96-Well Plate-to-Plate Gravity Fluorous Solid-Phase Extraction (F-SPE) for Solution-Phase Library Purification

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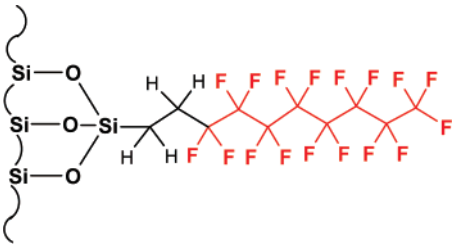
Large particle size (125–210 μm) fluorous silica gel bonded with a $-SiCH_2CH_2C_8F_{17}$ stationary phase has been employed for gravity-driven fluorous solid-phase extraction (F-SPE) on two types of 96-well plates. A 1 or 0.75 g portion of fluorous silica is packed to each well of the 3.5-mL Ex-Blok and the 2.2-mL deep-well filtration plates, respectively. Up to 50 mg of reaction mixture is loaded and then eluted with a fluorophobic solvent (DMSO, DMF, or 85:15 DMF−H_2O). Products collected in 96-well receiving plates are directly concentrated on a GeneVac vacuum centrifuge. This simple and highly efficient plate-to-plate F-SPE technique has been demonstrated in the purification of four 96-compound libraries produced by scavenging reactions with 1-(perfluoroctyl)propyl isatoic anhydride (F-IA), amide coupling reactions with 2-chloro-4,6-bis[(perfluorooctyl)propyloxy]-1,3,5-triazine (F-CDMT) or 2,4-dichloro-6-(perfluorooctyl)-propyloxy-1,3,5-triazine (F-DCT), and Mitsunobu reactions with fluorous diethyl azodicarboxylate (F-DEAD) and triphenylphosphine (F-TPP). Approximately 80% of products in each library have greater than 85% purity after F-SPE without conducting chromatography.

Introduction

Solid-phase extraction (SPE) is a powerful tool for analytical sample preparation and synthetic compound purification.[1] In addition to functionalized resins and silica gels for ionic- and polarity-based SPEs, fluorous silica gel has been developed for selective extraction of highly fluorinated (fluorous) molecules from a mixture containing nonfluorous compounds.[2] This unique separation technique has been widely used in fluorous chemistry.[3,4]

Fluorous chemistry as a new solution-phase technology has been integrated to many research areas such as the recovery of catalysts,[5] purification of small molecules,[6] immobilization of biomolecules,[7] and development of new microfluidic devices[8] and nanomaterials.[9] In our continuous effort in the advancement of fluorous silica gel-based "light fluorous" chemistry,[6e,10] we have reported the applications of 24-well plate-to-plate fluorous solid-phase extraction (F-SPE)[11] and automated F-SPE on the RapidTrace system[12] for purification of compound libraries. These two techniques employ a 40−60 μm particle size Fluoro*Flash* silica gel with a $-SiCH_2CH_2C_8F_{17}$ bonding phase.[13] Negative (for 24-well plate) or positive pressure (for the RapidTrace system) is required for the SPE process using 40–60 μm fluorous silica gel. We introduce here a specially designed large particle size (125−210 μm) fluorous silica gel for gravity-driven 96-well plate-to-plate F-SPE to further increase the throughput of fluorous purification. In addition to using water-blended elution solvents for F-SPE, we also tested DMSO and DMF as the elution solvents for water-free F-SPE, which is attractive for the separation of water-sensitive products. This simple and highly efficient 96-well F-SPE protocol could enhance the application of the F-SPE technique in solution-phase parallel and high-throughput synthesis.

Scheme 1. Bonding Phase of Fluorous Silica Gel



Results and Discussion

Large Particle Size Fluorous Silica Gel and 96-Well SPE Plates. The large fluorous silica gel is prepared by reaction of the $C_8F_{17}CH_2CH_2SiCl_3$ bonding agent with 125−210 μm silica gel following the procedure developed for the preparation of regular 40−60 μm particle size Fluoro*Flash* silica gel (Scheme 1).[13,14] Both endcapped and non-endcapped products can be readily prepared. The non-endcapped fluorous silica gel is used in this work.

