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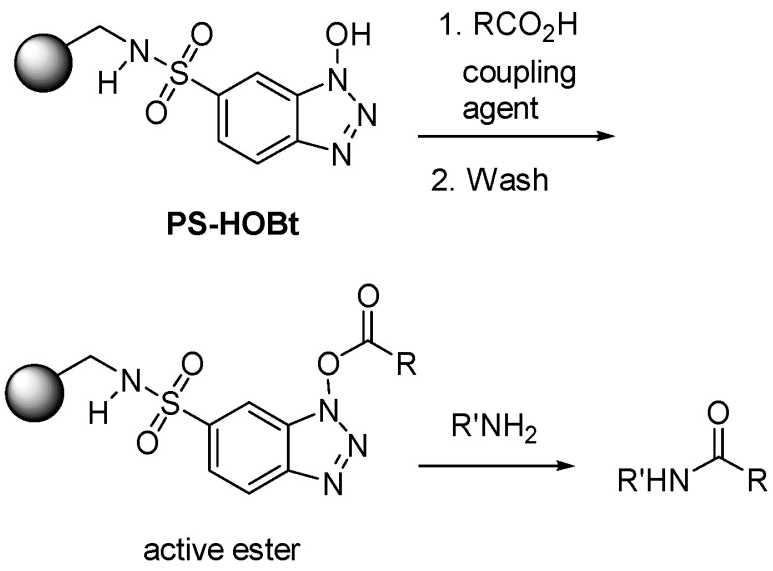

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Use of Statistical Design of Experiments in the Optimization of Amide Synthesis Utilizing Polystyrene-Supported *N*-Hydroxybenzotriazole Resin

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Two fields that routinely perform reaction optimization studies are chemical development (prior to scale-up) and medicinal or combinatorial chemistry (prior to analogue synthesis or library production). To date, the use of statistical design of experiments (DoE) in conjunction with automated synthesizers has been applied in process research to a greater extent than in the medicinal or combinatorial laboratories. We have applied DoE in conjunction with an automated synthesizer to optimize the synthesis of amides employing resin-bound *N*-hydroxybenzotriazole (PS−HOBt) active esters as intermediates. This methodology allowed the rapid development of an improved protocol for the parallel synthesis of amides by conversion of carboxylic acids to PS−HOBt esters followed by treatment with appropriate amines. Product isolation involved only simple filtration and evaporation.

Introduction

Statistical design of experiments (DoE)[1] is a recognized approach for optimizing chemical reactions during process development and improvement in the pharmaceutical and fine chemical industries. Recent advances in supporting software and automated synthesis instrumentation have led to the broader adoption of this approach in the pharmaceutical chemical development laboratory.[2] Many process groups routinely use these techniques for route scouting, parameter screening, and optimization prior to scale-up.[3] Here, the need to have a fully understood and optimal process prior to scale-up and manufacturing has driven the use of statistics and automation into the synthetic chemistry laboratory.

Another field where reaction parameter screening and optimization studies are conducted is in discovery research prior to analogue generation or library production. Here, robust and general chemistry is required to reliably produce pure compounds with diverse structural features for activity screening. By some accounts, the majority of time is spent developing reliable chemistry rather than conducting the actual library synthesis.[4] Advances in automated synthesis equipment have shifted the bottleneck from the actual synthesis to the task of designing and validating robust chemistry prior to library synthesis. Techniques to streamline this chemistry development activity are therefore needed. A novel approach is the application of DoE in conjunction with automated synthesis equipment to expedite chemistry development preceding library production. Recently Ley et al. reported on the use of DoE in an optimization study employing a resin-bound carbodiimide in amide synthesis.[5]

The use of polymer-supported reagents in organic syntheses is a powerful tool providing simplified workup and purification procedures so that parallel processing is facilitated.[6] A proven strategy for the synthesis of amide libraries employs resin-bound active esters as intermediates.[7] Advantages include the ability to preform active esters, store them in bulk or distribute them into blocks, and subsequently convert them to amides as new amines become available. Moreover, this methodology allows formation of amides simply by mixing the resin-bound active ester with appropriate amines followed by isolation of final products by filtration and evaporation. This protocol is highly amenable to automation and has been adopted by many high-throughput synthesis groups.

N-Hydroxybenzotriazole (HOBt) has been routinely used for decades to accelerate coupling reaction rates and reduce racemization in peptide synthesis.[8] A polystyrene-supported *N*-hydroxybenzotriazole first reported by Patchornik in 1975 was demonstrated to perform as an insoluble analogue to solution-phase HOBt in peptide synthesis.[9] This material was synthesized by Friedel−Crafts reaction directly onto the aromatic rings of the polystyrene resin. More recently Tartar reported a modified version (PS−HOBt) where the HOBt moiety was tethered from aminomethylpolystyrene through a sulfonamide bond.[10] In this later report, the PS−HOBt active ester formation was optimized with respect to activating agent, reagent equivalents, solvent, and time. Best conditions for ester formation employed bromotrispyrrolidinophosphonium hexafluorophosphate (PyBrOP) in the presence of diisopropylethylamine (DIEA). The procedure required a double coupling (2×3 equiv of carboxylic acid) in dimethylformamide (DMF) for 3 h each. Although effective, we found these conditions undesirable because of the high cost of PyBrOP and the need to perform a double coupling (a total of 6 equiv of acid) to effect reasonable loading. The procedure also called for premixing the coupling

