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Synthesis and Chiroptical Properties of Some Abbreviated NAD^+ Analogues

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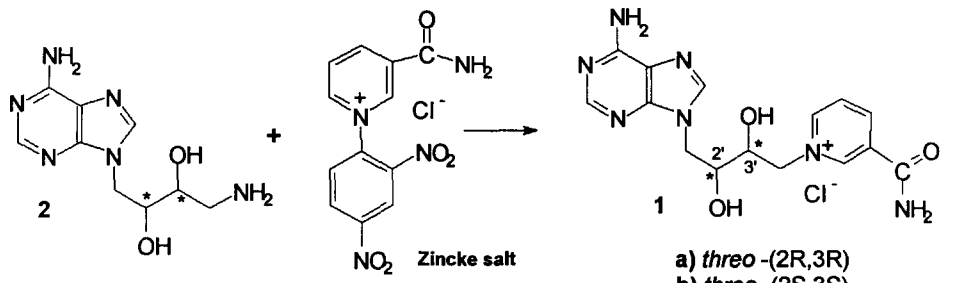
Abstract: Four novel "abbreviated" NAD^+ analogues were prepared by the Zincke reaction. The acyclic chain joining the bases of the analogues bears two stereogenic centers with hydroxyl groups in the both *erythro* and *threo* mutual relations. The CD spectra of all stereoisomers were measured and discussed.

Nicotinamide adenine dinucleotide, the biologically important compound combining adenine and nicotinamide nucleosides in one molecule, serves not only as a coenzyme of oxidation-reduction enzymes but also in several other reactions with no apparent redox change. This variety of functions can be attributed to the structural features of the NAD^+ molecule. Only the nicotinamide ring is involved in the biochemical redox reactions. The adenine part remains chemically unaltered during the process, but the interaction of the purine base and nicotinamide moiety is important for the conformation of coenzyme. The role of hydrophilic backbone joining the two bases is to bind the coenzyme into the active site of enzyme. To study the structure-activity relationship a great variety of NAD^+ analogues was synthesized[1].

We are investigating 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.[2] Circular dichroism, with its extreme sensitivity to conformational changes, should reflect the changes in intramolecular interaction of the bases and their dependence on external conditions.

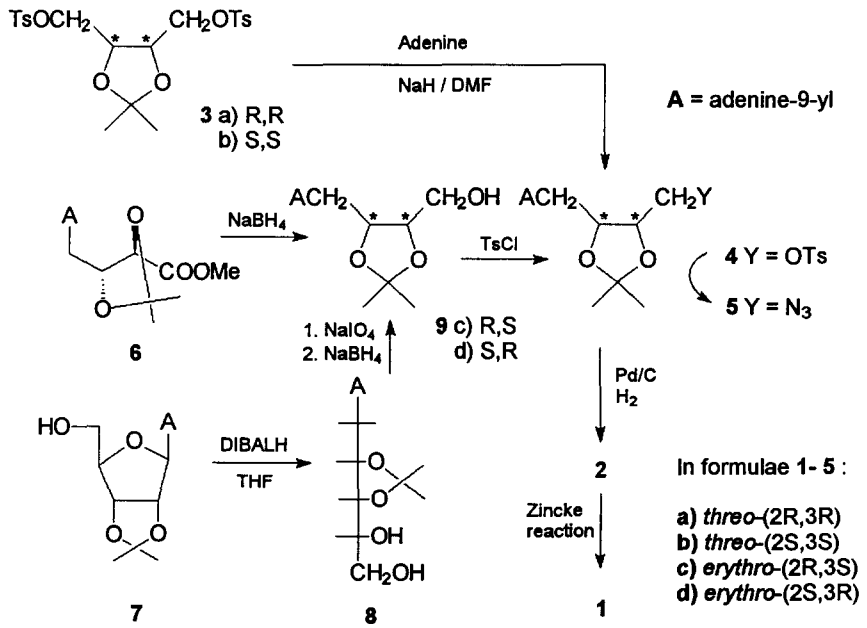
A series of four stereoisomeric abbreviated NAD^+ analogues 9-[4-(3-carbamoylpyridinium)-2,3-dihy-



Scheme 1

droxybutyl]adenine salts **1a-d** was prepared. Adenine and nicotinamide moieties of these analogues are linked by a four-carbon-atom acyclic chain bearing two stereogenic centers with hydroxyl groups to substitute the carbohydrate moiety. All four stereoisomers were obtained by the Zincke reaction[3] (**Scheme 1**) of the corresponding 9-(4-amino-2,3-dihydroxybutyl)adenines **2**, that were prepared from optically active starting materials by multistep syntheses (**Scheme 2**).

