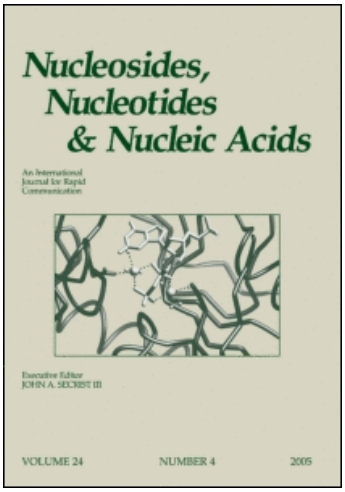
This article was downloaded by:
On: *26 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



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

Towards a Circular bis-Peptide Nucleic Acid

R. H. E. Hudson[a]; J. Tse[a]
[a] Department of Chemistry, The University of Western Ontario, Ontario, London, Canada

Online publication date: 09 August 2003

To cite this Article Hudson, R. H. E. and Tse, J.(2003) 'Towards a Circular **bis**-Peptide Nucleic Acid', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 1023 — 1027
To link to this Article: DOI: 10.1081/NCN-120022727
URL: http://dx.doi.org/10.1081/NCN-120022727

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1023–1027, 2003

Towards a Circular *bis*-Peptide Nucleic Acid

R. H. E. Hudson* and J. Tse

Department of Chemistry, The University of Western Ontario, London, Ontario, Canada

Downloaded At: 11:10 26 January 2011

ABSTRACT

En route to a circular bis-PNA molecule, we have synthesized and characterized the DNA binding of several ''clamp''-type bis-PNAs. In order to incorporate charge into a circular PNA, a new linker based on the achiral 2-aminoethylglycine has been used.

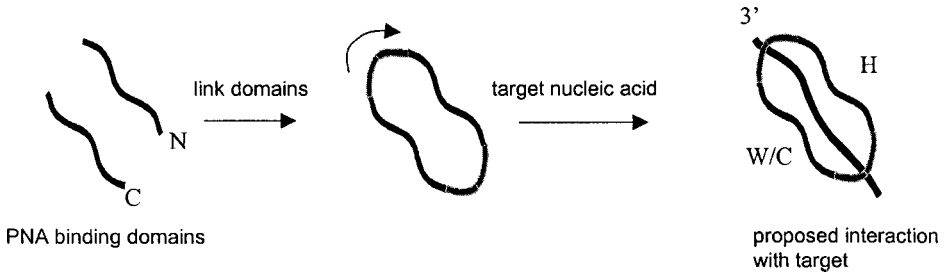
Various strategies have evolved in DNA chemistry to facilitate the formation of triple helices.[1] In this vein, Nielsen and coworkers originally developed peptide nucleic acid (PNA) as a triplex-forming ligand.[2] However, it was discovered that pyrimidine motif triple-helices tend to form by a strand invasion mechanism at A-rich target sequences to yield PNA(Y):DNA(R):PNA(Y) structures. To favour this interaction, ''clamp''-type bis-PNA molecules in which the PNA binding domains are covalently linked at *one end only* have been reported.[3] Our aim is to synthesize a circular bis-PNA molecule in which the PNA binding domains are covalently linked at *both ends*, Sch. 1. Such a molecule is hypothesized to be even more efficient at forming triple helices than ''clamp''-type bis-PNA molecules by analogy to DNA-based systems. Combined with internal modifications, especially cationic groups, this type of molecule may perform better than clamp-type bis-PNA under physiological ionic

*Correspondence: R. H. E. Hudson, Department of Chemistry, The University of Western Ontario, Room 209, N6A 5B7 London, Ontario, Canada; Fax: +1519 661 3022; E-mail: rhhudson@uwo.ca.

DOI: 10.1081/NCN-120022727
1525-7770 (Print); 1532-2335 (Online)
Copyright © 2003 by Marcel Dekker, Inc.
www.dekker.com

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

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



Scheme 1. Cartoon representation of the proposed biomolecular triplex formation by a circular PNA.

conditions. Study of the circular PNA will also allow us to investigate the contemporary thoughts on the mechanism of strand displacement triplex formation.

Peptide nucleic acid is a nucleic acid mimic based on a 2-aminoethylglycine polyamide with pendant nucleobase (B = Ade, Gua, Thy, Cyt, etc.) groups as shown in Fig. 1. Homopyrimidine PNA can form triple-helices of the composition PNA(Y):DNA(R):PNA(Y) at A-rich target sequences. If the target exists as a duplex, these triplexes will occur by strand invasion, under appropriate conditions. The formation of these structures is favoured by covalently linking the PNA binding domains together creating molecules known as bis-PNA. Several 'linker' structures have been used to connect the PNA domains that vary in length and composition e.g., the 27-atom tris(8-amino-3,5-dioxaoctanoic acid),[3] 23-atom based on lysine and 6-aminohexanoic acid,[4] 24-atom linker based on a trioxa-amino acid[4] and an 18-atom hexapeptide linkage.[5]

Starting with the available x-ray crystallographic structure,[5] we have designed a novel linker that is based on the backbone monomer of PNA, Sch. 2. Preliminary molecular modeling results using MM+ force field suggest that the energy of a linker having a composition of aeg-gly-aeg-gly-aeg (aeg = *N*-2-aminoethylglycine, gly = glycine) will be similar to those reported earlier. This linker offers the opportunity of studying the effect of placing positive charges in the linker region, without the introduction of chiral centers. Importantly, the linker has enough length and conformational flexibility to loop out of the way of a DNA strand that is longer than the bis-PNA molecule.

