CG base pair recognition within DNA triple helices by modified N-methylpyrrolo-dC nucleosides[†]

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3-Aminophenyl-modified analogues of the bicyclic nucleoside *N*-methyl-3*H*-pyrrolo[2,3*d*]pyrimidin-2(7*H*)-one were synthesised and incorporated directly into triplex-forming oligonucleotides in order to utilise their extended hydrogen bonding motif for recognition of the CG base pair. All analogues demonstrated strong binding affinity and very good selectivity for CG from pH 6.2 to 7.0; a marked improvement on previous modifications.

Introduction

Since their discovery in 1957,¹ the biomedical and biotechnological importance of DNA triple helices has been exploited. These structures have potential applications in gene therapy (antigene strategy),² site-directed DNA cleavage^{2a,3} and repair,^{2a,4} or as tools in molecular biology and biotechnology.^{2a,5} Binding of a triplex-forming oligonucleotide (TFO) to a specific region of the genome may block binding of the duplex by helicases. This inhibits gene expression, prevents DNA replication and interrupts cell division (chemotherapeutics).

DNA triple helices are formed by binding of a single-stranded TFO to a target duplex, *via* specific hydrogen bond interactions within the major groove. GC and AT base pairs form triplets with the natural bases C⁺ (protonated) and T respectively. Using modified nucleosides in TFOs,⁶ however, significantly enhances duplex binding affinity and selectivity.

Applications of TFOs require strong, selective binding to mixed-sequence DNA. However, triple helix-mediated recognition of Py.Pu base pairs (CG, TA) in double-stranded DNA is a significantly greater challenge than for the Pu.Py base pairs (GC, AT), as fewer hydrogen bonding residues are present within the major groove of the duplex.7 Thymine is the only natural base capable of binding to CG, but lacks selectivity, actually forming a more stable triplet with AT. The unnatural base ^{4H}T, one of several presented as a replacement for T (Fig. 1),8 is selective for CG, forming one weak C-H...O hydrogen bond and one conventional N-H ··· N hydrogen bond. Ranasinghe et al.9 examined the N-methylated base MP, an analogue of the bicyclic nucleoside, 6-methylpyrrolo-dC (MNHP), which has been investigated as a fluorescent mimic of C in duplex forming probes, the fluorescence of which is sensitive to hybridisation with G.¹⁰ The core structure maintains the hydrogen bonding motif of ^{4H}T,



Fig. 1 Binding models of T.CG, 4H T.CG, M P.CG and A PP.CG triplets. R = 2'-deoxyribofuranose sugar.

whilst contributing extra base-stacking *via* an extended conjugated system. Recognition was enhanced further by extending hydrogen bonding interactions across the CG base pair. This was achieved by replacing the 6-methyl group of ^MP with a 2-aminoethyl (^AEP) or 3-aminopropyl group (^APP) for binding to C=O⁶ and N⁷ of G, whilst introducing additional charge-stabilisation due to protonation of the pendant amine group. The 4*N*-methyl group was vital for maintaining selectivity, as it prevents this C-analogue from binding to GC base pairs.

These monomers demonstrated enhanced binding affinity to CG and improved selectivity compared to T, but were similar to ^{4H}T and ^MP, suggesting the aminopropyl group of ^APP does not contribute greatly to binding. From this work we hypothesised that reducing linker flexibility whilst allowing some rotational freedom around the aryl-C⁶ bond might position the amino group more precisely for effective hydrogen bonding.

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To this end, a number of TFOs containing substituted phenyl-*N*-methylpyrrolo-dC nucleotides (^xP) have been synthesised, with amino, acetamido, ureido, and guanidino groups in the 3-position (Fig. 2). Previously our approach has been to incorporate the furano-dT monomer in to TFOs and convert post-synthetically to *N*-methylpyrrolo-dC.¹¹ We now describe the direct incorporation of the *N*-methylpyrrolo-dC phosphoramidites in to TFOs, which is a higher yielding and simpler strategy.



Fig. 2 Putative ^{Ph}P.CG, ^AP.CG and ^UP.CG triplet binding motifs, and ^XP nucleotide. R = 2'-deoxyribofuranose, R' = H (^{Ph}P), NH_2 (^AP), $NHCOCH_3$ (^{Ac}P), $NHCONH_2$ (^UP), $NHC(NH_2^+)NH_2$ (^GP).

Results and discussion

The preliminary series of nucleosides, based on a 3H-furano[2,3-d]pyrimidin-2(7H)-one core, were constructed as described by Ranasinghe *et al.*^{9a} for the synthesis of ^AEP and ^APP. These monomers were incorporated into oligonucleotides and post-synthetically converted from furano- to *N*-methylpyrrolopyrimidines, as previously reported (ring-opening with methylamine, then subjected to acid-catalysed cyclisation with DOWEX-H⁺ resin).¹¹

Synthesis of N-methylpyrrolo-dC analogues

The alternative synthetic strategy, direct synthesis of *N*-methylpyrrolo-dC phosphoramidite monomers followed by incorporation into TFOs, obviates the need for tedious post-synthetic modification. This was adopted due to problems with the previous method caused by incomplete re-cyclisation of the *N*-methylpyrrolopyrimidine core (identified by HPLC), giving rise to secondary transitions in triplex melting studies. The direct synthetic route involved conversion of 4,4'-dimethoxytrityl (DMTr) protected 5-iodo-2'-deoxyuridine **2** into 5-iodo-4*N*-methyl-2'-deoxycytidine **3** *via* activation of the 4-carbonyl position, using

N-methylimidazole and POCl₃.¹² After methylamine insertion and deacetylation, the conversion product **3** was obtained in 90% yield (Scheme 1).



Five modified 6-phenyl-*N*-methylpyrrolo-dC phosphoramidite monomers were synthesised from this intermediate; trifluoroacetamido- (^AP, **12**), acetamido- (^{Ac}P, **8b**), ureido-(^UP, **8c**), guanidino- (^GP, **17**) and also 6-phenyl-*N*-methylpyrrolodC phosphoramidite (^{Ph}P, **8a**). Monomers **8a–c** were synthesised *via* Pd-catalysed cross-coupling with the appropriate phenyl acetylene (Scheme 2), followed by CuI-catalysed cyclisation¹³ then phosphitylation (Scheme 3). Monomers **12** and **17** required additional steps as described below (Schemes 4, 5).





Scheme 3 Synthesis of ^{Ph}P **8a**, ^{Ac}P **8b**, and ^UP **8c** phosphoramidites. i) alkyne **5a–c** (3.0 eq), Pd(PPh₃)₄ (0.1 eq), CuI (0.4 eq), Et₃N (5.0 eq), DMF, dark, rt, 1–3 h; ii) CuI (1.1 eq), Et₃N (10.0 eq), 4 Å sieves, DMF, dark, 125 °C, 0.5–2 h; iii) 2-cyanoethoxy-N,N-diisopropylamino chlorophosphine (1.1 eq), DIPEA (2.5 eq), CH₂Cl₂ or THF–DMF (1:2 v/v), rt, 1–2 h. R = H (a), NHCOCH₃ (b), NHCONH₂ (c).

Monomer ^AP **12** was synthesised by the route in Scheme 4, which involved trifluoroacetylation of ^AP monomer precursor **10** under forcing conditions, followed by phosphitylation. Monomer ^GP **17** was synthesised by guanidinylation of **10** using isothiourea **15**.



Scheme 4 Synthesis of ^AP phosphoramidite **12**. i) alkyne **4** (3.0 eq), Pd(PPh₃)₄ (0.1 eq), CuI (0.4 eq), Et₃N (5.0 eq), DMF, dark, rt, 1.5 h; ii) CuI (1.1 eq), Et₃N (10.0 eq), 4 Å sieves, DMF, dark, 125 °C, 1.5 h; iii) CF₃CO₂Et (20.0 eq), DMAP (1.0 eq), Et₃N (22.0 eq), CH₂Cl₂, 0 °C-rt, 35 min then 50 °C, overnight; iv) 2-cyanoethoxy-*N*,*N*-diisopropylamino chlorophosphine (1.1 eq), DIPEA (2.5 eq), CH₂Cl₂, rt, 5 h.

Guanidinylating reagent **15** was synthesised by a much improved synthetic route, in 89% yield compared to the literature yield of 35%.¹⁴ Guanidinylation followed by phosphitylation afforded the desired phosphoramidite monomer (Scheme 5). Careful handling minimised degradation of these acid- and base-sensitive protected guanidinyl-nucleosides.



Scheme 5 Synthesis of ^GP phosphoramidite 17. i) carbonate 14 (2.0 eq), NaHCO₃ (3.0 eq), degassed CH₂Cl₂–H₂O (2:1 v/v), rt, 8 h; ii) carbonate 14 (1.0 eq), CH₂Cl₂, rt, 14.5 h; iii) isothiourea 15 (2.4 eq), pyridine (4.8 eq), 4 Å sieves, CH₂Cl₂, reflux, 2 d; iv) 2-cyanoethoxy-*N*,*N*-diisopropylamino chlorophosphine (1.8 eq), DIPEA (2.0 eq), CH₂Cl₂, rt, 2 h. Compound 15 was synthesised by modification to literature route.¹⁴ Compound 14 synthesised according to literature method.¹⁵

Five monomers, ^{Ph}P, ^AP, ^{Ac}P, ^UP and ^GP (Fig. 3), and the natural nucleoside T, were thus incorporated into TFOs for UV and fluorescence melting studies, using standard solid-phase DNA synthesis, and standard deprotection conditions (conc. aq ammonia, rt or 55 °C, 4–24 h).

Melting studies

Preliminary DNA melting data¹¹ indicated high binding affinities and good selectivity for CG. In the present study, UV melting



Fig. 3 Monomers incorporated into oligonucleotides.

