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Note

Glycosylation of Pyrrolo[2,3-d]pyrimidines with 1-O-Acetyl-2,3,5-tri-O-benzoyl-#-D-ribofuranose: Substituents and Protecting Groups Effecting the Synthesis of 7-Deazapurine Ribonucleosides

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Glycosylation of Pyrrolo[2,3-*d*]pyrimidines with 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose: Substituents and Protecting Groups Effecting the Synthesis of 7-Deazapurine Ribonucleosides

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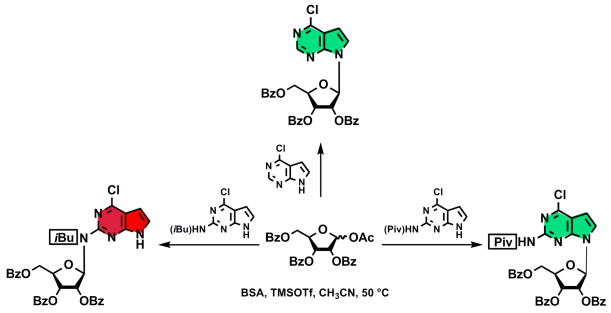


ABSTRACT

Glycosylation of non-functionalized 6-chloro-7-deazapurine with commercial available 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (45% yield) followed by amination and deprotection gave tubercidin in only two steps. Similar conditions applied for the synthesis of 7-deazaguanosine employing pivaloylated 2-amino-6-chloro-7-deazapurine gave 18% glycosylation yield. Less bulky isobutyryl or acetyl protected amino group directed the glycosylation toward the exocyclic amino substituent. 7-Halogenated intermediates were glycosylated followed by dehalogenation to overcome the low glycosylation yield in the synthesis of 7-deazaguanosine.

Pyrrolo[2,3-*d*]pyrimidine (7-deazapurines) are naturally occurring and have been isolated as monomeric nucleosides as well as constituents of nucleic acids.[1,2] Most of them are ribonucleosides such as tubercidin (**1a**) and its 7-substituted derivatives toyocamycin (**1b**) and sangivamycin (**1c**) (Figure 1) (if not otherwise stated purine numbering is used throughout the manuscript and systematic numbering in the Experimental Part). Other nucleosides such as 5'-deoxy-7-iodotubercidin have been discovered from marine organisms and can carry halogens in the 7-position.[3] Queuosine (**2b**) or archaeosine (**2c**) and related derivatives (**2d**) represent 7-subsituted 7-deazaguanine ribonucleosides, which are found in tRNA and formed by post-modification of ribonucleic acids *via* transglycosylation.[4] Dapiramicin A (**3**) represents a nucleoside related molecule with an unusual glycosylation site. Here, the sugar moiety is linked to the amino group of the 7-deazapurine base.[5]

As the shape of the 7-deazapurine nucleosides closely resembles that of purine nucleosides they have been incorporated in DNA and RNA by polymerases using triphosphates or in oligonucleotides by solid phase synthesis employing phosphoramidite chemistry.[6,7] 7-Deaza-2'-deoxyguanosine triphosphate replaced dGTP in the Sanger dideoxy sequencing and the pyrrolo[2,3-*d*]pyrimidine skeleton was used to introduce fluorescence dyes in chain terminators for conventional sequencing.[8] Also sequencing by synthesis makes use of 7-deazapurine nucleotides.[9]

A series of restriction enzymes inhibit DNA cleavage when purine bases were replaced by 7-deazapurines. Whereas many of them accepted 7-deazaadenosine in place of dA only a few could cleave sequences containing 7-deazaguanosine.[6a,b] Similar results observed on the same and on other restriction enzymes were reported more recently.[10a] Furthermore, poly(7-deazaguanylic) acid can code for poly (lysine) in a ribosomal translation assay.[10b] The literature of 7-deazapurine nucleoside synthesis and their occurrence has been reviewed.[11]

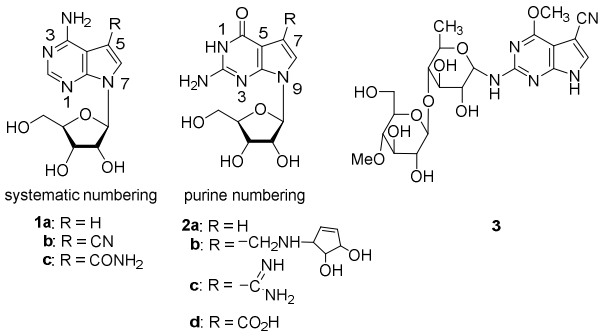


Figure 1. Naturally occurring 7-deazapurine nucleosides.

Various protocols have been developed for purine or pyrimidine nucleoside synthesis and were applied to 7-deazapurine nucleosides.[12] For 7-deazapurine 2'-deoxyribonucleosides the most efficient synthesis protocol uses nucleobase anion glycosylation.[13] The method developed in our laboratory, makes use of the increased reactivity of the pyrrolyl anion and proceeds in the stereo- and regioselective formation of *β*-D-2'-deoxyribonucleosides. This protocol was later utilized to a number of base-modified 2'-deoxyribonucleosides and is now the method of choice for their synthesis.[17a] However, the procedure could not be applied for the synthesis of ribonucleosides as long as acyl protecting groups are present at the 2-position of the sugar moiety. The use of common ribosugars such as 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (**4**) resulted in the undesired formation of orthoamides when the pyrrole ring was not functionalized.[14] The problem was not observed when benzyl protecting groups were employed, which do not participate in neighbour group interactions.[15] A stereocontrolled 7-deazapurine glycosylation was reported by Robins and Revankar using 5-TBDMS-2,3-isopropylidene protected ribofuranosyl chloride (Wilcox sugar) for the synthesis of *β*-D-ribonucleosides.[16a] Our laboratory prepared *β*-D and *α*-D-7-deazapurine ribonucleosides employing nucleobase anion glycosylation[16b,c] These protocols are now widely used for the

synthesis of base modified ribonucleosides. In some cases solvent and temperature were changed.[11b,16d-f] Although glycosylation with this sugar halide is high yielding the protocol is laborious.

