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RAPID OPTIMIZATION OF AN ICE INHIBITOR SYNTHESIS USING MULTIPLE REACTION CONDITIONS IN A PARALLEL ARRAY

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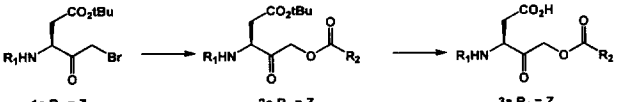
Abstract: Optimization of a 2-step reaction sequence was accomplished in *3–4 days*, with over *200 different reaction conditions* evaluated. Combinatorial arrays were performed using the optimized conditions to synthesize 590 new compounds which were tested for inhibition against N-His (D381E) ICE. Thirty-five compounds showed at least a tenfold improvement in activity compared to an initial standard.

Introduction

Reaction optimization is often the rate limiting step in a medicinal chemistry project. The traditional method of optimization involves repeated reactions, often changing a single variable at a time. This linear approach, while effective, is a time consuming endeavor. We report the use of a nonlinear, array approach to rapid reaction optimization, involving simultaneous investigation of reaction conditions.[1] This strategy was developed to provide rapid transition from traditional chemistry to automated synthesis. However, it is applicable for general optimization of reactions for traditional chemistry or reaction scale-up.

Interleukin-1β converting enzyme (ICE) is a cysteine protease that is implicated in a number of inflammatory diseases.[2] ICE is specific for Asp at P_1, and one class of inhibitors is (acyloxy)methylketones of aspartic acid.[3] In order to identify novel inhibitors of this class, a combinatorial approach utilizing novel carboxylic acid derivatives was undertaken.

Scheme 1



The standard literature procedure, which was adequate for traditional synthetic preparation, involved coupling of a β-*tert*-butyl aspartic acid bromomethylketone[2b] with a carboxylic acid using KF. Chromatography of the resulting acyloxyketone, deprotection of the β acid using TFA in dichloromethane, again followed by chromatography, gives the desired compounds.

Preparation of arrays by coupling **1** with various carboxylic acids from the Parke–Davis compound library following these procedures, without chromatography in between steps, gave complex mixtures. These

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were purified either by preparative thin layer chromatography or semi-preparative HPLC. In both cases, purification was tedious and led to a low percentage of success in the array (~10%). It was therefore necessary to optimize this reaction sequence to allow rapid preparation of the target compounds.

Two factors are important in optimization of chemistry for automated synthesis. First, the chemistry should be amenable to automation, that is, facile reagent handling with a robotic system that allows simultaneous preparation of 100 reactions at one time. Second, tedious purification, such as chromatography, at any step would ideally be avoided.

Chemistry

To investigate the coupling step, we chose **1a** and naphthyl acetic acid, which has the benefit of a good chromophore and lack of electronic influence from substitution. Only the base used and the solvent was varied. Other variables, such as temperature, concentration and time were kept constant (Table 1).[4]

A number of bases were chosen for investigation. The array was made by combining these bases with different solvents, mainly DMF, CH_3CN, and acetone. KF in DMF was used as a control. The solid supported reagents, KF on alumina or Celite, F on Amberlyst A-26 and the polymer bound morpholine[5] **4** were advantageous in the ability to remove reagent by a simple filtration as well as decreased hydroscopicity of the reagent. It was hoped that removal of BaF from these reactions would be effected by addition of $CaCO_3$ subsequent to the reaction, forming $BaCO_3$ and CaF, both of which could be removed by filtration.

In addition, a polymer supported quench reagent, polystyrene bound amino thiol[6] (see Table 1 ref c) **5**, was tested. A 1 mL aliquot of each reaction was transferred to a second vial and resin **5** was added in an attempt to remove any unreacted bromomethylketone. After further shaking of this second set of reaction 2 h, samples were taken for HPLC analysis.

Most of these reactions produced rather clean product. Many were incomplete, including BaF, K_2CO_3, solid supported fluoride sources and the polymer bound morpholine **4**. DBU caused decomposition, leading to little product. Three bases produced exceptionally clean reactions: KF, Hünig's base and Et_3N. DMF was seen as the solvent of choice, giving the cleanest reaction with both Et_3N and KF. In other solvents the reaction did not go to completion. DMF is also attractive since it will solubilize a broader range of acids than the other solvents tested. DMF can be removed under a stream of nitrogen at room temperature.

