View Online

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

Simon R. Gerrard,[a] Mastoura M. Edrees,[a] Imenne Bouamaied,[b] Keith R. Fox[c] and Tom Brown*[a]

Received 13th May 2010, Accepted 19th July 2010
DOI: 10.1039/c0ob00119h

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.

Downloaded by Institute of Organic Chemistry of the SB RAS on 22 December 2010
Published on 27 October 2010 on http://pubs.rsc.org | doi:10.1039/C0OB00119H

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 transcription factors and polymerases and prevent unwinding 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 ^{M}P, an analogue of the bicyclic nucleoside, 6-methylpyrrolo-dC (^{MNH}P), 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,

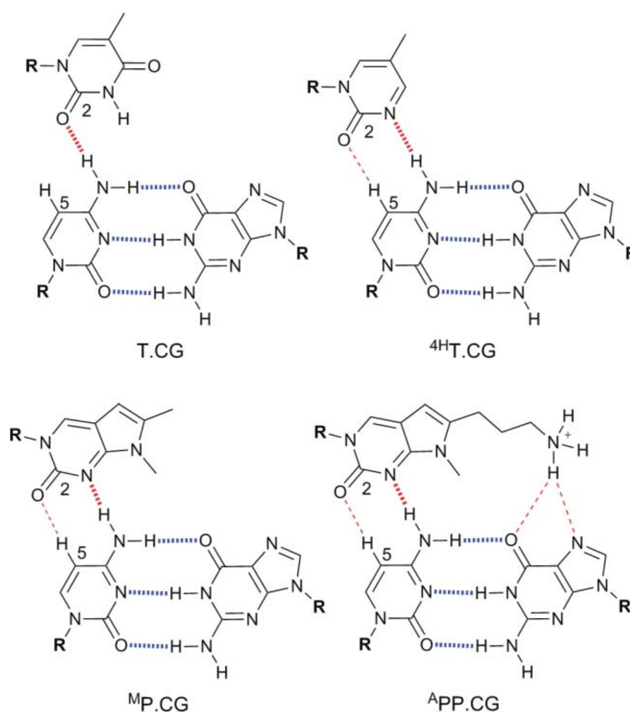


Fig. 1 Binding models of T.CG, ^{4H}T.CG, ^{M}P.CG and ^{A}PP.CG triplets. R = 2′-deoxyribofuranose sugar.

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 ^{M}P with a 2-aminoethyl (^{A}EP) or 3-aminopropyl group (^{A}PP) for binding to C═O^6 and N^7 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 ^{M}P, suggesting the aminopropyl group of ^{A}PP does not contribute greatly to binding. From this work we hypothesised that reducing linker flexibility whilst allowing some rotational freedom around the aryl-C^6 bond might position the amino group more precisely for effective hydrogen bonding.

[a]*School of Chemistry, University of Southampton, Highfield, Southampton, SO17 1BJ, UK. E-mail: tb2@soton.ac.uk*
[b]*ATDBio Ltd, School of Chemistry, University of Southampton, Highfield, Southampton, SO17 1BJ, UK*
[c]*School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX, UK*

† Electronic supplementary information (ESI) available: ^{13}C NMR, ^{1}H NMR (for phosphoramidites) and IR data, melting study protocols, melting data, example melting curves and derivatives, oligonucleotide sequences and ESMS data. See DOI: 10.1039/c0ob00119h

View Online

To this end, a number of TFOs containing substituted phenyl-*N*-methylpyrrolo-dC nucleotides (^{X}P) 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.

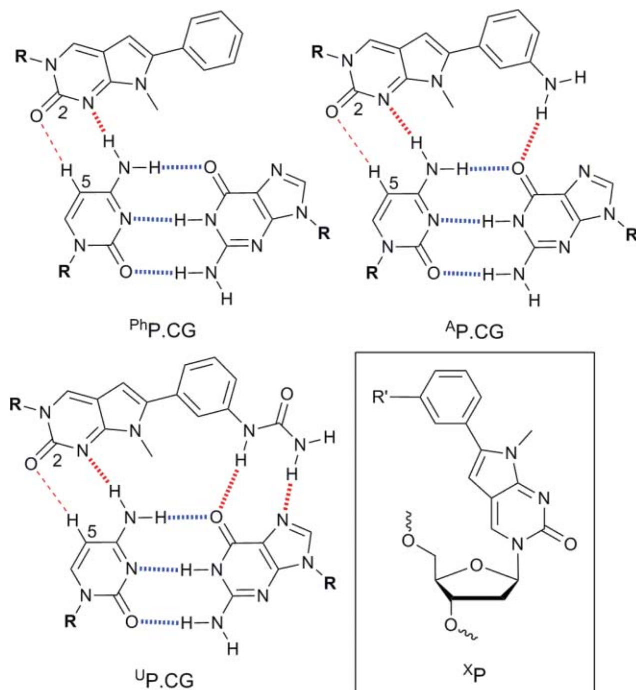


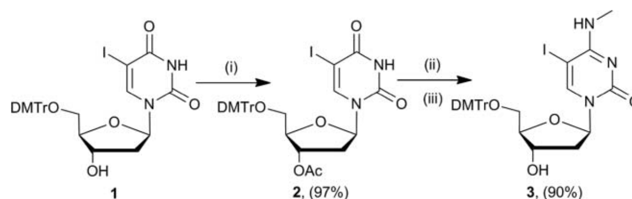
Fig. 2 Putative ^{Ph}P.CG, ^{A}P.CG and ^{U}P.CG triplet binding motifs, and ^{X}P nucleotide. R = 2′-deoxyribofuranose, R′ = H (^{Ph}P), NH_2 (^{A}P), $NHCOCH_3$ (^{Ac}P), $NHCONH_2$ (^{U}P), $NHC(NH_2^+)NH_2$ (^{G}P).

Results and discussion

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

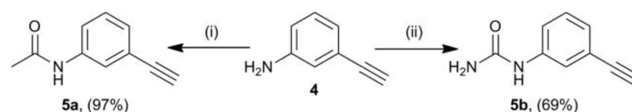
Synthesis of *N*-methylpyrrolo-dC analogues

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 $POCl_3$.[12] After methylamine insertion and deacetylation, the conversion product **3** was obtained in 90% yield (Scheme 1).

