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Triplex Stability of Oligodeoxynucleotides Containing Substituted quinazoline-2,4-(1*H*,3*H*)-dione.

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Abstract : Triple helical structures can be observed between double-stranded nucleic acids and a third strand through the formation of Hoogsteen hydrogen bond. We report here the use of quinazoline-2,4-dione derivatives as substitutes for thymine in TA*T triplets. The synthesis and the characterization of monochloro derivatives of quinazoline-2,4-dione as well as 5-fluoro and 6-nitro substituted quinazoline rings are described. The ability of the various modified bases to promote the formation of triplexes was reached by thermal denaturation studies. © 1997 Elsevier Science Ltd.

INTRODUCTION

The antisense strategy is based on the inhibition of gene expression resulting from the complementary hybridization of synthetic oligonucleotides to single-stranded RNA targets. The efficiency of the method is linked to the high specificity of the interaction between complementary nucleic acid sequences. The expression of numerous genes has been down-regulated in this way either in cell-free extract, in cultured cells or *in vivo* (for a review see [1]). Efficient inhibition of gene expression by synthetic oligonucleotides, has been reached by chemically modified oligomers which may exhibit, for instance, better nuclease resistance, improved uptake by cells or increased affinity for the targeted sequence [2]. The availability of the target for antisense hybridization has also to be taken into account. Cellular proteins may be bound to mRNA sites [3] or RNA folded back into secondary structures. Indeed, although mRNAs are often written as single chain nucleic acids, most of the time secondary or tertiary structures giving rise to various motifs (hairpins, bulges, pseudo-knots) are observed [4]. Such structures are formed through intramolecular pairing of complementary sequences scattered along the RNA strand. Therefore the antisense oligomer has to compete with intramolecular hybridization. To circumvent this difficulty an alternative strategy was developed where the antisense oligonucleotide is designed to interfere directly with a double-stranded region of mRNA via the formation of a local triple helix [5].

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Simultaneous targeting of a single-stranded and a double-stranded region of a hairpin by a unique antisense oligonucleotide sequence was able to impair *in vitro* translation (Le Tinévez and Toulmé, unpublished results).

Triple helix formation obeys structural features that do not accomodate every double-stranded sequence. The specificity in triplex formation is derived from third strand Hoogsteen hydrogen bonding by which thymine recognizes the TA base pair (TA*T triad) and protonated cytosine a CG base pair (CG*C+ triad) in the pyrimidine motif where the third strand is parallel to the purine strand of the Watson-Crick duplex (in this notation the third strand is written in the last position and * indicates Hoogsteen interaction type). Adenine recognition of TA base pair (TA*A triad) and guanine recognition of CG base pair (CG*G triad) occurs by reverse Hoogsteen mode in the purine motif, the purine third strand being antiparallel to the purine strand of the duplex. A common feature of these well established motifs is the necessity of a purine (A or G) in the central position of triplex triad, since only these provide two sets of hydrogen bonds (donors or acceptors) in the major groove of the double helix. Therefore triplex formation requires the occurence of homopurine stretches in double-stranded regions (for a review see [6,7]).

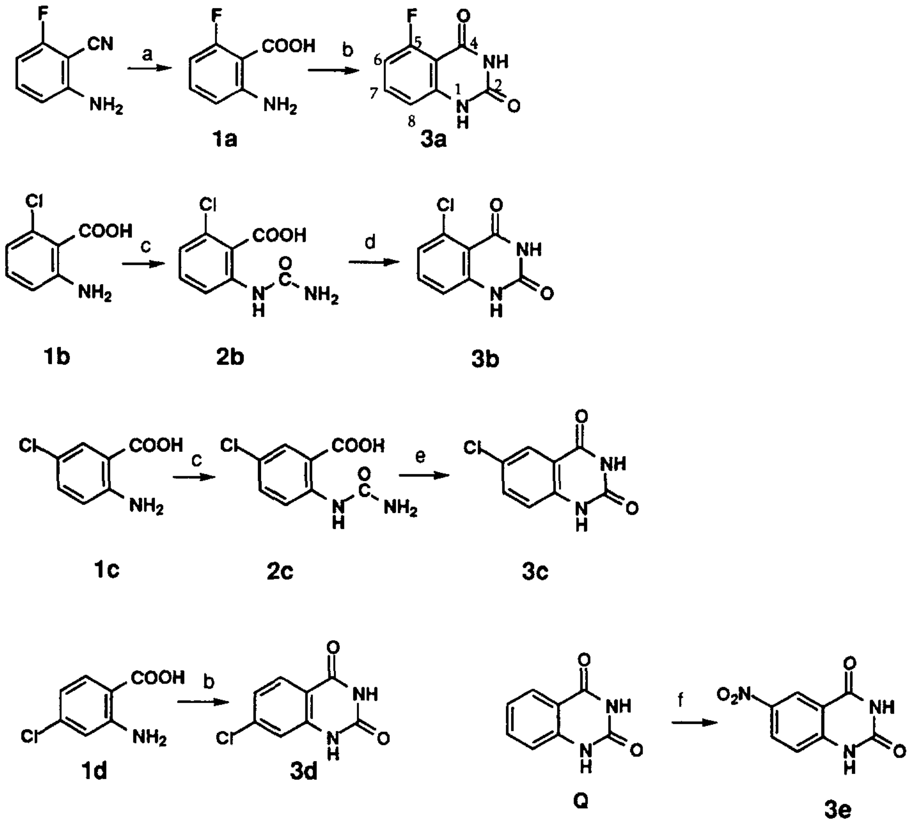
Strategies -such as the one used in double hairpin complexes [5]- which imply the formation of Watson Crick base pairs with the single-stranded part of the target, together with the formation of triplets for the double-stranded stem of the structured mRNA, would ideally require specific bases for single strand and double strand recognition in order to increase the overall specificity of the antisense oligonucleotide. In an attempt to design specific bases for triple helical structure formation, we explored the ability of extended aromatic domain of bases in forming triplexes. Stacking interactions between planar heterocyclic ring of nucleic acids contribute importantly to the stabilization of DNA and RNA duplexes [8]. Such interactions might also be of great importance for triplex formation. Increasing stacking interactions through chemical modification of nucleic acid bases may provide a mean to get more stable triplexes. As a first approach along this line we used a quinazoline nucleus (quinazoline-2,4(1*H*,3*H*)-dione : Q) as a substitute for thymine in DNA strands [9]. Previous results showed that duplexes are clearly destabilized by the substitution of a thymine by a quinazoline nucleus, whereas triplexes are less destabilized. We chose therefore to introduce various substituents on the quinazoline nucleus aiming at further increase in stacking interactions through dipolar contribution to Van der Waals forces and the extension of interacting domains. We report here the chemical synthesis of fluoro, chloro and nitro derivatives of quinazolines as well as their evaluation for preferential triple helix formation by thermal denaturation experiments.

RESULTS AND DISCUSSION

A - CHEMICAL SYNTHESIS

We chose to add substituents on the quinazoline nucleus at position 5, 6 or 7 which may be the most suitable area for overlapping of adjacent triplets [10]. The incorporation of the various quinazoline compounds as substitutes for thymine in antisense oligonucleotides was reached through the synthesis of their phosphoramidite derivatives and their use in automatic solid phase synthesizer. The general scheme of the synthesis includes, the glycosylation of the silylated substituted quinazoline-2,4-dione with 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-pentofuranosyl chloride, the purification and characterization of the resulting anomeric compounds, and the conversion of the β nucleoside isomers into the suitable phosphoramidite reagents.

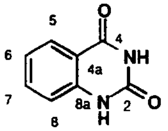
Synthesis of the quinazoline-2,4-dione derivatives. The chlorine atome was introduced at either 5,6 or 7 position (Scheme 1, compounds **3b,c,d**) and we chose to examine the influence of the variations in dipolar momentum by using fluoro and nitro substituents at position 5 and 6 respectively (**3a,e**). The synthetic schemes for the various quinazolines nucleus are presented in Scheme 1 and adapted from a previously published procedure [11].



Reagents and conditions : a) OH^-; b) Urea, H_2O 100°C; c) Cyanic acid; d) TFA; e) CH_3COOH; f)HNO_3, H_2SO_4 100°C

Scheme 1 : Synthesis of pyrimidine heterocycles

Condensation of commercially available substituted 2-aminobenzoic acids with urea or cyanic acid allowed us to reach the desired pyrimidine-2,4-dione moities. The 6-nitroquinazoline-2,4-dione **3e** was synthesized by direct nitration of quinazoline-2,4-dione (**Q**) according to Bogert *et al*[12]. The compounds were generally isolated by precipitation and crystallisation. When necessary, crude synthetic materials were purified by flash chromatography. Structural determinations were reached mainly by ^{1}H NMR and ^{13}C NMR spectroscopies (Table 1). Assignments reported in Table 1 arise from comparison with parent compounds and previously published data [9]. Multiplicity of ^{13}C signals were checked by DEPT experiments.



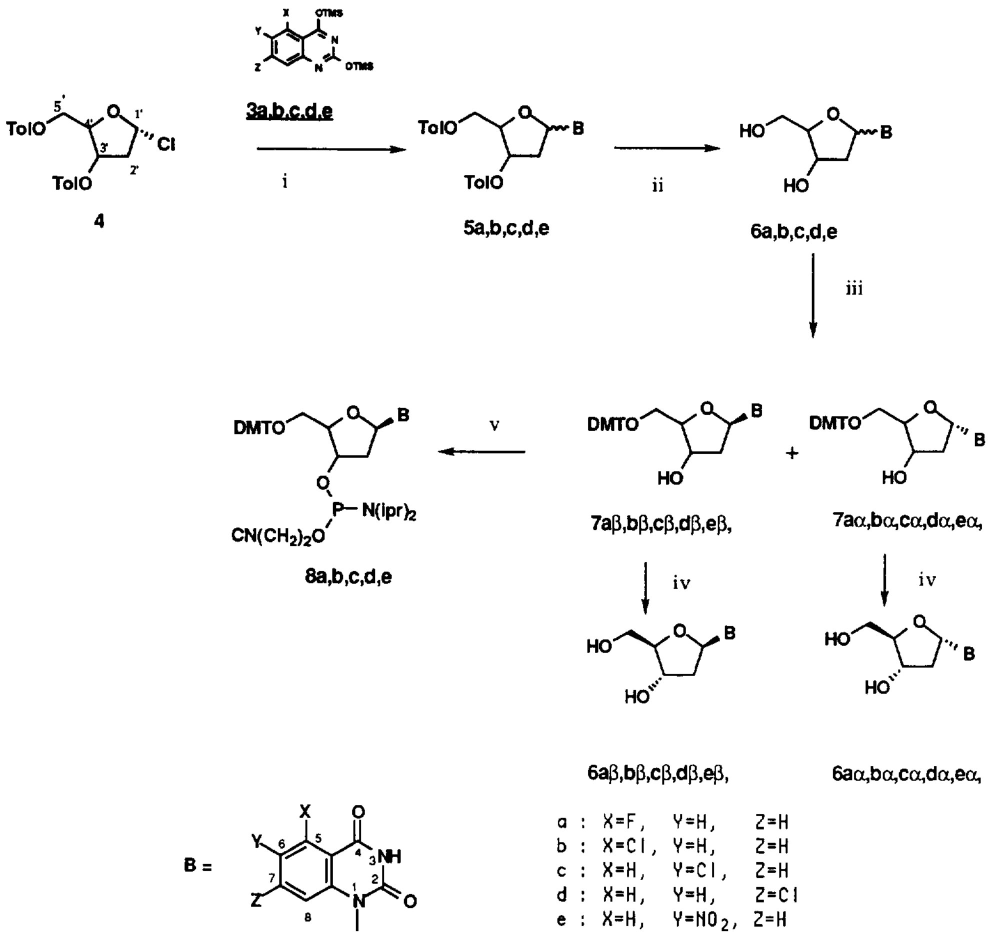
Numbering of the quinazoline nucleus

Compd.	C-2	C-4	C-4a	C-5	C-6	C-7	C-8	C-8a
Q	151.3	163.9	115.3	127.9	123.3	135.9	116.3	141.8
3a	150.1	160.1	104.0	161.4	109.0	135.6	111.6	143.0
3b	149.3	160.4	111.2	133.6	114.4	134.2	124.7	143.3
3c	150.0	161.8	115.7	125.9	126.2	134.8	117.5	139.7
3d	149.8	161.5	114.4	123.0	145.5	129.4	116.6	141.7
3e	150.3	162.1	113.3	122.0*	128.0*	142.1	114.7	139.2

* Assignments may be reversed.

