A Novel Cleavage Technique To Generate Small Molecule Compounds and Libraries via a Two-Resin System

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Received July 22, 1997

Application of organic synthesis to solid supports has led to the successful implementation of combinatorial chemistry in the drug discovery process. This paper describes a novel use of the Hofmann elimination of tetrasubstituted amine salts on solid-phase resin to generate diverse combinatorial libraries of trisubstituted amines. Highly pure compounds were isolated without further purification by the addition of a second resin as the source reagent to promote the required elimination. The use of mixed resin systems to generate compounds is a novel application of bead-based technologies.

Introduction

The emergence of combinatorial chemistry as a major contributor to the drug discovery process[1] has led to the very rapid development of novel synthetic methodologies[2] for the organic synthesis of nonpeptidyl small molecules on solid support resins. In addition, recent advances in the use of new types of linker strategies[2a,3] have expanded the diversity of compounds generated upon cleavage from the resin. In particular, a recent report[4] described the use of a linker strategy in which substituted amines were added in a Michael fashion[5] to an acrylate bound resin as seen in Figure 1.

Subsequent alkylation to quaternize the trisubstituted amine followed by the addition of a suitable base allowed for the Hofmann elimination of the desired product and the regeneration of the starting acrylate resin. A limitation in this effort was noted by the lack of diverse monosubstituted amines implemented and the cited need to extract each compound to ensure an acceptable level of purity;[4a] this is especially troublesome in the case of large library production[6] which might employ automation equipment. We report on concurrent efforts in our laboratories, in which we have expanded the scope of the inputs to attain an acceptable number of inputs to generate large libraries (>10 000) of trisubstituted amines. Figure 2 is an example of our early general synthesis methodology involving the use of monosubstituted amines. In addition, we will discuss our efforts in which we developed new methodology to bypass the problem of extraction but still maintain high yields and acceptable purity standards.

In an effort to define the generality of the Michael addition a large variety of amines were employed. The diversity of disubstituted amines, cyclic and noncyclic, was explored first as only a second step of alkylation was needed prior to elimination. These inputs were found to proceed smoothly. In addition, a number of monosubstitiuted amines were employed successfully. This was immediately followed by the reductive amination of aldehyde and ketone inputs which allowed for greater diversity. To examine the products of the Michael addition step, a select but diverse number of alkylating reagents were used to study the methodology necessary to generate the desired compounds. Table 1 contains a partial list of amines, aldehydes were applicable, and alkylating agents employed in this study.

As reported by Morphy et al.,[4a] we have also demonstrated that the Hofmann elimination could be utilized to generate the desired trisubstituted amines by the use of an excess of triethylamine (TEA). Unfortunately, after Hofmann elimination only the use of time-consuming aqueous extraction effectively provided for the full removal of the unwanted triethylamine hydrobromide or iodide salts formed in the elimination step. A recently published follow-up paper by Morphy,[4b] cited an improved extraction method by the use of solid-phase biphasic columns. However both of these techniques create a serious problem if one is making large libraries (>1000 compounds) or one uses automation equipment. An alternative strategy studied was the application of heat[7] which produced compounds that contained no TEA salts,

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(1) (a) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233−1251. (b) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1384−1401.

(2) For recent reviews on solid-phase organic synthesis, see (a) Thompson, M. A.; Ellman, J. A. *Chem. Rev. (Washington, D.C.)* **1996**, *96*, 555−600. (b) Terrett, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; Steele, J. *Tetrahedron* **1995**, *51*, 8135−8173. (c) Fruchtel, J. S.; Jung, G. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 17−42. (d) Balkenhohl, F.; von dem Bussche-Hunnefeld, C.; Lansky, A.; Zechel, C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2288−2337.

