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Solution-Phase Parallel Synthesis Using Ion-Exchange Resins

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Abstract: Ion-exchange resins are useful as scavengers in solution-phase parallel synthesis. Ester and amide libraries have been generated using basic ion-exchange resins to facilitate the formation of products and to remove reaction byproducts. Acidic ion-exchange resins have been used as selective amine scavengers in the synthesis of urea and amine libraries. Several compound libraries have been prepared using the basic ion-exchange resin Amberlyst 21 as part of our lead optimization program. The utility of ion-exchange resins as a means of generating both large and small focused libraries will be reviewed.

Introduction Combinatorial chemistry and related techniques have had a major impact on not only lead discovery but lead optimization as well.[1] These approaches have provided large diverse libraries of compounds to feed the demand created by advances in high throughput screening. Initial efforts in this area focused on creating large diverse libraries for random screening. However, if hit compounds were identified from other sources such as in-house libraries, natural products or structure-based drug design, the time required to develop the solid-phase chemistry needed for subsequent optimization was not acceptable.

As part of our drug discovery efforts, we were confronted with the task of optimizing compounds derived from a variety of commercial sources. The goal was to prepare small libraries of discrete compounds in milligram quantities and integrate the synthesis of the compounds with current in-house screening methodologies. As combinatorial chemistry matured, the usefulness of having available techniques to prepare small focused libraries was quickly realized. The ability to synthesize and evaluate structural changes rapidly, and develop structure-activity relationships was imperative for the success of the program.

Recently, several groups have reported on the use of solid-phase resins as "quench reagents" and resin bound reagents to expedite the synthesis and purification of derivatives.[2] In addition, acid/base extractions[3] and high speed purification procedures[4] have also been reported. These approaches are very useful and have been applied to several of our lead optimization programs in Inflammation and Bone Metabolism. They meet the requirements of being easily implemented and adapted to current medicinal chemistry procedures and have been integrated into our medicinal chemistry strategies when possible. However, in many instances, several reagents, scavengers or combinations must be used to produce compounds with the required purity. In certain circumstances, custom preparation of the scavengers or reagents with the needed properties was necessary.

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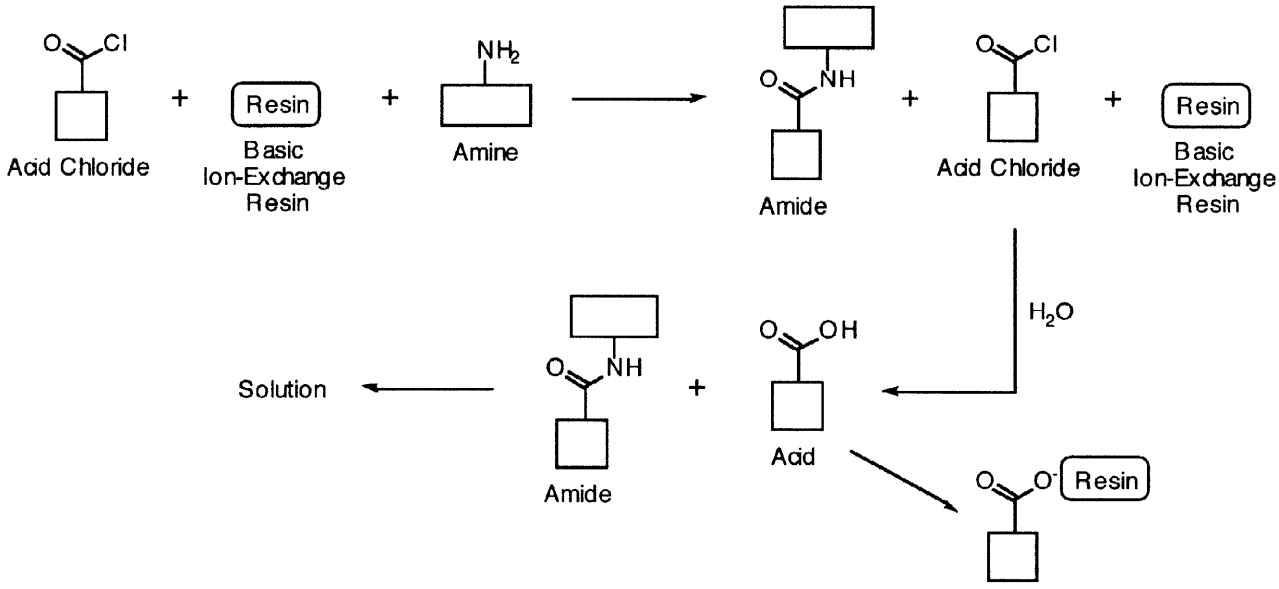
The goal was to devise easy to use, cost effective combinatorial methodologies. The ideal procedures would allow for complete conversion of reactants, require minimal handling of reactants and products and provide pure samples in high yields. The techniques needed to be adaptable to parallel synthesis production and simple to implement.