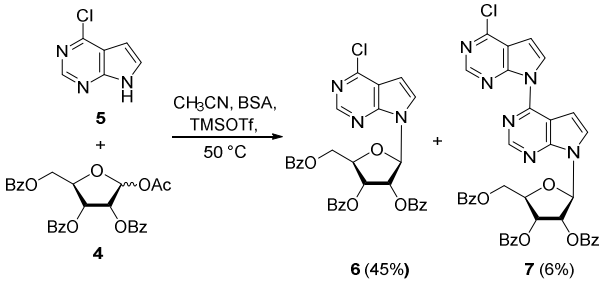
Later, we found that the commonly used 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (**4**) can be employed in the glycosylation of 7-deazapurines when 7-halogenated nucleobases are used.[17] We showed that 7-functionalized 7-deazapurine nucleosides can be synthesized in excellent yields in a stereo- and regio-controlled way when a one pot glycosylation reaction without isolation of the silylated 7-deazapurine bases is used and Vorbrüggen conditions are employed.[17] The 7-iodo derivatives open us the way to introduce clickable side chains in nucleosides and oligonucleotides. However, we failed to apply this route to non-functionalized 6-chloro-7-deazapurine[18] and 2-amino-6-chloro-7-deazapurine[19], the most suitable starting materials for the syntheses of tubercidin[20] or 7-deazaguanosine[21]. The reason is the reduced reactivity of the pyrrole nitrogen, which is increased by 7-halogen substituents making the system more purine like. Furthermore, glycosylation of pyrrole carbons of 7-deazaguanine can take place as shown by $SnCl_4$ promoted reactions.[22] As methylation experiments on the free 7-deazaguanine base demonstrated that under alkaline conditions the most reactive site is the lactam nitrogen. This position is also the privileged site for glycosylation.[23] Consequently we studied the trimethylsilyl trifluoromethanesulfonate (TMSOTf) promoted reaction in more detail and characterized side products effecting the glycosylation reactions.

The synthesis protocol for the glycosylation of 6-chloro-7-deazapurine starts with the silylation of the nucleobase with *N*,*O*-bis(trimethylsilyl)acetamide (BSA), followed by the addition of the sugar **4** and TMSOTf in a one-pot reaction. To this end, the reaction temperature was altered, the ratio of sugar *vs* nucleobase was changed and the reaction times

were varied. In more detail, the glycosylation of 6-chloro-7-deazapurine (**5**) with the ribosugar **4** proceeded most efficiently when the reaction time was limited to 16 h and the temperature to 50 °C (Scheme 1). The sugar *vs* base ratio was 2.5:1. Side products were removed after chromatographical work-up and could be easily separated as they showed completely different mobilities as the glycosylation product **6**. Applying these conditions the glycosylation yield was 45% (for details see the Experimental Section). Earlier studies without unambiguous assignment of nucleoside structure (**6**) used mercury oxide catalyzed glycosylation (18% yield).[23d] Previously, anhydro sugars have been used for the synthesis of pyrimidine ribonucleosides or those with an altered ribose moiety.[24a,b] Later, an anhydrosugar was used for the synthesis of the 7-deazapurine nucleoside **6** (49% glycosylation yield).[24c] However, in our work the standard sugar for ribonucleoside synthesis is used. As we show later only one more step is necessary to access tubercidin in high overall yield.

One side product of the glycosylation, which was formed to a minor extent (6%), was characterized (Scheme 1). Its structure was assigned to nucleoside **7** as the ^{1}H-NMR displayed two sets of signals for the nucleobase and only one for the sugar residue. Further evidence was made on the basis of ^{1}H-^{1}H-COSY, HSQC and HMBC spectra (see the Supporting Information). A similar compound was isolated after nucleobase anion glycosylation of a 2,6-disubstituted 7-deazapurine.[16c] The formation of **7** is the result of an addition elimination reaction.

