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2'-*O*-(Carbamoylmethyl)oligoribonucleotides

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Abstract: Fully modified oligonucleotides containing 2'-*O*-cyanomethylribonucleotides were synthesised on automated DNA synthesisers using protected 2'-*O*-cyanomethylribonucleoside-3'-*O*-phosphoramidite building blocks. The 2'-*O*-cyanomethyloligoribonucleotides were post-synthetically converted into 2'-*O*-carbamoylmethyloligoribonucleotides during standard deprotection conditions with aqueous ammonia. The complete conversion was proven using ion- exchange HPLC and MALDI mass spectrometry of the final oligomer. The 2'-*O*-carbamoylmethylribonucleotides showed an substantial increase the melting temperature (Tm) of duplexes with complementary RNA relative to the corresponding RNA homoduplex. Consequently these analogues should prove useful for a variety of antisense applications.

INTRODUCTION

2'-*O*-Methyl ethers[2-8] of ribonucleosides are natural modifications that are found *in vivo* as a minor component of many types of RNA. 2'-*O*-Methyloligoribonucleotides exhibit high resistance to both DNA and RNA specific nucleases and form hybrids of high thermal stability with complementary RNA.[9] These analogues, as well as the recently described 2'-*O*-allyloligoribonucleotides[10], have proven to be valuable compounds for antisense experiments and associated applications in biochemistry and molecular biology.[8] Following the encouraging results obtained with antisense probes made of 2'-*O*-methylated RNA, the use of other, non-naturally occurring, 2'-*O*-alkyl ethers of ribonucleotides has also been explored.[11] In general, other 2'-*O*-alkyl substitutions also stabilised the duplex, with smaller substituents resulting in greater duplex stability than larger ones.[11] The improved hybridisation of 2'-*O*-alkyl ethers of oligoribonucleotides to complementary RNA has been attributed to the tendency of these electronegative substituents to shift the conformational equilibrium in the sugar moiety towards the C3'-endo conformation consistent with the A-form geometry of RNA duplexes.[12-15] In the case of the 2'-*O*-(2-methoxyethyl) modification[16,17] further stabilisation is obtained due to the gauche effect between the two oxygens, that results in a conformation of the side chain consistent with duplex formation.[16,18,19] Destabilisation by larger 2'-*O*-alkyl groups can be explained by steric interference of the larger alkyl chains with other parts of the duplex, or disruption of water structure in the minor groove.[12]

A new interesting compound in this series is the 2'-*O*-carbamoylmethyl analogue. The reason for selecting the carbamoylmethyl group for the modification of the 2'-hydroxyl moiety is based on its small size, its electronegative character and the double bound character of the amide function. All of these together could

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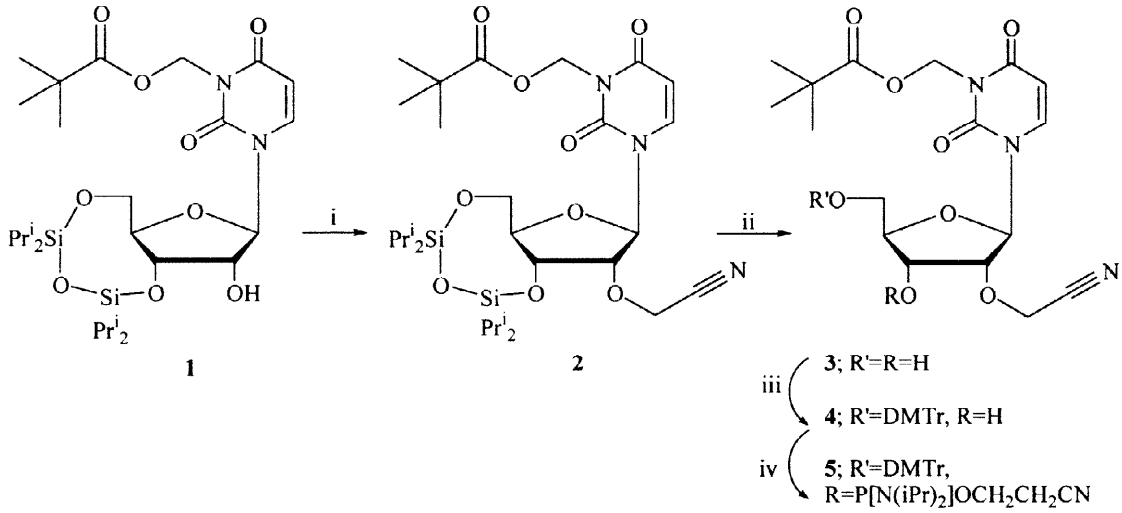
further enhance some of the desired properties observed with 2'-*O*-allylribonucleotides.[10] We thought that 2'-*O*-cyanomethyloligoribonucleotides could be a suitable starting material for post-synthetic conversion to 2'-*O*-carbamoylmethyloligoribonucleotides. This report describes the synthesis of 2'-*O*-cyanomethylribonucleoside-3'-*O*-phosphoramidites, the assembly of these monomers into 2'-*O*-cyanomethyloligoribonucleotides and post synthetic conversion of these oligonucleotides into 2'-*O*-carbamoylmethyloligoribonucleotides and their hybridisation properties.

