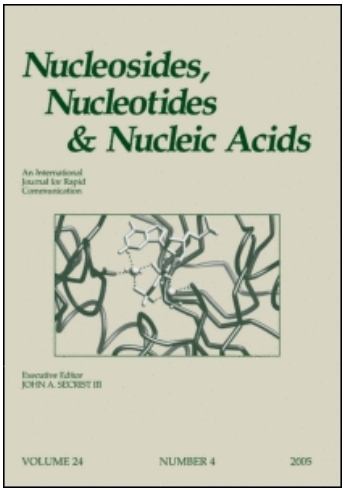


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# Diastereoselective Synthesis of 2'-Deoxy and 2'-O-Methyl Dinucleoside (3' 5')-Methylphosphonates via Alkoxymagnesium Chloride-Mediated Nucleoside Coupling

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# DIASTEREOSELECTIVE SYNTHESIS OF 2'-DEOXY AND 2'-O-METHYL DINUCLEOSIDE (3', 5')-METHYLPHOSPHONATES VIA ALKOXYMAGNESIUM CHLORIDE-MEDIATED NUCLEOSIDE COUPLING

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**Abstract:** A diastereoselective dinucleoside methylphosphonate synthetic method that features coupling of diastereomerically pure 1,1,1,3,3,3-hexafluoro-2-propyl nucleoside-3'-O-methylphosphonate monomers with 3'-O-protected nucleoside monomers mediated by alkoxymagnesium chloride reagents is described. This synthetic method was found to be diastereospecific in the synthesis of selected 2'-deoxy dinucleoside methyphosphonates and diastereoselective in the synthesis of all sixteen 2'-O-methyl dinucleoside methylphosphonates.

## INTRODUCTION

There has been much recent interest in the possibility of using oligonucleotides or oligonucleotide analogs to control gene expression in mammalian cells[1,2] and as potential therapeutic agents.[3-6] The phosphodiester linkages in naturally occuring oligonucleotides are susceptible to degradation by endogenous nucleases, and these oligonucleotides also show poor cellular uptake. To avoid the problem of susceptibility to nuclear degradation, a considerable amount of effort has been expended to the design and synthesis of oligonucleotides with neutral or nonionic linkages. Ts'o and Miller first proposed using the neutral methylphosphonate linkage, and they have since demonstrated improved *in vivo* stability with oligonucleotides containing this linkage.[7-9] Methylphosphonate oligonucleotides are readily synthesized from suitably protected methylphosphonamidite synthons on automated DNA synthesizers. The methylphosphonate linkage is chiral, and, consequently, linkages of Rp and Sp configurations are formed during each coupling step. Thus, each oligomer consists of a

mixture of $2^n$ diastereomers, where n is the number of linkages in the oligomer. It has been further demonstrated that methylphosphonate oligonucleotides containing linkages with Rp configuration have higher Tm values than the corresponding oligonucleotides containing linkages with the Sp configuration.[10-12] An efficient synthesis of methylphosphonate oligonucleotides containing purely Rp or Sp linkages would enable their affinities for complementary RNA or DNA and their antisense activities to be evaluated. Hence, a goal of this research is to develop an efficient method for synthesizing chiral dinucleoside methylphosphonate synthons in a stereospecific manner which would enable the synthesis of chirally pure methylphosphonate oligonucleotides on solid support.

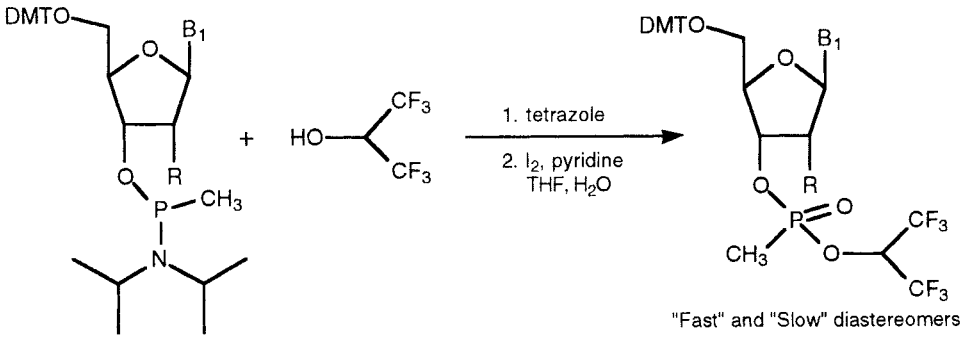
Nonstereospecific methylphosphonate oligonucleotide synthesis has been acheived using P(III) synthons (methylphosphonamidites) or with P(V) synthons.[10-12] Recently, stereospecific synthetic approaches employing P(V) synthons have been reported.[13-19] With this approach dinucleoside methylphosphosphonate synthesis has been accomplished by base-mediated coupling of an activated 5'-O-protected-3'-O-methylphosphonate nucleoside monomer with a suitably protected nucleoside 5'-OH component. Stec *et al*[13-16] and Noyori and Hayakawa,[20,21] have described the use of *tert*-butylmagnesium chloride, and more recently the use of DBU/ LiCl[17] has been described, for promoting this type of nucleoside coupling reaction. The methods using *tert*-butylmagnesium chloride are appropriate for solution-phase synthesis of dinucleoside methylphosphonates, but until very recently they have been considered too harshly basic to be used for solid phase oligonucleotide synthesis.[33,34] Here we describe a diastereoselective dinucleoside methylphosphonate synthesis which features coupling of diastereomerically pure 1,1,1,3,3,3-hexafluoro-2-propyl nucleoside-3'-O-methylphosphonate monomer **2** (R=H) or **4** (R=OMe) with 3'-O-protected nucleoside **5** (R=H) or **6** (R=OMe) in the presence of an alkoxymagnesium chloride reagent to give dinucleoside methylphosphonate **7** (R=H) or **8** (R=OMe) after 3'-O-silyl deprotection (Scheme 1). Thus, the use of alkoxymagnesium reagents in these nucleoside coupling reactions was found to be generally applicable to the diastereospecific synthesis of 2'-deoxy dinucleoside methylphosphonates and diastereoselective synthesis of 2'-O-methyl dinucleoside methylphosphonates.[22]

