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Gemcitabine, Pyrrologemcitabine and 2'-Fluoro- 2'-Deoxycytidines: Synthesis, Physical Properties and Impact of Sugar Fluorination on Silver Ion Mediated Base Pairing

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Abstract

The stability of silver mediated “dC-dC” base pairs relies not only on the structure of the nucleobase but also is sensitive to structural modification on the sugar moiety. 2'-Fluorinated 2'-deoxycytidines with fluorine atoms in the arabino (up) and the ribo (down) configuration, as well as with geminal fluorine substitution (anticancer drug gemcitabine) and the novel fluorescent phenylpyrrolo-gemcitabine (^{ph}PyrGem) were synthesized. All nucleosides display the recognition face of naturally occurring 2'-deoxycytidine. Nucleosides were converted into phosphoramidites and incorporated in 12-mer oligonucleotides by solid-phase synthesis. Addition of silver ions to DNA duplexes with a fluorine modified “dC-dC” pair near central position led to significant duplex stabilization. The stability increase was higher for duplexes with fluorinated sugar residues than those with the unchanged 2'-deoxyribose moiety. Similar observations were made on “dC-dT” pairs and to a minor extend on the “dC-dA” pairs. The increase of silver ion mediated base pair stability was reversed by annulation of a pyrrole ring to the cytosine moiety as shown for 2'-fluorinated ^{ph}PyrGem compared to phenylpyrrolo-dC (^{ph}PyrdC). The phenomenon results from stereoelectronic effects induced by fluoro substitution which are transmitted from the sugar moiety to the silver ion mediated base pairs. This depends on the number of fluorine substituents, their configuration and the structure of the nucleobase.

Introduction

Metal mediated base pairs can function as an alternative (orthogonal) pairing system to canonical Watson-Crick base pairing thereby forming double helical DNA and RNA.[1] The introduction of metal-based functionalities provide nucleic acids unique chemical, physical and biological properties.-It allows the development of metal ion sensors, molecular wires, DNA-based logic gates, and detection systems for single nucleotide polymorphisms.[2a-g] Furthermore, metal mediated base pairs can be used to alter molecular properties of nucleic acids such as electrical conductivity or charge-transfer.[2d,2h] The replacement of hydrogen bonds within DNA base pairs by metal interactions might be used to expand the genetic coding system.

As the persistence length of 50 nm makes DNA a rigid rod, metal DNA represents a unique hybrid biomaterial with application in biotechnology and for the construction of nanodevices. Recent developments on metal responsive systems demonstrated the utility of nucleic acid metal ion assemblies.[1,2a-b] Nevertheless, applications utilizing metal DNA are still in an initial state.

Amongst the various metal ions forming metal mediated base pairs, silver mediated base pairs found special attention.[3] Silver ions have the capacity to interact with DNA or RNA[4] and form extremely stable base pairs that surpass stability of canonical Watson-Crick pairs stabilized by hydrogen bonds. An interesting aspect of the Ag^+ ion is their selective binding to nucleobases instead to the phosphodiester backbone. Often, metal mediated base pairs are formed between identical nucleobases (homo base pairs).[5] In this regard, the dC-dC homo pair is of particular importance. It is naturally occurring and forms stable silver ion complexes [6] as well as stable silver mediated dC-dC homo base pairs and pairs between anomeric dC nucleosides.[7] One or two silver ions can be captured by one base pair[8] and silver-mediated base pairs are recognized by polymerases during DNA-amplification.[9] An X-ray structure of a RNA duplex gave a detailed picture on the molecular structure of the C-Ag^+-C interaction

within the RNA double helix.[10a] More recently, the crystal structure of a DNA duplex was reported which forms silver(I)-DNA hybrid nanowires consisting of silver-mediated base pairs formed by natural nucleobases.[10b]

Earlier work on the Watson-Crick base pairing system has shown that structural modifications on the nucleobases have a strong impact on the base pair stability. Regarding silver mediated base pairs similar observations have been made.[11] The annulation of a pyrrole ring or an imidazole moiety to the cytosine base led to very stable metal-mediated DNA. So far, pyrrolo-dC and imidazolo-dC form the most stable silver mediated "dC-dC" base pairs in double helical DNA.[12] As pyrrolo-dC and imidazolo-dC are strongly fluorescent, the silver mediated base pairs are also fluorescent providing the system interesting new properties for detection. Nevertheless, also ribose modification can affect base pair stability as it is reported for DNA *vs* RNA.[13] As almost nothing is known on the impact of sugar modification on silver mediated base pairs formed by canonical bases in DNA we anticipated that structural changes on the sugar moiety can alter the properties of silver mediated base pairing as well. This was considered as stereoelectronic effects induced in the sugar moiety might be transmitted to the nucleobase. To this end, we selected the fluorine atom to functionalize the 2'-position in the dC-Ag^+-dC pair. Fluorine substituents are known to stabilize the anomeric center with regard to glycosylic bond hydrolysis due to the negative inductive effect. Furthermore, the beneficial influence of fluorine substituents is well established in therapeutic applications of nucleosides and oligonucleotides. The 2'-difluoro-dC nucleoside gemcitabine **3** is used for therapeutic application as drug to treat pancreatic cancer. Antisense oligonucleotides containing fluorine atoms at the 2'-position of the ribose moiety have shown to develop remarkable properties and act as polymeric drugs.[14]

This work investigates the influence of 2'-fluoro substituents on the formation and stability of silver mediated homo "dC-dC" and "pyrrolo-dC-pyrrolo-dC" base pairs. The fluorinated nucleosides used in this study are shown in Figure 1. These nucleosides contain cytosine or

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cytosine derivatives as nucleobases that all show the recognition face of the naturally occurring nucleoside. Some of them were known (**1-4**)[15] others had to be synthesized (**5-6**). Phosphoramidites were prepared and used as building blocks for solid phase synthesis. The influence of 2'-fluoro up (arabino), 2'-fluoro down (ribo) and geminal fluorine functionalization was studied with regard to metal ion mediated base pairing. Also, an anticipated fluorine effect was compared between dC- and phenylpyrrolo-dC residues. This included the anticancer drug gemcitabine having two geminal fluorine atoms in the 2'-position.

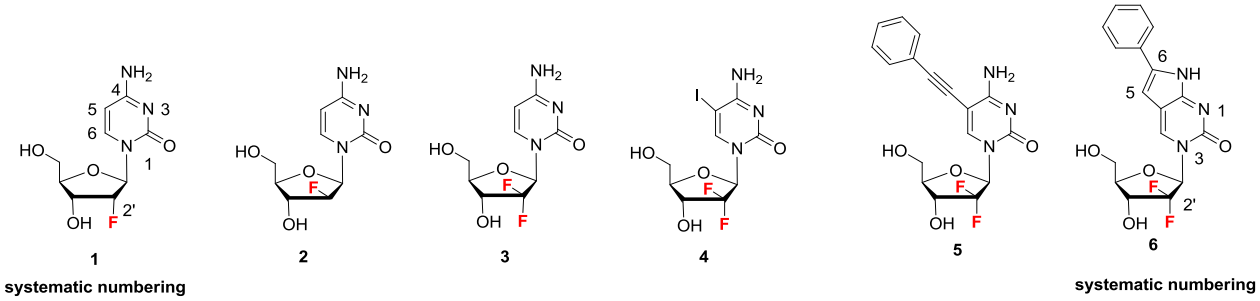

Figure 1. Structures of modified dC nucleosides.

Results and Discussion

1. Synthesis of Monomers and their Physical Properties.

For this study nucleosides **1** to **6** were synthesized. Syntheses of nucleosides **1-4**[15] were performed according to reported literature protocols whereas the syntheses of compounds **5** and **6** are described in this manuscript.

To access ^{ph}PyrGem (**6**) two different synthetic routes (Schemes 1 and 2) were exercised. In both cases gemcitabine (**3**) was used as starting material and iodinated to 5-iodo-gemcitabine (**4**). Iodination of **3** has been already reported[15f] but the reaction was performed on a milligram scale with moderate yield (58%). Moreover, glacial acetic acid was utilized as reaction medium which complicated work-up.[15f] Now, a multigram scale protocol was

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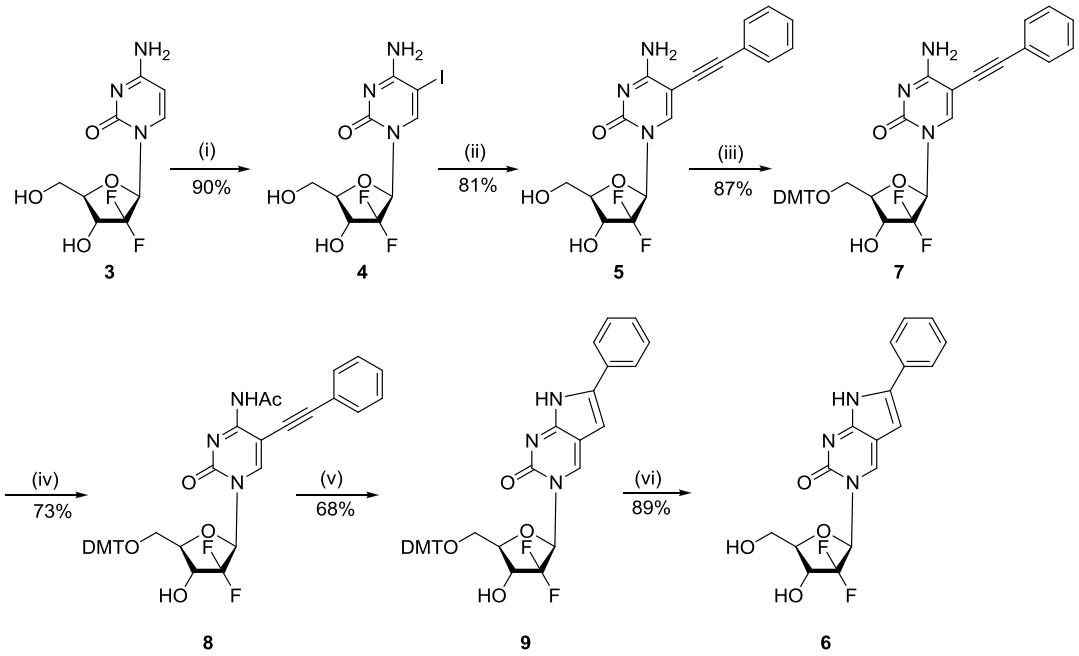
developed using ethanol as solvent. Due to this change, the yield was increased to 90%, the work up of the reaction mixture was simplified and gram amounts of 5-iodo-gemcitabine (**4**) became easily accessible (Scheme 1).

In the first route, 5-iodo-gemcitabine (**4**) was converted to **5** (81% yield) by *Sonogashira* cross-coupling (DMF, DIPEA, [$Pd(PPh_3)_4$], CuI), with a 4-fold excess of phenylacetylene (Scheme 1). Protection of the 5′-OH group of **5** with a 4,4’-dimethoxytrityl chloride followed by amino group blocking with an acetyl residue gave nucleoside **8**. Formation of the pyrrole system was achieved by intramolecular cyclization using CuI and triethylamine (→ **9**). Final cleavage of the DMT residue with trichloroacetic acid gave nucleoside **6** in 31% overall yield (5 steps) starting from 5-iodo-gemcitabine (**4**).



Scheme 1. Synthesis of nucleosides **4**-**6**. Reagents and conditions: (i) CCl_4, I_2, HIO_3, EtOH, 45 °C, 16 h; (ii) $Pd(PPh_3)_4$, CuI, DIPEA, phenylacetylene, DMF, rt, 16 h; (iii) DMT-Cl, pyridine, rt, 12 h; (iv) acetic anhydride, DMF, rt, 12 h; (v) CuI, triethylamine, DMF, 60 °C, 24 h; (vi) trichloroacetic acid (TCA), rt, 15 min.

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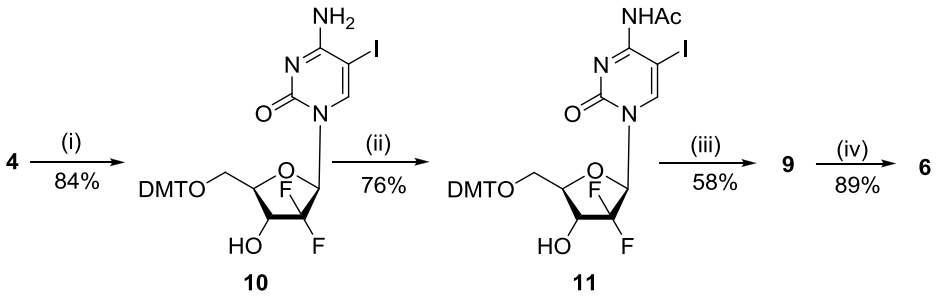
In the second route, the 5'-OH group of 5-iodo-gemcitabine (**4**) was protected with a DMT residue and the amino group with an acetyl group to yield the protected iodo nucleoside **11** (Scheme 2). Then, *Sonogashira* cross-coupling of **11** with phenylacetylene and subsequent cyclization gave nucleoside **9** in 58% in two steps. Cleavage of the DMT residue gave ^{ph}PyrGem (**6**) in 33% overall yield (4 steps). Both routes gave comparable overall yields but the second route is one step shorter.



