7-HALOGENATED 7-DEAZAPURINE 2′-*C*-METHYLRIBONUCLEOSIDES

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This article is dedicated to Professor Antonín Holý, a good friend and an excellent scientist, on the occasion of his 75th birthday.

7-Halogenated 7-deazapurine 2′-*C*-methylribonucleosides related to adenosine, inosine and guanosine were synthesized employing a "one-pot" glycosylation protocol (Vorbrüggen glycosylation). The immunosine 2′-*C*-methylribonucleoside **4** was prepared by the same glycosylation strategy. By this route, the synthesis of the anti-HCV active compound 7-fluoro-7-deaza-2′-*C*-methyladenosine (**1b**) was notably simplified, while other nucleosides were synthesized for the first time. A single-crystal X-ray analysis of 7-fluoro-7-deaza-2′-*C*-methylinosine (**2b**) was performed showing that the glycosylic bond adopts an *anti* orientation (χ = –139.9(2)°), while the sugar moiety has an *N* conformation (C3′-*endo*; *P* = 4.3(1)°, τ_m = 42.4(1)°).

Keywords: Nucleosides; 2′-*C*-Methylribonucleosides; 7-Deazapurines; Pyrrolo[2,3-*d*]pyrimidine; Glycosylation; Single-crystal X-ray analysis.

Hepatitis C virus (HCV) infections still represent an unsolved health problem inducing chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma[1]. Significant effort has been devoted to the development of active compounds of therapeutic value for efficient HCV infection treatment[1–3]. Virally encoded polymerases, which are essential for HCV replication, were established as drug targets. Among the nucleoside inhibitors, 2′-*C*-

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methylribonucleosides showed excellent chain-terminating properties, thus affecting RNA replication. In this context, 2′-*C*-methyladenosine and 2′-*C*-methylguanosine were identified as potent inhibitors of HCV RNA replication *in vitro*[4]. However, both compounds also suffer from significant drawbacks. 2′-*C*-Methyladenosine was found to serve as a substrate for adenosine deaminase and purine nucleoside phosphorylase, indicating that its restricted oral bioavailability results from metabolic conversion[4–6]. For 2′-*C*-methylguanosine, cellular uptake and intracellular metabolism to the corresponding triphosphate were limited[5,6]. Nucleosides employing modified nucleobases were expected to circumvent these drawbacks. Recently, the triphosphate of 7-deaza-2′-*C*-methyladenosine (**1a**) was identified as a potent inhibitor of HCV replication[7] and its 7-fluorinated derivative **1b** was even found to be the most potent compound[6] (purine numbering is used throughout the manuscript).

Due to our continuous efforts on the synthesis of 7-deazapurine nucleosides as structural analogues of natural purine nucleosides, we became interested in synthesizing novel 7-deazapurine 2′-*C*-methylribonucleosides and to study their conformational properties. Moreover, we devoted considerable effort towards the simplification of synthetic protocols of already reported compounds with notable anti-HCV activity making them more easily accessible.

In this work, we focus on the synthesis and properties of 7-halogenated 7-deazapurine 2′-*C*-methylribonucleosides related to adenosine, inosine and guanosine (Fig. 1). The immunosine 2′-*C*-methylribonucleoside **4** was prepared as well. A single-crystal X-ray analysis of compound **2b** was performed and conformational properties were studied in solution and in solid state.

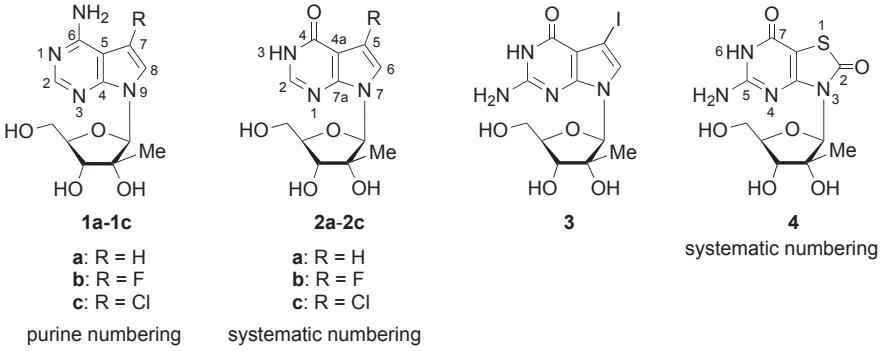


FIG. 1

RESULT AND DISCUSSION

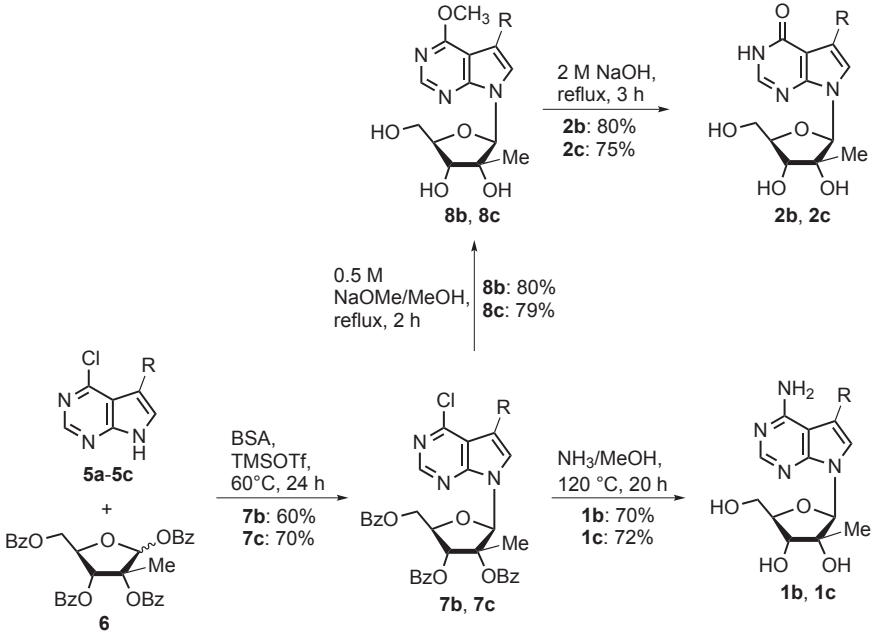
Synthesis of 7-Deazapurine 2′-C-Methylribonucleosides

Several strategies were developed for the synthesis of 7-deazapurine ribonucleosides[8]. Among them, the Silyl–Hilbert–Johnson reaction using a Lewis acid as catalyst (Vorbrüggen glycosylation[9]) performed in one step was established as a versatile protocol for the synthesis of a broad range of 7-deazapurine ribonucleosides[8]. Wolfe and co-workers used the "one-pot" protocol for the synthesis of pyrimidine and purine 2′-*C*-methylribonucleosides[10]. In their work, the heterocyclic moiety was silylated with BSA [*N*,*O*-bis(trimethylsilyl)acetamide] in MeCN and glycosylated with 2-*C*-methyl-1,2,3,5-tetra-*O*-benzoyl-D-ribofuranose (**6**) in the presence of $SnCl_4$ or trimethylsilyl trifluoromethanesulfonate (TMSOTf)[10]. Later, Ding et al. used a similar procedure for the synthesis of 2′-*C*-methyltoyocamycin and 7-functionalized 7-deaza-2′-*C*-methylinosine (MeCN, TMSOTf)[11]. However, all efforts to employ 6-chloro-7-deazapurine (**5a**) as nucleobase and the 2-*C*-methyl sugar donor **6** [10] in the "one-pot" glycosylation reaction failed[12]. A corresponding observation was made in the series of ribonucleosides by Seela and co-workers as well as by others[8,13]. On the contrary, in the laboratories of Merck, the nucleobase anion glycosylation (6-chloro-7-deazapurine (**5a**) as nucleobase and 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-1-*O*-methyl-α-D-ribofuranose as sugar component) was used to synthesize the 7-nonhalogenated 6-chloro-7-deazapurine 2′-*C*-methylribonucleoside as key intermediate[6]. Later it was found that the above mentioned nucleobase anion glycosylation reaction proceeds in fact *via* an α-epoxide intermediate as sugar donor[12].

