HIGH-THROUGHPUT SYNTHESIS

Principles and Practices

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Case Study 6-2

Solid-Phase Parallel Synthesis of Large Tetrahydroisoquinolinone Library

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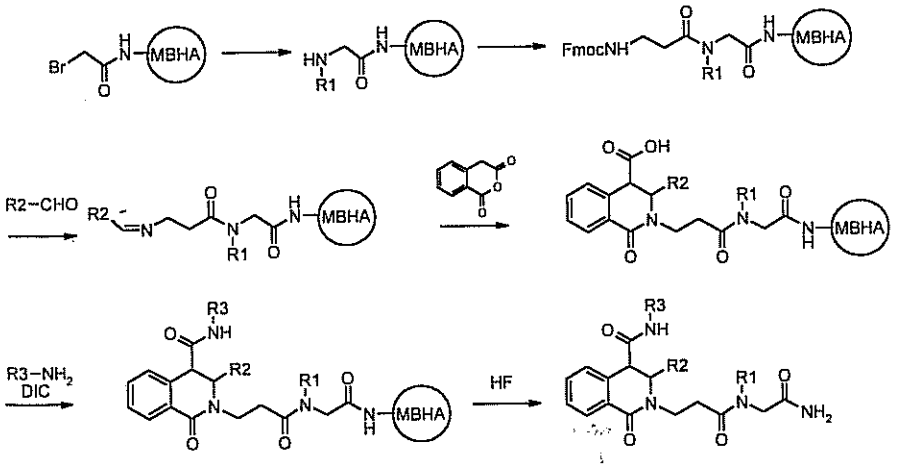
ABSTRACT

A library of 30,816 tetrahydroisoquinolinones was prepared using a combination of "tea-bag" synthesis and synthesis in microtiterplates with "surface suction" separation of solid and liquid phases.



Scheme 1

Chemicals

1. Bromoacetic acid
2. β-Alanine
3. Benzaldehyde
4. Salicylaldehyde
5. 1-Naphthaldehyde
6. 2-Chloro-5-nitrobenzaldehyde
7. 2-Chloro-6-fluorobenzaldehyde
8. 2-Cyanobenzaldehyde
9. 2-Imidazolecarboxaldehyde
10. *o*-Anisaldehyde
11. 2-Naphthaldehyde
12. 2-Pyridinecarboxaldehyde
13. 2-Quinolinecarboxaldehyde
14. Piperonal
15. 3,5-bis(trifluoromethyl)benzaldehyde
16. 5-Nitrovanillin
17. 3-Methylbenzaldehyde
18. 3-Nitrobenzaldehyde
19. 3-Phenoxybenzaldehyde
20. 3-Thiophenecarboxaldehyde
21. 4-(3-Dimethylaminopropoxy)benzaldehyde
22. 4-(Dimethylamino)benzaldehyde
23. 4-(Methylthio)benzaldehyde
24. Trifluoro-*p*-tolualdehyde
25. 4-Biphenylcarboxaldehyde
26. 4-Bromobenzaldehyde
27. 4-Hydroxybenzaldehyde
28. *p*-Tolualdehyde
29. 4-Propoxybenzaldehyde
30. 6-Methyl-2-pyridinecarboxaldehyde
31. 3,4,5-Trimethoxybenzaldehyde
32. 4-Ethylbenzaldehyde
33. *N*-(2-Aminoethyl)pyrrolidine
34. Pyrrolidine
35. *N,N*-Dimethylethylenediamine
36. 2-Methoxyethylamine
37. Cyclohexylamine
38. 1-Methylpiperazine
39. Tetrahydrofurfurylamine

