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High-Throughput ^{1}H NMR and HPLC Characterization of a 96-Member Substituted Methylene Malonamic Acid Library

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A solid phase organic synthesis method has been developed for the preparation of substituted methylene malonamic acids and malonic ester mono acids **5**. Two substituents are introduced into the core molecule **5** by preparation of unsymmetrical malonic acid derivatives **2**, followed by Knoevenagel condensation with aromatic or aliphatic aldehydes, giving resin-bound **4**. Evaluation of the scope of these reactions led to the preparation of a 96-member library from a set of eight amines/alcohols (seven amines and one alcohol) and 11 aldehydes leading to 88 substituted methylene malonamic/malonate mono acids **5** and eight unsymmetrical malonamic/malonate mono acids **3**. Structural validation and quantitation for every member of the library was obtained by evaluation of ^{1}H NMR and HPLC, respectively. The ^{1}H NMR data were obtained using automated delivery of DMSO solutions of every library member from a 96-deep well microtiter plate to a flow probe-equipped NMR spectrometer. HPLC data were used for determination of the extent of conversion of malonamic/malonate esters **2** to the products **5** by an external standard method. Summary information from the ^{1}H NMR and HPLC data is viewed as plate diagrams for analysis of the final library.

Introduction

Advances in parallel synthesis and combinatorial chemistry have provided discovery organizations with a powerful set of methods for preparation of libraries of compounds for specific screening needs.[1,2] Depending on the requirements of a particular screen, library size can range from very large (thousands to millions of members) for lead identification to small (fewer than a thousand) for lead optimization. One of the major challenges in preparation and evaluation of these libraries, whether large or small, has been validation of chemical structures within the set. In the case of large libraries used for lead identification, one is primarily interested in identification of the active members. Unless the "inactives" are used in evaluation of structure−activity relationships, structural validation of the inactive members is not critical. The need for structure validation of all individual members is greatest for the focused, lead optimization libraries. For these small libraries, a significant number of the library members can exhibit activity in the targeted assay or physical property test. Often, the structural identity of all members, including the inactive compounds, is necessary for analysis of structure−activity relationships. In this case, it is most desirable to validate the structure and amount of each member of the library.

A widespread practice is the use of chromatography (HPLC or GC) for the identification of library member purity.[3] While these techniques lend themselves to automation and collection of data, in most cases they do not provide precise indications of product concentrations or the relative amounts of the desired product and the impurities. These problems can be addressed by determining a response factor for model compounds in a library and using detectors that provide improved relative response of the analytes such as evaporative light scattering detectors or UV detection at 210 nm.[4] Even with better detection methods and response factors, chromatographic methods do not allow for determination of the structure of the product. As an alternative, ^{1}H NMR can be used for both the determination of yield and structure validation of a sample. We have previously reported the use of ^{1}H NMR with hexamethyldisiloxane (HMDS) as an internal standard as a means of establishing the yield and extent of conversion for products of solid phase synthesis reactions.[5] With the advent of benchtop LC−MS, mass spectral identification also provides a powerful means for structure validation in libraries.[6] However, LC−MS validation requires a molecular ion of diagnostic value for a significant percentage of the library members.

We have recently investigated the use of high-throughput ^{1}H NMR for the validation of targeted libraries in our discovery efforts. NMR can provide structural confirmation as well as product purity for members of a library. Substituted methylene malonamic acids **5**, which we have previously prepared from Knoevenagel condensation of aldehydes with unsymmetrical malonamic esters and malonates **2** on a solid support, seemed particularly well suited for high-throughput ^{1}H NMR analysis.[7] These compounds did not provide useful mass spectral data via a diagnostic molecular ion, using electrospray or APCI sources, due to decomposition and decarboxylation. However, malonamic acids **5** were remarkably stable at room temperature; they can be purified to provide stable solids and can be properly characterized by

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Scheme 1

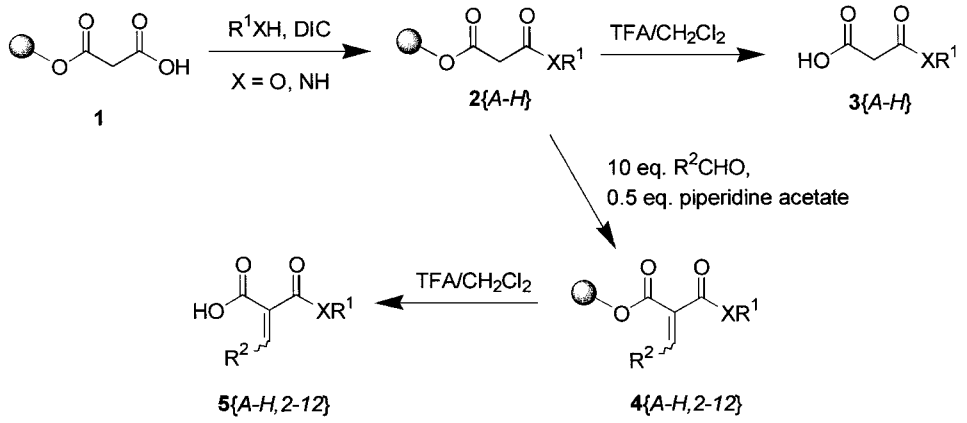
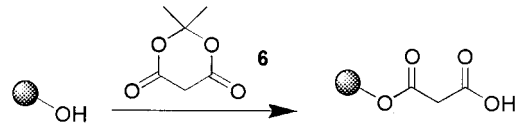


Table 1. Preparation of Wang Malonic Acid Resin **1**[a]



entry	resin[b]	conditions[c]	loading (mequiv/g)	yield (%)
a	Chem-Impex (0.95 mequiv/g)	(1)TMS-Cl; (2) **6**, 24 h	0.943	>95
b		(1)TMS-Cl; (2) **6**, 48 h	0.737	83.9
c		(1) **6**, 24 h	1.025	>95
d		(1) **6**, 48 h	0.685	78
e	Advanced Chemtech (0.88 mequiv/g)	(1)TMS-Cl; (2) **6**, 24 h	0.671	82
f		(1)TMS-Cl; (2) **6**, 48 h	0.542	66.2
g		(1) **6**, 24 h	0.518	63.3
h		(1) **6**, 48 h	0.461	56.4
i	ArgoGel (0.40 mequiv/g)	(1)TMS-Cl; (2) **6**, 48 h	0.166	42.9
j		(1) **6**, 24 h	0.258	66.7
k		(1) **6**, 48 h	0.169	43.7

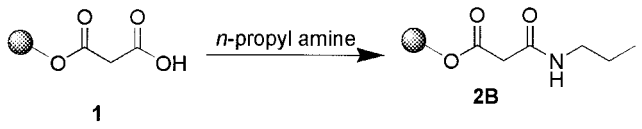
[a] Loading and yield were determined by direct cleavage [1]H NMR with 10 mM HMDS as an internal standard. The yields indicate the ratio of the observed loading to the theoretical loading for each resin. [b] Wang resins from three vendors were obtained and are listed with the vendors reported loading of Wang linker. Theoretical loadings of **1** for the Chem-Impex, Advanced Chemtech, and ArgoGel Wang resins are 0.878, 0.818, and 0.387 mequiv/g, respectively. [c] Each trial consisted of 0.1 mmol of resin. Conditions: TMS-Cl, 3 equiv of trimethylsilyl chloride and 2 equiv of Et_3N in 2 mL of THF for 8 h; Meldrum's acid, **6**, 4 equiv in 3 mL of THF at 65 °C for the specified time.

elemental analysis, NMR, and FAB-MS. Although few substituted methylene malonamic acids have been reported, they have been incorporated in tetrapeptide phosphotyrosine mimetics,[8] used to prepare malonyl derivatives of penicillanic acids,[9] investigated for effects on cerebral circulation,[10] and suggested as dehydropeptidase inhibitors.[11] The relative scarcity of these structural types in the literature encouraged us to prepare a library of these novel compounds for investigation in our screens. Using a 96-member malonamic acid sublibrary as an example, we have determined the extent of conversion, structural identity, and yield of all members of the library using a combination of [1]H NMR and HPLC data.

