New Approach to the Synthesis of Oligo(nucleoside methanephosphonate)s[1]

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A new method of preparation of 5′-*O*-DMT-(*N*-protected) nucleoside 3′-*O*-(methanephosphonofluoridates) **2** and their phosphonylating properties toward alcohols, in particular the 5′-hydroxyl groups of nucleosides (or nucleotides), is presented. Preparation of dinucleoside (3′,5′)-methanephosphonates **3** in solution and solid-phase synthesis of Me-Oligo **6** demonstrate the potential use of **2** as monomers.

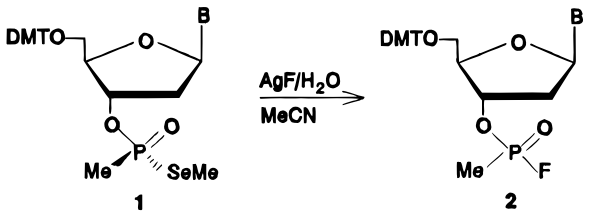
In the course of our studies on the synthesis and reactivity of 5′-*O*-DMT-nucleoside 3′-*O*-(*Se*-methyl methanephosphonoselenolates) **1**[2] we have found that they are readily converted to 5′-*O*-DMT-protected nucleoside 3′-*O*-methanephosphonofluoridates (**2**, B = Thy, Ade^{Bz}, Cyt^{Bz}, Gua^{ibu}) in quantitative yield by treatment at room temperature with a 20% molar excess of aqueous AgF (Scheme 1). This conversion, contrary to our expectations based upon stereochemical results of reactions of P-chiral phosphoryl aziridates with pyridine−x(HF)$_n$ complex,[3] but in agreement with results of other studies,[4,5] is not stereospecific.

Starting from any single diastereoisomer [R_P]-**1** or [S_P]-**1**, examination of this reaction by means of ^{31}P NMR and ^{19}F NMR established that complete disappearance of the signals for **1** was accompanied by the appearance of a nearly 1:1 ratio of a pair of dublets characteristic for the diastereomeric mixture **2**. This reaction was complete within 15 min. Dilution of the reaction mixture with chloroform and washing of the resulting solution with brine leaves a practically pure diastereomeric mixture of **2** in the organic phase. The identity of **2** has been also proved by FAB MS analysis.

It was found that **2** (B = Thy) diluted in EtOH in the presence of 10 mol equiv of DBU provides quantitatively 5′-*O*-DMT-thymidine 3′-*O*-(*O*-ethyl methanephosphonate) (**4**). The identity of **4** has been proved by MS analysis and by ^{31}P and ^{1}H NMR spectroscopy. In the light of this observation, nucleoside methanephosphonofluoridates **2** have been employed for the phosphonylation of 3′-*O*-protected nucleosides **5**. Reaction of **2** with **5** in acetonitrile solution, performed in the presence of 10−15 equiv of DBU, is usually accomplished within 15−20 min, with almost exclusive formation of dinucleoside (3′,5′)-methanephosphonates **3**. Diastereomeric ratios of **3** obtained, according to Scheme 2, and physicochemical characteristics of products are presented in Table 2.

Further simplification of this approach has been achieved reacting **1** with **5** (B = Thy) under anhydrous conditions in the presence of triethylamine trihydrofluoride and DBU. In this case, conversion of nucleoside 3′-*O*-(*Se*-methyl methanephosphonoselenolate)s **1** into **2** and their condensation with **5** leads with reasonable yields to dinucleoside (3′,5′)-methanephosphonates **3** in a one-pot reaction proceeding at room temperature. Independently, triethylamine trihydrofluoride (1 M) in dioxane was used as fluorinating agent for the conversion **1** → **2**. It was established that under the reaction conditions (3-fold molar excess of Et$_3$N·3HF, MeCN) but without DBU,

Scheme 1



1 2

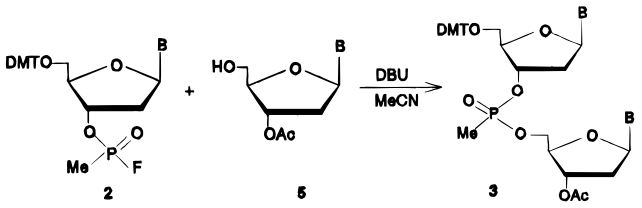
Reaction conditions: AgF (25% in water)- 1.2 eqiuv.; room temp.; 5 min.

