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Microwave-Accelerated SPOT-Synthesis on Cellulose Supports

Matthew D. Bowman, Ryan C. Jeske, and Helen E. Blackwell*

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

blackwell@chem.wisc.edu

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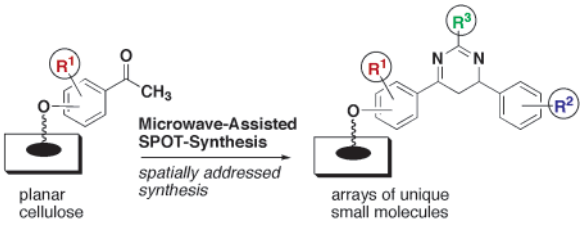
ABSTRACT



We demonstrate that microwave irradiation can dramatically accelerate reaction rates for spatially addressable library synthesis on planar membrane supports. The development of a robust support/linker system, microwave-assisted synthesis of small molecule test libraries, and methods for solid-phase scale-up on cellulose are described.

Combinatorial chemistry has become an important tool in many areas of research, including chemical biology and drug discovery.[1] As the demand for libraries of small organic molecules continues to grow, particularly due to the burgeoning fields of genomics and proteomics, the need to develop new synthesis platforms for the rapid generation and evaluation of "natural product-based" and "drug-like" compounds becomes more urgent.[2] Parallel and split-pool synthesis on polymeric solid supports are currently the most common strategies for the generation of combinatorial libraries.[3] While both of these approaches have enabled the synthesis of large libraries, performing reactions on conventional solid-phase resins can have significant drawbacks—the hydrophobic beads used are often expensive, difficult to manipulate, fragile, and not amenable to many types of biological assays. Furthermore, solid-phase reaction rates are frequently 10- to 100-fold slower than their solution-phase counterparts.[4] This problem frequently outweighs the purification benefits permitted by solid supports and requires significant time investment in reaction optimization. The development of an inexpensive and robust library synthesis platform that combines the purification benefits of solid-phase resins with enhanced reaction rates would impact significantly the role of combinatorial techniques in research today. Here, we demonstrate that the strategic combination of microwave-assisted organic reactions with SPOT-synthesis on planar supports presents a platform that can address many of these current limitations.[5]

SPOT-synthesis is a conceptually simple and flexible approach for parallel library synthesis on planar cellulose supports (Figure 1).[6] This method involves spatially addressed, solid-phase synthesis on derivatized cellulose sheets (readily prepared from inexpensive filter paper) to generate arrays of unique molecules (1−10,000 SPOTs).[7,8] In contrast

* To whom correspondence should be addressed.

(1) (a) Ley, S. V.; Baxendale, I. R. *Nat. Rev. Drug. Discov.* **2002**, *1*, 573−586. (b) Lam, K. S.; Renil, M. *Curr. Opin. Chem. Biol.* **2002**, *6*, 353−358. (c) Erhardt, P. W. *Pure Appl. Chem.* **2002**, *74*, 703−785.

(2) (a) Schreiber, S. L. *Science* **2000**, *287*, 1964−1969. (c) Seneci, P.; Miertus, S. *Mol. Divers.* **2000**, *5*, 75−89.

(3) (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 Discuss. Devel.* **2000**, *3*, 439−453.

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(5) For recent commentaries on microwave-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.

(6) Frank, R. *Tetrahedron* **1992**, *48*, 9217−9232.

(7) For a recent SPOT-synthesis review, see: Frank, R. *J. Immunol. Methods* **2002**, *267*, 13−26.

(8) We and others use Whatman 1Chr chromatography paper for SPOT-synthesis (thickness = 0.34 mm and cost ca. 0.5¢/cm^2).

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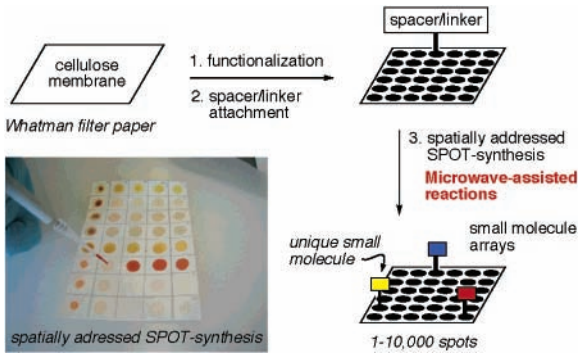


Figure 1. Schematic of the SPOT-synthesis process.