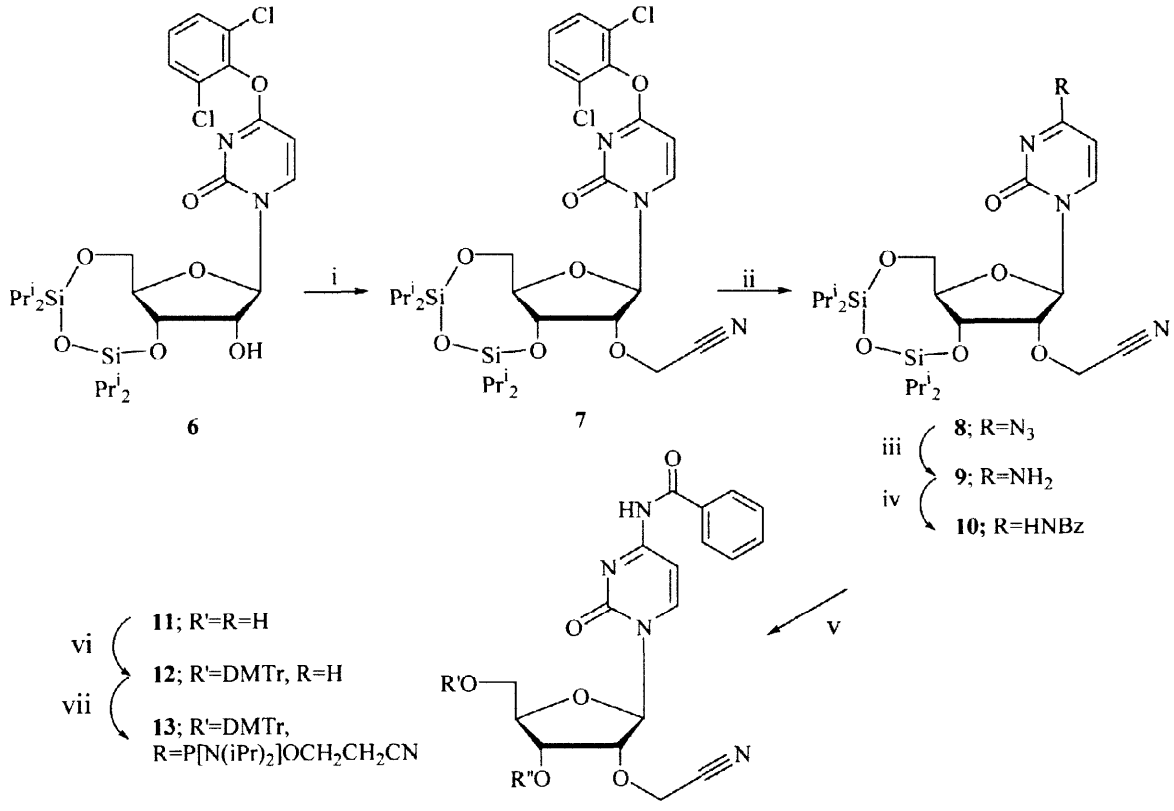
RESULTS AND DISCUSSION

The route to the 2'-*O*-cyanomethyluridine building block, compound **5**, is illustrated in **Scheme 1**. Compound **1** was synthesised according to Grøtli *et al.*[20] Alkylation of compound **1** with 2-*tert.*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorin (BEMP)[21] and bromoacetonitrile in anhydrous acetonitrile gave compound **2** in good yield. The ammonia labile Pom group not only protects the N-3-position from alkylation, but also increases the lipophilicity of the various intermediates and prevents undesirable side reactions during the assembly of the oligonucleotide. Subsequent desilylation followed by dimethoxytritylation and phosphitylation afforded the desired building block **5**. The overall yield of the 2'-*O*-cyanomethyluridine building block was 44 % for the 7 step synthesis starting from uridine.



Scheme 1. Preparation of the 2'-*O*-cyanomethyluridine monomer. Reagents: i, bromoacetonitrile and 2-*tert.*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorin in acetonitrile; ii, tetrabutylammonium fluoride in tetrahydrofuran; iii, 4,4'-dimethoxytrityl chloride and triethylamine in pyridine; iv, 2-cyanoethoxy *N*,*N*-diisopropylaminochlorophosphine and *N*,*N*-diisopropylethylamine in dichloroethane.

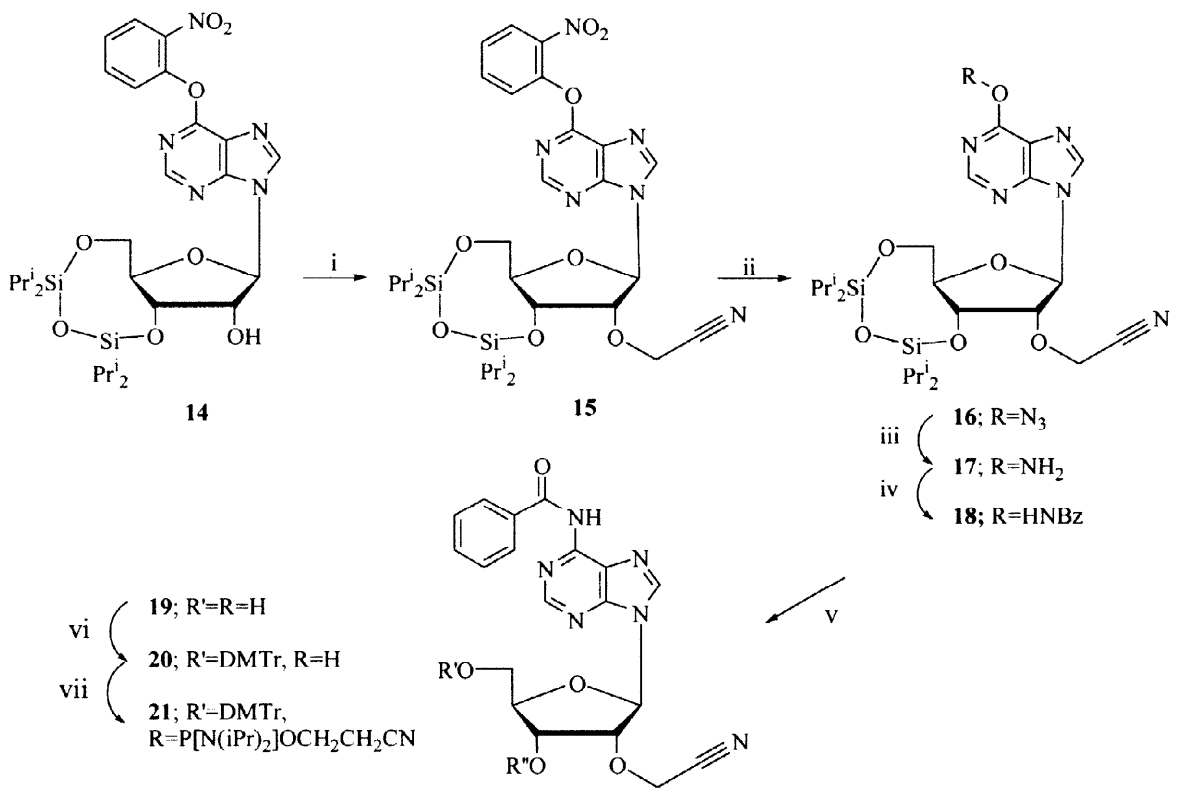
Our route to the 2'-*O*-cyanomethylcytidine-3'-*O*-phosphoramidite building block **13** is illustrated in **Scheme 2**. This compound was prepared in 29 % overall yield from uridine *via* 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-4-*O*-(2,6-dichlorophenyl)uridine (**6**). The latter compound was synthesised analogously to the 4-*O*-(2-nitrophenyl) derivative as described by Sproat.[21] The intermediate 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-cyanomethyl-4-*O*-(2,6-dichlorophenyl)uridine, compound **7**, was treated with 2.5 equivalents of sodium azide in DMF at 60 °C for 7h to give compound **8**. This material was smoothly reduced in excellent yield using anhydrous stannous chloride, thiophenol and triethylamine in acetonitrile and was then treated with benzoyl chloride in pyridine to give 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-N-4-benzoyl-2′-*O*-cyanomethylcytidine, compound **9**.



