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Regioisomers of 2′,3′-dideoxynucleosides related to 2-(phosphonylmethoxy)ethyl nucleosides

Thomas Kofoed[1], Abd El-Hamid AA Ismail[1], Erik B Pedersen[1]*, Claus Nielsen[2]

[1] *Department of Chemistry, Odense University, DK-5230 Odense M;*
[2] *Retrovirus Laboratory, Department of Virology, Statens Seruminstitut, Artillerivej 5, DK-2300 Copenhagen, Denmark*

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Summary — Methyl 2,3-dideoxy-5-iodo-D-pentofuranoside was synthesized in four steps from 2-deoxy-D-ribose. The 5-phosphonate nucleoside was synthesized by an Arbuzov reaction between the 5-iodo sugar and triethyl phosphite, followed by condensation with thymine and N^4-isobutyrylcytosine. The regioisomers with the phosphonate at the 1′-position and the nucleobases at the 5′-position were synthesized by reaction of the nucleobase with the 5-tosyl sugar and subsequent reaction with triethyl phosphite using TMS triflate as catalyst. The diethylphosphonates were deprotected using bromotrimethylsilane.

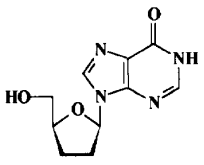
nucleoside synthesis / 2′,3′-dideoxy nucleoside / 5′-phosphonate nucleoside / glycosylphosphonate / PMEA analog

Résumé — **Régioisomères de 2′,3′-didéoxy nucléosides à partir de 2-(phosphonylméthoxy)éthyl nucléosides.** Le 2,3-didéoxy-5-iodo-D-pentafuranoside de méthyle a été synthétisé en quatre étapes à partir du 2-déoxy-D-ribose. Le nucléoside 5-phosphonate a été synthétisé par une réaction d'Arbuzov entre le 5-iodo sucre et le phosphite de triéthyle, suivie d'une condensation avec la thymine et la N^4-isobutyrylcytosine. Les régioisomères du phosphonate en position 1′ et les bases en position 5′ sont synthétisés par réaction de la base avec le sucre 5-tosyl suivie d'une réaction avec le phosphite de triéthyle, en utilisant le TMSOTf comme catalyseur. Les phosphonates de diéthyle sont ensuite libérés par utilisation du bromotriméthylsilane.

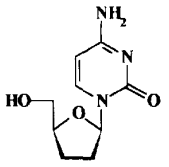
synthèse de nucléoside / 2′,3′-didéoxy nucléoside / 5′-phosphonate nucléoside / glycosylphosphonate / analogue PMEA

Introduction

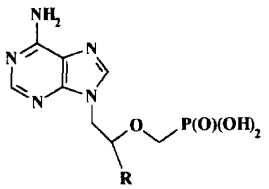
The finding that some nucleoside analogs possess activity against the human immunodeficiency virus (HIV) has triggered a huge interest in the development of novel nucleosides. As a general feature these analogs almost all contain a sugar and a nucleobase part, like naturally occurring nucleosides. The 2′,3′-dideoxynucleosides belong to a class of closely related analogs, in that the only modification in the sugar part lies in the missing 3′-hydroxy groups, eg, 2′,3′-dideoxyinosine (ddI) [1] and 2′,3′-dideoxycytidine (ddC) [2]. Among the acyclic nucleosides we find phosphonates 9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA) [3, 4] and 9-(2-phosphonylmethoxyethyl)adenine (PMEA) [5]. These compounds have only the adenine nucleobase intact compared to adenosine, but do however show great activity against HIV and also against other retroviruses, eg, herpesviruses.



ddI



ddC



PMEA R=H
HPMPA R=CH_2OH

The commonly used methods for the synthesis of modified nucleosides can be divided into two categories: (i) those using the naturally occurring ribo or 2′-deoxy nucleosides as starting material in a linear synthesis; and (ii) those which incorporate the nucleobase by a condensation reaction with a sugar derivative in a convergent synthesis. The main advantage of using a nucleoside as a starting material lies in the control of the stereochemistry at the anomeric carbon, but the

* Correspondence and reprints

method is limited by the relatively few naturally occurring nucleosides. The convergent synthesis opens a wealth of possibilities for introducing structural modifications into both the nucleobase and the sugar part of the molecule. A major drawback is the glycosylation reaction which normally results in an anomeric mixture, which is in many cases difficult to separate into isomers.

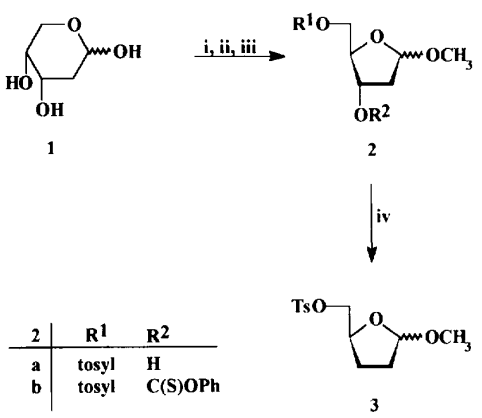
The 2′,3′-dideoxynucleosides have mainly been prepared by the first strategy through the deoxygenation of the two (ribo) or one (2′-deoxy) hydroxy groups. The acyclic phosphonates do not contain a naturally occurring sugar part and they are therefore synthesized following another strategy. Two principal methods have been used: introduction of the phosphonomethyl ether functionality into N^9-(2-hydroxyalkyl)adenine or by alkylation of adenine with a synthon already containing the phosphonomethyl group [4, 5]. The latter method is of advantage if a series of base-modified analogs is to be synthesized. PMEA has been synthesized by either of these two methods [3]; the second method afforded the highest yields. Several base-modified [6] and 2-substituted [7] PMEA analogs have been synthesized, but these analogs do not show the same activity as PMEA and HPMPA. PMEA recently entered clinical trials for the treatment of HIV infection. The early results were rather disappointing since the cellular uptake and bioavailability of PMEA after oral administration was found to be poor [8], probably due to the phosphonyl group exhibiting a negative charge at physiological pH. Therefore, bis(pivaloyloxymethyl)esters of PMEA (bis(pom) PMEA) have been examined as prodrugs, and the bis(pom) PMEA showed a 2.5-fold lower therapeutic index as compared to the unmodified analog [8]. Otmar et al [9, 10] have extended the PMEA research to cyclic analogs of PMEA and various stereomers of [5-adenin-9-yl-5-deoxyfuranosyl]phosphonates and its 2-deoxy analogs were synthesized.

