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Oligomeric Building Block Approach to the Synthesis of Diastereomerically Pure Pentathymidine 3′,5′-Methanephosphonates

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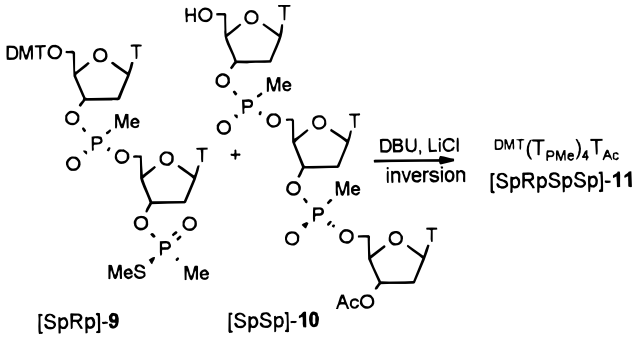
ABSTRACT



A method for a large-scale synthesis of stereodefined oligo(nucleoside 3′,5′-methanephosphonates) has been developed, based on transient 3′-O protection, which allows for the conversion of the protecting chirally defined methanephosphonanilidate group, located at the 3′ end of a stereoregular oligomer, into diastereomerically pure "*oligomeric building blocks*" for stereospecific coupling with the 5′-OH group of another oligonucleotide.

Oligo(nucleoside 3′,5′-methanephosphonates) (oligo-PMe) have been considered as potentially useful candidates for drugs in *antisense and antigene strategy*.[1] They are capable of sequence-specific recognition and binding to their target molecules (mRNA or DNA),[2] exhibit resistance toward *cellular nucleases*,[3] and are able to gain access to the intracellular environment.[4]

The methanephosphonate internucleotide phosphorus atom constitutes a chiral center; therefore, in the case of oligo-PMe obtained via elongation of the oligomer within $n - 1$ nonstereospecific coupling reactions, the final product consists of a mixture of 2^{n-1} P diastereomers. The strong influence of the P diastereomerism of oligo-PMe on their physicochemical properties[5] was first recognized for chimeric oligomers with alternating methanephosphonate/phosphodiester bonds[6] and further for stereoregular octathymidine methanephosphonates[7] obtained by the first stereospecific method.[8]

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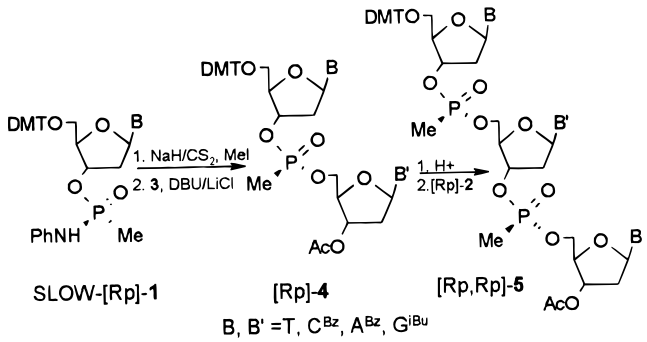
The synthesis of stereodefined oligo-PMe can be approached via nonstereospecific condensation reactions followed by separation of diastereomers[9] or via stereospecific coupling reactions of diastereomerically pure monomers, but both are limited to relatively short oligomers.[10]

As demonstrated earlier, condensation of a pure diastereomer of 5′-*O*-DMT-nucleoside 3′-*O*-(*S*-methyl methanephosphonothioate) (**2**; prepared from diastereomerically pure 5′-*O*-DMT-nucleoside-3′-*O*-methanephosphonanilidate (**1**)) with 3′-*O*-acetylthymidine (**3**) provides 5′-*O*-DMT-3′-*O*-acetyl dinucleoside 3′,5′-methanephosphonate (**4**).[11] This condensation performed in the presence 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU)/LiCl is stereospecific and occurs with inversion of configuration.[12] Removal of the 5′-O protective group and further condensation of the resulting 3′-*O*-acetyl dinucleoside 3′,5′-methanephosphonate with an appropriate *S*-alkyl methanephosphonothioate **2** allow for elongation of dinucleotide into 3′-*O*-Ac-protected tris-(nucleoside methanephosphonate) (**5**; Scheme 1). Performed

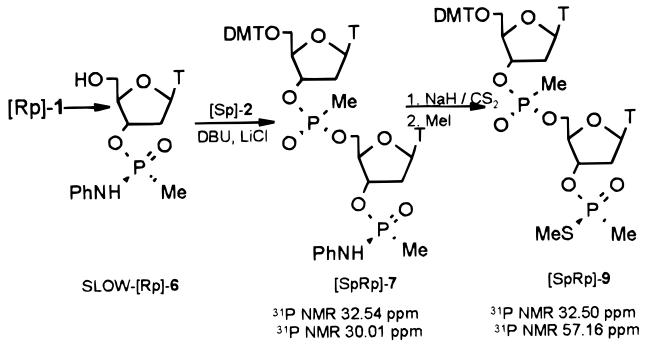
Scheme 1



in the same manner, further stepwise elongation of the oligonucleotide chain allowed us to obtain diastereomerically pure pentakis(nucleoside methanephosphonate) with predetermined absolute configuration at phosphorus of each internucleotide methanephosphonate linkage.[13]

