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Peptide Nucleic Acids (PNAs) with a Functional Backbone

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Abstract. The synthesis of 10 new T-PNA monomers derived from L-amino acids is presented. The monomers were incorporated into decameric PNA oligomers, and the hybridisation with RNA, DNA and PNA complements studied by thermal stability measurements.

PNA (peptide nucleic acid) is a DNA mimic with an achiral, uncharged pseudopeptide backbone[1-3] (**Figure**). Oligomer synthesis[4] and the properties of PNA containing several modified analogs have recently been reviewed.[5] Introduction of chirality and functionality into the backbone has been studied by substituting alanine[6], lysine, serine, glutamic acid (only D), aspartic acid (only L), or isoleucine (only L)[7] for glycine. From this work it was concluded that: 1) the glycine units in the PNA backbone can be replaced by chiral amino acids with only a moderate loss of binding affinity to DNA and RNA, 2) D-amino acids are better accommodated in the backbone of a PNA-DNA duplex than L-amino acids, 3) positively charged side chains seem to give higher Tm values than neutral side chains, which are better than negatively charged side chains. We are presently investigating the possibility of using a series of such monomers as building blocks in combinatorial libraries.[8] Here, we report the synthesis and hybridisation properties of ten new monomers (**4a-j**).

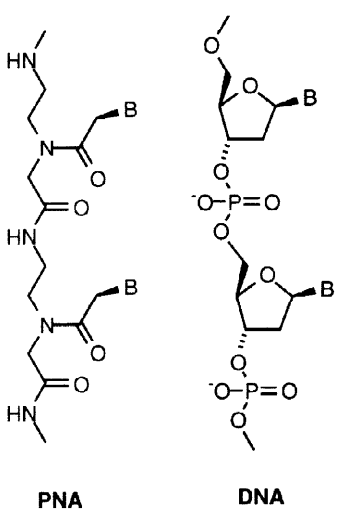

Figure : B=adenine, cytosine, guanine, or thymine.

Synthesis

The monomers **4a-j** were prepared from the protected L-amino acids as shown in the **Scheme**. The synthetic route[9] follows that used for the six previously described monomers[6,7], except that the backbones **2a-j** were purified by flash chromatography instead of by precipitating their hydrochloride salts. The average yields of step a-c were 67%, 74% and 87%, respectively. In step c it was preferable to avoid base as this led to 5% racemization in the case of alanine.[6] Alkaline hydrolysis could be avoided in all but two cases: the 2-Br-Z protecting group of the Tyr monomer **4i** is not compatible with either hydrogenation or the use of Pd(0); and the His monomer **4f** is so polar that barium hydroxide hydrolysis followed by sulphuric acid (precipitating barium sulphate) and freeze drying seemed to be the only possibility. The new monomers[9] were incorporated into PNA decamers by standard Boc synthesis.[4] For yet unknown reasons the Asn monomer **4e** coupled very poorly, and pure oligomers containing this monomer could not be obtained.

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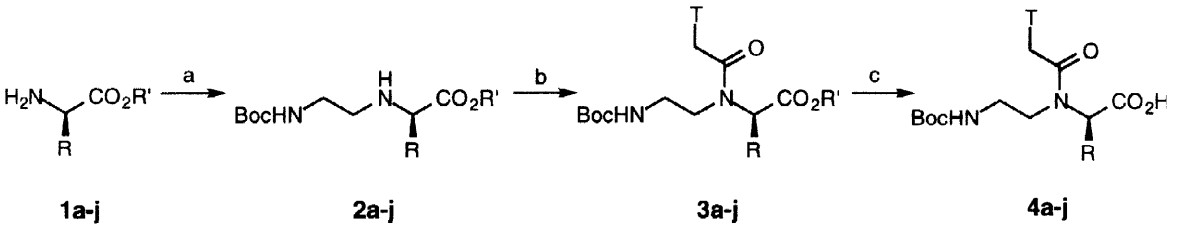

Scheme: Synthesis of the monomers **4a-j**. R is given in note 9. R' = benzyl in **a-e**, **j**. R' = allyl in **g**, **h**. R' = methyl in **f**, **i**. Reagents: a) $BocNHCH_2CHO$, $NaBH_3CN$, AcOH, MeOH; b) thymin-1-yl-acetic acid, DCC, DhbtOH, DMF; c) **3a-e**, **j**: H_2, 10% Pd/C, MeOH; **3g**, **h**: $Pd(PPh_3)_4$, morpholine, THF; **3f**: $Ba(OH)_2$, THF, then aq. H_2SO_4; **3i**: LiOH, THF, then HCl.

