Synthesis and Adenosine Deaminase Inhibitory Activity of 3′-Deoxy-1-deazaadenosines

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Dedicated to Prof. Dr. *Frank Seela* on the occasion of his 60th birthday

New 1-deazapurine nucleosides were synthesized by coupling 2,6-dichloro-1-deaza-9*H*-purine (= 5,7-dichloro-3*H*-imidazo[4,5-*b*]pyridine) with a 3-deoxyribose derivative by the acid-catalyzed fusion method. The condensation reaction gave an anomeric mixture of the N^9-β-D- and N^9-α-D-3′-deoxynucleosides, which were treated with methanolic ammonia at room temperature to obtain the deprotected derivatives. Reaction of the β-D-anomer with different amines gave 2-chloro-N^6-substituted nucleosides, which were dechlorinated to give the corresponding 3′-deoxy-1-deazaadenosines. Biological studies on adenosine deaminase from calf intestine showed that the new compounds are inhibitors of the enzyme, the 3′-deoxy-1-deazaadenosine being the most potent one with a K_i of 2.6 μM.

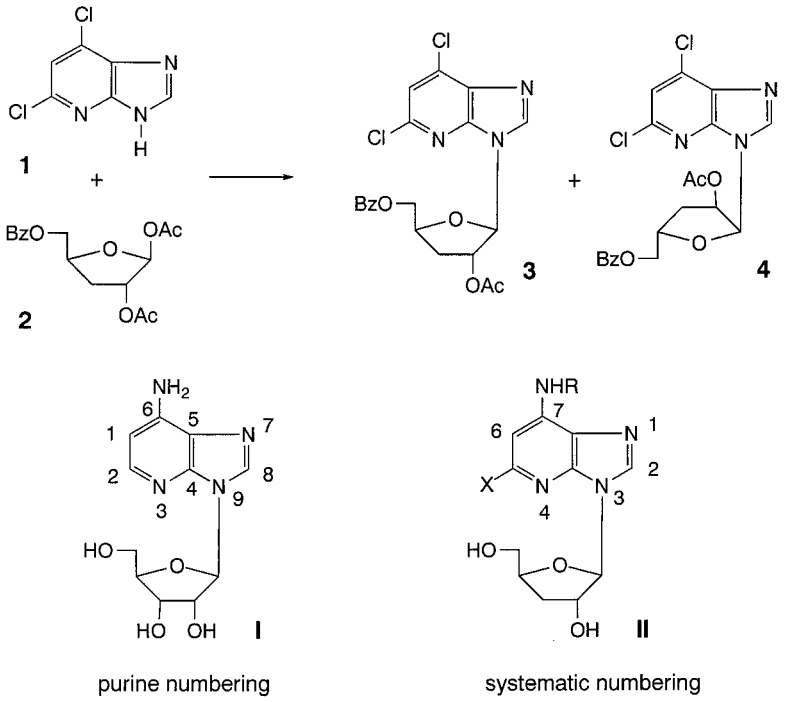
1. Introduction. – 1-Deazaadenosine (c^1A, **I**) is a nucleoside endowed with a number of pharmacological properties: it has been shown to possess cytotoxic activity [1], to inhibit platelet aggregation [2], and to act as an agonist of adenosine receptors [3]. Furthermore, 1-deazaadenosine analogues exhibit good inhibitory activity against adenosine deaminase (ADA) [4][5], an enzyme involved in the pathogenesis of the severe combined immunodeficiency disease (SCID) [6–8], and responsible for deamination of potentially useful nucleoside-based drugs [9]. Elevated serum ADA activity has been described in various autoimmune and inflammatory diseases, including systemic lupus erythematosus [10].

Recently *Seela et al.* described the incorporation of 1-deazaadenosine into a hammerhead ribozyme and the resulting catalytic activity [11].

In recent years, a number of 1-deazapurine nucleosides were synthesized by coupling 5,7-dichloro-3*H*-imidazo[4,5-*b*]pyridine (**1**; *Scheme 1*) [12] with ribose, 2-deoxyribose, and 2,3-dideoxyribose derivatives [13–16]. Some of these molecules exhibited *in vitro* activity against human immunodeficiency virus type-1 (HIV-1); in particular compounds bearing an N^6-cycloheptyl or a cyclooctyl ring were the most active ones in every series, while the N^6-unsubstituted derivatives proved to be good inhibitors of adenosine deaminase (ADA).