Basic Ion-Exchange Resins To this end, we explored the use of ion-exchange resins as a means of "purifying" products and as acidic or basic scavengers.[5] Ion-exchange resins seemed ideal in that they are available in a wide range of pH's and forms, thus eliminating the need to produce reagents. In addition, they are inexpensive compared to solid-phase resins and there use in synthetic chemistry to catalyze synthetic procedures is widely cited.[6]

We envisioned that in the formation of amides, which are a common structural motif in many drug molecules, a single ion-exchange resin could be used as a scavenger or "quench reagent" in a combinatorial or parallel synthesis array (Scheme 1). As illustrated, the reaction of an amine with a slight excess of an acid chloride in the presence of a basic ion-exchange resin should result in the conversion of the acid chloride to the appropriate amide. The ion-exchange resin functions as a solid-phase basic scavenger, soaking up the HCl produced and allowing the minimal amount of amine present to react. Adding a small amount of water to the reaction transforms any unreacted acid chloride to the corresponding acid and in effect transforms the ion-exchange resin into a quench reagent, since the acid is now adsorbed by the resin.

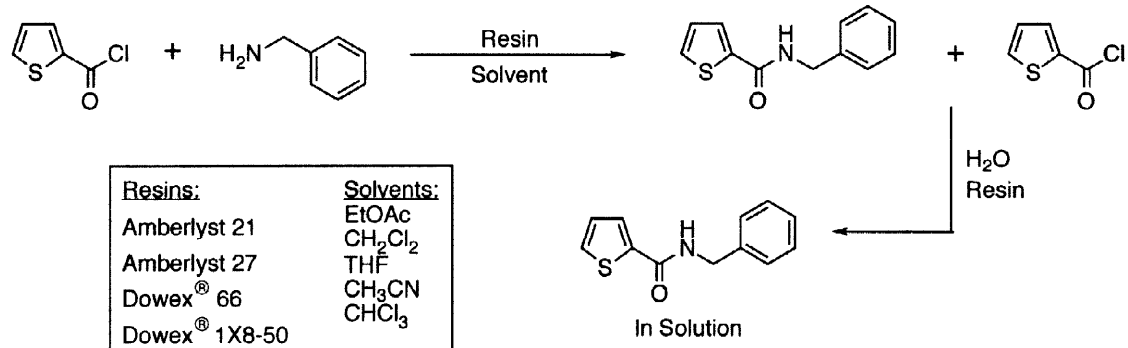
Scheme 1



The hypothesis was tested by reacting 2-thiophene carboxylic acid chloride with benzyl amine in the presence of several different basic ion-exchange resins. The resins were selected based upon their commercial

availability.[7] As reported previously, several of the resins functioned in the capacity just described. In addition, several solvents were also explored including THF, EtOAc, CH_2Cl_2, CH_3CN, and $CHCl_3$. It was determined that EtOAc was the solvent of choice due to the behavior of the resins in the solvent. In EtOAc, the resins remained evenly dispersed and the solvent was easily removed. This was not the case in the other solvents. The resin that provided the highest purity of products (>99%) was Amberlite IRA-68[5a]. It was also determined that aniline derivatives worked as well as benzyl amine, and a variety of aromatic acid chlorides could also be used. Each of the amides was isolated by simple concentration of the solvents, and in most cases the products were >95% pure by HPLC.

