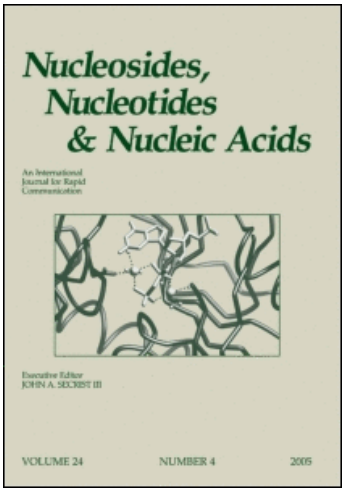

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Nucleosidyl-*O*-Methylphosphonates: A Pool of Monomers for Modified Oligonucleotides

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Nucleosidyl-*O*-Methylphosphonates: A Pool of Monomers for Modified Oligonucleotides

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ABSTRACT

An unique set of 5′-*O*- and 3′-*O*-phosphonomethyl derivatives of four natural 2′-deoxyribonucleosides, 1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine, 5′-*O*- and 2′-*O*-phosphonomethyl derivatives of 1-(3-deoxy-β-D-*erythro*-pentofuranosyl)thymine, and 1-(3-deoxy-β-D-*threo*-pentofuranosyl)thymine has been synthesized as a pool of monomers for the synthesis of modified oligonucleotides. The phosphonate moiety was protected with 4-methoxy-1-oxido-2-pyridylmethyl ester group, serving also as an intramolecular catalyst in the coupling step.

Key Words: Nucleoside phosphonates; P-C linkage; Nucleotide analogs; Pentofuranosylthymine derivatives; Protection; Regioizomers; Non-isosteric internucleotide linkage; Isopolar oligonucleotides.

INTRODUCTION

The solid-phase synthesis of isopolar phosphonate oligonucleotides employs a modern phosphotriester methodology for incorporation of the nucleoside phosphonic acid residues into the growing oligonucleotide chain.[1–4] The practical introduction of the

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4-methoxy-1-oxido-2-picolyl (Pic) group for the protection of phosphate and phosphonate moieties in nucleotides by Efimov[5] and Stawinski,[6] resp., enables both shortening the coupling time and considerably increase the yield of the coupling step due to an ability of the 4-methoxy-1-oxido-2-picolyl group to act as a powerful intramolecular catalyst on the activation of the phosphorus moiety by arylsulfonyl chlorides.

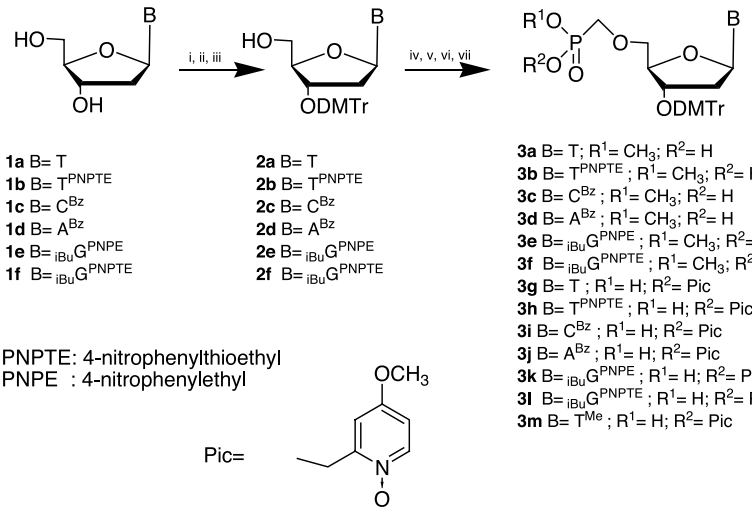
Recently we reported the phosphotriester solid-phase synthesis and properties of novel types of nuclease stable phosphonate oligonucleotides differing in the position of the bridging methylene group inserted into the internucleotide linkage between ester oxygen and phosphorus atom. Thus, two types regioisomeric phosphonate oligonucleotides containing either *3′-O-P-CH$_2$-O-5″* or *3′-O-CH$_2$-P-O-5″* internucleotide linkage were subjected to study on their hybridisation properties and ability to elicit RNase H activity. Obtained encouraging results, mainly for the altering oligonucleotides consisting of phosphonate and phosphodiester internucleotide bonds,[7] and also our further study on oligonucleotide-based HIV-I integrase inhibitors[8] containing the *3′-O-CH$_2$-P-O-5″* internucleotide linkages in the cleavage site, prompted us to continue the investigation in the area of phosphonate oligonucleotides. For a complex study on these compounds, we have synthesized the protected nucleoside phosphonic acids derived from four natural 2′-deoxynucleosides. Since there are two possible positions of an extra methylene group in the internucleotide linkage, both nucleoside 5′- and 3′-phosphonates are highly desirable. Moreover it was decided, to extend this group into thymine derivatives differing in configuration of its sugar moiety.

RESULTS AND DISCUSSION

Synthesis of 3′- and 5′-phosphonate monomers derived from 2-deoxy-β-D-*erythro*–pentofuranosylderivatives of nucleobases (Schemes 1 and 2).

3′-*O*-**2a–f** and 5′-*O*-dimethoxytrityl-2′-deoxynucleosides **4a–e** were used as starting compounds for the synthesis of final phosphonate monomers **3g–l** (Scheme 1) and **5f–j** (Scheme 2), respectively. Nucleosides **2a–f** protected with the 3′-*O*-dimethoxytrityl group were prepared in three steps including a regioselective silylation of the 5′-hydroxyl of *N*-protected 2′-deoxynucleosides (**1a**, **1b**,[9] **1c**,[10] **1d**,[10] **1e**,[11,12] **1f**,)[9] with *tert*-butyldiphenylsilyl chloride in pyridine, followed by the reaction of 3′-hydroxyl with dimethoxytrityl chloride in DCM in the presence of DBU, according to the procedure described for tritylation of secondary hydroxyl[13] and final treatment of fully protected compounds with TBAF in THF. 5′-*O*-Dimethoxytrityl-2′-deoxynucleosides **4a–e** (Scheme 2) were prepared by the reaction of nucleosides **1a–e** with dimethoxytrityl chloride in pyridine.[14]

Dimethyl esters **3** and **5** ($R^1 = R^2 = CH_3$), obtained from the reaction of alkoxides generated in situ from dimethoxytrityl derivatives **2**, **4** and sodium hydride with dimethyl-tosyloxymethylphosphonate[15] (DTMP), were selectively demethylated in 60% aqueous pyridine to monoesters **3a–f**, **5a–e**, and these compounds were esterified with 4-methoxy-1-oxido-2-pyridylmethanol (MOPM)[16] using 2-chloro-5,5-dimethyl-2-oxido-1,3,2-dioxaphosphinane (CDOP) as a condensing agent and 4-methoxy-1-oxidopyridine (MOP) as an *O*-nucleophilic catalyst.[11,12] Obtained mixed diesters were treated with 60% aqueous pyridine to cleave off remaining methyl ester group to afford final monomers **3g–l** and **5f–j** in 5′- and 3′-series, respectively.



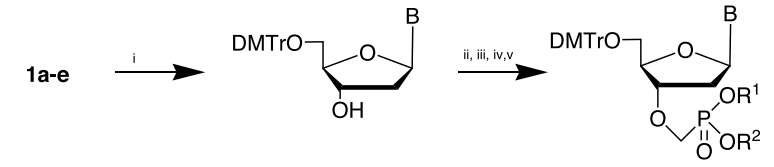
1a B= T
1b B= T^{PNPTE}
1c B= C^{Bz}
1d B= A^{Bz}
1e B= $_{iBu}G^{PNPE}$
1f B= $_{iBu}G^{PNPTE}$

2a B= T
2b B= T^{PNPTE}
2c B= C^{Bz}
2d B= A^{Bz}
2e B= $_{iBu}G^{PNPE}$
2f B= $_{iBu}G^{PNPTE}$

3a B= T; R^1= CH_3; R^2= H
3b B= T^{PNPTE} ; R^1= CH_3; R^2= H
3c B= C^{Bz} ; R^1= CH_3; R^2= H
3d B= A^{Bz} ; R^1= CH_3; R^2= H
3e B= $_{iBu}G^{PNPE}$; R^1= CH_3; R^2= H
3f B= $_{iBu}G^{PNPTE}$; R^1= CH_3; R^2= H
3g B= T ; R^1= H; R^2= Pic
3h B= T^{PNPTE} ; R^1= H; R^2= Pic
3i B= C^{Bz} ; R^1= H; R^2= Pic
3j B= A^{Bz} ; R^1= H; R^2= Pic
3k B= $_{iBu}G^{PNPE}$; R^1= H; R^2= Pic
3l B= $_{iBu}G^{PNPTE}$; R^1= H; R^2= Pic
3m B= T^{Me} ; R^1= H; R^2= Pic

Scheme 1. i. TBDPSCI, pyridine; ii. DMTrCl, DBU, DCM; iii. TBAF, THF; iv. NaH, DMF, $TsOCH_2P(O)(OCH_3)_2$; v. 60% pyridine; vi. 4-methoxy-1-oxido-2-pyridylmethanol, 2-chloro-5, 5-dimethyl-2-oxido-1,3,2-dioxaphosphinane, pyridine; vii. 60% pyridine.

In the case, of the reaction of thymine nucleosides **2a** and **4a**, and also **9a**, **15**, **19**, **23** and **27** with dimethyl tosyloxymethylphosphonate, the formation of substantial amount of 3-*N*-methyl derivatives was observed. This seems to be the main limitation of the use of dimethyl-tosyloxymethylphosphonate for introduction of *O*-phosphonomethyl moiety on the 3-*N*-unprotected thymine derivatives because of very difficult separation of 3-*N*-methyl and 3-*N*-unsubstituted derivatives.



