Studies on the synthesis of the derivatives of 5-(dihydroxyboryl)-cytosines and -isocytosines

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Boron derivatives of isocytosine, containing a dihydroxyboryl group in the 5-position, have been prepared for the first time. Reaction of appropriate pyrimidines with *n*-butyllithium and subsequent boronation at −100 °C with triethylborate, followed by catalytic hydrogenation, gave hydrolytically stable *N*,*N*-dimethyl-5-(dihydroxyboryl)isocytosine 8a and *N*-methyl-5-(dihydroxyboryl)isocytosine 8b. Theoretical calculations suggest that the corresponding boron derivatives of cytosine cannot be obtained as thermodynamically stable compounds.

Introduction

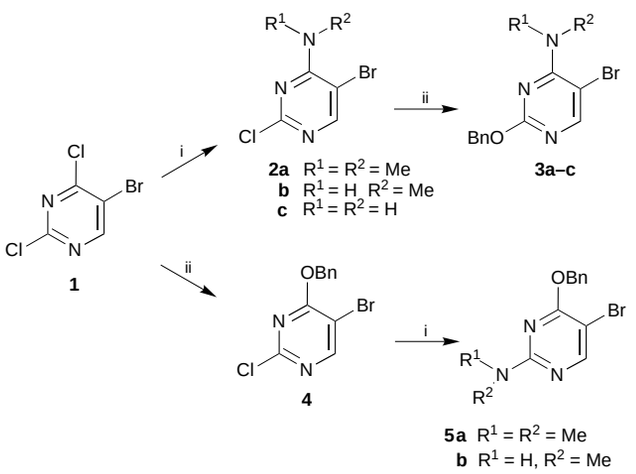
In the last decade, interest in organoboron chemistry has been directed to the synthesis of boron-containing pyrimidines, nucleotides, nucleosides and their analogs.[1–3] Various derivatives of pyrimidinylboronic acid have been synthesized for their potential anticancer and antiviral properties.[4–6] Although a dihydroxyborylation reaction of uracils has been described, no information has been provided on a dihydroxyborylation reaction for cytosines. Prompted by this fact we attempted to obtain 2- and 4-aminopyrimidones containing a boronic acid moiety. One of the most often used procedures for the preparation of boron-substituted pyrimidines is the reaction of the corresponding 5-halopyrimidines with butyllithium[7] and subsequent direct reaction of the carbanion with trialkyl borates at low temperature. The chemical nature of the carbanion generated from uracil should be similar to that generated from cytosines, but even in the case of uracils the desired coupling does not take place in certain cases, since the nucleophilicity of some carbanions is not great enough, or the carbon–boron bond of the product is not stable enough to be isolated.[8,9]

In this paper we discuss and report on attempts to obtain 5-(dihydroxyboryl)cytosines and 5-(dihydroxyboryl)isocytosines *via* nucleophilic substitution conducted at −100 °C.

