A Concise and Traceless Linker Strategy toward Combinatorial Libraries of 2,6,9-Substituted Purines

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Introduction

Despite concerns that it would be extremely difficult to design specific ATP competitive inhibitors of kinases, there have been a number of success stories including the p38 Map kinase, tyrosine kinases, and cyclin-dependent kinases.[1,3] Selective inhibitors of each of these kinases are in various stages of clinical testing. The ability to discriminate between extremely homologous kinases such as CDK1 vs CDK2 has been demonstrated by the development of novel thioflavopiridol derivatives that display enhanced selectivity for CDK1 relative to CDK2.[2]

To date, a variety of heterocyclic scaffolds including pyrimidines, indolines, pyrrolopyrimidines, indirubins, purines, quinazolines, trisubstituted imidazoles, pyrazolopyrimidines, flavones, and anilinoquinolines have been developed as kinase inhibitors.[1,3] As each scaffold presents unique opportunities for the presentation of functional groups to the kinase active site, there is a need for efficient and flexible methods for preparing libraries of each of these inhibitor classes. We have chosen to focus our development efforts toward the purine nucleus for several reasons: (1) solid-phase and solution-phase purine chemistries have been sufficiently explored such that unified schemes toward the derivatization of the 2-, 6-, 7-, 8-, and 9-positions[4] should be possible; (2) purines have been demonstrated to provide high-affinity ligands for a variety of proteins; and (3) solid-phase methods used to prepare purine libraries such as resin capture, nucleophilic aromatic substitution reactions, Mitsunobu alkylations, and palladium coupling reactions are readily generalized to other heterocyclic systems of interest.

Several solid- and solution-phase approaches for the synthesis of purine analogues have been reported in the literature over the past 5 years.[4] One limitation of these approaches is that one substituent is held invariant in order to anchor the purine ring to the solid phase (Scheme 1). To avoid this limitation, a "traceless" strategy was desired that would be compatible with production-scale library synthesis in spatially separate or divide−recombine formats.

Another limitation of previous synthetic approaches[4g] is the low reactivity of the 2-fluoro group once an amino substituent has been installed at C6. For example, complete displacement at C2 of a 2-fluoro-6-benzylaminopurine in solution requires heating at over 100 °C for 12 h using *n*-butanol as solvent. Complete aromatic substitution of 2-fluoro or 2-chloro purine compounds on solid support requires even higher temperatures and often results in significant side reactions. This limits the range of functional groups that can be installed at C2 and also creates difficulties in library production.

Results and Discussion

We found that 6-amino-2-fluoro-9-alkylpurines react with primary amines in methanol at room temperature. With slightly more forcing conditions (in refluxing methanol), sterically hindered amines such as the α-amino group of arginine can be successfully introduced at the C2-position (Scheme 2) with good yields. Unfortunately, these conditions failed to translate to solid support, presumably due to resin swelling problems in methanol. Despite testing a range of solvent systems (NMP, DMF, dioxane, DMSO, THF, and their combinations such as $DMF^{V}/MeOH^{V}$ 1/1), no solvent was found that allowed complete substitution below 100 °C.

One possible solution to this problem involves C2 substition prior to substitution at C6. This requires reversing the natural reactivity which favors initial substitution at C6. We found that a C6 sulfenylpurine, such as 2-fluoro-6-phenylsulfenyl (or a 6-benzylsulfenyl) purine, directs quantitative and selective substitution by an amine to the C2-position at 80 °C (Scheme 3). To develop this as a combinatorial scheme, we envisioned that we could subsequently substitute C6 after oxidation of the thioether to the sulfone as has been demonstrated in the synthesis of 2,4-diaminopyrimidine.[5] The 2-fluoro-6-thiophenylpurine was easily prepared by reacting excess thiophenol with 2-fluoro-6-chloropurine in methanol at 0 °C and then purifying by recrystallization.

We have previously demonstrated that the N9-position can be alkylated on solid support under Mitsunobu