Table 1 : Carbon NMR data of the quinazoline compounds

Chemical synthesis of nucleoside derivatives of quinazolines. Our general route to the phosphoramidite derivatives is shown in scheme 2. It was established in our previous work on quinazoline-2,4(1*H*,3*H*)-dione [9]. We used a direct glycosylation of silylated quinazoline-2,4-dione with 2-deoxyribofuranoside chloride via CuI catalyst in dry chloroform which favor the β orientation of the base. [13]. In the case of 7-chloroquinazoline, the best yield was obtained with anhydrous CH_3CN as solvent. The unresolvable anomeric mixtures of ditoluoyl nucleosides were isolated through flash chromatography over silica gel column. Removal of the toluoyl groups with sodium hydroxide in methanol gave excellent yields for the corresponding nucleosides (**6a, b, c, d, e**). The free nucleosides were then converted to the corresponding 5'-O-(4,4'-dimethoxytrityl) derivatives by treatment with 4,4'-dimethoxytritylchloride (DMTrCl) in anhydrous pyridine. The various anomeric mixtures were resolved at this step. The β anomers (slow moving species) were readily isolated by silica gel chromatography (relative yields range from 27 to 46 %) . These yields were lower than the one we got for quinazoline-2,4-dione (55%) [9].



a : X=F, Y=H, Z=H
b : X=Cl, Y=H, Z=H
c : X=H, Y=Cl, Z=H
d : X=H, Y=H, Z=Cl
e : X=H, Y=NO_2, Z=H

Reagents and conditions : i)CuI, dry $CHCl_3$ (CH_3CN for 5d) ; ii) CH_3OH, OH^- ; iii) DMTr,Pyr ; iv) CH_3COOH, H_2O ; v) 2-cyanoethyl-N,N-diisopropylchlorophosphite, N,N-diisopropylamine.

Scheme 2 : Synthesis of the various nucleosides and their corresponding phosphoramidites.

The β configurations were further demonstrated on detritylated compounds (**6aα,β** to **6eα,β**) at the exception of **6bβ** (unsufficient amount of **7bβ**). The full assignments of ^1H and ^{13}C NMR spectra of each anomeric compounds (α and β) **6a** to **6e** were reached by ^1H-^1H and ^1H-^{13}C correlation methods (COSY, HMQC [14], HMBC [15]) (data not shown). We have previously established the spectroscopic properties of α and β anomer of quinazoline-2,4-dione [9], and comparison with these data allowed us to unambiguously reach the configuration of all the new compounds studied here.

Compd	H-1'	H-2',2"	H-3'	H-4'	H-5'
Q β	6.62 (t) 7.8 Hz	1.90(m) 2.61(m)	4.35(m)	3.67(m)	3.59(m)
6aβ	6.60 (t)	1.90(m) 2.45(m)	4.40(m)	3.65(m)	3.65(m)
6bβ	nd	nd	nd	nd	nd
6cβ	6.65 (t) 7.8 Hz	1.91(m) 2.58(m)	4.37(m)	3.67(m)	3.67(m)
6dβ	6.60 (t) 7.8 Hz	1.91(m) 2.50(m)	4.40(m)	3.66(m)	3.66(m)
6eβ	6.70 (t) 7.8 Hz	1.90(m) 2.60(m)	4.40(m)	3.70(m)	3.60(m)

Compd	H-1'	H-2',2"	H-3'	H-4'	H-5'
Q α	6.68(d,d) 8.6; 7.8 Hz	2.39(m) 2.51(m)	4.35(m)	4.09(m)	3.53(m)
6aα	6.63(d,d) 8.5; 8.0 Hz	2.41(m)	4.35(m)	4.08(m)	3.51(m)
6bα	6.63(t) 8.5 Hz	2.40(m)	4.35(m)	4.10(m)	3.55(m)
6cα	6.66 (t) 8.0Hz	2.31(m) 2.45(m)	4.35 (q) 6.0Hz	4.08(q) 4.0Hz	3.53(d,d) 12.0; 5Hz
6dα	6.70(t)	2.20(m) 2.52(m)	4.38(m)	4;08(q) 4.0Hz	3.53(m)

Table 2 : Proton NMR data of the quinazoline nucleosides (nd = non determined)

Data in Table 2 reveal that H2',2" and H4' chemical shifts readily distinguish β from α anomers. Nevertheless, these assignments were further confirmed by nuclear Overhauser effects between proton H1' and H4' for β anomers and H1'-H3' for α anomers. A typical ROESY correlation map is given in Fig.1.

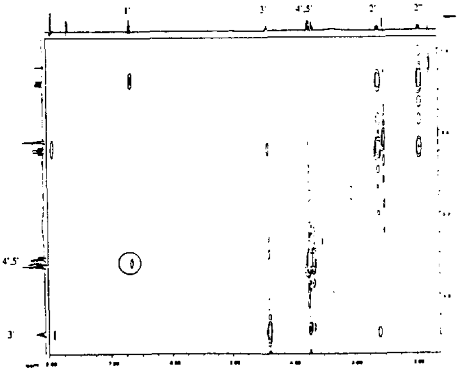


Figure 1 : Part of the 2-D ROESY correlation map of the β anomer of 6c (mixing time 450 ms). The open circle shows the correlation spot for H-1', H-4' as expected for the β configuration. No correlation is detected at H-1', H3' position.

These assignments of configuration at the anomeric carbon as α or β have often been based on the splitting pattern of H1' [16] : a pseudotriplet is observed for β 2'deoxyribonucleotides and a doublet of doublets is seen for α nucleosides. Nevertheless deviations from these simple rules have already been observed [17,18], which rely on changes in conformational equilibria and solvent dependence of coupling constants. Detection of dipolar relaxation between nearby protons through NOESY or ROESY 2D NMR experiments is therefore the most reliable method [18]. The chemical shift pattern observed for H2',2" and H4' protons in the quinazoline series must not be used as a general rule and may be correlated with syn-anti conformation of the base [9].

The β anomers were then converted to phosphoramidite derivatives using conventional phosphitylation reaction with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite, in dichloromethane in the presence of *N,N*-diisopropylethylamine. Phosphoramidites were isolated as white foams after two purification steps by silica gel flash chromatography using ethyl acetate/dichloromethane/triethylamine (45:45:10, v/v/v) as elution solvent. The yields obtained ranged from 23 to 81%.

Oligonucleotides synthesis. By using the phosphoramidites **8a,b,c,d,e** along with the commercially available A, T, G, C phosphoramidites, one or two modified bases were incorporated into oligodeoxynucleotides. For the modified phosphoramidites the coupling time was increased up to 15 minutes and the coupling yields were comparable to those of the unmodified ones (>98%).

B - BINDING PROPERTIES OF MODIFIED OLIGOMERS

The ability of modified quinazoline-containing oligomers to elicit the formation of duplexes or triplexes was evaluated by thermal denaturation studies. Single-stranded and double-stranded targets were the same as the ones we used for the evaluation of quinazoline-2,4 dione. Triplexes were designed in order to allow easy analysis of the effect of single or multiple substitutions of thymine by a modified quinazoline base within purine or pyrimidine motifs. In all cases the third strand was bridged by a pentanucleotide loop to one strand of the double-stranded target, leading to bimolecular complexes allowing fast kinetics for triplex formation. The thermal denaturation of such complexes generally occurs in a single transition from triple to random coil, making Tm determination more accurate. In order to compare thermal denaturation data, duplexes and triplexes were formed using a sodium cacodylate buffer (pH 7) which contained spermine as unique polycationic species.

1 - Single-stranded target. We first evaluated the ability of quinazoline derivatives to bind to a single-stranded DNA target. Results showed (Table 3) that the substitution of one T by the modified bases (duplexes D2 to D7) led to a significant decrease in Tm, compared with the unmodified duplex D1. Introduction of dipolar momentum on the quinazoline nucleus led to a slightly less destabilizing contribution than the one we observed for the parent quinazoline (D2), with the noticeable exception of the 5-chloro-quinazoline (D4) which largely destabilised double-stranded structures.

```
5'  A  C  A  A  C  A  C  A  C  C  A  C
    I  I  I  I  I  I  I  I  I  I  I  I
3'  T  G  T  T  G  B  G  T  G  G  T  G
```

Complexes	Sequences, B =	ΔTm (Tm) °C
D1	T	0.0 (56.0)
D2	Q	-5.0
D3	**3a**	-2.5
D4	**3b**	-8.0
D5	**3c**	-3.5
D6	**3d**	-4.0
D7	**3e**	nd
D8	Propynyl-U	+2.5

Table 3 : Oligonucleotides sequences. Vertical bars indicate Watson-Crick base-pairing. D1 to D8 are the studied duplexes (B = T, Q etc...) Tm = melting temperature (± 0.5°C). ΔTm = Tm from reference complex - Tm from modified duplex. nd = non determined.

2 - Double-stranded targets.

*PyPu*Py triplexes.*

The recognition of TA base pairs by modified quinazolines (B) resulting in the formation of TA*B triplets, by substitution of one or two Ts were evaluated from Tm determination for triplexes T1 to T15 (Table 4). The melting curves of these complexes showed a single transition from bound to dissociated structure (not shown).

```
   C   C  T  T  T  T   T   T  T  T  T   T  5'
A      I  I  I  I  I   I   I  I  I  I   I
C      G  A  A  A  A   A   A  A  A  A   A  3'
A      •  •  •  •  •   •   •  •  •  •   •
   C   C  T  T  T  B1  B2  T  T  T  B3  T  3'
```

Complexes	Sequences			ΔTm (Tm) (°C)
	B1	B2	B3	
T1	T	T	T	0.0 (41.5)
T2	Q	T	T	-2.5
T3	Q	Q	T	-3.5
T4	Q	T	Q	-4.5
T5	**3a**	T	T	-3.0
T6	**3b**	T	T	-4.0
T7	**3b**	**3b**	T	-7.0
T8	**3b**	T	**3b**	-11.0
T9	**3c**	T	T	-1.0
T10	**3c**	**3c**	T	-3.0
T11	**3c**	T	**3c**	-3.5

T12	3d	T	T	-1.5
T13	3d	3d	T	-1.5
T14	3e	T	T	-4.0
T15	Propynyl-U	T	T	-1.5

Complex	Sequence	Tm (°C)
D9	5' C T T T T T T T T T T I I I I I I I I I I I 3' G A A A A A A A A A A	33.0

Table 4 : Oligonucleotide sequences. Vertical bars and dots indicate Watson-Crick and Hoogsteen hydrogen bonding respectively. T1 to T15 are the studied triplexes ; D9 is the reference duplex. Tm = melting temperature ΔTm = Tm from reference complex - Tm from modified duplex or triplex. Tm were obtained under the conditions indicated in the experimental part.

Triplex formation was revealed by comparison of Tm data from corresponding double strand (Table 3, D9, 33°C) and complex T1 (41.5°C). Single or double substitutions of T by the various quinazoline derivatives led to a decrease in the Tm value. The slightest destabilizations were observed with 6-Cl and 7-Cl derivatives (T9-T11 and T12, T13). Double substitutions did not further destabilize the complexes with 7-Cl quinazoline nucleus since a ΔTm of -1.5°C were observed for T12 and T13. Comparison with previously published data on quinazoline (T1-T4) suggests that the stacking ability of aromatic cycles were improved in these last cases. In contrast the 5-chloro substituent clearly destabilized the third strand since double substitution led to a Tm of 30.5°C (T8 : ΔTm, -11°C) which is less than the Tm of the corresponding double strand (D9 : 33°C). These data can also be compared with a substitution of T by G (36°C) [9] which is the least stable base triplet in this context [19]. Fluoro and nitro compounds led also to noticeable destabilization and were not studied further.

*PyPu*Pu triplexes*

Similar experiments were conducted within the purine motif series. Complexes T16 to T21(Table 5) were designed to form CG*G and TA*T triplets, the Hoogsteen third strand exhibiting an antiparallel orientation relative to the purine strand. Triplex formation is revealed by a 8°C increase in Tm from double strand (D10) to triplex (T16).

```
   5' C T C C T C C C T C C T 3'
      I I I I I I I I I I I I
A  C  G A G G A G G G A G G A 5'
C     • • • • • • • • • • • •
A  C  G T G G B G G G T G G T 3'
```

Complexes	Sequences B=	ΔTm (Tm) (°C)
T16	T	0.0 (62.0)
T17	Q	-1.5
T18	**3a**	(40, 58)
T19	**3b**	(46)
T20	**3c**	-2.5
T21	6NO2-Q	(35, 60)

Complex	Sequence	Tm (°C)
D10	5' C T C C T C C C T C C T I I I I I I I I I I I I 3' G A G G A G G G A G G A	54.0

Table 5 : Oligonucleotide sequences. Vertical bars and dots indicate Watson-Crick and Hoogsteen hydrogen bonding respectively. T16 to T21 are the studied triplexes ; D10 is a reference duplex. Tm = melting temperature. ΔTm = Tm from reference complex - Tm from modified duplex or triplex. Tm were obtained under the conditions indicated in the experimental part.

