Convenient and Efficient Approach to Di- and Trideoxyribonucleotide N3′→P5′ Phosphoramidates

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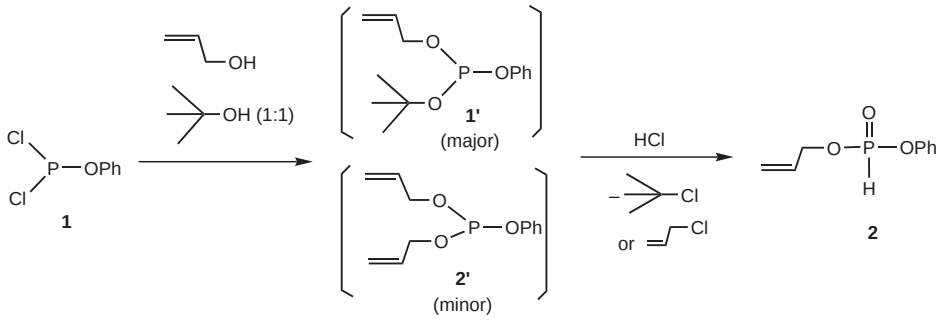
Abstract: Arbuzov reaction of phenyl phosphorodichloridite with an equivalent of allyl alcohol and *tert*-butyl alcohol produced phenyl allyl H-phosphonate and the subsequent ester-exchange with various nucleosides produced nucleoside allyl H-phosphonates. Antherton–Todd reaction of the nucleoside allyl H-phosphonates with 3′-amino-3′-deoxythymidine yielded dideoxyribonucleotide N3′→P5′ phosphoramidates, and the repetition of this procedure provided trideoxyribonucleotide N3′→P5′ phosphoramidates. The method can be used for the synthesis of oligodeoxyribonucleotide N3′→P5′ phosphoramidates without any protection for all nucleosides.

Key words: nucleotide N3′→P5′ phosphoramidate, H-phosphonate, ester-exchange, Antherton–Todd reaction

Oligonucleotide N3′→P5′ phosphoramidates have been attracted considerable attention as a class of compounds of potential therapeutic value,[1–6] since these oligonucleotide analogues are resistant towards various nucleases[2,7] and hybridize to complementary DNA or RNA targets with much higher affinity than their natural congeners do.[2] Some synthetic methods of these phosphoramidates have been developed using phosphotriester chemistry,[8] via the Staudinger type of reaction,[9] or by the phosphoramidite method.[4] Gryaznov and co-workers have prepared oligonucleotide N3′→P5′ phosphoramidates via oxidative coupling of aminonucleosides with H-phosphonate diesters under Antherton–Todd oxidation conditions.[4,10–13] Stec and co-workers synthesized a kind of compounds by using 2-alkylamino-2-thio-1,3,2-oxathiaphospholanes[14] as the starting material.[15] H-phosphonates are useful intermediates in chemistry, and to find the new synthetic routes to these compounds constitutes a valuable target. In the past two decades, studies on H-phosphonate derivatives have greatly progressed.[16–22] Advances on the development of a comprehensive H-phosphonate methodology and the underlying chemistry for the preparation of biologically important phosphate esters and their analogues have been discussed by Stawinski and Kraszewski.[20] Stawinski and co-workers have developed H-phosphonate methods to synthesize dideoxynucleotide N3′→P5′ phosphoramidates through reaction of nucleoside H-phosphonate monoester with aminonucleoside in the presence of Me_3SiCl and I_2[21] or exchange of aryl H-phosphonates with aminonucleoside and the following oxidation.[22] However, all these methods required the protection of functional groups in nucleosides. Here we would like to report a convenient and efficient synthesis of dideoxyribonucleotide N3′→P5′ phosphoramidates without any protection for nucleosides.

Reaction of phenyl phosphorodichloridite (**1**) with one equivalent of allyl alcohol and one equivalent of *tert*-butyl alcohol in CH_2Cl_2 at room temperature under nitrogen atmosphere provided phenyl allyl H-phosphonate diester (**2**) in 90% yield with minor diallyl H-phosphonate diester appearing (determined by ^{31}P NMR). The reaction could undergo the formation of the corresponding major *tert*-butyl phenyl allyl phosphite triester (**1′**) and phenyl diallyl phosphate triester (**2′**) intermediates, followed by Arbuzov rearrangement by the reaction of HCl (Scheme 1).



Scheme 1 Synthesis of phenyl allyl H-phosphonate

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Scheme 2 shows the synthetic route of di- and trideoxyribonucleotide N3′→P5′ phosphoramidates. The displacement reaction of **2** with nucleoside in dry pyridine at room temperature provided nucleoside allyl H-phosphonate diester (**4**) in 73–89% yield (determined by ^{31}P NMR). The chemoselectivity observed for 5′-hydroxyl in non-protected nucleosides **2**, leading to nucleoside allyl 5′-phosphonate diesters, is different from reactivity and steric hindrance among 5′-OH, 3′-OH and amino group of base. The $^{3}J_{P-H}$ coupling between 5′-CH_2 and P in ^{1}H NMR spectra of compounds **4** was observed.

