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A direct method for the synthesis of nucleoside 5′-methylenebis(phosphonate)s from nucleosides

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Abstract—An efficient method for the preparation of nucleoside 5′-methylenebis(phosphonate)s has been developed. Unprotected nucleosides are phosphonylated directly with methylenebis(phosphonic dichloride). Reaction conditions were optimized to prevent side product formation.

1. Introduction

Since ribonucleoside 5′-oligophosphates are involved in a large number of biochemical processes, there is a great demand for analogs, for example, nucleotides modified in the phosphate chain. The advantage of methylene bisphosphonates is the extreme stability of the P–C bonds, which precludes any enzymatic cleavage. Hence, such types of compound can serve as precursors for the preparation of non-hydrolyzable enzyme inhibitors and be exploited as drugs and as other tools for biochemical studies.[1–6]

Although extensive investigations have been carried out with the aim of synthesizing ribonucleoside 5′-methylenebis(phosphonate)s, the existing multi step methods afford the target products in only modest yields. Two main synthetic strategies have been applied. In one of them, a suitably protected nucleoside reacts with a strongly activated bisphosphonate.[6–8] The other strategy consists of the preparation of a nucleoside 5′-sulfonyl ester followed by its nucleophilic substitution with bisphosphonate.[9–11] Recently, the preparation of inosine 5′-methylenebis(phosphonate) by phosphonylation of inosine (suitably protected on ribose) with methylenebis(phosphonic dichloride) has been reported.[12]

In the present communication we describe a convenient and highly efficient method for direct phosphonylation of unprotected nucleosides with methylenebis(phosphonic dichloride) (Scheme 1). Our intention was to generalize a well known approach to phosphorylate the 5′-hydroxyl with phosphorus oxychloride in trimethyl phosphate, as first reported by Yoshikawa et al.[13] Such an approach should have several advantages over other methods in terms of mildness of the reaction conditions, selectivity, efficiency and convenience.

2. Results and discussion

However, due to the bulkiness of methylenebis(phosphonic dichloride) as compared to phosphorus oxychloride, some additional aspects should be taken into account in the case of the former reagent. Since the reaction proceeds via intermediate **2**, in addition to the risk of 2′- and 3′-phosphorylation, the formation of the dinucleotide **3** and the 5′,3′-cyclomethylenebisphosphonate **4** is also possible (Scheme 2).

As a starting point to study 5′-phosphonylation with methylenebis(phosphonic dichloride), we chose standard conditions for 5′-phosphorylation with $POCl_3$.[13] The reaction was carried out at 0 °C, with a 2-fold excess of $CH_2(POCl_2)_2$. The progress of the reaction was monitored using analytical HPLC. On disappearance of the starting material the reaction was quenched by addition of aqueous triethylammonium bicarbonate (TEAB).[14]

