

Resin-Capture and Release Strategy toward Combinatorial Libraries of 2,6,9-Substituted Purines

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A resin-capture and release strategy for making combinatorial 2,6,9-trisubstituted purine libraries is demonstrated by capturing N9-derivatized purines at the C6 position with a thio-modified polymer. The C2 fluoro group is subsequently substituted with primary and secondary amines followed by thioether oxidation and release by C6 substitution with amines and anilines. This approach complements a previously reported strategy where a 6-phenylsulfenylpurine scaffold was captured at the C2 position with a resin-bound amine.³

The sequencing of the human genome and the genomes of numerous pathogens has resulted in an explosion in the number of proteins of unknown function. The synthesis of small-molecule ligands that can be used to probe the activities of these proteins presents both huge opportunities and challenges for chemists. One of the most fruitful applications of combinatorial chemistry has been the design of flexible synthetic schemes that generalize a privileged scaffold from a particular protein to other members of that protein family. We have chosen to develop numerous routes^{1,3} to substituted purine libraries to explore the potential of this compound class to inhibit diverse members of the protein kinase family. Inhibitors² of protein kinases have proven to be invaluable tools in the elucidation of signal transduction networks, as well as promising clinical candidates for the treatment of cancer, cardiovascular disease, and inflammatory and neurological diseases.

Recently, we reported a concise and convergent traceless linker strategy for the solid-phase synthesis of combinatorial libraries of 2,6,9-trisubstituted purines³ (Scheme 1). While this approach is ideally suited for the traceless synthesis of focused C6 purine libraries, it suffers from two limitations: (1) because the C2 amino substituent is tethered to the solid support prior to being covalently attached to the scaffold, only primary amines can be installed at the C2 position; (2) it is inefficient to prepare C2-focused libraries because this substitution reaction is the first combinatorial step. To address these limitations, we have developed a complementary approach in which resin capture can be performed at the C6 position, resulting in a resin-bound purine that can be derivatized in the final step at C2.

It is known that the 2-sulfonyl group of 4-amino-substituted pyrimidines can be displaced by various amines.⁴ Because 2,4-dichloropyrimidine has similar amination reactivity as 2,6-dichloropurines under basic conditions (the 4

position of pyrimidine and the 6 position of purines undergo selective nucleophilic substitution first; complete substitution of the 2 position afterward requires elevated temperature and high concentrations of amine nucleophile), we speculated that the same would be true for the purine system. We therefore devised the synthetic approach shown in Scheme 2.

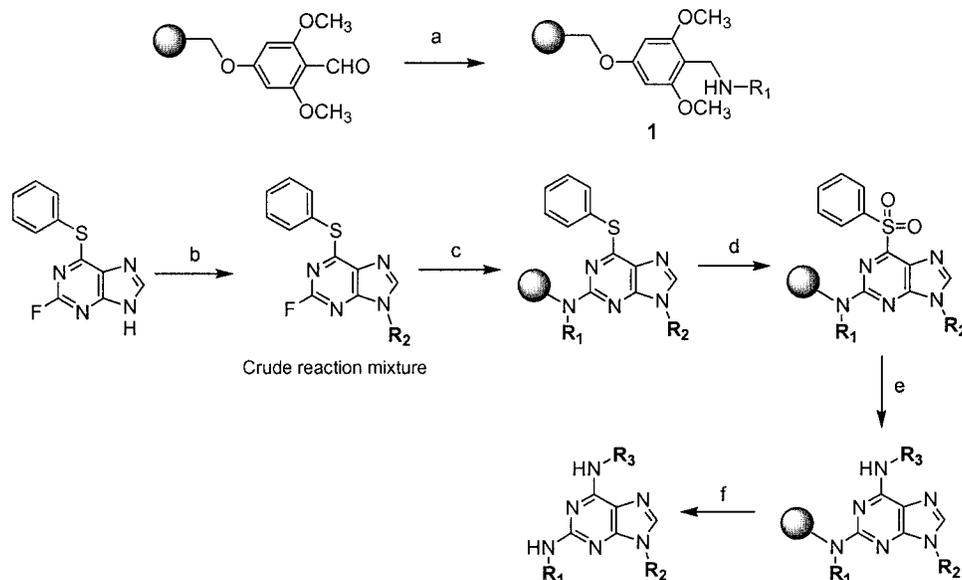
The starting scaffold, 2-phenylsulfenyl-6-chloropurine, can be obtained by thiophenol displacement of commercially available 2-bromohypoxanthine followed by chlorination with POCl₃ in 90% overall yield. The Mitsunobu reaction can first be used to carry out alkylation reactions at the N9 position. Resin-bound amine **1**, which is obtained by reductive amination of a 4-formyl-3,5-dimethoxyphenoxymethyl-functionalized polystyrene resin (PAL), is used to capture the N9-alkylated purine from the crude Mitsunobu reaction mixture by nucleophilic substitution at C6. Unfortunately, C2 oxidation to the sulfone and subsequent displacement with amines under a variety of conditions resulted in only the 2-phenylsulfonyl-6-amino-substituted purine product, consistent with the observation that nucleophilic displacement of C2 on a C6 amino-substituted purine is difficult.^{1,3}

