Synthesis of Nicotinamide Adenine Dinucleotide (NAD) Analogues with a Sugar Modified Nicotinamide Moiety

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The synthesis of nicotinamide adenine dinucleotide (NAD) analogues in which the ribose unit of the nicotinamide moiety is replaced by a hexitol, altritol, and cyclohexenyl sugar mimic is described.

Introduction. – Nicotinamide adenine dinucleotide (NAD^+) is a cofactor in more than 400 biological redox reactions and requires the presence of enzymes such as dehydrogenases [1]. As the most important role in these reactions is played by the nicotinamide moiety, this fragment of the molecule was intensively studied. NAD^+, however, is a labile molecule, and the nicotinamide glycosyl bond is easily cleaved, both chemically and enzymatically (by ADP ribosylating enzymes). Therefore, it would be desirable to have a 'sugar-modified' NAD^+ analogue available with a more stable nicotinamide glycoside.

In solution, NAD^+ occurs as a mixture of folded and unfolded forms with the aromate rings in close proximity in the folded forms [2]. In solid state (X-ray) and when bound to enzymes, NAD^+ adopts an extended conformation [3][4]. The ribose ring of the nicotinamide moiety adapt the *exo*-C(3′) conformation as well in solution (85% *S* conformation) as in solid phase [2]. Interestingly, the *S* conformation is also the most abundant (90% *S*) for the ribose ring when the nicotinamide moiety is reduced (*i.e.*, in $NADPH^+$) [5].

To find a sugar surrogate that can replace the ribose moiety of the nicotinamide part of NAD^+ (so that a more stable compound is obtained while keeping the catalytic activity), we should address questions about the importance of sugar conformation and flexibility of NAD^+ during the catalytic process, and the relative importance of the presence of an anomeric center for catalysis. Such questions can be approached by making sugar-modified analogues of NAD^+ available.

In this area of research, our interest is mainly focused on the study of catalytic activity of dehydrogenases. All protein structures containing NAD^+ (810) were retrieved from the *Protein Data Bank* [6]. Analysis of these complexes revealed that the ribose part of the nicotinamide moiety in NAD^+ predominantly adopts a conformation in the southern half of the pseudorotation wheel (89% = *S*-type). In all structures of NAD^+ co-crystallized with malate dehydrogenase (*e.g.*, 4MDH),

glucose-6-phosphate dehydrogenase (*e.g.*, 1H9A) and histidynol dehydrogenase (*e.g.*, 1KAE), the studied ribose is in an *S*-type conformation (*Fig.*).

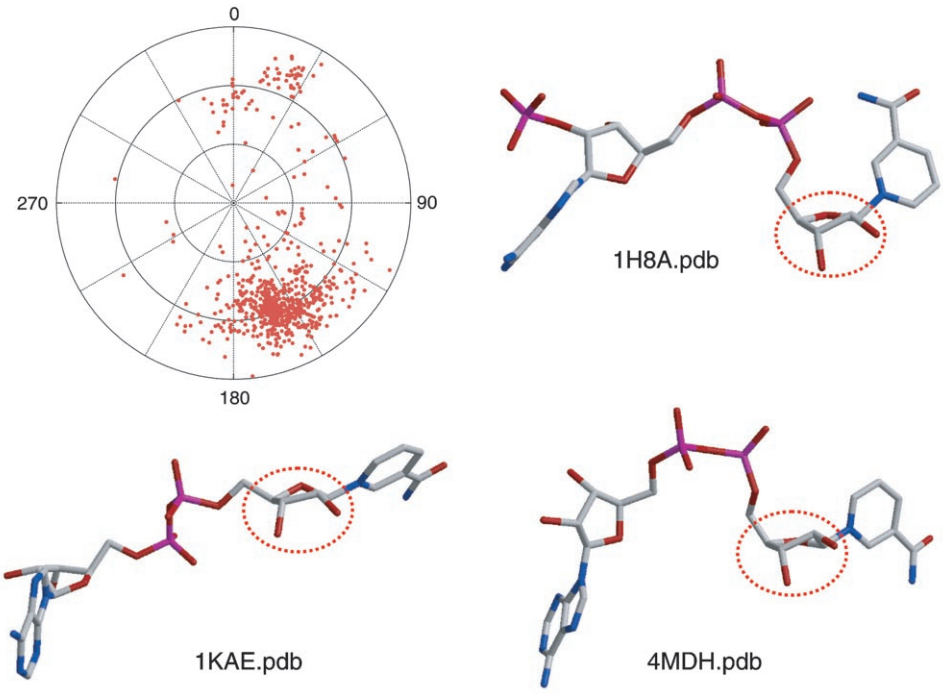
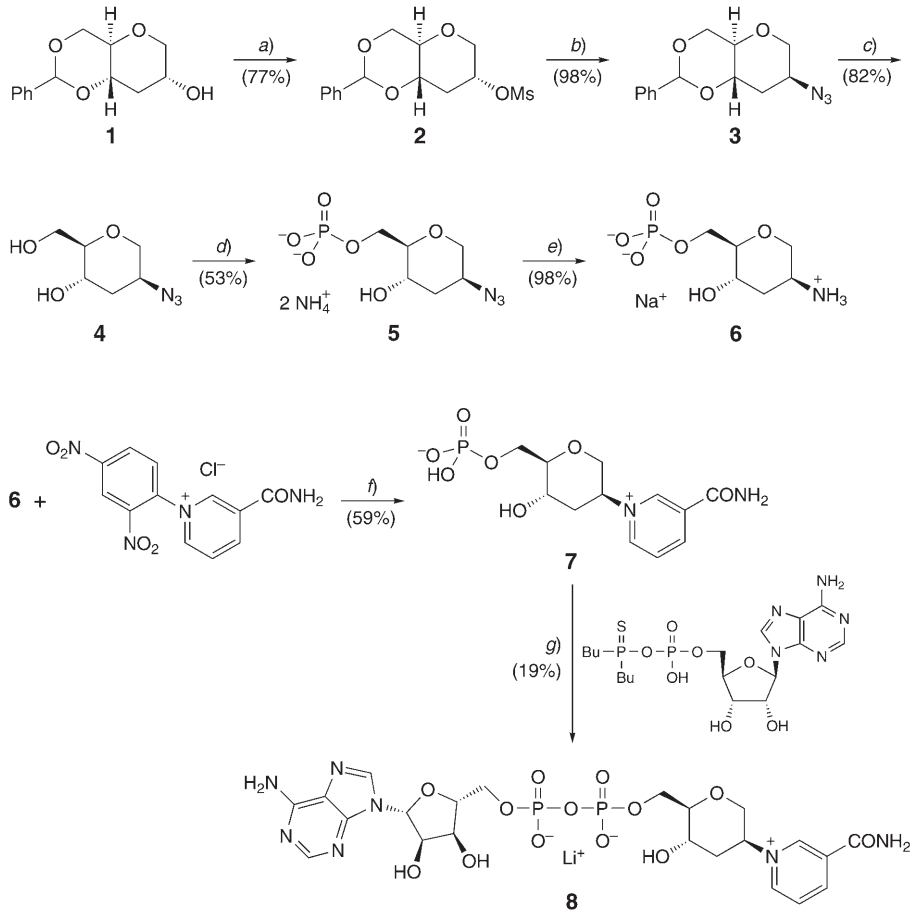


Figure. *The ribose part of the nicotinamide moiety in NAD⁺ predominantly adopts an* S*-type conformation in dehydrogenases*

It is clear that the chemical and enzymatic lability of NAD^+ is due to the presence of an anomeric center and thus can be overcome by synthesizing carbocyclic or hexitol-like analogues. The importance of having two free OH groups on the 'sugar' ring can be approached by synthesizing altritol analogues. However, these six-membered analogues are rigid mimics of a nucleoside in the *N*-type conformation, while, in the crystal structures with dehydrogenases, the ribose part of the nicotinamide moiety adopts an *S*-type conformation. Therefore, we also synthesized the cyclohexenyl analogue as a congener with more conformational diversity.

Results. – The NAD^+ analogue with a hexitol moiety was synthesized from 1,5-anhydro-4,6-*O*-benzylidene-3-deoxy-D-glucitol (*Scheme 1*) [7][8]. The N_3 group in the 2-position (*i.e.*, **3**) was introduced by mesylation of **1** (→**2**), followed by nucleophilic substitution with NaN_3 with inversion of configuration. The benzylidene protecting group was removed with acid (→**4**), and the primary OH group was phosphorylated using $POCl_3$ in trimethyl phosphate (→**5**). Then the N_3 group at C(2) was reduced to an amino group (→**6**), which was used as nucleophile in the *Zincke* reaction. The nicotinamide nucleotide **7** was obtained by reaction of **6** with 1-(2,4-dinitrophenyl)-3-carbamoylpyridinium chloride (NDC) (or tetrafluoroborate) and purification over 2-

