Pergamon

Bioorganic & Medicinal Chemistry, Vol. 3, No. 11, pp. 1493–1502, 1995

0968-0896/95 $9.50 + .00

0968-0896(95)00138-7

Oligonucleotide Analogues Containing 4'-*C*-(Hydroxymethyl)uridine: Synthesis, Evaluation and Mass Spectrometric Analysis

Kenneth Due Nielsen,[a] Finn Kirpekar,[b] Peter Roepstorff[b] and Jesper Wengel[a]*

[a]*Department of Chemistry;* [b]*Department of Molecular Biology, Odense University, DK-5230 Odense M, Denmark*

Abstract—2',3'-Di-*O*-*tert*-butyldimethylsilyl-4'-*C*-(hydroxymethyl)uridine was synthesized and converted into the phosphoramidite building blocks **9** and **13**. Novel oligodeoxynucleotide analogues containing 4'-*C*-hydroxymethyl linked phosphodiester internucleoside linkages and 3'-hydroxyl linked phosphodiester internucleotide linkages were synthesized on an automated DNA-synthesizer. The latter modification introduced an additional 4'-*C*-hydroxymethyl functionality. Oligodeoxynucleotides with one or two modifications in the middle or in the ends of 17-mers, 15-mers and 14-mers have been evaluated with respect to hybridization properties and enzymatic stability. Compared to unmodified oligomers, 3'-end-modified oligodeoxynucleotides were stabilized towards 3'-exonucleolytic degradation, but showed moderately to strongly lowered hybridization properties towards complementary DNA. However, more promising results were obtained in melting experiments with complementary RNA where only small decreases in melting temperature were detected. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was used to identify products from syntheses of the modified oligodeoxynucleotide analogues.

Introduction

Since the discovery that gene-specific antisense oligonucleotides were able to impede viral replication in Rous Sarcoma Virus infected chick embryo fibroblasts,[1,2] inhibition of protein synthesis by antisense oligonucleotides has emerged as an attractive alternative to classical drug design strategies.[3–5] The antisense approach is generally based on the sequence specific binding of a synthetic antisense molecule to the mRNA coding for disease-related proteins, which may result in suppression of translation. To act as an effective inhibitor of gene expression *in vivo*, an antisense oligonucleotide needs to (a) be stable towards intra- and extracellular nucleases, (b) hybridize with appropriate affinity and specificity to the target sequence, and (c) be able to penetrate cellular membranes.[4,6] Unmodified single stranded RNA and DNA do not fulfil these criteria, primarily because they suffer from rapid degradation by cellular nucleases. Therefore, it is necessary to use chemically modified oligonucleotides.

Recent work in this area from our laboratories includes incorporation of carbohydrate modified monomers into oligodeoxynucleotides.[7–11] As a continuation of the work with 3'-*O*-protected 4'-*C*-(hydroxymethyl)thymidine,[11] we here report the synthesis of 4'-*C*-modified nucleoside phosphoramidites **9** and **13** and their incorporation [linked through the 4'-hydroxymethyl (amidite **9**) and the 3'-hydroxyl (amidite **13**) functionality] into short oligodeoxynucleotide segments. The novel modified oligonucleotide analogues were studied with respect to their hybridization properties towards complementary DNA and RNA and their stability towards snake venom phosphodiesterase (3'-exonuclease). In addition, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was used to identify expected as well as unexpected products from syntheses of the modified oligonucleotides. The work described here was stimulated by the possibility to link reporter groups, intercalators or lipophilic carriers at the additional 4'-*C*-functionality facing the minor groove in a B-DNA helix. Furthermore, it was recently reported that oligodeoxynucleotides containing the reporter group biotin linked to the 4'-position of 4'-*C*-branched thymidine monomers were able to form stable duplexes with complementary DNA.[12]

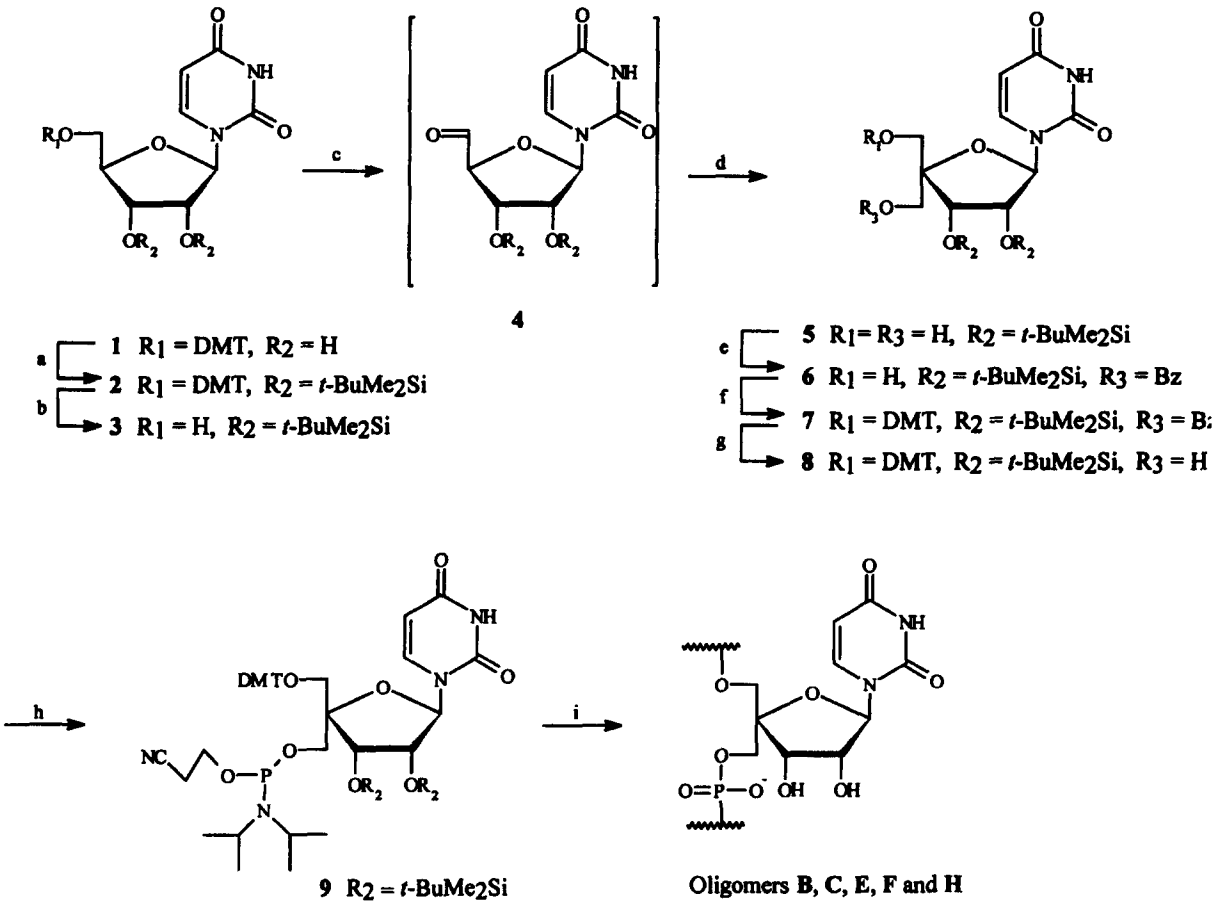
Results and Discussion

Chemistry

Uridine was reacted with 4,4'-dimethoxytritylchloride in dry pyridine to give the 5'-*O*-protected nucleoside **1**[13,14] which by use of *tert*-butyldimethylsilyl chloride (*t*-BuMe$_2$SiCl) and imidazole in DMF was silylated at the 2'-*O*- and 3'-*O*-positions affording compound **2**.[14] Subsequently, the 5'-*O*-dimethoxytrityl protective group was removed with 10% *p*-toluenesulfonic acid (PTSA) in CH_2Cl_2 to give **3**[15] in 36% yield based on uridine. For the transformation of nucleoside **3** to 4'-*C*-hydroxymethyl uridine derivative **5**, we used a Swern oxidation[16,17] [oxalyl chloride/DMSO/diisopropylethylamine (DIPEA)] followed by aldol condensation between the intermediate nucleoside aldehyde **4** and formaldehyde in the presence of aqueous sodium hydroxide with concomitant Cannizzaro reduction to

