Titanium(IV) Isopropoxide Mediated Solution Phase Reductive Amination on an Automated Platform: Application in the Generation of Urea and Amide Libraries

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Abstract*:* Amine libraries and their derivatives are important targets for high throughput synthesis because of their versatility as medicinal agents and agrochemicals. As a part of our efforts towards automated chemical library synthesis, a titanium(IV) isopropoxide mediated solution phase reductive amination protocol was successfully translated to automation on the Tridentä library synthesizer of Argonaut Technologies. An array of 24 secondary amines was prepared in high yield and purity from 4 primary amines and 6 carbonyl compounds. These secondary amines were further utilized in a split synthesis to generate libraries of ureas, amides and sulfonamides in solution phase on the Tridentä . The automated runs included 192 reactions to synthesize 96 ureas in duplicate and 96 reactions to synthesize 48 amides and 48 sulfonamides. A number of polymer-assisted solution phase protocols were employed for parallel work-up and purification of the products in each step.

INTRODUCTION

High throughput parallel solution phase strategy has recently emerged as a leading concept for the expedient generation of small molecule libraries [1]. In this context, solution phase protocols amenable to automation are of particular interest. Theparallel, automated synthesis of diverse amines and their derivatives is an objective of high priority from the perspective of drug discovery [2]. A recent paper [3] reports that a quarter of the registered drugs in the global market contain at least one amino group. The reductive amination of carbonyl compounds that allows an expedient access to amines is a widely applied reaction in organic synthesis [4]. The sequence involves the formation of an imine or iminium intermediate upon exposure of a carbonyl compound to a primary or secondary amine followed by *in situ* reduction to afford an alkylated amine. The traditional method for carrying out this transformation has been catalytic hydrogenation [5] which is, however, incompatible with a number of otherwise reducible functional groups such as nitro, cyano and 'C-C' multiple bonds. Among the hydride-based reagents, sodium cyanoborohydride [6] (Borch reduction) has found considerable application. The use of this reagent is, however, compromised by its expense and toxicity [7] that risks the presence of residual cyanide [8] in the product as well as in the work-up system. The other noteworthy hydride reagents are sodium triacetoxyborohydride [9] and pyridine-borane complex [10].

In connection with our ongoing studies on reductive amination reactions, we have earlier reported an efficient, one-pot method for the reductive amination of aldehydes and ketones with primary as well as secondary amines [11] using a combination of titanium(IV) isopropoxide and sodium borohydride. The protocol works well with a variety of amines and carbonyl compounds, including enolizable aldehydes and ketones. Steric hindrance seems to pose no problem. Unlike the other acid-catalyzed methods, the use of titanium(IV) isopropoxide permits the reductive amination of substrates containing potentially acid sensitive functional groups such as acetal, acetonide, and Boc derivatives [11]. Though many of the reported protocols for reductive amination reactions work well for the preparation of tertiary amines, synthesis of secondary amines by reductive alkylation of primary amines is, in many cases, compromised by over-alkylation reactions [12]. The formation of variable amounts of tertiary amines along with the desired secondary amines is common. The present reaction conditions, however, offer clean conversion affording only the desired secondary amines in high yields.

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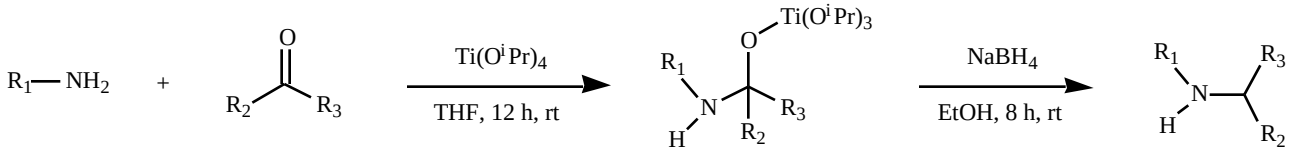
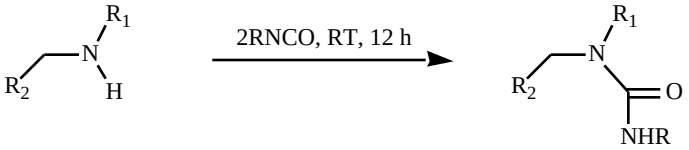


Scheme 1. Synthesis of seconday amines by titanium(iv) isopropoxide mediated reductive amination reaction.