A wide range of 96-well filtration plates are commercially available. Most of those plates have a standard footprint, but they differ in well shape (round or square) and volume (0.5−3.5 mL). For F-SPE applications, plates with a big well-volume could allow more packing materials for high sample loading. In this work, we tested two 96-well plates: 3.5-mL Ex-Blok from Exelixis and 2.2-mL deep-well plates from United Chemical Technologies, Inc. (UCT) (Figure 1). The

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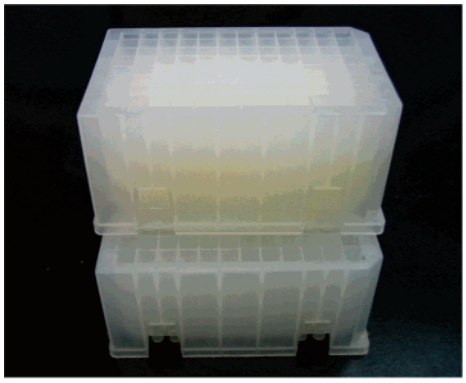
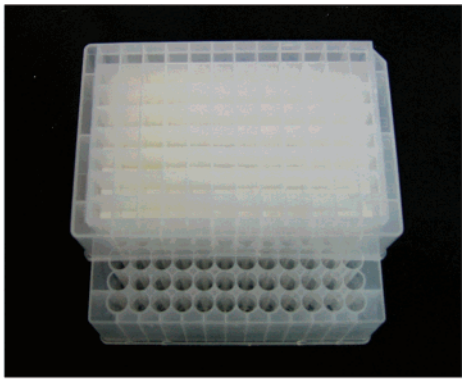


96-well Ex-Blok™ filtration (top) and receiving (bottom) plates



UCT 96 deep-well filtration (top) and receiving (bottom) plates

Figure 1. 96-Well Ex-Blok plate (left) and UCT deep-well plate (right).

Ex-Blok is a reaction block which has a puncture diaphragm inferior design for filtration.[15] The UCT plate has a standard deep-well format for filtration,[16] which is compatible with robotic and liquid handling devices from Advanced Chemtech, Beckman, Bodan, Gilson, Hamilton, Packard, Sagain, Tecan, Tomtec, Zinser, and Zymark.

General Considerations of F-SPE Plates.[17] Each well of the Ex-Blok is packed with 1.0 g of fluorous silica gel, and each well of the UCT deep-well plate is packed with 0.75 g of fluorous silica gel. A 5% sample loading is recommended,[2c] though up to 10% sample loading has been tested with success.

The fluorophilicity and the volume of the loading solvent are critical to the success of F-SPE. Common organic solvents have following order of fluorophilicity: THF > acetone, MeCN, MeOH > DMF > DMSO > H_2O.[2c] DMF and DMSO are good organic solvents and have relatively low fluorophilicity, they are ideal loading solvents for F-SPE. Since the resolution using the large particle size silica gel is lower than the regular 40−60 μm silica gel, we strongly suggest using DMSO or 85:15 DMF−H_2O for sample loading. With a small volume of these loading solvents, the sample may not always to be a clear solution, it could be a slurry. Since the majority of insoluble compounds are fluorous compounds, even the slurry loading works well without significant loss of organic products.

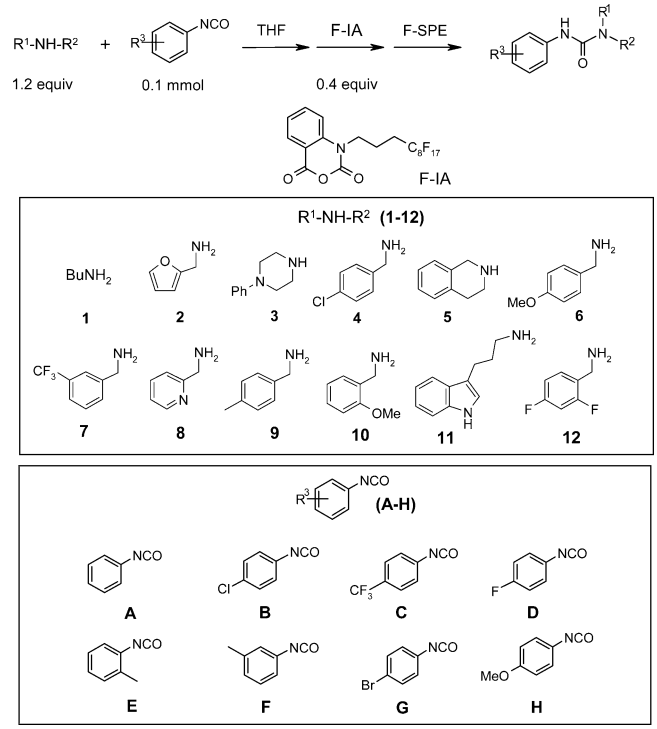
F-SPE consists of two elution passes. The first elution is with a fluorophobic solvent such as 80:20 MeOH−H_2O or 85:15 DMF−H_2O. In the current project, 100% DMSO and DMF are also used as fluorophobic elution solvents for water-free F-SPE. The non-fluorous components are washed out and the fluorous components retained on fluorous silica gel. Obviously, DMSO or DMF can only be used as the elution solvent for library compounds which have higher boiling points than the solvents so that products will not be lost during solvent evaporation. The second elution is for the fluorous components using more fluorophilic solvents such as MeOH, MeCN, or THF. In this work, the fluorous components are derivatives for the fluorous reagents and scavengers; they are not the desired products. After additional wash with acetone or THF, the SPE plate can be conditioned for reuse.

