"ABBREVIATED" NAD^+ ANALOGUES CONTAINING A PHOSPHONATE FUNCTION

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Dedicated to the memory of Dr Zdenek Arnold.

"Abbreviated" NAD^+ analogues with anionic phosphonate function as a part of the link between the adenine and nicotinamide moieties, 9-(2-phosphonomethoxyethyl)adenine 2-(3-carbamoylpyridinium)ethyl ester (**1a**), (*R*)- and (*S*)-9-(2-phosphonomethoxypropyl)adenine 2-(3-carbamoylpyridinium)ethyl ester (**1b** and **1c**), (*RS*)-9-(3-hydroxy-2-phosphonomethoxypropyl)adenine 2-(3-carbamoylpyridinium)ethyl ester (**1d**), and (*S*)- and (*R*)-1-[3-(adenin-9-yl)-2-phosphonatomethoxypropyl]-3-carbamoylpyridinium (**2a** and **2b**), were prepared by multistep syntheses using the Zincke reaction in the last step.

Key words: NAD^+ analogues; Phosphonate nucleotide analogues; Zincke reaction.

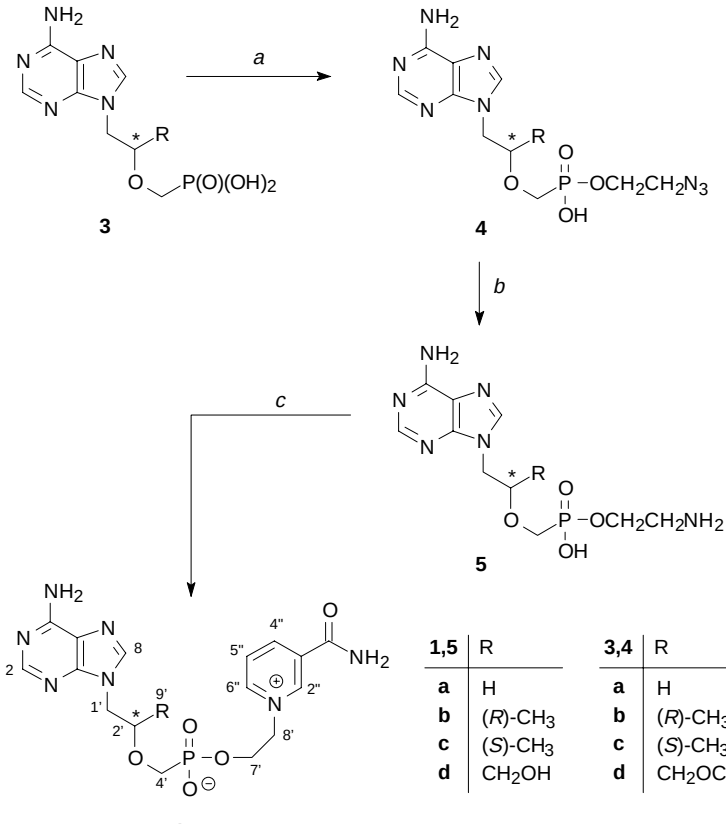
Nicotinamide adenine dinucleotide (NAD) and related nicotinamide adenine dinucleotide phosphate are involved in many enzymatic redox reactions catalyzed by dehydrogenases and reductases. The coenzymatic activity of NAD depends not only on the nicotinamide moiety (that is during the redox reaction reduced to its 1,4-dihydro form and *vice versa*) but also on the rest of the molecule. This "nonfunctional" part that remains chemically unaltered, can induce significant conformational changes and is important for the interaction with the enzyme. A great number of NAD analogues with modified various parts of the molecule has been prepared to investigate the structure–activity relationship. Considering all the metabolic roles of NAD, its analogues may have very interesting biological properties[1].

We studied such modification of the coenzyme, where the adenine and the nicotinamide moieties are preserved, but the ribosediphosphoribose link is replaced by an acyclic chain[2,3]. Functionalized alkyl chains were chosen as linking elements because of their flexibility and greater stability compared to ribose.

In this paper we report on compounds containing, contrary to the previous analogues[2], an anionic phosphonate function that either compensates the positive charge of the carbamoylpyridinium moiety (compounds **1a–1d**) or even changes the total charge

of the molecule to a negative one (compounds **2a** and **2b**), to some extent similarly as in the NAD^+ molecule. As parent structures for the design of such NAD^+ analogues we have chosen the acyclic phosphonate nucleotide analogues 9-(2-phosphonomethoxyethyl)adenine (PMEA, **3a**), 9-(2-phosphonomethoxypropyl)adenine (PMPA, **3b**, **3c**) and 9-(3-hydroxy-2-phosphonomethoxypropyl)adenine (HPMPA), that exhibit manifold biological effects[4,5].

Compounds of the first group (**1a–1d**) (Scheme 1) contain a phosphonomethyl monoester function as a part of the acyclic chain joining the adenine and nicotinamide moieties. Starting with the corresponding phosphonates **3a–3c** (PMEA, ref.[6], (*R*)- or (*S*)-PMPA, ref.[7]) and 3′-*O*-benzyl HPMPA (**3d**), prepared by alkylation of adenine with diisopropyl {[1-(benzyloxy)methyl]-2-(*p*-toluenesulfonyloxy)}ethoxymethylphosphonate[8] followed by deprotection of the phosphonate group, the 2-azidoethyl monoesters

