SYNTHESIS OF SOME "ABBREVIATED" NAD$^+$ ANALOGUES

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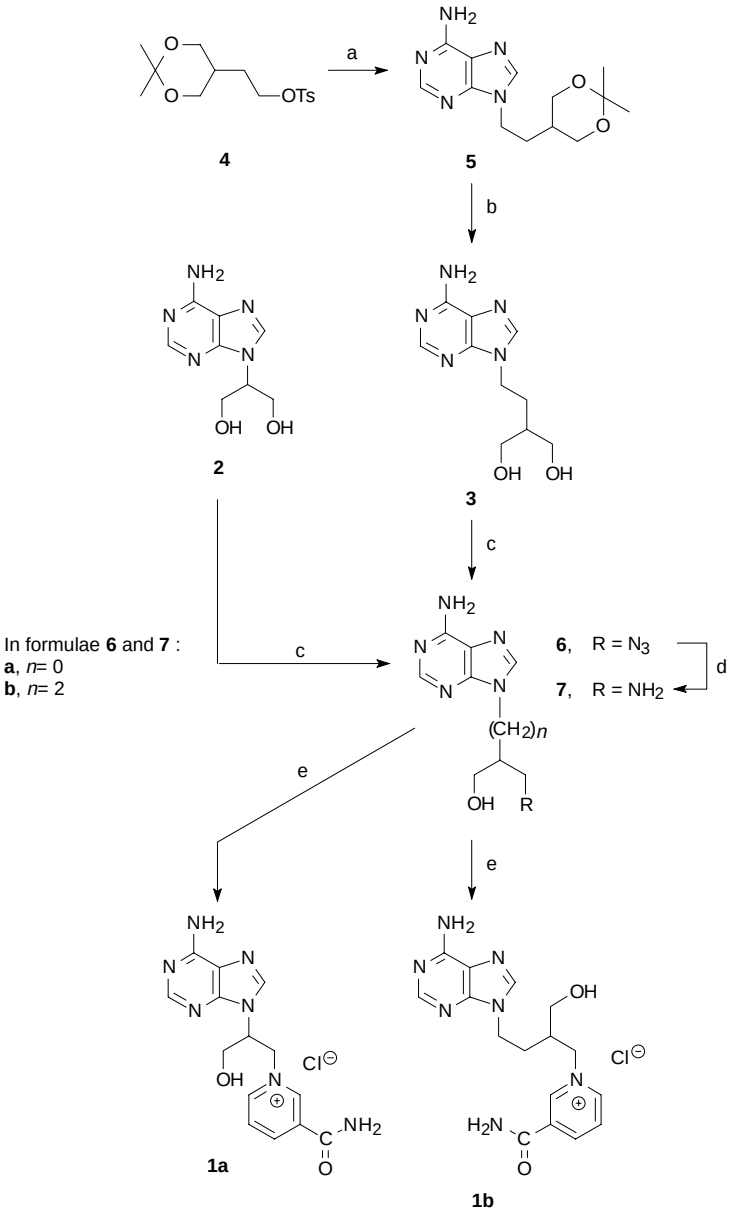
Four new "abbreviated" NAD$^+$ analogues with hydroxymethyl or carboxyl function as a substituent of an aliphatic chain linking the adenine and nicotinamide moieties were prepared using the Zincke reaction as the key step. As intermediates several new acyclic nucleoside analogues containing hydroxy, carboxyl, azido and amino group were prepared.

Key words: Nicotinamide adenine dinucleotide; Zincke reaction; Acyclic nucleoside analogues; Pyridinium salts.

Considering the broad biological role of NAD, its analogues may have very interesting biological applications[1]. A great number of model compounds have been prepared to investigate the structure–activity relationship in the series of NAD analogues. Our study concerns so-called "abbreviated" NAD$^+$ analogues, where the adenine and the nicotinamide moieties are preserved, but the ribosediphosphoribose link is replaced by a functionalized acyclic chain. This type of linking elements was chosen because of their flexibility and an increased stability compared to that of the natural skeleton. We have already reported on optically active compounds containing hydroxy groups[2,3] and NAD$^+$ analogues with phosphonate function[4] incorporated in the linkage. In this paper we report on very simplified model compounds with a two- or four-carbon-atom linking chain bearing the branching hydroxymethyl or carboxyl group.