40. Benzylamine
41. 3-Methylbenzylamine
42. 1-(3-Aminopropyl)imidazole
43. Tyramine
44. 2-Chlorobenzylamine
45. 4-Chlorobenzylamine
46. 3-(Trifluoromethyl)benzylamine
47. Cyclopropylamine
48. Propylamine
49. 4-(Aminomethyl)pyridine
50. Allylamine
51. Morpholine
52. 3-(Aminomethyl)pyridine
53. 2-Thiophenemethylamine
54. 4-(2-Aminoethyl)morpholine
55. 4-(Ethyleminomethyl)pyridine
56. 3-Methoxybenzylamine
57. 2-(4-Methoxyphenyl)ethylamine
58. 2,3-Dimethoxybenzylamine
59. 2,4-Dichlorophenethylamine
60. *N*,*N*-Diethyl-1,3-propanediamine
61. 3-Dimethylaminopropylamine
62. 1-(2-Aminoethyl)piperidine
63. *N*,*N*,*N*′,*N*′-Tetraethyldiethylenetriamine
64. Isoamylamine
65. Methylisobutylamine
66. 3-Ethoxypropylamine
67. Thiomorpholine
68. (*R*)-(−)-1-Cyclohexyethylamine
69. Isonipecotamide
70. *N*,*N*-Diethyl-*N*′-methylethylenediamine
71. 1,2,3,4-Tetrahydroisoquinoline
72. *N*,*N*,*N*′-Triethylethylenediamine
73. β-Alanine ethyl ester hydrochloride
74. 1-(3-Aminopropyl)-2-pipecoline
75. Ethyl-1-piperazinecarboxylate
76. 4-(Trifluoromethyl)benzylamine
77. 1-Benzylpiperazine
78. 1-(2-Furoyl)piperazine
79. L-Leucine methyl ester hydrochloride
80. 4-Bromopiperidine hydrobromide

81. 1-Bis(4-fluorophenyl)methylpiperazine
82. 4-(1-Pyrrolidinyl)piperidine
83. 3,3′-Dipicolylamine
84. Neopentylamine
85. *N*,*N*-Di-*N*-butylethylenediamine
86. Ethyl-3-aminobutyrate
87. 3-Butoxypropylamine
88. *N*-(3-Aminopropyl)morpholine
89. 4-*tert*-Butylcyclohexylamine
90. 4-Amino-2,2,6,6-tetramethylpiperidine
91. 2-Amino-5-diethylaminopentane
92. *N*-(2-Aminoethyl)-*N*-ethyl-*m*-toluidine
93. Dodecylamine
94. Acethydrazide
95. Methylhydrazinocarboxylate
96. 2-(2-Aminoethyl)pyridine
97. 2-(Aminomethyl)-1-ethylpyrrolidine
98. Piperazine
99. Diisopropylcarbodiimide
100. Bromophenol blue
101. HATU
102. *p*-Methylbenzhydrylamine resin
103. Trimethylorthoformate (TMOF)
104. Homophthalic anhydride
105. *N*-Hydroxybenzotriazole
106. *N*,*N*-Dimethylaminopyridine

Equipment and Supplies

1. Eppendorf Pipettman (1 mL)
2. Multichannel pipetor
3. GeneVac centrifugal evaporator
4. Yaskawa robotic platform
5. 96-channel distribution manifold
6. 96-channel suction manifold
7. Sealing device for sealing tea-bags
8. Platform shaker
9. Polypropylene bottles of various sizes
10. Automatic pipetor Multiprobe 104
11. 96-channel pipetor Quadra 96
12. Polypropylene HF chamber
13. HF apparatus

I. INTRODUCTION

As part of an ongoing drug discovery effort a procedure for the synthesis of large numbers of small heterocyclic molecules was needed. We have found the combination of manual synthesis of intermediates in "tea-bags" [1] followed by the synthesis in the wells of deep-well microtiterplates to be a very convenient method for preparation of large libraries. Hundreds of intermediates from tea-bags can be distributed into tens of thousands of wells which are processed on the robotic platform. The washing is achieved by "surface suction" [2]. However, it is not necessary to use the robot for the last step of the synthesis; manual washing is merely more tedious and time consuming. The cleavage from the solid support was accomplished by exposing the resin in the plates to gaseous hydrogen fluoride (HF) [3]. An example is the modification of the process presented earlier for the synthesis of mixture library [4].

II. GENERAL PROCEDURE

Solid support (*p*-methylbenzhydrylamine resin, 1.1 mmol/g, 130 μm, Chem-Impex, Wood Dale, IL) was distributed into polypropylene (mesh polypropylene, 100 mesh) bags (1.1 g) and sealed. Resin was swollen in dimethylformamide (DMF), and 780 bags were shaken for 20 min in 50% piperidine/DMF to neutralize the resin. The bags were then washed 4 times with DMF, once with 0.3 M HOBT/DMF, and then 4 more times with DMF (the last wash contained 0.01% bromophenol blue for monitoring the subsequent acylation step [5]).