Table 1. Synthesis and Physical Properties of 5′-*O*-DMT-(*N*-protected)-nucleoside 3′-*O*-(methanephosphonofluoridates) (2)

	substrate **1**		product **2**				
B′	^{31}P NMR: δ^a	J^1_{P-Se} (Hz)	^{31}P NMR: δ^a	J^1_{P-F} (Hz)	diast ratio	FAB MS	yield^b
T	49.7	430	30.7	1060	1/1[c]	623.4	96
			30.6	1059			
C^{Bz}	50.3	430	30.3	1060	1/1	712.2	95
			30.2	1059			
G^{iBu}	50.6	431	31.3	1058	1/1	718.2	90
			30.9	1060			
A^{Bz}	50.2	432	30.6	1057	1/1	736.2	95
			30.3	1059			

[a] All ^{31}P NMR measurements in $CDCl_3$. [b] Isolated by means of precipitation (see Experimental Section). [c] When the reaction was carried out at −40 °C (THF−MeCN), observed diastereomeric ratio of **2** was 3/2.

Scheme 2



2 5 3

Reaction conditions: 2 -2.5 equiv.; 5 -1 equiv.; DBU -10-15 fold excess; room temperature; time- 15-20 min.

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(1) This paper is dedicated to Professor Colin Reese on the occasion of his 65th birthday.

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Table 2. Synthesis and Properties of Dinucleoside (3′,5′)-Methanephosphonates 3

substrates		product **3**			
2	**5**	^{31}P NMR: δ[a]	diastereom ratio[b] fast/slow	HPLC t_R[c] (min)	FAB MS
G^{ibu}	T	32.47	55	11.28	982.4
		32.75	45	12.20	
	C^{Bz}	32.67	44	13.45	1071.4
		32.89	56		
	A^{Bz}	32.60	47(61)[d]	13.22	1167.6
		32.94	53(39)	13.79	
	G^{iBz}	32.39	50	9.68	1077.4
		33.15	50	9.86	
C^{Bz}	T	32.23	55	13.16	976.4
		32.55	45	13.79	
	C^{Bz}	31.79	45	12.09	1065.4
		32.22	55	13.09	
	A^{Bz}	32.43	55	14.54	1161.7
		32.67	45	15.54	
	G^{ibu}	32.04	57	10.74	1071.6
		33.03	43	11.05	
T	T	31.34	54	14.08	887.4
		31.71	46	14.36	
	C^{Bz}	32.63	54	12.81	976.4
		33.15	46	13.23	
	A^{Bz}	32.43	59	13.74	1072.7
		32.79	41	14.49	
	G^{ibu}	32.04	58	9.47	982.5
		33.46	42	9.92	
A^{Bz}	T	32.23	52	12.69	1000.5
		32.71	48	13.63	
	C^{Bz}	32.57	49	14.95	1089.6
		32.94	51	15.31	
	A^{Bz}	32.39	57	16.74	1185.8
		32.62	43	17.03	
	G^{ibu}	32.10	54	10.87	1095.4
		33.29	46	11.30	

[a] NMR data for fully protected dimers. [b] Diastereomeric ratio determined by means of integrated signals in ^{31}P NMR spectra. [c] HPLC data; deprotected dimers, ODS-HYPERSIL, 5 μm, 25 cm; conditions 4−40% MeCN/0.1 M TEAB, flow 1.5 mL/min, 1.5%/min. [d] HPLC integration, fully deprotected $G_{PMe}A$ (in brackets).

exclusive formation of **2** was observed (^{31}P NMR δ 30.8, 30.65, $^1J_{P-F}$ = 1060 Hz for both diastereomers, as measured in C_6D_6/MeCN).

Since DBU-resistant solid supports with anchored "starter" nucleoside are used in our laboratory for the synthesis of oligo(nucleoside phosphorothioate)s[6] and oligo(nucleoside phosphorodithioate)s[7] we have used **5** linked to LCA-CPG *via* a succinyl-sarcosinoyl linker[8] for solid-phase synthesis of oligo(nucleoside methanephosphonate)s **6**. The protocol used for automated synthesis of **6** is shown in Table 3, and compounds **6** obtained by this method are listed in Table 4.

It has to be mentioned that a step-yield of condensation *via* methanephosphonofluoridates is >92%, as measured by trityl assay, and preparative yield of isolated dimer $T_{Me}T$ by means of HPLC reaches 90%.