In the context of our solution phase high throughput chemistry approach, we describe herein the utility of the titanium isopropoxide mediated reductive amination protocol in the automated parallel synthesis of amines. A 24-member library of secondary amines has been synthesized in high yield and purity by reductive alkylation of 4 primary amines with 6 aldehydes and ketones. The reaction conditions are quite amenable to automated parallel synthesis in solution phase. All reagents including titanium(IV) isopropoxide and sodium borohydride can be delivered as solutions, which is a prerequisite for automated fluid handlers. The protocol permits the use of an excess of carbonyl compound with respect to primary amine to drive the reaction to completion without compromising the purity of the product secondary amine. The endurance of the synthetic sequence towards automation was further verified by carrying out reactions in duplicate. The secondary amines were subsequently converted, using a solution phase split [13] synthesis approach, to urea, amide and sulfonamide libraries. All reactions (Schemes 1, 2 and 3) were successfully carried out on the Tridentä automated library synthesizer of Argonaut Technologies. Polymer-assisted solution phase techniques were employed in each step for high throughput parallel work-up and purification.

RESULTS AND DISCUSSION

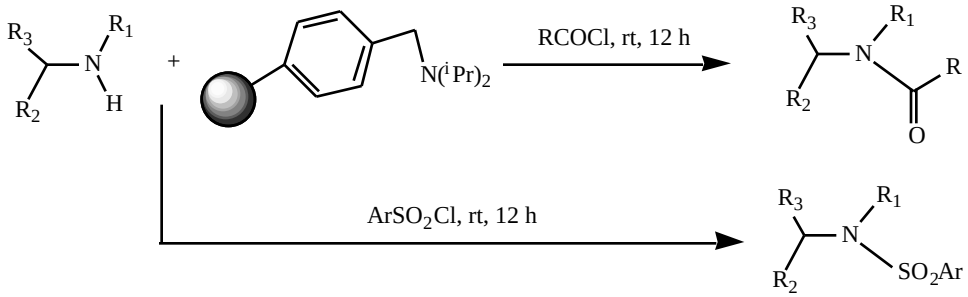
In the split synthesis approach [13] for the generation of compound libraries, the products from each diversity step are divided and used as a feedstock for the subsequent step of the synthesis. Accordingly, the strategy requires that all reactions at the early stages of the multi-step sequence be carried out on a larger scale. Thus, in our synthetic scheme, we required enough quantities of secondary amines from the first step to split for the subsequent step of the synthesis. We planned to synthesize ca 0.8 mmol (200-250 mg) of each of the amines to provide enough material for the syntheses of the targeted libraries.



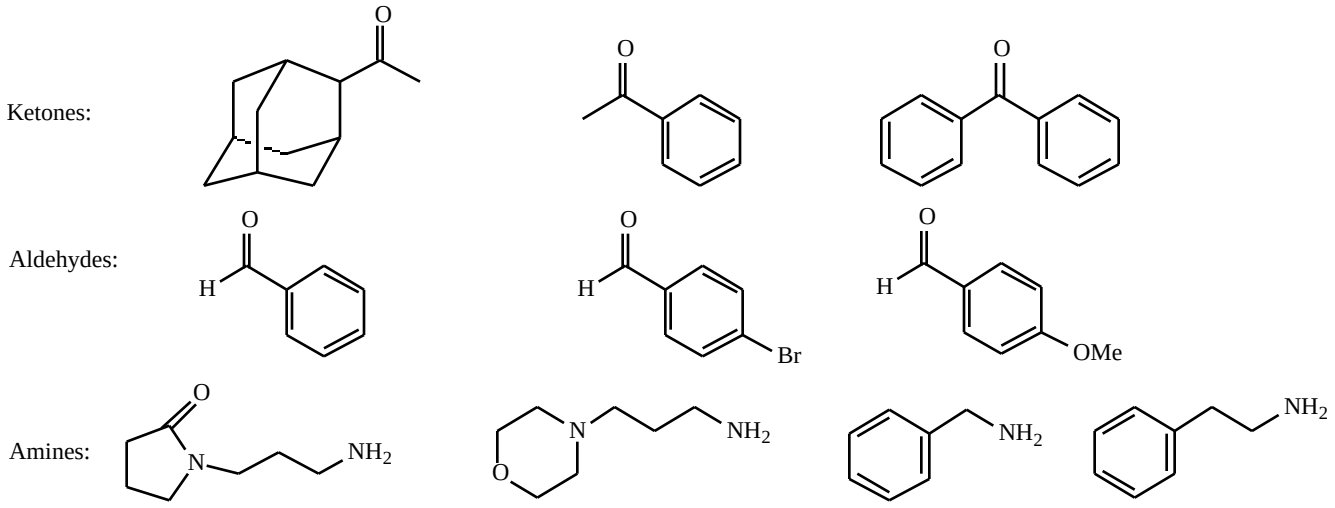
Scheme 2. Urea synthesis by condensation of secondary amines with alkyl isocyanates.