Scheme 1



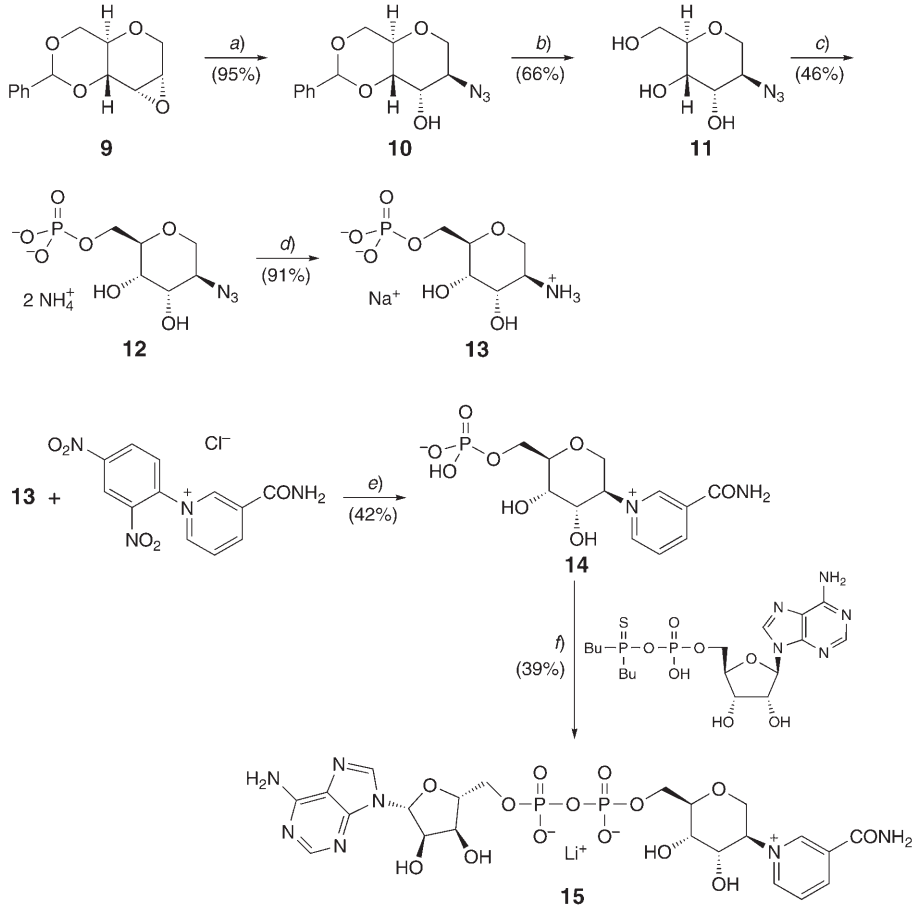
a) MsCl, 4-(dimethylamino)pyridine (DMAP), pyridine, r.t., 14 h. *b*) NaN_3, DMF, 80°, 31 h. *c*) 80% AcOH, 95°, 1 h. *d*) 1. $POCl_3$, $(MeO)_3PO$, 0°, 3 h; 2. H_2O, NH_4OH. *e*) 1. H_2 (30 psi), $PtO_2 \cdot H_2O$, H_2O/MeOH, r.t., 2 h; 2. *Dowex 50WX4-400* (Na^+). *f*) H_2O/MeOH, $Et_3NH^+HCO_3^-$, 40°, 4 d. *g*) 1. $AgNO_3$, DMF, pyridine, r.t., 40 h; 2. LiOH.

(diethylamino)ethyl (DEAE)-cellulose. The hexitol nicotinamide adenine dinucleotide (hNAD$^+$) **8** was obtained from the nucleotide **7** and adenosine 5′-(phosphoric dibutylphosphinothioic anhydride) using $AgNO_3$ as activating agent.

The synthesis of the altritol nicotinamide adenine dinucleotide (aNAD$^+$; **15**) started from the epoxide of 4,6-benzylidene-1,5-anhydro-D-allitol **9** (*Scheme 2*) [9]. The epoxide was opened using NaN_3 in methoxyethanol (→**10**), and the benzylidene protecting group was removed using AcOH (→**11**). After phosphorylation of the primary OH group (→**12**), the N_3 group was reduced (→**13**). The amino group was then reacted with NDC to obtain the nicotinamide nucleotide **14**. Reaction of **14** with

the activated adenosine monophosphate (AMP) yielded altritol nicotinamide adenine dinucleotide (aNAD$^+$; **15**).

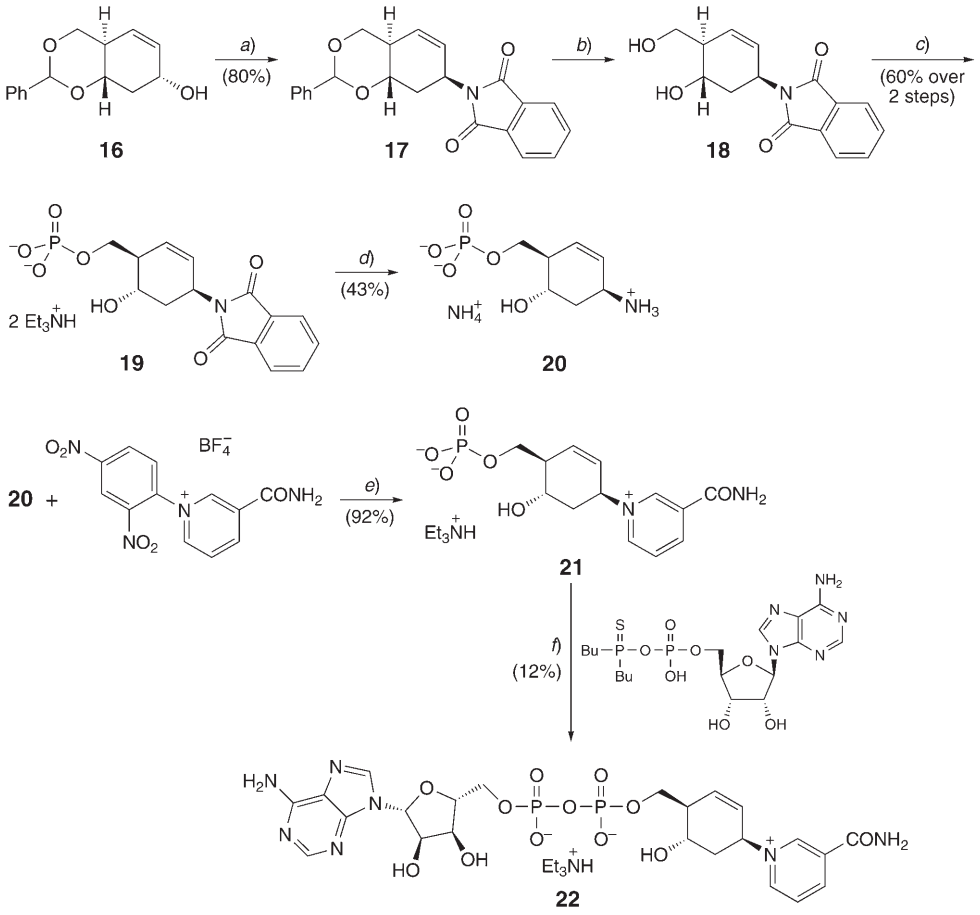
Scheme 2



a) NaN_3, NH_4Cl, $MeOCH_2OH/H_2O$, 100°, 18 h. *b*) 80% AcOH, 95°, 2 h. *c*) 1. $POCl_3$, $(MeO)_3PO$, 0°, 3 h; 2. H_2O, Et_3N. *d*) 1. H_2 (30 psi), $PtO_2 \cdot H_2O$, H_2O/MeOH, r.t., 85 h; 2. *Dowex 50X8-200* (Na^+). *e*) 1-(2,4-dinitrophenyl)-3-carbamoylpyridinium chloride (NDC), H_2O/MeOH, $Et_3NH^+HCO_3^-$, 40°, 6 d. *f*) 1. $AgNO_3$, DMF, pyridine, r.t., 65 h; 2. LiOH.

The synthesis of the cyclohexenyl congener **22** was somewhat more complex. It started from the chiral cyclohexenyl alcohol precursor **16** which was described before [10][11] (*Scheme 3*). The OH function was converted into an amino group with inversion of the configuration at the C-atom *via* the phtalimides **17** and **18** as intermediates. The primary alcohol **18** was first phosphorylated (→**19**) before the phtalimide group was removed using NH_2NH_2 (→**20**). Condensation reaction with NDC (→**21**) and with activated AMP was accomplished in a similar way as described before to obtain **22**. The obtained compounds **8**, **15**, and **22** are currently under

Scheme 3



a) Diisopropyl azodicarboxylate (DIAD), Ph_3P, phtalimide, THF, 1 h. *b*) 80% AcOH, 95°, 1 h. *c*) 1. $POCl_3$, $(MeO)_3PO$, 0°, 5 h; 2. triethylammonium bicarbonate (TEAB), 30 min. *d*) 1. NH_2NH_2, EtOH, 95°, 16 h; 2. *Dowex 400X*. *e*) 1. $^iPr_2EtN$, MeOH, 55° for 4 h, and 16 h at r.t.; 2. TEAB; 3. *Dowex 50X8-200*. *f*) 1. $AgNO_3$, DMF, pyridine, r.t., 16 h; 2. H_2S; 3. *Sephadex A25*.

evaluation as NAD^+ analogues in order to study the structural requirements for activity as cofactors for dehydrogenases.

Experimental Part

General. All solvents used for reactions are anal. grade or freshly distilled. Anh. pyridine was obtained by reflux on KOH and distilled. DMF was stored over *Linde* 4-Å molecular sieves, followed by distillation under reduced pressure. Precoated aluminum sheets (*Fluka* silica gel/TLC cards, 254 nm) were used for TLC, and spots were visualized with UV and anisaldehyde–sulfuric acid spray. Column chromatography (CC) was performed on silica gel (0.060–0.200 nm). UV: *UVIKON 940* apparatus; λ_{max}

in nm. NMR Spectra: 200- or 500-MHz *Varian* apparatus with TMS as internal standard for ^{1}H-NMR and (D_6)DMSO (39.6 ppm) for ^{13}C-NMR. ^{31}P-NMR Spectra: were obtained using 85% H_3PO_4 as external standard. Exact mass measurements were performed on a quadruple time-of-flight mass spectrometer (*Q-TOF-2*, *Micromass*), equipped with a standard electrospray-ionization (ESI) interface; in *m*/*z*.

