EXPERIMENTAL.

NMR Experiments. The NMR sample was prepared by mixing equimolar amounts of the two strands, each desalted on a Sephadex G15 column. The final concentration was ~3mM hybrid, 10mM sodium phosphate buffer, 100mM NaCl and 0.05 mM EDTA. We recorded standard NOESY, DQF-COSY-, TOCSY-, watergate NOESY-, and ^{1}H-^{13}C HSQC-spectra at either 500 or 800 MHz at 25 °C on either a Varian INOVA 800 Spectrometer or a Varian INOVA 500 Spectrometer employing the States phase cycling scheme.

NOESY spectra with mixing times of 250 ms and 80 ms were acquired in D_2O at 800 MHZ using spectral widths of 8000 Hz, 2048 complex points in t_2, and 512 t_1-experiments, each with 64 scans, with a repetition delay of 2.5 s. The residual signal from HDO was removed by presaturation. A watergate NOESY spectrum with a mixing time of 200 ms was acquired in H_2O/D_2O (9:1) at 500 MHZ using a spectral width of 5000 Hz, 2048 complex points in t_2, and 600 t_1-experiments, each with 96 scans, with a repetition delay of 3.5 s.

DQF-COSY and TOCSY spectra were acquired in D_2O at 800 MHZ using spectral widths of 8000 Hz, 2048 complex points in t_2, and 1024 and 512 t_1-experiments, respectively, each with 48/32 scans, with a repetition delay of 2.0 s. The residual signal from HDO was removed by presaturation. A DQF-COSY spectrum was obtained with a pulse sequence in which the first pulse was replaced with an E-BURP type selective pulse.[1] This spectrum was acquired in D_2O at 500 MHZ with spectral widths of 5000 Hz and 1200 Hz in F_2 and F_1, respectively. 2048 complex points were used in t_2, and 1024 t_1-experiments, each with 80 scans, with a repetition delay of 2.4 s. An E-COSY spectrum was obtained with a pulse sequence in which the first pulse was replaced with an E-BURP type selective pulse.[1] This spectrum was acquired in D_2O at 500 MHZ with spectral widths of 5000 Hz and 1200 Hz in F_2 and F_1, respectively. 2048 complex points were used in t_2, and 450 t_1-experiments, each with 96 scans, with a repetition delay of 2.4 s.

A ^{1}H, ^{13}C-HSQC spectrum was acquired in D_2O at 800 MHZ with spectral widths of 8000 Hz and 15000 Hz in F_2 and F_1, respectively. 2048 complex points were used in t_2, and 1024 t_1-experiments, each with 64 scans, with a repetition delay of 1.7 s.

A J-scaled ^{1}H, ^{31}P-HMBC spectrum was acquired in D_2O at 500 MHZ with spectral widths of 3000 Hz and 600 Hz in F_2 and F_1, respectively.[2] 2048 complex points were used in t_2,

and 56 t_1-experiments, each with 976 scans, with a repetition delay of 2.7 s. The scaling factor employed was ê = 5.

The acquired data were processed with FELIX (version 97.2, MSI/Biosym Technologies, San Diego, CA). All spectra were apodized by skewed sinebell squared in F_1 and F_2. NOESY spectra were baseline-corrected in F_2 by means of the FLATT routine.[3] The J-scaled ^{1}H, ^{31}P-HMBC spectrum was linear predicted from 28 to 56 points.

MD-Simulation. For the partly and fully modified hybrids, MD simulations were performed with standard A-form starting geometries. For reference, an MD simulation of the unmodified hybrid was also performed with standard A-form starting geometry. Partial atomic charges for the á-L-LNA nucleotides were determined by the RESP procedure.[4] The modified nucleotides were built in the *xleap* module of AMBER,[5] and the hybrids were subjected to 1000 steps of *in vacuo* energy minimisation (EM). Afterwards the nucleic acids were neutralised by placing sodium ions at a distance of 3 Å from the phosphorous atoms on the OPO-bisectors. The nucleic acids were then submerged in rectangular periodic boxes of TIP3P water stretching 10 Å beyond the duplexes, this resulted in the addition of ca. 4100 water molecules per duplex. The sizes of the boxes were approx. 46 x 46 x 58 Å after equilibration. Equilibration was performed by 1000 steps of EM with positional restraints (500 kcal/mol) on the nucleic acids atoms, followed by 70 ps of MD with gradually lowered positional restraints on the nucleic acid atoms. Production runs were performed over 1ns. All MD calculations were performed with the SANDER module of AMBER6.[5] at constant temperature/pressure (300K/1 atm) using 2 fs time steps. The SHAKE algorithm was applied to constrain all bonds to hydrogens. A non-bonded cut-off of 10 Å was applied and electrostatic interactions were calculated using the PME procedure with a grid spacing of ~1 Å. During the production runs, coordinates were dumped at every ps.

References.