Introduction of modified quinazolines in this antiparallel context led to separated transitions for the thermal denaturation of triplexes to duplexes and duplexes to single strands along with low hyperchromicity indicating a clear destabilization of the structures. The only exception was the 6-chloro quinazoline derivative which exhibited a ΔTm of -2.5°C (T20), however less favorable than the one we got with quinazoline in a previous study (-1.5°C, T17) [9]. We therefore did not introduce further substitutions in this context.

Whatever the context of the modified base, we did not observe any stabilization of triple stranded-structures. Few pyrimidines derivatives are able to enhance the stability of triplexes. C-5-(1-propynyl)-2'-deoxyuridine when incorporated into oligonucleotides was shown to enhance cooperative binding of adjacent third strands on DNA targets [10]. The effect of 5-propyne substitution on stability of triple helices was also determined by thermal denaturation experiments, and an increase in Tm of 2.4°C for each substitution was observed [20]. When we incorporated such a modification in triplex T15 we unexpectedly observed a 1.5°C decrease in Tm. However we observed an increase of 2.5°C for its introduction in duplex D8 (Table 3), in good agreement with the known properties of C-5 propyne analogues of 2'-deoxyuridine and 2'-deoxycytidine [21,22]. It has been proposed that a significant contribution to the triple helix stabilization of C-5 propyne derivatives arises from stacking interactions within the third strand [10]. Our results suggest that the base stacking configuration of TA*T triplets in triplexes T1 to T15 largely differs from the model proposed by Raghunathan *et al.* [23], and do not allow an efficient overlap of aromatic rings between consecutive triplets. They also suggest that the Hoogsteen third strand conformation does not significantly differ when a T is substituted by a propynyl derivative or a quinazoline ring, since we observed similar contributions to triplex stability.

CONCLUSION

Our goal was to design specific bases for triple helix formation through the extension of the heterocyclic rings. Preferential binding for double strand was simply evaluated through the analysis of ΔTm values for duplexes and triplexes (Table 6). Positive values for ΔΔTm (ΔΔTm= ΔTm (triplex)-ΔTm(duplex)) are indicative for preferential binding for double strands, on the opposite, negative values indicate a preferential binding to single strand.

Modified bases **B** =	T	Q	5FQ	5ClQ	6ClQ	7ClQ	Propy nyl-U
5' A C A A C A C A C C A C 3' I I I I I I I I I I I I 3' T G T T G **B** G T G G T G 5'	Tm 56	ΔTm - 5	ΔTm -2.5	ΔTm - 8	ΔTm - 3.5	ΔTm -4	ΔTm +2.5
C C T T T T T T T T T T 5' A I I I I I I I I I I I C G A A A A A A A A A A 3' A • • • • • • • • • • • C C T T T **B** T T T T T T 3'	Tm 41.5	ΔTm - 2.5	ΔTm - 3	ΔTm - 4	ΔTm -1	ΔTm - 1.5	ΔTm -1.5
Preferential binding for double strand : ΔΔTm= ΔTm (triplex)-ΔTm(duplex) *		2.5	-0.5	4	2.5	2.5	-4

Table 6 : ΔTm comparisons from duplexes and triplexes

As shown in Table 6, large variations were observed for ΔΔTm (-4 to +4 °C). Although we observed positive values for ΔΔTm, this only reveals in our case that triplexes are less destabilized than duplexes. Substitution in position 6 or 7 of the quinazoline ring do not allow to increase selectivity for triple strand formation when compared to quinazoline. The substitution in position 5 is dependent on the nature of the halogen and can lead to opposite effects : preferential formation of duplex (fluorine) or triplex (chlorine). However in this last case, the increase in selectivity is associated with a noticeable destabilization of both structures. This analysis outlines also that the substitution of thymidine by 5-(1-propynyl)-2'-deoxyuridine in antisense oligonucleotides targeted simultaneously to single-stranded and double-stranded region would lead to a loss of selectivity particularly when long sequences are used.

We supposed that Van der Waals contact between consecutive triplets are weak and arise mainly from poor overlap of aromatic rings. We are now investigating the properties of polycyclic aromatic quinazoline derivatives which may allow more efficient overlap.

EXPERIMENTAL

General

Thin layer chromatography was performed on Merck silica gel 60F254 aluminium backed-plates. Flash chromatography was carried out on Merck silica gel 60. Melting point were determined on Köfler bench. The IR and UV spectra were recorded on a Brucker IFS-25 and Kontron Uvikon 940. Mass spectra were recorded on a VG Autospec spectrometer. NMR measurements : ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a BRUCKER AC200 (except NOE, HMQC and HMBC experiments on nucleosides recorded on a BRUCKER AMX 500) spectrophotometer. The chemical shifts are expressed in p.p.m using TMS as internal standard (for ^{13}C and ^{1}H data) and 85% H_3PO_4 as external standard (^{31}P data). HPLC was carried out on KONTRON INSTRUMENT. Melting curves were recorded on a Varian Cary 1E spectrophotometer with a temperature controller unit.

2-amino-5-fluoro-benzoic acid (1a)

2-amino-6-fluoro-benzonitrile (10g, 0.25 mmol) and NaOH (10g, 0.25 mmol) was refluxed in water (150 ml) for 12h. The reaction was monitored by TLC. The crude was evaporated, salts were precipitated in EtOH and filtered off. The amino acid was obtained by evaporation of the ethanolic phase. Yield = 10.2g (90%). Rf = 0.34 (CH_3OH/CH_2Cl_2, 3:7, v:v). ^{1}H NMR (200 MHz, DMSOd_6) : 6.16 (dd, 1H, H_5, $^{3}J_{HH}$ = 8.3 Hz, $^{3}J_{HF}$ = 11.7 Hz) ; 6.44 (d, 1H, H_3, $^{3}J_{HH}$ = 8.7 Hz) ; 7.04 (td, 1H, H_4, $^{3}J_{HH}$ = 8.3 Hz, $^{4}J_{HF}$ = 6.4 Hz). ^{13}C NMR (50.32 MHz, DMSOd_6) : 100.74 (C_1, $^{2}J_{CF}$ = 15.2 Hz) ; 101.67 (C_5, $^{2}J_{CF}$ = 22.89 Hz) ; 111.9 (C_3) ; 133.3 (C_4, $^{3}J_{CF}$ = 12.2 Hz) ; 152.5 (C_2, $^{3}J_{CF}$ = 4.6 Hz) ; 162.7 (C_6, $^{1}J_{CF}$ = 253.3 Hz) ; 167.6 (Carbonyl).

5-fluoro-quinazoline-2,4-(1*H*,3*H*)-dione (3a)

1a (10g, 64.5 mmol) was dissolved in water (45 ml) with a few drops of 2M NaOH, and was refluxed with urea (30.9 g, 516 mmol) for 72h. The reaction was monitored by TLC (methanol : CH_2Cl_2, 10 : 90). The crude was then evaporated and **3a** was precipitated in ethanol (White powder) mp 250° (dec). Yield = 9.7 g (84%). Rf = 0.30 (CH_3OH/CH_2Cl_2, 1:9, v/v). MS (EI) : 180.0. IR (KBr) : 3495, 1731, 1705, 1680, 1626, 1442, 1404. UV (CH_3OH) : λmax 244nm (ε:1708), 218nm (ε:9250). ^{1}H NMR (200 MHz, DMSOd_6) : 6.87 (dd, 1H, H_6, $^{3}J_{HH}$ = 7.81 Hz, $^{3}J_{HF}$ = 11.2 Hz) ; 6.98 (d, 1H, H_8, $^{3}J_{HH}$ = 8.3 Hz) ; 7.56 (td, 1H, H_7, $^{3}J_{HH}$ = 7.8 Hz, $^{4}J_{HF}$ = 5.4 Hz). ^{13}C NMR (50.32 MHz, DMSOd_6) : 104.0 (C_{4a}, $J^{3}{}_{HF}$ = 10.5 Hz) ; 109.0 (C_6, $^{3}J_{HF}$ = 21.3 Hz) ; 111.4 (C_8) ; 135.6 (C_7, $^{4}J_{HF}$ = 10.7 Hz) ; 143.0 (C_{8a}) ; 150.1 (C_2) ; 160.1 (C_4) ; 161.37 (C_5, $^{1}J_{HF}$ = 260 Hz).

1-(3,5-di-O-p-toluoyl-2-deoxy-α,β-D-erythro-pentofuranosyl)-5-fluoro-quinazoline-2,4-(3*H*)-dione (5a)

A mixture of **3a** (1 g ; 6.8 mmol), a few cristals of ammonium sulfate and acetamide, a few drops of trimethylsilylchloride, and anhydrous pyridine (12ml) was refluxed in hexamethyldisilazane (HMDS) (25ml) for 24 hours. Excess of HMDS was removed in vacuo by co-evaporation with toluene. The residue was dissolved in dry chloroform (45ml), then **4** (1.6 g ; 4.15 mmol) and CuI (0.425 g ; 2.2 mmol) was added and stirred at RT for 24 hours. The reaction was monitored on TLC. The mixture was diluted in CH_2Cl_2 (80ml) and then washed twice by water, dried over $MgSO_4$ and evaporated. The anomeric mixture of nucleosides was purified by flash chromatography with a gradient of ethyl acetate in hexane (10-85%). Yield = 420mg (19%). Rf = 0,35 (ethyl acetate/cyclohexane, 5:5, v/v). ^{1}H NMR (200 MHz, $CDCl_3$) : 2.4 (s, 6H, CH_3 Tol) ; 3.1 (m, 2H, $H_{2'}$) ; 4.22 (m, 3H, $H_{4'}$ and $H_{5'}$) ; 5.7 (m, 1H, $H_{3'}$) ; 6.75-8.0 (m, 12H, Har Tol, $H_{1'}$, H_5, H_7 and H_8) ; 9.45 (bb, 1H, N<u>H</u>). ^{13}C NMR (50.32 MHz, $CDCl_3$) : 21.6 (CH_3

tol) ; 34.1-34.2 ($C_{2'}$) ; 63.5-64.7 ($C_{5'}$) ; 73.5-74.9 ($C_{3'}$) ; 81.3 ($C_{1'}$) ; 84.6-85.7 ($C_{4'}$) ; 106.60 (C4a) ; 111.4 (C_6, $J^2{}_{CF}$ = 21.4 Hz) ; 111.9 (C8) ; 126.3 (C_{quat} Tol) ; 129.1-129.6 (C_{ar} Tol) ; 135.3 (C_7, $J^3{}_{CF}$ = 10.7 Hz) ; 141.1 (C_{8a}) ; 144.0-144.3 (C_{quat} Tol) ; 149.7 (C_2) ; 158.6 (C_4) ; 162.5 (C_5, $J^1{}_{CF}$ = 265.5 Hz) ; 166.0 (Carbonyl Tol).

1-(2 deoxy-α, β-D-erythro-pentofuranosyl)-5-fluoro-quinazoline-2, 4-(3*H*)-dione (6a)

The product **5a** (420 mg, 0.79 mmol) was added to methanolic sodium (70 mg NaOH in 10 ml of anhydrous methanol) and stirred for 1h at RT. The solution was concentrated and then poured on a short silica gel column eluted with CH_2Cl_2 : MeOH (1:1) to afford **6a**. Yield = 222mg (95%). Rf = 0.06 (ethyl acetate/cyclohexane, 7:3, v:v).