Table 1 UV triplex melting experiment: \underline{XP} against \underline{YZ} at pH 6.2, 6.6 and7.0 (10 mM sodium phosphate, 200 mM NaCl, 1 mM Na₂EDTA)^{*a.b*}

5'-	5'- -GCT CGA	TTT AAA TTT	TTM AAG TTC	V <u>X</u> W AYA T <u>Z</u> T	MTM GAG CTC	TMT AGA TCT	TCG AGC-	5'	
VXW	YZ	pН	Т	^{Ph} I		Υ ^Λ	^{Ac} P	$^{\rm U}\mathbf{P}$	GР
TXT PXT PXT TXT TXP PXT PXT PXP TXT	CG GC AT TA CG CG	6.2 6.2 6.6 7.0	26.7 — — 30.2 20.1 n.d. 23.6 22.9	7 32 22 22 18 34 34 2 36 1 24 . 26 5 26 9 28	.2 .7 .9 .9 .6 1 .2 .9 .9 .9 .9 .2 .8 .8 .9 .6	31.5 22.5 22.6 n.d. 37.0 23.6 24.5 27.0 28.4 18 3	28.5 21.4 21.0 21.8 	30.9 21.2 22.2 22.3 	29.0 20.4 21.8 19.3
	5'. VXW TXT TXP PXT PXP TXT TXP PXT PXP TXT	5'-GCT CGA VXW YZ TXT CG GC AT TA TA TXP CG PXT PXP TXT CG TXP PXT PXP TXT CG	5'-TTT5'-GCT AAA CGA TTTVXWYZPHTXTCG6.2GCATTATAPCGACPXPTXTCGACTXPPXPTXTPXPTXTCG7.0	5'-TTT TTM 5'-GCT AAA AAG CGA TTT TTC VXW YZ PH T TXT CG 6.2 GC - AT - TXP CG 6.2 PXT - PXT - TXT CG 6.2 PXT - PXP 30.2 TXT CG 6.6 PXP 30.2 TXT CG 6.6 PXP 30.2 TXT CG 6.6 PXT 23.0 PXP 22.9 TXT CG 7.0	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	5'-TTT TTM VXW MTM 5'-GCT AAA AAG AYA GAG CGA TTT TTC TZT CTC VXW YZ pH T PhP C TXT CG 6.2 26.7 32.2 C GC — 22.7 C	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $

^{*a*} Average $T_{\rm m}$ values for triplex melt given in °C. Heating/cooling at 0.5 °C min⁻¹. n.d. = not determined ($T_{\rm m} < 17$ °C). ^{*b*} M = ^{5-Me}dC;P = 5-(3-aminoprop-1-ynyl)-dU (pdU), V, W = T or P, $\underline{X} = T$, ^{Ph}P, ^AP, ^{Ac}P, ^UP or ^GP; purine duplex strand ($\underline{Y} = C, G, A, T$); pyrimidine duplex strand ($\underline{Z} = G, C, T, A$).

analysis was conducted using modified TFOs (see ESI, Section B.3 and Table C1), targeting a single CG inversion in a homopurine tract, in a modified version of Leumann's triplex motif.⁸ UV melting was performed with a temperature gradient of $0.5 \,^{\circ}$ C min⁻¹ at pH 6.2–7.0 (Table 1).

The order of stability of analogues of the triplet in Fig. 2, based on hydrogen bonding, base-stacking capability and charge, is predicted to be ${}^{G}P > {}^{U}P > {}^{Ac}P > {}^{Ph}P > {}^{M}P > T ({}^{M}P$ examined in previous study¹¹). It was surprising therefore, that the simplest analogue, ${}^{Ph}P$, should match or outperform all other monomers against CG in all TFOs, under all buffer conditions (Table 1,



Fig. 4 UV melting curves (a/c) and derivatives (b/d). a/b: ^xP (TFO 1-6, TXT, see ESI, Table C1) against CG at pH 6.2 (10 mM sodium phosphate, 200 mM NaCl, 1 mM Na₂EDTA). See Table 1, entry 1. c/d: ^AP (TFO 3, TXT, see ESI, Table C1) against YZ at pH 6.2 (10 mM sodium phosphate, 200 mM NaCl, 1 mM Na₂EDTA). See Table 1, entries 1–4.

entries 1,7–12, see Fig. 4a,b). The acetamide, ^{Ac}P, demonstrated lowest binding affinity to CG (still higher than T) in all sequences and buffer conditions, also contradicting our expectations (entries 1,7–11).

Selectivity for CG was high for all monomers at pH 6.2 (Table 1, Fig. 4c,d). ^{Ac}P was least selective for CG, ($\Delta T_{\rm m}$ +6.7 °C vs. TA, entry 4), and ^{Ph}P demonstrated greatest selectivity (+9.3 °C vs. AT, entry 3), similar to ^AP (+8.9 °C vs. AT). ^{Ph}P would be expected to exhibit lowest selectivity due to lack of potential hydrogen bonding interactions with G, necessary for good discrimination between base pairs.

Sequence-dependence of binding was also assessed by varying the nucleotides on either side of monomer ^xP in the TFO. Thymine and 5-(3-aminoprop-1-ynyl)-2'-deoxyuridine (pdU) were used for recognition of the neighbouring AT base pairs, the latter of which significantly increases triplex stability due to introduction of charge-stabilising interactions and additional basestacking.^{7a}

TFOs containing central TFO trinucleotides, PXT and TXP, are expected to dissociate at a similar temperature (T_m), as only the sequence order is changed and differences in melting kinetics should be minimal. PXT-oligonucleotides, however, formed more stable triplexes than TXP-containing TFOs (pH 6.6, entries 9,10, see ESI, Fig. B1), by up to +2.5 °C (X = ^AP). This difference between sequences is probably due to specific electrostatic, hydrogen bonding or steric interactions between neighbouring pdU and ^xP nucleotides, which are difficult to predict.

Fluorescence-based triplex melting analysis¹⁶ was also conducted, using 5'-DABCYL-labelled, modified TFOs (see ESI, Section B.4 and Table C2), targeting a single CG inversion in a homopurine tract of a 5'-FAM-labelled hairpin duplex at pH 6.2–7.0 and at pH 7.0 with 2 mM spermine (Table 2).

These results correlate more closely with predictions based on the triplet binding model in Fig. 2. Under all buffer conditions, for TXT sequences (entries 1,11,18,20, see ESI, Fig. B2), ^GP demonstrated the highest T_m values by up to +2.9 °C (at pH 6.2), while ^{Ac}P performed poorest throughout, except at pH 7.0 where ^{Ph}P was poorest (<30 °C, entry 18). The neighbouring nucleotides to ^xP were varied as previously described for the UV melting study. It was noted that neighbouring protonated pdU nucleotides contributed less stabilisation to the ^GP triplex than the rest, possibly due to significant electrostatic destabilisation from the guanidinium moiety, which was also observed in the UV melting. Relative destabilisation from two neighbouring pdU nucleotides

Table 2 Fluorescence triplex melting experiment: \underline{XP} against \underline{YZ} at pH 6.2, 6.6 and 7.0, or 7.0 + 2 mM spermine* (10 mM sodium phosphate, 200 mM NaCl, 1 mM Na₂EDTA)^{*a,b*}

5'- F	5 -gt ca	' -Q - GTT CAA	PMM AGG TCC	TPM AAG TTC	V <u>X</u> W A <u>Y</u> A TZT	TPT AAA TTT	PTM AAG TTC	PT AAC TTG	TGG ACC	сн-т А-н
Entry	VXW	/ YZ	pН	Т	P	^h P	^P	AcP	^U P	GР
1	TXT	CG	6.2	44	3 4	4.0	44.0	43.9	44.3	3 47.2
2		GC		_	3	2.0	36.7	38.8	39.9	36.9
3		AT		_	3	4.6	33.9	35.0	35.1	37.0
4		TA		_	3	7.6	36.7	41.9	40.7	39.7
5	TXP	CG	6.2	47	5 5	0.8	51.0	50.3	52.0	53.0
6	PXT			48	4 4	9.6	49.1	51.0	50.8	51.3
7	PXP	CG	6.2	51	1 5	5.7	56.8	55.1	57.8	56.0
8		GC		_	4	2.1	45.8	46.9	46.0	45.0
9		AT		_	4	4.0	43.3	45.0	43.8	47.0
10		TA		_	4	8.9	47.2	50.4	49.6	5 51.0
11	TXT	CG	6.6	_	3	7.0	37.5	36.2	36.9	40.3
12		GC			3	1.0	n.d.	n.d.	n.d.	n.d.
13		AT			n	ı.d.	n.d.	n.d.	n.d.	30.9
14		TA			3	1.0	31.5	33.9	33.5	5 33.0
15	TXP	CG	6.6		4	4.0	43.2	43.8	44.2	2 46.2
16	PXT				4	3.0	42.2	43.8	43.0) 43.7
17	PXP				4	8.1	48.5	46.5	48.2	2 47.2
18	TXT	CG	7.0		n	ı.d.	32.0	30.9	31.6	5 32.7
19	PXP				3	9.9	40.6	39.9	39.9	38.5
20	TXT	CG	7.0	*	3	6.1	36.0	36.0	37.4	39.0

^{*a*} Average $T_{\rm m}$ values for triplex melt given in °C. n.d. = not determined $(T_{\rm m} < 30 \,^{\circ}{\rm C})$. ^{*b*} M = ^{5-Me}dC, P = 5-(3-aminoprop-1-ynyl)-dU (pdU), V,W = T or P, $\underline{X} = T$, ^{Ph}P, ^AP, ^{Ac}P, ^UP or ^GP, $\mathbf{Q} = \text{DABCYL}$, $\mathbf{F} = \text{FAM}$, H = hexa(ethylene glycol), HEG, hairpin duplex $\underline{YZ} = \text{CG}$, GC, AT, TA.

grows with increasing pH (entries 7,17,19), such that the relative binding affinity of ^GP for CG, drops from moderate to lowest. The binding affinity of ^UP for CG is also affected to a lesser extent.