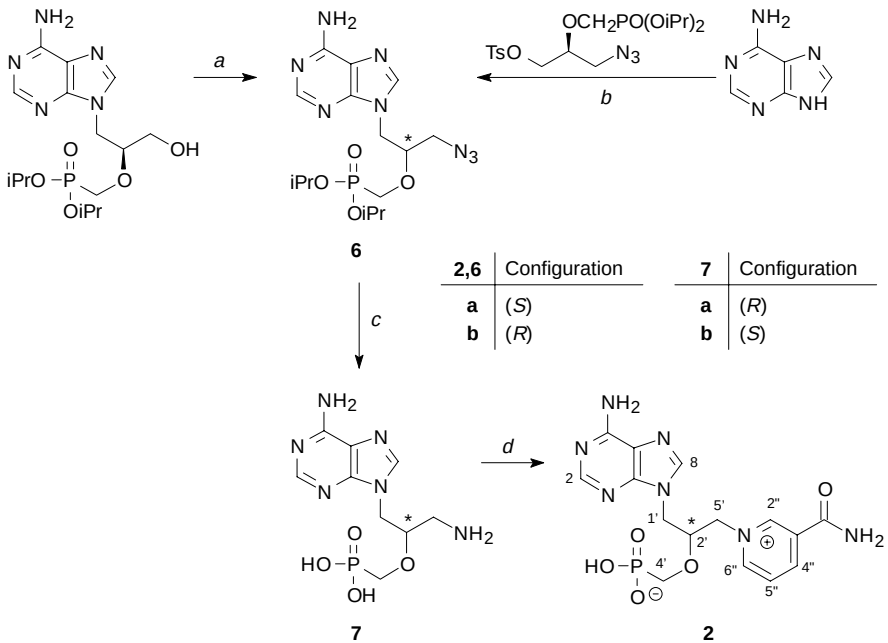


a) 1. $(Cl_3CO)_2CO$, 2.$HOCH_2CH_2N_3$; *b*) Pd-C/H_2;
c) DBU/3-carbamoyl-1-(2,4-dinitrophenyl)pyridinium chloride

SCHEME 1

4a–4d were prepared by the standard method using triphosgene/dimethylformamide for activation[9]. Reduction of the azido group (and simultaneous cleavage of the benzyl group in the derivative **4d**) over palladium on charcoal afforded the aminoethyl esters **5a–5d**. These amino derivatives were converted into the NAD^+ analogues **1a–1d** by reaction with 3-carbamoyl-1-(2,4-dinitrophenyl)pyridinium chloride in absolute methanol (Zincke reaction)[10]. Since in the presence of the acid group the amino function is protonated, the Zincke reaction, based on nucleophilic attack by amine at the position 2 of the activated pyridinium salt, had to be carried out in the presence of a strong non-nucleophilic amine base, e.g. 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).

In the stereoisomeric compounds **2a** and **2b** (Scheme 2) the acyclic chain is functionalized with a free phosphonomethoxy moiety. The first step in the synthesis of the (*R*)-enantiomer **2b** was the alkylation of adenine by diisopropyl (*S*)-[1-azidomethyl-2-(*p*-toluenesulfonyloxy)]ethoxymethylphosphonate[11] in the presence of cesium carbonate to obtain (*R*)-azido derivative **6b**. The (*S*)-enantiomer **6a** was prepared from diisopropyl (*S*)-HPMPA[12] via its *O*-tosyl derivative that was converted to the azido compound by



a) 1. TsCl/Py, 2. NaN_3/DMF; *b*) $CsCO_3$/DMF; *c*) 1. Pd-C/H_2, 2. Me_3SiBr/CH_3CN; *d*) DBU/3-carbamoyl-1-(2,4-dinitrophenyl)pyridinium chloride

SCHEME 2

reaction with sodium azide. After reduction of **6a** and **6b** over palladium on charcoal, the protecting isopropyl ester groups were removed by action of bromotrimethylsilane to form amino analogues of HPMPA **7a** and **7b**. The last step of the reaction sequence was the Zincke reaction of derivatives **7a** and **7b** that under the above-mentioned conditions gave the desired compounds **2a** and **2b**.

The structures of the resulting NAD^+ analogues **1a–1d**, **2a** and **2b** were confirmed by ^1H NMR (Table I) and ^{13}C NMR spectra (Table II). High resolution mass spectrometry was used to determine the molecular formulas instead of microanalyses, because of the extremely hygroscopic character of the compounds.

The cytostatic activity was tested on L-1210 mouse leukemia cells. Neither of the compounds exhibited significant cytostatic activity and neither was cytotoxic. *In vitro* activities against DNA viruses and retroviruses were determined at the Rega Institute for Medical Research (Professor E. De Clercq, Head), Catholic University Leuven, Belgium; these results, together with *in vitro* enzyme studies, will be published in detail and compared with other types of NAD^+ analogues in a separate communication.

EXPERIMENTAL

Unless stated otherwise, solvents were evaporated at 40 °C/2 kPa and compounds were dried at 2 kPa over P_2O_5. 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 carried out 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. NMR spectra were measured on a 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), or in deuterium oxide containing sodium deuteroxide with sodium 3-(trimethylsilyl)propanesulfonate as an internal standard for ^1H NMR and dioxane as an external standard for ^{13}C NMR (δ(dioxane) 66.86 ppm). Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using the FAB technique (ionization by Xe, accelerating voltage 8 kV, glycerol matrix). Dimethylformamide was distilled from P_2O_5 and stored over molecular sieves (4A). The cytostatic assays were performed in the laboratory of Dr I. Votruba at this Institute.

