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Synthesis of Dinucleoside (N3′→MeP5′) Methanephosphonamidates

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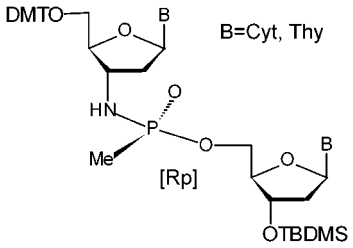
ABSTRACT



Three different approaches were used for the synthesis of dinucleoside methanephosphonamidates [3′-NH-P(O)(CH_3)O-5′], starting from dichloromethylphosphine or dichloromethanephosphonate as the phosphorus-containing moiety. 5′-DMT-3′-amino-3′-deoxythymidine and *N*(4)-benzoyl-5′-DMT-3′-amino-2′,3′-dideoxycytidine were used as the aminonucleoside precursors and the respective 3′-protected nucleosides (thymidine or *N*(4)-benzoyl-2′-deoxycytidine) as the 5′-hydroxyl reagents.

Oligonucleotide analogues possessing high affinity toward double-stranded DNA (dsDNA) are good candidates for the antigene[1] therapeutic strategy. Such DNA analogues are required to be stable to nucleolytic degradation and to exhibit enhanced cellular uptake and low affinity toward proteins.[2] To date, two DNA analogues—oligo(nucleoside methanephosphonate)s with an [R_P] configuration[3] (Figure 1a) and N3′→P5′-phosphoramidate oligonucleotides[4] (Figure 1b)—have been investigated as candidates for therapeutic applications. In view of the advantageous properties of the above DNA analogues, we have designed oligomers with the combined structural features of both classes. Thus, we synthesized thymidine dimers linked by a novel *P*-chiral methanephosphonamidate [3′-NH-P(O)(CH_3)O-5′] moiety (Figure 1c) and introduced them into a DNA chain in alternate positions.[5,6] The novel DNA analogues exhibit resistance to nucleolytic degradation and, for constructs with the [R_P] configuration, enhanced affinity toward double-stranded DNA. These features make them useful molecular tools for inhibition of expression of specific biological targets. We designed oligomers complementary to the polypurine tract sequence [PPT: 5′-d(AAA AGA AAA GGG GGG A)-3′] of HIV-1 viral RNA.[7] Such oligomers are composed of dimers of thymidine and/or cytidine linked via methanephosphonamidate groups. Thus our aim was to find an effective synthetic route to the dinucleoside methanephosphonamidates NnpmN, where N for the chosen sequence is T or dC, and npm represents the methanephosphonamidate linkage.

The formation of a methanephosphonamidate linkage between two nucleoside units was studied by three different synthetic approaches. As monomeric units we used 5′-(4,4′-dimethoxytrityl) (DMT)-protected 3′-aminonucleosides **1**[8]

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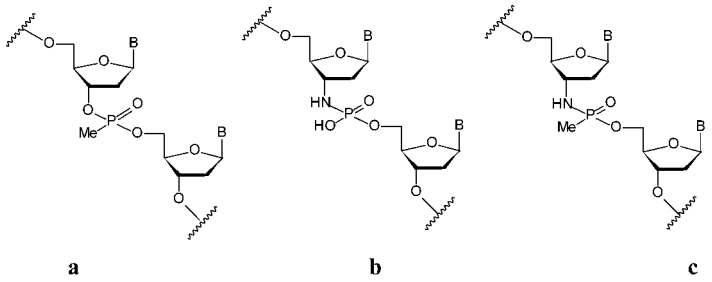


Figure 1. DNA analogues with a modified internucleotide linkage (B = nucleobase).

(3′-amino-3′-deoxythymidine **1a** or *N*(4)-benzoyl-3′-amino-2′,3′-dideoxycytidine **1b**) and 3′-protected nucleosides **2** (3′-*O*-acetyl- or 3′-*O*-*tert*-butyldimethylsilylthymidine **2a** and *N*(4)-benzoyl-3′-*O*-*tert*-butyldimethylsilyl-2′-deoxycytidine **2b**) (Scheme 1). As with the methanephosphonates, this nonstereospecific synthesis of the dinucleoside methanephosphonamidates led to a mixture of two diastereoisomers, [S_P] and [R_P].