Preconditioning of 96-well plates to remove air bubbles is crucial for the gravity F-SPE. Air bubbles in fluorous silica gel dramatically slow down the elution speed and cause the channel effect to interfere the separation. The preconditioning is accomplished by degassing under reduced pressure. The plate immersed in DMF is pumped under vacuum for several minutes to remove air bubbles. After degassing, the plate is kept wet with DMF before it is used for F-SPE or sealed for short-time storage. Details on preconditioning are described in the experimental section.

96-Well Ex-Blok F-SPE Plate. Each well of the Ex-Blok has about 3.5 mL of volume. After a packing with 1.0 g of fluorous silica gel is applied, it leaves ~2.0 mL open space for addition of elution solvents. The sample loading is suggested to be less than 50 mg. The utility of the Ex-Blok plate has been demonstrated in the purification of urea compounds generated from reactions involving a fluorous scavenger 1-(perfluoroctyl)propyl isatoic anhydride (F-IA). DMSO was used as the fluorophobic elution solvent for water-free gravity F-SPE. The plate has also been tested in the purification of amide products generated from the coupling reactions using 2-chloro-4,6-bis[(perfluorooctyl)-propyloxy]-1,3,5-triazine (F-CDMT). In this case, DMF was used as a fluorophobic elution solvent.

Compared to polymer-supported scavengers, fluorous scavengers are for the solution-phase reactions which have favored reaction kinetics and in which lower molar amounts are used.[18] In this project, F-IA was used as an amine scavenger[19] for the preparation of a 96-membered urea library (Table 1). Parallel reactions were conducted at 0.1-mmol scale using 8 aryl isocyanates and 12 amines. Amines were used in slight excess amount (1.2 equiv). Unreacted amines were scavenged by addition of F-IA (0.4 equiv). After concentration, 96 reaction mixtures were loaded onto a preconditioned Ex-Blok F-SPE plate with 0.5 mL of DMSO as a loading solvent. The plate was then washed three times with 1.25 mL of DMSO under gravity, and fractions were collected in three 2.0-mL receiving plates. After the SPE plate was thoroughly washed with 1:1:0.01 THF/MeOH/TFA, it was reconditioned with DMF for future reuse. Randomly selected fractions were analyzed by LC-MS, and the receiving plates containing the first and second fractions

Table 1. 96 Ureas Generated from F-IA Scavenging Reactions



	1		2		3		4		5		6		7		8		9		10		11		12	
A	89[a]	95[b]	93	97	85	98	88	97	87	97	94	97	85	95	97	99	88	98	94	97	97	95	95	97
B	93	99	96	99	95	99	99	99	87	99	99	99	95	99	99	99	66	99	99	99	89	99	98	99
C	99	93	74	99	74	99	88	93	91	97	74	96	97	93	88	94	94	97	86	97	98	97	82	96
D	95	95	98	99	97	99	97	97	96	97	95	99	96	99	98	98	93	99	91	98	94	97	96	99
E	87	99	83	99	75	99	44	99	90	99	81	99	97	99	87	99	71	99	78	99	99	99	87	99
F	87	93	96	98	92	96	77	96	90	93	78	94	97	94	83	92	94	96	78	96	99	96	83	97
G	95	99	90	99	94	99	97	99	96	99	91	99	99	99	90	99	97	99	95	99	98	99	96	99
H	95	97	89	96	99	92	86	98	89	98	98	92	96	98	86	97	96	95	98	74	84	99	99	98

[a] Percent yield. [b] Percent purity by UV210 detection.

