Chimeric (α-amino acid + nucleoside-β-amino acid)$_n$ peptide oligomers show sequence specific DNA/RNA recognition†

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An α/β-peptide backbone oligonucleotide comprising natural α-amino acids alternating with a β-amino acid component derived from thymidine sequence specifically recognizes and binds to deoxy- and ribo-oligoadenylates in triplex mode.

Novel oligonucleotide (ON) analogues that can form stable duplexes or triplexes with target nucleic acids (NA) are important synthetic objectives because of their use as therapeutic agents in antisense and antigene strategies.[1] Various types of modified oligonucleotides have been developed over the last two decades as potential diagnostic probes and antisense and antigene therapeutics.[2] The more recent developments such as splice correcting[3] and exon skipping[3] strategies require highly robust nucleic acid analogues that are stable under physiological conditions as single strands as well as in the form of duplexes with complementary RNA sequences. The study of unnatural de-phosphono nucleic acid oligomers is gaining in importance for such applications. The replacement of the internucleoside sugar–phosphate linkages by the robust sugar–amide bond could be advantageous as in PNA.[4,5] This would maintain the chirality and 3′–5′ directionality of the backbone, along with the enzymatic stability of the amide bond. Several four- and five-atom amide-linked (6/7 atom repeating units) deoxy[6,7] and ribo[8,9] ON analogues are known in the literature. The other relevant examples of 6/7-atom repeating units are those of pyrrolidine-based NA analogues[10] such as pyrrolidinyl[11]/POM-PNA[12] and *bep*PNA (Fig. 1)[13] or those comprising prolyl-aminopyrrolidine-2-carboxylic acid[14] and prolyl-2-aminocyclopentanecarboxylic acid (prolyl-ACPC backbone) (Fig. 1).[15,16]

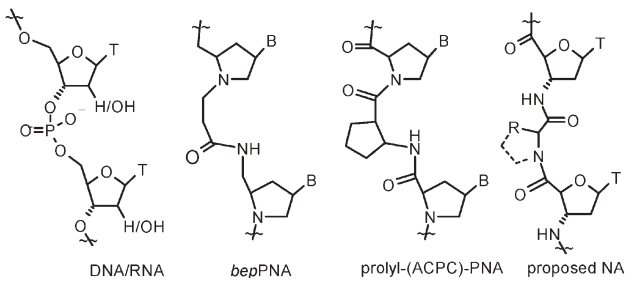

Fig. 1 Structure of DNA/RNA and amino acid backbone modified ONs.

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† Electronic supplementary information (ESI) available: Experimental procedures; ^{1}H, ^{13}C, mass spectra of compounds **7** and **8**; MALDI-TOF mass spectra of ONs **12–15**; UV melting experiments and CD curves. See DOI: 10.1039/b716835g

The prolyl-ACPC backbone seems very interesting as this alternating α/β amino acid backbone exhibited preferential binding with complementary DNA but not with complementary RNA sequences. We perceived that alternating nucleoside-β-amino acids with natural α-amino acids (Fig. 2) would give rise to similar alternating α/β amino acid backbone scaffolds. A literature search revealed that similar work on phosphate free oligonucleoside analogues is reported in a patent.[17] The internucleoside amino acids used were only glycine and lysine in that study and only binding studies with complementary DNA oligomers were reported. In the light of recent developments in this area, we thought it worthwhile to reconstitute the sugar–amino acid backbone incorporating different natural amino acids for RNA binding studies.

In this communication, we report the synthesis and DNA/RNA binding studies of amide linked oligothyminyl DNA analogues (Fig. 1) comprising conformationally constrained α-amino acids (L-proline **2** and sarcosine **3**, Fig. 2), a positively charged α-amino acid (L-lysine **4**, Fig. 2) and a neutral α-amino acid (L-methionine **5**, Fig. 2) in the backbone, alternating with a β-amino acid nucleoside component derived from thymidine (**1**, Fig. 2). The potential advantages are as follows. (a) In 3′-deoxy-3′-amino-2′-deoxyribose sugar the five-membered ring pucker is in a preferred 3′-endo conformation that is better suited for m-RNA recognition.[18] (b) The new synthesis of nucleoside-β-amino acid is quite simple using a recently reported TEMPO–BAIB method.[19] (c) The flexibility of the five atom internucleoside amide linker could be better fitting for replacement of a phosphate group for RNA binding taking into account the shorter amide bond compared to the phosphodiester linkage.[7] (d) A negatively charged phosphate group can be replaced by neutral (proline, sarcosine and methionine) or positively charged[20] (lysine) alternatives.