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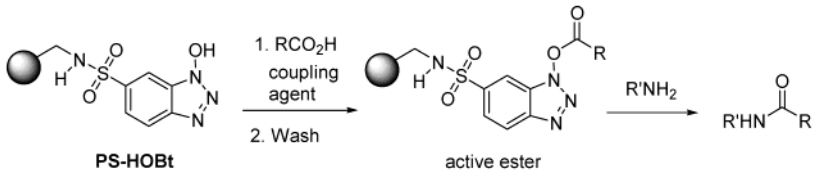
Scheme 1



agent, DIEA, and carboxylic acid and adding this solution to the resin. This was not ideal from an automation perspective where it is preferable to add the resin first followed by the other reagents consecutively in a single well. The need for 6 equiv of often-valuable carboxylic acids makes the procedure particularly unattractive for medicinal chemistry applications.

With these results in mind, we investigated alternative methods for the formation and reaction of PS−HOBt active esters. Our goal was to find a general procedure that would not require double coupling or a large excess of carboxylic acid. This paper describes the application of DoE in conjunction with automated synthesis equipment for the parameter screening and optimization. The optimized chemistry was then applied in the preparation of a small set of structurally diverse amides without the need for laborious and time-consuming purification.[11]

Results and Discussion

Active ester formation from PS−HOBt and carboxylic acids was first evaluated with several coupling agents (Scheme 1). After screening a standard set of coupling agents and conditions, we found that benzoic acid could be loaded using the relatively inexpensive diisopropylcarbodiimide (DIC) in the presence of catalytic 4-(dimethylamino)pyridine (DMAP) in place of PyBrOP/DIEA. The initial experimental protocol for determining the efficiency of ester formation consisted of treating PS−HOBt resin with excess carboxylic acid using DIC coupling agent and catalytic DMAP, affording the resin-bound activated ester intermediate. The excess acid and side products were then removed by washing the resin with solvent. Subsequent addition of excess nucleophilic amino compound effected a second coupling reaction, thereby releasing the desired amide from the resin. In this study, the use of excess amine was important to ensure complete release of the carboxylic acid so that the efficiency of its loading could be determined. The excess amino compounds were scavenged with sulfonic acid resin, MP−TsOH, providing the amides in pure form for quantitation of the acid loading efficiency based on the yield of amide.[12] To optimize this active ester formation, a number of experimental variables were examined using a statistical DoE software package[13] in concert with a Trident automated synthesizer.[14] The experimental variables initially suspected to be important in the process included reaction time, solvent composition, equivalents of reagents, and order of reagent addition. Order of addition was important from the standpoint of facilitating automation by avoiding the premixing of reagents in a separate vessel. A complete variable list including experimental ranges is shown in Table 1.

By use of the DoE software package, a 2^{5-1} fractional factorial experiment was constructed (design 1). This is a screening design employed to determine which primary variables and secondary variable interactions affect the response (resin loading). To limit the number of variables in this first experiment, benzoic acid and excess benzylamine (3 equiv) were used in each run. The product, benzylbenzamide, was quantitated by GC using an internal standard to allow calculation of the resin loading.[15] In addition to the 16 experimental runs required in a 2^{5-1} design, two replicates (to estimate error) and five centerpoints (to test for curvature in the experimental space) were added. The extra runs were not costly, since all 24 were conducted in a single experiment using the automated Trident synthesizer and workstation. A complete summary of experimental conditions and results is shown in Table 2.

Table 1. Independent Variable Settings for Design 1

variable name	variable units	range/levels
time	h	$1.0 \leq$ time ≤ 16
solvent ratio	DMF/DCM	$20 \leq$ % DMF ≤ 80
amount of carboxylic acid	equiv	$1.5 \leq$ equiv of acid ≤ 3.0
amount of DIC	equiv	$1.5 \leq$ equiv of DIC ≤ 3.0
order of addition	acid or DIC	acid added first or last

Statistical analysis of this data set was conducted and the Fusion Pro software developed a predictive model automatically. Examination of the replicate error statistics (Table 3) showed excellent reproducibility, indicating that the synthesizer had performed consistently and the analytical GC method for quantitation of product was sound. Minimal curvature was observed in this model.

One of the advantages of using a DoE software package is in viewing the results graphically as response surfaces that are created automatically. Response surfaces are graphs of the response (loading) on the *z* axis vs any two of the experimental variables on the *xy* axes. Figure 1 shows the responses predicted by the model generated through statistical analysis of the experimental data shown in Table 2. Parts A and B of Figure 1 show the effect of order of addition on the loading when reaction time and DIC equivalents are plotted. When the acid is added last, the loading declines steadily with longer reaction time and is independent of DIC equivalents. However, when the acid is added first, the loading remains constant over time and drops as the amount of DIC decreases. The highest loading is observed when the acid is added first.