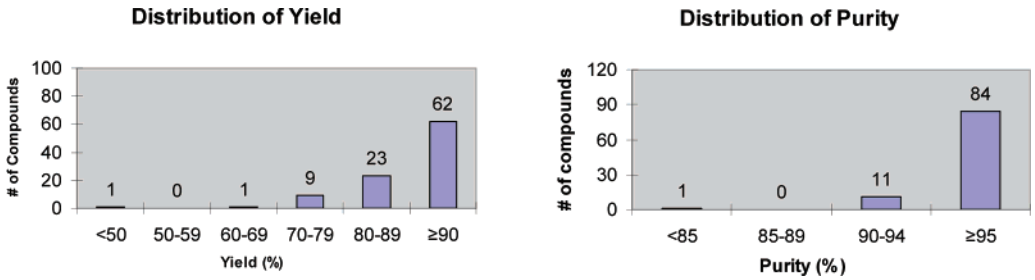
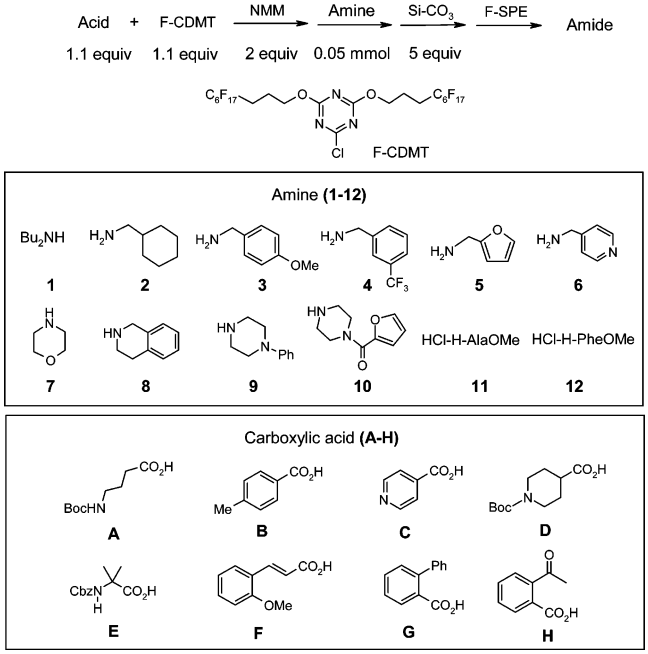


Figure 2. Yield (left) and purity (right) distributions of 96 urea products after F-SPE.

were concentrated on a GeneVac evaporator. Compounds in the plates were combined and resubmitted to LC-MS analysis. The yields and purity of 96 ureas are shown in Table 1, and their distributions are shown in Figure 2. Among 96 products, 95 products have yields greater than 50% and 95 products have purities greater than 90% detected by LC-MS at UV210.

CDMT is a well-documented reagent for amide coupling and peptide synthesis.[20] The fluorous version of CDMT has been developed for solution-phase library synthesis.[11,21] F-CDMT and its derivative have two C_6F_{17} tags. The high fluorine content make them easy to separate by F-SPE using 100% DMF as a fluorophobic elution solvent. In this project, a 96-membered amide compound library was prepared at 0.05-mmol scale by reactions of an array of 12 amines and 8 carboxylic acids (Table 2). The selection of amines included primary amines, secondary amines, and amino esters. The acids included aliphatic, aromatic, and Cbz-protected amino acids. The coupling reactions were conducted in the presence of *N*-methylmorpholine (NMM). After the reactions were over, the reaction mixtures were passed through a 96-well filtration plate charged with SAX ion-exchange silica gel ($Si-CO_3$) to free the NMM salt. After rinsing the silica gel with THF, the filtrate collected in the receiving plate was concentrated to dryness and then loaded onto an Ex-Blok plate with 0.5 mL of DMF. DMF (3×1.25 mL) was loaded for elution, and products were collected in three receiving plates. A similar post-SPE process

Table 2. Ninety-six Amide Products Generated Using F-CDMT as a Coupling Agent



	1		2		3		4		5		6		7		8		9		10		11		12	
A	72[a]	99[b]	79	99	76	99	95	99	86	98	68	99	70	99	72	98	67	99	74	98	74	82	78	98
B	49	99	90	99	89	99	77	99	92	99	88	93	43	99	96	89	88	98	57	99	86	91	93	99
C	34	72	92	99	99	96	93	99	89	94	85	92	63	92	92	99	90	99	91	99	54	90	0	0
D	89	94	92	99	83	98	89	99	86	99	78	98	80	99	85	80	86	99	86	99	87	99	88	98
E	35	99	91	99	87	99	91	99	69	98	83	92	30	99	35	91	36	99	30	99	85	99	90	99
F	84	95	73	98	76	97	87	99	73	98	78	95	81	98	82	88	80	99	92	98	66	96	91	83
G	63	99	91	98	89	98	91	98	80	96	59	55	59	99	83	87	67	98	61	97	85	99	90	98
H	76	99	89	97	88	99	73	99	88	99	79	99	73	98	75	98	90	97	84	98	94	86	95	57

[a] Percent yield. [b] Percent purity by UV210 detection.