Method Development

The use of a common intermediate, resin-bound malonic acid **1**, was desirable for development of a library synthesis method that would allow sequential incorporation of the R^1 and R^2 substituents of **5**, and as a means of providing protection of one carbonyl group via attachment to the support (Scheme 1). Solution phase addition of Meldrum's acid to silylated amines and alcohols is known to provide malonamic acids and malonate monoesters, respectively.[12] Our initial attempt at synthesis of the malonic acid derivative of Wang resin followed this literature precedent (Table 1, entry a). Treatment of Wang's resin with trimethylsilyl chloride in the presence of triethylamine provided the silylated resin as evidenced by FTIR microscopy which revealed disappearance of the O−H stretch and new peaks at 1247 (Si−O−C stretch), 872, and 841 cm^{-1}. The silylated Wang resin was converted to the malonic acid derivative by treatment with Meldrum's acid **6** in THF at reflux. We required a significant amount of this material for our studies, and although this method worked well on small scale, attempted larger-scale reactions did not provide consistent results. Formation of **1** was investigated in greater detail using an Argonaut Nautilus 2400 synthesizer to carry out 11 parallel reactions using three resins, different reaction times, and different silylation conditions. All resin loadings were determined by direct cleavage, followed by [1]H NMR

Table 2. Comparison of Conditions for Preparation of Resin **2B**[a]



1 **2B**

entry	reagents	equivalents	conversion (%)	yield (%)
a	HOBt/DIC/amine	4:4:4	97	78
b	HOBt/DIC/amine	4:4:4 (amine last)[b]	64	56
c	HOBt/DIC/amine	4:4:4 (DIC last)[b]	97	96
d	HOBt/DIC/amine	4:4:4 (HOBt last)[b]	97	96
e	HOBt/DIC/amine	4:4:8	20	18
f	HOBt/DIC/amine	4:4:2	97	96
g	$(COCl)_2$/amine	4:4	79	30
h	PyBOP/DIEA/amine	3:6:3	95	65
i	DIEA/DIC/amine	4:4:4	0	0
j	HATU/HOAt/NMM	5:5:8	89	28

[a] Yield was determined by direct cleavage ^{1}H NMR with 10 mM HMDS as an internal standard. All runs consisted of treatment of **1** (100 mg, 0.1 mmol) with the appropriate amount of reagents at room temperature for 18 h. [b] The last reagent was added after the reaction mixture was stirred for 1 h.

using hexamethyldisiloxane (HMDS) as an internal standard.[5] For all three resins, silylation did not have a significant effect on the conversion of the benzyl alcohol of Wang's resin to a monomalonate ester (compare entries a, e, and i with entries c, g, and k) and could be omitted in preparative reactions. Longer reaction times in the presence of Meldrum's acid led to lower loadings (entries a and b), presumably due to cleavage of malonic acid from the resin during the reaction. On the basis of these results, a bulk quantity of Wang malonic acid resin was prepared from 100 g of Wang resin (using the conditions corresponding to entry c) and a loading of 0.987 mequiv/g (96.5% conversion) was obtained.

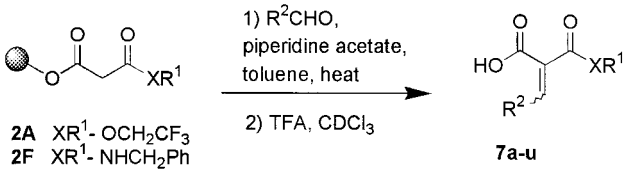
Preparation of the amide and ester derivatives was accomplished using standard coupling procedures from the corresponding amine or alcohol and malonic acid resin.[13] In the case of alcohols or anilines, large excesses of these reagents can be tolerated in the carbodiimide coupling reaction. However, for the more nucleophilic amines, inconsistent results were obtained following a standard treatment of 4 equiv each of HOBt, amine, and DIC (Table 2, entries a−f). For typical solid phase peptide synthesis, excess carboxylic acids or activated esters were added to resin-bound amines. However, for the preparation of **2**, excess amine was added to a resin-bound carboxylic acid. The coupling of *n*-propylamine was investigated in detail with 10 side-by-side reactions using conditions which have been reported for the coupling of amines to resin-bound carboxylic acids.[14] While the 18 h reaction time may not have been optimal for some of the conditions, the relative extents of conversion and yields are instructive. The first run (entry a) represents the standard conditions in which the three reagents are added sequentially and allowed to react for 18 h.[14a] The next three runs (entries b−d) show the effect of waiting 1 h prior to adding the final reagent. Adding the amine later than HOBt or DIC has the most profound effect, lowering both the the extent of conversion and yield. Excess amine (entry e) also lowers the yield of the desired malonamic ester, while just 2 equiv (entry f) provided excellent conversion and yield. Treatment of the resin with oxalyl chloride (entry g) followed by washing the resin and addition of amine gave a low yield of 30%.[14b] We have previously obtained good results from the oxalyl chloride treatment by using shorter reaction times.[7a] Although the extents of conversion for the PyBOP and HATU treatments were good (entries h and j), the overall yields of malonamic acid were poor.[14c,e] Presumably, the malonamic acid was cleaved prematurely during the reaction due to the long reaction times and subsequently washed away. On the basis of the optimized coupling conditions, eight resins were prepared (Table 3) which all gave quantitative conversion of the malonic acid and yields in the range of 84−94%.

Table 3. Preparation of Malonate and Malonamide Resins **2**{*A*−*H*}[a]

resin	R^1	loading (mequiv/g)	yield (%)
A	OCH_2CF_3	0.813	89
B	NH*n*-C_3H_7	0.867	91.3
C	$NHCH_2C(CH_3)_3$	0.755	81.7
D	$NHCH(CH_3)COOCH_3$	0.853	93.6
E	NH*c*-C_6H_{11}	0.793	86.8
F	NHbenzyl	0.814	89.7
G	NH(3-CF_3-4-Cl)Ph	0.712	84.8
H	NH(2,6-diOMe-4-Cl)Ph	0.714	84.5

[a] See Table 1. Conversions for all resins were greater than 95%.

Table 4. Preparation of Alkylidene Malonate and Malonamic Acids **7**[a]