(3) Most recently (a) Han, Y.; Walker, S. D.; Young, R. N. *Tetrahedron Lett.* **1996**, *37*, 2703−2706. (b) Beaver, K. A.; Seigmund, A. C.; Spear, K. L. *Tetrahedron Lett.* **1996**, *37*, 1145−1148. (c) Boehm, T. L.; Hollis Showalter, H. D. *J. Org. Chem.* **1996**, *61*, 6498−6499. (d) Ngu, K.; Patel, D. V. *Tetrahedron Lett.* **1997**, *38*, 973−976. (e) Routledge, A.; Abell, C.; Balasuramanian. *Tetrahedron Lett.* **1997**, *38*, 1227−1230. (f) See refs 2a and 2b for a list of other linkers and strategies.

(4) (a) Morphy, J. R.; Rankovic, Z.; Rees, D. C. *Tetrahedron Lett.* **1996**, *37*, 3209−3212. (b) Brown, A. R.; Rees, D. C.; Rankovic, Z.; Morphy, J. R. *J. Am. Chem. Soc.* **1997**, *119*, 3288−3295.

(5) (a) Ley, S. V.; Mynett, D. M.; Koot, W.-J. *Synlett.* **1995**, *60*, 6006−6007. (b) Cody, D. R.; DeWitt, S. H. H.; Hodges, J. C.; Kiely, J. S.; Moos, W. H.; Pavia, M. R.; Roth, B. D.; Schroeder, M. C.; Stankovic, C. J. PCT Int. App. WO 9408711; *Chem Abstr.* **1995**, *122*, 106536.

(6) ^{1}H NMR spectra of the compounds were found to contain a large excess of triethylamine. An example in the Supporting Information is provided.

(7) The resin with a quaternary ammonium compound in DMF was simply heated at 60−80 °C.

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Published on Web 01/28/1998

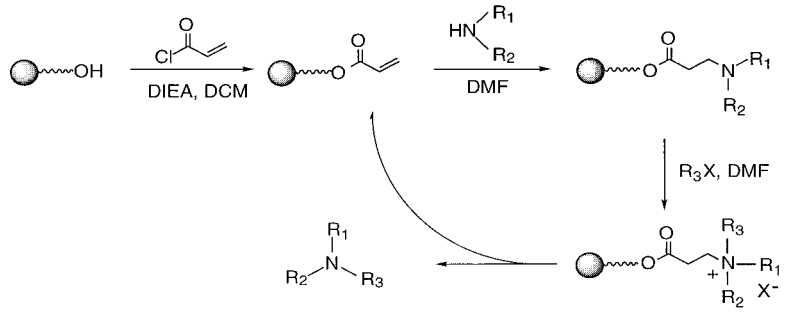


Figure 1. Synthesis of trisubstituted amines.

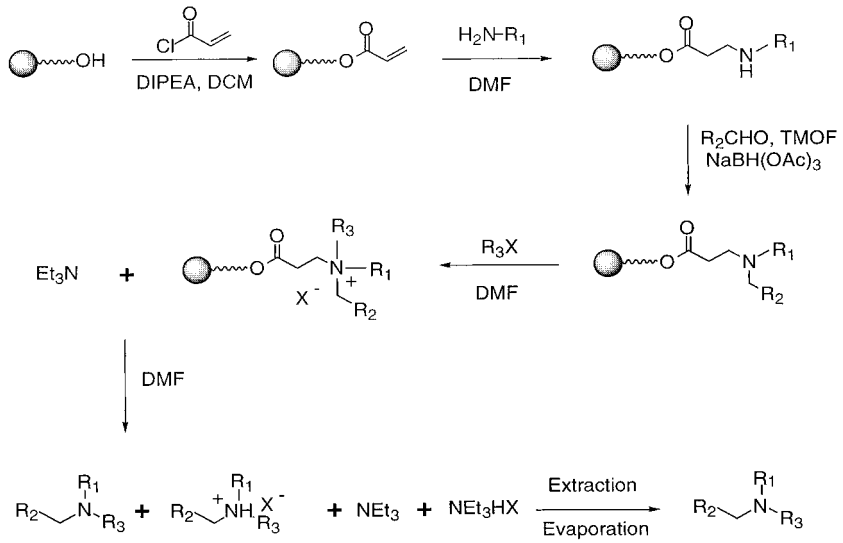
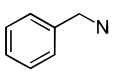
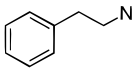
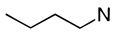
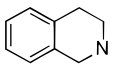
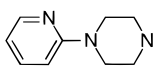
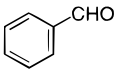
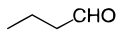
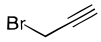
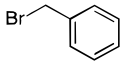
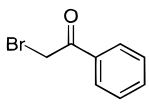


Figure 2. Combinatorial synthesis of trisubstituted amines using three building blocks.

