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# Combined solution-phase and solid-phase synthesis of 2-amino-7,8-dihydropteridin-6(5*H*)-ones

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ABSTRACT

An efficient and general synthesis of substituted 2-amino-7,8-dihydropteridin-6(5*H*)-ones using a combination of solution-phase and solid-phase chemistry is described. Solution-phase chemistry was used to produce two pyrimidine regioisomers that were separated by flash column chromatography. Utilizing the desired regioisomer, solid-phase chemistry was used to effect the rapid construction of the substituted 2-amino-7,8-dihydropteridin-6(5*H*)-one system in high overall yield and purity.

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The design and synthesis of scaffolds that possess drug-like characteristics and are amenable to the incorporation of a wide selection of substituents remain as ongoing challenges for the construction of useful compound collections in drug discovery.[1] An approach whereby key synthetic modifications of reagents and intermediates in solution are followed by further transformations on solid support is a viable methodology to meet these challenges. The use of both solution-phase and solid-phase reactions in a combined synthetic strategy allows some of the limitations inherent in either method to be circumvented. For example, chemo- and regioselectivity issues of precursors are better addressed using typical solution-phase methods, e.g., low temperatures and chromatographic purification methods. A solid-phase strategy is preferable for slow reactions where a large excess of reagents can be used to drive the transformation to completion with the excess of reagents easily being removed by washing with solvent.[2–4]

Dihydropteridinones have a well exemplified history as biologically active molecules, for example, as kinase inhibitors[5,6] and as inhibitors of the RNA interference pathway in human cells.[7] The dihydropteridinone scaffold has been named a privileged heterocyclic ring system.[8,9] The demonstrated varied biological activity makes this an ideal template for further investigation and warrants the development of additional synthetic strategies to access this motif. The work reported here describes an efficient and general synthesis of substituted 2-amino-7,8-dihydropteridin-6(5*H*)-ones (Fig. 1) using a combination of solution-phase and solid-phase chemistry.

Commercially available 2,4-dichloro-5-nitropyrimidine (**1**) was used as the starting point to synthesize the desired 2-amino-7,8-dihydropteridin-6(5*H*)-one core structure. Derivatization of **1** with an amino-acid methyl ester at 0 °C gave the two pyrimidine regioisomers **2** and **3** in a ratio of about 9:1, respectively (Scheme 1).[4] Separation of the desired regioisomer **2** from **3** was easily achieved by chromatography. This chromatographic purification necessitates that this step be carried out in solution and it resulted in high purity levels of the substituted 2-amino-7,8-dihydropteridin-6(5*H*)-ones at the end of the subsequent solid-phase synthesis.

The use of amino acid derivatives in the construction of biologically active compounds allows the incorporation of a broad variety of readily available functionalities.[10,11] A range of amino acid methyl esters derived from L-alanine, L-lysine, L-serine, L-tyrosine, and L-glutamic acid were used (Table 1). The lysine, serine, tyrosine, and glutamic acid-based intermediates incorporated acid-labile protecting groups on their side chains. All subsequent

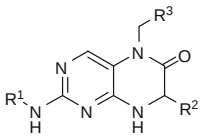


**Figure 1.** Substituted 2-amino-7,8-dihydropteridin-6(5*H*)-ones.

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[†] In 2008, Pharmacopeia, Inc. was acquired by Ligand Pharmaceuticals, Inc.