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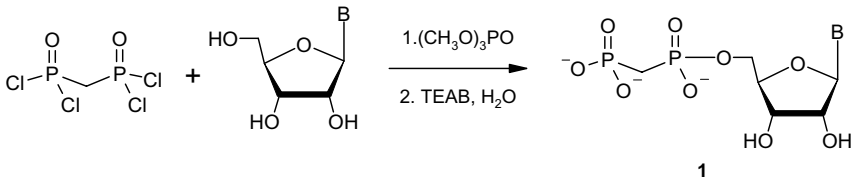
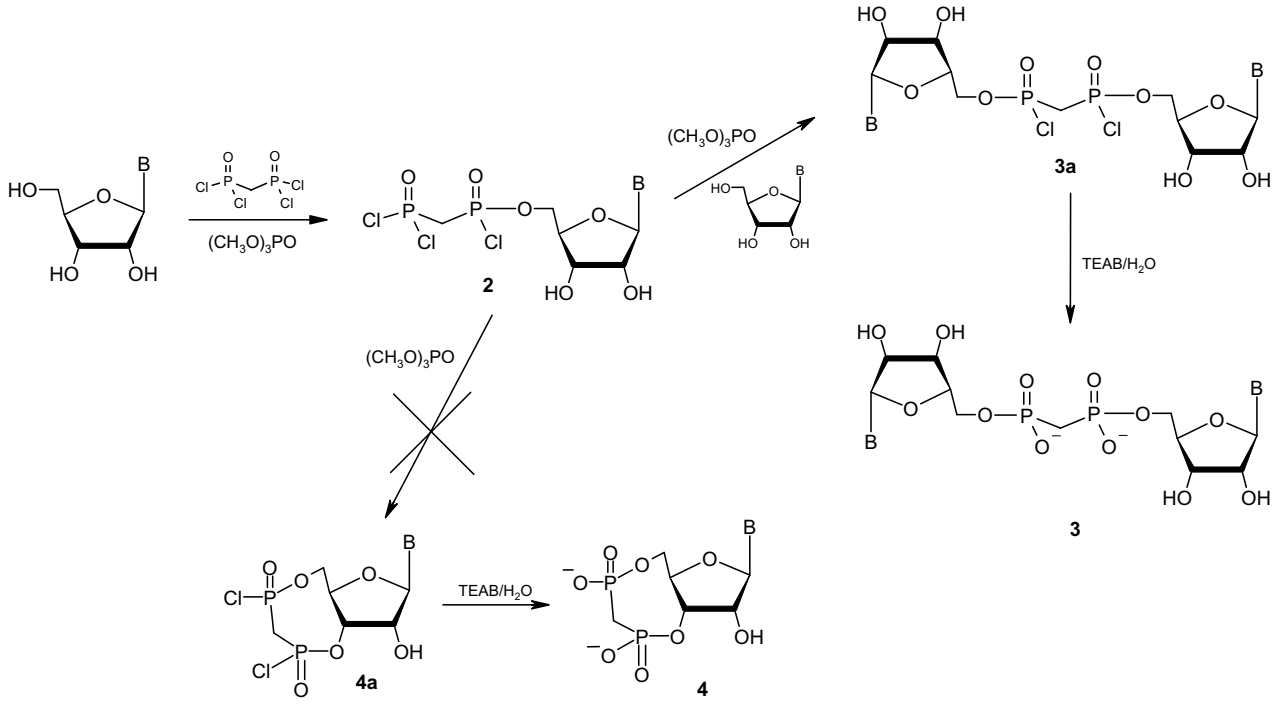


Scheme 1.

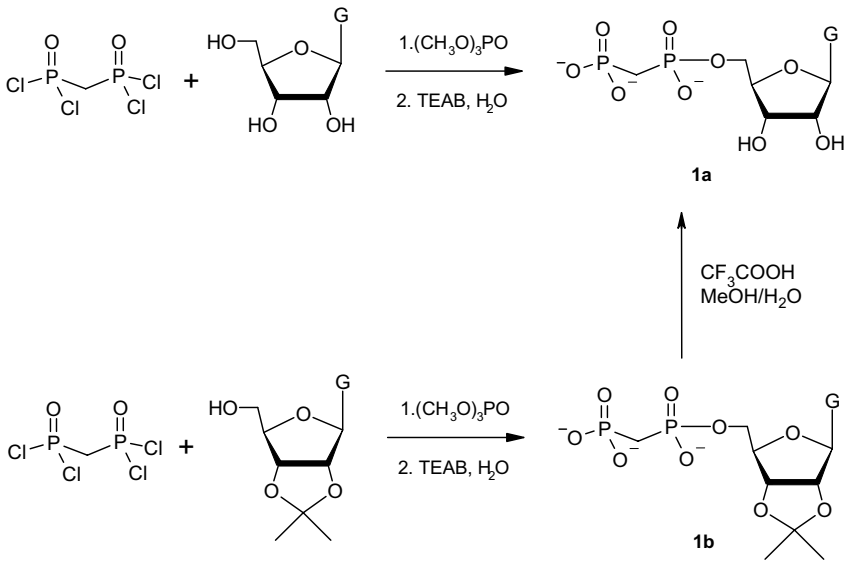


Scheme 2.

