Pergamon

Tetrahedron Letters, Vol. 37, No. 10, pp. 1539-1542, 1996

0040-4039/96 $15.00 + 0.00

S0040-4039(96)00066-4

N-pent-4-enoyl Nucleosides: Application in the Synthesis of Support-bound and Free Oligonucleotide Analogs by the *H*-phosphonate Approach

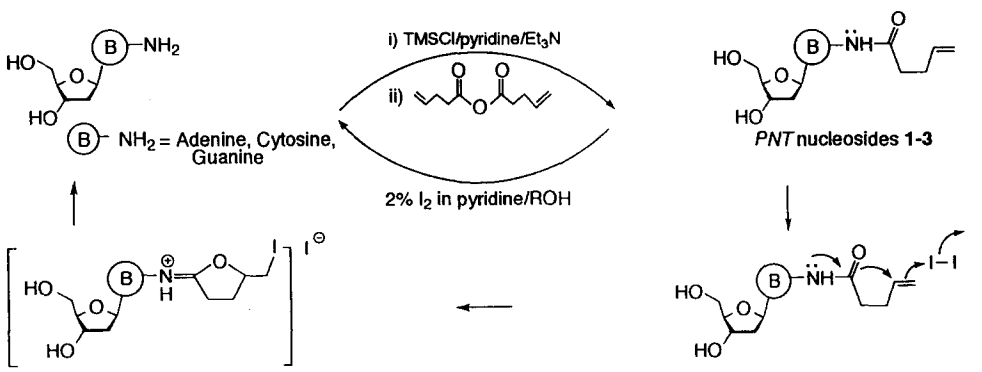
Radhakrishnan P. Iyer, Theresa Devlin, Ivan Habus, Nan-Hui Ho, Dong Yu and Sudhir Agrawal

Hybridon Inc., One Innovation Drive, Worcester, Massachusetts 01605, USA.

Abstract: *N*-pent-4-enoyl nucleoside *H*-phosphonates are versatile building blocks for the synthesis of support-bound and free oligonucleotide analogs.

Oligonucleotides, natural or suitably modified, are finding increasing applications as diagnostic and as potential therapeutic agents.[1a,b] The routine synthesis of oligonucleotides is generally carried out on a solid-support, in conjunction with phosphoramidite[2] or hydrogen-phosphonate (*H*-phosphonate) chemistry[3] wherein removal of the protecting groups and cleavage from the support is accomplished by treatment with aqueous NH_4OH (28%, 55 °C, 10 h). However, the synthesis of oligonucleotides bearing base-labile functionalities, e.g., methylphosphotriesters, RNA analogs, carboxylic ester and certain peptide-oligonucleotide conjugates is still a major challenge. Additionally, in certain nucleic acid-based applications, it is desirable to have natural and modified, *solid-support-bound* oligonucleotides which can also be rapidly cleaved. These considerations prompted us to search for a protecting group for the nucleobases that is compatible with solid-phase oligonucleotide synthesis, and one which could be removed expeditiously under mild conditions. We report herein that *N*-pent-4-enoyl (*PNT*) nucleosides **1-3,** in conjunction with *H*-phosphonate chemistry, can be used in the preparation of support-bound and free oligonucleotide analogs.[4]