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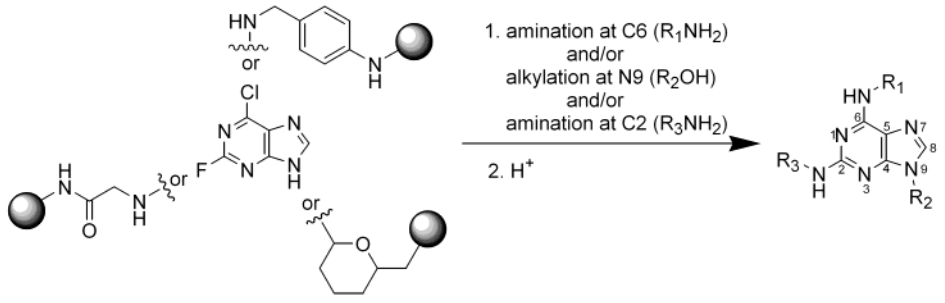
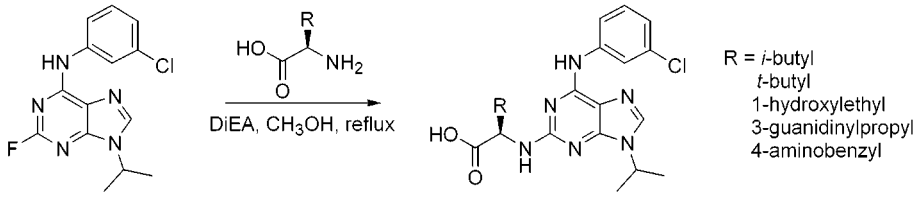
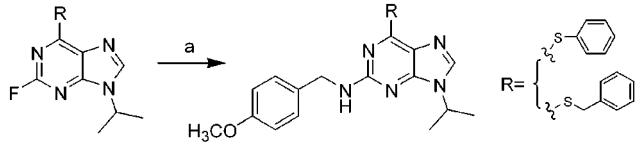
Scheme 1. Previously Described Combinatorial Strategies for 2,6,9-Trisubstituted Purines



Scheme 2. Coupling Amino Acids to Purine C2-Position



Scheme 3. Sulfenyl Group Protecting Purine C6-Position[a]

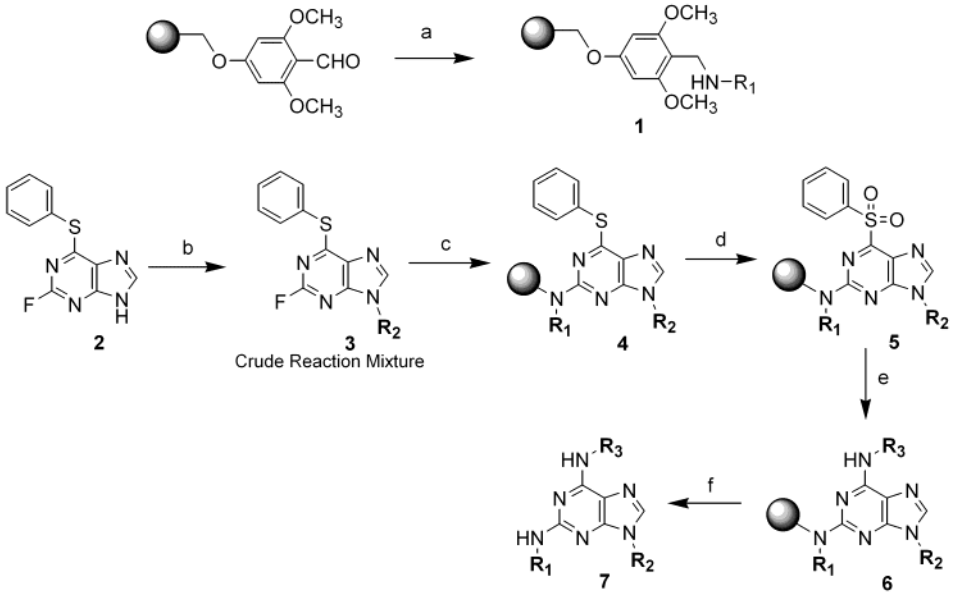


[a] (a) 2 equiv of 4-methoxybenzylamine, 3 equiv of DiEA, BuOH, 80 °C.

conditions with a variety of alcohols. However, performing the N9-modification reaction on support had several drawbacks, including incomplete alkylation with secondary alcohols, consumption of large excesses of reagent, and inconvenient handling of reaction in a 96-well format. To circumvent this problem, we devised a scheme whereby a resin-bound amine is used to capture a C6-phenylsulfenyl-N9-alkylpurine directly from the crude reaction mixture. This allows the moisture-sensitive Mitsunobu reaction to be performed as the first combinatorial step in solution, making the overall scheme more convergent.

