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Utility of 4,6-dichloro-2-(methylthio)-5-nitropyrimidine. Part 3: Regioselective solid-phase synthesis of a 2,6,8,9-tetrasubstituted purine library☆

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Abstract—The first regiocontrolled solid-phase synthesis of a 2,6,8,9-tetrasubstituted purine library was performed through on-resin elaboration of 4,6-dichloro-2-(methylthio)-5-nitropyrimidine. A series of primary amines were loaded on ArgoGel-MB-CHO resin via reductive amination to yield secondary amines. Subsequent attachment of the starting pyrimidine core unit and C6-chloride substitution by primary amines yielded the resin-bound 4,6-disubstituted-2-methylthio-5-nitropyrimidines. Oxone® mediated oxidation of the 2-methylthio moiety to the corresponding sulfone allowed facile substitution at the 2-position. $CrCl_2$ assisted reduction of the nitro group, followed by acid catalyzed orthoester cyclization and finally TFA mediated cleavage provided the tetrasubstituted purine final products. Most of the final purines were cleaved in good to excellent yield and purity, however, it was found that bulky groups at N9 hindered cyclization in C8-substituted derivatives. For these systems, LC purification of the crude cleavage products provided the target purines in high purity.

1. Introduction

Synthetic purines have been shown to exhibit a wide range of bioactivity and have been the focus of recent attempts to design selective ATP-site competitive inhibitors of kinases, such as cyclin-dependent kinases (CDKs). CDKs are responsible for a number of cell cycle and division processes and are thus interesting therapeutic targets for the treatment of several diseases.[2] Examples of purine derivatives which have shown promising bioactivity toward CDKs are olomoucine (**1**), roscovitine (**2**), and purvalanol A (**3**) (Fig. 1).[3] Due to the ubiquity of kinases in biological systems, however, selectivity of designed inhibitors is generally poor. For example, olomoucine has an IC_{50} value of 7 μM against CDK-1, but is equally active against CDK-2.[4] The same selectivity issue is associated with roscovitine. Despite these selectivity concerns, purines offer an exceptional scaffold for the synthesis of potent kinase inhibitors for two obvious reasons: (1) the structural similarity between designed targets and the purine base of ATP; and (2) the high level of regio-isolated functionalization that is possible on the purine core.

A number of methods have been presented in recent years for the solid-phase synthesis of substituted purine derivatives. Although individual variations have been advanced, these methods can be generally classified into two categories, the first of which is far more prevalent: (1) attachment of a polyhalogenated purine onto a prefunctionalized resin, followed by subsequent halide substitution and other functionalization, such as N9-alkylation under Mitsunobu-type conditions[5] and (2) a polyfunctional pyrimidine core is attached to the resin and is subsequently elaborated into the corresponding purine.[6] Both of these approaches allow for the preparation of 2,6,9-substituted purines. However, to our knowledge, no method has been set forth which allows for the solid-phase synthesis of 2,6,8,9-tetrasubstituted purine derivatives that is directly applicable to parallel synthesis.[7] In addition, previous methods face one or several limitations. Substitution of polyhalogenated purines is often nonregiospecific as are Mitsunobu-type alkylations, which also limits the range of substitutents that can be introduced at N9 into products derived from

☆ See Ref. 1.

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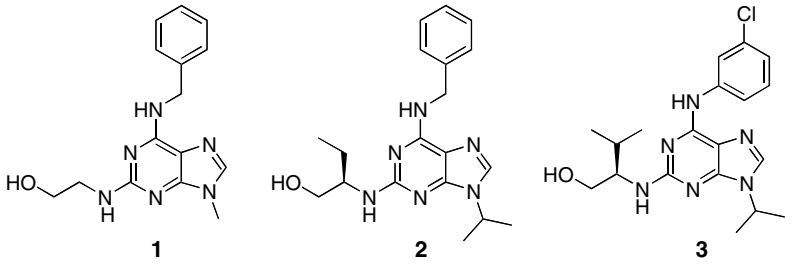


Figure 1. Biologically active purine compounds olomoucine (**1**), roscovitine (**2**), and purvalanol A (**3**).