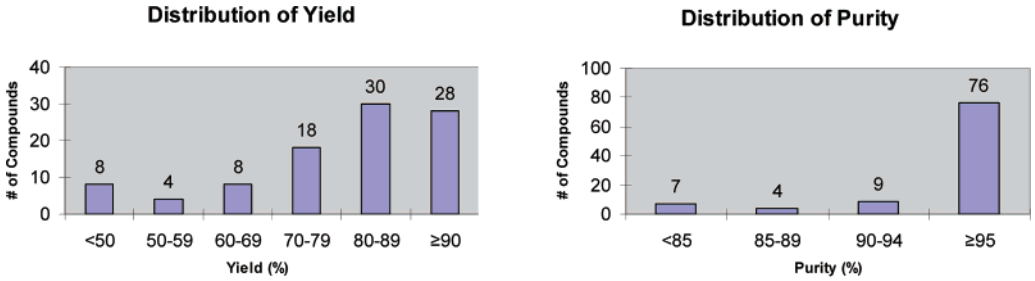


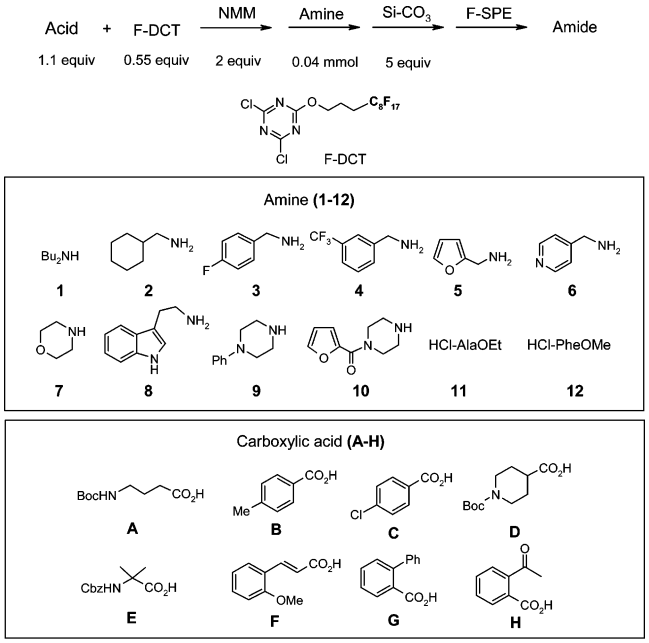
Figure 3. Yield (left) and purity (right) distribution of 96 amide products after F-SPE.

described in the F-IA scavenging reaction library was performed. Results listed in Table 2 and Figure 3 show that, among 96 products, 88 products have yields greater than 50% and 89 products have purities greater than 85% detected by LC-MS at UV210.

96-Well UCT Deep-Well F-SPE Plate. Each well of the 2.2-mL UCT plate is packed with 0.75 g of fluorous silica gel leaving ~1.0 mL of open space for the addition of elution solvents. The sample loading should be less than 40 mg. The 96-well UCT plate has been demonstrated in the purification of amide products generated from the coupling reactions using 2,4-dichloro-6-(perfluorooctyl)propyloxy-1,3,5-triazine (F-DCT) and in the purification of fluorous Mitsunobu reactions mixtures. In both cases, 85:15 DMF−H_2O was employed as the fluorophobic elution solvent for F-SPE.

F-DCT can be used as an amide coupling reagent[22] as well as a nucleophile scavenger for solution-phase library synthesis.[23] F-DCT has two active chlorines; therefore, it can produce up to two equivalents of amide products. A 96-membered amide compound library was prepared by reactions of an array of 12 carboxylic acids and 8 amines (Table 3). The selection of acids and amines as well as the reaction conditions were similar to reactions using F-CDMT described above. After reaction and removal of NMM, concentrated samples were loaded onto the UCT plate with 0.5 mL of 85:15 DMF−H_2O. The plate was eluted with 85:15 DMF−H_2O (3 × 1.0 mL), and fractions were collected in three 2.0-mL receiving plates for concentration and analysis. Results listed in Table 3 and Figure 4 show that, among 96 products, 84 products have yields greater than 50%

Table 3. Ninety-six Amide Products Generated Using F-DCT as a Coupling Agent



	1		2		3		4		5		6		7		8		9		10		11		12	
A	97[a]	99[b]	76	99	70	99	63	99	80	99	34	99	83	99	31	13	72	99	62	99	75	99	76	99
B	92	99	97	99	79	99	85	99	93	99	44	99	98	99	60	99	89	99	92	99	85	99	76	99
C	95	99	90	99	91	99	80	99	85	99	41	99	89	99	55	99	83	99	94	99	88	99	95	99
D	88	99	93	96	79	99	78	99	97	99	39	99	84	99	58	99	74	99	77	99	84	99	90	99
E	51	73	75	99	92	99	95	99	95	99	31	99	57	99	50	99	46	92	38	96	89	99	75	99
F	97	98	92	98	76	98	90	98	78	99	37	50	91	98	26	0	93	99	96	95	99	93	88	99
G	59	83	77	94	71	94	85	95	81	94	78	50	66	99	72	97	58	91	63	94	76	91	91	94
H	91	99	87	38	71	45	70	86	72	79	39	80	86	99	36	50	89	99	61	99	76	81	69	26

[a] Percent yield. [b] Percent purity by UV210 detection.

