A Structure-Based Library Approach to Kinase Inhibitors

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The purine ring system is a key structural element of the substrates and ligands of many biosynthetic, regulatory, and signal transduction proteins including cellular kinases, G proteins, and polymerases. Consequently, combinatorial libraries based on this scaffold should facilitate the search for inhibitors of many biomedically significant processes. We have begun to develop libraries around the purine scaffold in connection with our efforts to generate selective inhibitors of the cell cycle kinases. The cyclin-dependent kinases (CDKs) are the principal regulators of processes such as cell growth, DNA replication, and cell division.[1] In human cells, CDC2 and CDK2 have been implicated in the control of mitosis and DNA replication, respectively.[2,3] A number of studies have provided data that support the importance of these CDKs in human diseases such as cancer[4,5] and restinosis,[6,7] and have stimulated an active search for chemical inhibitors of these kinases.[8] While purine analogs were being screened for inhibition of various protein kinases, a relatively selective inhibitor, olomoucine (Figure 1), was identified[9] that competitively inhibits CDK2/cyclin A with an IC_{50} of 7 μM.

A comparison of the CDK2 crystal structures containing bound ATP and bound olomoucine confirms that olomoucine binds in the adenine binding pocket of CDK2, but its purine nucleus adopts an entirely different orientation than that observed for ATP.[10] In spite of the good shape complementarity shown by the olomoucine−CDK2 complex, structural variations at C-6, C-2, and N-9 might be expected to lead to enhanced affinity and selectivity for CDK2. The coupling of this structural information with combinatorial methods is an obvious strategy for optimizing olomoucine's potency. Herein we apply this approach to the solid-phase synthesis and screening of combinatorial libraries based on the purine scaffold found in olomoucine.

In order to facilitate both the chemical and biological evaluation of soluble olomoucine analogues, synthesis is performed in a spatially-separated fashion using Geysen's pin apparatus.[11] The purine scaffold is attached to the support by either a glycinamide installed at C-2 or a hydroxyethyl substituent installed at N-9. Initial synthetic efforts focused on preparing a 6-chloropurine derivative bearing an active ester that could be used to derivatize pins containing an acid-labile Rink[12] linker (Scheme 1). The sequence begins with the regioselective methylation of **1** which affords a separable 7:1 mixture of 9- and 7-methyl-2-amino-6-chloropurine isomers, respectively.[13] The exocyclic amine is trifluoroacetylated, alkylated with *tert*-butyl α-iodoacetate, and the alkylated trifluoroacetamide is saponified. Acid-catalyzed cleavage of **2** followed by PyBroP (bromotripyrrolidinophosphonium hexafluorophosphate)-mediated[14] activation of the free acid with *p*-nitrophenol (PNP) provides active ester **3** which can be stored indefinitely at 4 °C.[15] Coupling of **3** to support-bound free amine (1.1 μmol/pin) can be monitored by a quantitative ninhydrin procedure[16] and is typically complete within 12 h.

The first combinatorial step consists of acylating the exocyclic nitrogen of **4**. Treatment of the purine with a dichloromethane solution of the acid chloride in the presence of 2,6-di-*tert*-butyl-4-methylpyridine results in complete coupling after 12 h, providing tertiary amide **5**. Reversed-phase HPLC studies[17] established that even sterically congested groups can be attached to the purine scaffold using this protocol. The second combinatorial step is the nucleophilic aromatic substitution of chloropurine **5** by primary and secondary amines. Competitive

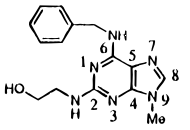
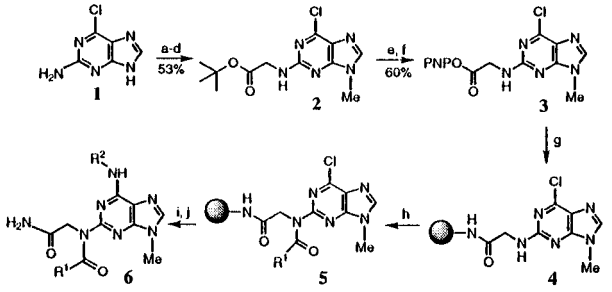


Figure 1. Structure of olomoucine and numbering scheme for purine nucleus.

