Nucleoside Oxazaphospholidines as Novel Synthons in Oligonucleotide Synthesis

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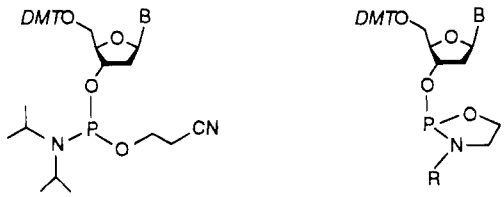
Since the introduction in 1981 of the nucleoside phosphoramidite synthons by Beaucage and Caruthers[1] and the subsequent refinement of this pioneering chemistry,[2] the synthesis of oligonucleotides for routine applications in molecular biology and in diagnostics, as well as for the large-scale manufacture of modified oligonucleotides[3] for antisense-based therapeutic applications,[4] has been greatly facilitated. In this context, the β-cyanoethyl phosphoramidite nucleoside phosphoramidite **1**[5] is the most widely used synthon. We considered the possibility



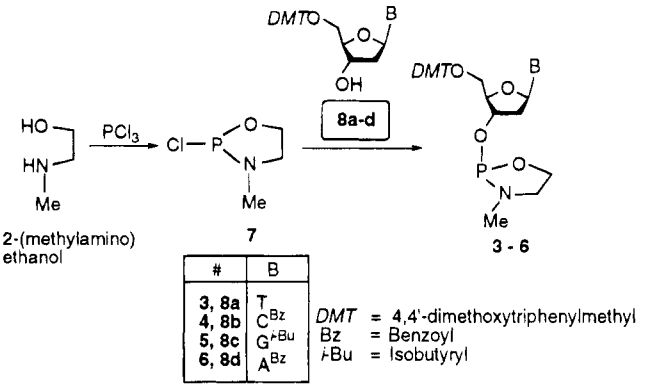
1 **2**

B = Thymin-1-yl (T)
= N^4-benzoylcytosin-1-yl (C^{bz})
= N^2-Isobutyrylguanin-9-yl ($G^{i\text{-}Bu}$)
= N^6-benzoyladenin-9-yl (A^{bz})

of using nucleoside oxazaphospholidines having the general structure **2**, readily derived from the corresponding β-amino alcohols, as alternative synthons. Our interest in nucleoside oxazaphospholidines was spurred by the availability of vast chiral pools of β-amino alcohols, the expectation being that the substituents in the chiral oxazaphospholidine ring can effect diastereofacial selectivity in internucleotidic coupling reactions. Our ongoing efforts in this latter area, as applied to the synthesis of oligonucleoside phosphorothioates, have been presented elsewhere in a preliminary paper.[6] In the present work, we demonstrate the generality in the use of simple nucleoside phosphoramidite synthons, such as 3-methyl-1,3,2-oxazaphospholidines **3**–**6**, in the synthesis of oligodeoxynucleotides.

The requisite oxazaphospholidines **3**–**6** are readily accessed by a two-step sequence as shown in Scheme 1. Thus, reaction of PCl_3 with 2-(methylamino)ethanol gave 2-chloro-3-methyl-1,3,2-oxazaphospholidine (**7**)[7] (^{31}P-NMR, δ = 167.8 ppm). Reaction of **7** with the corresponding 5′-*O*-[(4,4′-dimethoxytriphenyl)methyl (DMT)]-nucleosides **8a**–**d** gave the nucleoside oxazaphospholidines

Scheme 1



3–**6** as white foamy materials. The proton decoupled ^{31}P-NMR spectrum of each of the nucleoside oxazaphospholidines revealed two signals (almost equal integral ratio) at ca. δ 135–138 ppm corresponding to a pair of P-diastereomers. The high resolution FAB-MS of **3**–**6** gave the expected molecular ion peak, in each case, supporting the proposed structure.