Compounds **1a** and **1b** were prepared by multistep syntheses following a similar reaction pathway, with the Zincke reaction as the last step (Scheme 1). The bis-hydroxymethyl derivatives **2** (ref.[5]) and **3** (prepared by the standard alkylation of adenine with tosylate **4** and deprotection of the side chain of the resulting acyclic nucleoside **5**) were monotosylated and the products subsequently converted into azido derivatives **6a** and **6b** by the reaction with sodium azide. The azido group was catalytically hydrogenated over palladium on charcoal to give amino compounds **7a** and **7b**. The Zincke reaction[6] of these amino derivatives with 3-carbamoyl-1-(2,4-dinitrophenyl)-pyridinium chloride in dry methanol afforded the target compounds: 1-[2-(adenin-9-yl)-3-hydroxypropyl]-3-carbamoylpyridinium chloride (**1a**) and 1-[4-(adenin-9-yl)-



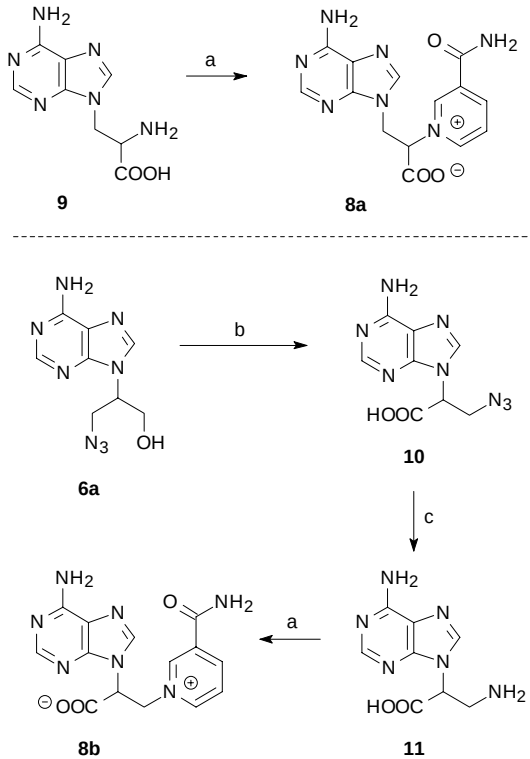
a) adenine, Cs_2CO_3, DMF; b) 0.25 M H_2SO_4; c) 1. TsCl, pyridine, 2. NaN_3, DMF; d) Pd/C, H_2, MeOH; e) 3-carbamoyl-1-(2,4-dinitrophenyl)pyridinium chloride, MeOH

SCHEME 1

2-(hydroxymethyl)butyl]-3-carbamoylpyridinium chloride (**1b**) – with diverse length of the linking chain and different position of the hydroxymethyl substituent.

The other pair of "abbreviated" NAD^+ analogues, **8a** and **8b**, contains a short two-carbon-atom link substituted in the both alternative positions by the carboxyl group, that compensates the positive charge of the 3-carbamoylpyridinium moiety.

3-(Adenin-9-yl)-2-(3-carbamoylpyridinium)propanoate (**8a**) was prepared from the known 3-(adenin-9-yl)-2-aminopropanoic acid[7] (**9**) (Scheme 2). The conditions of the Zincke reaction had to be suitably modified in accord with our recent finding[4]: To avoid the protonation of amino group, the acid carboxyl function was deprotonated by



a) 3-carbamoyl-1-(2,4-dinitrophenyl)pyridinium chloride, MeOH, DBU, pH 7.5; b) $KMnO_4$, NaOH; c) Pd/C, H_2, MeOH

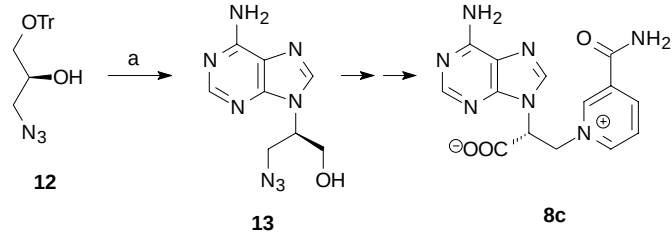
SCHEME 2

the addition of the non-nucleophilic base DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) to the reaction mixture.

The intermediate **6a** was used as the starting material for the synthesis of 2-(adenin-9-yl)-3-(3-carbamoylpyridinium)propanoate (**8b**). Its hydroxymethyl group was oxidized by potassium permanganate under basic conditions to form carboxylic acid **10**. Catalytic hydrogenation of azido group over palladium on charcoal afforded the

β-amino acid **11**. The Zincke reaction of this amino derivative was carried out in the presence of DBU to form the compound **8b**.