The pentamer TCCTG, formerly prepared as homochiral [*all*-R_P]- and [*all*-S_P]-constructs,[2] is presently being used in attempts at separation of the diastereomeric mixture (16 isomers) by chiral HPLC column (data not shown). Experiments toward improvement of the yield of oligo(nucleoside methanephosphonate)s prepared by the method presented are in progress.

Table 3. Synthetic Protocol for the Automated Solid-Phase Synthesis of Me-Oligos Using Monomers 2

step	reagent or solvent	volume (mL)	purpose	time (s)
1	(a) dichloroacetic acid in CH_2Cl_2	2.3	detritylation	50
	(b) acetonitrile	7.0		150
2	(a) DBU activated **2** in CH_3CN[a]	0.6	coupling	600
	(b) methylene chloride	3.33	wash	200
	(c) acetonitrile	7.0	wash	150
3	(a) DMAP/Ac_2O/lutidine in THF	0.33	capping	20
	(b) acetonitrile	7.0	wash	150

[a] For the 1 μmol scale, a mixture of 450 μL of 0.5 M DBU in CH_3CN and 150 μL of 0.1 M **2** in CH_3CN was used.

Table 4. Oligo-Me 6 prepared from 2 *via* the Solid-Phase Synthesis

sequence	average yield (trityl assay)	HPLC data (tR)[a] (min)
TT	92%	7.50
TTT	78%	8.80
TTTT	83%	9.60
TCCTG	81%	9.60
TCCTT	87%	9.35

[a] ODS HYPERSIL; gradient 5−40% MeCN in 0.1 M TEAB, pH = 7.0, flow = 1.5 mL/min, t = 15 min).

In the light of recent reports by Arnold[9] and Reynolds *et al.*[10] demonstrating that chimeric oligonucleotides containing [R_P]-methanephosphonates linked to phosphates or phosphorothioates at alternate positions are promising tools for an *antisense mRNA strategy*,[11] an easy access to **2**, which can be produced in solution in bulk quantities, represents a new source of dinucleoside (3,5)-methanephosphonates for a dimer-block synthesis of aforementioned chimeras. Also, in the context of reported results it has to be mentioned that ionic nucleoside 3′-*O*-phosphorofluoridates have been used for the synthesis of an oligonucleotide phosphate link by von Tigerstrom and Smith in the early 1970's.[12] Our studies on the "Revisited von Tigerstrom−Smith Approach" as applied to preparation of Me-oligos indicate that, in the presence of DBU, nucleoside 3′-*O*-methanephosphonofluoridates **2** are very efficient, as expected,[13] phosphonylating agents toward oxygen nucleophiles, including nucleosides, and can effectively be employed for the preparation of dinucleoside (3′,5′)-methanephosphonates.

Very recently, the reaction of **2** with 5′-*O*-silylated nucleosides performed in the presence of potassium fluoride, has been presented as a new approach to the synthesis of **3**.[14] Since the preparation of starting **1** is relatively simple, and their stability seems to be higher

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than that of nucleoside methanephosphonamidites,[15] the methodology presented here can be considered as an alternative to the widely applied phosphonamidite method.[16]

Experimental Section

Warning! Biological properties of nucleoside 3′-O-methanephosphonofluoridates have not been evaluated. Therefore, use caution to avoid skin contact in handling these compounds.

Chemical shifts (δ) are reported relative to TMS (^{1}H) and 85% H_3PO_4 (^{31}P) as external standards. Positive chemical shift values are assigned for compounds resonating at lower fields than standards. High-pressure liquid chromatography was run on a gradient system, equipped with reverse-phase column (ODS Hypersil, 5 μm, 25 cm, 4.6 mm). The solvent system used: 0.1 M triethylammonium bicarbonate (TAEB) at pH = 7.0 (A), and 40% MeCN in 0.1 M TEAB (B). Column chromatography and HPTLC analyses were performed on silica gel (240−400 mesh) and silica gel precoated F_{254} plates (E. Merck, Inc.), respectively. Solvents and reagents were purified according to standard laboratory techniques and stored under Ar.

AgF (25% weight in water) was purchased from POCH, Poland. Triethylamine trihydrofluoride was purchased from Aldrich Ltd. **1**, **2**, and **5** were dried before condensation by means of coevaporation with pyridine and left under high vacuum overnight. Condensation reactions were performed under dry argon.