2A XR^1- OCH_2CF_3
2F XR^1- $NHCH_2Ph$

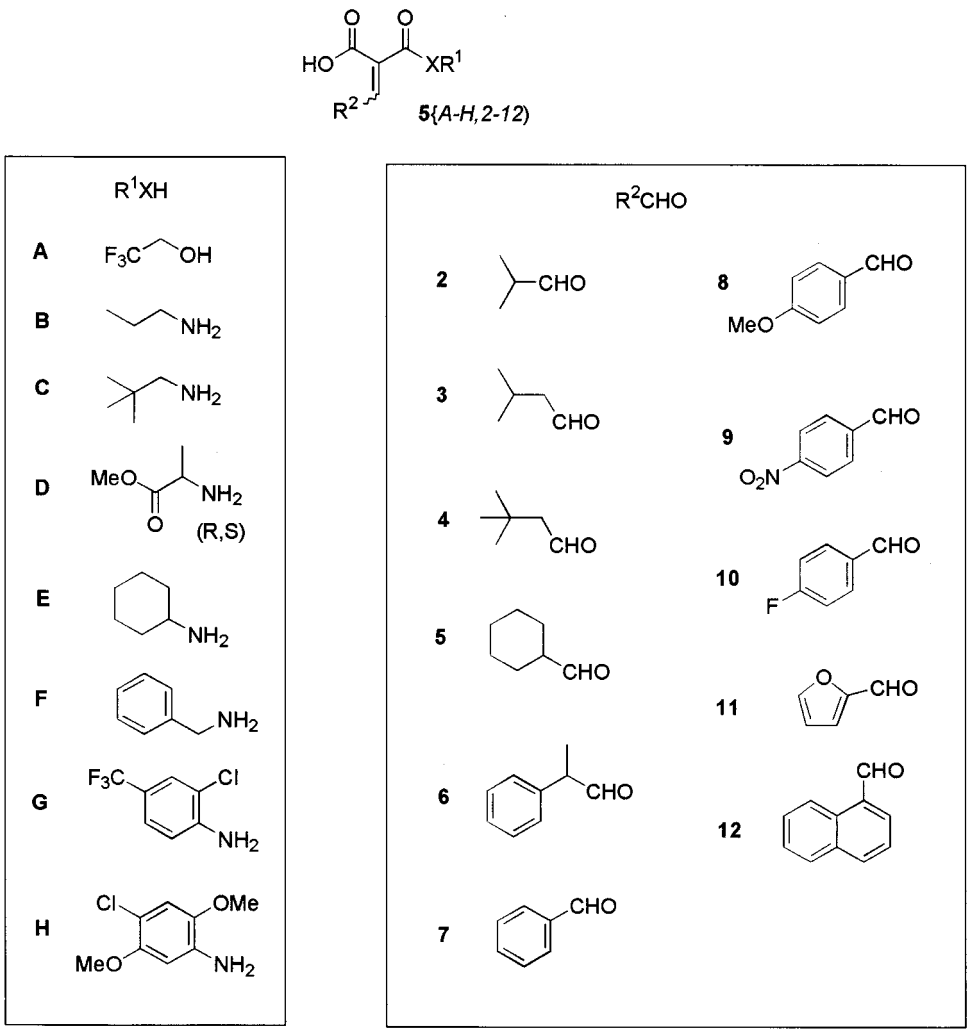
7a-u

compd	starting resin	R^2	conversion (%)	*E*:*Z* ratio	yield (%)
7a	2F	Ph	>95	64:36	45[b]
7b		4-(MeO)Ph	>95	53:47	48[b]
7c		4-(NO_2)Ph	>95	92:8	89[b]
7d		4-(F)Ph	>95	58:42	61[b]
7e		2-furyl	>95	90:10	65[b]
7f		$(CH_3)_2CH$	>95	45:55	45[b]
7g	2A	$(CH_3)_2CH$	92	56:44	97.1
7h		$(CH_3)_2CHCH_2$	>95	38:62	94.5
7i		$(CH_3)_3CCH_2$	>98	54:46	83.4
7j		$(CH_3)_3C$	3	−	−
7k		$PhCH_2$	28	−	−
7l		$PhCH(CH_3)$	53	62:38	56
7m		Ph_2CH	18	56:44	−
7n		cyclohexyl	>98	31:69	94
7o		5-norbornen-2-yl	96	38:62	>98
7p		Ph	93	55:45	90.7 (45[b])
7q		3-pyridyl	92	39:61	93
7r		1-naphthyl	>98	53:47	92.5
7s		4-(OMe)Ph	>98	70:30	90.5
7t		4-(F)Ph	93	50:50	77.4
7u		2-furyl	>98	55:45	78.3

[a] Yields and *E*:*Z* ratios were determined by direct cleavage ^{1}H NMR using 10 mM HMDS as an internal standard, unless otherwise noted. [b] Yield of isolated material. Following chromatographic purification, only one isomer was observed by NMR.

The Knoevenagel reaction was investigated for a series of aldehydes to determine the scope of the reaction for resin-

Scheme 2



bound malonic acid derivatives (Table 4).[15] Aromatic and branched aliphatic aldehydes gave good results, although the more hindered pivaloyl, phenylacetyl, and diphenylacetaldehyde (**7j,k,m**) gave less than 30% conversion. It is worth noting that the *E*:*Z* ratio was nearly 1:1 for the malonate and malonamic acid derivatives. Although the Knoevenagel reaction has been reported to proceed on the solid phase with active methylene substrates without removal of water,[7b,16] we have found anhydrous conditions with some means of removal of water to be advantageous. For larger-scale preparations, a Dean−Stark trap was employed which allowed facile conversion to **4** in most cases in less than 1 h. Two types of reactor vessels were investigated for the library or production runs: an Argonaut Nautilus 2400 vessel and a glass "syringe barrel" type vessel equipped with a lower luer fitting, a frit, and an upper nitrogen line. The Nautilus vessel was attached to a rocker for agitation, allowing complete mixing, while the glass syringe barrel was placed upright on a shaker. Water droplets, which were observed in the syringe reactors during the course of the reaction, remain in the upper condensation region of the vessel. Since these vessels were not inverted or rotated, the water was effectively removed during reaction, allowing complete conversions of **2** to **4**. The Nautilus vessels, due to the complete mixing, required the addition of molecular sieves (or other dehydrating agent) to achieve quantitative conversions, particularly for the malonate ester resin **2A**.

Cleavage of the products from the resin was significantly mild to allow isolation of the carboxylic acids **7** without decarboxylation to disubstituted olefins.[17] Mass spectra were collected by various techniques for **7a** and **7p** and found to provide the decarboxylation products, such as *N*-benzylcinnammide for **7a**, as the base peak. The expected molecular ions of **7a,c**−**e** were observed by FAB-MS, and **7p** was only observed by negative FAB-MS using a triethylamine matrix. Attempts were made to obtain confirmation of a molecular ion using direct infusion electrospray and APCI of acetonitrile/water solutions both with and without ammonium acetate buffer; however, the heated capillary (>200 °C) employed with these techniques led to decarboxylation or decomposition of all samples. NMR spectroscopy, however, clearly showed the malonate mono acid **7p** as the major product with less than 5% decarboxylation. Upon isolation, crystalline products were obtained which were further characterized (**7a,b,f,p**) by combustion analysis. It was apparent that we would not be able to obtain confirmation of structure of library members from our benchtop LC−MS by either electrospray or APCI. [1]H NMR, however, provided straightforward analysis of conversion to **7a**−**u** by monitoring the appearance of a vinyl proton and loss of the

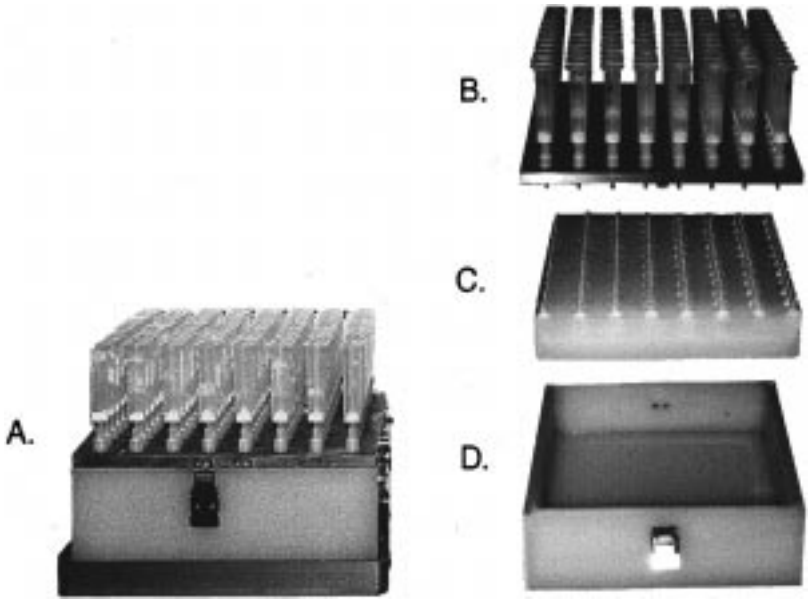


Figure 1. SynThesis Array Reactor (STAR) containing positions for 96 flow-through vessels in an 8 × 12 array. (A) The assembled reactor is placed on an orbital shaker for agitation. Components include (B) the manifold top with luer fittings for the bottom of each of the vessels, (C) the sample collection rack, and (D) the manifold for pressure regulation of the vessels.

methlyene resonance of **3**{*A*−*H*}. In addition, the presence of the decarboxylation product can be monitored by the presence of a set of doublets for the two vinyl protons.