To avoid substitution at the C6 position with an amine prior to derivatization of the C2 position, an approach was devised in which the purine is first linked to the solid support at the C6 via a thioether. The thioether-linked purine is obtained by resin capture of the crude N9 Mitsunobu alkylation product at C6 using a methylmercapto resin. The C2 position is subsequently derivatized by a nucleophilic substitution reaction with amines. The C6 substituent is then introduced by displacement of the sulfonyl group with amines after oxidative activation of the thioether linkage, and the final product is released into the reaction solution (Scheme 3). This approach offers the following advantages: (1) secondary amines can be introduced at the C2 position; (2) only the activated polymer-bound purine intermediate can be released; (3) the activated sulfone linker allows traceless cleavage, and (4) construction of focused C2 purine libraries

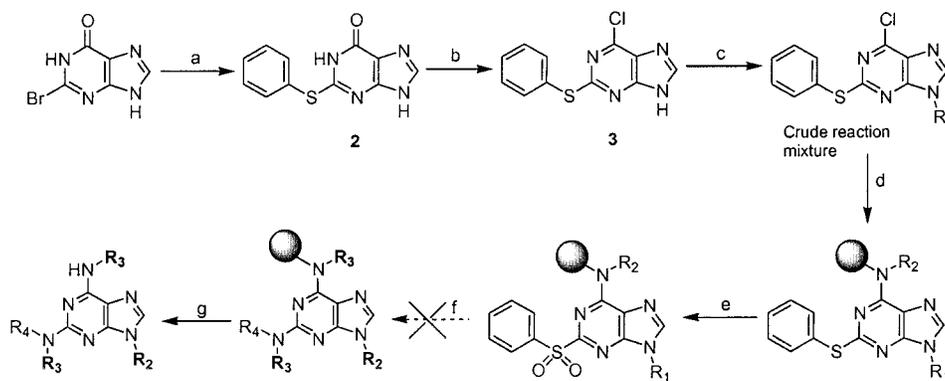
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Scheme 1. Traceless Combinatorial Approach toward 2,6,9-Trisubstituted Purine Libraries^a

^a (a) R_1-NH_2 , $NaBH(OAc)_3$, 1% HOAc, THF; (b) 1.5 equiv of R_2OH , 1.8 equiv of PPh_3 , 1.3 equiv of DiAD, THF, room temp; (c) 0.5 equiv of **1**, 1.5 equiv DiEA, BuOH, 80 °C; (d) 10 equiv of *m*-CPBA/NaOH (1:1), 1,4-dioxane with 10% H_2O ; (e) 2 equiv of R_3-NH_2 , anhydrous dioxane, 80 °C; (f) $CH_2Cl_2/TFA/Me_2S/H_2O$ 45:45:5:5.

Scheme 2. C2 Sulfon Group Cannot Be Displaced by Amines^a

^a (a) 2 equiv of thiophenol, 3 equiv of DiEA, MeOH, 80 °C; (b) 6 equiv of $POCl_3$, 2 equiv of Bu_4NCl , 1 equiv of *N,N*-dimethylaniline, anhydrous CH_3CN , reflux; (c) 1.5 equiv of R_1OH , 1.8 equiv of PPh_3 , 1.3 equiv of DiAD, THF, room temp; (d) 0.5 equiv of **1**, BuOH, 1 equiv of DiEA, 80 °C; (e) 10 equiv of *m*-CPBA/NaOH (1:1), dioxane; (f) 5 equiv of R_3R_4NH , 1,4-dioxane, 80 °C; (g) $CH_2Cl_2/TFA/Me_2S/H_2O$ 45:45:5:5.

