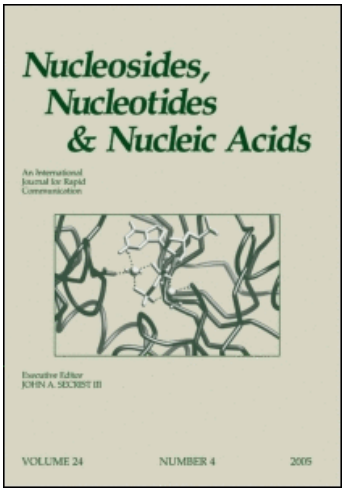


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis, Biophysical Properties, and Stability Studies of Mixed Backbone Oligonucleotides Containing Novel Non-Ionic Linkages

Radhakrishnan P. Iyer[a]; Dong Yu[a]; Theresa Devlin[a]; Nan-Hui Ho[a]; Suzanne Johnson[a]; Sudhir Agrawal[a]
[a] Hybridon Inc., Worcester, MA

To cite this Article Iyer, Radhakrishnan P. , Yu, Dong , Devlin, Theresa , Ho, Nan-Hui , Johnson, Suzanne and Agrawal, Sudhir(1997) 'Synthesis, Biophysical Properties, and Stability Studies of Mixed Backbone Oligonucleotides Containing Novel Non-Ionic Linkages', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 1491 — 1495
To link to this Article: DOI: 10.1080/07328319708006214
URL: http://dx.doi.org/10.1080/07328319708006214

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1491-1495 (1997)

SYNTHESIS, BIOPHYSICAL PROPERTIES, AND STABILITY STUDIES OF MIXED BACKBONE OLIGONUCLEOTIDES CONTAINING NOVEL NON-IONIC LINKAGES

Radhakrishnan P. Iyer, Dong Yu, Theresa Devlin, Nan-Hui Ho, Suzanne Johnson, and Sudhir Agrawal
Hybridon Inc., One Innovation Drive, Worcester, MA 01605.

ABSTRACT: Mixed backbone oligonucleotides (MBOs) (containing ionic and non-ionic internucleotidic linkages) in which the non-ionic segments are either methylphosphotriester (PO-OMe) or primary phosphoramidate (PO-NH_2) linkages have been prepared using the recently described *N*-pent-4-enoyl (*PNT*) nucleoside phosphoramidates and *H*-phosphonates. Biophysical properties and stability studies suggest that these MBOs are novel antisense molecules.

During the last decade, there has been considerable progress in the development of phosphorothioate oligonucleotides as potential therapeutic and diagnostic agents,[1a-c] and as unique tools in understanding cellular processes at the molecular level. The oligonucleotide-based drug design is characterized by its universal appeal and simplicity in that it employs well-understood principles governing molecular recognition. In this context, second generation analogs exemplifying the following properties are being sought: (a) higher selectivity; (b) enhanced resistance to cellular nucleases; (c) improved cellular uptake; (d) amplification of the antisense effect by induction of RNase H; (e) unique pharmaceutical properties which enable site- and tissue-specific delivery; (f) lesser polyanion-related side effects; and (g) oral bioavailability with favorable pharmacokinetic profile. Towards this goal, we have synthesized MBOs which contain methylphosphotriester (PO-OMe, PS-OMe)[2] and phosphoramidate (PO-NH_2)[3a-c] as the non-ionic linkages in conjunction with phosphoric diester (PO) and phosphorothioate (PS) as the ionic counterparts. Reported herein is the biophysical properties and stability studies of these MBOs.

MBOs with PO-OMe and PS-OMe linkages

Our synthetic strategy involves the use of phosphoramidite chemistry on controlled-pore glass support (CPG) (Scheme 1). Briefly, the PSOMe and POOMe linkages were constructed using *PNT* nucleoside methoxy phosphoramidite (*MEPNT*) synthons and the PO or PS segment using *PNT* nucleoside β-cyanoethyl phosphoramidite (*CEPNT*) synthons[4] respectively. Following the synthesis, the removal of the *PNT* groups, the β-cyanoethyl phosphate protecting groups, and cleavage of the oligonucleotide from the support were accomplished in one step by exposure to K_2CO_3/MeOH (0.05 M, rt, 30 to 36 h). The oligonucleotides were isolated by preparative polyacrylamide gel electrophoresis (PAGE) (7 M urea, 15-25 °C) using an assembly equipped for water circulation.[4b]

