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# Rapid Purification of Small Molecule Libraries by Ion Exchange Chromatography

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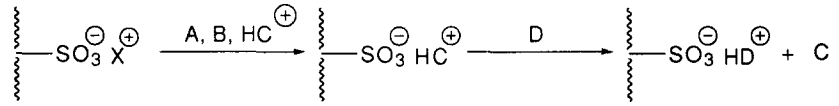
**Abstract:** Amines and acylated amines are synthesized in traditional solution phase reactions, then rapidly purified by ion exchange chromatography to yield pure products. In some instances, impurities devoid of ionizable functionality can be covalently modified prior to purification. The generic purification sequence is applicable to a variety of reactions, and is amenable to automation with commercially available equipment.


The generation of small molecule libraries for screening against biological targets has emerged as an area of intense interest in the pharmaceutical industry. These libraries have largely been synthesized using solid phase organic synthesis (SPOS) to minimize side products, expedite reaction work-up, and facilitate the application of automation.[1] More recently, workers have examined alternatives to SPOS for the production of large numbers of high purity compounds in parallel through a variety of techniques such as the use of solid supported reagents,[2,3] liquid/liquid extraction of solution phase reactions,[4] and fluorinated reagents for work-up simplification.[5] We have developed methodology for the expedited work-up and purification of traditional solution phase reactions using solid supported scavenging reagents.[6] Ideally, expedited synthesis methodology should be 1) applicable in a broad range of reactions; 2) tolerant of a variety of substrates within a reaction class; 3) amenable to automated synthesis. We now wish to present our results on the application of ion exchange chromatography in the expedited work-up and purification of organic molecules synthesized in solution, and in the automated construction of small molecule libraries.

**Figure 1**



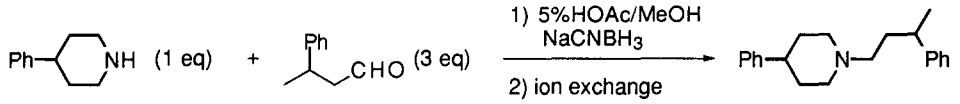

Strong Cation Exchange Column

The advantage of ion exchange chromatography over more traditional small molecule purification modes such as flash chromatography or HPLC is that one can reliably predict the elution characteristics of a broad range of molecules solely by the presence or absence of an ionizable site in the molecule. As illustrated simply in **Figure 1**, neutral molecules A and B pass through a sulfonic acid based cation exchange column, while protonated molecule C is retained. Reaction mixtures produced under a variety of conditions can be purified identically provided the desired products are either the only ionizable materials or the only non-ionizable materials

in solution, an ideal scenario for automated reaction purification. Ion exchange chromatography has been employed for many years in a wide variety of applications,[7] such as water purification,[8] protein purification,[9] and serum analysis.[10] Its potential as a method of purifying small organic molecules, however, has been largely unexplored, the principal application in this area being the purification of extremely hydrophilic molecules such as peptides and amino acids which can be difficult to purify by other means.[11,12]

Our initial experiment was the purification of a reductive amination reaction mixture using cation exchange chromatography (**Scheme 1**). In an illustrative example, 4-phenylpiperidine was combined with a 3-fold excess of 3-phenylbutyraldehyde in 5% acetic acid in methanol as solvent, and treated with an excess of sodium cyanoborohydride. When no starting secondary amine remained by TLC, the reaction mixture was poured over a Varian strong cation exchange (SCX) column.[13] The column was rinsed with methanol to remove neutral impurities, then treated with a 2M solution of anhydrous ammonia in methanol to elute the product tertiary amine in 83% yield and 86% purity by HPLC analysis. The method of purification allows for the addition of a large excess of one reagent (in this case aldehyde) to drive the reaction to completion, as in solid phase synthesis, without fear of work-up complications. Thus purity and yield of the final product are high.

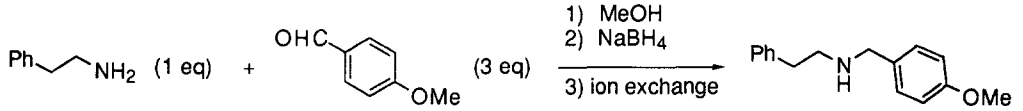
**Scheme 1**




yield: 83%
HPLC purity:86%

Similarly, secondary amines can be synthesized from the corresponding primary amines and aldehydes by employing a large excess of aldehyde in methanol to form imine nearly quantitatively with respect to primary amine, then reducing the mixture with sodium borohydride and passing the resulting solution over an SCX column (**Scheme 2**). These reductive amination procedures have been used in our labs to rapidly synthesize hundreds of secondary and tertiary amines in high purity.

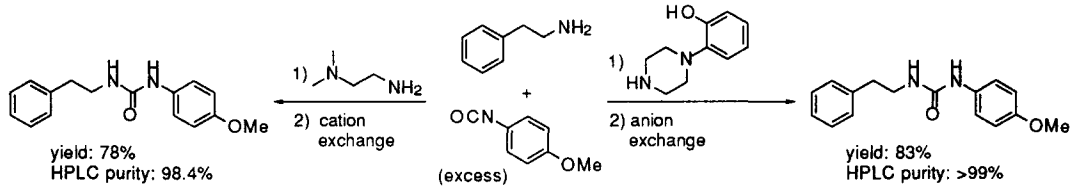
**Scheme 2**




yield: 86%
HPLC purity: 97%

We have also explored the possibility of covalently trapping a non-ionizable impurity with a solution phase scavenger to create an ionizable impurity for removal by ion exchange. The concept of solution phase scavengers is complimentary to our earlier work on solid phase covalent scavengers,[6] and is amenable to both anion and cation exchange chromatography depending on the scavenger employed. As an illustration, phenethylamine was reacted with 1.25 equivalents of 4-methoxyphenylisocyanate to form product urea plus starting isocyanate (**Scheme 3**). The isocyanate impurity was removed by quenching either with N,N-dimethylaminoethylamine followed by cation exchange, or by quenching with 1-(2-hydroxyphenyl)piperazine followed by anion exchange.[14,15]

