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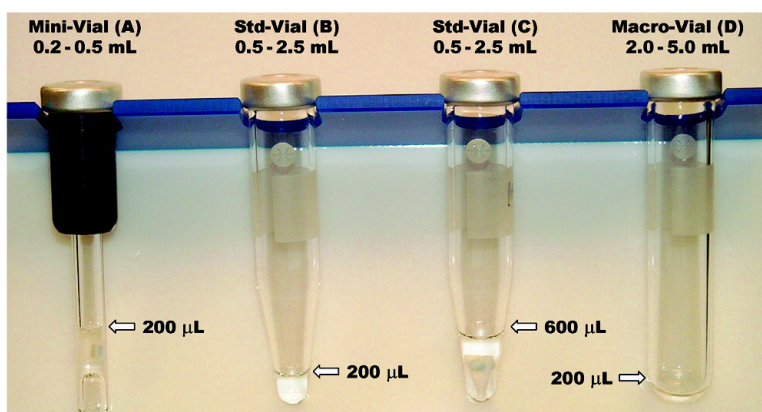
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Microwave-Assisted Organic Synthesis Using Minivials to Optimize and Expedite the Synthesis of Diverse Purine Libraries

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The new Personal Chemistry ultralow-volume (0.2−0.5 mL) minivials are shown to enable small-scale optimization and synthesis of purines at optimal reaction concentrations (0.1−0.4 M), thereby increasing the overall efficiency of this microwave-assisted library synthesis.

Introduction

Microwave-assisted organic synthesis (MAOS) has been widely employed to enable and expedite the synthesis of diverse heterocycles.[1−4] Microwave irradiation has been shown not only to reduce reaction times, but often to provide higher yields of the desired products, as compared to traditional heating methods. Standard small-scale microwave vial sizes provide adequate working volumes (0.5−5 mL) for reactions using 30−500 milligrams of starting materials. Although this scale is often desirable for the synthesis of intermediates, these reaction volumes limit reaction optimization and library synthesis to unnecessarily large scales. The volume of solvent necessary to absorb the microwave irradiation in Personal Chemistry's microwave cavity limits the reaction scale. Thus, a typical single experiment to optimize a chemical reaction requires 30 milligrams of a 300 molecular weight scaffold in 0.5 mL of solvent to achieve a typical reaction concentration of 0.2 M. Dilution of reagents to limit the use of valuable advanced intermediates or scaffolds often results in dramatically increased reaction times and lower yields. These small-scale limitations have been recently addressed by the new minivials from Personal Chemistry, which have a working volume of 0.2−0.5 mL. We demonstrate their utility herein with the optimization of microwave conditions to affect the S_N2Ar substitution of 2-fluoropurines with sterically hindered and electron-deficient amines in near quantitative yields. High reagent concentrations (0.36 M) in the minivials were crucial to the success of these microwave reactions.

Results and Discussion

Substituted purines, such as Purvalanol **1**, selectively inhibit protein kinases and therefore have been the focus of many library syntheses (Scheme 1).[5] Several different solution-phase syntheses[5,6] and solid-phase syntheses[7−9] have been described in the literature, each having their own attributes and limitations. In our application, we specifically desired access to a highly diverse set of amines at the C-2

position of the purine core. Therefore, we opted for a solution-phase route, with the final reaction being a microwave-assisted S_N2Ar substitution of the C-2 fluoropurine **3** with a diverse array of amines to facilitate increased yields of the desired C-2 aminopurines **2** in shorter reaction times.

The 2-fluoro-6-benzylamino-9-butylpurine core **5** was synthesized according to literature procedures and subsequently used in microwave optimization reactions (Scheme 2).[6] Previous reports demonstrate that S_N2Ar substitution of the 2-fluoropurine with amines usually requires high temperatures (>100 °C) and extended reaction times (12−24 h) to ensure complete substitution. These reactions are commonly performed in DMSO/*n*-butanol mixtures.[6,7] However, when this cosolvent was used in our initial microwave reactions, we observed significant amounts of the hydrolysis product **7**. To circumvent this side reaction, various solvents were tested using 2-fluoro-6-benzylamino-9-butylpurine **5**, 20 equiv of *N*-(2-hydroxyethyl)piperazine, and 3 equiv of DIEA.

