Stereoselective Synthesis of a Cyano Alkylphosphonate by Intramolecular Rearrangement

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Introduction

The functionalization of oligonucleotides with an alkyl group has led to improved properties such as enhanced lipophilicity for the potential diagnostic and therapeutic applications of these modified oligonucleotides. For the time being, only methylphosphonate oligonucleotides have been widely studied as modulators of gene expression.[1] An unappreciated problem concerning the use of alkylphosphonate in the antisense strategy is their polydiastereoisomerism. Now a common approach for the stereoselective synthesis of methylphosphonate is the nucleophilic displacement at tetracoordinated phosphorus center. It was reported that *p*-nitrophenyl,[2] methylselenyl,[3] and 1,1,1,3,3,3-hexafluoro-2-propanoxyl[4] groups can be replaced by nucleosides to give chiral methylphosphonates in the presence of *t*-BuMgCl or DBU. However, this methodology requires the chromatographic separation of the two diastereomers of the chiral precusor.

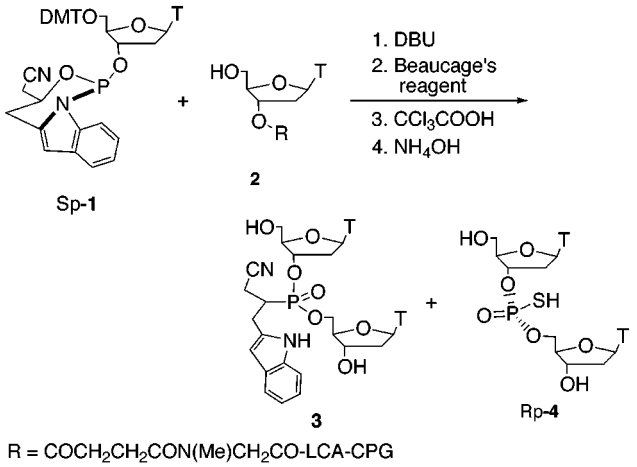
We wish to present our results using indolyl-oxazaphosphorine **1** in the stereoselective synthesis of cyano alkylphosphonate, which was discovered in its application to solid-phase synthesis.

Results and Discussion

As reported recently, reaction of 3 equiv of indolyl-oxazaphosphorine **1** with 3′-*O*-*tert*-butyldiphenylsilylthymidine **5** in the presence of several equivalents of DBU gave phosphorothioate **4** in very high diastereomeric excess[5] after sulfurization and removal of the chiral auxiliary. We therefore tried to adapt the procedure to solid support synthesis of nucleosides.

5′-*O*-DMT-thymidine **2** (28 mg, 1 μmol), obtained by immobilizing thymidine on controlled pore glass via a DBU-resistant sarcosinyl-succinoyl linker (37.9 μmol/g),[6]

Scheme 1



was reacted with 20 equiv of Sp-**1** in acetonitrile (0.2 mL, 0.1 M) and 200 equiv of DBU (30 μL, 0.2 mmol) (Scheme 1). After 5 min, the solid support was washed with acetonitrile, sulfurized with Beaucage's reagent, and treated with aqueous ammonia. To our great surprise, alkyl phosphonate **3** was obtained instead of the expected phosphorothioate $T_{ps}T$ dimer Rp-**4** (Figure 1a). Phosphonate **3** was characterized by HPLC−MS, and the intensities of its isotopic masses perfectly matched the theoretical ones.[7]

