Report

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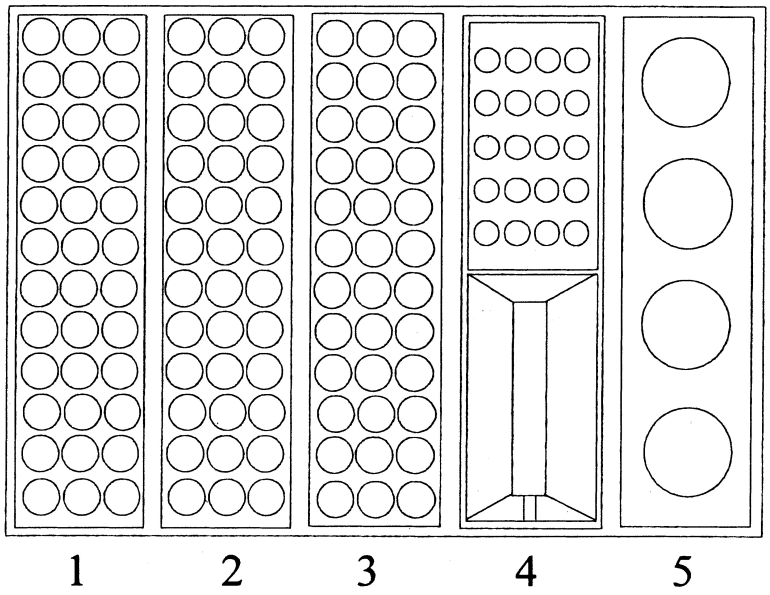

AutoChem: Automated Solution-Phase Parallel Synthesis and Purification via HPLC

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The advent of combinatorial techniques has had a tremendous impact on the way in which drug discovery is approached.[1] Combinatorial chemistry has been chiefly directed toward lead finding; its application to lead optimization has only recently gained attention.[2] Lead optimization demands the purest forms of the chemical entities for reliable determination of structure−activity relationships (SAR) and pharmacological properties. Significant effort has been ongoing to increase the speed and efficiency at which chemical reactions are performed. Automated solution-phase synthesis has been pioneered by Fuchs and co-workers[3] and reviewed by Lindsey.[4] Recently Kuwahara,[5] Lawrence,[6] and Whitten[7] have also reported methods for automated solution-phase syntheses. To date, these efforts have not effectively combined the synthesis and purification steps into a workable solution for automation. We report herein a practical method for performing parallel solution-phase synthesis with automated on-line purification on one HPLC system, and we have called this technique AutoChem. The key benefits of AutoChem are ease of use, purity of products, and facile sample tracking through the use of standard HPLC software for product purification.

One of the caveats of solution synthesis is that, normally, various workup techniques are required prior to the purification of compounds. Since the chemistry described herein is performed directly on an HPLC system, this bottleneck is circumvented by directly purifying reactions after they are complete. Our use of automated solution-phase parallel synthesis is geared toward the facile optimization of lead structures once the synthetic steps are optimized using traditional methods. In this manner, rapid generation of purified analogues can be used for reliable biological profiling. We have focused on solution-phase chemistry because it allows shorter development times compared to solid-phase synthesis. This can be accomplished because the XY-robot used to perform the chemistry is an HPLC autoinjector and the software for the HPLC system can directly access the reaction vessels after the preprogrammed reaction time has elapsed.[8] Chemistry using the AutoChem system is normally performed 16 reactions at a time on a 50−250 μmol scale. On the 50 μmol scale, 7−15 mg of purified products is isolated, whereas 40−80 mg can be realized on the 250 μmol scale. Typically, Wisp vials (4 mL capacity) capped with PTFE-faced silicone-lined septa/open-top polypropylene closures are used for reagent solutions and reactions. The autoinjector has room for up to five racks, as illustrated in Figure 1. The chemistry performed on the

