Synthesis and Properties of Oligoribonucleotide Analogs Having Formacetal Internucleoside Linkages

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Introduction

The possibility to use short oligonucleotides as therapeutic agents has recently received much attention.[1,2] To be efficient, such antisense agents should exhibit high enzymatic stability while retaining binding affinity and specificity to the intracellular target, usually a messenger RNA. Unmodified oligonucleotides are readily degraded by nucleases and, thus, are poor antisense compounds. There are many modifications (for recent reviews, see refs 1 and 2) that increase the enzymatic stability of oligonucleotides. However, in most instances the binding affinity decreases. Most of these studies were done on oligodeoxynucleotides or 2′-*O*-alkyl analogs.

The use of oligoribonucleotide analogs would have the advantages of higher stability of RNA−RNA duplexes than DNA−RNA hybrids, ready access to monomeric synthons (e.g., can be often readily prepared from modified sugars and heterocyclic bases), and less costly starting materials. Unfortunately, a free 2′-OH function causes cleavage of the phosphodiester linkage at a far too high rate under physiological conditions. Some 2′-O-alkylated analogs (2′-*O*-Me, 2′-*O*-allyl, 2′-*O*-CH_2CH_2-OCH_3) show both increased enzymatic stability and good affinity to complementary RNA, but the starting materials are less attractive because of higher price or elaborate synthesis.[1,2] However, oligoribonucleotide analogs with dephosphono internucleoside linkages can contain free 2′-hydroxyls and still have high enzymatic and chemical stability.

Dephosphono linkages in oligodeoxyribonucleotides that are reported to give stable duplexes are those having amide[3] 3′-CH_2CONH-5′ and 3′-CH_2NHCO-5′, methylene(methylimino)[4] 3′-$CH_2N(CH_3)$O-5′, methylene(dimethylhydrazo)[5] 3′-$CH_2N(CH_3)N(CH_3)$-5′, formacetal[6−9] 3′-OCH_2O-5′, and thioformacetal[9] 3′-SCH_2O-5′ linkages (for recent reviews, see ref 2).

There are only a few reports on such modifications in oligoribonucleotides. Damha et al.[10] have found that oligodeoxynucleotides containing sulfide-linked (3′-CH_2-CH_2S-5′) ribofuranosylthymine dimers exhibit higher affinity to the RNA target than their counterparts having the corresponding deoxy dimers. Cao and Matteucci[11] have incorporated a diribonucleoside thioformacetal analog in an oligodeoxyribonucleotide. If compared with the full phosphodiester DNA or the deoxythioformacetal modification, this substitution results in slightly decreased affinity toward the complementary RNA. Uniformly modified oligoribonucleotides with dimethylene sulfone linkages (3′-$CH_2SO_2CH_2$-5′) have been prepared, but the strong self-association of this analog prevents hybridization with both complementary RNA and DNA.[12] Thus, the effect of various dephosphono linkages on the stability of modified RNA−RNA duplexes still remains largely unexplored.

In this paper, we report the synthesis of a formacetal linked uridine−uridine dimer and its incorporation in oligoribonucleotides. The stability of modified RNA−RNA duplexes having formacetal linkages replacing natural phosphodiesters at selected positions was studied by UV melting experiments. The formacetal substitution caused a slight increase in duplex stability ($\Delta t_m = +0.2$ to $+0.8$ °C per modification).

Results and Discussion

The synthesis of oligoribonucleotides containing internucleoside formacetal linkages at defined positions is most conveniently done with suitably protected dimeric building blocks that can be used in standard oligonucleotide synthesis. Preparation of such ribonucleoside dimers requires synthesis of monomeric units (donor and acceptor) and coupling of them to form the formacetal linkage. Final protecting group manipulation is necessary to make the building block suitable for oligonucleotide synthesis.

For the formation of internucleosidic formacetal linkages we employed (methylthio)methylene (MTM) acetals that previously have been used in the DNA series.[6−9] The syntheses of MTM-donors are shown in Scheme 1. As starting material in the first experiments we used 2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(monomethoxytrityl)uridine[13] (**1**). Treatment of **1** with dimethyl sulfide/benzoyl

