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Insertion of 5-Methyl-N^4-(1-pyrenylmethyl)cytidine into DNA. Duplex, Three-way Junction and Triplex Stabilities

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Abstract: 5'-*O*-(4,4'-Dimethoxytrityl)-5-methyl-N^4-(1-pyrenylmethyl)cytidine (**5**) was prepared by reaction of 1-pyrenylmethylamine with an appropriate protected 4-(1,2,4-triazolyl)thymidine derivative which was synthesized from 5-*O*-DMT protected thymidine by acetylation with acetic anhydride and subsequent reaction with triethylamine, 1,2,4-triazole and $POCl_3$. Stabilities are reported for DNA duplexes, three-way junctions and triplexes when **5** is inserted. Most interestingly, the three-way junctions are stabilized when **5** is used for insertion into the junction region. This break-through for recognizing the foot of a stem could have far-reaching importance because new targets for antisense oligos is now rendered possible on intact secondary structures. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

RNA can adopt a large variety of secondary structures and antisense targeting of RNA requires consideration of the target structure. For example, for strand invasion of the HIV TAR element, one has to consider the thermodynamic penalty paid by disrupting the four base pairs of the stem between a six-base loop and a three-base bulge.[1] For this reason, the number of possible antisense targets is much lower than what is often believed. It would be possible to increase the number of antisense targets if the RNA stem junctions of internal loops could be recognized by an antisense oligodeoxynucleotide (ODN), e.g. by hybridizing to the foot of a stem across the stem without disrupting the secondary structure of the RNA. In this way one could think that targeting would be possible to the HIV RRE element which has been proposed to have a large internal loop with multi-stem junctions.[2] The group of Hélène[3] used a DNA model system to study the stability of complexes formed by a 17mer ODN with DNA fragments containing hairpin structures. The hairpin ODN forming the three-way junction exhibited only slightly stronger binding than an ODN with two parts of complementary sequences separated by a bulged sequence which could not form a hairpin. One, two or more unpaired nucleotides located in the three-way junction region did not result in any considerable stabilization of the duplex.[3-5] Formation of the three-way junction has been confirmed in NMR studies on complexes with two unpaired bases at the branch point.[6,7] The NMR work indicated a preferred base stacking interaction across the branch point. This observation has inspired us in this work to investigate the effect on the stability of the

three-way junction when an intercalating moiety is linked to an unpaired nucleobase in the junction region.

Decreased duplex stabilities have been determined from thermal melting studies of ODN's with an anthracene-9-ylmethyl group bound to the N^2 amino group of a 2'-deoxyguanosine residue.[8] The melting temperature increased 1 °C when 8,9-dihydro-9-hydroxyaflatoxin B_1 was attached to N^7 of a 2'-deoxyguanosine.[9] Extensive duplex studies have been reported with 4-aminobiphenyl and 2-aminofluorene bound with the amino group at the 8-position of a 2'-deoxyguanosine.[10,11] When the duplex contained a bulged guanine either unmodified or modified with the 2-aminofluorene, strong increases in the modified duplex melting temperatures were observed.[11] In structural studies of benzo[a]pyrene adducts to DNA,[12] increased stabilities were found when a bulged guanine had been modified at the N^2 amino group by reaction with 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene.[13,14] The NMR structural studies showed that the benzo[a]pyrene ring intercalates between the two base pairs around the bulge. Bishofberger and Matteucci[15] observed considerably increased stabilities when a nucleoside with a naphth[2',3':4,5]imidazo[1,2-f]pyrimidine base was inserted in one of the duplex strands, the stabilization being greatest when the extra base was present between the two terminal base pairs.

We now report about an easy synthesis of an 2'-deoxy-5-methyl-N^4-(1-pyrenylmethyl)cytidine derivative and how insertion of this compound into an ODN can stabilize a three-way junction when inserted into the junction region. Also we observed that insertion of this compound into a triplex forming ODN resulted in increased stabilises of the corresponding triplexes.