1,5-Anhydro-4,6-O-*benzylidene-3-deoxy-2*-O-*(methylsulfonyl)*-D-ribo-*hexitol* (**2**). To a soln. of *1,5-anhydro-4,6*-O-*benzylidene-3-deoxy*-D-*glucitol* [8] (**1**; 5.000 g, 21.16 mmol) and a cat. amount of 4-(dimethylamino)pyridine (DMAP) in 40 ml of pyridine at 0°, MsCl (3 ml, 38 mmol) was added. The mixture was stirred at r.t. for 14 h. After evaporation and co-evaporation with toluene, the residue was extracted three times with warm $CHCl_3$, and the combined extracts were filtered over a short path of silica gel. Evaporation of the solvent gave the crude product, which was washed with Et_2O and precipitated from MeOH to afford **2** (5.103 g, 77%). Oil. R_f (hexane/AcOEt 1:2) 0.8, R_f (hexane/AcOEt 2:1) 0.5. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.92 (*ddd*, $^2J(3_{ax},3_{eq}) = 12$, $^3J(3_{ax},2) = 11.5$, $^3J(3_{ax},4) = 11$, $H_{ax}-C(3)$); 2.68 (*ddd*, $^2J(3_{eq},3_{ax}) = 12$, $^3J(3_{eq},2) = 5.5$, $^3J(3_{eq},4) = 4$, $H_{eq}-C(3)$); 3.06 (*s*, Me); 3.32 (*ddd*, $^3J(5,6_{ax}) = 10$, $^3J(5,4) = 8$, $^3J(5,6_{eq}) = 5$, H–C(5)); 3.41 (*dd*, $^2J(1_{ax},1_{eq}) = {}^3J(1_{ax},2) = 11$, $H_{ax}-C(1)$); 3.59 (*ddd*, $^3J(4,3_{ax}) = 11$, $^3J(4,5) = 8$, $^3J(4,3_{eq}) = 4$, H–C(4)); 3.68 (*dd*, $^2J(6_{ax},6_{eq}) = {}^3J(6_{ax},5) = 10$, $H_{ax}-C(6)$); 4.21 (*ddd*, $^2J(1_{eq},1_{ax}) = 11$, $^3J(1_{eq},2) = 5.5$, $^4J(1_{eq},3_{eq}) = 2$, $H_{eq}-C(1)$); 4.33 (*dd*, $^2J(6_{eq},6_{ax}) = 10$, $^3J(6_{eq},5) = 5$, $H_{eq}-C(6)$); 4.72–4.90 (*m*, H–C(2)); 5.53 (*s*, PhC*H*), 7.33–7.51 (*m*, 5 arom. H). ^{13}C-NMR: see *Table 1*.

Table 1. *^{13}C-NMR Chemical Shifts* [ppm] *and Assignments for Compounds* **1**–**7**. *J* in Hz.

	1 ($CDCl_3$)	**2** ($CDCl_3$)	**3** ($CDCl_3$)	**4** ($CDCl_3$)	**5** (D_2O)	**6** (D_2O)	**7** (D_2O)[a])
C(1)	72.4	69.4	69.3	68.8	70.7	69.5	69.2 or 69.0
C(2)	65.6	72.3	57.4	57.2	59.4	50.0	69.0 or 69.2
C(3)	38.4	35.8	32.8	35.8	36.5	35.0	37.7
C(4)	73.1	73.2	74.0 or 74.2	63.0	63.8	62.2	62.2
C(5) (^3J(C,P))	76.4	75.7	74.2 or 74.0	81.7	83.3 (7.6)	83.7 (6.1)	83.2 (6.1)
C(6) (^3J(C,P))	69.2	68.9	68.9	62.8	66.4 (4.6)	65.7 (4.6)	65.3 (3.1)
Me		38.6					
Ph*C*	101.7	101.8	102.0				
C_{ipso}	137.4	137.1	137.4				
C_o	126.2	126.1	126.1				
C_m	128.4	128.4	128.4				
C_p	129.2	129.3	129.2				
C(2), C(4) (py)							146.3, 146.6
C(3) (py)							136.3
C(5) (py)							130.9
C(6) (py)							148.2
C=O							168.2

[a]) Position numbers of the glucitol part are primed in the text.

1,5-Anhydro-2-azido-4,6-O-*benzylidene-2,3-dideoxy*-D-arabino-*hexitol* (**3**). To a soln. of **2** (5.000 g, 15.91 mmol) in 120 ml of DMF, NaN_3 (2.1 g, 32 mmol) was added, and the mixture was heated at 80° under N_2 with stirring. After 31 h, the mixture was cooled to r.t., and the solvent was evaporated *in vacuo*. The residue was triturated with warm $CHCl_3$, and the extract was filtered over a short path of silica gel. The solvent was removed, and the resultant solid was precipitated from hexane to yield **3** (4.073 g, 98%). White powder. R_f (hexane/AcOEt 2:1) 0.9. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.91 (*ddd*, $^2J(3_{ax},3_{eq}) = 13$, $^3J(3_{ax},4) = 12$, $^3J(3_{ax},2) = 4$, $H_{ax}-C(3)$); 2.26–2.40 (*dm*, $^2J(3_{eq},3_{ax}) = 13$, $H_{eq}-C(3)$); 3.39 (*dd*, $^3J(5,6_{ax}) = 10$, $^3J(5,6_{eq}) = 5$, H–C(5)); 3.69 (*dd*, $^2J(1_{ax},1_{eq}) = 12$, $^3J(1_{ax},2) = 2$, $H_{ax}-C(1)$); 3.77 (*dd*,

$^2J(6_{ax},6_{eq}) = {}^3J(6_{ax},5) = 10$, $H_{ax}-C(6)$); 3.55–4.06 (*m*, $H_{eq}-C(1)$, H–C(2), H–C(4)); 4.28 (*dd*, $^2J(6_{eq},6_{ax}) = 10$, $^3J(6_{eq},5) = 5$, $H_{eq}-C(6)$); 5.60 (*s*, PhC*H*), 7.34–7.52 (*m*, 5 arom. H). ^{13}C-NMR: see *Table 1.*

1,5-Anhydro-2-azido-2,3-dideoxy-D-arabino-*hexitol* (**4**). A soln. of **3** (4.000 g, 15,31 mmol) in 180 ml of 80% AcOH was heated at 95° for 1 h. After evaporation, and co-evaporation with H_2O and then with toluene, the residue was purified by silica gel CC (0–10% AcOEt in hexane) and dried *in vacuo* over P_4O_{10} to afford **4** (2.174 g, 82%). Oil. R_f (hexane/AcOEt 1:2) 0.25. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.72 (*ddd*, $^2J(3_{ax},3_{eq}) = 14$, $^3J(3_{ax},4) = 11$, $^3J(3_{ax},2) = 4$, $H_{ax}-C(3)$); 2.25–2.40 (*dm*, $^2J(3_{eq},3_{ax}) = 14$, $H_{eq}-C(3)$); 2.67 (br. *s*, OH); 3.05 (br. *s*, OH); 3.12–3.28 (*dm*, $^3J(5,4) = 9$, H–C(5)); 3.59 (*dd*, $^2J(1_{ax},1_{eq}) = 12$, $^3J(1_{ax},2) = 2$, $H_{ax}-C(1)$); 3.74–4.04 (*m*, $H_{eq}-C(1)$, H–C(2), H–C(4), H–C(6a), H–C(6b)). ^{13}C-NMR: see *Table 1.*

1,5-Anhydro-2-azido-2,3-dideoxy-6-O-*phosphono*-D-arabino-*hexitol Diammonium Salt* (**5**). The azide **4** (1.004 g, 5.80 mmol) was dissolved under N_2 in 6 ml of $(MeO)_3PO$ (freshly distilled *in vacuo* over BaO), cooled in an ice bath, and then triturated with 1.7 ml of a 1:1 (*v*/*v*) mixture of $POCl_3$ (distilled immediately prior to use) and $(MeO)_3PO$. The $POCl_3$ soln. was prepared at 0° under N_2, stirred for 15 min, and added to the soln. of **4** in a single portion. After 3 h of stirring at 0°, the reaction was quenched by addition of 6 ml of ice water and 9 ml of cold Et_3N. The resulting mixture was evaporated to dryness *in vacuo*, the residue was washed with $(i\text{-}Pr)_2O$ and purified by silica-gel CC (0–35% NH_4OH (20% soln. in H_2O) in i-PrOH) to give **5** (0.677 g, 43%). Pale yellow oil. Mixed fractions were combined and repurified to give an additional portion of **5** (0.159 g, 10%). The total yield was 53%. R_f (i-PrOH/25% NH_4OH_{aq}/H_2O 6:3:1) 0.35. ^{1}H-NMR (200 MHz, D_2O): 1.80 (*ddd*, $^2J(3_{ax},3_{eq}) = 13.5$, $^3J(3_{ax},4) = 11$, $^3J(3_{ax},2) = 3.5$, $H_{ax}-C(3)$); 2.26–2.42 (*dm*, $^2J(3_{eq},3_{ax}) = 13.5$, $H_{eq}-C(3)$); 3.43 (*ddd*, $^3J(5,4) = 8.5$, $^3J(5,6a) = 5.5$, $^3J(5,6b) = 2$, H–C(5)); 3.68 (*dd*, $^2J(1_{ax},1_{eq}) = 12.5$, $^3J(1_{ax},2) = 1.5$, $H_{ax}-C(1)$); 3.77–4.24 (*m*, $H_{eq}-C(1)$, H–C(2), H–C(4), $H_a-C(6)$, $H_b-C(6)$). ^{13}C-NMR: see *Table 1.*