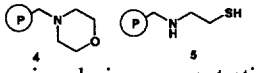
Interestingly, the thiol quench resin **5** was found to have little positive effect on product purity, and in many cases caused ester hydrolysis in the desired product.

A similar experiment was performed to examine the deprotection of the β-carboxylic acid (Table 2).[7] Different concentrations of TFA in CH_2Cl_2 were tried along with acidic ion exchange resins. Both strong and weak acidic ion exchange resins were also tried. These would have the advantage of removal of the acid source prior to concentration to obtain the final product.

Table 1

rxn	base	solvent	adjuvant	result[a]	rxn	base	solvent	adjuvant	result[a]
1	KF	DMF		95/60	42	$EtNiPr_2$	$CHCl_3$		97/67
2	KF	CH_3CN		88/48	43	**Et_3N**	**DMF**		**99/88**
3	KF	acetone		76/46	44	Et_3N	DMF	NaI (cat)	99/46
4	KF	acetone	NaI(1 eq)	97/20	45	Et_3N	THF		97/46
5	KF on alumina	DMF		66/37	46	Et_3N	THF	NaI (cat)	98/13
6	KF on alumina	CH_3CN		73/41	47	Et_3N	CH_3CN		87/13
7	KF on alumina	acetone		68/40	48	Et_3N	CH_3CN	NaI (cat)	99/46
8	KF on alumina	acetone	NaI(1 eq)	100/27	49	Et_3N	acetone		99/59
9	KF on Celite	DMF		76/41	50	Et_3N	acetone	NaI (cat)	99/59
10	KF on Celite	CH_3CN		72/43	51	Et_3N	dioxane		98/40
11	KF on Celite	acetone		77/46	52	Et_3N	$CHCl_3$		97/22
12	KF on Celite	acetone	NaI(1 eq)	85/38	53	DBU	DMF		decomp[b]
13	F on Amberlyst A-26	DMF		38/26	54	DBU	DMF	NaI (cat)	decomp
14	F on Amberlyst A-26	CH_2Cl_2		68/44	56	DBU	THF		decomp
15	F on Amberlyst A-26	THF		46/20	57	DBU	THF	NaI (cat)	decomp
16	K_2CO_3	DMF		98/71	58	DBU	CH_3CN		decomp
17	K_2CO_3	DMF	NaI (cat)	90/95	59	DBU	CH_3CN	NaI (cat)	decomp
18	K_2CO_3	THF		90/90	60	DBU	acetone		decomp
19	K_2CO_3	THF	NaI (cat)	90/35	61	DBU	acetone	NaI (cat)	62/8
20	K_2CO_3	acetone		89/69	62	**4**[c]	DMF		66/14
21	K_2CO_3	acetone	NaI(1 eq)	100/33	63	**4**	DMF	NaI (cat)	98/36
22	K_2CO_3	CH_3CN		95/90	64	**4**	THF		33/38
23	K_2CO_3	CH_3CN	NaI (cat)	62/26	65	**4**	THF	NaI (cat)	NR
24	K_2CO_3	CH_3CN	Et_4NBr	68/42	66	**4**	CH_3CN		79/35
25	K_2CO_3	H_2O/CH_2Cl_2	Et_4NBr	44/19	67	**4**	CH_3CN	NaI (cat)	81/50
26	BaF	DMF	$CaCO_3$	39/22	68	**4**	acetone		71/19
27	BaF	CH_3CN	$CaCO_3$	25/7	69	**4**	acetone	NaI (cat)	86/45
28	BaF	acetone	$CaCO_3$	25/7	70	**4**	dioxane		20/11
29	$AgCO_3$	DMF		85/80	71	**4**	$CHCl_3$		25/7
30	$AgCO_3$	THF		55/26	72	N-Me morpholine	DMF		92/29
31	$AgCO_3$	acetone		84/56	73	N-Me morpholine	dioxane		95/32
32	$AgCO_3$	CH_3CN		90/84	74	N-Me morpholine	THF		95/35
33	$EtNiPr_2$	DMF		95/95	75	N-Me morpholine	acetone		96/46
34	$EtNiPr_2$	DMF	NaI (cat)	98/90	76	N-Me morpholine	CH_3CN		97/49
35	$EtNiPr_2$	THF		97/90	77	N-Me morpholine	$CHCl_3$		94/26
36	$EtNiPr_2$	THF	NaI (cat)	86/46	78	pyridine	DMF		decomp
37	$EtNiPr_2$	CH_3CN		93/94	79	pyridine	dioxane		decomp
38	$EtNiPr_2$	CH_3CN	NaI (cat)	96/86	80	pyridine	THF		decomp
39	$EtNiPr_2$	acetone		93/72	81	pyridine	acetone		decomp
40	$EtNiPr_2$	acetone	NaI (cat)	92/80	82	pyridine	CH_3CN		decomp
41	$EtNiPr_2$	dioxane		97/35					