The sequence chosen for the DNA target strand is 5′-AAGGAAA-3′ that is amenable to triplex formation. For future comparison to an authentic closed circular bis-PNA, we have synthesized the sequences listed in Table 1 which include the components for a termolecular triplex and two structural isomers of a bimolecular

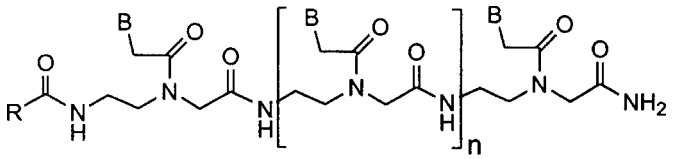
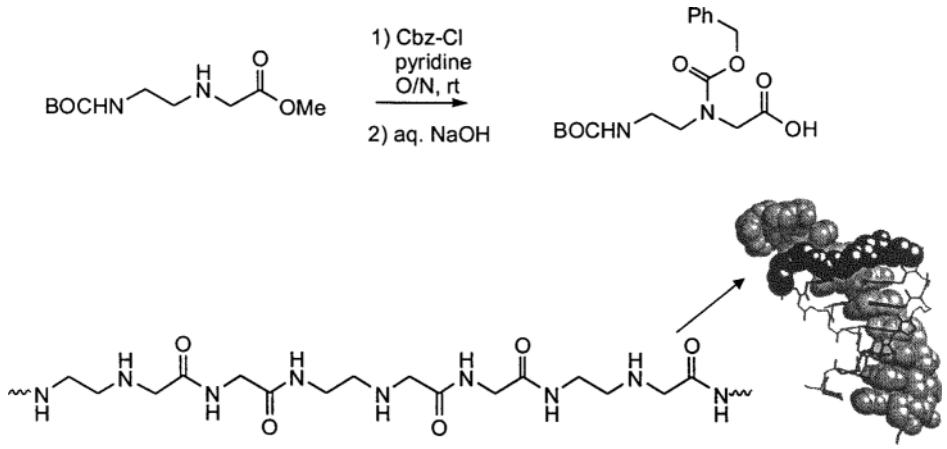


Figure 1. Generic structure of PNA.

Downloaded At: 11:10 26 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

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



Scheme 2. Linker synthesis and composition. Notes: Coordinates for bis-PNA complexed with DNA have been adapted from Ref.[8]. DNA strand rendered in space filling mode, bis-PNA rendered in wireframe (PNA sequences) and space filling (peptidic linker).

Table 1. Sequences used.

Number	PNA sequences
1[a]	H_2N-lys-TTCCTTT-lys-CO_2H
2[b]	H_2N-lys-TTTCCTT-lys-CO_2H
3[c]	H_2N-TCTCTTT-$(egl)_3$-TTTCTCT-lys-NH_2
4[c]	H_2N-TTCCTTT-$(egl)_3$-TTTCCTT-lys-NH_2
5[c]	H_2N-TCTCTTT-aeg-gly-aeg-gly-aeg-TTTCTCT-lys-NH_2
6[c]	H_2N-TTCCTTT-aeg-gly-aeg-gly-aeg-TTTCCTT-lys-NH_2
7[d]	H_2N-CTT-aeg-gly-aeg-gly-aeg-TTCCTTT- aeg-gly-aeg-gly-aeg-TTTC-CO_2H
8[d]	H_2N-CTTT-aeg-gly-aeg-gly-aeg-TTTCCTT- aeg-gly-aeg-gly-aeg-TTC-CO_2H
	DNA sequences
9	5′-CGCAAGGAAACGC-3′
10	5′-CGCAAAGGAACGC-3′
11	5′-CGCAGAGAAACGC-3′
12	5′-CGCAAAGAGACGC-3′
9X[e]	5′-CGCAAG***X***AAACGC-3′
10X[e]	5′-CGCAAA***X***GAACGC-3′
11X[e]	5′-CGCAGA***X***AAACGC-3′
12X[e]	5′-CGCAAA***X***AGACGC-3′

[a]Hoogsteen complementary strand.
[b]Watson-Crick complementary strand.
[c]Clamp bis-PNA.
[d]Pre-circular bis-PNA.
[e]DNA mismatch sequences (***X*** = A, C, T). PNA sequences were synthesized by standard manual synthesis utilizing the BOC/CBz strategy and their identity confirmed by MALDI-MS analysis. Definitions: aeg = 2-aminoethylglycine; egl = 8-amino-3,6-dioxaoctanoic acid.

