Pyrazolo[3,4-*d*]pyrimidine ribonucleosides related to 2-aminoadenosine and isoguanosine: synthesis, deamination and tautomerism†

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The syntheses and properties of 8-aza-7-deazapurine (pyrazolo[3,4-*d*]pyrimidine) ribonucleosides related to 2-aminoadenosine and isoguanosine are described. Glycosylation of 8-aza-7-deazapurine-2,6-diamine **5** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**12**) in the presence of $BF_3 \cdot Et_2O$ as a catalyst gave the N^8 isomer **14** (73%) with a trace amount of the N^9 isomer **13a** (4.8%). Under the same reaction conditions, the 7-halogenated 8-aza-7-deazapurine-2,6-diamines **6–8** afforded the thermodynamically more stable N^9 nucleosides **13b–d** as the only products (53–70%). Thus, a halogen in position 7 shifts the glycosylation from N^8 to N^9. The 8-aza-7-deazapurine-4,6-diamine ribonucleosides **1a–d** were converted to the isoguanosine derivatives **3a–d** by diazotization of the 2-amino group. Although compounds **1a,b** do not contain a nitrogen at position 7 (the enzyme binding site), they were deaminated by adenosine deaminase; however, their deamination occurred with a much slower velocity than that of the related purines. The pK_a values indicate that the 7-non-functionalized nucleosides **1a** (pK_a 5.8) and **15** (pK_a 6.4) are possibly protonated in neutral conditions when incorporated into RNA. The nucleosides **3a–d** exist predominantly in the keto (lactam) form with K_{TAUT} (keto/enol) values of 400–1200 compared to 10^3–10^4 for pyrrolo[2,3-*d*]pyrimidine isoguanosine derivatives **4a–c** and 10 for isoguanosine itself, which will reduce RNA mispairing with U.

Introduction

8-Aza-7-deazapurines (pyrazolo[3,4-*d*]pyrimidines) exhibit extraordinary physical and biological properties[1–4] if they are constituents of nucleosides or oligonucleotides. 8-Aza-7-deazapurines (purine numbering is used in the general discussion) closely resemble the structure of purines and make their nucleosides ideal mimics of the canonical purine constituents of DNA or RNA. Since there is an absence of *de novo* purine biosynthesis in most parasites, these organisms are wholly dependent in the salvage pathway for the purine nucleoside metabolism. However, they will accept certain 8-aza-7-deazapurines in place of purines. For example, allopurinol (pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one), which is an approved drug for the treatment of gout, was also shown to be active against several leishmania and trypanosome species.[5–7] In both species allopurinol is converted to allopurinol ribonucleoside 5′-phosphate.[8,9] Monomeric 8-aza-7-deazapurine nucleosides develop antiparasitic, antitumour and antiviral activity.[10–23] In particular, 8-aza-7-deazapurine-2,6-diamine and its riboside **1a** are powerful purine nucleoside antagonists.[24,25] In the form of their triphosphates these nucleosides are substrates of polymerases, thereby making significant contributions to DNA and RNA diagnostics.[26] The thermal stability of oligo-2′-deoxyribonucleotide duplexes increases significantly when 7-halogenated 8-aza-7-deazapurines replace purines.[27] It has been shown that the 7-halogenated 8-aza-7-deazapurine-2,6-diamine **2e** forms a base pair with thymine that is as stable as a dG–dC base pair,[28] while the base pair of thymine and the non-functionalized 8-aza-7-deazapurine-2,6-diamine is not stronger than the dA–dT pair.

Recently, it was shown that 2-amino-7-deaza-2′-deoxyadenosine (**2a**), the pyrrolo[2,3-*d*]pyrimidine analogue of **1a**, is protonated even under neutral conditions when it is a constituent of an oligonucleotide, thereby forming a stable mismatched base pair with dC. However, the halogenated derivatives **2b–d** with lower pK_a values do not pair with dC.[29] Base pairing ambiguity is also found for other nucleosides that generate sufficient amounts of rare tautomers such as 2′-deoxyisoguanosine, thereby forming strong base pairs not only with dC or $isoC_d$, but also stable mismatches with dT and dG. This pairing ambiguity is significantly diminished if the 7-deazapurines bear electron-withdrawing substituents in the 7-position.[30]

This manuscript investigates 7-halogenated 8-aza-7-deazapurine nucleosides related to 2-aminoadenosine and isoguanosine. The 8-aza-7-deazapurine-2,6-diamine ribonucleoside **1a** and its 7-substituted derivatives **1b–d** (Scheme 1) were synthesized. As compounds **1a–d** do not contain an N-7 nitrogen, they might not be deaminated by adenosine deaminase, as was observed for the pyrrolo[2,3-*d*]pyrimidine analogues **2a–d**. Chemical deamination of **1a–d** afforded the isoguanosine derivatives **3a–d**. As it has been reported that the tautomeric enol formation of isoguanine nucleosides results in base pairing with T (or U),[30] the ability to form rare tautomers was studied on compounds **3a–d** and compared with the data obtained for purine and 7-deazapurine nucleosides (**4a–c**).

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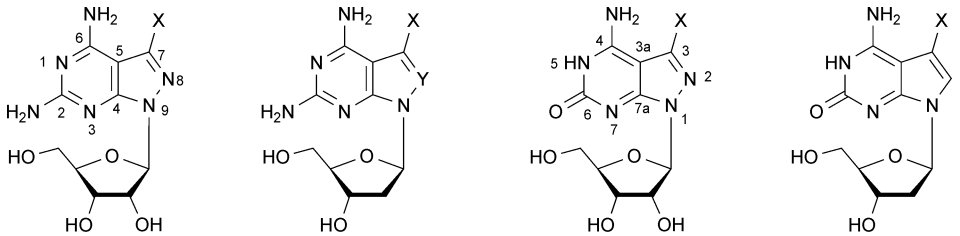
† Electronic supplementary information (ESI) available: pK_a measurements, relative initial velocities of the deamination reaction, and determination of keto/enol populations. See DOI: 10.1039/b708736e



a: X = H; **b**: X = Cl; **c**: X = Br; **d**: X = I

Scheme 1 Structures of the nucleosides.

Results and discussion

1. Synthesis of 8-aza-7-deazapurine ribonucleosides

The literature reports various synthetic routes to 8-aza-7-deazapurine ribonucleosides:

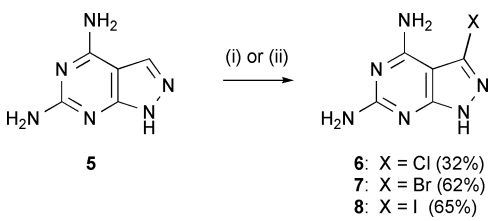
(i) Davoll described the syntheses of the 8-aza-7-deazapurine-2,6-diamine ribonucleosides **1a** and **15** using the chloromercury derivatives of the nucleobases and tri-*O*-benzoyl-D-ribofuranosyl chloride as the sugar component.[25] He obtained a mixture of N^8 and N^9 regioisomers in only 5–10% total yield.

(ii) The glycosylation of 2,6-substituted 8-aza-7-deazapurines *via* the corresponding trimethylsilyl intermediates with tetra-*O*-acetyl-β-D-ribofuranose and trimethylsilyl triflate as catalyst.[31]

(iii) Cottam *et al.*[10,23] used $BF_3{\cdot}Et_2O$ as the catalyst under reflux conditions, yielding various 8-aza-7-deazapurine ribonucleosides.

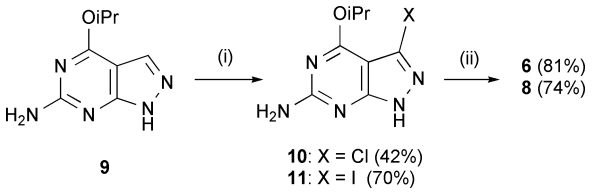
(iv) Recently, Bookser and Raffaele[32] investigated the direct glycosylation of 8-aza-7-deazapurine with microwave assistance.

Our laboratory has studied the synthesis of 2,6,7-trisubstituted 8-aza-7-deazapurine ribonucleosides using the corresponding 7-halogenated 8-aza-7-deazapurines as precursors.[33] As an efficient route for the synthesis of 7-halogenated 8-aza-7-deazapurine derivatives related to 2-aminoadenosine and isoguanosine does not exist, this manuscript investigates the synthesis of a series of 7-substituted 8-aza-7-deazapurine derivatives using commercially available 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**12**) for glycosylation. The starting material for the halogenated bases is 8-aza-7-deazapurine-4,6-diamine (**5**) prepared as described earlier from guanidine carbonate and 3-amino-4-cyanopyrazole in a ring-forming reaction.[25] Compound **5** was halogenated with bromine ($\rightarrow$ **7**)[34] or halosuccinimides (NXS, X = Cl, I), affording the 7-halogenated nucleobases **6–8** (Scheme 2).



