Synthesis and properties of 2'-O-neopentyl modified oligonucleotides†

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2'-O-Neopentyldeoxyuridine (Un) was synthesized and incorporated into a series of oligodeoxyribonucleotides. Single and triple incorporations in various arrangements were performed. The Watson and Crick pairing properties with complementary DNA and RNA were investigated by UV melting curves, CD spectroscopy, and molecular dynamic simulations. The results were compared to those obtained with DNA–DNA and DNA–RNA duplexes involving dU at the same positions. Oligonucleotides containing Un clearly demonstrated their ability to form duplexes with both complementary DNA and RNA but with higher stabilities for the DNA–RNA duplexes similar to the one of the parent DNA–RNA duplex. Investigations into the thermodynamic properties of these 17-base-pair duplexes revealed ΔG values (37 °C) that are in line with the measured Tm values for both the DNA–DNA and DNA–RNA duplexes. CD spectroscopic structural investigations indicated that the conformations of the DNA–DNA and DNA–RNA duplexes involving Un are similar to those of the dT–rA and dU–rA containing duplexes. Only small changes in intensities and weak blue shifts were observed when three Uns were incorporated into the duplexes. The results of the molecular dynamic simulations showed, for the six duplexes involving the modified nucleoside Un, calculated curvatures similar to those of the corresponding unmodified duplexes without base-pair disruption. The neopentyl group is able to be accommodated in the minor grooves of both the DNA–DNA and RNA–DNA duplexes. However, molecular dynamic simulations indicated that the Uns adopt a C2'-exo sugar pucker conformation close to an A-helix type without perturbing the C2'-endo sugar pucker conformations of their 2'-deoxynucleoside neighbours. These results confirm the potential of 2'-O-neopentyldeoxyuridine as a nucleoside surrogate for oligonucleotide based therapeutic strategies.

Introduction

Chemically modified oligonucleotides (ONs) are powerful research tools for gene structure, regulation and function analysis as well as promising therapeutic agents.1-13 It has been known for decades that modulation of gene expression can be achieved by targeting messenger RNAs with single-stranded chemically modified ONs (antisense-strategy).14 More recently, it has been demonstrated that inhibition of gene expression in mammalian cells can be efficiently achieved by double-stranded oligoribonucleotides via the interference (RNAi) pathway.15 RNA interference involves complex biological gene-silencing mechanisms and homogeneously increased stability of chemically modified oligoribonucleotides to their target may reduce their efficiency by impairing guide strand dissociation. In addition to the common properties (resistance to nucleases, cell-specific delivery, efficient uptake and adequate intracellular distribution), efficient double-stranded chemically modified siRNAs require fine tuning of their binding affinity along the sequences. The rapid development of this strategy has benefited from the different structural modifications of ONs previously selected for the antisense strategy and potent siRNA have been selected.16-33 However, this strategy triggers unintended gene silencing due to the inherent miRNA-like behaviour of double-stranded RNAs. It has been recently shown that chemical modifications of siRNAs at specific positions can reduce off-targeting.8,22-27,30,32 The most efficient is the strongly destabilizing unlocked nucleic acid (UNA).26 A few modifications have been tested. We do believe that other new chemically modified nucleosides, to

†Electronic supplementary information (ESI) available: 1H NMR, 13C NMR, 31P NMR spectra. HPLC and MS characterisations for ONs and Fig. 6–8. See DOI: 10.1039/c2ob26871j
be identified, may be used to finely tune the hybridization properties of chemically modified siRNA. Previous studies indicated that 2′-modified ONs appear to be promising due to their favourable hybridization with complementary RNA. More often, such modifications force the ONs to adopt a higher proportion of the northern conformation in a helical structure, which is one of the contributing factors of the stronger hybridization property of these modified ONs. The binding affinity of the modified ONs is also dependent on the size of the alkoxy group. We focused on the neopentyl group that to our knowledge has never been reported for 2′-substitution of nucleosides. We made the hypothesis that this bulky group could be accommodated in the minor groove upon hybridization of the ON with its RNA target.

Here, we report the synthesis of 2′-O-neopentyldeoxyuridine (Un) and study the effect of its single and multiple incorporations, in different arrangements, into a series of 17-mer oligodeoxynucleotides on their binding properties with DNA and RNA targets. The thermal and thermodynamic transition profiles of duplexes with complementary DNA and RNA were evaluated. Owing to the structural differences between Un and dT, in order to assess the contribution of the 2′-O-neopentyl group to duplex stability, a series of ONs involving dU in the same positions was also studied. A study of the helix conformation of duplexes with different Un contents was carried out by CD spectroscopy and molecular dynamic simulations.

Results and discussion

Structures and experimental design

The neopentyl group was linked to the 2′-position of 2′-deoxyuridine and the modified nucleoside was incorporated in various arrangements into a 17-mer sequence (Fig. 1 and 2). A single incorporation was performed at either the 5′-end or the internal position of the sequence (ONS 4 and 5). Multiple insertions (three) were performed either in a continuous row (ON 6) or spaced by natural nucleosides (ON 7). For a comprehensive comparison of the properties of the new modified nucleoside, the binding properties of ONs involving either one (ONS 8 and 9) or three dU incorporations (ONS 10 and 11) at the same positions along with the unmodified ON (ON 3) used as a reference were also studied. 23-mer DNA (ON 1) and RNA (ON 2) targets were chosen so that, upon hybridization, three nucleotides would overhang on each side of the duplexes in order to mimic the interaction of ONs with full length targets. The structures of the modified nucleosides Un and dU used in this study are shown in Fig. 1 and those of the ONs 1–11 are depicted in Fig. 2.

