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# Oligonucleotides with 3-hydroxy-*N*-acetylprolinol as Sugar Substitute

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**Abstract**: Fully modified oligonucleotides were synthesised from the 3-*O*-phosphoramidites of monomethoxytritylated *trans*-3-hydroxy-*N*-[($N^6$-benzoyladenin-9-yl)-acetyl]-prolinol [(2*S*,3*R*) and (2*R*,3*S*) series], *trans*-3-hydroxy-*N*-[(thymin-1-yl)-acetyl]-prolinol [(2*S*,3*R*) and (2*R*,3*S*) series], and *cis*-3-hydroxy-*N*-[($N^6$-benzoyl-adenin-9-yl)-acetyl]-L-prolinol (2R,3R). Remarkably, as well the L-*trans* (2*R*,3*S*) as the D-*trans* (2*S*,3*R*) all-adenine oligonucleotides are capable of hybridisation with complementary DNA and RNA. With modified all-thymine *trans*-oligomers no complexation with natural nucleic acids was observed. However, complex formation between two modified strands of the same sense of chirality does occur with formation of a triple stranded complex. The all-thymine oligonucleotides with *trans*-3-HO-*N*-acetylprolinol backbone are capable of hybridisation with *trans*-4-HO-N-acetylprolinol oligoadenylates of the same enantiomeric form in both the D and the L series, and inversely, the all-adenine oligonucleotide with the *trans*-3-HO conformation hybridises with the *trans*-4-HO oligothymidylates. While the former interactions have a triple stranded origin, the latter are 1:1 interactions. No interactions were noticed upon mixing oligonucleotide analogues of different sense of chirality. Modified mixed *trans*-3-HO A,T sequences display no hybridisation with complementary nucleic acids, nor homocomplex formation. The L-*cis* all-adenine oligonucleotide hybridises with its RNA complement. Several complexes were investigated by circular dichroism and microcalorimetry. In conclusion, the 3-hydroxy-*N*-acetylprolinol system represents an example of homochiral oligonucleotides built up from two enantiomeric forms and hybridizing both with natural nucleic acids.


## INTRODUCTION

The structure of RNA by virtue of its base pairing properties allows for perfect sequence selective interactions, as can be recognised e.g. in the folding of RNA into helix-stem structures (RNA-RNA interactions) or in the DNA-RNA interactions. Antisense therapeutics are meant to exploit this specificity in DNA or RNA base pairing[1]. The first generation antisense therapeutics can be easily recognised as chemically modified nucleic acids, since the introduced chemical alterations are relatively small with as a typical example the phosphorothioate oligonucleotides. More recent constructs evolve necessarily towards more aberrant structures in the search for the optimal antisense molecule, as evidenced in the development of the PNA as modified "oligonucleotide" where only the nucleobases are left as reminder of the original nucleic acid structure[2,3]. Some excellent reviews describing all possible modifications have been published[4-6].

Previously, we synthesised *trans*-4-HO-*N*-acetylprolinol (*trans*-4-HO-NAP) oligonucleotides (Figure 1) as potential analogues of 2'-5' connected ribonucleic acids[7]. The L-*trans*-all-adenine oligomer of this type is capable of stable hybridisation with natural nucleic acids, DNA as well as RNA, although in the backbone the 6-atom spacing as it can be found in the common nucleic acids, is not respected[7]. During the course of our work, proline based oligonucleotides with the bases directly attached to the proline ring have been described, but no hybridisation data were disclosed yet[8].

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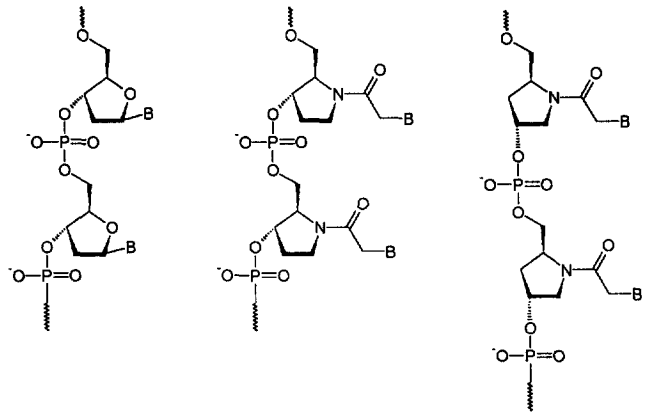



Fig. 1. Structural comparison between DNA, 3-HO-NAP and 4-HO-NAP oligonucleotides.

*Synthesis*

*Trans*-3-hydroxyproline is commercially available in mg quantities unsuitable for this project. However the interest in this compound has been quite high because of its presence in teleomycin antibiotics and several alkaloids as so called amino-sugar. Therefore, several synthetic schemes can be found in literature. Since the stereochemistry of the hydroxyproline unit differs in the diverse natural compounds, most synthetic schemes also take this feature into account.

In an attempt to further improve the hybridisation efficiency of the modified oligomer, especially the modified thymine oligonucleotide which only displays complexation with complementary RNA, we now prepared 3-hydroxy-*N*-acetylprolinol (Figure 2) derived nucleotide analogues, where phosphorus spacing is as in DNA (Figure 1).

## RESULTS AND DISCUSSION

3-Hydroxyprolinol can be prepared in an enantiomerically pure form, starting from enantiomerically pure serine[9-11], glutamic acid[12] or vinyl glycine[13]. The synthesis starting from cyclopentadiene[14] requires the enzymatic resolution of a racemic alcoholic intermediate. The most profitable synthetic scheme combines the advantages of common chemistry and a common starting material. The scheme as published by Rapoport[9] was therefore chosen with slight alterations (Scheme 1).

The *N*-(phenylsulfonyl)-L-serine[15] **1** was converted to the 5-hydro-3-pentenone derivative **2** as described[9] in 50% average yield. The addition reaction of hydrogen chloride to afford **3** was performed in THF to avoid formation of the methylether derivative which occurred when methanol/HCl(sat) was used. The product obtained after extraction was used in the next step without further purification.

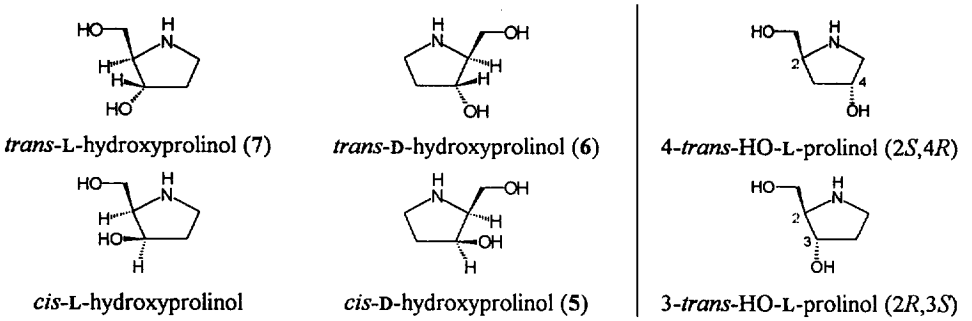
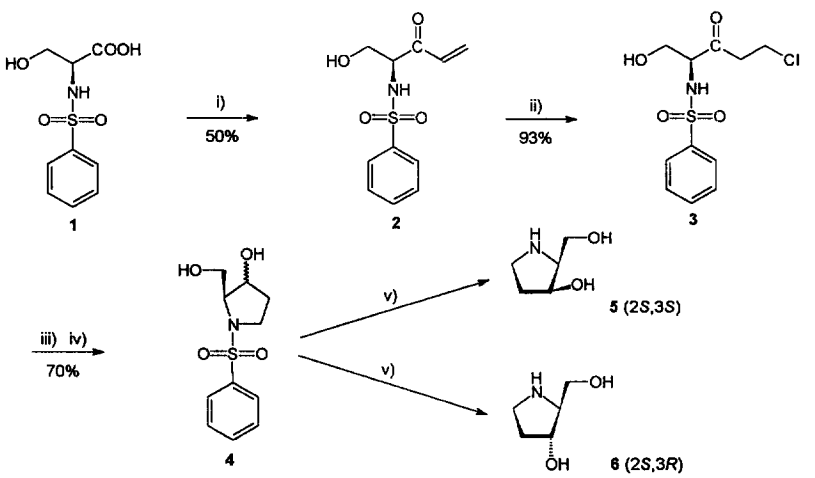



Fig. 2. Carbohydrate mimics used as sugar part of the oligonucleotides.