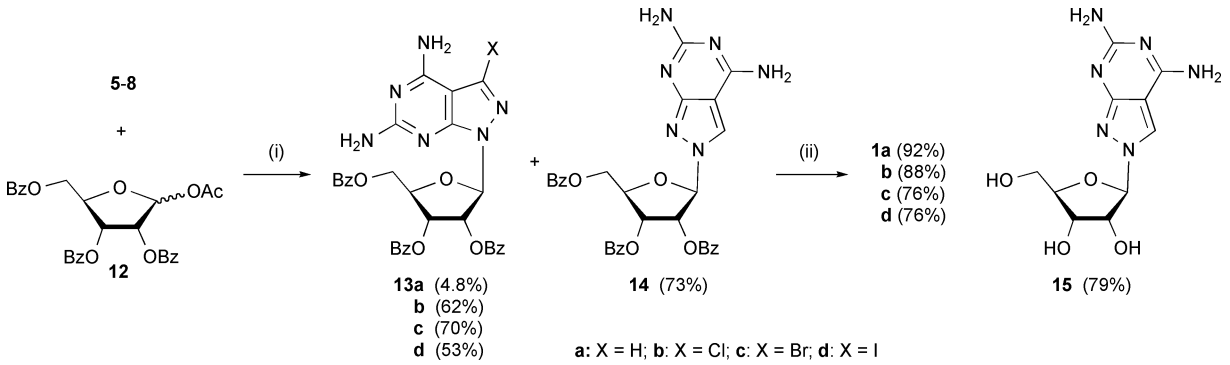
Scheme 2 *Reagents and conditions*: (i) **6**: *N*-chlorosuccinimide (NCS), DMF, 24 h, r.t.; **8**: *N*-iodosuccinimide (NIS), CH_2Cl_2, 3 d, reflux, (ii) **7**: Br_2, H_2O, r.t., 1 h, reflux, 1 h.

The low solubility of the base **5** in DMF or dichloromethane resulted in low halogenation yields (32–65%). Moreover, difficulties were encountered during the purification of **6** and **8** due to poor solubility. Thus, for large scale preparation an alternative route was used. Compound **9** bearing a hydrophobic isopropoxy group was employed as a starting material. The halogenated derivative **11**[35] was synthesized from **9**[36] with NIS under reflux in 1,2-dichloroethane. The former was converted to the diamino compound **8** (74%) in aqueous ammonia in an autoclave. In the same way, the chloro derivatives **10** and **6** were prepared (Scheme 3).



Scheme 3 *Reagents and conditions*: (i) **10**: NCS, $ClCH_2CH_2Cl$, 5 h, reflux; **11**: NIS, $ClCH_2CH_2Cl$, 2.5 h, reflux; (ii) 25% NH_4OH–dioxane (2 : 1), 120 °C, 24 h.

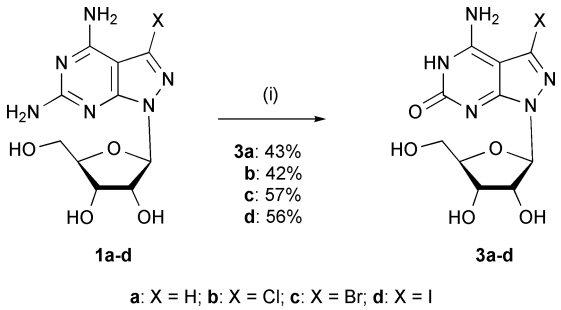
Next, the glycosylation of the nucleobases **5–8** was investigated. First, we tried to perform the glycosylation with the sugar moiety **12** in MeCN with HMDS (base silylation) and tin chloride as catalyst. This resulted in a complicated reaction mixture and very little of the desired product. Later on we applied the procedure of Cottam *et al.*,[10,23] with $BF_3{\cdot}Et_2O$ as catalyst under reflux, which gave satisfactory yields. According to an earlier observation,[25] two isomers are expected with N^8 and N^9 as the glycosylation sites, as was always found in the case of 2′-deoxyribonucleosides.[35] However, in the case of the 7-halogenated 8-aza-7-deazapurine-2,6-diamines **6–8**, only the N^9 isomers **13b–d** (53–70%) (Scheme 4) were obtained. In constrast, the glycosylation of the 7-non-functionalized compound **5** resulted in two products, which were assigned to be **13a** (N^9 4.8%) and **14** (N^8 73%). Therefore the 7-halogen substituent has a significant influence on the outcome of the glycosylation. The 7-non-functionalized compound **5** gave a higher total glycosylation yield (78%) than the halogenated bases **6–8** (53–70%), with the N^8 isomer in excess. The 7-halogen shifts the glycosylation site completely to N^9, while compound **5** (which is not hindered at the N^8 position by a 7-halogen substituent) can be more easily attacked at N^8. Also, the



Scheme 4 *Reagents and conditions*: (i) $BF_3{\cdot}Et_2O$, CH_3NO_2, 30 min, reflux; (ii) 0.5 M $NaOCH_3/CH_3OH$, overnight, r.t.

electron-withdrawing character of the halogen substituents operates in the same direction. As compound **5** reacts much faster with the sugar (5 min, TLC monitoring) than compounds **6–8** (20–30 min) the formation of **14** is kinetically controlled, while the N^9 isomer is the thermodynamically more stable product. The protected nucleosides **13a–d** and **14** were deblocked with sodium methoxide to give the free ribonucleosides **1a–d** and **15** (76–92%) (Scheme 4).

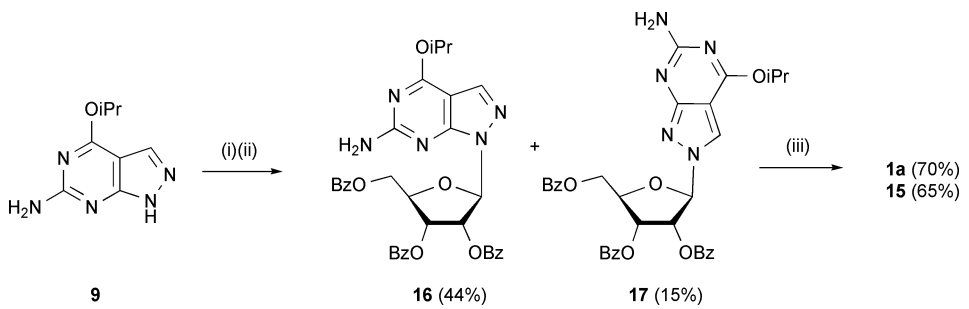
To access a higher yield of the N^9 isomer **1a**, the 2-amino-6-isopropoxy-8-aza-7-deazapurine **9**[36] (Scheme 5) was employed in another glycosylation protocol. Compound **9** was first silylated with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and then reacted with the sugar **12** in the presence of TMSOTf (trimethylsilyl trifluoromethanesulfonate) for 3 h at room temperature, affording compound **16** in 44% yield (Scheme 5). Here, the N^8 isomer **17** was isolated in only 15% yield. The moderate total glycosylation yield resulted from a partial cleavage of the isopropoxy group under the reaction conditions (HMDS, TMSOTf), which was confirmed by a nucleoside side-product isolated from the reaction mixture. ^{1}H NMR spectra showed the absence of the isopropoxy group, and a longer reaction time led to an increasing yield of that compound, which after deprotection with sodium methoxide (0.2 M) yielded 8-aza-7-deazaguanosine. Here the N^9 isomer is favoured from both the bulkiness and the electron-withdrawing effect of the isopropoxy group. Compounds **16** and **17** were converted to the nucleosides **1a** and **15** by heating in aqueous ammonia (Scheme 5). Next, compounds **1a–d** were deaminated selectively by diazotization at the 2-NH_2 group, affording the isoguanosine analogue **3a** and its halogenated derivatives **3b–d** in yields of 42–57% (Scheme 6).



Scheme 6 *Reagents and conditions*: (i) $NaNO_2$, AcOH, H_2O, 50 min, 50 °C.

2. Deamination with adenosine deaminase

Selective deamination of the 6-NH_2 group was expected by the action of adenosine deaminase (ADA). ADA catalyzes the irreversible hydrolysis of adenosine or 2′-deoxyadenosine to the corresponding inosine and ammonia. Enzymatic deamination by ADA has found many applications in nucleoside chemistry and has led to functionalized purine nucleoside analogues in drug discovery and medicinal chemistry. This reaction can be performed on a preparative scale.[37–41] As the ADA deaminates β-D isomers exclusively, anomeric mixtures can be also separated.[42] Considerable effort has been directed towards studies of the structural requirements for adenosine derivatives to act as substrates. When this method was applied to pyrrolo[2,3-*d*]pyrimidine nucleosides, *e.g.* tubercidin (7-deazaadenosine), it was shown that 7-deazapurines are not deaminated (Table 1). It was therefore



Scheme 5 *Reagents and conditions*: (i) HMDS, $(NH_4)_2SO_4$, 2 h, reflux; (ii) 1-*O*-acetyl-2,3,5-tri-*O*-benzyl-D-ribofuranose, TMSOTf, 3 h, r.t.; (iii) 28% NH_4OH–dioxane (4 : 1), overnight, 100 °C.

Table 1 Relative initial velocities for adenosine derivatives deaminated with adenosine deaminase[a]

Compound	Rel. V_{max}	Compound	Rel. V_{max}
1a	0.27 (± 0.02)	Adenosine[44]	100
1b	0.18 (± 0.02)	2-Aminoadenosine[44]	25
1c	0	8-Azaadenosine[44]	217
1d	0	7-Deazaadenosine[44]	0
8-Aza-7-deazaadenosine	1.32 (± 0.01)	8-Aza-7-chloro-7-deaza-2′-deoxyadenosine	0

[a] For conditions see Experimental section and Supplementary information†.

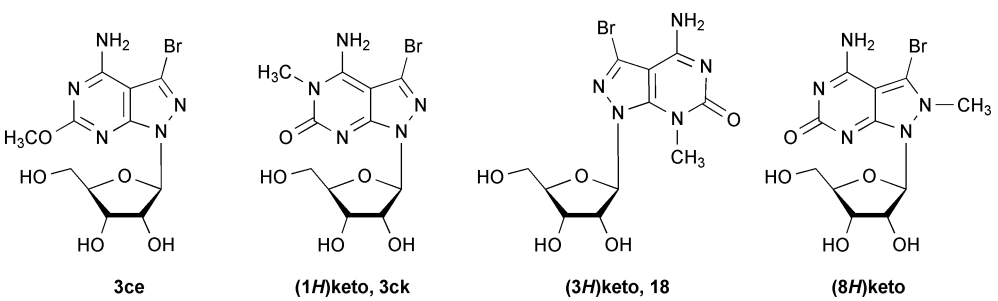
concluded that N-7 is an essential enzyme binding site that is necessary for the deamination reaction. This was supported by the observation that another modified nucleoside, namely 8-azaadenosine, is quickly deaminated (Table 1). As it is known that 8-aza-7-deaza-2′-deoxyadenosine (which does not contain a nitrogen at the 7-position) can be deaminated by adenosine deaminase, it was concluded that this nitrogen is not essential for deamination.[43] As nothing was known about the substrate properties of the diamino nucleoside **1a** or the halogenated derivatives **1b–d**, the conversion to the corresponding 8-aza-7-deazaguanosine by ADA was studied.