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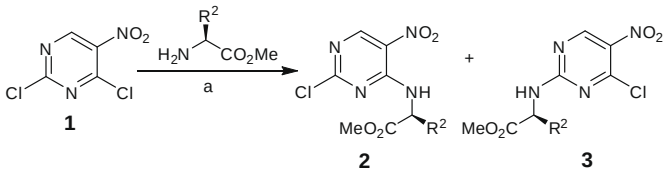
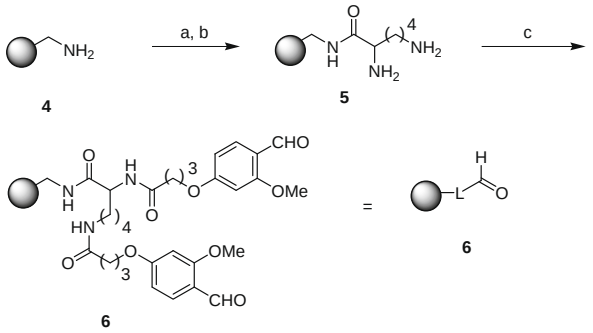


**Scheme 1.** Reagent and condition: (a) $(i\text{-Pr})_2$NEt, THF, 0 °C.

transformations in the synthesis were successfully performed on solid phase.

The solid-phase synthesis utilized aminomethyl-terminated Argogel® (**4**) as the solid support based on its favorable swelling characteristics in both polar and non-polar solvents. Prior to attaching a linker to the solid support resin **4** was acylated with *N*-α-*N*-ε-bis-Fmoc-lysine (Scheme 2). Subsequent Fmoc deprotection provided **5**, which effectively increases the loading capacity of the resin by doubling the number of amino groups for further functionalization. Acylation of **5** with the acid-cleavable linker 4-(4′-formyl-3′methoxy)phenoxybutyric acid gave **6**. This linker facilitates TFA-mediated release of intermediates and final products from the solid support. Any acid-labile protecting groups

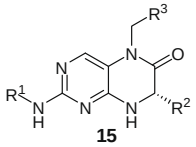


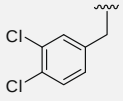
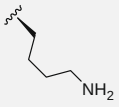
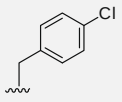
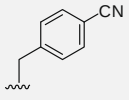
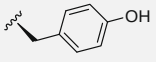
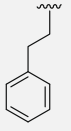
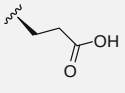
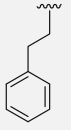
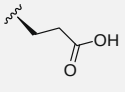
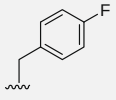
**Scheme 2.** Reagents and conditions: (a) *N*-α-*N*-ε-bis-Fmoc-lysine, DIC, HOBt monohydrate, 1:1 DMF:$CH_2Cl_2$, 25 °C; (b) 20% piperidine/DMF, 25 °C; and (c) 4-(4′-formyl-3′methoxy)phenoxybutyric acid, DIC, HOBt monohydrate, DMF, 25 °C.

incorporated during the solid-phase synthesis are also removed under these conditions.

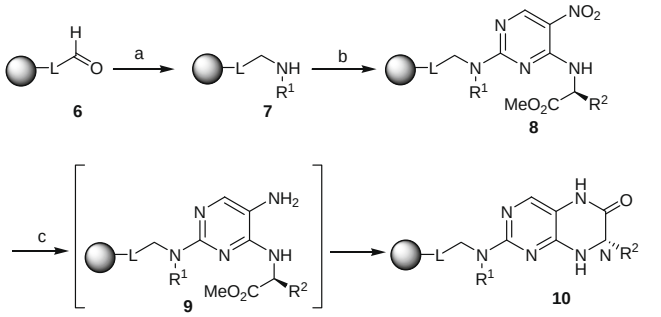
The resin-bound aldehyde **6** was derivatized by reductive amination with a series of primary amines ($R^1NH_2$) using sodium triacetoxyborohydride to give secondary amine **7** (Scheme 3). The set of primary amines successfully employed included a range

**Table 1**
Substituted 2-amino-7,8-dihydropteridin-6(5*H*)-ones synthesized on solid phase



| Compd | $R^1$ | $R^2$ | $R^3$ | (pmol)/bead[a] | Crude purity[b] (%) | Yield[c] (%) |
|---|---|---|---|---|---|---|
| **15a** |  |  |  | 1870 | 77 | 74 |
| **15b** |  |  |  | 1253 | 83 | 50 |
| **15c** |  |  |  | 986 | 89 | 39 |
| **15d** |  |  |  | 1808 | 95 | 72 |
| **15e** |  |  |  | 2411 | 86 | 96 |
| **15f** |  |  |  | 1136 | 81 | 45 |

[a] Observed (pmol) per bead based on comparison with an analytical reference.
[b] As determined by HPLC analysis of the crude bead eluent at 220 nm.
[c] Yield based on average loading per bead.

