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Synthesis of 3'-*C*-branched 1',5'-anhydromannitol Nucleosides as new Antiherpes Agents.

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Abstract: A series of 3'-β-*C*-branched anhydromannitol nucleosides were conveniently synthesized starting from commercially available D-ribose. The reaction sequences were: (i) conversion of the protected pentofuranose to the corresponding nitrohexopyranose; (ii) addition of the conjugated base of the nitrosugar to formaldehyde; (iii) removal of the nitro group by n-tributyltin hydride treatment and (iv) Mitsunobu type alkylation to introduce the nucleobase. The conformation of intermediates and final compounds were deduced from NMR analysis. The thymine congener showed potent activity against herpes simplex virus (HSV).

INTRODUCTION

Modification of the carbohydrate moiety of nucleosides has been the most successful strategy to discover antiviral nucleosides. In comparison to the efforts made to discover antiviral nucleosides with acyclic and cyclic four- and five-membered sugar moieties, very little research has been performed in the field of pyranose and hexitol nucleosides.[1] This is due to the fact that six-membered sugar rings are less flexible than five-membered rings and to the general belief that conformational flexibility is essential for an antiviral nucleoside. Indeed, the modified nucleoside itself is a prodrug and has to be converted to mono-, di- and triphosphate before being presented at its final target: the DNA polymerase. The conformation of the nucleoside at the level of the different activating enzymes may be different from each other, moreover, this conformation may change during the phosphorylation processes. However, it has recently been demonstrated that several nucleoside analogues with a six-membered carbohydrate moiety may exhibit activity against herpes simplex virus (HSV)[2-4] or human immunodeficiency virus (HIV).[1] By synthesizing the carbocyclic analogues of these nucleosides,[5] we became aware of the conformational requirements of these nucleoside analogues for antiviral activity: the active nucleosides have an axially oriented base moiety, while the inactive congeners have an equatorially oriented base moiety. To further explore the structural, stereochemical and conformational requirements which drive these nucleoside analogues to their biologically active form, we attempted the synthesis and investigated the properties of the 3'-*C* branched 1',5'-anhydrohexitol nucleosides in the 3'-(*S*) configuration. We demonstrated previously[4] that substitution of the 3'-up position with an hydroxyl function leads to retention of the biological activity spectrum, although with reduced potency. Introduction of an hydroxymethyl group may

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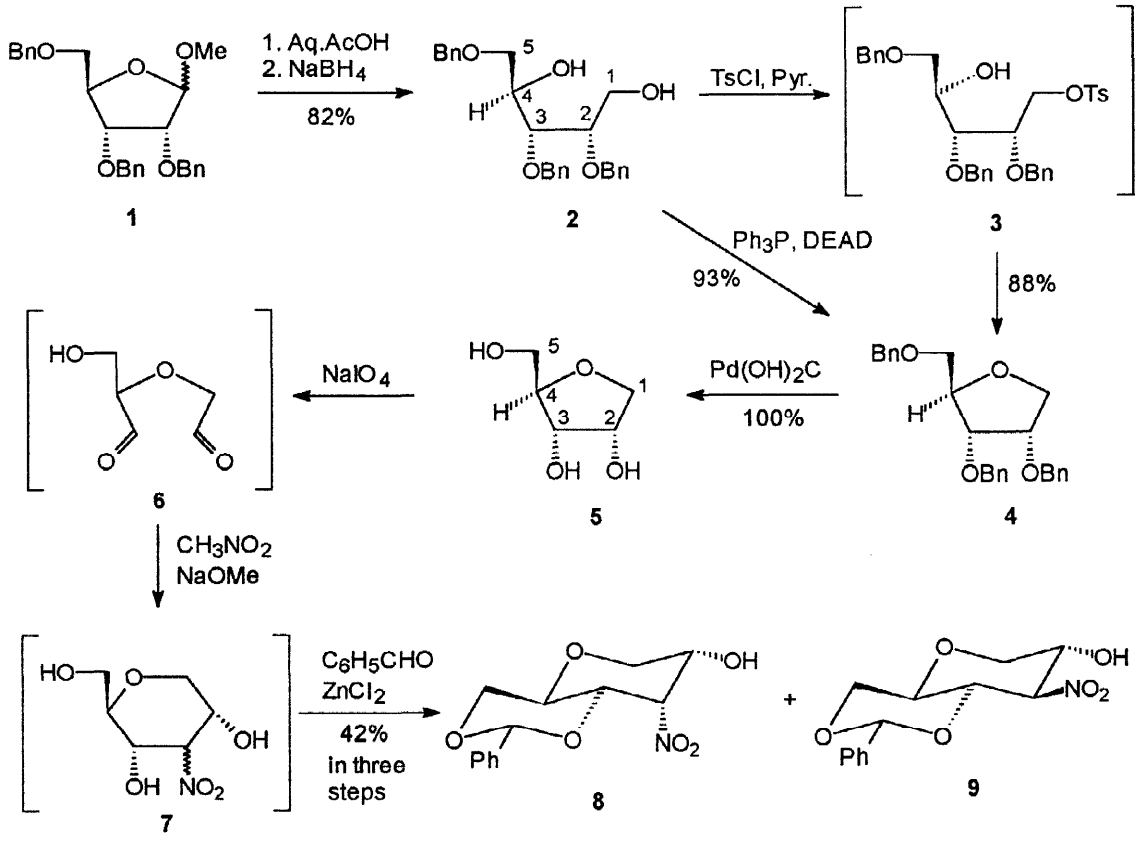
give us information about the available substitutional space at the 3'-position. As a 3'-(*S*)-hydroxymethyl group may occupy an equatorial position, we did not expect conformational alterations of the original anhydrohexitol nucleoside.

Introduction of branching at the carbon atom next to the heterocyclic base in a *cis*-configuration with regard to the base moiety is not an easy undertaking. To find synthetic methodology that provides smooth access to such nucleoside analogues, we decided to start from commercially available D-ribose and transform, in the first step, the furanose to a nitrohexitol. In the second step, we exploited the unique feature of a nitro group[6] for *C-C* bond formation reaction at its α-carbon. After formation of the *C*-branched nitrohexitol, the nitro group can easily be removed by n-tributyltin hydride[7] reduction and the reaction product might serve as a common starting material for the synthesis of various modified anhydrohexitol nucleosides. Herein, we report on a facile synthesis of previously unknown 3'-*C*-branched anhydrohexitol nucleosides as new potent antiviral entities.

RESULTS AND DISCUSSION

Conversion of D-ribose to the corresponding 1-O-methyl-D-ribose was acheived following a literature procedure.[8] Treatment of 1-O-methyl-D-ribose with benzyl bromide in the presence of sodium hydride in DMF gave 1-O-methyl-2,3,5-tri-O-benzyl-D-ribose (**1**) (Scheme 1) in 89% yield. Compound **1** was converted into the protected reduced open chain 2,3,5-tri-O-benzyl-D-ribitol (**2**) by acid hydrolysis[8] followed by $NaBH_4$ reduction in 82% total yield. Treatment of **2** with p-toluenesulfonyl chloride in pyridine at room temperature gave **4** in 88% yield. The acyclic intermediate **3** could not be isolated due to instantaneous cyclization. Alternatively, **2** was treated with Ph_3P and diethylazodicarboxylate (DEAD)[9] in dry dioxane at room temperature to give 1,4-anhydro-2,3,5-tri-O-benzyl-D-ribitol (**4**) in 93% isolated yield. The Mitsunobu-type reaction was carried out on 8 mmol scale, but was not attempted at large scale due to the precise conditions in which reagents have to be added in order to obtain reproducible results. Previous efforts on an analogous cyclization reaction using compound **2** with an additional unprotected hydroxyl function at position 2 was less successful.[10] The benzyl protecting groups of **4** were removed by catalytic hydrogenation to give 1,4-anhydro-D-ribitol (**5**) in 100% yield. At this stage we went over from the pentitol compound to the hexitol structure by insertion of an appropriately functionalized methylene unit between C-2 and C-3, which allows a branching reaction in a later step. Thus, **5** was treated with $NaIO_4$[11] in methanol and water at 0 °C to give the intermediate **6**, which, after usual work up, was treated with conjugated base of nitromethane in methanol to yield an epimeric mixture of 1,5-anhydro-3-deoxy-3-nitro-D-allitol and glucitol (**7**). After standard work up, **7** was treated with benzaldehyde and $ZnCl_2$[12] at room temperature to give 1,5-anhydro-4,6-O-benzylidene-3-deoxy-3-nitro-D-allitol (**8**) (8%) and 1,5-anhydro-4,6-O-benzylidene-3-deoxy-3-nitro-D-glucitol **9** (34%) in a combined yield of 42% (in three

