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Syntheses of 2′-*C*-amidoalkyl and 2′-*C*-cyanoalkyl containing oligodeoxyribonucleotides and assessment of their hybridisation affinity for complementary DNA and RNA

Lavinia Brennan,[a,*] Richard Cosstick,[a] Ian A. O'Neil[a] and Arthur Van Aerschot[b]

[a]*Robert Robinson Laboratories, Department of Chemistry, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK*
[b]*Rega Institute for Medical Research, Laboratory for Medicinal Chemistry, 10 Minderbroedersstraat, Leuven 3000, Belgium*

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Abstract—Oligodeoxynucleotides containing 2′-*C*-branched nucleosides with an amide or nitrile appended to either a one or two carbon alkyl chain have been synthesised. The phosphoramidites of the 2′-*C*-modified nucleosides were prepared and incorporated into the oligonucleotides using automated DNA synthesis. The duplex stability with complementary RNA and DNA was measured by UV melting experiments, in order to assess whether the amide/nitrile function could induce any duplex stability without the presence of the 2′-oxygen. The duplex stabilities of the oligonucleotides containing the 2′-*C*-modifications were decreased in the absence of the 2′-oxygen.

1. Introduction

Over the past decade a large number of nucleoside analogues has been synthesised with the aim of enhancing the properties of antisense oligonucleotides.[1–3] Ideally, a suitable antisense candidate should have some resistance towards nucleases, show increased duplex stability with complementary RNA[4,5] and have the ability to invoke RNase H activity.[6] Thus far, the most promising nucleic acid analogues for antisense therapy include LNA[7,8] and the 2′-*O*-(methoxyethyl) modification,[9] both of which display some nuclease resistance and an increased duplex stability with complementary RNA. However, such analogues fail to activate RNase H. The increased duplex stability displayed by these analogues has been attributed to these modifications causing a shift in the conformational equilibrium towards the C3′-*endo* sugar pucker. This shift is a result of the *gauche* effects between the ring oxygen and the electronegative 2′-*O*-substituent. This gives rise to a structure, which is more RNA-like, and thus forming stronger duplexes with complementary RNA.[5] In order to activate RNase H, the antisense oligonucleotide is required to be more DNA-like,[10,11] having a predominantly C2′-*endo* sugar pucker. This conformation is favoured when the 2′-oxygen is replaced by a less electronegative atom such as carbon. However, nucleoside analogues that adopt the DNA-type conformation have generally been shown to cause a decrease in duplex stability with complementary RNA.

Previously we have investigated the synthesis of several functionalised 2′-*C*-branched nucleosides and encouragingly, preliminary studies have shown that the incorporation of these 2′-*C*-modified residues into dinucleoside monophosphates confers considerable resistance to nucleases.[12] Related to this work, Sproat and co-workers have shown that 2′-*O*-(carbamoylmethyl) **1** containing oligonucleotides show a higher affinity for RNA than those containing simple 2′-*O*-alkyl or 2′-*O*-allyl modifications.[13] These results suggest that in addition to the known beneficial effect of the 2′-oxygen, the amide group itself has some stabilising effect on the duplex with complementary RNA. In order to further investigate this, we report the synthesis of 2′-*C*-branched nucleosides containing an amide appended to either a one **2** or two **3** carbon alkyl chain, followed by incorporation into oligonucleotides and assessment of the stability of duplexes formed with complementary RNA and DNA. This study should give an indication whether the effect of the amide alone is important for duplex stability or if the increased affinity is reliant on the presence of the 2′-oxygen.

Another recent report in the literature investigated the effect on binding affinity and nuclease resistance of 2′-*O*-cyanoethyl modifications.[14] Such modifications were found to be stabilising and it was suggested that this was due to the polarised nitrile bond inferring stability through H_2O bridge structures around the 2′-position.

Keywords: 2′-*C*-Modified nucleosides; Duplex stability; Antisense.
* Corresponding author at present address: Department of Chemistry, University of Southampton, Southampton SO17 1BJ, UK. Tel.: +44 23 8059 6786; fax: +44 23 8059 2291; e-mail: lb15@soton.ac.uk

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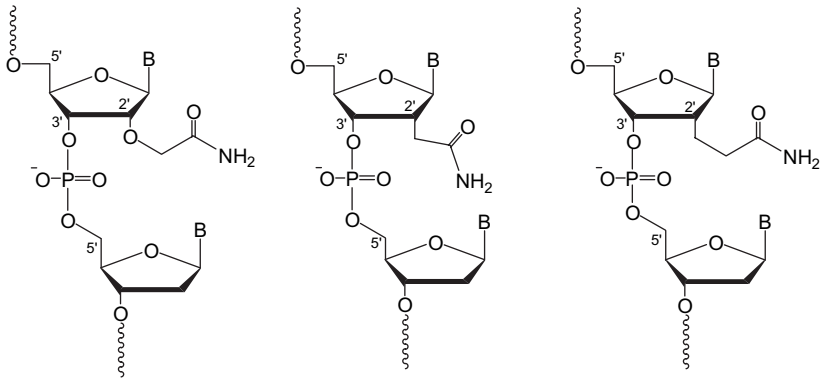


1 **2** **3**

B = uracil-1-yl

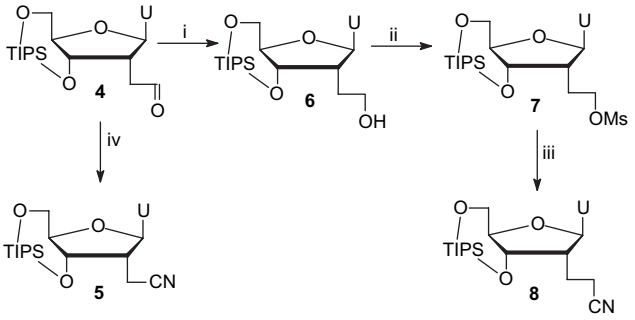
In this paper we also report the syntheses and binding studies of the 2′-*C*-cyanoethyl and 2′-*C*-cyanomethyl analogues and comment on the comparison between these and the corresponding 2′-*O*-cyanoethyl analogue.

2. Results and discussion

Previous attempts within this laboratory to prepare a 2′-*C*-amide-3′-phosphoramidite building block **24** for incorporation into oligonucleotides had given low yields. It was also observed that the amide group underwent dehydration in the presence of the oxidant during oligonucleotide synthesis.[12]

It has been shown by Sproat and co-workers[13] that oligonucleotides containing a 2′-*O*-nitrile can undergo aminolysis of the 2′-nitrile group by treatment with concentrated aqueous ammonia. In addition, it is possible to carry out this transformation under the standard conditions used for removal of base and phosphate protecting groups and simultaneous cleavage of the oligonucleotide from the solid-phase support. However, since completing this work, Sekine and co-workers[14] report that using a mixture of NH_4OH and NH_4OAc for deprotection and cleavage leaves the nitrile function intact.

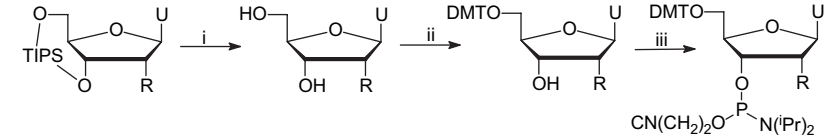
It was envisaged that a 2′-*C*-nitrile would be synthetically more accessible and that this may avoid any potential problems previously associated with the 2′-*C*-amide phosphoramidite. Post-synthetic treatment of the oligonucleotide with aqueous ammonia was expected to result in aminolysis of the 2′-*C*-nitrile to give the 2′-*C*-amide.



Scheme 1. Preparation of 2′-*C*-nitrile nucleosides. Reagents and conditions: (i) $NaBH_4$ in methanol, 99%; (ii) CH_3SO_2Cl in pyridine, 82%; (iii) NaCN in DMSO, 71% and (iv) (a) hydroxylamine hydrochloride in pyridine/chloroform (b) selenium dioxide 66%.