The 24 reductive amination reactions were performed in duplicate on the Trident to synthesize 24 secondary amines. The duplicate runs provided sufficient amounts of secondary amines to generate the urea, amide and sulfonamide libraries. The reagent matrix used 4 primary amines, 3 aldehydes and 3 ketones (Scheme 4).

The reductive amination protocol [11] involved two steps in one pot. The formation of an intermediate titanium(IV) complex from a mixture of primary amine, carbonyl compound, and



Scheme 3. Synthesis of amides and sulfonamides.



Scheme 4. Primary amines, aldehydes and ketones used in reductive amination.

titanium(IV) isopropoxide in THF was followed by the addition of sodium borohydride in ethanol (Scheme 1). The automated delivery of titanium(IV) isopropoxide proceeded smoothly without any blockage in the delivery line of the Trident synthesizer. A moisture sensitive reagent like titanium(IV) isopropoxide has the potential to form insoluble titanium dioxide by hydrolysis. The reaction contents remained clear throughout the process confirming the inertness of the reaction environment. The reduction step that involved the automated delivery of a freshly prepared solution of sodium borohydride in ethanol proceeded well even though some pressure built up in the reaction vessels. The synthesizer's reaction vessels can withstand up to 30 psi of internal pressure. After reduction, the reaction mixtures were drained to collection vials on the Autosampler and quenched with water. The resulting inorganic precipitate was removed by filtration. The product amines were isolated and purified by a "catch and release" protocol [14] using MP-TsOH, a sulfonic acid functionalized resin. The resin-amine salt, formed by 'catching' the amine on MP-TsOH, was washed several times with methanol. This process removed any non-basic impurities such as an alcohol generated by the reduction of an excess carbonyl compound used in the reductive amination reaction. The amines were next released by a 2 M solution of ammonia in methanol.

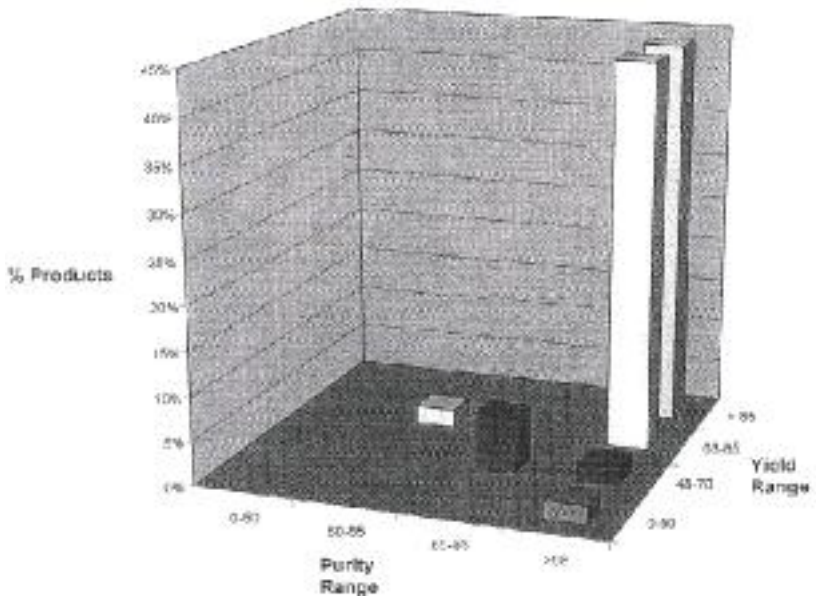
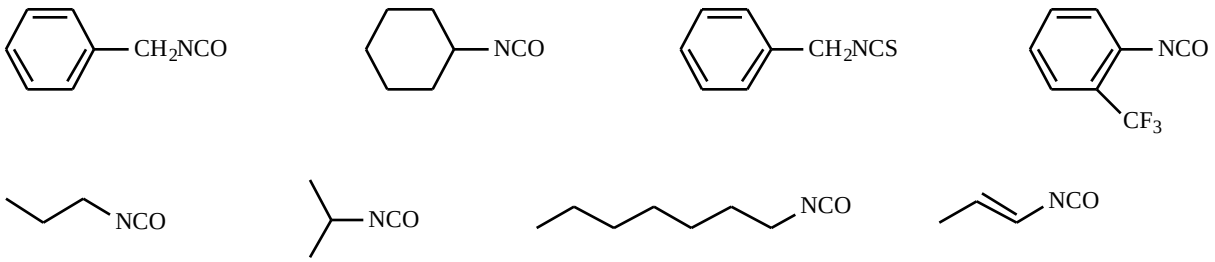