[a]%completion (based on **1a**)/%purity(corrected for unreacted **1a**); [b]decomposition; [c]



4 5

There was concern that decomposition of the product was occurring during concentration of the final reaction mixture. Aliquots of the reactions were taken for HPLC analysis both before and after concentration.

It was clear that TFA was an extremely poor choice for this deprotection, as every reaction showed complex mixtures. Unfortunately, the ion exchange resins also proved inadequate, showing little product by HPLC. A repeat of a portion of the array using only the ion exchange resins, but allowing a 12 h reaction time showed little increase in percent completion of the reactions. Both HCl in EtOAc and CSA in THF gave very clean product. Although CSA gave better yields, HCl is the preferred acid since CSA would be difficult to remove from the final product.

Table 2

rxn	Acid	solvent	adjuvant	conc	result[a]	rxn	Acid	solvent	result
1	TFA	CH_2Cl_2		20%	100/88	21	IRP-64	dioxane	NR
2	TFA	CH_2Cl_2		50%	100/58	22	IRP-64	THF	NR
3	TFA	CH_2Cl_2		90%	100/19	23	IRP-64	EtOAc	NR
4	TFA	CH_2Cl_2	PhSMe	50%	80/65	24	IRC-58	CH_2Cl_2	NR
5	TFA	CH_2Cl_2	Et_3SiH	50%	100/65	25	IRC-58	dioxane	NR
6	TFA	CH_2Cl_2	m-cresol	50%	100/49	26	IRC-58	THF	NR
7	**HCl**	**EtOAc**		**1M**	**48/98**	27	IRC-58	EtOAc	NR
8	HCl	PhMe		1M	37/97	28	DOWEX 50	CH_2Cl_2	NR
9	HCl	dioxane		1M	29/97	29	DOWEX 50	dioxane	NR
10	HCl	THF		1M	24/98	30	DOWEX 50	THF	NR
11	HCl	dioxane	10% H_2O	1M	NR[b]	31	DOWEX 50	EtOAc	NR
12	TsOH	PhMe			70/63	32	Amberlite IR120	CH_2Cl_2	NR
13	TsOH	acetone	H_2O		45/97	33	Amberlite IR120	dioxane	NR
14	TsOH	CH_2Cl_2	PhSMe		65/88	34	Amberlite IR120	THF	NR
15	CSA[c]	CH_2Cl_2			56/99	35	Amberlite IR120	EtOAc	NR
16	CSA	THF			74/98	36	Nafion	CH_2Cl_2	NR
17	CSA	CH_2Cl_2	PhSMe		56/99	37	Nafion	dioxane	NR
18	CSA	THF	H_2O		32/99	38	Nafion	THF	NR
19	CSA	THF	PhSMe		72/99	39	Nafion	EtOAc	NR
20	IRP-64	CH_2Cl_2			NR				

[a]%completion (based on **2a**) / %purity (corrected for unreacted **2a**); [b] No Reaction; [c]camphor sulfonic acid.

Discussion

While KF, Hünig's base and Et_3N all gave satisfactory results in the first step, Et_3N was chosen. Excess Et_3N is easily removed by evaporation and solutions are easily dispensed by a liquid handling robot. There were concerns over the hydroscopic nature of KF and the consequences of dispensing 100 samples of this solid in an automated environment. It was noted that **1** is not stable in the presence of Et_3N over time, presumably forming the quaternary amine salt. For this reason, a 20% excess of **1** was used in subsequent arrays. An aqueous extraction was added after the coupling step to remove this impurity and also any unreacted naphthyl acetic acid. Extractions of multiple samples are easily done on a Tecan liquid handling robot.