As we envisaged, the synthesis of 7-halogenated 7-deazapurine 2′-*C*-methylribonucleosides, we intended to employ the 7-fluorinated nucleobase **5b** [14] or the corresponding 7-chloro derivative **5c** [15] together with the commercially available sugar precursor 1,2,3,5-tetra-*O*-benzoyl-2-*C*-methyl-D-ribofuranose[10] (**6**) in the glycosylation reaction. From our experiences in the synthesis of 7-halogenated 7-deazapurine ribonucleosides, we were confident that the "one-pot" glycosylation protocol (Vorbrüggen glycosylation) would proceed smoothly with the halogenated nucleobases **5b** and **5c**. It should be noted that the 7-fluorinated 7-deazapurine derivative **1b** was also prepared under Mitsunobu conditions (triphenylphosphine, diethyl azodicarboxylate in THF) employing **5b** as nucleobase and 2,3,5-tri-*O*-benzoyl-2-*C*-methyl-D-ribofuranose as sugar precursor[6]. However, under the reported conditions, compound **1b** was only obtained in low overall

yield of 5% [6]. The same authors reported the synthesis of the 7-chloro analogue **1c** from **1a** employing *N*-chlorosuccinimide to introduce the chloro substituent[6].

Our synthesis route towards 7-halogenated 7-deazapurine 2′-*C*-methyl-ribonucleosides (**1b**, **1c**, **2b**, **2c**, **8b**, **8c**) using the "one-pot" glycosylation protocol and Vorbrüggen conditions is summarized in Scheme 1. Compound **5b** was first silylated with BSA (1.2 equiv.) in anhydrous acetonitrile at room temperature. Then, the sugar **6** was added in portions together with TMSOTf as catalyst, and the reaction was allowed to proceed overnight at elevated temperature. It was found that under the acidic conditions due to TMSOTf and elevated temperature (60 °C), the sugar precursor **6** was easily decomposed when long reaction times were applied. Consequently, it was necessary to use an excess of **6** (totally 2.5 equiv.) which had to be added in portions to the reaction mixture. By this method, the protected glycosylation product **7b** was obtained in 60% yield; the formation of other isomers was not observed. Deprotection of **7b** in 0.5 M NaOMe/MeOH afforded the deblocked nucleoside **8b** with simultaneous displacement of the 6-chloro substituent by a 6-methoxy group.

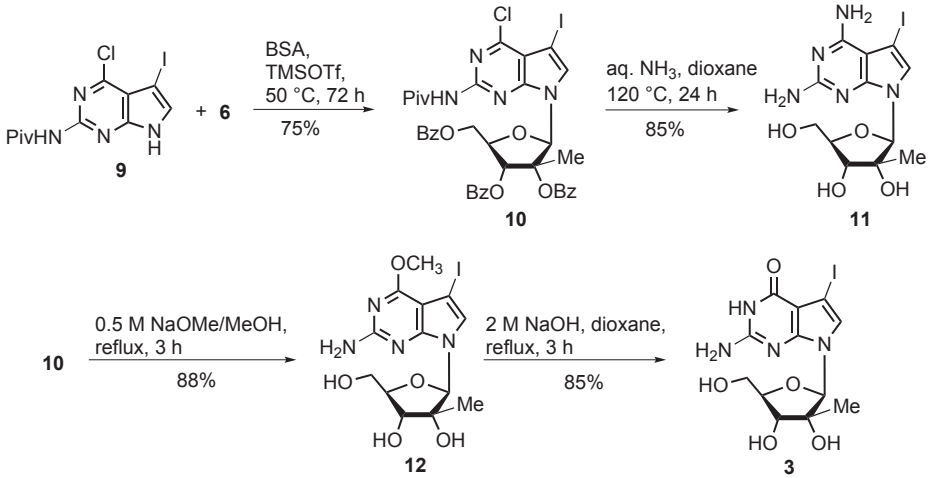


Scheme 1

Treatment of **8b** with 2 M aq. NaOH yielded 7-fluoro-7-deaza-2′-*C*-methylinosine (**2b**) in 80% yield. Treatment of compound **7b** with methanolic ammonia (autoclave) led to the deprotection of the sugar moiety and concomitant conversion of the chloro substituent to an amino group yielding 7-fluoro-7-deaza-2′-*C*-methyladenosine (**1b**) in a good 42% yield over two steps. Thus, the "one-pot" procedure reported herein represents a significant improvement over the earlier reported protocol employing Mitsunobu conditions[6] and allows the convenient synthesis of the HCV-active compound **1b** as well as its analogues **2b**, **7b** and **8b**.

By the same glycosylation strategy, the 7-chloro intermediate **7c** was obtained in even higher yield (70%). From the protected **7c**, the 7-chloro-6-methoxy compound **8c** (79%), the inosine derivative **2c** (75%) and the adenosine derivative **1c** (72%) were obtained in good yields. During all the reactions, the 7-fluoro or 7-chloro substituent was not affected.

In the series of halogenated 7-deaza-2′-*C*-methylguanosines, the 7-fluorinated derivative has been reported recently[16]. In our work, the "one-pot" method was applied to the synthesis of the 7-iodo-7-deaza-2′-*C*-methylguanosine **3**, the 6-methoxy analogue **12** and its 2,6-diamino derivative **11** (Scheme 2). For this, the silylated 2-pivaloylamino-6-chloro-7-iodo-7-deazapurine aglycon **9** [17] was employed in the glycosylation reaction together with the protected sugar **6** (TMSOTf, MeCN, 50 °C, 72 h) to give the fully protected nucleoside **10** in 75% yield. The protected compound **10** was transformed to the 2,6-diamino-7-iodo-7-deazapurine 2′-*C*-methylribonucleoside[18] (**11**) by treatment with aq. NH_3/dioxane at 120 °C

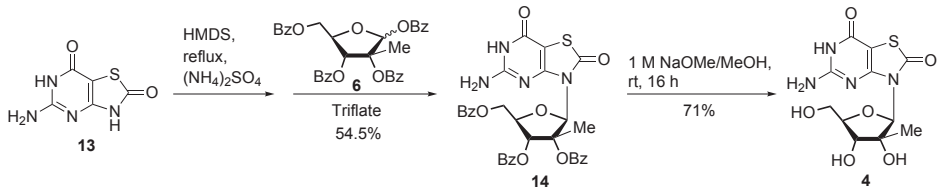


SCHEME 2

in a sealed vessel (85% yield), while the 6-methoxy compound **12** was obtained in 88% yield by refluxing **10** in 0.5 M NaOMe/MeOH. Nucleoside **12** was further converted (2 M NaOH, dioxane, reflux) to the 7-iodinated 2′-*C*-methylguanosine analogue **3** (85% yield).

