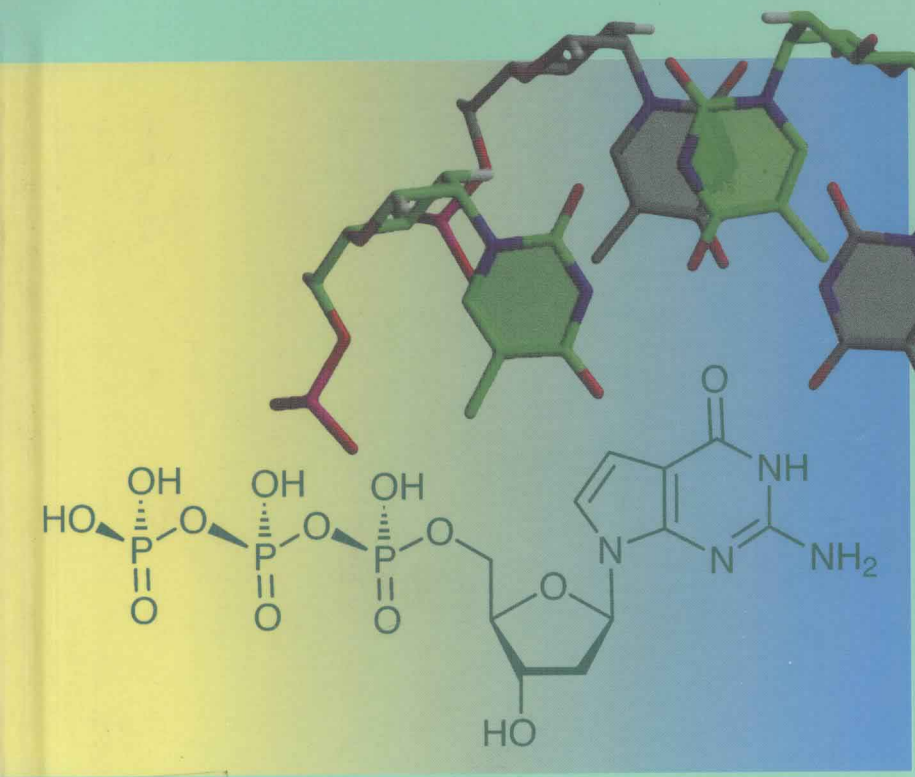
M. Volkan Kisakürek
Helmut Rosemeyer (Eds.)

Perspectives in Nucleoside and Nucleic Acid Chemistry



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Dr. M. Volkan Kisakürek
Organisch-chemisches Institut
der Universität Zürich
Winterthurerstrasse 190
CH-8057 Zürich

Dr. Helmut Rosemeyer
Laboratorium für Organische
und Bioorganische Chemie
Universität Osnabrück
Barbarastrasse 7
D-49069 Osnabrück

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Preface

The chemistry of nucleosides and nucleic acids continues to be a rapidly developing field of study. Many of the most important recent advances in medicinal chemistry, such as the application of the antisense and antigene concept to drug discovery, have occurred in this field with the development of novel nucleoside- and nucleotide-based antiviral and antitumor drugs. The realization of atypical base-pairing interactions involving natural as well as synthetic nucleosides has extended the genetic alphabet and expanded horizons in molecular-recognition applications. New synthesis strategies, novel protecting groups and solid supports, and combinatorial chemistry have helped spur progress in drug-development and genome-sequencing research.

This volume, comprised of contributions written by internationally recognized experts, covers cutting-edge developments in recent nucleoside and nucleic acid research. The majority of the manuscripts were originally dedicated to Prof. Dr. *Frank Seela*, University of Osnabrück, on the occasion of his 60th birthday and have appeared in *Helvetica Chimica Acta*. The topics covered by the contributions consist of the most-recent synthesis innovations, including novel protecting-group and solid-support strategies and combinatorial approaches, nucleic acid labeling for the development of reporter molecules, spectroscopy and structural studies, investigations of enzymatic reaction mechanisms, thermodynamic and computational investigations, stability assessments, and medicinal applications. The comprehensive scope of this collection will be valued by *synthetic*, *physical*, *organic*, *bioorganic*, and *medicinal* chemists working on all aspects of nucleoside and nucleic acid research. This book will also prove an invaluable introduction to the state-of-the-art in nucleoside and nucleic acid chemistry to aid researchers new to the field.

October 2000

M. Volkan Kısakürek
Helmut Rosemeyer

Contents

Part I. Nucleosides and Nucleotides

Part II. Oligonucleotides

The Synthesis of Bicyclic N^4-Amino-2′-deoxycytidine Derivatives

by **David Loakes**, **Rita Bazzanini**[1]), and **Daniel M. Brown***

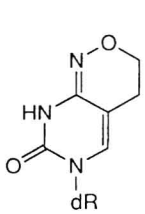
Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK
(Tel: (+ 44) 1223248011; Fax: (+ 44) 1223412178; e-mail: dmb@mrc-lmb.cam.ac.uk)

Nucleosides which have ambivalent tautomeric properties have value in a variety of nucleic acid hybridization applications, and as mutagenic agents. We describe here synthetic studies directed to stable derivatives of this kind of nucleoside based on N^4-aminocytosine. Treatment of the 4-(1*H*-1,2,4-triazol-1-yl)-5-(chloroethyl)pyrimidinone nucleoside derivative **5** with hydrazine leads to formation of the 6,6-bicyclic pyrimido-pyridazin-7-one **3**, and with methylhydrazine to the corresponding fixed tautomeric 1-methyl derivative **7** (*Scheme 1*). If these cyclization reactions are carried out in the presence of a base, the 6-ring bicyclic derivatives undergo rearrangement to their corresponding 5-ring pyrrolo-pyrimidin-2-one analogues **8** (*Scheme 2*). In the reaction of the triazolyl derivative **5** with 1-[(benzyloxy)carbonyl]-1-methylhydrazine, spontaneous cyclization gives the 5-ring derivative **13** related to **8** rather than the open-chain product **12** (*Scheme 4*). Reaction of an acetylated analogue of triazolyl derivative **5** with 1,1-dimethylhydrazine gives rise to some of the open-chain product **9**, but it too cyclizes to a product that we have assigned the structure of the 6,6-ring quaternary ammonium salt **11** (*Scheme 3*).

