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Modular Nucleic Acid Surrogates. Solid Phase Synthesis of α-Helical Peptide Nucleic Acids (αPNAs)

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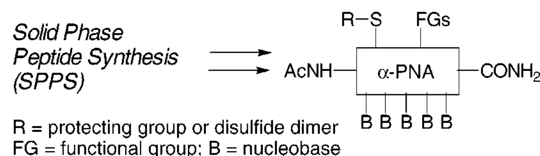
ABSTRACT



The synthesis and characterization of prototype α-helical peptide nucleic acid (αPNA) modules 1–3 as well as disulfide dimers 4 and 5 are reported. These molecules combine an α-helical peptidyl scaffold with well-defined nucleobase molecular recognition patterns and could serve as a basis for novel antisense and/or antigene agents. Structure assignments for these αPNAs were supported by MALDI-TOF mass spectrometry, and the α-helical nature of 4 in water was confirmed by circular dichroism (CD) spectroscopy.

In most DNA-regulatory proteins, α-helical subunits serve as the molecular scaffolding for presentation of key amino acid side chains to their specific nucleic acid binding sites.[1] Sequence-specific binding of these α-helical binding domains to dsDNA occurs in the major groove as a consequence of multiple interactions arrived at (combinatorially) through evolutionary selection. Despite work toward the elucidation of Nature's "code" for molecular recognition of dsDNA,[2] it is not yet feasible to rationally design a peptide structure that will bind to any particular DNA duplex.[3] On the other hand, molecular recognition of single-stranded nucleic acids via Watson–Crick (W–C) base pairing is quite predictable and provides the basis for antisense approaches to genetic manipulation.[4] Nielsen's pioneering discovery that the (2-deoxy)ribose phosphate framework of nucleic acids could be replaced by a much simpler polyamide backbone has spawned considerable interest in "peptide nucleic acids" (PNAs)[5] and related constructs.[6] However, the merger of an α-helical peptidyl scaffold with well-defined nucleobase molecular recognition patterns (W–C base pairing for ssRNA and Hoogsteen base pairing for dsDNA) has not been explored. A particular advantage of such hybrid structures, which we term as α-helical peptide-based nucleic acids or αPNAs,[7] would be the opportunity to functionalize the peptide scaffold itself to enhance transport, hybridization, and other properties.

We now report the synthesis and characterization of prototype αPNA modules Ac-C(Acm)-G-S^T-D-A-E-S^T-A-A-K-S^T-A-A-E-S^T-A-Aib-A-S^T-K-G-NH_2 (**1**), Ac-Aib-G-S^T-D-A-E-S^T-A-A-K-S^T-A-A-E–S^T-A-Aib-A-S^T-K-C(Acm)-NH_2 (**2**), and Ac-C(Acm)-G-S^C-D-A-E-S^C-A-A-K-S^C-A-A-E-S^C-A-Aib-A-S^C-K-G-NH_2 (**3**), as well as the tail-to-tail dimer [Ac-C-G-S^T-D-A-E-S^T-A-A-K-S^T-A-A-E-S^T-A-Aib-A-S^T-K-G-NH_2]$_2$ (**4**) and head-to-head dimer [Ac-Aib-G-S^T-D-A-E-S^T-A-A-K-S^T-A-A-E-S^T-A-Aib-A-S^T-K-C-NH_2]$_2$ (**5**).[8]

(1) Cf. Steitz, T. A. *Q. Rev. Biophys.* **1990**, *23*, 205.
(2) (a) Desjarlais, J. R.; Berg, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7345. (b) Desjarlais, J. R.; Berg, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 2256. (c) Rebar, E. J.; Pabo, C. O. *Science* **1994**, *263*, 671. (d) Jamieson, A. C.; Kim, S.-H.; Wells, J. A. *Biochemistry* **1994**, *33*, 5689. (e) Choo, Y.; Klug, A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11163.
(3) For an alternative approach to dsDNA recognition via the minor groove, see: Trauger, J. W.; Baird, E. E.; Dervan, P. B. *Nature* **1996**, *382*, 559.
(4) *Antisense Research and Application*; Crooke, S. T., Ed.; Springer-Verlag: Berlin, 1998.
(5) PNA reviews: (a) Dueholm, K. L.; Nielsen, P. E. *New J. Chem.* **1997**, *21*, 19. (b) Eriksson, M.; Nielsen, P. E. *Q. Rev. Biophys.* **1996**, *29*, 369.