Table 1. Sample of Building Blocks Used

1° Amines			
2° Amines			
Aldehydes Ketones			
Alkylating Agents			
			

but it was observed that the products were contaminated with other unidentified impurities.[8] Neither of these methods was found to give satisfactory levels of dependable yield and/or purity of the desired products.

(8) ^{1}H NMR spectra of the compounds were found to contain a unidentified polymeric contamination.

In an attempt to solve the paradigm of attaining high purity by an alternate means, we first attempted cleavage of the quaternary amine salts from resin by the use of 1 equiv of TEA. This proved to be helpful as the ^{1}H NMR spectra of the cleaved compounds contained less TEA salts.[11] However, there was still an undesired excess of the base still present.

At the same time a series of articles had described the use of resin bound scavengers which were able to sequester excess reagents and leave the desired pure products in the solution. Most notably, ion-exchange resins had been demonstrated to be useful as reagents and scavengers in a variety of solution-phase parallel syntheses so as to remove unwanted reaction byproducts and eliminate the need for aqueous workups.[9] More recently, applications involving specific functional groups have been introduced with outstanding results.[10]

Results and Discussion

It was postulated that the use of a basic ion-exchange resin might act as a regenerating agent for a solution

(9) (a) Parlow, J. J. *Tetrahedron Lett.* **1996**, *37*, 5257−5260. (b) Gayo, L. M.; Suto, M. J. *Tetrahedron Lett.* **1997**, *38*, 513−516.

(10) (a) Kaldor, S. W.; Siegel, M. G.; Fritz, J. E.; Dressman, B. A.; Hahn, P. J. *Tetrahedron Lett.* **1996**, *37*, 7193−7196. (b) Kaldor, S. W.; Fritz, J. E.; Tang, J.; McKinney, E. R. *Bio. Med. Chem. Lett.* **1996**, *6*, 3041−3044. (c) Flynn, D. L.; Crich, J. Z.; Devraj, R. V.; Hockerman, S. L.; Parlow, J. J.; South, M. S.; Woodard, S. *J. Am. Chem. Soc.* **1997**, *119*, 4874−4881. (d) Booth, R. J.; Hodges, J. C. *J. Am. Chem. Soc.* **1997**, *119*, 4882−4886.

(11) ^{1}H NMR spectra of various compounds were seen to contain TEA in a range of 5−60% of an equivalence. Recently Brown et al. utilized powdered K_2CO_3 to effectively deprotonate the diisopropylethylamine salt in situ. See ref 4b.

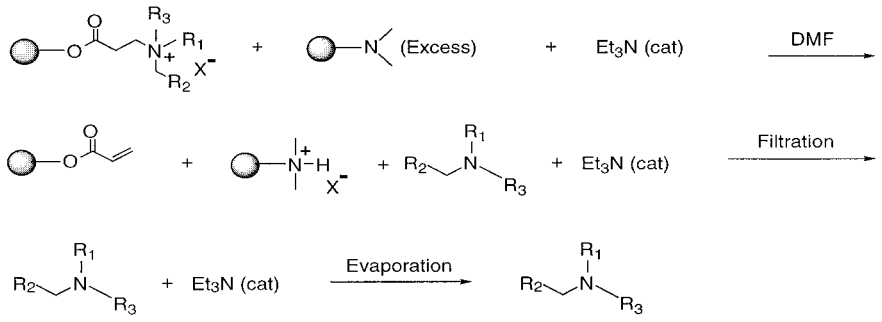


Figure 3. One-pot Hofmann elimination and extraction.

