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Incorporation and characterization of abasic and phenyl residues in peptide nucleic acids

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Abstract

The *N*-(2-aminoethyl)glycine backbone unit of PNA has been derivatized with phenylacetic acid and acetic acid moieties to produce monomers for incorporation into PNAs. As expected, oligomers containing these residues form PNA·DNA double helices that are destabilized compared to the unsubstituted duplexes. However, unlike the analogous substitutions in DNA, these residues show very little sequence specificity. © 1999 Elsevier Science Ltd. All rights reserved.

Peptide nucleic acids (PNAs) are surrogates for natural oligonucleotides constructed on uncharged pseudopeptide backbones.[1–3] PNA can hybridize with complementary sequences of DNA and RNA to form double- and triple-helical complexes. PNA can also readily hybridize with complementary DNA sequences with concurrent displacement of the DNA complement (strand invasion). The binding of PNAs is usually very tight, largely independent of salt concentration, and generally more sensitive to the presence of mismatches than DNA partners.[2] Because of these characteristics, PNA has great potential as an antisense/antigene reagent; this has been recently demonstrated by the inhibition of cellular telomerase activity.[4] Moreover, the examination of PNA·DNA and PNA·PNA complexes has afforded a unique opportunity to evaluate our understanding of the determinants of nucleic acid structure. For example, PNA·DNA and PNA·RNA double helices have structures similar to the natural complexes.[5,6] Complete replacement of the ribose-phosphodiester backbone in PNA·PNA complexes still produces helical structures (P-form) related to natural nucleic acids.[7] These results highlight the roles of base stacking and hydrogen bonding in the formation of helical nucleic acid structures.

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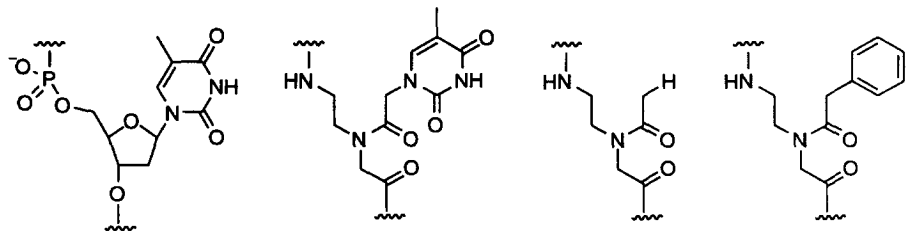


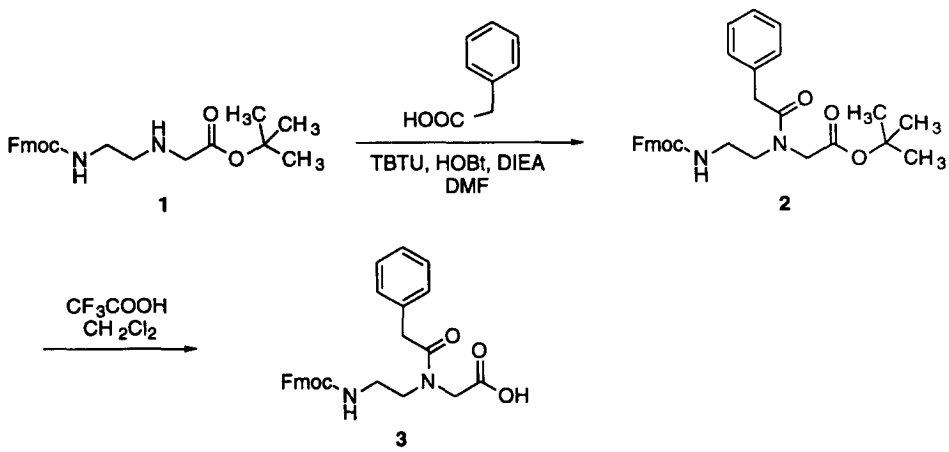
This laboratory has been interested in exploiting the relative ease at which the nucleobases of PNA can be replaced with other moieties.[8,9] Such modified PNAs can serve as powerful probes of the influence of the structure of individual residues upon the overall structure of double helical complexes. Described herein is an examination of the acetyl residue in a sequence where extensive stability data exists. In this way, the effect of the incorporation of this residue, the PNA equivalent of an abasic site, can be better evaluated. In addition, we report the incorporation of a phenyl residue into PNA. This residue is a simple aromatic system that is capable of stacking but not hydrogen bonding. The use of these residues will allow for direct comparisons with previous work incorporating similar substitutions in PNA·DNA[10] and DNA·DNA.[11]

