CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.201103270

Synthesis of Aldehyde-Linked Nucleotides and DNA and Their Bioconjugations with Lysine and Peptides through Reductive Amination

Veronika Raindlová,[a] Radek Pohl,[a] and Michal Hocek*[a, b]

Abstract: 5-(5-Formylthienyl)-, 5-(4-formylphenyl)- and 5-(2-fluoro-5-formylphenyl)cytosine 2′-deoxyribonucleoside mono- (**dC^{R}MP**) and triphosphates (**dC^{R}TP**) were prepared by aqueous Suzuki–Miyaura cross-coupling of 5-iodocytosine nucleotides with the corresponding formylarylboronic acids. The **dC^{R}TP**s were excellent substrates for DNA polymerases and were incorporated into DNA by primer extension or PCR. Reductive aminations of the model **dC^{R}MP**s with lysine or lysine-containing tripeptide were studied and optimized. In aqueous phosphate buffer (pH 6.7) the yields of the reductive aminations with tripeptide **III** were up to 25%. Bioconjugation of an aldehyde-containing DNA with a lysine-containing tripeptide was achieved through reductive amination in yields of up to 90% in aqueous phosphate buffer.

Keywords: aldehydes · DNA · nucleotides · peptides · reductive aminations

Introduction

Bioconjugation of nucleic acids with other biomolecules or unnatural compounds is of great importance in medicinal chemistry and chemical biology (e.g., for delivery of oligonucleotides)[1] or even in nanotechnology and material sciences.[2] In most cases, the (bio)molecule is linked to a sugar or phosphate at either the 3′ or 5′ end of an oligonucleotide (ON).[3] Linkage through a nucleobase offers better programmability of the position(s) and number of modifications, and their exposition to the major groove in DNA. It is usually done by attachment of a reactive group to a nucleobase in a nucleotide building block, incorporation to an ON and post-synthesis attachment of the target molecule. Introduction of an alkyne, alkene, azide or diene group or a cysteine residue either by chemical phosphoramidite synthesis or by enzymatic polymerase synthesis has been achieved and the modified DNA has been used for [2+3]-dipolar cycloadditions (triazole or isoxazoline formation),[4,5] Staudinger ligation,[6] Diels–Alder reactions[7] or native peptide ligations.[8]

An aldehyde function is a very attractive group because of its high and specific reactivity with diverse reagents. However, only several examples of nucleobase-linked aldehyde-modified ONs are known;[9–12] these are either prepared directly or indirectly by click reaction with azide derivatives of reducing sugars,[4f] or by introduction of 2,3-dihydroxypropyl or 3,4-dihydroxypyrrolidine moieties[13,14] followed by oxidative cleavage of the vicinal diols to (di)aldehydes. Kittaka et al.[12] reported several examples of DNA–protein cross-links through reductive amination of 5- or 6-formyluracil. Sugar-linked aldehyde- or ketone-modified nucleic acids have been more frequently explored;[1c,15] these have often served for conjugation through Schiff base, hydrazone or oxime formation or reductive amination. Recently, we have reported a facile and efficient construction of nucleobase-linked aldehyde-modified DNA in two steps (cross-coupling of a nucleoside triphosphate with formylthienylboronic acid followed by polymerase incorporation) and its further conjugation or staining by hydrazone formation in water.[16] The efficiency of this approach prompted us to explore the potential of the (now) easily available formylaryl–nucleobase linked DNA for conjugation with lysine and lysine-containing peptides through reductive amination.

Results and Discussion

For the bioconjugation or cross-linking of DNA with natural unmodified peptides or proteins, a reductive amination of an aldehyde group with nucleophilic amino group of lysine was envisaged. For the construction of the aldehyde-linked DNA,[16] we planned to use the above-mentioned two-step procedure[17] consisting of a cross-coupling of formylarylboronic acids with halogenated 2′-deoxyribonucleoside triphosphates (dNTPs) followed by polymerase incorporation[18] by

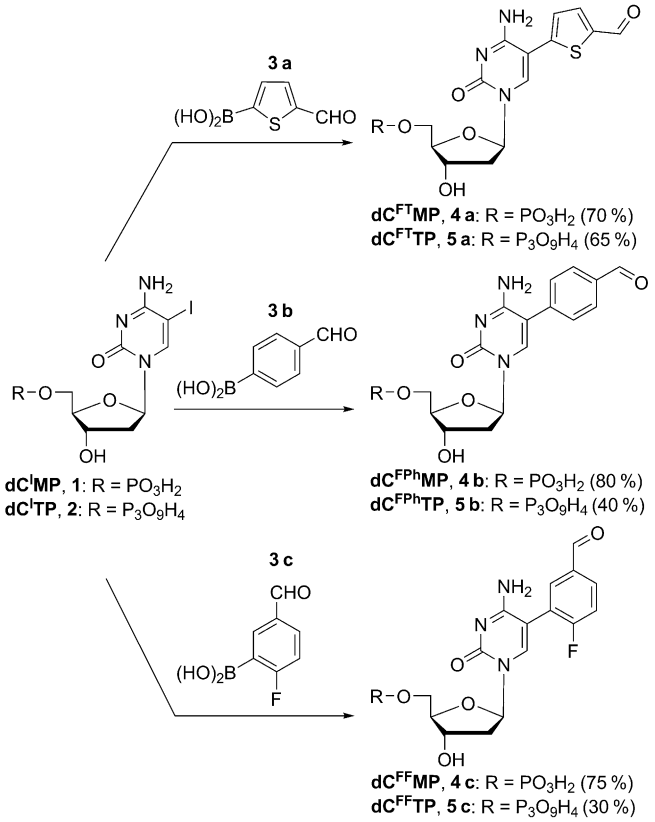
[a] V. Raindlová, Dr. R. Pohl, Prof. Dr. M. Hocek
Institute of Organic Chemistry and Biochemistry
Academy of Sciences of the Czech Republic
Gilead Sciences & IOCB Research Center
Flemingovo nam. 2, 16610 Prague 6 (Czech Republic)
Fax: (+420) 220-183-559
E-mail: hocek@uochb.cas.cz
Homepage: http://www.uochb.cas.cz/hocekgroup