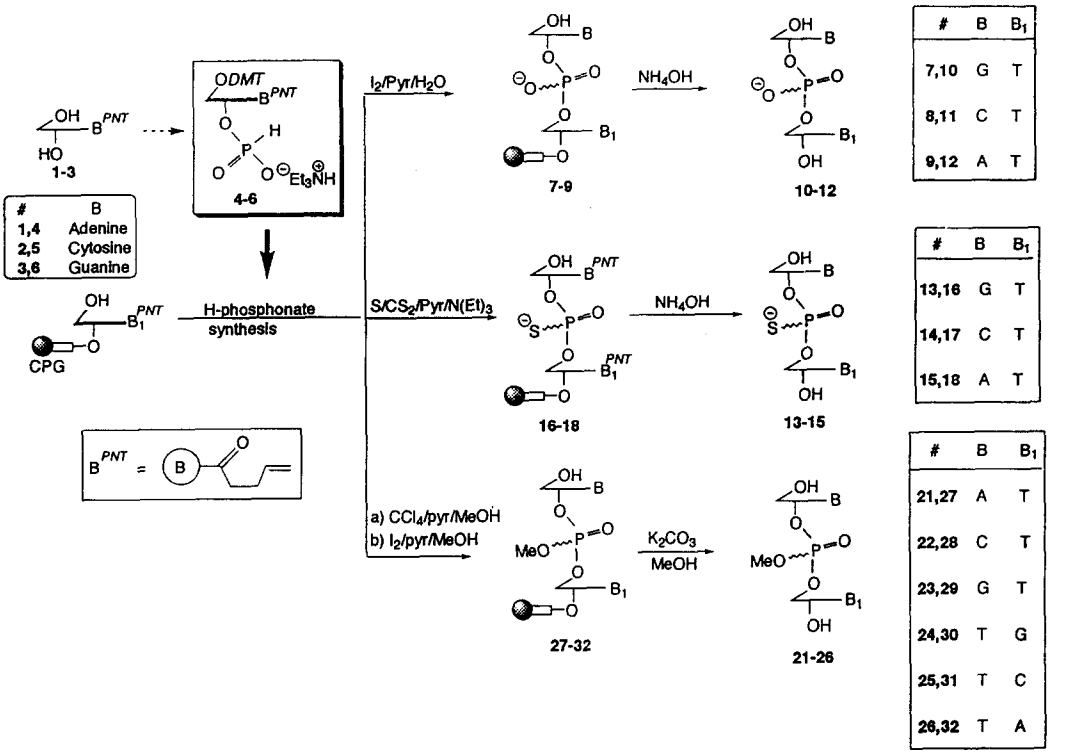
The *PNT nucleosides*, **1-3**,[5a-b] were prepared using pent-4-enoic anhydride following the transient protection of 3' and 5'-hydroxyl groups as their trimethylsilyl ethers (Scheme 1).[6a,b] Deprotection of **1-3**



Scheme 1

was readily effected using iodine (2% in pyridine/H_2O or pyridine/MeOH, 98/2, 30 min) or under basic but mild conditions.[6c] The versatility of the *PNT* group is illustrated by the preparation of different classes of oligonucleotides as below:

Support-bound and free Phosphodiester-Oligonucleotides: For the preparation of PO-dinucleosides by the *H*-phosphonate chemistry (Scheme 2), the requisite 5'-*O*-dimethoxytrityl (DMT)-3'-*H*-phosphonates **4-6** were synthesized from **1-3**.[3,7] The CPG-bound *H*-phosphonate dimers were then treated with iodine (2% in pyridine/H_2O, 98/2, 30 min), to oxidize the *H*-phosphonates as well as to effect chemoselective removal of the *PNT* group *in a single step* to give the CPG-bound phosphoric diesters **7-9** (Scheme 2). Finally, cleavage of the PO-dimers from the support, with aqueous NH_4OH (28%, ambient temperature, 1 h) or K_2CO_3 (0.05 M in MeOH, 8 h), furnished **10-12** (yields 96-98%).



Scheme 2. Oligonucleotide analogs from *PNT H*-phosphonates

The dimers **10-12** were found to be identical to authentic materials (prepared using dA^{Bz}, dC^{Bz} and dG^{iBu} monomers), as evaluated by reversed-phase HPLC (Fig. 1)[8] and UV spectra. *Importantly, these experiments indicated, for the first time, the potential for achieving simultaneous oxidation of the internucleotidic H-phosphonate linkages as well as the deprotection of the nucleobases, in oligonucleotide synthesis, using the H-phosphonate approach.*

The preparation of phosphorothioates (PS) oligonucleotides was carried out using H-phosphonate chemistry, as above, wherein at the chain of the chain assembly, the oxidative sulfurization of the internucleotidic linkages was carried out using elemental sulfur/CS_2/pyridine/$N(Et)_3$. Removal of the *PNT* group and cleavage from the support was readily effected with 28% NH_4OH (ambient temperature, 1-2 h).[9]

Fig. 1 shows the HPLC analysis of the crude PS dimers synthesized as above which indicates their formation in >96-98% yields.