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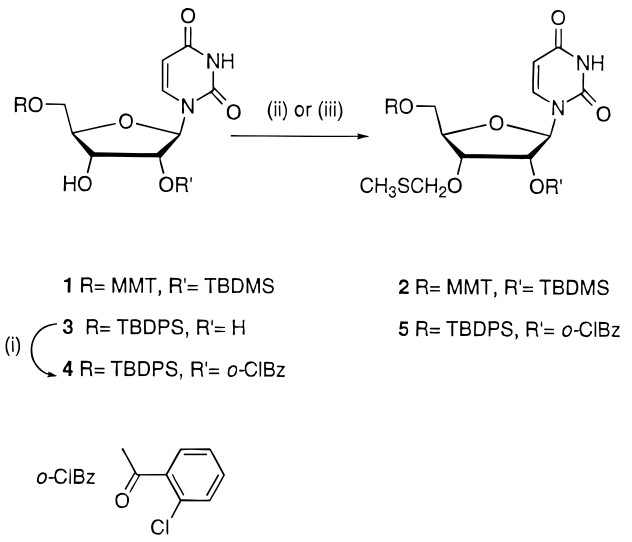
Scheme 1[a]



[a] Key: (i) *o*-Chlorobenzoyl chloride, 1.1 equiv, −78 °C (85%); (ii) dimethyl sulfide, benzoyl peroxide, 2,4,6-collidine (20%); (iii) DMSO, acetic anhydride, acetic acid (27%).

peroxide[6] gave the 3′-*O*-MTM derivative **2** in only 20% yield after silica gel column chromatography. The major product isolated (in 31% yield) was the 3′-keto derivative. To remove traces of non-nucleosidic byproducts, **2** was also crystallized from hexane−ether. Coupling of **2** with 2′,3′-*O*-bis(*tert*-butyldimethylsilyl)uridine[14] **6** using either *N*-bromosuccinimide[6a,8] (NBS) or *N*-iodosuccinimide[6a,7b] (NIS) as activators was not successful. Varying yields of the corresponding couplings in the deoxy series have been reported (NBS 45%[8] and 11%,[6a] NIS 30%,[7b] and 32%[6a]). Possibly the steric bulkiness of the 2′-*O*-TBDMS group complicated the synthesis further.

Recently, the activation with NIS in the presence of a catalytic amount of trifluoromethanesulfonic acid (TfOH)[15] was shown to be superior in the synthesis of formacetal-linked 2′-deoxynucleosides.[6a,7a,c] Since this method would not be compatible with the acid-labile MMT protection, we developed a new protecting group strategy. The key step was the selective 2′-*o*-chlorobenzoylation[16,17] of 5′-*O*-(*tert*-butyldiphenylsilyl) uridine[18] (**3**) (*tert*-butyldiphenylsilyl = TBDPS) (Scheme 1). Reaction of **3** with 1.1 equiv of *o*-chlorobenzoyl chloride at −78 °C gave selectively the 2′-acyl derivative **4** containing only traces of the possible 3′-acyl isomer. Reactions of **4** with dimethyl sulfide/benzoyl peroxide[6] or DMSO/acetic anhydride/acetic acid[19] gave after silica gel column chromatography and crystallization from hexane−ether the 3′-MTM donor **5** in 20 and 27% yield, respectively. In both cases unidentified more polar and more lipophilic by products were formed.

The selective 2′-benzoylation of 5′-*O*-dimethoxytrityl nucleosides has been previously reported.[16] The same selectivity could be expected with 5′-*O*-TBDPS uridine **3**. However, migration of the *o*-ClBz group to the 3′-OH in **4** could occur during the synthesis of **5**, thus yielding the 2′-*O*-MTM isomer. The correct structure of **5** was unambiguously assigned as the correct 3′-*O*-MTM isomer by a 2D long-range $^{13}C-^{1}H$ correlation NMR experiment (strong cross-peak signals corresponding to correlation from C3′ to OCH_2S protons and from OCH_2S carbon to H3′). Coupling of donor **5** with acceptor **6** using NIS/TfOH[6a,7a] activation gave dimer **7** in 55% isolated yield.

The synthesis of the selectively protected ribonucleoside dimer suitable for incorporation in oligonucleotides is complicated because of the need of selective protection of the terminal 2′-OH. This could be done by synthesizing a monomeric acceptor having different protecting groups at 2′, and 3′-OH and selectively removing the 3′-*O*-protection after the synthesis of the dimer. However, this approach would require more synthetic steps and additional protecting groups to achieve selective protection and deprotection. Instead we used a more straightforward protecting group manipulation based again on the selective 2′-*o*-chlorobenzoylatoin (Scheme 2).