In the present paper, we report for the first time the synthesis of a series of 1-deazaadenosine derivatives obtained by modifying the 3′-position of the sugar moiety. This goal was achieved by coupling 2,6-dichloro-1-deazapurine (**1**) with the 3-deoxy-β-D-ribose derivative **2** to obtain the 3′-deoxy-1-deazapurine nucleosides of the general formula **II**.

Scheme 1



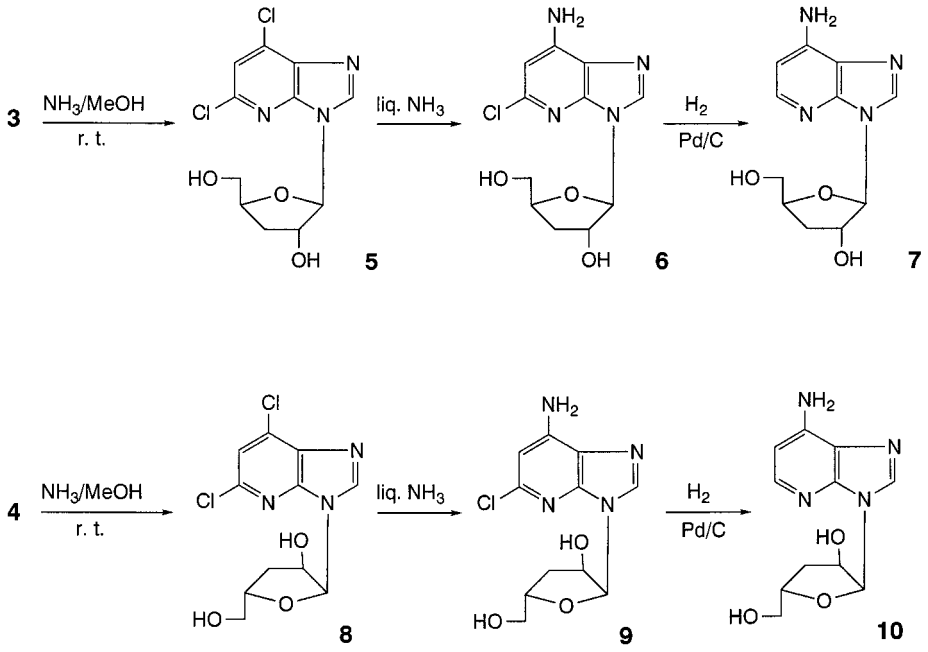
2. Results. – 5,7-Dichloro-3*H*-imidazo[4,5-*b*]pyridine (**1**) [12] was treated with 1,2-di-*O*-acetyl-5-*O*-benzoyl-3-deoxy-β-D-ribofuranose (**2**) [17] in the presence of a catalytic amount of TsOH at 160° *in vacuo* for 10 min. The coupling gave a mixture of the *N*-3-β-D-3′ and *N*-3-α-D-3′-deoxyribonucleosides **3** and **4** with a total yield of 81% (*Scheme 1*). These protected nucleosides were reacted with NH_3/MeOH at room temperature to give the compounds **5** and **8**, respectively, from which the 1-deazaadenosine derivatives **6** and **9**, respectively, were obtained by treatment with liquid NH_3 at 120° in a steel bomb (*Scheme 2*). Catalytic hydrogenolysis of the Cl-atom in **6** or **9** with 10% Pd/C in absolute EtOH and in the presence of 1N NaOH afforded the corresponding derivatives **7** and **10** (*Scheme 2*).

Compound **5** was also reacted with cycloheptyl amine or cyclooctylamine, at 105° for 16 h, to provide the 5-chloro-7-(cycloheptylamino)-1-(3-deoxy-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (**11**), and the 5-chloro-7-(cyclooctylamino)-1-(3-deoxy-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (**13**), which were dechlorinated under the above-mentioned conditions to give **12** and **14** (*Scheme 3*), respectively.