Table 1 Isolated Yields of Synthesized Di- and Trideoxyribonucleotide N3′→P5′ Phosphoramidates (**6** and **8**)[23]

Compound	Yield of **6** (%)[a]	Yield of **8** (%)[b]
a	70	64
b	78	70
c	64	61
d	60	58
e	76	69

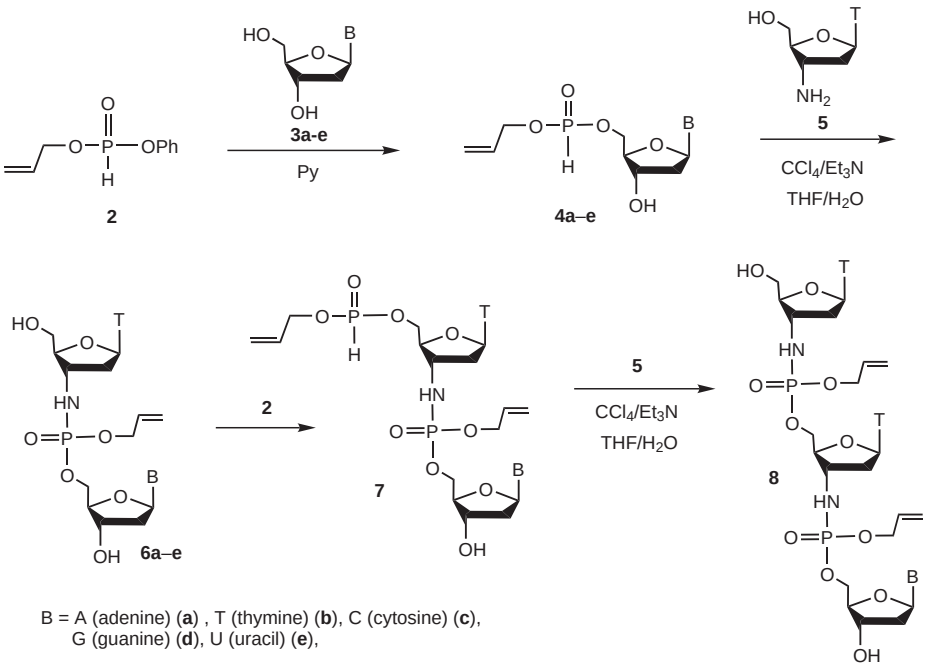
[a] Overall yield from **1** to **6**.
[b] Overall yield from **6** to **8**.

Antherton–Todd reaction of **4** with 3′-amino-3′-deoxythymidine **5** in THF, in the presence of carbon tetrachloride, trimethylamine and small amount of H_2O (almost quantitatively) yielded dinucleotide N3′→P5′ phosphoramidates (**6a**–**e**, Scheme 2) at room temperature, and purification by silica gel column chromatography using $CHCl_3$–MeOH (3:1) as eluent provided **6a**–**e** in 60–78% yields (see Table 1). The repetition of the above procedure gave trinucleotide N3′→P5′ phosphoramidate, i.e. ester exchange reaction of **2** with **6** yielded **7**, and Atherton–Todd reaction of **7** with 3′-amino-3′-deoxythymidine (**5**) produced trinucleotide N3′→P5′ phosphoramidate **8** in 58–70% yield after silica gel chromatography using $CHCl_3$–MeOH (3:1) as eluent.

In conclusion, we have developed a new convenient and efficient synthetic method that provides a facile access to di- and trideoxyribonucleotide N3′→P5′ phosphoramidates. In these reactions, phenyl allyl H-phosphonate is a good phosphonylating agent of nucleosides. The method is simple and efficient, and makes use of readily available nucleosides H-phosphonates without any protection for nucleosides. It is also suitable to the preparation of oligodeoxyribonucleotide N3′→P5′ phosphoramidates.

General Procedure for Preparation of Di- and Trideoxyribonucleotide N3′→P5′ Phosphoramidates

To a flask containing phenyl phosphorodichloridite (195 mg, 1 mmol) in 2 mL of CH_2Cl_2, mixture of allyl alcohol (1 mmol) and *tert*-butanol (1 mmol) in 3 mL of CH_2Cl_2 was added dropwise at r.t., and the solution was stirred at that temperature for 20 min. After evaporation of the solvent in vacuo, the residue was dissolved in 2 mL of pyridine and added to 3′-deoxynucleoside (1 mmol) in 2 mL of pyridine, and the mixture was stirred at r.t. for 2 h. Then, the solvent was removed by evaporation, the residue was dissolved in 2 mL of THF, and the solution was added dropwise to the mixture of 3′-amino-3′-deoxythymidine (241 mg, 1 mmol), carbon tetrachloride (308 mg, 2 mmol), Et_3N (202 mg, 2 mmol), small amount of H_2O (1 mL) and THF (4 mL). The resulting solution was stirred at r.t. for 30 min. Then, the solvent was removed in vacuo, and dideoxyribonucleotide N3′→P5′ phosphoramidates (**6a**–**e**) are purified over silica gel chromatography using $CHCl_3$–MeOH (3:1) as eluent. Repeating the above procedure, ester exchange reaction of **2** with **6** yielded **7**, and Atherton–Todd reaction of **7** with 3′-amino-3′-deoxythymidine produced **8**, and trinucleotide N3′→P5′ phosphoramidate was obtained in 58–70% yield after silica gel chromatography using $CHCl_3$–MeOH (3:1) as eluent.



