# Sugar–thioacetamide backbone in oligodeoxyribonucleosides for specific recognition of nucleic acids†

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**The amide linkage being shorter than the natural phosphate linkage, an additional atom is introduced into oligodeoxyribonucleosides (ODNs) with sugar–thioacetamide backbone that show very good RNA recognition properties.**

The use of modified nucleic acids as gene-targeted drugs and as tools in molecular biology is a developing field.[1] The designed oligodeoxyribonucleosides (ODNs) to be effective leads as drugs need to have enhanced strength of hybridization with target RNA, aqueous solubility, efficiency of cellular uptake, degradation of RNA by RNAs-H enzyme and also easy synthetic methodologies. The current phosphorothioate ODN drugs are a mixture of diastereomeric ODNs due to the chirality at the phosphorus. The design and synthesis should also fulfil the requirement for enantiomeric purity to obtain homogeneous ODNs. The backbone modifications being developed presently include the second and third generation antisense ODNs such as 2′-*O*-alkyl,[2] 2′-*O*-methylthioethyl,[3] LNA,[4] HeNA,[5] morpholino NA,[6] aminoethylglycyl PNA (Fig. 1, *aeg*PNA)[7] or several PNA modifications.[8] The polyamide backbone ODNs such as *aeg*PNA are promising because of the ease of their synthesis and strong binding to the target complementary nucleic acids. Among the sugar–amide backbones, there are many examples in the literature suggesting that a five-atom amide linker leading to a seven-atom repeating backbone may be more useful because of the reduced conformational flexibility of the amide relative to the six-atom phosphodiester backbone.[9] This postulate has also been supported by X-ray studies.[9] Some of the analogues with extended seven-atom backbones cross-pair with RNA with high affinity compared to DNA.[9] Our recent work on PNA modifications (Fig. 1, *bep*PNA) suggested that ODNs containing an extra atom in the pyrrolidine–amide backbone stabilized the complexes with RNA over DNA.[10] In the search for an ideal backbone, we comply that seven-atom extended sugar–amide backbones could be more uniform with respect to nucleobase orientation as well as distance complementarity to bind complementary RNA sequences. We employ a very easy synthetic methodology that fulfills the above mentioned criteria and is much simpler to execute compared to several other reported oligonucleotide analogues so far.[1,11] In this communication we present the synthesis of thioacetamido nucleic acids (TANA) and the thermal stability studies with complementary DNA and RNA sequences. The strategy of the design, synthesis of the monomer blocks, oligomer synthesis and their complementary RNA recognition using UV-$T_m$ measurements is discussed.

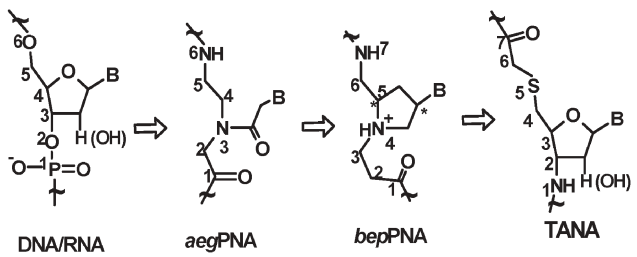
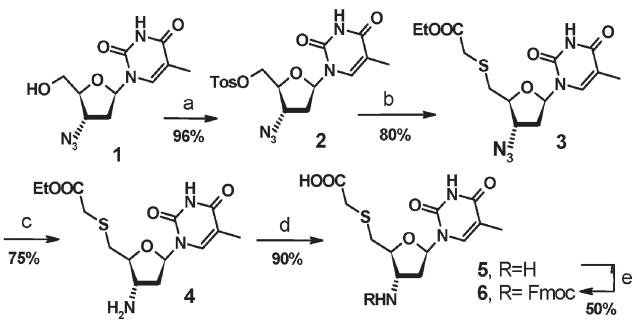



**Fig. 1** Designed TANA as a DNA/RNA mimic.

The design is based on the following considerations: (1) in 3′-deoxy-3′amino ribose sugar the five-membered heterocyclic ring pucker is preferred to be 3′-endo[12] which is better suited for mRNA recognition; (2) the solid phase synthesis and scale-up methodology for an amide bond formation are very well established; (3) the linker group has no chiral center; and (4) the flexibility of the phosphate group in DNA/RNA may be conserved by using a five-atom amide linker.