This NAD^+ analogue was prepared also in the form of the optically active (*R*)-enantiomer **8c**. Adenine was alkylated under Mitsunobu conditions with a homochiral hydroxy derivative **12** (Scheme 3). According to the literature[8], we assume that the reaction occurs with inversion of configuration to form derivative **13**. The subsequent synthetic



12 **13** **8c**

a) 1. adenine, PhP_3, DEAD, DMF; 2. H^+

SCHEME 3

sequence identical with that described for the racemic compound **8b** gave the (*R*)-enantiomer **8c**.

The structures of the resulting NAD^+ analogues **1a, 1b** and **8a**–**8c** were confirmed by ^1H NMR spectra. The CD spectra were measured to prove the optical activity of **8c**. High resolution mass spectrometry was used to determine the molecular formulae instead of microanalyses, because of the extremely hygroscopic character of all these compounds.

The target compounds were prepared within the framework of our studies of structure–activity relationships in the series of acyclic adenine nucleoside analogues. The cytostatic assays were performed by Dr I. Votruba at this Institute. Neither of the compounds exhibited significant cytostatic activity or cytotoxicity in L-1210 mouse leukemia cells. *In vitro* effects against the DNA viruses and retroviruses were examined at the Rega Institute for Medical Research (Prof. E. De Clercq, Head), Catholic University Leuven (Belgium). Antiviral activities of these and other "abbreviated" NAD^+ analogues as well as the results of their *in vitro* enzyme studies will be published in a separate communication.

EXPERIMENTAL

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa and compounds were dried at 2 kPa over phosphorus pentoxide. Melting points were determined on a Kofler block and are uncorrected. Analytical TLC were performed on Silufol UV_{254} plates (Kavalier Votice, Czech Republic). Preparative TLC were carried out on 40 × 17 × 0.4 cm loose layer plates of silica gel containing UV indicator. Paper electrophoresis was performed on a Whatman No. 3 MM paper at 40 V/cm for 1 h in 0.05 M triethylammonium hydrogen carbonate (TEAB) at pH 7.5; the electrophoretical mobilities are referenced to uridine 3′-phosphate. ^1H NMR spectra (δ, ppm; *J*, Hz) were measured on a Varian

Unity 500 spectrometer (500 MHz) in hexadeuteriodimethyl sulfoxide referenced to the solvent signals (2.5 ppm), or in deuterium oxide containing sodium deuteroxide, with sodium 3-(trimethylsilyl)propanesulfonate as an internal standard. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix). Dimethylformamide was distilled from phosphorus pentoxide and stored over molecular sieves (4 Å). CD spectra were measured on a Jobin–Yvon Dichrograph Mark V using software Dichrosoft Version A.

9-[2-(2,2-Dimethyl-1,3-dioxan-5-yl)ethyl]adenine (**5**)

A stirred mixture of adenine (3.0 g, 22 mmol), cesium carbonate (3.6 g, 11 mmol) and dimethylformamide (30 ml) was heated at 120 °C for 2 h. After addition of 2,2-dimethyl-5-[2-(*p*-toluenesulfonyloxy)ethyl]-1,3-dioxane (**4**) (ref.[9]; 10 g, 32 mmol) in dimethylformamide (5 ml), the heating at 120 °C was continued for 4 h. The reaction mixture was taken down, co-distilled with water and the product was isolated by preparative TLC on silica gel (15% methanol in chloroform). Yield: 0.9 g (15%), m.p. 145–147 °C. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.16 s, 1 H (H-2); 8.14 s, 1 H (H-8); 7.20 brs, 2 H (NH_2); 4.15 t, 2 H, J(1′,2′) = 7.1 (H-1′); 3.76 dd, 2 H, J(3′,4a′) = J(3′,5a′) = 5.4, J_g = 12.0 (H-4a′ and H-5a′); 3.53 dd, 2 H, J(3′,4b′) = J(3′,5b′) = 8.8, J_g = 12.0 (H-4b′ and H-5b′); 1.75 q, 2 H, J(1′,2′) = J(3′,2′) = 7.1 (H-2′); 1.55 m, 1 H, (H-3′); 1.32 s and 1.25 s, 2 × 3 H (CH_3). For $C_{13}H_{19}N_5O_2$ (277.3) calculated: 56.30% C, 6.91% H, 25.25% N; found: 55.98% C, 7.12% H, 24.89% N. Mass spectrum (FAB), *m/z* (rel.%): 278 (100) [M + H].