Among the guanosine analogues, immunosine (7-thia-8-oxoguanosine, TOG, isatoribine) possesses *in vivo* activity against a variety of DNA and RNA viruses[19,20]. It was found that immunosine exhibits a stimulatory effect on both cellular and humoral components of the immune response. The observed antiviral effect has been attributed primarily to the induction of α-interferon[20,21]. In this regard, we became interested in synthesizing the 2′-*C*-methylribonucleoside derivative of immunosine (**4**). A patent[22] describes the synthesis of **4**, using the same glycosylation conditions reported by Wolfe et al.[10] (BSA as silylating agent, MeCN, glycosylation of **13** and **6** in the presence of $SnCl_4$). By this route, the 2′-*C*-methylribonucleoside **4** was obtained in 14% overall yield over two steps[22].

However, we favoured the "one-pot" glycosylation protocol as an improved synthesis strategy as shown in Scheme 3. The nucleobase **13** employed in the glycosylation reaction was prepared in 5 steps from 2,4-diamino-5-hydroxypyrimidine according to Baker and Chatfield[23]. Due to the high reactivity of the 5-aminothiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (systematic numbering) moiety (**13**) compared to the inertness of the 7-deazapurines in Lewis acid catalyzed glycosylation reactions, the glycosylation reaction was carried out at room temperature. For that, the immunosine base **13** was first silylated with hexamethyldisilazane (HMDS) in the presence of ammonium sulfate and pyridine under reflux. Then, the silylated **13** was reacted with 1 equiv. of the 2-*C*-methyl sugar **6** in the presence of TMSOTf (1.4 equiv.) at room temperature to give **14**. It was found that excessive sugar caused multiple glycosylations confirmed by NMR spectra which showed more than one set of sugar signals. During the nucleobase anion glycosylation of 2′-deoxyimmunosine a similar observation was made[24]. An excess of more than 1.3 equivalents of the 2′-deoxy sugar component led to the introduction of two sugar residues (one at each



SCHEME 3

lactam moiety). This was attributed to the two immunosine lactam deprotonation sites (pK_a values of 7.2 and 10.0)[24]. After work-up, the 2′-*C*-methyl compound **14** was obtained in 54.5% yield. The benzoylated **14** was deprotected with 1 M NaOMe/MeOH to afford the free nucleoside **4** in 71%. By our route, the overall yield of 2′-*C*-methylimmunosine (**4**) over two steps was improved to 37%.

Physical Properties of 7-Deazapurine 2′-C-Methylribonucleosides

All compounds were characterized by ^{1}H and ^{13}C NMR spectra and by DEPT-135 (distortionless enhancement by polarization transfer) NMR as well as by ESI-TOF mass spectrometry or elemental analysis (see Experimental). The ^{13}C NMR chemical shifts are compiled in Table I. The sugar carbons were assigned according to ref.[10], and the nucleobase carbons were assigned according to refs[13,17,24–26]. ^{19}F NMR spectra were taken for the fluorinated compounds **1b**, **2b**, **7b** and **8b**. The position of the fluorine substituent at C-7 or C-8 for 2′-*C*-methylribonucleosides **1b**, **2b**, **7b** and **8b** was assigned from *J*(F,C) coupling constants given in Table I (values in parentheses; purine numbering is used throughout discussion). *J*(F,C-7) has a value of about 245–250 Hz showing ^1J coupling, while the ^2J coupling constants of *J*(F,C-8) and *J*(F,C-5) show values of about 25 and 15 Hz, respectively. In some case, ^3J couplings with C4 and C6 were observed (*J*(F,C-4) and *J*(F,C-6) around 3 Hz).

*Single-Crystal X-ray Analysis of Compound **2b***

Studies conducted towards the structure-activity relationship of purine ribonucleosides as inhibitors of HCV replication showed that, the ribo conformation of the 2′-hydroxy group is of particular importance for the activity of nucleosides[4]. Moreover, effective inhibition seems to require a sensitive balance of the bulkiness of the substituent at C2′; between too little (2′-H) and too much (2′-*C*-ethyl) steric bulkiness. In this regard, the size of the 2′-*C*-methyl substituent appeared to be nearly optimal[4]. Indeed, 2′-*C*-methylribonucleosides demonstrated promising activity in cell-based HCV RNA replication assays. On the other hand, modification of the nucleobase appeared to be tolerated to some extent by the HCV RNA polymerase (HCV NS5B)[4,6].

For a series of nucleosides, the preferred sugar conformation in solution was calculated on the basis of ^{1}H NMR coupling constants ($J_{1'2'}$, $J_{2'3'}$, $J_{3'4'}$). However, this calculation method cannot be applied to 2′-*C*-methylribo-

TABLE I
^{13}C NMR chemical shifts (δ, ppm) of nucleosides and nucleoside precursors[a,d]

Compd	C(2)[b] / C(2)[c]	C(4)[b] / C(7a)[c]	C(5)[b] / C(4a)[c]	C(6)[b] / C(4)[c]	C(7)[b] / C(5)[c]	C(8)[b] / C(6)[c]	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	Me
5b[25]	151.1	146.8	105.4	148.5	139.7	111.2	–	–	–	–	–	–
5c[25]	151.1	150.5	112.6	149.9	101.6	126.1	–	–	–	–	–	–
7b	151.9	146.5	107.0 (14.4)	149.5 (3.8)	140.5 (249.7)	112.3 (26.9)	87.7	84.4	75.4	79.4	63.9	17.8
7c	151.7	149.8	113.6	150.6	103.5	126.4	87.9	84.3	75.3	79.3	63.9	17.8
1b	152.8	145.6	92.0 (15.7)	155.8	142.5 (244.9)	103.9 (26.8)	90.0	78.7	71.7	82.2	59.4	19.5
1c	152.7	148.6	99.5	156.8	102.3	118.6	90.3	78.6	71.5	82.2	59.2	19.6
8b	151.5	146.6 (2.6)	94.5 (15.3)	161.4 (2.9)	141.1 (246.6)	106.8 (27.4)	90.3	78.7	71.5	82.4	59.3	19.4
8c	151.6	149.8	102.4	162.2	102.4	121.3	90.7	78.7	71.4	82.4	59.2	19.6
2b	144.8	142.8	97.0 (12.9)	156.5 (2.7)	144.8 (245.5)	103.6 (27.2)	90.4	78.8	71.5	82.4	59.3	19.6
2c	145.0	146.0	106.1	157.1	104.6	117.9	90.7	78.7	71.3	82.3	59.1	19.6
9[17]	152.5[e]	151.4[e]	112.3	150.6[e]	51.7	132.7	–	–	–	–	–	–
10	152.0[e]	151.6[e]	113.1	151.7[e]	54.0	128.6[e]	88.7	84.8	75.9	79.0	64.3	18.1
11	159.8[e]	152.6[e]	96.3	157.5	51.8	122.8	89.8	78.6	71.8	81.8	59.4	19.9
12	159.4[e]	154.0[e]	98.5	162.8[e]	51.1	124.4	90.0	78.6	71.6	81.9	59.3	19.7
3	152.6[e]	150.3[e]	99.7	158.1[e]	54.5	122.1	90.1	78.6	71.6	81.9	59.2	19.8
13[24,b]	155.7	156.5	87.6	157.2	–	171.2	–	–	–	–	–	–
14[b]	153.6	152.3	87.5	156.1	–	169.0	88.1	86.1	76.9	77.8	64.0	17.7
4[b]	155.9	154.1	85.8	157.2	–	169.1	91.2	78.0	74.4	84.0	62.4	20.3

[a] Measured in DMSO-d_6 at 298 K. [b] Purine numbering. [c] Systematic numbering. [d] Values given in parentheses are coupling constants between C and F. [e] Tentative.

nucleosides. The 2′-*C*-methyl substituent excludes the calculation of the $J_{1'2'}$ and $J_{2'3'}$ coupling constants, leaving only $J_{3'4'}$ as a clue to the sugar puckering. For compound **2b**, the $J_{3'4'}$ value of 7.8 Hz, obtained from ^{1}H NMR measurements in D_2O, points to a predominant *N* sugar pucker[27].