The monomer synthons 3′-Fmoc-amino-5′-thioacetic acid derivative of thymidine **6** and 3′-Fmoc-amino-5′-thioacetic acid derivative of 2′-deoxy cytidine **14** were synthesized from AZT as shown in Scheme 1 and Scheme 2, respectively. The AZT, **1** was converted to its 5′-tosyl derivative **2**. The 3′-azido-5′-tosyl thymidine **2** was converted to 5′-ethyl thioacetate **3** by nucleophilic displacement with ethyl thioacetate in the presence of sodium hydride in very good yields. The azide group was converted to a




**Scheme 1** Synthesis of the thyminyl–sugar–amino acid monomer unit. Reagents: (a) tosyl chloride–pyridine; (b) ethyl thioacetate, NaH, DMF; (c) $H_2S$–pyridine–15%TEA; (d) aqueous LiOH (2 M), MeOH; and (e) Fmoc–succinimide, $NaHCO_3$, acetone–water.

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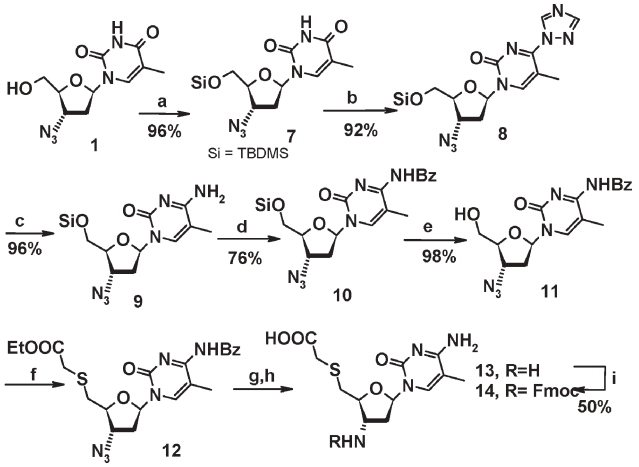
† Electronic supplementary information (ESI) available: $^1$H, $^{13}$C NMR, mass spectral data for compounds **6** and **14**, HPLC profiles and mass spectra for the modified oligomers **15–20**, and Job's plot and melting curves for the complexes with RNA. See DOI: 10.1039/b603958h




**Scheme 2** Synthesis of the 5-methylcytosinyl–sugar–amino acid monomer unit. Reagents: (a) TBDMSCl, imidazole, DMF; (b) 1,2,4 triazole, $POCl_3$, TEA, acetonitrile; (c) conc. $NH_3$, dioxane; (d) BzCl, pyridine; (e) TBAF, THF; (f) i. tosyl chloride–pyridine and ii. ethyl thioacetate, NaH, DMF; (g) $H_2S$–pyridine–15%TEA; (h) aqueous LiOH (2 M), MeOH; (i) Fmoc–succinimide, $NaHCO_3$, acetone–water.

3′-amino group in **4** using pyridine–$H_2S$ in 75% yield.[13] The ester function was then hydrolyzed to get the free sugar–amino acid **5**. The free amine group was then protected as Fmoc to get **6**.