Scheme 2. Synthesis of nucleoside **6**. Reagents and Conditions: (i) DMT-Cl, pyridine, rt, 12 h; (ii) acetic anhydride, DMF, rt, 12 h; (iii) $Pd(PPh_3)_4$, CuI, triethylamine, phenylacetylene, DMF, rt, 16 h; (iv) trichloroacetic acid (TCA), rt, 15 min.

Recently, Hudson et al reported that 4,4'-dimethoxytritylation of 6-arylpyrrolocytidine analogs resulted in a sluggish reaction.[16] Therefore, they added triethylamine to the reaction mixture. However, this gave C7-DMT-subtituted arylpyrrolocytidines as main products.[16] We found, when ^{ph}PyrGem (**6**) was treated with 3.5 eq. of DMT-Cl in the absence of triethylamine for 6 h the 5'-*O*-DMT protected nucleoside **9** was formed in 87% yield without formation of a C7-functionalized DMT product. Under the same reaction conditions the DMT protected nucleoside **13** was obtained from ^{ph}PyrdC (**12**) in 70% yield. Also in this case formation of a C-7-DMT product was not observed. Most probably, triethylamine activates the 7-position of the nucleobase for electrophilic attack as described for the Mannich reaction or for electrophilic halogenation.[17]

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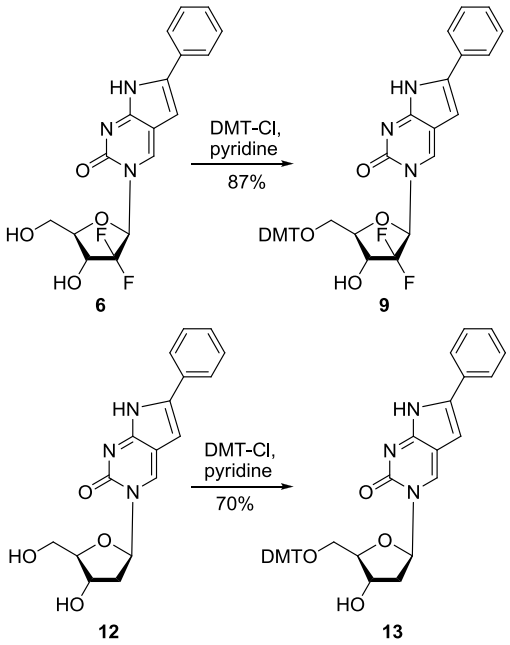

Scheme 3. Synthesis of DMT-protected nucleosides **9** and **13**.

Next, the physical properties of fluorinated nucleosides were investigated as they are considered to be of importance in regard to formation of silver mediated base pairs. Figure 2 shows the chromatographic behavior of nucleosides on hydrophobic RP-18 silica resin and demonstrates the influence of fluorine substituents on the lipophilicity of various cytidine (Figure 2a) and pyrrolo-dC derivatives (Figure 2b). In the cytidine series, the ribonucleoside is the most hydrophilic compound whereas gemcitabine the most lipophilic molecule. However, also antipodal changes of 2'-fluoro substituents have a significant impact. The 2'-fluoro-up nucleoside **2** is significantly more lipophilic as nucleoside **1** with the fluorine atom in down position. Figure 2b demonstrates the increasing lipophilicity of ^{ph}PyrdC (**12**) to ^{ph}PyrGem (**6**) and the even higher lipophilicity of the open chain ^{ph}PyrGem precursor **5**.

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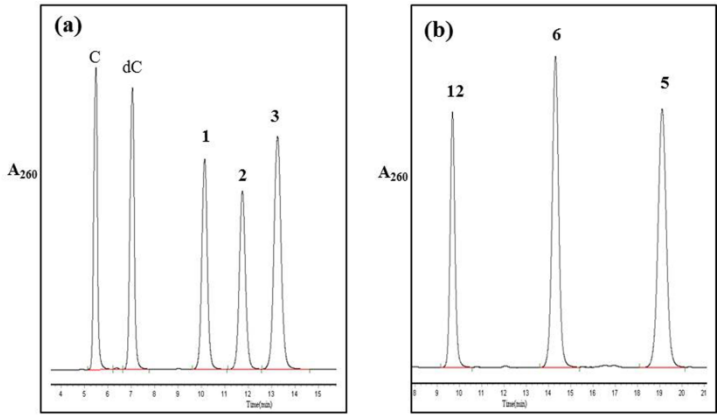

Figure 2. Reversed-phase HPLC elution profiles of nucleosides: a) artificial mixture of cytidine, 2'-deoxycytidine and 2'-deoxy-2'-fluorocytidine nucleosides **1**, **2**, and **3** monitored at 260 nm using gradient system III; b) artificial mixture of nucleosides **5**, **6**, and **12** monitored at 260 nm using gradient system IV.

Furthermore, it has been found that fluorine substituents on the sugar moiety change the conformation compared to ribo- or deoxyribonucleosides. Whereas a fluorine substituent in the 2'-fluoro-up position enhances the preference for the S-type pseudorotamers, the fluorine substituent in the down position increases the amount with N-type conformation. Geminal fluoro substituents enhance the preference of an S-type sugar pucker in the solid-state, whereas the N-type pucker is preferred within an oligonucleotide.[18] Changes on the pK_a value of the nucleobase are also observed going back to the fluorine modification of the sugar residue. As already reported 2'-deoxycytidine (dC, pK_a = 4.3)[12b] has a similar pK_a value as cytidine (C, pK_a = 4.3).[19a] However, mono-fluoro functionalization at the 2'-position reduces the pK_a value independently from fluoro-down (**1**, pK_a = 3.9)[15d] or fluoro-up (**2**, pK_a = 3.9)[19b] positioning. Gemcitabine (**3**, pK_a = 3.6)[19c] shows the lowest pK_a value due to the two geminal electronegative fluorine substituents. The same is true for the pK_a values of ^{ph}PyrGem (**6**, pK_a = 2.3 and 11.0, see Figure S1) which are lower than those of ^{ph}PyrdC (**12**, pK_a = 2.7 and 11.5).[12b]

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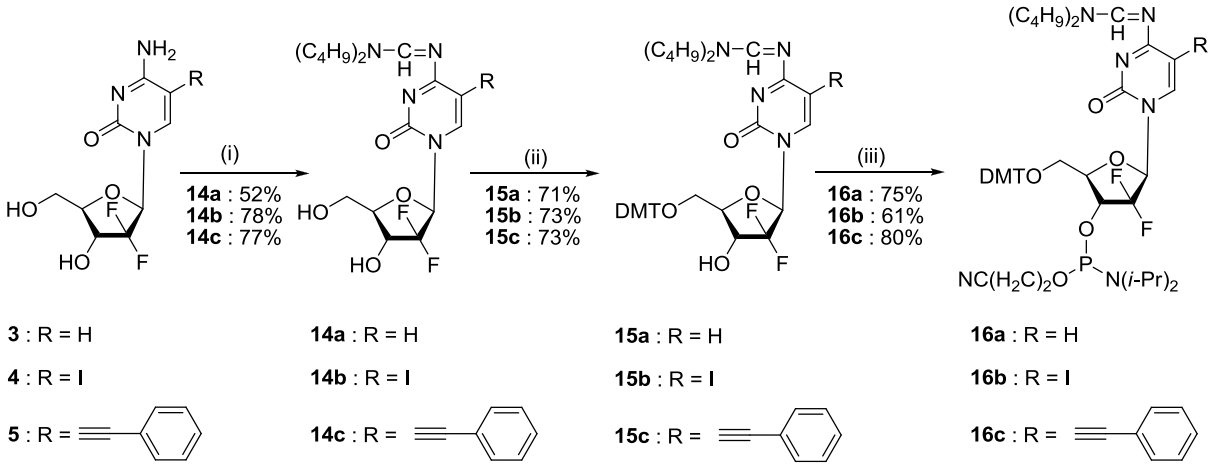
For oligonucleotides to be accessed, nucleosides **3**-**6** were converted into phosphoramidite building blocks **16a**-**16c** and **17** (Schemes 4 and 5) whereas the syntheses of phosphoramidites of nucleosides **1** and **2** were already reported (for structures see Figure S2).[20, 21] Also, a phosphoramidite building block of **3**[22] was described with a benzoyl protected amino group. For our study and better solubility we prepared dibutylformamidine derivatives. First, nucleosides **3**-**5** were protected at the 4-amino group with a dibutylformamidine residue affording the protected intermediates **14a** (52%), **14b** (78%) and **14c** (77%) respectively. Then, compounds **14a**-**14c** were converted to 5′-*O*-DMT derivatives **15a** (71%), **15b** (73%) and **15c** (73%), respectively. Finally, phosphitylation of nucleosides **15a**-**15c** under standard conditions yielded the phosphoramidites **16a** (75%), **16b** (61%) and **16c** (80%) respectively (Scheme 4).



Scheme 4. Synthesis of phosphoramidites **16a**-**16c**. Reagents and conditions: (i) *N*,*N*-dibutylformamid dimethylacetal, methanol, 30 °C; (ii) DMT-Cl, pyridine, rt, 12 h; (iii) DIPEA, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, CH_2Cl_2, rt, 30 min.

The 5′-*O*-DMT protected nucleoside **9** was converted to phosphoramidite **17** in 89% yield (Scheme 5). All new synthesized compounds were characterized by ^{1}H-, ^{13}C-NMR spectra as

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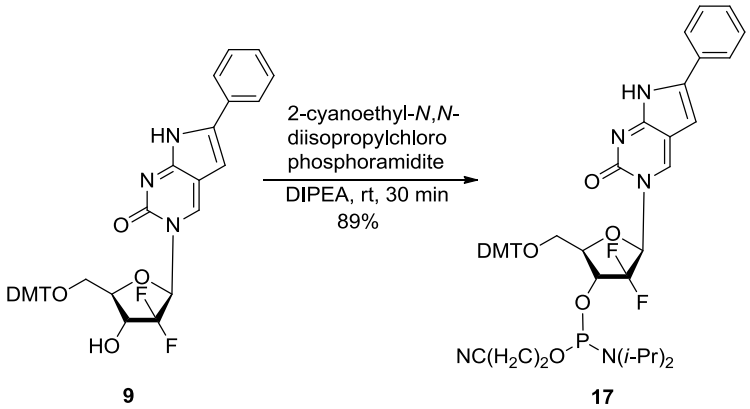
well as ESI-TOF mass spectra (see Experimental section). The ^{1}H-^{13}C correlated (HMBC and HSQC) NMR spectra were used to assign the ^{13}C NMR signals. For details see the Experimental section (for spectra, see the Supporting Information).



Scheme 5. Synthesis of phosphoramidite **17**.

2. Synthesis, Characterization and Photophysical Properties of Oligonucleotides.

Oligonucleotides (ODNs) containing nucleosides **1**-**6** were prepared on solid-phase using the phosphoramidites of nucleosides **1**-**2**, phosphoramidites **16a**-**16c** and **17** together with standard building blocks. Nucleosides **1**-**6** were incorporated in central positions of the oligonucleotides 5′-d(TAG GTC AAT ACT) (ODN-**1**) and 3′-d(ATC CAG TTA TGA) (ODN-**2**) replacing dC and dG residues. After solid-phase synthesis, the oligonucleotides were cleaved from the solid support and deprotected in concentrated 28% aqueous ammonia at 55 °C for 2 h. The coupling yields of the modified building blocks were always higher than 95%. The ODNs containing nucleoside **6** (^{ph}PyrGem) are not stable in ammonia at elevated temperature. Therefore, oligonucleotides containing nucleoside **6** were deprotected in 28% aqueous ammonia for 16 h at room temperature. All synthesized oligonucleotides were purified by reversed-phase HPLC (RP-18), detritylated with 2.5% dichloroacetic acid in dichloromethane and again purified by HPLC. The contents of single peaks were isolated in all cases (Figure S3, Supporting Information). Subsequently, the molecular masses were

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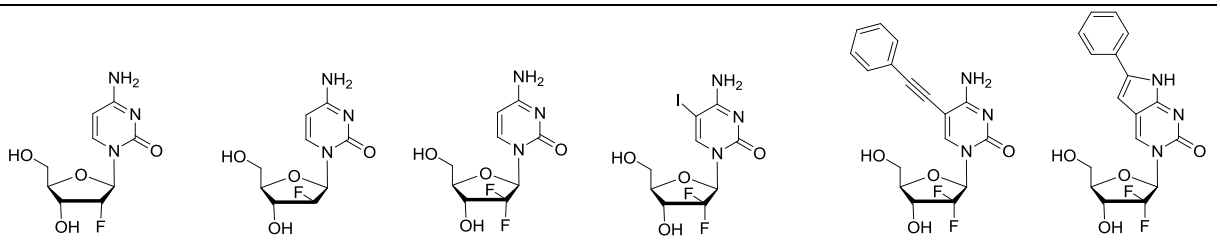
determined by MALDI-TOF mass spectra. Table 1 displays all modified oligonucleotides used in this study together with their mass spectrometric data.