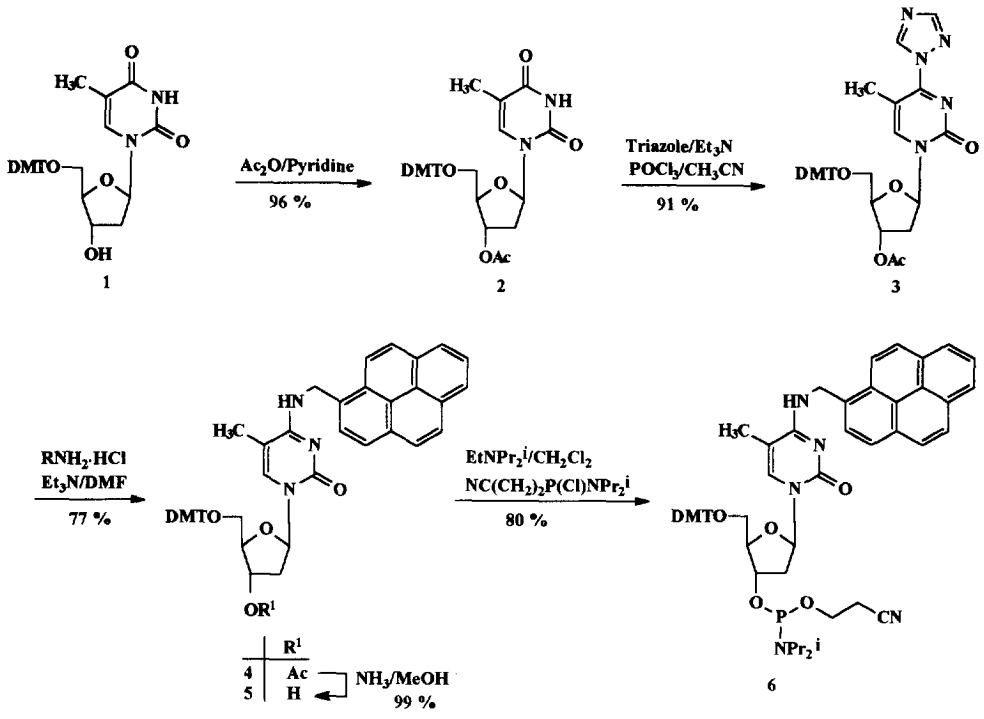
RESULTS

Nucleoside Synthesis

The protected nucleoside **2** was prepared in 96 % yield from 5'-*O*-(4,4'-dimethoxytrityl)thymidine. The 1-pyrenylmethylamino group was introduced in the 4-position of pyrimidine **2** via its corresponding triazolyl derivative. The protected thymidine **2** was treated with 1,2,4-triazole, triethylamine and phosphorus oxychloride in acetonitrile and the triazolyl derivative **3** was isolated by silica gel chromatography in 91 % yield. Commercial 1-pyrenemethylamine hydrochloride was allowed to react with **3** in the presence of triethylamine in DMF at 80 °C for 1h to afford **4** in 77 % yield. Deacetylation of compound **4** was carried out in saturated NH_3/MeOH at room temperature to give **5** in 99 % yield as a pale yellow foam.

DNA Synthesis and Thermal Denaturation Profiles

Compound **5** was reacted with 2-cyanoethyl-*N*,*N*-isopropylchlorophoshoramidite in the presence of *N*,*N*-diisopropylethylamine and CH_2Cl_2 to give the white phosphoramidite compound **6** in 80 % yield, and this compound was used for ODN synthesis. The purity of the phosphoramidite was 100 % according to ^{31}P NMR.



DMT = 4,4'-Dimethoxytrityl
R = 1-Pyrenylmethyl

Both unmodified and modified ODN's were synthesized on a Pharmacia Gene Assembler special DNA-synthesizer in 0.2 μmol-scale following standard phosphoramidite methodology[16] employing **6** and commercial 2'-deoxynucleoside β-cyanoethylphosphoramidites. The coupling efficiencies (2 min couplings) for the modified phosphoramidite **6** was approximately 60 % compared to approximately 99 % for commercial phosphoramidites (2 min couplings). The efficiency of each coupling step was monitored by release of the dimethoxytrityl cation after each coupling step. Removal from the solid support and deprotection was carried out at room temperature in 25 % ammonia at room temperature for four days. All ODN's were desalted using Pharmacia NAP-10 columns. The structural identity of the modified ODN's in Entry 4 and 6 (Table 1) was confirmed by matrix assisted laser desorption ionization (MALDI) mass spectrometry. The samples were prepared according to the fast evaporation surface technique[17] using Ferulic acid as matrix. The ODN's were observed in the MALDI mass spectra as singly charged sodium adducts. Good correlation was found between the expected and measured masses.