The relative initial velocity of the deamination was measured UV-spectrophotometrically. The enzyme was diluted to a concentration (0.004 units μl^{-1}) that warrants substrate saturation, thereby making the initial velocity concentration-independent. The initial velocities of compounds **1a,b** and 2-aminoadenosine were measured at 250 nm, and the data are compiled in Table 1. It can be seen that the non-functionalized compound **1a** and the chloro compound **1b** were deaminated, but with much lower velocities than adenosine, while the deamination of **1c** and **1d** does not occur. Consequently, N-7 is not essential for the adenosine deaminase; however, the positional change of N-7 (purine) to N-8 (pyrazolo[3,4-*d*]pyrimidine) leads to a 100-fold decrease of the reaction rate (25 for 2-aminoadenosine and 0.27 for **1a**). This trend is also observed in the series of adenosine derivatives (100 for adenosine and 1.32 for 8-aza-7-deazaadenosine). A 7-chloro substituent introduced in compound **1a** decreases the rate slightly (0.18 for **1b** *vs.* 0.27 for **1a**), while the 7-chlorinated 8-aza-7-deazaadenosine is not deaminated under the same conditions. Although compounds **1a** and **1b** are deaminated rather slowly, the reaction can be performed on preparative scale when a large excess of the enzyme is employed.

3. Preparation and assignment of fixed tautomers of 8-aza-7-deazaisoguanine nucleosides

As the enol content of isoguanosine and corresponding derivatives controls mismatch formation, the tautomeric keto–enol equilibrium of the isoguanosine derivatives **3a–d** was determined (see the following paragraph). For this it is necessary to correlate the UV spectra of the particular tautomers to the structures of the keto and enol forms. Consequently, the tautomeric species were fixed by replacing the corresponding protons by methyl groups. This will not influence UV spectra significantly, but will allow us to make this assignment. Compound **3c** was chosen as an example. Four possible methylated tautomers are shown in Scheme 7. As diazomethane directs the methylation to the oxo-group,[30] the fixed enol compound **3ce** (Scheme 7) will be accessible. However, two products were obtained when the reaction was performed in methanol with diazomethane freshly prepared from nitrosomethylurea (50% KOH in ether). According to the signals of a methoxy group identified by ^{1}H NMR and ^{13}C NMR, and a UV maximum at 264 nm (Fig. 1), one of the isomers was **3ce** (Scheme 7).

The other methylation product shows NMR signals of an N-methyl group and identical UV spectra in both water and dioxane (Fig. 1, compound **18**), which indicate a fixed keto form – (1*H*)keto, (3*H*)keto, or (8*H*)keto (Scheme 7). However, compound **18** is different from compound **3ck** (fixed (1*H*)keto form). Also, the N-8 methylated compound is excluded because there is no chemical shift change for C-7 in the ^{13}C NMR spectra (**18** *vs.* **3c**, Table 3), which would be an indication of N-8 methylation. Moreover, in the ^{13}C NMR spectra of compound **18**, C-4 has a ~10 ppm up-field shift compared to **3ce** and **3ck**. Therefore the methyl group has to be attached to N-3 (compound **18**, Scheme 7). When compound **3c** was treated with trimethylphosphate under



Scheme 7 Tautomers of compound **3c** fixed by methylation.

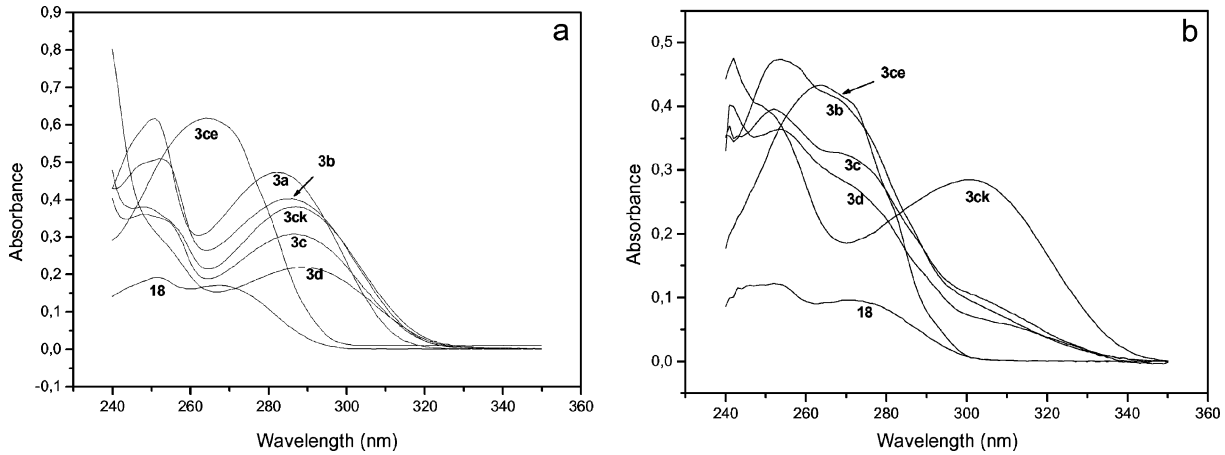


Fig. 1 UV spectra of compounds **3a–d**, **3ck**, **3ce** and **18** in water (pH ~6) (a) and in dioxane (containing 2% water) (b).

alkaline conditions, the fixed (1*H*)keto compound **3ck** (Scheme 7) was obtained together with compound **3ce**. Compound **3ck** carries a methyl group as shown by the NMR spectra, and represents a fixed keto form according to the UV absorption both in water and in dioxane (Fig. 1), resembling the fixed keto form of the pyrrolo derivative.[30] Moreover, compound **3ck** shows a 6 ppm up-field shift for C2 compared to compound **3c** (Table 3).

Fig. 1 shows the compilation of UV spectra of compounds **3a–d**, **3ck**, **3ce** and **18**. Compounds **3b–d** are derivatives of **3a** and show similar UV spectra (see Fig. 1 and Table 2). The UV spectra of the fixed tautomers **3ck** and **3ce** can be used to identify the fixed keto and fixed enol forms for the whole series of nucleosides **3a–d**.

4. Spectroscopic data

Table 3 compiles the ^{13}C NMR chemical shifts of all new compounds. The assignments of the ^{13}C NMR chemical data of the base residues are made according to the literature,[28,45,46] as are the assignments of the carbon resonances of the sugar residues.[47a] The table shows that compared to the 7-non-functionalized 8-aza-7-deazapurines (**5**, **1a**, **3a**, **13a**), the C-7 signals of the 7-chloro derivatives (**6**, **1b**, **3b**, **13b**) are shifted up-field (1–5 ppm). The shift for the 7-bromo derivatives (**7**, **1c**, **3c**, **13c**) is in the same direction but is stonger (13–15 ppm), while for the iodo compounds (**8**, **1d**, **3d**, **13d**) the up-field shift is the strongest (35–40 ppm). The anomeric configuration was assigned to be β-D in view of the small coupling constant ($^3J_{HH}$) for the anomeric proton[47b] (2–3 Hz for **13a–d**, see Experimental section) as well as the chemical shifts of C(1′), C(2′), and C(4′), which indicate the β-D anomers.[47c]

Table 2 UV absorption maxima of nucleosides in water and dioxane

Compound	λ_{max}/nm (water)	λ_{max}/nm (dioxane)[a]
3a	282, 251	262
3b	286, 252	265
3c	287, 249	266
3d	289	268
3ce	264	264
3ck	287	300
18	268, 252	270, 251

[a] 2% water was added for solubility.

5. Determination of pK_a values and the keto/enol content of nucleosides

Protonation and keto–enol tautomerism of nucleosides influence base pairing selectivity and mismatch formation of DNA and RNA. Thus, it is of importance to determine these physico-chemical parameters in solution. At first, the pK_a values of the ribonucleosides **1a–d**, **3a–d** and **15** were determined by spectrophotometric titration[48] (pH 1.2–13.5) at 220–350 nm (see Supplementary data†). As shown in Table 4, the pK_a value of the 7-non-functionalized nucleoside **1a** is about 2 units higher than those of the 7-halogenated compounds **1b–d**. Therefore, compound **1a** is easier to protonate than the derivatives carrying an electron-withdrawing halogen at the 7-position. The N^8 nucleoside **15** shows a pK_a value (6.4) which is higher than that of the N^9 compound **1a** (5.8). The table includes also the pK_a values of the corresponding purine and pyrrolo[2,3-*d*]pyrimidine nucleosides. The diamino compounds **1a** and **2a** show almost no difference (5.8 for **1a** and 5.7 for **2a**), while that of the 2-aminoadenosine is significantly lower (4.4). However, the halogenation has different effects on pyrazolo[3,4-*d*]pyrimidines and pyrrolo[2,3-*d*]pyrimidines. In the former series (**1a–d**), 7-halogenation decreases the pK_a from 5.8 (**1a**) to about 3.7 (**1b–d**), but in the latter case only from 5.7 (**2a**) to 4.7 (**2b–d**). In the series of the isoguanosine derivatives **3a**, **4a** and **isoG**, the pyrazolo analogue **3a** shows similar behaviour to the purine congener **isoG**. The pyrrolo isoguanosine derivative **4a** is more basic (pK_a 4.6) than the purine and pyrazolo analogues (3.4 and 3.7 respectively). It is also shown that 7-halogenation decreases the pK_a value of both protonation and deprotonation by about 1 unit in both the pyrrolo and pyrazolo derivatives (**3a–d**, **4a–c**). Furthermore, it indicates that there is not much difference between the various halogen substituents on the basicity of the nucleosides in certain series of compounds. Within the oligonucleotide chain the pK_a values of protonation can be significantly higher than those of the free nucleosides. Thus, the nucleosides **1a** and **15** might be protonated even under neutral conditions if they are constituents of nucleic acids. The base pairing properties of the 2′-deoxyribo derivatives of **1a** and **15** have already been studied;[27,49] however, the pH-dependent character needs further investigation.