Similar effects were observed for TFOs with PXT or TXP central trinucleotides. In contrast to UV melting data, PXT-containing TFOs exhibited a lower binding affinity to the CG duplex than the corresponding TXP-containing TFOs (entries 5,6,15,16). This is except for the acetamide (^{Ae}P), which exhibited the reverse at pH 6.2 (entries 5,6) and no difference at pH 6.6 (entries 18,19). ^GP displayed the largest difference between sequences (-2.8/-2.5 °C at pH 6.2/6.6, entries 5,6,15,16, see ESI, Fig. B5). With the exception of ^{Ae}P, this T_m difference did not appear to relate to pH.

Table 3 Fluorescence/UV triplex melting comparison experiment: X
against CG at pH 6.2 (10 mM sodium phosphate, 200 mM NaCl, 1 mM
Na2EDTA) in fluorescence melting motif (Table 2)^a

Method	Base pair	Buffer	${}^{\rm Ph} P$	^P	^{Ac} P	^U P	GР
Fluorescence UV	CG	6.2	44.0 ^b 45.1	44.0 ^b 45.2	43.9 ^b 44.6	44.3 ^b 45.7	47.2 ^b 48.6
$\Delta T_{ m m}$			+1.1	+1.2	+0.7	+1.4	+1.4

^{*a*} Average $T_{\rm m}$ values for triplex melt given in °C. Heating at 0.5 °C min⁻¹. $\underline{X} = {}^{\rm Ph}P, {}^{\rm A}P, {}^{\rm Ac}P, {}^{\rm U}P$ or "P. ^{*b*} Values from entry 1 of Table 2 for comparison.

The selectivity for CG at pH 6.2 (entries 1–4,7–10) and pH 6.6 (entries 11–14) varied to a greater extent than in the UV melting study. For the TXT sequence, ^GP is most selective (+7.3–7.5 °C *vs.* TA, see ESI, Fig. B4), and ^AP and ^{Ph}P also showed very good selectivity at pH 6.2 (+7.3/+6.4 °C respectively). The urea and acetamide, however, were less selective for CG.

Selectivity results for the PXP sequence, however, differed significantly, such that ^AP is now most selective (+9.6 °C), followed by ^UP (+8.2 °C) and ^{Ph}P (+6.8 °C). ^{Ac}P and ^GP are now of low to moderate selectivity. The relative destabilising electrostatic influence on ^GP of the neighbouring protonated pdU nucleotides has an effect on selectivity, in addition to binding affinity.

Differences in the DNA sequences were assumed to be responsible for the relative differences in triplex stability and selectivity between UV and fluorescence melting studies. In order to exclude the possibility that these differences were caused by the method of analysis, UV triplex melting experiments were conducted on the fluorescence melting motif (TXT sequence, pH 6.2, Table 3). These data prove that the method of analysis does not affect the relative recognition properties of the monomers. All UV melting $T_{\rm m}$ s were on average 1.2 °C higher than those observed by fluorescence melting.

In general, there appears to be an inverse correlation between steric bulk and CG-recognition capability. The unmodified phenyl monomer ^{Ph}P, despite lacking hydrogen bonding functionality for binding to G, formed the strongest and most selective triplexes in all UV-melting conditions, closely followed by the aniline ^AP. The guanidinium monomer ^GP, appeared most sequence dependent, exhibiting excellent binding affinity and selectivity in most fluorescence melting conditions, yet moderate to poor binding affinity and moderate to good selectivity in the UV melting triplex sequence. The acetamide performed poorest of all, both in terms of binding affinity and selectivity.

Choosing between recognition monomers depends strongly on TFO sequence. In sequences with few pdU modifications, ^AP and ^{Ph}P monomers are best. In pdU-rich sequences, where the modification is abutted by T, ^GP is best at all pHs. However, with neighbouring pdU nucleotides, the relative stability of the ^GP triplex drops, such that ^UP (pH 6.2) then ^AP (pH 6.6/7.0) produce the most stable triplexes. For these reasons, from these studies, the optimum monomer for CG recognition is the aniline, ^AP. This monomer has the highest overall selectivity and binding affinity, little pH dependence, and its recognition properties appear least sequence-specific.

Conclusion

Five CG recognition monomers were synthesised and incorporated into TFOs to recognise a CG inversion in a target duplex. Most demonstrated a high binding affinity/selectivity for CG, and would be suitable for use in synthesis of oligotherapeutics (gene therapy, chemotherapy) and other applications. TFO sequence, however, is the key factor governing the relative differences in triplex stability and selectivity between recognition monomers. This is of great importance in design of oligonucleotides for targeting mixed sequences, as sequence-effects are hard to predict. These modified nucleotides offer a significant improvement on current CG recognition monomers.

Experimental

General

Reagents for chemical synthesis were purchased from Sigma-Aldrich, Fluka, Acros Organics, Fisher Scientific or Alfa Aesar and used without further purification. Pyridine, dichloromethane (CH_2Cl_2) , triethylamine (Et_3N) and N,N-diisopropylethylamine (DIPEA) were distilled over calcium hydride; phosphorus oxychloride over sodium wire; tetrahydrofuran over sodium wire and benzophenone; and diethyl ether by passing through a column of dry alumina then degassing at low temperature, or by degassing with argon over activated 4 Å molecular sieves shortly before use. Phosphitylating reagents, DNA phosphoramidite monomers, other reagents and solid supports were purchased from Link Technologies Ltd. or Applied Biosystems Ltd. Deuterated NMR solvents were purchased from Apollo Scientific Ltd. All reactions requiring absence of oxygen were carried out under an atmosphere of argon, and glassware was dried overnight at 120 °C before use. Column chromatography was carried out under air or argon pressure using Fisher Scientific DAVISIL 60 Å (35–70 micron) silica gel, and reactions were monitored by TLC, using Merck Kieselgel 60 F254 or Machenary-Nagel Alugram Sil G/UV254 silica gel plates (0.22 mm thickness, aluminium backed). Compounds were visualised by irradiation at 254/365 nm, or by staining with *p*-anisaldehyde, potassium permanganate, ninhydrin, H_2SO_4 ethanol or ceric sulfate, followed by heating. Proton NMR spectra were recorded using either a Bruker AC300 or Bruker DPX400 spectrometer. Fluorine and phosphorus NMR spectra were recorded using a Bruker AC300 spectrometer. NMR spectra were recorded in deuterated chloroform or dimethylsulfoxide. Chemical shifts are given in ppm relative to tetramethylsilane, and spectra are calibrated to the appropriate residual solvent peak.¹⁷ J values are accurate to within 0.5 Hz. Assignment was aided by H-H/H-C correlation experiments. Compound numbering is shown in Fig. 5. Low-resolution mass spectra were recorded using electrospray ionisation (ES) on a Fisons VG platform instrument or on a Waters ZMD quadrupole mass spectrometer, in HPLC grade acetonitrile or methanol. High-resolution mass spectra were recorded in HPLC grade acetonitrile or methanol using electrospray ionisation on a Bruker APEX III FT-ICR mass spectrometer. Melting points were recorded on a Gallenkamp Electrothermal melting point apparatus and quoted uncalibrated. Elemental (CHN) Thermal Combustion Analysis was carried out by MEDAC Ltd., Egham, Surrey, UK.



Fig. 5 Example compound numbering (6c and 16).

Synthesis

3'-O-Acetyl-5'-O-(4,4'-dimethoxytrityl)-5-iodo-2'-deoxyuridine (2).¹⁸ To a stirred solution of 5'-O-(4,4'-dimethoxytrityl)-5-iodo-2'deoxyuridine[‡]1 (10.0 g, 15.2 mmol) in distilled pyridine (40.0 mL), under an argon atmosphere, at 0 °C was added acetic anhydride (7.20 mL, 76.3 mmol, 5.0 eq) dropwise over 20 min. The reaction was stirred at 0 °C for 15 min then allowed to warm to room temperature. After $2\frac{3}{4}$ hours, the reaction mixture was concentrated in vacuo and under high vacuum, co-evaporating with acetone $(2 \times 50 \text{ mL})$ and diethyl ether $(2 \times 50 \text{ mL})$, to give a white foam/syrup. The syrup was dissolved in ethyl acetate (300 mL) and washed with water (3 \times 75 mL). The aqueous was re-extracted (ethyl acetate, 40 mL) and combined organic fractions were washed with sat. aq KCl (120 mL), dried (Na₂SO₄) and concentrated in vacuo and under high vacuum to give a white foam. Following purification by column chromatography $(CH_2Cl_2-MeOH-Et_3N, 100:0:0.5\rightarrow 98:2:0.5)$, and drying under high vacuum, the desired product 2 was afforded as a white foam (10.3 g, 14.7 mmol, 97%). R_f 0.31 (CH₂Cl₂-acetone-Et₃N, 90:10:0.3), 0.48 (CH₂Cl₂-MeOH-Et₃N, 95:5:0.3), 0.33 (hexane-ethyl acetate-Et₃N, 70:30:0.3); ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 11.75 (1H, s, NH³), 8.05 (1H, s, H⁶), 7.39 (2H, d, J = 8.0 Hz, H¹³), 7.32 (2H, t, J = 7.3 Hz, H¹⁴), 7.28 (2H, d, J = 9.0 Hz, H⁹), 7.28 (2H, d, J = 9.0 Hz, H⁹), 7.23 (1H, tt, J =1.1, 7.2 Hz, H¹⁵), 6.89 (4H, d, J = 8.8 Hz, H¹⁰), 6.10 (1H, dd, J = 6.1, 8.2 Hz, $H^{1'}$), 5.23 (1H, td, J = 2.4, 6.4 Hz, $H^{3'}$), 4.07 (1H, td, J = 2.9, 4.5 Hz, H^{4'}), 3.74 (6H, s, OCH₃), 3.32 (1H, dd, J =4.9, 10.4 Hz, $H^{5'}$), 3.22 (1H, dd, J = 3.1, 10.4 Hz, $H^{5'}$), 2.44 (1H, td, J = 7.0, 11.5 Hz, H²), 2.33 (1H, ddd, J = 2.0, 6.0, 14.3 Hz, H^{2}), 2.03 (3H, s, COCH₃); MS (ES⁺) m/z: 721 ([M + Na]⁺, 12), 303 (DMTr⁺, 100); (ES⁻) *m*/*z*: 697 ([M – H]⁻, 100); HRMS (ES⁺): calcd for $C_{32}H_{31}N_2O_8I$ (M), $[M + Et_3N + H]^+ = 800.2402$, found 800.2369; $[M + Na]^+ = 721.1017$, found 721.1007.