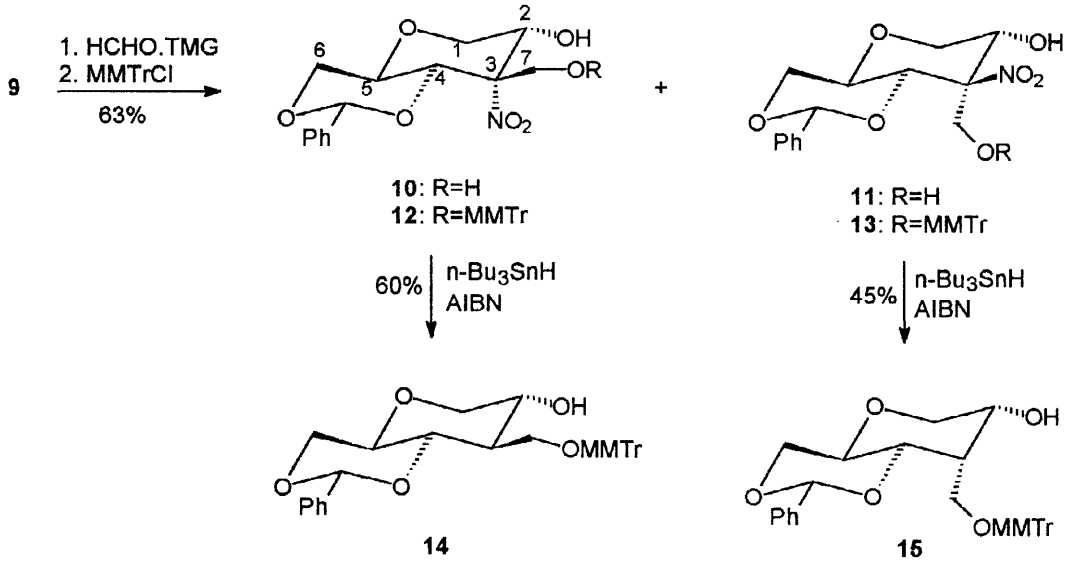
steps). Compound **8** was found to be easily isomerized to the thermodynamically more stable **9**. Thus, treatment of **8** with Et_3N in methanol at reflux temperature gave **9** (67%) and **8** (20%) after chromatographic separation.



Scheme 1

Treatment of **9** with aqueous formaldehyde in acetonitrile in the presence of a catalytic amount of *N,N*-tetramethylguanidine (TMG)[6,7c] for 30 min gave a mixture of 3-*C*-branched sugars 1,5(R)-anhydro-4(S),6-*O*-benzylidene-3-deoxy-3(S)-*C*-(hydroxymethyl)-2(R)-hydroxy-3-nitrohexitol (**10**) and 1,5(R)-anhydro-4(S),6-*O*-benzylidene-3-deoxy-3(R)-C-(hydroxymethyl)-2(R)-hydroxy-3-nitrohexitol (**11**) in 63% combined yield (Scheme 2). The separation of **10** and **11** was carried out on the protected analogues obtained after treatment with monomethoxytrityl chloride in pyridine at room temperature, giving 1,5(R)-anhydro-4(S),6-*O*-benzylidene-3-deoxy-3(S)-*C*-(MMTr-oxymethyl)-2(R)-hydroxy-3-nitrohexitol (**12**) (33%) and 1,5(R)-anhydro-4(S),6-*O*-benzylidene-3-deoxy-3(R)-*C*-(MMTr-oxymethyl)-2(R)-hydroxy-3-nitrohexitol (**13**) (47%) in a combined yield of 80%. The nitro group of **12** and **13** was substituted by a hydrogen atom using n-tributyltin hydride[13] as previously described for furanose nucleosides.[7] Thus, treatment of **12** with n-tributyltin hydride in

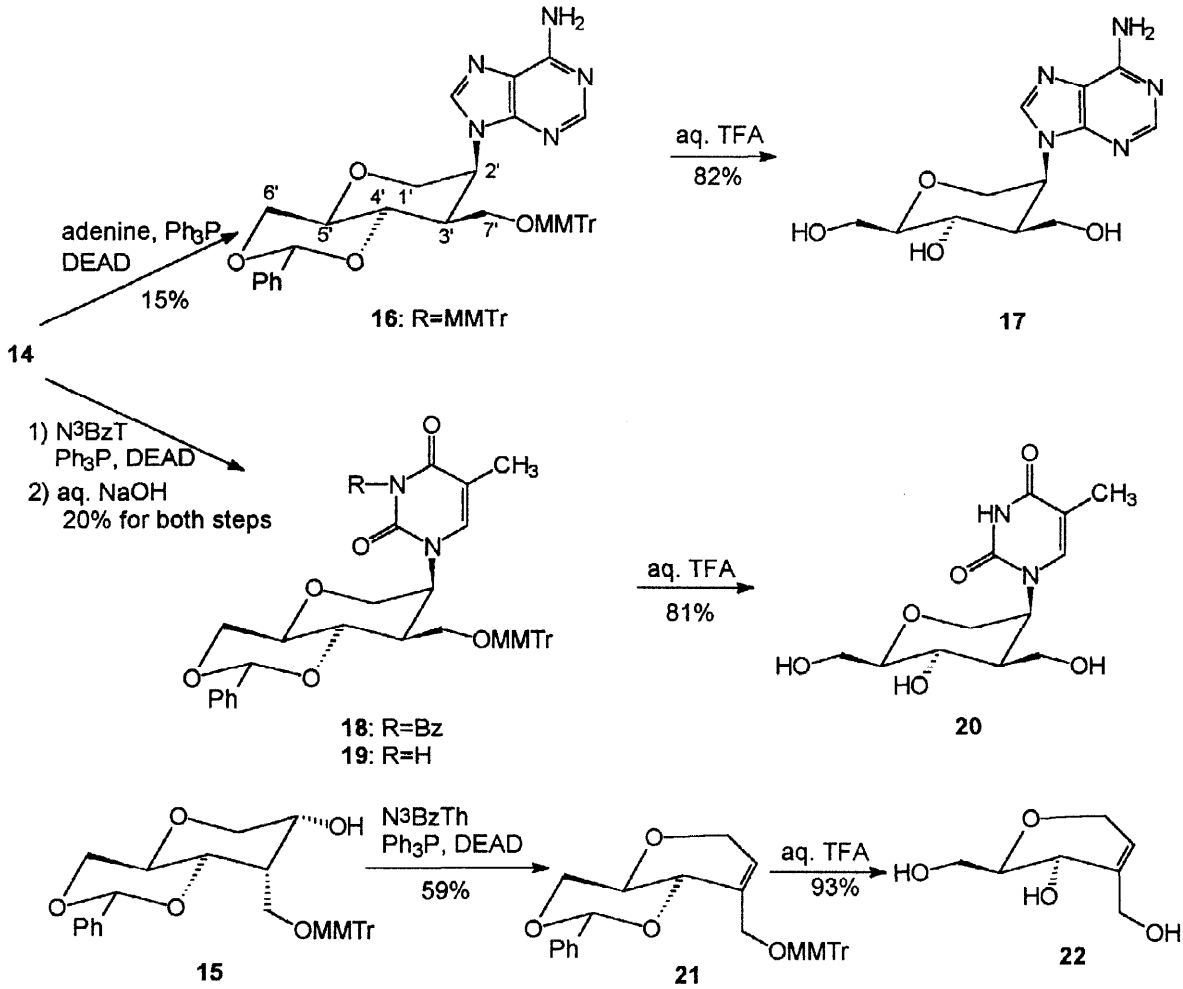
the presence of a catalytic amount of AIBN in toluene at 110 °C for 90 min gave 1,5-anhydro-4,6-*O*-benzylidene-3-deoxy-3-*C*-(MMTr-oxymethyl)-D-glucitol (**14**) in 60% isolated yield. Likewise, **13** afforded 1,5-anhydro-4,6-*O*-benzylidene-3-deoxy-3-*C*-(MMTr-oxymethyl)-D-allitol (**15**) in 45% yield. During the denitration of **12** or **13** upon treatment with tributyltin hydride, theoretically two products (*i.e* **14** and **15**) should be obtained. The C-3 radical generated from **12** or **13** can acquire a hydrogen atom from tributyltin hydride either at the α- or the β-face of the sugar moiety. In both cases, only the major isomer (i.e. **14** from **12** and **15** from **13**) was isolated. The other isomer could not be obtained in pure form due to the presence of similarly migrating side compounds. Generally, removal of a tertiary or secondary nitro group using n-tributyltin hydride is a high yielding reaction. When an alkoxy group is present in the β-position of a secondary nitro group, however, yields become lower.[7a] In the case of compounds **12** and **13**, the yield of the substitution of the tertiary nitro group by hydrogen atom is moderate due to the presence of an hydroxy group at the β-position of the nitro group. The effect of the presence of an alkoxy or hydroxy group at the β-position of a nitro group to give the corresponding denitrated product upon n-tributyltin hydride treatment needs further investigation.