[b] Prof. Dr. M. Hocek
Department of Organic and Nuclear Chemistry
Faculty of Science, Charles University in Prague
Hlavova 8, 12843 Prague 2 (Czech Republic)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201103270.

primer extension (PEX) or polymerase chain reaction (PCR). In this study, we focused on modifications of 2′-deoxycytidines bearing three different formylaryl groups at the position 5 to study the electronic effects of the aryl group on the reactivity of the aldehyde. At first, the synthesis and reductive aminations were tested and optimized on the corresponding nucleoside monophosphates (dNMPs) as models for ONs and then the procedures were applied for the synthesis of the modified dNTPs, their incorporations to DNA and its conjugations with a model peptide.

The desired modified cytidine monophosphates (**dC^{R}MP**s) bearing reactive aldehyde groups were prepared by aqueous Suzuki cross-coupling reaction of iodinated nucleoside monophosphate **dC^{I}MP** (**1**) with three different boronic acids: 5-formylthiophene-2-boronic acid (**3a**), 4-formylphenyl boronic acid (**3b**) and 2-fluoro-5-formylphenyl boronic acid (**3c**; Scheme 1). The reactions of **dC^{I}MP** (**1**) with **3a–c** were performed in ACN/H_2O in the presence of Cs_2CO_3, $Pd(OAc)_2$ and tris(3-sulfonatophenyl)phosphine sodium salt (TPPTS). The reaction with boronic acids **3a** and **b** proceeded smoothly at 100 or 90 °C, whereas the fluorinated boronic acid **3c** was less stable and the reaction temperature was decreased to 75 °C. The corresponding aldehyde-modified nucleoside monophosphates **dC^{FT}MP** (**4a**), **dC^{FPh}MP** (**4b**) and **dC^{FF}MP** (**4c**) were isolated in good yields of 70–80 % and were further used for the study of reductive aminations (vide infra).



Scheme 1. Reagents and conditions: **1** or **2**, **3a–c**, Cs_2CO_3, $Pd(OAc)_2$, TPPTS, ACN/H_2O. **dC^{FT}MP** (**4a**): 100 °C, 1 h; **dC^{FT}TP** (**5a**): 100 °C, 1 h; **dC^{FPh}MP** (**4b**): 90 °C, 2 h; **dC^{FPh}TP** (**5b**): 90 °C, 30 min; **dC^{FF}TP** (**4c**): 75 °C, 1.5 h; **dC^{FF}TP** (**5c**): 75 °C, 30 min.

The corresponding aldehyde-modified nucleoside triphosphates (**dC^{R}TP**s) were prepared (Scheme 1) analogously from **dC^{I}TP** (**2**). The Suzuki cross-coupling reactions of **2** with boronic acids **3a–c** gave the desired products **dC^{FT}TP** (**5a**),[16] **dC^{FPh}TP** (**5b**) and **dC^{FF}TP** (**5c**) in 65, 40 and 30 % yields, respectively, after isolation by semipreparative HPLC, conversion to sodium salts by ion exchange and freeze-drying.

These modified **dC^{R}TPs** (**5a–c**) served as substrates for polymerase incorporation into DNA. Seven different DNA polymerases were tested in PEX or PCR experiments. The enzymatic incorporation of **dC^{R}TPs** (**5a–c**) in PEX was studied by using Pwo, KOD XL, DeepVent(exo$^-$), DeepVent, DyNAzyme II and Vent(exo$^-$) DNA polymerases and two different templates (a 19-mer comprising single modification and a 31-mer with four modifications in separate positions). All of the tested **dC^{R}TP**s were successfully and efficiently incorporated into DNA by using both types of template, and all the above-mentioned polymerases gave full-length aldehyde modified 19- or 31-mer ONs (Figure 1a and b; Figures S1–S3 in the Supporting Information). The thermal denaturation study (Table S5 in the Supporting Information) showed slight destabilization of the duplex by formylthienyl or formylphenyl (−0.25 or −0.75 °C per modification) and significant (−2.75 °C per modification) destabilization for the fluoroformylphenyl groups.

In order to compare the efficiency of incorporation of the aldehyde-modified **dC^{R}TP**s with the natural dCTP, we also