Synthesis

Synthesis of the 2′-O-neopentyldeoxyuridine and its phosphoramidite building block. Several methods for the 2′-alkylation of uridine have been reported previously. Most of them include the protection of 5′- and 3′-hydroxyl groups as well as the N-3 position of the nucleic base to make the alkylation of the 2′-position specific. We chose to proceed without protection of the nucleoside following a strategy adapted from literature reports (Scheme 1). 2,2′-Anhydro-1-β-D-arabinofuranosyluracil 13 was obtained by reaction of uridine (12) with diphenylcarbonate and reacted with the trimethylsilyl ether of neopentyl 15 in the presence of BF3·Et2O as the Lewis acid for the activation of the cyclic ether bond (Scheme 1). By using two-fold excess of BF3·Et2O and five-fold excess of the trimethylsilyl ether in DMF at 120 °C for 5 days the Un 16 was obtained with an 18% yield after purification. The phosphoramidite building block 18 was obtained by selective 5′-O-dimethoxytritylation of the unprotected modified nucleoside 16 with DMTCl in pyridine (compound 17) followed by reaction with 2-cyanoethyketraisopropylphosphorodi- amide in the presence of 4,5-dicyanoimidazole with a 50% yield after purification. (Experimental procedures are given vide infra.)
Synthesis of the oligonucleotides 4–7. The structures of the ONs synthesized are given in Fig. 2. The coupling of 2′-O-neopentyldeoxyuridine phosphoramidite 18 was performed using standard procedures. The main change to the typical synthesis was the use of longer coupling times (10 min). The coupling yields were around 97–98% based on a trityl cation assay. The deprotection step was performed using standard conditions (see the Experimental section). After purification by reversed-phase chromatography, the purity of all oligomers described was verified by reversed-phase analysis. Retention times of modified ONs are higher than that of the unmodified ON and increased with the number of Un incorporations (ESI†). These results indicated the presence of more lipophilic compounds. The retention times are also dependent on the position of incorporation of the modified nucleoside. As previously reported with other nucleoside analogues46 the presence of the modified nucleoside at the 5′-end (ON 4) induces an increased retention time as compared to its incorporation at the internal position of the sequence (ON 5). The ON 6 involving three contiguous modified nucleosides is also largely more retained than ON 7 containing also three modified nucleosides but separated by at least two intervening natural nucleosides. The integrity of all ONs was confirmed by MALDI-TOF mass spectrometry (ESI†).

Thermal denaturation studies
The influence of dT replacement (one or three) by 2′-O-neopentyldeoxyuridine (Un) on duplex stability was investigated by thermal denaturation studies, followed by absorption spectroscopy, of DNA–DNA and RNA–DNA duplexes obtained by mixing modified ONs 4–7 with 23-mer single-stranded DNA (ON 1) and RNA (ON 2) target sequences (X = A) (Fig. 2). For a better understanding of the properties of the new modified nucleoside, the binding properties of ONs 8–11 involving dU incorporations at the same positions were also studied. The purity of ONs 1–3, 8–11 (from commercial sources) has been verified by reversed-phase chromatography and MALDI-TOF spectrometry before use (data not shown). The melting curves of the DNA–DNA and RNA–DNA duplexes containing the modified ONs 4–7 and the parent duplexes are shown in Fig. 3.

Fig. 3 UV melting curves (260 nm) for DNA–DNA duplexes (a): 1 [5′-d-(TAC ACT AAA ACT GGT AAC ACT TC)3] + ONs 3–7, and DNA–RNA duplexes (b): 2 [5′-r-(UAC ACU AAA ACU GGU AAC ACT UC)3] + ONs 3 (diamond), 4 (up triangle), 5 (square), 6 (circle) and 7 (cross) in a 10 mM sodium phosphate, pH 7, buffer containing 150 mM NaCl and 1 mM EDTA. Concentrations were 1 μM for each strand. T_m values are reported in Table 1.

Scheme 1 Synthesis of the modified nucleoside 16 and the phosphoramidite derivative 18. Reagents and conditions: (i) diphenylcarbonate, DMF, 85 °C; (ii) NaHCO3, 120 °C, 4 h; (iii) TMS-Cl, TEA, Et2O, 0 °C, 30 min; (iv) rt, 72 h; (v) B,F–Et2O, DMF, 120 °C, 120 h; (vi) DMTrCl, Py; (vii): 2-cyanoethyltetraisopropylphosphoro- diamidite, 4,5-dicyanoimidazole, CH3CN, CH2Cl2.
Experiments were performed in a 10 mM sodium phosphate, pH 7, bu
cation). In the same conditions, the incorporation of three dUs
more larger destabilizing e
ation of dU in place of thymidine led to a weak

table of the modified nucleoside inside the
duplexes (terminal versus internal position) and the target
considered (DNA versus RNA). The presence of the modified
nucleoside Un at the 5′-end of the duplex had a less destabiliz-
ing effect than its incorporation at the internal position. In the
presence of the DNA target (ON 1), single modifications at the
5′-end of the sequence (ONs 4 and 8) induced very weak devi-
ations in \( T_m \) compared to that of the unmodified duplex (\( \Delta T_m = -0.1 ^\circ C \) for Un and \( \Delta T_m = -0.5 ^\circ C \) for dU). A more pronounced effect was observed (\( \Delta T_m = -3.2 ^\circ C \)) when Un was located at the internal position of the sequence (ONs 5), while the desta-
bilization induced by the presence of dU (ON 9) at the same
position was weaker (\( \Delta T_m = -0.5 ^\circ C \)). In the presence of the
RNA target (ON 2), the 5′-end modification led to a weak stabiliz-
ation for Un (\( \Delta T_m = +0.1 ^\circ C \)) and a weak destabilization for
dU (\( \Delta T_m = -0.4 ^\circ C \)) while a more pronounced effect was observed for the internal nucleotide substitution (\( \Delta T_m = -1.8 ^\circ C \) for Un and \( -0.8 ^\circ C \) for dU).

**Table 1** \( T_m \) and thermodynamic parameter data for the duplexes

<table>
<thead>
<tr>
<th>ONs</th>
<th>( T_m ) (°C)</th>
<th>( \Delta T_m/mod ) (°C)</th>
<th>( -\Delta H^0 ) (kcal mol(^{-1}))</th>
<th>( -\Delta S^0 ) (eu)</th>
<th>( -\Delta G_{T=25}^0 ) (kcal mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>49.5</td>
<td>—</td>
<td>-132.6</td>
<td>-381.6</td>
<td>-14.2</td>
</tr>
<tr>
<td>4</td>
<td>49.4</td>
<td>-0.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>46.3</td>
<td>-3.2</td>
<td>-122.0</td>
<td>-352.5</td>
<td>-12.7</td>
</tr>
<tr>
<td>6</td>
<td>44.1</td>
<td>-1.8</td>
<td>-111.6</td>
<td>-322.9</td>
<td>-11.5</td>
</tr>
<tr>
<td>7</td>
<td>40.6</td>
<td>-2.9</td>
<td>-106.7</td>
<td>-311.2</td>
<td>-10.2</td>
</tr>
<tr>
<td>8</td>
<td>40.0</td>
<td>-0.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>47.4</td>
<td>-0.9</td>
<td>-126.2</td>
<td>-364.2</td>
<td>-13.25</td>
</tr>
<tr>
<td>11</td>
<td>48.2</td>
<td>-0.4</td>
<td>-126.4</td>
<td>-364.5</td>
<td>-13.35</td>
</tr>
</tbody>
</table>

In the presence of the RNA target, the incorporation of the
three Uns in a continuous way (ON 6) led to a \( T_m \) value similar
to that of the reference (\( \Delta T_m = -0.1 ^\circ C \)) while the incorpo-
ration of the three Un residues in a discontinuous way (ON 7)
had a destabilizing effect (\( \Delta T_m = -0.9 ^\circ C \) per modification).
In the same conditions, the incorporation of the three dUs either
in a row (ON 10) or in a discontinuous way (ON 11) had a
delaying effect (\( \Delta T_m = -0.8 ^\circ C \) and \( -0.5 ^\circ C \) per
modification, respectively).