5'-O-(4,4'-Dimethoxytrityl)-5-iodo-4N-methyl-2'deoxycytidine (3). To a stirred solution of acetylated nucleoside 2 (5.85 g, 8.38 mmol) and *N*-methylimidazole (8.64 mL, 108 mmol, 12.9 eq) in distilled pyridine (130.0 mL), under an argon atmosphere, at 0 °C, distilled phosphorous oxychloride (3.00 mL, 32.2 mmol, 3.8 eq) was added dropwise over 20 min. The yellow suspension was stirred for 20 min then allowed to warm to room temperature over a further 30 min. The mixture was cooled to 0 °C, 40 wt% MeNH₂-water solution (40.5 mL, 468 mmol MeNH₂, 55.9 eq MeNH₂) was added, and the cloudy solution was stirred at room temperature for 18 h. The mixture was concentrated under high vacuum, dissolved in CH₂Cl₂ (300 mL) and washed with water $(3 \times 75 \text{ mL})$. The combined aqueous fraction was re-extracted (CH₂Cl₂, 40 mL), the combined organic layer was washed with sat. aq KCl (120 mL), dried (Na₂SO₄) and concentrated to give a pale yellow foam. Following careful purification by column chromatography (CH₂Cl₂-MeOH-Et₃N, 98:2:0.5), and precipitation with Et_2O , the 4N-methyl-dC nucleoside 3 was afforded, as a white powder (4.92 g, 7.35 mmol, 88%). $R_{\rm f}$ 0.40 (CH₂Cl₂-MeOH-Et₃N, 90:10:0.3), 0.36 (CH₂Cl₂-acetone-Et₃N, 80:20:0.3), 0.57 (acetone-MeOH-Et₃N, 90:10:0.3); Mp 141-144 °C (ethyl acetate-diethyl ether); ¹H NMR (400 MHz, DMSO d_6) δ (ppm) 7.93 (1H, s, H⁶), 7.40 (2H, d, J = 7.5 Hz, H¹³), 7.32 (2H, t, J = 7.3 Hz, H¹⁴), 7.29 (4H, d, J = 9.0 Hz, H⁹), 7.22 (1H, tt, J = 2.0, 7.3 Hz, H¹⁵), 7.06 (1H, q, J = 4.5 Hz, NHCH₃), 6.89 (4H, d, J = 9.0 Hz, H¹⁰), 6.12 (1H, dd, J = 6.2, 7.4 Hz, H^{1'}), 5.25 (1H, d, J = 4.3 Hz, 3'-OH), 4.20 (1H, dt, J = 3.3, 6.4 Hz, H^{3'}), 3.91 (1H, dd, J = 3.3, 6.5 Hz, H⁴), 3.74 (6H, s, OCH₃), 3.20 (1H, dd, J = 3.3, 10.8 Hz, $H^{5'}$), 3.17 (1H, dd, J = 4.5, 10.8 Hz, $H^{5'}$), 2.80 (3H, d, J =4.5 Hz, NHCH₃), 2.21 (1H, ddd, J = 3.0, 6.0, 13.3 Hz, H^{2'}), 2.09 $(1H, td, J = 6.8, 13.6 Hz, H^2); MS (ES^+) m/z: 692 ([M + Na]^+, 12),$ 670 ($[M + H]^+$, 3), 303 (DMTr⁺, 100); (ES⁻) m/z: 668 ($[M - H]^-$, 100); HRMS (ES⁺): calcd. for $C_{31}H_{32}N_3O_6I$, $[M + K]^+$ 708.0967, found 708.0962, [M + Na]⁺ 692.1228, found 692.1219, [M + H]⁺ 670.1409, found 670.1414; Anal. calcd. for C₃₁H₃₂N₃O₆I (669.51) requires C, 55.61%; H, 4.82%; N, 6.27%. Found C, 54.80%; H, 4.75%; N, 6.05%.

3-Acetamidophenyl acetylene (5a).¹⁹ To a stirred solution of 3ethynylaniline 4 (3.50 mL, 31.1 mmol) and distilled Et₃N (4.37 mL, 31.4 mmol, 1.0 eq) in dry, degassed diethyl ether (15.0 mL), at 0 °C, under an argon atmosphere, was added acetyl chloride (2.23 mL, 31.4 mmol, 1.0 eq) in dry, degassed diethyl ether (25.0 mL), dropwise over 45 min. The reaction was stirred for a further 15 min then allowed to warm to room temperature over 30 min. The mixture was filtered, the white precipitate was washed with diethyl ether $(3 \times 35 \text{ mL})$ and combined ether fractions were concentrated in vacuo to give a cream-coloured solid. The solid was triturated with water (60 mL) to which diethyl ether (100 mL) was added, and the layers were separated. The ether layer was washed with water (50 mL), combined aqueous layers were re-extracted (diethyl ether, 20 mL), and combined ether fractions were concentrated in vacuo and dried under high vacuum to afford the desired product 5a as a pale brown oil, which crystallised on standing to an off-white waxy solid (4.79 g, 30.1 mmol, 97%). R_f 0.40 (CH₂Cl₂-CH₃OH-Et₃N, 95:5:0.3); Mp 92-94 °C (chloroform-hexane), lit.19 94-96 °C (CCl₄); ¹H NMR (300 MHz, DMSO- d_6) δ (ppm) 10.00 (1H, s, NHCOCH₃), 7.77 (1H, app. t, J = 1.7 Hz, H²), 7.54 (1H, ddd, J =1.1, 2.0, 8.1 Hz, H⁴), 7.30 (1H, t, J = 7.9 Hz, H⁵), 7.13 (1H, td, *J* = 1.3, 7.7 Hz, H⁶), 4.13 (1H, s, C=CH), 2.05 (3H, s, COCH₃); MS (ES⁺) m/z: 160 ([M + H]⁺, 100), 182 ([M + Na]⁺, 11), 201 ([M + CH₃CN + H]⁺, 7), 223 ([M + CH₃CN + Na]⁺, 17), 242 ([M + AcOH + Na]⁺, 12); (ES⁻) m/z: 158 ([M - H]⁻, 100); HRMS

[‡] Prepared according to literature procedure.18

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 (ES^+) : calcd. for $C_{10}H_9NO$, $[2M + Na]^+ 341.1260$, found 341.1239, $[M + Na]^+ 182.0576$, found 182.0565, $[M + H]^+ 160.0757$, found 160.0755.

3-Ureidophenyl acetylene (5b).²⁰ 3-Ethynylaniline 4 (3.00 mL, 26.6 mmol) and dry phenyl carbamate (7.31 g, 53.3 mmol, 2.0 eq) were heated in a sealed tube, under an argon atmosphere, excluding moisture, in dim light at 90 °C for 12 h. The mixture was cooled to room temperature, removed using warm methanolacetone, and dried under high vacuum. Following purification by column chromatography (CH₂Cl₂-acetone, 95: $5 \rightarrow 50:50$), and drying under high vacuum, the desired urea 5b was afforded, as an off-white, papery solid (2.93 g, 18.3 mmol, 69%). $R_{\rm f}$ 0.31 (CH₂Cl₂-CH₃OH-Et₃N, 90:10:0.3), 0.04 (CH₂Cl₂-acetone-Et₃N, 90:10:0.3); ¹H NMR (300 MHz, DMSO- d_6) δ (ppm) 8.61 (1H, s, NH), 7.64 (1H, t, J = 1.7 Hz, H²), 7.32 (1H, ddd, J = 1.1, 1.1)2.0, 8.2 Hz, H⁴), 7.22 (1H, t, J = 7.9 Hz, H⁵), 7.00 (1H, td J = 1.3, 7.3 Hz, H⁶), 5.89 (2H, s, NH₂), 4.08 (1H, s, C=CH); MS (ES⁺) m/z: 343 ([2M + Na]⁺, 21), 183 ([M + Na]⁺, 70), 102 ([Et₃N + H^{+} , 100); (ES⁻) m/z: 159 ([M – H]⁻, 100); HRMS (ES⁺): calcd. for $C_9H_8N_2O$, $[M + Na]^+$ 183.0529, found 183.0530, $[2M + Na]^+$ 343.1171, found 343.1170.

General procedure for Pd-catalysed cross-coupling of aromatic alkynes 4, 5a–c with 5-iodo-4*N*-methyl-2′-deoxycytidine derivative 3

To a solution of 5'-O-(4,4'-dimethoxytrityl)-5-iodo-4N-methyl-2'deoxyuridine 3 (0.8–2.5 mmol) in anhydrous DMF (2.5–6.0 mL), under an argon atmosphere, in absence of light, was added CuI (0.4 eq), distilled Et₃N (5.0 eq) and aromatic alkyne 4, **5a–c** (3.0 eq). The mixture was stirred for 15–20 min then *tetrakis*(triphenylphosphine) palladium (0) (0.1 eq) was added and the reaction was stirred at room temperature for 1–3 h. The reaction mixture was concentrated under high vacuum, co-evaporating with methanol-toluene or acetone-toluene to give an orange– brown to brown foam. Aqueous workup or filtration of product from acetone–methanol (1:1) and/or column chromatography afforded the desired product, **6a** and **9** (isolated pure), **6b** and **6c** (isolated with alkyne dimer impurity and used in following reaction where impurity was removed).