Results and discussion

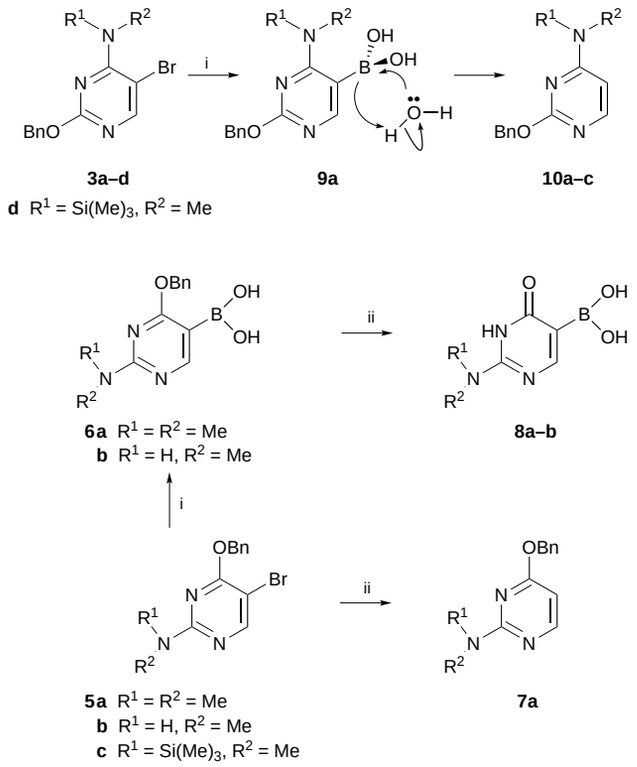
Synthesis

The synthesis of protected cytosines **3a**–**c** started with the 2,4-dichloro-5-bromopyrimidine **1**.[10] The 4-chloro atoms were converted into amino groups in **2a**–**c**, and subsequent reaction of **2a**–**c** and BnONa yielded **3a**–**c**. Similarly, protected isocytosines **5a**,**b** were obtained from **1** *via* conversion of the 4-chloro group to a benzyl group in **4**, followed by amination to give **5a**,**b**. Unfortunately, we were not able to obtain 2-amino-4-benzyloxy-5-bromo pyrimidine, a structural isomer of **3c**, *via* this route. Ammonia was too weak a base to replace the chlorine atom at the 2-position of the pyrimidine moiety, as was the case for compounds **5a**,**b**. Using more harsh conditions (under pressure or ammonia in water at 100 °C) we obtained amination products at the 4- and 5-positions. The general route is shown in Scheme 1. We employed an *O*-benzyl group as a good solubilizing and protecting group, as the amide function should thus be suitably protected during the introduction of the dihydroxyboryl group. Deprotection of the amide group had been achieved previously by catalytic hydrogenation without concomitant loss of the dihydroxyboryl group.[4]



Scheme 1 Synthesis of the 4-amino- and 4-alkylamino-2-benzyloxy-5-bromopyrimidines and 2-alkylamino-4-benzyloxy-5-bromopyrimidines: i, HNR^1R^2 in alcohol; ii, BnONa in dry toluene

In our synthesis we adopted a general method for preparing boronic acids, employing the reaction of the appropriate organometallic pyrimidines with esters of boronic acid, described by Schinazi and Prusoff[4] (Scheme 2). We followed their procedure, but unfortunately, for protected cytosines **3a**–**c** we were unable to obtain stable boron-containing pyrimidines, even when the temperature was in the range −100 to −110 °C and a large excess of triethyl borate was used. On hydrolysis and work up, the only compounds that could be isolated appeared to be 4-amino- and 4-alkylamino-2-benzyloxy pyrimidines **10a**–**c**. However, for compound **3a** we observed the formation of a carbon–boron bond and formation of boron-containing cytosine. As judged by ^{1}H NMR spectroscopy, immediately after work up and isolation of products, we obtained a mixture of the desired 4-dimethylamino-2-benzoxy-5-(dihydroxyboryl)pyrimidine and 4-dimethylamino-2-benzoxypyrimidine **10a**. No boron-containing pyrimidine was present in the NMR spectrum after a few hours; only compound **10a** was present, with resonances of increased intensity with respect to the internal standard. This led us to the conclusion that the nucleophilicity of the generated carbanion is strong enough to form a carbon–boron bond, but the 4-dimethylamino-2-benzyloxy-5-(dihydroxyboryl)pyrimidine obtained is not stable and undergoes a very fast process of deboronation (compound **9a**). The mechanism can be explained in terms of hydrolysis of the 5-dihydroxyboryl function and



Scheme 2 Boronation reactions of the 4-amino- and 4-alkylamino-2-benzyloxy-5-bromopyrimidines and 2-alkylamino-4-benzyloxy-5-bromopyrimidines: i, Bu^{n}Li in dry THF, then $B(OEt)_3$, −100 °C, then H^+; ii, Pd/H_2 in EtOH, pressure

formation of boric acid and **10a**. Surprisingly, a structural isomer, 2-dimethylamino-4-benzoxy-5-(dihydroxyboryl)pyrimidine **6a**, was obtained in excellent yield *via* a halogen–metal exchange reaction on **5a** followed by boronation. On hydrolysis and work up, stable **6a** was obtained as fine white crystals. Catalytic hydrogenation of **6a** using palladium on charcoal afforded the deblocked *N*,*N*-dimethyl-5-(dihydroxyboryl)-isocytosine **8a**, the first isocytosine containing a dihydroxyboryl function. Attempted application of this method to unprotected 2-methylamino-4-benzoxy-5-bromopyrimidine **5b** was unsuccessful, as in the case of **3a**–**c**—compound **7b** was obtained as the only product. However the use of a trimethylsilyl protecting group on the methylamino function (compound **5c**) and subsequent boronation gave the desired product **6b** in moderate yield. Catalytic hydrogenation furnished the *N*-methyl-5-(dihydroxyboryl)isocytosine **8b**.

