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Stereoselective Synthesis of Alkylphosphonates: A Facile Rearrangement of Cyanoethyl-Protected Nucleoside Phosphoramidites

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Abstract:

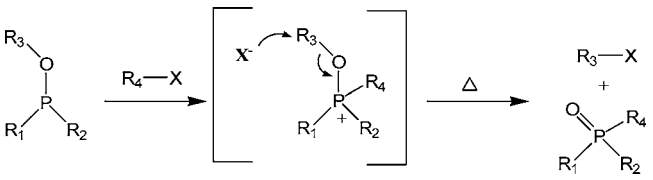
Cyanoethyl-protected nucleoside phosphoramidites undergo a facile Michaelis−Arbuzov reaction upon addition of acrylonitrile to afford cyanoethyl phosphonates. This rearrangement is stereoselective at room temperature, but racemization is observed at high temperatures, indicating two different pathways. A plausible mechanism is proposed for this reaction.

Introduction

The Michaelis−Arbuzov reaction is one of the most extensively investigated reactions in organophosphorus chemistry and is widely used to prepare phosphonates, phosphinates, and phosphine oxides.[1] This reaction involves the reaction of an ester of trivalent phosphorus species with an alkyl halide. The overall process ultimately results in the formation of a pentavalent P═O phosphorus species (Scheme 1). The second step of this general reaction is believed to have S_N2 character.[2] However, this concept has been questioned,[3] and it has been demonstrated that the reaction mechanism varies in character with the reagent and the solvent used. Caruthers and Nielsen have shown that alkyl deoxynucleoside phosphites having alkyl groups which stabilize the S_N1 character of the second stage of the Arbuzov reaction react selectively with most electrophiles and eliminate only the appropriate tertiary alkyl-protecting group.[4] Recently, we have observed that during synthesis of phosphorothioate oligonucleotides, inefficient sulfurization followed by acid treatment and subsequent ammonium hydroxide incubation led to the formation of 5′-terminal DMT C-phosphonate monoesters.[5]

Since introduction of the phosphoramidite method for the synthesis of oligonucleotides and their modified analogues,[6] evaluation of phosphorothioate oligonucleotides as new therapeutic drugs for treatment of diseases through an antisense mechanism has taken a new dimension. Multiple oligonucleotide drugs are being evaluated in human clinical trials against various targets.[7] Recently, extensive improvements have been reported by many laboratories,[8] including ours,[9] for efficient and economical synthesis of oligonucleotides. As a result, phosphorothioate oligonucleotides are routinely synthesized on scales up to 500−650 mmol using low excess (1.75−2.0 mol equiv) of phosphoramidites leading to high yield (3.5−3.7 g of purified drug/mmole) and high quality as judged by ion-pair LC−MS. Thus, currently β-cyanoethyl-protected 2′-deoxy- as well as 2′-*O*-methoxyethylribonucleoside phosphoramidites are routinely used as synthons for oligomerization. Upon storage of these phosphoramidites in

Scheme 1. **General mechanism of Arbuzov rearrangement**



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(7) A number of first- and second-generation phosphorothioate oligonucleotides are in various stages of clinical trials against PKCα, ICAM, TNFα, PTP-1B, VLA4, c-*raf*, survivin, BCLx, APOB-100, etc. for the treatment of a variety of diseases such as cancer, psoriasis, diabetes, asthma, arthritis, multiple sclerosis, diabetic retinapathy, etc.

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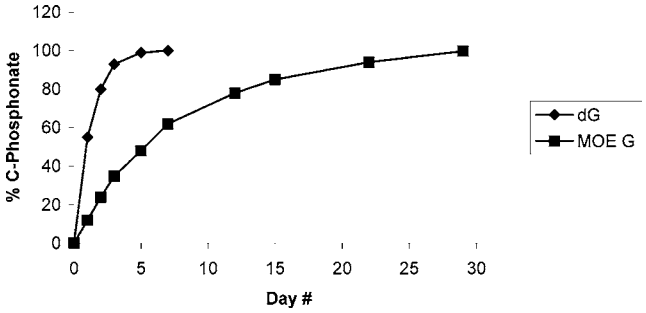


