

# Controlled stepwise conversion of 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine into 2,4,6,8-tetrasubstituted pyrimido[5,4-*d*]pyrimidines

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For the rational synthesis of 2,4,6,8-tetrasubstituted pyrimido[5,4-*d*]pyrimidines, required as purine mimetics, sequential nucleophilic substitutions of 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine have been investigated. Reaction conditions have been devised leading to 2,4,6,8-tetrasubstituted pyrimido[5,4-*d*]pyrimidines with patterns of substitution denoted as *abab* (reaction with nucleophile 1 at C-4 and C-8, followed by nucleophile 2 at C-2 and C-6) or *abac* (reaction with nucleophile 1 at C-4 and C-8, nucleophile 2 at C-2 and nucleophile 3 at C-6) or *abcd* (reaction with nucleophile 1 at C-4, nucleophile 2 at C-8, nucleophile 3 at C-2 and nucleophile 4 at C-6). The use of low temperature, relatively dilute solution and careful addition of the amine nucleophile can control the critical first step. The third step in the production of the *abcd* pattern leads to two regioisomers, which have been structurally characterised by <sup>1</sup>H NMR and a crystal structure analysis. Selected 2,4,6,8-tetrasubstituted pyrimido[5,4-*d*]pyrimidines were tested as inhibitors of the cyclin-dependent kinase 1 complex (cyclin B/CDK1), but none of the compounds showed significant activity.

## Introduction

Pyrimido[5,4-*d*]pyrimidines are a relatively little studied heterocyclic system, which are of interest in the context of drug development because of their structural similarity to purines. We are especially interested in inhibitors of cyclin-dependent kinases (CDKs) and have developed purine derivatives (*e.g.* 2-amino-6-cyclohexylmethoxypurine **1**) that inhibit the cyclin B1/CDK1 and cyclin A3/CDK2 complexes at concentrations in the low micromolar range.<sup>1</sup> These purines are analogues of the 'benchmark' inhibitors olomoucine **2a** and roscovitine **2b**,<sup>2,3</sup> although they bind to CDK2 in a different orientation.<sup>1</sup> The objective of the present study was to obtain pyrimido[5,4-*d*]pyrimidine analogues of the purine CDK inhibitors. The synthetic access to such molecules requires that the pyrimido[5,4-*d*]pyrimidine scaffold be manipulated in a rational way. To this end we have investigated how to control the substitution chemistry of 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine (**3**, TCPP).<sup>4</sup> This compound is readily prepared from 1,5-dihydropyrimido[5,4-*d*]pyrimidine-2,4,6,8-(3*H*,7*H*)-tetraone **4**.<sup>4</sup> Besides enabling the synthesis of individual purine mimetics, a better knowledge of the chemistry of 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidines would provide an opportunity for parallel or combinatorial syntheses of a diverse array of novel compounds.

TCPP is the classical precursor of 2,4,6,8-tetrasubstituted pyrimido[5,4-*d*]pyrimidines, including the useful coronary vasodilator dipyrindamole (**5**, Persantin).<sup>5</sup> The substitution pattern in dipyrindamole can be described as *abab* where *a* corresponds to a substituent at the 4- and 8-positions of the pyrimido[5,4-*d*]pyrimidine scaffold, whereas *b* corresponds to a

substituent at the 2- and 6-positions (Fig. 1). The formation of pyrimido[5,4-*d*]pyrimidines with an *abab* pattern of substitution is the typical outcome of two stepwise substitution reactions of TCPP, employing an excess of nucleophile in each step.<sup>6,7</sup> This is because the rates of substitution greatly favour the C-4 and C-8 positions over C-2 and C-6. For example, treatment of TCPP with an excess of benzylamine at ambient temperature led to the formation of 4,8-bis(benzylamino)-2,6-dichloropyrimido[5,4-*d*]pyrimidine **6a**. Further reaction of this compound with an excess of ethanolamine (2-aminoethanol) at 120 °C gave 4,8-bis(benzylamino)-2,6-bis(2-hydroxyethylamino)pyrimido[5,4-*d*]pyrimidine **6b** (Scheme 1). The observed regioselectivity relates to the ability to delocalise the negative charge arising from attack of a nucleophile at C-2/C-6 or C-4/C-8. Attack at C-4/C-8 gives a species in which negative charge on nitrogen (*e.g.* N-3) can be delocalised into an adjacent C=N bond, whereas attack at C-2/C-6 does not permit this stabilising interaction.

The reactions of 4,8-bis(benzylamino)-2,6-dichloropyrimido[5,4-*d*]pyrimidine with nucleophiles can be carried out in a stepwise fashion to afford 4,8-bis(benzylamino)-2,6-disubstituted pyrimido[5,4-*d*]pyrimidines, with different substituents at C-2 and C-6, *i.e.* an *abac* substitution pattern (*cf.* Fig. 1). Furthermore, we have found that the reaction of TCPP with benzylamine can be controlled to give the mono-substituted product, 4-benzylamino-2,6,8-trichloropyrimido[5,4-*d*]pyrimidine, in acceptable yield. This compound served as a valuable starting material for the preparation of 2,4,6,8-tetrasubstituted pyrimido[5,4-*d*]pyrimidines with an *abcd* pattern of substitution, *via* a sequence of controlled nucleophilic substitutions of

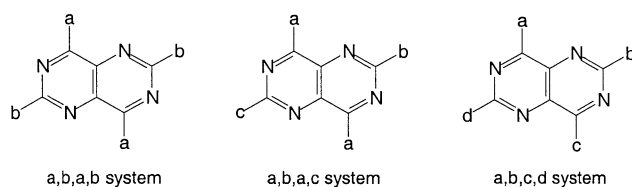
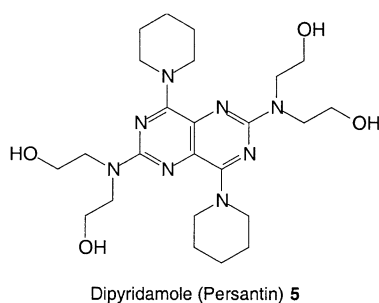
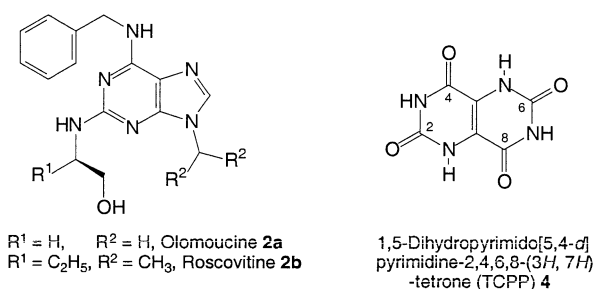
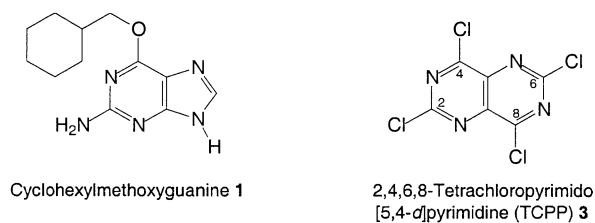
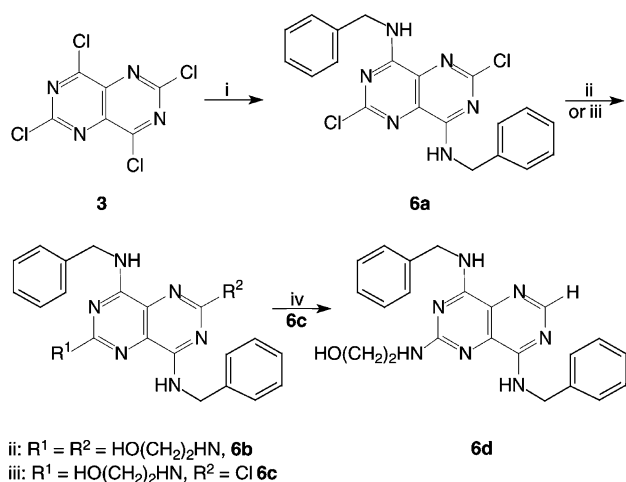


Fig. 1 Substitution patterns for pyrimido[5,4-*d*]pyrimidines.