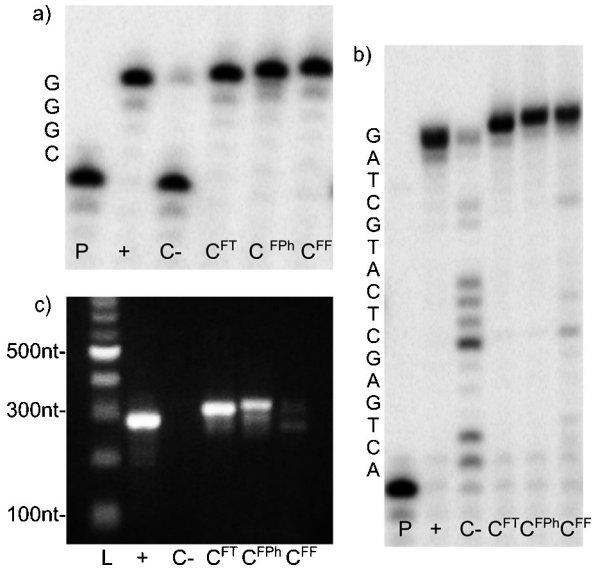


Figure 1. Primer extension and PCR incorporation of **dC^{FT}TP** (**5a**), **dC^{FPh}TP** (**5b**) and **dC^{FF}TP** (**5c**). a) PEX, 19-mer template, KOD XL DNA polymerase. C-: dGTP; C^{FT}: **dC^{FT}TP** (**5a**), dGTP; C^{FPh}: **dC^{FPh}TP** (**5b**), dGTP; C^{FF}: **dC^{FF}TP** (**5c**), dGTP. b) PEX, 31-mer template, DyNAzyme DNA polymerase; c) PCR, 297-mer template, KOD XL DNA polymerase. b) and c) C-: dTTP, dATP, dGTP; C^{FT}: **dC^{FT}TP** (**5a**), dTTP, dATP, dGTP; C^{FPh}: **dC^{FPh}TP** (**5b**), dTTP, dATP, dGTP; C^{FF}: **dC^{FF}TP** (**5c**), dTTP, dATP, dGTP. P: primer; L: oligonucleotide ladder; (+): natural dNTPs.

performed a simple kinetic study in the single nucleotide PEX experiment using two selected DNA polymerases (KOD XL and DeepVent(exo$^-$)). The incorporation of formylthienyl- and formylphenyl-linked **dC^{FT}TP** (**5a**) and **dC^{FPh}TP** (**5b**), respectively, proceeded with a rate comparable with unmodified dCTP and was finished within 30 seconds. The incorporation of fluorinated **dC^{FF}TP** (**5c**) took 2 min to completion. The enzymatic incorporation of **dC^{R}TP**s by DeepVent(exo$^-$) DNA polymerase proceeded with a similar rate and efficiency, but with extension of the reaction times (>5 min) formation of longer products by non-templated incorporation of one or two more nucleotides was observed (Figures S7–S9 in the Supporting Information).

Then we tested the aldehyde-modified **dC^{R}TP**s (**5a–c**) for the amplification of DNA by PCR with three templates (98, 297 and even 1162 nucleotides [nt]) using selected DNA polymerases (Phusion, Vent(exo$^-$) and KOD XL). All the PCR amplifications with **dC^{FT}TP** (**5a**) and **dC^{FPh}TP** (**5b**) were successful with all three polymerases and templates (Figure 1c and Figures S4–S6 in the Supporting Information) and clean (only slightly smeared) spots of the full-length products were observed on the gels in all cases. **dC^{FF}TP** (**5c**), however, was a far worse substrate in PCR reactions. The spots of the 98 and 287 nt DNA products were rather smeared and the 1162-mer was not detected at all. This is consistent with the lower efficiency of **dC^{FF}TP** (**5c**) revealed in the kinetic studies and thermal denaturation study (vide supra).

The next goal was to develop the procedure for the conjugation of the aldehyde-modified DNA through reductive amination. This reaction was first tested and optimized on all the three model **dC^{R}MP**s (**4a–c**) by using two amino acids, acetylated and free lysine (Ac-Lys-NH_2·HCl, **I**, and Lys·HCl, **II**) and one synthetically prepared lysine-containing tripeptide (Ac-Ala-Lys-Ala-NH_2, **III**). Reductive amination in water is challenging due to unfavorable equilibrium in the Schiff-base formation as it has been repeatedly shown in dynamic combinatorial chemistry studies.[19] Therefore, we tested the reactions in methanol, glycerol, aqueous glycerol (10%) and phosphate buffer (Scheme 2, Table 1). Several reducing agents ($Na_2S_2O_4$,[20] $NaBH(OAc)_3$,[21] H_2/Pd-C,[22] $NaBH_4/H_3BO_3$[23] and $NaBH_3CN$[11]) were tested under different conditions but, in most cases, the conversions were very low and only the reduction of the aldehydes to hydroxymethylcytosines **11a–c** was observed. Only the use of $NaBH_3CN$ gave acceptable conversions of the reductive aminations, and therefore, this reagent was used in the systematic screening of the conditions. As expected, both the conversions and the selectivities for reductive aminations versus aldehyde reductions were strongly solvent-dependent (Table 1 and Tables S1–S3 and S4 in the Supporting Information).