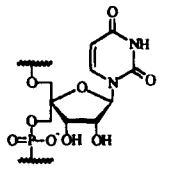
give **5** in 38% yield based on **3**. The strategy applied here for the synthesis of **5** was earlier developed for the synthesis of other 4'-*C*-hydroxymethyl substituted nucleosides.[18–20] Initially, we applied a Moffatt oxidation[18,19] for the synthesis of the unstable aldehyde **4**, but we generally obtained higher yields for the transformation of **3** to **5** when using a Swern oxidation. The 4'-*C*-hydroxymethyl functionality of **5** was regioselectively protected by reaction with benzoyl chloride (1.0 equivalent) in pyridine at 0 °C to give nucleoside **6** in 58% yield. This selectivity might be due to steric hindrance from the base moiety and it corresponds to results obtained earlier during dimethoxytritylation of an analogous thymidine nucleoside.[21] The structure of the monobenzoylated compound **6** was confirmed by ^{1}H–^{1}H COSY and NOE ^{1}H NMR experiments: Saturation of the signals at δ 3.73 and 4.07 ($HOCH_2$-) gave enhancement of the signals from 2'-H and 3'-H and no effect on the signal from 1'-H; no effect on 2'-H and 3'-H was observed when the signals from $BzOCH_2$ were saturated, but instead enhancement of 1'-H at δ 5.57. The free 5'-hydroxyl group of **6** was dimethoxytritylated to give intermediate **7**, and 5'-*O*-(4,4'-dimethoxytrityl)-4'-*C*-(hydroxymethyl)uridine derivative **8** was subsequently obtained in 44% yield (based on **6**) after debenzoylation in methanolic ammonia with NaOH added to complete the debenzoylation. Nucleoside **8** was reacted with 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite in the presence of DIPEA[22,23] to give the phosphoramidite building block **9** in 73% yield after precipitation in hexane (Scheme 1). The amidite **9** enables incorporation of 4'-*C*-(hydroxymethyl)uridine monomers containing unnatural 5'-hydroxyl to 4'-*C*-hydroxymethyl linked backbones into oligonucleotides (monomer **X**, Table 1).

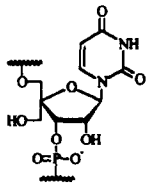
To synthesize the phosphoramidite building block **13** (Scheme 2), nucleoside **8** was desilylated with tetrabutylammonium fluoride (*n*-Bu_4NF) in THF to give triol **10** in 88% yield. Silylation of **10** with *t*-$BuMe_2SiCl$/$AgNO_3$/pyridine in THF afforded both a monosilylated nucleoside [tentatively assigned as the 4'-*C*-(*tert*-butyldimethylsilyl)oxymethyl derivative] and the desired disilylated nucleoside **11** in yields of 43 and 31%, respectively. After column chromatographic purification, the monosilylated compound was reacted with *t*-$BuMe_2SiCl$ (1.0 equivalent) to give additional **11** in 68% yield. To prove the structural assignment of the disilylated derivative, **11** was acetylated and the product (compound **12**) was analyzed by a ^{1}H–^{1}H COSY NMR experiment: the doublet originating from the 3'-H shifts downfield by 1.2 ppm after acetylation



Scheme 1. *Key: (a) *t*-$BuMe_2SiCl$, imidazole, DMF; (b) 10% PTSA in CH_2Cl_2; (c) oxalyl chloride, DMSO, DIPEA, CH_2Cl_2; (d) HCHO, 0.2 M NaOH, *p*-dioxane; (e) benzoyl chloride, pyridine; (f) DMTCl, pyridine; (g) NH_3, NaOH, MeOH; (h) 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite, DIPEA, CH_2Cl_2; (i) DNA-synthesizer.

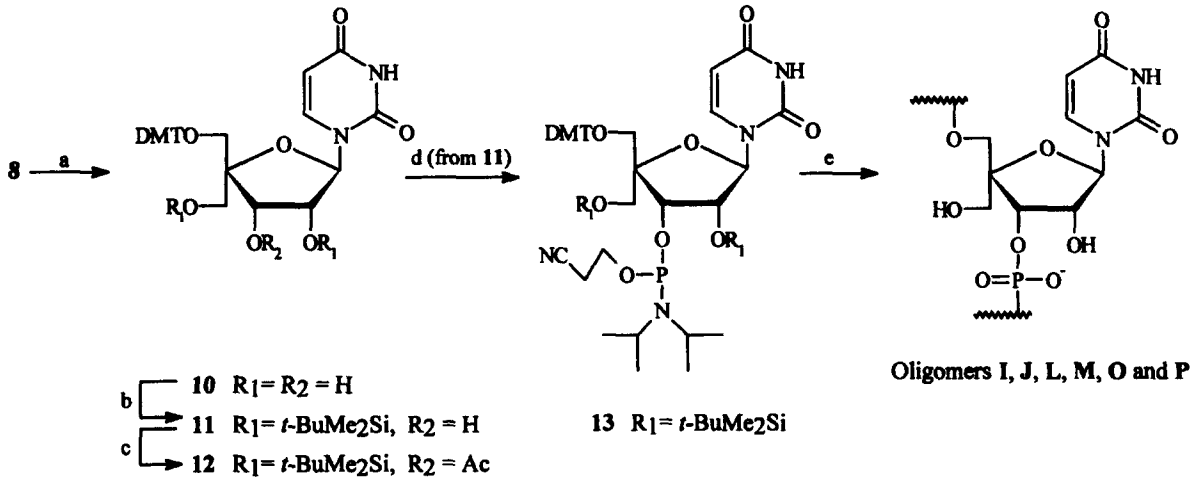
Table 1. Sequences synthesized and melting experiments

X = 

Y = 

	Sequence[a]	T_m(°C)[b]	ΔT_m(°C)[d]	T_m(°C)[c]	ΔT_m(°C)[d]
A	5'-TTAACTTCTTCACATTC-3'	55			
B	5'-TTAACTTCTTCACAT**X**C-3'	51	-4		
C	5'-TTAACTTCTTCACA**XX**C-3'	47	-4		
D	5'-CACCAACTTCTTCCACA-3'	64			
E	5'-CACCAAC**X**TCTTCCACA-3'	52	-12		
F	5'-CACCAAC**X**TCT**X**CCACA-3'	40	-12		
G	5'-TTTTTTTTTTTTTT-3'	40		33	
H	5'-TTTT**X**T**X**T**X**T**X**TTT-3'	<10			
A	5'-TTAACTTCTTCACATTC-3'	55			
I	5'-TTAACTTCTTCACAT**Y**C-3'	52	-3		
D	5'-CACCAACTTCTTCCACA-3'	64			
J	5'-CACCAAC**Y**TCTTCCACA-3'	57 (30[e])	-7		
K	5'-TTTTTTTTTTTTTTG-3'	41		31	
L	5'-TTTTTT**Y**TTTTTTTG-3'	32	-9	28	-3
M	5'-TTTTTTTTTTTTT**Y**T-3'	38	-2	32	-1
N	5'-UUUUUU**Y**UUUUUUUG-3'	-	-	-	-

[a]A = 2'-deoxyadenosine, C = 2'-deoxycytidine, G = 2'-deoxyguanosine, T = thymidine, U = uridine, X = nucleoside derived from **9**, Y = nucleoside derived from **13**.
[b]Melting point towards complementary DNA.
[c]Melting point towards complementary RNA.
[d]Change in melting point per modification.
[e]Melting point for the oligomer obtained by partial termination of the synthesis just prior to incorporation of modification Y.



