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USE OF THE HEXAFLUORO-2-BUTYL PROTECTING GROUP IN THE SYNTHESIS OF DNA FRAGMENTS VIA THE PHOSPHORAMIDITE APPROACH ON SOLID SUPPORTS†

Sung-Gug Kim, Kouichi Eida, and Hiroshi Takaku

Department of Industrial Chemistry, Chiba Institute of Technology, Tsudanuma, Narashino, Chiba 275, Japan

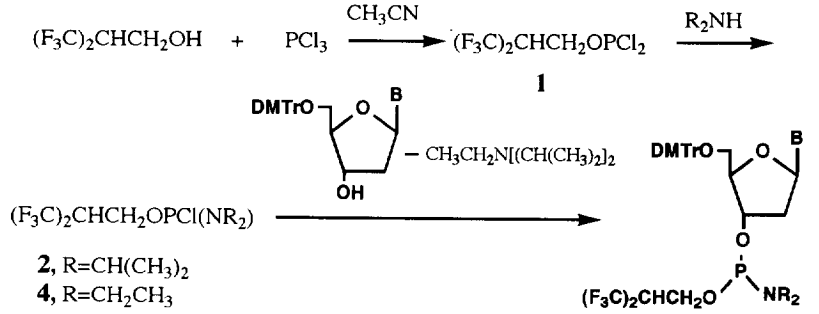
Summary: The hexafluoro-2-butyl (HFB) group is a new protecting group for internucleotidic bonds in the synthesis of oligodeoxyribonucleotides by the phosphoramidite approach. This group can be removed rapidly under the same condition as the 2-cyanoethyl group. Furthermore, we have found that the phosphitylating agent, hexafluoro-2-butyl-N.N-dialkylaminochlorophosphine, is easy to prepare in terms of its stability during purification by distillation, which does not require high vacuum.

The development of phosphoramidite[1] and H-phosphonate[2] approaches has enabled the rapid chemical synthesis of oligo- and poly-deoxyribonucleotides on solid supports. The phosphoramidite approach has been especially accessible and more successful in its application to molecular biology. However, pure 2-cyanoethyl-N,N-dialkylaminochlorophosphines are, in general, difficult to obtain because of their thermal decomposition during purification by distillation under very high vacuum.[3]

In order to overcome this problem, we describe two developments: the phosphitilating agent, hexafluoro-2-butyl-N,N-dialkylaminochlorophosphine can be prepared in good yield by distillation under low vacuum, and the hexafluoro-2-butyl (HFB) protecting group of the phosphate can be removed rapidly from the internucleotidic bonds by ammonia treatment for the synthesis of oligodeoxyribonucleotides by the phosphoramidite approach.

First, we examined the preparation of the phosphitylating agent (**2**) shown in Scheme 1. To a dry CH_3CN solution (30 ml) of PCl_3 (20.7 ml, 237 mmol) was added hexafluoro-2-butanol (6.3 g, 35 mmol) in dry CH_3CN (11 ml) at -40°C under nitrogen atmosphere. The mixture was gradually warmed to room temperature and stirred for an additional 13 h. The mixture was filtered and concentrated *in vacuo*. The residue was fractionally distilled to yield 7.9 g (85%) of hexafluoro-2-butoxydichlorophosphine (**1**): bp 45°C/25 mmHg; ^{31}P-NMR ($CDCl_3$, 85% H_3PO_4) 178.1 ppm. The compound **1** (3.3 g, 12 mmol) was treated with diisopropylamine (3.5 ml, 25 mmol) in dry ethyl ether (30 ml) at -78°C under a nitrogen atmosphere for 2 h. The mixture was gradually warmed to room temperature and was stirred for an additional 24 h. The mixture was filtered and concentrated *in vacuo*. The residue was fractionally distilled to give 3.4 g (83%) of **2**: bp 87°C/1.5 mmHg; ^{31}P-NMR ($CDCl_3$, 85% H_3PO_4) 177.9 ppm. This result suggests that the phosphitiylating agent, **2**, can be prepared in good yield without thermal decomposition during purification by distillation under low vacuum.

The procedure for the preparation of the phosphoramidite units is outlined in Scheme 1. The reaction of



3a, R=CH(CH$_3$)$_2$,B=T **5a**, R=CH$_2$CH$_3$,B=T
3b, R=CH(CH$_3$)$_2$, B=bzA **5b**, R=CH$_2$CH$_3$, B=bzA
3c, R=CH(CH$_3$)$_2$, B=ibuG **5c**, R=CH$_2$CH$_3$, B=ibuG
3d, R=CH(CH$_3$)$_2$,B=anC **5d**, R=CH$_2$CH$_3$,B=anC

Scheme 1

5'-O-dimethoxytrityl-N-protected nucleosides (1.0 molar equiv.) with **2** (1.5 molar equiv.) in the presence of diisopropylethylamine (3.0 molar equiv.) in a THF solution (5 ml/mmol) at room temperature under nitrogen gave the required phosphoramidite units **3a-d** in good yields.[4)]