Figure 1. **Time course of rearrangement of phosphoramidites 1a (dG) and 1g (MOE G) upon addition of acrylonitrile at room temperature.**

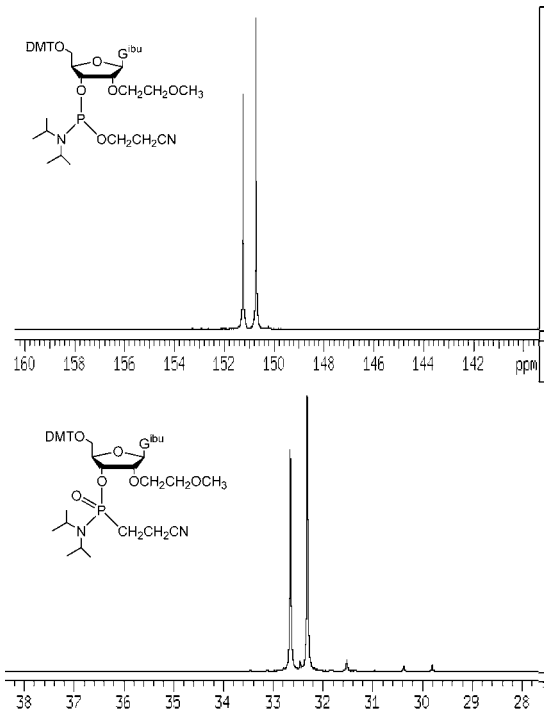


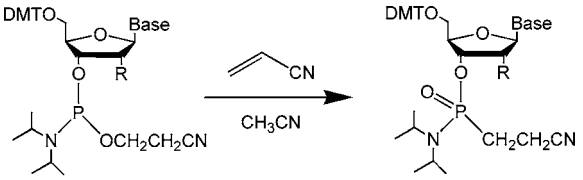
Figure 2. **^{31}P NMR ($CDCl_3$) spectra of compounds 1g and 2g.**

acetonitrile solution at room temperature for several days, we noticed the formation of low levels of a new compound that exhibited a pair of peaks with a chemical shift around 32−33 ppm by ^{31}P NMR spectroscopy.[10] The level of this nonreactive compound increased over time, and we reasoned that these compounds could be cyanoethyl phosphonates formed through a Michaelis−Arbuzov-like rearrangement.[11] Stimulated by this interesting observation, we investigated the transformation of various nucleoside cyanoethyl phosphoramidites. We report here the scope and limitations of this reaction as well as its mechanism.[12,13]

Results and Discussion

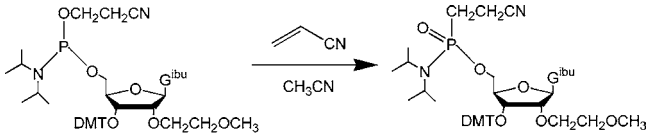
A crucial step during synthesis of oligonucleotides using phosphoramidite chemistry is the addition of an activated

Scheme 2



1 a-j **2 a-j**

Scheme 3



3 **4**

phosphoramidite synthon to the support-bound hydroxy functionality of the preceding residue. An excess of phosphoramidite over a support-bound hydroxy group is used to drive the reaction to completion. It is readily apparent that the use of a minimum amount of phosphoramidite needed to complete the reaction would lead to substantial savings in raw material cost. This assumes that these phosphoramidite monomers are stable in acetonitrile (MeCN) solution for several days before they are consumed. However, we and others in many laboratories have found that phosphoramidites degrade in solution even if stored under inert atmosphere. Initial investigation using ^{31}P NMR spectroscopy revealed that cyanoethyl-protected phosphoramidites when stored in anhydrous MeCN (<10 ppm water) solutions decompose slowly to give two main products, viz. *H*-phosphonate, possibly formed due to presence of water in solvent/amidite, and a hitherto unknown rearranged product as shown in Scheme 1. The formation of the P−C phosphonate bond is well characterized by its distinguished shift in ^{31}P NMR signal (Figure 2) as well as by ion-pair LC−MS−MS data. Reversed phase (RP)-HPLC shows two sets of signals which are more polar than the starting phosphoramidite peaks (Figure 3). The rate of formation of this product depends on the nucleoside (G ≫ A > C > T), with the fully protected deoxyguanosine amidite being the fastest. The order is the same for 2′-deoxy- as well as for 2′-*O*-methoxyethylribonucleoside phosphoramidites. Figure 1 shows the time course of rearrangement of **1a** and **1g** upon addition of 10% acrylonitrile in MeCN (v/v) at room temperature.