The reactions were microwave-irradiated for 15 min in 0.5 mL of various solvents in the standard (0.5−2.5 mL) microwave vials. Conversions were calculated on the basis of HPLC absolute area at 220 nM. Alcoholic solvents, such as ethanol (entry 2) afforded significantly less of the undesired side product **7**, but conversions were low. Acetonitrile gave only the desired product **6**; however, conversions were low, since reaction temperatures can reach only 170 °C in this solvent. In addition, the low volumes of solvent (0.5 mL) combined with the continuous maximum power output (300 W) of the microwave synthesizer to heat the poorly absorbing acetonitrile to maximum temperature resulted in sporadic overloading of the microwave instrument upon repeated use. Increasing the amount of solvent in the reaction vessel to 0.6 mL and minor instrument modifications eliminated this instrument error. *N*-Methylpyrrolidinone (NMP) as solvent also afforded only the desired product in moderate yield, but reached 220 °C. A 1:1 mixture of acetonitrile and NMP gave optimal results: 95% conversion to product **6** with no byproduct **7** observed. The acetonitrile/ NMP mixture provides an ideal solvent, since NMP strongly absorbs microwave energy and acetonitrile is a good solu-

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Scheme 1
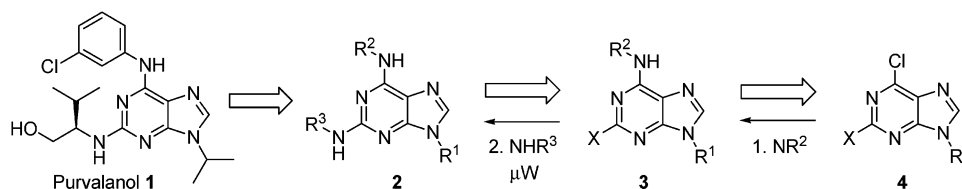
Scheme 2
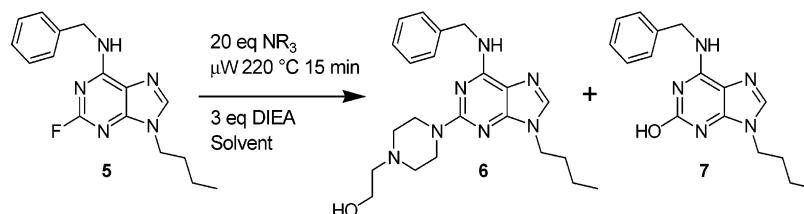
**Table 1.** Solvent Optimization Using Standard 0.5−2.5 mL Vials

entry	solvent	temp, °C[a]	pressure, bar	6/7	% conversion[b]
1	DMSO/*n*-BuOH	220	10	2:1	75
2	EtOH	180	15	9:1	60
3	ACN	170	12	1:0	60
4	NMP	220	5	1:0	75
5	ACN/NMP	220	9	1:0	95

[a] Maximum temperature limit of solvent was reached for all reactions. [b] Calculated on the basis of HPLC at 220 nM.

bilizing agent for purines. Acetonitrile may also enhance the reaction due to increased reaction pressure (2×) versus NMP alone (entry 5).

The scope and generality of this microwave-assisted S_N2Ar method was tested with a variety of amines (Table 2). Reactions were performed with merely 10 mg of compound **5** in 600 μL at 0.06 M in the standard vial (0.5−5.0 mL). In general, conversions were >95% with piperazino or unhindered primary amines (entries 1−3), but conversions were only moderate using sterically hindered or secondary amines (entries 4, 6). Poor conversions were observed using cyclic or very sterically hindered amines (entries 5, 8). Improving the conversions for these unreactive amines was critical to the success of our library synthesis, since these C-2 substituents are found in many kinase inhibitors. For instance, sterically hindered amine **4** is found in Purvalanol **1**.

Since the temperature of the reaction was at its maximum and we did not want to extend the synthesis time for our library by increasing the individual reaction times, we opted to investigate the concentration dependency of this microwave reaction with these very unreactive amines. As a beta test site for Personal Chemistry, we were supplied with ultralow-volume minivials A to evaluate in our chemistry application (Figure 1). The new minivials have a working volume of 0.2−0.5 mL and are fitted with adaptors so that a standard crimp cap can be attached and the minivial inserted into the standard microwave instrument cavity. The minivial should be advantageous for small-scale optimization and library synthesis of 10−50 mg of product, since one can achieve high concentrations (0.2−0.4 M) with limited amounts of valuable intermediates or scaffolds.

