Pergamon

Tetrahedron Letters, Vol. 37, No. 10, pp. 1543-1546, 1996

0040-4039/96 $15.00 + 0.00

S0040-4039(96)00067-6

Oligonucleoside phosphoramidates from *N*-Pent-4-enoyl Nucleoside *H*-Phosphonates

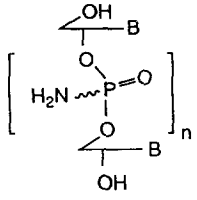
Radhakrishnan P. Iyer, Theresa Devlin, Ivan Habus, Dong Yu, Suzanne Johnson and Sudhir Agrawal

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

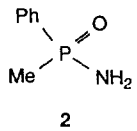
Abstract: *N*-pent-4-enoyl nucleoside *H*-phosphonates are versatile building blocks for the synthesis of oligonucleotide phosphoramidates.

The diagnostic use and therapeutic potential of oligonucleotides are well recognized.[1a,b] In this context, phosphorothioate analogs have already provided the necessary impetus for exploiting the therapeutic potential of oligonucleotides.[1b] It has been previously demonstrated that incorporation of the non-ionic oligonucleotide segments as flanking sequences in a phosphorothioate oligonucleotide changes the degradation, cellular uptake, affinity to target nucleic acid, pharmacokinetic profile and *in vivo* stability.[1b,c] Such ampiphilic oligonucleotides are expected to confer favorable pharmacophoric and pharmacodynamic attributes to an active agent.

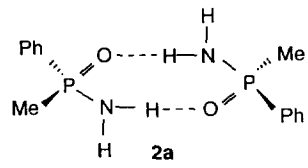
Many non-ionic oligonucleoside phosphoramidate analogs, have been previously studied.[1a,b] However, to the best of our knowledge, attempted preparation of analogs bearing a primary phosphoramidate ($PO\text{-}NH_2$) linkage, exemplified by the structure **1**, have not been successful.[2a] The $PO\text{-}NH_2$ linkage in **1** is isosteric with the phosphoric diester group but differs from methylphosphonates and morpholidates,[2b] in that $PO\text{-}NH_2$ group can potentially hydrogen bond with water thus allowing for increased water solubility of a chimeric oligonucleotide containing segments of these phosphoramidate linkages. Additionally, we were



1 B = nucleobase



2



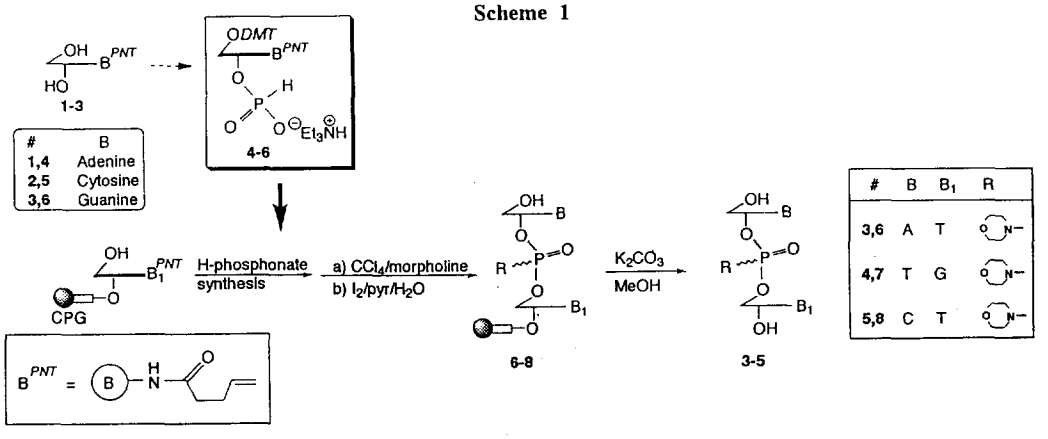
2a

intrigued by the possibility that the duplexes of oligonucleotides bearing $PO\text{-}NH_2$ linkages might exhibit enhanced stability through co-operative hydrogen-bonding interactions with the backbone of a complementary target, in addition to the well-known Watson-Crick or Hoogsteen base-pairing modes. These expectations were based on a report by Harger et al.,[3] who observed that simple phosphinic amides e.g., **2** had a tendency to form hydrogen-bonded dimer **2a** in non-polar solvents.