Scheme 2

Compounds **14** and **15** are used as starting materials for further modifications. Treatment of **14** with Ph_3P, adenine and DEAD in dry dioxane gave 1,5-anhydro-4,6-*O*-benzylidene-2-(adenin-9-yl)-2,3-dideoxy-3-*C*-(MMTr-oxymethyl)-D-mannitol (**16**) in 15% yield (Scheme 3). The benzylidene and MMTr protecting groups in **16** were removed by treatment with 80% aqueous CF_3COOH at room temperature to give 1,5-anhydro-2-(adenin-9-yl)-2,3-dideoxy-3-*C*-hydroxymethyl-D-mannitol (**17**) in 82% isolated yield. Compound **14** was

treated with Ph_3P, N^3-benzoylthymine and DEAD in dry dioxane to give 1,5-anhydro-4,6-*O*-benzylidene-2-(N^3-benzoylthynin-1-yl)-2,3-dideoxy-3-*C*-(MMTr-oxymethyl)-D-mannitol (**18**) which was directly treated with aqueous NaOH (N) in dioxane at room temperature to give 1,5-anhydro-4,6-*O*-benzylidene-2-(thymin-1-yl)-2,3-dideoxy-3-*C*-(MMTr-oxymethyl)-D-mannitol (**19**) in 20% yield (two steps). The coupling yield of both adenine and thymine derivatives (**16** and **18**) was not optimized. Treatment of **19** with aqueous CF_3COOH at room temperature gave 1,5-anhydro-2-(thymin-1-yl)-2,3-dideoxy-3-hydroxymethyl)-D-mannitol (**20**) in 81% yield. However, the introduction of the base moiety, using Mitsunobu reaction, in order to obtain the 3'-*C* branched nucleoside with a 3-R configuration failed. Treatment of **15** under identical reaction conditions as described for **14** gave 1,5(R)-anhydro-4(S),6-*O*-benzylidene-2,3-dideoxy-2-ene-3-*C*-(MMTr-oxymethyl)-D-hexitol (**21**) in 59% isolated yield. Finally, the benzylidene and MMTr protecting groups in **21** were removed by treatment with aqueous CF_3COOH at room temperature to give 1,5(R)-anhydro-2,3-dideoxy-2-ene-3-*C*-hydroxymethyl-4(S)-D-hexitol **22** (93%).



Scheme 3

Structure Determination of Compounds 9, 12, 13, 19-22 by NMR Spectroscopy.

The key step to the ring expansion from ribose to a 1,5-anhydrohexitol is the addition of nitromethane conjugated base to the bis-aldehyde sugar derivative **6** (Scheme 1). Similar synthetic routes have shown[11] that the insertion of nitromethane places the OH group at what here becomes C-4 of the hexitol at the α-face of the six-membered ring. From a synthetic viewpoint, the 2-OH and 3-NO_2 groups could each end up either on the α- or the β-face of the sugar moiety; thus, four diastereoisomers (**A**, **B**, **C**, and **D**; see Fig. 1) need to be considered as candidate products of the reaction. Apparently, only two of these are obtained, corresponding to structures **8** and **9** (Scheme 1). Furthermore, the six-membered ring in each product could theoretically adopt two conformations ($^4C_1$, as indicated in Fig. 1, and $^1C_4$).

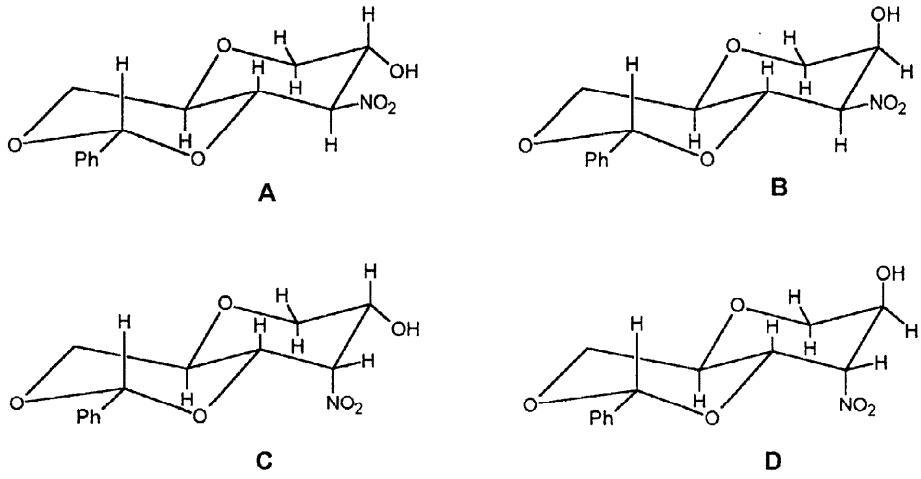


Fig. 1

The stereochemistry of structure **9** at C-2, C-3 and C-4 as well as its ring conformation were investigated by NMR spectroscopy and X-ray crystallography. The ^1H NMR spectrum recorded at 500 MHz was completely assigned by the combination of gradient-enhanced DQF-COSY and HSQC experiments; the latter experiment proved useful because the H-2 and H-4 signals virtually coincided while the C-2 and C-4 signals were clearly separated. The pertinent ^1H and ^{13}C NMR data are included in the Experimental Section (the numbering system used for the carbon atoms is given in Scheme 1). The vicinal coupling constants $^3J_{HH}$ were found to be: $^3J_{1,2} = 11.0$ Hz, $^3J_{1',2} = 5.8$ Hz, $^3J_{2,3} = 9.9$ Hz, $^3J_{3,4} = 9.8$ Hz, $^3J_{4,5} = 9.9$ Hz, $^3J_{5,6} = 10.2$ Hz and $^3J_{5,6'} = 5.0$ Hz. Relatively large (9.5-11 Hz) values for $^3J_{HH}$ are typically observed for diaxial pairs of vicinal protons in cyclohexyl rings and their analogues (having a torsion HCCH angle of approximately 180°);

therefore, the observed values indicate the axial orientation of H-1, H-2, H-3, H-4, and H-5 with respect to the anhydrohexitol ring, and the axial orientation of H-6 relative to the benzylidene six-membered ring system (and thus the equatorial orientation of H-1' and H-6'). Independently, the 4,6-*O*-benzylidene group was proven to originate from C-4 and C-5 in equatorial orientations; the H-4 and H-6 (and H-exo) atoms showed a strong NOE interaction, diagnostic for their 1,3-syn diaxial relative orientation. Therefore, **9** occurs in the $^4C_1$ chair conformation, in which all bulky substituent groups occur in equatorial positions. The collective set of NMR data identifies the stereochemistry of **9** as that of 1,5-anhydro-D-glucitol (**A** in Fig. 1), establishing its configuration as indicated in Scheme 1. Independent, unambiguous structural proof of **9** came from X-ray crystallography (details to be pusblished elsewhere).

The stereochemistry of **8** was inferred from its propensity to transform into **9** under basic conditions. When **8** was treated with Et_3N in methanol at reflux temperature, a mixture of **8** and **9** was obtained (for details, see the Experimental Section), from which **9** was isolated. Thus, **8** was concluded to be the C-3 epimer of **9** (**C** in Fig. 1), having the 1,5-anhydro-D-allitol configuration.

Compounds **12** and **13**, each obtained in pure state (Scheme 2), posed a particular challenge for NMR structural investigation. In this pair of C-3 epimers, no hydrogen atom is attached to C-3; rather, two bulky substituents, i.e., NO_2 and CH_2-*O*-MMTr (*i.e*,C7), are found at C-3. Therefore, the configurations of **12** and **13** could not be determined solely by the set of vicinal $^3J_{HH}$ scalar coupling constants obtained from their 500-MHz 1D ^1H NMR spectra. In addition, the use of NOE couplings was needed. First, three individual J-coupled ^1H networks were identified by gradient-enhanced DQF-COSY: (a) H-1, H-1', H-2 (and 2-OH); (b) H-4, H-5, H-6, H-6' and (c) the two non-equivalent protons H7 and H' of the CH_2-*O*-MMTr (*i.e* C7) group at C-3 (two doublets, sharing a geminal coupling constant of –8.8 Hz in **12** and –10.5 Hz in **13**, respectively). In DMSO-d_6 (but not in $CDCl_3$), the 2-OH signal was observed as a doublet, not correlated to a C-atom in the HSQC spectrum; this doublet served as the COSY entrance to sub-network (a). The only other doublet pattern in the spectrum was assigned to H-4, facilitating the assignment of sub-network (b) based on COSY data. Chemical shifts and coupling constants for **12** and **13** are listed in the Experimental Section. For both **12** and **13** the configuration of the 4,6-*O*-benzylidene ring with respect to the 1,5-anhydrohexitol ring was inferred to be as in structure **9**, based on the relatively large $^3J_{4,5}$ coupling (9.5 Hz, indicating that H-4 and H-5 are both in axial orientation), and strong NOE effects from H-4 to H-6 (axial) and H-exo (axial). Moreover, a large coupling was observed in **12**, between H-2 and H-1, pointing to diaxial orientation of H-2 and H-1 in **12**. However, examination of the relative orientation of each of the three sub-networks with respect to the other two required the use of further NOE spectroscopy. For example, selective inversion of the H-2 signal in the spectrum of **12** (at 4.56 ppm) yielded a clearly observable NOE effect on 2-OH (d at 6.08 ppm), H-4 (d at 4.13 ppm), H-1eq (dd at 3.95 ppm) and H7 (d, 3.72 ppm). The occurrence of the latter NOE determines the CH_2-OMMTr (*i.e* C7) at C-3 to be in equatorial position. (It should be noted that in $CDCl_3$, the H-6eq and H-2 signals were found to