Table 2. Reactivity of Various Amines with 2-Fluoro-6-benzylamino-9-butylpurine **5**[a]
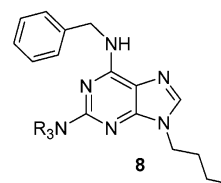

Entry	Product	Amines (H-NR$_3$)	% Conversion[b]
1	**8a**		95
2	**8b**		90
3	**8c**		90
4	**8d**		60
5	**8e**		50
6	**8f**		50
7	**8g**		50

[a] Standard conditions in 0.5−2.5 mL microwave vial were 20 equiv amine (H−NR$_3$) with compound **5** at 0.06 M in ACN/NMP (1:1), microwave 15 min at 220 °C. [b] Conversion calculated on the basis of HPLC integration at 220 nM.

The unreactive amine from entry 7 (Table 2) was used in a test experiment to determine the effect of increasing reaction concentration on product conversion. As a control, the reaction was performed in the standard 0.5−2.5 mL vials B using 2-fluoro-6-benzylamino-9-butylpurine **5**, 20 equiv of the amine of entry 7 (Table 2) and 3 equiv of DIEA in 0.06 M of acetonitrile/NMP and microwave-irradiated for 15 min at 220 °C. Conversions were monitored by HPLC at 1, 3, 8, and 15 min. At 15 min, a 50% conversion was observed (Figure 2). In contrast, the same reaction in the minivial A gave only 25% conversion to the desired product
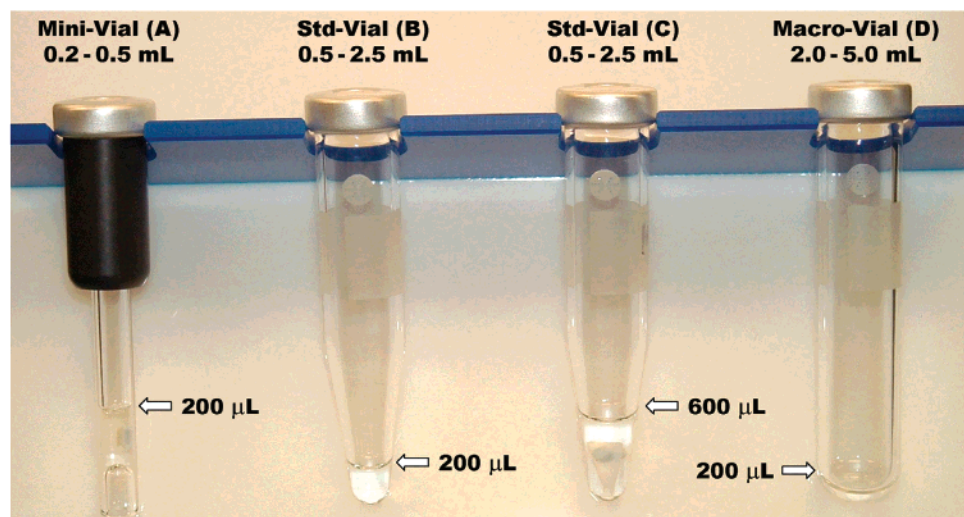
Figure 1. Comparison of various microwave vials available from Personal Chemistry: (A) 0.2−0.5-mL minivial with 200 μL of solvent, (B) 0.5−2.5-mL vial with 200 μL of solvent, (C) 0.5−2.5-mL vial with 600 μL (minimum recommended volume), and (D) 2.5−5.0-mL vial with 200 μL of solvent.
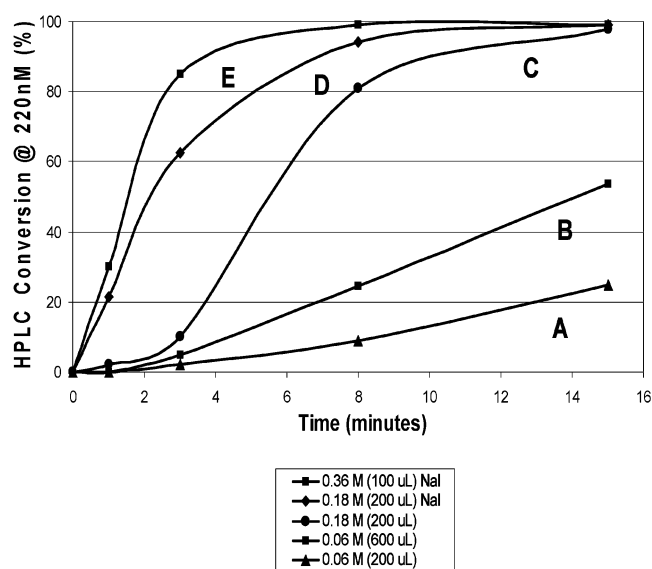
Figure 2. Graph comparing the rate of reaction of 2-fluoro-6-benzylamino-9-butylpurine **5** with amine **8g** (Table 2) upon microwave heating at different concentrations of reactants using the minivials and standard vials. Graphed lines: (A) 0.06 M in minivial (200 μL of solvent), (B) 0.06 M in standard vial (600 μL of solvent), (C) 0.18 M in minivial (200 μL of solvent), (D) 0.18 M in minivial (200 μL of solvent) with catalytic NaI, and (E) 0.36 M in minivial (100 μL of solvent) with catalytic NaI.