Next, the tautomeric equilibria of the isoguanine derivatives **3a–d** were investigated and compared with corresponding purine

Table 3 ^{13}C NMR chemical shifts (ppm) of pyrazolo[3,4-*d*]pyrimidines and related ribonucleosides **1–18**[a]

Compound	C-2 (C-6)[b]	C-4 (C-7a)[b]	C-5 (C-3a)	C-6 (C-4)[b]	C-7 (C-3)	C-1′	C-2′	C-3′	C-4′	C-5′
5	162.5	158.2	94.8	157.8	132.6					
6	163.0	158.5	91.6	157.3	130.9					
7	162.8	158.4	93.8	157.4	118.1					
8	162.1	157.8	89.5	157.6	97.5					
9[35]	162.1	159.1	95.8	162.9	131.7					
10	162.4	159.4	93.7	162.6	131.0					
11[35]	162.6	159.1	89.6	162.6	99.3					
1a	162.6	158.3	95.4	157.2	133.4	87.8	72.9	70.9	84.7	62.6
1b	163.0	158.3	92.2	157.3	128.6	87.1	72.5	70.7	84.7	62.3
1c	162.8	158.1	94.4	157.4	119.4	87.1	72.6	70.6	84.7	62.3
1d	162.3	157.7	91.6	157.5	98.3	87.4	72.6	70.7	84.8	62.4
3a	156.8	[c]	92.3	154.5	134.9	88.8	73.1	71.1	85.2	62.6
3b	157.0	[c]	89.6	154.1	133.2	87.2	72.7	70.7	85.1	62.3
3c	158.6	[c]	92.1	155.0	120.7	88.5	73.0	71.1	85.5	62.8
3d	156.3	[c]	92.8	154.4	95.2	88.2	72.7	70.7	85.2	62.4
3ce	165.3	157.5	97.0	158.5	119.8	88.1	72.9	70.8	85.3	62.4
3ck	152.8	156.6	90.4	153.6	120.0	87.7	72.7	70.8	85.1	62.5
13a	163.0	158.3	95.3	157.5	134.8	85.3	74.0	71.4	78.4	63.6
13b	163.3	158.4	92.2	157.3	133.6	85.0	73.6	71.1	78.7	63.3
13c	162.5	158.1	94.5	157.2	121.2	85.1	73.5	71.1	78.8	63.4
13d	162.5	157.8	93.5	157.7	98.3	85.1	73.5	71.2	78.8	63.5
14	162.4	162.2	98.2	159.6	126.4	91.3	75.0	71.3	79.0	63.5
15	162.7	162.5	97.6	159.5	124.5	94.4	75.0	70.7	85.6	62.2
16	162.6	158.5	96.4	163.0	133.5	85.5	74.0	71.3	78.5	63.5
17	161.9	164.0	98.5	164.3	125.8	91.5	75.0	71.2	79.1	63.4
18	158.6	148.9	95.1	157.3	120.6	90.7	73.2	70.2	85.5	61.7

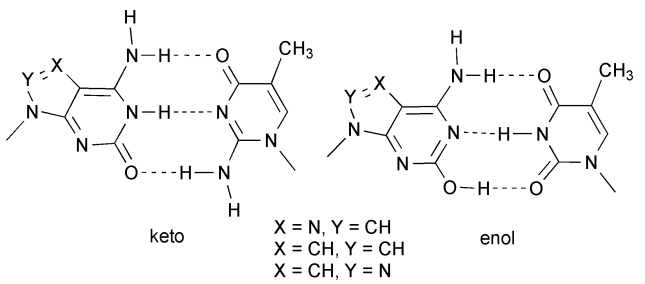
[a] Measured in DMSO-d_6 at 298 K. Carbons are labelled according to purine numbering; the labels in parentheses are the systematic numbers. [b] Tentative. [c] Not detected.

Table 4 pK_a values of nucleosides[a]

Compound	Wavelength[b]/nm	pK_a	Compound	Wavelength[b]/nm	pK_a
1a	257	5.8	**2a**[29]	285	5.7
1b	275	3.7	**2b**	285	4.9
1c	276	3.6	**2c**	286	4.8
1d	276	3.7	**2d**	288	4.9
3a	265 (276)	3.7 (9.7)	**4a**[50]	255 (275)	4.6 (10.5)
3b	295 (276)	2.7 (8.7)	**4b**[50]	264 (264)	3.9 (9.7)
3c	294 (275)	2.7 (8.6)	**4c**[50]	264 (264)	3.8 (9.8)
3d	266 (278)	2.7 (8.7)	2-Aminoadenosine	296	4.4
15	305	6.4	isoG	250 (265)	3.4 (9.5)

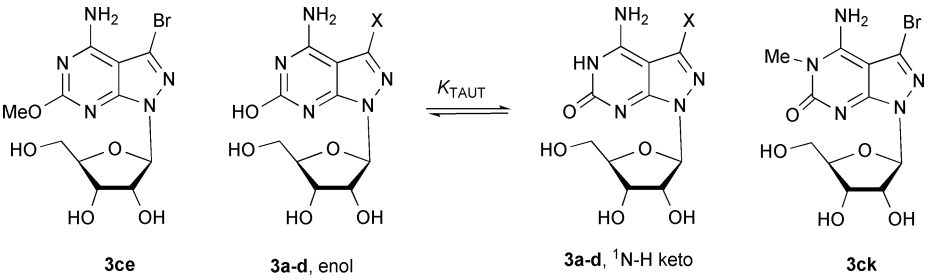
[a] Measured in phosphate buffer (7.8 g $NaH_2PO_4 \cdot H_2O$ in 500 ml H_2O) from pH 1.2 to pH 13.5. Values refer to protonation; those in parentheses refer to deprotonation. [b] Wavelength with the most significant absorbance change.

and 7-deazapurine congeners. In aqueous solution, the canonical nucleic acid constituents guanosine and 2′-deoxyguanosine exist predominantly in the keto (lactam) form ($K_{TAUT} \approx 10^4$–10^5),[51] which leads to an almost perfect Watson–Crick base pairing. As only one of 10^4–10^5 of the G_d molecules is enolized, mispairing is rare. Also, it was reported that iG_d favours the keto (N–H) form in aqueous solution and forms a stable base pair with iC_d (Scheme 8). However, the enol tautomer is present to a significant amount (10%).[52] This allows the formation of stable mismatches with dT or dU (Scheme 8) and decreases the discrimination ability of isoguanine during base pairing,[53] which limits the use of the iG_d in hybridization. Since the keto–enol equilibrium of iG_d depends on the polarity of its microenvironment, one can expect that the modification of nucleobases would influence this equilibrium, resulting in alteration of the coding properties. It has already been shown with pyrrolo[2,3-*d*]pyrimidine analogues of $isoG_d$ that the



Scheme 8 Keto and enol tautomers of isoguanine (and modified derivatives) pair with iso C (or C) and T (or U).

enol content is only about 1/1000,[54] and significantly decreased when a 7-halogenated 7-deazaisoguanine replaces isoguanine.[30] As a mimic of isoguanosine, compounds **3a–d** might also prefer



Scheme 9 Tautomeric equilibrium of nucleosides **3a–d**.

the keto form in the aqueous solution. To prove our hypothesis, the tautomeric equilibria between the keto and the enol forms of **3a–d** were determined UV-spectrophotometrically in aqueous and non-aqueous solutions.

As shown in Fig. 1 and Table 2, the UV spectra of compounds **3a–d**, measured in water, showed the same maxima (250 nm and 286 nm), resembling the fixed keto form **3ck** (Scheme 9). However, under non-aqueous conditions (dioxane with 2% of water for solubilization), the UV absorbance changes totally, showing the maxima of the fixed enol form **3ce** (264 nm). Therefore, it is reasonable to assume that in aqueous solution the compounds **3a–d** exist almost entirely in the keto form, while in dioxane solution the enol form is the predominant species.