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Figure Legends

Figure S1. *a)* The aromatic - H1' region of the 250 ms NOESY spectrum of d($C_1$$^{á L}$**T**^{L}$_2$$G_3A_4$$^{á L}$**T**^{L}$_5$$A_6$$^{á L}$**T**^{L}$_7$$G_8$ C_9):r($G_{10}C_{11}A_{12}U_{13}A_{14}U_{15}C_{16}A_{17}G_{18}$) obtained at 800 MHz. The sequential H1'(n) - H6/H8(n+1) connectivity pathway is shown (full line for the á-L-LNA strand and broken line for the RNA strand). The adenine H2 resonances are indicated with bold lines. *b)* Identical region as in a) of a 250 ms NOESY spectrum calculated from the last 500 ps of the MD simulation of d(C$^{á L}$**T**^{L}GA$^{á L}$**T**^{L}A$^{á L}$**T**^{L}GC):r(GCAUAUCAG).

Figure S2. *a)* The H1' - H2'/H2" region of an experimental selective DQF-COSY of the d($C_1$$^{á L}$**T**^{L}$_2$$G_3A_4$$^{á L}$**T**^{L}$_5$$A_6$$^{á L}$**T**^{L}$_7$$G_8C_9$):r($G_{10}C_{11}A_{12}U_{13}A_{14}U_{15}C_{16}A_{17}G_{18}$) duplex obtained at 500 MHz. The resonances of the six deoxyriboses are indicated. *b)* The DQF-COSY spectrum calculated with the parameters obtained by spectral simulation with the CHEOPS program.

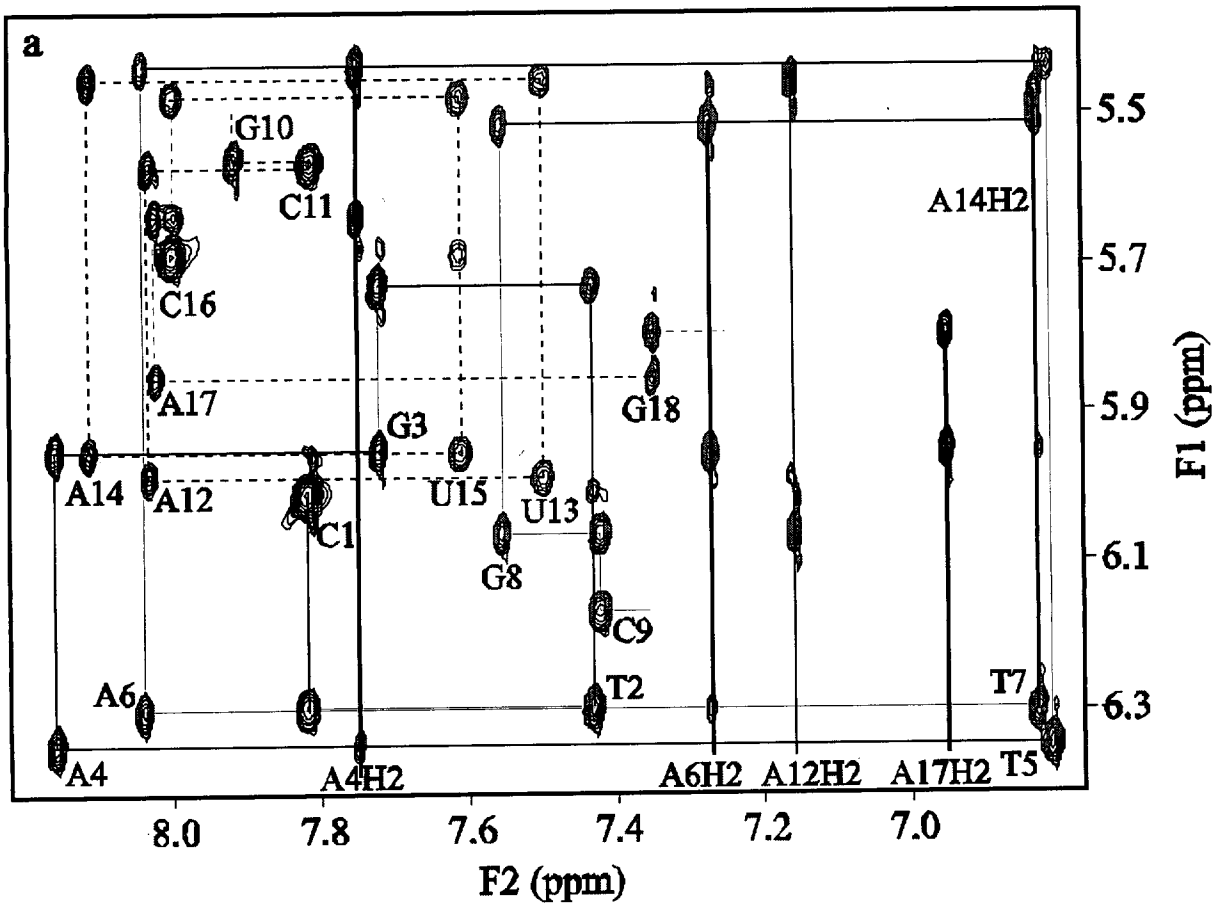
Figure S3. *a)* The H1' - H2'/H2" region of an experimental selective E-COSY of the d($C_1$$^{á L}$**T**^{L}$_2$$G_3A_4$$^{á L}$**T**^{L}$_5$$A_6$$^{á L}$**T**^{L}$_7$$G_8C_9$):r($G_{10}C_{11}A_{12}U_{13}A_{14}U_{15}C_{16}A_{17}G_{18}$) duplex obtained at 500 MHz. The resonances of the six deoxyriboses are indicated. *b)* The E-COSY spectrum calculated with the parameters obtained by spectral simulation with the CHEOPS program.

