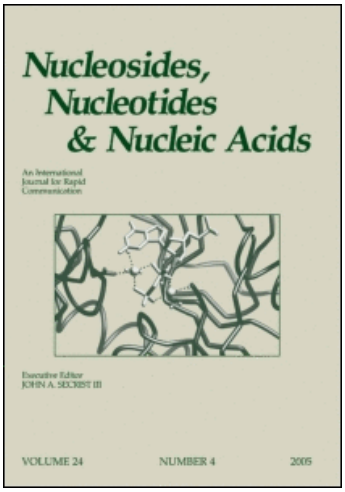

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Thermodynamic Studies on Hairpin Forming Antisense-Oligodeoxynucleotides Directed Against Hepatitis C Virus RNA

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To cite this Article Lehmann, Thomas J. , Eisenhardt, Stefan and Engels, Joachim W.(1999) 'Thermodynamic Studies on Hairpin Forming Antisense-Oligodeoxynucleotides Directed Against Hepatitis C Virus RNA', Nucleosides, Nucleotides and Nucleic Acids, 18: 6, 1689 — 1691
To link to this Article: DOI: 10.1080/07328319908044824
URL: http://dx.doi.org/10.1080/07328319908044824

NUCLEOSIDES & NUCLEOTIDES, 18(6&7), 1689–1691 (1999)

THERMODYNAMIC STUDIES ON HAIRPIN FORMING ANTISENSE-OLIGODEOXYNUCLEOTIDES DIRECTED AGAINST HEPATITIS C VIRUS RNA

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ABSTRACT: Phosphorothioate and benzyl-modified antisense-oligodeoxynucleotides directed against nucleotides 334-350 of the Hepatitis C Virus RNA form surprisingly stable hairpins. These data contribute to solve a structural detail information in search for a global secondary structure model of the Non Coding Region (NCR) of HCV.

Since the first cloning of the Hepatitis C Virus in 1989[1] several secondary structure models of its NCR have been published[2]. The NCR of the positive stranded HCV is 341 nts. in length, highly conserved and contains an internal ribosomal entry site (IRES) for the initiation of translation. Treatment of chronic HCV infection with alpha interferon is insufficient because of the high relapse rate. Antisense oligonucleotides are useful molecular tools for the treatment of viral diseases including HCV[3].

We synthesized terminally phosphorothioate and benzyl modified antisense oligonucleotides directed against the IRES of HCV. A proposed NCR secondary structure model from Honda[2c] postulates apart from a pseudo knot a hairpin structure with a five base pair forming stem and a heptaloop which contains the AUG initiation codon. Antisense oligodeoxynucleotides made for hybridization with the hairpin RNA sequence 5′-CGUGCACCAUGAGCACG-3′ (nts. 334-350) or truncated parts of it form surprisingly stable hairpin structures in the absence of the target RNA. Further investigations of the RNA sequence 5′-GUAGACCGUGC-3′ (nts. 328-338) designed following the structure of Brown[2a] yielded no self-assoziation. Determined sequences and T_m-values are shown in table 1.

TABLE 1. Melting temperatures of antisense ODN. Complementary sequences are underlined. Mismatched bases are in bold letters. Errors ± 0,5 °C. (n.d.= not detected)

sequences of phosphorothioate (°) and benzyl modified (*) ODN 350 345 340 335 330 (nts. of NCR of HCV)	**T_m-value [°C]**
3′- (segment of target HCV-RNA) C G U G C C A G A U G -5′	n.d.
5′- T°G°C°T C A T G G T G C A C G G T C T A°C°G°A -3′	42,4
5′- T°C°A°T G G T G C A C G G T C T A°C°G°A -3′	n.d.
5′- T°G°C°T C A T G G T G C A C G G T°C°T°A -3′	30,2
5′- T°G°C°T C A T G G T G C°A°C° -3′	n.d.
5′-C G T G C T C A T G G T G C A C G -3′	69,3
5′-C **C** T G C T C A T G G T G **G** A C G -3′	33,6
5′-C°G°T°G C T C A T G G T G C°A°C°G -3′	60,6
5′-C°G T°G C T C A T G G T G°C A°C G -3′	61,8
5′- G°T°G°C T C A T G G T G°C°A°C -3′	47,0
5′- T°G°C°T C A T G G T°G°C°A -3′	28,8
5′-C*G*T*G C T C A T G G T G C*A*C*G -3′	n.d.
5′-C*G T*G C T C A T G G T G*C A*C G -3′	56,0
5′- G*T*G*C T C A T G G T G*C*A*C -3′	n.d.
5′- T*G*C*T C A T G G T*G*C*A -3′	n.d.

Phosphate backbone modified oligonucleotides were synthesized using standard phosphoramidite chemistry. Characterization occurred by MALDI mass spectrometry. The T_m-values of the antisense oligonucleotides were determined by UV/VIS spectroscopy at 260 nm and 274 nm in phosphate buffer. To make sure not to measure also possible duplex hybridisation we found concentration independent T_m-values within a range from 1 μmol to 150 μmol as required for unimolecular transitions.

These results are in better agreement with the proposed secondary structure of Honda[2c] than with the published structures by Brown[2a] and Wang[2b] and contribute to answer a more detailed information in search for a global secondary structure model of the NCR of HCV.

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