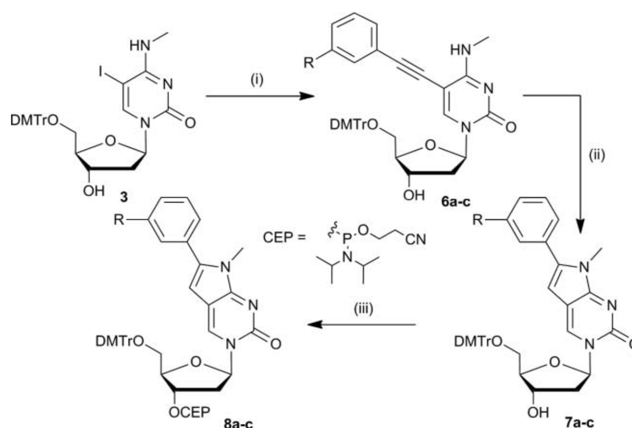


Scheme 1 Synthesis of 5-iodo-4*N*-methyl-2′-deoxycytidine substrate **1**. i) Ac_2O (1.7 eq), pyridine, 0 °C–rt, 3 h; ii) $POCl_3$ (3.8 eq), NMI (12.8 eq), pyridine, 0 °C–rt, 1 h; iii) 40 wt% CH_3NH_2–H_2O, 0 °C–rt, 16 h.

Five modified 6-phenyl-*N*-methylpyrrolo-dC phosphoramidite monomers were synthesised from this intermediate; trifluoroacetamido- (^{A}P, **12**), acetamido- (^{Ac}P, **8b**), ureido- (^{U}P, **8c**), guanidino- (^{G}P, **17**) and also 6-phenyl-*N*-methylpyrrolo-dC 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 cyclisation[13] then phosphitylation (Scheme 3). Monomers **12** and **17** required additional steps as described below (Schemes 4, 5).



Scheme 2 Synthesis of alkynes **5a,b** i) AcCl (1.0 eq), Et_3N (1.0 eq), Et_2O, 0 °C–rt, 1.5 h, ii) phenyl carbamate (2.0 eq), 90 °C, 12 h.

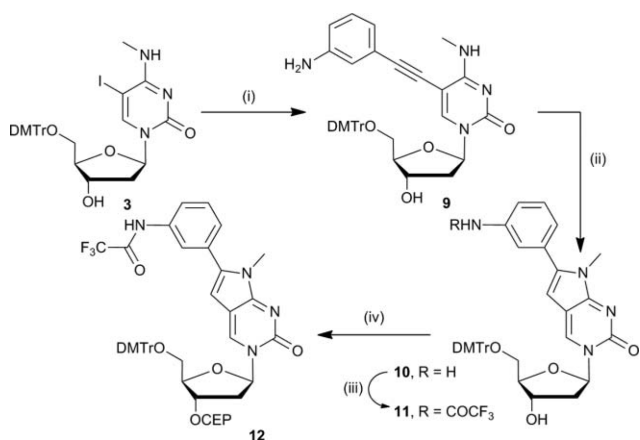


Scheme 3 Synthesis of ^{Ph}P **8a**, ^{Ac}P **8b**, and ^{U}P **8c** phosphoramidites. i) alkyne **5a–c** (3.0 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.4 eq), Et_3N (5.0 eq), DMF, dark, rt, 1–3 h; ii) CuI (1.1 eq), Et_3N (10.0 eq), 4 Å sieves, DMF, dark, 125 °C, 0.5–2 h; iii) 2-cyanoethoxy-*N*,*N*-diisopropylamino chlorophosphine (1.1 eq), DIPEA (2.5 eq), CH_2Cl_2 or THF–DMF (1 : 2 v/v), rt, 1–2 h. R = H (**a**), $NHCOCH_3$ (**b**), $NHCONH_2$ (**c**).

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

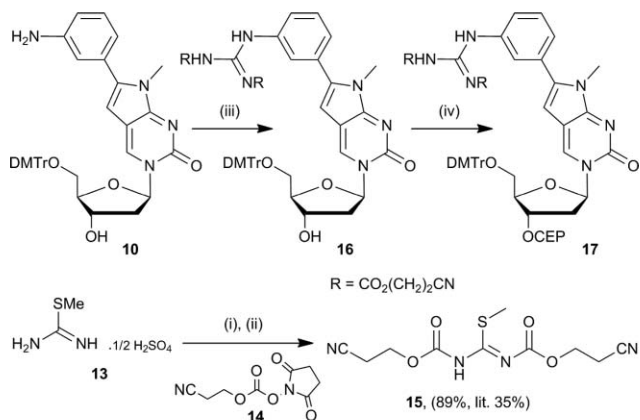
Published on 27 October 2010 on http://pubs.rsc.org | doi:10.1039/C0OB00119H
Downloaded by Institute of Organic Chemistry of the SB RAS on 22 December 2010

 This journal is © The Royal Society of Chemistry 2010



Scheme 4 Synthesis of ^{A}P phosphoramidite **12**. i) alkyne **4** (3.0 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.4 eq), Et_3N (5.0 eq), DMF, dark, rt, 1.5 h; ii) CuI (1.1 eq), Et_3N (10.0 eq), 4 Å sieves, DMF, dark, 125 °C, 1.5 h; iii) CF_3CO_2Et (20.0 eq), DMAP (1.0 eq), Et_3N (22.0 eq), CH_2Cl_2, 0 °C–rt, 35 min then 50 °C, overnight; iv) 2-cyanoethoxy-*N*,*N*-diisopropylamino chlorophosphine (1.1 eq), DIPEA (2.5 eq), CH_2Cl_2, 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 ^{G}P phosphoramidite **17**. i) carbonate **14** (2.0 eq), $NaHCO_3$ (3.0 eq), degassed CH_2Cl_2–H_2O (2 : 1 v/v), rt, 8 h; ii) carbonate **14** (1.0 eq), CH_2Cl_2, rt, 14.5 h; iii) isothiourea **15** (2.4 eq), pyridine (4.8 eq), 4 Å sieves, CH_2Cl_2, reflux, 2 d; iv) 2-cyanoethoxy-*N*,*N*-diisopropylamino chlorophosphine (1.8 eq), DIPEA (2.0 eq), CH_2Cl_2, 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, ^{A}P, ^{Ac}P, ^{U}P and ^{G}P (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 data[11] indicated high binding affinities and good selectivity for CG. In the present study, UV melting

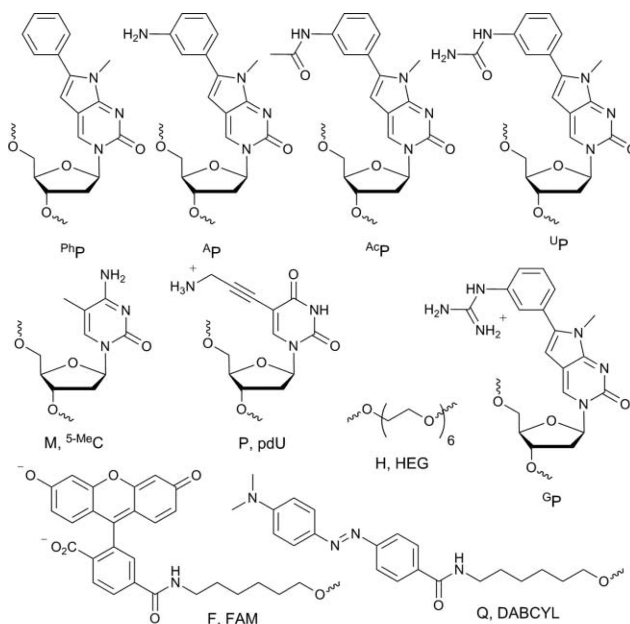


Fig. 3 Monomers incorporated into oligonucleotides.