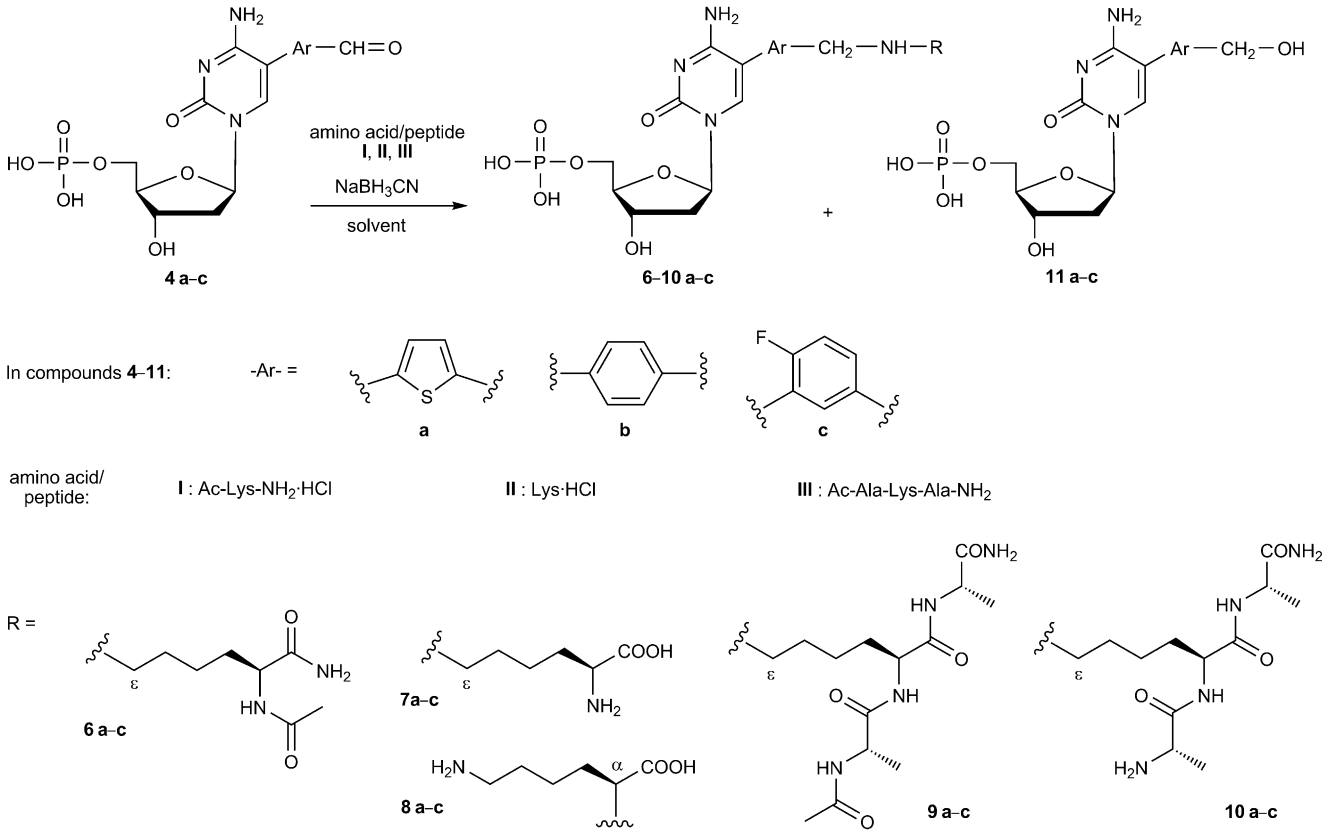
The reactions of **4a–c** with acetylated lysine (**I**) usually gave mixtures of the desired conjugates **6a–c** and the reduced hydroxymethyl derivatives **11a–c**. In methanol or glycerol, the yields of **6a–c** were good to excellent (55–88%) and the side-products **11a–c** were formed in negligible amounts. On the other hand, in 10% glycerol or phosphate buffer, the reduction of the aldehyde was the major reaction (yields of **11a–c**, 28–40%) and the desired products **6a–c** were only isolated in 18–33% yields. The yields of the reactions of formylthienyl derivative **4a** were significantly lower than those of the two formylphenyl derivatives **4b**,**c**.

Analogous reactions with lysine (**II**) were more complicated by the fact that both α- and ε-amino groups can react in the reductive amination with an aldehyde. Therefore, two types of products **7a–c** (ε-) and **8a–c** (α-) were observed in all the reductive aminations of **4a–c**. Moreover, in the reaction of **4b** with **II** in methanol, formation of a minor (3%) compound **12b** was observed as the product of double reductive aminations at both amino groups (Scheme 3). The combined yields of both reductive amination products (**7**+**8**) were comparable or slightly lower in organic solvents compared to acetylated lysine (**I**) but in aqueous solutions the conversions of reductive aminations were slightly higher. In methanol or glycerol, the ratio of ε- to α-substituted products **7a–c** to **8a–c** were 5:1 to 2:1, whereas in aqueous glycerol or phosphate buffer the α-substituted products prevailed (1:1 to 1:5).

The reactions of **4a–c** with the acetylated tripeptide amide **III** typically gave the desired products of the reductive amination **9a–c** (yields 22–52% in organic solvents and 10–25% in aqueous solutions) accompanied by either hydroxymethylcytosine byproducts **11a–c** (in water) or deacetylated products of reductive amination **10a–c** (in organic solvents). In the reaction of **4a** with **III** in glycerol, a minor byproduct **13a** was isolated in 13% yield as a product of deacetylation of **III** followed by reductive amination at the amino group at the N terminus of the peptide (Scheme 3).

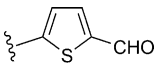
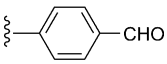
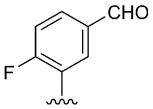
In general, all the reductive aminations gave mixtures of products and the yields were moderate to good (but never quantitative). Obviously, in methanol and glycerol, the yields of the products of the reductive aminations were higher than in aqueous glycerol or aqueous phosphate buffer. However, only the aqueous solutions are applicable for the intended bioconjugations of nucleic acids with peptides. The formylphenyl derivative **4b** gave the highest yields of reductive aminations with peptide **III** in the phosphate buffer at pH 6.7, which are the conditions suitable for reactions of nucleic acids and peptides. Therefore, we selected the 5-(4-formylphenyl)cytosine triphosphate, **dC^{FPh}TP** (**5b**) for further studies of the conjugations.

