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Small Molecule Macroarray Construction via Ugi Four-Component Reactions

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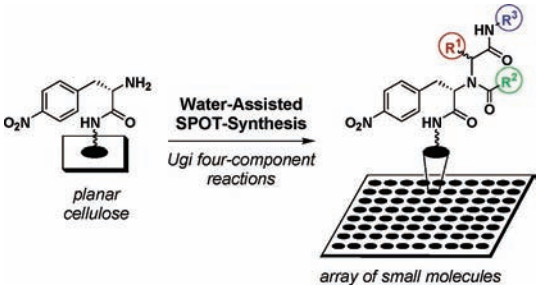
ABSTRACT



We report the construction of small molecule macroarrays via Ugi four-component reactions on planar cellulose supports. Array synthesis was enabled by the development of a high efficiency photocleavable linker system and the strategic use of both water- and microwave-assisted organic reactions.

Research in genomics and proteomics is delivering an increasing number of new biological targets.[1] The use of small molecule probes to study and further annotate these targets has attracted considerable interest.[2] Access to such probes is contingent on the development of robust methods for their synthesis and biological evaluation. Combinatorial synthesis on polymeric solid supports remains one of the most common routes for the construction of small molecule libraries.[3] Standard polystyrene resins, however, are often expensive and difficult to manipulate, and reactions performed on these supports can exhibit reduced rates. Further, these hydrophobic supports are frequently incompatible with biological assays performed directly on immobilized compounds. Recently, we developed a library synthesis platform using planar cellulose supports that addresses these challenges.[4] This synthetic approach is based on microwave-assisted SPOT-synthesis[5] and permits the rapid construction of small molecule "macroarrays". These lower density arrays are complementary to small molecule microarrays,[6] yet differ in two ways: (1) compound synthesis is performed directly on the planar support, and (2) sufficient compound is generated per spot for characterization and biological evaluation post-cleavage. Here, we report the use of multiple-component reactions for the efficient construction of small molecule macroarrays. Further, we demonstrate that the pretreatment of the support with small volumes of water in these reactions,[7] as opposed to the use of microwave irradiation, dramatically increases product conversion and purity.

We were interested in evaluating multiple-component reactions (MCRs) for macroarray construction because these reactions can introduce structural diversity with high efficiency, joining three or more reactants in a single step.

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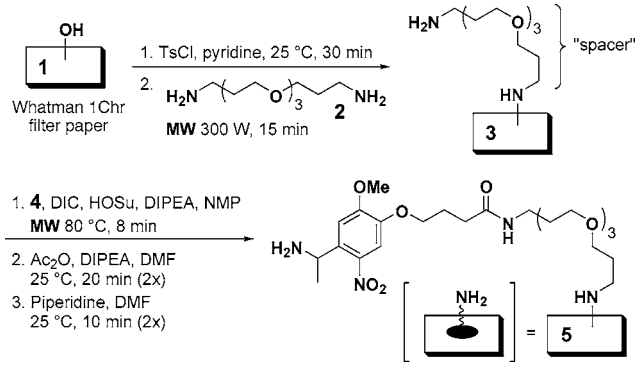
MCRs are suited uniquely for combinatorial library synthesis, as evidenced by considerable recent work in this area.[8] The Ugi 4CR represents one of the best studied MCRs and has been performed on traditional solid supports and in numerous combinatorial applications.[9] In the Ugi 4CR, an amine component reacts with an acid, an aldehyde or ketone, and an isocyanide to produce an α-acylamino amide. While commonly high yielding, this reaction can suffer from exceptionally long reaction times, especially when performed on solid-phase bound substrates (i.e., on the order of days).[10] To accelerate the reaction rate, Hoel and Nielsen recently applied microwave (MW) irradiation during Ugi 4CRs on solid-phase bound substrates and observed dramatically shortened reaction times (≤5 min).[11] MW irradiation continues to emerge as a powerful method to accelerate a broad range of solid-phase reactions.[12] Therefore, on the basis of these past reports, we chose to examine MW-assisted reaction conditions for Ugi 4CRs performed in a macroarray format.