The 5-methyl cytosine monomer **14** was synthesized starting from AZT (Scheme 2). AZT **1** was protected as 5′-*O*-*tert*-butyldimethylsilyl ether to get **7**. The nucleobase was then transformed to 5-methyl cytosine derivative **9** *via* $C^4$-1,2,4 triazolo derivative **8** followed by replacement of the triazolide with ammonia to get **9**.[14] The exocyclic amino group was benzoyl protected to get **10**. 5′-*O*-desilylation, tosylation and conversion to 5′-thioethylacetate derivative of cytosine **11** was accomplished as in the case of synthesis of thyminyl monomer. The azide group was converted to 3′-amino group using pyridine–$H_2S$ in 75% yield to get **12**.[13] The ester function was then hydrolyzed to get free sugar–amino acid **13**. It was found that during the basic hydrolysis of the ester group the exocyclic amino group was deprotected. Protection of the 3′-amino group was then accomplished to get Fmoc–amino acid 5-methyl cytosine derivative **14**. All the new compounds were characterized using spectroscopic techniques such as IR, $^1H$, $^{13}C$ NMR and MALDI-TOF mass spectral analysis.† The protected thymine monomer **6** and cytosine monomer **14** were utilized in the solid phase oligomer synthesis.

To test the sequence specific DNA/RNA recognition by the oligomers comprising the sugar–amino acids **6** and **14**, two 8-mer sequences **15** and **16** were synthesized using rink amide resin and Fmoc peptide synthesis strategy. During the synthesis of sequence **16**, excess of 5-methylcytosine monomer was used in the coupling stage and capping with acetic anhydride was done in order to ensure that the free $C^4$-amino group remains protected during further synthesis. In order to test the compatibility of these modified monomers in *aeg*PNA, three sequences with mixed TANA–PNA backbone (**17–19**) and control *aeg*PNA **20** were synthesized using Fmoc based solid phase peptide synthesis procedures. The oligomer sequences (Table 1) were cleaved from the solid support using standard conditions and the sequence **16** was further treated with concentrated $NH_3$ to deprotect the $C^4$-amino protecting group of the cytosine residue. The uncharged backbone polypyrimidine PNA sequences are known to bind to complementary DNA/RNA in a triplex mode.[15] To establish the binding stoichiometry of the TANA oligomers (**15** and **16**) Job's plot study[16] was undertaken with complementary DNA/RNA sequences.† The binding stoichiometry could be clearly established to be 2 : 1 between sequence **15** and RNA **22** using UV spectroscopy.† The TANA : DNA binding could not be observed in the Job's plot. All the binding studies of the sequences listed in Table 1 with complementary DNA/RNA were carried out in 2 : 1 stoichiometry using UV-$T_m$ experiments. The complexes of TANA **15** with DNA (3′-GCAAAAAAAACG-5′) **21** and RNA r(3′-GCAAAAAAAACG-5′) **22** were used to study their comparative binding efficiency. The TANA **16** has a 5-methyl cytosine unit and its binding with complementary DNA (3′-GCAAAAGAAACG-5′) and RNA sequence r(3′-GCAAAAGAAACG-5′) **23** was studied. For single nucleotide mismatch studies the UV-$T_m$ measurements were carried out using sequences **15** : **23** and **16** : **22** (see Supplementary information,† normalized UV absorbance *vs.* temperature plots). The results are summarized in Table 2. For control, the $T_m$ of complexes of DNA 3′-TTTTTTTT-5′ **24** and *aeg*PNA **20** with DNA **21** and RNA **22** were measured under identical buffer conditions. It is evident from the above experiments that the homopyrimidine TANA backbone oligomers (**15** and **16**) bind to the homopurine complementary RNA sequences (**22** and **23**, respectively) and show a single base-mismatch discrimination of about 10–14 °C. The thermal stability of **16** : **23** was increased by