To test the conjugations of aldehyde-modified nucleic acids with the peptide **III**, we incorporated one or four modified nucleotides using **dC^{FPh}TP** (**5b**) with either the 19- or 31-mer template in the presence of DeepVent(exo$^-$) DNA polymerase under standard the conditions discussed above. Then the resulting modified DNA, either unpurified or after purification, was treated with the peptide **III** in the presence of $NaBH_3CN$ in phosphate buffer. The reaction with **III** (3 equiv) showed only very minor conjugate formation. Only further increase in the excess of **III** (to 50-fold) gave high conversion of the conjugation. Figure 2 shows the



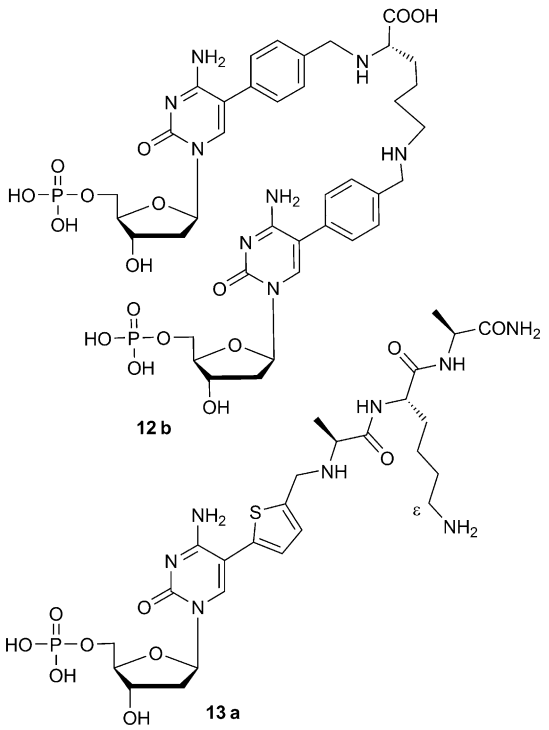
Scheme 2. Reductive aminations of **4a–c** with amino acids **I**, **II** and peptide **III** (3 equiv) in the presence of $NaBH_3CN$ (1 equiv).

Table 1. Yields of the reductive aminations of **4a–c** with amino acids **I**, **II** and peptide **III**.

		Ac-Lys-NH_2·HCl (**I**)		Lys·HCl (**II**)			Ac-Ala-Lys-Ala-NH_2 (**III**)	
Ar–CH=O	Solvent	Product	Yield [%]	Product	Yield [%]	(**7x**:**8x**)[a]	Product	Yield [%]
 **4a**	methanol	**6a**	55[b]	**7a+8a**	54[c]	4:1	**9a**	29[c,e]
	99% glycerol		63[b]		51[c]	2:1		36[c]
	10% glycerol		19[b]		25[c]	1:2		10[c]
	PO_4^{2-} buffer[d]		18[b]		26[b]	1:1		13[b]
 **4b**	methanol	**6b**	71[b]	**7b+8b**	44[c]	5:1	**9b**	52[c,f]
	99% glycerol		88[b]		50[c]	2:1		51[c,g]
	10% glycerol		28[b]		53[c]	1:1		23[c]
	PO_4^{2-} buffer[d]		24[b]		26[b]	1:2		25[b]
 **4c**	methanol	**6c**	76[b]	**7c+8c**	77[c]	5:1	**9c**	24[c,h]
	99% glycerol		79[b]		74[c]	2:1		22[c]
	10% glycerol		33[b]		35[c]	1:1		23[c]
	PO_4^{2-} buffer[d]		25[b]		44[b]	1:5		22[b]

[a] **x**=**a**, **b** or **c**. [b] 48 h. [c] 24 h. [d] phosphate buffer (50 mM, pH 6.7). [e] **10a** (6%). [f] **10b** (14%). [g] **10b** (6%). [h] **10c** (5%).

results of the reductive aminations of the 19-mer PEX product containing one formylphenyl modification with different modes of purification of the PEX products and/or the reductive amination products. In all cases, the spot of the PEX product (lane C^{FPh}) almost fully disappeared and an intense new band of the DNA–peptide conjugate was observed with slower mobility (lanes 1–4). The conversions of the reductive aminations were calculated from the PAGE by using the ImageJ quantification program (Figure S10 in the Supporting Information). The results showed that with unpurified PEX product the conversion was about 80% whereas with purified PEX product, the yield was 90%. Final purification of the DNA–peptide conjugate (NucAway spin columns) did not further improve the purity. The formation of this DNA–peptide conjugate was confirmed by MALDI-ToF MS spectrometry (found *m/z* 6369.5, calcd 6368.2; Fig-



Scheme 3. Minor byproducts in reductive amination reactions.

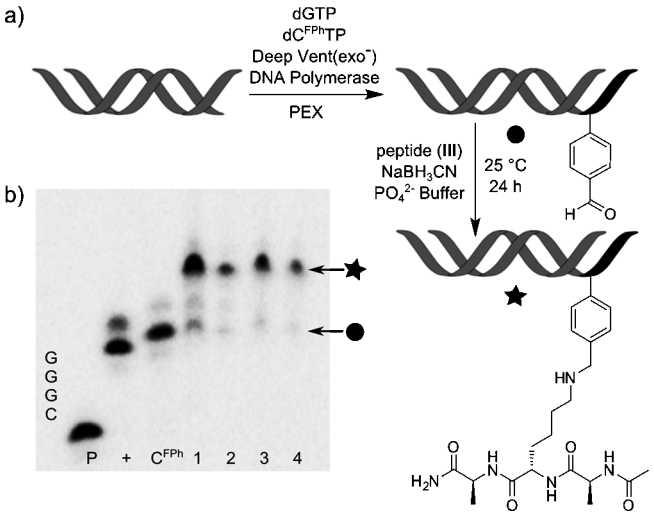


Figure 2. a) Scheme of peptide–DNA conjugation via a phenyl unit linker. b) PAGE analysis of PEX and the DNA–peptide conjugation by using the 19-mer template, DeepVent(exo$^-$) DNA polymerase and peptide **III**; P: primer; +: natural dNTPs; C^{FPh}: **dC^{FPh}TP** (**5b**), dGTP; lanes 1–4: **dC^{FPh}TP** (**5b**), dGTP, Ac-Ala-Lys-Ala-NH_2 (50 equiv), $NaBH_3CN$; lane 1: no purification; lanes 2, 3: PEX product purification (QIAquick nucleotide removal kit); lanes 2, 4: DNA–peptide conjugate purification (NucAway Spin columns).

ure S12 in the Supporting Information). In the case of the 31-mer PEX product containing four aldehydes, the reductive amination was quantitative (Figure S11 in the Supporting Information) but a mixture of several products (presumably of single and multiple conjugation) was observed.

