Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron 62 (2006) 4715–4727
Tetrahedron

Efficient synthesis of small molecule macroarrays: optimization of the macroarray synthesis platform and examination of microwave and conventional heating methods

Matthew D. Bowman, Megan M. Jacobson, Brian G. Pujanauski and Helen E. Blackwell*

Department of Chemistry, University of Wisconsin-Madison, 1101 University Avenue, Madison, WI 53706-1322, USA

Received 22 December 2005; revised 6 February 2006; accepted 6 February 2006
Available online 31 March 2006

Abstract—We report a method for the efficient construction of small molecule macroarrays using microwave-assisted SPOT-synthesis. Macroarrays of 1,3-diphenylpropenones (chalcones) were synthesized rapidly and in high purity starting from robust, Wang-linker-derivatized planar supports. We have optimized the entire chalcone macroarray construction process and evaluated the efficiency and utility of microwave-assisted reactions in array synthesis. Microwave heating was found to be most beneficial for reactions that require temperatures greater than the boiling points of the solvents. These microwave-assisted conditions permitted straightforward access to macroarrays of 2,4,6-triarylpyridines derived from the original chalcone scaffold.

1. Introduction

Combinatorial chemistry is now an established tool for the discovery of small molecules and materials with new properties. This approach has seen wide use in the fields of drug development,[1] chemical biology,[2] supramolecular chemistry,[3] material science,[4] and chemical sensor development.[5] With the advent of high-throughput screening and ultra-sensitive analytical techniques,[6,7] the amount of material required for evaluation has been reduced to the nanomolar and sub-nanomolar range. As a result, combinatorial synthesis techniques have been developed that provide maximum structural diversity at an exceedingly small scale.[8] These techniques have relied typically on solid-phase synthesis methods using polymeric supports;[9] however, the conventional beaded supports are frequently expensive, mechanically fragile, and incompatible with many on-bead assays. Further, the reaction rates for solid-phase reactions are often considerably slower than their homogeneous counterparts (e.g., 10–100 times).[10] These drawbacks have motivated the development of improved synthesis and screening platforms for combinatorial chemistry.

SPOT-synthesis represents an attractive alternative to the use of conventional polymeric resins for combinatorial synthesis.[11] Originally developed for peptide synthesis, this method involves spatially addressed synthesis on derivatized cellulose sheets (readily prepared from inexpensive laboratory filter or chromatography paper) to generate arrays of unique molecules (1–10,000 spots per array, Fig. 1).[12,13] In contrast to conventional polystyrene resins, the hydrophilic membrane sheets are easy to manipulate during synthesis and

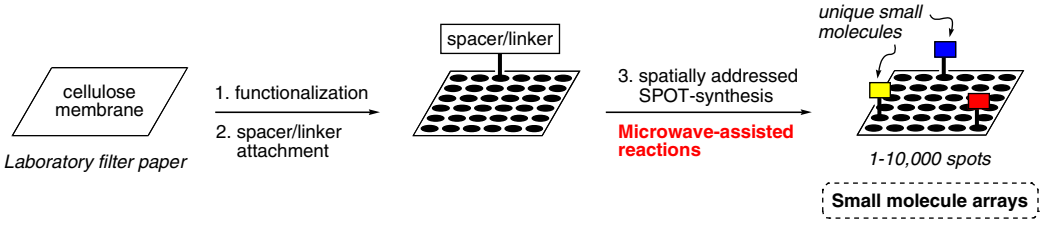


Figure 1. Schematic of the SPOT-synthesis process used to generate small molecule macroarrays.

Supplementary data associated with this article can be found in the online version, at doi: 10.1016/j.tet.2006.02.083.
* Corresponding author. Tel.: +1 608 262 1503; fax: +1 608 265 4534; e-mail: blackwell@chem.wisc.edu

0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.02.083

washing steps and are mechanically robust. Furthermore, planar cellulose supports are compatible with various on support biological screening methods, including protein-binding assays, enzyme-linked immunosorbent assays (ELISA), and agar overlays.[14] Assays also can be performed after compound cleavage—each spot of the macroarray commonly yields 50–200 nmol of compound, which is sufficient for evaluation in miniaturized solution-based bioassays.[15]

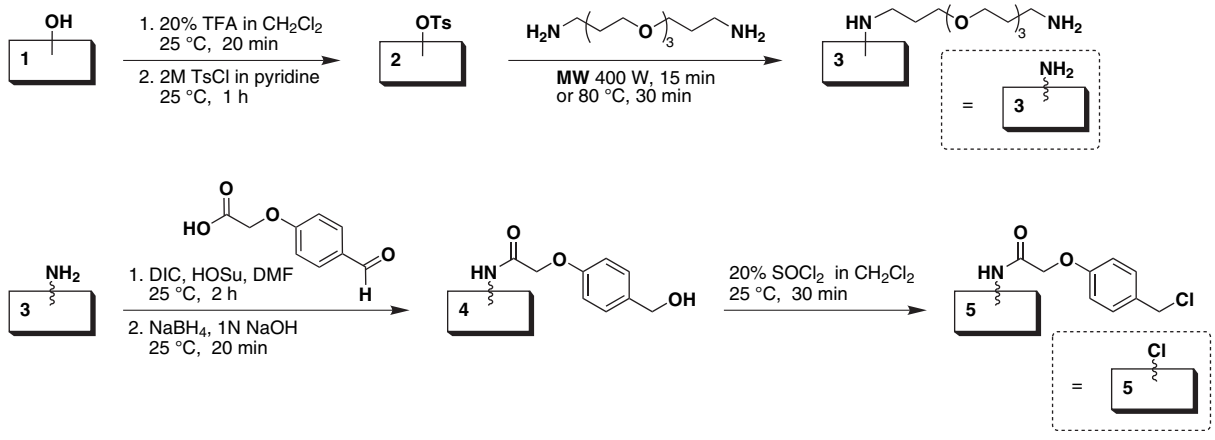
Examples of non-peptidic libraries generated via SPOT-synthesis are limited, and most have relied on simple acylation chemistry.[16] More demanding chemistry has not been pursued, in part, because (1) SPOT-synthesis suffers from slow reaction rates similar to conventional solid-phase synthesis, and (2) general, reproducible methods for heating spatially addressed reactions are not available. We reasoned that the application of microwave (MW) heating during SPOT-synthesis could address these two limitations and broaden the reaction scope of the method.[17] The use of MW irradiation as an unconventional heating method for organic synthesis has increased dramatically over the past decade, primarily due to the observed reductions in reaction times and concomitant enhancements in conversion and purity.[18] The wider availability of commercial MW synthesis reactors has also played a role in the expansion of this nascent field.

Recently, we demonstrated the successful union of these two technologies through the efficient MW-assisted synthesis of high purity 1,3-diphenylpropenone (chalcone),[19] dihydropyrimidine, and α-acylamino amide macroarrays.[20] These macroarrays represent some of the most complex small molecule libraries generated via SPOT-synthesis to date. In this preliminary work, we developed two robust cellulose support systems functionalized with orthogonal linker systems (Fig. 1): one acid-cleavable,[19] and the other photocleavable.[20] The application of MW irradiation to selected reactions in macroarray synthesis allowed for small molecule libraries (ca. 100-member) to be constructed at unprecedented rates (on the order of hours). This work sets the stage for the full integration of MW-assisted reactions in SPOT-synthesis to examine the scope and limitations of small molecule macroarrays as a new combinatorial chemistry platform.

Toward this goal, we have performed a systematic optimization of the MW-assisted SPOT-synthesis process using our chalcone macroarrays[19] as a lead example. Here, we report full details of this optimization study, including (1) the synthesis of the linker-derivatized support, (2) the loading of initial building blocks, and (3) the Claisen–Schmidt condensation to produce the chalcones. We also critically evaluated the efficiency and utility of MW-assisted reactions in array synthesis in comparison to conventional heating methods. Our optimization study ultimately revealed new modes of reactivity for certain library building blocks used in chalcone macroarray synthesis—this discovery permitted straightforward access to a set of 2,4,6-triarylpyridine macroarrays.

2. Results and discussion

Optimization of linker derivatization procedure. Our first goal was to reduce the total time required to generate the acid-cleavable (Wang-type)[21] linker-derivatized support used in macroarray synthesis. Our originally reported reaction sequence took over 21 h and was a considerable bottleneck in macroarray construction.[19] The overall procedure for preparation of linker-derivatized support **4** is outlined in Scheme 1. The first step was a pre-swelling of the cellulose support (**1**, 20 cm×20 cm sheet) using trifluoroacetic acid (TFA) to increase the surface area and thus the number of reactive sites for derivatization. We found that by doubling the concentration of TFA in the initial swelling step from 10% to 20% we could achieve better reproducibility in linker loading levels. Removing the residual TFA from the support prior to subsequent steps, however, required lengthy drying times in a vacuum oven (over 16 h). We were pleased to find that replacing the vacuum drying with two 5-min washes of anhydrous CH_2Cl_2 and a 20 min drying time using a stream of N_2 gave a support of equal quality. Notably, this simple procedural adjustment significantly reduced the synthesis time (by 15 h). Next, the pre-swelled paper (**1**) was reacted with tosyl chloride (TsCl) in pyridine (2.0 M) at room temperature following our original procedure to generate tosylated cellulose support **2**.[19] We found that subjecting the support to the TsCl solution for variable amounts of time gave different and reproducible tosylation



Scheme 1. Preparation of Wang-linker-derivatized support **4** and chloride-derivatized, 'activated linker', support **5**. Supports were washed with various solvents and dried routinely between each synthetic step.

levels (1 h ~3.8 μmol/cm^2, 10 h ~10.0 μmol/cm^2, as determined via subsequent amination reactions).[22] A flexible diamino 'spacer' unit, 4,7,10-trioxa-1,13-tridecanediamine, was then introduced to support **2** through standard nucleophilic substitution chemistry (Fig. 1). This 'spacer' unit has been shown to improve the accessibility of array-bound molecules for subsequent reactions and on support assays.[23] We found that the spacer could be coupled to tosylated support **2** by either MW-assisted conditions (400 W, 15 min in a Milestone MicroSYNTH Labstation multimode MW reactor)[24,25] or by conventional heating in an oven (80 °C, 30 min; see below) to achieve equivalent amine loadings (ca. 4–10 μmol/cm^2). Thus, use of the MW reactor in this displacement step provided only a minor reduction in synthesis time.

