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Synthesis and properties of novel pyrrolidinyl PNA carrying β-amino acid spacers

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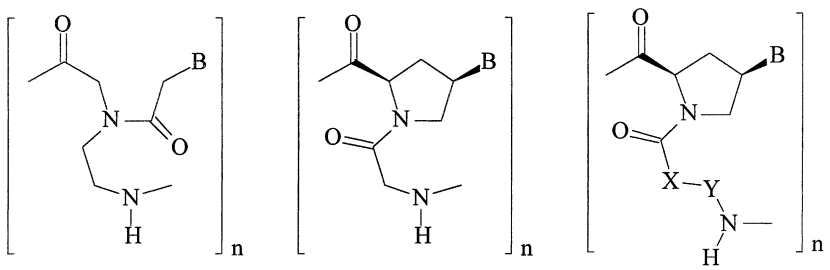
Abstract—Novel pyrrolidinyl peptide nucleic acids comprising alternate sequences of nucleobase-modified D-proline and β-amino acid spacers selected from L-aminopyrrolidine-2-carboxylic acid, D-aminopyrrolidine-2-carboxylic acid, (1*R*,2*S*)-2-aminocyclopentane carboxylic acid and β-alanine were synthesized using solid phase methodology. Gel-binding shift assay revealed that only the homothymine PNA decamer bearing D-aminopyrrolidine-2-carboxylic acid spacer binds with $(dA)_{10}$.

Nielsen and his collaborators have shown that oligonucleotide analogues in which the sugar-phosphate backbone is replaced by a poly(*N*-aminoethylglycine) with the nucleobases attached through a methylenecarbonyl group at the glycine nitrogen (**1**) strongly hybridize with complementary oligonucleotides.[1] The strong bonding of these polyamide nucleic acids (PNAs) was attributed, in part, to the lack of negative charge on the PNA.[2] In natural oligonucleotides or their phosphorothioate analogues, electrostatic repulsion between the negatively charged strands offsets the attractive forces from hydrogen bonding and π-stacking of the bases. PNAs have also been shown to be more selective in recognizing their complementary sequences, the greater discrimination against mismatched sequences arising from the greater binding contribution from each nucleobase. We have recently developed this concept further and generated a new class of oligonucleotide analogues derived from the pyrrolidine core structure (**2**).[3–5] A number of related pyrrolidine PNAs have been reported recently, many of which showed binding to their complementary oligonucleotides.[6–11]

We believe it should be possible to enhance further the binding of these PNAs to complementary oligonucleotides by replacing the glycine residue in (**2**) with an alternative spacer with an appropriate conformational rigidity. Molecular modeling suggested that if the glycine spacer in (**2**) is replaced by a β-amino acid in which the dihedral angle between the amino group and the carboxylic acid is close to 0°, a very favorable geometry for hybridization to the complementary



(**1**) (**2**) (**3**)

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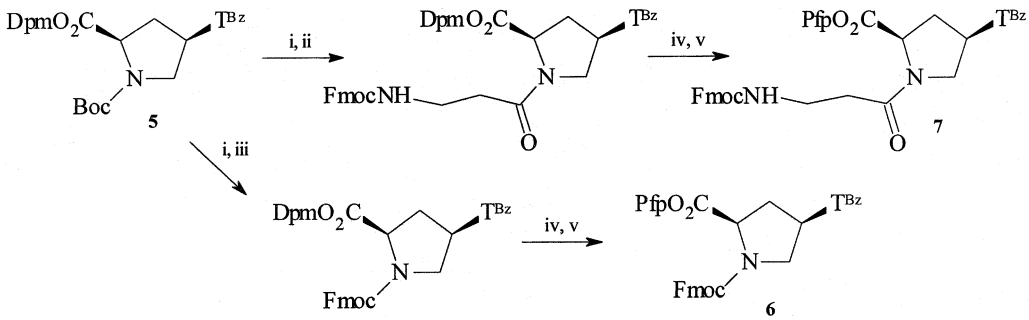
oligonucleotide should result.[12] Here we report the synthesis and binding studies of a series of novel pyrrolidinyl PNAs bearing various β-amino acid spacers (**3**). The selected spacers include L-aminopyrrolidine-2-carboxylic acid (L-Apc), D-aminopyrrolidine-2-carboxylic acid (D-Apc), (1*R*,2*S*)-2-aminocyclopentane carboxylic acid (L-Acpc) and β-alanine (β-ala). Initially four homothymine PNA decamers **3a**-T_{10}, **3b**-T_{10}, **3c**-T_{10} and **3d**-T_{10} were selected as synthetic targets.