Next, we examined the solid phase synthesis of DNA template (5'TAATTACGACTCACTATAGAAGATC-TGGAGGTCCTGTGTTCGATCCACAGAATTCGCACCA3') 60 mer, containing the T7 promoter sequence for the preparation of the half *t*-RNA^{Phe} [5)] using T7 RNA polymerase under the standard coupling protocols employed in automated nucleic acid synthesis techniques (Applied Biosystems DNA/RNA synthesizer, model 392). Solid phase synthesis was performed using a synthesis system on a 0.2 μM scale controlled-pore glass (CPG) support with the phosphoramidite units **3a-d**. After the synthetic cycles were completed, the CPG support was treated with conc. ammonia at 55°C for 5 h, which simultaneously cleaved the oligomer from the support and removed the base- and phosphate-protecting groups. The DNA solution was decanted from the CPG support and the solvent was removed *in vacuo*. The residue was dissolved in aqueous sodium acetate (pH 5.5) and the crude DNA was isolated by precipitation with ethanol. The length and purity of the crude DNA were analyzed by polyacrylamide gel electrophoresis (PAGE). However, when the phosphoramidite unit (**3**) was used to synthesize the template DNA 60 mer, we did not observe the expected template DNA 60 mer. Due to the HFB group on the phosphorus, the stepwise coupling efficiencies were <80%, thereby limiting the utility of this method to the preparation of relatively short sequences (e.g., ~20 mer).

There are studies of the improvement of coupling efficiencies by activation with a mixture of (dimethylamino)pyridine and tetrazole[6)] as well as with 4-nitrophenyltetrazole[7)]. Indeed, the synthesis of longer oligodeoxyribonucleotides requires the use of highly reactive phosphoramidite units, such as nucleoside 2-cyanoethyl-N,N-diethylamino-phosphoramidites.[8)] However, these reagents are not as stable as the corresponding N,N-diisopropylamino-phosphoramidite. Wishing to retain the desirable characteristics of the HFB substituent, while at the same time increasing the reactivity of the phosphoramidite, we reasoned that reducing the steric bulk of the nitrogen substituents would have the desired effect. We have therefore examined

the application of hexafluoro-2-butyl-N,N-diethylamino-phosphoramidites to the synthesis of longer oligonucleotides. The phosphitilating reagent (**4**) (87%, bp 89°C/1.5 mmHg; ^{31}P-NMR ($CDCl_3$, 85% H_3PO_4) 175.9 ppm), prepared by the procedure used the for the preparation of N,N-diispropylaminochlorophosphine **2**, can be obtained with high purity by simple vacuum distillation. As expected, **4** reacted with the 5'-O-dimethoxytrityl-N-protected nucleosides faster than **2** (3 times more rapidly), and gave good yields of the desired nucleoside phosphoramidite units (**5a-d**).[9] The phosphoramidite units **5** were compared with those of the corresponding 2-cyanoethyl-N,N-diisopropylamino-phosphoramidite units, which are commercially available. Solid phase synthesis was performed using a synthetic system on a 0.2 μM scale CPG support. After the synthetic cycles were completed, the CPG support was subjected to a deblocking procedure that led to the release of the synthetic oligonucleotides from the CPG support and to its deblocking. The length and purity of the crude DNA was analyzed by polyacrylamide gel electrophoresis (PAGE). The PAGE analysis (Figure 1) showed that the 60 mer made with the hexafluoro-2-butyl-N,N-diethylamino-phosphoramidites was slightly superior to that made with the 2-cyanoethyl-N,N-diisopropylamino-phosphoramidites. These reagents are as stable as the corresponding 2-cyanoethyl-N,N-diisopropylamino-phosphoramidites and very attractive for routine use.

1 2 3

Fig. 1. Analytical denaturing PAGE gel of the DNA template 60 mer containing the T7 promoter sequence for the preparation of the half *t*-RNA^{Phe}. The DNA was electrophoresed on a 15% gel run in 90mM TEAB buffer (pH 8.3) at 400V. Lane 1: crude fully deprotected DNA 60 mer with N,N-diisopropyllamino-phosphoramidites. Lane 2: crude fully deprotected the DNA 60 mer with N,N-diethylamino-phosphoramidites. Lane 3: the DNA 60 mer with N,N-diethylamino-phosphoramidites after partial purification by an OPC reverse phase cartridge.

It is concluded that a new phosphitilating reagent **4** is obtained easily and in good yield by distillation under lower vacuum than that required for 2-cyanoethyl-N,N-diisopropylchlorophosphine. This reagent can be used for the preparation of nucleoside phosphoramidite units as key intermediates in the synthesis of longer oligo-

nucleotides by the phosphoramidite approach on a solid support. Furthermore, the hexafluoro-2-butyl group can removed easily from the internucleotidic bonds via a ß-elimination mechanism under the same condition as the 2-cyanoethyl group.

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REFERENCES AND FOOTNOTES

†This article is dedicated to professor Tsujiaki Hata on the occasion of his sixtieth birthday.

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