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Commercially Available 5′-DMT Phosphoramidites as Reagents for the Synthesis of Vinylphosphonate-Linked Oligonucleic Acids

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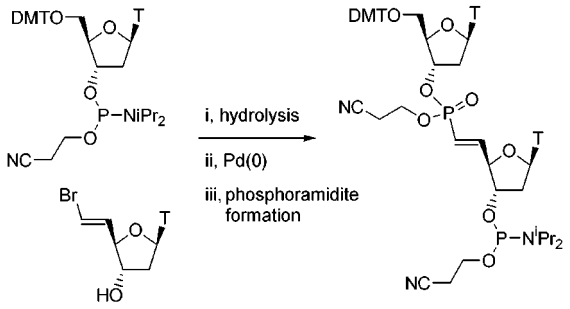
ABSTRACT



Commercially available cyanoethyl phosphoramidites derived from T, d(C), d(A), and d(G) were hydrolyzed (1*H*-tetrazole, MeCN/H_2O) to give the corresponding *H*-phosphonates in excellent yields. Palladium(0)-catalyzed cross-coupling of each of these with the thymidine-derived vinylbromide 2 afforded the corresponding vinylphosphonate-linked dimers T*T, d(C)*T, d(A)*T, and d(G)*T in modest to good yields. The T*T dimer was further elaborated to give a 5′-DMT-T*T-3′-CEP building block, and this was used in the automated synthesis of the TpT*TpT tetramer.

The ability of exogenous synthetic oligonucleotides to modulate the function of specific genes in vitro and in vivo has stimulated a considerable amount of research into their use as therapeutic agents and diagnostic tools.[1] A large amount of this work has focused upon chemical modification of the internucleotide linkage in order to address problems associated with nuclease degradation of the naturally occurring phosphodiester moiety. Recently we reported a method for the synthesis of modified T*T and d(A)*T dimers containing a hydrolytically stable vinylphosphonate internucleotide linkage **3**. The key step utilizes a palladium(0)-catalyzed coupling reaction between the thymidine-derived 1-bromo-1-alkene **2** and nucleotide-derived *H*-phosphonates (e.g., **1**) to yield the corresponding dimers **3** in moderate to high yield (Scheme 1).[2] We found that under our optimized conditions ($Pd(OAc)_2$, dppf, propylene oxide,[3] THF) a range of functional groups were tolerated, including the base-labile cyanoethyl group. We now wish to report further results in this area, which have led to the synthesis of building blocks suitable for oligonucleotide construction. Much of our early development work was performed on materials possessing silicon-based protecting groups on the 5′- and 3′-hydroxyls, but we were aware of the need to manipulate these termini into functionality that would be more compatible with

(1) (a) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543. (b) Szymkowski, D. E. *Drug Discovery Today* **1996**, *1*, 415. (c) Englisch, U.; Gauss, D. H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 613. (d) Uhlmann, E.; Peyman, A.; Breipohl, G.; Will, D. W. *Angew. Chem., Int. Ed.* **1998**, *37*, 2797.

(2) (a) Abbas, S.; Hayes, C. J. *Tetrahedron Lett.* **2000**, *41*, 4513. (b) Abbas, S.; Hayes, C. J., *Synlett* **1999**, 1124. For an alternative route to vinylphosphonate-linked nucleic acids, see also: (c) Zhao, Z.; Caruthers, M. H. *Tetrahedron Lett.* **1996**, *37*, 6239.

(3) Lei, H.; Stoakes, M. S.; Schwabacher, A. W. *Synthesis* **1992**, 1255.

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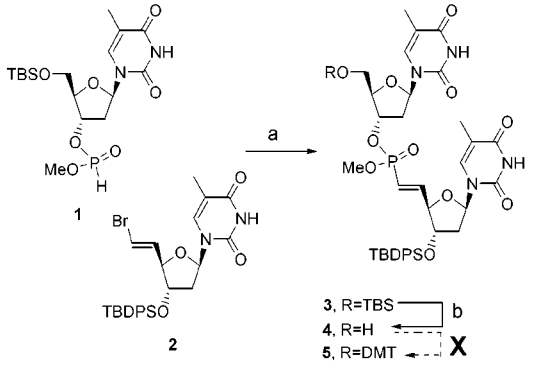
Scheme 1[a]



[a] Reagents: (a) $Pd(OAc)_2$, propylene oxide, THF, 70 °C (78%); (b) HF (48% aq), MeCN, rt (73%).