Figure S4. *a)* The deoxyribose sugar pucker distributions from the last 500 ps of the MD simulation of the unmodified duplex. *b)* The ribose sugar pucker distributions from the last 500 ps of the MD simulation of the unmodified duplex.

Figure S5. *a)* The deoxyribose sugar pucker distributions from the last 500 ps of the MD simulation of the partly modified duplex. *b)* The ribose sugar pucker distributions from the last 500 ps of the MD simulation of the partly modified duplex.

Figure S6. *a)* The deoxyribose sugar pucker distributions from the last 500 ps of the MD simulation of the fully modified duplex. *b)* The ribose sugar pucker distributions from the last 500 ps of the MD simulation of the fully modified duplex.

Figure S7. CD spectra of the unmodified hybrid (- - -·- - -), the partly modified á-L-LNA:RNA hybrid, d($C_1$$^{á L}$**T**^{L}$_2$$G_3A_4$$^{á L}$**T**^{L}$_5$$A_6$$^{á L}$**T**^{L}$_7$$G_8C_9$):r($G_{10}C_{11}A_{12}U_{13}A_{14}U_{15}C_{16}A_{17}G_{18}$) (———) and for comparison the partly modified LNA:RNA hybrid, d(C_1**T**^{L}$_2$$G_3A_4$**T**^{L}$_5$$A_6$**T**^{L}$_7$$G_8C_9$): r($G_{10}C_{11}A_{12}U_{13}A_{14}U_{15}C_{16}A_{17}G_{18}$) (·····).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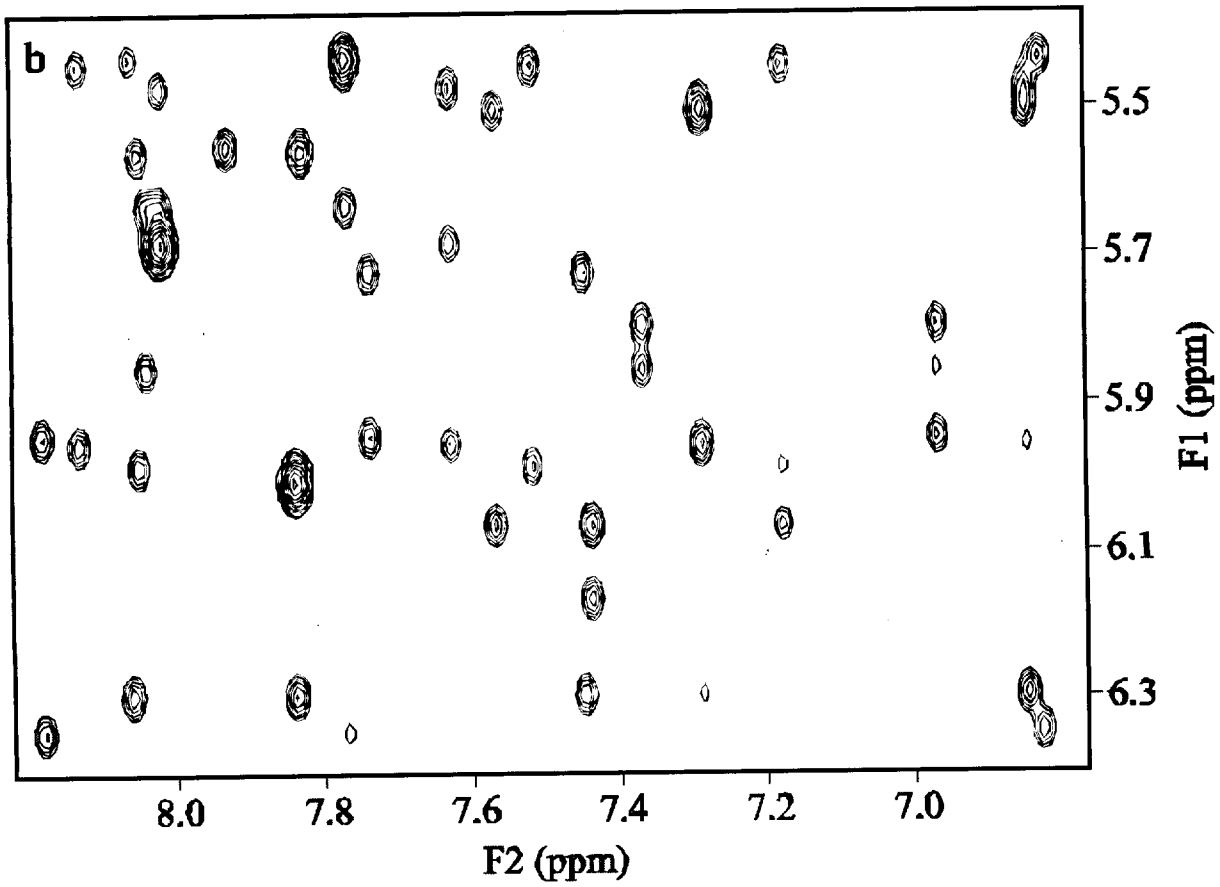
C16
A17
G3
A14
A12
C1
U15
U13
G8
G18
C9
T2
A6
A4
A4H2
A6H2
A12H2
A17H2
A14H2
T7
T5
5.5
5.7
5.9
6.1
6.3
F1 (ppm)
8.0
7.8
7.6
7.4
7.2
7.0
F2 (ppm)


b
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5.9
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F1 (ppm)
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7.8
7.6
7.4
7.2
7.0
F2 (ppm)

