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SYNTHESIS OF 2'-DEOXY-1-DEAZAWYOSINE

A STABILE 2'-DEOXYISOSTERE OF A RARE tRNA CONSTITUENT

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Abstract—2'-Deoxy-1-deazawyosine (**3a**), an isostere of the rare tRNA constituent wyosine (**1a**) and its α-anomer have been synthesized via glycosylation of the nucleobase **5a** with the halogenose **6**. In contrast to the labile parent **1a**, the isostere is highly stabile against hydrolysis even as 2'-deoxynucleoside. The stability of **3a** is due to the low susceptibility of the pyrrol ring to protonation. The rapid hydrolysis of wyosine (**1a**) compared to guanosine is explained by a favoured solvatization of the molecule being freezed in the *anti*-conformation.

The occurrence of rare nucleosides is a characteristic feature of tRNA.[1] It has been considered that these hypermodified molecules play an important role in the process of codon anticodon recognition[2] and in the interaction with nucleotide binding enzymes, such as synthetases.

Besides mono- and bicyclic structures the rare tricyclic nucleoside wyosine (**1a**)[3] has been isolated. It is located adjacent to the 3'-site of the anticodon of $tRNA^{Phe}$ from Turolopsis utilis. Related compounds like wybutosine (**1b**)[4] and its peroxide wybutoxosine (**1c**)[5] have been found at the same position of tRNAs of various sources including mammalian liver tissue. These nucleosides have been the subject of intense chemical and biochemical studies because of their intensive fluorescence[3] and extreme hydrolytic lability.[6] Moreover, a tRNA containing its own fluorescent probe is ideally suited to study conformational changes in the anticodon region.[7]

Structure elucidation of wyosine has been accomplished using its nucleobase wye (**2a**)[8,9] which is excits from tRNA by mild acid treatment.[10] Soon after the isolation of **2a** wyosine was suggested to have the N-3 ribosyl structure **1a**. Unfortunately, the synthesis of wyosine proved to be extremely difficult, because direct glycosylation of **2a**, led to the N-1 isomer.[3] It was finally elaborated by Goto's group[11] and by Itaya *et al.*[12] via N-3-methylguanosine[11,13] as the key intermediate thus confirming the proposed structure.

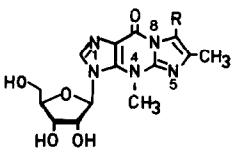
The selective excision of wye from tRNA which leaves the intact polynucleotide is one important example where just one nucleobase can be split off at its N-glycosidic bond. More recently replacement of guanine by 7-deaza-guanine derivatives in an enzymic transglycosylation has been observed which makes this class of heterocyclic bases particularly interesting.[14]

Our studies on 7-deazapurine (pyrrolo[2,3-d]pyrimidine) nucleosides like 7-deazaguanosine[15] or its 2'-deoxy derivative[16] have demonstrated them to have an extremely stabile N-glycosylic bond which even allows proton catalysed anomerisation and isomerisation of the sugar moiety.[17]. Therefore, we anticipated that a 1-deazaisostere of wyosine should have a stabile N-glycosylic bond as well, even when a 2'-deoxyfuranosyl residue as in **3a** is attached to the aglycon which normally decreases the hydrolytic stability of the nucleosides by two or three orders of magnitude.[18] In the following we present the synthesis of 1-deaza-2'-deoxywyosine (**3a**) and compare some of its properties with that of wyosine.

The main obstacle in the synthesis of wyosine, glycosylation of **2a** at the wrong position was not to be expected with the 1-deazaisostere, since we had learned from the synthesis of 7-deazaguanosine[15] and 7-deaza-2'-deoxyguanosine[16] that regioselective glycosylation at the pyrrolo nitrogen could be achieved by formation of a pyrrolyl anion.[19] This is generated by either sodium hydride or phase-transfer catalysis using a two-phasic mixture of dichloromethane and 50% aqueous sodium hydroxide.