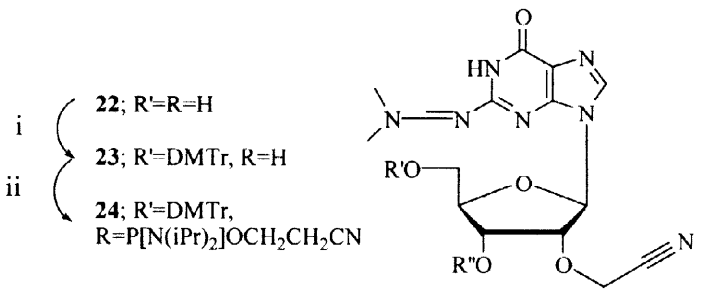
Scheme 2. The preparation of the 2'-*O*-cyanomethylcytidine monomer. Reagents: i, bromoacetonitrile and 2-*tert*.-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorin in acetonitrile; ii, sodium azide in dimethylformamide; iii, stannous chloride, thiophenol and triethylamine in acetonitrile; iv, benzoyl chloride in pyridine; v, tetrabutylammonium fluoride in tetrahydrofuran; vi, 4,4'-dimethoxytrityl chloride and triethylamine in pyridine; vii, 2-cyanoethoxy *N,N*-diisopropylaminochlorophosphine and *N,N*-diisopropylethylamine in dichloroethane.

Subsequent desilylation with tetrabutylammonium fluoride removed the disiloxane bridge to give compound **11**. Dimethoxytritylation and phosphitylation gave the desired 2'-*O*-cyanomethylcytidine monomer, compound **13**.

The 2'-*O*-cyanomethyladenosine monomer (**21**) was synthesised *via* alkylation of the previously described intermediate, 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-6-(2-nitrophenoxy)purine riboside, compound **14**[21] (**Scheme 3**). The 2'-*O*-cyanomethyl monomer was then obtained in analogous fashion to the 2'-*O*-methyl ones[7] except that the displacement of dichlorophenoxide was done with sodium azide in DMF followed by reduction with $[Sn(SPh)_3]^-$ and the base was protected with a benzoyl group. The overall yield of the 2'-*O*-cyanomethyladenosine monomer, compound **21**, was 21 % starting from 6-chloropurine riboside.



Scheme 3. The preparation of the 2'-*O*-cyanomethyladenosine monomer. Reagents: i, bromoacetonitrile and 2-*tert.*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorin in acetonitrile; ii, sodium azide in dimethylformamide; iii, stannous chloride, thiophenol and triethylamine in acetonitrile; iv, benzoyl chloride in pyridine; v, tetrabutylammonium fluoride in tetrahydrofuran; vi, 4,4'-dimethoxytrityl chloride and triethylamine in pyridine; vii, 2-cyanoethoxy *N*,*N*-diisopropylaminochlorophosphine and *N*,*N*-diisopropylethylamine in dichloroethane.



Scheme 4. The preparation of the 2'-*O*-cyanomethylguanosine monomer. i, 4,4'-dimethoxytrityl chloride and triethylamine in pyridine; ii, 2-cyanoethoxy *N*,*N*-diisopropylaminochlorophosphine and *N*,*N*-diisopropylethylamine in dichloroethane.

N-2-Dimethylaminomethylidene-2′-*O*-cyanomethylguanosine, compound **22** was made as previously described.[22] Subsequent dimethoxytritylation and phosphitylation afforded the desired building block **24**.

Table 1. Conversion of the cyanomethyl group during deprotection of the oligonucleotides.

Oligonucleotide	Deprotection conditions	mw; calculated	mw; found	Product
dT-U2′*O*CM (**25**)	30 % aqueous ammonia	586.5	604.9	**26**
dT-U2′*O*CM (**25**)	2M sodium hydroxide	586.5	604.8	**27**
dT-U2′*O*CM (**25**)	2M ammonia in methanol	586.5	587.0	**28**
dTdTdT U2′*O*CM (**29**)	30 % aqueous ammonia	1212.0	1212.9	**32**
dTdTdT U2′*O*CM (**29**)	2M sodium hydroxide	1213.0	1213.2	**33**
dTdT U2′*O*CM U2′*O*CM (**30**)	30 % aqueous ammonia	1270.0	1270.8	**34**
dTdT U2′*O*CM U2′*O*CM (**30**)	2M sodium hydroxide	1271.0	1271.7	**35**
dT U2′*O*CM U2′*O*CM U2′*O*CM (**31**)	30 % aqueous ammonia	1324.0	1324.9	**36**
dT U2′*O*CM U2′*O*CM U2′*O*CM (**31**)	2M sodium hydroxide	1325.1	1325.6	**37**

U2′*O*CM = 2′-O-cyanomethyluridine

The synthesis of oligonucleotides on solid support was performed using the phosphoramidite chemistry on a commercial DNA synthesiser. In order to examine the stability of the 2'-*O*-cyanomethyl group during the synthesis on solid phase a short oligonucleotide (**25**) was synthesised using a dT solid support and the 2'-*O*-cyanomethyluridine building block, compound **5**. The synthesised dimer was then exposed to different deprotection conditions to convert the cyanomethyl group to a carbamoylmethyl group and the dimer was analysed by means of mass spectrometry.In general, nitriles can be hydrolysed to give either amides or carboxylic acids. The amide is initially formed, but since amides are also hydrolysed by acide or basic treatment, the carboxylic acid is the more common product. When the acid is desired, the reagent of choice is aqueous sodium hydroxide.[23] Thus, treatment of the support bound dimer dTU_{CM} with 2 M sodium hydroxide for 12h at room temperature resulted in complete hydrolysis of the cyanogroup with simultaneously cleavage of the protecting groups (**Table 1**). However, there are a number of procedures for stopping at the amide stage, among them the use of concentrated H_2SO_4, formic acid and HCl or HBr and dry HCl followed by H_2O to mention a few.[24]