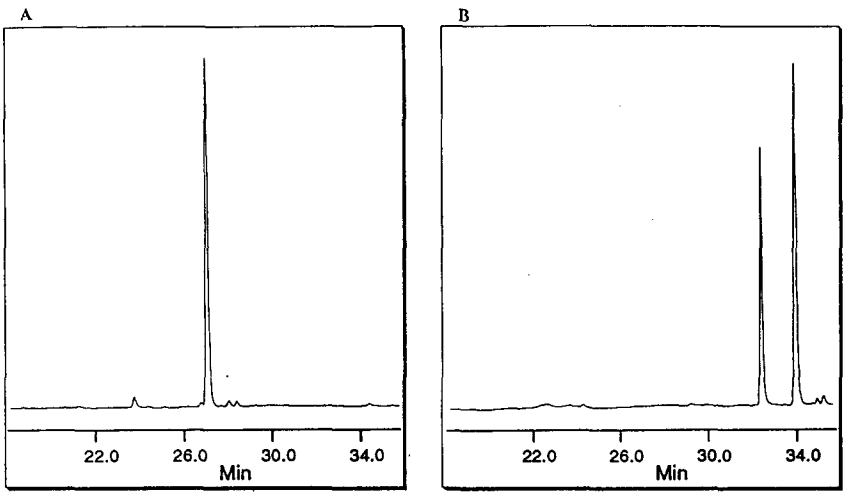


Figure 1. HPLC[8] profiles: **Panel A.** crude 5'[AT] (PO) dimer. **Panel B**. Crude 5'[AT] (PS) dimer.

Support-bound and free *O*- Methyl phosphotriester (PO-OMe) analogs: The versatility of the *PNT* group is further demonstrated by the facile preparation of methylphosphotriesters[10] e.g., **21-26** (Scheme 2). Thus, treatment of the appropriate CPG-bound *H*-phosphonates with CCl_4/pyridine/MeOH (8/1/1, 5 min), followed by exposure to I_2 solution (2%, in pyridine/*MeOH*, 98/2, 30 min)[11a] gave the CPG-bound methylphosphotriesters **27-32**. Cleavage from the support with K_2CO_3 (0.05 M in MeOH, 8 h),[12a,b] gave the methylphosphotriesters **21-26**. Analysis of the *crude* products, by reversed-phase HPLC and NMR (Fig. 2) indicated a pair of diastereomers (R_p, S_p) integrating to greater than 97% of the desired phosphotriester

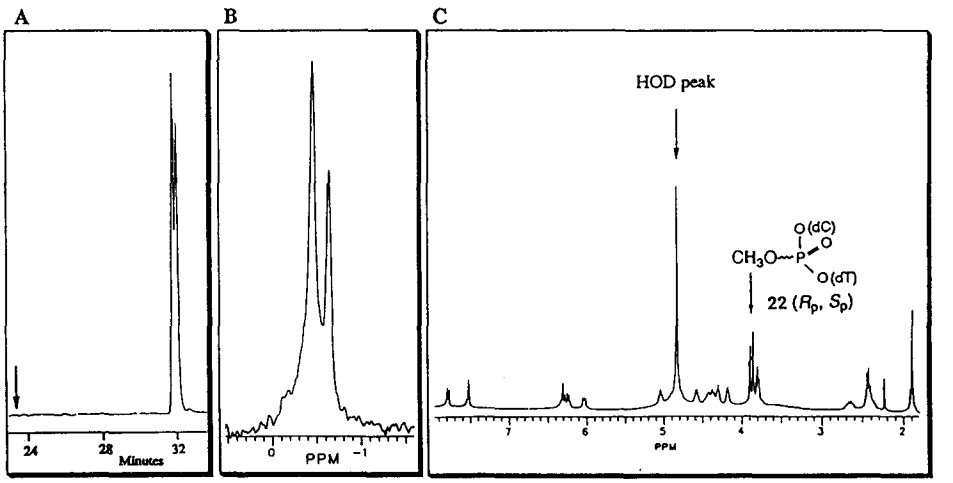


Fig.2. Panel A. HPLC profile[8] of 5'-CT (PO-OMe) (**22**); peaks at ca. 32 min represent R_p, S_p diastereomers. Arrow indicates the expected peak position of 5'-CT (PO). **Panel B**. ^{31}P-NMR spectrum of R_p, S_p **22**. **Panel C**. ^{1}H-NMR spectrum of **22**; Arrow indicates the -OCH_3 resonances.

product.[11] Under our synthesis conditions, the formation of the corresponding dinucleoside phosphoric *diesters* was minimal (< 2%) (Fig. 2). The above strategy was also employed in the synthesis of a pentanucleoside phosphotriester, 5'd[$Ap_{(OMe)}Tp_{(OMe)}Cp_{(OMe)}Tp_{(OMe)}G$] essentially as per the protocol described above and fully characterized (data not shown).

