Case Study 6-3

Solid-Phase Parallel Synthesis of a Large Array of Tetrahydroisoquinolinones

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ABSTRACT

An array of 768 tetrahydroisoquinolinones was prepared using a tilted-plate centrifugation technique. This technique allows for parallel processing of shallow-well microtiter plates used as a reaction vessel. Automated instrument simplifies the tedious task of manual distribution of building blocks and plate washing.

Chemicals

1. *N,N*-Dimethylethylenediamine
2. Benzylamine
3. 3-(Trifluoromethyl)benzylamine
4. Cyclopropylamine
5. Propylamine
6. Acethydrazide
7. Methyl hydrazinocarboxylate
8. Piperazine
9. Benzaldehyde
10. 1-Naphthaldehyde
11. o-Anisaldehyde
12. 2-Pyridinecarboxaldehyde

13. 3-Phenoxybenzaldehyde
14. 4-(3-Dimethylaminopropoxy) benzaldehyde
15. 4-(Methylthio)benzaldehyde
16. 4-Biphenylcarboxaldehyde
17. 4-Bromobenzaldehyde
18. 6-Methyl-2-pyridinecarboxaldehyde
19. 3,4,5-Trimethoxybenzaldehyde
20. 4-Ethylbenzaldehyde
21. 1-(2-Aminoethyl)pyrrolidine
22. Pyrrolidine
23. *N,N*-Dimethylethylenediamine
24. *N,N,N′,N′*-Tetraethyldiethylenetriamine
25. *N,N*-Diethyl-*N′*-methylethylenediamine
26. *N,N,N′*-Triethylethylenediamine
27. 1-(3-Aminopropyl)-2-pipecoline
28. 4-(Trifluoromethyl)benzylamine
29. Diisopropylcarbodiimide
30. Bromophenol blue
31. *N*-[(Dimethylamino)-1H-1,2,3-triasol[4,5-b]pyridylmethylene]-*N*-methylmethanammonium hexafluorophosphate *N*-oxide (HATU)
32. *p*-Methylbenzhydrylamine resin
33. Trimethylorthoformate (TMOF)
34. Homophthalic anhydride
35. *N*-Hydroxybenzotriazole (HOBt)
36. *N,N*-Dimethylaminopyridine (DMAP)
37. *N,N*-Dimethylformamide (DMF)
38. Di-isopropylethylamine (DEA)
39. Di-isopropylcarbodiimide (DIC)
40. Dimethylsulfoxide (DMSO)

Equipment and Supplies

1. Intelligent centrifuge Compas 768.2
2. Eppendorf Pipettman (1 mL)
3. Multichannel pipetor
4. Gene Vac centrifugal evaporator or lyophilizer
5. Platform shaker
6. Automatic pipetor Multiprobe 104 and 208
7. Polypropylene HF chamber
8. HF apparatus

I. INTRODUCTION

After the identification of a hit from large "screening" library, the logical next step is the "hit explosion"—synthesis of a large number of compounds with structure similar to the original which were not included in the original screening library. We have developed the technique of tilted centrifugation [1], which allows synthesis of an array of up to 768 members in one batch.

The principle of tilted centrifugation is shown in Fig. 1. A resin-containing vessel is attached in the tilted position at the perimeter of the centrifugal plate and spun. Resin, which has sedimented at the bottom of the vessel, does not remain at the bottom of the flask. As the surface of liquid supernatant moves, the solid support layer moves as well. If the speed of rotation is increased, the centrifugal force created by rotation (which depends on the radius of rotation and the speed) combines with gravitation and the resulting force causes the liquid surface to stabilize at an angle perpendicular to the resulting force vector. At the ratio of relative centrifugal force (RCF) to G of 3, the angle of the liquid surface will be about 61 degrees. If the speed is increased so that the ratio of these forces is more than 50, the situation is close to the RCF of infinity; therefore, the liquid (and resin layer) angle will be close to 90 degrees. The pocket created by the tilt now allows only solid phase to remain in the pocket and all of the liquid is expelled.