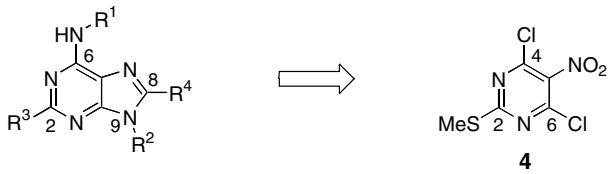


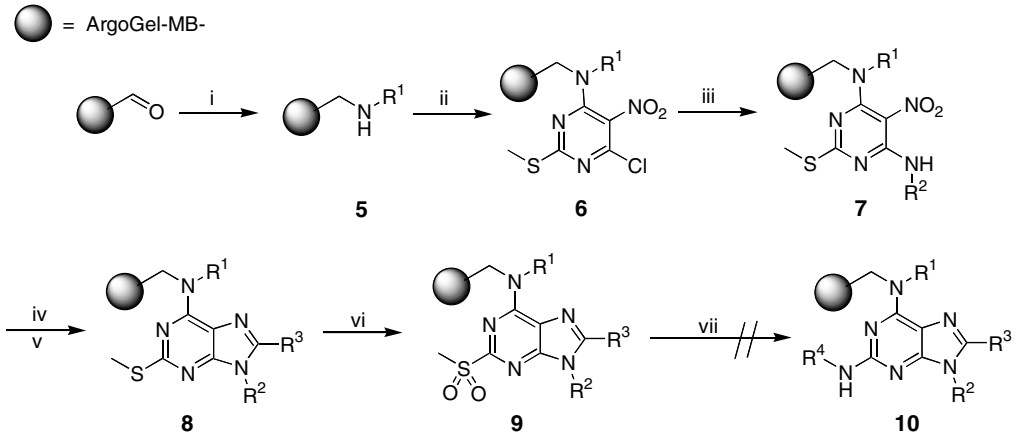
Figure 2. Numbering schemes of 2,6,8,9-tetrasubstituted purines accessible from the starting material 4,6-dichloro-2-methylthio-5-nitropyrimidine **4**.

primary or secondary alcohols. In addition to exacerbating the difficulty of synthesis, this severely limits the diversity space that can be explored by these methods. In order to find purine derivatives with greater selectivity it is necessary to allow for maximum diversity at all possible positions on the scaffold. The method by which the compounds are prepared must allow for complete regiocontrol and all steps must be facile and high yielding. With this in mind, we present a new method of solid-phase purine synthesis based on functionalization of the starting material 4,6-dichloro-2-(methylthio)-5-nitropyrimidine **4** (Fig. 2). Elaboration of this starting scaffold allows for the synthesis of structurally diverse 2,6,8,9-tetrasubstituted purines in seven on-resin steps with complete regiospecificity at every step. In addition to allowing variation at all four positions, this method allows for the incorporation of a wide variety of substituents, including phenyl and *t*-butyl, at the 9 position.