Removal of all silyl protecting groups with tetrabutylammonium fluoride gave dimer **8**, which was protected at the 5′-OH with a monomethoxytrityl (MMT) group to give **9**. Dimer **9** was reacted with 1.1 equiv of *o*-chlorobenzoyl chloride at −78 °C and successively phosphonylated[17] in the same reaction mixture using the PCl_3/imidazole reagent[20] to give the dimeric building block **10** for use in oligonucleotide synthesis. The correct structure of **10** was confirmed by ^{1}H NMR spectroscopy.[21] This protecting group strategy can be used as a general method for preparation of modified ribonucleoside dimers suitable for incorporation in oligonucleotides.

The isomeric purity of the product isolated is a crucial question when using the selective 2′-acylation of ribonucleosides. When this approach was used to synthesize protected ribonucleoside 3′-*O*-hydrogen phosphonates we found that the target products can be efficiently separated from the 2′-isomers using simple silica gel chromatography.[17b] This finding was important because even a small contamination would produce a nonhomogeneous product after multiple coupling steps. In the present work we incorporated the formacetal linked dimer **10** in oligoribonucleotides at selected sites. We used TLC and NMR to check the isomeric purity of compounds **5** and **7**−**10**. Potential contamination undetectable using these methods should have little influence on the evaluation of the properties of the modified oligoribonucleotides synthesized.

Three model oligoribonucleotides (Table 1) having two (**11**), three (**12**), and six (**13**) internucleoside formacetal linkages (**f**) were synthesized using the automated solid-

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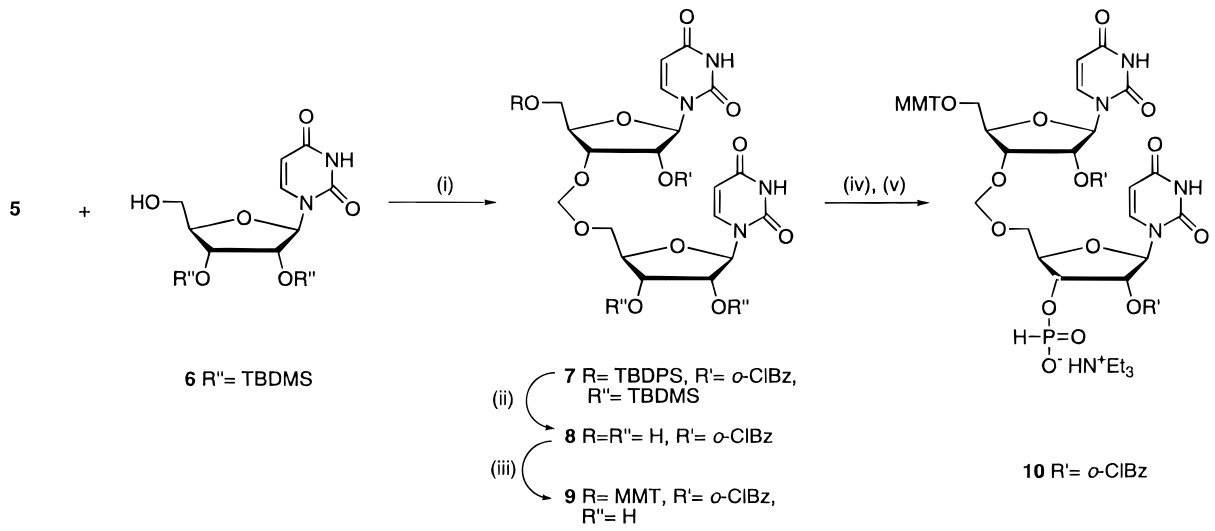
(21) The signal of H3′ in the 5′-yl uridine unit of **10** showed typical coupling pattern (pentet, 1:2:2:2:1) due to spin−spin interactions with H2′ and H4′ (J = ca. 5 Hz) and with P (J = ca. 10 Hz), thus confirming the correct structure of **10** as the 3′-*O*-hydrogenphosphonate.

Scheme 2[a]



[a] Key: (i) NIS, TfOH (cat.) (55%); (ii) tetrabutylammonium fluoride (84%); (iii) 4-monomethoxytrityl chloride (65%); (iv) *o*-chlorobenzoyl chloride, 1.1 equiv, −78 °C; (v) PCl_3, imidazole, NEt_3, −78 °C, triethylammonium bicarbonate (aqueous) (71%).

Table 1. Oligonucleotide Sequences and Affinity Toward Complementary RNA (t_m)

no.	oligonucleotides[a]	full ribo	modifd[b]
11	AAGCGAU$_f$UU$_f$UGACACU	56.7	57.2 (+0.3)
12	ACAU$_f$UCGU$_f$UGU$_f$UCGA	53.4	53.9 (+0.2)
13	(U$_f$U)$_6$U	13.8	18.8 (+0.8)

[a] **f** denotes position of the formacetal linkage. [b] Δt_m are given in parentheses.