Incorporation of the phenyl and abasic residues into PNAs required the preparation of appropriate monomers followed by assembly of the oligomers. The starting material for the syntheses of the required monomers, *tert*-butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl] glycinate (**1**), was synthesized by the method of Thomson and co-workers.[12] Condensation of **1** with commercially available phenylacetic acid produced the fully protected monomer Fmoc-Ph-*t*Bu (**2**) in 72% yield (Scheme 1). This compound was purified by chromatography and produced satisfactory NMR and mass spectra.† The acetyl monomer (Ac), a stable abasic residue, was synthesized from **1** as described previously.[8] Removal of the C-terminal *tert*-butyl esters was accomplished by brief treatment with trifluoroacetic acid, producing monomers for PNA synthesis. PNA oligomers were produced using the standard automated solid phase synthetic method[13] except that the novel residues were coupled manually. The PNAs were purified using HPLC, and oligomers containing the novel residues produced satisfactory MALDI-TOF mass spectra.‡

Hybridization of the 15-mer PNA oligomers with oligodeoxynucleotides was performed in PES buffer (10 mM phosphate, 0.1 mM EDTA, 100 mM NaCl, pH 7), and the stability of each of these complexes was evaluated by thermal denaturation experiments using absorbance spectroscopy. All of the absorbance versus temperature curves were sigmoidal, indicating that double helix melting is cooperative (Fig. 1). **PNA 1**, which contains the acetyl residue, showed T_ms ranging from 51.1°C (opposite dA) to 52.9°C (opposite dC) (Fig. 1A). A PNA·DNA duplex with the same sequence but containing a normal A-T pair in the variable position was found by Egholm and co-workers[2] to have a T_m of 68.5°C. Thus, inclusion of abasic sites destabilizes the complexes by approximately 16.5°C. **PNA 2**, which bears a phenyl residue, forms somewhat more stable PNA·DNA complexes than **PNA 1** (Fig. 1B). In these cases, the T_ms of the complexes ranged from 52.9°C (opposite dG) to 54.4°C (opposite dC). The average drop in T_m versus

† *tert*-Butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl]-*N*-[(phenyl)acetyl] glycinate (**2**): MS (FAB, MeOH/NBA) 515 $(M+H)^+$; ^1H NMR (360 MHz, $CDCl_3$, two rotomers) δ 7.78 (d, 2H), 7.62 (d, 2H), 7.36 (t, 2H), 7.25 (m, 7H), 4.43 and 4.35 (rotomer d, 2H), 4.22 (t, 1H), 4.05–3.29 (m, 8H), 1.50 and 1.47 (rotomer s, 9H).

‡ **PNA 1** (H-TGT ACG Ac CAC AAC TA-NH_2) *m/z*: 3924.3 $[M+H]^+$, calcd: 3923.6; **PNA 2** (H-TGT ACG Ph CAC AAC TA-NH_2) *m/z*: 3999.3 $[M+H]^+$, calcd: 3999.6.



Scheme 1.

a complex with an A-T pair was approximately 14.5°C. Notably, neither residue showed significant sequence selectivity; the ranges of T_ms for the acetyl residue is 1.8°C and for the phenyl residue is 1.5°C.

Comparison of the thermodynamic data for the substituted (Fig. 1) and unsubstituted PNA·DNA double helices[2] shows the expected changes: a large drop in enthalpy partly compensated by an increase in entropy. The enthalpy change is likely due to loss of hydrogen bonds and favorable stacking interactions. In this respect, the relatively small difference between the non-stacking acetyl residue and the phenyl residue highlights the importance of a polarized pi-system for favorable stacking.[11] The increase in entropy is probably due to solvation effects and the greater flexibility afforded by the removal of hydrogen bonds.