In this communication we present evidence that the 5′-O protective group of substrate **1** (B = Thy) can be selectively removed, and the resulting thymidine 3′-*O*-methanephosphonanilidate (**6**) can be coupled with *S*-methyl methanephosphonothioate **2** (B = Thy), providing dithymidine 3′,5′-methanephosphonate with a 3′-O pendant methanephosphonanilidate function (**7**; Scheme 2). The 3′-*O*-methanephosphonanilidate group of **7** can be converted into the corresponding methanephosphonothioate of **8** in a chemoselective manner (***PN*** → ***PS***),[14] providing, after alkylation, the

Scheme 2



appropriate dithymidine methanephosphonate with a pendant 3′-*O*-*S*-methyl methanephosphonothioate (**9**). Compound **9** can be used as a dimeric "building block" for condensation with the trimer $^{5'-OH}T_{PMe}T_{PMe}T_{3'-Oac}$ (**10**), giving pentakis-(thymidine methanephosphonate) (**11**) of predetermined configuration at the phosphorus atom of each internucleotide methanephosphonate linkage.

The key assumption of the present work was that the presence of a 3′-*O*-methanephosphonanilidate pendant group of mono- or oligonucleotide will not interfere with the process of nucleophilic substitution at the phosphorus atom of the *S*-Me methanephosphonothioate function attached at the 3′-O position of another mono- or oligonucleotide. Since the coupling process requires the presence of a strong organic base, namely DBU/LiCl, one may speculate that an excess of DBU may generate an anionic site at the pendant methanephosphonanilidate which competes with 5′-OH of the same molecule in the process of nucleophilic substitution at phosphorus. That speculation was not unmerited, since DBU can be used as an alternative to NaH base for ***PN*** → ***PX*** conversion.[15] Therefore, diastereomerically pure (R_P)-**1** (B = Thy; ^{31}P NMR δ 30.22 ppm; ^{1}H NMR ($CDCl_3$) δ (slow) 1.90 ppm (d, 3H, J_{P-H} = 16.90 Hz, P−Me)) was treated with 3% trichloroacetic acid in dichloromethane (15 min), providing (R_P)-5′-*OH*-thymidine 3′-*O*-methanephosphonanilidate (**6**: 86% yield; ^{31}P NMR δ 30.25 ppm; $C_{17}H_{22}O_6N_3P$, MS FAB [M − H] 395, calcd 395.3).

Condensation of (R_P)-**6** with (S_P)-**2** (B = Thy) (in a 3:1 molar ratio) in the presence of DBU (9 molar equiv) and LiCl (9 equiv) yielded dinucleotide **7** with an internucleotide methanephosphonate of S_P configuration possessing a 3′-O pendant methanephosphonanilidate group of R_P configuration. Compound **7** was purified by means of silica gel column chromatography (silica gel 60, 230−400 mesh, eluted with an increasing gradient of ethanol (0−4%) in chloroform; R_f = 0.5 in chloroform−methanol (9:1)), and the yield of pure compound was 53% (^{31}P NMR δ 32.54 ppm, 30.01 ppm; $C_{49}H_{55}O_{14}N_5P_2$, MS FAB [M − H] 998.9, calcd 998.94). Treatment of a DMF solution of **7** with NaH (2 equiv, 50% suspension in mineral oil) and CS_2 (10 equiv), followed by alkylation of the terminal sodium 3′-*O*-methanephosphonate

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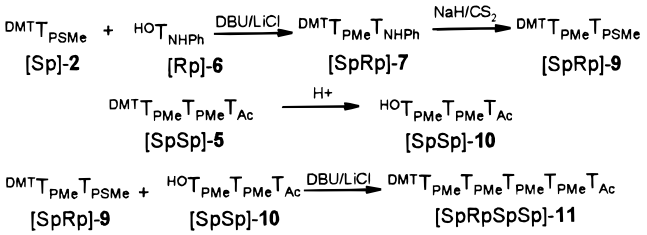
in **8** with methyl iodide (10 equiv), gave the compound (S_PR_P)-**9**, which was isolated by means of silica gel flash column chromatography, using a gradient of EtOH (1−3%) in $CHCl_3$ as the eluting system. The yield of (S_PR_P)-**9** was 75% (^{31}P NMR δ 32.50 ppm, 57.16 ppm; ^{1}H NMR ($CDCl_3$) δ 2.31 (d, 13.3, 3H, P−SMe), 1.57 (d, 17.59, 3H, P−Me), 1.84 (d, 15.63, 3H, Me-P−SMe); $C_{44}H_{52}O_{14}N_4P_2S$, MS FAB [M − H] 954.3, calcd 954.9).