It should be noted that \( T_m \) values for the DNA–DNA
(ONs 1 + 2) and DNA–RNA (ONs 1 + 3) duplexes used as refer-
ces are very close (\( \Delta T_m = 1 ^\circ C \)). These results are not
surprising since \( T_m \) values are largely dependent on
sequences.47–49 They are in accordance with the literature
data dealing with similar pyrimidine/purine ratios. The incorpo-
ration of dU in place of thymidine led to a weak \( T_m \) value
decrease similar to the literature report.50 DNA–DNA duplexes
containing Un are clearly less stable than the corresponding
duplexes involving dU. Conversely, the stability of DNA–RNA
duplexes with three Uns in a row is slightly superior to that of
the corresponding duplexes involving dU (\( \Delta T_m = +0.8 ^\circ C \) per
modification) while the incorporation of Uns in a discontinu-
ous way led to a slight destabilisation (\( \Delta T_m = -0.4 ^\circ C \) per
modification). These results show clearly a binding prefer-
ence for the RNA target that can be tuned by the number and
the positions of incorporation of the Un nucleoside.

**Mismatch discrimination for DNA–DNA and DNA–RNA
duplexes**

To determine the base-pairing selectivity in DNA and RNA recog-
nition by Un, ON 5 involving modification at the 11th posi-
tion was also hybridized with the targets DNA (ON 1) and RNA
(ON 2) involving cytosine (X = C), guanine (X = G) or thymine
(X = T) in the position opposite the modified nucleoside
(Table 2). The same experiments were also performed with the
ONs 3 and 9, involving respectively dT and dU, used as refer-
ces. Observation of the results indicated that in any case the
presence of mismatched base-pairs resulted in a dramatic

The melting temperatures (\( T_m \)) for all duplexes are reported in
Table 1. The results are an average of at least two series of
independent experiments.

**Duplexes with single nucleotide change**

Analysis of the data showed different results that were dependent
on both the position of the modified nucleoside inside the
duplexes (terminal versus internal position) and the target
considered (DNA versus RNA). The presence of the modified
nucleoside Un at the 5′-end of the duplex had a less destabiliz-
ing effect than its incorporation at the internal position. In the
presence of the DNA target (ON 1), single modifications at the
5′-end of the sequence (ONs 4 and 8) induced very weak devi-
ations in \( T_m \) compared to that of the unmodified duplex (\( \Delta T_m = -0.1 ^\circ C \) for Un and \( \Delta T_m = -0.5 ^\circ C \) for dU). A more pronounced effect was observed (\( \Delta T_m = -3.2 ^\circ C \)) when Un was located at the internal position of the sequence (ONs 5), while the desta-
bilization induced by the presence of dU (ON 9) at the same
position was weaker (\( \Delta T_m = -0.5 ^\circ C \)). In the presence of the
RNA target (ON 2), the 5′-end modification led to a weak stabiliz-
ation for Un (\( \Delta T_m = +0.1 ^\circ C \)) and a weak destabilization for
dU (\( \Delta T_m = -0.4 ^\circ C \)) while a more pronounced effect was observed for the internal nucleotide substitution (\( \Delta T_m = -1.8 ^\circ C \) for Un and \( -0.8 ^\circ C \) for dU).

**Duplexes with three nucleotide changes**

The \( T_m \) values were also dependent on both the positions of
the modified nucleosides and the target considered (DNA versus RNA). In the presence of the DNA target, the incorpo-
ration of three Uns in a continuous way (ON 6) was slightly less
destabilizing (\( \Delta T_m = -1.8 ^\circ C \) per modification) than a single
incorporation (ON 5) (\( \Delta T_m = -3.2 ^\circ C \)), while the incorporation of the
three Un residues in a discontinuous way (ON 7) had a
much larger destabilizing effect (\( \Delta T_m = -2.9 ^\circ C \) per modifi-
cation). In the same conditions, the incorporation of three dUs
either in a continuous (ON 10) or in a discontinuous way
(ON 11) had a similar destabilizing effect (\( \Delta T_m = -0.7 ^\circ C \) per
modification and \( \Delta T_m = -0.5 ^\circ C \) per modification, respectively).
decrease of $T_m$ values. However, several differences can be pointed out. When incorporated in DNA–DNA duplexes Un was slightly less efficient than dT and dU at discriminating the mismatches. Conversely, in RNA–DNA duplexes the $\Delta T_m$ observed were nearly equivalent in the three series except when rU was the opposite nucleoside. Importantly, the $\Delta T_m$ observed for the less destabilizing wobble base pair Un–dG was similar to those obtained for dU–dG and dT–dG. From these experiments, it appears that for the sequence studied ONs involving Un recognize the RNA target with selectivity equivalent to those obtained with the natural dT and the base-modified nucleoside dU.

Thermodynamic data of duplex formation

The thermodynamic data of duplex formation for the modified ONs (5–7 and 9–11) and the reference (ON 3) with both the DNA (ON 1) and RNA (ON 2) targets were determined from melting curves by the concentration variation method. The thermodynamic data obtained from $1/T_m$ versus log $[C_m]$ plots$^{31,52}$ are summarized in Table 1. In the presence of the DNA target, an important decrease of the enthalpy of duplex formation was observed for the three ONs involving Un (4–7) as compared to the unmodified ON (3) and to the ONs containing dU (9–11). This was to some extent counterbalanced by entropy compensation leading to standard enthalpies of duplex formation $\Delta G^\circ$ in agreement with the observed thermal stabilities of the different series of duplexes. In the presence of the RNA target, except for ON 5 involving a single Un incorporation, the enthalpy of duplex formation for ONs 6 and 7 involving three Us was only slightly inferior to that of the reference and slightly superior to those obtained for the corresponding ONs involving the dU nucleosides (9–10). For ONs 6 and 7 involving three Us the changes in entropy terms were also weak as compared to the reference values. The calculated standard enthalpies of DNA–RNA duplex formation $\Delta G^\circ$ are in line with the observed thermal stabilities.