be partially overlapping, making selective inversion of H-2 for an NOE experiment impossible.) Thus, in terms of Fig. 1, with H-4 fixed in axial position, the H-2/H-4 NOE (due to a 1,3-syn diaxial interaction) classifies compound **12** as either A or C (with H-2 axial); H-2 in axial orientation can only experience an NOE with one of the CH_2 protons of the CH_2-*O*-MMTr (*i.e* C7) group if that group is pointing in equatorial direction (establishing the structure of **12** as C, with the 3-NO_2 group axial).

The configurational assignment of **14** and **15**, the next pair of C-3 epimers in Scheme 2, by NMR spectroscopy was hampered by the limited stability of the compounds. Therefore, it was decided to retroactively confirm the stereochemistry of **14** after coupling the nucleobase at the C-2 position of the sugar moiety. For this purpose, the thymine derivatives **19** and **20** were studied by 500-MHz NMR spectroscopy. The ^1H spectra of **19** in $CDCl_3$ and **20** in D_2O were fully assigned by COSY and HSQC experiments. The ^1H and ^{13}C NMR data for these compounds are listed in the Experimental Section; the numbering of the carbon atoms is given in Scheme 3. The pertinent vicinal coupling constants for **19** were found to be: $^3J_{1',2'} = 3.8$ Hz, $^3J_{1'',2'} = 0.6$ Hz, $^3J_{2',3'} = 6.2$ Hz, $^3J_{3',4'} = 11.1$ Hz, $^3J_{4',5'} = 9.6$ Hz, $^3J_{5',6'} = 10.5$ Hz and $^3J_{5',6''} = 4.6$ Hz. The large values for $^3J_{3',4'}$ and $^3J_{4',5'}$ are indicative of two pairs of diaxially oriented vicinal protons, establishing H-3', H-4' and H-5' to be in axial orientation. The combination of the axial orientation of H-3' and the relatively small value of $^3J_{2',3'}$ points to the equatorial orientation of H-2'. The small values of $^3J_{1',2'}$ and $^3J_{1'',2'}$ are consistent with axial-equatorial and diequatorial couplings, respectively. From these data it is evident that the thymine group has been introduced at C-2' in axial position. After deprotection, compound **20** is soluble in D_2O. The vicinal $^3J_{HH}$ coupling constants of interest were found to be: $^3J_{1',2'} = 3.4$ Hz, $^3J_{1'',2'} = 0.5$ Hz, $^3J_{2',3'} = 3.3$ Hz, $^3J_{3',4'} = 10.1$ Hz, $^3J_{4',5'} = 10.1$ Hz, $^3J_{5',6'} = 3.4$ Hz and $^3J_{5',6''} = 2.4$ Hz. The large values for $^3J_{3',4'}$ and $^3J_{4',5'}$ imply that H-3', H-4' and H-5' are all in axial orientation. The combination of the axial orientation of H-3' and the relatively small value of $^3J_{2',3'}$ points to the equatorial orientation of H-2'. The small values of $^3J_{1',2'}$ and $^3J_{1'',2'}$ are consistent with axial-equatorial and diequatorial couplings, respectively. This set of coupling constants provides unambiguous evidence for the stereochemistry of **20** as the derivatized 1,5-anhydro-D-mannitol, occurring in the $^4C_1$ ring conformation, as depicted in Fig. 2A.

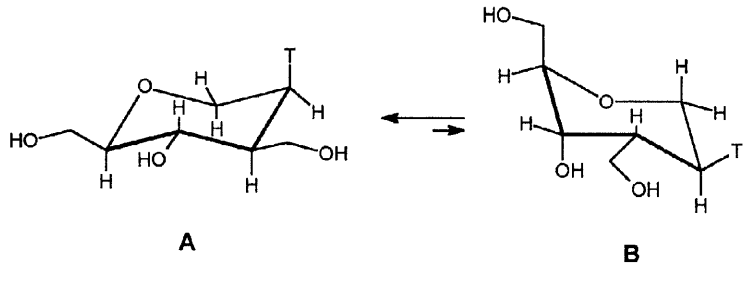


Fig. 2

The structure of **21** and **22** was determined on the basis of their ^{1}H, ^{13}C and ATP (attached proton test) spectra. Compound **21** was obtained from **15**, and was converted to **22** upon aq. CF_3COOH treatment. The ^{13}C-spectrum of **15** revealed that C3 resonates at 44.0 ppm. In ^{13}C-spectrum of **22** two new signals at 139.6 and 120.5 ppm were observed. These down field chemical shifts are expected for sp^2 hybridized carbons. These signals were assigned as C3 and C2. The APT experiment revealed that the signal at 139.6 ppm belongs to a quaternary carbon atom, suggesting it can be assigned to C3. If the double bond were present at the 2-position, two carbons (C2 and C3) must be attached to a proton. The ^{1}H-NMR spectrum of **22** also reveals that only one proton signal associated with sp^2 carbon resonates at 5.65 ppm. This down field chemical shift is expected for a proton covalently bonded to sp^2 hybridized carbon.

The antiviral activity of **17** and **20** was determined against herpes simplex virus-1, herpes simplex virus-2, vaccinia virus, vesicular stomatitis virus, Coxsackie virus B4, parainfluenza-3 virus, reovirus-1, Sindbis virus and Punta Toro virus. Activity was found for the adenine analogue **17** against vaccinia virus (70 μg/mL) and for the thymine analogue **20** against herpes simplex virus-1 (4 μg/mL) and herpes simplex virus-2 (70 μg/mL). These concentrations correspond to the EC_{50} values or concentrations required to reduce virus-induced cytopathogenicity by 50 %. Neither compound **17** nor **20** showed cytotoxicity (E_6SM cell cultures) at the highest concentration tested (400 μg/mL). The fact that no antiviral activity was noted with **20** against thymidine kinase deficient HSV-1 strains (data not shown), suggests that phosphorylation by the virus-encoded thymidine kinase may be a crucial step in the metabolic activation of these nucleoside analogues. The antiviral activity of **20** is remarkable in view of the lower activity of the 2'-deoxy-1',5'-anhydromannitol congeners[4] with a pyrimidine base moiety. It demonstrates that the structure-activity relationship of the 1,5-anhydrohexitol nucleosides is different from that of the furanose nucleosides.

EXPERIMENTAL SECTION

All concentrations were performed in vacuum. Filtrates were dried over dry Na_2SO_4. Column chromatography was performed on silica gel (0.060-0.200 nm or 0.030-0.075 nm). Melting points were determined in capillary tubes with a Buchi-Tottoli apparatus and are uncorrected. High resolution mass spectra (HRMS) were recorded on a Kratos Concept 1H mass spectrometer. Except for the NMR experiments, all other general procedures were described previously.[3]

NMR Experiments.

Standard one-dimensional (1D) ^{1}H and ^{13}C NMR spectra of compounds **2**, **4**, **5**, **8**, **9**, **12**, **13**, **14**, **15**, **16**,

17, **19**, **20**, **21** and **22**, dissolved in suitable solvents, were recorded with a Varian Gemini 200 spectrometer (operating at 200 MHz for ^1H and 50 MHz for ^{13}C), at ambient temperature. The ^{13}C spectra were acquired under WALTZ-16 composite-pulse ^1H decoupling conditions. ^1H NMR chemical shifts were referenced to internal tetramethylsilane (TMS) at 0 ppm (either directly, or indirectly against the residual solvent signals, $CHCl_3$ in $CDCl_3$ δ 7.26, and DMSO-d_5 in DMSO-d_6 δ 2.49) or to DSS (for the samples in D_2O, by measuring against the residual HDO signal as secondary reference, δ 4.76 ppm at 26 °C and δ 4.69 ppm at 33 °C). ^{13}C NMR chemical shifts for samples in organic solvents were referenced to internal TMS (0 ppm) (indirectly, measured against the CD_3 signal of DMSO-d_6 at δ 39.6; or the signal of $CDCl_3$ at δ 76.9). ^{13}C NMR chemical shifts for samples in D_2O were referenced to internal DSS (0 ppm) (indirectly, relative to the CH_2 signal of dioxane in D_2O at 67.4 ppm). The symbols s, d, dd, ddd, t, m, or br s are used for signals in ^1H spectra, as follows: s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublet of doublets, t = triplet, m = multiplet, and br s = broad singlet.