Scheme 2



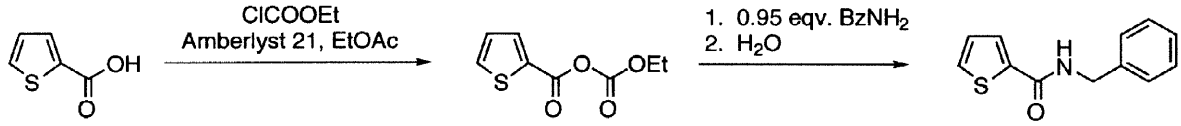
Resins:	Solvents:
Amberlyst 21	EtOAc
Amberlyst 27	CH_2Cl_2
Dowex® 66	THF
Dowex® 1X8-50	CH_3CN
Dowex® 1X2-100	$CHCl_3$
Amberlite® IRA-68	
Amberlite® IRA-900	
Amberlite® IRA-904	
Amberjet™ 4200	

We were highly encouraged by these results and wanted to explore the versatility of this reaction further. In many instances, the preparation of the acid chloride adds a step to the synthesis. Some of the acid chlorides are difficult to prepare or they are unstable when isolated. In addition, certain acid chlorides are difficult to hydrolyze to the carboxylic acids. One way to circumvent this is to use ion-exchange resins for the preparation of an activated acid, which can directly be used in the amide forming step. This "in situ" activation has been used in both solution and solid phase reactions on peptides and small organic molecules, but under more classical conditions requiring a workup and excess reagents.[8] We believed that a basic ion-exchange resin could be used to facilitate the formation of a mixed anhydride starting with an aromatic acid and ethylchloroformate. The basic resin would expedite the formation of the anhydride (soak up HCl) and absorb any unreacted acid prior to and after addition of the amine.

As shown in Scheme 3, reaction of 2-thiophene carboxylic acid with ethylchloroformate in the presence of Amberlyst 21, followed by the addition of benzyl amine and water resulted in the clean formation of the corresponding amide. The mixed anhydride formed is not isolated but used directly in the next step. This

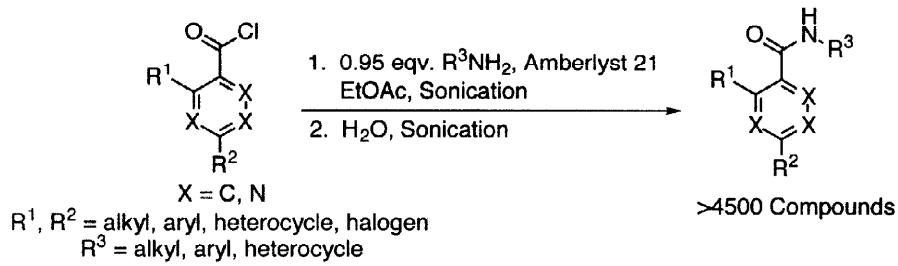
procedure eliminates the need to use the acid chloride. In many cases, the mixed anhydride is more easily hydrolyzed when needed.

Scheme 3



Having developed these procedures, we were now in a position to apply them to the preparation of libraries for lead optimization. The compounds would need to be run and formatted in a microtiter format. In our HTS assays, we identified a compound in which these new procedures seemed ideal.[9] The goal was to prepare libraries of compounds where we varied in a uniform way the amide portion, as we made structural changes to the pyrimidine portion. Scheme 4 illustrates the compounds examined. A series of substituted pyrimidine, benzene and related molecules (28 -30) were synthesized and converted to their corresponding acid chlorides. In a microtiter format, all of these were reacted with 160 commercially available amines using two plates (80 compounds per plate). In total, >4500 compounds were made and screened in a 3 point dose-response assay. The synthesis involved adding 1.05 equivalents of each acid chloride to a microtiter plate containing 80 different amines in EtOAc (400 μL) in the presence of Amberlyst 21 resin. The microtiter plate was sonicated to effect mixing and then 30 μL of H_2O was added to each well to effect hydrolysis of any unreacted acid chloride. The plates were sonicated for 5 minutes more and then 280 μL of the organic layer was removed with an 8-channel pipet and transferred to a set of tared test tubes. The samples were concentrated, solvated in DMSO at 5 mg/mL and sent for analysis and screening. Prior to screening, each of the samples was analyzed by TLC and random samples were analyzed by HPLC 15 -20 samples (purity >85%). Figure 1 contains examples of the type of acid chlorides that were used in these studies.