The syntheses of the target 2′-*C*-nitrile nucleosides **5** and **8** are illustrated in Scheme 1. Starting from the previously reported 2′-*C*-aldehyde[12] **4**, 2′-*C*-cyanomethyl nucleoside **5** was prepared using hydroxylamine hydrochloride followed by treatment with selenium dioxide.[15] Alcohol **6** was prepared by simple sodium borohydride reduction in methanol of the aldehyde **4**. Initial attempts to access nitrile **8** via a direct route using Mitsunobu conditions gave poor yields and generated many side products making purification difficult. It was found that accessing compound **8** via mesylate **7**, proved to be more facile and gave an overall higher yield.

The 2′-*C*-nitrile nucleosides **5** and **8** were prepared for incorporation into oligonucleotides by conversion to the corresponding 5′-*O*-dimethoxytrityl-3′-*O*-phosphoramidite monomers. The synthesis is shown in Scheme 2.



5 R = CH_2CN
8 R = $(CH_2)_2CN$
9 R = CH_2CONH_2
10 R = $(CH_2)_2CONH_2$
11 R = CH_2CO_2H

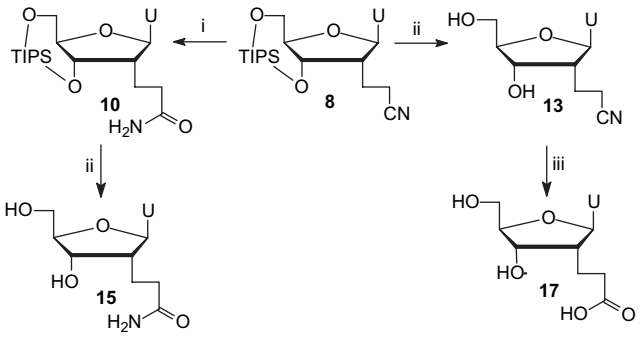
12 R = CH_2CN
13 R = $(CH_2)_2CN$
14 R = CH_2CONH_2
15 R = $(CH_2)_2CONH_2$
16 R = CH_2CO_2H

18 R = CH_2CN
19 R = $(CH_2)_2CN$
20 R = CH_2CONH_2
21 R = $(CH_2)_2CONH_2$

22 R = CH_2CN
23 R = $(CH_2)_2CN$
24 R = CH_2CONH_2
25 R = $(CH_2)_2CONH_2$

Scheme 2. Preparation of 5′-*O*-dimethoxytrityl-3′-*O*-phosphoramidite nucleosides. Reagents and conditions: (i) $NEt_3 \cdot 3HF$ in THF, 52–92%; (ii) dimethoxytrityl chloride in pyridine/DCM, 45–79% and (iii) 2-cyanoethyl-bis-*N*,*N*,*N*,*N*-diisopropylaminophosphoramidite, diisopropylammonium tetrazolide in CH_3CN, 44–56%.

The 2′-*C*-nitrile-5′-*O*-dimethoxytrityl-3′-*O*-phosphoramidites **22** and **23** were incorporated into deoxyoligonucleotides using standard automated solid-phase synthesis with the phosphoramidite method.[16–18] As previously mentioned, it has been reported in the literature[12] that by using the standard deprotection conditions (concentrated aqueous ammonia at 55 °C, 6 h is normal, although for convenience 16 h is often used), a 2′-*O*-nitrile undergoes aminolysis, to give the corresponding 2′-*O*-amide. Thus, we expected the same reaction to happen with our 2′-*C*-nitrile modification. Model studies performed on the free 2′-*C*-nitrile modified nucleoside had shown that when treated with aqueous ammonia (16 h), the nitrile had been converted to a mixture of the corresponding amide and carboxylic acid. Thus, to confirm successful aminolysis of the nitrile groups, within the oligonucleotides, the final deprotected oligonucleotides were analysed by high-resolution mass spectrometry as well as by enzyme digestion of the oligonucleotide to its constituent nucleosides, followed by HPLC analysis. For the HPLC analysis, standards of the 2′-*C*-nitriles, 2′-*C*-amide and 2′-*C*-carboxylic acids were required. The preparation of the 2′-*C*-nitriles, **12** and **13** has already been discussed. The syntheses of the 2′-*C*-carboxymethyluridine **16** and 2′-*C*-amidomethyluridine **14** are described in a previous publication.[12] The syntheses of the standards **15** and **17** are shown below in Scheme 3.



Scheme 3. Preparation of standards for HPLC. Reagents and conditions: (i) MnO_2/SiO_2 in toluene, 40%; (ii) $NEt_3 \cdot 3HF$ in THF, 92% and (iii) 2 M NaOH.

The purified unmodified oligonucleotide **26**, together with the oligonucleotides prepared with the phosphoramidites containing the 2′-*C*-nitrile modification **27** and **28** were characterised by high-resolution mass spectrometry (Table 1). In the case of oligonucleotides **27** and **28**, the mass spectrum data indicates that the nitrile function remains intact during the extended deprotection and does not undergo aminolysis.

To confirm the presence of the 2′-*C*-nitrile-containing nucleosides, oligonucleotides **27** and **28** were then treated with snake venom phosphodiesterase (SVPDE) and alkaline phosphatase (AP). The resulting mixture of nucleosides was analysed by reverse-phase HPLC, using conditions that had been found to separate all the constituent nucleosides (see Supplementary data). Previously within the group dinucleotides containing either a 2′-*C*-amide or 2′-*C*-nitrile function were found to have an increased resistance to hydrolysation by SVPDE as compared to natural dinucleotides.[12]

Table 1. Characterisation of oligonucleotides by electrospray mass spectrometry

Oligo No.	DNA sequence	Mass calcd	Mass found
26	5′-CCTAAAT-U-TGCC-3′	3564.6	3564.5
27	5′-CCTAAAT-X_1-TGCC-3′	3603.6	3603.6
28	5′-CCTAAAT-X_2-TGCC-3′	3617.6	3617.8
29	5′-CCTAAAT-X_3-TGCC-3′	3635.7	3635.8

26 U=2′-deoxyuridine, **27** X_1=2′-CH_2CN, **28** X_2=2′-CH_2CH_2CN, **28** X_3=2′-$CH_2CH_2CONH_2$. Oligonucleotides **27**, **28** and **29** result from the incorporation of phosphoramidites **22**, **23** and **25**, respectively.

The HPLC analysis of both oligonucleotides **27** and **28**, confirmed that in both cases the nitrile functions had not undergone aminolysis. The transformation was also attempted with methanolic ammonia, however, again no conversion of the nitrile was evident. Initially it was hoped that employing the standard deprotection condition of 33% aqueous ammonia at 55–60 °C for 16 h would be sufficient enough to transform the nitrile into an amide. However, this was clearly not the case and the results were surprising, since preliminary HPLC analysis of the monomer 2′-*C*-nitrile had shown evidence of partial aminolysis followed by complete saponification upon treatment with 33% aqueous ammonia. In addition, it had been previously reported that 2′-*O*-nitrile, which had been incorporated into an oligonucleotide and treated in the same manner had in fact, undergone aminolysis to give the 2′-*O*-amide.

It is well documented that the presence of the carbon at the 2′-position—as opposed to oxygen—gives the sugar pucker of the modified nucleoside a predominantly C2′-*endo* conformation due to loss of *gauche* interactions between O3′ and O4′. The difference in conformation is likely to change the orientation of the nitrile group from that in the 2′-*O*-analogue. It may be noted that this change in orientation is enough to prevent nucleophilic attack of ammonia, for example, electrostatic repulsion may come from the anionic oxygen of the phosphate linkage. Although problems were envisaged with the incorporation of a 2′-*C*-amide into an oligonucleotide, it was decided to synthesise and directly incorporate the 2′-*C*-amide-5′-*O*-dimethoxytrityl-3′-*O*-phosphoramidites. Thus the compounds **24** and **25** were synthesised as shown in Scheme 2.

Once the 2′-*C*-phosphoramidites had been successfully prepared, incorporation into the oligonucleotide of the same sequence (3′-CCGTUTAAATCC-5′) as used for the 2′-*C*-nitriles was attempted, using automated solid-phase synthesis as before. The incorporation of the 2′-*C*-amidoethyl **25** proved to be successful, although the trityl assays showed that the coupling efficiency was quite low. However, coupling of the 2′-*C*-methylamide proved to be unsuccessful.