Parts C−F of Figure 1 show the effect of order of addition and solvent ratio on the loading when DIC equivalents and benzoic acid equivalents are varied. When the solvent ratio is 20:80 DMF/DCM and the acid is added first (Figure 1C), the loading remains uniformly high when 3.0 equiv of DIC are used. This trend is independent of the benzoic acid equivalents. With the same solvent ratio and the acid added last (Figure 1D), the loading is relatively independent of

Table 2. Experimental Design and Results for Design 1 (2^{5-1} Fractional Factorial Experiment)

run no.	time (h)	solvent ratio (DMF/DCM)	amount of acid (equiv)	amount of DIC (equiv)	order of addition	resin loading
1	9	50:50	2.3	2.3	acid first	0.89
2	16	20:80	3.0	1.5	acid last	0.76
3	16	80:20	3.0	3.0	acid last	0.00
4	1	20:80	1.5	1.5	acid last	0.81
5	1	80:20	3.0	3.0	acid first	0.85
6	16	80:20	1.5	1.5	acid last	0.00
7	9	50:50	2.3	2.3	acid first	0.89
8	16	20:80	1.5	3.0	acid last	0.63
9	9	50:50	2.3	2.3	acid first	0.91
10	1	80:20	1.5	1.5	acid first	0.41
11	16	20:80	1.5	1.5	acid first	0.72
12	1	20:80	3.0	1.5	acid first	0.86
13	1	80:20	3.0	1.5	acid last	0.71
14	9	50:50	2.3	2.3	acid first	0.88
15	1	20:80	1.5	3.0	acid first	0.93
16	1	20:80	1.5	1.5	acid last	0.82
17	1	80:20	1.5	3.0	acid last	0.70
18	16	20:80	3.0	3.0	acid first	1.08
19	16	20:80	1.5	1.5	acid first	0.73
20	16	80:20	3.0	1.5	acid first	0.59
21	9	50:50	2.3	2.3	acid first	0.89
22	1	20:80	3.0	3.0	acid last	1.09
23	16	80:20	1.5	3.0	acid first	0.66
24	16	20:80	3.0	3.0	acid first	1.05

Table 3. Replicate Error Statistics for Design 1

replicate group run no.	response (resin capacity)	std dev	group *F* ratio[a]	*P* value[b]
1	0.89	0.0110	0.65	0.6630
7	0.89			
9	0.91			
14	0.88			
21	0.89			
4	0.81	0.0071	0.31	0.6001
16	0.82			
11	0.72	0.0071	0.31	0.6001
19	0.73			
18	1.08	0.0212	4.66	0.0743
24	1.05			

[a] This is the first standard deviation of the overall experimental error variance computed from the group response data divided by the average of the overall error computed from the remaining groups. [b] The *P* value is the probability the group is not different from the other groups. If the reported *P* value associated with a replicate group is 0.05 or less, then the individual replicate group error variance is defined as statistically different from the pooled error variance estimated from the remaining groups. Here, none of the replicate groups are statistically different from the remaining groups.

equivalents of benzoic acid and DIC used. Again, the highest loading is observed when the acid was added first. A similar trend was observed in parts E and F of Figure 1, where the solvent ratio was 80:20 DMF/DCM and the acid was added first and last, respectively. Here, with the higher concentration of DMF, the loading decreased dramatically when the benzoic acid was added last.

Figure 1G shows a predicted loading response surface where the solvent ratio is 50:50 DMF/DCM, the benzoic acid is added first, and the equivalents of DIC and benzoic acid are varied. Highest loadings are predicted where DIC is 3.0 equiv and is largely independent of benzoic acid equivalents. Figure 1H shows a predicted response surface where the amount of benzoic acid is constant (2.3 equiv) and added first and the solvent ratio and DIC equivalents are varied. Here, the loading is predicted to steadily decline as the percentage of DMF increases or the DIC equivalents decrease.

In summary, on the basis of the data in Table 2, the main variable effects and interactions were determined to be (1) the interaction of time and order of addition (only when the acid was added last), (2) the solvent ratio, (3) the amount of DIC, and (4) the order of addition (only when the acid was added last). In all cases, higher loading was observed when the acid was added first. This order of addition also caused reaction time to become an insignificant variable. This is important because when robotics are used to process large numbers of reactions, it is difficult to meet rigorous time constraints.[16] For these reasons, the acid first order of addition was adopted in future designs. From examination of the solvent ratio effects, it was clear that high DMF compositions correlated with reduced loading in all cases. DMF is often used in this type of coupling reaction because of the poor solubility of many carboxylic acids and intermediates formed in situ. In the previous study[10a] of PS−HOBt resin, DMF was adopted on the basis of solubility considerations. From these results, DMF levels should be kept to a minimum for highest loading. It was also apparent that the amount of acid was not a significant variable provided that sufficient DIC was employed. On the basis of this first design model, the most favorable conditions were found to be acid added first, 20:80 DMF/DCM, reaction time of 1 h (provided acid is added first), 3 equiv of DIC, 1.5 equiv of carboxylic acid (provided 3 equiv of DIC is used).