Compounds **9**, **12**, **13**, **19** and **20** were analyzed more in depth by NMR spectroscopy at higher magnetic field (corresponding to a ^1H frequency of 500 MHz). Aliquots (typically, 5-10 mg) of these samples were dissolved in 0.7 mL of either DMSO-d_6 (**9**), or $CDCl_3$ (**12**, **13**, and **19**) or D_2O (**20**). The solutions were transferred into 5-mm Wilmad 528-PP tubes. High-field NMR spectra were recorded on a Varian UNITY 500 spectrometer, operating at 499.627 MHz for ^1H NMR and at 125.643 MHz for ^{13}C NMR, and running under VNMR software version 5.1A. The instrument was equipped with a 5-mm ID PFG (inverse-detection with pulsed magnetic field *z*-gradients) $^1H\{X\}$ probe, tuned for ^{13}C in the "decoupling" X-channel, a Varian Performa II PFG source and a waveform generator. All experiments were conducted at a sample temperature of 33 °C; the samples were not spun. ^1H chemical shifts and coupling constants were deduced by first-order interpretation of the spectra. Two-dimensional (2D) homonuclear, gradient-enhanced[14] double-quantum filtered COSY[15] experiments, in concert with ^1H-detected (^1H-^{13}C) heteronuclear gradient-enhanced[16] HSQC[17] experiments, were used for the complete assignment of the ^1H and ^{13}C signals. During HSQC acquisition, GARP-1 decoupling[18] was applied in the ^{13}C channel. The 1D NOESY experiments on compounds **12** and **13** were conducted using the DPFG-NOE pulse sequence[19] with a mixing time of 2 s duration. Selective inversion of the pertinent resonances was achieved using 180° I-SNOB-3 pulses[20] with typical band widths of 25 to 35 Hz (equivalent to a B_1 field of 30 Hz during 80 to 115 ms). The I-SNOB-3 pulses were generated by the Varian waveform generator using the excitation profiles calculated by Pandora's Box program (version 4.1), a part of Varian's VNMR software[21]. All data were processed with standard VNMR 5.1A software routines[5].

Compound **9** was crystallized from MeOH and CH_2Cl_2 by slow evaporation of the solvents. X-ray diffraction intensities were measured on a Stoe STADI4 diffractometer.

1-*O*-Methyl-2,3,5-tri-*O*-benzyl-D-ribose (1). Compound **1** was prepared following similar reaction

condition described[8] for 1-*O*-methyl-3,5-di-*O*-benzyl-2-deoxy-D-ribose using 1-*O*-methyl-D-ribose (30.6 g, 186.6 mmol), benzyl bromide (81.2 mL, 682.7 mmol), NaH (80%, 21.0 g, 698.6 mmol) in DMF (400 mL) to give **1** (72.0 g, 89%). Major isomer, ^{1}H-NMR ($CDCl_3$). 7.41-7.20 (m, 15H) arom; 4.90-4.48 (m, 6H) 3 x $ArC\underline{H}_2$; 4.35 (m, 2H) H1 and H4; 4.02 (dd, J = 4.6, 7.1 Hz, 1H) H2/H3; 3.83 (br. d, J = 4.7 Hz, 1H) H2/H3; 3.61 (dd, $J_{4,5}$ = 3.9 Hz, $J_{5,5'}$ = 10.6 Hz, 1H) H5; 3.50 (dd, $J_{4,5'}$ = 5.6 Hz, $J_{5,5'}$ = 10.6 Hz, 1H) H5'; 3.30 (s, 3H) OMe. ^{13}C-NMR ($CDCl_3$). 138.2, 137.7, 128.2, 127.9, 127.7, 127.5, 127.4, 106.3, 80.4, 79.6, 78.3, 73.3, 72.3, 72.2, 71.2, 55.0.

2,3,5-Tri-*O*-benzyl-D-ribitol (2). Compound **1** (68.0 g, 156.5 mmol) was treated with 80% acetic acid in water (700 mL) at 90 °C for 9 h. The reaction mixture was cooled to room temperature, the solvent was removed, coevaporated with toluene and methanol. The residue was dissolved in methanol (800 mL) and cooled to 0 °C. To this cold solution $NaBH_4$ (6.29 g, 166.3 mmol) was added and the reaction mixture was kept at 0 °C for 30 min. The reaction mixture was adjusted to pH 7.0 by addition of acetic acid. The solvent was removed, the residue dissolved in dichloromethane (950 mL) and washed successively with aqueous saturated $NaHCO_3$ (1 x 50 mL) and water (1 x 50 mL). The organic layer was dried, filtered and concentrated. The residue was purified by silica gel column chromatography (0-50% EtOAc in hexane) to give **2** (54.0 g, 82%) as a colourless oil. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.39-7.18 (m, 15 H) arom; 4.77-4.45 (m, 6H) 3 x $ArCH_2$; 3.99 (m, 1H) H4; 3.86-3.72 (m, 4H) H1, H1', H2 and H3; 3.57 (br d, 2H) H5, H5'; 2.98 (br s, 1H) OH; 2.62 (br s, 1H) OH. ^{13}C-NMR ($CDCl_3$, 50 MHz): 138.0, 137.9, 128.4, 128.0, 127.8; (arom); 79.4, 79.3, (C2 and C3); 73.9, 73.4, 72.0, 71.0, (C5 and 3 x $Ar\underline{C}H_2$) 70.6 (C4)and 61.0(C1). HRMS calcd. for $C_{26}H_{31}O_5$ $(M+H)^+$ 423.2171, found 423.2171.

1,4-Anhydro-2,3,5-tri-*O*-benzyl-D-ribitol (4). A mixture of **2** (50.0 g, 118.3 mmol) and p-toluenesulfonyl chloride (27.0 g, 141.6 mmol) in pyridine (600 mL) was kept at room temperature for 48 h. The solvent was removed. The residue was dissolved in dichloromethane (900 mL), washed successively with aqueous saturated $NaHCO_3$ (150 mL) and water (50 mL). The organic layer was dried, filtered and concentrated. The residue was purified by silica gel column chromatography (0-3% MeOH in CH_2Cl_2) to give **4** (42.0 g, 88%) as a colorless oil. Compound **4** was also prepared by a Mitsunobu reaction. DEAD (2.56 mL) in dioxane (40 mL) was added to a mixture of alcohol **2** (3.5 g, 8.28 mmol), Ph_3P (4.36 g, 16.62 mmol) in 40 mL of dioxane over a period of 90 min and the reaction mixture was kept at room temperature overnight. The solvent was removed and the residue was purified by silica gel column chromatography (0-30% EtOAc in hexane) to give **4** (3.1 g, 93%) as a colorless oil. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.39-7.19 (m, 15 H) arom; 4.68-4.44 (m, 6H) 3 x $ArC\underline{H}_2$; 4.15 (m, $J_{3,4}$ = 6.2 Hz, 1H) H4; 4.05-3.88 (m, 4H) H3, H2 and H1'; 3.62 (dd, $J_{4,5}$ = 3.3 Hz, J5,5' = 10.6 Hz, 1H) H5; 3.51 (dd, $J_{4,5'}$ = 4.5, 10.6 Hz, 1H) H5'. ^{13}C-NMR ($CDCl_3$, 50 MHz): 138.1, 138.0 137.9, 128.3, 127.9, 127.8, 127.7, 127.6 (arom); 80.4, 78.3, 76.5 (C4, C3 and C2); 73.4, 72.1, 71.8, 70.6 and 70.0 (C1, C5 and 3 x $Ar\underline{C}H_2$). HRMS calcd. for $C_{26}H_{28}O_4Na$ $(M+Na)^+$ 427.1885, found 427.1890.

1,4-Anhydro-D-ribitol (5). **Compound 4** (36.0 g, 88.99 mmol) was treated with $Pd(OH)_2$ on C (20%) (10.0 g), methanol (600 mL) and cyclohexene (200 mL) at reflux temperature overnight. The catalyst was removed by filtration. The filtrate was concentrated to give pure **5** (11.93 g, 100%) as a colourless oil. ^{1}H-NMR (DMSO-d_6, 200 MHz): 3.95 (m, 1H); 3.87-3.70 (m, 2H); 3.60-3.43 (m, 3H); 3.33 (dd, J = 4.9 Hz, J = 11.6 Hz, 1H). ^{13}C-NMR (DMSO-d_6, 50 MHz): 83.4 (C4), 72.2 (C1), 71.8, 70.7 (C2 and C3) and 62.0 (C5). HRMS calcd. for $C_5H_{11}O_4$ $(M+H)^+$ 135.0657, found 135.0623.