Table 1. Synthesized oligonucleotides and their molecular masses measured by MALDI-TOF mass spectra.

Entry	Oligonucleotide	M.W. calcd.[a] M.W. found [b]	Entry	Oligonucleotide	M.W. calcd.[a] M.W. found [b]
ODN-**1**	5'-d-(TAG GT**C** AAT ACT)	3603.6 3604.4	ODN-**11**	5'-d-(TAG GT**4** AAT ACT)	3806.3 3806.3
ODN-**2**	5'-d(AGT ATT **G**AC CTA)	3643.6 3643.7	ODN-**12**	5'-d(AGT ATT **4**AC CTA)	3766.3 3765.8
ODN-**3**	5'-d(AGT ATT **A**AC CTA)	3627.6 3629.2	ODN-**13**	5'-d-(TAG GT**5** AAT ACT)	3780.5 3780.2
ODN-**4**	5'-d(AGT ATT **T**AC CTA)	3618.6 3619.3	ODN-**14**	5'-d(AGT ATT **5**AC CTA)	3740.5 3740.4
ODN-**5**	5'-d-(TAG GT**1** AAT ACT)	3662.4 3662.3	ODN-**15**	5'-d-(TAG GT**6** AAT ACT)	3780.5 3781.5
ODN-**6**	5'-d(AGT ATT **1**AC CTA)	3622.4 3622.8	ODN-**16**	5'-d(AGT ATT **6**AC CTA)	3740.5 3739.9
ODN-**7**	5'-d-(TAG GT**2** AAT ACT)	3662.4 3664.3	ODN-**17**	5'-d-(TAG GT**12** AAT ACT)[12a]	
ODN-**8**	5'-d(AGT ATT **2**AC CTA)	3622.4 3622.8	ODN-**18**	5'-d(AGT ATT **12**AC CTA)[12a]	
ODN-**9**	5'-d-(TAG GT**3** AAT ACT)	3680.4 3680.3	ODN-**19**	5'-d(AGT ATT **C**AC CTA)[11b]	
ODN-**10**	5'-d(AGT ATT **3**AC CTA)	3640.4 3640.6			



1 **2** **3** **4** **5** **6**

[a] Calculated on the basis of molecular weight as $[M+H]^+$. [b] Determined by MALDI-TOF mass spectrometry as $[M+H]^+$ in the linear positive mode.

Phenylpyrrolo-dC (^{ph}PyrdC) is commonly known as a strongly fluorescent nucleoside containing a 2-oxopyrrolo[2,3-*d*]pyrimidine "isohypoxanthine" system.[23] Therefore, it is widely used to explore the structure and dynamics of nucleic acids.[24] Consequently, we investigated the fluorescence properties of the 2'-difluoro modified ^{ph}PyrGem **6**. Fluorescence measurement of **6** was performed and compared to ^{ph}PyrdC (**12**).[12b] Resulting spectra are

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displayed in Figure 3. Phenylpyrrolo nucleosides **6** and **12** show almost identical UV maxima (359 nm for **6**, and 358 nm for **12**, Figure S4), fluorescence maxima (461 nm for **6**, and 458 nm for **12**) and Stoke's shift (102 nm for **6**, and 100 nm for **12**). The fluorescence intensity of ^{ph}PyrGem (**6**) is about 1.4 fold lower than that of ^{ph}PyrdC (**12**). Stereoelectronic effects induced by geminal fluorine atoms might be responsible for these changes.

Next, the photophysical properties of ^{ph}PyrGem (**6**) in a single-stranded (ss) oligonucleotide and an oligonucleotide duplex (ds) were investigated. Identical concentrations in aqueous buffer were used. A significant UV/vis bathochromic shift (11 nm) is observed when phenylpyrrolo gemcitabine (**6**) is incorporated in single-stranded ODN-**15** and a 7 nm UV/vis bathochromic shift is observed when the duplex ODN-**15**•ODN-**2** is formed (Figure S4). Compound **6** and single-stranded ODN-**15** show almost identical fluorescence maxima (461 nm) with no fluorescence quenching among nucleoside **6** and ODN-**15**. In contrary, the fluorescence is strongly decreased upon oligonucleotide duplex formation (ODN-**15**•ODN-**2**) because of increased stacking interactions with neighboring bases. The duplex ODN-**15**•ODN-**2** containing **6** shows fluorescence at a wavelength similar to that of the monomeric nucleoside **6** and ODN-**15**.

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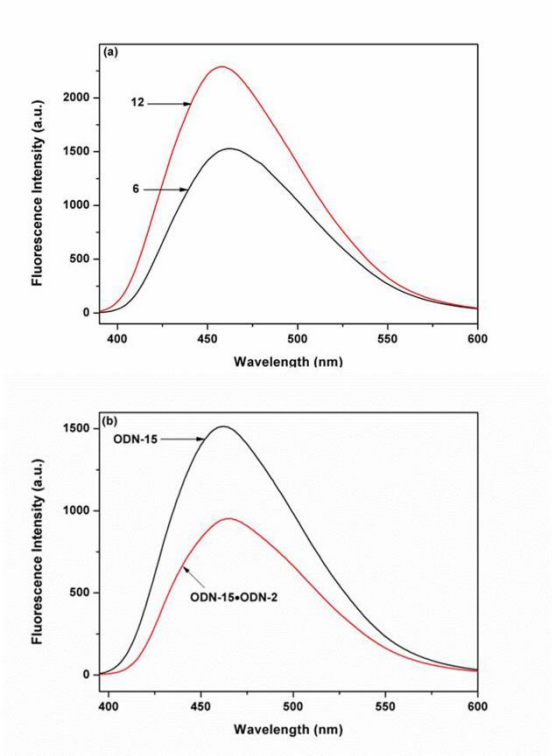

Figure 3. Fluorescence spectra of a) monomeric nucleosides **6** and **12**; b) single-stranded ODN-**15** and duplex ODN-**15**•ODN-**2**. All measurements were performed in 100 mM NaOAc, 10 mM $Mg(OAc)_2$ buffer (pH 7.4) at 5 µM concentration. Excitation wavelength: 368 nm. For solubility reasons 0.4% DMSO was used in case of monomeric nucleoside.

3. Influence of 2'-Fluorination on the Stability of Canonical and Silver Mediated Base Pairs

The fluorine-carbon bond stability (109 kcal/mol)[25] makes fluorine substituents rather resistant to metabolic transformations. The hydrophobic, but polar fluorine atom has approximately the same atomic radius as hydrogen. Therefore, it is well accommodated in DNA and DNA-RNA hydrids.[20,21,26] However, the fluorine van-der-Waals radius is larger (1.47 Å) than that of hydrogen (1.20 Å) and similar to that of oxygen (1.52 Å).[27] Fluorine has the highest electronegativity of all elements. Consequently, fluorine has been incorporated in nucleosides, nucleotides and nucleic acids to alter chemical and medicinal properties.[28] Gemcitabine (**3**) is a prominent example and one of the widely used anticancer drugs. It is

incorporated in DNA and inhibits chain elongation.[29] The modification of the sugar by fluorine functionalization has also a large impact on DNA stability and activity.[20,21,26] Modification of the sugar moiety effects base pair stability by stereoelectronic effects, increases lipophilicity and causes conformational changes.[30] Stereoelectronic effect is a term combining steric effects and electronic properties. Both effects can be induced by fluorine functionalization of the sugar moiety. Steric effects depend on the steric demand of substituents whereas the electronic effects depend on the electronegativity of substituents. Both phenomena can influence sugar conformation and base pair stability.

As nothing is known on the influence of ribose functionalization on the stability of silver mediated base pairs formed by dC-dC mismatches and closely related derivatives we decided to study this matter and to investigate the influence of antipodal and bis-fluorine functionalization of dC (nucleosides **1**-**6** and **12)**. This includes functionalization of the canonical dC-dG pair, base pair mismatches of dC-dA and dC-dT and most importantly, the homo dC-dC pair. All investigations were performed on the standard duplex 5'-d-(TAGGTCAATACT) • 3'-d-(ATCCAGTTATGA) ODN-**1**•ODN-**2** containing the modified base pairs in a near central position embedded in dA-dT pairs and replacing the canonical dC-dG pair. The studies used T_m measurements to determine the duplex stability in the presence and absence of silver ions.

In the first series of experiments (Table 2, left column, Table S1 and Figure S5) the dC residues in the dC-dG pair were replaced by nucleosides **1**-**6** and **12** within 12-mer duplexes. Without silver ions most duplexes showed very similar T_m values with regard to the unmodified counterpart (around 47°C) indicating that a fluorine mono-functionalization in the up or down position has very little influence on the base pair stability. However, geminal fluorine functionalization (gemcitabine, **3**) destabilizes the duplex, a 5-iodo substituent (nucleoside **4**) compensates the decrease, whereas a phenylacetylene residue in 5-position (nucleoside **5**) has a negative impact. Replacing dC with ^{ph}PyrdC (**12**) or ^{ph}PyrGem (**6**) in the

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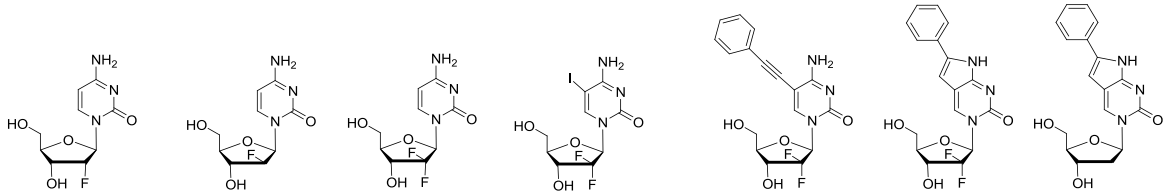
"dC-dG" pair leads to base pair stabilization and significantly higher T_m values. When silver ions were added the duplex stability did not change in any case. Therefore, it was concluded that silver ions do not participate in "dC- dG" base pair formation neither in the case of unmodified nor in 2'-fluorine modified oligonucleotides.

Next, the impact of fluorine modification was studied on duplexes with dC-dA and dC-dT hetero base pairs (mismatches, Table 2, middle and right column, Figures S6 and S7). Also in these cases duplexes incorporating nucleosides **1**-**6** and **12** showed very similar stabilities in the absence of silver ions except those with the pyrrolo-dC skeleton that are more stable. Most probably the increased duplex stability of pyrrolo-dC mismatches in the absence of silver ions relies on increased stacking interactions between the phenyl residues of the phenylpyrrolo-dC skeleton with nucleobase neighbors. Addition of silver ions leads to a T_m increase in almost every case but not for the pyrrolo-dC derivatives **6** and **12** and the precursor **5**. The stability of fluorine modified "dC-dA" and "dC-dT" base pairs is similar to that of the unmodified base pairs for compound **1**-**3** showing that fluorine modification has very little influence on mismatch stability. Moreover, for "dC-dA" mismatches two silver ions were required to obtain the highest duplex stability (T_m). Taken together, increase of T_m values in the presence of silver ions points to the formation of silver mediated base pairs which is in line with observations made on other silver mediated "dC-dA" and "dC-dT" mismatches without fluorine modification.[9c,31] Substituents at the 5-position of the base destabilizes the silver ion mediated base pairs.

Table 2. T_m values (°C) of duplexes with modified dC residues opposite to dG, dA and dT in the absence and presence of silver ions.

5'-d-(TAG GTX AAT ACT) 3'-d-(ATC CAY TTA TGA)	T_m [°C] with n equiv of Ag^+/modified base pair[a]								
	Y= dG (ODN-**2**)			Y= dA (ODN-**3**)			Y= dT (ODN-**4**)		
	n=0	n=1 (ΔT_m)[b]	n=2 (ΔT_m)[b]	n=0	n=1 (ΔT_m)[b]	n=2 (ΔT_m)[b]	n=0	n=1 (ΔT_m)[b]	n=2 (ΔT_m)[b]
X = dC (ODN-1)	47.0	47.5 (+0.5)	47.0 (+0.0)	24.5	29.0 (+4.5)	32.0 (+7.5)	25.5	34.5 (+9.0)	35.5 (+10.0)
X= **1** (ODN-**5**)	46.5	47.0 (+0.5)	47.5 (+1.0)	23.5	27.5 (+4.0)	30.5 (+7.0)	26.0	31.0 (+5.0)	32.5 (+6.5)
X= **2** (ODN-**7**)	47.0	47.5 (+0.5)	47.5 (+0.5)	24.0	30.0 (+6.0)	32.5 (+8.5)	26.0	34.0 (+8.0)	35.5 (+9.5)
X= **3** (ODN-**9**)	43.5	44.5 (+1.0)	44.5 (+1.0)	22.5	30.0 (+7.5)	32.0 (+9.5)	25.5	35.5 (+10.0)	34.0 (+8.5)
X= **4** (ODN-**11**)	47.5	48.0 (+0.5)	48.0 (+0.5)	23.5	25.0 (+1.5)	29.0 (+5.5)	28.0	29.0 (+1.0)	30.0 (+2.0)
X= **5** (ODN-**13**)	43.5	45.0 (+1.5)	45.0 (+1.5)	24.0	24.5 (+0.5)	26.0 (+2.0)	26.5	26.5 (0.0)	27.5 (+1.0)
X= **6** (ODN-**15**)	49.5	50.5 (+1.0)	50.0 (+0.5)	28.0	31.0 (+3.0)	30.0 (+2.0)	33.5	36.0 (+2.5)	36.0 (+2.5)
X= **12** (ODN-**17**)	51.0	52.0 (+1.0)	52.0 (+1.0)	31.0	30.5 (-0.5)	30.5 (-0.5)	34.5	35.0 (+0.5)	36.0 (+1.5)



1 **2** **3** **4** **5** **6** **12**

[a] Measured at 260 nm with 5 µM + 5 µM single-strand concentration at a heating rate of 1.0 °C/min in 100 mM NaOAc, 10 mM $Mg(OAc)_2$, pH = 7.4 in the presence of various concentrations of $AgNO_3$ (0–2.0 equiv.). [b] $\Delta T_m = T_m$ (after addition of 1 or 2 equiv $AgNO_3$) - T_m (before addition of $AgNO_3$).