Scheme 2. [a]Key: (a) *n*-Bu_4NF, THF; (b) *t*-$BuMe_2SiCl$, $AgNO_3$, pyridine, THF; (c) Ac_2O, triethylamine, 4-(*N*,*N*-dimethylamino)pyridine, CH_2Cl_2; (d) 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite, DIPEA, CH_2Cl_2; (e) DNA-synthesizer.

hereby confirming silylation of the 2'-hydroxy- and 4'-*C*-hydroxymethyl functionalities in compound **11**. By using the same reagents as described earlier for the synthesis of **9**, the phosphoramidite building block **13**, suitable for incorporation of 4'-*C*-hydroxymethyluridine with a natural 5'-hydroxyl to 3'-hydroxyl backbone (monomer **Y**, Table 1) into an oligonucleotide, was obtained from **11** in 86% yield.

Synthesis of oligonucleotides

The oligonucleotides **A–N** (Table 1) were synthesized by standard phosphoramidite methodology[24] on an automated DNA-synthesizer using the appropriate building blocks (**9**, **13**, commercial 2'-deoxynucleoside β-cyanoethylphosphoramidites and commercial 2'-*O*-*t*-BuMe$_2$Si protected ribonucleoside β-cyanoethyl-phosphoamidites). The coupling efficiencies (12 min couplings) for the modified phosphoramidites **9** and **13** were approximately 85 and 25%, respectively, compared to approximately 99% (deoxyamidites) and 95% (*ribo*-amidites) for commercial phosphoramidites (2 min couplings) as evaluated by monitoring the release of the dimethoxytrityl cation after each coupling step. We attribute the very low coupling yield achieved for amidite **13** to steric hindrance from the 2'-hydroxyl- and 4'-*C*-hydroxymethyl silyl-protective groups. The oligonucleotides were removed from the solid support and deblocked with concentrated ammonia at 20 °C for 3 days. Removal of the silyl groups was done by treatment with tetrabutylammonium fluoride in THF. Desalting of the oligomers was performed by filtration through NAP-10 columns and purification of the oligomers was performed using disposable reversed-phase chromatography cartridges.

Matrix assisted laser desorption/ionization mass spectrometry

The composition of the oligonucleotides **E**, **F**, **I** and **J** were analyzed by MALDI-MS. Oligomer **E** containing one modification and oligomer **F** containing two modifications gave relative molecular masses of 5067.1 (calc. 5066.4) and 5097.2 (calc. 5097.3), respectively. Because of the homogeneous results obtained from trityl assays during syntheses of the modified oligonucleotides **B**, **C**, **E**, **F** and **H** (modification **X**), we hereby consider their composition verified. Oligomer **I** with one modification in the 3'-end gave a relative molecular mass of 5102.4 (calc. 5102.4), which analogously is considered as a confirmation of the composition of oligonucleotide analogues **I**, **L** and **M** (modification **Y**). Oligomer **J** containing one modification gave a relative molecular mass of 5066.8 (calc. 5066.5), and an additional minor peak with a relative molecular mass of 2633.5 (calc. 2633.8) corresponding to a 9-mer oligomer where the synthesis has been terminated just before incorporation of modification **Y**. The result of the mass analysis of oligomer **J** was confirmed by the melting point experiment, as two melting points were observed. The presence of the impurity may be explained by the low coupling yield (25%) of amidite **13** leading to a crude oligonucleotide mixture containing approximately 70% of the unwanted 9-mer which was not completely removed during cartridge purification. A post-synthetic strand cleavage by nucleophilic attack from the 4'-*C*-hydroxymethyl on phosphorus could be another explanation for the formation of this impurity. However, as no indication of strand cleavage was observed for the corresponding 4'-*C*-(hydroxymethyl)thymidine analogue[25] and as significant strand cleavage requires the presence of a cyclic vicinal diol system,[26] this explanation seems improbable. We did not observe analogous impurities for any other modified oligonucleotide.

Calibration of the spectra is often a problem in MALDI-MS analysis of oligonucleotides. However, if two internal standards with masses flanking the 'unknown' are included, a mass accuracy better than ±1 Da is routinely obtained. In Figures 1 and 2, the internal standards are PNAs which consist of nucleobases attached to a polyamide backbone.[27] PNAs have several advantages when analyzing oligonucleotides: first, they are compatible with the 3-hydroxypicolinic acid (3-HPA) matrix which is by far the most commonly used for MALDI-MS analysis of oligonucleotides. Second, due to the absence of a labile *N*-glycosidic bond, loss of nucleobases is not observed, as it is for nucleic acids.[28] Third, PNAs have reduced tendency to form salts with alkali ions, which is a major problem for MALDI-MS of oligonucleotides.[28] As a result, PNAs give well-resolved and symmetric molecular-ion peaks, which makes them very suitable as internal mass calibration standards.

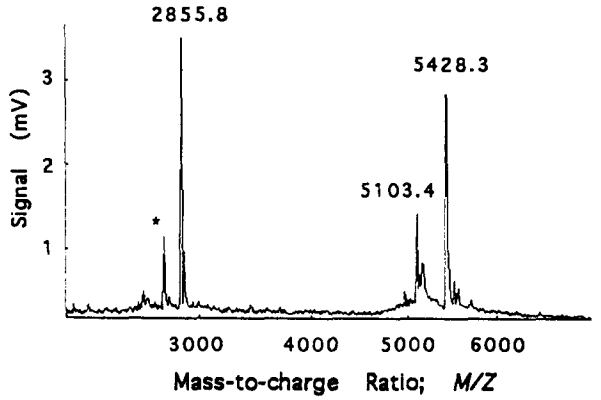


Figure 1. MALDI mass spectrum of oligonucleotide **I** (m/z = 5103.4). The compounds m/z 2855.8 and 5428.3 are PNAs used as internal standards. The peak marked with an asterix corresponds to a doubly charged species of the largest PNA. All m/z given are for singly protonated species.

The masses given in this work were normally obtained with a reflector time-of-flight (RTOF) mass analyzer because the mass resolution and consequently the mass accuracy is better than with a linear time-of-flight analyzer. However, care should be taken when interpreting RTOF spectra because some analytes under-

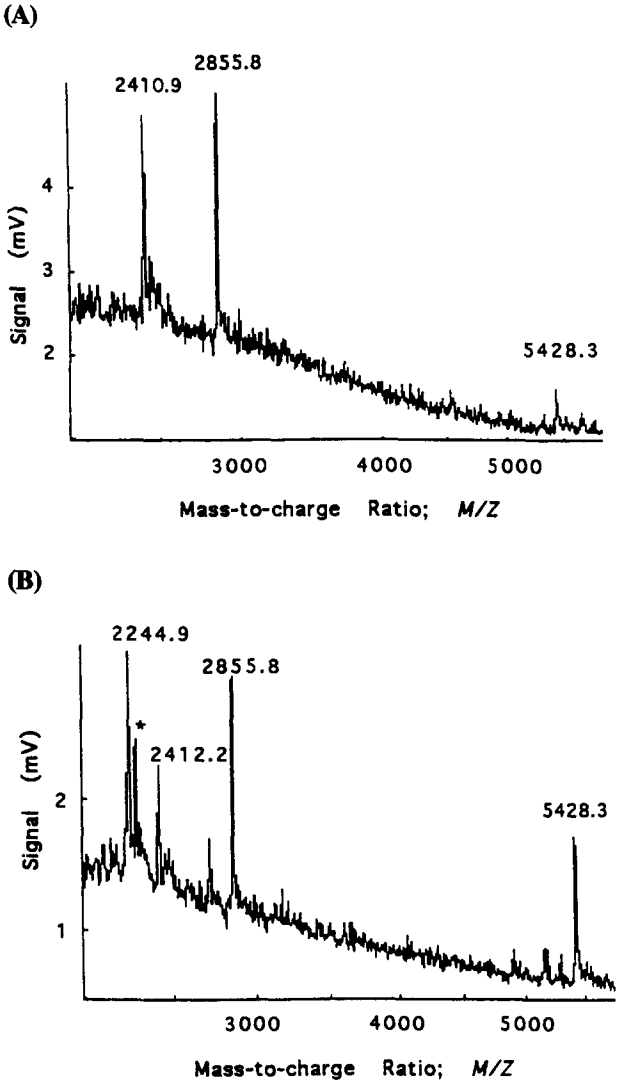


Figure 2. MALDI mass spectra of oligonucleotide **N**. Internal standards and given m/z as in Figure 1. (A) Recorded with a linear time-of-flight analyzer. (B) Recorded with a reflector time-of-flight analyzer. The peak marked with an asterix corresponds to a K^+ adduct of the component at m/z 2244.9..