Since nucleosides showing activity against HIV need to be phosphorylated in vivo, there would be a chance of enhancing the biological activity of 2′,3′-dideoxynucleosides by introducing a phosphonate group at the 5′-position. An example of this idea was reported by Hakimelahi et al [11] who in a linear synthesis obtained 3′,5′-dideoxythymidine-5′-phosphonic acid and found moderate activity for this compound against HIV-1.

In our effort to find new anti-HIV agents, we were interested in the synthesis of 2′,3′-dideoxy cyclic analogs of PMEA. Here we report that the same sugar synthon can be used for both the synthesis of such PMEA analogs and a convergent synthesis of 2′,3′,5′-trideoxynucleoside-5-phosphonates.

Results and discussion

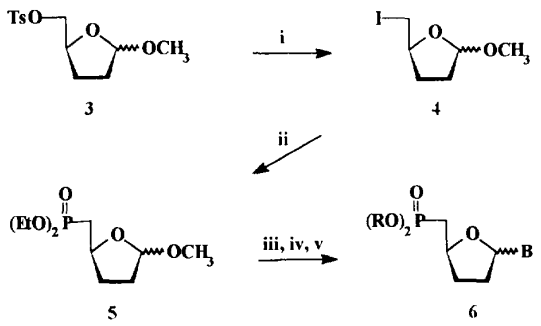
2-Deoxy-D-ribose **1** was used as the starting material. The methyl furanoside **2** was prepared by the treatment of **1** with 1% HCl in methanol [12, 13] and was selectively 5-*O*-protected with *p*-toluenesulfonyl chloride in pyridine to give methyl 2-deoxy-5-*O*-(*p*-toluenesulfonyl)-α,β-D-*erythro*-pentofuranoside **2a**. The 3′-hydroxy group was deoxygenated in two steps. Treatment with phenyl chlorothiocarbonate in dry acetonitrile in the presence of 4-(dimethylamino)pyridine



Scheme 1. (i) MeOH, HCl; (ii) TsCl, pyridine; (iii) PhOC(S)Cl, DMAP, CH_3CN, (iv) Bu_3SnH, AIBN, toluene.

(DMAP) for 4 h at room temperature gave the thionocarbonate **2b** (77%), which on tributyltin hydride mediated cleavage of the phenyl thionocarbonate as described by Barton [14] furnished the methyl 2,3-dideoxy-5-*O*-tosyl-α,β-D-pentofuranoside **3** in 73% yield (scheme 1).

The 5′-C-P bond was formed via an Arbuzov reaction. The 5-iodo compound **4** was prepared by reaction of **3** with a mixture of sodium iodide in butanone at reflux for 16 h. After column chromatography **4** was obtained in 67% yield. This compound was heated at 120 °C for 48 h with triethyl phosphite in an Arbuzov reaction to furnish the methyl 5-diethylphosphono-2,3,5-trideoxy-α,β-pentofuranoside **5** in 61% yield. The standard procedure involved in the glycosylation reaction developed by Vorbrüggen et al utilizes 1,1,1,3,3,3-hexamethyldisilazane (HMDS) as a silylating agent and trimethylsilyl trifluoromethanesulfonate (TMS triflate) as a Lewis acid. TMS triflate is normally preferred as a Lewis acid as it is considered less acidic than $SnCl_4$, but we were very successful in using $SnCl_4$ as Lewis acid and *N*,*O*-bis(trimethylsilyl) acetamide (BSA) as silylating agent. A condensation reaction with BSA and $SnCl_4$ can be done in less than 3 h compared to 1 or 2 days when using the HMDS/TMS triflate procedure. The condensation of the sugar derivative **5** with the nucleobases was achieved by activating the base with BSA in acetonitrile. The silyl derivative thus obtained was condensed in situ with the sugar derivative **5**, as described by Vorbrüggen et al [15, 16] in the presence of $SnCl_4$. As nucleobases we used thymine and N^4-isobutyrylcytosine, and we obtained the 5′-diethylphosphono-2′,3′,5′-trideoxy nucleosides **6a** and **6b** in 73–81% yields as anomeric mixtures (1:1). We were unable to separate the anomers by silica-gel column chromatography, and the nucleotides were therefore deprotected as an anomeric mixture with bromotrimethylsilane in acetonitrile [17, 18]. This gave the final nucleotides 5′-phosphono-2′,3′,5′-trideoxy-α,β-D-nucleosides **6c** in 94% yield and **6d** in 76% yield using an additional step with methanolic amonia for deprotection of the amino group. All the final deprotected