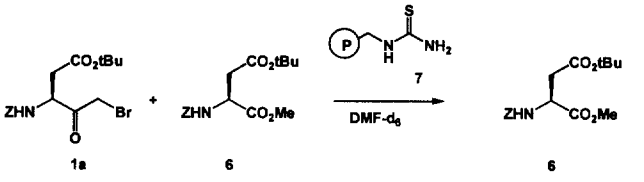
In order to test this procedure, the optimized 2-step reaction sequence was carried out on a 0.1 mmol scale. The reactions proceeded cleanly. Only desired product **3a** and protected *tert*-butyl ester **2a** were isolated (HPLC analysis showed an 86:14 ratio). The isolated yield of **3a** was 72%. Reaction time for the deprotection was increased to 5 h in subsequent arrays in order to completely deprotect intermediate **2a**.

A further concern was that any unreacted **1** would contaminate the samples, causing false positives in the in vitro assays. Subsequent to running this array, a thiourea resin **7** was developed[8] as a polymer-supported quench reagent.[5] Addition of this resin to a 1:1 mixture of **1a** and ZAspOMe **6** (Scheme 2) in DMF and heating for 3 h at 60 °C removed all of **1a**. The ester hydrolysis found with **5** was not encountered with **7**. The resin quench with **7** was used prior to extractive workup in the production arrays.

Using these optimized reaction conditions, ZAspCH_2Br **1a** and AcValAspCH_2Br **1b** were each coupled to 50 acids per array. It was expected that derivatives made with **1b**, having a longer address, would bind more tightly with ICE, while derivatives made with **1a** would be a more sensitive probe of ICE activity. Following

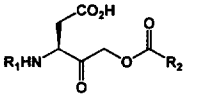
these optimized conditions, 850 syntheses were attempted, resulting in 590 new chemical entities of sufficient purity (cutoff > 75%).

Scheme 2



Biological Results

We chose **7** as a benchmark for this work. The 3-phenylpropionyl moiety was shown by Merck[3b] to be a potent P_1 substituent. Of the 415 Z-substituted Asp derivatives tested for inhibition of N-His (D381E) ICE,[9] 35 showed at least a ten-fold improvement in activity. In order to validate the arrays, the biological activity of the combinatorially synthesized compounds was compared with that of several resynthesized pure compounds.[10] In these cases, the activity was confirmed.



	R_1	R_2	IC_{50} (μM)[a]	IC_{50} (μM)[b]	K_i (μM)[b]
7	Z	2-phenylethyl	12.0	35.0	4.5
8	Z	2,6-dichlorobenzyl	0.742	--	--
9	AcVal	2,6-dichlorobenzyl	**0.005**	**0.008**	0.082
10	Z	2,3,6-trichlorobenzyl	0.936	--	--
11	AcVal	2,3,6-trichlorobenzyl	**0.005**	**0.007**	0.113

[a]data for combinatorially prepared compounds; [b]data for resynthesized compounds.

Summary

We have demonstrated the benefits of batch optimization of reaction conditions. A two step reaction sequence was optimized in *3–4 days*, with over *200 different reaction conditions* evaluated. This was easily accomplished without the use of robotics. Work is currently underway to automate the optimization procedures.[11] While the aim of this procedure was to optimize a reaction sequence for automated synthesis, a similar strategy can be used for a reaction sequence utilized by standard synthetic practices.

