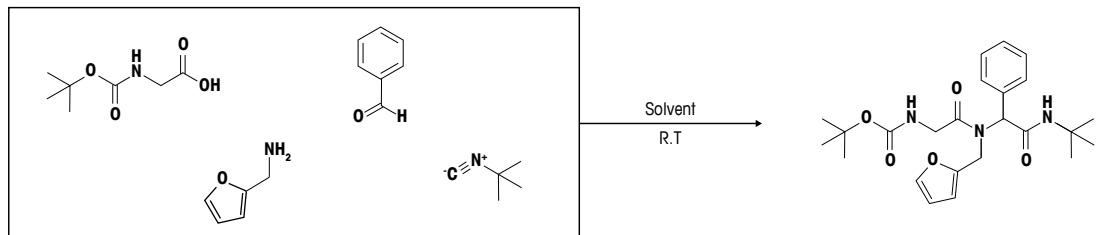


Optimization of the Ugi Reaction Using Parallel Synthesis and Liquid Handling

Optimization of the Ugi Reaction Using Parallel Synthesis and Automated Liquid Handling

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<http://www.jove.com/index/details.stp?ID=942>



Introduction

The Ugi reaction has proved to be a convenient way to quickly create diverse libraries of compounds¹⁻³. It involves the reaction of an amine, an aldehyde, a carboxylic acid and an isonitrile typically in methanol at room temperature. The Ugi reaction has often been used as a tool in the synthesis of pharmacologically active molecules and we have exploited it to quickly access compounds in the search for new anti-malarial agents⁴. It has been observed that Ugi products sometimes precipitate in pure form from the reaction mixture^{1,5}. This is a very fortunate outcome since the reaction can then be easily scaled up without requiring costly purification procedures such as chromatography. It would be most beneficial to optimize the Ugi product yield as obtained directly from filtering the reaction mixture without further treatment. To this

end we utilized a 48-position METTLER TOLEDO MiniBlock[®] equipped with filtration tubes⁶. A METTLER TOLEDO MiniMapper, automated liquid handler⁷, was used to deliver the reagents and solvent. The parameters of interest were the concentration, the solvent composition and the excess of some of the reagents.

Experimental

The MiniMapper was programmed to deliver liquids in the following sequence to empty filter tubes in a 48-position MiniBlock[®]: additional solvent, furfurylamine (2M in methanol), benzaldehyde (2M in methanol), boc-glycine (2M in methanol) and t-butylisocyanide (2M in methanol). The default addition volume was 100 microliters. If a reagent is noted as being added in excess, 120 microliters were delivered. The MiniBlock[®] was then placed on a

shaker for 16 hours before being filtered using house vacuum. Two washes are performed by adding methanol (1ml) to each tube followed by 15min shaking before filtering off. The tubes were then dried under high vacuum in a desiccator for at least 30min. The yield was calculated from the increase in weight of the filter tubes. The purity was assessed by H NMR for one sample from each solvent system and concentration. The reactions were run in triplicate⁸⁻¹⁰ and the average yields are reported in Table 1.

Characterization of the Ugi

product 'tert-butyl (2-[[2-(tert-butylamino)-2-oxo-1-phenylethyl] (furan-2-ylmethyl)amino]-2-oxoethyl) carbamate': white solid; m.p.(11) 202-204 C; ¹H NMR (12, spectrum) (500MHz, ppm, CDCl₃) 1.33 (s, 9H), 1.45 (s, 9H), 4.21 (m, 2H), 4.49 (d, J=18Hz, 1H),

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4.50 (d, $J=18\text{Hz}$, 1H), 5.47 (s, 1H), 5.60 (s, 1H), 5.62 (s, 1H), 5.89 (s, 1H), 6.10 (s, 1H), 7.19 (s, 1H), phenyl 7.21-7.37 (m, 5H); ^{13}C NMR (13, spectrum) (500MHz, ppm, CDCl_3) 28.3, 28.6, 42.3, 42.8, 51.7, 62.9, 79.5, 107.7, 110.4, 128.5, 128.7, 129.6, 134.7, 141.9, 149.8, 155.7, 168.4 170.2; IR (14, spectrum) ($-\text{max cm}^{-1}$ ATR): 1645,1673,1699, 3331; FAB-HRMS (15, spectrum) (calculated for $\text{C}_{24}\text{H}_{33}\text{N}_3\text{O}_5$ m/z 444.2498 $[\text{M}+\text{H}]$, obtained 444.2517).