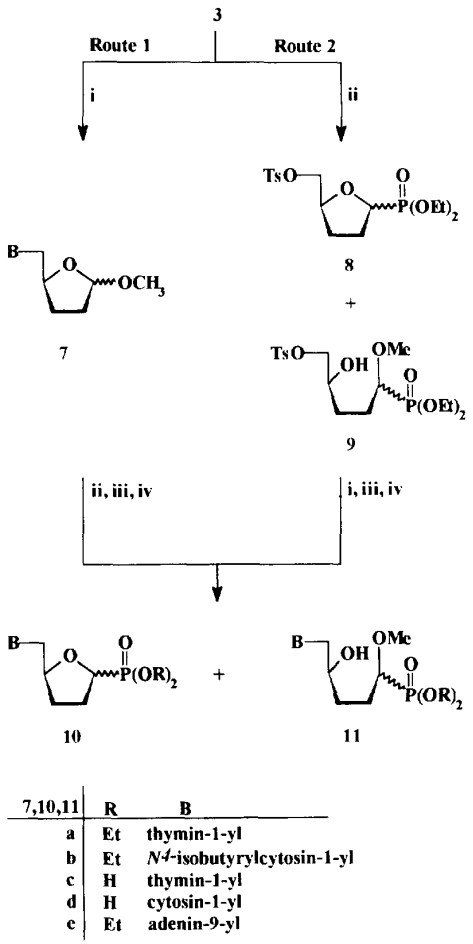


6	R	B
a	Et	thymin-1-yl
b	Et	N^4-isobutyrylcytosin-1-yl
c	H	thymin-1-yl
d	H	cytosin-1-yl

Scheme 2. (i) NaI, butanone; (ii) $P(OEt)_3$; (iii) silylated nucleobase, $SnCl_4$, CH_3CN; (iv) $(CH_3)_3SiBr$, CH_3CN; (v) NH_3, CH_3OH.

compounds were purified by ion-exchange column chromatography using Dowex CCR-2 (Na^+-form), which gave the final product as the disodium salts (scheme 2).

The cyclic PMEA analogs **10** were synthesized in two ways. The first approach consists of the condensation of the sodium salt of thymine, N^4-isobutyrylcytosine or adenine with the 2,3-dideoxy-5-tosyl sugar synthon **3** using the method developed by Holý et al [19, 20]. The nucleobase was treated with sodium hydride in DMF at 80 °C for 1 h, and after addition of the sugar **3** the mixture was stirred at 80–90 °C for 15–18 h. The mixture was concentrated and purified by column chromatography on silica gel giving **7** in 47–60% yield. The adenyl derivative **7e** was isolated as a pure anomer. Selective decoupling experiments confirmed N-9 glycosylation of adenine. Selective decoupling of H-5′ changed the coupling pattern of C-8 from a doublet of triplets into a doublet, only, and C-4 from a multiplet into a doublet of doublets. The introduction of the phosphono group is based on the known reaction of carbocations with trialkyl phosphite leading to dialkyl phosphonates. Treatment of a mixture of the furanoside **7** and triethyl phosphite (1.5 equiv) in methylene chloride or acetonitrile with TMS triflate (1.2 equiv) at room temperature gave an anomeric mixture (3:2) of the glycosylphosphonate **10** in 55–76% yield. It was not possible to separate the two anomers by simple column chromatography and the anomeric mixture was deprotected as described for **6**. Only for **10e** did we fail to obtain the corresponding deprotected compound. To our surprise we also isolated a small amount of the ring open analog **11a** (24%) in the synthesis of **10a**. We [21, 22] and others [23] have previously observed a similar ring open product during coupling reaction of methyl furanoside with a silylated nucleobase. This side product is most likely formed via silylation of the ring oxygen followed by ring opening. The compound **10a** was also synthesized by introducing the phosphono group in the first step giving **8** (60%), followed by the condensation with the sodium salt of thymine giving **10a** in 34% yield. The ring open analog **9** was also isolated in 26% yield. This strategy is of advantage if a large number of derivatives with various bases must be synthesized. Unfortunately, the overall yield was not as good compared to route 1, and the purification of the final compound was more troublesome due to the formation of several side products. Compound **10e** was also synthesized according to route 2 but the purity of the raw product was rather low when compared to the synthesis of **10e** in route 1 (scheme 3).



7,10,11	R	B
a	Et	thymin-1-yl
b	Et	N^4-isobutyrylcytosin-1-yl
c	H	thymin-1-yl
d	H	cytosin-1-yl
e	Et	adenin-9-yl

Scheme 3. (i) Nucleobase, DMF, NaH; (ii) $P(OEt)_3$, CH_2Cl_2 or MeCN, TMS-triflate; (iii) $(Me_3)_3SiBr$, CH_3CN; (iv) NH_3, CH_3OH.

The new compounds were identified by NMR and by FAB-positive, negative mass spectra and compared with similar compounds [9–11, 24, 25]. The biological test results showed that the nucleosides **6c,d** and **10c,d** did not show any significant activity at 100 μM against HIV-1 in MT-4 cells. Compound **10e** showed toxicity against the MT-4 cells at 100 μM, but no activity was observed against HIV-1 at lower concentrations. Expression of HIV in culture medium was quantified by HIV antigen detection ELISA.

Experimental section

^{1}H NMR spectra were recorded at 250 MHz, ^{13}C NMR spectra at 62.5 MHz and ^{31}P NMR spectra at 101.3 MHz on a Bruker AC 250 spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane for ^{1}H NMR, relative to DMSO-d_6 or $CDCl_3$ for ^{13}C NMR and relative to 85% H_3PO_4 for ^{31}P NMR. FAB mass spectra were recorded on a Kratos MS 50 RF spectrometer. Silica gel Merck 230–400 Mesh was used for column chromatography. Microanalysis was carried out at the HC Ørsted Institute, Universitetsparken, Copenhagen Ø, DK-2100. All new compounds were obtained as oils.

Methyl 5-iodo-2,3,5-trideoxy-α,β-D-pentofuranoside ***4***

Methyl 2,3-dideoxy-5-*O*-tosyl-α,β-D-pentofuranoside **3** (6.0 g, 21.0 mmol) was dissolved in a mixture of sodium iodide (15 g, 100 mmol) and butanone (300 mL) and refluxed at 85 °C for 16 h. After filtration and concentration, the resulting residue was dissolved in a mixture of H_2O (100 mL) and Et_2O (100 mL). The water phase was extracted with Et_2O and the combined organic fractions were washed with saturated aqueous sodium thiosulfate (100 mL) and with water (100 mL), followed by drying ($MgSO_4$), filtration and concentration. The product **4** was purified by column chromatography (200 g silica gel) using CH_2Cl_2 as eluent. Yield 2.8 g (67%).