1-(2-deoxy-5-(4,4'-dimethoxytrityl)-α,β-D-erythro-pentofuranosyl)-5-fluoro-quinazoline-2,4-(3*H*)-dione (7a)

The product **6a** (222 mg, 0.75 mmol) was carefully dried by three co-evaporations with pyridine and then dissolved in anhydrous pyridine (10 ml). Dimethoxytritylchloride (278 mg, 0.82 mmol), triethylamine (0.150 ml, 1 mmol) and dimethyl-amino-pyridine (5 mg, 0.03 mmol) were added under an argon atmosphere and mixed for 12h at RT. The reaction was poured on $CHCl_3$ (100ml), the organic phase was washed by $NaHCO_3$ solution, dried and evaporated. The two anomers were then purified by flash chromatography with a gradient of ethyl acetate in hexane (50-100%) and then CH_3OH in CH_2Cl_2 (0-20%) in presence of 1% triethylamine. Yield = 256.5 mg (57% , α:β = 69:31). Rf 7aα=0,58, Rf 7aβ = 0.45 (ethyl acetate/cyclohexane, 70:30, v:v). **7aα** (amorphous) : ^1H NMR (200 MHz, $CDCl_3$) : 2.8 (m, 2H, $H_{2'}$) ; 3.25 (m, 2H, $H_{5'}$) ; 3.8 (s, 6H, OCH_3) ; 4.4 (m, 2H, H3' and $H_{4'}$) ; 6.35 (m, 1H, $H_{1'}$) ; 6.7-7.5 (m, 16H, Har), 7.7 (bb, 1H, N<u>H</u>). ^{13}C NMR (50.32 MHz, $CDCl_3$) : 39.0 ($C_{2'}$) ; 55.1 (CH_3 DMT) ; 64.9 ($C_{5'}$) ; 74.0 ($C_{3'}$) ; 86.5 - 88.2 ($C_{1'}$, $C_{4'}$) ; 88.4 (Cquat DMT) ; 106.0 (C_{4a}) ; 111.0 (C_8) ; 111.5-111.9 (d, C_6, $J^2{}_{CF}$ = 20.2 Hz) ; 113.1 (C_{ar} DMT) ; 126.8-127.8-127.9-129.9 (C_{ar} DMT) ; 135.8 (C_{quat} DMT,) ; 135.0 (d, C_7,$^3J_{CF}$ = 10.6 Hz) ; 143.0-144.6 (C_{quat} DMT, C_{8a}) 149.2 (C_2) ; 158.5 (Cquat DMT, C_4) ; 162.6 (d, C_5, $J^1{}_{CF}$ = 265 Hz). **7aβ** (amorphous) : ^1H NMR (200 MHz, $CDCl_3$) : 2.45 (2m, 2H, $H_{2'}$) ; 3.45 (m, 2H, $H_{5'}$) ; 3.7 (s, 6H, OCH_3) ; 3.95 (m, 1H, $H_{4'}$) ; 4.75 (m, 1H, $H_{3'}$) ; 6.6-7.4 (m, 17H, H_{ar}, $H_{1'}$, H_5, H_7, H_8) ; 7.8 (bb, 1H, N<u>H</u>). ^{13}C NMR (50.32 MHz, $CDCl_3$) : 36.6 ($C_{2'}$) ; 55.1 (CH_3 DMT) ; 62.4 ($C_{5'}$) ; 70.3 ($C_{3'}$) ; 84.0-84.9 ($C_{1'}$, $C_{4'}$) ; 86.3 (C_{quat} DMT) ; 106.9 (C_{4a}) ; 110.7 (d, C_6, J^2CF = 20.6 Hz) ; 113.0 (C_{ar} DMT, C_8) ; 126.8-127.7-128.2-129.0 (C_{ar} DMT) ; 134.8 (d, C_7, $J^3{}_{CF}$ = 11.1 Hz) ; 135.5 (C_{quat} DMT) ; 141.3 (C_{8a}) ; 144.5 (C_{quat} DMT) ; 150.9 (C_2) ; 158.4 (Cquat DMT) ; 160.2 (C_4) ; 162.3 (d, C_5, $J^1{}_{CF}$ = 263.5 Hz).

1-(2-deoxy-α-D-erythro-pentofuranosyl)-5-fluoro-quinazoline-2,4-(3H)-dione (6aα)

The product **7aα** (80 mg, 0.13 mmol) was stirred for 30 min. at RT with 80% acetic acid aqueous solution (4ml). Then, the mixture was evaporated and poured on a short silica gel column eluted with CH_3OH:CH_2Cl_2 (50:50) to afford 28 mg (yield = 73%) of α nucleoside (amorphous) . ^1H NMR (500 MHz, $DMSOd_6$), d (ppm) : 2.41 (m, 2H, H2') ; 3.51 (m, 2H, $H_{5'}$) ; 4.08 (m, 1H, $H_{4'}$) ; 4.35 (m, 1H, $H_{3'}$) ; 6.63 (dd, 1H, $H_{1'}$, ^3J = 8.5Hz) ; 7.03 (m, 1H, H_6) , 7.68 (m, 2H, H_7 and H_8). ^{13}C NMR (125.0 MHz, $DMSOd_6$), d (ppm) : 35.6 ($C_{2'}$) ; 61.2 ($C_{5'}$) ; 69.9 ($C_{3'}$) ; 81.2 ($C_{1'}$) ; 85.5 ($C_{4'}$) ; 105.7 (d, C_{4a}, $^2J_{CF}$ = 8.4 Hz) ; 109.8 (d, C_6, $^2J_{CF}$ = 20.6 Hz) ; 112.2 (d, C_8, $^4J_{CF}$ = 2.9 Hz) ; 134.5 (d, C_7, $^3J_{CF}$ = 11.1 Hz) ; 140.4 (C_{8a}) ; 149.6 (C_2) ; 158.5 (C_4) ; 161.0 (d, C_5, $^1J_{CF}$ = 261 Hz).

1-(2-deoxy-β-D-erythro-pentofuranosyl)- 5-fluoro-quinazoline-2,4-(3*H*)-dione (6aβ)

The β analogue **6aβ** was prepared as described for **6aα** using 0.1 mmol of 7aβ. Yield = 25 mg (84%). ^1H NMR (200 MHz, DMSOd6) : 1.9 (m, 1H, $H_{2'a}$) ; 2.6 (m, 1H, $H_{2'b}$) ; 3.65 (m, 3H, $H_{5'}$, $H_{4'}$) ; 4.4 (m, 1H, $H_{3'}$) ; 6.62 (t, 1H, $H_{1'}$, 3J = 7.8Hz) ; 7.0 (m, 1H, H_6) ; 7.6 (m, 2H, H_7, H_8). ^{13}C NMR (50.32 MHz, DMSOd6) : 36.0 ($C_{2'}$) ; 60.8 ($C_{5'}$) ; 69.5 ($C_{3'}$) ; 83.7 ($C_{1'}$) ; 86.5 ($C_{4'}$) ; 106.2 (C_{4a}) ; 110.4 (d, C_6,, $2J_{CF}$ = 20.6 Hz) ; 113.1 (C_8) ; 134.9 (d, C_7, $3J_{CF}$ = 10.1 Hz) ; 141.3 (C_{8a}) ; 150.2 (C_2) ; 159.3 (C_4) ; 161.5 (d, C_5, $J^1{}_{CF}$ = 260.3 Hz). HR-FAB Cacld for $C_{13}H_{14}N_2O_5F$ (M^++1) : 297.0886 ; Found : 297.0878

1-(2-deoxy-3-O-(2-cyanoethoxy(diisopropylamino)-phosphino)-5-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl)-5-fluoro-quinazoline-2,4-(3*H*)-dione (8a)

The product **7aβ** (80 mg, 0.13 mmol) was dried carefully by three co-evaporations with pyridine and dissolved in anhydrous CH_2Cl_2 (1 ml). Then, distilled N,N-diisopropylethylamine (86 ml, 0.52 mmol) was added followed by slow addition of 2-cyano-N,N-diisopropylphosphoramidochloridite (46 mg, 0.195 mmol dissolved in 200 ml dry CH_2Cl_2) under an argon atmosphere and stirred for 1h at RT. The reaction was monitored by TLC, quenched with methanol (1.4 ml) and diluted in ethyl acetate (15 ml). The organic phase was washed with saturated solution of $NaHCO_3$ and NaCl, then dried and evaporated. Crude phosphoramidite was purified twice by flash chromatography with ethyl acetate : hexane : triethylamine (45:45:10) as eluent. Yield = 48 mg (46%). Rf = 0.42 (ethyl acetate/cyclohexane, 50:50, v/v). ^1H NMR (200 MHz, $CDCl_3$) : 1.2 (m, 12H, iPr) ; 2.4 (2m, 2H, $H_{2'}$) ; 2.9 (m, 2H, $C\underline{H}_2CN$) ; 3.55 (m, 6H, $C\underline{H}_2OP$, $C\underline{H}iPr$, $H_{5'}$) ; 3.75 (s, 6H, OCH_3) ; 4.07 (m, 1H, $H_{4'}$) ; 4.9 (m, 1H, $H_{3'}$) ; 6.7-7.4 (m, 17H, H_{ar}, $H_{1'}$, H_6, H_7, H_8) ; 7.9 (m, 1H, $N\underline{H}$). ^{13}C NMR (50.32 MHz, $CDCl_3$) : 19.5-19.9 ($\underline{C}H_2CN$) ; 24.4 ($\underline{C}H_3iPr$) ; 35.5 ($C_{2'}$) ; 42.9 ($\underline{C}HiPr$) ; 55.1 (CH_3DMT) ; 57.8-58.2 ($\underline{C}H_2OP$) ; 61.7 ($C_{5'}$) ; 67.6-70.7 ($C_{3'}$) ; 84.0 ($C_{1'}$, $C_{4'}$) ; 86.3 (C_{quat} DMT) ; 107.0 (C_{4a}) ; 111.4 (C_6) ; 113.0 (C_{ar} DMT, C_8) ; 126.9-127.7-128.3-130.2 (C_{ar} DMT) ; 135.4 (C_{ar} DMT, C_7) ; 140.8 (C_{8a}) ; 144.4 (C_{quat} DMT) ; 149.7 (C_2) ; 158.5 (C_{quat} DMT, C_4), 162.0 (d, C_5, $J^1{}_{CF}$ = 264 Hz). ^{31}P NMR (200 MHz, $CDCl_3$) d (ppm) : 147.34, 147.7.

2-ureido-6-chloro-benzoic acid (2b)

2-amino-6-chloro-benzoic acid **1b** (4g, 23.3 mmol) was dissolved in water (40 ml) by adding NaOH 10N (2.4 ml, 24 mmol). The solution was stirred10h with Na cyanate (1.68g, 25.6 mmol) and acetic acid (1.46 ml, 25.6 mmol). The reaction was monitored on TLC. The pure product was then precipitated in HCl 5N. Yield = 3.22g (65%). Rf = 0.65 ($CH_3OH/CHCl_3$, 50:50, v/v). ^1H NMR (200 MHz, DMSOd6) : 6.35 (bb, 2H, $N\underline{H}_2$) ; 7.09 and 7.8 (d, 2H, H_3, H_5 unresolved, 3J = 8 Hz) ; 7.3 (t, 1H, H_4, 3J = 8 Hz,) ; 8 (s, 1H, $N\underline{H}$). ^{13}C NMR (50.32 MHz, DMSOd6) : 120 (C_5) ; 122 (C_3) ; 125 (C_1) ; 129.4(C_6) ; 130 (C_4) ; 138 (C_2) ; 155 (Carbonyl urea) ; 166.7 (Carbonyl acid).

5-chloro-quinazoline-2, 4-(3*H*,4*H*)-dione (3b)

2b (3 g, 14 mmol) was refluxed in trifluoroacetic acid (40 ml) for 24h. The reaction was monitored by TLC. The crude was then evaporated. The main part of the cyclized product was filtered off in hot ethanol, and the minor part, still contained in ethanol, was purified by flash chromatography with a gradient of methanol in CH_2Cl_2 (0-100%). Yield = 0.982 g (35%). Rf = 0,25 ($CH_3OH/CHCl_3$, 20:80, v/v). MS (EI) : 196.1. IR (KBr) : 3449, 3219, 2361, 1735, 1718, 1693, 1676. UV (CH_3OH) : λmax 318 nm (ε = 3495), 228 nm (ε = 13636), 223 nm (ε = 19090). ^1H NMR (200 MHz, DMSOd6) : 7.14 (m, 2H, H_6, H_8) ; 7.5 (t, 1H, H_7, 3J = 8.2 Hz). ^{13}C NMR (50.32 MHz, DMSOd6) : 111.2 (C_{4a}) ; 114.4 (C6) ; 124.7 (C_8) ; 133.6 (C_5) ; 134.2 (C_7) ; 143.3 (C_{8a}) ; 149.3 (C_2) ; 160.4 (C_4).