For the synthesis of the both *threo* amino derivatives **2a,b** the earlier described procedure was used.[4] Sodium salt of adenine was alkylated by ditosyl threitol derivatives **3a** and **3b** prepared from D-mannitol and



Scheme 2

L-tartaric acid, respectively. The resulting compounds **4** on treatment with sodium azide, subsequent catalytic hydrogenation of the intermediates **5** and acid hydrolysis afforded the enantiomeric *threo* amino derivatives **2a,b.**

The *erythro* amino derivative **2c** was obtained from methylester of D-eritadenine[4] **6** that was transformed to its 2',3'-O-isopropylidene derivative, reduced with sodium borohydride and the intermediate **9c** was further tosylated to form compound **4c**. The transformation of tosyl derivative **4c** to **2c** via azido derivative **5c** was analogous to that of the *threo* derivatives.

Recently published[5] reductive cleavage of the ribose moiety in 2',3'-O-isopropylidene adenosine was applied to obtain compound **8**. Side-chain degradation and further modification of the chain as described above afforded the *erythro* amino derivative **2d**. Resulting compounds **2a-d** were treated with 3-carbamoyl-1--(2,4-dinitrophenyl)pyridinium chloride (Zincke salt) in methanol to form abbreviated NAD^+ analogues **1a-d**.

NMR SPECTRA

Table 1: ^1H NMR Spectra in $(CD_3)_2SO$.

	1a	**1b**	**1c**	**1d**
H - 2	8.16s	8.16 s	8.21 s	8.21 s
H - 8	8.05 s	8.06 s	8.16 s	8.17 s
HN_2	7.21 brs	7.21 brs	7.62 brs	7.90 br
H - 1a'	4.30 dd (4.4, 13.9)	4.30 dd (4.6, 13.9)	4.51 dd (3.0, 13.9)	4.51 dd (3.2, 14.2)
H - 1b'	4.20 dd (8.5, 13.9)	4.20 dd (8.5, 13.9)	4.16 dd (8.0, 13.9)	4.17 dd (8.3, 14.2)
H - 2'	4.05 m	4.05 m	3.85 m	3.84 m
H - 3'	3.93 m	3.93 m	3.77 m	3.76 m
H - 4a'	4.87 dd (2.2, 12.9)	4.87 dd (2.2, 12.9)	4.99 dd (2.7, 12.9)	4.98 dd (3.2, 13.2)
H - 4b'	4.64 dd (9.8, 12.9)	4.64 dd (9.8, 12.9)	4.64 dd (8.8, 12.9)	4.62 dd (8.8, 13.2)
OH	5.66, 5.51 2xd (7.3, 6.8)	5.67, 5.52 2xd (7.3, 6.8)	5.95, 5.78 2xbr	5.87, 5.73 2xbr
H - 2"	9.57 brs	9.52 brs	9.45 brs	9.40 brs
H - 4"	8.99 dt (1.5, 1.5, 8.1)	8.99 dt (1.5, 1.5, 8.1)	8.99 dt (1.5, 1.5, 8.1)	8.97 dt (1.5, 1.5, 8.1)
H - 5"	8.26 dd (6.1, 8.1)	8.26 dd (6.1, 8.1)	8.27 dd (6.1, 8.1)	8.27 dd (6.1, 8.1)
H - 6"	9.14 dt (1.2, 1.2, 6.1)	9.14 dt (1.2, 1.2, 6.1)	9.12 brd (1.2, 1.2, 6.1)	9.09 dt (1.2, 1.2, 6.1)
$CONH_2$	8.66, 8.16 2xbrs	8.68, 8.17 2xbrs	8.67, 8.17 2xbrs	8.58, 8.16 2xbrs

Table 2: ^{13}C NMR Spectra in $(CD_3)_2SO$.

