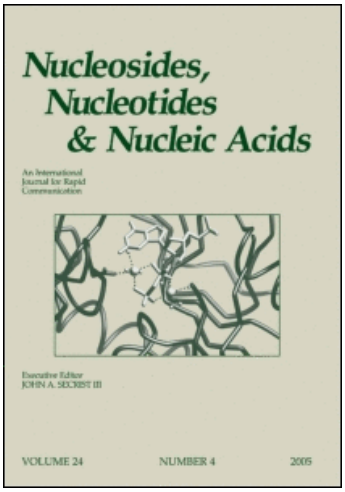


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis, Biophysical, and Biochemical Properties of PNA-DNA Chimeras

Zoltán Bajor[ab]; Gyula Sági[a]; Zsuzsanna Tegyey[a]; László Ötvös[a]
[a] Chemical Research Center, Institute of Chemistry, Hungarian Academy of Sciences, Budapest, Hungary [b] Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary

Online publication date: 09 August 2003

To cite this Article Bajor, Zoltán , Sági, Gyula , Tegyey, Zsuzsanna and Ötvös, László(2003) 'Synthesis, Biophysical, and Biochemical Properties of PNA-DNA Chimeras', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 1215 — 1217
To link to this Article: DOI: 10.1081/NCN-120022839
URL: http://dx.doi.org/10.1081/NCN-120022839

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1215–1217, 2003

Synthesis, Biophysical, and Biochemical Properties of PNA-DNA Chimeras

Zoltán Bajor,* Gyula Sági, Zsuzsanna Tegyey, and László Ötvös

Chemical Research Center, Institute of Chemistry,
Hungarian Academy of Sciences,
Budapest, Hungary

ABSTRACT

Three PNA-DNA chimeric dimer synthons (**tT**, **upT** and **uhT**, see Sch. 1) have been synthesized in solution and used to make T_{20}-analogue chimeras applying standard solid-phase DNA synthesis protocol. Duplex forming ability of chimeras with dA_{20} and their hydrolyses by 3′- and 5′-exonucleases (snake venom and bovine spleen phosphodiesterase, respectively) have been investigated.

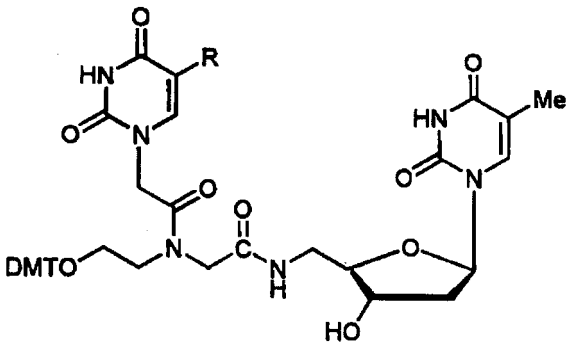
Key Words: 5-Alkynl-uracils; Dimer synthons; Chimeras; Thermal stability; Exonucleases.

According to the extended studies of Uhlmann et al.[1] PNA-DNA chimeras, consisting of PNA and DNA blocks, retain the RNase H inducing ability of natural DNA, in addition, they have higher thermal and enzymatic stability compared to the corresponding DNA or RNA counterparts. In order to avoid the difficulties coming from the different conditions of solid-phase PNA and DNA syntheses we prepared

*Correspondence: Zoltán Bajor, Hungarian Academy of Sciences, Chemical Research Center, 1025 Pusztaszeri út 59-67, H-1025 Budapest, Hungary; Fax: +36 14 38 4134; E-mail: bajor@chemres.hu.

DOI: 10.1081/NCN-120022839
1525-7770 (Print); 1532-2335 (Online)

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016



Scheme 1. **tT**: R = Me, **upT**: R = propyn-1-yl, **uhT**: R = 1-hexyn-1-yl.

the 3′-P-amidites and the LCAA-CPG bound 3′-succinates of 3 chimeric dimers (**tT**, **upT** and **uhT**, see Sch. 1) in solution and incorporated them into T_{20}, varying their number and position. Application of this strategy[2] made possible the exclusive use of routine oligonucleotide synthesis protocol for the preparation of PNA-DNA chimeras.