Facile rearrangement occurs at elevated temperature (55 °C), and nearly quantitative product formation is observed with various cyanoethyl-protected nucleoside phosphoramidites (**1a**−**1j**) within 14−120 h, depending upon the nucleoside (Scheme 2). The rearranged products (**2a**−**2j**) were purified by silica gel flash chromatography using ethyl acetate−hexane and were obtained as colorless amorphous solids (Table 1).[14]

Rearrangement of a Primary Cyanoethyl Phosphoramidite. The rate of rearrangement of cyanoethyl-protected

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(14) Both phosphoramidite **3** (purchased from ChemGenes) and its rearranged product **4** were found to be difficult to purify.

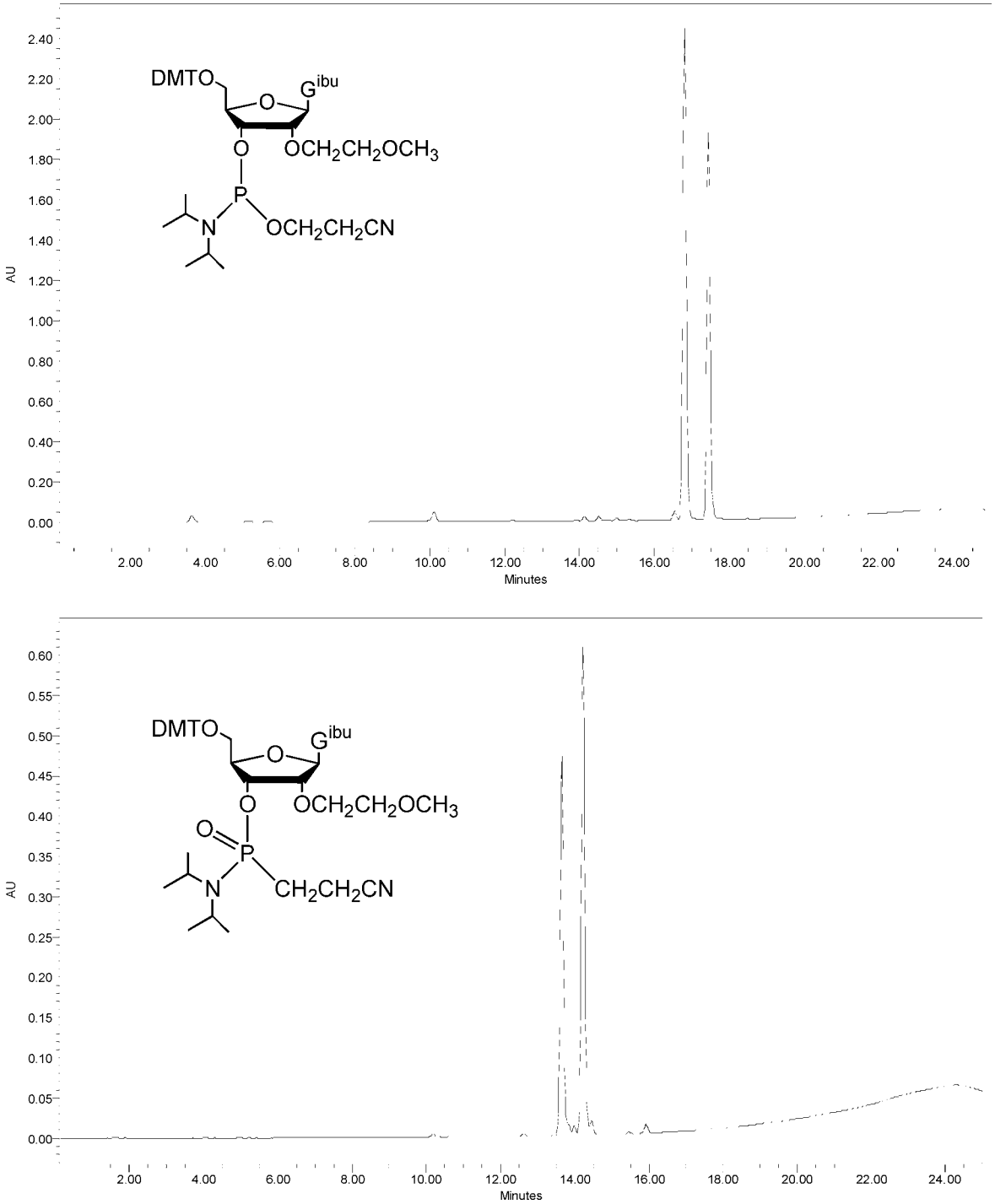


Figure 3. **HPLC analysis of phosphoramidite (1g) and its corresponding cyanoethyl phosphonate (2g).**