Table 1 UV triplex melting experiment: $^{X}\underline{P}$ against $\underline{YZ}$ at pH 6.2, 6.6 and 7.0 (10 mM sodium phosphate, 200 mM NaCl, 1 mM Na_2EDTA)[a,b]

```
    5'-TTT TTM VXW MTM TMT
5'-GCT AAA AAG AYA GAG AGA TCG
    CGA TTT TTC TZT CTC TCT AGC-5'
```

Entry	VXW	YZ	pH	T	^{Ph}P	^{A}P	^{Ac}P	^{U}P	^{G}P
1	TXT	CG	6.2	26.7	32.2	31.5	28.5	30.9	29.0
2		GC		—	22.7	22.5	21.4	21.2	20.4
3		AT		—	22.9	22.6	21.0	22.2	21.8
4		TA		—	18.6	n.d.	21.8	22.3	19.3
5	TXP	CG	6.2	—	34.2	—	—	—	—
6	PXT			—	34.9	—	—	—	—
7	PXP			30.2	36.9	37.0	31.6	34.3	32.1
8	TXT	CG	6.6	20.1	24.9	23.6	21.6	23.6	21.9
9	TXP			n.d.	26.2	24.5	22.7	24.7	22.3
10	PXT			23.6	26.8	27.0	23.8	25.6	23.2
11	PXP			22.9	28.9	28.4	24.2	26.4	23.7
12	TXT	CG	7.0	—	18.6	18.3	n.d.	n.d.	n.d.

[a] Average T_m values for triplex melt given in °C. Heating/cooling at 0.5 °C min^{-1}. n.d. = not determined (T_m < 17 °C). [b] M = $^{5\text{-Me}}$dC;P = 5-(3-aminoprop-1-ynyl)-dU (pdU), V, W = T or P, $\underline{X}$ = T, ^{Ph}P, ^{A}P, ^{Ac}P, ^{U}P or ^{G}P; 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 °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 > ^{A}P > ^{Ph}P > ^{M}P > T (^{M}P examined in previous study[11]). 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,

Downloaded by Institute of Organic Chemistry of the SB RAS on 22 December 2010
Published on 27 October 2010 on http://pubs.rsc.org | doi:10.1039/C0OB00119H

View Online

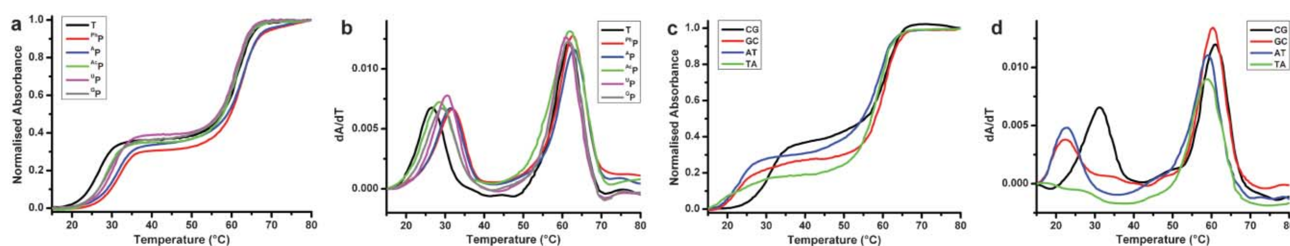


Fig. 4 UV melting curves (**a/c**) and derivatives (**b/d**). **a/b**: ^{X}P (TFO 1-6, TXT, see ESI, Table C1) against CG at pH 6.2 (10 mM sodium phosphate, 200 mM NaCl, 1 mM Na_2EDTA). See Table 1, entry 1. **c/d**: ^{A}P (TFO 3, TXT, see ESI, Table C1) against YZ at pH 6.2 (10 mM sodium phosphate, 200 mM NaCl, 1 mM Na_2EDTA). See Table 1, entries 1–4.

Downloaded by Institute of Organic Chemistry of the SB RAS on 22 December 2010
Published on 27 October 2010 on http://pubs.rsc.org | doi:10.1039/C0OB00119H

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, (ΔT_m +6.7 °C *vs.* TA, entry 4), and ^{Ph}P demonstrated greatest selectivity (+9.3 °C *vs.* AT, entry 3), similar to ^{A}P (+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 ^{X}P 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 base-stacking.[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 = ^{A}P). This difference between sequences is probably due to specific electrostatic, hydrogen bonding or steric interactions between neighbouring pdU and ^{X}P nucleotides, which are difficult to predict.

Fluorescence-based triplex melting analysis[16] 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), ^{G}P 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 ^{X}P were varied as previously described for the UV melting study. It was noted that neighbouring protonated pdU nucleotides contributed less stabilisation to the ^{G}P 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 grows with increasing pH (entries 7,17,19), such that the relative binding affinity of ^{G}P for CG, drops from moderate to lowest. The binding affinity of ^{U}P 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 (^{Ac}P), which exhibited the reverse at pH 6.2 (entries 5,6) and no difference at pH 6.6 (entries 18,19). ^{G}P 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 ^{Ac}P, this T_m difference did not appear to relate to pH.

Table 2 Fluorescence triplex melting experiment: $\underline{^{X}P}$ 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 Na_2EDTA)[a,b]

```
        5'-Q-PMM TPM VXW TPT PTM PT
5'-F-GT GTT AGG AAG AYA AAA AAG AAC TGG T-H
     CA CAA TCC TTC TZT TTT TTC TTG ACC A-H
```

Entry	VXW	YZ	pH	T	^{Ph}P	^{A}P	^{Ac}P	^{U}P	^{G}P
1	TXT	CG	6.2	44.3	44.0	44.0	43.9	44.3	47.2
2		GC		—	32.0	36.7	38.8	39.9	36.9
3		AT		—	34.6	33.9	35.0	35.1	37.0
4		TA		—	37.6	36.7	41.9	40.7	39.7
5	TXP	CG	6.2	47.5	50.8	51.0	50.3	52.0	53.0
6	PXT			48.4	49.6	49.1	51.0	50.8	51.3
7	PXP	CG	6.2	51.1	55.7	56.8	55.1	57.8	56.0
8		GC		—	42.1	45.8	46.9	46.0	45.0
9		AT		—	44.0	43.3	45.0	43.8	47.0
10		TA		—	48.9	47.2	50.4	49.6	51.0
11	TXT	CG	6.6	—	37.0	37.5	36.2	36.9	40.3
12		GC		—	31.0	n.d.	n.d.	n.d.	n.d.
13		AT		—	n.d.	n.d.	n.d.	n.d.	30.9
14		TA		—	31.0	31.5	33.9	33.5	33.0
15	TXP	CG	6.6	—	44.0	43.2	43.8	44.2	46.2
16	PXT			—	43.0	42.2	43.8	43.0	43.7
17	PXP			—	48.1	48.5	46.5	48.2	47.2
18	TXT	CG	7.0	—	n.d.	32.0	30.9	31.6	32.7
19	PXP			—	39.9	40.6	39.9	39.9	38.5
20	TXT	CG	7.0*	—	36.1	36.0	36.0	37.4	39.0

