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Solution-Phase Synthesis of 2,6,9-Trisubstituted Purines

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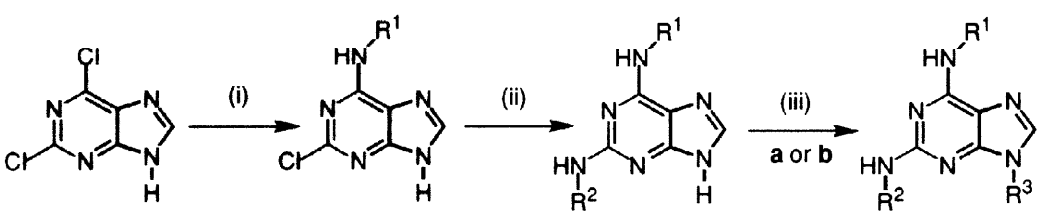
Abstract: A simple three-step method for the solution-phase combinatorial synthesis of 2,6,9-trisubstituted purines from 2,6-dichloropurine is described. The synthesis exploits the use of resin capture to remove excess reagent used in the final step.

Protein kinases catalyse the transfer of the γ-phosphate of ATP to a serine, threonine or tyrosine residue of an acceptor protein. They are attracting a great deal of interest because of their critical role in cell cycle regulation.[1-4] In designing inhibitors for protein kinases attention has been focused on the ATP binding site, and particularly molecules including a purine motif. Recently it was shown that olomoucine, a trisubstituted purine, is a potent, selective inhibitor of a cyclin-dependent kinase complex $p33^{cdk2}$/cyclin A (IC_{50} = 7 μM).[5-7] This has prompted several groups to report syntheses of substituted purines libraries on the solid-phase.[8-10] Here we report on a complementary solution phase synthesis of trisubstituted purines which exploits the use of resin capture to scavenge excess reagent.

The use of 2,6-dichloropurine as a template



1 **2** **3** **4**

Scheme 1 (i) amine R^1NH_2 (1.05 eq), Et_3N (1.1 eq), DMF, 80-100 °C, 2-24 h. (ii) amine R^2NH_2 (5 eq), DMF, Et_3N (1.1 eq), 150 °C, 30 h, then formylpolystyrene beads (25 eq), DMF , 60 °C, 24 h. (iii) **a** benzyl chloride (1.05 eq), K_2CO_3, dry DMF, rt, 24 h, or **b** R^3OH (1.05 eq), DEAD (3 eq), PPh_3 (3 eq), dioxane, rt, 6 h. See Tables 1, 2, and 3 for the identities of R^1, R^2 and R^3.

The starting point for our synthesis of 2,6,9-trisubstituted purines was the commercially available 2,6-dichloropurine **1** (Scheme 1, shown for reaction with primary amines). Initial substitution at the 6-position[11] was carried out by refluxing 2,6-dichloropurine **1** separately with 1.05 equivalents of a series of primary amines, secondary amines and hydrazine. Amines were chosen to represent a range of functionality and a selection of those used are shown in Table 1. Due to differences in reactivities of the various amines, reaction times varied from 2–24 hours. When the reactions were seen to be complete by tlc, they could either be worked

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up to isolate the monosubstituted purines **2** (Table 1), or the next step in the synthesis could be performed directly to form the 2,6-disubstituted purines **3** (Table 2) without workup. This strategy was developed so that the synthesis could be transferred onto a robotic synthesiser. For the isolated compounds **2**, each was fully characterised by melting point, ^{1}H NMR, ^{13}C NMR, IR spectroscopy and electrospray mass spectrometry (ESMS). The melting points were all above 200 °C.

