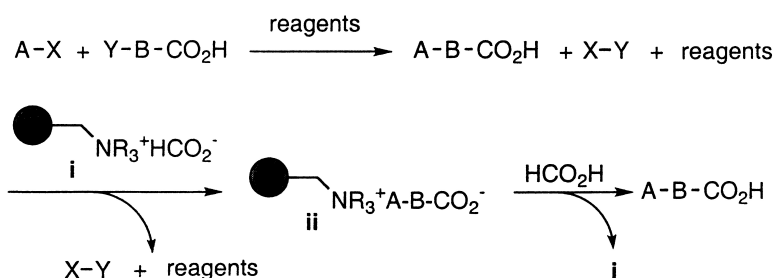


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# Solid Phase Extraction Purification of Carboxylic Acid Products from 96-Well Format Solution Phase Synthesis with DOWEX 1×8-400 Formate Anion Exchange Resin

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The anion exchange resin DOWEX 1×8-400 formate has been developed for the isolation or resin capture of carboxylic acids from solution phase reactions in a 96-well format using a batchwise solid phase extraction technique. Eleven different anion exchange resins (formate forms) were evaluated for their efficiency at scavenging aryl and aliphatic carboxylic acids from solution. The model carboxylic acids had  $pK_a$ s ranging from 3.40 to 4.89. Exchange efficiency onto the resin was  $pK_a$  dependent with the carboxylic acids but not with their diisopropylethylammonium salts. Exchange off of the resin also showed  $pK_a$  dependence with the stronger acids requiring more concentrated solvent acid for exchange. DOWEX 1×8-400 formate was determined to have superior capacity and the fastest exchange rate. Solvents suitable for exchanging the acids onto the resin were  $CH_2Cl_2$ , methanol, and various solvent/water mixtures. Solvents suitable for exchanging the carboxylic acids off of the resin were TFA/solvent or  $HCO_2H$ /solvent mixtures. The resin was found to swell best in  $CH_2Cl_2$  and in polar protic solvents such as water, alcohols, and acids. Application of this technique to the crude product mixtures from an arrayed reductive amination and an arrayed Stille reaction provided product carboxylic acids in yields averaging 57% and purities averaging 89%.

## Introduction

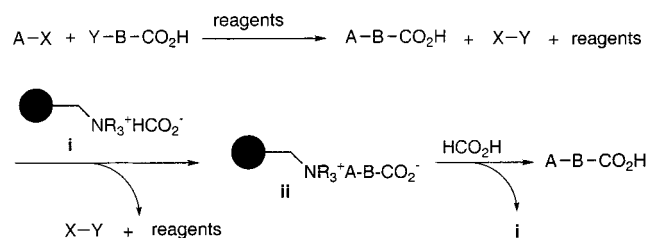
Purification methods are key to successful parallel synthesis of discrete compounds. Solid phase chemistry was emphasized early in combinatorial chemistry because of the ease of separation of the resin bound products from the contaminating excess reagents and byproducts which remained in solution.<sup>1</sup> More recently, solution phase combinatorial methods have gained in popularity as the techniques available for obtaining pure products have increased in number. Solution phase combinatorial chemistry offers the advantage of easier reaction monitoring (by MS for example), decreased stoichiometric excesses, and the elimination of two reactions from the reaction sequence, i.e., attachment to and detachment from the support resin. Solution phase combinatorial chemistry techniques often rely on a resin bound reagent to scavenge excess reactants or byproducts from reaction mixtures.<sup>2</sup> In particular, ion exchange resins in limited occasions have been used in solid phase extraction (SPE) protocols for this purpose.<sup>3,4</sup> Liquid–liquid extraction methods have also been used for parallel purification.<sup>5,6</sup> Even more rare is the application of ion exchange resins for the purification of products of a solution phase reaction.<sup>7,8</sup> In principle, any reaction which involves compounds with readily ionizable groups should be amenable to the separation of those compounds from the greater reaction mixture in a SPE process using ion exchange resins. When these ionic compounds are the desired products, they can be exchanged off of the resin with an appropriate volatile acid or base after

the resin is washed free of contaminating nonionic excess reagents and byproducts.<sup>9</sup>

The carboxylic acid moiety ( $CO_2H$ ) and other acidic functionalities are an integral part of many pharmacophores and are often present in reactants and intermediates. Thus a method which provides for the high throughput selective removal of compounds with acidic functionality from reaction mixtures should have broad applicability to solution phase combinatorial chemistry. Fifty years ago, Cohn published the first report on the use of DOWEX 1 200-400 mesh formate anion exchange resin for column chromatographic purification of nucleotides,<sup>10</sup> and the method became popular for the purification of phosphates and phosphonates.<sup>11</sup> More recently DOWEX 1×8-400 acetate anion exchange resin was found useful for the purification of  $CO_2H$  functionalized nucleotide mimetics.<sup>12</sup> Herein we describe the adaptation of this SPE method for the 96-well format isolation of  $CO_2H$ -containing reaction products. The batchwise technique employed is ideal for the high throughput parallel requirements of combinatorial chemistry.

The concept behind the ion exchange purification is illustrated in abstract form in Scheme 1. This strategy allows for large excesses of the nonionic reagent to be utilized in order to drive the reaction to completion. While solid phase combinatorial chemistry always utilizes large excesses of solution phase reactants for this purpose, it is often inconvenient to employ large excesses in solution phase combinatorial chemistry. This is especially true when expensive resins are employed to scavenge the excess reagents. Thus, often synthetic routes are devised which are as close to

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Scheme 1<sup>a</sup>

<sup>a</sup> A reaction between compound A-X and Y-B-CO<sub>2</sub>H in the presence of certain reagents results in a mixture containing the product A-B-CO<sub>2</sub>H as well as reagent and X-Y byproducts. Treatment of the mixture with anion exchange resin **i** extracts the CO<sub>2</sub>H compound from the mixture (SPE) to produce the resin bound A-B-CO<sub>2</sub><sup>-</sup> (**ii**). The nonionic impurities can be removed by filtration and solvent washing of the resin. Finally, treatment of the resin **ii** with a volatile solvent acid such as HCO<sub>2</sub>H releases the product acid A-B-CO<sub>2</sub>H from the resin in purified form.

Table 1. Anion Exchange Resins

resin	matrix <sup>a</sup>	group <sup>b</sup>	mesh size	cross-linking (%)	total capacity <sup>c</sup>	
					mequiv/mL	mequiv/g
DOWEX						
1×8-400 ( <b>A</b> )	gel	I	200–400	8	1.2	3.5
1×2-100 ( <b>B</b> )	gel	I	50–100	2	0.7	3.5
2×8-200 ( <b>C</b> )	gel	II	100–200	8	1.2	<i>d</i>
66 ( <b>D</b> )	mp	III	16–50	<i>d</i>	1.4	4.0
Marathon A ( <b>E</b> )	gel	I	30–40	<i>d</i>	1.2	4.0
MSA-1 ( <b>F</b> )	mp	I	20–50	<i>d</i>	1.0	4.0
Amberlite						
IRA-400 ( <b>G</b> )	gel	I	16–50	8	1.4	3.8
IRA-900 ( <b>H</b> )	mp	I	16–50	<i>d</i>	1.0	4.2
IRA-67 ( <b>I</b> )	gel	III	16–50	<i>d</i>	1.6	5.6
Amberjet 4200 ( <b>J</b> )	gel	I	16–50	<i>d</i>	1.3	<i>d</i>
Amberlyst 21 ( <b>K</b> )	mp	IV	16–50	<i>d</i>	1.3	4.7

<sup>a</sup> mp = macroporous. <sup>b</sup> I = RPhCH<sub>2</sub>NMe<sub>3</sub><sup>+</sup>. II = RPhCH<sub>2</sub>N-((CH<sub>2</sub>)<sub>2</sub>OH)Me<sub>2</sub><sup>+</sup>. III = RPhCH<sub>2</sub>NMe<sub>2</sub>. IV = freebase “alkylamine” (see footnote d). <sup>c</sup> The total capacity is the total concentration of exchangeable groups per unit of resin in milliequivalents which equates to millimoles. The capacity in mequiv/mL is the concentration per milliliter of water wet resin. The capacity in mequiv/g is the concentration per gram of dry resin. <sup>d</sup> Exact information is proprietary to the manufacturer.

stoichiometric equivalence as possible. However, as a synthesis sequence becomes more complex, it becomes difficult if not impossible to know what stoichiometric equivalence is for each individual reaction well. This should not be an issue if the product is ionic. In which case, large excesses of nonionic reagents can be employed since SPE can be used to isolate the product. Furthermore, ion exchange resins are generally inexpensive and consequently can also be considered for the removal of ionic reagents in order to isolate a neutral product.