In the first method, we used dichloromethanephosphonate (P^V) as a precursor of the internucleotide linkage. The synthesis was accomplished by a one-pot, two-step strategy.[5] First, the 3′-terminal nucleoside (free 5′-OH group) was reacted with a 1.5 molar excess of Me-P(O)Cl_2 in anhydrous pyridine to give nucleosid-5′-yl-methanephosphonochloridate **3**, which, without isolation, was subjected to coupling with the 3′-aminonucleoside **1**. A pair of diastereoisomers, designated by their mobility on silica gel as the FAST-migrating (**4**) and the SLOW-migrating (**5**) isomers, was formed in ca. 40% yield, in a 1:1 ratio, in addition to the symmetrical dinucleoside methanephosphonate side product **6** (up to 20%). We could not improve the yield of the reaction either by using 2 molar equiv of Me-P(O)Cl_2 and 5 molar equiv of 3′-aminonucleoside **1** to 1 molar equiv of the 3′-protected nucleoside **2** or by changing the order of the two-step process, namely, first forming the methanephosphonamidochloridate and then coupling it with the 3′-protected nucleoside **2**. Diastereomers **4** and **5** and the side product **6** were separated by HPLC or column chromatography on silica gel with a methanol/chloroform gradient (Table 1).

The second approach was realized by coupling dichloromethylphosphine (P^{III}) with the protected 3′-terminal nucleoside **2** followed by condensation of the intermediate product **7** with the 3′-aminonucleoside **1** (Scheme 2, Route B).[9] The reaction was carried out under dry argon in the presence of collidine with equimolar amounts of substrates. The coupling products were oxidized with *tert*-butylhydroperoxide (TBHP) and separated as described above. The yield of the desired products (diastereomers **4** and **5**) was less than 40%. The side product **6** was again isolated in ca. 20% yield.

The low yield of desired products in the above approaches as well the need to separate the diastereomers from the side product **6** and from the residual substrate (3′-aminonucleoside

Scheme 1. Synthesis of Dinucleoside Methanephosphonamidates **4** (FAST) and **5** (SLOW) according to Route A