4a B= T
4b B= T^{PNPTE}
4c B= C^{Bz}
4d B= A^{Bz}
4e B= $_{iBu}G^{PNPTE}$

PNPTE: 4-nitrophenylthioethyl
PNPE : 4-nitrophenylethyl

5a B= T; R^1= CH_3; R^2= H
5b B= T^{PNPTE} ; R^1= CH_3; R^2= H
5c B= C^{Bz} ; R^1= CH_3; R^2= H
5d B= A^{Bz} ; R^1= CH_3; R^2= H
5e B= $_{iBu}G^{PNPTE}$; R^1= CH_3; R^2= H
5f B= T ; R^1= H; R^2= Pic
5g B= T^{PNPTE} ; R^1= H; R^2= Pic
5h B= C^{Bz} ; R^1= H; R^2= Pic
5i B= A^{Bz} ; R^1= H; R^2= Pic
5j B= $_{iBu}G^{PNPTE}$; R^1= H; R^2= Pic
5k B= T^{Me} ; R^1= H; R^2= Pic

Scheme 2. i. DMTrCl, pyridine; ii. NaH, DMF, $TsOCH_2P(O)(OCH_3)_2$; iii. 60% pyridine; iv. 4-methoxy-1-oxido-2-pyridylmethanol, 2-chloro-5,5-dimethyl-2-oxido-1,3,2-dioxaphosphinane, pyridine; v. 60% pyridine.

At the same end of this work we found that diisopropyl tosyloxymethylphosphonate[17] is much advantageous reagent than DTMP. This agent does not alkylate thymine and 2-*N*-protected guanine moieties at the 3-*N* and 1-*N* positions, respectively. Resulting diisopropyl ester is stable under workup and chromatography, and the removal of both diisopropyl ester groups is accomplish using bromotrimethylsilane. Re-esterification of free phosphonate moiety with 4-methoxy-1-oxido-2-pyridylmethanol in the presence of DCC yielded mostly monoester **3g** and **5f** and traces of diester that was transformed into monoester by treatment with aqueous pyridine. This improved method will be published afterwards in the work dealing with different structures.

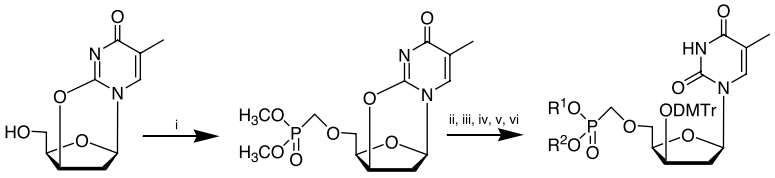
Another difficulty was observed during the alkylation of guanine derivative **2e** with dimethyl tosyloxymethylphosphonate in the presence of sodium hydride. In this case, the 6-*O*-[2-(4-nitrophenyl)ethyl] protecting group of compound **2e** underwent very fast β-elimination reaction (interestingly, under the same reaction conditions, the 6-*O*-[2-(4-nitrophenylthio)ethyl] protecting group in compounds **2f** and **4e** was stable). Therefore, the phosphonate **3** (B = 2-*N*-isobutyrylguanine) was isolated as dimethyl ester ($R^1 = R^2 = Me$), and this compound was protected again with 2-(4-nitrophenyl)ethyl group using 2-(4-nitrophenyl)ethanol, triphenylphosphine and DEAD.

Synthesis of 5′-phosphonate monomer derived from 1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine (Scheme 3).

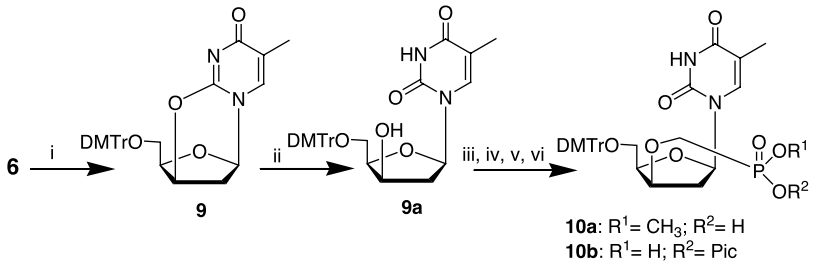
Sodium salt of 2,3′-anhydrothymidine (**6**), generated in situ with sodium hydride at −30°C was condensed with DTMP in DMF. Also in this case, the alkylation was accompanied by the formation of unwanted 3-*N*-methyl derivative. Obtained dimethyl ester **7** was hydrolysed in 0.5 M aqueous sodium hydroxide giving, after dimethoxytritylation of the 3′-hydroxy group with dimethoxytrityl chloride in pyridine and in presence of silver triflate,[18] monomethyl ester **8a**. Following esterification with 4-methoxy-1-oxido-2-pyridylmethanol[16] led to mixed diester **8** ($R^1 = CH_3$; R^2 = Pic), which was selectively hydrolysed, in aqueous pyridine giving 5′-monomer **8b**.

Synthesis of 3′-phosphonate monomer derived from 1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine (Scheme 4).

5′-Hydroxyl group of 2,3′-anhydrothymidine (**6**) was tritylated using dimethoxytrityl chloride in pyridine and the anhydro ring was subsequently opened in 0.5 M sodium hydroxide in aqueous pyridine. Obtained 1-(2-deoxy-5-*O*-dimethoxytrityl-β-D-*threo*-pentofuranosyl)thymine was alkylated with DTMP as described above. In this case, desired product **10a** was obtained only in 21% yield and unwanted 3-*N*-



Scheme 3. i. NaH, DMF, $TsOCH_2P(O)(OCH_3)_2$; ii. NaOH; iii. DMTrCl, TfOAg, pyridine; iv. 4-methoxy-1-oxido-2-pyridylmethanol, 2-chloro-5,5-dimethyl-2-oxido-1,3,2-dioxaphosphinane, pyridine; iv. 60% aq. pyridine.

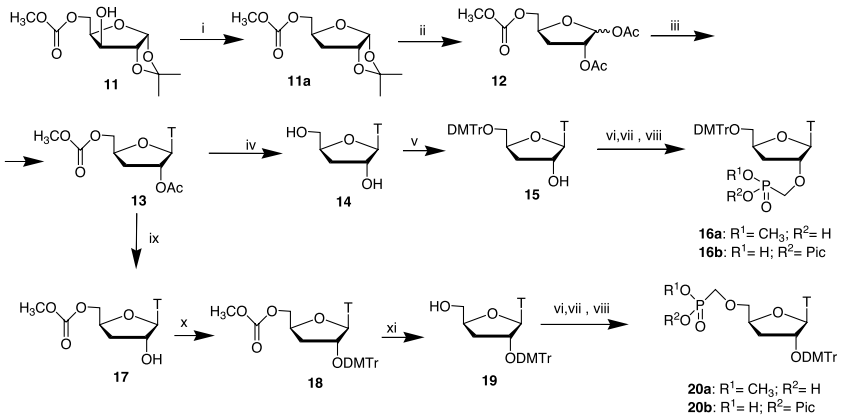


Scheme 4. i. DMTrCl, pyridine; ii. NaOH, H_2O, pyridine; iii. NaH, DMF, $TsOCH_2P(O)(OCH_3)_2$; iv. 60% aq. pyridine; v. 4-methoxy-1-oxido-2-pyridylmethanol, 2-chloro-5,5-dimethyl-2-oxido-1,3,2-dioxaphosphinane, pyridine; vi. 60% aq. pyridine.

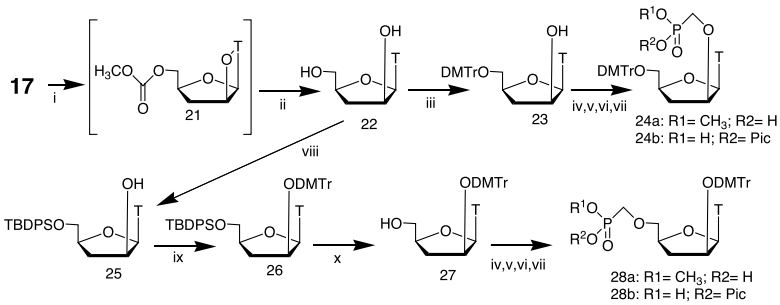
methylderivative (a little higher R_f on TLC) in 18% yield. Product **10a** was further transformed into 3′-phosphonate monomer **10b** as mentioned above.

Synthesis of 2′- and 5′-phosphonate monomers derived from 1-(3-deoxy-β-D-*erythro*-pentofuranosyl)thymine (**14**) (Scheme 5).

Acetolysis of 3-deoxy-1,2-*O*-isopropyliden-5-*O*-methoxycarbonyl-β-D-*erytro*-pentofuranose (**11a**), prepared by radical deoxygenation[19] of 1,2-*O*-isopropyliden-5-*O*-methoxycarbonyl-β-D-*xylo*-pentofuranose[20,21] (R. Liboska, unpublished results) (**11**) via its immidazoylthiocarbonyl derivative, in acetic anhydride-acetic acid-sulphuric acid mixture led to the precursor **12**. Nucleosidation reaction of this compound with 2,4-*O*-bis-trimethylsilylthymine in acetonitrile under tin tetrachloride catalysis afforded 1-(2-*O*-acetyl-3-deoxy-5-*O*-methoxycarbonyl-β-D-*erythro*-pentofuranosyl)thymine[22,23] (**13**). This compound was used as a starting material for the synthesis of both key dimethoxytrityl derivatives **15** and **19**. 2′-*O*-Acetyl and 5′-*O*-methoxycarbonyl groups were removed from nucleoside **13** by its heating with Dowex 1 (OH^- form) in ethanol.



