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Solid-phase synthesis of N-9-substituted 2,8-diaminopurines

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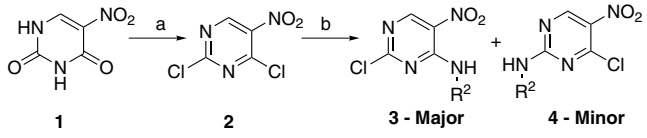
Abstract—A general and efficient solid-phase synthesis of N-9-substituted 2,8-diaminopurines from 5-nitrouracil is described. The key synthetic transformation employs a carbodiimide-mediated cyclization of a thiourea. Thiourea formation on solid phase is performed using both thermal and microwave reaction conditions. Regiospecific solution-phase synthesis of key building blocks allows the incorporation of desired substituents at N-9 of the purine nucleus.

Purines represent an important class of compounds from a biochemical perspective with a myriad of physiological processes being controlled or supported by agents based on the purine core. The diversity of the purine nucleus within the complex science of nature ranges from inclusion in nucleic acids to molecules controlling key neurological functions.[1] In addition, the natural diversity present within the ATP binding sites of kinases provides the opportunity to develop selective purine-based kinase inhibitors with enormous therapeutic potential.[2,3] The importance of purines and purine mimics in pharmaceutical research necessitates the development of efficient and versatile syntheses of such molecules.[4]

In recent years, there have been significant advances in solid-phase chemistry, facilitating the synthesis of combinatorial and parallel compound collections.[5–7] A number of solid-phase syntheses of purine-based compounds have been reported, using either a scaffold-based approach[8] or an on-resin strategy for the formation of these purine derivatives.[9] The aim of this project was to develop a general and efficient solid-phase synthesis of N-9-substituted 2,8-diaminopurines that allows diversity elements to be incorporated at C-2, C-8 and N-9. The development of a robust solid-phase synthesis would subsequently be used to generate parallel and combinatorial N-9-substituted 2,8-diaminopurine compound collections.[10]

5-Nitrouracil (**1**) was selected as the basis for construction of the purine ring system, fixing the C-6 substituent of the generated purine as hydrogen. This also allows structural diversity to be incorporated at the 2-, 8- and 9-positions of the purine core. The initial conversion of **1** to the common intermediate 2,4-dichloro-5-nitropyrimidine (**2**) was performed using phosphorus oxychloride. Solution-phase amination of **2** allows regioselective nucleophilic displacement to be conducted at C-4 based on the increased reactivity of the 4-chloro position. The reaction of **2** with a selection of primary amines and primary anilines in THF at −78 °C resulted in the formation of **3** and **4** with the ratios of regioisomers between 10:1 and 20:1 (Scheme 1). The desired major regioisomers **3** were readily isolated by flash chromatography to provide the key intermediates for incorporation into the solid-phase synthesis.

The development of a viable solid-phase synthesis necessitates a connection point to the solid support. Using intermediates **3**, solid-phase attachment can efficiently be achieved through C-2 of the pyrimidine via chlorine displacement with a resin-bound secondary amine **8**



Scheme 1. Reagents and conditions: (a) $POCl_3$, *i*-Pr_2NEt, 0–25 °C, 70%; (b) R^2NH_2, *i*-Pr_2NEt, THF, −78°C.

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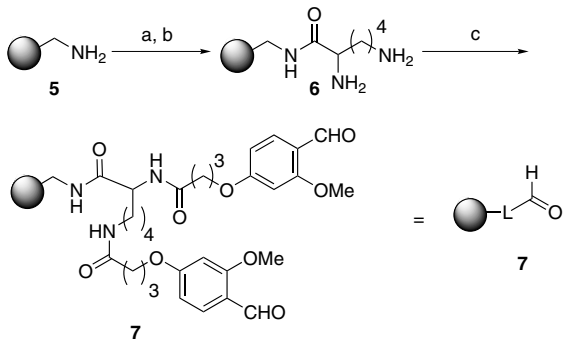
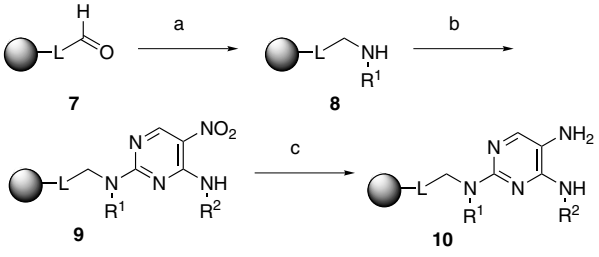