The ability of ODN's to hybridize to their complementry DNA strand and to form triplexes by hybridizing to duplexes was examined by UV melting measurements. The melting points T_m were determined as the maximum of the first derivative of the melting curve. DNA duplexes (Table 1) and DNA three-way junctions (Tabel 2 and 3) were formed from equimolar amounts in each strand at pH 7.0 in 2 mM EDTA, 20 mM Na_2HPO_4 and 280 mM NaCl. Two three-way junctions were investigated and **6** was used for insertion at five different positions around the junction. The triplexes (Table 4) were formed from equimolar amounts in each strand in a slightly acidic salt buffer pH 5.5 in 10 mM Na-acetate and 0.5 M NaCl.

DISCUSSION

We were looking for an easily available nucleoside which can increase duplex and triplex stabilities when inserted as a bulged nucleoside into the duplex or triplex forming ODN. We assumed that covalent bonding of an intercalated pyrene to the bulged nucleoside could result in such a stabilization even though a bulge normally should result in destabilization of the hybridized complexes. We found that 5-*O*-DMT protected 5-methyl-N^4-(1-pyrenylmethyl)cytidine could be synthesized in a high overall yield (49 %) from thymidine and could be used for the synthesis of the required ODN's on a DNA synthesizer after conversion into adequate phosphoramidite. 5-*O* DMT protected N^6-(1-pyrenylmethyl)adenosine,[18] previously synthesized and used in DNA synthesis, could also be a promising candidate for the present type of work, but has the disadvantage that a suitable starting material, such as 6-chloropurine 2-deoxyriboside, is not commercially available.

That intercalation contributed to duplex stabilization when **6** was incorporated as a bulge into an ODN, could be confirmed by characteristic observations similar to those previously reported. We observed a positional effect of inserted 5-methyl-N^4-(1-pyrenylmethyl)cytidine. As seen from Table 1, the highest stabilization occurred when the base was inserted between the two terminal base pairs into the sequence 5'-AT (compare Entry 2 and 6) or 5'-TA (compare Entry 4 and 7). This observation is parallel to the observation of Bischofberger and Matteucci[14] who inserted the naphth-[2',3':4,5]-imidazo-[1,2-f]-pyrimidine base and found stabilization being the greatest when the extra base was present between the two terminal base pairs. Also it should be mentioned that X-ray diffraction analysis revealed intercalation of daunomycin in the terminal CG base pair of the self-complementary ODN 5'-CGTACG.[19] The insertion of an extra base is believed to cause destabilizing geometric distortions to the double helix. From the same entries as above it is noticed that insertion of the natural base cytosine in the middle of a sequence causes a considerable destabilization, but not when inserted in the two terminal base pairs. Stabilization due to the base stacking properties of the extra pyrenylmethyl group is estimated by comparing the melting temperatures of the insertion of modified bases with those of cytosine insertions.

Table 1. Hybridization data (T_m °C) of ODN's. $\mathbf{X_0}$: With complementary DNA of same length without insertion. $\mathbf{X_1}$: With **6** used for insertion. **X=C**: Insertion of cytidine. $\mathbf{X_1}$ **(M-A)**: Mismatching nucleoside in the complementary strand around the inserted nucleoside. $\mathbf{X_0}$ **(M-A)**: No insertion, but only mismatching nucleoside in the complementary strand at the same position as in the previous column. $\mathbf{X_1}$-$\mathbf{X_0}$ and $\mathbf{X_1}$-**(X=C)**: Differences in T_m.

Entry	*ODN*	X_o	X_1	*X=C*	X_1 *(M-A)*	X_0 *(M-A)*	X_1-X_o	X_1-*(X=C)*
1	5'-$C_4T_3CX_nT_5A$	47.2	43.2	37.6	36.4 (A-A)	31.6 (A-A)	-4.0	5.6
2	5'-$C_4T_3AT_4AX_nT$	44.4	46.0	42.8			1.6	3.2
3	5'-$C_4T_4X_nGT_4A$	33.6	28.8	31.6			-4.8	-2.8
4	5'-$C_4T_3AT_5X_nA$[a]	44.0	51.6	43.0			7.6	8.6
5	5'-$C_4T_3AX_nT_5X_nA$	44.0	40.4	33.5			-3.6	6.4
6	5'-$C_4T_3AX_nT_5A$[b]	44.0	36.4	32.9	35.6 (G-A)	36.0 (G-A)	-7.6	4.5
7	5'-$C_4T_4X_nAT_4A$	42.4	41.6	33.4			-0.8	8.2
8	5'-$C_4T_4X_nT_5A$	45.0	39.6	32.0	34.8 (G-A)	35.6 (G-A)	-5.4	7.6