**Scheme 1** Conversion of 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine **3** (TCPP) into **6a–6d**. Reagents and conditions: (i) 4 equiv. benzylamine, THF, rt, 20 min; (ii) ethanolamine, 120 °C 12 h; (iii) 64 equiv. ethanolamine, THF, 75 °C, 6 h; (iv) 3.9 equiv. KOH,  $\text{H}_2$ , 30–40 psi, 10% Pd/C, THF, 20 h.

the remaining three chloro functions. The structures of the *abac* and *abcd* tetrasubstituted pyrimido[5,4-*d*]pyrimidines described herein were validated by spectroscopic characterisation of all compounds, and for the *abcd* type by an X-ray analysis of a representative end-product. The *abcd* pattern of substitution was of particular interest in the context of analogues of purine CDK inhibitors and so selected compounds were evaluated as inhibitors of the cyclin B/CDK1 complex.

## Results and discussion

**Symmetrical and non-symmetrical 4,8-bis(benzylamino)-pyrimido[5,4-*d*]pyrimidines (*abab* and *abac* substitution pattern e.g. *a* = benzylamino, *b* = 2-hydroxyethylamino, *c* = H)**

Various methods to access TCPP **3** have been described.<sup>4</sup> We found that a satisfactory method is to heat an aqueous solution of 5-aminoorotic acid at 210 °C in the presence of ammonium chloride and potassium cyanate to give tetraone **4**.<sup>4</sup> This was isolated as its disodium salt, which was converted into **3** by treatment with a mixture of phosphorus oxychloride and phosphorus pentachloride. Reaction of 4 mol equivalents of benzylamine with TCPP in THF at ambient temperature rapidly gave 4,8-bis(benzylamino)-2,6-dichloropyrimido[5,4-*d*]pyrimidine **6a** in good yield, without formation of the tri- and tetra-substituted benzylamino derivatives (Scheme 1). The procedure used was a modification of that reported,<sup>7</sup> employing reduced quantities of benzylamine to improve the isolation and purification of compound **6a**.

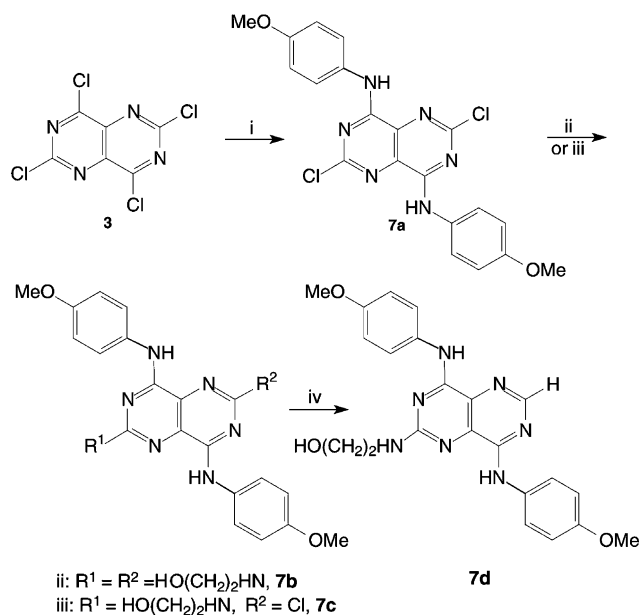
For the synthesis of symmetrical (*abab*) and non-symmetrical (*abac*) 2,6-disubstituted 4,8-bis(benzylamino)pyrimido[5,4-*d*]pyrimidines, nucleophilic substitutions at the C-2 and C-6 positions were required. Heating **6a** with neat ethanolamine at 100 °C gave 4,8-bis(benzylamino)-2,6-bis(2-hydroxyethylamino)pyrimido[5,4-*d*]pyrimidine **6b** (Scheme 1). Substitution at the 2- (or -6) position of **6a** with an amine should result in deactivation of the pyrimido[5,4-*d*]pyrimidine system to further substitution. By carefully controlling the reaction temperature and the concentration of amine used, it was possible to introduce an amino function selectively at C-2 (or C-6). Thus, when compound **6a** was heated with ethanolamine in THF at 75 °C 4,8-bis(benzylamino)-6-chloro-2-(2-hydroxyethylamino)pyrimido[5,4-*d*]pyrimidine **6c** was obtained in good yield.

The reductive dehalogenation of **6c** was performed using Pd/C, KOH and dry THF in a pressure vessel under a hydrogen atmosphere of 30–40 psi.<sup>8</sup> This method produced compound **6d** cleanly and in good yield, with no apparent debenzylation (Scheme 1). Having demonstrated a selective method for the synthesis of the *abac* substituted pyrimido[5,4-*d*]pyrimidines with benzylamino substituents at C-4 and C-8, the methodology was applied to the generation of analogous *p*-methoxyanilino compounds. Reaction of 4-methoxyaniline with TCPP (2.1 : 1 molar ratio) in THF at ambient temperature gave 4,8-bis(4-methoxyanilino)-2,6-dichloropyrimido[5,4-*d*]pyrimidine **7a** in good yield (Scheme 2). Subsequent reaction with ethanolamine in THF selectively produced 4,8-bis(4-methoxyanilino)-6-chloro-2-(2-hydroxyethylamino)pyrimido[5,4-*d*]pyrimidine **7c**, whereas the use of neat ethanolamine gave 4,8-bis(4-methoxyanilino)-2,6-bis(2-hydroxyethylamino)pyrimido[5,4-*d*]pyrimidine **7b**. Reductive dehalogenation of **7c** furnished 4,8-bis(4-methoxyanilino)-2-(2-hydroxyethylamino)pyrimido[5,4-*d*]pyrimidine **7d** (Scheme 2).

Both **7c** and **7d** displayed marked fluorescence in organic solvents and were also highly photosensitive. Aerial photosensitivity has been reported for dipyridamole **5**, whereby one of the piperidine rings is oxidised to give 2,6-bis(2-hydroxyethylamino)-4-(2-oxopiperidinyl)-8-piperidinylpyrimido[5,4-*d*]pyrimidine.<sup>9</sup>

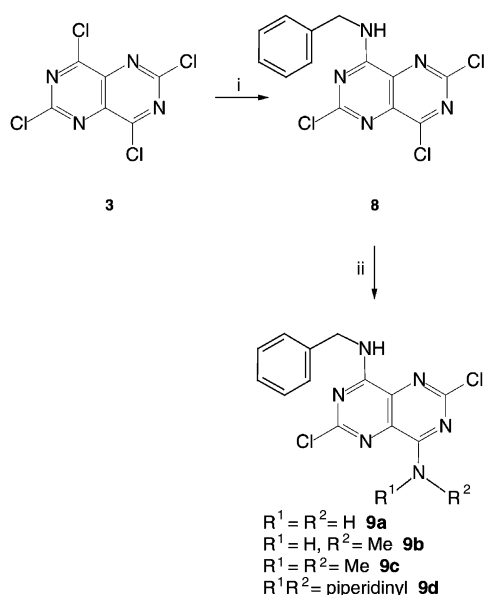
**C-4 Monobenzylaminopyrimido[5,4-*d*]pyrimidine analogues (*abcd* substitution pattern, *a* = benzylamino, *b* = e.g. methylamino, *c* = 2-hydroxyethylamino, *d* = H)**

The synthesis of homopurine analogues of olomoucine or roscovitine requires selective monosubstitution of the 4 (or 8) chloro group of TCPP **3** with benzylamine. A variety of reaction parameters were investigated in order to achieve this goal. Reproducible results were obtained using a 1.1 : 1 molar ratio of benzylamine to TCPP in an equal volume of THF at



**Scheme 2** Conversion of TCPP into **7a–d**. *Reagents and conditions:* (i) 2.1 equiv. 4-methoxyaniline, 2.4 equiv.  $\text{K}_2\text{CO}_3$ , THF, rt, 20 min; (ii) ethanolamine, 100 °C, 12 h; (iii) 146 equiv. ethanolamine, THF, 65 °C, 6 h; (iv) 4.6 equiv. KOH,  $\text{H}_2$ , 30–40 psi, 10% Pd/C, THF, 7 days.

