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3'-C-Phosphonates as Nucleotides Analogues Synthesis Starting from Original C-Phosphonosugars (in ribo- and deoxyribo- series)

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dedicated to Pr Leroy B. Townsend for his 60th birthday.

Key words: 3-C-phosphonosugar, 3'-C-phosphononucleoside, Pudovic reaction, α-hydroxyphosphonate

Abstract: 3-C-phosphonosugars were synthetised starting from 2-deoxy-D-ribose and α-D-xylofuranose *via* the Pudovic reaction followed by reduction. Their condensation with nucleobases (thymine and adenine) gave original 3'-C-phosphononucleosides in ribo- and 2-deoxyribo- series, with a stereocontrol of the reactions.

Phosphate esters are ubiquitous compounds in biological systems. Chemists and biologists have identified them as prototypes for the design and synthesis of analogues in order to study biological processes and to prepare drugs.

The replacement of the O-phosphate group in a biologically active molecule by a phosphonic acid group [1,2] might be expected to have interesting biological effects, because these compounds are structurally similar to phosphate compounds and are highly stable. The C-P bond which replaces the C-O-P bond cannot be hydrolysed by phosphatases.

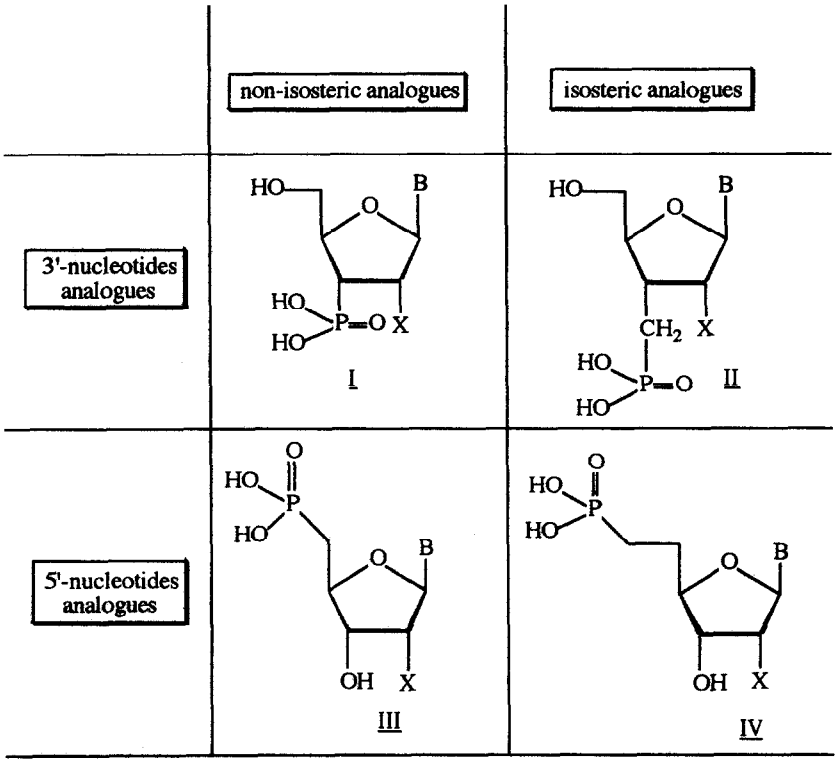
Nucleosides analogues are important as potential antimetabolites. Formation of their active triphosphate derivatives, which subsequently enter the natural nucleotide pool, requires a free 5'-OH. Therefore, most chemical modifications of the sugar moiety have involved the other positions. Among them a number of 3'-substitued nucleosides have been synthetized, for example AZT and ddI, currently used for anti-HIV chemotherapy.

We have been interested in the synthesis of phosphonate analogues of 3'-nucleotides (Structure I, Figure 1). In addition to their antimetabolitic potential, such compounds might be useful as new synthons for the elaboration of antisense modified oligonucleotides for antiviral or antitumoral chemotherapy.

Several approaches to the synthesis of isosteric (Figure 1, Structures II and IV) and non-isosteric phosphonates (Figure 1, Structure III) analogues of nucleotides have been reported [3] . Methods involving a stabilised Wittig reagent or the Arbuzov reaction with an halogen derivative have most often been employed. But these routes are not applicable to structure I (Figure 1). Therefore we have developed an alternative

synthesis via the Pudovic reaction [4-6] which gives the corresponding α-hydroxyphosphonate. This paper describes the synthesis of 3'-deoxy-3'-phosphononucleosides of thymine and adenine in the DNA and RNA series (Structure I with B = thymine or adenine, X = H or OH) by condensation of the appropriate phosphonosugar and the aglycon. This approach is of considerable interest because it is general, starting from a phosphonosugar precursor it is possible to obtain numerous phosphononucleosides by condensation with natural or modified bases.

- Figure 1: Phosphonates Analogues of Nucleotides-



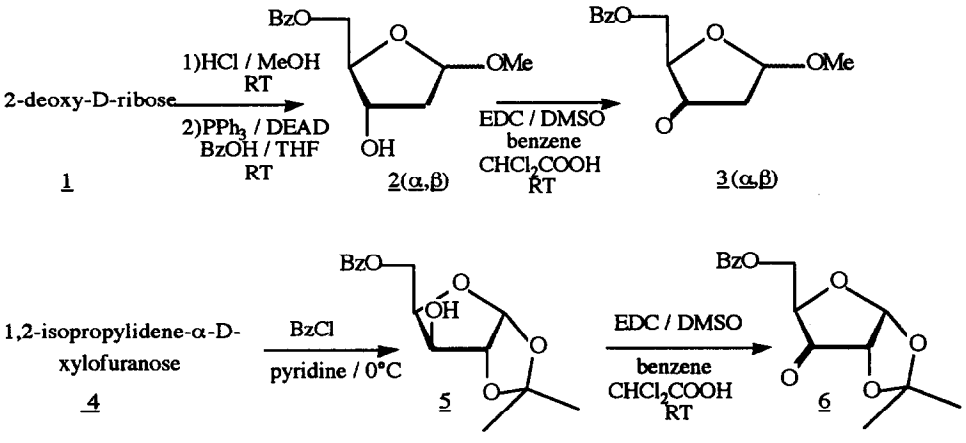
(B = puric, pyrimidic or modified nucleobase
X = H or OH)

SYNTHESIS:

Starting from 2-deoxy-D-ribose, we have developed a route to 3'-phosphono-2'-deoxyribonucleosides. Alternatively, starting from 1,2-isopropylidene-α-D-xylofuranose, the ribonucleosides can be obtained and subsequently transformed into 2'-deoxyribonucleosides [7].

2-Deoxy-D-ribose was cyclized by the C.C. Bhat procedure[8] and then selectively protected using the Mitsunobu reaction [9] to give compound 2 (mixture α/β, proportions 42/58) with an overall yield of 72%. Condensation of a 2-deoxy-sugar with a nucleobase generally gives an anomeric mixture of nucleosides, therefore we have not separated the 2α and 2β anomers. Compound 5 was obtained in 90% yield from commercially available 1,2-isopropylidene-α-D-xylofuranose. Moffatt oxidation [10] of 2 and 5 afforded the ketosugars 3 and 6, respectively, in quantitative yield (Scheme 1).

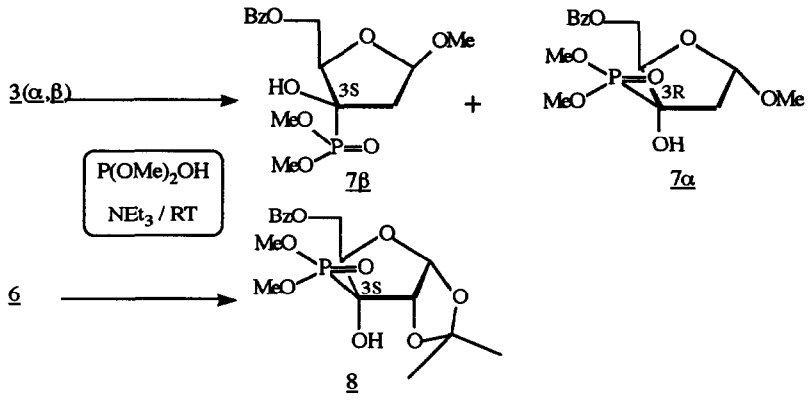
- Scheme 1 -



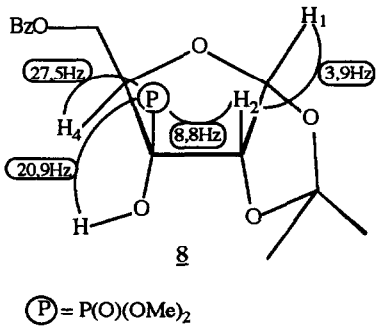
(DEAD: diethylazodicarboxylate
EDC: N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride)

Conversion of 3 and 6 to the corresponding α-hydroxyphosphonates (7 and 8) by means of the Pudovic reaction [5] (Scheme 2) occurred in a stereocontrolled manner. The 3α and 3β anomers gave respectively 7α and 7β which were separated. Stereocontrol presumably results in part from the presence of the anomeric OCH_3 groups, the reagent approaching from opposite side of the OCH_3, since it is reasonable to assume that the α and β anomers of 3 give rise to compounds 7α and 7β with the 3R and 3S configurations respectively. Product 8, on the other hand, was shown to possess only the 3S configuration; the stereoselectivity was due to the adjacent isopropylidene group, which prevents the phosphite from approaching above the plane of the sugar. Products 7α, 7β and 8 were obtained in 19%, 37% and 95% yields, respectively. D.F. Wiemer and coworkers have described the hydrophosphonylation of nucleotides and observed stereocontrol resulting from the presence of the nucleobase. In these instances the phosphonate group was above the plane of the sugar [11].