go extensive metastable fragmentation (i.e. fragmentation in the first flight tube) which will give rise to additional and often well-defined peaks. This is exemplified in Figure 2, where the same sample preparation of oligonucleotide **N** is analyzed in linear and reflector mode. Only one component at a molecular mass of 2409.9 corresponding to 5'-U_7G-3' (calc. 2410.4), which arise from complete termination of the oligonucleotide synthesis just prior to the incorporation of modification **Y**, is present in the linear mode spectrum (Fig. 2A). In addition to the signal from 5'-U_7G-3' at m/z = 2412.2, a peak at m/z = 2244.9 is observed in the RTOF spectrum (Fig. 2B). Since the latter peak is not present in the linear spectrum, it can be attributed to metastable decay of the 5'-U_7G-3'. However, if the analysis only included the RTOF spectrum, it could easily and erroneously be concluded that the sample contained an additional component. The fragmentation leading to the peak at m/z = 2244.9 in Figure 2B may be explained by some special characteristics of the 5'-U_7G-3': because uracil lacks a basic functionality, the charge-giving proton will be located on the guanine and this protonation will weaken the corresponding *N*-glycosidic bond and promote loss of the nucleobase.[29] The loss of this 3'-terminal guanine promotes an additional 1,2-*cis*-elimination of the 3'-hydroxyl group and the 4'-hydrogen because a stable furan ring is then formed at the 3'-end.[30] The mass difference between the 5'-U_7G-3' peak and the fragment ion peak (167.3 Da observed, 168.1 Da calc.) confirms this suggested fragmentation scheme.

Melting experiments

The hybridization properties of the modified oligonucleotides were measured as previously described.[31] The melting temperatures (T_m) and the differences between modified and unmodified oligomers as the change in melting temperature per modification (ΔT_m) are listed in Table 1. Incorporation of the modified nucleotide derived from **9** (**X**, 5'-hydroxyl to 4'-*C*-hydroxymethyl backbone) one and two times in the middle of a 17-mer oligodeoxynucleotide (**E** and **F**) leads to ΔT_m of –12 °C per modification, whereas one or two modified monomers incorporated in the 3'-end region (**B** and **C**) lowers T_m by 4 °C per modification. The destabilizing effect of **X** is larger than observed for the corresponding 4'-*C*-(hydroxymethyl) linked 3'-*O*-ethylthymidine analogue,[11] which induced decreases in ΔT_m of –8 and –3 °C for modifications incorporated at the same positions in identical sequences. This additional destabilization may be a result of increased unfavorable steric interactions or conformational effects caused by the presence of the 2'-hydroxyl in **X**. The data obtained here indicate that modification with **X** in the middle of a sequence causes appreciable distortion of the resulting DNA:DNA duplex compared to the unmodified duplex, while 3'-end modifications cause only minor distortion of the duplex structure. Accordingly, no cooperative melting was observed above 10 °C for the 14-mer **H** containing four modifications in the middle.

Incorporation of 4'-*C*-hydroxymethyluridine by use of the 3'-*O*-phosphoramidite **13** (**Y**, 5'-hydroxyl to 3'-hydroxyl backbone) induced a change in T_m of –7 °C for one modification in the middle (**J**) and of –3 °C for one 3'-end modification (**I**). These decreases are smaller than the ones observed above for **X** in identical sequences, but still relatively large compared to results obtained recently for 4'-*C*-hydroxymethyl thymidine monomers incorporated into oligomers in an analogous way (5'-hydroxyl to 3'-hydroxyl backbone).[25] The T_m-data obtained indicate that the conformations of the pentofuranose ring in the modified nucleotides **X** and **Y** are far from ideal when optimal DNA:DNA duplex stability is considered. This was confirmed by modeling studies (Hyperchem™ Program, Amber Force Field, Polak-Ribiere Algorithm) which revealed distorsions in the sugar–phosphate backbone, especially for modification **X**.

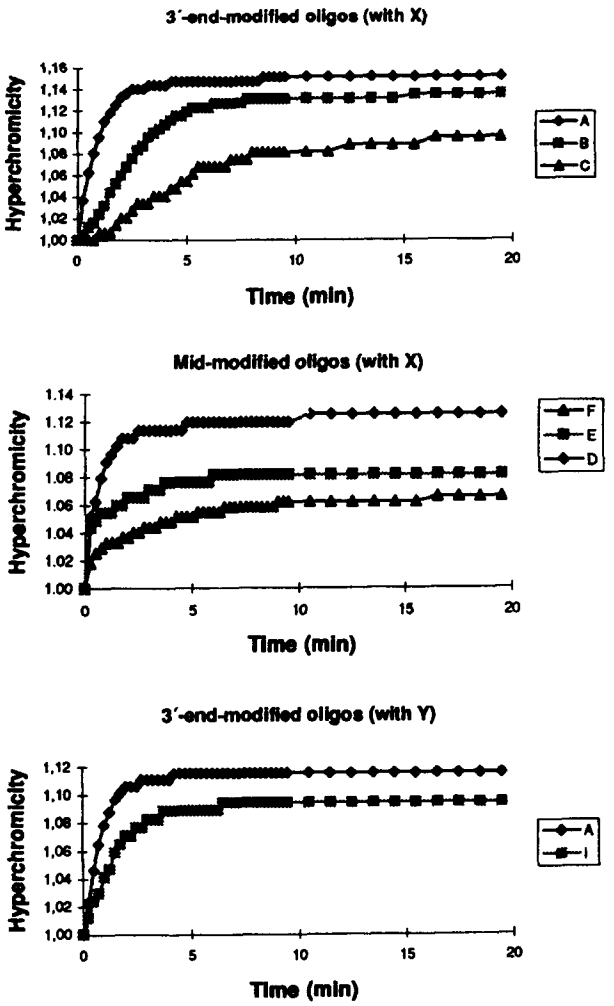


Figure 3. Time course of snake venom phosphodiesterase digestion of modified (**B**, **C**, **E**, **F** and **I**) and unmodified (**A** and **D**) oligonucleotides. The difference in the final hyperchromicity (1.12/1.15) during digestion of unmodified oligonucleotide **A** is due to the use of different batches of enzyme.

Incorporation of **Y** in the middle of a $T_{14}G$ strand (**L**) and in the 3'-end of a T_{14} strand (**M**) induces approximately the same change in melting temperature (–9 and –2 °C, respectively) as observed above when the complementary strand was DNA. However, when RNA is used as target, the change in T_m is only –3 and –1 °C, respectively. Monomer **Y** probably adopts a 3'-*endo*-like conformation (this has been supported by modeling studies) which is known to be the dominant ring-conformation in DNA:RNA duplexes.[32] After having obtained these results, we attempted to synthesize modified 14-mer oligo-*ribo*-nucleotides to evaluate the effect of 4'-*C*-hydroxymethyluridine monomers on the melting temperature of RNA:RNA duplexes. This should enable more information to be gained about the conformation of **X** and **Y**. We were unable to isolate the desired oligomers, however, because of very low coupling yields for amidite **Y** during these syntheses.

Enzymatic stability of the oligomers

On the basis of earlier experiments showing that 3'-phosphodiesterases play a predominant role in the *in vivo* degradation of natural oligonucleotides,[33] we decided to evaluate the enzymatic stability of oligonucleotides (**A**–**F** and **I**) towards snake venom phosphodiesterase (SVPDE, 3'-exonuclease). The increase in absorbance (hyperchromicity) at 260 nm was monitored during digestion with SVPDE.[34,35] Earlier work in our laboratory,[7] where results from hyperchromicity experiments of SVPDE digested oligonucleotides were compared with results from digestion of the corresponding 5'-end ^{32}P-labelled oligonucleotides using denaturating gel electrophoresis, has shown that hyperchromicity experiments can be used to obtain reliable information about the influence of modified monomers on the enzymatic stability of oligonucleotides. On the basis of the results depicted in Figure 3, it is clear that especially the anomalously linked modification **X** induces a considerable resistance towards 3'-exonucleolytic degradation. Thus, incorporation of **X** two times in the 3'-end induces a significant increase in half-life (~ five-fold), while incorporation of **Y** once in the 3'-end results in an approximate doubling of the half-life. The curves for the mid-modified oligomers **E** and **F** indicate rapid digestion from the 3'-end until the enzyme encounters a modified monomer **X**. Subsequent degradation of the shorter (now 3'-end protected) oligomers is hampered. This is reflected in the reduced slopes of the curves for **E** and **F** after approximately 1 min digestion.