Figure S1

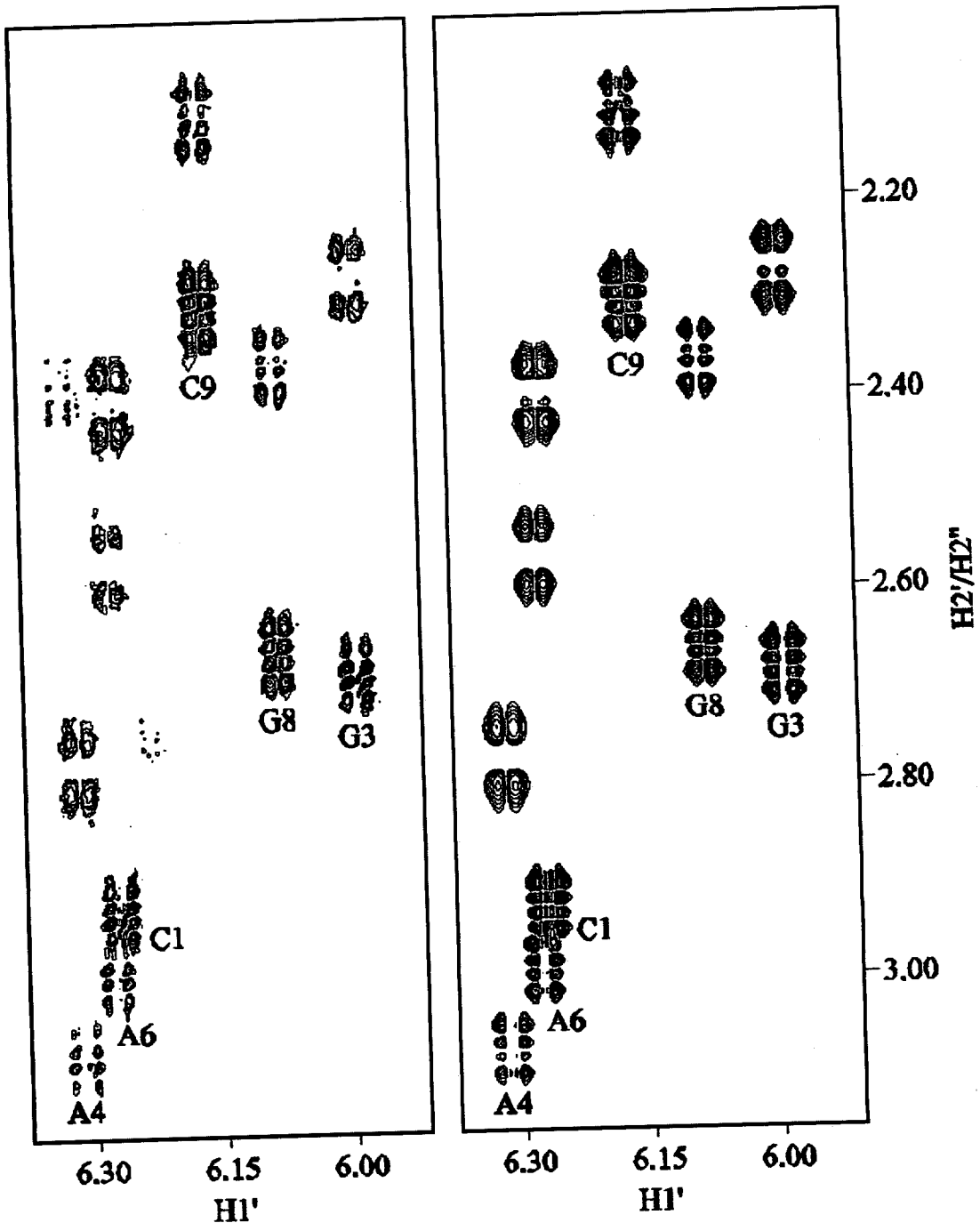


Figure S2