9-(3-Benzyloxy-2-phosphonomethoxypropyl)adenine (**3d**)

A stirred mixture of adenine (1.0 g, 7.4 mmol), cesium carbonate (1.2 g, 3.7 mmol) and dimethylformamide (10 ml) was heated at 120 °C for 1 h. After addition of diisopropyl {[1-(benzyloxy)methyl]-2-(*p*-toluenesulfonyloxy)}ethoxymethylphosphonate[8] (2.0 g, 3.9 mmol) in dimethylformamide (15 ml), the heating at 120 °C was continued for 4 h. The reaction mixture was taken down, and the product of alkylation was isolated by preparative thin-layer chromatography on silica gel (15% of methanol in chloroform). Then acetonitrile (60 ml) and bromotrimethylsilane (5 ml) were added to the dried residue and the mixture was stirred at room temperature for 24 h. The solvent was evaporated, the residue was codistilled with acetonitrile, dissolved in water and made alkaline with triethylamine. After evaporation in vacuo the residue was deionized on Dowex 50X8 (H^+ form, 70 ml). The product-containing fraction was evaporated and applied onto a column of Dowex 1 (acetate form, 50 ml). The column was washed with water and 0.25 M acetic acid (0.5 l), then the Dowex was stirred with hot 5 M acetic acid and

TABLE I
^{1}H NMR spectra (δ, ppm) of compounds **1** and **2**

Proton	**1a**[a]	**1b**[b]	**1c**[b]	**1d**[a,c]	**2a**[b]	**2b**[a]
H-2	8.46 s, 1 H	8.20 s, 1 H	8.13 s, 1 H	8.11 s, 1 H	8.24 s, 1 H	8.16 s, 1 H
H-8	8.40 s, 1 H	8.14 s, 1 H	8.10 s, 1 H	8.10 s, 1 H	8.21 s, 1 H	8.12 s, 1 H
NH_2	7.27 s, 2 H			7.19 s, 2 H		7.25 brs, 2 H
H-1′	4.38 t, 2 H *J*(1′,2′) = 5.1	4.32 dd, 1 H *J*(1′a,2′) = 3.2 *J*(gem) = 14.9 4.17 dd, 1 H *J*(1′b,2′) = 7.6 *J*(gem) = 14.9	4.28 dd, 1 H *J*(1′a,2′) = 3.0 *J*(gem) = 14.9 4.14 dd, 1 H *J*(1′b,2′) = 7.5 *J*(gem) = 14.9	4.21 dd, 1 H *J*(1′a,2′) = 4.2 *J*(gem) = 14.2 4.10 dd, 1 H *J*(1′b,2′) = 7.3 *J*(gem) = 14.2	4.70 dd, 1 H *J*(1′a,2′) = 3.6 *J*(gem) = 15.4 4.55 dd, 1 H *J*(1′b,2′) = 4.6 *J*(gem) = 15.4	4.57 dd, 1 H *J*(1′a,2′) = 4.0 *J*(gem) = 14.4 4.21 dd, 1 H *J*(1′b,2′) = 5.4 *J*(gem) = 14.4
H-2′	3.81 t, 2 H *J*(1′,2′) = 5.1	3.94 m, 1 H	3.92 m, 1 H	3.71 m, 1 H	4.45 m, 1 H	4.44 m, 1 H
H-4′	3.66 d, 2 H *J*(P,CH) = 8.3	3.68 dd, 1 H *J*(P,CHa) = 8.8 *J*(gem) = 13.4 3.46 dd, 1 H *J*(P,CHb) = 9.7 *J*(gem) = 13.4	3.69 dd, 1 H *J*(P,CHa) = 8.8 *J*(gem) = 13.5 3.46 dd, 1 H *J*(P,CHb) = 9.7 *J*(gem) = 13.5	3.37 dd, 1 H *J*(P,CHa) = 9.0 *J*(gem) = 13.4 3.35–3.25 m, 1 H	3.71 dd, 1 H *J*(P,CHa) = 9.0 *J*(gem) = 12.9 3.39 dd, 1 H *J*(P,CHb) = 9.8 *J*(gem) = 12.9	3.42 dd, 1 H *J*(P,CHa) = 8.3 *J*(gem) = 13.2 3.25 dd, 1 H *J*(P,CHb) = 8.1 *J*(gem) = 13.2
H-5′	–	–	–	–	5.08 dd, 1 H *J*(5′a,2′) = 2.9 *J*(gem) = 13.9 4.58 dd, 1 H *J*(5′b,2′) = 8.3 *J*(gem) = 13.9	5.07 dd, 1 H *J*(5′a,2′) = 2.4 *J*(gem) = 13.6 4.55 dd, 1 H *J*(5′a,2′) = 6.8 *J*(gem) = 13.6

TABLE I
(*Continued*)