Two additional sets of reaction conditions were then investigated for the solid-phase synthesis. In the second set (Figure 1b), polymer-supported thymidine **2** was first mixed with DBU, followed by addition of Sp-**1** in acetonitrile. As shown in the Figure 1b, all thymidine **2** reacted, and phosphonate **3** was formed as the major product, in addition to 10−20% of the desired dimer Rp-**4**. Finally, when the above procedure was repeated but the solid support was washed with acetonitrile before addition of a solution of Sp-**1**, 10−20% of the desired dimer Rp-**4** was obtained, in addition to unreacted thymidine (**T**). No phosphonate **3** was detected in this run (Figure 1c). By repeating procedure c four times before sulfurization, the ratio of dimer Rp-**4** to unreacted thymidine increased from 0.30:1 to 1.06:1. These results can be interpreted as follows: (a) Reaction of polymer-supported **2** with monomer **1** activated by DBU is slow as compared to DBU-induced β-elimination of **1** to **8**. (b) Equilibration of CPG-bound thymidine **2** with DBU probably binds a certain proportion of the DBU on the solid support, providing 10−20% of activated **2**. This activated **2** is responsible for the formation of 10−20% of the desired Rp-**4** found both in runs b and c (Figure 1).

In our previous studies with the solution phase,[5] indolyloxazaphosphorine **1** was first mixed with 3′-*O*-*tert*-butyldiphenylsilylthymidine **5**, and then DBU was introduced. Only phosphite triester was obtained. To clarify the reaction path leading to phosphonate **3**, the reaction was carried out in solution with a different mixing order.

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(7) HPLC-MS (FAB) *m/z*: 735 (M + Na^+, 4.52), 713 (M + H^+, 100), 714 (42.95), 715 (9.40).

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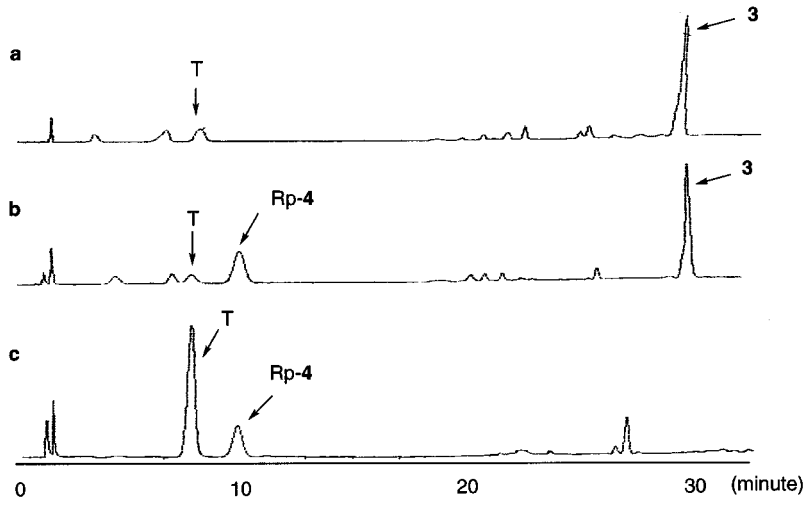


Figure 1. HPLC analysis for the reaction of thymidine **2** with Sp-**1**. HPLC: HP 1090 Serial II; Waters C4 column (3.9 × 300 mm); solvent A, water; B: acetonitrile; 1.5 mL/min flow rate; 3% B increase linearly to 7% B for the first 15 min, then increase to 40% B during the next 20 min. (a) As described in text. (b) To the sintered glass funnel were added thymidine **2** (1 μmol) and 30 μL of DBU (0.2 mmol) in 0.1 mL of acetonitrile, and then a solution of Sp-**1** in acetonitrile (0.2 mL, 0.1 M) was added by a syringe. After 5 min, the solid support was washed with acetonitrile (3 × 2 mL) and sulfurized with Beaucage's reagent (0.1 mL, 0.1 M in THF). After detritylation, the solid support was cleaved with NH_4OH (28%) at 50 °C for 2 h. (c) To the sintered glass funnel was added **2** (1 μmol) and 30 μL of DBU (0.2 mmol) in 0.1 mL of acetonitrile. After 3 min, the solid support was washed with acetonitrile (2 × 1 mL), and then a solution of Sp-**1** in acetonitrile (0.2 mL, 0.1 M) was added by a syringe. The following is same as in procedure b.