## RESULTS AND DISCUSSION

Our method was first evaluated in the synthesis of several 2'-deoxy dinucleoside methylphosphonates. To that end methylphosphonate monomers **2a-c** were made in

straight-forward manner from readily available methylphosphonamidites **1**[23] and 1,1,1,3,3,3-hexafluoro-2-propanol as depicted in Scheme 1. In each case a nearly equal mixture of "slow" and "fast" diastereomers were obtained, as indicated by $^{31}P$ NMR analysis of crude product. We found that the "fast" and "slow" diastereomers of **2a-c** could be readily separated by flash chromatography. The alkoxymagnesium chloride-mediated nucleoside coupling reaction conditions were initially investigated and optimized for the synthesis of 2'-deoxy dinucleoside methylphosphonates **7a-e**. Results for these 2'-deoxy dinucleoside syntheses are shown in Table 1. In general, reactions mediated by 3-ethyl-3-pentanoxymagnesium chloride (generated *in situ* from *tert*-butylmagnesium chloride and 3-ethy-3-pentanol) were cleaner (fewer side products) than reactions mediated by *tert*-butoxymagnesium chloride (generated *in situ* from *tert*-butylmagnesium chloride and *tert*-butanol). In addition, the 2:1 stoichiometry of **"slow"-2:5** in reactions run at 50 °C (Reaction method C) appeared to be more efficient (shorter reaction times and higher yields) than the 1.3:1 stoichiometry of **"slow"-2:5** in reactions run at room temperature (Reaction method B). Coupling reactions mediated by the alkoxymagnesium chloride reagents proceeded with inversion of configuration at phosphorus,[13] yielding a single diastereomer of the dinucleoside methylphosphonates **7**. Thus, the "slow" diastereomer of methylphosphonate monomers **2a-c** upon coupling gave rise to the "fast" diastereomer of dinucleoside methylphosphonates **7**.[13] We have made tentative dinucleoside methylphosphonate stereochemical assignments at phosphorus that are based on and in agreement with the previously observed correlation between the $^{31}P$ chemical shift and the elution order from silica gel chromatography with absolute configurations at the phosphorus center of dinucleoside methylphosphonates.[24-26] Thus, the fast-eluting diastereomer[27] of dinucleoside methylphosphonates **7a-e** (tentatively assigned Rp) resonated at higher field in $^{31}P$ NMR in $CDCl_3$, whereas the slow-eluting diastereomer of dinucleoside methylphosphonates **7a-e** (tentatively assigned Sp) resonated at lower field in $^{31}P$ NMR in $CDCl_3$. Verification of absolute configuration at phosphorus of monomers **2a-c** and dinucleoside methylphosphonates **7a-e** on the basis of of 2D-ROESY NMR studies[24-26] or x-ray crystallography was not pursued.

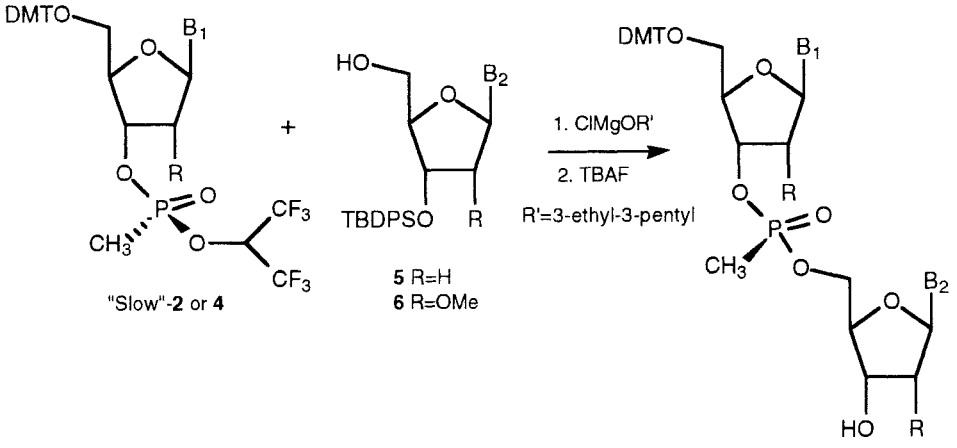
Similar nucleoside coupling reactions with reagents consisting of other metals, such as zinc and aluminum, were very slow or unreactive (see Table 2). Reactions with potassium *tert*-butoxide and potassium hydride were rapid and efficient but gave a mixture of dinucleoside methylphosphonate diastereomers, presumably due to P-epimerization of methylphosphonate monomer **2** during the reaction. Thus, of the




**1a** R=H, $B_1=A^{Bz}$
**1b** R=H, $B_1=T$
**1c** R=H, $B_1=C^{i\text{-}Bu}$
**3a** R=OMe, $B_1=A^{Bz}$
**3b** R=OMe, $B_1=C^{i\text{-}Bu}$
**3c** R=OMe, $B_1=G^{i\text{-}Bu}$
**3d** R=OMe, $B_1=U$

"Fast" and "Slow" diastereomers

**2a** R=H, $B_1=A^{Bz}$
**2b** R=H, $B_1=T$
**2c** R=H, $B_1=C^{i\text{-}Bu}$
**4a** R=OMe, $B_1=A^{Bz}$
**4b** R=OMe, $B_1=C^{i\text{-}Bu}$
**4c** R=OMe, $B_1=G^{i\text{-}Bu}$
**4d** R=OMe, $B_1=U$




"Slow"-**2** or **4**

**5** R=H
**6** R=OMe

"Fast" -**7** (R=H)
"Fast" and "Slow"-**8** (R=OMe)

**7a** $B_1=A^{Bz}$; $B_2=G^{i\text{-}Bu}$
**7b** $B_1=A^{Bz}$; $B_2=T$
**7c** $B_1=T$; $B_2=C^{i\text{-}Bu}$
**7d** $B_1=T$; $B_2=T$
**7e** $B_1=C^{i\text{-}Bu}$; $B_2=C^{i\text{-}Bu}$

**8a** $B_1=A^{Bz}$; $B_2=Gi\text{-}Bu$
**8b** $B_1=B_2=A^{Bz}$
**8c** $B_1=A^{Bz}$; $B_2=C^{i\text{-}Bu}$
**8d** $B_1=A^{Bz}$; $B_2=U$
**8e** $B_1=C^{i\text{-}Bu}$; $B_2=G^{i\text{-}Bu}$
**8f** $B_1=C^{i\text{-}Bu}$; $B_2=A^{Bz}$
**8g** $B_1=B_2=C^{i\text{-}Bu}$
**8h** $B_1=C^{i\text{-}Bu}$; $B_2=U$

**8i** $B_1=B_2=G^{i\text{-}Bu}$
**8j** $B_1=G^{i\text{-}Bu}$; $B_2=A^{Bz}$
**8k** $B_1=G^{i\text{-}Bu}$; $B_2=C^{i\text{-}Bu}$
**8l** $B_1=G^{i\text{-}Bu}$; $B_2=U$
**8m** $B_1=U$; $B_2=G^{i\text{-}Bu}$
**8n** $B_1=U$; $B_2=A^{Bz}$
**8o** $B_1=U$; $B_2=C^{i\text{-}Bu}$
**8p** $B_1=B_2=U$