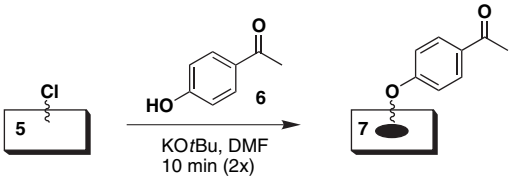
We found that the Wang-linker-derivatized membrane **4** could be prepared from amino support **3** using two different, yet complimentary, methods. Both methods involve two stages: first, the 'pro-linker', 4-formylphenoxyacetic acid, was coupled to support **3** via a standard carbodiimide (DIC) coupling at room temperature. Next, the resulting aldehyde support was reduced using sodium borohydride ($NaBH_4$) at room temperature to yield benzylalcohol-derived support **4**. The two methods only differed in *how* the pro-linker was physically applied to the planar support in the first step. In the first method, the entire membrane was immersed in the pro-linker coupling solution for 2 h in a 'blanket-type' functionalization. This method gave the highest linker loading overall (2.6 μmol/cm^2).[26] However, this bulk procedure required a large quantity of pro-linker reagent, and does not represent a general linker loading strategy if the linker reagent is either expensive or difficult to synthesize. In the second method, the pro-linker was applied in a spatially addressed, or 'spotted', manner, delivering 6.0 μL aliquots of the activated solution to individual spots on amino support **3** (applied twice over 2 h). Spatially addressed delivery only required 20% of the quantity of linker relative to the blanket-type method; however, linker loadings were diminished (1.8 μmol/cm^2). Since 4-formylphenoxyacetic acid is readily available either synthetically[19,27] or from commercial sources, we chose to use the blanket-type functionalization technique to generate support **4** throughout the present study. Using this optimized method, we routinely prepared four 20 cm×20 cm sheets of linker-derived support **4**, each with 120 spot-capacity, in less than 8 h. This represents a significant improvement in synthesis efficiency, as our original method required over 21 h to generate a single sheet of support **4**. The sheets were found to be stable at room temperature for at least one month (stored in a desiccator) and could be used in an 'off-the-shelf' manner for small molecule macroarray construction.

Benzylic alcohol-derived support **4** requires activation prior to the attachment of macroarray building blocks; we found conversion of support **4** to the benzylic chloride **5** to be an effective activation approach (Scheme 1). In our initial work, we examined chlorination of support **4** with TsCl in anhydrous DMF.[19] This method was extremely sensitive to the level of moisture in the DMF solvent. Careful optimization of the chlorination procedure revealed that chlorination with $SOCl_2$ in anhydrous CH_2Cl_2 gave comparable chlorination levels to our original method (based on the loading of 4′-hydroxyacetophenone (**6**), see below), yet was far more reproducible. We ascribe this to the relative ease of maintaining an anhydrous environment with CH_2Cl_2. After chlorination, activated membranes **5** were stored under N_2 and had to be submitted promptly to macroarray construction (within 15 min of preparation) in order to minimize hydrolysis.

Optimization of initial building block loading. We next examined the loading of hydroxyacetophenone building blocks onto activated support **5** under a variety of conventional and MW heating conditions to determine if MW-assisted reactions provided an advantage for this step in macroarray construction (Table 1). We selected 4′-hydroxyacetophenone (**6**) as a model substrate, and identical conditions were used for all reactions to facilitate direct comparison between the two heating methods (3.0 μL aliquot of 2.0 M **6** and 2.0 M KO*t*Bu in anhydrous DMF, 10 min). Application of this volume of reagent to support **5** provided a spot with a 0.3 cm^2 area. Conventional heating conditions were examined in a standard laboratory drying oven (VWR model #13OOU, for ≥80 °C) or a laboratory incubator (Lab-Line Imperial II, for 40 °C reactions). MW heating conditions for this step were examined in the Milestone MicroSYNTH multimode MW reactor. We discovered that the temperature of the planar support surface could be measured easily during these reactions using a non-contact IR thermometer (Craftsman model # 82327) positioned in apertures at the top of either the oven or MW reactor.

The first challenge that needed to be overcome in this study was the inconsistency of heating in a drying oven. We found that, depending on the position in the oven, the temperature could vary easily by up to ±10 °C. This made the uniform heating of 20 cm×20 cm planar support sections in the oven difficult, especially over short times (e.g., 5–10 min).

Table 1. Optimization of loading of model acetophenone substrate **6** onto planar support **5**



Entry	Conditions (final membrane temperature)	Substrate loading (nmol/cm^2)[a]
1	Room temperature (22 °C)	60
2	Oven (40 °C)[b]	80
3	Oven (80 °C)	260
4	MW 500 W (40 °C)[c]	120
5	MW 500 W, on Pyrex dish (80 °C)[d]	120
6[e]	Oven (80 °C)	190

[a] Determined by integration of the HPLC trace with UV detection at 254 nm. Integration values were compared to a UV calibration curve generated for **6**. Error ±10 nmol/cm^2.
[b] Performed in a laboratory incubator.
[c] Support suspended in the middle of the MW reactor cavity using tape to minimize heating due to conduction from a surface.
[d] Support placed in flat 2.6 L Pyrex dish and then on rotor in MW cavity. Pyrex dish thickness=0.5 cm.
[e] Prepared using support **4** on which the linker was applied in a spatially addressed manner.

We solved this homogeneity problem by placing a large sand bath in the oven that was allowed to equilibrate to the target temperature overnight prior to a conventional heating experiment. The sand bath was found to give stable and reproducible temperatures that varied only by ±1 °C. The planar membranes were heated by simply placing the membrane on top of the sand. The sand bath provided an added benefit to conventional heating in the oven, as we found its presence minimized dramatic temperature changes that accompanied the opening and closing of the oven door.

Not surprisingly, the loading of model acetophenone **6** increased steadily with increasing temperature using conventional heating methods, with the highest achievable loading obtained at 80 °C (Table 1, entries 1–3). Our original heating method using the MW oven (entries 4 and 5), however, resulted in an intermediate loading level relative to conventional heating, even when the final temperature of the membrane also reached 80 °C (entry 5).[25] The rapid heat transfer observed from the pre-heated sand bath in the oven to the support could explain this difference, in part. After 30 s of heating on the sand bath, the temperature of the support was measured to be 80 °C. In contrast, the temperature of the Pyrex dish used in the MW-assisted reaction only reached 80 °C (from room temperature) after 20 min of constant irradiation in the MW reactor (500 W).

Interestingly, the loading values achieved when the support was heated either in or outside of a Pyrex dish in the MW reactor were equivalent (entries 4 and 5). This suggests that a different heating mechanism than simple conduction could be operative for these MW-assisted reactions. Quantifying such a heating mechanism is difficult using bulk temperature measurements such as IR, as often these measurements do not reflect microenvironments of higher temperatures in a material (or 'hotspots').[28] The presence of such hotspots could explain the higher loading achieved using the MW reactor (without the Pyrex dish, entry 4) versus heating in the oven at 40 °C (entry 2), even though the bulk temperatures of the membranes were measured to be identical. Finally, comparison of entries 3 and 6 illustrates the lower hydroxyacetophenone loadings obtained when the pro-linker is applied in a spatially addressed manner as opposed to in a blanket-type functionalization (see above).

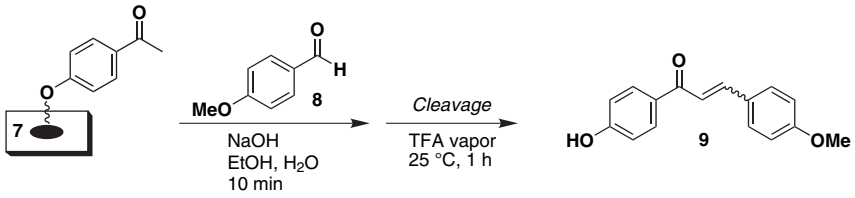
This study revealed that heating in an 80 °C oven was the highest yielding method for initial hydroxyacetophenone substrate attachment. We note, however, that this procedure only gave hydroxyacetophenone loading values equal to 10% of the available reactive linker sites (260 nmol/cm^2 vs 2.6 μmol/cm^2).[22] Repetitive applications of reagents and heating failed to show a significant improvement in loading for this reaction. We believe that there are two possible causes for this outcome. First, the benzylic chlorides of activated support **5** could simply react with residual water in the membrane or with free hydroxyls on the cellulose surface as opposed to substrate **6**. The use of non-anhydrous DMF or older bottles of KO*t*Bu has been observed to reduce loading. Second, native hydroxyl groups on the cellulose paper also could be activated during the chlorination protocol and compete with the activated linker for substrate. Evidence for this latter theory was obtained when fluorescent hydroxyacetophenones were coupled to **5** and repeated exposure to cleavage and elution conditions failed to remove the substrates completely from the surface (i.e., fluorescence was observed when the membranes were irradiated with UV light). Ongoing efforts are focused on developing alternate activation protocols for linker-derivatized support **4** to surmount these two obstacles and increase loadings.