Hybridisation properties

The modified PNA decamers were examined for their binding to complementary, antiparallel RNA, DNA and PNA decamers by thermal stability (Tm) measurements (**Table**). In entry 2-11 each modified monomer was incorporated once in the middle of the PNA, and in entry 12-15 the modified monomer was incorporated in three positions. It is clear from the data that the effect of substituents on Tm is not additive, and the results indicate that the major contribution is due to the modifications in the middle of the sequence (compare entries 3 and 14, 5 and 13, and 7 and 15). The results also show a tendency for sterically demanding substituents (Tyr, His, Trp, Phe and Val) to be less well tolerated than smaller ones although this effect is small. Examination of the available three dimensional structures of PNA-RNA[10] , PNA-DNA[11] and PNA-PNA[12] duplexes also indicates that substituents at the glycine α-positions should be easily accommodated.

Table. Melting temperatures (°C) of PNA-RNA and PNA-DNA duplexes.[a, b]

Entry	Backbone modification[c]	MS,found (calc.)	T_m (RNA, antiparallel)	T_m (DNA, antiparallel)	T_m (PNA, antiparallel)
1	Gly	2727.1 (2725.1)	54.0	50.5	69.0
2	Arg (**5h**)	2822.6 (2824.2)	49.0	45.5	67.0
3	Leu (**5b**)	2781.8 (2781.2)	49.0	45.5	65.5
4	Gln (**5d**)	2796.1 (2796.1)	49.0	43.5	66.5
5	Lys (**5l**)	2794.9 (2796.2)	48.5	46.0	68.0
6	Tyr (**5i**)	2830.0 (2831.1)	47.5	42.5	66.5
7	His (**5f**)	2807.1 (2805.1)	47.5	42.5	65.5
8	Thr (**5g**)	2769.5 (2769.1)	47.5	44.0	65.5
9	Trp (**5j**)	2856.4 (2854.1)	47.0	42.5	67.0
10	Phe (**5c**)	2814.7 (2815.1)	46.5	42.0	67.0
11	Val (**5a**)	2764.9 (2767.1)	46.5	42.0	61.0
12	Ala (**6k**)	2768.0 (2767.1)	49.0	46.5	65.5
13	Lys (**6l**)	2938.3(2938.3)	47.5	47.0	62.5[d]
14	Leu (**6b**)	2896.8 (2893.3)	47.0	45.0	60.5
15	His (**6f**)	2965.9 (2965.2)	46.0	42.0	60.0[d]

(a) Measured in aqueous buffer containing 100 mM NaCl, 10 mM phosphate, 0.1 mM EDTA, pH 7.0; heating rate: 1 K min^{-1}. UV absorbance measured at 260 nm. (b) The PNA sequence was H-GTAGAT$_x$CACT-NH_2 in entry 1-11 and H-GT$_x$AGAT$_x$CACT$_x$-NH_2 in entry 12-15. The complementary RNA, DNA and PNA sequences were: 5'-AGUGAUCUAC, 5'-d(AGTGATCTAC) and H-AGTGATCTAC-NH_2. (c) The backbone at the T_x position was constructed with the monomer derived from the indicated amino acid.[9] (d) The complementary PNA sequence was: H-AGTGATCTAC-Lys-NH_2.

Previous results have shown that incorporation of D-amino acids into the PNA backbone gave rise to PNAs that hybridise better to DNA and RNA than PNAs with the corresponding L-amino acids.[6,7] We would also expect that the D-forms of the presently described monomers would hybridise better. This is also in accord with the observation that the modified PNAs bind relatively better to the achiral PNA complement then to the chiral DNA or RNA complement. Therefore, we conclude that a great variety of functionalisation of the PNA

backbone at the glycine α-positions can be done without severely compromising the hybridisation properties of the PNA.