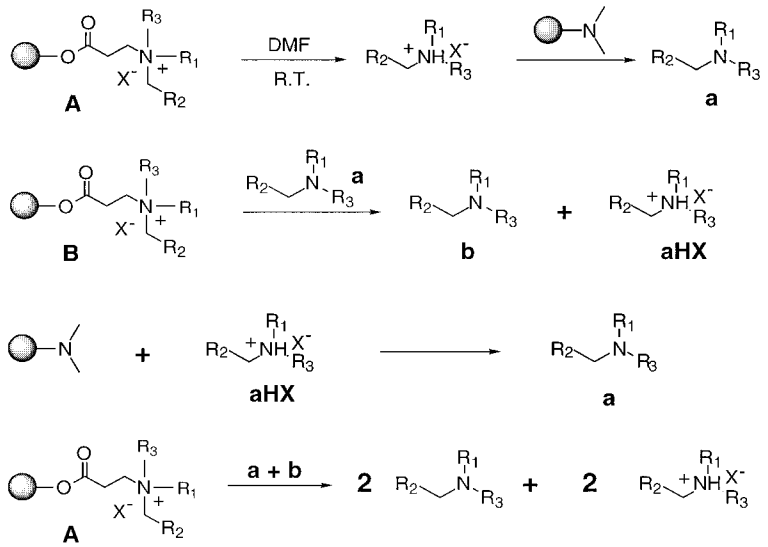


Figure 4. Possible mechanism for Amberlite-promoted Hofmann elimination.

amine catalyst which could promote the Hofmann elimination reaction. In this process, one could theoretically accomplish the cleavage step of the product amine and at the same time remove undesired the salt with an excess of a second resin bound amine. Evaporation of the filtrate would afford one pure product free of the undesired amine salts as indicated by Figure 3.

Upon implementation of this protocol, this strategy was found to be quite successful. Simple agitation overnight of the resin bound quaternary ammonium compound, a trace amount of TEA and the weakly basic ion-exchange resin Amberlite in DMF, produced the highly pure desired product with good yields after filtration and evaporation of the filtrate.

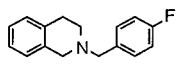
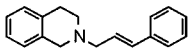
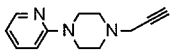
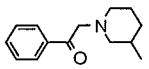
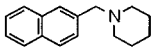
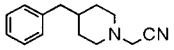
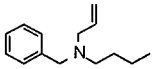
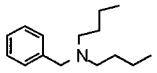
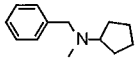
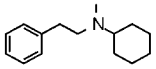
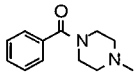
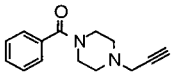
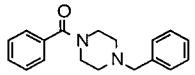
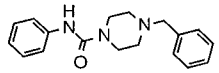
Further experimentation with Amberlite weakly basic ion-exchange resin in DMF and the resin bound quaternary ammonium compounds, in the *absence* of any base, proved to be successful. Highly pure products were isolated after routine filtration and evaporation of the excess filtrate. It was found that yield was a function of time with the optimal reaction length 18 h for library production. Lastly, we performed the same experiment (no base) using deprotected rink amide resin. To our surprise we obtained identical results to those of highly purified products. Less efficient yields were observed though an extended reaction time to give comparable results.

A quick review of the literature revealed only one other example on the use of more than one polymeric reagent in the same vessel.[12] *The remarkable aspect of this reaction is that the presence of two polymeric reagents is the only requirement to effect the transformation.* Two possible explanations for this effect can be reasoned. First, the resin containing the quaternary ammonium compounds undergoes a thermal elimination of a small amount of product. The newly created amine hydrobromide salt acts upon the ion-exchange Amberlite, or the rink resin, losing the salt and transforming to the salt free amine in a catalytic like cycle. This derived amine can then act as the base, promoting Hofmann elimination to provide more product as shown in Figure 4. An alternative explanation could be that there remains a trace amount of residual base from previous handling in the matrix of the resins which could initiate the elimination reaction.