Conclusion

4'-*C*-(Hydroxymethyl)uridine phosphoramidite derivatives **9** and **13** have been synthesized and applied in automated syntheses of novel oligonucleotide analogues. Moderate to strong destabilization of duplexes with complementary DNA was observed compared to unmodified controls. However, promising results were obtained for oligomers **L** and **M** containing modification **Y** (5'-hydroxyl to 3'-hydroxyl backbone) in melting experiments with complementary RNA where only small decreases in melting temperature were obtained. These results, together with the enhanced stability towards 3'-exonucleolytic degradation and the presence of the additional 4'-*C*-hydroxymethyl functionality, suggest that 4'-*C*-(hydroxymethyl)uridine, incorporated as modification **Y** (5'-hydroxyl to 3'-hydroxyl backbone), should be useful as a monomeric substitute in biologically active antisense oligonucleotides.

Experimental

NMR spectra were recorded at 250 MHz for ^1H NMR and 62.9 MHz for ^{13}C NMR on a Bruker AC 250 spectrometer, and at 202.3 MHz for ^{31}P NMR on a Varian Unity 500 spectrometer; δ-values are in ppm relative to tetramethylsilane as internal standard (^1H NMR and ^{13}C NMR) and relative to 85% H_3PO_4 as external standard (^{31}P NMR). EI mass spectra were recorded on a Varian Mat 311A spectrometer and FAB mass spectra on a Kratos MS 50 RF spectrometer.

Oligonucleotides were synthesized on a Pharmacia Gene Assembler Special® DNA-Synthesizer. Purification of 5'-*O*-DMT-ON oligonucleotides was accomplished using Oligopurification Cartridges (Cruachem Inc.) and desalting was performed using NAP-10 columns (Pharmacia). The silica gel (0.040–0.063 mm) used for column chromatography was purchased from Merck. Snake venom phosphodiesterase (*Crotalus adamanteus*) was obtained from Pharmacia. Melting profiles were obtained on a Perkin-Elmer UV/VIS spectrometer fitted with a PTP-6 Peltiér temperature programming element.

Matrix assisted laser desorption/ionization mass spectrometry

Mass spectra were obtained on a prototype laser desorption mass spectrometer from Applied Biosystem AB, Uppsala, Sweden, or a Bruker Reflex® time-of-flight instrument (Bruker-Franzen Analytik, Bremen, F.R.G.). All spectra were recorded in positive ion detection mode. Sample preparation with a 3-hydroxypicolinic acid matrix and NH_4^+-loaded cation exchange beads was done as previously described.[10] The two PNAs[27] used as internal standards have the compositions lysinylamide-TCACTAGATG-H (MW = 2854.80 Da) and lysinylamide-ACTCCTCGCTACTGCCTTAT-H (MW = 5427.30 Da), respectively. Between 10 and 50 pmol of analyte as well as standards were used in typical sample preparation.

2',3'-Di-O-(tert-*butyldimethylsilyl)-5'*-O-*(4,4'-dimethoxytrityl)uridine (2).*[14] Compound **2** was prepared as previously described.[14] ^{1}H NMR data were identical with published data.[14] ^{13}C NMR ($CDCl_3$): δ –5.06, –5.02, –4.53, –4.21 ($Si(\underline{C}H_3)_2$), 17.84, 17.87 ($\underline{C}(CH_3)_3$), 25.54, 25.59 ($C(\underline{C}H_3)_3$), 55.12 (OCH_3), 61.44 (C-5'), 70.65 (C-2'), 76.05 (C-3'), 82.78 (C-1'), 87.26 ($\underline{C}(Ar)_3$), 89.59 (C-4'), 101.80 (C-5), 113.12, 113.16, 127.16, 127.68, 128.28, 130.20, 131.01, 134.85, 140.35 (DMT), 143.93 (C-6), 150.19 (C-2), 163.41 (C-4). R_f 0.56 (5% CH_3OH in CH_2Cl_2).

2',3'-Di-O-(tert-*butyldimethylsilyl)uridine (3).*[14,15] A solution of **2** (8.13 g, 10.52 mmol) in CH_2Cl_2 (150 mL) was cooled to 0 °C, followed by dropwise addition of 10% *p*-toluenesulfonic acid dissolved in a mixture of CH_2Cl_2 (30 mL) and CH_3OH (3 mL). After stirring for 30 min, the reaction mixture was neutralized with 32% aqueous ammonia and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (30 mL) and washed with H_2O (3 × 20 mL), dried (Na_2SO_4) and purified by silica gel CC (0–4% CH_3OH in CH_2Cl_2, v/v) to give **3** (3.23 g, 66%) as a white solid material. ^{1}H NMR and ^{13}C NMR data were identical with published data.[15]

2',3'-Di-O-(tert-*butyldimethylsilyl)-4'*-C-*(hydroxymethyl)uridine (5).* Oxalyl chloride (5.36 g, 42.2 mmol) was dissolved in anhydrous CH_2Cl_2 (20 mL) and the solution was stirred at –70 °C. Anhydrous DMSO (6.58 g, 84.4 mmol), dissolved in anhydrous CH_2Cl_2 (5 mL) was added dropwise over 15 min and stirring was continued for 1 h at –70 °C. Nucleoside **3** (10.0 g, 21.1 mmol), dissolved in anhydrous CH_2Cl_2 (10 mL), was added dropwise over 30 min followed by further stirring for 1 h. Diisopropylethylamine (13.63 g, 105.5 mmol) was added and after additional stirring for 30 min at –70 °C, the solution was allowed to warm to room temperature. H_2O (100 mL) and CH_2Cl_2 (100 mL) were added and the organic layer was separated, dried (Na_2SO_4) and evaporated. The residue was redissolved in a mixture of *p*-dioxane (100 mL) and 37% aqueous formaldehyde (5.2 mL). NaOH (21.0 mL) was added dropwise and the solution was stirred at 5 °C for 28 h. The reaction mixture was neutralized with acetic acid:pyridine (4:1, v/v), diluted with CH_2Cl_2 (100 mL) and washed with saturated aqueous solutions of $NaHCO_3$ (3 × 25 mL) and NaCl (3 × 25 mL), and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue purified by silica gel CC (0–5% CH_3OH in CH_2Cl_2, v/v) to give **5** (4.05 g, 38%) as a white solid material. ^{1}H NMR (CD_3OD): δ 0.13–0.25 (*m*, 12H, $SiMe_2$), 0.92–1.04 (*m*, 18H, *tert*-butyl), 3.65–4.12 (*m*, 4H, H-5'a, H-5'b, H-5"a, H-5"b), 4.51–4.60 (*m*, 2H, H-2', H-3'), 5.80 (*d*, 1H, J = 8.1 Hz, H-5), 6.05 (*d*, 1H, J = 4.8 Hz, H-1'), 8.17 (*d*, 1H, J = 8.1, H-6). ^{13}C NMR (CD_3OD): δ –4.22, –3.86, –3.80, –3.31 ($Si(\underline{C}H_3)_2$), 19.26, 19.36 ($\underline{C}(CH_3)_3$), 26.69, 26.88, 26.94, 27.17 ($C(\underline{C}H_3)_3$), 63.72, 64.54 (C-5', C-5"), 74.06 (C-2'), 77.47 (C-3'), 89.75, 90.45 (C-1', C-4'), 103.05 (C-5), 143.53 (C-6), 152.58 (C-2), 166.36 (C-4). R_f 0.25 (5% CH_3OH in CH_2Cl_2, v/v); R_f 0.44 (10% CH_3OH in CH_2Cl_2, v/v). MS (FAB) m/z 503 ($[M+H]^+$, 4.0%). Anal. calcd for $C_{22}H_{42}O_7N_2Si_2 \cdot 0.75H_2O$: C, 51.18; H, 8.50; N, 5.44. Found: C, 51.09; H, 8.28; N, 5.49.