Scheme 2. Reagents and conditions: (a) *N*-α-*N*-ε-bis-Fmoc-Lys, HOBt monohydrate, DIC, CH_2Cl_2, DMF, 25 °C; (b) piperidine, DMF, 25 °C; (c) 4-(4′-formyl-3′-methoxy)phenoxybutyric acid, HOBt monohydrate, DIC, CH_2Cl_2, DMF, 25 °C.

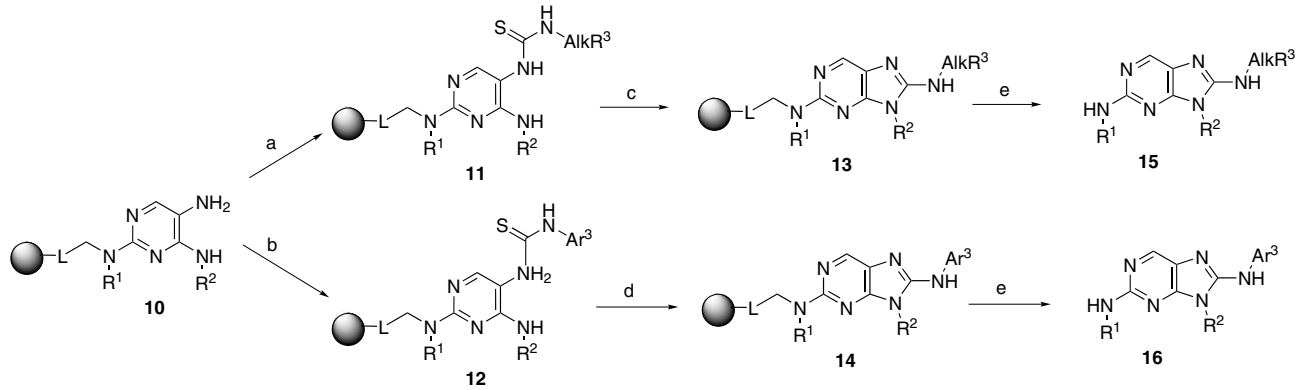
(vide infra). Solid-phase synthesis was initiated by acylating aminomethyl-terminated Argogel® (**5**) with *N*-α-*N*-ε-bis-Fmoc-lysine followed by Fmoc deprotection to generate **6** (Scheme 2). This increases the loading capacity of the resin by doubling the number of amino groups for further functionalization. This was followed by acylation with 4-(4′-formyl-3′-methoxy)phenoxybutyric acid. This linker allows acid-mediated cleavage of the final compounds from solid phase, and is compatible with the chemistry required to conduct the purine synthesis. The generation of a resin-bound secondary amine **8** was achieved by the reductive alkylation of a primary amine (R^1NH_2) with **7** using sodium triacetoxyborohydride in 1,2-dichloroethane (Scheme 3). The resin-bound 5-nitro-pyrimidine **9** was then formed by N-arylation of **8** with **3**.



Scheme 3. Reagents and conditions: (a) R^1–NH_2, $Na(OAc)_3BH$, 1,2-dichloroethane, 25 °C; (b) **3**, *i*-Pr_2NEt, DMF, 25 °C; (c) $Na_2S_2O_4$, NH_4OH, *p*-dioxane, H_2O, 25 °C.