Thermal melting points (T_m) of all the chimera·dA_{20} duplexes were lower compared to that of T_{20}·dA_{20} ($T_m = +57.4°C$ in physiological solution), but the degree of T_m-drops strongly depended on the number and position of chimeric dimers incorporated. Placing of one single **tT**, **upT** or **uhT** block at the 3′-terminus of T_{20} resulted in a relatively small ($\Delta T_m = -1.8 - -2.7°C$) drop of T_m value while insertion of these building units into the middle of the sequence led to a significant decrease ($\Delta T_m = -9.1 - -7.6°C$). Consecutive incorporation of 5 **tT** blocks into the 5′-terminus brought about considerable T_m-drop ($\Delta T_m = -20.7°C$). The T_m value of (**tT**)$_5$-T_{10} (+36.7°C) was even lower than that of T_{11}·dA_{11} ($T_m = +38.6°C$) indicating that the chimeric 5′-half of this analogue is not involved in cooperative binding to the complementary strand, at all. On the other hand, if the 5 **tT** units are replaced by **upT** blocks a noticable duplex stabilization ($T_m = +41.6°C$) was observed, which correlates with the known duplex stabilizing effect of the short 5-alkynyl-substituted pyrimidine bases.[3] However, symmetrical positioning of the 5 **tT** blocks in T_{20} (the case of (T_2-**tT**)$_5$) led to complete disruption of the duplex structure ($T_m = +11.3°C$). Thermal stabilities of analogues with **uhT** building blocks are practically identical with those of the **tT** containing counterparts. In these cases the duplex stabilizing effect of triple bond is compensated by the hydrophobic destabilizing effect of long side-chain, which protrudes into the major groove and disrupts the water-structure around the phosphate moiety.

Analogues, containing one **tT**, **upT** or **uhT** unit at the 3′-terminus of T_{20}, proved 33–53 times more resistant to the hydrolysis by snake venom phosphodiesterase (SV PDE) than T_{20}. The degree of resistance unambiguously correlates with the increasing length of 5-alkynyl side-chain. Similar effect was observed for the hydrolysis of (**tT**)$_5$-T_{10}, (**upT**)$_5$-T_{10} and (**uhT**)$_5$-T_{10} as well, which were digested by bovine spleen phosphodiesterase (BS PDE). Surprisingly, in spite of the much longer chimeric strands the hydrolysis half times ($t_{1/2}$) were only 5–8 times higher relative to the

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

$t_{1/2}$ value of T_{20}. It can likely be attributed to the greater endonuclease activity of BS PDE compared to that of SV PDE.

ACKNOWLEDGMENTS

Financial support of OTKA (Hungarian Scientific Research Fund, project no.: T026472) is gratefully acknowledged. The authors are indebted to Mrs. Emma Belinszki and Mrs. Mária Baraczka for their valuable technical assistance.

REFERENCES

1. Uhlmann, E.; Will, D.W.; Breipohl, G.; Langner, A.; Ryte, A. Synthesis and properties of PNA/DNA chimeras. Angew. Chem. Int. Ed. Engl. **1996**, *35* (22), 2632–2635.
2. Wenninger, D.; Seliger, H. Synthesis and hybridization properties of modified oligonucleotides with PNA-DNA dimer blocks. Nucleosides & Nucleotides **1997**, *16*, 977–980.
3. Sági, J.; Szemz, A.; Ebinger, K.; Szabolcs, A.; Sági, Gy.; Ruff, É.; Ötvös, L. Base-modified oligodeoxynucleotides. I. Effect of 5-alkyl, 5-(1-alkenyl) and 5-(1-alkynyl) substitution of the pyrimidines on duplex stability and hydrophobicity. Tetrahedron Lett. **1993**, *34* (13), 2191–2194.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016