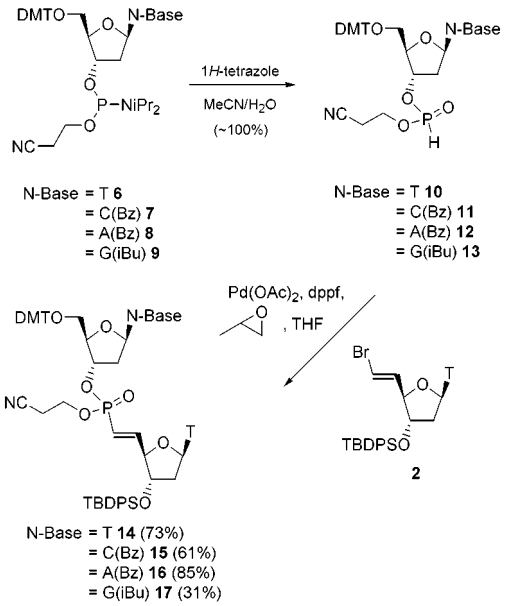
oligonucleotide synthesis (i.e., 5′-DMT and 3′-phosphoramidite). To this end, we quickly found that the 5′-TBS group could be removed cleanly from **3** using HF (48% aqueous) in MeCN, thus revealing the free 5′-hydroxyl group **4**. We hoped to protect **4** as the corresponding 5′-DMT derivative **5**, but after much effort we were unable to find suitable conditions to achieve this transformation (Scheme 1). Initially we found this result very surprising, especially since very similar protections have been reported in the literature, but further experimentation showed the 5′-hydroxyl of **4** to be resistant to reaction with a range of other electrophiles.[4] The reason for this lack of reactivity was not particularly clear, but comparison to successful DMT-protections on analogous substrates seemed to indicate that the 3′-TBDPS group was somehow responsible.

To overcome the frustration of these initial results, we decided to examine a much more direct solution to the "5′-DMT problem". We wondered whether *H*-phosphonates that already contained the 5′-DMT moiety could be used as coupling partners in the cross-coupling reaction, as this would avoid the troublesome protecting group swap. This was particularly attractive as the corresponding cyanoethyl-protected phosphoramidite precursors were commercially available, and the use of these materials would remove two unnecessary steps from the synthetic sequence. We were pleased to find that hydrolysis (1*H*-tetrazole, $MeCN/H_2O$) of each of the four commercially available[5] 5′-DMT-cyanoethyl phosphoramidites derived from thymidine **6**, 2′-deoxycytidine **7**, 2′-deoxyadenosine **8**, and 2′-deoxyguanosine **9**, provided the corresponding *H*-phosphonates **10**, **11**, **12**, and **13**, respectively, in uniformly high yield (~100%). As a result of the efficiency of this hydrolysis reaction, purification of the *H*-phosphonates was not necessary and these materials could be used in crude form for the subsequent cross-coupling reactions (Scheme 2).[6]

(4) Caruthers and Zhao (ref 2c) have reported the 5′ -DMT protection of a vinylphosphonate-linked nucleic acid. Other attempts were made to acylate (Ac_2O or AcCl, pyridine, DMAP) and TBS protect (TBS-Cl, imidazole, DMF, and TBS-OTf, $^iPr_2NEt$, CH_2Cl_2) the 5′-OH, but these efforts were unsuccessful.

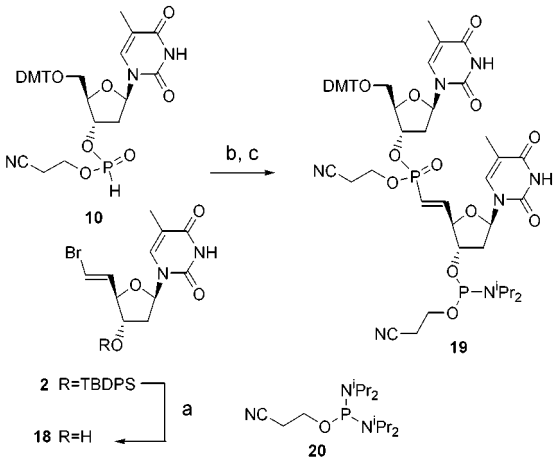
(5) Materials purchased from Cruachem Ltd. and used without further purification.

Scheme 2



Coupling of each of these *H*-phosphonates (1.4 equiv) with the thymidine-derived vinyl bromide **2**, using our previously developed conditions ($Pd(OAc)_2$ (0.2 equiv), dppf (0.4 equiv), propylene oxide (10 equiv), THF), provided the corresponding vinylphosphonate-linked dimers (T*T **14**, d(C)*T **15**, d(A)*T **16**, and d(G)*T **17**)[7] in modest to good yields (Scheme 2). The all pyrimidine-containing dimers **14**

Scheme 3[a]



[a] Reagents: (a) TBAF, THF (99%); (b) $Pd(OAc)_2$ (0.2 equiv), dppf (0.4 equiv), propylene oxide (10 equiv), THF, 70 °C (59%); (c) 1*H*-tetrazole, **20**, CH_2Cl_2 (70%).