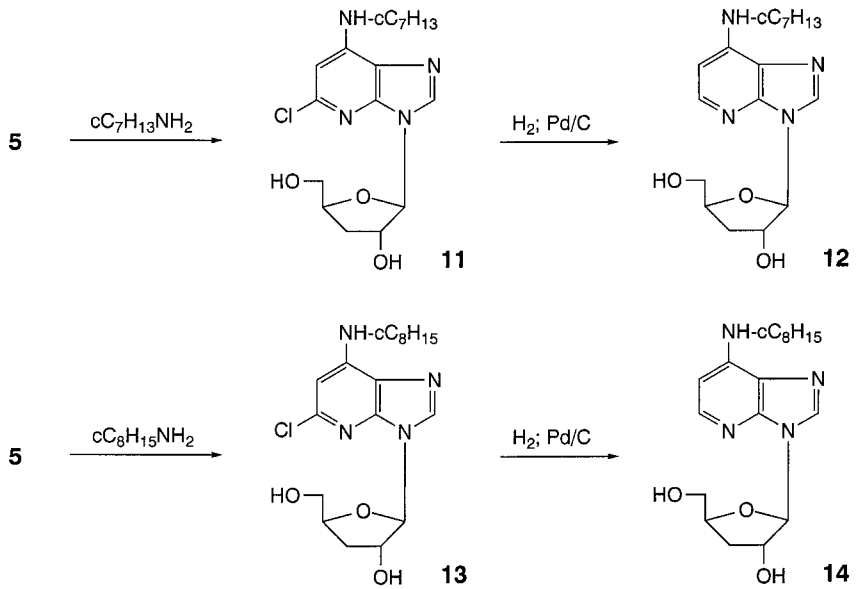
It is worth noting that previously reported coupling of the same sugar, **2**, with 2,6-dichloro-3-deazapurine afforded, in addition to the β-D- and α-D-anomers of the 3′-deoxyribonucleoside, the α-anomer of the 3′-deoxyarabino-nucleoside, derived from epimerization at C(2) of the sugar [18].

Structural Assignment. The glycosylation site and the anomeric configuration were assigned on the basis of UV data and ^{1}H-NMR spectra, including 1D-^{1}H-NOE difference spectra, of the deprotected nucleosides **5**–**9**.

Scheme 2



Scheme 3



The UV spectra of nucleosides **5** and **8** as **6** and **9** were essentially identical (see *Exper. Part*).

An NOE at the H–C(2) upon saturation of H–C(1′) indicated N(1) or N(3) as glycosylation site in the case of all compounds **5**–**9** (see Table 1).

However, since no NOE was observed on the 7-NH_2 group in compounds **6** and **9**, the presence of N(1)-regioisomers can be excluded [19][20]. This assignment was also unequivocal for the chloro compounds **5** and **8**, as the amino compounds were obtained from the chloro derivatives. Furthermore, the lack of an NOE on H–C(5) upon irradiation of H–C(1′) in compound **7** confirmed N(3) as glycosylation site, excluding the presence of N(4)-regioisomers.

Table 1. *NOE Data* [%] *of Imidazo[4,5-*b*]pyridine Nucleosides in (D_6)DMSO at 23°*

Compound	Irradiated proton	Observed NOE [%]
5	H–C(1′)	H–C(2) (2.3), H–C(2′) (1.7), H–C(4′) (1.0), H_b–C(3′) (0.4)
	H–C(4′)	H–C(2) (0.3), H–C(6) (1.4), H–C(1′) (1.3), H_b–C(3′) (3.1), H–C(5′) (1.7), H–C(5′) (1.7)
8	H–C(1′)	H–C(2) (1.4), H–C(2′) (and H–C(4′)) (2.1), H_a–C(3′) (0.7)
6	H–C(1′)	H–C(2) (2.6), H–C(2′) (1.2), H–C(4′) (1.1), H_b–C(3′) (0.4)
9	H–C(1′)	H–C(2) (1.5), H–C(2′) (and H–C(4′)) (2.2), H_a–C(3′) (0.7)
7	H–C(1′)	H–C(2) (4.0), H–C(2′) (2.0), H–C(4′) (1.0)

The NOEs on H–C(4′) upon irradiation of H–C(1′) in compounds **5**, **6**, and **7** established the β-D-configuration in all three cases; NOEs on H_b–C(3′) were observed in compounds **5** and **6** and not in **7**. On the other hand, irradiation of H–C(1′) in compounds **8** and **9** gave NOEs on H_a–C(3′) and none on H–C(4′), establishing the α-D-configuration [21].