Scheme 1. Glycosylation of 6-Chloro-7-deazapurine

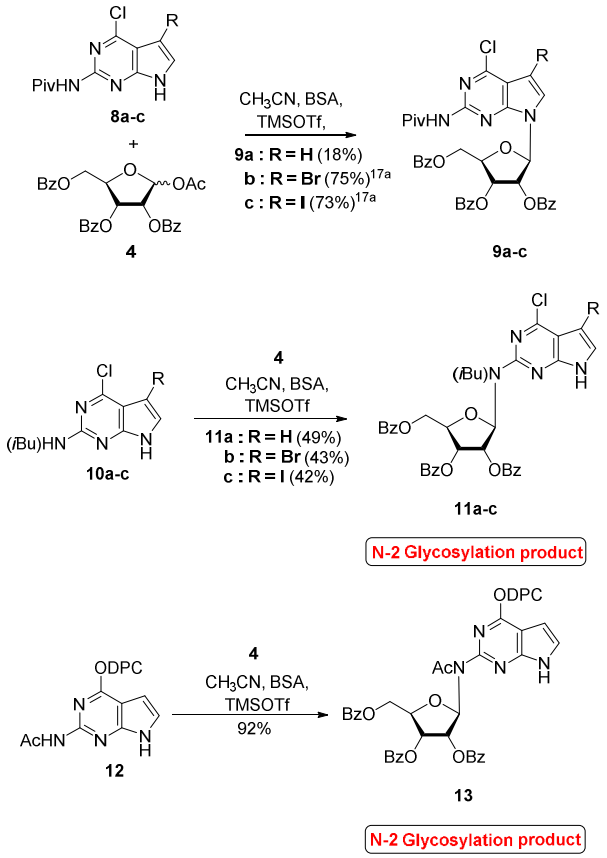


Encouraged by these results and the high yields reported for the glycosylation of halogenated nucleobases **8b,c**[17a,25] with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (**4**) the conditions were used for the glycosylation of pivaloyl protected 2-amino-6-chloro-pyrrolo[2,3-*d*]pyrimidine (**8a**)[25], which is the key intermediate to access 7-deazaguanosine. A 2-fold excess of ribosugar **4** over the pivaloylated nucleobase **8a** was employed, TMSOTf was used as catalyst and the temperature was kept at 50 °C (Scheme 2, for details see the Experimental Section). TLC monitoring showed that the glycosylation reaction proceeds much slower in comparison to 6-chloro-7-deazapurine **5**. Therefore, the reaction time was extended to 24 h. After chromatographical work-up the glycosylation product **9a** was isolated in 18%. No side product formation was observed but a significant amount of starting material was not consumed. Anyhow, due to the facile work-up this route enables a quick preparation of the nucleoside.

To improve reactivity of the base the influence of amino protecting groups was evaluated and the isobutyryl group was chosen as an alternative (→**10a**[26]). For comparison, the 7-halogenated nucleobases **10b** and **10c** were also investigated (see the Experimental Section and Scheme S1 in the Supporting Information). Glycosylation of **10a-c** was performed under the same reaction conditions as described for **9a** (Scheme 2). As a result, glycosylation did not take place at the pyrrole nitrogen nor at heterocyclic nitrogens as reported for other cases.

Instead, the isobutyrylated amino group was glycosylated (→**11a-c**). Such side product formation was already reported occurring in low yield for guanine and 7-deazapurine 2'-deoxyribonucleosides.[17a,27] A further change of protecting groups – an acetyl protected amino group and a diphenylcarbamoyl- (DPC) protected oxo group (**12**)[28]- gave also the amino glycosylated nucleoside (→**13**) (Scheme 2). Accordingly, only the bulky pivaloyl group protects the amino nitrogen from the side reaction. In order to mask the amino group more efficiently phthaloyl protection was chosen. To this end, 2-phthalimido-6-chloro-7-deazapurine (**14**) was synthesized and employed in the glycosylation reaction (for details see the Experimental Section and Scheme S2 in the Supporting Information). Unfortunately, this group was not stable during the procedure. As shown later a combination of the pivaloyl group together with a halogen in 7-position has to be used to drive the glycosylation to the pyrrole nitrogen resulting in efficient synthesis.[17a]

Scheme 2. Glycosylation of Protected 7-Deazapurines



Earlier work on 7-deazapurines reported on the glycosylation of pyrimidine nitrogens or pyrrole carbons.[22,23] Hence, the glycosylation positions were confirmed with the help of two dimensional NMR spectra. In general a shift of C1' signal (1.1-1.5 ppm downfield) and the C4' (1.5-1.8 ppm upfield) are shifted when the glycosylation position was altered form pyrrole nitrogen to the amino group (Table S1, Supporting Information). For nucleoside **9a** glycosylated at the pyrrole nitrogen cross-peaks of C-4 and C-8 of the nucleobase with the anomeric sugar proton C-1' are observed in the HMBC spectra. These cross-peaks are not obtained in case of the amino glycosylated nucleosides **11a-c** and **13**. In these cases, cross-peaks appeared from C-2 of the nucleobase to the anomeric proton at C-1' of the sugar moiety

(Figure 2). Furthermore, a cross-peak between the carbonyl carbon of the acyl protecting group and the anomeric proton was obtained excluding glycosylation of pyrimidine nitrogens. This was further evidenced as no significant differences were observed in the UV-spectra of the compounds as well as for the chemical shifts of the nucleobase carbons.

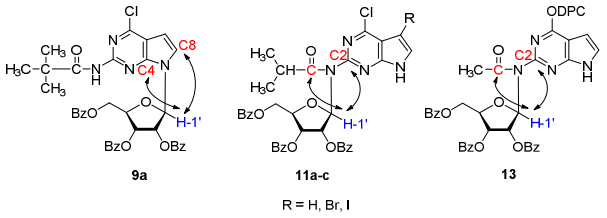
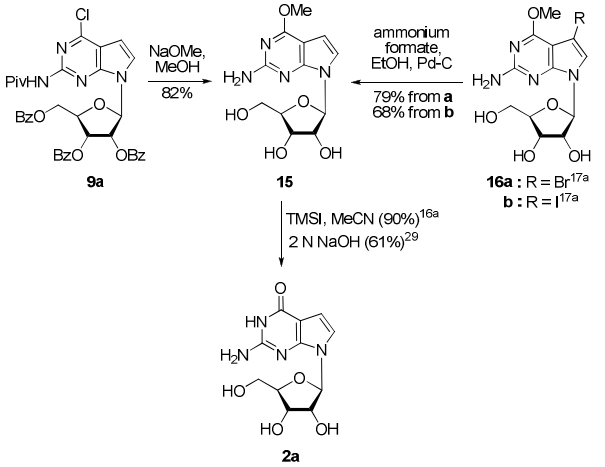


Figure 2. Cross-peaks obtained by 2D-NMR spectra confirming the glycosylation position.

