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# 2'-*O*-Propargyl Oligoribonucleotides: Synthesis and Hybridisation

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**Abstract**: Fully modified oligonucleotide sequences containing 2'-*O*-propargylribonucleotides were synthesised on automated DNA-synthesisers using the phosphoramidite approach. A highly selective alkylation procedure was used to introduce the propargyl functionality, thereby enabling the synthesis of protected 2'-*O*-propargyl-3'-*O*-phosphoramidites, building blocks for the assembly of 2'-*O*-propargyl oligoribonucleotides. The suitability of phosphoramidite chemistry for the introduction of this modified nucleoside was proven using MALDI or ES mass spectrometry of the final oligomer. The 2'-*O*-propargyl oligoribonucleotides showed an increase in the Tm of duplexes with complementary RNA relative to the corresponding RNA homoduplex. These analogues should prove useful for a variety of antisense applications. 

## INTRODUCTION

The appearance of oligonucleotide analogues as promising therapeutic agents has stimulated much effort towards the development of novel nuclease resistant oligonucleotides, which are capable of hybridising with appropriate specificity and affinity to complementary sequences thus acting as effective inhibitors of gene expression.[3,4] To avoid rapid degradation by cellular nucleases, synthetic oligonucleotides need to be modified.[3,5,6] Chemical strategies to improve oligonucleotide stability are being explored, including modifications of the deoxy/ribo sugar and the heterocyclic base as well as modification or replacement of the internucleotide phosphodiester linkage.[7,8] We have concentrated our research on synthesising oligonucleotide analogues with modified sugars, *viz*. 2'-*O*-alkyl ribonucleotides.[9] These compounds possess properties that render them ideally suited for studying RNA processing. The synthesis of several 2'-*O*-alkyl ethers of ribonucleosides has been explored. This has included the synthesis of compounds in which alkyl is methyl[10-16], ethyl[17], allyl[18], propyl and pentyl[19], aminopropyl[20], butyl[21], octyl[22] or 2-methoxyethyl[23] with most studies being carried out with the methyl and allyl analogues. In addition to nuclease resistance, 2'-*O*-alkyl RNA's have an enhanced affinity to complementary RNA that decreases when the size of the alkyl group increases,[9,19,22,24] the

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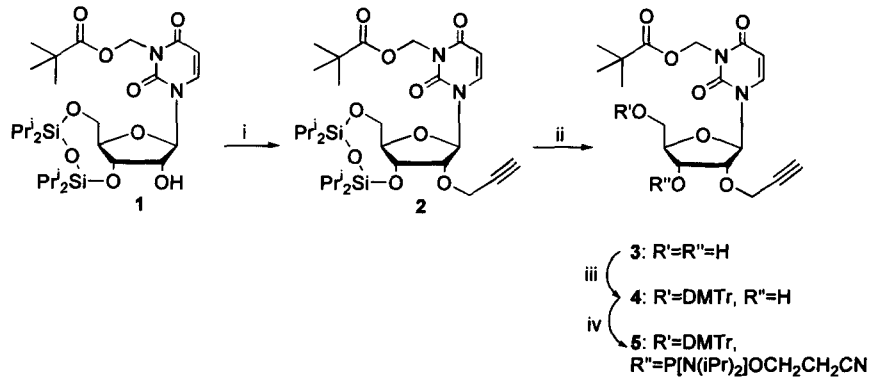
exception being the 2'-O-methoxyethyl derivative and related analogues. [23] Also, long alkyl groups stimulate the undesirable homoduplex formation and non-specific lipid binding.[9,19,24] On the other hand, nuclease stability is increased by increasing the size of the alkyl group.[19,24] Alkyl groups of three C atoms are a good compromise between nuclease stability on one hand and the duplex stabilisation and non-specific binding on the other. In this sense the 2'-*O*-allyl oligoribonucleotides have proved important for *in vitro* studies of RNA processing, and for the *in vitro* and *in vivo* localisation of small nuclear ribonucleic acids.[9]

Encouraged by the excellent results above, we have now turned to 2'-*O*-propargyl oligoribonucleotides. Propargyl groups are similar to allyl groups in size but the triple bond could enhance some of the desired properties as has been observed with C-5 propyne pyrimidines.[25] This report describes the synthesis of protected 2'-*O*-propargyl phosphoramidites and the preparation of 2'-*O*-propargyl oligoribonucleotides. The hybridisation properties of these modified oligonucleotides are also described.

## RESULTS AND DISCUSSION

Our route to the 2'-*O*-propargyluridine-3'-*O*-phosphoramidite building block **5** is illustrated in **Scheme 1**.