The starting material for the synthesis of **3a**, N-3-methyl-7-deazaguanine (**4**) hydrochloride,[19,20] is formed regioselectively by condensation of N-methylguanidine with ethyl 2-cyano-2-(2',2'-diethoxyethyl)butyrate followed by cyclization of the pyrimidine intermediate. Condensation of 3-methyl-7-deazaguanine (**4**) with chloroacetone should give the tricyclic heterocycles **5a** or **5b**. The reaction was performed in anhydrous hexamethylphosphoric acid triamide at room temperature with potassium carbonate as base resulting in a smooth conversion to one single reaction product that was crystallized from methanol. The ^{1}H-NMR data of this material exhibit close resemblance for the imidazole and Me signals with those of wye (**2a**). This implied that condensation of chloroacetone with 3-methyl-7-deazaguanine (**4**) occurred regioselectively under formation of **5a**. However, a reaction of **4** to the isomer **5b** could not be excluded by this analogy.

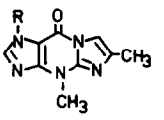
Thus, we carried out the synthesis of the isomeric **5b**. Condensation of 3-methyl-7-deazaguanine (**4**) with α-chloropropionaldehyde yields a reaction product that was different from that prepared with chloroacetone. This is revealed clearly by ^{1}H-NMR spectroscopy (Table 1) which furthermore allows to assign the position of the Me group at C-2 or C-3. The C-Me group which is identified by its long range coupling constant (^{3}J) with the vicinal olefinic proton of about 1 Hz, is found at 2.25 ppm in the condensation product of chloroacetone and at 2.66 ppm in the isomeric compound; the opposite trend is found in the chemical shifts of the imidazole proton. This is due to the deshielding effect of the carbonyl group and



1a: R = H

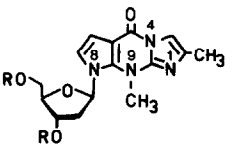
b: R = $CH_2CH_2CHCO_2CH_3$ (NHCO$_2$CH$_3$)

c: R = $CH_2CHCHCO_2CH_3$ (HOO, NHCO$_2$CH$_3$)



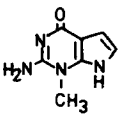
2a: R = H

b: R = CH_3



3a: R = H

b: R = Tol



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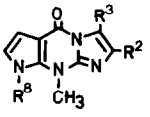
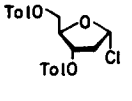


	R^2	R^3	R^8
5a	CH_3	H	H
b	H	CH_3	H
c	CH_3	H	CH_3



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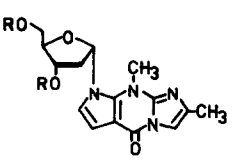
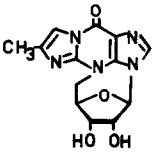


7a: R = H

b: R = Tol



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demonstrates that the product of condensation with chloroacetone has the structure **5a** and the other is the isomer **5b**. These results are in agreement with those reported for wye (**2a**) and its isomer.[3]

Further structural proof came from the ^{13}C-NMR spectra (Table 2). For the assignment of the ^{13}C resonances the fully coupled spectrum of **5a** was measured. Therefrom (Table 3) the carbons bearing a proton are readily identified by their large one-bond coupling constants ^1J. The resulting doublets are further split due to long-range coupling. C-3 is thus found from the quadruplet due to the coupling to the vicinal Me group (^3J = 4.8 Hz). The pyrrol carbons C-6 and C-7 show coupling to their respective neighbor protons and furthermore to the exchangeable imino proton NH which hence is linked to N-8; these couplings disappear after H/D exchange (D_2O). The signal of C-5a which appears at highest field of the quarternary carbons also shows a coupling to exchangeable NH besides two others with 6-H and 7-H. C-2 appears as a doublet of quartets (dq, ^2J = 8.9 and 7.0 Hz) due to coupling with 3-H and 2-CH_3, it is distinguished from the dq of C-9a (^3J = 3.2 and 7.8 Hz) because the three-bond coupling constant of a methyl group is usually about 3.5 Hz and not 7.0 Hz which is therefore a two-bond coupling. The very complex coupling pattern of C-8a is not resolved; the remaining signal is the broad singlet of C-5 whose two long-range couplings, with 3-H and 6-H are obviously small (< 3 Hz) as is expected for a *cis* coupling.[21]