With this information in hand, a second experimental design was assembled as shown in Table 4. In this plan the solvent ratio was varied between 20:80 and 50:50 DMF/DCM. This range was chosen because higher levels of DMF had been shown to give inferior results in design 1, although some DMF is usually required to make a solution of

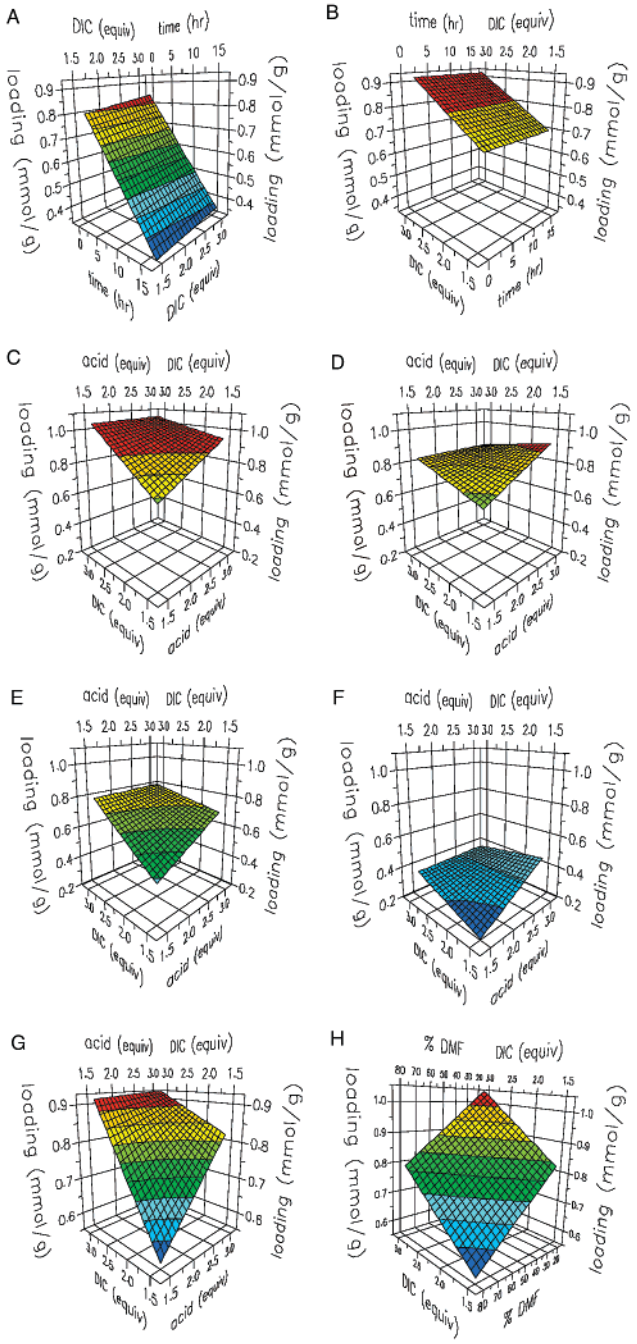


Figure 1. Loading response surfaces: (A) order of addition = acid last; (B) order of addition = acid first; (C) solvent ratio = 20:80 DMF/DCM, order of addition = acid first; (D) solvent ratio = 20:80 DMF/DCM, order of addition = acid last; (E) solvent ratio = 80:20 DMF/DCM, order of addition = acid first; (F) solvent ratio = 80:20 DMF/DCM, order of addition = acid last; (G) computed center point, order of addition = acid first; (H) computed center point, order of addition = acid first.

Table 4. Independent Variable Settings for Design 2

variable name	variable units	range/levels
time	h	1.0 ≤ time ≤ 16
solvent ratio	DMF/DCM	20 ≤ % DMF ≤ 50
amount DIC	equiv	2.0 ≤ equiv of DIC ≤ 6.0
acid type	substitution	aryl or alkyl

carboxylic acid. The amount of DIC was varied between 2.0 and 6.0 equiv because in design 1 the highest level setting (3.0 equiv) gave the highest loading, so the effect of even higher levels needed to be explored. A new variable, acid type, was added because we sought general conditions independent of carboxylic acid structure. Benzoic acid (aryl) and cyclopropylpropionic acid (alkyl) were used at a constant level of 1.5 equiv and were added first. Time was kept as a variable because it could not be eliminated as important when other acids are employed. In all runs, benzylamine was used in excess (3 equiv) and the products were analyzed for loading calculations as per design 1.

Table 5. Experimental Results for Design 2 (Model-Robust-Process-Optimization)

run no.	time (h)	solvent ratio (DMF/DCM)	amount DIC (equiv)	acid type[a]	loading
1	16.0	20:80	4.0	aryl	0.78
2	1.0	50:50	5.0	alkyl	0.68
3	16.0	20:80	2.0	alkyl	0.79
4	1.0	50:50	4.0	alkyl	0.68
5	16.0	50:50	2.0	aryl	0.68
6	16.0	50:50	2.0	alkyl	0.70
7	1.0	50:50	4.0	alkyl	0.66
8	1.0	20:80	4.0	alkyl	0.92
9	1.0	50:50	2.0	aryl	0.61
10	1.0	50:50	4.0	aryl	0.79
11	1.0	20:80	4.0	alkyl	1.02
12	16.0	50:50	5.0	aryl	0.66
13	1.0	20:80	3.0	aryl	0.92
14	16.0	50:50	6.0	alkyl	0.67
15	16.0	50:50	6.0	alkyl	0.67
16	1.0	20:80	6.0	aryl	0.84
17	1.0	20:80	4.0	aryl	0.91
18	1.0	20:80	2.0	alkyl	0.75
19	16.0	20:80	2.0	aryl	0.77
20	1.0	50:50	4.0	aryl	0.81
21	16.0	20:80	5.0	alkyl	0.76
22	1.0	20:80	2.0	alkyl	0.53
23	1.0	20:80	4.0	aryl	0.90
24	16.0	20:80	6.0	aryl	0.75
25	1.0	50:50	6.0	alkyl	0.79

[a] Alkyl = 3-cyclopentylpropionic acid; aryl = benzoic acid.