Fig. (1). Population map of secondary amines as a function of purity and yield range.



Scheme 5. Isocyanates used for urea library.

Both aldehydes and ketones were reductively aminated to produce the secondary amines in good to excellent yields. The titanium(IV) mediated reaction conditions offered clean conversions affording only the desired secondary amines in high yields; no over-alkylation of the product amines was observed. An excess of aldehydes and ketones (1.1 molequiv) was used to drive the reaction to completion. The purity (GC area percent) and yield of the secondary amines are shown in Fig. (**1**). The reaction conditions were found suitable for reductive amination of sterically hindered and enolizable aldehydes and ketones. The graph gives the percentage of products formed within defined purity and yield ranges. The results show that 92% of the products were formed in greater than 98% purity. Among the products formed in greater than 98% purity, 45% were isolated in greater than 85% yield, and 42% more products in greater than 70% yield. These results are consistent with the trial runs performed on a Questä semi-manual synthesizer (Argonaut Technologies). Lower purity was observed in samples derived from benzophenone due to the presence of residual primary amines. These products were further purified with a resin bound aldehyde to scavenge residual primary amines. The consistency in purity and yield for the replicate samples (two per compound) was excellent; the difference in percent purity for most of the replicates was within 4%.

The twelve secondary amines, derived from four primary amines and three aldehydes, were employed for the generation of the urea library. The 192 reactions performed on the Trident involved the synthesis of 96 ureas in duplicate. The reactions were run in duplicate to test the robustness of the automated sequence. The reagent matrix used twelve secondary amines, seven isocyanates and one isothiocyanate (Scheme 5). The reaction was carried out by stirring a solution of the amine and the isocyanate in DCM for 12 h at 24 °C. Excess isocyanates were used to drive the reactions to completion. Purification of the product ureas was performed by scavenging excess isocyanates using PS-trisamine, a polymer-bound primary amine.

The purity (HPLC area percent) and yield of the ureas are shown in Fig. (**2**). The population graph shows the percentage of products formed within

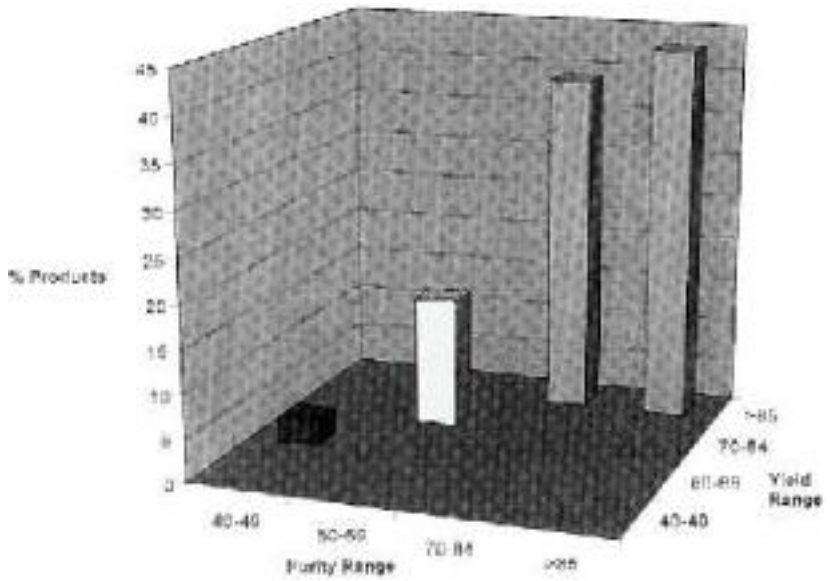
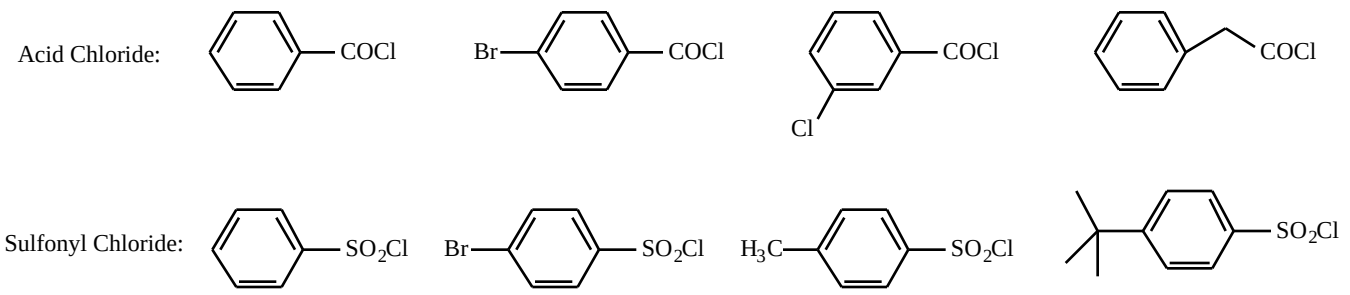