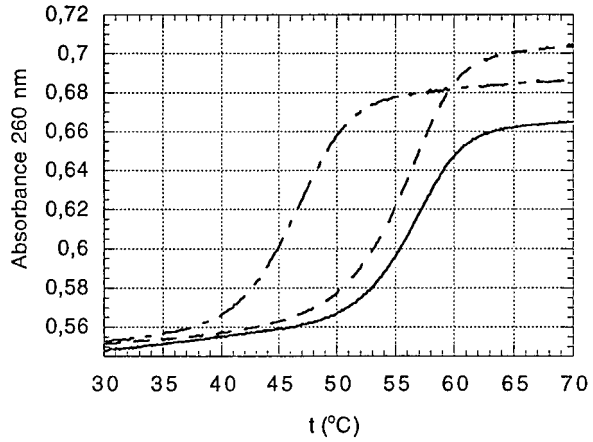


Figure 1. UV melting curves of duplexes formed by **11** (—) and full ribo (- - -) and full deoxy (—•—) versions of **11** with complementary RNA.

phase H-phosphonate method for RNA synthesis[22] and 2′-*O*-*o*-ClBz protection.[17] Dimer **10** was used with standard coupling conditions.[22b] Full ribo versions of sequences **11**−**13** and the complementary oligoribonucleotides were also synthesized. The stability of the corresponding duplexes were characterized by UV melting experiments (see Table 1 and Figure 1). The correct nucleoside residue composition of all oligonucleotides was confirmed by enzymatic degradation followed by RP HPLC analysis. The molecular weight of **11** was also confirmed by matrix-assisted laser desorption ionization time-of-flight MS.[23]

The effect of formacetal replacement in our models was an increase of duplex stability of +0.2 to +0.8 °C per modification (Table 1). This is in contrast to incorporation of formacetal linkages in oligodeoxynucleotides that is reported to decrease the stability of the DNA−RNA duplexes by −0.7 °C per modification (ref 9a, seven modifications in a 15mer). A decrease is also reported for DNA−DNA duplexes: Δt_m from −1.4 to −2.4 °C (ref 7a, one to three modifications in a 10mer).

Replacement of the phosphodiester moiety with a formacetal linkage should weaken the O4′−C4′−C3′−O3′ gauche effect,[24] which drives the ribose conformation toward 2′-endo. This in turn should stabilize the RNA−RNA duplex (A type, 3′-endo sugars), whereas DNA−DNA duplexes (B type, 2′-endo sugars) should be destabilized.[25] The effects in DNA−RNA duplexes where the DNA strand has modified internucleoside linkages and may adopt intermediate equilibria or other conformations are more difficult to predict. Destabilization of such hybrids is reported.[8,9a]

The reduction of the unfavorable O4′−C4′−C3′−O3′ gauche effect apparently compensated for changes in bond length when the phosphodiester linkages were replaced by formacetals (C−O compared to P−O). Thus, a slight stabilization was observed in our models. The CD spectra of RNA−RNA duplexes containing formacetal linkages were practically identical with those of unmodified ones and consistent with an A form helix (see the Supporting Information, Figure 2). This is in good agreement with NMR studies[26] and molecular dynamics simulations[27] showing that the perturbation of the DNA−DNA duplex caused by a single formacetal linkage is minimal.

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Although there are many different sides to developing antisense compounds, favorable duplex formation is likely to be of major importance. Because RNA−RNA duplexes are generally more stable than DNA−RNA hybrids, oligoribonucleotide analogs having chemically and enzymatically stable internucleoside linkages may be better second generation antisense compounds than their deoxy counterparts. For instance, the duplex formed by the full deoxy version of **11** with complementary RNA had t_m = 46.9 °C (Figure 1), i.e., 9.8 °C lower than the duplex formed by the full ribo oligomer. The replacement of selected phosphodiester linkages with formacetal groups further increased the affinity of the oligoribonucleotides towards their RNA complements. Thus, oligonucleotides containing ribonucleoside formacetal units are of potential use as second-generation antisense compounds. The protecting group manipulations reported herein should be also useful for synthesis of ribonucleoside dimers having other modified linkages and thus allow synthesis and more extensive studies of oligonucleotides carrying such linkages.