Table 1. **Rearrangment of phosphoramidites to cyanoethyl *C*-phosphonates**

			^{31}P NMR ($CDCl_3$) ppm		RP-HPLC (min)[15]	
cmpd	R	base	**1**	**2**	**1**	**2**
a	H	G^{ibu}	149.61,148.97	31.97, 31.64	16.45, 16.93	13.02, 13.51
b	H	A^{bz}	149.93, 149.81	32.02, 31.67	17.37, 17.83	14.07. 14.63
c	H	T	149.89, 149.49	32.58, 32.11	16.80, 17.43	13.65, 14.28
d	H	C^{bz}	150.31, 149.76	32.82, 32.29	18.22, 18.88	15.02, 15.68
e	OCH_3	G^{ibu}	151.39, 151.20	33.11, 31.98	16.72, 17.29	14.29
f	OCH_3	A^{bz}	152.18, 151.43	32.37, 31.59	17.74, 18.19	14.59, 14.91
g	$OCH_2CH_2OCH_3$	G^{ibu}	151.24, 150.71	32.65, 32.31	16.80, 17.44	13.65, 14.22
h	$OCH_2CH_2OCH_3$	A^{bz}	150.04, 149.77	32.22, 31.70	17.77, 18.36	14.65, 15.13
i	$OCH_2CH_2OCH_3$	$^{me}C^{bz}$	150.16, 149.71	32.59, 32.38	21.60, 22.12	19.58, 20.13
j	$OCH_2CH_2OCH_3$	^{me}U	149.76, 149.60	32.62, 32.28	17.16, 18.09	13.89, 14.49