Table 1. ^{1}H-NMR data of imidazo[1,2-a]pyrrolo[2,3-d]pyrimidines in Me_2SO-d_6[a]

Compd	H-3	H-8	H-9	N—CH_3	CH_3	Ring NH
5a	7.32 (q, 0.8)	6.55 (d, 3.6)	6.95 (d, 3.6)	3.87	2.25 (d, 0.8)	11.8
5c	7.29 (q, 1.2)	6.48 (d, 3.6)	6.83 (d, 3.6)	4.13 (N-2)	2.25 (d, 1.2)	4.03 (N-1 CH_3)
7a	7.28 (q, 0.9)	6.54 (d, 3.7)	7.36 (d, 3.7)	4.11	2.20 (d, 0.9)	—
3a	7.30 (q, 0.9)	6.56 (d, 3.6)	7.19 (d, 3.6)	4.13	2.20 (d, 0.9)	—
5b	6.71 (q, 1.1)[b]	6.51 (d, 3.5)	6.91 (d, 3.5)	3.79	2.66 (d, 1.1)	n.d.
	H-1′	H-2′$_a$	H-2′$_b$	H-3′	H-4′	2 H-5′
7a	6.59 (dd, 3.6, 7.2)[c]	2.77	2.33	4.36	3.72	3.5–3.7
3a	6.54 (t, 6.4)[c]	2.61	2.35	4.35	3.86	3.46

[a] Chemical shifts are given as δ values relative to TMS as internal standard. Coupling pattern and constants in Hertz (Hz) are given in parentheses: d, doublet; q, quartet; t, triplet; n.d., not detected.

[b] H-2.

[c] Couplings were determined in D_2O solution where 1′-H and 8-H do not overlap.

Table 2. ^{13}C-NMR data of imidazo[1,2-a]pyrrolo[2,3-d]pyrimidines and N^3-methyl-7-deazaguanine hydrochloride (**4** · HCl) in hexadeuterio-dimethylsulfoxide[a]

Compd	C-2	C-3	C-5	C-5a	C-6	C-7	C-8a	C-9a	CH_3	9-CH_3	C-1'	C-2'	C-3'	C-4'	C-5'
5a	136.7	104.7	153.2	99.7	103.2	117.4	139.9	142.3	13.9	31.8					
5b	125.2	121.8	156.0	98.0	103.4	117.1	140.3	143.4	12.1	31.8					
5c	136.7	104.5	152.6	99.7	101.7	124.3	138.6	143.0	13.8	33.3	36.1[b]				
3a	138.8	104.6	152.7	99.8	102.4	119.6	138.8	143.2	13.8	34.6	85.5	40.2	70.4	88.0	61.4
7a	138.8	104.6	152.7	99.9	103.0	118.0	138.8	143.2	13.8	34.2	84.6	40.1	70.2	87.4	61.4
4 · HCl[c]			156.0	101.1	103.9	118.8	138.0	150.8		33.2					

[a] Chemical shifts given as δ values relative to TMS as internal standard.
[b] N^8-CH_3.
[c] The numbering follows the imidazo[1,2-a]pyrrolo[2,3-d]pyrimidine system instead of the pyrrolo[2,3-d]pyrimidine system.

The UV spectra of c^{1}wye (**5a**) and its isomer **5b** are similar under neutral and alkaline conditions (Table 4). In acidic media, however, the isomer **5b** exhibits a shoulder at 345 nm which is not found in the spectrum of **5a**. The long wavelength absorption of c^{1}wye (**5a**) is shifted hypsochromically compared to the isomer **5b** at pH 7.0, the same is true for wye (**2a**) and isowye.