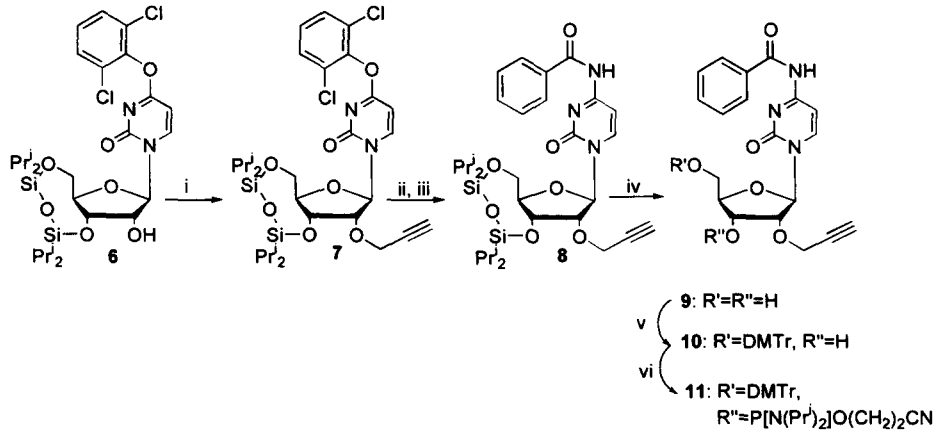


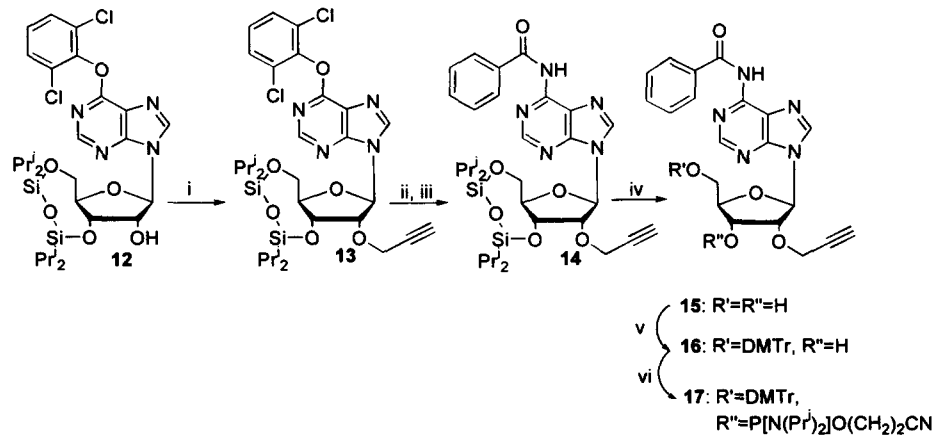

**Scheme 1**. Reaction scheme for the preparation of 2'-*O*-propargyluridine-3'-*O*-phosphoramidite building block **5**. Reagents and conditions: i, propargyl bromide, BEMP in acetonitrile, 0 °C, 15 min then room temp., 90 min; ii, TBAF in THF, room temp., 15 min; iii, DMTrCl, triethylamine in pyridine, room temp., 5h; iv, 2-cyanoethoxy *N,N*-diisopropylaminochlorophosphine, *N,N*-diisopropylethylamine in dichloromethane, room temp., 1h.

Compound **1** was synthesised according to Grøtli *et al.*[26] The propargyl group was then introduced using one equivalent of propargyl bromide and BEMP,[27] giving compound **2** in reasonable yield (48 %). Subsequent desilylation gave compound **3** in 97 % yield. Dimethoxytritylation and phosphitylation proceeded in yields of 92 and 86 % respectively, affording the desired 2'-*O*-propargyluridine building block **5**.

Our route to the 2'-*O*-propargylcytidine-3'-*O*-phosphoramidite is illustrated in **Scheme 2**. 3',5'-*O*-

(Tetraisopropyldisiloxane-1,3-diyl)-4-*O*-(2,6-dichlorophenyl)uridine **6** was prepared according to the procedure of Sproat *et al.*[27] Compound **6** was then converted into compound **7** in 61 % yield using the alkylation procedure described above. Displacement of dichlorophenoxide from compound **7** by ammonia/tetrahydrofuran in a stainless steel bomb for 72 h at 90°C, followed by acylation of the resultant 4-amino moiety gave the 2'-*O*-propargylcytidine derivative **8** in 89% isolated yield. Subsequent desilylation, dimethoxytritylation and phosphitylation yielded the desired 2'-*O*-propargylcytidine building block **11** in excellent overall yield.

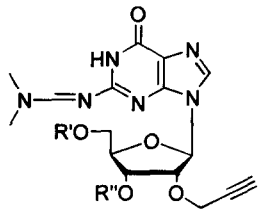



**Scheme 2.** Reaction scheme for the preparation of 2'-*O*-propargylcytidine-3'-*O*-phosphoramidite building block **11** and 2'-*O*-propargyladenosine 3'-*O*-phosphoramidite building block **17**. Reagents and conditions: i, propargyl bromide, BEMP in acetonitrile, 0 °C, 15 min then room temp., 90 min; ii, $NH_3$ in THF, 70 °C, 72 h; iii, BzCl in pyridine, room temp., 1h; iv, TBAF in THF, room temp., 15 min; v, DMTrCl, triethylamine in pyridine, room temp., 5h; vi, 2-cyanoethoxy *N,N*-diisopropylaminochlorophosphine, *N,N*-diisopropylethylamine in dichloromethane, room temp., 1h.

Alkylation of 3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-6-(2,6-dichlorophenoxy)purine riboside **12**[27] using propargyl bromide and BEMP gave compound **13** in 57 % yield (**Scheme 2**). Treatment with ammonia/tetrahydrofuran in a stainless steel bomb and subsequent acylation gave an excellent yield (90 %) of the disiloxane protected 2'-*O*-propargyl-$N^6$-benzoyladenosine **14**. Subsequent desilylation, dimethoxytritylation and phosphitylation yielded the desired 2'-*O*-propargyladenosine building block **17** in good overall yield.

$N^2$-Dimethylaminomethylidene-2'-*O*-propargylguanosine, compound **18**, was prepared from guanosine according to the procedure of Grøtli *et al.*[28,29] (**Scheme 3**). Subsequent dimethoxytritylation and phosphitylation gave the desired 2'-*O*-propargylguanosine building block **20**.




i **18**: R'=R"=H
**19**: R'=DMTr, R"=H
ii **20**: R'=DMTr,
R"=P[N(Pr$^i$)$_2$]O(CH$_2$)$_2$CN

**Scheme 3**. Reaction scheme for the preparation of $N^2$-dimethylaminomethylidene-2'-*O*-propargylguanosine, compound **18**. Reagents and conditions: i, DMTrCl, triethylamine in pyridine, room temp., 5h; vi, 2-cyanoethoxy *N,N*-diisopropylaminochlorophosphine, *N,N*-diisopropylethylamine in dichloromethane, room temp., 1h

Compounds **4**, **10**, **16** and **19** were also succinylated on the 3'-hydroxyl groups, enabling subsequent preparation of activated esters for coupling to long chain aminoalkyl-controlled pore glass (LCAA-CPG, 500Å) to give the desired supports for solid phase synthesis (data not shown).