The determination of optimal conditions for the reduction of the nitro group was subsequently examined. A number of methods have been used to conduct such reductions on the solid phase, including tin(II) chloride,[11] chromium(II) chloride[12] and lithium aluminum hydride/aluminum chloride.[9] However, the reduction conditions that generate an acidic medium were excluded due to the potential for undesired premature cleavage of the pyrimidine intermediate from the acid-cleavable linker. Also, reduction conditions compatible with a variety of protecting groups that would be included within the R^1 and R^2 substituents were also required. The ability of polyethyleneglycolated resins such as Argogel® to swell in alcoholic and aqueous solvents provided the opportunity to employ a sodium hydrosulfite-based reduction under mildly basic conditions in aqueous media[13] (Scheme 3). A variety of hydrosulfite concentrations was investigated, in conjunction with different bases and solvent systems. The use of 0.5 M sodium hydrosulfite and 0.5 M ammonium hydroxide in 2:1 v/v water/*p*-dioxane provided good results, allowing a clean conversion of **9** to triaminopyrimidine **10**.

Cyclization to the diaminopurine derivative was envisaged via a two-step process involving reaction with an isothiocyanate, followed by carbodiimide activation of the resulting thiourea with subsequent cyclization to the purine (Scheme 4).[14] The functionalization of triaminopyrimidine **10** with an isothiocyanate required forcing conditions due to its poor nucleophilicity. Alkyl thiourea **11** formation with alkyl isothiocyanates (AlkNCS) occurred under thermal conditions via reaction of **10** with a 0.5 M solution of an alkyl isothiocyanate in DMF/EtOH at 80 °C. However, heating **10** with aryl isothiocyanates (ArNCS) showed incomplete aryl thiourea formation. Microwave conditions were then investigated to perform this reaction. Microwave irradiation of **10** with 0.5 M aryl isothiocyanate in CH_2Cl_2/DMF resulted in the complete conversion of **10** to aryl thiourea[15] **12**. The conditions to facilitate purine formation



Scheme 4. Reagents and conditions: (a) AlkNCS, DMF, EtOH, 80 °C; (b) ArNCS, DMF, CH_2Cl_2, microwave; (c) DIC, DMF, EtOH, 80 °C; (d) DIC, CH_2Cl_2, DMF, EtOH, 25 °C; (e) TFA, CH_3CN, 25 °C.

from the thiourea through carbodiimide-mediated cyclization were subsequently investigated. The reaction of alkyl thiourea intermediate **11** with diisopropylcarbodiimide (DIC) at room temperature resulted in minimal cyclization. However, thermal cyclization to **13** occurred smoothly in DMF/EtOH at 80 °C. The cyclization of aryl thiourea **12** to purine **14** was more facile, occurring at room temperature on treatment with DIC. The final cleavage of **13** and **14** from the solid support was conducted using TFA in acetonitrile to provide 8-alkyl diaminopurines **15** and 8-aryl diaminopurines **16**, respectively.

Using the reaction conditions developed for the solid-phase synthesis, the formation of both 8-alkylamino diaminopurines **15** and 8-arylamino diaminopurines **16** provided consistently good results with a wide range of R^1, R^2 and R^3 components. Alkyl-, heteroalkyl-, benzyl- and aryl-substitution could efficiently be incorporated at all three diversity points (Table 1). The functionalities containing acid-labile protecting groups were also included in the solid-phase synthesis, with **15b** and **16a** incorporating *N*-Boc and *O*-*t*-Bu groups, respectively. The removal of these protecting groups was achieved concomitantly with the acid cleavage of the diaminopurines from the solid support.