Calculations

As shown by these experiments, the boron derivative of *N*,*N*-dimethylcytosine was probably formed during the reaction but it was unstable, unlike the derivative of *N*,*N*-dimethylisocytosine **6a**. Similarly, we could not obtain boron derivatives of *N*-methylcytosine or cytosine, whereas the boron derivative of *N*-methylisocytosine **6b** is a stable compound. Our guess was that boron derivatives of cytosine could undergo the hydrolysis reaction more easily then derivatives of isocytosine. The results of calculations seems to corroborate this hypothesis. The ion formed after the attack of *n*-butyllithium on the bromine atom at C(5) is less stable (heat of formation is 2.4 kcal mol^{-1} higher) in cytosine than in isocytosine (see ions 1 and 6 in Table 2). Moreover, hydrolysis of the boron derivative of cytosine should be much faster then the hydrolysis of the analogous derivative of isocytosine. Structures of substrates, transition states and products of one of those reactions are shown in Fig. 1. The reaction starts when the water molecule complexed near the boron atom (about 0.18 nm) starts to move towards it [Fig. 1(*a*)]. One of the water hydrogen atoms then moves towards

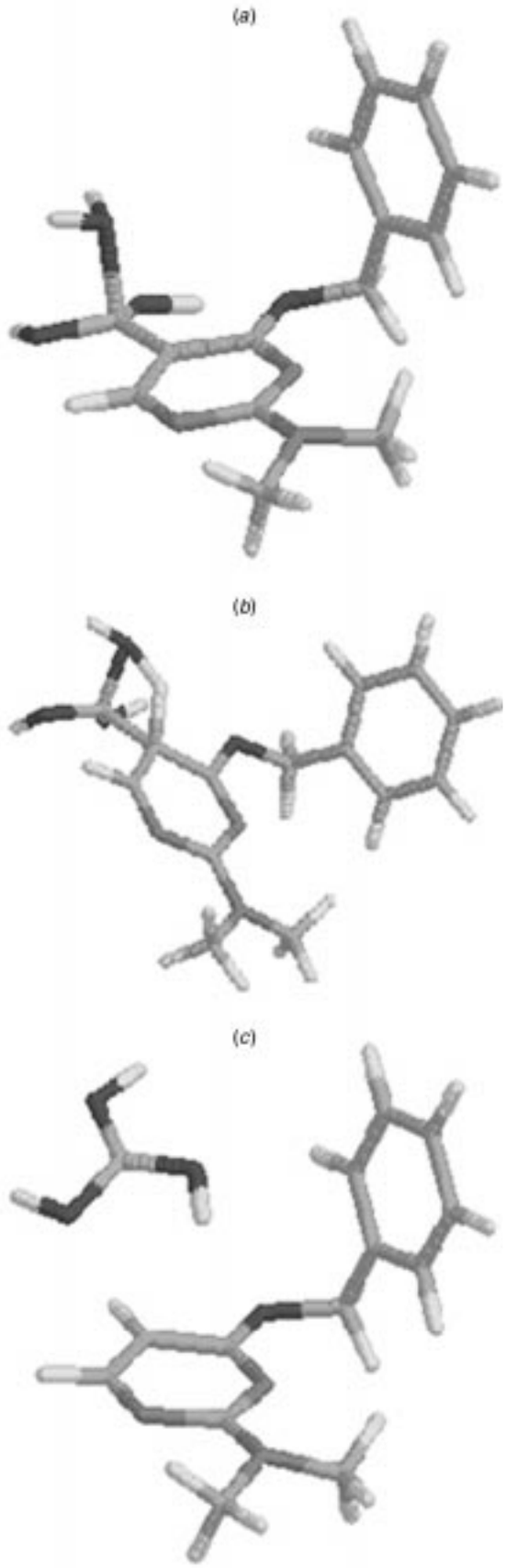


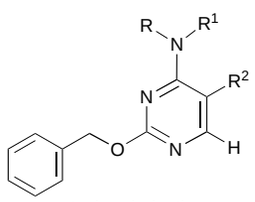
Fig. 1 Structure of (*a*) substrate, (*b*) transition state and (*c*) product of the selected hydrolysis reaction

C(5) in cytosine (or isocytosine ring) and in the transition state it is about half way between oxygen and C(5) [Fig. 1(*b*)]. Finally, the oxygen atom reaches the distance typical for a HO–B bond, and the whole $B(OH)_3$ molecule moves away from the cytosine (or isocytosine ring) [Fig. 1(*c*)].