Finally, the influence of fluorine modification on metal mediated "dC-dC" homo base pair was studied in the absence and presence of one or two equivalents of silver ions. Oligonucleotides incorporating nucleosides **1**-**6** and **12** were utilized and were hybridized with complementary strands containing the same modified "dC" residues in opposite position. The resulting duplexes contain the same fluorine modification in both strands (homo base pairs). Base pairing was also investigated on hetero base pairs with duplexes incorporating nucleosides **1**-**6** and **12** opposite to dC (Table 3, Figure S9). In this case only one strand of the duplexes contains a fluorine modified sugar moiety. Non-modified duplexes containing dC-dC base pairs were used as a control.

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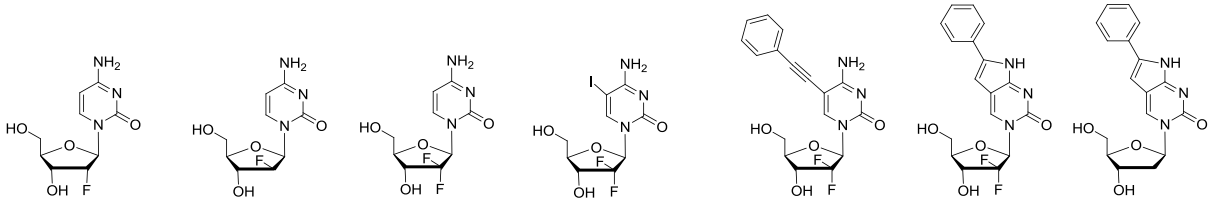
Table 3. T_m values (°C) of duplexes containing modified dC residues in homo base pairs and opposite to dC in the absence and presence of silver ions.[a]

Duplexes	T_m [°C] with n equiv of Ag^+/modified base pair n=0	n=1 (ΔT_m)[b]	n=2 (ΔT_m)[b]	Duplexes	T_m [°C] with n equiv of Ag^+/modified base pair n=0	n=1 (ΔT_m)[b]	n=2 (ΔT_m)[b]
5'-d-(TAG GTC AAT ACT) (ODN-**1**) 3'-d-(ATC CAC TTA TGA) (ODN-**19**)	26.5	34.0 (+7.5)	35.5 (+9.0)				
5'-d-(TAG GT1 AAT ACT) (ODN-**5**) 3'-d-(ATC CA1 TTA TGA) (ODN-**6**)	23.0	33.5 (+10.5)	34.5 (+11.5)	5'-d-(TAG GT1 AAT ACT) (ODN-**5**) 3'-d-(ATC CAC TTA TGA) (ODN-**19**)	25.5	34.5 (+9.0)	35.5 (+10.0)
5'-d-(TAG GT2 AAT ACT) (ODN-**7**) 3'-d-(ATC CA2 TTA TGA) (ODN-**8**)	22.0	36.5 (+14.5)	37.5 (+15.5)	5'-d-(TAG GT2 AAT ACT) (ODN-**7**) 3'-d-(ATC CAC TTA TGA) (ODN-**19**)	20.5	36.0 (+15.5)	37.0 (+16.5)
5'-d-(TAG GT3 AAT ACT) (ODN-**9**) 3'-d-(ATC CA3 TTA TGA) (ODN-**10**)	20.0	31.5 (+11.5)	33.0 (+13.0)	5'-d-(TAG GT3 AAT ACT) (ODN-**9**) 3'-d-(ATC CAC TTA TGA) (ODN-**19**)	<20	33.0 (>+13.0)	34.5 (>+14.5
5'-d-(TAG GT4 AAT ACT) (ODN-**11**) 3'-d-(ATC CA4 TTA TGA) (ODN-**12**)	21.0	28.0 (+7.0)	28.5 (+7.5)	5'-d-(TAG GT4 AAT ACT) (ODN-**11**) 3'-d-(ATC CAC TTA TGA) (ODN-**19**)	22.0	28.0 (+6.0)	29.5 (+7.5)
5'-d-(TAG GT5 AAT ACT) (ODN-**13**) 3'-d-(ATC CA5 TTA TGA) (ODN-**14**)	24.0	20.5 (-3.5)	21.5 (-2.5)	5'-d-(TAG GT5 AAT ACT) (ODN-**13**) 3'-d-(ATC CAC TTA TGA) (ODN-**19**)	29.5	33.0 (+3.5)	32.0 (+2.5)
5'-d-(TAG GT6 AAT ACT) (ODN-**15**) 3'-d-(ATC CA6 TTA TGA) (ODN-**16**)	34.0	46.5 (+12.5)	47.0 (+13.0)	5'-d-(TAG GT6 AAT ACT) (ODN-**15**) 3'-d-(ATC CAC TTA TGA) (ODN-**19**)	31.0	37.0 (+6.0)	37.5 (+6.5)
5'-d-(TAG GT12 AAT ACT) (ODN-**17**) 3'-d-(ATC CA12 TTA TGA) (ODN-**18**)	35.0[12b]	30/58[c]	57.5 (+22.5)	5'-d-(TAG GT12 AAT ACT) (ODN-**17**) 3'-d-(ATC CAC TTA TGA) (ODN-**19**)	33.0	35.5 (+2.5)	36.5 (+3.5)



[a] Measured at 260 nm with 5 µM + 5 µM single-strand concentration at a heating rate of 1.0 °C/min in 100 mM NaOAc, 10 mM $Mg(OAc)_2$, pH = 7.4 in the presence of various concentrations of $AgNO_3$ (0–2.0 equiv.). [b] $\Delta T_m = T_m$ (after addition of 1 or 2 equiv $AgNO_3$) - T_m (before addition of $AgNO_3$). [c] Biphasic melting.

At first homo base pairs (left hand side of Table 3, Figure S8) were studied. In general the T_m values of duplexes containing homo base pairs in the absence of silver are significantly lower than the T_m of the reference duplex. For duplexes incorporating the phenylpyrrolo nucleosides **6** and **12** the highest T_m values were observed. After addition of silver the duplex stability increased significantly (7.5°C to 22.5°C) except for duplex ODN-**13**•ODN-**14** containing 5-phenylacetylene-gemcitabine (**5**). Duplexes with hetero base pairs with only one dC modification in the silver mediated base pair (Table 3, right side) show low T_m values in the

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absence of silver ions as it was observed for the homo base pairs. Addition of silver ions leads to a significant increase of duplex stability pointing to silver mediated base pair formation. The ΔT_m changes resulting from the addition of silver ions are shown in Table 3 and are also displayed in the bar diagram of Figure 4. From this, it can be concluded that dC residues bearing fluorine atoms in 2-position of the sugar residues either in "up", "down" or "geminal" positioning strengthen the stability of silver-mediated "dC-dC" homo base pairs (fluorine substituent in both strands) or "dC-dC" hetero base pairs (fluorine substituent in one strand). Interestingly, nucleosides **1**-**4** show similar T_m values no matter if incorporated in silver mediated "dC-dC" homo or "dC-dC" hetero base pairs. Thus, fluorine modification in one strand leads already to significant duplex stabilization whereas modification in both strands did not lead to a substantial additional increase. Substituents in 5-position of gemcitabine have a negative impact on these base pairs (homo and hetero pair). For ^{ph}PyrGem **6** the situation is different. Geminal difluoro substituents introduced to silver mediated ^{ph}PyrdC homo base pairs are destabilizing. Apparently, the two electronegative fluorine substituents on the sugar moiety have a different impact on the electronic properties of the cytosine base of gemcitabine (**3**) and the phenylpyrrolopyrimidine base of ^{ph}PyrGem (**6**).

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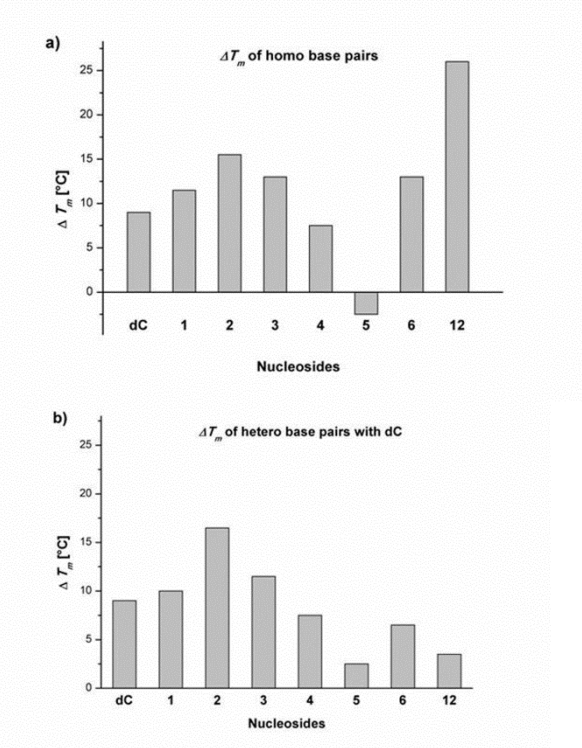

Figure 4. Bar diagrams showing ΔT_m values of the DNA duplex 5'-d-(TAG GTC AAT ACT) • 3'-d-(ATC CAC TTA TGA) ODN-**1**•ODN-**19** modified with dC analogs **1**-**6** and **12**. ΔT_m corresponds to T_m after addition of 2 equiv $AgNO_3$ - T_m before addition of $AgNO_3$.

In order to verify the formation of silver-mediated "dC-dC" pairs, and to determine the amount of silver ions bound to the homo base pairs and hetero base pairs, stoichiometric titration experiments were performed using UV absorbance or fluorescence changes (Figure 5 a-f). From the titration curves it can be concluded that homo base pairs of **1** (fluoro-down dC) and **2** (fluoro-up dC) bind one silver ion per base pair. In case of ^{ph}PyrGem (**6**) two silver ions are bound as reported for ^{ph}PyrdC (**12**).[12b] For gemcitabine (**3**) the situation is more complex. Contrary to oligonucleotide duplexes incorporating the mono fluoro nucleosides **1** or **2** the duplex with gemcitabine **3** binds two silver ions. However, a break is observed during the titration. After addition of one silver ion the second silver ion is bound less strongly (Figure 5d). Apparently, two electronegative fluorine substituents alter the electronic surface potential of the duplex. This electronic perturbation has been already discussed in other cases[18b] and is

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the source of the one electron oxidation of gemcitabine and the formation of C-2' and C-3' sugar radicals.[32] Furthermore, the sugar moiety of gemcitabine adopts the N-conformation in oligonucleotide duplexes whereas dC displays an S-sugar pucker.[18b] The high electronegativity of two fluorine substituents might be the reason for the special behavior of gemcitabine. Additional factors such as the enhanced lipophilicity of fluorinated nucleoside residues have to be considered. Nevertheless, a base pair stability increase is not observed when the second silver ion is bound. Thus, the second silver ion is not participating in base pairing or is necessary to compensate the negative impact of geminal fluorine modification.