2-Amino-1,5-anhydro-2,3-dideoxy-6-O-*phosphono*-D-arabino-*hexitol Sodium Salt* (**6**). The azide **5** (0.677 g, 2.51 mmol) was dissolved in 75 ml of H_2O and 25 ml of MeOH. *Adams* catalyst ($PtO_2 \cdot H_2O$, 0.068 g, 0.28 mmol) was added, and the mixture was shaken at r.t. for 2 h in a *Parr* hydrogenation apparatus (30 psi). After removal of the catalyst, the filtrate was concentrated to dryness, dissolved in H_2O, and applied to a column of *Dowex 50WX4-400* (Na^+) ion-exchange resin with H_2O as the eluent. Evaporation and drying the residue in a vacuum dessicator over P_4O_{10} provided **6** (0.666 g, 98%). Pale yellow glass. R_f (i-PrOH/25% NH_4OH_{aq}/H_2O 6:3:1) 0.2. ^{1}H-NMR (500 MHz, D_2O): 1.93 (*ddd*, $^2J(3_{ax},3_{eq}) = 14.4$, $^3J(3_{ax},4) = 12.1$, $^3J(3_{ax},2) = 4.1$, $H_{ax}-C(3)$); 2.34 (*dddd*, $^2J(3_{eq},3_{ax}) = 14.4$, $^3J(3_{eq},4) = 5.1$, $^4J(3_{eq},1_{eq}) = {}^3J(3_{eq},2) = 2.6$, $H_{eq}-C(3)$); 3.41 (*dddd*, $^3J(5,4) = 9.6$, $^3J(5,6a) = 4.1$, $^3J(5,6b) = 2.2$, $^4J(5,P) = 0.5$, H–C(5)); 3.73 (*dddd*, $^3J(2,3_{ax}) = 4.1$, $^3J(2,3_{eq}) = 2.6$, $^3J(2,1_{ax}) = 2.0$, $^3J(2,1_{eq}) = 1.7$, H–C(2)); 3.78 (*dd*, $^2J(1_{ax},1_{eq}) = 13.2$, $^3J(1_{ax},2) = 2.0$, $H_{ax}-C(1)$); 3.97 (*ddd*, $^2J(1_{eq},1_{ax}) = 13.2$, $^4J(1_{eq},3_{eq}) = 2.6$, $^3J(1_{eq},2) = 1.7$, $H_{eq}-C(1)$); 3.98 (*ddd*, $^3J(4,3_{ax}) = 12.1$, $^3J(4,5) = 9.6$, $^3J(4,3_{eq}) = 5.1$, H–C(4)); 4.01 (*ddd*, $^2J(6b,6a) = 11.8$, $^3J(6b,P) = 5.4$, $^3J(6b,5) = 2.2$, $H_b-C(6)$); 4.05 (*ddd*, $^2J(6a,6b) = 11.8$, $^3J(6a,P) = 6.9$, $^3J(6a,5) = 4.1$, $H_a-C(6)$). ^{13}C-NMR: see *Table 1.* ^{31}P-NMR (202 MHz, D_2O): 3.51. Anal. calc. for $C_6H_{13}O_6NNa$ (218.162): C 33.03, H 6.01, N 6.42; found: C 33.20, H 5.79, N 6.22.

1-(2,4-Dinitrophenyl)-3-carbamoylpyridinium tetrafluoroborate was prepared according to the combination of protocols reported by *Slama et al.* [12] and *Walt et al.* [13]. Nicotinamide (5.0 g, 40.9 mmol) and 1-chloro-2,4-dinitrobenzene (20.0 g, 98.7 mmol) were placed in 1-l round-bottom flask equipped with a magnetic stirring bar. The mixture was melted and heated to 105° with stirring under an inert atmosphere for 1 h to form a dark red solid. After cooling to around 30–40°, the solid was dissolved in 250 ml of reagent-grade MeOH, followed by addition of 190 ml of Et_2O. The liquid was decanted from the oily precipitate, and the procedure was repeated two times. The residue was dissolved in 125 ml of H_2O, extracted with $CHCl_3$ (8 × 20 ml) and treated with activated charcoal (1 g).

After filtration through *Celite*, the soln. was concentrated *in vacuo* at 30° to 30 ml and mixed with a soln. of $NaBF_4$ (9.0 g, 82.0 mmol) in 20 ml of H_2O. An oily material was formed, which was redissolved in 350 ml of H_2O by heating on a steam bath and cooled to give yellow crystals. After two recrystallizations from MeOH, the fine pale yellow crystals were collected, washed with Et_2O, and dried *in vacuo*. M.p. 166° ([12]: 167–169°). R_f (BuOH/AcOH/H_2O 5:2:3) 0.6. The yield of 1-(2,4-dinitrophenyl)-3-carbamoylpyridinium tetrafluoroborate was 8.8 g (57%).

For the preparation of *1-(2,4-dinitrophenyl)-3-carbamoylpyridinium chloride* (NDC), the soln. obtained after filtration over charcoal was concentrated to a viscous oil, which was mixed while still warm with 30 ml of BuOH. The flask was stopped with rubber septum, flushed with N_2 and kept at 5° for 2 d. The pale yellow crystals of NDC were isolated by removal of the mother liquor using a syringe, followed by washing with dry Et_2O. Traces of solvent were removed under vacuum. This procedure was required in order to minimize exposure of NDC to moisture.

*1,5-Anhydro-2-(3-carbamoylpyridinium)-2,3-dideoxy-6-*O*-phosphono-*D*-glucitol* (**7**). To a stirred soln. of **6** (0.100 g, 0.40 mmol) in 6 ml of H_2O and 4 ml of MeOH a soln. of NDC (0.148 g, 0.46 mmol) in 1.5 ml of H_2O was added dropwise over several hours. After the addition was complete, the reaction was stirred for 4 d at 40°, 0.3 ml of 0.5M aq. triethylammonium bicarbonate (TEAB) being added over this period. The reaction was monitored by TLC. When quant. conversion of starting amine **6** was achieved, the mixture was triturated with TEAB soln. and cooled to 0°. The colored precipitate was separated by centrifugation. The soln. was concentrated *in vacuo* and passed through a column (21 mm × 14 cm) packed with 2-(diethylamino)ethyl (DEAE) *Whatman DE-52* cellulose for crude purification. The column was washed with 0.01M TEAB. Pure nucleotide **7** was isolated by HPLC on DEAE *Sephadex A-25* cellulose (column dimensions 18 mm × 24 cm), pre-equilibrated with 0.01M TEAB, eluting with TEAB soln. (0.01 → 0.5M). The desired fractions were collected and concentrated to dryness. TEAB was removed by repeated co-evaporation with H_2O, and the residue was dried *in vacuo* over P_4O_{10} to yield **7** (0.083 g, 59%). Yellow glass. R_f (i-PrOH/25% NH_4OH_{aq}/H_2O 6:4:1) 0.15. ^{1}H-NMR (200 MHz, D_2O): 2.40 (*ddd*, $^2J(3'_{ax},3'_{eq}) = 15$, $^3J(3'_{ax},4') = 12$, $^3J(3'_{ax},2') = 5$, $H_{ax}-C(3')$); 2.58–2.73 (*dm*, $^2J(3'_{eq},3'_{ax},) = 15$, $H_{eq}-C(3')$); 3.59–3.72 (*dm*, $^3J(5',4') = 9$, H–C(5′)); 3.95 (*ddd*, $^3J(4',3'_{ax}) = 12$, $^3J(4',5') = 9$, $^3J(4',3'_{eq}) = 5$, H–C(4′)); 4.06–4.26 (*m*, $CH_2(6')$); 4.26 (*dd*, $^2J(1'_{ax},1'_{eq}) = 14$, $^3J(1'_{ax},2') = 3$, $H_{ax}-C(1')$); 4.63 (br. *d*, $^2J(1'_{eq},1'_{ax}) = 14$, $H_{eq}-C(1')$); 5.26 (*m*, H–C(2′)); 8.27 (*dd*, $^3J(5,4) = 8$, $^3J(5,6) = 6$, H–C(5)); 8.94 (*d*, $^3J(4,5) = 8$, H–C(4)); 9.39 (*d*, $^3J(6,5) = 6$, H–C(6)); 9.49 (*s*, H–C(2)). ^{13}C-NMR: see *Table 1*. ESI-MS (pos.): 333.1 (M^+). ESI-MS (neg.): 331.4 ($[M-2H]^-$).