By use of the DoE software package, a model-robust-process-optimization experiment (design 2) was constructed for the variables and ranges in Table 4. This was an optimization design that allowed the determination of the best settings for the primary variables and secondary variable interactions found to be important in the first screening experiment (design 1). The runs were conducted in a single automated experiment, and the results are shown in Table 5.

Statistical analysis of this data set was conducted, and the Fusion Pro software developed a refined predictive model automatically. The resulting response surface grafts are presented in Figure 2. Parts A and B of Figure 2 show the effect of varying the DIC equivalents and time when the solvent ratio is 20:80 DMF/DCM with benzoic acid and cyclopentylpropionic acid, respectively. Inspection of the data in Table 4 showed maximum loading where an amount of 4 equiv of DIC was used with either acid. Higher amounts of DIC, i.e., 6 equiv, were deleterious to the loading. From the loading response surface in Figure 2A, the maximum loading is predicted to be where an amount of 4.4 equiv of DIC is used. This result is independent of time, with 1 h being sufficient for ester formation. Comparable loading is observed with either acid type in Figure 2A vs Figure 2B. Parts C and D of Figure 2 are similar grafts, with the only

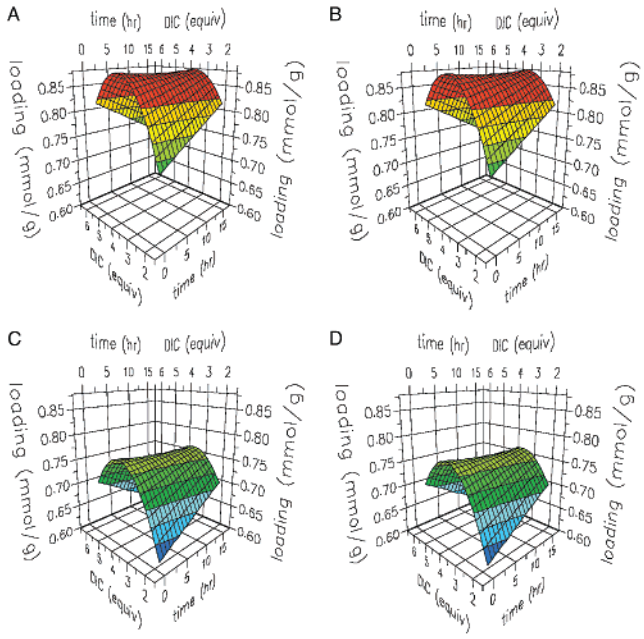


Figure 2. Loading response surfaces: (A) solvent ratio = 20:80 DMF/DCM, acid type = aryl; (B) solvent ratio = 20:80 DMF/DCM, acid type = alkyl; (C) solvent ratio = 50:50 DMF/DCM, acid type = aryl; (D) solvent ratio = 50:50 DMF/DCM, acid type = alkyl.

difference being solvent ratio at 50:50 DMF/DCM. The same trend is observed with a maximum loading predicted at 4.4 equiv of DIC independent of time. Again, comparable capacities are observed with either acid type. Maximum loading is predicted with the 20:80 DMF/DCM ratio. This solvent ratio effect is consistent with the predictions from design 1.

In summary, statistical analysis of this data set revealed the following (in order) as the only significant variables: (1) solvent ratio, with lower DMF/DCM ratios preferred; (2) amount of DIC, with 4.4 equiv giving maximum loading. Time and acid type are predicted to be insignificant. It is interesting to note that an amount greater than 4.4 equiv of DIC is deleterious to the reaction. This goes against conventional "solid-phase" chemistry wisdom that a large excess is always better. It was gratifying to see that the results were more or less the same with either acid type (alkyl or aryl). This was desirable from the standpoint of a robust process for library synthesis. The optimum conditions for realizing maximum resin loading were predicted to be 4.4 equiv of DIC, 20:80 DMF/DCM, and reaction time in the range of 1−12 h.

With these refined conditions in hand, we proceeded to test the generality with a larger set of acid types. This was intended to validate the generality of the conditions prior to the production of a larger set of amide analogues. A third model-robust-process-screening design was constructed (design 3) as described in Table 6. In this screening experiment, the primary variable was acid type. Five structurally different acids were selected. The time variable ranged between 2 and 6 h because of the possibility of reactivity differences among the five different acid types. A new variable, resin lot, was added to exclude the possibility of resin lot-to-lot variability prior to library production. The solvent ratio (20:80 DMF/DCM), DIC equiv (4.4), carboxylic acid equiv (1.5), and order of addition (acid first) were held constant in all cases. Benzylamine was used in excess (3 equiv) and the products were analyzed for loading calculations as per the two previous designs.