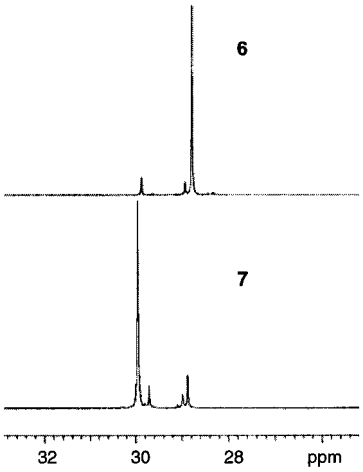
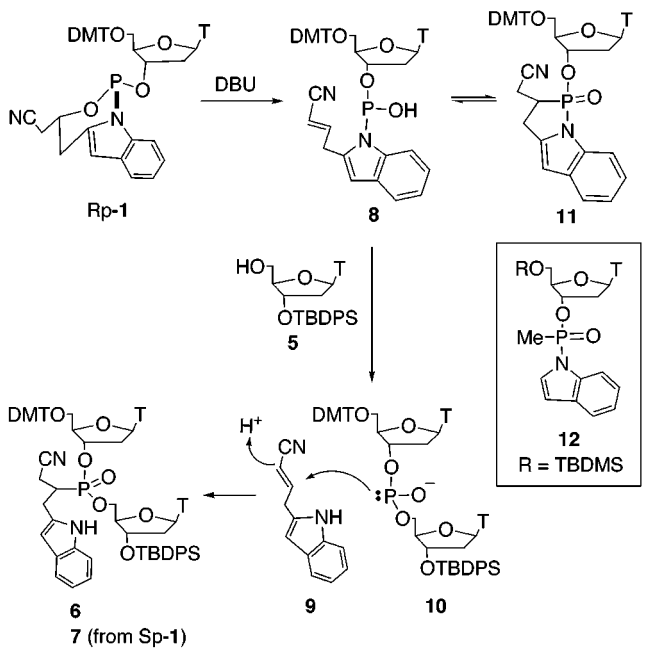


Figure 2. ^{31}P NMR of alkylphosphonate **6** and **7**.

First, 5 equiv of DBU was added to a $CDCl_3$ solution of Rp-**1** synthesized from (*R*)-3-hydroxyl-4-(2-indolyl)butyronitrile and consisting of two diastereomers in a ratio of 40:1. After several minutes, two peaks in ^{31}P NMR were observed at 32.55 and 32.26 ppm with a ratio of 1:10. Then 1 equiv of 3′-*O*-*tert*-butyldiphenylsilylthymidine **5** was added. A major peak around 29.08 ppm appeared, and the peaks around 32 ppm disappeared. After chromatography, alkyl phosphonate **6** was obtained as a mixture of four diastereomers, with a ^{31}P NMR signal at 29.88, 29.63, 28.93, and 28.79 ppm. The ratio of these four peaks was 13.5:1.0:10.8:139.9, as shown in Figure 2. The intermediates corresponding to the peaks at 32 ppm could not be isolated.

In a parallel run, the reaction of a less pure Sp-**1** (mixture of two diastereomers in a ratio of 13:1) with DBU and thymidine **5** provided alkylphosphonate **7.** Four similar peaks in the ^{31}P NMR of **7** were observed as in **6** (Figure 2), but the peak at 29.95 ppm became a major

Scheme 2



one. The ratios of these four peaks were 13.5:1.3:1.0: 1.9.The configuration of these compounds has not been elucidated.

The phosphonate formation is best explained by postulating a β-elimination to form phosphite **8** or its anion, followed by formation of **11**, 32 ppm. We first thought that reaction of **11** with thymidine derivative **5** or **2** would then directly provide phosphonates **6**, **7**, and hence, **3**. However, the analogous phosphonate **12** did not react with **5** under the conditions used. We therefore think that **11** is in equilibrium with **8**, which then reacts with **2** or **5** as outlined to provide phosphite **10** or its anion. The latter then adds to the α,β-unsaturated nitrile **9** to give

alkylphosphonates. The latter reaction is well established.[8]

Although four diastereomeric phosphonates **6** or **7** were formed, one diastereomer predominated both when Sp-**1** and Rp-**1** were used as starting materials. Further investigations on the mechanism and usefulness of the reaction will be reported in due course.

