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Rapid Microscale Synthesis: Solution Phase Parallel Synthesis of a Library of Piperazines and Piperidines Using a Water Soluble Base

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Abstract: A library of 1086 piperazines and 835 piperidines was prepared *via* Rapid Microscale Synthesis (RMS) using the water soluble base 1,1,3,3-tetramethylguanidine to catalyze the reactions and to simplify purification.

With the rapid development of automated high-throughput screening, combinatorial chemistry and parallel synthesis have attracted considerable attention in recent years as a tool to accelerate the drug discovery process.[1] Techniques for the rapid synthesis of analogs on a solid support have received considerable recent attention as a method to enhance the discovery of new drugs.[1-4] Initially, the major emphasis was on the synthesis of peptide libraries. Methods used to obtain these libraries have been automated in many cases and include synthesis on beads,[5] chips,[6] and pins.[7,8] With the disclosure of the synthesis of small organic molecules on solid support by Bunin and Ellman[3] and the subsequent report of the "diversomers" approach by DeWitt and co-workers,[4] a significant effort toward the discovery and optimization of biological activity using the solid phase approach has shifted to small organic molecules.[9-13] Also the synthesis of "peptoids" has received considerable recent attention.[14] These approaches rely on the attachment of a starting material or a core organic molecule to a solid support (bead) with a linker before performing organic reactions and, in most cases, removal of the product from the bead before biological testing. The attachment of the product to the beads is advantageous in that excess reagents used in the reaction can be removed by filtration of the beads before removal of the product from the bead, thus providing a convenient purification procedure. The removal of the linker from the product,[12, 15] or utilization of the linker as part of the product,[4] has been addressed in some very elegant ways. DeWitt has recently reviewed the status of automated instrumentation for solid phase synthesis of small organic molecules.[16]

In contrast to solid phase synthesis of large numbers of small organic molecules, solution phase synthesis of libraries has received substantially less attention and many libraries prepared by this method are obtained as mixtures of compounds.[17, 18] Recently, soluble polymer supports have been reported as a promising approach for solution phase synthesis followed by precipitation of the polymer and subsequent removal of the product from the polymer.[13] Automated solution phase organic synthesis has focused on the synthesis of single compounds (as opposed to mixtures) and was pioneered by Fuchs and co-workers[19] for optimization of reaction conditions with a Zymark robot.

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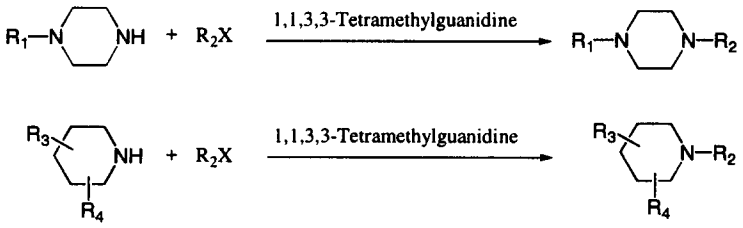
Piperazines and piperidines form a backbone to many interesting biologically active molecules.[20] This has led to several library syntheses of piperazine and piperidine containing compounds using both solid phase[21] and solution phase techniques.[22] However, these libraries are small. We wanted to generate larger libraries by solution phase synthesis with sufficient quantities of the end compounds for multiple biological screens. An important criteria for these libraries was that they should consist of single compounds that could be used in multiple screens. We also set a criteria for these libraries that they should consist of single compounds with purities which are in excess of 70%. We have recently reported successful optimization of a Corticotropin Releasing Factor (CRF) receptor lead heterocyclic compound using a solution phase Rapid Microscale Synthesis (RMS) automated technique that generated hundreds of compounds and have expanded this to other heterocycles.[23] Herein, we report the generation of a large library of N-alkyl and N-acylpiperazines and piperidines using RMS. To enhance the purification process for the automated synthesis, we used a base that was not only effective for catalyzing the reaction but additionally was easily removed by liquid/liquid extraction. The use of conventional bases such as diisopropylethylamine was handicapped by its poor extraction into water. 1,1,3,3-Tetramethylguanidine was found to catalyze the alkylations and acylations very cleanly and was completely removed from the product by a water extraction.