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

This journal is © The Royal Society of Chemistry 2010

View Online

Downloaded by Institute of Organic Chemistry of the SB RAS on 22 December 2010
Published on 27 October 2010 on http://pubs.rsc.org | doi:10.1039/C0OB00119H

Table 3 Fluorescence/UV triplex melting comparison experiment: X against CG at pH 6.2 (10 mM sodium phosphate, 200 mM NaCl, 1 mM Na_2EDTA) in fluorescence melting motif (Table 2)[a]

Method	Base pair	Buffer	^{Ph}P	^{A}P	^{Ac}P	^{U}P	^{G}P
Fluorescence	CG	6.2	44.0[b]	44.0[b]	43.9[b]	44.3[b]	47.2[b]
UV			45.1	45.2	44.6	45.7	48.6
ΔT_m			*+1.1*	*+1.2*	*+0.7*	*+1.4*	*+1.4*

[a] Average T_m values for triplex melt given in °C. Heating at 0.5 °C min^{-1}. X = ^{Ph}P, ^{A}P, ^{Ac}P, ^{U}P or ^{G}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, ^{G}P is most selective (+7.3–7.5 °C *vs.* TA, see ESI, Fig. B4), and ^{A}P 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 ^{A}P is now most selective (+9.6 °C), followed by ^{U}P (+8.2 °C) and ^{Ph}P (+6.8 °C). ^{Ac}P and ^{G}P are now of low to moderate selectivity. The relative destabilising electrostatic influence on ^{G}P 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_ms 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 ^{A}P. The guanidinium monomer ^{G}P, 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, ^{A}P and ^{Ph}P monomers are best. In pdU-rich sequences, where the modification is abutted by T, ^{G}P is best at all pHs. However, with neighbouring pdU nucleotides, the relative stability of the ^{G}P triplex drops, such that ^{U}P (pH 6.2) then ^{A}P (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, ^{A}P. 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 F_{254} or Machenary-Nagel Alugram Sil G/UV_{254} 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.

View Online

Downloaded by Institute of Organic Chemistry of the SB RAS on 22 December 2010
Published on 27 October 2010 on http://pubs.rsc.org | doi:10.1039/C0OB00119H

Compound numbering

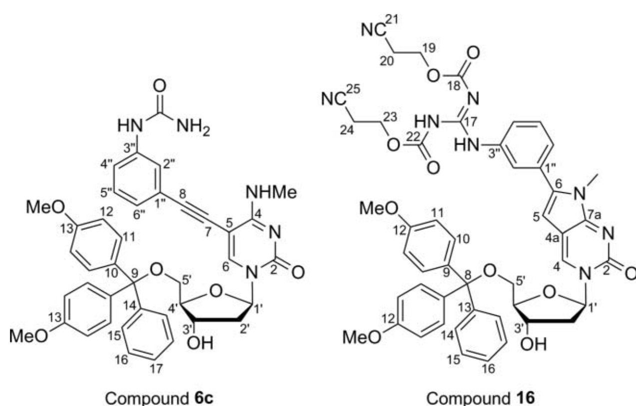


Fig. 5 Example compound numbering (**6c** and **16**).

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 × 50 mL) and diethyl ether (2 × 50 mL), to give a white foam/syrup. The syrup was dissolved in ethyl acetate (300 mL) and washed with water (3 × 75 mL). The aqueous was re-extracted (ethyl acetate, 40 mL) and combined organic fractions were washed with sat. aq KCl (120 mL), dried (Na_2SO_4) 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→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 (CH_2Cl_2–acetone–Et_3N, 90 : 10 : 0.3), 0.48 (CH_2Cl_2–MeOH–Et_3N, 95 : 5 : 0.3), 0.33 (hexane–ethyl acetate–Et_3N, 70 : 30 : 0.3); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 11.75 (1H, s, NH^3), 8.05 (1H, s, H^6), 7.39 (2H, d, J = 8.0 Hz, H^{13}), 7.32 (2H, t, J = 7.3 Hz, H^{14}), 7.28 (2H, d, J = 9.0 Hz, H^9), 7.28 (2H, d, J = 9.0 Hz, H^9), 7.23 (1H, tt, J = 1.1, 7.2 Hz, H^{15}), 6.89 (4H, d, J = 8.8 Hz, H^{10}), 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, OCH_3), 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, $H^{2'}$), 2.33 (1H, ddd, J = 2.0, 6.0, 14.3 Hz, $H^{2'}$), 2.03 (3H, s, $COCH_3$); 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-4*N*-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% $MeNH_2$–water solution (40.5 mL, 468 mmol $MeNH_2$, 55.9 eq $MeNH_2$) was added, and the cloudy solution was stirred at room temperature for 18 h. The mixture was concentrated under high vacuum, dissolved in CH_2Cl_2 (300 mL) and washed with water (3 × 75 mL). The combined aqueous fraction was re-extracted (CH_2Cl_2, 40 mL), the combined organic layer was washed with sat. aq KCl (120 mL), dried (Na_2SO_4) and concentrated to give a pale yellow foam. Following careful purification by column chromatography (CH_2Cl_2–MeOH–Et_3N, 98 : 2 : 0.5), and precipitation with Et_2O, the 4*N*-methyl-dC nucleoside **3** was afforded, as a white powder (4.92 g, 7.35 mmol, 88%). R_f 0.40 (CH_2Cl_2–MeOH–Et_3N, 90 : 10 : 0.3), 0.36 (CH_2Cl_2–acetone–Et_3N, 80 : 20 : 0.3), 0.57 (acetone–MeOH–Et_3N, 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, H^6), 7.40 (2H, d, J = 7.5 Hz, H^{13}), 7.32 (2H, t, J = 7.3 Hz, H^{14}), 7.29 (4H, d, J = 9.0 Hz, H^9), 7.22 (1H, tt, J = 2.0, 7.3 Hz, H^{15}), 7.06 (1H, q, J = 4.5 Hz, N*H*CH_3), 6.89 (4H, d, J = 9.0 Hz, H^{10}), 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, $H^{4'}$), 3.74 (6H, s, OCH_3), 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, $NHCH_3$), 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 $C_{31}H_{32}N_3O_6I$ (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 3-ethynylaniline **4** (3.50 mL, 31.1 mmol) and distilled Et_3N (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 × 35 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 (CH_2Cl_2–CH_3OH–Et_3N, 95 : 5 : 0.3); Mp 92–94 °C (chloroform–hexane), *lit.*[19] 94–96 °C (CCl_4); ^1H NMR (300 MHz, DMSO-d_6) δ (ppm) 10.00 (1H, s, N*H*$COCH_3$), 7.77 (1H, app. t, J = 1.7 Hz, H^2), 7.54 (1H, ddd, J = 1.1, 2.0, 8.1 Hz, H^4), 7.30 (1H, t, J = 7.9 Hz, H^5), 7.13 (1H, td, J = 1.3, 7.7 Hz, H^6), 4.13 (1H, s, C≡CH), 2.05 (3H, s, $COCH_3$); MS (ES^+) m/z: 160 ($[M + H]^+$, 100), 182 ($[M + Na]^+$, 11), 201 ($[M + CH_3CN + H]^+$, 7), 223 ($[M + CH_3CN + Na]^+$, 17), 242 ($[M + AcOH + Na]^+$, 12); (ES^-) m/z: 158 ($[M - H]^-$, 100); HRMS