Qualitatively, these T_m data mirror the results of Millican et al.[11] for the similar base substitutions in DNA·DNA double helices. This observation is not surprising because PNA·DNA double helices are structurally very similar to DNA·DNA duplexes.[3,5] However, the lack of sequence specificity in the PNA case differs from the all-DNA system where the T_m ranges were considerably larger (4°C for abasic, 6.5°C for phenyl).[11] This observation is especially surprising because of the greater mismatch specificity seen in PNA·DNA helices with the same sequence.[2] However, a similar lack of base specificity has been reported by Schuster et al.[10] for PNAs bearing acetyl and anthraquinone residues. Overall, the replacement of a nucleobase in a PNA·DNA double helix with groups as structurally varied as hydrogen atoms, phenyl groups and anthraquinones surprisingly appears to produce nearly complete base-pairing degeneracy at that site. Further experiments are currently underway to probe this phenomenon.

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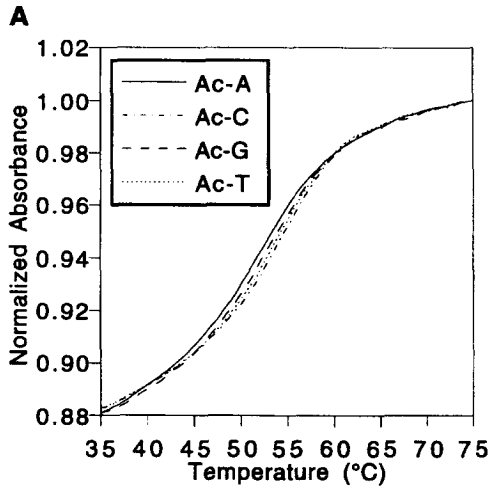
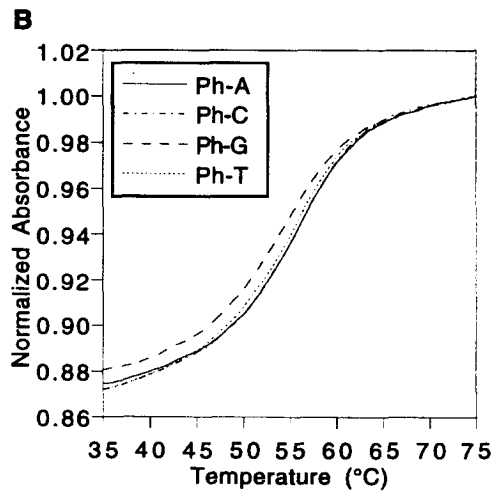


H-TGT ACG Ac CAC AAC TA-NH_2 **PNA 1**
3'-ACA TGC Y GTG TTG AT-5' *DNA*

Y	T_m (°C)	ΔH (kJ/mol)	ΔS (kJ/mol•K)
A	51.1	-261	-0.69
G	52.0	-291	-0.78
C	52.9	-306	-0.83
T	52.5	-297	-0.80



H-TGT ACG Ph CAC AAC TA-NH_2 **PNA 2**
3'-ACA TGC Y GTG TTG AT-5' *DNA*

Y	T_m (°C)	ΔH (kJ/mol)	ΔS (kJ/mol•K)
A	54.3	-298	-0.79
G	52.9	-295	-0.79
C	54.4	-305	-0.82
T	53.9	-318	-0.86

Figure 1. Thermal denaturation curves for PNA·DNA double helices. Absorbance was measured at 260 nm in PES buffer. The concentration of each strand was 4 μM. T_ms were determined by multiplying the maxima of the derivative plots (T_{max}) by 0.971);[14] the estimated error in each reported value is ±0.5°C. Thermodynamic parameters were calculated by the method of Gralla and Crothers.[15] For comparison, Egholm et al. report a T_m of 68.5°C, ΔH=–446 kJ/mol, and ΔS=–1.20 kJ/mol K for the unmodified PNA·DNA double helix (PNA residue=T, Y=A) in the same buffer conditions.[2] (A) **PNA 1** (acetyl)+complementary DNAs. (B) **PNA 2** (phenyl)+complementary DNAs

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