Pergamon

Tetrahedron, Vol. 53, No. 8, pp. 2731-2750, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00048-3

N-pent-4-enoyl (*PNT*) Group As a Universal Nucleobase Protector: Applications in the Rapid and Facile Synthesis of Oligonucleotides, Analogs, and Conjugates

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Abstract: Rapid synthesis of phosphoric diester and phosphorothioate oligonucleotides in 1 micromol to 1 millimol scale has been achieved using *PNT* nucleoside phosphoramidites. Furthermore, facile synthesis of mixed backbone oligonucleotides (MBOs) as antisense agents can be carried out using *PNT* nucleoside phosphoramidites in conjunction with *PNT* nucleoside *H*-phosphonates and *PNT* nucleoside phosphonamidites. The versatility of the *PNT* group is further demonstrated by its use in the preparation of bioreversible oligonucleotide conjugates.

INTRODUCTION

The advent of the solid-phase phosphoramidite[1] and *H*-phosphonate chemistries[2] has accelerated the development of oligonucleotides as potential therapeutic and diagnostic agents,[3] as well as, for applications in molecular biology.[3b] As oligonucleotide-based applications continue to evolve, synthetic methodologies need to be refined to meet these needs. For example, the phosphoramidite chemistry had been initially tailored to utilize nucleobase (dC^{Bz}, dA^{Bz}, and dG^{iBu}) and phosphate (β-cyanoethyl) protecting groups, as well as, solid-support anchoring groups (succinyl linkage) which are removable only under strongly basic conditions (28% aq. NH_4OH, 55 °C, 12 h). At least three limitations have become apparent with these robust protecting groups: (a) long turn around time in the availability of oligonucleotides for routine applications, and in particular, for biological evaluation, (b) difficulty in synthesizing oligonucleotide analogs, DNA/RNA mimics, and conjugates which contain heat/base-sensitive functionalities and, (c) prolonged deprotection time diminishes the production capacity during large-scale manufacture.

In connection with antisense applications of oligonucleotides, we needed rapid and ready access to: (a) phosphorothioate (PS) and phosphoric diester (PO) oligonucleotides, (b) non-ionic phosphoric diester isosteres, useful in the design of mixed-backbone oligonucleotides (MBOs) as second generation antisense compounds (Figure 1), and (c) oligonucleotide conjugates[4] (e.g., prodrugs) representing single or multiple

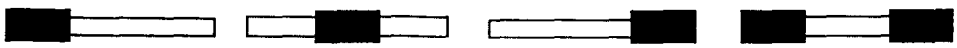


Fig. 1. Examples of MBOs; The dark area represent non-ionic segments, while the open area represent ionic segments.[3d]

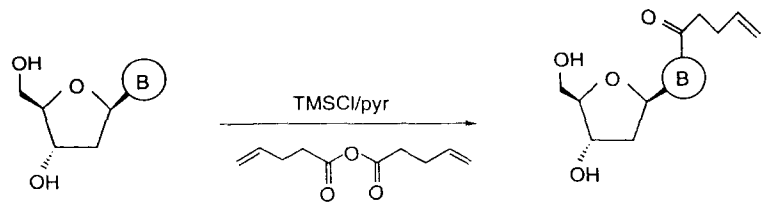
site-specific modifications in an oligonucleotide. To fulfill these objectives, we considered the evaluation of an alternate nucleobase protecting group, which would be compatible with phosphoramidite and *H*-phosphonate chemistries. Although a number of protecting groups have been reported for the preparation of oligonucleotides,[5] only limited studies exist regarding their applicability in the synthesis of modified oligonucleotides, and in the rapid synthesis of PO and PS oligonucleotides, the exceptions being the (*tert*-butyl) phenoxyacetyl (*t*-PAC)-, phenoxyacetyl (PAC)-, and *N,N*-dimethylformamidino-protected nucleosides.[6] We have reported preliminary studies of *PNT* as a nucleobase protecting group in the synthesis of MBOs containing triesters,[7a,e] methylphosphonates[7b], and amidates[7c,d]. The *PNT* group is a versatile nucleobase protecting group because it can be removed using multiple deprotection protocols ranging from neutral to basic conditions. We report here that the *PNT* nucleoside monomers can also be employed in the synthesis of PS, and PO oligonucleotides and MBOs containing methylphosphonate and PS linkages, on small, as well as, large-scale, but in a rapid synthesis mode when compared to dA^{Bz}, dC^{Bz} and $dG^{i\text{-}Bu}$ monomers. Described herein is the preparation of bioreversible oligonucleotide conjugates using *PNT* phosphoramidites.

RESULTS AND DISCUSSION

Recently, the *PNT* group was used as an aliphatic amine-protecting group in the synthesis of aminoglycosides.[8] As a nucleoside protecting group, *PNT* seemed attractive because it could be installed under mild conditions, and the deprotection could be achieved in a chemoselective manner under neutral conditions. To the best of our knowledge, the *PNT* group had not been previously employed as a nucleobase protecting group. Consequently, the synthesis and stability of *PNT* nucleosides, as well as, the conditions for deprotection of (dC^{PNT}[1], dA^{PNT} [2], and dG^{PNT} [3]) (Scheme 1) had to be established before evaluating their application in the synthesis of oligonucleotides using solid-phase phosphoramidite and *H*-phosphonate chemistry.

Synthesis of PNT nucleosides

The synthesis of the *PNT* nucleosides (Scheme 1) was carried out by applying the Jones's strategy[9] of transient silyl protection of the 3' and 5' hydroxyl groups of the corresponding nucleosides followed by acylation of the exocyclic amino group using pent-4-enoic anhydride.[10] The preparation of dC^{PNT} (**1**) and dG^{PNT} (**3**) was uneventful, and the products were isolated by direct crystallization of the crude (yields of 80 to 90% on



Scheme 1: Synthesis of *PNT* nucleosides

100 g scale). For the preparation of dA^{PNT} (**2**), initial purification of the crude product using a short silica gel column was required to remove the unreacted anhydride/acid, following which, **2** could be isolated in yields of 75 to 85% by crystallization and characterized.

The *PNT* nucleosides could be stored at room temperature or preferably at 0 to 5 °C. In a limited stability study using **1**, it was found that storage at room temperature in a closed container for 6 months did not cause any detectable decomposition, and more than 97% of the material was found to be intact as evaluated by reversed-phase HPLC (data not shown).

Deprotection of PNT nucleosides

To develop deprotection protocols, *PNT* nucleosides, either free or controlled-pore-glass (CPG)-anchored, were used in model studies.

(a) Neutral conditions. The use of iodine/THF/H_2O or *N*-iodosuccinimide/THF/H_2O system has been recommended[8a,b] for the removal of *PNT* groups from aliphatic amines. After considerable experimentation, we found that exposure of the individual nucleosides to I_2 (0.45 M in pyridine) for 30 min, followed by treatment with pyridine/H_2O, or pyridine/MeOH, resulted in complete deprotection to the corresponding free nucleosides. The rate of deprotection was dC~dA>dG.

In conjunction with *PNT* amidities and *PNT H*-phosphonates (*vide infra*), the potential for the preparation of base-deprotected support-bound, normal and modified oligonucleotides for various applications in molecular biology and biotechnology is being evaluated.

(b) Aqueous base conditions: use of 28% NH_4OH. Since the *PNT* group is an acyl type protecting group, we investigated whether one-step rapid deprotection, and cleavage from the support can be achieved. When CPG-bound *PNT* nucleosides *per se* were exposed to NH_4OH (28%, 55 °C, 15 min to 1 h), complete deprotection of the nucleoside and simultaneous cleavage from the support was observed in each case as monitored by reversed-phase HPLC. The rate of deprotection was **1>2>3**. As expected, under identical conditions, the deprotection of the dA^{Bz} and dG^{iBu} was incomplete (ca. < 20 to 30% deprotected) (data not shown).