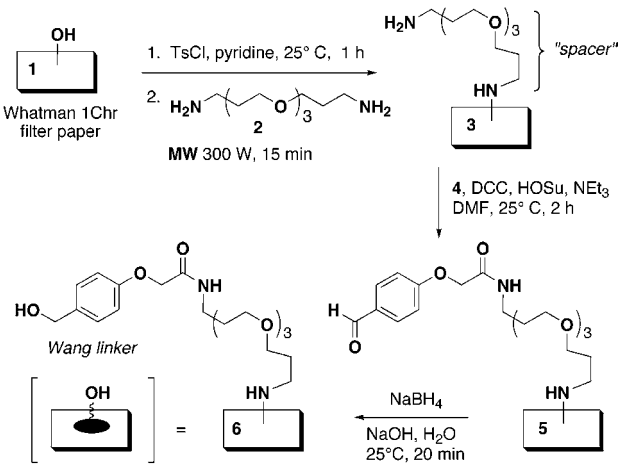
to conventional polystyrene resins, the hydrophilic, mechanically robust membrane sheets are easy to manipulate during synthesis and washing steps. Furthermore, cellulose membranes are compatible with various on-support biological screening methods, including protein-binding assays, enzyme-linked immunosorbent assays (ELISA), and agar overlay.[7]

Despite the advantages outlined above, the application of the SPOT-synthesis technique has been largely restricted to the construction of simple peptide libraries.[7] Examples of nonpeptide libraries generated via SPOT-synthesis are scarce, and most have been based on simple acylation chemistry.[9] More challenging 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 hypothesized that microwave (MW) assistance could broaden the reaction scope of SPOT-synthesis and facilitate the generation of complex small molecule libraries. The use of MW irradiation as a non-conventional heating method for organic synthesis has increased over the past decade, primarily due to marked reductions in reaction times and enhancements in conversion and purity.[10] To date, however, the generality and utility of MW-assisted reactions on planar polymeric supports have not been explored.[5a,11]

We chose to examine the scope and limitations of MW-assisted SPOT-synthesis through the preparation of libraries of chalcone-derived molecules, as chalcone synthesis via Claisen−Schmidt condensation has been performed with success in solution under MW conditions previously.[10,12] Chalcones can be derivatized easily to generate diverse second-generation libraries.[13,14] Further, chalcones present a proven molecular scaffold for biological evaluation, as certain derivatives exhibit potent and specific activities, e.g., activation of Sirtuin proteins[15] and broad anticancer activity.[16]

We initiated our investigations through the development of a robust, linker-functionalized planar cellulose support (Scheme 1). Previous cellulose derivatization methods for

Scheme 1. Support Modification and Linker Installation



SPOT-synthesis have relied on reaction of cellulose with epibromohydrin.[7,11] We chose to avoid this toxic, expensive reagent and found direct tosylation of support **1**[8] to be an effective method to activate cellulose prior to introduction of diamine **2** as a "spacer" element. By controlling the concentration of TsCl and reaction time, the loading level of the support could be varied from 50 nmol/cm^2 to 10 μmol/cm^2.[17] Diamine **2** was installed as a "spacer" since this unit has been shown to improve the accessibility of support-bound molecules for subsequent reactions and on-support assays.[11] MW irradiation of tosyl cellulose in neat diamine **2** (300 W) significantly expedited spacer incorporation; support **3** was generated in 15 min as opposed to the 6 h required for similar incorporation using traditional methods (100 °C, in a drying oven). We found MW heating of membranes in shallow glass vessels to be straightforward and reproducible using a commercial multimodal MW reactor.[18] In contrast, a household MW oven (Kenmore model 721; 300 W) gave irreproducible results for heating planar support reactions and underscores the importance of using dedicated, commercial MW reactors for synthesis.[5,10]

We next introduced a versatile acid-cleavable, Wang-type linker system onto amino-cellulose support **3** (Scheme 1).[19]

(9) 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.

(10) For a recent MW review, see: Lidstrom, P.; Tierney, J.; Wathey, B.; Westman, J. *Tetrahedron* **2001**, *57*, 9225−9283.

(11) Scharn, D.; Wenschuh, H.; Reineke, U.; Schneider-Mergener, J.; Germeroth, L. *J. Comb. Chem.* **2000**, *2*, 361−369.

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(13) Katritzky, A. R.; Serdyuk, L.; Chassaing, C.; Toader, D.; Wang, X.; Forood, B.; Flatt, B.; Sun, C.; Vo, K. *J. Comb. Chem.* **2000**, *2*, 182−185 and references therein.