Next, the tautomeric equilibria (K_{TAUT} = [keto]/[enol]) of compounds **3a–d** were determined in dioxane–water mixtures ranging from 98 : 2 to 5 : 95. The ratio of the tautomers was determined by the multiwavelength method of Dewar and Urch.[55] Data were collected at around 265 and 286 nm, where the extinction coefficients of both forms show the highest difference. When the water–dioxane ratio increases, the UV spectra for compound **3a–d** shifted gradually, more and more resembling the UV spectra of the keto form. When the water content was higher than 55%, the spectra became solvent-independent and the band at 265 nm nearly disappeared, while the absorbance at 286 nm increased to the level of the pure keto form. By adding more water, the whole spectrum is moved to shorter wavelength due to the increase of the polarity of the solvent system (see Supplementary data†). The composition of keto and enol forms was maintained at the same level. From these data, the K_{TAUT} values were determined quantitatively by the method of Shugar *et al.*[52] and Voegel *et al.*[56] A plot of the logarithm of the tautomeric equilibrium constant for **3a–d** *versus* the polarity parameter $E_T(30)$[57–59] of a set of the dioxane–water mixtures is shown in Fig. 2. When $E_T(30)$ is in the range 38–50, the relationship between log[K_{TAUT}] and $E_T(30)$ is linear. This can be used to estimate the value of K_{TAUT} in aqueous solution. By extrapolating this linear relationship to the $E_T(30)$ value of water (63.1),[59] the tautomeric equilibrium constants for compound **3a–d** were calculated as 400 for **3a**, 750 for **3b**, 550 for **3c** and 1200 for **3d**. These values are smaller than those of the corresponding pyrrolo[2,3-*d*]pyrimidine analogues **4a–d** (10^3–10^4)[30,54] but larger than that of isoguanosine (10)[52] (Table 5). This means that the pyrazolo[3,4-*d*]pyrimidines **3a–d** are more enolized than the pyrrolo[2,3-*d*]pyrimidines **4a–d**, while isoguanosine itself shows the highest enol content. Enol formation of isoguanosine is

Table 5 The tautomerism equilibrium constants for isoguanosine derivatives

Compound	K_{TAUT}	Compound	K_{TAUT}
3a	400	**4a**[30]	1880
3b	750	**4b**[30]	12 800
3c	550	**4c**[30]	19 400
3d	1200	iGd[52]	10

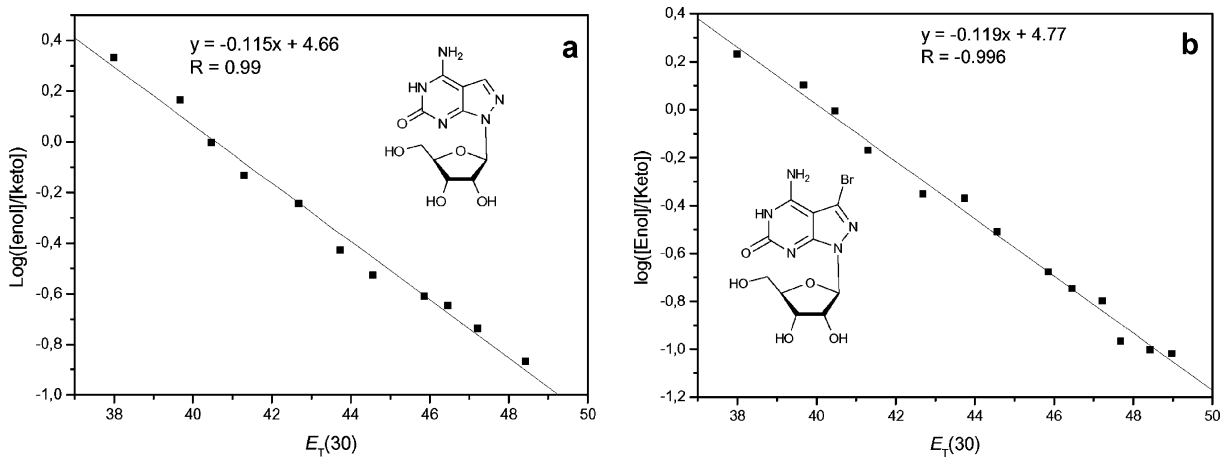


Fig. 2 Plot of log([enol]/[keto]) *versus* $E_T(30)$ for compound **3a** (a) and **3c** (b) in mixtures of dioxane ($E_T(30)$ = 36.0) and water ($E_T(30)$ = 63.1).

thought to be the reason for mismatch formation, and therefore the position exchange of N-7 and C-8 results in a decrease of the mispairing of isoguanosine. Moreover, the halogenated derivatives **3b–d** have almost the same ratio of enol content as **3a**. Thus the halogens should not change the base recognition of the 8-aza-7-deazaisoguanosine derivative (**3a**) but enhance the base pair stability even in mismatches, which is consistent with observation in the case of oligonucleotides.[46]

Conclusions

7-Halogenated 8-aza-7-deazapurine-2,6-diamines and the corresponding ribonucleosides (**1a–d**, **15**) were synthesized. The glycosylation of the 7-non-functionalized 8-aza-7-deazapurine-2,6-diamine **5** yielded the N^8 nucleoside (73%) as main product with very little of the N^9 compounds (4.8%). A 7-halogen substituent shifts the glycosylation reaction exclusively to N^9. The resulting diamino compounds **1a–d** were further converted to the isoguanosine derivatives **3a–d**. 8-Aza-7-deazapurine-2,6-diamine ribonucleoside **1a** and 7-chloro derivative **1b**, which do not contain a nitrogen in position 7, are substrates of ADA, but with significantly lower relative V_{max} values than the purine congener 2-aminoadenosine, while the bromo and iodo derivatives (**1c** and **1d**) could not be deaminated with detectable velocities. Compound **1a** and **15** have pK_a values as high as 5.8 and 6.4, which indicates possible protonation in oligonucleotides under neutral conditions, resulting in mismatch formation. For compounds **3a–d** the keto–enol equilibrium constant K_{TAUT} is between 400–1200. Thus, it is expected that they should show better mismatch discrimination than isoguanosine. As protonation of **15** occurs at pH 6.4, protonation of oligonucleotides might occur under neutral conditions. As a consequence, compounds **1a–d** and **3a–d** might be used as pH-dependent molecular switches. The synthesis and properties of the corresponding oligonucleotides is currently being evaluated.

Experimental

General

All chemicals were purchased from Acros, Fluka, or Sigma-Aldrich. TLC: aluminium sheets coated with silica gel 60 F_{254} (0.2 mm, VWR International). Flash chromatography (FC): 0.4 bar, silica gel 60 (Merk, Darmstadt, Germany). Mp: Linström apparatus, not corrected. UV spectra: U-3200 UV-Vis spectrometer (Hitachi, Japan). NMR spectra: Avance-250 or AMX-500 spectrometers (Bruker); δ values in ppm relative to Me_4Si as internal standard. J values are in Hz. Elemental analyses: performed by the Mikroanalytisches Laboratorium Beller, Göttingen, Germany. Adenosine deaminase (EC 3.5.4.4), Type V from bovine spleen. Solution in 50% glycerol, 50 mM potassium phosphate, pH 6.0, 160 units per ml (Sigma). The adenosine deaminase was diluted with 0.06 M Sørensen buffer (pH 7.0). A 40-fold diluted solution (0.004 units per µl) was used. The substrate was dissolved in the same buffer, after the deaminase (1 µl, 0.004 units) was added; the UV absorbance was detected and recorded at certain wavelengths where the UV spectra have the maximal changes. The absorption at this wavelength *vs.* the time was plotted. The slope of the linear relationship between the absorbance and the time was taken as S. The extinction coefficients of the substrate and the product at certain wavelengths were measured as ε_{sub} and ε_{pro}; the difference between them is defined as $\Delta\varepsilon = \varepsilon_{pro} - \varepsilon_{sub}$. The relative V_{max} of the sample was calculated according to the equation

$$V_{max(sample)} = V_{max(standard)} \times (S_{sample}\Delta\varepsilon_{standard} / S_{standard}\Delta\varepsilon_{sample}).$$

2-Aminoadenosine was taken as standard substrate with a relative V_{max} of 25.[44] For details see Supplementary data†.

3-Chloro-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (6)

From 5. To a solution of 1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (**5**)[26] (1.5 g, 10 mmol) in anhydrous DMF (50 cm^3), *N*-chlorosuccinimide (2.02 g, 15 mmol) was added. The mixture was kept stirred at room temperature. After 24 h, the mixture was evaporated to dryness. The remaining residue was adsorbed on silica gel (15 g) and applied to a flash chromatography (FC) silica gel column (10 × 6 cm, CH_2Cl_2–CH_3OH, 20 : 1) to yield a slightly yellow solid; recrystallization from MeOH afforded yellowish crystals (0.59 g, 32%).

From 10. 6-Amino-3-chloro-4-isopropoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (**10**) (100 mg, 0.44 mmol) was suspended in conc. aq. NH_3–dioxane (2 : 1, 30 cm^3) and placed in an autoclave. The suspension was stirred at 120 °C for 24 h. The clear solution was evaporated until precipitation occurred. The precipitate was filtrated and washed with acetone, affording compound **6** as slightly yellow amorphous solid (66 mg, 81%) (Found: C, 32.60; H, 2.80; N, 45.21. $C_5H_5ClN_6$ requires C, 32.53; H, 2.73; N, 45.53%); mp > 300 °C (from MeOH); TLC (silica gel, CH_2Cl_2–CH_3OH, 10.1): R_f 0.20; λ_{max}(MeOH)/nm 275 (ε/dm^3 mol^{-1} cm^{-1} 6900), 257 (7000) and 224 (29 200); δ_H(250 MHz; DMSO-d_6; Me_4Si) 6.19 (2 H, br s, NH_2); 6.79 (2 H, br s, NH_2), 12.61 (1 H, br s, NH).

3-Iodo-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (8)

From 5. To a suspension of 1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (**5**)[26] (0.75 g, 5 mmol) in anhydrous 1,2-dichloroethane (200 cm^3), *N*-iodosuccinimide (1.35 g, 6 mmol) was added. The mixture was kept stirring under reflux for 3 d. The mixture was cooled and evaporated to dryness and purified by FC (silica gel column, 10 × 5 cm, CH_2Cl_2–CH_3OH, 20 : 1) to yield a slightly yellow solid (0.9 g, 65%).