and **15** were produced in very pleasing 73% and 61% yields, respectively. Gratifyingly, coupling of the adenosine-derived *H*-phosphonate **12** with **2** afforded the mixed purine-pyrimidine vinylphosphonate-linked dimer **16** in an excellent 85% yield. As we anticipated at the outset of this work, the

coupling of the 2′-deoxyguanosine-derived *H*-phosphonate **13** proved to be the most challenging. After a reaction time of 7 h we were able to isolate 31% (67% based on recovered **2**) of the desired coupled product **17**. We are currently examining the use of alternative catalyst systems and protecting group strategies in order to improve this 2′-deoxyguanosine coupling, and these results will be reported in due course. Having demonstrated that the 5′-DMT *H*-phosphonates could be utilized as coupling partners in palladium(0)-catalyzed P−C couplings, we were anxious to see whether the resulting dimers could be used as building blocks in oligonucleic acid synthesis as originally intended. We therefore decided to examine the construction of the suitably functionalized 5′-DMT-T*T-3′-CEP building block **19**.[8] In anticipation of encountering problems with removal

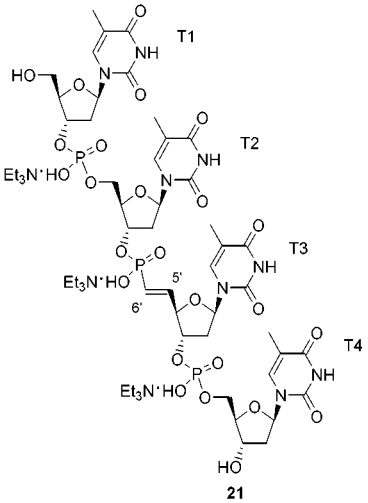


Figure 1.

of the 3′-TBDPS protecting group from the acid- and base-sensitive dimer **14**, we decided to deprotect the vinyl bromide **2** and couple the resulting free alcohol **18** with the *H*-phosphonate **10** (Scheme 3). Thus, treatment of **2** with TBAF in THF cleanly gave the desired product **18** in excellent yield. Pleasingly, coupling of **18** and **10**, using our standard conditions (vide supra), provided the desired 3′-OH dimer in 59% isolated yield. Tetrazole-mediated reaction of this material with 2-cyanoethyl-tetraisopropylphosphoramidite **20**[9] cleanly afforded the desired phosphoramidite building block **19** as a 1:1:1:1 mixture of diastereoisomers.[10] With access to this material we were now in a position to examine the incorporation of the modified T*T dimer into an oligonucleic acid using standard solid-phase synthesis techniques.[11] As an illustration, a 10 μmol synthesis of the TpT*TpT tetramer **21** was performed (Figure 1). To ensure good incorporation of the dimer **19**, the coupling time for this step was extended from 60 to 180 s, but the rest of the automated synthesis remained as standard. Cleavage from the solid support and deprotection of the cyanoethyl protecting groups was achieved by treatment with NH_4OH,[12] and purification of the crude material by reverse phase HPLC provided the desired tetramer **21** as the major product.[13]

At the outset of this project we were concerned that the vinylphosphonate may not withstand the NH_4OH deprotection conditions, as both Michael additions and cleavage of the 3′−O−P phosphate bond are possible. The identity of **21** was firmly established by NMR spectroscopy, with the 1D ^{1}H NMR spectrum of **21** clearly showing all four H1′ protons and both of the olefinic (H5′ (6.56 ppm) and H6′ (6.11 ppm)) protons associated with the vinylphosphonate internucleotide linkage. The proton decoupled ^{31}P NMR spectrum of **21** shows three distinct peaks, two characteristic of phosphodiesters (δ 0.01 and 0.13 ppm) and one of the vinylphosphonate (δ 13.64 ppm).[14]

In summary, we have shown that commercially available phosphoramidites can serve as precursors to a range of vinylphosphonate-containing dimers. We have shown that both acid- and base-sensitive functional groups are tolerated in the palladium-catalyzed coupling reaction when propylene oxide is used as the HBr scavenger. Furthermore, we have shown that the T*T dimer **19** synthesized using this methodology can be used successfully in the automated synthesis of oligonucleic acids. Work is currently in progress to extend this work to the incorporation of modified purine-containing dimers and the synthesis of longer oligonucleic acid sequences of biological importance.

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

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(6) Purification by SiO_2 gel column chromatography lead to substantial decomposition of the *H*-phosphonates and provided only poor yields of the desired coupling partners.

(7) The asterisk (*) signifies the position of the vinylphosphonate internucleotide linkage.

(8) McBride, L. J.; Caruthers, M. H. *Tetrahedron Lett.* **1983**, *24*, 245.

(9) Bannwarth, W.; Trzeciak, A. *Helv. Chim. Acta* **1987**, *70*, 175.

(10) As judged by proton-decoupled ^{31}P NMR.

(11) Caruthers, M. H. *Science* **1985**, *230*, 281.

(12) Caruthers and Zhao have also reported the solid-phase synthesis of vinylphosphonate linked oligonucleic acids (see ref 2c). In their case, however, they used an *o*-chlorophenyl group for protection of the phosphonate internucleotide linkage and an extra synthetic step was needed for its deprotection.

(13) The only other product to be isolated from the automated synthesis was thymidine, which results from the incomplete coupling of the phosphoramidite **20** to the 5′-OH of the initial CPG-supported thymidine (corresponding to T4 in **22** (Figure 2)).

(14) For copies of relevant spectra see Supporting Information.