## Anion Exchange Optimization Results

Eleven anion exchange resins were compared for their efficiency at exchanging four carboxylic acids for the resin bound formate ion. Table 1 lists the resins and their vendor specified properties. This is not an exhaustive selection of all possible anion exchange resins but is broad enough so that a range of properties can be surveyed for their effect on CO<sub>2</sub>H exchange. The resins are all polystyrene based with different benzylic amino functional groups. Groups I and II are quaternary amines and are strong anion exchangers while groups III and IV are freebase amines and are weak anion

Table 2. Carboxylic Acids

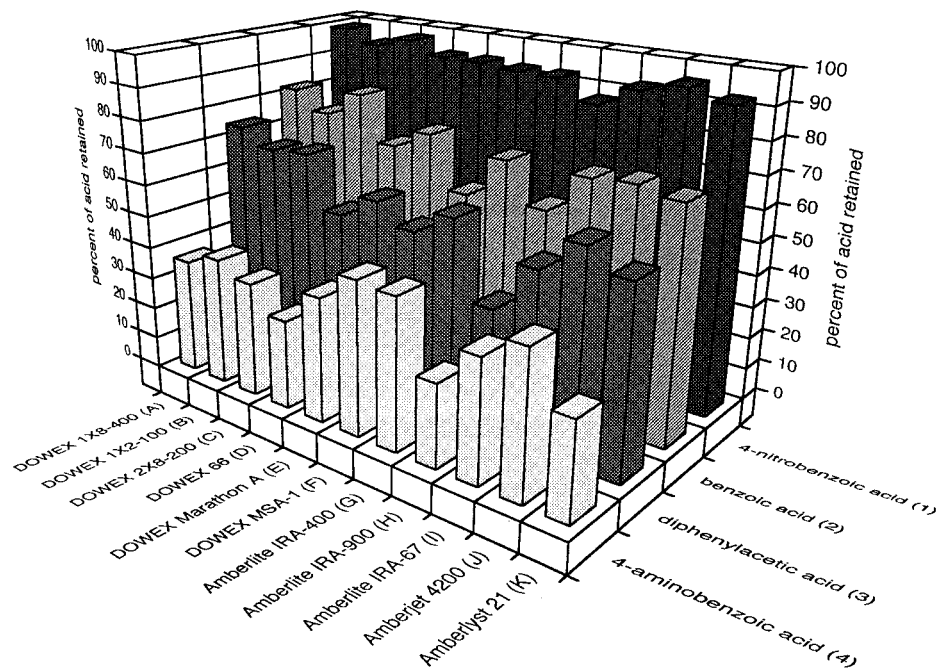
example acid	FW	pK <sub>a</sub>	solvent acid	FW	pK <sub>a</sub>
4-nitrobenzoic acid ( <b>1</b> )	167.1	3.40	CF <sub>3</sub> CO <sub>2</sub> H	114.0	0.30
benzoic acid ( <b>2</b> )	122.1	4.20	HCO <sub>2</sub> H	46.0	3.74
diphenylacetic acid ( <b>3</b> )	212.2	3.96	CH <sub>3</sub> CO <sub>2</sub> H	60.1	4.74
4-aminobenzoic acid ( <b>4</b> )	137.1	4.89			

exchangers. The range of resin water retention is about 2-fold with the 2% cross-linked resin (65–75% water retention) holding about twice as much water as the 8% cross-linked resin (34–40% water retention).<sup>9b</sup> For practical purposes, in this study, each experiment utilized 100 mg of each water saturated resin for the exchange of 0.020 mmol of each carboxylic acid unless specified otherwise. Therefore, by taking into consideration water retention and mequiv/g total capacity for the variety of anion exchange resins, 100 mg of water wet resin equates to ca. 5- to 13-fold excess in exchange sites available for a 0.020 mmol sample of a carboxylic acid.

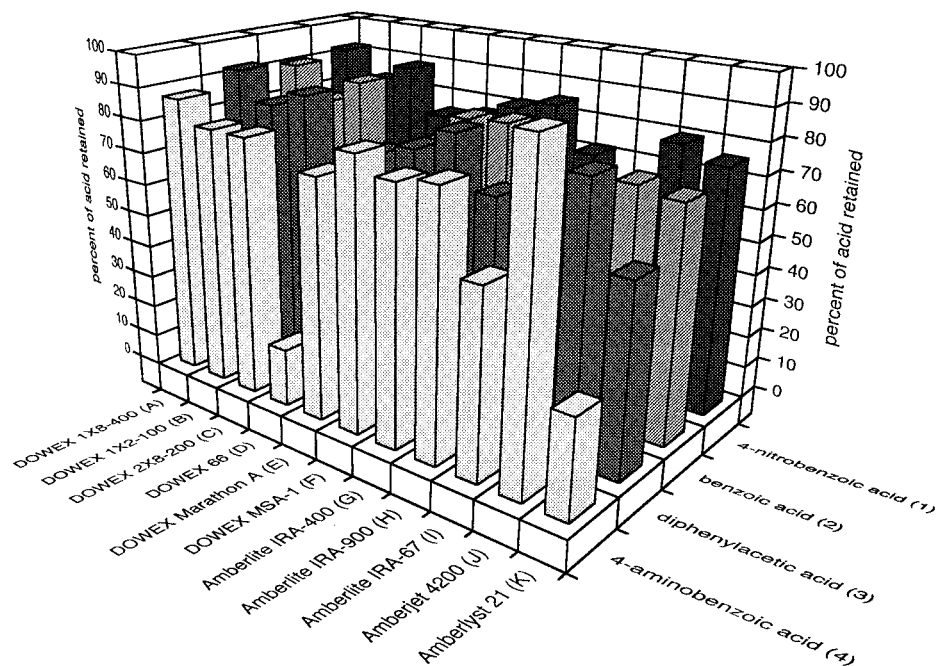
The model carboxylic acids and the volatile solvent acids are listed in Table 2. The three aryl- and one alkyl-CO<sub>2</sub>H compounds range in acidity from pK<sub>a</sub> 3.40–4.89. All experiments were batchwise ion exchange SPE operations. They were performed in 96-well filterplates and involved mixing at 450 rpm 100 mg of water saturated resin with 0.02 mmol of a CO<sub>2</sub>H compound in 0.5 mL of 9:1 DMSO/water for at least 1 h unless specified otherwise. The percent of CO<sub>2</sub>H compound remaining was determined by HPLC comparison of a reference solution to the filtrate from a resin treated solution.

A comparison of the percent exchange of the four CO<sub>2</sub>H compounds onto the eleven formate resins demonstrated that exchange efficiency is pK<sub>a</sub> dependent (Figure 1). The strongest acid, 4-nitrobenzoic acid (**1**), exchanged nearly quantitatively on all resin types whereas the weakest acid, 4-aminobenzoic acid (**4**), exchanged poorly across all resin types. A different result was observed for the CO<sub>2</sub>H diisopropylethylammonium (DIEA) salts (Figure 2). The DIEA salts allowed for a differentiation of the resins. Compared to other resins, the three DOWEX resins **A**, **B**, and **C** were less sensitive to pK<sub>a</sub> differences, and all four acid DIEA salts exchanged well with the formate on these resins. Other resins were less consistent. The weak anion exchangers, i.e., resins **D** and **K**, again showed pK<sub>a</sub> dependence in their exchange properties, favoring stronger over weaker acid DIEA salts.<sup>13</sup>

The three DOWEX resins **A**, **B**, and **C** were studied further for their working exchange capacity (Figure 3). The exchange of 0.020 mmol of each acid DIEA salt was tested onto 200 mg, 100 mg, 50 mg, and 25 mg of each of these three resins (water saturated). Resins **A** and **C** exchanged over 50% of each acid even at the lowest concentration of exchange sites (25 mg). In their water saturated forms, resins **A**, **B**, and **C** were determined to contain 47%, 70%, and 41% water, respectively. Assuming that their total dry weight capacity in their formate forms does not vary greatly from their chloride forms (Table 1), then 25 mg of the water saturated resins **A** and **B** contains, respectively, approximately 0.046 and 0.026 mmol of exchange sites available for the exchange



**Figure 1.** Percent exchange of carboxylic acids onto ion exchange resins (formate forms).



**Figure 2.** Percent exchange of carboxylic acid DIEA salts onto ion exchange resins (formate forms).

of 0.020 mmol of each example  $\text{CO}_2\text{H}$  compound. The capacity for resin **C** should be similar to **A** based on its similar water retention and mequiv/mL total capacity. Thus the resins were moderately effective at exchange even when used at only 1- to 3-fold in excess. While at 10–20-fold in excess, they were close to 100% effective at exchanging  $\text{CO}_2\text{H}$  compounds.