Previous attempts had been made to couple the 2′-*C*-amidomethyl-phosphoramidite **24** into a dinucleotide using solution-phase chemistry. This study had shown that during the oxidation step, partial dehydration of the 2′-*C*-amide had resulted in the 2′-*C*-nitrile.[12] A mechanism for this reaction had been proposed whereby a seven-membered ring intermediate had been formed in the presence of an activator such as tetrazole or DCI. Such a side reaction could clearly interfere with the coupling of the 2′-*C*-amidomethyl-3′-*O*-phosphoramidite **24**, and could therefore account for the low yield obtained.

A similar mechanism can be proposed for the 2′-*C*-amidoethyl-3′-phosphoramidite nucleoside, however, in this case intramolecular cyclisation would lead to the less stable eight-membered ring and would therefore be less favoured. Nevertheless it cannot be ruled out since it would explain why the coupling efficiency for the incorporation of the 2′-*C*-amidoethyl nucleoside was lower than expected. The fact that the 2′-*C*-cyanoethyl coupled well, indicates that factors other than steric hindrance are involved.

Duplex formation with the modified sequences was examined by mixing each oligomer with its complementary DNA or RNA strand and determining the melting temperatures (T_m)[19] of the hybrids by temperature-dependent UV spectrophotometry. Table 2 shows the T_m after incorporation of the single modification X_1, X_2 or X_3 into DNA oligomers.

Table 2. Sequences (5′-CCTAAATXTGCC-3′, 3′-GGATTTAAACGG-5′) and stability studies of the different duplexes carrying a single sugar modification

	DNA complement		RNA complement (0.1 M)
	0.1 M	1.0 M	
26	46.0	54.4	39.0
27	32.5	41.4	30.5
28	33.7	42.0	31.6
29	34.4	42.8	31.4

T_m values were determined at 260 nm in KH_2PO_4 buffer (20 mM, pH 7.5) containing EDTA (0.1 mM) and NaCl (either 0.1 M or 1.0 M), with 4 μM of each strand. Oligo **26**, X=U; Oligo **27**, X=2′-CH_2CN; **28**, X=2′-CH_2CH_2CN and **29** X=2′-$CH_2CH_2CONH_2$.

The data show that, oligonucleotides **27**–**29** incorporating the 2′-*C*-modifications, result in a decrease in duplex stability with complementary DNA and RNA as compared to the unmodified sample **26**. This is consistent with other studies, which have shown that in general 2′-*C*-modifications lead to a lower T_m. This is known to be as a result of the absence of the 2′-*O* (or similar electronegative group), which is able to adopt a favourable C3′-*endo* conformation as a result of *gauche* interaction with O4′. In the DNA–DNA duplex at low salt concentration the amidoethyl modification **29** gives a slightly higher T_m than the corresponding nitrile **28**. Interestingly, comparing samples **27** and **28** the longer chain length does not seem to have a destabilising effect than for the same modification with one carbon less. This is not in agreement with previous studies on 2′-*O*-alkyl modifications, which have shown an inverse relationship between chain length and binding affinity for complementary RNA.[20] The oligos **26**–**29** have higher duplex stabilities with their DNA complement than with their RNA complement. Generally the stability of duplexes is in the order RNA/RNA>DNA/RNA>DNA/DNA,[21] however, this can be sequence dependent. Often oligonucleotides containing AT rich stretches—as in this case and others[22,23]—have been found to show greater stability with their DNA complement than with their RNA complement.

These results are in agreement with other studies, which have shown that 2′-*O* atom is essential for an increase in duplex stability and highlight that either an amide group or a nitrile group alone seem to have no significant effect.

Some modifications such as the 2′-*O*-methoxyethyl[9] are known to result in a significantly higher duplex stability, thought to be due to an extended *gauche* effect between O4′–2′-*O* and the oxygen of the methoxyethyl chain. This 2′-substituent is also thought to aid hydration, adding further stabilisation.[24] Given that the 2′-*O*-amide and 2′-*O*-nitrile have also resulted in a significant increase in duplex stability, there could be a possibility that of such modifications to contribute to such an extended *gauche* effect or similar stabilising effect due to an increase in hydration.

3. Experimental

Most of the general analytical procedures have been described in an earlier publication.[12] NMR spectra were recorded at the field strength indicated, and peaks displaying a multiplicity due to the presence of two diastereoisomers are denoted with an asterisk. HPLC was conducted using a Gilson HPLC, equipped with a Gilson 321 pump, Gilson 170 diode array detector, recording at 260 nm and a Gilson 234 auto injector. Operated using Unipoint version 3.0 software. Unless otherwise stated, analyses were performed on a Hichrom (250×4.6 mm) column packed with kromasil 100-C_{18}. Eluent gradient employed *A*=50 mM potassium phosphate (pH 7), *B*=60% A/40% CH_3CN.

Snake venom phosphodiesterase (*Crotalus adamanteus*) was purchased as lyophilised powder containing ~35% Tris buffer salts from Sigma (0.5 units) dissolved in 100 μl of Tris buffer prior to use. Alkaline phosphatase (*Bovine Intestinal Mucosa*) was purchased as a solution (1,000 DEA units containing 50% glycerol, pH 7). Dissolved in 80 μl of 50:50 glycerol/water prior to use.

3.1. Synthesis of monomers

3.1.1. 2′-Deoxy-2′-α-*C*-(2-oxoethyl)-3′,5′-*O*-(1,1,3,3,-tetraisopropyldisiloxy)uridine (4). As described in previous publication.[12] 81%; ^{1}H NMR δ (200 MHz, $CDCl_3$) 0.92–1.08 (28H, m, 4×^{i}Pr), 2.67 (1H, m, H6′), 2.91–3.03 (2H, m, H2′, H6″), 3.90–3.98 (1H, m, H4′), 4.03–4.97 (2H, m, H5′, H5″), 4.55 (1H, t, H3′), 5.72–5.76 (2H, m, H1′, H5), 7.65 (1H, d, J_{6-5}=8.24 Hz), 9.55 (1H, br s, NH), 9.80 (1H, s, H7″). ^{13}C NMR δ (75 MHz, $CDCl_3$) 12.63–13.31 (*C*H(CH_3)$_2$×4), 16.84–17.94 (*C*H_3×8), 40.38 (C6′), 43.48 (C2′), 61.20 (C5′), 69.51 (C3′), 84.03 (C4′), 88.49 (C1′), 102.33 (C5), 139.14 (C6), 150.32 (C2), 163.08 (C4), 194.46 (C7′). Found HRMS *m/z* (FAB+) $[M+H]^+$ 513.2451, $C_{23}H_{41}N_2O_7Si_2$ requires 513.2452. Found C, 53.76; H, 7.93; N, 5.38. $C_{23}H_{40}N_2O_7Si_2$ requires C, 53.88; H, 7.87; N, 5.47.

3.1.2. 2′-Deoxy-2′-α-*C*-(cyanomethyl)-3′,5′-*O*-(1,1,3,3,-tetraisopropyldisiloxy)uridine (5). Compound **4** (800 mg; 1.56 mmol) was dissolved in chloroform (~3 ml). To this was added pyridine (1 equiv; 1.56 mmol; 0.13 ml) drop wise with stirring. Hydroxylamine hydrochloride was added (1.1 equiv; 1.72 mmol; 119 mg) and the mixture heated to reflux. After 3 h TLC [hexane/EtOAc (50%)] showed no starting material present. SeO_2 (1 equiv; 1.56 mmol; 173 mg) was added and the reaction was left to cool to room temperature and then stirred overnight. After which time TLC

[hexane/EtOAc (50%)] showed the reaction to be complete. Solvent was removed in vacuo and EtOAc (50 ml) was added, this was washed with $NaHCO_3$ (2×50 ml) and NaCl (2×50 ml). All organic layers were dried over $MgSO_4$. Solvent was removed in vacuo to give crude product as a yellow foam, which was then purified by column chromatography on silica gel, eluting with [hexane/EtOAc (50%)] to give product **5** as a white foam (523 mg; 66%). ^{1}H NMR δ (200 MHz, $CDCl_3$) 1.00–1.08 (28H, m, 4×^{i}Pr), 2.72–2.75 (3H, m, H2′, H6′, H6″), 4.05 (3H, m, H4′, H5′, H5″), 4.58 (1H, m, H3′), 5.75 (1H, d, H5, J_{5-6}=7.7 Hz, 1H, d, H1′), 7.67 (1H, d, H6, J_{6-5}=8.24 Hz). ^{13}C NMR δ (75 MHz, $CDCl_3$) 13.04–13.75 (*C*H(CH$_3$)$_2$×4), 15.75 (C6′), 17.18–17.79 (*C*H$_3$×8), 45.86 (C2′), 61.06 (C5′), 69.13 (C3′), 83.87 (C4′), 88.64 (C1′), 102.66 (C5), 118.18 (*C*N), 139.21 (C6), 150.64 (C2), 163.62 (C4). Found HRMS *m/z* [M+H$^+$], 510.2447, $C_{23}H_{40}N_3O_6Si_2$ requires 510.2455.