Introduction. – Nucleosides which are capable of base-pairing with more than one of the natural DNA/RNA bases are mutagenic. Such analogues are of use not only to explore aspects of chemical mutagenesis [1][2], but also as tools in molecular biology. We have extensively examined the mutagenic nucleoside **1**, which behaves as both thymidine and deoxycytidine. Its 5′-triphosphate has been used for random mutagenesis [3][4], whilst in oligonucleotides, it has been used in primers for PCR [5] and to study H-bonding patterns in DNA duplexes [6][7]. The analogue, however, does not behave indiscriminately as either T or C, but has a bias towards T. For this reason, we have for some time been examining alternative analogues that may shift this balance. The 5,6-ring (ribo) analogue **2** was prepared [8] in the expectation that the smaller ring size might have an effect on the tautomeric ratio. This, whilst appearing to behave more as a cytidine analogue in its ^{1}H-NMR spectrum, proved to be too unstable to investigate further.

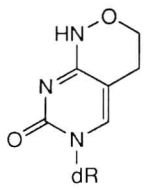
N^4-Hydroxycytosine derivatives have tautomeric constants (K_T) of the order of 10 with the imino (thymine-like) form predominating [9]. N^4-Aminocytosine derivatives exist predominately in the amino form, with K_T of around 30 in H_2O. Whilst these compounds are highly mutagenic *in vivo*, N^4-(alkylamino)cytosine derivatives are significantly less mutagenic [10]. This suggests that bicyclic analogues should show ambivalent base-pairing, but be less potent chemical mutagens. We, therefore, chose to

[1]) Present address: Consiglio Nazionale delle Recerche, I.Co.C.E.A. Area della Ricerca, Via P. Gobetti, 101, I-40129, Bologna.

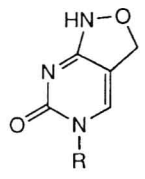


T-like

1



C-like



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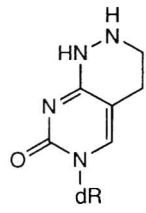


3

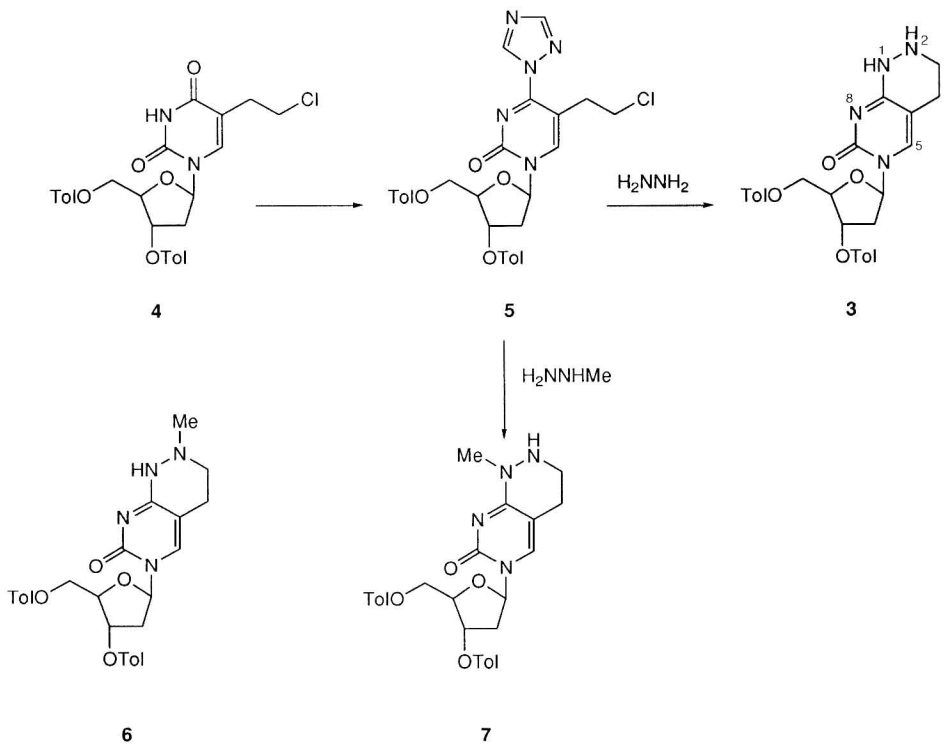
investigate N^4-amino bicyclic analogues, such as **3**. The parent bicyclic compound **3** proved unstable; presumably it is susceptible to aerial oxidation. Therefore, we have attempted to prepare alkylated derivatives in the expectation that they would be stable, and that such compounds could then be used to investigate their ambivalent H-bonding behaviour in oligonucleotides.