**Table 1** The TANA and TANA–PNA oligomer sequences

| Sequence[a] | RP-HPLC[b] | $(Mass_{calc})/(Mass_{obs})$ |
|---|---|---|
| H-**t t t t t t t t**-βala **15** | 12.7 | 2467.6/2491.0 (M + $Na^+$) |
| H-**t t t t c t t t**-βala **16** | 12.3 | 2466.1/2487.9 (M + $Na^+$) |
| H-t t t t t t t **t**-βala **17** | 10.0 | 2249.4/2251.7 (M + $H^+$) |
| H-t t t **t** t t t t-βala **18** | 8.3 | 2249.4/2252.6 (M + $H^+$) |
| H-t **t** t t t t t **t**-βala **19** | 10.1 | 2342.9/2367.9 (M + $Na^+$) |
| H-t t t t t t t t-βala **20** | 7.6 | 2218.2/2218.9 (M + $H^+$) |

[a] **t** and **c** denote TANA backbone, t denotes *aeg*PNA backbone. [b] Retention time in minutes.

**Table 2** UV-$T_m$ values[a] in °C of $(TANA)_2/(TANA\text{-}PNA)_2$ : DNA/RNA triplexes[b]

| No | Sequence | DNA **21** | RNA **22** | RNA **23** |
|---|---|---|---|---|
| 1 | H-**t t t t t t t t**-βala **15** | nd[d] | 63.8 (63.5)[c] | 49.8 |
| 2 | H-**t t t t c t t t**-βala **16** | nd[e] | 52.9 | 63.1 (67.1)[c] |
| 3 | H-t t t t t t **t**-βala **17** | nd | 53.5 | — |
| 4 | H-t t t **t** t t t t-βala **18** | nd | 32.4 | — |
| 5 | H-t **t** t t t t **t**-βala **19** | nd | 25.5 | — |
| 6 | H-t t t t t t t t-βala **20** | 42.6 | 61.5 | 52.5 |
| 7 | 3′-TTTTTTTT-5′ **24** | nd | 20 | nd |

[a] $T_m$ = melting temperature (measured in buffer: 10 mM sodium phosphate, pH 7.0 with 100 mM NaCl and 0.1 mM EDTA). Measured from 10 to 90 °C at ramp 0.2 °C $min^{-1}$. UV-absorbance measured at 260 nm. All values are an average of 3 independent experiments and accurate to within ±0.5 °C. [b] T denotes DNA backbone. [c] Values in parentheses denote melting temperature at pH 5.5. [d] nd = not detected. [e] With complementary DNA sequence (3′-GCAAAAGAAACG-5′).

4 °C at pH 5.5 in which a $C^+GC$ base triple is formed.[15] The complex of TANA oligomer **15** with cRNA **22** was highly stable compared to the DNA : RNA complex (**24** : **22**) and the stability was comparable with that of *aeg*$PNA_2$ : RNA (**20** : **22**). The formation of cDNA : TANA complexes was not observed. To test the compatibility of the TANA backbone in *aeg*PNA : TANA mix-backbone oligomers, the sequences **17–19** were synthesized. Sequence **17** has a TANA monomer unit at C-terminus, sequence **18** has the modified unit at the central position and sequence **19** is an alternate *aeg*-TANA sequence. All the complexes of oligomers **17–19** with RNA **22** were destabilized compared to the control *aeg*PNA : RNA complex **20** : **22**. Contrary to the earlier reported *bep*PNA backbone,[11] the mixed backbone containing sugar amide and *aeg*PNA seems to be incompatible to form regular helical structures and mixed backbone ODNs **17–19** show significant decrease in binding with cRNA. The stability of the complexes of homogeneous backbone TANA with RNA over DNA is a very valuable result from an application perspective. The synthesis of mixed purine–pyrimidine backbone sequences and the study of compatibility of the TANA dimers in a regular phosphodiester backbone will be interesting and the work in this direction is currently in progress.

This communication presents the results of selective RNA recognition by a homogeneous DNA analogue and the ease of its synthesis that has potential to be extended to purine nucleosides. The thio function in the backbone may have an additional advantage for better bioavailability of these modified oligodeoxyribonucleosides.[3] The simplicity of the approach described here is equally appealing as is the selectivity.

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