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

A solution of bromoacetic acid in DMF (1 M and 8.7 equivalents) was added to DIC (1.2 M) and the mixture was shaken in a polypropylene flask for 1–2 h. After disappearance of the blue coloration, the bags were washed 3 times with DMF and separated into groups of 30 bags. Each group was washed twice with dimethylsulfoxide (DMSO) and the solution of an amine (1 M) in DMSO was added. The bags were shaken for 18–24 h, washed once with DMSO, and then 5 times with DMF (last wash containing bromophenol blue).

B. Coupling of β-Alanine

A solution of Fmoc–β-alanine was added (0.3 M and 3 equivalents) to DIC (0.3 M, 3 equivalents), HOBT (0.3 M, 3 equivalents), and a 1/10 equivalent of

DMAP. After disappearance of the blue coloration in bags not containing tertiary amine groups (these bags remain blue), the bags were washed 3 times by DMF and 4 times with dichloromethane. The bags were then laid out in the hood to air-dry. When the resin was dry, a small sample of resin from each amine group was taken, cleaved with HF, and analyzed. Only the tea-bags that had the expected material in a purity of 90% or greater were carried through to the next step (Fmoc deprotection). To remove the Fmoc group from the β-alanine, a solution of 50% piperidine in DMF was added. After 20 min of incubation, the bags were washed with DMF 5 times and separated into groups of 24 (3 amines in the R1 position did not pass quality control, total number of processed bags dropped to 690).

C. Schiff Base Formation and Cyclization

The appropriate aldehyde solutions (0.8 M in DMF) were combined with an equal volume of 1.6 M trimethylorthoformate (TMOF) in DMF and then were added to each group of 24 bags. After 3 h incubation, the liquid was removed and two washes with 0.2 M TMOF/DMF were performed. A solution of homophthalic anhydride (0.4 M in DMF and 5 ×) with DIEA (0.03 M) was added to each bottle and bags were shaken overnight. The liquid was removed and six washes with DMF were performed, followed by three washes with *t*-butyl methyl ether. At this stage the bags were dried again, and a sample from each bag was taken, placed into a well of a microtiterplate, and exposed to gaseous HF for 2 h. Samples were extracted and analyzed by LC-MS. Only the bags containing expected product in purity better than 85% (evaporative light-scattering detection) were taken to the next step of synthesis (48 bags were removed and 642 bags were processed further).

D. Coupling of Amine

In this step the content of each bag was divided into 48 wells of a deep-well microtiter plate. A set of "master plates" of 48 amine solutions (1 M in DMF) was created in deep-well microtiter plates by automatic pipetting from stock solutions (Multiprobe 104, Packard Canberra, Meriden, CT). A solution of HATU (0.3 M in DMF) was added to all wells of each plate by multichannel pipetting (Quadra 96, Tomtec), and after 20 min incubation, the appropriate amine solutions were added to each well by pipetting from the master plates (the HATU solution remains in the wells during this reaction). After overnight incubation of closed microtiter plates on the shaker, the solution was removed by surface suction and incubation with HATU and amine solution was repeated overnight, once again. The solution was removed by surface suction (Fig. 1) and microtiter plates

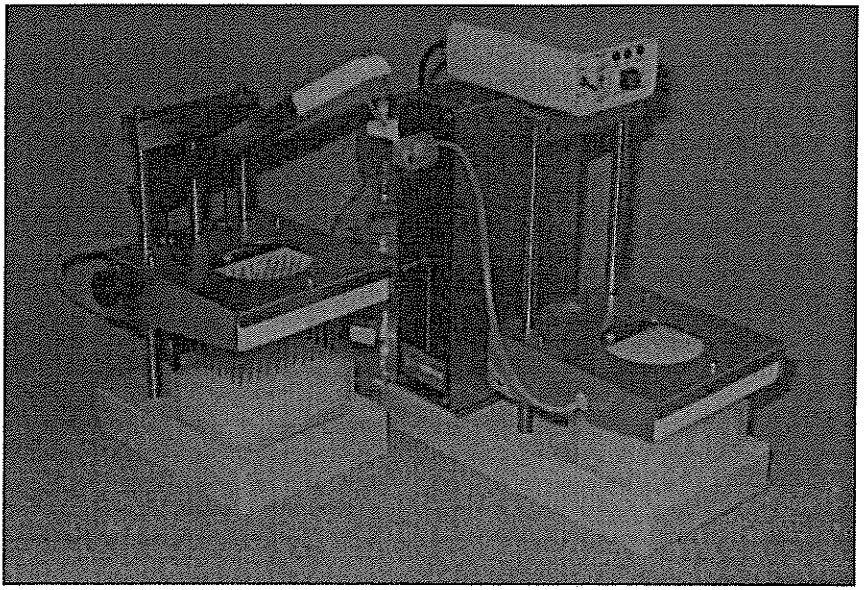


Figure 1 Manual 96-channel delivery (right) and suction (left) manifolds used for surface suction washing of solid phase distributed in microtiter plates.