In order to access 7-deazaguanosine (**2a**) compound **9a** was treated with 0.5 M NaOMe to afford the 6-methoxy nucleoside **15**[16c] (82%), from which 7-deazaguanosine **2a** was obtained according to the literature (Scheme 3).[16a,29] Following this route an overall yield of 13% starting from **8a** was obtained. The corresponding 7-deazaadenosine (**1a**, tubercidin) was obtained by treatment of compound **6** with NH_3/MeOH (85%, Scheme 4). Thus, tubercidin was prepared from 6-chloro-7-deazapurine (**5**) and commercially available 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (**4**) in 38% overall yield in 2 steps (Scheme 1 and 4).

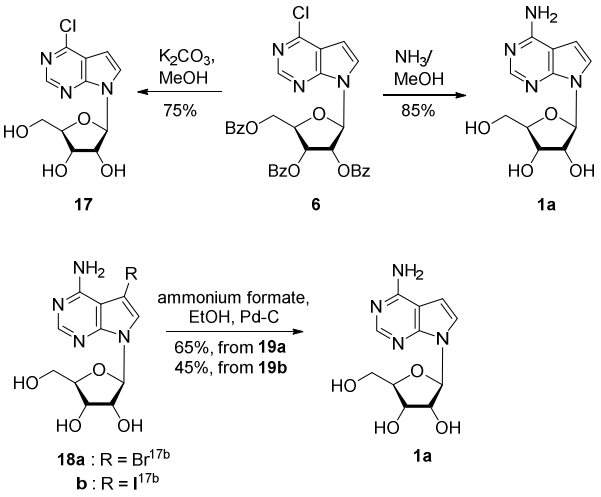
According to the high glycosylation yields already reported for the halogenated nucleobases **8b,c** an alternative strategy was employed to access 7-deazaguanosine (**2a**). For this **16a,b** were prepared according to the literature.[17a] Then, the halogenated nucleosides (**16a,b**) were treated with ammonium formate/Pd to remove the halogen (Scheme 3). Following this route 7-deazaguanosine (**2a**) was obtained in 36% via the bromo compound **8b** and 30% via the iodo compound **8c**.

Scheme 3. Synthesis of 7-Deazaguanosine (2a)



For comparison, tubercidin was synthesized by dehalogenation[20b,30] using compounds **18a,b**[17b]. The dehalogenation yields were 65% using **18a** and 45% employing **18b**. Formation of the protected chloro compound **6** from the protected 6-chloro-7-iodo-7-deazapurine ribonucleoside gave quantitative yields when the Grignard reagent iPrMgCl•LiCl was used.[30c] Here, the overall yields were 17% (bromo) and 18% (iodo) which is lower than obtained via direct glycosylation (Scheme 1 and 4). Overall yields are calculated on the nucleobases as starting materials. From **6** the deprotected 6-chloro-7-deazapurine ribonucleoside (**17**) was prepared by treatment with K_2CO_3/MeOH (75%) (Scheme 1 and 4). Overall yields of tubercidin synthesized *via* glycosylation of 7-halogenated-6-chloro-7-deazapurines followed by dehalogenation were 17% from **18a** and 18% for **18b**. The reaction routes require always four reaction steps (additional halogenation and dehalogenation) and yields differ between the methods[30a-c]. The direct glycosylation of the 6-chloro-7-deazapurine with the ribosugar **4** gives tubercidin in 38% overall yield and requires only two steps.

Scheme 4. Synthesis of Tubercidin (1a)



In conclusion, glycosylation yield and glycosylation position of 7-deazapurines with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (**4**) depend strongly on 7-substituents (H vs halogen) and amino protecting groups. Up to now, established one pot glycosylation protocols could not efficiently be applied to 6-chloro-7-deazapurine and 2-amino-6-chloro-7-deazapurine the most suitable starting materials for the syntheses of tubercidin or 7-deazaguanosine. Now, glycosylation of 6-chloro-7-deazapurine (**5**) with **4** (45% yield) followed by amination and sugar deprotection gave tubercidin (**1a**) in 38% yield using controlled reaction conditions. This represents the method of choice for the synthesis of tubercidin as glycosylation uses cheap sugar sources requires only two steps and can be performed in multigram scale.

Similar conditions applied to 7-deazaguanosine (**2a**) using the pivaloylated 2-amino-6-chloro-7-deazapurine (**8a**) gave 18% glycosylation yield. To improve the glycosylation less bulky isobutyryl or acetyl residues were used. As a result the glycosylation was directed to the exocyclic amino group. The resulting products are related to the antibiotic dapiramicin. 7-

deazaguanosine (**2a**) was obtained by an alternative route using glycosylation of 7-halogenated derivatives followed by dehalogenation in good overall yield (36%).