–78 °C, with a controlled, dropwise addition of the benzylamine solution from a syringe at a rate of 1 ml per minute. After stirring the mixture for 5 minutes, addition of water precipitated **8** (Scheme 3) in acceptable yield and sufficiently pure by TLC and HPLC analysis to be used directly.



**Scheme 3** Conversion of TCPP into **8** as an intermediate in the synthesis of **9a–d**. *Reagents and conditions:* (i) 1.14 equiv. benzylamine,  $\text{K}_2\text{CO}_3$ , THF, –78 °C, 15 min; (ii) excess  $\text{R}^1\text{R}^2\text{NH}$ , THF, 0 °C, 40 min.

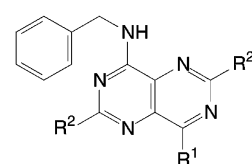
Treatment of the monobenzylamino derivative **8** with ammonia, methylamine, dimethylamine or piperidine in THF at 0 °C gave the corresponding 4,8-disubstituted pyrimido[5,4-*d*]pyrimidines **9a–d** (Scheme 3). Heating each of compounds **9a–d** with ethanolamine in THF afforded the corresponding trisubstituted pyrimido[5,4-*d*]pyrimidine derivatives **10a–d** (Scheme 4). In the absence of THF and with an excess of ethanolamine the corresponding tetrasubstituted pyrimido[5,4-*d*]pyrimidines **11a–d** were obtained.

Dehalogenation of compounds **10a–d** (Scheme 5) gave the corresponding homopurines **12a–d** in good yield. Only com-

**Table 1** Selected  $^1\text{H}$  NMR chemical shifts for **12bi** and **12bii**

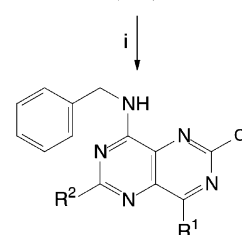
Group	<b>12bi</b> <sup>a</sup>	<b>12bi</b> <sup>b</sup>	<b>12bii</b> <sup>a</sup>	<b>12bii</b> <sup>b</sup>
$\text{NCH}_3$	2.97	3.06	3.03	3.08
$\text{ArCH}_2\text{N}$	4.76	4.78	4.69	4.72
$\text{N}=\text{C}-\text{H}$	8.17	8.42	8.14	8.42

<sup>a</sup>  $d_6$ -DMSO. <sup>b</sup> +DCI/ $\text{D}_2\text{O}$ .



$R^1 = \text{NH}_2$ ,  $R^2 = \text{NH}(\text{CH}_2)_2\text{OH}$  **11a**  
 $R^1 = \text{NHMe}$ ,  $R^2 = \text{NH}(\text{CH}_2)_2\text{OH}$  **11b**  
 $R^1 = \text{NMe}_2$ ,  $R^2 = \text{NH}(\text{CH}_2)_2\text{OH}$  **11c**  
 $R^1 = \text{piperidinyl}$ ,  $R^2 = \text{NH}(\text{CH}_2)_2\text{OH}$  **11d**

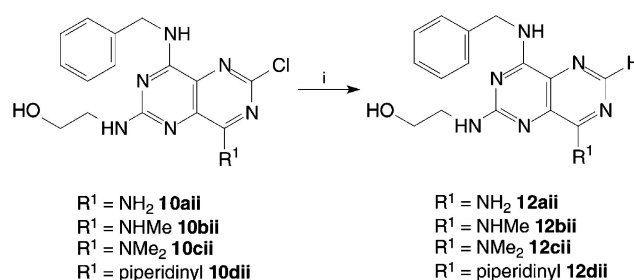
ii  
 (9a-d)



$R^1 = \text{NH}_2$ ,  $R^2 = \text{NH}(\text{CH}_2)_2\text{OH}$  **10aii**  
 $R^1 = \text{NHMe}$ ,  $R^2 = \text{NH}(\text{CH}_2)_2\text{OH}$  **10bii**  
 $R^1 = \text{NMe}_2$ ,  $R^2 = \text{NH}(\text{CH}_2)_2\text{OH}$  **10cii**  
 $R^1 = \text{piperidinyl}$ ,  $R^2 = \text{NH}(\text{CH}_2)_2\text{OH}$  **10dii**

(for isomers **10ai**, **10bi**, **10ci** and **10di**  
 the Cl and  $\text{R}^2$  group change places)

**Scheme 4** Mono and disubstitution of **9a–d** with ethanolamine. *Reagents and conditions:* i) ethanolamine, THF, 65 °C, 6 h; ii) excess ethanolamine, 100 °C.

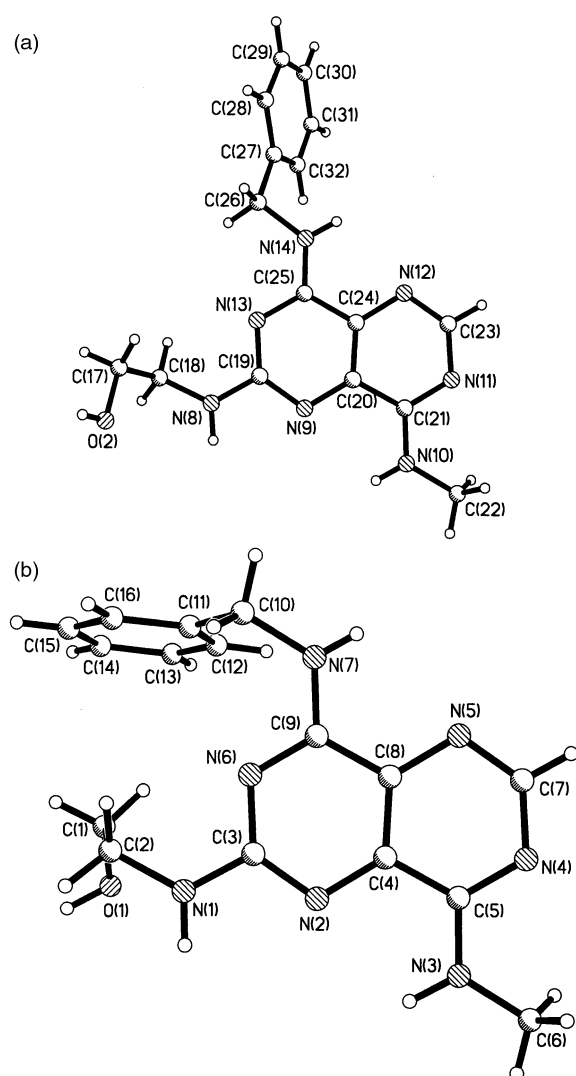


**Scheme 5** Reductive dehalogenations of compounds **10aii–dii** [note these reactions were carried out on mixtures of **10ai** and **10aii**, **10bi** and **10bii**, **10ci** and **10cii**, **10di** and **10dii** leading to mixtures of **12ai** and **12aii**, **12bi** and **12bii**, **12ci** and **12cii**, **12di** and **12dii**, respectively; only the structures of the **ii** isomers are shown (the **i** isomers have the Cl or H at C-2 and the  $\text{CH}_2\text{CH}_2\text{OH}$  at C-6)]. *Reagents and conditions:* i) 6.1 equiv. KOH,  $\text{H}_2$ , 10% Pd/C, THF, 20 h.

pound **11b** in the 2,6-bis(2-hydroxyethylamino) series could be obtained in a pure form. Similarly, compound **12b** was the only member of the dehalogenated derivatives to be obtained in a pure enough form for characterisation and biological evaluation. This was due to the instability of the compounds and difficulties encountered with purification.