Fidelity of Oligonucleotide Synthesis

In order to ascertain the structural integrity of the PO-OMe group in the MBO, representative MBOs (having PO and POOMe linkages) were prepared and subjected to enzymatic digestion with *snake venom phosphodiesterase* and *bacterial alkaline phosphatase*. These studies revealed that the POOMe linkage was present to the extent of 97% and the presence of diester resulting from demethylation during purification and isolation were less than 3%. In the case of the MBOs having PS-OMe linkages, the expected integral ratio of the PSOMe/PS segment was observed in each case.[4a,b]

Biophysical properties

The thermal denaturation temperatures (T_ms) of complexes formed between MBOs (25-mers), and complementary DNA and RNA were determined as before. Representative data is revealed in Table 1. It is clear that in the case of the MBO analogs **1-7**, the incorporation of the triester segment into GEM 91® (**8**) only marginally affects the T_m values.

MBOs with phosphoramidate linkages

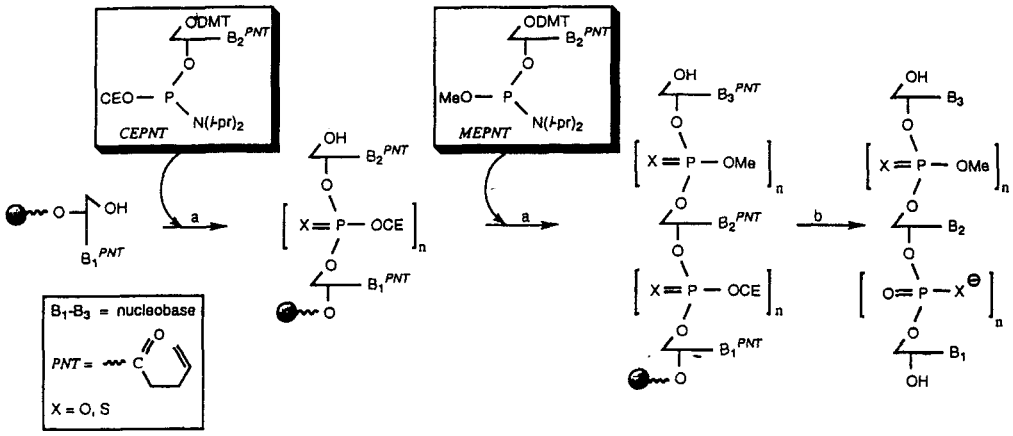
The P[O]NH_2 linkage is a novel non-ionic linkage as it is: (a) isosteric with phosphoric diester; and (b) is expected to make the MBO water soluble because of its potential to hydrogen-bond with water. As before, phosphoramidite chemistry was used to build the PO/PS linkage, and H-phosphonate chemistry for the PO-NH_2 linkage.

Fidelity of oligonucleotide synthesis

To ascertain that complete base and phosphate deprotection had occurred without any attendant base-modifications, enzymatic digestion of representative MBOs (having PO and PO-NH_2 linkages) was carried (*vide supra*). Our studies reveal that complete deprotection had been achieved while preserving the integrity of the PO-NH_2 linkage.[6a,b]

Biophysical properties

The thermal denaturation temperatures (T_ms) of complexes formed between MBOs (25-mers), and complementary DNA and RNA were determined. Representative data is revealed



SCHEME 1. Synthesis of MBOs; key: (a) phosphoramidite cycle using *tert*-butyl hydroperoxide as the oxidizing agent and 3*H*-1,2-benzodithiole-3-one-1,1-dioxide[6] as the sulfurizing reagent; (b) K_2CO_3 (0.05 M in MeOH).

TABLE 1. Comparative T_m Data of GEM 91® Analogs 1-7.