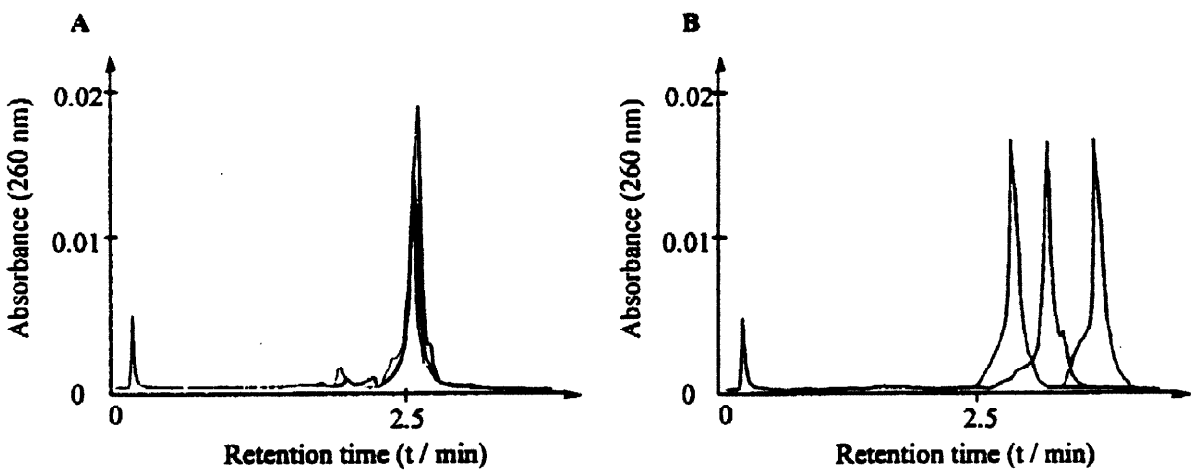


Figure 1. Chromatograms of compounds **33**, **34** and **35** treated with aqueous ammonia (panel **A**) or with 2 M sodium hydroxide (panel **B**); analysed on a POROS 20HQ (4.6x100 mm) column; starting buffer (A): 0.0 M NaCl and 10 mM NaOH (pH 10); eluent (B) : 1.5 M NaCl and 10 mM NaOH (pH 10); gradient 0-70 % buffer B in 4 min, flow rate 15 ml min^{-1}.

The same result can also be obtained by using water and certain metal ions or complexes.[24] To investigate if this also would be the case with aqueous ammonia the support bound dimer dTU_{CM} was treated with 32% aqueous ammonia for 12h at 60 °C, standard deprotection conditions for oligonucleotides. As expected the

cyanomethyl function was not stable under these conditions (**Table 1**). However, the difference in mass between the carboxymethyl and the carbamoylmethyl functionality is only 1.01 and could not be distinguished in the current experiment. Treatment of the same dimer with methanolic ammonia left the 2'-*O*-cyanomethyl functionality intact (**Table 1**).Anion-exchange chromatography is the predominant method used for nucleic acid purification, due to the high negative charge density from the backbone phosphate. We reckoned that this would be a way to analyse potential mixtures of carboxymethyl and the carbamoylmethyl carrying oligoes since each 2'-*O*-carboxymethyl residue will give an additional negative charge at high pH. Thus, dTdTdTU^{CM} (**29**), dTdTU^{CM}U^{CM} (**30**) and dTU^{CM}U^{CM}U^{CM} (**31**) were synthesised, deprotected with aqueous ammonia under standard conditions and the crude material analysed by ion-exchange at pH 10 (**Fig 1A**). All three oligonucleotides gave similar retention time. As a control, the same sequences as above were deprotected with 2 M sodium hydroxide for 12h at room temperature. Ion-exchange chromatography at pH 10 gave different retention times for the three oligonucleotides (**Fig 1B**). The collected product fractions were analysed by matrix assisted laser desorption mass spectrometry (**Table 1**). Therefore we could conclude that the cyano group is hydrolysed to the amide function when treated with aqueous ammonia.

The new 2'-*O*-cyanomethyl building blocks were further used in solid-phase phosphoramidite chemistry to prepare a dodecamer oligonucleotide and its complementary sequence (**Table 2**). The coupling efficiency of the 2'-*O*-cyanomethyl-3'-*O*-phosphoramidite building blocks were routinely > 97 % using 1*H*-tetrazole as activator, as monitored by release of the dimethoxytrityl cation after each coupling step. This coupling efficiency is similar to that usually observed in the condensation of 2'-*O*-allylribonucleoside-3'-*O*-phosphoramidites.

Table 2. 2'-*O*-Carbamoyloligoribonucleotides for Tm studies.

Oligo-nucleotide	Deprotection conditions	Yield (crude) A_{260}	Yield (pure) A_{260}	Mass calc.	Mass Found	Compound
36	30 % aq. NH_3	58	42	4480.04	4481.1	**37**
38	30 % aq. NH_3	55	36	4480.04	4481.2	**39**

36 & **37** 5'-GAG GCU UAU CCA-3'; **38** & **39**: 5'-UGG AUA AGC CUC-3'.

Upon completion of assembly the controlled-pore glass carrier was treated with conc. ammonia for 24 h at 60 °C to cleave the oligomer from the carrier and remove the β-cyanoethyl, acyl and amidine protecting groups, plus hydrolyse the cyano group. The prolonged reaction with ammonia was necessary to hydrolyse all the cyano groups, and this is not detrimental to the oligonucleotide. The crude 5'-*O*-dimethoxytrityl protected oligomer was then analysed and purified by reversed phase HPLC on a Nucleosil 120, C18 steel column.
The product peak was then lyophilised and the residue treated with 80 % acetic acid to remove the remaining protecting groups. The composition of the oligonucleotides was verified by matrix assisted laser desorption mass spectrometry (**Table 2**).

The hybridisation properties of the modified ribonucleotides towards their complementary strands were checked by UV melting point (Tm) measurements. The results are given in **Table 3**.