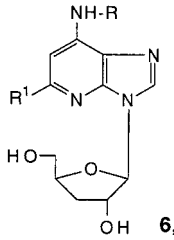
Discussion. – The new compounds **6**, **7**, and **11**–**14** were evaluated as inhibitors of adenosine deaminase from calf intestine [22], and the results are listed in *Table 2*. The 2′-deoxy-1-deazaadenosine (**16**) and the corresponding 2-Cl derivative **15** were reported as reference compounds [14–16]. The results show that none of the tested compounds were substrates of the enzyme, and the 3′-deoxy-1-deazaadenosine (**7**), although less potent than the corresponding 2′-deoxy derivative **16** ($K_i = 0.19$ μM), was still a good ADA inhibitor with a K_i of 2.6 μM. The presence of a Cl-atom at C(2) of 1-deazapurine produced a decrease of activity (**7**, $K_i = 2.6$ μM *vs.* **6**, $K_i = 35$ μM) compared to 2′-deoxy-1-deazaadenosines **16** and **15** (*Table 2*) and the corresponding ribose- and 2′,3′-dideoxyribose derivatives [16]. As expected, substitution at N(6) with bulky and hydrophobic cycloalkyl rings brought about a relevant decrease of ADA inhibitory activity (**11**–**14**, K_i from 38 to 115 μM). In these cases, the Cl-atom at C(2) does not significantly affect the activity. All these findings are in agreement with our previous hypothesis of a direct interaction of the 1-deazaadenosine derivatives with the catalytic site of the enzyme, involving 6-NH_2 group as a H-bond donor [16]. Studies on human immunodeficiency virus type-1 (HIV-1) are in progress and will be reported elsewhere.

Experimental Part

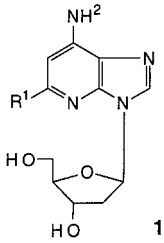
General. TLC: precoated TLC plates with silica gel *60 F-254* (*Merck*). FC: silica gel *60* (*Merck*). M.p.: *Büchi* apparatus; uncorrected. UV Spectra: *Perkin-Elmer Coleman 575* spectrophotometer. ^{1}H-NMR Spectra: *Varian VXR* 300-MHz spectrometer; δ in ppm, J in Hz. Elemental analyses were determined on a *Carlo Erba* model *1106* analyser, exper. values within ±0.4% of calc. values.

*Glycosylation of 5,7-Dichloro-3*H*-imidazo[4,5-*b*]pyridine* (**1**) *with 1,2-*O*-Diacetyl-5-*O*-benzoyl-3-deoxy-β-*D*-ribofuranose* (**2**). An intimate mixture of **1** [12] (100 mg, 0.53 mmol), **2** [17] (342 mg, 1.06 mmol), and TsOH (cat. amount) was heated at 160°; when the fusion started, vacuum from a water aspirator (25 nm) was applied until bubbling ceased (5–10 min). The resulting solid was submitted to FC (silica gel; cyclohexane AcOEt

Table 2. *ADA Inhibitory Activity of 1-Deazapurine Nucleosides*



6, 7, 11-14



15, 16

Compound	R	R^1	K_i [μM]
6	H	Cl	35
7	H	H	2.6
1	cC_7H_{13}	Cl	115
12	cC_7H_{13}	H	93
13	cC_8H_{15}	Cl	38
14	cC_8H_{15}	H	89
15		Cl	23
16		H	0.19

70:30) to obtain a mixture of the two nucleosides **3** and **4** (195 mg; 81%). Anal. samples of **3** and **4** were obtained as amorphous solids by separating the mixture on prep. TLC ($CHCl_3$/MeOH 98:2).

*3-(2-*O*-Acetyl-5-*O*-benzoyl-3-deoxy-β-*D*-ribofuranosyl)-5,7-dichloro-3*H*-imidazo[4,5-*b*]pyridine* (**3**): Yield 52%. ^{1}H-NMR ((D$_6$)DMSO): 2.13 (*s*, MeCO); 2.28–2.42 (*m*, 1 H–C(3′)); 2.72–2.89 (*m*, 1 H–C(3′)); 4.40–4.78 (*m*, 2 H–C(5′), H–C(4′)); 5.79 (*d*, *J* = 6.7, H–C(2′)); 6.27 (*s*, H–C(1′)); 7.53–7.43 (*m*, 2 arom. H); 7.69–7.60 (*m*, 1 arom. H); 7.70 (*s*, H–C(6)); 7.80–7.88 (*m*, 2 arom H); 8.77 (*s*, H–C(2)). Anal calc. for $C_{20}H_{17}Cl_2N_3O_5$ (450.27): C 53.35, H 3.81, N 9.33; found: C 53.50, H 3.92, N 9.12.