Reduction of the carbonyl with $LiBH_4$ afforded mainly the trans diastereoisomer as reported[9], and the obtained mixture was cyclized to obtain **4**. After separation by flash chromatography, the free amino diols **5** and **6** were obtained upon cleavage of the sulphonamide by $NH_3$(liq)/Na[16,17]. Deprotection of β-hydroxy-amino acid sulphonamides has been described to be difficult[18]. Deprotection with HBr/acetic acid was possible but provided the desired compound only in low yield. Isolation of the free amine went with difficulty. Most of the added buffering salt, sodium acetate, was removed by several precipitations with ethanol and the obtained oil was used as such for the condensation reaction.



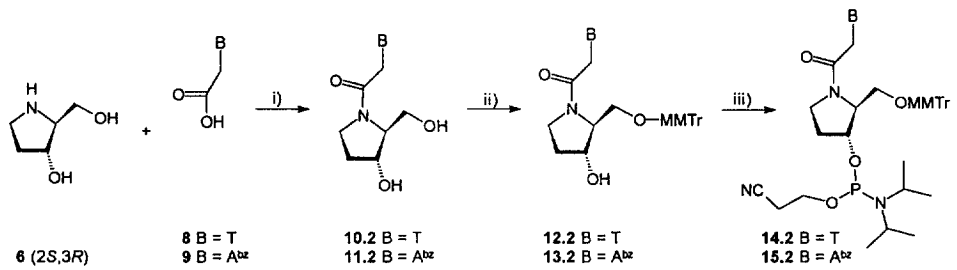

i) THF, BuLi, vinylMgBr, -78°C; ii) THF, HCl gas; iii) isopropanol, $LiBH_4$ (2 M) in THF; iv) $K_2CO_3$, $CH_3OH$; separation of diastereoisomers (isolated yield: trans 47%, cis 23%); v) $NH_3$(liq)/Na.

Scheme 1. Synthesis of enantiomerically pure 3-HO-D-prolinol as prepared from L-serine.

The synthesis starting from L-serine afforded the 2(*S*) stereoisomers (or D-prolinol derivatives), with a ratio of 3*R*/3*S* (or *trans*/*cis*) of about 2/1 after reduction with $LiBH_4$ in isopropanol and subsequent cyclisation.

Starting from D-serine the opposite enantiomers were obtained. Stereochemical assignment places the 4-HO-L-prolinol derivatives (2*S*) in the opposite enantiomeric class as the 3-HO-L-prolinol congeners (2*R*). However, because of a change in priority of the substituents at the asymmetrical carbon atom upon reduction of 3-hydroxy-L-proline (2*S*,3*S*), a hydroxyprolinol ring with 2(*R*) configuration is obtained. The stereoisomers 4-HO-prolinol (2*S*) and 3-HO-prolinol (2*R*) have the primary alcohol functions oriented similarly.

The amino diols **5**, **6** and **7** were condensed respectively with thymin-1-yl acetic acid[19] (**8**) or $N^6$-benzoyladenin-9-yl acetic acid[7] (**9**), monomethoxytritylated at the primary alcohol and converted to the 3-*O*-phosphoramidites, **14.y** and **15.y** respectively, as described before (Scheme 2)[7], with suffix y indicating the respective isomers (Figure 3). The monomers were oligomerised applying standard phosphoramidite chemistry making use of a 1,3-propanediol modified solid support to avoid synthesis of several differently functionalised supports[20]. The 1,3-propanediol tail has no influence on the pairing properties of oligonucleotides[20].




**6** (2*S*,3*R*) **8** B = T **9** B = $A^{bz}$ **10.2** B = T **11.2** B = $A^{bz}$ **12.2** B = T **13.2** B = $A^{bz}$ **14.2** B = T **15.2** B = $A^{bz}$

i) DCC or HBTU, HOBt, NMM, pyridine/DMF; ii) *p*-anisylchlorodiphenylmethane, pyridine, 40°C; iii) *N*,*N*-diisopropylethylamine, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, $CH_2Cl_2$. T = thymin-1-yl; $A^{bz}$ = $N^6$-benzoyladenin-9-yl.

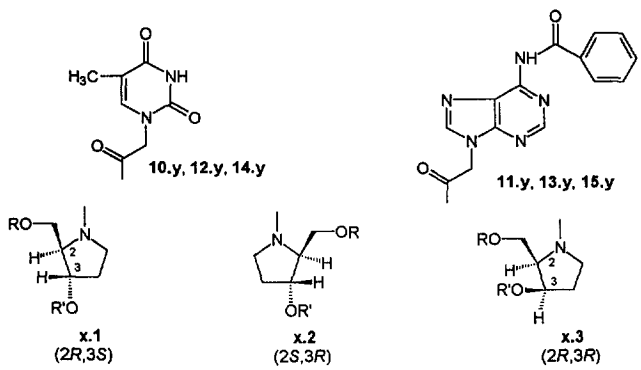
Scheme 2. Synthesis of nucleotide building blocks in the *trans*-3-HO-D-prolinol series.

Stepwise coupling yields (95-97% average) and total synthesis yield of oligomers were only slightly lower than for natural sequences. To all fully modified oligonucleotides two additional natural dC's were coupled at the 5'-terminus, allowing radioactive labelling. The additional bases were presumed to have no influence on the pairing properties of the oligonucleotide analogues.

*Analysis of the monomeric units and of oligonucleotides*

*NMR-analysis.* The geometrical assignment of the 3-HO-prolinol compounds was based on the comparison of the $^1$H NMR spectra with those published by Rapoport, using NOE experiments for determination of the configurations.

As noticed before in the 4-HO-prolinol series, the *N*-acetyl-3-HO-prolinol nucleosides likewise have doubled $^{13}$C signals in their NMR spectra. By analogy, the occurrence of two rotamers for these products seems reasonable due to restricted rotation of the nitrogen-carbon bond of the amide group. This phenomenon likewise recently was noticed by Dell'Uomo et al.[11]. All monomers were fully characterised using MS, UV and elemental analysis.




| X.Y | R | R' | X.Y | R | R' | X.Y | R | R' |
|---|---|---|---|---|---|---|---|---|
| **10.1** | | | **12.1** | | | **14.1** | | |
| **10.2** | | | **12.2** | | | **14.2** | | |
| **11.1** | H | H | **13.1** | MMTr[a] | H | **15.1** | MMTr[a] | P[b] |
| **11.2** | | | **13.2** | | | **15.2** | | |
| **11.3** | | | **13.3** | | | **15.3** | | |

a) MMTr stands for monomethoxytrityl ether protection;
b) P indicates the diisopropylamino-β-cyanoethoxyphosphinyl moiety.

Fig. 3. Overview of all synthesised nucleoside analogues.

*Mass spectrometry.* Electrospray ionisation (ESI) mass spectrometry of fully modified oligonucleotides confirmed the identity of the obtained products (data see experimental part).

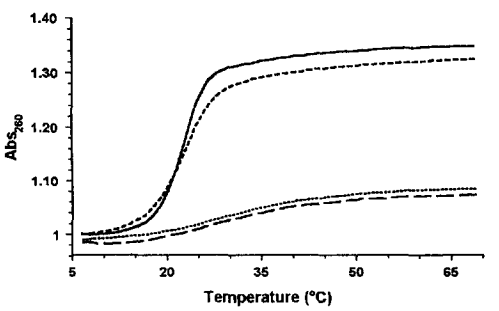



Fig. 4. Melting curves for the interactions of 3-HO-*trans*-adenine NAP oligonucleotides with tridecathymidine.
3-HO-*trans*-L-$A^*_{13}$: dashed ---, single strand dotted ···;
3-HO-*trans*-D-$A^*_{13}$: solid line, single strand dashed – – – (all normalised).

*Evaluation of oligonucleotide characteristics*

UV-melting curves were run to gain preliminary insight into the pairing properties of the newly designed oligomers. Intra-strand base stacking is rather pronounced for the *trans*-adenine oligomers, hyperchromicity is 6% over the 5-65°C range, compared to 14% for the control $(dA)_{13}$ oligomer. In contrast with the 4-HO-prolinol series, however, some kind of self-pairing can be detected as upon heating a slight sigmoidal curve is produced with the transition ("Tm") around 28 °C for both the D and L series (Figure 5 - bottom curves).