We have built the computer-driven centrifuge with eight positions for tilted microtiter plates. A 96-channel distributor connected to six-port selector valve performs the delivery of washing solvents and common reagents. The centrifuge

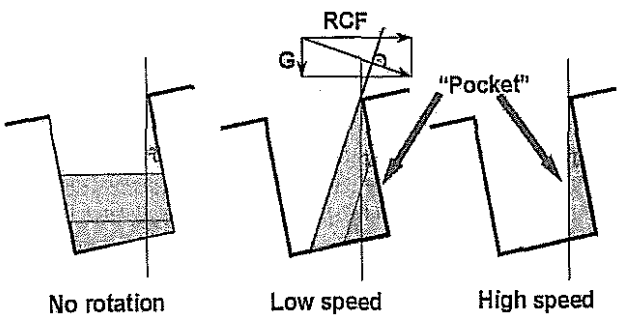
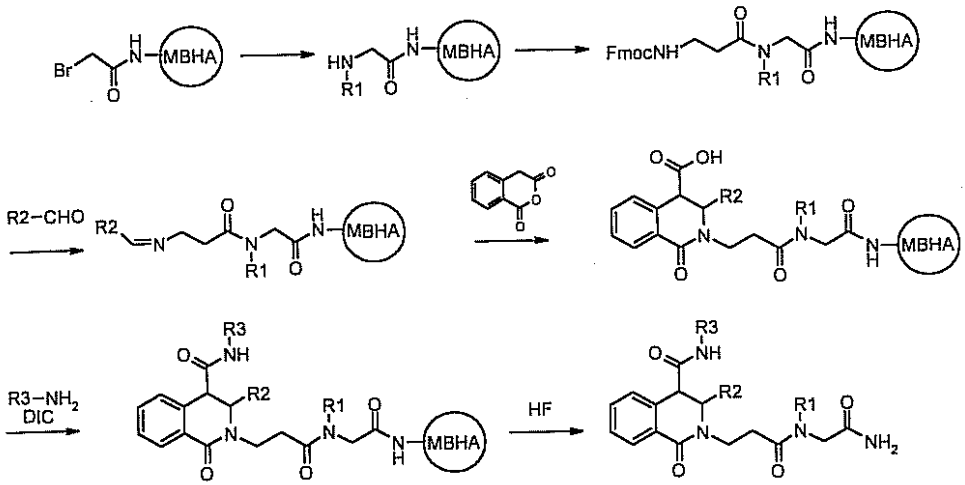


Figure 1 Principle of tilted-plate centrifugation. Formation of the pocket in the well of a tilted plate during centrifugation (direction: left to right). The solid support (lower layer) is collected in the pocket, while the liquid (upper layer) is expelled from the well. The liquid surface angle is perpendicular to the resulting force vector of the relative centrifugal force (RCF) and gravity (G).

Figure 2 Compas 768.2 integrated with Packard Canberra Multiprobe 104.

was integrated with the Packard Multiprobe 104 liquid distribution system for the delivery of individual building blocks and reagents. Inclusion of the pipetting system allows us to perform the whole synthesis in a completely automatic regimen. Figure 2 shows the view of this instrument.

The synthesis is performed in the following way. A microtiter plate with a slurry of solid support distributed into it is placed on the perimeter of a rotor with a permanent tilt of 9 degrees. The rotor is rotated at the speed required for complete removal of the liquid portion of the well content. After stopping the rotation, the microtiter plate is placed (rotor is turned) under the multichannel (96-channel) liquid delivery head. The solvent selector valve is turned into the appropriate position and the washing solvent is delivered by actuating the syringe pump. This operation is repeated until all plates are serviced. The rotor is spun at the speed at which the liquid phase is just reaching the edge of the well, thus wetting all solid support in the "pocket," and after reaching this speed rotation is stopped. The cycle of slow rotation and stopping is repeated, thus gently mixing the slurry of solid support in the liquid phase. After shaking for the appropriate time, the plates are spun at high speed. The process of addition and removal of washing solvent is repeated for as many washes as are required. The plates are then consecutively placed under the array of 96 openings in the centrifuge cover, and appropriate building block solutions and coupling reagents are delivered by



Scheme 1

pipetting (Multiprobe 104) through the openings from the stock solutions placed on the centrifuge cover. Alternatively, building blocks are delivered by manual pipetting with a multichannel pipetor from a trough or a prepared "master plate." This alternative is a faster option in the case where the number of building blocks used in the particular step is compatible with logical division of the microtiter plate into rows and columns (4,6,8,12), or when only one building block is distributed over the large part of the plate. When incubation at the elevated temperature is required, plates are removed from the centrifuge, stoppered with the cap mats, and incubated in the shaker oven. After the final wash and drying of the resin in the plate, cleavage can be performed by gaseous hydrogen fluoride (HF) as described in the communication about library synthesis [2].

