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Syntheses and Oligonucleotide Incorporation of Nucleoside Analogues Containing Pendant Imidazolyl or Amino Functionalities – The Search for Sequence-Specific Artificial Ribonucleases

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Dedicated to Prof. Dr. Wojciech Stec on the occasion of his 65th birthday

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Oligonucleotide derivatives containing imidazolyl and amino functionalities have been proposed as artificial ribonucleases that might mimic the activities of RNase A but with greater sequence specificity. Such oligonucleotide reagents would have important applications as enhanced antisense reagents that obviate the need to recruit a cellular nuclease or nuclease complex. We present the syntheses of six nucleoside phosphoramidite analogues containing pendant protected imidazolyl or amino groups and their incorporation into oligonucleotides. We show that the six functionalised phosphoramidites have similar efficiencies of incorporation and present mass spectral evidence that the composition of an oligonucleotide library of 81 components is consistent with the expected mass mixture distribution. Such phosphoramidites are thus suitable starting materials for construction of mixed oligonucleotide libraries containing a region of imidazolyl and amino-modified nucleotides ready for screening for sequence-specific ribonuclease activity.

Introduction

Synthetic oligonucleotides and their analogues are used widely to block gene expression in cells both as therapeutics and for target validation.[1] The most common mechanism for the inhibition of gene expression is for the binding of the oligonucleotide to induce RNA cleavage by the recruitment of an endogenous nuclease, such as RNase H (antisense)[2] or the RISC complex (siRNA).[3] In recent years there have been attempts to develop oligonucleotide-based artificial nucleases that contain motifs capable of inducing sequence-specific RNA cleavage without the requirement for protein recruitment. Such artificial nucleases have usually included chemically modified residues in order to enhance the catalytic cleavage rates.[4] Some approaches to artificial nucleases have employed a heavy metal ion,[5] or a redox reagent bound to an oligonucleotide[6] to achieve RNA targeted cleavage. However, it is unclear whether these will be useful in vivo since RNA cleavage may require the addition of exogenous co-factors.

The protein ribonuclease A (RNase A) achieves highly efficient RNA cleavage by use of a protonated imidazole group (His-119) and a free imidazole group (His-12) which act in a general acid/base catalytic mechanism. In addition, a positively charged amine side chain (Lys-41) is thought to stabilise the pentacoordinate transition state of the scissile phosphate.[7] With RNase A as a model, Bashkin et al. were first to propose the imidazolyl modification of oligonucleotides as hydrolytic ribonuclease mimics where the RNA hydrolysis activity of imidazole is combined with the ability of oligonucleotides to bind RNA in a sequence-specific manner.[8] A 2′-deoxyuridine derivative modified at the 5-position was synthesised that incorporated a long linker arm and a *tert*-butoxycarbonyl (BOC) protected imidazole residue.[8,9] The unit was incorporated successfully into an 11-mer oligonucleotide, but no results were published with regard to RNA hydrolysis. Ushijima et al. used the same side arm modification at the 5′-end of an antisense phosphorothioate oligonucleotide to target a gag mRNA sequence from the HIV-1 genome.[10] Use of this modification resulted in a higher *anti* HIV-1 activity than that from the unmodified phosphorothioate oligonucleotide.

More recently, several studies have focussed on the incorporation of amino/imidazolyl (lysyl/histidyl) functionalities into different locations in an oligonucleotide. For example, Reynolds et al. incorporated a non-nucleotidic internal linker into a methyl phosphonate oligonucleotide and then modified it with bis-amino, amino plus imidazolyl, or bis-imidazolyl units.[11] Low levels of site-specific cleavage of an RNA target were observed with units containing imidazole

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modifications after a 5 day incubation period. However, some other reported bis-imidazolyl oligonucleotide conjugates have shown no hydrolytic activity.[12] Beban and Miller used post-synthetic modification to conjugate imidazole units to a 2′-modified 2′-deoxyuridine nucleoside,[13] whilst Polushin conjugated histidine or imidazoleacetic acid to the 3′-position of thymidine for incorporation at the 3′-terminus of oligonucleotides.[14] Unfortunately, no RNA cleavage assays were reported using these derivatives.

The most successful oligonucleotide-based imidazolyl artificial nucleases to date have targeted specific RNA sequences within structured tRNAs. Ushijima et al.[15] and Beloglazova et al.[16] showed that imidazole units attached to the ends of oligonucleotides can cleave at particular "fragile" regions within tRNAs, usually between CpA sequences. A number of non-oligonucleotide imidazole-containing small molecules have also been designed.[17] Such artificial nucleases can cleave RNA sequences site-selectively at susceptible scissile phosphates, but lack the extended sequence specificity of oligonucleotides.

Site-specific cleavage of RNA has been achieved through the use of amine modifications alone. Endo et al. have synthesised a DNA oligoamine conjugate[18] and showed it possessed modest RNA hydrolysis capabilities. Verheijen et al. tethered ethylenediamine to the 5′-end of a PNA oligomer and also showed RNA hydrolysis activity.[19] Most recently an $(ArgLeu)_4$-Gly peptide conjugated to a short DNA was revealed to have RNA hydrolysis activity when targeting specific HIV-1 and tRNA sequences.[20] Taken together with Komiyama and Inokawa's earlier results,[21] the idea that there are highly susceptible sequences within structured nucleic acids is reinforced.

Examples of imidazolyl- and amino-modified nucleosides harnessed for use in RNA catalysis also exist through their conversion into deoxynucleotide triphosphate (dNTP) analogues and enzymatic incorporation into modified DNAzymes selected through in vitro selection experiments.[22] The research groups of Perrin,[23] Williams[24] and Beigelman[25] have reported the synthesis of dNTPs modified with pendant imidazole and amino functionalities. Deoxynucleoside analogues containing imidazole and amino functions have also been designed for use in oligonucleotide synthesis.[26] Although to date the cleavage rates of such DNAzymes have not proved to be particularly high, the principle of utilisation of imidazoles and amino functionalities in an oligonucleotide background is now well established.

Our aim is to chemically synthesise small synthetic libraries of oligonucleotides containing amino- and imidazolyl-modified nucleosides and then to screen these sequences for their ability to cleave a desired RNA substrate. A particular advantage of this approach over enzymatic selection methods is that there is no limitation to those few nucleotide analogues that act as substrates for a polymerase-based reaction. Thus it should be possible in principle to explore a wider chemical space and functionality.

We have already reported the synthesis of some *seco*-pseudonucleoside analogues potentially suitable for imidazole and amino functionalisation.[27] In addition, we have reported recently the synthesis of oligonucleotides containing imidazole and amino side chains at the 2′-position of uridine.[28] In this paper we describe the synthesis of a number of nucleoside phosphoramidites functionalised with imidazolyl and amino groups on heterocyclic base moieties and their successful incorporation with similar coupling efficiencies into 2′-deoxyribo- and into 2′-*O*-methyl ribo-oligonucleotides. These modified nucleosides are suitable for use in the synthesis of mixed libraries of functionalised oligonucleotides for the purpose of screening them for RNA cleavage activity.

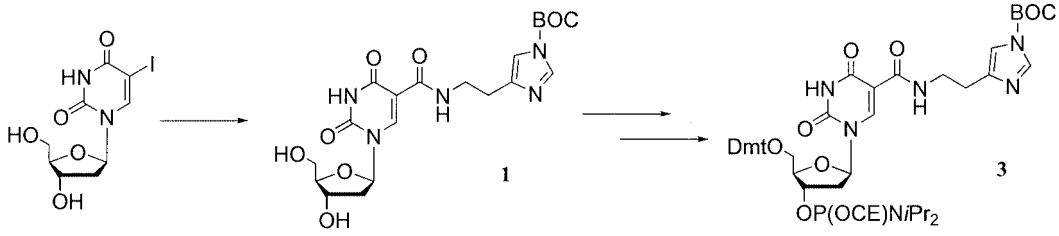
Results and Discussion

Our concept for design of an artificial ribonuclease is that such a molecule should contain both RNA substrate binding region(s) and a catalytic region which would contain the modified nucleosides. To maximise the opportunities to select "active" molecules (i.e. those capable of catalysing RNA hydrolysis), the catalytic region would consist of combinatorial mixtures of differently modified nucleosides, whereas binding regions would be exactly matched to the RNA target. Each library of oligonucleotides must contain an unbiased population of modified and/or unmodified nucleotides and thus the first requirement is to ensure that all phosphoramidites used in library synthesis have similar coupling efficiencies. For example, it is well established that coupling efficiencies differ markedly between 2′-deoxynucleoside, ribonucleoside or 2′-OMe ribonucleoside phosphoramidites. In modifying the heterocyclic base, rather than the sugar moiety, we aimed to minimise these differences in coupling efficiencies.

Synthesis of 5-Carboxamide-Modified Imidazolyl and Amino dU Analogues

A facile synthesis for the incorporation of a number of carboxamido modifications at the 5-position of uridine has been published previously by Dewey et al.[29] In place of formation of a carboxy ester intermediate and then reaction with an amine,[30] Dewey et al. used a one-pot carboxamidation procedure. 5-Iodouridine carrying various sugar protecting groups was reacted with a number of different amines to give the desired 5-carboxamido products. However, not all products were isolated and yields were not optimised. Other 5-carbamoyl-2′-deoxyuridine analogues, such as the ethylenediamine derivative, may be synthesised post-synthetically after the incorporation of 5-methoxycarbonyl-2′-deoxyuridine phosphoramidite into oligonucleotides.[31]

By use of completely unprotected 5-iodo-2′-deoxyuridine, we were able to incorporate successfully a histamine group through a one-pot carboxamidation procedure. After several attempts at the reaction, it became apparent that the product could be formed in consistently good yield with different palladium catalysts, but purification by



Scheme 1.

chromatography was problematic due to the polar nature of the product. By protection of the imidazole ring of the histamine with a base-labile *tert*-butoxycarbonyl group (BOC) the molecule became more hydrophobic and this facilitated ready purification. The BOC group is required to ensure compatibility with oligonucleotide synthesis and prevent the bis-dimethoxytritylation of the 5′-hydroxy and the imidazole later in the synthesis. The molecule was then easily converted into the required phosphoramidite in good yield (Scheme 1).