9-[4-Hydroxy-3-(hydroxymethyl)butyl]adenine (**3**)

A solution of compound **5** (0.9 g, 3.2 mmol) in 0.25 M sulfuric acid (60 ml) was allowed to stand for 3 days at room temperature and deionized on Dowex 50X8 column (H^+ form, 70 ml). The pure product was obtained after crystallization from water–ethanol. Yield: 0.5 g (66%), m.p. 161–162 °C. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.14 s, 1 H (H-2); 8.13 s, 1 H (H-8); 7.17 brs, 2 H (NH_2); 4.44 brt, 2 H, J(OH,4′) = J(OH,5′) = 4.5 (OH); 4.20 dd, 2 H, J(1′,2′) = 6.8 (H-1′); 3.43 dd, 2 H, J(3′,4a′) = J(3′,5a′) = 5.9, J_g = 10.5 (H-4a′ and H-5a′); 3.35 dd, 2 H, J(3′,4b′) = J(3′,5b′) = 5.6, J_g = 10.5 (H-4b′ and H-5b′); 1.79 dt, 2 H, J(1′,2′) = J(3′,2′) = 6.8 (H-2′); 1.43 m, 1 H, ΣJ = 36.6 (H-3′). For $C_{10}H_{15}N_5O_2$. H_2O (255.3) calculated: 47.05% C, 6.71% H, 27.43% N; found: 46.72% C, 6.83% H, 26.96% N. Mass spectrum (FAB), *m/z* (rel.%): 238 (100) [M + H].

Azido Derivatives **6**. General Procedure

p-Toluenesulfonyl chloride (1.0 g, 5.25 mmol) and 4-(dimethylamino)pyridine (5 mg) were added at –10 °C to a stirred solution of 9-[2-hydroxy-1-(hydroxymethyl)ethyl]adenine[5] (**2**) or 9-[4-hydroxy-3-(hydroxymethyl)butyl]adenine (**3**) (4.75 mmol) in pyridine (20 ml). After standing at room temperature overnight, water (5 ml) was added and the mixture was taken down. The residue was taken up in ethyl acetate (40 ml), washed with water and dried over magnesium sulfate. After filtration and evaporation of the solvent, sodium azide (0.8 g, 12.5 mmol) and dimethylformamide (20 ml) were added, the mixture was stirred for 6 h at 100 °C, filtered while hot and the filtrate taken down. The residue was co-distilled with toluene and product was purified by chromatography on silica gel column (methanol–chloroform).

9-[2-Azido-1-(hydroxymethyl)ethyl]adenine (**6a**). Yield: 0.49 g (44%), m.p. 150–151 °C. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.22 s, 1 H (H-2); 8.14 s, 1 H (H-8); 7.24 brs, 2 H (NH_2); 5.25 t, 1 H, J(3′,OH) = 5.5 (OH); 4.66 m, 1 H (H-1′); 4.03 dd, 1 H, J(1′,2a′) = 9.0, J_g = 12.9 (H-2a′); 3.85 dd, 1 H, J(1′,2b′) = 4.9, J_g = 10.7 (H-2b′); 3.87 pent, 1 H, J(1′,3a′) = 6.1, J(3a′,OH) = 5.6, J_g = 12.0 (H-3a′); 3.79 pent, 1 H, J(1′,3b′) = J(3b′,OH) = 5.4, J_g = 12.0 (H-3b′). For $C_8H_{10}N_8O$ (234.2) calculated:

41.02% C, 4.30% H, 47.84% N; found: 40.83% C, 4.39% H, 46.96% N. Mass spectrum (FAB), *m/z* (rel.%): 235 (100) [M + H].

9-[4-Azido-3-(hydroxymethyl)butyl]adenine (**6b**). Yield: 0.39 g (31%), m.p. 139–141 °C; TLC (chloroform–methanol 7 : 3), R_F = 0.49. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.16 s, 1 H (H-2); 8.13 s, 1 H (H-8); 7.19 brs, 2 H (NH_2); 4.70 t, 1 H, *J*(OH,4′) = *J*(OH,5′) = 5.4 (OH); 4.20 t, 2 H, *J*(1′,2′) = 7.1 (H-1′); 3.42 d, 2 H, *J*(3′,4′) = 5.9 (H-4′); 3.39 t, 2 H, *J*(3′,5′) = *J*(5′,OH) = 5.4 (H-5′); 1.84 sept and 1.79 sept, 2 × 1 H, *J*(1′,2′) = *J*(3′,2′) = 7.1, J_g = 13.9 (H-2′); 1.54 m, 1 H, Σ*J* = 36.8 (H-3′). Mass spectrum (FAB), *m/z* (rel.%): 263 (60) [M + H].