5-Phenylethynyl-5'-O-(4,4'-dimethoxytrityl)-4N-methyl-2'-deoxycytidine (6a). Obtained from 3 (0.55 g, 0.82 mmol) and alkyne 5a (2.64 mmol, 3.0 eq), after filtration and column chromatography (CH₂Cl₂-MeOH-Et₃N, 90:10:0.5) as a pale orange foam (0.47 g, 0.74 mmol, 90%). R_f 0.66 (ethyl acetate-MeOH-aq NH₃, 5:1:1); ¹H NMR (400 MHz, CDCl₃) δ (ppm) 8.20 (1H, s, H⁶), 7.45 (2H, dd, J = 1.2, 8.6 Hz, H¹⁵), 7.35 (2H, d, $J = 9.1 \text{ Hz}, \text{H}^{11}$), 7.35 (2H, d, $J = 8.9 \text{ Hz}, \text{H}^{11}$), 7.31 (1H, tt, J = 1.5, 7.3 Hz, $H^{4''}$), 7.25 (2H, t, J = 7.1 Hz, $H^{3''}$), 7.25 (2H, t, J = 7.9 Hz, H^{16}), 7.17–7.12 (2H, m, H^{17} , $H^{2''}$), 6.79 (2H, d, J = 8.9 Hz, H^{12}), 6.77 (2H, d, J = 8.9 Hz, H¹²), 6.38 (1H, dd, J = 6.1, 6.8 Hz, H^{1'}), 5.77 (1H, q, J = 4.8 Hz, NHCH₃), 4.58 (1H, td, J = 2.8, 5.5 Hz, $H^{3'}$), 4.19 (1H, dd, $J = 3.2, 6.2 \text{ Hz}, H^{4'}$), 3.70 (3H, s, OCH₃), 3.69 $(3H, s, OCH_3), 3.41 (1H, dd, J = 3.1, 10.6 Hz, H^{5'}), 3.34 (1H, dd, Hz, H$ J = 3.8, 10.6 Hz, H^{5'}), 3.11 (3H, d, J = 5.0 Hz, NHCH₃), 3.01 (1H, br s, 3'-OH), 2.76 (1H, ddd, J = 3.1, 5.9, 13.8 Hz, H^{2'}), 2.28 $(1H, J = 6.7, 13.4 \text{ Hz}, \text{H}^2); \text{ MS } (\text{ES}^+) m/z; 745 ([M + \text{Et}_3\text{N} + \text{H}]^+,$ 100), 666 ($[M + Na]^+$, 52); HRMS (ES⁺): calcd. for C₃₉H₃₇N₃O₆,

 $[M + K]^+$ 682.2314, found 682.2303; $[M + Na]^+$ 666.2575, found 666.2565; $[M + H]^+$ 644.2755, found 644.2755.

5-(3-Acetamidophenyl)ethynyl-5'-O-(4,4'-dimethoxytrityl)-4Nmethyl-2'-deoxycytidine (6b). Obtained from 3 (1.69 g, 2.53 mmol) and alkyne 5b (7.60 mmol, 3.0 eq), after aqueous workup with 5% w/v aq Na2EDTA (pH 9) and column chromatography (CH₂Cl₂-acetone-pyridine, $90:10:1 \rightarrow 30:70:1$), partially purified as a yellow glassy foam (1.99 g). $R_{\rm f}$ 0.16 (CH₂Cl₂-acetone-Et₃N, 40:60:0.3), 0.04 (CH₂Cl₂-acetonepyridine, 80:20:0.3), 0.28 (CH₂Cl₂-acetone-Et₃N, 20:80:0.3), 0.32 (CH₂Cl₂-acetone-pyridine, 20:80:1); ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 9.97 (1H, s, NHCOCH₃), 8.00 (1H, s, H⁶), 7.79 (1H, s, $H^{2''}$), 7.46 (1H, ddd, J = 1.0, 2.0, 8.5 Hz, $H^{4''}$), 7.43 $(1H, q, J = 5.0 \text{ Hz}, \text{NHCH}_3), 7.40 (2H, dd, J = 1.3, 8.3 \text{ Hz}, H^{15}),$ 7.29 (2H, d, J = 9.0 Hz, H¹¹), 7.29–7.25 (1H, m, H^{5"}), 7.28 (2H, d, J = 8.5 Hz, H¹¹), 7.24 (2H, t, J = 7.8 Hz, H¹⁶), 7.15 (1H, tt, J =1.3, 7.3 Hz, H^{17}), 6.90 (1H, td, J = 1.3, 7.8 Hz, $H^{6''}$), 6.84 (2H, d, J = 9.0 Hz, H¹²), 6.83 (2H, d, J = 9.0 Hz, H¹²), 6.16 (1H, t, J =6.8 Hz, H^{1'}), 5.28 (1H, d, J = 4.5 Hz, 3'-OH), 4.26 (1H, dt, J = 3.5, 6.7 Hz, $H^{3'}$), 3.97 (1H, td, J = 3.0, 5.0 Hz, $H^{4'}$), 3.66 (6H, s, OCH_3), 3.23 (1H, dd, J = 5.0, 10.5 Hz, $H^{5'}$), 3.15 (1H, dd, J = 2.5, 10.5 Hz, $H^{5'}$), 2.86 (3H, d, J = 4.5 Hz, NHCH₃), 2.27 (1H, ddd, $J = 3.5, 6.3, 13.6 \text{ Hz}, \text{H}^2$), 2.16 (1H, td, $J = 6.7, 13.6 \text{ Hz}, \text{H}^2$), 2.06 (3H, s, COCH₃); MS (ES⁺) m/z: 723 ([M + Na]⁺, 100), 303 $(DMT^+, 29); (ES^-) m/z: 699 ([M - H]^-, 100); HRMS (ES^+): calcd.$ for C₄₁H₄₀N₄O₇, [M + H]⁺ 701.2970, found 701.2974.

5-(3-Ureidophenyl) ethynyl-5' - *O*-(4, 4' - dimethoxytrityl) - 4*N*methyl-2'-deoxycytidine (6c). Obtained from 3 (1.25 g, 1.87 mmol) and alkyne 5c (5.61 mmol, 3.0 eq), after filtration and column chromatography (CH₂Cl₂-acetone–Et₃N, 20:80:0.5 →0:100:0.5), partially purified as a pale brown foam (1.49 g) and used without further purification. $R_{\rm f}$ 0.18 (CH₂Cl₂-MeOH– Et₃N, 90:10:0.3), 0.11 (CH₂Cl₂-acetone–Et₃N, 20:80:0.3), 0.44 (acetone–MeOH–Et₃N, 90:10:0.3).

5-(3-Aminophenyl) ethynyl - 5' - O-(4, 4' - dimethoxytrityl) - 4Nmethyl-2'-deoxycytidine (9). Obtained from 3 (0.97 g, 1.45 mmol) and alkyne 4 (4.35 mmol, 3.0 eq), after filtration and column chromatography (CH₂Cl₂–MeOH–Et₃N, 100:0:0.5 \rightarrow 97:3:0.5) as a pale orange foam (0.91 g, 1.39 mmol, 95%). R_f 0.31 (CH₂Cl₂-MeOH-Et₃N, 90:10:0.3); 0.31 (CH₂Cl₂-acetone-Et₃N, 20:80:0.3), 0.03 (CH₂Cl₂-acetone-Et₃N, 80:20:0.3); ¹H NMR (400 MHz, DMSO- d_6) δ (ppm) 7.94 (1H, s, H⁶), 7.40 (2H, dd, J = 1.1, 8.4 Hz, H¹⁵), 7.35 (1H, q, J = 4.8 Hz, NHCH₃), 7.30–7.25 (2H, m, H¹⁶), 7.29 (2H, d, J = 8.0 Hz, H¹¹), 7.29 (2H, d, J = 9.0 Hz, H¹¹), 7.17 (1H, tt, J = 1.0, 7.3 Hz, H¹⁷), 6.96 (1H, t, J = 7.8 Hz, $H^{5''}$), 6.86 (2H, d, J = 8.5 Hz, H^{12}), 6.83 (2H, d, J = 8.8 Hz, H^{12}), 6.61 (1H, t, J = 1.6 Hz, $H^{2''}$), 6.57 (1H, ddd, J = 1.1, 2.4, 8.0 Hz, H^{4"}), 6.46 (1H, ddd, J = 1.1, 2.5, 7.5 Hz, H^{6"}), 6.15 (1H, app. t, J = $6.7 \text{ Hz}, \text{H}^{1'}$), 5.30 (1 H, d, J = 4.3 Hz, 3'-OH), $5.11 (2 \text{H}, \text{br s}, \text{NH}_2)$, 4.25 (1H, td, J = 3.1, 9.0 Hz, H^{3'}), 3.97 (1H, td, J = 2.4, 4.8 Hz, H^{4'}), 3.68 (3H, s, OCH₃), 3.67 (3H, s, OCH₃), 3.23 (1H, dd, J = 5.0, 10.5 Hz, $H^{5'}$), 3.14 (1H, dd, J = 2.5, 10.3 Hz, $H^{5'}$), 2.86 (3H, d, J = 4.8 Hz, NHC H_3), 2.27 (1H, ddd, J = 3.0, 5.9, 13.4 Hz, H^{2'}), 2.13 (1H, td, J' 6.8, 13.6 Hz, $H^{2'}$); MS (ES⁺) m/z: 681 ([M + Na]⁺, 100); HRMS (ES⁺): calcd. for $C_{39}H_{38}N_4O_6$, [M + Na]⁺ 681.2684, found 681.2671; [M + H]⁺ 659.2864, found 659.2847.

General procedure for Cu-catalysed cyclisation of 5-alkynyl-4*N*-methyl-2'-deoxycytidine nucleosides (6a–c, 9)

To a solution of nucleoside **6a-c**, **9** (0.8–3.4 mmol) and distilled Et_3N (10.0 eq) in anhydrous DMF (2.0–10.0 mL) with activated 4 Å molecular sieves, under an argon atmosphere, in absence of light was added CuI (1.1 eq). The reaction mixture was stirred at 125 °C for 0.5–2 h, then cooled to rt, and the solution was removed and dried under high vacuum to give a dark brown foam/syrup. Aqueous workup with 5% w/v aq Na₂EDTA (pH 9) and sat. aq KCl or filtration of impurities, then column chromatography afforded the desired product **7a–c**, **10**.