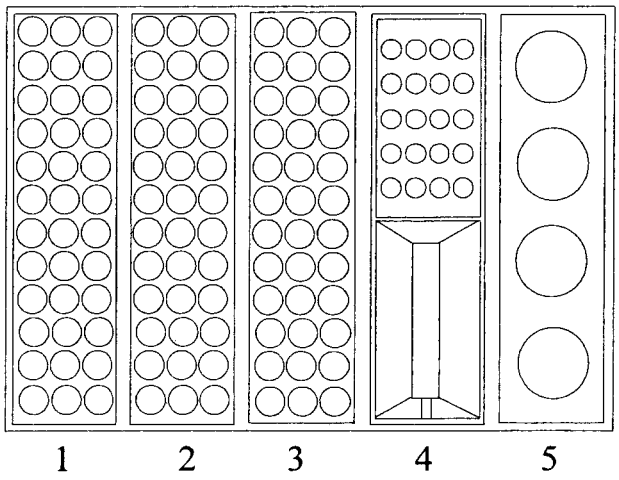

Figure 1. Layout of the autoinjector workbed.

AutoChem system is organized by using the first rack for diverse reagents, the second rack for starting materials and reagents common to all reactions (or a second set of diversity reagents), and the third rack for reaction mixtures. The fourth and fifth racks are utilized for solid-phase extraction prior to column loading as necessary. The fifth rack can alternatively hold 14 scintillation vials for common reagents. Large quantities of common reagents can be delivered from this rack.

Parallel solution-phase reactions are set up by the HPLC autoinjector via the use of five simple pipetting routines[9] combined with a wait step. This strategy provides a simple user interface, which we desired so that various chemistries could be automated without the need for reprogramming any software. Two pipetting routines, AddCom[10] and AddMix, were written which transfer common reagents from the starting material/common reagent rack (position 2) to the reaction rack (position 3). The AddCom routine serves to charge each reaction vessel with starting material solution with no needle rinsing since all the vessels receive the same reagent. The AddMix routine aspirates common reagent and dispenses to one vial at a time, mixing each reaction and rinsing the needle between vials. Similarly, two routines for pipetting diversity reagents from rack 1 to the reaction rack were developed. The AddDiv routine adds diversity reagent with mixing, while the DivAdd routine functions without mixing. This latter step is desirable when the diverse reagent is required to be the first reagent added to the reaction vessel. A fifth routine was also added, Matrix, which allows for easy setup of reactions involving multiple starting materials and diversity reagents. An example of such a setup would be the reductive amination of a set of aldehydes with a set of amines (vide infra).

Purification of reaction mixtures was accomplished on C-8 columns (YMC) using a generic gradient with acetoni-

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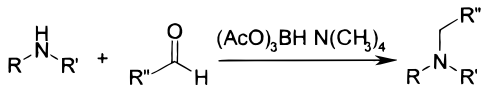
Scheme 1



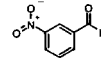
trile and water (0.025% TFA).[11] Typically the column size is 20 × 50 mm for the 50 μmol scale chemistry, while the larger scale requires columns of 30 × 75 mm. In all cases, the best packing material in our experience is of 5 μm particle size. The choice of C-8 over C-18 was made due to the tendency of many samples to elute either too early or too late in the gradient. Using this gradient method with the C-8 column resulted in substantially more of the samples eluting during the gradient, providing optimal use of the chromatographic conditions.

To highlight the utility of this technique, the Borch reduction, Scheme 1, was selected as a test case. This reaction was chosen because it would challenge the system, since purification of related compounds would be required. Several reducing agents and conditions were profiled including sodium borohydride, sodium triacetoxyborohydride,[12] and triethyl borate with lithium borohydride ($LiBH_4$). We have experienced difficulties transferring suspensions of sodium triacetoxyborohydride such that reactions with this reagent gave variable results including incomplete reactions. In some cases considerable bis-alkylated product and the alcohol from the reduction of the aldehyde were also isolated. Measures such as grinding the reagent to a fine powder and filtration of the suspension through glass wool did not preclude these difficulties. None of these problems were observed when tetramethylammonium triacetoxyborohydride[13] (Aldrich) was utilized in the reactions instead. The solubility and reactivity differences between these two reagents have been observed in directed reductions of β-hydroxy ketones.[14]