Modified all-thymine *trans*-oligomers display no melting upon annealing with complementary DNA or RNA, whereas the all-adenine *trans*-oligomers (L or D $A^*_{13}$) show hybridisation at nearly all conditions studied (Table 1, lines 3 and 8 and Figure 5). Stereochemistry (L or D analogues) in the case of these 3-hydroxyprolinol derived oligonucleotides is at first sight unimportant even for interaction with DNA which is in contrast with the hybridisation characteristics of the 4-hydroxyprolinol oligomers[7]: the stability of the complexes composed of DNA or RNA with L or D complementary adenine oligomers respectively, is similar (compare lines 3 and 8 left and right, respectively). The destabilisation upon hybridisation of LA* or DA* with DNA or RNA is equal for both modified strands, as compared to their equivalent natural systems, duplex DNA and hybrid DNA:RNA respectively (compare line 3 with 1 and line 8 with 6).

Table 1. Tm values (°C) of oligonucleotides incorporating *trans*-3-HO-NAP as sugar substitute.[a]

| | | Tm[b] | | | Tm[b] | |
|---|---|---|---|---|---|---|
| | L-*trans* | 0.1 M NaCl | 1 M NaCl | D-trans | 0.1 M NaCl | 1 M NaCl |
| | **Complement DNA** | | | | | |
| **1** | $T_{13}/A_{13}$ (duplex) | 34 | 48 | $T_{13}/A_{13}$ (duplex) | 34 | 48 |
| **2** | $LT^*_{13}/A_{13}$ | *c* | *c* | $DT^*_{13}/A_{13}$ | *c* | *c* |
| **3** | $T_{13}/LA^*_{13}$ (triplex) | *c* | 21 | $T_{13}/DA^*_{13}$ (triplex) | *c* | 21 |
| **4** | mixed LT*,LA*[d]/DNA | n.a. | *c* | mixed DT*,DA*[d]/DNA | n.a. | *c* |
| | **Complement RNA** | | | | | |
| **5** | $T_{13}$/polyA | 29 | 47 | $T_{13}$/polyA | 29 | 47 |
| **6** | $A_{13}$/polyU | 29 | 61 | $A_{13}$/polyU | 29 | 61 |
| **7** | $LT^*_{13}$/polyA | n.a. | *c* | $DT^*_{13}$/polyA | n.a. | *c* |
| **8** | $LA^*_{13}$/polyU | 18 | 40 | $DA^*_{13}$/polyU | 19 | 39 |
| **9** | mixed LA*,LT*[d]/RNA | n.a.[e] | *c* | mixed DA*,DT*[d]/RNA | n.a.[e] | *c* |
| | **Homocomplexes** | | | | | |
| **10** | mixed LA*,LT* | *c* | *c* | mixed DA*,DT* | *c* | *c* |

a) to all fully modified oligonucleotides two additional natural dC's were coupled at the 5'-terminus. When known through calorimetric titration, the hybridization state (duplex or triplex) is mentioned between brackets. b) Tm were measured in a buffer containing 0.02 M $KH_2PO_4$, pH 7.5, 0.1 mM EDTA with 0.1 or 1 M NaCl and 4 μM of each oligonucleotide at a heating rate of 0.2 °C/min. c) No hypochromicity detectable. d) sequence TAATAATATAAATTTT. e) n.a.: not assessed.

In general, the complexes formed with *trans*-3-HO-NAP oligonucleotides are considerably less strong than those of the previously described *trans*-4-HO-NAP molecules[7]. Where a Tm of 48 °C was obtained only for the *trans*-4-HO-L-NAP $A^*_{13}$ oligonucleotide versus $(dT)_{13}$ in 1 M NaCl, and no interaction was detected for the corresponding D-series, here a Tm of 21 °C was recorded for both series of *trans*-3-HO-NAP $A^*_{13}$ oligonucleotides. Calorimetric investigation, however, proved both interactions to be triple stranded complexes containing two pyrimidine strands (Figure 5).

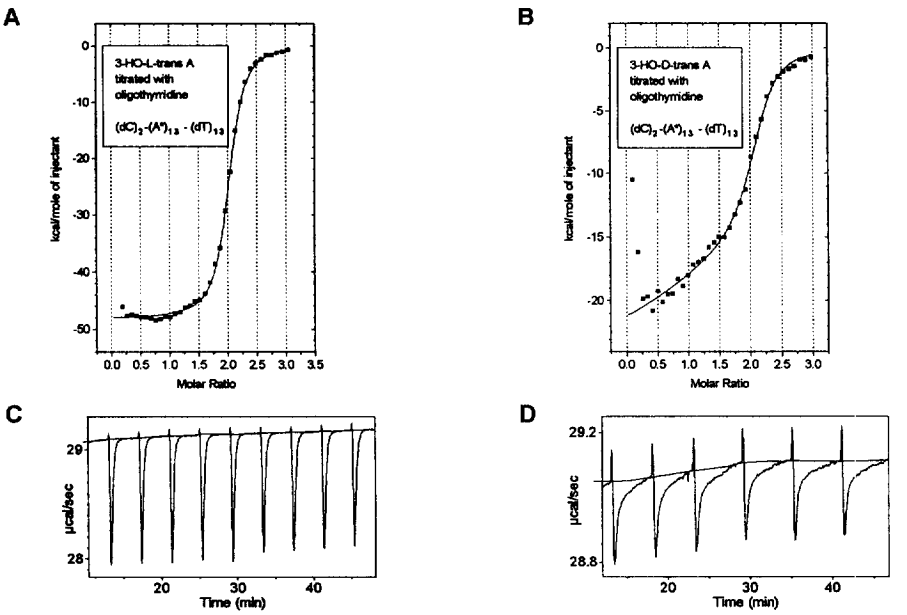



Fig. 5. Microcalorimetric titration of 3-HO-*trans*-adenine NAP oligonucleotides with tridecathymidine. Panels C and D display the actual raw data from time 12 to 47 min, with injections every 4 min in the L series (panel C) and every 6 min in the D series (panel D).

Remarkably, only a single transition is seen going from a single stranded $A^*_{13}$ to the triple stranded complex. In the D-series, the interaction is very slow as indicated as well by the UV studies (hysteresis) as by microcalorimetric titrations (compare panels C and D of Figure 5). In addition, the enthalpy change is considerably lower than for the corresponding L-NAP oligonucleotide. The calorimetric titration curve also hints at two contiguous slow processes: the heat produced upon the first 10-15 injections is gradually diminishing, where a clear "all or none" process would result in a S shape titration curve as in panel A. The accuracy of this titration curve (panel B) is somewhat lower, as a slight shift of the baseline over the long equilibration time after each injection (360 sec) causes deviations of the obtained enthalpy value. The apparently same type of interaction was noticed for 3 pairs of 4-HO-NAP oligos: complex formation of 4-HO-*trans*-L-$A^*_{13}$ with its congener 4-HO-*trans*-L-$T^*_{13}$ and the corresponding A*-T* interaction in the D-series, as well as the 4-

HO-*cis*-L-$A^*_{13}$ interaction with $(dT)_{13}$ all afforded a triple stranded T-A-T complex with a single transition[7], but all three proved to be slowly forming complexes.

We also investigated the hybridisation potential of mixed, non self-complementary sequences of the *trans*-configuration with their natural complement for which the sequence 5'-T*A*A*T*A*A*T*A*T*A*A*A*T*T*T*T*T*-3' (16-mer) was chosen. No hybridisation could be detected with the complementary natural nucleic acids (line 4 and 9), nor could the formation of a homo-complex be shown (line 10). The Tm of the equivalent natural DNA complex was determined as 48.5 °C (1 M NaCl).

In addition a homo-complex, composed of two complementary strands with the same sense of chirality and both having the *trans*-3-HO-NAP structure, is only forming at high salt concentration (Table 2, line 1 and Figure 6 panel A), and the hysteresis effect indicates these complexes to be formed very slowly.