Data Analysis

The precipitate yield data was analyzed using the single-factor or two-factor (with replicates) analysis of variance (ANOVA) tools available in Microsoft Excel.¹⁶ For those variables found to be statistically significant in the ANOVA analysis, Fisher's Least Significant Difference (LSD) test was used to determine the specific experimental settings exhibiting significant differences in yield of the Ugi precipitate.¹⁷ All significance tests were performed at the 95% confidence level.

Results and Discussion

Figure 1 summarizes the effect of solvent composition on the yield of the Ugi precipitate. A single factor ANOVA indicates a significant difference in average yield for the four solvents studied at a reagent concentration of 0.2M.

Fisher's LSD test indicates that the precipitate yields obtained in methanol and ethanol are not statistically different at the 95% confidence level (signified by the bar over the names of solvents in the figure); the yield of Ugi precipitate is significantly greater in ethanol and methanol than in acetonitrile, which in turn is significantly greater than

that in THF.

Considering the effect of reagent concentration, the data in Table 1 shows a precipitous drop in yield when going from 0.2M to 0.07M using methanol, ethanol and acetonitrile as the solvent; the yields were comparable in methanol at the 0.2M and 0.4M concentrations. Note that Ugi reactions are generally run in the 0.5-2M range.² However, when using an automated liquid handler, it may be difficult to start with reagent solutions much more concentrated than 2M, thus placing a practical upper limit on the final concentration at about 0.5M. The results of this optimization study suggest caution in interpreting the success or failure of Ugi reactions below 0.2M, since non-linear effects are apparent.

Figure 2 summarizes the effect of solvent composition and reagent excess on the yield of Ugi precipitate at a reagent concentration of 0.2M. The two-factor ANOVA results indicate a statistically significant solvent and reagent excess effect. In addition, there is a statistically significant interaction between the two variables. Details of the interaction are uncovered in Figure 2 which shows that the yield of precipitate is affected by the solvent used. Again note that the bars over the factors in the figure indicate the yields are not statistically different at the 95% confidence level. For both ethanol and methanol the best yields are obtained with the imine or isonitrile in excess, while for acetonitrile, significantly improved results are obtained with the amine, aldehyde or isonitrile in excess. As noted above in the discussion of the results in Table 1, the overall yield of precipitate is low for THF as the solvent, and only an excess

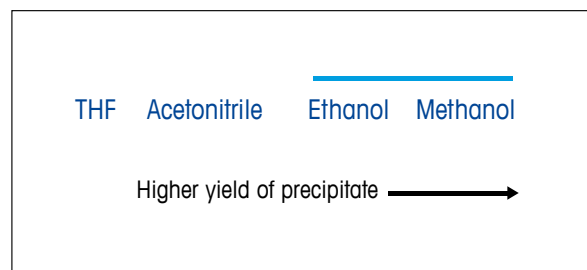


Figure 1. Summary of the effect of solvent composition on the yield of Ugi precipitate at a reagent concentration of 0.2M. The listed solvents include 40% methanol at this concentration.