Results and Discussion. – The 5-(2-chloroethyl)-2′-deoxyuridine derivative **4** [11] was converted to its C^4-triazolyl derivative **5**, which was then subjected to a series of reactions with various hydrazines. Thus, reaction with anhydrous hydrazine led to the rapid displacement of the C^4-triazolyl group, followed by a slower cyclization with the 5-(2-chloroethyl) group to give the bicyclic product **3** (*Scheme 1*). The product is rather unstable, readily degrading to a number of products, even if stored at 4°. Treatment of the C^4-triazolyl derivative **5** with methylhydrazine under similar conditions led to an essentially single product, though in a slower reaction. Although in equivalent displacement reactions the N-atom carrying the Me group is the more nucleophilic, we hoped to obtain some of the desired **6** [10]. The structure of the 3′,5′-di-*O*-acetyl rather than 3′,5′-di-*O*-toluoyl derivative was elucidated by NOE experiments (no NOE on irradiation of MeN (3.14 ppm), irradiation of CH_2N (*t*, 2.97 ppm) → enhancement of the exchangeable signal and NOE at $CH_2(4)$ (2.51 ppm)). From this data, we deduced that the structure was not that of **6**, but of the regioisomer **7**.

It was observed that if the above cyclization reactions were carried out in the presence of a base, a second minor product was also formed (this product was also slowly formed on standing, particularly in solution). These minor products were rather difficult to separate from the first-formed 6,6-bicyclic products **3** and **7**. Therefore, **3** and **7** were treated with Et_3N or pyridine whereupon each was converted into this second product **8a** and **8b**, respectively, the methylated derivative **7** rearranging much slower than **3** (*Scheme 2*). Thus, the transformation of **3** in pyridine at 50° was complete after 16 h, whereas, under the same conditions, **7** had only reacted to *ca.* 50%. The rearrangement also occurred in 2,6-lutidine. The structures of **8a**,**b** were established as the 5-membered ring isomers from their ^{1}H-NMR spectra. The isomer **8a** was further characterized by conversion to its crystalline hydrazone with benzaldehyde, thus confirming the presence of the free NH_2 group.

The product **8a**, derived from **3**, showed a *s* (2 H) at 4.82 ppm for an unsubstituted NH_2 group. For the Me-substituted product **8b**, MeN group was a *d* (3.34 ppm), whilst the NH proton was a *q* (5.30 ppm). The MeN *d* collapsed to a *s* in the presence of D_2O.

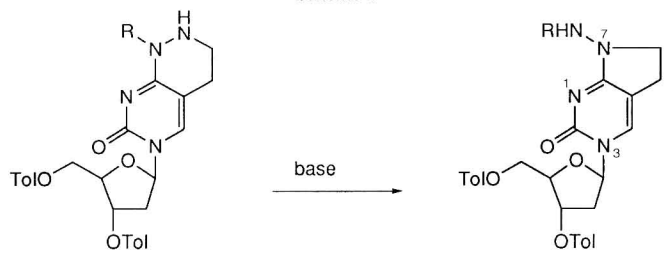
Scheme 1



4 **5** **3**

6 **7**

Scheme 2

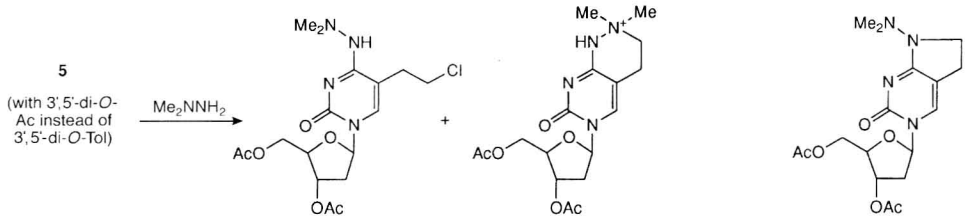


3 R = H
7 R = Me

8a R = H
b R = Me

To investigate the formation of the 5-membered ring bicyclic analogues further, the 3′,5′-di-*O*-acetyl-substituted analogue of **5** was reacted with 1,1-dimethylhydrazine (*Scheme 3*). This was expected to give **9**, which we then planned to use to examine whether cyclization would occur leading to the 5-ring **10**. Displacement of the triazolyl group of the di-*O*-acetyl analogue of **5** by 1,1-dimethylhydrazine in tetrachloroethane at 100° overnight gave the expected product **9** in low yield, besides a major product which was polar, water-soluble, and very difficult to isolate in pure form. In an earlier

Scheme 3



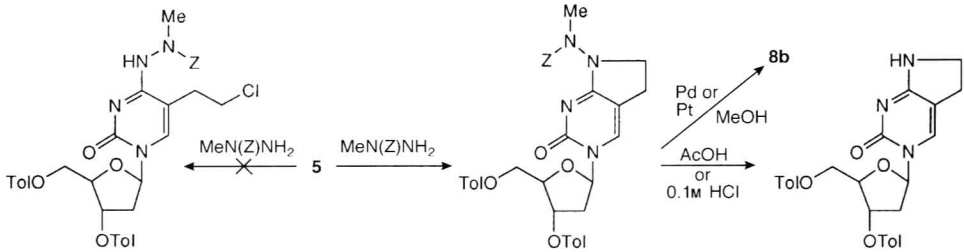
preliminary report [12], we suggested that this product was the quaternary ammonium salt **11**. This assignment was based on the fact that the product is water-soluble and polar: it has a mass spectrum with M^+ at m/z 381 and a ^{1}H-NMR spectrum corresponding to the proposed structure **11**. Isolation of the pure product has been attempted by a variety of methods including ion-exchange and reversed-phase HPLC, but has so far eluded us and is the subject of further work. It is anticipated that we may be able to demethylate the purified product to give the desired bicyclic di-*O*-acetyl analogue of **6**. Interestingly, we have no evidence, despite attempting the reaction many times under a variety of conditions, that ring contraction to the 5-ring analogue **10** occurred.