The phosphoramidate **1**, presents a formidable synthetic challenge because of its extreme lability to acidic and aqueous alkaline conditions[2a,4] that are normally employed in the synthesis and processing of oligonucleotides. We have recently reported that pent-4-enoyl group is a versatile nucleobase protecting group which is readily removed under mild conditions.[5,6] We report here the synthesis and spectral characteristics of di- and tri-nucleoside phosphoramidates, having the general structure **1**, using *N*-pent-4-enoyl (*PNT*) nucleoside *H*-phosphonates.

As a model study for the preparation of **1**, using *PNT*-*H*-phosphonates, the synthesis of the well-known morpholidate analogs **3-5** was undertaken. For the preparation of **3-5** (Scheme 1), the requisite *PNT* nucleoside 5'-*O*-dimethoxytrityl (5'-DMT)-3'-*H*-phosphonates **6-8** were synthesized as reported previously.[6]

Scheme 1



Next, the appropriate CPG-bound *H*-phosphonate dimers were synthesized and then treated with CCl_4/morpholine (90/10, 30 min).[2] Removal of the *PNT* group was achieved by exposure to iodine (2% *w/v*, pyridine/H_2O, 98/2, 30 min), to give the base deprotected support-bound morpholidates **6-8**. Finally, cleavage from the support using either NH_4OH (28%, 1-2 h, ambient temperature) or K_2CO_3 (0.05 M in MeOH, 8 h) furnished the diastereomeric dinucleoside morpholidates **3-5** which were fully characterized (Fig. 1).

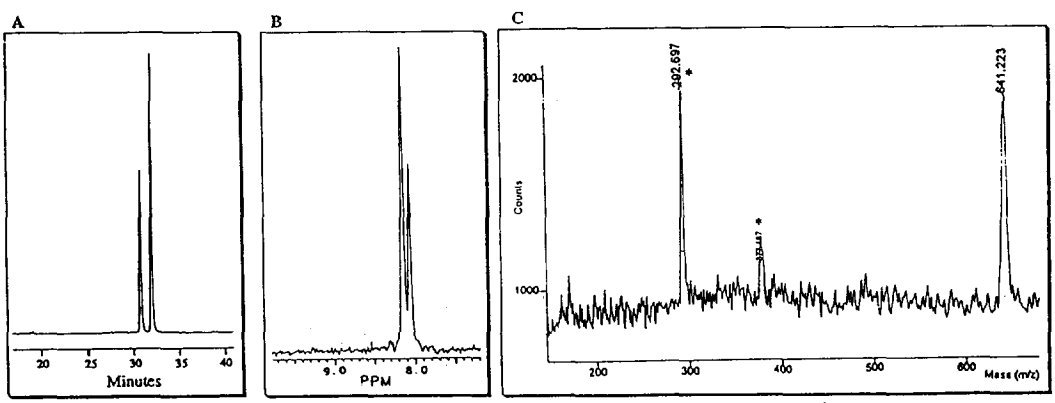
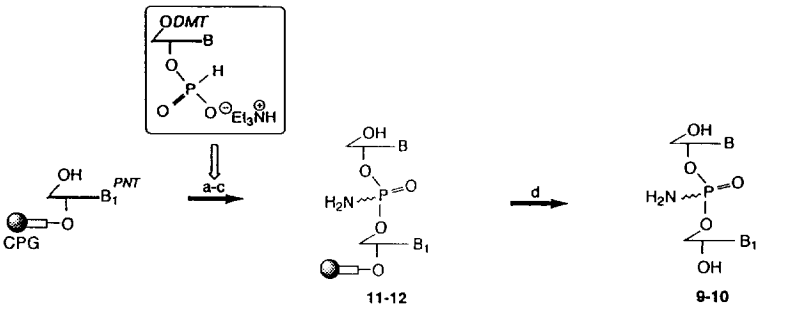


Figure 1. **Panel A**. HPLC[7] profile of morpholidate **4**; **Panel B** ^{31}P-NMR (D_2O, H_3PO_4) of **4**; **Panel C**. MALDI-TOF mass spectrum of **4**. The peak at *m/z* 641.223 corresponds to the $(M+H)^+$ of **4**.