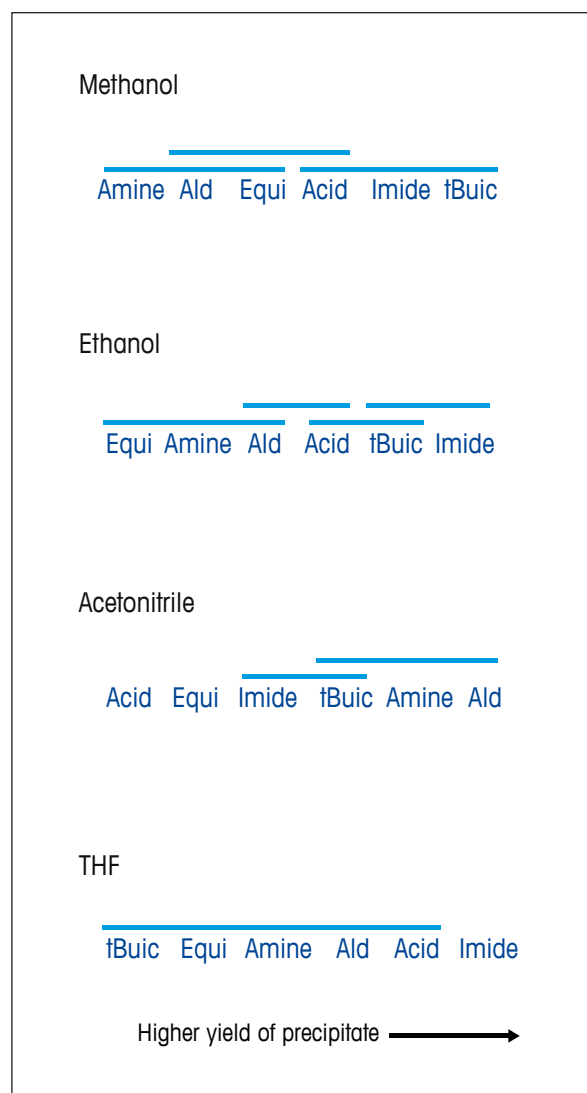


Figure 2. Summary of the effect of solvent composition and reagent excess on the yield of Ugi precipitate at a reagent concentration of 0.2M. The listed solvents include 40% methanol at this concentration.

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of imine leads to a statistically significant improvement in precipitate yield. These different patterns of improved yield for the four solvents is what leads to the statistically significant interaction effect and may be an indicator of different interaction of the Ugi product with the different solvents.

Although the numerical results in Table 1 show the best yield was obtained in 0.4M methanol with 1.2 equivalents of excess imine, 0.2M acetonitrile/methanol and 0.2M ethanol/methanol mixtures also provided significant yields. Note particularly that all of the THF/methanol mixtures at 0.2M gave very poor results. The Ugi reaction has been successfully carried out in a variety of solvents, including THF.² Note that the low yields here do not imply that the Ugi reaction did not take place in THF mixtures, only that a precipitate was not formed.

Figure 3 summarizes the effect of reagent concentration and reagent excess on the yield of Ugi precipitate using methanol as the solvent. The two-factor ANOVA results indicate a statistically significant concentration and reagent excess effect. As described above, the yield

of precipitate at reagent concentrations of 0.2M and 0.4M are significantly greater than at 0.07M (but do not differ from each other). In addition, there is a statistically significant interaction between the two variables under study. Note that the pattern of the improvement in precipitate yield with identity of the excess reagent is generally the same at the higher reagent concentrations.

Overall, the results described in Figures 2 and 3 demonstrate that there were interactions between the solvent choice, reagent concentration and identity of the reagent in excess, but overall these were more subtle than the straight solvent and concentration effects. Others have found similar results when optimizing a Ugi reaction.¹⁸

Conclusion

The optimization experiment found the highest yield of the 48 runs to be 66% from 0.4M methanol with 1.2 eq. of imine. This is significantly above the 49% yield obtained from the initial reaction under equimolar concentration at

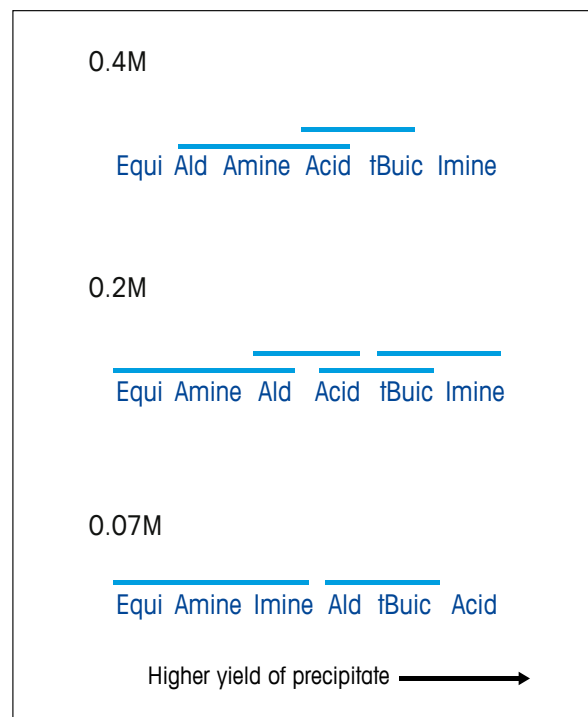


Figure 3. Summary of the effect of concentration and reagent excess on the yield of Ugi precipitate in methanol solvent.

0.4M in methanol. Good reproducibility of the precipitate yields was obtained in these replicate experiments, allowing for subtle interaction effects to be positively identified.