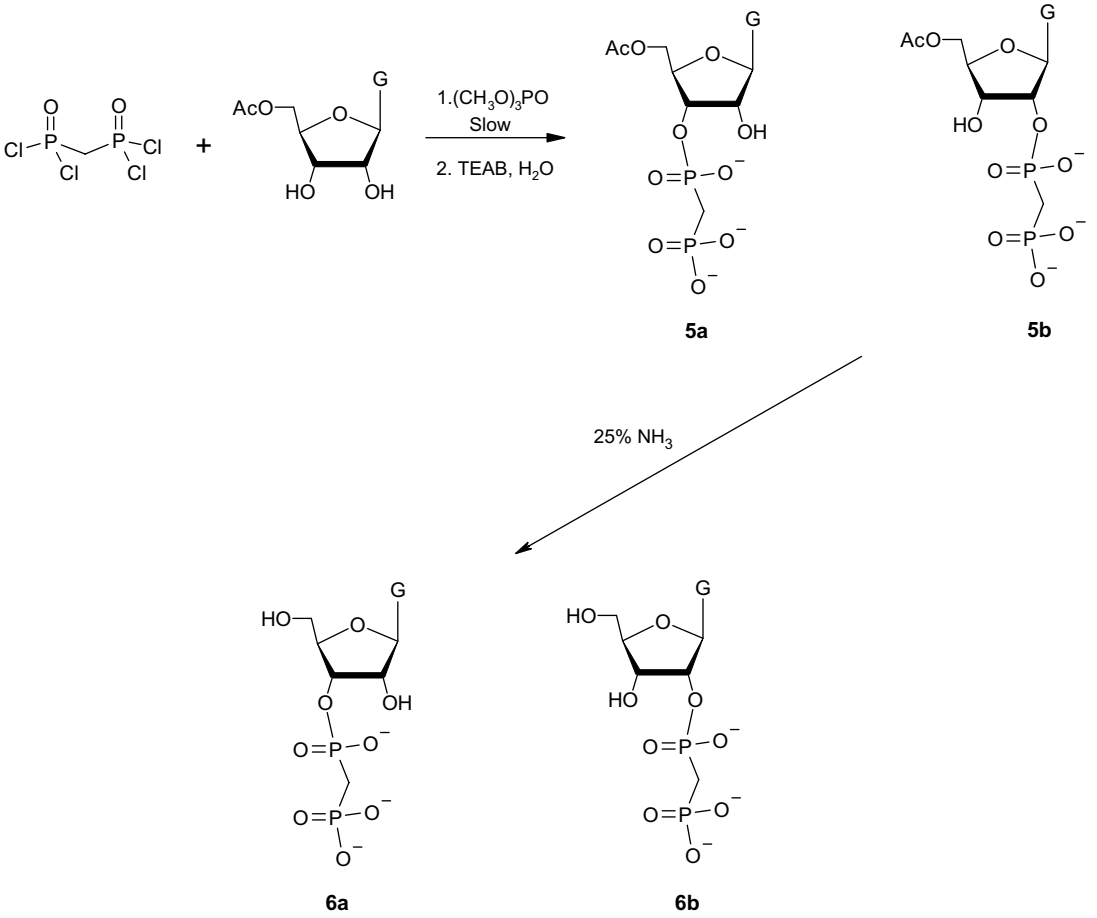
We have observed that $CH_2(POCl_2)_2$ is more reactive than $POCl_3$, that is, all reactions with the former reagent have higher rates and are completed faster in comparison to those when $POCl_3$ was used. This is not surprising, since a lack of electron back-donation from the CH_2 group makes the phosphorus centre more electrophilic, even though the relative reactivities of the particular nucleosides are similar to those for standard phosphorylation.

