Use of 2-Cyano-1-*t*-butylethyl or 2-Cyano-1-(1,1-diethyl-3-butenyl)ethyl as a Phosphorus Protecting Group in Oligonucleotide Synthesis via *in situ* Phosphoramidite Methods

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2-Cyano-1-*t*-butylethyl or 2-cyano-1-(1,1-diethyl-3-butenyl)ethyl 3'-*O*-phosphorimidazolidite of 5'-*O*-protected nucleoside was prepared *in situ* by the use of 2-cyano-1-*t*-butylethyl or 2-cyano-1-(1,1-diethyl-3-butenyl)ethyl phosphorobisimidazolidite as a new phosphitylating reagent. The phosphorimidazolidites were found to be key intermediates for preparing 5'-*O*-protected nucleoside 3'-*O*-monoalkylphosphoramidites *in situ* useful in the solid-phase oligonucleotide synthesis, and for synthesizing conveniently 3'-OH free dinucleoside phosphates and phosphorothioates in solution.

The phosphoramidite method is most widely used for the synthesis of oligonucleotide in solution and on a solid support.[1] In this approach, the 2-cyanoethyl group has been proved to be an useful protecting group for the internucleotide linkage.[2]

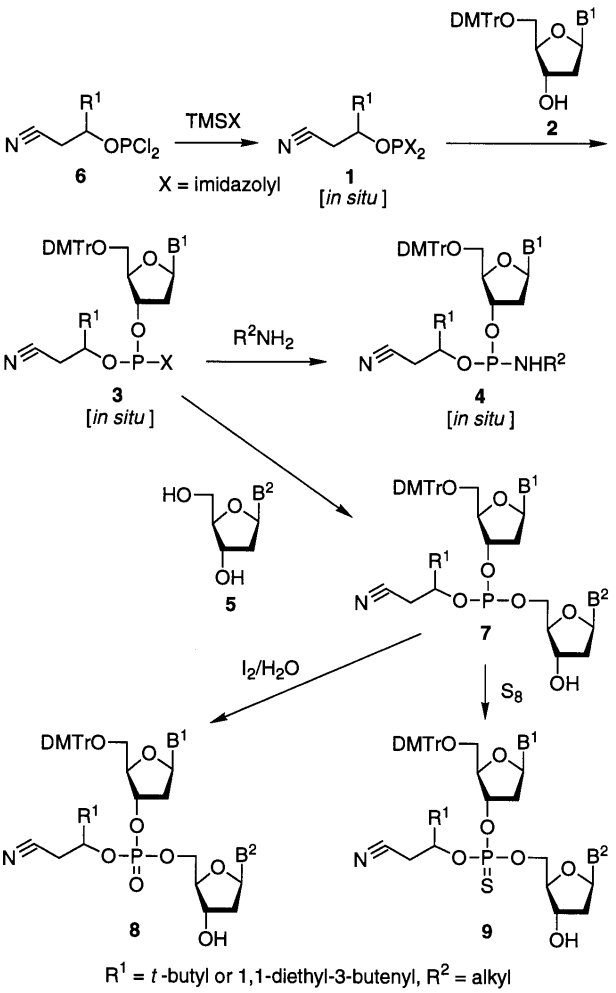
We wish to present here three developmental works for broadening the scope in the 2-cyanoethyl phosphoramidite chemistry: (i) a new phosphitylating reagent, 2-cyano-1-*t*-butylethyl or 2-cyano-1-(1,1-diethyl-3-butenyl)ethyl phosphorobisimidazolidite **1**, is used in *in situ* generation of 5'-*O*-protected nucleoside 3'-*O*-phosphorimidazolidite **3**; (ii) the phosphorimidazolidite **3** serves as a key intermediate for the preparation of a new type of the nucleoside 3'-*O*-phoshoromonoalkylamidite **4**; (iii) the imidazolidite **3** is used for the selective introduction of the 3'-5' internucleotide linkage by the reaction with a 3',5'-*O*,*O*-unprotected nucleoside **5**.