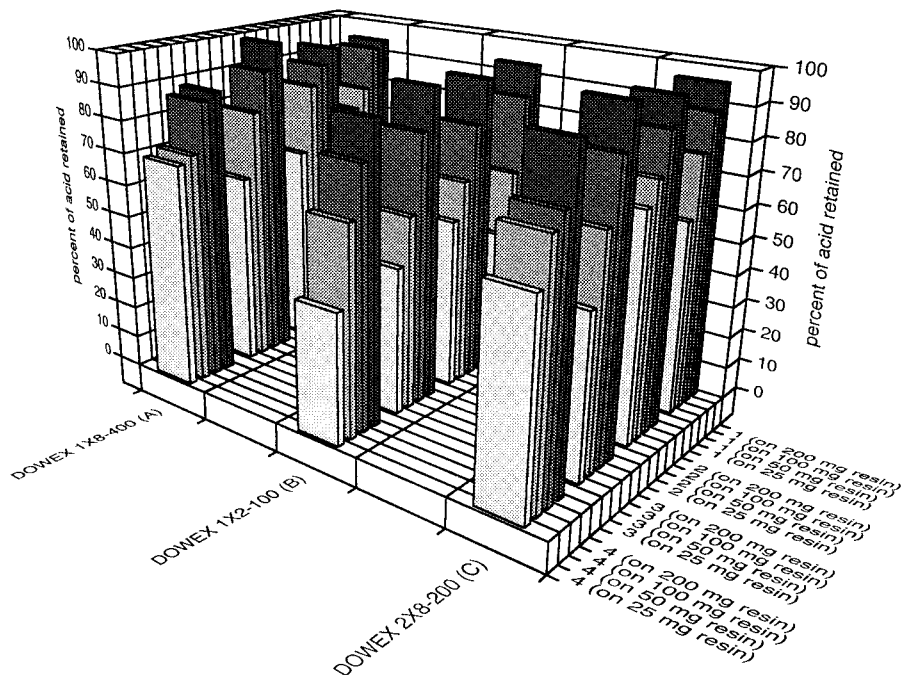
Since resins **A** and **C** had a slightly better working capacity than **B**, they were examined next in an arrayed experiment to determine the kinetics of the exchange (Figure 4). Comparison of the exposure of each acid DIEA salt to these resins for time periods of 3, 15, and 60 min revealed a noticeable difference in kinetics of exchange. DOWEX 1x8-

400 formate (**A**) was faster to exchange and consistent across the  $\text{CO}_2\text{H}$   $\text{pK}_a$  range. It was chosen for all further studies.

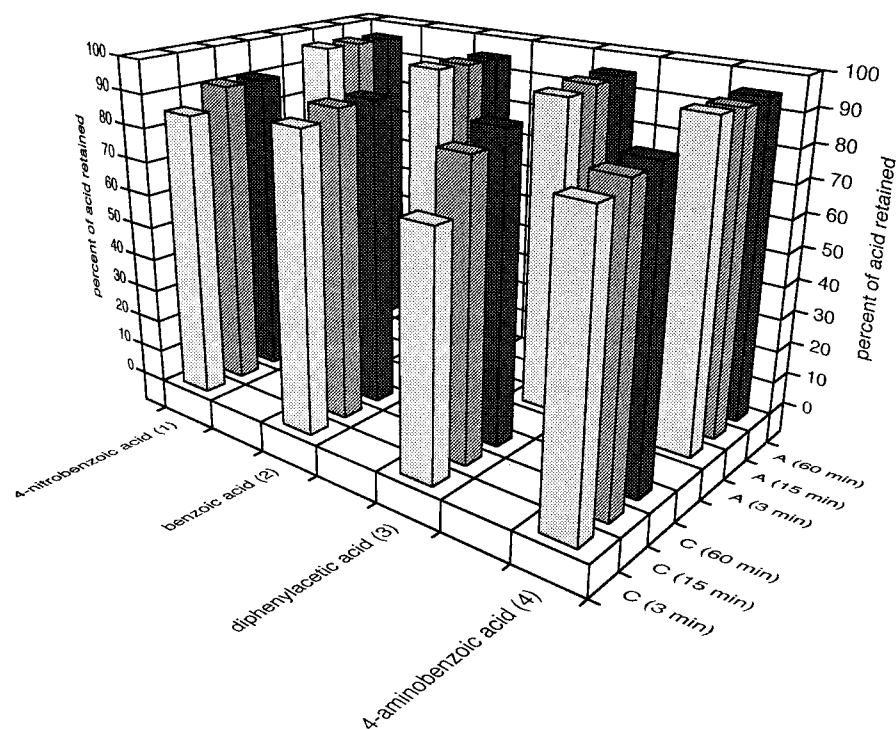
The choice of solvent for the SPE operation is critical to its success. A variety of solvents were tested as mediums for the transfer of DIEA salts of acids **2** and **3** onto resin **A** (Figure 5). In this experiment, the resin was dried and then preswollen in the solvent tested prior to exposure to the acid. The best solvents for exchange were  $\text{CH}_2\text{Cl}_2$ , methanol, and various solvent water mixtures. Anhydrous polar solvents were especially poor for the SPE of the aliphatic acid **3**.

Next, several solvent/acid combinations were investigated in order to discover the variety of systems useful for exchanging the  $\text{CO}_2\text{H}$  compounds off of DOWEX 1x8-400





**Figure 3.** Working resin capacity for carboxylic acid DIEA salts.



**Figure 4.** Speed of carboxylic acid DIEA salt exchange onto ion exchange resins.

(A) (Figures 6 and 7). Figure 6 shows the optimal solvent systems for exchange from the resin with 90:10 TFA/water and 90:10 formic acid/water being the most effective. For all but the strongest acid **1**, 95:5 methanol/TFA and 95:5 methanol/formic acid also provided a high recovery of the example carboxylic acids. Other systems tested included  $\text{CH}_2\text{-Cl}_2$  and/or acetic acid as components of the exchange cocktail (Figure 7). In some cases, e.g., 95:5  $\text{CH}_2\text{Cl}_2/\text{AcOH}$ , this permitted a complete differentiation of diphenylacetic acid (**3**) from benzoic acid (**2**).

The DOWEX 1x8-400 anion exchange resin was converted to several different anionic forms: bicarbonate,

carbonate, hydroxide, acetate, and chloride. These were used to evaluate the importance of the counterion to the SPE of  $\text{CO}_2\text{H}$  compounds (Figures 8 and 9). The counterions bicarbonate, carbonate, and hydroxide all exchanged quantitatively with acids (Figure 8) and acid DIEA salts (Figure 9) and thus showed no  $\text{pK}_a$  dependence in their exchange efficiency. The acetate counterion was similar to the formate ion in exchange efficiency. The chloride ion exchanged poorly with the free acids (Figure 8) but exchanged well with the DIEA salts (Figure 9). Thus several counterions can serve as alternatives to the formate ion.

Swelling of the gel phase in any matrix-supported puri-

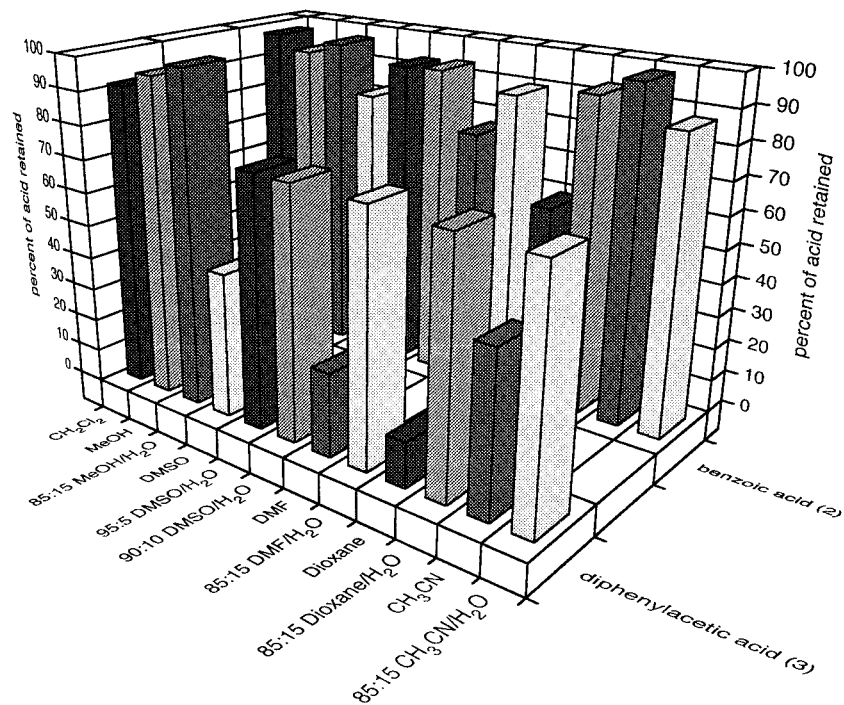


Figure 5. Solvent optimization for exchange of DIEA salts onto DOWEX 1×8-400 formate resin (A).