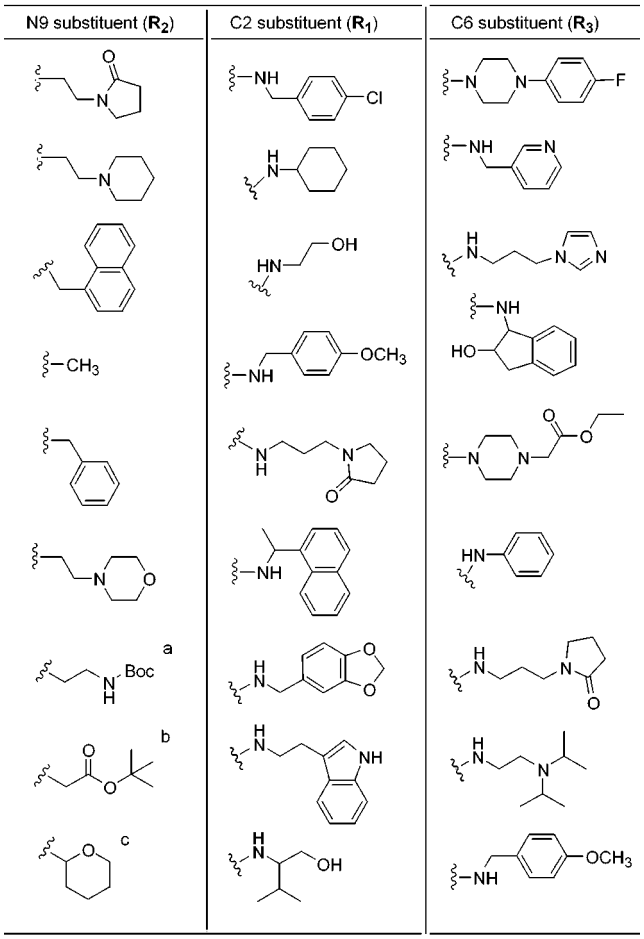
To achieve a "traceless" linkage to solid support, primary amines were coupled by reductive amination using sodium triacetoxyborohydride to a 4-formyl-3,5-dimethoxyphenoxymethyl-functionalized polystyrene resin (PAL).[6] The purine ring was then captured at the C2-position by reacting the PAL-amine resin with 1.5 equiv of the crude N9-alkylated 2-fluoro-6-phenylsulfenylpurine and 3 equiv of diisopropylethylamine in *n*-butanol at 80 °C. The C6-position could then be substituted following oxidation−activation of the thioether to the sulfone (Scheme 4). The use of *m*-chloroperbenzoic acid to oxidize the thioether linkage resulted in the premature cleavage from solid support, presumably as a result of acid and

Scheme 4. Traceless Combinatorial Approach toward 2,6,9-Trisubstituted Purine Library[a]



[a] (a) 5 equiv of R_2-NH_2, 3 equiv of $NaBH(OAc)_3$, 1% HOAc, THF; (b) 1.5 equiv of R_2OH, 1.8 equiv of PPh_3, 1.3 equiv of DiAD, THF, rt; (c) 0.5 equiv of **1**, 1.5 equiv of 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.

Table 1. N-9 Substituent (R_2) Introduced by Alkylation; C-2 Substituent (R_1) Introduced through Resin-Bound Amines; C-6 Substituent (R_3) Introduced by Displacement with Amines[a]



[a] Except c, which was made by reacting purine with 3 equiv of DHP and catalytic PPTS, and b, which was made by alkylation with 3 equiv of *tert*-butyl bromoacetate and 4 equiv of K_2CO_3 in DMF, all other substituents (R_2) were introduced by Mitsunobu alkylation. It should be noted that after final TFA cleavage, a, b, and c gave rise to the corresponding deprotected forms.

oxidant sensitivity of the benzylic PAL-amine linkage. This problem was overcome by performing the oxidation in buffered solution with *m*-chloroperbenzoic acid that had been neutralized with a stoichiometric amount of sodium hydroxide.

This combinatorial scheme has the following merits: (1) the difficulty of C2 substitution is overcome by "directing" the first substitution to C2 by using a phenylsulfenyl group as a "protective" group at the C6-position; (2) N9 modification is accomplished in solution and purified by resin-capture; (3) the PAL linker allows traceless cleavage; and (4) construction of focused C6 purine libraries can readily be accomplished because this is the last step in the combinatorial synthesis scheme.

The scope of the chemistry has been validated for a range of substituents. Analysis of the final products following the general protocol described above by LC-MS revealed greater than 95% conversion of the starting materials with crude HPLC purities over 85%. Most primary and secondary alcohols lacking additional acidic hydrogens work well in the Mitsunobu reaction at N9 (Table 1). As the Mitsunobu is performed on the C6-phenylsulfenylpurine there is very little detectable contamination from the N7 regioisomer. Alkylation can also be performed with active alkyl bromides (such as *tert*-butyl bromoacetate) or N9 can be temporally protected with the THP group to provide additional diversity. C2 substituents are limited to primary amines (Table 1) due to the need for a reaction site to attach the purine to solid support. Immoblized anilines only result in partial capture of the purine from solution. Because *m*-chloroperbenzoic acid is used to convert the C6 thioether to a sulfone in the final activation step, substituents having functional groups prone to oxidation cannot be used in the first two derivatization steps. C6 displacement of the sulfone works for primary amines, cyclic secondary amines (such as piperazines), and electron-rich anilines (Table 1). The displacement reaction fails for many acyclic, sterically hindered secondary amines and electron-poor anilines.