1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-3-nitro-D-allitol (8) and **1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-3-nitro-D-glucitol (9)**. Compound **5** (11.9 g, 88.71 mmol) was dissolved in water (200 mL) and cooled to 0 °C. To this cold solution $NaIO_4$ (20.9 g) in 200 mL water was slowly added. The reaction mixture was kept at 0 °C for 60 min. After addition of ethanol (400 mL) the reaction mixture was stirred for an additional 10 min. The precipitate was filtered. The filtrate was concentrated and coevaporated with ethanol. The residue was dissolved in dry ethanol (200 mL). The undissolved material was removed by filtration. The filtrate was concentrated. The residue was dissolved in dry methanol (300 mL) and cooled to 0 °C. To this cold solution nitromethane (9 mL) was added, followed by NaOMe (1.9 g, Na metal in 15 mL dry methanol) and the reaction mixture was kept at room temperature for 2 h. The pH of the reaction mixture was adjusted to 7.0 by addition of glacial acetic acid, and was concentrated. The residue was treated with benzaldehyde (200 mL) and $ZnCl_2$ (27.13 g) at room temperature for 24 h. The excess benzaldehyde was removed under reduced pressure. The residue was dissolved in ethyl acetate (900 mL) and aqueous saturated $NaHCO_3$ (300 mL) was added. The precipitate was filtered and the organic layer was separated. The aqueous layer was re-extracted with ethyl acetate (400 mL). The combined organic layer was washed with water (1 x 100 mL), dried and concentrated. The residue was purified by silica gel column chromatography (0-35% EtOAc in hexane) to give **8** (2.0 g, 8%) and **9** (8.5 g, 34%) as a white solid. **Compound 8**: ^{1}H-NMR (DMSO-d_6, 200 MHz): 7.40 (s, 5H); 5.84 (d, J= 5.4 Hz, 1H); 5.79 (s, 1H); 5.11 (dd, J = 3.4, 10.6 Hz, 1H); 4.51 (t, J = 10.0 Hz, 1H); 4.32 (br. s, 1H); 4.21 (dd, J = 4.70, 10.2 Hz, 1H); 3.80 (m, 3H); 3.42 (m, 1H). ^{13}C-NMR (DMSO-d_6, 50 MHz): 137.4, 129.1, 128.3, 126.2 (arom); 100.9 (benzylidene), 86.0 (C3); 73.9 (C4); 71.3 (C5); 70.5 (C2); 68.2 (C1) and 67.9 (C6). HRMS calcd. for $C_{13}H_{16}N_1O_6$ $(M+H)^+$ 282.0977, found 282.0961. **Compound 9**: ^{1}H-NMR (DMSO-d_6, 500 MHz): 7.35 (br. s, 5H) arom; 6.00 (d, $J_{2,2\text{-OH}}$ = 6.0 Hz, 1H) 2-OH; 5.66 (s, 1H) benzylidene, 4.81 (t, $J_{3,4}$ = 9.8 Hz, 1H) H3; 4.23 (dd, $J_{5,6}$ = 5.0 Hz, $J_{6,6'}$ = 10.2 Hz, 1H)H6'; 4.08 (m, $J_{2,3}$ = 9.9 Hz, 1H) H2; 4.06 (t, $J_{4,5}$ = 9.9 Hz, 1H) H4; 3.92 (dd, $J_{1,1'}$ = 11.0 Hz, $J_{1,2}$ = 5.8 Hz, 1H) H1', 3.75, (t, $J_{6,6'}$ = 10.2 Hz, 1H) H6', 3.52 (m, $J_{5,6}$ = 10.2 Hz, $J_{5,6'}$ = 5.0 Hz, 1H) H5; 3.37 (t, $J_{1,1'}$ = 11.0 Hz, $J_{1',2}$ = 11.0 Hz, 1H) H1'. ^{13}C-NMR (DMSO-d_6, 125 MHz): 137.3, 129.1, 128.1, 126.1, (arom) 100.4 (benzylidene), 91.5 (C3), 77.2 (C4), 69.9 (C5), 69.5 (C2), 68.1 (C1), 67.7 (C6). HRMS calculated for $C_{13}H_{16}N_1O_6$ $(M+H)^+$ 282.0977, found 282.0996. Mp: 141-142 °C. Anal. calcd for $C_{13}H_{15}NO_6.0.9H_2O$: C, 52.49; H, 5.69; N, 4.71. Found: C, 52.71; H, 5.71; N, 5.06.

1,5(R)-Anhydro-4(S),6-*O*-benzylidene-3-deoxy-3(S)-*C*-(MMTr-oxymethyl)-2(R)-hydroxy-3-nitrohexitol (12) and 1,5(R)-Anhydro-4(S),6-*O*-benzylidene-3-deoxy-3(R)-*C*-(MMTr-oxymethyl)-2(R)-hydroxy-3-nitrohexitol (13). A mixture of **9** (5.7 g, 20.26 mmol), 35% aqueous formaldehyde (20 mL) and *N,N*-tetramethylguanidine (635 μL) in acetonitrile (20 mL) was kept at room temperature for 40 min. The pH of the reaction mixture was adjusted to 7.0 by addition of acetic acid. The solvent was removed and the residue was dissolved in ethyl acetate (500 mL), washed with aqueous saturated $NaHCO_3$ (10 mL) and water (10 mL). The organic layer was dried, filtered and concentrated. The residue was purified by silica gel column chromatography (0-3% MeOH in CH_2Cl_2) to give a mixture of **10** and **11** (4.0 g, 63%). The mixture of **10** and **11** (3.2 g, 10.3 mmol) was treated with monomethoxytrityl chloride (4.8 g, 15.50 mmol) in pyridine (40 mL) at room temperature for 7 days. The reaction mixture was poured into ice water (30 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic layer was dried, filtered and concentrated. The residue was purified by silica gel column chromatography (0-50% EtOAc in hexane) to give **12** (2.0 g, 33%) and **13** (2.8 g, 47%) as white foam. **Compound 12:** ^{1}H-NMR (DMSO-d_6, 500 MHz): 7.50-7.10 (m, 17H) arom; 6.85 (m, 2H) arom; 6.08 (d, J = 5.8 Hz, 1H) 2OH; 5.55 (s, 1H) benzylidene; 4.55 (m, 1H) H2; 4.29 (dd, $J_{5,6}$ = 4.7 Hz, $J_{6,6'}$ = 10.3 Hz, 1H) H6; 4.16 (m, $J_{5,6}$ = 10.2 Hz, $J_{5,6'}$ = 4.7 Hz, 1H) H5; 4.13 (d, $J_{4,5}$ = 9.5 Hz, 1H) H4; 3.98 (t, $J_{1,1'}$ = 11.0 Hz, $J_{1,2}$ = 11.0 Hz, 1H) H1; 3.95 (dd, $J_{1,1'}$ = 11.0 Hz, $J_{1',2}$ = 6.8 Hz) H1'; 3.75 (dd, $J_{6,6'}$ = 10.3 Hz, $J_{5,6'}$ = 10.2 Hz, 1H), H6'; 3.72 (d, $J_{7,7'}$ = 8.8 Hz, 1H) H7; 3.64 (s, 3H) MMTr; 3.60 (d, $J_{7,7'}$ = 8.8 Hz, 1H) H7'. 13C NMR ($CDCl_3$): 159.0, 144.1, 143.8, 137.4, 134.8, 131.2, 129.0, 128.1, 127.3, 126.8, 113.5 (arom); 102.2 (benzylidene); 91.7 (C3); 87.0 (MMTr); 76.3, 69.6, 67.0, 66.6, 65.7, 59.3 (C7, C6, C5, C4, C2 and C1); 55.2 (MMTr). HRMS calcd. for $C_{34}H_{33}N_1O_8Na$ $(M+Na)^+$ 606.2104, found 606.2083. **Compound 13**: ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.51-7.18 (m, 17 H) arom; 6.78 (m, 2H) arom; 5.67 (s, 1H) benzylidene; 4.61 (m, 2H); 4.15 (m, 1H); 4.05 (br. d, 1H); 3.91-3.56 (m, 7H); 3.21 (br. d, 1H); 2.81 (d, J = 6.7 Hz, 1H). ^{13}C-NMR ($CDCl_3$, 125 MHz): 158.8, 143.4, 143.1, 136.7, 134.2, 130.3, 129.0, 128.3, 128.0, 127.9, 127.3, 126.0, 113.3 (arom); 102.0 (benzylidene); 91.7 (C3); 87.6 (MMTr); 77.2, 70.0, 69.4, 69.1, 69.0, 61.0 (C7, C6, C5, C4, C2, C1); 55.2 (MMTr). HRMS calcd. for $C_{34}H_{33}N_1O_8Na$ $(M+Na)^+$ 606.2104, found 606.2112.