^{1}H NMR ($CDCl_3$) predominant anomer: δ 1.54–2.25 (m, 4H, H-2, H-3), 3.12–3.45 (m, 2H, H-5), 3.35 (s, 3H, OCH_3), 4.24–4.35 (m, 1H, H-4), 5.06–5.11 (m, 1H, H-1).

^{13}C NMR ($CDCl_3$) predominant anomer: δ 11.03 (C-5), 29.62 (C-3), 33.44 (C-2), 54.57 (OCH_3), 80.21 (C-4), 105.64 (C-1).

Methyl 5-diethylphosphono-2,3,5-trideoxy-α,β-D-pentofuranoside ***5***

Compound **4** (2.8 g, 11.57 mmol) was dissolved in freshly distilled $P(OEt)_3$ (25 mL, 24.23 g, 146 mmol) and stirred at 120 °C for 48 h under nitrogen. The phosphite was removed in vacuo at 40 °C and the crude product purified by column chromatography (150 g silica gel) using a gradient of CH_2Cl_2 in methanol (1 → 5%). Yield 1.78 (61%).

^{1}H NMR ($CDCl_3$) predominant anomer: δ 1.30–1.37 (m, 6H, 2 × CH_3), 1.62–2.30 (m, 6H, H-2, H-3, H-5), 3.33 (s, 3H, OCH_3), 4.04–4.35 (m, 1H, H-4), 5.06–5.11 (m, 1H, H-1).

^{13}C NMR ($CDCl_3$) predominant anomer: δ 16.26, 16.35 (2 × Et), 30.35 (d, J = 7.7 Hz, C-3), 32.00 (C-2), 32.22 (d, J = 139 Hz, C-5), 54.56 (OCH_3), 61.43 (m, 2 × Et), 72.45 (C-4), 104.79 (C-1).

^{31}P NMR ($CDCl_3$): δ 28.40 major (28.50 minor).

FAB(positive) MS: m/z 253 ($M + H^+$).

1-[5-(Diethylphosphono)-2,3,5-trideoxy-α,β-D-pentofuranosyl]thymine ***6a***

Thymine (330 mg, 2.62 mmol) was dissolved in a mixture of CH_3CN (8 mL) and *N*,*O*-bis(trimethylsilyl) acetamide (BSA) (1.07 g, 1.3 mL, 5.24 mmol). After heating at 80 °C for 30 min the mixture was cooled at 0 °C and the 5′-phosphono sugar **5** (300 mg, 1.19 mmol) dissolved in CH_3CN (2 mL) was added to the silylated thymine. To this clear mixture was added $SnCl_4$ dropwise (3.1 g, 1.4 mL, 11.96 mmol), and after stirring at room temperature for 1 h the reaction was quenched by addition of EtOAc (50 mL) and washing with saturated aqueous $NaHCO_3$ (10 mL) and with H_2O (10 mL) followed by drying ($MgSO_4$) and concentration. The pure product was obtained after silica gel column chromatography (100 g) using CH_2Cl_2/CH_3OH (95:5) as eluent. This gave **6a** as a 1:1 anomeric mixture (300 mg, 73%).

^{1}H NMR ($CDCl_3$) predominant anomer: δ 1.34, 1.35 (2 × t, 6H, J = 7.1 Hz, 2 × Et), 1.80–2.61 (m, 6H, H-2′, H-3′, H-5′), 1.94 (d, 3H, J = 1.1 Hz, 5-CH_3), 4.07–4.21 (m, 4H, 2 × Et), 4.64 (m, 1H, H-4′), 6.08 (t, 1H, J = 5.7 Hz, H-1′), 7.20 (d, 1H, J = 1.1 Hz, H-6).

^{13}C NMR ($CDCl_3$) predominant anomer: δ 12.48 (5-CH_3), 16.23, 16.33 (2×Et), 31.14 (d, J = 7.7 Hz, C-3′), 32.30 (d, J = 130 Hz, C-5′), 32.59 (C-2′), 61.80 (m, 2 × Et), 76.18 (C-4′), 86.57 (C-1′), 110.53 (C-5), 134.85 (C-6), 150.43 (C-2), 164.12 (C-4).

^{31}P NMR ($CDCl_3$) predominant anomer: δ 27.1 major (27.3 minor).

FAB (positive) MS: m/z 347 ($M + H^+$).

Anal $C_{14}H_{23}N_2O_6P \cdot 0.25H_2O$, calc: C 47.93, H 6.75, N 7.98. Found: C 47.82, H 6.78, N 7.91.

1-[5-(Diethylphosphono)-2,3,5-trideoxy-α,β-D-pentofuranosyl]-N^4-isobutyrylcytosine ***6b***

The same procedure as for the synthesis of **6a** was used, except that isobutyrylcytosine (480 mg, 2.65 mmol) was used instead of thymine. Yield 452 mg (81%).

^{1}H NMR ($CDCl_3$) predominant anomer: δ 1.14, 1.17 (2 × s, 6H, 2 × CH_3), 1.29, 1.30 (2 × t, 6H, J = 7.1 Hz, 2 × Et), 1.84–2.74 (m, 7H, CH, H-2′, H-3′, H-5′), 4.04–4.17 (m, 4H, 2 × Et), 4.64 (m, 1H, H-4′), 6.00 (m, 1H, H-1′), 7.40 (d, 1H, J = 3.0 Hz, H-5), 7.84 (d, 1H, J = 3.0 Hz, H-6).