Adenosine 5′-(Phosphoric dibutylphosphinothioic anhydride) was synthesized by the reaction of adenosine monophosphate with dibutylphosphinothioyl bromide and isolated as a white solid (90% yield) according to the procedure of *Slama et al.* [14]. R_f (i-PrOH/25% NH_4OH_{aq}/H_2O 6:3:1) 0.9. For potassium salt: ^{1}H-NMR (500 MHz, D_2O): 0.76–0.80 (*m*, 2 Me); 1.24–1.30 (*m*, 2 CH_2 (Bu)); 1.43–1.48 (*m*, 2 CH_2 (Bu)); 2.00–2.07 (*m*, 2 CH_2 (Bu)); 4.15–4.19 (*m*, 1 H); 4.34–4.39 (*m*, 1 H); 4.54 (*dd*, *J* = 5.1, 3.7, 1 H); 4.80–4.85 (*m*, 1 H); 6.12 (*d*, *J* = 5.9, H–C(1′)); 8.24 (*s*, H–C(2)); 8.46 (*s*, H–C(8)). ^{13}C-NMR (125 MHz, D_2O): 12.9 (Me); 12.9 (Me); 23.0 (Me*C*H_2); 24.2 (Et*C*H_2); 33.8 (*d*, ^1J(C,P) = 68.4, C–P); 33.8 (*d*, ^1J(C,P) = 68.4, C–P); 65.7 (*d*, ^2J(C,P) = 5.8, C(5′)); 70.7 (C(3′)); 74.1 (C(2′)); 83.9 (*d*, ^3J(C,P) = 9.8, C(4′)); 87.1 (C(1′)); 118.8 (C(5)); 140.1 (C(8)); 149.4 (C(4)); 155.9 (C(2)); 160.4 (C(6)). ^{31}P-NMR (202 MHz, D_2O): −9.2 (*d*, ^2J(P,P) = 34.2, P=O); 103.9 (*d*, ^2J(P,P) = 34.2, P=S).

Dibutylphosphinothioyl bromide was prepared by bromination of tetrabutyldiphosphine disulfide according to the procedure of *Furusawa et al.* [15] (see also [14]). The product was isolated as a pale yellow liquid. B.p. 96°/0.1 mm Hg [15][16].

Tetrabutyldiphosphine disulfide was prepared in 42% yield by the reaction of $PSCl_3$ with BuMgBr according to the procedure of *Furusawa et al.* [15] with slight modification: 2.5M soln. of BuMgBr was used.

Hexitol Nicotinamide Adenine Dinucleotide Lithium Salt **8**. Nucleotide **7** (0.067 g, 0.2 mmol) and adenosine 5′-(phosphoric dibutylphosphinothioic anhydride) (0.211 g, 0.4 mmol) were dissolved in 1 ml of dry, amine free DMF and 3 ml of pyridine (distilled over BaO immediately prior to use). The solvents were evaporated *in vacuo* to remove traces of moisture, and the procedure was repeated three times. The final residue was dissolved in 2.4 ml of DMF and 3 ml of pyridine. The flask was wrapped in aluminum foil for protection from light, and $AgNO_3$ (0.274 g., 1.61 mmol) was added in one portion. The flask was immediately capped with a septum, charged with N_2, and the mixture was stirred at r.t. After 40 h, 15 ml of H_2O was added, and H_2S was bubbled into the mixture. The Ag_2S precipitate was removed by filtration through *Celite*. The filtrate was extracted by $CHCl_3$ (3 × 5 ml). Combined org. extracts were washed with 15 ml of H_2O. The combined aq. extracts were concentrated *in vacuo* at r.t. The product **8** was isolated by HPLC in three steps. First on DEAE *Sephadex A-25* cellulose (column dimensions 18 mm × 24 cm, pre-equilibrated with 0.01M TEAB, eluting with TEAB soln. (0.01 → 0.5M). The desired fractions were

collected and evaporated *in vacuo* at r.t. TEAB was removed by repeated co-evaporation with H_2O. The second column with DEAE *Whatman DE-52* cellulose (18 mm × 25 cm) was pre-equilibrated with H_2O, eluting with aq. HCOOH ($0 \rightarrow 0.3$M). The desired fractions were collected and lyophilized twice. The residue was dissolved in H_2O. The soln. was adjusted to pH 6 with 0.1M LiOH and applied to a column with *Sephadex LH-20* cellulose (18 mm × 31 cm) with H_2O as eluent. Two lyophilizations yielded **8** (0.026 g, 19%). Fine white powder. R_f (i-PrOH/25% NH_4OH_{aq}/H_2O 6:4:1) 0.5. UV (H_2O): 259. ^{1}H-NMR (500 MHz, D_2O): 2.37 (*ddd*, $^2J(3'_{ax},3'_{eq}) = 15.0$, $^3J(3'_{ax},4') = 11.5$, $^3J(3'_{ax},2') = 5.2$, $H_{ax}-C(N3')$); 2.62 (*ddd*, $^2J(3'_{eq},3'_{ax}) = 15.0$, $^2J(3'_{eq},4') = 4.8$, $^3J(3'_{eq},2') = 2.8$, $^4J(3'_{eq},1'_{eq}) = 2.8$, $H_{eq}-C(N3')$); 3.66 (*dt*, $^3J(5',4') = 9.2$, $^3J(5',6') = 3.1$, H–C(N5′)); 3.95 (*ddd*, $^3J(4',3'_{ax}) = 11.5$, $^3J(4',5') = 9.2$, $^3J(4',3'_{eq}) = 4.8$, H–C(N4′)); 4.22 (*dd*, $^2J(1'_{ax},1'_{eq}) = 14.4$, $^3J(1'_{ax},2') = 3.1$, $H_{ax}-C(N1')$); 4.24 (*dd*, $^2J(5'a,5'b) = 12.1$, $^3J(5'a,4') = 3.1$, $H_a-C(A5')$); 4.24–4.30 (*m*, CH_2(N6′)); 4.28 (*dd*, $^2J(5'b,5'a) = 12.1$, $^3J(5'b,4') = 3.1$, $H_b-C(A5')$); 4.40 (*ddd*, $^3J(4',3') = {}^3J(4',5'a) = {}^3J(4',5'b) = 3.1$, $^4J(4',P) = 1.9$, H–C(A4′)); 4.53 (*dd*, $^3J(3',2') = 4.7$, $^3J(3',4') = 3.1$, H–C(A3′)); 4.57 (*ddd*, $^2J(1'_{eq},1'_{ax}) = 14.4$, $^4J(1'_{eq},3'_{eq}) = 2.8$, $^3J(1'_{eq},2') = 2.6$, $H_{eq}-C(N1')$); 4.74 (*dd*, $^3J(2',1') = 5.4$, $^3J(2',3') = 4.7$, H–C(A2′)); 5.20 (*dddd*, $^3J(2',3'_{ax}) = 5.1$, $^3J(2',1'_{ax}) = 3.1$, $^3J(2',3'_{eq}) = 2.8$, $^3J(2',1'_{eq}) = 2.6$, H–C(N2′)); 6.14 (*d*, $^3J(1',2') = 5.4$, H–C(A1′)); 8.25 (*dd*, $^3J(5,4) = 8.3$, $^3J(5,6) = 6.1$, H–C(N5)); 8.40 (*s*, H–C(A2)); 8.60 (*s*, H–C(A8)); 8.89 (*dd*, $^3J(4,5) = 8.3$, $^4J(4,2) = 1.5$, H–C(N4)); 9.37 (*d*, $^3J(6,5) = 6.1$, $^4J(6,2) = 1.5$, H–C(N6)); 9.45 (*t*, $^4J(2,4) = {}^4J(2,6) = 1.5$, H–C(N-2)). ^{13}C-NMR (125 MHz, D_2O): 38.4 (NC(3′)); 62.6 (NC(4′)); 67.5 (*dd*, $^2J(6',P) = 4.9$, $^4J(6',P) = 2.8$, NC(6′)); 67.9 (*dd*, $^2J(5',P) = 3.7$, $^4J(5',P) = 2.8$, AC(5′); 69.6 (NC(2′)); 69.8 (NC(1′)); 73.0 (AC(3′)); 77.4 (AC(2′)); 83.2 (*d*, $^3J(5',P) = 7.9$, NC(5′)); 86.9 (*d*, $^3J(4',P) = 8.7$, AC(4′)); 90.5 (AC(1′)); 121.20 (AC(5)); 131.5 (NC(5)); 136.8 (NC(3)); 144.7 (AC(8)); 146.7 (NC(2); 147.0 (NC(4)); 148.7 (NC(6)); 149.2 (AC(4)); 151.1 (AC(2)); 153.4 (AC(6)); 168.5 ($CONH_2$). ^{31}P-NMR (202 MHz, D_2O): −10.7 (*d*, $^2J(P,P) = 20.5$, A–P); −10.3 (*d*, $^2J(P,P) = 20.5$, N–P). ESI-Q-TOF-MS: 660.1229 ($[M-2H]^+$, $C_{22}H_{28}N_7O_{13}P_2^+$; calc. 660.1220).