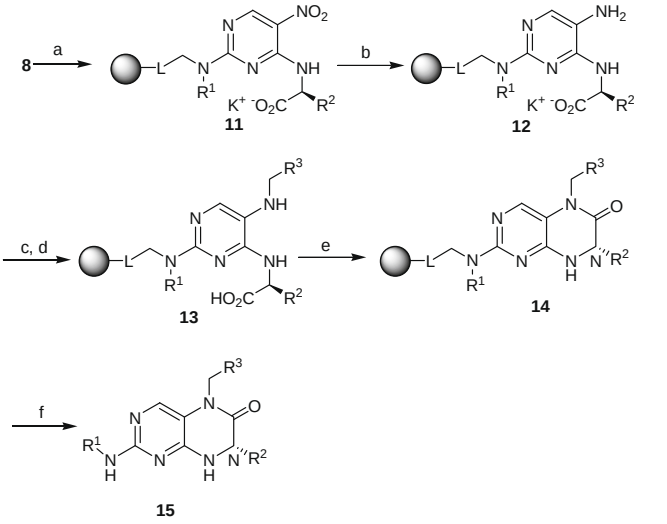


**Scheme 3.** Reagents and conditions: (a) $R^1NH_2$, $Na(OAc)_3BH$, 1,2-dichloroethane, 25 °C; (b) **2**, $(i\text{-}Pr)_2NEt$, DMF, 25 °C; (c) 0.15 M $Na_2S_2O_4$, $NH_4OH$, $H_2O$/1,4-dioxane, 25 °C.

of alkyl, aryl, and arylalkylamines (Table 1). Subsequent N-arylation of **7** with **2** provided resin-bound nitropyrimidine **8**. This was followed by reduction of the nitro group to give the corresponding aminopyrimidine **9**. Two methods were investigated to effect this reduction. Tin(II) chloride in DMF reduces the nitro group, but requires extended reaction time to facilitate complete reduction. This method also generates HCl which can result in the premature cleavage of the ligand from the acid-labile linker. Sodium hydrosulfite in a 1:1 mixture of water and 1,4-dioxane was ultimately chosen as the preferred reduction condition.[4] This reaction works efficiently under basic conditions, but the use of a variety of bases and reagent concentrations had to be investigated to minimize the formation of undesired by-products. The use of ammonium hydroxide as a base in conjunction with a low concentration of sodium hydrosulfite facilitates clean nitro group reduction, but the low reagent concentration necessitates the use of two reduction cycles to drive the reaction to completion.

During the course of the nitro reduction, premature cyclization of **9** from intramolecular reaction of the resulting amino group with the methyl ester occurred, yielding 2-amino-7,8-dihydropteridin-6(5*H*)-one **10** with no substituent at the *N*-5 position (Scheme 3). Even the increased steric bulk of a *tert*-butyl ester did not prevent this premature cyclization. Attempts to introduce the desired substituent at *N*-5 after cyclization gave desired product in low yield and purity. This premature cyclization was prevented by saponification of the ester **8** to the corresponding carboxylate **11** prior to nitro reduction to give **12** (Scheme 4). The resulting carboxylate anion and amino group in **12** do not react with each other. Resin-bound primary aminopyrimidine **12** was subsequently derivatized at *N*-5 with an aromatic aldehyde via reductive alkylation to give **13**. Initial studies showed that an in situ imine formation followed by imine reduction results in some undesired double alkylation. A two-step procedure involving initial imine formation followed by washing of the resin and subsequent imine reduction was therefore developed to ensure that only single alkylation occurs. Optimized conditions incorporate two cycles of imine formation. A second cycle is necessary to drive imine formation to completion, presumably because washing after the first cycle helps to force this equilibrium reaction in the required direction by removal of the water by-product. A range of aromatic and heteroaromatic aldehydes were successfully utilized in this transformation (Table 1).