The synthetic route to prepare alkylpiperazines and alkylpiperidines is outlined in Scheme 1. A modified version of the HP7686 Prep Station was required to carry out the automated synthesis and workup of the products.[23] Reaction and workup conditions were programmed on a PC computer terminal using a Windows®-based program. All additions were performed by the robots in series, but reactions were run in parallel in groups of 50 per robot.



X=Br, Cl

Scheme 1

Starting materials were commercially available. Piperazine and piperidine compounds were made as 0.5 M stock solutions in either toluene or toluene/DMF. The alkyl halides were made as 1 M stock solutions in toluene. Diisopropylethylamine was initially used as the base required for the reaction, but was found to be difficult to remove from the reaction products. However, by using the water soluble base 1,1,3,3-tetramethylguanidine, purification of the product and removal of the base by liquid/liquid extraction was readily accomplished with the robot. Boger[24] and ourselves[23] have reported liquid/liquid extraction as a

method to purify reaction products prepared by parallel synthesis, but we believe that this is the first report of the specific use of 1,1,3,3-tetramethylguanidine as a water soluble base for parallel synthesis.

All reactions were carried out in 1.8 ml reaction vials on 0.05 mmol scale and provided 10-20 mg of final products. For a typical reaction, 100 μl of the starting piperazine or piperidine was transferred by the robot from a stock solution into the reaction vial, followed by the addition of 60 μl alkyl halide (1.2 equivalents) and 8 equivalents of 1,1,3,3-tetramethylguanidine. The reaction mixtures were stirred and heated at 90 °C on the robot's tray overnight. After the reactions were complete, the robot dispensed EtOAc to each reaction vial and performed a water wash. The organic layers were transferred to new tared product vials. Evaporation of the solvent by a speed-vac gave the final products. All the library products were checked by TLC and were homogeneous. About 5% of the products were randomly selected for analysis by GC-MS, and most of the products were greater than 70% pure as estimated by the area under the curve for the GC trace. It should be emphasized that liquid/liquid extraction removed excess base by the water wash. Trace amounts of alkyl halide were detected in only a few of the final products obtained from the alkylation reactions by GC-MS analysis. 1.2 equivalents of alkyl halide were required for reactions to proceed to completion and the speed-vac portion of the reaction work up removed any volatile alkyl halide. It was observed on optimization of the reaction conditions that reactions generally did not proceed to completion with only 1.0 to 1.1 equivalents of alkyl halide.

A total of 712 alkyl piperazines and 543 alkyl piperidines were prepared. Table 1 summarizes some of the piperazines and piperidines and alkyl halides used in the reaction and the product purity as estimated by GC-MS. In general, the alkylating reagents with electron-donating groups on the aromatic ring gave better yields than the alkylating agents with electron-withdrawing groups.

Table 1. Examples of the alkylation reaction for the generation of a library of piperazines and piperadines.

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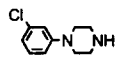
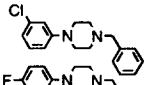
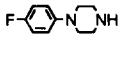
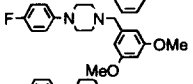
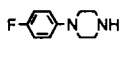
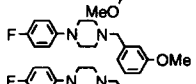
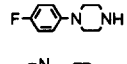
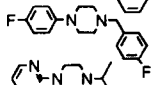
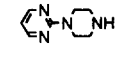
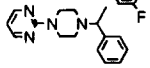
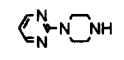
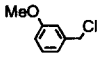
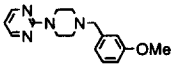
			100
			78
			85
			77
			82
			95

Table1(continued)