Scheme 5. i. Bisimidazoylthiocarbonate, Bu_3SnH, toluene; ii. Ac_2O, AcOH, DCM, H_2SO_4; iii. 2,4-O-bistrimethylsilylthymine, $SnCl_4$, acetonitrile; iv. Dowex 1 OH^-, methanol; v. DMTrCl, pyridine; vi. 60% pyridine; vii. 4-methoxy-1-oxido-2-pyridylmethanol, 2-chloro-5,5-dimethyl-2-oxido-1,3,2-dioxaphosphinane, pyridine; viii. 60% pyridine; ix. HCl, methanol; x. DMTrCl, DBU, DCM; xi. aq. NH_3.



Scheme 6. i. MsCl, pyridine; ii. Dowex 50 OH^-, methanol; iii. DMTrCl, pyridine; iv. NaH, DMF, $TsOCH_2P(O)(OCH_3)_2$; v. 60% pyridine; vi. 4-methoxy-1-oxido-2-pyridylmethanol, 2-chloro-5,5-dimethyl-2-oxido-1,3,2-dioxaphosphinane, pyridine; vii. 60% pyridine; viii. TBDPSCI, pyridine; ix. DMTRCI, DBU, pyridine; x. TBAF, THF.

Obtained nucleoside **14** was dimethoxytritylated at the 5′-position by currently used procedure giving 5′-protected compound **15**.

The synthesis of the 3′-*O*-DMTr derivative **19** started also from precursor **13**. Its selective deacetylation by treatment with 1M hydrogen chloride in methanol afforded in a high yield 3′-deoxy-5′-*O*-methoxycarbonylthymidine (**17**), and this compound was dimethoxytritylated using dimethoxytrityl chloride and DBU in DCM. 2′-*O*-Dimethoxytrityl derivative **19** was obtained by removal of methoxycarbonyl group in methanolic ammonia.

Both protected nucleoside derivatives **15** and **19** were transformed into phosphonate monomers **16b** and **20b** employing described procedure.

Synthesis of 2′- and 5′-phosphonate monomers derived from 1-(3-deoxy-β-D-*thre*o-pentofuranosyl)thymine (**22**) (Scheme 6).

Inversion of configuration on 2′ carbon atom of compound **17** was carried out via anhydro intermediate **21** obtained by cyclisation of 2′-*O*-mesylderivative of compound **17**. The anhydro ring of compound **21** was opened by treatment with Dowex 1 (OH^- form), and simultaneously the 5′-*O*-methoxycarbonyl group was cleaved off giving nucleoside **22**.[24] This compound was transformed into both 5′-*O*-dimethoxytrityl derivative **23**[25] and 2′-*O*-dimethoxytrityl derivative **27**. The compound **27** was obtained as described for compound **2**. Dimethoxytrityl derivatives **23** and **27** were converted into 5′-phosphonate **28b** and 2′-phosphonate **28b**, respectively, as described above.

EXPERIMENTAL

Unless stated otherwise, all used solvents were anhydrous. Final products were lyophilized from dioxane or benzene, and dried over phosphorus pentoxide at 40°C and 13 Pa. TLC was performed on silica gel pre-coated aluminium plates Silufol UV 254 foils (Kavalier Glassworks, Votice, Czech Republic), and compounds detected by UV light (254 nm), by heating (detection of dimethoxytrityl group; orange color), and by spraying with 1% solution of 4-(4-nitrobenzyl)pyridine in ethanol followed by heating and treating with gaseous ammonia (blue color of mono- and diesters of

phosphonic acid). Preparative column chromatography was carried out on silica gel (40–60 μm; Fluka) neutralized with triethylamine (1 ml/100 g), and elution was performed at the flow rate of 40 ml/min. The following solvent systems were used for TLC and preparative chromatography: toluene-ethyl acetate 4:1 (T1), 1:1 (T2); toluene-acetone 1:1 (T3); chloroform-ethanol 9:1 (C1); ethyl acetate-acetone-ethanol-water 4:1:1:1 (H1), 12:2:2:1 (H3). The concentrations of solvent systems are stated in volume percents (%, v/v). Mass spectra were recorded on ZAB-EQ (VG Analytical) instrument, using FAB (ionization with Xe, accelerating voltage 8 kV). Glycerol and thioglycerol were used as matrices. NMR spectra were measured on Varian Unity 500 instrument (^{1}H at 500 MHz, ^{13}C at 125.7 MHz) in deuterated chloroform or hexadeuteriodimethylsulfoxide (DMSO-d_6).

General Methods

Method A. Preparation of methyl esters **3a–f**, **5a–e**, **10a**, **16a**, **20a**, **24a**, **28a** (Tables 1 and 2).

Sodium hydride (60% suspension in mineral oil; 3 equiv) was added at −30°C, under argon atmosphere, to a solution of protected nucleoside and DTMP (1.1 equiv) in DMF (5 ml/mmol of nucleoside). The suspension was vigorously stirred at r.t. for 3–16 h until the starting nucleoside disappeared (TLC in H-3). The reaction mixture was then cooled to −30°C, and carefuly neutralised with glacial acetic acid (2 equiv) in DMF (1 ml/mmol). The temperature was increased to r.t., and the solution was concentrated at 40°C in vacuo (13 Pa). The residue was dissolved in chloroform (50 ml/mmol) and the solution was washed with 1M solution of sodium hydrogen carbonate. Organic layer was dried over sodium sulfate, evaporated, and the crude product was treated in 60% aqueous pyridine (30 ml/mmol) at 50°C for 16 h. After evaporation, the crude monomethyl ester was dissolved in chloroform, and washed with 1M aqueous TEAB to remove methylpyridinium cation. Organic layer was dried over sodium sulfate, evaporated, and the desired product was obtained by purification on silica gel column using a linear gradient from ethyl acetate to solvent system H3 followed by H3 to solvent system H1.

Method B. Synthesis of 4-methoxy-1-oxido-2-pyridylmethyl esters **3g–l**, **5f–e**, **8b**, **10b**, **16b**, **20b**, **24b**, **28b** (Tables 3 and 4).

Table 1. Alkylation of 3′-*O*-dimethoxytritylnucleosides.

DMTr-Nucleoside			DMTOMP[a]		NaH		DMF	Conditions	Yield	
	g	mmol	g	mmol	g	mmol	(ml)	(Temp./time)	(%)	Product
2a	5.45	10	3.24	11	1.22	30	50	20°C/24 h	37	**3a**
2b	1.7	2.2	0.9	3	0.26	6.6	15	10°C/3 h	35	**3b**
2c	2.4	3.8	1.24	4.2	0.46	11.4	20	20°C/24 h	72	**3c**
2d	5.14	7.8	2.53	8.6	0.94	23.4	40	20°C/20 h	50	**3d**
2e	3.94	5	1.62	5.5	0.6	15	25	20°C/24 h	5.7	**3e**
2f	2.69	3.28	1.17	4	0.4	10	30	10°C/4 h	18	**3f**

[a]Dimethyl tosyloxymethylphosphonate.

Table 2. Alkylation of 5′-*O*-dimethoxytritylnucleosides.

DMTr-Nucleoside			DMTOMP[a]		NaH		DMF	Conditions	Yield	
	g	mmol	g	mmol	g	mmol	(ml)	(temp./time)	(%)	Product
4a	5.5	10	3.24	11	1.22	30	50	20°C/20 h	50	**5a**
4b	4.5	6.2	2	7	0.68	17	50	20°C/24 h	51	**5b**
4c	6.13	9.7	3.24	11	1.22	30	50	20°C/20 h	38	**5c**
4d	3.84	5.84	1.9	6.43	0.7	18	30	20°C/24 h	60	**5d**
4e	5.9	7.2	2.75	9.36	0.86	21.6	50	10°C/3 h	14	**5e**

[a]Dimethyl tosyloxymethylphosphonate.

A mixture of monomethyl ester, obtained according to Method A, 4-methoxy-1-oxido-2-pyridylmethanol (1.1 eq.), and 4-methoxy-1-oxido-2-pyridine (3 eq.) dried by co-evaporation with pyridine was dissolved in pyridine (10 ml/mmol of methyl ester), and the solution was treated with 2-chloro-5,5-dimethyl-2-oxido-1,3,2-dioxaphosphinane (CDOP) (3 eq.) under argon atmosphere and vigorous stirring at r.t. for 6–16 h (TLC in H1). An excess of CDOP was decomposed by addition of small amount of water, and the reaction mixture was concentrated in vacuo at low temperature in a bath. The residue was partitioned between chloroform and 1M TEAB, organic layer was evaporated, and the residue was treated in 60% aqueous pyridine (~30 ml/mmol) at 50–60°C for 16 h. After evaporation of the reaction mixture, the residue was partitioned between chloroform and 1M TEAB. The organic layer was evaporated, and crude product was purified on silica gel, using a linear gradient of solvent system H3 in ethyl acetate followed by gradient of solvent system H1 in H3.

4-Methoxy-1-oxido-2-pyridylmethyl 2-deoxy-3-*O*-dimethoxytrityl-1-(thymin-1-yl)-β-D-*erythro*-pentofuranos-5-yloxymethylphosphonate (3g). Compound **3g** was prepared according to **Method B** (Table 5) from monomethyl ester **3a** obtained according to **Method A** (Table 3).