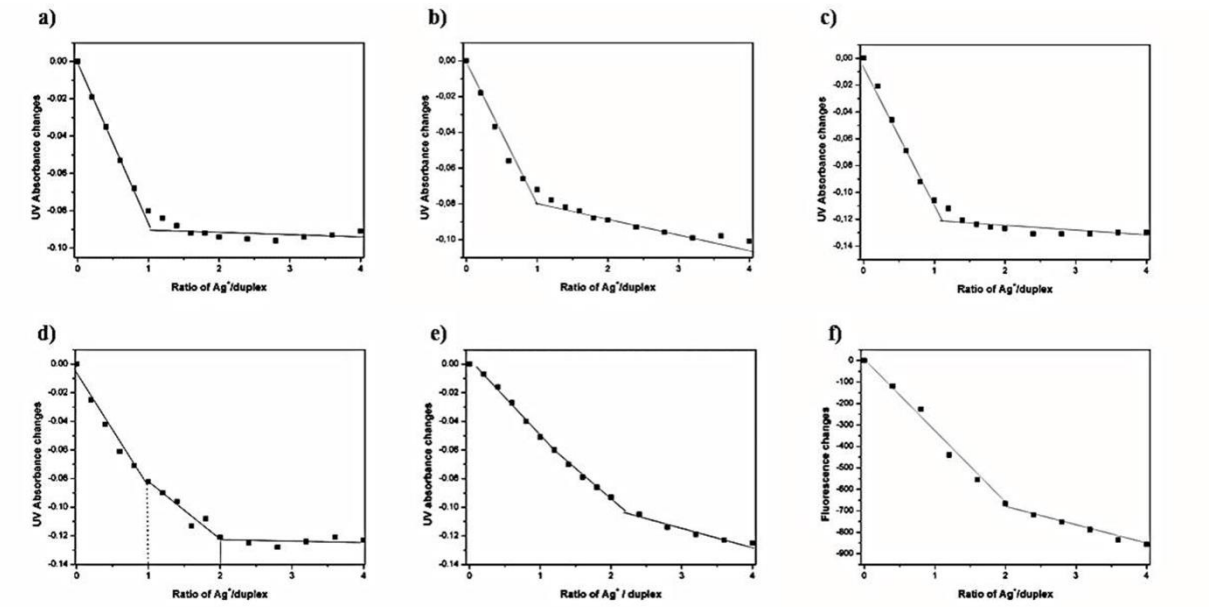

Figure 5. Titration graphs displaying consumption of equivalents of silver/duplex *vs* changes in UV absorbance (measured at 260 nm) or fluorescence emission (measured at 460 nm): a) ODN-**1**•ODN-**19** (dC-dC); b) ODN-**5**•ODN-**6** (**1**-**1**); c) ODN-**7**•ODN-**8** (**2**-**2**); d) ODN-**9**•ODN-**10** (**3**-**3**); e) ODN-**15**•ODN-**16** (**6**-**6**); f) fluorescence titration for duplex ODN-**15**• ODN-**16** (**6**-**6**). All experiments were performed in aqueous 100 mM NaOAc, 10 mM $Mg(OAc)_2$, pH = 7.4.

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6. Conclusion

DNA functionalization at the 2'-position of the sugar moiety has a strong impact on the formation and stability of silver mediated "dC-dC" base pairs. In this study, dC analogs with fluorine in the arabino (up) and ribo (down) configuration, as well as the anticancer drug gemcitabine **3** and the new fluorescent ^{ph}PyrGem **6** analog both with two geminal fluorine substituents were incorporated in 12-mer DNA fragments near central positions utilizing phosphoramidites (**16a-c**, **17**) and solid-phase synthesis. All modified nucleosides display the same recognition face as the naturally occurring 2'-deoxycytidine. To this end, a series of new 2'-deoxycytidine analogs were synthesized; one with an annulated pyrrole ring (**6**) and two 5-substituted derivatives (**4**, **5**). Starting material was 5-iodogemcitabine (**4**) which was converted to the 5-phenylacetylene derivative **5** by *Sonagashira* cross coupling. After cyclization ^{ph}PyrGem (**6**) was obtained. An alternative route utilizing a different sequence of reaction steps was established. Photophysical data of nucleosides, oligonucleotides and duplexes demonstrate reduced fluorescence of ^{ph}PyrGem (**6**) with two geminal fluorine atoms in the sugar moiety compared to the unmodified ^{ph}PyrdC (**12**).

The impact of antipodal (fluoro-up/fluoro-down) and geminal 2'-fluorine modification (gemcitabine and phenylpyrrolo-gemcitabine) was studied by hybridization of complementary strands (12-mers) with mismatches in the absence and presence of silver ions. Addition of silver ions to fluorine modified "dC-dC" pairs led to significant duplex stabilization. The increase measured by T_m changes was significantly higher for duplexes with fluorinated sugar residues than for those with 2'-deoxycytidine. Similar observations found for "dC-dC" pairs were made on "dC-dT" pairs and to a minor extend on the "dC-dA" pair. The increase of silver ion mediated base pair stability was reversed by annulation of a pyrrole ring to the cytosine moiety as shown for 2'-fluorinated ^{ph}PyrGem (**6**) compared to ^{ph}PyrdC (**12**). Apparently, the nucleobase structure is of utmost importance for the transmission of electronic effects from the sugar moiety to the metal mediated base pair.

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Herein, we report for the first time on stereoelectronic effects induced by 2'-fluorination of the sugar moiety that are transmitted to the silver-mediated base pair. The impact depends on the number of substitutions, the configuration and the structure of the nucleobase. Consequently 2' fluorine modification on the sugar moiety can be used to modulate silver mediated base pair stability depending on the configuration of the fluorine atom and the number of fluorine replacements. All structural modifications did not change the recognition face of 2'-deoxycytidine. One or two silver ions were bound by the particular base pair. Figure 6 summarizes possible silver mediated base pair structures.

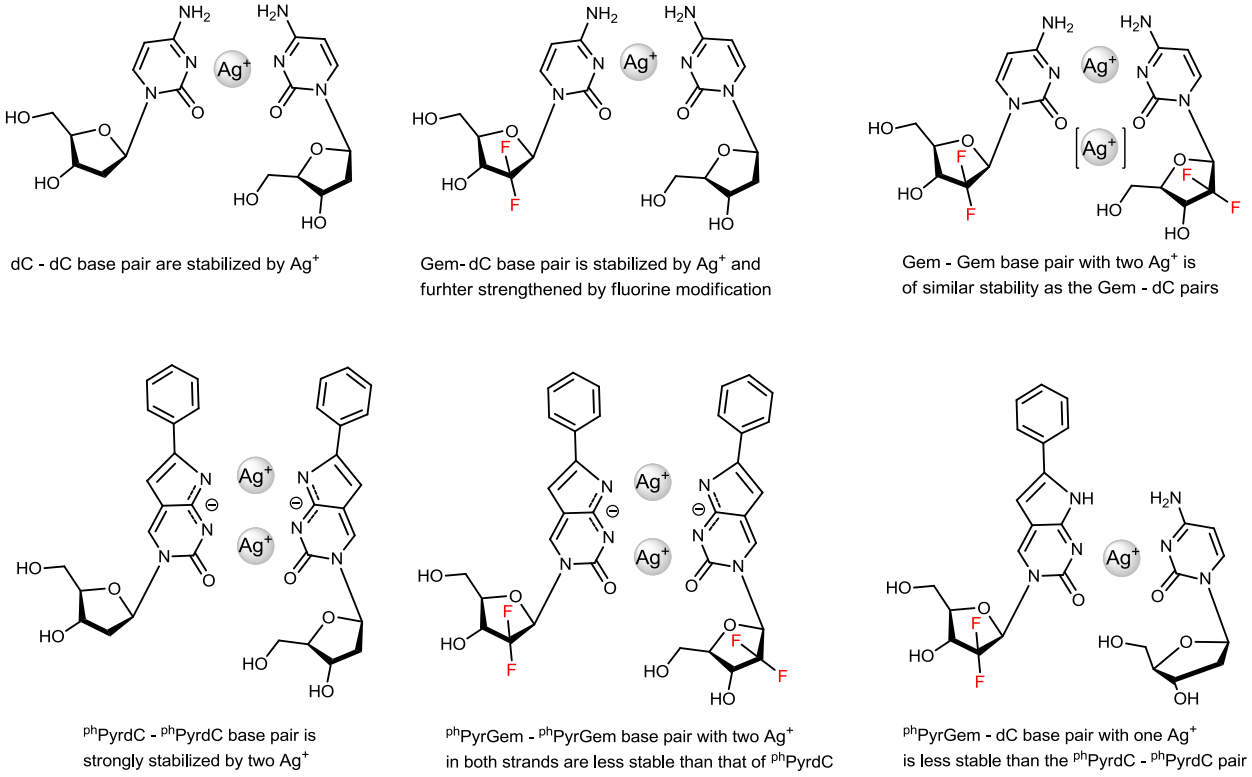

Figure 6. Proposed structures of silver mediated base pairs demonstrating the fluorine effect on the base pair stability.

Experimental Section

General methods and materials

All chemicals and solvents were of laboratory grade as obtained from commercial suppliers and were used without further purification. Reversed-phase HPLC was carried out on a 4 x 250 mm RP-18 (10 μm) LiChrospher 100 column with a HPLC pump connected with a variable wavelength monitor, a controller and an integrator. The molecular masses of the oligonucleotides were determined by MALDI-TOF mass spectrometry in the linear positive mode with 3-hydroxypicolinic acid (3-HPA) as a matrix. Fluorescence spectra were measured with a fluorescence spectrophotometer in the wavelength range between 220 to 600 nm at room temperature using standard quartz cuvettes with a path length of 1 cm. The thermal melting curves were measured with a UV-vis spectrophotometer equipped with a thermoelectrical controller. The temperature was measured continuously in the reference cell with a Pt-100 resistor with a heating rate of 1 °C min^{-1}. T_m values were determined from the melting curves using the software MELTWIN, version 3.0.[33]

Oligonucleotide syntheses and characterization

Solid-phase oligonucleotide syntheses were performed on an ABI 392-08 synthesizer at 1 μmol scale (trityl-on mode) employing the phosphoramidites of nucleosides **1**, **2**[20,21], phosphoramidites **16a**-**16c**, **17** as well as the standard building blocks with an average coupling yield over 95%. After cleavage from the solid support, oligonucleotides containing nucleosides **1**-**5** were deprotected in 28% aqueous ammonia at 55 °C for 2 h. While oligonucleotides containing nucleoside **6** were deprotected in 28% aqueous ammonia for overnight at room temperature. The DMT-containing oligonucleotides were purified by reversed-phase HPLC (RP-18) with the gradient system at 260 nm: (A) MeCN, (B) 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN, 95:5; gradient *I*: 0-3 min 10-15% A in B, 3-15 min 15-50% A

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in B; flow rate 0.8 mL/min. The purified "trityl-on" oligonucleotides were treated with 2.5% $CHCl_2COOH/CH_2Cl_2$ for 2 min at 0 °C to remove the 4,4′-dimethoxytrityl residues. The detritylated oligomers were purified again by reversed-phase HPLC with gradient *II*: 0-20 min 0-20% A in B; 20-25 min, 20% A in B; flow rate 0.8 mL/min. For Figure 2 following gradient system was used, gradient *III*: (B) 0.1 M $(Et_3NH)OAc$ (pH 7.0)/MeCN, 98:2; 0-25 min 100% B; flow rate 0.8 mL/min; gradient *IV*: (A) MeCN, (B) 0.1 M $(Et_3NH)OAc$ (pH 7.0)/MeCN, 95:5; 0-25 min 20% A in B; flow rate 0.8 mL/min. The oligonucleotides were desalted on a short column (RP-18) using water for elution of salt, while the oligonucleotides were eluted with H_2O/MeOH (2:3). The oligonucleotides were lyophilized on a Speed-Vac evaporator to yield colorless solids which were frozen at -24 °C. The purity of all oligonucleotides were confirmed by RP-18 HPLC (Figure S3, Supporting Information) and MALDI-TOF mass spectrometry (see Tables 1). The extinction coefficients ε_{260} (H_2O) of the nucleosides are: dA 15400, dG 11700, dT 8800, dC 7300, **3** 11900 (MeOH), **4** 4400 (MeOH), **5** 15100 (MeOH) and **6** 27700 (MeOH). The extinction coefficients of the oligonucleotides were calculated from the sum of the extinction coefficients of nucleoside constituents.