The ^{1}H-NMR spectrum of the salt **11** shows a *s* (6 H) at 3.15 ppm for Me_2N^+, whereas for the dimethylhydrazine product **9**, there is a *s* (6 H) at 2.50 ppm. This is consistent with the change in chemical shift for Me_2N to Me_2N^+. Thus, the data are entirely consistent with the structure of **11** being the 6-ring quaternary ammonium derivative.

As the methylamino residue of methylhydrazine is evidently the more nucleophilic group [13], it was decided to use a protected methylhydrazine derivative as an alternative route to produce the analogue **6**. Once deprotected, the methylamino residue could undergo cyclization with the 5-(chloroethyl) side chain. Thus, reaction of the triazolyl derivative **5** with 1-[(benzyloxy)carbonyl]-1-methylhydrazine [14] gave a product that we initially believed to be the expected compound **12** in a slow reaction (*Scheme 4*). On this assumption, we reductively removed the (benzyloxy)carbonyl (Z) group using either Pt or Pd in MeOH. However, the product that we obtained was not the desired product but again the 5-membered-ring derivative **8b**, as established by its ^{1}H-NMR spectrum, and comparison with the product **8b** obtained by ring contraction from **7** (see above). We therefore assumed that the product had preferentially cyclized or rearranged with N^4 as the (benzyloxy)carbonyl protecting group was being removed. Thus, the reduction was carried out in acid (AcOH or 0.1M HCl), as we hoped that protonation of the amino group would prevent ring closure after removal of the Z protecting group. This would then enable cyclization to occur to give the desired regiospecific product following neutralization. However, the product **14** obtained was again a 5-ring derivative related to **8**; moreover, under these conditions, the methylamino group had been reductively cleaved (*Scheme 4*).

Subsequently, we found that the intermediate (Z-protected) reaction product, initially assigned the structure **12**, was in fact the cyclized reaction product **13** (see

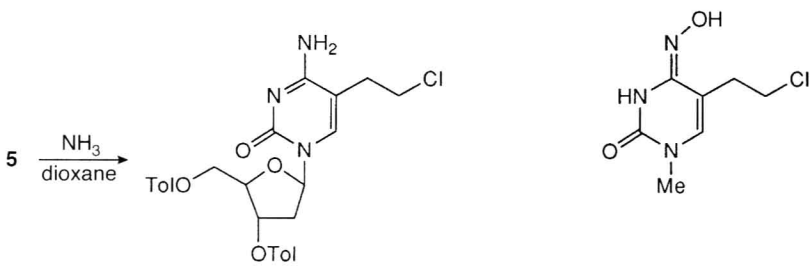
Scheme 4



12 **13** **14**

Scheme 4). This was confirmed by the MS of **13**, which showed an ion at *m/z* 675 ($[M + Na]^+$), corresponding to the loss of HCl from **12**. Thus cyclization must have occurred after displacement of the triazole moiety and prior to reduction. The open-chain intermediate **12** was never observed in this reaction despite using a variety of alternative reaction conditions. The formation of the 5-membered ring bicyclic product is surprising. In this connection, when the triazole **5** was treated with ammonia, the cytidine derivative **15** was formed which did not undergo cyclization (*Scheme 5*). Neither did the *N*-hydroxycytidine derivative **16** [15] cyclize to give the corresponding bicyclic product. The presence of the Z group at the methylhydrazine renders it particularly unreactive, both in terms of the initial nucleophilic displacement of the C^4-triazolyl group and towards further cyclization.

Scheme 5

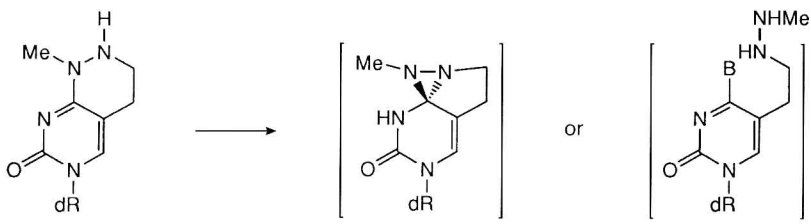


15 **16**

It is of interest to speculate on the nature of the formation of the bicyclic compound **13**. We have previously experienced the fact that the chloroethyl side chain is particularly unreactive towards nucleophilic displacement reactions, except for intramolecular cyclization. Therefore, the formation of **13** probably does not arise by first displacing chloride, followed by cyclization. However, the cyclization of the chloroethyl group to form a bicyclic compound is, nevertheless, a slow reaction, compared to the nucleophilic displacement of the C^4-triazolyl group. Although the Z-protected hydrazine is particularly unreactive, it appears that, once the initial reaction to displace the triazole moiety has occurred, cyclization with the chloroethyl group is spontaneous.