From the distinct differences of the UV spectra the pK_a values of **5a** and **5b** were determined in Teorell/Stenhagen buffer solutions. Both compounds exhibit two pK_a values: one for the protonation (pK_a = 4.2; **5a** and **5b**) and the other for the formation of the anion; pK_a = 11.1 (**5a**) and pK_a = 10.8 (**5b**).

When the pK values of N-3-methyl-7-deazaguanine (**4**) and 7-deazaguanine are compared it can be seen that N-3 methylation strongly enhances the basicity of the heterocycle and the pK_{a1} value is raised almost three pH-units. Part of this basicity is lost on annelation of the imidazole ring as shown by compounds **5a–c** (Table 4).

The ease of the formation of the pyrrolyl anion in c^{1}wye compared to other pyrrolo[2,3-d]pyrimidines should allow efficient N-3 alkylation when alkaline conditions are employed. To elaborate reaction conditions and to establish the site of electrophilic attack we checked this by methylation. The reaction of **5a** in DMF with methyl iodide in the presence of sodium hydride leads to one reaction product. This was considered to have structure **5c** because the UV spectrum was very similar to that of the starting material (Table 4). In contrast to **5a**, **5c** exhibits almost identical UV spectra under neutral and alkaline conditions, thus no acidic pK_a value was found. The methylation position of **5c** was confirmed by the proton-coupled ^{13}C-NMR spectrum which shows a coupling of C-7 with the protons of the N-8 Me group (^3J = 3.6 Hz; Table 3).

After the position of alkylation had been confirmed, glycosylation was performed under similar conditions using the protected halogenose **6**. As expected

Table 3. ^{13}C-, ^{1}H-NMR coupling constants for imidazo[1,2-a]pyrrolo[2,3-d]pyrimidines in Me_2SO-d_6[a]

Compd	C-2			C-3			C-5			C-5a		
		$^2J_{3\text{-H}}$	$^2J_{2\text{-CH}_3}$		$^1J_{3\text{-H}}$	$^3J_{2\text{-CH}_3}$	$^3J_{3\text{-H},6\text{-H}}$		$^2J_{6\text{-H}}$	$^3J_{7\text{-H}}$	$^3J_{8\text{-NH}}$
5a	dq[b]	8.9	7.0	dq	194.6	4.8	br[c] s < 3	dd	4.5	7.5	4.5
5a[f]	dq	9.0	7.4	dq	195.8	4.9	br s < 3	dd	4.5	7.9	
5b	dq	14.8[g]	7.3[g]	dq	187.2[g]	5.0[g]	br s < 3	dd	4.8	7.2	
5c	dq	8.9	6.9	dq	194.8	4.7	br s < 3	dd	4.2	8.1	
3a	dq	8.9	7.0	dq	194.9	4.7	br s < 3	dd	4.0	7.9	
7a	dq	8.5	6.8	dq	195.3	4.7	br s < 3	dd	4.1	8.0	

Compd	C-6				C-7				C-9a		C-8a
		$^1J_{6\text{-H}}$	$^2J_{7\text{-H}}$	$^3J_{8\text{-NH}}$		$^1J_{7\text{-H}}$	$^2J_{6\text{-H}}$	$^{2,3}J$		$^3J_{3\text{-H}}$	$^3J_{9\text{-CH}_3}$	
5a	ddd	176.1	7.8	5.4	ddd	189.4	7.5	1[h]	dq	7.6	3.2	m n.r.[e]
5a	dd	175.8	9.2		dd	191.2	7.1			n.d.[d]		m n.r.
5b	dd	175.9	8.0		dd	189.3	8.2		dq	12.1	3.4	m n.r.
5c	dd	178.0	7.1		ddq	191.4	7.2	3.6[i]	dq	7.6	3.8	m n.r.
3a	dd	177.9	6.8		ddd	192.4	8.2	4.0[k]	dq	7.7	3.0	m n.r.
7a	dd	177.7	7.0		ddd	197.5	8.1	4.0[k]	dq	7.7	3.0	m n.r.