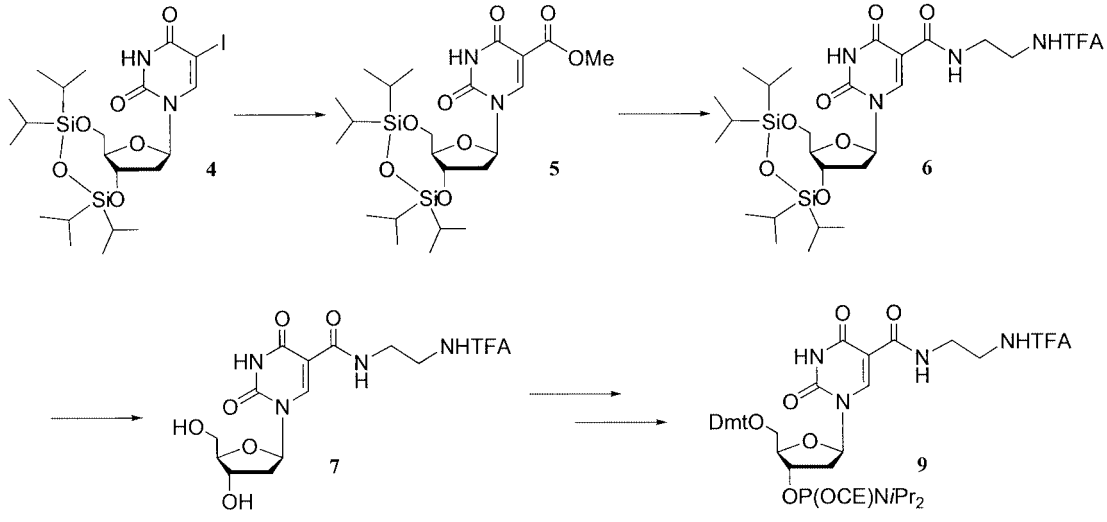
By contrast, and analogous to previously published work,[29] the incorporation of an ethylenediamine group by 5-carboxamidation was found to be less straightforward. Efforts using the unprotected 5-iodo-2′-deoxyuridine resulted in large amounts of unknown nucleosidic by-products from the reaction and attempts to optimise the reaction by using different palladium(II) and palladium(0) catalysts proved ineffective. To avoid these problems, the synthesis was effected via the 3′,5′-(tetraisopropyldisiloxane-1,3-diyl)-protected 5-methyl ester **5**, which was formed in quantitative yield (Scheme 2). This derivative was then reacted with ethylenediamine and the resultant amine protected with a trifluoroacetyl group in a one-pot synthesis. Protection of the terminal amine at this stage also allowed the desilylation product to be purified easily by silica gel chromatography. The product was then easily converted into the desired phosphoramidite.

Synthesis of 6-Modified Imidazolyl and Amino dA Analogues

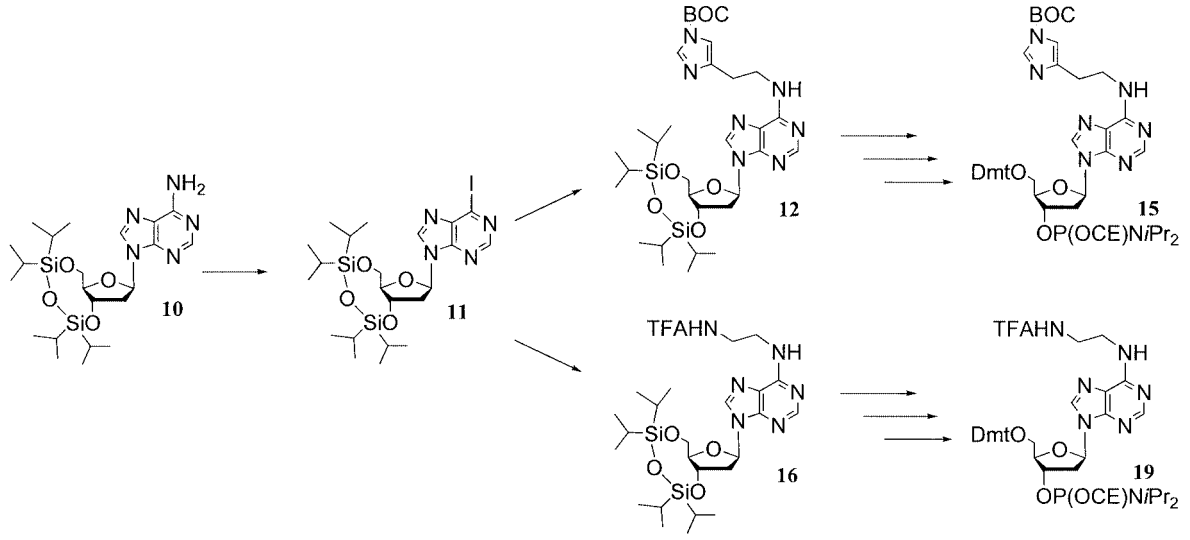
Cosstick and Douglas have synthesised previously the N^6-(ethylamino)-2′-deoxyadenosine phosphoramidite for incorporation into a nucleotide dimer for potential cross-linking studies.[32] Starting with a 6-chloro-3′,5′-bis(toluoyl) derivative,[33] we tried to synthesise the N^6-ethylimidazole derivative, but found difficulty with purification following sugar deprotection. To avoid this problem, we synthesised 6-iodo-3′,5′-(tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyadenosine following the photo-induced diazotisation procedure developed by Nair and Richardson,[34] which was also utilised by Cosstick and Douglas in their synthesis. Substitution with either histamine or ethylenediamine proceeded well and the amino derivatives were protected without prior purification (Scheme 3). Desilylation, dimethoxytritylation and conversion into the desired phosphoramidites were completed in reasonable yields.

Synthesis of 4-Modified 6-Methylimidazolyl and Amino dC Analogues

The incorporation of 6-Me-2′-deoxyuridine (6-Me-dU) into oligonucleotides has been found to destabilize DNA duplexes by 2–3 °C per modification.[35] In ^{1}H NMR (NOE) and molecular modeling experiments, it was re-



Scheme 2.



Scheme 3.

vealed that this was because 6-Me-dU has a preference to adopt a *syn* conformation of the base rather than the *anti* conformation required for Watson–Crick base-pairing in both A and B form duplexes.[35,36]

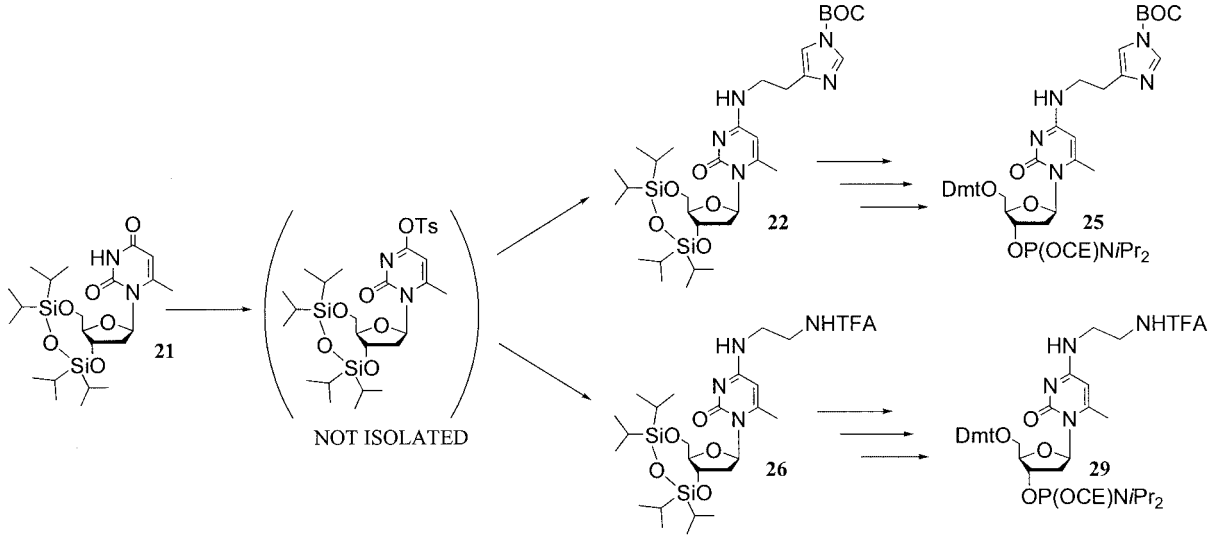
By prevention of non-Watson–Crick base pairing within our catalytic regions, through incorporation of the 6-methyl modification, we aim to encourage the formation of alternative, less rigid bulge structures within otherwise duplex regions.

6-Methyl-2′-deoxyuridine was first synthesised by Holy in 1973.[37] However, since then a more convenient synthesis has been published that proceeds by a lithiation of 3′,5′-(tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyuridine.[38] We followed this procedure to give the 6-methyl derivative in good yield.

There are numerous methods of activating the 4-position of pyrimidines for nucleophilic displacement by amines. Miah et al. showed a one-pot strategy where the 4-triazole derivative was formed in good yield through transient silyl protection of the sugar hydroxy groups.[39] This method was attempted with the free 6-methyl-dU nucleoside, but poor yields resulted after amine substitution and relevant protecting group incorporation (BOC or TFA). As an alternative, the 4-tosyl derivative was formed directly from the silylated nucleoside and taken in crude form into the next step. Amino substitution and protection followed (Scheme 4). The three-step reaction proceeded in good yield and it was found that purification could be deferred until after the desilylation step. The synthesis was also attempted via the 4-chloro derivative, but no increase in overall yield was attained. Again, conversion into the desired phosphoramidites was carried out easily.

Synthesis of Modified Oligonucleotides

All six phosphoramidites were incorporated successfully into a central position within a 15-mer oligodeoxynucleo-



Scheme 4.

tide sequence using standard coupling times and reagents. The analogues in each case coupled with >95% efficiency. The oligonucleotides were deprotected by use of saturated methanolic ammonia overnight at room temperature. Ion-exchange HPLC of the oligonucleotides showed practically single product peaks and MALDI-TOF mass spectrometry revealed all oligonucleotides had the desired mass (Table 1).

Table 1. Calculated and observed masses for 15-mer deoxy oligonucleotides containing a single incorporation of each of the modified nucleoside phosphoramidites.