(14) Powers, D. G.; Casebier, D. S.; Fokas, D.; Ryan, W. J.; Troth, J. R.; Coffen, D. L. *Tetrahedron* **1998**, *54*, 4085−4096.

(15) Howitz, K. T.; Bitterman, K. J.; Cohen, H. Y.; Lamming, D. W.; Lavu, S.; Wood, J. G.; Zipkin, R. E.; Chung, P.; Kisielewski, A.; Zhang, L. L.; Scherer, B.; Sinclair, D. A. *Nature* **2003**, *425*, 191−196.

(16) Lawrence, N. J.; Rennison, D.; McGown, A. T.; Ducki, S.; Gul, L. A.; Hadfield, J. A.; Khan, N. *J. Comb. Chem.* **2001**, *3*, 421−426.

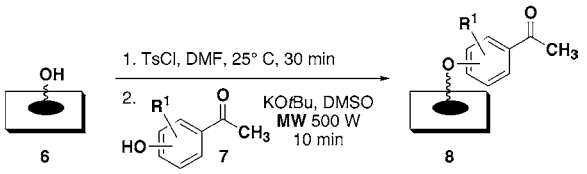
(17) Functionalization levels were determined by quantitation of Fmoc-derivatized amino-support **3** using UV spectroscopy (at 296 nm).

(18) All MW-assisted reactions on cellulose supports were performed in a Milestone Ethos Microsynth multimodal reactor using power (wattage) control. Temperature control was not possible due to the low solvent volumes used. Supports were washed with various solvents and dried routinely between each synthesis step; see the Supporting Information.

First, 4-formylphenoxyacetic acid (**4**) was coupled to support **3** via a standard carbodiimide coupling. Aldehyde support **5** was then subjected to $NaBH_4$ to yield benzyl alcohol-derived support **6**. Notably, this four-step reaction sequence did not impact the integrity of the cellulose membrane; we routinely generate support **6** on large scale (ca. 300 cm^2 sheets) and find this support/linker system to be highly robust for SPOT-synthesis.

We evaluated the feasibility of MW-assisted synthesis on support **6** through the generation of select chalcones. First, we attached various hydroxyacetophenones **7** to support **6** via MW-assisted nucleophilic substitution (Scheme 2).

Scheme 2. Initial Building Block Loading

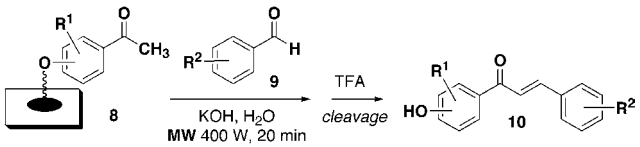


Support **6** was activated immediately prior to use by treatment with TsCl in DMF. Solutions of acetophenones **7** and base (KO-*t*-Bu, in DMSO) were then spotted onto activated support **6** in a spatially addressable manner (SPOT size = 0.28 cm^2) and subjected to MW irradiation at 500 W for 10 min.[18] Reproducible acetophenone (**7**) loadings ranging from 100 to 200 nmol/cm^2 were obtained via this protocol (as determined after physically punching-out the SPOT and TFA-mediated cleavage).[20] This short reaction time contrasts with the 5−10 h required for analogous solid-phase nucleophilic displacements using traditional heating methods (50 °C).[13] Detailed optimization work revealed that high-boiling polar solvents, such as DMSO, and concentrated building block solutions (ca. 2.0 M **7**) were required for high loadings (data not shown). These conditions allow for sustained MW heating[5,10] and were used for all subsequent MW-assisted SPOT-syntheses.

We next investigated the feasibility of MW-assisted Claisen−Schmidt condensations in a SPOT format (Table 1). In preliminary experiments, spotting of support **8** with various benzaldehydes and base in polar solvents (e.g., KOH in H_2O) followed by MW irradiation at 400 W for 20 min generated chalcone species **10** (after TFA cleavage) with good to excellent conversions (60−98%).[18,21] This represents a marked reduction in reaction time and a concomitant enhancement in yield; these condensation reactions typically require ca. 2−48 h for lower conversions using conventional solid-support synthesis methods.[13,22]

With these two reactions optimized, we synthesized a test library of chalcones on support **6** (Table 1). Six acetophenones **7** and eight benzaldehydes **9** were selected as library building blocks, based on their solubility in polar solvents and the presence of functional group handles, i.e., hydroxyl and/or halogen, for the incorporation of additional library diversity elements.[2b] The building blocks were combined to yield a parallel array of 40 unique chalcones (**10a**−**10l′**) with good to excellent conversions in almost all cases (as determined after TFA cleavage).[20,21] Different basic reaction conditions were required depending on the benzaldehyde species (**9**). More reactive benzaldehydes, such as 3-hydroxybenzaldehyde, underwent efficient condensation at 1.0 M in

