Pergamon

Tetrahedron Letters, Vol. 38, No. 7, pp. 1161-1164, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00014-2

Combinatorial Synthesis of 2,9-Substituted Purines

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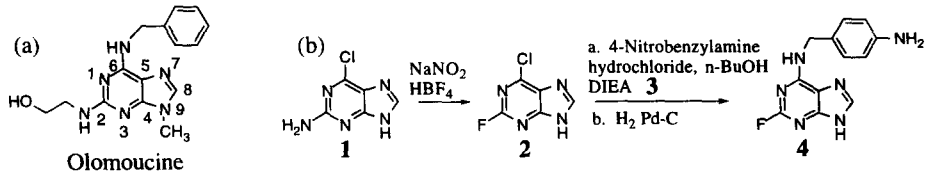
Abstract: A method for the combinatorial synthesis of 2,9-substituted purines using a Mitsunobu reaction to alkylate the N-9 position and an amination reaction to install amines at the C-2 position has been developed. © 1997, Elsevier Science Ltd. All rights reserved.

The purine ring is a common structural element of a large number of agonists, antagonists, substrates and effectors that play key roles in many cellular processes. It is therefore reasonable to expect that combinatorial libraries of purine derivatives may provide inhibitors of these processes that are useful biological probes or lead molecules for drug development efforts. An example of one such inhibitor is olomoucine (Scheme Ia) which exhibits moderate inhibitory activity (IC-50 = 7 μM) but good selectivity for the CDK/cyclin protein kinases[1]. A 2.4 Å crystal structure of olomoucine bound to CDK2[2] reveals that the purine portion of olomoucine binds in the conserved ATP binding pocket, while the benzylamino group extends into a region of the active site unique to the CDK2 kinases. Our goal has been to use combinatorial chemisty to increase the affinity and specificity of olomoucine through the introduction of diversity at the 2, 6, and 9-positions of the purine ring. We have previously reported the combinatorial synthesis of substituted 2-acylamino-6-amino- and 2,6-diaminopurines[3]. This paper describes a method for the synthesis of combinatorial libraries of 9-alkyl-2-amino purines for screening against CDK2, as well as other cellular kinases, G-proteins and polymerases.

Previous work has established that the 6-benzylamino group contributes significantly to the specificity and binding affinity of olomoucine. We therefore decided to incorporate the benzylamino substituent into a 2-fluoro-6-(4-aminobenzylamino)purine(**4**) core. Synthesis of **4** is accomplished by converting commercially available 2-amino-6-choloropurine(**1**) to **2** by diazotization in aqueous fluoroboric acid with sodium nitrite followed by monoamination at the 6-position with 4-nitrobenzylamine and hydrogenation(Scheme Ib)[4].

Scheme I.



Olomoucine **1** **2** **4**

Solid phase synthesis begins with the coupling of 5-(4-formyl-3,5-dimethoxyphenyloxy)valeric acid(PAL)[5] to amine derivatized crowns[6] using diisopropylcarbodiimide-hydroxybenzotriazole in DMF. The purine core **4** is coupled by reductive amination using sodium triacetoxyborohydride in DMF containing 1% acetic acid (Scheme II). The first combinatorial step consists of alkylation of the N-9 position with a variety of alcohols using Mitsunobu conditions[7] to yield **8**. The alkylation reaction is monitored by cleavage of the product from support followed by analytical reverse-phase HPLC[8] and characterization by FAB-MS(Table I)[9].

Primary and secondary aliphatic alcohols alkylate exclusively at the N-9 position while benzylic alcohols result in partial alkylation of the N-6 position. Good conversion to product is observed (HPLC yield: 73-88%) with a variety of alcohols including sterically hindered secondary alcohols such as 3-hydroxytetrahydrofuran.

Because mild reaction conditions are employed, alcohols containing base sensitive functional groups can be incorporated. Additionally, the large number of commerically available alcohols offers considerable diversity in the library at the N-9 position.

Scheme II.