Sequence 5′-ATC TGT AXT CCG TCA	Calcd. mass	Obsd. mass
Oligo 1: X = nucleoside derivative **3**	4640	4639
Oligo 2: X = nucleoside derivative **9**	4589	4591
Oligo 3: X = nucleoside derivative **15**	4668	4669
Oligo 4: X = nucleoside derivative **19**	4617	4619
Oligo 5: X = nucleoside derivative **25**	4612	4610
Oligo 6: X = nucleoside derivative **29**	4561	4559

To investigate the coupling efficiencies of the modified phosphoramidites, an equimolar ratio of modified phosphoramidite together with 2′-deoxyuridine phosphoramidite was mixed together and used in a single coupling reaction within a 15-mer oligonucleotide. Because the inclusion of the amino or imidazole functionalities reduces retention of the oligonucleotide on ion-exchange HPLC, the ratio of modified oligonucleotide to dU-containing oligonucleotide can be observed by integration of the two separate peaks. All six modified phosphoramidites were shown to have slightly reduced coupling efficiencies when compared to 2′-deoxyuridine phosphoramidite. For example, with dU phosphoramidite and the phosphoramidite **29**, the integrated ratio of peak areas was 1.6:1 (Figure 1). All other

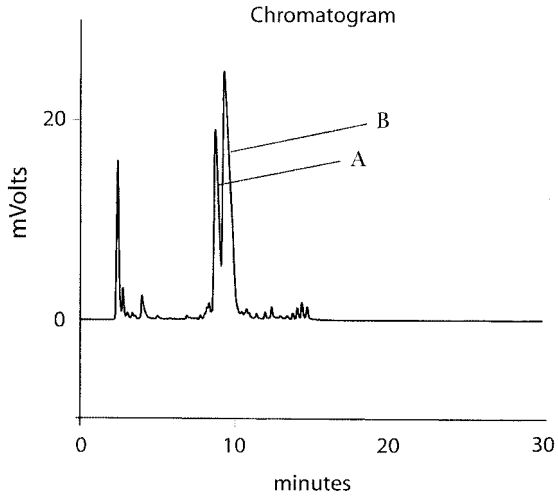


Figure 1. Ion-exchange HPLC chromatograph showing the relative incorporation of phosphoramidite **29** and 2′-deoxyuridine (dU) phosphoramidite into a 15-mer DNA sequence. Peak A contains the amino-modified nucleoside and the larger peak B contains the unmodified dU. There is an integrated peak area ratio of about 1:1.6.

Table 2. The 81 different sequences formed from a mixture of three different phosphoramidites (represented here as 1, 2, 3) inserted over four degenerate positions and their respective masses. The theoretical masses are calculated for the oligonucleotide sequence 2′-OMe-GUC CUN NNN ACU CC where N contains a mixture of dU and the 5-carboxamide-modified amino and imidazolyl dU analogues (represented by 1, 2 and 3, respectively). Although there are 81 different sequences, there are only 15 different masses.

Degenerate sequence	Theoretical mass	Degenerate sequence	Theoretical mass	Degenerate sequence	Theoretical mass
1111	4363	2111	4449	3111	4500
1112	4449	2112	4535	3112	4586
1113	4500	2113	4586	3113	4637
1121	4449	2121	4535	3121	4586
1122	4535	2122	4621	3122	4672
1123	4586	2123	4672	3123	4723
1131	4500	2131	4586	3131	4637
1132	4586	2132	4672	3132	4723
1133	4637	2133	4723	3133	4774
1211	4449	2211	4535	3211	4586
1212	4535	2212	4621	3212	4672
1213	4586	2213	4672	3213	4723
1221	4535	2221	4621	3221	4672
1222	4621	2222	4707	3222	4758
1223	4672	2223	4758	3223	4809
1231	4586	2231	4672	3231	4723
1232	4672	2232	4758	3232	4809
1233	4723	2233	4809	3233	4860
1311	4500	2311	4586	3311	4637
1312	4586	2312	4672	3312	4723
1313	4637	2313	4723	3313	4774
1321	4586	2321	4672	3321	4723
1322	4672	2322	4758	3322	4809
1323	4723	2323	4809	3323	4860
1331	4637	2331	4723	3331	4774
1332	4723	2332	4809	3332	4860
1333	4774	2333	4860	3333	4911

coupling ratios were between 1.5–1.7:1 (unmodified: modified).

Synthesis of an 81-Component Oligonucleotide Library

To ensure the suitability of our modified phosphoramidites in the production of oligonucleotide libraries, a number of simplified test oligonucleotide libraries were synthesized by the standard oligonucleotide synthesis procedures used previously. A selection of the modified phosphoramidites and dU were coupled in a number of degenerate positions within 2′-O-methyl oligonucleotides using a 1.6:1 (modified:unmodified phosphoramidite) molar ratio. To enable reversed phase cartridge purification of the mixtures, each test library was synthesised with the terminal dimethoxytrityl group left on which was then removed during the course of purification.

Although a library containing a mixture of three phosphoramidites inserted over four degenerate positions creates a possible 81 different sequences, the members of that library only possess 15 different masses (Table 2). This makes characterization of the libraries a realistic possibility. Although the theoretical and experimental MALDI-TOF mass spectra of each test library were not identical they revealed qualitative consistencies with the masses and their associated relative intensities (An example is shown: Figure 2). This suggests the phosphoramidites were all incorporated and with similar coupling efficiencies. It is hoped

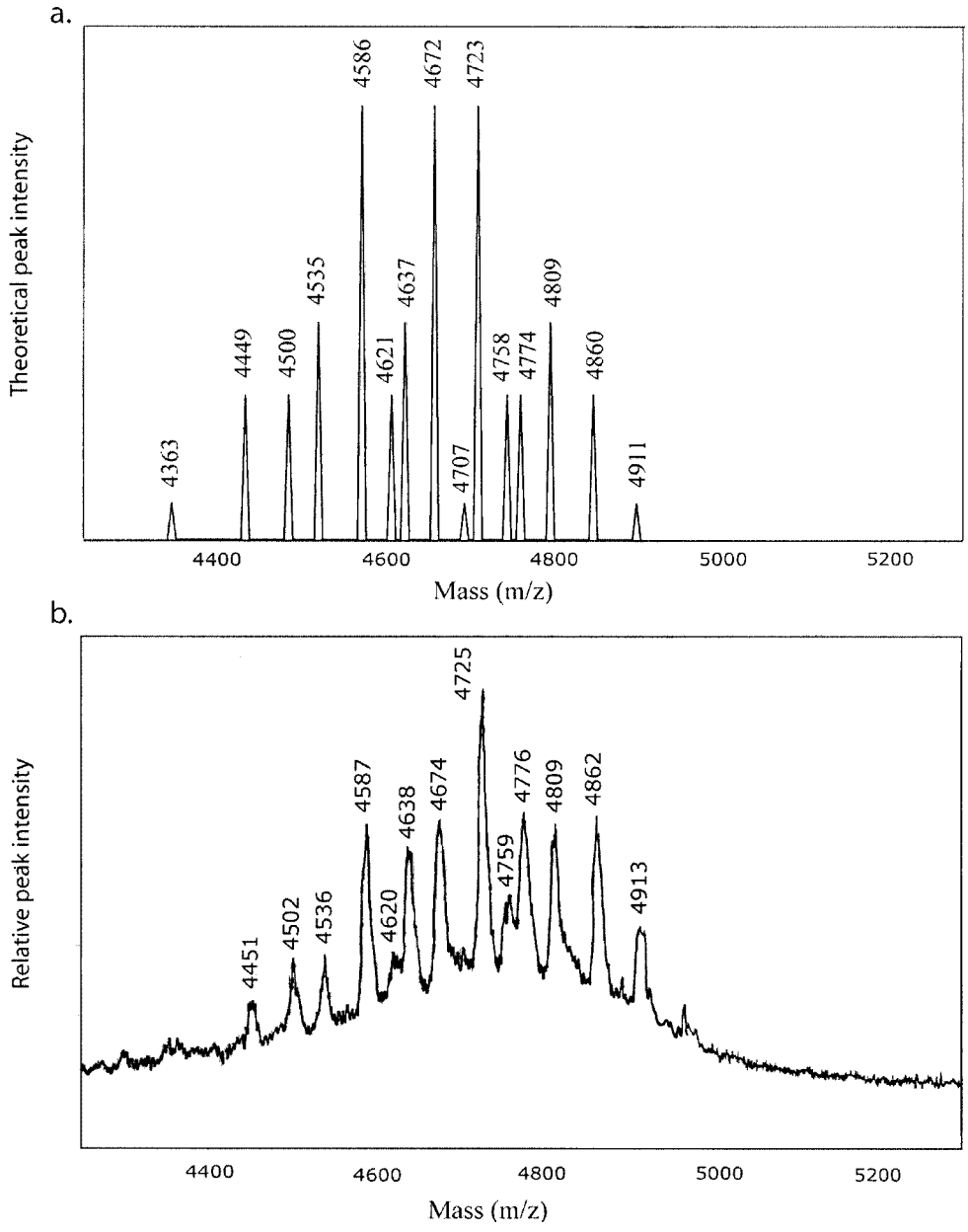


Figure 2. Theoretical (a) and experimental (b) mass spectra of an 81 member test library having the sequence 2′-OMe-GUC CUN NNN ACU CC where N contains a mixture of dU and the 5-carboxamide-modified amino and imidazolyl dU analogues. The theoretical masses are calculated in Table 2.

that they can therefore be used in more complicated library syntheses.

Conclusions

We have synthesised phosphoramidites of six nucleoside analogues containing protected imidazolyl or amino functionalities on the heterocyclic bases and shown efficient incorporation into oligonucleotides with similar efficiencies. These nucleoside analogues are also shown to be suitable in principle for use in synthesis of modified oligonucleotide libraries of mixed sequences. The synthesis of appropriate oligonucleotide libraries in various formats and testing for RNA cleavage is currently underway.

Experimental Section

All reagents and solvents used in chemical synthesis were purchased from Sigma–Aldrich or Lancaster. Methanol and ethanol were distilled from over magnesium and stored with 4-Å molecular sieves under argon. Dimethylformamide (DMF), pyridine and dichloromethane (DCM) were purchased in their anhydrous form and used as received. TLC was carried out on pre-coated F254 silica plates and column chromatography with BDH "Silica gel for flash chromatography". ^{1}H and ^{13}C NMR spectra were recorded with a Bruker DRX 300 spectrometer. Chemical shifts (δ) for ^{1}H and ^{13}C are referenced to internal solvent resonances and reported relative to $SiMe_4$. High resolution mass spectra were recorded with a Bio-Apex II FT-ICR spectrometer.

Oligonucleotide Synthesis and Characterisation: Oligonucleotide synthesis was carried out with an Applied Biosystems 394 DNA/RNA synthesiser on a 1 μM scale under standard synthetic procedures based on phosphoramidite chemistry. Purification was carried out by ion-exchange HPLC using a Dionex NucleoPac PA-100 column with a linear gradient of 15–55% buffer B over 20 minutes [Buffer A: sodium perchlorate (1 mM), Tris·HCl pH 6.8 (20 mM), 25% formamide. Buffer B: Sodium perchlorate (400 mM), Tris·HCl pH 6.8 (20 mM), 25% formamide]. Samples were then desalted by dialysis and lyophilised. Analytical reversed-phase HPLC was carried out, where necessary, with a Phenomonex Luna C-18(2) column eluting with a gradient of acetonitrile in 0.1 M triethylammonium acetate buffer (pH, 7.0). MALDI-TOF mass spectra were obtained with a Voyage-DE BioSpectrometry Workstation (PerSeptive Biosystems) in positive ion mode using a 1:1 mixture of 2,6-(dihydroxy)acetophenone (40 mg mL^{-1} in MeOH) and aqueous diammonium citrate (80 mg mL^{-1}) as matrix.