EXPERIMENTAL SECTION

General Methods and Materials. All chemicals and solvents were of laboratory grade as obtained from commercial suppliers and were used without further purification. Thin-layer chromatography (TLC) was performed on TLC aluminium sheets covered with silica gel 60 F254 (0.2 mm). Flash column chromatography (FC): silica gel 60 (40-60 μM) at 0.4 bar. UV-spectra were recorded on a UV-spectrophotometer: λ_{max} (ε) in nm, ε in dm^3 mol^{-1} cm^{-1}. NMR spectra were measured at 599.74 MHz for ^1H and 150.82 MHz for ^{13}C. ^1H-^{13}C correlated (HMBC, HSQC) NMR spectra were used for the assignment of the ^{13}C signals (Table S1, Supporting Information). The *J* values are given in Hz; δ values in ppm relative to Me_4Si as internal standard. For NMR spectra recorded in DMSO-d_6, the chemical shift of the solvent peak was set to 2.50 ppm for ^1H NMR and 39.50 ppm for ^{13}C NMR. ESI-TOF mass spectra of nucleosides were recorded on a Micro-TOF spectrometer.

Glycosylation of 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (5).

N,*O*-Bis(trimethylsilyl)acetamide (BSA, 5.007 g, 6.0 mL, 24.61 mmol) was added to a stirred suspension of 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**5**)[18] (3.0 g, 19.53 mmol) in anh. MeCN (10 mL). The solution became clear and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (**4**, 24.619 g, 48.80 mmol) was added followed by the addition of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 5.894 g, 4.8 mL, 26.52 mmol). The reaction mixture was stirred at 50 °C for 16 h, cooled to rt and diluted with CH_2Cl_2 (50 mL). The organic phase was washed with saturated $NaHCO_3$, dried and applied to FC (silica gel, CH_2Cl_2/MeOH, 99:0.5).

4-Chloro-7-[(2,3,5-tri-*O*-benzoyl)-*β*-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (6). From the fastest migrating zone compound **6** was obtained as colorless foam (5.2 g, 45%). TLC (silica gel, CH_2Cl_2/MeOH, 99:1) R_f 0.4. λ_{max} (MeOH)/nm 226 (ε/dm^3 mol^{-1} cm^{-1} 54900), 275 (8100), 280 (7500). ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.66-4.71 (m, 1H, H-5'), 4.79-4.89 (m, 2H, H-5', H-4'), 6.18 (t, J = 5.4 Hz, 1H, H-3'), 6.41 (t, J = 5.7 Hz, 1H, H-2'), 6.73 (d, J = 5.1 Hz, 1H, H-1'), 6.77 (d, J = 3.6 Hz, 1H, H-5), 7.40-7.53 (m, 6H, Ar-H), 7.59-7.67 (m, 3H, Ar-H), 7.83-7.90 (m, 2H, Ar-H), 7.95-7.97 (m, 4H, Ar-H), 8.04 (d, J = 3.9 Hz, 1H, H-6), 8.57 (s, 1H, H-2). ESI-TOF m/z calcd for $C_{32}H_{24}ClN_3O_7Na$ [M + Na$^+$] 620.1195, found 620.1213.

4-(4"-Chloro-7"*H*-pyrrolo[2,3-*d*]pyrimidin-7"-yl)-7-(*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (7). From the slower migrating zone compound **7** was obtained as colorless foam (0.890 g, 6%). TLC (silica gel, CH_2Cl_2/acetone, 98:2) R_f 0.4. λ_{max} (MeOH)/nm 226 (ε/dm^3 mol^{-1} cm^{-1} 69400), 274 (13600), 280 (13200), 300 (12500). ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.68-4.73 (m, 1H, H-5'), 4.80-4.85 (m, 1H, H-5'), 4.88-4.92 (m, 1H, H-4'), 6.19 (t, J = 5.7 Hz, 1H, H-3'), 6.48 (t, J = 6.0 Hz, 1H, H-2'), 6.81 (d, J = 6.0 Hz, 1H, H-1'), 6.94 (d, J = 3.6 Hz, 1H, H-5"), 7.00 (d, J = 3.9 Hz, 1H, H-5), 7.43-7.55 (m, 7H, Ar-H), 7.60-7.70 (m, 3H, Ar-H), 7.85-7.88 (m, 2H, Ar-H), 7.97-8.02 (m, 3H, Ar-H), 8.03 (d, J = 3.0 Hz, H-6"), 8.30 (d, J = 3.0 Hz, H-6), 8.76 (s, 1H, H-2), 8.78 (s, 1H, H-2"). ESI-TOF m/z calcd for $C_{38}H_{27}ClN_6O_7Na$ [M + Na$^+$] 737.1522, found 737.1551.