The reactions described for compounds **9a–d** leading to **10a–d** and **12a–d** produced an equimolar mixture of C-2 and C-6 regioisomers, as gauged by  $^1\text{H}$  NMR analysis (Table 1). The respective isomers were separated chromatographically for compounds **10b** (i and ii) and **12b** (i and ii).  $^1\text{H}$  NMR analysis of the separated isomers allowed their assignment as **12bi** ('C-6 isomer') for the less polar compound and as **12bii** ('C-2 isomer') for the more polar compound. The assignment was based on the positions of the resonances from the *N*-alkyl group and benzyl methylene. Elucidation of the structures of the isomers of compound **12b** (i and ii) was aided by their protonation with DCI (Table 1). The *N*-methyl resonance furthest upfield should correspond to the pyrimidine ring with the (2-hydroxyethylamino) group attached. In contrast, the ring bearing no substituent at C-2 or C-6 should exhibit a downfield shift for either the *N*-methyl or *N*-benzyl methylene resonance. Protonation of the pyrimido[5,4-*d*]pyrimidine system (most likely on an endocyclic nitrogen) resulted in a downfield shift of all resonances and especially the resonance for H-2/H-6.

Proof of the structure of **12bii**, and hence **12bi**, was obtained by X-ray crystal structure analysis which confirmed compound **12bii** as the C-2 isomer (for data see Fig. 2). Two conformers,



**Fig. 2** The *anti* (a) and *gauche* (b) conformers observed in the crystal structure of **12bii**.

*anti* and *gauche* with respect to the N(14)–C(26) (Fig. 2a) and N(7)–C(10) (Fig. 2b) bonds, were observed in the crystallographic asymmetric unit of the structure. These are linked together by a network of hydrogen bonds involving all eight potential O–H and N–H donors, with N atoms of the pyrimido[5,4-*d*]pyrimidine systems as acceptors, O...N and N...N distances ranging from 2.72 to 3.17 Å. There is also approximately parallel stacking of pyrimido[5,4-*d*]pyrimidine and phenyl rings in various directions in the crystal structure. This combination of hydrogen bonding and hydrophobic stacking interactions may help to explain the poor solubility of the pyrimido[5,4-*d*]pyrimidine analogues.

All of the exocyclic C–N bonds connecting to the pyrimido[5,4-*d*]pyrimidine ring are relatively short [*ca.* 1.34 Å: N<sup>14</sup>–C<sup>25</sup> (1.333), N<sup>10</sup>–C<sup>21</sup> (1.333), N<sup>8</sup>–C<sup>19</sup> (1.355); atom numbers refer to the crystal structure assignment], and are approximately the same length as the carbon–carbon double bond of ethene (1.33 Å), suggesting partial double bond character. These data are in accord with the observation of rotameric species in the  $^1\text{H}$  NMR spectra of certain compounds, in particular **8** and **9c**. The spectra displayed two broad singlets for both the benzyl methylene and dimethylamino groups, each pair collapsing to a broad singlet upon heating the samples to coalescence. The corresponding figures for the C-alkyl–N bonds, by comparison, are greater [by *ca.* 0.1 Å: N<sup>14</sup>–C<sup>26</sup> (1.464), N<sup>10</sup>–C<sup>22</sup> (1.464), N<sup>8</sup>–C<sup>18</sup> (1.450)], as would be expected for a non-conjugated carbon–nitrogen single bond.

### Inhibition of cyclin B/CDK1

Three initial series of pyrimido[5,4-*d*]pyrimidine analogues have been synthesised in order to determine the inhibitory potential of the pyrimido[5,4-*d*]pyrimidine pharmacophore against cyclin B/CDK1. The activity of the cyclin B/CDK1 complex, in the presence of seven selected pyrimido[5,4-*d*]pyrimidines (**6b–6d**, **7c**, **7d**, **10bi**, **12bi**), was measured by its ability to phosphorylate a cellular substrate using radiolabelled  $^{32}\text{P}$  ATP.<sup>10</sup> Difficulties were encountered in obtaining accurate data because of the low solubility of all of the compounds in aqueous media. This necessitated the inclusion of either 1% or 10% DMSO in the assay medium, although even with 10% DMSO some compounds could not be assayed at a concentration of 100 μM. It should be noted that a 10 μM concentration of the reference standard olomoucine gave 64% inhibition (IC<sub>50</sub> 9 μM; note IC<sub>50</sub> is the concentration of drug required to reduce the activity of the enzyme by 50%) in 1% DMSO, whereas the same concentration of drug in 10% DMSO gave 31% inhibition. The results indicated that none of the pyrimido[5,4-*d*]pyrimidines tested exhibited significant activity (*i.e.* IC<sub>50</sub> < 100 μM) against cyclin B/CDK1.

### Conclusions

The work described in this paper has shown how reactions of 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine with nucleophiles can be controlled to give structurally characterised tetrasubstituted pyrimido[5,4-*d*]pyrimidines with *abab*, *abac* or *abcd* substitution patterns. The methodology described in this paper should facilitate the design of pyrimido[5,4-*d*]pyrimidine analogues, which are more soluble in aqueous media and more active as CDK inhibitors. Furthermore, the results presented indicate how combinatorial approaches could be used for the development of biologically active compounds based on the pyrimido[5,4-*d*]pyrimidine scaffold.

### Experimental

All techniques were performed as described in earlier work,<sup>11</sup> except where indicated below. Combustion analyses were carried out on a Carlo-Erba Instrumentazione 1106 analyser

or by Butterworths Laboratories Ltd, 54–56 Waldergrave Road, Teddington, Middlesex, UK TW11 8LG. All proton and carbon nuclear magnetic resonance ( $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR) spectra were obtained in deuteriated dimethyl sulfoxide ( $d_6$ -DMSO) except where indicated and exchangeable protons were validated using a deuterium oxide ( $\text{D}_2\text{O}$ ) 'shake'. Carbon spectra were recorded using a Bruker AMX 500 spectrometer and are broad band decoupled. The following abbreviations are used to describe peak patterns in proton spectra: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet; and combinations thereof, e.g. dd (double doublet).

The general starting material 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine and all products derived from the pyrimido[5,4-*d*]pyrimidine nucleus were protected from direct light sources due to their potential photosensitive nature.<sup>9</sup> Tetrahydrofuran was distilled from sodium and benzophenone, and used immediately. Benzylamine was dried over calcium hydride and distilled. Dimethylacetamide was dried over barium oxide and distilled. Distilled solvents were stored over 4 Å molecular sieves under an inert gas atmosphere.<sup>12</sup>

#### 2,4,6,8-Tetrachloropyrimido[5,4-*d*]pyrimidine 3<sup>4</sup>

A solution containing 5-aminoorotic acid † (1.06 g, 6.19 mmol), potassium cyanate (2.54 g, 31.36 mmol) and ammonium chloride (3.35 g, 63.2 mmol) in water (19 ml) was stirred for 0.5 h. The mixture was heated to 140 °C whilst the water was allowed to evaporate, giving a thick paste. Heating the paste at 210 °C for 0.5 h gave a beige solid which was cooled, triturated with water and filtered. After drying, the solid was added to a boiling solution of sodium hydroxide (0.1 M, 125 ml), and the yellow solution was left to cool. The product was removed by filtration to yield pale orange crystals, which were dried and used directly (0.88 g, 73%). 3,7-Disodio-1,5-dihydropyrimido[5,4-*d*]pyrimidine-2,4,6,8-(3*H*,7*H*)-tetraone (6.0 g, 25 mmol) was mixed with phosphorus oxychloride (250 ml) and phosphorus pentachloride (30 g) under nitrogen and refluxed for 15 h. Phosphorus oxychloride was removed by short-path distillation to give a brown solid to which crushed ice (350 ml) was added. The slurry was filtered and the remaining brown solid was dried and extracted with chloroform using a Soxhlet apparatus. Removal of the solvent *in vacuo* gave the product as a pale yellow powder which was used directly (3.44 g, 51%);  $\lambda_{\text{max}}/\text{nm}$  329 (7400), 282 (2800), 258 (12300); EIMS  $m/z$  274 ( $\text{M}^+$  + 6, 11%), 272 ( $\text{M}^+$  + 4, 49), 270 ( $\text{M}^+$  + 2, 100), 268 ( $\text{M}^+$ , 83).