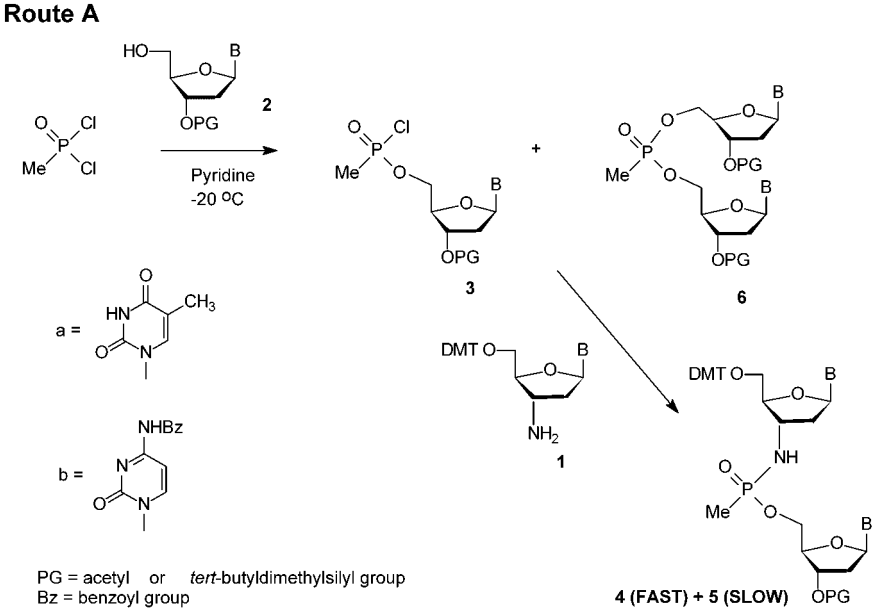


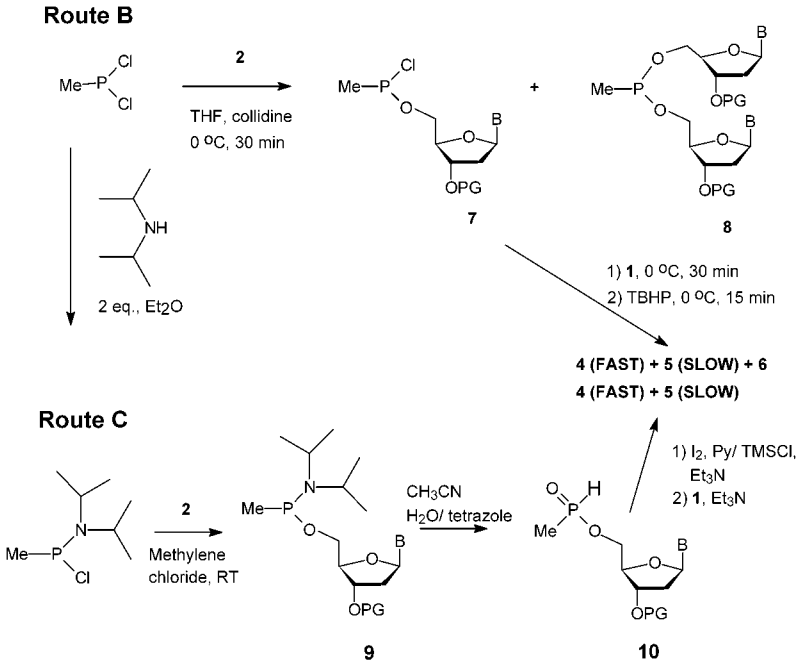
Table 1. Spectral and Chromatographic Characteristics of Fully Protected Di(deoxyribonucleoside)methanephosphonamidates **4** and **5** and the 3′- Deprotected **11** and **12** (**a** and **b** Are Defined in Scheme 1)

compd no.	structure	mobility (TLC R_f or HPLC t_R)	MALDI-TOF calcd	MALDI-TOF exptl	^{31}P NMR δ [ppm]	J_{P-Me} (Hz)
4aa	TmpnT[a] F	FAST (R_f 0.60)[b]	887	887[c]	34.19	16.57
5aa	TmpnT[a] S	SLOW (R_f 0.51)[b]	887	887[c]	34.78	16.48
4ab	TnpmC F	FAST (R_f 0.38)[d]	1049	1051 $[M + H]^+$	34.09	16.47
5ab	TmpnC S	SLOW (R_f 0.39)[d]	1049	1073 $[M + H + Na]^+$	34.28	16.53
4bb	CmpnC F	FAST (t_R 14.8 min)	1139	1139 $[M]^+$,	33.32, 33.57	16.41, 16.46
5bb	CmpnC S	SLOW (t_R 14.9 min)	1139	1162 $[M + Na]^+$		
4ba	CmpnT F	FAST (R_f 0.20)[d]	1049	1048 $[M - H]^-$	32.99	16.70
5ba	CmpnT S	SLOW (R_f 0.18)[d]	1049	1048 $[M - H]^-$	33.74	16.46
11aa	TmpnT-OH F	FAST (R_f 0.29)[b]	844	844.3[c]	35.13	16.70
12aa	TmpnT-OH S	SLOW (R_f 0.29)[b]	844	844.3[c]	36.15	16.55
11ab	TmpnC-OH F	FAST (t_R 15.4 min)	935	933.6[c]	35.89	16.38
12ab	TmpnC-OH S	SLOW (t_R 16.8 min)	935	933.4[c]	34.72	16.44
11ba	CmpnT-OH F	FAST (R_f 0.30)[b]	935	933.9[c]	34.28	16.68
12ba	CmpnT-OH S	SLOW (R_f 0.29)[b]	935	933.5[c]	35.25	16.41
11bb	CmpnC-OH F	FAST (t_R 14.1 min)	1025	1022.8	35.52, 34.54	16.20, 16.78
12bb	CmpnC-OH S	SLOW (t_R 14.5 min)	1025	1022.8		

[a] 3′-OAc. [b] Chloroform/methanol 90:10 (v/v). [c] FAB MS. [d] Chloroform/methanol 95:5 (v/v).

