SYNTHESIS OF BASE-MODIFIED "ABBREVIATED" NAD ANALOGUES*

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Dedicated to Professor Dr Fritz Sauter on the occasion of his 65th birthday.

The "abbreviated" model of NAD, 1-[3-(adenin-9-yl)-2-hydroxypropyl]-3-carbamoylpyridinium chloride (*VIIIa*), and its 2,6-diaminopurine (*VIIIb*), 3-deazaadenine (*VIIIc*), guanine (*VIIId*) and cytosine (*VIIIe*) analogues were prepared by the Zincke reaction. The (*R*)-isomer of the adenine model *VIIIa* (compound *IX*) was prepared for chiroptical studies. As shown by NMR, UV and CD spectra, neither in dimethyl sulfoxide nor in water any intramolecular π–π interactions exist between the heteroaromatic systems.

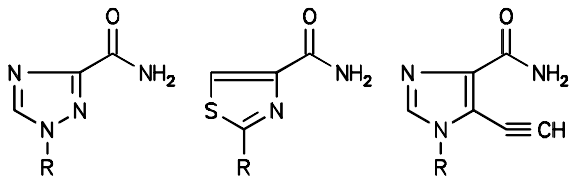
In living systems, pyridine nucleosides are most often encountered in the form of 1-substituted nicotinamide or 1,4-dihydronicotinamide that are constituents of the coenzyme nicotinamide adenine dinucleotide (NAD) or its 2′-phosphate (NADP). An important NAD-containing enzyme is IMP-dehydrogenase[2], active in the biosynthesis of purines. This enzyme catalyzes the conversion of inosine 5′-phosphate (IMP) into xanthosine 5′-phosphate (XMP) that involves addition of water molecule to the C2–N3 bond in the heterocyclic base of IMP followed by loss of two hydrogen atoms. IMP-dehydrogenase is often mentioned in connection with transformed cells. Its inhibitors, such as ribavirin[3] (*I*), inhibit effectively the synthesis of RNA and DNA in virus-infected cells. A cytostatic effect was described for other IMP-dehydrogenase inhibitors – tiazofurin (*II*) and EICAR (*III*) (refs[4–6]). The inhibition can be evoked either by the compound itself or by a modified-base analogue of NAD arising from it by metabolic transformation. It is worth to notice that all the three above-mentioned inhibitors contain a five-membered heterocyclic system and a carbamoyl group in β-position to the atom bearing

* This paper was in part published in a preliminary form[1].

the sugar moiety. Still more conspicuous is the analogy with the natural nicotinamide mononucleoside in the case of 3-β-D-ribofuranosylbenzamide which is a highly effective inhibitor of IMP-dehydrogenase[7].

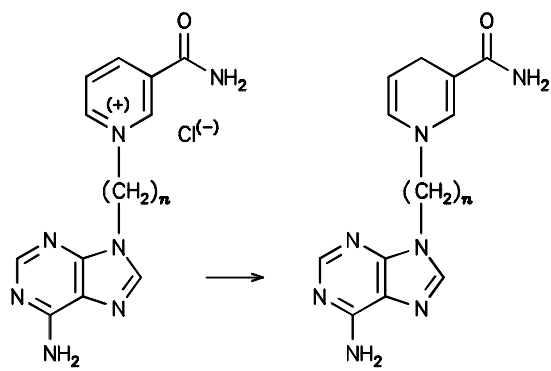
Since the correct function of the NAD coenzyme depends not only on the nicotinamide part as such but also on the sugar phosphate and adenine parts (because of interaction with the apoenzyme), we studied such modification of the coenzyme that consists in replacement of the sugar moieties in both nucleosides by an acyclic polar chain (2-hydroxy-1,3-propylidene) and in replacement of adenine by another purine, modified purine, or pyrimidine base. This model contains two heterocyclic systems whose mutual π–π interaction can stabilize its molecule in various preferred conformations and thus more or less effectively substitute NAD in the coenzyme role. Depending on pH, temperature and solvent, molecules of the true coenzyme NAD can exist in, or between, two limiting conformations: folded (stacked) (Fig. 1) and extended[8,9].

The conformation of NAD and some of its analogues was intensively studied, particularly in connection with coenzyme–apoenzyme or coenzyme–substrate interactions[10,11].



I *II* *III*

In formulae *I* – *III* : R = β-D-ribofuranosyl



IV *V*

In formulae *IV*, *V* : *a*, *n* = 3

b, *n* = 6

Relatively few studies, however, concern intramolecular interactions of the coenzyme proper. Compounds *IV* and *V* were prepared[9] as models of NAD or NADH for studies of intramolecular interactions in water and dimethyl sulfoxide. It has been found that for aqueous media, where the coenzyme molecule exists mainly in the folded form, compounds *IVa* and *Va* (with the three-membered bridge) are better models, whereas in dimethyl sulfoxide the extended conformation is favoured and thus the models *IVb* and *Vb* (with the 1,6-hexylidene bridge) are more appropriate.

Apart from the already mentioned studies[8,9] determining the NAD conformations by CD spectra or fluorimetry, also ultraviolet[12] and NMR spectroscopy[13] has been used.

Other models, 1-phenyl- or 1-benzyl-3-carbamoylpyridinium salts[14] which served as models for oxidoreductase coenzyme in oxidation-reduction reactions, were prepared using nucleophilic attack by amine in the position 2 of an activated pyridinium salt (the so-called Zincke reaction[15]). Another model[16] of the NAD coenzyme contains quaternized nicotinamide on the 5′-carbon atom of adenosine; this compound was already prepared by the Zincke reaction.

RESULTS AND DISCUSSSION

The starting *N*-(3-amino-2-hydroxypropyl) derivatives *VIa – VIe* of heterocyclic bases were prepared according to two general procedures (Scheme 1): either by catalytic hydrogenation of the azido derivatives obtained by alkylation of the corresponding bases with azidomethyloxirane[17,18], or by hydrazinolysis of *N*-(2-hydroxy-3-phthalimidopropyl) derivatives prepared by reaction of the bases with *N*-oxiranylmethylphthalimide[18]. Zincke reaction of the obtained *N*-(3-amino-2-hydroxypropyl) derivatives *VIa – VIe* with 1-(2,4-dinitrophenyl)-3-carbamoylpyridinium chloride (*VII*) afforded the desired compounds *VIIIa – VIIIe* containing nicotinamide and the nucleoside base linked by the 2-hydroxy-1,3-propylidene group (Scheme 2).