Proton	**1a**[a]	**1b**[b]	**1c**[b]	**1d**[a,c]	**2a**[b]	**2b**[a]
H-7′	4.40 m, 2 H *J*(P,OCH) = 7.8	4.14 m, 2 H *J*(7′,8′) = 4.7 *J*(P,OCH) = 7.0	4.15 m, 2 H	4.19 m, 2 H		
H-8′	4.91 t, 2 H *J*(7′,8′) = 4.9	4.78 t, 2 H *J*(7′,8′) = 4.7	4.80 t, 2 H *J*(7′,8′) = 4.5	4.78 t, 2 H *J*(7′,8′) = 4.5	–	–
H-9′	–	1.17 d, 3 H *J*(9′,2′) = 6.3	1.17 d, 3 H *J*(9′,2′) = 6.2	3.19 dd, 1 H *J*(9′,2′) = 6.2 *J*(gem) = 13.6 3.35–3.25 m, 1 H	–	–
H-2″	9.51 s, 1 H	9.17 s, 1 H	9.18 s, 1 H	9.41 s, 1 H	9.28 s, 1 H	9.75 s, 1 H
H-4″	9.00 dt, 1 H *J*(4″,2″) = *J*(4″,6″) = 1.2 *J*(4″,5″) = 8.1	8.86 dt, 1 H *J*(4″,2″) = *J*(4″,6″) = 1.0 *J*(4″,5″) = 8.1	8.86 dt, 1 H *J*(4″,2″) = *J*(4″,6″) = 1.0 *J*(4″,5″) = 8.1	8.85 dt, 1 H *J*(4″,2″) = *J*(4″,6″) = 1.0 *J*(4″,5″) = 8.1	8.87 dt,1 H *J*(4″,2″) = *J*(4″,6″) =1.0 *J*(4″,5″) = 8.1	8.83 dt, 1 H *J*(4″,2″) = *J*(4″,6″) =1.5 *J*(4″,5″) = 8.1
H-5″	8.29 dd, 1 H *J*(5″,4″) = 8.1 *J*(5″,6″) = 6.1	8.17 dd, 1 H *J*(5″,4″) = 8.1 *J*(5″,6″) = 6.1	8.18 dd, 1 H *J*(5″,4″) = 8.1 *J*(5″,6″) = 6.0	8.21 dd, 1 H *J*(5″,4″) = 8.1 *J*(5″,6″) = 6.1	8.15 dd, 1 H *J*(5″,4″) =8.1 *J*(5″,6″) = 6.1	8.15 dd, 1 H *J*(5″,4″) = 8.1 *J*(5″,6″) = 6.1
H-6″	9.15 dt, 1 H *J*(6″,2″) = *J*(6″,4″) = 1.2 *J*(6″,5″) = 6.1	8.94 dt, 1 H *J*(6″,2″) = *J*(6″,4″) = 1.0 *J*(6″,5″) = 6.1	8.95 dt, 1 H *J*(6″,2″) = *J*(6″,4″) = 1.0 *J*(6″,5″) = 6.0	9.12 dt, 1 H *J*(6″,2″) = *J*(6″,4″) = 1.0 *J*(6″,5″) = 6.1	9.03 dt, 1 H *J*(6″,2″) = *J*(6″,4″) = 1.0 *J*(6″,5″) = 6.1	9.19 dt, 1 H *J*(6″,2″) = *J*(6″,4″) = 1.5 *J*(6″,5″) = 6.1
NH_2″	8.65 s, 1 H 8.16 s, 1 H	–	–	8.00 s, 1 H 9.10 s, 1 H	–	7.81 s, 1 H 10.26 s, 1 H

[a] In $(CD_3)_2SO$. [b] In D_2O. [c] Additional signal: 5.92 brs, 1 H (OH).

filtered off. The filtrate was evaporated and the residue codistilled with water to obtain the pure compound **3d** (1.05 g, 68%), m.p. 240–243 °C. ^{1}H NMR spectrum (D_2O): 8.09 s, 1 H (H-2); 8.18 s, 1 H (H-8); 7.32–7.24 m, 3 H and 7.1–6.9 m, 2 H (arom. H); 4.43 dd, 1 H, *J*(1′a,2′) = 4.4, *J*(gem) = 14.7 (Ha-1′); 4.39 dd, 1 H, *J*(1′b,2′) = 5.6, *J*(gem) = 14.7 (Hb-1′); 4.34 d, 1 H, *J*(gem) = 11.5 (CH_2Ph); 3.95 m, 1 H (H-2′); 3.66 dd, 1 H, *J*(P,CHa) = 9.5, *J*(gem) = 12.0 (Ha-4′); 3.63 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 12.0 (Hb-4′); 3.61 dd, 1 H, *J*(5′a,2′) = 4.5, *J*(gem) = 11.0 (Ha-5′); 3.48 dd, 1 H, *J*(5′b,2′) = 3.9, *J*(gem) = 11.0 (Hb-5′). Mass spectrum (FAB), *m/z* (rel.%): 394 (80) $[M + H]^+$. For $C_{16}H_{20}N_5O_5P$ (393.3) calculated: 48.86% C, 5.13% H, 17.80% N, 7.87% P; found: 48.53% C, 5.07% H, 17.69% N, 8.25% P.

TABLE II
^{13}C NMR spectra (δ, ppm) of compounds **1** and **2**

Atom	**1a**[a]	**1b**[b]	**1c**[b]	**1d**[a]	**2a**[b]	**2b**[a]
C-2	150.80	152.01	151.84	152.54	152.35	152.69
C-4	148.79	148.81	148.42	149.89	148.60	149.96
C-5	118.11	117.60	117.28	118.63	117.67	118.54
C-6	151.16	154.98	154.75	156.12	155.18	156.19
C-8	143.01	142.96	142.71	141.79	142.80	142.98
C-1′	43.25	47.50	47.45	44.05	43.10	43.06
C-2′	70.26 d *J*(P,C) = 11.7	75.44 d *J*(P,C) = 11.8	75.37 d *J*(P,C) = 11.7	80.59 d *J*(P,C) = 10.0	77.55 d *J*(P,C) = 11.7	76.90 d *J*(P,C) = 6.3
C-4′	64.90 d *J*(P,C) = 160.2	62.71 d *J*(P,C) = 160.2	62.67 d *J*(P,C) = 161.1	66.40 d *J*(P,C) = 156.3	66.61 d *J*(P,C) = 155.3	68.28 d *J*(P,C) = 150.5
C-5′	–	–	–	–	62.04	61.76
C-7′	61.52 d *J*(P,C) = 4.0	62.28 d[c] *J*(P,C) = 5.9	62.26 d *J*(P,C) = 4.0	62.33	–	–
C-8′	63.53 d *J*(P,C) = 6.0	62.01 d[c] *J*(P,C) = 5.9	62.03 d *J*(P,C) = 6.0	62.80 d *J*(P,C) = 5.9	–	–
C-9′	–	15.51	15.49 d *J*(P,C) = 3.1	60.75	–	–
C-2″	147.20	146.63	146.52	146.93	147.14	147.43
C-3″	133.87	133.18	133.18	134.11	133.24	134.32
C-4″	146.16	144.30[c]	144.22[c]	145.20	144.56	145.89
C-5″	127.88	127.89	127.84	127.79	127.80	127.56
C-6″	144.09	144.04[c]	143.94[c]	143.94	144.38	144.68
C=O	162.96	165.03	164.88	163.82	165.36	163.79

[a] $(CD_3)_2SO$ or [b] D_2O. [c] Signals may be reversed.