**Scheme 1**

**TABLE 1. Results of Rp 2'-deoxy dinucleoside methylphonate syntheses using alkoxymagnesium chloride reagents**

| 2'-deoxy Dinucleoside | Base/metal Reagents (Equivalents) | Reaction method[a] | Reaction time | Reaction yield |
|---|---|---|---|---|
| **7a** | *tert*-Butoxymagnesium chloride (5.3) | A | 17 h | 76%[b] |
| **7a** | 3-Ethyl-3-petanoxymagnesium chloride (5.0) | A | 17 h | 60%[c] |
| **7a** | 3-Ethyl-3-petanoxymagnesium chloride (4.5) | C | 4 h | 74%[c] |
| **7b** | 3-Ethyl-3-petanoxymagnesium chloride (3.5) | C | 5 h | 55%[c] |
| **7c** | 3-Ethyl-3-petanoxymagnesium chloride (3.5) | B | 17 h | 18%[c] |
| **7d** | 3-Ethyl-3-petanoxymagnesium chloride (3.5) | B | 17 h | 42%[c] |
| **7e** | 3-Ethyl-3-petanoxymagnesium chloride (3.2) | C | 4 h | 73%[c] |

[a] Method A: Reactions were run in THF at RT using 2:1 stoichiometry of **"Slow"-2** : **5**. Method B: Reactions were run in THF at RT using 1.3:1 stoichiometry of **"Slow"-2** : **5**. Method C: Reactions were run in THF at 50 °C using 2:1 stoichiometry of **"Slow"-2** : **5**. [b] Yield of isolated product after flash chromatography on silica gel. [c] Yield estimated by $^{31}$P NMR integration.



**Table 2. Results of Rp 2'-deoxy dinucleoside methylphonate syntheses using other base/metal reagents**

| 2'-deoxy Dinucleoside | Base/metal Reagent (Equivalents) | Reaction method[a] | Reaction time | Ratio Rp/ Sp (yield) |
|---|---|---|---|---|
| **7a** | *tert*-Butanol, DIBAL-H (5.3) | A | 15 h | 0/0 (0%)[b] |
| **7a** | Sodium hydride, zinc (II) chloride (5.0) | A | 70 h | 1/ 0 (18%)[c] |
| **7a** | Potassium hydride (4.5) | B | 0.5 h | 7.8: 1 (ND)[c] |
| **7d** | Potassium hydride (4.5) | B | 0.5 h | 3/ 2 (ND)[c] |
| **7a** | Potassium tert-butoxide (5.0) | A | 1 h | 4.3/ 1 (ND)[c] |
| **7d** | Potassium tert-butoxide (5.0) | B | 1 h | 3/ 2 (ND)[c] |

[a] Method A: Reactions were run in THF at RT using 2:1 stoichiometry of **"Slow"-2:5**. Method B: Reactions were run in THF at RT using 1.3:1 stoichiometry of **"Slow"-2:5**. [b] This reaction showed no detectable dinucleoside methylphosphonate **7a** by $^{31}$P NMR [c] Diastereomer ratio estimated by $^{31}$P NMR integration. ND=yield not determined.

reagents described above it was clear that the alkoxymagnesium chloride reagents, 3-ethyl-3-pentanoxymagnesium chloride in particular, were superior, at least in terms of yield and diastereoselectivity.

We next turned our attention to the 2'-O-methyl dinucleoside methyphosphonate series. Hence, 2'-O-methyl methylphosphonate monomers **4a-d** were made in straight forward manner from the corresponding readily available 2'-O-methyl methylphosphonamidites **3**[28] and 1,1,1,3,3,3-hexafluoro-2-propanol as depicted in Scheme 1. In each case diastereoselectivity for the desired "slow" diastereomer was observed. Diastereomer ratios, $R_f$ and $^{31}P$ data for these compounds are listed in Table 3. As in the case of 2'-deoxy methylphosphonate monomers **2a-c**, the diastereomers of 2'-O-methyl methylphosphonate monomers **4a-d** could be separated by flash chromatography on silica gel.[29] The 3-ethyl-3-pentanoxymagnesium chloride reagent was employed for all sixteen 2'-O-methyl dinucleoside methylphosphonate syntheses. Yields and spectral data for dinucleoside methylphosphonates **8a-p** (3'-O-silyl deprotected) are shown in Table 4. We found that these coupling reactions required heating at 55-65 °C, whereas the 2'-deoxy dinucleoside syntheses proceeded at room temperature. We also observed formation of various amounts of the undesired "slow" dinucleoside in each of the 2'-O-methyl dinucleoside syntheses, which was most likely due to partial P-epimerization of methylphosphonate monomer **4** during the reaction. The amount of "slow" dinucleoside methylphosphonate obtained from coupling reactions is consistent with the extent of P-epimerization of methylphosphonate monomer **4** during the reaction. Support for this claim was obtained from P-epimerization studies on monomer **4d**. Both diastereomers of uridine monomer **4d** were independently subjected to 1.5 equivalents of 3-ethyl-3-pentanoxymagnesium chloride in THF at 55 °C for 17 h (essentially the conditions of the coupling reaction). In the case of the "fast" diastereomer we observed 22% P-epimerization and in the case of the "slow" diastereomer we observed 13% P-epimerization (determined by $^{31}P$ NMR integration). Thus, the extent of P-epimerization was consistent with the amount of "slow" dimer formation observed in the syntheses of the 2'-O-methyl uridine dinucleoside methylphosphonates **8m-p** (see Table 4).

For the 2'-O-methyl dinucleoside methylphosphonates **8a-p** we have also made tentative stereochemical assignments at phosphorus that are also based on the aforementioned correlation between the $^{31}P$ chemical shift and the elution order from the silica gel column with absolute configurations at the phosphorus center of 2'-deoxy dinucleoside methylphosphonates.[24-26] Thus, the fast-eluting diastereomer[27] of 2'-O-methyl dinucleoside methylphosphonates **8a-p** (tentatively assigned Rp) resonated at

**Table 3. Experimental data for 2'-O-Me phosphonate monomer synthons 4a-d**

| Compound | Ratio Slow:Fast | $R_f$ (Fast/ Slow), method[a] | $^{31}P$ NMR δ (Fast/Slow)[b] |
|---|---|---|---|
| **4a** | 2.8 : 1[c] | 0.27/ 0.18, A | 34.10/35.05 |
| **4b** | 1.2 : 1[d] | 0.42/ 0.21, B | 33.73/ 36.00 |
| **4c** | 2.9 : 1[d] | 0.32/ 0.21, B | 35.01/ 34.88 |
| **4d** | 1.6 :1[d] | 0.26/ 0.17, A | 34.53/ 35.20 |

[a] Method A: 6:1 Methylene chloride-acetone on silica gel 60 $F_{254}$ TLC plates; Method B: 5:2 methylene chloride-acetone on silica gel 60 $F_{254}$ TLC plates. [b] $^{31}P$ chemical shifts in ppm, with two percent $H_3PO_4$ in $D_2O$ as external reference. [c] Determined by $^{31}P$ NMR integration. [d]



higher field in $^{31}P$ NMR in $CDCl_3$, whereas the slow-eluting diastereomer (tentatively assigned Sp) resonated at lower field in $^{31}P$ NMR in $CDCl_3$. Verification of absolute configuration at phosphorus of monomers **4** and 2'-O-methyl dinucleoside methylphosphonates **8** on the basis of 2D-ROESY NMR studies[24-26] and/or x-ray crystallography is desirable and is being pursued in our laboratories.