[a] Values are given in Hertz (Hz) and are estimated to be accurate to ±0.2 Hz.
[b] Multiplicities: d, doublet; q, quartet; m, multiplet; s, singlet.
[c] br, broad.
[d] n.d. not detected due to overlapping signals.
[e] n.r. not resolved.
[f] After addition of D_2O.
[g] Coupling constants of C-2 and C-3 are listed under C-3 and C-2, respectively.
[h] $^2J_{8\text{-NH}}$.
[i] $^3J_{8\text{-CH}_3}$.
[k] $^3J_{1'\text{-H}}$.

Table 4. Physical properties of imidazo[1,2-a]pyrrolo[2,3-d]pyrimidines

Compd	pK_{a1}	pK_{a2}	UV spectra H_2O (pH 1.0) λ_{max} (nm)	UV spectra H_2O (pH 7.0) λ_{max} (nm)	UV spectra H_2O (pH 13.0) λ_{max} (nm)	Fluorescence data Emission λ_{max} (nm)	Fluorescence data Excitation λ_{max} (nm)
5a	4.2	11.0	282, 226	302, 262, 228	316, 275, 236	415, 475 (sh)	306
5b	4.2	10.8	345 (sh), 272, 221	307, 269, 224	319, 270, 238	419, 480 (sh)	305
5c	4.1	—	280, 233	305, 270, 233	306, 267, 233	412, 475 (sh)	310
3a	3.75	—	277, 230	304, 270, 234	303, 265, 230	400	303
7a			278, 232	305, 270, 234	304, 265, 235		
4	5.3	11.1	262, 220	272	280, 231		
c[7]G	2.5	10.3					
2a[3]	3.7	8.5	284, 255, 231	306, 265, 231[a]	301, 274, 231[b]	450, 470[30]	318
Isowye[3]	3.8	8.7	288, 251 (sh), 232	313, 256, 232[a]	307, 264, 233[b]		
2b[31]	3.4	—	277, 228	297, 264, 233	297, 264, 233		
1a[27]	3.1	—	277, 230	296, 236	295, 236		

[a] pH 5.8.
[b] pH 11.0.
[c] [7]G, 7-deazaguanine; isowye, 7-methyl isomer of compound **2a**.

glycosylation results in the formation of an anomeric mixture of fluorescent material. Chromatography on silica gel separated by-products and the mixture of anomers **3a** and **7a** was obtained in 43% yield. The moderate yield of glycosylation may be due to orthoamides that can be formed under these conditions.[22] This is supported by the finding that the crude product mixture which did not contain the starting material **5a** (TLC) gave rise to the formation of the chromophore **5a** after treatment with sodium methylate. The anomers **3a** and **7a** are not cleaved under these conditions.

Since the protected nucleosides **7b** and **3b** were tedious to separate the material was deprotected and then subjected to chromatography. Two zones were obtained and the material of the faster migrating zone is assigned the β-configuration (**3a**) from its ^{1}H-NMR spectrum (pseudo triplet of the anomeric proton;[23] Table 1). The material of the slower migrating zone with a doublet of doublets for the 1'-H signal is the α-anomer **7a**. This assignment is also confirmed by the distance of the 2'-H_a and 2'-H_b signals which is more pronounced in the α- than in the β-anomer.[24] 1-Deazawye (**5a**) and its derivatives **5b** and **5c** exhibit fluorescence but the intensity is not as pronounced as that of their natural counterparts. As is expected the excitation maxima (Table 4) are almost identical to the absorption maxima and furthermore similar to that of wye (**2a**).

In comparison to wye (**2a**) the fluorescence emission of the 1-deaza isosteres are hypsochromically shifted (Table 4). An even more pronounced shift is observed for the nucleoside **3a**.

The stability of the N-glycosylic bond of the isostere **3a** is remarkably increased in comparison to wyosine (**1a**). No cleavage is observed in 0.1 N HCl at room temperature overnight. By raising the temperature to 70° slow release of the nucleobase can be observed which is complete within 24 hr.