*1,5-Anhydro-2-azido-4,6-*O*-benzylidene-2-deoxy-*D*-altritol* (**10**). To a soln. of *1,5 : 2,3-anhydro-4,6-*O*-benzylidene-*D*-allitol* [9] (**9**; 1.000 g, 4.27 mmol) in a mixture of 2-methoxyethanol/H_2O 5:1 (240 ml) were added NaN_3 (1.500 g, 23.07 mmol) and NH_4Cl (1.500 g, 28.04 mmol), and the mixture was stirred at 100° for 18 h under N_2. After evaporation, the residue was treated with warm $CHCl_3$ (100 ml) and CH_2Cl_2 (100 ml), and extracts were filtered over a short path of silica gel to remove inorganic salts. The solvents were removed *in vacuo* to yield pure **10** (1.126 g, 95%). Pale yellow oil. R_f (CH_2Cl_2/MeOH 98:2) 0.7. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.46 (*d*, $^3J = 1.5$, OH); 3.68–3.94 (*m*, 5 H); 4.05 (*dd*, $J = 13$, 2, H–C); 4.13 (br. *s*, H–C); 4.28–4.36 (*m*, H–C); 5.65 (*s*, PhC*H*); 7.35–7.55 (*m*, 5 arom. H). ^{13}C-NMR: see *Table 2*. ESI-MS (pos.): 278.0 ($[M+H]^+$), 300.0 ($[M+Na]^+$).

*1,5-Anhydro-2-azido-2-deoxy-*D*-altritol* (**11**). A soln. of **10** (1.116 g, 4.02 mmol) in 80% AcOH (60 ml) was heated at 95° for 2 h under N_2. After evaporation and co-evaporation with H_2O, then with toluene, the residue was purified by silica gel CC (30–90% AcOEt in hexane). The obtained colorless solid was washed with $CHCl_3$ and dried *in vacuo* over P_4O_{10}: **11** (0.505 g, 66%). R_f (hexane/AcOEt 1:2) 0.1. ^{1}H-NMR (500 MHz, (D_6)DMSO): 3.38–3.45 (*m*, H–C(4), H–C(5), $H_a-C(6)$); 3.61–3.65 (*m*, $H_b-C(6)$, $H_{eq}-C(2)$); 3.66 (*d*, $^2J(1_{ax},1_{eq}) = 12.2$, $H_{ax}-C(1)$); 3.72 (br. *s*, $\Delta\nu_{1/2} = 10$, $H_{eq}-C(3)$); 3.75 (*dd*, $^2J(1_{eq},1_{ax}) = 12.2$, $^3J(1_{eq},2) = 1.5$, $H_{eq}-C(1)$); 4.46 (br. *t*, HO–C(6)); 4.69 (*d*, $J(4,OH) = 4.6$, HO–C(4)); 5.21 (*d*, $J(3,OH) = 4.1$, HO–C(3)). ^{13}C-NMR (125 MHz, (D_6)DMSO): 61.2 (C(2)); 61.5 (C(6)); 63.1 (C(1)); 65.8 (C(4)); 68.2 (C(3)); 77.2 (C(5)); for other solvents, see also *Table 2*.

*1,5-Anhydro-2-azido-2-deoxy-6-*O*-phosphono-*D*-altritol Diammonium Salt* (**12**). Compound **12** was obtained in an analogous way as phosphate **5**. Azide **11** (0.503 g, 2.66 mmol) was dissolved under N_2 in 3 ml of $(MeO)_3PO$, cooled in an ice bath, and triturated with 1.7 ml of a 1:1 (*v*/*v*) mixture of $POCl_3$ and $(MeO)_3PO$. After 3 h of stirring at 0°, the reaction was quenched by addition of 3 ml of ice-water and 4 ml of cold Et_3N. The resulting mixture was evaporated *in vacuo* to dryness, and the residue was washed with $(i\text{-}Pr)_2O$ and purified by silica gel CC (0.35% NH_4OH (20% soln. in H_2O) in i-PrOH) to give **12** (0.353 g, 46%). Pale yellow oil. R_f (i-PrOH/25% NH_4OH_{aq}/H_2O 6:4:1) 0.4. This compound was used directly in the next step. ^{13}C-NMR: see *Table 2*.

*1,5-Anhydro-2-amino-2-deoxy-6-*O*-phosphono-*D*-altritol Sodium Salt* (**13**). Azide **12** (0.353 g, 1.31 mmol) was dissolved in 10 ml of H_2O and 25 ml of MeOH. *Adams* catalyst ($PtO_2 \cdot H_2O$, 0.069 g, 0.28 mmol) was added, and the mixture was shaken for 85 h in a *Parr* hydrogenation apparatus (30 psi).

Table 2. *^{13}C-NMR Chemical Shifts* [ppm] *and Assignments for Compounds* **10**–**14**

	10 ($CDCl_3$)	**11** ($CDCl_3$)	**11** (D_2O)	**12** (D_2O)	**13** (D_2O)	**14** (D_2O)[a]
C(1)	64.9	63.9	65.7	65.9	65.0	66
C(2)	60.6	60.6	63.0	63.0	53.6	72.7
C(3)	66.9 or 66.7	68.6	70.1	70.1	68.6	71.6
C(4)	76.9	66.0	66.9	66.4	65.4	67.8
C(5) (^3J(C,P))	66.9 or 66.7	74.8	78.4	77.5 (7.6)	78.2 (7.6)	79.7 (7.6)
C(6) (^2J(C,P))	68.9	63.3	63.2	66.1 (4.6)	65.6 (3.5)	65.3 (3.0)
Ph*C*	102.1					
C_{ipso}	137.1					
C_o	126.2					
C_m	128.4					
C_p	129.4					
C(2) (py)						146.4
C(3) (py)						136.4
C(4) (py)						147.3
C(5) (py)						131.2
C(6) (py)						148.6
C=O						168.0

[a]) Position numbers of the altritol part are primed in the text.

After removal of the catalyst, the filtrate was concentrated to dryness, dissolved in H_2O, and applied to a column of *Dowex 50X8-200* (Na^+) ion-exchange resin with H_2O as the eluent. Evaporation and keeping the residue in a vacuum dessicator over P_4O_{10} gave **13** (0.342 g, 91%). Pale yellow amorphous powder. R_f (i-PrOH/25% NH_4OH_{aq}/H_2O 6:4:1) 0.25. ^{1}H-NMR (500 MHz, D_2O): 3.47–3.51 (*m*, H–C(2)); 3.72 (*dt*, ^3J(5,4) = 9.1, ^3J(5,6) = 3.4, H–C(5)); 3.82 (*d*, $^2J(1_{ax},1_{eq}) = 13.2$, H_{ax}–C(1)); 3.86 (*dd*, ^3J(4,5) = 9.1, ^3J(4,3) = 3.2, H–C(4)); 3.94 (*dd*, J(6,5) = 3.4, J(6,P) = 6.0, CH_2(6)); 3.97 (*dd*, $^2J(1_{eq},1_{ax}) = 13.2$, $^3J(1_{eq},2) = 1.7$, H_{eq}–C(1)); 4.20 (*dd*, ^3J(3,2) = ^3J(3,4) = 3.2, H–C(3)). ^{13}C-NMR (125 MHz, D_2O): 53.6 (CH, C(2)); 65.0 (CH_2, C(1)); 65.4 (CH, C(4)); 65.6 (CH_2, C(6)); 68.6 (CH, C(3)); 78.2 (*d*, ^3J(5,P) = 7.6, CH, C(5)); see also *Table 2*. ^{31}P-NMR (202 MHz, D_2O): 3.92. Anal. calc. for $C_6H_{13}O_7NNa$ (234.162): C 30.78, H 5.60, N 5.98; found: C 30.93, H 5.41, N 5.74.

*1,5-Anhydro-2-(3-carbamoylpyridinium)-2-deoxy-6-*O*-phosphono-*D*-altritol* (**14**) was prepared in 42% yield analogously to **7** from **13** (0.100 g, 0.35 mmol, soln. in 4 ml of MeOH and 5 ml of H_2O), NDC (0.201 g, 0.62 mmol, soln. in 2.1 ml of H_2O) and 0.6 ml of 0.5M aq. TEAB. R_f (i-PrOH/25% NH_4OH_{aq}/H_2O 6:4:1) 0.15. ^{1}H-NMR (200 MHz, D_2O): 4.02–4.23 (*m*, 4 H); 4.40 (*dd*, $^2J(1'_{ax},1'_{eq}) = 13$, $^3J(1'_{ax},2') = 4$, H_{ax}–C(1')); 4.54 (*dd*, $^2J(1'_{ax},1'_{eq}) = 13$, $^3J(1'_{eq},2') = 4$, H_{eq}–C(1')); 4.60 (*dd*, ^3J(2',3') = 6.5, ^3J(3',4') = 2, H–C(3')); 5.04 (*ddd*, ^3J(2',3') = 6.5, $^3J(1'_{ax},2') = {}^3J(1'_{eq},2') = 4$, H–C(2')); 8.30 (*dd*, ^3J(4,5) = 8, ^3J(5,6) = 7, H–C(5)); 8.99 (*d*, ^3J(4,5) = 8, H–C(4)); 9.37 (*d*, ^3J(5,6) = 7, H–C(6)); 9.51 (*s*, H–C(2)). ^{13}C-NMR: see *Table 2*.