Scheme 1. Glycinamide-Based Synthesis of 2-(Acylamino)-6-aminopurines[a]



[a] Conditions: (a) 1.1 equiv of NaH, 1 equiv of MeI, DMF; (b) 3 equiv of trifluoroacetic anhydride, CH_2Cl_2; (c) 1.1 equiv of NaH, 2 equiv of *tert*-butyl α-iodoacetate, DMF; (d) aqueous K_2CO_3, MeOH; (e) TFA, 1,4-dimethoxybenzene; (f) 1 equiv of PyBroP, 1 equiv of *p*-nitrophenol, 3 equiv of DIEA, CH_2Cl_2; (g) 0.05 M **3**, Rink-derivatized solid support, 0.06 M DIEA, DMF, 37 °C, 12 h; (h) 0.2 M R^1COCl, 0.25 M 4-methyl-2,6-di-*tert*-butylpyridine, CH_2Cl_2, 37 °C, 12 h; (i) 0.25 M R^2NH_2, DMF/DMSO, 1:1 (v/v), 4 °C, 16 h; (j) CH_2Cl_2/TFA/Me_2S (v/v), rt, 2 h.

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(13) This reaction is particularly regioselective for alkylation at N-9 and typically affords a 5:1 mixture of N-9/N-7 alkylation products (85% yield) which can be separated by flash chromatography.
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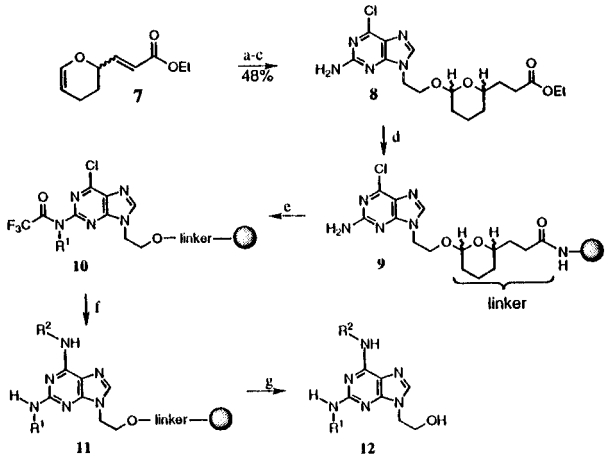
(17) HPLC analysis was performed using a Rainin C_{18} column, running a 40−100% gradient of methanol in water buffered with 0.5% triethylammonium acetate (pH 8.0). UV detection of peaks was monitored at either 254 or 310 nm.

Scheme 2. 9-(2-Hydroxyethyl)-Linked Synthesis of 2,6-Diaminopurines[a]



[a] Conditions: (a) 1.1 equiv of 2-(benzyloxy)ethanol, 10 mol % *p*-TsOH, CH_2Cl_2, −10 °C to rt, 6 h; (b) H_2, 10% Pd/C, EtOAc, rt, 24 h; (c) 1 equiv of 2-amino-6-chloropurine, 2 equiv of PPh_3, 1 equiv of diethylazodicarboxylate (DEAD), THF, −10 °C to rt, 12 h; (d) (i) 1 equiv of 1:1 2 N NaOH dioxane, rt, 12 h, (ii) 2 equiv of pentafluorophenol, 1.1 equiv of diisopropylcarbodiimide, DMF, 0 °C to rt, 6 h; (iii) 0.05 M PFP ester, aminoalkyl support, 0.06 M DIEA, DMF, 37 °C, 12 h; (e) (i) 0.2 M trifluoroacetic anhydride, 0.30 M 4-methyl-2,6-di-*tert*-butylpyridine, CH_2Cl_2, 37 °C, 4 h, (ii) 0.2 M R^1OH, 0.4 M PPh_3, 0.2 M DEAD, THF, −10 °C to rt, 6 h; (f) 0.25 M R^2NH_2, DMSO, 70 °C, 12 h; (g) TFA/H_2O, 90:10 (v/v), rt, 1 h.

deacylation of R^1 is minimized by performing the amination in a 1:1 mixture of DMF/DMSO (0.25 M in amine) at 4 °C. After cleavage from the support using 80:15:5 dichloromethane/trifluoroacetic acid/dimethyl sulfide, this four-step sequence results in purine derivatives having the general structure **6**.