Our synthetic strategy was aimed at assembling these molecules on a solid support, for its subsequent adaptation to well established conventional peptide chemistry.[21] Oxidation of the 5′-hydroxy moiety of 3′-azidothymidine **6** to obtain **7** was reported earlier[17,22a] by Varma *et al.* using $RuCl_3$–$Na_2S_2O_8$–KOH. The use of strongly alkaline conditions in this reaction makes it unsuitable

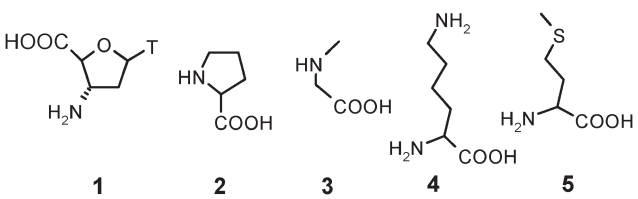
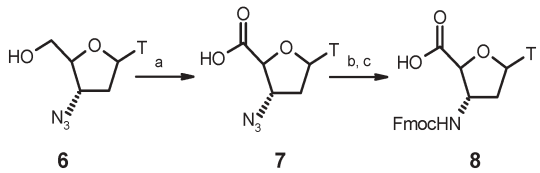


Fig. 2 Structures of monomer units.



Scheme 1 Synthesis of thyminyl sugar amino acid monomer unit. *Reagents and conditions*: (a) TEMPO–BAIB, acetonitrile–water; (b) Pd/C–H_2, methanol; (c) Fmoc-succinimide, $NaHCO_3$, acetone–water. TEMPO 2,2,6,6-tetramethyl-1-piperidinyloxyl; BAIB [bis(acetoxy)-iodo]benzene; Fmoc fluorenylmethoxycarbonyl.

for adaptation to other amino-protected nucleosides. The products were also often contaminated with inorganic salts. Oxidation using Sharpless conditions works well only for purine nucleosides and not with pyrimidine nucleosides.[22b] A recently published TEMPO–BAIB method[19a] used for 2′,3′-isopropylidene ribonucleosides could be a more general procedure for nucleosides[19b] as it is reported to be compatible with several protecting groups and other functionalities such as azide. The products isolated are not contaminated with any inorganic salts. Thus, **6** was easily converted to the corresponding acid **7** under TEMPO–BAIB oxidation conditions and the product was recovered by trituration with ether and acetone (Scheme 1). The 3′-azido group in **7** was then hydrogenated using Pd–C to an amine which was subsequently Fmoc protected to give the monomer building block **8**. The monomer **8** along with protected α-L-amino acids (**2**, **3**, **4** and **5**) was used to synthesize four octameric NA sequences (**12**, **13**, **14** and **15**) on a rink amide resin support using an Fmoc peptide synthesis strategy. For sequences **12**, **13** and **15**, β-alanine was used as a linker to the resin and for sequence **14** no linker was used.[23] All the coupling reactions were monitored by the Kaiser test. The oligomers were cleaved from the solid support using standard conditions and were purified by gel-filtration followed by RP-HPLC. Finally the purity and integrity of the oligomers were established by MALDI-TOF mass spectrometry (ESI†).

The hybridization properties of the amino acid modified oligomers **12**, **13**, **14** and **15** with complementary DNA or RNA ONs GCAAAAAAAACG **9** or r(GCAAAAAAAACG) **10** were determined by thermal denaturation studies.[24] UV melting experiments indicate that oligomers **12**, **13**, **14** and **15** hybridized to complementary DNA and RNA with melting temperatures

Table 1 UV-T_m[a] values (in °C) of chimeric modified ONs:complementary DNA/RNA

No.	Sequence	DNA **9**	RNA **10**	ΔT_mRNA–DNA
1	TTTTTTTT **11**	17.8	15.6	—
2	β-Ala-(Pro-**t**)$_8$-H **12**	49.0	60.8	+11.8
3	β-Ala-(Sar-**t**)$_8$-H **13**	49.1	61.6	+12.5
4	(Lys-**t**)$_8$-H **14**	56.3[b]	69.0[b]	+12.7
5	β-Ala-(met-**t**)$_8$-H **15**	47.3	57.3	+10.0

[a] T_m = melting temperature measured in 10 mM sodium phosphate buffer, pH 7.0 with 100 mM NaCl and 0.1 mM EDTA, from 5 to 85 °C at ramp 0.5 °C. All values are an average of three independent experiments and accurate to within ±0.5 °C. T denotes DNA backbone. **t** denotes amide backbone. DNA **9**: 5′ GCAAAAA-AAACG 3′, RNA **10**: r(5′ GCAAAAAAAACG 3′). [b] UV melting experiments were performed at pH 5.5 also but no significant change in melting temperatures was detected.