To confirm the 5′-regioselectivity of the reaction we carried out the phosphonylation reaction with guanosine and obtained product **1a**. The same reaction with 2′,3′-*O*-isopropylidene guanosine gave **1b**. Following isolation and purification of **1b**, the isopropylidene group was removed under acidic conditions and the resulting product appeared identical to **1a** by means of ESI MS, NMR and HPLC (Scheme 3). We also conducted the phosphonylation using 5′-protected (acetylated) guanosine, anticipating slow formation of 2′ and 3′ phosphonylated products (Scheme 4). After 3 h less than half of the nucleoside had reacted which confirmed our prediction. Two products, having very similar retention times, were formed in ca. 2:1 ratio. We were unable to separate them by RP or ion-exchange HPLC, hence they were isolated as a mixture. Mass spectroscopy proved their structures as 5′-*O*-acetyl guanosine 3′-methylenebis(phosphonate) (**5a**) and 5′-*O*-acetyl guanosine 2′-methylenebis(phosphonate) (**5b**) (*m*/*z* calcd 482.00. Found 482.00). After deprotection in aqueous ammonia, the mixture was analyzed by NMR to identify the major and the minor products: 3′-methylenebis(phosphonate) (**6a**) and guanosine 2′-methylenebis(phosphonate) (**6b**), respectively.[15]

During phosphonylation of the nucleosides dinucleotide **3** was consistently obtained in varying quantities, increasing with the time of the reaction, however its formation was significantly slower than that of the desired product **1**. At the end of most of the reactions only a percentage of **3** was observed. Unfortunately, less reactive nucleosides were transformed into a nearly equimolar mixture of **1** and **3**, because the initially formed **2** further reacted with the nucleoside, still present in the reaction mixture. We thought that this problem might be overcome by using a larger amount of $CH_2(POCl_2)_2$ in the reaction. The higher $CH_2(POCl_2)_2$ concentration should increase the rate of formation of **1** and thus lessen the amount of **3**. It was rewarding to see that addition of 4–6 equiv of $CH_2(POCl_2)_2$ to the reaction mixture of less reactive nucleosides, afforded almost exclusively the desired mononucleotide diphosphate **1**.



Scheme 3.



Scheme 4.

In contrast, when 0.4 equiv of $CH_2(POCl_2)_2$ was used for the reaction with guanosine, compound **3** was the only product formed.[16]

We did not observe the formation of the cyclic compound of type **4** in any of the cases examined. Moreover, the products of diphosphonylation: 5′,3′- or 5′,2′-*O*-di-[methylenebis(phosphonate)]s were observed only after long periods of time or when a very high (4–10 equiv) excess of $CH_2(POCl_2)_2$ was used. The role of trimethyl phosphate in this reaction is probably the same as in the original Yoshikawa phosphorylation with $POCl_3$,

but the reaction mechanism is not yet fully understood. No adduct of $CH_2(POCl_2)_2$ and $(CH_3O)_3PO$ could be detected directly by ^{31}P NMR in CH_2Cl_2 solution. However, the selectivity of the reactions towards 5′-primary hydroxyl groups might be explained by the steric requirements of such a short-lived intermediate.

To demonstrate the versatility of this new methodology, a series of nucleosides were utilized as substrates to produce a number of nucleoside 5′-methylenebis(phosphonate)s **1a**–**h**, which are presented in Table 1. The yields are based on integration of the HPLC peaks,[17] since the extinction coefficients of the substrate and of the product were almost identical. Samples of products were isolated by preparative HPLC[18] and their structures were confirmed by ^1H and ^{31}P NMR and mass spectra (Table 2). In preparative runs, the scale of experiments could be increased even up to 1.5 mmol of the substrate and the isolation could be performed using ion-exchange column chromatography (see Section 2). Unfortunately, the yields are then lowered by ca. 10% in comparison with those from Table 1.

In summary, the methodology presented for the preparation of nucleoside 5′-methylenebis(phosphonate)s of type **1** represents a new, efficient and general entry to this class of compounds. It makes use of readily available starting materials and does not require the use of any protecting groups. More detailed studies on this reaction are currently in progress.