Syntheses of **3a**-T_{10}, **3b**-T_{10} and **3c**-T_{10} require the protected building blocks including Fmoc-L-aminopyrrolidine-2-carboxylic acid pentafluorophenyl ester (**4a**), Fmoc-D-aminopyrrolidine-2-carboxylic acid pentafluorophenyl ester (**4b**), Fmoc-(1*R*,2*S*)-2-aminocyclopentane carboxylic acid pentafluorophenyl ester (**4c**) and Fmoc-protected PNA monomer (**6**). Both D- and L-aminopyrrolidine-2-carboxylic acid were synthesized from D- and L-proline via the corresponding *N*-nitrosoprolines.[13] Protection of D- and L-aminopyrrolidine-2-carboxylic acid with Fmoc-Cl followed by activation with pentafluorophenyl trifluoroacetate/DIEA[14] gave pentafluorophenyl esters (**4a**) and (**4b**), respectively. (–)-Cispentacin [(1*R*,2*S*)-2-aminocyclopentane carboxylic acid][15] was similarly protected and activated to give the pentafluorophenyl ester (**4c**). Boc-protected thymine monomer (**5**)[16] was converted to the activated Fmoc-protected PNA monomer (**6**) in four steps (Scheme 1). The PNA syntheses were performed in a stepwise fashion without pre-formation of the dipeptide building blocks. For comparison, the flexible β-alanine was also used as spacer. In this case the dipeptide building block was synthesised by selective deprotection of the *N*-Boc group in the thymine monomer (**5**) by *p*-toluenesulfonic acid-acetonitrile[16] followed by *N*-ethyl-*N*′-dimethylaminopropyl-carbodiimide·HCl (EDC·HCl) mediated coupling with commercially available Fmoc-β-alanine. The dipeptide was treated with 4 M HCl in dioxane to deprotect the diphenylmethyl ester followed by treatment with pentafluorophenyl trifluoroacetate/DIEA to give the activated dipeptide (**7**) in 43% overall yield (Scheme 1).

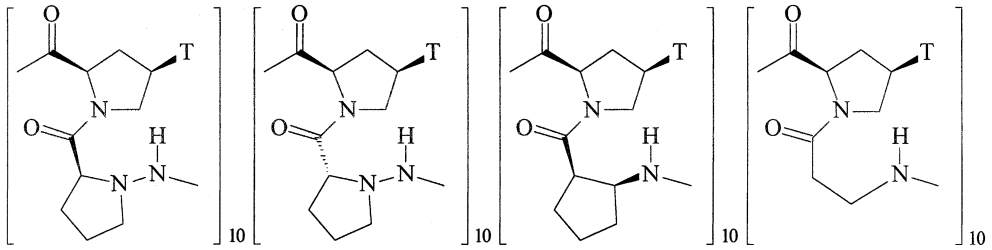
Oligomerization of these building blocks was performed on Novasyn TGR resin (2.5 μmol scale for PNA **3a–c** and 5 μmol scale for PNA **3d**) as previously described.[17] Lysine amide was included at the C-temini of all PNA for comparison with previous PNA in this series. Each pentafluorophenyl activated monomer was attached to the resin using 4 equivalents of the monomer and 4 equivalents of 1-hydroxy-7-azabenzotriazole (HOAt) in DMF (60 min, single coupling). Capping (Ac_2O/DIEA) was performed after each step. After removal of the *N*-Fmoc group by treatment with 20% piperidine in DMF, the synthesis cycle was repeated until the complete sequences of **3a**-T_{10}, **3b**-T_{10}, **3c**-T_{10} and **3d**-T_{10} were obtained. Quantitative monitoring of the dibenzofulvene-piperidine adduct released during Fmoc group deprotection showed that all coupling reactions proceeded efficiently (average coupling efficiency per cycle: **3a**-T_{10} 98.0, **3b**-T_{10} 99.2%, **3c**-T_{10} 99.8, **3d**-T_{10} 96.4%). The crude PNAs were released from the resin by treatment with trifluoroacetic acid without prior deprotection of the Fmoc group in order to use it as a purification handle.[18] After purification by reverse phase HPLC, the Fmoc-ON PNAs were treated with 20% piperidine in DMF to give the fully deprotected PNAs which were characterized by ESI-mass spectrometry (Table 1).