^{13}C NMR ($CDCl_3$) predominant anomer: δ 16.27, 16.37 (2 × Et), 18.96, 18.99 (2 × CH_3), 31.51 (d, J = 7.9 Hz, C-3′), 32.11 (d, J = 133 Hz, C-5′), 36.29 (C-2′), 61.75 (m, 2 × Et), 76.64 (C-4′), 88.59 (C-1′), 96.00 (C-5), 143.13 (C-6), 155.06 (C-2), 162.62 (C-4), 177.36 (C=O).

^{31}P NMR ($CDCl_3$): δ 27.3 major (27.1 minor).

FAB (positive) MS: m/z 402 ($M + H^+$).

Anal $C_{17}H_{28}N_3O_6P \cdot H_2O$, calc: C 48.68, H 7.21, N 10.24. Found: C 49.05, H 7.22, N 9.88.

1-[5-Phosphono-2,3,5-trideoxy-α,β-D-pentofuranosyl]-thymine ***6c***

5′-(Diethylphosphono)thymidine **6a** (110 mg, 0.34 mmol) was dissolved in CH_3CN (2 mL) and at 0 °C was added dropwise bromotrimethylsilane (0.2 mL, 1.1 mmol). The mixture was stirred at room temperature for 4 h, followed by concentration. The residue was taken up in H_2O (2 mL) and washed with EtOAc (3 × 1 mL). The aqueous phase was added to a Dowex (Na^+ form) column, followed by concentration. This gave **6c** as an anomeric mixture (100 mg, 94%).

^{1}H NMR (D_2O) predominant anomer: δ 1.73–2.47 (m, 6H, H-2′, H-3′, H-5′), 1.84 (s, 3H, 5-CH_3), 4.17–4.64 (m, 1H, H-4′), 6.03 (m, 1H, H-1′), 7.50 (s, 1H, H-6).

^{13}C NMR (D_2O): δ 14.17 (5-CH_3), 31.14 (d, J = 7.7 Hz, C-3′), 33.98 (C-2′), 35.21 (d, J = 138.3 Hz, C-5′), 77.50 (C-4′), 89.32 (C-1′), 113.56 (C-5), 140.27 (C-6), 154.28 (C-2), 169.30 (C-4).

^{31}P NMR (D_2O): δ 24.1 major (23.4 minor).

FAB (negative) MS: m/z 289 ($M - H^+$).

1-[5-Phosphono-2,3,5-trideoxy-α,β-D-pentofuranosyl]-cytosine ***6d***

The same procedure as for the synthesis of **6c** was used, except that (diethylphosphono)cytosine **6b** (375 mg, 0.93 mmol) was used instead of **6a** and that the product

obtained was treated with concentrated aqueous ammonia. Yield 195 mg (76%).

^{1}H NMR (D_2O) predominant anomer: δ 1.61–2.24 (m, 6H, H-2′, H-3′, H-5′), 4.05–4.27 (m, 1H, H-4′), 5.99 (m, 1H, H-1′), 6.09 (d, 1H, J = 7.7 Hz, H-5), 7.83 (d, 1H, J = 7.1 Hz, H-6).

^{13}C NMR (D_2O) predominant anomer: δ 31.33 (d, J = 9.9 Hz, C-3′), 33.40 (d, J = 132 Hz, C-5′), 35.27 (C-2′), 79.24 (C-4′), 88.96 (C-1′), 95.59 (C-5), 144.13 (C-6), 152.52 (C-2), 162.60 (C-4).

^{31}P NMR ($CDCl_3$): δ 25.6 major (23.6 minor).

FAB (negative) MS: m/z 274 ($M - H^+$).

Methyl 5-C-(thymin-1-yl)-2,3,5-trideoxy-α,β-D-pentofuranoside **7a**

Thymine (750 mg, 6.0 mmol) was added to a suspension of NaH (300 mg of a 60% oil dispension, 7.5 mmol) in dry DMF (50 mL). The mixture was heated at 80 °C for 1 h. A solution of the sugar **3** (1.7 g, 6.0 mmol) in dry DMF (7.5 mL) was slowly added over 1 h and the resulting mixture was stirred at 90 °C for 15 h. After concentration in vacuo, the resulting residue was dissolved in hot $CHCl_3$ (2 × 100 mL), filtered and concentrated. Product **7** was purified by silica-gel column chromatography (150 g) using a linear gradient of CH_2Cl_2/MeOH (1 → 5%). Yield 705 mg (49%).

^{1}H NMR ($CDCl_3$) predominant anomer: δ 1.69–2.09 (m, 4H, H-2′, H-3′), 1.92 (d, 3H, J = 1.1 Hz, 5-Me), 3.39 (s, 3H, OMe), 3.48 (dd, 1H, J = 7.5, 14.2 Hz, H-5′), 4.13 (dd, 1H, J = 2.8, 14.1 Hz, H-5′), 4.28–4.37 (m, 1H, H-4′), 4.99 (m, 1H, H-1′), 7.17 (d, 1H, J = 1.2 Hz, H-6).

^{13}C NMR ($CDCl_3$) predominant anomer: δ 13.12 (5-Me), 26.76 (C-2′), 32.98 (C-3′), 54.29, 55.18 (C-5′, OMe), 78.20 (C-4′), 105.23 (C-1′), 109.02 (C-5), 139.80 (C-6), 151.75 (C-2), 163.86 (C-4).

Methyl 5-C-(N^4-isobutyrylcytosin-1-yl)-2,3,5-trideoxy-α,β-D-pentofuranoside **7b**

The same method as for **7a** was employed except that N^4-isobutyrylcytosine (1.09 g, 6.0 mmol) was used instead of thymine. This gave **7b** in 47% yield after column chromatography.