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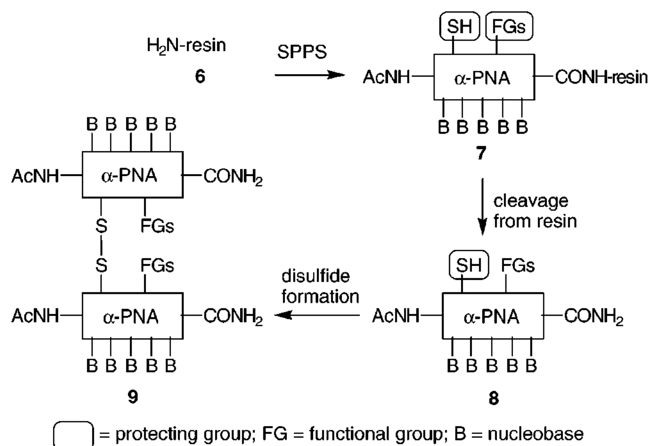
The primary amino acid sequences of these prototype αPNA modules **1−3** incorporate residues known to favor α-helix formation.[9] Specifically, these peptide backbones include hydrophobic amino acids (A and Aib), internal salt bridges (E-$(aa)_3$-K-$(aa)_3$-E), a macrodipole (D-$(aa)_{15}$-K), and an *N*-acetyl cap.[10] The C-termini of these αPNA modules end in a carboxamide function to preclude any potential intramolecular end effects. Cysteine residues allow for the implementation of a "disulfide stitchery" strategy to connect the αPNA modules at either termini.[11] Finally, each αPNA module incorporates five properly spaced nucleobases for Watson−Crick base-pairing to a target nucleic acid sequence. These nucleobases are attached via a methylene link to serine hydroxyls in order to preserve the *N*-glycoside (O−C−N) substructure found in nucleic acids.[12] A molecular model of **4** bound to B-helical dA_{10} suggests that, despite some expected deformation after energy minimization (Insight II/CVFF−see Supporting Information for details), this αPNA design is geometrically reasonable (Figure 1).

Figure 1. Ribbon representation of energy-minimized molecular model of αPNA(T10) "tail-to-tail" dimer **4** bound to B-helical $(dA)_{10}$.

The general synthetic route to dimeric αPNAs is depicted in Scheme 1 and consists of three distinct stages: (1) solid-phase peptide synthesis (SPPS) of the resin-bound B5 module **7**, (2) cleavage these peptides from the resin with concomitant deprotection of all aa residues except cysteine to give thiol-protected αPNA module **8**, and (3) thiol deprotection and disulfide bond formation to produce the B5 dimer **9**. Because the modified serine residue is potentially both acid- and base-labile, the strategic and tactical issues associated with the SPPS of these αPNAs resemble those associated with glycopeptides,[13] making the endeavor nontrivial. Accordingly, we chose the base-labile Fmoc protecting group (PG) for the N-terminal amines and acid-labile PGs (BOC and *tert*-butyl ester) for all side chain functionality excepting C. The I_2-labile acetamidomethyl (Acm) PG was chosen for the C residue to facilitate a separate disulfide formation

Scheme 1. General Protocol for αPNA Synthesis



step.[14] The nucleobase-modified serine derivatives **14** and **15** were prepared by the route shown in Scheme 2. The acid-labile Rink amide linker[15] was employed so that release of a fully extended peptide amide from the resin and removal of the acid-labile PGs could be achieved at the same time.

(6) (a) Weller, D. D.; Daly, D. T.; Olsen, W. K.; Summerton, J. E. *J. Org. Chem.* **1991**, *56*, 6000. Huang, S.-B.; Nelson, J. S.; Weller, D. D. *J. Org. Chem.* **1991**, *56*, 6007. (These papers predate Nielsen's original publication.) (b) Garner, P.; Yoo, J. U. *Tetrahedron Lett.* **1993**, *34*, 1275. (c) Lewis, I. *Tetrahedron Lett.* **1993**, *34*, 5697. (d) Almarsson, O.; Bruice, T. C.; Kerr, J.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7518. (e) Lenzi, A.; Reginato, G.; Taddei, M. *Tetrahedron Lett.* **1995**, *36*, 1713. Lenzi, A.; Reginato, G.; Taddei, M.; Trifilieff, E. *Tetrahedron Lett.* **1995**, *36*, 1717. See also: Ceulemans, G.; Khan, K.; Van Schepdael, A.; Herdewijn, P. *Nucleosides Nucleotides* **1995**, *14*, 813. (f) Loy, E.; Kessler, H. *Liebigs Ann.* **1996**, 201. (g) Jordon, S.; Schwemler, C.; Kosch, W.; Kretschmer, A.; Schwenner, E.; Stropp, U.; Mielke, B. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 681. See also: Gangamani, B. P.; Kumar, V. A.; Ganesh, K. N. *Tetrahedron* **1996**, *52*, 15017. (h) Lowe, G.; Vilaivan, T. J. *J. Chem. Soc., Perkin Trans. 1* **1997**, 539; 547; 555. (i) Begmeier, S. C.; Fundy, S. L. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3135. (j) Goodnow, R. A., Jr.; Richou, A.-R.; Tam, S. *Tetrahedron Lett.* **1997**, *38*, 3195. Goodnow, R. A., Jr.; Tam, S.; Pruess, D. L.; McComas, W. W. *Tetrahedron Lett.* **1997**, *38*, 3199. (k) Altmann, K.-H.; Chiesi, C. S.; García-Echeverría, C. *Biorg. Med. Chem. Lett.* **1997**, *7*, 1119. García-Echeverría, C.; Hüsken, D.; Chiesi, C. S.; Altmann, K.-H. *Biorg. Med. Chem. Lett.* **1997**, *7*, 1123. Kuwahara, M.; Arimitsu, M.; Sisido, M. *J. Am. Chem. Soc.* **1999**, *121*, 256. (l) Cantin, M.; Schütz, R.; Leumann, C. J. *Tetrahedron Lett.* **1997**, *38*, 4211. (m) Howarth, N. M.; Wakelin, L. P. G. *J. Org. Chem.* **1997**, *62*, 5441. (n) Tsantrizos, Y. S.; Lunetta, J. F.; Boyd, M.; Fader, L. D.; Wilson, M.-C. *J. Org. Chem.* **1997**, *62*, 5451.