Lastly, we turn to the question of the mechanism whereby the 6,6-ring hydrazine derivatives of type **7** rearrange to 5,6-ring isomers, carrying an N^4-amino function in the cytosine moiety (see *Scheme 2*). The evidence is strong that the first-formed products are the 6,6-ring bicyclic compounds of type **7** and result from a displacement first of the triazolyl residue of **5** followed by ring closure. Rearrangement is base-catalysed and occurs in the cases where the N^4-amino N-atom carries a proton. Two mechanisms suggest themselves (*Scheme 6*). In the first, an intramolecular displacement at C(4) by the N^4-amino group occurs, *via* a strained transition entity **17**. Alternatively, if the base is acting as a nucleophile, an intermediate **18** may be postulated which would lead in a second displacement reaction to the thermodynamically and kinetically favoured 5-membered ring product. The fact that the rearrangement occurs in 2,6-lutidine strongly suggests that the first mechanism is the more likely route.

Scheme 6



7 **17** **18**

Experimental Part

General. Unless otherwise stated, reactions were worked up as follows: After removal of the solvent, the product was dissolved in $CHCl_3$ and washed with aq. sodium hydrogen carbonate soln. The combined org. fractions were dried (Na_2SO_4) and evaporated. TLC: pre-coated F_{254} silica gel plates. Column chromatography (CC): *Merck* silica gel *60* or reversed-phase column *LiChroprep RP-18* (*Merck*). M.p.: *Gallenkamp* melting point apparatus; uncorrected. UV Spectra: *Perkin-Elmer-Lambda-2* spectrophotometer; in 10% MeOH/H_2O unless otherwise stated; λ_{max} (ε) in nm. ^{1}H-NMR Spectra: *Bruker DRX 300*; in (D_6)DMSO, unless otherwise stated; δ in ppm, J values in Hz. NOE Experiments: *Bruker-AMX-500* spectrometer. Mass spectra: *Kratos MS890*; in *m/z* (rel. %).

*5-(2-Chloroethyl) 1-[2-deoxy-(3,5-di-*O*-(*p*-toluoyl)-β-*D*-ribofuranosyl]-4-(1*H*-1,2,4-triazol-1-yl)pyrimidin-1(1*H*)-one* (**5**). To a suspension of 1*H*-1,2,4-triazole (4.72 g, 68.3 mmol) in dry MeCN (100 ml) at 0°, phosphoric trichloride (1.27 ml, 13.7 mmol) was added dropwise, and the mixture was stirred at 0° for 15 min. After this time, dry Et_3N (11.5 ml, 82 mmol) was added and the mixture stirred at 0° for a further 20 min, then at r.t. for 10 min. A soln. of 5-(2-chloroethyl)-1-[2-deoxy-3,5-di-*O*-(*p*-toluoyl)-β-D-ribofuranosyl]pyrimidine-2,4-(1*H*,3*H*)-dione [11] (2.4 g, 4.5 mmol) in dry MeCN (10 ml) and DMF (10 ml) was then added dropwise with vigorous stirring. The mixture was kept under Ar at r.t. overnight and then evaporated. The residue was dissolved in $CHCl_3$, the soln. washed with aq. $NaHCO_3$ soln., dried, and evaporated, and the orange syrup purified by CC (silica gel, AcOEt/hexane 1:1): 2.27 g (86%) of **5**. White solid. UV: 323 (5200), 242 (33200), min. 216 (14200). M.p. 155–157°. ^{1}H-NMR: 2.28 (*s*, *Me*C_6H_4); 2.39 (*s*, *Me*C_6H_4); 2.59–2.68 (*m*, 1 H–C(2')); 2.87–3.17 (*m*, 1 H–C(2'), CH_2CH_2Cl); 3.57 (*t*, J = 7.1, $CH_2CH_2Cl_2$); 4.56–4.74 (*m*, H–C(4'), 2 H–C(5')); 5.62–5.65 (*m*, H–C(3')); 6.28 (*t*, J = 6.5, H–C(1')); 7.22, 7.36, 7.76, 7.93 (4 *d*, 8 arom. H (Tol)); 8.37 (*s*, H–C(6)); 8.40 (*s*, CH(triazole)); 9.36 (*s*, CH (triazole)). FAB-MS: 578.8 ([M + H]$^+$). HR-MS: 578.18268 ([M + H]$^+$, $C_{29}H_{29}N_5O_6{}^{35}Cl^+$; calc. 578.18066; deviation = 3.50 ppm).

6-[2-Deoxy-3,5-di-O-(p-toluoyl)-β-D-ribofuranosyl]-2,3,4,6-tetrahydropyrimido[4,5-c][1,2]pyridazin-7(1H)-one (**3**). To a soln. of **5** (0.25 g, 0.43 mmol) in dry MeCN (10 ml), anh. hydrazine (20 µl, 0.64 mmol) was added, and the soln. was stirred at r.t. for 2 h. The soln. was concentrated and chromatographed (5% MeOH/$CHCl_3$): 0.19 g (87%) of **3**. White solid. UV: 278 (10900), 244 (33300), min. 269 and 218; pH 1: 274 (10600), 240 (31850), min. 269 and 233; pH 12: 274 (10500), 240 (31750), min. 268 and 233. ^{1}H-NMR: 2.37 (*s*, *Me*C_6H_4); 2.38 (*s*, *Me*C_6H_4); 2.43–2.63 (*m*, CH_2, 2 H–C(2′)); 3.32 (br. *s*, NH); 3.49–3.69 (*m*, CH_2N); 4.44–4.48 (*m*, H–C(4′)); 4.50–4.63 (*m*, 2 H–C(5′)); 5.56 (br. *s*, H–C(3′)); 6.28–6.37 (*m*, H–C(1′)); 7.10 (*s*, H–C(5)); 7.31–7.36 (*m*, 4 arom. H (Tol)); 7.86–8.31 (*m*, 4 arom. H (Tol)); 9.64 (br. *s*, NH). FAB-MS: 505.9 ([*M* + H]$^+$). HR-MS: 505.20813 ([*M* + H]$^+$, $C_{27}H_{29}N_4O_6^+$; calc. 505.20871; deviation 1.10 ppm).

