(3-oxo-hexanoyl)-L-homoserine lactone (OHHL; Figure 2).

Preliminary assays revealed five stilbenes (**11a-10**, **11a-12**, **11a-20**, **11a-37**, and **11b-9**) with inhibitory activities in the

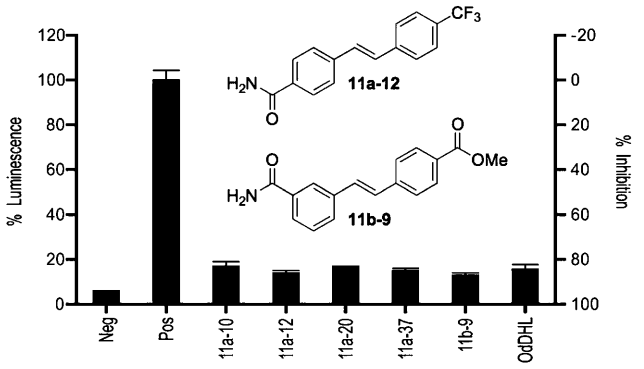


Figure 2. Luminescence (LuxR) inhibition data for the five most potent stilbenes in library **11a**,**b** (1:1 against OHHL; 5 μM compound). Two of the most active stilbenes are shown. Strain: *V. fischeri* ES114 (Δ-*luxI*). Neg. control = DMSO. Pos. control = OHHL. Assays performed in triplicate (see Supp. Info. for assay protocol and full stilbene structures).

V. fischeri mutant strain comparable to some of the most potent known native and non-native LuxR antagonists (e.g., *N*-(3-oxo-dodecanoyl)-L-homoserine lactone (OdDHL); Figure 2).[19] Indeed, these stilbenes were capable of inhibiting LuxR by ≥ 80% at a 1:1 ratio against OHHL. Additional experiments are required to elucidate the mechanism by which these stilbenes elicit their LuxR inhibitory effects. Nevertheless, these screening data suggest that stilbenes with structures beyond the resveratrol (**1**) scaffold are capable of modulating QS responses. Few compounds with structures dissimilar to native QS signals have been shown to interfere with QS;[18,20] therefore, the discovery of these active ligands from such a small library of compounds (100-member) is noteworthy.

In summary, we have demonstrated that palladium-mediated, carbon–carbon bond forming reactions are compatible with the small molecule macroarray approach. As a test case, a 100-member macroarray of unique stilbenes was constructed via spatially addressed Heck reactions in high conversions and purities. Subsequent biological testing of the stilbene library (**11a**,**b**) in a bacterial QS assay revealed several new and potent stilbene-derived inhibitors of a QS receptor in the Gram-negative bacterium, *V. fischeri*. Overall, this work serves to highlight the emerging potential of the small molecule macroarray platform for chemical biology research. Ongoing work is directed at further extending the scope of metal-mediated couplings on planar cellulose supports, as well as a thorough biological evaluation of the stilbene library, and will be reported in due course.

Experimental Section

Representative Heck reaction protocol on styrene-derivatized cellulose support 8a,**b**: Spotting solutions were prepared by dissolving aryl iodides (**9**, 0.25 mmol), $Pd(OAc)_2$ (50 μL of a 0.166 M $Pd(OAc)_2$ solution in NMP), and Na_2CO_3 (31.8 mg, 0.30 mmol) in NMP (50 μL). Aliquots (3.0 μL) of this solution were pipetted onto the 100 spots of styrene-functionalized support **8a**,**b**. The support was heated at 70 °C for 12 min on a sand bath and then washed by immersion and swirling in 150 mL portions

of DMF (2×), H_2O (2×), MeOH (2×), hexanes (2×) and CH_2Cl_2 (2×). Thereafter, stilbene macroarray **10a**,**b** was dried on a sand bath at 70 °C for 6 min prior to TFA vapor cleavage (60 min, 25 °C) to yield cleaved stilbenes **11a**,**b**. See Supporting Information for full synthetic details.

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