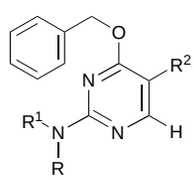
Table 1 Heats of formation of the hydrolysis reaction critical points and energetic barrier for the hydrolysis reaction (kcal mol^{-1})

	Isocytosine derivatives			Cytosine derivatives		
	NH_2	*N*-Methyl	*N*,*N*-Dimethyl	NH_2	*N*-Methyl	*N*,*N*-Dimethyl
$H_{f(substr.)}$	−197.6	−194.1	−187.0	−194.6	−191.2	−175.7
$H_{f(prod.)}$	−217.2	−213.7	−207.2	−214.9	−211.1	−203.8
$H_{f(TS)}$	−150.5	−147.0	−140.4	−152.3	−149.0	−135.4
$H_{f(TS)} - H_{f(substr.)}$	47.1	47.1	46.6	42.3	42.1	40.2
ΔH_f	−19.6	−19.6	−20.2	−20.3	−20.0	−28.1

Table 2 Heat of formation of anions which can be formed after an organolithium reagent attack onto the bromine atom in position 5 in the cytosine (or isocytosine) ring



cytosine derivatives



iso-cytosine derivatives

	Anion				
Entry	Derivative	R	R^1	R^2	Heat of formation/kcal mol^{-1}
1	cytosine	CH_3	CH_3	anion	68.1
2	cytosine	CH_3	H	anion	61.7
3	cytosine	CH_3	anion	H	20.6
4	cytosine	H	H	anion	58.8
5	cytosine	H	anion	H	21.2
6	isocytosine	CH_3	CH_3	anion	65.7
7	isocytosine	CH_3	H	anion	66.0
8	isocytosine	CH_3	anion	H	23.6
9	isocytosine	H	H	anion	62.8
10	isocytosine	H	anion	H	24.2

The heats of formation of substrates $H_{f(substr.)}$, transition states $H_{f(TS)}$ and products $H_{f(prod.)}$ are given in Table 1. One can see that the hydrolysis reaction of any of the cytosine and isocytosine derivatives is energetically beneficial because ΔH_f (*i.e.* $H_{f(prod.)} - H_{f(substr.)}$) is negative, but all the reactions of the cytosine derivatives have an energetic barrier $H_{f(TS)} - H_{f(substr.)}$ about 5 kcal mol^{-1} lower then the analogous reaction of isocytosine. This means that the hydrolysis reaction of cytosine derivatives should be much faster than that of isocytosine derivatives. In this model we assumed a water molecule as the hydrolytic factor, but we feel that this model can be extrapolated to other hydrolytic factors.

The rate of hydrolysis explains why it was not possible to obtain cytosine derivatives, but the experiment shows that the procedure proposed enables a synthesis of the boron derivative of *N*,*N*-dimethylisocytosine only, and does not lead to the desired products in the case of *N*-methylisocytosine derivatives. To explain this phenomenon one should compare stabilities (expressed by heats of formation) of ions which can be formed after attack of *n*-butyllithium onto the bromine atom at C(5) in the cytosine or isocytosine ring. These heats of formation are given in Table 2. When comparing anions **2** ($H_f = 61.7$ kcal mol^{-1}) and **3** ($H_f = 20.6$ kcal mol^{-1}) one can see that the latter is much more stable (lower heat of formation). The difference between these two ions lies in where the negative charge is situated. In anion **2** the charge is on C(5), while in anion **3** it is on the amine nitrogen. Even assuming that anion **2** is formed during reaction with *n*-butyllithium, it will promptly rearrange into **3** (1,5-hydrogen shift). This explains why the product of this reaction has a hydrogen atom at C(5). An analogous situation can be observed when comparing anions **4** and **5**, **7** and **8**, **9** and **10**. In all these cases the anion with charge positioned at C(5) is much less stable than the one with charge at the amine nitrogen.

In conclusion, it is not possible to obtain boron derivatives of cytosine and isocytosine using the proposed procedure if there is a possible 1,5-hydrogen shift for the cytosines (or a 1,3-hydrogen shift for the isocytosines). It is possible to obtain boron derivatives when the amine nitrogen is methylated, but the cytosine derivative is obtained in lower yields (less stable anion) and decomposes much more rapidly (hydrolysis) than the one based on isocytosine.