To obtain additional information on the conformation, we determined the solid-state structure of compound **2b** by single-crystal X-ray crystallography. To the best of our knowledge, only three 2′-*C*-methylribonucleoside crystal structures have been reported in the literature so far. These are 2′-*C*-methyltubercidin (**1a**)[6], 2′-*C*-methylsangivamycin (**15**)[28] and 2′-*C*-methyluridine (**16**)[29,30] (Fig. 2).

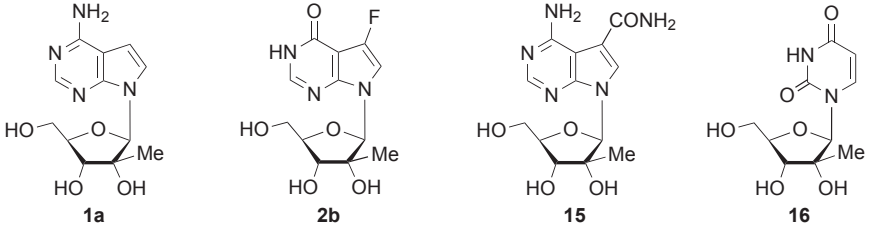


FIG. 2
2′-*C*-methylribonucleosides analyzed by single-crystal X-ray analysis

The crystal structure of 7-fluoro-7-deazainosine 2′-*C*-methylribonucleoside (**2b**) is shown in Fig. 3 and characteristic crystal data are summarized in Table II. In the crystal structure of **2b**, the glycosylic bond adopts a torsion angle of $\chi = -139.9(2)°$. According to the *syn/anti* definition of the torsion angle χ (O4′–C1′–N9–C4) referring to the orientation of the nucleobase relative to the sugar moiety[31], the glycosylic bond of **2b** is in the *anti*

TABLE II
Selected crystal data obtained from the single-crystal X-ray analysis of compound **2b**

Empirical formula	$C_{12}H_{14}FN_3O_5$	Unit cell dimensions	a = 4.8617(2) Å
			b = 16.0982(6) Å
			c = 16.8400(6) Å
Formula weight	299.26	Volume	V = 1317.98(9) Å^3
Crystal size	0.20 × 0.18 × 0.10 mm	Z value	Z = 4
Crystal system	orthorhombic	Calculated density	1.508 g cm^{-3}
Space group	$P2_12_12_1$		

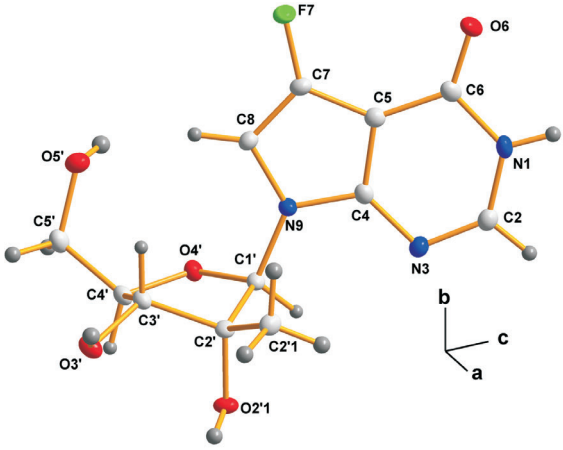


FIG. 3
ORTEP presentation of the single crystal X-ray structure of 7-fluoro-7-deaza-2′-*C*-methylinosine (**2b**), showing the crystallographic numbering scheme. Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as spheres of arbitrary size

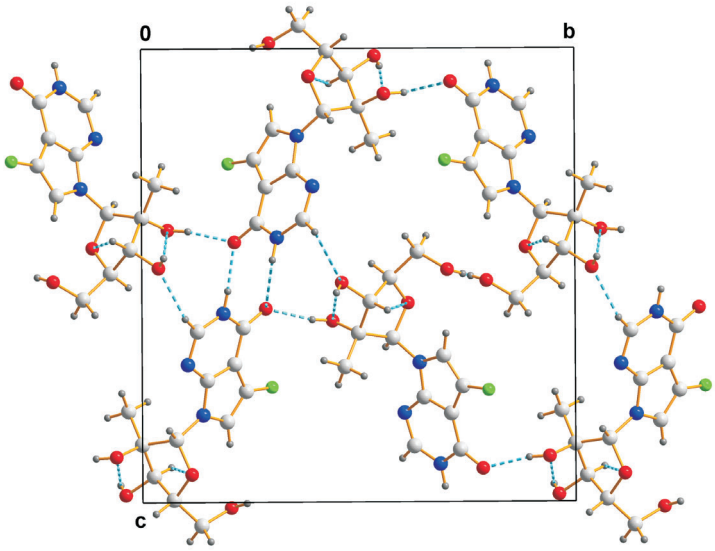


FIG. 4
The crystal packing of **2b**, showing the intermolecular hydrogen-bonding network (projection parallel to the *bc* plane)

conformation. Also, the 2′-*C*-methylribonucleosides **1a**, **15** and **16** show *anti* conformation[6,28–30]. The glycosylic bond length (N9–C1′) of **2b** is 1.462(2) Å, which is slightly longer than that of **1a** (1.460(2) Å)[6] but shorter than N9–C1′ of **16** (1.479(2) Å)[28–30].

The sugar moiety of compound **2b** shows an *N* conformation (unsymmetrical twist of C3′-*endo*–C2′-*exo*, $^3T_2$), with a pseudorotation phase angle of P = 4.3(1)° and a maximum puckering amplitude of τ_m = 42.4(1)°. This is in line with the *N* sugar conformations (C3′-*endo*) found for the reported 2′-*C*-methylribonucleosides **1a**, **15** and **16** [6,28–30] and is rational for 2′-*C*-methylribonucleosides, which have the preference to keep the bulky methyl group in the equatorial position[4]. The conformation around the C4′–C5′ bond of **2b** is +*sc* (*gauche*, *gauche*) with the torsion angle γ(O5′–C5′–C4′–C3′) = 65.2(2)°, which is similar to that of compound **16** (γ = 60.8°)[29].

In the solid state, the 7-deazapurine moiety of **2b** is nearly planar. The deviations of the ring atoms from the least-squares plane (N9/C8–C4/N3/C2/N1) are in the range of –0.0150 Å (atom C6) to 0.0118 Å (atom N1), with a rms deviation of 0.0090 Å. The 7-fluoro substituent and atom O6 lie 0.0089 and 0.0500 Å, respectively, below this plane.

The crystal structure of the 7-fluorinated 2′-*C*-methylribonucleoside **2b** is stabilized by three-dimensional hydrogen bond network involving the H atoms of the heteroatoms (N1–H1···O6^{i}, O2′1–H2′O···O6^{ii}, O3′–H3′O···O2′1^{iii}, O5′–H5′O···O5′^{iv}; for symmetry codes see Table III, Fig. 4). The fluoro substituent (F-7) does not take part in the hydrogen bonding as observed in other fluorinated compounds[32].