### CD spectra of modified duplexes

In order to investigate the influence of the incorporation of Un on the duplex structures, we recorded CD spectra of modified ONs involving either one modification (ONs 4, 5, 8 and 9) or three modifications (ONs 6, 7, 10 and 11) in the presence of either their complementary DNA or RNA target sequences (ONs 1 and 2). The unmodified duplexes (ONs 1 + 3 and 2 + 3) were used as references. The results are shown in Fig. 4. In the presence of DNA as a complement (Fig. 4a), and compared with the unmodified duplex, the introduction of a single Un (ONs 4 and 5) resulted in unchanged CD spectra. However, spectra modifications were observed for the duplexes involving three Un nucleosides (ONs 6 and 7). The presence of three consecutive Uns (ON 6) resulted in a 30% intensity increase of the positive peak around 275 nm together with a blue shift of about 5 nm (between 270 and 215 nm). The intensity of the negative peak at 245 nm was also slightly reduced. The CD spectra of the DNA–DNA duplex involving the three Us at non-consecutive positions was also changed as compared to that of the unmodified duplex. A blue shift was also observed as well as a reduced negative peak at 245 nm slightly more pronounced than for the duplex involving the contiguous Uns. These changes reflect differences in the duplex structures due to the presence of the neopentyl group at the 2′-position of the 2′-deoxyribose. The CD spectra of the duplexes involving dU are identical to that of the unmodified DNA–DNA duplex (Fig. 4c).

As observed for the DNA–DNA duplexes the presence of a single Un in the DNA–RNA duplexes did not result in modifications in the CD spectra (Fig. 4b). But changes were observed for the duplexes involving three Us. Once again, a slight blue shift and also intensity changes were observed for both duplexes. A small intensity increase at 260 nm and an important decrease at 220 nm were observed for the duplex with three contiguous Us, and only a very weak intensity decrease at 270 nm for the duplex with three non-contiguous Us. The CD spectra of the DNA–RNA duplexes containing dU were identical to that of the DNA–RNA unmodified duplex except in the case of the presence of three contiguous dUs for which a small blue shift was observed between 255 and 215 nm (Fig. 4d). Comparison with the data obtained for the DNA–RNA duplexes involving dU incorporations in the same positions allowed the conclusion that the observed weak spectra modifications reflect small structural changes of the duplexes that are mainly due to the presence of the neopentyl group.
Although weak spectra changes were observed for the duplexes involving three Uns, their general shapes were similar to those of the B-type DNA–DNA and A/B type RNA–DNA unmodified duplexes.

**Molecular dynamic simulations**

In order to correlate the thermal and thermodynamics properties of the different series of duplexes with structures on a more detailed level, we performed molecular dynamic simulations of 5 ns on the DNA–DNA and DNA–RNA duplexes involving a single internal incorporation of Un as well as those involving three Un incorporations. The structures of the corresponding duplexes involving dU were also investigated. Simulations of the unmodified DNA–DNA and DNA–RNA duplexes were used as references (see the Experimental section for conditions).

**DNA–DNA duplexes**
The six DNA–DNA duplexes containing Un and dU modified nucleosides showed a stable helical structure during the 5 ns of simulation without any base-pair disruption and showed an average value of their calculated curvature comparable to the reference. The RMSD times-series were quickly stabilized around values from 2.4 Å to 3.4 Å. For the three duplexes containing the modified nucleoside Un, we observed that the neopentyl group remained stable and localized in the minor groove. The positioning of the neopentyl group is shown in Fig. 5a. The variations of the sugar pucker conformation of each residue during the simulation in the form of a heat map for the DNA–DNA duplex involving a single Un are shown in ESI (Fig. 6a†). We observed that all residues conserved a C2'-endo sugar pucker conformation, B-helix type, during the entire simulation time except the Un residue that adopted a C2'-exo sugar pucker conformation (close to an A-helix) (data not shown). The same result was observed for the DNA–DNA duplexes containing three Uns where all residues adopted a C2'-endo sugar pucker conformation (B-helix) except the modified nucleoside Uns that adopted a C2'-exo sugar pucker conformation (close to an A-helix) (data not shown). For the three simulations involving dU nucleosides, all the residues adopt a C2'-endo sugar pucker conformation and the heat maps obtained cannot be distinguished from the reference (data not shown).
We also studied the evolution of major and minor groove width of each duplex during the simulation. The results obtained for the minor groove width measured on unmodified DNA–DNA duplex are shown in Fig. 7a (ESI†). A narrowing of the minor groove width (around 8–10 Å in blue, instead of 12–15 Å in green) due to the presence of an A-tract in the target sequence was observed. This phenomenon has been largely described in the literature.53–57 The incorporation of a single Un nucleotide in the T-tract sequence also caused a narrowing of the minor groove (data not shown). However, the presence of three Un nucleosides, contiguous or not, prevented the narrowing of the minor groove probably due to the presence of the neopentyl groups localized in the minor groove. Fig. 7b in ESI† shows the heat map for the DNA–DNA duplex involving three non-contiguous Uns. Finally, we estimated the average solvent-accessible surface area (SASA) of the neopentyl residues for the three duplexes containing the modified nucleoside Un in order to correlate their exposure to the solvent with their number and position in the sequence. We observed a lower neopentyl exposure to the solvent (∼240 Å²) for a single and three contiguous modifications. In the first case, the low exposure was probably due to the groove narrowness that tends to bury the neopentyl group while in the second case, the decrease of exposure was due to the proximity of the three neopentyl groups. On the contrary, the introduction of three non-contiguous Un nucleosides increased the solvent exposure of the neopentyl groups (∼260 Å²). This is consistent with the fact that the presence of a second Un nucleoside within the T tract prevents the narrowing of the minor groove.