Conclusion

An efficient and straightforward synthesis of three different 5-formylaryl-modified cytidine mono- and triphosphates was developed by using the aqueous cross-coupling reactions of 5-iodocytosine nucleotides with formylarylboronic acids. The **dC^{R}TP**s were excellent substrates for DNA polymerases and were incorporated into DNA by means of PEX, and two of them also by PCR, with an efficiency close to natural dCTP. In this way, different aldehyde-modified DNAs were prepared, ranging from short ONs bearing one or several modifications up to large DNA duplexes (100–1200 nt) bearing many aldehyde groups (40–580) at specific positions. The reactivity toward reductive aminations with lysine, N-protected lysine and a lysine-containing tripeptide were first tested on model nucleotides **dC^{R}MP**s (**4a–c**) and the effect of solvent was studied. Obviously, the reductive aminations proceeded much better in methanol or glycerol than in aqueous mixtures. In the case of lysine, the solvent also influenced the ratio of ε- to α-amino group chemoselectivity. In phosphate buffer, as the only suitable medium for bioconjugations, the maximum yields of reductive aminations with tripeptide **III** were about 25% for formylphenyl derivative **4b**. This modification was then incorporated into DNA and the PEX product was subjected for the reductive amination with tripeptide **III**. This reaction gave 80–90% conversion but only in the presence of large (50-fold) excess of the tripeptide. Apparently, the efficiency of the reductive amination cannot match the success of Cu-mediated triazole formation used in click chemistry.[4] However, the reductive amination can be performed with native peptides and proteins containing natural lysine (no incorporation of any unnatural amino acid is needed in contrast to the click reaction).[4] Therefore, we believe that this method has promising potential in nucleic acid–peptide or –protein conjugations. In particular, cross-linking[24] with some sequence-specific DNA-binding proteins could be improved by specific interactions between the DNA and protein. On the other hand, the high natural abundance of lysine in proteins could impair the specificity of the bioconjugation. Studies of the reductive aminations of aldehyde-modified ONs with longer peptides and proteins, as well as their applications in cross-linking assays for protein binding are under way in our laboratory.

Experimental Section

General chemistry: NMR spectra were measured on a Bruker Avance 500 at 500 MHz for ^{1}H and 125.7 MHz for ^{13}C, or on Bruker Avance at 600 MHz for ^{1}H and 150.9 MHz for ^{13}C, instruments in D_2O (reference to dioxane as internal standard, $\delta_H = 3.75$ ppm, $\delta_C = 67.19$ ppm) or in [D_4]methanol (reference to TMS as internal standard). Chemical shifts are given in ppm (δ scale), coupling constants (J) in Hz. Complete assignment of all NMR signals were achieved by using a combination of H,H COSY, H,C HSQC and H,C HMBC experiments. Semi-preparative separation of nucleoside triphosphates and monophosphates was performed by HPLC on a column packed with 10 μm C18 reversed

phase (Phenomenex, Luna C18 (2)). IR spectra were measured either on Bruker Alpha FT-IR spectrometer by using ATR technique or by using KBr tablets. Mass spectra were measured by ESI. High resolution mass spectra were measured on a LTQ Orbitrap XL (Hermo Fischer Scientific) spectrometer by using ESI ionization technique. Mass spectra of functionalized DNA were measured by MALDI-ToF, Reflex IV (Bruker) with nitrogen laser. UV/Vis spectra were measured on a Varian CARY 100 Bio spectrophotometer at room temperature. Known starting compounds were either purchased from suppliers and used without any further treatment (5-formylthiophene-2-boronic acid (**3a**), 4-formylphenyl boronic acid (**3b**) and 2-fluoro-5-formylphenyl boronic acid (**3c**) from Frontier Scientific) or prepared by literature procedures (compounds **dC^{I}MP** (**1**),[16] **dC^{I}TP** (**2**),[17c] **dC^{FT}MP** (**4a**)[16] and **dC^{FT}TP** (**5a**)[16]).