4-Chloro-2-(N^2-pivaloyl)amino-7-[(2,3,5-tri-*O*-benzoyl)-*β*-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (9a). To a stirred suspension of compound **8a**[25] (0.505 g, 2.00 mmol) in anhydrous MeCN (14 mL) was added BSA (0.488 g, 0.59 mL, 2.40 mmol) at room temperature. After stirring for 30 min, TMSOTf (0.578 g, 0.47 mL, 2.60 mmol) was introduced. Then, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**4**) (2.018 g, 4.00 mmol)

was added in three portions (every 4 h) and the reaction mixture was stirred at 50 °C for 24 h. The solution was cooled to room temperature and diluted with CH_2Cl_2 (50 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by FC (silica gel, CH_2Cl_2/MeOH, 99.8:0.2) to give **9a** (0.250 g, 18%) as yellowish foam. TLC (CH_2Cl_2/MeOH, 98:2) R_f 0.42. λ_{max} (MeOH)/nm 234 (ε/dm^3 mol^{-1} cm^{-1} 51600), 276 (10000), 281 (9800). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.19 (s, 9H, 3 x CH_3), 4.66-4.69 (m, 1H, H-5'), 4.79-4.87 (m, 2H, H-5', H-4'), 6.40-6.42 (m, 1H, H-2'), 6.49 (t, J = 6.0 Hz, 1H, H-3'), 6.56 (d, J = 4.2 Hz, 1H, H-1'), 6.66 (d, J = 4.2 Hz, 1H, H-7), 7.43-7.49 (m, 6H, ArH), 7.61-7.67 (m, 3H, ArH), 7.81 (d, J = 3.6 Hz, 1H, H-8), 7.89-7.95 (m, 6H, ArH), 10.26 (s, 1H, NH). ESI-TOF m/z calcd for $C_{37}H_{33}ClN_4O_8Na$ [M + Na]$^+$ 719.1879, found 719.1889.

4-Chloro-5-bromo-2-(*N*^{2}-isobutyryl)amino-7*H*-pyrrolo[2,3-*d*]pyrimidine (10b). A stirred solution of **10a**[26] (1.250 g, 5.24 mmol) in anhydrous DMF (30 mL) was treated with *N*-bromosuccinimide (1.119 g, 6.29 mmol) and the reaction mixture was stirred at rt for 16 h. Then reaction mixture was poured into ice water (300 mL), the precipitate was filtered, washed with water (3 x 30 mL) and recrystallized from MeOH to give compound **10b** (1.231 g, 74%) as a colorless solid. TLC (silica gel, CH_2Cl_2/MeOH, 98:2) R_f 0.19. λ_{max} (MeOH)/nm 248 (ε/dm^3 mol^{-1} cm^{-1} 38400), 275 (6800). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.08 (d, J = 6.6 Hz, 6H, 2 x CH_3), 2.74-2.81 (m, 1H, CH), 7.72 (s, 1H, H-8), 10.65 (s, 1H, NH), 12.70 (s, 1H, NH). ESI-TOF m/z calcd for $C_{10}H_{10}BrClN_4ONa$ [M + Na]$^+$ 338.9619, found 338.9619.

4-Chloro-5-iodo-2-(*N*^{2}-isobutyryl)amino-7*H*-pyrrolo[2,3-*d*]pyrimidine (10c). As described for **10b** with **10a** (2.470 g, 10.35 mmol), anhydrous DMF (100 mL) and *N*-iodosuccinimide (2.800 g, 12.45 mmol). Compound **10c** (3.050 g, 81%) was obtained as a colorless solid. TLC (silica gel, CH_2Cl_2/MeOH, 98:2) R_f 0.21. λ_{max} (MeOH)/nm 251 (ε/dm^3 mol^{-1} cm^{-1} 38400),

275 (9500). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.08 (d, J = 6.6 Hz, 6H, 2 x CH_3), 2.74-2.81 (m, 1H, CH), 7.72 (s, 1H, H-8), 10.61 (s, 1H, NH), 12.69 (s, 1H, NH). ESI-TOF *m*/*z* calcd for $C_{10}H_{10}ClIN_4ONa$ $[M + Na]^+$ 386.9480, found 386.9482.

4-Chloro-2-[(*N*^{2}-isobutyryl){2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl}]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine (11a). As described for **9a** with **10a** (0.477 g, 2.00 mmol), MeCN (14 mL), BSA (0.977 g, 1.17 mL, 4.80 mmol), TMSOTf (0.578 g, 0.47 mL, 2.60 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**4**) (2.018 g, 4.00 mmol). Purification by FC (silica gel, CH_2Cl_2/MeOH, 99.5:0.5) gave **11a** (0.670 g, 49%) as a yellowish foam. TLC (CH_2Cl_2/MeOH, 98:2) R_f 0.23. λ_{max} (MeOH)/nm 231 (ε/dm^3 mol^{-1} cm^{-1} 56000), 274 (7500), 281(6900). ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.93 (d, J = 6.6 Hz, 3H, CH_3), 1.10 (d, J = 6.6 Hz, 3H, CH_3), 2.60-2.67 (m, 1H, CH), 4.33-3.36 (m, 1H, H-5'), 4.52-4.54 (m, 1H, H-5'), 4.62-4.64 (m, 1H, H-4'), 5.83 (t, J = 6.6 Hz, 1H, H-3'), 6.04-6.06 (m, 1H, H-2'), 6.35 (d, J = 3.6 Hz, 1H, H-1'), 6.55 (d, J = 3.6 Hz, 1H, H-7), 7.37-7.47 (m, 6H, ArH), 7.59-7.66 (m, 4H, ArH, H-8), 7.77-7.79 (m, 2H, ArH), 7.83-7.85 (m, 2H, ArH), 7.90-7.91 (m, 2H, ArH), 12.64 (s, 1H, NH). ESI-TOF *m*/*z* calcd for $C_{36}H_{31}ClN_4O_8Na$ $[M + Na]^+$ 705.1723, found 705.1744.