It is known that 2'-deoxynucleosides are less stabile than ribonucleosides by about two orders of magnitude.[6] This might impair an isolation of a 2'-deoxynucleoside of wye, as has been proposed by Reese[6] and demonstrates that introduction of the nucleobase **5a** dramatically strengthens the N-glycosylic bond. A mechanistical interpretation of this fact can be given as follows: According to pK_a measurements (Table 4) the isostere **3a** ($pK_a = 3.75$) has a similar pK of protonation as wyosine ($pK_a = 3.1$). It seems likely that the position of protonation is the same in both molecules. This is supported by the UV spectra of **1a** and **3a**, which exhibit almost the same shifts in going from neutral to acidic medium.

From other pyrrolo[2,3-d]pyrimidines it is known that protonation of the pyrrol system is most difficult to achieve.[25] We therefore suggest that protonation of **3a** occurs either at O-5 or at one of the nitrogens of the guanidine moiety. The latter confines the positive charge within this part of the molecule. Protonation of O-5, however, would allow location of the positive charge at the nitrogen of the glycosylic centre which is a prerequisite for hydrolytic cleavage of **3a** and **1a** as well. We believe that the hydrolysis of 1-deazawyosine (**3a**) is initiated by O-5 protonation. As this is done at the cost of dearomatization of the pyrrol moiety the reaction is not favoured.

A similar situation is found in N-6-dimethyl-3-methyladenosin.[26] In contrast to **3a** wyosine (**1a**) can also be protonated at N-1. Protonation at that position can easily initiate hydrolysis of the glycosylic bond and would explain the differences in the stability of wyosine (**1a**) and its 1-deaza isostere (**3a**). A still open question is the extraordinary rate of hydrolysis of wyosine (**1a**) or 3-methyl-guanosine compared to guanosine.

The methylation at N-4 in wyosine or N-3 in 3-methylguanosine must account for this phenomenon but the reason for this is hitherto far from known. The +I-effect of the N-4-Me group cannot account for the destabilization of the glycosylic bond as it is demonstrated by the hydrolytic stability of 4,5'-cyclowyosine (**8**) which is comparable to that of guanosine; the same is true for 3,5'-cycloguanosine.[6]

Itaya *et al.*[27] have suggested that the enhancement of hydrolysis is due to steric assistance of the Me substituent, supported by the finding that bulkier alkyl groups increase the rate of hydrolysis. From model building studies of **1a** it is evident that the nucleobase is forced into a rather narrow range of conformations in the anti region. The *syn* conformation can be achieved only if considerable stress is posed onto the glycosylic bond. That the *anti* conformation is predominant in **3a**

also is reflected by its ^{13}C-NMR coupling constant with the anomeric proton ($^3J_{C\text{-}7,1'\text{-}H}$) of 4 Hz giving a torsion angle of $\phi = 140°$[28] which corresponds to a glycosylic torsion angle χ of $-20°$ or $-100°$, both in the *anti* range.[29]

This *anti* orientation is the most striking difference between the hydrolytic labile wyosine (**1a**) and the relatively stabile cyclonucleoside **8**. We would like to suggest that the extreme enhancement of hydrolysis of **1a** is due to the *anti* orientation of the sugar moiety. In this extended form the molecule is much more susceptible to solvation by water molecules than in the compact *syn* form. If the hydrolysis follows a A_1^{35} mechanism the charged sugar intermediate is much better stabilized in the highly hydrated *anti* than in the poorly solvated *syn* conformation.

EXPERIMENTAL

General

M.ps were determined on a Berl apparatus (Wagner and Munz, FRG) and are not corrected. Elemental analyses were performed by Mikroanalytisches Labor Beller (Göttingen, FRG). NMR spectra were recorded on Varian EM 390 or Bruker WM 250 spectrometers. Unless otherwise stated spectra were measured in $(CD_3)SO$; δ values are in ppm with Me_4Si as internal standard. UV spectra were recorded on a Uvicon 810 (Kontron, Switzerland) or Shimadzu UV 210 A spectrometer and fluorescence spectra with a Perkin–Elmer MPF 4 spectrometer. Mass spectra were measured by a Varian MAT 311 A spectrometer. The p*K* values were determined spectrophotometrically at 20° in Teorell–Stenhagen buffer.[32] TLC was carried out on silica gel SilG-25 UV 254 plates (Macherey–Nagel, FRG). Column chromatography was performed on silica gel 60, 230–400 mesh ASTM (Merck, FRG). Solvent systems: (A) CH_2Cl_2–MeOH (95:5), (B) $CHCl_3$–MeOH (9:1), (C) $CHCl_3$–MeOH (4:1), (D) $CHCl_3$, (E) CH_2Cl_2–MeOH (98:2).