Table 3. Effect of 2'-*O*-carbamoylmethyl substitution on Tm in homo- and heteroduplexes

Duplex	Tm (°C)
37 + 39	**74.01**
37 + 41	**66.06**
37 + 43	**46.08**
39 + 40	**65.63**
39 + 41	**45.69**
40 + 41	**56.90**
42 + 43	**44.80**

Tm= melting temperature in °C determined under the following conditions: 3 μM of each strand in tris(hydroxy methyl)amino methane·HCl (10 mM, pH 7) containing KCl (0.1 M) and EDTA (1mM). 5'-GAG-GCX-XAX-CCA-3'; 2'-*O*-carbamoylmethylRNA, **37**: X=$U_{2'Ocarbamoylmetyl}$; RNA, **40**: X=U; DNA, **42**:X=T. 5'-XGG-AXA-AGC-CXC-3'; 2'-*O*-carbamoylmethylRNA, **39**: X=$U_{2'Ocarbamoylmetyl}$; RNA, **41**: X=U; DNA, **43**:X=T.

The data show that 2'-*O*-carbamoylmethyl modifications increases the ability to form stable hybrids with RNA. The 2'-*O*-carbamoylmethylRNA:RNA heteroduplex is more than 7°C more stable than the 2'-*O*-allyl RNA:RNA heteroduplex of identical sequence.[25] Furthermore, the 2'-*O*-carbamoylmethyl:RNA heteroduplex is more than 20°C more stable than the 2'-*O*-carbamoylmethylRNA:DNA heteroduplex, showing excellent discrimination between RNA and DNA targets. This is comparable with the data for 2'-*O*-propargyloligoribonucleotides.[26] It is also important to point out that the 2'-*O*-carbamoylmethylRNA:DNA heteroduplex has a similar stability to the DNA:DNA heteroduplex of identical sequence. Finally, the 2'-*O*-

carbamoylmethylRNA homoduplex is more than 8°C more stable than the 2'-*O*-carbamoylmethylRNA:RNA heteroduplex and more than 17°C more stable than the corresponding RNA homoduplex.

CONCLUSION

The synthesis of 2'-*O*-carbamoylmethyl oligoribonucleotides has been described. The 2'-*O*-cyanomethyl building blocks could be incorporated into oligonucleotides and converted into 2'-*O*-carbamoylmethyl functionalities by treatment with aqueous ammonia; standard deprotection conditions for oligonucleotides. Moreover, the 2'-*O*-carbamoylmethyloligoribonucleotides form stable duplexes with complementary RNA. They show good discrimination between RNA and DNA targets and forms remarkably stable homoduplexes.

The use of the 2'-*O*-cyanomethyl building blocks to generate deprotected 2'-*O*-cyanomethyloligoribonucleotides and 2'-*O*-carbamoylmethyloligoribonucleotides is currently in progress. In addition it is expected that the 2'-*O*-cyanomethyl building blocks will find applications in the preparation of oligonucleotide-peptide conjugates.

EXPERIMENTAL

^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker AM 250 spectrometer using tetramethylsilane and trimethyl phosphate as references. Chemical shifts are reported in parts per million (ppm) downfield relative to the internal standard. Mass spectra were recorded using either electrospray ionisation (ESI) or Matrix Assisted Laser Desorption Ionisation (MALDI). The silica gel (35-70 mm) used for column chromatography was purchased from SDS. TLC was carried out on Merck DC Kieselgel 60 F_{254} aluminium sheets. All reagents used were of the highest available purity. Anhydrous solvents were purchased from SDS. Compounds **1**[20], **6**[21], **14**[21] and **22**[22] were synthesised according to literature procedures.

Synthesis of monomers

3-N-Pivaloyloxymethyl-2'-O-cyanomethyluridine (3)

Compound **1** (4.25 g, 7.07 mmol) was dried by evaporation of dry acetonitrile (10 ml) and dissolved in anhydrous acetonitrile (30 ml) under argon. BEMP (2.15 ml, 7.43 mmol) followed immediately by bromoacetonitrile (1.0 ml, 14.36 mmol) were added with stirring and exclusion of moisture. TLC showed complete reaction after 3 h. The reaction was quenched with methanol (2 ml) and solvent evaporated *in vacuo*. The residue was dissolved in dichloromethane (100 ml), washed with phosphate buffer (100 ml), the organic layer separated, dried (Na_2SO_4), filtered and evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (125 g) eluting with hexane/ethyl acetate (4:1 and 3:1v/v). Pure product **2** (2.94 g, 65.0 %) was obtained as a white foam. This material (2.55 g, 3.99 mmol) was dissolved in dry

tetrahydrofuran (30 ml) and a 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran (4 ml) was added with stirring. TLC (ethanol/dichloromethane 1:9 by vol.) showed complete reaction after 5 min. The reaction mixture was quenched with pyridine / methanol / water (20 ml, 3:1:1 by volume), and the solution was poured into stirred pyridinium form Dowex 50 W x4-200 resin (15 g) suspended in pyridine / methanol / water (20 ml, 3:1:1 by volume). The mixture was stirred for 30 min, the resin filtered off and washed with the above solution (3x 20ml). Combined filtrate and washings were evaporated to dryness *in vacuo*, and residual pyridine was removed by addition and evaporation of toluene. The crude product was purified on silica gel (35 g) eluting with a gradient of ethanol from 0 to 6 % in dichloromethane. The product was obtained as a solid white foam (1.51 g, 95.5 %) of R_f 0.16 on TLC in ethanol-dichloromethane (5:95 v/v). ^{1}H NMR δ($CDCl_3$): 8.33 (1H, d, *J*7.8 Hz, H-6), 7.49 (1H, d, *J*7.8 Hz, H-5), 5.81 (1H, s, *J*5.7 Hz, H-1'), 4.59-4.31 (5H, m, H-2', H-3', H-4', H-5'), 4.27 (2H, AB, *J*15.5 Hz, CH_2CN), 3.86 (2H, s, Pom CH_2) and 1.09p.p.m. (9H, m, Pom CH_3's). ^{13}C NMR , δ($CDCl_3$): 177.82 (Pom C=O), 161.90 (C-4), 150.37 (C-2), 139.50 (C-6), 115.87 (cyanomethyl CN), 101.50 (C-5), 88.44 (C-1'), 84.15 (C-4'), 82.59 (C-2'), 67.92 (C-3'), 64.31 (C-5), 60.03 (Pom CH_2), 55.85 (cyanomethyl CH_2), 38.74 (Pom tBu, q) and 28.80 (Pom CH3's). Analysis of $C_{17}H_{23}N_3O_8$ requires C, 51.37; H, 5.85; N, 10.58; found C, 51.39; H, 5.86; N, 10.60.