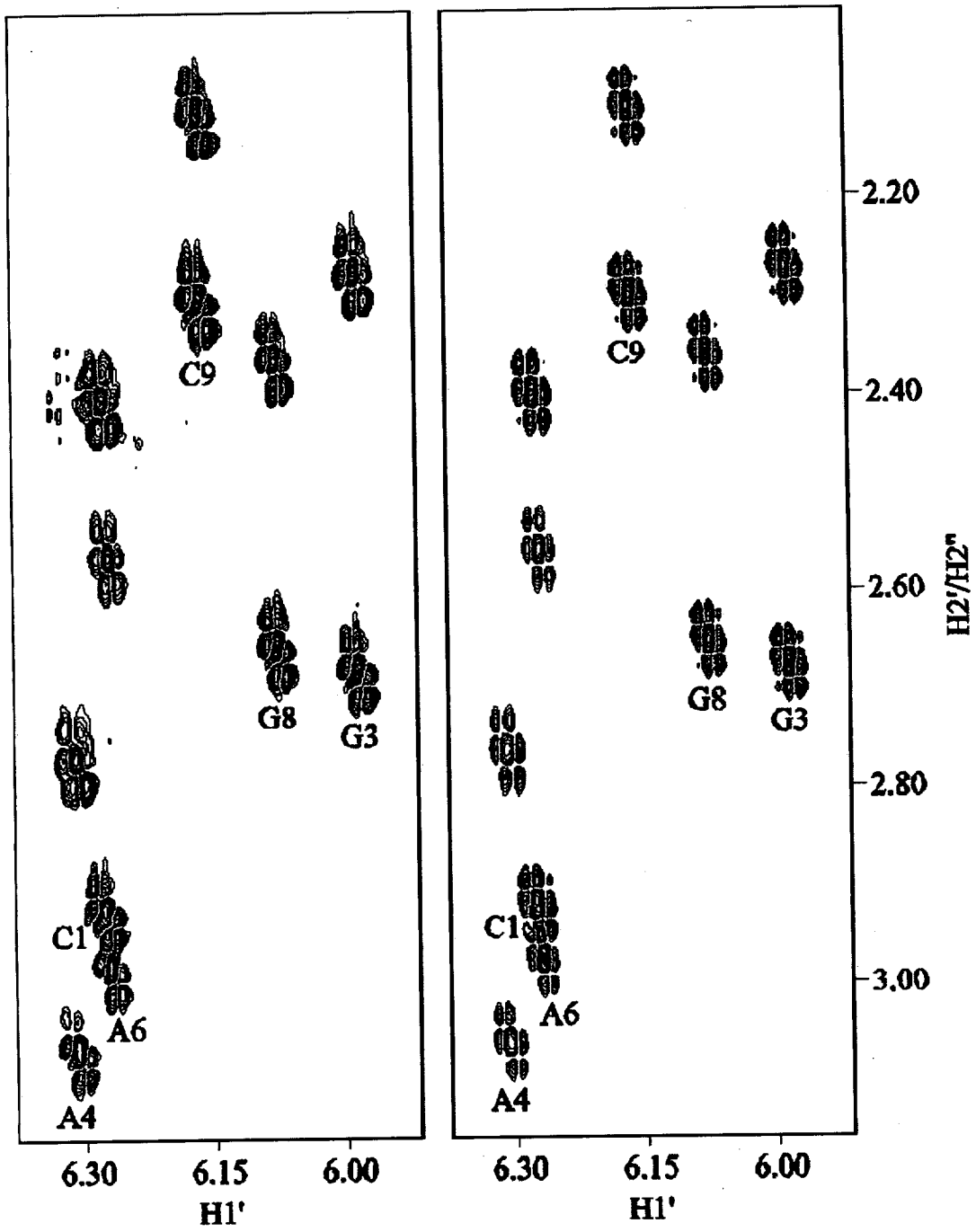


Figure S3

