# *N*-Trifluoroacetylamino Alcohols as Phosphodiester Protecting Groups in the Synthesis of Oligodeoxyribonucleotides

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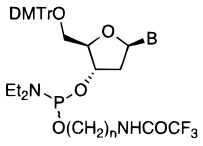
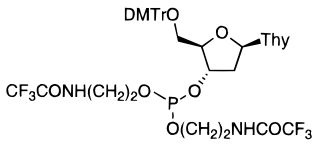
*Received June 2, 1997*

The functionalization of oligonucleotides with specific labels has, over the years, considerably increased our knowledge of nucleic acid structure and function, and led to an increased interest in the potential diagnostic and therapeutic applications of these modified oligonucleotides. Aminoalkyl groups have been particularly useful for the functionalization of oligonucleotides with a variety of reporter groups, intercalators, cross-linkers, and DNA/RNA cleaving agents.[2] While 5′-aminoalkyl phosphodiester functions of oligonucleotides are relatively stable, little is known about the properties of oligonucleotidic aminoalkyl phosphotriesters. We have recently discovered that after standard detritylation of the solid phase-linked dinucleoside phosphotriester **5** (Scheme 1), treatment with concentrated ammonium hydroxide produced the dinucleotide **12** in near quantitative yields.[3] The expected aminoalkylated dinucleoside phosphotriester **9** could not be isolated. These unexpected findings prompted us to investigate the applicability of *N*-trifluoroacetylamino alcohols as phosphodiester protecting groups to the synthesis of oligodeoxyribonucleotides. We now report the synthesis of the novel deoxyribonucleoside phosphoramidites **1**−**3** and their application to the solid phase synthesis of model oligonucleotides. The mechanism and kinetics of conversion of aminoalkylated phosphotriesters to phosphodiester functions will also be discussed.



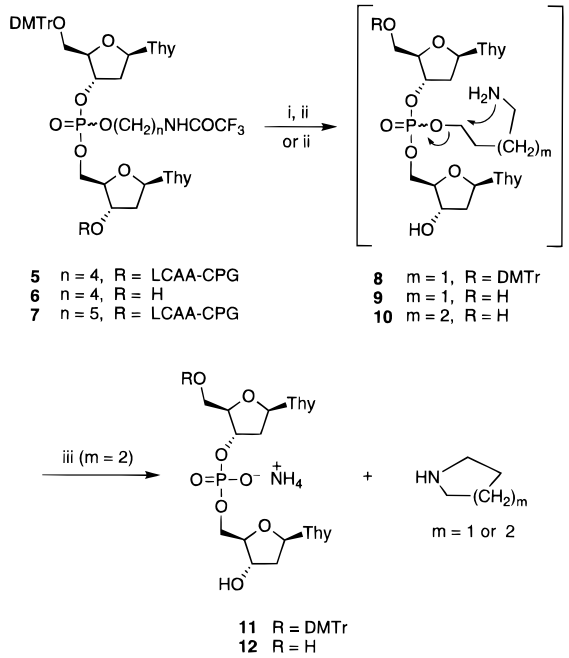

**1** n = 2, B = Thy
**2a** n = 4, B = Thy
**2b** n = 4, B = bzCyt
**3** n = 5, B = Thy




**4**

Typically, 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxythymidine or 5′-*O*-(4,4′-dimethoxytrityl)-*N*[4]-benzoyl-2′-deoxycytidine (1.0 mmol) is reacted with hexaethylphosphorus triamide[4] (1.0 mmol) and the *N*,*N*-diethylammonium salt of 1*H*-tetrazole (1.0 mmol) in methylene chloride (5 mL) for 30 min at 25 °C. Without isolation of the nucleoside 3′-*O*-phosphordiamidite that is generated, an equimolar amount of the desired *N*-trifluoroacetylamino alcohol is added to the reaction mixture and stirred for 6 h at ambient temperature. This one-pot synthesis produced the phosphoramidites, **2a** or **2b**, and **3** in yields exceeding 90%. However, the preparation of thymidine phosphoramidite **1** under these conditions is, unlike that of **2a** and **3**, less efficient (*ca*. 50% yield) due to the concomitant formation of the symmetrical phosphite **4**. The purified deoxyribonucleoside phosphoramidites **1**−**3** were characterized by $^1$H- and $^{31}$P-NMR spectroscopy.[5] Each phosphoramidite has been employed in the solid phase synthesis of a dinucleotide and an octadecanucleotide. Under standard synthesis conditions, the coupling efficiencies of the phosphoramidites **2a**, **2b**, or **3** are similar (99%) to that of commercial 2-cyanoethyl deoxyribonucleoside phosphoramidites. Much like these commonly used phosphoramidites, **2a**, **2b**, and **3** retained their coupling efficiencies even after being left in acetonitrile solutions for at least one week. Surprisingly, the coupling efficiency of the phosphoramidite **1** is considerably lower (*ca*. 85%) than that of **2a** or **3** under similar conditions and, hence, unsuitable for the synthesis of large oligonucleotides. $^{31}$P-NMR analysis of the activation of **1** with 1*H*-tetrazole revealed the rapid formation of an, as yet, unidentified side product and a commensurately rapid decrease in the concentration of **1**. Less than optimal concentration of **1** may be responsible for its poor coupling efficiency.