[a]MS of 5'-$C_4T_3AT_5X_1A$: Found *m/z* 4677.9 ($M+Na^+$). Calcd *m/z* 4679.3. [b]MS of 5'-$C_4T_3AX_1T_5A$: Found *m/z* 4677.8 ($M+Na^+$). Calcd *m/z* 4679.3.

From the X_1-(X=C) column in Table 1 it is now seen for both the 5'-AT sequence and the 5'-TA sequence that the positional effects of the base stacking is rather small. Clearly, the unfavourable steric contribution due to the bulge is the sole factor to contributing to the positional effect on duplex stabilities when the modified base is inserted into the ODN's. This unfavourable steric contribution is diminished when the extra base is located close to the end. For all sequence we observed the difference in melting temperatures in the range of -2.8 - 8.6 °C on going from the control with cytosine insertion to the pyrenylmethyl modified base [X_1-(X=C)]. These results are comparable to the 6 °C increase for the duplex melting temperature which has been reported on going from guanine insertion to insertion of the corresponding (+)-trans-anti-benzo[a]pyrene N^2-guanine adduct.[13] For the isomeric (+)-cis-anti-benzo[a]pyrene adduct the increase was

25 °C.[14] It is a reasonable supposition that a bulged duplex can be stabilized by a linked pyrene intercalator. There are two reports describing that bulges in double-strand DNA can be stabilized by intercalating drugs such as ethidium.[20,21]

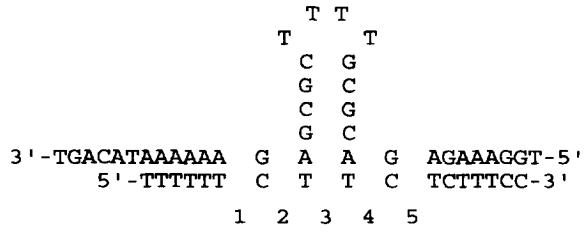
Table 2. Hairpin 1: Hybridization data (T_m °C) when hybridized at the foot with a complementary DNA which has been inserted at positions 1-5 using **6**.

```
                T T
              T     T
                C  G
                G  C
                C  G
                G  C
3'-TAGGGG  T  A  A  G  AAAAAAT-5'
  5'-CCCC  A  T  T  C  TTTTTT-3'
           1  2  3  4  5
```

Entry	*ODN*	*Insertion Position*	T_m	ΔT_m
9	5'-$C_4AT_2CT_6$		25.2	
10	5'-$C_4XAT_2CT_6$	1	25.6	0.4
11	5'-$C_4AXT_2CT_6$	2	28.8	3.6
12	5'-$C_4ATXTCT_6$	3	34.8	9.6
13	5'-$C_4AT_2XCT_6$	4	29.2	4.0
14	5'-$C_4AT_2CXT_6$	5	25.6	0.4
15	5'-$C_4XAT_2CXT_6$	1 and 5	22.6	-2.6
16	5'-$C_4AXT_2XCT_6$	2 and 4	29.2	4.0

Intercalation of the pyrene moiety is also supported by the sequence specificity of the inserted unnatural base. It has been reported that pyrene exhibit a distinct preference for intercalating within A-T sequences in double strand DNA.[22,23] From the X_1-(X=C) column in Table 1, Entry 4 and 7, we also observe a high stabilization due to the pyrene modification for the 5'-TA sequence. In fact, the stability is directional since the 5'-AT sequence gives a more moderate stabilization of the duplex (Entry 2 and 6).

Table 3. Hairpin 2: Hybridization data (T_m °C) when hybridized at the foot with a complementary DNA which has been inserted at positions 1-5 using **6**.