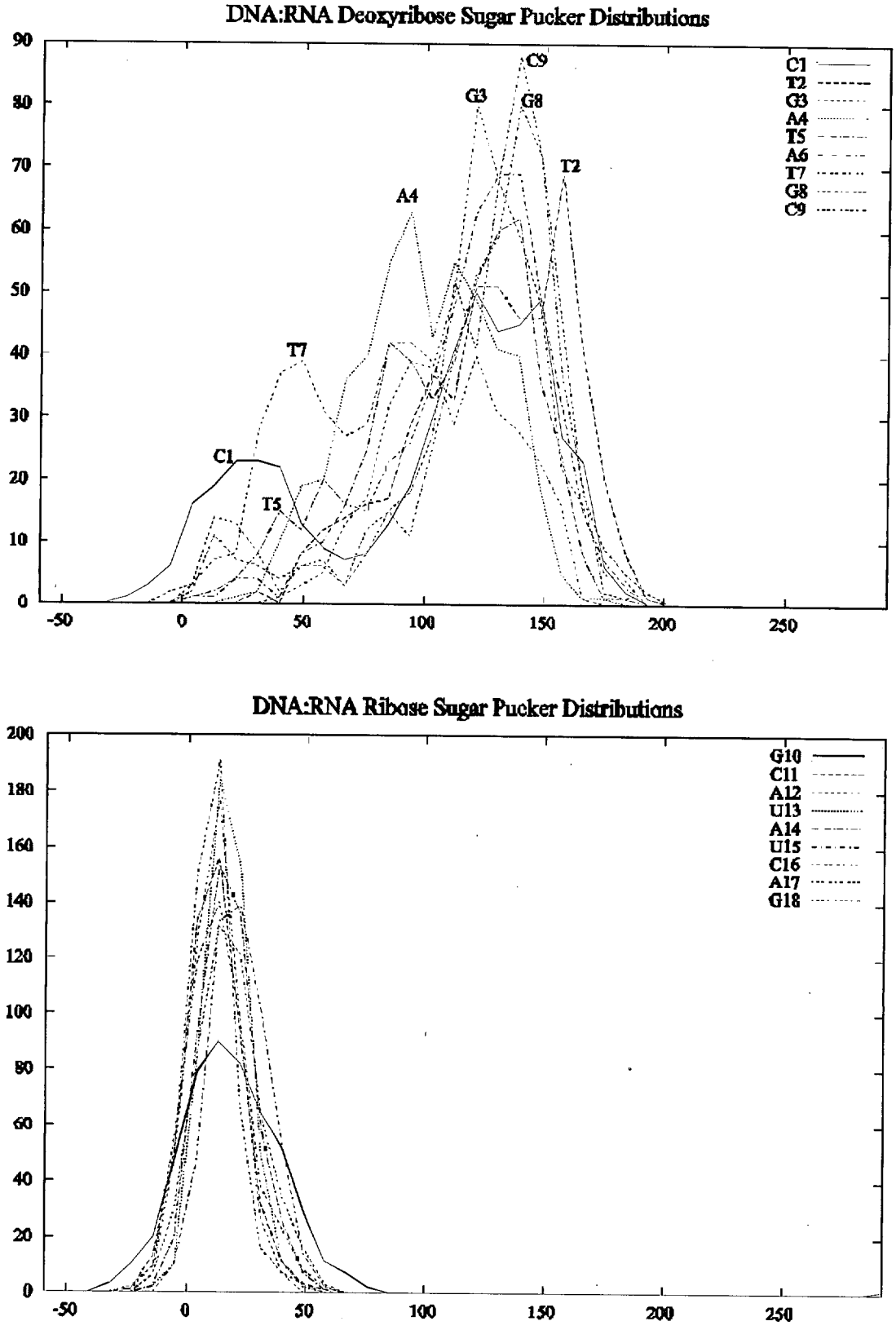
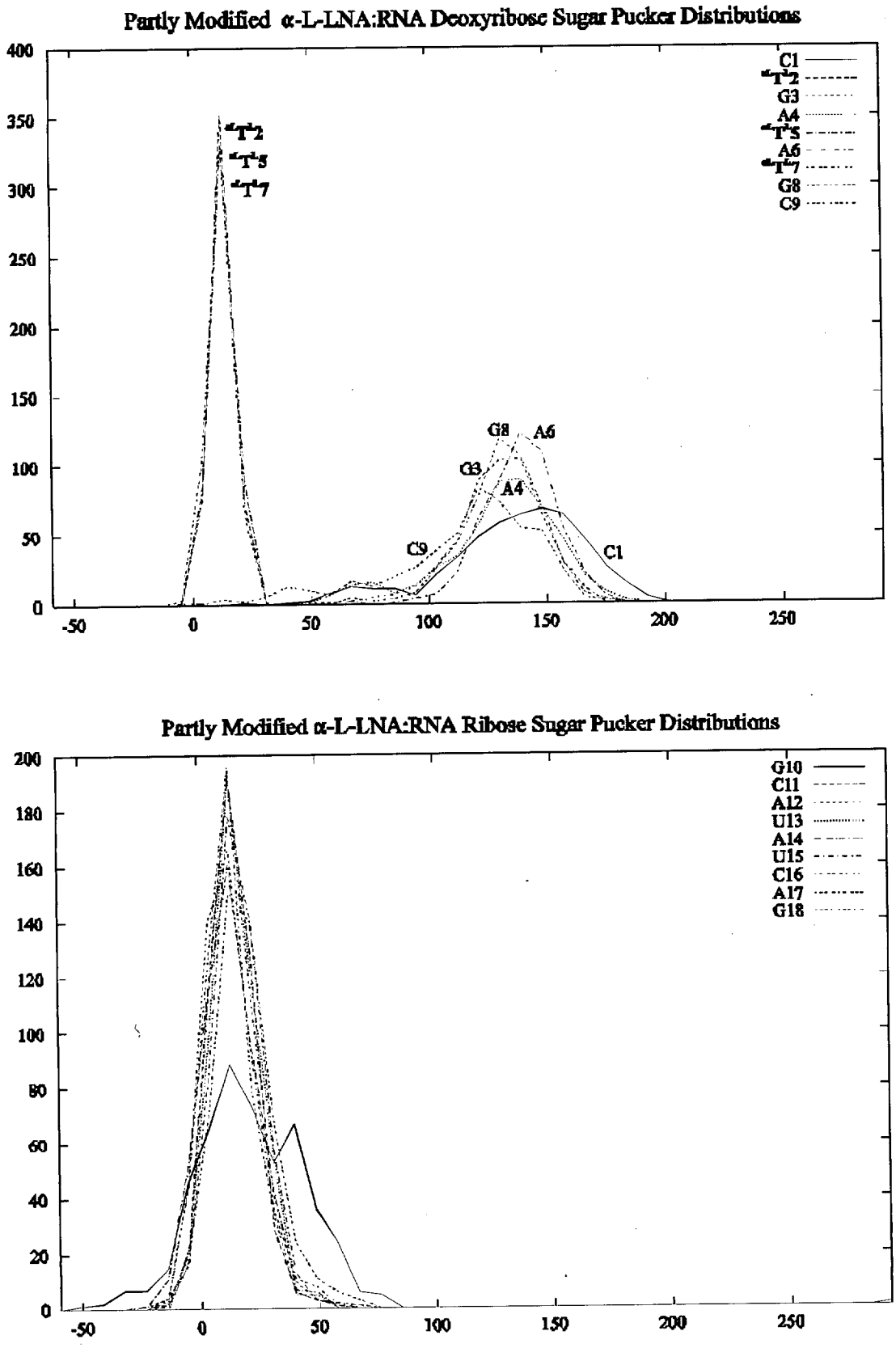