Library Production

On the basis of the method development work, sets of alcohols/amines and aldehydes were chosen for the production library (Scheme 2). While a variety of reactor formats and systems are now available, we prefer to use a production device which allows facile layout of the reagents in either manual or automated means. A 96-vessel SynThesis Array Reactor (STAR) has been developed for production using flow-through reaction vessels which allows for washing of the resins and direct cleavage of the products into individual sample vessels (Figure 1).[18] Custom-made glass vessels (12 mL) were prepared which have a luer tip outlet at the bottom, a glass frit and a screw fitting at the top for addition of a setum cap, and a nitrogen line. The 96 vessels are arranged in an 8 × 12 array which is a larger-scale format (12 in. × 12 in. plate) of a standard 96-well plate. This format allows facile manual filling of each of the vessels with resins and reagents. Once the vessels are treated with the appropriate reagents, the structural identity of any library member can be determined directly from the position in the array. Rows A−H and columns 2−12 correspond to the R^1 and R^2 substituents, respectively (Scheme 2). An isopycnic slurry of polymer-bound N-substituted malonamic acids and malonate esters **2**{*A*−*H*} was prepared and added to the corresponding rows A−H of the STAR vessels such that each vessel contained 0.1 mmol of the substrate. After thorough washing of the resins, the glass vessels were purged with nitrogen and each column in the reactor block was treated with a solution of the appropriate aldehyde. Column 1 (samples A1−H1) was left untreated to provide starting material within the sample set as an external standard for both analytical and biological testing. The reactor array was fitted with an aluminum heater block set at 80 °C and allowed to heat for 18 h. After a suitable wash protocol, the resins were cleaved directly with TFA/CH_2Cl_2 (1:1) into a set of 96 vials placed directly beneath the glass reactors and dried to constant weight.

Using a Bohdan workstation, solutions of each sample in acetonitrile were prepared and sample aliquots were transferred to analytical vials for HPLC analysis. Chromatographic analysis was carried out using both UV detection and an evaporative light scattering detector (ELSD). While the ELSD can provide a better indication of the purity of samples,[4] either detector is suitable for measurement of conversion from the starting resins **2**{*A*−*H*}. Due to the setup of the reactor block, the samples in column 1 (**5**{*A*−*H,1*}) correspond to 0.1 mmol samples of cleaved malonamic acids **3**{*A*−*H*}. The *N*-cyclohexylmalonamic acid, **5**{*E,1*}, gives a single chromatographic peak at 1.5 min using our standard reverse phase gradient (Figure 2). The conversion to **5**{*E,3*} is complete as evidenced by the presence of two peaks (3.1 and 3.6 min) corresponding to the *E* and *Z* isomers. By comparison, partial conversion to **5**{*E,6*} is observed as evidenced by a significant starting material peak and two new product peaks (3.5 and 3.9 min). Although the response factors of the products are unknown, the conversion can be measured by analysis of the peak area of the starting material. In the case of sample **5**{*E,6*}, the starting material represents 49% of the area obtained for a 0.1 mmol sample **5**{*E,1*}. Therefore, the maximum possible extent of conversion to product(s) is 51%, using the starting material as an internal standard. Since each of the 96 samples in this set consisted of 0.1 mmol of material prior to the Knoevenagel reaction, the remaining starting material can be used as an internal standard for determination of the extent of conversion of reaction. This assumes that the desired products are obtained and that the reaction provides few side products. In addition, the chromatographic properties of the products must be such that they do not interfere with the measurement of starting material. These potential interferences can be addressed by

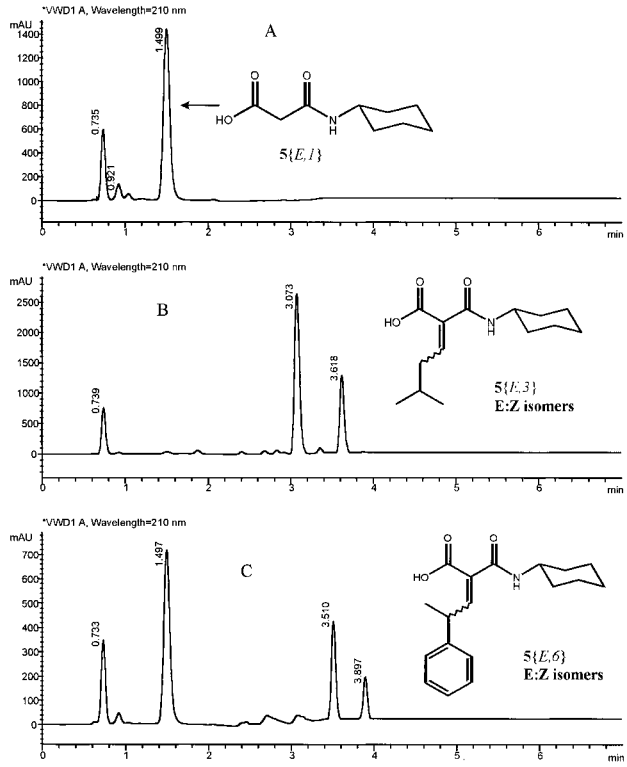


Figure 2. Chromatographic HPLC analysis of three members of the 96-component library **5**: (A) malonamic acid **5**{*E,1*}, (B) mixture of *E* and *Z* isomers of **5**{*E,3*}, and (C) incomplete reaction of **5**{*E,6*}. Samples were analyzed using a standard gradient and conditions (see the Experimental Section) and 210 nm UV detection.

using an alternative method for analysis for the set of library products, such as mass spectrometry or NMR. Extents of conversion as measured with the HPLC method were good for most of the samples (Figure 3) with only seven of the 88 samples providing less than 75% conversion. A "plate view" with shading for samples failing the 75% conversion criteria shows that the aldehyde used in column 6 did not provide adequate conversion, except for malonate resin **2A** and malonamic acid **2G**. In addition, row H had significant amounts of starting material in all samples except **5**{*H,11*}.

[1]H NMR spectra were obtained for protonated DMSO solutions of each of the samples using a flow-through probe interfaced with an automated sample injector. The samples were prepared as 50 mM solutions in DMSO and transferred to a 96-deep well plate. Each sample was collected with both DMSO and water suppression. Although this eliminated analysis of the 2.4−3.6 ppm region of the proton spectra, most of the analyte resonances reside outside this region. Sample **5**{*A,12*} clearly shows the presence of two isomers in a 2:3 ratio (Figure 4). The methylene resonance of the starting material **5**{*A,1*} (4.59 ppm) is not visible in the product spectrum, although a small amount of this material may be obscured by the methylene quartet (4.75 ppm) of the major isomeric product. Relative integrations of the vinyl protons (8.36 and 8.43 ppm) and the methylene quartets (4.75 and 4.90 ppm) are supportive of a single set of isomers without a significant amount of starting malonate **5**{*A,1*}, since both of these pairs of resonances exhibit a 2:3 ratio. The conversion of **5**{*A,1*} to product was also confirmed by the HPLC analysis of **5**{*A,12*} which showed an absence (less than 5%) of the starting material.