	1a	1b	1c	1d
C - 2	152.62	152.42	150.94	150.62
C - 4	150.08	150.03	149.64	149.53
C - 5	118.99	118.88	118.61	118.54
C - 6	156.17	155.99	154.97	154.47
C - 8	142.29	142.30	142.57	142.50
C - 1'	46.52	46.55	46.79	46.92
C - 2'	71.03	71.01	71.33	71.34
C - 3'	69.67	69.66	7078	70.77
C - 4'	64.72	64.72	64.05	64.09
C - 2"	147.72	147.67	147.51	147.53
C - 3"	133.85	133.85	133.49	133.53
C - 4"	146.06	146.05	145.78	145.81
C - 5"	127.79	127.76	127.54	127.57
C - 6"	143.89	143.90	143.69	143.66
C-O	163.24	163.23	162.28	163.01

CD SPECTRA - RESULTS AND DISCUSSION

The CD spectra of the enantiomeric *threo* compounds **1a** and **1b** in water are shown in Fig.1A. The spectra are approximately mirror images within the scope of experimental error. Both spectra differ from the sum of the spectra of its components ((S)-9-(2,3-dihydroxypropyl)adenine[6] and (S)-1-(2,3-dihydroxypropyl) -3-carbamoylpyridinium perchlorate[2] for **1b**, see Fig. 1B). The CD spectrum of **1a** is similar to the CD spectrum of NAD^+ (Fig. 1A) and the spectrum of **1b** to the CD spectrum of adenosine mononicotinate under the conditions which do not favor stacking (i.e. low pH or the presence of dioxane).[7-9] The CD spectra of the enantiomeric *erythro* compounds **1c** and **1d** (Fig. 2A) differ substantially from the spectra of **1a** and **1b** and are very similar to the spectra calculated as the sum of the spectra of its constituents (Fig.2B).

The CD spectra of all stereoisomers are not much sensitive to the changes of the experimental conditions which can decrease the stacking forces. The changes in CD spectra due to acidification by HCl or addition of dioxane (not shown) are small and cannot be interpreted as changes in stacking but more probably reflect the changes in UV absorption spectra under these experimental conditions. These results indicate that, in water, these compounds are probably not stacked or show only a low degree of stacking.

The ability of the compounds **1a-d** to form a stacked conformation was further investigated using the addition of salts which can increase the degree of stacking. In a high salt concentration the conformation of these compounds depends on the nature and concentration of salts. In 2M NaCl and 4.5M LiCl only small

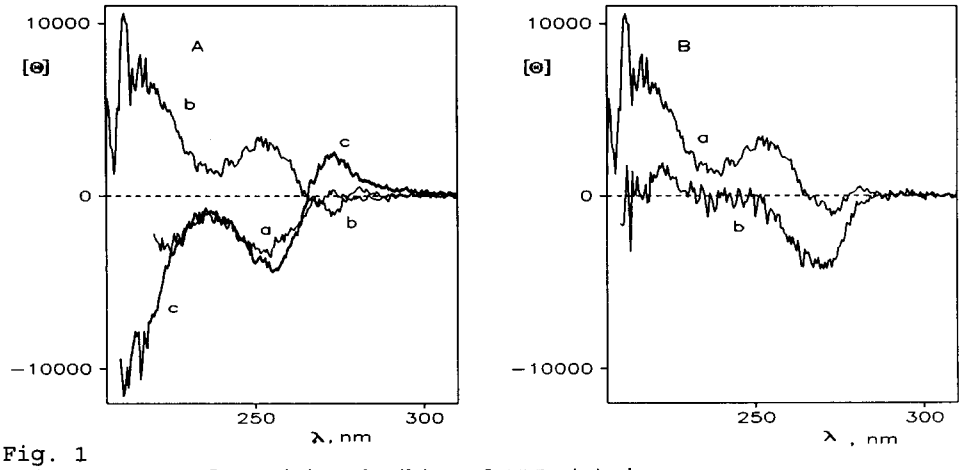


Fig. 1
A.CD spectra of 1a (a), 1b (b) and NAD (c) in water
B.CD spectra of 1b (a) and the sum of its constituents ((S)-9-(2,3-dihydroxypropyl)adenine and (S)-1-(2,3-dihydroxypropyl)-3-carbamoyl pyridinium perchlorate) (b) in water