Table 2. Tm values (°C) of homo-complexes of trans-3-HO-NAP oligonucleotides.[a]

| | L-trans | Tm[b] | D-trans | Tm[b] |
|---|---|---|---|---|
| 1 | 3-HO-$T^*_{13}$/3-HO-$A^*_{13}$ (triplex) | 17/14[c] (23%) | 3-HO-$T^*_{13}$/3-HO-$A^*_{13}$ (triplex) | 17/14[c] (24%) |
| 2 | 3-HO-$T^*_{13}$/4-HO-$A^*_{13}$ (triplex) | 30/25[c] (26%) | 3-HO-$T^*_{13}$/4-HO-$A^*_{13}$ (triplex) | 31/25[c] (27%) |
| 3 | 3-HO-$A^*_{13}$/4-HO-$T^*_{13}$ (duplex) | 25 (17%) | 3-HO-$A^*_{13}$/4-HO-$T^*_{13}$ (duplex) | 25 (18%) |
| 4 | 4-HO-L-$A^*_{13}$/4-HO-L-$T^*_{13}$ (triplex) | 28.5 (28%) | 4-HO-D-$A^*_{13}$/4-HO-L-$T^*_{13}$ (triplex) | 25 (28%) |
| 5 | 3-HO-L-$T^*_{13}$/3-HO-D-$A^*_{13}$ | 28[d] (5%) | 3-HO-D-$T^*_{13}$/3-HO-L-$A^*_{13}$ | 28[d] (5%) |
| 6 | 3-HO-L-$A^*_{13}$/4-HO-D-$T^*_{13}$ | 28[d] (5%) | 3-HO-D-$A^*_{13}$/4-HO-L-$T^*_{13}$ | 28[d] (5%) |
| 7 | $T_{13}/A_{13}$ | 48 (28%) | | |

a) to most of the fully modified oligonucleotides two additional natural dC's were coupled at the 5'-terminus. When known through calorimetric titration, the hybridization state (duplex or triplex) is mentioned between brackets, only the *trans*-NAP oligonucleotides (3-HO and 4-HO) have been studied. b) Tm were measured in a buffer containing 0.02 M $KH_2PO_4$, pH 7.5, 0.1 mM EDTA with 1 M NaCl and 4 μM of each oligonucleotide at a heating rate of 0.2 °C/min. c) The first number applies to the Tm obtained upon heating, the second one to the cooling experiment. d) melting of the 3-HO-$A^*_{13}$ single strand.

When melting curves were run at 0.1 °C/min, still a 2 °C difference in Tm was obtained between the heating and cooling experiment. When the adenine strand is replaced by its *trans*-4-HO-NAP equivalent[7], stability is reinstalled to approximate that of a *trans*-4-NAP homo-complex (Table 2 lines 2 and 4 and Figure 6 panel B) but equilibration remains very slow. However, a large discrepancy is noticed with the inverse complex. While for the complex with the 3-HO-NAP thymine oligonucleotide a large hysteresis effect is seen, this is absent for the corresponding complex with the 3-HO-NAP adenine oligonucleotide. The latter also displays about 18% hypochromicity, while for the former this amounts to 27%.

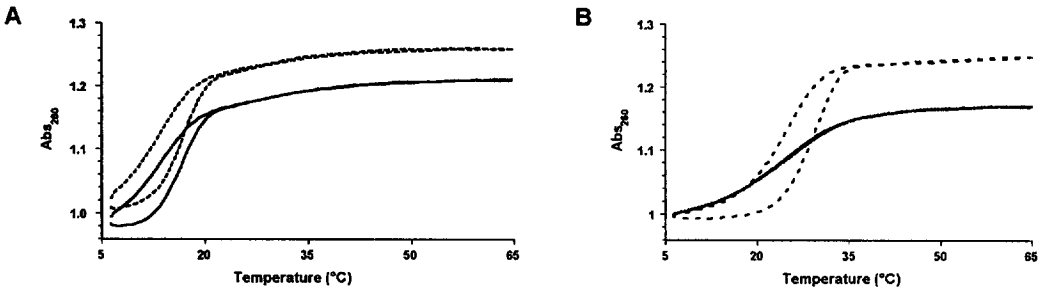



Fig. 6. Melting curves for the homo-complexes.

Panel A: Within the trans-3-HO-NAP series (heating + cooling): 3-HO-L-$T^*_{13}$/3-HO-L-$A^*_{13}$ solid, and 3-HO-D-$T^*_{13}$/3-HO-L-$A^*_{13}$ dashed. The substantial hysteresis effect can be clearly noticed (curves normalised).
Panel B: Between a 3-HO and 4-HO-trans-NAP oligonucleotide: 3-HO-L-$A^*_{13}$/4-HO-L-$T^*_{13}$ solid (dotted for cooling experiment) and 3-HO-L-$T^*_{13}$/4-HO-L-$A^*_{13}$ dashed (with hysteresis effect) (curves normalised).

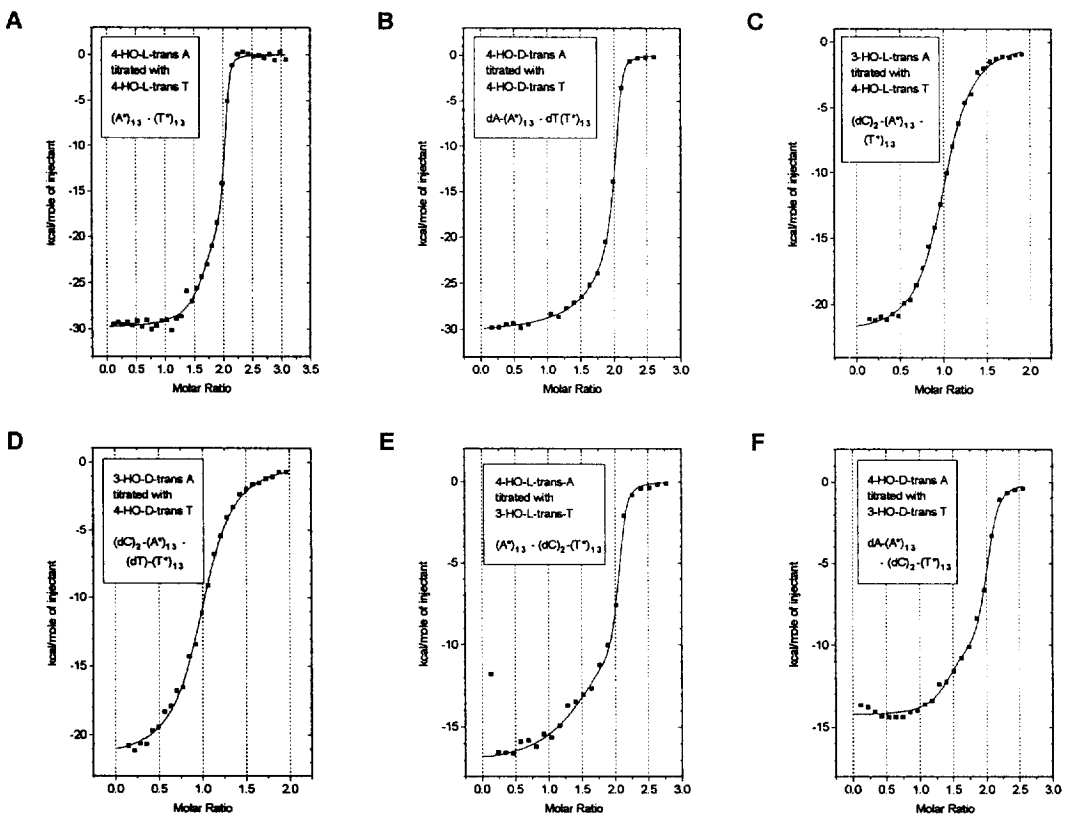



Fig. 7. Microcalorimetric titrations of different homo-complexes.