 $3-N-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-\beta-D-ribofuranosyl]-$ 6-phenyl-(2,3H)-N-methylpyrrolo[2,3-d]pyrimidine-2(7H)-one (7a). Obtained from 6a (0.52 g, 0.80 mmol) after aqueous workup and column chromatography (CH₂Cl₂-MeOH-Et₃N, 90:10:0.5), as a pale orange foam (0.37 g, 0.57 mmol, 71%). $R_{\rm f}$ 0.67 (ethyl acetate–MeOH–aq NH₃, 5:1:1), ¹H NMR (400 MHz, DMSO- d_6) δ (ppm) 8.80 (1H, s, H⁴), 7.49–7.44 (1H, m, H^{4''}), 7.46– 7.42 (2H, m, $H^{2''}$), 7.45 (2H, dd, J = 1.8, 8.1 Hz, H^{14}), 7.41 (2H, dt, J = 1.9, 7.9 Hz, $H^{3''}$), 7.35 (2H, d, J = 8.9 Hz, H^{10}), 7.34 (2H, d, J = 9.1 Hz, H¹⁰), 7.29 (2H, tt, J = 1.5, 7.4 Hz, H¹⁵), 7.23 (1H, tt, J = 1.3, 7.2 Hz, H¹⁶), 6.83 (2H, d, J = 8.9 Hz, H¹¹), 6.82 (2H, d, J =8.9 Hz, H¹¹), 6.50 (1H, dd, J = 5.2, 6.1 Hz, H^{1'}), 5.56 (1H, s, H⁵), 4.70 (1H, dd, J = 5.3, 10.6 Hz, H^{3'}), 4.21 (1H, td, J = 2.9, 4.9 Hz, H^{4'}), 3.76 (3H, s, OCH₃), 3.75 (3H, s, OCH₃), 3.57 (3H, s, NCH₃), $3.56 (1H, dd, J = 2.9, 10.7 Hz, H^{5'}), 3.51 (1H, dd, J = 3.3, 10.8 Hz,$ $H^{5'}$), 3.36 (1H, br s, 3'-OH), 2.86 (1H, td, $J = 6.1, 13.7 \text{ Hz}, H^{2'}$), 2.42 (1H, ddd, $J = 5.3, 6.1, 13.7 \text{ Hz}, \text{H}^2$); MS (ES⁺) m/z: 745 ([M + Et₃N + H]⁺, 81), 666 ([M + Na]⁺, 100), 644 ([M + H]⁺, 12); HRMS (ES^{+}) : calcd. for $C_{39}H_{37}N_{3}O_{6}$, $[M + K]^{+}$ 682.2314, found 682.2304; [M + Na]⁺ 666.2575, found 666.2567; [M + H]⁺ 644.2755, found 644.2754.

3-N-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-6 - (3 - acetamidophenyl) - (2, 3H) - N - methylpyrrolo [2, 3 - d] pyri midine-2(7H)-one (7b). Obtained from 6b (1.92 g of 1.97 g) after filtration from CH₂Cl₂ then aqueous workup and column chromatography (acetone-MeOH-Et₃N, $100:0:0.5 \rightarrow 90:10:0.5$), as a yellow powder (1.43 g, 2.04 mmol, 84% over two steps). $R_{\rm f}$ 0.09 $(4:1, CH_2Cl_2$ -acetone-Et₃N, 20:80:0.3), 0.14 (CH₂Cl₂-acetone-Et₃N) pyridine, 20:80:1); ¹H NMR (400 MHz, DMSO- d_6) δ (ppm) 10.08 (NHCOCH₃), 8.65 (1H, s, H⁴), 7.79 (1H, br s, H^{2"}), 7.61 $(1H, br d, J = 8.0 Hz, H^{4''}), 7.42 (1H, t, J = 8.0 Hz, H^{5''}), 7.40 (2H, H^{5''})), 7.40 (2H, H^{5''}), 7.40 (2H, H^{5''})), 7.40 (2H, H^{5''}))$ d, J = 7.0 Hz, H¹⁴), 7.32 (2H, t, J = 7.0 Hz, H¹⁵), 7.29 (2H, d, J =8.5 Hz, H¹⁰), 7.28 (2H, d, J = 9.0 Hz, H¹⁰), 7.25 (1H, t, J = 7.0 Hz, H^{16}), 7.16 (1H, br d, J = 8.0 Hz, $H^{6''}$), 6.90 (2H, d, J = 9.0 Hz, H^{11}), 6.89 (2H, d, J = 9.0 Hz, H^{11}), 6.26 (1H, dd, J = 5.3, 6.3 Hz, $H^{1'}$), 5.62 (1H, s, H^{5}), 5.39 (1H, d, J = 5.0 Hz, 3'-OH), 4.43 (1H, td, J = 5.5, 11.0 Hz, H^{3'}), 4.01 (1H, dd, J = 4.0, 8.0 Hz, H^{4'}), 3.70 (3H, s, OCH₃), 3.69 (3H, s, OCH₃), 3.47 (3H, s, NCH₃), 3.40 (1H, dd, $J = 4.0, 11.0 \text{ Hz}, \text{H}^{5'}$), 3.30 (1H, dd, $J = 3.0, 11.0 \text{ Hz}, \text{H}^{5'}$), 2.45 $(1H, td, J = 6.5, 13.6 Hz, H^2), 2.19 (1H, ddd, J = 5.0, 6.5, 13.6 Hz,$ H^{2'}), 2.08 (3H, s, COCH₃); MS (ES⁺) *m*/*z*: 803 ([M + Et₃N + H]⁺, 14), 723 ($[M + Na]^+$, 100); (ES⁻) m/z: 699 ($[M - H]^-$, 100); HRMS (ES⁺): calcd. for C₄₁H₄₀N₄O₇, [M + H]⁺ 701.2970, found 701.2965.

 $3-N-[2'-\text{Deoxy}-5'-O-(4,4'-\text{dimethoxytrity}])-\beta-D-\text{ribofuranosy}]-6-(3-\text{ureidopheny}])-(2,3H)-N-\text{methylpyrrolo}[2,3-d]pyrimidine-$

2(7H)-one (7c). Obtained from 6c (1.48 g of 1.49 g) after filtration from methanol, column chromatography \times 2 (acetone–MeOH–Et₃N, $100:0:0.5\rightarrow 90:10:0.5$), and precipitation (MeOH-acetone-Et₂O) as a very pale yellow powder (1.03 g, 1.47 mmol, 79% over two steps). R_f 0.24 (acetone–MeOH–Et₃N, 90:10:0.3), 0.49 (CH₂Cl₂-MeOH-Et₃N, 80:20:0.3); ¹H NMR (400 MHz, DMSO-d₆) δ (ppm) 8.71 (1H, s, NHCONH₂), 8.63 (1H, s, H⁴), 7.63 (1H, t, J = 1.8 Hz, H^{2"}), 7.41 (1H, ddd, J =1.0, 2.0, 7.0 Hz, $H^{4''}$), 7.41 (2H, d, J = 7.0 Hz, H^{14}), 7.34 (1H, t, J = 8.0 Hz, H^{5"}), 7.32 (2H, tt, J = 1.5, 7.0 Hz, H¹⁵), 7.29 (2H, d, J = 9.0 Hz, H¹⁰), 7.28 (2H, d, J = 8.5 Hz, H¹⁰), 7.28 (1H, t, J =8.0 Hz, H¹⁶), 7.02 (1H, td, J = 1.3, 7.5 Hz, H^{6"}), 6.89 (2H, d, J =9.0 Hz, H¹¹), 6.89 (2H, d, J = 9.0 Hz, H¹¹), 6.26 (1H, dd, J = 5.0, 6.5 Hz, H^{1'}), 5.92 (2H, s, CONH₂), 5.59 (1H, s, H⁵), 5.40 (1H, d, J = 4.8 Hz, 3'-OH), 4.43 (1H, qn, J = 5.5 Hz, H^{3'}), 4.00 (1H, dd, $J = 4.0, 8.0 \text{ Hz}, \text{H}^{4'}$), 3.70 (3H, s, OCH₃), 3.69 (3H, s, OCH₃), 3.46 $(3H, s, NCH_3)$, 3.40 (1H, dd, J = 4.0, 10.5 Hz, H^{5'}), 3.29 (1H, dd, $J = 2.5, 10.5 \text{ Hz}, \text{H}^{5'}$, 2.44 (1H, td, $J = 6.3, 13.1 \text{ Hz}, \text{H}^{2'}$), 2.18 $(1H, ddd, J = 5.0, 6.5, 13.6 \text{ Hz}, \text{H}^2); \text{ MS (ES}^+) m/z: 1425 ([2M + 10.05]); m/z: 142$ Na]⁺, 8), 724 ([M + Na]⁺, 100), 702 ([M + H]⁺, 50); HRMS (ES⁺): calcd. for C₄₀H₃₉N₅O₇, [M + Na]⁺ 724.2742, found 724.2733; Anal. calcd. for C₄₀H₃₉N₅O₇ (701.77) requires C, 68.46%; H, 5.60%; N, 9.97%. Found C, 65.70%; H, 5.82%; N, 9.35%.