(c) Anhydrous conditions: (i) use of K_2CO_3/MeOH. Recently, K_2CO_3/MeOH (0.05 M) has been used as the reagent for the deprotection and cleavage, during the preparation of methylphosphotriester oligonucleotides,[11] a challenging synthetic target.[12] In the event, exposure of the CPG-bound *PNT* nucleosides to K_2CO_3/MeOH (0.05 M, 3 to 4 h, rt) resulted in their simultaneous cleavage from the support and complete nucleobase deprotection (as monitored by HPLC). This reagent was used for the synthesis of MBOs containing methylphosphotriester and PS linkages.[7c]

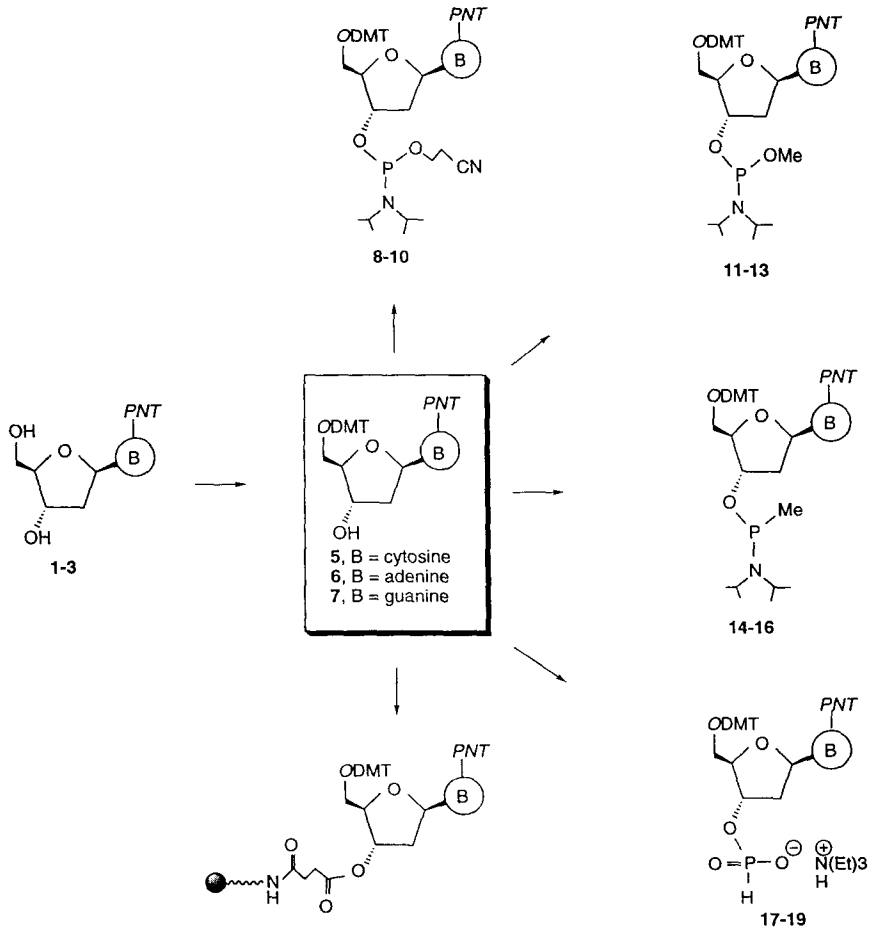
(ii) Use of NH_3/DMF. The CPG-bound *PNT* nucleosides could also be simultaneously deprotected and cleaved from the support using saturated NH_3 in DMF (65 °C, 20 h). This observation paved the way for the synthesis of MBOs containing primary phosphoramidate (PO-NH_2) linkages[7c,d,13a] using *PNT H*-phosphonates. Incidentally, the synthesis of these oligonucleotide analogs are reportedly difficult due to the lability of this linkage to aqueous NH_4OH.[13b,c]

Incidentally, the role, if any, of the γ, δ olefinic bond in effecting the facile base-mediated cleavage of the *PNT* group in the case of the nucleosides **1-3** has not yet been ascertained. It is also pertinent to mention here that the actual conditions for the deprotection in the case of solid-phase synthesis of oligonucleotides may

vary from that employed in the case of the *PNT* nucleosides themselves,[6d] and will depend on: (a) the scale of synthesis, (b) the length and composition of the sequence, and (c) the type of chemistry employed, i.e., phosphoramidite vs. *H*-phosphonate.

Synthesis of monomer synthons

(a) PNT phosphoramidites. The *PNT* nucleosides **1-3** were converted to the corresponding 5'-*O*-[4,4'-dimethoxytriphenyl]methyl (hereafter named 5'-DMT) derivatives **5-7**, and subsequently to the β-cyanoethyl- (**8-10**), methoxy-3'-*O*-(phosphoramido)-5'-DMT monomers (**11-13**) (hereafter named *CEPNT* and *MEPNT* respectively), as well as, methyl phosphonamidite monomers (*MPNT*) (**14-16**) (Scheme 2).[14]



Scheme 2. Synthesis of *CEPNT*, *MEPNT*, *MPNT*, and *H*-phosphonate monomer synthons and CPG-anchored *PNT* nucleosides.

(b) PNT-H-phosphonates. The 5'-DMT *PNT H*-phosphonates **17-19** were prepared (Scheme 3) as their triethylammonium salts using standard procedures,[15a] and were obtained as white foams. However, the dG^{PNT} *H*-phosphonate **19** was somewhat hygroscopic and had to be handled under anhydrous conditions.

Support-anchored PNT nucleosides.

The individual *PNT* nucleosides were anchored on to controlled-pore-glass (CPG) supports *via* the succinylated CPG (Scheme 3).[15b] The loading was in the range of 40-60 μmol/g when employing CPG of grain size 100 to 130 μm.

Rapid synthesis of phosphodiester oligonucleotides

(a) Using CEPNT amidites. In order to evaluate the potential of *CEPNT* amidites **8-10** in enabling the rapid synthesis of phosphodiester oligonucleotides, we prepared 20-mer (**20**), 30-mer (**21**) and 50-mer (**21a**) oligonucleotides on a 1 μmol scale. All the synthesis were performed using the recommended cycles in standard phosphoramidite chemistry except that for the oxidation of the phosphite linkage, *tert*-butyl hydroperoxide (see Table 1, Experimental) was employed when using **8-10**. Interestingly, in the case of the *PNT* amidites, the step-wise coupling efficiency (Figure 2, for **21a**) appeared to be better than that of the commercially available β-cyanoethyl dC^{Bz}, dA^{Bz}, and dG^{iBu} amidites (hereafter referred to as "standard amidites") as monitored by "trityl yields" (also, see phosphorothioates *vide infra*). This factor presumably contributed at least partly to improvements in the quality of the product (*vide infra*).

5'-TCC-GCT-ACT-TAC-TGT-CTC-ATC-GTT-ATC-TC T-GTA-CCT-GAA-TCG-TCC-GTC-AT **(21a)**

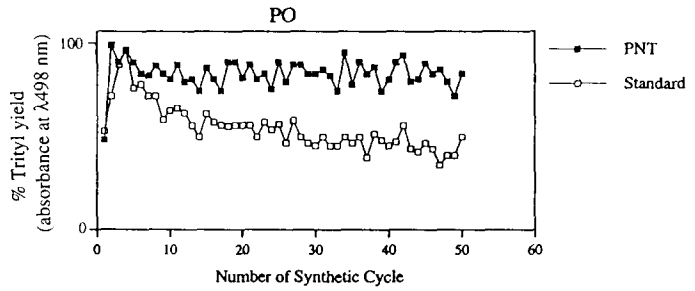


Fig. 2. Trityl monitoring during the synthesis of the 50-mer oligonucleotide (**21a**) using *PNT* amidites and standard amidites.

Following the synthesis, the CPG-bound oligonucleotides were treated with 28% NH_4OH (55 °C, 1.5 h for 20-mer, 2 h for 30 mer) to effect in one step: (a) removal of the *PNT* group; (b) removal of the β-cyanoethyl phosphate protecting group; and (c) cleavage of the oligonucleotide from the support. Representative sequences were subjected to base composition analysis after enzymatic digestion with nucleases (Figure 3). In each case, the nucleosides were present in the expected integral ratio. Figure 4 shows the comparative ion-exchange chromatography (IE) profiles[16] of oligonucleotide **20**, prepared using *PNT* amidites **8-10**, and those prepared using standard amidites. The overall yield of the N-mer, measured as the ratio of the product peak to the combined areas of all peaks including the product peak, was also higher when *PNT* amidites were employed in synthesis as compared to that with standard amidites. Importantly, the presence of

N - 1 fragment appeared to be lower (by ca. 30%) in the case of oligonucleotides prepared using *PNT* amidites as compared to that with standard amidites). Incidentally, the N - 1 peak is a heterogeneous mixture of sequences, that is difficult to remove by HPLC compared to other truncated sequences. Thus, the use of *CEPNT* amidites, allowed rapid synthesis of PO oligonucleotides, in better quality (i.e., less N - 1 and truncated sequences).