Altritol Nicotinamide Adenine Dinucleotide Lithium Salt **15**. Nucleotide **14** (0.051 g, 0.15 mmol) was dissolved in 2.5 ml of formamide (*p.a.* grade). Pyridine (1 ml; distilled over BaO immediately prior to use) was added and evaporated *in vacuo* to remove the traces of moisture. The procedure was repeated three times. Adenosine 5'-(phosphoric dibutylphosphinothioic anhydride) (0.155 g, 0.30 mmol) was added, and the resulting mixture was co-evaporated with pyridine three times. The final residue was dissolved in 2 ml of pyridine. The flask was wrapped in aluminum foil for protection from light, and $AgNO_3$ (0.200 g, 1.18 mmol, dried at 120° for 2 h) was added in one portion. The flask was immediately capped with a septum, charged with N_2, and the mixture was stirred at r.t. for 65 h. Separation of the dinucleotide was performed as described for compound **8**. Lithium salt **15** (0.039 g, 39%) was obtained as fine white powder. R_f (i-PrOH/25% NH_4OH_{aq}/H_2O 6:4:1) 0.4. UV (H_2O): 259. ^{1}H-NMR (500 MHz,

D_2O): 4.06–4.13 (*m*, H–C(N5′)); 4.09–4.11 (*m*, H–C(N4′)); 4.24 (*dd*, ^2J(5′a,5′b) = 11.7, ^3J(5′a,4′) = 3.3, H_a–C(A5′)); 4.27 (*dd*, ^2J(5′b,5′a) = 11.7, ^3J(5′b,4′) = 2.6, H_b–C(A5′)); 4.27–4.31 (*m*, CH_2(N6′)); 4.36 (*dd*, $^2J(1'_{ax},1'_{eq})$ = 13.2, $^3J(1'_{ax},2')$ = 4.1, H_{ax}–C(N1′)); 4.39–4.43 (*m*, H–C(A4′)); 4.46 (*dd*, $^2J(1'_{eq},1'_{ax})$ = 13.2, $^3J(1'_{eq},2')$ = 4.6, H_{eq}–C(N1′)); 4.53 (*dd*, ^3J(3′,4′) = 5.8, ^3J(3′,2′) = 2.7, H–C(N3′)); 4.53 (*dd*, ^3J(3′,2′) = 5.1, ^3J(3′,4′) = 3.2, H–C(A3′)); 4.77 (*dd*, ^3J(2′,1′) = 5.7, ^3J(2′,3′) = 5.1 , H–C(A2′)); 5.02 (*ddd*, $^3J(2',1'_{eq})$ = 4.6, $^3J(2',1'_{ax})$ = 4.1, ^3J(2′,3′) = 2.7, H–C(N2′)); 6.13 (*d*, ^3J(1′,2′) = 5.7, H–C(A1′)); 8.25 (*dd*, ^3J(5,4) = 8.3, ^3J(5,6) = 6.0, H–C(N5)); 8.31 (*s*, H–C(A2)); 8.53 (*s*, H–C(A8)); 8.91 (*dd*, ^3J(4,5) = 8.3, ^4J(4,2) = 1.5, H–C(N4)); 9.30 (*dd*, ^3J(6,5) = 6.0, ^4J(6,2) = 1.5, H–C(N6)); 9.44 (*t*, ^4J(2,4) = ^4J(2,6) = 1.5, H–C(N2)). ^{13}C-NMR (125 MHz, D_2O): 66.4 (NC(1′)); 67.7 (*d*, ^2J(6′,P) = 5.0, NC(6′)); 67.9 (*d*, ^2J(5′,P) = 6.4, AC(5′)); 68.0 (NC(4′)); 72.1 (NC(3′)); 73.1 (NC(2′)); 73.2 (AC(3′)); 77.1 (AC(2′)); 79.4 (*d*, ^3J(5′,P) = 6.9, NC(5′)); 86.7 (*d*, ^3J(4′,P) = 7.5, AC(4′)); 89.9 (AC(1′)); 121.2 (AC(5)); 131.6 (NC(5)); 136.8 (NC(3)); 144.3 (AC(8)); 146.8 (NC(2)); 147.7 (NC(4)); 148.9 (NC(6)); 151.2 (AC(4)); 151.5 (AC(2)); 153.4 (AC(6)); 165.7 ($CONH_2$). ^{31}P-NMR (202 MHz, D_2O): −10.68. ESI-MS (neg.): 676 ($[M-2H]^-$), 698 ($[M-3H+Na]^-$). ESI-MS (pos.): 678 (M^+), 700 ($[M-H+Na]^+$). ESI-Q-TOF-MS: 676.1147 ($[M-2H]^+$, $C_{22}H_{28}N_7O_{14}P_2^+$; calc. 676.1169).

N-*[(4a*R,*7*S,*8a,*S*)-4a,7,8,8a-Tetrahydro-2-phenyl-4*H*-1,3-benzodioxin-7-yl]phtalimide* (**17**). A soln. of diisopropyl azodicarboxylate (DIAD; 5.3 ml, 25.52 ml) in dry THF (50 ml), was slowly added to a stirred suspension of Ph_3P (7 g, 26.68 mmol) [10–11], **16** (4.2 g, 18.08 mmol) and phtalimide (4 g, 27.18 mmol) in dry THF (170 ml) at r.t. under N_2. After 1 h, the solvent was removed under reduced pressure, and the crude material was purified by CC using a gradient of AcOEt/hexane (4 : 6, 5 : 5, 6 : 4) to afford **17** (5.23 g, 80%). White solid. ^{1}H-NMR (500 MHz, $CDCl_3$): 2.14–2.18 (*m*, 1 H, CH_2(8)); 2.26–2.32 (*m*, 1 H, CH_2(8)); 2.50–2.53 (*m*, H–C(4a)); 3.86 (*d*, ^2J = 11.1, 1 H, CH_2(4)); 4.36 (*dd*, *J* = 10.7, 4.4, 1 H, CH_2(4)); 4.44–4.49 (*m*, H–C(8a)); 5.08–5.12 (*m*, H–C(7)); 5.65–5.70 (*m*, H–C(5), H–C(6), PhC*H*); 7.34–7.83 (*m*, 9 arom. H). ^{13}C-NMR: see *Table 3*. ESI-MS: 362 ($[M+H]^+$), 384 ($[M+Na]^+$).

Table 3. *^{13}C-NMR Chemical Shifts* [ppm] *and Assignments for Compounds* **17**–**21**

	17[a]) ($CDCl_3$)	**18** (DMSO)	**20** (D_2O)	**21** (D_2O)[b])
C(1)	45.0	45.3	48.1	69.8
C(2)	126.1	127.5	126.4	125.2
C(3)	134.1	134.4	135.5	139.6
C(4) (^3J(C,P))	39.1	44.0	46.2 (7.5)	46.2 (7.5)
C(5)	75.9	64.6	67.5	66.3
C(6)	34.4	30.4	34.2	38.6
C(7) (^2J(C,P))	70.6	62.8	66.6 (3.4)	67.4 (3.2)
Ph*C*	102.1			
arom. C (phth)	123.2, 126.4, 127.0, 128.2, 128.8, 131.7, 138.5	122.9, 128.1, 131.6		
C(2) (py)				146.2
C(3) (py)				136.5
C(4) (py)				147.3
C(5) (py)				131.3
C(6) (py)				148.2
C=O	168.0	167.7		164.0

[a]) Arbitrary numbering. [b]) Position numbers of the 4-(phosphonomethyl)cyclohex-2-enyl part are primed in the text.

N-*[(1*S,*4*R,*5*S*)-5-Hydroxy-4-(hydroxymethyl)cyclohex-2-en-1-yl]phtalimide* (**18**). A suspension of **17** in an 80% soln. of AcOH was heated at 95°. When the entire solid disappeared (no more starting material on TLC), the volatiles were removed and co-evaporated with H_2O to give **18**, after

chromatography. ^{1}H-NMR (500 MHz, (D_6)DMSO): 1.74–1.78 (*m*, H–C(4)); 2.14–2.18 (*m*, CH_2(6)); 3.39–3.43 (*m*, CH_2(7)); 4.02–4.06 (*m*, H–C(5)); 4.69 (br. *s*, HO–C(7)); 4.76 (*d*, *J* = 3.5, HO–C(5)); 4.93–4.96 (*m*, H–C(1)); 5.61–5.65 (*m*, H–C(3)); 5.65–5.69 (*m*, H–C(2)); 7.80–7.86 (*m*, 4 arom. H). ^{13}C-NMR: see *Table 3*. ESI-MS ($C_{15}H_{15}NO_4$): 274 ($[M+H]^+$), 296 ($[M+Na]^+$).

*(1*S*,4*R*,5*S*)-1-Amino-5-hydroxy-4-*O*-(phosphonomethyl)cyclohexene Ammonium Salt* (**20**). To a suspension of **18** (3 g, 10.98 mmol) in 25 ml of $(MeO)_3PO$ at 0° was added $POCl_3$ (0.8 ml) in one portion. The mixture was stirred for 5 h at 0° until the mixture became homogenous. The reaction was quenched with 250 ml of cold TEAB and stirred for 30 min. The volatiles were removed under reduced pressure, and the crude mixture was adsorbed on a silica column and eluted using a gradient of CH_2Cl_2/MeOH (100:0, 95:5, 90:10, 80:20 and 50:50). The desired fractions were evaporated to yield the crude phosphate **19** as a white solid (2.8 g, 60%).