Experimental Section

General Methods. NMR spectra were recorded at 500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR, and 202 MHz for ^{31}P NMR. Chemical shifts are reported (δ) relative to TMS (^{1}H) and 85% H_3PO_4 (^{31}P) as external standards. THF was dried by distillation on sodium benzophenone ketyl, acetonitrile, and triethylamine on calcium hydride, pyridine on barium oxide. DBU was distilled under vacum and then stored over 4 Å Linde molecular sieves under argon. PCl_3 was first degassed by refluxing for 2 h under argon followed by fractional distillation and stored under argon. Water was HPLC grade from Aldrich Chemical Co. Inc.; 3′-*O*-TBDPS-thymidine, Beaucage's reagent, and solid support CPG were generously provided by ISIS Pharmaceuticals (Carlsbad, CA).

Indolyloxazaphosphorine (Sp-1). A dry 100-mL round-bottomed flask containing 20 mL of dry THF was flushed with argon and sealed with a septum, and then 1.3 mL of PCl_3 (15 mmol) was introduced via a syringe. The flask was cooled to −78 °C in a dry ice/acetone bath, and a solution of (*S*)-3-hydroxy-4-(2-indolyl)butyronitrile (3.0 g, 15 mmol) in THF (15 mL) containing triethylamine (6.9 mL, 50 mmol) was added via a syringe. The reaction mixture was stirred for 30 min at −78 °C and then warmed to 0 °C for 1 h. The flask was cooled to −78 °C again, and a solution of 5-*O*′-TBDMS-thymidine (8.2 g, 15 mmol) in THF (20 mL) was added via a syringe. The reaction mixture was stirred at −78 °C for 30 min, the cooling bath was removed, and the solution was warmed to room temperature. The triethylammonium chloride was filtered off and washed with CH_2Cl_2 (2 × 10 mL). The filtrate was concentrated and purified by silica gel chromatography (CH_2Cl_2/CH_3CN 1:10) to afford white solid indolyloxazaphosphorine Sp-**1** (5.43 g) in 47% yield, mp 112−113 °C. Two diastereomers of indolyloxazaphosphorine Sp-**1** were obtained in a ratio of 13:1 as established by ^{31}P NMR: ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 121.58 ppm (93%), 122.17 ppm (7.0%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.93 (br s, 1H, NH), 7.55 (s, 1H, H-6), 7.24, 6.83 (m, 17H, aromatic H), 6.40 (m, 2H, H-3-indole, H-1′), 4.95 (m, 1H, H-3′), 4.41 (m, 1H, CHOP), 4.04 (m, 1H, H-4′), 3.80 (ss, 6H, 2 × OCH_3), 3.50, 3.31 (m, 2H, HH′-5′), 3.10 (m, 2H, CH_2C-indole), 2.72 (m, 2H, CH_2CN), 2.49, 2.24 (m, 2H, HH′-2′), 1.41 (s, 3H, CH_3C-5); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.44, 158.62, 150.20, 143.92, 137.74, 137.61, 135.22, 134.98, 134.93, 133.58, 129.96, 128.00, 127.85, 127.09, 122.55, 121.63, 120.44, 115.76, 113.13 (d, J = 1.9 Hz), 111.33, 110.30 (d, J = 10.0 Hz), 104.17, 86.89, 85.18 (d, J = 3.6 Hz), 84.49, 75.07 (d, J = 10.0 Hz), 70.20 (d, J = 7.3 Hz), 62.75, 55.15, 39.45 (d, J = 3.6 Hz), 30.75 (d, J = 5.5 Hz), 29.52, 25.14 (d, J = 2.6 Hz), 11.56; MS (FAB, nitrobenzyl alcohol) *m/e* 795 (M + Na^+, 4.6), 773 (M + H^+, 1.0), 772 (1.8); HRMS (FAB, glycerol) *m/e* calcd for $C_{43}H_{42}N_4O_8P$ [MH^+] 773.274 02, found 773.273 95.