Table 1: Isolated 6-substituted purines (2)

substituent	Yield[a]	substituent	Yield[a]
4-amino-*N*-benzylpiperidino	70	3-fluoroanilino	82
4-cyanobenzylamino	63	5-indanamino	54
benzylamino	76	4-isopropylanilino	47
4-butoxyanilino	62	2-methoxyanilino	56
butylamino	86	phenethylamino	92
cyclohexylamino	72	morpholino	88
2,4-dichlorobenzylamino	86	hydrazino	86

[a] yield after recrystallisation from ethanol/water

Some of the isolated monosubstitued purines were used to make a small library. Equimolar quantities of three purines **2** were mixed and divided into 3 "pots", following the method of Furka[12] to produce a 3 x 3 component library. Each pot was heated at 150 °C for 30 hours in DMF with 5 equivalents of a primary amine, secondary amine or hydrazine,[13] in the presence of triethylamine to give 3 pots each containing 3 compounds **3**. At this stage it was necessary to remove the excess amine added to force the formation of **3** to completion. This problem was most conveniently overcome by using formyl-polystyrene beads[14] which were shown to react preferentially with the added amines (albeit slowly with the secondary amines) and immobilised them on the resin. The treatment was continued at 60 °C for 24 hours. The resin beads were filtered off, washed with DMF, and the DMF removed to yield mixtures of purines with no traces of amine impurity detectable by LCMS. The presence of all the expected compounds was comfirmed for each sublibrary by ESMS, HPLC and LCMS.

Individual 2,6-disubstituted purines **3** were prepared directly from 2,6-dichloropurine without isolation of the intermediate 6-substituted purines **2** (Table 2). Excess amine was removed by treatment with formyl-polystyrene beads, and the final compounds were isolated by precipitation with diethyl ether.

Table 2: 2,6-Disubstituted purines (3)

6-substituent	2-substituent	Yield %	Purity[a] %
benzylamino	benzylamino	77	95
benzylamino	ethanolamino	77	49[b]
benzylamino	*N*-morpholino	62	98
cyclohexylamino	benzylamino	62	87
N-morpholino	*N*-morpholino	57	97
hydrazino	hydrazino	72	95

[a]Purity determined by C-18 reverse phase HPLC using a Columbus 50 x 2 mm C18 column (15-75% CH_3CN in H_2O containing 0.1 % TFA), monitored at 254 nm using a UV detector and by a SEDEX Evaporative Light Scattering Detector.
[b] formation of unidentified byproduct in a 1:1 ratio.

A selection of 2,6-disubstituted purines **3** were further elaborated by benzylation at the N-9 position. This could be achieved either by using benzyl choride with anhydrous potassium carbonate in DMF,[15] or by using the Mitsunobu reaction (exemplified with 2,3-difluorobenzyl alcohol).[16] The 2,6,9-trisubstituted purines **4** were purified chromatographically (Table 3). The regioselectivity observed in this reaction is thought to be due to steric blocking of the N-7 position by the C-6 substitutent.

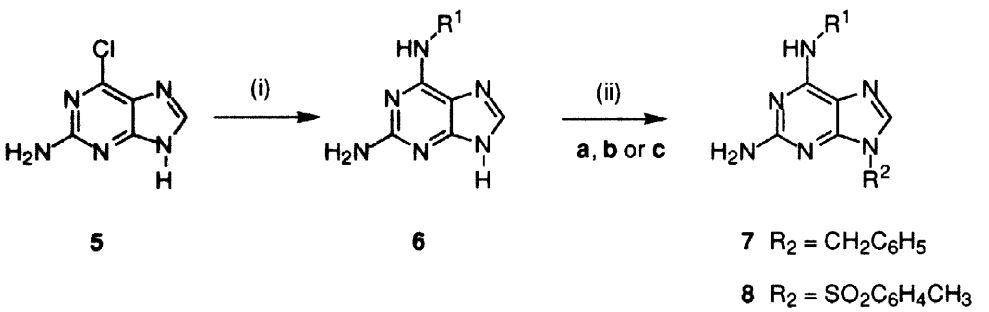
Table 3: 2,6,9-Trisubstituted purines (4)

6-substituent	2-substituent	9-substituent	Yield %	Purity[a] %
benzylamino	benzylamino	benzyl	61	92
butylamino	*N*-morpholino	benzyl	94	87
N-morpholino	*N*-morpholino	benzyl	84	90
N-morpholino	*N*-morpholino	2,3-difluorobenzyl	86	93

[a]Purity determined by C-18 reverse phase HPLC using a Columbus 50 x 2 mm C18 column (15-75% CH_3CN in H_2O containing 0.1% TFA), monitored at 254 nm using a UV detector and by a SEDEX Evaporative Light Scattering Detector.