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REFERENCES AND NOTES

1. Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497.
2. Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 1895.
3. Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 9677.
4. Christensen, L.; Fitzpatrick, R.; Gildea, B.; Petersen, K. H.; Hansen, H. F.; Koch, T.; Egholm, M.; Buchardt, O.; Nielsen, P. E.; Coull, J.; Berg, R. H. *J. Peptide Sci.* **1995**, *1*, 175.
5. Nielsen, P. E.; Haaima, G. *Chem. Soc. Rev.* **1997**, 73.
6. Dueholm, K. L.; Petersen, K. H.; Jensen, D. K.; Egholm, M.; Nielsen, P. E.; Buchardt, O. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1077.
7. Haaima, G.; Lohse, A.; Buchardt, O.; Nielsen, P. E. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1939.
8. Nielsen, P. E. *Methods Enzymol.* **1996**, *267*, 426.
9. *Example procedures:* **Glutamine monomer (4d).** Cs_2CO_3 (5.54 g, 17.0 mmol) was added to a stirred solution of Boc-Gln-OH (4.00 g, 16.2 mmol) in DMF (58 ml). After 30 min benzyl bromide (2.31 ml, 19.5 mmol) was added and the mixture was stirred for 2 hr and then filtered through celite. The filtrate was evaporated *in vacuo*, and the residue dried *in vacuo*. Sat. aq. $NaHCO_3$ (75 ml) was added, and the mixture was extracted with AcOEt (150 ml). The organic phase was washed with brine (80 ml), dried over Na_2SO_4, and evaporated *in vacuo* to give 5.25 g of crude product. Recrystallisation from hexane/ethyl acetate (2:1, 120 ml) resulted in a white precipitate of Boc-Gln-OBzl, yield 4.97 g (91%), m.p. 107-108 °C. Anal. calc. for $C_{17}H_{24}N_2O_5$: C 60.70, H 7.19, N 8.33; found: C 60.88, H 7.02, N 8.25.
TFA (4.5 ml) was added at 0 °C to Boc-Gln-OBzl (1.50 g, 4.46 mmol). The solution was stirred for 35 min at rt. and then evaporated *in vacuo*. Sat. aq. $NaHCO_3$ (30 ml) was added, and the mixture extracted with AcOEt (3 x 50 ml) and CH_2Cl_2 (3 x 50 ml). The combined extracts were dried (Na_2SO_4), and the solvent was evaporated *in vacuo* to give 0.84 g (88%) of **1d** as an oil.
A solution of **1d** (1.52 g, 6.45 mmol) and Boc-aminoacetaldehyde (1.23 g, 7.74 mmol) in MeOH (45 ml) were stirred at 0 °C for 15 min. $NaBH_3CN$ (243 mg, 3.87 mmol) followed by AcOH (0.44 ml, 7.74 mmol) were added and the solution was stirred for 1 hr at rt. The solution was evaporated *in vacuo*, sat $NaHCO_3$ (50 ml) was added, and the mixture extracted with AcOEt (200 ml). The organic phase was washed with brine (50 ml), dried over Na_2SO_4, and the solvent evaporated *in vacuo* to give 2.53 g of **2d** which was purified by flash chromatography (10% MeOH in AcOEt). Yield: 1.20 g of **2d** as an oil (49%).
DCC (1.03 g, 4.98 mmol) was added to a stirred solution of thymin-1-yl-acetic acid (0.92 g, 4.98 mmol) and DhbtOH (0.81 g, 4.98 mmol) in dry DMF (9 ml) at rt, and the solution was stirred for 40 min at rt. **2d** (1.18 g, 3.11 mmol) dissolved in dry DMF (14 ml) was added and the solution was stirred overnight. DCU was removed by filtration, and the DMF was removed *in vacuo*. The remaining oil was dissolved in AcOEt (200 ml), and the organic phase was washed with sat. aq. $NaHCO_3$ (50 ml). The H_2O phase was extracted with AcOEt (2 x 50 ml). The combined organic phases were dried ($MgSO_4$), and evaporated *in vacuo* to give 2.32 g which was purified by flash chromatography (eluent: 10% MeOH in AcOEt). Yield: 1.39 g of **3d** as a white foam (82%). Anal. calc. for $C_{26}H_{35}N_5O_8.0.5\ H_2O$: C 56.30, H 6.56, N 12.63; found: C 56.65, H 6.68, N 12.15.
3d (1.30 g) in MeOH (17 ml) was hydrogenated at 1 atm over 10 % Pd-C (203 mg) for 1.5 hr at rt. The solution was filtered through celite, and then evaporated *in vacuo*. Yield: 0.96 g of **4d** as a white solid (88%. Total yield from **1d** 35%). Anal. calc. for $C_{19}H_{29}N_5O_8.H_2O$: C 48.20, H 6.60, N 14.79; found: C 48.52, H 6.71, N 14.62. $[\alpha]_D^{28} = -18.1$ (c = 3 in