 $3-N-[2'-\text{Deoxy}-5'-O-(4,4'-\text{dimethoxytrity}])-\beta-D-ribofuranosyl]-$ 6 - (3 - aminophenyl) - (2, 3H) - N - methylpyrrolo[2, 3-d] - pyrimidine -2(7H)-one (10). Obtained from 6c (2.21 g, 3.36 mmol) after filtration from CH2Cl2, column chromatography (CH2Cl2-acetone-Et₃N, 50:50:0.5 \rightarrow 0:100:0.5), as a pale brown foam (0.71 g, 1.07 mmol, 80%). R_f 0.08 (CH₂Cl₂-MeOH-Et₃N, 95:5:0.3), 0.14 (CH₂Cl₂-acetone-Et₃N, 20:80:0.3); ¹H NMR (400 MHz, DMSO- d_6) δ (ppm) 8.63 (1H, s, H⁴), 7.41 (2H, d, J = 7.3 Hz, H¹⁴), 7.32 (2H, t, J = 7.0 Hz, H¹⁵), 7.29 (4H, d, J = 8.8 Hz, H¹⁰), 7.25 (1H, tt, J = 1.8, 7.3 Hz, H¹⁶), 7.13 (1H, t, J = 7.8 Hz, H^{5"}), 6.90 (2H, d, J = 8.8 Hz, H¹¹), 6.89 (2H, d, J = 9.0 Hz, H¹¹), 6.67 (1H, t, J = 1.8 Hz, H²), 6.64 (1H, ddd, J = 0.8, 2.2, 8.0 Hz, H⁴), 6.60 (1H, td, J = 1.3, 8.0 Hz, H^{6"}), 6.26 (1H, dd, J = 5.0, 6.3 Hz, H^{1'}), 5.51 $(1H, s, H^5)$, 5.42 (1H, d, J = 4.8 Hz, 3'-OH), 5.26 (2H, br s, NH₂), 4.44 (1H, td, *J* = 5.3, 10.5 Hz, H^{3'}), 4.00 (1H, dd, *J* = 3.9, 7.9 Hz, H^{4'}), 3.71 (3H, s, OCH₃), 3.70 (3H, s, OCH₃), 3.44 (3H, s, NCH₃), $3.39 (1H, dd, J = 3.9, 10.7 Hz, H^{5'}), 3.30 (1H, d, J = 2.6, 10.9 Hz,$ $H^{5'}$), 2.44 (1H, td, J = 6.5, 13.1 Hz, $H^{2'}$), 2.19 (1H, ddd, J = 5.1, 6.4, 13.4 Hz, $H^{2'}$); MS (ES⁺) m/z: 681 ([M + Na]⁺, 100); HRMS (ES⁺): calcd. for $C_{39}H_{38}N_4O_6$, $[M + Na]^+$ 681.2684, found 681.2677; Anal. calcd. for C₃₉H₃₈N₄O₆ (658.74) requires C, 71.11%; H, 5.81%; N, 8.50%. Found C, 69.90%; H, 6.11%; N, 8.41%.

3-*N*-[**2**'-Deoxy-5'-*O*-(**4**,4'-dimethoxytrityl)-β-D-ribofuranosyl]-**6**-(**3**-trifluoroacetamidophenyl)-(**2**,3*H*)-*N*-methylpyrrolo[**2**,3-*d*]pyrimidine-**2**(7*H*)-one (**11**). To a stirred solution of nucleoside **10** (1.50 g, 2.28 mmol) in distilled CH₂Cl₂ (8.0 mL), under an argon atmosphere, at room temperature, was added distilled Et₃N (50.1 mmol, 22.0 eq), activated 4 Å molecular sieves and DMAP (0.76 mmol, 0.3 eq). After cooling in ice, ethyl trifluoroacetate (45.5 mmol, 20.0 eq) was added dropwise over 20 min. The reaction was stirred at 0 °C for a further 10 min, allowed to warm to room temperature then refluxed under argon for 17 h. On cooling, the mixture was concentrated to a mobile syrup then redissolved in MeCN and filtered. The filtrate was dried, redissolved in CH₂Cl₂ (150 mL), washed with water (60 mL) and

the aqueous was re-extracted with CH_2Cl_2 (2 × 50 mL). Combined organic fractions were dried (Na₂SO₄), and concentrated in vacuo and under high vacuum to give a yellow gum. Following column chromatography (ethyl acetate-MeOH-Et₃N, 95:5:0.5), the product 11 was afforded as a pale yellow foam (1.11 g, 1.47 mmol, 65%). $R_{\rm f}$ 0.31 (CH₂Cl₂-acetone-Et₃N, 30:70:0.3), 0.08 (CH₂Cl₂-acetone-Et₃N, 80:20:0.3); ¹⁹F NMR (282 MHz, DMSO- d_6) δ (ppm) -73.6 (NHCOCF₃); ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 11.36 (1H, s, NHCOCF₃), 8.68 (1H, s, H⁴), 7.83 (1H, br s, $H^{2''}$), 7.75 (1H, td, J = 0.9, 8.3 Hz, $H^{4''}$), 7.55 (1H, t, J = 8.0 Hz, $H^{5''}$), 7.40 (2H, d, J = 8.0 Hz, H^{14}), 7.37 (1H, d, J =8.2 Hz, H^{6"}), 7.32 (2H, t, J = 7.3 Hz, H¹⁵), 7.29 (2H, d, J = 8.3 Hz, H^{10}), 7.28 (2H, d, J = 9.0 Hz, H^{10}), 7.24 (1H, t, J = 7.5 Hz, H^{16}), 6.89 (2H, d, J = 8.5 Hz, H¹¹), 6.88 (2H, d, J = 9.0 Hz, H¹¹), 6.25 (1H, app. t, J = 5.7 Hz, H¹), 5.65 (1H, s, H⁵), 5.42 (1H, d, J =4.3 Hz, 3'-OH), 4.42 (1H, qn, J = 5.0 Hz, H^{3'}), 4.00 (1H, dd, J =3.9, 7.4 Hz, H4'), 3.69 (3H, s, OCH₃), 3.69 (3H, s, OCH₃), 3.49 $(3H, s, NCH_3), 3.39 (1H, d, J = 7.3 Hz, H^{5'}), 3.35 (1H, d, J =$ 7.3 Hz, H^{5'}), 2.45 (1H, td, J ' 6.5, 13.1 Hz, H^{2'}), 2.19 (1H, ddd, $J = 5.5, 6.1, 13.2 \text{ Hz}, \text{H}^2$; MS (ES⁺) m/z: 793 ([M + K]⁺, 15), 777 ([M + Na]⁺, 99), 303 (DMT⁺, 100); HRMS (ES⁺): calcd. for $C_{41}H_{37}N_4O_7F_3$, $[M + Na]^+$ 777.2507, found 777.2509.

N, *N'* - *Bis* - [(2 - cyanoethoxy) carbonyl] - *S* - methylisothiourea (15).^{14,21} A mixture of *S*-methylisothiourea hemisulfate 13 (6.03 g, 43.3 mmol) and NaHCO₃ (10.9 g, 130 mmol, 3.0 eq) in argon-degassed, distilled water (50.0 mL) was stirred, under an argon atmosphere, at room temperature, for 30 min. To this was added degassed CH₂Cl₂ (150.0 mL) followed by (2-cyanoethyl)-*N*-succinimidyl carbonate (CEOC-succinimide)§ 14 (18.9 g, 86.6 mmol, 2.0 eq) and the mixture was stirred vigorously, under an argon atmosphere, at room temperature for 8 h. The mixture was diluted with CH₂Cl₂ (100 mL) and water (100 mL), agitated and separated. The aqueous layer was re-extracted (CH₂Cl₂, 3×100 mL) and combined CH₂Cl₂ fractions were dried (Na₂SO₄) and concentrated *in vacuo* and under high vacuum to give a mixture of *mono*- and *bis*-modified isothiourea as a viscous, cloudy yellow syrup.

The syrup was re-dissolved in distilled CH₂Cl₂ (65.0 mL), and further treated with CEOC-succinimide 14 (9.47 g, 43.3 mmol, 1.0 eq), and the mixture was stirred under an argon atmosphere, at room temperature for $14\frac{1}{2}$ hours. The yellow solution was diluted with CH₂Cl₂ (135 mL) then washed with sat. aq KCl (80 mL). The aqueous fraction was re-extracted (CH₂Cl₂, 2×30 mL) and the combined organic fractions were dried (Na2SO4) and concentrated in vacuo to give a cloudy, yellow syrup. Following purification by column chromatography (CH₂Cl₂-ethyl acetate, 95:5), and drying under high vacuum, the desired bis-modified product 30 was afforded as a hard, powdery white solid (11.0 g, 38.7 mmol, 89%). R_f 0.46 (CH₂Cl₂-ethyl acetate, 75:25), 0.28 (CH₂Cl₂-ethyl acetate, 90 : 10); ¹H NMR (400 MHz, CDCl₃) δ (ppm) 11.77 (1H, s, NH), 4.40 (2H, t, J = 6.3 Hz, H³), 4.36 (2H, t, J = 6.0 Hz, H³), 2.78 (4H, t, J = 6.5 Hz, CH₂CN), 2.44 (3H, s, SCH₃); MS (ES⁺) *m*/*z*: 307 ([M + Na]⁺, 100), 285 ([M + H]⁺, 12).

 $3-N-[2'-\text{Deoxy}-5'-O-(4,4'-\text{dimethoxytrity})-\beta-\text{D-ribofuranosy}]-6-(3-{N,N'-bis-[(2-cyanoethoxy)carbony]]guanidiny]}phenyl)-(2,3H)-N-methylpyrrolo[2,3-d]pyrimidine-2(7H)-one (16). To a$