In order to study intramolecular interactions by analysis of chiroptical properties of "abbreviated" NAD models, we prepared the optically pure (*R*)-isomer of compound *VIIIa*, 1-[(2*R*)-3-(adenin-9-yl)-2-hydroxypropyl]-3-carbamoylpyridinium chloride (*IX*). The starting compound in the synthesis (Scheme 3), (2*R*)-1-chloro-2,3-propanediol, on reaction with sodium azide afforded (2*S*)-3-azido-1,2-propanediol (*X*). This compound

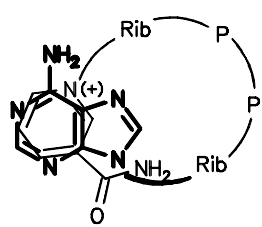
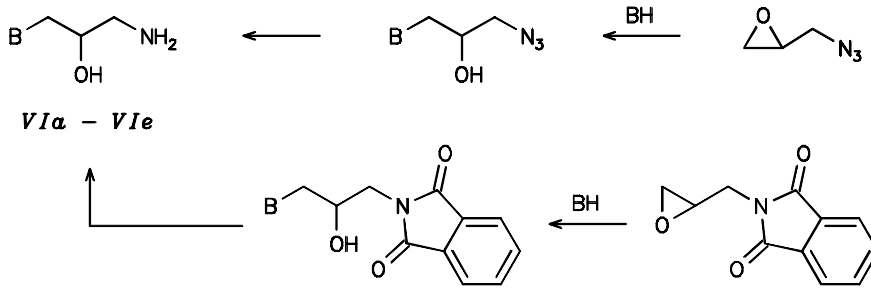
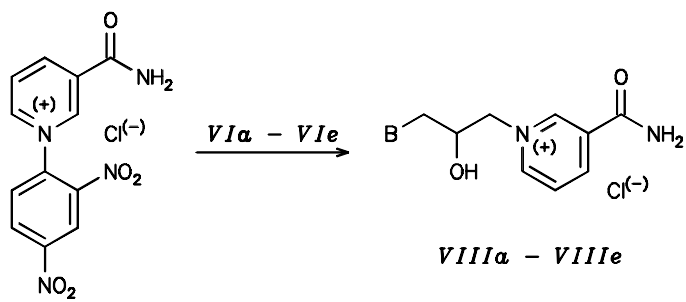


FIG. 1
Possible "folded" conformation of the NAD molecule



In formulae *VI* : ***a***, B = adenin-9-yl
b, B = 2,6-diaminopurin-9-yl
c, B = 3-deazaadenin-9-yl
d, B = guanin-9-yl
e, B = cytosin-1-yl

SCHEME 1



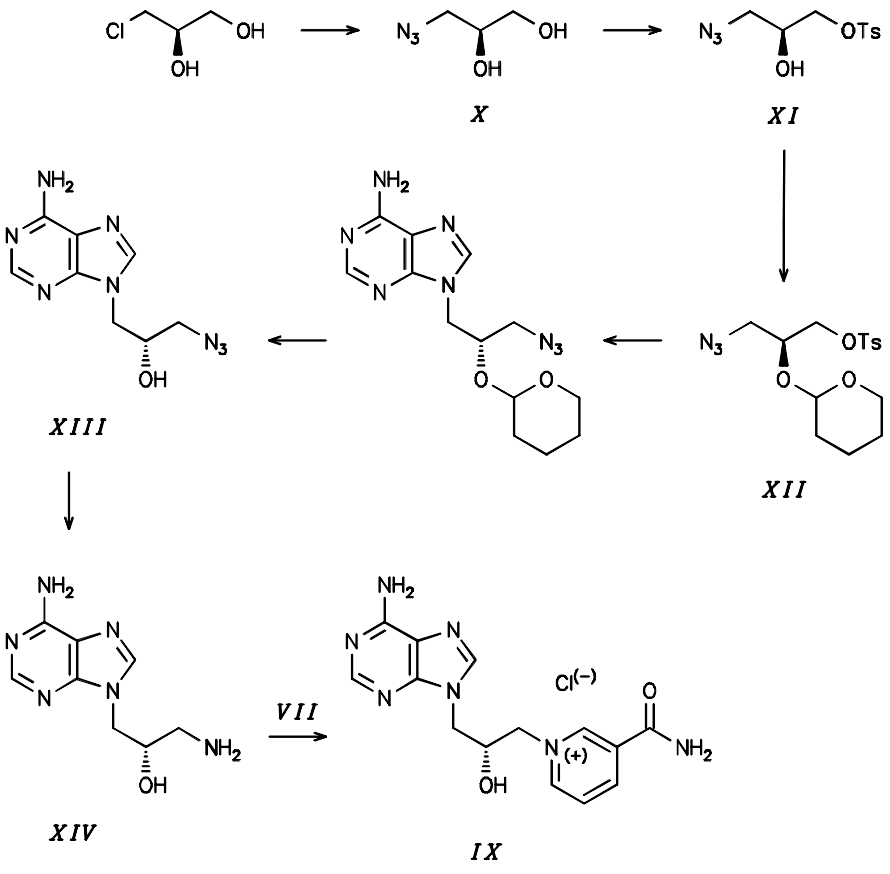
VII *VIIIa – VIIIe*

In formulae *VIII* : ***a***, B = adenin-9-yl
b, B = 2,6-diaminopurin-9-yl
c, B = 3-deazaadenin-9-yl
d, B = guanin-9-yl
e, B = cytosin-1-yl

SCHEME 2

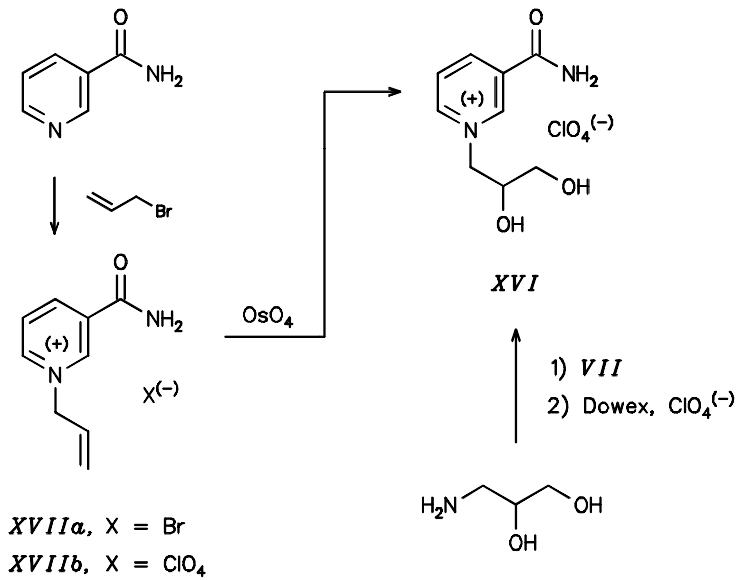
was tosylated to give the (*S*)-tosylate *XI* in which the free hydroxyl was protected as tetrahydropyranyl group. The obtained derivative *XII* on reaction with adenine and subsequent deprotection afforded (*R*)-azido derivative *XIII*. Catalytic hydrogenation of *XIII* furnished (*S*)-3-amino-2-hydroxypropyl derivative *XIV* which upon Zincke reaction with pyridinium salt *VII* gave the optically active model *IX* with absolute configuration (*R*).