We selected the photolabile linker 4-{4-[1-(Fmoc-amino)-ethyl]-2-methoxy-5-nitrophenoxy}butanoic acid (**4**, Scheme 1) for macroarray construction, as this linker has been well

Scheme 1. Support Modification and Linker Installation[a]



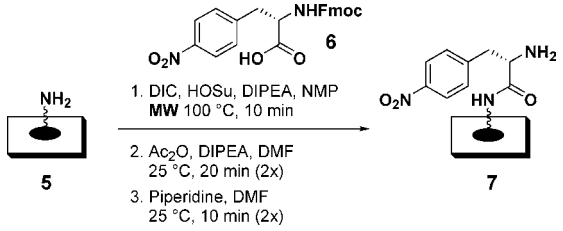
[a] Reagents and conditions: TsCl = tosyl chloride; MW = microwave irradiation; DIC = *N*,*N*′-diisopropylcarbodiimide; HOSu = hydroxy succinimide; DIPEA = diisopropylethylamine; NMP = 1-methyl-2-pyrrolidinone; Ac_2O = acetic anhydride; DMF = dimethylformamide.

studied and has proven to be effective in prior SPOT-syntheses.[13] In addition, photolabile linkers provide advantages for postsynthetic macroarray screening, as library members remain spatially addressed after solvent-free cleavage with UV light. For example, this "dry-state" cleavage permits bacteriological agar overlay assays to be performed directly on the array. Prior to the attachment of photolabile linker **4** to planar cellulose, we derivatized the cellulose (Whatman 1Chr filter paper, **1**) with a flexible diamine spacer unit (**2**) to enhance its reactivity (Scheme 1).[4,14] The spacer was attached via tosylation of cellulose membrane **1** followed by submersion of the membrane in neat diamine **2** and subjection to MW heating (300 W, 15 min) in a commercial MW reactor.[15] This MW-assisted displacement reaction generated support **3** with reproducible amine spacer loadings of ca. 4 μmol/cm^2 (as determined by UV Fmoc quantitation).[16]

Next, photolabile linker **4** was attached to amine spacer support **3** via a standard carbodiimide-mediated coupling reaction (Scheme 1). We applied linker **4** to support **3** in an arrayed "spot" format (0.3 cm^2/spot), as opposed to submersing the entire membrane in a solution of **4**, to minimize the use of this expensive reagent. Immediately after linker application, the membrane was placed between two conductive, polymer composite plates (Weflon)[17] and subjected to MW irradiation for 8 min at 80 °C.[18] We have found that this Weflon sandwiching procedure is a convenient method to heat planar polymeric membranes and can enhance conversion in numerous MW-assisted reactions. Using this method, linker loadings of ca. 450 nmol/cm^2 were generated (as determined by UV Fmoc quantitation).[16,19] Following linker loading, the membrane was acetylated (to "cap" any unreacted spacer amines) and subjected to Fmoc deprotection to generate **5**.

We chose to immobilize the amine building block on support **5** for the Ugi 4CR and found *N*-Fmoc-4-nitro-L-phenylalanine (Fmoc-Nph (**6**), Scheme 2) to be an excellent

Scheme 2. Loading of Fmoc-Nph onto Support **5**



amine substrate, as its strong absorbance at 280 nm allowed for straightforward UV quantification. Fmoc-Nph (**6**) was coupled efficiently to the linker spots of support **5** using a MW-assisted protocol similar to that used for linker attach-