Table 1. Chalcone Test Library Synthesized on Support **6**



entry[a]	R^1 (**7**)	R^2 (**9**)	purity[b] (%)
10a	4-OH	3-OH	92
10b	4-OH	3-Br	89
10c	4-OH	4-Cl	99
10d	4-OH	3-OH, 4-NO_2	93
10e	4-OH	4-OH	70
10f	4-OH	4-OH, 3-Br	69
10g	4-OH	4-OH, 3-OMe	79
10h	4-OH	5-I, 4-OH, 3-OMe	75
10i	3-OH	3-OH	90
10j	3-OH	3-Br	>99
10k	3-OH	4-Cl	92
10l	3-OH	3-OH, 4-NO_2	86
10m	3-OH	4-OH	84
10n	3-OH	4-OH, 3-Br	81
10o	3-OH	4-OH, 3-OMe	91
10p	3-OH	5-I, 4-OH, 3-OMe	90
10q	4-OH, 2-Me	3-OH	63
10r	4-OH, 2-Me	3-Br	67
10s	4-OH, 2-Me	4-Cl	90
10t	4-OH, 2-Me	3-OH, 4-NO_2	74
10u	4-OH, 2-Me	4-OH	43
10v	4-OH, 2-Me	4-OH, 3-Br	62
10w	4-OH, 2-Me	4-OH, 3-OMe	68
10x	4-OH, 2-Me	5-I, 4-OH, 3-OMe	66
10y	4-OH, 3-OMe	3-OH	95
10z	4-OH, 3-OMe	3-Br	90
10a′	4-OH, 3-OMe	4-Cl	92
10b′	4-OH, 3-OMe	3-OH, 4-NO_2	96
10c′	4-OH, 3-OMe	4-OH	87
10d′	4-OH, 3-OMe	4-OH, 3-Br	81
10e′	4-OH, 3-OMe	4-OH, 3-OMe	84
10f′	4-OH, 3-OMe	5-I, 4-OH, 3-OMe	87
10g′	4-OH, 3-NO_2	3-OH	66
10h′	4-OH, 3-NO_2	3-Br	65
10i′	4-OH, 3-NO_2	4-Cl	51
10j′	4-OH, 3-NO_2	3-OH, 4-NO_2	59
10k′	4-OH, 2-OH	3-OH	40
10l′	4-OH, 2-OH	3-Br	52

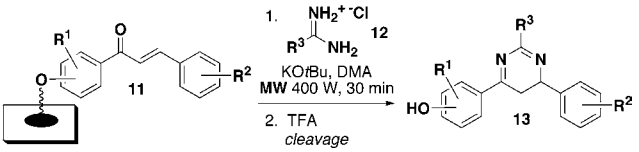
[a] MW reaction conditions, solvent volumes, and base concentration vary for certain substrates. See text and Supporting Information. [b] Determined by integration of the HPLC trace at 254 nm.

(19) Wang, S. S. *J. Am. Chem. Soc.* **1973**, *95*, 1328−1333.
(20) Compounds were cleaved quantitatively from the Wang linker by treatment with TFA vapor (in a vacuum desiccator, 25 °C, 60 min). See refs 11 and 19.
(21) Both cis and trans chalcone isomers were observed in certain cases; see the Supporting Information.
(22) Control SPOT reactions (30 min, 25 °C) generated chalcones in low yields (ca. 10%); see the Supporting Information.

a 6 N aq KOH solution, while less reactive substrates, such as vanillin, required a more concentrated solution of both aldehyde (2.0 M) and base (12 N aq KOH) to achieve good conversion. Preparation of the library in a spatially addressed format permitted the application of different reaction conditions in parallel and expedited library synthesis. *Indeed, this library was synthesized in only 60 min* (for three reaction steps starting from support **6**). These results underscore the utility of MW-assisted reactions in library synthesis.[5]

To further investigate the scope of MW-assisted synthesis on support **6**, we examined several heterocycle-generating reactions on support-bound chalcones.[13] We discovered that the condensation of chalcones **11** with either acetamidine or benzamidine hydrochloride (**12**) yielded dihydropyrimidines **13** with good to excellent conversions under MW-assisted conditions (Table 2).[23] We then prepared a small library of