#### 4-Benzylamino-2,6,8-trichloropyrimido[5,4-*d*]pyrimidine 8

To TCPP (0.10 g, 0.37 mmol) and potassium carbonate (0.10 g, 0.74 mmol) in THF (6 ml), stirred under nitrogen at –78 °C, was added benzylamine (0.04 g, 0.42 mmol) in THF (6 ml) dropwise from a syringe at a rate of 1 ml per min. A cloudy yellow solution was obtained which was allowed to warm up for 10 min. Addition of an excess of water gave a fine yellow precipitate which was extracted with ethyl acetate. After drying of the organic extract over  $\text{MgSO}_4$ , removal of the solvent gave a yellow glass. Trituration with petrol, filtration and drying *in vacuo* gave the title compound as a pale yellow solid, which was used without further purification (0.09 g, 72%);  $\delta_{\text{H}}$  4.69–4.72 (2 H, d,  $J$  6.1 Hz,  $\text{ArCH}_2\text{N}$ ), 4.72–4.81 (2 H, d,  $J$  6.4 Hz,  $\text{ArCH}_2\text{N}$ ), 7.31–7.50 (5 H, m, *Ar-H*), 9.20 (1 H, br s,  $\text{ArCH}_2\text{NH}$ ), 10.19 (1 H, br s,  $\text{ArCH}_2\text{NH}$ );  $m/z$  345 ( $\text{M}^+$  + 6, 3%), 343 ( $\text{M}^+$  + 4, 23), 341 ( $\text{M}^+$  + 2, 73), 339 ( $\text{M}^+$ , 79), 308 (3), 306 (12), 304 (18), 233 (40), 91 (100). Calculated for  $\text{C}_{13}\text{H}_8\text{N}_5\text{Cl}_3$ ;  $m/z$  338.98. Found 338.98.

† The IUPAC name for orotic acid is 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylic acid.

#### Synthesis of *abab* substituted-pyrimido[5,4-*d*]pyrimidines (a = NHR, b = Cl); standard procedure A

To TCPP (2.90–7.40 mmol) in dry THF (50–60 ml) stirred under  $\text{N}_2$  at 25 °C, was added an appropriate amine (4.0 mol equiv. or 2.1 mol equiv. with 2.40 equiv. potassium carbonate). The resulting suspension was agitated for 20 min. Addition of water (80 ml) precipitated the product. The solid was filtered off, washed with water and dried. The resulting product was either used as such or recrystallised from hot ethyl acetate.

#### 4,8-Bis(benzylamino)-2,6-dichloropyrimido[5,4-*d*]pyrimidine

**6a.** TCPP (2.0 g, 7.40 mmol), benzylamine (3.23 g, 3.24 ml, 29.60 mmol) and dry THF (60 ml) were mixed according to standard procedure A. Recrystallisation from hot ethyl acetate gave the title compound as a fine yellow powder (2.10 g, 69%); mp 229–230 °C [lit.,<sup>7</sup> 228–229 °C];  $\lambda_{\text{max}}/\text{nm}$  348 (14516), 341 (11262), 304 (23950), 218 (21573);  $\delta_{\text{H}}$  4.74 (4 H, d,  $J$  6.1 Hz,  $\text{ArCH}_2\text{NH}$ ), 7.32–7.47 (10 H, m, *Ar-H*), 9.40 (2 H, br s,  $\text{ArCH}_2\text{NH}$ );  $\delta_{\text{C}}$  (200 MHz) 43.7 ( $\text{CH}_2\text{N}$ ), 127.0, 127.4, 128.3, 132.2, 138.2, 155.4, 159.1.

#### 2,6-Dichloro-4,8-bis(4-methoxyanilino)pyrimido[5,4-*d*]pyrimidine 7a.

TCPP (0.80 g, 2.96 mmol), potassium carbonate (1.0 g, 7.24 mmol) and 4-methoxyaniline (0.76 g, 6.20 mmol) were mixed in THF (50 ml) according to standard procedure A to yield the title compound as a crude yellow solid (0.80 g, 62%);  $\delta_{\text{H}}$  3.88 (6 H, s, 2 × MeO), 7.01–7.12 (4 H, d,  $J$  9.0 Hz, *Ar-H*<sup>3/5</sup>), 7.80–7.84 (4 H, d,  $J$  9.0 Hz, *Ar-H*<sup>2/6</sup>);  $m/z$  442 ( $\text{M}^+$ , 20%), 427 (10).

#### Reaction of 4-benzylamino-2,6,8-trichloropyrimido[5,4-*d*]pyrimidine (8) with amines; standard procedure B

To a stirred solution of 4-benzylamino-2,6,8-trichloropyrimido[5,4-*d*]pyrimidine (0.077 M) in dry THF (13 ml) at 0 °C was added a solution of the appropriate amine (> 2.0 mol equiv.) in THF (13 ml) over a 10 min period. In all cases a precipitate formed and the suspension was left to stir for 10–30 min. Water (20 ml) was added and the THF was removed *in vacuo*. Purification of the product was achieved either by filtration and washing with water or by extraction with ethyl acetate. The crude material was dried and the product was either used directly or purified by silica column chromatography.

#### 8-Amino-4-benzylamino-2,6-dichloropyrimido[5,4-*d*]pyrimidine 9a.

Aqueous ammonia (4.0 ml) in THF (12 ml) was added to 4-benzylamino-2,6,8-trichloropyrimido[5,4-*d*]pyrimidine **8** (0.23 g, 0.69 mmol) in THF (12 ml) according to standard procedure B. Filtration of the reaction mixture, followed by washing with water and drying, gave the title compound as a beige solid (0.10 g, 46%);  $\delta_{\text{H}}$  4.69–4.72 (2 H, d,  $J$  6.0 Hz,  $\text{ArCH}_2\text{NH}$ ), 7.31–7.41 (5 H, m, *Ar-H*), 8.29 (2 H, br s,  $\text{NH}_2$ ), 9.32 (1 H, t,  $\text{ArCH}_2\text{NH}$ );  $m/z$  320 ( $\text{M}^+$ , 95%), 91 (100). Calculated for  $\text{C}_{13}\text{H}_{10}\text{N}_6\text{Cl}_2$ ;  $m/z$  320.0. Found 320.0.

#### 4-Benzylamino-2,6-dichloro-8-methylaminopyrimido[5,4-*d*]pyrimidine 9b.

4-Benzylamino-2,6,8-trichloropyrimido[5,4-*d*]pyrimidine **8** (0.30 g, 0.88 mmol), THF (12 ml) and ethanolic methylamine (33%, 0.8 ml) in THF (12 ml) were mixed according to standard procedure B. Purification of the crude product by extraction into ethyl acetate, followed by chromatography employing 12% ethyl acetate–petrol as eluent, gave the title compound as a white powder (0.12 g, 41%);  $\delta_{\text{H}}$  3.03 (3 H, d,  $J$  4.2 Hz,  $\text{NHCH}_3$ ), 4.73 (2 H, d,  $J$  4.7 Hz,  $\text{ArCH}_2\text{NH}$ ), 7.32–7.48 (5 H, m, *Ar-H*), 8.82 (1 H, m,  $\text{NHCH}_3$ ), 9.37 (1 H, m,  $\text{ArCH}_2\text{NH}$ );  $m/z$  334 ( $\text{M}^+$ , 100%), 299 (10), 91 (70). Calculated for  $\text{C}_{14}\text{H}_{12}\text{N}_6\text{Cl}_2$ ;  $m/z$  334.0. Found 334.0.

**4-Benzylamino-2,6-dichloro-8-dimethylaminopyrimido[5,4-*d*]-pyrimidine 9c.** Ethanolic dimethylamine (0.5 ml, 33%) in THF (2 ml) was added to 4-benzylamino-2,6,8-trichloropyrimido[5,4-*d*]pyrimidine **8** (0.05 g, 0.15 mmol) in THF (2 ml) according to standard procedure B. Filtration of the reaction mixture followed by washing with water and drying gave the title compound as a beige solid (0.04 g, 67%);  $\delta_{\text{H}}$  (CDCl<sub>3</sub>) 3.30–3.90 (6 H, br d, (CH<sub>3</sub>)<sub>2</sub>N), 4.74–4.76 (2 H, d, *J* 5.9 Hz, ArCH<sub>2</sub>NH), 7.25–7.60 (5 H, m, Ar-*H* × 5); *m/z* 348 (M<sup>+</sup>, 61%), 304 (20), 106 (77), 91 (100). Calculated for C<sub>15</sub>H<sub>14</sub>N<sub>6</sub>Cl<sub>2</sub>: *m/z* 348.1. Found 348.1.