Synthesis of 5-{2-[1-(*tert*-Butoxycarbonyl)imidazol-4-yl]ethylaminocarbonyl}-2′-deoxyuridine (1): 5-Iodo-2′-deoxyuridine (3.0 g; 8.47 mmol) was suspended in anhydrous DMF (30 mL) and histamine (2.82 g; 25.4 mmol; 3.0 equiv.), triethylamine (7.1 mL; 50.8 mmol; 6.0 equiv.) and tetrakis(triphenylphosphane)palladium (489 mg; 0.42 mmol; 0.05 equiv.) were added. The reaction mixture was incubated in a Parr High-Pressure reaction vessel at 70 °C under 90 psi carbon monoxide for 18 h. The reaction mixture was then cooled to room temperature before being filtered through celite and the filtrate concentrated under vacuum to an oil. The oil was then taken up in anhydrous DMF (50 mL) and to the stirring solution under argon was added triethylamine (3.54 mL; 25.4 mmol; 3.0 equiv.) and *tert*-butyl dicarbonate (2.78 g; 12.7 mmol; 1.5 equiv.). After 15 minutes anhydrous methanol (1 mL) was added and the reaction mixture again concentrated to an oil under vacuum before being re-dissolved in the minimum amount of a 2% solution of methanol in DCM and applied to a silica gel column. The product was eluted using a gradient of 3–8% methanol in DCM. Desired fractions were pooled and concentrated under vacuum to give a pale yellow powder (2.41 g; 5.18 mmol; 62%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 11.81 (br., 1 H, NH-3), 8.77 (t, $^3J_{H,H}$ = 5.51 Hz, 1 H, N*H*–CH_2), 8.67 (s, 1 H, H-6), 8.09 (s, 1 H, N*CH*N), 7.29 (s, 1 H, N*CH*C), 6.10 (t, $^3J_{H,H}$ = 6.6 Hz, 1 H, H-1′,), 5.25 (d, $^3J_{H,H}$ = 4.1 Hz, 1 H, 3′-OH), 4.97 (t, $^3J_{H,H}$ = 4.7 Hz, 1 H, 5′-OH), 4.21 (m, 1 H, H-4′), 3.84 (m, 1 H, H-3′), 3.53 (m, 2 H, H-5′, H5′′), 3.27 (m, 2 H, NHC$H_2$$CH_2$), 2.67 (t, $^3J_{H,H}$ = 6.8 Hz, 2 H, NHCH_2CH_2), 2.19–2.11 (m, 2 H, H2′, H2′′), 1.55 [m, 9 H, C(CH_3)$_3$] ppm. ^{13}C NMR (300 MHz, DMSO, 25 °C): δ = 162.4 (C-4), 154.4 (NH–CO), 150.1 (C-6), 146.6 (CH_2*C*), 141 (N*CH*N), 114.4 (N*CH*C), 106 (C-5), 88 (C-4′), 86 (C-1′), 86.2 [*C*(CH_3)$_3$], 91 (C-3′), 62.0 (C-5′), 46.6 (C-2′), 38.7 (NH*C*$H_2$$CH_2$), 28.6 (NHC$H_2$*C*$H_2$), 28 [C($CH_3$)$_3$]. MS (ES$^+$): *m*/*z* = 466 [MH$^+$], 366, 242, 229, 102. HRMS: *m*/*z* calculated for $C_{20}H_{28}N_5O_8$ [MH$^+$]: 466.1938, found 466.1949. TLC (DCM/methanol, 9:1): R_f = 0.7.

Synthesis of 5-{2-[1-(*tert*-Butoxycarbonyl)imidazol-4-yl]ethylaminocarbonyl}-2′}-5′-dimethoxytrityl-2′-deoxyuridine (2): The starting nucleoside **1** (2.0 g; 4.30 mmol) was dissolved in anhydrous pyridine (20 mL) and placed in a dry flask under argon. Dimethoxytrityl chloride (1.60 g; 4.73 mmol; 1.1 equiv.), dissolved in a 1:1 mixture of anhydrous DCM and pyridine (10 mL), was slowly added to the stirring nucleoside solution. After 18 hours the reaction was quenched by the addition of anhydrous methanol (1 mL) and the reaction mixture was concentrated to an oil under vacuum. The oil was then re-dissolved in DCM (100 mL) and washed with saturated sodium hydrogen carbonate solution (satd. $NaHCO_3$) (100 mL) and brine (100 mL). The organics were dried with sodium sulfate and then re-concentrated under vacuum to an oil before being purified by silica gel flash column chromatography eluting with a gradient of 1–4% methanol in DCM. Desired fractions were concentrated under vacuum to give a white foam (2.0 g; 2.62 mmol; 61%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 11.71 (br., 1 H, NH-3), 8.80 (t, $^3J_{H,H}$ = 5.5 Hz, 1 H, N*H*CH_2), 8.41 (s, 1 H, H-6), 8.11 (s, 1 H, N*CH*N), 7.36–7.18/6.91–6.85 (m, 14 H, 13 × Ar-H, 1 × N*CH*C), 6.05 (t, $^3J_{H,H}$ = 6.6 Hz, 1 H, H-1′), 5.34 (br., 1 H, 3′-OH), 4.24 (m, 1 H, H-4′), 3.90 (m, 1 H, H-3′), 3.69 (s, 6 H, 2 × Ar–OCH_3), 3.52 (m, 2 H, H-5′, H5′′), 3.16 (m, 2 H, NHC$H_2$$CH_2$), 2.68 (t, $^3J_{H,H}$ = 6.8 Hz, 2 H, NHCH_2CH_2), 2.22–2.12 (m, 2 H, H2′, H2′′), 1.54 [m, 9 H, C(CH_3)$_3$] ppm. MS (ES$^+$): *m*/*z* = 768 [MH$^+$], 668, 303, 242, 102. HRMS: *m*/*z* calculated for $C_{41}H_{46}N_5O_{10}$ [MH$^+$] 768.3245, found 768.3250. TLC (DCM/methanol, 9:1): R_f = 0.7.

Generic Synthesis of 3′-*O*-ß-Cyanoethyl-*N*,*N*-diisopropylphosphoramidites 3, 9, 15, 19, 25 and 29 from the Corresponding 5′-Dimethoxytrityl Compounds 2, 8, 14, 18, 24 and 28: The starting dimethoxytritylated compound (≈ 0.5–0.6 mmol) was dried in vacuo overnight before being dissolved in anhydrous DCM (5 mL) and diisopropylethylamine (4 equiv.). The solution was then placed under argon, before the addition of 2-cyanoethyl diisopropylchlorophosphoramidite (1.5 equiv.). After 20 minutes the reaction was quenched by the addition of distilled methanol (0.2 mL) and the organics diluted by the addition of ethyl acetate (15 mL). The solution was then washed with 10% sodium carbonate solution (15 mL) and brine (15 mL) before being separated, dried with sodium sulfate and concentrated in vacuo. The residue was purified by silica gel chromatography eluting with a mixture of DCM, ethyl acetate and triethylamine (45:45:10 to 80:10:10). Desired fractions

were combined and concentrated under vacuum yielding a white foam (75–90%).

Synthesis of 5-Iodo-3′,5′-*O*-[(tetraisopropyl)disiloxane-1,3-diyl]-2′-deoxyuridine (4): 5-Iodo-2′-deoxyuridine (5.0 g; 14.2 mmol) was dried by repeated co-evaporation from anhydrous pyridine (3 × 50 mL) before being dissolved in a further portion of the same solvent (50 mL) and sealed in a flask under argon. To the stirring solution was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (5.0 mL; 15.63 mmol; 1.1 equiv.) and the reaction was left at room temperature overnight. After concentrating to an oil under vacuum, the reaction mixture was re-dissolved in DCM (100 mL) and washed with satd. $NaHCO_3$ solution (100 mL). The organics were separated and then washed with brine (100 mL). After a second separation, the organics were dried with anhydrous sodium sulfate and then concentrated under vacuum to a foam. Purification was performed by silica-gel flash column chromatography eluting with a gradient of 1–4% methanol in DCM. The desired fractions were concentrated under vacuum to yield a white foam (8.2 g; 13.8 mmol; 97%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 11.70 (s, 1 H, NH), 7.94 (s, 1 H, H-6), 5.92 (t, $^3J_{H,H}$ = 3.0 Hz, 1 H, H-1′), 4.51 (m, 1 H, H-4′), 4.00–3.91 (m, 2 H, H5′, H5′′), 3.69 (m, 1 H, H-3′), 2.35–2.20 (m, 2 H, 2 × H-2′), 1.08 [m, 4 H, 4 × *CH*(CH$_3$)$_2$], 1.05–0.96 [m, 24 H, 4 × CH(*CH*$_3$)$_2$] ppm. ^{13}C NMR (300 MHz, DMSO, 25 °C): δ = 161.4 (C-4), 150.7 (C-2), 145.6 (C-6), 85.3 (C-5), 84.9 (C-4′), 70.2 (C-5′), 61.9 (C-3′), 40.2 (C-2′), 17.9 [*C*H(CH$_3$)$_2$], 13.2 [CH(*C*H$_3$)$_2$] ppm. HRMS: *m*/*z* calculated for $C_{21}H_{38}IN_2O_6Si_3$ [MH$^+$] 597.1313, found 597.1305. TLC (DCM/methanol, 19:1): R_f = 0.9.

Synthesis of 5-Methoxycarbonyl-3′,5′-*O*-[(tetraisopropyl)disiloxane-1,3-diyl]-2′-deoxyuridine (5): The starting nucleoside **4** (3.0 g; 5.03 mmol) was dissolved in anhydrous methanol (30 mL) and triethylamine (1.5 mL) and (bis-benzonitrile)palladium dichloride (50 mg) was added to the solution. The reaction mixture was heated at 50 °C under 80 psi carbon monoxide in a Parr pressure reactor for 18 hours. After this time the reaction mixture was filtered through celite to remove the palladium catalyst and the filtrate concentrated under vacuum to give a white powder. No further purification was required (5.0 mmol; 100% yield). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 9.02 (br., 1 H, NH), 8.55 (s, 1 H, H-6), 5.99 (m, 1 H, H-1′), 4.46 (m, 1 H, H-4′), 4.00–3.91 (m, 2 H, H5′, H5′′), 3.82 (s, 3 H, CH$_3$), 3.18 (m, 1 H, H-3′), 2.55–2.52 and 2.37–2.34 (m, 2 H, H2′, H2′′), 1.10–1.00 [m, 28 H, 4 × *CH*(CH$_3$)$_2$, 4 × CH(*CH*$_3$)$_2$] ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 25 °C): δ = 159.6 (C-4), 149.5 (C-2), 147.5 (C-6), 86.0 (C-5), 85.7 (C-4′), 68.5 (C-5′), 60.9 (C-3′), 52.9 (CH$_3$), 40.2 (C-2′), 17.5 [*C*H(CH$_3$)$_2$], 13.4 [CH(*C*H$_3$)$_2$] ppm. HRMS: *m*/*z* calculated for $C_{23}H_{40}N_2O_8$ [MH$^+$] 551.2221, found 551.2212. TLC (DCM/methanol, 19:1): R_f = 0.75.