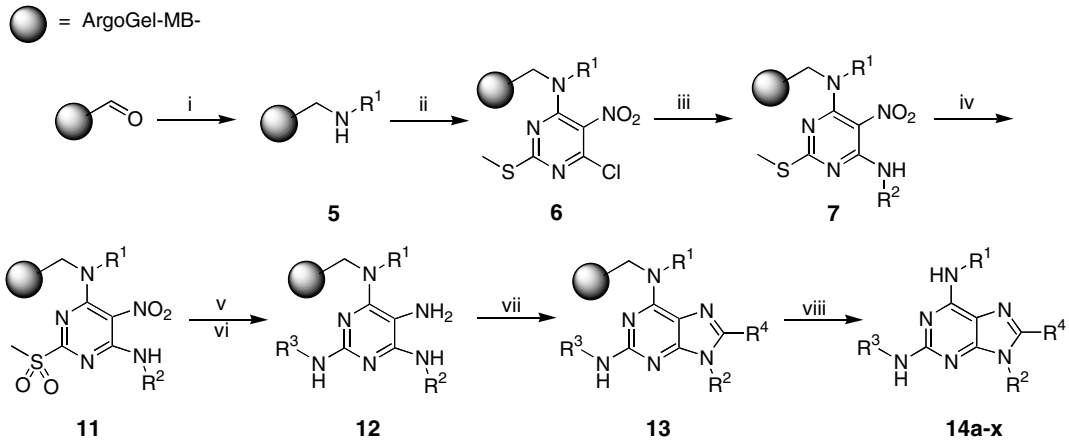
2. Results and discussion

Our original solid-phase synthetic route was based on attachment of **4** to a prefunctionalized resin, which was prepared by reductive amination of ArgoGel-MB-CHO with amine R^1–NH_2, substitution at C6 with amine R^2–NH_2, and subsequent oxidation/substitution at the 2-position after reduction/cyclization had taken place according to Scheme 1. However, as has been shown in previous similar attempts,[5n] substitution of an alkyl- or arylsulfone from the 2-position of a 6-aminosubstituted purine is exceedingly difficult with nitrogen nucleophiles. Even the use of sodium or lithium amide salts required several days at elevated temperatures (130–150 °C) to realize 30% C2-substitution. This observation was confirmed on-resin as well as in solution.

To avoid the difficulty of substitution at the 2-position, the synthetic route was redesigned to take advantage of the activating effect of the 5-nitro substituent, thus leaving reduction/cyclization to the final on-resin step of the synthesis (Scheme 2). Using this approach, substitution of the 4-chloro, 6-chloro, and 2-methylsulfonyl positions could be realized at room temperature within 3 h. Due to the acid-labile nature of the linker used, it was necessary to carefully choose the oxidation method of the 2-methylthio substituent. *m*-Chloroperbenzoic acid was found to partially cleave the substrate from the resin, as was Oxone® alone. However, $NaHCO_3$ buffered



Scheme 1. Original synthetic approach to 2,6,8,9-tetrasubstituted purines. Reagents and conditions: (i) R_1–NH_2 (3 equiv), $Na(OAc)_3BH$ (3 equiv), DCE, 24 h, rt; (ii) **4** (3 equiv), DIEA (3 equiv), THF, 1 h, rt; (iii) R_2–NH_2 (3 equiv), DIEA (3 equiv), THF, 1 h, rt; (iv) $CrCl_2$ (10 equiv), 20:1 DMF/MeOH, 4 h, rt; (v) R_3–$C(OCH_3)_3$, $MeSO_3H$ (cat.), 24 h, 80 °C; (vi) Oxone® (2.5 equiv), $NaHCO_3$ (5 equiv), 20:10:1 MeOH/DCM/H_2O, 24 h, rt; (vii) R_4–NH_2 or R_4–NHM (M = Na, Li), DMF, 130 °C, 7 days.



Scheme 2. Revised synthetic approach to 2,6,8,9-tetrasubstituted purines. Reagents and conditions: (i) R_1–NH_2 (3 equiv), $Na(OAc)_3BH$ (3 equiv), DCE, 24 h, rt; (ii) **4** (3 equiv), DIEA (3 equiv), THF, 1 h, rt; (iii) R_2–NH_2 (10 equiv), DIEA (3 equiv), THF, 1 h, rt; (iv) Oxone® (2.5 equiv), $NaHCO_3$ (5 equiv), 20:10:1 MeOH/DCM/H_2O, 24 h, rt; (v) R_3–NH_2 (3 equiv), DIEA (3 equiv), 1 h, rt; (vi) $CrCl_2$ (15 equiv), 20:1 DMF/MeOH, 4 h, rt; (vii) R_4–$C(OCH_3)_3$, $MeSO_3H$ (cat.), 48 h, 80 °C; (viii) 9:1 TFA/H_2O, rt, 3 h.