RNA–DNA duplexes

We still observed that the six DNA–RNA duplexes containing modifications remained stable with a conserved helical structure during the 5 ns of simulation without any base-pair disruption or significant curvature. The RMSD times-series were stabilized around higher values than the DNA–DNA duplexes, from 2.7 Å to 5.0 Å, which was probably caused by a longer adaptation time for the DNA–RNA hybridization. However, for the three duplexes containing the modified nucleoside Un, the neopentyl group was stable and well-localized in the minor groove. The positioning of the neopentyl group is shown in Fig. 5b. Heat maps of the sugar puckering variations of paired residues during the simulation showed two distinct areas. The RNA strand showed the characteristic sugar puckering conformations of an A-helix whereas those obtained for the DNA strand were characteristic of a B-helix. As for DNA–DNA duplexes, the Un modifications inserted on the DNA strand adopted the characteristic sugar puckering conformations of an A-helix without disturbing their neighboring sugar puckering conformations. The heat map of the sugar puckering variation of paired residues for the DNA–RNA duplex containing a single modified nucleoside Un is shown in Fig. 6b in ESI†. The A-tract influence on the minor groove width is less visible on the DNA–RNA hybrids than on the DNA–DNA hybrids (Fig. 7 in ESI†). A slight effect is however still discernible on the minor groove of the unmodified DNA–RNA duplex (Fig. 7c†) and the modified DNA–RNA duplex involving a single Un that is not observed for the DNA–RNA duplexes involving three Un modifications, contiguous or not. The heat map for the DNA–RNA duplex involving three non-contiguous Uns is shown in Fig. 7d in ESI†. Finally, the solvent exposure of the Un nucleosides was generally higher in DNA–RNA duplexes than in DNA–DNA duplexes, which could be explained by the widening of the A-tract minor groove in DNA–RNA duplexes compared to DNA–DNA duplexes. However, we always observed a decrease of solvent exposure for the three contiguous Uns. So we noticed a slight increase of the solvent exposure dependent on the number and positioning of the Un nucleosides. The most reduced solvent exposure occurred with the insertion of three contiguous Uns (∼255 Å²) followed by the insertion of one Un modification (∼270 Å²) which is comparable with the insertion in the A-tract of three discontinuous Un modifications (∼275 Å²).

The results of the melting studies and CD experiments are consistent with those of the molecular simulation studies indicating that either a single or three neopentyl groups, in different arrangements, were able to be accommodated in the minor groove of both the DNA–DNA and RNA–DNA duplexes. However, an important difference was observed between the structures involving dU and Un modified nucleosides in the simulations. While a C2'-endo sugar puckering was conserved in all the dU residues, all the Un residues adopted a C2'-exo sugar puckering conformation, close to an A-helix type. The results of the melting studies indicated a binding preference of the ONs 5, 6 and 7, involving Un residues, for the RNA over the DNA complement, whatever the number and positions of modifications. The selectivity of Un base-pairing in RNA–DNA duplexes was equivalent to those of dT and dU and slightly weaker in the presence of the DNA targets. Another observation concerns the non-additive effect of three Un incorporations. While a single Un incorporation at the internal
position of the sequence (ON 5) had a destabilizing effect (relative to dU) ($\Delta T_m = -1 \, ^\circ C$ per modification for the RNA–DNA duplex and $\Delta T_m = -2.7 \, ^\circ C$ per modification for the DNA–DNA duplex), the presence of three consecutive Uns in ONs modified the RNA–DNA duplex ($\Delta T_m = +0.8 \, ^\circ C$ per modification) and destabilized the DNA–DNA duplex ($\Delta T_m = -1.1 \, ^\circ C$ per modification). When the three Un insertions were spaced by at least two natural 2′-deoxynucleosides both the RNA–DNA and DNA–DNA duplexes were destabilized ($\Delta T_m = -0.4 \, ^\circ C$ per modification and $\Delta T_m = -2.5 \, ^\circ C$ per modification, respectively). Although a single Un incorporation in both the DNA–DNA and RNA–DNA duplexes was destabilizing, their CD spectra were indistinguishable from those reported for the unmodified duplexes and those containing dU at the same position. Only a small blue shift at 275 nm and reduced intensity at 220 nm were observed for the CD spectra of the most stable RNA–DNA duplex involving three contiguous Uns. The most important changes in CD spectra were observed for the DNA–DNA duplexes involving three non-consecutive Uns (corresponding to the less stable duplex). They consisted of a slight blue shift associated with 30% increase at 275 nm when Uns were consecutive and a reduced negative peak at 245 nm in the presence of non-consecutive Uns.

On the basis of these results and the literature reports on modified oligonucleotides involving different series of 2′-modified nucleosides several observations can be made and hypotheses suggested to explain the different binding abilities observed for our modified ONs involving the modified 2′-O-neopentyldeoxyuridine. Different classes of 2′-modified nucleosides have been incorporated into ONs $^6,16,18,24,36,37,40,58-66$ Among them, those involving O-methyl $^{34,40}$ O-2′-O-methoxyethyl $^{31,36}$ 2′-O-[2-(methyl-amino)-2-oxoethyl] $^{36}$ 2′-O[3-amino-propyl] $^{50,61}$ 2′-O-[2-(guanidinium)ethyl] $^{62}$ and 2′-fluor $^{18,24,58,59}$ substituents have been reported to improve the biochemical properties of ONs. A specific mention must be made of the 2′,4′-C-methylene bicyclonucleosides $^{6,25,26,28,64,65}$ among the most useful modified nucleosides to date exhibiting unique binding properties on the basis of their use in a great variety of applications. The improved binding affinities of these different classes of modified ONs have been reported to rely mainly on their tendency to adopt the 2′-exo pucker conformation. The binding affinities of the 2′-modified ONs are also influenced by other different parameters: the size of the 2′-O-substituent, the number and positions of the modified nucleosides, the sequence effect, the hydration changes in the minor groove and potential additional interactions between heteroatoms present in the substituents and the ON backbone.

More specifically, comparison of our results with the literature reports on ONs involving a few nucleosides modified at the 2′-position with aliphatic alkyl groups in a background of 2′-deoxynucleosides showed similar behaviors. $^{16,35}$ These modified ONs were able to form DNA–DNA and RNA–DNA hybrids with stabilities dependent on the size and structure of the pendent groups at the 2′-position. The thermal stability of the complexes was reduced as the length of the 2′-O-alkyl chain increased. It has also been shown that the binding affinities of these modified ONs were largely sequence dependent and also dependent on the number and positions of the modified nucleosides inside the sequence. So the comparison of the $\Delta T_m$ per modification cannot be quantitative. However, it has been reported that substitutions smaller than 2′-O-propyl stabilized the RNA–DNA duplexes. The literature report on modified ONs involving 2′-O-modified adenosines indicated that the incorporation of five 2′-O-alkylated adenosines involving methyl, ethyl, propyl, butyl or pentyl groups in purine-rich ONs did not change the CD spectra of the RNA–DNA duplexes compared to the parent while a bulker group induced important changes. $^{35}$ In addition, similarly to our finding, the effects of several incorporations were not additives. It has been reported that for almost every modification (including O-ethyl) the $\Delta T_m$ per modification was negative in ONs that contained a single substitution and three consecutive 2′-substitutions were less destabilizing (or more stabilizing) than isolated substitutions spaced by unmodified deoxynucleosides. As observed with our modified ONs, 2′-O-substitutions on adenosine have been reported to induce a binding preference for their RNA over DNA complements.