Based on the model reactions, the preparation of the dinucleoside phosphoramidates **9-10** was undertaken (Scheme 2). As before, the appropriate H-phosphonate dimers (5'-DMT off) were prepared and



Scheme 2. a) H-phosphonate synthesis; b) CCl_4/0.5M NH_3 in dioxane; c) I_2/pyr/MeOH; d) Satd. NH_3/DMF/55°C.

treated with a solution of ammonia in dioxane (0.5 M in dioxane/CCl_4, 1/1, 30 min) followed by iodine (2% *w/v* in pyridine/MeOH, 98/2, 30 min) to give the corresponding CPG-bound base-deprotected phosphoramidates **11-12**. Subsequently, the CPG-bound phosphoramidates **11-12**, were cleaved under the same conditions as employed for morpholidates (*vide supra*). However, analysis of the dinucleotides by HPLC,[7] revealed mainly the presence of nucleoside and unidentified products presumably resulting from the decomposition of the phosphoramidates upon exposure to alcoholic base or aqueous ammonia. Attempted cleavage of the CPG-bound dimers from the support using *N,N*-diisopropylethylamine in CH_3CN or DBU in CH_3CN were also unsuccessful. The 5'-DMT-on dimers[8] also gave the same results. Quite clearly, unlike the morpholidate linkage, the primary phosphoramidate linkages in **9-10** are unstable towards aqueous or alcoholic bases or even hindered bases. However, treatment of the CPG-bound phosphoramidates **11-12** with a saturated solution of anhydrous ammonia in dioxane (55 °C, 12-16 h) or anhydrous ammonia in dimethylformamide furnished the amidates **9-10**, as diastereomeric pairs (95% yields, as evaluated by recovered AU_{260} units and HPLC), which were fully characterized (Figure 2).

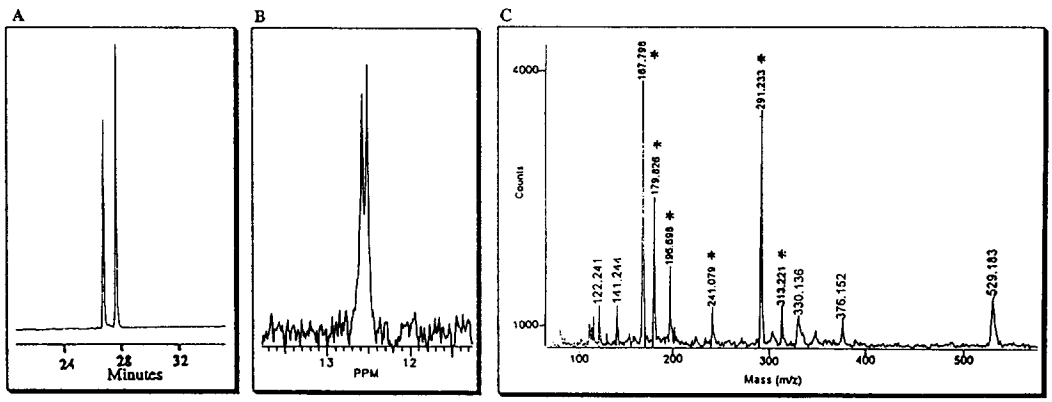


Figure 2. **Panel A**. HPLC[7] profile of amidate analog 5'-TC (PO-NH_2) (**9**). **Panel B** ^{31}P-NMR of **9**. **Panel C**. MALDI-TOF mass spectrum of **9**. The peak at *m/z* 529.183 corresponds to $(M-H)^-$. *represent matrix-associated peaks.