Fig. (2). Population map of ureas as a function of purity and yield range.



Scheme 6. Acid chlorides and aryl sulfonyl chlorides used for amide/sulfonamide library.

defined purity and yield ranges. The results show that 43% of the products had greater than 85% purity. Thirty-nine percent of the products had a purity range of 70-84%; these products were mainly obtained from benzyl isocyanate, 2-trifluorophenyl isocyanate and benzyl isothiocyanate. Analysis of the HPLC data of these products revealed that the impurities were mostly from residual isocyanates and symmetrical ureas. In these cases, an incubation time of longer than 2 h with PS-Trisamine is warranted. HPLC analysis of these three commercial isocyanates used in this sequence also confirmed that they contained 10-15% symmetrical ureas as impurities. The isolated yield of urea was generally high, with 82% of the products formed in greater than 85% yield. The consistency in purity and yield for the replicate samples (two per compound) was very good. The results again demonstrate high reproducibility in the synthesis.

The twelve more hindered secondary amines derived from ketones were used for coupling with acyl chlorides and sulfonyl chlorides to synthesize 96 amides and sulfonamides. These 96 reactions used twelve secondary amines, four acyl chlorides and four aryl sulfonyl chlorides (Scheme 6).

The synthesis was performed by stirring a mixture of the amine, the acid chloride and a polymer bound tertiary amine, PS-DIEA for 12 h at 24 ^{o}C. PS-DIEA was used to trap hydrogen chloride formed during the reaction. The product amides and sulfonamides were purified through the use of PS-Trisamine, a resin bound primary amine to scavenge excess acyl/sulfonyl chloride. The purity (HPLC area percent) and yield of the amides/sulfonamides are shown in Fig. (**3**). The graph shows the percentage of products formed within defined purity and yield ranges. The results show that 71% of the products had greater than 85% purity. Lower purity and yield were observed