5-Bromo-4-chloro-2-[(*N*^{2}-isobutyryl){2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl}]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine (11b). As described for **9a** with **10b** (0.635 g, 2.00 mmol), MeCN (14 mL), BSA (0.488 g, 0.59 mL, 2.40 mmol), TMSOTf (0.578 g, 0.47 mL, 2.60 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**4**) (2.018 g, 4.00 mmol). Purification by FC (silica gel, CH_2Cl_2/MeOH, 99.5:0.5) gave **11b** (0.660 g, 43%) as a yellowish foam. TLC (CH_2Cl_2/MeOH, 98:2) R_f 0.23. λ_{max} (MeOH)/nm 232 (ε/dm^3 mol^{-1} cm^{-1} 53700), 274 (8100), 281 (7000). ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.93 (d, J = 6.6 Hz, 3H, CH_3), 1.10 (d, J = 6.6 Hz, 3H, CH_3), 2.60-2.67 (m, 1H, CH), 4.33-4.36 (m, 1H, H-5'), 4.57-4.59 (m, 1H, H-5'), 4.63-4.65 (m, 1H, H-4'), 5.85 (t, J = 6.6 Hz, 1H, H-3'), 6.01-6.03 (m, 1H, H-2'), 6.36 (d, J =

4.2 Hz, 1H, H-1'), 7.38-7.46 (m, 6H, ArH), 7.59-7.65 (m, 3H, ArH), 7.76-7.77 (m, 2H, ArH), 7.85-7.91 (m, 5H, ArH, H-8), 12.99 (s, 1H, NH). ESI-TOF *m*/*z* calcd for $C_{36}H_{30}BrClN_4O_8Na$ $[M + Na]^+$ 783.0828, found 783.0811.

4-Chloro-5-iodo-2-[(N^2-isobutyryl){2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl}]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine (11c). As described for **9a** with **10c** (0.729 g, 2.00 mmol), MeCN (14 mL), BSA (0.488 g, 0.59 mL, 2.40 mmol), TMSOTf (0.578 g, 0.47 mL, 2.60 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**4**) (2.018 g, 4.00 mmol). Purification by FC (silica gel, CH_2Cl_2/MeOH, 99.5:0.5) gave **11c** (0.685 g, 42%) as a yellowish foam. TLC (CH_2Cl_2/MeOH, 98:2) R_f 0.27. λ_{max} (MeOH)/nm 232 (ε/dm^3 mol^{-1} cm^{-1} 46800), 274 (8400), 281 (7200). ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.92 (d, J = 6.6 Hz, 3H, CH_3), 1.09 (d, J = 6.6 Hz, 3H, CH_3), 2.59-2.65 (m, 1H, CH), 4.33-3.36 (m, 1H, H-5'), 4.55-4.58 (m, 1H, H-5'), 4.62-4.65 (m, 1H, H-4'), 5.84 (t, J = 6.0 Hz, 1H, H-3'), 6.01 (t, J = 5.4 Hz, 1H, H-2'), 6.35 (d, J = 4.2 Hz, 1H, H-1'), 7.38-7.46 (m, 6H, ArH), 7.60-7.65 (m, 3H, ArH), 7.76 (d, J = 7.8 Hz, 2H, ArH), 7.85-7.90 (m, 5H, ArH, H-8), 12.97 (s, 1H, NH). ESI-TOF *m*/*z* calcd for $C_{36}H_{30}ClIN_4O_8Na$ $[M + Na]^+$ 831.0689, found 831.0669.

2-{[N^2-Acetyl]-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)}amino-4-(O^4-diphenylcarbamoyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (13). As described for **9a** with **12**[28] (0.150 g, 0.39 mmol), MeCN (5 mL), BSA (0.142 g, 170 µL, 0.70 mmol), TMSOTf (0.112 g, 91 µL, 0.50 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**4**) (0.391 g, 0.77 mmol). The reaction mixture was stirred at 50 °C for 6 h, cooled to room temperature, and diluted with CH_2Cl_2 (30 mL). The solution was washed with aqueous saturated $NaHCO_3$ and brine. The combined extracts were dried (Na_2SO_4), and the solvent was evaporated. Purification by FC (silica gel, CH_2Cl_2/MeOH, 99.3:0.7) gave **13** (0.295 g, 92%) as yellowish foam. TLC (CH_2Cl_2/MeOH, 98:02) R_f 0.19. λ_{max} (MeOH)/nm 231 (ε/dm^3 mol^{-1} cm^{-1} 69600), 274 (11900), 281(11300). ^{1}H

NMR (600 MHz, DMSO-d_6) δ 2.10 (s, 3H, CH_3), 4.32-3.35 (m, 1H, H-5'), 4.43-4.45 (m, 1H, H-5'), 4.62-4.65 (m, 1H, H-4'), 5.85 (t, J = 6.6 Hz, 1H, H-3'), 6.16 (dd, J = 6.3 Hz, J = 3.6 Hz, 1H, H-2'), 6.42 (d, J = 3.6 Hz, 1H, H-1'), 6.59 (d, J = 3.6 Hz, 1H, H-7), 7.27-7.30 (m, 4H, ArH), 7.38-7.76 (m, 16H, ArH, H-8), 7.72-7.74 (m, 2H, ArH), 7.82-7.83 (m, 2H, ArH), 7.91-7.93 (m, 2H, ArH), 12.49 (s, 1H, NH). ESI-TOF *m*/*z* calcd for $C_{47}H_{37}N_5O_{10}Na$ $[M + Na]^+$ 854.2433, found 854.2442.