It is interesting to note that the resin-bound aminopyrimidine **13** did not spontaneously cyclize to give the desired resin-bound 2-amino-7,8-dihydropteridin-6(5*H*)-one **14**. No cyclization was observed even upon heating. Activation of the carboxyl group with DIC was required to effect the formation of **14**. Release of the desired 2-amino-7,8-dihydropteridin-6(5*H*)-one **15** from solid support and removal of any acid-labile protecting group was accomplished by treatment of **14** with 60% trifluoroacetic acid in



**Scheme 4.** Reagents and conditions: (a) 1.0 M KOH, $H_2O$/MeOH, 25 °C; (b) 0.15 M $Na_2S_2O_4$, $NH_4OH$, $H_2O$/1,4-dioxane, 25 °C; (c) 0.5 M $R^3CHO$, THF, 25 °C (two cycles), (d) 0.5 M $NaCNBH_3$, 2% AcOH/MeOH, 25 °C; (e) 0.15 M DIC, $CH_2Cl_2$, 25 °C; (f) 60% TFA/$CH_3CN$, 25 °C.

acetonitrile. 2-Amino-7,8-dihydropteridin-6(5*H*)-ones **15** were successfully produced using a range of combinations of $R^1$, $R^2$, and $R^3$ groups (Table 1).

To determine the yield of **15a–f** in the acid-mediated cleavage from solid phase, the combined eluent from 20 solid-phase beads of each compound was quantitatively analyzed versus an analytically pure sample of the corresponding 2-amino-7,8-dihydropteridin-6(5*H*)-one. Released compound yields range from 39% to 96% over seven solid-phase synthetic steps (Table 1). The purity levels of crude **15a–f** were exceptionally high. This is exemplified in Figure 2, which shows the HPLC profile at 220 nm of the purified analytical standard of **15d** in comparison with the crude bead eluent of **15d**.

In conclusion, a general combined solution-phase and solid-phase method exploiting the advantages of each technique for the synthesis of substituted 2-amino-7,8-dihydropteridin-6(5*H*)-ones has been developed. The synthesis performed well with a wide range of $R^1$, $R^2$, and $R^3$ components, and provided a high level of purity. The synthetic methodology developed is robust and can be used to efficiently generate parallel and combinatorial 2-amino-7,8-dihydropteridin-6(5*H*)-one compound collections.

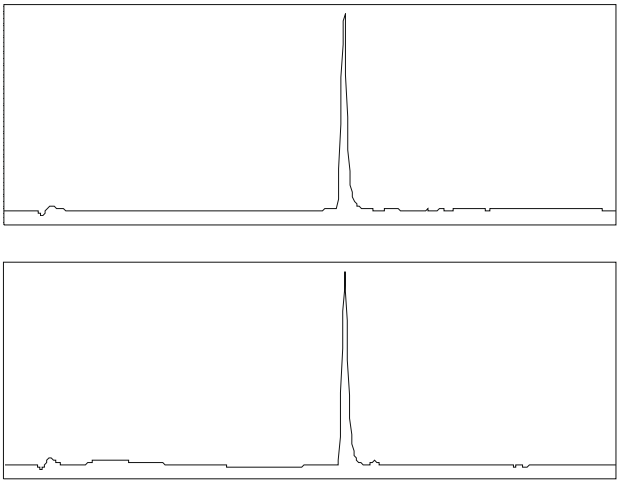


**Figure 2.** HPLC profiles at 220 nm of purified **15d** (upper chromatogram) and crude bead eluent of **15d** (lower chromatogram).

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