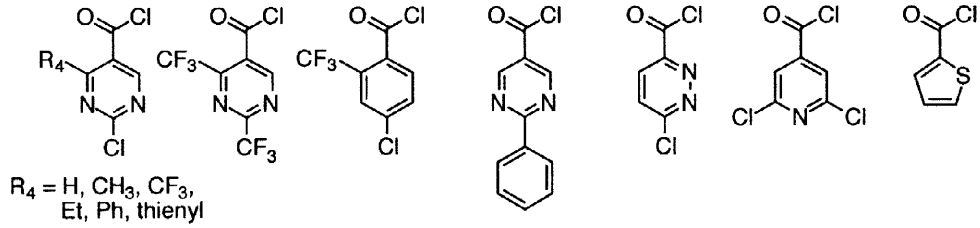
Scheme 4



Overall, this approach proved to be useful and resulted in the optimization of this series of compounds. The techniques allowed for the preparation of a large number of compounds focused around a hit molecule and provided a great deal of structure-activity information in a relatively short period of time. These techniques have

also been applied to the preparation of ester libraries by simply substituting various alcohols in place of the amines described above.

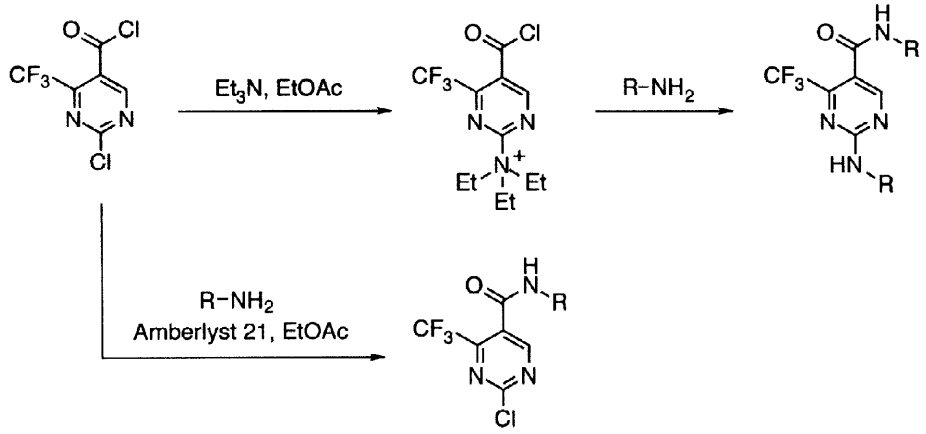
Figure 1



The power and potential utility of this procedure using ion-exchange resins was demonstrated on derivatives in which R^2 was chlorine or fluorine. In most circumstances, one could simply react the acid chloride in the presence of a base with the appropriate amine. However, when triethylamime is added to the 2-chloropyrimidine acid chlorides, the quaternary salt at the 2-position is readily formed and easily displaced with amines (Scheme 5). We have used this procedure to make 2-substiuted pyrimidines. However, in the presence of Amberlyst 21 resin in EtOAc, the reaction cleanly proceeds to give only the amide. These conditions have been used to prepare larger quantities of several of the compounds identified in our assays.

In this same series of compounds, we examined the use of Amberlyst 27 as the catalyst. It was found that the reactions proceeded as expected, but the products obtained were not as pure as determined by HPLC and TLC. It was surmised that the increased basicity of Amberlyst 27 catalyzed the formation of additional products. The selectivity seen in this case has been difficult to repeat using more standard chemical techniques.