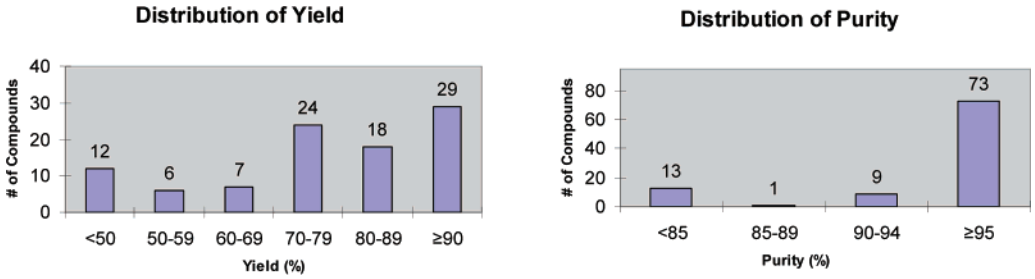


Figure 4. Yield (left) and purity (right) distributions of 96 amide products after F-SPE.

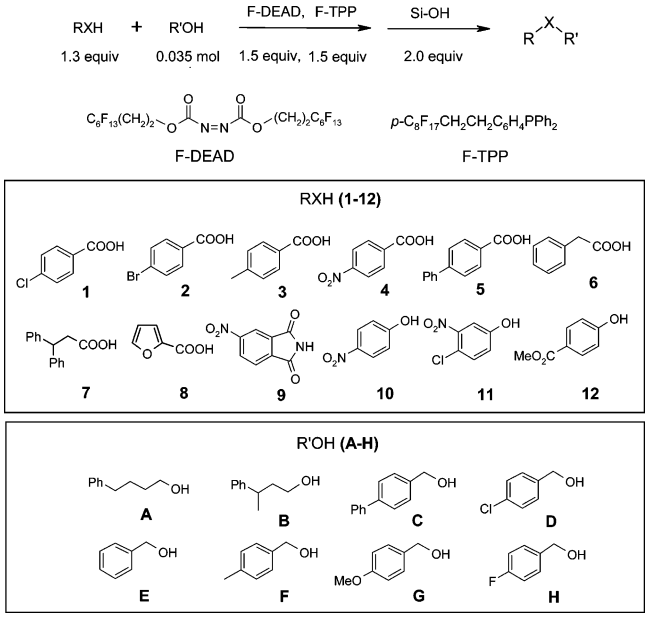
and 83 products have purities greater than 85% detected by LC-MS at UV220.

The Mitsunobu reaction is a powerful tool to generate C−O and C−N bonds. This reaction requires the use of dialkyl azodicarboxylate and triphenylphosphine as the coupling agents. Removal of derivatives from the coupling agents is not always an easy task. Fluorous Mitsunobu reactions developed by Curran and Dandapani provide a easy solution to separate the derivatives.[24] We employed the literature protocol to produce a 96-membered Mitsunobu library at 0.035-mmol scale (Table 4) using excess amounts of nucleophile (1.3 equiv), F-DEAD (1.5 equiv), and F-TPP (1.5 equiv). The reactions were completed in 1 h at room temperature. The reaction mixtures were transferred to a 96-well plate charged with SAX ion exchange silica gel (Si−OH) to remove unreacted nucleophiles. After rinsing the silica gel with THF, filtrates collected in a plate were concentrated to dryness and loaded onto the UCT F-SPE plate with 0.5 mL of 85:15 DMF−H_2O. The plate was eluted with 85:15 DMF−H_2O (3 × 1.0 mL), and fractions were collected in three 2.0-mL receiving plates for concentration and analysis. Results listed in Table 4 and Figure 5 show that, among 96 products, 90 products have yields greater than 50% and 85 products have purities greater than 85% detected by LC-MS at UV220.