‡ Prepared according to literature procedure.[18]

This journal is © The Royal Society of Chemistry 2010

View Online

(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 methanol–acetone, and dried under high vacuum. Following purification by column chromatography (CH_2Cl_2–acetone, 95 : 5→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_f 0.31 (CH_2Cl_2–CH_3OH–Et_3N, 90 : 10 : 0.3), 0.04 (CH_2Cl_2–acetone–Et_3N, 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, H^2), 7.32 (1H, ddd, J = 1.1, 2.0, 8.2 Hz, H^4), 7.22 (1H, t, J = 7.9 Hz, H^5), 7.00 (1H, td J = 1.3, 7.3 Hz, H^6), 5.89 (2H, s, NH_2), 4.08 (1H, s, C≡CH); MS (ES+) m/z: 343 ($[2M + Na]^+$, 21), 183 ($[M + Na]^+$, 70), 102 ($[Et_3N + H]^+$, 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-4*N*-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 Et_3N (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)-4*N*-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 (CH_2Cl_2–MeOH–Et_3N, 90 : 10 : 0.5) as a pale orange foam (0.47 g, 0.74 mmol, 90%). R_f 0.66 (ethyl acetate–MeOH–aq NH_3, 5 : 1 : 1); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.20 (1H, s, H^6), 7.45 (2H, dd, J = 1.2, 8.6 Hz, H^{15}), 7.35 (2H, d, J = 9.1 Hz, H^{11}), 7.35 (2H, d, J = 8.9 Hz, 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, H^{12}), 6.38 (1H, dd, J = 6.1, 6.8 Hz, $H^{1'}$), 5.77 (1H, q, J = 4.8 Hz, N*H*CH_3), 4.58 (1H, td, J = 2.8, 5.5 Hz, $H^{3'}$), 4.19 (1H, dd, J = 3.2, 6.2 Hz, $H^{4'}$), 3.70 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 3.41 (1H, dd, J = 3.1, 10.6 Hz, $H^{5'}$), 3.34 (1H, dd, J = 3.8, 10.6 Hz, $H^{5'}$), 3.11 (3H, d, J = 5.0 Hz, NHCH_3), 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 Hz, $H^{2'}$); MS (ES+) m/z: 745 ($[M + Et_3N + H]^+$, 100), 666 ($[M + Na]^+$, 52); HRMS (ES+): calcd. for $C_{39}H_{37}N_3O_6$, $[M + K]^+$ 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)-4*N*-methyl-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 Na_2EDTA (pH 9) and column chromatography (CH_2Cl_2–acetone–pyridine, 90 : 10 : 1 →30 : 70 : 1), partially purified as a yellow glassy foam (1.99 g). R_f 0.16 (CH_2Cl_2–acetone–Et_3N, 40 : 60 : 0.3), 0.04 (CH_2Cl_2–acetone–pyridine, 80 : 20 : 0.3), 0.28 (CH_2Cl_2–acetone–Et_3N, 20 : 80 : 0.3), 0.32 (CH_2Cl_2–acetone–pyridine, 20 : 80 : 1); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.97 (1H, s, N*H*$COCH_3$), 8.00 (1H, s, H^6), 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 Hz, N*H*CH_3), 7.40 (2H, dd, J = 1.3, 8.3 Hz, H^{15}), 7.29 (2H, d, J = 9.0 Hz, H^{11}), 7.29–7.25 (1H, m, $H^{5''}$), 7.28 (2H, d, J = 8.5 Hz, H^{11}), 7.24 (2H, t, J = 7.8 Hz, H^{16}), 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, H^{12}), 6.83 (2H, d, J = 9.0 Hz, H^{12}), 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, NHCH_3), 2.27 (1H, ddd, J = 3.5, 6.3, 13.6 Hz, $H^{2'}$), 2.16 (1H, td, J = 6.7, 13.6 Hz, $H^{2'}$), 2.06 (3H, s, $COCH_3$); MS (ES+) m/z: 723 ($[M + Na]^+$, 100), 303 (DMT^+, 29); (ES−) m/z: 699 ($[M - H]^-$, 100); HRMS (ES+): calcd. for $C_{41}H_{40}N_4O_7$, $[M + H]^+$ 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 (CH_2Cl_2–acetone–Et_3N, 20 : 80 : 0.5 →0:100 : 0.5), partially purified as a pale brown foam (1.49 g) and used without further purification. R_f 0.18 (CH_2Cl_2–MeOH–Et_3N, 90 : 10 : 0.3), 0.11 (CH_2Cl_2–acetone–Et_3N, 20 : 80 : 0.3), 0.44 (acetone–MeOH–Et_3N, 90 : 10 : 0.3).

5-(3-Aminophenyl)ethynyl-5′-*O*-(4,4′-dimethoxytrityl)-4*N*-methyl-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 (CH_2Cl_2–MeOH–Et_3N, 100 : 0 : 0.5→ 97 : 3 : 0.5) as a pale orange foam (0.91 g, 1.39 mmol, 95%). R_f 0.31 (CH_2Cl_2–MeOH–Et_3N, 90 : 10 : 0.3); 0.31 (CH_2Cl_2–acetone–Et_3N, 20 : 80 : 0.3), 0.03 (CH_2Cl_2–acetone–Et_3N, 80 : 20 : 0.3); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 7.94 (1H, s, H^6), 7.40 (2H, dd, J = 1.1, 8.4 Hz, H^{15}), 7.35 (1H, q, J = 4.8 Hz, N*H*CH_3), 7.30–7.25 (2H, m, H^{16}), 7.29 (2H, d, J = 8.0 Hz, H^{11}), 7.29 (2H, d, J = 9.0 Hz, H^{11}), 7.17 (1H, tt, J = 1.0, 7.3 Hz, H^{17}), 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 Hz, $H^{1'}$), 5.30 (1H, d, J = 4.3 Hz, 3′-OH), 5.11 (2H, br s, 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, OCH_3), 3.67 (3H, s, OCH_3), 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, NHCH_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.