Scheme 2 Synthetic route of di- and trideoxyribonucleotide N3′→P5′ phosphoramidates

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(23) Spectral data for the representative products **6b** and **8b**. Compound **6b**: ^{31}P NMR (121.5 MHz, DMSO-d_6): δ = 10.37, 10.26 ppm. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.76 (s, 3 H, 5-CH_3 of 3′-amino-3′-deoxythymidine), 1.78 (s, 3 H, 5-CH_3 of 3′-deoxythymidine), 2.11–2.17 (m, 5 H, 2′-H of 3′-amino-3′-deoxythymidine and 3′-deoxythymidine and 4′-H of 3′-amino-3′-deoxythymidine), 3.50–3.65 (m, 2 H, 3′-H of 3′-amino-3′-deoxythymidine and 3′-H of 3′-deoxythymidine), 3.65–3.93 (m, 2 H, 5′-H of 3′-amino-3′-deoxythymidine), 4.01 (m, 1 H, 4′-H of 3′-deoxythymidine), 4.01–4.11 (m, 2 H, 5′-H of 3′-deoxythymidine), 4.21–4.30 (m, 1 H, 3′-NH of 3′-amino-3′-deoxythymidine), 4.39–4.47 (m, 2 H, OCH_2-CH=CH_2), 5.17–5.35 (m, 2 H, OCH_2-CH=CH_2), 5.88–6.00 (m, 1 H, OCH_2-CH=CH_2), 6.12 (t, 1 H, ^{3}J = 6.18 Hz, 1′-H of 3′-amino-3′-deoxythymidine), 6.19 (t, 1 H, ^{3}J = 6.87 Hz, 1′-H of 3′-deoxythymidine), 7.52 (s, 1 H, 6-H of 3′-amino-3′-deoxythymidine), 7.73 (s, 1 H, 6-H of 3′-deoxythymidine), 11.29 (s, 1 H, 3-H of 3′-amino-3′-deoxythymidine), 11.32 (s, 1 H, 3-H of 3′-deoxythymidine) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 12.11, 12.26, 60.39, 65.58, 65.97, 66.05, 70.21, 70.30, 83.24, 83.88, 84.69, 85.80, 109.21, 109.83, 117.04, 133.53, 133.62, 135.81, 136.18, 150.35, 150.42, 163.70, 163.76 ppm. ESI-MS: *m/z* = 586.4 $[M + H]^+$, 608.2 $[M + Na]^+$.
Compound **8b**: ^{31}P NMR (121.5 MHz, DMSO-d_6): δ = 10.08, 10.30 ppm. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.76 (s, 3 H, 5-CH_3 of 3′-amino-3′-deoxythymidine), 1.78 (s, 6 H, 5-CH_3 of 3′-deoxythymidine and 3′-amino-3′-deoxythymidine), 1.99–2.35 (m, 6 H, 6 × H-2′), 3.54–3.86 (m, 5 H, H-4′ of 3′-deoxythymidine and 2 × H-5′), 3.87–4.33 (m, 8 H, 2 × H-4′, 4 × H-5′ and 2 × H-3′ of 3′-amino-3′-deoxythymidine), 4.33–4.50 (m, 3 H, 2 OCH_2-CH=CH_2 and H-3′ of 3′-deoxythymidine), 5.10–5.38 (m, 4 H, 2 OCH_2-CH=CH_2), 5.83–6.02 (m, 2 H, 2 OCH_2-CH=CH_2), 6.04–6.26 (m, 3 H, H-1′), 7.54 (s, 2 H, H-6 of 3′-deoxythymidine and 3′-amino-3′-deoxythymidine), 7.74 (s, 1 H, H-6 of 3′-amino-3′-deoxythymidine), 11.23–11.36 (m, 3 H, 3 × H of NH-3) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 12.60, 12.73, 50.82, 51.22, 60.76, 65.61, 65.76, 66.21, 66.51, 66.57, 66.74, 66.80, 70.59, 70.61, 79.75, 83.72, 83.97, 84.40, 85.03, 85.10, 86.35, 86.49, 109.72, 110.25, 110.34, 117.57, 117.74, 133.90, 133.97, 136.30, 136.72, 150.75, 150.84, 164.18 ppm. ESI-MS: *m/z* = 951.9 $[M + Na]^+$.