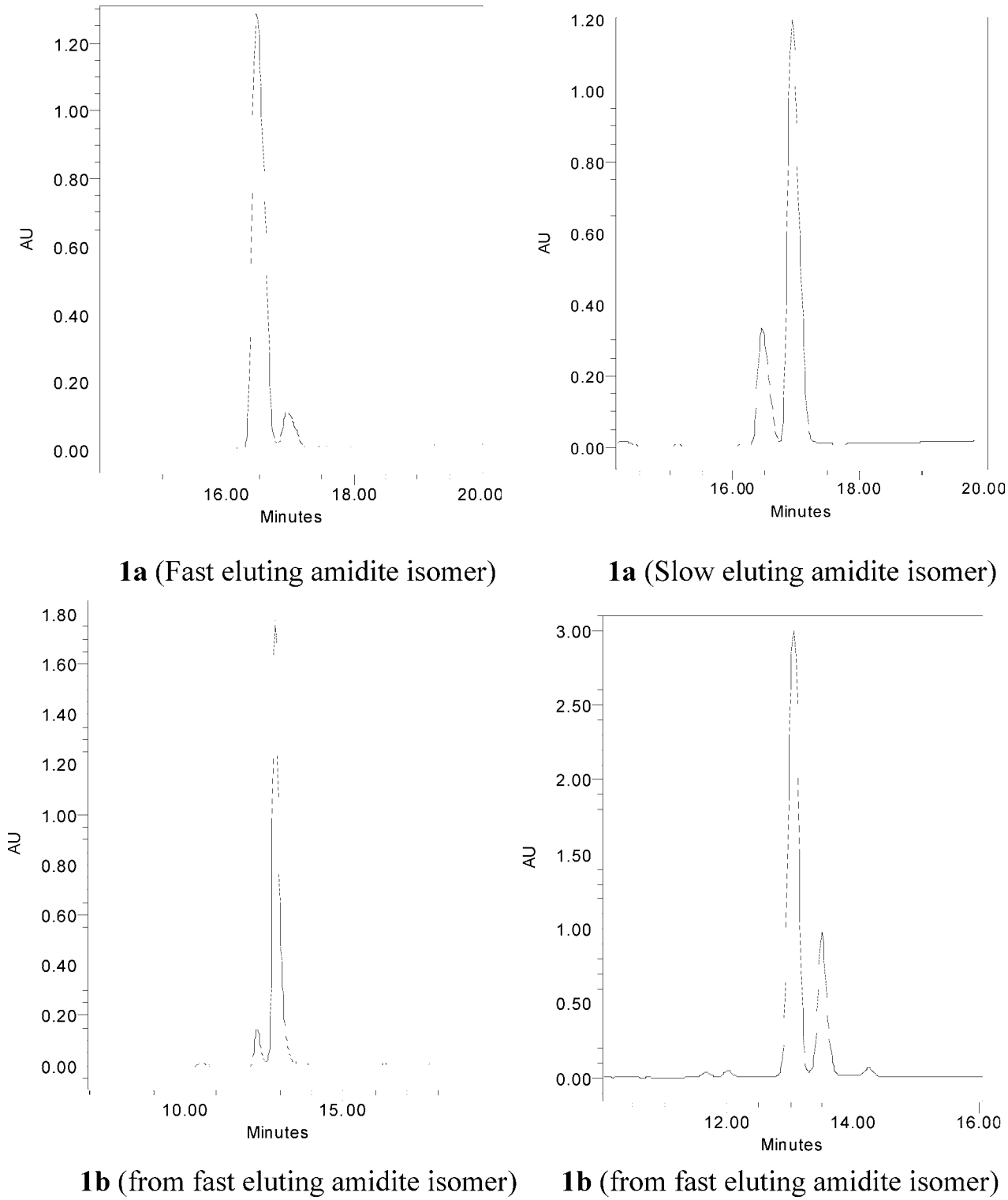
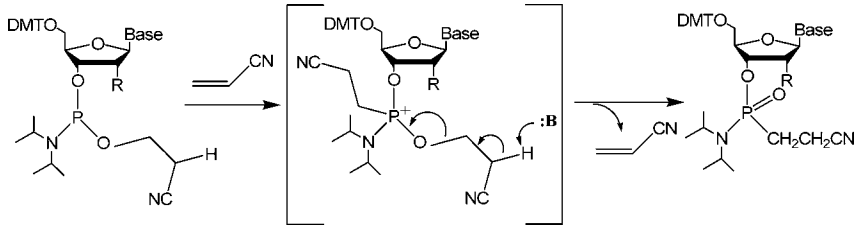


Figure 4. **RP-HPLC analysis of stereo-enriched dG phosphoramidite isomers and their corresponding rearranged products.**

phosphoramidite on a primary hydroxy group was studied (Scheme 3). Treatment of compound **3** (^{31}P NMR ($CDCl_3$) δ 150.10, 150.03 ppm; RP-HPLC 16.56, 17.06 min) with acrylonitrile in anhydrous MeCN under the above reaction conditions gave product **4** as a colorless amorphous foam (^{31}P NMR ($CDCl_3$) δ 31.32, 31.19 ppm; RP-HPLC 14.36, 15.62 min). The rearrangement was found to be approximately twice as fast as for **1g** under identical conditions.