The synthesis of oligonucleotides on solid support was performed using phosphoramidite chemistry on an Applied Biosystem-394 DNA synthesiser. In order to examine the stability of the 2'-*O*-propargyl group during synthesis on solid phase, two model oligonucleotides (**21** and **22**) were synthesised (**Table 1**). Our main concern was the potential sensitivity of the propargyl group to the oxidation conditions used in standard phosphoramidite chemistry. The C-5 propyne analogs of deoxycytidine have been introduced into oligonucleotides using H-phosphonate chemistry.[25] However, no stability problems with the propargyl moiety were reported. Thus, oligonucleotides **21** and **22** (**Table 1**) were synthesised on a 0.2 μmol scale by using supports derived from the 3'-*O*-succinates of $N^6$-benzoyl-2'-*O*-propargyl-5'-*O*-dimethoxytrityladenosine, and $N^4$-benzoyl-2'-*O*-propargyl-5'-*O*-dimethoxytritylcytidine, and 5'-*O*-dimethoxytritylthymidine-3'-*O*-

phosphoramidite using a standard DNA synthesis cycle (25 sec. coupling time). Oxidation (**Table 1**) was carried out with 0.1 M iodine,[30] 0.02 M iodine[31] or 1M tBuOOH.[32-38]

**Table 1**. Oxidation test

| Oligonucleotides | Oxidation reagent | Oxidation time | Calculated mass (Dalton) | Found mass (Dalton) |
|---|---|---|---|---|
| **21** | 0.1 M iodine | 15 sec | 3953.76 | 3952.9 |
| **22** | 0.1 M iodine | 15 sec | 3929.73 | 3929.1 |
| **21** | 0.02 M iodine | 15 sec | 3953.76 | 3953.1 |
| **22** | 0.02 M iodine | 15 sec | 3929.73 | 3929.3 |
| **21** | 1.0 M tBuOOH | 60 sec | 3953.76 | 3953.3 |
| **22** | 1.0 M tBuOOH | 60 sec | 3929.73 | 3929.4 |

Oligonucleotide **21**: 5'-$(dT)_{11}$-A-3' Oligonucleotide **22**: 5'-$(dT)_{11}$-C-3'

After standard deprotection with ammonia the crude products were analysed by reversed phase HPLC. In all cases a major peak was obtained and collected. The purified oligonucleotides were analysed by ESMS (**Table 1**). All products gave the expected mass with an accuracy of < 0.02 %, and we therefore concluded that the molecular structure of the synthesised products corresponds to the intact 12 mer containing one 2'-*O*-propargyl ribo unit. This proved that the 2'-*O*-propargyl building blocks could be incorporated without side reactions or degradation. Oxidation is usually carried out by treatment with iodine-water rather than with tBuOOH. However, the use of tBuOOH has the advantage that the column is excluded from any contact with water and we decided to synthesise the remaining 2'-*O*-modified oligonucleotides using this reagent.

The two 2'-*O*-propargyloligoribonucleotides **23** and **24** (**Table 2**) were then prepared in order to carry out melting experiments.

**Table 2**. 2'-*O*-Propargyloligoribonucleotides **23** and **24**

| Oligonucleotides | Yield, crude ($A_{260}$) | Yield, purified ($A_{260}$) | Calculated mass (Dalton) | Found mass (Dalton) |
|---|---|---|---|---|
| **23** | 56.3 | 36 | 4251.92 | 4250.86 |
| **24** | 63.4 | 48 | 4251.92 | 4250.66 |

Oligonucleotide **23**: 3'-ACC UAC UCG GAG-5' Oligonucleotide **24**: 3'-CUC CGA AUA GGU-5'

A coupling time of 15 min using 1*H*-tetrazole as activator was used to ensure high yield coupling of the protected 2'-*O*-propargyl ribonucleotide monomers. The average coupling yields (as determined by DMTr cation assay) were 98 %. Upon completion of the synthesis the support bound oligonucleotide was kept in ammonia at 55 °C for 12h to cleave the oligonucleotide from the carrier and remove the β-cyanoethyl and base protecting groups. The oligonucleotides were purified by reverse-phase HPLC and characterised by MALDI (**Table 2**). Again, the measured values corresponded to the mass calculated for the two oligomers and confirmed the composition.

Hybridisation of the modified oligonucleotides (**23** and **24**) to their unmodified RNA (**25** and **26**) or unmodified DNA (**27** and **28**) was evaluated spectrophotometrically. The Tm's for the various duplexes are listed in **Table 3**. Three main conclusions can be drawn from the data. First, these data show that the 2'-*O*-propargyl modification increases the ability to form stable hybrids with RNA. The 2'-*O*-propargyl:RNA heteroduplex is slightly more stable than the 2'-*O*-allyl RNA:RNA heteroduplex of identical sequence.[24] Second, the 2'-*O*-propargyl-RNA:RNA heteroduplex is more than 20°C more stable than the 2'-*O*-propargyl-RNA:DNA heteroduplex, showing excellent discrimination between RNA and DNA targets. Third, the 2'-*O*-propargyl RNA homoduplex is only 2 °C more stable than the 2'-*O*-propargyl-RNA:RNA heteroduplex of identical sequence. This is in contrast to the 2'-*O*-methyl and 2'-*O*-allyl-RNA homoduplexes which are in turn about 5 and 9 °C more stable than the corresponding heteroduplexes with RNA.[24] This reduced stabilisation of the 2'-*O*-propargyl-RNA homoduplex relative to 2'-*O*-methyl and 2'-*O*-allyl-RNA homo duplexes, can be especially valuable in reducing self-hybridisation of partially complementary 2'-*O*-alkyl antisense probes, or chemically modified ribozymes.