3. Experimental

Regarding the described method in general, the following comments should be made. To prevent the formation of nucleoside 5′,3′- and 5′,2′-di-[methylenebis(phosphonate)]s, especially when a large excess of $CH_2(POCl_2)_2$ is used, the reaction progress should be constantly monitored (HPLC), in order to quench the reaction before the diphosphonylation products are formed. Some nucleosides, like uridine, may be less reactive and hence a large excess of $CH_2(POCl_2)_2$ may have to be used to suppress dinucleotide **3** formation. A typical experimental procedure is exemplified below by the preparation of

Table 1. Phosphonylation of nucleosides with methylenebis(phosphonic dichloride)

Nucleoside 5′-methylenebis(phosphonate) (product)	Nucleoside (substrate)	Equivalents of $CH_2(POCl_2)_2$	Reaction time	HPLC yield (%)
1a	Guanosine	2	1 h	85
1b	2′,3′-*O*-Isopropylidene guanosine	2	1 h	89
1c	3′-*O*-Methyl guanosine	2	1 h	81
1d	7-Methyl guanosine	4	45 min	81
1e	Adenosine	2	4 h	77
1f	Inosine	2	35 min	75
1g	Uridine[a]	6	9 h	65
1h	Cytidine	2	1 h	89

Conditions: nucleoside—0.04 mmol; $CH_2(POCl_2)_2$—as given; trimethyl phosphate—1 mL; temperature—0 °C.
[a] The unusual resistance of uridine towards phosphonylation is in accordance with its lower reactivity, compared to other ribonucleosides, in the classic Yoshikawa phosphorylation.[13]

Table 2. NMR and MS data of compounds **1a**–**1h** in D_2O

	Compound							
	1a	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**
H1′	5.92	6.10	5.91	6.07	6.14	6.14	5.99	5.96
H2′	4.76	5.38	4.92	4.68	4.80	4.80	4.41[a]	4.37[a]
H3′	4.55	5.21	4.20	4.53	4.56	4.55	4.39[a]	4.36[a]
H4′	4.33	4.65	4.43	4.40	4.39	4.38	4.28	4.28
H5′	4.17[a]	4.14[a]	4.15[a]	4.31	4.18[a]	4.18	4.20[a]	4.24
H5″	4.15[a]	4.12[a]	4.14[a]	4.20	4.19[a]		4.18[a]	4.16
H2	—	—	—	—	8.57	8.50	—	—
H5	—	—	—	—	—	—	8.02	8.15
H6	—	—	—	—	—	—	5.97	6.20
H8	8.12	8.29	8.17	—[b]	8.27	8.22	—	—
CH_3	—	1.45; 1.66	3.53	4.13	—	—	—	—
CH_2	2.14	2.12	2.17	2.21	2.19	2.18	2.19	2.19
Pα	19.23	18.93	16.17	19.18	19.32	19.27	19.24	19.23
Pβ	15.67	15.42	16.32	15.11	15.59	15.70	15.55	15.60
$^2J_{P-CH_2}$	19.6	19.9	19.9	19.6	19.9	19.9	19.9	20.0
ESI MS $[M-H]^-$	440.00	480.01	454.04	454.02	424.02	425.01	401.00	400.01
Calcd mass	440.00	480.00	454.00	454.00	424.01	425.00	401.00	400.00

^1H and ^{31}P NMR chemical shifts are in parts per million (±0.01) versus the standard references; coupling constants are in hertz (±0.2).
[a] Approximate value due to signal overlapping.
[b] Exchangeable proton.

guanosine 5′-methylenebis(phosphonate), obtained as the triethylammonium salt.

A solution of methylenebis(phosphonic dichloride) (353 mg, 1.4 mmol) in trimethyl phosphate[19] (10 mL), cooled to 0 °C was added to a suspension of guanosine (200 mg, 0.7 mmol) in trimethyl phosphate at 0 °C. The reaction mixture was stirred at 0 °C and samples were analyzed by HPLC after 15 min intervals. After 1 h, on disappearance of guanosine, 0.7 M aqueous TEAB[14] was added to pH 7. The mixture was separated by chromatography on DEAE Sephadex (A-25, HCO_3^- form), using 0–1 M linear gradient of aqueous TEAB. Fractions containing the product were pooled and evaporated to dryness, with ethanol added repeatedly to remove TEAB buffer. Guanosine 5′-methylenebis(phosphonate) triethylammonium salt **1a** (379 mg, 73%), was obtained as a glassy solid.