General Procedure for Preparation of 5′-*O*-DMT-nucleoside 3′-*O*-Methanephosphonofluoridates 2. Into a stirred solution of **1**[2] (0.1mmol) in acetonitrile (MeCN) (5 mL) a solution of AgF (0.125 mmol, 25% in water) was added in one portion. The reaction was performed at ambient temperature. Stirring was continued for 5 min, followed by dilution of the reaction mixture with $CHCl_3$ (25 mL) and extraction of the resulting mixture with brine (twice, 10 mL). The organic fraction was dried with $MgSO_4$, concentrated to dryness, redissolved in a small volume of dry $CHCl_3$, and precipitated from petroleum ether (bp 40−60 °C). Pure **2** were stored as white powders for several weeks. Yields and analytical data are collected in Table 1.

Reaction between 5′-*O*-DMT-Thymidine 3′-*O*-Methanephosphonofluoridate (2) and Ethanol. Into a solution of **2** (31 mg, 0.05 mmol) in freshly distilled EtOH (0.2 mL) a solution of DBU in MeCN (10% v/v, 0.2 mL) was added. The ^{31}P NMR spectrum, recorded after 10 min, confirmed formation of an exclusive product **4** as a mixture of diastereomers: δ 32.01 (42%) and 31.83 (58%) ($EtOH/C_6D_6$). After short column chromatography (2% EtOH in $CHCl_3$), **4** was analyzed by MS: FAB $[M - H]^-$ 649.5. Yield 27 mg (85%).

General Procedure for Coupling of 5′-*O*-DMT-Nucleoside 3′-*O*-Methanephosphonofluoridates (2) with 3′-*O*-Protected Nucleosides 5. **2** (0.05 mmol, 2.5 mol equiv) and **5** (0.02 mmol, 1 equiv) were dissolved in MeCN (2.5 mL), followed by addition of DBU (10−15 mol equiv). The progress of condensation was followed by TLC. After the reaction was complete (usually 15−20 min), the reaction mixture was diluted with $CHCl_3$ (20 mL) and washed twice with 0.1 M citric acid (10 mL). After drying the organic phase over anhydrous $MgSO_4$, concentration, and precipitation from petroleum ether, the crude product was analyzed by means of ^{31}P NMR. Purification of dimers **3** as well as separation of diastereomers were performed by means of silica gel column chromatography [Kiesegel 60 or 60H, eluent system: chloroform−methanol (0−5%)]. Yields of **3** varied from 95% for $T_{PMe}T$ to 80% for $G_{PMe}A$. Removal of base-labile protective groups and detritylation were performed as described earlier.[2]

Preparation of 5′-*O*-DMT thymidylyl (3′,5′)-3′-*O*-Acetylthymidine 3′-methanephosphonate (3) *via* Activation of 1 with Triethylamine Trihydrofluoride and DBU. **1** (45 mg, 0.065 mmol)) and **5** (14 mg, 0.05 mmol) were dissolved in MeCN (1 mL) with DBU (0.05 mL) added to this solution, followed by triethylamine trihydrofluoride (0.025 mL, 1 M in dioxane). After 10 min the reaction was complete (HPTLC assay). The reaction mixture was diluted with $CHCl_3$ (10 mL), washed twice with 0.1 M citric acid, dried with $MgSO_4$, and concentrated. ^{31}P NMR spectrum confirmed the formation of dimer $T_{PMe}T$, δ 32.07 and 31.80 in the ratio $[S_P]$:$[R_P]$ = 46:54, with total yield 70%.

Solid-Phase Synthesis. Solid-phase experiments were carried out on an Applied Biosystems 391 PCR-Mate Synthesizer, using protocol described in Table 3. Columns (1 μmol) were prepared using CPG (controlled pore glass) support with the succinyl-sarcosinoyl linkers with a loading: for 5′-*O*-DMT-N^{iBu}-Gua 32.12 μmol/g; for 5′-*O*-DMT-Thy - 29.38 μmol/g.[17]

Prepared compounds **6** are listed in Table 4. Their release from support and deprotection was performed as follows: the solid support was transferred to the Eppendorf tube and treated with a solution of NH_3/H_2O:EtOH:MeCN (10:45:45) (0.5 mL) for 30 min. Solvents were evaporated to dryness, followed by addition of 1:1 mixture of EtOH and ethylenediamine (0.5 mL). After 6−10 h the solution was separated from solid CPG, concentrated to dryness, and dissolved in $EtOH:H_2O$ (1:1), pH = 7 (adjusted). Products **6** were purified by means of HPLC (data included in Table 4).

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