**Table 3.** Hybridisation of 2'-*O*-propargyloligoribonucleotides to their complementary sequences

| Duplex | Tm (°C) |
|---|---|
| **23** + **24** | 60.90 |
| **23** + **26** | 58.88 |
| **23** + **28** | 37.44 |
| **25** + **26** | 56.90 |
| **27** + **28** | 44.73 |
| **24** + **25** | 58.82 |
| **24** + **27** | 37.58 |

Tm= melting temperature in °C determined under the following conditions:
3 μM of each strand in tris(hydroxymethyl)amino methane HCl (10 mM, pH 7) containing KCl (0.1 M) and EDTA (1mM). **23** and **24**: 2'-*O*-propargyl-oligoribonucleotides, **25** and **26**: unmodified RNA, **27** and **28**: unmodified DNA

## CONCLUSION

The synthesis of 2'-*O*-propargyl oligoribonucleotides has been described. The 2'-*O*-propargyl building blocks could be incorporated into oligonucleotides without side reactions and degradation. Moreover, the 2'-*O*-propargyloligoribonucleotides form stable duplexes with complementary RNA and show good discrimination between RNA and DNA targets. The relatively small difference between homoduplex and RNA heteroduplex stability associated with the 2'-*O*-propargyl oligonucleotides may also prove useful in reducing self-hybridisation of partially self-complementary 2'-*O*-alkyl RNA probes.

## EXPERIMENTAL SECTION

$^{1}H$, $^{13}C$ and $^{31}P$ NMR spectra were recorded on a Bruker AM 250 spectrometer using tetramethylsilane and external 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 (ES) ionisation or Matrix Assisted Laser Desorption Ionisation (MALDI). The silica gel (35-70mm) 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**[26], **6**[27], **12**[27] and **18**[28,29] were synthesised according to literature procedures.

*Synthesis of monomers*

**3',5'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-$N^3$-pivaloyloxymethyl-2'-*O*-propargyluridine (2)** Compound **1** (10.0 g, 16.6 mmol) was dried by evaporation of anhydrous acetonitrile (100 ml) . The foam was dissolved in anhydrous acetonitrile (100 ml) under argon and the solution cooled in an ice-bath. Propargyl bromide (3.2 ml, 21.6 mmol) and BEMP (5.8 ml, 20.0 mmol) were added with stirring and exclusion of moisture. The solution was kept for 15 min at 0 °C and then stirred for 90 min at room temperature. Silica gel TLC showed complete reaction. The mixture was concentrated to dryness *in vacuo*. The residue was dissolved in ethyl acetate and washed with 0.5 M phosphate buffer (pH 7, 200 ml) and saturated brine (200 ml). The separated organic phase was dried ($Na_2SO_4$), filtered and solvent was removed *in vacuo*. The crude product was purified by column chromatography on silica gel (200 g) using chloroform as eluant. Pure title compound was obtained as a white foam (5.1 g, 48 %) of $R_f$ 0.45 in methanol/chloroform (2:98 v/v). $^{1}H$ NMR, δ($CDCl_3$): 8.36 (1H, d, *J* 7.8 Hz, H-6), 7.56 (1H, d, *J* 7.8 Hz, H-5), 5.82 (1H, s, *J* 5.7, H-1'), 4.67 (2H, m, propargyl $CH_2$), 4.52-4.19 (5H, m, H-2', H-3', H-4', H-5'), 3.87 (2H, s, Pom $CH_2$), 2.50 (1H, s, propargyl CH), 1.14 (9H, m, Pom $CH_3$'s) and 1.07 p.p.m. (28H, m, $Pr^i$'s). $^{13}C$ NMR, δ($CDCl_3$): 177.29 (Pom C=O), 161.59 (C-4), 149.91 (C-2), 138.20 (C-6), 101.06 (C-5), 89.32 (C-1'), 81.71 (C-2'), 80.47 (C-4'), 79.20 (propargyl C), 74.68 (propargyl =CH), 68.26 (C-3'), 64.42 (Pom, $CH_2$), 59.35 (C-5'), 58.00 (propargyl $OCH_2$), 36.75 (Pom C), 26.95 (Pom $CH_3$'s), 17.34-16.71 (isopropyl $CH_3$,s), 13.39, 13.07, 12.83 and 12.36 p.p.m (isopropyl CH's). Analysis of $C_{30}H_{50}N_2O_9Si_2$ requires

C, 56.39; H, 7.90; N, 4.38; found C, 56.43; H, 7.91; N, 4.39.

**$N^3$-Pivaloyloxymethyl-2'-*O*-propargyluridine (3)**

Compound **2** (5.0 g, 7.8 mmol) was dissolved in dry tetrahydrofuran (25 ml) and 1 M tetrabutylammonium fluoride in tetrahydrofuran (20 ml) was added. After 5 min stirring TLC showed complete reaction. The reaction mixture was quenched with pyridine/methanol/water (10 ml, 3:1:1 by volume), and the solution was poured into stirred pyridinium form Dowex 50W x4-200 resin (40 g) suspended in pyridine/methanol/water (25 ml, 3:1:1 by volume). The mixture was stirred for 30 min, the resin filtered off and washed with the above solution (3x25 ml). 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 by column chromatography on silica gel (100 g) eluting with methanol/dichloromethane (1:9 v/v). Pure title compound was obtained as a white foam (3.0 g, 97 %) of $R_f$ 0.15 in methanol/dichloromethane (1:9 v/v). $^1$H NMR, δ($CDCl_3$): 8.31 (1H, d, *J* 7.8 Hz, H-6), 7.52 (1H, d, *J* 7.8 Hz, H-5), 5.84 (1H, s, *J* 5.7, H-1'), 4.61 (2H, m, propargyl $CH_2$), 4.57-4.26 (5H, m, H-2', H-3', H-4', H-5'), 3.88 (2H, s, Pom $CH_2$), 2.48 (1H, s, propargyl CH) and 1.08 p.p.m. (9H, m, Pom $CH_3$'s). $^{13}$C NMR, δ($CDCl_3$): 177.55 (Pom C=O), 161.70 (C-4), 150.38 (C-2), 140.05 (C-6), 101.75 (C-5), 89.68 (C-1'), 84.85 (C-4'), 80.89 (C-2'), 78.72 (propargyl C), 75.75 (propargyl =CH), 68.79 (C-3'), 64.55 (Pom, $CH_2$), 61.15 (C-5'), 58.34 (propargyl $OCH_2$), 36.61 (Pom C) and 26.94 p.p.m. (Pom $CH_3$'s). Analysis of $C_{18}H_{24}N_2O_8$ requires C, 54.53; H, 6.11; N, 7.06; found C, 54.57; H, 6.13; N, 7.07.