5'-O-Dimethoxytrityl-3-N-pivaloyloxymethyl-2'-O-cyanomethyluridine-3'-O-(2-cyanoethyl N,N-diisopropylphosphoramidite) (5)

Compound **3** (1.48 g, 3.72 mmol) was dried by evaporation of pyridine *in vacuo*. Anhydrous pyridine (20 ml), triethylamine (0.83 ml, 6 mmol) and 4,4'-dimethoyxtrityl chloride (2.25 g, 6.65 mmol) were added with stirring and exclusion of moisture. TLC (dichloromethane/ethanol 9:1v/v) showed complete reaction after 3 h with a new spot of R_f 0.58. The reaction was quenched by addition of ethanol (2 ml) and the solvent was removed *in vacuo*. The residual syrup was dissolved in dichloromethane (150 ml) and the solution washed with 1M aqueous sodium bicarbonate (150 ml). The organic phase was separated, dried (Na_2SO_4), filtered and solvent removed *in vacuo*. The crude product was purified on silica (100 g) eluting with dichloromethane/ethyl acetate (1:1). Pure compound **4** (2.45 g, 94.2 %) was obtained as a yellow solid foam of R_f 0.51 on TLC in ethanol-dichloromethane (5:95 v/v). This material (2.35 g, 3.36 mmol) was dried by evaporation from toluene *in vacuo*. The residual foam was dissolved in dry 1,2-dichloroethane (30 ml) containing *N,N*-diisopropylethylamine (1.66 ml, 9.72 mmol) and the solution cooled on ice under argon. 2-Cyanoethoxy *N,N*-diisopropylaminochlorophosphine (1.07 ml, 4.86 mmol) was added dropwise with stirring. The reaction was then stirred for 1 h at room temperature. Dichloromethane (100 ml) was added and the solution was washed with 1 M aqueous sodium bicarbonate (100 ml), dried (Na_2SO_4), filtered and the solvent removed *in vacuo*. The crude product was purified on silica (100 g) eluting with hexane-dichloromethane (2:1) containing 2% triethylamine. Removal of solvent *in vacuo* and lyophilisation from benzene gave the title compound (2.93 g, 96.7 %) as an solid off-white foam. ^{31}P NMR δ(CH_2Cl_2: concentric external D_2O lock): 145.42 and 144.98.

Analysis of $C_{47}H_{56}N_5O_{11}P$ requires C, 62.86; H, 6.30; N, 7.80; found C, 62.89; H, 6.31; N, 7.81. ES-MS m/z 899.2 (M+).

3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-2′-O-cyanomethyl-4-O-(2,6-dichlorophenyl)uridine (7)

Compound **6** (12.51 g, 19.80 mmol) was treated with bromoacetonitrile (1.4 ml, 20.10 mmol) and BEMP (5.45 ml, 19.98 mmol) in acetonitrile (100 ml) according to the procedure for compound **2**. The crude product was purified by chromatography on silica gel (400 g) and eluted with hexane-ethyl acetate (3:1 and 2:1 v/v). Pure title compound was obtained as a solid white foam (8.51 g, 64.1 %) of R_f 0.31 on TLC in hexane-ethyl acetate (2:1 v/v). ^{1}H NMR δ($CDCl_3$): 8.43 (1H, d, *J*8 Hz, H-6), 7.49-7.20 (3H, m, Ph, H-3, H-4, H-5), 6.29 (1H, d, *J* 8.0 Hz), 5.85 (1H, s, H-1'), 4.43-4.04 (7H, m, H-2', H-3', H-4', H-5' and CH_2CN) and 1.14 p.p.m. (28H, m, ^{i}Pr's). ^{13}C NMR , δ_C($CDCl_3$): 176.99 (Pom C=O), 161.35 (C-4), 149.73 (C-2), 136.84 (C-6), 100.99 (C-5), 91.45 (C-1'), 81.76 (C-4'), 74.91 (C-2'), 69.03(C-3'), 64.31 (C-5), 60.23 (Pom CH_2), 38.52 (Pom tBu, q), 26.73 (Pom CH_3's), 17.19-16.58 (isopropyl CH_3's) and 13.13-12.31 (isopropyl CH's). Analysis of $C_{29}H_{41}Cl_2N_3O_7Si_2$ requires C, 51.92; H, 6.17; N, 6.27; found C, 51.95; H, 6.18; N, 6.28.

4-N-Benzoyl-2′-O-cyanomethylcytidine (11)

Compound **7** (8.4 g, 12.52 mmol) and sodium azide (1.22 g, 18.78 mmol) were stirred in dry *N,N*-dimethylformamide (200 ml) under argon for 7 h at 60°C. The solution was allowed to cool overnight and solvent was removed *in vacuo*. The residue was dissolved in dichloromethane and washed with 1M aqueous sodium bicarbonate, dried (Na_2SO_4), filtered and solvent removed *in vacuo*. TLC in petrol/ethyl acetate (1:1 v/v) showed complete reaction. Anhydrous stannous chloride (3.09 g, 16.3 mmol) was dissolved with stirring in dry acetonitrile (200 ml) and then thiophenol (6.7 ml, 65.22 mmol) and triethylamine (6.8 ml, 48.91 mmol) were added. Crude compound **8** was dissolved in dry acetonitrile (40 ml) was added to the above yellow solution. The reaction was left stirred at room temperature overnight. TLC in dichloromethane/ethanol (95:5 v/v) showed complete reaction. Solvent was removed *in vacuo* and the yellow residue was dissolved in dichloromethane (300 ml). The solution was washed withe 1 M sodium hydroxide (300 ml) and the aqueous phase was backwashed with dichloromethane (2x200 ml). The combined organic layers were dried (Na_2SO_4), filtered and solvent was removed *in vacuo*. The crude product (compound **9**) was dried by evaporation from pyridine and then dissolved in dry pyridine (70 ml) and the solution cooled in ice. Benzoyl chloride (5.11 ml, 44 mmol) was added during 5 min with stirring and exclusion of moisture. Stirring was continued for 1.5 h at room temperature whereupon TLC showed complete reaction with a new spot R_f 0.64 in dichloromethane/ethanol (19:1). The mixture was cooled in ice and the reaction quenched by addition of water (2.7 ml) followed after 5 min by 25% ammonia solution (11 ml). Stirring was continued for 20 min at room temperature and then solvent was removed *in vacuo*. The residual gum was dissolved in dichloromethane (250 ml) and washed with 1 M aqueous sodium bicarbonate (2x200 ml). The organic phase was dried (Na_2SO_4), filtered and solvent removed *in vacuo*. This material