In an effort to evaluate different Hofmann elimination conditions, we picked representative examples from several trisubstituted amine libraries and conducted Hofmann elimination reactions using TEA, Amberlite IRA-95 basic ion-exchange resin, and deprotected rink amide polystyrene resin. It must be noted that the yields are based upon the reported loading of hydroxymethyl resin (a single batch was used for this experiment). As shown Table 2, compounds **1**−**6** were synthesized by the Michael addition of a secondary amine to the acrylate resin, followed by quarternization employing various alkylating agents (alkyl, allyl, propargyl, and benzyl

(12) Parlow, J. J. *Tetrahedron Lett.* **1995**, *36*, 1395−1396 and references therein.

Table 2. Comparing Different Hofmann Elimination Conditions

Trialkylamine	A. TEA (EXTRACTED) yield (%)[a]	purity (%)[b]	B. AMBERLITE yield (%)	purity (%)	C. RINK AMIDE yield (%)	purity (%)
1.[c] 	68	95	70	95	35	95
2. 	65	95	69	95	38	95
3. 	59	95	61	95	33	95
4.[d] 	31	95	45	95	35	95
5. 	58	95	65	95	26	95
6. 	10	95	12	95	5	95
7.[e] 	32	95	37	95	16	95
8.[f] 	25	95	26	95	23	95
9.[g] 	51	95	40	95	14	95
10.[h] 	58	95	63	95	56	95
11.[i] 	43	95	55	95	24	95
12. 	50	95	52	95	41	95
13. 	51	95	58	95	37	95
14.[j] 	41	95	51	95	57	95

[a] Yield based upon use of 1.00 g of hydroxymethyl resin with a labeled loading of 1.2 mmol/g. The Hofmann elimination for all three experiments was carried out for 18 h. Evaporation of solvents was conducted at the same length of time for accurate comparison of three experiments. *Note*: increased reaction times for Amberlite and rink amide results in substantially higher yields. [b] Purity was estimated from the crude ^{1}H NMR (±2.5%). [c] Reaction sequence: 1,2,3,4-tetrahydroisoquinoline and 4-fluorobenzyl bromide. [d] Reaction sequence: 3-methylpiperidine and bromoacetophenone. [e] Reaction sequence: butylamine, benzaldehyde, and allyl bromide. [f] Reaction sequence: butylamine, butyraldehyde, and benzyl bromide. [g] Reaction sequence: benzylamine, cyclopentanone, and methyl iodide. [h] Reaction sequence: phenethylamine, cyclohexanone, and methyl iodide. [i] Reaction sequence: piperazine, benzoyl chloride, and methyl iodide. [j] Reaction sequence: piperazine, phenyl isocyanate, and benzyl bromide.

bromides and α-bromo ketones) and subsequent Hofmann elimination. The Hofmann precursor for compounds **7**−**10** was synthesized by Michael addition of primary amines, benzylamine and phenethylamine, followed by reductive alkylation with aliphatic, aromatic aldehydes and cyclic ketones. Quarternization and subsequent Hofmann elimination provided the desired compounds. Compounds **11**−**14** were generated by the simply use of piperazine as a linker, followed by acylation or urea formation; alkylation, and elimination thus provided the unsymmetrical piperazine derivatives.

In general, the Amberlite method provided slightly higher yields than that involving the use of the TEA/ extraction method. The rink amide method displayed slightly lower yields in some of the examples, which indicated lower efficiency of ion exchange with the HBr salt, which could due to the lower loading of the amine functionality on rink amide than Amberlite or the basicity of the resin.

To compare and contrast the effects of different Hofmann elimination conditions on resin, compound **2** was studied under five different conditions. The ^{1}H NMR of the Hofmann elimination products in deuterated Methanol is displayed in Figure 5.