Conclusion

Results reported in this paper provide conclusive evidence that derivatives of cytosine and their isocytosine isomers show very different behavior towards the dihydroxyborylation reaction. The direct reaction of isocytosine carbanions with triethyl borate at low temperature followed by catalytic hydrogenation led to stable *N*,*N*-dimethyl-5-(dihydroxyboryl)isocytosine **8a** and *N*-methyl-5-(dihydroxyboryl)isocytosine **8b**. The same reaction conducted on cytosines led only to isolation of the hydrolysis products. Theoretical calculations demonstrated that the instability of cytosine derivatives bearing a boronic moiety can be explained in terms of a lower energy barrier to hydrolysis with respect to the analogous reactions of isocytosine derivatives.

Experimental

Methods

Melting points were determined on a Boetius apparatus and are reported uncorrected. All ^{1}H NMR and ^{13}C NMR spectra were performed on a Varian Gemini 300 MHz spectrometer in $CDCl_3$ and $(CD_3)_2SO$. Chemical shifts (δ) are reported in ppm downfield from an internal $SiMe_4$ standard. Mass spectra were recorded on an AMD 402 spectrometer, ionization was through

electron impact (EI). Elemental analyses were determined on Perkin-Elmer apparatus. TLC was carried out on commercially available plates coated with silica gel 60 F_{254} (Merck).

Method of calculation

The calculations were performed using AM1 semiempirical hamiltonians, with the MOPAC 6.0 program. The transition states (TS) of the hydrolysis reactions were found with the reaction path method, and were then optimized using Baker's algorithm.[11] After each TS was found it was checked to confirm that it had one and only one negative normal mode, and then the TS was decomposed using the IRC method to give substrates and products. Those species were optimized using Baker's method. All the calculations were carried out on an SGI PowerChallenge computer.

Materials

Synthesis of 4-amino- and 4-alkylamino-2-chloro-5-bromopyrimidines 2a–c. Reaction of 5-bromo-2,4-dichloropyrimidine **1** with dimethylamine is representative. Two equivalents of 33% dimethylamine in MeOH (12.0 g, 87.8 mmol) were slowly added to a MeOH solution (100 ml) of 5-bromo-2,4-dichloropyrimidine (10.0 g, 43.9 mmol) at 0 °C. The mixture was stirred at 0 °C for 2 h and then overnight at a room temperature. The MeOH was evaporated under reduced pressure, the residue taken into 100 ml of water, extracted with $CHCl_3$ (3 × 100 ml) and the combined filtrates were dried over $MgSO_4$ and concentrated almost to dryness. Then 100 ml of hexane was added and the pure crystalline precipitate of 4-dimethylamino-2-chloro-5-bromopyrimidine **2a** was collected and used in the next reaction without further purification (7.57 g, 73%). Compounds **2b** (82%) and **2c** (71%), precipitated from the reaction, were filtered off, washed with water, dried and used without purification in the following reaction.

4-*Dimethylamino*-2-*chloro*-5-*bromopyrimidine* **2a**.—$\delta_H(CDCl_3)$ 8.17 (1H, s, C^6-H), 3.26 (6H, s, CH_3); *m*/*z* 235 (M^+, 46%).

4-*Methylamino*-2-*chloro*-5-*bromopyrimidine* **2b**.—$\delta_H(CDCl_3)$ 8.11 (1H, s, C^6-H), 5.59 (1H, br s, N-H), 3.09 (3H, d, *J* 5 Hz, CH_3); *m*/*z* 221 (M^+, 76%).

4-*Amino*-2-*chloro*-5-*bromopyrimidine* **2c**.—$\delta_H(CDCl_3)$ 8.24 (1H, s, C^6-H), 5.76 (2H, br s, NH_2); *m*/*z* 207 (M^+, 75%).