Scheme 5

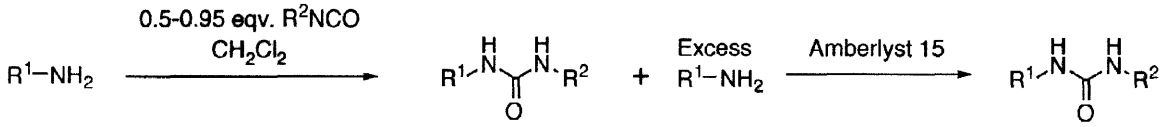


Acidic Ion-Exchange Resins The use of acidic resins has also been explored in several different types of chemistry. In these cases, the resins have been examined for their ability to scavenge reactants from the reactions and eliminate the need for a purification step. We have not yet examined the use of acidic resins in a

similar role to that described above, only because the opportunity has not presented itself. However, the use of acidic resins as reagents in many types of reactions is well documented.[10]

One of the first applications of acidic resins we examined is shown in Scheme 6.[5] The reaction of isocyanates with amines is a common method of building combinatorial libraries of various magnitudes. Several groups have published their results on the generation and use of urea libraries[11]. Some of the groups have prepared these libraries using aminomethyl or polyamine resins as scavengers of the excess isocyanates used.[2,12] We have also successfully applied these techniques to the generation of focused libraries in our inflammation program. However, we wanted to determine if acidic ion-exchange resins could function as effectively. In particular, it would allow one to use an excess of the amines, not the precious isocyanates. In some situations, the amines are more readily available and usually more stable. As shown, the generation of ureas proceeds cleanly. Addition of Amberlyst 15 resin to the reaction mixture followed by removal of the solvent provides the desired ureas in >95% purity.

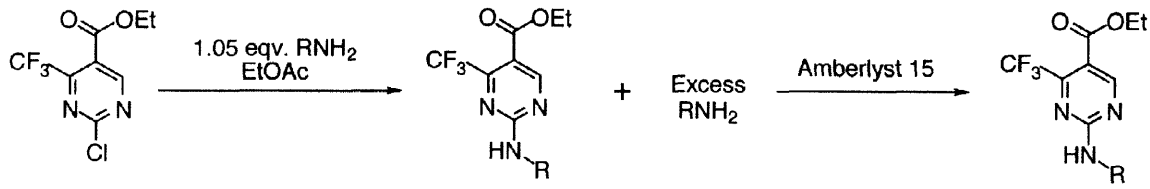
Scheme 6



In addition to their use in the formation of ureas, they can also be used to form amides as described above, if the acid chloride is difficult to obtain. However, in these situations the resin could not be added until after the reaction was complete and some type of basic resin/catalyst would still be required.

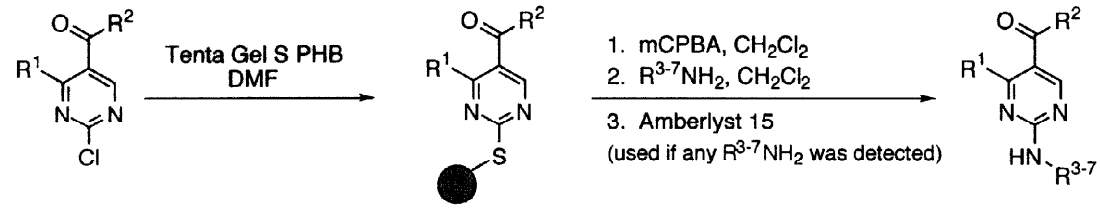
One other reaction that acidic resins are useful is nucleophilic displacement on heterocycles (Scheme 7). Treatment of ethyl 2-chloro-4-trifluoromethyl-pyrimidine-5-carboxylate with amines results in clean conversion to the 2-amino derivative. To drive this reaction to completion, a slight excess of the amine is needed. Under normal conditions, if these compounds were used in the preparation of a library, the presence of the excess amine would not be tolerable. Therefore, adding a small amount of Amberlyst 15 provides clean products. Again, the resin functions as a selective scavenger in that none of the less basic 2-aminopyrimidines are scavenged.