Spectrum B (yield 65.2%) demonstrates that aqueous extraction effectively remove the undesired TEA salts

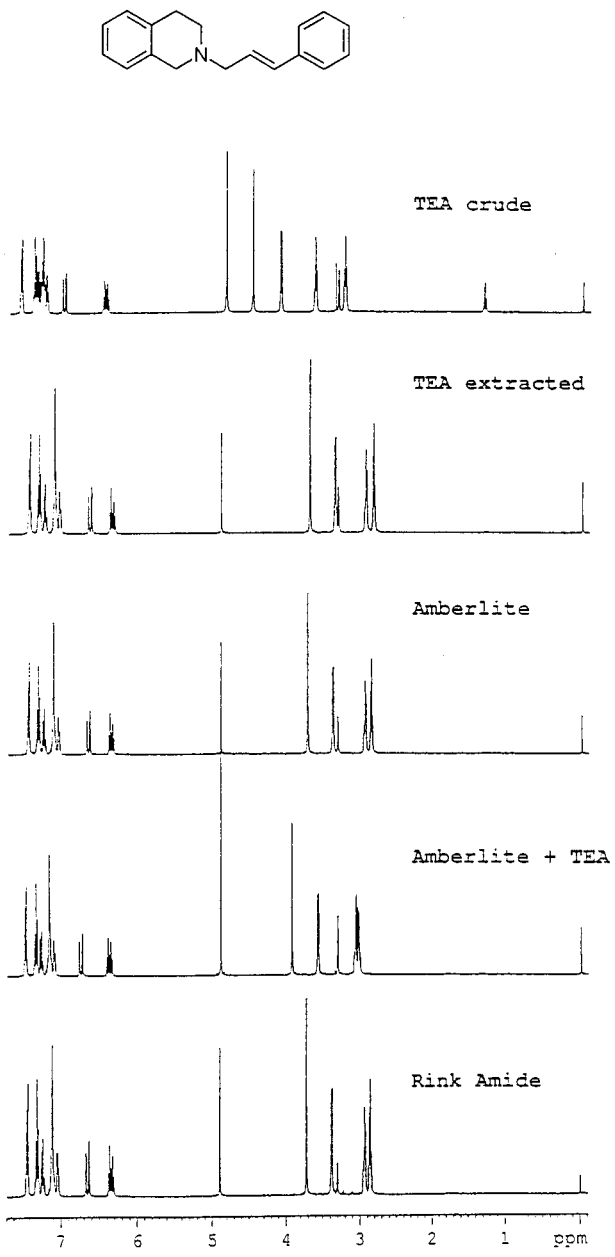


Figure 5. Comparison of methods to promote Hofmann elimination.

that are observed in the crude product A (yield 108.7%). [Note: spectrum A contains an unusually low amount of the base triethylamine; examples of ^{1}H NMR spectra containing large amounts of TEA in the crude products are found in Supporting Information.] The products exposed to a basic resin, C (yield 68.6%) and E (yield 37.8%), can be seen to be identical in quality with respect to B. Spectrum D (yield 82.9%) indicates a highly pure product, but a slight shift of some proton signals is noted. This shift effect was seen in other products under similar conditions.

Conclusion

In summary, we have developed a route to generate large diverse libraries of trisubstituted amines under very mild conditions which gives the desired products in high yields and eliminates the need for additional time-consuming purification steps. In addition, we have discovered a novel application of solid−solid reactions by employing the ion-exchange resin Amberlite. The rink amide resin is also effective, albeit at lower efficiency. The Amberlite method has proven to be most successful for our library production. Amberlite-promoted Hofmann eliminations in solution-phase organic syntheses have proven to be successful, as several examples have shown. Results on this application and other novel solid−solid interactions will be forthcoming.

Experimental Section

Materials. All solid-phase reactions were carried out at room temperature. Reagents were purchased from Aldrich and Acros and used without further purification. Amberlite IRA-95 weakly basic ion exchange was purchased from Sigma and was washed before use. (Hydroxymethyl and rink amide) polystyrene resins were purchased from Novabiochem with a loading of 1.20 and 0.68 mmol/g, respectively.