An attractive feature of this synthetic method is that the undesired "fast" diastereomer of methylphosphonate monomer **2** or **4** can be recycled. Treatment of the "fast" diastereomer of **2a** or **4a** with 0.25 equivalents of the sodium salt of 1,1,1,3,3,3-hexafluoro-2-propanol (generated *in situ* from sodium hydride and 1,1,1,3,3,3-hexafluoro-2-propanol) resulted in P-epimerization to give a 1:1 mixture of diastereomers in the case of **2a** and a 2:1 mixture of "slow" and "fast" diastereomers, respectively, in the case of **4a** after 1 h in acetonitrile at room temperature.

The "fast" dinucleoside methylphosphonates **8a-p** described above were purified by preparative reverse-phase HPLC and converted to the corresponding 3'-O-(methyl-N,N-diisopropylphosphonamidite) or 3'-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite) synthons that we have used to prepare novel methylphosphonate oligonucleotides, some of the details of which have been described elsewhere.[30-32]

Application of this chemistry to solid-phase dinucleoside synthesis is under investigation. We believe that the mild alkoxymagnesium chloride reagents may be suitable for solid phase synthesis as long as base-resistent support and linker chemistry is employed, such as the PEG support recently described by Wickstrom and Le Bec.[33,34]

**Table 4. Results of "Fast" (Rp) 2'-O-Me dinucleoside methylphonate syntheses**

| 2'-O-Me Dinucleoside | Overall Yield[a] | Ratio %Rp/%Sp[b] | $^{31}$P NMR, δ(Rp/Sp)[c] | Prep. HPLC Purity[d] | Exact Mass calc'd(adduct) | HRMS FAB$^+$ found (adduct)[e] |
|---|---|---|---|---|---|---|
| **8a** | 42% | 98.0/0.2 | 31.70/ 33.41 | 97.3% | 1115.4017 $(M+H)^+$ | 1115.4077 $(M+H)^+$ |
| **8b** | 32% | 90.0/9.0 | 31.77/ 33.50 | 92.5% | 1265.2899 $(M+Cs)^+$ | 1265.2935 $(M+Cs)^+$ |
| **8c** | 64% | 97.4/2.2 | 31.44/ 33.46 | 95.3% | 1207.2943 $(M+Cs)^+$ | 1207.2895 $(M+Cs)^+$ |
| **8d** | 27% | 97.2/2.6 | 31.26/ 33.14 | 93.2% | 1138.2364 $(M+Cs)^+$ | 1138.2325 $(M+Cs)^+$ |
| **8e** | 15% | 96.1/ 3.2 | 31.42/ 32.99 | 98.7% | 1189.3048 $(M+Cs)^+$ | 1189.3094 $(M+Cs)^+$ |
| **8f** | 22% | 89.1/ 6.5 | 32.27/ 33.69 | 93.8% | 1207.2943 $(M+Cs)^+$ | 1207.2890 $(M+Cs)^+$ |
| **8g** | 38% | 91.5/ 5.0 | 32.13/ 34.21 | 97.8% | 1149.2987$(M+Cs)^+$ | 1149.2948 $(M+Cs)^+$ |
| **8h** | 28% | 83.4/ 1.2 | 32.01/ 33.53 | 99.0% | 1080.2408 $(M+Cs)^+$ | 1080.2373 $(M+Cs)^+$ |
| **8i** | 25% | 95.9/ 1.9 | 31.09/ 32.71 | 99.6% | 1097.4134 $(M+H)^+$ | 1097.4156 $(M+H)^+$ |
| **8j** | 39% | 95.8/ 2.9 | 32.40/ 33.32 | 96.9% | 1247.3004 $(M+Cs)^+$ | 1247.3025 $(M+Cs)^+$ |
| **8k** | 46% | 94.3/ 4.5 | 31.45/ 32.92 | 98.3% | 1057.4072 $(M+H)^+$ | 1057.4092 $(M+H)^+$ |
| **8l** | 28% | 93.8/ 5.6 | 32.37/ 33.22 | 93.7% | 1010.3313 $(M+Na)^+$ | 1010.3333 $(M+Na)^+$ |
| **8m** | 15% | 88.0/ 11.5 | 31.54/ 33.37 | 96.1% | 1120.2470 $(M+Cs)^+$ | 1120.2429 $(M+Cs)^+$ |
| **8n** | 40% | 92.0/ 6.8 | 32.21/ 33.68 | 90.9% | 1138.2364 $(M+Cs)^+$ | 1138.2328 $(M+Cs)^+$ |
| **8o** | 26% | 89.0/ 11.0 | 31.93/ 33.13 | 99.2% | 1080.2408 $(M+Cs)^+$ | 1080.2420 $(M+Cs)^+$ |
| **8p** | 36% | 78.0/ 20.0 | 31.67/ 33.30 | 92.8% | 1011.1830 $(M+Cs)^+$ | 1011.1794 $(M+Cs)^+$ |

[a] Isolated overall yield from **"slow"-4** after flash chromatograhy on silica gel. [b] determined by reverse phase HPLC integration (area percent). [c] $^{31}$P chemical shifts in ppm, with two percent $H_3PO_4$ in $D_2O$ as external reference. [d] Purity of **"fast"-8** after preparative reverse-phase HPLC. [e] High resolution FAB$^+$ data was determined by peak matching.