The RNA binding preference of ONs involving Un nucleosides is likely due to the tendency of the 2′-modified nucleosides to adopt a 2′-exo geometry inconsistent with B-form DNA–DNA geometry. Furthermore, the presence of the neopentyl groups could induce more perturbations of the hydration in the minor groove of the DNA–DNA duplex than in the minor groove of the RNA–DNA duplex. Concerning the variations in $\Delta T_m$ per modification, although the same trend was observed in the presence of both the DNA and the RNA targets, several observations can be made. A single incorporation of Un (ON 5) was performed at the end of a tract of four consecutive A–T base-pairs. It is known that this structure facilitates formation of a water spine in the minor groove of the B-DNA stabilizing the duplex. Furthermore the minor groove width of the unmodified dA–dT tract was reduced. $^{33-37}$ The results of molecular simulation studies indicated that the incorporation of a single Un nucleotide in the T-tract sequence also caused a narrowing of the minor groove. However, the presence of three Un nucleosides, contiguous or not, prevented the contraction of the minor groove. It should be noted that in ON 7 involving three non-contiguous Uns, both of them were located in the T-tract and separated by two natural 2′-deoxynucleosides. In the RNA–DNA duplexes the A-tract influence on the minor groove width was still present but less visible than on the DNA–DNA hybrids. In addition, the decrease of solvent exposure observed when three Uns were consecutive suggested that interactions between them were possible on the surface of the minor groove which could also be a source of additional stabilization.

We have reported the ability of modified ONs, involving either one or three modified 2′-O-neopentyluridines, to hybridize preferentially with their RNA target sequences with a stability increase of +0.8 °C per modification when the modified
nucleosides are consecutive. This affinity increase is nearly similar to that observed for fully modified 2’-O-methyl and 2’-O-propyl ONs.\textsuperscript{36} It is likely that the incorporation of 2’-O-neopentyluridines in different numbers and arrangements in other sequences could lead to slightly different binding properties. However, the incorporation of a greater number of 2’-O-neopentyluridines introducing more A-character for the duplex formed with the RNA complement could contribute to an increased stabilization of the duplex as previously reported for ONs involving other 2’-O-alkylated nucleosides.\textsuperscript{36} In addition, Un nucleosides could be used in association with other modified nucleosides able to adopt a 2’-exo geometry to obtain a modulation of the binding affinity of modified ONs.

**Conclusion**

A 2’-deoxyuridine analogue involving a neopentyl group at the 2’-position Un was synthesized and incorporated into a series of 17-mer ONs. The modified ONs involved either one (at the 5’-end or the internal position) or three (either in a contiguous fashion or separated by at least two natural nucleosides) modified Un nucleosides. The corresponding series of 17-mer ONs involving dU at the same positions was also used for comparison. The pairing ability of these two series of ONs with their DNA and RNA complements was investigated by thermal denaturation and circular dichroism measurements. The results showed clearly the ability of all modified ONs to specifically bind their DNA and RNA targets with the formation of duplex structures similar to those of the B-type DNA–DNA and A-B type RNA–DNA references. Small changes in the CD spectra were observed only for the duplexes containing three Un nucleosides. Different duplex stabilities were observed. The presence of Un at the terminal position of the duplexes had no effect on their stability. However, a single incorporation at the internal position had a destabilizing effect in the presence of both the DNA and RNA complements ($\Delta T_m = -2.7$ °C and $\Delta T_m = -1$ °C, respectively relative to the corresponding duplexes containing dU). The presence of three Uns led to different duplex stability changes dependent on both their arrangements and the target considered. In all cases, the presence of three Uns in DNA–DNA duplexes had a destabilizing effect. However, the presence of three contiguous Uns had a less important effect ($\Delta T_m = -1.1$ °C per modification relative to dU) as compared to their incorporation in a non-contiguous way ($\Delta T_m = -2.5$ °C per modification relative to dU). Three consecutive Un incorporations led to the RNA–DNA duplex with a stability slightly superior to that of the RNA–DNA duplex containing dU–rA pairs ($\Delta T_m = +0.8$ °C per modification) while a slight destabilization was observed ($\Delta T_m = -0.4$ °C per modification relative to dU) when the three Uns were inserted in a non-contiguous fashion. This trend was confirmed by the results of the thermodynamic investigations. To rationalize these differences in stability, the behaviour of the modified nucleosides Un and dU in the different duplexes was evaluated by molecular dynamic simulations. The results showed for all duplexes stable helical structures during the 5 ns of simulation, similar to the reference, without any base-pair disruption. For the duplexes containing the modified nucleoside Un, the neopentyl group remained stable and localized in the minor groove. However, an important difference was observed between the structures involving dU and Un modified nucleosides. While in the modified ONs containing the dU modified nucleosides all the residues adopted a C2’-endo sugar pucker conformation, in the modified ONs involving the new modified Un nucleoside all residues conserved a C2’-endo sugar pucker conformation, except the Un residues which adopted a C2’-exo sugar pucker conformation, close to an A-helix type. The differences in stability observed for the RNA–DNA and RNA–DNA duplexes, involving three Uns in different arrangements, can be explained by the sequence context, the local minor structure changes as well as by the hydration changes. On the basis of these results, Un represents an interesting dU analogue that could be used for tuning the hybridization properties of ONs and due to this is worthy of further exploration for applications in oligonucleotide therapeutic based strategies.

**Experimental**

**General methods**

All solvents used were of the highest purity and did not contain more than 10 ppm H$_2$O. All chemicals were used as obtained unless otherwise stated. Analytical TLC was performed on pre-coated alumina plates (Merck silica gel 60F 254, ref. 5554), and preparative TLC on glass-backed silica plates (60F 254, ref. 5717). For flash chromatography, Merck silica gel 60 (70–230 mesh, ref. 7734) was used. Compounds were directly visualized on the plates by UV-shadowing. ODN syntheses were performed on an Expedite Nucleic Acid Synthesis system 8909 from PerSeptive Biosystems. Reversed-phase chromatography was performed on a 600E (System Controller) equipped with a photodiode array detector (Waters 990) using a Lichrospher 100 RP 18 (5 mm) column (125 mm × 0.4 mm) from Merck with a linear gradient of CH$_3$CN in 0.1 M aqueous triethylammonium acetate, pH 7, with a flow rate of 1 cm$^3$ min$^{-1}$. Oligonucleotides containing dU were from Eurogentec and RNA sequence from Biomers. $^1$H NMR, $^{13}$C NMR and $^{31}$P NMR were recorded at 400 MHz, 100 MHz and 162 MHz, respectively. $^1$H chemical shifts are reported in ppm relative to either residual solvent peak DMSO (2.54 ppm) or Me$_4$Si. $^{31}$P chemical shifts were referenced to H$_3$PO$_4$ (external reference). The following abbreviations are used for the proton spectra multiplicities: s: singlet, d: doublet, t: triplet, q: quartet, q: quintuplet, m: multiplet, br: broad, dd: doublet of doublet, dt: doublet of triplet. Coupling constants (J) are reported in Hz. Absorption spectra were recorded with an Uvikon 860 spectrophotometer. The molar extinction coefficient of the modified nucleosides was considered to be equivalent to that of the 2’-deoxyuridine. Consequently, the molar...
extinction coefficients of ONs were determined according to the literature.67