Optimization of the Claisen–Schmidt condensation. We have shown previously that the Claisen–Schmidt condensation proceeds smoothly under MW-assisted conditions on planar support-bound acetophenones (400 W, 20 min).[19] For the present study, we were interested in systematically comparing the MW-assisted reaction to that performed under conventional heating, using the analogous drying oven conditions and non-contact temperature measurements described above for acetophenone loading. Support-bound acetophenone **7** was selected as the substrate for optimization studies (Table 2). We found that a wide variety of substituted benzaldehydes were reactive with **7** under the following spatially addressed, Claisen–Schmidt condensation conditions: 6.0 μL aliquot of 1.0 M benzaldehyde and 1.5 N NaOH in 50% aq EtOH, 10 min. We chose *p*-anisaldehyde (**8**) as a condensation partner for further optimization studies, as this substrate was found to react to give chalcone product **9** at a rate that was convenient for repeated analyses (e.g., entry 1, 22% conversion to **9** after 10 min at room temperature). Again, macroarray spots of 0.3 cm^2 area were examined. Conversion and yield of chalcone **9** were determined after TFA vapor compound cleavage from the support (see Section 4 for full details of the cleavage protocol).

Similar to our observations for acetophenone **6** loading, conversion to chalcone product **9** increased steadily with increasing temperature using conventional heating and one application of anisaldehyde (**8**) (entries 1–4). Application of a second aliquot of anisaldehyde (**8**) and heating again at either 80 °C or 120 °C were observed to increase conversion (entries 5 and 6); however, substantial byproducts appeared and the yield of chalcone **9** was diminished at the higher temperature. MW-assisted conditions (500 W) with one application of anisaldehyde (**8**) (entry 7) gave similar conversions to chalcone product **9** as conventional heating in the oven at 40 °C (entry 2).[25] Here, as expected, heating to 40 °C using either the MW reactor or oven gave similar results. Again, the use of the Pyrex dish did not significantly impact conversion for the MW-assisted reaction (entry 8). Analogous to conventional heating, a double application of anisaldehyde (**8**) in the MW-assisted reaction gave a marked increase in the product conversion (entry 9) that was comparable to double coupling and conventional heating at 80 °C (entry 5). However, the yield of chalcone **9** was slightly reduced. Finally, careful optimization of both heating methods revealed that spotting the anisaldehyde (**8**) solution and heating at 80 °C in the oven three times gave the highest yield and purity of chalcone product **9** (entry 10). Conventional heating methods for the Claisen–Schmidt condensation therefore appear slightly superior for this step in macroarray synthesis.

We next investigated the potential use of hydroxybenzaldehydes, as opposed to hydroxyacetophenones, as the planar support-bound substrate in the Claisen–Schmidt condensation. This approach was attractive because a substantially

Table 2. Optimization of Claisen–Schmidt condensation of support-bound acetophenone **7**



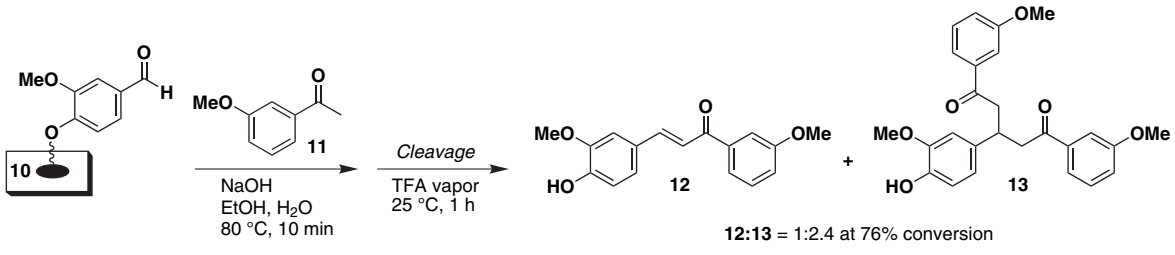
Entry	Conditions (final temperature)	Conversion (%)[a]	Purity of **9** (%)[b]	Yield of **9** (%)[c]
1	Room temperature (22 °C)	22	13	16
2	Oven (40 °C)[d]	46	29	30
3	Oven (80 °C)	81	62	48
4	Oven (120 °C)	90	76	59
5[e]	Oven (80 °C)	95	83	72
6[e]	Oven (120 °C)	97	69	49
7	MW 500 W (40 °C)[f]	45	26	26
8	MW 500 W Pyrex dish (70 °C)	47	24	23
9[g]	MW 500 W Pyrex dish (80 °C)	92	81	62
10[g]	Oven (80 °C)	>99	97	87

[a] Based on residual **6** observed in HPLC spectra and quantified using a UV calibration curve at 254 nm. Error ±3%.
[b] Determined by integration of HPLC spectra with UV detection at 254 nm. Error ±3%.
[c] Quantified using a UV calibration curve at 254 nm. Error ±5%.
[d] Performed in a laboratory incubator.
[e] Anisaldehyde (**8**)/base solution was applied a second time and the support was heated for an additional 10 min.
[f] Support suspended in the middle of the MW reactor cavity using tape to minimize heating due to conduction from a surface.
[g] Anisaldehyde (**8**)/base solution was applied a third time and the support was heated for an additional 10 min.

larger number of substituted hydroxybenzaldehyde building blocks are commercially available relative to substituted hydroxyacetophenones, and could permit the construction of chalcone macroarrays with increased structural complexity. We were pleased to observe that our optimized loading conditions for hydroxyacetophenones described above were directly translatable to hydroxybenzaldehydes, achieving analogous compound loadings (ca. 290 nmol/cm^2). However, problems arose during the subsequent Claisen–Schmidt condensation step between support-bound hydroxybenzaldehydes and solution-phase acetophenones, due to two competing reaction pathways. A representative reaction of support-bound vanillin (**10**) with 3′-methoxyacetophenone (**11**) is shown in Scheme 2. We discovered that the desired Claisen–Schmidt condensation reaction between **10** and **11** was in competition with Michael addition of **11** to the newly formed chalcone. Even after one application of the acetophenone (**11**) solution, three distinct species could be observed after 10 min at 80 °C (in the oven): (1) the starting material vanillin, (2) the target chalcone **12**, and (3) a byproduct **13** (as determined post-cleavage). The latter compound was the major product (2.4:1 **13**:**12** at 76% conversion). Its identity as 1,5-diketone **13** was confirmed by comparison to an authentic sample of **13** that was synthesized and characterized separately (see Section 4). Further optimization of this reaction to give chalcone **12** selectively is ongoing in our laboratory.

The side reaction to generate diketone **13**, while initially unwanted, proved fortuitous. By spotting vanillin-derived support **10** with acetophenone **11** and heating four times in succession (80 °C in the oven, 10 min each), we were able to drive the reaction predominantly to the 1,5-diketone product **13** (88% conversion). In our previous work, we had already demonstrated the versatility of the chalcone scaffold for the generation of second-generation heterocyclic macroarrays.[19] We sought to use this new reaction pathway to our advantage for the construction of small molecule macroarrays with increased structural complexity. Thus, we submitted support-bound 1,5-diketones to a variety of reaction conditions to explore their reactivity in macroarray synthesis.

Synthesis of triarylpyridine macroarrays. Diketones such as **13** have been used previously as precursors to 2,4,6-triarylpyridines.[29,30] Triarylpyridines have found wide use as building blocks for supramolecular chemistry[31] and as chemosensors,[32] and the development of efficient methods to generate this class of compounds has attracted considerable interest. Traditional syntheses of triarylpyridines involving acetophenones and benzaldehydes are low yielding, due to



Scheme 2. Reaction of support-bound vanillin **10** gives two products (**12** and **13**).

the intermediate dihydropyridine reducing the intermediate chalcone.[33] To alleviate this unwanted side reaction, Kröhnke developed an elegant alternative utilizing pyridinium salts.[29] Recent reports of MW-assisted and solid-phase synthetic routes to triarylpyridines from chalcone precursors[34,35] inspired us to attempt their synthesis from support-bound diketones (e.g., compound **14** in Scheme 3). We chose to examine MW-assisted reaction conditions with **14** first to establish if the chemistry was feasible. Ammonium acetate (NH_4OAc) has been used frequently in conjunction with acetic acid in this reaction; however, we believed that the acid-cleavable linker on the macroarray could be unstable under these conditions, especially at elevated temperatures. Instead, we chose to use a neutral, concentrated solution (3 M) of NH_4OAc in water for this condensation reaction.

Our initial screen of MW-assisted reaction conditions to generate 2,4,6-triarylpyridine **15** from support-bound diketone **14** revealed that the reaction proceeded best when the entire membrane was submerged in the aq NH_4OAc solution, that is, under blanket-type reaction conditions. To examine reactions on small sections of support **14** (e.g., several punched-out spots), we found that performing the reaction in sealed 10 mL glass reaction vessels in a CEM Explorer monomodal MW system was most convenient.[36] These sealed-tube MW reaction conditions had two positive attributes: they permitted (1) automated temperature control during the MW reaction and (2) heating over the boiling point of the solvent (i.e., 100 °C for water). The latter feature was important, as high temperatures were required for this reaction to proceed. Different temperatures were evaluated (120–180 °C) over 20 min reaction times. We found that 160 °C afforded the highest purity of the triarylpyridine product **16r** product (82%), as compared to the 70% purity achieved at either 120 °C or 180 °C (as determined post-cleavage).[37] We were pleased to observe that the cellulose support was physically stable under these more forcing reaction conditions.

For the synthesis of full triarylpyridine macroarrays (40 spots), we found that the standard, 10 mL glass MW reaction tubes were not large enough to accommodate the planar support (6 cm×15 cm, rolled into a tube). Instead, using a 70 mL Teflon/polyetheretherketone (PEEK) reaction vessel (shown in Fig. 2) in the Milestone MW multimode reactor proved more suitable for full macroarray synthesis. The temperature of the MW-assisted reaction could be controlled using a fiber-optic probe threaded into the vessel. In general, MW-assisted conditions directly translated from the monomodal to multimodal MW systems, if the reactions were performed under temperature control.[38] The only minor change we incorporated was a slower 10 min ramp time to 160 °C in the Milestone MW reactor relative to the CEM MW reactor, in order to protect the integrity of the reusable Teflon inserts. The total reaction time was 1 h, consisting of a 10 min ramp, a 20 min hold time, and a 30 min cool down period (no MW) to 70 °C, after which the vessel could be safely opened to retrieve the membrane.