Spectroscopic study of mutual interactions between the bases required a comparison of both hypothetical chiral components of the system. Whereas 9-[(2*R*)-2,3-dihydroxypropyl]adenine ((*R*)-DHPA, *XV*) is already known[19], the other component, 1-(2,3-dihydroxypropyl)-3-carbamoylpyridinium perchlorate (*XVI*) was hitherto undescribed. We prepared this compound by two pathways (Scheme 4): either by Zincke reaction of



SCHEME 3

3-amino-1,2-propanediol with 1-(2,4-dinitrophenyl)-3-carbamoylpyridinium chloride (*VII*) or by hydroxylation of the double bond in 1-allyl-3-carbamoylpyridinium perchlorate (*XVIIb*) with osmium tetroxide in the presence of sodium chlorate. The use of perchlorate *XVIIb* instead of the originally obtained bromide *XVIIa* excluded undesired oxidation of the anion during the hydroxylation.



SCHEME 4

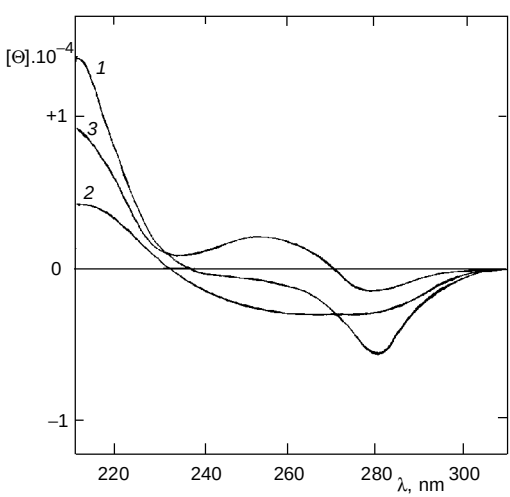
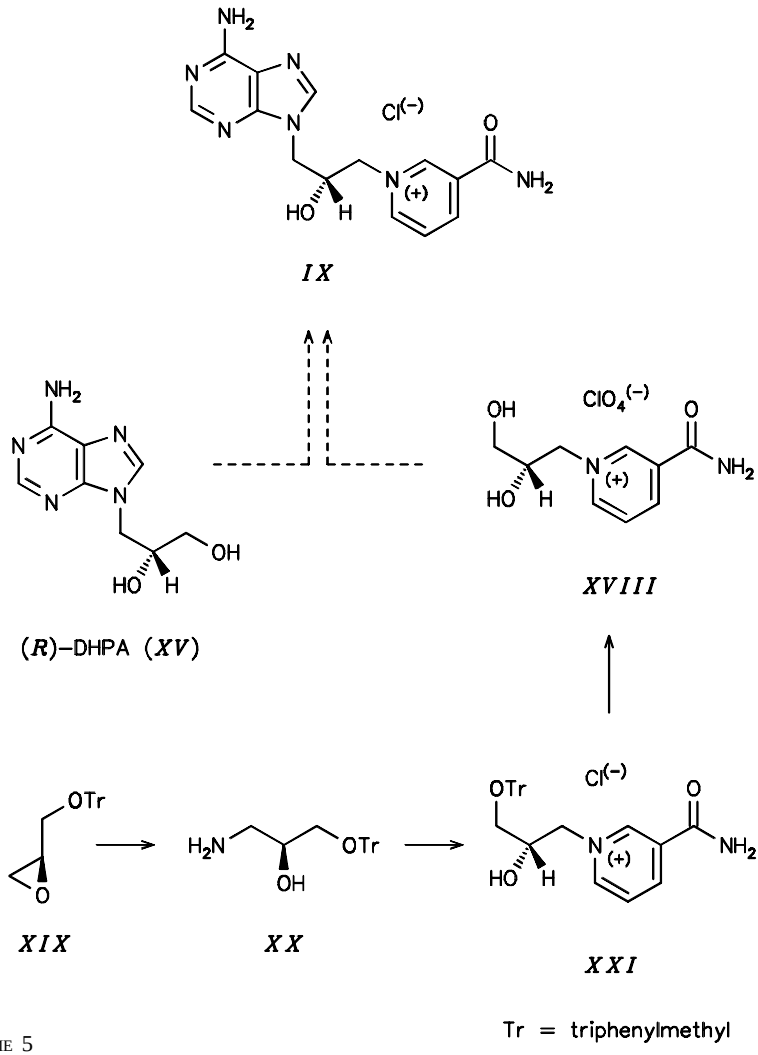


FIG. 2
CD spectra of compounds *IX* (*1*), *XV* (*2*) and *XVIII* (*3*)

For chiroptical studies we needed the optically active isomer of *XVI*, 1-[(2*S*)-2,3-dihydroxypropyl]-3-carbamoylpyridinium perchlorate (*XVIII*) which, together with (*R*)-DHPA (*XV*), has been taken as model of the chiral part of molecule *IX* (Scheme 5). Compound *XVIII* was also prepared by the Zincke reaction. To avoid complications due to desalting, we chose the following procedure. Reaction of (*S*)-trityloxymethyloxirane (*XIX*) with methanolic ammonia gave (*S*)-3-amino-1-trityloxy-2-propanol (*XX*) which on reaction with compound *VII* afforded the trityl derivative *XXI*. After deprotection with acetic acid, the product was finally converted into perchlorate *XVIII* (Scheme 5).