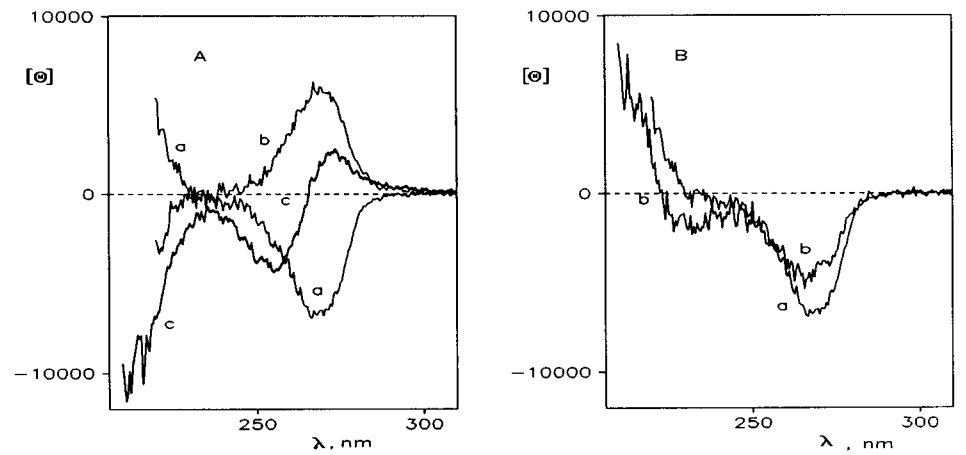


Fig. 2
A.CD spectra of 1c (a), 1d (b) and NAD (c) in water
B.CD spectra of 1c (a) and the sum of its constituents((R)-9-(2,3-dihydroxypropyl)adenine and (S)-1-(2,3-dihydroxypropyl)-3-carbamoylpyridinium perchlorate) (b) in water

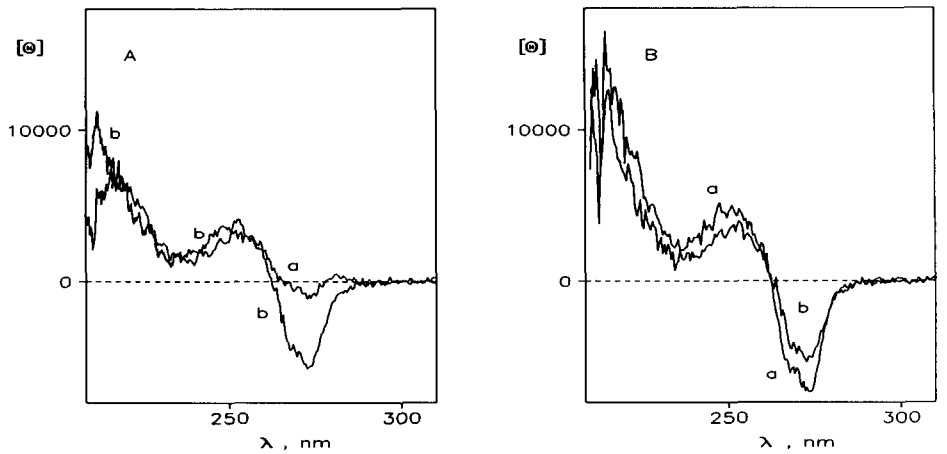


Fig. 3
A. CD spectra of 1b in water (a) and in 5M $NaClO_4$ (b)
B. CD spectra of 1b in 5M $NaClO_4$ at -30 °C (a) and at 40 °C (b)