Comparison of 12 stacked [1]H NMR spectra shows significant conversion of the starting material **5**{*A,1*} to Knoevenagel products, **5**{*A,2*−*12*}, for each of the 11 reaction mixtures in row A of the STAR block (Figure 5). The malonate monoester **5**{*A,1*} exhibits two resonances for the methylene groups (4.72 and 3.70 ppm); however, only the CH_2CF_3 methylene group has diagnostic value, since the higher-field malonate methylene resonance is partly obscured by the solvent suppression. Each of the 11 product spectra shows significant changes in the methylene resonance as well as the addition of signals from the aldehyde component of the reaction. The four samples **5**{*A,2*−*5*} exhibit the expected aliphatic signals from incorporation of the aliphatic aldehydes in the products. Compound **5**{*A,6*} shows significant amounts of starting material as well as the expected product. The products prepared from the six aromatic aldehydes (**5**{*A,7*−*12*}) all show incorporation of the aromatic groups (7.00−

	1	2	3	4	5	6	7	8	9	10	11	12
A	100	0	0	0	0	0	10	5	0	15	5	0
B	100	0	0	0	0	100	0	0	0	0	0	0
C	100	19	0	0	7	94	6	5	0	6	0	0
D	100	0	0	0	0	90	0	0	0	0	0	0
E	100	7	3	0	4	49	0	0	0	2	2	2
F	100	14	11	4	12	100	11	8	3	3	5	5
G	100	13	8	5	16	24	8	10	5	8	0	5
H	100	35	22	22	20	100	18	16	12	21	0	22

Figure 3. Plate view of the HPLC data for conversion of starting materials in column 1 (**5**{*A-H,1*}) to products in columns 2−12 (**5**{*A-H,2*−*12*}). Cells shaded in gray indicate reaction mixtures containing greater than 20% starting material.

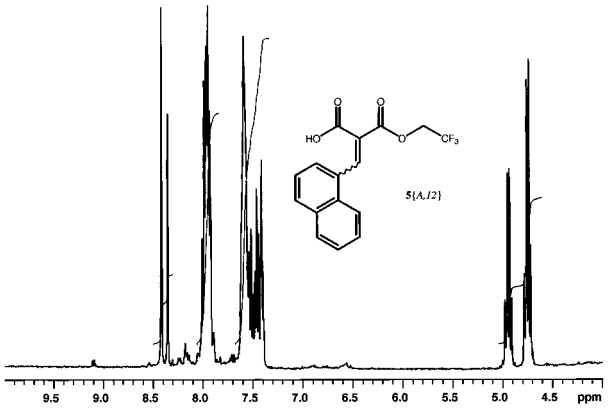


Figure 4. Flow-through probe ^{1}H NMR of library sample **5**{*A,12*} in DMSO obtained from a 96-deep well plate. The spectrum was acquired with DMSO and water solvent suppression.

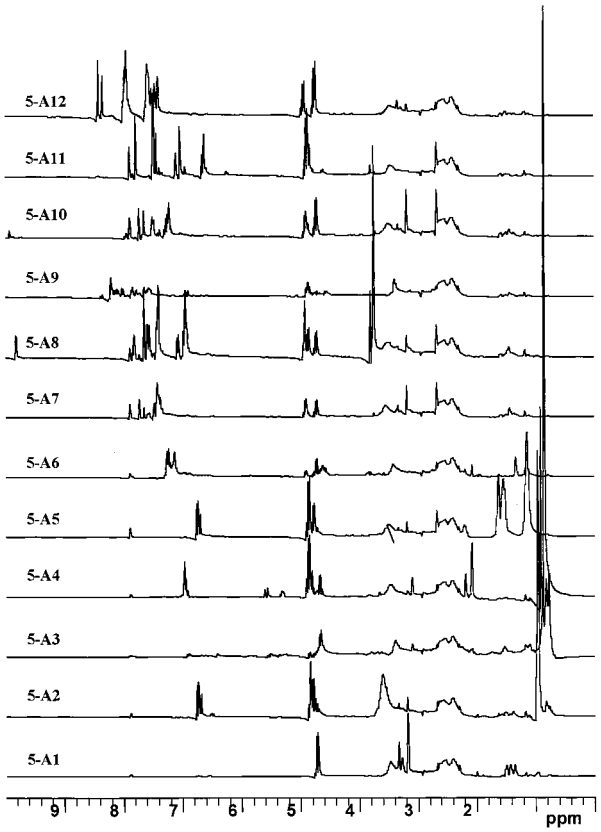


Figure 5. Flow-through probe ^{1}H NMR spectra of library samples **5**{*A,1*−*12*} in DMSO obtained from a 96-deep well plate. The spectrum was acquired with DMSO and water solvent suppression.

8.50 ppm) as well as changes in the malonate methylene ester resonances. Over time, we noted that some of the samples underwent a retro Knoevenagel reaction due to the presence of adventitious water in the DMSO solutions to give small amounts of the aldehyde and the unsubstituted malonamic or malonate mono acids. This can be clearly seen in samples **5**{*A,8*} and **5**{*A,10*} which contain aldehyde impurities as evidenced by the aldehyde proton resonance (9.50−10.00 ppm). On further investigation of freshly prepared samples, we found that hydrolysis can be avoided by keeping the samples rigorously dry.

For each of the 96 reaction products **5**{*A*−*H,1*−*12*}, two ^{1}H NMR spectra were obtained: one without and one with solvent suppression. The nonsuppressed spectra were autophased and the phasing parameters applied to the solvent-suppressed spectra. This method offered the most consistent automated phasing of the spectra, but precluded the use of integration due to slight phasing inconsistency from sample to sample. Using sets of 12 stacked ^{1}H NMR spectra for each of rows A−H, all 96 reactions products **5**{*A*−*H,1*−*12*} were qualitatively analyzed for the presence of the desired product and given a value of 0 for no product, 50 for mixtures of product and starting material, or 100 for greater than 80% product (Figure 6). On the basis of the plate view, all but five of the samples contain product. Some contain observable starting material (rating of 50); however, these products have been observed to convert back to the starting aldehyde and malonamic acids **3** in the presence of adventitious water. Particularly striking is the correspondence between the plate view diagrams of the HPLC conversion data (Figure 3) and the NMR structure confirmation (Figure 6). Both methods clearly indicate the lack of products or partial conversion for the compounds in row 6 (**5**{*B*−*H,6*}). The lack of reactivity of aldehyde {*6*} in the Knoevenagel reaction is not surprising, since it has a highly enolizable proton α to the carbonyl group. It is readily apparent that the results of the 96 parallel reactions can be used to determine reaction scope and used in the next iteration to optimize conditions for various substrates employed in followup libraries.

Plate view analysis, particularly when different analytical techniques are employed, is a powerful method for validating the chemical structures within combinatorial libraries. Using automated data collection methods and computational tools for preparing plate view analyses, combinatorial libraries can be validated with a minimum of individual spectral or chromatographic analysis. The plate view can be used to focus on analytical information which appears to be outside of the desired purity range. Each set of compounds will have particular requirements for optimization of the analytical methods, although this need can be addressed in the method development phase prior to production of a library. With proper analytical methods in place, one can readily collect and analyze all the required data for characterization of moderate-sized libraries by NMR and HPLC. The utility of the plate view technique for validation of libraries will have a direct impact on the determination of reaction scope used in library synthesis and on evaluation of the physical and biological properties of library members. Determination of structure−activity relationships is also facilitated, since structural validation of both active and inactive members can be achieved. Application of plate view NMR and HPLC techniques is currently being extended to larger libraries via more sophisticated methods for automated analysis of individual library members.

Experimental Section

General Procedures. Polymer-supported reactions were carried out using flasks fitted with a glass frit at the bottom and a sidearm connected to a needle valve (Aldrich, Z28,-330-4). Prior to the reactions being carried out, the polymer-bound starting material was allowed to swell in the reaction solvent for 30−60 min in an inert atmosphere. Moderate-

	1	2	3	4	5	6	7	8	9	10	11	12
A	100	100	50	100	100	50	50	100	100	50	100	100
B	100	100	100	100	100	0	50	100	50	50	50	50
C	100	50	50	50	50	0	50	100	100	50	100	100
D	100	50	50	50	50	0	100	100	100	100	100	100
E	100	100	100	100	100	50	100	100	100	100	100	100
F	100	50	50	100	50	0	100	100	100	100	100	100
G	100	50	100	50	50	50	100	50	50	50	100	100
H	100	50	50	100	100	0	100	100	100	100	100	100

Figure 6. Plate view of scoring of [1]H NMR data of library compounds **5**{*A*−*H*,*1*−*12*}. Cells shaded in gray indicate reaction mixtures which do not contain the expected resonances of the products.

scale reactions (50−500 mg of resin) were carried out using an Argonaut Nautilus 2400. For library production (100 μmol/reaction), we utilized a custom-designed SynThesis Array Reactor (STAR) using 15 mL glass syringe barrel reactors equipped with a luer fitting at the bottom and a screw fitting on the top.[18] Each vessel was fitted with a septum cap and nitrogen line. Wang or *p*-alkoxybenzyl alcohol resin was obtained from Advanced ChemTech (Louisville, KY), Argonaut Technologies (San Carlos, CA), and Chem-Impex International (Wood Dale, IL). Preparative and analytical reversed phase liquid chromatographic separations were achieved using aqueous 0.1% TFA/acetonitrile as the eluant. Analytical HPLC was carried out using a Rexchrom (Regis Technology) C18 ODS column (10 cm × 4.6 mm inside diameter; 5 μm particles) using a gradient from 30 to 80% acetonitrile over the course of 5 min at a flow rate of 2 mL/min. Detection was achieved using either UV detection (210 and 254 nm) or evaporative light scattering detection (ELSD).

