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Convenient Synthesis of *N*-Unprotected Deoxynucleoside 3′-Phosphoramidite Building Blocks by Selective Deacylation of *N*-Acylated Species and Their Facile Conversion to Other *N*-Functionalized Derivatives

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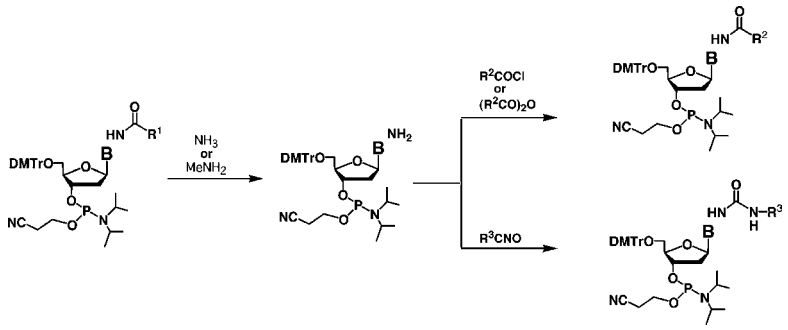
ABSTRACT



A new route to *N*-unprotected deoxynucleoside 3′-phosphoramidite building blocks by use of highly selective *N*-deacylation of commercially available *N*-acylated deoxynucleoside 3′-phosphoramidites is described. These compounds could be readily converted to other types of *N*-protected species by facile *N*-acylations with acylating reagents.

For more than two decades, various methods and reactions for the chemical synthesis of oligodeoxynucleotides have been developed.[1] For example, the phosphoramidite method[2] established by Caruthers and Beaucage allowed us to synthesize oligonucleotides rapidly and has exercised a significant influence in the fields of not only organic chemistry but also biotechnology and medicinal applications. Afterward, Letsinger and Gryaznov demonstrated for the first time DNA synthesis without protection of nucleobases in the phosphoramidite method.[3] In 1997, Hayakawa and Kataoka reported another procedure for the synthesis of DNA fragments without base protection by use of imidazolium

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(1) Beaucage, S. L., Bergstrom, D. E., Glick, G. D., Jones, R. A., Eds. *Current Protocols in Nucleic Acid Chemistry*; John Wiley & Sons: New York, 2000 and references therein

(2) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859−1862.

(3) Gryaznov, S. M.; Letsinger, R. L. *J. Am. Chem. Soc.* **1991**, *113*, 5876−5877.

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triflate as a promoter for the activation of deoxynucleoside 3′-phosphoramidite building blocks.[4] Moreover, we recently reported the first development of an almost completely *O*-selective phosphorylation procedure, i.e., "the activated phosphite method", by use of reactive phosphite intermediates generated from *N*-unprotected phosphoramidites.[5] Our new strategy proved to be advantageous over the previous ones because the cleavage step of the N−P (III) bonds which could be generated by the phosphitylation of the amino groups of the nucleobases could be omitted.

The activated phosphite method must be very attractive for the high-throughput synthesis of DNA molecules and the synthesis of alkali-labile modified DNAs such as oligonucleotides containing DNA lesions.[6] To demonstrate the usefulness in the latter application, we have already reported the synthesis of oligonucleotides incorporating alkali-labile modified bases such as 4-*N*-acetylcytosine by the combined use of this activated phosphite method and new silyl-type linkers that could be cleaved under neutral conditions.[5a]

To expand the activated phosphite method in commercial custom DNA synthesis, convenient protocols for the synthesis of *N*-unprotected deoxynucleoside 3′-phosphoramidite units should be developed. Jones and co-workers reported a general method for the synthesis of 5′-*O*-dimethoxytrityldeoxyribonucleosides, which were prepared by dimethoxytritylation of *N*-dimethylaminoamidinodeoxynucleosides followed by removal of the *N*-protecting groups.[7] More straightfoward methods for the synthesis of 5′-*O*-tritylated deoxyribonucleosides directly from deoxynucleosides have been reported by several research groups.[8] In the case of 2′-deoxycytidine and 2′-deoxyadenosine, the 5′-*O*-DMTr-*N*-free deoxynucleoside derivatives can be obtained in approximately 70% yields by the dimethoxytritylation of deoxynucleosides in the presence of dichroloacetic acid and triethylamine.[4] However, the isolated yield was very low in the tritylation of *N*-free deoxyguanosine because the *O*-selectivity of the tritylation was very poor. The *O*-selective tritylation of deoxyguanosine in the presence of imidazole, methanesulfonic acid, and triethylamine reported by Kataoka[8b] gave unsatisfactory results in our large-scale synthesis of 5′-*O*-DMTr-deoxyguanosine. For these reasons, most of the previous methods in the synthesis of *N*-unprotected deoxynucleoside 3′-phosphoramidite units are unsuitable for the large-scale synthesis of DNA.