1-(3,5-di-O-p-toluoyl-2-deoxy-α,β-D-erythro-pentofuranosyl)-5-chloro-quinazoline-2,4-(3*H*)-dione (5b)

The analogue **5b** was prepared as described for **5a** using 1.8 mmol of **3b**. The nucleoside was purified by flash chromatography in a gradient of methanol in CH_2Cl_2 (0-20%). Yield = 436 mg (50%). Rf = 0,27 (CH_3OH /$CHCl_3$, 5/95, v/v). ^1H (200 MHz, $CDCl_3$) : 2.3 (s, 6H, CH_3 Tol) ; 2.8 (m, 2H, $H_{2'}$) ; 4.6 (m, 3H, $H_{4'}$ and $H_{5'}$) ; 5.7 (m, 1H, $H_{3'}$) ; 6.8-8.0 (m, 12H, Har Tol, $H_{1'}$, H_5, H_7 and H_8) ; 9.6 (bb, 1H, N$\underline{H}$). ^{13}C (50.32 MHz, $CDCl_3$) : 21.5 (CH_3 tol) ; 34.2 ($C_{2'}$) ; 63.4-64.6 ($C_{5'}$) ; 73.4-74.8 ($C_{3'}$) ; 81.2 ($C_{1'}$) ; 84.6-85.7 ($C_{4'}$) ; 114.0 (C4a) ; 114.9 (C6) ; 126.2-126.4 (C_{quat} Tol) ; 127,0 (C8) ; 129.0-129.5 (C_{ar} Tol) ; 133.6 (C5) ; 136.4 (C7) ; 141.7 (C8a) ; 143.8-144.3 (C_{quat} Tol) ; 149.6 (C_2) ; 159.3 (C_4) ; 165.9 (Carbonyl Tol).

1-(2-deoxy-α, β-D-erythro-pentofuranosyl)-5-chloro-quinazoline-2, 4-(3*H*)-dione (6b)

The analogue **6b** was prepared exactly as described for **6a**. Yield = 234 mg (95%). Rf = 0.06 (CH_3OH/$CHCl_3$, 5:95, v/v).

1-(2-deoxy-5-(4,4'-dimethoxytrityl)-α,β-D-erythro-pentofuranosyl)-6-chloro-quinazoline-2,4-(3H)-dione (7b)

The analogue **7b** was prepared exactly as described for **7a**. Yield = 337 mg (73% , α:β = 73:27). Rf=0.66 for **7bα** and Rf=0.46 for **7bβ** (ethyl acetate/cyclohexane, 70:30, v/v). **7bα :** ^1H NMR (200 MHz, $CDCl_3$) : 2.73 (m, 2H, $H_{2'}$) ; 3.3 (m, 2H, $H_{5'}$) ; 3.77 (s, 6H, OCH_3) ; 4.4 (m, 2H, $H_{3'}$, $H_{4'}$) ; 6.3 (bb, 1H, O$\underline{H}$) ; 6.8 (m, 1H, $H_{1'}$) ; 7.1-7.55 (m, 16H, Har), 9.1 (bb, 1H, N$\underline{H}$). ^{13}C NMR (50.32 MHz, $CDCl_3$) : 38.7 ($C_{2'}$) ; 55.1 (CH_3 DMT) ; 64.9 ($C_{5'}$) ; 73.7 ($C_{3'}$) ; 86.3 (C_{quat} DMT) ; 87.9 - 88.3 ($C_{1'}$, $C_{4'}$) ; 113.0 (C_{ar} DMT) ; 114.1 (C_6) : 114.4 (C_{4a}) ; 126.7 (C_8) ; 127.7-127.9-129.9 (C_{ar} DMT) ; 133.6 (C5) ; 135.7-135.8 (C_{quat} DMT) ; 136.0 (C7) ; 144.2 (C_{8a}) ; 144.6 (C_{quat} DMT) ; 150.8 (C_2) ; 158.4 (Cquat DMT) ; 161.3 (C_4). **7bβ:** ^1H NMR (200 MHz, $CDCl_3$) : 2.15 (m, 1H, $H_{2'a}$) ; 2.9 (m, 1H, $H_{2'b}$) ; 3.5 (m, 2H, $H_{5'}$) ; 3.7 (s, 6H, OCH_3) ; 4.0 (m, 1H, $H_{4'}$) ; 4.82 (m, 1H, $H_{3'}$) ; 6.2 (bb, 1H, O$\underline{H}$) ; 6.7-7.5 (m, 17H, H_{ar}, $H_{1'}$, H_5, H_7, H_8) ; 8.0 (bb, 1H, N$\underline{H}$). ^{13}C NMR (50.32 MHz, $CDCl_3$) : 36.5 ($C_{2'}$) ; 55.1 (CH_3 DMT) ; 62.4 ($C_{5'}$) ; 71.1 ($C_{3'}$) ; 84.3 -85.0 ($C_{1'}$, $C_{4'}$) ; 86.4 (C_{quat} DMT) ; 113.0 (C_{ar} DMT) ; 113.8 (C_{4a}) : 116.3 (C_6) ; 126.8 (C_8) ; 127.1-127.7-128.1-130.1 (C_{ar} DMT) ; 134.1 (C5) ; 135.4 (C_{quat} DMT) ; 136.1 C7) ; 141.9 (C_{8a}) ; 144.4 (C_{quat} DMT) ; 149.7 (C_2) ; 158.4 (C quat DMT) ; 159.5 (C_4).

1-(2-deoxy-α-D-erythro-pentofuranosyl)-5-chloro-quinazoline-2,4-(3H)-dione (6bα)

The analogue **6bα** was prepared as described for **6aα** using 0.13 mmol of **7bα**. Yield : 40 mg (95%). Rf = 0.3 (CH_3OH/$CHCl_3$, 1:9, v/v). ^1H NMR (500 MHz, $DMSOd_6$) : 2.4 (m, 2H, $H_{2'}$) ; 3.55 (m, 2H, $H_{5'}$) ; 4.1 (m, 1H, $H_{4'}$) ; 4.35 (m, 1H, $H_{3'}$) ; 6.6 (t, 1H, $H_{1'}$, 3J = 8.5 Hz) ; 7.3-7.84 (2d, 2H, H_6 and H_8 unresolved, 3J = 8Hz) ; 7.62 (t, 1H, H_7, 3J = 8Hz). ^{13}C NMR (125 MHz, $DMSOd_6$) : 35.7 ($C_{2'}$) ; 61.1 ($C_{5'}$) ; 69.9 ($C_{3'}$) ; 84.4($C_{1'}$) ; 85.5 ($C_{4'}$) ; 113.1 (C_{4a}) ; 115.6 (C_8) ; 125.6 (C_6) ; 133.3 (C_7) ; 133.6 (C_5) ; 141.3 (C_{8a}) ; 149.1 (C_2) ; 158.9 (C_4). HR-FAB Cacld for $C_{13}H_{14}N_2O_5Cl$ (M^++1) : 313.0591 ; Found : 313.0584

1-(2-deoxy-3-O-(2-cyanoethoxy(diisopropylamino)-phosphino)-5-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl)-5-chloro-quinazoline-2,4-(3*H*)-dione (8b)

The analogue **8b** was prepared as described for **8a** using 0.15 mmol of 7bβ. Yield = 63 mg (67%). Rf = 0.53 (ethyl acetate/cyclohexane/triethylamine, 45:45:10, v/v/v). MS FAB^- = 813.13 $(M-H)^{-1}$. ^1H NMR (200 MHz, $CDCl_3$) : 1.1 (m, 12H, iPr) ; 2.2-2.9 (m, 2H, $H_{2'}$) ; 2.3 (m, 2H, $C\underline{H}_2CN$) ; 3.55 (m, 6H, $C\underline{H}_2OP$, $C\underline{H}iPr$, $H_{5'}$) ; 3.77 (s, 6H, OCH_3) ; 4.05 (m, 1H, $H_{4'}$) ; 4.9 (m, 1H, $H_{3'}$) ; 6.6-7.4 (m, 17H, H_{ar}, $H_{1'}$, H_6, H_7, H_8) ; 8.0 (m, 1H, N$\underline{H}$). ^{13}C NMR (50.32 MHz, $CDCl_3$) : 20.2 ($C\underline{H}_2CN$) ; 24.4 ($\underline{C}H_3iPr$) ; 35.5 ($C_{2'}$) ; 43.0 ($\underline{C}HiPr$) ; 55.1 (CH_3DMT) ; 57.9-58.3 ($\underline{C}H_2OP$) ; 61.8 ($C_{5'}$) ; 72.0 ($C_{3'}$) ; 84.2 ($C_{1'}$) ;

84.3 (C4') ; 86.4 (C_{quat} DMT) ; 113.0 (C_{ar} DMT, C_{4a}) ; 116.3 (C_6) ; 126.9-127.7-128.3 (C_{ar} DMT, C_8) ; 130.2 (C_{ar} DMT) ; 134.0 (C5) ; 135.4 (C_{quat} DMT) ; 136.1 (C7) ; 141.9 (C_{8a}) ; 144.4 (C_{quat} DMT) ; 149.8 (C_2) ; 158.5 (C_{quat} DMT) ; 159.7(C_4). ^{31}P NMR (200 MHz, $CDCl_3$) d (ppm) : 147.34, 147.69.

2-ureido-5-chloro-benzoic acid (2c)

5-chloroanthranilic acid **1c** (4.65 g, 27.1 mmol) was dissolved in 100 ml of water by adding NaOH 5N (7.4 ml). To this, was added sodium cyanate (1.9 g, 29.8 mmol) and acetic acid (1 ml, 29.8 mmol). Stirring was continued for 3 hours at RT. **2c** was precipitated with HCl 1N (20 ml). Yield : 5.26 g (91%). ^{1}H (200 Mhz, DMSOd$_6$) : 6.47 (bb, 1H, OH) ; 7.39 (dd, 1H, H_4, ^3J = 8.8 and ^4J = 2.9 Hz) ; 7.83 (d, 1H, H_6, ^4J = 2.9 Hz) ; 8.37 (d, 1H, H_3, ^3J = 9.2 Hz). ^{13}C (50.32 MHz, DMSOd$_6$) : 117.0 (C_1) ; 121.5 (C_6) ; 124.4 (C_5) ; 130.5 (C_6) ; 133.2 (C_4) ; 142.0 (C_2) ; 155.4 (carbonyl ureido) ; 168.8 (carbonyl acid).

6-chloro-Quinazoline-2,4-(1*H*,3*H*)-dione (3c)

2c (1.77 g, 8.2 mmol) was dissolved in acetic acid (170 ml), and stirred at 55°C. The reaction was monitored by TLC, and stopped after 24 hours. Acetic acid was evaporated and quinazoline was cristallized from methanol. Yield : 600 mg (35%). Rf = 0.68 ($CH_3OH/CHCl_3$, 20:80, v/v). MS (EI) : 196.1. IR (KBr) : 3460, 3209, 2351, 1747, 1715, 1664, 1479. UV (CH3OH) : λmax 322nm (ε = 3209), 253nm (ε = 6423), 245nm (ε = 5303), 220nm (ε = 32056). ^{1}H (200MHz, DMSOd$_6$) : 7.14 (d, 1H, H_8, ^3J = 8.8 Hz) ; 7.64 (dd, 1H , H_7, ^3J = 8.8 and ^4J = 2.4 Hz) ; 7.78 (d, 1H, H_5, ^4J = 1,9 Hz) ; 11.2 and 11.4 (2s, NH unresolved). ^{13}C (50.32 MHz, DMSOd$_6$) : 115.7 (C_{5a}) ; 117.5 (C_8) ; 125.9 (C_5) ; 126.2 (C_6) ; 134.8 (C_7) ; 139.7 (C_{8a}) ; 150.0 (C_2), 161.8 (C_4).

1-(3,5-di-O-p-toluoyl-2-deoxy-α,β-D-erythro-pentofuranosyl)-6-chloro-quinazoline-2,4-(3H)-dione (5c)

The analogue **5c** was prepared as described for **5a** using 2.9 mmol of **3c**. The cooper salt was filtered off and then the mixture was washed by $NaHCO_3$, NaCl solution and water, dried over $MgSO_4$ and evaporated. The nucleoside was purified by flash chromatography in a gradient of ether oxyde in hexane (0-80%). Yield : 882 mg (50%). Rf = 0.67 (ethyl acetate/cyclohexane, 50:50, v/v). ^{1}H (200 MHz, $CDCl_3$) : 2.4 (s, 6H, CH_3 Tol) ; 2.85 (m, 2H, $H_{2'}$) ; 4.65 (m, 3H, $H_{4'}$ and $H_{5'}$) ; 5.7 (m, 1H, $H_{3'}$) ; 7.4 (m, 12H, $H_{1'}$, Har Tol, H_5, H_7 and H_8) ; 10.04 (bb, 1H, NH). ^{13}C (50.32 MHz, $CDCl_3$) : 21.5 (CH_3 tol) ; 34.0-34.4 ($C_{2'}$) ; 63.1-64.7 ($C_{5'}$) ; 73.2-75.0 ($C_{3'}$) ; 81.2-81.4 ($C_{1'}$) ; 84.1-85.3 ($C_{4'}$) ; 117.6 (C_8) ; 118.0-117.8 (C_{4a}) ; 126.2 (C_6) ; 126.5-126.7(C_{quat} Tol) ; 128.0 (C_5) ; 129.1-129.5 (C_{ar} Tol) ; 134.5 (C_7) ; 137.5-137.7 (C_{8a}) ; 144.2-144.4 (C_{quat} Tol) ; 149.8 (C_2) ; 160.7 (C_4) ; 165.9 (Carbonyl Tol).