4'-C-(Benzoyloxymethyl)-2',3'-di-O-(tert-*butyldimethylsilyl)uridine (6).* 4'-*C*-Hydroxymethyl derivative **5** (0.50 g, 1.0 mmol) was co-evaporated with anhydrous pyridine (3 × 10 mL) and dissolved in anhydrous pyridine (7 mL) at 5 °C with stirring. Benzoyl chloride (0.14 g, 1.0 mmol) was added dropwise over 5 min. After 1 h, the mixture was heated to room temperature and stirred for an additional 1 h. The reaction was quenched with *n*-butanol (0.2 mL), and after dilution with CH_2Cl_2 (30 mL), the reaction mixture was washed with saturated aqueous solutions of $NaHCO_3$ (30 mL) and NaCl (30 mL), dried (Na_2SO_4) and evaporated to dryness under reduced pressure. After purification by silica gel CC (0–3% CH_3OH in CH_2Cl_2, v/v), nucleoside **6** (0.35 g, 58%) was isolated as a white foam. ^{1}H NMR ($CDCl_3$): δ 0.07–0.23 (*m*, 12H, $SiMe_2$), 0.86–0.95 (*m*, 18H, *tert*-butyl), 3.73 (*d*, 1H, J = 12.0 Hz, H-5'a), 4.07 (*d*, 1H, J = 12.0 Hz, H-5'b), 4.35 (*d*, 1H, J = 12.5 Hz, H-5"a), 4.56 (*d*, 1H, J = 5.2 Hz, H-3'), 4.63–4.66 (*m*, 1H, H-2'), 4.91 (*d*, 1H, J = 12.5 Hz, H-5"b), 5.57 (*d*, 1H, J = 4.3 Hz, H-1'), 5.72 (*d*, 1H, J = 7.0 Hz, H-5), 7.26–8.05 (*m*, 6H, H-6, benzoyl), 9.16 (*s*, 1H, NH). ^{13}C NMR ($CDCl_3$): δ –4.94, –4.59, –4.54, –4.06 ($Si(\underline{C}H_3)_2$), 17.91, 18.02 ($\underline{C}(CH_3)_3$), 25.84, 25.88, ($C(\underline{C}H_3)_3$), 62.60, 64.88 (C-5', C-5"), 71.78 (C-2'), 74.35 (C-3'), 87.27 (C-1'), 94.39 (C-4'), 102.15 (C-5), 128.30, 129.60, 129.78, 133.02 (benzoyl), 143.06 (C-6), 150.16 (C-2), 163.11 (C-4),

166.39 (C=O). R_f 0.57 (10% CH_3OH in CH_2Cl_2, v/v). MS (FAB) m/z 607 ([M+H]$^+$, 17.0%). Anal. calcd for $C_{29}H_{46}O_8N_2Si_2$: C, 57.40; H, 7.64; N, 4.62. Found: C, 57.45; H, 7.54; N, 4.72.

2',3'-Di-O-(tert-*butyldimethylsilyl)-5'*-O-*(4,4'-dimethoxytrityl)-4'*-C-*(hydroxymethyl)uridine (8).* Nucleoside **6** (0.51 g, 0.84 mmol) was dried by co-evaporation with anhydrous pyridine and dissolved in anhydrous pyridine (12 mL), and 4,4'-dimethoxytrityl chloride (0.36 g, 1.07 mmol) was added. The resulting mixture was stirred at 20 °C for 20 h. After dilution with CH_2Cl_2 (35 mL), the reaction mixture was washed with saturated aqueous solutions of $NaHCO_3$ (30 mL) and NaCl (30 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was dissolved in saturated methanolic ammonia (60 mL) and stirred at 20 °C for 12 h. To complete debenzoylation, a solution of NaOH (250 mg, 6.2 mmol) in CH_3OH (35 mL) was added and stirring was continued for 50 min. The solvent was removed under reduced pressure, and the residue purified by silica gel CC (0–2% CH_3OH, 0.5% pyridine in CH_2Cl_2, v/v/v)) to give **8** (296 mg, 44%) as a white solid material. ^{1}H NMR ($CDCl_3$): δ 0.07–0.23 (*m*, 12H, $SiMe_2$), 0.80–0.93 (*m*, 18H, *tert*-butyl), 3.35 (*d*, 1H, *J* = 10.5 Hz, H-5"a), 3.54 (*d*, 1H, *J* = 10.5 Hz, H-5"b), 3.76–3.85 (*m*, 8H, 2 × OCH_3, H-5'a, H-5'b), 4.26–4.29 (*m*, 1H, H-2'), 4.41 (*d*, 1H, *J* = 5.6 Hz, H-3'), 5.25 (*d*, 1H, *J* = 8.0 Hz, H-5), 5.91 (*d*, 1H, *J* = 2.5 Hz, H-1'), 6.80–7.38 (*m*, 13H, DMT), 7.82 (*d*, 1H, *J* = 8 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ –4.84, –4.51, –4.48, –3.98 ($Si(\underline{C}H_3)_2$), 17.87, 17.91 ($\underline{C}(CH_3)_3$), 25.72, 25.80 ($C(\underline{C}H_3)_3$), 62.89, 63.97 (C-5', C-5"), 72.40 (C-2'), 76.32 (C-3'), 87.21 ($\underline{C}(Ar)_3$), 87.77 (C-1'), 91.62 (C-4'), 102.15 (C-5), 113.12, 113.15, 127.16, 127.70, 127.74, 128.39, 130.26, 130.29, 134.82, 134.98, 141.00 (DMT), 143.87 (C-6), 149.89 (C-2), 158.73 (DMT), 163.92 (C-4). R_f 0.67 (10% CH_3OH in CH_2Cl_2, v/v). MS (EI) m/z = 804 ([M]$^+$, 1.0%). Anal. calcd for $C_{43}H_{60}O_9N_2Si_2 \cdot 0.25H_2O$: C, 63.79; H, 7.53; N, 3.46. Found: C, 63.52; H, 7.51; N, 3.47.

4'-C-((O-(2-Cyanoethoxy)diisopropylaminophosphino)-hydroxymethyl)-2',3'-di-O-(tert-*butyldimethylsilyl)-5'*-O-*(4,4'-dimethoxytrityl)uridine (9).* Compound **8** (114 mg, 0.14 mmol) was dried by co-evaporation with anhydrous acetonitrile (3 × 2 mL) and dissolved under N_2 in anhydrous CH_2Cl_2 (0.6 mL). *N,N*-Diisopropylethylamine (0.14 mL) was added followed by dropwise addition of 2-cyanoethyl *N,N*-diisopropylphosphoamidochloridite (0.053 mL, 0.23 mmol). After 1 h, the reaction was quenched with CH_3OH (0.03 mL) and diluted with EtOAc (3 mL). The mixture was washed with saturated aqueous solutions of $NaHCO_3$ (2 × 3 mL) and NaCl (2 × 3 mL), dried (Na_2SO_4) and evaporated under reduced pressure. The residual gum was dissolved in toluene (1 mL) and precipitated in hexane (150 mL) at –20 °C. The product was collected by filtration and dried under vacuum to give **9** (104 mg, 73%) as white crystals. ^{31}P NMR ($CDCl_3$): δ 149.8, 150.2. R_f 0.67 (10% CH_3OH in CH_2Cl_2, v/v). MS (FAB) m/z 1043 ([M+K]$^+$, 6.0%).