(T_m) higher than those of the complexes formed by oligothymidinyl-DNA fragment **11** of the same length (Fig. 3, Table 1). Complexes formed by all the modified oligomers **12**, **13**, **14** and **15** with the RNA target showed higher stability than similar complexes formed with the DNA target (Table 1). The positively charged lysine oligomer **14** formed the most stable complexes with both DNA (**14:9**) and RNA (**14:10**). The UV melting experiments of ON **14** with complementary DNA **9** and RNA **10** were performed at pH 5.5 but no significant change in melting temperature could be detected, indicating that the side chain lysine amine group could be protonated even at physiological pH. A single base mismatch caused a fall in T_m (11–18 °C) in the case of either DNA or RNA targets (ESI†) similar to the case of PNA.[4c] A UV–Job's plot of the modified oligomers with complementary DNA and RNA clearly showed a 2 : 1 stoichiometry of the complex (ESI†).

The CD spectra of single strands **12–15** showed ellipticity minima at ~280 nm with no positive CD band (ESI†). The CD spectrum of the 2 : 1 hybrid formed between **12** and complementary RNA **10** showed ellipticity minima at 246 nm and ellipticity maxima at 260 and 281 nm (Fig. 4). The CD spectra of the complexes of **13**, **14** and **15** with complementary RNA showed a similar CD signature (Fig. 4), substantiating the formation of triple helical structures.[25]

Thus, the complexes between chimeric (α-amino acid + nucleoside-β-amino acid) backbone ONs (**12**, **13**, **14** and **15**) and their

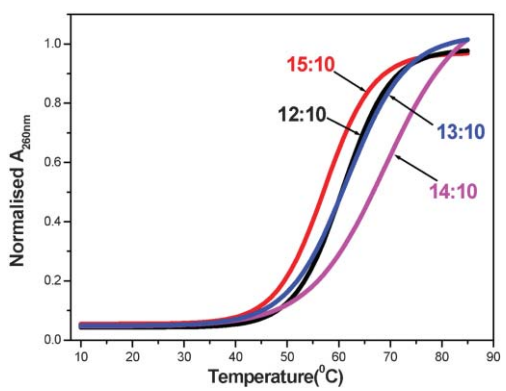


Fig. 3 UV melting graph of ONs **12**, **13**, **14** and **15** with RNA **10** (in 10 mM sodium phosphate buffer, pH = 7.0, 100 mM NaCl, and 0.1 mM EDTA). RNA **10**: r(5′ GCAAAAAAAACG 3′).

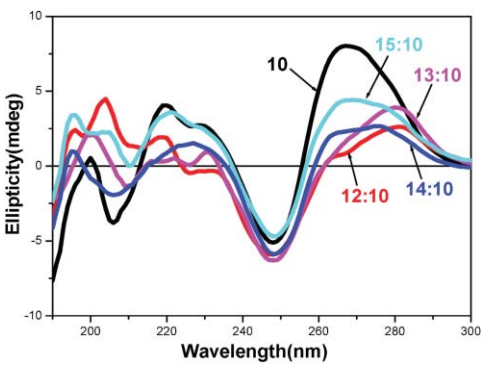


Fig. 4 CD graph of ONs **12**, **13**, **14** and **15** with RNA **10** (in 10 mM sodium phosphate buffer, pH = 7.0, 100 mM NaCl, and 0.1 mM EDTA). RNA **10**: r(5′ GCAAAAAAAACG 3′).

complementary DNA/RNA (**9**/**10**) oligonucleotides were found to be highly stable as compared to DNA:DNA (**11**:**9**) or DNA:RNA (**11**:**10**) duplexes. The presence of a positively charged α-amino acid (L-lysine) contributes further to the strong binding properties (7–8 °C). The stability of complexes of sarcosine and proline analogues, similar to the prolyl-ACPC PNA, in which one amide hydrogen is absent, suggests the absence of a contribution from the known secondary structures of the (α-amino acid + β-amino acid) peptide scaffolds[26] to the overall stability of the complexes. The 5-atom amide linked oligomers in this simple (α-amino acid + nucleoside-β-amino acid) backbone exhibit much higher stability of complexes with both complementary DNA and RNA, complexation with RNA being favoured over DNA. The basis of this selectivity could be similar to that for the other 5-atom internucleoside amide linkages.[7,27]

The use of naturally occurring α-amino acids in conjunction with an easily accessible nucleoside derived β-amino acid described in this communication thus provides a very simple peptide scaffold to create a nucleobase sequence for DNA/RNA recognition. Further studies on the mixed purine–pyrimidine sequences as well as incorporation of negatively charged and D-amino acids into the backbone to study the stereoelectronic requirements of the complexes are currently in progress in our laboratory.

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23 Stereoelectronically benign β-alanine was used as a spacer from the support during the synthesis of sequences **12**, **13**, and **15** for exerting a uniform steric effect at the C-terminus where amino acids were uncharged. In sequence **14**, positively charged L-lysine was preferred at the C-terminus because L-lysine at the C-terminus of peptide nucleic acids is known to have stabilizing effects on complexes with DNA and RNA due to electronic contributions (see reference 4).

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