Oxone® was found to be satisfactorily mild to avoid cleavage, while allowing for clean conversion to the corresponding sulfone. In contrast to the results obtained using the resin-bound 2-methylsulfonyl purine **9** (Scheme 1), displacement proceeded smoothly using the resin-bound 2-methylsulfonyl pyrimidine **11** (Scheme 2). We were initially frustrated with the reduction of the C5-nitro group. Reduction of resin-bound aryl nitro groups has been reported to be problematic due to incomplete reduction, premature cleavage of the substrate from the resin, or subsequent contamination with large amounts of inorganic salts. This step is further complicated by the apparent susceptibility of the intermediate tetraaminopyrimidine to reoxidize to the corresponding hydroxylamino, nitroso, or nitro in air upon washing of the resin.

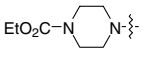
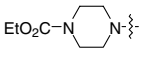
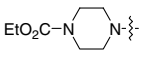
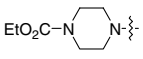
A large number of reagents and conditions were attempted to realize this crucial step, including $SnCl_2$, $SnCl_2{\cdot}H_2O$, and $Na_2S_2O_4$. Some success was realized through the use of dioctylviologen dihydrobromide in concert with $Na_2S_2O_4$ according to the methods of Park[8a] and Makara,[8b] however, the best reproducible results were found utilizing a variation of the method reported by Hari and Miller[9] ($CrCl_2$/DMF/MeOH). Interestingly, the use of anhydrous $CrCl_2$ in DMF without the presence of MeOH results in almost quantitative cleavage of the substrate from the resin. Presumably, the presence of a protic solvent does not allow a strong Lewis acid interaction of the chromium with the resin-bound purine, which may lead to premature release from the acid-labile methoxybenzyl linker. Following the successful reduction of the nitro group, purine formation (**13**) was realized by acid catalyzed cyclization in the presence of an orthoester. Finally, cleavage to afford **14** was effected in the standard fashion using TFA in water.

Table 1 shows a representative library that was prepared using the above described method. While only a small set is shown as being used for R^1 in the initial reductive amination, we have also applied this methodology using anilines to create compounds similar to purvalanol A. We have also used less nucleophilic amines (including primary amines) as R^3 in the sulfone displacement, as has been demonstrated in our earlier work.[1] As one would expect, purity is lower in compounds incorporating a bulky substituent at N9, such as a *t*-butyl group, as these substrates show slower cyclization kinetics than smaller substituents. The lower purity of these targets is due to significant contamination from the noncyclized acetamide in the crude cleavage products (for example, when $R^4 = Me$; see table entry **14l**). This was not observed with the corresponding formamides ($R^4 = H$, see table entry **14k**). A similar phenomenon was observed with N9-phenyl substituted derivatives (e.g., **14e** vs **14f**), presumably due to steric effects in addition to the lower nucleophilicity of the aniline-like nitrogen. These limitations could likely be overcome by optimization of the reaction time for those substrates with bulky substitutions at N9 when $R^4 \neq H$. Despite these limitations, access to the targeted purines in acceptable purity was readily realized following LC purification of the crude cleavage products.

3. Conclusion

We have reported on an example of our solid-phase methodology to the library synthesis of highly substituted purines. For most of the library members, the overall yield and purity of the crude cleavage products was acceptable. In the case of the more highly substituted compounds it was sometimes necessary to resort to LC purification of the crude cleavage product to achieve good purity. Despite this limitation, the method allows for access to fully substituted purines which are not readily available by other methods. Coupled with our solution phase protocol,[1a] the utility of the title compound **4** with respect to purine synthesis has been firmly established.

Table 1. Synthesized 2,6,8,9-tetrasubstituted purine derivatives **14a**–**x** prepared according to the presented method

Compound	R^1	R^2	R^3	R^4	Purity[a]
14a					95
14b					87
14c					98
14d					85
14e					74 (100)
14f					57 (96)
14g					65 (100)
14h					50 (87)
14i					89
14j					26 (93)
14k					92
14l					18 (100)
14m					97
14n					91
14o					94
14p					78 (100)
14q					59 (100)
14r					36 (95)
14s					47 (98)
14t					33 (91)
14u					91
14v					33 (100)
14w					92
14x					21 (91)

[a] Crude purity. Number in parentheses represents purity after prep LC purification.

Supplementary data

Experimental details including full characterization for compound **14a** as a representative example can be found in the supplementary data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.02.104.

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