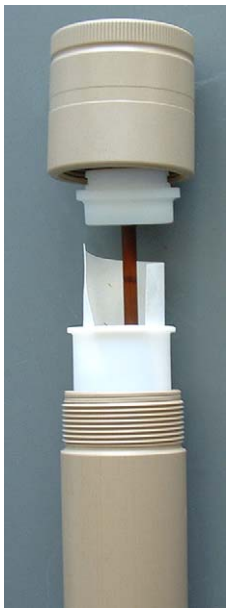
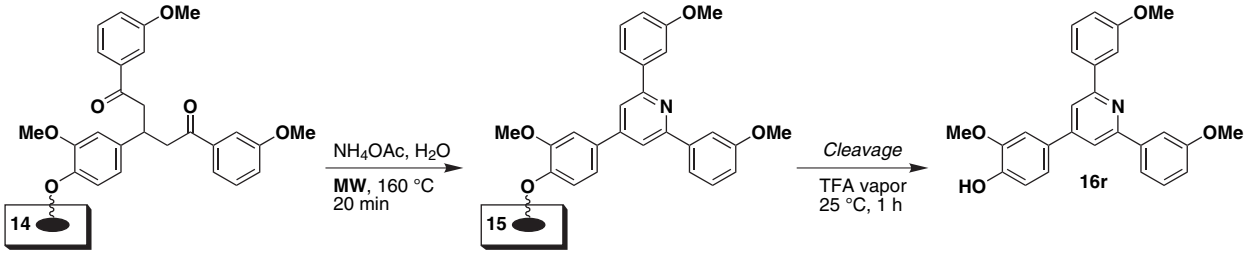


Figure 2. Reaction format for MW-assisted macroarray reactions. Shown is a macroarray that was rolled and placed into a 70 mL Milestone Teflon/PEEK reaction vessel. Reactions are performed in reusable Teflon inserts (white) that fit inside the PEEK outer casing (beige). A ceramic sheath in the center (brown) houses a fiber-optic probe for direct temperature measurement during the reaction. Vessel dimensions: 12 cm tall with an internal diameter of 3 cm.

In analogy to the optimization studies above, we also examined this reaction under conventional heating conditions. Heating the sealed polymeric vessel to 160 °C, however, was problematic for two reasons. First, the outer PEEK vessel acts as a highly efficient insulator; for example, in the MW-assisted triarylpyridine condensation reaction, the temperature of this outer vessel was measured to be only 120 °C (using a non-contact IR thermometer) even after the contents had been heated at 160 °C for 20 min (as determined by using a fiber-optic probe). This insulation made it extremely difficult to mimic the MW temperature gradient inside the vessel using an external, conventional heating source (e.g., an oil bath). Matching heating gradients is a frequent challenge when performing comparisons of conventionally heated processes with MW-assisted variants.[18a] Second, we

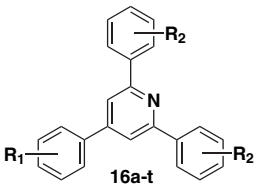


Scheme 3. Representative MW-assisted synthesis of 2,4,6-triarylpyridines (**16**) from support-bound diketones (**14**).

had safety concerns about the prolonged conventional heating of closed vessels for macroarray synthesis. Thus, we examined this reaction at 80 °C under atmospheric conditions in the drying oven. These conditions gave lower conversion: for example, triarylpyridine **16r** was generated in only 55% conversion when membrane **14** was heated in aq NH_4OAc in the oven at 80 °C for 1 h. However, complete conversion (>99%) and high purity product (89%) were obtained after 12 h at 80 °C in the oven. Therefore, application of MW heating to this condensation reaction provides a clear advantage over conventional heating in terms of reaction time. These benefits are achievable, in part, because the MW reactor and specialized MW vessels allow the safe and reproducible heating of solvents over their boiling points.[18] Indeed, we believe that this is one area of chemistry where MW-assisted reactions are poised to make a large impact.

We used the optimized MW-assisted method developed above to construct a 40-member macroarray of symmetrical 2,4,6-triarylpyridines (**16**) using four hydroxybenzaldehyde and 10 acetophenone building blocks. Starting from linker-derived support **4**, the macroarray was synthesized and the array compounds were cleaved in only 12 h (Table 3). Analysis of 20 randomly selected macroarray members (50% of the library) by LC–MS indicated good to excellent product purities (63%–91%). Purities were largely substrate dependent, with the lowest purities occurring when a fluoro- or methoxy-substituent was in the *para* position of the incoming acetophenone. We speculate that this reduction in purity could be due to positive mesomeric effects deactivating the chalcone for subsequent Michael addition. Two triarylpyridines (**16l** and **16r**) were selected for synthesis in solution as controls in order to determine representative overall reaction yields on the triarylpyridine macroarray. Examination of calibration curves generated for **16l** and **16r** showed product yields of 78% and 88%, respectively, after cleavage of these compounds from the macroarray. These results demonstrate that macroarray synthesis represents a rapid and high yielding approach for the generation of symmetrical 2,4,6-triarylpyridines (**16**).

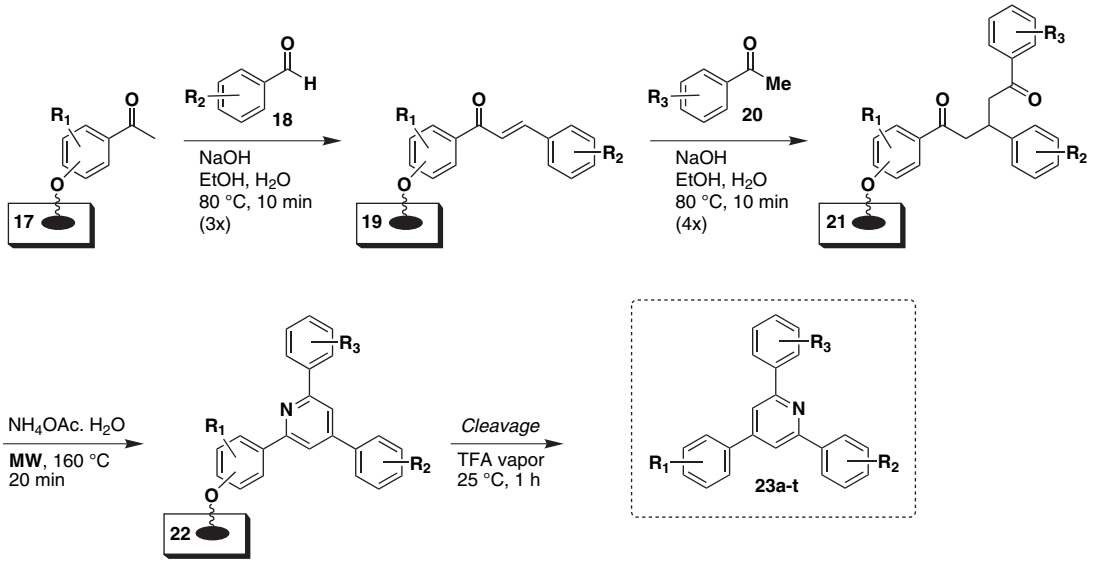
Table 3. Purity data for selected members of symmetrical 2,4,6-triarylpyridine macroarray **16**



Entry	Compound	R_1	R_2	Purity (%)[a]
1	**16a**	3-OH	3-F	85
2	**16b**	3-OH	4-F	75
3	**16c**	3-OH	3-Br	84
4	**16d**	3-OH	3-CF_3	85
5	**16e**	3-OH	4-CF_3	83
6	**16f**	4-OH	H	91
7	**16g**	4-OH	4-Br	83
8	**16h**	4-OH	3-MeO	86
9	**16i**	4-OH	3,4-MeO	63
10	**16j**	4-OH	3-CF_3	85
11	**16k**	4-OH, 3-OMe	3-F	76
12	**16l**	4-OH, 3-OMe	3-Br	78
13	**16m**	4-OH, 3-OMe	4-Br	84
14	**16n**	4-OH, 3-OMe	3-OMe	90
15	**16o**	4-OH, 3-OMe	4-OMe	63
16	**16p**	3-OH, 4-OMe	3-F	75
17	**16q**	3-OH, 4-OMe	4-F	67
18	**16r**	3-OH, 4-OMe	3-OMe	88
19	**16s**	3-OH, 4-OMe	3-CF_3	73
20	**16t**	3-OH, 4-OMe	4-CF_3	74

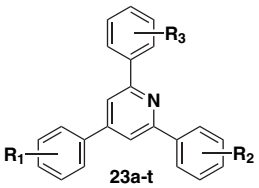
[a] Crude purity determined by integration of the HPLC trace with UV detection at 254 nm. Error ±3%.

Further examination of triarylpyridine synthesis on our planar support platform revealed that unsymmetrical triarylpyridines were also accessible. Starting with support-bound acetophenones, rather than benzaldehydes, we found that a far greater diversity of triarylpyridine products (**23**) could be achieved, as the products could be constructed in a step-wise manner (Scheme 4). In an initial step, bound acetophenone building blocks (**17**) could be reacted with a series of benzaldehydes (**18**) to form chalcone macroarrays (**19**),



Scheme 4. Synthesis of unsymmetrical 2,4,6-triarylpyridine macroarray **23**.