The crude **19** (0.34 g, 0.6 mmol) was dissolved in 22 ml of EtOH and NH_2NH_2 (0.06 ml, 1.23 mmol) was added. The mixture was heated at 95° overnight. EtOH was evaporated, and the residue was dissolved in H_2O and washed with AcOEt. The aq. layers were collected and evaporated under reduce pressure. The residue was purified using an ion exchange-resin (*Dowex 400X* (Na^+)) CC packed with i-PrOH and eluted with i-PrOH/NH_4OH/H_2O (9:1:0 → 6:3:1). Evaporation gave **20** (0.07 g, 43%). White solid. ^{1}H-NMR (500 MHz, D_2O): 2.03 (*ddd*, *J*(6a,6b) = 13.9, *J*(6a,5) = 6.6, *J*(6a,1) = 3.2, H_a–C(6)); 2.15 (*ddd*, *J*(6b,6a) = 13.9, *J*(6b,5) = 8.1, *J*(6b,1) = 5.8, H_b–C(6)); 2.41–2.45 (*m*, H–C(4)); 3.86 (*ddd*, *J*(7a,7b) = 10.2, *J*(7a,5) = *J*(7a,P) = 5.6, H_a–C(7)); 3.98 (*ddd*, *J*(7b,7a) = 10.2, *J*(7b,5) = *J*(7b,P) = 5.1, H_b–C(7)); 4.01–4.05 (*m*, H–C(1)); 4.15 (*ddd*, *J*(5,6b) = 8.2, *J*(5,6a) = 6.6, *J*(5,4) = 5.1, H–C(5)); 5.86 (*ddd*, *J*(3,2) = 10.2, *J*(3,4) = *J*(3,1) = 2.5, H–C(3)); 6.00 (*dm*, *J*(2,3) = 10.2, H–C(2)). ^{13}C-NMR: see *Table 3*. ^{31}P-NMR (202 MHz, D_2O): 1.95 (*s*). ESI-Q-TOF-MS: 222.0523 ($C_7H_{13}NO_5P^+$; calc. 222.1477).

*(1*S*,4*R*,5*S*)-1-(Carbamoylpyridinium)-5-hydroxy-4-(phosphonomethyl)cyclohex-2-ene Triethylammonium Salt* (**21**). Compound **20** (0.07 g, 0.26 mmol) was dissolved in a mixture of MeOH (4.5 ml) and dry EtN(i-Pr)$_2$ (0.09 ml, 0.52 mmol). The mixture was stirred for 30 min. at r.t. under N_2. The *Zincke* salt (0.1 g, 0.28 mmol) was added in one portion. A deep red color was formed immediately. The mixture was then heated at 55° for 4 h and overnight at r.t. After one night, the starting material was not totally disappeared, and another 0.03 g of the tetrafluoroborate salt was added. The mixture was stirred for an additional 2 h. The reaction was quenched with 50 ml of 1M TEAB soln., and the volatiles were removed. The crude material was purified over *Dowex 50X8-200* ion-exchange column (Na^+) and eluted with H_2O. After evaporation of the desired fractions, the residue was dissolved in H_2O and purified with HPLC using a column of DEAE *Sephadex A-25* and an eluting system of 0.01M TEAB → 0.5M TEAB. Evaporation of the pure fractions gave **21** (0.09 g, 92%). White powder. ^{1}H-NMR (500 MHz, D_2O): 2.31–2.35 (*ddd*, *J*(6′a,6′b) = 13.8, *J*(6′a,5′) = 6.6, *J*(6′a,1′) = 3.1, H_a–C(6′)); 2.48–2.54 (*dm*, *J*(6′b,6′a) = 13.8, H_b–C(6′)); 2.57–2.61 (*m*, H–C(4′)); 4.05–4.11 (*m*, CH_2(7′), H–C(5′)); 5.65–5.69 (*m*, H–C(1′)); 6.03–6.07 (*m*, H–C(3′)); 6.41 (*dm*, *J*(2′,3′) = 11.5, H–C(2′)); 8.22 (*dd*, *J*(5,4) = 8, *J*(5,6) = 6, H–C(5)); 8.92 (*d*, *J*(4,5) = 8, H–C(4)); 9.12 (*d*, *J*(6,5) = 6.1, H–C(6)); 9.35 (*s*, H–C(2)). ^{13}C-NMR: see *Table 3*. ^{31}P-NMR (202 MHz, D_2O): 1.02 (*s*). ESI-Q-TOF-MS: 327.0739 ($C_{13}H_{16}N_2O_6P^+$; calc. 327.2514).

Cyclohexenyl Nicotinamide Adenine Dinucleotide Triethylammonium Salt **22**. To a mixture of **21** (0.1 g, 0.23 mmol) and adenosine 5′-(phosphoric dibutylphosphinothioic anhydride) (0.24 g, 0.4 mmol) were added 2 ml of DMF and 4 ml of dry pyridine, and the solvents were removed under reduced pressure. This procedure was repeated two times. The residue was dried on an oil pump and then dissolved under N_2 in a mixture of DMF/pyridine 1:1. The mixture was stirred for 20 min at r.t. The flask was wrapped with an aluminium foil to protect from light. $AgNO_3$ (0.32 g, 1.87 mmol) was added, and the mixture was stirred overnight at r.t. 30 ml of H_2O was added, and H_2S was bubbled into the mixture for 20 min. The black precipitate was filtered, and the filtrate was extracted 3 times with $CHCl_3$. Combined org. extracts were washed with H_2O, and the aq. layers were concentrated under reduced pressure (*T*(bath) < 25°). The product was purified by HPLC on *Sephadex-A25* cellulose using 0.01M TEAB → 0.5M TEAB in 280 min. The desired fractions were collected and lyophilized to give **22** as a solid TEA salt (0.018 g, 12%). ^{1}H-NMR (500 MHz, D_2O): 2.24 (*ddd*, *J*(N6′a,N6′b) = 14, *J*(N6′a,N5′) = 8.3, *J*(N6′a,N1′) = 6, H_a–C(N6′)); 2.4 (*ddd*, *J*(N6′b,N6′a) = 14.2, *J*(N6′b,N1′) = 6.2, *J*(N6′b,N5′) = 3.1,

H_b–C(N6′)); 2.47 (*ddddd*, *J*(N4′,N5′) = 5.5, *J*(N4′,N7′a) = 4.5, *J*(N4′,N7b′) = 3.8, *J*(N4′,N3′) = 3.1, *J*(N4′,N2′) = 2.8, H–C(N4′)); 4.04 (*ddd*, *J*(N5′,N6′a) = 8.3, *J*(N5′,N4′) = 5.5, *J*(N5′,N6′b) = 3.1, H–C(N5′)); 4.09–4.16 (*dAB*, *J*(N7′a,N7′b) = 10.6, *J*(N7′a,N4′) = 4.5, *J*(N7′b,N4′) = 3.8, H_a–C(N7′), H_b–C(N7′)); 4.20–4.23 (*dAB*, *J*(A5′a,A5′b) = 11.9, *J*(A5′a,A4′) = 3.1, *J*(A5′b,A4′) = 3.5, H_a–C(A5′), H_b–C(A5′)); 4.36 (*ddd*, *J*(A4′,A3′) = 3.2, *J*(A4′,A5′a) = 3.1, *J*(A4′,A5′b) = 3.5, H–C(A4′)); 4.49 (*dd*, *J*(A3′,A2′) = 5.6, *J*(A3′,A4′) = 3.2, H–C(A3′)); 4.75 (*dd*, *J*(A2′,A1′) = 5.9, *J*(A2′,A3′) = 5.6, H–C(A2′)); 5.55 (*dddd*, *J*(N1′,N6′b) = 6.2, *J*(N1′,N6′a) = 6.0, *J*(N1′,N2′) = 2.8, *J*(N1′,N3′) = 1.6, H–C(N1′)); 5.89 (*ddd*, *J*(N2′,N3′) = 10.2, *J*(N2′,N4′) = *J*(N2′,N1′) = 2.8 , H–C(N2′)); 6.05 (*d*, *J*(A1′,A2′) = 5.9, H–C(A1′)); 6.28 (*ddd*, *J*(N3′,N2′) = 10.2, *J*(N3′,N4′) = 3.1, *J*(N3′,N1′) = 1.6, H–C(N3′); 8.14 (*dd*, *J*(N5,N4) = 8.1, *J*(N5,N6) = 6.2, H–C(N5)); 8.15 (*s*, H–C(A2)); 8.43 (*s*, H–C(A8)); 8.78 (*dd*, *J*(N4,N5) = 8.1, *J*(N4,N2) = 1.5, H–C(N4)); 8.99 (*dd*, *J*(N6,N5) = 6.2, *J*(N6,N2) = 1.5, H–C(N6)); 9.26 (*dd*, *J*(N2,N4) = 1.5, *J*(N2,N6) = 1.5, H–C(N2)). ^{13}C-NMR (125 MHz, D_2O): 38.5 (NC(6′)); 46.2 (*d*, *J*(NC4′,NP) = 8.8, NC(4′)); 66.0 (NC(5′)); 68.0 (*d*, *J*(NC7′,NP) = 5.8, NC(7′)); 68.2 (*d*, *J*(AC5′,AP) = 4.8, AC(5′)); 69.8 (NC(1′)); 73.1 (AC(3′)); 76.7 (AC(2′)); 86.5 (*d*, *J*(AC4′,AP) = 8.7, AC(4′)); 89.4 (AC(1′)); 121.1 (AC(5)); 125.0 (NC(2′)); 131.2 (NC(5)); 136.4 (NC(3)); 139.6 (NC(3′)); 142.4 (AC(8)); 146.0 (NC(2)); 147.1 (NC(4)); 148.0 (NC(6)); 151.7 (AC(4)); 155.6 (AC(2)); 158.1 (AC(6)); 168.7 ($CONH_2$). ^{31}P-NMR (202 MHz, D_2O): –10.9 (*d*, *J*(NP,AP) = 21.5, NP); –10.6 (*d*, *J*(NP,AP) = 21.5, AP). ESI-Q-TOF-MS: 657.1313 ($C_{23}H_{29}N_7O_{12}P_2^+$; calc. 657.471).

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