SCHEME 5

As seen from the NMR spectra of compounds *VIIIa – VIIIe* in hexadeuteriodimethyl sulfoxide, as well as from the UV spectra taken in water, there is no intramolecular π–π interaction between the aromatic moieties: neither change in chemical shifts of aromatic protons nor marked hypochromism have been observed. The CD spectrum of compound *IX* is practically the exact sum of the spectra of compounds *XVIII* and (*R*)-DHPA (*XV*) representing chiral parts of the "abbreviated" chiral model, each bearing only one base (Fig. 2); no additional bands were observed.

In spite of claims[20] that the interaction between the aromates is optimal if they are linked with a three-carbon fragment, we observed no interaction either in the NMR and UV spectra or in the CD spectra. One of the reasons may probably be the polarity of the hydroxyl which may render these interactions impossible due to solvation in water or dimethyl sulfoxide connected with formation of strong hydrogen bonds.

The cytostatic activity of the synthesized NAD analogues and their inhibitory effects on IMP dehydrogenase are now under study.

EXPERIMENTAL

Methods

The temperature data are uncorrected. Melting points were determined on a Boetius block. Thin-layer chromatography was performed on Silufol UV_{254}, column chromatography on Silpearl (both Kavalier, The Czech Republic) or on octadecyl silica gel (20 μm, Laboratorni Pristroje, Prague, The Czech Republic); detection on a Uvicord 4701 A instrument (LKB, Sweden) at 254 nm. Analytical HPLC was performed on an Altech 200 × 4 mm column packed with Separon SGX-RPS 10 μm. NMR spectra (δ, ppm; *J*, Hz) were measured on a Varian UNITY-200 spectrometer (^{1}H at 200 MHz and ^{13}C at 50.3 MHz) or UNITY-500 (^{1}H at 500 MHz and ^{13}C at 125.7 MHz) in hexadeuteriodimethyl sulfoxide with tetramethylsilane as internal standard. Ultraviolet absorption spectra (λ_{max}, nm (ε_{max})) were taken on a Beckmann DU-65 spectrometer, mass spectra (*m*/*z*) on a ZAB-EQ (VG Analytical) instrument using the FAB technique (Xe ionization, accelerating voltage 8 kV). Optical rotation was determined on a Perkin–Elmer 141 MCA polarimeter at 20 °C, CD spectra were obtained with a Jobin–Yvon Mark V instrument.

Materials

(*R*)-DHPA was prepared according to a described procedure[21]. The preparation of *N*-(3-amino-2-hydroxypropyl) derivatives *VIa – VIe* is also described[19,20]. (*S*)-Trityloxymethyloxirane was obtained from Sipsy company (France); its optical purity was stated to be >96%.

1-(2,4-Dinitrophenyl)-3-carbamoylpyridinium Chloride (*VII*)

The title compound was prepared as described[14] except that a solution of the crude product in methanol was added dropwise under vigorous stirring and cooling into ether. Decanting and washing with ether afforded the product *VII* in 64% yield; m.p. 134 – 136 °C (reported[14] m.p. 115 °C for a 1 : 1 methanol solvate). For $C_{12}H_9ClN_4O_5$. 0.5 H_2O (333.7) calculated: 43.19% C, 3.02% H, 16.78% N; found: 43.06% C, 2.89% H, 16.45% N. Mass spectrum (*m*/*z*): 289 (M – Cl), 123 (Nic + H).

Synthesis of "Abbreviated" NAD Models *VIIIa – VIIIe* and *IX*. General Procedure

1-(2,4-Dinitrophenyl)-3-carbamoylpyridinium chloride (*VII*; 0.325 g, 1 mmol) was added to a stirred solution of *N*-(3-amino-2-hydroxypropyl) derivative *VIa – VIe* or *XIV* (1 mmol) in dry methanol (30 ml) and the reaction mixture was stirred at ambient temperature for 90 min. During this time the colour changed from dark red to yellow. The deposited crystals were collected, washed with ether and dried.

In the case of guanine derivative *VIIId* this characteristic change of colour did not occur, however, the formation of the product was proven by TLC (R_F 0.02 in 2-propanol–25% aqueous ammonia–water (7 : 1 : 2)). The products were isolated in the following manner. After evaporation of the solvent, water (40 ml) was added to the residue and the undissolved 2,4-dinitroaniline was filtered off. The filtrate was washed with chloroform (3 × 20 ml) and the unreacted Zincke salt *VII* was removed by reaction with concentrated ammonia (20 ml). After evaporation and precipitation with acetone, the oily residue afforded lightly coloured crystals of compound *VIIId*.

For yields, elemental analyses, UV and mass spectra of compounds *VIIIa – VIIIe* and *IX* see Table I.

1-[3-(Adenine-9-yl)-2-hydroxypropyl]-3-carbamoylpyridinium chloride (VIIIa). ^{1}H NMR spectrum: 9.54 brs, 1 H, J(2,4) = J(2, 6) > 0 (H-2, Nic); 9.15 dt, 1 H, J(6,2) = J(6,4) = 1.2, J(6,5) = 6.1 (H-6, Nic); 9.02 dt, 1 H, J(4,2) = J(4,6) = 1.5, J(4,5) = 8.1 (H-4, Nic); 8.74 brs and 8.15 brs, 2 × 1 H (NH_2, Nic); 8.25 dd, 1 H, J(5,4) = 8.1, J(5,6) = 6.1 (H-5, Nic); 8.16 s and 8.12 s, 2 × 1 H (H-2, H-8, base); 7.25 brs, 2 H (NH_2, base); 6.04 d, 1 H, J(OH,CH) = 5.0 (OH); 4.97 brd, 1 H, J = 11.3 and 4.22 – 4.52 m, 4 H (2 × NCH_2 + OCH).

1-[3-(2,6-Diaminopurin-9-yl)-2-hydroxypropyl]-3-carbamoylpyridinium chloride (VIIIb). ^{1}H NMR spectrum: 9.62 brs, 1 H, (H-2, Nic); 9.16 brd, 1 H, J(6,5) = 6.1 (H-6, Nic); 9.02 brd, 1 H, J(4,5) = 8.1 (H-4, Nic); 8.27 dd, 1 H, J(5,4) = 8.1, J(5,6) = 6.1 (H-5, Nic); 8.73 brs and 8.18 brs, 2 × 1 H (NH_2, Nic); 7.71 s, 1 H (H-8, base); 6.71 brs and 5.79 brs, 2 H (NH_2, base); 6.10 d, 1 H, J(OH,CH) = 5.4 (OH); 4.88 dd, 1 H, J = 2.7 and 12.9 (NCH_2); 4.49 dd, 1 H, J = 8.8 and 12.9 (NCH_2); 4.36 m, 1 H (OCH); 4.19 dd, 1 H, J = 4.6 and 14.2 (NCH_2); 4.08 dd, 1 H, J = 6.3 and 14.2 (NCH).