Amino derivatives **7**. General Procedure

Compound **6** (0.92 mmol) was hydrogenated in methanol (40 ml) over 10% palladium on charcoal (0.36 g) with addition of $PdCl_2$ (40% solution in HCl, 0.2 ml) under stirring for 20 h at room temperature. The mixture was filtered through a pad of Celite. The catalyst was washed with hot methanol and hot water (100 ml each). The filtrate was evaporated and the residue was deionized on Dowex 50X8 (H^+ form, 40 ml).

9-[2-Amino-1-(hydroxymethyl)ethyl]adenine (**7a**). Yield: 0.12 g (60%), m.p. 158–160 °C. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.11 s, 1 H (H-2); 8.10 s, 1 H (H-8); 7.15 br, 2 H (NH_2); 5.0 br, 1 H (OH); 4.40 qd, 1 H, *J*(1′,2a′) = *J*(1′,3′) = 6.9, *J*(1′,2b′) = 4.9 (H-1′); 3.87 dd, 1 H, *J*(1′,2a′) = 6.9, J_g = 12.2 (H-2a′); 3.77 dd, 1 H, *J*(1′,2b′) = 4.9, J_g = 11.2 (H-2b′); 3.50 brs, 2 H (NH_2′); 3.04 d, 2 H, *J*(1′,3′) = 6.9 (H-3′). For $C_8H_{12}N_5O$ (208.2) calculated: 46.15% C, 5.81% H, 40.36% N; found: 45.41% C, 5.70% H, 39.62% N. Mass spectrum (FAB), *m/z* (rel.%): 209 (40) [M + H].

9-[4-Amino-3-(hydroxymethyl)butyl]adenine (**7b**). Yield: 0.20 g (92%), strongly hygroscopic. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.15 s, 1 H (H-2); 8.12 s, 1 H (H-8); 7.16 brs, 2 H (NH_2); 4.80 brs, 1 H (OH); 4.20 t, 2 H, *J*(1′,2′) = 7.1 (H-1′); 2.87 dd, 1 H, *J*(3′,4a′) = 7.3, J_g = 12.9 (H-4a′); 2.82 dd, 1 H, *J*(3′,4b′) = 5.3, J_g = 12.9 (H-4b′); 3.52 dd, 1 H, *J*(3′,5a′) = 4.4, J_g = 11.0 (H-5a′); 3.46 dd, 1 H, *J*(3′,5b′) = 6.1, J_g = 11.0 (H-5b′); 1.84 m, 2 H (H-2′); 1.65 m, 1 H (H-3′). Mass spectrum (FAB), *m/z* (rel.%): 237 (40) [M + H].

NAD+ Analogues **1**. General Procedure

3-Carbamoyl-1-(2,4-dinitrophenyl)pyridinium chloride[2] (0.14 g, 0.43 mmol) was added to a solution of compound **7** (0.42 mmol) in dry methanol (10 ml) and the mixture was stirred for 4 h. The crude product was precipitated by addition of ether and filtered off. The precipitate was dissolved in water (10 ml) and washed with ether (10 × 10 ml). The aqueous solution was evaporated *in vacuo*, the residue was dissolved in methanol and the product was precipitated by addition of ether.

1-[2-(Adenin-9-yl)-3-hydroxypropyl]-3-carbamoylpyridinium chloride (**1a**). Yield: 97 mg (66%) , E_{Up} –0.63. ^{1}H NMR spectrum ($(CD_3)_2SO$): 9.58 t, 1 H, *J*(2″,4″) = *J*(2″,6″) = 1.5 (H-2″); 8.90 dd, 2 H *J*(2″,4″) = *J*(2″,6″) = 1.5, *J*(4″,6″) = 7.3 (H-4″ and H-6″); 8.68 brs, 1 H (NH_2″); 8.30 s, 1 H (H-2); 8.07 t, 1 H, *J*(5″,6″) = *J*(5″,4″) = 7.3 (H-5″); 8.13 brs, 1 H (NH_2″); 7.88 s, 1 H (H-8); 7.26 brs, 2 H (NH_2); 5.69 t, 1 H, *J*(OH,3′) = 5.4 (OH); 5.38 m, 1 H (H-1′); 5.32 dd, 1 H, *J*(1′,2a′) = 3.1, J_g = 12.0 (H-2a′); 5.24 dd, 1 H, *J*(1′,2b′) = 10.0, J_g = 12.0 (H-2b′); 4.09 pent, 1 H, *J*(1′,3a′) = *J*(3a′,OH) = 5.4, J_g = 11.7 (H-3a′); 3.79 ddd, 1 H, *J*(1′,3b′) = 3.9, *J*(3b′,OH) = 5.1, J_g = 11.7 (H-3b′). Exact mass (FAB HRMS): calculated for $C_{14}H_{16}N_7O_2$: 314.1403; found: 314.1384. Mass spectrum (FAB), *m/z* (rel.%): 314 (70) [M – Cl].