For characterization purposes, seven purine analogues were prepared on Rink-derivatized resin (0.59 mmol/g) and evaluated by reversed-phase HPLC analysis.[17] The compound corresponding to the major peak[18] was isolated, and the structure of the purine derivative was verified by high-resolution spectroscopy (^{1}H NMR and FAB-MS).[19] Following this, a small library of 36 purine derivatives was prepared on pins using six acid chlorides and five amines, plus five aminated pins having no acyl group. The entire library was evaluated by reversed-phase HPLC which in all cases indicated that very few, if any, purine-containing side products were produced during the sequence. UV analysis of material cleaved from pins (289 nm, $\epsilon = 12\,000$ M^{-1} cm^{-1}) indicated a 30−75% overall yield for the solid-phase chemistry.

In order to expand the chemistry that can be carried out at the C-2 amino group to include alkylation reactions, a strategy was devised to attach the purine core to the support through N-9. Initial efforts to load 2-amino-6-chloro-9-(2-hydroxyethyl)purine to a solid support functionalized with an acid-labile dihydropyran linker resulted in the exclusive attachment of the purine to the linker via the exocyclic amine at C-2. For this reason, solution-phase chemistry was developed to attach the purine core to the tetrahydropyranyl linker prior to loading (Scheme 2).[20] The modified purine is then attached to the solid support by reacting the aminoalkyl-derivatized pins (1.1 μmol/pin) with the pentafluorophenyl ester corresponding to **8**.

Library synthesis begins with the acylation of **9** with trifluoroacetic anhydride and is followed by alkylation with the desired alcohol under Mitsonobu[21] conditions to afford trifluoroacetamide **10**. Subsequent amination is accompanied by aminolysis of the trifluoroacetamide and provides purines having the structure **11**. Purine alcohol **12** is cleaved from the support using 90:10 trifluoroacetic acid/water. Six purine analogues were prepared on aminoalkyl-derivatized resin (0.87 mmol/g) using this five-step sequence with overall isolated yields of 75−85%. Following this, a small library of 16 alkylated aminopurines was prepared on pins using primary and benzylic alcohols. Again HPLC analysis of the cleaved material revealed few, if any, side products.

As a first step in the combinatorial optimization of olomoucine, we targeted the C-6 position of the purine core for substitution with a wide variety of amines.[22] Using the glycinamide-linked purine scaffold, a library of 348 purine derivatives was prepared using 5 acid chlorides and 58 amines plus 58 aminated pins bearing no acyl group. Evaluation of the library was carried out using a microtiter-based solution-phase assay[23] for protein kinase[24] activity which identified CDKZ inhibitors containing *meta*- and *para*-substituted benzylamines that appeared to be more active than olomoucine. Solution-phase synthesis and characterization of one such derivative, 2-((2-hydroxyethyl)amino)-6-((4-methoxybenzyl)-amino)-9-(isopropylamino)[9] provided an inhibitor having an IC_{50} (600 nM) more than an order of magnitude lower than that measured for oloumucine (7 μM).

Using the acylation and Mitsonobu chemistry described above, we are currently constructing larger libraries (>5000) in which a variety of structures are appended to the amino group at C-2. New strategies are also being developed to introduce N-9 substituents in a combinatorial format and to construct macrocycles between these substituents and those at C-2. The iteration of library synthesis with structural analysis of the optimized leads should provide an effective strategy for the development of more potent and selective inhibitors of CDK2. In addition, libraries containing purine derivatives may prove useful in the search for inhibitors of a large number of cellular processes.

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Supporting Information Available: Experimental procedures and analytical data for the synthesis and chracterization of the compounds, description of the solid-phase chemistry depicted in Schemes 1 and 2, listing of the building blocks used in the 2-(acylamino)-6-aminopurine library, and analytical evaluation of the 2-(acylamino)-6-aminopurine library (10 pages). See any current masthead page for ordering and Internet access instructions.

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(18) In most instances only a single peak was detected.
(19) Yields for the four-step sequence on resin ranged from 60 to 85%.
(20) Dihydropyran **7** was prepared in three steps and 44% overall yield from commercially available 3,4-dihydro-2*H*-pyran-2-carboxylic acid, sodium salt.
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(24) Crude lysates containing activated CDK2/cyclin A (Δ171, modified with His-6) were obtained from Professor David O. Morgan and purified to homogeneity.