stirred solution of nucleoside 10 (2.07 g, 3.14 mmol) in distilled CH₂Cl₂ (15 mL), under an argon atmosphere, at room temperature, was added activated 4 Å molecular sieves. After 15 min, guanidinylating reagent 15 (7.59 mmol, 2.4 eq) then distilled pyridine (15.2 mmol, 4.8 eq) were added, and the mixture was refluxed under argon for 2 d. Argon-degassed CH₂Cl₂ (200 mL) was added, the solution was washed with sat. aq KCl (100 mL), dried (Na₂SO₄), and concentrated *in vacuo* and under high vacuum to give a pale yellow foam. Following purification by column chromatography (CH₂Cl₂-acetone, $50:50 \rightarrow 90:10$), on silica gel pre-equilibrated with pyridine, the desired guanidinylated product 16 was afforded, as a pale yellow foam (1.25 g, 1.40 mmol, 44%). $R_{\rm f}$ 0.37 (CH₂Cl₂-acetone, 20:80); ¹H NMR (400 MHz, CDCl₃) δ (ppm) 11.57 (1H, s, CONH), 9.91 (1H, s, Aryl-NH), 8.52 (1H, s, H⁴), 7.48 (1H, t, J = 1.8 Hz, H^{2"}), 7.23 (1H, ddd, J = 1.0, 2.0,8.0 Hz, $H^{4''}$), 7.18–7.15 (1H, m, $H^{5''}$), 7.15 (2H, d, J = 7.0 Hz, H^{14}), 7.04 (4H, d, J = 9.0 Hz, H¹⁰), 7.04–6.99 (1H, m, H¹⁶), 6.98 (2H, t, J = 9.0 Hz, H¹⁵), 6.95 (1H, br d, J = 8.5 Hz, H^{6"}), 6.54 (2H, d, J =9.0 Hz, H¹¹), 6.53 (2H, d, J = 9.0 Hz, H¹¹), 6.19 (1H, t, J = 5.5 Hz, $H^{1'}$), 5.29 (1H, s, H^{5}), 4.39 (1H, q, J = 5.5 Hz, $H^{3'}$), 4.19 (2H, br t, J = 5.8 Hz, H²⁴), 4.00 (2H, br t, J = 5.8 Hz, H²⁰), 3.90 (1H, td, J = 3.0, 5.0 Hz, H^{4'}), 3.46 (3H, s, OCH₃), 3.45 (3H, s, OCH₃), 3.34 $(3H, s, NCH_3), 3.26 (1H, dd, J = 3.0, 11.0 Hz, H^{5'}), 3.21 (1H, dd, J = 3$ J = 3.3, 10.8 Hz, H⁵), 2.59–2.52 (1H, m, H²), 2.53 (2H, br t, J =6.0 Hz, H²³), 2.41 (2H, br t, J = 5.8 Hz, H¹⁹), 2.11 (1H, ddd, J =5.5, 6.5, 13.6 Hz, H^{2'}); MS (ES⁺) *m*/*z*: 918 ([M + Na]⁺, 100), 895 $([M + H]^+, 10);$ HRMS (ES⁺): calcd. for $C_{48}H_{46}N_8O_{10}, [M + Na]^+$ 917.3229, found 917.3234.

General procedure for phosphitylation of 6-aryl-*N*-methylpyrrolodC nucleosides 7a–c, 11, 16

To a solution of nucleoside **7a–c**, **11**, **16** (0.25–1.95 mmol) in distilled CH_2Cl_2 , THF or THF–DMF, strictly under an argon atmosphere and excluding moisture, distilled DIPEA (2.0–2.5 eq) was added, followed by chloro-phosphitylating reagent, 2-cyanoethoxy-*N*,*N*-diisopropylaminochlorophosphine (1.1–1.5 eq) dropwise and the reaction was stirred at room temperature for 1–5 h. The mixture was diluted with distilled DCM or argon-degassed ethyl acetate and washed with degassed sat. aq KCl, dried under argon (Na₂SO₄) and concentrated *in vacuo*. Purification by column chromatography using degassed solvents, on silica gel pre-equilibrated with Et₃N or pyridine, under argon pressure, afforded the desired air- and acid-sensitive phosphoramidites **8a–c**, **12**, **17**. Product **8c** was further purified by precipitation (distilled CH₂Cl₂–degassed hexane).

3-*N*-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-6-phenyl-(2,3*H*)-*N*-methylpyrrolo[2,3-*d*]pyrimidine-2(7*H*)-one-3'-*O*-(2-*O*-cyanoethyl-*N*,*N*-diisopropyl) phosphoramidite (8a). Obtained from 7a (0.37 g, 0.57 mmol) and chloro-phosphitylating reagent (0.63 mmol, 1.1 eq), with DIPEA (1.42 mmol, 2.5 eq) in CH₂Cl₂, after column chromatography (ethyl acetate–MeCN– Et₃N, 50:50:0.1), as a distereomeric mixture (*ca.* 4:3), as a pale yellow foam (0.32 g, 0.38 mmol, 66%). *R*_f 0.44 (ethyl acetate–MeCN, 1:1); ³¹P NMR (121 MHz, CDCl₃) δ (ppm) 150.3, 149.5 (P^{III}); MS (ES⁺) *m/z*: 945 ([M + Et₃N + H]⁺, 74), 866 ([M + Na]⁺, 100), 844 ([M + H]⁺, 71).

[§] Prepared according to literature procedure.15,21b

3-*N*-[**2**'-Deoxy-5'-*O*-(**4**,**4**'-dimethoxytrityl)-β-D-ribofuranosyl]-**6**-(**3**- acetamidophenyl) - (**2**,**3***H*) - *N* - methylpyrrolo[**2**,**3**-*d*]pyrimidine-**2**(*7H*)-one-**3**'-*O*-(**2**-*O*-cyanoethyl-*N*,*N*-diisopropyl) phosphoramidite (**8b**). Obtained from **7b** (1.37 g, 1.95 mmol) and chloro-phosphitylating reagent (2.15 mmol, 1.1 eq), with DIPEA (4.91 mmol, 2.5 eq) in THF, after column chromatography (CH₂Cl₂-acetone-Et₃N, 30:70:0.5), as a distereomeric mixture (*ca*. 1:1), as a pale yellow foam (1.51 g, 1.68 mmol, 86%). *R*_f 0.33, 0.46 (CH₂Cl₂-acetone-Et₃N, 20:80:0.3); ³¹P NMR (121 MHz, DMSO-*d*₆) δ (ppm) 149.2, 149.0 (P^{III}); MS (ES⁺) *m/z*: 939 ([M + K]⁺, 5), 923 ([M + Na]⁺, 100), 901 ([M + H]⁺, 9); HRMS (ES⁺): calcd. for C₅₀H₅₇N₆O₈P, [M + Et₃N + H]⁺ 1002.5253, found 1002.5261; [M + Na]⁺ 923.3868, found 923.3886; [M + H]⁺ 901.4048, found 901.4033.

3-N-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-6 - (3 - ureidophenyl) - (2,3H) - N - methylpyrrolo[2,3 - d]pyrimidine phospho-2(7H)-one-3'-O-(2-O-cyanoethyl-N,N-diisopropyl) ramidite (8c). Obtained from 7b (0.92 g, 1.31 mmol) and chloro-phosphitylating reagent (1.46 mmol, 1.1 eq), with DIPEA (3.30 mmol, 2.5 eq) in THF-DMF (5:1, v/v), after column chromatography (acetone–MeOH–Et₃N, $100:0:0:0.5 \rightarrow 98:2:0.5$), as a distereomeric mixture (*ca.* 1:1), as a pale yellow foam (0.60 g, 0.67 mmol, 51%). R_f 0.30 (acetone-CH₂Cl₂-Et₃N, 90:10:0.3), 0.24, 0.31 (methanol-acetone-Et₃N, 5:95:0.3), 0.17 (acetone-Et₃N, 100:0.3); ³¹P NMR (121 MHz, DMSO- d_6) δ (ppm) 149.2, 149.0 (P^{III}); MS (ES⁺) *m/z*: 940 ([M + K]⁺, 8), 924 ([M + Na]⁺, 45), 902 ([M + H]⁺, 78), 303 (DMT⁺, 100); HRMS (ES⁺): calcd. for $C_{49}H_{56}N_7O_8P$, $[M + Na]^+$ 924.3820, found 924.3825; $[M + H]^+$ 902.4001, found 902.4000.

3-N-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-6 - (3 - trifluoroacetamidophenyl) - (2, 3H) - N - methylpyrrolo[2, 3 d]pyrimidine-2(7H)-one-3'-O-(2-O-cyanoethyl-N,N-diisopropyl) phosphoramidite (12). Obtained from 11 (0.19 g, 0.25 mmol) and chloro-phosphitylating reagent (0.29 mmol, 1.2 eq), with DIPEA (0.63 mmol, 2.5 eq) in CH₂Cl₂, after column chromatography $(CH_2Cl_2-acetone-Et_3N, 99:1:0.5\rightarrow 95:5:0.5)$, as a distereometric mixture (ca. 2:1), as a pale yellow foam (0.17, 0.18 mmol, 70%). $R_{\rm f}$ 0.50, 0.57 (CH₂Cl₂-acetone-Et₃N, 50:50:0.3), 0.29, 0.34 (CH₂Cl₂-acetone-Et₃N, 80:20:0.3), 0.18, 0.25 (CH₂Cl₂-Et₃N, 100:0.3); ³¹P NMR (121 MHz, DMSO-*d*₆) δ (ppm) 149.1, 148.9 (P^{III}); ¹⁹F NMR (282 MHz, DMSO- d_6) δ (ppm) -73.5 (NHCOCF₃); MS (ES⁺) m/z: 977 ([M + Na]⁺, 100), 955 ([M + H^{+} , 13); (ES⁻) m/z: 953 ([M – H]⁻, 100); HRMS (ES⁺): calcd. for $C_{50}H_{54}N_6O_8F_3P$, [M + Na]⁺ 977.3585, found 977.3591; [M + H]⁺ 955.3766, found 955.3783.

3-*N*-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-**6**-(3-{*N*,*N'*-*bis*-[(2-cyanoethoxy)carbonyl]guanidinyl}phenyl)-(2,3*H*)-*N*-methylpyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one-3'-*O*-(2-*O*cyanoethyl-*N*,*N*-diisopropyl) phosphoramidite (17). Obtained from 16 (0.50 g, 0.56 mmol) and chloro-phosphitylating reagent (0.84 mmol, 1.5 eq), with DIPEA (1.15 mmol, 2.0 eq) in CH₂Cl₂, after column chromatography (ethyl acetate–acetone–pyridine, 5:95:1), as a distereomeric mixture (*ca.* 1:1), as a pale yellow foam (0.29 g, 0.27 mmol, 47%). *R*_f 0.68, 0.69, (CH₂Cl₂-acetone, 20:80); ³¹P NMR (121 MHz, CDCl₃) δ (ppm) 150.3, 149.4 (P^{III}); MS (ES⁺) m/z: 1197 ([M + Et₃N + H]⁺, 48), 1118 ([M + Na]⁺, 58), 1096 ([M + H]⁺, 100).

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Notes and references

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