Indolyloxazaphosphorine (Rp-1). Using the same procedure as for the synthesis of indolyloxazaphosphorine Sp-**1**, the reaction of (*R*)-3-hydroxy-4-(2-indolyl)butyronitrile (30 mg, 0.15 mmol) with PCl_3 (13 μL, 0.15 mmol) and 5′-*O*-DMT-thymidine (81.6 mg, 0.15 mmol) afforded a crude product. Purification with thin-layer chromatography gave 42 mg of indolyloxazaphosphorine Rp-**1** in 36% yield. Two diastereomers of indolyloxazaphosphorine Rp-**1** were obtained in a ratio of 40:1 as established by ^{31}P NMR: ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 120.10 ppm (2.4%), 119.68 ppm (97.6%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (br s, 1H, NH), 7.53−6.80 (m, 18H, H-6, aromatic H), 6.41 (dd, 1H, J = 8.0, 6.0 Hz, H-1′), 6.27 (s, 1H, H-3-indole), 5.05 (m, 1H, H-3′), 4.43 (m, 1H, CHOP), 4.00 (m, 1H, H-4′), 3.80 (ss, 6H, 2 × OCH_3), 3.32, 2.87 (m, 2H, HH′-5′), 3.17, 3.06 (m, 2H, CH_2C-indole), 2.81, 2.72 (m, 2H, CH_2CN), 2.40 (m, 2H, HH′-2′), 1.32 (s, 3H, CH_3C-5); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.51, 158.57, 150.15, 143.81, 137.72, 137.60, 135.36, 134.90, 134.84, 133.05, 130.00, 129.95, 129.32, 128.97, 128.05, 127.93, 127.81, 127.03, 122.56, 121.65, 120.42, 115.66, 113.08 (d, J = 1.9 Hz), 111.29, 110.24 (d, J = 11.0 Hz), 104.27, 86.77, 84.71 (d, J = 2.8 Hz), 84.22, 74.55 (d, J = 6.4 Hz), 69.70 (d, J = 8.2 Hz), 62.59, 55.11, 39.62, 30.48 (d, J = 5.5 Hz), 25.36 (d, J = 2.8 Hz), 11.40; HRMS (FAB, glycerol) *m/e* calcd for $C_{43}H_{42}N_4O_8P$ [MH^+] 773.27402, found 773.27395.

Immobilized Thymidine (2). To a dry 6 mL Hypovials were added 5′-*O*-DMT-thymidine (109 mg, 0.2 mmol), CPG with sarcosinyl-succinonyl linker (1.0 g), 4-DMAP (12 mg, 0.1 mmol), triethylamine (80 μL), DEC (384 mg, 2.0 mmol), and anhydrous pyridine (5 mL). The mixture was shaken at room temperature for 24 h. Pentachlorophenol (134 mg, 0.5 mmol) was added, and the mixture was shaken for an additional period of 16 h. The CPG was filtered off and washed successively with pyridine, CH_2Cl_2, and ether. Then the CPG was treated with reagent grade piperidine (5 mL), and the slurry was shaken for 10 min. The result CPG was filtered off, washed successively with CH_2Cl_2 and ether, and dried under vacuum. The dried CPG was mixed with equal parts of two solutions of 0.5 M acetic anhydride in THF and 0.5 M 4-DMAP/2,4,6-trimethylpyridine in THF (4 mL each). The slurry was shaken for 2 h and then washed successively with pyridine, CH_2Cl_2, THF, and ether. The loading amount was measured by Trityl Analysis, 37.9 μmol/g. Detritylation with 3% trichloroacetic acid in 1,2-dichloroethane afforded the immobilized thymidine **2**.