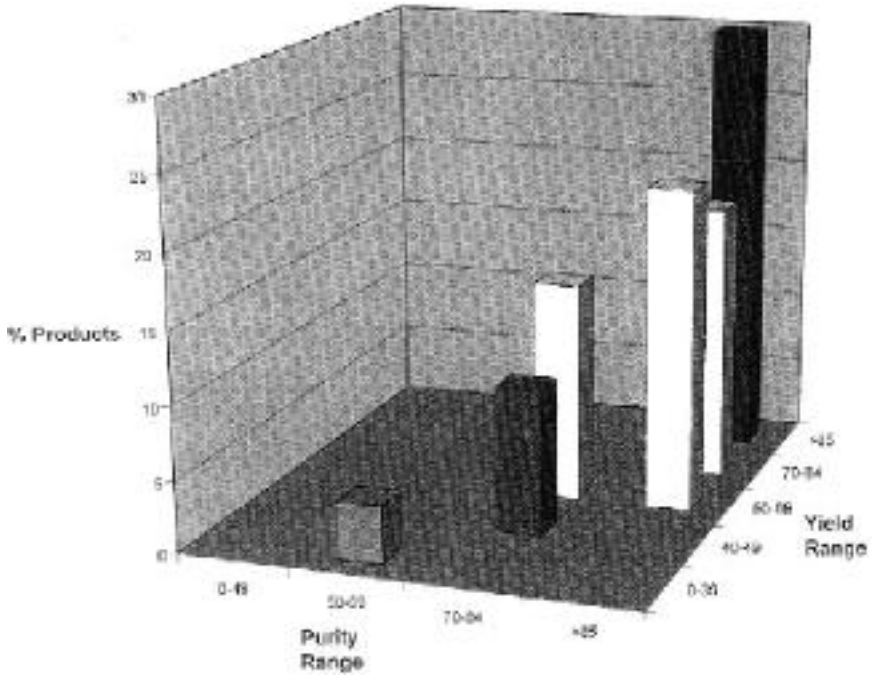


Fig. (3). Population map of amides and sulfonamides as a function of purity and yield range.

in samples derived from phenylacetyl chloride. This was probably due to the formation of side products *via* a ketene intermediate generated from phenylacetyl chloride.

In conclusion, we have demonstrated that the titanium isopropoxide/sodium borohydride reductive amination protocol can be well adapted for robust automated synthesis of secondary amines in solution phase on the Trident automated library synthesizer. The procedure allows a broad scope of carbonyl compounds ranging from reactive aldehydes to relatively unreactive ketones. Twenty-four secondary amines were prepared in duplicate from 4 primary amines and 6 aldehydes and ketones using this protocol. These amines were used in subsequent automated runs to generate a 96-member urea library in duplicate and a 96-member amide library. Excellent consistency in purity and yield among duplicate samples further proves the robustness of the sequence towards automation. Polymer-assisted solution phase techniques are employed in each step for product purification and isolation.

EXPERIMENTAL SECTION

General

Starting materials were used as received from their respective suppliers. PS-DIEA, PS-Trisamine, PS-Benzaldehyde and MP-TsOH were obtained from Argonaut Technologies (San Carlos, CA). All automated runs were performed on the Trident library synthesizer (Argonaut technologies). Parallel concentration of the product solutions was done on a Savant Speedvac Model SC 250DDA. GC analysis was performed on a Hewlett-Packard 6890 GC with a HP-5 phenylmethylsilicone capillary column (30 m x 0.32 mm x 0.25 μm). GC purity was determined by peak area using a thermal conductivity detector (TCD). HPLC analysis was carried out on a Hewlett-Packard 1050 HPLC with a Microsorb MV C18 column. HPLC purity was determined by area percent using a UV detector at 223 nm. ^{1}H NMR spectra were recorded on a Varian 300 spectrometer.

Synthesis of an Array of 24 Secondary Amines in Duplicate by Reductive Amination

THF solutions (1 M) of the carbonyl compounds and 2 M THF solutions of the primary amines were prepared and placed on the Trident autosampler. The synthesis involved delivery of 0.45 mL (0.45 mmol) of the three aldehyde and three ketone solutions to the reaction cassette (8 deliveries for each carbonyl compound) followed by the delivery of 0.2 mL (0.4 mmol) of the four primary amine solutions (12 deliveries for each amine). A solution (2 M, 0.3 mL) of titanium(IV) isopropoxide in THF was next delivered to all 48 reaction vessels from a common reagent position and the reaction cassette was agitated for 12 h at 24 ^{o}C. A 0.75 M solution of sodium borohydride in absolute ethanol was prepared, placed in a common reagent position and 1.5 mL of this solution was delivered to all 48 reaction vessels. The reaction cassette was further agitated at 24 ^{o}C for 8 h and the reaction contents were drained into collection vials. Water (0.5 mL) was added to each vial to quench the reaction and the resulting white precipitate was removed by filtration. The product amines were purified by "catching" them on a sulfonic acid functional resin (MP-TsOH, 0.65 g, 0.95 mmol) in 6 mL SPE cartridges, followed by 3 x 4 mL methanol washes to remove non-basic impurities. The products were released from the polymer support with 3 x 3 mL of 2 M ammonia solution in methanol. After removal of the solvent using the Speedvac, weight yields of the products were obtained and the purity analyzed by GC. A few samples were randomly selected and their purity was further assessed by ^{1}H NMR. This procedure afforded ca 0.7 mmol of each of the secondary amines. The products derived from