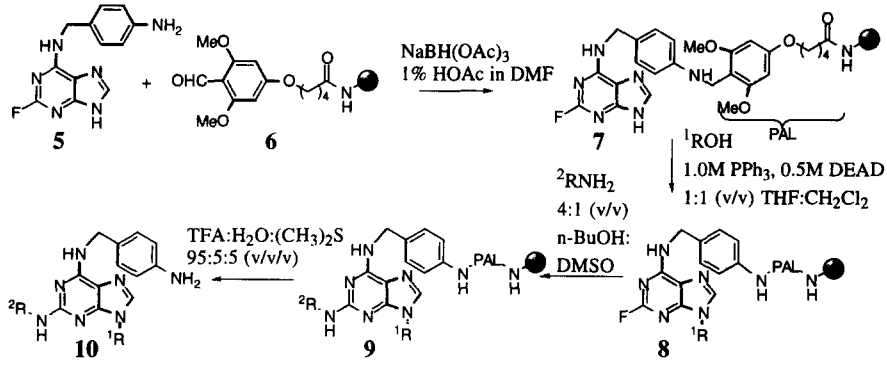


Table I. Products of N-9 alkylation reaction.

Alcohol (^{1}R)	Yield (%) Starting Material	Yield (%) Product	Yield (%) Other
Ethanol	11	88	1
N,N-Dimethylethanolamine	15	84	1
Isopropanol	11	87	2
Benzyl alcohol	14	30(35*)	21
2-(3-Thienyl)ethanol	17	83	0
3-Hydroxytetrahydrofuran	18	74	8
4-(2-Hydroxyethyl)morpholine	11	88	1
(+/-)-1-Phenyl-1-propanol	24	25(50*)	1
2-(2-Thienyl)ethanol	21	78	1

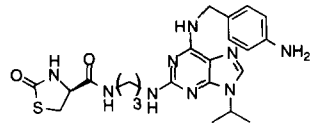
*Yield of 6,9-dialkylated purine

The second combinatorial step involves substitution of the fluorine at the C-2 position of the purine ring with amines to yield **9**. The final product **10** is cleaved from the solid support and analyzed by reverse-phase HPLC[8] and FAB-MS(Table II)[9]. Solution phase chemisty has demonstrated that a fluoro group is more readily displaced than the corresponding chloride or bromide.

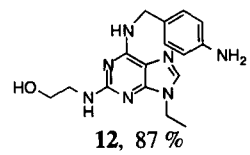
Table II. Products of C-2 substitution reaction.

Amine Name (^{2}R)	Alcohol (^{1}R) 2-(3-thienyl) ethanol % **10**	Alcohol (^{1}R) 3-hydroxy-tetrahydro-furan % **10**	Alcohol (^{1}R) 4-(2-hydroxy-ethyl)-morpholine % **10**
Butylamine	83	85	69
2-Aminoethanol	76	80	82
1,3-Diaminopropane	55	75	72
4-Methoxybenzylamine	77	76	78
2-Fluorobenzylamine	73	66	76
2-(2-Aminoethyl)pyridine	76	77	83
S-(-)-2-Amino-3-phenyl-1-propanol	55	60	61
Cyclohexylamine	60	57	56
2-(Methylamino)ethanol	ND	75	80
(+/-)-2-Amino-3-methyl-1-butanol	ND	54	51

HPLC yields of the desired products are good (51-85%, average 70%) with minor biproducts consisting primarily of starting material from either the alkylation or amination reactions. The amination chemistry is particularly versatile because primary and secondary amines bearing a wide range of unprotected functional groups are acceptable building blocks. Due to the small quantity of compound (1.6 μmol) released from a crown, two purines were synthesized on larger scale for characterization purposes on PAL derivatized MBHA resin (0.87 mmol/g) by Scheme II.



11, 74%



12, 87 %

After cleavage from resin using the TFA cocktail, both compounds were purified by preparative TLC and characterized by reverse phase HPLC, ^{1}H NMR, and high-resolution mass spectrometry[10]. Synthesis and biological evaluation of larger libraries (500-1000 compounds) of purine derivatives using Scheme II is currently in progress. We are also investigating the possibility of directly linking the purine core to solid support via an amino or oxygen substitutent at the 6-position thereby allowing the combinatorial synthesis of adenine or guanine derivatives.

Acknowledgement. This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Division of Material Sciences, and also by the Division of Energy Biosciences of U.S. Department of Energy under Contract No. DE-AC03-76SF00098. PGS is a Howard Hughes Medical Institute Investigator. NSG thanks the National Science Foundation for a predoctoral fellowship. We wish to thank Professor David Morgan for the cdk2/cyclinA, Professor Sung-Hou Kim for the Cdk/olomoucine crystal structure coordinates, and Dr. Andrew Bray of Chiron Mimeotopes for a generous gift of the crowns, and Thea Norman for advice and the synthesis of 2-fluoro-6-chloropurine.