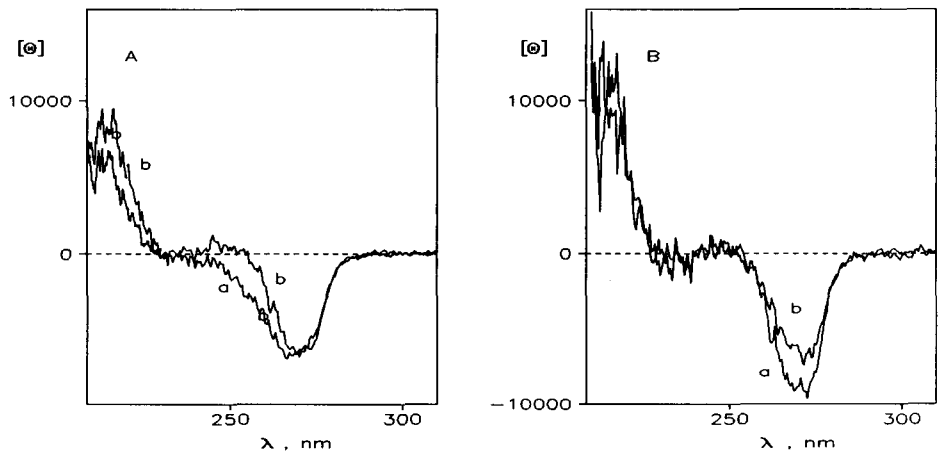


Fig. 4
A. CD spectra of 1c in water (a) and in 5M $NaClO_4$ (b)
B. CD spectra of 1c in 5M $NaClO_4$ at -20 °C (a) and 40 °C

changes in CD were observed. In the case of **1a** and **1b** these salts increase slightly the intensity of CD band at 272 nm, in the case of **1c** and **1d** they slightly increase the intensity of CD band in the region of 270 nm and shift of its maximum to higher wavelengths (not shown). Of the salts tested only $NaClO_4$ has a substantial effect on CD spectra of these compounds. Figure 3A showing the CD spectra of **1b** indicates that $NaClO_4$ substantially increases the intensity of CD bands at 272 nm and 215 nm and slightly increases the intensity of CD band at 252 nm. The effect of $NaClO_4$ on **1a** is qualitatively the same. For **1c** and **1d** the increase of the $NaClO_4$ concentration affects the shape of the CD band at about 270 nm and the intensity of the band at 212 nm (Fig. 4A); these effects are temperature-sensitive (Fig. 2B, 4B). These results indicate that at high $NaClO_4$ concentrations the NAD^+ analogues **1a-d** are at least partly in a stacked conformation. The CD spectra indicate that only the conformation of **1a** is to some extent similar to NAD^+, however, its degree of stacking is lower.

It is well known[10] that stacking of nucleotides is reflected by the decrease in absorption coefficient. The comparison of UV spectra of **1b** and **1c** in water and in 5M $NaClO_4$ has shown about 3% decrease of absorbance at 260 nm in 5M $NaClO_4$. This observation further supports our conclusion that these compounds can form at least partly stacked conformation. However, their degree of stacking under comparable conditions is lower compared to NAD^+ or adenine mononicotinate.

EXPERIMENTAL

Unless otherwise stated, solvents were evaporated at 40°C/2kPa and compounds were dried at 60°C/2kPa over P_2O_5. TLC was performed on Silufol UV 254 plates (Kavalier Votice, Czech Republic). Column chromatography was performed on silica gel (30 mm) of the same source. NMR spectra were measured on Varian Unity 500 spectrometer (500 MHz for ^1H and 125.7 MHz for ^{13}C NMR) in hexadeuteriodimethyl sulfoxide referenced to the solvent signals 2.5 ppm for ^1H and 39.7 ppm for ^{13}C NMR. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix) technique. CD spectra were measured on a Jobin-Yvon Dichrographe MarkV using software Dichrosoft Version A written by Dr. P. Maloň of our Institute. The cell length was 0.02 - 1 cm. The measurement of CD as a function of temperature (temperature range from +40°C to -30°C) was carried out using Jobin-Yvon Variokryostatin 0.1 cm cell. The concentration of samples was calculated from the absorbance values at absorption maximum. The absorbance was measured on a spectrophotometer Unicam PU8800 using 0.1 cm cells. The value of ε_{max} determined in our Laboratory, ($\varepsilon(260)$ =22225 for **1a-d** was used for calculations). Concentration of NAD^+, (S)-9-(2,3-dihydxypropyl)-adenine and (S)-1-(2,3-dihydroxypropyl)-3-carbamoylpyridinium perchlorate was determined from the weighed amounts. Satisfactory microanalyses were obtained for all compounds, except for extremely hygroscopic compounds **8**, **2c,d** and **1a-d**.