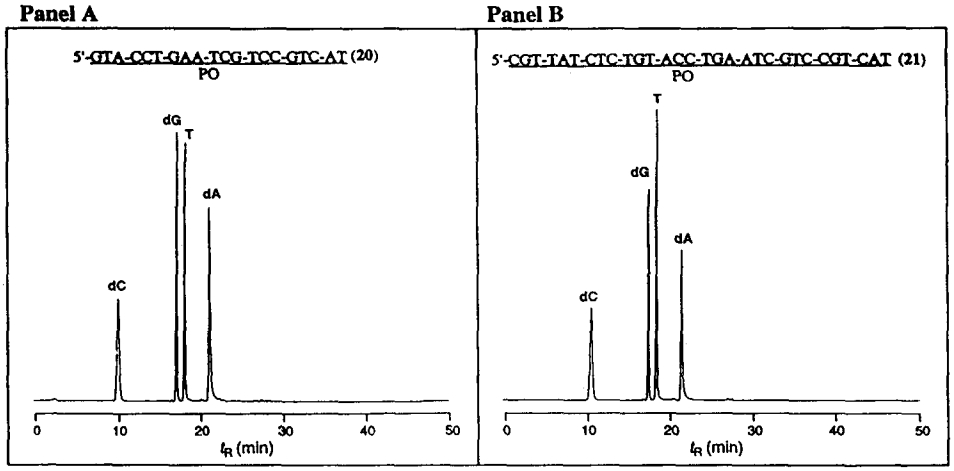


Fig. 3. Reversed-phase HPLC analysis of the *snake venom phosphodiesterase* and *alkaline phosphatase* digests of **20** (20-mer) **(Panel A)** and **21** (30-mer) **(Panel B)**.

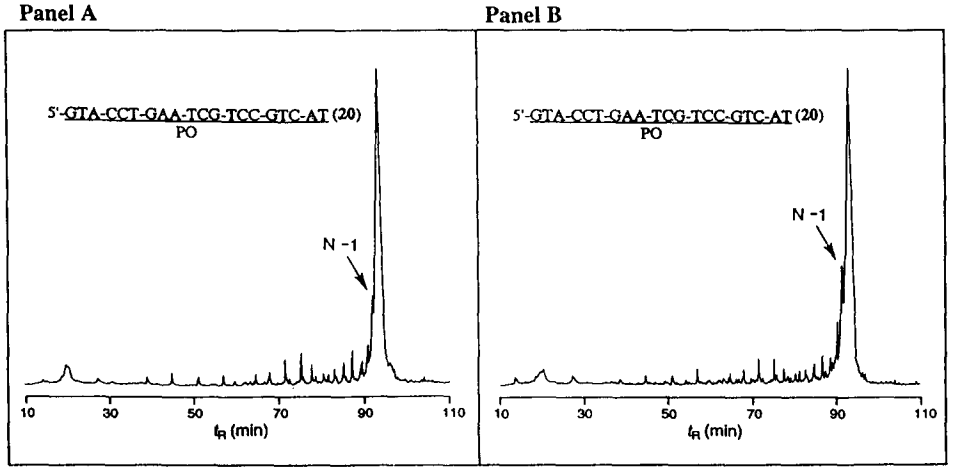


Fig. 4. Ion-exchange chromatographic profiles of crude **20** prepared using *CEPNT* amidites **(Panel A)** and that prepared using standard amidites **(Panel B)**.

(b) Using PNT H-phosphonates. In *H*-phosphonate chemistry,[2] the iodine-mediated oxidation of the *H*-phosphonate linkage to phosphodiester group is usually done at the end of the synthesis. Since the

PNT group is removed using iodine/pyridine/H_2O reagent, the possibility of achieving simultaneous oxidation and deprotection seemed logical. Accordingly, di-, tri-, and deca-oligonucleoside *H*-phosphonates were treated with iodine reagent for 1 h and the oligonucleotide cleaved from the support (28% NH_4OH, 1 h). Reversed-phase HPLC analysis indicated that under these conditions, the di- and tri-nucleotides had been formed in > 90% yields. The PAGE analysis of the decamer oligonucleotide showed the presence of the desired product decamer but along with the presence of a minor slow-moving band (ca. 10% of the major product), and truncated sequences. In order to establish its identity, the slow moving band was isolated, and subjected to enzymatic digestion. The HPLC analysis of the enzymatic digest revealed that the ratio of the nucleosides was different compared to that from an authentic decamer (data not shown). Additionally, other minor unidentified peaks were seen. These results suggested that perhaps, chain-branched products were generated during the synthesis/oxidation step or that the oxidation reaction was incomplete. It has been reported that the oxidation of *H*-phosphonate with I_2/pyridine/H_2O system is slow and it is preferable to do it in an automated mode using a three-reagent combination in the DNA synthesizer.[15a] Further work is under way to devise an optimal reagent combination to achieve the goal of simultaneous oxidation and deprotection in the routine rapid synthesis of PO oligonucleotides of longer length.

Rapid synthesis of phosphorothioates

Procedures which enable rapid synthesis of phosphorothioates should accelerate the screening and evaluation of these oligonucleotides for *in vivo* biological activity. Therefore, the evaluation of *PNT* monomers for rapid synthesis of phosphorothioates appeared timely and significant.

(a) Using CEPNT amidites. Several oligonucleotides (**22-25**) (Figure 5) of varying lengths and base compositions were prepared using *CEPNT* amidites **8-10** on a 10 μmol scale using standard phosphoramidite chemistry. In all cases, the oxidative sulfurization of the internucleotidic phosphite linkage was carried out using 3*H*-benzodithiole-3-one-1,1-dioxide.[18] For comparison, **22-25** were also prepared using the standard amidites under standard synthesis conditions. As before, the step-wise coupling efficiency when using *PNT* amidites was better than that of the standard amidites as monitored by trityl yields (see Figure 5 for an example) which perhaps contributed to the improvement in yields and quality of the product. It is

22 5'GCG TGC CTC CTC ACT GGC (18-mer)
23 5'GTA CCT GAA TCG TCC GTC AT (20-mer)
24 5'CTC TCG CAC CCA TCT CTC TCC TTC T (25-mer)
25 5'CGT TAT CTC TGT ACC TGA ATC GTC CGT CAT (30-mer)

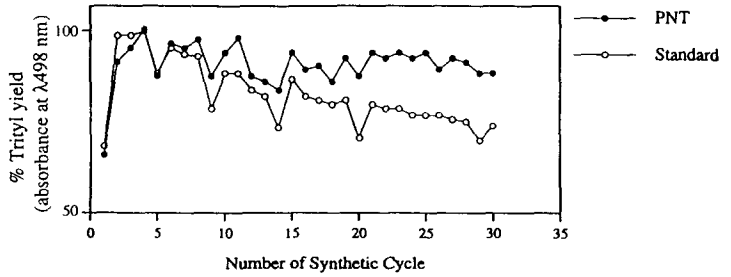
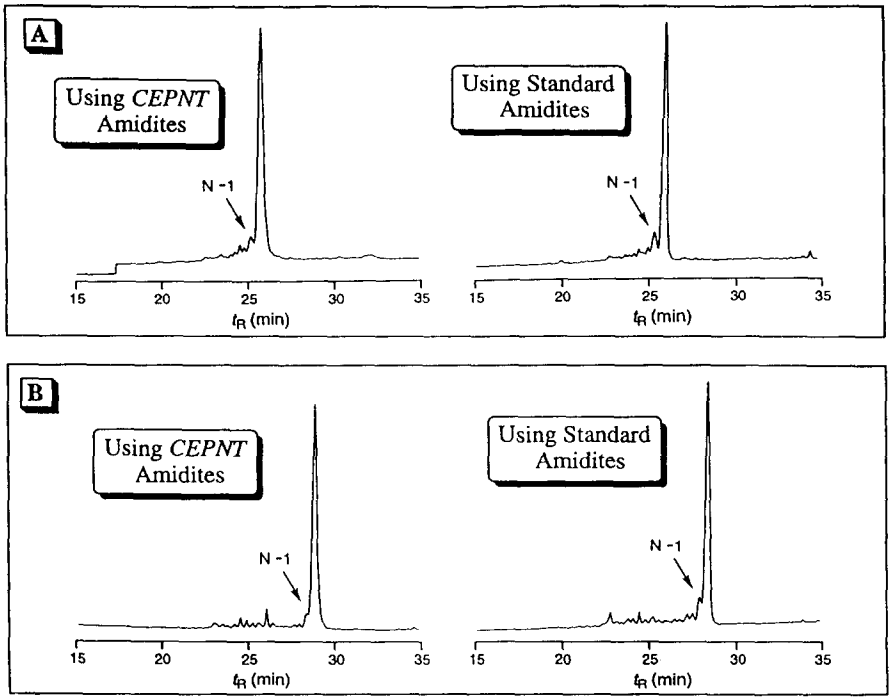