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4. **2-Fluoro-6-chloropurine (2).** A 0.3 M aqueous solution of sodium nitrite (200 mL) is added dropwise to a cooled (-15 °C), vigorously stirred suspension of 2-amino-6-chloropurine (6.0 g, 35.4 mmol) in fluoroboric acid (120 mL, 48 weight % in water) over 75 min. The pale yellow reaction mixture is stirred at room temperature for 20 min and then recooled (-15 °C) and neutralized to pH = 6.0 with aq. NaOH (50 weight % in water). The water is removed *in vacuo* and the resulting orange solid is purified by chromatography (5 cm x 10 cm silica, eluted with 90:10 CH_2Cl_2:MeOH, R_f = 0.50; compound is dry loaded in 250 mL silica) to yield 3.7 g (61%) of **2** as a white solid. mp = 161-162 °C, ^{1}H NMR (400 MHz, 1:1 (v/v) CD_3CN:d_6-DMSO, 373 K): δ 8.63; ^{13}C (101 MHz, 1:1 (v/v) CD_3CN:d_6-DMSO): δ 129.1, 150.1, 156.7, 158.9, 163.9; ^{19}F (376 MHz, TFA standard, d_6-DMSO): δ 22.8; mass spectrum (FAB$^+$) m/e 173 $(MH)^+$; HRMS Calcd for $(C_5H_2N_4ClF)H^+$: 173.0030, Found 173.0032.
2-Fluoro-6-(4-nitrobenzylamino)purine (3). 2-Fluoro-6-chloropurine (1.0 g, 5.81 mmol) is combined with 4-nitrobenzylamine hydrochloride (1.1 g, 5.81 mmol) and diisopropylethylamine (2.8 mL, 1.63 mmol) in n-butanol (80 mL). The resulting slurry is heated at 50 °C for 12h and then at 90 °C for 12h. Removal of solvent *in vacuo* and purification by chromatography (3 cm x 10 cm silica eluted with 90:10 CH_2Cl_2:MeOH, R_f = 0.45) yields 1.5 g (90%) of **3** as a faintly yellow solid. mp = 277-278 °C (decomp.); ^{1}H NMR (400 MHz, d_6-DMSO, 373 K): δ 4.74 (bs, 2H), 7.57 (d, 2H, 8.3 Hz), 8.13 (s, 1H), 8.17 (d, 2H, 8.3 Hz), 8.92 (bs, 1H); ^{13}C (101 MHz, d_6-DMSO): δ 42.9, 123.4, 123.5, 127.8, 128.2, 139.5, 146.5, 147.5, 157.5, 159.5; ^{19}F (376 MHz, TFA standard, d_6-DMSO): δ 22.8; mass spectrum (FAB$^+$) m/e 289 $(MH)^+$; HRMS Calcd for $(C_{12}H_9N_6O_2F)H^+$: 289.0849, Found: 289.0850.

2-Fluoro-6-(4-aminobenzylamino)purine (4). 2-Fluoro-6-(4-nitrobenzylamino)purine (700 mg, 2.43 mmol) is suspended in ethyl acetate (100 mL) and the flask is purged with N_2 for 10 min. To this suspension is added 10% Pd/C catalyst (300 mg). The flask is purged with N_2 for another 10 min and placed under an H_2 atmosphere (1 atm). After 30 h the reaction flask is purged with N_2 and the catalyst removed by filtration through celite. The celite is washed extensively with methanol. Removal of the solvent *in vacuo* yields 510 mg (81 %) of **4** as a faintly yellow solid: m.p. glass; R_f = 0.23 (95:5 CH_2Cl_2:MeOH); ^1H NMR (400 MHz, d_6-DMSO, 373K): δ 4.44 (bs, 2H), 4.94 (bs, 2H), 6.48 (d, 2H, 8.3 Hz), 7.00 (d, 2H, 8.3 Hz), 8.02 (s, 1H), 8.36 (bs, 1H); ^{13}C (101 MHz, d_6-DMSO) δ 43.1, 112.3, 112.8, 113.8, 126.2, 128.4, 128.5, 147.6, 157.8, 159.8; mass spectrum (FAB^+) m/e 398 $(MH)^+$; HRMS Calcd for $(C_{12}H_{12}N_6O)H^+$: 258.1029, Found: 258.1025.