Scheme 1.

Table 1. ESI-MS spectra of the PNAs **3a**–**3d**

PNA	M_r found	M_r calcd
3a-T_{10}	3476.25, 3515.00, 3553.75	3478.69 (M), 3516.79 (M-H+K), 3554.88 (M-2H+2K)
3b-T_{10}	3478.54, 3516.70, 3539.92, 3554.69	3478.69 (M), 3516.79 (M-H+K), 3538.77 (M-2H+Na+K), 3554.88 (M-2H+2K)
3c-T_{10}	3469.08, 3492.56, 3507.13, 3531.48 and 3544.16	3468.82 (M); 3490.80 (M-H+Na); 3506.91 (M-H+K), 3528.89 (M-2H+K+Na), 3545.01 (M-2H+2K)
3d-T_{10}	3067.00, 3089.13 and 3104.17	3068.16 (M); 3090.14 (M-H+Na); 3106.25 (M-H+K)



3a-T_{10} **3b**-T_{10} **3c**-T_{10} **3d**-T_{10}

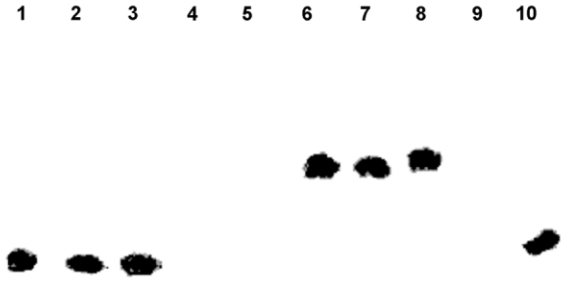


Figure 1. Gel hybridization experiment: Lane 1: FdA_{10}+**3d**-T_{10}; Lane 2: FdA_{10}+**3c**-T_{10}; Lane 3: FdA_{10}+**3a**-T_{10}; Lanes 4–5: not used; Lane 6–8: FdA_{10}+**3b**-T_{10} 1:1, 1:2, 1:5; Lane 9: not used; Lane 10: FdA_{10} (negative control). Conditions: the electrophoresis experiments were carried out on 15% polyacrylamide gel in 90 mM TBE buffer pH 8.3 at a constant DC voltage of 100 V.

Preliminary binding studies of the four novel β-PNAs to oligodeoxyribonucleotides were carried out by the polyacrylamide gel binding shift technique using fluorescently labelled $(dA)_{10}$ [FdA_{10}] as a probe. After a brief incubation of 1:1 mixtures of the PNA and DNA at 20°C, the samples were electrophoresed in 15% polyacrylamide gel at the same temperature. Only PNA **3b**-T_{10} bearing a D-2-aminopyrrolidine-2-carboxylic acid spacer showed positive results as evidenced by the presence of a new slow-moving fluorescent band and the disappearance of the fluorescent $(dA)_{10}$ band (Fig. 1). Furthermore, only **3b**-T_{10} showed observable melting with poly(dA) with a T_m value greater than 80°C at 150 mM NaCl and pH 7. Investigation of the detailed binding properties of PNA **3b** to its complementary oligonucleotides will be published elsewhere.