**5'-*O*-Dimethoxytrityl-$N^3$-pivaloyloxymethyl-2'-*O*-propargyluridine-3'-*O*-(2-cyanoethyl *N,N*-diisopropylphosphoramidite) (5)**

Compound **3** (2.9 g, 6.0 mmol) was dried by evaporation of anhydrous pyridine (25 ml). Anhydrous pyridine (50 ml), triethylamine (1.7 ml, 12.0 mmol) and 4,4'-dimethoxytrityl chloride (2.5 g, 7.2 mmol) were added with stirring and exclusion of moisture. The reaction was left at room temperature for 5h. TLC shoved complete reaction. The reaction was quenched with methanol (3 ml) and the solvent was removed *in vacuo*. The residual syrup was dissolved in ethyl acetate (200 ml) and the solution was washed with 1 M aqueous sodium bicarbonate (200 ml), the organic phase separated, dried ($Na_2SO_4$), filtered and solvent was removed *in vacuo*. The crude product was purified by column chromatography on silica gel (100 g) using dichloromethane containing 1 % triethylamine as eluent. Pure compound **4** was obtained as a white foam (3.9 g, 92 %) of $R_f$ 0.11 in hexane/ethyl acetate (2:1 v/v). This material (1.8 g, 2.6 mmol) was dried by evaporation of anhydrous acetonitrile and dissolved in dry 1,2-dichloroethane (20 ml) containing *N,N*-diisopropylethylamine (1.2 ml, 6.5 mmol). The solution was cooled on ice and 2-cyanoethoxy *N,N*-diisopropylaminochlorophosphine (0.7 ml, 3.1 mmol) was added dropwise with stirring and exclusion of moisture. The reaction was then stirred for 1h at room temperature. TLC showed complete reaction. The reaction mixture was diluted with dichloromethane (100 ml), washed with 1 M aqueous sodium hydrogen carbonate (120 ml), the organic layer separated, dried

($Na_2SO_4$), filtered and evaporated to dryness under reduced pressure. The crude product was purified by chromatography on silica gel (50 g) eluting with hexane-dichloromethane (2:1 v/v) containing 3 % triethylamine. Product fractions were pooled and solvent was evaporated *in vacuo*. Lyophilisation from benzene afforded the title compound as a solid white foam (2.0 g, 86 %) of $R_f$ 0.27 on TLC (pre-incubated with triethylamine) in hexane-ethyl acetate (2:1 v/v). Analysis of $C_{48}H_{63}N_4O_{11}P$ requires C, 64.13; H, 7.08; N, 6.23; found C, 6471; H, 7.26; N, 6.26. ES-MS m/z 900.3 (M+). $^{31}P$ NMR, δp($CH_2Cl_2$, concentric external $D_2O$ lock): 147.15 and 146.94 p.p.m.

**3',5'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-4-*O*-(2,6-dichlorophenyl)-2'-*O*-propargyluridine (7)**

3',5'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-4-*O*-(2,6-dichlorophenyl)uridine (8.0 g, 12.7 mmol) was alkylated as described for the synthesis of compound **2** above. The crude product was purified by column chromatography on silica gel (180 g) using hexane/ethyl acetate (3:1 v/v) as eluant. Pure title compound was obtained as a white foam (5.2 g, 61 %) of $R_f$ 0.37 in hexane/ethyl acetate (2:1 v/v). $^1H$ NMR, δ($CDCl_3$): 8.43 (1H, d, *J* 8.0 Hz, H-6), 7.46-7.20 (3H, m, Ph, H-3, H-4, H-5), 6.29 (1H, d, *J* 8.0 Hz, H-5), 5.87m (1H, s, H-1'), 4.88-4.60 (2H, m, propargyl $CH_2$), 4.40-4.06 (5H, m, H-2', H-3', H-4', H-5'), 2.50 (1H, s, propargyl CH), and 1.16 p.p.m. (28H, m, $^i$Pr's). $^{13}C$ NMR, δ($CDCl_3$): 169.65 (C-4), 154.41 (C-2), 144.60 (Ph, C-1), 144.31 (C-6), 128.73 (Ph, C-2, C-6), 128.41 (Ph, C-3, C-5), 126.87 (Ph, C-4), 93.57 (C-5), 89.27 (C-1'), 82.99 (C-4'), 81.40 (C-2'), 79.17 (propargyl C), 74.34 (propargyl =CH), 67.89 (C-3'), 59.22 (C-5'), 58.00 (propargyl $OCH_2$), 17.29-16.72 (isopropyl CH3's), 13.29, 13.11, 12.92 and 12.24 p.p.m. (isopropyl CH's). Analysis of $C_{30}H_{42}Cl_2N_2O_7Si_2$ requires C, 53.79; H, 6.33; N, 4.18; found C, 53.83; H, 6.34; N, 4.20.