1-[3-(3-Deazaadenin-9-yl)-2-hydroxypropyl]-3-carbamoylpyridinium chloride (VIIIc). ^{1}H NMR spectrum: 9.60 brs, 1 H, (H-2, Nic); 9.18 brd, 1 H, J(6,2) = J(6,4) = 1.0, J(6,5) = 6.1 (H-6, Nic); 9.03 brd, 1 H, J(4,2) = J(4,6) = 1.0, J(4,5) = 8.1 (H-4, Nic); 8.79 brs and 8.17 brs, 2 × 1 H (NH_2, Nic); 8.37 s, 1 H (H-8, base); 8.27 dd, 1 H, J(5,4) = 8.1, J(5,6) = 6.1 (H-5, Nic); 8.14 brs, 2 H (NH_2, base); 7.78 d, 1 H, J(2,3) = 6.6 (H-2, base); 7.38 d, 1 H, J(3,2) = 6.6 (H-3 base); 6.07 d, 1 H, J(OH,CH) = 5.9 (OH); 5.07 dd, 1 H, J = 1.0 and 12.7 (NCH_2); 4.61 dd, 1 H, J = 2.0 and 13.0 (NCH_2); 4.57 dd, 1 H, J = 9.0 and 12.7 (NCH_2); 4.38 dd, 1 H, J = 8.1 and 13.0 (NCH_2); 4.36 m, 1 H (OCH).

1-[3-(Guanin-9-yl)-2-hydroxypropyl]-3-carbamoylpyridinium chloride (VIIId). ^{1}H NMR spectrum: 10.81 br, 1 H, (NH_2, base); 9.58 brs, 1 H (H-2, Nic); 9.15 brd, 1 H, J(6,5) = 6.1 (H-6, Nic); 9.03 brd, 1 H, J(4,5) = 8.1 (H-4, Nic); 8.77 brs and 8.18 brs, 2 × 1 H (NH_2, Nic); 8.26 dd, 1 H, J(5,6) = 6.1, J(5,4) = 8.1 (H-5, Nic); 7.68 s, 1 H (H-8, base); 6.64 brs, 2 H (NH_2, base); 6.03 d, 1 H, J(OH,CH) = 6.1 (OH); 4.88 dd, 1 H, J = 2.9 and 12.9 (NCH_2); 4.50 dd, 1 H, J = 9.0 and 12.9 (NCH_2); 4.36 m, 1 H (OCH); 4.18 dd, 1 H, J = 4.6 and 13.2 (NCH_2); 4.07 dd, 1 H, J = 6.4 and 13.2 (NCH_2).

1-[3-(Cytosin-1-yl)-2-hydroxypropyl]-3-carbamoylpyridinium chloride (VIIIe). ^{1}H NMR spectrum: 9.50 brs, 1 H (H-2, Nic); 9.14 dt, 1 H, J(6,2) = J(6,4) = 1.0, J(6,5) = 6.1 (H-6, Nic); 9.01 dt, 1 H, J(4,2) = J(4,6) = 1.5, J(4,5) = 8.1 (H-4, Nic); 8.71 brs and 8.16 brs, 2 × 1 H (NH_2, Nic); 8.25 dd, 1 H, J(5,6) = 6.1, J(5,4) = 8.1 (H-5, Nic); 7.54 d, 1 H, J(6,5) = 7.3 (H-6, base); 7.29 brs and 7.08 brs, 2 × 1 H (NH_2, base); 5.87 d, 1 H, J(OH,CH) = 6.1 (OH); 5.70 d, 1 H, J(5,6) = 7.3 (H-5, base); 4.88 dd, 1 H, J = 2.5 and 13.2 (NCH_2); 4.47 dd, 1 H, J = 8.8 and 12.7 (NCH_2); 4.19 m, 1 H (OCH); 3.97 dd, 1 H, J = 4.2 and 12.7 (NCH_2); 3.72 dd, 1 H, J = 6.8 and 13.2 (NCH_2).

1-[(2R)-3-(Adenin-9-yl)-2-hydroxypropyl]-3-carbamoylpyridinium chloride (IX). ^{1}H NMR spectrum: 9.53 brs, (H-2, Nic); 9.14 brd, 1 H, J(6,2) = J(6,4) = 1.0, J(6,5) = 6.1 (H-6, Nic); 9.01 brd, 1 H,

TABLE I
Physico-chemical characteristics of the "abbreviated" NAD models *VIII, IX*

Compound	M.p., °C Yield, %	Formula (M.w.)	Calculated/Found			Spectrum	
			% C	% H	% N	UV	MS
VIIIa	282 – 283 52	$C_{14}H_{16}ClN_7O_2 . 0.5\ H_2O$ (358.8)	46.86 46.87	4.77 4.59	27.33 27.37	259 (18 600)	314 (M – Cl), 192 (M – Nic – Cl) 135 (base), 122 (Nic)
VIIIb	285 – 287 47	$C_{14}H_{17}ClN_8O_2 . H_2O$ (382.8)	43.92 44.05	5.00 4.58	29.27 29.63	291 (10 780) 273 (9 500) 253 (13 800)	329 (M – Cl), 151 (base + H) 123 (Nic + H)
VIIIc	205 – 207 73	$C_{15}H_{17}ClN_6O_2 . 2\ H_2O$ (384.9)	46.82 46.35	5.50 5.12	21.84 20.95	265 (8 200)	313 (M – Cl)
VIIId	189 – 191 64	$C_{14}H_{16}ClN_7O_2 . 3\ H_2O$ (419.8)	40.05 39.92	5.28 4.84	23.35 23.30	255 (12 300)	330 (M – Cl), 137 (Nic + H + CH_2)
VIIIe	261 – 264 68	$C_{13}H_{16}ClN_5O_3 . H_2O$ (343.8)	45.42 45.20	5.28 4.82	20.37 20.76	275 (13 100)	290 (M – Cl), 168 (M – Nic – Cl) 123 (Nic + H), 112 (base + H)
IX	167 – 169 73	$C_{14}H_{16}ClN_7O_2$ (349.8)	48.07 47.87	4.61 4.92	28.03 27.65	261 (16 500)	314 (M – Cl)

J(4,2) = *J*(4,6) = 1.0, *J*(4,5) = 8.1 (H-4, Nic); 8.73 brs and 8.16 brs, 2 × 1 H (NH_2, Nic); 8.25 dd, 1 H, *J*(5,4) = 8.1, *J*(5,6) = 6.1 (H-5, Nic); 8.16 s and 8.11 s, 2 × 1 H (H-2, H-8, base); 7.24 brs, 2 H (NH_2, base); 6.03 d, 1 H, *J*(OH,CH) = 4.6 (OH); 4.96 dd, 1 H, *J* = 2.2 and 13.2 (NCH_2); 4.42 dd, 1 H, *J* = 4.2 and 13.4 (NCH_2); 4.39 m, 1 H (OCH); 4.28 dd, 1 H, *J* = 6.1 and 13.4 (NCH_2).