Table 4. Purity data for selected members of unsymmetrical 2,4,6-triarylpyridine macroarray **23**



23a-t

Entry	Compound	R_1	R_2	R_3	Purity (%)[a]
1	**23a**	4-OH	H	4-Br	81
2	**23b**	4-OH	H	4-CF_3	79
3	**23c**	4-OH	3-Br	3-CF_3	80
4	**23d**	4-OH	3-Br	3-OMe	75
5	**23e**	4-OH	4-OMe	4-Br	70
6	**23f**	4-OH	4-Cl	3-OMe	67
7	**23g**	3-OH	H	3-OMe	72
8	**23h**	3-OH	H	3-CF_3	81
9	**23i**	3-OH	3-Br	4-Br	78
10	**23j**	3-OH	3-Br	4-CF_3	76
11	**23k**	3-OH	4-OMe	H	76
12	**23l**	3-OH	4-Cl	4-Br	76
13	**23m**	3-OH	4-Cl	3-CF_3	80
14	**23n**	3-OH	4-OMe	3-OMe	74
15	**23o**	4-OH, 3-OMe	H	3-OMe	70
16	**23p**	4-OH, 3-OMe	3-Br	4-Br	79
17	**23q**	4-OH, 3-OMe	3-Br	4-CF_3	82
18	**23r**	4-OH, 3-OMe	4-OMe	H	70
19	**23s**	4-OH, 3-OMe	4-OMe	3-CF_3	77
20	**23t**	4-OH, 3-OMe	4-Cl	3-CF_3	84

[a] Crude purity determined by integration of the HPLC trace with UV detection at 254 nm. Error ±3%.

using the optimized reaction conditions described above. In a subsequent step, the chalcones were treated with a second set of acetophenones to generate diketones (**21**). Here, we utilized conventional heating conditions to achieve full conversion to the diketones (see above). Finally, diketones (**21**) could be condensed with NH_4OAc using our optimized MW-assisted conditions in the Milestone MW reactor to give triarylpyridine arrays (**22**). This step-wise synthesis is noteworthy, as it allows for control of the substituents on each phenyl ring of the triarylpyridine (**23**), and thus is amenable to the construction of triarylpyridine macroarrays (**23**) of high structural complexity.

To evaluate the feasibility of this reaction strategy in macroarray synthesis, we constructed a 60-member macroarray of unsymmetrical 2,4,6-triarylpyridines (**22**) using three hydroxyacetophenone, four benzaldehyde, and five acetophenone building blocks (Table 4). Macroarrays of this size were readily constructed and cleaved in less than one day. Analysis of a random sampling of a third of the library members by LC–MS showed moderate to good purities (67%–84%). Overall, the ease of macroarray synthesis, along with the wide availability of numerous acetophenones and benzaldehydes, renders this method a powerful new technique for the rapid synthesis of 2,4,6-triarylpyridines (**23**).

3. Conclusions and outlook

We have performed a systematic optimization of the MW-assisted SPOT-synthesis process using chalcone macroarrays as our primary focus. Each step in the small molecule macroarray synthesis was critically examined and streamlined, including: (1) the synthesis of the linker-derivatized support, (2) the loading of initial building blocks, and (3) the Claisen–Schmidt condensation to give the chalcones. This work allowed for a dramatic reduction in synthesis time required to generate the linker-derivatized support **4** (by over 15 h); improved accessibility of this support will advance the examination of additional library pathways on the macroarray platform. Our optimization study also revealed new modes of reactivity for support-bound benzaldehydes and acetophenones used in chalcone macroarray synthesis—this discovery permitted straightforward production of a set of novel 2,4,6-triarylpyridine macroarrays, **15** and **22**, respectively.

This study also inspired a detailed comparison of MW heating versus conventional heating in macroarray synthesis. For both of the steps in chalcone synthesis, conventional heating methods gave equal, if not slightly improved, results relative to MW-assisted conditions. However, we found that for reactions that require temperatures higher than the boiling points of the solvents, i.e., in triarylpyridine synthesis, the use of the MW reactor provided more convenient and safe access to these temperatures and pressures relative to conventional heating. These results indicate that, while small molecule macroarray synthesis benefits from MW-assisted reactions, the technique is not fully reliant on them. This discovery is important, as it broadens the availability of our synthesis platform to researchers who may not have access to a commercial MW reactor.

Overall, this study has extended the scope of the small molecule macroarray synthesis technique and streamlined the construction process. The stage is now set for the systematic exploration of the chemical reactions and screening formats that are compatible with the small molecule macroarray platform. These studies are underway in our laboratory. In the future, the synthetic route to 2,4,6-triarylpyridines (**16** and **23**) described herein could be developed further to generate arrays of different, and potentially useful, molecules. For example, terpyridines and bipyridines, which have found widespread application in chemical sensors[39] and organic light emitting diodes,[40] could be synthesized readily using this method through the incorporation of acetyl pyridine building blocks. The planar array format could facilitate a rapid screening of their sensing or photophysical properties while bound to the support.[41] Current efforts in our laboratory are focused broadly in this area and will be reported in due course.

4. Experimental

4.1. General

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-300 spectrometer in deuterated solvents at 300 MHz and 75 Hz, respectively. Chemical shifts are reported in parts per million (ppm, δ) using tetramethyl silane (TMS) as a reference (0.0 ppm). Coupling constants are reported in Hertz. LC–MS (ESI) was obtained using a Shimadzu LCMS-2010 (Columbia, MD) equipped with two pumps (LC-10ADvp), controller (SCL-10Avp), autoinjector (SIL-10ADvp), UV diode array detector (SPD-M10Avp), and single quadrupole

analyzer (by electrospray ionization, ESI). The LC–MS is interfaced with a PC running the Shimadzu LC–MS solution software package (Version 2.04 Su2-H2). A Supelco (Bellefonte, PA) 15 cm×2.1 mm C-18 wide-pore reverse phase column was used for all LC–MS work. Standard reverse phase HPLC conditions for LC–MS analyses were as follows: flow rate=200 μL/min; mobile phase A=0.4% formic acid; mobile phase B=0.2% formic acid in acetonitrile. HPLC analyses were performed using a Shimadzu HPLC equipped with a single pump (LC-10ATvp), solvent mixer (FCV-10ALvp), controller (SCL-10Avp), autoinjector (SIL-10AF), and UV diode array detector (SPD-M10Avp). A Shimadzu Premier 25 cm×4.6 mm C-18 reverse phase column was used for all HPLC work. Standard reverse phase HPLC conditions were as follows: flow rate=1.0 mL/min; mobile phase A=0.1% trifluoroacetic acid (TFA); mobile phase B=0.1% TFA in acetonitrile. UV detection at 254 nm was used for all HPLC analyses. Compound purities were determined by integration of the peaks in HPLC traces measured at this wavelength.

Attenuated total reflectance (ATR)-IR spectra were recorded with a Bruker Tensor 27 spectrometer, outfitted with a single reflection MIRacle Horizontal ATR by Pike Technologies. A ZnSe crystal with spectral range 20,000–650 cm^{-1} was used. UV spectra were recorded using a Cary 50 Scan UV–Vis spectrometer running Cary WinUV 3.00 software. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (E-5715-7, Merck). All reported melting points are uncorrected. Reactions subjected to oven heating were performed on a pre-heated bed of sand in a VWR 13OOU drying oven. Temperature measurements of planar surfaces were acquired using a Craftsman (model # 82327) non-contact IR thermometer with an error of ±2.5%. An Eppendorf pipette with a calibrated range between 0.5 μL and 10.0 μL outfitted with disposable plastic tips was used to 'spot' or apply reagents onto the membrane in a spatially addressed manner.

4.2. Microwave synthesis instrumentation

MW reactions involving macroarrays of greater than five spots were performed in a Milestone MicroSYNTH Labstation multimode MW synthesis reactor.[24] This instrument is equipped with a continuous power source (1000 W max) and interfaced with an Ethos MicroSYNTH Lab Terminal PC running EasyWave reaction monitoring software. Using this reactor system, MW irradiation can be applied to reactions using either power (wattage) control or temperature control. The MW reactor is equipped with a fiber-optic temperature sensor that allows direct monitoring of the internal temperature of reaction vessels, and an infrared sensor (installed in the side wall of the reactor cavity) that can monitor the surface temperature of reaction vessels inside the cavity. Solvent depths of ca. 1 cm in the reaction vessel are required for accurate temperature monitoring using the submerged fiber-optic temperature probe.

MW reactions involving macroarrays of less than five spots were performed in a CEM Discover MW reactor.[36] This monomode MW reactor is interfaced with a laptop PC running CEM ChemDriver software (v. 3.5.4) and is equipped with an autosampler (CEM Explorer) capable of holding 24×10 mL thick-walled Pyrex tubes. An external IR sensor is used to monitor the temperature. For each MW-assisted reaction, the ramp time to reach the target temperature was set to 40 min. However, in all of the reactions studied herein, the Discover MW reactor required no more than 2 min to reach the target temperature. Once the target temperature is reached, the MW system automatically starts to count down the hold time at this temperature. A non-invasive pressure sensor is used to monitor the pressure. The upper pressure limit was set to 280 psi: note, this pressure limit was never reached during the reactions outlined herein.

MW-assisted solution-phase reactions were performed in the Milestone MicroSYNTH MW reactor using specialized 70 mL Teflon/PEEK vessels (shown in Fig. 2). Reactions are performed in Teflon inserts that fit inside the PEEK outer casing that is designed to withstand temperatures of up to 200 °C and pressures of up to 280 psi. The internal temperature of the vessel can be monitored using a fiber-optic temperature sensor in a protective ceramic sheath. At pressures above the 280-psi limit, the vessels are designed to release excess pressure by venting and then reseal themselves. No evidence for the venting was observed during the course of the reactions described herein.

4.3. Materials

All reagents were purchased from commercial sources (Alfa-Aesar, Aldrich, and Acros) and used without further purification. Solvents were purchased from commercial sources (Aldrich and J. T. Baker) and used as obtained, with the exception of dichloromethane (CH_2Cl_2), which was distilled over calcium hydride immediately prior to use. Planar cellulose membranes (Whatman 1Chr chromatography paper, 20×20 cm^2) were purchased from Fisher Scientific and stored in a desiccator at room temperature until ready for use.