6-[2-Deoxy-3,5-di-O-(p-toluoyl)-β-D-ribofuranosyl]-2,3,4,6-tetrahydro-1-methylpyrimido[4,5-c][1,2]pyridazin-7(1H)-one (**7**). To a soln. of **5** (0.25 g, 0.43 mmol) in MeCN (10 ml), methylhydrazine (35 µl, 0.66 mmol) was added and the soln. stirred at r.t. for 8 h. The soln. was evaporated and the residue chromatographed (5% MeOH/$CHCl_3$): 0.19 g (85%) of **7**. Off-white foam. UV: 285 (12100), 243 (38700), min. 218 and 273; pH 1: 303 (14950), 244 (41700), min. 216 and 269; pH 12: 285 (13200), 239 (37600), min 229 and 266. ^{1}H-NMR: 2.22–2.32 (*m*, 2 H–C(4)); 2.37 (*s*, *Me*C_6H_4); 2.38 (*s*, *Me*C_6H_4); 2.37–2.54 (*m*, 2 H–C(2′)); 2.88 (*m*, CH_2N); 3.12 (*s*, MeN); 4.46–4.48 (*m*, H–C(4′)); 4.51–4.65 (*m*, 2 H–C(5′)); 5.52 (*t*, *J* = 6.9, NH); 5.56–5.59 (*m*, H–C(3′)); 6.33 (*t*, *J* = 6.3, H–C(1′)); 7.31–7.37 (*m*, 4 arom. H (Tol)); 7.34 (*s*, H–C(5)); 7.86-7.92 (*m*, 4 arom. H (Tol)). FAB-MS: 519.8 ([*M* + H]$^+$). HR-MS: 519.22802 ([*M* + H]$^+$, $C_{28}H_{31}N_4O_6^+$; calc. 519.22437; deviation −7.0 ppm).

In the same manner the 3′,5′-di-*O*-acetyl instead of 3′,5′-di-*O*-toluoyl derivative was prepared. White foam. UV: 292, 229, min. 250; pH 1: 305, min. 252. ^{1}H-NMR: 2.06 (*s*, Ac); 2.07 (*s*, Ac); 2.24–2.30 (*m*, 2 H–C(2′)); 2.51 (*m*, 2 H–C(4)); 2.97 (*t*, *J* = 5.7, CH_2N); 3.14 (*s*, MeN); 4.12–4.17 (*m*, H–C(4′)); 4.22–4.25 (*m*, 2 H–C(5′)); 5.15–5.19 (*m*, H–C(3′)); 5.59 (*t*, *J* = 5.7, NH); 6.24 (*t*, *J* = 6.7, H–C(1′)); 7.38 (*s*, H–C(5)). EI-MS: 366 ([*M* + H]$^+$). HR-MS: 366.1572 ([*M* + H]$^+$, $C_{16}H_{22}N_4O_6^+$; deviation 3.2 ppm).

7-Amino-3-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-ribofuranosyl]-3,5,6,7-tetrahydro-2H-pyrrolo[2,3-d]pyrimidin-2-one (**8a**). A soln. of **3** (200 mg, 0.6 mmol) in MeCN (10 ml) and Et_3N (1 ml) was heated under reflux overnight. Alternatively, a soln. of **3** was stirred in pyridine at r.t. overnight. After evaporation, the product was chromatographed (5% MeOH/$CHCl_3$): 146 mg (73%) of **8a**. White foam. UV: 283 (10400), 245 (32000), min. 270 and 215; pH 1: 286 (11100), 244 (29800), min. 270 and 215. ^{1}H-NMR: 2.37 (*s*, *Me*C_6H_4); 2.38 (*s*, *Me*C_6H_4); 2.44–2.58 (*m*, 2 H–C(2′), CH_2); 3.58 (*t*, *J* = 7.5, CH_2N); 4.22–4.23 (*m*, H–C(4′)); 4.50–4.63 (*m*, 2 H–C(5′)); 4.82 (*s*, NH_2); 5.54–5.56 (*m*, H–C(3′)); 6.36 (*t*, *J* = 7.8, H–C(1′)); 7.27 (*s*, H–C(4)); 7.32–7.36 (*m*, 4 arom. H (Tol)); 7.86–7.91 (*m*, 4 arom. H (Tol)). FAB-MS: 505.9 ([*M* + H]$^+$), 527.9 ([*M* + Na]$^+$). HR-MS: 527.18983 ([*M* + Na]$^+$, $C_{27}H_{28}N_4O_6Na^+$; calc. 527.19067; deviation 1.60 ppm).