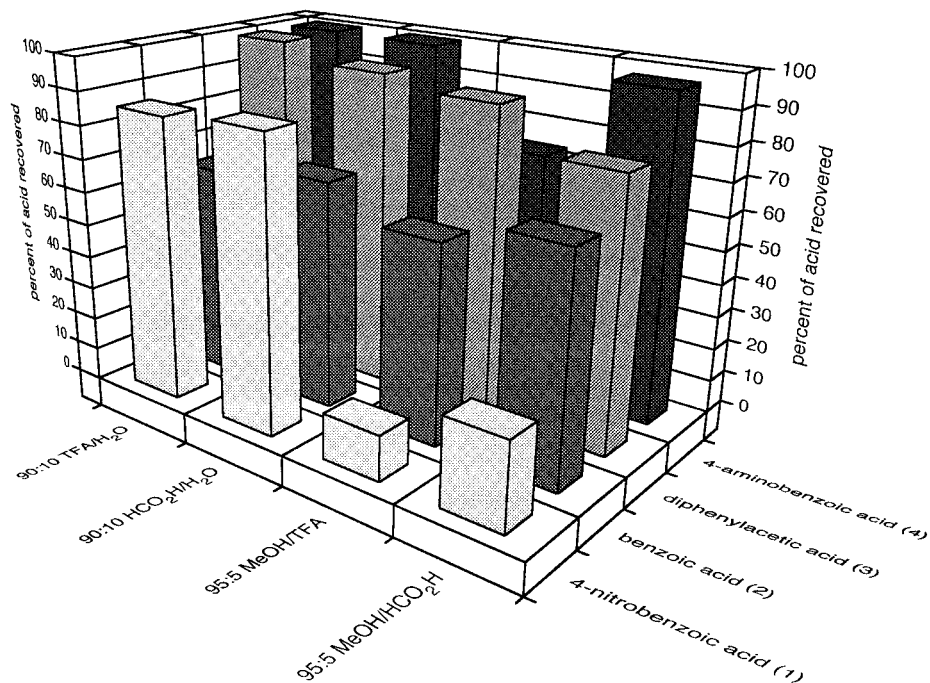


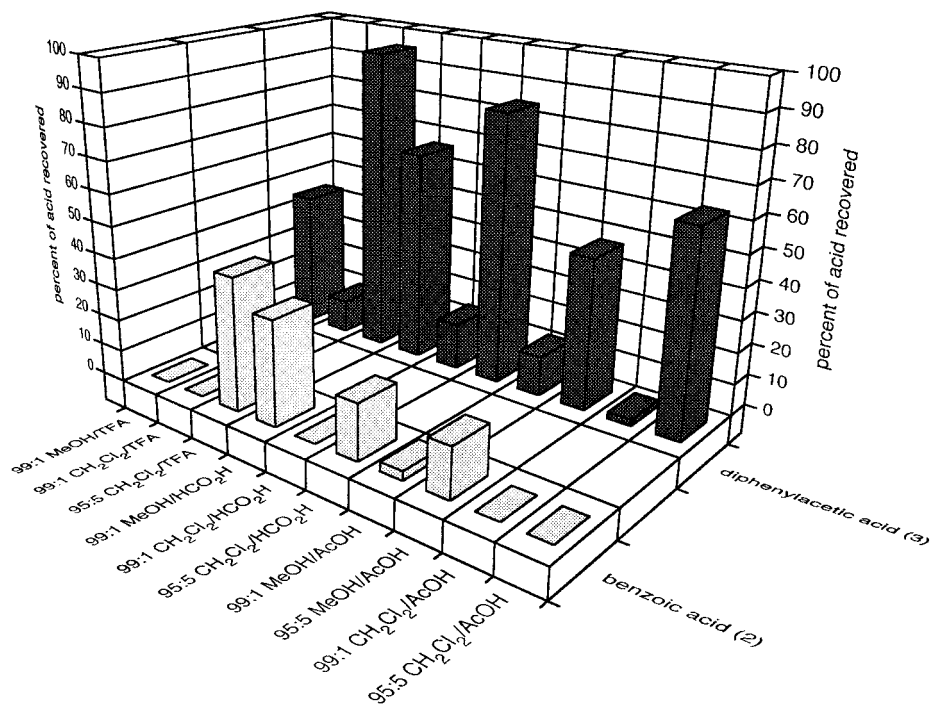
Figure 6. Optimal solvents for exchange off of DOWEX 1×8-400 resin (A).

fication or reaction is very important to the efficiency of that operation. Success or failure can often be related to the ability of the gel to swell in the solvent medium utilized. Table 3 lists the swelling of DOWEX 1×8-400 formate anion exchange resin in various solvent systems. The solvents that worked the best as mediums for exchanging CO<sub>2</sub>H DIEA salts onto the resin (Figure 5) were in general those that swelled the resin most efficiently. Solvent acids TFA and formic acid induced the most swelling followed by alcohols, CH<sub>2</sub>Cl<sub>2</sub>, and various solvent/water mixtures. These trends, with the exception of the swelling in CH<sub>2</sub>Cl<sub>2</sub>, are essentially

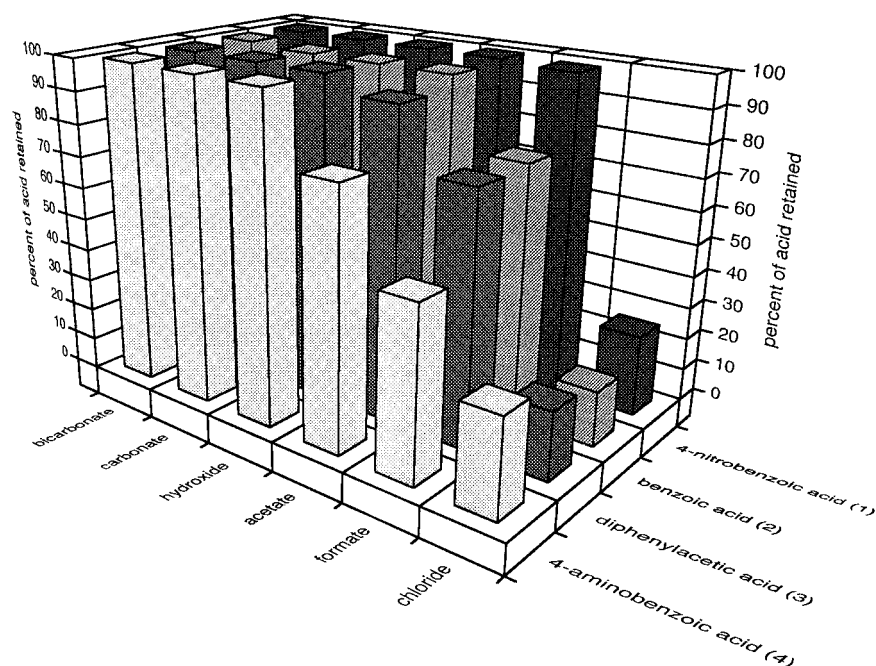
the reverse of what is seen with neutral polystyrene resins traditionally used in solid phase synthesis.<sup>14</sup>

### Synthesis Example Results

This optimized SPE procedure was applied to two reactions which are popular in combinatorial chemistry because of their robustness and diversity potential: the reductive amination<sup>1</sup> and the Stille coupling.<sup>15</sup> In the reductive amination, three amines in 4-fold excess, benzylamine, *n*-propylamine, and aniline, were combined with *o*-, *m*-, and *p*-carboxybenzaldehyde (5) in an array format at 0.020 mmol