Experimental Section

Pyridine and acetonitrile were dried over 3 Å molecular sieves. Methylene chloride was dried over 4 Å molecular sieves. Triethylamine and DMSO were dried by refluxing with CaH_2 overnight followed by distillation. PCl_3 was distilled. Triethylammonium bicarbonate buffer (pH ca. 7.5) was prepared by passing CO_2 (g) through a mixture of triethylamine and water until saturation. Acetic acid (pa), acetic anhydride (pa), hexane (pa), 32% aqueous NH_3 (pa), and trifluoromethanesulfonic acid were purchased from Merck, tetrabutylammonium fluoride, *o*-chlorobenzoyl chloride, and 4-methoxytrityl chloride were purchased from Aldrich, and imidazole, *N*-iodosuccinimide were purchased from Fluka and used without further purification. NMR spectra were recorded on a JEOL GSX-270 spectrometer at 25 °C. Chemical shifts are given in ppm relative to tetramethylsilane (^{1}H), $CDCl_3$ (δ 77.17 ppm, ^{13}C) and 2% H_3PO_4 in D_2O (coaxial inner tube, ^{31}P). Signals were assigned by ^{1}H−^{1}H and ^{13}C−^{1}H COSY. Long-range ^{13}C−^{1}H correlation was done using JEOL pulse sequence and parameters for standard ^{13}C−^{1}H COSY experiment except that the delay time was 60 ms. FAB mass spectra were recorded on a JEOL SX-102 instrument with *m*-nitrobenzyl alcohol (*m*-NBA) or glycerol as a matrix. TLC was done on Merck silica gel 60 F_{254} precoated plates using solvents A ($CHCl_3$/methanol, 19:1, v/v), B (toluene/ethyl acetate, 1:1, v/v), C ($CHCl_3$/methanol, 4:1, v/v), D ($CHCl_3$/methanol, 9:1, v/v), E (2-propanol/water/25% aqueous NH_3, 85:5:10, v/v/v). Silica gel (35−70 μm) from Amicon Europe was used for column chromatography, and the columns were run in the flash mode; chloroform was passed through basic Al_2O_3 prior to use.

2′-*O*-*o*-(Chlorobenzoyl)-5′-*O*-(*tert*-butyldiphenylsilyl)uridine (4). 5′-*O*-(*tert*-Butyldiphenylsilyl)uridine (**3**)[18] (2.41 g, 5 mmol) was coevaporated with dry pyridine (2 × 50 mL) and dissolved in CH_2Cl_2/pyridine (19:1, 80 mL). The reaction mixture was cooled to −78 °C (acetone−dry ice), a solution of *o*-chlorobenzoyl chloride (0.70 mL, 5.5 mmol, 1.1 equiv) in CH_2Cl_2 (5 mL) was added during 15 min, and the mixture was stirred for 1 h at −78 °C. CH_3OH (0.5 mL) was added, and the mixture was extracted with saturated $NaHCO_3$ (aqueous) (100 mL). The organic layer was separated, dried over Na_2SO_4, evaporated to ca. 20 mL and precipitated in hexane (500 mL). The precipitate was filtered and dried in vacuo, yield 2.42 g, 85%, R_f = 0.46 (solvent A). Trace amounts of the possible 3′-*O*-(*o*-chlorobenzoyl) isomer could be detected R_f = 0.39 (solvent A). To avoid migration of the *o*-chlorobenzoyl group this crude material was used without further purification. The trace impurities were removed during subsequent synthetic steps. ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 8.92 (s, 1H, NH), 7.92 (d, J = 7.7 Hz, 1H, *o*-ClBz), 7.82 (d, J = 8.1 Hz, 1H, H6), 7.68 (m, 4H, Ar), 7.44 (m, 9H, Ar), 6.32 (d, J = 4.4 Hz, 1H, H1′), 5.54 (m, 1H, H2′), 5.43 (d, 1H, H5), 4.67 (m, 1H, H3′), 4.17 (m, 1H, H4′), 4.12 and 3.90 (ABX system, $J_{H5'-H5''}$ = 12.0 Hz, $J_{H5'-H4'}$ = 1.2 Hz, 2H, H5′), 1.12 (s, 9H, CH_3). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ: 164.80, 163.08 (C4, C=O in *o*-ClBz), 150.26 (C2), 139.85 (C6), 135.77, 135.53, 132.80, 132.18, 130.38, 130.30, 128.19 (TBDPS), 134.05, 133.56, 132.43, 131.37, 128.65, 127.06 (*o*-ClBz), 102.88 (C5), 86.67 (C1′), 84.75 (C4′), 77.21 (C2′), 69.72 (C3′), 63.14 (C5′), 27.15 (CH_3), 19.49 (quaternary C in *t*-Bu).