**Scheme 1**[a,b]




[a] Conditions: (i) 3% TCA/$CH_2Cl_2$, 25 °C, 5 min; (ii) concd $NH_4OH$, 25 °C, 2 h; (iii) concd $NH_4OH$, 56 °C, 2 h. [b] Key: DMTr, 4,4′-dimethoxytrityl; Thy, thymin-1-yl; LCAA-CPG, long chain alkylamine controlled-pore glass; TCA, trichloroacetic acid.

The CPG-linked dimers **5** and **7** (Scheme 1) were detritylated and then released from their respective support by treatment with concd $NH_4OH$ for 2 h at 25 °C. Reversed-phase (RP) HPLC analysis of the deprotected dimers indicated that **5** was exclusively converted

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(2) For a comprehensive review on the subject, see: Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 1925−1963.

(3) In agreement with our findings is the decomposition of *H*-phosphonate diesters bearing 4-aminobutyl groups to *H*-phosphonate monoesters; see Sobkowski, M; Stawinski, J.; Sobkowska, A.; Kraszewski. A. *J. Chem. Soc., Perkin Trans. I* **1994**, 1803−1808.

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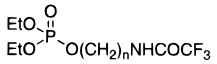
(5) Proton-decoupled $^{31}$P-NMR (121 MHz, $CDCl_3$): **1**, 147.3 and 146.5 ppm; **2a**, 145.9 and 145.7 ppm; **2b**, 145.4 and 144.7 ppm; **3**, 146.0 and 145.8 ppm. $^1$H-NMR spectra are provided as Supporting Information.

S0022-3263(97)00962-6 CCC: $14.00 

to thymidylyl-(3′→5′)-thymidine (TpT) **12** which was identical to TpT prepared by conventional 2-cyanoethyl phosphoramidite chemistry. The deprotection kinetics of the (*N*-trifluoroacetylamino)butyl phosphate protecting group from the dimer **6** has been evaluated by $^{31}$P-NMR spectroscopy.[6] The NMR data are consistent with an initial rate-limiting cleavage of the *N*-trifluoroacetyl group, followed by a rapid cyclodeesterification of the intermediate **8** to produce pyrrolidine and the dinucleoside phosphodiester **11**. The cyclodeesterification step proceeded so readily that only a very small accumulation of **8** was detected during the first 30 min of the deprotection reaction. Complete conversion of **6** to the dinucleotide **11** occurred within 2 h at 25 °C.[6] Interestingly, deprotection of the dimer **7** under the conditions used for **5** gave the dithymidine (5-aminopentyl) phosphate triester **10** as a major product (*ca*. 90%). Complete deprotection of the dinucleotide **7** to TpT required treatment with concd $NH_4OH$ at 56 °C for 2 h. No evidence of internucleotidic phosphodiester cleavage was detected under the conditions used for the deprotection of either **5** or **7**. Phosphoramidites **2a**, **2b**, and **3** have also been applied to the solid phase synthesis of octadecathymidylic ($dT_{18}$) and octadecadeoxycytidylic ($dC_{18}$) acids using standard protocols. These polynucleotides were cleaved from their respective CPG support upon treatment with concd $NH_4OH$ for 2 h at 25 °C. The oligodeoxycytidylate was further treated with concd $NH_4OH$ at 55 °C (10 h) to ensure complete nucleobase deprotection. The overall synthetic quality and recovery of $dT_{18}$ and $dC_{18}$ were comparable to that obtained by the use of 2-cyanoethyl phosphoramidites.[6] These crude oligonucleotides were completely hydrolyzed by snake venom phosphodiesterase (SVP) and bacterial alkaline phosphatase (BAP) to their corresponding nucleosides. Neither thymine or cytosine modification nor incomplete deprotection of phosphotriester functions were detected by RP-HPLC analysis of the hydrolysates.[6] These results also suggest that conventional nucleobase protecting groups do not interfere with deprotection of the (*N*-trifluoroacetylamino)butyl phosphotriester function.