- Scheme 2 -



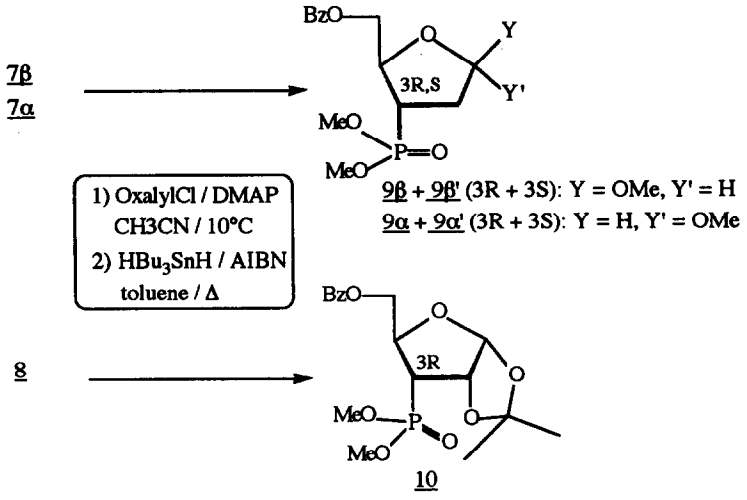
- Figure 2: Coupling Constants in the ^{1}H NMR Spectrum of 8 -



The stereochemistry of 8 was unambiguously established from the ^{1}H NMR spectrum by the cis coupling between H2 and P (8.8 Hz) and the trans coupling between H4 and P (27.5Hz) [6]. The position of H2 and H4 was known and therefore the observed couplings between H2 and P, and H4 and P confirmed structure 8 (Figure 2)with the phosphonate group above the plane of the sugar.

Reduction of 7α, 7β and 8, using the modification of the Barton reaction developed by Dolan and Mc Millan [12], produced the deoxygenated products. Epimerization was observed with 7α and 7β, each affording a C3 epimeric mixture, respectively 9α + 9'α and 9β + 9'β The epimers could not be separated. The yields were respectively 86 and 60%. However 8 underwent stereoselective deoxygenation, which resulted from the presence of isopropylidene group, giving only 10 with a 3R configuration in 93% yield.

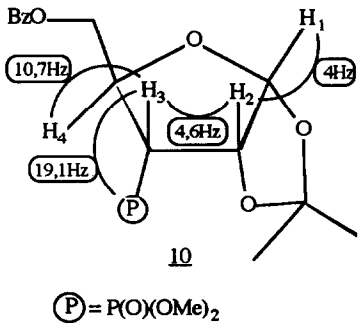
-Scheme 3-



(OxalylCl: monomethyloxalyl chloride; DMAP: dimethylaminopyridine; AIBN: αα'-azobisisobutyronitrile)

The position of the phosphonate group was established unambiguously from the observed cis coupling between H2 and H3 and trans coupling between H3 and H4: the phosphonate group is located below the plane of the sugar (Figure 3).

- Figure 3: Coupling Constants in the ^{1}H NMR Spectrum of 10 -



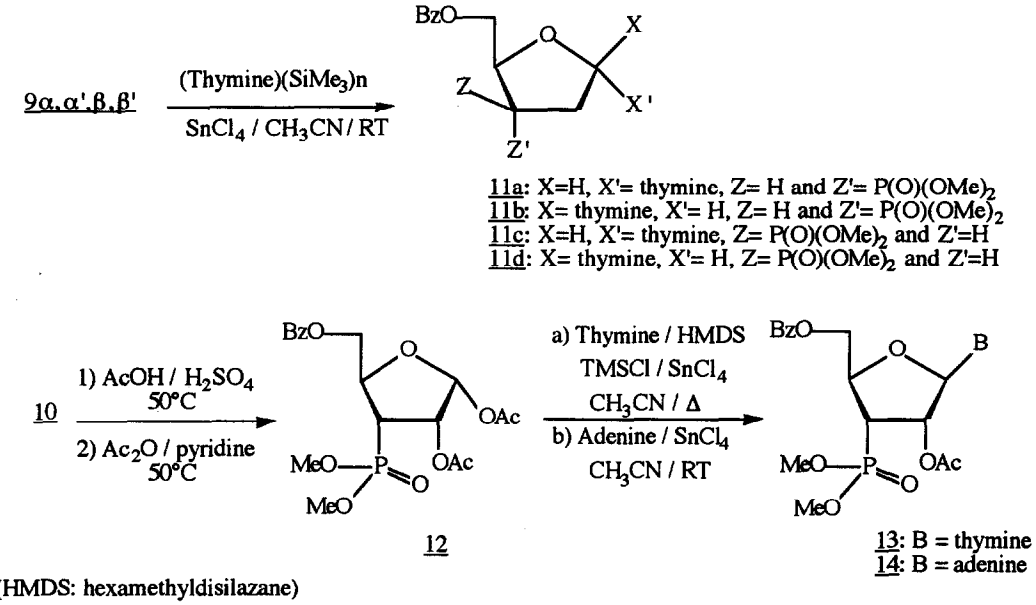
Condensation of 9 (mixture of the 4 stereoisomers) with thymine [13] led to the phosphononucleoside 11 as a mixture of 4 stereoisomers (60%) (Scheme 4). Separation of the 4 isomers 11 (a, b, c and d) by HPLC gave analytical samples of each compound and the ^{1}H NMR spectra allowed structure assignments.

Diacetylation of 10 gave the phosphonosugar 12 in 76% yield. The coupling constants observed on its ^{1}H NMR spectrum confirmed also the position of the phosphonate group. Condensation of 12 with thymine by the Vorbrüggen process [14,15] and with adenine by the Saneyoshi process [16,17] gave products 13 (29%) and 14

(28%). However, nucleosides 13 and 14 were exclusively the β anomers due to the participation of the acetate group at C2 (Scheme 4).

On the other hand, the reduction of the α-hydroxyphosphonates described by D.F.Wiemer [11] would probably give the corresponding C3 epimeric phosphonates. This seems to be confirmed by the work of Kakefuda who has studied this type of reduction and described an "inversion" on the carbon bearing the hydroxyl [18].

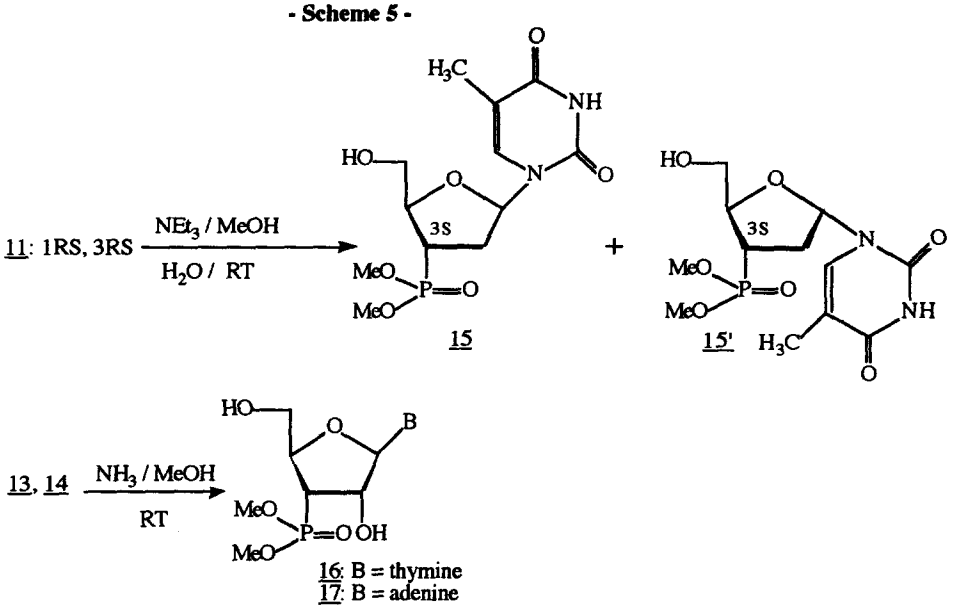
- Scheme 4 -



(HMDS: hexamethyldisilazane)