In this paper, we report a convenient method for the synthesis of *N*-unprotected deoxynucleoside 3′-phosphoramidite units by treatment of commercially available *N*-protected deoxynucleoside 3′-phosphoramidite units with ammonia or methylamine.

First, we studied the selective removal of the acetyl group from the 4-*N*-acetyldeoxycytidine 3′-phosphoramidite unit to prepare *N*-free deoxycytidine 3′-phosphoramidite (Scheme 1 and Table 1). As a result, it was found that the 4-*N*-acetyl group of the cytosine base could be cleaved completely by treatment with 2 M NH_3/MeOH for 2 h. Although there was some concern about β-elimination of the 2-cyanoethyl group of the phosphoramidite unit, this functional group was found to be stable in 2 M NH_3/MeOH for 2 h because the phosphorus atom of the phosphoramidite unit is trivalent so that it has poor leaving group ability. The *N*-free deoxycytidine 3′-phosphoramidite unit was obtained in 94% yield, as shown in entry 1 of Table 1. Similarly, the *N*-free deoxyadenosine 3′-phosphoramidite derivative was obtained in 98% yield by treatment of the 6-*N*-phenoxyacetyldeoxadenosine 3′-phosphoramidite derivative with 2 M NH_3/MeOH for 1 h (entry 2). The *N*-free deoxyguanosine 3′-phosphoramidite derivative was also obtained in 88% yield by using deprotection of 2-*N*-(4-isopropylphenoxyacetyl)-deoxyguanosine 3′-phosphoramidite derivative (entry 3). In contrast, the *N*-benzoyl group of *N*-benzoyldeoxycytidine was more stable under the same conditions using NH_3−MeOH. Since the cleavage reaction required 12 h, side reactions such

Scheme 1. Deacylation of *N*-Acylated Phosphoramidite Derivatives

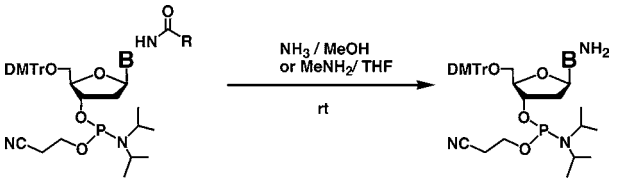


Table 1. Preparation of *N*-Unprotected Phosphoramidite Derivatives

entry	condition	nucleobase	protecting group	time (h)	yield[a] (%)
1	2 M NH_3/ MeOH	cytosine	acetyl	2	94
2	2 M NH_3/ MeOH	adenine	phenoxyacetyl	1	98
3	2 M NH_3/ MeOH	guanine	4-isopropyl-phenoxyacetyl	2	88
4	2 M NH_3/ MeOH	cytosine	benzoyl	12	68
5	2 M NH_3/ MeOH	adenine	benzoyl	50	56
6	2 M NH_3/ MeOH	guanine	isobutyryl	22	72
7	7 M NH_3/ MeOH	guanine	isobutyryl	6	62
8	2 M $MeNH_2$/ THF	adenine	benzoyl	2	89
9	2 M $MeNH_2$/ THF	cytosine	benzoyl	12	85
10	2 M $MeNH_2$/ THF	guanine	isobutyryl	14	75
11	2 M $MeNH_2$/ THF	cytosine	acetyl	2	96
12	2 M $MeNH_2$/ THF	adenine	phenoxyacetyl	1	97
13	2 M $MeNH_2$/ THF	guanine	4-isopropyl-phenoxyacetyl	2	90

[a] Isolated yields of *N*-free phosphoramidite compounds.

(4) Hayakawa Y.; Kataoka, M. *J. Am. Chem. Soc.* **1998**, *120*, 12395−12401.