4.4. Methods

4.4.1. Preparation of cellulose supports.

4.4.1.1. Representative synthesis of amino cellulose support (3). The cellulose amination procedure was adapted from a literature protocol.[16c] Spots were marked on a 15 cm×18 cm sheet of Whatman 1Chr paper (**1**) at distances 1.4 cm apart using a #2 lead pencil. In this format, 120 spots (0.3 cm^2) can be accommodated on a single sheet without any detectable cross contamination. The sheet was immersed in 100 mL of 20% TFA in CH_2Cl_2 for 10 min in a covered 2.6 L Pyrex dish. The acid solution was decanted carefully away from the sheet. The sheet was washed by adding 60 mL of CH_2Cl_2, allowing the sheet to soak for 5 min, and then decanting the CH_2Cl_2. This washing procedure was repeated an additional time, then the membrane was dried under a stream of air for 20 min. TsCl (19.0 g, 50 mmol) was dissolved in 50 mL pyridine in a covered 2.6 L Pyrex dish. The solution was swirled for 5 min, after which the acid-swelled sheet was added. The sheet was swirled for 1 h, after which the TsCl solution was decanted. The support was washed by immersion in two consecutive baths of EtOH (100 mL, 5 min each), followed by immersion in CH_2Cl_2 for 5 min. Tosylated support **2** was dried under a stream of N_2 for 20 min.

A 60 mL aliquot of 4,7,10-trioxa-1,13-tridecanediamine was added to a covered 2.6 L Pyrex dish and heated in an oven to 80 °C. Tosylated support **2** was immersed in the pre-heated amine solution and heated for 30 min at 80 °C. The amine solution was carefully decanted from the paper. The support then was washed by adding and then decanting 70 mL portions of DMF, EtOH, 1.0 N NaOH, H_2O, EtOH (2×), and CH_2Cl_2 (5 min in each wash). Amino support **3** was dried under a stream of N_2 for 10 min.

4.4.1.2. Representative Fmoc quantitation protocol on cellulose supports. The amine loading of support **3** was quantified according to standard UV Fmoc analysis procedures.[22] A spot (6 mm diameter) was punched from amino support **3** using a desktop hole punch and immersed in 200 μL of 0.60 M *N*-(9-fluorenylmethoxycarbonyloxy) succinimide (Fmoc–OSu) in DMF for 2 h. The spot was washed with 10 mL of EtOH, 10 mL of acetone, and 10 mL of CH_2Cl_2. The spot was allowed to air dry for 20 min in a glass vial, after which 960 μL of DMF and 40 μL of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were added. The spot was swirled in this mixture for 30 s and then allowed to stand for 15 min. The mixture was swirled again for 30 s, then 100 μL of this solution was removed and diluted with 2.0 mL of DMF. The solution was swirled again for 30 s. The absorbance was read at 296 nm (ε_{296}=9500 M^{-1} cm^{-1}) in a quartz cuvette. The value was multiplied by 21 to account for the dilution. Loadings of 1.0–10 μmol/cm^2 were obtained using this method. Longer tosylation reaction times gave higher levels of functionalization (e.g., 1 h= 3.8 μmol/cm^2, 12 h=10 μmol/cm^2).

4.4.1.3. Representative synthesis of Wang-type linker functionalized cellulose support (4). 4-Formylphenoxyacetic acid[19] (5.40 g, 30.0 mmol), diisopropylcarbodiimide (DIC, 4.7 mL, 30.0 mmol), *N*-hydroxysuccinimide (HOSu, 3.45 g, 30.0 mmol), NEt_3 (4.2 mL, 30.0 mmol), and DMF (50 mL) were combined in a 2.6 L Pyrex dish. The dish was covered and swirled for 30 min at room temperature. A 15 cm×18 cm sheet of amino cellulose support (**3**) was added. The dish was covered again and the mixture was swirled at room temperature for 2 h on a rotary shaker. The coupling solution was decanted. The support was then washed by adding and then decanting 70 mL portions of DMF (2×), EtOH (2×), and CH_2Cl_2 (5 min in each wash). The aldehyde-derivatized support was dried under a stream of N_2.

A 100 mL aliquot of 1.0 M $NaBH_4$ in 1.0 M aq NaOH was added to the aldehyde-derivatized support. The mixture was swirled for 20 min, after which the $NaBH_4$ solution was decanted. The support was washed by adding and then decanting 70 mL portions of H_2O (2×), EtOH (2×), and CH_2Cl_2 (5 min in each bath). The benzyl alcohol-derivatized support (**4**) was dried under a stream of N_2. To approximate the linker loading, the amount of residual amine on support **4** was measured by Fmoc quantitation as described above, except an 11-fold dilution was used instead. Residual amine loadings on support **4** were found to be ca. 600–800 nmol/cm^2.

4.4.2. Preparation of small molecule macroarrays.

4.4.2.1. Preparation of chloride-derivatized support (5). A 15 cm×18 cm sheet of benzyl alcohol-derivatized support **4** was immersed in a solution of $SOCl_2$ (10 mL, 137 mmol) in CH_2Cl_2 (40 mL) in a 2.6 L Pyrex dish. The dish was covered and swirled for 30 min at room temperature. The $SOCl_2$ solution was carefully decanted, and the support was washed by immersing and decanting 100 mL portions of CH_2Cl_2 (3×, 2 min in each wash). The chloride-derivatized, or 'activated linker', support (**5**) was placed under a stream of N_2 to dry for 10 min and then used immediately in the next step.

4.4.2.2. Synthesis of hydroxybenzaldehyde/hydroxyacetophenone macroarrays (10 and 17). Solutions of various hydroxyacetophenones or hydroxybenzaldehydes (2.0 M) and KO*t*Bu (2.0 M) were prepared in anhydrous DMF in 4 mL vials and quickly sealed with Teflon caps. Aliquots (3.0 μL) of these solutions were applied to the appropriate spots on a 15 cm×18 cm sheet of activated linker support **5**. The spotted support then was placed on a bed of pre-heated sand in a drying oven set to 80 °C for 10 min. The spotting and heating steps were repeated (1×). The support was removed and washed by adding and decanting 100 mL portions of 1.0 N aq NaOH, H_2O, EtOH (2×), and CH_2Cl_2 (5 min in each wash). The resulting hydroxybenzaldehyde (**10**) or hydroxyacetophenone macroarrays (**17**) were dried under a stream of air for 20 min.

4.4.2.3. Representative synthesis of chalcone macroarray (19). A ca. 300–500 μL solution of substituted benzaldehyde (**18**, 1.0 M) and NaOH (1.5 N) was prepared in 50% aq EtOH in a 4 mL vial and sealed with a Teflon cap. (A 0.5 M solution of substituted benzaldehyde (**18**) in 1.5 N NaOH in 50% aq EtOH can also be substituted in cases where preparing a 1.0 M solution of benzaldehyde substrates is impossible due to solubility reasons. For example, 4-chlorobenzaldehyde and *m*-anisaldehyde require these alternate conditions). A 6.0 μL aliquot of this solution was applied to the appropriate spots on a 15 cm×18 cm sheet of hydroxyacetophenone macroarray **17**. The support then was placed on a bed of pre-heated sand in a drying oven set to 80 °C for 10 min. The spotting and heating steps were repeated (2×). The support was removed and washed by adding and decanting 100 mL portions of 1% aq AcOH, DMSO, EtOH (2×), and CH_2Cl_2 (5 min in each wash). The resulting chalcone macroarray (**19**) was dried under a stream of air for 20 min.

4.4.2.4. Representative synthesis of diketone macroarrays (15 and 22). A ca. 300–500 μL solution of substituted acetophenone (1.0 M) and NaOH (1.5 N) was prepared in 66% aq EtOH in a 4 mL vial and sealed with a Teflon cap. (A 0.5 M solution of acetophenone in 1.5 N NaOH in 66% aq EtOH can be substituted in cases where preparing a 1.0 M solution of acetophenone substrate is impossible due to solubility reasons. For example, 4′-bromoacetophenone and 3′-bromoacetophenone require these alternate conditions). A 6.0 μL aliquot of this solution was applied to the appropriate spots on hydroxybenzaldehyde macroarray **10** to generate symmetrical diketones or chalcone macroarray **19** to generate unsymmetrical diketones. The support was placed on a bed of pre-heated sand in the oven set to 80 °C for 10 min. The spotting and heating steps were repeated (3×). The membrane was removed and washed by adding and decanting 100 mL portions of 1% aq

AcOH, DMSO, EtOH (2×), and CH_2Cl_2 (5 min in each wash). The resulting symmetrical diketone (**15**) and unsymmetrical diketone macroarrays (**22**) were dried under a stream of air for 20 min.

4.4.2.5. Representative synthesis of triarylpyridine macroarrays (16 and 23). A 3.1 M stock solution of aq NH_4OAc was prepared by dissolving NH_4OAc (77.08 g, 1.0 mol) in 250 mL of water. A dried diketone macroarray (**15** or **22**) measuring 15 cm×6 cm (40 spots) was gently rolled into a tube and placed inside a 70 mL Teflon/PEEK MW reaction vessel. A 50 mL portion of the aq NH_4OAc solution was added to the vessel, and the vessel was sealed. Using this method, multiple macroarrays could be prepared simultaneously using multiple Teflon/PEEK reaction vessels. The vessels were placed on a rotating base inside the Milestone MW reactor, and the fiber-optic probe was introduced into one of the vessels. Using a maximum wattage of 800 W, the reaction mixtures were heated from room temperature to 160 °C over 10 min, held at 160 °C for 20 min, and allowed to cool for 30 min. The supports were removed, unrolled, and washed by adding and decanting 100 mL portions of H_2O, EtOH (2×), and CH_2Cl_2 (5 min in each wash). The resulting triarylpyridine macroarrays (**16** and **23**) were dried under a stream of N_2 for 20 min.