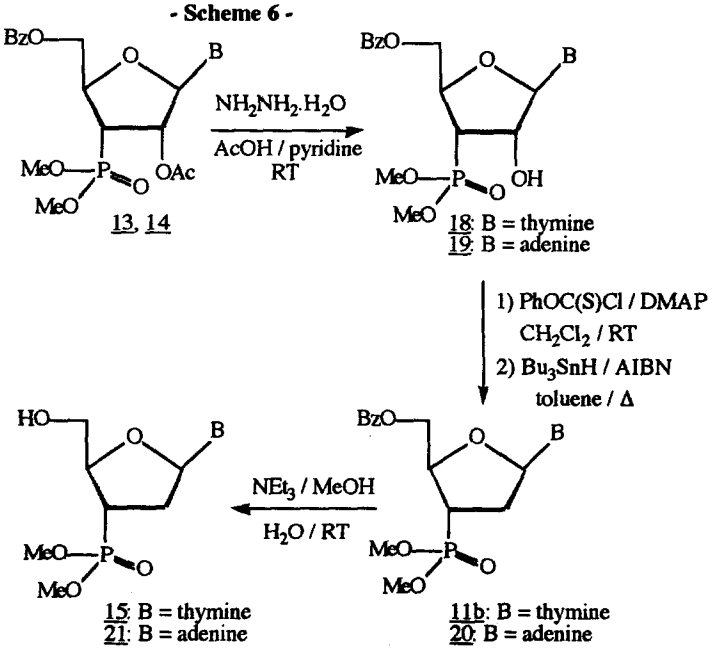
Deprotection of 11 (mixture of 4 stereoisomers) under basic conditions [19] (Scheme 5) gave 15 and 15' in equal amounts with an overall yield of 50%. Partial degradation of 11 was also observed. Surprisingly, the mixture of 4 stereoisomers (11) produced only two isomers 15 and 15' whose stereochemistry was established by ^{1}H NMR, indicating inversion in the course of reaction for the two compounds possessing the 3R configuration. This type of inversion has been noted previously with 3'-cyanonucleosides [20]. On the other hand, basic deprotection [15] of 13 and 14 afforded only the corresponding products 16 (75%) and 17 (85%).

- Scheme 5 -



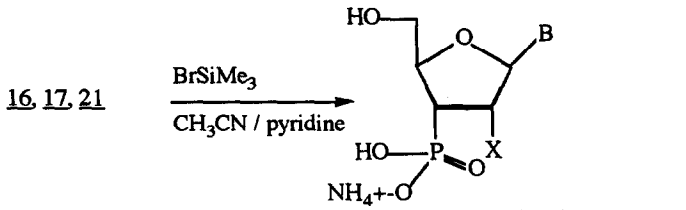
Selective deprotection of the hydroxyl at C2 by the Ishido proceeding [21] in compounds 13 and 14 (70 and 67% yields respectively), followed by deoxygenation by the Barton reaction [22-24], resulted in compounds 11b and 20, in 83 and 52% yields, respectively. Basic hydrolysis [19] then afforded 15 and 21 in 55% yields (Scheme 6).

- Scheme 6 -



Dealkylation of the phosphonate esters 16, 17 and 21 with tribromomethylsilane [25] gave the corresponding phosphonic acids 22, 23, and 24, isolated as hygroscopic monoammonium salts (Scheme 7).

- Scheme 7 -



22: B = thymine, X = OH
23: B = adenine, X = OH
24: B = adenine, X = H

Compounds 15-17 and 21-24 were screaned for anti-HIV activity in two cell lines: MT4 and CEM (tests conducted in the laboratory of Professor Kirn, University of Strasbourg, France). No significant activity was found, although cytotoxicity was observed at concentrations greater than 10^{-5}M. The inhibitatory properties of 23 and 24 against adenylate cyclase are currently under investigation.

CONCLUSION:

A direct synthesis of 2',3'-dideoxy-3'-phosphonothymidine (15) starting from 2-deoxy-D-ribose has been achieved. However, the overall yield was low due to the instability of some intermediates, as well as the lack of stereochemical selectivity inherent in this route. The use of 1,2-isopropylidene-α-D-xylofuranose as starting material affords exclusively β ribonucleosides with the desired (R) configuration at C3 (phosphonate group below the plane of the sugar). Furthemore, the method is more general in that it may be used to prepare both ribo- and 2'-deoxyribonucleosides with various nucleobases. Work in progress is directed towards the application of the synthesis to other nucleosides and the further functionalization of the 3'-phosphonate group.

EXPERIMENTAL SECTION:

Melting points were determined in open capillary tubes on a Thermotechnal apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer spectrophotometer. Microanalysis were performed in the microanalysis laboratory of ENSCM (Montpellier). The HPLC analysis and separations were performed with a Waters-Millipore system (C18 Nucleosil column, eluent: H_2O / CH_3CN). Ultra-violet spectra (UV) were recorded on a Cary 1186 spectrophotometer. Proton and carbon nuclear magnetic resonances were determined with a AC 250 Bruker spectrometer. Chemical shifts are expressed in parts per million, with TMS as reference. The multiplicity was indicated as: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet) and l (large). Phosphorus nuclear magnetic resonances were determined with a WP 200 SY Bruker spectrometer, chemical shifts are expressed in parts per million, with phosphoric acid as reference. Fast-atom bombardment mass spectra (FAB-MS) were recorded in the positive or negative ion mode on a JEOL DX 300 mass spectrometer and the matrix were glycerol (G), a mixture of glycerol and thioglycerol (50/50 v/v) (GT) or metanitrobenzyl alcohol (NBA). Optical rotations were mesured in a 1 cm cell on a Perkin-Elmer Model 241 spectropolarimeter. Thin layer chromatography (TLC) was performed on a precoated aluminium sheets of silica

gel 60 F_{254} (Merk n° 5554), visualization of products being accomplished by UV absorbance or by charring with 10% ethanolic sulfuric acid and heating. Column chromatography was performed with silica gel 60 (Merk n°9385) under atmospheric pressure. Preparative layer chromatography was performed by plates of silica gel 60 F_{254} (Merk n° 13895) (PLC). All the solvents used for the reactions were anhydrous.

1-O-Methyl-2-deoxy-5-O-benzoyl-αβ-D-ribofuranose 2.

2-deoxy-D-ribose 1 (10 g, 74.4 mmoles) in MeOH (180 ml) was treated with MeOH satured with HCl (1 mL) at room temperature. After 15 mn of stirring the reaction mixture was neutralised with Ag_2CO_3, filtered through celite, concentrated under vacuum and coevaporated with toluene.

The resulting oil was dissolved in THF (20 ml), then PPh_3 (19.5 g, 81.8 mmoles), benzoic acid (9 g, 81.8 mmoles) and DEAD (11.5 ml, 81.8 mmoles) were added successively. After stirring for 2 days at room temperature, the mixture was concentrated. The residue was purified by column chromatography over silica gel, eluted with AcOEt / hexane (gradient 30/70 to 40/60) and gave 2 (12.2 g, 72%) as an oil.

Rf = 0.7 (AcOEt)

MS m/z (FAB > 0, NBA): 253 $(M+H)^+$, 275 $(M+Na)^+$, 221 $(M-OCH_3)^+$, 105 $(PhCO)^+$, 441 $(M-OCH_3+H)^+$

^1H NMR ($CDCl_3$) δ 2.15 (m, 2H, H2 and H2'), 3.18 (m, 1H, OH), 3.28 and 3.38 (s and s, 3H, OCH_3, α/β: 42/58), 4-4,6 (m, 4H, H3, H4, H5 and H5'), 5.07 and 5.11 (dd and d, 1H, H1, α/β: 42/58), 7.4 (m, 2H, arom meta), 7.55 (m, 1H, arom para), 8 (m, 2H, arom ortho)

1-O-Methyl-2-deoxy-5-O-benzoyl-αβ-D-furan-3-ulose 3.

The sugar 2 (10 g, 39.7 mmoles) was dissolved in DMSO (75 ml) and benzene (75 ml), EDC (14.4 g, 79.4 mmoles) was added followed by dichloroacetic acid (1.6 ml, 19.8 mmoles) with stirring at room temperature. After 3 hours the reaction was complete. The reaction mixture was diluted with CH_2Cl_2 and washed successively with brine and water, dried over Na_2SO_4 and concentrated. The oil obtained was used for the next step without further purification because of its instability on silica gel.

IR: 1765 cm^{-1}

1,2-Isopropylidene-5-O-benzoyl-α-D-xylofuranose 5.

In a solution of 1,2-isopropylidene-α-D-xylofuranose (10g, 52.6 mmoles) in pyridine (230 ml) was added BzCl (6.1 ml, 52.6 mmoles) at 0°C. After stirring for 1 hour, the solution was concentrated and coevaporated with toluene. The crude product was dissolved in AcOEt and the organic phase was washed with H_2O, saturated aqueous $NaHCO_3$, H_2O and dried over Na_2SO_4. The oil was purified by column chromatography over silica gel eluted with CH_2Cl_2 / MeOH 98/2. The product was precipited in a mixture of AcOEt and hexane to give a white solid (14 g, 91%).

mp 85°C

Rf = 0.74 (AcOEt)

MS m/z (FAB > 0, GT): 295 $(M+H)^+$, 237 $(M-C_3H_6O+H)^+$

^1H NMR ($CDCl_3$) δ 1.4 (s, 3H, CH_3), 1.6 (s, 3H, CH_3), 3.4 (d, 1H, OH, J_{OH-H3}=4Hz), 4.25 (m, 1H, H3), 4.4 (m, 2H, H5 and H4), 4.65 (d, 1H, H2, J_{H1-H2}=3.63Hz), 4.8 (dd, 1H, H5', $J_{H5-H5'}$=12.7Hz and $J_{H5'-H4}$=9.19Hz), 6.1 (d, 1H, H1, J_{H1-H2}=3.63Hz), 7.5 (m, 2H, arom meta), 7.65 (m, 1H, arom para), 8.15 (m, 2H, arom ortho)

1,2-Isopropylidene-5-O-benzoyl-α-D-furanos-3-ulose 6.