Synthesis of 3′,5′-*O*-[(Tetraisopropyl)disiloxane-1,3-diyl]-5-[2-(trifluoroacetylamino)ethylaminocarbonyl]-2′-deoxyuridine (6): The starting nucleoside **5** (2.0 g; 3.8 mmol) was dissolved in anhydrous DMF (50 mL) and placed under argon. To the stirring solution was added ethylenediamine (2.5 mL; 38 mmol; 10.0 equiv.) and a catalytic amount of (dimethylamino)pyridine (DMAP). After 18 hours the reaction mixture was concentrated to an oil under vacuum by co-evaporation with toluene. The oil was then re-dissolved in anhydrous methanol (10 mL) and triethylamine (2.1 mL; 15.2 mmol; 4.0 equiv.) and ethyl trifluoroacetate (0.9 mL; 7.58 mmol; 2.0 equiv.) added. The solution was stirred for 10 minutes before being concentrated to an oil under vacuum. The oil was then re-dissolved in DCM (50 mL) and washed with satd. $NaHCO_3$ solution. The organic layer was separated, dried with anhydrous sodium sulfate and re-concentrated. Purification was performed by silica gel flash column chromatography eluting the column in 2% methanol in DCM. Desired fractions were concentrated to give a white foam (1.7 g; 2.78 mmol; 73%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 11.88 (br., 1 H, 3-NH), 9.46 (br. t, 1 H, 5-CONH), 8.78 (br. t, 1 H, NHCOCF$_3$), 8.40 (s, 1 H, H-6), 5.98 (d, 1 H, H-1′), 4.53 (m, 1 H, H-4′), 3.99–3.95 (m, 2 H, H5′, H-5′′), 3.74 (m, 1 H, H-3′), 3.40 (t, $^3J_{H,H}$ = 5.4 Hz, 2 H, CF$_3$CONH*CH*$_2$), 3.31 (t, $^3J_{H,H}$ = 5.3 Hz, 2 H, 5-CONH*CH*$_2$), 2.49–2.37 (m, 2 H, H2′, H2′′), 1.08–0.96 [m, 28 H, 4 × CH(CH$_3$)$_2$]. ^{13}C NMR (300 MHz, DMSO, 25 °C): δ = 163.9 (C-4), 162.7 (NHCO–Ar), 152.2 (NH*C*OCF$_3$), 150.1 (C-2), 147.1 (C-6), 105.8 (C-5), 86.2 (C-4′), 85.4 (C-1′), 70.7 (C-3′), 62.3 (C-5′), 38.1 (CH$_2$–CH$_2$), 17.9/13.5 [*C*H(CH$_3$)$_2$], 12.8 [CH(*C*H$_3$)$_2$] ppm. MS (ES$^+$): *m*/*z* = 653 [MH$^+$], 242, 180. HRMS: *m*/*z* calculated for $C_{26}H_{44}F_3N_4O_8Si_2$ [MH$^+$] 653.2650, found 653.2646. TLC (DCM/methanol, 49:1): R_f = 0.25.

Synthesis of 5-[2-(Trifluoroacetylamino)ethylaminocarbonyl]-2′-deoxyuridine (7): The starting nucleoside **6** (1.7 g; 2.61 mmol) was dissolved in anhydrous THF (34 mL) and to the stirring solution was added TBAF (1 M in THF) (6.3 mL; 6.3 mmol; 2.4 equiv.). The reaction was carefully monitored by TLC and after 2 hours water (1 mL) was added and the solution partially concentrated under vacuum before being applied directly to a silica gel column for purification. The desired product was slowly eluted from the column using a gradient of 5–10% methanol in DCM (750 mg; 1.83 mmol; 70%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 11.88 (br., 1 H, NH-3), 9.50 (t, $^3J_{H,H}$ = 4.8 Hz, 1 H, NHCOCF$_3$), 8.82 (t, $^3J_{H,H}$ = 5.6 Hz, 1 H, 5-CONH), 8.69 (s, 1 H, H-6), 6.11 (t, $^3J_{H,H}$ = 6.6 Hz, 1 H, H-1′), 5.28 (br., 1 H, 5′-OH), 5.01 (br., 1 H, 3′-OH), 4.22 (m, 1 H, H-4′), 3.84 (m, 1 H, H-3′), 3.42 (m, 2 H, 2 × H-5′), 3.40 (m, 2 H, *CH*$_2$NHCOCF$_3$), 3.32 (m, 2 H, 5-CONH*CH*$_2$), 2.19–2.09 (m, 2 H, 2 × H-2′). ^{13}C NMR (300 MHz, DMSO, 25 °C): δ = 163.9 (C-4), 162.8 (CONH), 150.4 (C-2), 146.8 (C-6), 111.0 (CF$_3$), 106.1 (C-5), 89.7 (C-4′), 87.1 (C-1′), 71.3 (C-3′), 62.1 (C-5′) ppm. MS (ES$^+$): *m*/*z* = 433 [MNa$^+$], 314, 242, 186. HRMS: *m*/*z* calculated for $C_{14}H_{17}F_3N_4O_7Na$ [MNa$^+$] 433.0947, found 433.0957. TLC (DCM/methanol, 9:1): R_f = 0.25.

Synthesis of 5′-Dimethoxytrityl-5-[2-(trifluoroacetylamino)ethylaminocarbonyl]-2′-deoxyuridine (8): The starting nucleoside **7** (1.12 g; 2.73 mmol) was dissolved in anhydrous pyridine (20 mL) and placed in a dry flask under argon. Dimethoxytrityl chloride (1.02 g; 3.0 mmol; 1.1 equiv.), pre-dissolved in a 1:1 mixture of anhydrous DCM and pyridine (8 mL), was slowly added to the stirring nucleoside solution. After 18 hours the reaction was quenched by the addition of anhydrous methanol (1 mL) and the reaction mixture was concentrated to an oil under vacuum. The oil was then re-dissolved in DCM (100 mL) and washed with satd. $NaHCO_3$ solution (100 mL) and brine (100 mL). The organics were dried with sodium sulfate and then re-concentrated under vacuum to an oil before being purified by silica gel flash column chromatography eluting with a gradient of 1–4% methanol in DCM. Desired fractions were concentrated under vacuum to give a white foam (1.52 g; 2.14 mmol; 78%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 9.49 (br., 1 H, NHCOCF$_3$), 8.82 (t, $^3J_{H,H}$ = 5.5 Hz, 1 H, 5-CONH), 8.42 (s, 1 H, H-6), 7.36–7.18/6.88–6.85 (m, 13 H, Ar-H), 6.08 (t, $^3J_{H,H}$ = 6.2 Hz, 1 H, H-1′), 5.34 (d, $^3J_{H,H}$ = 4.4 Hz, 1 H, 3′-OH), 4.08 (m, 1 H, H-4′), 3.71 (m, 1 H, H-3′), 3.39 (m, 2 H, *CH*$_2$NHCO), 3.31 (m, 2 H, 6-NH*CH*$_2$), 3.17 (d, $^3J_{H,H}$ = 3.9 Hz, 2 H, 2 × H-5′), 2.24–2.17 (m, 2 H, 2 × H-2′) ppm. MS (ES$^+$): *m*/*z* = 735 [MNa$^+$], 529, 490, 380, 303, 284, 143, 102. HRMS: *m*/*z* calculated for $C_{35}H_{35}F_3N_4O_9Na$ [MNa$^+$] 735.2254, found 735.2245. TLC (DCM/methanol, 9:1): R_f = 0.6.

Synthesis of 3′,5′-*O*-[(Tetraisopropyl)disiloxane-1,3-diyl]-2′-deoxyadenosine (10): 2′-Deoxyadenosine (10.0 g; 35.2 mmol) was dried by

co-evaporation from anhydrous pyridine (3×100 mL) before being dried overnight in a vacuum oven at 50 °C. The nucleoside was then suspended in anhydrous pyridine (100 mL) and placed under argon. 1,3-Dichloro-1,1,3,3-(tetraisopropyl)disiloxane (11.5 mL; 36 mmol; 1.02 equiv.) was carefully added to the suspension and was left to stir vigorously for two hours. The reaction mixture was then concentrated to an oil before being re-dissolved in DCM (200 mL) and washed with satd. $NaHCO_3$ solution. The organic layer was separated, washed with brine and dried with anhydrous sodium sulfate. After the organics were re-concentrated under vacuum, purification was performed by silica gel flash column chromatography eluting in a gradient of 0–2% methanol in DCM. The desired faction were combined and concentrated under vacuum to give a white foam (17.7 g; 33.6 mmol; 96%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 8.20 (s, 1 H, H-2), 8.06 (s, 1 H, H-8), 7.29 (br., 2 H, NH_2), 6.26 (d, 1 H, H-1′), 5.18 (m, 1 H, H-4′), 3.88 (m, 2 H, H-5′, H5′′), 3.77 (m, 1 H, H-3′), 2.64–2.52 (m, 2 H, H-2′, H-2′′), 1.10–1.00 [m, 28 H, 4×CH(CH$_3$)$_2$]. HRMS: *m*/*z* calculated for $C_{22}H_{40}N_5O_4Si_2$ [MH$^+$] 494.2619, found 494.2621. TLC (DCM/methanol, 19:1): R_f = 0.4.

Synthesis of 6-Iodo-3′,5′-*O*-[(tetraisopropyl)disiloxane-1,3-diyl]-2′-deoxypurine (11): The starting nucleoside **10** (10.0 g; 19 mmol) was partially dissolved in a mixture of diiodomethane (106 mL; 1.33 mol; 70 equiv.) and isoamyl nitrite (51.2 mL; 380 mmol; 20 equiv.). The reaction mixture was irradiated at 70 °C for one hour with "white" light. After this time the reaction mixture was cooled and was concentrated under vacuum before being redisssolved in DCM (250 mL) and washed with satd. sodium metabisulfite solution (150 mL) and brine (150 mL) before being dried with sodium sulfate and concentrated. Purification was performed by silica gel chromatography eluting with a gradient of 0–2% methanol in DCM. The desired organics were pooled and concentrated to give a yellow foam (7.4 g; 11.6 mmol; 61%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 8.72 (s, 1 H, H-2), 8.54 (s, 1 H, H-8), 6.37 (d, 1 H, H-1′), 5.10 (m, 1 H, H-4′), 3.89 (m, 2 H, 2×H-5′), 3.82 (m, 1 H, H-3′), 2.94–2.82/2.62–2.57 (m, 2 H, H-2′, H-2′′), 1.04–1.00 [m, 28 H, 4×CH(CH$_3$)$_2$] ppm. ^{13}C NMR (300 MHz, DMSO, 25 °C): δ = 147.7 (C-4), 139.6 (C-6), 123.9 (C-5), 85.3 (C-4′), 83.7 (C1′), 72.0 (C-2′), 63.2 (C-5′), 18.0/17.7 [*C*H(CH$_3$)$_2$], 13.5/13.0 [CH(*C*H$_3$)$_2$] ppm. HRMS: *m*/*z* calculated for $C_{22}H_{38}IN_4O_4Si_2$ [MH$^+$] 605.1476, found 605.1486. TLC (DCM/methanol, 19:1): R_f = 0.95.