Conclusions

In summary, we report a concise and traceless linker strategy toward making 2,6,9-trisubstituted combinatorial purine libraries. Resin-bound amines are used to capture a C6-phenylmecapto-9-alkylated purine directly from the crude Mitsunobu alkylation reaction mixture. The C6-position is then subsituted following oxidation−activation of the thioether to the sulfone. This strategy overcomes the difficulty of C2 substitution by "directing" the first substitution to C2 by using a phenylsulfenyl group to protect the C6-position. A 1000-compound combinatorial purine library has been synthesized using this approach in a 96-well format and will be reported elsewhere.

Experimental Section

General. N9 Mitsunobu alkylation reactions and C6 sulfone displacement reactions were carried out under anhydrous conditions under an argon atmosphere. C2 resin capture reactions were carried out in 4 mL scintillation glass vials unless otherwise noted. Anhydrous tetrahydrofuran and 1,4-dioxane were obtained by passing them through commercially available alumina columns. All other reagents, resins, and solvents were purchased at highest commercial quality and used without further purification. Purity of compounds was assessed by reverse-phase liquid chromatography−mass spectrometry (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 instruments and 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 × 2.00 mm 5 μm C18 column): (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) 16 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 14 min, followed by 2 min elution with 95% A.

General Procedure for the Combinatorial Synthesis of 2,6,9-Trisubstituted Purines. (a) Reductive Amination for the Synthesis of PAL-Resin-Bound Amine (1). To a suspension of 4-formyl-3,5-dimethoxyphenoxymethyl-functionalized polystyrene resin (PAL) (10.0 g, 11.3 mmol) in DMF (350 mL) was added a primary amine (56.5 mmol), followed by addition of

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sodium triacetoxyborohydride (7.18 g, 33.9 mmol) and acetic acid (6.52 mL, 113 mmol). The mixture was shaken gently at room temperature for 12 h and then washed with methanol (300 mL × 4) and dichloromethane (300 mL × 4) and dried under vacuum. The complete conversion of PAL aldehyde to resin-bound amine was confirmed by disappearance of the aldehyde stretch.

(b) 2-Fluoro-6-phenylsulfenylpurine (2). To a solution of 2-fluoro-6-chloropurine (10.0 g, 57.9 mmol) in methanol (200 mL) was added diisopropylethylamine (25.2 mL, 144.7 mmol). The mixture was cooled to 0 °C and followed by slow addition of thiophenol (11.9 mL, 115.8 mmol) via an addition funnel over 1 h. The reaction was stirred at 0 °C for 12 h. The solvent was then removed under reduced pressure, and the solid was collected by filtration and washed twice with hexanes. The collected solid was further purified by recrystallization from methonal to afford the desired product (11.8 g, 83% yield). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.54 (m, 3H), 7.66(m, 2H), 8.49(s, 1H); MS $C_{11}H_7FN_4S$ [MH^+] 246.04, found 247.05

(c) 2-Fluoro-6-phenylsulfenyl-9-alkylpurine (3). To a flame-dried round-bottom flask (500 mL) were added 2-fluoro-6-phenylsulfenylpurine (10.0 g, 40.6 mmol), triphenylphosphine (19.2 g, 73.1 mmol), and alcohol (52.8 mmol), followed by dissolution 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 was allowed to warm to room temperature and stirred under argon. After overnight stirring, the solvent was removed under reduced pressure and the crude material was directly used in the next step without further purification.

Resin Capture of 3 at C2 from Crude Mitsunobu Reaction 4. To a solution of crude 2-fluoro-6-phenylsulfenyl-9-alkylpurine (0.15 mmol) in *n*-butanol (1.0 mL) was added PAL-resin-bound amine **1** (0.10 mmol), followed by addition of diisopropylethylamine (0.30 mmol). The suspension was heated to 80 °C under argon. After 12 h, the resin was washed with methanol (3 mL × 4) and dichloromethane (3 mL × 4) and dried under vacuum. The complete conversion of secondary amine (PAL-amine) to tertiary amine was confirmed using the bromophenol blue test.[7]

Activation of C6 by Oxidation of Thioether to Sulfone (5). 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, 1M, 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 (6) and Product Cleavage (7). The resin **5** (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 washed with methanol (1 mL × 4) and dichloromethane (1 mL × 4) and dried under vacuum to afford resin **6**. Resin **6** was subsequently cleaved using CH_2Cl_2:TFA:Me_2S:H_2O 45:45:5:5/v:v:v:v (0.5 mL) to afford desired product **7** (in average >85% HPLC purity, 80% purified yield).

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Supporting Information Available: Detailed experimental procedures and spectra data of the compounds disclosed. This material is available free of charge via the Internet at http://pubs.acs.org.

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