5,9 - Dihydro - 2,9 - dimethyl - 5 - oxo - 8,9H - imidazo[1,2-a] pyrrolo[2,3-d]pyrimidine (**5a**)

Compound **4**[19,20] (1.45 g, 7.2 mmol) was dissolved in 1 N NaOH (20 ml) at elevated temp. After cooling the soln was adjusted to pH 8.0 with 1 N AcOH leading to precipitation of colourless material which was complete after 5 hr at 4°. It was filtered off, dried and suspended in HMPA (50 ml). K_2CO_3 (1.4 g, 13 mmol) and chloroacetone (2 ml, 2.3 g, 25 mmol) were added under stirring. Stirring was continued for 5 hr at ambient temp, solid material was filtered off and the filtrate was evaporated *in vacuo*. The resultant was crystallized from MeOH (150 ml) to yield colourless crystals (500 mg). Another 90 mg were obtained after concentration of the mother liquor raising the total yield to 590 mg (40%). Recrystallization from $CHCl_3$ gave colourless needles, m.p. 282° (dec); TLC (C) R_f 0.8; UV (MeOH): λ_{max} 300, 262, 230 nm (ε 8000, 3800, 31 800); MS (*m/e*) 202 (M^+), 187, 173, 153, 135, 119. (Found: C, 59.36; H, 5.07; N, 27.64. Calc for $C_{10}H_{10}N_4O$ (202.2): C, 59.40; 4.98; N, 27.71%.)

*5,9 - Dihydro - 3,9 - dimethyl - 5 - oxo - 8,9H - imidazo[1,2-a] pyrrolo[2,3-d]*pyrimidine (**5b**)

Compound **5b** was prepared as described for **5a** from 4-hydrochloride (122 mg, 0.61 mmol) but instead of chloroacetone α-chloropropionaldehyde[34] (1 ml, 1.18 g, 13 mmol) was used. The residue obtained from evaporation of HMPA was dissolved in CH_2Cl_2–MeOH (4:1, 10 ml) and adsorbed on silica gel (5 g). From the total the solvent was evaporated and the silica gel suspended in solvent A (10 ml) and the slurry applied to the top of a silica gel column (30 × 2 cm). Elution with the same solvent afforded a main zone. After pooling the fractions and evaporation a yellowish material (55 mg, 45%) was obtained. Recrystallization from $CHCl_3$ yielded colourless needles, m.p. 306° (dec.); TLC (C) R_f 0.8; UV (MeOH) λ_{max} 304, 271 (sh), 264, 227 nm (ε 7800, 4500, 4800, 30 000). (Found: C, 59.50, H, 5.11, N, 27.64. Calc for $C_{10}H_{10}N_4O$ (202.2): C, 59.40; H, 4.98; N, 27.7%.)

5,9 - Dihydro - 2,8,9 - trimethyl - 5 - oxo - 8,9H - imidazo[1,2-a] pyrrolo[2,3-d]pyrimidine (**5c**)

A suspension of **5a** (202 mg, 1.0 mmol) and NaH in oil (60 mg of a 80% mixture, 2 mmol) in DMF was stirred for 10 min at room temp until the evolution of H_2 had ceased. MeI (100 μl, 228 mg, 1.6 mmol) was added under stirring and stirring was continued for another 30 min at ambient temp. Then DMF was evaporated *in vacuo* and the solid residue was partitioned between CH_2Cl_2 (95 ml) and water (5 ml). The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was chromatographed in solvent E on a silica gel column (30 × 1 cm) affording a main zone which yielded colourless crystals (89 mg, 41%). Recrystallization from MeOH afforded colourless needles, m.p. 247–249°. TLC (C) R_f 0.87; UV (MeOH) λ_{max} 302, 261, 234 nm (ε 7200, 4200, 36 100). MS (*m/e*) 216 (M^+), 201, 187, 173, 149. (Found: C, 61.11; H, 5.53; N, 25.92. Calc for $C_{11}H_{12}N_4O$ (216.2): C, 61.10; H, 5.59; N, 25.91%.)