at the identical reaction concentration. An observed lower reaction pressure in the minivials versus the standard vials may account for this discrepancy. The same reaction was then performed in the minivials at 3 times the concentration (0.18 M) of the initial experiments. Complete conversion to product was observed after the 15-min irradiation. Addition of a catalytic amount of NaI further reduced the reaction time to completion to ~11 min. This reaction presumably proceeds through the 2-iodopurine intermediate, which has previously been shown to be more reactive than the starting 2-fluoro or 2-chloro species.[8] To our knowledge, this is the first described use of NaI to catalyze an S_N2Ar reaction on a halopurine. Pushing beyond the recommended limit (0.2 mL) of the minivial, the reaction concentration was increased

Table 3. Optimized S_N2Ar Reactions in 0.2−0.5 mL Minivials[a]

Entry	Product	Amines (H-NR$_3$)	% Yield[c]
1	**8a**		95
2	**8d**		77
3	**8g**		89[b]

[a] Standard conditions in 0.2−0.5 mL minivials were 20 equiv amine (H−NR$_3$) with compound **5** at 0.18 M in ACN/NMP (1:1), microwave 15 min at 220 °C for compounds **8a** and **8d**. [b] A catalytic amount of NaI was added to entry 3 and the mixture was microwave-irradiated for 8 min at 220 °C. [c] Isolated yields from microwave reaction in minivials.

6 times from the original concentration to 0.36 M by lowering the total solvent volume in the minivial to 0.1 mL. Microwave irradiation for only 8 min gave complete conversion to the desired product **8g** with an 89% isolated yield (Table 3). The generality of these new microwave conditions was demonstrated for compounds **8a** and **8d** with isolated yields of 95 and 77%, respectively.

Conclusion

Microwave-assisted S_N2Ar conditions were optimized using minivials to efficiently incorporate a diverse collection of sterically hindered and nonnucleophilic amines at the C-2 position of the purine scaffold. The new minivials allowed us to minimize reaction times and dramatically increase yields of this S_N2Ar reaction by increasing reaction concentrations with limited amounts of scaffolds and reagents. We

believe that these minivials are ideal reaction vessels for the optimization of difficult chemistries on valuable intermediates and for efficiently producing libraries of 5−50 mg of final product by MAOS.

Experimental Section

General Details. Commercially available reagents were used without further purification. Anhydrous solvents were purchased from Aldrich. NMR spectra were recorded using a Varian Mercury 400 instrument operating at 400 MHz for ^{1}H spectra and 377 MHz for ^{19}F spectra. LC/MS data were determined using an Agilent 1100 LC/MSD. Purification was performed using a Waters FractionLynx Preparative LC/MS system using a Waters C_{18} symmetry column with ESI detection. Personal Chemistry Optimizer and Liberator were used for microwave reactions. Minivials (Part no. 354832) and standard vials were provided by Personal Chemistry.

General Procedure for Preparation of 2-Fluoro-6-benzylamino-9-butylpurine (Compound 5). 2-Fluoro-6-benzylaminopurine (1.0 g, 4.1 mmol) was dissolved in 0.2 M dry THF. A solution of triphenylphosphine (1.4 g, 5.3 mmol) and 1-butanol (0.56 mL, 6.2 mmol) in 0.2 M dry DCM was added. The flask was purged with nitrogen and cooled to 0 °C. Diisopropylazodicarboxylate (1.1 mL, 5.3 mmol) was added dropwise via syringe. The mixture was stirred at 0 °C for 1 h, then warmed to room temperature overnight. Solid white precipitate was filtered and recrystallized from MeOH to afford compound **5** (0.99 g, 81% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.87 (bt, J = 6.1 Hz, 1H), 8.13 (s, 1H), 7.32 (d, J = 7.8 Hz, 2H), 7.29 (t, J = 7.8 Hz, 2H), 7.21 (m, 1H), 4.61 (d, J = 6.4 Hz, 2H), 4.17 (t, J = 6.9 Hz, 2H), 1.73 (m, 2H), 1.22 (m, 2H), 0.86 (t, J = 7.5 Hz, 3H). ^{19}F NMR (reference $CFCl_3$): δ −51.3; MS $C_{16}H_{18}FN_5$ [M + H]$^+$ 299.15. Found 300.1.