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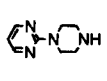
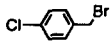
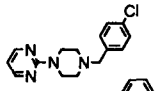
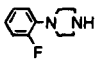
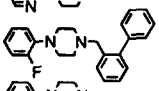
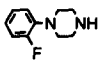
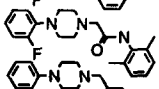
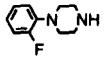
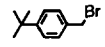
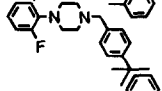
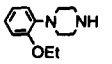
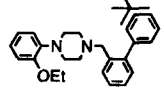
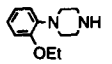
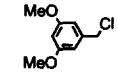
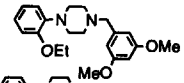
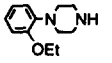
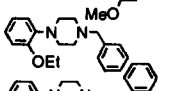
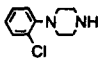
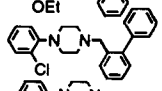
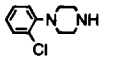
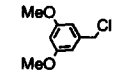
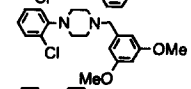
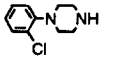
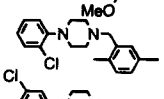
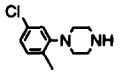
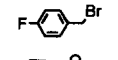
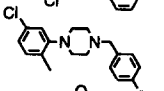
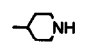
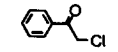
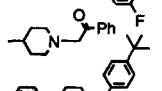
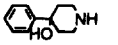
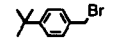
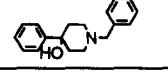
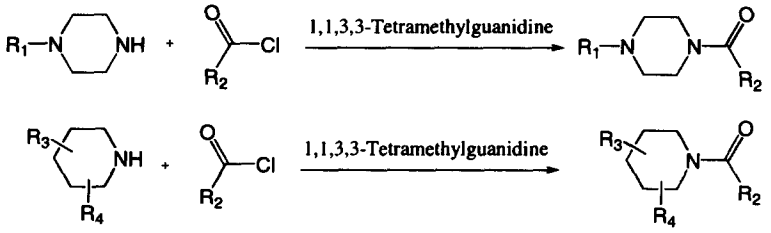
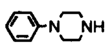
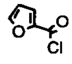
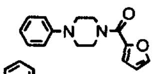
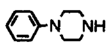
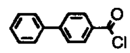
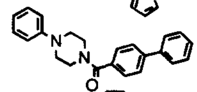
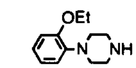
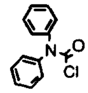
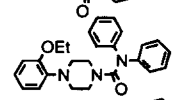
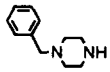
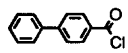
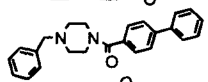
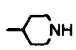
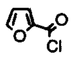
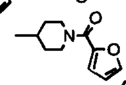
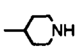
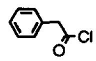
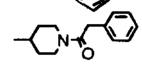
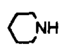
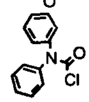
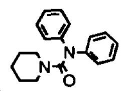
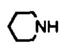
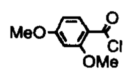
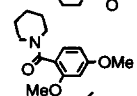
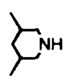
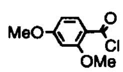
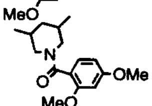
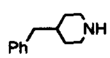
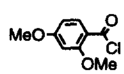
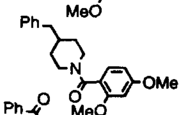
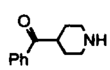
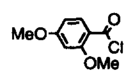
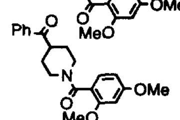
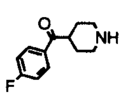
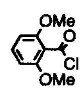
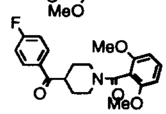
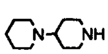
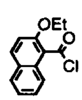
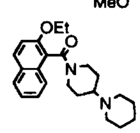
			72
			72
			85
			77
			79
			100
			96
			71
			94
			100
			98
			100
			97

[a] Based on the GC-MS data



Scheme 2

Table 2. Examples of the acylation reaction for the generation of a library of piperazines and piperadines.