(2*S*)-3-Azido-1,2-propanediol (*X*)

The title compound was prepared from (2*R*)-1-chloro-2,3-propanediol as described for the racemic derivative. Yield 48%, b.p. 81 – 90 °C/17 Pa (reported[21] 86 °C/16 Pa). Mass spectrum (*m*/*z*): 118 (M + H). $[\alpha]_D$ −14.52° (*c* 0.574, 0.1 M HCl).

(2*S*)-3-Azido-2-hydroxypropyl *p*-Toluenesulfonate (*XI*)

A solution of tosyl chloride (26.45 g, 128 mmol) in acetonitrile (78 ml) was added to a solution of (2*S*)-3-azido-1,2-propanediol (*X*; 15.0 g, 128 mmol) in pyridine (11.5 ml) and acetonitrile (52 ml) under stirring and cooling with ice. After 24 h, water (15 ml) was added and the mixture was set aside for 90 min at room temperature. The solvent was evaporated to half of the original volume, ethyl acetate (140 ml) was added and the mixture was washed with water. After drying and evaporation of the solvent, the residue was chromatographed on silica gel in chloroform–methanol (19 : 1) to give product *XI* as a viscous oil (16.34 g, 44%). For $C_{10}H_{13}N_3O_4S$ (271.2) calculated: 44.28% C, 4.83% H, 15.49% N; found: 44.59% C, 4.85% H, 15.58% N. ^{1}H NMR spectrum: 7.80 d, 2 H, *J* = 8.3 (arom.); 7.48 d, 2 H, *J* = 8.3 (arom.); 5.68 brs, 1 H (OH); 4.03 dd, 1 H, *J* = 4.1 a 10.0 (OCH_2); 3.96 dd, 1 H, *J* = 5.6 and 10.0 (OCH_2); 3.90 dd, 1 H, *J* = 5.9 and 10.0 (OCH_2); 3.89 m (OCH); 3.56 dd and 3.52 dd, 2 H, *J* = 5.4 and 12.2 (NCH_2); 2.41 s, 3 H (CH_3). Mass spectrum (*m*/*z*): 272 (M + H); 155 (tosyl).

(2*S*)-3-Azido-2-(tetrahydropyran-2-yloxy)propyl *p*-Toluenesulfonate (*XII*)

3,4-Dihydro-2*H*-pyran (9.93 g, 118 mmol) and a small amount of hydrochloric acid in dimethylformamide (3 ml) were added to an ice-cooled and stirred solution of (2*S*)-3-azido-2-hydroxypropyl *p*-toluenesulfonate (*XI*, 16.3 g, 60 mmol) in chloroform (31 ml). After 24 h the mixture was made alkaline with triethylamine, the solvent was evaporated and the residue was dissolved in ethyl acetate (150 ml). The solution was washed with water, dried and the solvent was evaporated to give the oily product *XII* (21.65 g, quantitative yield) which was used directly in the next step.

9-[(2*R*)-3-Azido-2-hydroxypropyl]adenine (*XIII*)

A solution of adenine (6.75 g, 50 mmol) and sodium hydride (60% suspension in oil; 2.0 g, 50 mmol) in dimethylformamide (20 ml) was stirred at 80 °C for 1 h. Compound *XII* (21.65 g, 60 mmol) in dimethylformamide (20 ml) was then added and the reaction mixture was heated at 100 °C for 30 h. After evaporation of the solvent and codistillation with toluene (3 × 40 ml), the residue was extracted with boiling chloroform. The solvent was evaporated and the residue was subjected to column chromatography on silica gel in chloroform–methanol (0 – 5%). After crystallization from ethyl acetate, the obtained intermediate (6.82 g, 43%, m.p. 131 – 134 °C) was deprotected by heating with 0.25 M sulfuric acid (50 ml) at 80 °C for 30 h. The reaction mixture was neutralized with barium hydroxide to pH 7, filtered through Celite and the solid on the filter was washed with boiling water. The filtrate was evaporated to dryness and the residue was crystallized from water to give compound *XIII* (2.1 g, 42%), m.p. 171 – 173 °C. For $C_8H_{10}N_8O$ (234.2) calculated: 41.02% C, 4.30% H, 47.84% N; found: 40.99% C, 4.32% H, 47.73% N. ^{1}H NMR spectrum: 8.13 s and 8.05 s, 2 × 1 H (H-2, H-8, base); 7.21 brs, 2 H (NH_2, base); 5.65 d, 1 H, *J*(CH,OH) = 5.1 (OH); 4.23 – 4.17 m and 4.13 – 4.08 m, 1 H

and 2 H (H-1′ and H-2′); 3.35 dd and 3.24 dd, 2 × 1 H, $J(3',2')$ = 3.4 and 6.1, $J(g)$ = 12.7 (H-3′). Mass spectrum (*m/z*): 235 (M + H); 192 (M – N_3); 178 (M – CH_2N_3); 136 (adenine + H). $[\alpha]_D$ +30.98° (*c* 0.008, 0.1 M HCl).

9-[(2*S*)-3-Amino-2-hydroxypropyl]adenine (*XIV*)

A mixture of compound *XIII* (1.4 g, 6 mmol), methanol (350 ml), water (40 ml), concentrated hydrochloric acid (3.5 ml) and 10% Pd/C (0.52 g) was hydrogenated at 50 °C for 72 h. The reaction mixture was filtered through Celite and the solid on the filter was washed with water. The aqueous solution was desalted on Dowex 50 X 8 (H^+ form). After washing out the inorganic salts with water, the product was eluted with water–ammonia mixture (10 : 1). Evaporation of the solvent and codistillation of the residue with ethanol (3 × 50 ml) afforded amorphous colourless compound *XIV* in quantitative yield (1.262 g). ^{1}H NMR spectrum: 8.12 s and 8.03 s, 2 × 1 H (H-2, H-8, base); 7.18 brs, 2 H (NH_2, base); 4.32 dd and 4.05 dd, 2 × 1 H, $J(1',2')$ = 4.2 and 7.3, $J(g)$ = 13.9 (H-1′); 3.74 m, 1 H, ΣJ = 23.0 (H-2′); 2.47 dd and 2.44 dd, 2 × 1 H, $J(3',2')$ = 5.6 and 5.9, J = 13.2 (H-3′); 3.36 br, 2 H (NH_2). $[\alpha]_D$ +17.93° (*c* 0.009, 0.1 M HCl).