(5) (a) Ohkubo, A.; Ezawa, Y.; Seio, K.; Sekine, M. *J. Am. Chem. Soc.* **2004**, *126*, 10884−10896. (b) Ohkubo, A.; Seio, K.; Sekine, M. *Tetrahedron Lett.* **2004**, *45*, 363−366.

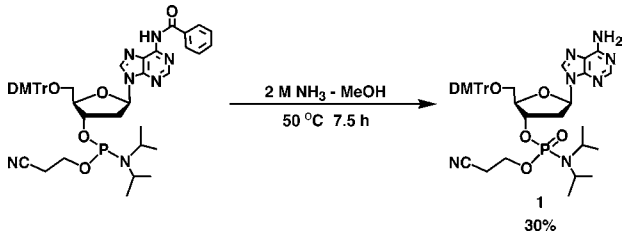
(6) (a) Roupioz, Y.; Lhomme, J.; Kotera, M. *J. Am. Chem. Soc.* **2002**, *124*, 9129−9135. (b) Kim, J.; Gil, J. M.; Greenberg, M. M. *Angew. Chem., Int. Ed.* **2003**, *42*, 5882−5885. (c) Zheng, Y.; Sheppard, L. T. *Chem. Res. Toxicol.* **2004**, *17*, 197−207.

(7) Kung, P.-P.; Jones, R. A. *Tetrahedron Lett.* **1992**, *33*, 5869−5872.

(8) (a) Adamiak, R. W.; Biala, E.; Grzeskowiak, K.; Kierzek, R.; Kraszewski, A.; Markiewicz, W. T.; Okupniak, J. Stawinski, J. Wiewiorowski, M. *Nucleic Acids* Res. **1978**, *5*, 1889−1905. (b) Kataoka, M.: Hayakawa, Y. *J. Org. Chem.* **1999**, *64*, 6087−6089. (c) Nishino, S.; Nagato, Y.; Hasegawa, Y.; Yamamoto, H.; Kamaike, K.; Ishido, Y. *Heteroatom Chem.* **1991**, *2*, 187−196.

as hydrolysis of the phosphoramidite gradually increased so that the yield of the target *N*-free compound was lower than that obtained by use of the 4-*N*-acetyldeoxycytidine derivative as a starting material (entry 4). The deacylation of 6-*N*-benzoyldeoxyadenosine and 2-*N*-isobutyryldeoxyguanosine required prolonged periods of time as shown in entries 5 and 6, respectively. The *N*-benzoyl group of the *N*-benzoyldeoxyadenosine 3′-phosphoramidite especially had the highest stability. An attempt to accelerate the reaction by elevating the temperature to 50 °C failed because of the unexpected oxidation of the phosphorus atom during the deprotection of the 6-*N*-benzoyldeoxyadenosine 3′-phosphoramidite derivative, as shown in Scheme 2. The phosphoramidate compound was isolated in 30% yield.

Scheme 2. Deacylation and Oxidation of *N*-Acylated Phosphoramidite Derivatives



Although the increase of the NH_3 concentration could accelerate the deprotection of 2-*N*-isobutyryldeoxyguanosine, the yield was still low, as shown in entry 7.

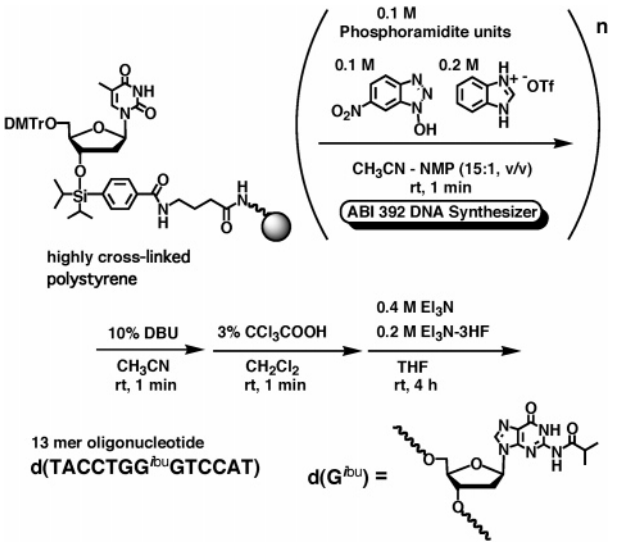
To improve the yield of the protocols by use of 4-*N*-benzoyldeoxycytidine, 6-*N*-benzoyldeoxyadenosine, and 2-*N*-isobutyryldeoxyguanosine, we tried to enhance the nucleophilicity of NH_3 by replacement of MeOH as a protic solvent to THF as an aprotic solvent. The deprotection of 6-*N*-benzoyl dA was initially treated with 2 M $MeNH_2$/THF. The reaction required only 2 h and the yield was 89%, as shown in entry 8. In 2 M $MeNH_2$/THF, the side reactions such as hydrolysis could not be observed.