The ketone 6 was prepared according to the same procedure as 3 with the 5-O-benzoyl-1,2-isopropylidene-α-D-xylofuranose 5 (14 g, 47.6 mmoles) in DMSO (100 ml) and benzene (100 ml) with EDC (12.7 g, 66.6 mmoles) and dichloroacetic acid (2 ml, 23.8 mmoles). The yield of white solid 6 was 87% (12 g).

m.p. 85 °C (Et_2O)

MS m/z (FAB > 0, GT): 293 $(M+H)^+$, 235 $(M-C_3H_6O+H)^+$

^{1}H NMR ($CDCl_3$) δ 1.45 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 4.45 (d and dd, 2H, H2 and H5, J_{H1-H2}=4.37Hz, $J_{H5-H5'}$=11.7Hz and J_{H5-H4}=4.3 Hz), 4.7 (m, 2H, H5' and H4), 6.15 (d, 1H, H1, J_{H1-H2}=4.37Hz), 7.45 (m, 2H, arom meta), 7.6 (m, 1H, arom para), 8 (m, 2H, arom ortho)

Analysis: cal. for $C_{15}H_{16}O_6$: C 61.64, H 5.48; found: C 61.70, H 5.83%

1-O-Methyl-2-deoxy-3-(R)-C-dimethylphosphono-5-O-benzoyl-α-D-furanose 7α and 1-O-methyl-2-deoxy-3-(S)-C-dimethylphosphono-5-O-benzoyl-β-D-furanose 7β.

The ketosugar 3 (39.7 mmoles) was dissolved in dimethyl phosphite (50 ml) and NEt_3 (5.4 ml, 39.7 mmoles) was added at room temperature. After stirring 4 hours the crude product was concentrated under vacuum. The resulting oil was dissolved in AcOEt and washed with saturated aqueous $NaHCO_3$ and with H_2O. The organic phase was dried over Na_2SO_4 and concentrated under vacuum. Purification by column chromatography over silica gel eluted with AcOEt afforded an anomeric mixture 7α+7β. The anomers were separated by the crystallization of 7α in Et_2O: 7α (2.7 g, 19%), 7β (5.3 g, 37%).

7α : m.p. 121-122°C (Et_2O)

Rf = 0.3 (AcOEt)

0.6 (CH_2Cl_2 / MeOH, 93/7)

MS m/z (FAB > 0, NBA): 361 $(M+H)^+$, 329 $(M-OCH_3)^+$, 105 $(PhCO)^+$, 720 $(2M)^+$

^{31}P NMR ($CDCl_3$) δ 23.4

^{1}H NMR ($CDCl_3$) δ 2.3 (d, 1H, H2), 2.6 (m, 1H, H2'), 3.35 (s, 3H, anomeric OCH3), 3.7 (d, 1H, OH, J_{HP}=25.3 Hz), 3.85 (2d, 6H, $P(OCH_3)_2$, J_{HP}=10.5Hz), 4.55 (m, 1H, H4), 4.7 (m, 2H, H5 and H5'), 5.10 (dd, 1H, H1, J_{H1-H2}=1.12 and $J_{H1-H2'}$=4.6Hz), 7.45 (m, 2H, arom meta), 7.55 (m, 1H, arom para), 8.1 (m, 2H, arom ortho)

Analysis: cal. for $C_{15}H_{21}O_7P$: C 50.00, H 5.83; found: C 49.90, H 5.63%

7β : Rf = 0.3 (AcOEt)

Rf = 0.5 (CH_2Cl_2 / MeOH, 93/7)

^{31}P NMR ($CDCl_3$) δ 22.7

^{1}H NMR ($CDCl_3$) δ 2.2 (m, 1H, H2), 2.6 (m, 1H, H2'), 3.4 (s, 3H, anomeric OCH3), 3.8 (m, 6H, $P(OCH_3)_2$), 3.95 (d, 1H, OH, J_{HP}=25Hz), 4.4 (m, 1H, H4), 4.65 (m, 2H, H5 and H5'), 5.15 (d, 1H, H1, J=5.3Hz), 7.45 (m, 2H, arom meta), 7.55 (m, 1H, arom para), 8.1 (m, 2H, arom ortho)

Analysis: cal. for $C_{15}H_{21}O_7P$: C 50.00, H 5.83; found: C 49.60, H 5.65%

1,2-Isopropylidene-3-C-dimethylphosphono-5-O-benzoyl-α-D-ribofuranose 8.

The synthesis of 8 starting from 6 (10 g, 34.2 mmoles) was carried out by the same reaction and work up procedure used for 7. The residue was purified by column chromatography over silica gel eluted with CH_2Cl_2 / MeOH (100/0 and 99/1) to give 8 (13.1 g, 95%)) as a white solid.

m.p. 58 °C

Rf = 0.25 (CH_2Cl_2 / MeOH, 97/3)

$[\alpha]_D^{20}$ = +5,7 (c=1.4, $CHCl_3$)

MS m/z (FAB > 0, NBA): 403 $(M+H)^+$, 425 $(M+Na)^+$, 345 $(M-C_3H_6O+H)^+$, 805 $(2M+H)^+$

^{31}P NMR ($CDCl_3$) δ 21.85

^{1}H NMR ($CDCl_3$) δ 1.4 (s, 3H, CH_3), 1.6 (s, 3H, CH_3), 3.3 (d, 1H, OH, J_{H-P}=20.9 Hz), 3.88 (d, 3H, $POCH_3$, J_{H-P}=10.6Hz), 3.89 (d, 3H, $POCH_3$, J_{H-P}=10.6Hz), 4.3 (ddd, 1H, H4, J_{H4-P}=27.5Hz, J_{H4-H5}=8.8 Hz and $J_{H4-H5'}$=2.78Hz), 4.6 (dd,1H, H5, J_{H5-H4}=8.8Hz and $J_{H5-H5'}$=12Hz), 4.85 (dd, 1H,

H5', $J_{H5'-H4}$=2.8Hz and $J_{H5-H5'}$=12Hz), 4.78 (dd, 1H, H2, J_{H1-H2}=3.9Hz and J_{H2-P}=8.8Hz), 5.95 (d, 1H, H1, J_{H1-H2}=3.9Hz), 7.4 (m, 2H, arom meta), 7.6 (m,1H, arom para), 8.1 (m, 2H, arom ortho)

Analysis: cal. for $C_{17}H_{23}O_9P$: C 50.75, H 5.72; found: C 50.56, H 5.86%

Epimeric mixture of 1-O-methyl-2,3-dideoxy-3-(R and S)-C-dimethylphosphono-5-O-benzoyl-α-D-furanoses 9α and 9α'.

The alcohol 7α (2.2 g, 6.1 mmoles) was dissolved in CH_3CN (40 ml) and 4-(DMAP) (3.74 g, 30.5 mmoles) was added, followed by monomethyloxalyl chloride (2.9 ml, 30.5 mmoles) by drops at 10°C. After 5 mn of stirring the reaction mixture was poured onto H_2O and AcOEt. The aqueous phase was extracted with AcOEt. The combined organic phases were washed with satured aqueous $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concentrated under vacuum. The oil was coevaporated with toluene and dissolved in the same solvent (30 ml), Bu_3SnH (2.4 ml, 9.1 mmoles) was added followed by AIBN (220 mg) under an argon atmosphere. The mixture was stirred at 100°C for 2 hours and than concentrated. The residue was purified by column chromatography over silica gel (eluent: gradient of MeOH 0% to 2% in CH_2Cl_2) to give 9α + 9α' (1.8 g, 86%).

Rf = 0.3 (AcOEt)

MS m/z (FAB > 0, NBA): 345 $(M+H)^+$, 313 $(M-OCH_3)^+$, 105 $(PhCO)^+$

^{31}P NMR ($CDCl_3$) δ 28.85 and 32.01

^1H NMR ($CDCl_3$) δ 2.6 (m, 1H, H2), 2.9 (m, 1H, H2'), 3.15 (m, 1H, H3), 3.65 and 3.70 (s and s, 3H, anomeric OCH3, 50/50), 4.15 (m, 6H, $P(OCH_3)_2$), 4.9 (m, 3H, H4, H5 and H5'), 5.4 and 5.5 (m and m, 1H, H1, 50/50), 7.8 (m, 2H, arom meta), 7.9 (m, 1H, arom para), 8.45 (m, 2H, arom ortho)

Analysis: cal. for $C_{15}H_{21}O_7P$: C 52.32, H 6.10; found: C 52.04, H 6.15%

Epimeric mixture of 1-O-methyl-2,3-dideoxy-3-(R and S)-C-dimethylphosphono-5-O-benzoyl-β–D-furanoses 9β and 9β'.