(7) Wakelin (ref 6m) has proposed the term "α-PNA" to describe peptide nucleic acids made up of α-amino acid building blocks. We feel that his usage of the α-designation is somewhat redundant (since a peptide is, by definition, made up of α-amino acids) and less descriptive (it could be applied to many of the systems described in ref 6).

(8) Amino acid abbreviations: aa = generic amino acid, A = Ala, Aib = 2-aminoisobutyric acid, C = Cys, D = Asp, E = Glu, G = Gly, K = Lys, S^T = 1-[(Ser)methyl]thymine, S^C = 1-[(Ser)methyl]cytosine.

(9) Our prototype αPNA design was based on the amphiphilic helix sequence contained in Baltzer's SA-42 helix-loop-helix: Olofsson, S.; Johansson, G.; Baltzer, L. *J. Chem. Soc., Perkin Trans. 2* **1995**, 2047.

(10) Cf. Regan, L.; DeGrado, W. F. *Science* **1988**, *241*, 976.

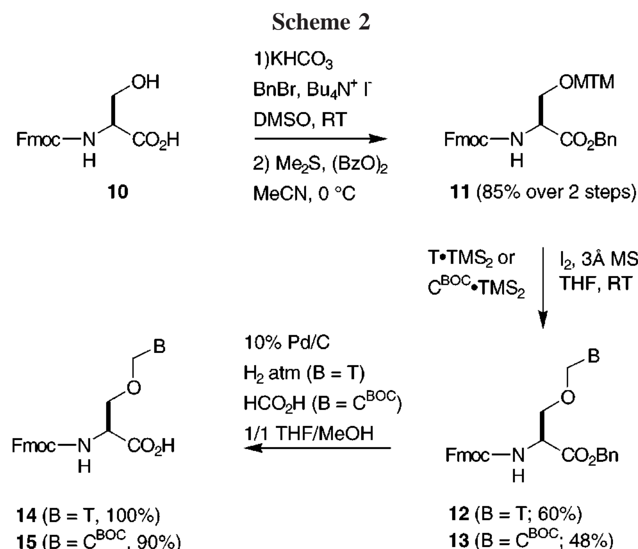
(11) Park, C.; Campbell, J. L.; Goddard, W. A., III. *J. Am. Chem. Soc.* **1995**, *117*, 6287.

(12) Since our original publication on peptide-based nucleic acid surrogates (ref 6b), we discovered that a synthesis of racemic Bz-Ser[CH_2U]-OMe had been reported in the Russian chemical literature: Timoshchuk, V. A.; Olimpieva, T. I. *Zh. Obsh. Khim.* **1988**, *58*, 2404.

(13) Cf. Paulsen, H.; Schleyer, A.; Mathieux, N.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1997**, 281.

(14) Veber, D. F.; Milkowski, J. D.; Varga, S. L.; Denkewalter, R. G.; Hirschmann, R. *J. Am. Chem. Soc.* **1972**, *94*, 5456.

Scheme 2



Finally, DBU[16] was used for Fmoc deprotection and Carpino's HATU[17] reagent was used for all peptide couplings.