It has to be pointed out that the ^{31}P NMR spectrum of the reaction mixture between *S*-methyl methanephosphonothioate **2** and methanephosphonanilidate **6** did not contain any resonance signals indicating the formation of a P−N(Ph)−P bond. Moreover, the conversion **6** → **7** was stereospecific (no epimerization at the phosphorus of the phosphonanilidate function in **7**), since for the intermediary dithymidine methanephosphonate with pendant 3′-*O*-sodium methanephosphonothioate (**8**) only two resonance lines at 32.21 ppm (internucleotide methanephosphonate) and at 76.38 ppm (3′-*O*-sodium methanephosphonothioate) were observed. Consequently, alkylation of **8** with methyl iodide gave the single compound **9**. Overall, **7** → **9** conversion occurred with retention of configuration.[14]

The second component for the *in-solution block condensation*, trimer **10**, was prepared as follows: 3′-*O*-acetyl thymidine **3** was condensed with (S_P)-5′-*O*-DMT-thymidine 3′-*O*-(*S*-methyl methanephosphonothioate) (**2**), providing (S_P)-5′-*O*-DMT-3′-*O*-Ac-dithymidine 3′,5′-methanephosphonate (**4**; ^{31}P NMR δ 32.23 ppm; MS FAB$^-$ [M − H] 887.3, calcd 887.23). After the standard purification and the removal of the 5′-*O*-DMT group (S_P)-3′-*O*-acetyl dithymidine 3′,5′-methanephosphonate (^{31}P NMR δ 33.12 ppm) was condensed with (S_P)-**2** (B = T), and that condensation afforded (S_PS_P)-5′-*O*-DMT-3′-*O*-acetyl tris(thymidine methanephosphonate) (**5**; ^{31}P NMR δ 31.29, 31.32 ppm; $C_{55}H_{64}O_{20}N_6P_2$, MS FAB$^-$ [M − H] 1188.7, calcd 1191.1). Treatment of fully protected trimer **5** (B = Thy) with 3% trichloroacetic acid in dichloromethane for 5 min provided (S_PS_P)-3′-*O*-acetyl tris(thymidine methanephosphonate) (**10**; ^{31}P NMR δ 31.16, 31.25 ppm in $CDCl_3$). The total yield of the partially deprotected trimer **10** was 64%.

The crucial step of condensation of the dimeric building block **9** (2.7 equiv) and 5′-O deprotected trimer **10** (21 mg, 1 equiv) was performed in 0.2 M pyridine solution in the presence of LiCl (10 equiv) and DBU (20 equiv) at room temperature (1 h) (Scheme 3). The excess DBU was first removed via precipitation of the concentrated (to one-fourth of the total volume) reaction mixture with hexane, followed by extraction of the reaction mixture, dissolved in chloroform, with a 0.05 M solution of citric acid. The final product was purified by means of silica gel column chromatography,

Scheme 3



using as eluent a gradient of EtOH (5−10%) in chloroform. Compound **11** was isolated in 18% yield (6.3 mg; ^{31}P NMR δ 33.08, 32.81, 32.50 ppm in the ratio 1:2:1, respectively; $C_{54}H_{74}O_{29}N_{10}P_4$, MALDI TOF (after deprotection) 1450, calcd 1450.4; HP TLC (10% MeOH in $CHCl_3$) R_f 0. 31; HPLC Econosphere C18 column, 0.1 M triethylammonium bicarbonate (A, TEAB; pH 7.5), 80% MeCN in TEAB (B), gradient 30−100% B, 2% B/min, R_t = 16.4 min).

The corollary to this work was the proof that condensation of **2** + **6** occurs without active participation of the pendant 3′-*O*-methanephosphonanilidate group of **6** and that this group can be converted to 3′-*O*-*S*-methyl methanephosphonothioate **9**. The overall process of preparation of **9** is stereospecific. Because of the presence of the good *S*-Me leaving group at the terminal phosphonate in **9**, condensation with the 5′-OH trinucleotide **10** was feasible. The preparation of pentamer **11,** although in modest yield (18%) of the final coupling, provided a new example of stereospecific chemical ligation of two oligonucleotide components. Since the scale-up of the demonstrated process is achievable, this demonstrates the possibility of the in-solution preparation of longer oligo(nucleoside methanephosphonates) via the block-condensation approach.

The advantage of the method presented here is the relatively large scale of the synthesis and *separation of diastereomeric species only at the stage of monomeric precursors*, while the drawback of the presented strategy is low yield (not optimized yet). Further studies involving the application of this method to the preparation of heteronucleoside sequences, with nucleobases other than thymidine, are in progress.

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