Synthesis of N^6-{2-[1-(*tert*-Butoxycarbonyl)imidazol-4-yl]ethyl}-3′,5′-*O*-[(tetraisopropyl)disiloxane-1,3-diyl]-2′-deoxyadenosine (12): The starting nucleoside **11** (3.0 g; 5.06 mmol) was dissolved in anhydrous DMF (30 mL) and placed under argon. To the stirring solution was added histamine (1.68 g, 15.18 mmol; 3.0 equiv.) and triethylamine (3.06 mL; 30.36 mmol; 6.0 equiv.) and the reaction mixture left to stir overnight. Di-*tert*-butyl dicarbonate (5.28 g; 30.36 mmol; 6.0 equiv.) was then added and after 30 minutes anhydrous methanol (1 mL) was added to quench the reaction. The solution was concentrated under vacuum to give an oil before being taken up in DCM (100 mL) and washed with satd. $NaHCO_3$ solution. The organics were separated, dried with anhydrous sodium sulfate and then re-concentrated. Purification was performed by silica-gel flash column chromatography eluting with a gradient of 0–4% methanol in DCM. Desired fractions were pooled and concentrated under vacuum to yield a pale yellow foam (3.41 g; 3.64 mmol; 72%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 8.35 (br., 1 H, NH), 8.02 (s, 1 H, H-8), 7.93 (s, 1 H, H-2), 7.26 (s, 1 H, NC*H*N), 7.17 (s, 1 H, NC*H*C), 6.27 (d, 1 H, H-1′), 4.97 (m, 1 H, H-4′), 4.04 (m, 2 H, NHCH_2CH$_2$), 3.89 (m, 3 H, 2×H-5′, H-3′), 2.93 (t, $^3J_{H,H}$ = 6.4 Hz, 2 H, NHCH$_2$CH_2), 2.69–2.64 (m, 2 H, 2×H-2′), 1.63 (s, 9 H, 3×CH$_3$), 1.12–1.05 [m, 28 H, 4×CH(CH$_3$)$_2$] ppm. ^{13}C NMR (300 MHz, DMSO, 25 °C): δ = 155.3 (C-6), 153.1 (C-2), 148.5 (C=O), 147.5 (C-4), 141.8 (CH2-*C*), 114.3 (Im-CH), 85.8 (*C*CH$_3$), 85.2 (C-4′), 83.1 (C1′), 72.4 (C-2′), 63.5 (C-5′), 28.2 (C*C*H3), 27.9 (NHCH$_2$*C*H$_2$), 18.5/17.9 [CH(*C*H$_3$)$_2$], 13.5/12.9 [*C*H(CH$_3$)$_2$] ppm. MS (ES$^+$): *m*/*z* = 688 [MH$^+$], 579, 454, 342, 229, 217. HRMS: *m*/*z* calculated for $C_{32}H_{54}N_7O_6Si_2$ [MH$^+$] 688.3674, found 688.3678. TLC (DCM/methanol, 9:1): R_f = 0.9.

Synthesis of N^6-{2-[1-(*tert*-Butoxycarbonyl)imidazol-4-yl]ethyl}-2′-deoxyadenosine (13): The starting nucleoside **12** (2.1 g; 3.06 mmol) was dissolved in anhydrous THF (10 mL) and to the stirring solution was added a 1 M solution of tetrabutylammonium fluoride (TBAF) in THF (7.3 mL; 7.3 mmol; 2.4 equiv.). The reaction was carefully monitored by TLC and after 20 minutes the solution was partially concentrated to approximately one quarter of its volume before being applied directly to a silica gel column for chromatographic purification. After washing the column with DCM, the product was purified using a gradient of 4–8% methanol in DCM. Desired fractions were combined and concentrated under vacuum to give a white foam (1.0 g; 2.21 mmol; 72%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 8.11 (s, 1 H, H-8), 7.69 (br., 1 H, NH), 7.28 (s, 1 H, H-2), 7.20 (s, 1 H, NC*H*N), 6.52 (s, 1 H, NC*H*C), 6.43 (t, $^3J_{H,H}$ = 6.6 Hz, 1 H, H-1′), 4.89 (d, $^3J_{H,H}$ = 5.5 Hz, 1 H, 3′-OH), 4.89 (t, $^3J_{H,H}$ = 5.4 Hz, 1 H, 5′-OH), 4.31 (m, 1 H, H-4′), 3.77 (m, 1 H, H-3′), 3.67 (m, 2 H, NHCH_2CH$_2$), 3.52–3.30 (m, 2 H, 2×H-5′), 2.79 (t, $^3J_{H,H}$ = 7.0 Hz, 2 H, NHCH$_2$CH_2), 2.14–2.10 (m, 2 H, 2×H-2′), 1.56 (s, 9 H, 3×CH$_3$) ppm. MS (ES$^+$): *m*/*z* = 446 [MH$^+$], 346, 284, 242, 186, 143. HRMS: *m*/*z* calculated for $C_{20}H_{28}N_7O_5$ [MH$^+$] 446.2152, found 446.2162. TLC (DCM/methanol, 9:1): R_f = 0.5.

Synthesis of N^6-{2-[1-(*tert*-Butoxycarbonyl)imidazol-4-yl]ethyl}-5′-dimethoxytrityl-2′-deoxyadenosine (14): The starting nucleoside **13** (880 mg; 1.77 mmol) was dried by repeated co-evaporation from anhydrous pyridine (3×20 mL) before being dissolved in a further portion of the solvent (10 mL). Dimethoxytrityl chloride (687 mg; 2.03 mmol; 1.15 equiv.) was added to the stirring solution under argon before being left for 48 hours. After this period, a further portion of dimethoxytrityl chloride (0.1 equiv.) was added and the reaction monitored by TLC over 4 hours. As no further reaction seemed to be taking place, the reaction was quenched by the addition of methanol (1 mL) and the solution was concentrated under vacuum to give an oil. After dissolving the oil in DCM (50 mL) it was washed with satd. $NaHCO_3$ solution (50 mL) and re-concentrated before being purified by silica gel flash column chromatography eluting the desired product in a gradient of 0–4% methanol (1.12 g; 14.8 mmol; 83%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 8.11 (s, 1 H, H-8), 7.69 (br., 1 H, NH), 7.36 (s, 1 H, H-2), 7.33–7.20/6.84–6.79 (m, 13 H, Ar–H), 7.05 (s, 1 H, NC*H*C), 6.51 (s, 1 H, NC*H*N), 6.45 (t, 1 H, H-1′), 5.32 (d, $^3J_{H,H}$ = 5.3 Hz, 1 H, 3′-OH), 4.32 (m, 1 H, H-4′), 3.90 (m, 1 H, H-3′), 3.70 (s, 6 H, 2×CH$_3$), 3.32 (m, 2 H, NHCH_2CH$_2$), 3.10 (m, 2 H, 2×H-5′), 2.79 (t, $^3J_{H,H}$ = 7.0 Hz, 2 H, NHCH$_2$CH_2), 2.22–2.18 (m, 2 H, 2×H-2′), 1.56 (s, 9 H, 3×CH$_3$) ppm. MS (ES$^+$): *m*/*z* = 748 [MH$^+$], 648, 546, 380, 303, 189. HRMS: *m*/*z* calculated for $C_{41}H_{45}N_7O_7Na$ [MNa$^+$] 770.3278, found 770.3270. TLC (DCM/methanol, 19:1): R_f = 0.5.

Synthesis of 3′,5′-*O*-[(Tetraisopropyl)disiloxane-1,3-diyl]-N^6-[2-(trifluoroacetylamino)ethylamino]-2′-deoxyadenosine (16): The starting nucleoside **11** (3.0 g; 5.1 mmol) was dissolved in anhydrous DMF (40 mL) and placed under argon. To the stirring solution was added ethylenediamine (6.8 mL; 102 mmol; 2.0 equiv.) and the reaction carefully monitored by TLC. After 1 hour the solution was

concentrated to an oil under vacuum and the residue re-dissolved in DCM (100 mL). The organics were washed with satd. $NaHCO_3$ (100 mL) and brine (100 mL) before being dried with sodium sulfate and concentrated to an oil under vacuum. The oil was then taken up in anhydrous methanol (40 mL) and triethylamine (14.2 mL; 102 mmol; 20.0 equiv.) and ethyl trifluoroacetate (3.0 mL; 2.5 mmol; 5.0 equiv.) were added. After 20 minutes the reaction mixture was once again concentrated under vacuum before being taken up in DCM (100 mL) and washed with satd. $NaHCO_3$ solution. After the organics were separated, dried with sodium sulfate and concentrated to an oil under vacuum, they were purified by silica gel flash column chromatography eluting with a gradient of 0–2% methanol in DCM. The desired fractions were concentrated to give a pale yellow foam (2.4 g; 3.87 mmol; 76%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 9.49 (br., 1 H, $NHCOCF_3$), 8.23 (s, 1 H, C-8), 8.12 (s, 1 H, C-2), 7.97 (br., 1 H, 6-NH), 6.27 (d, 1 H, H-1′), 5.22 (m, 2 H, 6-NHCH_2), 3.90 (m, 2 H, H-5′), 3.79 (m, 1 H, H-4′), 3.62 (m, 2 H, 6-$NHCH_2CH_2$), 3.41 (m, 1 H, H-3′), 2.85–2.81/2.58–2.55 (m, 2 H, H2′, H2′′), 1.10–1.01 [m, 28 H, 4×$CH(CH_3)_2$] ppm. ^{13}C NMR (300 MHz, DMSO, 25 °C): δ = 155.5 (C=O / C-6), 152.9 (C-2), 148.6 (C-4), 118.3 (C-5), 85.2 (C-4′), 83.1 (C-1′), 72.5 (C-3′), 63.6 (C-5′), 38.2 ($CONHCH_2$), 17.9–17.7/13.5–12.9 [$CH(CH_3)_2$, $CH(CH_3)_2$] ppm. MS (ES$^+$): *m*/*z* = 633 [MH$^+$], 522, 377, 342, 229, 185, 143. HRMS: *m*/*z* calculated for $C_{28}H_{42}F_3N_6O_5Si_2$ 655.2707, found 655.2710. TLC (DCM/methanol, 19:1): R_f = 0.1.