Table 6. Independent Variable Settings for Design 3

variable name	variable units	range/levels
acid	type	alkyl aryl cinnamic heterocyclic amino
time	h	2 < time < 6
resin	lot	lot 1 lot 2

Table 7. Experimental Results for Design 3 (Acid Type and Resin Lot Screening)

run no.	acid type[a]	time (h)	resin lot	loading
2	alkyl	2.0	lot 2	0.80
18	alkyl	2.0	lot 1	0.91
12	alkyl	2.0	lot 2	0.93
26	alkyl	6.0	lot 2	1.00
3	alkyl	2.0	lot 1	1.18
1	amino	6.0	lot 1	0.70
10	amino	2.0	lot 2	0.70
14	amino	2.0	lot 1	0.70
24	amino	2.0	lot 2	0.70
25	amino	2.0	lot 1	0.70
27	amino	6.0	lot 1	0.70
21	aryl	6.0	lot 2	0.89
9	aryl	2.0	lot 2	0.92
19	aryl	2.0	lot 1	0.92
22	aryl	2.0	lot 1	0.92
13	aryl	2.0	lot 2	1.06
6	cinnamic	6.0	lot 2	0.66
11	cinnamic	2.0	lot 1	0.70
8	cinnamic	2.0	lot 1	0.71
5	cinnamic	2.0	lot 2	0.74
23	cinnamic	2.0	lot 2	0.76
20	heterocyclic	6.0	lot 1	0.50
15	heterocyclic	6.0	lot 1	0.56
7	heterocyclic	2.0	lot 1	0.57
17	heterocyclic	2.0	lot 2	0.61
4	heterocyclic	2.0	lot 1	0.69
16	heterocyclic	2.0	lot 2	0.72

[a] Alkyl = 3-cyclopentylpropionic acid; amino = *N*-Boc-phenylalanine; aryl = benzoic acid; cinnamic = cinnamic acid; heterocyclic = quinaldic.

Table 8. Optimal General Conditions for Amide Synthesis

time, h	2
solvent ratio	20:80 DMF/DCM
amount of carboxylic acid, equiv	1.5
amount of DIC, equiv	4.4
order of addition	acid before DIC

Statistical analysis of the data in Table 7 indicated that the only significant variable affecting the loading was the acid type.[17] As observed in the two previous experiments, time had no independent statistical effect. In this case, resin lot also had no significant effect. Satisfied with the general applicability of our optimized protocol, we were confident in proceeding to the validation stage. The optimal conditions discovered are shown in Table 8. In comparison with the previously known literature conditions,[10a] this represented considerable improvement. The expensive PyBrOP coupling

Table 9. Results of the 3 × 16 Amide Library Synthesis

		benzylamine (A)		1-phenylpiperizine (B)		aniline (C)	
entry	carboxylic acid	% yield[a]	% purity[b]	% yield[a]	% purity[b]	% yield[a]	% purity[b]
1	2-naphthoic acid	92	89	89	82	92	87
2	3-phenylpropionic acid	70	99	69	81	91	95
3	3-bromo-4-methylbenzoic acid	93	61	84	67	91	72
4	3-cyclopentylpropionic acid	80	98	77	99	90	99
5	3-phenylpropionic acid	82	98	62	81	88	98
6	benzoic acid	93	92	87	96	86	94
7	cyclohexanecarboxylic acid	92	96	92	99	83	99
8	4-biphenylcarboxylic acid	90	92	80	96	82	90
9	Boc-alanine	56	93	71	58	61	97
10	3,5-difluorobenzoic acid	80	76	67	64	60	81
11	5-hydantoinacetic acid[c,d]	61	nd	62	nd	55	nd
12	cinnamic acid	73	98	65	97	55	95
13	Boc-phenylalanine	54	98	58	65	54	97
14	4-bromophenylacetic acid[d]	39	88	67	63	40	95
15	4-iodophenoxyacetic acid[d]	15	nd	27	59	32	90
16	quinaldic acid[d]	71	87	37	72	16	97

[a] Refers to the isolated mass yield based on 0.7 equiv of amine used in cleavage. [b] Refers to purity as determined by HPLC analysis with UV detection. [c] Product could not be detected by UV. [d] These acids were selected on the basis of previous knowledge that they were difficult cases; see text.

agent (6 equiv) was replaced with inexpensive DIC (4.4 equiv), and the need for a double coupling was eliminated. Time was reduced from 6 to 2 h. The requirement for 6 equiv of carboxylic acid was reduced to 1.5 equiv. The requirement to premix the acid and the coupling agent was eliminated, making the procedure more amenable to automation.

With this optimized and generally applicable protocol in hand, we went on to build a small library (16 × 3) of known amides as described in Table 9. The 16 acids chosen represented broad structural diversity, including the heterocylic acids (5-hydantoinacetic and quinaldic) and phenylacetic acids that were known to be difficult cases.[18] By use of the Trident synthesizer, the 16 carboxylic acids were converted into the corresponding active esters. After resin washing to remove excess reagents and side products, the active esters were treated with one of three different amines (primary, secondary, or aniline) to afford a 48-member set of amides. To minimize purification requirements, amines were used in substoichiometric amounts (0.7 equiv) and the yields were calculated on the basis of this limiting reagent. The amine treatment was done for 3 h at 25 °C except for the aniline cases that were done at 63 °C. Following filtration and resin washing, the products were isolated by simple concentration. Mass yield was determined, purity was established through HPLC analysis, and structures were confirmed by ^{1}H NMR.[19] Results are summarized in Table 9. With benzylamine cleavage, isolated yields ranged from 54% to 93% with purities greater than 87% (with the exception of substrates containing phenylacetic acid moieties). Similar results were obtained with 1-phenylpiperizine where yields ranged from 37% for quinaldic acid up to 92% for cyclohexane carboxylic acid. Purities were somewhat lower, ranging from 59% to 99%. For aniline, yields were moderate to excellent, with purities being >90% in all but three cases. The synthetic efficiency followed the order aryl ∼ alkyl > cinnamic ∼ amino > heterocyclic. In keeping with previous reports, 18 carboxylic acids containing phenylacetic or phenoxyacetic acids generally give inferior results.