**4-Benzylamino-2,6-dichloro-8-piperidinopyrimido[5,4-*d*]-pyrimidine 9d.** 4-Benzylamino-2,6,8-trichloropyrimido[5,4-*d*]pyrimidine **8** (0.13 g, 0.37 mmol), THF (5 ml) and piperidine (0.04 g, 0.44 mmol) in THF (5 ml) were mixed according to standard procedure B. Extraction with ethyl acetate, drying (Na<sub>2</sub>SO<sub>4</sub>), and removal of the solvent *in vacuo* gave the title compound as a crude solid (0.09 g, 63%);  $\delta_{\text{H}}$  1.76 (6 H, br s, -(CH<sub>2</sub>)<sub>3</sub>-), 4.30 (4 H, br s, CH<sub>2</sub>NCH<sub>2</sub>), 4.72–4.75 (2 H, d, *J* 6.4 Hz, ArCH<sub>2</sub>NH), 7.32–7.44 (5 H, m, Ar-*H*), 9.35 (1 H, t, *J* 6.4 Hz, ArCH<sub>2</sub>NH); *m/z* 392 (M + 4, 9%), 390 (M + 2, 50), 388 (M<sup>+</sup>, 75), 297 (8), 106 (20), 91 (90), 84 (100). Calculated for C<sub>18</sub>H<sub>18</sub>N<sub>6</sub>Cl<sub>2</sub>: *m/z* 388.1. Found 388.1.

#### Reaction of *abab* and *abcc* substituted-pyrimido[5,4-*d*]-pyrimidines with ethanolamine (mono substitution); standard procedure C

To the appropriate 2,6-dichloro-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidine (0.15–1.13 mmol) in THF (5–12 ml) under nitrogen was added ethanolamine (1.25–10 ml). The resultant yellow solution was heated at 65 °C for 2–6 h (unless stated otherwise), over which period a fine precipitate of ethanolamine hydrochloride formed. The suspension was cooled and water (50 ml) was added, precipitating the product and dissolving the salt. The resultant mixture was filtered, to give an off-white solid which was retained. The filtrate was extracted with ethyl acetate and the organic layer was washed with water, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated, producing a secondary yield of product. Column chromatography and/or recrystallisation from a suitable solvent afforded the desired compound.

**4,8-Bis(benzylamino)-6-chloro-2-(2-hydroxyethylamino)-pyrimido[5,4-*d*]pyrimidine 6c.** 4,8-Bis(benzylamino)-2,6-dichloropyrimido[5,4-*d*]pyrimidine **6a** (0.31 g, 0.74 mmol), dry THF (12 ml) and ethanolamine (3.0 ml, 48 mmol) were heated at 75 °C for 6 h following standard procedure C. The product was obtained by recrystallisation from hot ethyl acetate to yield the title compound as a fine white powder (0.12 g, 61%); mp 180–181 °C:  $\nu_{\text{max}}$ /cm<sup>-1</sup> 3584 (NH), 3354 (OH), 3029 (CH), 2936 (CH), 1590, 1568, 1534, 1508;  $\delta_{\text{H}}$  3.50 (4 H, m, NH(CH<sub>2</sub>)<sub>2</sub>OH), 4.75 (5 H, m, ArCH<sub>2</sub>NH × 2, CH<sub>2</sub>OH), 6.81 (1 H, br s, CH<sub>2</sub>NH), 7.41 (10 H, m, Ar-*H*), 8.44 (2 H, br m, ArCH<sub>2</sub>NH); *m/z* 437 (M + 2, 19%), 435 (M<sup>+</sup>, 60), 91 (100) (Found: C 60.73; H 4.95; N 22.38; C<sub>22</sub>H<sub>22</sub>N<sub>7</sub>ClO requires: C 60.58; H 5.09; N 22.50%).

**6-Chloro-2-(2-hydroxyethylamino)-4,8-bis(4-methoxyanilino)-pyrimido[5,4-*d*]pyrimidine 7c.** 2,6-Dichloro-4,8-bis(4-methoxyanilino)pyrimido[5,4-*d*]pyrimidine **7a** (0.50 g, 1.13 mmol), THF (10 ml) and ethanolamine (10.12 g, 10 ml) were stirred for 2 h following standard procedure C. Recrystallisation from ethyl acetate gave the product as a yellow solid (0.48 g, 91%); mp 198–200 °C:  $\delta_{\text{H}}$  3.69 (4 H, m, NH(CH<sub>2</sub>)<sub>2</sub>OH), 3.87 (3 H, s, CH<sub>3</sub>O), 3.89 (3 H, s, CH<sub>3</sub>O), 4.81 (1 H, br s, CH<sub>2</sub>OH), 7.01–7.05 (3 H, d, *J* 8.8 Hz, Ar-*H*<sup>3/5</sup>, CH<sub>2</sub>NH), 7.09–7.14 (2 H, d, *J* 8.8 Hz, Ar-*H*<sup>3/5</sup>), 7.83–7.87 (2 H, d, *J* 8.8 Hz, Ar-*H*<sup>2/6</sup>), 7.99–8.04 (2 H, d, *J* 8.8 Hz, Ar-*H*<sup>2/6</sup>), 9.50 (2 H, br s, ArCH<sub>2</sub>NH);

*m/z* 467 (M<sup>+</sup>, 100%), 452 (20), 436 (89) (Found: C 56.59; H 4.62; N 20.93; C<sub>22</sub>H<sub>22</sub>ClN<sub>7</sub>O<sub>3</sub> requires: C 56.56; H 4.75; N 20.98%).

**8-Amino-4-benzylamino-2-chloro-6-(2-hydroxyethylamino)-pyrimido[5,4-*d*]pyrimidine 10ai and 8-amino-4-benzylamino-6-chloro-2-(2-hydroxyethylamino)pyrimido[5,4-*d*]pyrimidine 10aai.** Following standard procedure C; 8-amino-4-benzylamino-2,6-dichloropyrimido[5,4-*d*]pyrimidine **9a** (0.20 g, 0.63 mmol), THF (10 ml) and ethanolamine (5 ml) were stirred for 3 h. Purification of the crude product by column chromatography using 3% methanol–dichloromethane as eluent, followed by recrystallisation from ethyl acetate, gave a mixture of the title compounds as a yellow solid (0.10 g, 47%); mp 164–166 °C:  $\delta_{\text{H}}$  3.46–3.57 (4 H, m, NH(CH<sub>2</sub>)<sub>2</sub>OH), 4.70–4.78 (3 H, m, ArCH<sub>2</sub>NH, CH<sub>2</sub>OH), 6.79 (1 H, br s, CH<sub>2</sub>NH), 7.31–7.48 (6 H, m, Ar-*H*, HNH), 8.41 (1 H, br s, HNH), 8.43 (1 H, br s, ArCH<sub>2</sub>NH); *m/z* 345 (M<sup>+</sup>, 60%), 329 (15), 314 (45), 91 (100) (Found: C 52.18; H 4.48; N 27.99; C<sub>15</sub>H<sub>16</sub>N<sub>7</sub>ClO requires: C 52.17; H 4.67; N 28.39%).