Table 3. Synthesis of 4-methoxy-1-oxido-2-pyridylmethyl esters of protected nucleosid-5′-phosphonic acids.

Methyl ester			MOPM[a]		MOP[b]		CDOP[c]		Py	Temp./time	Yield	
	g	mmol	g	mmol	g	mmol	g	mmol	(ml)	(°C/min)	(%)	Product
3a	1.34	1.78	0.3	1.96	0.7	5.4	1	5.4	25	20/60	87	**3g**
3b	0.71	0.84	0.15	0.84	0.3	2.3	0.5	2.3	15	20/60	64	**3h**
3c	1.99	2.36	0.4	2.6	0.9	7.1	1.31	7.1	20	20/30	27	**3i**
3d	6.93	8	1.37	8.8	3	24	4.5	24	120	20/120	31	**3j**
3e	0.09	0.09	0.02	0.1	0.03	0.27	0.05	0.27	3	20/60	89	**3k**
3f	0.58	0.61	0.11	0.7	0.26	2.1	0.39	2.1	10	20/90	64	**3l**

[a]4-methoxy-1-oxido-2-pyridylmethanol.
[b]4-methoxy-1-oxidopyridine.
[c]2-chloro-5,5-dimethyl-2-oxido-1,3,2-dioxaphosphinane.

Table 4. Synthesis of 4-methoxy-1-oxido-2-pyridylmethyl esters of protected nucleosid-3′-phosphonic acids.

Methyl ester		MOPM[a]		MOP[b]		CDOP[c]		Py	Temp./time	Yield	
	g	mmol	g	mmol	g	mmol	g	mmol	(ml)	(°C/min)	(%)	Product
5a	0.81	1.07	0.21	1.37	0.47	3.72	0.69	3.72	15	20/50	70	**5f**
5b	2.98	3.18	0.6	3.5	1.2	9.54	1.8	9.54	40	20/60	81	**5g**
5c	3.6	4.37	0.74	4.19	1.67	13.15	2.43	13.15	40	20/60	40	**5h**
5d	3.04	3.5	0.6	3.85	1.3	10.5	1.9	10.5	50	20/50	52	**5i**
5e	0.97	1.02	0.2	1.23	0.4	3.1	0.58	3.1	15	20/40	39	**5j**

[a]4-methoxy-1-oxido-2-pyridylmethanol.
[b]4-methoxy-1-oxidopyridine.
[c]2-chloro-5,5-dimethyl-2-oxido-1,3,2-dioxaphosphinane.

HR FAB calcd for $C_{39}H_{41}N_3O_{12}P$ 774.2428 $(M\text{-}H)^-$, found 774.2460.
NMR: Tables 5, 6, and 7.

4-Methoxy-1-oxido-2-pyridylmethyl 2-deoxy-3-*O*-dimethoxytrityl-1-(4-*O*-(2-(4-nitrophenylthio)ethyl)thymin-1-yl)-β-D-*erythro*-pentofuranos-5-yloxymethyl-phosphonate (3h). Compound **3h** was prepared according to **Method B** (Table 5) from monomethyl ester **3b** obtained according to **Method A** (Table 3).

HR FAB calcd for $C_{47}H_{48}N_4O_{14}PS$ 955.2625 $(M\text{-}H)^-$, found 955.2545.
NMR: Tables 5, 6, and 7.

4-Methoxy-1-oxido-2-pyridylmethyl 2-deoxy-3-*O*-dimethoxytrityl-1-(4-*N*-benzoylcytosin-1-yl)-β-D-*erythro*-pentofuranos-5-yloxymethylphosphonate (3i). Compound **3i** was prepared according to **Method B** (Table 5) from monomethyl ester **3c** obtained according to **Method A** (Table 3).

HR FAB calcd for $C_{45}H_{44}N_4O_{12}P$ 863.2693 $(M\text{-}H)^-$, found 863.2693.
NMR: Tables 5, 6, and 7.

4-Methoxy-1-oxido-2-pyridylmethyl 2-deoxy-3-*O*-dimethoxytrityl-1-(6-*N*-benzoyladenin-9-yl)-β-D-*erythro*-pentofuranos-5-yloxymethylphosphonate (3j). Compound **3j** was prepared according to **Method B** (Table 5) from monomethyl ester **3d** obtained according to **Method A** (Table 3).

HR FAB calcd for $C_{46}H_{44}N_6O_{11}P$ 887.2806 $(M\text{-}H)^-$, found 887.2760.
NMR: Tables 5, 6, and 7.

4-Methoxy-1-oxido-2-pyridylmethyl 2-deoxy-3-*O*-dimethoxytrityl-1-(2-*N*-isobutyryl-6-*O*-(2-(4-nitrophenyl)ethyl)guanin-9-yl)-β-D-*erythro*-pentofuranos-5-yloxymethylphosphonate (3k). Compound **3k** was prepared according to **Method B** (Table 5) from monomethyl ester **3e**. Dimethoxytrityl derivative **2e** (3.94 g; 5 mmol) was treated with DTMP and sodium hydride according to **Method A** (Table 3). The reaction mixture was neutralized by glacial acetic acid at low temperature and concentrated in vacuo at 40°C. Residue in chloroform was applied on silica gel column

Table 5. ^{1}H NMR spectra of 4-methoxy-1-oxido-2-pyridylmethyl- and methyl esters of 5′-phosphonates derived from 2-deoxy-β-D-*erythro*-pentofuranosyl derivative of nucleobases and from 1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine.

										Base				
	H-1′	H-2′	H-2″	H-3′	H-4′	H-5′	H-5″	P-CH_2		H-2, H-6	H-8, H-5, CH_3	NH	POH	Note
3a	6.26	1.93	1.63	4.19	3.67	3.31	3.10	3.30	3.23	7.63	1.80	11.25	10.00	a
3b	6.29	1.92	1.65	4.21	3.70	3.29	3.11	3.40	3.32	7.55	1.85	–	10.50	a, e
3c	6.34	1.84	2.02	4.22	3.68	3.38	3.11	3.30	3.26	8.51	7.34	11.20	10.40	a, b
3d	6.59	2.65	2.11	4.35	3.68	3.28	3.15	3.27 (2H)		9.06	8.75	11.20	10.20	a, b
3e	6.36	2.63	1.85	4.33	3.71	3.39	3.20	3.38	3.33	–	8.57	11.05	10.10	a, c, d
3f	6.38	2.63	1.88	4.33	3.70	3.35	3.21	3.40	3.37	–	8.45	10.90	10.45	a, d, e
3g	6.24	1.91	1.61	4.16	3.66	3.33	3.10	3.32 (2H)		7.66	1.76	11.25	10.00	f
3h	6.28	1.91	1.61	4.16	3.73	3.35	3.13	3.47	3.42	7.70	1.82	–	10.50	e, f
3i	6.31	1.81	1.96	4.16	3.67	3.36	3.10	3.39	3.32	8.53	7.26	11.12	10.70	b, f
3j	6.56	2.54	2.04	4.33	3.71	3.33	3.20	3.37	3.32	9.05	8.74	11.18	10.70	b, f
3k	6.33	2.82	1.67	4.32	3.76	3.58	3.22	3.32 (2H)		–	8.61	11.05	10.10	c, d, f
3l	6.35	2.69	1.79	4.32	3.70	3.47	3.25	3.43	3.40	–	8.59	11.03	10.20	d, e, f
8a	5.75	1.09	1.72	4.32	3.80	3.93	3.89	3.65	3.61	7.67	1.67	11.20	10.10	a
8b	5.73	1.07	1.70	4.30	3.76	3.92 (2H)		3.68 (2H)		7.68	1.65	11.20	10.70	f

a: P-OCH_3: 3.32 d, 3H, J(P, OCH) = 10.2 (5′-P); 3.40 d, 3H, J = 10.2 (3′-P).
b: Bz: 8.05 d, 2H, 7.64 t, 1H, 7.55 t, 2H.
c: PNPE: 8.16 d, 2H, 7.65 d, 2H (arom H); 4.76 t, 2H, J = 7.0 (O-CH_2); 3.30 t, 2H, J = 7.0 (CH_2-arom).
d: IBU: 2.86 sept, 1H, J = 6.8 (CH); 1.08 d, 6H, J = 6.8 (CH_3).
e: PNPTE: 8.11 d, 2H, 7.59 d, 2H (arom H); 4.07 t, 2H, J = 7.2 (O-CH_2); 3.28 t, 2H, J = 7.2 (S-CH_2).
f: PIC: 8.03 d, 1H, J = 7.1, 7.06 d, 1H, J = 3.5, 6.83 dd, 1H, J = 3.5 a 7.1 (arom H); 4.78 d, 2H, J(P, OCH) = 8.1 (P-OCH_2); 3.71 s, 3H (OCH_3).
DMTr: 7.42 d, 2H, 7.34 t, 2H, 7.29 d, 4H, 7.26 t, 1H, 6.92 d, 4H (arom H); 3.73 s, 6H (OCH_3).

Table 6. Interaction constants for Table 5.