In an effort to detect the formation of pyrrolidine or piperidine during the deprotection of (*N*-trifluoroacetylamino)butyl- or (*N*-trifluoroacetylamino)pentyl phosphate protecting groups, the non-nucleosidic phosphotriesters **13** and **14** were prepared, and each treated with deuterium oxide saturated with ammonia until complete cyclodeesterification to *O*,*O*-diethyl phosphoric acid diester was observed by $^{31}$P-NMR spectroscopy. The generation of pyrrolidine and piperidine during the deprotection of **13** and **14**, respectively, was unambiguously confirmed by $^{1}$H-NMR spectroscopy.[6] These findings strongly support the cyclodeesterification mechanism depicted in Scheme 1 and are consistent with related literature data.[7−10]



**13** n = 4
**14** n = 5

The (*N*-trifluoroacetylamino)butyl and (*N*-trifluoroacetylamino)pentyl phosphate protecting groups reported herein can serve as alternatives to the traditional 2-cyanoethyl phosphate protecting group in the solid-phase synthesis of oligonucleotides and may be superior in specific circumstances. Since the (*N*-trifluoroacetylamino)butyl group can be removed by concd $NH_4OH$ at ambient temperature, this phosphate protecting group could be as effective as the 2-cyanoethyl group in the synthesis of oligonucleotides carrying base-sensitive nucleobases. In addition, the (*N*-trifluoroacetylamino)butyl or the (*N*-trifluoroacetylamino)pentyl phosphate protecting group may be useful in the synthesis of oligoribonucleotides when the 5′-hydroxy function is protected with groups such as the (fluoren-9-ylmethoxy)carbonyl (Fmoc)[11] or the (2-dansylethoxy)carbonyl (Dnseoc)[12] group. The stepwise removal of these groups is typically carried out under non-nucleophilic basic conditions (0.1 M DBU in MeCN) which are more compatible with the (*N*-trifluoroacetylamino)alkyl phosphate protecting groups than the 2-cyanoethyl group. Another potential limitation of the 2-cyanoethyl group for phosphate protection is the release of acrylonitrile during its deprotection. Depending on the base used, addition of acrylonitrile to thymine/uracil at N-3 has been well-documented.[13] By comparison, the release of pyrrolidine or piperidine during the deprotection of the (*N*-trifluoroacetylamino)butyl or (*N*-trifluoroacetylamino)pentyl phosphate protecting group is quite innocuous.

The strategy we have outlined for the protection of phosphodiesters is not limited to the use of the trifluoroacetyl group for masking the amino function of the 4-aminobutyl and 5-aminopentyl groups. Other groups requiring very unique deprotection conditions may conceivably be employed. In this context, the Fmoc[14] and the (benzyloxy)carbonyl[7] groups have been applied to the protection of an aminoethyl phosphotriester function. Thus, the application of *N*-protected 4-aminobutyl or *N*-protected 5-aminopentyl groups to phosphate protection in oligonucleotide synthesis may potentially provide a much greater versatility in deprotection conditions than currently possible with the conventional 2-cyanoethyl group.

The synthetic approach to the phosphoramidites **2a**, **2b**, and **3** is being extended to the other nucleobases, and *N*-protecting groups other than trifluoroacetyl for the 4-aminobutyl function will be tested in the solid phase synthesis of oligonucleotides. Results of these findings will be reported in due course.

**Supporting Information Available:** NMR spectra of **1**−**3**, **13**, and **14**; mechanistic study of phosphate deprotection using **13** and **14** as models; comparative polyacrylamide gel electrophoresis of $dT_{18}$ that has been prepared from **2a**, **3**, and the parent 2-cyanoethyl phosphoramidite; and HPLC analysis of SVP and BAP hydrolysates of unpurified $dT_{18}$ and $dC_{18}$ (13 pages).

JO970962X

(6) Data shown as Supporting Information.
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