1) forced us to choose a longer synthetic pathway via oxidative amidation of a P−H bond. This approach has been successfully used for the synthesis of dinucleoside 3′P→5′N and N3′→5′P phosphoramidates and phosphothioamidates via oxidative coupling of a nucleoside 3′- or 5′-H-phosphonate or its thio analogue with, respectively, a 5′- or 3′-aminonucleoside in the presence of trimethylsilyl chloride and an excess of iodine.[10] It was also suggested that a nucleoside-3′-phosphinate may undergo an iodine-promoted oxidative coupling with a 5′-aminonucleoside to give a dinucleoside 3′CH_3P→5′N methanephosphonamidate.[11] We prepared the 5′-methanephosphinamidite derivative **9**[12] and transformed it in situ, by treatment with tetrazole in aqueous acetonitrile,[13] into the 5′-methanephosphinate **10** (Scheme 2, Route C). All our efforts to purify product **10** failed because of its instability under the purification conditions (silica gel, $CHCl_3$/MeOH). Instead, we decided to purify substrate **9** (by silica gel column chromatography) to

Scheme 2. Route B, Synthesis of Dinucleoside Methanephosphonamidates **4** (FAST) and **5** (SLOW) Using Dichloromethylphosphine. Route C, Synthesis of Dimers **4** and **5** via Oxidative Amidation. Abbreviations Are as In Scheme 1



maximize the purity of the unstable compound **10** and to use it crude for the subsequent reaction. Oxidative coupling of **10** and the 3′-aminonucleoside **1**, carried out in the presence of trimethylsilyl chloride (TMS-Cl) and a 5-fold molar excess of iodine, resulted in the formation of the desired diastereomers **4** and **5** in a 1/1 molar ratio. To prevent protonation of the aminonucleoside under the reaction conditions, resulting in an inhibition of the nucleophilic substitution at phosphorus, we used an excess of triethylamine in addition to pyridine as solvent. Reaction products were isolated by aqueous workup and separated by column or HPLC silica gel chromatography in a chloroform/methanol solvent system (Table 1). The yield of the oxidative amidation process was in the range 50−60%.

By comparison with Routes A and B, the third approach seems to be the most attractive with respect to the overall yield and the consumption of aminonucleoside **1**, which is obtained by a rather time-consuming chemical synthesis.[4] Moreover, despite being more laborious than Routes A and B, it yields exclusively a mixture of diastereomers **4** and **5**, uncontaminated with the side product **6**. We tried to shorten this approach by direct synthesis of the H-phosphonate derivative **10** by means of a reaction of dichloromethylphosphine with nucleoside **2** and with water. The reaction resulted in the formation of the desired compound **10**, but all our efforts of purification by column chromatography failed.

The structures of the dinucleoside methanephosphonamidates **4**, **5**, and **6** were confirmed by NMR, MS, and CD analysis. Selected spectral data (^{31}P and ^{1}H NMR and MS) are given in Table 1. The absolute configurations at phosphorus of the diastereomers **4** and **5** have not been assigned, except for compounds **4aa** and **5aa**, for which absolute configurations have already been assigned.[5] By analogy with these compounds, the fast migrating dimer **4** (FAST) should be the [R_P] diastereomer and slow migrating dimer **5** (SLOW) should be the [S_P] diastereomer.

All the dinucleoside diastereomers **4** and **5** were deprotected under basic conditions (for removal of the acetyl protecting groups) or in the presence of fluoride anion[14] (for removal of the *tert*-butyldimethylsilyl group) in yields of >80%. The dinucleoside methanephosphonamidate units **11** (originating from diastereomers **4**, FAST) and **12** (originating from diastereoisomer **5**, SLOW) (Table 1)[15] with a free 3′-OH group were subjected to phosphitylation with chloro-2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite and then successfully introduced into long-sequence chimeric oligonucleotides by automated solid-phase synthesis.[5,16] The physicochemical properties of these oligomers are presently under investigation.

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Supporting Information Available: Detailed synthetic procedures A, B, and C as well spectral characterization of the products. This material is available free of charge via the Internet at http://pubs.acs.org.

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