## EXPERIMENTAL SECTION

**General Methods** All reactions were run under a positive pressure of dry argon. Reactions requiring anhydrous conditions were performed in flame-dried glassware which was cooled under argon. Anhydrous solvents and reagent solutions were transferred using oven-dried syringes. Tetrahydrofuran (THF) was distilled from potassium/benzophenone ketyl immediately prior to use; 3-ethyl-3-pentanol was dried over 3A or 4A molecular sieves for 24 hours prior to use. Methylene chloride, pyridine, acetonitrile, tetrazole reagent and 2-methyl-2-propanol were obtained as anhydrous reagent (<0.005% water) and were used without further purification. Reagent grade solvents were used for chromatography without further purification. TLC was performed on 0.2mm E. Merck precoated silica gel 60 $F_{254}$ TLC plates (20 x 20 cm aluminum sheets). Flash chromatography was performed using E. Merck 230-400 mesh silica gel (60 $F_{254}$). Analytical Reverse-phase HPLC was performed using a Waters 3.9 mm x 300 mm Bondpak™ C-18 column on a Beckman System Gold equipped with a model 125 programmable solvent module, a model 168 diode-array detector module and a model 502 autosampler. The solvents used were A: 50% acetonitrile in 0.10 M triethylammonium acetate (TEAA), pH 7.0-7.2; and B: 100% acetonitrile. The gradient system was 0-100% B over 20 minutes at a flow rate of 1.0 mL/min. All analytical reverse-phase chromatograms were monitored at 260nm. Preparative reverse-phase HPLC was performed using an Elka Nobel 2.0 inch x 250 mm Kromasil® column on a Beckman System Gold equipped with a model 126 programmable solvent module fitted with m-Flow™ preparative pump heads, a model 166 programmable detector module and a Rheodyne 7125 injector fitted with a 20-mL injector loop. An isocratic solvent system composed of 39-50% acetonitrile in water (depending on the particular dinucleoside) at a flow rate of 60 mL/min was used. The preparative reverse-phase chromatograms were monitored at either 283 nm or 295 nm or 300 nm depending on the particular dinucleoside. 300 MHz $^1H$ NMR and 121 MHz $^{31}P$ spectra were recorded on a Bruker ARX 300 Spectrometer. All $^1H$ and $^{31}P$ were obtained in $CDCl_3$ unless otherwise indicated. FAB Mass spectra were provided by The Scripps Research Institute Mass Spectrometry Facility of San Diego, California. The spectra were obtained using a Cs ion gun and were recorded on a FISONS/VG-ZAB-VSE High Resolution Mass Spectrometer. All $^1H$ NMR, $^{31}P$ NMR, and MS spectra were consistent with assigned structure.

**General procedure for the synthesis of "Fast" (Rp) 2'-deoxy dinucleoside (3',5')-methylphosphonates (7a-e)**. To a flame-dried one-neck round-bottomed flask were added the metal/base reagents (additional THF solvent was added as needed). A THF solution of the 2'-deoxy nucleoside 5'-OH component **5** (1.0 equivalent) was then added at room temperature via syringe. The reaction mixture was stirred at room temperature for 20 min. Then a THF solution of the 1,1,1,3,3,3-hexafluoro-2-propyl nucleoside 3'-methylphosphonate component **2** (2.0 or 1.3 equivalents, see Table 1 or 2) was added via syringe and the reaction mixture was stirred at either room temperature or 50 °C (see Table 1 or 2 for reaction temperature and duration). The reactions were quenched with saturated aqueous ammonium chloride solution and extracted with ethyl acetate. Organic extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a yellow-orange foam. Yields of dinucleosides were generally estimated by $^{31}P$ NMR integration (see Table 1 or 2). Where applicable, purification of the dinucleoside was performed by flash chromatography on silica gel using a solvent mixture composed of 75:25:3 ethyl acetate-methylene chloride-methanol, increasing to 75:25:10 ethyl acetate-methylene chloride-methanol.



**General procedure for the preparation of "Fast"- and "Slow"-1,1,1,3,3,3-Hexafluoro-2-propyl 2'-O-methyl nucleoside 3'-methylphosphonates (4a-d)**. To a solution of the 2'-O-methyl nucleoside 3'-O-methylphosphonamidite **3** (1.0 equivalent) in dry acetonitrile (4 mL per mmol of methylphosphonamidite) at room temperature were added via syringe 1,1,1,3,3,3-hexafluro-2-propanol (2.0 equivalents) and then tetrazole reagent (0.45 M solution in acetonitrile, 2 equivalents). The reaction was stirred at room temperature for 3 min and then transferred to a round-bottomed flask containing $I_2$/pyridine/THF/$H_2O$ (0.1 M $I_2$ in 74.75% THF, 25% pyridine and 0.25% $H_2O$ (v/v), 2 equivalents). The resulting mixture was stirred at room temperature for 20 min. The reaction was quenched by adding 10% aqueous sodium bisulfite solution and after 10 min the resulting bilayer was extracted with ethyl acetate. The organic extracts were washed twice with 10% aqueous citric acid solution, once with saturated aqueous sodium bicarbonate solution and once with brine. The organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford a pale yellow foam. The diastereomers were separated by flash chromatography on silica gel (230-400 mesh) using a mixture of methylene chloride-acetone (see particular compound for solvent ratio).

**"Fast"- and "Slow"-1,1,1,3,3,3-Hexafluoro-2-propyl $N^6$-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-O-methyladenosine 3'-methylphosphonate (4a).** Following the general procedure with 5.55 g (6.66 mmol) of methylphosphonamidite **3a** provided a 1:2.8 mixture (determined by $^{31}P$ NMR integration) of the "fast" and "slow" diastereomers, respectively. Flash chromatography on silica gel using 6:1 methylene chloride-acetone gave 0.58 g (9%) of the "fast" diastereomer, 1.94 g (32%) of the "slow" diastereomer and 2.99 g (49%) of a mixture of the two diastereomers (ratio not determined).

"Fast" diastereomer: mp: 108-110 °C; $R_f$ 0.27 (6:1 methylene chloride-acetone); $^1H$ NMR (300 MHz) d 9.29 (br s, 1H), 8.67 (s, 1H), 8.16 (s, 1H), 8.02 (d, $J$ = 7.5 Hz, 2H), 7.60-7.41 (m, 5H), 7.32-7.22 (m and overlapping d, $J$ = 8.7 Hz, 7H), 6.81 (d, $J$ = 8.7 Hz, 4H), 6.18 (d, $J$ = 6.6 Hz, 1H), 5.37 (septet, $J$ = 6.0 Hz, 1H), 5.26 (ddd, $J$ = 8.7, 4.8, 2.7 Hz, 1H), 4.89 (t, $J$ = 5.4 Hz, 1H), 4.51 (d, $J$ = 2.70 Hz, 1H), 3.77 (s, 6H), 3.56 (dd, $J$ = 10.8, 3.9 Hz, 1H), 3.46-3.41 (s, 3H and overlapping dd, $J$ = 10.8, 3.6 Hz, 1H), 1.71 (d, $J$ = 18.0 Hz, 3H); $^{31}P$ NMR (121 MHz) d 34.10; HRMS ($FAB^+$) calcd for $C_{43}H_{40}F_6N_5O_9P$+H 916.2546, found 916.2569.