Downloaded by Institute of Organic Chemistry of the SB RAS on 22 December 2010
Published on 27 October 2010 on http://pubs.rsc.org | doi:10.1039/C0OB00119H

View Online

Downloaded by Institute of Organic Chemistry of the SB RAS on 22 December 2010
Published on 27 October 2010 on http://pubs.rsc.org | doi:10.1039/C0OB00119H

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 Na_2EDTA (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)-*β*-D-ribofuranosyl]-6-phenyl-(2,3*H*)-*N*-methylpyrrolo[2,3-*d*]pyrimidine-2(7*H*)-one (7a). Obtained from **6a** (0.52 g, 0.80 mmol) after aqueous workup and column chromatography (CH_2Cl_2–MeOH–Et_3N, 90 : 10 : 0.5), as a pale orange foam (0.37 g, 0.57 mmol, 71%). R_f 0.67 (ethyl acetate–MeOH–aq NH_3, 5 : 1 : 1), ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.80 (1H, s, H^4), 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, H^{10}), 7.29 (2H, tt, J = 1.5, 7.4 Hz, H^{15}), 7.23 (1H, tt, J = 1.3, 7.2 Hz, H^{16}), 6.83 (2H, d, J = 8.9 Hz, H^{11}), 6.82 (2H, d, J = 8.9 Hz, H^{11}), 6.50 (1H, dd, J = 5.2, 6.1 Hz, $H^{1'}$), 5.56 (1H, s, H^5), 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, OCH_3), 3.75 (3H, s, OCH_3), 3.57 (3H, s, NCH_3), 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 Hz, $H^{2'}$), 2.42 (1H, ddd, J = 5.3, 6.1, 13.7 Hz, $H^{2'}$); MS (ES$^+$) m/z: 745 ([M + Et_3N + H]$^+$, 81), 666 ([M + Na]$^+$, 100), 644 ([M + H]$^+$, 12); HRMS (ES$^+$): calcd. for $C_{39}H_{37}N_3O_6$, [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)-(2,3*H*)-*N*-methylpyrrolo[2,3-*d*]pyrimidine-2(7*H*)-one (7b). Obtained from **6b** (1.92 g of 1.97 g) after filtration from CH_2Cl_2 then aqueous workup and column chromatography (acetone–MeOH–Et_3N, 100 : 0 : 0.5→90:10 : 0.5), as a yellow powder (1.43 g, 2.04 mmol, 84% over two steps). R_f 0.09 (4 : 1, CH_2Cl_2–acetone–Et_3N, 20 : 80 : 0.3), 0.14 (CH_2Cl_2–acetone–pyridine, 20 : 80 : 1); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 10.08 (N*H*$COCH_3$), 8.65 (1H, s, H^4), 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, d, J = 7.0 Hz, H^{14}), 7.32 (2H, t, J = 7.0 Hz, H^{15}), 7.29 (2H, d, J = 8.5 Hz, H^{10}), 7.28 (2H, d, J = 9.0 Hz, H^{10}), 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, OCH_3), 3.69 (3H, s, OCH_3), 3.47 (3H, s, NCH_3), 3.40 (1H, dd, J = 4.0, 11.0 Hz, $H^{5'}$), 3.30 (1H, dd, J = 3.0, 11.0 Hz, $H^{5'}$), 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, $COCH_3$); MS (ES$^+$) m/z: 803 ([M + Et_3N + H]$^+$, 14), 723 ([M + Na]$^+$, 100); (ES$^-$) m/z: 699 ([M – H]$^-$, 100); HRMS (ES$^+$): calcd. for $C_{41}H_{40}N_4O_7$, [M + H]$^+$ 701.2970, found 701.2965.

3-*N*-[2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*β*-D-ribofuranosyl]-6-(3-ureidophenyl)-(2,3*H*)-*N*-methylpyrrolo[2,3-*d*]pyrimidine-2(7*H*)-one (7c). Obtained from **6c** (1.48 g of 1.49 g) after filtration from methanol, column chromatography × 2 (acetone–MeOH–Et_3N, 100 : 0 : 0.5→90 : 10 : 0.5), and precipitation (MeOH–acetone–Et_2O) as a very pale yellow powder (1.03 g, 1.47 mmol, 79% over two steps). R_f 0.24 (acetone–MeOH–Et_3N, 90 : 10 : 0.3), 0.49 (CH_2Cl_2–MeOH–Et_3N, 80 : 20 : 0.3); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.71 (1H, s, N*H*$CONH_2$), 8.63 (1H, s, H^4), 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, H^{15}), 7.29 (2H, d, J = 9.0 Hz, H^{10}), 7.28 (2H, d, J = 8.5 Hz, H^{10}), 7.28 (1H, t, J = 8.0 Hz, H^{16}), 7.02 (1H, td, J = 1.3, 7.5 Hz, $H^{6''}$), 6.89 (2H, d, J = 9.0 Hz, H^{11}), 6.89 (2H, d, J = 9.0 Hz, H^{11}), 6.26 (1H, dd, J = 5.0, 6.5 Hz, $H^{1'}$), 5.92 (2H, s, $CONH_2$), 5.59 (1H, s, H^5), 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 Hz, $H^{4'}$), 3.70 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 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 Hz, $H^{5'}$), 2.44 (1H, td, J = 6.3, 13.1 Hz, $H^{2'}$), 2.18 (1H, ddd, J = 5.0, 6.5, 13.6 Hz, $H^{2'}$); MS (ES$^+$) m/z: 1425 ([2M + Na]$^+$, 8), 724 ([M + Na]$^+$, 100), 702 ([M + H]$^+$, 50); HRMS (ES$^+$): calcd. for $C_{40}H_{39}N_5O_7$, [M + Na]$^+$ 724.2742, found 724.2733; Anal. calcd. for $C_{40}H_{39}N_5O_7$ (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′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*β*-D-ribofuranosyl]-6-(3-aminophenyl)-(2,3*H*)-*N*-methylpyrrolo[2,3-*d*]-pyrimidine-2(7*H*)-one (10). Obtained from **6c** (2.21 g, 3.36 mmol) after filtration from CH_2Cl_2, column chromatography (CH_2Cl_2–acetone–Et_3N, 50 : 50 : 0.5→0 : 100 : 0.5), as a pale brown foam (0.71 g, 1.07 mmol, 80%). R_f 0.08 (CH_2Cl_2–MeOH–Et_3N, 95 : 5 : 0.3), 0.14 (CH_2Cl_2–acetone–Et_3N, 20 : 80 : 0.3); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.63 (1H, s, H^4), 7.41 (2H, d, J = 7.3 Hz, H^{14}), 7.32 (2H, t, J = 7.0 Hz, H^{15}), 7.29 (4H, d, J = 8.8 Hz, H^{10}), 7.25 (1H, tt, J = 1.8, 7.3 Hz, H^{16}), 7.13 (1H, t, J = 7.8 Hz, $H^{5''}$), 6.90 (2H, d, J = 8.8 Hz, H^{11}), 6.89 (2H, d, J = 9.0 Hz, H^{11}), 6.67 (1H, t, J = 1.8 Hz, $H^{2'}$), 6.64 (1H, ddd, J = 0.8, 2.2, 8.0 Hz, $H^{4''}$), 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, NH_2), 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, OCH_3), 3.70 (3H, s, OCH_3), 3.44 (3H, s, NCH_3), 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 $C_{39}H_{38}N_4O_6$ (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 CH_2Cl_2 (8.0 mL), under an argon atmosphere, at room temperature, was added distilled Et_3N (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 CH_2Cl_2 (150 mL), washed with water (60 mL) and