(2R,3S)-*erythro*-9-(4-O-Toluenesulfonyl-2,3-O-isopropylidene-2,3,4-trihydroxybutyl)adenine (4c)
(2S,3R)-*erythro*-9-(4-O-Toluenesulfonyl-2,3-O-isopropylidene-2,3,4-trihydroxybutyl)adenine (4d)

(General procedure): p-Toluenesulfonylchloride (1.36 g, 7.0 mmol) and 4-dimethylaminopyridine (30 mg) was added at -10°C to a stirred solution of *erythro*-9-(2,3-O-isopropylidene-2,3,4-trihydroxybutyl)adenine (9) (1.60 g, 5.7 mmol) in pyridine (20 ml). After standing at room temperature overnight, water (10 ml) was added and the mixture was taken down. The residue was taken up in chloroform (100 ml), washed with water and dried over magnesium sulfate. Pure product was obtained after crystallization from ethanol. Yield: 1.8 g (73%) of compound **4c**, m.p. 281-283°C. ^{1}H NMR, δ: 8.12 (s, 1H, H-2), 8.04 (s, 1H, H-8), 7.80 and 7.49 (2xd, 2x2H, OTs), 7.24 (brs, 2H, NH_2), 4.64 (ddd, 1H, J=3.7, 6.3 and 9.1, H-2'), 4.43 (br td, 1H, J=3.4, 6.3 and 7.3, H-3'), 4.30 (dd, 1H, J=3.7, Jg=14.0, H-1'), 4.28 (dd, 1H, J=3.4, Jg=10.4, H-4'), 4.15 (dd, 1H, J=9.1, Jg=14.0, H-1'), 4.09 (dd, 1H, J=7.3, Jg=10.4, H-4'), 2.43 (s, 3H, OTs), 1.34 and 1.19 (2xs, 2x3H, CH_3). Anal. calcd. for $C_{19}H_{23}N_5O_5S$: C, 52.64; H, 5.12; N, 16.16; found: C, 52.47; H, 5.35; N, 16.13. Yield: 2.0 g (80%) of compound **4d**, 282-284°C. ^{1}H NMR, δ: 8.12 (s, 1H, H-2), 8.04 (s, 1H, H-8), 7.80 and 7.49 (2xd, 2x2H, OTs), 7.23 (brs, 2H, NH_2), 4.63 (ddd, 1H, J=3.7, 6.3 and 9.1, H-2'), 4.42 (ddd, 1H, J=3.4, 6.3 and 7.3, H-3'), 4.29 (dd, 1H, J=3.4, Jg=14.2, H-1'), 4.28 (dd, 1H, J=3.4, Jg=10.5, H-4'), 4.16 (dd, 1H, J=9.5, Jg=14.2, H-1'), 4.09 (dd, 1H, J=7.6, Jg=10.5, H-4'), 2.43 (s, 3H, OTs), 1.34 and 1.19 (2xs, 2x3H, CH_3). Anal. calcd. for $C_{19}H_{23}N_5O_5S$: C, 52.64; H, 5.12; N, 16.16; found: C, 52.74; H, 5.39; N, 15.88.