The yield and reaction rate in deprotection of 4-*N*-benzoyldeoxycytidine also increased by use of 2 M $MeNH_2$/THF compared with that of 2 M NH_3/MeOH, as shown in entry 9. Similarly, the deprotection of 2-*N*-isobutyryldeoxyguanosine was improved (entry 10).

To confirm the effectiveness of the aprotic conditions, we also examined the deacylation under aqueous conditions by use of 28% NH_3(aq)−pyridine (1:1, v/v) instead of NH_3−MeOH. Under these conditions, the deprotection of 6-*N*-benzoyldeoxyadenosine became rather slow (data not shown), and the corresponding hydrolyzed compound, deoxyadenosine 3′-*H*-phosphonate derivative, was generated as the most significant side product.

A modified oligonucleotide, d[TACCTGG^{ibu}GTCCAT] incorporating a 2-*N*-isobutyrylguanine (G^{ibu}) as a base-labile functional group was synthesized by using these *N*-free phosphoramidite units to examine the chemical reactivity of these reagents, as shown in Scheme 3. Elongation of the

Scheme 3. Synthesis of Alkaline-Labile Oligonucleotide Having 2-*N*-Isobutyrylguanine



oligomer was carried out on highly cross-linked polystyrene[9] beads by use of 0.2 M benzimidazolium triflate (BIT)[10] and 1-hydroxy-6-nitrobenzotriazole[11] as a second activator. The silyl-type linker[5a,12] that could be cleaved under nearly neutral conditions such as $Et_3N{\cdot}3HF$ was used to anchor the oligonucleotide to the resin. The manipulations of detritylation, coupling, and oxidation were carried out on an automated DNA synthesizer. The capping reaction step was omitted to avoid the undesired acylation of the amino groups of the nucleobases. After elongation, all the 2-cyanoethyl groups on the phosphate groups were cleaved by treatment with 10% DBU in CH_3CN for 1 min. Subsequently, the DMTr group of the terminal 5′-hydroxyl group was removed by the acid treatment using CCl_3COOH. The 13mer oligonucleotide was released from the resin by treatment with 0.2 M $Et_3N{\cdot}3HF$ and 0.4 M Et_3N for 4 h and analyzed by HPLC. Figure 1 shows both the excellent *O*-selectivity and the high coupling efficiency. The modified 13mer was isolated in 39% yield. This oligomer was characterized by MALDI-TOF mass spectroscopy. These results showed that *N*-free phosphoramidite units prepared by the deprotection of *N*-acylated phosphoramidite units had high enough reactivity to synthesize oligonucleotides in the *N*-unprotected phosphoramidite method which was identical to that of the same *N*-unprotected phosphoramidite units prepared according to the previous methods.[5a]

Interestingly, it was also found that the phosphoramidite skeleton was sufficiently stable under the conditions for acylation of the unprotected bases. For example, the 4-amino

(9) McCollum, C.; Andrus, A. *Tetrahedron Lett.* **1991**, *32*, 4069−4072.
(10) Hayakawa, Y.; Kataoka, M.: Noyori, R. *J. Org. Chem.* **1996**, *61*, 7996−7997.
(11) (a) Marugg, J. E.; van der Bergh, C.; Tromp, M.; van der Marel, G. A.; van Zoest, W. J.; van Boom, J. H. *Nucleic Acids Res.* **1984**, *12*, 9095−9110. (b) de Vroom, E.; Fidder, A.; Marugg, J. E.; van der Marel, G. A.; van Boom, J. H. *Nucleic Acids Res.* **1986**, *14*, 5885−5900.
(12) Kobori, A.; Miyata, K.; Ushioda, M.; Seio, K.; Sekine, M. *Chem. Lett.* **2002**, 16−17.