Synthesis of 4-amino- and 4-alkylamino-2-benzyloxy-5-bromopyrimidines 3a–c. Reaction of 4-dimethylamino-2-chloro-5-bromopyrimidine **2a** with BnONa is representative. A stirred solution of benzyl alcohol (3.52 g, 32.6 mmol) in anhydrous toluene (50 ml) was treated with 60% w/w NaH in oil (1.3 g, 32.6 mmol). The mixture was warmed to about 50 °C to facilitate the formation of the sodium salt. After all the NaH had reacted, **2a** (7.0 g, 29.6 mmol) was slowly added to avoid a rapid exothermic reaction and the mixture was stirred at about 100 °C overnight. The precipitate of NaCl was filtered off, and the filtrate was then evaporated under reduced pressure. The resulting oil was dissolved in 100 ml of water and extracted with $CHCl_3$ (3 × 100 ml). The combined organic fractions were dried over $MgSO_4$ and concentrated. Crystallisation from a mixture of diethyl ether and hexane gave 4-dimethylamino-2-benzyloxy-5-bromopyrimidine **3a** (5.9 g, 65%). The same procedure gave 4-methylamino-2-benzyloxy-5-bromopyrimidine **3b** (78%). Pure 4-amino-2-benzyloxy-5-bromopyrimidine **3c** was separated by column chromatography on silica gel with hexane–$CHCl_3$ (using a gradient from 3 : 7 to 1 : 9 v/v) (35%).

4-*Dimethylamino*-2-*benzyloxy*-5-*bromopyrimidine* **3a**.—Mp 56–57 °C (Found: C, 50.46; H, 4.41; N, 13.37. $C_{13}H_{14}N_3OBr$ requires C, 50.66; H, 4.58; N, 13.64%); $\delta_H(CDCl_3)$ 8.13 (1H, s, C^6-H), 7.45–7.26 (5H, m, C_6H_5), 5.34 (2H, s, CH_2), 3.20 (6H, s, CH_3); *m*/*z* 307 (M^+, 55%).

4-*Methylamino*-2-*benzyloxy*-5-*bromopyrimidine* **3b**.—Mp 86–88 °C (Found: C, 48.93; H, 4.00; N, 14.05. $C_{12}H_{12}N_3OBr$ requires C, 49.00; H, 4.11; N, 14.28%); $\delta_H(CDCl_3)$ 8.03 (1H, s, C^6-H), 7.48–7.26 (5H, m, C_6H_5), 5.35 (1H, br s, N-H), 5.36 (2H, s, CH_2), 3.04 (3H, d, *J* 5 Hz, CH_3); *m*/*z* 293 (M^+, 74%).

4-*Amino*-2-*benzyloxy*-5-*bromopyrimidine* **3c**.—(Found: C, 46.86; H, 3.42; N, 15.07. $C_{11}H_{10}N_3OBr$ requires C, 47.16; H, 3.60; N, 15.00%); $\delta_H(CDCl_3)$ 8.15 (1H, s, C^6-H), 7.46–7.26 (5H, m, C_6H_5), 5.34 (2H, s, CH_2), 5.30 (2H, br s, NH_2); *m*/*z* 279 (M^+, 33%).

Synthesis of 2-chloro-4-benzyloxy-5-bromopyrimidine 4. To a stirred solution of the benzyl alcohol (5.22 g, 48.3 mmol) in anhydrous toluene (100 ml) was added 60% NaH in oil (1.93 g, 48.3 mmol). The mixture was warmed to about 50 °C to facilitate formation of the sodium salt. After 30 min the suspension was cooled to −20 °C and 5-bromo-2,4-dichloropyrimidine (10 g, 43.9 mmol) was added dropwise so that the temperature did not rise. Then the mixture was stirred at room temperature overnight. The precipitate of NaCl was filtered off, and the filtrate was then evaporated under reduced pressure. The resulting oil was dissolved in water (100 ml) and extracted with $CHCl_3$ (3 × 100 ml). The combined organic fractions were dried over $MgSO_4$ and concentrated. Then 100 ml of hexane was added and the pure crystalline precipitate of 2-chloro-4-benzyloxy-5-bromopyrimidine **4** (7.02 g, 54%) was collected and used in the next reaction without further purification: $\delta_H(CDCl_3)$ 8.45 (1H, s, C^6-H), 7.50–7.27 (5H, m, C_6H_5), 5.51 (2H, s, CH_2); *m*/*z* 298 (M^+, 57%).

Synthesis of 2-alkylamino-4-benzyloxy-5-bromopyrimidines 5a,b. Three equivalents of 33% dimethylamine in MeOH (5.02 g, 30 mmol) were added to a solution of **4** (3 g, 10 mmol) in methanol (100 ml) and the reaction was refluxed for 2 h. The solution was evaporated under reduced pressure, and then water (50 ml) was added. The solution was extracted with $CHCl_3$ (3 × 50 ml) and the combined chloroform extracts were dried over $MgSO_4$. The solvent was removed and the residue was separated by column chromatography on silica gel using hexane–chloroform (1 : 1 v/v) to yield crude 2-dimethylamino-4-benzyloxy-5-bromopyrimidine. Crystallisation from EtOH gave white crystals of **5a** (2.6 g, 84%). 2-Methylamino-4-benzyloxy-5-bromopyrimidine **5b** was obtained in a similar reaction of **4** with 2.5 equiv. of methylamine (65%).