**3',5'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-$N^4$-benzoyl-2'-*O*-propargylcytidine (8)**

Compound **7** (5.0 g, 7.5 mmol) in dry tetrahydrofuran (50 ml) was treated with dry ammonia (10 g) in a Teflon lined stainless steel bomb at 70°C for 72 h. The cooled bomb was opened carefully in a well ventilated fume cupboard. Solvent was removed *in vacuo* and the residue dried by evaporation of pyridine (2x75 ml). The residue was dissolved in dry pyridine (100 ml) and the solution cooled on an ice-bath. Benzoyl chloride (1.9 ml, 16.4 mmol) was added dropwise with stirring and exclusion of moisture. Stirring was continued for 1 h at room temperature whereupon TLC showed complete reaction. The mixture was cooled in ice and the reaction quenched by addition of water (10 ml) followed by 25 % ammonia solution (8 ml). Stirring was continued for 20 min at room temperature and then the solvent was removed *in vacuo*. The residual gum was dissolved in ethyl acetate (200 ml) and the solution washed with 1M sodium bicarbonate (2x200 ml). The organic layer was separated, dried ($Na_2SO_4$), filtered and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography on silica gel (120 g) using hexane/ethyl acetate (2:1 and 1:1 v/v) as eluant. Pure title compound was obtained as a white foam (4.2 g, 89 %) of $R_f$ 0.21 in hexane/ethyl acetate (12:1 v/v). $^1H$ NMR, δ($CDCl_3$): 8.36 (1H, d, *J* 7.7 Hz, H-6), 7.81-7.41 (6H, m, benzoyl and H-5), 5.85 (1H, s, *J* 7.9, H-1'), 4.72-4.58 (2H, m, propargyl $CH_2$), 4.42-4.11 (5H, m, H-2', H-3', H-4', H-5'), 2.43 (1H, s, propargyl CH), and

1.08 p.p.m. (28H, m, $Pr^i$'s). $^{13}C$ NMR, δ($CDCl_3$): 166.80 (benzoyl, C=O), 162.75 (C-4), 154.40 (C-2), 144.19 (C-6), 132.98 (Ph, C-1), 132.73 (Ph, C-4), 128.57 (Ph, C-2, C-6), 127.58 (Ph, C-3, C-5), 95.99 (C-5), 89.77 (C-1'), 81.91 (C-4'), 80.54 (C-2'), 79.19 (propargyl C), 74.31 (propargyl =CH), 67.69 (C-3'), 59.32 (C-5'), 57.93 (propargyl $OCH_2$), 17.33-16.75 (isopropyl CH3's), 13.24, 13.13, 12.89 and 12.29 p.p.m. (isopropyl CH's). Analysis of $C_{31}H_{45}N_3O_8Si_2$ requires C, 57.81; H, 7.06; N, 6.53; found C, 57.87; H, 7.09; N, 6.55.

**$N^4$-Benzoyl-2'-*O*-propargylcytidine (9)**

Compound **7** (4.0 g, 6.4 mmol) was desilylated and worked up according to the procedure used to prepare compound **3** above. The crude product was purified by column chromatography on silica gel (100 g) using methanol/dichloromethane (7:93 v/v) as eluant. Pure title compound was obtained as a white foam (2.2 g, 91 %) of $R_f$ 0.18 in methanol/dichloromethane (1:9 v/v). $^1H$ NMR, δ( DMSO-$d_6$): 8.39 (1H, d, *J* 7.6 Hz, H-6), 7.89-7.45 (6H, m, benzoyl and H-5), 6.01 (1H, s, H-1'), 4.97-4.69 (2H, m, propargyl $CH_2$), 4.62-4.13 (5H, m, H-2', H-3', H-4', H-5') and 2.57 p.p.m. (1H, s, propargyl CH). $^{13}C$ NMR, δ(DMSO-$d_6$): 167.00 (benzoyl, C=O), 162.84 (C-4), 154.39 (C-2), 144.21 (C-6), 133.09 (Ph, C-1), 132.79 (Ph, C-4), 128.65 (Ph, C-2, C-6), 127.56 (Ph, C-3, C-5), 96.05 (C-5), 88.97 (C-1'), 83.87 (C-4'), 81.54 (C-2'), 79.16 (propargyl C), 74.27 (propargyl =CH), 67.39 (C-3'), 59.02 (C-5') and 57.90 p.p.m. (propargyl $OCH_2$). Analysis of $C_{19}H_{19}N_3O_7$ requires C, 56.85; H, 4.78; N, 10.47; found C, 56.89; H, 4.79; N, 10.49.

**5'-*O*-Dimethoxytrityl-$N^4$-benzoyl-2'-*O*-propargylcytidine-3'-*O*-(2-cyanoethyl *N,N*-diisopropyl-phosphoramidite) (11)**

Compound **9** (2.2 g, 5.7 mmol) was dimethoxytritylated and worked up according to the procedure used to prepare compound **4** above. The crude product was purified by column chromatography on silica gel (100 g) using ethyl acetate/dichloromethane (7:1 v/v) containing 1% triethylamine as eluent. Pure compound **9** was obtained as a white foam (3.72 g, 93 %) of $R_f$ 0.37 in methanol/dichloromethane (5:95 v/v). This material (1.0 g, 1.5 mmol) was phosphitylated and worked up according to the procedure used to prepare compound **5** above The crude product was purified by chromatography on silica gel (50 g) eluting with hexane-dichloromethane (1:1 v/v) containing 2 % triethylamine. Product fractions were pooled and solvent was evaporated *in vacuo*. Lyophilisation from benzene afforded the title compound as a solid white foam (1.1 g, 85 %) of $R_f$ 0.43 on TLC (pre-incubated with triethylamine) in ethanol/dichloromethane (5:95 v/v). Analysis of $C_{49}H_{58}N_5O_9P$ requires C, 66.27; H, 6.60; N, 7.89; found C, 66.94; H, 6.63; N, 7.92. ES-MS m/z 889.2 (M+). $^{31}P$ NMR, δp($CH_2Cl_2$, concentric external $D_2O$ lock): 147.17 and 146.69 p.p.m.