(2R,3S)-*erythro*-9-(4-Azido-2,3-O-isopropylidene-2,3-dihydroxybutyl)adenine (5c)
(2S,3R)-*erythro*-9-(4-Azido-2,3-O-isopropylidene-2,3-dihydroxybutyl)adenine (5d)

(General procedure): A mixture of compound **4** (1.5 g, 3.5 mmol) and sodium azide (1.6 g, 25 mmol) in dimethylformamide (30 ml) was stirred for 4h at 100°C, filtered while hot and the filtrate taken down. The residue was extracted with chloroform (100 ml), filtered and taken down. Pure product was obtained after crystallization from ethyl acetate (light petroleum added). Yield: 0.62 g (56%) of compound **5c**, m.p. 194-196° C. ^{1}H NMR, δ: 8.14 (s, 1H, H-2), 8.09 (s, 1H, H-8), 7.24 (brs, 2H, NH_2), 4.62 (ddd, 1H, J=3.2, 6.3 and 10.0, H-2'), 4.44 (td, 1H, J=4.4, 6.6 and 6.6, H-3'), 4.33 (dd, 1H, J=3.2, Jg=14.0, H-1'), 4.24 (dd, 1H, J=10.0, Jg=14.0, H-1'), 3.61 (dd, 1H, J=4.4, Jg=13.2, H-4'), 3.58 (dd, 1H, J=7.1, Jg=13.2, H-4'), 1.47 and 1.24 (2xs, 2x3H, CH_3). Anal. calcd. for $C_{12}H_{16}N_8O_2$: C, 47.36; H, 5.30; N, 36.83; found: C, 47.12; H, 5.25; N, 37.05. Yield: 0.51 g (47%) of compound **5d**, m.p. 194-197°C. ^{1}H NMR, δ: 8.14 (s, 1H, H-2), 8.09 (s, 1H, H-8), 7.23 (brs, 2H, NH_2), 4.61 (ddd, 1H, J=3.2, 6.3 and 10.0, H-2'), 4.44 (td, 1H, J=4.4, 6.6 and 6.6, H-3'), 4.34 (dd, 1H, J=3.0, Jg=14.1, H-1'), 4.23 (dd, 1H, J=10.2, Jg=14.1, H-1'), 3.60 (dd, 1H, J=4.4, Jg=13.2, H-4'), 3.58 (dd, 1H, J=7.1, Jg=13.2, H-4'), 1.47 and 1.24 (2xs, 2x3H, CH_3). Anal. calcd. for $C_{12}H_{16}N_8O_2$: C, 47.36; H, 5.30; N, 36.83; found: C, 47.09; H, 5.28; N, 37.16.

(2R,3S)-*erythro*-9-(4-Amino-2,3-dihydroxybutyl)adenine (2c)

(2S,3R)-*erythro*-9-(4-Amino-2,3-dihydroxybutyl)adenine (2d)

(General procedure): Compound **5** (0.50 g, 1.6 mmol) was hydrogenated in acetic acid (50 ml) over 10% palladium on charcoal (0.40 g) with stirring for two days at room temperature. The mixture was filtered through Celite, evaporated and codistilled with water. The residue was dissolved in 0.25M H_2SO_4 (20 ml). After standing at room temperature for 24h the mixture was deionized on Dowex 50 X 8 column (40 ml, H^+ form). The crude product was purified by crystallization from water-ethanol. Yield: 0.25 g (61%) of compound **2c**, m.p. 127-129°C. ^{1}H NMR, δ: 8.13 (s, 1H, H-2), 8.02 (s, 1H, H-8), 7.19 (brs, 2H, NH_2), 4.42 (dd, 1H, J=2.7, Jg=13.9, H-1'), 4.04 (dd, 1H, J=8.1, Jg=13.9, H-1'), 3.65 (td, 1H, J=2.7, 7.8 and 8.1, H-2'), 3.17 (m,1H, ΣJ=18.1, H-3'), 2.75 (dd, 1H, J=3.9, Jg=12.7, H-4'), 2.61 (dd, 1H, J=6.6, Jg=12.7, H-4'), 5.10 (br, 2H, OH), 3.40 (br, 2H, NH_2). Yield: 0.29 g (71%) of compound **2d**, m.p. 124-127°C. ^{1}H NMR, δ: 8.13 (s, 1H, H-2), 8.02 (s, 1H, H-8), 7.18 (brs, 2H, NH_2), 4.41 (dd, 1H, J=2.7, Jg=14.0, H-1'), 4.05 (dd, 1H, J=8.1, Jg=14.0, H-1'), 3.64 (td, 1H, J=2.7, 7.8 and 8.1, H-2'), 3.16 (m,1H, ΣJ=18.1, H-3'), 2.75 (dd, 1H, J=3.9, Jg=12.8, H-4'), 2.61 (dd, 1H, J=6.7, Jg=12.8, H-4'), 5.09 (br, 2H, OH), 3.39 (br, 2H, NH_2).