3.1.3. 2′-Deoxy-2′-α-*C*-(2-hydroxyethyl)-3′,5′-*O*-(1,1,3,3,-tetraisopropyldisiloxy)uridine (6). Compound **4** (4.46 g; 8.71 mmol) was dissolved in MeOH (50 ml) and the solution was cooled to 0 °C. $NaBH_4$ (3 equiv; 26.31 mmol; 0.99 g) was added in small portions. The mixture was stirred for 25 min at 0 °C, after this time TLC [DCM/MeOH (10%)] showed the reaction to be complete. The reaction was quenched by addition of citric acid crystals until neutral pH was reached. Solvent was removed in vacuo to give a white foam (4.44 g; 99%). ^{1}H NMR revealed no further purification was required. ^{1}H NMR δ (200 MHz, $CDCl_3$) 0.99–1.09 (28H, m, 4×^{i}Pr), 1.66 (1H, m, H6′), 2.14 (1H, m, H6″), 2.46 (1H, m, H2′), 3.88–3.93 (3H, m, H4′, H7′, H7″), 3.99 (1H, m, H5′), 4.22 (1H, d, H5″), 4.43 (1H, t, H5′), 5.72 (1H, d, H5, J_{5-6}=8.2 Hz), 5.87 (1H, s, H1′), 7.98 (1H, d, H6, J_{6-5}=7.96 Hz), 9.25 (1H, br s, NH). ^{13}C NMR δ (75 MHz, $CDCl_3$) 12.87–13.77 (*C*H(CH$_3$)$_2$×4), 17.32–17.92 (*C*H$_3$×8), 28.18 (C6′), 47.17 (C2′), 60.10 (C7′), 61.07 (C5′), 67.57 (C3′), 83.38 (C4′), 89.39 (C1′), 102.15 (C5), 140.04 (C6), 151.32 (C2), 163.91 (C4). Found HRMS *m/z* (FAB+) [M+H]$^+$ 515.2606, $C_{23}H_{43}N_2O_7Si_2$ requires 515.2609. Found C, 53.64; H, 8.25; N, 5.38. $C_{23}H_{42}N_2O_7Si_2$ requires C, 53.67; H, 8.23; N, 5.45.

3.1.4. 2′-Deoxy-2′-α-*C*-(2-methylsulfonyloxyethyl)-3′,5′-*O*-(1,1,3,3,-tetraisopropyldisiloxy)uridine (7). Compound **6** (4.44 g; 8.63 mmol) was co-evaporated with dry pyridine (2×10 ml) and then dissolved in dry pyridine (50 ml) and cooled to 0 °C under argon. Mesyl chloride (2 equiv; 12.27 mmol; 1.34 ml) was added drop wise and the solution was stirred at room temperature for 1 h (by which time the solution had become dark brown in colour). After this time TLC [DCM/MeOH (10%)] showed the reaction to be complete. A few drops of H_2O were added to quench the reaction and then the pyridine was removed in vacuo by co-evaporation with toluene. The resulting residue was taken up in DCM (50 ml) and washed with $NaHCO_3$ (2×50 ml) and NaCl (2×50 ml). All organic layers were dried over $MgSO_4$. Solvent was removed in vacuo to give crude product as an orange oil, which was then purified by column chromatography on silica gel, eluting with [DCM/MeOH (0→3%)] to give product **7** as a white foam (4.2 g; 82%). ^{1}H NMR δ (200 MHz, $CDCl_3$) 1.00–1.08 (28H, m, 4×^{i}Pr), 1.88 (1H, m, H2′), 2.37 (2H, m, H6′, H6″), 3.05 (3H, s, S–C*H*$_3$), 3.85–3.93 (1H, m, H4′), 4.00 (1H, d, H5′), 4.18 (1H, dd, H5″), 4.42–4.49 (3H, m, H3′, H7′, H7″), 5.70 (1H, d, H5, J_{5-6}=8.24 Hz), 5.75 (1H, s, H1′), 7.82 (1H, d, H6, J_{6-5}=8.24 Hz), 9.37 (1H, br s, NH). ^{13}C NMR δ (75 MHz, $CDCl_3$) 12.55–13.37 (*C*H(CH$_3$)$_2$×4), 16.96 (*C*H$_3$×8), 25.95 (C6′), 37.35 (C2′), 45.21 (*C*H$_3$–S), 60.11 (C5′), 67.95 (C3′), 68.13 (C7′), 83.13 (C4′), 88.74 (C1′), 101.94 (C5), 139.38 (C6), 150.37 (C2), 163.40 (C4). Found HRMS *m/z* (FAB+) [M+H]$^+$ 593.2375, $C_{24}H_{45}N_2O_9SSi_2$ requires 593.2384.

3.1.5. 2′-Deoxy-2′-α-*C*-(2-cyanoethyl)-3′,5′-*O*-(1,1,3,3,-tetraisopropyldisiloxy)uridine (8). Compound **7** (315 mg; 0.53 mmol) was dissolved in the minimum amount of dry DMSO (~3 ml). The solution was stirred under argon and heated to 60 °C then NaCN (2 equiv; 1.06 mmol; 52 mg) was added and the mixture heated to 80 °C. After 15 min TLC [hexane/EtOAc (40%)] revealed the reaction to be complete. The reaction was allowed to cool and EtOAc (30 ml) was added, this was washed with $NaHCO_3$ (2×20 ml) and NaCl (2×20 ml). All organic layers were dried over $MgSO_4$. Solvent was removed in vacuo to give crude product as a yellow oil, which was then purified by column chromatography on silica gel, eluting with [hexane/EtOAc (0→40%)] to give product **8** as a white foam (200 mg; 71%). ^{1}H NMR δ (300 MHz, $CDCl_3$) 0.79–0.88 (28H, m, 4×^{i}Pr), 1.56–1.62 (2H, m, H7′, H7″), 2.00–2.11 (2H, m, H6′, H6″), 2.38–2.57 (1H, m, H2′), 3.65 (1H, d, H4′, $J_{4'-5'}$=8.7 Hz), 3.77 (1H, dd, H5′, $J_{5'-5''}$=13.5 Hz, $J_{5'-4'}$=8.7 Hz), 3.98 (1H, d, H5″ $J_{5''-5'}$=13.2 Hz), 4.27 (1H, t, H3′), 5.46 (1H, s, H1′), 5.59 (1H, d, H5, J_{5-6}=8.1 Hz), 7.60 (1H, d, H6, J_{6-5}=8.1 Hz), 8.86 (1H, br s, NH). ^{13}C NMR δ (75 MHz, $CDCl_3$) 12.56–13.38 (*C*H(CH$_3$)$_2$×4), 15.46 (C7′), 16.83–17.44 (*C*H$_3$×8), 22.44 (C6′), 47.89 (C2′), 59.91 (C5′), 67.90 (C3′), 83.02 (C4′), 88.48 (C1′), 101.95 (C5), 119.60 (*C*N), 139.12 (C6), 150.16 (C2), 163.08 (C4). Found HRMS *m/z* [M+H$^+$], 524.2608, $C_{24}H_{42}N_3O_6Si_2$ requires 524.2612. IR (CH_2Cl_2) 3398 cm^{-1} (NH), 2872 cm^{-1} (CH), 2250 cm^{-1} (CN), 1695 cm^{-1} (CO).