A general method of preparation of **1** and **3** is represented in Scheme 1. The reaction of the phosphorodichloridite **6** (R^1 = *t*-butyl or 1,1-diethyl-3-butenyl)[3] with 1-(trimethylsilyl)imidazole in a 1 : 2.2 ratio in toluene, followed by evaporation under reduced pressure to dryness, gave **1**[4] quantitatively as an oil.[5]

The treatment of **1** with 1.03 equivalents of 5'-*O*-(4,4'-dimethoxytrityl(DMTr))-nucleoside **2** for 1 h at room temperature produced the corresponding imidazolidite **3** in ca. 97% yield (based on **1**).[6] This indicates that the reaction of **1** with **2** proceeds selectively before the produced **3** reacts with **2** to give the 3'-3' dinucleoside phosphite. This would be caused by the steric hindrance of the phosphorus protecting group.[7]

The compound **3** was converted to the phosphoromonoalkylamidite **4** quantitatively by adding 1 equivalent of the corresponding primary amine (Scheme 1). The monoalkylamidite **4** obtained was useful for the solid-phase oligonucleotide synthesis using an automated synthesizer[8] without any purification. For example, the thymidine icosamer (d-T_{20}) was synthesized in an average coupling yield of 99.1% by the use of *in situ* prepared **4** (R^1 = *t*-C_4H_9, R^2 = *i*-C_3H_7)[9] as a monomer unit.

In addition, **3** was selectively coupled with the 5'-hydroxyl group of a 3',5'-*O*,*O*-unprotected nucleoside **5** to give the corresponding triester **7**[10] (Table 1). Thus, the reaction of **3** with



R^1 = *t*-butyl or 1,1-diethyl-3-butenyl, R^2 = alkyl

Scheme 1.

1.1-1.3 equivalents of **5** in chloroform/pyridine (1/2, v/v) (room temperature, several hours) afforded **7** in good yields (based on **3**). The phosphite triester **7** was readily oxidized with iodine/water to give the phosphate derivatives. Thus, the treatment of crude **7** prepared as mentioned above with 1.2 equivalents of iodine in water/THF (1/10, v/v) for 0.5 h at room temperature produced the phosphate **8** essentially quantitatively.[11] Furthermore, the reaction of crude **7** with elemental sulfur gave the phosphorothioate derivatives **9** (Table 1).[12]

The 2-cyano-1-*t*-butylethyl or 2-cyano-1-(1,1-diethyl-3-butenyl)ethyl group of the internucleotide phosphate and phosphorothioate was removed as readily as the 2-cyanoethyl group in concetrated aqueous ammonia/pyridine (1/1, v/v) (room temperature, < 1 h).

Table 1. The *in situ* coupling reaction of **3** with **5**

Entry	R^1	$B^{1\,a}$	$B^{2\,a}$	^{31}P NMR[b] of **7** δ / ppm	Yield[c] of **7** / %	Selectivity[d] / %
1	*t*-C_4H_9	A^{Bz}	G^{iBu}	e	94[f] (80)	96[g]
2	*t*-C_4H_9	T	G^{iBu}	e	94[f] (90)	97[g]
3	$C(C_2H_5)_2$-CH_2CHCH_2[h]	C^{Bz}	C^{Bz}	140.8, 141.0 141.7	89	92
4	$C(C_2H_5)_2$-CH_2CHCH_2	T	T	140.6, 140.7 141.0, 141.8	>88	>91
5	$C(C_2H_5)_2$-CH_2CHCH_2	A^{Bz}	A^{Bz}	140.0, 141.1 141.3, 141.7	91 (88)	94
6	$C(C_2H_5)_2$-CH_2CHCH_2	G^{iBu}	G^{iBu}	140.7, 141.3 142.0, 142.8	93	96