Calorimetric titration proved the former to be a triple stranded complex, while clearly duplex formation is noticed for the latter for both the L and D series. Thus, where the *trans*-4HO-NAP homo-complexes (*trans*-4-L-A*/*trans*-4-L-T* and *trans*-4-D-A*/*trans*-4-D-T*, Figure 7 panels A and B) proved to be 1:2 complexes with a single transition, in contrast, the *trans*-3HO-L-A*/*trans*-4-HO-L-T* and the corresponding interaction in the D series clearly are 1:1 interactions (panels C and D), albeit forming at a slow rate. The latter can be seen at the heat dissipation rate level, with comparison of a few calorimetric injections for the different interactions studied (not shown). The complex formation of the *trans*-3HO-thymine oligonucleotide with its *trans*-4-HO-adenine counterpart is less clear-cut with apparently at first the formation of an intermediate, but finalizing as a triple stranded complex (panels E and F). The impression is that from the hypochromicity values as well one can deduce whether the obtained complex is triple stranded or not with values of 24% to 28% compared to only 18% for the duplex of line 3 (Table 2). For the natural duplex, however, the hypochromicity amounts to 28% as well. No substantial interactions could be seen upon mixing either one of the L-NAP oligonucleotides with a counterpart of the D-series. Caution had to be payed when evaluating an oligo of both 3-HO-$A^*_{13}$ series, which as a single strand already display a sigmoidal curve with 5% hypochromicity (Table 2, lines 5 and 6).

For the *cis*-3-HO-NAP oligonucleotides only the oligomer composed of L-proline related compounds has been investigated (Table 3). This adenine oligomer does not hybridise with complementary DNA but retains the capacity of complex formation with complementary RNA. Stability of the complexes is low and approximates those found for the *cis*-4-HO-L-NAP oligonucleotide with RNA[7].

Table 3. Tm values of oligonucleotides with *N*-acetyl-*cis*-L-HO-prolinol as sugar substitute.

| | **Complement DNA** | 0.1 M NaCl | 1 M NaCl | | **Complement RNA** | 0.1 M NaCl | 1 M NaCl |
|---|---|---|---|---|---|---|---|
| **1** | $A_{13}/T_{13}$ | 35[a] | 48 | **4** | $dA_{13}$/polyU | 29 | 61 |
| **2** | 3-HO-L-$A^*_{13}/T_{13}$ | *b* | *b* | **5** | 3-HO-L-$A^*_{13}$/polyU | 10/6[c] | 37/32[c] |
| **3** | 4-HO-L-$A^*_{13}/T_{13}$ | *b* | 20/21[c] | **6** | 4-HO-L-$A^*_{13}$/polyU | 7 | 33 |

a) Tm were measured in a buffer containing 0.02 M $KH_2PO_4$, pH 7.5, 0.1 mM EDTA with 0.1 or 1 M NaCl and 4 μM of each oligonucleotide. b) No hypochromicity detectable. c) The first number applies to the Tm obtained upon heating, the second one to the cooling experiment.

Figure 8 summarises representative CD spectra of the duplexes. As was noticed for the *trans*-4-HO-NAP oligomers, association with polyU results in A-like conformations. Modified single strands have no clear profile. The fully artificial systems, homo-complex as well as complex between *trans*-4-HO-L-NAP-$A_{13}$ (L$A^*_{13}$) and *trans*-3-HO-L-NAP-$T_{13}$ (L$T^*_{13}$) display an unrelated low intensity spectrum. The complexes formed with $T_{13}$

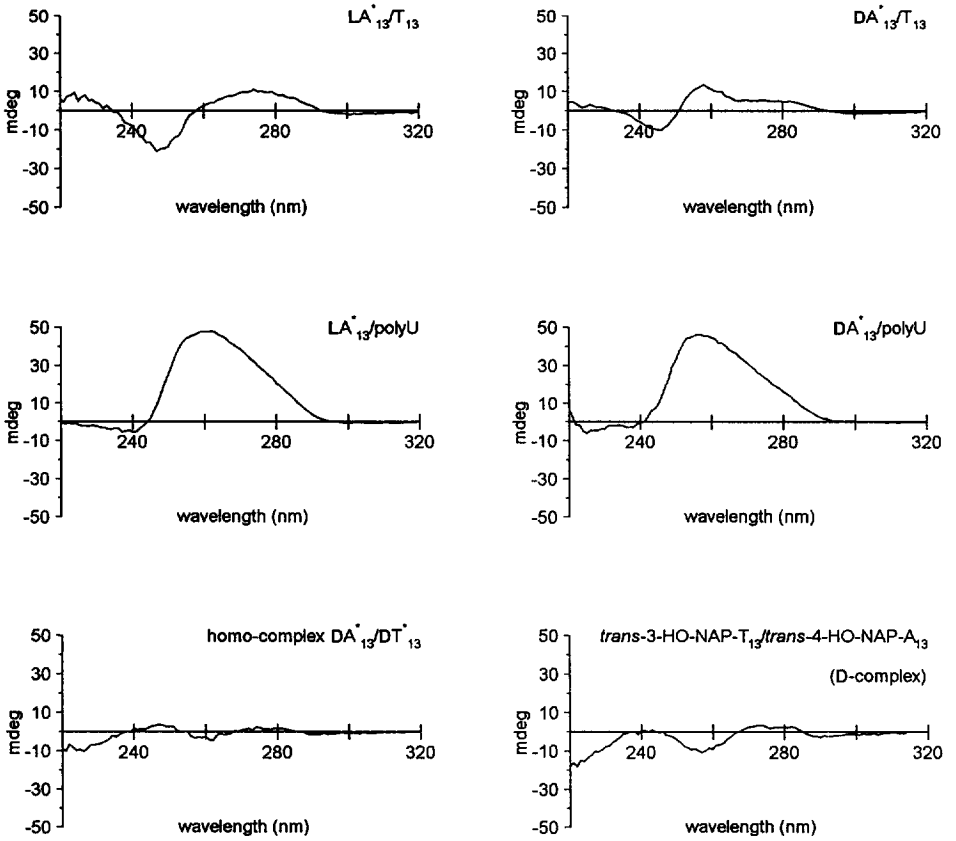



Fig. 8. CD-spectra of oligonucleotides incorporating *trans*-3-HO-NAP as sugar substitute.

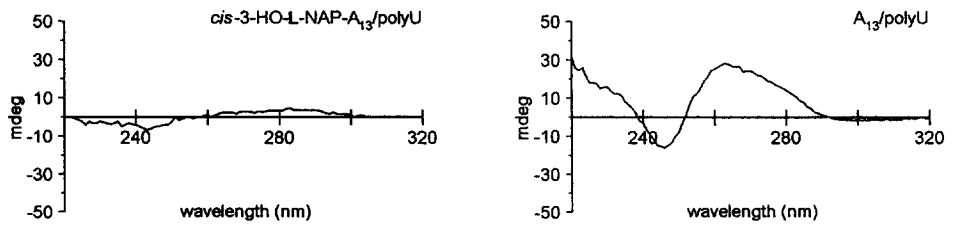



Fig. 9. CD-spectra of complexes with *cis*-oligomers.

show serious conformational alterations as compared to normal A or B-type helices. In this series the *trans*-3-HO-D-NAP-A oligonucleotide interacts with complementary DNA. It is the only D-oligonucleotide (stereochemically related to L-DNA instead of natural D-DNA) so far detected to interact with natural DNA (albeit with formation of a triple stranded complex, vide infra). The complex spectrum is comparable to the one recorded for the *trans*-4-HO-NAP-$T_{13}$/polyrA system[7] but has an extremely low intensity.

For the *cis*-3-HO-NAP all-adenine oligonucleotide, CD-spectra indicate a seriously altered helix conformation (Figure 9). The spectrum resembles the one obtained for a single stranded modified oligomer. Apparently the natural polyU strand has to adapt its conformation to the more rigid modified oligonucleotide.

## CONCLUSIONS

Although the 3-HO-NAP oligonucleotides were developed to increase pairing efficiency with complementary ssDNA and RNA, this structure does not live up to the expectations. The stabilities of the complexes formed are considerably lower than those previously detected with 4-HO-NAP structures. On the other hand, a notable broadening of the pairing interactions was observed, allowing L- as well as D-compounds to interact with complementary RNA as well as ssDNA. Many different homo-complexes could be formed as well with most of them resulting from triple stranded interactions.

## EXPERIMENTAL

Mass spectra were recorded using liquid secondary ion mass spectrometry (LSIMS) with thioglycerol (Thgly) as matrix. Most important fragments were as for 4-HO-trans-nucleosides[7]. Reaction circumstances, equipment and methods are exactly the same as reported for the synthesis of the 4-hydroxyprolinol analogues[7]. Therefore, only analytical data of newly synthesised compounds are given together with the equivalents used in the reactions and yields obtained. The first numbers of the $^{13}C$ NMR data indicate the shift for the major rotamer, except for signals assigned to aromates.