	1′2′	1′2″	2′2″	2′3′	2″3′	3′4′	4′5′	4′5″	5′5″	P-CH_2	
3a	9.8	5.4	13.9	5.6	1.0	1.0	2.7	3.4	10.7	8.3	8.8
3b	9.5	5.5	13.7	5.5	1.0	1.0	2.8	3.0	10.5	8.7	9.3
3c	7.5	5.6	14.2	5.6	1.0	1.0	2.4	3.2	10.7	8.1	8.5
3d	9.3	5.9	13.9	5.6	1.0	1.0	2.9	3.1	10.7	8.1	–
3e	9.5	5.6	13.9	5.0	1.0	1.0	3.2	3.4	10.5	8.5	9.2
3f	9.6	5.6	13.9	5.1	1.0	1.0	3.0	4.0	10.5	8.2	8.8
3g	9.5	5.5	13.2	5.2	1.0	1.0	2.5	3.0	10.5	8.3	–
3h	9.3	5.5	13.2	5.5	1.0	1.0	2.3	3.7	10.6	8.8	8.8
3i	8.4	5.8	13.4	5.6	1.7	1.7	2.6	3.6	10.5	8.6	8.8
3j	9.2	5.8	13.4	5.4	1.5	1.5	3.0	4.0	10.8	8.6	9.2
3k	9.5	5.4	13.2	5.4	1.0	1.0	4.0	4.5	10.5	8.2	–
3l	9.8	5.6	13.4	5.5	1.0	1.0	3.2	4.3	10.6	9.0	9.0
8a	5.4	6.8	13.6	5.4	6.8	5.0	4.5	5.4	10.7	8.6	8.8
8b	5.5	6.7	13.6	5.4	6.5	5.0	4.5	4.5	–	8.6	–

and phosphonate **3** (B = 2-*N*-isobutyrylguanine; $R^1 = R^2 = CH_3$) 0.4 g (0.525 mmol; 10.5%) eluted with a linear gradient of ethanol in chloroform (up to 10%). This compound, 2-(4-nitrophenyl)ethanol (0.3 g; 1.84 mmol) and triphenylphosphine (0.525 g; 2 mmol) were dried by co-evaporation with dioxane, and this mixture treated with DEAD (0.32 ml; 1.84 mmol) in dioxane (3 ml) at r.t. overnight. The solution was concentrated in vacuo, the residue was partitioned between chloroform and 1M sodium hydrogencarbonate, organic layer was evaporated and crude product was heated in 60% aqueous pyridine ($\sim$ 30 ml/mmol) at 50–60°C for 16 h. After evaporation, the residue was partitioned between chloroform and 1M TEAB, organic layer was evaporated and crude product was purified on silica gel, using a linear gradient of solvent system H3 in ethyl acetate followed by a gradient of solvent system H1 in H3. Obtained monomethyl ester **3e** was then converted into the 4-methoxy-1-oxido-2-pyridylmethyl ester **3k** according to **Method B**.

HR FAB calcd for $C_{51}H_{54}N_7O_{14}PNa$ 1042.3363 $(M + Na)^+$, found 1042.3364.

NMR: Tables 5, 6, and 7.

4-Methoxy-1-oxido-2-pyridylmethyl 2-deoxy-3-*O*-dimethoxytrityl-1-(2-*N*-isobutyryl-6-*O*-(2-(4-nitrophenylthio)ethyl)guanin-9-yl)-β-D-*erythro*-pentofuranos-5-yloxymethylphosphonate (3l). Compound **3l** was prepared according to **Method B** (Table 5) from monomethyl ester **3f** obtained according to **Method A** (Table 3).

HR FAB calcd for $C_{51}H_{53}N_7O_{14}PS$ 1050.3109 $(M-H)^-$, found 1050.3095.

NMR: Tables 5, 6, and 7.

4-Methoxy-1-oxido-2-pyridylmethyl 2-deoxy-5-*O*-dimethoxytrityl-1-(thymin-1-yl)-β-D-*erythro*-pentofuranos-3-yloxymethylphosphonate (5f). Compound **5f** was prepared according to **Method B** (Table 8) from monomethyl ester **5a** obtained according to **Method A** (Table 4).

HR FAB calcd for $C_{45}H_{58}N_4O_{12}P$ 877.3789 $(M + Et_3N + H)^+$, found 877.3788.

NMR: Tables 8, 9, and 10.

Table 7. ^{13}C NMR spectra of 4-methoxy-1-oxido-2-pyridylmethyl- and methyl esters of 5′-phosphonates derived from 2-deoxy-β-D-*erythro*-pentofuranosyl derivatives of nucleobases and from 1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine.

	C-1′	C-2′	C-3′	C-4′	C-5′	P-C	C-2	C-4	C-5	C-6	C-8, CH_3	Note
3a	83.92	37.97	75.29	84.56	72.49 (10.7)	67.92 (154.3)	150.84	163.92	110.35	136.20	12.02	a
3b	85.99	39.28	75.66	86.57	73.09 (9.8)	67.23 (157.2)	151.58	164.29	110.37	136.12	13.59	a, e
3c	85.17	39.20	75.21	86.21	72.14 (9.8)	67.46 (153.3)	154.65	163.08	97.17	146.19	–	a, b
3d	83.50	38.82	75.73	85.20	72.68 (12.6)	67.78 (154.3)	151.30	150.38	125.64	152.47	144.90	a, b
3f	84.08	38.54	75.90	85.35	70.08 (10.7)	67.12 (159.2)	159.90	152.54	117.65	153.42	141.96	a, f, g
3g	84.21	38.24	75.18	84.71	72.73 (11.7)	67.85 (158.2)	150.89	163.99	110.51	136.13	12.22	f
3h	84.88	38.42	75.13	85.32	72.63 (10.7)	67.83 (158.2)	150.86	162.94	109.73	135.11	12.83	e, f
3i	85.18	39.20	75.10	86.16	72.19 (11.7)	68.27 (153.3)	154.18	163.10	96.84	146.40	–	b, f
3j	83.47	38.67	75.52	85.15	72.67 (12.6)	68.24 (156.25)	151.78	150.36	125.64	152.41	143.56	b, f
3k	84.11	38.56	75.59	85.23	72.11 (10.7)	68.05 (158.2)	159.76	151.66	117.54	152.98	144.31	f, g, h
3l	84.16	38.20	75.77	85.38	72.76 (10.7)	67.25 (158.0)	159.85	152.42	117.73	153.39	141.97	e, f, g
8a	80.67	37.25	75.25	82.64	67.85 (8.6)	67.23 (159.0)	150.59	164.07	109.39	136.71	12.30	a
8b	82.37	34.54	71.96	82.48	66.85 (8.6)	65.88 (159.2)	150.39	163.88	109.12	139.34	12.14	f

DMTr: 158.80, 158.76, 145.77, 136.28, 136.22, 130.50, 2C, 130.40, 2C, 128.23, 2C, 128.07, 2C, 127.12, 113.64, 2C, 113.58, 2C, 87.02, 55.36, 55.34.
a: P-OCH_3: 51.04 d, J(P,C) = 5.9 (5′-P); 51.37 d, J(P,C) = 5.9 (3′-P).
b: N-Bz: 167.61, 133.57, 128.64, 4C, 132.59.
e: PNPTE: 146.85, 145.06, 126.74, 2C, 124.29, 2C, 66.72, 28.17.
f: PIC: 158.32, 151.42 d, J(P,C) = 5.9, 140.32, 110.80, 108.74, 61.15 d, J(P,C) = 3.9, 56.16.
g: iBu: 34.71, 19.55, 2C.
h: PNPE: 146.80, 146.45, 130.58, 2C, 123.58, 2C, 57.59, 34.39.

Table 8. ^{1}H NMR spectra of 4-methoxy-1-oxido-2-pyridylmethyl- and methyl esters of 3′-phosphonates derived from 2-deoxy-β-D-*erythro*-pentofuranosyl derivatives of nucleobases and from 1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine.

									Base				
	H-1′	H-2′	H-2″	H-3′	H-4′	H-5′	H-5″	P-CH_2	H-2, H-6	H-8, H-5, CH_3	NH	POH	Note
5a	6.14	2.24	2.32	4.32	4.03	3.27	3.16	3.38 3.35	7.51	1.43	11.38	10.00	a
5b	6.18	2.25	2.35	4.32	4.05	3.28	3.18	3.53 3.49	7.55	1.44	11.40	10.20	a, e
5c	6.11	2.20	2.49	4.26	4.14	3.32	3.26	3.40 3.36	8.17	7.19	11.35	10.20	a, b
5d	6.43	3.01	2.55	4.41	4.16	3.23	3.18	3.44 (2H)	8.64	8.61	11.20	10.00	a, b
5e	6.30	2.82	2.45	4.61	4.04	3.25	3.11	3.51 (2H)	–	8.35	10.90	10.50	a, d, e
5f	6.10	2.22	2.30	4.36	4.01	3.24	3.11	3.40 (2H)	7.50	1.38	11.32	10.00	f
5g	6.14	2.23	2.34	4.32	4.02	3.27	3.14	3.58 (2H)	7.52	1.42	–	10.60	e, f
5h	6.07	2.18	2.49	4.27	4.13	3.29	3.23	3.48 (2H)	8.14	7.18	11.28	10.95	b, f
5i	6.39	3.01	2.83	4.39	4.15	3.22	3.16	3.58 (2H)	8.61	8.56	11.20	10.42	b, f
5j	6.22	2.84	2.42	4.52	4.02	3.24	3.10	3.57 (2H)	–	8.52	11.00	10.05	d, e, f
10a	6.21	2.12	2.43	4.07	4.11	3.38	3.22	3.45 3.30	7.60	1.68	11.25	10.00	a
10b	6.18	2.14	2.48	4.16	4.01	3.35	3.30	3.55 3.48	7.58	1.61	11.21	10.50	f

a: P-OCH_3: 3.32 d, 3H, J(P, OCH) = 10.2 (5′-P); 3.40 d, 3H, J = 10.2 (3′-P).
b: Bz: 8.05 d, 2H, 7.64 t, 1H, 7.55 t, 2H.
c: PNPE: 8.16 d, 2H, 7.65 d, 2H (arom H); 4.76 t, 2H, J = 7.0 (O-CH_2); 3.30 t, 2H, J = 7.0 (CH_2-arom).
d: IBU: 2.86 sept, 1H, J = 6.8 (CH); 1.08 d, 6H, J = 6.8 (CH_3).
e: PNPTE: 8.11 d, 2H, 7.59 d, 2H (arom H); 4.07 t, 2H, J = 7.2 (O-CH_2); 3.28 t, 2H, J = 7.2 (S-CH_2).
f: PIC: 8.03 d, 1H, J = 7.1, 7.06 d, 1H, J = 3.5, 6.83 dd, 1H, J = 3.5 a 7.1 (arom H); 4.78 d, 2H, J(P, OCH) = 8.1 (P-OCH_2); 3.71 s, 3H (OCH_3).
DMTr: 7.42 d, 2H, 7.34 t, 2H, 7.29 d, 4H, 7.26 t, 1H, 6.92 d, 4H (arom H); 3.73 s, 6H (OCH_3).