Table 1. Reagents for Reductive Amination

Reagent	Solvent	Concentration	Reagent Type	mmol	Volume/Mass
Titanium(IV) Isopropoxide	THF	2.0 M	Common	0.6	0.3 mL
Carbonyl Compound	THF	1.0 M	Diversity	0.45	0.45 mL
Primary Amine	THF	2.0 M	Diversity	0.4	0.2 mL
Sodium Borohydride	EtOH	0.75 M	Common	1.12	1.5 mL
MP-TsOH		1.46 mmol/g	Common	0.95	0.65 g

Table 2. Reagents for the Urea Library

Reagent	Solvent	Concentration	Reagent Type	mmol	Volume/Mass
Secondary Amine	DCM	0.1 M	Diversity	0.04	0.4 mL
Alkyl Isocyanate	DCM	0.1 M	Diversity	0.08	0.8 mL
PS-Trisamine		3.27 mmol/g	Common	0.1	0.03 g

benzophenone had residual primary amines that were further purified by scavenging off primary amines using a polymer bound aldehyde, PS-Benzaldehyde. These samples were dissolved in anhydrous THF, added PS-Benzaldehyde (0.33 g, 0.4 mmol), incubated for 6 h at 24 °C, filtered and concentrated to afford pure secondary amines. The reagents and stoichiometry used for the reductive amination reaction are summarized in Table 1.

Synthesis of an Array of 96 Ureas in Duplicate

The 12 secondary amines derived from the reductive amination of aldehydes with primary amines were used in the split synthesis of ureas. The condensation reaction of these amines with isocyanates were run in duplicate using 8 isocyanates (12 x 8 x 2). The reagents and stoichiometry used for the urea synthesis are summarized in Table 2. Solutions of the amines (0.1 M) and isocyanates (0.1 M) in DCM were prepared and placed on the autosampler. The synthesis involved delivery of 0.4 mL (0.04 mmol) of the 12 amine solutions to the 4 reaction cassettes (16 deliveries for each amine) followed by the delivery of 0.8 mL (0.08 mmol) of the 8 isocyanate solutions (24 deliveries for each isocyanate). The reaction cassettes were then agitated at room temperature for 12 h. The reaction contents were drained into collection vials containing a resin bound primary amine (PS-Trisamine, 0.03 g, 0.1 mmol), incubated for 2 h, filtered into vials and concentrated. The urea samples were weighed, and their purity was analyzed by using HPLC.

Synthesis of an Array of 96 Amides and Sulfonamides

The 12 secondary amines derived from the reductive amination of ketones with primary amines were used for the synthesis of 96 amides and sulfonamides. The reagent matrix involved 12 amines, 4 acid chlorides and 4 arylsulfonyl chlorides. The reagents and stoichiometry used for the urea synthesis are summarized in Table 3. PS-DIEA resin (0.1 g, 0.38 mmol) was loaded into each of the 96 reaction vessels before cassette assembly. Solutions of the amines (0.16 M), acyl chlorides (0.32 M) and aryl sulfonyl chlorides (0.32 M) in DCM were prepared in vials and placed on the autosampler. The synthesis involved delivery of 0.5 mL (0.08 mmol) of the 12 amine solutions to the 2 reaction cassettes (8 deliveries for each amine) followed by the delivery of 0.5 mL (0.16 mmol) of the 8 acyl/aryl sulfonyl chloride solutions (12 deliveries for each acyl/aryl sulfonyl chloride). The reaction cassettes were then agitated at room temperature for 12 h. The reaction contents were drained into collection vials containing a resin bound primary amine (PS-Trisamine, 0.06 g, 0.2 mmol), incubating for 2 h, filtered into pre-weighed vials and concentrated. The amide and sulfonamide samples were weighed and their purity was analyzed by HPLC.

Table 3. Reagents for the Amide/Sulfonamide Library

Reagent	Solvent	Concentration	Reagent Type	mmol	Volume/Mass
PS-DIEA		3.8 mmol/g	Common	0.38	0.1 g
Secondary Amine	DCM	0.16 M	Diversity	0.08	0.5 mL
Acid Chloride	DCM	0.32 M	Diversity	0.16	0.5 mL
Sulfonyl Chloride	DCM	0.32 M	Diversity	0.16	0.5 mL
PS-Trisamine		3.27 mmol/g	Common	0.2	0.06 g

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