3.1.6. 2′-Deoxy-2′-α-*C*-(amidomethyl)-3′,5′-*O*-(1,1,3,3,-tetraisopropyldisiloxy)uridine (9). As described in previous publication.[12] ^{1}H NMR δ (400 MHz, $CDCl_3$) 1.01–1.10 (28H, m, 4×^{i}Pr), 2.47 (1H, dd, H6′, $J_{6'-2'}$=10.34 Hz, $J_{6'-6''}$=17.4 Hz), 2.63–2.73 (2H, m, H2′, H6″), 3.90 (1H, dd, H5′, $J_{5'-4'}$=7.3 Hz, $J_{5'-5''}$=11.6 Hz), 3.97 (1H, m, H4′), 4.08 (1H, dd, H5″, $J_{5''-4'}$=3.4 Hz, $J_{5''-5'}$=11.5 Hz), 4.53 (1H, dd, H3′, $J_{3''-4'}$=5.0 Hz, $J_{3'-2'}$=7.1 Hz), 5.75 (1H, d, H5, J_{5-6}=8.3 Hz), 5.95 (1H, d, H1′, $J_{1'-2'}$=6.1 Hz), 6.00 (1H, br s, NH-amide), 6.70 (1H, br s, NH-amide), 7.47 (1H, d, H6, J_{6-5}=8.1 Hz), 9.89 (1H, br s, NH-uracil). ^{13}C NMR δ (75 MHz, $CDCl_3$) 12.61–13.53 (*C*H(CH$_3$)$_2$×4), 16.92–17.53 (*C*H$_3$×8), 31.74 (C6′), 44.93 (C2′), 62.84 (C5′), 72.10 (C3′), 85.30 (C4′), 88.14 (C1′), 103.15 (C5), 139.18 (C6), 151.40 (C2), 163.19 (C4), 173.88 (C7′). Found HRMS *m/z* [M+H$^+$], 528.2557, $C_{23}H_{41}N_2O_8Si_2$ requires 528.2561.

3.1.7. 2′-Deoxy-2′-α-*C*-(2-amidoethyl)-3′,5′-*O*-(1,1,3,3,-tetraisopropyldisiloxy)uridine (10). Compound **8** (200 mg; 0.38 mmol) was dissolved in toluene (~5 ml) and a freshly prepared sample of $MnO_2/SiO_2\cdot H_2O$* (9.2 equiv; 3.48 mmol; 4.2 g) was added. The mixture was heated to

reflux and stirred under argon for 24 h. After this time, TLC [DCM/MeOH (10%)] showed reaction to be complete. Solvent was evaporated in vacuo and the residue was dissolved in MeOH and filtered under suction and then re-filtered under gravity to remove as much of the silica as possible. Solvent was removed in vacuo to give crude product as a white foam, which was then purified by column chromatography on silica gel, eluting with [DCM/MeOH (0→4%)] to give product **10** as a white foam (81 mg; 40%). According to Lui et al.[25] each gram of supported reagent is found to contain 0.83 mmol of MnO_2 (as determined by titration against sodium oxalate and potassium permanganate) and 3.1 g of free H_2O (as determined by Karl–Fischer method). ^{1}H NMR δ (400 MHz, $CDCl_3$) 1.00–1.09 (28H, m, 4×^{i}Pr), 1.71 (2H, m, H7′, H7″), 2.17 (1H, m, H6′), 2.25 (1H, m, H6″), 2.56 (1H, m, H2′), 2.70 (1H, m, H4′), 3.94 (1H, d, H5′, $J_{5'-5''}$=16 Hz), 4.21 (1H, d, H5″, $J_{5''-5'}$=16 Hz), 4.42 (1H, t, H3′), 5.57 (1H, br s, NH), 5.59 (1H, d, H5, J_{5-6}=8 Hz), 5.74 (1H, s, H1′), 6.68 (1H, br s, NH), 7.96 (1H, d, H6, J_{6-5}=8 Hz), 10.68 (1H, br s, NH). ^{13}C NMR δ (75 MHz, $CDCl_3$) 12.05–13.38 (*C*H(CH_3)$_2$×4), 16.44–17.08 (*C*H_3×8), 19.72 (C6′), 32.10 (C7′), 48.37 (C2′), 59.44 (C5′), 66.99 (C3′), 82.52 (C4′), 8.60 (C1′), 101.75 (C5), 139.47 (C6), 151.37 (C2), 163.61 (C4), 175.62 (C8′). Found HRMS *m*/*z* [M+Na$^+$], 564.25542, $C_{24}H_{43}N_3O_7Si_2Na$ requires 564.25373.

3.1.8. 2′-Deoxy-2′-α-*C*-(carboxymethyl)-3′,5′-*O*-(1,1,3,3,-tetraisopropyldisiloxy)uridine (11). As described in previous publication.[12] ^{1}H NMR δ (400 MHz, $CDCl_3$) 1.02–1.10 (28H, m, 4×^{i}Pr), 2.46 (1H, dd, H6′, $J_{6'-2'}$=10.8 Hz, $J_{6'-6''}$=14.9 Hz), 2.74–2.84 (2H, m, H2′, H6″), 3.87 (1H, m, H4′), 4.00–4.10 (2H, m, H5′, H5″), 4.50 (1H, t, H3′, $J_{3'-2'}$=7.7 Hz, $J_{3'-4'}$=3.9 Hz), 5.76 (1H, d, H5, J_{5-6}=8.1 Hz), 5.93 (1H, d, H1′, $J_{1'-2'}$=3.2 Hz), 7.65 (1H, d, H6, J_{6-5}=8.1 Hz), 10.60 (1H, br s, NH). ^{13}C NMR δ (75 MHz, $CDCl_3$) 12.99–13.79 (*C*H(CH_3)$_2$×4), 17.24–17.81 (*C*H_3×8), 31.56 (C6′), 46.34 (C2′), 61.32 (C5′), 69.47 (C3′), 84.04 (C4′), 88.95 (C1′), 102.96 (C5), 139.66 (C6), 151.51 (C2), 164.34 (C4). Found HRMS *m*/*z* [M+H$^+$], 529.2396, $C_{23}H_{42}N_2O_8Si_2$ requires 529.2401.

3.2. General procedure for the removal of the 1,1,3,3-tetraisopropyldisiloxy protecting group

The TIPS-protected nucleoside (0.45 mmol) was dissolved in dry THF (30 ml), and to this was added $NEt_3\cdot 3HF$ (0.08 mL, 5 mmol). The solution was left for 8–16 h and monitored by TLC. The solution was concentrated in vacuo, diluted with methanol (20 ml) and evaporated onto silica gel; the product was subsequently isolated by column chromatography (DCM containing an increasing gradient of MeOH from 0–10%) as a colourless oil or white foam.

3.2.1. 2′-Deoxy-2′-α-*C*-(cyanomethyl)uridine (12). 52%; ^{1}H NMR δ (200 MHz, MeOD) 2.59–2.79 (3H, m, H2′, H6′, H6″), 3.75 (2H, d, H5′, H5″, $J_{5'-4'}$=3.28 Hz), 4.03 (1H, m, H4′), 4.32 (1H, m, H3′), 5.74 (1H, d, H5, J_{5-6}=8.24 Hz), 6.07 (1H, d, H1′, $J_{1'-2'}$=8.24 Hz), 7.97 (1H, d, H6, J_{6-5}=8.24 Hz). ^{13}C NMR δ (100 MHz, MeOD) 14.11 (C6′), 47.13 (C2′), 63.55 (C5′), 73.93 (C3′), 89.27 (C4′), 89.47 (C1′), 103.85 (C5), 119.96 (*C*N), 142.49 (C6), 153.06 (C2), 166.41 (C4). Found HRMS *m*/*z* [M+H$^+$], 268.0933, $C_{11}H_{14}N_3O_5$ requires 268.0931.