Zincke reaction (synthesis of 9-[4-(3-carbamoylpyridinium)-2,3-dihydroxybutyl]adenine chlorides **1a-d**)

(General procedure): To the solution of compound **2** (0.20 g, 0.8 mmol) in dry methanol (20 ml) 3-carbamoyl-1-(2,4-dinitrophenyl)pyridinium chloride (Zincke salt, 0.27 g, 0.83 mmol) was added. The mixture was stirred for 5h, a crude product was precipitated by ether and filtered off. The precipitate was dissolved in water (20 ml) and washed by ether (10 x 20 ml). After evaporation of the aqueous solution, the residue was dissolved in methanol and the product was precipitated by an addition of ether. Yields: 0.17 g (56%) of compound **1a**, 0.19 g (63%) of compound **1b**, 0.20 g (67%) of compound **1c**, 0.20 g (67%) of compound **1d.** For ^{1}H and ^{13}C NMR spectra see Table 1 and 2. FABMS: 344.3 $[M - Cl^-]^+$.

9-(2,3-O-Isopropylidene-1-deoxy-D-ribityl)adenine (8)

To the suspension of 2',3'-O-isopropylideneadenosine (5 g, 16.3 mmol) in dry tetrahydrofuran (100 ml) under argon 1M solution of diisobutylaluminium hydride in THF (100 ml) was slowly added at -20°C. The mixture was stirred for 24 h at room temperature, decomposed successively with ethyl acetate (150 ml) and 4M NaOH (150 ml), and filtered. The filtrate was taken down in vacuo, ethanol was added and the mixture was concentrated and filtered again. The residue was purified by the column chromatography on silicagel (50 ml). Pure product (2.7 g, 54%) was obtained after crystallization from ethanol (light petroleum added), m.p. 230-232°C. ^{1}H NMR, δ: 8.14 (s, 1H, H-2), 8.08 (s, 1H, H-8), 7.20 (brs, 2H, NH_2), 5.0 (br, 1H, OH-C_4), 4.6 (br, 1H, OH-C_5), 4.55 (m, 2H, H-1' and H-2'), 4.21 (dd, 1H, J= 11.5 and 13.9, H-1'), 4.11 (dd,1H, J= 5.9 and 9.3, H-3'), 3.64 (m, 2H, H-4' and H-5'), 3.43 (m, 1H, H-5'), 1.43 and 1.19 (2xs, 2x3H, CH_3).

(2S,3R)-*erythro*-9-(2,3-O-Isopropylidene-2,3,4-trihydroxybutyl)adenine (9d)

To the solution of $NaIO_4$ (1.84g) in 70% acetone (100 ml) the compound **8** (2.5 g, 8.1 mmol) was added at 0°C and the mixture was stirred at the same temperature for 2.5h. Acetone was evaporated, water (20 ml) and Dowex 1 (BH_4^- form, 20ml) was added at 0°C and the mixture was stirred at room temperature for 2 h and filtered. The filtrate was taken down and the product (1.8 g, 83%) was crystallized from ethanol; m.p. 187-188°C. ^{1}H NMR, δ: 8.13 (s, 1H, H-2), 8.08 (s, 1H, H-8), 7.20 (brs, 2H, NH_2), 5.05 (br s, 1H, OH), 4.57 (ddd, 1H, J=3.2, 6.4 and 10.0, H-2'), 4.37 (dd, 1H, J=2.9, Jg=14.2, H-1'), 4.26 (q, 1H, J=6.2, H-3'), 4.21 (dd, 1H, J=10.3, Jg=14.2, H-1'), 3.63 (d, 2H, J=6.1, H-4'), 1.43 and 1.21 (2xs, 2x3H, CH_3). Anal. calcd. for $C_{12}H_{17}N_5O_3$: C, 51.60; H, 6.14; N, 25.08; found: C, 50.78; H, 6.14; N, 24.89.

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