Having obtained the nucleoside phosphoramidites **3**–**6** in preparative-scale reactions, the stage was set for their use in the solid-phase coupling reactions with the controlled-pore-glass (CPG)-bound[2a] nucleoside. Initially, we prepared the dimers TT (**9**), CT (**10**), GT (**11**), and AT (**12**) containing phosphoric diester linkages (Scheme 2). Thus, contacting a solution of each monomer nucleoside **3**–**6** with the support-bound T-nucleoside, in the presence of tetrazole, as the coupling reagent, followed by oxidation with iodine solution and "capping" (with acetic anhydride), gave the corresponding support-bound dimer products. The coupling efficiency, in each case, was greater than 98% as assessed by trityl assay. Following the synthesis, standard deprotection (28% NH_4OH, 55 °C, 12 h) was sufficient to cleave the dimer from the support, completely remove the base protecting groups, and remove the β-alkylamino appendage on the phosphate, to give the nucleoside dimers **9**–**12**, identical in all respects (evaluated by ^{31}P-NMR and reversed-phase HPLC) with authentic materials prepared from the corresponding cyanoethyl phosphoramidite synthons. Using a similar sequence of reactions, the dimers TT (**13**), CT (**14**), GT (**15**), and AT (**16**), containing phosphorothioate (Ps) linkages, were obtained, as a mixture of diastereomers (^{31}P-NMR and HPLC, Figure 1), by conducting the oxidative sulfurization of the phosphite **17** with 3*H*-benzodithiol-3-one 1,1-dioxide (**18**).[8] Similar synthesis of the Ps trimer T–T–T and analysis of the crude product by ion-exchange HPLC revealed that the desired trimer product had formed in greater than 95% yield (as revealed by integrated area under the peaks) corresponding to a stepwise coupling yield of greater than 98%.

The formation of the internucleotidic phosphite linkage in **17** (Scheme 2), anticipated from the coupling reaction, can be envisioned to occur by initial protonation of the oxazapholidines **3**–**6**, by tetrazole, followed by nucleophilic attack of the 5′-OH group of the support-bound nucleoside, thereby yielding the ring-opened intermediate

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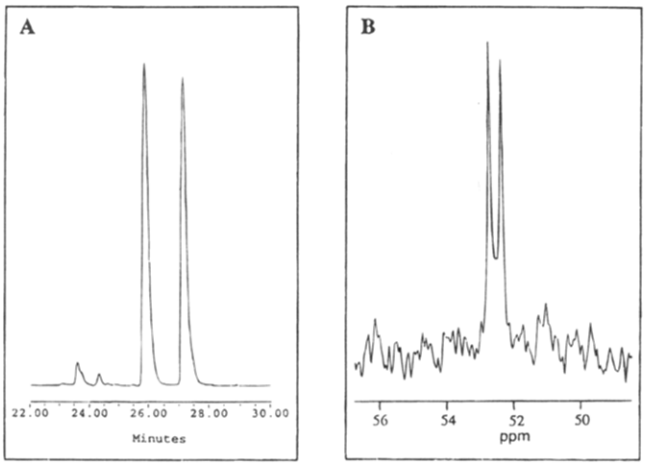
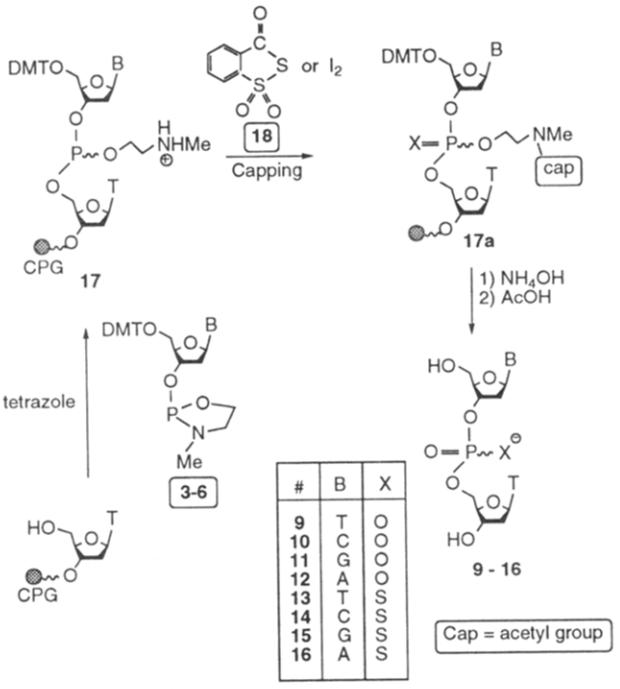


Figure 1. (A) HPLC profile of the crude phosphorothioate dimer **13**; the peak at 26 min is the R_p isomer and that at 27.7 min is the S_p isomer. (B) ^{31}P-NMR spectrum of the crude phosphorothioate dimer **13**; R_p is at δ 52.6 ppm and S_p isomer is at δ 52.2 ppm.

Scheme 2



17 (which presumably exists in the protonated form). During the next sequence in the synthesis cycle, which involves the "capping" of any unreacted 5′-OH group of the nucleoside, the secondary amino function in **17** is also simultaneously capped, rendering the latter unavailable to participate in any subsequent undesired side reactions. Following the synthesis, the removal of this appendage from the phosphate backbone is accomplished by treatment with 28% ammonium hydroxide at (55 °C, 10 h), under conditions, as employed in oligonucleotide chemistry.[9,10] Under these conditions of deprotection, the exclusive formation of the desired phosphodiesters was observed with no evidence for the presence of the corresponding phosphoramidates resulting from competing reactions.