The use of 2-amino-6-chloropurine as a template

We have also investigated making a solution phase library of trisubstituted purines using 2-amino-6-chloropurine as the template (Scheme 2, shown for reaction with a primary amine). The first reaction, nucleophilic attack at C-6 with a primary or secondary amine, proceeded smoothly to form **6** under similar conditions used for the conversion of **1** to **2**. By first substituting at the 6-position, N-7 is sterically less accessible and clean benzylation on N-9 was achieved either using benzyl chloride and potassium carbonate, or using Mitsunobu conditions with benzyl alcohol. If N-6 is not substituted first, alkyation at both N-9 and N-7 is observed.[9] The 6-substituted aminopurines **6** reacted slowly with tosyl chloride to make **8**, the sulphonamide at N-9.[17] Representative examples of **7** and **8** are shown in Table 4. Attempts to further functionalise **7** or **8** by reductive alkylation or sulphonylation on the 2-amino group failed due to the very low reactivity of this group.



5 **6** **7** $R_2 = CH_2C_6H_5$ **8** $R_2 = SO_2C_6H_4CH_3$

Scheme 2 (i) amine R^1NH_2 (1.05 eq), Et_3N (1.1 eq), DMF, 80-100 °C, 24 h. (ii) **a** benzyl chloride (1.05 eq), K_2CO_3, DMF, rt, 24 h; or **b** benzyl alcohol (1.05 eq), DEAD (3 eq), PPh_3(3 eq), dioxane, rt, 6 h; or **c** tosyl chloride (1.05 eq), Et_3N (1.1 eq), DMF, 0 °C to rt, 24 h.

Table 4: 6,9-disubstituted 2-aminopurines (7) and (8)

6-substituent	9-substituent	Yield %	Purity[a] %
butylamino	benzyl	95	90
3-fluorobenzylamino	*p*-toluenesulphonamido	78	67

[a]Purity determined by C-18 reverse phase HPLC using a Columbus 50 x 2 mm C18 column (15-75% CH_3CN in H_2O containing 0.1 % TFA), monitored at 254 nm using a UV detector and by a SEDEX Evaporative Light Scattering Detector.