Conclusion

A general and robust process for preparing amides utilizing polymer-bound HOBt was rapidly developed utilizing DoE in concert with an automated synthesizer. In the first experiment, the reaction parameters thought to be important were screened to determine which were most influential. Statistical analysis narrowed this list down to order of addition, solvent ratio, and amount of DIC. Furthermore, the model predicted that adding the acid first was superior. In the second experiment, the solvent ratio, amount of DIC, and time were investigated further with the ranges shifted toward the region of maximum loading as predicted by the first design model. Here, an additional variable (acid type) was added to ensure that general conditions were found. Analysis of these data showed an optimal level of DIC and the trend that lower DMF ratios gave consistently good results for both acids. A third experiment was designed to validate generality and to test lot-to-lot variability prior to full library production. Our new reaction conditions represented considerable improvement over those originally reported. The expensive PyBrOP coupling agent (6 equiv) was replaced with inexpensive DIC (4.4 equiv), and the need for a double coupling was eliminated. The requirement for 6 equiv of carboxylic acid was reduced to 1.5 equiv, and the reaction time was reduced from 6 to 2 h. The requirement to premix the acid and the coupling agent was eliminated, simplifying the procedure and making it more amenable to automation. The optimized protocol was then applied to the production of a 48-member set of structurally diverse amides. The synthesis was successful, with yields ranging from 54% to 93% with purities greater than 87% (with the exception of substrates containing phenylacetic acids). These results were comparable to those previously reported[10a] utilizing the less efficient PyBrOP/DIEA procedure. Through the conducting of three statistically designed experiments over the course of approximately 2 weeks, the chemistry was rigorously explored and characterized to the point where we could proceed to library production with a high degree of confi-

dence. The methodology of utilizing automated experimentation guided by statistical experimental design is a powerful and efficient tool for rapid chemistry development preceding analogue synthesis or library production.

Experimental Section

Solvents and reagents were used as received from general suppliers. PS−HOBt resin was obtained from Argonaut Technologies. HPLC was performed on an HP 1050 system using a platinum EPS C18 100A 3 μm rocket column from Alltech and a UV detector (223 nm). GC was performed on an HP 5890 with an HP-5 column from Agilent (5% cross-linked PH ME siloxane). Statistical experimental planning and analysis of data were done with FusionPro version 7.0 from S-Matrix. All synthetic experiments were performed on a Trident library synthesizer and a Trident workstation from Argonaut Technologies.

Procedure for Design 1 (2^{5-1} Fractional Factorial Experiment). The following stock solutions were freshly prepared: 2.442 g of benzoic acid in 20.00 mL of DMF (1.000 M), 2.524 g of 1,3-diisopropylcarbodiimide and 1.466 g of 4-(dimethylamino)pyridine in 20.00 mL of dichloromethane (1.000 M DIC and 0.6 M DMAP), 3.215 g of benzylamine, and 0.9253 g of biphenyl and 3.878 g of diisopropylethylamine in 100 mL of THF (0.03 M benzylamine, 0.06 M biphenyl, 0.03 M DIEA). Three Trident cassettes (one for each time of study) were prepared by predrying 24 reaction vessels (RVs) and charging 0.20 g of PS−HOBt(HL) resin (∼0.20 mmol). The cassettes were placed in a Trident workstation, and the appropriate solutions were manually added to the 24 RVs in the order and amounts indicated in Table 2. Depending on the specified order of addition, either the acid solution or the DIC/DMAP solution was added in the appropriate order. Finally DCM or DMF was added to adjust the solvent ratio to the specified range. The cassettes were transferred to a Trident synthesizer, and ester formation was allowed to proceed with agitation for 1, 9, or 16 h at 25 °C. After this period, the vessels were automatically drained and washed three times with 4 mL portions of DMF, THF, DMF, and DCM to remove side products and unreacted starting materials. For the amide formation, the cassette was transferred to a Trident workstation and each vessel was treated with 2.0 mL of benzylamine/biphenyl solution. The exact mass of the solution was recorded to enable loading calculations based on response factors. The cassettes were agitated for 3 h at 25 °C. Each vessel was then drained into a collection vial, and the resin was rinsed three times with 2 mL portions of THF. The solutions were then analyzed by GC to determine the amount of benzylbenzamide present relative to biphenyl using the response factor method. The loading of the active ester resin intermediate was then calculated on the basis of the yield of benzylbenzamide.