To determine the yield of **15a**–**16d** released from the solid support upon acid cleavage, the combined eluent from 20 beads of each compound was quantitatively analyzed versus an analytically pure sample of the corre-

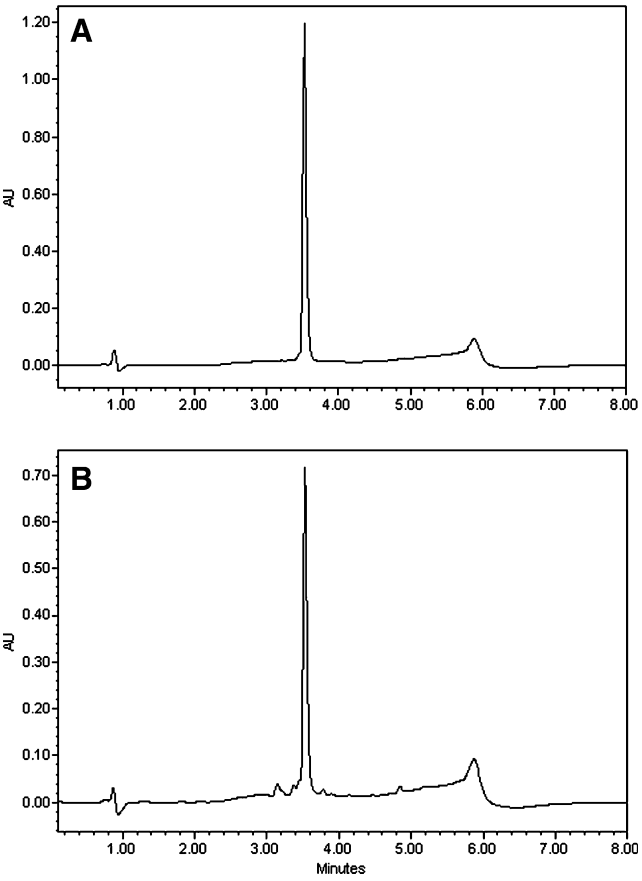
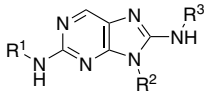
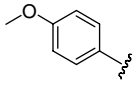
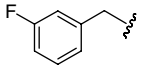
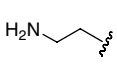
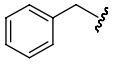
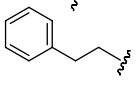
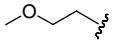
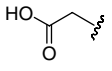
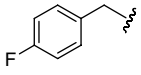
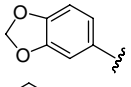
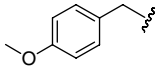
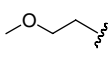
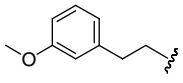
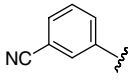
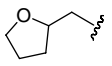
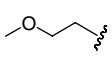


Figure 1. (A) HPLC profile of purified **16a** at 220 nm. (B) HPLC profile of the crude bead eluent of **16a** at 220 nm.

Table 1. N-9-substituted 2,8-diaminopurines synthesized on solid phase



Compound	R^1	R^2	R^3	nmol per bead[a]	Yield[b] (%)	Purity[c] (%)
15a				2.1	67	81
15b				2.8	89	88
15c				1.7	54	77
16a				1.7	55	83
16b				2.5	81	89
16c				1.9	61	80
16d				2.3	75	79

[a] Observed nmol per bead based on comparison with an analytical reference.
[b] % Yield based on average loading per bead.
[c] % Purity based on analytical HPLC analysis at 220 nm.

sponding purine derivative. The released quantities ranged from 1.7 to 2.8 nmol per bead, corresponding to yields of 54–89% (Table 1). In addition to good yields, the purity level of the crude N-9-substituted diminopurines was exceptionally high.

This is exemplified by Figure 1, which shows the HPLC profile at 220 nm of the purified analytical standard of **16a** in comparison to the crude bead eluent (Fig. 1).[16]

In conclusion, a general and efficient solid-phase synthesis of N-9-substituted 2,8-diaminopurines has been developed. The synthesis performs well with a wide range of R^1, R^2 and R^3 components, and provides a high level of both yield and purity. This route provides an effective method for the construction of both parallel and combinatorial N-9-substituted 2,8-diaminopurine libraries.

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15. Microwave reactions were performed using a LAVIS-1000 multiQUANT irradiating at 500 W for 5×1 min with 15 min intervals between each irradiation.
16. Analytical HPLC analysis was conducted using a PDA linked Waters Millenium 2290 and Phenomenex Luna 3u 50×3 mm C8 column.