^{1}H NMR ($CDCl_3$) predominant anomer: δ 1.19 (s, 6H, 2 × CH_3), 1.63–2.15 (m, 4H, H-2′, H-3′), 3.37 (s, 3H, OMe), 3.82 (dd, 1H, J = 7.2, 13.7 Hz, H-5′), 4.24 (dd, 1H, J = 2.8, 13.7 Hz, H-5′), 4.34–4.45 (m, 2H, H-4′, CH), 4.99 (m, 1H, H-1′), 7.42 (d, 1H, J = 7.3 Hz, H-5), 7.76 (d, 1H, J = 7.3 Hz, H-6), 9.29 (br s, 1H, NH).

^{13}C NMR ($CDCl_3$) predominant anomer: δ 18.88 (2 × CH_3), 26.61 (C-2′), 32.41 (C-3′), 36.17 (CH), 54.52, 55.44 (C-5′, OCH_3), 77.23 (C-4′), 95.88 (C-5), 105.84 (C-1′), 149.73 (C-6), 155.86 (C-2), 162.67 (C-4), 177.33 (C=O).

Methyl 5-C-(adenin-9-yl)-2,3,5-trideoxy-α,β-D-pentofuranoside **7e**

The same method as for **7a** was employed except that adenine (810 mg, 6.0 mmol) was used instead of thymine. This gave **7e** in 60% yield after column chromatography.

^{1}H NMR (DMSO-d_6): δ 1.65–1.97 (m, 4H, H-2′, H-3′), 3.24 (s, 3H, OMe), 4.16 (dd, 1H, J = 7.3, 14.0 Hz, H-5′), 4.29 (dd, 1H, J = 4.4, 14.0 Hz, H-5′), 4.40–4.45 (m, 1H, H-4′), 4.92 (m, 1H, H-1′), 7.22 (s, 2H, NH_2), 8.11 and 8.15 (2 × s, 2H, H-2 and H-8).

^{13}C NMR (DMSO-d_6): δ 26.35 (C-2′), 32.16 (C-3′), 48.06 (C-5′), 54.07 (OCH_3), 77.58 (C-4′), 104.78 (C-1′), 118.41 (C-5), 141.03 (C-8), 149.54 (C-4), 152.28 (C-2), 155.84 (C-6).

FAB (positive) MS: m/z 250 ($M + H^+$).

Anal $C_{11}H_{15}N_5O_2$, calc: C 53.00, H 6.07, N 28.09. Found C 52.58, H 5.98, N 28.04.

Diethyl [2,3-dideoxy-5-O-(p-toluenesulfonyl)-α,β-D-pentofuranosyl]phosphonate **8** *and diethyl (4S)-4-hydroxy-1-methoxy-5-[(p-toluenesulfonyl)-oxy]pentylphosphonate* **9**

Methyl 2,3-dideoxy-5-*p*-toluenesulfonyl-α,β-D-pentofuranoside **3** (500 mg, 1.74 mmol) was dissolved in a mixture of CH_2Cl_2 (4 mL) and $P(OEt)_3$ (0.46 mL). The solution was cooled at 0 °C and TMS triflate (0.4 mL, 2.21 mmol) was added dropwise. After stirring at 0 °C for 1 h the mixture was quenched with H_2O (0.5 mL) and diluted with EtOAc (500 mL), followed by washing with saturated aqueous $NaHCO_3$ (2 × 200 mL) and with H_2O (2 × 200 mL). The organic phase was concentrated in vacuo and purified by silica-gel column chromatography (200 g) using CH_2Cl_2/MeOH (19:1) as eluent. This gave 410 mg of **8** (60%) as an anomeric mixture (1:1) and the ring open analog **9** in 190 mg (26%).

• *Compound* **8**

^{1}H NMR ($CDCl_3$) predominant anomer: δ 1.32–1.39 (m, 6H, 2 × Et), 1.90–2.22 (m, 4H, H-2 and H-3), 2.44 (s, 3H, 5-CH_3), 3.95–4.22 (m, 8H, H-1, H-4, H-5 and 2 × Et), 7.32–7.79 (m, 4H, tosyl).

^{13}C NMR ($CDCl_3$) predominant anomer: δ 16.2, 16.4 (2 × d, J = 6 Hz, 2 × Et), 21.5 (5-CH_3), 27.0 (d, J = 17 Hz, C-3), 28.0 (d, J = 5 Hz, C-2), 62.2 (m, Et), 70.77 (C-5), 76.81 (d, J = 125 Hz, C-1), 77.9 (C-4), 127.8, 129.8, 133.7, 144.8 (tosyl).

^{31}P NMR ($CDCl_3$): δ 23.16, 23.72.

FAB (positive) MS: m/z 393 ($M + H^+$).

• *Compound* **9**

^{1}H NMR ($CDCl_3$) predominant epimer: δ 1.33 (t, 6H, J = 7.1 Hz, 2 × Et), 1.50–1.98 (m, 4H, H-2, H-3), 2.45 (s, 3H, tosyl), 3.13–3.46 (m, 2H, H-5), 3.50 (s, 3H, OCH_3), 3.79–4.09 (m, 2H, H-1, H-4), 4.08–4.21 (m, 4H, 2 × Et), 7.30–7.36 (m, 2H, tosyl), 7.77–7.81 (m, 2H, tosyl).

^{13}C NMR ($CDCl_3$) predominant epimer: δ 16.36 (Et), 21.51 (tosyl), 26.25 (d, J = 2.7 Hz, C-2), 29.00 (d, J = 11.8 Hz, C-3), 60.04 (OCH_3), 62.31 (m, Et), 68.99 (C-5), 73.61 (C-4), 77.47 (d, J = 164.46 Hz, C-1), 127.85, 129.81, 132.70, 144.86 (tosyl).

^{31}P NMR ($CDCl_3$): δ 23.89, 23.99.

FAB (positive) MS: m/z 425 ($M + H^+$).