Substrate	Acid Chlorides	Products	Purity (%)[b]
			72
			85
			96
			100
			100
			100
			100
			100
			100
			85
			85
			96
			95

[b] Based on GC-MS data

In the case of the acylation reaction sequence (Scheme 2), the same reaction conditions were initially tried as were used for alkylation but the purities of the products were not satisfactory. However, by reacting the starting piperidine or piperazine and an acid chloride (1:1.2 ratio) and 8 equivalents of 1,1,3,3-tetramethylguanidine in anhydrous toluene at room temperature (instead of 90 °C) overnight with stirring, the reactions proceeded efficiently and in high yield. The reactions were purified by washing with 1 M Na_2CO_3 to remove excess acid chloride and with water to remove 1,1,3,3-tetramethylguanidine. Table 2 summarizes a portion of the piperazines and piperidines and acid chlorides used for RMS, and the purity of the product as estimated by GC-MS analysis. A total of 374 acyl piperazines and 292 acyl piperidines were prepared for the library.

In summary, a robotics-driven solution phase method was developed for the parallel synthesis of alkyl and acylpiperazines and piperidines using RMS. The water-soluble base 1,1,3,3-tetramethylguanidine catalyzed the reactions and was readily removed from the products by liquid/liquid extractive workup carried out by the robot. Additional work is ongoing to utilize RMS for the synthesis of pure compound libraries.

Experimental

All the starting materials were commercially available. The standard 1.8-ml reaction vials were purchased from Hewlett-Packard or National Scientific Company. All GC-MS data were performed on a Hewlett-Packard 5890 GC and 5972 MS detector.

The Prep Stations were modified by heating two of the sample trays with hot water from a Heating Bath Circulator. This allows as many as 50 reactions to be heated to 90 °C at any one time per robot. The original Prep Station was upgraded with an additional 10 heating positions that are capable of heating to 125 °C. The Prep Station was interfaced to a HP5890 GC with a 30-m capillary column (packed with crosslinked 5% Phenyl Methyl Silicone) and a HP5972 EI MS detector. The interface programs used were HP Bench Supervisor (version A4.02) and HP Chem Station with sample delivery *via* the HP 7673A Sample Tray and HP 18593B Autoinjector in the standard setups. Data listing the sample identity, reaction conditions, GC Chromatogram and percent purity for each sample is automatically reported from the HP Chem Station software.

General Procedure for alkylation of piperidines and piperazines : The robot transferred 100 µl 1-(2-chlorophenyl)piperazine (0.05 mmol) from 0.5 M stock solution in toluene (REAGENT1) to a reaction vial (RXNVIAL), and then transferred 60 ul 2,5-dimethylbenzylchloride (0.06 mmol) from a 1 M stock solution (REAGENT2) and 50 µl (50 mg, 0.4 mmol) 1,1,3,3-tetramethylguanidine (REAGENT3) to the same reaction vial. The vial was picked up by the robot arm and mixed for two minutes and heated at 90 °C for 15 hours. After the reaction, the robot dispensed 1.0 ml EtOAc and 0.45 ml water to the reaction vial. After the rapid mixing, the organic layer was transferred to a product vial and dried under a speed-vac to give N-2- chlorophenyl-N'-2,5-dimethylbenzylpiperazine (13 mg, 83%); ^1H NMR ($CDCl_3$) δ 2.32 (s, 3H), 2.35 (s, 3H), 2.65 (broad t, 4H), 3.06 (broad t, 4H), 3.51 (s, 2H) 6.85-7.40 (m, 7H). Anal. Calcd. for $C_{19}H_{23}N_2Cl$: C, 72.48; H, 7.36; N, 8.90. Found: C, 72.25; H, 7.31; N, 8.68.