Entry	*ODN*	*Insertion Position*	T_m	ΔT_m
17	5'-$T_6CT_2CT_3C_2$		28.4	
18	5'-$T_6XCT_2CT_3C_2$	1	28.4	0.0
19	5'-$T_6CXT_2CT_3C_2$	2	32.8	4.4
20	5'-$T_6CTXTCT_3C_2$	3	34.8	6.4
21	5'-$T_6CT_2XCT_3C_2$	4	36.0	7.6
22	5'-$T_6CT_2CXT_3C_2$	5	28.4	0.0
23	5'-$T_6XCT_2CXT_3C_2$	1 and 5	22.0	-6.4
24	5'-$T_6CXT_2XCT_3C_2$	2 and 4	34.8	6.4

Whereas a mismatch has a dramatic effect on duplex stability, a much less pronounced effect was observed when a mismatch was introduced at the site of intercalation (Entry 1, 6 and 8 in Table 1). One could infer a considerable distortion of the duplex structure at the site of intercalation resulting in reduced hybridization of the neighbouring nucleosides.

For our investigation on recognition of hairpin structures in ODN's by oligodeoxy-nucleotides we selected a sequence (Hairpin 2, Table 3) which previously has been investigated[3] by spectroscopic measurements (melting curves) and chemical reactions (osmium tetroxide reaction, copper-phenanthroline cleavage). Therefore we are sure that our reference ODN can form a three-way junction. Also we used Hairpin 1 (Table 2) in order to evaluate sequence specificity. For both hairpins we observed a considerable stabilization (Table 2 and 3) when the pyrenylmethyl modified base was inserted in the targeting ODN at the middle of the

three-way junction or at the adjacent sites. Further away from the stem at insertion position 1 and 5, no stabilization was observed. The largest increase (9.6 °C) in melting temperature for Hairpin 1 was observed when the modified base was inserted into the middle of the three-way junction at position 3 whereas for Hairpin 2 the largest increase (7.6 °C) was on insertion adjacent to the stem. This positional effect may be related to coaxial stacking since a determining factor for this could be sequence of the base pairs immediately flanking the junction region.[24]

The stabilization due to insertion of the modified base into the three-way junction region is rather remarkable when compared with previous results for Hairpin 2. In this case only a 1.5 °C increase in melting temperature was observed when thymidine was inserted into the middle of the three-way junction region. The stabilization is also remarkable when compared with insertion of the modified base into the middle of double stranded DNA (Table 1). This means that inserted intercalating bases preferentially improve recognition of stem regions. Others have also targeted oligonucleotides against hairpin structures, but only antisense oligonucleotides targeted to the open regions of the loop had nearly equal affinity for the transcript compared to the complement.[25]

If it is a general feature that nucleobase linked to intercalators can recognize a junction region, there are many questions to be answered in molecular biology. Will the benzo[a]pyrene guanine adduct in DNA interfere with three-strand DNA junctions which are believed to play a role in certain recombination events[26]? Furthermore, since three-way junctions can serve as a model for interactions in four-way junctions, or Holiday junctions,[27] is it then possible that benzo[a]pyrene guanine adducts will influence Holiday junctions during meiotic recombination, a process which allows the cell to shuffle genetic material among homologous chromosomes[28]?

In recent years there has also been an interest in conjugation of DNA intercalators to triple helix forming ODN's in order to stabilize the triplex under physiological conditions. Nearly all publications in this area described intercalators such as acridines,[29a] oxazolopyridocarbazole[30] or psoralen[31] linked by a polymethylene linker to the 5'-end of the triplex forming ODN utilizing the 5' triplex-duplex junction as a strong binding site for the conjugated intercalator. When using absorption spectroscopy as a function of temperature at pH 5.5 we observed as expected two transions. The one at the higher temperature reflects the dissociation of the double-stranded targets while the one at lower temperature existed only in the presence of the triplex forming ODN (Table 4). At pH 5.0 we observed a higher triplex melting temperature which could not be determined exactly without curve analysis for the ODN's containing intercalating pyrene. On adding **6** at 5'-end, we observed at pH 5.5 an increased triplex melting of 6.4 °C which is considerably less than the one observed when a cytidine at the 5'-end of the triplex forming ODN is replaced with a 5-methylcytidine linked at N^4 to an appropriately substituted acridine.[29b] Unlike earlier reports,[32] we observed a considerable increase in the triplex stability on conjugation to an intercalator in the middle of the triplex forming ODN. Insertion of **6** in the middle of the strand afforded a 10.4 °C increase in the melting temperature when