"Slow" diastereomer: mp: 112-114 °C; $R_f$ 0.18 (6:1 methylene chloride-acetone); $^1H$ NMR (300 MH) d 9.08 (s, 1H), 8.70 (s, 1H), 8.17 (s, 1H), 8.03 (d, $J$ = 7.2 Hz, 2H), 7.61-7.40 (m, 5H), 7.33-7.22 (m and overlapping d, $J$ = 8.7 Hz, 7H), 6.82 (d, $J$ = 8.7 Hz, 4H), 6.14 (d, $J$ = 7.2 Hz, 1H), 5.25-5.13 (m and overlapping septet, $J$ = 6.3 Hz, 2H), 4.91 (dd, $J$ = 7.2, 4.5 Hz, 1H), 4.38 (d, $J$ = 1.5 Hz, 1H), 3.78 (s, 6 H), 3.55 (dd, $J$ = 10.8, 3.6 Hz, 1H), 3.50 (s, 3H) 3.37 (dd, $J$ = 10.8, 3.6 Hz, 1H), 1.79 (d, $J$ = 18.6 Hz, 3H); $^{31}P$ NMR (121 MHz) d 35.05; HRMS ($FAB^+$) calcd for $C_{43}H_{40}F_6N_5O_9P$+H 916.2546, found 916.2570.

**"Fast"- and "Slow"-1,1,1,3,3,3-Hexafluoro-2-propyl $N^2$-isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-O-methylguanosine 3'-methylphosphonate (4c).** Following the general procedure with 47.86 g (58.7 mmol) of phosphoramidite **3c** provided a 1:2.9 mixture (determined by HPLC integration) of "fast" and "slow" diastereomers, respectively. Flash chromatography on silica gel using 6:1 methylene chloride-acetone gave 8.6 g (16 %) of the "fast" diastereomer, 15.7 g (30 %) of "slow" diastereomer and 8.4 g (16 %) of a mixture of the two diastereomers (ratio not determined).

"Fast" diastereomer: $R_f$ 0.32 (5:2 methylene chloride-acetone); $^1H$ NMR (300 MHz) d 11.99 (s, 1H), 8.77 (br s, 1H), 7.79 (s, 1H), 7.41-7.20 (m, 9H), 6.76 (m, 4H), 5.92-5.86

(dd, $J$ = 9.3, 4.5 Hz and overlapping d, $J$ = 5.1 Hz, 2H), 5.24 (septet, $J$ = 5.7 Hz, 1H), 4.89 (t, $J$ = 4.8 Hz, 1H), 4.41 (m, 1H), 3.76 (s, 6 H), 3.55 (dd, $J$ = 11.1,1.8 Hz, 1H), 3.48 (s, 3H), 3.13 (dd, $J$ = 11.1, 2.7 Hz, 1H), 1.98 (septet, $J$ = 6.6 Hz, 1H), 1.65 (d, $J$ = 18.0 Hz, 3H), 1.01 (d, $J$ = 6.9 Hz, 3H), 0.87 (d, $J$ = 6.9 Hz, 3H); $^{31}$P NMR (121 MHz) d 35.01; HRMS (FAB$^+$) calcd for $C_{40}H_{42}F_6N_5O_{10}P$+H 898.2652, found 898.2675.

"Slow" diastereomer: $R_f$ 0.21 (5:2 methylene chloride-acetone); $^1$H NMR (300 MHz) d 12.00 (s, 1H), 7.85 (s, 1H), 7.80 (s, 1H), 7.52 (d, $J$ = 6.0 Hz, 2H), 7.38 (d, $J$ = 9.0 Hz, 4H), 7.27-7.13 (m, 3H), 6.79 (app t, $J$ = 9.0 Hz, 4H), 5.79 (d, $J$ = 7.5 Hz, 1H), 5.36 (dd, $J$ = 9.0, 6.0 Hz, 1H), 5.14 (m, 2H), 4.28 (s, 1H), 3.75 (s, 6H), 3.54 (m, 1H and overlapping s, 3H), 3.06 (dd, $J$ = 11.1, 2.7 Hz, 1H), 1.75 (d, $J$ = 18.6 Hz, 3H), 1.54 (septet, $J$ = 6.6 Hz, 1H), 0.88 (d, $J$ = 6.9 Hz, 3H), 0.67 (d, $J$ = 6.9 Hz, 3H); $^{31}$P NMR (121 MHz) d 34.88; HRMS (FAB$^+$) calcd for $C_{40}H_{42}F_6N_5O_{10}P$+H 898.2652, found 898.2673.



**"Fast"- and "Slow"-1,1,1,3,3,3-Hexafluoro-2-propyl N$^4$-isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-O-methylcytidine 3'-methylphosphonate (4b).** Following the general procedure with 58.0 g (74.8 mmol) of methylphosphonamidite **3b** provided a 1:1.2 mixture (determined by HPLC integration) of the "fast" and "slow" diastereomers, respectively. Flash chromatography on silica gel using 6:1 methylene chloride-acetone gave 22.5 g (35 %) of the "fast" diastereomer and 25.7 g (40 %) of the "slow" diastereomer.

"Fast" diastereomer: $R_f$ 0.42 (5:2 methylene chloride-acetone); $^1$H NMR (300 MHz) d 9.41 (br s, 1H), 8.50 (d, $J$ = 7.5 Hz, 1H), 7.44-7.27 (m, 9H), 7.09 (d, $J$ = 7.5 Hz, 1H), 6.87 (d, $J$ = 8.7 Hz, 4H), 6.01 (s, 1H), 5.85 (m, 1H), 5.24 (ddd, $J$ = 9.3, 9.3, 4.5 Hz, 1H), 4.37 Hz, 1H), 4.37 (d, $J$ = 8.7 Hz, 1H), 4.30 (d, $J$ = 4.5 Hz, 1H), 3.84 (s, 6H), 3.70 (m, 1H and overlapping s, 3H), 3.41 (d, $J$ = 10.5 Hz, 1H), 2.54 (septet, $J$ = 6.9 Hz, 1H), 1.48 (d, $J$ = 18.3 Hz, 3H), 1.15 (d, $J$ = 6.9 Hz, 3H), 0.97 (d, $J$ = 6.9 Hz, 3H); $^{31}$P NMR (121 MHz) d 33.73; HRMS (FAB$^+$) calcd for $C_{39}H_{42}F_6N_3O_{10}P$+Cs 990.1566, found 990.1525.