Seq. #	Sequence	T_m (°C)[¶]	T_m (°C)[§]
1	5' C•T•C TCG CAC CCA TCT CTC TCC TTC T (PSOMe / PS)	52.5	62.5
2	5' C•T•C•T CGC AC CCA TCT CTC TCC TTC T (PSOMe / PS)	51.5	61.8
3	5' C•T•C•T•C GCAC CCA TCT CTC TCC TTC T (PSOMe / PS)	50.7	60.6
4	5' CTC TCG CAC CCA TCT CTC TCCT T•C•T (PS / PSOMe)	51.0	62.0
5	5' CTC TCG CAC CCA TCT CTC TCC T•T•C•T (PS / PSOMe)	50.3	61.1
6	5' C•T•C TCG CAC CCA TCT CTC TCC T T•C•T (PSOMe / PS / PSOMe)	49.1	60.5
7	5' CTC TCG CAC CCA T•C•T•C TCT CCT TCT (PS / PSOMe / PS)	50.8	60.3
8	5' CTC TCG CAC CCA TCT CTC TCC TTC T (PS)	54.0	64.2

[¶] Against complementary DNA (30-mer) (PO); [§] Against complementary RNA (25-mer) (PO); All T_ms represent the average of at least two determinations.

TABLE 2. Comparative T_m Data of MBO Analogs 9 - 12 of GEM 91® (8)

Seq. #	Sequence	T_m¶	T_m§
9	5' C•T•C•T•C•GCA CCC ATC TCT CTC CTT CT PO-NH_2 PO	61.3	-
10	5' C•T•C•T•CGC ACC CAT CTC TCT CCT TCT PO-NH_2 PS	53.1	63.3
11	5' C•T•C TCG CAC CCA TCT CTC TCC TT•C•T PO-NH_2 PS PO-NH_2	55.3	63.8
12	5' CTC TCG CAC CC•A•T•C•T CTC TCC TTC T PS PO-NH_2 PS	52.0	59.8
8	5' CTC TCG CAC CCA TCT CTC TCC TTC T PS	55.0	65.1
13	5' CTC TCG CAC CCA TCT CTC TCC TTC T PO	64.8	-

¶ Against complementary DNA (30-mer) (PO); §Against complementary RNA (25-mer) (PO). All T_ms represent the average of at least two determinations forward and backward.

in Table 2. Our data shows that in the case of GEM 91® analogs 9-12, the incorporation of the phosphoramidate segment into GEM 91® (8) only marginally affects the T_m values.

Enzymatic stability of MBOs

Preliminary studies revealed that incorporation of non-ionic linkages (PSOMe, POOMe, or $PONH_2$) increases the stability of these MBOs against degradation by nucleases depending on the site of incorporation and the length of the non-ionic segment.[4b,6b]

Thus the MBOs with methylphosphotriester and phosphoramidate linkages represent novel antisense molecules. The evaluation of biological activity, and pharmacokinetics of the MBOs is in progress, and will be reported in due course.

REFERENCES

1. (a) Uhlmann E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 544. (b) Thuong, N. T.; Helene, C. *Angew. Chem. Intl. Ed. Engl.* **1993**, *32*, 666. (c) Agrawal, S.; Iyer, R. P.; *Curr. Op. Biotech.* **1995**, *6*, 12.
2. Miller, P. S.; Fang, K. N.; Kondo, N. S.; Tso, P. O. P. *J. Am. Chem. Soc.* **1971**, *93*, 6657.
3. (a) Tomasz, J. *Nucleosides & Nucleotides* **1983**, *2*, 51. (b) Letsinger, R. L.; Bach, S. A.; Eaddie, J. S. *Nucl. Acids Res.* **1986**, *8*, 3487. (c) Peyrottes, S.; Vasseur, J. J.; Imbach, J. L.; Rayner, B. *Nucl. Acids Res.* **1996**, *24*, 1841.

4. (a) Iyer, R. P.; Yu, D.; Ho, N. -H.; Devlin, T.; Agrawal, S. *J. Org. Chem.* **1995**, *60*, 8132. (b) For details, see: Iyer, R. P.; Yu, D.; Jiang, Z.; Agrawal, S. *Tetrahedron* **1996** (in press).
5. Iyer, R. P.; Egan, W.; Regan, J. B. *J. Am. Chem. Soc.* **1990**, *112*, 1253.
6. For details see: (a) Iyer, R. P.; Devlin, T.; Habus, I.; Yu, D.; Johnson, S.; Agrawal, S. *Tetrahedron Lett.* **1996**, *37*, 1543. (b) Devlin, T.; Iyer, R. P.; Johnson, S.; Agrawal, S. *Bioorg. Med. Chem. Lett.* **1996**, *24* (in press).