4.4.2.6. TFA vapor compound cleavage procedure. Compound spots were punched out using a standard desktop hole punch (0.6 cm diameter) and placed in individual 4 mL vials. A 10.0 mL portion of TFA was added to the bottom of a glass vacuum desiccator. The vials containing the spots were placed on a perforated ceramic platform in the desiccator that was situated 7 cm above the TFA. The desiccator was evacuated to 60 mm Hg over a 10 min period. The desiccator was disconnected from the vacuum, sealed, and allowed to stand for an additional 50 min at room temperature. The vials were removed from the desiccator and 1.0 mL of CH_3CN was added to each vial. The vials were sealed and shaken for 15 min, after which the spots were removed and the CH_3CN was concentrated under reduced pressure. The resulting residue was dissolved in 150 μL 50% aq CH_3CN and analyzed by HPLC.

4.4.3. Preparation of solution-phase standard compounds.

4.4.3.1. 4′-Hydroxy-4-methoxychalcone (9). 4′-Hydroxyacetophenone (**6**, 1.63 g, 12 mmol) and *p*-anisaldehyde (**8**, 1.45 mL, 12 mmol) were dissolved in MeOH (30 mL) in a 70 mL Teflon Milestone MW reaction vessel. A 2 mL aliquot of 50% (w/v) aq NaOH was added, and the solution was stirred until the reactants had dissolved fully. The reaction vessel was closed tightly and heated with stirring in a Milestone MW reactor from room temperature to 150 °C over 15 min, held at 150 °C for 20 min, and allowed to cool to room temperature over 20 min. The reaction mixture was poured over ca. 30 g of ice and acidified to pH 1.0 with 1.0 N HCl, forming a yellow precipitate. This solid was isolated by filtration and recrystallized from MeOH to afford 900 mg of golden crystals of **9** (30% yield). TLC: R_f=0.25 (hexane/EtOAc 3:2); melting point: 185–188 °C; ^{1}H NMR: (300 MHz, DMSO-d_6) δ 10.35 (br s, 1H), 8.05, 6.89 (AA′XX′, $J_{AA'}=J_{XX'}=2.4$, $J_{AX}=8.6$, $J_{AX'}=0.2$ Hz, 4H), 7.92, 7.00 (AA′XX′, $J_{AA'}=J_{XX'}=2.4$, $J_{AX}=8.6$, $J_{AX'}=0.2$ Hz, 4H), 7.80, 7.60 (AB peak, J=15.5 Hz, 2H); ^{13}C NMR: (75 MHz, DMSO-d_6) δ 187.7, 162.7, 161.8, 143.3, 131.7, 131.2m, 130.0, 128.207, 120.3, 116.0, 115.0, 56.0; IR (ATR): 3200, 2990, 1643, 1602, 1562, 1512, 1430, 1352, 1286, 1223, 1165, 1046 cm^{-1}; ESI-MS: expected, 254.1; observed, *m*/*z* 254.8 [M+H$^+$].

4.4.3.2. 3,3′-Dimethoxy-4-hydroxychalcone (12). 3-Methoxy-4-[(tetrahydro-2*H*-pyran-2-yl)-oxy]-benzaldehyde (hereafter called THP-vanillin,[42] 500 mg, 2.1 mmol), 3′-methoxyacetophenone (315 mg, 2.1 mmol), NaOH (84 mg, 2.1 mmol), and 5.0 mL of MeOH were combined in a 20 mL round-bottom flask equipped with a magnetic stirring bar and stirred for 12 h at room temperature. Acetyl chloride (0.5 mL, 7.0 mmol) was then added quickly to the flask. After stirring for 10 min, the mixture was poured into 20 mL of 1.0 N HCl and 20 mL of CH_2Cl_2. The solution was extracted twice with CH_2Cl_2. The organic phases were combined and extracted with 40 mL of 1.0 N NaOH. The aq phase was washed twice with CH_2Cl_2, acidified to pH 1.0 with 1.0 N HCl, and then extracted with 20 mL of CH_2Cl_2 (2×). The organic fractions were combined, dried over $MgSO_4$, filtered, and concentrated to a brown oil. The oil was purified by flash silica gel chromatography (CH_2Cl_2/EtOAc 4:1) to give 120 mg of **12** as a yellow oil (20% yield). TLC: R_f=0.69 (CH_2Cl_2/EtOAc 4:1); ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.78 (d, J=15.6 Hz, 1H), 7.57 (dt, J=7.7, 1.3 Hz, 1H), 7.52 (dd, J=4, 1.3 Hz), 7.37 (t, J=7.9 Hz, 1H), 7.34 (d, J=15.6 Hz, 1H), 7.18 (dd, J=8.2, 2.0 Hz, 1H), 7.10 (br s, 1H), 7.09 (ddd, J=8.2, 2.6, 1.0 Hz, 1H), 6.94 (d, J=8.2 Hz, 1H), 6.30 (s, 1H), 3.90 (s, 3H), 3.84 (s, 3H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 190.6, 160.1, 148.7, 147.2, 145.6, 140.1, 129.7, 127.6, 123.7, 121.2, 119.9, 119.1, 115.2, 113.2, 110.4, 56.2, 55.7; IR (ATR): 3394, 3055, 2940, 2836, 1657, 1577, 1513, 1487, 1465, 1452, 1431, 1376, 1321, 1200, 1174, 1158, 1123 cm^{-1}; ESI-MS: expected, 284.1; observed, *m*/*z* 285.0 [M+H$^+$].

4.4.3.3. 1,5-Di-(3-methoxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)-1,5-pentanedione (13). THP-vanillin[42] (500 mg, 2.1 mmol), 3′-methoxyacetophenone (630 mg, 4.2 mmol), and NaOH (168 mg, 4.2 mmol) were ground together in a mortar and pestle for 30 min. The resulting yellow paste was allowed to stand at room temperature for 12 h. The paste then was dissolved in 20 mL of 1.0 N HCl and 20 mL of CH_2Cl_2. The mixture was stirred for 1 h, after which the organic phase was separated. The aq phase was extracted two times with CH_2Cl_2. The organic phases were combined, dried over $MgSO_4$, filtered, and concentrated to give a brown oil. The oil was purified using flash silica gel chromatography (CH_2Cl_2/EtOAc 4:1) to give 30 mg of **13** as a colorless oil (0.5% yield). TLC: R_f=0.72 (CH_2Cl_2/EtOAc 4:1); ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.54 (dt, J=8.0, 1.2 Hz, 2H), 7.46 (dd, J=2.5, 1.2 Hz, 2H), 7.35 (t, J=8.0 Hz), 7.09 (ddd, J=8.0, 2.5, 1.2 Hz, 2H), 6.78 (m, 3H), 5.50 (s, 1H), 3.99 (p, J=7.0 Hz, 1H), 3.42, 3.38 (AB component of ABX system, J_{AB}=16.5, $J_{AX}=J_{BX}$=7.0 Hz, 2H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 198.6, 159.8, 146.4, 144.3, 138.4, 135.7, 129.6, 120.8, 119.6, 119.5, 114.5, 112.3, 110.7, 55.9, 55.4, 45.3, 37.2; IR (ATR): 3727, 3703, 3629, 3595, 3054, 1684, 1597, 1583, 1517, 1486, 1465, 1452, 1430, 1361, 1288, 1210, 1160, 1125 cm^{-1}; ESI-MS: expected, 434.2; observed, *m*/*z* 435.1 [M+H$^+$].

4.4.3.4. 2,6-Di-(3-bromophenyl)-4-(4-hydroxy-3-methoxyphenyl)-pyridine (16l). THP-vanillin[42] (500 mg, 2.1 mmol), 3′-bromoacetophenone (836 mg, 4.2 mmol), and NaOH (168 mg, 4.2 mmol) were ground together in a mortar and pestle for 30 min. The resulting orange paste was allowed to stand at room temperature for 1 h. The paste was then transferred to a 70 mL Teflon MW reaction vessel along with a magnetic stirring bar. A 0.5 mL aliquot of acetic acid was added to the reaction vessel, followed by butanol (5.0 mL) and hydroxylamine hydrogen chloride (510 mg, 7.3 mmol). The reaction vessel was closed tightly and heated with stirring in the Milestone MW reactor from room temperature to 170 °C over 10 min, held at 170 °C for 10 min, and allowed to cool to room temperature over ca. 30 min. A 20 mL portion of water was added to the vessel, and the reaction mixture was stirred for 8 h at room temperature. A white solid gradually formed. This solid was filtered to give 86 mg of triarylpyridine **16l** (8% yield). TLC: R_f=0.55 (1% AcOH in CH_2Cl_2). ^{1}H NMR: (300 MHz, $CDCl_3$) δ 8.31 (t, J=1.8 Hz, 2H), 8.11 (ddd, J=7.8, 1.5, 1.2 Hz, 2H), 7.81, (s, 2H), 7.59 (ddd, J=8.0, 2.2, 1.1 Hz, 2H), 7.40 (t, J=8.0 Hz, 2H), 7.29 (dd, J=8.3, 1.9 Hz, 1H), 7.20 (d, J=1.9 Hz, 1H), 7.08 (d, J=8.3 Hz, 1H), 5.81 (s, 1H), 4.04 (s, 3H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 156.3, 150.8, 147.3, 147.2, 141.7, 132.3, 130.5, 130.3, 125.9, 123.2, 120.9, 117.6, 115.3, 109.6, 56.5; IR (ATR): 3509, 3061, 1601, 1567, 1548, 1518, 1478, 1469, 1442, 1422, 1387, 1369, 1350, 1271, 1241, 1212, 1177, 1120 cm^{-1}; ESI-MS: expected, 509.0; observed, *m/z* 509.9 [M+H$^+$].