Procedure for Design 2 (Process Optimization Experiment). The following stock solutions were freshly prepared: 0.9159 g of benzoic acid in 50.00 mL of 20:80 DMF/DCM *and* 50:50 DMF/DCM (0.150 M), 1.0665 g of 3-cyclopentylpropionic acid in 50 mL of 20:80 DMF/DCM *and* 50:50 DMF/DCM (0.150 M), 2.524 g of 1,3-diisopropylcarbodiimide in 20.00 mL of DCM (0.100 M), 0.5864 g of 4-(dimethylamino)pyridine in 20.00 mL of dichloromethane (0.240 M), 3.2168 g of benzylamine, and 0.9268 g of biphenyl and 3.8810 g of diisopropylethylamine in 100 mL of THF (0.03 M benzylamine, 0.06 M biphenyl, 0.03 M DIEA). Two Trident cassettes (one for each time of study) were prepared by predrying 25 reaction vessels and charging 0.20 g of PS−HOBt(HL) resin (∼0.20 mmol). The cassettes were placed in a Trident workstation, and the appropriate solutions were manually added to the 24 RVs in the order and amounts indicated in Table 5. The DMAP solution (0.50 mL) was added first followed by the appropriate acid solution (2.00 mL) and finally the DIC solution (0.20 mL). The ester formation was allowed to proceed with agitation for 1 or 16 h at 25 °C. The washing, cleavage, and analysis were conducted as described for design 1 above.

Procedure for Design 3 (Acid-Type Screening Experiment). The following stock solutions were freshly prepared and loaded onto the Trident synthesizer: 0.9159 g of benzoic acid in 20.00 mL of DMF (0.375 M), 1.0665 g of 3-cyclopentylpropionic acid in 20 mL of DMF (0.375 M), 1.1112 g of cinnamic acid in 20 mL of DMF (0.375 M), 1.2988 g of quinaldic acid in 20 mL of DMF (0.375 M), 1.9898 g of *N*-Boc-L-phenylalanine in 20 mL of DMF (0.375 mL), 6.0576 g of 1,3-diisopropylcarbodiimide in 30.00 mL of DCM (1.60 M), 0.8145 g of 4-(dimethylamino)pyridine in 150.00 mL of dichloromethane (0.044 M), and 3.2148 g of benzylamine, 0.9253 g of biphenyl, and 3.8775 g of diisopropylethylamine in 100 mL of THF (0.03 M benzylamine, 0.06 M biphenyl, 0.03 M DIEA). Two Trident cassettes were prepared by predrying 24 reaction vessels and charging 0.20 g of PS−HOBt(HL) resin (∼0.20 mmol). The run was programmed to deliver reagents in the order and amounts indicated in Table 7. The DMAP solution (2.70 mL) was added first followed by the acid solution (0.80 mL) and finally the DIC solution (0.50 mL). The ester formation was allowed to proceed with agitation for 2 or 6 h at 25 °C. The washing, coupling, and analysis were conducted as described for the first experiment described above.

Procedure for Library Production. The following stock solutions were freshly prepared and loaded onto the Trident synthesizer: all 16 carboxylic acids listed in Table 9, 0.375 M in DMF, 10.00 mL each, 20.823 g of 1,3-diisopropylcarbodiimide in 100.00 mL of DCM (1.60 M), 2.199 g of 4-(dimethylamino)pyridine in 400.00 mL of dichloromethane (0.044 M), 0.5626 g of benzylamine and 1.9388 g of DIEA in 100.00 mL of THF (0.0525 M benzylamine and 0.150 M DIEA), 0.8518 g of 1-phenylpiperazine and 1.9388 g of DIEA in 100 mL of THF (0.0525 M 1-phenylpiperazine and 0.150 M DIEA), 0.4889 g of aniline and 1.9388 g of DIEA in 100.00 mL of THF (0.0525 M aniline and 0.150 M DIEA). Two Trident cassettes were prepared by predrying 48 reaction vessels and charging 0.15 g of PS−HOBt(HL) resin (∼0.15 mmol). The run was programmed to deliver reagents in the order and amounts indicated in Table 9. The DMAP solution (2.00 mL) was added first followed by the appropriate acid solution (0.60 mL) and the DIC solution (0.40 mL). The ester formation was allowed to proceed with agitation for 3 h at 25 °C, and the washing was completed as described above.

Coupling was conducted by adding the appropriate amine/DIEA solution (2.00 mL) for 3 h at the specified temperature. The products were collected and concentrated in vacuo (Genevac) at 25 °C. The residues were weighed to determine mass yield and were characterized by HPLC (purity) and ^{1}H NMR (identity).

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Supporting Information Available. ^{1}H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Here, the loading refers to the efficiency of loading the acids onto the PS−HOBt resin to form the active esters.

(13) The Fusion Pro package available from S-Matrix Corp. was used (www.s-matrix-corp.com).

(14) The Trident synthesizer (Argonaut Technologies) was used in this work.

(15) Resin loading refers to the efficiency of loading the acid onto the PS−HOBt resin. Because excess benzylamine was used, it was assumed that 100% of the loaded acid was released as benzylbenzamide.

(16) We also found that order of addition was especially important when conducting larger scale reactions where active esters were prepared in bulk so that they could split out for subsequent reactions. In the case of 10 g and larger preparations, it was advantageous to allow the reaction mixtures to mix for 10−15 min prior to final addition of the carboxylic acid solution.

(17) Response surface graphs could not be generated here because there is less than two continuous (numeric) variables.

(18) In ref 7, Salvino et al. comment on the difficulty in forming active esters with substrates containing basic amine or phenylacetic acid.

(19) ^{1}H NMR spectra were obtained on 38% of the samples to confirm structural assignments. Because the compounds are known and amide synthesis via this methodology is well established, this was viewed as sufficient characterization.

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