**3',5'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-4-*O*-(2,6-dichlorophenoxy)-2'-*O*-propargylpurine riboside (13)**

3',5'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-4-*O*-(2,6-dichlorophenoxy)purine riboside, compound **12** (10.0 g, 15.3 mmol) was alkylated as described for the synthesis of compound **2** above. The crude product was purified

by column chromatography on silica gel (200 g) using hexane/ethyl acetate (3:1 v/v) as eluant. Pure title compound was obtained as a white foam (6.3 g, 57 %) of $R_f$ 0.51 in hexane/ethyl acetate (2:1 v/v). $^1$H NMR, δ( $CDCl_3$): 8.31 (1H, s, H-8), 8.18 (1H, s, H-2), 8.01-7.26 (4H, m, Ph), 5.97 (1H, s, H-1'), 4.71 (2H, m, propargyl $CH_2$), 4.52-3.85 (5H, m, H-2', H-3', H-4', H-5'), 2.41 (1H, s, propargyl CH) and 0.98 (28H, m, $^i$Pr's ). $^{13}$C NMR, δ($CDCl_3$): 158.30 (C-6), 152.30 (C-4), 151.79 (C-2), 145.57 (Ph, C-1), 141.84 (C-8), 129.52 (Ph, C-2, C-6), 128.79 (Ph, C-3, C-5), 127.15 (Ph, C-4), 121.80 (C-5), 88.86 (C-1'), 81.44 (C-4'), 81.23 (C-2'), 78.96 (propargyl C), 75.48 (propargyl =CH), 69.79 (C-3'), 59.75 (C-5'), 58.48 (propargyl $OCH_2$), 17.35-16.82 (isopropyl $CH_3$'s), 13.33, 12.85 and 12.49 p.p.m. (isopropyl CH's). Analysis of $C_{31}H_{42}Cl_2N_4O_6Si_2$ requires C, 53.66; H, 6.11; N, 8.08; found C, 53.71; H, 6.13; N, 8.09.

**3',5'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-$N^6$-benzoyl-2'-*O*-propargyladenosine (14)**

Compound **13** (6.2 g, 8.5 mmol) was treated with anhydrous ammonia and worked up according to the procedure given for the synthesis of compound **7** above. The $N^6$-acylation was performed with benzoyl chloride and the workup was as for compound **8**. The crude product was purified by column chromatography on silica gel (200 g) using hexane/ethyl acetate (1:1 v/v) as eluant. Pure title compound was obtained as a white foam (5.0 g, 90 %) of $R_f$ 0.21 in hexane/ethyl acetate (1:1 v/v). $^1$H NMR, δ( $CDCl_3$): 8.63 (1H, s, H-8), 8.17 (1H, s, H-2), 7.80-7.39 (5H, m, Ph), 5.98 (1H, s, H-1'), 4.76 (2H, m, propargyl $CH_2$), 4.41-3.89 (5H, m, H-2', H-3', H-4', H-5'), 2.29 (1H, s, propargyl CH) and 1.03 (28H, m, $^i$Pr's ). $^{13}$C NMR, δ($CDCl_3$): 166.84 (benzoyl C=O), 158.33 (C-6), 152.38 (C-4), 151.83 (C-2), 141.89 (C-8), 133.23 (benzoyl, C-1), 132.91 (benzoyl, C-4), 128.53 (benzoyl, C-3, C-5), 127.41 (benzoyl, C-2, C-6), 121.83 (C-5), 88.87 (C-1'), 81.53 (C-2'), 81.18 (C-2'), 78.90 (propargyl C), 75.53 (propargyl =CH), 69.83 (C-3'), 59.65 (C-5'), 58.43 (propargyl $OCH_2$), 17.11-16.42 (isopropyl $CH_3$'s), 13.33, 13.02, 12.39 and 12.32 p.p.m. (isopropyl CH's). Analysis of $C_{32}H_{45}N_5O_6Si_2$ requires C, 58.94; H, 6.97; N, 10.74; found C, 58.98; H, 7.00; N, 10.76.

**$N^6$-Benzoyl-2'-*O*-propargyladenosine (15)**

Compound **14** (5.0 g, 7.6 mmol) was desilylated and worked up according to the procedure used to prepare compound **3** above. The crude product was purified by column chromatography on silica gel (100 g) using methanol/dichloromethane (8:92 v/v) as eluant. Pure title compound was obtained as a white foam (2.8 g, 90 %) of $R_f$ 0.17 in methanol/dichloromethane (1:9 v/v). $^1$H NMR, δ(DMSO-$d_6$): 8.62 (1H, s, H-8), 8.13 (1H, s, H-2), 7.77-7.40 (5H, m, Ph), 5.95 (1H, s, H-1'), 4.77 (2H, m, propargyl $CH_2$), 4.40-3.85 (5H, m, H-2', H-3', H-4', H-5') and 2.32 p.p.m. (1H, s, propargyl CH). $^{13}$C NMR, δ(DMSO-$d_6$): 166.89 (benzoyl C=O), 158.39 (C-6), 152.31 (C-2), 151.74 (C-4), 141.81 (C-8), 133.11 (benzoyl, C-1), 132.83 (benzoyl, C-4), 128.53 (benzoyl, C-3, C-5), 127.55 (benzoyl, C-2, C-6), 121.83 (C-5), 88.79 (C-1'), 82.65 (C-4'), 81.34 (C-2'), 78.95 (propargyl C), 75.59 (propargyl =CH), 69.89 (C-3'), 61.42 (C-5'), and 58.49 p.p.m. (propargyl $OCH_2$). Analysis of $C_{20}H_{19}N_5O_5$ requires C, 58.67; H, 4.69; N, 17.11; found C, 58.71; H, 4.70; N, 17.13.

**5'-*O*-Dimethoxytrityl-$N^6$-benzoyl-2'-*O*-propargyladenosine-3'-*O*-(2-cyanoethyl *N,N*-diisopropyl-phosphoramidite) (17)**

Compound **15** (2.7 g, 6.6 mmol) was dimethoxytritylated and worked up according to the procedure used to prepare compound **4** above. The crude product was purified by column chromatography on silica gel (100 g) using ethyl acetate/dichloromethane (1:1 v/v) containing 1% triethylamine as eluent. Pure compound **14** was obtained as a white foam (4.2 g, 90 %) of $R_f$ 0.27 in methanol/dichloromethane (5:95 v/v). This material (2.1 g, 3.0 mmol) was phosphitylated and worked up according to the procedure used to prepare compound **5** above The crude product was purified by chromatography on silica gel (75 g) eluting with hexane-dichloromethane (1:1 v/v) containing 3 % triethylamine. Product fractions were pooled and solvent was evaporated *in vacuo*. Lyophilisation from benzene afforded the title compound as a solid white foam (2.3 g, 86 %) of $R_f$ 0.43 and 0.36 on TLC (pre-incubated with triethylamine) in ethyl acetate/dichloromethane (4:1 v/v). Analysis of $C_{50}H_{58}N_7O_5P$ requires C, 65.84; H, 6.42; N, 10.75; found C, 65.90; H, 6.43; N, 10.78. ES-MS m/z 913.1 (M+). $^{31}P$ NMR, δp($CH_2Cl_2$, concentric external $D_2O$ lock): 147.32 and 147.07 p.p.m.

