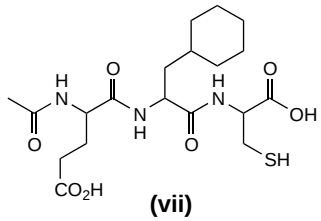
amino acids from peptide **(vi)** [K_i = 0.041 and 0.025 μM (Ref. 11) against full-length and truncated NS3, respectively] yielded a tripeptide inhibitor, **(vii)** (K_i = 4.1 and 230 μM against full-length and truncated NS3, respectively), which was more active against the full-length enzyme than the truncated enzyme. These results suggest that the full-length peptide is a better target with which to screen potential inhibitors against.



(vii)

8 Walker, M.A. (1999) Hepatitis C virus: an overview of current approaches and progress. *Drug Discov. Today* 4, 518–529

9 Yao, N. *et al.* (1999) Molecular views of viral polyprotein processing revealed by the crystal structure of the hepatitis C virus bifunctional protease-helicase. *Structure* 7, 1353–1363

10 Johansson, A. *et al.* (2001) Inhibition of hepatitis C virus NS3 protease activity by product-based peptides is dependent on helicase domain. *Bioorg. Med. Chem Lett.* 11, 203–206

11 Cicero, D.O. *et al.* (1999) Structural characterization of the interactions of optimized product inhibitors with the N-terminal proteinase domain of the hepatitis C virus (HCV) NS3 protein by NMR and modelling studies. *J. Mol. Biol.* 289, 385–396

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Gene therapy

DNA dumbbells: decoys for gene-regulatory proteins

The relative simplicity of synthesis, together with high affinity and specificity for a variety of targets, indicates that oligonucleotide-based therapeutics could be of considerable interest as an approach to rational drug design. Regulation of gene expression by targeting DNA and RNA with complementary single-stranded or folded (catalytic) oligonucleotides forms the basis of well-known antisense, antigene and ribozyme technologies in gene therapy[1–3]. Recently, the first antisense drug, phosphorothioate oligonucleotide fomivirsen, gained approval in the USA and Europe for the treatment of cytomegalovirus infections, earning the Galenus Prize. Furthermore, several anti-HIV hammerhead ribozymes are now in clinical trials.

There is another, less-studied gene-therapeutic strategy termed the 'sense approach', which is based on the targeting of another participant of the genetic machinery, regulatory proteins, with double-stranded oligonucleotides[4]. It is assumed that oligodeoxynucleotide duplexes that carry specific sites for binding with transcription factors might act as decoys at micromolar to nanomolar concentrations[4,5]. Hence, DNA duplexes closed at both termini by stretches of single-stranded nucleic acids or synthetic linkers are of substantial interest[5–11]. These nucleic acid constructs are known as DNA dumbbells because of their similarity in shape with a piece of sports equipment. DNA dumbbells have been considered to be promising oligonucleotide therapeutics mainly because of their increased nuclease resistance and the fact that they more readily enter cells than common DNA duplexes with open termini do[5].

Two general strategies for the preparation of DNA dumbbells have been elaborated. Biochemical syntheses were described on the basis of enzymatic ligation of either a pair of hairpin-like oligonucleotides with short dangling sticky ends[6] or a single, self-complementary, circularizable oligonucleotide[7]. Both these approaches are effective for the assembly of DNA dumbbells with 8–25 bp duplex stems.

However, they experience problems beyond this size range. The upper limit is caused by incorrect annealing of longer self-circularizable and hairpin-like oligonucleotides into non-circularized DNA duplexes, which result in decreased yield of large DNA dumbbells. The lower limit is imposed by steric and conformational constraints in small DNA dumbbells that compromise the efficiency of DNA ligase.

More recently, purely chemical syntheses have been developed[7,8]. Although both enzymatic and chemical approaches for DNA dumbbell assembly are robust, the latter might be preferred for large-scale production because it is faster and less costly[7]. Importantly, chemical syntheses enable DNA dumbbells to be designed that carry chemically reactive groups for covalent affinity modification (crosslinking) of DNA-recognizing proteins[8], as well as to produce DNA dumbbells with non-nucleotide loops, which modulate their lipophilicity and enhance biostability[9]. Using chemical syntheses, researchers have also been able to overcome the problems associated with producing very small DNA dumbbells intrinsic to biochemical approaches, and have yielded DNA dumbbells with duplex stems as short as 3 bp.