Table 9. Interaction constants for Table 8.

	1′2′	1′2″	2′2″	2′3′	2″3′	3′4′	4′5′	4′5″	5′5″	P-CH_2	
5a	8.3	5.9	13.7	6.1	2.4	3.0	4.4	3.2	10.5	9.0	8.8
5b	7.9	6.1	13.7	6.2	2.2	3.0	4.5	3.0	10.5	9.4	9.2
5c	6.6	6.1	13.9	6.0	3.4	3.0	4.6	3.4	10.5	8.8	8.8
5d	7.4	6.3	13.9	6.1	3.2	2.9	5.8	4.6	10.3	8.3	–
5e	6.6	6.2	13.8	6.5	4.4	3.2	6.0	3.2	10.5	9.0	–
5f	8.4	5.9	13.7	6.0	2.0	2.5	3.9	3.1	10.5	9.0	–
5g	7.7	6.2	13.6	6.0	2.0	2.5	3.5	2.8	10.3	9.0	–
5h	6.3	6.3	13.9	6.3	3.5	3.0	4.7	3.4	10.5	8.7	–
5i	7.6	6.3	13.9	6.0	2.8	2.6	6.0	4.8	10.2	8.6	–
5j	7.2	6.5	13.7	6.0	2.5	3.0	6.3	5.0	10.2	8.4	–
10a	2.4	8.7	15.1	1.0	5.3	3.5	7.6	3.4	10.2	9.8	9.1
10b	2.7	8.8	14.9	1.0	5.5	4.4	6.7	4.6	10.0	9.6	8.5

2-Deoxy-5-*O*-dimethoxytrityl-1-(4-*O*-(2-(4-nitrophenylthio)ethyl)thymin-1-yl)-β-D-*erythro*-pentofuranos-3-yloxymethyl-phosphonate (5g). Compound **5g** was prepared according to **Method B** (Table 8) from monomethyl ester **5b** obtained according to **Method A** (Table 4).

HR FAB calcd for $C_{47}H_{48}N_4O_{14}PS$ 955.2625 $(M\text{-}H)^-$, found 955.2500.

NMR: Tables 8, 9, and 10.

4-Methoxy-1-oxido-2-pyridylmethyl 2-deoxy-5-*O*-dimethoxytrityl-1-(4-*N*-benzoylcytosin-1-yl)-β-D-*erythro*-pentofuranos-3-yloxymethylphosphonate (5h). Compound **5h** was prepared according to **Method B** (Table 8) from monomethyl ester **5c** obtained according to **Method A** (Table 4).

HR FAB calcd for $C_{45}H_{46}N_4O_{12}P$ 865.2850 $(M + H)^-$, found 865.2849.

NMR: Tables 8, 9, and 10.

4-Methoxy-1-oxido-2-pyridylmethyl 2-deoxy-5-*O*-dimethoxytrityl-1-(6-*N*-benzoyladenin-9-yl)-β-D-*erythro*-pentofuranos-3-yloxymethylphosphonate (5i). Compound **5i** was prepared according to **Method B** (Table 8) from monomethyl ester **5d** obtained according to **Method A** (Table 4).

HR FAB calcd for $C_{52}H_{61}N_6O_{11}P$ 990.4167 $(M + Et_3N + H)^+$, found 990.4335.

NMR: Tables 8, 9, and 10.

4-Methoxy-1-oxido-2-pyridylmethyl 2-deoxy-5-*O*-dimethoxytrityl-1-(2-*N*-isobutyryl-6-*O*-(2-(4-nitrophenylthio)ethyl)guanin-9-yl)-β-D-*erythro*-pentofuranos-3-yloxymethylphosphonate (5j). Compound **5j** was prepared according to **Method B** (Table 8) from monomethyl ester **5e** obtained according to **Method A** (Table 4).

HR FAB calcd for $C_{51}H_{53}N_7O_{14}PS$ 1050.3109 $(M\text{-}H)^-$, found 1050.2983.

NMR: Tables 8, 9, and 10.

4-Methoxy-1-oxido-2-pyridylmethyl 2-deoxy-3-*O*-dimethoxytrityl-1-(thymin-1-yl)-β-D-*threo*-pentofuranos-5-yloxymethylphosphonate (8b). A mixture of 2,3′-anhydrothymidine (**6**) (4.36 g; 19.45 mmol) and dimethyl tosyloxymethylphosphonate

Table 10. ^{13}C NMR spectra of 4-methoxy-1-oxido-2-pyridylmethyl- and methyl esters of 3′-phosphonates derived from 2-deoxy-β-D-*erythro*-pentofuranosyl derivatives of nucleobases and from 1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine.

	C-1′	C-2′	C-3′	C-4′	C-5′	P-C	C-2	C-4	C-5	C-6	C-8, CH_3	Note
5a	83.07	36.42	80.68 (9.8)	83.97	64.59	65.35 (153.3)	150.63	163.85	109.87	135.79	11.89	a
5b	83.76	36.97	81.27 (11.7)	85.64	62.05	64.95 (158.2)	150.88	163.31	109.38	135.48	12.70	a, e
5c	83.78	38.05	79.91 (11.7)	86.48	63.62	65.59 (153.3)	154.38	163.16	96.28	144.61	–	a, b
5d	83.74	35.51	80.81 (9.3)	84.11	64.34	65.51 (153.3)	151.62	150.56	126.10	152.15	144.78	a, b
5e	83.87	38.34	79.34 (10.7)	83.98	60.76	65.03 (156.25)	159.69	152.19	117.72	152.87	141.63	a, e, g
5f	83.48	38.36	80.84 (10.7)	85.30	64.51	65.47 (156.0)	150.85	163.13	108.98	134.39	12.18	f
5g	83.52	36.87	81.36 (11.7)	85.31	64.39	63.82 (156.4)	150.59	162.84	109.28	134.82	12.47	e, f
5h	83.83	38.15	80.26 (10.7)	86.59	63.61	65.91 (153.7)	154.22	163.30	96.19	144.49	–	b, f
5i	83.72	35.39	81.34 (11.7)	84.24	64.28	65.55 (156.25)	151.64	150.57	126.14	152.15	143.42	b, f
5j	83.64	35.78	80.11 (11.7)	83.87	64.57	66.01 (156.2)	159.52	152.27	117.51	152.73	141.78	e, f, g
10a	82.84	36.69	80.21 (11.7)	83.89	62.96	65.65 (158.2)	151.24	164.43	110.19	137.40	12.54	a
10b	82.11	36.33	79.14 (9.8)	82.90	61.97	66.16 (159.3)	150.89	163.97	109.91	137.39	12.08	f

DMTr: 158.80, 158.76, 145.77, 136.28, 136.22, 130.50, 2C, 130.40, 2C, 128.23, 2C, 128.07, 2C, 127.12, 113.64, 2C, 113.58, 2C, 87.02, 55.36, 55.34.
a: P-OCH_3: 51.04 d, J(P,C) = 5.9 (5′-P); 51.37 d, J(P,C) = 5.9 (3′-P).
b: N-Bz: 167.61, 133.57, 128.64, 4C, 132.59.
e: PNPTE: 146.85, 145.06, 126.74, 2C, 124.29, 2C, 66.72, 28.17.
f: PIC: 158.32, 151.42 d, J(P,C) = 5.9, 140.32, 110.80, 108.74, 61.15 d, J(P,C) = 3.9, 56.16.
g: iBu: 34.5, 19.55, 2C.
h: PNPE: 146.80, 146.45, 130.58, 2C, 123.58, 2C, 57.59, 34.39.

(6.5 g, 22 mmol) dried by co-evaporation with DMF was treated under vigorous stirring with sodium hydride (60% suspension in mineral oil; 2.34 g; 58.5 mmol) in DMF (100 ml) at −15°C for 3 h, and then at r.t. overnight (TLC in H1). The reaction mixture was cooled to −10°C, carefully neutralized with glacial acetic acid (2.4 ml; 40 mmol), and DMF was evaporated at 40°C (13 Pa). The residue was partitioned between chloroform and 1M aqueous sodium hydrogencarbonate. The organic phase was concentrated in vacuo, oily residue dissolved in 1M aqueous solution of sodium hydroxide (100 ml), and the solution stirred at r.t. for 2 h. The solution was neutralized to pH 7 with Dowex 50 (H^+ cycle), the resin was filtered off, and obtained solution was concentrated. The residue was co-evaporated several times with pyridine, dissolved in pyridine (100 ml), and treated with dimethoxytrityl chloride (8.1 g; 24 mmol) and silver triflate (6.2 g; 24 mmol) at r.t. for 16 h. The reaction was quenched by addition of small amount of methanol, and the solution was concentrated. Compound **8a** was obtained by silica gel chromatography using a linear gradient of solvent system H3 in ethyl acetate followed by a linear gradient of solvent system H1 in H3. Yield 3.66 g (4.86 mmol; 25%) of **8a**. This compound was transformed into 4-methoxy-1-oxido-2-pyridylmethyl ester **8b** according to **Method B**. Compound **8b** was obtained in 42% yield (1.81 g; 2.064 mmol).