**Scheme 3**




Since the ability to utilize ion exchange chromatography as a separation method relies on the nature of the products formed and is nearly independent of the reaction conditions, reactions conducted under a variety of conditions can be purified in a nearly identical manner. This is an ideal scenario for the application of simple robotics for purification. As an illustration, we synthesized three secondary amines by reductive amination using ion exchange purification (amine **1** from p-tolualdehyde and 3,3-diphenylpropylamine: 92% purity by HPLC; amine **2** from α-methylphenylacetaldehyde and 4-fluorobenzylamine: 94%; amine **3** from α-methylphenylacetaldehyde and 3,3-diphenylpropylamine: 91%) and subjected each with no further purification to reaction in a 3 x 3 matrix with three different substrates (aldehyde **A**, epoxide **B**, and isocyanate **C**). Each substrate required different reaction conditions: reductive amination with an aldehyde in acidic methanol, epoxide opening in methanol under neutral conditions, and acylation in dry chloroform (**Figure 2**). An excess of reagents was employed in such a way as to ensure that the desired product was the only ionizable component (for alkylation with substrates **A** or **B**) or the only non-ionizable component (for acylation with isocyanate **C**) present in the final reaction mixture. Thus, for reductive amination a two-fold excess of aldehyde **A** was employed, for epoxide opening a two-fold excess of epoxide **B** was employed, and for acylation with **C** a two fold excess of amines **1-3** was employed. When the reactions were complete by TLC as judged by disappearance of the limiting reagent, the nine solutions were subjected to ion exchange in an automated fashion using a commercially available Hamilton Microlab 2200 robot with SPE capability to separate ionizable from non-ionizable materials. The robot successfully purified all nine reactions independent of reaction conditions to a purity after two steps of greater than 80% in all cases and in excess of 90% in the majority of cases, with yields ranging from 71-92%.[16]

**Figure 2: 3x3 matrix of reactions purified by ion exchange**[a, b]

| | 1 | 2 | 3 |
|---|---|---|---|
| A | yield: 72%<br>HPLC purity: 98% | yield: 84%<br>HPLC purity: 88% | yield: 71%<br>HPLC purity: 95% |
| B | yield: 85%<br>HPLC purity: 99% | yield: 81%<br>HPLC purity: 80% | yield: 80%<br>HPLC purity: 93% |
| C | yield: 90%<br>HPLC purity: 91% | yield: 92%<br>HPLC purity: 86% | yield: 87%<br>HPLC purity: 87% |

[a] purities listed are for the two step sequence with only ion exchange purification

[b] HPLC conditions: NovaPak 3.9 x 150 mm C18 column, 50-100% acetonitrile/0.1% TFA, 20 min.

In summary, we have explored the use of ion exchange chromatography for the automated purification of a variety of amine functionalizations and found it to be an effective method for the synthesis of high purity small molecule libraries in an automated fashion. Further applications of ion exchange chromatography for library synthesis will be reported in due course.

**General Procedure for Reductive Amination**: To a 4-mL vial was added 4-phenylpiperidine (20 mg, 0.124 mmol), 3-phenylbutyraldehyde (55 mg, 0.37 mmol), and 250 μL 10% acetic acid in methanol. To this solution was added 250 μL of a 1N solution of sodium cyanoborohydride in methanol. The vial was sealed and shaken at room temperature for 4 hours. The solution was applied to a 500 mg SCX column (Varian), and the column flushed with 3 mL methanol. The product was then eluted with 1 mL of 2 M ammonia in methanol to give 30 mg (83% yield) of the desired tertiary amine.

**Acknowledgments**

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11. Some workers have explored the use of ion exchange resins in the production of combinatorial libraries; see Gayo, L. M.; Suto, M. J. *Tetrahedron Lett.* **1997,** *38*, 513-516; Parlow, J. J. *Tetrahedron Lett.* **1996,** *37*, 5257-5260; Lawrence, R. M.; Biller, S. A.; Fryszman, O. M.; Poss, M. A.; Weller, H. A. A Simple Procedure for the Automated Solution Phase Synthesis and Purification of Non-Peptidic Amides. In *Conference on Molecular Diversity and Combinatorial Chemistry*; January 28, 1996: San Diego, CA, 1996.
12. For a recent and somewhat novel example of the use of solid phase extraction in the purification of combinatorial libraries, see: Virgilio, A. A.; Schürer, S. C.; Ellman, J. A. *Tetrahedron Lett.* **1996**, *37*, 6961-6964.
13. Varian SCX columns are sulfonic acid residues covalently linked to silica gel. These prepacked columns are particularly convenient in that they readily fit off the shelf automated solid phase extraction equipment.
14. Analytical data for urea: $^1$H NMR (300 MHz, $CDCl_3/CD_4OD$): 7.1-7.3 (m, 7H); 6.84 (d, 2H, J=9 Hz); 3.81 (s, 3H); 3.50 (t, 2H, J=6.8 Hz); 2.83 (t, 2H, J=6.8 Hz). MS (M+1): 271.1.
15. We have found it necessary to purify commercially available 1-(2-hydroxyphenyl)piperazine by flash chromatography in order to achieve optimal results
16. All compounds gave mass spectral data consistent with the desired structure.

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