"Slow" diastereomer: $R_f$ 0.21 (5:2 methylene chloride-acetone); $^1$H NMR (300 MHz) d 9.17 (br s, 1H), 8.45 (d, $J$ = 7.5 Hz, 1H), 7.40-7.27 (m, 9H), 7.07 (d, $J$ = 7.5 Hz, 1H), 6.86 (d, $J$ = 8.7 Hz, 4H), 6.08 (d, $J$ = 1.8 Hz, 1H), 5.10-4.98 (m, 2H), 4.35-4.29 (m, 2H), 3.83 (2s, 6H), 3.72 (s, 3H), 3.67 (dd, $J$ = 11.4, 2.0 Hz, 1H), 3.43 (dd, $J$ = 11.4, 2.0 Hz, 1H), 2.57 (septet, $J$ = 6.9 Hz, 1H), 1.86 (d, $J$ = 18.6 Hz, 3H), 1.17 (d, $J$ = 6.9 Hz, 3H), 1.04 (d, $J$ = 6.9 Hz, 3H); $^{31}$P NMR (121 MHz) d 36.00; HRMS (FAB$^+$) calcd for $C_{39}H_{42}F_6N_3O_{10}P$+Cs 990.1566, found 990.1607.

**"Fast"- and "Slow"-1,1,1,3,3,3-Hexafluoro-2-propyl 5'-O-(4,4'-dimethoxytrityl)-2'-O-methyluridine 3'-methylphosphonate (4d).** Following the general procedure with 25.21 g (35.7 mmol) of methylphosphonamidite **3d** provided a 1:1.6 mixture (determined by HPLC integration) of "fast" and "slow" diastereomers, respectively. Flash chromatography on silica gel using 6:1 methylene chloride-acetone gave 8.7 g (31 %) of the "fast" diastereomer, and 14.0 g (49 %) of the "slow" diastereomer.

"Fast" diastereomer : $R_f$ 0.26 (6:1 methylene chloride-acetone); $^1H$ NMR (300 MH) d 9.21 (br s, 1H), 7.85 (d, J = 8.1 Hz, 1H), 7.37-7.25 (m, 9H), 6.85 (d, *J* = 8.7 Hz, 4H), 6.07 (d, *J* = 3.9 Hz, 1H), 5.38 (septet, *J* = 6.0 Hz, 1H), 5.27 (d, *J* = 8.1 Hz, 1H), 5.15 (dt, *J* = 8.4, 5.1 Hz, 1H), 4.35 (t, *J* = 6.9 Hz, 1H), 4.03 (t, *J* = 7.5 Hz, 1H), 3.80 (s, 6H), 3.61-3.55 (m, 1H and overlapping s, 3H), 3.46 (dd, *J* = 11.1, 1.8 Hz, 1H), 1.62 (d, *J* = 18.0 Hz, 3H); $^{31}P$ NMR (121 MHz) d 34.53; HRMS (FAB$^+$) calcd for $C_{35}H_{35}F_6N_2O_{10}P$+Cs 921.0988, found 921.1011.

"Slow" diastereomer: $R_f$ 0.17 (6:1 methylene chloride-acetone); $^1H$ NMR (300 MHz) d 9.59 (br s, 1H), 7.81 (d, *J* = 8.1 Hz, 1H), 7.37-7.23 (m, 9H), 6.85 (d, *J* = 8.4 Hz, 4H), 6.08 (d, *J* = 4.5 Hz, 1H), 5.38-5.26 (septet, *J* = 6.0 Hz, 1H and overlapping d, *J* = 8.1 Hz, 1H), 5.06 (dd, *J* = 11.4, 5.1 Hz, 1H), 4.24 (m, 1H), 4.11 (t, *J* = 4.8 Hz, 1H), 3.80 (s, 6H), 3.60-3.56 (s, 3H and overlapping m, 1H), 3.42 (dd, *J* = 11.1, 1.8 Hz, 1H), 1.75 (d, *J* = 18.6 Hz, 3H); $^{31}P$ NMR (121 MHz) d 35.20; HRMS (FAB$^+$) calcd for $C_{35}H_{35}F_6N_2O_{10}P$+Cs 921.0988, found 921.0952.

**General procedure for the synthesis of "Fast"- and "Slow"- 2'-O-methyl dinucleoside (3', 5')-methylphosphonates (8a-p)** A solution of the 2'-O-methyl nucleoside-5'-OH component **6** (1.0 equivalent) in THF (15 mL) was dried over 3Å molecular sieves at 50 °C (oil bath temperature) under a positive pressure of argon for 12 h prior to reaction. A solution of the "slow" 1,1,1,3,3,3-hexafluoro-2-propyl 2'-O-methyl nucleoside 3'-methylphosphonate component **4** (1.2 equivalents) in THF (15 mL) was dried over 3Å molecular sieves at room temperature under a positive pressure of argon for 12 h prior to reaction. A flame-dried 100 mL one-neck round-bottomed flask was charged with *tert*-butylmagnesium chloride (1.0 M solution in THF; 5.9 equivalents for **8i**; 4.9 equivalents for **8j**, **8k** and **8l**; 4.7 equivalents for **8a**, **8e** and **8m**; and 3.7 equivalents for all other dinucleosides) via syringe. The solution was cooled to 0 °C (ice-water bath) and then 3-ethyl-3-pentanol was added (5.9 equivalents for **8i**; 4.9 equivalents for **8j**, **8k** and **8l**; 4.7 equivalents for **8a**, **8e** and **8m**; and 3.7 equivalents for all other dinucleosides) via syringe. The resulting mixture was warmed to room temperature over 20 min to give a grey suspension. To this suspension was added via syringe the pre-dried

THF solution of the 2'-O-methyl nucleoside 5'-OH component **6**. The molucular sieves were rinsed with THF (2 x 4 mL) and the washings were added to the reaction flask. The yellow reaction mixture was stirred at room temperature for 20 min. Then the pre-dried THF solution of the "slow" 1,1,1,3,3,3-hexafluoro-2-propyl 2'-O-methyl nucleoside 3'-methylphosphonate component **4** was added via syringe. The molecular sieves were washed with THF (2 x 4 mL) and the washings were added to the reaction flask. The reaction flask was fitted with a condenser and then immersed in an oil bath which was pre-heated at 60-65 °C. The reaction was heated at this temperature under argon for 12-66 h depending on the particular dinucleoside(see Table 4). The reactions were quenched with saturated aqueous ammonium chloride solution and extracted with ethyl acetate (3 x 150 mL). Organic extracts were washed once with brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a yellow-orange foam. The crude product was purified by flash chromatography on silica gel (30 g silica gel/ g of crude product) using 3:1 ethyl acetate-methylene chloride to elute unreacted 2'-O-methyl nucleoside-5'-OH component **6** and then 75:25:10 ethyl acetate-methylene chloride-methanol to elute the 2'-O-methyl dinucleoside methylphosphonate 3'-OTBDPS derivative.