Conclusion

In this project, we have evaluated 96-well plates charged with 125−210 μm fluorous silica gel for parallel SPE of fluorous reaction mixtures. The F-SPE process is driven by gravity without the use of special pressure devices. The simplicity and efficiency of 96-well F-SPE have been demonstrated in the purification of four demonstration libraries. Water-free F-SPEs with DMSO or DMF as the

Table 4. Ninety-six Fluorous Mitsunobu Reactions



	1		2		3		4		5		6		7		8		9		10		11		12	
A	71[a]	98[b]	80	96	77	92	73	94	86	93	65	84	48	79	94	92	94	99	94	94	97	97	87	91
B	95	90	92	94	99	91	99	90	97	91	67	92	58	85	98	92	99	98	97	92	99	93	90	92
C	96	99	90	96	97	93	94	97	83	91	88	66	71	70	95	96	53	99	55	95	96	49	58	93
D	51	95	28	91	87	94	45	98	77	98	56	93	60	91	61	96	36	0	92	99	99	99	96	99
E	98	95	87	97	88	92	93	96	79	91	57	95	51	85	81	58	97	99	96	99	92	98	76	99
F	88	96	91	98	76	95	98	99	89	97	54	97	47	86	99	91	84	99	93	95	95	95	95	99
G	97	90	96	90	78	85	98	92	87	86	9	84	57	72	97	87	97	81	97	85	92	91	98	80
H	78	95	78	95	90	95	90	99	89	94	71	99	57	87	96	92	93	99	94	99	93	97	91	99

[a] Percent yield. [b] Percent purity by UV210 detection.

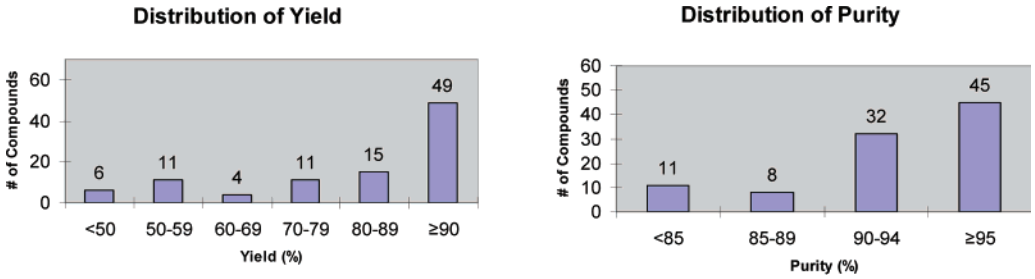


Figure 5. Yield (left) and purity (right) distributions of 96 Mitsunobu products after F-SPE.

fluorophobic elution solvents have been evaluated in two cases. Compared to previously reported 24-well plate and automated SPE on the RapidTrace system, the 96-well plate-to-plate method has higher purification throughput, though less quantity of product (0.02−0.1 mmol) can be obtained, which is an alternative method for purification of solution-phase libraries.

Experimental Section

Material and Methods. All fluorous reagents and silica gel are available from Fluorous Technologies Inc. as commercial products.[13] Other reagents and solvents used in this project were also obtained from the commercial sources. The 96-well Ex-Blok plates were obtained from Exelixis, and the deep-well filtration plates were purchased from United Chemical Technologies, Inc.[16] The 2.0-mL 96-well receiving plates were purchased from Fisher Scientific.[25] LC-MS spectra were obtained on an Agilent 1100 system with a Gemini C18 column (4.6 × 50 mm). A GeneVac HT-4 high vacuum centrifuge was used for DMSO removal, and a GeneVac EZ-2 plus evaporator was used for evaporation of other solvents.

General Procedures for Plate-to-Plate SPE. (A) Degassing of a New F-SPE Plate. A new F-SPE plate was washed with THF (1.0 mL) and DMF (3 × 1.0 mL) and then immersed in a container filled with DMF. The container was pumped under vacuum (<20 mmHg) for 5−10 min.

(B) General F-SPE Procedure for Ex-Blok. The plate was conditioned by washing with DMSO or DMF (3 × 1.25 mL). The concentrated reaction mixture was mixed with 0.5 mL of DMSO or DMF and then sonicated or shaken until a solution or suspension was formed. The samples, as a solution or a suspension, were loaded onto the F-SPE plate with a multichannel pipet and eluted under gravity. Each round of gravity elution takes about 10 min. The eluent collected during the loading was discarded. The sample

containers were rinsed with 1.25 mL of DMSO or DMF. The liquid was loaded onto the F-SPE plate with a multichannel pipet and eluted under gravity. The collected eluent was the first fraction. DMSO or DMF (1.25 mL) was added, and the second fraction was collected. The process was repeated, and the third fraction was collected. The SPE plate was washed with 1:1:0.01 THF/MeOH/TFA (5 × 1.25 mL) and DMF (2 × 1.25 mL) and then stored for future reuse. LC-MS analysis of randomly selected fractions indicated that the majority of products were in the first and second fractions, which were concentrated on a GeneVac evaporator. The products were combined, weighed, and analyzed by LC-MS.