Figure S4


Partly Modified α-L-LNA:RNA Deoxyribose Sugar Pucker Distributions
C1
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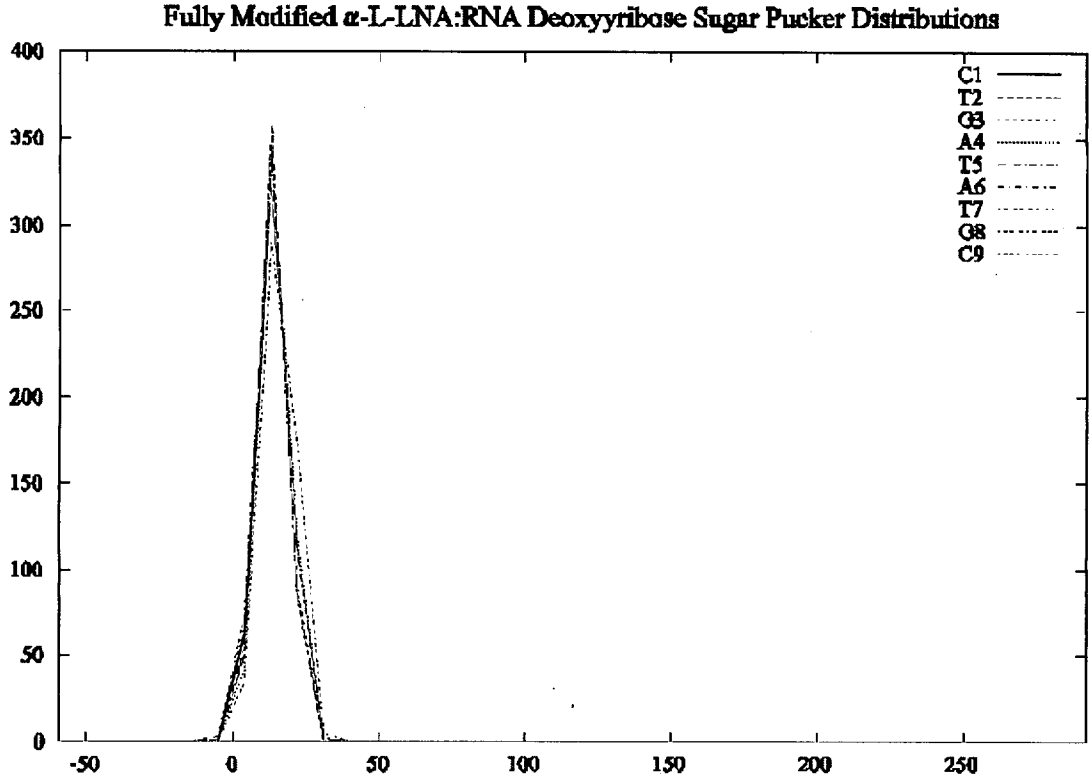
G3
A4
αT5
A6
αT7
G8
C9
αT2
αT5
αT7
G8
A6
G3
A4
C9
C1
Partly Modified α-L-LNA:RNA Ribose Sugar Pucker Distributions
G10
C11
A12
U13
A14
U15
C16
A17
G18

Figure S5


Fully Modified α-L-LNA:RNA Deoxyyribose Sugar Pucker Distributions
C1
T2
G3
A4
T5
A6
T7
G8
C9
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350
300
250
200
150
100
50
0
-50
0
50
100
150
200
250