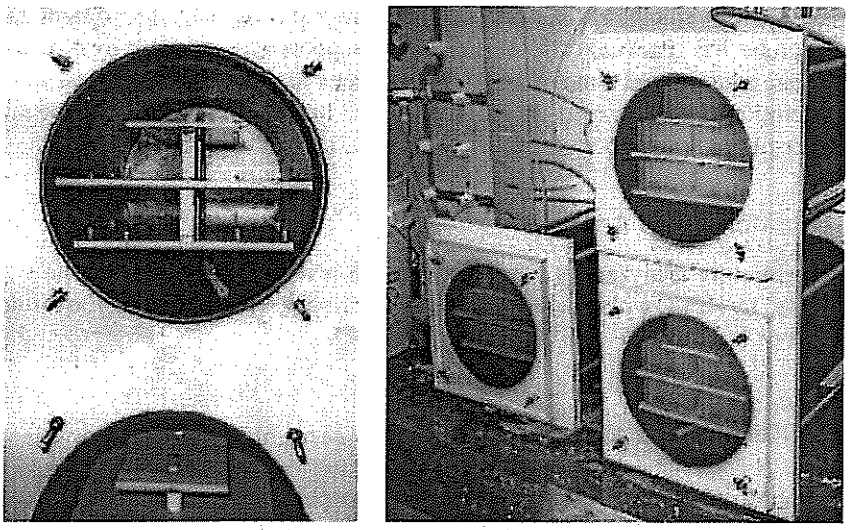


Figure 2 Polypropylene chambers used for cleavage of compounds from benzhydrylamine resin by exposure to gaseous hydrogen fluoride. The window is made of polymethacrylate covered with polypropylene foil.

Table 1 Purities of Tetrahydroisoquinolinones Synthesized by Combination of Tea-bag and Surface Suction Techniques[a]

Plate classification	Number of plates	MS (%)	HPLC (%)
QC passed	263	90.52	80.64
QC failed	58	48.63	78.49
Total	321	82.17	80.21

[a] MS, average percent of wells in the plate in which the correct molecular weight was identified; HPLC, average purity of 12 wells analyzed in each plate. QC = Quality Control.

were washed with DMF 10 times and with 50% *tert*-butyl methyl ether/DCM 4 times.

E. Cleavage from the Solid Support

The plates were air-dried for 2 days and placed in a polypropylene chambers (Fig. 2). The chamber was flushed with nitrogen for 30 min and then filled with gaseous hydrogen fluoride. After 2 h at room temperature, the chamber was flushed with nitrogen overnight, and plates were removed and placed in the desiccator. After overnight evacuation the plates were placed onto the table of the Multiprobe 208 (Packard Canberra) and solid support was extracted by repeated (4 times) addition and removal of 165 μL of acetic acid into the individual wells of microtiter plate. The extracts were transferred to deep-well polypropylene microtiter plates and evaporated in the Gene Vac, or lyophilized.

F. Postcleavage Analysis

All wells were analyzed by flow injection MS and one row of each plate was analyzed by HPLC with evaporative light-scattering detector (ELSD). The results are given in Table 1.

III. COMMENTARY

Separation of solid and liquid phases is the major problem in automation of solid-phase synthesis. We have found as a most convenient the use of "surface suction" method. This method does not require the use of any porous material for separation of liquid and solid phases. The manifold of 96 needles is slowly lowered against the surface of the liquid with continuous suction so that the liquid

is shaved from the surface without disturbing the settled resin beads. Therefore, the needles can go very close to the layer of sedimented particles without removing them from the mixture. Obviously, this method requires that the solid phase sediment in the washing step. Even though it is convenient to perform this step robotically, it is feasible even for the manual synthesis as the suction and delivery manifolds are available commercially.*

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* Spyder Instruments, Inc. (*http://www.5z.com/spyder*), Torviq (*http://www.torviq.com*).