A trinucleotide phosphoramidate **13** was also prepared in a similar manner. The expected four diastereomers of **13** were well separated by HPLC (Fig. 3). Interestingly, ^{31}P-NMR of **13** (Fig. 3) revealed the presence of six peaks for the four diastereomers of **13** at ca. δ 12-13 ppm. In analogy with Harger's rationalization[3] that multiple peaks in the ^{1}H-NMR of simple phosphinic amides is due to the presence of dimeric species (**2a**) in solution, the potential presence of multimeric species of **13** can also be suggested. However, alternate interpretations are possible to explain the ^{31}P-NMR of spectrum of **13** and a distinction between the various possibilities must await further experiments that are currently under way.

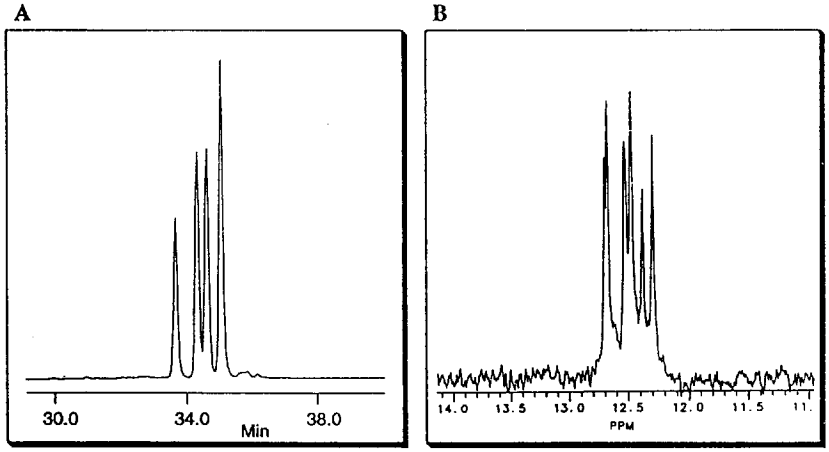


Figure 3. **Panel A**. HPLC profile of $[T]_3(PO\text{-}NH_2)$ (**13**); **Panel B**. ^{31}P-NMR (D_2O) of **13**.

Biophysical and biochemical studies of oligonucleoside phosphoramidates and the chimeric oligonucleotides[1b,c] bearing segments of phosphoramidates are also under way and will be reported in due course.

References and Notes

1. For reviews see: (a) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 544-584; (b) Agrawal, S, Iyer, R. P. *Curr. Op. Biotech.* **1995**, *6*, 12-19; (c) Agrawal, S.; Temsamani, J.; Galbraith, W.; Tang, J. *Clin. Pharmacokinet.* **1995**, *28*, 7-16.
2. (a) Froehler, B. C. *Tetrahedron Lett.* **1986**, *27*, 5575-78; (b) Agrawal, S.; Goodchild, J.; Civiera, M. P.; Thornton, A. H.; Sarin, P. S.; Zamecnik, P. C. *Proc. Natl. Acad. Sci. USA*, **1988**, *85*, 7079-83.
3. Harger, M. J. P. *J. Chem. Soc. Chem. Commun.* **1976**, 555-56.
4. Shabarova, Z.; Bogdanova, A. *Advanced Organic Chemistry of Nucleic Acids*, VCH Publishers Inc.: New York, 1994; pp. 169-180.
5. 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.
6. Iyer, R. P.; Devlin, T.; Habus, I., Ho, N-H.; Yu, D.; Agrawal, S. *Tetrahedron Lett.* (preceding paper).
7. For details of HPLC conditions and analysis, see Iyer, R. P.; Yu, D.; Agrawal, S. *Bioorg. Chem.* **1995**, *23*, 1-21.
8. The 5'-DMT group is conveniently removed by brief treatment using Dowex® see Iyer, R. P.; Jiang, Z.; Yu, D.; Tan, W.; Agrawal, S. *Synth. Commun.* **1995**, *25*, 3611-3623.

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