However, until recently, no developments have been reported to enlarge the size of DNA dumbbells. Larger DNA dumbbells could have significant advantages as evidently they might carry longer DNA sequences of biological relevance and hence, could be used in gene therapy for intracellular delivery of larger chunks of nuclease-resistant foreign DNA to more effectively regulate gene expression. Recently, Kuhn *et al.* reported a new biosynthetic approach for the high-yield and high-purity preparation of a record-length DNA dumbbell featuring a duplex stem of 94 bp (Ref. 10). The protocol involved enzymatic ligation between a pair of hairpin-like oligonucleotides with long dangling sticky ends followed by multiple biotinylation via nick-translation and/or

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primer-extension of any unligated precursors and mono-ligated byproducts. These were readily removed from the DNA dumbbell preparation using a mild biomagnetic separation procedure.

Besides DNA dumbbells, hybrid DNA–RNA dumbbells consisting of a sense RNA sequence and corresponding antisense DNA sequence could also find use in gene therapy[11]. It is assumed that these stable chimeric constructs, when delivered to cells, will be digested by intracellular Rnase H, thus liberating an antisense DNA sequence to enable it to perform its function. Purely RNA dumbbell-like constructs have also been shown to be efficient for therapeutic intervention of infections caused by RNA viruses acting as decoys for virus-activating proteins[12].

Based on the promising data presented to date in numerous papers on DNA dumbbells and other decoys, together with the significant progress in cellular delivery of oligonucleotides[3], there is reasonable optimism that these agents might soon be used to inhibit transcription of disease-causing genes.

1 Crooke, S.T. (2000) Progress in antisense technology: the end of the beginning. *Methods Enzymol.* 313, 3–45

2 Winters, T.A. (2000) Gene targeted agents: new opportunities for rational drug development. *Curr. Opin. Mol. Ther.* 2, 670–681

3 Hughes, M.D. *et al.* (2001) The cellular delivery of antisense oligonucleotides and ribozymes. *Drug Discov. Today* 6, 303–315

4 Bielinska, A. *et al.* (1990) Regulation of gene expression with double-stranded phosphorothioate oligonucleotides. *Science* 250, 997–1000

5 Clusel, C. *et al.* (1993) *Ex vivo* regulation of specific gene expression by nanomolar concentration of double-stranded dumbbell oligonucleotides. *Nucleic Acids Res.* 21, 3405–3411

6 Wemmer, D.E. and Benight, A.S. (1985) Preparation and melting of single strand circular DNA loops. *Nucleic Acids Res.* 23, 8611–8621

7 Lim, C.S. and Hunt, C.A. (1997) Synthesis of DNA dumbbells: chemical vs. enzymatic ligation of self-complementary oligonucleotides. *Nucleosides Nucleotides* 16, 41–51

8 Kuznetsova, S.A. *et al.* (1998) Design and synthesis of double-stranded oligonucleotides containing reactive acylphosphate internucleotide groups. *FEBS Lett.* 431, 453–456

9 Kozerski, L. *et al.* (2001) A nicked duplex decamer DNA with a PEG6 tether. *Nucleic Acids Res.* 29, 1132–1143

10 Kuhn, H. *et al.* (2001) High-purity preparation of a large DNA dumbbell. *Antisense Nucleic Acid Drug Dev.* 11, 149–153

11 Yamakawa, H. *et al.* (1998) Properties of nicked and circular dumbbell RNA/DNA chimeric oligonucleotides containing antisense phosphodiester oligodeoxynucleotides. *Bioorg. Med. Chem.* 6, 1025–1032

12 Ma, M.Y-X. *et al.* (1993) Design and synthesis of RNA miniduplexes via a synthetic linker approach. 2. Generation of covalently closed, double-stranded cyclic HIV-1 TAR RNA analogs with high Tat-binding affinity. *Nucleic Acids Res.* 21, 2585–2589

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