MeOH). ^{1}H-NMR (DMSO-d_6,, major rotamer only): δ 11.27 (s, 1H), 7.36-6.75 (m, 4H), 4.63-4.22 (m, 3H); 3.80-2.72 (m, 4H), 2.20-2.06 (m, 3H), 1.93 (m, 1H), 1.76 (s, 3H), 1.38 (s, 9H). FAB$^+$MS: 456.2 (M+H$^+$).

The following monomers were prepared analogously:

Valine monomer (4a). Yield: 72%. $[\alpha]_D^{24}$= -33.7 (c = 3 in MeOH). ^{1}H-NMR (major rotamer only): δ 11.25 (s, 1H), 7.40 (s, 1H), 6.91 (s, 1H), 4.66 (s, 2H), 4.06 (m, 1H), 3.39 (m, 2H), 3.18 and 3.01 (m x 2, 2H); 2.20 (m, 1H); 1.76 (s, 3H), 1.36 (s, 9H), 0.96 (m, 6H). FAB$^+$ HRMS: *m/z* calc. for $C_{19}H_{31}N_4O_7$: 427.2193, found: 427.2191.

Leucine monomer (4b). Yield: 39%. Anal. calc. for $C_{20}H_{32}N_4O_7$: C 54.53, H 7.32, N 12.72; found: C 54.22, H 7.13, N 12.57. $[\alpha]_D^{24}$= -18.9 (c = 3 in MeOH). ^{1}H-NMR (major rotamer only): δ 11.26 (s, 1H), 7.39 (s, 1H), 6.88 (s, 1H), 4.7-4.3 (m, 4H), 3.5-2.9 (m, 4H), 1.8-1.4 (m, 3H) 1.76 (s, 3H), 1.38 (s, 9H), 0.88 (m, 6H). FAB$^+$MS: 441.1 (M+H$^+$).

Phenylalanine monomer (4c). Yield: 71%. Anal. calc. for $C_{23}H_{30}N_4O_7.0.50\ H_2O$: C 57.13, H 6.48, N 11.59; found: C 57.47, H 6.41, N 11.55. $[\alpha]_D^{24}$= -129.8 (c = 3 in MeOH). ^{1}H-NMR (major rotamer only): δ 11.29 (s, 1H), 7.34-7.19 (m, 6H), 6.55 (s, 1H), 4.58-4.45 (m, 2H), 4.16 (m, 1H), 3.40-2.60 (m, 6H), 1.76 (s, 3H), 1.33 (s, 9H). FAB$^+$MS: 497.2 (M+Na$^+$).

Asparagine monomer (4e). Yield: 48%. Anal. calc. for $C_{18}H_{27}N_5O_8.H_2O$: C 47.06, H 6.36, N 15.24; found: C 47.30, H 6.29, N 15.15. $[\alpha]_D^{28}$= -24.2 (c = 3 in MeOH). ^{1}H-NMR (major rotamer only): δ 11.26 (s, 1H), 8.00-6.80 (m, 4H), 4.58 (s , 2H), 4.30 (t, J=6.2 Hz, 1H), 3.60-2.40 (m, 6H); 1.75 (s, 3H), 1.38 (s, 9H). FAB$^+$MS: 442.2 (M+H$^+$).