*3-(2-*O*-Acetyl-5-*O*-benzoyl-3-deoxy-α-*D*-ribofuranosyl)-5,7-dichloro-3*H*-imidazo[4,5-*b*]pyridine* (**4**): Yield 29%. ^{1}H-NMR ((D$_6$)DMSO): 1.78 (*s*, MeCO); 2.42–2.58 (*m*, 2 H–C(3′)); 4.38–4.54 (*m*, 2 H–C(5′)); 4.97–5.09 (*m*, H–C(4′)); 5.59–5.70 (*m*, H–C(2′)); 6.69 (*d*, *J* = 4.8, H–C(1′)); 7.42–7.60 (*m*, 3 arom. H); 7.71 (*s*, H–C(6); 7.99–8.10 (*m*, 2 arom. H); 8.76 (*s*, H–C(2)). Anal calc. for $C_{20}H_{17}Cl_2N_3O_5$ (450.27): C 53.35, H 3.81, N 9.33; found: C 53.55, H 4.03, N 9.17.

*5,7-Dichloro-3-(3-deoxy-β-*D*-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine* (**5**) *and 5,7-Dichloro-3-(3-deoxy-α-*D*-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine* (**8**). To the mixture **3**/**4** (526 mg, 1.17 mmol) was added MeOH sat. at 0° with NH_3 (30 ml), and the mixture was allowed to stand at r.t. for 16 h. After evaporation, the residue was separated by FC ($CHCl_3$/MeOH 98:2) to obtain **5** (198 mg, 56%) and **8** (102 mg, 29%) as white solids.

Data of **5**: M.p. 164–165°. UV (EtOH): 260 (9800), 287 (15500). ^{1}H-NMR ((D$_6$)DMSO): 1.75–2.00 (*m*, 1 H–C(3′)); 2.17–2.36 (*m*, 1 H–C(3′)); 3.49–3.66 (*m*, 1 H–C(5′)); 3.68–3.83 (*m*, 1 H–C(5′)); 4.35–4.50 (*m*, H–C(4′)); 4.53–4.64 (*m*, H–C(2′)); 6.00 (*s*, H–C(1′)); 7.70 (*s*, H–C(6)); 8.89 (*s*, H–C(2)). Anal. calc. for $C_{11}H_{11}Cl_2N_3O_3$ (304.13): C 43.44, H 3.65, N 13.82; found: C 43.55, H 3.70, N 13.71.

Data of **8**: M.p. 182–183°. UV (EtOH): 260 (6600), 287 (9800). ^{1}H-NMR ((D$_6$)DMSO): 2.04–2.29 (*m*, 2 H–C(3′)); 3.38–3.63 (*m*, 2 H–C(5′)); 4.49–4.64 (*m*, H–C(4′), H–C(2′)); 6.33 (*d*, *J* = 4.4, H–C(1′)); 7.68 (*s*, H–C(6)); 8.61 (*s*, H–C(2)). Anal. calc. for $C_{11}H_{11}Cl_2N_3O_3$ (304.13): C 43.44, H 3.65, N 13.82; found: C 43.48, H 3.72, N 13.70.

*7-Amino-5-chloro-3-(3-deoxy-β-*D*-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine* (**6**) *and 7-Amino-5-chloro-3-(3-deoxy-α-*D*-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine* (**9**). To compound **5** or **8** (100 mg, 0.33 mmol) liq. NH_3 (10 ml) was added, and the mixture was heated in a steel bomb at 120° for 12 h. After evaporation of the NH_3, the residue was chromatographed (silica gel; $CHCl_3$/MeOH 92:8) to obtain **6** (48 mg, 52%) or **9** (46 mg, 49%), as chromatographically pure solids.

Data of **6**: M.p. 204–205°. UV (EtOH): 266 (19600). ^{1}H-NMR ((D$_6$)DMSO): 1.85–1.98 (*m*, 1 H–C(3′)); 2.17–2.33 (*m*, 1 H–C(3′)); 3.47–3.59 (*m*, 1 H–C(5′)); 3.62–3.78 (*m*, 1 H–C(5′)); 4.29–4.40 (*m*, H–C(4′)); 4.50–4.60 (*m*, H–C(2′)); 5.85 (*d*, *J* = 1.5, H–C(1′)); 6.39 (*s*, H–C(6)); 6.79 (br. *s*, NH_2); 8.34 (*s*, H–C(2)). Anal. calc. for $C_{11}H_{13}ClN_4O_3$ (284.70): C 46.41, H 4.60, N 19.68; found: C 46.50, H 4.71, N 19.61.