In conclusion, the use of the *PNT*-protecting group, which is *readily installed and expeditiously removed, under mild conditions*, should provide access to a variety of *support-bound and free, natural as well as modified,* oligonucleotides, oligonucleotide conjugates and RNA. The *PNT* group is compatible with solid-phase oligonucleotide synthesis using both *H*-phosphonate and phosphoramidite chemistry.[4] The use of the *PNT* nucleosides, in the synthesis of "chimeric" and "hybrid" oligonucleotides[13] incorporating different segments of modified oligonucleotides and their evaluation as modulators of gene expression will be reported in due course. In addition, the application of the support-bound oligonucleotides as affinity columns[14] and in oligonucleotide-based combinatorial libraries can be anticipated.

References and Notes

1. (a) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 544-584; (b) Agrawal, S, Iyer, R. P. *Curr. Op. Biotech.* **1995**, *6*, 12-19.
2. Beaucage, S. L. in *Methods in Molecular Biology: Protocols for Oligonucleotides and Analogs*; Agrawal, S., Ed.; Humana Press: Totowa, New Jersey, 1993, Vol. 20, pp. 33-61.
3. Froehler, B. C. in *Methods in Molecular Biology: Protocols for Oligonucleotides and Analogs*; Agrawal, S., Ed.; Humana Press: Totowa, New Jersey, 1993, Vol. 20, pp. 63-80.
4. For application of *PNT* nucleosides in phosphoramidite chemistry, see Iyer, R. P.; Yu, D.; Ho, N-H.; Devlin, T.; Agrawal S. *J. Org. Chem.* **1995**, *60*, 8132-33.
5. (a) For elegant applications of the *PNT* group in the synthesis of carbohydrates see: Debenham, J. S.; Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 3302-03 and references therein; (b) For a review of protecting groups in oligonucleotide synthesis, see: Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223-2311.
6. (a) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316-19; (b) The analogs **1-3** were fully characterized (see ref. 4); (c) Removal of the *PNT* group could also be carried out using 28% NH_4OH (1-2 h, ambient temperature) or anhydrous K_2CO_3 (0.05 M in MeOH, 3-4 h);[12] Deprotection conditions are being optimized.
7. The H-phosphonates **4-6** were characterized by ^{31}P-NMR and FAB-MS.
8. For details of HPLC conditions and analysis, see Iyer, R. P.; Yu, D.; Agrawal, S. *Bioorg. Chem.* **1995**, *23*, 1-21.
9. Deprotection and cleavage conditions are being optimized; Anhydrous K_2CO_3 (0.05 M in MeOH) can also be used for this purpose.
10. For recent efforts in the synthesis of methylphosphotriester oligonucleotides see: Hayakawa, Y.; Hirose, M.; Hayakawa, M.; Noyori, R. *J. Org. Chem.* **1995**, *60*, 925-930 and references therein.
11. (a) It was necessary to use I_2/pyridine/*MeOH* because deprotection with I_2/pyridine/*water* reagent resulted in the formation of ca. 10% of the corresponding phosphoric diester product; (b) Treatment of the triester (2 AU_{260} units) with *t*-butylamine/water, 1/1 (1 ml, 55 °C, 4 h) or thiophenol resulted in its conversion, exclusively, to the corresponding diester product.
12. (a) Kuijpers, W. H. A.; Huskens, J.; Koole, L. H.; van Boeckel, C. A. A. *Nucl. Acids Res.* **1990**, *18*, 5197-5205; (b) Conditions for cleavage from the support are being optimized.
13. Agrawal, S.; Mayrand, S.; Zamecnik, P. C.; Pederson T. *Proc. Natl. Acad. Sci. (USA)* **1990**, *87*, 1401-05.
14. T. Atkinson, S. Gillam, M. Smith, *Nucl. Acids Res.* **1988**, *16*, 6232.

(*Received in USA* 22 *November* 1995; *revised* 19 *December* 1995; *accepted* 21 *December* 1995)