The synthesis of 9β was carried out by the same reaction and work up procedure used for 9α starting from 7β (5.15 g, 14 mmoles). The mixture of the sugars 9β and 9β' was obtained as a yield of 60% (2.85 g).

Rf = 0.30 (AcOEt)

MS m/z (FAB > 0, NBA): 313 $(M- OCH_3)^+$, 105 $(PhCO)^+$

^{31}P NMR ($CDCl_3$) δ 30.63 and 30.78

^1H NMR ($CDCl_3$) δ 2.6 (m, 1H, H2), 2.8 (m, 1H, H2'), 3.1 (m, 1H, H3), 3.3 (2s, 3H, anomeric OCH3, 55/45), 4.1 (m, 6H, $P(OCH_3)_2$), 5 (m, 3H, H4, H5 and H5'), 5.3 (m, 1H, H1), 7.8 (m, 2H, arom meta), 7.9 (m, 1H, arom para), 8.4 (m, 2H, arom ortho)

1,2-Isopropylidene-3-deoxy-3-C-dimethylphosphono-5-O-benzoyl-α-D-ribofuranose 10.

The synthesis of 10 was carried out by the same reaction and work up procedure used for 9 starting from 8 (13g, 32.3 mmoles). In this reaction only 1.5 equivalents of 4-(DMAP) and monomethyloxalyl chloride was employed. The sugar 10 was obtained with a yield of 93% (11.7g).

Rf = 0.35 (CH_2Cl_2 / MeOH, 97/3)

$[\alpha]_D^{20}$ = +53.6 (c=1.4, $CHCl_3$)

MS m/z (FAB > 0, NBA): 387 $(M+H)^+$, 329 $(M-C_3H_6O+H)^+$, 105 $(PhCO)^+$

^{31}P NMR ($CDCl_3$) δ 25.06

^1H NMR ($CDCl_3$) δ 1.35 (s, 3H, CH_3), 1.6 (s, 3H, CH_3), 3.7 (d, 3H, $POCH_3$, J_{H-P}=11Hz), 3.85 (d, 3H, $POCH_3$, J_{H-P}=11Hz), 2.6 (ddd, 1H, H3, J_{H3-P}=19.1Hz, J_{H3-H4}=10.7Hz, J_{H3-H2}=4.6Hz), 4.35 (dd, 1H, H5, J_{H5-H4}=4.5Hz and $J_{H5-H5'}$=12.3Hz), 4.6 (ddd, 1H, H4, J_{H5-H4}=4.5Hz, $J_{H5'-H4}$=1.8Hz, J_{H3-}

H_4=10.7Hz), 4.7 (dd, 1H, H5', $J_{H5'-H5}$=12.5Hz), 4.98 (d, 1H, H2, J_{H1-H2}=4.16Hz), 5.88 (d, 1H, H1, J_{H1-H2}=3.67Hz), 7.45 (m, 2H, arom meta), 7.6 (m,1H, arom para), 8 (m, 2H, arom ortho)

Analysis: cal. for $C_{17}H_{23}O_8P$: C 52.85, H 5.96; found: C 52.38, H 6.07%

N1-(2,3-dideoxy-3-C-dimethylphosphono-5-O-benzoyl-furanosyl)-thymine 11.

A suspension of thymine (700 mg) and a catalytic amount of ammonium sulfate in HMDS (30 ml) was heated under reflux for 24 hours. After cooling, the excess of HMDS was removed under vacuum. The resulting silylated thymine was dissolved in CH_3CN (2 ml), then a solution of sugar 9 (mixture of the 4 stereoisomers) (1 g, 2.9 mmoles) and $SnCl_4$ (0.7 ml, 5.8 mmoles) were added successively. The reaction mixture was heated under reflux for 15 mn, cooled and neutralised with satured aqueous $NaHCO_3$. The solution was filtered through celite, and the product was extracted with AcOEt. The organic phases were dried under Na_2SO_4 and concentrated under vacuum. Chromatography of the residue on a silica gel column (eluent: CH_2Cl_2 and CH_2Cl_2 / MeOH 97/3) led the isolation of 11 as a mixture of 4 stereoisomeric compounds (1RS, 3RS) as a foam (760 mg, 60%). A separation by HPLC yield an analytical sample of each isomer.

Mixture of the 4 isomers.

Rf = 0.35 (CH_2Cl_2 / MeOH, 93/7)

MS m/z (FAB > 0, NBA): 439 $(M+H)^+$, 313 $(M-Base)^+$

N1-(2,3-dideoxy-3-(S)-C-dimethylphosphono-5-O-benzoyl-α-D-ribofuranosyl)-thymine 11a.

^1H NMR ($CDCl_3$) δ 1.75 (d, 3H, CH_3, J_{H5-H6}=0.7Hz), 2.25 (m, 1H, H2'), 2.75 (m, 1H, H3'), 2.95 (m, 1H, H2"), 3.8 (2d, 6H, $P(OCH_3)_2$, J_{H-P}=10Hz), 4.65 (m, 3H, H4', H5" and H5"), 6.14 (dd, 1H, H1', $J_{H1'-H2'}$= 6 and $J_{H1'-H2"}$= 7.9 Hz), 7.5 (m, 4H, 3H arom and H6), 8.05 (m, 2H, arom ortho), 8.9 (sl, 1H, NH)

^{31}P NMR ($CDCl_3$) δ 29.96

N1-(2,3-dideoxy-3-(S)-C-dimethylphosphono-5-O-benzoyl-β-D-ribofuranosyl)-thymine 11b.

^1H NMR ($CDCl_3$) δ 1.6 (d, 3H, CH_3 en 5, J_{H5-H6}=1Hz), 2.4 (m, 1H, H2'), 2.8 (m, 2H, H2" and H3'), 3.85 (d, 6H, $P(OCH_3)_2$, J_{H-P}=10.8Hz), 4.6 (m, 2H, H4' and H5'), 4.85 (dd, 1H, H5", $J_{H5"-H5'}$=13.2Hz and $J_{H5"H4'}$=2.9Hz), 6.1 (dd, 1H, H1', $J_{H1'-H2'}$=6.7Hz and $J_{H1'-H2"}$=4Hz), 7.25 (d, 1H, H6, J_{H5-H6}=1.08Hz), 7.45 (m, 2H, arom meta), 7.6 (m, 1H, arom para), 8.05 (m, 2H, arom ortho), 8.6 (sl, 1H, NH)

^{31}P NMR ($CDCl_3$) δ 29.1

N1-(2,3-dideoxy-3-(R)-C-dimethylphosphono-5-O-benzoyl-α-D-ribofuranosyl)-thymine 11c.

^1H NMR ($CDCl_3$) δ 1.93 (d, 3H, CH_3, J_{H5-H6}=1Hz), 2.5 (m, 1H, H2'), 2.85 (m, 2H, H2" and H3'), 3.75 (2d, 6H, $P(OCH_3)_2$, J_{H-P}=10.9Hz), 4.6 (m, 2H, H4' and H5'), 4.9 (m, 1H, H5"), 6.11 (dd, 1H, H1', $J_{H1'-H2'}$= 2.3 and $J_{H1'-H2"}$= 6.3 Hz), 7.1 (d, 1H, H6, J_{H6-H5}= 1.1Hz), 7.5 (m, 2H, arom meta), 7.6 (m, 1H, arom para), 8.1 (m, 2H, arom ortho), 8.9 (sl, 1H, NH)

^{31}P NMR ($CDCl_3$) δ 27.56

N1-(2,3-dideoxy-3-(R)-C-dimethylphosphono-5-O-benzoyl-β-D-ribofuranosyl)-thymine 11d.

^1H NMR ($CDCl_3$) δ 2 (d, 3H, CH_3, J_{H5-H6}=0.7Hz), 2.3 (m, 1H, H2'), 2.8 (m, 2H, H2" and H3'), 3.8 (2d, 6H, $P(OCH_3)_2$, J_{H-P}=10.8Hz), 4.4 (dd, 1H, H5', $J_{H4'-H5'}$=4.6Hz and $J_{H5"-H5'}$=12Hz), 4.6 (dd, 1H, H5", $J_{H4'-H5"}$=2.6Hz and $J_{H5"-H5'}$=12Hz), 4.75 (m, 1H, H4'), 6.3 (t, 1H, H1', $J_{H1-H2'}$= $J_{H1-H2"}$=6.4Hz), 7.35 (d, 1H, H6, J_{H6-H5}= 1.1Hz), 7.45 (m, 2H, arom meta), 7.6 (m, 1H, arom para), 8.05 (m, 2H, arom ortho), 8.5 (sl, 1H, NH)

^{31}P NMR ($CDCl_3$) δ 27.93

1,2-Di-O-acetyl-3-deoxy-3-C-dimethylphosphono-5-O-benzoyl-α-D-ribofuranose 12.