Fig. 5. Step-wise trityl monitoring during the synthesis of **25**.

pertinent to mention that phosphoramidites, derived from subtle modifications in the nucleobase or phosphate protecting groups or the sugar residue (deoxyribofuranosyl- vs. ribofuranosyl- vs. cyclopentano-), seem to couple with different efficiencies with a support-bound nucleoside. However, the mechanisms which explain these differences is poorly understood.[17]

In each case, rapid deprotection (28% NH_4OH, 55 °C, 2 h) was employed. For evaluation of the products, (capillary gel electrophoresis) (CE) was employed.[19] Figure 6 shows the comparative CE profiles of the representative *crude* **22** and **24** prepared using *PNT* and normal amidites respectively. The proportion of truncated sequences was lower in the case of oligonucleotides prepared using *PNT* amidites as compared to that with commercial amidites. Importantly, the presence of N - 1 fragment was reduced to the extent of 30 to 50% when using *PNT* amidites. Furthermore, in preparative HPLC, the ratio of the product peaks to the truncated sequences was higher by 8 to 10% with *PNT* amidites compared to standard amidites (data not shown). Thus, by using *PNT* amidites, rapid synthesis of PS oligonucleotides was achieved, and additionally the products were obtained in higher yields and better quality (less truncated sequences in the final product). To evaluate the



5'-GCG-TGC-CTC-CTC-ACT-GGC (**22**)
PS

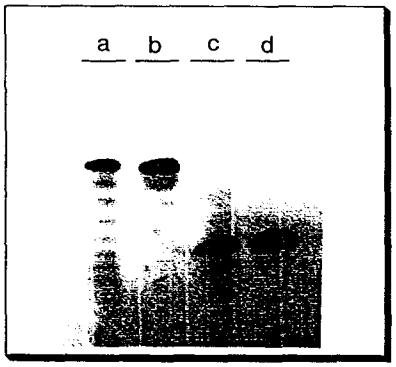
5'-CTC-TCG-CAC-CCA-TCT-CTC-TCC-TTC-T (**24**)
PS

Fig. 6. Comparative CE profiles of crude **22** (18-mer) (**Panel A**) and crude **24** (25-mer) (**Panel B**) prepared using *PNT* and normal amidites respectively.

applicability of the *PNT* amidites for large-scale synthesis, a pilot study was undertaken on the synthesis of **24** on a 600 μmol scale using OLIGO PILOT II® synthesizer. Following the synthesis, deprotection and cleavage was effected within 3 h using 28% NH_4OH (55 °C). Product evaluation was carried out by comparative CE analysis of the crude prepared using *CEPNT* and standard amidites. The results of this study was similar to those obtained on 10 μmol scale.

A variety of mechanisms appear to contribute to the formation of truncated sequences, principal among them being: (a) the presence of uncapped sites on the CPG carrying the leader nucleoside; (b) incomplete coupling during synthesis; (c) inefficient capping of unreacted sites during chain elongation; and (d) base-induced hydrolysis of the backbone (as well as base-induced hydrolysis of the apurinic sites) during deprotection.[20] Our results suggest that higher step-wise coupling efficiency (*vide supra*), and rapid deprotection could be the contributing factors for improved synthesis (higher yields and better quality) of PS-oligonucleotides using *CEPNT* amidites.

(b) Using PNT H-phosphonates. As before, both short (di- and trinucleotides) and longer oligonucleotides (10- and 15-mers) were prepared on a 1 micromol scale using *PNT-H*-phosphonates **17-19**. Typically e.g., following the assembly of the sequences, the oligonucleotide was treated with a solution of elemental sulfur in CS_2/pyridine//$N(Et)_3$. The removal of the *PNT* group and cleavage from the support was done using 28% NH_4OH (55 °C, 1 h). In this case, a shorter deprotection time was sufficient because *H*-phosphonate chemistry does not employ any phosphate protecting groups. For comparison, identical sequences were prepared using commercially available dC^{Bz}, dA^{Bz}, and dG^{iBu} *H*-phosphonates. Figure 7 shows the PAGE profile of the crude oligonucleotides **26** (10-mer) and **27** (15-mer) prepared using *PNT H*-phosphonates. For comparison, authentic samples of **26** and **27** were included. Thus, rapid synthesis of phosphorothioates can be achieved using *PNT H*-phosphonates.



26 5' TCA GCT CAT G
27 5' TGT ACT CAG CTC ATG

Fig. 7. PAGE profiles of the phosphorothioates **26** and **27** prepared using *PNT H*-phosphonates.

Lanes a, c: Crude **26** and **27** prepared using *PNT H*-phosphonates
Lanes b, d: Authentic markers, **26** and **27**.

Synthesis of MBOs

(a) MBOs with methylphosphonate, and PS segments. The methylphosphonate [P(O)Me] linkages have been found to be inherently susceptible to hydrolytic cleavage upon prolonged treatment with 28% NH_4OH.[21] Consequently, for the synthesis of [P(O)Me] oligonucleotides, a mild two-step prolonged

deprotection protocol is being followed. We have previously reported the synthesis of smaller MBOs having internucleotidic methylphosphonates P(O)Me or methylphosphonothioates P(S)Me as the non-ionic segments in conjunction with PO/PS segments using a short one-step deprotection protocol.[7b] In the present study, the MBO **28** (a GEM 91® analog, a 25-mer anti-HIV agent[22]) was prepared on a 10 micromol scale using the appropriate *CEPNT* and *MPNT* monomer synthons. A one step protocol using aqueous NH_4OH (28%, 1 h, 37 °C) was used to achieve deprotection, as well as, the cleavage of the oligonucleotide from the support. For comparison, identical sequences were prepared using commercially available dC^{iBu}, dA^{Bz}, and dG^{iBu} methylphosphonamidites. In this case, the deprotection was effected by an extended two-step protocol: (a) initial exposure of the oligomer to 28% NH_4OH (1 h, rt) followed by, (b) treatment with ethylene diamine/ethanol,1/1 (rt, 6 h). For evaluation, the samples of each oligonucleotide was subjected to CE (Figure 8). The oligonucleotides prepared using *PNT* amidites were identical to those prepared using commercial amidites.

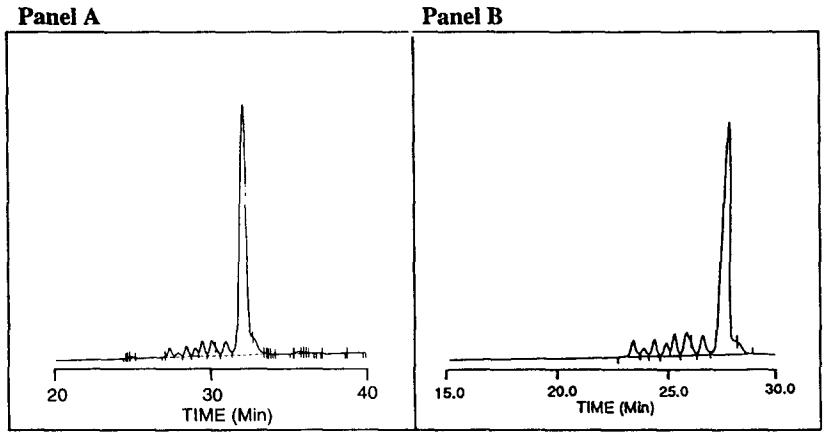


Fig. 8. Comparative CE profiles of 28 prepared using *MPNT* amidites (Panel A) and commercial amidites (Panel B).

Oligonucleotide conjugates

As part of a strategy to facilitate tissue- and site-specific delivery and to enhance cellular uptake, we have recently designed and synthesized acyloxyaryl prodrug derivative (e.g., **29**) of oligonucleotides.[23] These prodrugs were designed with an intent to facilitate intracellular esterase-mediated release of the oligonucleotide (Figure 9). The conjugating ligands were expected to serve multiple functions: (a) reduction of polyanion-related side effects due to reduced charges on the oligo; (b) enable tissue and target-specific delivery; (c) facilitate cellular uptake; and (d) harness the intracellular enzymes to release the oligonucleotide from the conjugating ligand. Thus far, the synthesis of the bioreversible conjugates, in which conjugating moieties are appended in a site-specific manner, has been difficult because of their labile nature under DNA synthesis

conditions when using standard monomers. A successful strategy of using *CEPNT* amidites for this purpose is illustrated by the following example.