compared with the wild strand. This increase in triplex stability is impressive when compared with a propanediol linked acridine in the middle of the triplex forming ODN which did not result in any significant increase of the triplex stability.[32a] It has been known for a long time[33] that the thermal dissociation (heating) curves of triplex helices are shifted toward higher temperatures with respect to the association (cooling) curves. This type of hysteresis is strongly dependent on pH and not present at lower pH. Like an earlier investigation[34] with no hysteresis at pH 5.8 we did not observe any appreciable hysteresis at pH 5.5, except when the nucleoside with the conjugated pyrene was inserted in the middle of the triplex forming ODN. In these cases we observed a hysteresis of 5 °C. If the nucleation-zipping model[34,35] is used for triplex formation one could imagine in the fast zipping up of the rest of the triplex after the unfavourable formation of the first base triplets, the zipping would be retarded because of the required reorganization of the duplex to allow intercalation in the middle of the duplex after formation of a minor number of base triplets.

Table 4. Hybridization data (T_m °C) of triplexes with insertions into the triplex forming ODN using **6** at pH 5.5.

Entry	*ODN*	T_m *(20→80 °C)*	T_m *(80→20 °C)*
25	5'-GACG$_4$A$_3$GA$_6$ 3'-CTGC$_4$T$_3$CT$_6$	60.4[a]	60.8[a]
26	5'-C$_4$T$_3$CT$_6$	31.2	30.0
27	5'-XC$_4$T$_3$CT$_6$	37.6	38.8
28	5'-C$_4$T$_2$XTCT$_6$	41.6	36.8
29	5'-XC$_4$T$_2$XTCT$_6$	35.6	29.6

[a]Duplex melting point.

CONCLUSION

What has emerged from the present study is that insertion of an 5-methyl-N^4-(1-pyrenylmethyl)cytidine within an oligonucleotide can stabilize duplex and triplex structures, and most interestingly, also a three-way junction when the insertion is made in the junction region. Such a stabilization of a three-way junction will have far-reaching importance because it is now rendered possible that the foot of a stem can be used as a target

for an antisense oligonucleotide. We anticipate this finding will start the interest of elucidating exact structures of three-way junctions with conjugated intercalators in order to design new intercalators and linkers to obtain further stabilization of the three-way junction.

EXPERIMENTAL

NMR spectra were recorded at 250 MHz for ^{1}H NMR, 62.9 MHz for ^{13}C NMR and 101.3 MHz for ^{31}P NMR on a Bruker AC-250FT spectrometer; δ-values are in ppm relative to tetramethylsilane as internal standard (^{1}H NMR and ^{13}C NMR), relative to 85 % H_3PO_4 as internal standard in ^{31}P NMR. Positive FAB mass spectra were recorded on a Kratos MS 50 RF spectrometer. Analytical silica gel TLC was performed on Merck precoated 60 F_{254} plates. The silica gel (0.040-0.063 mm) used for column chromatography was purchased from Merck. Matrix assisted laser desorption ionization mass (MALDI) mass spectra were obtained on a Bruker Reflex mass spectrometer. Melting experiments were carried out on a Perkin-Elmer UV/VIS spectrometer Lamda 2 fitted with a PTP-6 Peltier temperature programming element. The absorbance 260 nm was increased 1 °C/min in 1 cm cuvette. DNA syntheses were performed on a Pharmacia Gene Assembler Special® DNA-synthesizer. Complementary oligoribonucleotides were purchased from DNA Technology, Aarhus, Denmark.

3'-*O*-Acetyl-5'-*O*-(4,4'-dimethoxytrityl)thymidine (2)