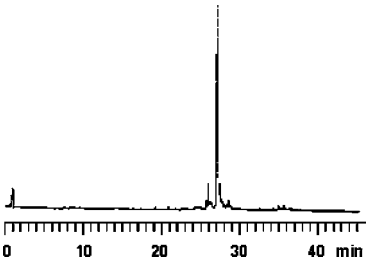
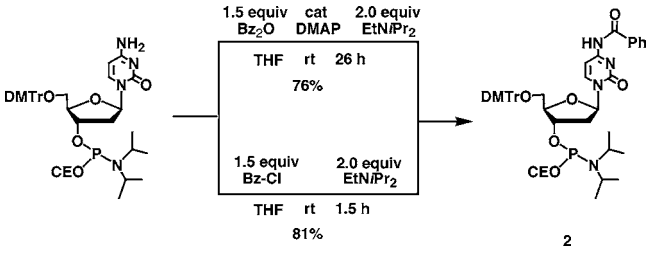


Figure 1. Anion-exchange HPLC of the crude mixture obtained in the synthesis of d[TACCTGG^{ibu}GTCCAT].

group of the *N*-unprotected deoxycytidine 3′-phosphoramidite derivative could be benzoylated by treatment with either Bz_2O (81%) or benzoyl chloride (76%) without significant side reactions, as shown in Scheme 4. In addition, the

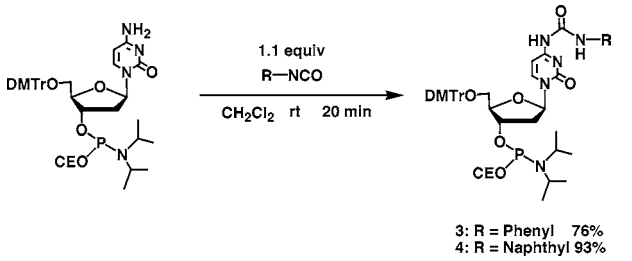
Scheme 4. Introduction of a Benzoyl Group into the *N*-Unprotected Deoxycytidine 3′-Phosphoramidite Derivative



reactions of the amino group with phenyl isocyanate and naphthyl isocyanate could be carried out successfully, as shown in Scheme 5. It was reported that such *N*-arylcarbamoylated nucleobases can serve as base pair mimics in DNA duplexes.[13] These results suggest that the phosphoramidite structure is tolerant under both the deacylation and reacylation conditions.

(13) (a) Miyata, K.; Tamamnushi, R.; Ohkubo, A.; Taguchi, H.; Seio, K.; Sekine, M. *Tetrahedron. Lett.* **2004**, *45*, 9365−9368. (b)Nakano, S.; Uotani, Y.; Nakashima, S.; Anno, Y.; Fujii, M.; Sugimoto, N. *J. Am. Chem. Soc.* **2003**, *125*, 8086−8087.

Scheme 5. Introduction of an *N*-Arylcarbamoyl Group into the *N*-Unprotected Deoxycytidine 3′-Phosphoramidite Derivative



3: R = Phenyl 76%
4: R = Naphthyl 93%

In summary, we have developed convenient and efficient alternative approaches to *N*-unprotected phosphoramidite units by the deprotection of *N*-acylated phosphoramide units using 2 M $MeNH_2$/THF. By use of this method, the synthesis of *N*-unprotected phosphoramidite units might be carried out in large-scale in the future.

It should be also emphasized that the present two-step strategy involving the *N*-deprotection and the reacylation of the *N*-acylated phosphoramidite units provide a new tool to obtain the desired functionalized oligonucleotides by using *N*-acylated deoxynucleoside 3′-phosphoramidite derivatives that are commercially available or are abundant in the laboratory. This process would be used for more specific purposes such as *N*-unprotected DNA synthesis and DNA modifications, and such protocols will be practically used for the facile synthesis of oligonucleotides having DNA lesions, DNA probes for DNA microarray and modified oligonucleotides having useful functional groups such as fluorescent dyes at the amino groups.

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Supporting Information Available: Experimental procedures and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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