FT-IR Microspectroscopy. The samples were prepared for analysis by transferring the sample with a stainless steel probe onto a clean microscope slide. A roller knife was then used to flatten the sample. One bead of sample was then transferred onto a KBr chip using a tungsten probe. Each spectrum was recorded at 4 cm^{-1} resolution on a Nicolet 800 Fourier transform interferometer equipped with a Spectra-Tech IR plan microscope. The spectra analyzed clearly showed the expected bands for the polystyrene polymer. The frequencies reported are indicative of the polymer-bound product.

Direct Cleavage of Resins for [1]H NMR Analysis and Determination of Polymer Loading. A standard cleavage solution of 10.98 mM hexamethyldisiloxane (HMDS) in 100 mL of TFA/$CDCl_3$ (1:1) was prepared and used for all of the evaluations of polymeric loading. The molarity of the HMDS solution was evaluated over time to determine the stability of the stock solution by treating a weighed amount (about 20 mg) of 4-hydroxybenzaldehyde with 1.00 mL of 10.98 mM HMDS. Measurement of the [1]H NMR integral of the HMDS peak (0.421 ppm) relative to that of either the aromatic or aldehyde resonances of 4-hydroxybenzaldehyde allowed an independent measure of the molarity of the HMDS solution. For determination of polymer loadings, a weighed amount of polymer (100 mg) was treated with 1.00 mL of 10.98 mM HMDS and the mixture allowed to shake for 30 min. The filtrate was collected and the resin washed three times with a minimal amount of $CDCl_3$. The filtrates were combined and placed in an NMR tube for direct evaluation of the purity of the cleaved product and measurement of the level of loading of the polymeric resin. The chemical shift of HMDS (0.421 ppm) in this solvent mixture was determined by comparison to added TMS. Loadings with this method are indicated for the cleaved products from resins as NMR loading and compared with the theoretical value (calcd) for determination of yield. Chemical shifts obtained using this solvent mixture (TFA/$CDCl_3$) are indicated as direct cleavage NMR.

Wang Malonic Acid Resin (1). An oven-dried 1 L glass polymer reactor was fitted with a condensor, and 100 g of Wang resin (Chem-Impex, catalog no. 01927, lot no. N12270, 1.12 mequiv/g) was added. The polymer was pretreated with 700 mL of anhydrous THF and allowed to soak for 1 h. THF was removed with positive N_2 pressure to keep the vessel dry, and the resin was subsequently treated with 61.0 g of freshly recrystallized Meldrum's acid (acetone/water, mp 92−93 °C) and 300 mL of dry THF. The mixture was heated to 65 °C with an oil bath for 24 h. After the suspension was allowed to cool, the solvent was removed and the resin washed three times each with THF, MeOH, and CH_2Cl_2, filtered, and allowed to dry overnight in vacuo to yield 109 g of a light brown resin: FTIR (microscopy) 3350, 3030, 2920 (C−H), 1729 (C═O), 1612 cm^{-1}. The average of two direct cleavage NMR analyses of the resin using 92.0 (0.968 mequiv/g) and 92.8 mg (1.006 mequiv/g) of dry resin, respectively, and 1.0 mL of 10.98 mM HMDS standard cleavage solution provided an average loading of 0.987 mequiv/g: direct cleavage [1]H NMR ($CDCl_3$/TFA) δ 3.65 (s, 2H).

NMR loading: calcd 1.022, found 0.987 (96.5% yield).

2,2,2-Trifluoroethyl Malonate Resin (2A). A vacuum-dried sample of 5.14 g (5 mmol) of **1** was weighed into an oven-dried glass polymer resin vessel and treated with 40 mL of anhydrous DMA. To this slurry were added 5.0 g (50 mmol) of trifluoroethanol and 0.12 g (1 mmol) of 4-(dimethylamino)pyridine (DMAP). After the mixture was agitated for 5 min under N_2, the mixture was treated with 6.31 g (50 mmol) of diisopropylcarbodiimide (DIC) and allowed to agitate under N_2 for 1 h. The treatment was repeated, and the resin was washed three times each with DMF, methanol, and CH_2Cl_2. After the mixture was allowed to dry overnight, 5.38 g of a yellow resin was obtained: FTIR (microscopy) 3031, 2920, 1773 (C═O), 1742 (C═O), 1600 cm^{-1}; direct cleavage ^{1}H NMR ($CDCl_3$/TFA) δ 3.70 (s, 2H), 4.59 (q, J = 8.1 Hz, 2H).

NMR loading: calcd 0.913, found 0.813 (89% yield).

***N*-(*n*-Propyl)malonamic Acid Resin (2B).** A vacuum-dried sample of 2.53 g (2.5 mmol) of **1** was weighed into an oven-dried glass polymer resin vessel and treated with 10 mL of anhydrous DMA. To this slurry were added 1 M HOBt/DMA (10 mL, 10 mmol) and *n*-propylamine (0.41 mL, 5 mmol). After the mixture was agitated for 5 min under N_2, the mixture was treated with 1 M diisopropylcarbodiimide (DIC)/DMA (10 mL, 10 mmol) and allowed to agitate under N_2 for 72 h. The resin was washed three times each with DMF, methanol, and CH_2Cl_2. After the mixture was allowed to dry overnight, 2.36 g of a yellow resin was obtained: direct cleavage ^{1}H NMR ($CDCl_3$/TFA) δ 0.97 (t, J = 7.2 Hz, 3H), 1.64 (sx, J = 7.2 Hz, 2H), 3.37 (q, J = 7.2 Hz, 2H), 3.67 (s, 2H), 7.75 (bs, 1H).

NMR loading: calcd 0.949, found 0.867 (91.3% yield).

***N*-(2,2-Dimethylpropyl)malonamic Acid Resin (2C).** Treatment of 2.07 g (2 mmol) of **1** by the general procedure afforded 2.20 g of a yellow resin: direct cleavage ^{1}H NMR ($CDCl_3$/TFA) δ 0.97 (s, 9H), 3.22 (d, J = 6.2 Hz), 3.73 (s, 2H), 7.90 (brs, 1H).

NMR loading: calcd 0.924, found 0.755 (81.7% yield).

***N*-[2-(Methyl propionate)]malonamic Acid Resin (2D).** Treatment of 2.07 g (2 mmol) of **1** by the general procedure afforded 2.15 g of a yellow resin: direct cleavage ^{1}H NMR ($CDCl_3$/TFA) δ 1.52 (d, 3H, J = 7.2 Hz), 3.64 (s, 2H), 3.88 (s, 3H), 4.72 (quint, 1H, J = 7.1 Hz), 7.94 (brs, 1H).

NMR loading: calcd 0.911, found 0.853 (93.6% yield).