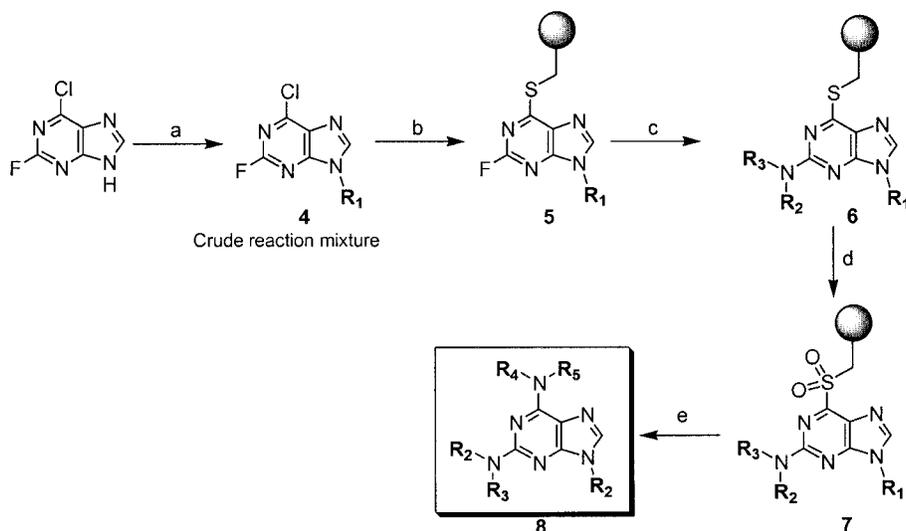
can readily be accomplished by coupling different amines at the C2 and using a single amine for the final displacement. This presents an advantage relative to scheme 1 due to the technical difficulty in preparing Pal-amine resins by reductive amination in an array format.

This chemistry has been validated for a broad range of substituents on the purine ring. Most primary and secondary alcohols lacking additional acidic hydrogens work well in the N9 Mitsunobu reaction.³ Quantitative alkylation of the starting purine can be achieved by using excess alkylating reagent (1.5 equiv of alcohol, 1.6 equiv of diisopropyl azodicarboxylate, and 2.0 equiv of triphenylphosphine). The Mitsunobu alkylation step can also be performed on solid support after the resin capture of 2-fluoro-6-chloropurine. This procedure resulted in very small quantities of the minor N7 Mitsunobu regioisomer but required considerably more alkylating reagents.

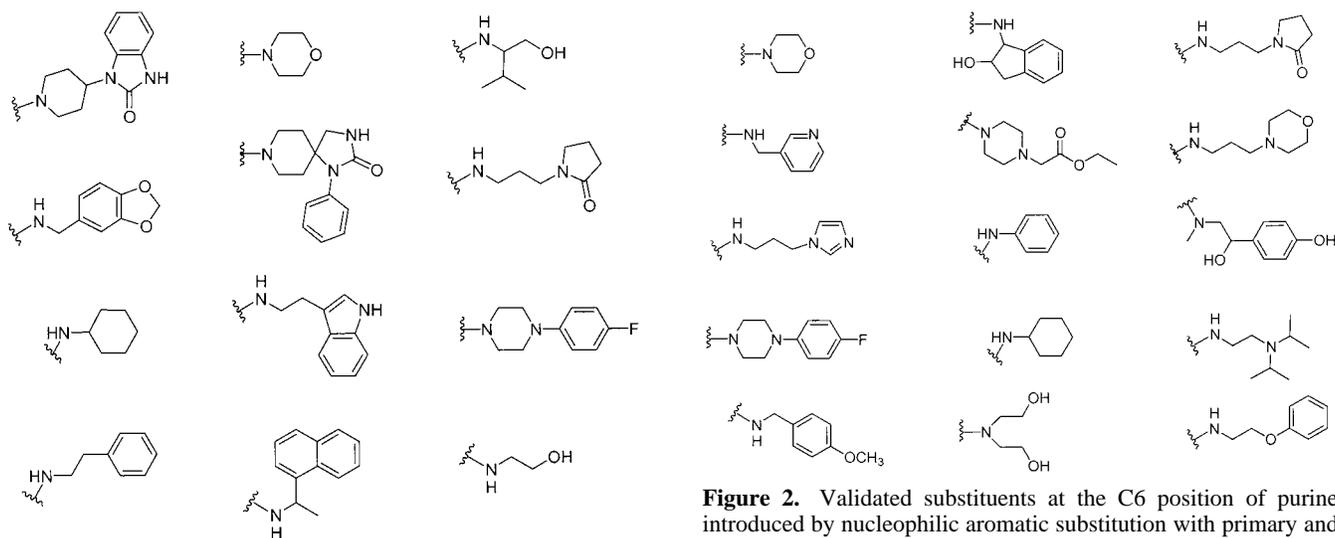
The C2 position can be substituted with variety of primary amines, such as sterically hindered 2-amino-3-methylbutanol, and cyclic and acyclic secondary amines (Figure 1). Normally