Synthesis of Azidoethyl Phosphonates **4**. General Procedure

Triphosgene (3.12 g, 10.5 mmol) was slowly added at room temperature to a mixture of compound **3** (3.5 mmol) and dimethylformamide (100 ml). After stirring for 1 h at room temperature, 2-azidoethanol (10 ml) was added and the mixture was allowed to stand for 20 h. The solution was neutralized with TEAB and then concentrated ammonia (30 ml) was added. After standing overnight and filtration, the solvent was evaporated, the residue was codistilled with water and applied onto a Dowex 50X8 column (H^+ form, 100 ml). The column was washed with water and the product was eluted with aqueous ammonia (1 : 20). The fraction containing the product was evaporated and applied on a column of Dowex 1 (acetate form, 50 ml). Elution with a gradient of 0–0.5 M acetic acid afforded the compound **4**.

9-(2-Phosphonomethoxyethyl)adenine 2-azidoethyl ester (**4a**): Yield 1.02 g (85%), m.p. 183–185 °C. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.185 s, 1 H (H-2); 8.18 s, 1 H (H-8); 7.73 brs, 2 H (NH_2); 4.34 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 3.94 dt, 2 H, *J*(7′, 8′) = 4.9, *J*(POCH) = 7.3 (H-7′); 3.89 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 3.70 d, 2 H, *J*(P,CH) = 8.3 (H-4′); 3.38 t, 2 H, *J*(8′,7′) = 4.9 (H-8′). Mass spectrum (FAB), *m/z* (rel.%): 343 (100) $[M + H]^+$. For $C_{10}H_{15}N_8O_4P$. 1/3 H_2O (348.3) calculated: 34.48% C, 4.53% H, 32.17% N, 8.89% P; found: 34.87% C, 4.65% H, 32.00% N, 8.50% P.

(R)-9-(2-Phosphonomethoxypropyl)adenine 2-azidoethyl ester (**4b**): Yield 1.13 g (90%), m.p. 136–138 °C. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.35 s, 1 H (H-2); 8.37 s, 1 H (H-8); 7.68 brs, 2 H (NH_2); 4.45 dd, 1 H, *J*(1′a,2′) = 3.0, *J*(gem) = 14.9 (Ha-1′); 4.29 dd, 1 H, *J*(1′b,2′) = 7.6, *J*(gem) = 14.9 (Hb-1′); 4.00 m, 1 H (H-2′); 3.79–3.85 m, 2 H, (H-7′); 3.75 dd, 1 H, *J*(P,CHa) = 9.1, *J*(gem) = 13.4 (Ha-4′); 3.53 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 13.4 (Hb-4′); 3.35 m, 1 H and 3.30 m, 1 H (H-8′); 1.25 d, 3 H, *J*(9′, 2′) = 6.3 (H-9′). Mass spectrum (FAB), *m/z* (rel.%): 357 (100) $[M + H]^+$.

(S)-9-(2-Phosphonomethoxypropyl)adenine 2-azidoethyl ester (**4c**): Yield 1.20 g (96%), m.p. 137–138 °C. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.37 s, 2 H (H-2 and H-8); 7.70 brs, 2 H (NH_2); 4.46 dd, 1 H, *J*(1′a,2′) = 2.9, *J*(gem) = 14.7 (Ha-1′); 4.29 dd, 1 H, *J*(1′b,2′) = 7.8, *J*(gem) = 14.7 (Hb-1′); 4.01 m, 1 H (H-2′); 3.77-3.86 m, 2 H, (H-7′); 3.76 dd, 1 H, *J*(P,CHa) = 9.0, *J*(gem) = 13.4 (Ha-4′); 3.50 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 13.4 (Hb-3′); 3.35 m, 1 H and 3.30 m, 1 H (H-5′); 1.24 d, 3 H, *J*(6′,2′) = 6.3 (H-6′). Mass spectrum (FAB), *m/z* (rel.%): 357 (100) $[M + H]^+$.

(RS)-9-(3-Hydroxy-2-phosphonomethoxypropyl)adenine 2-azidoethyl ester (**4d**): Yield 1.26 g (77%), m.p. >250 °C. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.23 s, 1 H (H-2); 8.13 s, 1 H (H-8); 7.36–7.20 m, 5 H (arom. H); 4.46 s, 2 H (CH_2Ph); 4.38 dd, 1 H, *J*(1′a,2′) = 3.9, *J*(gem) = 14.2 (Ha-1′); 4.25 dd, 1 H, *J*(1′b,2′) = 6.3, *J*(gem) = 14.2 (Hb-1′); 3.96 m, 1 H (H-2′); 3.79 m, 2 H, (H-7′); 3.58 m, 2 H (H-8′); 3.50–3.30 m, 4 H (H-4′) and (H-9′). Mass spectrum (FAB), *m/z* (rel.%): 463 (40) $[M + H]^+$. For $C_{18}H_{23}N_8O_5P$ (462.4) calculated: 46.75% C, 5.01% H, 24.24% N, 6.70% P; found: 46.51% C, 5.26% H, 23.97% N, 6.50% P.