2-*Dimethylamino*-4-*benzyloxy*-5-*bromopyrimidine* **5a**.—Mp 70–72 °C (Found: C, 50.89; H, 4.31; N, 13.40. $C_{13}H_{14}N_3OBr$ requires C, 50.66; H, 4.58; N, 13.64%); $\delta_H(CDCl_3)$ 8.13 (1H, s, C^6-H), 7.46–7.26 (5H, m, C_6H_5), 5.44 (2H, s, CH_2), 3.12 (6H, s, CH_3); *m*/*z* 307 (M^+, 40%).

2-*Methylamino*-4-*benzyloxy*-5-*bromopyrimidine* **5b**.—Mp 115–116 °C (Found: C, 48.80; H, 3.81; N, 14.13. $C_{12}H_{12}N_3OBr$ requires C, 49.00; H, 4.11; N, 14.28%); $\delta_H(CDCl_3)$ 8.12 (1H, s, C^6-H), 7.46–7.26 (5H, m, C_6H_5), 5.44 (2H, s, CH_2), 5.00 (1H, br s, N-H), 2.96 (3H, d, *J* 5 Hz, CH_3); *m*/*z* 293 (M^+, 27%).

Synthesis of 2-dimethylamino-4-benzyloxy-5-(dihydroxyboryl)pyrimidine 6a. 2-Dimethylamino-4-benzyloxy-5-bromopyrimidine **5a** (1.75 g, 5.68 mmol) was dissolved in dry, freshly distilled THF (70 ml) and cooled to −80 °C under an argon atmosphere, using a N_2–hexane bath. A 15% solution of *n*-butyllithium in hexane (4.4 ml, 6.92 mmol) was injected through a septum at such a rate that the internal temperature did not exceed −70 °C. The temperature was decreased to −100 °C, the yellow solution was stirred for an additional 5 min and triethyl borate (1.3 ml, 7.65 mmol) was injected. The clear reaction mixture was kept at the same temperature for 30 min and then was allowed to warm to room temperature over a period of 1.5 h. The solution was evaporated to dryness under reduced pressure and then water was added (40 ml). The aqueous solution was acidified with 1 M HCl to pH 2–3 and a white precipitate was formed. The precipitated compound was filtered off and washed with water. The filtrate was neutralised with saturated aqueous $NaHCO_3$, and a second crop of compound, precipitated as white fine crystals, was filtered off and washed with water. The precipitates were combined, washed with water, filtered and dried to give chromatographically pure 2-

dimethylamino-4-benzyloxy-5-(dihydroxyboryl)pyrimidine **6a** (1.29 g, 83%): mp 146–147 °C (Found: C, 57.34; H, 5.85; N, 15.12. $C_{13}H_{16}N_3O_3B$ requires C, 57.17; H, 5.91; N, 15.39%); δ_H[$(CD_3)_2SO$] 8.33 (1H, s, C^6-H), 7.49–7.28 (5H, m, C_6H_5), 7.40 [2H, s, $B(OH)_2$], 5.44 (2H, s, CH_2), 3.10 (6H, s, CH_3); δ_C[$(CD_3)_2SO$] 172.0 (s), 165.4 (s), 162.4 (s), 137.2 (s), 128.3 (s), 127.8 (s), 127.7 (s), 95.3 (s), 66.4 (s) and 36.4 (s); *m*/*z* 273 (M^+, 20%), 229 ($M^+ - BO_2H$, 68%).