2′-*O*-(*o*-chlorobenzoyl)-5′-*O*-(*tert*-butyldiphenylsilyl)-3′-*O*-[(methylthio)methyl]uridine (5). 2′-*O*-(*o*-Chlorobenzoyl)-5′-*O*-(*tert*-butyldiphenylsilyl)uridine (**4**) (2.30 g 3.7 mmol) was dissolved in a freshly prepared mixture of DMSO (12.5 mL), acetic anhydride (7.5 mL), and acetic acid (2.5 mL). The mixture was stirred at room temperature for 24 h (TLC, solvent B) and evaporated. The residue was dissolved in toluene (100 mL) and extracted with saturated $NaHCO_3$ (aqueous) (3 × 100 mL) and saturated NaCl (aqueous) (100 mL). The organic layer was separated, dried over Na_2SO_4, evaporated, and purified by silica gel column chromatography (0−40% of ethylacetate in toluene). The fractions containing product were pooled, evaporated and crystallized from hexane−ether to give **5** as a white powder. Yield: 0.68 g, 27% (unidentified trace contamination was detected by ^{1}H NMR). R_f = 0.62 (solvent B). ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 9.06 (s, 1H, NH), 7.91 (d, J = 7.0 Hz, 1H, *o*-ClBz), 7.80 (d, J = 8.0 Hz, 1H, H6), 7.74−7.65 and 7.46−7.30 (m, 13H, Ar), 6.33 (d, J = 4.0 Hz, 1H, H1′), 5.49 (t, 1H, H2′), 5.35 (dd, J_{H5-NH} = 2.0 Hz, 1H, H5), 4.72 (m, 1H, H3′), 4.68 and 4.60 (AB system, J = 11.7 Hz, 2H, OCH_2S), 4.20 (m, 1H, H4′), 3.94 and 3.70 (ABX system, $J_{H5'-H5''}$ = 11.7 Hz, $J_{H5'-H4'}$ = 1.9 Hz, 2H, H5′), 2.02 (s, 3H, SCH_3), 1.14 (s, 9H, CH_3). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ: 164.30, 163.11 (C4, C=O in *o*-ClBz), 150.25 (C2), 139.65 (C6), 135.84, 135.55, 132.93, 132.14, 130.39, 130.28, 128.22, 128.17 (TBDPS), 134.36, 133.36, 132.14, 131.37, 128.82, 126.93 (*o*-ClBz), 103.01 (C5), 87.12 (C1′), 83.42 (C4′), 75.93 (OCH_2S), 75.50 (C2′), 73.04 (C3′), 62.60 (C5′), 27.21 (CH_3), 19.49 (quaternary C in *t*-Bu), 14.23 (SCH_3). Positive FAB MS (mNBA matrix) m/z: 703 (M + Na), 681 (M + H), 633 (M − SCH_3), 623 (M − *t*-Bu), 569 (M − Ura).

Synthesis of Dimer 7. 2′,3′-*O*-Bis(*tert*-butyldimethylsilyl)-uridine (**6**)[14] (0.38 g, 0.81 mmol) was coupled with 2′-*O*-(*o*-chlorobenzoyl)-5′-*O*-(*tert*-butyldiphenylsilyl)-3′-*O*-[(methylthio)-methyl]uridine (**5**) (0.61 g, 0.9 mmol, 1.2 equiv) using the NIS/TfOH activation procedure previously reported.[6a,7a] The product was purified by silica gel column chromatography (0−5% of CH_3OH in $CHCl_3$). Yield: 0.49 g, 55% (based on **6**, no contamination could be detected by TLC and ^{1}H NMR). R_f = 0.50 (solvent B). ^{1}H NMR ($CDCl_3$, 270 MHz)[28] δ: 9.58 and 9.48 (2s, 2H, NH), 7.91 (d, J = 7.3 Hz, 1H, *o*-ClBz), 7.71−7.35 (m, 17H, Ar, H6*, H6), 6.27 (d, J = 5.1 Hz, 1H, H1′), 5.66−5.43 (m, 4H, H1′*, H2′, H5, H5*), 4.82 and 4.70 (AB system, J = 6.6 Hz, 2H, OCH_2O), 4.57 (m, 1H, H3′), 4.26−3.58 (m, 8H, H4′, H2′*, H3′*, H4′*, H5′, H5′*), 1.13 (s, 9H, CH_3 in TBDPS), 0.88 and 0.87 (2s, 18H, *t*-Bu in TBDMS), 0.09−0.04 (4s, 12H, $SiCH_3$). ^{13}C NMR ($CDCl_3$, 67.9 MHz)[28] δ: 164.46, 163.62, 163.32 (C4, C4*, C=O in *o*-ClBz), 150.41, 150.32 (C2, C2*), 140.30, 139.95 (C6, C6*), 135.78, 135.51, 132.70, 132.16, 130.41, 130.33, 128.19 (TBDPS), 134.27, 133.49, 132.08, 131.43, 128.68, 127.00 (*o*-ClBz), 103.11, 102.11 (C5, C5*), 96.60 (OCH_2O), 90.49 (C1′*), 87.25 (C1′), 83.69, 82.33, 75.71, 75.36, 75.17, 70.98 (C2′, C2′*, C3′, C3′*, C4′, C4′*), 67.33, 63.36 (C5′, C5′*), 27.17, 25.90 (CH_3), 19.44, 18.12 (quaternary C in *t*-Bu), −4.23, −4.42, −4.72, −4.83 ($SiCH_3$). Positive FAB MS (mNBA matrix) m/z: 1127 (M + Na), 1105 (M + H), 1047 (M − *t*-Bu), 993 (M − Ura), 633 (M − Urd,2 × TBDMS), 383 (Urd, *o*-ClBz).