We chose as our prodrug targets **30** and **31** which carry one, and two aryl ester ligands respectively at the backbone of the oligonucleotide. Scheme 3 illustrates the synthesis of **30** and **31**. The requisite phosphoramidite **32** was prepared and incorporated in a site-specific manner in an oligonucleotide. Following the synthesis, the deprotection of the oligonucleotide was carried out using K_2CO_3/MeOH (0.05 M, 12 h).

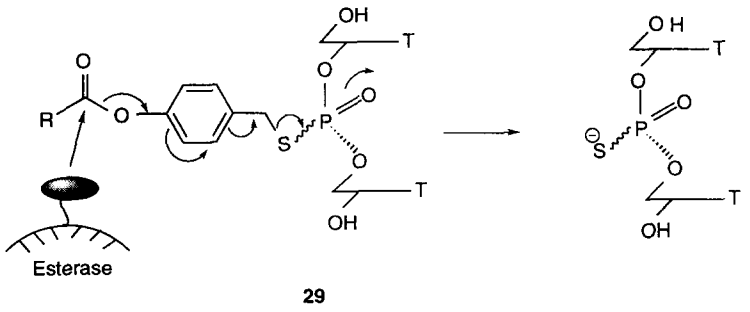
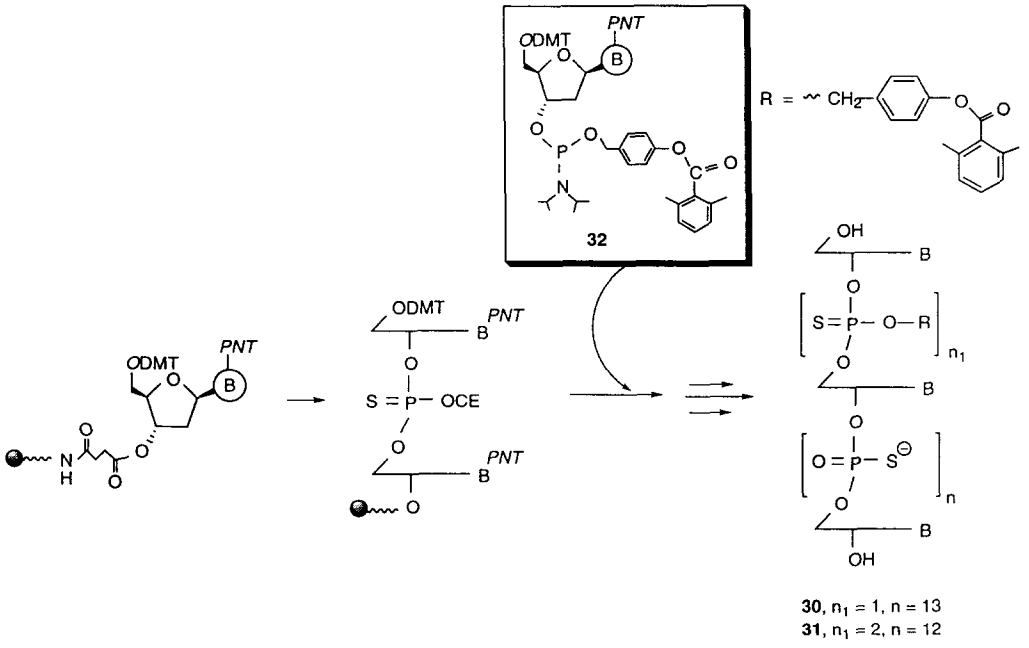


Fig. 9. Esterase-mediated bioreversibility of acxyloxyaryl derivative of oligonucleotide



Scheme 3. Examples of site-specific incorporation of bio-reversible conjugates in an oligonucleotide using the corresponding *PNT* phosphoramidite **32**; synthesis of **30** and **31**. Deprotection and cleavage from the support was accomplished using K_2CO_3/MeOH (0.05 M).

Earlier, model studies on dinucleoside analogs had established that under these conditions more than 90% of the product conjugate could be recovered intact with less than 10% of the product having suffered methanolysis of the ester group. The oligonucleotide conjugates **30** and **31** were isolated, and was shown to revert to the parent oligonucleotide by *chymotrypsin*-mediated hydrolysis. Details of these studies will be reported elsewhere.

CONCLUSION

Our studies suggest that, like other base-labile protecting groups,[6] the *PNT* group can be employed as a universal nucleobase protector for the rapid synthesis of PO, PS, oligonucleotides and MBOs containing P-Me linkages. Because the *PNT* group can be removed using multiple deprotection strategies, the use of this protecting group allows flexibility in the synthesis of oligonucleotide analogs, MBOs incorporating novel non-ionic phosphodiester isosteres, and oligonucleotide conjugates, for various applications in therapeutics and diagnostics. Although the usefulness of iodine-mediated deprotection of the *PNT* group has not been fully exploited in these studies, it should become apparent in the preparation of base-deprotected, support-bound oligonucleotides, and in combinatorial libraries. Such studies are in progress. Additional applications of the *PNT* group in the synthesis and large-scale manufacture of oligonucleotides, as well as, the biological evaluation of these oligonucleotides will be reported in the near future.

EXPERIMENTAL SECTION

Chemicals and, high purity and/or anhydrous, solvents were purchased from commercial vendors and used without further purification. 2'-Deoxyribonucleosides were purchased from Mallinckrodt Chemicals and used as such. DNA synthesis reagents and chemicals, were purchased from commercial sources.

Snake venom phosphodiesterase (Boehringer and Mannheim) and *alkaline phosphatase* (Pharmacia) were purchased and used as recommended by the manufacturer.

Electrophoretic analysis (PAGE) was carried out on 10 to 20% polyacrylamide-8 M urea gels (40 X 20 X 0.75 mm) using electrophoresis purity reagents (National Diagnostics, Atlanta, GA). Upon exposure to UV light (λ_{254} nm) and visualization against a fluorescent background (silica gel F_{254} TLC plate), the oligonucleotides were seen as dark bands.

Analytical reversed-phase HPLC (Waters 600E instrument equipped with a 996 photodiode Array UV detector) was done using a 8NV C-18, 4 μm Radial Pak cartridge column, a gradient of 100% A to 60% B over 70 min of buffer A (0.1 M NH_4OAc) and buffer B (80/20, CH_3CN/0.1 M NH_4OAc), and a flow rate of 1.5 mL/ min.

NMR spectra were recorded on a Varian 300 spectrometer operating in the presence of broad-band decoupling at 7.05 Tesla. ^1H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6(TMS as internal standard) or D_2O (TSP as internal standard) (δ = 0) as the case warranted. ^{31}P NMR spectra were recorded in $CDCl_3$ (trimethylphosphate as the external reference, δ = 0) or in D_2O (H_3PO_4 as the external reference, δ = 0).

Fast-atom bombardment mass spectra (FAB-MS) were recorded in both negative and positive modes and were performed by M-Scan Inc. (West Chester, PA). A cesium gun was used to generate ions from samples using a 2-nitrophenyl octyl ether matrix. Mass calibrations were performed using cesium iodide.

Solid-phase syntheses of oligonucleotides using phosphoramidite chemistry was done on a BIOSEARCH 8700 DNA synthesizer or EXPEDITE synthesizer (PerSeptive Biosystems). The synthesis was performed using the synthesis cycles recommended by the manufacturer. Table 1 shows a representative 1 μm-scale synthesis cycle. The *PNT* phosphoramidites were used as a solution in acetonitrile (0.08 M). 1*H*-tetrazole (0.5 M in acetonitrile) was employed as the activator. The capping step was done using two reagents CAP A (Ac_2O/2,6 lutidine/THF, 1/1/8) and CAP B (*N*-methyl-imidazole solution in THF, 16% *w/v*). The synthesis was carried out on CPG (500 Å) solid support. Oxidation of the internucleotidic phosphite linkage was carried out using *tert*-butyl hydroperoxide (1 M in toluene) over a period of 1 min. In all cases, the oxidative sulfurization reaction was effected by a 2 to 5% solution of the crystalline 3*H*-benzodithiol-3-one-1,1-dioxide (R. I. Chemical., Costa Mesa, CA) in acetonitrile (Burdick & Jackson, 0.01% water). The sulfurization reaction was performed over a period of 8 (1 μmol) to 45 sec depending on the scale of synthesis. The solid-phase syntheses of oligonucleotides using *H*-phosphonate chemistry was done using a BIOSEARCH 8700 DNA synthesizer employing synthesis programs recommended by the manufacturer

Synthesis of PNT nucleosides 1-3.