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8. Analytical HPLC was performed on a Rainin UV-1 system using a Vydac C-18 peptide column (4.6 x 250 mm, 10 μm particle size) with a gradient elution (solvent A: 1% H_3PO_4/Et_3N pH = 7.0; solvent B: CH_3CN) of 20-100% B over 20 min monitoring at 254 nm. HPLC yields are obtained by integration at 254 nm.

9. Experimental for Solid Phase Synthesis:
Loading PAL linker: Approximately 80 crowns are swollen in dry DMF (20 mL) containing 1% acetic acid by volume. 2-Fluoro-6-(4-aminobenzylamino)purine (250 mg, 0.97 mmol) and sodium triacetoxyborohydride (226 mg, 1.1 mmol) are added to the solution. The flask is purged extensively with nitrogen and placed on a wrist action shaker for 12 h. The solvent is removed with a filtration cannula and the crowns are washed with DMF (7x), 1:1 (v/v) CH_2Cl_2:THF (3x), CH_2Cl_2 (3x) using enough solvent to completely submerge the crowns and vortexing for 2 min. per rinse. Crowns are dried under a stream of N_2 and then under vacuum (*ca.* 1mm Hg).
Mitsunobu alkylation. A solution of 1.0 M triphenylphosphine in dry 1:1 (v/v) THF:CH_2Cl_2 is cooled to 0 °C under nitrogen. Diethylazodicarboxylate (DEAD) is added dropwise until a 0.5 M solution is attained. The solution is stirred at 0 °C for 1h. The triphenylphosphine/DEAD solution is added via a teflon cannula to a dry flask containing the alcohol (final concentration 0.4 M) and crowns. The flask is shaken at room temperature for 48 h. (addition of small amounts of DEAD after 12 hours improve yields). The crowns are washed as described above.
Amination. The crowns are swollen in a 0.5 M solution of amine in a 4:1 (v/v) n-butanol:DMSO solution and heated for 48h at 90-100 °C. The crowns are washed as described above.
Cleavage. The crowns are immersed in enough 95:5:5 (v/v/v) TFA:H_2O: dimethylsulfide (ca 200 μL) such that they are completely submerged. After 1h the crowns are removed from the solution and solvent removed *in vacuo*.

10. Characterization data for compounds **11** and **12** synthesized on solid support. Yields are based on mass balance of purified product relative to the resin substitution level.
2-(3-(S-2-Oxo-4-thiazolidinecarboxamide)propylamino)-6-(4-aminobenzylamino)-9-isopropylpurine (11). ^1H NMR (400 MHz, 1:1 (v/v) CD_3OD:CD_3CN, 373 K): δ 1.48 (d, 6H, 6.7 Hz), 1.72-1.75 (m, 2H), 3.23-3.28 (m, 4H), 3.36-3.43 (m, 3H), 3.65-3.69 (m, 1H), 4.24-4.27 (m, 1H), 4.54-4.59 (m, 3H), 6.60 (d, 2H, 8.3 Hz), 7.09 (d, 2H, 8.3 Hz), 7.65 (s, 1H); mass spectrum (FAB^+) m/e 484 $(MH)^+$; HRMS Calcd for $(C_{22}H_{29}N_9O_2S)H^+$: 484.224318, Found: 484.2235.
2-(2-Hydroxyethyl)-6-(4-aminobenzylamino)-9-ethylpurine (12). ^1H NMR (400 MHz, d_6-DMSO, 373 K): δ 1.32 (t, 3H, 7.2 Hz), 3.30-3.33 (m, 2H), 3.50 (bs, 2H), 3.96 (q, 2H, 7.2 Hz), 4.42 (bs, 2H), 4.67 (bs, 1H), 4.90 (bs, 2H), 6.16 (bs, 1H), 6.45 (d, 2H, 8.2 Hz), 7.00 (d, 2H, 8.2 Hz), 7.51 (bs, 1H), 7.69 (s, 1H); mass spectrum (FAB+) m/e 328 $(MH)^+$; HRMS Calcd for $(C_{16}H_{21}N_7O)H^+$: 328.1886, Found: 328.1882.

(*Received in USA* 12 *November* 1996; *accepted* 30 *December* 1996)