Synthesis of Aminoethyl Phosphonates **5**. General Procedure

The corresponding compound **4** (2 mmol) was hydrogenated in methanol (70 ml) over 10% palladium on charcoal (0.6 g) with stirring for 24 h at room temperature. The mixture was filtered through a Celite pad. The catalyst was washed with hot methanol and hot water. The filtrate was evaporated and compounds **5a–5c** were obtained as colourless foams, **5d** after crystallization from aqueous ethanol as a solid.

9-(2-Phosphonomethoxyethyl)adenine 2-aminoethyl ester (**5a**): Yield 0.57 g (90%). ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.18 s, 1 H (H-2); 8.13 s, 1 H (H-8); 7.19 brs, 2 H (NH_2); 4.29 t, 2 H, *J*(1′,2′) = 5.2 (H-1′); 3.84 m, 2 H (H-7′); 3.82 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 3.50 brs, 2 H (NH_2); 3.47 d, 2 H, *J*(P,CH) = 8.3 (H-4′); 2.85 t, 2 H, *J*(7′,8′) = 5.0 (H-8′). Mass spectrum (FAB), *m/z* (rel.%): 317 (100) $[M + H]^+$.

(R)-9-(2-Phosphonomethoxypropyl)adenine 2-aminoethyl ester (**5b**): Yield 0.41 g (62%). ^{1}H NMR spectrum (D_2O): 8.23 s, 1 H (H-2); 8.20, 1 H (H-8); 4.38 dd, 1 H, *J*(1′a,2′) = 3.2, *J*(gem) = 14.9 (Ha-1′); 4.24 dd, 1 H, *J*(1′b,2′) = 7.6, *J*(gem) = 14.9 (Hb-1′); 4.05–3.97 m, 3 H (H-2′ and H-7′); 3.75 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 13.4 (Ha-4′); 3.58 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 13.4 (Hb-4′); 3.06 t, 2 H, *J*(7′,8′) = 5.1 (H-8′); 1.23 d, 3 H, *J*(9′,2′) = 6.3 (H-9′). Mass spectrum (FAB), *m/z* (rel.%): 331 (60) $[M + H]^+$.

(S)-9-(2-Phosphonomethoxypropyl)adenine 2-aminoethyl ester (**5c**): Yield 0.49 g (75%). ^{1}H NMR spectrum (D_2O): 8.21 s, 1 H (H-2); 8.18, 1 H (H-8); 7.70 brs, 2 H (NH_2); 4.36 dd, 1 H, *J*(1′a,2′) = 3.1, *J*(gem) = 14.7 (Ha-1′); 4.22 dd, 1 H, *J*(1′b,2′) = 7.8, *J*(gem) = 14.7 (Hb-1′); 3.98 m, 1 H (H-2′); 3.72 dd, 1 H, *J*(P,CHa) = 9.0, *J*(gem) = 13.4 (Ha-4′); 3.56 q, 2 H, *J*(7′,8′) = 5.2, *J*(POCH) = 6.6 (H-7′); 3.46 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 13.4 (Hb-4′); 2.57 t, 2 H, *J*(7′,8′) = 5.2 (H-8′); 1.23 d, 3 H, *J*(9′,2′) = 6.3 (H-9′). Mass spectrum (FAB), *m/z* (rel.%): 331 (100) $[M + H]^+$.

(RS)-9-(3-Hydroxy-2-phosphonomethoxypropyl)adenine 2-aminoethyl ester (**5d**): Yield 0.37 g (53%), m.p. 225–227 °C. ^{1}H NMR spectrum (D_2O): 8.17 s, 1 H (H-2); 8.16, 1 H (H-8); 4.41 dd, 1 H, *J*(1′a,2′) = 4.0, *J*(gem) = 14.9 (Ha-1′); 4.35 dd, 1 H, *J*(1′b,2′) = 7.6, *J*(gem) = 14.9 (Hb-1′); 3.89 m, 1 H (H-2′); 3.85 m, 3 H (Ha-9′ and H-7′); 3.76 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 13.4 (Ha-4′); 3.59 dd, 1 H, *J*(9′b,2′) = 4.3, *J*(gem) = 12.5 (Hb-9′); 3.53 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 13.4 (Hb-4′); 3.10 t, 2 H, *J*(7′,8′) = 5.0 (H-8′). Mass spectrum (FAB), *m/z* (rel.%): 347 (30) $[M + H]^+$. For $C_{11}H_{19}N_6O_5P$. 1.5 H_2O (373.3) calculated: 35.39% C, 5.94% H, 22.51% N; found: 35.06% C, 5.68% H, 22.54% N.