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Table 4. ^{13}C-NMR data of gemcitabin derivatives[a]

	C2[b] C2[c]	C4[b] C7a[c]	C5[b] C4a[c]	C6[b] C4[c]	C7[b] C5[c]	C8[b] C6[c]	C1'	C2'	C3'	C4'	C5'	N=CH	C≡C
3	154.7	165.7	94.6	140.8	-	-	83.5 (32.9)	123.1 (258)	68.7 (22.6)	80.4 (4.5)	58.9	-	-
4	153.4	163.8	57.7	146.4	-	-	83.5 (32.3)	122.9 (258)	67.9 (22.4)	80.5 (4.5)	58.4	-	-
5	153.0	163.8	94.0	144.3	-	-	83.8 (30.0)	122.9 (256)	68.2 (23.0)	80.7 (4.0)	58.6	-	80.9 90.6
6	153.6	160.5	109.9	140.6	96.8	136.0	84.8 (34.2)	123.1 (259)	68.3 (23.1)	80.8 (5.0)	58.7	-	-
14a	154.7	171.5	102.2	141.6	-	-	83.9 (38.8)	123.0 (258)	68.4 (22.1)	80.5 (4.2.)	58.7	157.9	-
15a	154.5	171.4	102.3	141.4	-	-	84.1 (32.0)	122.7 (258)	69.2 (22.5)	78.7 (4.0)	61.6	158.0	-
14b	153.8	168.3	70.0	146.3	-	-	83.9 (33.1)	122.9 (258)	67.9 (21.1)	80.6 (4.5)	58.3	158.3	-
15b	153.7	168.4	70.3	146.2	-	-	84.4 (n.d.)	122.6 (258)	69.8 (23.1)	78.8 (n.d.)	61.9	158.3	-
14c	153.1	169.8	98.4	144.6	-	-	84.1 (29.2)	122.9 (260)	68.1 (22.1)	80.7 (n.d.)	58.5	157.9	83.8 91.9
15c	153.4	170.4	99.2	144.5	-	-	83.8 (n.d.)	123.1 (257)	70.2 (22.1)	79.6 (4.0)	62.4	158.5	83.8 92.5
7	152.9	163.8	94.2	144.5	-	-	84.2 (n.d.)	122.7 (257)	69.7 (22.0)	80.4 (n.d.)	62.0	-	80.4 90.9
8	152.1	161.5	94.3	144.4	-	-	85.1 (n.d.)	122.7 (258)	69.5 (23.1)	79.5 (2.0)	61.9	-	79.7 95.3
10	153.3	163.9	58.0	146.4	-	-	84.0 (n.d.)	122.6 (258)	69.9 (23.1)	78.9 (n.d.)	62.0	-	-
11	152.6	162.0	61.8	149.5	-	-	85.6 (n.d.)	122.5 (258)	69.7 (22.0)	79.5 (n.d.)	62.0	-	-
9	153.6	160.5	109.8	140.3	96.2	135.5	84.8 (n.d.)	122.8 (258)	68.4 (21.1)	78.8 (n.d.)	60.9	-	-

[a] Measured in DMSO-d_6 at 298 K. [b] Pyrimidine numbering. [c] Systematic numbering. n.d.: not detected.

1-(2-Deoxy-2,2'-difluoro-β-D-erythro-pentofuranosyl)-5-iodopyrimidin-2H-2-one (4)

A mixture of ethanol (40 mL), carbon tetrachloride (40 mL) and iodine (1.730 g, 6.82 mmol) was stirred for 10 min at room temperature. 2'-Deoxy-2',2'-difluorocytidine (4.600 g, 17.48 mmol) was added. Then reaction mixture was warmed at 45°C and treated with a solution of iodic acid (1.73 g, 9.83 mmol) in water (4.6 mL). Afterward reaction mixture was stir at 45°C for 16 h (TLC monitoring). After completion of reaction, the mixture was allowed to cool.

Then solvent was evaporated and the remaining residue was adsorbed on silica gel and applied to FC (silica gel, column: 15 x 5 cm, CH_2Cl_2/MeOH 90:10). Evaporation of the main zone afforded compound **4** as colorless solid (6.100 g, 90%). Analytical data were in accordance to the literature.[15f]

4-Amino-1-(2-deoxy-2,2'-difluoro-β-D-erythro-pentofuranosyl)-5-(phenylethynyl)pyrimidin-2H-2-one (5)

5-Iodo-2,2'-difluoro-2'-deoxycytidine (**4**) (963 mg, 2.47 mmol) was dissolved in dry DMF (10 mL), then CuI (96 mg, 0.50 mmol), tetrakis(triphenylphosphine) Pd(0) (288 mg, 0.25 mmol), *N*,*N*-diisopropylethylamine (732 mg, 963 µL, 5.66 mmol) and phenylacetylene (1.08 mL, 1.0 g, 9.8 mmol) were introduced. The solution was stirred at rt for overnight. The solvent was removed under vacuo, and the resulting oily residue was co-evaporated with toluene twice (2 x 20 mL), adsorbed on silica gel and applied to FC (silica gel, column: 15 x 4 cm, CH_2Cl_2/MeOH, 85:15). Evaporation of the solvent from the main zone afforded **5** as yellowish foam (728 mg, 81%). R_f = 0.6 (CH_2Cl_2/MeOH, 85:15); ^{1}H NMR (300 MHz, [D_6]DMSO, 25 °C): δ = 3.63-3.68 (m, 2H, 2 x H-5'), 3.81-3.93 (m, 1H, H-4'), 4.16-4.29 (m, 1H, H-3'), 5.40 (t , *J* = 4.8 Hz, 1H, HO-5'), 5.68 (t, *J* = 6.9 Hz, 1H, H-1'), 6.27 (d, *J* = 6.3 Hz, HO-3'), 7.29 (br, 1H, NH), 7.39-7.41 (m, 3H, Ar-H), 7.60-7.63 (m, 2H, Ar-H), 8.04 (br, 1H, NH), 8.26 (s, 1H, H-6); ^{19}F NMR (376 MHz, [D_6]DMSO, 25 °C): δ = -116.14 to -117.56; UV/Vis (MeOH): λ_{max} (ε) = 305 (13300), 283 (16300), 270 nm (17100 dm^3 mol^{-1} cm^{-1}); HRMS (ESI-TOF) *m/z*: calcd. For $C_{17}H_{15}F_2N_3O_4Na$: 386.0923 [*M*+Na]$^+$; found 386.0924.

4-Amino-1-(2-deoxy-2,2'-difluoro-5-O-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl)-5-(phenylethynyl)pyrimidin-2H-2-one (7)

Compound **5** (1.0 g, 2.75 mmol) was dried by repeated co-evaporation with dry pyridine (3 x 10 mL) and dissolved in dry pyridine (15 mL). To the solution was added 4,4'-dimethoxytrityl chloride (1.5 g, 4.43 mmol) and the reaction mixture was stirred at rt for 12 h.

The solution was diluted with CH_2Cl_2 (100 mL) and poured into 5% $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4, evaporated and applied to FC (silica gel, column: 15 x 5 cm, CH_2Cl_2/acetone 4:1). Evaporation of the main zone afforded compound **7** as colorless foam (1.6 g, 87%). R_f = 0.30(CH_2Cl_2/acetone, 85:15); ^{1}H NMR (300 MHz, [D_6]DMSO, 25 °C): δ = 3.40-3.44 (m, 2H, H-5'), 3.66 (s, 6H, OCH_3), 4.03-4.07 (m, 1H, H-4'), 4.31-4.42 (m, 1H, H-3'), 6.18 (t, *J* = 8.0 Hz, 1H, H-1'), 6.33 (d, *J* = 8.0 Hz, 1H, HO-3'), 6.86-6.88 (m, 6H, Ar-H), 7.14-7.45 (m, 13H, Ar-H), 7.97 (s, 1H, H-6), 8.08 (s, 1H, NH); ^{19}F NMR (376 MHz, [D_6]DMSO, 25 °C): δ = -114.77 to 116.77 (m, CF_2); UV/Vis (MeOH): λ_{max} (ε) = 307 (12700), 282 (18400), 270 (20300), 234 nm (32300 dm^3 mol^{-1} cm^{-1}); HRMS (ESI-TOF) *m/z*: calcd. for $C_{38}H_{33}F_2N_3O_6Na$: 688.2229 [*M*+Na]$^+$; found 688.2225.

4-(Acetyl)amino-1-(2-deoxy-2,2'-difluoro-5-O-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl)-5-(phenylethynyl)pyrimidin-2H-2-one (8)

Compound **7** (710 mg, 1.07 mmol) was dissolved in DMF (5 mL), then acetic anhydride (1.501 g, 1.39 mL, 14.70 mmol) was added and the solution was stirred for 12 h at rt. The solvent was evaporated and the remaining residue was co-evaporated with MeOH (3 x 3 mL) and applied to FC (silica gel, column: 8 x 3 cm, CH_2Cl_2/acetone 4:1). Evaporation of the main zone afforded compound **8** as yellowish foam (552 mg, 73%). R_f = 0.53(CH_2Cl_2/acetone, 8:2); ^{1}H NMR (300 MHz, [D_6]DMSO, 25 °C): δ = 2.35 (s, 3H, CH_3), 3.27-3.31 (m, 1H, H-5'), 3.45-3.51 (m, 1H, H-5'), 3.66 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 4.11-4.15 (m, 1H, H-4'), 4.34-4.36 (m, 1H, H-3'), 6.20 (t, *J* = 7.2 Hz, 1H, H-1'), 6.38 (d, *J* = 6.6 Hz, 1H, HO-3'), 6.86-6.88 (m, 4H, Ar-H), 7.14-7.45 (m, 14H, Ar-H), 8.29 (s, 1H, H-6), 9.87 (s, 1H, NH); ^{19}F NMR (376 MHz, [D_6]DMSO, 25 °C): δ = -114.94 to -116.41 (m, CF_2); UV/Vis (MeOH): λ_{max} (ε) = 298 (15600), 282 (20600), 275 (19300), 232 nm (33200 dm^3 mol^{-1} cm^{-1}); HRMS (ESI-TOF) *m/z*: calcd. for $C_{40}H_{35}F_2N_3O_7Na$: 730.2335 [*M*+Na]$^+$; found 730.2335.

6-(3-Phenyl)-3-[2-deoxy-2,2'-difluoro -5-O-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl]pyrrolo-[2,3-d]pyrimidin-2H-2-one (9)

From compound 8: A mixture of **8** (420 mg, 0.59 mmol), CuI (154 mg, 0.81 mmol) in triethylamine (10 mL) and DMF (10 mL) was heated at 60 °C for 24 h. The solvent was evaporated and the remaining residue was adsorbed on silica gel (20 g) and applied to FC (silica gel, column: 8 x 3 cm, CH_2Cl_2/MeOH 95:5). Evaporation of the main zone afforded compound **9** as yellowish foam (267 mg, 68%). R_f = 0.33 (CH_2Cl_2/MeOH, 95:5); ^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 3.41-3.43 (m, 1H, H-5'), 3.54-3.58 (m, 1H, H-5'), 3.73 and 3.74 (2s, 6H, 2 x OCH_3), 4.09-4.13 (m, 1H, H-4'), 4.52-4.62 (m, 1H, H-3'), 5.71 (s, 1H, H-5), 6.38 (t, *J* = 6.8 Hz, 1H, H-1'), 6.48 (d, *J* = 6.4 Hz, 1H, HO-3'), 6.94-6.96 (m, 4H, Ar-H), 7.28-7.48 (m, 12H, Ar-H), 7.69 (d, *J* = 7.6 Hz, 2H, Ar-H), 8.58 (s, 1H, H-6), 11.93 (s, 1H, NH); ^{19}F NMR (376 MHz, [D_6]DMSO, 25 °C): δ = -117.8; UV/Vis (MeOH): λ_{max} (ε) = 370 (5200), 268 (27000), 261 (27200), 235 nm (31000 dm^3 mol^{-1} cm^{-1}); HRMS (ESI-TOF) *m/z*: calcd. for $C_{38}H_{33}F_2N_3O_6Na$: 688.2230 $[M+Na]^+$; found 688.2247.

From compound 11: Compound **11** (500 mg, 0.68 mmol) was dissolved in dry DMF (5 mL) and triethylamine (4 mL). Then, CuI (30 mg, 0.16 mmol), tetrakis(triphenylphosphine) Pd(0) (90 mg, 0.08 mmol), and phenylacetylene (300 µL, 2.7 mmol) were introduced. The solution was stirred at rt overnight. The solvent was removed under vacuo, and the resulting oily residue was co-evaporated with toluene twice (2 x 20 mL), adsorbed on silica gel and applied to FC (silica gel, column: 15 x 4 cm, CH_2Cl_2/MeOH, 95:5). Evaporation of the solvent from the main zone afforded **9** (262 mg, 58%) as yellowish foam.

From compound 6: Compound **6** (50 mg, 0.14 mmol) was dried by repeated co-evaporation with dry pyridine (3 x 2 mL) and dissolved in dry pyridine (1 mL). To the solution was added 4,4'-dimethoxytrityl chloride (167 mg, 0.49 mmol) in four portions and the reaction mixture was stirred at rt for 6 h (TLC-control). The solution was diluted with CH_2Cl_2 (20 ml) and

poured into 5% $NaHCO_3$ soln. (20 ml). The org. layer was dried over Na_2SO_4 evaporated and applied to FC (silica gel, column: 8 x 3 cm, CH_2Cl_2/MeOH 95:5). Evaporation of the main zone afforded compound **9** as yellowish foam (80 mg, 87%).