To a stirred solution of **1** (5.98 g, 11 mmol) in dry pyridine was added acetic anhydride (2.13 mL, 22 mmol) at 0 °C. The reaction mixture was stirred for 4 h at 0 °C and 22 h at room temperature. The solvent was removed *in vacuo* and the resulting gum was coevaporated with dry toluene (2 x 20 mL). The product was purified by silica gel column using 0-1 % MeOH/$CHCl_3$ to obtain **2** as a white foam in 96 % yield. ^{1}H NMR ($CDCl_3$): δ 1.40 (s, 3H, CH_3), 2.07 (s, 3H, Ac), 2.45 (m, 2H, 2'-H), 3.46 (m, 2H, 5'-H), 3.78 (s, 6H, 2 x OCH_3), 4.13 (m, 1H, 4'-H), 5.44 (m, 1H, 3'-H), 6.43 (dd, 1H, J = 6.2 and 8.3 Hz, 1'-H), 6.84 (d, 4H, J = 8.8 Hz, arom), 7.21-7.33 (m, 7H, arom), 7.37-7.41 (m, 2H, arom), 7.61 (s, 1H, 6-H), 9.41 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 11.48 (CH_3), 20.83 (Ac), 37.83 (C-2'), 55.09 (2 x OMe), 63.54 (C-5'), 75.20 (C-3'), 83.88 (C-1'), 84.21 (C-4'), 87.03 (trityl), 111.52 (C-5), 113.17, 125.13, 127.06, 127.87, 127.99, 128.05, 128.87, 129.92, 135.14, 135.27, 144.07, 158.82 (arom), 135.05 (C-6), 150.47 (C-2), 163.80 (C-4), 170.31 ($COCH_3$). MS (FAB) ($CHCl_3$+3-nitrobenzylalcohol) *m/z*: 587 ($M+H^+$).

1-[3-*O*-Acetyl-2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-β-D-pentafuranosyl]-4-(1,2,4-triazol-1-yl)pyrimidin-2(1H)-one (3)

Triethylamine (6.48 g, 46 mmol) was added dropwise to a stirred cooled (ice-water bath) mixture of 1,2,4-

triazole (3.4 g, 48 mmol), $POCl_3$ (0.97 mL, 10 mmol) and MeCN (28 ml). A solution of **2** (2.93 g, 5 mmol) in MeCN (17 mL) was added and the reaction mixture was stirred at room temperature for 3 h. Triethylamine (4.5 ml, 32 mmol) and water (1.2 mL) were added. After 10 min, the solvent was evaporated in vacuo. The residue was partitioned between $CHCl_3$ (150 mL) and saturated aqueous $NaHCO_3$ (100 mL) and the phases were separated. The aqueous phase was extracted with $CHCl_3$ (100 mL). The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. The residue was chromatographed on silica gel column using 0-1 % CH_3OH/CH_2Cl_2 to give **3** (2.95 g, 91 %) as a pale orange foam. ^1H NMR ($CDCl_3$): δ 2.01 (s, 3H, CH_3), 2.09 (s, 3H, Ac), 2.40 (m, 1H, 2'-H), 2.87 (m, 1H, 2'-H), 3.47 (m, 2H, 5'-H), 3.77 (s, 6H, 2 x OCH_3), 4.28 (m, 1H, 4'-H), 5.44 (m, 1H, 3'-H), 6.40 (m, 1H, 1'-H), 6.81 (d, J = 8.7, 4H, arom), 7.26-7.4 (m, 9H, arom), 8.08 (s, 1H, 6-H), 8.31 (s, 1H, H-3, triazole), 9.28 (s, 1H, H-5, triazole). ^{13}C NMR ($CDCl_3$): δ 16.39 (CH_3), 20.81 (Ac), 39.46 (C-2'), 55.11 (2 x OMe), 63.28 (C-5'), 74.78 (C-3'), 84.91 (C-1'), 87.13 (C-4'), 87.28 (trityl), 105.93 (C-5), 113.23, 127.12, 127.92, 129.87, 135.08, 144.01, 144.94, 158.69 (arom), 135.01 (C-6), 153.30 (C-2), 146.33, 153.81 (triazole), 158.17 (C-4), 170.19 ($COCH_3$). MS (FAB) ($CHCl_3$+3-nitrobenzylalcohol) *m/z*: 638 ($M+H^+$).