Alkylphosphonate (3). To the sintered glass funnel were added **2** (27 mg, 1 μmol) and a solution of Sp-**1** in acetonitrile (0.2 mL, 0.1 M), and then 30 μL of DBU (0.2 mmol) was added by a syringe. After 5 min, the solid support was washed with acetonitrile (3 × 2 mL), and then Beaucage's reagent (0.1 mL, 0.1 M in THF) was added. After detritylation, the solid support was cleaved with NH_4OH (28%) at 50 °C for 2 h. The solution was evaporated to dryness, and the residue was dissolved in water (1 mL) and filtered. The solution was ready for HPLC analysis, and the result was shown in Figure 1a: HPLC-MS (FAB) *m/e* 735 (M + Na^+, 4.52), 713 (M+H^+, 100), 714 (42.95), 715 (9.40).

Alkylphosphonate (6). To a solution of Rp-**1** (34 mg, 0.044 mmol) in dry THF (2.5 mL) was added 5 equiv of DBU (32 μL). After 5 min, 1 equiv of 3′-*O*-TBDPS-thymidine (21 mg) was added. The mixture was stirred for 5 min. The solution was evaporated, and the crude product was purified by silica gel column chromatography (EtOAc) to give 25 mg of alkylphosphonate **6** as light yellow solid in 45% yield, mp 127−128 °C. Four isomers were observed from its ^{31}P NMR: ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 29.88 ppm (8.1%), 29.63 ppm (0.6%), 28.93 ppm (6.6%), 28.79 ppm (84.7%). The following NMR spectra were for the major isomer.: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.39, 9.32, 9.08 (3 × s, 3H, NH-3-T$^{5'}$, NH-3-T$^{3'}$, NH), 7.62−6.77 (m, 27H, aromatic H), 7.48 (s, 1H, H-6-T$^{5'}$), 7.12 (s, 1H, H-6-T$^{3'}$), 6.30 (m, 2H, H-1′-T$^{3'}$, H-3-indole), 6.12 (m, 1H, H-1′-T$^{5'}$), 5.16 (m, 1H, H-3′-T$^{3'}$), 4.29 (m, 1H, H-3′-T$^{5'}$), 4.10 (m, 1H, H-4′-T$^{5'}$), 4.06 (m, 1H, H-4′-T$^{3'}$), 3.88, 3.82 (m, 2H, HH′-5′-T$^{3'}$), 3.73, 3.72 (ss, 6H, 2 × CH_3O), 3.43, 3.29 (m, 2H, HH′-5′-T$^{5'}$), 3.17, 2.90 (m, 2H, CCH_2), 2.50 (m, 1H, H-2′-T$^{3'}$), 2.36 (m, 4H, H′-2′-T$^{3'}$, PCH, $CNCH_2$), 2.24, 2.06 (m, HH′-2′-T$^{5'}$), 1.77 (s, 3H, CH_3C-5-T$^{5'}$), 1.43 (s, 3H, CH_3C-5-T$^{3'}$), 1.06 (s, 9H, $SiC(CH_3)_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.62, 158.62, 150.28, 150.14, 143.86, 136.54, 136.24, 135.54 (d, J = 1.9 Hz), 135.10, 134.79, 132.75, 132.51, 130.06, 129.92 (d, J = 3.8 Hz), 128.02, 127.91, 127.88, 127.86, 127.83, 127.13, 121.69, 119.92, 119.71, 116.75 (d, J = 11.3 Hz), 113.16, 111.44, 111.03, 110.69, 102.40, 87.09, 86.76, 84.76 (d, J = 5.5 Hz), 84.19, 83.98 (d, J = 7.3 Hz), 77.54 (d, J = 6.4 Hz), 72.25, 65.86 (d, J = 7.3 Hz), 62.79, 55.07, 39.11, 33.98, 32.84, 29.52, 26.65, 26.19, 18.83, 16.27, 12.03, 11.60; MS (FAB, nitrobenzyl alcohol) *m/e* 1253 (M + H^+, 0.9), 1189 (4.2); HRMS (FAB, glycerol) *m/e* calcd for $C_{69}H_{74}N_6O_{13}SiP$ [MH^+] 1253.482 07, found 1253.482 50.

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Supporting Information Available: ^{1}H NMR, COSY, ^{13}C NMR, and ^{31}P NMR of compounds Rp-**1**, Sp-**1,** and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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