6-(3-Phenyl)-3-[2-deoxy-2,2'-difluoro-β-D-erythro-pentofuranosyl]pyrrolo-[2,3-d]pyrimidin-2H-2-one (6)

A solution of **9** (318 mg, 0.48 mmol) in CH_2Cl_2 was treated with trichlorocetic acid in dry CH_2Cl_2 (5 mL). The reaction mixture was stirred at rt for 15 min and neutralized with triethylamine (5 mL). The solvent was evaporated and the remaining residue was applied to FC (silica gel, column: 8 x 3 cm, CH_2Cl_2/MeOH 9:1). Evaporation of the main zone afforded compound **6** as yellowish solid (154 mg, 89%). R_f = 0.36 (CH_2Cl_2/MeOH, 9:1); ^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 3.70-3.74 (m, 1H, H-5'), 3.86-3.95 (m, 2H, H-4', H-5'), 4.24-4.32 (m, 1H, H-3'), 5.45 (br, 1H, HO-5', 1H,), 6.36-6.40 (m, 2H, H-1', HO-3'), 6.75 (s, 1H, H-5), 7.35-7.48 (m, 3H, Ar-H), 7.84-7.86 (d, *J* = 7.6 Hz, 2H, Ar-H), 8.61 (s, 1H, H-4), 11.90 (br, 1H, NH); ^{19}F NMR (376 MHz, [D_6]DMSO, 25 °C): δ = -116.77; UV/Vis (MeOH): λ_{max} (ε) = 370 (5200), 268 (27200), 260 nm (27700 dm^3 mol^{-1} cm^{-1}); HRMS (ESI-TOF) *m/z*: calcd. for $C_{17}H_{16}F_2N_3O_4$: 364.1103 $[M+H]^+$; found 364.1121.

6-(3-Phenyl)-3-[2-deoxy-5-O-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl] pyrrolo[2,3-d]pyrimidin-2H-2-one (13)

Compound **12**[23d] (0.050 g, 0.15 mmol) was dried by repeated co-evaporation with dry pyridine (3 x 2 mL) and dissolved in dry pyridine (1 mL). To the solution was added 4,4'-dimethoxytrityl chloride (148 mg, 0.44 mmol) in two portions and the reaction mixture was stirred at rt for 5 h (TLC-control). The solution was diluted with CH_2Cl_2 (20 ml) and poured into 5% $NaHCO_3$ soln. (20 mL). The org. layer was dried over Na_2SO_4 evaporated and applied to FC (silica gel, column: 9 x 3 cm, CH_2Cl_2/MeOH 95:5). Evaporation of the main

zone afforded compound **13** as yellowish foam (67 mg, 70%). Analytical data were in accordance to the literature.[17]

4-Amino-1-(2-deoxy-2,2'-difluoro-5-O-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl)-5-iodopyrimidin-2H-2-one (10)

Compound **4** (1.2 g, 3.08 mmol) was dried by repeated co-evaporation with dry pyridine (3 x 10 mL) and dissolved in dry pyridine (10 mL). To the solution was added 4,4'-dimethoxytrityl chloride (1.8 g, 5.31 mmol) and the reaction mixture was stirred at rt for 12 h. The solution was diluted with CH_2Cl_2 (100 mL) and poured into 5% $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4 evaporated and applied to FC (silica gel, column: 15 x 5 cm, CH_2Cl_2/MeOH 98:2). Evaporation of the main zone afforded compound **10** as colorless solid (1.8 g, 84%). R_f = 0.5 (CH_2Cl_2/acetone, 85:15); ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ = 3.26-3.33 (m, 2H, H-5'), 3.74 (s, 6H, 2 x OCH_3), 3.98-401 (m, 1H, H-4'), 4.23-4.34 (m, 1H, H-3'), 6.13 (t, *J* = 8.4 Hz, 1H, H-1'), 6.25 (d, *J* = 6.6 Hz, 1H, HO-3'), 6.89-6.92 (m, 5H, Ar-H), 7.21-7.42 (m, 9H, Ar-H, NH), 7.89 (s, 1H, H-6), 8.14 (s, 1H, NH); ^{19}F NMR (376 MHz, [D$_6$]DMSO, 25 °C): δ = -114.27 to -116.74 (m, CF_2); UV/Vis (MeOH): λ_{max} (ε) = 300 (sh, 4800), 282 (6800), 275 nm (6900 dm^3 mol^{-1} cm^{-1}); HRMS (ESI-TOF) *m/z*: calcd. for $C_{30}H_{28}F_2IN_3O_6Na$: 714.0883 [*M*+Na]$^+$; found 714.0890.

4-(Acetyl)amino-1-[2-deoxy-2,2'-difluoro-5-O-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-5-iodopyrimidin-2H-2-one (11)

Compound **10** (730 mg, 1.06 mmol) was dissolved in DMF (5 mL), then acetic anhydride (394 mg, 365 µL, 3.86 mmol) was added and the solution was stirred for 12 h at rt. The solvent was evaporated and the remaining residue was co-evaporated with MeOH (3 x 3 mL) and applied to FC (silica gel, column: 10 x 3 cm, CH_2Cl_2/acetone 8:2). Evaporation of the main zone afforded compound **11** as yellowish foam (591 mg, 76%). R_f = 0.6 (CH_2Cl_2/acetone, 8:2); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 2.27 (s, 3H, CH_3), 3.26-

3.30 (m, 1H, H-5'), 3.38-3.42 (m, 1H, H-5'), 3.75 (s, 6H, 2 x OCH_3), 4.06-4.10 (m, 1H, H-4'), 4.30-4.41 (m, 1H, H-3'), 6.15 (t, *J* = 8.0 Hz, 1H, H-1'), 6.32 (d, *J* = 6.8 Hz, 1H, HO-3'), 6.90-6.93 (m, 4H, Ar-H), 7.22-7.43 (m, 9H, Ar-H), 8.21 (s, 1H, H-6), 9.43 (s, 1H, NH); ^{19}F NMR (282 MHz, [D_6]DMSO, 25 °C): δ = -115.14; UV/Vis (MeOH): λ_{max} (ε) = 300 (sh, 4200), 282 (6200), 275 nm (6400 dm^3 mol^{-1} cm^{-1}); HRMS (ESI-TOF) *m/z*: calcd. for $C_{32}H_{30}F_2IN_3O_7Na$: 756.0989 [*M*+Na]$^+$; found 756.0992.

4-(Dibutylaminomethylidene)amino-1-(2-deoxy-2,2'-difluoro -β-D-erythro-pentofuranosyl)pyrimidin-2H-2-one (14a)

2,2'-Difluorocytidine (**3**) (1.0 g, 3.8 mmol) was dissolved in MeOH (50 mL) and treated with *N*,*N*-dibutylformamid dimethylacetal (3 mL). The reaction mixture was stirred for 1 h at 30 °C. The solvent was evaporated and the remaining oily residue was adsorbed on silica gel (50 g) and applied to FC (silica gel, column: 15 x 5 cm, CH_2Cl_2/MeOH 95:5). Evaporation of the main zone afforded compound **14a** as amorphous solid (800 mg, 52%). R_f = 0.60 (CH_2Cl_2/MeOH, 95:5); ^{1}H NMR (300 MHz, [D_6]DMSO, 25 °C): δ = 0.88 (q, *J* = 7.3 Hz, 6H, 2 x CH_3), 1.23-1.29 (m, 4H, 2 x CH_2), 1.50-1.58 (m, 4H, 2 x CH_2), 3.42-3.52 (m, 4H, 2 x NCH_2), 3.76-3.85 (m, 3H, H-4', H-5'), 4.11-4.20 (m, 1H, H-3'), 5.28 (br, 1H, HO-5'), 6.00 (d, *J* = 7.3 Hz, H-5), 6.16 (t, *J* = 7.8 Hz, 1H, H-1'), 6.28 (br, 1H, HO-3'), 7.90 (d, *J* = 7.3 Hz, 1H, H-6), 8.67 (s, 1H, N-C=H); ^{19}F NMR (282 MHz, [D_6]DMSO, 25 °C): δ = -117.211; UV/Vis (MeOH): λ_{max} (ε) = 320 nm (29000 dm^3 mol^{-1} cm^{-1}). HRMS (ESI-TOF) *m/z*: calcd. for $C_{17}H_{15}F_2N_3O_4Na$: 386.0923 [*M*+Na]$^+$; found 386.0924.

4-(Dibutylaminomethylidene)amino-1-[2-deoxy-2,2'-difluoro-5-O-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl]pyrimidin-2H-2-one (15a)

Compound **14a** (500 mg, 1.24 mmol) was dried by repeated co-evaporation with dry pyridine (3 x 10 mL) and dissolved in dry pyridine (15 mL). To the solution was added 4,4'-dimethoxytrityl chloride (550 mg, 1.62 mmol) and the reaction mixture was stirred at rt for 12

h. The solution was diluted with CH_2Cl_2 (100 mL) and poured into 5% $NaHCO_3$ soln. The organic layer was dried over Na_2SO_4 evaporated and applied to FC (silica gel, column: 15 x 5 cm, CH_2Cl_2/acetone 4:1). Evaporation of the main zone afforded compound **15a** as colorless foam (624 mg, 71%). R_f = 0.50 (CH_2Cl_2/acetone, 85:15); ^{1}H NMR (300 MHz, [D_6]DMSO, 25 °C): δ = 0.90 (q, *J* = 6.9 Hz, 6H, 2 x CH_3), 1.25-1.30 (m, 4H, 2 x CH_2), 1.53-1.58 (m, 4H, 2 x CH_2), 3.32 (m, 2H, NCH_2), 3.42-3.50 (m, 4H, H-5', NCH_2), 3.72 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 4.01-4.05 (m, 1H, H-4'), 4.28-4.32 (m, 1H, H-3'), 5.75 (d, *J* = 7.3 Hz, H-5), 6.19 (t, *J* = 8.0 Hz, 1H, H-1'), 6.34 (d, *J* = 6.7 Hz, 1H, HO-3'), 6.89-6.92 (m, 4H, Ar-H), 7.24-7.41 (m, 9H, Ar), 7.81 (d, *J* = 7.3 Hz, 1H, H-6), 8.67 (s, 1H, N-C=H); ^{19}F NMR (376 MHz, [D_6]DMSO, 25 °C): δ = -116.93; UV/Vis (MeOH): λ_{max} (ε) = 320 (28500), 233 nm (26600 dm^3 mol^{-1} cm^{-1}); HRMS (ESI-TOF) *m/z*: calcd. for $C_{39}H_{47}F_2N_4O_6$: 705.3458 [*M*+H]$^+$; found 705.3454.

4-[(Dibutylaminomethylidene)amino]-1-[2-deoxy-2,2'-difluoro-5-O-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-pyrimidin-2H-2-one 3'-[(2-Cyanoethyl)-N,N-(diisopropyl)]phosphoramidite (16a)

To a solution of compound **15a** (700 mg, 1 mmol) in anhydrous CH_2Cl_2 (10 mL) *N*,*N*-diisopropylethylamine (243 mg, 320 µL, 1.8 mmol) and chloro-(2-cyanoethoxy)-(diisopropylamino)phosphine (339 mg, 320 µL, 1.4 mmol) were added. After stirring for 30 min at rt, the solution was diluted with CH_2Cl_2 (50 mL) and poured into 5% aq. $NaHCO_3$ solution (60 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL), dried over Na_2SO_4, filtered and evaporated. FC (silica gel was pre-washed with traces of triethylamine) (silica gel, CH_2Cl_2/acetone, 85:15) gave compound **16a** (680 mg, 75%) as a mixture of diastereoisomers. R_f = 0.7 (CH_2Cl_2/acetone, 85:15); ^{31}P-NMR (121 MHz, $CDCl_3$, 25 °C, TMS): δ = 154.18 (t, *J* = 6.5), 152.46 (dd, *J* = 24.3, *J* = 11.3); ^{19}F NMR (376 MHz, $CDCl_3$, 25

°C, TMS): δ = -114.07 to 116.51 (m, CF_2); HRMS (ESI-TOF) *m/z*: calcd. for $C_{48}H_{64}F_2N_6O_7P$: 905.4537 $[M+H]^+$; found 905.4507.

4-(Dibutylaminomethylidene)amino-1-(2-deoxy-2,2'-difluoro-β-D-erythro-pentofuranosyl)-5-iodopyrimidin-2H-2-one (14b)

5-Iodo-2,2'-difluorocytidine (**4**) (500 mg, 1.3 mmol) was dissolved in MeOH (20 mL) and treated with *N,N*-dibutylformamid dimethylacetal (2 mL). The reaction mixture was stirred for 30 min at 30°C. The solvent was evaporated and the remaining oily residue was adsorbed on silica gel (50 g) and applied to FC (silica gel, column: 15 x 5 cm, CH_2Cl_2/MeOH 92:8). Evaporation of the main zone afforded compound **14b** as amorphous solid (531 mg, 78%). R_f = 0.60 (CH_2Cl_2/MeOH, 95:5); ^{1}H NMR (300 MHz, [D_6]DMSO, 25 °C): δ = 0.92 (q, *J* = 7.5 Hz, 6H, CH_3), 1.21-1.39 (m, 4H, 2 x CH_2), 1.52-1.69 (m, 4H, 2 x CH_2), 3.46 (t, *J* = 7.2 Hz, 4H, NCH_2), 3.55-3.67 (m, 3H, H5', NCH_2), 3.78-3.86 (m, 2H, H-4', H-5'), 4.14-4.28 (m, 1H, H-3'), 5.44 (t, *J* = 4.8 Hz, 1H, HO-5'), 6.10 (t, *J* = 7.2 Hz, 1H, H-1'), 6.26 (d, *J* = 6.6 Hz, 1H, HO-3'), 8.38 (s, 1H, H-6), 8.62 (s, 1H, N-C=H); ^{19}F NMR (376 MHz, [D_6]DMSO, 25 °C): δ = -117.17; UV/Vis (MeOH): λ_{max} (ε) = 334 nm (26200 dm^3 mol^{-1} cm^{-1}); HRMS (ESI-TOF) *m/z*: calcd. for $C_{18}H_{28}F_2IN_4O_4$: 529.1118 $[M+H]^+$; found 529.1120.