These were synthesized using pent-4-enoic anhydride, and the appropriate nucleoside by slight modification of the protocols developed by Ti et al[9] as described below:

Typical Procedure: Preparation of dC^{PNT} (1). 2'-Deoxycytidine hydrochloride (5 g, 19 mmol) was dried by evaporation from a suspension in pyridine (100 mL) and taken up in dry pyridine (100 mL). To the suspension, trichloromethylsilane (10.33 g, 95.05 mmol) was added and the mixture was stirred at ambient temperature for 30 minutes under argon. Then *PNT* anhydride (8.18 g, 11 mL) was introduced slowly into the reaction mixture and stirring continued. After 2 to 3 h, triethylamine (5 mL), was added to the cooled reaction mixture (0 °C) and stirring continued for a further 90 min. The reaction mixture was cooled in an ice-bath and was quenched by the addition of water (20 mL) followed 5 minutes later by the addition of 28% NH_4OH (20 mL). After stirring for an additional 30 min, the mixture was evaporated to dryness (caution need to be exercised in this step to avoid deacylation of the PNT nucleoside). To the residue was added water (150 mL) and the solution was extracted with ethyl acetate (2 X 50 mL). The aqueous layer which contained the *PNT* nucleoside was separated, and in some runs of the reaction, the product dC^{PNT} nucleoside spontaneously crystallized out from the aqueous medium. If spontaneous crystallization did not occur, the aqueous layer was allowed to stand for 6 to 8 h at 5 to 10 °C, for crystallization to take place. The crystals were filtered off and dried *in vacuo* (1 - 2 mm Hg) to give the product dC^{PNT} in 80 to 85% yields and was suitable for the preparation of *MEPNT* and *CEPNT* nucleoside phosphoramidite synthons.

The above procedure worked well for the preparation of dC^{PNT} and dG^{PNT}. As the dA^{PNT} was slightly water soluble, a modification of the work-up protocol was required for isolating it in good yields. Thus, following the evaporation of the reaction mixture (pH 8.0), as above, the residue was chromatographed on a short silica column, and eluted with ethyl acetate/MeOH/$N(Et)_3$, 80/10/10, *v/v*. The material thus obtained was dissolved in methanol and EtOAc/hexane, 80/20 was added for crystallization to occur. The desired product dA^{PNT} was obtained in 70 to 80% yields. Alternatively, for large-scale preparation, salting out technique was employed.

Spectral and analytical data of dA^{PNT} dC^{PNT}, and dG^{PNT}.

dC^{PNT} (1). ^{1}H NMR (DMSO-d_6): δ 10.86 (s, 1H), 8.34 (d, 1H, J = 7 Hz), 7.23 (d, 1H, J = 7 Hz), 6.12 (t, 1H, J = 6 Hz), 5.74-5.9 (m, 1H) 5.27 (bd, 1H), 4.9 - 5.15 (m, 3H), 4.25 (s, 1H), 3.7-3.95 (m, 1H), 3.5-3.7 (m, 2H), 2.55 (t, 2H, J = 7 Hz), 2.2-2.4 (m, 3H), 1.9-2.1 (m, 1H) ppm; ^{13}C NMR (DMSO-d_6): δ 28.2, 35.5, 40.9, 60.9, 69.9, 86.1, 87.9, 95.2, 115.3, 137.1, 144.9, 154.4, 162.2, 173.0 ppm; Analysis calcd for $C_{14}H_{19}N_3O_5$: C, 54.36; H, 6.2; N, 13.58; Found: C, 54.3; H, 6.11, N, 13.42.

dA^{PNT} (2). ^{1}H NMR (DMSO-d_6): δ 10.73 (s, 1H), 8.7 (s, 1 H), 8.7 (s, 1H), 6.5 (t, 1H, J = 7 Hz), 5.8 -6.0 (m, 1H) 5.39 (d, 1H, J = 4 Hz), 4.8-5.2 (m, 3H), 4.5 (bs, 1H), 3.95 (dd, 1H), 3.5-3.8 (m, 2H), 2.6-2.9 (m, 3H), 2.3-2.5 (m, 3H) ppm; ^{13}C NMR (DMSO-d_6): δ 28.5, 35.3, 39.6, 61.6, 70.6, 83.8, 87.9, 115.2, 123.8, 137.4, 142.6, 149.5, 151.4, 151.5, 170.9 ppm. Analysis calcd for $C_{15}H_{19}N_5O_4$: C, 54.04; H, 5.76; N, 21.0. Found: C, 54.01; H, 5.56; N, 20.73.

dG^{PNT}(3). ^{1}H NMR (DMSO-d_6): δ 12.1 (s, 1H), 11.8 (s, 1H), 8.27 (s, 1H), 6.24 (t, 1H, J = 7 Hz), 5.7-6.0 (m, 1 H), 5. 39 (d, 1H, J = 5 Hz), 4.9-5.2 (m, 3H), 4.45 (bs, 1H), 3.9 (m, 1H), 3.5-3.8 (m, 2H), 2.5-2.8 (m, 3H), 2.2-2.5 (m, 3H) ppm; ^{13}C NMR (DMSO-d_6): δ 28.2, 35.1, 39.8, 61.5, 70.5, 83.1, 87.7, 115.6, 120.2, 136.8, 137.4, 147.8, 148.3, 154.8, 175.5 ppm; Analysis calcd for $C_{15}H_{19}N_5O_5$: C, 51.57; H, 5.49; N, 20.04. Found: C, 51.5; H, 5.41, N, 19.67.

Synthesis of CEPNT (8-10) and MEPNT (11-13) nucleoside monomers.

Typical procedure: Synthesis of 8 from 1.

a) Tritylation. The dC^{PNT} nucleoside (**1**) (1.5 g, 5.05 mmol) was dissolved in dry pyridine (30 mL) and co-evaporated three times and taken up in dry pyridine (20 mL). A solution of 4,4-dimethoxytrityl chloride (2.6 g, 7.57 mmol) in dry pyridine (10 mL) was added over a period of 30 min. After stirring at ambient temperature for 1.5 h, the solvent was evaporated. The residue was chromatographed on a silica gel column and eluted with CH_2Cl_2/N(Et)$_3$/EtOH (100/3/3) to give 2.2 g (73%) of the DMT derivative **5**. For purification of 5'-*O*-DMT-dA^{PNT} (**6**), the eluent used was CH_2Cl_2/EtOAc/N(Et)$_3$ (19/1/1), and for **7**, elution was with CH_2Cl_2/EtOAc/EtOH (100/100/1). The 5'-*O*-DMT-*PNT* nucleosides (**5-7**), thus obtained, were used in the phosphitylation reactions.

b) Phosphitylation. To a solution of 5'-*O*-DMT dC^{PNT} (**5**) (2.24 g, 3.7 mmoles) in dry methylene chloride (50 mL) and triethylamine (5 mL) was added 2-cyanoethyl-*N,N'*-diisopropyl chlorophosphoramidite (0.94 g, 4.0 mmol) and stirred at room temperature for 3 h. The reaction mixture was quenched by adding ice-cold $NaHCO_3$ solution (5%, 40 mL). The solution was extracted with CH_2Cl_2 and the methylene chloride layer dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue chromatographed on a silica gel column. Elution with CH_2Cl_2/EtOAc/N(Et)$_3$, 6/4/0.5 gave 2.5 g of the *CEPNT* amidite **8**, (85% yield) as a white foam.

For the preparation of *MEPNT* amidites **11-13**, *N,N'*-diisopropyl methylphosphonamidic chloride was substituted in place of 2-cyanoethyl N,N'-diisopropyl chlorophosphoramidite in the above protocol.

CEPNT (dC) (8). Yellow foam; overall yield 60 to 65% based on **1**. ^{31}P NMR ($CDCl_3$): δ 147.42, 146.81 ppm (ca. R_p:S_p, 1:1 mixture); ^{1}H NMR ($CDCl_3$): δ 9.75 (1H, br), 8.20 (1H, d, J = 7.3 Hz),

7.43-7.20 (9H, m), 7.24 (1H, d, J = 7.3 Hz), 6.75-6.56 (4H, m), 6.22 (1H, t, J = 6.1 Hz), 5.8 (1H, ddt, J = 6.3, 10.2, 16.6 Hz), 5.05 (1H, dd, J = 1.4, 17.1 Hz), 4.98 (1H, dd, J = 1.4, 10.3 Hz), 4.60 (1H, m), 4.23-4.12 (3H, m), 3.76 (6H, s), 3.66-3.33 (6H, m), 2.58 (2H, t, J = 6.6 Hz), 2.41 (3H, m), 2.3 (1H, m), 1.1 (12H, d, J = 6.3 Hz) ppm; FAB-MS: Calcd for $C_{44}H_{54}N_5O_8P$, 812. 3788 $(M+H)^+$; Found *m/z* 812.3798.