Mechanism of Rearrangement. To understand the steps involved during the formation of cyanoethyl phosphonate product, the two diastereoisomers of **1g** were separated by the use of flash silica gel chromatography with ethyl acetate and hexane (1% triethylamine added). Completely pure diastereomers could not be obtained. The fast- and slow-eluting isomers were obtained in 92% and 79% purity based on RP-HPLC (see Figure 4) and ^{31}P NMR analyses (see

Scheme 4



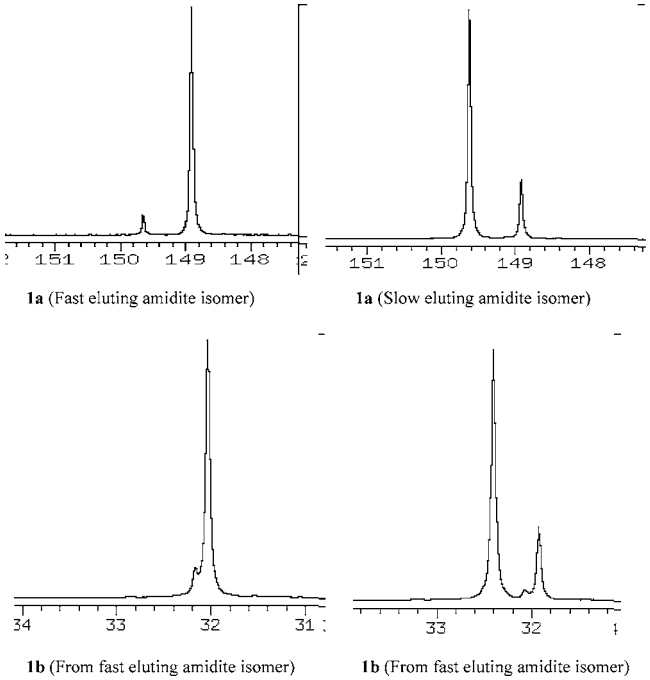


Figure 5. **^{31}P NMR ($CDCl_3$) analysis of stereo-enriched dG phosphoramidite isomers and their corresponding rearranged products.**

Figure 5) and were assigned Sp and Rp configurations, respectively, on the basis of our recent IR-VCD investigation[16] as well as another literature report.[17] Treatment of Sp-enriched diastereomers with 10 mol equiv of acrylonitrile at room temperature for 72 h afforded the same ratio of rearranged products as that of the starting phosphoramidite isomers. Similarly, treatment of Rp-enriched diastereomers under identical conditions afforded the same ratio of rearranged products as that for the starting phosphoramidite isomers after only 42 h, indicating that the two diastereomers rearrange at different rates. The retention of diastereomeric ratio indicates that the reaction is concerted and stereospecific. Surprisingly, in RP-HPLC, the polarity of the rearranged products (compared to that for phosphoramidite) is altered, reversing the elution pattern (Figure 4). This reversal is not fully understood at this time.

Table 2. **Analysis of dG phosphoramidite isomers and their corresponding rearranged products**

isomer	RP-HPLC (% enriched isomer)	^{31}P NMR ($CDCl_3$) % enriched isomer
fast eluting amidite	94	92
slow eluting amidite	79	78
rearranged product isomer #1	94	93
rearranged product isomer #2	78	78

However, treatment of the above two enriched diastereomers individually with acrylonitrile at 55 °C for 24 h gave a nearly 1:1 mixture of two rearranged products. As an another example, two diastereoisomers of **1j** were carefully separated by flash silica gel chromatography using ethyl acetate and hexane (1% triethylamine added). Treatment of the pure fast-eluting diastereomer with 10 mol equiv of acrylonitrile at 55 °C for 140 h gave a nearly 1:1 mixture of two compounds by RP- HPLC. Similarly, treatment of the pure slow-eluting diastereomer under identical conditions afforded two products whose peaks by RP-HPLC were superimposable with those of the products obtained from the fast-eluting isomer (Table 2). A plausible mechanism is proposed (Scheme 4). This proposed mechanism is supported by the fact that addition of 5 mol % DBU to a solution of 2′-deoxyguanosine, as well as to a solution of 2′-*O*-methoxyethylguanosine phosphoramidite in acetonitrile, accelerated formation of the rearranged product faster by two times as compared to control, indicating that liberation of acrylonitrile from phosphoramidite is the initial rate-determining step.

Experimental Section

General Methods. NMR spectra were recorded at 200 MHz for ^{1}H NMR, 50 MHz for ^{13}C NMR, and 80 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. Low-water acetonitrile (<10 ppm water) was purchased from Burdick & Jackson, NJ, and then stored over activated 4 Å Linde molecular sieves. All cyanoethyl-protected nucleoside phosphoramidites were purchased either from Pierce Inc., Milwaukee, WI, or ChemGenes Inc., MA, as colorless amorphous solids. Mass spectra were acquired using a LCQ quadrupole ion trap mass spectrometer equipped with an electrospray ionization source (Finnigan MAT, San Jose, CA).