A solution of 10 (13g, 33.7 mmoles) in 85% aqueous acetic acid (36 ml) was heated at 50°C and concentrated H_2SO_4 (1 ml) was added. After 5 hours the reaction mixture was concentrated and pyridine (7.6 ml, 67.4 mmoles) was added followed by acetic anhydride (61 ml). After stirring at 50°C for 30 mn the reaction was complete and the mixture was concentrated under vacuum. The resulting oil was dissolved in AcOEt and the organic phase was washed with satured aqueous $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concentrated. Purification by chromatography over silica gel (eluent CH_2Cl_2/ MeOH 97/3) gave 12 (11 g, 76%).

Rf = 0.35 (CH_2Cl_2 / MeOH, 97/3)

$[\alpha]_D^{20}$ = -10 (c=1, $CHCl_3$)

MS m/z (FAB > 0, NBA): 431 $(M+H)^+$, 371 $(M-OAc)^+$, 329 $(M-2\ OAc)^+$, 105 $(PhCO)^+$

^{31}P NMR ($CDCl_3$) δ 24.64

^1H NMR ($CDCl_3$) δ 1.85 (s, 3H, OAc), 2.15 (s, 3H, OAc), 3.05 (ddd, 1H, H3, J_{H3-P}=14.7Hz, J_{H3-H4}=10.2Hz, J_{H3-H2}=4.7Hz), 3.75 (t, 6H, $P(OCH_3)_2$, J_{H-P}=10.8Hz), 4.3 (dd, 1H, H5, J_{H5-H4}=4.4Hz and $J_{H5-H5'}$=12.3Hz), 4.75 (dd,1H, H5', $J_{H5-H5'}$=12.3Hz and $J_{H4-H5'}$=2.4Hz), 4.85 (m, 1H, H4), 5.5 (d, 1H, H2, J_{H2-H3}=4.6Hz), 6.1 (d, 1H, H1, J_{H1-P}=2Hz), 7.4 (m, 2H, arom meta), 7.5 (m, 1H, arom para), 8,1 (m, 2H, arom ortho)

Analysis: cal. for $C_{18}H_{23}O_{10}P$: C 50.23, H 5.35; found: C 50.60, H 5.58%

N1-(2-O-acetyl-3-deoxy-3-C-dimethylphosphono-5-O-benzoyl-β-D-ribofuranosyl)-thymine 13.

To a mixture of 12 (2.7 g, 6.3 mmoles) and thymine (790 mg, 6.3 mmoles) in CH_3CN (20 ml) were added HMDS (0.96 ml, 5 mmoles), TMSCl (0.63 ml, 5 mmoles) and $SnCl_4$ (0.9 ml, 7.6 mmoles). The solution was refluxed for 30 mn, cooled, neutralised quickly with satured aqueous $NaHCO_3$ and filtered through celite. The product was extracted with CH_2Cl_2. The organic phases were dried over Na_2SO_4 and concentrated under vacuum. The crude material was purified by silica gel column chromatography (gradient of methanol 0 to 2% in CH_2Cl_2) to led 13 as a foam (900 mg, 29%).

Rf = 0.35 (CH_2Cl_2 / MeOH, 93/7)

$[\alpha]_D^{20}$ = -7 (c=1, $CHCl_3$)

MS m/z (FAB > 0, NBA): 497 $(M+H)^+$, 371(M-base+H)$^+$, 437 $(M-OAc)^+$

^{31}P NMR ($CDCl_3$) δ 23.9

^1H NMR ($CDCl_3$) δ 1.65 (d, 3H, CH_3, J_{H6-H5}=1Hz), 2.15 (s, 3H, OAc), 3.3 (m, 1H, H3'), 3.75 (d, 3H, $POCH_3$, J_{H-P}=11.09Hz), 3.8 (d, 3H, $POCH_3$, J_{H-P}=11.03Hz), 4.45 (dd, 1H, H5', $J_{H5'-H4'}$=4.41Hz and $J_{H5''-H5'}$=12.8Hz), 4.75 (m, 2H, H5'' and H4'), 5.7 (m, 2H, H1' and H2'), 7 (d, 1H, H6, J_{H5-H6}=1Hz), 7.5 (m, 2H, arom meta), 7.6 (m,1H, arom para), 8.05 (m, 2H, arom ortho), 9.7 (sl, 1H, NH)

^{13}C NMR ($CDCl_3$) δ 12.2 (C5-$\underline{C}H_3$), 20.9 (CH_3 acetyl), 40 (d, C3', J_{C-P}= 150.3Hz), 53.1 (m, $P(O\underline{C}H_3)_2$), 63.9 (C5'), 75.8 (d, C2', J_{C-P}= 6.23 Hz), 78.2 (d, C4', J_{C-P}= 2.45Hz), 91.6 (d, C1', J_{C-P}= 8.36Hz), 111.5 (C5), 128.7 (arom meta), 129.5 (arom ipso), 129.6 (arom ortho), 133.5 (arom para), 136.2 (C6), 150.0, 163.7, 166.0, 169.8 (4 CO)

Analysis: cal. for $C_{21}H_{25}N_2O_{10}P$: C 50.80, H 5.04, N 5.64; found: C 50.43, H 5.00, N 5.41%

UV (EtOH 95%): λmin = 228 nm (ε=8250), λmax = 262 nm (ε=11040); (HCl 1M): λmin = 231 nm, λmax = 265 nm; (KOH 1M): λmin = 224 nm, λmax= 267 nm

N9-(2-O-acetyl-3-deoxy-3-C-dimethylphosphono-5-O-benzoyl-β-D-ribofuranosyl)-adenine 14.

The phosphonosugar 12 (3 g, 7 mmoles) was dissolved in CH_3CN (15 ml), adenine (940 mg, 7 mmoles) in CH_3CN (10 ml) and $SnCl_4$ (1.2 ml, 10.5 mmoles) were added successively at room temperature. The reaction mixture was stirred for 5 days and concentrated under vacuum. Then it was diluted with AcOEt, washed with satured aqueous $NaHCO_3$ and filtered through celite. The organic phase was washed with H_2O,

dried under Na_2SO_4 and evaporated. The resulting crude material was purified by silica gel column chromatography (eluent: CH_2Cl_2 / MeOH 100/0 and 98/2) to give 13 (1g, 28%) as a white foam.

Rf = 0.22 (CH_2Cl_2 / MeOH, 93/7)

$[\alpha]_D^{20}$ = +5 (c=1, $CHCl_3$)

MS m/z (FAB > 0, NBA): 506 $(M+H)^+$, 371 $(M\text{-base}+H)^+$, 105 (PhCO)

^{31}P NMR ($CDCl_3$) δ 24.1

^1H NMR ($CDCl_3$) δ 2.2 (s, 3H, OAc), 3.8 (t, 6H, $P(OCH_3)_2$, J_{H-P}=11,2Hz), 4.5 (ddd, 1H, H3', $J_{H3'-H4'}$=10.1Hz, $J_{H3'-P}$=19.3Hz and $J_{H3'-H2'}$=5.8Hz), 4.45 (dd, 1H, H5', $J_{H5'-H4'}$=4.6Hz and $J_{H5''-H5'}$=12.5Hz), 4.75 (dd, 1H, H5", $J_{H5''-H4'}$=2.1Hz and $J_{H5''-H5'}$=12.5Hz), 4.88 (m, 1H, H4'), 6 (sl, 1H, H1'), 6.2 (m, 1H, H2'), 6.55 (sl, 2H, NH_2), 7.35 (m, 2H, arom meta), 7.5 (m, 1H, arom para), 7.85 (m, 2H, arom ortho), 7.9 (s, 1H, H8), 8.2 (s, 1H, H2)

^{13}C NMR ($CDCl_3$) δ 21.3 (CH_3 acetyl), 39 (d, C3', J_{C-P}= 150Hz), 52-53 (m, $P(OCH_3)_2$), 63.6 (C5'), 76 (d, C2', J_{C-P}= 5.8Hz), 79 (d, C4', J_{C-P}= 4.3Hz), 90 (d, C1', J_{C-P}= 11Hz), 120.24 (C6), 128.41 (arom meta), 129.42 (arom ipso), 129.71 (arom ortho), 133.29 (arom para), 140.3 (C8), 148.98 (ethylenic C4 or C5), 152.44 (C2), 155.36 (ethylenic C4 or C5), 166.05 and 169.89 (2 CO)

Analysis: cal. for $C_{21}H_{24}O_8N_5P$: C 50.00, H 4.70, N:13.90; found: C 50.21, H: 4.82, N 13.30%

UV (EtOH 95%): λmin = 233 nm (ε=10400), λmax = 258.5 nm (ε=14000); (HCl 1M): λmin = 238 nm, λmax = 258 nm; (KOH 1M): λmin = 233 nm, λmax= 259 nm

N1-(2,3-dideoxy-3-C-dimethylphosphono-β-D-ribofuranosyl)-thymine 15 and N1-(2,3-dideoxy-3-C-dimethylphosphono-α-D-ribofuranosyl)-thymine 15' (method 1).