5'-O-(4,4'-Dimethoxytrityl)-4'-C-(hydroxymethyl)uridine (10). Compound **8** (288 mg, 0.360 mmol) was co-evaporated with anhydrous THF (2 × 4 mL) and dissolved under N_2 in anhydrous THF (10 mL). Tetra-*n*-butylammonium fluoride (1.1 M) in THF (1.3 mL, 1.47 mmol) was added. After stirring for 2 h, the mixture was diluted with CH_2Cl_2 (40 mL), washed with saturated aqueous solutions of $NaCO_3$ (2 × 20 mL) and NaCl (2 × 20 mL) and dried (Na_2SO_4). After evaporation of the solvents, the residue was purified by silica gel CC (0–7% CH_3OH, 0.5% triethylamine in CH_2Cl_2, v/v/v) to give **10** (182 mg, 88%) as a white foam. ^{1}H NMR ($CDCl_3$): δ 3.33–3.41 (*m*, 2H, H-5'a, H-5'b), 3.65–3.74 (*m*, 8H, 2 × OCH_3, H-5"a, H-5"b), 4.34 (*t*, 1H, *J* = 5.5 Hz, H-2'), 4.49 (*d*, 1H, *J* = 5.5 Hz, H-3'), 5.33 (*d*, 1H, *J* = 8.0 Hz, H-5), 6.03 (*d*, 1H, *J* = 5.5 Hz, H-1'), 6.80–7.38 (*m*, 13H, DMT), 7.61 (*d*, 1H, *J* = 8.0 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 55.09 (OCH_3), 63.01, 65.13 (C-5', C-5"), 71.96 (C-2'), 75.29 (C-3'), 87.07, 88.00, 89.34 ($\underline{C}(Ar)_3$, C-4', C-1'), 102.41 (C-5), 113.21, 123.85, 127.71, 127.74, 128.40, 130.03, 134.97, 135.15, 140.32 (DMT), 144.07 (C-6), 149.12 (C-2), 158.55 (DMT), 163.77 (C-4). R_f 0.44 (10% CH_3OH in CH_2Cl_2, v/v). MS (EI) m/z 576 ([M]$^+$, 1.2%). Anal. calcd for $C_{31}H_{32}O_9N_2Si_2 \cdot 0.6(CH_3CH_2)_3N$: C, 65.21; H, 6.48; N, 5.71. Found: C, 66.48; H, 5.91; N, 5.71.

*2'-O-(*tert*-Butyldimethylsilyl)-4'-C-((*tert*-butyldimethylsilyl)oxomethyl)-5'-O-(4,4'-dimethoxytrityl)uridine (11).* Nucleoside **10** (200 mg, 0.347 mmol) was dried by co-evaporation with anhydrous pyridine (3 × 7 mL) and dissolved under argon in anhydrous THF (6 mL). Anhydrous pyridine (0.14 mL) and $AgNO_3$ (74 mg, 0.434 mmol) was added, and stirring was continued for 5 min until almost all $AgNO_3$ was dissolved. *tert*-Butyldimethylsilyl chloride (105 mg, 0.730 mmol) was added and the resulting mixture was stirred at room temperature. After 4 h, analytical TLC indicated formation of monosilylated and disilylated products and no more starting material was detected. The solution was filtered into a 5% aqueous solution of $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phase was dried (Na_2SO_4), filtered and evaporated. The residue was purified by silica gel CC (0–5% CH_3OH, 0.5% pyridine in CH_2Cl_2, v/v/v) to give **11** (87 mg, 31%). Besides, a monosilylated product was isolated in 43% yield. This product was reacted with another equivalent of *tert*-butyldimethylsilyl chloride under analogous conditions to give additional **11** in 69% yield. ^{1}H NMR ($CDCl_3$): δ –0.08–0.17 (*m*, 12H, $SiMe_2$), 0.76–0.98 (*m*, 18H, *tert*-butyl), 3.35 (*d*, 1H, *J* = 10.5 Hz, H-5'a), 3.53 (*d*, 1H, *J* = 10.5 Hz, H-5'b), 3.62 (*d*, 1H, *J* = 10.5 Hz, H-5"a), 3.79 (*m*, 6H, 2 × OCH_3), 3.93 (*d*, 1H, *J* = 10.5 Hz, H-5"b), 4.40–4.51 (*m*, 2H, H-3', H-2'), 5.31 (*d*, 1H, *J* = 8.0 Hz, H-5), 6.06 (*d*, 1H, *J* = 5.6 Hz, H-1'), 6.81–7.37 (*m*, 13H, DMT), 7.60 (*d*, 1H, *J* = 8.0 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ –5.76, –5.71, –5.10, –5.06 ($Si(\underline{C}H_3)_2$), 17.88, 17.93 ($\underline{C}(CH_3)_3$), 25.48, 25.56, 25.63, 25.67 ($C(\underline{C}H_3)_3$), 55.12 (OCH_3), 64.32, 65.93 (C-5', C-5"), 72.30 (C-2'), 76.40 (C-3'), 87.29, 87.49, 87.72 ($\underline{C}(Ar)_3$), C-1', C-4'), 102.33 (C-5), 113.25, 127.08,

127.93, 128.02, 130.02, 130.09, 134.92, 135.12, 140.28 (DMT), 144.25 (C-6), 150.10 (C-2), 158.66 (DMT), 162.72 (C-4). R_f 0.81 (10% CH_3OH in CH_2Cl_2, v/v). MS (FAB) m/z 827 ([M+Na]$^+$, 3%). Anal. calcd for $C_{43}H_{60}O_9N_2Si_2 \cdot 4.0H_2O$: C, 58.88; H, 7.81. Found: C, 58.53; H, 8.04. There was not sufficient material for nitrogen analysis.

3'-O-Acetyl-2'-O-(tert-butyldimethylsilyl)-4'-C-((tert-butyldimethylsilyl)oxomethyl)-5'-O-(4,4'-dimethoxytrityl)-uridine (12). Nucleoside **11** (20 mg, 0.025 mmol) was dissolved in anhydrous CH_2Cl_2 (0.5 mL). Acetic anhydride (5 mg, 0.05 mmol), triethylamine (0.01 mL) and a few crystals of 4-(*N,N*-dimethylamino)pyridine were added and the solution was stirred at room temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with saturated aqueous solutions of $NaHCO_3$ (10 mL) and H_2O (10 mL), dried (Na_2SO_4) and evaporated under reduced pressure. Purification by silica gel CC (0–3% CH_3OH, 0.5% pyridine in CH_2Cl_2, v/v/v) afforded the monoacetylated product in sufficient quantity for NMR analysis. ^{1}H NMR ($CDCl_3$): δ 0.11–0.18 (*m*, 12H, $Si(CH_3)_2$), 0.71–0.96 (*m*, 18H, *tert*-butyl), 2.12 (*s*, 3H, Ac), 3.34 (*d*, 1H, *J* = 10.5 Hz, H-5'a), 3.44 (*d*, 1H, *J* = 10.5 Hz, H-5"a), 3.58 (*d*, 1H, *J* = 10.5 Hz, H-5'b), 3.74 (*d*, 1H, *J* = 10.5 Hz, H-5"b), 3.79 (*s*, 6H, 2 × OCH_3), 4.62 (*t*, 1H, *J* = 5.2 Hz, H-2'), 5.34 (*d*, 1H, *J* = 8.0 Hz, H-5), 5.65 (*d*, 1H, *J* = 5.2 Hz, H-3'), 6.06 (*d*, 1H, *J* = 5.2 Hz, H-1'), 6.82–7.37 (*m*, 13H, DMT), 7.80 (*d*, 1H, *J* = 8.0 Hz, H-6). R_f = 0.80 (5% CH_3OH in CH_2Cl_2).

2'-O-(tert-Butyldimethylsilyl)-4'-C-(tert-butyldimethylsilyloxomethyl)-3'-O-((2-cyanoethoxy)diisopropylamino-phosphino)-5'-O-(4,4'-dimethoxytrityl)uridine (13). Compound **11** (112 mg, 0.14 mmol) was dried by co-evaporation with anhydrous acetonitrile (3 × 2 mL) and dissolved under N_2 in anhydrous CH_2Cl_2 (0.6 mL). *N,N*-Diisopropylethylamine (0.14 mL) was added followed by dropwise addition of 2-cyanoethyl *N,N*-diisopropyl-phosphoramidochloridite (0.053 mL, 0.23 mmol). After 1 h, the reaction was quenched with CH_3OH (0.03 mL) and diluted with EtOAc (3 mL). The mixture was washed with saturated aqueous solutions of $NaHCO_3$ (2 × 3 mL) and NaCl (2 × 3 mL), dried (Na_2SO_4) and evaporated under reduced pressure. The residual gum was dissolved in toluene (1 mL) and precipitated in hexane (150 mL) at –20 °C. The product was collected by filtration and dried under vacuum to give **13** (120 mg, 86%) as white crystals. ^{31}P NMR ($CDCl_3$): δ 151.6, 151.7. R_f 0.65 (5% CH_3OH in CH_2Cl_2, v/v).