**4-Benzylamino-2-chloro-6-(2-hydroxyethylamino)-4-methylaminopyrimido[5,4-*d*]pyrimidine 10bi and 4-benzylamino-6-chloro-2-(2-hydroxyethylamino)-4-methylaminopyrimido[5,4-*d*]pyrimidine 10bii.** 4-Benzylamino-2,6-dichloro-8-methylaminopyrimido[5,4-*d*]pyrimidine **9b** (0.50 g, 0.15 mmol), THF (5 ml) and ethanolamine (1.25 ml) were stirred for 75 min according to standard procedure C. Column chromatography using 65% ethyl acetate–petrol as eluent gave a mixture of the title compounds as a white solid (0.04 g, 65%); mp 121–122 °C:  $\delta_{\text{H}}$  2.97–2.99 and 3.02–3.05 (3 H, 2 × d, *J* 4.7 Hz, NHCH<sub>3</sub>), 3.56–3.62 (4 H, m, NH(CH<sub>2</sub>)<sub>2</sub>OH), 4.68–4.70 and 4.71–4.77 (3 H, 2 × m, ArCH<sub>2</sub>NH, CH<sub>2</sub>OH), 6.75 (1 H, m, NHCH<sub>3</sub>), 7.31–7.48 (5 H, m, Ar-*H*), 7.86–8.02 (1 H, br d, CH<sub>2</sub>CH<sub>2</sub>NH), 8.38 (1 H, br d, ArCH<sub>2</sub>NH); *m/z* 361 (M + 2, 8%), 359 (M<sup>+</sup>, 22), 328 (15), 91 (100) (Found: C 53.52; H 4.84; N 26.93; C<sub>16</sub>H<sub>17</sub>N<sub>7</sub>ClO requires: C 53.48; H 5.05; N 27.28%).

**4-Benzylamino-2-chloro-6-(2-hydroxyethylamino)-8-dimethylaminopyrimido[5,4-*d*]pyrimidine 10ci and 4-benzylamino-6-chloro-2-(2-hydroxyethylamino)-8-dimethylaminopyrimido[5,4-*d*]pyrimidine 10cii.** Following standard procedure C; 4-benzylamino-2,6-dichloro-8-dimethylaminopyrimido[5,4-*d*]pyrimidine **9c** (0.30 g, 0.86 mmol), ethanolamine (10 ml) and THF (10 ml) were stirred for 3 h. Purification by column chromatography using 2% methanol–dichloromethane, followed by 6% methanol–dichloromethane, produced a mixture of the title compounds as a white solid (0.08 g, 25%); mp 106–108 °C:  $\delta_{\text{H}}$  3.50–3.63 (10 H, m, NH(CH<sub>2</sub>)<sub>2</sub>OH, (CH<sub>3</sub>)<sub>2</sub>N), 4.68–4.77 (3 H, m, ArCH<sub>2</sub>NH, CH<sub>2</sub>OH), 6.78 (1 H, m, CH<sub>2</sub>NH), 7.32–7.44 (5 H, m, Ar-*H*), 8.50 (1 H, m, ArCH<sub>2</sub>NH); *m/z* 373 (M<sup>+</sup>, 45%), 329 (18), 91 (100) (Found: C 54.99; H 5.35; N 25.79; C<sub>17</sub>H<sub>20</sub>N<sub>7</sub>ClO requires: C 54.68; H 5.40; N 26.26%).

**4-Benzylamino-2-chloro-6-(2-hydroxyethylamino)-8-piperidinopyrimido[5,4-*d*]pyrimidine 10di and 4-benzylamino-6-chloro-2-(2-hydroxyethylamino)-8-piperidinopyrimido[5,4-*d*]pyrimidine 10dii.** Following standard procedure C; 4-benzylamino-2,6-dichloro-8-piperidinopyrimido[5,4-*d*]pyrimidine **9d** (0.09 g, 0.23 mmol), THF (6 ml) and ethanolamine (1.50 ml) were stirred for 5 h. Purification by column chromatography using 40–50% ethyl acetate–petrol produced a mixture of the title compound as a crude yellow solid (0.05 g, 56%);  $\delta_{\text{H}}$  1.73 (6 H, br s, -(CH<sub>2</sub>)<sub>3</sub>-), 3.36–3.45 (2 H, m, NHCH<sub>2</sub>), 3.57–3.60 (2 H, m, CH<sub>2</sub>OH), 4.36 (4 H, br s, CH<sub>2</sub>NCH<sub>2</sub>), 4.67–4.75 (3 H, m, ArCH<sub>2</sub>NH, CH<sub>2</sub>OH), 6.85 (1 H, t, CH<sub>2</sub>NH), 7.31–7.45 (5 H, m, Ar-*H*), 8.36 (1 H, br s, ArCH<sub>2</sub>NH); *m/z* 413 (M<sup>+</sup>, 43%), 382 (15), 106 (10), 91 (100), 84 (30).

### Reaction of *abab* and *abcc* substituted-pyrimido[5,4-*d*]-pyrimidines with ethanolamine (disubstitution); standard procedure D

The appropriate pyrimido[5,4-*d*]pyrimidine was slurried in ethanolamine (2–5 ml) under nitrogen and heated at 80–120 °C for 1–3 days. After cooling the solution, water (20 ml) was added and an extraction was performed using ethyl acetate (100 ml). The organic solvent was dried (Na<sub>2</sub>SO<sub>4</sub>), and removed *in vacuo*. Purification of the crude product was achieved either by recrystallisation from hot ethyl acetate or by column chromatography on silica, to give the product as a pale yellow solid.

**4,8-Bis(benzylamino)-2,6-bis(2-hydroxyethylamino)pyrimido[5,4-*d*]pyrimidine 6b.** Following standard procedure D; 4,8-bis(benzylamino)-2,6-dichloropyrimido[5,4-*d*]pyrimidine **6a** (0.10 g, 0.24 mmol) and ethanolamine (2 ml) were heated at 120 °C for 20 h. The crude solid was recrystallised from ethyl acetate, producing the title compound as yellow crystals (0.08 g, 73%); mp 223–224 °C;  $\nu_{\max}/\text{cm}^{-1}$  3396 (NH), 3359 (OH), 2919 (CH), 1564, 1525;  $\delta_{\text{H}}$  3.55 (8 H, m, NH(CH<sub>2</sub>)<sub>2</sub>OH), 4.73 (6 H, m, ArCH<sub>2</sub>NH × 2, CH<sub>2</sub>OH), 6.12 (2 H, t, *J* 5.2 Hz, CH<sub>2</sub>NH × 2), 7.32–7.77 (10 H, m, Ar-*H*), 8.15 (2 H, m, ArCH<sub>2</sub>NH × 2); *m/z* 460 (M<sup>+</sup>, 100%) (Found: C 62.89; H 6.20; N 24.68; C<sub>24</sub>H<sub>28</sub>N<sub>8</sub>O<sub>2</sub> requires: C 62.57; H 6.13; N 24.34%).

**4-Benzylamino-2,6-bis(2-hydroxyethylamino)-8-methylamino-pyrimido[5,4-*d*]pyrimidine 11b.** Following standard procedure D; 4-benzylamino-2,6-dichloro-8-methylaminopyrimido[5,4-*d*]pyrimidine **9b** (0.02 g, 0.07 mmol) and ethanolamine (5 ml) were heated at 80 °C for 3 days. Purification using 6–10% methanol–dichloromethane gave the title compound as a yellow solid (0.13 g, 56%);  $\delta_{\text{H}}$  3.00–3.02 (3 H, d, *J* 4.9 Hz, NHCH<sub>3</sub>), 3.23–3.75 (8 H, m, NH(CH<sub>2</sub>)<sub>2</sub>OH), 4.68–4.75 (4 H, m, ArCH<sub>2</sub>NH, CH<sub>2</sub>OH × 2), 6.05–6.12 (1 H, q, *J* 4.9 Hz, NHCH<sub>3</sub>), 7.24–7.44 (7 H, m, Ar-*H* × 5, CH<sub>2</sub>NH × 2), 7.68–7.74 (1 H, t, *J* 5.6 Hz, ArCH<sub>2</sub>NH); *m/z* 386 (M + 2, 25%), 384 (M<sup>+</sup>, 79), 91 (100) (Found: C 56.64; H 6.18; N 29.13; C<sub>18</sub>H<sub>24</sub>N<sub>8</sub>O<sub>2</sub> requires: C 56.24; H 6.29; N 29.15%).