**Figure 7.** Comparison of other solvents for exchange off of DOWEX 1×8-400 resin (A).



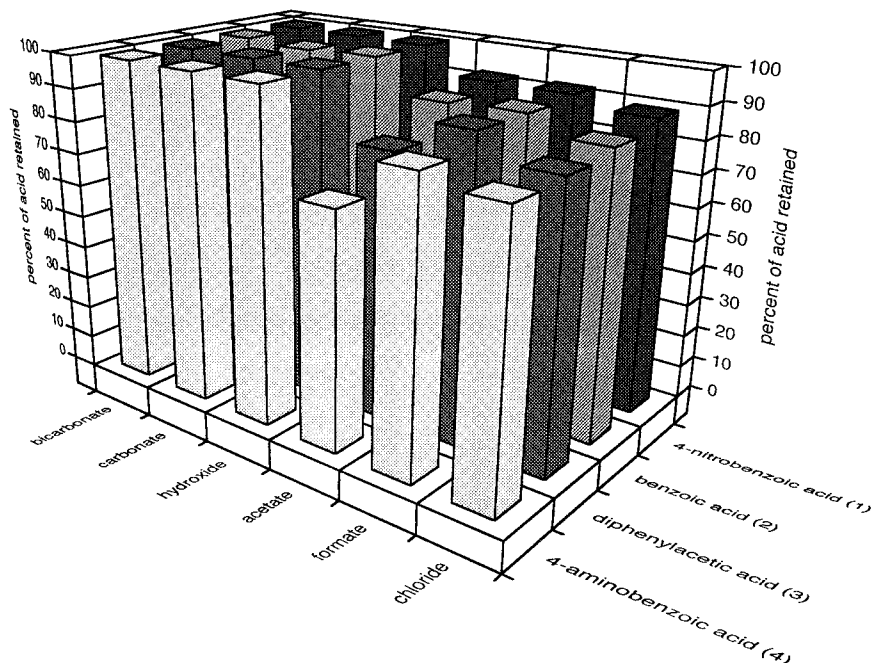
**Figure 8.** Counterion effect on percent exchange of carboxylic acids onto DOWEX 1×8-400 resin (A).

scale to produce the amino acids **6** (Scheme 2). As part of the workup procedure, water equaling 10% of the solution volume was added to the product mixture. This served to quench excess  $\text{NaBH}_4$  and to aid in the exchange of the products onto DOWEX 1×8-400 formate resin (A) (see Figure 5). The products were exchanged from the resin with 95:5 methanol/TFA. The products, yields, and HPLC (250 nm) purities are listed in Chart 1 ( $^1\text{H}$  NMR spectra and HPLC traces are included in the Supporting Information). The yields were determined by  $^1\text{H}$  NMR using acetic acid ( $\text{CH}_3$  resonance at  $\delta$  1.89 in  $\text{DMSO}-d_6$ ) as a 1.33 mM internal standard. The average yield for the nine reactions was 67%, and the average purity was 80%. However, the HPLC purity

determination of the propylamine adducts was hindered by the weak chromophoric nature of these products, and  $^1\text{H}$  NMR spectra indicated that all compounds were >95% pure with no traces of starting  $\text{CO}_2\text{H}$  compound present.

One notable product appeared in the aniline series. The bicyclic compound **6g** was formed as a result of the acidic treatment necessary for the exchange from the resin. The desired uncyclized reductive amination product was detected in small amounts in the initial  $^1\text{H}$  NMR study of the reaction mixture. After a short period of time, complete cyclization to **6g** was effected. Thus a new neutral product library is possible as a result of a post ion exchange compound modification.



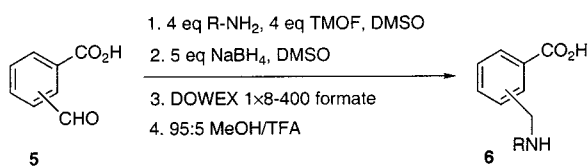


**Figure 9.** Counterion effect on percent exchange of carboxylic acid DIEA salts onto DOWEX 1×8-400 resin (A).

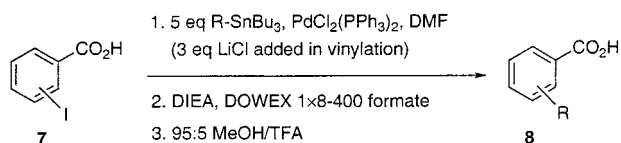
**Table 3.** DOWEX 1×8-400 Formate Resin (A) Swelling Properties

solvent	mL/g	solvent	mL/g
TFA	2.8	CH <sub>3</sub> CN	1.6
90:10 TFA/water	2.7	85:15 CH <sub>3</sub> CN/water	2.0
HCO <sub>2</sub> H	2.5	<i>i</i> -PrOH	2.2
90:10 HCO <sub>2</sub> H/water	2.5	CH <sub>2</sub> Cl <sub>2</sub>	2.0
MeOH	2.4	water	1.9
85:15 MeOH/water	2.2	THF	1.5
DMSO	1.5	toluene	1.5
85:15 DMSO/water	1.8	Et <sub>2</sub> O	1.3
DMF	1.7	dioxane	1.2
85:15 DMF/water	2.0	NMP	1.1

### Scheme 2

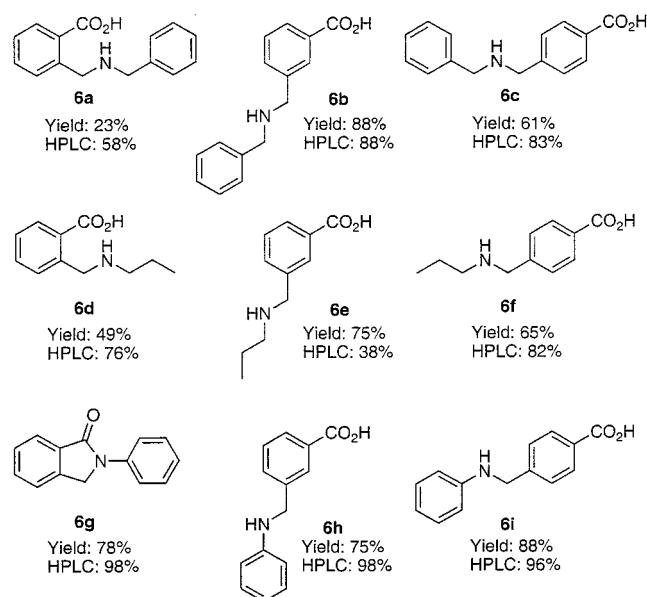


### Scheme 3



While reductive amination has a solution phase combinatorial chemistry equivalent,<sup>8</sup> the reported Stille coupling solution phase application is more specialized.<sup>16</sup> The following example is also specialized with respect to the use of the CO<sub>2</sub>H functionality as a handle to purify the coupling product away from the nonpolar organic impurities by SPE. In this reaction, 2-, 3-, and 4-iodobenzoic acids (7) were cross-coupled with 5 equiv each of phenyltributylstannane, 2-(tributylstannyl)furan, 2-(tributylstannyl)thiophene, and tributyl(vinyl)tin in discrete wells with the iodobenzoic acid as the limiting reagent set at 0.020 mmol (Scheme 3). These

### Chart 1



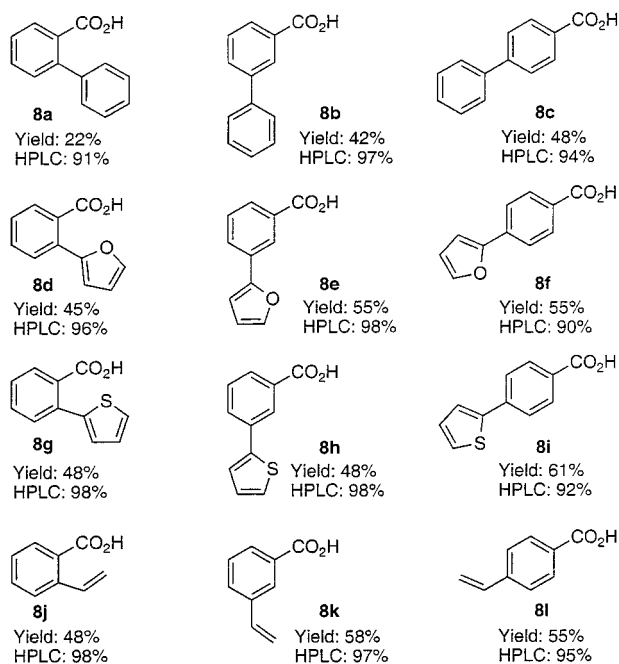
reagents were mixed at 85 °C in the presence of 0.10 equiv of PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> in DMF with 3 equiv of LiCl added for the vinylation examples.<sup>17</sup> After SPE purification on DOWEX 1×8-400 formate resin (A), good yields (<sup>1</sup>H NMR determined yield average = 49%) and excellent purities (HPLC determined purity average = 95%) of the desired products were obtained (Chart 2, <sup>1</sup>H NMR and HPLC traces are included in the Supporting Information). <sup>1</sup>H NMR spectra confirmed that tributyltin derived impurities were in general <5 mol % except for product **8a** which contained approximately 20 mol % tributyltin impurity.