1-(2 deoxy-α, β-D-erythro-pentofuranosyl)-6-chloro-quinazoline-2, 4(3*H*) dione (6c)

The analogue **6c** was prepared as described for **6a** using 1.6 mmol of **5a**. Yield : 475 mg (95%). Rf = 0.3 ($CH_3OH/CHCl_3$, 10:90, v/v).

1-(2-deoxy-5-(4,4'-dimethoxytrityl)-α,β-D-erythro-pentofuranosyl)-6-chloro-quinazoline-2,4-(3*H*)-dione (7c)

The analogue **7c** was prepared as described for **7a** using 1.5 mmol of **6c**. Yield : 510 mg (55,3%, α:β = 62:38). Rf=0.78 for **7ca** and Rf=0.63 for **7cb** (ethyl acetate/cyclohexane, 70:30, v/v).**7ca** : ^{1}H NMR (200 MHz, $CDCl_3$) : 2.75 (m, 2H, $H_{2'}$) ; 3.29 (m, 2H, $H_{5'}$) ; 3.77 (s, 6H, OCH_3) ; 4.0 (m, 1H, $H_{4'}$) ; 4.43 (m, 1H, $H_{3'}$) ; 6.84 (m, 1H, $H_{1'}$) ; 7.17-7.65 (m, 16H, Har), 8.9 (bb, 1H, N$\underline{H}$). ^{13}C NMR (200 MHz, $CDCl_3$) : 38.2 ($C_{2'}$) ; 54.9 (CH_3 DMT) ; 64.7 ($C_{5'}$) ; 73.1 ($C_{3'}$) ; 87.0 ($C_{4'}$) ; 86.1 (C_{quat} DMT) ; 86.5 ($C_{1'}$) ; 112.9 (C_{ar} DMT) ; 116.8 (C_8) : 118.5 (C_{4a}) ; 126.5 (C_5) ; 127.3-127.6-127.8 (C_{ar} DMT) ; 128.4 (C_6) ; 129.7 (C_{ar} DMT) ; 133.8 (C_7) ; 135.6 (C_{quat} DMT) ; 139.5 (C_{8a}) ; 144.5 (C_{quat} DMT) ; 151.9 (C_2) ; 158.2

(C_{quat} DMT) ; 163.3 (C_4). **7cb** : ^{1}H NMR (200 MHz, $CDCl_3$) : 2.22 (m, 1H, $H_{2'a}$) ; 2.85 (m, 1H, $H_{2'b}$) ; 3.61 (m, 3H, $H_{4'}$, $H_{5'}$) ; 3.82 (s, 6H, OCH_3) ; 4.86 (m, 1H, $H_{3'}$) ; 5.94 (m, 1H, $H_{1'}$) ; 6.74-7.5 (m, 16H, H_{ar}) ; 8.8 (bb, 1H, $N\underline{H}$). ^{13}C NMR (200 MHz, $CDCl_3$) : 36.7 ($C_{2'}$) ; 55.1 (OCH_3) ; 62.3 ($C_{5'}$) ; 70.5 ($C_{3'}$) ; 83.8 ($C_{1'}$) ; 85.0 ($C_{4'}$) ; 86.5 (C_{quat}DMT) ; 113.1 (C_{ar} DMT) ; 118.1 (C_{4a}) ; 119.0 (C_8) ; 127.0 (C_5) ; 127.5-127.8-128.3 (C_{ar} DMT) ; 129.1 (C_6) ; 130.2 (C_{ar} DMT) ; 134.7 (C_7) ; 135.5 (C_{quat} DM) ; 137.8 (C_{8a}) ; 144.3 (C_{quat} DMT) ; 150.6 (C_2) ; 158.5 (C_{quat} DMT) ; 161.5 (C_4).

1-(2-deoxy-α-D-erythro-pentofuranosyl)-6-chloro-quinazoline-2,4-(3H)-dione (6cα)

The analogue **6cα** was prepared as described for **6aα** using 0.1 mmol of **7cα**. Yield : 26 mg (85%). ^{1}H NMR (500 MHz, $DMSOd_6$) : 2.31 (m, 1H,$H_{2'a}$) ; 2.45 (m, 1H, $H_{2'b}$) ; 3.53 (dd, 2H, $H_{5'}$, ^2J = 12Hz, ^3J = 5Hz) ; 4.08 (q, 1H, $H_{4'}$, 3J = 4Hz) ; 4.35 (q, 1H, $H_{3'}$, ^3J = 6Hz) ; 4.8 (bb, 1H, $O\underline{H}$) ; 5.4 (bb, 1H, $O\underline{H}$) ; 6.66 (t, 1H, $H_{1'}$, ^3J = 8Hz) ; 7.74 (dd, 1H, H_8, 3J = 9.1Hz, ^5J = 2.6Hz) ; 7.93 (m, 2H, H_5 and H_7) ; 11.7 (bb, 1H, $N\underline{H}$). ^{13}C NMR (125.0 MHz, $DMSOd_6$) : 36.4($C_{2'}$) ; 61.7 ($C_{5'}$) ; 70.4 ($C_{3'}$) ; 84.4 ($C_{1'}$) ; 86.1 ($C_{4'}$) ; 118.2 (C_{4a}) ; 119.0 (C_7) ; 126.4 (C_5) ; 127.3 (C_6) ; 134.0 (C_8) ; 137.9 (C_{8a}) ; 149.8 (C_2) ; 160.5 (C_4). HR-FAB Cacld for $C_{13}H_{14}N_2O_5Cl$ (M^++1) : 313.0591 ; Found : 313.0605

1-(2-deoxy-β-D-erythro-pentofuranosyl)-6-chloro-quinazoline-2,4-(3H)-dione (6cβ)

The analogue **6cβ** was prepared as described for **6aα** using 0.1 mmol of **7cβ**. Yield : 26 mg (85%). ^{1}H NMR (500 MHz, $DMSOd_6$) : 1.91 (m, 1H, $H_{2'a}$) ; 2.58 (m, 1H, $H_{2'b}$) ; 3.67 (m, 3H, $H_{5'}$, $H_{4'}$) ; 4.37 (m, 1H, $H_{3'}$) ; 4.96 (bb, 1H, $O\underline{H}$) ; 5.24 (bb, 1H, $O\underline{H}$) ; 6.65 (t, 1H, $H_{1'}$, ^3J = 7.8Hz) ; 7.65 (dd, 1H, H_8, ^3J = 9.1Hz, ^5J = 2.7Hz) ; 7.91 (m, 2H, H_5, H_7) ; 11.8 (bb, 1H, $N\underline{H}$). ^{13}C NMR (125.0 MHz, $DMSOd_6$) : 36.1 ($C_{2'}$) ; 60.7 ($C_{5'}$) ; 69.5 ($C_{3'}$) ; 83.4 ($C_{1'}$) ; 86.5 ($C_{4'}$) ; 118.2 (C_{4a}) ; 119.4 (C_8) ; 126.3 (C_5) ; 127.3 (C6) ; 133.9 (C_7) ; 138.2 (C_{8a}) ; 149.8 (C2) ; 160.7 (C4). HR-FAB Cacld for $C_{13}H_{14}N_2O_5Cl$ (M^++1) : 313.0591 ; Found : 313.0605

1-(2-deoxy-3-O-(2-cyanoethoxy(diisopropylamino)-phosphino)-5-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl)-6-chloro-quinazoline-2,4-(3H)-dione (8c)

The analogue **8c** was prepared as described for **8a** using 0.51 mmol of 7cβ. Crude phosphoramidite was purified twice by flash chromatography with ethyl acetate:hexane:triethylamine (50:40:10) as eluent. Yield : 99 mg (48%). Rf = 0.44 (ethyl acetate/cyclohexane/triethylamine, 50:40:10, v/v/v). MS (FAB-) : 938.9 $(M-H)^{-1}$. ^{1}H NMR (200 MHz, $CDCl_3$) : 1.2 (m, 12H, iPr) ; 2.25 (m, 1H, $H_{2'}$) ; 2.5 (m, 2H,$\underline{CH_2}CN$) ; 2.6 (m, 2H, $\underline{CH_2}OP$) ; 2.8 (m, 1H, $H_{2'}$) ; 3.5 (m, 4H, $\underline{CH}iPr$, $H_{5'}$) ; 3.77 (s, 6H, OCH_3) ; 4.1 (m, 1H, $H_{4'}$) ; 4.9 (m, 1H, $H_{3'}$) ; 6.8 (m, 1H, $H_{1'}$) ; 7.15-7.48 (m, 16H, H_{ar}) ; 8.0 (m, 1H, $N\underline{H}$). ^{13}C NMR (50.32 MHz, $CDCl_3$) : 20.1 ($\underline{C}H_2CN$) ; 24.4 ($\underline{C}H_3iPr$) ; 35.6 ($C_{2'}$) ; 43.0-43.3 ($\underline{C}HiPr$) ; 55.1 (CH_3DMT) ; 57.9-58.3 ($\underline{C}H_2OP$) ; 61.6 ($C_{5'}$) ; 71.5 ($C_{3'}$) ; 83.8 ($C_{4'}$) ; 84.3 ($C_{1'}$) ; 86.5 (C_{quat} DMT) ; 113.1 (C_{ar} DMT) ; 117.3 (CN) ; 117.9 (C_{4a}) ; 119.1 (C_8) ; 127.0 (C_5) ; 127.1-127.8-128.1 (C_{ar} DMT) ; 128.4 (C_6) ; 130.2 (C_{ar} DMT) ; 135.0 (C_7) ; 135.3 (C_{quat} DMT) ; 137.6 (C_{8a}) ; 144.2 (C_{quat} DMT) ; 150.0 (C_2) ; 128.6 (C_{quat} DMT) ; 160.8(C_4).

7-chloroquinazoline-2, 4-(1*H*,3*H*)-dione (3d)

2-amino-4-chloro-benzoic acid **1d** (10 g, 58.4 mmol) was dissolved in water (90 ml) with NaOH 10N (6.5 ml, 42.2 mmol), and was then refluxed with urea (28 g, 460 mmol) for 5 days. The reaction was monitored by TLC. The precipitate formed was filtered off. Yield : 3.7 g (33%). Rf = 0.72 ($CH_3OH/CHCl_3$, 10:90, v/v). MS (EI) : 196.1. IR (KBr) : 3027, 3305, 1739, 1680, 1613. UV (CH_3OH): λmax = 309 nm (ε = 3540) ; λ = 224 nm (ε = 36000). ^{1}H NMR (200 MHz, $DMSOd_6$) : 6.15 (d, 1H, H_8, 4J = 3 Hz) ; 6.18 (dd, 1H, H_6, ^4J = 3 Hz, 3J = 11Hz) ; 7.85 (d, 1H, H_5, 3J = 11 Hz). ^{13}C NMR (50.32 Hz, $DMSOd_6$) : 113.3 (C_{4a}) ; 114.7 (C_8) ; 122-128 (C_5, C_6) ; 139.2 (C_{8a}) ; 142.1 (C7) ; 150.3 (C_2) ; 162.1 (C_4).