Table 2. Dihydropyrimidines Synthesized on Support **6**



entry	R^1	R^2	R^3	purity[a] (%)
13a	4-OH	3-Br	Me	88
13b	4-OH	3-Br	Ph	80
13c	4-OH	4-Cl	Me	83
13d	4-OH	4-Cl	Ph	69
13e	3-OH	3-Br	Me	86
13f	3-OH	3-Br	Ph	86
13g	3-OH	4-Cl	Me	73
13h	3-OH	4-Cl	Ph	84
13i	3-OMe, 4-OH	3-Br	Me	79
13j	3-OMe, 4-OH	3-Br	Ph	76
13k	3-OMe, 4-OH	4-Cl	Me	82
13l	3-OMe, 4-OH	4-Cl	Ph	83

[a] Determined by integration of the HPLC trace at 254 nm.

12 dihydropyrimidines (**13a**−**l**) using MW-assisted SPOT-synthesis. Interestingly, we observed the formation of only trace amounts of the related pyrimidine derivatives under these reaction conditions (30 min, MW 400 W, air atmosphere).[18] This is in contrast to previous pyrimidine syntheses on solid-phase (ca. 20 h, 100 °C in oil bath)[13] where no dihydropyrimidine intermediates were isolated. These data demonstrate how MW-assisted SPOT methods may complement current solid-phase techniques.

Representative LC traces for a three-step sequence of single SPOT reactions (ca. 100 nmol/SPOT) are shown in Figure 2 and reveal the efficient conversions achievable using MW-assisted SPOT-synthesis on support **6**.

One advantage of the SPOT-synthesis technique is that it provides rapid access to compounds in small quantities

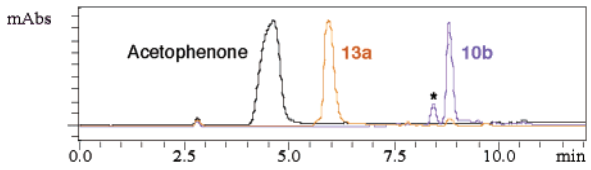


Figure 2. Overlaid LC traces for crude 4-hydroxyacetophenone, chalcone **10b**, and dihydropyrimidine **13a** cleaved from individual SPOTs of support **6**. UV detection at 254 nm. The smaller peak (*) is the cis isomer of **10b**.[21]

(nanomolar to micromolar scale) that are sufficient for characterization and biological evaluation. The ability to translate SPOT-synthesis protocols directly from membrane supports to another form of cellulose that is amenable to larger scale synthesis would expand the utility of this methodology further. We examined MW-assisted synthesis on cellulose powder, fibers, and Perloza beads[24,25] as potential scale-up synthesis methods. We found inexpensive Perloza beads (100 μm diameter) to be mechanically robust and readily functionalized with our Wang-type linker/spacer system using chemistry analogous to that outlined in Scheme 1 for support **6** (ca. 10−80 μmol/g loadings). In initial studies, this alternate support system was compatible with MW-assisted chalcone synthesis,[18] yielding chalcone **10e**, for example, in good purity (74%) at a loading of 4.3 μmol/g. We note that large-scale synthesis on Perloza (10−100 g) will require different MW reaction conditions; current work involves evaluation of continuous flow MW methods.[5]

In summary, we have demonstrated that MW-assisted SPOT-synthesis is a viable approach for the generation of parallel combinatorial libraries. We have developed a robust support/linker system for SPOT-synthesis (**6**), established its compatibility with a range of MW-assisted organic reactions, and extended this methodology to another cellulose support type for larger scale reactions. This combination of techniques presents an accessible and versatile platform for combinatorial discovery and should allow parallel libraries of moderate size to be prepared *and* screened on the order of days. Ongoing work is directed at the synthesis and biological evaluation of larger, second-generation chalcone libraries.

Acknowledgment. This work was supported by generous start-up funds from UW—Madison and CEM Corp. We thank Prof. Laura Kiessling for helpful discussions and CEM Corp. and Milestone, Inc. for technical assistance with MW synthesis instrumentation.

Supporting Information Available: Full experimental details for support derivatization and MW-assisted synthesis. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) The absolute tautomeric form of **13** remains unknown. Dihydropyrimidines can exist as 1,4 or 1,6 annular tautomers, see: Weis, A. L. *Tetrahedron Lett.* **1982**, *23*, 449−452.

(24) Perloza MT-100 is commercially available from Iontosorb Co.

(25) De Luca, L.; Giacomelli, G.; Porcheddu, A.; Salaris, M.; Taddei, M. *J. Comb. Chem.* **2003**, *5*, 465−471.