a total of 5 equiv of amines is used at a concentration of 2 M in butanol to ensure quantitative substitution of 2-chloropurine. Finally the C6 displacement of the sulfonyl group can be carried out with diverse primary and secondary amines and electron-rich anilines (Figure 2). Rather than use excess amine to quantitatively release resin-bound purine, which requires follow-up purification by solid-supported liquid extraction (SLE),⁵ a limited amount of amine (0.8 equiv) was used. This procedure essentially gave the pure purine product without the need of SLE purification. It should be noted that the final derivatization step must be carried under anhydrous conditions, since water can also react with the sulfonyl purine to release guanine as a byproduct.

In summary, we have developed an alternative approach for making combinatorial 2,6,9-trisubstituted purine libraries by capturing an N9-substituted 2-fluoro-6-chloropurine at the C6 position via a thioether linkage and subsequently modifying the polymer-bound purine intermediate at the C2 position, followed by substitution at the C6 position with concomitant release.

Scheme 3. Resin-Capture and Release Strategy toward Combinatorial Libraries of 2,6,9-Trisubstituted Purines^a

^a 1.5 equiv of R_1OH , 1.8 equiv of PPh_3 , 1.3 equiv of DiAD, THF, room temp; (b) 0.5 equiv of mercaptomethyl polystyrene resin, BuOH, 1 equiv of DiEA, 80 °C; (c) 3 equiv of R_2R_3NH , 4 equiv of DiEA, BuOH, 80 °C; (d) 10 equiv of *m*-CPBA/NaOH (1:1), dioxane; (e) 0.9 equiv of R_4R_5NH , anhydrous 1,4-dioxane, 80 °C.

**Figure 1.** Validated substituents at the C2 position of purine introduced by nucleophilic aromatic substitution with primary and secondary amines.

Experimental Section

General. The purity of compounds was assessed by reverse-phase liquid chromatograph and mass spectrometer (Agilent series 1100 LC-MS) with a UV detector at $\lambda = 255$ nm (reference at 360 nm) and an API-ES ionization source. NMR spectra were recorded on Bruker 400 and 500 MHz instrument that was calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to designate the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. LC elution methods (using a Phenomenex Luna 50 mm \times 2.00 mm, 5 μ m C18 column) were the following: (1) 10 min method, starting from 5% solvent A (acetonitrile) in solvent B (water with 0.5% acetic acid) and running the gradient to 95% A in 8 min, followed by 2 min elution with 95% A; (2) 6 min method, starting from 5% solvent A (acetonitrile) in solvent B (water with 0.5% acetic acid) and

Figure 2. Validated substituents at the C6 position of purine introduced by nucleophilic aromatic substitution with primary and secondary amines and anilines.

running the gradient to 95% A in 5 min, followed by 1 min elution with 95% A.

Representative Experimental Procedures. 2-Phenylsulfenylhypoxanthine (2). To a solution of 2-bromohypoxanthine (10.0 g, 46.5 mmol) in methanol (200 mL) was added thiophenol (9.55 mL, 93.0 mmol) and diisopropylethylamine (20.2 mL, 116.3 mmol). The reaction was stirred at 90 °C overnight. The solvent was removed under reduced pressure, and the solid was collected by filtration and washed with hexanes (100 mL \times 2). The collected solid was further purified by recrystallization from methanol to afford the desired product (10.1 g, 89% yield). ¹H NMR (400 MHz, $(CD_3)_2SO$): δ 7.50 (m, 3H), 7.62 (m, 2H), 7.97 (s, 1H). MS (ES) *m/z* calcd for $C_{11}H_8N_4OS$ [MH^+], 245.05; found, 245.05.