Glycosylation of compound **5a** *with 1-chloro-2-deoxy-3,5-di-O-p-toluoyl-α-D-erythro-pentofuranose* (**6**)

A suspension of **5a** (500 mg, 2.5 mmol) in anhyd DMF (15 ml) was treated with NaH in oil (200 mg of a 50% mixture, 4.2 mmol) and the mixture was stirred for 10 min at room temp until evolution of H_2 had ceased. Then **6**[33] (1.18 g, 3.0 mmol) was added in one portion under stirring. Stirring was continued for 1 hr and another portion of **6** (300 mg, 0.77 mmol) was added. After stirring for 1 hr the solvent was evaporated and the remaining solid residue was taken up in EtOAc (100 ml). Insoluble material was filtered off and the filtrate washed with water (3 × 10 ml). The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was chromatographed on a silica gel column (25 × 5 cm) with CH_2Cl_2 affording a fluorescent main zone. After pooling and evaporation a colourless amorphous foam (590 mg, 43%) was obtained exhibiting two zones on TLC (B) R_f 0.67 and 0.73. The ratio of the content was *ca* 3:2, determined by ^1H-NMR. The total was dissolved in 1/10 M NaOMe in MeOH (60 ml) and stirred for 1 hr at room temp. The solvent was evaporated and the residue taken up in water (25 ml); the pH was adjusted to 5 with 1 N AcOH and precipitated *p*-toluic acid was filtered off. The remaining *p*-toluic acid was separated by applying the filtrate to a column of hydrophobic resin (Amberlite XAD 2, 20 × 1 cm) and by stepwise elution with water and 20% aqueous MeOH, which yielded the mixture of anomers from the slow migrating zone. After pooling the main fraction, the solvent was evaporated, the residue dissolved in MeOH (25 ml) and adsorbed on silica gel (10 g). The mixture was evaporated suspended in solvent A and the slurry loaded on the top of a silica gel column (20 × 2.5 cm). Elution with solvent A separated the two main zones partially. Full separation was achieved by repeated chromatography.

8 - (2' - Deoxy - β - D - erythro - pentofuranosyl) - 5,9 - dihydro - 2,9 - dimethyl - 5 - oxo - 8,9H - imidazo[1,2-a]pyrrolo[2,3-d] pyrimidine (**3a**)

The faster migrating zone yielded a colourless foam (138 mg, 18%) after evaporation which crystallized from MeOH to give colourless needles, m.p. 203–205°; TLC (C) R_f 0.56; UV (MeOH) λ_{max} 303, 264, 235 nm (ε 8700, 5600, 36 000). (Found: C, 56.75; H, 5.86; N, 17.64. Calc for $C_{15}H_{18}N_4O_4$ (318.3): C, 56.60; H, 5.70; N, 17.60%.)

8 - (2' - Deoxy - α - D - erythro - pentofuranosyl) - 5,9 - dihydro - 2,9 - dimethyl - 5 - oxo - 8,9H - imidazo[1,2-a]pyrrolo[2,3-d] pyrimidine (**7a**)

From the slower migrating zone a colourless foam (182 mg, 23%) was obtained after evaporation. TLC (C) R_f 0.49; UV (MeOH) λ_{max} 303, 265, 235 nm (ε 7400, 4700, 32 300). (Found:

C, 56.57; H, 5.68, N, 17.41. Calc for $C_{15}H_{18}N_4O_4$ (318.3): C, 56.60, H, 5.70; N, 17.60%.)

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