4-(Dibutylaminomethylidene)amino-1-[2-deoxy-2,2'-difluoro-5-O-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-5-iodopyrimidin-2H-2-one (15b)

Compound **14b** (200 mg, 0.39 mmol) was dried by repeated co-evaporation with dry pyridine (3 x 10 mL) and dissolved in dry pyridine (15 mL). To the solution was added 4,4'-dimethoxytrityl chloride (167 mg, 0.49 mmol) and the reaction mixture was stirred at rt for 12 h. The solution was diluted with CH_2Cl_2 (30 mL) and poured into 5% $NaHCO_3$ solution (20 mL). The organic layer was dried over Na_2SO_4 evaporated and applied to FC (silica gel, column: 15 x 5 cm, CH_2Cl_2/acetone 85:15). Evaporation of the main zone afforded compound **15b** as colorless foam (228 mg, 73%). R_f = 0.30 (CH_2Cl_2/acetone, 85:15); ^{1}H NMR (400

MHz, [D_6]DMSO, 25 °C): δ = 0.93 (q, *J* = 8 Hz, 6H, CH_3), 1.25-1.38 (m, 4H, CH_2), 1.55-1.69 (m, 4H, CH_2), 3.27-3.38 (m, 2H, H-5'), 3.48-3.61 (2m, 4H, 2 x NCH_2), 3.74 (s, 6H, OCH_3), 4.00-4.04 (m, 1H, H-4'), 4.27-4.37 (m, 1H, H-3'), 6.15 (d, *J* = 8.0 Hz, 1H, H-1'), 6.91 (d, *J* = 8.0 Hz, 1H, HO-3'), 6.90-6.92 (m, 4H, Ar-H), 7.21-7.43 (m, 9H, Ar-H), 8.04 (d, *J* = 7.3 Hz, 1H, H-6), 8.64 (s, 1H, N-C=H); ^{19}F NMR (376 MHz, [D_6]DMSO, 25 °C): δ = -114.54 to 116.44 (m, CF_2); UV/Vis (MeOH): λ_{max} (ε) = 334 nm (26200 dm^3 mol^{-1} cm^{-1}). HRMS (ESI-TOF) *m/z*: calcd. for $C_{39}H_{46}F_2IN_4O_6$: $^+$ 831.2425 [*M*+H]$^+$; found 831.2401.

4-(Dibutylaminomethylidene)amino-1-[2-deoxy-2,2'-difluoro-5-O-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-5-iodopyrimidin-2H-2-one 3'-[(2-Cyanoethyl)-N,N-(diisopropyl)]phosphoramidite (16b)

To a solution of compound **15b** (150 mg, 0.18 mmol) in anh. CH_2Cl_2 (10 mL) *N,N*-diisopropylethylamine (0.041 g, 54 µL 0.32 mmol) and chloro-(2-cyanoethoxy)-(diisopropylamino)phosphine (0.057 g, 54 µL, 0.24 mmol) were added. After stirring for 30 min at rt, the solution was diluted with CH_2Cl_2 (5 mL) and poured into 5% aq. $NaHCO_3$ solution (50 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 30 ml), dried over Na_2SO_4, filtered and evaporated. FC (silica gel was pre-washed with traces of triethylamine) (silica gel, CH_2Cl_2/acetone, 9:1) gave compound **16b** (113 mg, 61%) as a mixture of diastereoisomers. R_f = 0.7 (CH_2Cl_2/acetone, 9:1); ^{31}P-NMR (121 MHz, $CDCl_3$, 25 °C, TMS): δ = 153.94 (t, *J* = 6.5), 152.63 (dd, *J* = 24.3, *J* = 11.3); ^{19}F NMR (376 MHz, $CDCl_3$, 25 °C, TMS): δ = -112.86 to 116.81 (m, CF_2); HRMS (ESI-TOF) *m/z*: calcd. for $C_{48}H_{63}F_2IN_6O_7P$: 1031.3503 [*M*+H]$^+$; found 1031.3481.

4-(Dibutylaminomethylidene)amino-1-(2-deoxy-2,2'-difluoro-β-D-erythro-pentofuranosyl)-5-(phenylethynyl)pyrimidin-2H-2-one (14c)

Compound **5** (262 mg, 0.72 mmol) was dissolved in MeOH (5 mL) and treated with *N,N*-dibutylformamid dimethylacetal (700 µL). The reaction mixture was stirred for 30 min at

30°C. The solvent was evaporated and the remaining oily residue was adsorbed on silica gel (20 g) and applied to FC (silica gel, column: 10 x 5 cm, CH_2Cl_2/MeOH 95:5). Evaporation of the main zone afforded compound **14c** as amorphous solid (280 mg, 77%). R_f = 0.60 (CH_2Cl_2/MeOH, 95:5); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 0.80-0.93 (m, 3H, CH_3), 1.22-1.33 (m, 3H, CH_3), 1.54-1.58 (m, 4H, 2 x CH_2), 1.60-1.67 (m, 4H, 2 x CH_2), 3.47-3.70 (m, 5H, H-5', NCH_2), 3.82-3.90 (m, 3H, H-4'), H-5'), 4.20-4.30 (m, 1H, H-3'), 5.44 (t, *J* = 4.0 Hz, 1H, HO-5'), 6.17 (t, *J* = 7.9 Hz, 1H, H-1'), 6.26 (d, *J* = 7.9 Hz, 1H, HO-3'), 7.37-7.45 (m, 4H, Ar-H), 8.38 (s, 1H, H-6), 8.71 (s, 1H, N-C=H); ^{19}F NMR (376 MHz, [D$_6$]DMSO, 25 °C): δ = -116.91; UV/Vis (MeOH): λ_{max} (ε) = 310 (23400), 286 (28900), 253 nm (20700 dm^3 mol^{-1} cm^{-1}); HRMS (ESI-TOF) *m/z*: calcd. for $C_{26}H_{32}F_2N_4O_4Na$: 525.2284 [*M*+Na]$^+$; found 525.2284.

4-(Dibutylaminomethylidene)amino-1-[2-deoxy-2,2'-difluoro-5-O-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-5-(phenylethynyl)pyrimidin-2H-2-one (15c)

Compound **14c** (227 mg, 0.45 mmol) was dried by repeated co-evaporation with dry pyridine (3 x 5 mL) and dissolved in dry pyridine (5 mL). To the solution was added 4,4'-dimethoxytrityl chloride (220 mg, 0.65 mmol) and the reaction mixture was stirred at rt for 4 h. The solution was diluted with CH_2Cl_2 (50 mL) and poured into 5% $NaHCO_3$ soln. The organic layer was dried over Na_2SO_4 evaporated and applied to FC (silica gel, column: 15 x 5 cm, CH_2Cl_2/acetone 4:1). Evaporation of the main zone afforded compound **15c** as colorless foam (264 mg, 73%). R_f = 0.5 (CH_2Cl_2/acetone, 4:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 0.82 (t, *J* = 8.0 Hz, 3H, CH_3), 0.92 (t, *J* = 8.0 Hz, 3H, CH_3), 1.24-1.34 (m, 4H, CH_2), 1.55-1.68 (m, 4H, CH_2), 3.43-3.61 (m, 8H, 2 x NCH_2, 2 x H-5'), 3.66 and 3.67 (2s, 6H, 2 x OCH_3), 4.05-4.09 (m, 1H, H-4'), 4.33-4.43 (m, 1H, H-3'), 6.21 (d, *J* = 8.0 Hz, 1H, H-1'), 6.34 (d, *J* = 4.0 Hz, 1H, HO-3'), 6.86-6.89 (m, 3H, Ar-H), 7.14-7.18 (m, 9H, Ar-H), 7.29-7.32 (m, 4H, Ar-H), 7.44-7.46 (m, 2H, Ar-H), 8.07 (s, 1H, H-6), 8.72 (s, 1H, N-C=H); ^{19}F NMR (376

MHz, [D_6]DMSO, 25 °C): δ = -114.91 to 116.63 (m, CF_2); UV/Vis (MeOH): λ_{max} (ε) = 311 (24700), 286 (30100), 236 nm (34800 dm^3 mol^{-1} cm^{-1}); HRMS (ESI-TOF) *m/z*: calcd. for $C_{47}H_{51}F_2N_4O_6$: 805.3771 [*M*+H]$^+$; found 805.3763.

4-(Dibutylaminomethylidene)amino-1-[2-deoxy-2,2'-difluoro-5-O-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-5-(phenylethynyl)pyrimidin-2H-2-one 3'-[(2-Cyanoethyl)-N,N-(diisopropyl)]phosphoramidite (16c)

To a solution of compound **15c** (210 mg, 0.26 mmol) in anhydrous CH_2Cl_2 (5 mL) *N*,*N*-diisopropylethylamine (59 mg, 78 µL, 0.46 mmol) and chloro-(2-cyanoethoxy)-(diisopropylamino)phosphine (83 mg, 78 µL, 0.35 mmol) were added. After stirring for 30 min at rt, the solution was diluted with CH_2Cl_2 (5 mL) and poured into 5% aq. $NaHCO_3$ solution (50 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL), dried over Na_2SO_4, filtered and evaporated. FC (silica gel was pre-washed with traces of triethylamine) (silica gel, CH_2Cl_2/acetone, 9:1) gave compound **16c** (209 mg, 80%) as a mixture of diastereoisomers. R_f = 0.7 (CH_2Cl_2/acetone, 9:1). ^{31}P-NMR (121 MHz, $CDCl_3$, 25 °C, TMS): δ = 153.86 (t, *J* = 5.7 Hz), 152.57 to 152.75 (m); ^{19}F NMR (376 MHz, $CDCl_3$, 25 °C, TMS): δ = -112.95 to 116.76 (2m, CF_2); HRMS (ESI-TOF) *m/z*: calcd. for $C_{56}H_{68}F_2N_6O_7P$: 1005.4850 [*M*+H]$^+$; found 1005.4852.

6-(3-Phenyl)-3-[2-deoxy-2,2'-difluoro-5-O-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl]pyrrolo[2,3-d]pyrimidin-2H-2-one 3'-[(2-Cyanoethyl)-N,N-(diisopropyl)]phosphoramidite (17)

To a soluton of compound **9** (316 mg, 0.47 mmol) in anh. CH_2Cl_2 (5 mL), *N*,*N*-diisopropylethylamine (108 mg, 142 µL, 0.83 mmol) and chloro-(2-cyanoethoxy)-(diisopropylamino)phosphine (150 mg, 142 µL, 0.64 mmol) were added. After stirring for 30 min at rt, the solution was diluted with CH_2Cl_2 (5 mL) and poured into 5% aq $NaHCO_3$ solution (50 mL). The aq layer was extracted with CH_2Cl_2 (2 x 30 mL), dried over Na_2SO_4,

filtered and evaporated. FC (silica gel was pre-washed with traces of triethylamine) (silica gel, CH_2Cl_2/acetone, 1:1) gave compound **17** (361 mg, 89%) as a mixture of diastereoisomers. R_f 0.5 (CH_2Cl_2/acetone, 1:1); ^{31}P-NMR (161 MHz, $CDCl_3$, 25 °C, TMS): δ = 155.0 (t, *J* = 6.5 Hz), 152.7 (t, *J* = 9.7 Hz);^{19}F NMR (376 MHz, $CDCl_3$, 25 °C, TMS): δ = -115.7 to 116.4 (m, CF_2); HRMS (ESI-TOF) *m/z*: calcd. for $C_{47}H_{51}F_2N_5O_7P$: 888.3308 $[M+H]^+$; found 888.3303.

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Key Words: DNA, gemcitabine, silver ions, base pairing, duplex stability

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Table of contents entry

Gemcitabine, Pyrrologemcitabine and 2'-Fluoro- 2'-Deoxycytidines: Synthesis, Physical Properties and Impact of Sugar Fluorination on Silver Ion Mediated Base Pairing

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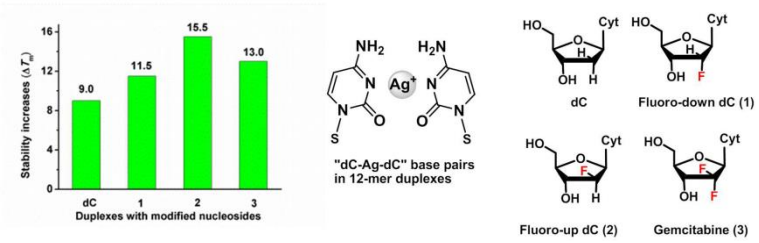

Talking between sugar residues and silver mediated cytosine – cytosine base pairs. Stereoelectronic effects induced by DNA ribose fluorination lead to stability changes of metal DNA.