4.4.3.5. 2,6-Di-(3-methoxyphenyl)-4-(4-hydroxy-3-methoxyphenyl)-pyridine (16r). Vanillin (1.00 g, 6.57 mmol), 3′-methoxyacetophenone (1.97 g, 6.57 mmol), NH_4OAc (2.00 g, 25.9 mmol), and 30 mL of acetic acid were combined in a 70 mL Teflon MW reaction vessel. A magnetic stirring bar was added to the vessel. The vessel was closed tightly and heated with stirring in the Milestone MW reactor from room temperature to 180 °C over 10 min, held at 180 °C for 20 min, and allowed to cool to room temperature over ca. 30 min. The MW heating sequence was repeated (1×). The reaction mixture was concentrated to an oil under reduced pressure. The oil was dissolved in 50 mL of EtOAc and washed with satd aq $NaHCO_3$ (2×), brine, and H_2O. The organic phase was concentrated under reduced pressure to give a brown oil. The oil was purified by flash silica gel column chromatography (1% AcOH in CH_2Cl_2) to give 65 mg of triarylpyridine **16r** as a colorless oil (2% yield). TLC: R_f=0.26 (1% AcOH in CH_2Cl_2); ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.81 (s, 2H), 7.79 (dd, J=2.6, 1.4 Hz, 2H), 7.74 (dt, J=8.0, 1.4 Hz, 2H), 7.42 (t, J=8.0 Hz, 2H), 7.29 (dd, J=8.2, 2.0 Hz, 1H), 7.21 (d, J=2.0 Hz, 1H), 7.07 (d, J=8.2 Hz, 1H), 7.00 (dd, J=8.0, 2.6 Hz, 2H), 5.79 (s, 1H), 4.01 (s, 3H), 3.92 (s, 6H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 160.2, 157.4, 150.3, 147.2, 147.0, 141.4, 131.5, 129.9, 120.8, 119.8, 117.3, 115.2, 114.8, 113.0, 109.7, 56.4, 55.6; IR (ATR): 3727, 3600, 3052, 1599, 1583, 1547, 1515, 1492, 1401, 1288, 1169, 1125 cm^{-1}; ESI-MS: expected, 413.2; observed, *m/z* 414.1 [M+H$^+$].

Acknowledgments

We thank the Donors of the ACS Petroleum Research Fund (41332-G1), the National Science Foundation (CHE-0449959), CEM Corporation, and the UW-Madison for financial support of this work and the W. M. Keck Foundation for support of instrumentation at the UW Center for Chemical Genomics. B.G.P. acknowledges support during the 2004–2005 academic year through a Wayland Noland Undergraduate Chemistry Research Fellowship.

References and notes

1. Ley, S. V.; Baxendale, I. R. *Nat. Rev. Drug Discov.* **2002**, *1*, 573–586 and references therein.
2. Lam, K. S.; Renil, M. *Curr. Opin. Chem. Biol.* **2002**, *6*, 353–358.
3. Newkome, G. R.; Childs, B. J.; Rourk, M. J.; Baker, G. R.; Moorefield, C. N. *Biotechnol. Bioeng.* **1999**, *61*, 243–253.
4. Wang, J.; Yoo, Y.; Gao, C.; Takeuchi, I.; Sun, X.; Chang, H.; Xiang, X.-D.; Schultz, P. G. *Science* **1998**, *279*, 1712–1714.
5. Mello, J. V.; Finney, N. S. *J. Am. Chem. Soc.* **2005**, *127*, 10124–10125.
6. (a) Winssinger, N.; Harris, J. L.; Backes, B. J.; Schultz, P. G. *Angew. Chem. Int. Ed.* **2001**, *40*, 3152–3155; (b) Hergenrother, P. J.; Depew, K. M.; Schreiber, S. L. *J. Am. Chem. Soc.* **2000**, *122*, 7849–7850.
7. Figeys, D.; Ducret, A.; Yates, J. R., III; Aebersold, R. *Nat. Biotechnol.* **1996**, *11*, 1544.
8. Burke, M. D.; Berger, E. M.; Schreiber, S. L. *J. Am. Chem. Soc.* **2005**, *126*, 1973–1996.
9. (a) Boyle, N. A.; Janda, K. D. *Curr. Opin. Chem. Biol.* **2002**, *6*, 339–346; (b) Tan, D. S.; Burbaum, J. J. *Curr. Opin. Drug Discov. Devel.* **2000**, *3*, 439–453.
10. Li, W. B.; Yan, B. *J. Org. Chem.* **1998**, *63*, 4092–4097.
11. Frank, R. *Tetrahedron* **1992**, *48*, 9217–9232.
12. For recent reviews of SPOT-synthesis, see: Frank, R. *J. Immunol. Methods* **2002**, *267*, 13–26; (b) Reineke, U.; Volkmer-Engert, R.; Schneider-Mergener, J. *Curr. Opin. Biotechnol.* **2001**, *12*, 59–64.
13. We use Whatman 1Chr chromatography paper for macroarray construction (thickness=0.18 mm and cost ca. 0.5 US ¢/cm^2).
14. Reineke, U.; Volkmer-Engert, R.; Schneider-Mergener, J. *Curr. Opin. Biotechnol.* **2001**, *12*, 59–64 and references therein.
15. Hilpert, K.; Volkmer-Engert, R.; Walter, T.; Hancock, R. E. W. *Nat. Biotechnol.* **2005**, *23*, 1008–1012.
16. For selected examples, see: (a) Scharn, D.; Germeroth, L.; Schneider-Mergener, J.; Wenschuh, H. *J. Org. Chem.* **2001**, *66*, 507–513; (b) Jobron, L.; Hummel, G. *Angew. Chem. Int. Ed.* **2000**, *39*, 1621–1624; (c) Ast, T.; Heine, N.; Germeroth, L.; Schneider-Mergener, J.; Wenschuh, H. *Tetrahedron Lett.* **1999**, *40*, 4317–4318.
17. For recent commentaries on MW-assisted combinatorial chemistry, see: (a) Blackwell, H. E. *Org. Biomol. Chem.* **2003**, *1*, 1251–1255; (b) Kappe, C. O. *Curr. Opin. Chem. Biol.* **2002**, *6*, 314–320.
18. For recent reviews on MW-assisted organic chemistry, see: (a) Kappe, C. O. *Angew. Chem. Int. Ed.* **2004**, *43*, 6250–6284; (b) Santagada, V.; Frecentese, F.; Perissutti, E.; Favretto, L.; Caliendo, G. *QSAR Comb. Sci.* **2004**, *23*, 919–944.
19. Bowman, M. D.; Jeske, R. C.; Blackwell, H. E. *Org. Lett.* **2004**, *6*, 2019–2022.
20. Lin, Q.; O'Neill, J. C.; Blackwell, H. E. *Org. Lett.* **2005**, *7*, 4455–4458.
21. Wang, S. S. *J. Am. Chem. Soc* **1973**, *95*, 1328–1333.

22. Amine functionalization levels were determined by derivatization of support **3** with Fmoc–OSu, cleavage of the N-Fmoc group with piperidine, and quantitation of released fulvene product using UV spectroscopy (at 296 nm) according to standard procedures. See: Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404–3409.
23. Scharn, D.; Wenschuh, H.; Reineke, U.; Schneider-Mergener, J.; Germeroth, L. *J. Comb. Chem.* **2000**, *2*, 361–369.
24. For further information about this commercial MW reactor system, see: http://www.milestonesci.com/index2.php.
25. Performing this MW-assisted reaction using automated temperature control during the reaction was not possible due to the low solvent volumes used.
26. Linker loading on support determined by quantifying the amount of free amine (using standard UV Fmoc analysis; see Ref. 22) on the support before and after coupling of the prolinker unit and its reduction. The difference between these two values was indicative of the amount of linker coupled to the support.
27. Sheppard, R. C.; Williams, B. J. *Int. J. Pept. Protein Res.* **1982**, *20*, 451–454.
28. Stuerga, D.; Gaillard, P. *Tetrahedron* **1996**, *52*, 5505–5510.
29. Kröhnke, F. *Synthesis* **1976**, 1–24.
30. Cave, G. W. V.; Raston, C. L. *Chem. Commun.* **2000**, 2199–2200.
31. (a) Constable, E. C.; Harverson, P.; Smith, D. R.; Whall, L. A. *Tetrahedron* **1994**, *50*, 7799–7806; (b) Zimmerman, S. C.; VanZyl, C. M.; Hamilton, G. S. *J. Am. Chem. Soc.* **1989**, *111*, 1373–1381.
32. (a) See Ref. 5; (b) Fang, A. G.; Mello, J. V.; Finney, N. S. *Tetrahedron* **2004**, *60*, 11075–11087.
33. Weiss, M. *J. Am. Chem. Soc.* **1952**, *74*, 200–202.
34. Tu, S.; Li, T.; Shi, F.; Fang, F.; Zhu, S.; Wei, X.; Zong, Z. *Chem. Lett.* **2005**, *34*, 732–733.
35. Grosche, P.; Höltzel, A.; Walk, T. B.; Trautwein, A. W.; Jung, G. *Synthesis* **1999**, *11*, 1961–1970.
36. For further information about this commercial MW reactor system, see: http://www.cem.com/.
37. Purity and yields determined by integration of HPLC spectra at 254 nm and comparison to a UV calibration curve generated for triarylpyridine **16r**. See Supplementary material.
38. For a recent commentary on this process, see: Alcázar, J. *J. Comb. Chem.* **2005**, *7*, 353–355.
39. Padilla-Tosta, M. E.; Lloris, J. M.; Martinez-Màñez, R.; Marcos, M. D.; Miranda, M. A.; Pardo, T.; Sancenón, F.; Soto, J. *Eur. J. Inorg. Chem.* **2001**, 1475–1482.
40. Gao, F. G.; Bard, A. J. *J. Am. Chem. Soc.* **2000**, *122*, 7426–7427.
41. Bowman, M. D.; Jacobson, M. M.; Blackwell, H. E. *Org. Lett.* **2006**, in press.
42. Webb, T. R. *Synthesis* **1984**, 213–214.