### Discussion

The comparison of 11 different anion exchange resins in their formate anion forms indicated that DOWEX 1×8-400 formate resin (A) had the most favorable properties for SPE



Chart 2



purification of CO<sub>2</sub>H compounds. An acid with a pK<sub>a</sub> lower than the conjugate acid of the anion on the resin will exchange well in acid or salt form. However, weaker acids need to be in their DIEA salt form to effectively exchange with formate.

Exchanging the free acids onto the resin involves a proton exchange equilibration between the acid in solution and the acid anion ionically bound to the resin. In this setting, equilibration will favor the weaker acid being protonated in solution while making a resin bound salt of the stronger acid. This effect was repeated with various other counterions (see Figure 8). The DIEA salt exchange is essentially equivalent across the pK<sub>a</sub> range studied because DIEAH<sup>+</sup> is the acid exchanging the proton. In this exchange, the formic acid DIEA salt stays in solution while the more hydrophobic carboxylic acids (**1–4**, **6**, and **8**) are preferentially salt-paired with the resin ammonium cation. Why the more hydrophobic CO<sub>2</sub>H anions are preferentially salt-paired with the resin ammonium cation may be related to a resin surface solvation phenomenon. Czarnik, Yan, and co-workers have proposed the view of the resin bead as a micro solvent environment based on studies of kinetics on synthesis supports.<sup>18</sup> By extending this premise to ion exchange resins, the DIEA salt exchange can be interpreted to favor the more hydrophobic acid anions being retained on the resin because of a microenvironment solvation by the phenyl groups of the polystyrene resin. The weak anion exchangers, i.e., resins **D**, **I**, and **K**, are generally not as efficient at exchange with the DIEA salts when compared to the quaternary amine salt functionalized resins (strong anion exchangers) because they have to undergo an unfavorable proton exchange with the strong base DIEA.

Mesh size and cross-linking also play critical roles in the effectiveness of the ion exchange resin. The 8% cross-linked resins retain less water and thus have almost twice the total capacity compared to 2% cross-linked resins (compare resins **A** and **C** to resin **B** in Table 1). Resins **A** and **C** were

determined to have a slightly better working capacity relative to resin **B** (Figure 3). Smaller bead sizes have previously been found to provide faster reactions.<sup>18c</sup> The 200–400 mesh size resin **A** is slightly faster at ion exchange compared to the 100–200 mesh size resin **C** (Figure 4). Also, from a technical handling standpoint, the smaller bead size (200–400 mesh) makes resin **A** much easier to distribute as a suspension in water into the wells of a 96-well filterplate using a multichannel pipet.<sup>19</sup>

The best solvents for ion exchange, not unexpectedly, are those which swell the gel most effectively (compare Figures 5 and 6 with Table 3). Solvents that solvate a negative charge work the best and swell the resin most effectively. These tend to be alcohols, solvent acids, water, and homogeneous water/solvent mixtures. The solvent CH<sub>2</sub>Cl<sub>2</sub> also swells the resin better than other aprotic solvents which is not surprising since it is known to swell polystyrene synthesis resins very effectively.<sup>14</sup> However, we have found that the heterogeneous two-phase CH<sub>2</sub>Cl<sub>2</sub>/water solvent systems do not work well for ion exchange presumably due to preferential resin solvation by water. Some solvent acid systems show selectivity for exchanging off CO<sub>2</sub>H compounds (see Figure 7). Whether this feature can be exploited for the batchwise separation of acids in 96-well format is a subject of further investigation.

The synthesis examples illustrate the high levels of purity that can be achieved by this capture/release product purification strategy when the reaction goes to completion and a single acidic product is formed. While the examples presented are model case studies, this approach has been used extensively to isolate larger, more complex molecules including compounds containing nitrogen-heteroaryl and other various functionalities.<sup>20</sup> This technology has also proven to be useful for scavenging CO<sub>2</sub>H byproducts from reactions, leaving the desired neutral reaction products purified in solution.<sup>19</sup> The SPE method is superior to liquid–liquid extraction since careful individual pipetting of separated layers or liquid handlers are not necessary to remove extracted layers. Also, emulsions and solubilities of products in the organic phase are not issues. The synthesis of the amino acids (Scheme 2) could not have been done easily using liquid–liquid extraction since the products would have had some water solubility at all pHs. Last but not least, ion exchange resins are much less expensive compared to scavenging resins typically used in solution phase combinatorial chemistry. These studies suggest that SPE with ion exchange resins can be a useful purification procedure whenever the separation of ionic compounds from reaction mixtures is required.

## Experimental Section

**General Methods.** A Polyfiltronics Multichem 96 × 2 mL reaction block was used for the reductive amination and a Charybdis 96 × 2 mL solution phase reaction block was used for the Stille reaction. Filtration was performed in a Polyfiltronics PKB 96 × 2 mL filterblock. Shaking was performed by an Innova 2100 shaker from New Brunswick, Inc. Centrifugal evaporations were performed in Multichem blocks by a GeneVac HT-4. Anhydrous solvents were

purchased from Aldrich and stored over 4 Å molecular sieves.  $^1\text{H}$  NMR spectra were obtained on a Varian Gemini-200 spectrometer operating at 200 MHz and were recorded in  $\delta$  units with DMSO- $d_6$  ( $\delta$  2.54) as a reference line internal standard. HPLC traces were performed using a YMC ODS-Aq, Aq-303-5,  $50 \times 4.6$  mm ID, S-5  $\mu\text{m}$ , 120 Å column with the UV detector set at 250 nm eluting with a 0.1% aqueous AcOH/CH<sub>3</sub>CN gradient at a 2.5 mL/min flow rate with a program of %buffer (time): 90 (0 min), 0 (4 min), 0 (6 min), 90 (6.1 min), and 90 (8 min). Low resolution mass spectral (LRMS) analyses were performed at Mass Consortium, San Diego, CA. For NMR analysis, the purified reaction products were dissolved in 0.65 mL of 90:10 DMSO- $d_6$ /D<sub>2</sub>O containing 1.33 mM acetic acid as an NMR internal reference standard. Concentration was determined by comparing a single proton integration from the product to the 4 mM internal standard signal at  $\delta$  1.89, and this information was used to calculate the product yield. For HPLC and LRMS analyses, samples were made by combining 0.050 mL of the solution of interest with 1 mL of methanol.

**DOWEX 1×8-400 Formate Resin (A).** DOWEX 1×8-400 ion exchange resin (DOWEX-1-chloride) was first slurried with 3–4 volumes of water. Fine particles which did not settle rapidly were decanted off and discarded. This was repeated until no significant suspension of fine particles was evident (3–4 times). The resin was transferred as an aqueous slurry to a coarse glass fritted funnel and gravity-eluted with 10 volumes of 3 M aqueous NaOH, 10 volumes of water, 10 volumes of 3 M aqueous formic acid, and 20 volumes of water (final pH 6–7). The resin was suction-filtered and, in general, used in water saturated (water wet) form.

The formate forms of resins **B–K** were prepared as described for resin **A**.

For specific solvent effect studies, 50 g of water wet resin **A** was slurried in 400 mL of methanol for 14 h and then filtered (2×). After drying under high vacuum at room temperature for 48 h, 26.5 g of anhydrous resin **A** was obtained.

**DOWEX 1×8-400 Bicarbonate Resin.** DOWEX 1×8-400 formate ion exchange resin was slurried with 1 M aqueous NaHCO<sub>3</sub> for 15 min and then filtered. After repeating this treatment twice, the resin was gravity-eluted with 20 volumes of water. The resin was stored at 5 °C in water.

This method was used to prepare other anionic forms of DOWEX 1×8-400: carbonate (from 3 M Na<sub>2</sub>CO<sub>3</sub>), hydroxide (from 3 M NaOH), acetate (from 3 M acetic acid), and chloride (from 3 M HCl).

**Determination of Percent Exchange of Carboxylic Acids onto Ion Exchange Resins (Figure 1).** In rows A–D of a filterblock were added water wet formate resins **A–K** (100 mg/well) into columns 1–11, respectively, and then 0.04 M solutions of acids **1–4** in 9:1 DMSO/water (0.5 mL/well) into rows A–D, respectively. The mixtures were sealed in a metal frame with a Teflon lined septa, shaken (450 rpm) for 16 h at room temperature, and then filtered. The filtrates were analyzed by HPLC and compared to reference 0.04 M

solutions to determine the percent of CO<sub>2</sub>H compound exchanged onto an ion exchange resin.