Preparation of dC^{FPh}TP (5b) by the Suzuki cross-coupling reaction: A water/acetonitrile mixture (2:1, 1 mL) was added through a septum to an argon-purged vial containing 5-iodo-2′-deoxycytidine triphosphate (**dC^{I}TP**, (**2**); 0.047 g, 0.071 mmol), 4-formylphenyl boronic acid (**3b**; 3 equiv, 0.017 g, 0.213 mmol) and Cs_2CO_3 (5 equiv, 0.062 g, 0.355 mmol). After the solids had dissolved, a solution of $Pd(OAc)_2$ (0.9 mg, 10 mol%) and TPPTS (5 equiv, 0.011 g, 0.020 mmol) in water/acetonitrile mixture (2:1, 1 mL) was injected into the reaction mixture and the resulting sample was stirred at 90 °C for 30 min. The reaction mixture was extracted with $CHCl_3$ (2×4 mL). The water layer containing the product was concentrated by evaporation and product was isolated by semipreparative HPLC on a C18 column with the use of a linear gradient starting from TEAB (0.1 M; triethylammonium bicarbonate) in H_2O/MeOH 3:1 to 0.1 M TEAB in H_2O/MeOH 1:1 as eluent. Several co-distillations with water, conversion to sodium salt form (Dowex 50WX8 in Na^+ cycle) followed by freeze drying from water, gave the product **dC^{FPh}TP** (**5b**) as a white powder (0.009 g, 40%). ^{1}H NMR (499.8 MHz, D_2O, $ref_{dioxane}$ = 3.75 ppm, pD = 7.1): δ = 2.37 (ddd, 1H, J_{gem} = 14.1 Hz, $J_{2'b,1'}$ = 7.1 Hz, $J_{2'b,3'}$ = 6.4 Hz, H-2′b), 2.46 (ddd, 1H, J_{gem} = 14.1 Hz, $J_{2'a,1'}$ = 6.3 Hz, $J_{2'a,3'}$ = 3.5 Hz, H-2′a), 4.12–4.20 (m, 2H, H-5′), 4.22 (m, 1H, H-4′), 4.63 (dt, 1H, $J_{3',2'}$ = 6.4, 3.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3′), 6.35 (dd, 1H, $J_{1'2'}$ = 7.1, 6.3 Hz, H-1′), 7.68 (m, 2H, H-*o*-phenylene), 7.89 (s, 1H, H-6), 8.05 (m, 2H, H-*m*-phenylene), 9.98 ppm (s, 1H, CHO); ^{13}C NMR (125.7 MHz, D_2O, $ref_{dioxane}$ = 69.3 ppm, pD = 7.1): δ = 41.90 (CH_2-2′), 67.92 (d, $J_{C,P}$ = 5.7 Hz, CH_2-5′), 73.23 (CH-3′), 88.6 (d, $J_{C,P}$ = 8.8 Hz, CH-4′), 88.90 (CH-1′), 112.41 (C-5), 132.76 (CH-*o*-phenylene), 133.66 (CH-*m*-phenylene), 138.20 (C-*p*-phenylene), 141.97 (C-*i*-phenylene), 143.21 (CH-6), 159.72 (C-2), 166.85 (C-4), 198.77 ppm (CHO); ^{31}P{^{1}H} NMR (202.3 MHz, D_2O, $ref_{phosphate\ buffer}$ = 2.35 ppm, pD = 7.1): δ = −21.70 (t, J = 19.6 Hz, P_β), −10.99 (d, J = 19.6 Hz, P_α), −6.57 ppm (bd, J = 19.6 Hz, P_γ); IR (KBr): $\tilde{\nu}$ = 3428, 2435, 2366, 1742, 1700, 1652, 1603, 1490, 1367, 1312, 1159, 1085, 981 cm^{-1}; MS (ESI$^-$): *m/z* (%): 613.9 (20) $[M-Na]^-$, 658.2 (5) $[M-H+Na]^-$, 284.6 (66) $[M+H-3Na]^{2-}$, 512.0 (32) $[M+2H-Na-HPO_3Na]^-$, 490.1 (31) $[M+3H-2Na-HPO_3Na]^-$; HRMS (ESI$^-$): *m/z* $[M-Na]^-$ calcd for $C_{16}H_{17}O_{14}N_3Na_2P_3$: 613.97242, found 613.97192; UV/Vis (H_2O, 100 μM) λ_{max1} (ε) = 286 (3198), λ (ε) = 254 nm (2146 mol^{-1} dm^3 cm^{-1}).

General procedure for reductive aminations of dC^{R}MPs (4a–c) with amino acids I, II and peptide III (methods A, B, C): Amino acid or peptide (3 equiv) was added in one portion to a stirred solution of aldehyde derivative **dC^{R}MP** (**4a–c**) in 2.5 mL of different solvents (MeOH, 99% glycerol, 10% glycerol or 50 mM phosphate buffer, pH 6.7) under an atmosphere of argon. After 1 h, $NaBH_3CN$ (1 equiv) was added through a syringe in the corresponding solvent (0.5 mL). After being stirred for 24 h (48 h) at room temperature under an atmosphere of argon the crude products and also side-products were isolated by semipreparative HPLC on a C18 column with the use of linear gradient of TEAB (triethylammonium bicarbonate, 0.1 M) in H_2O to 0.1 M TEAB in H_2O/MeOH (1:1) as eluent. Several co-distillations with water, conversion to sodium salt form (Dowex 50WX8 in Na^+ cycle) followed by freeze drying from water gave the products and side-products as white powders. Method A: amino acid **I**, 48 h, products **6a–c**, byproducts **11a–c**; method B: amino acid **II**, 24 h (48 h in phosphate buffer), products **7a–c**, **8a–c**, byproducts **11a–c**, **12b**; method C: peptide **III**, 24 h (48 h in phosphate buffer), products **9a–c**, byproducts **10a–c**, **11a–c**, **13a**.