Diisopropyl (*S*)-9-(3-Azido-2-phosphonomethoxypropyl)adenine (**6a**)

p-Toluenesulfonyl chloride (1.48 g, 7.8 mmol) and 4-dimethylaminopyridine (10 mg) were added at –10 °C to a stirred solution of diisopropyl (*S*)-9-(3-hydroxy-2-phosphonomethoxypropyl)adenine (1.5 g, 3.9 mmol) in pyridine (30 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. After filtration, the solvent was evaporated and sodium azide (1.4 g, 22 mmol) and dimethylformamide (30 ml) were added. The mixture was stirred for 4 h at 100 °C, filtered while hot and the filtrate taken down. The residue was codistilled with toluene and the product was purified by preparative thin-layer chromatography. Yield 0.8 g (50%) of compound **6a**, m.p. 110–112 °C. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.14 s, 1 H (H-2); 8.08, 1 H (H-8); 7.22 brs, 2 H (NH_2); 4.53 dsept, 1 H and 4.48 dsept, 1 H, *J*(CH,CH_3) = 6.1, *J*(P,OCH) = 7.6 (2 × POCH); 4.35 dd, 1 H, *J*(1′a,2′) = 4.2, *J*(gem) = 14.6 (Ha-1′); 4.29 dd, 1 H, *J*(1′b,2′) = 6.6, *J*(gem) = 14.6 (Hb-1′); 4.04 m, 1 H (H-2′); 3.89 dd, 1 H, *J*(P,CHa) = 9.0, *J*(gem) = 13.7 (Ha-4′); 3.85 dd, 1 H, *J*(P,CHb) = 9.5, *J*(gem) = 13.7 (Hb-4′); 3.65 dd, 1 H, *J*(5′a,2′) = 4.2, *J*(gem) = 13.4 (Ha-5′); 3.30 dd, 1 H, *J*(5′b,2′) = 5.4, *J*(gem) = 13.4 (Hb-5′); 1.21 d, 3 H, 1.18 d, 3 H, 1.17 d, 3 H and 1.13 d, 3 H, *J*(CH_3,CH) = 6.1 (6 × CH_3). Mass spectrum (FAB), *m/z* (rel.%): 413 (100) $[M + H]^+$. For $C_{15}H_{25}N_8O_4P$ (412.4) calculated: 43.68% C, 6.11% H, 27.18% N; found: 43.72% C, 5.88% H, 26.61% N.

Diisopropyl (*R*)-9-(3-Azido-2-phosphonomethoxypropyl)adenine (**6b**)

A stirred mixture of adenine (1.3 g, 9.6 mmol), cesium carbonate (1.6 g, 4.9 mmol) and dry dimethylformamide (40 ml) was heated at 120 °C for 1 h. After addition of diisopropyl (*S*)-[1-azidomethyl-(2-*p*-toluenesulfonyloxy)]ethoxymethylphosphonate[11] (4.4 g, 9.8 mmol) in dimethylformamide (20 ml), the heating at 120 °C was continued for 6 h. The reaction mixture was taken down and the product **6b** (2.0 g, 50%) was obtained by chromatography on a column of silica gel (150 ml, 2% of methanol in chloroform), m.p. 107–110 °C. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.15 s, 1 H (H-2); 8.10, 1 H (H-8); 7.30 brs, 2 H (NH_2); 4.53 dsept, 2 H, *J*(CH,CH_3) = 6.1, *J*(P,OCH) = 7.6 (2 × POCH);

4.36 dd, 1 H, *J*(1′a,2′) = 4.2, *J*(gem) = 14.6 (Ha-1′); 4.29 dd, 1 H, *J*(1′b,2′) = 6.6, *J*(gem) = 14.6 (Hb-1′); 4.04 m, 1 H (H-2′); 3.89 dd, 1 H, *J*(P,CHa) = 9.0, *J*(gem) = 13.9 (Ha-4′); 3.85 dd, 1 H, *J*(P,CHb) = 9.5, *J*(gem) = 13.9 (Hb-4′); 3.65 dd, 1 H, *J*(5′a,2′) = 4.1, *J*(gem) = 13.4 (Ha-5′); 3.30 dd, 1 H, *J*(5′b,2′) = 5.1, *J*(gem) = 13.4 (Hb-5′); 1.21 d, 3 H, 1.18 d, 3 H, 1.16 d, 3 H and 1.12 d, 3 H, *J*(CH_3,CH) = 6.1 (6 × CH_3). Mass spectrum (FAB), *m/z* (rel.%): 413 (80) $[M + H]^+$. For $C_{15}H_{25}N_8O_4P$ (412.4) calculated: 43.68% C, 6.11% H, 27.18% N; found: 43.52% C, 5.99% H, 26.93% N.

(*R*)-9-(3-Amino-2-phosphonomethoxypropyl)adenine (**7a**)
and (*S*)-9-(3-Amino-2-phosphonomethoxypropyl)adenine (**7b**)

Compound **6a** (0.82 g, 2 mmol) was hydrogenated in methanol (70 ml) over 10% palladium on charcoal (0.6 g) with 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 dried in vacuo and codistilled with acetonitrile. Acetonitrile (20 ml) and bromotrimethylsilane (2 ml) were added and the mixture was stirred at room temperature for 24 h. The solvent was evaporated, the residue was codistilled with acetonitrile, dissolved in water and made alkaline with triethylamine. After evaporation in vacuo the residue was deionized on Dowex 50X8 (H^+ form, 70 ml). The product-containing fraction was evaporated and applied on a column of Dowex 1 (acetate form, 50 ml). Elution with a linear gradient of acetic acid (0–0.5 M, 1 l each) and evaporation afforded compound **7a** (0.4 g, 66%). ^{1}H NMR spectrum (D_2O): 8.23 s, 1 H (H-2); 8.21, 1 H (H-8); 7.30 brs, 2 H (NH_2); 4.57 dd, 1 H, *J*(1′a,2′) = 4.1, *J*(gem) = 15.1 (Ha-1′); 4.43 dd, 1 H, *J*(1′b,2′) = 5.4, *J*(gem) = 15.1 (Hb-1′); 4.16 m, 1 H (H-2′); 3.77 dd, 1 H, *J*(P,CHa) = 9.5, *J*(gem) = 12.7 (Ha-4′); 3.56 dd, 1 H, *J*(P,CHb) = 9.5, *J*(gem) = 12.7 (Hb-4′); 3.28 dd, 1 H, *J*(5′a,2′) = 2.9, *J*(gem) = 13.4 (Ha-5′); 2.86 dd, 1 H, *J*(5′b,2′) = 9.8, *J*(gem) = 13.4 (Hb-5′). Mass spectrum (FAB), *m/z* (rel.%): 303 (100) $[M + H]^+$.