TABLE III
Hydrogen bond geometry of **2b**

D–H···A	D–H, Å	H···A, Å	D···A, Å	D–H···A, °
N1–H1···O6^{i}	0.88	1.91	2.792(2)	176.5
O2′1–H2′O···O6^{ii}	0.84	1.87	2.702(2)	168.7
O3′–H3′O···O2′1^{iii}	0.84	1.96	2.794(2)	169.9
O5′–H5′O···O5′^{iv}	0.84	2.06	2.863(1)	160.1

Symmetry codes: (i) x + 1/2, –y + 3/2, –z + 2; (ii) –x + 1, y – 1/2, –z + 3/2; (iii) x – 1, y, z; (iv) x + 1/2, –y + 3/2, –z + 1.

CONCLUSION

A series of 7-deazapurine 2′-*C*-methylribonucleosides carrying a 7-fluoro, 7-chloro and 7-iodo substituent were prepared employing a "one-pot" glycosylation protocol. The immunosine 2′-*C*-methylribonucleoside **4** was prepared by the same strategy. The "one-pot" procedure reported herein represents a significant improvement over the earlier reported protocol[6] for the synthesis of 7-fluoro-7-deaza-2′-*C*-methyladenosine (**1b**) and allows the convenient synthesis of the HCV-active compound **1b** as well as its 7-fluoro and 7-chloro analogues **1c**, **2b**, **2c**, **7b**, **7c**, **8b** and **8c**. Also, the 7-iodinated guanosine derivatives **3**, **11** and **12** as well as the 2′-*C*-methylimmunosine **4** were obtained in good yields. Moreover, from 7-fluoro-7-deaza-2′-*C*-methylinosine (**2b**), a single-crystal X-ray analysis was performed showing that the glycosylic bond has an *anti* conformation (χ = –139.9(2)°), while the sugar moiety adopts an *N* conformation (C3′-*endo*; P = 4.3(1)°, τ_m = 42.4(1)°).

EXPERIMENTAL

General

All chemicals are purchased from Acros, Fluka, or Sigma–Aldrich and were used without further purification. Thin layer chromatography (TLC) was performed on TLC aluminium sheets covered with silica gel 60 F_{254} (0.2 mm, VWR International). Flash chromatography (FC): silica gel 60 (40–60 μM, VWR International, Darmstadt, Germany) at 0.4 bar. Melting point (m.p.): Electrothermal 9200, uncorrected. UV-spectra were recorded on a U-3000 UV-Vis spectrophotometer (Hitachi, Japan); λ_{max} (ε) in nm, ε in l mol^{-1} cm^{-1}. NMR spectra: Avance DPX 250 spectrometer (Bruker, Germany) measured at 250.13 MHz for ^{1}H, 62.90 MHz for ^{13}C and 235.33 MHz for ^{19}F, and DPX 300 spectrometer (Bruker, Germany) measured at 300.15 MHz for ^{1}H, 75.48 MHz for ^{13}C and 282.40 MHz for ^{19}F. Chemical shifts are given in ppm (δ-scale) and coupling constants (*J*) in Hz. For NMR spectra recorded in DMSO, the chemical shift of the solvent peak was set to 2.50 ppm for ^{1}H and 39.50 ppm for ^{13}C. Elemental analyses were performed by the Mikroanalytisches Laboratorium Beller, Göttingen, Germany. ESI-TOF mass spectra were performed on a MicroTOF Bruker Daltonics mass spectrometer in the electropositive mode.

Single-Crystal X-ray Analysis of **2b**

The solid state structure of compound **2b**, $C_{12}H_{14}FN_3O_5$, was determined by single-crystal X-ray analysis. Crystals of **2b** were grown by slow evaporation from methanol. The obtained crystals of **2b** (colorless, block) were orthorhombic with the space group $P2_12_12_1$ and cell dimensions of a = 4.8617(2) Å, b = 16.0982(6) Å, c = 16.8400(6) Å, V = 1317.98(9) Å^3, and Z = 4. The calculated density was 1.508 g cm^{-3}.

For the diffraction experiment, a single-crystal was mounted on a MiTeGen MicroMountsfibre in a thin smear of oil. All diffraction measurements were made using

monochromatized MoKα radiation (λ = 0.71073 Å) on a CCD area detector equipped diffractometer (Bruker, Germany), at T = 100(2) K, with a θ range 1.75–28.52°. 1836 independent reflections out of 26.587 reflections were measured and 1655 reflections observed at the $I > 2\sigma(I)$ level (R_{int} = 0.0397).

The known configuration of the parent precursor was used to define the enantiomer employed in the refined model. In the absence of suitable anomalous scattering, Friedel equivalents could not be used to determine the absolute structure. Refinement of the Flack parameter[33] led to inconclusive values for this parameter [0.0 (8)]. Therefore, Friedel equivalents (1171) were merged before the final refinement. All H atoms were found in a difference Fourier synthesis. In order to maximize the data/parameter ratio, the H atoms were placed in geometrically idealized positions C–H = 0.95–1.00 Å and N–H = 0.88 Å (AFIX 93) and constrained to ride on their parent atoms with U_{iso}(H) = 1.2U_{eq}(C) = U_{eq}(N). The OH groups were refined as rigid groups allowed to rotate but not tip (AFIX 147) with O–H = 0.84 Å and U(H) = 1.5U_{eq}(O). The structure was refined using full-matrix least-squares on F^2 using 194 parameters and all independent reflections. The refinement converged with agreement statistics of R [$F^2 > 2\sigma(F^2)$] = 0.031, wR = (F^2) 0.077, S = 1.05.

CCDC 836029 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

4-Chloro-5-fluoro-7-(2,3,5-tri-*O*-benzoyl-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**7b**)

4-Chloro-5-fluoro-7*H*-pyrrolo[2,3-*d*]pyrimidine[14] (**5b**; 343 mg, 2 mmol) was suspended in dry acetonitrile (15 ml) and *N*,*O*-bis(trimethylsilyl)acetamide (BSA; 0.6 ml, 2.42 mmol) was added to the mixture. The mixture became clear, and after 10 min 1,2,3,5-tetra-*O*-benzoyl-2-*C*-methyl-D-ribofuranose[10] (**6**; 1.74 g, 3 mmol) and trimethylsilyl trifluoromethanesulfonate (TMSOTf; 0.54 ml, 2.80 mmol) were added. The mixture was stirred at r.t. for 10 min, and then stirring was continued at 60 °C. Another portion of compound **6** (1.16 g, 2 mmol) and TMSOTf (0.34 ml, 1.76 mmol) was added after 6 h. The reaction mixture was stirred overnight. The mixture was diluted with dichloromethane (100 ml) and washed with 5% aq. $NaHCO_3$. The organic layer was dried (Na_2SO_4) and filtered. The filtrate was evaporated to dryness and the residue was applied to FC (silica gel, column 5 × 15 cm, eluted with CH_2Cl_2). The main zone afforded **7b** as a colorless foam (0.75 g, 60%). TLC (silica gel, CH_2Cl_2): R_F 0.25. UV (MeOH): λ_{max} (ε) = 227 (48 800), 274 (5 400), 282 (5 000). ^{1}H NMR (250 MHz, DMSO-d_6): 1.59 s, 3 H (CH_3); 4.73–4.86 m, 3 H (H-4′, 2 × H-5′); 5.89–5.90 m, 1 H (H-3′); 7.04 s, 1 H (H-1′); 7.31–8.04 m, 15 H (H-arom); 8.12 d, 1 H, J(C,F) = 1.75 (H-6); 8.88 s, 1 H (H-2). ^{19}F NMR (235 MHz, DMSO-d_6): –169.24 (F-5). MS ESI-TOF calculated for $C_{33}H_{25}ClFN_3O_7Na$ $[M + Na]^+$: 652.1263; found: 652.1262.