Synthesis of Dimer 8. Dimer **7** (1.33 g, 1.2 mmol) was dissolved in dry acetonitrile (25 mL), and tetrabutylammonium fluoride (3.12 g, 12 mmol, dried by coevaporation with acetonitrile, 2 × 100 mL) was added. The mixture was stirred at room temperature for 3 h (TLC, solvents A and C), evaporated to ca. 10 mL, and extracted with $CHCl_3$ (50 mL) and saturated $NaHCO_3$ (aqueous) (50 mL). The aqueous layer was extracted with $CHCl_3$ (50 mL), organic layers were separated, dried over

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Na_2SO_4, and evaporated, and the residue was purified by silica gel column chromatography (0−16% of CH_3OH in $CHCl_3$). Yield: 0.65 g, 84%. R_f = 0.46 (solvent C). Positive FAB MS (glycerol matrix) *m/z*: 639 (M + H), 527 (M − Ura), 395 (M − Urd).

Synthesis of Dimer 9. Dimer **8** (0.64 g, 1 mmol) was reacted with 4-monomethoxytrityl chloride (0.34 g, 1.1 mmol) according to the standard procedure,[29] and the product was purified using silica gel column chromatography (0−10% of CH_3OH in $CHCl_3$). Yield: 0.59 g, 65%. R_f = 0.37 (solvent D). Positive FAB MS (mNBA matrix) *m/z*: 933 (M + Na), 910 (M), 666 (M − Urd).

Synthesis of Dimer 10. Dimer **9** (0.27 g, 0.3 mmol) was coevaporated with dry pyridine (2 × 20 mL) and dissolved in dry CH_2Cl_2/pyridine (19:1, 20 mL). The reaction mixture was cooled to −78 °C (acetone−dry ice), and a solution of *o*-chlorobenzoyl chloride (43 μL, 0.33 mmol) in CH_2Cl_2 (2 mL) was added during 15 min. The mixture was stirred at −78 °C for 30 min (TLC, solvent D) and then added dropwise to a stirred and cooled (−78 °C) mixture of imidazole (0.24 g, 3.5 mmol), PCl_3 (0.1 mL, 1.1 mmol), and triethylamine (0.5 mL, 3.6 mmol) in dry CH_2Cl_2 (30 mL). The reaction mixture was stirred at −78 °C for 30 min and then poured onto and extracted with 1.0 M TEAB (aqueous) pH 7.5 (60 mL). The organic layer was separated, dried over Na_2SO_4, and evaporated, and the residue was purified by silica gel column chromatography (0−15% of CH_3OH in $CHCl_3$ containing 0.1% of triethylamine). Yield: 0.26 g, 71%. R_f = 0.27 (solvent C), 0.65 (solvent E). ^{1}H NMR ($CDCl_3$, 270 MHz)[28] δ: 7.94, 7.88 (2m, 2H, *o*-ClBz), 7.70, 7.47−7.22 (m, 20H, H6, H6*, Ar), 6.88 (d, *J* = 630 Hz, 1H, PH), 6.85 (m, 2H, MMT), 6.20 (d, *J* = 4.4 Hz, 1H, H1′), 6.14 (d, *J* = 5.1 Hz, 1H, H1′*), 5.68 (bt, 1H, H2′), 5.60, 5.36 (2d, *J* = 8.0 Hz, 2H, H5, H5*), 5.43 (bt, 1H, H2′*), 4.99 (p, 1H, H3′*), 4.75 (m, 2H, OCH_2O), 4.67 (bt, 1H, H3′), 4.31 (m, 2H, H4′, H4′*), 3.84−3.50 (2m, 4H, H5′, H5′*), 3.77 (s, 3H, OCH_3), 2.77 (q, *J* = 7.3 Hz, 6H, NCH_2), 1.11 (t, 9H, CH_3). ^{13}C NMR ($CDCl_3$, 67.9 MHz)[28] δ: 164.40, 164.08, 163.54, 163.30 (C4, C4*, C═O in *o*-ClBz), 150.56 (C2, C2*), 158.92, 143.79, 143.57, 130.62, 128.62, 128.19, 127.46 (MMT), 140.28, 140.04 (C6, C6*), 134.67, 134.13 (MMT, *o*-ClBz), 133.40, 133.10, 132.45, 132.08,, 131.35, 131.05, 129.00, 128.76, 127.00, 126.87 (*o*-ClBz), 113.46 (MMT), 103.21, 102.96 (C5, C5*), 96.41 (OCH_2O), 87.64, 87.37, 87.07 (C1′, C1′*, MMT), 82.97, 82.57 (C4′, C4′*), 75.55, 75.28, 75.04 (C2′, C2′*, C3′), 70.78 (C3′*), 67.89, 62.71 (C5′, C5′*), 55.37 (OCH_3), 45.80 (NCH_2), 9.69 (CH_3). ^{31}P NMR (CH_3CN:pyridine, 3:1, 109.4 MHz) δ: 1.75. Negative FAB MS (mNBA matrix) *m/z*: 1111 (M − H).