1-Allyl-3-carbamoylpyridinium Bromide (*XVIIa*)

The title compound was prepared according to a described procedure[22]. Crystallization from ethanol afforded the allyl derivative in 63% yield; m.p. 149 – 150 °C (reported[22] m.p. 150 °C). ^{1}H NMR spectrum: 9.50 brs, 1 H, $J(2,4)$ = $J(2,6)$ = 1.0 (H-2); 9.20 brd, 1 H, $J(6,5)$ = 6.1 (H-6); 9.00 dt, 1 H, $J(4,2)$ = $J(4,6)$ = 1.5, $J(4,5)$ = 8.0 (H-4); 8.64 brs and 8.20 brs, 2 × 1 H (NH_2); 8.30 dd, 1 H, $J(5,4)$ = 8.0, $J(5,6)$ = 6.1 (H-5); 6.19 ddt, 1 H, ΣJ = 39.8, $J(2',1')$ = 6.35, $J(2',3'cis)$ = 10.2, $J(2',3'trans)$ = 16.9 (H-2′); 5.48 brd, 1 H, $J(3',2'trans)$ = 17.0, $J(g)$ = 1.5, $J(3',1')$ = 1.0 (H-3′); 5.47 brd, 1 H, $J(3',2'cis)$ = 10.2, $J(g)$ = 1.5, $J(3',1')$ = 1.0 (H-3′); 5.36 brd, 2 H, $J(1',2')$ = 6.35, $J(1',3')$ = 1.0 (H-1′).

1-Allyl-3-carbamoylpyridinium Perchlorate (*XVIIb*)

1-Allyl-3-carbamoylpyridinium bromide (*XVIIa*; 2.0 g, 8 mmol) was dissolved in a small amount of water, applied onto a column of Dowex 1 (ClO_4^- form) and the UV-absorbing fraction was collected. The solvent was evaporated to give colourless crystals (2.1 g, quantitative yield), m.p. 122 – 124 °C. For $C_9H_{11}ClN_2O_5$ (262.7) calculated: 41.16% C, 4.23% H, 10.86% N; found: 41.06% C, 4.11% H, 10.44% N. ^{1}H NMR spectrum: 9.42 brs, 1 H, $J(2,4)$ = $J(2,6)$ = 1.0 (H-2); 9.13 dt, 1 H, $J(6,2)$ = $J(6,4)$ = 1.2, $J(6,5)$ = 6.1 (H-6); 8.96 dt, 1 H, $J(4,2)$ = $J(4,6)$ = 1.5, $J(4,5)$ = 8.1 (H-4); 8.29 dd, 1 H, $J(5,4)$ = 8.1, $J(5,6)$ = 6.1 (H-5); 8.57 brs and 8.17 brs, 2 × 1 H (NH_2); 6.18 ddt, 1 H, ΣJ = 39.8, $J(2',1')$ = 6.35, $J(2',3'cis)$ = 10.3, $J(2',3'trans)$ = 16.8 (H-2′); 5.48 dq, 1 H, $J(3',2'cis)$ = 10.3, $J(g)$ = 1.5, $J(3',1')$ = 1.0 (H-3′); 5.46 dq, 1 H, $J(3',2'trans)$ = 16.8, $J(g)$ = 1.5, $J(3',1')$ = 1.2 (H-3′); 5.32 brd, 2 H, $J(1',2')$ = 6.35, $J(1',3')$ = 1.0 (H-1′).

1-(2,3-Dihydroxypropyl)-3-carbamoylpyridinium Perchlorate (*XVI*)

A. By Zincke reaction. 1-(2,4-Dinitrophenyl)-3-carbamoylpyridinium chloride (*VII*; 4.10 g, 13 mmol) was added to a stirred solution of 3-amino-1,2-propanediol[23] (1.15 g, 13 mmol) in methanol (90 ml) and the stirring was continued at room temperature for 6 h. During this time, a typical change from the deep-red to orange colour occurred. The solvent was evaporated, water (50 ml) was added and the insoluble 2,4-dinitroaniline was removed by filtration. The filtrate was washed with ether, water was evaporated and the residue was mixed with aqueous ammonia (30 ml). After stirring for 30 min, the reaction mixture was taken down and the above-described purification procedure was repeated.

Yield 3.02 g (93%) of deeply coloured oily product. For $C_9H_{13}ClN_2O_3 . H_2O$ (250.7) calculated: 43.12% C, 6.03% H, 11.17% N; found: 42.82% C, 6.37% H, 11.17% N. ^{1}H NMR spectrum: 9.50 brs, 1 H (H-2); 9.14 dt, 1 H, *J*(6,4) = *J*(6,2) = 1.2, *J*(6,5) = 6.1 (H-6); 9.03 dt, 1 H, *J*(4,2) = *J*(4,6) = 1.5, *J*(4,5) = 8.1 (H-4); 8.80 br and 8.17 brs, 2 × 1 H (NH_2); 8.26 dd, 1 H, *J*(5,4) = 8.1, *J*(5,6) = 6.1 (H-5); 5.59 d, 1 H, *J*(OH,CH) = 6.1 (OH); 5.14 t, 1 H, *J*(OH,CH) = 5.5 (OH); 4.87 dd and 4.56 dd, 2 × 1 H, *J*(1′,2′) = 3.1 and 8.5, *J*(*g*) = 12.8 (H-1′); 3.95 m, 1 H (H-2′); 3.53 dt and 3.35 dt, 2 × 1 H, *J*(3′,OH) = *J*(3′,2′) = 5.0 and 6.0, *J*(*g*) = 11.3 (H-3′). Mass spectrum (*m*/*z*): 197 (M – Cl); 123 (Nic + H). The obtained 1-(2,3-dihydroxypropyl)-3-carbamoylpyridinium chloride was converted into perchlorate *XVI* as described for compound *XVIIb*. ^{1}H NMR spectrum of compound *XVI*: 9.44 brs, 1 H (H-2); 9.11 dt, 1 H, *J*(6,4) = *J*(6,2) = 1.2, *J*(6,5) = 6.1 (H-6); 9.00 dt, 1 H, *J*(4,2) = *J*(4,6) = 1.5, *J*(4,5) = 8.1 (H-4); 8.69 brs and 8.17 brs, 2 × 1 H (NH_2); 8.26 dd, 1 H, *J*(5,4) = 8.1, *J*(5,6) = 6.1 (H-5); 5.50 brs, 2 H (OH); 4.86 dd and 4.05 dd, 2 × 1 H, *J*(1′,2′) = 3.2 and 8.5, *J*(*g*) = 12.8 (H-1′); 3.94 m, 1 H (H-2′); 3.53 and 3.35 2 × dd, 2 × 1 H, *J*(3′,OH) = *J*(3′,2′) = 4.9 and 6.4, *J*(*g*) = 11.3 (H-3′). Mass spectrum (*m*/*z*): 197 (M – ClO_4); 123 (Nic + H).