### Reductive dehalogenation of *abac* and *abcd* substituted-pyrimido[5,4-*d*]pyrimidines; standard procedure E

The appropriate pyrimido[5,4-*d*]pyrimidine (0.23–1.10 mmol) in dry THF (20–40 ml) was added under nitrogen to a pressure vessel containing 10% Pd/C (0.1–0.24 g) and potassium hydroxide (3.86–6.07 equiv.). The flask was evacuated and charged with hydrogen before pressurising to 40 psi and agitating for 1–7 days. On completion, the flask contents were filtered through Celite and eluted with ethyl acetate (100 ml). The solvent was dried (Na<sub>2</sub>SO<sub>4</sub>) and removed *in vacuo* to produce a crude solid. Purification was achieved by recrystallisation from hot ethyl acetate (or ethyl acetate–petrol) and/or silica column chromatography using methanol–dichloromethane as eluents, to give the desired product as a white solid.

**4,8-Bis(benzylamino)-2-(2-hydroxyethylamino)pyrimido[5,4-*d*]pyrimidine 6d.** 4,8-Bis(benzylamino)-2-chloro-6-(2-hydroxyethylamino)pyrimido[5,4-*d*]pyrimidine **6c** (0.10 g, 0.23 mmol), 10% Pd/C catalyst (0.11 g), KOH (0.05 g) and THF (20 ml) were mixed for 20 h according to standard procedure E. Recrystallisation of the solid from hot ethyl acetate gave the title compound as a white powder (0.04 g, 46%); mp 141–142 °C;  $\nu_{\max}/\text{cm}^{-1}$  3395 (NH), 3274 (OH), 3028 (CH), 2916 (CH), 1585, 1568, 1537, 1516;  $\delta_{\text{H}}$  3.00 (4 H, m, NH(CH<sub>2</sub>)<sub>2</sub>OH), 4.72–4.82 (5 H, m, ArCH<sub>2</sub>NH × 2, CH<sub>2</sub>OH), 6.65 (1 H, br s, CH<sub>2</sub>NH), 7.27–7.48 (10 H, m, Ar-*H*), 8.00 (1 H, br s, ArCH<sub>2</sub>NH), 8.22 (1 H, s, N=C–H), 8.52 (1 H, br s, ArCH<sub>2</sub>NH); *m/z* 401 (M<sup>+</sup>, 60%), 91 (100) (Found: C 65.64; H 5.59; N 23.98; C<sub>22</sub>H<sub>23</sub>N<sub>7</sub>O requires C 65.82; H 5.77; N 24.42%).

**2-(2-Hydroxyethylamino)-4,8-bis(4-methoxyanilino)pyrimido[5,4-*d*]pyrimidine 7d.** Following standard procedure E; 6-chloro-2-(2-hydroxyethylamino)-4,8-bis(4-methoxyanilino)pyrimido[5,4-*d*]pyrimidine **7c** (0.25 g, 0.50 mmol), 10% Pd/C (0.24 g), KOH (0.13 g) and THF (40 ml) were agitated for 7 days. Purification by silica column chromatography using 4% methanol–dichloromethane as eluent, followed by recrystallisation from ethyl acetate produced the title compound as a white solid (0.10 g, 45%); mp 166–167 °C;  $\delta_{\text{H}}$  3.69 (4 H, m, NH(CH<sub>2</sub>)<sub>2</sub>OH), 3.88 (3 H, s, CH<sub>3</sub>O), 3.91 (3 H, s, CH<sub>3</sub>O), 4.81 (1 H, br s, CH<sub>2</sub>OH), 7.01–7.11 (5 H, m, Ar-*H*<sup>3/5</sup> × 2, CH<sub>2</sub>NH), 7.92–7.96 (2 H, d, *J* 8.6 Hz, Ar-*H*<sup>2/6</sup>), 7.67–7.81 (2 H, d, *J* 8.6 Hz, Ar-*H*<sup>2/6</sup>), 8.43 (1 H, s, N=C–H), 9.20 (2 H, br s, ArCH<sub>2</sub>NH); *m/z* 433 (M<sup>+</sup>, 40%), 418 (10), 402 (20) (Found: C 61.12; H 5.16; N 22.37; C<sub>22</sub>H<sub>23</sub>N<sub>7</sub>O<sub>3</sub> requires: C 60.96; H 5.35; N 22.62%).

**4-Benzylamino-6-(2-hydroxyethylamino)-8-methylamino-pyrimido[5,4-*d*]pyrimidine 12bi and 4-benzylamino-2-(2-hydroxyethylamino)-8-methylaminopyrimido[5,4-*d*]pyrimidine 12bii.** A mixture of 4-benzylamino-2-chloro-6-(2-hydroxyethylamino)-8-methylaminopyrimido[5,4-*d*]pyrimidine **10bi** and 4-benzylamino-6-chloro-2-(2-hydroxyethylamino)-8-methylaminopyrimido[5,4-*d*]pyrimidine **10bii** (0.40 g, 1.10 mmol), 10% Pd/C (0.20 g), KOH (0.34 g) and THF (40 ml) were agitated for 72 h according to standard procedure E. Purification by column chromatography using 70–90% ethyl acetate–petrol gave a mixture of the title compounds as a white solid (0.15 g, 42%); mp 142–144 °C; *m/z* 325 (M<sup>+</sup>, 50%), 219 (90), 91 (100%). Repeated chromatography gave homogeneous samples (LCMS analysis) of the title compounds from the early and late column fractions.

**12bi** (white solid):  $\delta_{\text{H}}$  2.97 (3 H, distorted d, *J* 5 Hz, NHCH<sub>3</sub>), 3.5–3.6 (4 H, m, NH(CH<sub>2</sub>)<sub>2</sub>OH), 4.66 (1 H, br t, *J* 5 Hz, CH<sub>2</sub>OH), 4.76 (2 H, m, ArCH<sub>2</sub>NH), 6.6 (1 H, m, NHCH<sub>3</sub>), 7.3–7.4 (5 H, m, Ar-*H*), 7.9–8.0 (2 H, 2 × m, ArCH<sub>2</sub>NH and NH(CH<sub>2</sub>)<sub>2</sub>), 8.17 (1 H, s, N=C–H) [note on addition of D<sub>2</sub>O, the resonances at 4.66 and 7.9–8.0 disappeared, whilst those at 2.97 and 4.76 collapsed to singlets] (Found: C 58.95; H 5.78; N 29.64; C<sub>16</sub>H<sub>19</sub>N<sub>7</sub>O requires: C 59.06; H 5.89; N 30.13%).

**12bii** (yellow solid):  $\delta_{\text{H}}$  3.03 (3 H, distorted d, *J* 5 Hz, NHCH<sub>3</sub>), 3.5–3.6 (4 H, m, NH(CH<sub>2</sub>)<sub>2</sub>OH), 4.66 (1 H, m, CH<sub>2</sub>OH), 4.69 (2 H, m, ArCH<sub>2</sub>NH), 6.6 (1 H, m, NHCH<sub>3</sub>), 7.3–7.4 (5 H, m, Ar-*H*), 7.5 (1 H, br m, NH), 8.14 (1 H, s, N=C–H), 8.4 (1 H, br m, NH) [note on addition of D<sub>2</sub>O, the resonances at 4.66 and 7.9–8.0 disappeared, whilst those at 2.97 and 4.76 collapsed to singlets] (Found: C 58.95; H 5.78; N 29.64; C<sub>16</sub>H<sub>19</sub>N<sub>7</sub>O requires: C 59.06; H 5.89; N 30.13%).

### X-Ray crystallography

Crystal data for **12bii**: C<sub>16</sub>H<sub>19</sub>N<sub>7</sub>O, *M* = 325.4, orthorhombic, space group *Pbcn*, *a* = 38.754(8), *b* = 7.9580(16), *c* = 20.954(4) Å, *V* = 6462(2) Å<sup>3</sup>, *Z* = 16 (2 independent molecules in the asymmetric unit), synchrotron radiation ( $\lambda$  = 0.6889 Å),<sup>13</sup> *T* = 160 K; conventional *R*(*F*, *F*<sup>2</sup> > 2σ) = 0.0558, *R*<sub>w</sub>(*F*<sup>2</sup>, all data) = 0.1386 for 460 parameters and 6638 unique data. Programs: standard Bruker diffractometer control and integration software, and SHELXTL.<sup>14</sup> CCDC reference number(s) 161624. See <http://www.rsc.org/suppdata/p1/b1/b102224p/> for crystallographic files in .cif or other electronic format.

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