Synthesis of *N*^{6}-[2-(Trifluoroacetylamino)ethyl]-2′-deoxyadenosine (17): The starting nucleoside **16** (2.25 g; 3.56 mmol) was dissolved in anhydrous THF (25 mL) and a 1 M solution of TBAF in THF (7.8 mL; 7.8 mmol; 2.2 equiv.) added. The reaction mixture was left to stir at room temperature for approximately 30 minutes before being concentrated under vacuum to about one quarter of its original volume. This was then applied directly to a silica gel flash column chromatography column for purification. After washing the column with DCM, the product was eluted in a gradient of 4–7% methanol in DCM (1.2 g; 3.01 mmol; 85%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 9.5 (br., 1 H, $NHCOCF_3$), 8.36 (s, 1 H, H-8), 8.20 (s, 1 H, H-2), 7.99 (br., 1 H, 6-NH), 6.34 (t, $^3J_{H,H}$ = 7.3 Hz, 1 H, H-1′), 5.31 (d, $^3J_{H,H}$ = 3.9 Hz, 1 H, 3′-OH), 5.21 (t, $^3J_{H,H}$ = 5.3 Hz, 1 H, 5′-OH), 4.39 (m, 1 H, H-4′), 3.86 (m, 1 H, H-3′), 3.62–3.50 (m, 6 H, 2×H-5′, 2×CH_2), 2.71 (m, 1 H, H-2′), 2.26 (m, 1 H, H-2′′) ppm. MS (ES$^+$): *m*/*z* = 391 [MH$^+$], 280, 275, 229, 164. HRMS: *m*/*z* calculated for $C_{14}H_{18}F_3N_6O_4$ [MH$^+$] 391.1342, found 391.1331. TLC (DCM/methanol, 9:1): R_f = 0.25.

Synthesis of 5′-Dimethoxytrityl-*N*^{6}-[2-(trifluoroacetylamino)ethyl]-2′-deoxyadenosine (18): The starting nucleoside **17** (1.0 g; 2.51 mmol) was dried by repeated co-evaporation form anhydrous pyridine (3×20 mL) before being re-dissolved in a further portion of the solvent (10 mL). Dimethoxytrityl chloride (978 mg; 2.89 mmol; 1.15 equiv.), pre-dissolved in a 1:1 mixture of anhydrous pyridine and DCM (5 mL), was slowly added to the stirring nucleoside solution under argon. After 6 hours the reaction was quenched by the addition of anhydrous methanol (1 mL) and the reaction mixture concentrated to an oil under vacuum before being re-dissolved in DCM (50 mL). This organic solution was then washed with satd. $NaHCO_3$ solution (50 mL) and brine (50 mL) before being dried with sodium sulfate and re-concentrated to an oil. Purification was performed by silica gel flash column chromatography eluting with a gradient of 0–4% methanol in DCM. Desired fractions were combined and concentrated under vacuum to yield a pale yellow foam (1.4 g; 2.0 mmol; 80%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 9.50 (br., 1 H, $NHCOCF_3$), 8.13 (s, 1 H, H-8), 8.14 (s, 1 H, H-2), 7.95 (br., 1 H, 6-NH), 7.33–7.30, 7.23–7.17/6.81–6.76 (m, 13 H, Ar–H), 6.36 (t, $^3J_{H,H}$ = 6.5 Hz, 1 H, H-1′), 5.37 (d, $^3J_{H,H}$ = 4.4 Hz 1 H, 3′-OH), 4.46 (m, 1 H, H-4′), 3.96 (m, 1 H, H-3′), 3.70 (s, 6 H, 2×OCH_3), 3.62, 3.42/3.16 (m, 6 H, 2×H-5′, 2×CH_2), 2.85/2.34 (m, 2 H, H-2′, H-2′′) ppm. MS (ES$^+$): *m*/*z* = 693 [MH$^+$], 492, 303, 242, 102. HRMS: *m*/*z* calculated for $C_{15}H_{36}F_3N_6O_6$ [MH$^+$] 693.2648, found 693.2618. TLC (DCM/methanol, 9:1): R_f = 0.65.

Synthesis of 3′,5′-*O*-[(Tetraisopropyl)disiloxane-1,3-diyl]-2′-deoxyuridine (20): 2′-Deoxyuridine (6.89 g; 30.2 mmol) was dried by repeated co-evaporation from anhydrous pyridine (3×50 mL) before being suspended in a further portion of the solvent (100 mL). 1,3-Dichloro-1,1,3,3-(tetraisopropyl)disiloxane (10 g; 31.7 mmol; 1.05 equiv.) was slowly added to the stirring suspension under argon and left for two hours. The pyridine was removed under vacuum and the remaining white solid dissolved in DCM (250 mL) before being washed with satd. $NaHCO_3$ (250 mL) and brine (250 mL). The organics were separated, dried with sodium sulfate and concentrated to an oil. Purification was performed by silica gel flash column chromatography eluting in a gradient of 0–3% methanol in DCM (13.2 g; 28.3 mmol; 94%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 10.19 (br., 1 H, NH), 7.76 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H, H-6), 6.03 (d, $^3J_{H,H}$ = 6.2 Hz, 1 H, H1′), 5.68 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H, H-5), 4.41 (m, 1 H, H-4′), 4.13–4.09/3.99–3.95 (m, 2 H, H-5′, H-5′′), 3.73 (m, 1 H, H-3′), 2.53–2.46/2.27–2.20 (m, 2 H, H-2′, H-2′′), 1.14–0.98 [m, 28 H, 4×$CH(CH_3)_2$] ppm. HRMS: *m*/*z* calculated for $C_{21}H_{39}N_2O_6Si_2$ [MH$^+$] 471.2347, found 471.2352. TLC (DCM/methanol, 9:1): R_f 0.95

Synthesis of 6-Methyl-3′,5′-*O*-[(tetraisopropyl)disiloxane-1,3-diyl]-2′-deoxyuridine (21): The starting nucleoside **20** (14.5 g; 30.8 mmol) was dissolved in anhydrous THF (100 mL) and sealed in a flask under argon before being cooled to –78 °C. To the stirring solution was added lithium diisopropylamide (1.5 M solution in hexane) (100 mL; 154 mmol; 5.0 equiv.) in a manner so as to maintain the reaction temperature below –70 °C. The solution was then stirred for one hour before the addition of iodomethane (2.9 mL; 46.2 mmol; 1.5 equiv.) and stirred for a further 2 hours. After this time, glacial acetic acid (5 mL) was added and the solution warmed up to room temperature before being concentrated under vacuum. After re-dissolving the residue in DCM, a sodium hydrogen carbonate work-up was performed and the organics re-concentrated under vacuum. Purification was performed by silica gel flash column chromatography eluting the column in 30% Ethyl acetate in hexane and the after the elution of the faster running starting material, 60% ethyl acetate in hexane. The desired fractions were concentrated to yield a white solid (8.65 g; 17.9 mmol; 58%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 9.38 (br., 1 H, NH), 5.95 (m, 1 H, H-1′), 5.51 (s, 1 H, H-5), 4.91 (m, 1 H, H-4′), 3.98 (m, 2 H, 2×H-5′), 3.75 (m, 1 H, H-3′), 2.87/2.33 (m, 2 H, H-2′, H-2′′), 2.29 (s, 3 H, 6-CH3), 1.54 (s, 9 H, 3×BOC-CH_3), 1.11–0.95 [m, 28 H, 4×$CH(CH_3)_2$] ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 25 °C): δ = 163.5 (C-4), 153.7 (C-2), 150.4 (C-6), 103.4 (C-5), 86.1 (C-4′), 84.7 (C-1′), 73.4 (C-3′), 64.0 (C-5′), 39.6 (C-2′), 21.0 (6-CH3), 17.8 [$CH(CH_3)_2$], 13.6 [$CH(CH_3)_2$] ppm. HRMS: *m*/*z* calculated for $C_{22}H_{40}N_2O_6NaSi_2$ [MNa$^+$] 507.2323, found 507.2325. TLC (DCM/methanol, 19:1): R_f = 0.8.

Synthesis of *N*^{4}-{2-[1-(*tert*-Butoxycarbonyl)imidazol-4-yl]ethyl}-6-methyl-3′,5′-*O*-[(tetraisopropyl)disiloxane-1,3-diyl]-2′-deoxycytidine (22): The starting nucleoside **21** (1.1 g; 2.28 mmol) was dissolved in anhydrous DCM and placed in a flask under argon. To the stirring solution was added triethylamine (0.35 mL; 5.48 mmol; 2.4 equiv.) tosyl chloride (433 mg; 2.74 mmol; 1.2 equiv.) and a catalytic amount of 4-(dimethylamino)pyridine. The solution was left to stir

overnight. The reaction mixture was then washed with saturated $NaHCO_3$ solution, dried with sodium sulfate and concentrated to a white foam. The tosylated nucleoside was re-dissolved in anhydrous DMF before histamine (760 mg; 6.84 mmol; 3.0 equiv.) and triethylamine (1.9 mL; 13.7 mmol; 6.0 equiv.) were added and the solution stirred at room temperature for 2 hours. Di-*tert*-butyl dicarbonate (3.0 g; 13.7 mmol; 6.0 equiv.) was then added to the reaction mixture with a further portion of triethylamine (0.95 mL; 6.84 mmol; 3.0 equiv.). After 30 minutes the reaction was quenched by the addition of a small amount of anhydrous methanol (1 mL) and the reaction mixture concentrated to an oil under vacuum. The oil was taken up in DCM (50 mL) before being washed with satd. $NaHCO_3$ solution (50 mL) and brine (50 mL). The organics were separated, dried and concentrated under vacuum. Purification was performed by silica-gel flash column chromatography eluting with a gradient of 0–4% methanol in DCM (944 mg; 1.46 mmol; 64%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 8.11 (s, 1 H, N*CH*N), 7.75 (br., 1 H, NH), 7.27 (s, 1 H, N*CH*C), 5.98 (m, 1 H, H-1′), 5.47 (s, 1 H, H-5), 5.01 (m, 1 H, H-4′), 3.97/3.84 (m, 2 H, H-5′, H-5′′), 3.85 (m, 1 H, H-3′), 3.45 (m, 2 H, NH*CH*$_2$), 2.71 (t, 2 H, NHCH$_2$*CH*$_2$), 2.22 (m, 2 H, 2×H-2′), 2.18 (s, 3 H, 6-CH_3), 1.52 (s, 9 H, 3×BOC-CH_3), 1.14–0.98 [m, 28 H, 4×CH(CH_3)$_2$] ppm. ^{13}C NMR (300 MHz, DMSO, 25 °C): δ = 163.9 (C-4), 156.1 (C-6), 153.2 (C-2), 147.6 (NCO), 141.7 (CH_2CH_2C), 130.3 (Im-N*C*HN), 128.4 (Im-C*C*HN), 96.0 (C-5), 86.2 (C-4′), 84.7 (C-1′), 79.4 [*C*(CH_3)$_3$], 75.4 [C-3′], 65.3 (C-5′), 28.4 (6-CH_3), 17.6 [*C*H(CH_3)$_2$], 13.6 [CH(*C*H$_3$)$_2$] ppm. MS (ES$^+$): *m*/*z* = 678 [MH$^+$], 578, 488, 410, 320, 229, 143. HRMS: *m*/*z* calculated for $C_{32}H_{56}N_5O_7Si_2$ [MH$^+$], 678.3718, found 678.3735. TLC (19:1 DCM: Methanol): R_f 0.25.