As an example of the tilted-place centrifugation technique, we present here the explosion of the hit from large library synthesized by combination of tea-bag and surface suction techniques, presented earlier in this book [2]. The chemistry is illustrated in Scheme 1.

II. GENERAL PROCEDURE

The solid support (*p*-methylbenzhydrylamine resin, 1.1 mmol/g, 130 μm, Chem-Impex, Wood Dale, IL) was allowed to swell in DMF. The resin slurry was then distributed into the wells of eight polypropylene shallow-well microtiter plates (5 mg of resin per well). The microtiter plates were placed on the centrifugal

rotor in a tilted position (9-degree tilt) and solvent was removed by centrifugation at 350 rpm in the Compas 768.2 (Spyder Instruments, San Diego, CA) intelligent centrifuge. The resin was neutralized with a 5% DIEA/DMF solution and washed 6 times with DMF (the last wash contained 0.01% bromophenol blue for monitoring the subsequent acylation step [3]).

A. Attachment of Bromoacetic Acid and Formation of *N*-Substituted Glycine

After six additional DMF washes, a solution of bromoacetic acid (1 M) and DIC (1.2 M) in DMF (100 μL/well) was added, and the mixture was oscillated for 2 h. After the disappearance of blue coloration, the plates were washed 6 times with DMF, 4 times with DMSO, and a solution of an amine (amines 1–8, 1 M) in DMSO was added. The plates were shaken overnight and then washed once with DMSO and 5 times with DMF (last wash containing bromophenol blue).

B. Coupling of β-Alanine

A solution of Fmoc–β-alanine in 0.3 M HOBT and DIC was added (100 μL, 0.3 M). After disappearance of the blue coloration in plate wells not containing tertiary amine groups (these wells remain blue), the plates were washed 4 times with DMF and a solution of 50% piperidine in DMF was added. After 20 min of incubation, the plates were washed 5 times with DMF.

C. Schiff Base Formation and Cyclization

The aldehyde solutions (0.8 M) were combined with an equal volume of 1.6 M TMOF/DMF and added to the appropriate wells of each plate (100 μL). After 3 h incubation, the liquid was removed and two washes with 0.2 M TMOF/DMF were performed. A solution of homophthalic anhydride (75 μL, 0.4 M in DMF) with DIEA (0.03 M) was added and plates were shaken overnight. The liquid was removed and six washes with DMF were performed.

D. Coupling of Amine

A solution of HATU (0.3 M in DMF, 75 μL) was added to each well. After 20 min incubation, a solution of an appropriate amine (amines 21–28, 1 M in DMF, 75 μL) was added. After overnight incubation of closed microtiter plates on the shaker, the solution was removed by centrifugation and the process of preincubation with HATU and incubation with amine solution was repeated and allowed to react overnight once again. The solution was removed by centrifugation and