The stereoisomeric mixture 11 (40 mg, 0.09 mole) was dissolved in a solution of H_2O and MeOH (2 ml, v/v 1/1), and treated with NEt_3 (0.1 ml). The solution was stirred for 24 hours, and then concentrated under vacuum. Two isomers 15 and 15' were formed and separated by PLC (eluent: CH_2Cl_2/ MeOH 90/10) with several migrations (yield 50%, proportions 1/1).

NB: The treatment of 10 with methanolic ammonia or MeONa in MeOH gave the same mixture 15 and 15'.

15 and 15':

Rf = 0.15 (CH_2Cl_2 / MeOH, 93/7)

MS m/z (FAB > 0, NBA): 335 $(M+H)^+$

15:

^{31}P NMR ($CDCl_3$) δ 30.1

^1H NMR ($CDCl_3$) δ 1.8 (d, 3H, CH_3 en 5), 2.3 (m, 1H, H3'), 2.7 (m, 2H, H2' and H2"), 3.7 (2d , 6H, $P(OCH_3)_2$, J_{H-P}=13Hz and m, 1H, H5'), 4 (dd, 1H, H5", $J_{H5''-H5'}$=12Hz, $J_{H5''-H4'}$=2.6Hz), 4.2 (m, 1H, H4'), 5.95 (q, 1H, H1', J=3.5Hz), 7.55 (d, 1H, H6, J_{H5-H6}=1Hz), 9 (sl, 1H, NH)

15':

^{31}P NMR ($CDCl_3$) δ 30.4

^1H NMR ($CDCl_3$) δ 1.9 (d, 3H, CH_3, J_{H5-H6}=1Hz), 2.2 (m, 1H, H2'), 2.7 (m, 2H, H2" and H3'), 3.6 (dd, 1H, H5', $J_{H5'-H5''}$= 12.2 Hz and $J_{H5'-H4'}$= 2.9Hz), 3.75 (m, 6H, $P(OCH_3)_2$), 3.85 (dd, 1H, H5", $J_{H5'-H5''}$= 12.2Hz and $J_{H5''-H4'}$= 3.3Hz), 4.5 (m, 1H, H4'), 6.15 (m, 1H, H1'), 7.1 (d, 1H, H6), 8.1 (sl, 1H, NH)

N1-(3-deoxy-3-C-dimethylphosphono-β-D-ribofuranosyl)-thymine 16.

The phosphononucleoside 13 (380 mg, 0.8 mmole) was treated with a 0.3M solution of MeONa in MeOH (8 ml). After stirring at room temperature for 35 mn, the exces of MeONa was neutralised with dowex 50W2 resin in pyridinium form. The reaction mixture was filtered, the resin was washed with hot MeOH. Then

the solution was concentrated and coevaporated with toluene. Purification by chromatography on a silica gel column eluted with CH_2Cl_2 / MeOH 95/5 gave 16 (200 mg 75%) as a foam.

Rf = 0.14 (CH_2Cl_2 / MeOH, 90/10)

$[\alpha]_D^{20}$ = +14.5 (c = 1, DMSO)

MS m/z (FAB > 0, NBA): 351 $(M+H)^+$, 225 $(M\text{-base})^+$, 373 $(M+Na)^+$

^{31}P NMR (DMSO d6) δ 28.1

^1H NMR (DMSO d6) δ 1.7 (s, 3H, CH_3), 2.7 (ddd, 1H, H3'), 3.5 (dl, 1H, H5', $J_{H5'-H5''}$=10Hz), 3.6 (d, 6H, $P(OCH_3)_2$, J_{H-P}=11Hz), 3.8 (dl, 1H, H5", $J_{H5'-H5''}$=12Hz), 4.3 (m, 2H, H2' and H4'), 5.2 (sl, 1H, OH5'), 5.7 (s, 1H, H1'), 6.2 (d, 1H, OH3', $J_{OH-H3'}$=5.3Hz), 7 85 (s, 1H, H6), 11.3 (s, 1H, NH)

Analysis: cal. for $C_{12}H_{19}N_2O_8P$: C 41.14, H 5.43, N 8.00; found: C 41.10, H 5.75, N 7.70%

N9-(3-deoxy-3-C-dimethylphosphono-β-D-ribofuranosyl)-adenine 17.

The synthesis of 17 was carried out by the same reaction and work up procedure used for 16 starting from 14 (280 mg, 0.5 mmole). The nucleoside 17 was obtained as a foam (170 mg, 85%).

Rf = 0.1 (CH_2Cl_2 / MeOH, 93/7)

$[\alpha]_D^{20}$ = -27.2 (c = 1, DMSO)

MS m/z (FAB > 0, NBA): 360$(M+H)^+$, 225 $(M\text{-base})^+$, 382 $(M+Na)^+$

^{31}P NMR ($CDCl_3$) δ 28.42

^1H NMR (DMSO d6) δ 3.1 (ddd, 1H, H3'), 3.5 (m, 1H, H5'), 3.7 (2d , 6H, $P(OCH_3)_2$, J_{H-P}=10.7Hz), 3.8 (m, 1H, H5"), 4.4 (m, 1H, H4'), 4.8 (m, 1H, H2'), 5.25 (t, 1H, OH5', $J_{OH-H5'}$ and $J_{OH-H5''}$ =5.5Hz), 5.95 (sl, 1H, H1'), 6.35 (d, 1H, OH3', $J_{OH-H3'}$ =5.3Hz), 7.3 (sl, 2H, NH_2), 8.1 (s, 1H, H8), 8.4 (s, 1H, H2)

Analysis: cal. for $C_{12}H_{18}N_5O_6P$: C 40.11, H 5.01, N 19.50; found: C 39.97, H 5.42 , N 19.30%

N1-(3-deoxy-3-C-dimethylphosphono-5-O-benzoyl-β-D-ribofuranosyl)-thymine 18.

Hydrazine monohydrate (2.4 mmoles) in solution in acetic acid and pyridine (22 mmoles of $NH_2NH_2.H_2O$ for 66 ml of mixture pyridine-acetic acid, proportions v/v 4/1) was added to 13 (780 mg, 1.6 mmoles). After stirring 24 hours at room temperature, acetone (10 ml) was added, and the stirring was continued for 2 hours. Then the solution was evapored and partitioned between H_2O and AcOEt. The organic phase was washed with satured aqueous $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concentrated. Chromatography on a silica gel column (eluent: gradient of MeOH 2 to 5% in CH_2Cl_2) gave 18 (450 mg, 70%) as a foam.

Rf = 0.23 (CH_2Cl_2 / MeOH, 93/7)

$[\alpha]_D^{20}$ = +30 (c=1, $CDCl_3$)

MS m/z (FAB > 0, NBA): 455 $(M+H)^+$, 329(M-base+H)$^+$, 105 $(PhCO)^+$

^{31}P NMR ($CDCl_3$) δ 25.37

^1H NMR ($CDCl_3$) δ 1.6 (s, 3H, CH_3), 2.75 (ddd, 1H, H3'), 3.7 (d, 3H, $POCH_3$, J_{H-P}=11.17Hz), 3.85 (d, 3H, $POCH_3$, J_{H-P}=11.09Hz), 4.5 (dd, 1H, H5', $J_{H5'-H4'}$=3.67Hz and $J_{H5''-H5'}$=13.12Hz), 4.7 (sl, 1H, H2'), 4.8 (m, 2H, H5" and H4'), 5.7 (sl, 2H, H1' and OH), 7.4 (s, 1H, H6), 7.5 (m, 2H, arom meta), 7.6 (m, 1H, arom para), 8 (m, 2H, arom ortho), 10.55 (sl, 1H, NH)

Analysis: cal. for $C_{19}H_{23}N_2O_9P$: C 50.22, H 5.07, N 6.17; found: C 50.00, H 5.02, N 6.33%

N9-(3-deoxy-3-C-dimethylphosphono-5-O-benzoyl-β-D-ribofuranosyl)-adenine 19.

The synthesis of 19 was carried out by the same reaction and work up procedure used for 18 starting from 14 (850 mg, 1.7 mmoles). The nucleoside 19 was obtained as a foam (520 mg, 67%).