By a similar method the following were also determined: the percent exchange of carboxylic acid DIEA salts onto ion exchange resins (Figure 2); the working resin capacity for CO<sub>2</sub>H DIEA salts (Figure 3, resin amounts of 25, 50, 100, and 200 mg tested); the speed of the CO<sub>2</sub>H DIEA salt exchange onto ion exchange resin (Figure 4, 200 mg of resin tested in slurries with the acids for time periods of 3, 15, and 60 min); the counterion effects on acid exchange (Figure 8); and the counterion effects on acid DIEA salt exchange (Figure 9). For DIEA salt experiments, the acid solutions were 0.04 M in acid and 0.08 M in DIEA.

**Solvent Optimization for Exchange of DIEA Salts onto DOWEX 1×8-400 Formate Resin (Figure 5).** Into each well of rows A–B and columns 1–12 of a filterblock was added 53 mg of dry DOWEX 1×8-400 formate ion exchange resin (**A**) (equivalent to 100 mg of water wet resin). The resin in each well was preswollen for 12 h with the specific solvent used for the exchange and then excess solvent removed by filtration. Into row A wells were added, 0.5 mL/well, specific solvent solutions which were 0.04 M in benzoic acid (**2**) and 0.08 M in DIEA. The specific solvent systems used in these solutions were CH<sub>2</sub>Cl<sub>2</sub> (col 1), methanol (col 2), 85:15 methanol/water (col 3), DMSO (col 4), 95:5 DMSO/water (col 5), 90:10 DMSO/water (col 6), DMF (col 7), 85:15 DMF/water (col 8), dioxane (col 9), 85:15 dioxane/water (col 10), CH<sub>3</sub>CN (col 11), and 85:15 CH<sub>3</sub>CN/water (col 12). In the same manner, into row B wells were added specific solvent solutions of diphenylacetic acid (**3**). The filterblock was sealed in a metal frame with a Teflon lined septa, shaken (450 rpm) at room temperature for 1 h, and then filtered. The filtrates were analyzed by HPLC and compared to reference 0.04 M solutions in corresponding solvent systems to determine the percent of a CO<sub>2</sub>H compound exchanged onto the ion exchange resin.

**Determination of Optimal Solvents for the Exchange off of DOWEX 1×8-400 (Figure 6).** Into each well of rows A–D and columns 1–4 of a filterblock was added 53 mg of dry DOWEX 1×8-400 formate ion exchange resin (**A**) (equivalent to 100 mg of water wet resin). Into row A wells were added, 0.5 mL/well, methanol solutions which were 0.04 M in 4-nitrobenzoic acid (**1**) and 0.08 M in DIEA. Likewise, into rows B, C, and D were added benzoic acid (**2**)/DIEA solutions, diphenylacetic acid (**3**)/DIEA solutions, and 4-aminobenzoic acid (**4**)/DIEA solutions, respectively. The filterblock was sealed, shaken (450 rpm) for 60 min, and then filtered. Sample filtrates from each row contained <5% of the respective acids. The resin in all wells was washed with methanol (450 rpm, 2 × 15 min) followed by 85:15 CH<sub>3</sub>CN/water (450 rpm, 2 × 15 min) for columns 1 and 2 and methanol (450 rpm, 2 × 15 min) for columns 3 and 4. Into specific columns were added specific solvent acid solutions (0.5 mL/well): 90:10 TFA/water (col 1), 90:10 formic acid/water (col 2), 95:5 methanol/TFA (col 3), and 95:5 methanol/formic acid (col 4), and the filterblock was sealed and shaken (450 rpm) for 1 h. The solutions were filtered, and the resin was washed with 85:15 CH<sub>3</sub>CN/water (0.4 mL/well) for columns 1 and 2 and methanol (0.4 mL/

well) for columns 3 and 4 (450 rpm, 15 min). The washings were collected, combined with the cleavage solution filtrates, and centrifugally evaporated to provide residues of the recovered acids **1–4**. To each sample well was added 0.5 mL of 0.08 M DIEA in methanol, and the block shaken (450 rpm, 15 min) to dissolve the residues. Analysis of these solutions by HPLC in comparison with reference solutions provided the percent recovery.

In a similar manner, other solvent systems were examined for their efficiency at exchanging acids off of DOWEX 1×8-400 (Figure 7). In rows A and B, acids **2** and **3**, respectively, were exchanged onto resin. Prior to adding cleavage solvent to the resin, wells in columns 1, 4, 7, and 8 were washed with methanol and wells in columns 2, 3, 5, 6, 9, and 10 were washed with CH<sub>2</sub>Cl<sub>2</sub>. The following solvent systems were tested for cleavage in the respective columns: 99:1 methanol/TFA (col 1); 99:1 CH<sub>2</sub>Cl<sub>2</sub>/TFA (col 2); 95:5 CH<sub>2</sub>Cl<sub>2</sub>/TFA (col 3); 99:1 methanol/formic acid (col 4); 99:1 CH<sub>2</sub>Cl<sub>2</sub>/formic acid (col 5); 95:5 CH<sub>2</sub>Cl<sub>2</sub>/formic acid (col 6); 99:1 methanol/acetic acid (col 7); 95:5 methanol/acetic acid (col 8); 99:1 CH<sub>2</sub>Cl<sub>2</sub>/acetic acid (col 9); 95:5 CH<sub>2</sub>Cl<sub>2</sub>/acetic acid (col 10).

**Measurement of DOWEX 1×8-400 Formate Ion Exchange Resin (A) Swelling in Various Solvents.** This was performed as previously described<sup>14</sup> with 500 mg quantities of dry DOWEX 1×8-400 formate ion exchange resin (**A**). The volume of 1.00 g of dry resin was determined to be 0.8 mL.

**Reductive Amination Procedure.** In separate wells of the reaction block were added 0.2 mL of a 0.1 M aldehyde solution in DMSO (*o*- (A1–C1), *m*- (A2–C2), and *p*-carboxybenzaldehyde (A3–C3) (**5**)), 0.2 mL of a 0.4 M amine solution in DMSO (benzylamine (A1–A3), propylamine (B1–B3) and aniline (C1–C3)), and 0.080 mL of a 1.0 M trimethyl orthoformate (TMOF) solution in DMSO (all reaction wells). The block was sealed with a Teflon lined septa in a metal frame and shaken at 350 rpm for 6 h. Then to each reaction well was added 0.4 mL of a 0.26 M solution of NaBH<sub>4</sub> in DMSO, and the block was shaken at 350 rpm for 48 h. Next, 0.15 mL of water was added to each reaction well, and the block was shaken at 350 rpm for 0.5 h. The reaction mixtures were then transferred to a filterblock previously filled in each corresponding well with 0.22 g of water wet DOWEX 1×8-400 formate ion exchange resin. The block was shaken at 450 rpm for 1 h, and the solvents were removed by filtration. The resin was subjected to 15 min, 450 rpm wash cycles with 85:15 DMSO/water, methanol, CH<sub>3</sub>CN, and methanol (all at 0.6 mL/well). Then to each well was added 0.6 mL of 95:5 methanol/TFA, and the mixtures were shaken at 450 rpm for 1 h and then filtrates collected in a Multichem block. Each well was rinsed with 0.4 mL of methanol. The combined filtrates were centrifugally evaporated to provide the products **6**. Each sample LRMS M-1 matched the calculated value. <sup>1</sup>H NMR spectra and HPLC traces for each compound **6** are included in the Supporting Information.

**Stille Coupling Procedure.** All solutions were degassed for 5 min with N<sub>2</sub> prior to addition to the reaction block. In separate wells of the reaction block were added 0.2 mL of

a 0.1 M aryl iodide solution in DMF (2-iodo- (A1–D1), 3-iodo- (A2–D2), and 4-iodobenzoic acid (A3–D3) (**7**)), 0.2 mL of a 0.5 M aryl- or vinyl-stannane solution in DMF (phenyltributylstannane (A1–A3), 2-(tributylstannyl)furan (B1–B3), 2-(tributylstannyl)thiophene (C1–C3), and tributyl(vinyl)tin (D1–D3)), 0.1 mL of a 0.6 M solution of LiCl in DMF (D1–D3 only), and 0.1 mL of a 0.02 M PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> solution in DMF (all reaction wells). The block was sealed with a Teflon lined septum in a metal frame, shaken (450 rpm) at room temperature for 5 min, incubated in an oven at 85 °C for 1 h, shaken (450 rpm) at room temperature for 5 min, reincubated in an oven at 85 °C for 2 h, shaken (450 rpm) at room temperature for 5 min, and finally incubated in an oven at 85 °C for 5 h. After shaking (450 rpm) at room temperature for 30 min, 0.1 mL of a 0.4 M DIEA solution in DMF and 0.1 mL of water were added to each reaction well, and the mixtures were shaken (450 rpm) for 5 min. The reaction mixtures were then transferred to a filterblock previously filled in each corresponding well with 0.25 g (wells A1–A3, B1–B3, and C1–C3) or 0.35 g (wells D1–D3) of water wet DOWEX 1×8-400 formate ion exchange resin. The block was shaken at 450 rpm for 1 h, and the solvents were removed by filtration. The resin was subjected to 15 min, 450 rpm wash cycles with 85:15 DMSO/water, isopropyl alcohol (3×), and methanol (2×). Then to each well was added 0.5 mL of 95:5 methanol/TFA, the mixtures were shaken at 450 rpm for 30 min, and then the filtrates were collected in a Multichem block. This 95:5 methanol/TFA treatment was repeated with 0.4 mL per well. The combined filtrates were centrifugally evaporated to provide the products **8**. Each sample LRMS M-1 matched the calculated value. <sup>1</sup>H NMR spectra and HPLC traces for each compound **8** are included in the Supporting Information.