(8) For a review, see: Ugi, I. *Pure Appl. Chem.* **2001**, *73*, 187−191.
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(13) See: Scharn, D.; Germeroth, L.; Schneider-Mergener, J.; Wenschuh, H. *J. Org. Chem.* **2001**, *66*, 507−513 and references therein.
(14) See Supporting Information for full experimental details of MW-assisted reactions, array construction, and compound characterization.
(15) A multimodal MW reactor was used throughout this work.
(16) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404−3409.
(17) Plate size: 20 × 20 × 0.5 cm. Weflon is a composite of Teflon and carbon black particles and commercially available from Milestone, Inc.
(18) This protocol was developed using temperature-controlled MW heating methods by sandwiching a fiber optic temperature probe between two Weflon plates (one with a groove that fit the probe), ramping to a desired temperature using MW irradiation, and then holding at that temperature for a specified amount of time. The average wattages obtained during the ramp and hold times were then used in SPOT reactions to reproduce these heating curves.
(19) Control reactions performed at 80 °C in an oven for 10 min gave ca. 50% lower loadings.

ment (10 min at 100 °C, on Weflon). This extended MW-assisted coupling reaction gave consistent loadings of **6** at ca. 400 nmol/cm^2.[16,18,19] Subsequent acetylation of unreacted amine linker and Fmoc deprotection of Nph yielded amine support **7**.

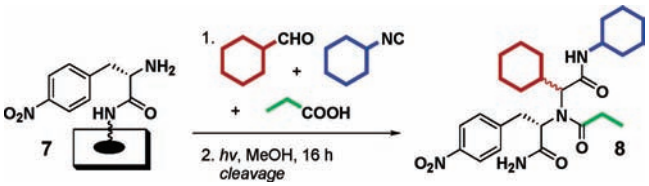
Prior to macroarray construction, tests were performed to examine the efficiency of H-Nph-NH_2 photocleavage from planar support **7**. We examined the photocleavage of *N*-acetyl-Nph-NH_2, as this derivative exhibited enhanced stability to cleavage conditions relative to that of H-Nph-NH_2. Various solvents, times, and light sources were evaluated; optimal cleavage conditions were achieved when "punched-out" spots (isolated with a desktop hole punch) were irradiated at 366 nm in methanol for 16 h using an upturned hand-held UV lamp.[20] Using this solution-phase method, cleavage efficiencies of 85−93% were achieved for support **7** (ca. 100 nmol/spot, as determined by UV).[14] Performing cleavage in the absence of methanol, that is, "dry state" cleavage, provided a reduced yet acceptable amount of material (ca. 55%). Both cleavage efficiencies are notable, as photocleavage from polymeric supports is commonly low yielding.[21] For example, we observed only 18% cleavage of *N*-acetyl-Nph-NH_2 from traditional polystyrene (PS) resin (100−200 mesh) with the identical photolinker using our solution-phase cleavage method.[22] We speculate that the higher photocleavage efficiency for planar supports could be due, in part, to the reduced light scattering caused by planar supports relative to PS beads. This high cleavage efficiency further underscores the value of planar supports for solid-phase synthesis.[4,5,13]

We selected cyclohexane carboxaldehyde, propionic acid, and cyclohexylisocyanide as components to test the Ugi 4CR on amine support **7**, as these three reactants have been shown previously to give high conversion to Ugi product **8** on solid support (Table 1).[11] We initially attempted the synthesis of model compound **8** by individually spotting the three components neat onto amine support **7** and allowing the reaction to proceed for 10 min at room temperature. These reaction conditions gave poor conversion and low purity (13%; Table 1, entry 1). We found that product purity quadrupled upon premixing the aldehyde and carboxylic acid components in DMF prior to spotting (entry 2).[23] Longer reaction times or multiple applications of reagents failed to improve conversion to **8**, however (data not shown). Surprisingly, subjecting our model Ugi 4CR to MW irradiation also failed to increase product conversion (entry 3).[17,18] This result differed from those of Hoel and Nielsen[11] and prompted the examination of alternate Ugi 4CR conditions for macroarray construction.