The binding of β-PNAs to their complementary oligonucleotides is remarkable since this appeared to violate Nielsen's '6+3' principle.[19] It is also of interest to note that only PNA **3b**-T_{10} bearing the D-aminopyrrolidine-2-carboxylic acid spacer gave positive results, while the PNA bearing L-aminopyrrolidine-2-carboxylic acid spacer did not. Recent work involving β-amino-acid oligomers[20–23] has indicated that the stereochemistry of the building blocks has a great influence on the conformation of the oligomers which may account for the results. Incidentally, PNA **3c** bearing (1*R*,2*S*)-2-aminocyclopentane carboxylic acid with the L-configuration at the α-carbon of the spacer also did not show evidence of binding to $(dA)_{10}$. PNA **3d** bearing a relatively flexible β-alanine spacer, should, in principle, be able to adopt any conformation required for binding to its complementary DNA. The lack of binding to DNA in this case may arise from the fact that entropy loss during the binding process would be high. At present it is not yet clear whether the presence of a rigid spacer amino acid with the correct stereochemistry alone is responsible for the unique binding behavior of β-PNA **3b** or whether there is a positive contribution to the binding resulting from attraction between the negatively charged phosphate groups of DNA and the protonated pyrrolidines in **3a** and **3b** as well.

In conclusion, we have developed a synthetic route for novel pyrrolidine PNAs bearing four different β-amino acid spacers. Preliminary binding studies of these PNAs using a gel electrophoresis technique revealed that the structure of the amino acid spacer plays an important role in determining the ability of these PNAs to bind to their complementary oligonucleotide.

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References

1. Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497–1500.
2. Uhlmann, E.; Peyman, A.; Breipohl, G.; Will, D. W. *Angew. Chem., Int. Ed.* **1998**, *37*, 2797–2823 and references there cited.
3. Lowe, G.; Vilaivan, T. *J. Chem. Soc., Perkin Trans. 1* **1997**, 539–546.
4. Lowe, G.; Vilaivan, T.; Westwell, M. S. *Bioorg. Chem.* **1997**, *25*, 321–329.

5. Vilaivan, T.; Khongdeesameor, C.; Wiriyawaree, W.; Mansawat, W.; Westwell, M. S.; Lowe, G. *Science Asia* **2001**, *27*, 113–120.
6. Jordan, S.; Schwemler, C.; Kosch, W.; Kretschmer, A.; Schwenner, E.; Stropp, U.; Mielke, B. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 681–686.
7. D'Costa, M.; Kumar, V. A.; Ganesh, K. N. *Organic Letters* **1999**, *1*, 1513–1516.
8. Vilaivan, T.; Khongdeesameor, C.; Harnyuttanakorn, P.; Westwell, M. S.; Lowe, G. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 2541–2545.
9. Puschl, A.; Tedeschi, T.; Nielsen, P. E. *Organic Letters* **2000**, *2*, 4161–4163.
10. Hickman, D. T.; King, P. M.; Cooper, M. A.; Slater, J. M.; Micklefield, J. *Chem. Commun*. **2000**, 2251–2252.
11. Altmann, K.-H.; Husken, D.; Cuenoud, B.; Garcia-Echeverria, C. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 929–933.
12. Westwell, M. S. unpublished work.
13. Klosterman, H. J.; Lamoureus, G. L.; Parsons, J. L. *Biochemistry* **1967**, *6*, 170–177.
14. Green, M.; Berman, J. *Tetrahedron Lett*. **1990**, *31*, 5851–5852.
15. Davies, S. G.; Ichihara, O.; Lenoir, I.; Walters, I. A. S. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1411–1415.
16. Lowe, G.; Vilaivan, T. *J. Chem. Soc., Perkin Trans. 1* **1997**, 547–554.
17. Lowe, G.; Vilaivan, T. *J. Chem. Soc., Perkin Trans. 1* **1997**, 555–560.
18. Thomson, S. A.; Josey, J. A.; Cadilla, R.; Gaul, M. P.; Hassman, C. F.; Luzzio, M. J.; Pipe, A. J.; Reed, K. L.; Ricca, D. J.; Wiethe, R. W.; Noble, S. A. *Tetrahedron* **1995**, *51*, 6179–6194.
19. Nielsen, P. E.; Haaima, G. *Chem. Soc. Rev*. **1997**, 73–78.
20. Gellman, S. H. *Acc. Chem. Res*. **1998**, *31*, 173–180 and references there cited.
21. Appella, D. H.; Christianson, L. A.; Klein, D. A.; Richards, M. R.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc*. **1999**, *121*, 7574–7578.
22. Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc*. **1996**, *121*, 13071–13072.
23. Ryan, K.; Gershell, L. J.; Still, W. C. *Tetrahedron* **2000**, *56*, 3309–3318.