General Methods. All reactions were carried out in peptide synthesis vessels and agitated on an orbit shaker. Concentration of solutions after workup was performed by reduced pressure rotary evaporation. NMR spectra were obtained on a 500 MHz instrument with $CDCl_3$ as the solvent unless noted. Infrared spectra were obtained on a Perkin-Elmer IR spectrometer Spectra 2000. Data were reported in % transmittance. High-resolution mass spectra (HRMS) were obtained from electron spray mass spectrometer. Gas chromatography was performed on a Hewlett-Packard HP 6890 series GC system with a HP G1099 column and a HP 5973 mass selective detector.

General Procedure for Preparing Acrylate Resin on Solid Phase. A typical experimental procedure is as follows: hydroxymethyl polystyrene resin (35 g, 42 mmol) [1.2 mmol/g] was added to a 1 L reaction vessel. After the addition of dichloromethane (250 mL) and diisopropylethylamine (73 mL, 420 mmol), followed by four equal portion additions of acryloyl chloride (34 mL, 420 mmol). The mixture was slowly stirred for 4 h. The resin was then washed with dichloromethane (500 mL) and methanol (500 mL) for four cycles. The procedure was repeated, and the acylated resin was air-dried.

General Procedure for Preparing Trisubstituted Amine Synthesis on Solid Phase. All trisubstituted amines gave clean 400 MHz ^{1}H NMR, ^{13}C NMR, and GC-MS. A portion of the acrylate resin (5 g, 6 mmol) was mixed with 50 mL of DMF and butylamine (11.9 mL, 120 mmol) and agitated over 18 h. The resin was washed with DMF (1 × 50 mL), dichloromethane (1 × 50 mL), methanol (2 × 50 mL), and ether (1 × 50 mL) and dried in vacuo. To the resin were added 80 mL of DMF, benzaldehyde (12.2 mL, 120 mmol), and glacial acetic acid (200 μL), and the mixture was shaken for 0.5 h. $NaBH(OAc)_3$ (25 g, 120 mmol) was added to the mixture and left to agitate for 18 h. The resin was washed with methanol (2 × 50 mL), DMF (2 × 50 mL), and methanol (2 × 50 mL) and subsequently dried. The dry resin was then suspended in a solution of allyl bromide (10.4 mL, 120 mmol) in DMF (50 mL) and agitated over another 18 h. Filtration was followed by rinsing with DMF (1 × 50 mL), dichloromethane (2 × 50 mL), and methanol (2 × 50 mL) and dried in vacuo. To three peptide vessels were added exactly 1.00 g of the dry resin with tetra-substituted amine salts. To the first vessel were added 167 μL of TEA (1.2 mmol) and 40 mL of DMF, to the

second vessel were added 3.0 g of pre-washed Amberlite IRA-95 ion-exchange resin (4.7 mequiv/g) and 40 mL of DMF, and to the third vessel were added 3.0 g of deprotected rink amide resin [Novabiochem, 0.68 mmol/g] and 40 mL of DMF. All three vessels were agitated over 18 h. Vessel one was filtered and evaporated to give a white solid which was partitioned between 10 mL of DCM and 10 mL of 5% aqueous sodium bicarbonate. The organic layer was dried over $MgSO_4$, passed through a frit filter, and rotary evaporated. A colorless thick oil was obtained (79 mg, 32% overall yield). Vessels two and three were both filtered and evaporated. The Amberlite method yielded 91 mg (37% yield, 99% purity by GC/MS) of material. The rink amide method yielded 40 mg (16% yield, 99% purity by GC/MS) of material. All compounds were characterized by proton and carbon NMR, GC-MS, and HR-MS.

Acknowledgment. We thank Alex Virgillio and Alex Kiselyov for key suggestions on ion-exchange resins. In addition we thank Sherri Lee, Wade Russu, Marcus Strawn, Tom Huang, and Paul Johnston for implementation of library synthesis and Ying Luo for assistance on NMR.

Supporting Information Available: Characterization data for all compounds, including 400 and 500 MHz ^{1}H NMR and ^{13}C NMR data and spectra, FT-IR, GC-MS, and HR-MS data, compounds **1**−**14** (66 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO971347W