Downloaded At: 11:10 26 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

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

triplex namely the clamp and pre-circular bis-PNAs. Hybridization studies with fully matched DNA (Table 2) and target sequences containing a single mismatch for complexes with either a 5′- or 3′-linker crossover indicate excellent sequence discrimination (data not shown). In general, a lower buffer pH favors both duplex and triplex formation, presumably based on a higher net charge on the PNA components and protonation of the cytosine nucleobase (for triplex formation). Two sequences and two linkers were examined in the context of a clamp-type bis-PNA, that was previously reported by Nielsen and coworkers [3] termed Nielsen sequence (NS), Nielsen linker (NL) and our sequence (HS) and linker (HL). Each PNA domain has the same composition but slightly different sequence. Within the experimental error, there is no sequence effect (cf. NS, NL complex 3:12 and HS, NL complex 4:10) nor a substantial effect for 3′-crossover vs. 5′-crossover. These data also show that the aeg-gly (HL) linker is comparable to the established egl (NL) linker, offering clearly better stability only under acidic conditions. This suggests quaternarizing the secondary amino group may offer a significant stabilizing effect even at neutral pH. The stability of the complex formed by the pre-circular ligand is somewhat less stable than the

Table 2. Thermal denaturation studies.

Complex composition	Binding mode	Tm, °C	
		pH = 5	pH = 7
Linear			
1:9	parallel duplex	58.2	44.4
1:10	antiparallel duplex	59.5	45.3
2:9	antiparallel duplex	50.2	35.0
2:10	parallel duplex	47.4	30.7
1:2:9	triplex	61.0, 49.0 (b)	46.5, 31.0 (b)
1:2:10	triplex	60.0, 49.5 (b)	46.6, 35.8 (b)
Clamp			
3:12	5′-crossover NS, NL	68.0	55.5
4:10	5′-crossover HS, NL	67.0	53.5
5:12	5′-crossover NS, HL	73.5	54.5
6:10	5′-crossover HS, HL	72.0	53.5
3:11	3′-crossover NS, NL	69.0	54.6
4:9	3′-crossover HS, NL	71.5	55.6
5:11	3′-crossover NS, HL	74.5	56.0
6:9	3′-crossover HS, HL	78.0	57.5
Pre-circular			
7:9	W/C break	64.0	47.0
8:9	Hoogsteen break	55.0	36.5
7:10	Hoogsteen break	60.0	46.0
8:10	W/C break	58.0	36.2

Note: All thermal denaturation measurements were done in the following buffer: 10 mM PO_3^{4-}, 100 mM Na^+, 0.1 mM EDTA, pH 7. pH adjusted with HCl. Tm values were estimated by the first derivative method (± 0.5°C). b = biphasic.

Downloaded At: 11:10 26 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

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

clamp-type bis-PNAs as judged by their respective Tm values. This is probably due to the break in one of the binding domains. However, the thermal stability of these complexes, in the best cases, are comparable to that of the termolecular triplex but exhibit a monophasic melting transition suggesting that both PNA domains are binding in a cooperative manner.

We are currently pursuing carbodiimide-mediated ligation of the pre-circular bis-PNA assembled on a DNA template,[6,7] and will present these results in due course.

ACKNOWLEDGMENTS

The authors wish to thank NSERC (Canada) for support of this work (RHEH). NSERC and the Ontario Graduate Scholarship Program are thanked for support (JT). We are also grateful for support from the University of Western Ontario through the Academic Development Fund.

Downloaded At: 11:10 26 January 2011

REFERENCES

1. Kool, E.T. Recognition of DNA, RNA, and proteins by circular oligonucleotides Acc. Chem. Res. **1998**, *31*, 502–510.
2. Nielsen, P.E.; Egholm, M.; Berg, R.H.; Buchardt, O. Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. Science **1991**, *254*, 1497–1500.
3. Egholm, M.; Christensen, L.; Dueholm, K.L.; Buchardt, O.; Coull, J.; Nielsen, P.E. Efficient pH-independent sequence-specific DNA binding by pseudoisocytosine-containing bis-PNA. Nucleic Acids Res. **1995**, *23*, 217–222.
4. Griffith, M.C.; Risen, L.M.; Greig, M.J.; Lesnik, E.A.; Sprankle, K.G.; Griffey, R.H.; Kiely, J.S.; Freier, S.M. Single and bis peptide nucleic acids as triplexing agents: Binding and stoichiometry. J. Amer. Chem. Soc. **1995**, *117*, 831–832.
5. Betts, L.; Josey, J.A.; Veal, J.M.; Jordan, S.R. A nucleic-acid triple-helix formed by a peptide nucleic-acid DNA complex. Science **1995**, *270*, 1838–1841.
6. Schmidt, J.G.; Christensen, L.; Nielsen, P.E.; Orgel, L.E. Information transfer from DNA to peptide nucleic acids by template-directed syntheses. Nucleic Acids Res. **1997**, *25*, 4792–4796.
7. Mattes, A.; Seitz, O. Sequence fidelity of a template-directed PNA-ligation reaction. Chem. Comm. **2001**, *20*, 2050–2051.
8. Betts, L.; Josey, J.A.; Veal, J.M.; Jordan, S.R. The Protein Data Bank, 1PNN. Science **1995**, *270*, 1838–1841.

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

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

Downloaded At: 11:10 26 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

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