2-Phenylsulfenyl-6-chloropurine (3). To a flame-dried round-bottom flask (200 mL) was added 2-phenylsulfenylhypoxanthine **2** (1.22 g, 5.0 mmol), tetrabutylammonium chloride (anhydrous, 2.78 g, 10.0 mmol), and *N,N*-dimethylaniline (0.63 mL, 5.0 mmol), followed by dissolving them

in acetonitrile (anhydrous, 50 mL). To the solution phosphorus oxychloride (2.8 mL, 30.0 mmol) was added dropwise. The reaction was refluxed under argon. After 2 h, the reaction mixture was cooled to 0 °C on an ice bath, and the reaction was quenched by addition of saturated sodium bicarbonate solution. The aqueous layer was extracted with ethyl acetate (4 × 100 mL). The combined organic layers were dried over sodium sulfate and were concentrated on the rotary evaporator. The crude product was further purified by recrystallization from methanol to afford the desired product **3** as a white solid (0.94 g, 72%). ¹H NMR (400 MHz, (CD₃)₂SO): δ 7.50 (m, 3H), δ 7.65 (m, 2H), 8.53 (s, 1H). MS (ES) *m/z* calcd for C₁₁H₇ClN₄S [MH⁺], 263.02; found, 263.00.

Solution-Phase N9 Mitsunobu Alkylation of Purine (4).

To a flame-dried round-bottom flask (500 mL) was added 2-fluoro-6-chloropurine (7.0 g, 40.6 mmol), triphenylphosphine (19.2 g, 73.1 mmol), and alcohol (52.8 mmol), followed by dissolving them in THF (anhydrous, 350 mL). The solution was cooled to -30 °C, and diisopropyl azodicarboxylate (12.0 mL, 60.9 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred under argon. After overnight, the solvent was removed under reduced pressure and the crude material was directly used in the next step without further purification.

Resin Capture of N9-Alkylated Purine Scaffold at C6 (5).

To a solution of crude 2-fluoro-6-chloro-9-alkylpurine (15.0 mmol) in *n*-butanol (200 mL) was added mercaptomethylpolystyrene resin (10.0 mmol, Midwest Biotech), followed by addition of diisopropylethylamine (5.2 mL, 30.0 mmol). The suspension was heated to 80 °C under argon. After overnight, the resin was washed with methanol (200 mL × 4) and dichloromethane (200 mL × 4) and dried under vacuum.

C2 Amination of Captured Purine (6).

Resin **5** (0.10 mmol) was suspended in *n*-butanol (1.0 mL), followed by addition of an amine (0.30 mmol) and diisopropylethylamine (0.40 mmol). After overnight shaking at 80 °C, the resin was washed with methanol (3 mL × 4) and dichloromethane (3 mL × 4) and dried under vacuum.

Activation of C6 by Oxidizing Sulfonyl Group to Sulfonyl Group (7).

To a solution of *m*-CPBA (0.23 g, 75%, 1.0 mmol) in 1,4-dioxane (9 mL) cooled to 0 °C was added an NaOH (1 mL, 1 M, 1.0 mmol) aqueous solution, followed

by addition of resin **4** (0.10 mmol). The suspension was shaken gently at room temperature. After 8 h the resin was washed with methanol (3 mL × 4) and dichloromethane (3 mL × 4) and dried under vacuum.

C6 Displacement with Amines and Product Releasing (8).

Resin **7** (0.05 mmol) was suspended in anhydrous 1,4-dioxane (0.6 mL), followed by addition of an amine (0.1 mmol). After overnight shaking at 80 °C, the resin was filtered using a polypropylene cartridge (45 μm PTFE frit), and the flow-through solution was collected. The resin was subsequently washed with dichloromethane (0.5 mL × 3), the flow-through was combined, and the solvent was removed under reduced pressure to afford the desired product **8** (on average, >85% HPLC purity, 80% purified yield).

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Supporting Information Available.

Detailed experimental procedures and spectral data of the compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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