Run	Solvent	Concentration	Ratios	Avg. Yield	Std Dev
1	Methanol	0.4M	Eqimolar	49.41	1.18
2	Methanol	0.4M	Amine xs	57.31	2.58
3	Methanol	0.4M	Ald xs	58.62	2.66
4	Methanol	0.4M	Acid xs	55.39	5.69
5	Methanol	0.4M	tBUIC xs	58.66	1.86
6	Methanol	0.4M	Imine xs	66.17	3.17
7	Methanol	0.2M	Eqimolar	50.84	1.37
8	Methanol	0.2M	Amine xs	47.20	2.29
9	Methanol	0.2M	Ald xs	50.47	0.97

Table 1. Yield of Ugi precipitate as a function of solvent composition, reagent concentration and reagent excess. All averages and standard deviations are for N=3 replicate reactions.

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Run	Solvent	Concentration	Ratios	Avg. Yield	Std Dev	Table 1. (continued)
10	Methanol	0.2M	Acid xs	54.15	1.21	
11	Methanol	0.2M	tBUIC xs	57.83	4.06	
12	Methanol	0.2M	Imine xs	57.49	2.59	
13	Methanol	0.07M	Eqimolar	0.53	0.42	
14	Methanol	0.07M	Amine xs	1.09	0.23	
15	Methanol	0.07M	Ald xs	9.06	1.49	
16	Methanol	0.07M	Acid xs	15.03	2.08	
17	Methanol	0.07M	tBUIC xs	9.88	1.53	
18	Methanol	0.07M	Imine xs	3.23	2.37	
19	Ethanol/Methanol (60/40)	0.2M	Eqimolar	47.99	3.67	
20	Ethanol/Methanol (60/40)	0.2M	Amine xs	48.29	1.73	
21	Ethanol/Methanol (60/40)	0.2M	Ald xs	50.39	1.46	
22	Ethanol/Methanol (60/40)	0.2M	Acid xs	54.04	0.90	
23	Ethanol/Methanol (60/40)	0.2M	tBUIC xs	55.12	2.51	
24	Ethanol/Methanol (60/40)	0.2M	Imine xs	57.91	2.50	
25	Ethanol/Methanol (87/13)	0.07M	Eqimolar	2.25	1.96	
26	Ethanol/Methanol (87/13)	0.07M	Amine xs	1.61	0.68	
27	Ethanol/Methanol (87/13)	0.07M	Ald xs	7.18	0.69	
28	Ethanol/Methanol (87/13)	0.07M	Acid xs	1.43	1.05	
29	Ethanol/Methanol (87/13)	0.07M	tBUIC xs	1.80	1.66	
30	Ethanol/Methanol (87/13)	0.07M	Imine xs	0.41	0.43	
31	Acetonitrile/Methanol (60/40)	0.2M	Eqimolar	37.09	1.86	
32	Acetonitrile/Methanol (60/40)	0.2M	Amine xs	41.00	3.01	
33	Acetonitrile/Methanol (60/40)	0.2M	Ald xs	42.88	1.50	
34	Acetonitrile/Methanol (60/40)	0.2M	Acid xs	32.09	3.22	
35	Acetonitrile/Methanol (60/40)	0.2M	tBUIC xs	41.00	0.85	
36	Acetonitrile/Methanol (60/40)	0.2M	Imine xs	37.95	1.36	
37	Acetonitrile/Methanol (87/13)	0.07M	Eqimolar	0.94	0.66	
38	Acetonitrile/Methanol (87/13)	0.07M	Amine xs	1.84	0.17	
39	Acetonitrile/Methanol (87/13)	0.07M	Ald xs	0.90	0.74	
40	Acetonitrile/Methanol (87/13)	0.07M	Acid xs	0.98	0.68	
41	Acetonitrile/Methanol (87/13)	0.07M	tBUIC xs	1.20	0.43	
42	Acetonitrile/Methanol (87/13)	0.07M	Imine xs	1.01	0.88	
43	THF/Methanol (87/13)	0.2M	Eqimolar	7.89	1.35	
44	THF/Methanol (87/13)	0.2M	Amine xs	8.15	0.98	
45	THF/Methanol (87/13)	0.2M	Ald xs	8.45	1.58	
46	THF/Methanol (87/13)	0.2M	Acid xs	10.75	0.72	
47	THF/Methanol (87/13)	0.2M	tBUIC xs	7.29	0.91	
48	THF/Methanol (87/13)	0.2M	Imine xs	11.76	0.62	

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