Histidine(Bom) monomer (4f). Yield: 51%. Anal. calc. for $C_{28}H_{36}N_6O_8.2H_2O$: C 54.19, H 6.50, N 13.54; found: C 54.02, H 6.11, N 13.23. $[\alpha]_D^{24}$= -36.2 (c = 3 in MeOH). ^{1}H-NMR (major rotamer only): δ 11.32 (s, 1H), 8.11 (s, 1H), 7.38-7.29 (m, 6H), 6.96 (s, 1H), 6.70 (s, 1H), 5.52 (m, 2H), 4.66-4.45 (m, 4H), 4.20 (m, 1H), 3.29-2.86 (m, 6H), 1.77 (s, 3H), 1.36 (s, 9H). FAB$^+$MS: 585.1 (M+H$^+$).

Threonine(Bzl) monomer (4g). Yield: 53%. Anal. calc. for $C_{25}H_{34}N_4O_8$: C 57.90, H 6.61, N 10.80; found: C 57.90, H 6.55, N 10.52. $[\alpha]_D^{24}$= -23.6 (c = 3 in MeOH). ^{1}H-NMR (major rotamer only): δ 11.30 (s, 1H), 7.70-7.00 (m, 6H), 6.85 (s, 1H), 4.73-4.10 (m, 5H), 3.55-3.00 (m, 5H), 1.76 (s, 3H), 1.37 (s, 9H), 1.19 (d, J=6.2 Hz, 3H). FAB$^+$MS: 541.0 (M+Na$^+$).

Arginine(di-Z) monomer (4h). Yield: 40%. Anal. calc. for $C_{36}H_{45}N_7O_{11}.1/2\ H_2O$: C 56.83, H 6.11, N 12.89; found: C 56.76, H 6.13, N 12.63. $[\alpha]_D^{24}$= -15.6 (c = 3 in MeOH). ^{1}H-NMR (major rotamer only): δ 11.26 (s, 1H), 9.15 (broad s, 2H), 7.60-7.20 (m, 11 H), 6.81 (m, 1H), 5.25 and 5.05 (2 x s, 4H), 4.65-4.32 (m, 3H), 3.96-3.85 (m, 2H), 3.60-2.93 (m, 4H), 2.00-1.40 (m, 4H), 1.72 (s, 3H), 1.37 (s, 9H). FAB$^+$MS: 752.2 (M+H$^+$).

Tyrosine(2-Br-Z) monomer (4i). Yield: 16%. Anal. calc. for $C_{31}H_{35}BrN_4O_{10}.H_2O$: C 51.60, H 5.17, N 7.76, Br 11.07; found: C 51.83, H 4.96, N 7.70, Br 10.58. $[\alpha]_D^{28}$= -104.4 (c = 3 in MeOH). ^{1}H-NMR (major rotamer only): δ 11.31 (s, 1H), 7.71-7.15 (m, 9H), 6.64 (broad s, 1H), 5.32 (s, 2H), 4.56 (m, 2H), 4.20 (m, 1H), 3.30-2.60 (m, 6H), 1.77 (s, 3H), 1.35 (s, 9H). FAB$^+$MS: 703.1 and 705.1 (M+H$^+$).

Tryptophan(For) monomer (4j). Yield: 26%. Anal. calc. for $C_{26}H_{31}N_5O_8.0.75\ H_2O$: C 56.25, H 5.91, N 12.62; found: C 56.38, H 5.97, N 12.23. $[\alpha]_D^{28}$= -115.3 (c = 3 in MeOH). ^{1}H-NMR (major rotamer only): δ 11.34 (s, 1H), 9.80-7.00 (m, 7H), 6.67 (s, 1H), 4.56 (m, 3H), 3.60-2.80 (m, 6H), 1.78 (s, 3H), 1.32 (s, 9H). FAB$^+$MS: 563.9 (M+H$^+$).

10. Brown, S. C.; Thomson, S. A.; Veal, J. M.; Davis, D. G. *Science* **1994,** *265,* 777.
11. Eriksson, M.; Nielsen, P. E. *Nature Struct. Biol.* **1996,** *3,* 410.
12. Rasmussen, H.; Kastrup, J. S.; Nielsen, J. N.; Nielsen, J. M.; Nielsen, P. E. *Nature Struct. Biol.* **1997,** *4,* 98.