1-[3-*O*-Acetyl-2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-β-D-pentofuranosyl]-4-(1-pyrenylmethylamino)pyrimidin-2(1H)-one (4)

A solution of **3** (1.27 g, 2 mmol) and 1-pyrenylmethylamine hydrochloride (1.07 g, 4 mmol) in triethylamine (10 mL) and DMF (20 mL) was stirred at 80 °C for 1 h. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel column using $CHCl_3$ to give **4** (616 mg, 77 %) as a yellow foam. ^1H NMR ($CDCl_3$): δ 1.32 (s, 3H, CH_3), 2.07 (s, 3H, Ac), 2.34 (m, 1H, 2'-H), 2.63 (m, 1H, 2'-H), 3.44 (m, 2H, 5'-H), 3.74 (s, 6H, 2 x OMe), 4.15 (m, 1H, 4'-H), 5.06 (m, 1H, 3'-H), 5.40 (d, 2H, J = 4.4 Hz, NH-CH_2), 6.60 (m, 1H, 1'-H), 6.82 (d, 4H, J = 8.8 Hz, arom), 7.15-7.40 (m, 9H, arom), 7.67 (s, 1H, 6-H), 7.96-8.26 (m, 9H, arom). ^{13}C NMR ($CDCl_3$): δ 12.17 (CH_3), 20.91 (Ac), 38.77 (C-2'), 43.75 (CH_2-NH), 55.09 (2 x OMe), 63.63 (C-5'), 75.29 (C-3'), 83.67 (C-1'), 85.48 (C-4'), 86.86 (trityl), 101.88 (C-5), 113.15 (trityl), 122.78-131.33 (Arom. C), 135.33 (C-6), 137.33, 144.27, 158.59 (trityl), 156.16 (C-2), 162.51 (C-4), 170.39 (CH_3CO). MS (FAB) ($CHCl_3$+3-nitrobenzylalcohol) *m/z*: 800 ($M+H^+$).

1-[2-Deoxy-5-*O*-(4,4'-dimethoxytrityl)-β-D-pentofuranosyl]-4-(1-pyrenylmethylamino)pyrimidin-2(1H)-one (5)

A solution of saturated ammonia in methanol (20 mL) was added to a stirred solution of **4** (470 mg) in methanol (20 mL) at 0 °C and stirred for 3 h at room temperature. The solvent was removed *in vacuo* and the residue chromatographed on silica gel column using 0-5 % MeOH in $CHCl_3$ to give **5** (440 mg, 99 %) as a pale yellow foam. ^1H NMR ($CDCl_3$): δ 1.33 (s, 3H, CH_3), 2.85 (m, 1H, 2'-H), 2.73 (m, 1H, 2-H'), 3.45 (m, 2H, 5'-H), 3.69 (s, 6H, 2 x OMe), 4.00 (m, 1H, 3'-H), 4.18 (m, 1H, 4'-H), 4.62 (br. s, 1H, OH), 5.14 (m, 1H, NH), 5.35 (d, 2H, J = 4.4, CH_2NH), 6.56 (m, 1H, 1'-H), 6.79 (d, 4H, J = 8.7, arom), 7.14-7.41 (m, 9H, arom),

7.72 (s, 1H, 6-H), 7.93-8.22 (m, 9H, arom). ^{13}C NMR ($CDCl_3$): δ 12.23 (CH_3), 42.05 (C-2'), 43.6 (CH_2NH), 55.02 (2 x OMe), 63.55 (C-5'), 72.00 (C-3'), 85.89, 85.96 (C-1', C-4'), 86.52 (trityl), 101.74 (C-5), 113.06 (trityl), 122.77-131.18 (arom), 135.48 (C-6), 135.53, 137.61, 144.42, 158.42 (trityl), 156.40 (C-2), 162.54 (C-4). MS (FAB) ($CHCl_3$+3-nitrobenzylalcohol) *m/z*: 758 ($M+H^+$).

Phosphoramidite 6

Compound **5** (320 mg, 0.42 mmol) was coevaporated with dry MeCN and dissolved in a mixture of *N,N*-diisopropylethylamine (0.4 mL) and dry CH_2Cl_2 (1.2 mL). 2-Cynoethyl-*N,N*-diisopropylchlorophosphoramidite (0.17 mL, 0.76 mmol) was added dropwise and the reaction was stirred at room temperature for 2 h. The reaction mixture was quenched by addition of CH_3OH (0.95 mL). Ethylacetate (15 mL) was added to the reaction mixture followed dy washing with $NaHCO_3$ (3 x 15 mL). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The product was purified by silica gel column chromatography ($EtOAc/CH_2Cl_2/Et_3N$ 45:45:10). The resulting gum was dissolved in 5 mL of toluene. This solution was added dropwise to cold pet. ether (200 mL) with stirring. The solid product was filtered off to give a white compound (282 mg, 80 %). ^{31}P NMR ($CDCl_3$): δ 149.27 and 149.84.

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