Data of **9**: M.p. 189–191°. UV (EtOH): 265 (14300). ^{1}H-NMR ((D$_6$)DMSO): 2.02–2.26 (*m*, 2 H–C(3′)); 3.38–3.60 (*m*, 2 H–C(5′)); 4.40–4.55 (*m*, H–C(4′), H–C(2′)); 6.18 (*d*, *J* = 4.3, H–C(1′)); 6.38 (*s*, H–C(6)); 6.76 (br. *s*, NH$_2$); 8.12 (*s*, H–C(2)). Anal. calc. for $C_{11}H_{13}ClN_4O_3$ (284.70): C 46.41, H 4.60, N 19.68; found: C 46.53, H 4.69, N 19.62.

*7-Amino-3-(3-deoxy-β-*D*-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine* (**7**) *and 7-Amino-3-(3-deoxy-α-*D*-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine* (**10**). To compound **6** or **9** (30 mg, 0.11 mmol) in abs. EtOH (10 ml), 1N NaOH (1 ml) and a cat. amount of 10% Pd/C were added. The mixture was hydrogenated at 40 psi for 6 h. The catalyst was removed by filtration and the filtrate evaporated. Purification by FC (CHCl$_3$/MeOH 94:6) gave **7** (20 mg, 73%) or **10** (16 mg, 55%) as colorless solids.

Data of **7**: M.p. 183–185°. UV (H$_2$O): 262 (17000). ^{1}H-NMR ((D$_6$)DMSO): 1.87–2.03 (*m*, 1 H–C(3′)); 2.18–2.36 (*m*, 1 H–C(3′)); 3.42–3.58 (*m*, 1 H–C(5′)); 3.62–3.77 (*m*, 1 H–C(5′)); 4.27–4.42 (*m*, H–C(4′)); 4.57–4.70 (*m*, H–C(2′)); 5.87 (*d*, *J* = 2.9, H–C(1′)); 6.32–6.48 (*m*, H–C(6), NH$_2$); 7.80 (*d*, *J* = 5.5, H–C(5)); 8.27 (*s*, H–C(2)). Anal. calc. for $C_{11}H_{14}N_4O_3$ (250.25): C 52.79, H 5.64, N 22.39; found: C 52.91, H 5.70, N 22.22.

Data of **10**: M.p. 177–179°. UV (H$_2$O): 261 (13400). ^{1}H-NMR ((D$_6$)DMSO): 2.02–2.25 (*m*, 2 H–C(3′)); 3.32–3.62 (*m*, 2 H–C(5′)); 4.38–4.56 (*m*, H–C(4′), H–C(2′)); 6.23–6.40 (*m*, H–C(1′), H–C(6), NH$_2$); 7.80 (*d*, *J* = 5.3, H–C(5)); 8.10 (*s*, H–C(2)). Anal. calc. for $C_{11}H_{14}N_4O_3$ (250.25): C 52.79, H 5.64, N 22.39; found: C 52.88, H 5.68, N 22.18.

*5-Chloro-(cycloheptylamino)-3-(3-deoxy-β-*D*-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine* (**11**) *and 5-Chloro-7-(cyclooctylamino)-3-(3-deoxy-β-*D*-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine* (**13**). To **5** (125 mg, 0.41 mmol), cycloheptylamine or cyclooctylamine (10 ml) was added, and the mixture was heated at 105° for 16 h. The excess amine was evaporated and the residue purified by FC (CHCl$_3$/MeOH 99:1) to give colorless solids which were crystallized from Et$_2$O to yield **11** (115 mg, 73%) and **13** (122 mg, 76%).