 This journal is © The Royal Society of Chemistry 2010

View Online

Downloaded by Institute of Organic Chemistry of the SB RAS on 22 December 2010
Published on 27 October 2010 on http://pubs.rsc.org | doi:10.1039/C0OB00119H

the aqueous was re-extracted with CH_2Cl_2 (2 × 50 mL). Combined organic fractions were dried (Na_2SO_4), and concentrated *in vacuo* and under high vacuum to give a yellow gum. Following column chromatography (ethyl acetate–MeOH–Et_3N, 95 : 5 : 0.5), the product **11** was afforded as a pale yellow foam (1.11 g, 1.47 mmol, 65%). R_f 0.31 (CH_2Cl_2–acetone–Et_3N, 30 : 70 : 0.3), 0.08 (CH_2Cl_2–acetone–Et_3N, 80 : 20 : 0.3); ^{19}F NMR (282 MHz, DMSO-d_6) δ (ppm) −73.6 ($NHCOCF_3$); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 11.36 (1H, s, $NHCOCF_3$), 8.68 (1H, s, H^4), 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, H^{15}), 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, H^{11}), 6.88 (2H, d, J = 9.0 Hz, H^{11}), 6.25 (1H, app. t, J = 5.7 Hz, $H^{1'}$), 5.65 (1H, s, H^5), 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, $H^{4'}$), 3.69 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 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 Hz, $H^{2'}$); 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 $NaHCO_3$ (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 CH_2Cl_2 (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 CH_2Cl_2 (100 mL) and water (100 mL), agitated and separated. The aqueous layer was re-extracted (CH_2Cl_2, 3 × 100 mL) and combined CH_2Cl_2 fractions were dried (Na_2SO_4) 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 CH_2Cl_2 (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 CH_2Cl_2 (135 mL) then washed with sat. aq KCl (80 mL). The aqueous fraction was re-extracted (CH_2Cl_2, 2 × 30 mL) and the combined organic fractions were dried (Na_2SO_4) and concentrated *in vacuo* to give a cloudy, yellow syrup. Following purification by column chromatography (CH_2Cl_2–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 (CH_2Cl_2–ethyl acetate, 75 : 25), 0.28 (CH_2Cl_2–ethyl acetate, 90 : 10); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 11.77 (1H, s, NH), 4.40 (2H, t, J = 6.3 Hz, H^3), 4.36 (2H, t, J = 6.0 Hz, $H^{3'}$), 2.78 (4H, t, J = 6.5 Hz, CH_2CN), 2.44 (3H, s, SCH_3); MS (ES^+) m/z: 307 ($[M + Na]^+$, 100), 285 ($[M + H]^+$, 12).

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*]pyrimidine-2(7*H*)-one (16). To a stirred solution of nucleoside **10** (2.07 g, 3.14 mmol) in distilled CH_2Cl_2 (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 CH_2Cl_2 (200 mL) was added, the solution was washed with sat. aq KCl (100 mL), dried (Na_2SO_4), and concentrated *in vacuo* and under high vacuum to give a pale yellow foam. Following purification by column chromatography (CH_2Cl_2–acetone, 50 : 50→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_f 0.37 (CH_2Cl_2–acetone, 20 : 80); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 11.57 (1H, s, CONH), 9.91 (1H, s, Aryl-NH), 8.52 (1H, s, H^4), 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, H^{10}), 7.04–6.99 (1H, m, H^{16}), 6.98 (2H, t, J = 9.0 Hz, H^{15}), 6.95 (1H, br d, J = 8.5 Hz, $H^{6''}$), 6.54 (2H, d, J = 9.0 Hz, H^{11}), 6.53 (2H, d, J = 9.0 Hz, H^{11}), 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, H^{24}), 4.00 (2H, br t, J = 5.8 Hz, H^{20}), 3.90 (1H, td, J = 3.0, 5.0 Hz, $H^{4'}$), 3.46 (3H, s, OCH_3), 3.45 (3H, s, OCH_3), 3.34 (3H, s, NCH_3), 3.26 (1H, dd, J = 3.0, 11.0 Hz, $H^{5'}$), 3.21 (1H, dd, J = 3.3, 10.8 Hz, $H^{5'}$), 2.59–2.52 (1H, m, $H^{2'}$), 2.53 (2H, br t, J = 6.0 Hz, H^{23}), 2.41 (2H, br t, J = 5.8 Hz, H^{19}), 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.

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

General procedure for phosphitylation of 6-aryl-*N*-methylpyrrolo-dC 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 (Na_2SO_4) and concentrated *in vacuo*. Purification by column chromatography using degassed solvents, on silica gel pre-equilibrated with Et_3N 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 CH_2Cl_2–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 CH_2Cl_2, after column chromatography (ethyl acetate–MeCN–Et_3N, 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); ^{31}P NMR (121 MHz, $CDCl_3$) δ (ppm) 150.3, 149.5 (P^{III}); MS (ES^+) m/z: 945 ($[M + Et_3N + H]^+$, 74), 866 ($[M + Na]^+$, 100), 844 ($[M + H]^+$, 71).

View Online

Downloaded by Institute of Organic Chemistry of the SB RAS on 22 December 2010
Published on 27 October 2010 on http://pubs.rsc.org | doi:10.1039/C0OB00119H