Benzaldehyde Hydrazone of **8a**: After treatment of **8a** with benzaldehyde in CH_2Cl_2, the soln. was evaporated and the residue chromatographed (2% MeOH/$CHCl_3$): pale yellow solid which recrystallized from EtOH. M.p. 200–202°. UV: 327 (23900), 240 (21200), min. 272 and 222; pH 1: 334 (17800), 247 (19600), min. 296 and 226; pH 12: 328 (24200), 240 (21000), min. 272. ^{1}H-NMR: 2.35 (*s*, *Me*C_6H_4); 2.39 (*s*, *Me*C_6H_4); 2.43–2.63 (*m*, 2 H–C(2′)); 2.71–2.88 (*m*, CH_2); 3.94–4.00 (*m*, CH_2(6)), 4.51–4.58 (*m*, H–C(4′)); 4.59–4.67 (*m*, 2 H–C(5′)); 5.57–5.60 (*m*, H–C(3′)); 6.36 (*t*, *J* = 7.5, H–C(1′)); 7.31–7.37 (*m*, 4 arom. H (Tol)); 7.40–7.47 (3 H, *m*, 3 arom. H (Ph)); 7.63 (*s*, H–C(4)); 7.74 (1 H, *s*, 1 arom. H (Ph)); 7.77 (*s*, N=CH); 7.90 (*s*, 1 arom. H(Ph)); 7.86–7.95 (*m*, 4 arom. H (Tol)). FAB-MS: 593.24 ([*M* + H]$^+$). HR-MS: 593.23926 ([*M* + H]$^+$, $C_{34}H_{33}N_4O_6^+$; calc. 593.23999; deviation 1.20 ppm).

3-[2-Deoxy-3,5-di-O-(p-toluoyl)-β-D-ribofuranosyl]-3,5,6,7-tetrahydro-7-(methylamino)-2H-pyrrolo[2,3-d]pyrimidin-2-one (**8b**) (*Method A*). A soln. of **7** (150 mg, 0.29 mmol) in MeCN (10 ml) and Et_3N (0.5 ml) was heated under reflux overnight. After evaporation, the product was chromatographed (5% MeOH/$CHCl_3$) to give a white solid which was recrystallized from EtOH: 124 mg (83%) of **8b**. UV: 283 (10800), 244 (31900), min. 269 and 217; pH 1: 293 (12800), 244 (32900), min. 268 and 216; pH 12: 283 (11900), 240 (31700), min. 265 and 229. M.p. 175–176°. ^{1}H-NMR: 2.37 (*s*, *Me*C_6H_4); 2.38 (*s*, *Me*C_6H_4); 2.49–2.65 (*m*, 2 H–C(2′), CH_2(5)); 3.34 (*d*, MeN, *s* after D_2O wash); 3.59 (*t*, *J* = 7.5, CH_2(6)); 4.43–4.63 (*m*, H–C(4′), 2 H–C(5′)); 5.30 (*q*, *J* = 5.7, exchangeable NH); 5.50–5.60 (*m*, H–C(3′)); 6.34 (*t*, *J* = 7.6, H–C(1′)); 7.31–7.36 (*m*, H–C(4), 4 arom. H (Tol)); 7.85–7.91 (*m*, 4 arom. H (Tol)). FAB-MS: 519.3 ([*M* + H]$^+$). HR-MS: 519.22380 ([*M* + H]$^+$, $C_{28}H_{31}N_4O_6^+$; calc. 519.22437; deviation 1.10 ppm). Anal. calc. for $C_{28}H_{30}N_4O_6$: C 64.9, H 5.8, N 10.8; found: C 64.93, H 5.87, N 10.80.

6-(3,5-Di-O-acetyl-2-deoxy-β-D-ribofuranosyl)-1,2,3,4,6,7-hexahydro-2,2-dimethyl-7-oxopyrimido[4,5-c][1,2]pyridazinium (**11**). To a soln. of the 3′,5′-di-*O*-acetyl-substituted analog of **5** [16] (1 g, 1.7 mmol) in tetrachloroethane (25 ml), 1,1-dimethylhydrazine (0.4 ml, 5.3 mmol) was added and the soln. heated at 100° overnight (TLC: two main products, one with R_f 0). The product was extracted (H_2O/$CHCl_3$), and each of the

two layers was evaporated. The org. layer was chromatographed (5% MeOH/$CHCl_3$) to give an off-white foam, which was characterized as *5-(2-chloroethyl)-1-(3,5-di-*O*-acetyl-2-deoxy-β-*D*-ribofuranosyl)-*N^4*-(dimethylamino)cytosine* (**9**; 0.32 g, 33%). Off-white foam. ^{1}H-NMR: 2.06 (*s*, Ac); 2.07 (*s*, Ac), 2.22–2.36 (*m*, 2 H–C(2′)); 2.50 (*s*, Me_2N); 2.95–3.05 (*m*, CH_2); 3.14 (*s*, CH_2Cl); 4.12–4.19 (*m*, H–C(4′)); 4.23–4.25 (*m*, 2 H–C(5′)); 5.14–5.19 (*m*, H–C(3′)); 5.59 (br. *s*, NH); 6.24 (*t*, *J* = 6.7, H–C(1′)); 7.38 (*s*, H–C(6)). EI-MS: 417 (M^+).

The aq. layer was evaporated and purified by CC (reversed-phase *C-18* silica gel, $H_2O \rightarrow 25\%$ MeOH/H_2O): 0.28 g (31%) of **11**. Brown foam. ^{1}H-NMR: 2.06 (*s*, Ac); 2.07 (*s*, Ac); 2.12–2.32 (*m*, 2 H–C(2′)); 2.79 (*t*, *J* = 5.3, CH_2); 3.15 (*s*, Me_2N^+); 3.44 (*t*, *J* = 5.6, CH_2N); 4.06–4.10 (*m*, H–C(4′)); 4.20–4.27 (*m*, 2 H–C(5′)); 5.14–5.16 (*m*, H–C(3′)); 6.24–6.30 (*m*, H–C(1′)); 7.23 (*s*, H–C(6)); 8.24 (*s*, NH). EI-MS: 381 (M^+).