(C) General F-SPE Procedure for UCT Deep-Well Plate. The plate was conditioned with 85:15 DMF−H_2O (3 × 1.0 mL). Each crude sample was mixed with 0.5 mL of 85:15 DMF−H_2O and sonicated or shaken until a solution or suspension was formed. The samples were loaded as a solution or a suspension onto the F-SPE plate with a multichannel pipet and eluted under gravity. The eluent collected during loading was discarded. The sample containers were rinsed with 1.0 mL of 85:15 DMF−H_2O. The liquid was loaded onto the F-SPE plate with a multichannel pipet and eluted under gravity. The collected eluent was the first fraction. A portion of 85:15 DMF−H_2O (1.0 mL) was added to each well, and the second fraction was collected. The process was repeated, and the third fraction was collected. The plate was washed with 1:1:0.01 THF/MeOH/TFA (5 × 1.0 mL) and DMF (2 × 1.0 mL) and then stored for future reuse. LC-MS analysis of randomly selected fractions indicated that the majority of products were in the first and second fractions, which were concentrated on a GeneVac evaporator. The products were combined, weighed, and analyzed by LC-MS.

(D) Procedure for Parallel Urea Formation Reactions (Table 1). Each isocyanate was distributed into a row of the 96-well reaction plate (0.1 mmol in 0.1 mL of THF for each well). Each amine was distributed into a column of the same plate (0.12 mmol in 0.1 mL of THF for each well). The plate was shaken at 600 rpm for 1 h before fluorous isatoic anhydride (F-IA) (0.04 mmol in 0.1 mL of THF) was added to each well. The reaction plate was shaken at 600 rpm for 2 h at room temperature and then concentrated on a GeneVac evaporator. Plate-to-plate F-SPE was performed following the general F-SPE procedure for Ex-Blok using DMSO as the loading and eluting solvent.

(E) Procedure for Parallel Amide Coupling Reactions Using F-CDMT (Table 2). Each carboxylic acid was distributed into a row of the 96-well reaction plate (0.055 mmol in 0.1 mL of THF for each well). NMM (0.1 mmol in 0.1 mL of THF) and F-CDMT (0.055 mmol in 0.2 mL of THF) were added to each well, and the plate was shaken at 600 rpm for 30 min at room temperature. Each amine was distributed into a column of the same plate (0.05 mmol in 0.1 mL of THF for each well). The reaction plate was shaken at 600 rpm for 1 h at room temperature before being transferred onto a 96-well plate charged with ion-exchange silica gel (SAX, CO_3 counterion, 0.2 mmol for each well). The plate was washed with THF (1.25 mL), and the filtrates in the receiving plate were concentrated on a GeneVac evaporator. Plate-to-plate F-SPE was performed following the general F-SPE procedure for Ex-Blok using DMF as the loading and eluting solvent.

(F) Procedure for Parallel Amide Coupling Reactions Using F-DCT (Table 3). Each carboxylic acid was distributed into a row of the 96-well reaction plate (0.044 mmol in 0.1 mL of THF for each well). NMM (0.08 mmol in 0.1 mL of THF) and F-DCT (0.022 mmol in 0.1 mL of THF) was added to each well, and the plate was shaken at 600 rpm for 30 min at room temperature. Each amine was distributed into a column of the same plate (0.04 mmol in 0.1 mL of THF for each well). The reaction plate was shaken at 600 rpm for 1 h at room temperature before being transferred onto a 96-well plate charged with ion-exchange silica gel (SAX, CO_3 counterion, 0.2 mmol for each well). The plate was washed with THF (1.25 mL), and the filtrates in the receiving plate were concentrated on a GeneVac evaporator. Plate-to-plate F-SPE was performed following the general F-SPE procedure for the UCT deep-well plate.

(G) Procedure for Fluorous Mitsunobu Reactions (Table 4). Each nucleophile was distributed into a column of the 96-well reaction plate (0.044 mmol in 0.1 mL of THF for each well). Each alcohol was distributed into a row of the same plate (0.035 mmol in 0.1 mL of THF for each well). F-TPP (0.05 mmol in 0.1 mL of THF) and F-DEAD (0.05 mmol in 0.1 mL of THF) were added to each well sequentially. The plate was shaken at 600 rpm for 1 h at room temperature before being transferred onto a 96-well plate charged with ion-exchange silica gel (SAX, OH counterion, 0.07 mmol for each well). The plate was washed with THF (1.25 mL), and the filtrates in the receiving plate were concentrated on a GeneVac evaporator. Plate-to-plate F-SPE was performed following the general F-SPE procedure for UCT deep-well plate.

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Supporting Information Available. LC-MS of representative compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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