Acknowledgements

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14. TEAB solution was obtained by acidification of aqueous triethylamine to pH 7.5 using CO_2.
15. Compound **6a:** $C_{11}H_{17}N_5O_{10}P_2$, ESI MS: 440.00 $[M-H]^-$ (calcd 440.00); ^{1}H NMR (400 MHz, D_2O): δ 8.00 (1H, s, H8), 5.97 (1H, d, H1′, $J_{1'-2'}$ = 6.0 Hz), 5.24 (1H, ddd, H3′, $J_{2'-3'}$ = 6.0 Hz, $J_{3'-4'}$ = 3.0 Hz, $J_{3'-P}$ = 3.5 Hz), 4.80 (1H, t, H2′, $J_{1'-2'} = J_{2'-3'}$ = 6.0 Hz), 4.40 (1H, m, H4′), 3.89 (2H, m, H5′,5″), 2.23 (2H, t, P–CH_2–P, J = 19.7 Hz); ^{31}P NMR (162 MHz, D_2O): 18.65 (1P, m, Pα), 15.36 (1P, m, Pβ). Compound **6b:** $C_{11}H_{17}N_5O_{10}P_2$, ESI MS: 440.00 $[M-H]^-$ (calcd 440.00); ^{1}H NMR (400 MHz, D_2O): δ8.00 (1H, s, H8), 6.06 (1H, d, H1′, $J_{1'-2'}$ = 5.7 Hz), 5.24 (1H, dt, H2′, $J_{1'-2'}$ = 5.7 Hz, $J_{2'-3'} = J_{2'-P}$ = 4.7 Hz), 4.54 (1H, t, H3′, $J_{2'-3'} = J_{3'-4'}$ = 4.7 Hz), 4.26 (1H, m, H4′), 3.93–3.82 (2H, m, H5′, 5″), 2.15 (2H, t, P–CH_2–P, J = 19.7 Hz); ^{31}P NMR (162 MHz, D_2O): δ 18.10 (1P, m, Pα), ~15.4 (1P, m, Pβ).
16. P^1,P^2-bis(5′-guanosine) methylenebis(phosphonate), $C_{21}H_{28}N_{10}O_{14}P_2$, ESI MS: 705.01 $[M-H]^-$ (calcd 705.00); ^{1}H NMR (400 MHz, D_2O): δ 8.20 (2H, s, H8), 5.94 (2H, d, H1′, $J_{1'-2'}$ = 5.3 Hz), 4.76 (2H, dt, H2′, $J_{1'-2'}$ = 5.3 Hz, $J_{2'-3'}$ = 4.3 Hz), 4.54 (2H, t, H3′, $J_{2'-3'} = J_{3'-4'}$ = 4.3 Hz), 4.34 (2H, m, H4′), 4.20–4.16 (4H, m, H5′, 5″), 2.19 (2H, t, P–C**H**$_2$–P, J = 19.7 Hz); ^{31}P NMR (162 MHz, D_2O): δ 18.30 (m).
17. Column 25 cm SUPELCOSIL™ LC-SAX (Supelco). Eluents: A: 0.01 M AcOH, 0.006 M KH_2PO_4, pH 4; B: 0.6 M KH_2PO_4. Elutions were performed at room temperature, with a 1.0 mL/min flow rate, in a linear gradient from 100% A to 100% B for 20 min (detection at 260 nm).
18. Semi-preparative column 19 × 300 mm, Nova-Pak HR C18 6 μm (waters). Elution at room temperature, with a 5 mL/min flow rate, in isocratic 0.05 M $AcONH_4$, pH 5.9.
19. Commercial trimethyl phosphate was distilled under reduced pressure.