Synthesis and Purification of Oligonucleotides. Solid-phase synthesis[22] and N-protecting[30] and 2′-O-*o*-ClBz protecting groups[17] have been previously reported. Synthesized oligonucleotides were cleaved from the support and deprotected using 32% NH_3/EtOH 3:1 for 8 h at rt. The ammonia solutions were lyophilized, and the residue was dissolved in 30% CH_3CN, passed through a disposable C-18 cartridge (Waters Sep Pac), filtered through a disposable syringe filter (Millex GV13, 0.22 μm), and purified on a Dionex NucleoPac PA-100 (4 × 250 mm) column using a linear gradient of 0−90 mM $LiClO_4$ in 20 mM sodium acetate (pH 6.5), 10% CH_3CN for 45 min. The collected fractions were lyophilized and further purified on a Supelcosil LC-18 (3 μm, 4.6 × 150 mm) column using a linear gradient of 0−25% CH_3CN in 50 mM triethylammonium acetate (pH 6.5) for 40 min. The oligonucleotides were collected, lyophilized, dissolved in water, and lyophilized again.

Enzymatic Cleavage. Oligonucleotides (0.5 A_{260} units) were dissolved in buffer (100 mM Tris−HCl, 12 mM $MgCl_2$, 100 mM NaCl, pH 8.6, 0.5 mL), snake venom phosphodiesterase (25 μL, 0.1 units/ml, Sigma) and calf intestine phosphatase (10 μL, 50 units/mL, Boehring Mannheim) were added, and reactions were incubated at 37 °C for 5 h. Aliquots (100 μL) were withdrawn, filtered (ultrafree-MC 10000 NMWL filter unit, Millipore), and analyzed on a Supelcosil LC-18 (3 μm, 4.6 × 150 mm) column using a linear gradient of 0−5% CH_3CN in 50 mM triethylammonium acetate (pH 6.5) for 40 min. Deprotected (32% NH_3/EtOH 3:1 for 8 h at rt) dimer **8** was used as standard.

Hybridization Thermodynamics. Absorbance vs temperature profiles were measured at 260 nm on a Varian Cary 3 spectrophotometer in a buffer containing 100 mM NaCl, 10 mM sodium phosphate (pH 7.2), 0.1 mM EDTA, and 2 μM oligonucleotides. Extinction coefficients were calculated from nearest neighbor approximation.[31] The temperature was increased at a rate of 0.2 °C. The melting temperatures were obtained by fitting the melting profile to a two-state transition model, with linear sloping lower and upper base lines and also using the Varian Cary software (Version 2.5). Reported values are the average of at least three experiments. CD spectra were recorded on a JASCO Model 700 spectropolarimeter at 20 or 4 °C (for **13** duplexes) using the same samples.

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Supporting Information Available: Spectral data of **8** and **9** and CD spectra of oligonucleotide duplexes formed by **11**−**13** with their complementary RNA sequences (2 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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