The 2'-O-methyl dinucleotide methylphosphonate 3'-OTBDPS derivatives were desilylated by treatment with tetrabutylammonium fluoride (TBAF) reagent (1.0 M in THF, 2.0 equivalents) at room temperature for 2 h. The reaction mixtures were concentrated under reduced pressure to give a yellow foam, which was purified by flash chromatography on silica gel using a solvent mixture composed of 75:25:5 ethyl acetate-methylene chloride-methanol, gradually increasing to 75:25:15 ethyl acetate-methylene chloride-methanol to provide the 2'-O-methyl dinucleotide methylphosphonate 3'-OH derivatives **8a-p** as pale yellow solids. Overall yields, diastereomer ratios and spectral data for compounds **8a-p** are shown in Table 4. The "fast" diastereomer of **8a-p** was obtained in 90-99% purity after purification by reverse-phase HPLC (described in the General Methods section). The principal contaminant was residual pyridine from coevaporations following the reverse-phase HPLC operation.



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## REFERENCES


1. Oligodeoxyribonucleotides. Antisense Inhibitors of Gene Expression; Cohen, J. S.; Ed.; MacMillan Press: London, 1989; Topics in Molecular and Structural Biology, Vol. 12.
2. Stein, C. A.; Cohen, J. S. *Cancer Res.* **1988**, *48*, 2659-2668.
3. Tidd, D. M. *Br. J. Cancer* **1991**, *63*, 6-8.
4. Riorden, M. L.; Martin, J. C. *Nature* **1991**, *350*, 442-443.
5. Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 544-579.
6. Miller, P. S.; Ts'o, P. O. P. *Annual Reports Med. Chem.* **1988**, *23*, 295-304.
7. Miller, P.S.; Cushman, C. D.; Levis, J. T. In *Oligonucleotides and Analogues*; Eckstein, F.; Ed.; Oxford University Press: Oxford, 1991; pp 137-159.
8. Miller, P. S.; Ts'o, P. O. P.; Hogrefe, R. I.; Reynolds, M. A.; Arnold, L. J., Jr. In *Antisense Research and Applications*; Crooke, S. T., Lebleu, B., Eds.; CRC Press, Inc.: Boca Raton, 1993; pp 189-203.
9. Miller, P. S. *Bio/technology* **1991**, 9, 358-362.
10. Miller, P. S.; Dreon, N.; Pulford, S. M.; McParland, K. B. *J. Biol. Chem.* **1980**, *235*, 9659-9665.
11. Lesnikowski, Z. J.; Jaworska, M.; Stec, W. J. *Nucleic Acids Res.* **1990**, *18*, 2109-2115.
12. Vyazovkina, E. V.; Savchenko, E. V.; Lokhov, S. G.; Engels, J. W.; Wickstrom, E.; Lebedev, A. V. *Nucleic Acids Res.* **1990**, *22*, 2404-2409.
13. Lesnikowski, Z. J.; Jaworska-Maslanka, M. M.; Stec, W. J. *Nucleosides & Nucleotides* **1991**, *10*, 733-736.
14. Lesnikowski, Z. J.; Jaworska, M.; Stec, W. J. *Nucleic Acids Res.* **1988**, *16*, 11675.
15. Lesnikowski, Z. J.; Wolkanin, P. J.; Stec, W. J. *Tetrahedron Lett.* **1987**, *28*, 5535-5538.
16. Cormier, J. F.; Pannunzio, T. *Tetrahedron Lett.* **1991**, *32*, 7161-7164.
17. Wozniak, L. A.; Pyzowski, J.; Wieczorek, M.; Stec, W. J. *J. Org. Chem.* **1994**, *59*, 5843-5846.
18. Lesnikowski, Z.J. *Bioorg. Chem.* **1993**, *21*, 127-155.
19. Lesnikowski, Z.J.; Stec, W. J. In *Methods in Biology, Vol. 20: Protocols for Oligonucleotides and Analogs*, Agrawal, S. Ed., Humana Press Inc., Totowa, NJ, 1993, p 285.
20. Noyori, R.; Uchiyama, M.; Kato, H.; Wakabayashi, S.; Hayakawa, Y. *Pure & Appl. Chem.* **1990**, *62*, 613-622.
21. Uchiyama, M.; Aso, Y.; Noyori, R.; Hayakawa, Y. *J. Org. Chem.* **1993**, *58*, 373-379.

22. To our knowledge this presented work constitutes the first report on diastereoselective 2'-O-Methyl dinucleoside (3', 5')-methylphosphonate synthesis.
23. The appropriately protected nucleoside methylphosphonamidite synthons are commercially available from American Bionetics Inc. or from Glen Research Inc.
24. Lebedev, A. V.; Frauendorf, A.; Vyazovkina, E. V.; Engels, J. W. *Tetrahedron* **1993**, *49(5)*, 1043-1052.
25. Seela, F.; Kretschmer, U. *J. Org. Chem.* **1991**, *56*, 3861-3869.
26. Löschner, T.; Engels, J. W. *Nucleic acids Res.* **1990**, *18*, 5083-5088 and references cited therein.
27. The diastereomer with greater $R_f$ value in silica gel thin layer chromatography in the protected form obtained from the coupling reaction.
28. These compounds were prepared by 5'-O-DMT protection followed by 3'-O-phosphitylation of heterocycle-protected 2'-O-methyl nucleoside monomers available from Monomer Sciences or Yamasa.
29. Resolution of these diastereomers was more difficult than in the 2'-deoxy series. IMPAX® silica gel from Dupont gave us best resolution and recovery of product.
30. Reynolds, M. A.; Schwartz, D. A.; Riley, T. A.; Vaghefi, M. M.; Hogrefe, R. I.; Jaeger, J. A.; Lebedev, A.; Arnold, L. J., Jr. *First International Antisense Conference of Japan*, Kyoto, Abstract P1-06, 1994.
31. Arnold, L. J., Jr. *First International Antisense Conference of Japan*, Kyoto, Abstract OP-31, 1994.
32. Daily, W. J.; Riley, T. A.; Atkins, M. B.; Scurria, M. A.; Marvin, W. B.; Schwartz, D. A.; Pirrung, M. C. 209th ACS National Meeting, Division of Organic Chemistry, Abstract No. 406, Anaheim, CA, April 2-6, 1995.
33. Le Bec, C.; Wickstrom, E. *J. Org. Chem.* **1996**, *61*, 510-513.
34. Le Bec, C.; Wickstrom, E. *Tetrahedron Lett.* **1994**, *51*, 9525-9528.

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