1-[4-(Adenin-9-yl)-2-(hydroxymethyl)butyl]-3-carbamoylpyridinium chloride (**1b**). Yield: 140 mg (88%), E_{Up} –0.61. ^{1}H NMR spectrum ($(CD_3)_2SO$): 9.46 brs, 1 H (H-2″); 9.14 d, 1 H, *J*(6″,5″) = 6.1 (H-6″); 8.93 d, 1 H, *J*(4″,5″) = 8.1 (H-4″); 8.24 dd, 1 H, *J*(5″,6″) = 6.1, *J*(5″,4″) = 8.1 (H-5″); 8.55 s and 8.18 s, 2 × 1 H (NH_2″); 8.17 s, 1 H (H-2); 8.16 s, 1 H (H-8); 7.39 brs, 2 H (NH_2); 4.90 brs, 1 H (OH); 4.77 dd, 1 H, *J*(3′,4a′) = 3.0, J_g = 13.0 (H-4a′); 4.68 dd, 1 H, *J*(3′,4b′) = 9.0, J_g = 13.0 (H-4b′);

4.25 m, 2 H (H-1′); 3.45 m, 2 H (H-5″); 2.14 m, 1 H (H-3′); 1.86 m, 2 H (H-2′). Exact mass (FAB HRMS): calculated for $C_{16}H_{20}N_7O_2$: 342.1678; found: 342.1622. Mass spectrum (FAB), *m/z* (rel.%): 342 (30) [M – Cl].

(*R*)-9-[2-Azido-1-(hydroxymethyl)ethyl]adenine (**13**)

Diethyl azodicarboxylate (5 ml, 15 mmol) was added to a mixture of adenine (2 g, 14.8 mmol), triphenylphosphine (7.7 g, 29.6 mmol) and (*S*)-3-azido-1-*O*-triphenylmethylpropane-1,2-diol[10] (**12**; 5.3 g, 14.8 mmol) in dimethylformamide (120 ml). The reaction mixture was stirred for 2 days at room temperature and taken down. The residue was refluxed for 2 h in 80% acetic acid (50 ml) and, after addition of water (100 ml) and ethanol (100 ml), the heating was continued for additional 6 h. Reaction mixture was taken down, the residue was dissolved in water (200 ml) and washed with chloroform. The aqueous solution was deionized on Dowex 50X8 (H^+ form, 80 ml). The pure product was obtained after preparative TLC on silica gel. Yield: 1.6 g (33%). ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.20 s, 1 H (H-2); 8.12 s, 1 H (H-8); 7.25 brs, 2 H (NH_2); 5.25 t, 1 H, J(3′,OH) = 5.5 (OH); 4.65 m, 1 H (H-1′); 4.03 dd, 1 H, J(1′,2a′) = 9.0, J_g = 12.8 (H-2a′); 3.85 dd, 1 H, J(1′,2b′) = 4.9, J_g = 12.8 (H-2b′); 3.82 pent, 1 H, J(1′,3a′) = 6.1, J(3a′,OH) = 5.6, J_g = 12.0 (H-3a′); 3.79 pent, 1 H, J(1′,3b′) = J(3b′,OH) = 5.4, J_g = 12.0 (H-3b′). Mass spectrum (FAB), *m/z* (rel.%): 235 (100) [M + H].