B. By hydroxylation of double bond. Sodium chlorate (4.28 g, 40 mmol), followed by osmium tetroxide (0.1 g, 0.4 mmol), was added to a saturated solution of 1-allyl-3-carbamoylpyridinium perchlorate (*XVIIb*; 4.50 g, 17 mmol) in water. The reaction mixture was stirred at 40 °C for 100 h. Water was evaporated, the residue was codistilled with ethanol, dissolved in ethanol and filtered. After evaporation, the residue was dissolved in a minimum amount of water and chromatographed on C_{18}-silica gel; yield 0.98 g (20%) of lightly coloured oil whose NMR and mass spectra were identical with those of the product prepared according to procedure *A*.

1-[(2*S*)-2-Hydroxy-3-trityloxypropyl]-3-carbamoylpyridinium Chloride (*XXI*)

A solution of (*S*)-trityloxymethyloxirane (*XIX*; 4.0 g, 12.6 mmol) in a mixture of methanol (30 ml) and chloroform (20 ml) was mixed with 30% methanolic ammonia (50 ml) and the solution was set aside at room temperature overnight. After evaporation of the solvent, the residue was chromatographed on a column of silica gel (200 g) in methanol–chloroform (1 : 4). The obtained (*S*)-3-amino-1-trityloxy-2-propanol (*XX*; 2.2 g, 6.6 mmol, 52%, R_F 0.4 in methanol–chloroform 1 : 9) was dissolved in methanol (50 ml) and mixed with 1-(2,4-dinitrophenyl)-3-carbamoylpyridinium chloride (*VII*; 2.05 g, 6.1 mmol). The reaction mixture was stirred overnight under exclusion of moisture, adsorbed on silica gel (100 ml) and applied onto a column of silica gel (200 ml). After washing the column with chloroform (2 liters), the product was eluted with methanol–chloroform (1 : 9). It was obtained as an amorphous foam, R_F 0.1 (methanol–chloroform 1 : 9); yield 1.68 g (54%). For $C_{28}H_{27}ClN_2O_2$ (459.0) calculated: 73.27% C, 5.93% H, 7.72% Cl, 6.10% N; found: 72.93% C, 5.82% H, 7.42% Cl, 5.92% N. ^{1}H NMR spectrum: 9.67 brs, 1 H (H-2); 9.18 d, 1 H (H-6); 9.12 d, 1 H (H-4); 9.00 brs and 8.20 brs, 2 × 1 H (NH_2); 7.44 d, 6 H and 7.33 t, 6 H and 7.24 t, 3 H, *J* = 7.6 (arom.); 5.92 d, 1 H, *J* = 5.0 (OH); 4.96 brd, 1 H, *J*(1′,2′) < 1.0, *J*(*g*) = 11.5 (H-1′); 4.69 brd, 1 H, *J*(1′,2′) = 8.5, *J*(*g*) = 11.5 (H-1′); 4.24 m, 1 H (H-2′); 3.16 dd and 2.92 dd, 2 × 1 H, *J*(3′,2′) = 4.4 and 5.8, *J*(*g*) = 9.5 (H-3′).

1-[(2*S*)-2,3-Dihydroxypropyl]-3-carbamoylpyridinium Perchlorate (*XVIII*)

A solution of compound *XXI* (1.42 g, 3 mmol) in 80% acetic acid was refluxed for 30 min. After evaporation to dryness and addition of water (100 ml), the mixture was washed with ether (2 × 50 ml) and evaporated. The residue was codistilled with water (3 × 50 ml), applied onto a column of Dowex 1X2 (ClO_4^- form) and eluted with water. The fraction absorbing at 254 nm was evaporated, the residue was codistilled with ethanol and crystallized from ethanol. Yield 0.75 g (89%) of compound *XVIII*, m.p. 130 – 131 °C. For $C_9H_{13}ClN_2O_7$ (296.7) calculated: 36.43% C, 4.41% H, 11.95% Cl,

9.44% N; found: 36.69% C, 4.39% H, 11.71% Cl, 9.33% N. Electrophoretic mobility (0.05 M triethylammonium hydrogen carbonate pH 7.5, 20 V/cm, referenced to uridine 3′-phosphate): 0.78. ^{1}H NMR spectrum: 9.38 brs, 1 H (H-2); 9.08 dt, 1 H, *J*(2,6) = 1.0, *J*(5,6) = 6.1 (H-6); 8.96 dt, 1 H, *J* = 1.2 and 1.5, *J*(4,5) = 8.1 (H-4); 8.26 dd, 1 H, *J*(5,4) = 8.1, *J*(5,6) = 6.1 (H-5); 8.58 brs and 8.16 brs, 2 × 1 H (NH_2); 5.54 d, 1 H, *J*(OH,CH) = 5.6 (OH); 5.05 brt, 1 H, *J* = 5.0 (OH); 4.84 dd, 1 H, *J*(1′,2′) = 3.1, *J*(*g*) = 12.9 (H-1′); 4.53 dd, 1 H, *J*(1′,2′) = 8.5, *J*(*g*) = 12.9 (H-1′); 3.93 m, 1 H (H-2′); 3.53 dt and 3.35 dt, 2 × 1 H, *J*(3′,2′) = 5.0 and 5.5, *J*(*g*) = 11.5 (H-3′). UV spectrum, λ_{max} (ε_{max}): (pH 7) 265 (4 500), (pH 12) 265 (4 400).

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