Scheme 7



Along these same lines, we were curious as to how effective these resins could be in building libraries containing mixtures. Previously, we reported on the development of a novel traceless linker for solid-phase chemistry.[13] Preparation of clean mixtures of compounds using 5 different amines was possible (Scheme 8). However, if all of the amines did not react, they were detected in the final products. If after the reaction is completed, Amberlyst 15 resin is used, only the desired mixtures are obtained (HPLC and TLC). The use of less than an equivalent of each amine partially solves this problem. If however, the material on the resin is very valuable and difficult to prepare, one may not want to use it in excess and then have to dispose of the remaining resin-bound material.

Scheme 8



Conclusions In summary, we have demonstrated the utility and versatility of ion-exchange resins for the generation of focused libraries around lead molecules. The techniques described are easily implemented and can be done in a parallel synthesis format. The use of basic ion-exchange resins as scavengers offers many advantages over conventional procedures, such as the ease of workup and availability. Also, acidic ion-exchange resins offer similar advantages, but the biggest advantage in the applications just described include selectivity and cost. Our future studies are focused on the exploration of these resins further. In particular, we are interested in their usefulness in multi-step reactions. Preliminary work in this area has looked promising.

EXPERIMENTAL SECTION

Procedure for the evaluation of ion-exchange resins (Scheme 2): Approximately 25 mg of ion-exchange resin was placed into each well of a 96-well plate (2 mL capacity) as follows: Amberlyst 21 (wells A-C of column 1), Amberlyst 27 (A-C of 2), Dowex® 66 (A-C of 3), Dowex® 1X8-50 (A-C of 4), Dowex® 1X2-100 (A-C of 5), Amberlite® IRA-68 (A-C of 6), Amberlite® IRA-900 (A-C of 7), Amberlite® IRA-904 (A-C of 8), and AmberJet™ 4200 (A-C of 9). Resin was not placed in column 10 (columns 11 and 12 were not used). Six solutions were prepared as follows: benzylamine (0.049 mL, 0.45 mmol) in EtOAc (4 mL), in THF (4 mL), and in CH_2Cl_2 (4 mL); 2-thiophenecarbonyl chloride (0.069 g, 0.47 mmol) in EtOAc (4 mL), in THF (4 mL), and in CH_2Cl_2 (4 mL). Into each well of rows A and D was placed 0.20 mL of the benzylamine in EtOAc solution. Benzylamine/THF (0.20 mL) was placed into rows B and E, and benzylamine/CH_2Cl_2 into rows C and F. The corresponding thiophenecarbonyl chloride solutions (0.20 mL) were then added. The plate was covered and sonicated for 45 min. Water (0.05 mL) was added to each well, and the reactions sonicated for another 30 min. The solutions in rows 1-3 were then transferred to tared test tubes using the same solvent (0.60

mL) employed in the reaction. Approximately 25 mg of ion-exchange resin was placed into wells D-F as follows: Amberlyst 21 (column 1), Amberlyst 27 (2), Dowex® 66 (3), Dowex® 1X8-50 (4), Dowex® 1X2-100 (5), Amberlite® IRA-68 (6), Amberlite® IRA-900 (7), Amberlite® IRA-904 (8), and AmberJet™ 4200 (9). After sonicating 30 min., the solutions were transferred to tared test tubes as described above, and all 60 reaction mixtures were evaporated to dryness. 3 mL of CH_3CN (0.1% TFA) was added to each, and the products were evaluated by HPLC [isocratic, CH_3CN/H_2O, 65:35 (1% TFA)]. Products prepared using Amberlite® 68 and Dowex® 1X8-50 were also analyzed by ^1H NMR and GCMS, and yields were determined.

Procedure for the preparation of N-Benzylthiophene-2-carboxamide (Scheme 3): To a solution of 2-thiophenecarboxylic acid (10 mg, 78.0 μmol) in CH_2Cl_2 (1 mL) was added Amberlyst 21 (50 mg) and ethyl chloroformate (7.5 μL, 78.8 μmol). After the mixture was shaken for 2 hours, n-butylamine (7.6 μL, 77.2 μmole) was added, and the reaction shaken for an additional 2 hours. Water (10 μL) was added, and the reaction shaken for 1 hour. Filtration and concentration provided the title compound (12 mg, 85% yield) in 90% purity with all spectra in agreement with literature (*Tetrahedron*, **1973**, *29(8)*, 1073-1081).