*(2S)-2-[(Phenylsulfonyl)amino]-5-chloro-1-hydroxy-3-pentanone (3).* The solid **2** (10 g, 39.2 mmol) obtained upon Grignard reaction was dissolved in anhydrous THF (500 mL). At 0 °C, HCl was bubbled through for 30 min, after which TLC (hexane-$Et_2O$ 6/4) indicated the reaction to be complete. The mixture was evaporated to dryness, and the residue was dissolved in saturated $NaHCO_3$ and extracted with $CH_2Cl_2$ (3 x 300 mL). The organic layer was washed once more with saturated $NaHCO_3$ and with brine. The extracts were combined, dried and evaporated to afford 93% of the chloro ketone **3** (10.63 g, 36.5 mmol). $^1H$ NMR analysis as reported[9].

*General procedure for removal of the N-phenylsulfonyl protecting group.* In each case, the *N*-phenylsulfonyl prolinol analogue was dissolved in ammonia (75 mL per gram) to which Na was added till persistence of a blue color (6 to 8 eq.), which turned green after 15 min. An excess of NaOAc was added and

the ammonia was evaporated. The residue was dissolved in a minimal amount of water and washed with $CH_2Cl_2$. The aqueous layer was treated with EtOH to precipitate excess of NaOAc and was acidified with HCl and evaporated. The residue contains NaCl which does not interfere with the coupling reaction. An analytical sample could be obtained upon ion exchange chromatography. $^1H$ and $^{13}C$ NMR as reported[21].

*Condensation reactions*

*3(S)-Hydroxy-2(R)-hydroxymethyl-N-[(thymin-1-yl)-acetyl]-pyrrolidine (10.1).* Synthesised from *trans*-L-3-hydroxyprolinol (0.62 g) and (thymin-1-yl)-acetic acid (1.2 eq.) in 80% yield.

$^1H$ NMR ($CD_3OD$): δ = 1.9 (s, 3H, $CH_3$), 1.9-2.4 (m, 2H, H-4), 3.4-3.8 (m, 5H, $CH_2OH$, H-5, H-2), 3.9-4.0 (brs, 1H, H-3), 4.3 (d, 2H, $CH_2$), 7.3 (s, 1H, H-6 of thymine). $^{13}C$ NMR ($CD_3OD$): δ = 12.2 ($CH_3$), 33.4 and 31.2 (C-4), 45.7 ($CH_2$), 50.3 (C-5), 61.3 and 63.1 ($CH_2OH$), 69.6 and 68.9 (C-2), 73.1 and 74.2 (C-3), 111.0 (C-5 of thymine), 143.7 and 143.9 (C-6 of thymine), 153.1 (C-2 of thymine), 167.0 (C-4 of thymine), 168.2 and 168.7 (amide). UV (MeOH): $\lambda_{max}$ (ε) = 269 nm (9400). LSIMS (Thgly) m/z: 284 $[M+H]^+$, 167 $[BCH_2CO]^+$, 118 [aminodiol + 2H]$^+$; HRMS $C_{12}H_{18}N_3O_5$ calculated 284.1246: found 284.1258. Elem. anal. $C_{12}H_{17}N_3O_5$: calculated C: 50.88, H: 6.05, N: 14.83; found C: 50.82, H: 6.02, N: 14.69.

*3(R)-Hydroxy-2(S)-hydroxymethyl-N-[(thymin-1-yl)-acetyl]-pyrrolidine (10.2).* Synthesised from *trans*-D-3-hydroxyprolinol (0.46 g) and (thymin-1-yl)-acetic acid (1.5 eq.) in 90% yield.

$^1H$ NMR ($CD_3OD$): δ = 1.9 (s, 3H, $CH_3$), 1.9-2.4 (m, 2H, H-4), 3.4-3.8 (m, 5H, $CH_2OH$, H-5, H-2), 3.9-4.0 (brs, 1H, H-3), 4.3 (d, 2H, $CH_2$), 7.3 (s, 1H, H-6 of thymine). $^{13}C$ NMR ($CD_3OD$): δ = 12.2 ($CH_3$), 33.4 and 30.7 (C-4), 45.7 ($CH_2$), 50.3 (C-5), 61.3 and 63.1 ($CH_2OH$), 69.6 (C-2), 73.1 and 74.2 (C-3), 111.0 (C-5 of thymine), 143.7 (C-6 of thymine), 153.2 (C-2 of thymine), 167.0 (C-4 of thymine), 168.2 (amide). UV (MeOH): $\lambda_{max}$ (ε) = 270 nm (8400). LSIMS (Thgly) m/z: 284 $[M+H]^+$, 167 $[BCH_2CO]^+$, 118 [aminodiol + 2H]$^+$; HRMS $C_{12}H_{18}N_3O_5$ calculated 284.1246: found 284.1242. Elem. anal. $C_{12}H_{17}N_3O_5$: calculated C: 50.88, H: 6.05, N: 14.83; found C: 50.78, H: 5.83, N: 14.77.

*3(S)-Hydroxy-2(R)-hydroxymethyl-N-[($N^6$-benzoyladenin-9-yl)-acetyl]-pyrrolidine (11.1).* The compound was synthesised from 0.61 g *trans*-L-hydroxyprolinol using 1.2 eq. ($N^6$-benzoyladenin-9-yl) acetic acid in 50% yield.

$^1H$ NMR (DMSO-$d_6$): δ = 1.7-2.3 (m, 2H, H-4), 3.1-3.8 (m, 5H, $CH_2OH$, H-5, H-2), 4.0 (brs, 1H, H-3), 4.2-4.3 (brs, 2H, $CH_2$), 7.5-7.7 (m, 3H, aryl), 7.9-8.1 (m, 2H, aryl), 8.4 (2xs, 1H, H-8 of adenine), 8.7 (s, 1H, H-2 of adenine). $^{13}C$ NMR (DMSO-$d_6$): δ = 32.1 and 30.2 (C-4), 44.3 ($CH_2$), 45.0 and 44.7 (C-5), 59.7 and 61.9 ($CH_2OH$), 68.2 and 67.4 (C-2), 70.8 and 72.1 (C-3), 125.1 (C-5 of adenine), 128.6, 132.5 and 133.6 (aryl), 145.8 (C-8 of adenine), 150.1 (C-4 of adenine), 151.5 (C-2 of adenine), 152.9 (C-6 of adenine), 165.1 (CO), 165.4 and 165.7 (amide). UV (MeOH) $\lambda_{max}$ (ε): 278 nm (11900). LSIMS (Thgly) m/z: 397 $[M+H]^+$, 240 $[BCH_2CO]^+$. HRMS $C_{19}H_{21}N_6O_4$ calculated 397.1624: found 397.1625. Elem. anal. $C_{19}H_{20}N_6O_4$: calculated C: 57.57, H: 5.09, N: 21.20; found C: 57.42, H: 4.92, N: 21.25.

*3(R)-Hydroxy-2(S)-hydroxymethyl-N-[($N^6$-benzoyladenin-9-yl)-acetyl]-pyrrolidine (11.2).* Starting from 1.0 g of *trans*-D-hydroxyprolinol and using 1.2 eq. of ($N^6$-benzoyladenin-9-yl)-acetic acid, the title compound was obtained in 54% yield.

$^1$H NMR ($CD_3OD$): δ = 1.9-2.5 (m, 2H, H-4), 3.5-4.0 (m, 5H, $CH_2OH$, H-5, H-2), 4.0 (brs, 1H, H-3), 4.3-4.5 (m, 2H, $CH_2$), 7.4-7.8 (m, 3H, aryl), 8.0-8.2 (m, 2H, aryl), 8.5 (2xs, 1H, H-8 of adenine), 8.7 (2xs, 1H, H-2 of adenine). $^{13}$C NMR ($CD_3OD$): δ = 33.5 and 31.4 (C-4), 45.9 ($CH_2$), 46.4 (C-5), 61.2 and 63.3 ($CH_2OH$), 69.8 and 69.2 (C-2), 73.1 and 74.1 (C-3), 122.9 (C-5 of adenine), 129.5 and 134.6 (aryl), 146.1 and 147.0 (C-8 of adenine), 150.1 (C-4 of adenine), 152.7 (C-2 of adenine), 153.8 (C-6 of adenine), 167.1 (CO), 167.6 and 168.5 (amide). UV (MeOH) $\lambda_{max}$ (ε): 278 nm (14200). LSIMS (Thgly) m/z: 397 $[M+H]^+$, 240 $[BCH_2CO]^+$. HRMS $C_{19}H_{21}N_6O_4$ calculated 397.1624: found 397.1646. Elem. anal. $C_{19}H_{20}N_6O_4$: calculated C: 57.57, H: 5.09, N: 21.20; found C: 57.71, H: 5.21, N: 20.99.