From 11. 6-Amino-3-iodo-4-isopropoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (**11**)[35] (5.5 g, 17.2 mmol) was suspended in 28% NH_3–dioxane (2 : 1, 350 cm^3) and placed in a steel bomb. The suspension was stirred at 120 °C for 24 h. The clear solution was evaporated until precipitation occurred. The precipitate was filtrated and washed with acetone, affording compound **8** as a colourless amorphous solid (3.5 g, 74%) (Found: C, 21.68; H, 1.82; N, 30.26. $C_5H_5IN_6$ requires C, 21.76; H, 1.83; N, 30.45%); TLC (silica gel, CH_2Cl_2–CH_3OH, 10.1): R_f 0.23; λ_{max}(MeOH)/nm 271 (ε/dm^3 mol^{-1} cm^{-1} 7800) and 224 (29 200); δ_H(250 MHz; DMSO-d_6; Me_4Si) 6.17 (2 H, br s, NH_2), 6.53 (2 H, br s, NH_2), 12.85 (1 H, br s, NH).

6-Amino-3-chloro-4-isopropoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (10)

Compound **9**[36] (400 mg, 2.07 mmol) was suspended in 1,2-dichloroethane (200 cm^3), *N*-chlorosuccinimide (NCS) (300 mg, 2.25 mmol) was added, and the mixture was stirred under reflux for 5 h. The solution was cooled, evaporated to dryness and the residue purified by FC (silica gel column, 10 × 5 cm, elution with CH_2Cl_2–EtOAc 2 : 1) to yield a colourless solid (200 mg, 42%). Recrystallization from methanol afforded colourless needles (Found: C, 42.10; H, 4.36; N, 30.35. $C_8H_{10}ClN_5O$ requires C, 42.21; H, 4.43; N, 30.76%); mp 253 °C (From MeOH); TLC (silica gel, CH_2Cl_2–CH_3OH, 10 : 1): R_f 0.44; λ_{max}(MeOH)/nm 278 (ε/dm^3 mol^{-1} cm^{-1} 7300), 248 (5700) and 224 (29 200); δ_H(250 MHz; DMSO-d_6; Me_4Si) 1.35 (6 H, m, 2 × CH_3), 5.46 (1 H, m, CH_3CHCH_3), 6.79 (2 H, br s, NH_2), 12.92 (1 H, br s, NH).

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (13a) and 2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (14)

1*H*-Pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (**5**)[26] (1.00 g, 6.67 mmol) was suspended in nitromethane (20 cm^3), and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**12**, 5.04 g, 10 mmol) was added. The mixture was brought to reflux (130 °C) and then $BF_3{\cdot}OEt_2$ (2.12 cm^3, 16.67 mmol) was added. Immediately, the mixture became clear and then slowly became dark. The mixture was kept stirred at this temperature for 15 min. After being cooled to r.t. (ice bath), the reaction mixture was evaporated, and the remaining residue was purified by FC (silica gel column, 12 × 5 cm, CH_2Cl_2–CH_3OH 50 : 1 → 20 : 1); compound **13a** was obtained (190 mg, 4.8%) as a colourless solid from the faster migrating zone (Found: C, 62.65; H, 4.50; N, 14.10. $C_{31}H_{26}N_6O_7$ requires C, 62.62; H, 4.41; N, 14.13%); TLC (silica gel, CH_2Cl_2–CH_3OH, 10 : 1): R_f 0.59; λ_{max}(MeOH)/nm 275 (ε/dm^3 mol^{-1} cm^{-1} 13 000), 258 (12 100) and 226 (59 500); δ_H(250 MHz; DMSO-d_6; Me_4Si) 4.52–4.55 (1 H, m, 5′-H), 4.61–4.64 (1 H, m, 5′-H), 4.84 (1 H, m, 4′-H), 6.18–6.22 (2 H, m, 3′-H and 2′-H), 6.26 (2 H, s, NH_2), 6.46 (1 H, d, *J* 2.5, 1′-H), 7.25 (2 H, br s, NH_2); 7.45–8.03 (17 H, m, 3 × Ph and NH_2), 8.35 (1 H, s, 3-H).

Compound **14** was collected from the second zone as a colourless solid (2.9 g, 73%), recrystallization from CH_2Cl_2–CH_3OH gave pale red crystals (Found: C, 62.64; H, 4.35; N, 14.15. $C_{31}H_{26}N_6O_7$ requires C, 62.62; H, 4.41; N, 14.13%); mp 165 °C (from CH_2Cl_2 and MeOH); TLC (silica gel, CH_2Cl_2–CH_3OH, 10 : 1): R_f 0.42; λ_{max}(MeOH)/nm 297 (ε/dm^3 mol^{-1} cm^{-1} 6400), 283 (7200), 266 (9300) and 227 (51 100); δ_H(250 MHz; DMSO-d_6; Me_4Si) 4.52–4.67 (2 H, m, 2 × 5′-H), 4.89 (1 H, m, 4′-H), 6.10 (1 H, m, 3′-H), 6.24 (1 H, m, 2′-H) 6.37 (2 H, br s, NH_2), 6.63 (1 H, s, 1′-H), 7.43–7.99 (17 H, m, 3 × Ph and NH_2), 8.41 (1 H, s, 3-H).

3-Chloro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (13b)

As described for **13a**, 3-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (**6**) (230 mg, 1.25 mmol) was converted to **13b**. Colourless foam (485 mg, 62%) (Found: C, 59.23; H, 3.96; N, 13.46. $C_{31}H_{25}ClN_6O_7$ requires C, 59.19; H, 4.01; N, 13.36%); TLC (silica gel, CH_2Cl_2–CH_3OH, 10 : 1): R_f 0.49; λ_{max}(MeOH)/nm 275 (ε/dm^3 mol^{-1} cm^{-1} 12 300), 262 (12 200) and 227 (68 700); δ_H(250 MHz; DMSO-d_6; Me_4Si) 4.53–4.65 (2 H, m, 2 × 5′-H), 4.82 (1 H, m, 4′-H), 6.07 (1 H, m, 3′-H), 6.19 (1 H, m, 2′-H), 6.43 (1 H, d, *J* 3.35, 1′-H), 6.53 (2 H, br s, NH_2), 7.02 (2 H, br s, NH_2), 7.46–8.07 (15 H, m, 3 × Ph).

3-Bromo-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (13c)

As described for **13b**, with 3-bromo-1*H*-pyrazolo[3,4-*d*]-pyrimidine-4,6-diamine (**7**)[34] (2.29 g, 10 mmol) and **12** (7.56 g, 15 mmol), **13c** was obtained (4.69 g, 69.7%) as a colourless foam (Found: C, 55.10; H, 3.82; N, 12.40. $C_{31}H_{25}BrN_6O_7$ requires C, 55.29; H, 3.74; N, 12.48%); TLC (silica gel, CH_2Cl_2–CH_3OH, 20 : 1): R_f 0.33; λ_{max}(MeOH)/nm 275 (ε/dm^3 mol^{-1} cm^{-1} 12 000), 263 (12 000) and 229 (66 500); δ_H(250 MHz; DMSO-d_6; Me_4Si) 4.59 (2 H, m, 2 × 5′-H), 4.83 (1 H, m, 4′-H), 6.07 (1 H, m, 3′-H), 6.21 (1 H, m, 2′-H), 6.44 (1 H, d, *J* 3.55, 1′-H), 6.57 (4 H, br s, 2 × NH_2), 7.44–8.08 (15 H, m, 3 × Ph).

3-Iodo-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (13d)

As described for **13b**, 3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (**8**) (552 mg, 2 mmol) and **12** (1.52 g, 3 mmol) gave **13d** (770 mg, 53.5%) as a colourless foam (Found: C, 51.95; H, 3.35; N, 11.82. $C_{31}H_{25}IN_6O_7$ requires C, 51.68; H, 3.50; N, 11.66%); TLC (silica gel, CH_2Cl_2–CH_3OH, 20 : 1): R_f 0.34; λ_{max}(MeOH)/nm 275 (ε/dm^3 mol^{-1} cm^{-1} 12 100), 264 (12 400) and 230 (67 600); δ_H(250 MHz; DMSO-d_6; Me_4Si) 4.58 (2 H, m, 2 × 5′-H), 4.82 (1 H, m, 4′-H), 6.03 (1 H, t, *J* 5.6, 3′-H), 6.23 (1 H, m, 2′-H), 6.42 (1 H, d, *J* 3.83, 1′-H), 6.49 (2 H, br s, NH_2), 6.75 (2H, br s, NH_2), 7.43–8.07 (15 H, m, 3 × Ph).

1-(β-D-Ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (1a)

From 13a. 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (**13a**) (183 mg, 0.31 mmol) was suspended in 0.5 M $NaOCH_3$/MeOH (5 cm^3) and the mixture was kept stirred at r.t. overnight. The mixture was evaporated to dryness. The residue was adsorbed on a small amount of silica gel (2 g) and loaded on the top of a FC silica gel column (5 × 2.5 cm), eluting with CH_2Cl_2–CH_3OH (10 : 1 → 2 : 1), furnishing compound **1a** (80 mg, 92%) as a colourless solid.

From 16. Compound **16** (see below) (650 mg, 1.02 mmol) was dissolved in 30 cm^3 dioxane and then 120 cm^3 aqueous ammonia (28%) was added. The mixture was kept stirred in an autoclave at 100 °C overnight. The reaction mixture was evaporated and the residue was adsorbed on silica gel (3 g) and applied to a silica gel column (8 × 2.5 cm). Elution with CH_2Cl_2–CH_3OH (20 : 1 → 5 : 1) gave compound **1a** (200 mg, 69%) as a colourless solid (Found: C, 42.40; H, 4.90; N, 29.90. $C_{10}H_{14}N_6O_4$ requires C, 42.55; H, 5.00; N, 29.77%); TLC (silica gel, CH_2Cl_2–CH_3OH, 4 : 1): R_f 0.14; λ_{max}(MeOH)/nm 276 (ε/dm^3 mol^{-1} cm^{-1} 8300), 259 (7800) and 224 (28 600); δ_H(250 MHz; DMSO-d_6; Me_4Si) 3.49–3.58 (2 H, m,

2 × 5′-H), 3.83 (1 H, m, 4′-H), 4.24 (1 H, m, 3′-H), 4.48 (1 H, m, 2′-H), 4.88 (1 H, t, *J* 5.6, 5′-OH), 5.02 (1 H, d, *J* 5.2, 3′-OH), 5.26 (1 H, d, *J* 5.6, 2′-OH), 5.90 (1 H, d, *J* 4.5, 1′-H), 6.22 (2 H, br s, NH_2), 7.16 (2H, br s, NH_2), 7.86 (1 H, s, 3-H).