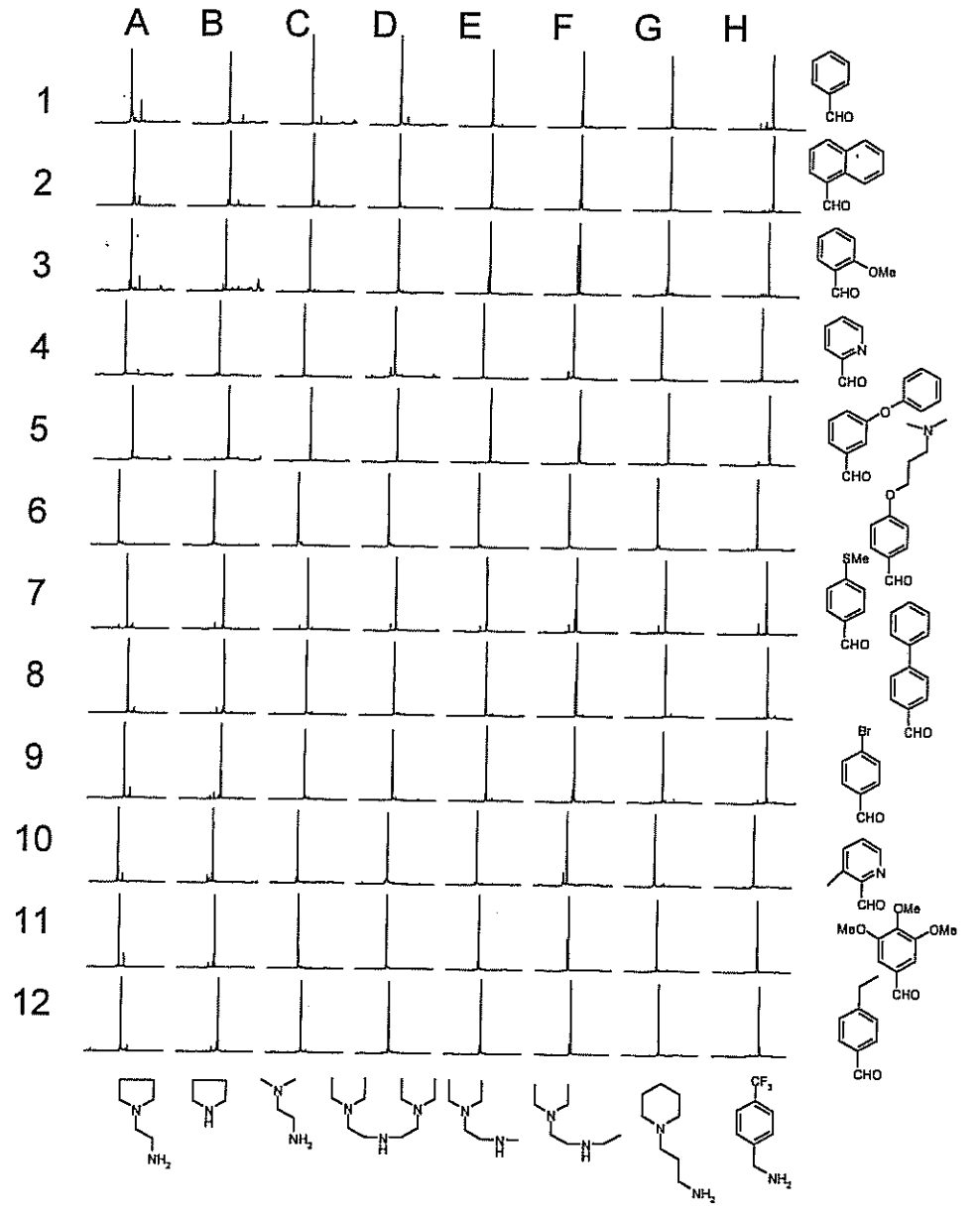


Figure 3 HPLCs of products from one plate from the synthesis of 768 tetrahydroisoquinolinones (R1 cyclopropylamine, R2 in rows, R3 in columns). Please note that products are diastereoisomers—in some cases separated by HPLC (column F).

all microtiter plates were washed with DMF 12 times and with *tert*-butyl methyl ether 6 times.

E. Cleavage from the Solid Support

The plates were air-dried for 3 days and placed in a polypropylene chamber [4]. The chamber was flushed with nitrogen for 30 min and then filled with gaseous hydrogen fluoride. After 2 h at room temperature, chamber was flushed with nitrogen overnight and the plates were removed and placed in the desiccator. After overnight evacuation the plates were placed onto the table of the Multiprobe 208 (Packard Canberra, Meriden, CT) and the solid support was extracted by repeated (4 times) addition and removal of 150 μL of acetic acid into the individual wells of the microtiter plate. The extracts were transferred to deep-well polypropylene microtiter plates, the content was frozen, and acetic acid was removed in the lyophilizer.

F. Postcleavage Analysis

All wells were analyzed by LC-MS. The average purity of the prepared compounds in plates 1–6 was 87%. Plates 7 and 8, in which hydrazine derivative was used in the first step, did not produce acceptable products. The HPLC traces of the compounds synthesized in one plate (plate 4, R1 cyclopropylamine) are shown in Fig. 3.

III. COMMENTARY

We have described the new technique for very simple separation of solid and liquid phases in solid-phase synthesis that uses standard polypropylene microtiter plates as reaction vessels. This technique allows the building of very simple instruments in which all filtration problems are avoided and which can be completely automated. The obvious limitation of this technique is the requirement that the solid support must sediment in the given solvent system; however, solvents of higher density than the used solid support can always be diluted or evaporated prior to the application of tilted centrifugation. Our enthusiasm for the new technique is best illustrated by the fact that we quit our jobs at Trega Biosciences, where this technology was developed, and are now commercializing the new synthesizer in our company, Spyder Instruments, dedicated to supplying every chemist with his own Compas 768.2. Application of this technology for smaller scale, higher density (up to the level of synthesis on individual beads), or larger scale, lower density synthesis is obvious.

ACKNOWLEDGMENT

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