Rf = 0.26 (CH_2Cl_2 / MeOH, 90/10)

$[\alpha]_D^{20}$ = -7 (c=1, DMSO)

MS m/z (FAB > 0, NBA): 464 $(M+H)^+$, 329 $(M\text{-base}+H)^+$, 105 $(PhCO)^+$

^{31}P NMR ($CDCl_3$) δ 26.8

^1H NMR ($CDCl_3$) δ 3.4 (ddd, 1H, H3'), 3.75 (d, 3H, $POCH_3$, $J_{H\text{-}P}$=11.1Hz), 3.98 (d, 3H, $POCH_3$, $J_{H\text{-}P}$=11.02Hz), 4.55 (dd, 1H, H5', $J_{H5'\text{-}H4'}$=4.3Hz and $J_{H5''\text{-}H5'}$=12.6Hz), 4.75 (d, 1H, H5", $J_{H5''\text{-}H5'}$=12.5Hz), 4.95 (m, 1H, H4'), 5.15 (sl, 1H, H2'), 6.05 (s, 1H, H1'), 6.5 (sl, 2H, NH_2), 7.12 (sl, 1H, OH), 7.45 (m, 2H, arom meta), 7.5 (m, 1H, arom para), 7.9 (m, 2H, arom ortho), 8.05 (s, 1H, H8), 8.18 (s, 1H, H2)

Analysis: cal. for $C_{19}H_{22}O_7N_5P$: C 49.24, H 4.75, N 15.18; found: C 49.10, H 4.61, N 15.00%

N1-(2,3-dideoxy-3-C-dimethylphosphono-5-O-benzoyl-β-D-ribofuranosyl)-thymine 11b (method 2).

To a solution of 18 (400 mg, 0.9 mmole) in CH_2Cl_2 (9 ml) were added phenoxythiocarbonyl chloride (0.25 ml, 1.8 mmoles) and 4-(DMAP) (440 mg, 3.6 mmoles). After stirring 1 hour at room temperature the reaction mixture was diluted with CH_2Cl_2. The organic phase was washed successively with H_2O, 1N aqueous hydrochloric acid, H_2O, satured aqueous $NaHCO_3$ and H_2O, then dried over Na_2SO_4 and evapored to dryness. The residue was coevaporated with toluene and dissolved in the same solvent (22 ml). The solution was treated with Bu_3SnH (0.64 ml, 2.4 mmoles) and AIBN (50 mg, 0.3 mmole) and stirred at 80°C for 1 hour. After evaporation, the crude product was purified by chromatography on a silica gel column with a gradient of MeOH, 0 to 2% in CH_2Cl_2 to give 11b (385 mg, 83%) as a foam.

Rf = 0.35 (CH_2Cl_2 / MeOH, 93/7)

$[\alpha]_D^{20}$ = 0 (c=1, $CHCl_3$)

Analysis: cal. for $C_{19}H_{23}N_2O_8P$: C 52.05, H 5.25, N 6.39; found: C 52.20, H 5.30, N 6.50%

N9-(2,3-dideoxy-3-C-dimethylphosphono-5-O-benzoyl-β-D-ribofuranosyl)-adenine 20.

The synthesis of 20 was carried out by the same reaction and work up procedure used for 11b (method 2) starting from 19 (450 mg, 0.97 mmole). The nucleoside 20 was obtained as a foam (230 mg, 52%).

Rf = 0.47 (CH_2Cl_2 / MeOH, 90/10)

$[\alpha]_D^{20}$ = 0 (c=1 , DMSO)

MS m/z (FAB > 0, NBA): 448 $(M+H)^+$, 313 $(M\text{-base}+H)^+$, 105 $(PhCO)^+$

^{31}P NMR ($CDCl_3$) δ 29.64

^1H NMR ($CDCl_3$) δ 2.8 (m, 1H, H2'), 3.1 (m, 1H, H2"), 3.3 (m, 1H, H3') , 3.8 (d, 6H, $P(OCH_3)_2$, $J_{H\text{-}P}$=10.8Hz), 4.5 (dd, 1H, H5', $J_{H5'\text{-}H4'}$=4.8Hz and $J_{H5''\text{-}H5'}$=12.1Hz), 4.65 (m, 2H, H5" and H4'), 6.1 (sl, 2H, NH_2), 6.3 (m, 1H, H1'), 7.4 (m, 2H, arom meta), 7.6 (m, 1H, arom para), 7.9 (m, 2H, arom ortho), 8.0 (s, 1H, H8), 8.35 (s, 1H, H2)

Analysis: cal. for $C_{19}H_{22}O_6N_5P$: C 51.00, H 4.92, N 15.66; found: C 49.80, H 4.72, N 15.51%

N1-(2,3-dideoxy-3-C-dimethylphosphono-β-D-ribofuranosyl)-thymine 15 (method 2).

The phosphononucleoside 11b (synthetised by method 2) (80 mg, 0.18 mmole) was dissolved in a mixture of H_2O and MeOH (proportions v/v 1/1, 4 ml), NEt_3 was added (100 µl) and the reaction mixture was concentrated after 30 mn of stirring. The product was purified by PLC (eluent CH_2Cl_2 / MeOH 90/10) and was obtained as an oil (32 mg , 55%).

Rf = 0.4 (CH_2Cl_2 / MeOH, 90/10)

MS m/z (FAB > 0, NBA): 335 $(M+H)^+$, 209 $(M\text{-base})^+$,357 $(M+Na)^+$, 669 $(2M+H)^+$

^{31}P NMR ($CDCl_3$) and ^1H NMR ($CDCl_3$): see above.

N9-(2,3-dideoxy-3-C-dimethylphosphono-β-D-ribofuranosyl)-adenine 21.

The synthesis of 21 was carried out by the same reaction and work-up procedure used for 22 starting from 20 (100 mg, 0.22 mmole). The nucleoside 21 was obtained as an oil (52 mg, 55%).

Rf = 0.25 (CH_2Cl_2 / MeOH, 90/10)

MS m/z (FAB > 0, NBA): 344 $(M+H)^+$, 366 $(M+Na)^+$

^{31}P NMR (DMSO d6) δ 31.80

^1H NMR ($CDCl_3$) δ 2.7 (m, 2H, H2' and H3'), 3.1 (m, 1H, H2"), 3.5 (m, 2H, H5' and H5"), 3.7 (d, 6H, $P(OCH_3)_2$, J_{H-P}=10.7Hz), 4.2 (m, 1H, H4'), 5.2 (sl, 2H, NH_2), 6.3 (q, 1H, H1', J=3.7Hz), 8.1 (s, 1H, H8), 8.4 (s, 1H, H2)

General procedure for the deprotection of the phosphonic acids for 16, 17 and 21.

The phosphononucleosides 16, 17 and 21 were dissolved in CH_3CN (2 ml for 50 mg of nucleoside), a catalytic amount of pyridine was added, then the reaction mixture was treated with $BrSiMe_3$ (10 equivalents) under an argon atmosphere, and stirred at room temperature for 14 hours. Pyridine (0.5 ml for 50 mg de nucleoside) and water (1 ml for 50 mg) were added. The stirring was continued for 2 hours, and the solution was washed two times with Et_2O and concentrated. The products, as hygroscopic ammonium salts 22, 23 and 24, were obtained as white hygroscopic solids after a purification with PLC (eluent: 2-propanol/ NH_4OH/ H_2O, 7/1/2) and lyophilisation.

N1-(2-hydroxy-3-deoxy-3-C-dihydroxyphosphono-β-D-ribofuranosyl)-thymine (ammonium salt) 22.

Rf = 0.5 (2-propanol / NH_4OH / H_2O, 7/1/2)

MS m/z (FAB < 0, G): 343 (anion+Na^+), 321 (anion)

^{31}P NMR (D_2O) δ 16.80

^1H NMR (D_2O) δ 1.9 (d, 3H, CH_3), 2.6 (m, 1H, H3'), 3.9 (d, 1H, H5', $J_{H5"-H5'}$= 12Hz), 4.15 (d, 1H, H5", $J_{H5"-H5'}$= 12.3Hz), 4.6 (m, 2H, H2' and H4'), 5.85 (s, 1H, H1'), 7.9 (s, 1H, H6), 9 (sl, 1H, NH),

N9-(3-deoxy-3-C-dihydroxyphosphono-β-D-ribofuranosyl)-adenine (ammonium salt) 23.

Rf = 0.3 (2-propanol / NH_4OH / H_2O, 7/1/2)

MS m/z (FAB < 0, G): 330 (anion)

RMN ^{31}P (D_2O), δ: 14.66

RMN ^1H (D_2O), δ: 3.2 (ddd, 1H, H3'), 4.5 (dd, 1H, H5', $J_{H5'-H5"}$=12.3Hz and $J_{H4'-H5'}$=4.6Hz), 4.7 (dd, 1H, H5", $J_{H5'-H5"}$=12.37Hz and $J_{H4'-H5'}$=3.5Hz), 5.3 (m, 1H, H4'), 5.6 (m, 1H, H2'), 6.8 (s, 1H, H1'), 9 and 9.1 (2s, 2H, H2 and H8)

N9-(2,3-dideoxy-3-C-dihydroxyphosphono-β-D-ribofuranosyl)-adenine (ammonium salt) 24.

Rf = 0.3 (2-propanol / NH_4OH / H_2O, 7/1/2)

MS m/z (FAB < 0, G): 314 (anion)

RMN ^{31}P (D_2O), δ: 17.66

RMN ^1H (D_2O), δ: 2-3 (3sl, 3H, H2', H2" and H3'), 3.7 (d, 1H, H5', $J_{H5'-H5"}$=12.4), 3.9 (d, 1H, H5", $J_{H5'-H5"}$=12.2Hz), 4.4 (m, 1H, H4'), 6.3 (s, 1H, H1'), 8.2 (s, 1H, H8), 8.4 (s, 1H, H2)

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