(compound **10**) was desilylated and worked up according to the procedure used to prepare compound **3** above. The crude product was dissolved in the minimum amount of dichloromethane/ethanol (9:1) and the product precipitated by addition of diethyl ether. The precipitate was filtered off and washed with diethyl ether. Compound **11** (3.76 g, 75%) was obtained as a white solid of R_f 0.46 in dichloromethane/ethanol (9:1). ^{1}H NMR δ(DMSO-d_6): 8.40 (1H, d, *J*7.7 Hz, H-6), 7.87-7.43 (6H, m, benzoyl and H-5), 5.98 (1H, s, H-1') and 4.63-4.11 (7H, m, H-2', H-3', H-4', H-5' and CH_2CN). ^{13}C NMR , δ_C($CDCl_3$): 167.42 (benzoyl C=O), 163.32 (C-4), 155.90 (C-2), 144.80 (C-6), 132.85 (phenyl C-1 and C-4), 128.53 (phenyl C-3 and C-5), 127.69 (phenyl C-2 and C-6), 115.92 (CN), 97.22 (C-5), 88.99 (C-1'), 83.72 (C-4'), 82.99 (C-2'), 66.85 (C-3'), 58.44 (C-5'), 55.53 p.p.m. (CH_2CN). Analysis of $C_{18}H_{18}N_4O_6$ requires C, 55.94; H, 4.70; N, 14.50; found C, 55.97; H, 4.71; N, 14.52.

5'-O-Dimethoxytrityl-N-4-benzoyl-2'-O-cyanomethylcytidine-3'-O-(2-cyanoethyl N,N-diisopropylphosphoramidite) (13).

Compound **11** (3.76 g, 9.80 mmol) was dimethoxytritylated according to the procedure given for the preparation of compound **6** above. The crude product was purified on silica (150 g) eluting with dichloromethane/ethyl acetate (1:1) containing 0.5% triethylamine. Pure compound **12** (5.62 g, 84%) was obtained as an off white foam of R_f 0.83 in dichloromethane/ethanol (9:1). This material (4.54 g, 6.91 mmol) was phosphitylated according to the procedure used to prepare compound **7** above. The crude product was purified on silica (100 g) eluting with dichloromethane/petrol (1:2 to 1:1) containing 2% triethylamine. After removal of solvent *in vacuo* and lyophilisation from benzene the title compound (5.54g, 89%) was obtained as an solid off-white foam of R_f 0.55 and 0.51 in dichloromethane/ethanol (19:1). ^{31}P NMR. δp(CH_2Cl_2: concentric external D_2O lock): 147.91 and 146.39 p.p.m. Analysis of $C_{48}H_{53}N_6O_9P$ requires C, 64.84; H, 6.02; N, 9.46; found C, 64.87; H, 6.04; N, 9.49. ES-MS m/z 890.1 (M+).

3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-2'-O-cyanomethyl-6-(2-nitrophenoxy)purine riboside (15)

Compound **14** (15.29 g, 23.31 mmol) was treated with bromoacetonitrile and BEMP in acetonitrile according to the procedure given for the preparation of compound **2**. The crude product was purified by column chromatography on silica gel (20 g) eluting with hexane/ethyl acetate (2:1 v/v) The pure product was obtained as a white foam (9.91 g, 61.2 %) of R_f 0.11 on TLC in hexane/ethyl acetate (2:1 v/v). ^{1}H NMR δ($CDCl_3$): 8.44 (1H, s, H-8), 8.18(1H, s, H-2), 8.0-7.28 (3H, m, Ph), 5.89 (1H, s, H-1'), 4.50-3.87 (7H, m, H-2', H-3', H-4', H-5' and CH_2CN) and 1.09 p.p.m. (28H, m, ^{i}Pr's). ^{13}C NMR , δ_C($CDCl_3$): 158.91 (C-6), 152.04 (C-4), 151.68 (C-2), 145.31 (Ph C-1), 142.09 (C-8), 141.77 (Ph C-2), 134.87 (Ph C-5), 126.57 (Ph C-3), 125.80 (Ph C-4), 125.36 (Ph C-6), 121.98 (C-5), 115.79 (cyanomethyl CN), 88.09 (C-1'), 81.94 (C-4'), 81.14 (C-2'), 69.60 (C-3'), 60.27 (C-5'), 56.19 (cyanomethyl CH_2), 17.07-16.87 (isopropyl CH_3s) and 13.19-12.38 (isopropyl CHs). Analysis of

$C_{30}H_{41}Cl_2N_5O_6Si_2$ requires C, 50.97; H, 5.86; N, 19.82; found C, 51.02; H, 5.87; N, 19.85.

4-N-Benzoyl-2′-O-cyanomethyladenosine (19)

Compound **15** (8.71 g, 12.98 mmol) was treated for 72 h at 60°C with sodium azide according to the procedure used for the preparation of compound **8** above. The crude compound **16** was then reduced using a reaction time of 15 min only according to the procedure used to prepare compound **9** above. This material (crude compound **17**) was benzoylated and desilylated according to the procedure used to prepare compounds **10** and **11** above. Compound **19** (3.24 g, 95%) was obtained as a white solid of R_f 0.36 in dichloromethane/ethanol (9:1). ^{1}H NMR δ(DMSO-d_6): 8.63 (1H, s, H-8), 8.14 (1H, s, H-2), 7.79-7.43 (5H, m, benzoyl), 5.97 (1H, s, H-1') and 4.45-3.87.11 (7H, m, H-2', H-3', H-4', H-5' and CH_2CN). ^{13}C NMR (DMSO-d_6) : 165.68 (C=O benzoyl), 151.94 (C-6), 151.62 (C-2), 150.48 (C-4), 142.87 (C-8), 133.35 (phenyl C-1), 132.37 (phenyl C-4), 128.40 (phenyl C-2, C-3, C-5 and C-6), 117.01 (CN), 86.13 (C-1′), 85.59 (C-4′), 81.46 (C-2′), 68.63 (C-3′), 60.97 (C-5′) and 55.63 (CH_2CN). Analysis of $C_{19}H_{18}N_6O_5$ requires C, 55.87; H, 3.96; N, 20.58; found C, 55.90; H, 3.98; N, 20.61.