Acknowledgements

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References and Notes

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4. A solution of **1a** (0.5 M) was prepared in CH_2Cl_2 and 1.4 mL (1.2 equiv) of this solution added to each vial. The vials were concentrated under a stream of nitrogen. Samples of naphthylacetic acid were dissolved in the appropriate solvent (0.3 M) and 2 mL added to the correct vials. The base (2 equiv, 0.5 M solution where appropriate) and any adjuvant was added. The reactions were shaken for 12 h. At this point, aliquots were taken for analytical HPLC analysis.
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7. Prepared acyloxyketone **5** was dissolved in CH_2Cl_2 to prepare a 0.5 M solution and dispensed to all vials and concentrated under a stream of nitrogen. Solutions of the acids in appropriate solvents were added and the reactions were shaken for 2 h. An aliquot was taken for HPLC analysis. The reactions were concentrated under a stream of nitrogen and analyzed by HPLC.
8. Aminomethyl polystyrene (2 g, 3.66 mmol N/g) was suspended in 25 mL of toluene and 25 mL of tBu-isothiocynate was added. The reaction was refluxed 24h, at which point the resin was filtered and washed with CH_2Cl_2 (5×) and MeOH (5×). The resin was dried at 50 °C under vacuum. The resin was suspended in a solution of 1 g phenol in 20 mL TFA and shaken for 8 h. The resin was filtered and washed with CH_2Cl_2 (5×) and MeOH (5×), then dried at 50 °C under vacuum. Analysis showed the loading of the resin to be 2.17 mmol S/g.
9. Compounds were dissolved in DMSO to a concentration of 10 mM. Serial dilutions onto a 96-well microtitre plate were performed using a robotic liquid-handler (Packard Multiprobe) to generate duplicate wells containing 20 mL of diluted compound in HGE buffer (100 mM HEPES pH 7.5, 0.5 mM EDTA, 20% glycerol, 0.1% BSA). N-His (D381E) ICE (Dang, L. C., Talanian, R. V., Banach, D., Hackett, M. C., Gilmore, J. L., Hays, S. J., Mankovich, J. A.; Brady, K. D. *Biochemistry* **1996**, *35*, 14910) was diluted in HGDE buffer (HGE + 5 mM DTT) to approximately 24 nM, and 60 mL was added to each well. Enzyme was incubated with inhibitor for 1 h at 30 °C, and substrate was added in 20 mL to a final concentration of 50 mM. Inhibitor concentrations in the final reaction were 0.2, 2.0, 20, or 200 mM, and DMSO was present at 5% in all wells. Five minutes after addition of substrate, absorbance (405 nm) was monitored for 60 min. Eliminating wells displaying erratic absorbances, reaction velocities were determined as the mean slope (DA_{405}/Dt) of the duplicate wells. Vehicle controls were included on each plate. Percentage activities were calculated as $A_{\%} = 100*(\text{inhibited velocity})/(\text{control velocity})$. IC_{50} was evaluated as $IC_{50} = (I)*A_{\%}/(100-A_{\%})$ for all $A_{\%}$ in the range $5\% < A_{\%} < 95\%$. Compound IC_{50}s were reported as the mean of the (up to 4) independent determinations.
10. Selected data: **7**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.31–7.13 (complex m, 10H), 5.87 (br d, 1H), 5.09 (s, 2H), 4.91 (m, 2H), 4.61 (m, 1H), 2.70 (complex m, 6H). MS (ES) for $C_{22}H_{23}NO_7$ 412.4 (M^+ + 1). **9**: ^{1}H NMR (300 MHz, DMSO-d_6) 12.41 (s, 1H), 8.52 (m, 1H), 7.92 (m, 1H), 7.49 (d, J = 7.9 Hz, 2H), 7.35 (t, J = 7.5 Hz, 1H), 4.89 (m, 2H), 4.53 (m, 1H), 4.05 (s, 2H), 4.02 (m, 1H), 2.67 (m, 1H), 2.50 (m, 1H), 1.91 (m, 1H); 1.84 (s, 3H); 0.82 (br s, 6H). MS (APCI) for $C_{20}H_{24}Cl_2N_2O_7$ 475 (M^+ + 1). **11**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (broad d, 1H), 7.88 (d, J = 8.3 Hz, 1H), 7.61 (d, J = 8.6 Hz, 1H), 7.51 (d, J = 8.6 Hz, 1H), 4.87 (broad s, 2H), 4.50 (broad d, J = 6.5 Hz, 1H), 4.08 (s, 2H), 4.04 (broad s, 1H), 3.33 (complex m, 3H), 2.68 (m, 1H), 2.48 (m, 1H), 1.88 (m, 1H), 1.81 (s, 3H), 0.78 (complex m, 6H). MS (APCI) for $C_{20}H_{23}Cl_3N_2O_7$ 511 (M^+ + 1); Anal. calcd C 47.12, H 4.55, N5.50. Found C 46.77, H 4.69, N 5.69.
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