3.2.2. 2-Deoxy-2′-α-*C*-(2-cyanoethyl)uridine (13). 83%; ^{1}H NMR δ (400 MHz, MeOD) 1.73–1.77 (1H, m, H7′), 2.05–2.10 (1H, m, H7″), 2.36–2.44 (1H, m, H6′), 2.54–2.56 (2H, m, H2′, H6″), 3.37 (2H, m, H5′, H5″), 4.01 (1H, m, H4′), 4.34 (1H, d, H3′, $J_{3'-4'}$=4.92 Hz), 4.52 (1H, br s, OH), 5.76 (1H, d, H5, J_{5-6}=8.08 Hz), 6.05 (1H, d, H1′, $J_{1'-2'}$=8.56 Hz), 8.03 (1H, d, H6, J_{6-5}=7.96 Hz). ^{13}C NMR δ (75 MHz, MeOD) 16.03 (C6′), 22.15 (C7′), 48.78 (C2′), 63.66 (C5′), 73.33 (C3′), 89.33 (C4′), 89.54 (C1′), 103.79 (C5), 121.19 (*C*N), 142.73 (C6), 153.05 (C2), 166.38 (C4). Found HRMS *m*/*z* [M+H$^+$], 282.1091, $C_{12}H_{16}N_3O_5$ requires 282.1090.

3.2.3. 2-Deoxy-2′-α-*C*-(amidomethyl)uridine[12] (14). 92%; ^{1}H NMR δ (200 MHz, MeOD) 2.43 (1H, m, H6′), 2.68 (2H, m, H2′, H6″), 3.75 (2H, d, H5′, H5″, $J_{5'-4'}$=3.3 Hz), 3.99 (1H, m, H4′), 4.34 (1H, m, H3′), 5.72 (1H, d, H5, J_{5-6}=8.24 Hz), 6.04 (1H, d, H1′, $J_{1'-2'}$=8.26 Hz), 7.98 (1H, d, H6 J_{6-5}=7.98 Hz).

3.2.4. 2-Deoxy-2′-α-*C*-(2-amidoethyl)uridine (15). 83%; ^{1}H NMR δ (400 MHz, MeOD) 1.73–1.77 (1H, m, H7′), 2.05–2.10 (1H, m, H7″), 2.36–2.44 (1H, m, H6′), 2.54–2.56 (2H, m, H2′, H6″), 3.37 (2H, m, H5′, H5″), 4.01 (1H, m, H4′), 4.34 (1H, d, H3′, $J_{3'-4'}$=4.92 Hz), 4.52 (1H, br s, OH), 5.76 (1H, d, H5, J_{5-6}=8.08 Hz), 6.05 (1H, d, H1′, $J_{1'-2'}$=8.56 Hz), 8.03 (1H, d, H6, J_{6-5}=7.96 Hz). ^{13}C NMR δ (75 MHz, MeOD) 16.03 (C6′), 22.15 (C7′), 48.78 (C2′), 63.66 (C5′), 73.33 (C3′), 89.33 (C4′), 89.54 (C1′), 103.79 (C5), 121.19 (*C*N), 142.73 (C6), 153.05 (C2), 166.38 (C4). Found HRMS *m*/*z* [M+H$^+$], 282.1091, $C_{12}H_{16}N_3O_5$ requires 282.1090.

3.2.5. 2-Deoxy-2′-α-*C*-(carboxymethyl)uridine[12] (16). 67%; ^{1}H NMR δ (400 MHz, MeOD) 2.37 (1H, dd, H6′, $J_{6'-2'}$=6.52 Hz, $J_{6'-6''}$=16 Hz), 2.65 (1H, dd, H6″, $J_{6''-2'}$=7.80 Hz, $J_{6''-6'}$=16 Hz), 2.72–2.76 (1H, m, H2′), 3.76 (2H, d, H5′, H5″, $J_{5'-4'}$=3.68 Hz), 4.00–4.02 (1H, m, H4′), 4.33 (1H, dd, H3′, J=1.28 Hz, J=2 Hz), 4.52 (1H, br s, OH), 5.73 (1H, d, H5, J_{5-6}=8.12 Hz), 6.07 (1H, d, H1′, $J_{1'-2'}$=8.5 Hz), 7.95 (1H, d, H6, J_{6-5}=8.08 Hz). ^{13}C NMR δ (75 MHz, MeOD) 31.82 (C6′), 47.06 (C2′), 63.95 (C5′), 74.69 (C3′), 89.43 (C4′), 89.88 (C1′), 103.65 (C5), 143.05 (C6), 152.65 (C2), 166.50 (C4), 176.97 (C7′).

3.2.6. 2-Deoxy-2′-α-*C*-(2-carboxyethyl)uridine (17). Compound **13** (10 mg) was dissolved in 2 M aqueous sodium hydroxide solution (1 ml) and stirred at room temperature for 7 days. The reaction progress was followed by reverse-phase HPLC. After one week no starting material remained and HPLC revealed a single peak with a retention time of 8.74 min. The product **17** was collected (~10 mg). ^{1}H NMR [†]δ (400 MHz, MeOD) 1.08–1.14 (1H, m, H7′), 1.30–1.38 (3×[HNCH$_2$C*H*$_3$]$^+$), 1.63 (1H, br s, H7″), 1.98–2.27 (3H, m, H6′, H6″, H2′), 3.17–3.21 (3×[HNC*H*$_2$CH$_3$]$^+$),

[†] Product was characterised by accurate mass spectrometry and ^{1}H NMR. ^{13}C NMR data were also collected, however due to the weak sample concentration quaternary carbons were not seen on the spectra and therefore only selected data are reported.

3.73 (2H, d, H5′, H5″, $J_{5'-4'}$=2.16 Hz), 4.00 (1H, s, H4′), 4.30 (1H, s, H3′), 5.74 (1H, d, H5, J_{5-6}=6.42 Hz), 6.04 (1H, s, H1′), 7.97 (1H, d, H6, J_{6-5}=6.70 Hz). ^{13}C NMR †δ (75 MHz, MeOD) 31.11 (C6′), 48.27 (C2′), 50.80 (C7′), 64.10 (C5′), 73.95 (C3′), 89.14 (C4′), 90.00 (C1′), 103.64 (C5), 143.07 (C6). Found HRMS *m/z* (FAB−) $[M-H]^+$ 299.08876, $C_{12}H_{14}N_2O_7$ requires 299.08793.

3.3. General procedure for the preparation of 5′-*O*-dimethoxytrityl ethers

The nucleoside (150 mg; 0.61 mmol) was dissolved in dry pyridine (1.5 ml). To this, a solution of dimethoxytrityl chloride (1.7 equiv; 1.03 mmol; 349 mg) in dry pyridine and dry DCM (1:3.5) (1 ml) was added drop wise over a period of 20 min. The solution was stirred under argon for 4 h, after this time, TLC [DCM/MeOH (10%)] showed the reaction to be complete. The reaction was quenched by addition of methanol (~0.5 ml), followed by addition of $NaHCO_3$ (0.5 ml). Solvent was removed in vacuo and residue was taken up in EtOAc (~5 ml). The mixture was then washed with $NaHCO_3$ (2×5 ml) and NaCl (2×5 ml). All organic layers were dried over $MgSO_4$. Solvent was removed in vacuo to give crude product, which was purified by column chromatography on silica gel, eluting with [DCM/MeOH (0→3%)] to give the product as a white foam.

3.3.1. 2′-Deoxy-2′-α-*C*-(cyanomethyl)-5′-*O*-dimethoxytrityluridine (18). 58%; ^{1}H NMR δ (400 MHz, MeOD) 2.64 (2H, m, H2′, H6′), 2.82 (1H, m, H6″), 3.47 (3H, m, H4′, 2×H5′), 3.71 (1H, m, H3′), 3.79 (6H, s, OCH_3), 5.48 (1H, d, H5, J_{5-6}=8.1 Hz), 6.07 (1H, d, H1′, $J_{1'-2'}$= 7.6 Hz), 6.85 (4H, d, ArH *o*-OCH_3, *J*=7.1 Hz), 7.22–7.40 (9H, m, ArH), 7.65 (1H, d, H6, J_{6-5}=8.3 Hz), 9.20 (1H, br s, NH). ^{13}C NMR δ (100 MHz, MeOD) 13.79 (C6′), 46.80 (C2′), 55.68 (O*C*H_3), 63.66 (C5′), 72.84 (C3′), 86.53 (C4′), 87.73 (O*C*Ar_3), 87.80 (C1′), 103.55 (C5), 113.69 (ArC), 113.79 (ArC), 118.48 (*C*N), 127.68 (ArC), 128.43 (ArC), 128.52 (ArC), 130.43 (ArC), 130.46 (ArC), 135.36 (ArC), 139.84 (C6), 144.43 (ArC), 151.10 (C2), 159.16 (ArC), 163.25 (C4). Found HRMS *m/z* $[M+H^+]$, 570.2235, $C_{33}H_{33}N_3O_7$ requires 570.2240.