2-(6-Benzylamino-9-butyl-9*H*-purine-2-yl)-piperazin-1-yl-ethanol (Compound 8a, Table 3). 2-Fluoro-6-benzylamino-9-butylpurine **5** (13 mg, 0.04 mmol) was dissolved in 200 μL of 1:1 (v/v) acetonitrile/NMP in a minivial microwave tube with a stir bar. Diisopropylethylamine (23 μL, 0.13 mmol) was added, followed by *N*-(2-hydroxyethyl)-piperazine (113 mg, 0.87 mmol). The reaction was microwave-irradiated at 220 °C for 15 min. The crude material was purified by HPLC using mass-directed fractionation to give compound **8a** (Table 3). (16 mg, 95%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.76 (bs, 1H), 8.21 (bs, 1H), 7.86 (s, 1H), 7.35 (d, J = 7.3 Hz, 2H), 7.27 (t, J = 7.3 Hz, 2H), 7.19 (m, 1H), 4.59 (m, 2H), 4.58 (bs, 2H), 4.0 (t, J = 6.9 Hz, 2H), 3.75 (t, J = 4.3 Hz, 2H), 3.48 (m, 2H), 3.22 (m, 2H), 3.18 (bs, 2H) 2.96 (bs, 2H), 1.72 (m, 2H), 1.20 (m, 2H), 0.87 (t, J = 7.3 Hz, 3H). MS $C_{22}H_{31}N_7O$ [M + H]$^+$ 409.26. Found 410.2.

2-(6-Benzylamino-9-butyl-9*H*-purin-2-ylamino)-3-methyl-butan-1-ol (Compound 8d, Table 3). 2-Fluoro-6-benzylamino-9-butylpurine **5** (13 mg, 0.04 mmol) was dissolved in 200 μL of 1:1 (v/v) acetonitrile/NMP in a minivial microwave tube with a stir bar. Diisopropylethylamine (23 μL, 0.13 mmol) was added, followed by DL-2-amino-3-methyl-1-butanol (91 mg, 0.88 mmol) and catalytic NaI. The reaction was microwave-irradiated at 220 °C for 15 min. The crude material was purified by HPLC using mass-directed fractionation to give product **8d** (Table 3) (13.0 mg, 77%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.81 (bs, 1H), 7.31 (d, J = 8.0 Hz, 2H), 7.30 (t, J = 7.3 Hz, 2H), 7.22 (t, J = 7.3 Hz, 1H), 4.83 (d, J = 14.9 Hz, 1H) 4.77 (d, J = 14.9, 1H), 4.01 (t, J = 7.0 Hz, 2H), 3.84 (m, 1H), 3.55 (d, J = 5.6 Hz, 2H), 1.79 (m, 2H), 1.31 (m, 3H), 0.92 (d, J = 7.2 Hz, 3H), 0.91 (t, J = 7.2 Hz, 3H), 0.90 (d, J = 7.2 Hz, 3H). MS $C_{21}H_{30}N_6O$ [M + H]$^+$ 382.25. Found 383.2.

2-(6-Benzylamino-9-butyl-9*H*-purin-2-ylamino)-2-methyl-propan-1-ol) (Compound 8g, Table 3). 2-Fluoro-6-benzylamino-9-butylpurine **5** (12.8 mg, 0.04 mmol) was dissolved in 100 μL of 1:1 (v/v) acetonitrile/NMP in a minivial microwave tube with a stir bar. Diisopropylethylamine (23 μL, 0.13 mmol) was added, followed by 2-amino-2-methyl-1-propanol (76.6 mg, 0.86 mmol) and catalytic NaI. The reaction was microwave-irradiated at 220 °C for 8 min.

The crude material was purified by HPLC using mass-directed fractionation to give product **8g** (Table 3) (14 mg, 89%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ 9.28 (bs, 1H), 8.06 (s, 1H), 7.32 (m, 4H), 7.24 (m, 1H), 7.0 (bs, 1H), 5.22 (bs, 1H), 4.67 (bs, 2H), 4.05 (t, J = 6.9 Hz, 2H), 3.45 (bs, 2H), 1.72 (m, 2H), 1.27 (bs, 6H), 1.24 (s, 2H), 0.87 (t, J = 7.3 Hz, 3H). MS $C_{20}H_{28}N_6O$ [M + H]$^+$ 368.2. Found 369.2.

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