1-(3,5-di-O-p-toluoyl-2-deoxy-α,β-D-erythro-pentofuranosyl)-7-chloro-quinazoline-2,4-(3*H*)-dione (5d)

The analogue **5d** was prepared a described for **5a** using 15.3 mmol of **3d** and 400 ml of dry acetonitrile for condensation. The salty precipitate was filtered off, then the crude was concentrated and diluted in 100 ml of CH_2Cl_2, washed twice by water, dried over $MgSO_4$ and evaporated. The nucleoside was purified by flash chromatography by a gradient of ethyl acetate in hexane (0-65%). Yield : 3 g (38%). Rf = 0,63 (ethyl acetate/cyclohexane, 50:50, v/v). ^1H NMR (200 MHz, $CDCl_3$) : 2.3 (s, 6H, CH_3 Tol) ; 2.96 (m, 2H, $H_{2'}$) ; 4.6 (m, 3H, $H_{4'}$ and $H_{5'}$) ; 5.65 (m, 1H, $H_{3'}$) ; 6.75 (m, 1H, $H_{1'}$) ; 7.1-8.2 (m, 11H, Har Tol, H_5, H_7 and H_8) ; 9.45 (bb, 1H, N$\underline{H}$). ^{13}C NMR (50.32 MHz, $CDCl_3$) : 21.6 (CH_3 tol) ; 34.6 ($C_{2'}$) ; 64.1-64.9 ($C_{5'}$) ; 74.0-75.0 ($C_{3'}$) ; 81.6 ($C_{4'}$) ; 85.0-85.5 ($C_{1'}$) ; 115.7 (C_{4a}) ; 115.8 (C_8) ; 124.1 (C_6) ; 126.1-126.6 (C_{quat} Tol) ; 129.1(C_{ar} Tol) ; 129.2 (C_5) ; 129.6-129.9 (C_{ar} Tol) ; 139.9 (C_{8a}) ; 140.9 (C_7) ; 144.0-144.4 (C_{quat} Tol) ; 149.8 (C_2) ; 160.8 (C_4) ; 166.3 (Carbonyl Tol).

1-(2-deoxy-α,β-D-erythro-pentofuranosyl)-7-chloro-quinazoline-2,4(3*H*)-dione (6d)

5d (3 g, 5.4 mmol) was dissolved in 20 ml CH_3OH:$CHCl_3$ (3:1) and stirred with NaOH 5N (2.4 ml, 12 mmol) for 1.5 h at RT. The reaction was monitored by TLC. The crude was concentrated, and the pure anomeric nucleoside (**6d**) was precipitated in ether oxyde. Yield : 1.2 g (72%). Rf = 0.28 (ethyl acetate/cyclohexane, 50:50, v/v).

1-(2-deoxy-5-(4,4'-dimethoxytrityl)-α,β-D-erythro-pentofuranosyl)-7chloro-quinazoline-2,4-(3*H*)-dione (7d)

The analogue **7d** was prepared as described for **7a** using 3.36 mmol of **6d**. Yield : 931 mg (45%, α:β = 77:23). Rf = 0,45 for **7dα** and Rf=0.3 for **7dβ** (ethyl acetate/cyclohexane, 50:50, v/v). **7dα** : ^1H NMR (200 MHz, $CDCl_3$) : 2.9 (m, 2H, $H_{2'}$) ; 3.3 (m, 2H, $H_{5'}$) ; 3.7 (s, 6H, OCH_3) ; 4.1 (m, 2H, $H_{4'}$) ; 5.0 (m, 1H, $H_{3'}$) ; 6.4 (m, 1H, $H_{1'}$) ; 6.8-7.5 (m, 16H, Har), 8.1 (bb, 1H, N$\underline{H}$). ^{13}C NMR (50.32 MHz, $CDCl_3$) : 38.8 ($C_{2'}$) ; 55.0 (CH_3 DMT) ; 64.7 ($C_{5'}$) ; 73.8 ($C_{3'}$) ; 86.3 (C_{quat} DMT) ; 87.6 - 88.0 ($C_{1'}$, $C_{4'}$) ; 113.0 (C_{ar} DMT) ; 114.8 (C_{4a}) ; 115.3 (C_8) ; 124.2 (C_6) ; 126.7-127.7 (C_{ar} DMT) ; 129.8 (C_{ar} DMT; C_5) ; 135.6 (C_{quat} DMT,) ; 141.7 (C_{8a}) ; 142.1 (C_7) ; 144.4 (C_{quat} DMT,) ; 149.8 (C_2) ; 158.3 (C quat DMT) ; 161.1 (C_4). **7dβ** : ^1H NMR (200 MHz, $CDCl_3$) : 2.2-2.8 (m, 2H, $H_{2'}$) ; 3.4 (m, 2H, $H_{5'}$) ; 3.7 (s, 6H, OCH_3) ; 3.9 (m, 1H, $H_{4'}$) ; 4.6 (m, 1H, $H_{3'}$) ; 6.6 (m, 1H, $H_{1'}$) ; 6.7-7.4 (m, 16H, H_{ar}, H_5, H_7, H_8) ; 8.1 (d, 1H, N$\underline{H}$). ^{13}C NMR (50.32 MHz, $CDCl_3$) : 36.7 ($C_{2'}$) ; 55.1 (CH_3 DMT) ; 63.9 ($C_{5'}$) ; 72.5 ($C_{3'}$) ; 84.3 ($C_{1'}$, $C_{4'}$) ; 86.5 (C_{quat} DMT) ; 113.1 (C_{ar} DMT) ; 114.9 (C_{4a}) ; 116.0 (C_8) ; 124.0 (C_6) ; 126.8-127.8-128.0 (C_{ar} DMT) ; 130.0 (Car DMT, C_5) ; 135.7 (C_{quat} DMT) ; 140.8 (C_{8a}) ; 141.4 (C_7) ; 144.5 (C_{quat} DMT) ; 149.4 (C_2) ; 158.4 (Cquat DMT) ; 160.9 (C_4).

1-(2-deoxy-α-D-erythro-pentofuranosyl)-7-chloro-quinazoline-2,4-(3H)-dione (6dα)

The analogue **6dα** was prepared as described for **6aα** using 0.3 mmol of **7dα**. Instead of a short silica gel column, **6dα** was precipitated in $CHCl_3$. Yield : 91 mg (90%). ^1H NMR (500MHz, $DMSOd_6$) : 2.28-2.52 (2m, 2H, $H_{2'}$) ; 3.53 (q, 2H, $H_{5'}$, ^3J = 3.4Hz) ; 4.08 (q, 1H, $H_{4'}$, ^3J = 4Hz) ; 4.38 (m, 1H, $H_{3'}$) ; 4.9-5.5 (2bb, 2H, OH) ; 7.33 (d, 1H, H_6, ^3J = 8.4Hz) ; 8.0 (dd, 1H, H_5, ^3J = 8.4Hz, ^5J = 1.5Hz) ; 8.08 (m, 1H, H_8). ^{13}C NMR (125 MHz, $DMSOd_6$) : 36.0 ($C_{2'}$) ; 61.3 ($C_{5'}$) ; 70.1 ($C_{3'}$) ; 84.0 ($C_{1'}$) ; 86.1 ($C_{4'}$) ; 114.8 (C_{4a}) ; 116.0 (C_8) ; 122.5 (C_6) ; 128.7 (C_5) ; 138.7 (C_7) ; 139.4 (C_{8a}) ; 149.5 (C_2) ; 160.2 (C_4).

1-(2-deoxy-β–D-erythro-pentofuranosyl)-7-chloro-quinazoline-2,4-(3H)-dione (6dβ)

The analogue **6dβ** was prepared as described for **6aα** using 0.04 mmol of **7dβ**. **6dβ** was purified on a short silica gel column eluted by methanol : ethyl acetate (60:40). Yield : 10.6 mg (85%). ^{1}H NMR (500 MHz, $DMSOd_6$) : 1.91 (m, 1H, $H_{2'a}$) ; 2.5 (m, 1H, $H_{2'b}$) ; 3.66 (m, 3H, $H_{5'}$, $H_{4'}$) ; 4.4 (q, 1H, $H_{3'}$, $^{3}J = 4$ Hz) ; 6.6 (t, 1H, $H_{1'}$, $^{3}J = 7.8$Hz) ; 7.3 (d, 1H, H_6, $^{3}J = 8.4$ Hz) ; 7.9 (d, 2H, H_5, $^{3}J = 8.4$ Hz) ; 8.0 (s, 1H, H8) . ^{13}C NMR (125.0 MHz, $DMSOd_6$) : 35.5 ($C_{2'}$) ; 59.9 ($C_{5'}$) ; 68.9 ($C_{3'}$) ; 83.0 ($C_{1'}$) ; 85.9 ($C_{4'}$) ; 114.9 (C_{4a}) ; 116.2 (C_8) ; 122.5 (C_6) ; 128.5 (C_5) ; 138.5 (C_7) ; 139.8 (C_{8a}) ; 149.8 (C2) ; 160.8 (C_4). HR-FAB Cacld for $C_{13}H_{14}N_2O_5Cl$ (M^{+}+1) : 313.0591 ; Found : 313.0596

1-(2-deoxy-3-O-(2-cyanoethoxy(diisopropylamino)-phosphino)-5-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl)-7-chloro-quinazoline-2,4-(3*H*)-dione (8d)

The analogue **8d** was prepared as described for **8a** using 0.316 mmol of **7dβ**. Yield : 210 mg (81%). Rf = 0.91 (ethyl acetate/cyclohexane/triethylamine, 45:45:10, v/v/v). MS (FAB^{-}) : 813.1 $(M\text{-}H)^{-1}$. ^{1}H NMR (200 MHz, $CDCl_3$) : 1.2 (m, 12H, iPr) ; 2.5 (m, 4H, $H_{2'}$, $C\underline{H}_2CN$) ; 3.6 (m, 6H, $C\underline{H}_2OP$, $C\underline{H}iPr$, $H_{5'}$) ; 3.72 (s, 6H, OCH_3) ; 4.0 (m, 1H, $H_{4'}$) ; 4.8 (m, 1H, $H_{3'}$) ; 6.7 (m, 1H, $H_{1'}$) ; 7.0-7.4 (m, 16H, H_{ar}, H_5, H_6, H_8) ; 8.1 (m, 1H, $N\underline{H}$). ^{13}C NMR (50.32 MHz, $CDCl_3$) : 20.2-21.1 ($\underline{C}H_2CN$) ; 24.5 ($\underline{C}H_3iPr$) ; 36.0 ($C_{2'}$) ; 43.0-43.3 ($\underline{C}HiPr$) ; 55.1 (CH_3DMT) ; 57.9-58.0 ($\underline{C}H_2OP$) ; 63.5 ($C_{5'}$) ; 73.0-73.1 ($C_{3'}$) ; 84.5 ($C_{1'}$, $C_{4'}$) ; 86.2 (C_{quat} DMT) ; 112.9 (C_{ar} DMT, C_8) ; 115.1 (C_{4a}) ; 116.2 (C_8) ; 124.0 (C_6) ; 126.6-127.6-128.2-130.1 (C_{ar} DMT) ; 130.2 (C_5) ; 135.7 (C_{quat} DMT) ; 140.9 (C_{8a}) ; 141.4 (C_7) ; 144.6 (C_{quat} DMT) ; 149.5 (C_2) ; 158.3 (C_{quat} DMT,), 161.1 (C_4). ^{31}P NMR (200 MHz, $CDCl_3$) : 147.7, 147.2.

6-Nitro-quinazoline-2,4-(1*H*,3*H*)-dione (3e)

Quinazoline-2,4-dione (4 g, 24.6 mmol) was dissolved in H_2SO_4 (8 ml), and HNO_3 65% (2.5 g, 25 mmol) was added slowly. The reaction was then poured on ice, the precipitate was filter off, washed with water, and cristallized from acetone:water (1:1, 100 ml/g). Yield : 3 g (57%). MS (EI) : 207.2. IR (KBr) : 3439, 3188, 3021, 1714, 1680, 1622. UV (CH_3OH) : λmax 312nm (ε = 7486). ^{1}H NMR (200 MHz, $DMSOd_6$) : 7.28 (d, 1H, H_8, $^{3}J = 8.8$ Hz) ; 8.4 (dd, 1H, H_7, $^{3}J = 8.8$ and $^{4}J = 2.3$ Hz) ; 8.55 (d, 1H, H_5, $^{4}J = 2.3$ Hz). ^{13}C (200 MHz, $DMSOd_6$) : 114.4 (C_{4a}) ; 116.6 (C_8) ; 123.0 (C_5) ; 129.4 (C_7) ; 141.7 (C_{8a}) ; 145.5 (C6) ; 149.8 (C2) ; 161.5 (C4).