**5'-*O*-Dimethoxytrityl- $N^2$-dimethylaminomethylidene-2'-*O*-propargylguanosine -3'-*O*-(2-cyanoethyl *N,N*-diisopropylphosphoramidite) (20)**

Compound **18**[25,26] (2.1 g, 5.6 mmol) was dimethoxytritylated and worked up according to the procedure used to prepare compound **4** above. The crude product was purified by column chromatography on silica gel (80 g) using ethyl acetate/dichloromethane (1:1 v/v) containing 1% triethylamine and then a gradient of ethanol (0-8%) in ethyl acetate containing 1% triethylamine. Pure compound **19** was obtained as a white foam (3.5 g, 92 %) of $R_f$ 0.10 in methanol/dichloromethane (5:95 v/v). This material (2.2 g, 3.2 mmol) was phosphitylated and worked up according to the procedure used to prepare compound **5** above. The crude product was purified by chromatography on silica gel (70 g) eluting with a gradient of ethanol (0-3%) in dichloroethane containing 2 % triethylamine. Product fractions were pooled and solvent was evaporated *in vacuo*. Lyophilisation from benzene afforded the title compound as a solid white foam (2.5 g, 91 %) of $R_f$ 0.57 on TLC (pre-incubated with triethylamine) in ethanol /dichloromethane (1:9 v/v). Analysis of $C_{46}H_{59}N_8O_8P$ requires C, 62.85; H, 6.78; N, 12.75; found C, 62.97; H, 6.81; N, 12.79. ES-MS m/z 880.2 (M+). $^{31}P$ NMR, δp($CH_2Cl_2$, concentric external $D_2O$ lock): 147.37 and 147.20 p.p.m.

*Synthesis and purification of oligonucleotides*

Oligodeoxyribonucleotides and oligoribonucleotides were prepared by automated solid phase synthesis on a 1 μmol scale using the phosphoramidites from Applied Biosystems following the standard protocols for DNA and RNA synthesis respectively. After assembly of the desired sequence, the controlled-pore glass (LCAA-CPG, 500Å) with attached fully protected oligonucleotide was transferred to a vial and conc. aq. ammonia (30 %, 2 ml) was added. The vial was heated at 55 °C for 10 h. The cooled products were then evaporated to dryness and redissolved in water (1.5 ml) and filtered (Nylon Acrodisc 0.2 μm pore size). Purification of the

deoxyoligonucleotides was carried out on COP cartridges.[39] The partly protected oligoribonucleotides were deprotected according to Wincott *et al.*[40] The crude sequences were then purified by reversed-phase HPLC 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 Eppendorf tube; it was then evaporated under reduced pressure. The residue was dissolved in sterile, deionised water (1 ml) and re-evaporated (3x). The samples were characterised by MS (data not shown).

The oligonucleotides **21** and **22** were prepared on controlled glass supports derivatized with protected 2'-*O*-propargylribonucleosides using dT phosphoramidite. The syntheses were carried out on a 0.2 μmol scale using 25 sec coupling time. Oxidation was carried out with 0.1 M iodine,[30] 0.02 M iodine[31] or 1M tBuOOH[32-38] (see Table 1). The protected oligonucleotides were cleaved from the support and deprotected as above. 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 treated and characterised as above.

The 2'-*O*-propargyloligoribonucleotides **23** and **24** were synthesised using a 1 μmol β-cyanoethyl phosphoramidite DNA cycle with an increased condensation wait time of 15 min and 0.5 M 1*H*-tetrazole in acetonitrile as activator. The oxidation step was carried out with 1M tBuOOH in isooctane/dichloroethane (1:2 v/v) with an oxidation time of 1 min.[41] After assembly of the desired sequence, the controlled-pore glass with attached fully protected oligonucleotides were transferred to a vial and conc. aq. ammonia (30 %, 2ml) was added. The vial was heated at 55 °C for 10 h. The cooled products were evaporated to dryness and dissolved in water (1.5 ml) and filtered (Nylon Acrodisc 0.2 μm pore size). The crude yields of 2'-*O*-propargyloligoribonucleotides were estimated spectrophotometrically to be 56 (**23**) and 63 ( **24**) $A_{260}$ units. The crude sequences were then purified by reversed-phase HPLC 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 Eppendorf tube; it was then evaporated under reduced pressure. The yields of **23** and **24** were estimated spectrophotometrically to be 36 and 48 $A_{260}$ units respectively. The residue was dissolved in sterile, deionised water (1 ml) and re-evaporated (3x). 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 sterile water (0.4 ml), extracted with ether (3x5 ml) and the water phase separated and evaporated under reduced pressure. The residue was dissolved in sterile, de-ionised water (1 ml) and re-evaporated (3x). The samples were characterised by MS (Table 2).

*Melting experiments*

Melting experiments were carried out in 1 ml of aq tris(hydroxymethyl)aminomethane·hydrochloride (10 mM, pH 7) containing KCl (0.1 M) and EDTA (1mM) using 3μM of each strand. The samples were preannealed by heating to 90 °C, then cooling slowly to room temperature. The melting transitions were measured using a Cary 3E UV/vis spectrophotometer fitted with a thermostatted cell block and temperature controller. 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 were obtained by taking the first derivative of the curves.

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