*2(R)-Hydroxy-3(R)-hydroxymethyl-N-[($N^6$-benzoyladenin-9-yl)-acetyl]-pyrrolidine (11.3).* The title compound was obtained in 85% starting from 0.5 g of *cis*-L-hydroxyprolinol and 1.2 eq. of ($N^6$-benzoyladenin-9-yl)-acetic acid.

$^1$H NMR ($CD_3OD$): δ = 1.9-2.3 (m, 2H, H-4), 3.4-4.1 (m, 5H, $CH_2OH$, H-5, H-2), 4.2-4.6 (m, 4H, H-3 and $CH_2$), 7.4-7.8 (m, 3H, aryl), 8.0-8.2 (m, 2H, aryl), 8.4 (2xs, 1H, H-8 of adenine), 8.6 (2xs, 1H, H-2 of adenine). $^{13}$C NMR ($CD_3OD$): δ = 33.6 and 30.4 (C-4), 45.6 and 44.3 ($CH_2$), 46.6 and 46.2 (C-5), 60.7 and 61.3 ($CH_2OH$), 64.3 and 65.1 (C-2), 71.8 and 72.0 (C-3), 124.0 and 123.9 (C-5 of adenine), 129.7 and 134.6 (aryl), 147.1 and 147.0 (C-8 of adenine), 150.6 (C-4 of adenine), 153.1 (C-2 of adenine), 153.9 (C-6 of adenine), 167.7 (CO), 168.4 (amide). UV (MeOH) $\lambda_{max}$ (ε): 279 nm (16500). LSIMS (Thgly) m/z: 397 $[M+H]^+$, 240 $[BCH_2CO]^+$. HRMS $C_{19}H_{21}N_6O_4$ calculated 397.1624: found 397.1638. Elem. anal. $C_{19}H_{20}N_6O_4$: calculated C: 57.57, H: 5.09, N: 21.20; found C: 57.41, H: 5.15, N: 21.08.

*Monomethoxytritylation reactions*

*3(S)-Hydroxy-2(R)-monomethoxytrityloxymethyl-N-[(thymin-1-yl)-acetyl] pyrrolidine (12.1).* Starting from 0.66 g of **10.1** and using 1.2 eq. of *p*-anisylchlorodiphenylmethane, the title compound was obtained in 20% yield.

$^{13}$C NMR ($CDCl_3$): δ = 12.2 ($CH_3$), 30.7 and 32.6 (C-4), 44.4 and 44.2 ($CH_2$), 49.2 and 48.6 (C-5), 55.1 ($OCH_3$), 63.9 and 62.0 ($CH_2OH$), 65.8 and 66.3 (C-2), 73.3 and 72.3 (C-3), 87.7 and 86.4 (C), 110.5 and 110.3 (C-5 of thymine), 113.2, 113.0, 126.8-130.4, 135.2, 143.5, 158.7 (MMTr), 141.2 and 141.4 (C-6 of thymine), 151.7 and 151.4 (C-2 of thymine), 164.6 (C-4 of thymine), 166.1 and 165.5 (amide). UV (MeOH) $\lambda_{max}$ (ε): 231 nm (sh) (16500), 269 nm (11800). LSIMS (Thgly, NaOAc) m/z: 578 $[M+Na]^+$, 600 $[M+2Na]^+$, 273 $[MMTr]^+$. HRMS $C_{32}H_{33}N_3O_6Na$ calculated 578.2267; found 578.2241. Elem. anal. $C_{39}H_{37}N_6O_5.0.5H_2O$: calculated C: 68.07, H: 6.07, N: 7.44; found C: 68.27, H: 6.03, N: 7.50.

*3(R)-Hydroxy-2(S)-monomethoxytrityloxymethyl-N-[(thymin-1-yl)-acetyl] pyrrolidine (12.2).* Starting from 0.31 g of **10.2** and 3.5 eq. of *p*-anisylchlorodiphenylmethane, 43% of the title compound was obtained.

$^{13}C$ NMR ($CDCl_3$): δ = 12.3 ($CH_3$), 30.8 and 32.7 (C-4), 44.2 and 44.5 ($CH_2$), 49.3 and 48.6 (C-5), 55.2 ($OCH_3$), 64.0 and 62.0 ($CH_2OH$), 66.0 and 66.4 (C-2), 73.4 and 72.5 (C-3), 87.7 and 86.5 (C), 110.6 and 110.5 (C-5 of thymine), 113.3, 113.1, 126.9-130.5, 134.7, 143.7, 158.7 (MMTr), 141.2 (C-6 of thymine), 151.7 and 151.3 (C-2 of thymine), 164.5 (C-4 of thymine), 166.1 and 165.5 (amide). UV (MeOH) $\lambda_{max}$ (ε): 231 nm (sh) (17300), 270 nm (11900). LSIMS (Thgly, NaOAc) m/z: 578 $[M+Na]^+$, 600 $[M+2Na]^+$, 273 $[MMTr]^+$. HRMS $C_{32}H_{33}N_3O_6Na$ calculated 578.2267; found 578.2275. Elem. anal. $C_{39}H_{37}N_6O_5.0.7H_2O$: calculated C: 67.64, H: 6.10, N: 7.39; found C: 67.68, H: 6.01, N: 7.51.

*3(S)-Hydroxy-2(R)-monomethoxytrityloxymethyl-N-[($N^6$-benzoyladenin-9-yl)-acetyl]-pyrrolidine (13.1).* Starting from 0.60 g of **11.1** and using 2.5 eq. of *p*-anisylchlorodiphenylmethane, **13.1** was obtained in 65% yield.

$^{13}C$ NMR (CDCl3): δ = 32.6 and 30.7 (C-4), 44.8 and 44.6 ($CH_2$), 44.7 (C-5), 55.1 ($OCH_3$), 61.8 and 63.8 ($CH_2OH$), 66.6 and 66.3 (C-2), 72.5 and 73.5 (C-3), 86.6 and 87.7 (C), 113.1, 113.2, 126.9-130.3, 135.1, 143.7, 158.7 (MMTr), 132.6 and 133.6 (aryl), 121.6 (C-5 of adenine), 144.0 (C-8 of adenine), 149.0 (C-4 of adenine), 152.2 (C-2 of adenine), 151.9 (C-6 of adenine), 164.7 (CO), 165.0 (amide). UV (MeOH) $\lambda_{max}$ (ε): 232 nm (28000), 282 nm (22200). LSIMS (Thgly, NaOAc) m/z: 691 $[M+Na]^+$, 273 $[MMTr]^+$. HRMS $C_{39}H_{36}N_6O_5Na$ calculated 691.2645; found 691.2661. Elem. anal. $C_{39}H_{37}N_6O_5.0.4H_2O$: calculated C: 69.13, H: 5.49, N: 12.43; found C: 69.13, H: 5.68, N: 12.66.

*3(R)-Hydroxy-2(S)-monomethoxytrityloxymethyl-N-[($N^6$-benzoyladenin-9-yl)-acetyl]-pyrrolidine (13.2).* Starting from 1.5 g of **11.2** and using 5.5 eq. of *p*-anisylchlorodiphenylmethane, the title compound was obtained in 48% yield.