Our optimized experimental protocol could be applied to either the manual or semiautomated SPPS of αPNAs and provided peptides **1** and **2** in 16% and 15% overall yields, respectively, from commercially available Fmoc-Rink-MBHA resin after purification by gradient reverse-phase HPLC. Subsequent I_2-mediated Acm deprotection/disulfide formation[18] of T5 monomer **1** provided the tail-to-tail αPNA dimer **4** in 84% yield after HPLC purification. Similar treatment of module **2** resulted in a 49% purified yield of the head-to-head dimer **5**. Structure assignments for the T5 modules **1** and **2**, C5 module **3**, and the homodimers **4** and **5** were supported by MALDI-TOF mass spectrometry: ([**1**•H]$^+$, calcd mass 2685.12, found 2683.06; [**2**•H]$^+$, calcd mass 2714.1627, found 2713.15; [**3**•H]$^+$, calcd mass 2714.1627, found 2713.15; [**4**•H]$^+$, calcd mass 5225.15, found 5222.40; [**5**•H]$^+$, calcd mass 5281.21, found 5282.26).

The α-helical nature of **4** in water was confirmed by circular dichroism (CD) spectroscopy (cf. Figure 2). The double minimum at 220 and 206 nm and maximum at 193 nm are characteristic of an α-helix[19] while the isodichroic point at 202 nm was suggestive of a temperature-dependent

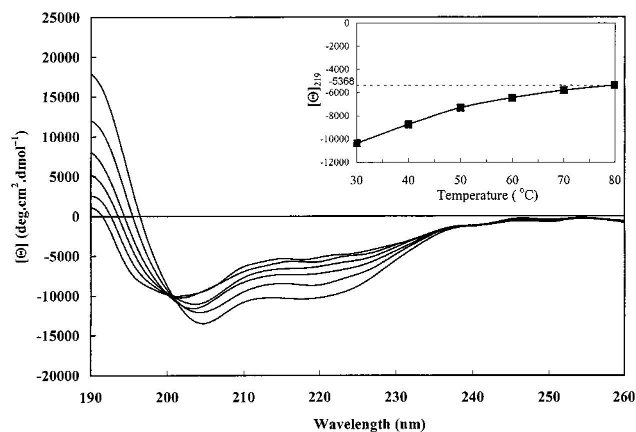


Figure 2. CD spectra of tail-to-tail dimer **4** (21 μM in H_2O) at 30, 40, 50, 60, 70, and 80 °C.

α-helix to random coil transition. The helical content of **4** at 20 °C in water was estimated to be 26%.[20] In conclusion, a practical solid-phase synthesis of prototype α-helical peptide nucleic acids (αPNAs) has been achieved and their propensity toward α-helix formation confirmed. Binding studies are now underway in our laboratories. Preliminary UV melting experiments show that αPNAs do form complexes with complementary DNA targets (**4**•dA_{10}, T_m = 51 °C; **5**•dA_{10}, T_m = 39 °C), thus validating the general concept.

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Supporting Information Available: Experimental procedures and characterization data for compounds **1**−**10** as well as UV melting experiments on **4**•dA_{10} and **5**•dA_{10}. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) (Rink amide linker = 4-(2′,4′-dimethoxyphenylaminomethyl)-phenoxyacetamido) Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787.

(16) (DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.) Wade, J. D.; Bedford, J.; Sheppard, R. C. Tregear, G. W. *Pept. Res.* **1991**, *4*, 194. A control experiment with Fmoc-Ser[CH_2T]-OMe indicated that the use of 20% piperidine led to the elimination of free thymine.

(17) (HATU = *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.

(18) Kamber, B.; Hartmann, A.; Eisler, K.; Riniker, B.; Rink, H.; Sieber, P.; Rittel, W. *Helv. Chim. Acta* **1980** *63*, 899.

(19) Holzwarth, G.; Doty, P. *J. Am. Chem. Soc.* **1965**, *87*, 218.

(20) Helical content was estimated by taking the ratio of ($[\theta]_{219} - [\theta]_0$)/$[\theta]_{100}$, where $[\theta]_{219}$ is the mean residual helicity at 219 nm (= −14850 deg cm^2 $dmol^{-1}$) and $[\theta]_0$ is the "background" mean residue ellipticity at 0% helicity (= −5368 deg cm^2 $dmol^{-1}$) as determined by a melting experiment (Figure 2, inset). To account for the length dependence of CD for an α-helix, the formula $[\theta]_{100} = [\theta]_H^\infty(1 - k/n)$ was used to calculate $[\theta]_{100}$ according to the following: Chen, Y.-H.; Yang, J. T.; Chau, K. H. *Biochemistry* **1974**, *13*, 3350. (We have not rigorously excluded the possibility that thymine chromophores might be contributing to the α-helix circular dichroism at 219 nm. For a discussion of analogous aromatic side-chain effects on CD, see: Chakrabartty, A.; Kortemme, T.; Padmanabhan, S.; Baldwin, R. L. *Biochemistry* **1993**, *32*, 5560.)