*7-{[(Benzyloxy)carbonyl]methylamino}-3-[2-deoxy-3,5-di-*O*-(*p*-toluoyl)-β-*D*-ribofuranosyl]-3,5,6,7-tetrahydro-2*H*-pyrrolo[2,3-*d*]pyrimidin-2-one* (**13**). To a soln. of **5** (0.5 g, 0.865 mmol) in tetrachloroethane (10 ml), 1-[(benzyloxy)carbonyl]-1-methylhydrazine [14] (0.38 g, 2.14 mmol) was added and the soln. heated at 85° overnight. After dilution with $CHCl_3$ and workup as described, the product was chromatographed (AcOEt/hexane/MeOH 1:1:0 → 7:3:0.1): 0.52 g (97%) of **13**. Off-white solid. UV: 283 (10400), 241 (34800), min. 267 and 219; pH 1: 300 (9900), 245 (24200), min. 269. ^{1}H-NMR: 2.37 (*s*, MeC_6H_4); 2.39 (*s*, MeC_6H_4); 2.53–2.72 (*m*, 2 H–C(2′), CH_2(5)); 3.08 (*s*, MeN); 3.60–3.82 (*m*, CH_2(6)); 4.48–4.63 (*m*, H–C(4′)); 2 H–C(5′)); 5.09–5.14 (*m*, CH_2); 5.56–5.58 (*m*, H–C(3′)); 6.33 (*t*, *J* = 7.3, H–C(1′)); 7.22–7.39 (*m*, 8 arom. H); 7.56 (*s*, H–C(4)); 7.84–7.92 (*m*, 5 arom. H). FAB-MS: 675.2448 ($[M+Na]^+$). HR-MS: 675.2448 ($[M+Na]^+$, $C_{36}H_{36}N_4O_8Na^+$; calc. 652.2431; deviation 2.50 ppm).

Pyrrolopyrimidinone **8b** (*Method B*). To a soln. of **13** (0.4 g, 0.6 mmol) in dry MeOH (20 ml), 10% Pd/C (or PtO_2) catalyst (50 mg) was added and the soln. stirred under H_2 for 2 h. The suspension was filtered through *Celite* and the cake washed with MeOH. The solvent was evaporated and the residue chromatographed (5% MeOH/$CHCl_3$): 0.25 g (79%) of **8b**. White solid. For data, see above (*Method A*).

*3-[2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-β-*D*-2-ribofuranosyl]-3,5,6,7-tetrahydro-2*H*-pyrrolo[2,3-*d*]pyrimidin-2-one* (**14**). To a soln. of **13** (0.4 g, 0.6 mmol) in AcOH (20 ml), *Adam*'s catalyst (50 mg) was added and the soln. stirred under H_2 for 4 h. Workup and FC as described for **8b** (*Method B*) gave **16** (0.13 g, 43%). White solid. UV: 277 (8400), 244 (32800), min. 265 and 216; pH 1: 285 (9500), 244 (31600), min. 269 and 220. ^{1}H-NMR: 2.37 (*s*, MeC_6H_4); 2.38 (*s*, MeC_6H_4); 2.42–2.49 (*m*, 2 H–C(2′)); 2.61–2.69 (*m*, CH_2); 3.49 (*t*, *J* = 7.8, CH_2(6)); 4.43–4.44 (*m*, H–C(4′)); 4.50–4.63 (*m*, 2 H–C(5′)); 5.55–5.57 (*m*, H–C(3′)); 6.34 (*t*, *J* = 7.9, H–C(1′)); 7.32–7.37 (*m*, H–C(6), 4 arom. H (Tol)); 7.86–7.92 (*m*, 4 arom. H (Tol)); 7.99 (*s*, NH). FAB-MS: 490.7 ($[M+H]^+$). HR-MS: 490.19792 ($[M+H]^+$, $C_{27}H_{28}N_3O_6^+$; calc. 490.19781; deviation −0.20 ppm).

*5-(2-Chloroethyl)-1-[2-deoxy-3,5-di-*O*-(*p*-toluoyl)-β-*D*-ribofuranosyl]cytosine* (**15**). A soln. of **5** (0.25 g, 0.43 mmol) in ammonia-saturated dioxane (10 ml) was stirred at r.t. overnight. The soln. was evaporated and the product chromatographed (5% MeOH/$CHCl_3$) to give a white solid. Attempts to recrystallize resulted in gel formation: 0.22 g (97%) of **15**. UV: 248 (23000), 272 (sh), min. 232; pH 1: 285 (11000), 245 (29300), min. 220 and 268. ^{1}H-NMR: 2.37 (*s*, MeC_6H_4); 2.39 (*s*, MeC_6H_4); 2.44–2.57 (*m*, 2 H–C(2′), CH_2); 3.53 (*t*, *J* = 7, CH_2Cl); 4.47–4.64 (*m*, H–C(4′), 2 H–C(5′)); 5.57–5.59 (*m*, H–C(3′)); 6.31 (*t*, *J* = 7.6, H–C(1′)); 7.09 (br. *s*, NH_2); 7.30–7.36 (*m*, 4 arom. H (Tol)); 7.47 (*s*, H–C(6)); 7.85–7.92 (*m*, 4 arom. H (Tol)). FAB-MS: 526.9 ($[M+H]^+$), 548.9 ($[M+Na]^+$). HR-MS: 548.15620 ($[M+Na]^+$, $C_{27}H_{28}N_3O_6{}^{35}ClNa^+$; calc. 548.15643; deviation 0.40 ppm).

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