1-(3,5-di-O-p-toluoyl-2-deoxy-α,β-D-erythro-pentofuranosyl)-6-nitro-quinazoline-2,4-(3H)-dione (5e)

The analogue **5e** was prepared as described for **5a** using 4.58 mmol of **3e**. Yield : 755 mg (50%). Rf = 0.14 (ethyl acetate/cyclohexane, 30:70, v/v). ^{1}H (200 MHz, $CDCl_3$) : 2.38 (s, 6H, CH_3 Tol) ; 2.9 (m, 2H, $H_{2'}$) ; 4.7 (m, 3H, $H_{4'}$ and $H_{5'}$) ; 5.6 (m, 1H, $H_{3'}$) ; 6.85 (m, 1H, $H_{1'}$) ; 7.45 (m, 11H, Har Tol, H_5, H_7 and H_8) ; 8.8 (bb, 1H, $N\underline{H}$). ^{13}C (50.32 MHz, $CDCl_3$) : 21.5 (CH_3 tol) ; 34.2-34.7 ($C_{2'}$) ; 62.9-64.5 ($C_{5'}$) ; 73.1-74.9 ($C_{3'}$) ; 81.5-81.7 ($C_{1'}$) ; 84.3-85.7 ($C_{4'}$) ; 117.1 (C_8) ; 124.4 (C_5) ; 126.1 (C_{4a}) ; 126.1-126.4 (C_{quat} Tol) ; 129.0 (C_7) ; 129.2-129.4 (C_{ar} Tol) ; 142.9 (C8a) ; 143.3-143.4 (C_{quat} Tol) ; 144.6 (C6) ; 149.5 (C_2) ; 160.0 (C_4) ; 165.8 (Carbonyl Tol).

1-(2-deoxy-α, β-D-erythro-pentofuranosyl)-6-nitro-quinazoline-2, 4-(3*H*)-dione (6e)

The analogue **6e** was prepared as described for **6a** using 1.3 mmol of **5e**. Yield : 400 (95%). Rf = 0.23 ($CH_3OH/CHCl_3$, 10:90, v/v).

1-(2-deoxy-5-(4,4'-dimethoxytrityl)-α,β-D-erythro-pentofuranosyl)-6-nitro-quinazoline-2,4-dione (7e)

The analogue **7e** was prepared as described for **7a** using 1.23 mmol of **6e**. The two anomers were purified by flash chromatography with a gradient of methanol in CH_2Cl_2 (0-6%) in presence of 1% triethylamine. Yield : 525 mg (72%, α:β = 53.5:46.5). Rf = 0,46 for 7eα and Rf = 0.21 for 7eβ (ethyl acetate/cyclohexane, 50:50, v/v).**7eα**: ^1H NMR (200 MHz, $CDCl_3$) : 2.7 (m, 2H, $H_{2'}$) ; 3.25 (m, 2H, $H_{5'}$ and $H_{4'}$) ; 3.77 (s, 6H, OCH_3) ; 4.4 (m, 1H, $H_{3'}$) ;5.9 (bb, 1H, O$\underline{H}$) ; 6.8 (m, 1H, $H_{1'}$) ; 7.1-7.6 (m, 16H, Har), 9.0 (bb, 1H, N$\underline{H}$). ^{13}C NMR (200 MHz, $CDCl_3$) : 38.8 ($C_{2'}$) ; 55.1 (CH_3 DMT) ; 65.0 ($C_{5'}$) ; 74.0 ($C_{3'}$) ; 86.5 (C_{quat} DMT) ; 87.4 ($C_{1'}$) ; 88.1 ($C_{4'}$) ; 113.1 (C_{ar} DMT) ; 115.0 (C_8) : 118.4 (C_{4a}) ; 124.2 (C_5) ; 126.8 (C_7) ; 127.8-128.0-129.9 (C_{ar} DMT) ; 135.7-135.9 (C_{quat} DMT) ; 142.3 (C_{8a}) ; 144.6 (C_{quat} DMT) ; 146.9 (C_6) ; 155.0 (C_2) ; 158.5 (Cquat DMT) ; 166.9 (C_4). **7eβ** :^1H NMR (200 MHz, $CDCl_3$) : 2.2 (m, 1H, $H_{2'a}$) ; 2.75 (m, 1H, $H_{2'b}$) ; 3.5 (m, 3H, $H_{5'}$) ; 3.7 (s, 6H, OCH_3) ; 4.0 (m, 1H, $H_{4'}$) ; 4.85 (m, 1H, $H_{3'}$) ; 6.2 (bb, 1H, O$\underline{H}$) ; 6.7-7.5 (m, 17H, H_{ar}, $H_{1'}$, H_5, H_7, H_8) ; 8.9 (bb, 1H, N$\underline{H}$). ^{13}C NMR (200 MHz, $CDCl_3$) : 36.8 ($C_{2'}$) ; 55.1 (CH_3 DMT) ; 62.1 ($C_{5'}$) ; 70.4 ($C_{3'}$) ; 84.0 ($C_{1'}$) ; 85.1 ($C_{4'}$) ; 86.7 (C_{quat} DMT) ; 113.1 (C_{ar} DMT) ; 117.2 (C_{4a}) : 118.6 (C_8) ; 124.1 (C_5) ; 127.1 (C_7) ; 127.8-128.4-128.9-130.2 (C_{ar} DMT) ; 135.4 (C_{quat} DMT) ; 142.9 (C_{8a}) ; 143.9 (C_6) ; 149.9 (C_{quat} DMT) ; 150.5 (C_2) ; 158.6 (Cquat DMT) ; 161.2 (C_4).

1-(2-deoxy-α-D-erythro-pentofuranosyl)-6-nitro-quinazoline-2,4-(3*H*)-dione (6eα)

The analogue **6eα** was prepared as described for **6aα** using 0.1 mmol of 7eα. Yield : 30 mg (93%). Rf = 0.2 ($CH_3OH/CHCl_3$, 10:90, v/v). ^1H NMR (200 MHz, $DMSOd_6$) : 2.39 (m, 2H, $H_{2'}$) ; 3.5 (m, 2H, $H_{5'}$) ; 4.1 (m, 1H, $H_{4'}$) ; 4.3 (m, 1H, $H_{3'}$) ; 6.0 (bb, 2H, O$\underline{H}$) ; 6.6 (t, 1H, $H_{1'}$, ^3J = 7.3 Hz) ; 8.0 (d, 1H, H_8, ^3J = 9.8 Hz) ; 8.37 (dd, 1H, H_7, ^3J = 9.3 Hz, ^4J = 3 Hz) ; 8.67 (d, 1H, H_5, 4J = 3 Hz). ^{13}C NMR (50.32 MHz, $DMSOd_6$) : 36.7 ($C_{2'}$) ; 61.7 ($C_{5'}$) ; 70.4 ($C_{3'}$) ; 84.7-86.2 ($C_{1'}$, $C_{4'}$) ; 117.6 (C_8, C_{4a}) ; 122.9 (C_5) ; 127.9 (C_7) ; 141.7 (C_{8a}) ; 144.2 (C_6) ; 151.3 (C_2) ; 162.0 (C_4).

1-(2-deoxy-β-D-erythro-pentofuranosyl)-6-nitro-quinazoline-2,4-(3H)-dione (6eβ)

The analogue **6eβ** was prepared as described for **6aα** using 0.1 mmol of 7eβ. Yield : 30 mg (93%). Rf = 0.2 ($CH_3OH/CHCl_3$, 10:90, v/v). ^1H NMR (500 MHz, $DMSOd_6$) : 1.95-2.6 (2m, 2H, $H_{2'}$) ; 3.7 (m, 3H, $H_{5'}$, $H_{4'}$) ; 4.4 (m, 1H, $H_{3'}$) ; 5.9 (bb, 2H, O$\underline{H}$) ; 6.7 (t, 1H, $H_{1'}$, ^3J = 7.3 Hz) ; 8.0 (d, 1H, H_8, ^3J = 9.8 Hz) ; 8.3 (dd, 1H, H_7, ^3J = 9.8 Hz, ^4J = 3 Hz) ; 8.65 (d, 1H, H_5, 4J = 3 Hz). ^{13}C NMR (125.0 MHz, $DMSOd_6$) : 36.2 ($C_{2'}$) ; 60.6 ($C_{5'}$) ; 69.4 ($C_{3'}$) : 83.7-86.5 ($C_{1'}$, $C_{4'}$) ; 117.6 (C_{4a}) ; 117.7 (C_8) ; 122.6 (C_5) ; 127.3 (C_7) ; 141.5 (C_{8a}) ; 144.3 (C_6) ; 151.2 (C_2) ; 162.0 (C_4). HR-FAB Cacld for $C_{13}H_{14}N_3O_7$ (M^++1) : 324.0831 ; Found : 324.0818

1-(2-deoxy-3-O-(2-cyanoethoxy(diisopropylamino)-phosphino)-5-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl)-6-nitro-quinazoline-2,4-(3H)-dione (8e)

The analogue **8e** was prepared as described for **8a** using 0.33 mmol of 7eβ. Crude phosphoramidite was purified twice by flash chromatography with ethyl acetate:triethylamine:CH_2Cl_2 (45:10:45) as eluent. Yield : 63 mg (23%). Rf = 0.63 (ethyl acetate/cyclohexane, 50:50, v/v). MS (FAB^-) = 824.5 $(M-H)^{-1}$
^1H NMR (200 MHz, $CDCl_3$) : 1.1 (m, 12H, iPr) ; 2.15-3.3 (m, 6H, $H_{2'}$, C$\underline{H_2}$CN, C$\underline{H_2}$OP) ; 3.6 (m, 4H, C$\underline{H}$iPr, $H_{5'}$) ; 3.77 (s, 6H, OCH_3) ; 4.1 (m, 1H, $H_{4'}$) ; 5.0 (m, 1H, $H_{3'}$) ; 6.8-7.4 (m, 17H, Har, $H_{1'}$.); 8.9(m, 1H, N$\underline{H}$). ^{13}C NMR (50.32 MHz, $CDCl_3$) : 20.4 ($\underline{C}H_2CN$) ; 23.42 ($\underline{C}H_3$iPr) ; 35.7 ($C_{2'}$) ; 43.3 ($\underline{C}$HiPr) ; 55.1 (CH_3DMT) ; 57.9 (C$\underline{H_2}$OP) ; 61.4 ($C_{5'}$) ; 72.0 ($C_{3'}$) ; 83.8 ($C_{4'}$) ; 84.5 ($C_{1'}$) ; 86.8 (C_{quat} DMT) ; 113.1 (C_{ar} DMT) ; 117.3 (C_{4a}, CN) ; 118.6 (C_8) ; 124.3 (C_5) ; 127.2-127.9-128.5 (C_{ar} DMT) ;

129.1 (C_7) ; 130.2 (C_{ar} DMT) ; 135.1 (C_{quat} DMT) ; 143.0 (C_{8a}) ; 143.5 (C_{quat} DMT) ; 143.6 (C6) ; 150.3 (C_2) ; 158.7 (C_{quat} DMT) ; 160.9(C_4). ^{31}P NMR (200 MHz, $CDCl_3$) : 147.5, 147.7.

Synthesis of oligonucleotides

All oligonucleotides are synthesized at 0.2 µmol-scale on a Millipore Expedite 8909 synthesizer using conventional β-cyanoethyl phosphoramidite chemistry. Standard phosphoramidites were purchased from Perseptive Biosystem, C-5 propynyl-2'-deoxyuridine phosphoramidite was from Glen Research. Coupling time was increased to 15 min. for all modified phosphoramidites. The coupling efficiency was the same as unmodified amidites as juged by detritylation profiles (>98%). All oligomers were synthesized 'trityl on' and were deprotected overnight by fresh concentrated NH_4OH at 55°C. The crude tritylated oligonucleotides were purified by reverse-phase HPLC (column Nucleoside 300-5 C18) using the following gradient system : A (triethylammonium acetate 0.1 M, pH 7) ; B (0.1 M triethylammonium acetate in 80% acetonitrile). A linear gradient of 0-60% buffer B over 60min at a flow of 1ml/min. was used. Detection was done at 260 nm for analytical runs and 290 nm for preparative ones. After collection oligomers were evaporated and detritylated for 1 h by 1 ml of 80% acetic acid solution. The solution was then evaporated, the residue resuspended in 1ml of water and extracted by ethyl acetate. Finally oligonucleotides wre precipitated using n-butanol. When required aliquots aliquots of purified oligonucleotides were analysed by polyacrylamide/urea gel electrophoresis to confirm the expected length and purity.

Melting experiments

0.5 nmoles of each purified oligonucleotide were dissolved in 0.5 ml of the appropriate buffer and boiled for 2 minutes. The buffer used was : 10 mM sodium cacodylate (pH = 7), 100 mM NaCl, 10 mM magnesium acetate, 1 mM spermine. Samples were kept at least 30 minutes at 4°C and were then heated from 4°C to 90°C at a rate of 0.5°C/min, the absorbance at 260 nm was measured every 30 seconds. {Oligonucleotides (0,25 nmol of each) were mixed together with the buffer and boiled 3 min. and then cooled down. The solution were then heated from 4 to 80°C at a rate of 0.5°C per minute and A_{260} was recorded against temperature. }

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