3.3.2. 2′-Deoxy-2′-α-*C*-(2-cyanoethyl)-5′-*O*-dimethoxytrityluridine (19). 79%; ^{1}H NMR δ (400 MHz, MeOD) 1.77 (1H, m, H6′), 2.15 (1H, m, H6″), 2.53 (3H, m, H2′, H7′, H7″), 3.47 (2H, 2×H5′, $J_{5'-4'}$=2.28 Hz), 3.79 (6H, s, OCH_3), 4.09 (1H, m, H4′), 4.51 (1H, m, H3′), 5.38 (1H, d, H5, J_{5-6}=8.4 Hz), 6.11 (1H, d, H1′, $J_{1'-2'}$=7.84 Hz), 6.85 (4H, d, ArH *o*-OCH_3, *J*=8.8 Hz), 7.31 (9H, m, ArH), 7.78 (1H, d, H6, J_{6-5}=8.1 Hz), 9.18 (1H, br s, NH). ^{13}C NMR δ (100 MHz, MeOD) 14.59 (C6′), 15.87 (C7′), 48.53 (C2′), 55.67 (O*C*H_3), 63.78 (C5′), 72.67 (C3′), 86.67 (C4′), 87.72 (O*C*Ar_3), 88.23 (C1′), 103.42 (C5), 113.77 (ArC), 119.73 (*C*N), 127.65 (ArC), 128.52 (ArC), 130.51 (ArC), 135.36 (ArC), 135.54 (ArC), 140.48 (C6), 144.44 (ArC), 151.19 (C2), 159.14 (ArC), 163.00 (C4). Found HRMS *m/z* $[M+NH_4^+]$, 601.2663, $C_{33}H_{41}N_5O_7$ requires 601.2662.

3.3.3. 2′-Deoxy-2′-α-*C*-(amidomethyl)-5′-*O*-dimethoxytrityluridine (20). 45%; ^{1}H NMR δ (400 MHz, $CDCl_3$) 2.57–2.70 (3H, m, H2′, H6′, H6″), 3.38 (2H, d, 2×H5′, $J_{5'-4'}$=2.68 Hz), 3.75 (6H, s, OCH_3), 4.14 (1H, d, H4′, $J_{4'-5'}$=2.36 Hz), 4.33 (1H, br s, H3′), 4.45 (1H, br s, 3′-OH), 5.41 (1H, d, H5, J_{5-6}=8.08 Hz), 6.06 (1H, d, H1′, $J_{1'-2'}$=7.44 Hz), 6.44 (1H, br s, NH-amide), 6.72 (1H, br s, NH-amide), 6.81 (4H, d, ArH *o*-OCH_3, *J*=8.92 Hz), 7.16–7.39 (9H, m, ArH), 7.64 (1H, d, H6, J_{6-5}=8.12 Hz), 10.37 (1H, br s, NH-uracil). ^{13}C NMR δ (100 MHz, $CDCl_3$) 31.26 (C6′), 46.85 (C2′), 55.25 (O*C*H_3), 63.80 (C5′), 73.45 (C3′), 85.96 (C4′), 86.91 (O*C*Ar_3), 88.24 (C1′), 102.97 (C5), 113.34 (ArC), 127.09 (ArC), 128.01 (ArC), 130.13 (ArC), 135.36 (ArC), 135.51 (ArC), 140.30 (C6), 144.36 (ArC), 151.74 (C2), 158.69 (ArC), 163.67 (C4), 175.02 (*C*ONH_2). Found HRMS *m/z* [M+Na$^+$], 610.2137, $C_{32}H_{33}N_3O_{78}Na$ requires 610.2165.

3.3.4. 2′-Deoxy-2′-α-*C*-(2-amidoethyl)-5′-*O*-dimethoxytrityluridine (21). 70%; ^{1}H NMR δ (400 MHz, $CDCl_3$) 1.62–1.66 (1H, m, H6′), 2.03 (1H, br s, H6″), 2.21–2.25 (1H, m, H2′), 2.35–2.46 (H, m, H7′, H7″), 3.40 (2H, s, 2×H5′), 3.74 (6H, s, OCH_3), 4.10 (1H, d, H4′, $J_{4'-5'}$= 2.72 Hz), 4.38 (1H, br s, H3′), 4.58 (1H, br s, OH), 5.34 (1H, d, H5, J_{5-6}=8.12 Hz), 5.99 (1H, d, H1′, $J_{1'-2'}$= 7.28 Hz), 6.19 (1H, br s, NH-amide), 6.44 (1H, br s, NH-amide), 6.81 (4H, d, ArH *o*-OCH_3, *J*=8.88 Hz), 7.16–7.36 (9H, m, ArH), 7.75 (1H, d, H6, J_{6-5}=8.08 Hz), ~10 (1H, br s, NH-uracil). ^{13}C NMR δ (100 MHz, $CDCl_3$) 31.25 (C6′), 33.57 (C2′), 50.38 (C7′), 55.62 (O*C*H_3), 64.00 (C5′), 72.23 (C3′), 85.64 (C4′), 87.40 (O*C*Ar_3), 88.66 (C1′), 103.12 (C5), 113.76 (ArC), 127.48 (ArC), 128.36 (ArC), 130.50 (ArC), 135.65 (ArC), 135.84 (ArC), 140.84 (C6), 144.75 (ArC), 151.62 (C2), 159.06 (ArC), 164.02 (C4), 176.78 (*C*ONH_2).

3.4. General procedure for the preparation of nucleoside-3′-[(2-cyanoethyl)-*N*,*N*-diisopropyl]phosphoramidites

The 5′-*O*-tritylated nucleoside (235 mg; 0.43 mmol) and diisopropylammonium tetrazolide (0.5 equiv; 0.21 mmol; 36.39 mg) were dissolved in dry CH_3CN (4 ml). To this, 2-cyanoethyltetraisopropylphosphoramidite (1.1 equiv; 0.47 mmol; 0.15 ml) was added drop wise. The solution was stirred under argon for 16 h, after this time, TLC [DCM/MeOH (10%)] showed the reaction to be complete. The reaction mixture was diluted with EtOAc (~20 ml) and then washed with $NaHCO_3$ (2×10 ml) and NaCl (2×10 ml). All organic layers were dried over $MgSO_4$. Solvent was removed in vacuo to give crude product, which was purified by column chromatography on silica gel, eluting with [hexane/EtOAc/NEt_3 (50:49:1%)] to give product as a white oil. This was then precipitated from hexane to give a white foam.

3.4.1. 2′-Deoxy-2′-α-*C*-2′-(cyanomethyl)-5′-*O*-dimethoxytrityluridine-[(2-cyanoethyl)-*N*,*N*-diisopropyl]phosphoramidite (22). 56%; ^{1}H NMR δ (400 MHz, MeOD) 1.11–1.31 (12H, m, 4×CH_3), 2.48–2.72 (5H, m, CH_2CN, H2′, H6′, H6″), 3.42–3.71 (6H, m, 2×Me_2C*H*N, NCCH_2CH_2O, 2×H5′), 3.81 (6H, s, OCH_3), 4.29†, 4.39† (1H, br s, H4′), 4.51†, 4.68† (1H, m, H3′) 5.44†, 5.50† (1H, d, H5, J_{5-6}=8.2 Hz, J_{5-6}=8.1 Hz), 6.04†, 6.05† (1H, d, H1′, $J_{1'-2'}$=4.1 Hz, $J_{1'-2'}$=3.7 Hz), 6.85–6.89 (4H, m, ArH *o*-OCH_3), 7.26–7.39 (9H, m, ArH), 7.72†, 7.69† (1H, d, H6, J_{6-5}=8.1 Hz, J_{6-5}=8.1 Hz). ^{31}P NMR δ (161 MHz, MeOD) 152.25, 153.47. Found HRMS *m/z* $[M+H^+]$, 770.3324, $C_{41}H_{50}N_5O_8P$ requires 770.3319.