Synthesis of 2-methylamino-4-benzyloxy-5-(dihydroxyboryl)-pyrimidine 6b. 2-Methylamino-4-benzyloxy-5-bromopyrimidine (1 g, 3.4 mmol) **5b** was suspended in hexamethyldisilazane (HMDS, 3 ml), a catalytic amount of $(NH_4)_2SO_4$ was added and the mixture was gently refluxed for 3 h. The excess of HMDS was evaporated under reduced pressure. The oily residue of silyl derivative of **5b** was dissolved in freshly distilled dry THF and cooled to −80 °C. Under an argon atmosphere a 15% solution of *n*-butyllithium in hexane (4.4 ml, 6.8 mmol) was injected through a septum and the resulting yellow solution was stirred for an additional 5 min. The temperature was decreased to −100 °C, triethyl borate (1.3 ml, 7.5 mmol) was added, the reaction mixture was stirred at −100 °C for 30 min and was then allowed to warm to room temperature over a period of 2 h. The solution was evaporated almost to dryness under reduced pressure and then water was added. The solution was acidified to pH with 1 M HCl, extracted with CH_2Cl_2 (3 × 25 ml) and the combined extracts were dried over $MgSO_4$. The solvent was removed to furnish an oil which was separated by column chromatography on a silica gel with $CHCl_3$–MeOH (using a gradient from 10:0 to 8:2 v/v). Pure 2-methylamino-4-benzyloxy-5-(dihydroxyboryl)pyrimidine **6b** was obtained as oil and solidified after 24 h (171 mg, 20%): (Found: C, 55.91; H, 5.26; N, 15.95. $C_{12}H_{14}N_3O_3B$ requires C, 55.63; H, 5.45; N, 16.22%); δ_H[$(CD_3)_2SO$] 8.31 and 8.26 (1H, s, tautomeric C^6-H), 7.49–7.29 [8H, m, C_6H_5 + N-H + $B(OH)_2$], 5.44 and 5.38 (2H, s, tautomeric CH_2), 2.78 (3H, d, *J* 5 Hz, CH_3); δ_C[$(CD_3)SO$] 172.7 and 172.1 (tautomeric s), 165.6 (s), 163.7 and 163.3 (tautomeric s), 137.1 and 137.2 (tautomeric s), 128.4 (s), 128.0 (s), 127.8 (s), 97.0 and 99.0 (tautomeric s), 66.3 (s) and 27.7 (s); *m*/*z* 215 ($M^+ - BO_2H$, 48%).

Synthesis of *N*,*N*-dimethyl-5-(dihydroxyboryl)isocytosine 8a and *N*-methyl-5-(dihydroxyboryl)isocytosine 8b. The debenzylation reaction of 2-dimethylamino-4-benzyloxy-5-(dihydroxyboryl)pyrimidine **6a** is representative. 10% Palladium on charcoal (400 mg) was added to **6a** (1.20 g, 4.4 mmol), dissolved in EtOH (150 ml) and the compound was hydrogenated at room temperature for 30 min under pressure (0.4 MPa). The mixture was then filtered through a Celite pad. After evaporation of the solvent the desired compound was obtained as white powder. The product was crystallized from ethyl acetate—methanol (2:1), yielding white prisms of *N*,*N*-dimethyl-5-(dihydroxyboryl)isocytosine **8a** (440 mg, 56%). *N*-Methyl-5-(dihydroxyboryl)isocytosine **8b** was obtained from **6b** in the same way (62%).

N,*N*-*Dimethyl*-5-(*dihydroxyboryl*)*isocytosine* **8a**.—Mp 176–178 °C (slow decomp.) (Found: C, 39.09; H, 5.24; N, 22.69. $C_6H_{10}N_3O_3B$ requires C, 39.38; H, 5.51; N, 22.96%); δ_H[$(CD_3)_2SO$] 11.23 (1H, br s, N-H), 8.09 [3H, br s, C^6-H + $B(OH)_2$], 3.08 (6H, s, CH_3); δ_C[$(CD_3)_2SO$] 170.1 (s), 163.9 (s), 155.6 (s), 100.4 (s) and 37.2 (s); *m*/*z* 139 ($M^+ - BO_2H$, 100%).

N-*Methyl*-5-(*dihydroxyboryl*)*isocytosine* **8b**.—Mp 183 °C (decomp.) (Found: C, 32.45; H, 5.10; N, 22.31. $C_5H_8N_3O_3B \cdot H_2O$ requires C, 32.21; H, 5.39; N, 22.48%); δ_H[$(CD_3)_2SO$] 11.31 (1H, br s, N-H), 8.08 [3H, br s, C^6-H + $B(OH)_2$], 6.75 (1H, br s, N-H), 2.81 (3H, s, CH_3); δ_C[$(CD_3)_2SO$] 170.0 (s), 164.4 (s), 156.4 (s), 101.8 (s) and 27.5 (s); *m*/*z* 125 ($M^+ - BO_2H$, 100%).

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