[a]T, C^{Bz}, A^{Bz} and G^{iBu} represent 1-thyminyl, 1-(N^4-benzoylcytosinyl), 9-(N^6-benzoyladeninyl) and 9-(N^2-isobutyrylguaninyl), respectively. [b]$(MeO)_3P$ = 140 ppm as an external standard. [c]Yield determined by ^{31}P NMR. Isolated yields after the sulfurization of **7** are presented in parentheses. [d]The selectivity is defined according to the following equation 1, where [**7**] and [3'-3' dimer] represent the respective molar composition ratios of **7** and the 3'-3' dimer formed as a by-product in the coupling reaction.

$$\text{Selectivity (\%)} = \{[\mathbf{7}] / ([\mathbf{7}] + [3'\text{-}3'\ \text{dimer}])\} \times 100 \quad (1)$$

[e]Not measured. [f]The yields were determined after the sulfurization of **7**. [g]The selectivities were determined after the sulfurization of **7**. [h]1,1-Diethyl-3-butenyl.

In conclusion, the *in situ* preparation of the phosphitylating reagents and the high selectivities in the phosphitylating reactions would make this methodology afford a new route to a facile oligonucleotide synthesis.

References and Notes

1 For example: S. L. Beaucage and R. P. Iyer, *Tetrahedron*, **49**, 6123 (1993) and references cited therein.

2 For example: N. D. Sinha, J. Biernat, and H. Köster, *Tetrahedron Lett.*, **24**, 5843 (1983); S. L. Beaucage and R. P. Iyer, *Tetrahedron*, **48**, 2223 (1992) and references cited therein.

3 2-Cyano-1-*t*-butylethanol was first treated with phosphorus trichloride, but desired **6** (R^1 = *t*-C_4H_9) was not obtained in high purity. Compound **6** was satisfactorily synthesized by the method of Hata et al., where phosphorus trichloride was allowed to react with the corresponding alkoxytrimethylsilanes. See: H. Nagai, T. Fujiwara, M. Fujii, M. Sekine, and T. Hata, *Nucleic Acids Res.*, **17**, 8581 (1989). **6** (R^1 = *t*-C_4H_9): yield 89%; bp 79–80 °C/0.1 mmHg (1 mmHg = 133.322 Pa); ^{31}P NMR (161.7 MHz, $CDCl_3$, $(MeO)_3P$) δ 177.9. **6** (R^1 = $C(C_2H_5)_2CH_2CHCH_2$): yield 78%; bp 119-120 °C/0.1 mmHg; ^{31}P NMR (161.7 MHz, $CDCl_3$, $(MeO)_3P$) δ 181.9.

4 Alkyl phosphorobisimidazolidite was previously used by Hata et al. for the preparation of alkyl nucleoside 3'-*O*-phosphonates. See: T. Wada, R. Kato, and T. Hata, *J. Org. Chem.*, **56**, 1243 (1991).

5 ^{31}P NMR (161.7 MHz, $CDCl_3$, $(MeO)_3P$): **1** (R^1 = *t*-C_4H_9) δ 109.4; **1** (R^1 = $C(C_2H_5)_2CH_2CHCH_2$) δ 109.4.

6 ^{31}P NMR of the reaction mixture displayed essentially four peaks (127–135 ppm, ca. 97%) for the desired imidazolidites **3**, and two signals (140-142 ppm, ca. 3%) for the 3'-3' dinucleoside phosphites formed from **1** and excess **2**. ^{31}P NMR (161.7 MHz, $CDCl_3$, $(MeO)_3P$): **3** (B^1 = T, R^1 = *t*-C_4H_9) δ 127.3, 127.5, 128.4, 130.6; **3** (B^1 = T, R^1 = $C(C_2H_5)_2CH_2CHCH_2$) δ 126.9, 129.5, 130.2, 133.5; **3** (B^1 = C^{Bz}, R^1 = $C(C_2H_5)_2CH_2CHCH_2$) δ 127.5, 131.1, 131.8, 134.8; **3** (B^1 = A^{Bz}, R^1 = $C(C_2H_5)_2CH_2CHCH_2$) δ 129.5, 130.3, 131.1, 134.3; **3** (B^1 = G^{iBu}, R^1 = $C(C_2H_5)_2CH_2CHCH_2$) δ 128.3, 128.6, 131.8, 132.6.