General Procedure for Rearrangement of Nucleoside Phosphoramidites (1a−1j). To a solution of cyanoethyl-protected phosphoramidite (**1a−1j**) (10 mmol) in anhydrous

(15) Reversed-phase HPLC was performed on a Waters system (600E system controller, 996 photodiode array detector, 717 autosampler). Conditions: Phenomenex Luna C18, 5 μ, 250 mm × 4.6 mm, A 0.1 M triethylammonium acetate (A)/acetonitrile (B) gradient was used as follows: starting with 50% A and 50% B to 100% B at 15 min; hold at 100% B for 5 min; then 50% A and 50% B at 25 min; flow rate = 1.5 mL/min.

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MeCN (100 mL) contained in a 250-mL screw-cap bottle was added acrylonitrile (5 mL). The solution was incubated in an oven at 55 °C for the required period of time. The reaction was monitored by RP-HPLC for the disappearance of starting material. All volatiles were removed under reduced pressure. The crude product was purified by silica gel flash chromatography and eluted with CH_2Cl_2:MeOH:NEt_3, 94:5:1, to obtain the product as a colorless foam.

5′-*O*-DMT-*N*^{2}-Isobutyryl-2′-deoxyguanosine-3′-*O*-[*P*-(2-cyanoethyl)-*N*,*N*-diisopropylaminophosphonate] (2a): ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.59, 18.91, 19.03, 22.37, 23.68, 36.01, 45.97, 46.07, 55.37, 55.42, 86.48, 113.34, 113.39, 121.98, 127.27, 128.04, 128.13, 128.21, 130.09, 135.51, 135.55, 135.62, 135.72, 144.49, 144.59, 148.05, 148.18, 148.23, 148.58, 156.12, 158.76, 158.85, 179.39, 179.72; ^{31}P NMR (80 MHz, $CDCl_3$) δ 31.97, 31.64; HRMS (FAB) Calcd for $C_{44}H_{54}N_7O_8P$ 839.901, found 839.433.

5′-*O*-DMT-*N*^{2}-Isobutyryl-2′-*O*-methoxyguanosine-3′-*O*-[*P*-(2-cyanoethyl)-*N*,*N*-diisopropylaminophosphonate] (2e): ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.59, 18.85, 18.90, 19.18, 19.25, 22.32, 23.53, 46.31, 55.29, 58.71, 86.60, 113.29, 113.39, 118.76, 119.19, 119.24, 119.61, 121.34, 121.85, 127.17, 127.28, 128.07, 128.20, 130.08, 135.09, 135.53, 135.66, 138.51, 144.17, 144.58, 148.09, 148.21, 148.37, 148.82, 155.78, 156.31, 158.78, 158.81, 179.31, 180.09; ^{31}P NMR (80 MHz, $CDCl_3$) δ 33.11, 31.98; HRMS (FAB) Calcd for $C_{45}H_{56}N_7O_9P$ 869.448, found 869.721.

5′-*O*-DMT-*N*^{2}-Isobutyryl-2′-*O*-methoxyethylguanosine-3′-*O*-[*P*-(2-cyanoethyl)-*N*,*N*-diisopropylaminophosphonate] (2g): ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.67, 11.77, 22.51, 23.64, 23.80, 45.96, 46.06, 55.46, 59.21, 70.60, 70.70, 72.65, 72.73, 86.33, 86.41, 87.54, 111.62, 111.69, 113.54, 127.49, 128.26, 128.41, 128.45, 130.38, 135.14, 135.19, 135.29, 135.31, 135.69, 144.18, 144.23, 151.01, 151.14, 159.02, 159.03, 164.28; ^{31}P NMR (80 MHz, $CDCl_3$) δ 32.65, 32.31; HRMS (FAB) Calcd for $C_{47}H_{60}N_7O_9P$ 897.481, found 897.636.

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Supporting Information Available

Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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