1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-3-*C*-(MMTr-oxymethyl)-D-glucitol (14). Compound **12** (900 mg, 1.54 mmol) was treated with n-tributyltin hydride (1.24 mL, 4.63 mmol) in the presence of AIBN (63 mg, 0.38 mmol) in toluene (15 mL) at 110 °C for 90 min. The solvent was removed. The residue was purified by silica gel column chromatography (0-2% MeOH in CH_2Cl_2) to give **14** (500 mg, 60%) as a slightly sticky light yellow foam. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.50-7.18 (m, 17 H) arom; 6.76 (m, 2H) arom; 5.49 (s, 1H) benzylidene; 4.31 (dd, J = 4.6, 10.4 Hz, 1H); 4.50-3.87 (m, 2H); 3.80-3.24 (m, 9H); 1.98 (m, 1H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 158.6, 144.3, 144.0, 137.3, 135.2, 130.2, 128.8, 128.1, 127.9, 127.1, 127.0, 126.0, 113.2, 101.0, 86.9, 76.4, 72.7, 71.5, 69.2, 68.3, 60.5, 55.2, 47.7. HRMS calcd. for $C_{34}H_{34}O_6Na$ $(M+Na)^+$ 561.2253, found 561.2264.

1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-3-*C*-(MMTr-oxymethyl)-D-allitol (15). A mixture of **13** (2.4 g, 4.1 mmol), n-tributyltin hydride (3.3 mL) and AIBN (200 mg, 1.21 mmol) in toluene (40 mL) was heated at 110 °C for 90 min. The solvent was removed. The residue was purified by silica gel column chromatography (0-40% EtOAc in hexane) to give **15** (1.0 g, 45%) as a colorless foam. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.50-7.18 (m, 17H) arom; 6.75 (m, 2H) arom; 5.71 (s, 1H) benzylidene; 4.33 (dd, $J_{5,6}$ = 4.8 Hz, $J_{6,6'}$ = 10.4 Hz, 1H) H6; 4.32 (t, $J_{4,5}$ = 10.4 Hz, 1H) H4; 4.09 (br s, $J_{2,3}$ = 2.6 Hz, 1H) H2; 3.99 (dd, $J_{1,1'}$ = 12.3 Hz, $J_{1,2}$ = 1.8 Hz, 1H) H1; 3.92 (t, $J_{6,6'}$ = 10.4 Hz, 1H) H6'; 3.80 (dd, $J_{3,7}$ = 3.6 Hz, $J_{7,7'}$ = 9.7 Hz, 1H) H7; 3.75 (br s, 1H) 2OH; 3.73 (s, 3H) MMTr; 3.61 (dt, $J_{1,1'}$ = 12.3 Hz, $J_{1',2}$ = 1.8 Hz, 1H) H1'; 3.41 (m, $J_{5,6}$ = 10.4 Hz, $J_{5,6'}$ = 4.8 Hz, 1H) H5; 3.28 (dd, $J_{7,7'}$ = 9.7 Hz, $J_{7',3}$ = 2.7 Hz, 1H) H7'; 2.01 (m, $J_{3,4}$ = 10.4 Hz, $J_{3,7'}$ = 2.7 Hz, $J_{3,7}$ = 3.6 Hz, 1H) H3. ^{13}C-NMR ($CDCl_3$, 125 MHz): 158.8, 144.4, 137.5, 135.4, 130.2, 129.0, 128.3, 128.2, 128.0, 127.2, 127.0, 126.2, 113.4 (arom); 101.8 (benzylidene), 87.0 (MMTr), 74.7 (C4), 74.0 (C5), 72.5 (C1), 70.5 (C2), 69.2 (C6), 61.4 (C7), 55.2 (MMTr), 44.0(C3). HRMS calcd. for $C_{34}H_{34}O_6Na$ $(M+Na)^+$ 561.2253, found 561.2241.

1,5-Anhydro-4,6-*O*-benzylidene-2-(adenin-9-yl)-2,3-dideoxy-3-*C*-(MMTr-oxymethyl)-D-mannitol (16). DEAD (309 μL) in 20 mL dioxane was added to a mixture of **14** (538.6 mg, 1.0 mmol), Ph_3P (524.6 mg, 2.0 mmol) and adenine (270.3 mg, 2.0 mmol) in dioxane (20 mL) over a period of 2 h and the reaction mixture was kept at room temperature for 36 h. The solvent was removed. The residue was purified by silica gel column chromatography (0-5% MeOH in CH_2Cl_2) to give **16** (100 mg, 15%) as a white solid. ^{1}H-NMR ($CDCl_3$, 200 MHz): 8.27 (s, 1H); 8.08 (s, 1H) H2 and H8; 7.50-6.50 (m, 19H) arom; 5.80 (br. s, 2H) NH_2; 5.61 (s, 1H) benzylidene; 5.35 (m, 1H) H2'; 4.42 (dd, $J_{5',6'}$ = 4.7, $J_{6',6''}$ = 10.3 Hz, 1H) H6'; 4.22-3.88 (m, 4H) H1', H1'', H4' and H6'; 3.73-3.58 (m, 4H) H5' and MMTr; 3.31 (dd, $J_{3',7'}$ = 2.6, $J_{7',7''}$ = 9.5 Hz, 1H) H7'; 2.95 (t, $J_{7',7''}$ = $J_{3',7''}$ = 9.5 Hz, 1H) H7'' and 2.62 (m, 1H) H3'. ^{13}C-NMR ($CDCl_3$, 50 MHz): 158.2 (MMTr), 155.3 (C6), 152.8 (C2), 150.4 (C4), 143.9, 143.6 (MMTr), 140.1 (C8), 137.1, 134.8, 133.4, 133.3, 131.9, 130.1, 129.2, 128.3, 128.2, 127.4, 126.6, 126.3 (MMTr), 118.5 (C5), 112.7 (MMTr), 102.0 (benzylidene), 86.6 (MMTr), 75.6, 74.5 (C4' and C5'), 72.3 (C1'), 69.1 (C6'), 59.7 (C7'), 55.1 (MMTr), 51.6 (C2') and 43.4 (C3'). HRMS calcd. for $C_{39}H_{37}N_5O_5Na$ $(M + Na)^+$ 678.2692, found 678.2685.

1,5-Anhydro-2-(adenin-9-yl)-2,3-dideoxy-3-*C*-hydroxymethyl-D-mannitol (17). Compound **16** (60 mg, 0.09 mmol) was treated with 80% trifluoroacetic acid in water (5 mL) at room temperature overnight. The solvent was removed. The residue was dissolved in methanol (3 mL) and treated with NH_4OH (1 mL). The solvent was removed, coevaporated with methanol and toluene. The residue was purified by crystallization (MeOH / CH_2Cl_2) to give **17** (22 mg, 81%). ^{1}H-NMR (DMSO-d_6, 200 MHz): 8.39, 8.17 (2 x s, 2H); 7.39 (br s, 2H); 5.07 (d, J = 6.3 Hz, 1H); 4.88 (m, 3H); 3.96 (m, 2H); 3.66 (m, 3H); 3.36 (m, 2H); 2.72 (m, 1H) and 2.08 (m, 1H). ^{13}C-NMR (DMSO-d_6, 50 MHz): 156.3 (C6), 152.4 (C2), 150.2 (C4), 140.5 (C8), 117.7 (C5), 82.4 (C5'), 69.8 (C1'), 62.0 (C4'), 60.3, 59.1 (C6' and C7'), 50.3 (C2') and 47.5 (C3'). HRMS calcd. for $C_{12}H_{18}N_5O_4$ $(M+H)^+$ 296.1358, found 296.1355.