9-(2-Azido-1-carboxyethyl)adenine (**10**)

A mixture of compound **6a** (1 g, 4.3 mmol), potassium permanganate (1.8 g), 2 M sodium hydroxide solution (1 ml) and water (20 ml) was stirred for 2 days at room temperature. After addition of another potassium permanganate portion (0.8 g) and 2 M sodium hydroxide solution (1 ml), the mixture was stirred for 4 days at ambient temperature, filtered and concentrated. The residue was deionized on Dowex 50X8 (H^+ form, 50 ml). The product-containing fraction was evaporated and applied on a Dowex 1 column (acetate form, 20 ml). Elution with a linear gradient 0–2 M acetic acid solution (1 l each), evaporation and crystallization from ethanol–water afforded pure product **10**. Yield: 0.55 g (52%), E_{Up} 0.65. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.24 s, 1 H (H-2); 8.14 s, 1 H (H-8); 7.35 brs, 2 H (NH_2); 5.52 dd, 1 H, J(1′,2a′) = 9.0, J(1′,2b′) = 4.4 (H-1′); 4.31 dd, 1 H, J(1′,2′a) = 9.0, J_g = 13.2 (H-2′a); 4.08 dd, 1 H, J(1′,2′a) = 4.4, J_g = 13.2 (H-2′). For $C_8H_8N_8O_2$. H_2O (266.2) calculated: 36.09% C, 3.79% H, 42.08% N; found: 36.06% C, 3.76% H, 41.80% N. Mass spectrum (FAB), *m/z* (rel.%): 249 (40) [M + H].

(R)-9-(2-Azido-1-carboxyethyl)adenine ((*R*)-**10**). Starting from compound **13**. Yield: 0.42 g (39%), E_{Up} 0.66. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.22 s, 1 H (H-2); 8.13 s, 1 H (H-8); 7.35 brs, 2 H (NH_2); 5.51 dd, 1 H, J(1′,2a′) = 9.0, J(1′,2b′) = 4.4 (H-1′); 4.33 dd, 1 H, J(1′,2′a) = 9.0, J_g = 13.1 (H-2′a); 4.09 dd, 1 H, J(1′,2′a) = 4.4, J_g = 13.1 (H-2′). Mass spectrum (FAB), *m/z* (rel.%): 249 (60) [M + H].

9-(2-Amino-1-carboxyethyl)adenine (**11**)

Compound **10** (0.25 g, 1 mmol) was hydrogenated in methanol (40 ml) over 10% palladium on charcoal (0.2 g) (0.1 ml 40% $PdCl_2$ in HCl added) under stirring for 24 h at room temperature. The mixture was filtered through a pad of Celite. The catalyst was washed with hot methanol and hot water (100 ml each). The filtrate was evaporated and the residue was deionized on Dowex 50X8 (H^+ form, 20 ml). The product-containing fraction was evaporated and applied on column of Dowex 1 (acetate form, 15 ml). Elution with a linear gradient 0–0.2 M acetic acid solution and evaporation of the product-containing fractions afforded pure product. Yield: 0.12 g (54%), E_{Up} 0.59. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.55 brs, 1 H (COOH); 8.12 s, 1 H (H-2); 8.10 s, 1 H (H-8); 7.15 brs, 2 H (NH_2); 4.91 dd, 1 H, J(1′,2′a) = 9.3, J(1′,2′b) = 5.6 (H-1′); 3.59 dd, 1 H, J(1′,2′a) = 9.3, J_g = 12.1 (H-2′a); 3.34 brs,

2 H (NH_2); 3.24 dd, 1 H, *J*(1′,2′b) = 5.6, J_g = 12.1 (H-2′b). Mass spectrum (FAB), *m/z* (rel.%): 223 (100) [M + H].

(R)-9-(2-Amino-1-carboxyethyl)adenine ((*R*)-**11**). Yield: 0.15 g (68%), E_{Up} 0.57. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.50 brs, 1 H (COOH); 8.10 s, 2 H (H-2, H-8); 7.16 brs, 2 H (NH_2); 4.90 dd, 1 H, *J*(1′,2′a) = 9.3, *J*(1′,2′b) = 5.6 (H-1′); 3.59 dd, 1 H, *J*(1′,2′a) = 9.3, J_g = 12.2 (H-2′a); 3.34 brs, 2 H (NH_2); 3.25 dd, 1 H, *J*(1′,2′b) = 5.6, J_g = 12.2 (H-2′b). Mass spectrum (FAB), *m/z* (rel.%): 223 (100) [M + H]. CD spectrum: $[\Theta]_{205}$ –7 370, $[\Theta]_{220}$ +1 190 (*c* 7.48 . 10^{-4} mol/l, water).