The Prep Station runs Bench Method or Bench Sequence to control the actions of the robots. A Bench Method consists of a series of instrument methods and a Bench Sequence consists of a series of Bench Methods. The following is the instrument method the robot performed to run the above reaction:

1) Rinse_System with 5.000 ml of Ethanol using Entire System Flow Path.
2) Rinse_System with 5.000 ml of Toluene using Entire System Flow Path.
3) Transfer 0.100 ml from REAGENT1 to RXNVIAL and 0.010 ml from Sample Loop.
4) Transfer 0.060 ml from REAGENT2 to RXNVIAL and 0.010 ml from Sample Loop.
5) Transfer 0.050 ml from REAGENT3 to RXNVIAL and 0.010 ml from Sample Loop.
6) Mix RXNVIAL at Medium speed for 2 minutes.
7) Heat RXNVIAL at 90 deg C for 900 minutes.
8) Wait for 10.00 minutes.
9) Rinse_System with 3.000 ml of Ethanol using Entire System Flow Path.
10) Rinse_System with 3.000 ml of Water using Entire System Flow Path.
11) Dispense 0.450 ml of Water into RXNVIAL.
12) Dispense 1.000 ml of EtOAc into RXNVIAL
13) Mix RXNVIAL at Medium Speed for 2 minutes.
14) Transfer 1.100 ml from RXNVIAL to Product Vial and 0.05 ml from Sample Loop.
15) Rinse_System with 5.000 ml of Ethanol using Entire System Flow Path.

General Procedure for acylation of piperidines and piperazines : The robot transferred 100 µl 1-(2-ethoxyphenyl)piperazine (0.05 mmol) from a 0.5 M stock solution in toluene (REAGENT1) to a reaction vial (RXNVIAL), and then transferred 60 µl diphenylcarbamyl chloride (0.06 mmol) from a 1 M stock solution (REAGENT2) and 40 µl (35 mg, 0.4 mmol) 1,1,3,3-tetramethylguanidine (REAGENT3) to the same reaction vial. The reaction vial was mixed at room temperature for 15 hr. After the reaction, the robot dispensed 1.0 ml EtOAc and 0.45 ml BASE (1 M Na_2CO_3) to the reaction vial. After rapid mixing, the organic layer was transferred to a Wash Vial and washed with 0.5 ml water. The organic layer was transferred to a tared Product Vial and dried under a speed-vac to give N-2-ethoxyphenyl-N'-diphenylcarbamyl piperazine (17 mg, 85%). ^{1}H NMR ($CDCl_3$) δ 1.42 (t, J=7.2 Hz, 3H), 2.94 (t, J=5.4 Hz, 4H), 3.56 (t, J=5.4 Hz, 4H), 4.03 (q, J=7.2 Hz, 2H), 6.8-7.4 (m, 14H). Anal. Calcd. for $C_{25}H_{27}N_3O_2$: C, 74.79; H, 6.78; N, 10.47. Found: C, 74.49; H, 7.00; N, 10.44.

The program the robot performed to run the above reaction is as follows:

1) Rinse_System with 5.000 ml of Ethanol using Entire System Flow Path.
2) Rinse_System with 5.000 ml of Toluene using Entire System Flow Path.
3) Transfer 0.100 ml from REAGENT1 to RXNVIAL and 0.010 ml from Sample Loop.
4) Transfer 0.060 ml from REAGENT2 to RXNVIAL and 0.010 ml from Sample Loop.
5) Transfer 0.040 ml from REAGENT3 to RXNVIAL and 0.010 ml from Sample Loop.
6) Mix RXNVIAL at Medium speed for 2 minutes.
7) Wait for 900 minutes.
8) Rinse_System with 3.000 ml of Ethanol using Entire System Flow Path.
9) Rinse_System with 3.000 ml of Water using Entire System Flow Path.

10) Dispense 0.500 ml of BASE into RXNVIAL.
11) Rinse_System with 3.000 ml of Water using Entire System Flow Path.
12) Dispense 1.000 ml of EtOAc into RXNVIAL
13) Mix RXNVIAL at Medium Speed for 2 minutes.
14) Transfer 1.100 ml from RXNVIAL to WASHVIAL and 0.05 ml from Sample Loop.
15) Dispense 0.500 ml of Water into WASHVIAL.
16) Mix WASHVIAL at Medium Speed for 2 minutes.
17) Tranfer 1.100 ml from WASHVIAL to PRODUCT and 0.05 ml from Sample Loop.
18) Rinse_System with 5.000 ml of Ethanol using Entire System Flow Path.

References and Notes

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