CEPNT (dA) (9). White foam; overall yield of 60 to 65% based on **2**. ^{31}P NMR ($CDCl_3$): δ 146.9, 146.81 ppm (ca. R_p:S_p, 1:1 mixture); ^1H NMR ($CDCl_3$): δ 8.60 (1H, br), 8.58 (1H, s), 8.15 (1H, s), 7.40-7.25 (9H, m), 6.81-6.70 (4H, m), 6.43 (1H, dd, J = 2.4, 6.6 Hz), 5.90 (1H, ddt, J = 6.5, 10.3, 16.9 Hz), 5.1 (1H, dd, J = 1.5, 17.1 Hz), 5.02 (1H, dd, 1.5, J = 10 Hz), 4.78 (1H, m), 4.30 (1H, m), 4.20-4.07 (2H, m), 3.74 (6H, s), 3.66-3.54 (2H, m), 3.48 (2H, m), 3.40-3.31 (2H, m), 2.98 (2H, t, J = 7.3 Hz), 2.6 (1H, m), 2.53-2.41 (3H, m), 1.16 (12H, d, J = 6.6 Hz) ppm; FAB-MS: Calcd for $C_{45}H_{54}N_7O_7P$, 836.3900 $(M+H)^+$; Found, *m/z*, 836.3899.

CEPNT (dG) (10). White foam; overall yield of 62 to 64% based on **7**. ^{31}P NMR ($CDCl_3$): δ 146.89, 146.83 ppm (ca. R_p:S_p, 1:1 mixture); ^1H NMR ($CDCl_3$): δ 11.8 (1H, br), 8.04 (1H, br), 7.82 (1H, s), 7.43-7.25 (9H, m), 6.82-6.69 (4 H, m), 6.25 (1H, dd, J = 5.6, 7.8 Hz), 5.70 (1H, m), 5.00 (1H, dd, J = 1.5, 17 Hz), 4.95 (1H, dd, J = 1.5, 9.5 Hz), 4.70-4.60 (1H, m), 4.15-4.06 (3H, m), 3.65 (6H, s), 3.58-3.20 (6H, m), 2.60 (2H, t, J = 6.6 Hz), 2.45 (1H, m), 2.28 (3H, m), 1.09 (12H, d, J = 6.4 Hz) ppm; FAB-MS: Calcd for $C_{45}H_{54}N_7O_8P$, 852.3850 $(M+H)^+$; Found *m/z*, 852.3869.

MEPNT (dC) (11). Pale yellow foam; overall yield of 74 to 76% based on **1**. ^{31}P NMR ($CDCl_3$): δ 147.49, 146.81 ppm (ca. R_p:S_p, 1:1 mixture); ^1H NMR ($CDCl_3$): δ, 10.0 (1H, br), 8.24 (1H, d, J = 7.4 Hz), 8.18 (1H, d, J = 7.4 Hz), 7.40-7.08 (9H, m), 6.84-6.76 (4H, m), 6.17 (1H, dd, J = 6.3, 5.1 Hz), 5.78 (1H, ddt, J = 6.4, 10, 16.9 Hz), 5.02 (1H, dd, J = 1.4, 17.3 HZ), 4.94 (1H, dd, J = 1.4, 10.2 Hz), 4.62-4.54 (1H, m), 4.08 (1H, m), 3.61 (6H, s), 3.56-3.40 (4H, m), 3.26 (3H, d, $^3J_{P-H}$ = 13.2 Hz), 2.88-2.57 (3H, m), 2.40-2.34 (2H, m), 2.24-2.18 (1H, m), 1.02 (12H, d, J = 6.7 Hz) ppm; FAB-MS: Calcd. for $C_{42}H_{53}N_4O_8P$, 773 $(M+H)^+$; Found *m/z*, 773.

MEPNT (dA) (12). White foam; overall yield of 60 to 62% based on **2**. ^{31}P NMR ($CDCl_3$): δ 147.04, 146.90 ppm (ca. R_p:S_p, 1:1 mixture); ^1H NMR ($CDCl_3$): 8.61 (1H, s), 8.55 (1H, br), 8.17 (1H, s), 7.42-7.19 (9H, m), 6.82-6.75 (4H, m), 6.48 (1H, dd, J = 2.9, 6.4 Hz), 5.93 (1H, ddt, J = 6.5, 10.3, 17 Hz) 5.13 (1H, dd, J = 17.0, 1.4 Hz), 5.04 (1H, dd, J = 1.4, 10.3 Hz), 4.82-4.70 (1H, m), 4.38-4.28 (1H, m), 3.8 (6H, s), 3.58 (2H, m), 3.49 (2H, m, $^3J_{P-H}$ = 18.1 Hz, J = 6.8 Hz), 3.35 (3H, d, $^3J_{P-H}$ = 13.4 Hz), 3.0 (2H, t, J = 7.4 Hz), 2.87 (1H, m), 2.66 (1H, m), 2.53 (2H, m), 1.17 (12H, dd, J = 6.8 Hz, $^4J_{P-H}$ = 2.4 Hz); FAB-MS: Calcd for $C_{43}H_{53}N_6O_7P$, 797 $(M+H)^+$; Found *m/z*, 797.

MEPNT (dG) (13). White foam; overall yield of 60 to 64% based on **3**. ^{31}P NMR ($CDCl_3$): δ 146.78, 146.74 ppm (ca. R_p:S_p, 1:1 mixture); ^1H NMR ($CDCl_3$): δ 11.8 (1H, br), 8.02 (1H, br), 7.80 (1H, s), 7.43-7.20(9H, m), 6.80-6.69 (4H, m), 6.20 (1H, dd, J = 5.6, 7.9 Hz), 5.68 (1H, m), 4.96 (1H, dd, J = 1.5, 17.1 Hz), 4.94 (1H, dd, J = 1.5, 9.3 Hz), 4.72-4.63 (1H, m), 4.14-4.07 (1H, m), 3.63 (6H, s), 3.57-3.36 (4H, m), 3.29 (3H, d, $^3J_{P-H}$ = 13.2 Hz), 3.08 (2H, m), 2.84-2.76 (1H, m), 2.59-2.46 (1H, m), 2.24 (2H, m), 1.02 (12H, d, J = 6.7 Hz) ppm; FAB-MS: Calcd for $C_{43}H_{53}N_6O_8P$, 813 $(M+H)^+$; Found *m/z*, 813.0.

Synthesis of MPNT monomers (14-16). These were prepared as previously reported.[7b]

Synthesis of PNT H-phosphonate monomers 17-19.

These monomers were synthesized by adaptation of the standard procedures as reported.[15a] For the synthesis of the dG^{PNT} *H*-phosphonate **19**, the following purification procedure was followed. Thus following its obtention by the standard protocol, the crude *H*-phosphonate (670 mg, 0.82 mmol) was dissolved in dichloromethane (5 mL) and precipitated in n-hexanes (1000 mL containing 5 mL of triethylamine) to give a yellow powder (560 mg). The precipitated product was further purified on reversed-phase column (C-18 silica gel, 125 Å, 55 to 105 μm, elution with a mixture of acetonitrile/methanol, 1/1) to give **19** (495 mg), (R_f = 0.64, CH_3OH/CH_3CN, 1/1, on R_p-18 F_{254} silica). The product was co-evaporated with a mixture of dichloromethane/triethylamine, 4/1 to give a white foamy product. Prior to its use in synthesis, the product was co-evaporated with anhydrous pyridine, and dried over P_2O_5 overnight.

The *H*-phosphonates **17-19** were obtained in yields of 60 to 65% (based on **1-3**).

H-phosphonate 17. ^{31}P NMR ($CDCl_3$): δ 3.57 ppm (dd, $^1J_{P\text{-}H}$ = 609.84 Hz, $^3J_{P\text{-}H}$ = 7 Hz); FAB-MS: Calcd for $C_{42}H_{52}N_4O_9P$, 777.86 $(M + H)^+$; Found *m/z*, 778.0

H-phosphonate 18. ^{31}P NMR ($CDCl_3$): 3.34 ppm (dd, $^1J_{P\text{-}H}$ = 613.47 Hz, $^3J_{P\text{-}H}$ = 7 Hz); FAB-MS: Calcd for $C_{42}H_{53}N_6O_8P$, 801.89 $(M+H)^+$; Found, *m/z* 802.0

H-phosphonate 19. ^{31}P NMR ($CDCl_3$): 3.26 ppm (br d, $^1J_{P\text{-}H}$ = 613.47 Hz); FAB-MS: Calcd for $C_{42}H_{52}N_6O_9P$, 816.89 $(M)^+$; Found, *m/z* 817.0

Preparation of CPG-bound dC^{PNT}, dA^{PNT} and dG^{PNT} nucleosides.