1,5-Anhydro-4,6-*O*-benzylidene-2-(thymin-1-yl)-2,3-dideoxy-3-*C*-(MMTr-oxymethyl)-D-mannitol (19). The aforementioned reaction described for formation of **16** was performed again using very similar conditions, starting from **14** (300 mg, 0.56 mmol), Ph_3P (294 mg, 1.12 mmol), N^3-benzoylthymine (256 mg, 1.12 mmol), DEAD (174 μL) and dioxane (2 x 6 mL) to give **18**, which was directly treated with aqueous NaOH (N) (5 mL) in dioxane (5 mL) overnight at room temperature. The pH of the reaction mixture was adjusted to 7.0 by addition of dilute aqueous HCl. The volume of the reaction mixture was reduced to half its size, followed by extraction with ethyl acetate (3 x 10 mL). The combined organic layer was washed with water (3 mL). The organic layer was dried, filtered and concentrated. The residue was purified by silica gel column chromatography (0-3% MeOH in CH_2Cl_2) to give **19** (70 mg, 20% in two steps). ^{1}H-NMR ($CDCl_3$, 500 MHz): ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.98-7.10 (m, 18H) arom, 6.68 (m, 2H) arom, 5.87 (s, 1H) benzylidene, 5.21 (m, $J_{2',3'}$ = 6.2 Hz, 1H) H2', 4.54 (dd, $J_{5',6'}$ = 4.6 Hz, $J_{6',6''}$ = 10.5 Hz, 1H) H6'; 4.39 (dd, $J_{4',5'}$ = 9.6 Hz, $J_{3',4'}$ = 11.1 Hz, 1H) H4'; 4.20 (br d, $J_{1',1''}$ = 13.3 Hz, $J_{1',2'}$ = 0.6 Hz, 1H) H1'; 4.15 (dd, $J_{1',1'}$ = 13.3 Hz, $J_{1'',2'}$ = 3.8 Hz, 1H) H1"; 4.07 (t, $J_{6',6''}$ = $J_{6'',5'}$ = 10.5 Hz, 1H) H6"; 3.83 (s, 3H) MMTr; 3.70 (ddd, $J_{5',6''}$ = 10.5 Hz, $J_{5',6'}$ = 4.6, $J_{4',5'}$ = 9.6 Hz, 1H) H5'; 3.67 (dd, $J_{3',7'}$ = 1.5 Hz, $J_{7',7''}$ = 9.5 Hz, 1H) H7'; 3.54 (dd, $J_{7',7''}$ = 9.5 Hz, $J_{3',7''}$ = 5.9 Hz, 1H), 2.65 (m, $J_{3',4'}$ = 11.1 Hz, $J_{3',7''}$ = 5.9 Hz, $J_{3',7'}$ = 1.5 Hz) H3'; 2.08 (s, 3H) $5CH_3$. ^{13}C-NMR ($CDCl_3$, 125 MHz): 162.9 (C4), 158.4 (MMTr), 150.8 (C2), 144.0, 143.4, 137.1, 134.8 (MMTr), 138.9 (C6), 130.2, 129.3, 128.7, 128.2, 127.5, 127.0, 126.7, 126.5, 113.0 (MMTr), 110.5 (C5), 102.4 (benzylidene), 86.3 (MMTr) 74.6 (C4' and C5'), 72.1 (C1'), 69.2 (C6'), 58.8 (C7'), 55.2 (MMTr), 51.8 (C2'), 43.2 (C3'), 12.9 ($5CH_3$). HRMS calcd. for $C_{39}H_{38}N_2O_7Na$ $(M+Na)^+$ 669.2576, found 669.2579.

1,5-Anhydro-2-(thymin-1-yl)-2,3-dideoxy-3-*C*-hydroxymethyl-D-mannitol (20). Compound **19** (50 mg, 0.8 mmol) was treated with trifluoroacetic acid (80% in water, 5 mL) at room temperature overnight. The solvent was removed. The residue was dissolved in methanol (3 mL) and treated with NH_4OH (1 mL). The solvent was removed, coevaporated with methanol and toluene. The residue was purified over a short silica gel column (0-10% MeOH in CH_2Cl_2) to give **20** (18 mg, 81%) as a colorless oil. ^{1}H-NMR (D_2O, 500 MHz): 7.96 (s, 1H) H6; 4.96 (br m, $J_{2',3'}$ = 3.3 Hz, 1H) H2'; 4.13 (br d, $J_{1',2'}$ <0.5 Hz, $J_{1',1''}$ = 13.2 Hz, 1H) H1', 3.98 (dd, $J_{1',1''}$ = 13.2 Hz, $J_{1'',2'}$ = 3.4 Hz, 1H) H1"; 3.88 (dd, $J_{5',6'}$ = 2.4 Hz, $J_{6',6''}$ = 12.6 Hz, 1H) H-6'; 3.83 (dd, $J_{3',7'}$ = 3.4 Hz, $J_{7',7''}$ = 11.6 Hz, 1H) H7'; 3.81 (dd, $J_{6',5'}$ = 3.4 Hz, $J_{6',6''}$ = 12.6 Hz, 1H) H6', 3.78 (t, $J_{4',5'}$ = $J_{3',4'}$ = 10.1 Hz) H4'; 3.52 (dd, $J_{7',7''}$ = 11.6 Hz, $J_{7'',3'}$ = 7.6 Hz, 1H) H7"; 3.37 (m, $J_{5',6'}$ = 3.4 Hz, $J_{5',6''}$ = 2.4 Hz, 1H) H5'; 2.18 (m, $J_{3',4'}$ = 10.1 Hz, $J_{3',7''}$ = 7.6 Hz, $J_{3',7'}$ = 3.4 Hz, 1H) H3'; 1.83 (s, 3H) $5CH_3$. ^{13}C-NMR (D_2O, 125 MHz): 164.5 (C4), 156.3 (C2), 142.0 (C6), 111.8 (C5), 82.2 (C5'), 70.5 (C1'), 62.6 (C4'), 61.4 (C6'), 59.7 (C7'), 52.1 (C2'), 47.0 (C3'), 12.5 ($5CH_3$). HRMS calcd. for $C_{12}H_{19}N_2O_6$ $(M+H)^+$ 287.1243, found 287.1241.

1,5(R)-anhydro-4(S),6-*O*-benzylidene-2,3-dideoxy-2-ene-3-*C*-(MMTr-oxymethyl)-D-hexitol (21). The reaction was performed using a reaction condition described for **16** using **15** (175 mg, 0.32 mmol), Ph_3P (168 mg, 0.64 mmol), N^3-benzoylthymine (146 mg, 0.64 mmol) and DEAD (100 μL) in dioxane (2 x 5 mL) to give

21 (100 mg, 59%). **^{1}H-NMR ($CDCl_3$, 200 MHz)**: 7.56-7.18 (m, 17 H); 6.78 (m, 2H); 5.88 (m, 1H); 5.58 (s, 1H); 4.38-4.26 (m, 4H); 3.87-3.46 (m, 7H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 158.5, 144.6, 144.5, 137.6, 135.6, 134.4, 130.3, 128.8, 128.4, 128.3, 128.1, 127.7, 126.8, 126.2, 122.5, 113.0, 101.6, 86.5, 75.8, 70.0, 69.5, 66.4, 61.8 and 55.2. HRMS calcd. for $C_{34}H_{32}O_5Na$ $(M+Na)^+$ 543.2147, found 543.2149.

1,5(R)-anhydro-4(S)-2,3-dideoxy-2-ene-3-*C*-hydroxymethyl-D-hexitol (22). Compound **21** (70 mg, 0.13 mmol) was treated with trifluoroacetic acid (2 mL) in CH_2Cl_2 (2 mL) at room temperature overnight. The solvent was removed, coevaporated with methanol and toluene. The residue was purified by a short silica gel column chromatography (0-8% MeOH in CH_2Cl_2) to give **22** (20 mg, 93%) as a colorless oil. ^{1}H-NMR (DMSO-d_6, 200 MHz): 5.65 (m, 1H) H2; 4.84 (d, J = 7.3 Hz, 1H) 4OH; 4.62 (t, J = 5.4 Hz, 1H) 6OH; 4.59 (t, J = 5.2 Hz, 1H) 7OH; 4.41 (m, 4H); 3.80 (m, 1H) H4; 3.63 (ddd, J = 2.5 Hz, J = 5.4 Hz, J = 11.6 Hz, 1H) H6; 3.42 (m, 1H); 3.18 (m, 1H). ^{13}C-NMR (DMSO-d_6, 50 MHz): 139.6 (C3); 120.5 (C2); 80.2 (C5); 64.4 (C1); 63.0 (C4); 61.6, 60.7 (C6 and C7). HRMS calcd. for $C_7H_{13}O_4$ $(M+H)^+$ 161.0813, found 161.0851.

Isomerization of 1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-3-nitro-D-allitol (8) to 1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-3-nitro-D-glucitol (9): Compound **8** (1.8 g, 6.4 mmol) was treated with Et_3N (3 mL) in methanol (60 mL) at reflux temperature overnight. The solvent was removed and the residue was purified by silica gel column chromatography to give **9** (1.2 g, 67%) and **8** (400 mg, 22%). Further prolongation of the reaction time revealed that the product distribution of **9** and **8** did not alter. In a similar manner pure **9** (450 mg, 1.6 mmol) was treated with Et_3N (750 μL) in methanol (15 mL) at reflux temperature overnight. The solvent was removed and the residue was purified by silica gel column chromatography (0-35% EtOAc in hexane) to give **9** (300 mg, 67%) and **8** (90 mg, 20%) as a white solid.

Biological activity assays. Antiviral activity was determined based on virus-induced cytopathicity assays as described previously.[22,23]

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