The use of water as a solvent in organic reactions has received considerable attention over the past 25 years,[7] due to impressive reaction rate enhancements and its obvious safety and environmental benefits. Reagent heterogeneity appears to play a prominent, yet poorly understood, role in rate acceleration.[7a] Recently, Pirrung and Das Sarma reported that water can appreciably accelerate the rates of isocyanide-based MCRs in solution.[24] We reasoned that these conditions could be applied to heterogeneous, solid-phase Ugi 4CRs on support **7**, and we found that the use of water as a solvent gave dramatically improved product purity (91%; Table 1, entry 4). Applying MW irradiation during this reaction caused a substantial decrease in conversion to **8** (entry 5); this correlates with data that show MCR rates are slower in water at elevated temperatures.[24] The poor solubility of the building blocks in water, however, hindered the reproducible delivery of reagents from aqueous suspensions during SPOT-syntheses. Further adjustment of reagent ratios and conditions revealed that simply prespotting support **7** with water (3 μL) prior to spotting the neat building blocks in a 1:6:3 ratio gave the highest product purity (95%, entry 7). Finally, to minimize the evaporation of reagents during macroarray construction, we found that spotting water and reagents at 4 °C (in a cold room) and then moving the array to room temperature for 15 min gave comparable product purity (entry 8). Ongoing studies in our laboratory are focused on fully characterizing the advantageous role of water in these solid-phase reactions.[25]

Using these aqueous Ugi 4CR conditions, we systematically examined library building blocks to determine the most reactive set for macroarray synthesis. As others have

Table 1. Selected Reaction Conditions for Ugi 4CR on Nph Support **7** to Generate Compound **8**



entry	temp (°C)	solvent[a]	pre-spot H_2O	mol ratio[b] R^1:R^2:R^3	purity (%)[c]
1	25	*d,e*	−	1:1:2	13
2	25	DMF	−	1:1:2	51
3	MW: 80[f]	DMF	−	1:1:2	47
4	25	H_2O	−	1:1:2	91
5	MW: 80[f]	H_2O	−	1:1:2	37
6	25	*d*	+	1:1:2	72
7	25	*d*	+	1:6:3	**95**
8	4[g]	*d*	+	1:6:3	**93**

[a] Used to dissolve or suspend building blocks prior to spotting (1 M aldehyde; 4 M acid; 4 M isocyanide). Aldehyde and acid premixed. Reaction times were 10 min unless noted. [b] R^1 = aldehyde; R^2 = carboxylic acid; R^3 = isocyanide. [c] Based on integration of LC spectra with UV detection (280 nm); error = ±2%. [d] No solvent used. [e] No premixing. [f] MW conditions: 8 min at 80 °C on Weflon. [g] Reaction mixture spotted at 4 °C and moved to 25 °C for 15 min.

(20) C. Entela Mineralight Lamp; Model UVGL-58.
(21) James, I. W. *Tetrahedron* **1999**, *55*, 4855−4946.
(22) Our cleavage method is similar to photocleavage protocols used for PS beads. See ref 22.
(23) The isocyanide was not premixed with the other components, to avoid formation of the Passerini 3CR product, and was applied to the support after the aldehyde/carboxylic acid mixture. We attribute the improved product purity to better mixing and the order of addition.
(24) Pirrung, M. C.; Sarma, K. D. *J. Am. Chem. Soc.* **2004**, *126*, 444−445.
(25) These water-accelerated Ugi 4CR conditions were directly translatable to PS resin, generating compound **8** in excellent purity (ca. 95%). See Supporting Information for details.

reported, we found that the reaction was most sensitive to the nature of the aldehyde component;[10a] aliphatic-type aldehydes gave the purest products, while aromatic aldehydes routinely gave reduced purities. The three sets of building blocks chosen for the construction of a 96-compound macroarray are shown in Figure 1; the amine building block was maintained as Nph.

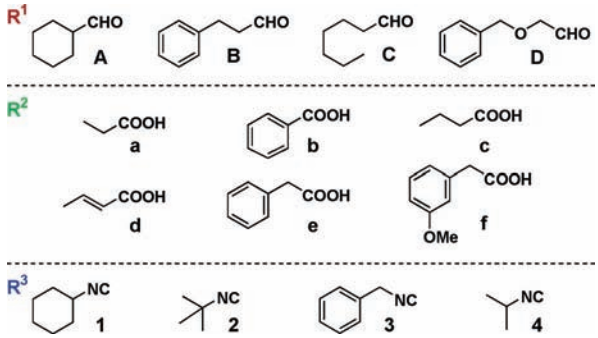


Figure 1. The three sets of building blocks used for the construction of a 96-member Ugi 4CR library.