Data of **11**: M.p. 140–142°. UV (EtOH): 271 (16000), 286 (15200). ^{1}H-NMR ((D$_6$)DMSO): 1.40–1.75 (*m*, 10 cycloheptyl H); 1.82–2.01 (*m*, 2 cycloheptyl H) 1 H–C(3′)); 2.16–2.32 (*m*, 1 H–C(3′)); 3.46–3.59 (*m*, 1 H–C(5′)); 3.62–3.77 (*m*, 1 H–C(5′)); 3.98–4.22 (*m*, 1 cycloheptyl H); 4.28–4.42 (*m*, H–C(4′)); 4.47–4.59 (*m*, H–C(2′)); 5.85 (*s*, H–C(1′)); 6.35 (*s*, H–C(6)); 6.93 (*d*, *J* = 8.4, NH$_2$); 8.35 (*s*, H–C(2)). Anal. calc. for $C_{18}H_{25}ClN_4O_3$ (380.87): C 56.76, H 6.62, N 14.71; found: C 56.88, H 6.79, N 14.60.

Data of **13**: M.p. 131–132°. UV (EtOH): 271 (20100), 286 (19200). ^{1}H-NMR ((D$_6$)DMSO): 1.43–1.99 (*m*, 14 cyclooctyl H, 1 H–C(3′)); 2.16–2.32 (*m*, 1 H–C(3′)); 3.45–3.60 (*m*, 1 H–C(5′)); 3.64–3.79 (*m*, 1 H–C(5′)); 4.01–4.26 (*m*, 1 cyclooctyl H); 4.29–4.42 (*m*, H–C(4′)); 4.48–4.61 (*m*, H–C(2′)); 5.86 (*s*, H–C(1′)); 6.36 (*s*, H–C(6)); 6.95 (*d*, *J* = 8.4, NH$_2$); 8.36 (*s*, H–C(2)). Anal. calc. for $C_{19}H_{27}ClN_4O_3$ (394.90): C 57.79, H 6.89, N 14.19; found: C 57.91, H 6.97, N 14.28.

*7-(Cycloheptylamino)-3-(3-deoxy-β-*D*-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine* (**12**) *and 7-(Cyclooctylamino)-3-(3-deoxy-α-*D*-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine* (**14**). As described for **6** or **9**, with **11** or **13** (0.17 mmol), abs. EtOH (15 ml), 1N NaOH (2 ml) and a cat. amount of 10% Pd/C. Purification by FC (CHCl$_3$/MeOH 99:1) gave **12** (36 mg, 59%) or **14** (38 mg, 65%), both as colorless solids.

Data of **12**: M.p. 140–142°. UV (EtOH): 267 (12100), 288 (14500). ^{1}H-NMR ((D$_6$)DMSO): 1.40–1.79 (*m*, 10 cycloheptyl H); 1.81–2.10 (*m*, 2 cycloheptyl H, 1 H–C(3′)); 2.19–2.39 (*m*, 1 H–C(3′)); 3.43–3.60 (*m*, 1 H–C(5′)); 3.62–3.80 (*m*, 1 H–C(5′)); 3.92–4.15 (*m*, 1 cycloheptyl H); 4.27–4.46 (*m*, H–C(4′)); 4.56–4.71 (*m*, H–C(2′)); 5.89 (*s*, H–C(1′)); 6.34 (*d*, *J* = 4.6, H–C(6)); 6.49 (*d*, *J* = 8.5, NH$_2$); 7.87 (*d*, *J* = 4.6, H–C(5)); 8.30 (*s*, H–C(2)). Anal. calc. for $C_{18}H_{26}N_4O_3$ (346.42): C 62.41, H 7.56, N 16.17; found: C 62.58, H 7.77, N 16.01.

Data of **14**: M.p. 136–137°. UV (EtOH): 267 (15000), 288 (18000). ^{1}H-NMR ((D$_6$)DMSO): 1.48–2.06 (*m*, 14 cyclooctyl H, 1 H–C(3′)); 2.16–2.36 (*m*, 1 H–C(3′)); 3.42–3.59 (*m*, 1 H–C(5′)); 3.62–3.79 (*m*, 1 H–C(5′)); 3.98–4.22 (*m*, 1 cyclooctyl H); 4.28–4.34 (*m*, H–C(4′)); 4.55–4.71 (*m*, H–C(2′)); 5.88 (*s*, H–C(1′)); 6.34 (*d*, *J* = 4.5, H–C(6)); 6.48 (*d*, *J* = 8.5, NH$_2$); 7.86 (*d*, *J* = 4.5, H–C(5)); 8.29 (*s*, H–C(2)). Anal. calc. for $C_{19}H_{28}N_4O_3$ (360.45): C 63.31, H 7.83, N 15.54; found: C 63.42, H 7.94, N 15.25.

Enzyme Assay. The method used for determination of the activity against ADA has been described in a preceding paper [22].

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