4,5-Dichloro-7-(2,3,5-tri-*O*-benzoyl-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**7c**)

4,5-Dichloro-7*H*-pyrrolo[2,3-*d*]pyrimidine[15] (**5c**; 200 mg, 1.06 mmol) was suspended in dry acetonitrile (10 ml) and *N*,*O*-bis(trimethylsilyl)acetamide (BSA; 0.42 ml, 1.70 mmol) was added to the mixture. The mixture became clear and after 10 min 1,2,3,5-tetra-*O*-benzoyl-2-*C*-methyl-D-ribofuranose[10] (**6**; 1.0 g, 1.72 mmol) and trimethylsilyl trifluoromethane-

sulfonate (TMSOTf; 0.3 ml, 1.6 mmol) were added. The mixture was stirred at 60 °C. After 5 h, compound **6** (800 mg, 1.38 mmol) and TMSOTf (0.23 ml, 1.19 mmol) were added. The reaction mixture was stirred overnight. The mixture was diluted with dichloromethane (50 ml) and washed with 5% aq. $NaHCO_3$. The organic layer was dried (Na_2SO_4) and filtered. The filtrate was evaporated to dryness, and the residue was applied to FC (silica gel, column 5 × 15 cm, eluted with CH_2Cl_2) affording **7c** as a colorless foam (0.48 g, 70%). TLC (silica gel, CH_2Cl_2): R_F 0.26. UV (MeOH): λ_{max} (ε) = 230 (26 300), 275 (2 500), 282 (2 300). ^{1}H NMR (300 MHz, DMSO-d_6): 1.58 s, 3 H (CH_3); 4.77–4.87 m, 3 H (H-4′, 2 × H-5′); 5.92–5.93 m, 1 H (H-3′); 7.03 s, 1 H (H-1′); 7.37–8.04 m, 15 H (H-arom); 8.29 s, 1 H (H-6); 8.88 s, 1 H (H-2). MS ESI-TOF calculated for $C_{33}H_{25}Cl_2N_3O_7Na$ $[M + Na]^+$: 668.0967; found: 668.0960.

5-Fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (**8b**)

Compound **7b** (630 mg, 1 mmol) was suspended in 0.5 M NaOMe/MeOH (50 ml), and the mixture was refluxed for 2 h. Then, the solvent was evaporated and the residue was adsorbed on silica gel and applied to FC (silica gel, column 8 × 5 cm, eluted with CH_2Cl_2/MeOH, 30:1→20:1). The main zone afforded **8b** as a colourless solid. Recrystallization from CH_2Cl_2 gave **8b** as colourless crystals (250 mg, 80%). M.p. 102 °C. TLC (silica gel, CH_2Cl_2/MeOH, 10:1): R_F 0.34. UV (MeOH): λ_{max} (ε) = 221 (19 000), 261 (sh, 4 700), 280 (5 700). ^{1}H NMR (300 MHz, DMSO-d_6): 0.67 s, 3 H (CH_3); 3.64–3.70 2 m, 1 H (H-5′b); 3.81–3.88 m, 2 H (H-5′a, H-4′); 3.92–3.97 m, 1 H (H-3′); 4.06 s, 3 H (OMe); 5.17 d, 1 H, J(3′,OH) = 6.30 (3′-OH); 5.20 s, 1 H (2′-OH); 5.23 t, 1 H, J(5′,OH) = 4.80 (5′-OH); 6.25 d, 1 H, J(H,F) = 1.5 (H-1′); 7.79 d, 1 H, J(H,F) = 1.8 (H-6); 8.44 s, 1 H (H-2). ^{19}F NMR (282 MHz, DMSO-d_6): –167.73 (F-5). MS ESI-TOF calculated for $C_{13}H_{16}FN_3O_5Na$ $[M + Na]^+$: 336.0966; found: 336.0964.

5-Chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (**8c**)

Compound **7c** (323 mg, 0.5 mmol) was suspended in 0.5 M NaOMe/MeOH (25 ml), and the mixture was refluxed for 2 h. Then, the mixture was evaporated, and the residue was adsorbed on silica gel and applied to the top of a column (silica gel, column 8 × 5 cm, eluted with CH_2Cl_2/MeOH, 30:1→20:1). The main zone afforded **8c** as colourless solid. Recrystallization from CH_2Cl_2 gave **8c** as colourless crystals (130 mg, 79%). M.p. 98 °C. TLC (silica gel, CH_2Cl_2/MeOH, 10:1): R_F 0.36. UV (MeOH): λ_{max} (ε) = 222 (18 300), 261 (sh, 4 200), 281 (4 900). ^{1}H NMR (300 MHz, DMSO-d_6): 0.68 s, 3 H (CH_3); 3.64–3.71 2 m, 1 H (H-5′b); 3.82–3.89 m, 2 H (H-5′a, H-4′); 3.94–3.99 m, 1 H (H-3′); 4.06 s, 3 H (OMe); 5.18 d, 1 H, J(3′,OH) = 6.30 (3′-OH); 5.22 s, 1 H (2′-OH); 5.26 t, 1 H, J(5′,OH) = 4.80 (5′-OH); 6.21 s, 1 H (H-1′); 7.99 s, 1 H (H-6); 8.47 s, 1 H (H-2). MS ESI-TOF calculated for $C_{13}H_{16}ClN_3O_5Na$ $[M + Na]^+$: 352.0676; found: 352.0682.

5-Fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (**2b**)

A solution of **8b** (156 mg, 0.5 mmol) in 2 M NaOH (3 ml) was heated under reflux for 3 h. The mixture was cooled, diluted with water (15 ml) and neutralized with 1 M AcOH to pH 7.

The solution was filtered, and the filtrate was applied to a column (15 × 2 cm, Serdolit AD-4, resin 0.1–0.2 mm). The column was washed with H_2O (200 ml), and the product was eluted with H_2O/*i*-PrOH (95:1, 300 ml). The product containing fractions were collected, and the solvent was evaporated yielding **2b** as a colorless solid. Recrystallization from methanol afforded **2b** as colorless needles (120 mg, 80%). M.p. 269 °C. TLC (silica gel, CH_2Cl_2/MeOH, 10:1): R_F 0.10. UV (MeOH): λ_{max} (ε) = 216 (21 600), 264 (8 200). ^{1}H NMR (250 MHz, DMSO-d_6): 0.71 s, 3 H (CH_3); 3.63–3.67 m, 1 H (H-5′b); 3.80–3.97 m, 3 H (H-5′a, H-4′, H-3′); 5.20 br s, 3 H (2′-OH, 3′-OH, 5′-OH); 6.10 d, 1 H, *J*(H,F) = 1.63 (H-1′); 7.49 d, 1 H, *J*(H,F) = 1.63 (H-6); 7.93 s, 1 H (H-2). ^{19}F NMR (235 MHz, DMSO-d_6): –166.13 (F-5). MS ESI-TOF calculated for $C_{12}H_{14}FN_3O_5Na$ $[M + Na]^+$: 322.0815; found: 322.0808.