NAD^+ Analogues **8**. General Procedure

DBU 1,8-diazabicyclo[5.4.0]undec-7-ene was added dropwise to a suspension of 3-(adenin-9-yl)-2-aminopropanoic acid[7] (**9**) or 9-(2-amino-1-carboxyethyl)adenine (**11**) (0.1 g, 0.45 mmol) in dry methanol (10 ml) until the pH reached 7–8 (wet pH-paper). Then the 3-carbamoyl-1-(2,4-dinitrophenyl)pyridinium chloride[2] (0.16 g, 0.49 mmol) was added and the mixture was stirred for 4 h. The solvent was evaporated and the residue dissolved in water (10 ml). In the case of compound **8a**, its aqueous solution was applied on the column of Amberlite IRC-50 (H^+ form, 20 ml). After washing with water, the resin was mixed with water (100 ml), the pH adjusted to 7–8 with ammonia and the suspension filtered. After evaporation of water, the residue was dissolved in methanol and the product was precipitated by an addition of ether. In the case of compound **8b**, its aqueous solution was washed with ether (10 × 10 ml) and the product was purified by preparative HPLC.

3-(Adenin-9-yl)-2-(3-carbamoylpyridinium)propanoate (**8a**). Yield: 97 mg (60%). ^{1}H NMR spectrum ($(CD_3)_2SO$): 9.40 brs, 1 H (H-2″); 8.99 d, 1 H, *J*(6″,5″) = 6.1 (H-6″); 8.80 d, 1 H, *J*(4″,5″) = 8.1 (H-4″); 8.50 s and 8.07 s, 2 × 1 H (NH_2″); 8.05 s, 1 H (H-2); 8.04 dd, 1 H, *J*(5″,6″) = 6.1, *J*(5″,4″) = 8.1 (H-5″); 7.77 s, 1 H (H-8); 5.61 dd, 1 H, *J*(3′a,2′) = 3.4, *J*(3′b,2′) = 10.5 (H-2′); 5.15 dd, 1 H, *J*(3′a,2′) = 3.4, J_g = 15.4 (H-3′a); 5.01 dd, 1 H, *J*(3′b,2′) = 10.5, J_g = 15.4 (H-3′b). Exact mass (FAB HRMS): calculated for $C_{14}H_{14}N_7O_3$: 328.1158; found: 328.1139. Mass spectrum (FAB), *m/z* (rel.%): 328 (50) [M + H].

2-(Adenin-9-yl)-3-(3-carbamoylpyridinium)propanoate (**8b**). Yield: 90 mg (61%). ^{1}H NMR spectrum (D_2O): 8.94 brs, 1 H (H-2″); 8.85 d, 1 H, *J*(6″,5″) = 6.1 (H-6″); 8.81 d, 1 H, *J*(4″,5″) = 8.1 (H-4″); 8.20 s, 1 H (H-2); 8.02 dd, 1 H, *J*(5″,6″) = 6.1, *J*(5″,4″) = 8.1 (H-5″); 7.99 s, 1 H (H-8); 5.70 dd, 1 H, *J*(2′,3′a) = 4.9, *J*(2′,3′b) = 9.5 (H-2′); 5.62 dd, 1 H, *J*(2′,3′a) = 4.9, J_g = 13.8 (H-3′a); 5.34 dd, 1 H, *J*(2′,3′b) = 9.5, J_g = 13.8 (H-3′b). Exact mass (FAB HRMS): calculated for $C_{14}H_{14}N_7O_3$: 328.1158; found: 328.1203. Mass spectrum (FAB), *m/z* (rel.%): 328 (30) [M + H].

(R)-2-(Adenin-9-yl)-3-(3-carbamoylpyridinium)propanoate (**8c**). Yield: 120 mg (81%). ^{1}H NMR spectrum (D_2O): 8.95 brs, 1 H (H-2″); 8.84 d, 1 H, *J*(6″,5″) = 6.1 (H-6″); 8.83 d, 1 H, *J*(4″,5″) = 8.1 (H-4″); 8.21 s, 1 H (H-2); 8.04 dd, 1 H, *J*(5″,6″) = 6.1, *J*(5″,4″) = 8.1 (H-5″); 7.97 s, 1 H (H-8); 5.70 dd, 1 H, *J*(2′,3′a) = 4.9, *J*(2′,3′b) = 9.5 (H-2′); 5.61 dd, 1 H, *J*(2′,3′a) = 4.9, J_g = 13.7 (H-3′a); 5.35 dd, 1 H, *J*(2′,3′b) = 9.5, J_g = 13.7 (H-3′b). Exact mass (FAB HRMS): calculated for $C_{14}H_{14}N_7O_3$: 328.1158; found: 328.0829. Mass spectrum (FAB), *m/z* (rel.%): 328 (30) [M + H]. CD spectrum: $[\Theta]_{214}$ –10 570, $[\Theta]_{270}$ +14 380 (*c* 6.24 . 10^{-4} mol/l, water).

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