2-(β-D-Ribofuranosyl)-2*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (15)

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-pyrimidine-4,6-diamine (**14**) (180 mg, 0.30 mmol) was suspended in 0.5 M $NaOCH_3$/MeOH (5 cm^3) and the mixture was kept stirred at r.t. overnight. The solvent was evaporated and the residue was dissolved in distilled water (30 cm^3). The resulting solution was applied to the top of a Serdolit AD-4 column (20 × 4 cm) and the compound eluted with water. The fractions containing the desired compound were combined and condensed to furnish compound **15** (67 mg, 79%) as a colourless solid (Found: C, 42.80; H, 4.93; N, 29.59. $C_{10}H_{14}N_6O_4$ requires C, 42.55; H, 5.00; N, 29.77%); λ_{max}(MeOH)/nm 297 (ε/dm^3 mol^{-1} cm^{-1} 6900), 265 (7600) and 225 (26 400); δ_H(250 MHz; DMSO-d_6; Me_4Si) 3.46–3.58 (1 H, m, 5′-H), 3.64–3.67 (1 H, m, 5′-H), 3.97 (1 H, m, 4′-H), 4.13 (1 H, m, 3′-H), 4.35 (1 H, m, 2′-H), 5.02–5.53 (3 H, br m, 5′-OH, 3′-OH and 2′-OH), 5.70 (1 H, d, *J* 3.6, 1′H), 5.77 (2 H, br s, NH_2), 7.21 (2 H, br s, NH_2), 8.26 (1 H, s, 3-H).

3-Chloro-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-pyrimidine-4,6-diamine (1b)

As described for **1a** (from **13a**), starting from 3-chloro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (**13b**, 180 mg, 0.29 mmol), compound **1b** (80 mg, 88%) was furnished as a colourless solid (Found: C, 37.91; H, 4.27; N, 26.43. $C_{10}H_{13}ClN_6O_4$ requires C, 37.92; H, 4.14; N, 26.54%); TLC (silica gel, CH_2Cl_2–CH_3OH, 5 : 1): R_f 0.30; λ_{max}(MeOH)/nm 276 (ε/dm^3 mol^{-1} cm^{-1} 8600), 261 (9300) and 227 (34 900); δ_H(250 MHz; DMSO-d_6; Me_4Si) 3.38–3.54 (2 H, m, 2 × 5′-H), 3.82 (1 H, m, 4′-H), 4.10 (1 H, m, 3′-H), 4.44 (1 H, m, 2′-H), 4.77 (1 H, t, *J* 5.5, 5′-OH), 5.06 (1 H, d, *J* 3.9, 3′-OH), 5.31 (1 H, d, *J* 5.6, 2′-OH), 5.86 (1 H, d, *J* 4.7, 1′-H), 6.44 (2 H, br s, NH_2), 6.97 (2 H, br s, NH_2).

3-Bromo-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-pyrimidine-4,6-diamine (1c)

As described for **1a,b**, but starting from 3-bromo-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (**13c**, 1.59 g, 2.36 mmol), compound **1c** was obtained as a colourless solid (648 mg, 76%). Recrystallization from H_2O gave colourless crystals (Found: C, 33.66; H, 3.64; N, 23.67. $C_{10}H_{13}BrN_6O_4$ requires C, 33.26; H, 3.63; N, 23.27%); mp 236 °C (from H_2O); TLC (silica gel, CH_2Cl_2–CH_3OH, 5 : 1): R_f 0.30; λ_{max}(MeOH)/nm 278 (ε/dm^3 mol^{-1} cm^{-1} 7300), 262 (7600) and 228 (29 400); δ_H(250 MHz; DMSO-d_6; Me_4Si) 3.50 (2 H, m, 2 × 5′-H), 3.83 (1 H, m, 4′-H), 4.10 (1 H, m, 3′-H), 4.45 (1 H, m, 2′-H), 4.79 (1 H, m, 5′-OH), 5.07 (1 H, d, *J* 4.1, 3′-OH), 5.32 (1 H, d, *J* 4.8, 2′-OH), 5.87 (1 H, d, *J* 4.4, 1′-H), 6.42 (2 H, br s, NH_2), 6.84 (2 H, br s, NH_2).

3-Iodo-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-pyrimidine-4,6-diamine (1d)

As described for **1a–c**, from 3-iodo-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (**13d**) (1.1 g, 1.53 mmol), compound **1d** was obtained (474 mg, 76%) as a colourless solid. Recrystallisation from MeOH–H_2O afforded colourless needles (Found: C, 29.72; H, 3.32; N, 20.39. $C_{10}H_{13}IN_6O_4$ requires C, 29.43; H, 3.21; N, 20.59%); mp 248 °C (dec.) (from MeOH–H_2O); TLC (silica gel, CH_2Cl_2–CH_3OH, 5 : 1): R_f 0.33; λ_{max}(MeOH)/nm 279 (ε/dm^3 mol^{-1} cm^{-1} 8000), 263 (9000) and 230 (29 900); δ_H(250 MHz; DMSO-d_6; Me_4Si) 3.49–3.63 (2 H, m, 2 × 5′-H), 3.89 (1 H, m, 4′-H), 4.17 (1 H, m, 3′-H), 4.54 (1 H, m, 2′-H), 4.68 (1 H, t, *J* 5.2, 5′-OH), 5.11 (1 H, d, *J* 5.0, 3′-OH), 5.36 (1 H, d, *J* 5.8, 2′-OH), 5.91 (1 H, d, *J* 4.8, 1′-H), 6.43 (2 H, br s, NH_2), 6.72 (2 H, br s, NH_2).

6-Amino-4-isopropoxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (16) and 6-amino-4-isopropoxy-2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2*H*-pyrazolo[3,4-*d*]pyrimidine (17)

A suspension of 6-amino-4-isopropoxy-1*H*-pyrazolo[3,4-*d*]-pyrimidine (**9**,[36] 386 mg, 2 mmol) and a catalytic amount of $(NH_4)_2SO_4$ in HMDS (10 cm^3) was refluxed for 2 h. The excess of HMDS was removed by evaporation and the residue was dissolved in 1,2-dichloroethane (6 cm^3). To this solution compound **12** (1.21 g, 2.4 mmol) was added followed by the addition of trimethylsilyl trifluoromethanesulfonate (0.6 cm^3, 5 mmol). The mixture was kept stirred at r.t. After 3 h, the mixture was diluted with CH_2Cl_2 (20 cm^3) and washed with 5% aqueous $NaHCO_3$. The organic layer was dried and filtered. The filtrate was evaporated to a dryness and dissolved in dichloromethane (4 ml) and applied to the top of a FC silica gel column (10 × 2.5 cm). Elution with CH_2Cl_2–CH_3OH (100 : 1 → 20 : 1) yielded two compounds; the compound eluting first was **16** (566 mg, 44%), obtained as a colourless foam (Found: C, 64.00; H, 5.10; N, 10.74. $C_{34}H_{31}N_5O_8$ requires C, 64.00; H, 4.90; N, 10.98%); TLC (silica gel, CH_2Cl_2–CH_3OH, 20 : 1): R_f 0.66; λ_{max}(MeOH)/nm 275 (ε/dm^3 mol^{-1} cm^{-1} 11 800) and 255 (53 500); δ_H(250 MHz; DMSO-d_6; Me_4Si) 1.33 (3 H, s, CH_3), 1.36 (3 H, s, CH_3), 4.51–4.61 (3 H, m, 2 × 5′-H and 4′-H), 4.84 (1 H, m, 3′-H), 5.48 (1 H, m, CH_3CHCH_3), 6.16–6.21 (2 H, m, 2′-H and 3′-H), 6.51 (1 H, d, *J* 2.4, 1′-H), 6.93 (2 H, br s, NH_2), 7.42–8.01 (16 H, m, 3 × Ph and 3-H).

The compound eluting last was **17** (185 mg, 15%), obtained as a pale yellow foam (Found: C, 63.92; H, 5.24; N, 10.88. $C_{34}H_{31}N_5O_8$ requires C, 64.00; H, 4.90; N, 10.98%); TLC (silica gel, CH_2Cl_2–CH_3OH, 20 : 1): R_f 0.27; λ_{max}(MeOH)/nm 282 (ε/dm^3 mol^{-1} cm^{-1} 7700) and 268 (8400); δ_H(250 MHz; DMSO-d_6; Me_4Si) 1.32–1.40 (6 H, m, 2 × CH_3), 4.56–4.63 (2 H, m, 2 × 5′-H), 4.90 (1 H, m, 4′-H), 5.45–5.50 (1 H, m, CH_3CHCH_3), 6.12 (1 H, m, 3′-H), 6.27 (1 H, m, 2′-H), 6.50 (1 H, d, *J* 1.7, 1′-H), 6.56 (2 H, s, NH_2), 7.43–8.02 (15 H, m, 3 × Ph), 8.49 (1 H, s, 3-H).