These were prepared using the succinylated CPG as described.[15b] Typical nucleoside loadings were 40-60 μmol/g. The CPG-bound nucleosides were stored at -20 °C until ready to use.

Rapid synthesis of PO oligonucleotides

(a) Using CEPNT amidites and 28% NH_4OH for fast deprotection. For the rapid synthesis of PO oligonucleotides **20, 21 and 21a** on a 1 μmol scale, the synthesis cycle shown in Table 1 was followed. Following the synthesis, the CPG was treated with aqueous NH_4OH (28%, 10 mL, 55 °C, 2 h) and the solution evaporated on a speed vac to obtain the crude product. For comparison identical sequences were prepared using standard amidites. In this case, at the end of the synthesis cycle, the CPG was treated with NH_4OH (28%, 55 °C, 10 h). The ammoniacal solution was processed as above. The samples were analyzed by CE and ion-exchange chromatography.

(b) Using PNT H-phosphonates; simultaneous oxidation and deprotection using I_2/pyridine. For the rapid synthesis of PO oligonucleotides on a 1 μmol scale, standard *H*-phosphonate cycle was followed as recommended by the manufacturer. The *H*-phosphonates were used as a 1% solution in anhydrous pyridine/acetonitrile, 1/1. Adamantanecarbonyl chloride (2% in pyridine/CH_3CN, 1/1) was used as the activator. Following the synthesis, the CPG-bound oligonucleoside *H*-phosphonate was flushed back and forth with I_2 reagent (2% in pyridine/H_2O, 98/2) for 1 h. The CPG was then treated with aqueous NH_4OH (28%, 5 mL, 55 °C, 1 h) to obtain the crude products. For comparison, authentic samples were prepared using standard amidites. The crude samples were analyzed by PAGE.

Base composition analysis:

To ca. 1.5 units of the oligonucleotide in sterile water was added Tris.HCl buffer (250 nm, pH 9.0, 100 μL) and 0.015 units of *snake venom phosphodiesterase*. The mixture was incubated for 3 h at 37 °C. To

the above incubate was added 10 mL $MgCl_2$ (0.5 M), *alkaline phosphatase* (2 μL, 4 units), and incubated at 37 °C for 12 h. The reaction mixture was analyzed by reversed-phase HPLC using 8NV C18 4 μm Radial Pak Cartridge column using a gradient of 100% A to 60% B over 70 minutes at a flow rate of 1.5 mL/min (solvent A: 0.1 M NH_4OAc; solvent B: CH_3CN/solvent A, 80/20). The elution order of the nucleosides under these conditions was dC, dG, T, and dA.

Rapid synthesis of PS oligonucleotides 22-25, using 28% NH_4OH for rapid deprotection

(a) Using CEPNT amidites; (i) 1 to 10 μmol scale. For the rapid synthesis of PS oligonucleotides on a 1 μmol scale synthesis cycle shown in Table 1 was followed. For 10 μmol scale, the synthesis cycle recommended by the manufacturer was followed. Following the synthesis, the CPG was treated with aqueous NH_4OH (28%, 12 mL, 55 °C, 2 h) to obtain the crude product. In each case, identical sequences were prepared using standard amidites. Comparative CE analysis[19] of the oligonucleotides was carried out.

(b) Using PNT H-phosphonates. For the rapid synthesis of PS oligonucleotides on a 1 μmol scale, standard *H*-phosphonate cycle was followed as recommended by the manufacturer. The *H*-phosphonates were used as a solution in anhydrous pyridine/acetonitrile, 1/1. Following the synthesis, the oxidative sulfurization was carried out using elemental sulfur/CS_2/pyridine/$N(Et)_3$. The CPG was treated with aqueous NH_4OH (28%, 5 mL, 55 °C, 1 h) to obtain the crude product which was analyzed by PAGE.

Large-scale rapid synthesis of 22 using 28% NH_4OH for deprotection; A pilot study on 600 μmol scale using CEPNT amidites.

The synthesis was carried out on an Oligo Pilot II® synthesizer (Pharmacia Biotech) using standard 1 millimol scale PS synthesis program recommended by the manufacturer. The *CEPNT* amidites were used as 0.2 M solutions in acetonitrile. CPG-anchored dC^{PNT} (15 g, 40 μmol/g) was used as the solid support. For detritylation, contact time with CPG was 4 min. A solution of 1*H*-tetrazole (0.4 M in acetonitrile) was used as the activator (contact time 4 min). The oxidative sulfurization was carried out using 3*H*-1,2-benzodithiole-3-one-1,1-dioxide (5% *w/v*)[18] (contact time 50 sec). Capping was performed using Cap A (*N*-methyl imidazole 20% (*w/v*) in THF and Cap B (Ac_2O, 20% *v/v* in THF and *sym*-collidine, 30% *v/v* in THF) (contact time 50 sec). Following the synthesis, the CPG was treated with 28% NH_4OH (55 °C, 3 to 4 h). The oligonucleotide was purified by reversed-phase HPLC. The DMT group was removed using 80% acetic acid followed by extraction with ethyl acetate. Dialysis, followed by lyophilization gave ca. 1.6 to 2.2 g of the product.

Synthesis of MBOs

(a) MBOs with P(O)Me and PS linkages; one step rapid deprotection using 28% NH_4OH. The sequence **28** was synthesized on a 10 μmol scale using phosphoramidite chemistry. All the amidites were used as a solution in acetonitrile. The P(O)Me segment was built up using *PNT* phosphonamidite monomers while the PS segment was constructed using *CEPNT* monomers. Following the synthesis, the CPG was treated with 28% NH_4OH (37 °C, 1 h) to obtain the product. The identical sequence was synthesized using dC^{iBu}, dA^{Bz}, and dG^{iBu} phosphonamidites. In this case, the deprotection was effected by a two step-protocol. Comparative CE analysis of the oligonucleotides were done (Figure 8).

Oligonucleotide conjugates; Synthesis of 30-31 using K_2CO_3/MeOH for deprotection.

Synthesis of the phosphoramidite 32. To a solution of 5'-DMT-T (0.226 g, 0.415 mmol) in CH_2Cl_2 (5 mL) and triethylamine (1 mL) was added diisopropylphosphoramidous dichloride (0.1 g, 0.498 mmol) at 0 °C. After 5 min, 4-(2,6-dimethyl benzoyloxy)-benzyl alcohol (106.2 mg, 0.415 mmol) in CH_2Cl_2 (1 mL) was added. The reaction mixture was allowed to warm up to room temperature, and stirring continued for an additional 30 min. Following work-up, the product was isolated by column chromatography (silica gel, EtOAc/CH_2Cl_2/hexane/N(Et)$_3$, 0.5/1/1/0.1) to obtain **32** in 90% yield as a white foam. ^{31}P NMR ($CDCl_3$): δ 146.16, 146.0 ppm.

The phosphoramidite **32** was used to couple to the 5'-OH group of the growing oligonucleotide chain. Following the synthesis on a 1μm scale, the CPG-bound oligonucleotide conjugate was exposed to K_2CO_3/MeOH (0.05 M, 12 h). Further processing and purification afforded **30** and **31**.

Table 1

Step	Reagent/solvent	Function	Time (sec)	Quantity of reagent delivered (mL)
1.	3% TCA/DCM	Detritylation	50	0.75
2.	CH_3CN	Wash	20	0.82
3.	0.08 M phosphoramidite/tetrazole	coupling	45	0.22
4.	CH_3CN	wash	56	0.50
5.	*tert*-BuOOH (1 M in toluene)	oxidation	60	0.50
	or			
	3*H*-BD (2% in CH_3CN)	sulfurization	8	0.30
6.	CH_3CN	wash	98	1.50
7.	Cap A, Cap B	capping	15	0.30
8.	CH_3CN	wash	98	1.50

TCA = trichloroacetic acid; DCM = dichloromethane; 3*H*-BD = 3*H*-benzodithiole-3-one-1,1-dioxide. CAP A contains acetic anhydride, 2,6-lutidine and THF; CAP B contains *N*-methyl imidazole in THF.

Acknowledgement: We thank the referees for their comments and suggestions.

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(*Received in USA* 5 *May* 1996; *revised* 7 *January* 1997; *accepted* 9 *January* 1997)