5-Chloro-3,7-dihydro-7-(2-*C*-methyl-β-D-ribofuranosyl)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (**2c**)

A solution of **8c** (70 mg, 0.21 mmol) in 2 M NaOH (3 ml) was heated under reflux for 3 h. The mixture was cooled, diluted with water (10 ml) and neutralized with 1 M HCl to pH 7. The solution was evaporated to dryness, and the residue was applied to FC (silica gel, eluted with CH_2Cl_2/MeOH, 10:1→5:1). Compound **2c** was obtained as a colorless solid. Recrystallization from methanol afforded **2c** as colourless needles (50 mg, 75%). M.p. 250 °C (dec.). TLC (silica gel, CH_2Cl_2/MeOH, 10:1): R_F 0.11. UV (MeOH): λ_{max} (ε) = 218 (15 600), 264 (6 700). ^{1}H NMR (300 MHz, DMSO-d_6): 0.71 s, 3 H (CH_3); 3.62–3.67 2 m, 1 H (H-5′b); 3.80–3.94 m, 3 H (H-5′a, H-4′, H-3′); 5.15 d, 1 H, *J*(3′,OH) = 3.60 (3′-OH); 5.18 s, 1 H (2′-OH); 5.21 t, 1 H, *J*(5′,OH) = 4.65 (5′-OH); 6.06 s, 1 H (H-1′); 7.68 s, 1 H (H-6); 7.94 s, 1 H (H-2); 12.12 br s, 1 H (NH). MS ESI-TOF calculated for $C_{12}H_{14}ClN_3O_5Na$ $[M + Na]^+$: 338.0520; found: 338.0523.

4-Amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**1b**)

Compound **7b** (900 mg, 1.43 mmol) was suspended in methanolic ammonia (saturated at 0 °C, 80 ml) and stirred at 120 °C for 20 h. The mixture was evaporated to dryness and the residue was applied to FC (silica gel, column 5 × 8 cm, eluted with CH_2Cl_2/MeOH, 50:1→10:1) yielding **1b** as a colorless solid. Compound **1b** [6] was crystallized from methanol affording **1b** as colorless crystals (300 mg, 70%). M.p. 219 °C. TLC (silica gel, CH_2Cl_2/CH_3OH, 10:1): R_F 0.13. UV (MeOH): λ_{max} (ε) = 211 (23 600), 281 (8 600). ^{1}H NMR (250 MHz, DMSO-d_6): 0.68 s, 3 H (CH_3); 3.62–3.68 m, 1 H (H-5′b); 3.80–3.83 m, 2 H (H-5′a, H-4′); 3.90–3.93 m, 1 H (H-3′); 5.10–5.17 m, 3 H (2′-OH, 3′-OH, 5′-OH); 6.16 d, 1 H, *J*(H,F) = 1.65 (H-1′); 7.00 br s, 2 H (NH_2); 7.49 d, 1 H, *J*(H,F) = 1.62 (H-6); 8.07 s, 1 H (H-2). ^{19}F NMR (235 MHz, DMSO-d_6): –168.00 (F-5). MS ESI-TOF calculated for $C_{12}H_{15}FN_4O_4Na$ $[M + Na]^+$: 321.0970; found: 321.0971.

4-Amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**1c**)

Compound **1c** was prepared as described for **1b** with **7c** (200 mg, 0.31 mmol). After FC (silica gel, eluted with CH_2Cl_2/MeOH, 50:1→10:1), **1c** [6] was obtained as a colorless solid (70 mg, 72%). M.p. 153 °C (dec.). TLC (silica gel, CH_2Cl_2/MeOH, 10:1): R_F 0.15. UV (MeOH): λ_{max} (ε) = 210 (20 300), 281 (7 700). ^{1}H NMR (300 MHz, DMSO-d_6): 0.69 s, 3 H (CH_3);

3.63–3.68 2 m, 1 H (H-5′b); 3.80–3.85 m, 2 H (H-5′a, H-4′); 3.92 d, 1 H, *J*(3′,OH) = 6.88 (H-3′); 5.12 d, 1 H, *J*(3′,OH) = 6.76 (3′-OH); 5.14 s, 1 H (2′-OH); 5.20 t, 1 H, *J*(5′,OH) = 4.62 (5′-OH); 6.13 s, 1 H (H-1′); 6.87 br s, 2 H (NH_2); 7.74 s, 1 H (H-6); 8.11 s, 1 H (H-2). MS ESI-TOF calculated for $C_{12}H_{15}ClN_4O_4Na$ $[M + Na]^+$: 337.0674; found: 337.0682.

4-Chloro-5-iodo-2-pivaloylamino-7-[(2,3,5-tri-*O*-benzoyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (**10**)

Into a stirred suspension of 4-chloro-5-iodo-2-pivaloylamino-7*H*-pyrrolo[2,3-*d*]pyrimidine[17] (**9**; 757 mg, 2.0 mmol) in anhydrous MeCN (14 ml), *N*,*O*-bis(trimethylsilyl)acetamide (BSA; 1.0 ml, 4.04 mmol) was added at room temperature. After stirring for 5 min, trimethylsilyl trifluoromethanesulphonate (TMSOTf; 0.75 ml, 3.9 mmol) was added and 1,2,3,5-tetra-*O*-benzoyl-2-*C*-methyl-D-ribofuranose[10] (**6**; 3.02 g, 5.2 mmol) was introduced in three portions (once per 24 h). In total, the reaction was stirred at 50 °C for 72 h, cooled to room temperature, and diluted with CH_2Cl_2 (80 ml). The solution was washed with aq. saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated under reduced pressure to give a syrup, which was applied to FC (silica gel, column 4 × 12 cm, eluted with CH_2Cl_2/petroleum ether, 2:1→100:0). The main zone afforded compound **10** as a yellowish foam (1.26 g, 75%). TLC (silica gel, CH_2Cl_2/MeOH, 99:1): R_F 0.44. ^{1}H NMR (300 MHz, DMSO-d_6): 1.27 s, 9 H (3 × CH_3); 1.66 s, 3 H (CH_3); 4.74–4.85 m, 3 H (H-4′, 2 × H-5′); 6.15 br s, 1 H (H-3′); 6.87 s, 1 H (H-1′); 7.36–8.05 3 m, 15 H (arom-H); 8.08 s, 1 H (H-6); 10.42 s, 1 H (NH). For $C_{38}H_{34}ClIN_4O_8$ (837.06) calculated: 54.53% C, 4.09% H, 6.69% N; found: 54.63% C, 4.15% H, 6.54% N.

5-Iodo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2,4-diamine (**11**)

A suspension of **10** (418 mg, 0.50 mmol) in dioxane (30 ml) and 25% aq. NH_3 (70 ml) was introduced into an autoclave and stirred at 120 °C for 24 h. The reaction mixture was evaporated under reduced pressure to give a syrup, which was adsorbed on silica gel, and applied on the top of a column (silica gel, column 3 × 8 cm, CH_2Cl_2/MeOH, 95:5). Fractions containing compound **11** were collected and evaporated to dryness to give **11** as a yellowish solid (179 mg, 85%). TLC (silica gel, CH_2Cl_2/MeOH, 9:1): R_F 0.17. UV (MeOH): λ_{max} (ε) = 232 (31 100), 270 (8 500), 289 (7 600). ^{1}H NMR (300 MHz, DMSO-d_6): 0.73 s, 3 H (CH_3); 3.57–3.64 2 m, 1 H (H-5′b); 3.76–3.81 m, 2 H (H-5′a, H-4′); 3.90 dd, 1 H, *J*(3′,OH) = 6.60, *J*(3′,4′) = 8.70 (H-3′); 4.88 s, 1 H (2′-OH); 5.11 t, 1 H, *J*(5′,OH) = 4.80 (5′-OH); 5.16 d, 1 H, *J*(3′,OH) = 6.60 (3′-OH); 5.85 br s, 2 H (NH_2); 5.95 s, 1 H (H-1′); 6.19 s, 2 H (NH_2); 7.34 s, 1 H (H-6). For $C_{12}H_{16}IN_5O_4$ (421.19) calculated: 34.22% C, 3.83% H, 16.63% N; found: 34.19% C, 3.90% H, 16.45% N.