Synthesis

2',2'-Anhydrouridine 13.44 To a suspension of uridine 12 (40 g, 0.16 mol) into dry DMSO (44 cm³) was added diphenyl carbonate (38.6 g, 0.18 mol) under argon at room temperature. The mixture was stirred at 85 °C until the slurry became clear, then sodium hydrogen carbonate (0.21 g, 2.50 mmol) was added. After stirring at 120 °C for 4 hours, the reaction was diluted with MeOH (60 cm³) and then filtered through a bunker funnel and the solid washed with MeOH. The solid was re-suspended into MeOH (60 cm³) and stirred for 30 min at room temperature. The suspension was again filtered to yield 13 (27.43 g, 0.12 mol, 74%) as white solid. Rf13 0.12 (CHCl₃–MeOH, 95 : 5, v/v), δH (400 MHz, DMSO-d₆), 3.18 [1H, m, H–C(5)]; 3.28 [1H, m, H–C(5)]; 4.06 [1H, dt, J = 0.8, 4.8 Hz, H–C(4)]; 4.38 [1H, s, H–C(3)]; 4.97 [1H, s, HO–C(5)]; 5.19 [1H, d, J = 6.0 Hz, H–C(2)]; 5.83 [1H, d, J = 7.6 Hz, H–C(5)]; 5.88 [1H, d, J = 7.2 Hz, HO–C(3)]; 6.30 [1H, d, J = 5.6 Hz, H–C(1)]; 7.83 [1H, d, J = 8.4 Hz, H–C(6)], δC (100 MHz, DMSO-d₆), 60.7 [1t, C(5)]; 74.6 [1d, C(3)]; 88.6 [1d, C(2)]; 89.1 [1d, C(4)]; 89.9 [1d, C(1)]; 108.5 [1d, C(5)]; 136.7 [1d, C(6)]; 159.7 [1s, C(4)]; 171.1 [1s, C(2)]. HRMS (ESI–TOF): m/z, found 227.0664 ([M + Na]⁺, C₇H₁₂N₂O₅Na⁺ calc. 337.1376).

5'-O-Dimethoxytrityl-2',2'-O-neopenylicuridine 17. To a solution of 2',2'-O-neopenylicuridine 16 (1.59 g, 5.05 mmol) into dry pyridine (40 cm³) was added dropwise at 0 °C a solution of dimethoxytrityl chloride (2.05 g, 6.06 mmol) into dry pyridine (10 cm³) over 1 hour. After stirring for 5 hours at room temperature, the reaction was quenched with MeOH (0.5 cm³). The reaction was again stirred for 30 minutes and then diluted with CH₂Cl₂ (100 cm³). The mixture was washed successively with cold water (3 × 50 cm³), cold 5% aq. NaHCO₃ (2 × 50 cm³) and cold brine (50 cm³). The organic phase was dried over Na₂SO₄ and concentrated to dryness under reduced pressure. The residue was co-evaporated with toluene (2 × 25 cm³) and purified by silica gel chromatography (the column was packed with eluent containing 5% TEA to prevent detritylation, eluent: hexane–EtOAc–TEA, 80 : 19 : 1 v/v/v) to yield 17 (1.78 g, 2.88 mmol, 57%) as a white foam. Rf17 = 0.59 (CDCl₃–MeOH, 9:1, v/v), δH (400 MHz, CDCl₃), 0.95 [9H, s, –CH₃(CH₂)₂]; 2.62 [1H, d, J = 9.6 Hz, HO–C(3)]; 3.29 [1H, d, J = 9.2 Hz, –OCH₂CH(CH₃)₂]; 3.53 [1H, dd, J = 2.8, 11.2 Hz, H–C(5)]; 3.58 [1H, dd, J = 2.0, 11.2 Hz, H–C(5)]; 3.66 [1H, d, J = 9.2 Hz, –OCH₂CH(CH₃)₂]; 3.79, 3.80 (6H, 2s, –OCH₃); 3.87 [1H, d, J = 5.2 Hz, H–C(2)]; 4.47 [1H, dt, J = 5.2, 8.8 Hz, H–C(3)]; 5.28 [1H, d, J = 8.0 Hz, H–C(5)]; 5.93 [1H, s, H–C(1′)]; 6.83–6.86 (4H, m, arom. H); 7.22–7.32 (7H, m, arom. H); 7.38–7.41 (2H, m, arom. H); 8.04 [1H, d, J = 8.4 Hz, H–C(6)]; 9.30 (1H, br s, –NH), δC (100 MHz, CDCl₃), 26.5 [q, –C(CH₃)₂]; 32.8 [2s, –C(CH₃)₂]; 55.2 [q, –OCH₃]; 61.1 [t, –OCH₂CH₂CH₃]; 68.4, 80.9, 82.7, 83.3, 87.1 [4d, 1t, C(1’), C(2’), C(3’), C(4’), C(5’)]; 87.4 [s, –Ar]; 101.9 [1d, C(4)]; 113.3 [d, arom. CH], 127.1, 127.9, 128.1, 130.1, 130.2, 131.8, 135.0, 135.3 (2s, arom. Cq), 140.0 [d, C(6)]; 144.3 (s, arom. Cq), 150.0 [s, C(2)]: 158.6, 158.7 (2s, arom. Cq); 163.4 [s, C(4)]. HRMS (ESI–TOF): m/z, found 639.2675 ([M + Na]⁺, calc. 639.2682).