Table 1 summarizes our results using tetramethylammonium triacetoxyborohydride to prepare 32 secondary and tertiary amines from four primary and secondary amines and eight aldehydes, respectively. All of these 32 individual reactions were performed and the products purified by automating two runs of 16 reactions, with each run being a (4 × 4) matrix of amines and aldehydes, set up using the Matrix routine. As illustrated in Table 1, the yields of these reactions varied from ~20% to ~90%. Ninety percent of the compounds were isolated with >99% purity as determined by GC-MS. ^{1}H NMR spectral analysis was used to substantiate the purity determination. With one exception, the remaining compounds were determined to be ~95% pure. In some cases, bis-alkylated byproducts were observed in small amounts and also isolated in pure form from the chromatography. In one of the reactions only bis-alkylated byproduct was isolated.

We have illustrated the first example of a fully automated solution-phase parallel synthesis method including online product purification, AutoChem. The versatile generic pipetting routines, user-friendly software, and simple organization by racks of common reagents, diversity reagents, and reaction vessels allows the chemist to perform different chemistries in a straightforward fashion. The preparation of 32 pure products from Borch reductions in one week exemplify the utility of this method.

Table 1. Yields[a] and GC Purity of Borch Reductions Using AutoChem

R'-CHO \ R—NH$_2$				
	16 (99)	53 (96)	62 (99)	42 (91)
	32 (93) [58]	34 (99) [59]	79 (99)	89 (99)
	63 (99)	[66]	76 (99)	93 (97)
	76 (97)	53 (99) [39]	74 (99)	72 (99)
	76 (99)	51 (99) [19]	45 (99)	68 (99)
	38 (99)	42 (99) [24]	63 (99)	53 (99)
	70 (96)	40 (99)	36 (99)	57 (99)
	74 (60)	45 (99)	76 (99)	77 (99)

[a] Except for the aniline products, all yields are based on TFA salts of the desired amine product, as determined by ^{1}H NMR. Numbers in brackets indicate the yield of dialkylated byproduct.

Above all, AutoChem eliminates the bottleneck involved in the generation of pure compounds using solution-phase chemistry. Currently, this technique is being employed in our in-house drug discovery programs. Efforts are also underway to expand the possible chemistries that may be amenable to this method. Results of these efforts will be communicated in due course.

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Supporting Information Available. In depth description of the pipetting methods and GC-MS and ^{1}H NMR data for all compounds prepared. This material is free of charge via the Internet at http://pubs.acs.org.

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(8) The system used is a Gilson HPLC setup consisting of the following devices: An Aspec XL autoinjector, a 305 pump, a 306 pump, a 204 fraction collector and a 119 dual wavelength UV detector. The software used to organize the chemistry routines and then the purification is the Gilson Unpoint 1.7 system (or later). Total cost of the system was $75,000.
(9) The routines described herein were written in Pascal, using the 719 developers kit, which is available from Gilson, Inc.
(10) The AddCom routine performs the following operations: For *n* reactions and *m* μL of solution needed per reaction, $n \times m$ μL of solution is aspirated from the common reagent rack in one step and then *m* μL is dispensed equally into each of *n* vials in the reaction rack.
(11) Mobile phase: A = 0.1% TFA in acetonitrile; B = 0.025% TFA in water. Gradient: 10% A for 3 min; 10% to 90% A during 6.7 min; 90% A for 4.6 min; 90% to 10% A in 0.7 min. For an example of a gradient similar to this which was used recently for high throughput library purification, see: Zeng, L.; Wang, X.; Wang, T.; Kassel, D. B. *Comb. Chem. High Throughput Screening* **1998**, *1*, 101−111.
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