HR FAB calcd for $C_{39}H_{41}N_3O_{12}P$ 774.2428 $(M\text{-}H)^-$, found 774.2402.

NMR: Tables 5, 6, and 7.

4-Methoxy-1-oxido-2-pyridylmethyl 2-deoxy-5-*O*-dimethoxytrityl-1-(thymin-1-yl)-β-D-*threo*-pentofuranos-3-yloxymethylphosphonate (10b). Dimethoxytrityl chloride (23 g; 68 mmol) was added in six portions during 6 h to a stirred solution of thymidine (15 g; 62 mmol) in pyridine (150 ml), and the solution was set aside for 24 h. Mesyl chloride (6.24 ml; 80.60mmol) was added at 0 °C, the reaction mixture was stirred at r.t. for 2 h and then diluted with 2M aqueous sodium hydroxide (300 ml). Obtained dark solution was stirred 2 d at r.t., neutralized to pH 7 with Dowex 50 (H^+ form), and after filtration, extracted with chloroform. Organic layer was concentrated *in vacuo* and residue purified on silica gel using a linear gradient of acetone in toluene to afford pure compound **9a** in 99% yield (33.5 g; 61.5 mmol). This compound **9a** (11 g; 20 mmol) was alkylated with DTMP to the methylester **10a** according to **Method A** in 21% yield (2.7 g; 4.12 mmol). The obtained compound **10a**, was further transformed to monomer **10b** according to **Method B** in 60% yield (2.18 g; 2.49 mmol).

HR FAB calcd for $C_{39}H_{41}N_3O_{12}P$ 774.2428 $(M\text{-}H)^-$, found 774.2431.

NMR: Tables 8, 9, and 10.

1-(2-*O*-Acetyl-3-deoxy-5-*O*-methoxycarbonyl-β-D-*erythro*-pentofuranosyl)thymine (13). A solution of 1,2-*O*-isopropyliden-5-*O*-methoxycarbonyl-D-xylofuranose (**11**) (55 g; 221 mmol) and bis-imidazoylthiocarbonate (237 g; 465 mmol) in DCM (1000 ml) was stirred under argon atmosphere at r.t. for 48 h. The reaction mixture was concentrated to a half volume, extracted first with 1 M HCl (2x500 ml) and then with saturated aqueous solution of sodium bicarbonate (2 × 500 ml). The organic layer was evaporated, the residue was dissolved in diethyl ether (300 ml), filtered through celite and filtrate was evaporated. Yellow solid was dissolved in toluene (2200 ml) and the solution was heated to reflux. The solution of tributyltin hydride (98 ml; 332 mmol) in

toluene (500 ml) was added to the gently refluxing solution during 6 h, and the reaction mixture was refluxed for additional 2 h (TLC in S1). The reaction mixture was concentrated and residue was partitioned between acetonitrile and hexane at 40°C. The acetonitrile layer was evaporated, and crude product **11a** was purified on silica gel using a linear gradient of ethyl acetate in toluene giving 59% (30.3 g; 130.5 mmol) of **11a**. Concentrated sulfuric acid (2.42 ml) was added to a mixture of **11a** (14.05 g; 60.5 mmol), acetic anhydride (25 ml), and acetic acid (15 ml) in DCM (180 ml) at 0°C. The reaction mixture was stirred at r.t. overnight. Anhydrous sodium acetate (8.2 g) was added, and the suspension was stirred vigorously for 30 min. The solution was concentrated at first in the vacuum of the water pump followed by oil pump. The residue was partitioned between chloroform and water. The organic layer was washed with saturated solution of potassium chloride, dried with sodium sulfate, evaporated, and finally co-evaporated with xylene (3 × 50 ml) and DCM (3 × 50 ml). Diacetyl derivative **12** was obtained in 64% yield (10.62 g; 38.45 mmol).

A solution of **12** (18.6 g; 67.33 mmol) in acetonitrile (400 ml) was added through a teflon tubing via argon pressure to crystalline 2,4-*O*-bis(trimethylsilyl)thymine (18.2 g; 67.33 mmol). After complete dissolution of thymine derivative, anhydrous tin tetrachloride (16 ml; 135 mmol) was added. The reaction mixture was stirred at r.t. for 20 h. Pyridine (45 ml; 550 mmol) was added and the mixture was concentrated in vacuo. The residue was diluted with chloroform, and resulting suspension was filtered through celite. The filtrate was evaporated, and pure compound **13** was obtained in 40% yield (9.32 g; 27.23 mmol) by chromatography on silica gel using a linear gradient of ethyl acetate in toluene.

HR FAB calcd for $C_{14}H_{19}N_2O_8$ 343.1141 $(M + H)^+$, found 343.1210.

^{1}H NMR: 1.78 (d, 3H, J(CH3,6) = 1.2, CH3); 2.05 (s, 3H, OAc); 2.06 (ddd, 1H, J (3″, 2′) = 2.2, J (3″, 4′) = 6.2, J (3″, 3′) = 13.9, H-3″); 2.27 (ddd, 1H, J (3′, 2′) = 6.9, J (3′,4′) = 8.9, J (3′, 3″) = 13.9, H-3); 3.72 (s, 3H, OCH3); 4.24 (dd, 1H, J (5′, 4′) = 5.6, J (5′, 5″) = 11.9, H-5′); 3.36 (dd, 1H, J (5″, 4′) = 2.8, J (5″, 5′) = 11.9, H-5″); 4.39 (m, 1H, H-4′); 5.24 (dt, 1H, J (3′, 1′) ∼ J (2′, 3″) = 2.3, J (2′, 3′) = 6.9, H-2′); 5.80 (d, 1H, J (1′, 2′) = 2.5, H-1′); 7.46 (brq, 1H, J (6, CH3) = 1.2, H-6); 11.38 (s, 1H, NH).

^{13}C NMR: 12.24 (CH3); 20.90 (CH3); 32.36 (C-3′); 55.11 (OCH3); 68.15 (C-5′); 76.97, 2C (C-2′ a C-4′); 90.14 (C-1′); 110.00 (C-5); 136.74 (C-6); 150.42 (C-2); 155.25 (OC(O)O); 163.95 (C-4); 170.03 (C=O).

4-Methoxy-1-oxido-2-pyridylmethyl 3-deoxy-5-*O*-dimethoxytrityl-1-(thymin-1yl)-β-D-*erythro*-pentofuranos-2-yloxymethylphosphonate (16b). Dowex 1 (OH^- form) was added to a solution of compound **13** (3 g; 8.76 mmol) in methanol (200 ml). The suspension was stirred at r.t. for 24 h, neutralized with acetic acid to pH 7 and filtered off. The filtrate was evaporated, and the residue was co-evaporated several times with water (50 ml) and ethanol (50 ml) to remove traces of acetic acid, and finally dried with pyridine (2 × 50 ml). Obtained nucleoside **14** in pyridine (60 ml) was treated with dimethoxytrityl chloride (4.8 g; 8.76 mmol) which was added in 10 potions during 6 h. The reaction mixture was left at r.t. overnight, concentrated, and dimethoxytrityl derivative **15** was purified on silica gel using a linear gradient of ethanol in chloroform giving 63% yield (3 g; 5.5 mmol) of **15**. The compound **15** (3 g; 5.5 mmol) was transformed to methylester **16a** in 66% yield (2.79 g; 3.64 mmol)

Table 11. ^{1}H NMR spectra of 4-methoxy-1-oxido-2-pyridylmethyl- and methyl esters of 5′- and 3′-phosphonates derived from 1-(3-deoxy-β-D-*erythro*-pentofuranosyl) derivatives of thymine and from 1-(3-deoxy-β-D-*threo*-pentofuranosyl)thymine.

									Base				
	H-1′	H-2′	H-3′	H-3″	H-4′	H-5′	H-5″	P-CH_2	H-2, H-6	H-8, H-5, CH_3	NH	POH	Note
20a	5.88	4.24	1.45	1.30	4.13	3.47	3.28	3.43 (2H)	7.21	1.70	11.30	10.20	a
20b	5.80	4.25	1.50	1.35	4.13	3.50	3.37	3.52 (2H)	7.09	1.63	11.30	10.00	c
16a	5.79	4.19	2.14	1.98	4.33	3.25	3.22	3.68 3.56	7.46	1.89	11.40	10.25	a
16b	5.75	4.18	2.11	1.91	4.23	3.20	3.15	3.62 3.59	7.42	1.40	11.35	10.20	c
28a	6.10	4.41	1.34	1.00	3.73	3.56	3.50	3.43 3.41	7.67	1.91	11.35	11.00	a
28b	6.07	4.39	1.31	0.98	3.70	3.58	3.52	3.51 (2H) 7.66	1.90	11.30	10.90	c	
24a	6.08	4.38	2.30	1.86	4.06	3.22	3.18	3.33 (2H) 7.37	1.54	11.35	10.95	a	
24b	6.07	4.45	2.29	1.89	4.01	3.21	3.18	3.43 3.38	7.36	1.47	11.25	10.90	c

a: P-OCH_3: 3.32 d, 3H, J(P, OCH) = 10.2 (5′-P); 3.40 d, 3H, J = 10.2 (3′-P).
b: Bz: 8.05 d, 2H, 7.64 t, 1H, 7.55 t, 2H.
c: PIC: 8.03 d, 1H, J = 7.1, 7.06 d, 1H, J = 3.5, 6.83 dd, 1H, J = 3.5 a 7.1 (arom H); 4.78 d, 2H, J(P, OCH) = 8.1 (P-OCH_2); 3.71 s, 3H (OCH_3).
DMTr: 7.42 d, 2H, 7.34 t, 2H, 7.29 d, 4H, 7.26 t, 1H, 6.92 d, 4H (arom H); 3.73 s, 6H (OCH_3).