5'-O-Dimethoxytrityl-3',3'-diacytoxy-N,N'-diisopropylphosphorodicarboximido-2',2'-O-neopenylicuridine 18. To a mixture of 5'-O-dimethoxytrityl-2',2'-O-neopenylicuridine 17 (2.10 g, 3.41 mmol) and 4,5-dicyanoimidazole (0.27 g, 2.28 mmol, 0.25 M in CH₂Cl₂) into dry CH₂Cl₂ (43 cm³) was added dropwise 2-cyanoethyl-N,N',N'-tetraisopropylphosphorodiamidite (1.6 cm³, 5.12 mmol) over 10 minutes under argon. The reaction was stirred for 1 hour at room temperature and then diluted with CH₂Cl₂ (100 cm³). The mixture was washed with cold 5% aq. NaHCO₃ (2 × 50 cm³) and cold brine (50 cm³). The organic phase was dried over Na₂SO₄ and evaporated to dryness under reduced pressure. The residue was purified by silica gel chromatography (the column was packed with eluent containing 5%
TEA to prevent detritylation, eluent: CH₂Cl₂–TEA (99 : 1, v/v) to CH₂Cl₂–EtOAc–TEA, 70 : 29 : 1, v/v/v) to yield 18 (1.81 g, 2.22 mmol, 65%) as a white foam. R_{f} = 0.35, 0.42 (CH₂Cl₂–EtOAc; 8 : 2, v/v). δ_{NMR} (400 MHz, CDCl₃): 0.91, 0.93 [9H, 2S, –C(CH₃)₃]; 1.04–1.30 [6H, m, 3H, –CH(CH₃)₂]; 2.43 (1H, t, J = 6.0 Hz, –OCH₂CH₂CH₃); 2.61 (1H, t, J = 6.0 Hz, –OCH₂CH₂CN); 3.32–3.99 [7H, m, –OCH₂C(CH₃)₃]; –CH₂(CH₃)₃]; 3.78, 3.79, 3.80, 3.81 [6H, 4S, –OCH₂]; 3.97, 3.98 [1H, 2m, –C(3)]; 4.23, 4.28 [1H, 2m, H–C(4)]; 4.39–4.56 [1H, 2m, H–C(5)]; 5.20, 5.22 [1H, 2d, J = 8.2 Hz, H–C(5)]; 5.91, 5.94 [1H, 2d, J = 2 Hz, H–C(1)]; 6.82–6.86 [4H, m, arom. H]; 7.33–7.38 (7H, m, arom. H); 7.38–7.43 (2H, m, arom. H); 8.01, 8.08 [1H, 2d, J = 8 Hz, H–C(6)]; 8.67 (br s, 1H, –NH). δ_C (100 MHz, CDCl₃): 20.2, 20.4 [2dt, J = 6.9 Hz, –OCH₂CH₂CN]; 24.5–24.8 [m q, –CH(CH₃)₂]; 26.5, 26.6 [2q, –CH₂C(CH₃)₃]; 32.1, 32.2 [2S, –CH₂C(CH₃)₃]; 43.1, 43.2 [2dd, J = 12.7 Hz, –CH–CH(CH₃)₂]; 55.2, 55.3 [2q, –OCH₃]; 57.8, 58.1 [2dt, J = 18.3 Hz, –OCH₂CH₂CN]; 60.7, 61.1 [2t, –CH₂C(CH₃)₃]; 69.9–87.1 [1t, 4d, C(1′), C(2′), C(3′), C(4′), C(5′)]; 87.9, 88.1 [2S, –C(3′)]; 101.7, 101.8 [2d, C(5)]; 113.2 (d, arom. CH); 117.4, 117.5 (2S, –CN); 127.1, 127.9, 128.0, 128.2, 128.3, 130.3 (6 d, arom. CH); 134.9, 135.1, 135.2, 135.3 [4S, arom. Cq]; 140.1, 140.2 [2d, C(6)]; 144.2, 144.3 [2S, arom. Cq]; 149.9, 150.0 [2S, C(2)]; 158.6, 158.7 [2S, arom. Cq]; 163.2, 163.3 [2S, C(4)].^33P NMR (162 MHz, CDCl₃), δ = 149.6, 149.8. C₄H₆H₂N₄O₆P (816.92). HRMS (ESI+): m/z, found 839.3773 [(M + Na)^+], C₄₂H₅₇N₄O₉NaP^+, calc. 839.3761.

Oligonucleotide synthesis, purification and characterization. All ONs were prepared from modified nucleoside phosphoramidite 18 and commercially available dA, dC, dG, and dT phosphoramidites (Biosolve) and deoxynucleoside-CPG targets were determined by a plot of 1/λ_{m} versus log [C_m].^51,52 C_m is the concentration of free ONs 5–7 and 9–11 at the melting temperature. ON concentrations were 10^{-5} M, 5 × 10^{-6} M, 2.5 × 10^{-6} M, and 10^{-6} M in a 10 mM NaH₂PO₄ buffer (pH 7.0) containing 150 mM NaCl and 1 mM EDTA. The CD spectra were recorded between 215 and 315 nm. The reported spectra correspond to the average of three scans.

Molecular dynamic simulations. Initial structures of the DNA–DNA duplexes were generated with the nucleic acid builder (NAB) module of AMBER11^69 into a uniform ideal Watson/Crick B-form DNA duplex. Regarding the RNA–DNA duplex of the unmodified sequence, each strand was built following its ideal geometry (A-helix and B-helix for the RNA and Watson/Crick B-form DNA duplex. The initial structures of the modified RNA–DNA duplexes were extracted from the MD simulation of the parent RNA–DNA duplex to allow the adjustment of the two strands together before inserting modifications. Geometry and charge distribution of modified nucleotides (Un and dU) were computed using the Jaguar program^70 at the HF/6-31G* level and fitted with the Resp program for compatibility with the Wang et al. force field^71 including parmbsc0 modifications.^72

The SANDER module of the AMBER11 package was used for the simulations. All systems were neutralized with Na+ counter-ions and solvated with TIP3P water molecules. After 4000 steps of energy minimization, the system was heated from 0 K to 300 K with restraints applied on atomic positions of the oligomer and the counter-ions, then relaxed during 250 ps with gradual removal of the atomic restraints. A final
relaxation step of 10 ps was performed without any restraint, followed by 5 ns of free MD simulation in an NPT ensemble with periodic boundary conditions. The conformational parameters of nucleic acids were analyzed as time series using the program CURVESP+ and the PTRAJ module of the AMBER11 package. To follow the evolution of the neopentyl group positioning during the simulation, we defined the $\Omega$ angle as the torsion angle $\{P_A, P_{Un}, O^3_{Un}, C_{Un}\}$ (Fig. 8 in ESI†).

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Notes and references
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