***N*-Cyclohexylmalonamic Acid Resin (2E).** Treatment of 2.53 g (2.5 mmol) of **1** with cyclohexylamine (495 mg, 5 mmol) by the general procedure afforded 2.48 g of a yellow resin: direct cleavage ^{1}H NMR ($CDCl_3$/TFA) δ 1.22−1.45 (m, 5H), 1.65−1.69 (m, 1H), 1.76−1.80 (m, 2H), 1.93−1.96 (m, 2H), 3.67 (s, 2H), 3.85 (m, 1H), 7.63 (bs, 1H).

NMR loading: calcd 0.914, found 0.793 (86.8% yield).

***N*-Benzylmalonamic Acid Resin (2F).** Treatment of 2.07 g (2 mmol) of **1** by the general procedure afforded 2.21 g of a yellow resin: direct cleavage ^{1}H NMR ($CDCl_3$/TFA) δ 3.68 (s, 2H), 4.56 (d, 2H, J = 5.5 Hz), 7.29 (m, 2H), 7.37 (m, 3H), 7.75 (brs, 1H).

NMR loading: calcd 0.907, found 0.814 (89.7% yield).

***N*-[3-(Trifluoromethyl)-4-chlorophenyl]malonamic Acid Resin (2G).** Treatment of 2.07 g (2 mmol) of **1** by the general procedure afforded 2.41 g of a yellow resin: direct cleavage ^{1}H NMR ($CDCl_3$/TFA) δ 3.81 (s, 2H), 7.54 (d, 1H, J = 8.7 Hz), 7.69 (dd, 2H, J = 8.7, 2.4 Hz), 7.81 (d, 1H, J = 2.4 Hz), 9.23 (brs, 1H).

NMR loading: calcd 0.84, found 0.712 (84.8% yield).

***N*-(2,5-Dimethoxy-4-chlorophenyl)malonamic Acid Resin (2H).** Treatment of 2.07 g (2 mmol) of **1** by the general procedure afforded 2.21 g of a yellow resin: direct cleavage ^{1}H NMR ($CDCl_3$/TFA) δ 3.81 (s, 2H), 3.91 (s, 3H), 3.95 (s, 3H), 7.02 (s, 1H), 8.01 (s, 1H), 9.53 (s, 1H).

NMR loading: calcd 0.845, found 0.714 (84.5% yield).

Preparation of Methylene-Substituted Malonamic Acids. *N*-Benzyl-2-benzylidenemalonamic Acid (7a). Polymer-bound *N*-benzyl malonamide **2F** (0.33 g, 0.276 mmol) was slurried with 3.5 mL of anhydrous toluene and treated with 7 μL (0.069 mmol) of piperidine, 4 μL (0.069 mmol) of acetic acid, and 84 μL (0.83 mmol) of benzaldehyde. The mixture was heated to 85 °C for 24 h with an overhead stirrer being used for agitation. After filtration of the mixture, the resin was washed three times each with toluene, methanol, CH_2Cl_2, and Et_2O to give a light tan resin: FTIR (microscopy) 3305 (N−H), 3030, 2922, 1712 (C═O, ester), 1670 cm^{-1} (C═O, amide). The resulting polymer was cleaved with 1 mL of 95% TFA/H_2O and agitated for 1 h. After collection of the filtrate, the polymer was washed three times with 50% TFA/CH_2Cl_2 and the combined filtrates were concentrated in vacuo to yield 69 mg (89% crude material) of a yellow oil which was >90% pure as determined by ^{1}H NMR. Chromatographic purification (silica, 0.1% acetic acid/1% MeOH/CH_2Cl_2) afforded 35 mg (45%) of an off-white solid: mp 155−157 °C; ^{1}H NMR ($CDCl_3$) δ 4.51 (d, 2H, J = 5.7 Hz), 6.22 (brs, 1H), 7.17−7.44 (m, 10H), 8.07 (s, 1H), 9.60 (brs, 1H); MS (FAB) *m*/*z* (relative intensity) 288 (M + Li, 100), 282 (M + H, 61). Anal. Calcd for $C_{17}H_{15}NO_3 \cdot 0.25H_2O$: C, 71.44; H, 5.47; N, 4.90. Found: C, 71.34; H, 5.52; N, 4.70.

***N*-Benzyl-2-(4-methoxy)benzylidenemalonamic Acid (7b).** Treatment of 1.0 g (0.81 mmol) of **2F** afforded after cleavage 196 mg (78%) of a yellow oil. Chromatographic purification (silica, 0.1% acetic acid/1% MeOH/CH_2Cl_2) afforded 121 mg (48%) of an off-white solid: mp 209−211 °C; ^{1}H NMR (acetone-d_6) δ 2.08 (q, 6H), 3.86 (s, 3H), 4.55 (d, J = 5.7 Hz, 2H), 6.87 (d, J = 8.7 Hz, 1H), 7.32 (m, 5H), 7.41 (d, J = 7.5 Hz, 2H), 7.57 (t, J = 8.3 Hz, 3H). Anal. Calcd for $C_{18}H_{17}NO_4$: C, 69.38; H, 5.46; N, 4.50. Found: C, 69.19; H, 5.56; N, 4.42.

***N*-Benzyl-2-(4-nitro)benzylidenemalonamic Acid (7c).** Treatment of 1.0 g (0.81 mmol) of **2F** afforded after cleavage 135 mg (72.7%) of a yellow oil: ^{1}H NMR (CD_3OD) δ 4.41 (d, 2H, J = 5.5 Hz), 7.25−7.40 (m, 7H), 7.55−7.75 (m, 2H), 7.99 and 8.02 (set of singlets, 1H); MS (FAB) *m*/*z* (relative intensity) 327 (M + H, 15), 279 (15), 194 (100).

***N*-Benzyl-2-(4-fluoro)benzylidenemalonamic Acid (7d).** Treatment of 1.0 g (0.81 mmol) of **2F** afforded after cleavage 103 mg (89%) of a yellow oil. Chromatographic purification (silica, 0.1% acetic acid/1% MeOH/CH_2Cl_2) afforded 71 mg (48%) of a light yellow solid: mp 156−160 °C; ^{1}H NMR (CD_3CN) δ 1.96 (m, 3H), 4.44 (d, 2H, J = 5.4 Hz), 7.04 (t, 2H, J = 8.4 Hz), 7.32 (m, 7H), 7.36 (brs, 2H), 7.67 (s, 1H);

^{19}F NMR (CD_3CN) δ −111.7; MS (FAB) *m*/*z* (relative intensity) 300 (M + H, 100).

***N*-Benzyl-2-(4-nitro)benzylidenemalonamic Acid (7e).** Treatment of 1.0 g (0.81 mmol) of **2F** afforded after cleavage 175 mg (quantitative) of a yellow oil: ^{1}H NMR ($CDCl_3$) δ 4.66 (d, 2H, J = 5.5 Hz), 6.54 (brs, 1H), 7.25−7.40 (m, 8H), 7.86 (s, 1H); MS (FAB) *m*/*z* 272 (M + H), 278 (M + Li).

***N*-Benzyl-2-isopropylmethylenemalonamic Acid (7f).** Treatment of 1.0 g (0.81 mmol) of **2F** afforded 166 mg (83%) of a yellow oil. Chromatographic purification (silica, 0.1% acetic acid/1% MeOH/CH_2Cl_2) followed by recrystillization from ethyl acetate/hexanes afforded 90 mg (45%) of an off-white solid: mp 111−112.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.10 (q, 6H), 3.24 (m, 1H), 3.53 (m, 1H), 4.58 (q, 2H), 6.97 (s, 1H), 7.20 (d, J = 10.2 Hz, 1H), 7.15 (d, J = 10.5 Hz, 1H), 7.33 (m, 5H). Anal. Calcd for $C_{14}H_{17}NO_3$: C, 67.99; H, 6.93; N, 5.66. Found: C, 67.80; H, 6.80; N, 5.61.