3.4.2. 2′-Deoxy-2′-α-*C*-2′-(2-cyanoethyl)-5′-*O*-dimethoxytrityluridine-[(2-cyanoethyl)-*N*,*N*-diisopropyl]phosphoramidite (23). 45%; ^{1}H NMR δ (400 MHz, MeOD) 1.11–1.33 (12H, m, 4×CH_3), 1.80 (1H, m, H6′), 2.34 (1H, m, H6″), 2.48–2.76 (5H, m, CH_2CN, H2′, H7′, H7″), 3.40–3.73 (6H, m, 2×Me$_2$CHN, NCCH$_2$CH_2O, 2×H5′), 3.79†, 3.80† (6H, s, OCH_3), 4.19†, 4.30† (1H, br s, H4′), 4.53†, 4.65† (1H, m, H3′), 5.33†, 5.40† (1H, d, H5, J_{5-6}=8.2 Hz, J_{5-6}=8.1 Hz), 6.04†, 6.06† (1H, d, H1′, $J_{1'-2'}$=6.6 Hz, $J_{1'-2'}$=8.1 Hz), 6.84–6.89 (4H, m, ArH *o*-OCH$_3$), 7.25–7.39 (9H, m, ArH), 7.80†, 7.83† (1H, d, H6, J_{6-5}=8.3 Hz, J_{6-5}=8.1 Hz), 9.30 (1H, br s, NH). ^{31}P NMR δ (161 MHz, MeOD) 150.67, 152.73. Found HRMS *m*/*z* [M+H$^+$], 784.3477, $C_{42}H_{52}N_5O_8P$ requires 784.3475.

3.4.3. 2′-Deoxy-2′-α-*C*-2′-(amidomethyl)-5′-*O*-dimethoxytrityluridine-[(2-cyanoethyl)-*N*,*N*-diisopropyl]phosphoramidite (24). 52%; ^{1}H NMR δ (400 MHz, CDCl$_3$) 1.12–1.29 (12H, m, 4×CH_3), 2.47–2.80 (4H, m, CH_2CN, H2′, H6′), 2.96 (1H, m, H6″), 3.37–3.88 (6H, m, 2×Me$_2$CHN, NCCH$_2$CH_2O, 2×H5′), 3.79† (2×6H, s, OCH_3†), 4.27†, 4.38† (1H, br s, H4′), 4.43†, 4.54† (1H, m, H3′) 5.46†, 5.47† (1H, d, H5, J_{5-6}=8.08 Hz, J_{5-6}=8.08 Hz), 5.59 (1H, br s, NH-amide), 5.83 (1H, br s, NH-amide), 6.15†, 6.18† (1H, d, H1′, $J_{1'-2'}$=9.68 Hz, $J_{1'-2'}$=9.40 Hz), 6.83–6.85 (4H, m, ArH *o*-OCH$_3$), 7.22–7.42 (9H, m, ArH), 7.59†, 7.67† (1H, d, H6 J_{6-5}=8.12 Hz, J_{6-5}=8.08 Hz), ~10 (1H, br s, NH-uracil). ^{13}P NMR δ (161 MHz, CDCl$_3$) 150.36, 151.36. Found HRMS *m*/*z* [M+Na$^+$], 810.3257, $C_{41}H_{50}N_5O_9PNa$ requires 810.3244.

3.4.4. 2′-Deoxy-2′-α-*C*-2′-(2-amidoethyl)-5′-*O*-dimethoxytrityluridine-[(2-cyanoethyl)-*N*,*N*-diisopropyl]phosphoramidite (25). 44%; ^{1}H NMR δ (400 MHz, CDCl$_3$) 1.11–1.27 (12H, m, 4×CH_3), 1.76–1.83 (1H, m, H6′), 2.01–2.10 (1H, m, H6″), 2.34–2.64 (5H, m, CH_2CN, H2′, H7′, H7″), 3.39–3.88 (6H, m, 2×Me$_2$CHN, NCCH$_2$CH_2O, 2×H5′), 3.80† (2×6H, s, OCH_3†), 4.20†, 4.22† (1H, m, H4′), 4.57–4.62† (2×1H, m, H3′†) 5.33†, 5.34† (1H, d, H5, J_{5-6}=8.12 Hz, J_{5-6}=8.28 Hz), 5.46 (1H, br s, NH-amide), 5.90 (1H, br s, NH-amide), 6.02†, 6.03† (1H, d, H1′, $J_{1'-2'}$=7.28 Hz, $J_{1'-2'}$=7.00 Hz), 6.83–6.86 (4H, m, ArH *o*-OCH$_3$), 7.26–7.40 (9H, m, ArH), 7.79†, 7.86† (1H, d, H6, J_{6-5}=8.24 Hz, J_{6-5}=8.24 Hz), 9.25 (1H, br s, NH-uracil). ^{31}P NMR δ (161 MHz, CDCl$_3$) 149.73, 152.05. Found HRMS *m*/*z* [M+Na$^+$], 824.3405, $C_{42}H_{52}N_5O_9PNa$ requires 824.3400.

3.5. Syntheses and purification of oligonucleotides (26–29)

Oligonucleotides were prepared using an expedite automated solid-phase synthesiser on a 1 μmol scale following standard protocols. The exocyclic amine groups of adenosine and cytidine were protected with benzoyl groups, while that of guanine was protected with an isobutryl group. Dimethoxytrityl groups were removed with 5% dichloroacetic acid. The protected oligonucleotides were cleaved from the support and protecting groups removed using aqueous ammonia, 55 °C for 16 h. Final dimethoxytrityl groups were removed following purification by HPLC, using 80% acetic acid/H_2O.

Further purification was done on a Mono Q®-HR 5/5 anion exchange column with the following gradient system (*A*=10 mM NaOH, pH 12.0, 0.1 M NaCl; *B*=10 mM NaOH, pH 12.0, 0.9 M NaCl). The low-pressure liquid chromatography system consisted of a Merck-Hitachi L 6200 A intelligent pump, a Mono Q®-HR 10/10 column (Pharmacia), a Uvicord SII 2138 UV detector (Pharmacia-LKB) and a recorder. The product-containing fraction was desalted on a NAP-25® column and lyophilised.

Oligonucleotides were characterised and their purity was checked by HPLC–MS on a capillary chromatograph (CapLC, Waters, Milford, MA). Columns of 150 mm×0.3 mm length (LCPackings, San Francisco, CA) were used. Oligonucleotides were eluted with a triethylammonium/1,1,1,3,3,3-hexafluoro-2-propanol/acetonitrile solvent system. Flow rate was 5 μl/min. Electrospray spectra were acquired on an orthogonal acceleration/time-of-flight mass spectrometer (Q-Tof-2, Micromass, Manchester, UK) in negative ion mode. Scan time used was 2 s. The combined spectra from a chromatographic peak were deconvoluted using the MaxEnt algorithm of the software (Masslynx 3.4, Micromass, Manchester, UK). Theoretical oligonucleotide masses were calculated using the monoisotopic element masses.

3.6. Melting temperatures

Oligomers were dissolved in 0.1 M NaCl, 0.02 M potassium phosphate, pH 7.5, 0.1 mM EDTA. The concentration was determined by measuring the absorbance in MilliQ water at 260 nm at 80 °C and assuming the sugar modified nucleoside analogues to have the same extinction coefficients in the denatured state as 2′-deoxyuridine (ε=9660). The concentration in all experiments was 4 μM for each strand unless otherwise stated. Melting curves were determined with a Varian Cary 300 BIO spectrophotometer. Cuvettes were maintained at constant temperature by means of water circulation through the cuvette holder. The temperature of the solution was measured with a thermistor directly immersed in the cuvette. Temperature control and data acquisition were done automatically with an IBM-compatible computer using Cary WinUV thermal application software. Following a quick heating and cooling cycle to allow proper annealing of both strands, the samples were heated at a rate of 0.2 °C/min starting at 5 °C up to 80 °C and cooling again at the same speed. Melting temperatures were determined by plotting the first derivative of the absorbance versus temperature curve and are the average of two runs. Up and down curves in general showed identical T_m values.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.11.024.

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