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**Supporting Information Available.** <sup>1</sup>H NMR spectra and HPLC traces for each compound **6** and **8**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References and Notes

- (1) (a) Terret, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; Steele, J. *Tetrahedron* **1995**, *51*, 8135–8173. (b) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555–600. (c) Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. *Tetrahedron* **1996**, *52*, 4527–4554. (d) Balkenhohl, F.; von dem Bussche-Hunnefeld, C.; Lansky, A.; Zechel, C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2288–2337. (e) Lebl, M. J. *Comb. Chem.* **1999**, *1*, 3–24.
- (2) (a) Recent multifaceted application: Ley, S. V.; Massi, A. *J. Comb. Chem.* **2000**, *2*, 104–107. (b) Kaldor, S. W.; Siegel, M. G.; Fritz, J. E.; Dressman, B. A.; Hahn, P. J. *Tetrahedron Lett.* **1996**, *37*, 7193–7196. (c) Review: Flynn, D. L.; Devraj, R. V.; Parlow, J. J. *Curr. Opin. Drug Discovery Dev.* **1998**, *1*, 41–50. (d) Review: Booth, R. J.; Hodges, J. C. *Acc. Chem. Res.* **1999**, *32*, 18–26. (e) Review: Parlow, J. J.; Devraj, R. V.; South, M. S. *Curr. Opin. Chem. Biol.* **1999**, *3*, 320–336.



- (3) (a) Use of Amberlyst 21 (freebase tertiary amine) anion exchange resin for scavenging of HCl and carboxylic acids from a combinatorial acylation reaction: Suto, M. J.; Gayo-Fung, L. M.; Palanki, M. S. S.; Sullivan, R. *Tetrahedron* **1998**, *54*, 4141–4150. (b) Use of Amberlyst A-26 bicarbonate anion exchange resin for scavenging HBr: Stauffer, S. R.; Katzenellenbogen, J. A. *J. Comb. Chem.* **2000**, *2*, 318–329.
- (4) (a) Use of a mixed bed of DOWEX 50W×8-200 strong acid cation exchange resin and Amberlite IRA-400 strong anion exchange resin to scavenge triethylammonium iodide: Boger, D. L.; Goldberg, J.; Andersson, C.-M. *J. Org. Chem.* **1999**, *64*, 2422–2427. (b) Use of Amberlyst A-15 calcium salt for the scavenging of tetrabutylammonium fluoride: Parlow, J. J.; Flynn, D. L. *Tetrahedron* **1998**, *54*, 4013–4031. (c) Use of Amberlite IRA-95 weak base (freebase tertiary amine) anion exchange resin to freebase catalytic quantities of tertiary amine HBr and HI salts: Ouyang, X.; Armstrong, R. W.; Murphy, M. M. *J. Org. Chem.* **1998**, *63*, 1027–1032.
- (5) Acid/base aqueous extraction methods for combinatorial product purification: (a) Cheng, S.; Comer, D. D.; Williams, J. P.; Myers, P. L.; Boger, D. L. *J. Am. Chem. Soc.* **1996**, *118*, 2567–2573. (b) Neuville, L.; Zhu, J. *Tetrahedron Lett.* **1997**, *38*, 4091–4094. (c) Solid supported aqueous extraction: Johnson, C. R.; Zhang, B.; Fantauzzi, P.; Hocker, M.; Yager, K. M. *Tetrahedron* **1998**, *54*, 4097–4106.
- (6) Fluorous phase extractive isolation: Studer, A.; Hadida, S.; Ferritto, R.; Kim, S.-Y.; Jeger, P.; Wipf, P.; Curran, D. P. *Science* **1997**, *275*, 823–826.
- (7) Use of Amberlyst A-26 (hydroxide) anion exchange resin to effect combinatorial condensation and purification of 2,4-pyrrolidinones: Kulkarni, B. A.; Ganesan, A. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2454–2455.
- (8) Use of Varian SCX column (strong acid cation exchanger) for the combinatorial purification of amines: Siegel, M. G.; Hahn, P. J.; Dressman, B. A.; Fritz, J. E.; Grunwell, J. R.; Kaldor, S. W. *Tetrahedron Lett.* **1997**, *38*, 3357–3360.
- (9) Technical information bulletins on ion exchange chromatography. (a) *Supelco Bulletin 910, Guide to Solid Phase Extraction*, 1998. Supelco, Supelco Park, Bellefonte, PA 16823-0048, Tel: 800-247-6628. (b) *DOWEX Ion Exchange Resins, Powerful Chemical Processing Tools*, 1998. (c) *DOWEX Fine Mesh Spherical Ion Exchange Resins*, 1998. Dow North America, P.O. Box 1206, Midland, MI 48641-1206, Tel: 800-447-4369. (d) *Rohm and Haas Ion Exchange Laboratory Guide*, 1993. Rohm and Haas, Philadelphia, PA 19105, Tel: 800-221-8992. (e) *Aldrich Technical Information Bulletin No. 142*, 1987. Aldrich Chemical Co., Milwaukee, WI 53233, Tel: 800-231-8327.
- (10) Cohn, W. E. *J. Am. Chem. Soc.* **1950**, *72*, 1471–1478.
- (11) See, for example: Holy, A.; Rosenberg, I. *Collect. Czech. Chem. Commun.* **1987**, *52*, 2801–2809.
- (12) Bookser, B. C.; Kasibhatla, S. R.; Appleman, J. R.; Erion, M. D. *J. Med. Chem.* **2000**, *43*, 1495–1507.
- (13) The usefulness of resin **K** in its freebase form has already been demonstrated for the scavenging of HCl and carboxylic acids; see ref 3.
- (14) Santini, R.; Griffith, M. C.; Qi, M. *Tetrahedron Lett.* **1998**, *39*, 8951–8954.
- (15) (a) Deshpande, M. S. *Tetrahedron Lett.* **1994**, *35*, 5613–5614. (b) Forman, F. W.; Sucholeiki, I. *J. Org. Chem.* **1995**, *60*, 523–528. (c) Plunkett, M. J.; Ellman, J. A. *J. Org. Chem.* **1997**, 2885–2893. (d) Chamoin, S.; Houldsworth, S.; Snieckus, V. *Tetrahedron Lett.* **1998**, *39*, 4175–4178. (e) Sieber, F.; Wentworth, P., Jr.; Janda, K. D. *J. Comb. Chem.* **1999**, *1*, 540–546. (f) Brody, M. S.; Finn, M. G. *Tetrahedron Lett.* **1999**, *40*, 415–418.
- (16) Purification by the fluororous phase extraction technique: Hoshino, M.; Degenkolb, P.; Curran, D. P. *J. Org. Chem.* **1997**, *62*, 8341–8349.
- (17) The LiCl additive was only necessary in the reaction with tributyl(vinyl)tin. In its absence, the vinyl coupling did not go to completion. While the mechanism for why LiCl is beneficial in some Stille couplings is still unclear, it has been seen previously to aid in driving reactions to completion; see refs 15b, 15e, and 15f.
- (18) (a) Czarnik, A. W. *Biotech. Bioeng. (Comb. Chem.)* **1998**, *61*, 77–79. (b) Li, W.; Yan, B. *J. Org. Chem.* **1998**, *63*, 4092–4097. (c) Li, W.; Xiao, X.; Czarnik, A. W. *J. Comb. Chem.* **1999**, *1*, 127–129.
- (19) It is convenient to transfer ion exchange resin into 96-well plates as a 3:1 water:resin slurry using a Labsystems 12 channel (50–300  $\mu$ L) Finnpiptette fitted with Geno-DNA-Tip S 10–200  $\mu$ L polypropylene pipette tips.
- (20) Bookser, B. C.; Zhu, S.; Raffaele, N. B. Unpublished results.

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