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(7*H*)-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 (CH_2Cl_2–acetone–Et_3N, 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 (CH_2Cl_2–acetone–Et_3N, 20 : 80 : 0.3); ^{31}P 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 $C_{50}H_{57}N_6O_8P$, $[M + Et_3N + H]^+$ 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)-(2,3*H*)-*N*-methylpyrrolo[2,3-*d*]pyrimidine-2(7*H*)-one-3′-*O*-(2-*O*-cyanoethyl-*N*,*N*-diisopropyl) phosphoramidite (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–Et_3N, 100 : 0 : 0.5→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–CH_2Cl_2–Et_3N, 90 : 10 : 0.3), 0.24, 0.31 (methanol–acetone–Et_3N, 5 : 95 : 0.3), 0.17 (acetone–Et_3N, 100 : 0.3); ^{31}P 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, 3*H*)-*N*-methylpyrrolo[2,3-*d*]pyrimidine-2(7*H*)-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 CH_2Cl_2, after column chromatography (CH_2Cl_2–acetone–Et_3N, 99 : 1 : 0.5→95 : 5 : 0.5), as a distereomeric mixture (*ca.* 2 : 1), as a pale yellow foam (0.17, 0.18 mmol, 70%). R_f 0.50, 0.57 (CH_2Cl_2–acetone–Et_3N, 50 : 50 : 0.3), 0.29, 0.34 (CH_2Cl_2–acetone–Et_3N, 80 : 20 : 0.3), 0.18, 0.25 (CH_2Cl_2–Et_3N, 100 : 0.3); ^{31}P NMR (121 MHz, DMSO-d_6) δ (ppm) 149.1, 148.9 (P^{III}); ^{19}F NMR (282 MHz, DMSO-d_6) δ (ppm) −73.5 (NHCOCF_3); 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 CH_2Cl_2, 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, (CH_2Cl_2–acetone, 20 : 80); ^{31}P NMR (121 MHz, $CDCl_3$) δ (ppm) 150.3, 149.4 (P^{III}); MS (ES^+) m/z: 1197 ($[M + Et_3N + H]^+$, 48), 1118 ($[M + Na]^+$, 58), 1096 ($[M + H]^+$, 100).

Acknowledgements

We gratefully acknowledge the BBSRC and The Ministry of Higher Education (Egypt) for funding, and ATDBio Ltd. for synthesis and purification of oligonucleotides.

Notes and references

1 G. Felsenfeld, D. R. Davies and A. Rich, *J. Am. Chem. Soc.*, 1957, **79**, 2023–2024.
2 (*a*) T. A. Winters, *Curr. Opin. Mol. Ther.*, 2000, **2**, 670–681; (*b*) D. Praseuth, A. L. Guieysse and C. Helene, *BBA-Gene Struct. Expr.*, 1999, **1489**, 181–206; (*c*) A. Majumdar, N. Puri, B. Cuenoud, F. Natt, P. Martin, A. Khorlin, N. Dyatkina, A. J. George, P. S. Miller and M. M. Seidman, *J. Biol. Chem.*, 2003, **278**, 11072–11077; (*d*) V. Karympalis, K. Kalopita, A. Zarros and H. Carageorgiou, *Biochemistry (Moscow)*, 2004, **69**, 855–860; (*e*) G. M. Carbone, S. Napoli, A. Valentini, F. Cavalli, D. K. Watson and C. V. Catapano, *Nucleic Acids Res.*, 2004, **32**, 4358–4367; (*f*) S. Buchini and C. J. Leumann, *Curr. Opin. Chem. Biol.*, 2003, **7**, 717–726.
3 (*a*) H. E. Moser and P. B. Dervan, *Science*, 1987, **238**, 645–650; (*b*) S. Antony, P. B. Arimondo, J. S. Sun and Y. Pommier, *Nucleic Acids Res.*, 2004, **32**, 5163–5173; (*c*) J. M. Woynarowski, *Adv. DNA Seq. Spec. Agents*, 2002, **4**, 1–27.
4 (*a*) M. M. Seidman and P. M. Glazer, *J. Clin. Invest.*, 2003, **112**, 487–494; (*b*) F. Guillonneau, A. L. Guieysse, S. Nocentini, C. Giovannangeli and D. Praseuth, *Nucleic Acids Res.*, 2004, **32**, 1143–1153; (*c*) J. M. Kalish, M. M. Seidman, D. L. Weeks and P. M. Glaze, *Nucleic Acids Res.*, 2005, **33**, 3492–3502.
5 (*a*) Z. Ma and J.-S. Taylor, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 11159–11163; (*b*) M. Bello-Roufai, T. Roulon and C. Escude, *Chem. Biol.*, 2004, **11**, 509–516.
6 S. Hildbrand, A. Blaser, S. P. Parel and C. J. Leumann, *J. Am. Chem. Soc.*, 1997, **119**, 5499–5511.
7 (*a*) K. R. Fox, *Curr. Med. Chem.*, 2000, **7**, 17–37; (*b*) D. M. Gowers and K. R. Fox, *Nucleic Acids Res.*, 1999, **27**, 1569–1577.
8 I. Prévot-Halter and C. J. Leumann, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 2657–2660.
9 (*a*) R. T. Ranasinghe, D. A. Rusling, V. E. C. Powers, K. R. Fox and T. Brown, *Chem. Commun.*, 2005, 2555–2557; (*b*) D. A. Rusling, V. E. C. Powers, R. T. Ranasinghe, Y. Wang, S. D. Osborne, T. Brown and K. R. Fox, *Nucleic Acids Res.*, 2005, **33**, 3025–3032.
10 (*a*) D. A. Berry, K.-Y. Jung, D. S. Wise, A. D. Sercel, W. H. Pearson, H. Mackie, J. B. Randolph and R. L. Somers, *Tetrahedron Lett.*, 2004, **45**, 2457–2461; (*b*) R. H. E. Hudson and A. Ghorbani-Choghamarani, *Synlett*, 2007, 870–873.
11 S. R. Gerrard, N. Srinivasan, K. R. Fox and T. Brown, *Nucleosides, Nucleotides Nucleic Acids*, 2007, **26**, 1363–1367.
12 P. Herdewijn, J. Balzarini, M. Baba, R. Pauwels, A. V. Aerschot, G. Janssen and E. D. Clercq, *J. Med. Chem.*, 1988, **31**, 2040–2048.
13 *Japan Pat.*, JP99-255499.
14 T. P. Prakash, A. Püschl and M. Manoharan, *Nucleosides, Nucleotides Nucleic Acids*, 2007, **26**, 149–159.
15 M. Manoharan, T. P. Prakash, I. Barber-Peoc'h, B. Bhat, G. Vasquez, B. S. Ross and P. D. Cook, *J. Org. Chem.*, 1999, **64**, 6468–6472.
16 R. A. J. Darby, M. Sollogoub, C. McKeen, L. Brown, A. Risitano, N. Brown, C. Barton, T. Brown and K. R. Fox, *Nucleic Acids Res.*, 2002, **30**, 39e.
17 H. E. Gottlieb, V. Kotlyar and A. Nudelman, *J. Org. Chem.*, 1997, **62**, 7512–7515.
18 B. Classon and B. Samuelsson, *Acta Chem. Scand., Ser. B*, 1985, **39b**, 501–504.
19 *USA Pat.*, US 4162265.
20 S. J. Vadhia, PhD, University of Southampton, 2007.
21 (*a*) T. P. Prakash, A. Puschl, E. Lesnik, V. Mohan, V. Tereshko, M. Egli and M. Manoharan, *Org. Lett.*, 2004, **6**, 1971–1974; (*b*) *USA Pat.*, US 20060160763.

 This journal is © The Royal Society of Chemistry 2010