The synthesis of a 17-mer Po-oligonucleotide (**19**) (DMT-on) was next undertaken on a 10 μmol scale according to the modified synthesis cycle.[11] Following the synthesis, the oligonucleotide was deprotected (28% NH_4-OH, 55 °C, 12 h) and purified by preparative reversed-phase HPLC[12] and isolated by standard protocols.[3,10] Figure 2 shows the ^{31}P-NMR spectrum and analytical polyacrylamide gel electrophoretic profile of the oligonucleotide **19**. A sample of the oligonucleotide **19**, subjected to nucleobase composition analysis,[13] revealed the presence of the bases in the expected ratio, with no detectable modification.

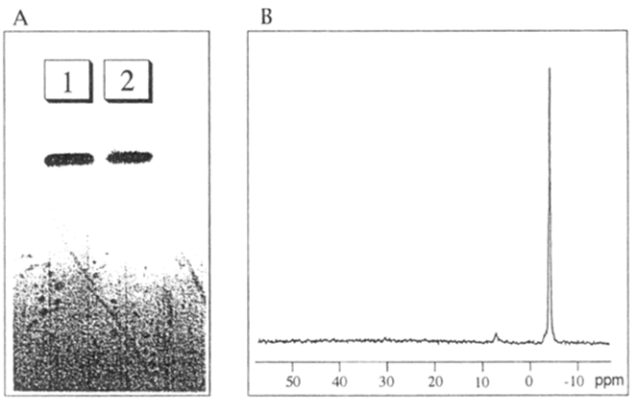


Figure 2. (A) PAGE profile of an HPLC-purified 17-mer (Po) [sequence 5′-ATGCGTGCAATAGCCTT-3′] (**19**). Lane 1: **19** synthesized using amidite **1** (10 μmol, overall yield 85%). Lane 2: **19** synthesized using oxazaphospholidines **3**–**6** (10 μmol, overall yield 80%). (B) ^{31}P NMR spectrum of **19**.

The accessibility of the nucleoside oxazaphospholidines of the general structure **2**—from readily available, structurally divergent, cheaper starting materials[14] —in a sequence involving fewer synthetic steps, as compared to the cyanoethyl phosphoramidite synthons—render them as potential alternative synthons in oligonucleotide synthesis.[15]

Supporting Information Available: Experimental procedures, NMR spectral data, and FAB-MS for compounds **3**–**7**, as well as HPLC profile of $(dA)_5$ (Po), TpsTpsT trimer synthesized using **3**–**6** and base composition analysis of **19** (7 pages).

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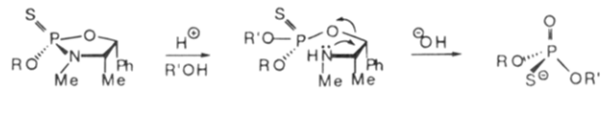
(9) The removal of the β-(alkylamino) appendage from **17a** is expected to occur by a process similar to that described by Cooper et al., who found that oxazaphospholidin-2-one **20**, derived from (1*R*,2*S*)-ephedrine, underwent ring opening with P–N bond cleavage, under acidic conditions, to give **21**. Base-catalyzed fragmentation of **21** yields **22** and 1,2-dimethyl-3-phenylaziridine. See: Cooper, D. B.; Hall, C. R.; Harrison, J. M.; Inch, T. D. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1969.



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(11) The coupling time in oligonucleotide synthesis is longer (by about 30%) than that with the amidite monomer **1**. The capping, oxidation, and detritylation cycle times were the same as those normally employed in standard oligonucleotide synthesis by phosphoramidite chemistry. We are currently optimizing the synthesis parameters and reagents to get maximum efficiency in the solid-phase synthesis of oligonucleotides.

(12) Reversed-phase HPLC was performed as described; see: Iyer, R. P.; Yu, D.; Agrawal, S. *Bioorg. Chem.* **1995**, *23*, 1.

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(14) 2-(Methylamino)ethanol, required for the synthesis of nucleoside monomers **3**–**6**, is cheaper than 3-hydroxypropionitrile and *N*,*N*-diisopropylamine, the raw materials required for the synthesis of the nucleoside monomers exemplified by the structure **1**.

(15) This paper is dedicated to the late Professor R. B. Woodward.