5′-O-Dimethoxytrityl-N-6-benzoyl-2′-O-cyanomethyladenosine-3′-O-(2-cyanoethyl N,N-diisopropylphosphoramidite) (21)

Compound **19** (3.24 g, 7.90 mmol) was dimethoxytritylated according to the procedure used to prepare compound **6** above. The crude product was purified on silica (100 g) eluting with dichloromethane/ethyl acetate (1.1 v/v) containing 0.5% triethylamine. Pure compound **20** (3.83 g, 68%) was obtained an off-white foam of R_f 0.42 in dichloromethane/ethanol (19:1 v/v). This material was phosphitylated according to the procedure used to prepare compound **7** above. The crude product was purified on silica (100 g) eluting with dichloromethane/petrol (1:1 v/v) containing 5% triethylamine. The title compound (4.06 g, 83%) was obtained as a solid white foam of R_f 0.61 and 0.54 in dichloromethane/ethanol (19:1 v/v). ^{31}P NMR spectrum (CH_2Cl_2), concentric external D_2O lock) : +147.77 and 147.24 p.p.m. Analysis of $C_{49}H_{53}N_8O_8P$ requires C, 64.45; H, 5.86; N, 12.28; found C, 64.49; H,5.88; N,12.30. ES-MS m/z 914.8(M+).

5′-O-Dimethoxytrityl-N-2-dimethylaminomethylidene-2′O-cyanomethylguanosine-3′-O-(2-cyanoethyl N,N-diisopropylphosphoramidite) (24)

Compound **22** (2.68 g, 7.10 mmol) was dimethoxytritylated according to the procedure used to prepare compound **6** above. The crude product was purified on silica (100 g) eluting with dichloromethane containing 0 to 6% ethanol and 0.5% triethylamine. Compound **23** (4.28 g, 89%) was obtained as a solid white foam of R_f 0.42 in dichloromethane/ethanol (9:1). Compound **23** (4.28 g, 6.30 mmol) was phosphitylated according to the procedure used to prepare compound **7** above. The crude product was purified on silica (90 g) eluting first with dichloromethane/petrol (3:1) containing 2% triethylamine then with dichloromethane containing 2% ethanol and 2% triethylamine. Compound **24** (4.51 g, 81%) was obtained as a solid white foam of R_f 0.41 in

dichloromethane/ethanol (13:1 v/v). ^{31}P NMR. $\delta p(CH_2Cl_2$: concentric external D_2O lock) 148.02 and 146.83 p.p.m. Analysis of $C_{45}H_{54}N_9O_8P$ requires C, 61.45; H,6.20; N,14.33; found C, 61.50; H, 6.21; N, 14.37. ES-MS m/z 881.5(M+).

Synthesis and purification of oligonucleotides (25-43)

Oligodeoxyribonucleotides (**42** and **43**) and oligoribonucleotides (**40** and **41**) were prepared by automated solid phase synthesis on a 1 μmol scale following standard protocols for DNA and RNA synthesis respectively. The protected oligonucleotides were cleaved from the support, deprotected and purified as previously described.[26] The samples were characterised by MS (data not shown).

The oligonucleotides (**25**, **29**, **30** and **31**) were prepared on controlled glass supports derivatised with dT. The syntheses were carried out on a 0.2 μmol scale using a 900 sec coupling time for the 2′-*O*-cyanomethyluridine phosphoramidite. The protected oligonucleotides were cleaved from the support and deprotected with conc. aq. ammonia (30 %), 2M methanolic ammonia or 2M aq. sodium hydroxide (see **Table 1**). The crude sequences were then purified by reversed-phase HPLC, with the dimethoxytrityl group still attached, on a μBondapak C18 column with a gradient of acetonitrile in 0.1 M aq. triethylammonium acetate, pH 6.5 as eluent. The eluate containing the desired component was collected and transferred to a sterile, deionised water (1ml) and reevaporated (3x). The samples were characterised by MS (**Table 1**).

The two 2′-*O*-cyanomethyloligoribonucleotides (**36** and **38**) were synthesised using a standard 1 μmol β-cyanoethyl phosphoramidite RNA cycle with the condensation wait time set to 900 sec with 1H-tetrazole as the activator. The oxidation step was carried out with 0.02 M iodine. After assembly of the desired sequence, the controlled-pore glass with attached fully protected oligoribonucleotides were transferred to a vial and conc. aq. ammonia (30%, 2 ml) was added. The vial was heated at 55 °C for 24 h. The cooled products were then evaporated to dryness and the redissolved in water (1.5 ml) and filtered (Nylon Acrodisc 0.2μm pore size). The crude yields of **37** and **39** are shown in **Table 2**. The crude sequences were then purified by reversed-phase HPLC on a μBondapak C_{18} column with a gradient of acetonitrile in 0.1 M aq triethylammonium acetate, pH 6.5 as eluent. The eluate containing the desired component was collected and transferred to an sterile Eppendorf tube; it was then evaporated under reduced pressure. The yields of **37** and **39** are shown in **Table 2**. The residue was dissolved in sterile, deionised water (1 ml) and re-evaporated. The purified oligonucleotides were dissolved in 80 % aq. acetic acid (0.4 ml) and left at room temp for 30 min. The samples were then diluted with 0.4 ml sterile water, extracted with ether (3x5 ml) and the water phase separated and evaporated under reduced pressure. The residue was dissolved in sterile, deionised water (1 ml) and re-evaporated (3x). The samples were characterised by MS (see Table 2).

Melting experiments

Melting experiments were performed with 3 μM of each strand in Tris(hydroxymethyl)amino methane·HCl (10 mM, pH 7) containing KCl (0.1 M) and EDTA (1mM). The samples were preannealed by heating to 90° C, then cooling slowly to room temperature. Melting curves were obtained by measuring the increase of absorbance at 260 nm with temperature using a temperature gradient of 0.3° C min^{-1} starting at 20° C and finishing at 85° C. The melting temperatures (Table 3) were obtained by taking the first derivative of the curves.

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