Synthesis of oligonucleotides

Synthesis of oligonucleotides **A–N** were performed in 0.2 μmol scale for deoxyribonucleotides and 1.0 μmol scale for ribonucleotides. The coupling efficiencies (12 min couplings) for the modified phosphoramidites **9** and **13** were approximately 85 and 25%, respectively, compared to approximately 99% (deoxyamidites) and 95% (*ribo*-amidites) for commercial phosphoamidites (2 min couplings) as judged by quantification of the dimethoxytrityl cation released after each coupling step. The 5'-*O*-DMT-ON oligonucleotides were removed from the solid support by treatment with concentrated ammonia at room temperature for 72 h, which also removed the nucleobase and phosphate protective groups. Subsequent purification using disposable reversed-phase chromatography cartridges, detritylation, desilylation and desalting as previously described,[9] afforded the pure oligomers.

Melting experiments

Melting experiments were carried out by mixing each oligomer with its complementary DNA or RNA strand in medium salt buffer (1 mM EDTA, 10 mM Na_3PO_4, 140 mM NaCl, pH 7.2) as previously described.[31] The melting temperature of the duplex was determined as the local maximum of the first derivative plot of the melting curve.

Enzymatic stability of oligonucleotides

A solution of oligonucleotides (0.2 OD) in 2 mL of the following buffer (0.1 M Tris–HCl, pH 8.6, 0.1 M NaCl, 14 mM $MgCl_2$) was digested with 1.2 U SVPDE (snake venom phosphodiesterase; 34 μL of a solution of the enzyme in the following buffer: 5 mM Tris–HCl, pH 7.5, 50% glycerol (v/v)) at 25 °C. During digestion, the increase in absorbance at 260 nm was followed. The absorption vs time curve of the digestion was plotted.

Acknowledgements

The Danish Natural Science Research Council, The Carlsberg Foundation and The Novo Nordisk Foundation are acknowledged for generous financial support. The mass spectrometry investigations were supported by the commission of the European Communities (grant no. GENO-CT93-0082) under the research program Biomed I. The authors are grateful to the late Professor Ole Buchardt, University of Copenhagen, for supplying PNAs.

References and Notes

1. Zamecnik, P. C.; Stephenson, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 280.

2. Stephenson, M. L.; Zamecnik, P. C. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 285.

3. Cohen, J. S. *Antiviral Res.* **1991**, *16*, 121.

4. Hélène, C.; Toulmé, J.-J. *Biochem. Biophys. Acta.* **1990**, *1049*, 99.

5. Milligan, J. F.; Matteucci, M. D.; Martin, J. C. *J. Med. Chem.* **1993**, *36*, 1923.

6. Stein, C. A.; Cohen, J. S. *Cancer Res.* **1988**, *48*, 2659.

7. Nielsen, P.; Kirpekar, F.; Wengel, J. *Nucleic Acids Res.* **1994**, *22*, 703.

8. Nielsen, P.; Dreiøe, L. H.; Wengel, J. *Bioorg. Med. Chem.* **1995**, *3*, 19.

9. Jørgensen, P. N.; Stein, P. C.; Wengel, J. *J. Am. Chem. Soc.* **1994**, *116*, 2231.

10. Svendsen, M. L.; Wengel, J.; Dahl, O.; Kirpekar, F.; Roepstorff, P. *Tetrahedron* **1993**, *49*, 11341.

11. Dreiøe, L. H.; Wengel, J. *Nucleosides Nucleotides* **1994**, *13*, 1939.

12. Maag, H.; Schmidt, B.; Rose, S. J. *Tetrahedron Lett.* **1994**, *35*, 6449.

13. Smith, M.; Rammler, D. H.; Goldberg, I. H.; Khorana, H. G. *J. Am. Chem. Soc.* **1962**, *84*, 430.

14. Myers, A. G.; Gin, D. Y.; Rogers, D. H. *J. Am. Chem. Soc.* **1994**, *116*, 4697.

15. Prepared earlier by slightly different procedures: (a) Ogilvie, K. K.; Beaucage, S. L.; Schifman, A. L.; Theriault, N. Y.; Sadana, K. L. *Can. J. Chem.* **1978**, *56*, 2768; (b) Flockerzi, D.; Silber, G.; Charubala, R.; Schlosser, W.; Varma, R. S.; Creegan, F.; Pfleiderer, W. *Liebigs Ann. Chem.* **1981**, 1568.

16. Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651.

17. Mancuso, A. J.; Swern, D. *Synthesis* **1981**, 165.

18. Youssefyeh, R. D.; Verheyden, J. P. H.; Moffatt, J. G. *J. Org. Chem.* **1979**, *44*, 1301.

19. Jones, G. H.; Taniguchi, M.; Tegg, D.; Moffatt, J. G. *J. Org. Chem.* **1979**, *44*, 1309.

20. O-Yang, C.; Wu, H. Y.; Fraser-Smith, E. B.; Walker, K. A. M. *Tetrahedron Lett.* **1992**, *33*, 37.

21. O-Yang, C.; Kurz, W.; Eugui, E. M.; McRoberts, M. J.; Verheyden, J. P. H.; Kurz, L. J.; Walker, K. A. M. *Tetrahedron Lett.* **1992**, *33*, 41.

22. Sinha, N. D.; Biernat, J.; Köster, H. *Tetrahedron Lett.* **1983**, *24*, 5843.

23. McBride, L. J.; Caruthers, M. H. *Tetrahedron Lett.* **1983**, *24*, 245.

24. Caruthers, M. H. *Acc. Chem. Res.* **1991**, *24*, 278.

25. Fensholdt, J.; Thrane, H.; Wengel, J. *Tetrahedron Lett.* **1995**, *36*, 2535.

26. Hardy, P. M.; Holland, D.; Scott, S.; Garman, A. J.; Newton, C. R.; McLean, M. J. *Nucleic Acids Res.* **1994**, *22*, 2998.

27. Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497.

28. Nordhoff, E.; Cramer, R.; Karas, M.; Hillenkamp, F.; Kirpekar, F.; Kristiansen, K.; Roepstorff, P. *Nucleic Acids Res.* **1993**, *21*, 3347.

29. Kirpekar, F.; Norhoff, E.; Kristiansen, K.; Roepstorff, P.; Hahner, S.; Hillenkamp, F. *Rapid Commun. Mass. Spectrom.* **1995**, in press.

30. Nordhoff E.; Karas, M.; Cramer, R.; Hahner, S.; Hillenkamp, F.; Lezius, A.; Kirpekar, F.; Muth, J.; Meier, C.; Engels, J. W. *J. Mass. Spectrom.* **1995**, *30*, 99.

31. Bjergårde, K.; Dahl, O. *Nucleic Acids Res.* **1991**, *19*, 5843.

32. Dickerson, R. E.; Drew, H. R.; Conner, B. N.; Wing, R. M.; Fratini, A. V.; Kopka, M. L. *Science* **1982**, *216*, 475.

33. Tidd, D. M.; Warenius, H. M. *Br. J. Cancer* **1989**, *60*, 343.

34. Augustyns, K.; Vandendriessche, F.; Aerschot, A. V.; Busson, R.; Urbanke, C.; Herdewijn, P. *Nucleic Acids Res.* **1992**, *20*, 4711.

35. Newman, P. C.; Nwosu, V. U.; Williams, D. M.; Cosstick, R.; Seela, F.; Connolly, B. A. *Biochemistry* **1990**, *29*, 9891.

(*Received in U.S.A.* 24 *April* 1995; *accepted* 1 *August* 1995)