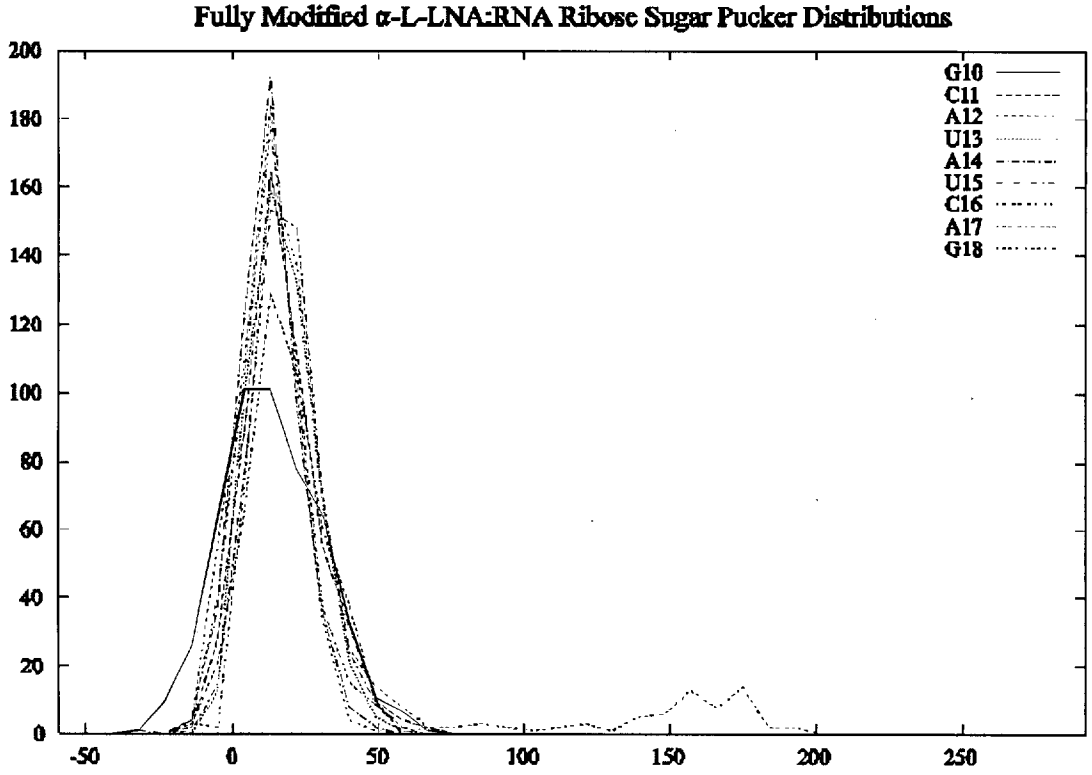
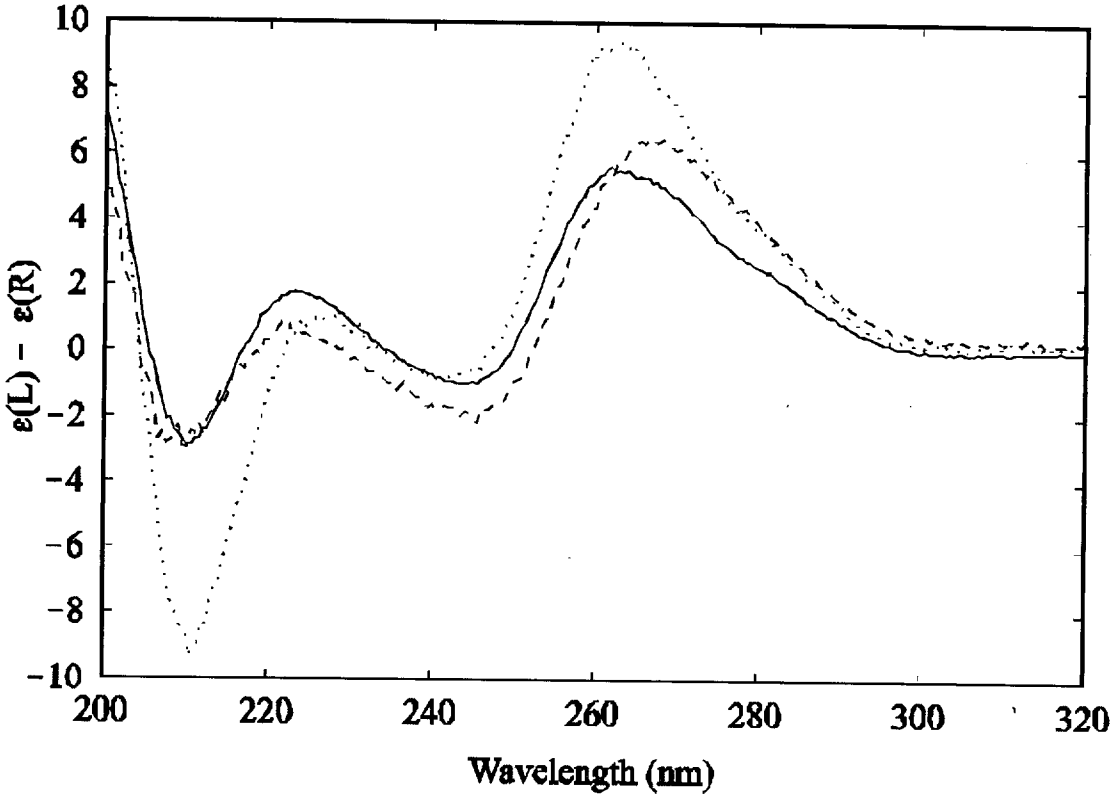

Fully Modified α-L-LNA:RNA Ribose Sugar Pucker Distributions
G10
C11
A12
U13
A14
U15
C16
A17
G18
200
180
160
140
120
100
80
60
40
20
0
-50
0
50
100
150
200
250

Figure S6


10
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6
4
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0
−2
−4
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−10
ε(L) − ε(R)
200
220
240
260
280
300
320
Wavelength (nm)

Figure S7