$^{13}C$ NMR ($CDCl_3$): δ = 32.7 and 30.9 (C-4), 44.9 and 44.8 ($CH_2$), 44.7 and 44.4 (C-5), 55.2 ($OCH_3$), 61.9 and 63.8 ($CH_2OH$), 66.7 and 66.4 (C-2), 72.6 and 73.6 (C-3), 86.7 and 87.8 (C), 113.1, 113.3, 127.0-130.4, 135.2, 144.1, 158.8 (MMTr), 132.7 and 133.7 (aryl), 121.9 (C-5 of adenine), 144.2 and 144.0 (C-8 of adenine), 149.4 and 149.3 (C-4 of adenine), 152.7 and 152.5 (C-2 of adenine), 152.0 and 151.9 (C-6 of adenine), 164.7 (CO), 165.1 (amide). UV (MeOH) $\lambda_{max}$ (ε): 232 nm (27500), 282 nm (21900). LSIMS (Thgly, NaOAc) m/z: 691 $[M+Na]^+$, 273 $[MMTr]^+$. HRMS $C_{39}H_{36}N_6O_5Na$ calculated 691.2645; found 691.2653. Elem. anal. $C_{39}H_{37}N_6O_5.2.6H_2O$: calculated C: 65.46, H: 5.80, N: 11.74; found C: 65.40, H: 5.20, N: 11.68.

*3(R)-Hydroxy-2(R)-monomethoxytrityloxymethyl-N-[($N^6$-benzoyladenin-9-yl)-acetyl]-pyrrolidine (13.3).* The title compound was synthesised in 50% yield starting from 0.31 g of **11.3** and using 2.0 eq. of p-anisylchlorodiphenylmethane.

$^{13}C$ NMR ($CDCl_3$): δ = 32.7 and 29.6 (C-4), 44.6 ($CH_2$), 44.6 and 43.0 (C-5), 55.0 ($OCH_3$), 59.2 and 60.5 ($CH_2OH$), 60.7 and 61.9 (C-2), 70.9 (C-3), 87.0 and 88.0 (C), 113.2, 127.8-130.3, 134.7, 143.6, 158.5 (MMTr), 133.5 and 132.5 (aryl), 121.8 (C-5 of adenine), 143.8 (C-8 of adenine), 149.4 and 149.2 (C-4 of adenine), 152.4 and 152.2 (C-2 of adenine), 151.8 (C-6 of adenine), 164.2 (CO), 164.9 (amide). UV (MeOH) $\lambda_{max}$ (ε): 232 nm (24800), 282 nm (20100). LSIMS (Thgly, NaOAc) m/z: 691 $[M+Na]^+$, 273 $[MMTr]^+$. HRMS $C_{39}H_{36}N_6O_5Na$ calculated 691.2645; found 691.2622. Elem. anal. $C_{39}H_{37}N_6O_5.0.2H_2O$: calculated C: 69.67, H: 5.46, N: 12.50; found C: 69.63, H: 5.56, N: 12.53.

*Phosphoramidite synthesis*

The phosphoramidite synthesis was carried out using 1 mmol of starting material with 3 eq. of N,N-diisopropylethylamine and 1.5 eq. of N,N-diisopropylchlorophosphoramidite. Yields were as follows: **14.1** (99%), **14.2** (90%), **15.1** (96%), **15.2** (94%), **15.3** (97%).

Phosphoramidite analyses:

| | TLC-system[a] | Rf | MS | $^{31}$P NMR[b] | |
|---|---|---|---|---|---|
| **14.1** | 28/70/2 | 0.58 | 754 $[M-H]^-$ | 149.32 (148.70) | 148.09 (148.68) |
| **14.2** | 28/70/2 | 0.63 | 754 $[M-H]^-$ | 149.32 (148.70) | 148.10 (148.68) |
| **15.1** | 28/70/2 | 0.58 | 867 $[M-H]^-$ | 149.05 (149.83) | 149.02 (148.42) |
| **15.2** | 28/70/2 | 0.63 | 867 $[M-H]^-$ | 149.05 (149.83) | 149.02 (148.42) |
| **15.3** | 28/70/2 | 0.56 | 867 $[M-H]^-$ | 150.34 (150.043) | 149.85 (150.16) |

a) the ratios given are those for the system n-hexane/acetone/triethylamine. b) the numbers between brackets are the minor peaks.

*Oligonucleotide synthesis*

Oligonucleotide synthesis was carried out on an automated DNA-synthesizer, model ABI 392 purchased from Applied Biosystems, using the phosphoramidite approach. Condensations were run at 0.13 M of the respective modified building block for 3 min to ensure adequate coupling yields. Intermediate deprotection of the monomethoxytrityl protecting group was performed by prolonged acid treatment (90 seconds). The obtained sequences were deprotected and cleaved of the solid support by treatment with concentrated ammonia at 55 °C for 16 hours. After a first purification on a NAP-10$^R$ column (Sephadex G25-DNA grade), a Mono-Q$^R$ HR 10/10 anion exchange column (Pharmacia) was used with the following gradient system: A: NaOH, pH 12.0 (10 mM), NaCl (0.1 M); B: NaOH, pH 12.0 (10 mM), NaCl (0.9 M). The gradient used depended on the oligonucleotide. Flow rate 2 mL/min. Product containing fractions were immediately neutralised by addition of aqueous ammonium acetate. The purification of the *trans*-3-hydroxyprolinol based oligoadenylates (for both D and L series), which seem to aggregate, could not be done under the usual conditions at pH 12. Therefore a denaturing gradient was run from A to B with A: 50 mM TrisHCl, 0.05 M NaCl, 5 M urea and 20% MeOH; B: 50 mM TrisHCl, 1 M NaCl, 5 M urea and 20% MeOH. Following concentrations, the eluent was desalted on a NAP-10$^R$ column and lyophilised.

*UV-melting experiments and wavelength scans*

UV-melting experiments and wavelenth scans were recorded using a Uvikon 940 spectrophotometer. Samples were dissolved in a buffer solution containing 0.1 or 1 M NaCl, 0.02 M potassium phosphate pH 7.5, 0.1 mM EDTA. The oligomer concentration was determined by measuring the absorbance at 80 °C and assuming the following extinction coefficients in the denatured state: T = 8500, A = 13000 and C = 7500. The concentration in all experiments was 4 μM for each strand. Cuvettes were thermostated with water circulating through the cuvette holder and the temperature was measured with a thermistor immersed directly in the cuvette.

For the melting experiments, temperature control and data acquisition were done automatically with an IBM/PC AT compatible computer. The samples were heated and cooled at a rate of 0.2 °C/min with data

sampling every 30 seconds. Tm values were determined taking the maximum of the first derivative curve. If a substantial difference was detected between heating and cooling melting curves, both recorded Tm are given (first figure relating to heating experiment).

*Titration microcalorimetry*

The calorimetric cell was filled with 5 μM solution of the respective modified $A_{13}$-oligo and titrated with a 100 μM solution of tridecathymidine or the tridecathymidine analogue with both solutions made up in 1 M NaCl, 0.02 M potassium phosphate pH 7.5 and 0.1 mM EDTA, analogous to the buffer used for Tm determination. An injection was made either every 240 or 360 sec depending on the kinetics of the interaction.

*Mass spectrometric analysis of oligonucleotides*

Five nanomol of the respective oligonucleotide were taken up in 0.5 ml of 0.2 M triethylammonium bicarbonate (TEAB) pH 8 and loaded on a C18 cartridge (Waters) pre-equilibrated with aqueous TEAB. After washing the cartridge with 2 ml of TEAB solution, the oligo was eluted with MeOH:TEAB 0.2 M 1:1 and the eluate was lyophilised.

Immediately before use, the samples were dissolved in 300 μl of a mixture of acetonitrile:0.01 M ammonium acetate 1:1 (final concentration of the oligonucleotide about 16 pmol/μl). Electrospray ionisation mass spectra were recorded in negative mode on a VG Quattro II mass spectrometer (Micromass, Manchester, UK) equipped with a Mass Lynx data system. The sample spray flow was set to 10 μl/min. Five spectra were acquired and summed in MCA mode in the m/z range 500 to 1500. The molecular weights were determined applying the maximum entropy algorithm.

| | | Calcd. | Found |
|---|---|---|---|
| 3-HO-L-*trans*-A | CC $A^{*}_{13}$-propanediol | 5259.8 | 5259.4 |
| 3-HO-L-*cis*-A | CC $A^{*}_{13}$-propanediol | 5259.8 | 5259.0 |
| 3-HO-L-*trans*-T | CC $T^{*}_{13}$-propanediol | 5142.6 | 5142.5 |

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