Table 12. Interaction constants for Table 11.

	1′2′	2′3′	2′3″	3′3″	3′4′	3″4′	4′5′	4′5″	5′5″	P-CH_2	
20a	4.6	6.8	4.0	13.4	6.8	4.8	2.8	6.0	10.6	8.6	–
20b	4.0	7.7	3.8	13.4	7.7	6.7	2.7	5.0	10.7	8.2	–
16c	3.6	6.3	4.0	13.6	6.8	5.0	2.8	6.4	10.7	8.8	9.0
16b	3.0	6.9	3.0	13.4	6.9	4.8	3.3	4.4	10.8	8.4	8.4
28a	1.0	5.5	1.0	13.2	5.5	5.2	4.0	4.6	10.7	9.6	9.0
28b	1.0	5.5	1.0	13.2	5.5	5.0	4.0	4.5	10.7	10.0	10.0
92d	1.0	5.5	1.0	13.2	5.5	5.0	3.0	4.0	10.5	9.8	9.8
24a	7.1	8.5	7.1	n*	n*	n*	3.2	4.5	11.0	8.3	8.5
24b	7.2	8.7	7.2	n*	n*	n*	2.6	4.5	11.0	8.3	–

*Unclear multiplet—unable to determine interaction constant.

according to **Method A**, and final picolyl ester **16b** was prepared from **16a** according to **Method B** in 31% yield (1.01 g; 1.134 mmol).

HR FAB calcd for $C_{39}H_{41}N_3O_{12}P$ 774.2428 $(M\text{-}H)^-$, found 774.2408.

NMR: Tables 11, 12, and 13.

4-Methoxy-1-oxido-2-pyridylmethyl 3-deoxy-2-*O*-dimethoxytrityl-1-(thymin-1-yl)-β-D-*erythro*-pentofuranos-5-yloxymethylphosphonate (20b). A solution of compound **13** (3 g; 8.76 mmol) in 1M HCl in methanol (220 ml) was left at r.t. for 3 h and evaporated. Compound **17** was obtained after chromatohraphy on silica gel using a linear gradient of ethanol in chloroform in 71% yield (1.96 g; 6.24 mmol).

The mixture of **17** (1.96 g; 6.24 mmol), DBU (1.4 ml; 9 mmol), and dimethoxytritylchloride (3 g; 9 mmol) in DCM (20 ml) was stirred ar r.t. for 48 h. The reaction was quenched with methanol (5 ml), the solution was evaporated, and the residue was partitioned between saturated aqueous solution of citric acid and chloroform. Dimethoxytrityl derivative **18** was purified on a silica gel using a linear gradient of acetone in toluene. Compound **18** was dissolved in methanol, the solution was saturated with gaseous ammonia at 15°C, and left at r.t. for 24 h. After evaporating methanol, obtained dimethoxyderivative **19** was purified by chromatography on silica gel using a linear gradient of acetone in toluene to obtain 38% yield (1.3 g; 2.39 mmol).

The compound **19** (1.3 g; 2.39 mmol) was transformed to the methylester **20a** in 21% yield (0.64 g; 0.834 mmol) according to **Method A**, and this compound to picolyl ester **20b** in 81% yield (0.526 g; 0.678 mmol) by **Method B**.

HR FAB calcd for $C_{39}H_{41}N_3O_{12}P$ 774.2428 $(M\text{-}H)^-$, found 774.2407.

NMR: Tables 11, 12, and 13.

4-Methoxy-1-oxido-2-pyridylmethyl 3-deoxy-5-*O*-dimethoxytrityl-1-(thymin-1-yl)-β-D-*threo*-pentofuranos-2-yloxymethylphosphonate (24b). Dimethoxytrityl chloride (2 g; 6 mmol) was added in 8 portions during 6 h to a solution of compound **22**[24] (1.4 g; 5.4 mmol) in pyridine (25 ml). The reaction mixture was stirred at r.t. overnight, quenched with methanol (0.5 ml), and evaporated. The residue was partitioned between chloroform and 1M sodium hydrogencarbonate. 5′-*O*-Dimethoxytrityl

Table 13. ^{13}C NMR spectra of 4-methoxy-1-oxido-2-pyridylmethyl- and methyl esters of 5′- and 3′-phosphonates derived from 1-(3-deoxy-β-D-*erythro*-pentofuranosyl) derivatives of thymine and from 1-(3-deoxy-β-D-*threo*-pentofuranosyl)thymine.

	C-1′	C-2′	C-3′	C-4′	C-5′	P-C	C-2	C-4	C-5	C-6	CH_3	Note
20a	90.18	76.14	34.63	77.24	74.33 (10.3)	67.32 (157.2)	150.94	164.12	110.49	137.06	12.17	a
20b	91.03	76.48	34.74	77.47	74.13 (9.8)	67.64 (155.8)	150.87	164.15	110.44	137.33	12.20	c
16a	89.92	84.86 (10.0)	31.00	79.62	64.33	65.22 (159.3)	150.46	164.10	109.49	135.98	12.15	a
16b	89.67	84.03 (10.7)	32.29	79.33	64.06	66.82 (160.0)	150.25	163.92	109.26	139.35	11.99	c
28a	76.66	70.29	35.13	70.29	74.15 (9.8)	67.34 (156.3)	151.54	165.12	108.70	139.11	12.97	a
28b	75.46	73.07	33.10	73.07	72.52 (9.8)	67.87 (161.1)	151.11	163.94	108.70	139.38	12.30	c
24a	83.74	79.63 (9.8)	32.90	75.57	64.91	66.58 (157.2)	150.99	164.17	108.50	137.91	12.30	a
24b	83.33	79.27 (9.8)	32.81	74.78	64.57	67.28 (154.3)	150.81	163.78	108.34	139.41	11.95	c

DMTr: 158.56, 2C, 145.10, 135.82, 135.68, 130.12, 4C, 128.27, 2C, 128.09, 2C, 127.13, 113.60, 4C, 86.05, 55.34, 2C.
a: P-OCH_3: 51.82 d, J(P,C) = 5.8.
b: N-Bz: 167.71, 133.61, 133.08, 128.82, 4C.
c: PIC: 158.65, 151.13 d, J(P,C) = 5.9, 140.07, 110.87, 108.84, 61.06 d, J(P,C) = 3.9, 56.46.

derivative **23** was obtained by purification on silica gel using a linear gradient of acetone in toluene in 99% yield (3 g; 5.37 mmol).

Compound **23** (0.56 g; 1 mmol) was transformed to the methylester **24a** in 73% yield (0.56 g; 0.73 mmol) according to **Method A**. Compound **24a** afforded a final monomer **24a** by **Method B** in 44% yield (0.39 g; 0.44 mmol).

HR FAB calcd for $C_{45}H_{58}N_3O_{12}P$ 877.3789 $(M + H + TEA)^+$, found 877.3715.

NMR: Tables 11, 12, and 13.

4-Methoxy-1-oxido-2-pyridylmethyl 3-deoxy-2-*O*-dimethoxytrityl-1-(thymin-1-yl)-β-D-*threo*-pentofuranos-5-yloxymethylphosphonate (28b). *Tert*-butyldiphenylsilyl chloride (1.73 ml; 6.74) was added to a solution of nucleoside **22**[25] (1.57 g; 6.48 mmol) in pyridine (25 ml). The reaction mixture was stirred at r.t. for 48 h, quenched with methanol (0.5 ml), and evaporated. The residue was partitioned between chloroform and 1M sodium hydrogencarbonate. The organic layer was dried with sodium sulfate, evaporated, and dissolved in DCM (20 ml). DBU (1.21 ml; 8 mmol) and dimethoxytrityl chloride (2.73 g; 8 mmol) were added to the solution, and the mixture was stirred at r.t. for 24 h, quenched with methanol (0.5 ml), and evaporated. The residue was partitioned between chloroform and 10% aqueous citric acid. The organic layer was dried with sodium sulfate, evaporated, the residue was co-evaporated with toluene (50 ml), THF (50 ml), and treated with 0.678 M TBAF in THF (30 ml) at r.t. for 16 h. Dowex 50 (Et_3NH^+ form) (30 ml) and methanol (50 ml) were added, and, after short stirring, the suspension was filtered, and the filtrate was evaporated. 2′-O-Dimethoxytrityl derivative **27** was obtained by purification on silica gel using a linear gradient of acetone in toluene in 17% yield (0.62 g; 1.1 mmol). Compound **27** (0.62 g; 1.1 mmol) was transformed to the methylester **28a** in 42% yield (0.35 g; 0.46 mmol) according to **Method A**, and this compound afforded a final monomer **28b** in 80% yield (0.33 g; 0.37 mmol) by **Method B**. HR FAB calcd for $C_{45}H_{58}N_3O_{12}P$ 877.3789 $(M + H + TEA)^+$, found 877.38610.

NMR: Tables 11, 12, and 13.

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