Compound **7b** was obtained by the same procedure; yield 0.3 g (50%). ^{1}H NMR spectrum (D_2O): 8.24 s, 1 H (H-2); 8.22, 1 H (H-8); 7.32 brs, 2 H (NH_2); 4.58 dd, 1 H, *J*(1′a,2′) = 4.2, *J*(gem) = 15.1 (Ha-1′); 4.43 dd, 1 H, *J*(1′b,2′) = 5.4, *J*(gem) = 15.1 (Hb-1′); 4.18 m, 1 H (H-2′); 3.76 dd, 1 H, *J*(P,CHa) = 9.4, *J*(gem) = 12.7 (Ha-4′); 3.58 dd, 1 H, *J*(P,CHb) = 9.5, *J*(gem) = 12.7 (Hb-4′); 3.29 dd, 1 H, *J*(5′a,2′) = 2.9, *J*(gem) = 13.4 (Ha-5′); 2.86 dd, 1 H, *J*(5′b,2′) = 9.8, *J*(gem) = 13.4 (Hb-5′). Mass spectrum (FAB), *m/z* (rel.%): 303 (100) $[M + H]^+$.

NAD^+ Analogues **1** and **2**. General Procedure

DBU was added dropwise to a suspension of compound **5** or **7** (1 mmol) in dry methanol (20 ml) until the pH reached 7.5. Then Zincke salt[3] (3-carbamoyl-1-(2,4-dinitrophenyl)pyridinium chloride, 0.34 g, 1.05 mmol) was added and the mixture was stirred for 5 h. The crude product was precipitated with ether (50 ml) and filtered off. The precipitate was dissolved in water (20 ml) and washed with ether (10 × 20 ml). In the case of **1a**, **2a** and **2b**, water was evaporated, the residue was dissolved in methanol and the product was precipitated by addition of ether. In the other cases, the aqueous solution of **1b** or **1c** was applied onto a column of Amberlite (H^+, 20 ml). After washing with water the resin was mixed with water (100 ml), the mixture adjusted to pH 7.5 with ammonia and the suspension filtered. After evaporation of water, the residue was dissolved in methanol and the product was precipitated by addition of ether.

9-(2-Phosphonomethoxyethyl)adenine 2-(3-carbamoylpyridinium)ethyl ester (**1a**): Yield 0.30 g (68%). Mass spectrum (FAB), *m/z* (rel.%): 422 (20) $[M + H]^+$. For $C_{16}H_{20}N_7O_5P$. 3 H_2O (475.4) calculated: 40.42% C, 5.51% H, 20.62% N, 6.52% P; found: 40.42% C, 5.72% H, 20.43% N, 6.13% P. Exact mass (FAB HRMS) for $C_{16}H_{21}N_7O_5P$ calculated: 422.1342; found: 422.1295.

(R)-9-(2-Phosphonomethoxypropyl)adenine 2-(3-carbamoylpyridinium)ethyl ester (**1b**): Yield 0.31 g (71%). Mass spectrum (FAB), *m/z* (rel.%): 436 (20) $[M + H]^+$. For $C_{17}H_{22}N_7O_5P$. 2.5 H_2O (480.4) calculated: 42.50% C, 5.67% H, 20.41% N; found: 42.24% C, 5.65% H, 20.13% N. Exact mass (FAB HRMS) for $C_{17}H_{23}N_7O_5P$ calculated: 436.1498; found: 436.1493.

(S)-9-(2-Phosphonomethoxypropyl)adenine 2-(3-carbamoylpyridinium)ethyl ester (**1c**): Yield 0.22 g (50%). Mass spectrum (FAB), *m/z* (rel.%): 436 (40) $[M + H]^+$. For $C_{17}H_{22}N_7O_5P$. 2.5 H_2O (480.4) calculated: 42.50% C, 5.67% H, 20.41% N; found: 42.35% C, 5.79% H, 20.03% N. Exact mass (FAB HRMS) for $C_{17}H_{23}N_7O_5P$ calculated: 436.1498; found: 436.1606.

(RS)-9-(3-Hydroxy-2-phosphonomethoxypropyl)adenine 2-(3-carbamoylpyridinium)ethyl ester (**1d**): Yield 0.32 g (70%). Mass spectrum (FAB), *m/z* (rel.%): 452 (40) $[M + H]^+$. Exact mass (FAB HRMS) for $C_{17}H_{23}N_7O_6P$ calculated: 452.1447; found: 452.1299.

(S)-1-[3-(Adenin-9-yl)-2-phosphonatomethoxypropyl]-3-carbamoylpyridinium (**2a**): Yield 0.31 g (76%). Mass spectrum (FAB), *m/z* (rel.%): 408 (60) $[M + H]^+$. Exact mass (FAB HRMS) for $C_{15}H_{19}N_7O_5P$ calculated: 408.1185; found: 408.1147.

(R)-1-[3-(Adenin-9-yl)-2-phosphonatomethoxypropyl]-3-carbamoylpyridinium (**2b**): Yield 0.25 g (61%). Mass spectrum (FAB), *m/z* (rel.%): 408 (90) $[M + H]^+$. Exact mass (FAB HRMS) for $C_{15}H_{19}N_7O_5P$ calculated: 408.1185; found: 408.1241.

For ^1H and ^{13}C NMR spectra of compounds **1** and **2** see Tables I and II.

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