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REFERENCES AND NOTES

1. Grant, B. D.; Tsigelny, I.; Adams, J. A.; Taylor, S. S. *Prot. Sci.* **1996**, *5*, 1316-1324.
2. Schulzegahmen, U.; Debondt, H. L.; Kim, S. H. *J. Med. Chem.* **1996**, *39*, 4540-4546.
3. Songyang, Z.; Lu, K. P.; Kwon, Y. T.; Tsai, L. H.; Filhol, O.; Cochet, C.; Brickey, D. A.; Soderling, T. R.; Bartleson, C.; Graves, D. J.; Demaggio, A. J.; Hoekstra, M. F.; Blenis, J.; Hunter, T.; Cantley, L. C. *Mol. Cell. Biol.* **1996**, *16*, 6486-6493.
4. Norbury, C.; Nurse, P. *Ann. Rev. Biochem.* **1992**, *61*, 441-470.
5. Vesely, J.; Havlicek, L.; Strnad, M.; Blow, J. J.; Donelladeana, A.; Pinna, L.; Letham, D. S.; Kato, J.; Detivaud, L.; Leclerc, S.; Meijer, L. *Eur. J. Biochem.* **1994**, *224*, 771-786.
6. Misteli, T.; Warren, G. *J. Cell Sci.* **1995**, *108*, 2715-2727.
7. Glab, N.; Labidi, B.; Qin, L. X.; Trehin, C.; Bergounioux, C.; Meijer, L. *FEBS Lett.* **1994**, *353*, 207-211.
8. Gray, N. S.; Kwon, S.; Schultz, P. G. *Tetrahedron Lett.* **1997**, *38*, 1161-1164.
9. Norman, T. C.; Gray, N. S.; Koh, J. T.; Schultz, P. G. *J. Amer. Chem. Soc.* **1996**, *118*, 7430-7431.
10. Nugiel, D. A.; Cornelius, L. A. M.; Corbett, J. W. *J. Org. Chem.* **1997**, *62*, 201-203.
11. *General protocol for the preparation of 6-amino-substituted purines* **2.** 2,6-Dichloropurine (100 mg) was added to a solution of the corresponding amine (1.05 eq) in DMF (1 ml) and Et_3N (1.1 eq) and the resulting mixture was stirred at 80 °–100 °C for 2-24 h. To isolate the product **2** the solution was allowed to cool, evaporated to dryness, taken up in ethyl acetate (5 ml), washed with water (2 x 5 ml), brine (1 x 5 ml), dried over anhydrous magnesium sulphate and concentrated *in vacuo*. Diethyl ether (1 ml) was added and a precipitate formed which was filtered, washed with cold water (2 ml) and cold ethanol (1 ml) and dried *in vacuo*. The 6-aminopurine **2** was then recrystallied from ethanol/water. This protocol can also be used for the conversion of **5** to **6**.
12. Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Pept. Prot. Res.* **1991**, *37*, 487-493.
13. *General protocol for preparation of 2,6-diaminosubstituted purines* **3.** 2-Chloro-6-amino-substituted purine **2** (120 mg –either a single compound or a three component equimolar mixture) was refluxed in excess amine (5 eq) in DMF (2 ml) with Et_3N (1.1 eq) at 150 °C for 30 h. Once cooled, the mixture was evaporated to dryness, taken up into DMF (5 ml) and formylpolystyrene (CHO terminating) resin (25 eq) added. The reaction was stirred at 60 °C for 24 h. The resin was then washed with DMF (3 x 3 ml) and the filtrate evaporated to dryness. The remaining mixture was evaporated to dryness, taken up in ethyl acetate, washed with water (2 x 5 ml), and brine (1 x 5 ml), dried over anhydrous magnesium sulphate and concentrated *in vacuo*. Diethyl ether (1 ml) was added and a precipitate formed which was filtered, washed with cold water (2 ml) and cold ethanol (1 ml) and dried *in vacuo*.
14. Kaldor, S. W.; Seigel, M. G. *Tetrahedron Lett.* **1996**, *37*, 7193-7196.
15. *Benzylation of* **3.** Anhydrous potassium carbonate (4.6 eq) was added to a solution of the disubstituted purine (100 mg) in dry DMF (1 ml). The mixture was stirred at rt for 30 min followed by the addition of benzyl chloride (1.05 eq) The reaction was left stirring for 24 h at rt after which time the potassium carbonate was filtered off, water (10 ml) was added and a precipitate formed which was collected, washed with cold diethyl ether (5 ml) and dried *in vacuo*.
16. *Mitsunobu reaction on* **3.** To a cool solution (5 °C) of the disubstituted purine (100 mg), benzyl alcohol (1.05 eq) and DEAD (3 eq) in dry dioxane (2 ml) was added PPh_3 (3 eq) slowly over 10 min. The mixture was then allowed to warm to rt. with stirring for 6 h after which time it was evaporated to dryness. The dihydroDEAD and Ph_3PO were removed by passing the mixture through a short column of silica gel using AcOEt:hexane (1:1) as eluant.
17. *Preparation of 9-sulphonamidopurines* **8.** To a cold solution (0 °C) of purine (100 mg) in dry DMF (1 ml) containing dry Et_3N (1.1 eq) was added tosyl chloride (1.05 eq) over 10 min. The reaction was kept at 0 °C for 2 h and left at rt for a further 24 h. The solution was then diluted with water (1 ml) and a precipitate formed which was collected by filtration, washed with cold diethyl ether and dried *in vacuo*.