Example Procedure for the preparation 2-Chloro-4-trifluoromethy-5-pyrimidine carboxamides (Scheme 4): Amberlyst A-21 ion exchange resin (5-10 beads) was placed into 80 wells of a 1 mL deep well microtiter plate (rows 1 and 12 open). The 80 individual amines (100 μL, 22.4 mmol in EtOAc) were added to the individual wells and diluted with additional EtOAc (0.2 mL). Then to each well was added a solution of 2-chloro-4-trifluoromethyl-5-pyrimidine acid chloride (100 mL, 24.6 mmol) in EtOAc. The wells were capped and the plate was sonicated for 0.25 h. To each well was added H_2O (30 μL) and the plate was sonicated for an additional 0.1 h. The organic layer (280 μL) of each well was transferred into a tared test tube and concentrated to provide 80 individual compounds. Each compound was analyzed by TLC (1/1 hexanes/EtOAc) and 15 random samples were analyzed by HPLC. This procedure was used to prepare derivatives of the acid chlorides illustrated in Figure 1.

Example Procedure for the preparation of ureas (Scheme 6): 1-Benzyl-3-phenylurea: To aniline (5 μL, 55 μmol) in CH_2Cl_2 (0.5 mL) was added benzylisocyanate (6 μL, 52 μmol) in CH_2Cl_2 (0.5 mL). After sonication for 1 hour, Amberlyst 15 (50 mg) was added, and the reaction mixture was sonicated for an additional 20 min. Filtration and concentration of the filtrate provided the title compound (11 mg, 92% yield) in 98% purity with all spectra in agreement with literature (Wolman, Y.; Gallop, P. M. *J. Org. Chem.*, **1962**, *27*, 1902-1903). Other amines that were examined include, butylamine, aniline, cyclohexylamine and 3,5-dichloroaniline

Procedure for the preparation of Ethyl 2-arylamino-4-trifluoromethylpyrimidine-5-carboxylate (Scheme 7): To a solution of ethyl 2-chloro-4-trifluoromethylpyrimidine-5-carboxylate (7 mg, 27.6 μmol) in THF (0.4 mL) was added a mixture of 5 different amines (5.8 μmol each of 2-naphthylamine, 4-cyanoaniline, 3-ethylaniline, 2,3-difluoroaniline, and 3,4-dimethoxyaniline) in THF (0.2 mL) and aminomethylmorpholine resin (39 mg, 29 μmol). The mixture was shaken for 24 hours and monitored using thin-layer chromatography. Amberlyst 15 (100 mg) was added, and the reaction was shaken for an additional 3

hours. The reaction mixture was then filtered and concentrated to provide the title compounds. Analysis of these compounds using GCMS showed five products in equal amounts and no starting materials.

Procedure for the preparation of Ethyl 2-alkyl/arylamino-4-trifluoromethylpyrimidine-5-carboxylate (Scheme 8): A mixture of Tenta Gel S PHB (15 g, 3.6 mmol) and 2-chloro-4-trifluoromethylpyrimidine-5-carboxylate (4.6 g, 18 mmol) in DMF (100 mL) was shaken for 24 hours. Triethylamine (1.8 g, 18 mmol) was added and the mixture shaken for 1 hour. The resin was filtered, washed with DMF, water, EtOH and $CHCl_3$, and dried. ^{13}CNMR and ^{19}FNMR of the resin confirmed the compound was attached to the resin. The resin (400 mg, 0.1 mmol) was shaken with mCPBA (173 mg, 1 mmol) in CH_2Cl_2 (5 mL) for 12 hours. The resin was filtered, washed with CH_2Cl_2, and dried. The resin (80 mg, 0.02 mmol) was then shaken with a mixture of amines (0.0038 mmol each of 2-naphthylamine, 4-cyanoaniline, 3-ethylaniline, 2,3-difluoroaniline, and 3,4-dimethoxyaniline) in CH_2Cl_2 (0.4 mL) for 24 hours. The resin was filtered and the solvent evaporated with a stream of nitrogen. The structures were identified using GCMS.

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