The final macroarray was constructed on a 15 × 18 cm piece of support **7**, with 0.3 cm^2 spots spaced 1.2 × 1.8 cm apart in a grid. Multichannel pipets were used to expedite the manual delivery of reagents onto the planar support, and the total setup and synthesis time was less than 30 min. A subset of the library (30%) was cleaved and analyzed by LC−MS, and all of these compounds showed purities greater than 85%, with 37% of the samples tested having purities greater than 95% (Table 2). This array format provided ca. 50 μg of compound per spot at ca. 90% cleavage efficiency. A representative LC trace used for analysis is shown in Figure 2. For products where the diastereomeric products

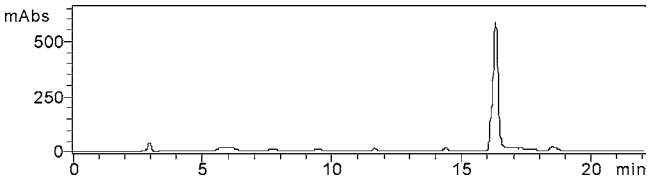
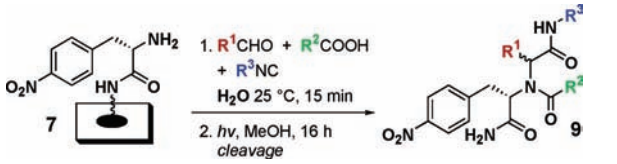


Figure 2. Representative LC trace of macroarray member **9a** cleaved from a single spot. UV detection at 280 nm.

could be resolved by LC, their ratios ranged from 3:1 to 6:1. These diastereoselectivities could be inverted when the Ugi 4CR was performed on PS support with an alternate linker (Rink amide),[14] suggesting that the linker environment

Table 2. Purity Data for Selected Ugi 4CR Library Members[a]



entry	R^1	R^2	R^3	purity (%)[b]	entry	R^1	R^2	R^3	purity (%)[b]
8/9a	A	a	1	93	**9o**	C	c	2	98
9b	A	b	3	97	**9p**	C	d	3	89
9c	A	c	2	96	**9q**	C	e	4	97
9d	A	d	1	97	**9r**	C	f	2	93
9e	A	e	3	90	**9s**	C	f	3	93
9f	A	f	4	96	**9t**	D	a	1	92
9g	B	a	4	96	**9u**	D	a	4	97
9h	B	b	1	88	**9v**	D	b	1	98
9i	B	c	4	97	**9w**	D	c	3	92
9j	B	d	2	95	**9x**	D	d	2	90
9k	B	e	3	90	**9y**	D	d	3	92
9l	B	f	1	93	**9z**	D	e	4	93
9m	C	a	2	93	**9a′**	D	f	2	89
9n	C	b	4	87	**9b′**	D	f	3	96

[a] Reaction conditions: see Table 1, entry 8. [b] Based on integration of LC spectra with UV detection at 280 nm; error = ±2%.

may play a role in the reaction. Future efforts are directed at improving these diastereoselectivities, generating Ugi products with increased structural diversity, and producing larger macroarrays.

In conclusion, we have shown that Ugi 4CRs are compatible with small molecule macroarray construction on planar cellulose supports. We have developed a robust photolabile linker/support system that gives exceptionally high cleavage efficiencies relative to traditional polymeric supports. Further, we have shown that water also can accelerate heterogeneous solid-phase MCR rates in analogy to those of solution-phase MCRs. This approach permitted the rapid production of a 96-member small molecule macroarray in excellent purity. The incorporation of MCRs further expands the versatility of the SPOT-synthesis technique and should find use in numerous small molecule synthesis and screening efforts.

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Supporting Information Available: Full experimental details for macroarray construction. This material is available free of charge via the Internet at http://pubs.acs.org.

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