Diethyl [5-C-(thymin-1-yl)-2,3,5-trideoxy-α,β-D-pentofuranosyl]phosphonate **10a** *and diethyl (4S)-4-hydroxy-1-methoxy-5-(thymin-1-yl)pentylphosphonate* **11a**

• *Route 1*

Methyl 5-*C*-(thymin-1-yl)-2,3,5-trideoxy-α,β-D-pentofuranoside **7a** (300 mg, 1.25 mmol) was dissolved in a mixture of CH_2Cl_2 (3 mL) and $P(OEt)_3$ (0.3 mL, 291 mg, 1.75 mmol). After cooling to 0 °C, TMS triflate (0.3 mL, 368 mg, 1.66 mmol) was added dropwise and the mixture was stirred at room temperature for 1 h. The mixture was quenched by addition of H_2O (0.5 mL) and diluted with EtOAc (500 mL) followed by washing with saturated aqueous $NaHCO_3$ (2 × 200 mL) and with H_2O (200 mL). The water phase was extracted with EtOAc (2 × 200 mL) and the combined organic phases were dried ($MgSO_4$) and concentrated. After

column chromatography (100 g) using a gradient of CH_3OH in CH_2Cl_2 (0 → 5%) gave **10a** as an anomeric mixture (3:2) in 55% yield and the ring-opened product **11a** in 24% yield.

• *Route 2*

A suspension of thymine (65 mg, 0.52 mmol) and NaH (30 mg of a 60% oil dispension, 0.75 mmol) in dry DMF (5 mL) was heated at 80 °C for 1 h, followed by dropwise addition of diethyl [2,3-dideoxy-5-*O*-(*p*-toluenesulfonyl)-α,β-D-pentofuranosyl]-phosphonate **8** (200 mg, 0.51 mmol) (dissolved in 2 mL DMF). After stirring at 90 °C for 15 h, the solution was concentration in vacuo and the resulting residue was dissolved in hot $CHCl_3$ (2 × 10 mL), filtered and concentrated. The product **10a** was obtained in pure form after silica gel column chromatography (150 g) using a linear gradient of CH_2Cl_2/MeOH (1 → 5%). Yield 34%.

• *Compound* ***10a***

^{1}H NMR ($CDCl_3$) predominant anomer: δ 1.33 (t, 6H, J = 7.0 Hz, 2×Et), 1.90 (d, 3H, J = 1.0 Hz, 5-CH_3), 2.06–2.53 (m, 4H, H-2′, H-3′), 3.70 (dd, 1H, J = 6.6, 14.4 Hz, H-5′), 4.01 (dd, 1H, J = 2.9, 14.4 Hz, H-5′), 4.10, 4.45 (m, 6H, H-1′, H-4′, 2×Et), 7.17 (d, 1H, J = 1.2 Hz, H-6), 9.61 (br s, 1H, NH).

^{13}C NMR ($CDCl_3$) predominant anomer: δ 12.14 (5-CH_3), 16.40 (d, J = 5.7 Hz, Et), 27.17 (C-2′), 28.86 (d, J = 5.8 Hz, C-3′), 50.12 (C-5′), 62.34 (m, Et), 73.75 (d, J = 172 Hz, C-1′), 79.11 (d, J = 5.7 Hz, C-4′), 110.13 (C-5), 141.24 (C-6), 151.30 (C-2), 164.37 (C-4).

^{31}P NMR ($CDCl_3$): δ 23.61 major (24.75 minor).

FAB (positive) MS: m/z 347 ($M + H^+$).

• *Compound* ***11a***

^{1}H NMR ($CDCl_3$) predominant epimer: δ 1.33 (t, 6H, J = 7.1 Hz, 2 × Et), 1.86 (5-CH_3), 1.51–2.03 (m, 4H, H-2′, H-3′), 3.52 (s, 3H, OCH_3), 3.30–4.39 (m, 8H, H-1′, H-4′, H-5′, 2 × Et), 7.18 (d, 1H, J = 1.0 Hz, H-6), 9.94 (br s, 1H, NH).

^{13}C NMR ($CDCl_3$) predominant epimer: δ 12.05 (5-CH_3), 16.40 (d, J = 5.3 Hz, Et), 26.33 (d, J = 2.4 Hz, C-2′), 30.76 (d, J = 12.4 Hz, C-3′), 54.48 (OCH_3), 59.96 (C-5′), 62.3 (m, Et), 69.10 (C-4′), 77.52 (d, J = 164 Hz, C-1′), 109.20 (C-5), 142.48 (C-6), 151.42 (C-2), 164.81 (C-4).

^{31}P NMR ($CDCl_3$): δ 24.11 major (24.25 minor).

FAB (positive) MS: m/z 379 ($M + H^+$).

Diethyl [5-C-(N^4-isobutyrylcytosin-1-yl)-2,3,5-trideoxy-α,β-D-pentofuranosyl]phosphonate ***10b***

Compound **7b** (300 mg, 1.0 mmol) was dissolved in a mixture of CH_2Cl_2 (2.5 mL) and $P(OEt)_3$ (0.26 mL, 1.5 mmol, 253 mg). After cooling at 0 °C, TMS triflate (0.22 mL, 267 mg, 1.2 mmol) was added dropwise, and the mixture stirred at room temperature for 2 h. The reaction was quenched by addition of H_2O (0.5 mL), and diluted with EtOAc (500 mL). The resulting solution was washed with saturated aqueous $NaHCO_3$ (3 × 100 mL) and with H_2O (2×100 mL). The organic phase was dried over $MgSO_4$, concentrated and purified by column chromatography on silica gel giving **10b** in 64% yield (255 mg).

^{1}H NMR ($CDCl_3$) predominant anomer: δ 1.22, 1.24 (2 × s, 6H, 2 × CH_3), 1.32 (t, 6H, J = 7.1 Hz, 2 × Et), 1.84–2.74 (m, 5H, CH, H-2′, H-3′), 4.04–4.17 (m, 6H, H-5′, 2 × Et), 4.64 (m, 1H, H-4′), 6.00 (m, 1H, H-1′), 7.36 (d, 6H, J = 7.3 Hz, H-6), 7.73 (d, 6H, J = 7.3 Hz, H-5).