4-Amino-1-(β-D-ribofuranosyl)-1*H*-pyrazolo-[3,4-*d*]pyrimidine-6-one (3a)

To a stirred solution of $NaNO_2$ (69 mg, 1 mmol) in H_2O (2.9 cm^3) at 50 °C, 1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (**1a**) (70 mg, 0.25 mmol) was added followed by AcOH

(0.16 cm^3, 2.8 mmol) dropwise. The mixture became a clear solution within 5 min. The reaction was kept stirred at 50 °C and monitored by TLC; after 50 min, the mixture was diluted with H_2O (10 cm^3) and concentrated aq. NH_3 was added until pH 8 was reached. The solution was applied to a column (20 × 2 cm, Serdolit AD-4 resin). The column was washed with H_2O (200 cm^3), and the product was eluted with H_2O–*i*PrOH (95 : 1, 300 cm^3), yielding **3a** (30 mg, 43%) as a colourless solid (Found: C, 42.34; H, 4.73; N, 24.80. $C_{10}H_{13}N_5O_5$ requires C, 42.40; H, 4.63; N, 24.73%); λ_{max}(MeOH)/nm 282 (ε/dm^3 mol^{-1} cm^{-1} 5600) and 262 (7300); δ_H(250 MHz; DMSO-d_6; Me_4Si) 3.52–3.58 (2 H, m, 2 × 5′-H), 3.85 (1 H, m, 4′-H), 4.12 (1 H, m, 3′-H), 4.47 (1 H, m, 2′-H), 5.07–5.30 (3 H, m, 5′-OH, 3′-OH and 2′-OH), 5.80 (1 H, d, *J* 4.5, 1′-H), 7.93 (1 H, s, 3-H), 8.67 (2 H, br s, NH_2).

4-Amino-3-chloro-1-(β-D-ribofuranosyl)-1*H*-pyrazolo-[3,4-*d*]pyrimidine-6-one (3b)

As described for **3a**, 3-chloro-1-(β-D-ribofuranosyl)-1*H*-pyrazolo-[3,4-*d*]pyrimidine-4,6-diamine (**1b**) (120 mg, 0.38 mmol) was converted to **3b**, yielding a colourless solid (50 mg, 42%) (Found: C, 37.68; H, 3.65; N, 21.89. $C_{10}H_{12}ClN_5O_5$ requires C, 37.81; H, 3.81; N, 22.04%); λ_{max}(MeOH)/nm 293 (ε/dm^3 mol^{-1} cm^{-1} 4600), 250 (7300) and 229 (23 800); δ_H(250 MHz; DMSO-d_6; Me_4Si) 3.44–3.56 (2 H, m, 2 × 5′-H), 3.84 (1 H, m, 4′-H), 4.08 (1 H, m, 3′-H), 4.41 (1 H, m, 2′-H), 4.90–5.34 (3 H, m, 5′-OH, 3′-OH and 2′-OH), 5.81 (1 H, d, *J* 4.8, 1′-H), 7.56 (2 H, br s, NH_2), 11.23 (1 H, br s, 5-NH).

4-Amino-3-bromo-1-(β-D-ribofuranosyl)-1*H*-pyrazolo-[3,4-*d*]pyrimidine-6-one (3c)

As described for **3a**, compound **1c** (361 mg, 1 mmol) was converted to **3c** (206 mg, 57%), which was obtained as a yellowish solid (Found: C, 32.95; H, 3.35; N, 19.15. $C_{10}H_{12}BrN_5O_5$ requires C, 33.17; H, 3.34; N, 19.34%); λ_{max}(MeOH)/nm 293 (ε/dm^3 mol^{-1} cm^{-1} 4200), 248 (5800) and 231 (22 400); δ_H(250 MHz; DMSO-d_6; Me_4Si) 3.44–3.57 (2 H, m, 2 × 5′-H), 3.85 (1 H, m, 4′-H), 4.08 (1 H, m, 3′-H), 4.45 (1 H, m, 2′-H), 5.01–5.60 (3 H, m, 5′-OH, 3′-OH and 2′-OH), 5.80 (1 H, d, *J* 4.9, 1′-H), 7.66 (2 H, br s, NH_2).

4-Amino-1-(β-D-ribofuranosyl)-3-iodo-1*H*-pyrazolo-[3,4-*d*]pyrimidine-6-one (3d)

As described for **3a**, compound **1d** (143 mg, 0.35 mmol) was converted to **3d**, which was obtained as a white powder (80 mg, 56%) (Found: C, 29.46; H, 3.05; N, 17.27. $C_{10}H_{12}IN_5O_5$ requires C, 29.36; H, 2.96; N, 17.12%); λ_{max}(MeOH)/nm 295 (ε/dm^3 mol^{-1} cm^{-1} 4200) and 235 (19 500); δ_H(250 MHz; DMSO-d_6; Me_4Si) 3.40–3.57 (2 H, m, 2 × 5′-H), 3.85 (1 H, m, 4′-H), 4.09 (1 H, m, 3′-H), 4.45 (1 H, m, 2′-H), 4.80–5.55 (3 H, m, 5′-OH, 3′-OH and 2′-OH), 5.77 (1 H, s, 1′-H), 7.51 (2 H, br s, NH_2).

4-Amino-3-bromo-6-methoxy-1-(β-D-ribofuranosyl)-1*H*-pyrazolo-[3,4-*d*]pyrimidine (3ce) and 4-amino-3-bromo-7-methyl-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-one (18)

Compound **3c** (130 mg, 0.36 mmol) was suspended in methanol (8 cm^3) and CH_2N_2 in ether (freshly prepared, 1 cm^3) was added. The mixture was kept stirred at r.t. for 20 min and then evaporated. The residue was adsorbed to silica gel (1.5 g) and applied to the top of a FC silica gel column (10 × 2.5 cm), elution with CH_2Cl_2–CH_3OH (50 : 1 → 5 : 1), affording compound **3ce** (39 mg, 29%) and **18** (30 mg, 22%).

Compound **3ce** was recrystallized from MeOH–CH_2Cl_2, giving colourless crystals (Found: C, 35.36; H, 4.00; N, 18.51. $C_{11}H_{14}BrN_5O_5$ requires C, 35.12; H, 3.75; N, 18.62%); mp 226 °C (from MeOH and CH_2Cl_2); TLC (silica gel, CH_2Cl_2–CH_3OH, 10 : 1): R_f 0.29; λ_{max}(MeOH)/nm 263 (ε/dm^3 mol^{-1} cm^{-1} 8300); δ_H(250 MHz; DMSO-d_6; Me_4Si) 3.33–3.58 (2 H, m, 2 × 5′-H), 3.86 (4 H, m, 4′-H and OCH_3), 4.13 (1 H, m, 3′-H), 4.54 (1 H, m, 2′-H), 4.77 (1 H, t, *J* 5.6, 5′-OH), 5.16 (1 H, d, *J* 5.2, 3′-OH), 5.39 (1 H, d, *J* 5.9, 2′-OH), 5.95 (1 H, d, *J* 4.8, 1′-H), 8.05 (2 H, br s, NH_2).

Compound **18** was recrystallized from MeOH, giving colourless crystals (Found: C, 35.02; H, 4.12. $C_{11}H_{14}BrN_5O_5$ requires C, 35.12; H, 3.75); mp 205 °C (dec.) (from MeOH–CH_2Cl_2); TLC (silica gel, CH_2Cl_2–CH_3OH, 5 : 1): R_f 0.47; λ_{max}(MeOH)/nm 269 (ε/dm^3 mol^{-1} cm^{-1} 8500) and 252 (10 300); δ_H(250 MHz; DMSO-d_6; Me_4Si) 3.27–3.54 (2 H, m, 2 × 5′-H), 3.61 (3 H, s, CH_3), 3.90 (1 H, m, 4′-H), 4.18 (1 H, m, 3′-H), 4.70 (2 H, m, 2′-H and 5′-OH), 5.21 (1 H, d, *J* 5.9, 3′-OH), 5.43 (1 H, d, *J* 5.4, 2′-OH), 6.00 (1 H, d, *J* 3.4, 1′-H), 6.56 (1 H, br s, NH of NH_2), 7.97 (1 H, br s, NH of NH_2).

4-Amino-3-bromo-5-methyl-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-one (3ck)

To a solution of compound **3c** (120 mg, 0.33 mmol) in H_2O (2.5 cm^3), trimethylphosphate (2.1 cm^3, 18.3 mmol) was added. The mixture was stirred at 50 °C and kept alkaline by addition of 2 N NaOH. After 20 h, the mixture was cooled and diluted with water (10 cm^3) and neutralized. The mixture was then evaporated and co-evaporated several times with MeOH, and the residue was adsorbed on silica gel (3 g) and applied to a FC silica gel column (5 × 2.5 cm, CH_2Cl_2–CH_3OH 50 : 1 → 5 : 1), to give compound **3ce** (20 mg, 16%) from the early fractions, and **3ck** (40 mg, 32%) from the later fractions as a colourless solid (Found: C, 35.30; H, 4.11; N, 18.55. $C_{11}H_{14}BrN_5O_5$ requires C, 35.12; H, 3.75; N, 18.62); TLC (silica gel, CH_2Cl_2–CH_3OH, 5 : 1): R_f 0.35; λ_{max}(MeOH)/nm 295 (ε/dm^3 mol^{-1} cm^{-1} 3500) and 248 (3700); δ_H(250 MHz; DMSO-d_6; Me_4Si) 3.36 (3 H, s, CH_3), 3.43–3.66 (2 H, m, 2 × 5′-H), 3.83 (1 H, m, 4′-H), 4.07 (1 H, m, 3′-H), 4.43 (1 H, m, 2′-H), 4.90 (1 H, m, 5′-OH), 5.08 (1 H, d, *J* 4.8, 3′-OH), 5.30 (1 H, d, *J* 6.1, 2′-OH), 5.76 (1 H, d, *J* 3.4, 1′-H), 8.01 (2 H, br s, NH_2).

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