Synthesis of *N*^{4}-{2-[1-(*tert*-Butoxycarbonyl)imidazol-4-yl]ethyl}-6-methyl-2′-deoxycytidine (23): The starting nucleoside **22** (4.75 g; 7.0 mmol) was dissolved in anhydrous THF (80 mL) and a 1 M solution of TBAF in THF (16.8 mL; 16.8 mmol; 2.4 equiv.) added. After 30 minutes the reaction mixture was concentrated to an oil and then loaded directly onto a silica gel chromatography column for purification. The desired compound was eluted using a gradient of 4–7% methanol in DCM. Desired fractions were combined and concentrated under vacuum to yield a white solid (2.0 g; 4.47 mmol; 64%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 8.11 (s, 1 H, N*CH*N), 7.61 (t, $^3J_{H,H}$ = 8.0 Hz, 1 H, NH), 7.28 (s, 1 H, N*CH*C), 6.11 (m, 1 H, H-1′), 5.52 (s, 1 H, H-5), 5.07 (d, $^3J_{H,H}$ = 4.8 Hz 1 H, 3′-OH), 4.80 (m, 1 H, 5′-OH), 4.31 (m, 1 H, H-4′), 3.65–3.45 (m, 5 H, 2×H-5′, H-3′, NH*CH*$_2$), 2.71 (m, 2 H, NHCH$_2$*CH*$_2$), 2.26 (s, 3 H, CH_3), 1.93 (m, 2 H, 2×H-2′), 1.54 (s, 9 H, 3×BOC-CH_3) ppm. MS (ES$^+$): *m*/*z* = 436 [MH$^+$], 343, 320, 229, 186. HRMS: *m*/*z* calculated for $C_{20}H_{29}N_5O_6$ [MH$^+$] 458.2016, found 458.2031. TLC (DCM/methanol, 9:1): R_f = 0.25.

Synthesis of *N*^{4}-{2-[1-(*tert*-Butoxycarbonyl)imidazol-4-yl]ethyl}-5′-dimethoxytrityl-6-methyl-2′-deoxycytidine (24): The starting nucleoside **23** was dried overnight in a vacuum oven before being dried further by repeated co-evaporation from anhydrous pyridine (2×20 mL). It was then dissolved in a further portion of anhydrous pyridine (10 mL) and sealed in a vessel under argon. Dimethoxytrityl chloride (1.45 g; 4.30 mmol; 1.1 equiv.), pre-dissolved in a 1:1 mixture of anhydrous pyridine and DCM (10 mL), was slowly added to the stirring solution. After 6 hours the reaction was quenched by the addition of anhydrous methanol (1 mL) and the solution concentrated to an oil under vacuum. The oil was taken up in DCM (50 mL) and washed with satd. $NaHCO_3$ solution (50 mL) and brine (50 mL) before being separated, dried with sodium sulfate and concentrated to a foam under vacuum. Purification was performed by silica gel flash column chromatography eluting with a gradient of 0–4% methanol in DCM to yield a yellow foam on drying (2.28 g; 3.04 mmol; 78%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.98 (s, 1 H, N*CH*N), 7.43–7.19/6.80–6.77 (m, 13 H, Ar-H), 7.18 (s, 1 H, N*CH*C), 6.22 (m, 1 H, H-1′), 5.39 (s, 1 H, H-5), 4.68 (m, 1 H, H-4′), 3.80 (m, 1 H, H-3′), 3.71 (s, 6 H, 2×OCH_3), 3.69 (m, 2 H, 4-NH*CH*$_2$), 3.48–3.40 (m, 2 H, 2×H-5′), 2.78 (m, 2 H, 4-NHCH$_2$*CH*$_2$), 2.61–2.56 (m, 5 H, 6-CH_3, 2×H-2′), 1.60 (s, 9 H, 3×BOC-CH_3) ppm. MS (ES$^+$): *m*/*z* = 760 [MNa$^+$], 738 [MH$^+$], 660, 529, 380, 303, 284, 143. HRMS: *m*/*z* calculated for $C_{41}H_{48}N_5O_8$ [MH$^+$] 738.3503, found 738.3525. TLC (DCM/methanol, 19:1): R_f = 0.3.

Synthesis of 6-Methyl-3′,5′-*O*-[(tetraisopropyl)disiloxane-1,3-diyl]-*N*^{4}-[2-{trifluoroacetylamino}ethyl]-2′-deoxycytidine (26): The starting nucleoside **21** (1.2 g; 2.51 mmol) was dissolved in anhydrous DCM and placed in a flask under argon. To the stirring solution was added triethylamine (0.39 mL; 6.02 mmol; 2.4 equiv.) tosyl chloride (476 mg; 3.01 mmol; 1.2 equiv.) and a catalytic amount of dimethylaminopyridine. The solution was left to stir overnight at room temperature. The reaction mixture was then washed with saturated $NaHCO_3$ solution, dried with sodium sulfate and concentrated to white foam. The solid was re-dissolved in anhydrous DMF and ethylenediamine (1.0 mL; 15.06 mmol; 6.0 equiv.) and triethylamine (2.1 mL; 15.1 mmol; 6.0 equiv.) were added and the solution stirred at room temperature for 30 minutes. The reaction mixture was concentrated to an oil and then co-evaporated with portions of toluene (3×20 mL) to dryness. The solid was re-dissolved in anhydrous methanol and ethyl trifluoroacetate (3.0 mL; 25.1 mmol, 10.0 equiv.) and triethylamine added (1.05 mL; 7.52 mmol; 3.0 equiv.). After 20 minutes the reaction mixture was concentrated to an oil under vacuum. The oil was taken up in DCM (100 mL) before being washed with satd. $NaHCO_3$ solution (100 mL) and brine (100 mL). The organics were separated, dried and concentrated under vacuum. Purification was performed by silica-gel flash column chromatography eluting with a gradient of 0–4% methanol in DCM (1.24 g; 1.91 mmol; 76%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 9.49 (br., 1 H, NHCOCF_3), 7.77 (br., 1 H, 4-NH), 5.99 (m, 1 H, H-1′), 5.48 (s, 1 H, H-5), 5.00 (m, 1 H, H-4′), 3.98/3.94 (m, 2 H, 2×H-5′), 3.30 (m, 1 H, H-3′), 2.71 (m, 2 H, 4-NH*CH*$_2$), 2.26 (m, 2 H, H-2′), 2.20 (s, 3 H, 6-CH_3), 1.11–0.96 [m, 28 H, 4×CH(CH_3)$_2$] ppm. MS (ES$^+$): *m*/*z* = 645 [MNa$^+$], 623 [MH$^+$], 454, 342, 265, 229, 185, 143. HRMS: *m*/*z* calculated for $C_{26}H_{45}F_3N_4O_6NaSi_2$ [MNa$^+$] 645.2727, found 645.2719. TLC (DCM/methanol, 9:1): R_f = 0.8.

Synthesis of 6-Methyl-*N*^{4}-[2-(trifluoroacetylamino)ethyl]-2′-deoxycytidine (27): The starting nucleoside **26** (1.90 g; 3.04 mmol) was dissolved in anhydrous THF (40 mL) and a 1 M solution of TBAF in THF (7.3 mL; 7.30 mmol; 2.4 equiv.) was added and the solution stirred for 10 minutes. The reaction mixture was then partially concentrated under vacuum and then applied directly to a silica gel chromatography column for purification. After eluting the column with a large volume of DCM (400 mL) the desired product was eluted using a gradient of 6–8% methanol in DCM. Desired fractions were combined and concentrated under vacuum to yield a white solid (920 mg; 2.36 mmol; 78%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 9.49 (br., 1 H, NHCOCF_3), 7.71 (br., 1 H, 4-NH), 6.12 (m, 1 H, H-1′), 5.53 (s, 1 H, H-5), 5.08 (d, 1 H, 3′-OH, *J* = 4.9), 4.78 (t, $^3J_{H,H}$ = 5.4 Hz, 1 H, 5′-OH), 4.32 (m, 1 H, H-4′), 3.65–3.57/3.51–3.47 (m, 5 H, 2×H-5′, H-3′, 2×NHCH$_2$*CH*$_2$), 2.25 (s, 3 H, 6-CH_3), 1.94 (m, 2 H, 2×H-2′) ppm. MS (ES$^+$): *m*/*z* = 403 [MNa$^+$], 288, 265, 186. HRMS: *m*/*z* calculated for $C_{14}H_{20}F_3N_4O_5$ [MH$^+$], 338.1386, found 338.1371. TLC (DCM/methanol, 9:1): R_f = 0.4.

Synthesis of 5′-Dimethoxytrityl-6-methyl-N^4-[2-(trifluoroacetylamino)ethyl]-2′-deoxycytidine (28): The starting nucleoside **27** (850 mg; 2.18 mmol) was dried by repeated co-evaporation form anhydrous pyridine (3 × 20 mL) before being dissolved in a further portion of the solvent (10 mL). Dimethoxytrityl chloride (811 mg; 2.40 mmol; 1.1 equiv.) was added and the reaction mixture stirred under argon at room temperature overnight. The reaction was quenched by the addition of anhydrous methanol (1 mL) and concentrated to an oil under vacuum. After taking the oil up in DCM (50 mL) it was washed with saturated $NaHCO_3$ solution and brine. The organics were dried with sodium sulfate and then concentrated to a foam. Purification was performed by silica gel flash column chromatography eluting with a gradient of 0–4% methanol in DCM (1.35 g; 1.95 mmol; 89%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 9.83 (br., 1 H, $NHCOCF_3$), 7.43–7.40, 7.28–7.19, 6.8–6.77 (m, 13 H, Ar–H), 6.19 (m, 1 H, H-1′), 5.52 (s, 1 H, H-5), 4.62 (m, 1 H, H-4′), 3.94 (m, 1 H, H-3′), 3.77 (s, 6 H, 2 × OCH_3), 3.53–3.47 (m, 2 H, 2 × H-5′), 3.23–3.14, 2.71–2.68 (m, 4 H, $NHCH_2CH_2$), 2.19 (s, 3 H, 6-CH_3), 1.92 (m, 2 H, 2 × H-2′) ppm. MS (ES^+): *m*/*z* = 683 [MH^+], 492, 436, 303, 242, 150, 102. HRMS = *m*/*z* calculated for $C_{35}H_{38}F_3N_4O_7$ [MH^+], 683.2720, found 683.2722. TLC (DCM/methanol, 19:1): R_f = 0.25.

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