^{13}C NMR ($CDCl_3$) predominant anomer: δ 16.37, 16.45 (2 × Et), 18.91 (2 × CH_3), 27.27 (C-2′), 28.83 (d, J = 5.7 Hz, C-3′), 36.64 (CH), 52.15 (C-5′), 62.30, 62.59 (2 × d, J = 6.9 Hz, 2 × Et), 73.76 (d, J = 172 Hz, C-1′), 78.62 (d, J = 5.7 Hz, C-4′), 95.94 (C-5), 149.76 (C-6), 156.02 (C-2), 162.34 (C-4), 177.38 (C=O).

^{31}P NMR ($CDCl_3$): δ 23.6 major (24.0 minor).

FAB (positive) MS: m/z 402 ($M + H^+$).

[5-C-(Thymin-1-yl)-2,3,5-trideoxy-α,β-D-pentofuranosyl]phosphonic acid ***10c***

Diethyl [5-*C*-(thymin-1-yl)-2,3,5-trideoxy-α,β-D-pentofuranosyl]phosphonate **10a** (120 mg, 0.35 mmol) was dissolved in CH_3CN (2 mL) and cooled to 5 °C followed by addition of bromotrimethylsilane (0.15 mL, 0.83 mmol). After 20 min at room temperature the mixture was concentrated in vacuo and a mixture of H_2O (3 mL) and pyridine (1.5 mL) was added to the residue and stirred at room temperature for 1 h. The final product was concentrated and then desalted by running it through a Dowex column (Na^+ form). Yield 71 mg (70%).

^{1}H NMR (D_2O) predominant anomer: δ 1.60–2.24 (m, 4H, H-2′, H-3′), 1.85 (s, 3H, 5-CH_3), 3.80–4.35 (m, 4H, H-1′, H-4′, H-5′), 7.49 (d, 1H, J = 1.0 Hz, H-6).

^{13}C NMR (D_2O) predominant anomer: δ 12.11 (5-CH_3), 28.02 (C-2′), 29.57 (d, J = 6.0 Hz, C-3′), 51.67 (C-5′), 76.00 (d, J = 161.6 Hz, C-1′), 78.92 (d, J = 7.6 Hz, C-4′), 111.20 (C-5), 144.56 (C-6), 153.25 (C-2), 167.72 (C-4).

^{31}P NMR ($CDCl_3$): δ 21.57 major (20.96 minor).

FAB (positive) MS: m/z 291 ($M + H^+$).

[5-C-(Cytosin-1-yl)-2,3,5-trideoxy-α,β-D-pentofuranosyl]phosphonic acid ***10d***

The same procedure as for **10c** was employed except that diethyl [5-*C*-(isobutyrylcytosin-1-yl)-2′,3′,5′-trideoxy-α,β-D-pentofuranosyl]phosphonate **10b** (80 mg, 0.20 mmol) was used and that the product was treated with concentrated aqueous ammonia. Yield 35 mg (64%).

^{1}H NMR (D_2O) predominant anomer: δ 1.55–2.22 (m, 4H, H-2′, H-3′), 3.71–4.34 (m, 4H, H-1′, H-4′, H-5′), 5.99 (d, 1H, J = 7.5 Hz, H-5), 7.67 (d, 6H, J = 7.5 Hz, H-6).

^{13}C NMR (D_2O) predominant anomer: δ 28.11 (C-2′), 29.61 (d, J = 6.9 Hz, C-3′), 52.93 (C-5′), 75.90 (d, J = 162.4 Hz, C-1′), 78.86 (d, J = 7.6 Hz, C-4′), 95.61 (C-5), 149.76 (C-6), 154.92 (C-2), 163.93 (C-4).

^{31}P NMR ($CDCl_3$): δ 22.20 major (22.05 minor).

FAB (positive) MS: m/z 276 ($M + H^+$).

Diethyl [5-C-(adenin-9-yl)-2,3,5-trideoxy-α,β-D-pentofuranosyl]phosphonate ***10e***

Compound **7e** (120 mg, 0.48 mmol) was dissolved in a mixture anhydrous CH_3CN (20 mL) and $P(OEt)_3$ (0.2 mL, 1.2 mmol). After cooling to 0 °C, TMS triflate (0.2 mL, 1 mmol) was added dropwise under nitrogen and the mixture was stirred at room temperature for 2 h. Water (0.2 mL) was added and the solution diluted with EtOAc and washed with saturated aqueous $NaHCO_3$ (50 mL). The organic phase was concentrated and chromatographed on a silica-gel column with 1–5% MeOH in CH_2Cl_2 to give **10e** as an anomeric mixture (1:1). Yield 135 mg (76%).

^{1}H NMR ($CDCl_3$): δ 1.21–140 (m, 6H, 2 × Et), 1.85–2.38 (m, 4H, H-2′, H-3′), 4.09–4.65 (m, 8H, H-1′, H-4′, H-5′, 2×Et), 5.76 (s, 2H, NH_2), 7.93 and 8.11 (2×s, 1H, H-2), 8.33 and 8.34 (2 s, 1H, H-8).

^{13}C NMR ($CDCl_3$): δ 16.45, 16.54 (2 × Et), 27.02, 27.24 (C-2′), 29.66 (C-3′), 46.16, 47.33 (C-5′), 62.26, 62.78 (2 × m, 2 × Et), 74.08, 74.17 (2 × d, J = 172 Hz, C-1′), 79.07 (m, C-4′), 119.11, 119.38 (C-5), 141.48,

142.30 (C-8), 150.53, 150.70 (C-4), 152.77, 153.03 (C-2), 155.39, 155.44 (C-6).

FAB (positive) MS: m/z 356 (M + H^+).

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