4-Chloro-2-phthalimido-7*H*-pyrrolo[2,3-*d*]pyrimidine (14). To a solution of 2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine[19] (1.000 g, 5.93 mmol) in DMF (10 mL) was added *N*-carbethoxyphthalimide (9.752 g, 44.49 mmol), and the reaction mixture was heated at 100 °C for 24 h. To the reaction mixture was added ice-water (30 mL) and the resulting precipitate was collected and purified by FC (silica gel, CH_2Cl_2/MeOH, 99:1) to give **14** (0.690 g, 39%) as colorless solid. TLC (CH_2Cl_2/MeOH, 95:05) R_f 0.51. ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.76 (d, J = 3.6 Hz, 1H, H-7), 7.88 (d, J = 3.6 Hz, 1H, H-8), 7.96-7.97 (m, 2H, Ar-H), 8.03-8.04 (m, 2H, Ar-H), 12.96 (s, 1H, NH). ESI-TOF *m*/*z* calcd for $C_{14}H_7ClN_4O_2Na$ $[M + Na]^+$ 321.0150, found 321.0158.

2-Amino-4-methoxy-7-(*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (15).

Method A (from compound 9a). Compound **9a** (0.310 g, 4.45 mmol) was dissolved in 0.5 M $NaOCH_3$/MeOH (7 mL) and the reaction mixture was refluxed for 3 h. After completion of the reaction (TLC monitoring), the solvent was evaporated and the residue was purified by FC (silica gel, CH_2Cl_2/MeOH, 96:4) to give the product **15** (0.108 g, 82%) as a colorless solid.

Method B (by dehalogenation of compounds 16a or 16b). Compound **16a**[17a] (0.221 g, 0.59 mmol) or **16b**[17a] (0.250 g, 0.59 mmol) was suspended in EtOH (15 mL), then Pd on activated charcoal (0.030 g) and ammonium formate (0.372 g, 5.90 mmol) were added. The reaction mixture was refluxed for 1 h in case of **16a** and for 20 h in case of **16b**. After completion of

the reaction (TLC monitoring), the hot reaction mixture was filtered and washed with hot EtOH. The combined filtrates were evaporated and the residue was purified by FC (silica gel, CH_2Cl_2/MeOH, 96:4) to give **15** (0.137 g, 79%, from compound **16a** and 0.119 g, 68%, from compound **16b**) as a colorless solid. Analytical data were identical to those reported in the literature.[16c]

4-Chloro-7-(*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (17). Compound **6** (0.500 g, 0.83 mmol) was dissolved in MeOH (10 mL). Then, K_2CO_3 (0.020 g) was added and the reaction mixture stirred for 30 min at rt (TLC-monitoring). The solvent was evaporated and the remaining residue was applied to FC (silica gel, CH_2Cl_2/MeOH, 9:1). After evaporation of the solvent and crystallization from H_2O compound **17** (0.180 g, 75%) was obtained as colorless solid. Analytical data were identical to data reported in the literature.[16b]

4-Amino-7-(*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (Tubercidin, 1a)

Method A (from compound 6). Compound **6** (0.75 g, 1.25 mmol) was treated with NH_3/MeOH (200 mL) at 130 °C in a steel bomb for 16 h. Then, the solvent was evaporated and the remaining residue was applied to FC (silica gel, CH_2Cl_2/MeOH, 3:2). The solvent was evaporated and the remaining residue was crystallized from MeOH to give compound **1a** as colorless solid (0.284 g, 85%). Analytical data were identical to data reported in the literature.[16b,20b]

Method B (by dehalogenation of compound 18a). Compound **18a** (2.0 g, 5.79 mmol), was suspended in EtOH (100 mL), then Pd on activated charcoal (0.100 g) and ammonium formate (0.600 g, 9.51 mmol) were added and the mixture was stirred under reflux for 3 h.

The hot reaction mixture was filtered and the filter cake was washed several times with hot MeOH (10 x 10 mL). The combined filtrates were evaporated to dryness and the remaining residue was triturated with a small amount of MeOH (~ 2-3 mL). The resulting precipitate was filtered, washed with acetone and dried. The resulting amorphous solid (1.8 g containing salt) was recrystallized from H_2O to give colorless crystals of **1a** (1.0 g, 65%). Analytical data were identical to data reported in the literature.[16b,20b]

Method B (by dehalogenation of compound 18b). Compound **18b** (0.250 g, 0.64 mmol) was suspended in EtOH (15 mL), then Pd on activated charcoal (0.030 g) and ammonium formate (0.402 g, 6.38 mmol) were added. Afterwards, the reaction mixture was refluxed for 5 h. After complete conversion (TLC monitoring), the hot reaction mixture was filtered and washed with hot EtOH. The combined filtrate was evaporated and the residue was purified by FC (silica gel, CH_2Cl_2/MeOH, 90:10) to give **1a** (0.077 g, 45%) as a colorless solid. Analytical data were identical to data reported in the literature.[16b,20b]

ASSOCIATED CONTENT

Supporting Information

^{1}H-, ^{13}C, ^{1}H-^{1}H-COSY, HSQC and HMBC NMR spectra of all compounds.

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Notes

The authors declare no competing financial interest.

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