2,2,2-Trifluoroethyl 2-Benzylidenemalonate Monoacid (7p). To an oven-dried three-neck flask equipped with an overhead stirrer, Dean−Stark trap, and N_2 line was added 1.07 g (0.87 mmol) of **2A**. The resin was suspended in 20 mL of anhydrous benzene and treated with 0.88 mL (8.7 mmol) of benzaldehyde, 43 μL (0.43 mmol) of piperidine, and 20 μL (0.43 mmol) of acetic acid. The stirred mixture was heated to reflux. After 1 h, the distillate was clear. After 3 h, the bulk mixture was allowed to cool and washed three times each with CH_2Cl_2, MeOH, and CH_2Cl_2. The resin was allowed to air-dry overnight to give 1.07 g (theoretical value of 1.15 g) of a yellow resin: direct cleavage ^{1}H NMR ($CDCl_3$/TFA) δ 4.68 and 4.69 (q, 2H, J = 8.1 Hz), 7.44−7.60 (m, 5H), 8.08 and 8.128 (s, 1H).

NMR loading: calcd 0.76, found 0.58 (76% yield). To 0.98 g (0.57 mmol) of resin was added 10 mL of TFA/CH_2Cl_2 (1:1) for 30 min. The resin was filtered and washed three times with CH_2Cl_2, and the filtrates were combined and concentrated. An oil was obtained which was dissolved in ether, extracted four times with water, dried, and concentrated to give an orange-yellow oil. Chromatographic purification (C18, 45% acetonitrile/water) afforded 160 mg (quantitative) of an off-white solid. Trituration with hexanes gave 70 mg (45%) of a white-yellow solid: mp 105−108 °C dec; ^{1}H NMR ($CDCl_3$) δ 4.63 and 4.64 (pair of q, 2H, J = 8.4 Hz), 7.25−7.58 (m, 5H), 7.90 and 7.98 (pair of s, 1H), 10.80 (brs, 1H); ^{19}F NMR ($CDCl_3$) δ −74.4 and −74.0 (pair of t, 3F, J = 8.4 Hz); MS (FAB, Et_3N) *m*/*z* (relative intensity) 273 (M − H, 100), 229 (M − CO_2, 78). Anal. Calcd for $C_{12}H_9F_3O_4 \cdot 0.25H_2O$: C, 51.71; H, 3.44. Found: C, 51.75; H, 3.45.

Preparation of Alkylidene-Substituted Malonate Acids (7g−u). 2,2,2-Trifluoroethyl malonate resin **2A** was suspended in dry toluene under N_2, and a 1 M solution of aldehyde in toluene was added. Each resin was then treated with 0.1 M piperidinium acetate in toluene (0.5 mL, 0.05 mmol), and a few molecular sieves were added. Each reaction mixture was heated to 80 °C for 18 h while it was shaken. After filtration of the mixtures, the resins were washed three times each with toluene, DMF, methanol, and CH_2Cl_2 and dried. The resulting polymers were treated with 1.00 mL of 50% TFA/$CDCl_3$ and agitated for 1 h. After collection of the filtrate, the polymer was washed three times with a minimal amount of $CDCl_3$ and the combined filtrates were analyzed by ^{1}H NMR.

Library Preparation. Preparation of Methylene-Substituted Malonamic and Malonate Acids (5{*A*−*H*,*1*−*12*}). Each of the polymer-bound N-substituted malonamic acids and malonate esters **2**{*A*−*H*} (1.2 mmol) was slurried in a mixture of CH_2Cl_2/DMF (60:40) and portioned by repeater pipet into an appropriate row of 12 glass reaction vessels in a 96-vessel STAR reactor block (0.1 mmol/vessel). The resins were washed three times each with DMF, MeOH, and DCM and air-dried. The resins were suspended in dry toluene (1 mL), and 1 M solutions of aldehydes {*2*−*12*} in toluene (1 mL, 1 mmol) were added to the appropriate column. Each resin was then treated with 0.1 M piperidinium acetate in toluene (0.5 mL, 0.05 mmol), and a few 4 Å molecular sieves were added. Each reaction mixture was placed under N_2 and heated to 80 °C for 18 h while it was being shaken. After filtration of the mixtures, the resins were washed three times each with toluene, DMF, methanol, and CH_2Cl_2 and dried. The resulting polymers were treated with 1 mL of 50% TFA/CH_2Cl_2 and agitated for 1 h. After collection of the filtrate, the polymer was washed three times with CH_2Cl_2 and the combined filtrates were concentrated to oils. Each sample was contained in a 1 dram screw cap vial in a STAR block collection rack organized as an 8 × 12 array. A Bohdan (ALD-200) workstation has been configured to hold the STAR collection rack, an orbital shaker, HPLC analytical vial racks, a balance, and a bar code reader. The STAR collection rack was placed in the Bohdan workstation, and each sample was diluted with 1 mL of acetonitrile and allowed to dissolve with an orbital shaker. A 100 μL aliquots of each sample was transferred to analytical HPLC vials and diluted with 0.4 mL of acetonitrile to provide a 20 mM solution of each sample.

NMR Sample Preparation. The samples in the vials contained in the STAR block collection rack were concentrated, redissolved in 2 mL of DMSO per vial (50 mM solution), and transferred to a Tecan RSP 5072 liquid handling robot. The bed of this robot has been modified to hold a variety of containers, from STAR inserts and STAR blocks to deep well and microtiter plates. The Tecan robot is controlled by a Compaq Proline A computer running version 1.90 Tecan Logic software. The flexibility of this software allows the programming of a variety of liquid handling tasks. Transfer of 1.0 mL of solution to fill each of the 96 wells of the plate for the NMR (Beckman model 267006) was accomplished in 48 min. The transfer needle of the robot was flushed with 1.0 mL of fresh DMSO and then washed with 5.0 mL of fresh DMSO between each transfer. After the plate was capped (Beckman model 267002), it was taken to the NMR spectrometer. Once the NMR spectra were obtained, the deep well plate was returned to the Tecan robot so that the solutions could be transferred back into their respective vials. Using the same wash protocol, each solution was transferred to its respective vial. Using this program in test runs with water, we found that 98% of the solutions (by weight) could be recovered. Transfer back to the vials in the STAR rack took 48 min.

NMR Flow-Through Probe Setup. A 400 MHz Varian Inova NMR spectrometer equipped with a Varian's VAST (Versatile Automated Sample Transport) automated sampling system was used to acquire data from the 96-well plate through a direct-injection NMR method.[19] The three-nucleus (^{1}H, ^{31}P, and ^{13}C), direct-detection, NMR flow probe had a deuterium lock and a 240 μL flow cell. Prior to data acquisition, a 775 μL sample was withdrawn from its well and injected into the probe. After examination, it was blown out of the probe with 90 psi dry nitrogen gas and returned to the well from which it came. One rinse cycle was performed between samples. ^{1}H NMR spectra were acquired for 1.8 s following an approximately 30° tip-angle pulse. Varian's WET solvent suppression sequence[20] was used to reduce signals from DMSO, water, and an unidentified impurity present in the DMSO ($d_H = 2.9$ ppm). In addition, Varian's solvent subtraction was employed for the DMSO and water peaks during postacquisition data processing. We acquired 128 transients for each spectrum, which provided better than necessary signal-to-noise ratios. A sample transport cycle of injection and retrieval required about 3 min. Thus, a full series of removing the previous sample, rinsing the probe, injecting the next sample, allowing for sample thermal equilibration, performing a SCOUT scan (used to determine the appropriate solvent suppression parameters), and acquiring data was performed in approximately 11 min. Using these parameters, the ^{1}H NMR spectra of all 96 samples **5**{*A*−*H*,*1*−*12*} were obtained. Analysis consisted of a comparison of the disappearance of the resonances of the unsubstituted malonamic/malonate mono acids **5**{*A*−*H*,*1*} with the appearance of substituted products **5**{*A*−*H*,*2*−*12*}.

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Supporting Information Available. Calculation of theoretical loadings for resins and ^{1}H NMR spectra for all 96 library members, **5**{*A*−*H*,*1*−*12*}. This material is available free of charge via the Internet at http://pubs.acs.org.

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