7 R. L. Letsinger, E. P. Groody, N. Lander, and T. Tanaka, *Tetrahedron*, **40**, 137 (1984); J. E. Marugg, C. E. Dreet, G. A. van der Marel, and J. H. van Boom, *Recl. Trav. Chim. Pays-Bas*, **103**, 97 (1984).

8 The chain elongation was achieved on a 0.2 μmol scale following the standard protocol by using a PerSeptive Biosystems Expedite™ 8909 automated synthesizer. The reagents except for **4** and solvents used were purchased from PerSeptive Biosystems, Inc.

9 The reagent **4** (B^1 = T, R^1 = *t*-C_4H_9, R^2 = *i*-C_3H_7) used in solid-phase synthesis was prepared by the reaction of **3** (B^1 = T, R^1 = *t*-C_4H_9) with *i*-propylamine in a 1 : 1 ratio in chloroform followed by dilution to a 0.1 mol dm^{-3} solution with acetonitrile. The reagent **4** was stable for at least one month when stored at –20 °C under an inert atmosphere. ^{31}P NMR (161.7 MHz, $CDCl_3$, $(MeO)_3P$): δ 141.8, 142.4, 144.5, 145.8.

10 The related reaction, in which the nucleoside 3'-*O*-phosphorochloridites were treated with the 3',5'-*O*,*O*-unprotected nucleosides in the presence of a base, followed by oxidation with iodine/water, afforded the 3'-OH free dinucleoside phosphates in ca. 65% yields.[7]

11 After the usual work-up, the crude product (B^1 = B^2 = T, R^1 = *t*-C_4H_9, 4.5 mmol based on **1**) was chromatographed on a column of silica gel (150 g) with chloroform/methanol (100/1 to 100/7, v/v) to give **8** (3.49 g, 81%): ^{31}P NMR (161.7 MHz, $CDCl_3$, $(MeO)_3P$): δ –4.2, –3.4; R_f silica (chloroform/methanol = 10/1, v/v): 0.33.

12 In a typical case, elemental sulfur (0.26 g, 8 mmol) was added to the reaction mixture of **7** (B^1 = T, B^2 = G^{iBu}, R^1 = *t*-C_4H_9, 4 mmol based on **1**) prepared as mentioned above. The resulting mixture was stirred for 2 h at room temperature, poured into water (100 mL) and then extracted with chloroform (100 mL × 2). The combined organic layer was washed with 5% $NaHCO_3$ (40 mL × 2) and brine (50 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was chromatographed on a column of silica gel (100 g) with chloroform/methanol (100/1 to 100/7, v/v) to give **9** (3.85 g, 90%): ^{31}P NMR (161.7 MHz, $CDCl_3$, $(MeO)_3P$): **9** (B^1 = T, B^2 = G^{iBu}, R^1 = *t*-C_4H_9): δ 65.6, 66.1, 66.2; R_f silica (chloroform/methanol = 7/1, v/v): 0.42. ^{31}P NMR and TLC analysis data for the other dinucleoside phosphorothioates **9** were as follows: **9** (B^1 = A^{Bz}, B^2 = G^{iBu}, R^1 = *t*-C_4H_9), ($CDCl_3$): δ 65.4, 65.9, 66.0, 66.2; R_f silica (chloroform/methanol = 7/1, v/v): 0.38, **9** (B^1 = B^2 = A^{Bz}, R^1 = $C(C_2H_5)_2CH_2CHCH_2$), ($CDCl_3$/pyridine = 1/2, v/v): δ 65.2, 65.5, 65.8, 65.9; R_f silica (chloroform/methanol = 7/1, v/v): 0.48.