2-Amino-5-iodo-4-methoxy-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**12**)

A solution of **10** (837 mg, 1.0 mmol) in 0.5 M NaOMe/MeOH (20 ml) was heated under reflux for 3 h. The reaction mixture was neutralized with glacial acetic acid, the crude product was adsorbed on silica gel, and applied on the top of a column (silica gel, column 3 × 8 cm, CH_2Cl_2/MeOH, 95:5). Fractions containing compound **12** were collected and evaporated to dryness to give **12** as a colorless solid (384 mg, 88%). TLC (silica gel, CH_2Cl_2/MeOH, 9:1): R_F 0.36. UV (MeOH): λ_{max} (ε) = 234 (29 700), 267 (6 500), 289 (6 200). ^{1}H NMR (250 MHz,

DMSO-d_6): 0.71 s, 3 H (CH_3); 3.64–3.80 2 m, 4 H (2 × H-5′, H-4′, H-3′); 3.92 s, 3 H (OMe); 4.92 s, 1 H (2′-OH); 5.13–5.19 m, 2 H (5′-OH, 3′-OH); 5.98 s, 1 H (H-1′); 6.40 s, 2 H (NH_2); 7.45 s, 1 H (H-6). For $C_{13}H_{17}IN_4O_5$ (436.20) calculated: 35.80% C, 3.93% H, 12.84% N; found: 35.69% C, 3.83% H, 12.63% N.

2-Amino-5-iodo-7-(2-*C*-methyl-β-D-ribofuranosyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (**3**)

Compound **12** (174 mg, 0.40 mmol) was dissolved in 2 M NaOH (40 ml) and 1,4-dioxane (6 ml). The mixture was stirred under reflux for 3 h. After neutralization with 2 M HCl, the volume was reduced by 50%. The solution was applied to a Serdolit AD-4 column (3 × 12 cm, resin 0.1–0.2 mm; Serva, Germany). Salt was removed by elution with H_2O (150 ml), and the product was eluted with H_2O/*i*-PrOH (9:1→5:1). Fractions containing compound **3** were combined, and the solvent was evaporated to afford **3** as a colorless solid (144 mg, 85%). TLC (silica gel, CH_2Cl_2/MeOH, 5:1): R_F 0.33. UV (MeOH): λ_{max} (ε) = 227 (18 600), 267 (10 500), 289 (6 600). ^{1}H NMR (300 MHz, DMSO-d_6): 0.74 s, 3 H (CH_3); 3.57–3.64 2 m, 1 H (H-5′b); 3.75–3.81 m, 2 H (H-5′a, H-4′); 3.88 dd, 1 H, *J*(3′,OH) = 6.15, *J*(3′,4′) = 8.85 (H-3′); 4.87 s, 1 H (2′-OH); 5.10 t, 1 H, *J*(5′,OH) = 4.80 (5′-OH); 5.19 d, 1 H, *J*(3′,OH) = 6.30 (3′-OH); 5.86 s, 1 H (H-1′); 6.35 br s, 2 H (NH_2); 7.28 s, 1 H (H-6); 10.44 s, 1 H (NH). For $C_{12}H_{15}IN_4O_5$ (422.18) calculated: 34.14% C, 3.58% H, 13.27% N; found: 34.26% C, 3.69% H, 13.20% N.

5-Amino-3-(2,3,5-tri-*O*-benzoyl-2-*C*-methyl-β-D-ribofuranosyl)-thiazolo[4,5-*d*]pyrimidin-2,7(3*H*,6*H*)-dione (**14**)

A mixture of dry 5-aminothiazolo[4,5-*d*]pyrimidin-2,7(3*H*,6*H*)-dione[23] (**13**; 184 mg, 1.0 mmol), hexamethyldisilazane (HMDS; 3.3 ml), ammonium sulfate (2 mg), and pyridine (0.33 ml) was heated under reflux overnight with the exclusion of moisture. Excess HMDS was removed by evaporation to provide the silylated derivative as a syrup. To the silylated intermediate were added dry acetonitrile (10 ml) and 1,2,3,5-tetra-*O*-benzoyl-2-*C*-methyl-D-ribofuranose[10] (**6**; 580 mg, 1.0 mmol). To the formed suspension, trimethylsilyl trifluoromethanesulfonate (0.31 ml, 1.4 mmol) was added. The suspension became a clear solution, and the reaction mixture was stirred at ambient temperature for 16 h. The solution was evaporated to dryness, and the residual syrup was dissolved in EtOAc (30 ml). The solution was washed with 5% aq. $NaHCO_3$ solution (2 × 10 ml), and dried (Na_2SO_4). The solvent was evaporated to yield a syrup. The syrup was dissolved in dichloromethane (2 ml) and applied to the top of a column (silica gel, 12 × 2.5 cm, eluted with CH_2Cl_2/EtOAc, 4:1→2:1). Compound **14**[22] (350 mg, 54.5%) was obtained as a colorless foam. TLC (silica gel, CH_2Cl_2/MeOH, 10:1): R_F 0.45. NMR data in analogy to ref.[26]. ^{1}H NMR (300 MHz, DMSO-d_6): 1.80 s, 3 H (CH_3); 4.55–4.60 m, 2 H (2 × H-5′); 4.76–4.82 m, 1 H (H-4′); 6.13–6.27 m, 1 H (H-3′); 6.68 s, 1 H (H-1′); 7.06 br s, 2 H (NH_2); 7.40–8.09 m, 15 H (H-arom); 11.40 br s, 1 H (NH). MS ESI-TOF calculated for $C_{32}H_{26}N_4O_9SNa$ $[M + Na]^+$: 665.1313; found: 665.1326.

5-Amino-3-(2-*C*-methyl-β-D-ribofuranosyl)-thiazolo[4,5-*d*]pyrimidin-2,7(3*H*,6*H*)-dione (**4**)

To a suspension of **14** (220 mg, 0.34 mmol) in methanol (2.6 ml), 1 M NaOMe/MeOH (1.75 ml) was added. The mixture became clear and was stirred at r.t. for 16 h. The reaction mixture was neutralized with Dowex-50$^+$ resin and filtered. The filtrate was evaporated to

dryness. The residue was triturated with ether (2 × 2.5 ml) to yield **4** [22] as a slight yellow solid (80 mg, 71%). NMR data in analogy to ref.[26]. ^{1}H NMR (300 MHz, DMSO-d_6): 1.05 s, 3 H (CH_3); 3.60 br s, 2 H (2 × H-5′); 3.74–3.80 m, 1 H (H-4′); 3.96 br s, 1 H (H-3′); 4.60 s, 1 H (3′-OH); 4.80 s, 1 H (2′-OH); 5.25 br s, 1 H (5′-OH); 5.98 s, 1 H (H-1′); 7.08 br s, 2 H (NH_2); 11.02 br s, 1 H (NH). MS ESI-TOF calculated for $C_{11}H_{14}N_4O_6SNa$ $[M + Na]^+$: 353.0526; found: 353.0541.

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