Compound 9b: This was prepared from **dC^{FPh}MP** (**4b**) according to the general procedure (method C) in phosphate buffer (50 mM, pH 6.7). After being stirred for 48 h the product was isolated as white powder (7 mg, 25%): ^{1}H NMR (499.8 MHz, D_2O, $ref_{dioxane}$ = 3.75 ppm): δ = 1.35 (d, 3H, $J_{3,2}$ = 7.2 Hz, H-3-AcAla), 1.39 (d, 3H, $J_{3,2}$ = 7.2 Hz, H-3-AlaNH$_2$), 1.46 (m, 2H, H-4-Lys), 1.73 (m, 2H, H-5-Lys), 1.76, 1.85 (2×bm, 2×1H, H-3-Lys), 2.00 (s, 3H, CH_3CO), 2.33 (ddd, 1H, J_{gem} = 14.1 Hz, $J_{2'b,1'}$ = 7.9 Hz, $J_{2'b,3'}$ = 6.2 Hz, H-2′b), 2.43 (ddd, 1H, J_{gem} = 14.1 Hz, $J_{2'a,1'}$ = 6.1 Hz, $J_{2'a,3'}$ = 3.4 Hz, H-2′a), 3.07 (t, 2H, $J_{6,5}$ = 7.8 Hz, H-6-Lys), 3.85, 3.88 (2×dt, 2×1H, J_{gem} = 11.3 Hz, $J_{H,P}$ = $J_{5',4'}$ = 5.3 Hz, H-5′), 4.15 (td, 1H, $J_{4',5'}$ = 5.3 Hz, $J_{4',3'}$ = 3.4 Hz, H-4′), 4.21 (s, 2H, CH_2N), 4.24 (q, 1H, $J_{2,3}$ = 7.2 Hz, H-2-AcAla), 4.27 (q, 1H, $J_{2,3}$ = 7.2 Hz, H-2-AlaNH$_2$), 4.31 (dd, 1H, $J_{2,3}$ = 8.7, 5.7 Hz, H-2-Lys), 4.51 (dt, 1H, $J_{3',2'}$ = 6.2, 3.4 Hz, $J_{3',4'}$ = 3.4 Hz, H-3′), 6.34 (dd, 1H, $J_{1',2'}$ = 7.9, 6.1 Hz, H-1′), 7.51 (m, 2H, H-*o*-phenylene), 7.56 (m, 2H, H-*m*-phenylene), 7.76 ppm (s, 1H, H-6); ^{13}C NMR (125.7 MHz, D_2O, $ref_{dioxane}$ = 69.3 ppm): δ = 19.16 (CH_3-3-AcAla), 19.38 (CH_3-3-AlaNH$_2$), 24.26 (CH_3CO), 24.89 (CH_2-4-Lys), 28.03 (CH_2-5-Lys), 33.01 (CH_2-3-Lys), 41.66 (CH_2-2′), 49.89 (CH_2-6-Lys), 52.15 (CH-2-AlaNH$_2$), 52.52 (CH-2-AcAla), 53.59 (CH_2NH), 55.93 (CH-2-Lys), 66.35 (d, $J_{C,P}$ = 4.4 Hz, CH_2-5′), 73.94 (CH-3′), 88.69 (CH-1′), 88.80 (d, $J_{C,P}$ = 8.5 Hz, CH-4′), 112.74 (C-5), 132.83 (CH-*o*-phenylene), 133.18 (CH-*m*-phenylene), 134.96 (C-*p*-phenylene), 136.00 (C-*i*-phenylene), 142.71 (CH-6), 159.81 (C-2), 167.17 (C-4), 176.30 (C-1-Lys), 176.92 ($COCH_3$), 178.29 (C-1-AcAla), 180.31 ppm (C-1-AlaNH$_2$); ^{31}P{^{1}H} NMR (202.3 MHz, D_2O): δ = 4.50 ppm; IR (KBr): $\tilde{\nu}$ = 3387, 2937, 1652, 1543, 1479, 1416, 1374, 1313, 1265, 1073, 975 cm^{-1}; MS (ESI$^+$): *m/z* (%): 725.3 (12) $[M+H]^+$, 747.3 (63) $[M+Na]^+$, 769.3 (52) $[M-1H+2Na]^+$, 770.2 (19) $[M+2Na]^+$, 791.2 (10) $[M-2H+3Na]^+$; HRMS (ESI$^+$): *m/z* $[M+H]^+$ calcd for $C_{30}H_{46}O_{11}N_8P$: 725.30182, found 725.30218; UV/Vis (H_2O, 100 μM) λ_{max1} (ε) = 238 (10126), λ_{max2} (ε) = 277 (6193), λ (ε) = 254 nm (8459 mol^{-1} dm^3 cm^{-1}).

Primer extension experiment (single incorporation): The reaction mixture (20 μL) contained KOD XL DNA polymerase (Merc4biosciences, Novagen, 2.5 U μL^{-1}, 0.02 μL), dGTP (4 mM, 0.05 μL) and dCTP (4 mM, 0.05 μL), **dC^{FT}TP** (**5a**), **dC^{FPh}TP** (**5b**) or **dC^{FF}TP** (**5c**; 4 mM, 1 μL), primer (3 μM, 1 μL, Prim248 short: 3′-GGGTACGG CGGGTAC-5′) and 19-mer template (3 μM, 1.5 μL, Oligo1C: 5′-CCCGCCCAT GCCGCCCATG-3′) in KOD XL reaction buffer (2 μL) supplied by the manufacturer. Prim248 short was labeled by the use of [γ^{32}P]-ATP according to standard techniques. Reaction mixtures were incubated for 12 min at 60 °C in a thermal cycler and were stopped by addition of stop solution (40 μL, 80% [v/v] formamide, 20 mM EDTA, 0.025% [w/v] bromophenol blue, 0.025% [w/v] xylene cyanol) and heated for 5 min at 95 °C. Reaction mixtures were separated by the use of a 12.5% denaturing PAGE. Visualization was performed by phosphorimaging.

Polymerase chain reaction (287 nt): The PCR reaction mixture (20 μL) contained KOD XL DNA polymerase (Merc4biosciences, Novagen, 2.5 U μL^{-1}, 0.8 μL), DMSO (100%, 1 μL), formamide (5%, 1 μL), betaine (0.75 M, 1 μL), TMAC (50 mM, 1 μL), natural dNTPs (10 mM, 0.5 μL) functionalized **dC^{FT}TP** (**5a**), **dC^{FPh}TP** (**5b**) or **dC^{FF}TP** (**5c**) (10 mM, 2 μL), primers (20 μM, 2 μL, PrimS1-HIV1: 5’-GATCACTCTT TGGCAGCGAC CCCTCGTCAC-3’, and 20 μM, 2 μL, Prim S2-HIV1: 5’-TTAAAGTGCA GCCAATCTGA GTCAACAGAT-3‘), and a 297-mer template (74.02 ng μL^{-1}, 0.3 μL, wt-HIV-1 PR: 5’-CCTCAGATCA CTCTTTGGCA GCGACCCCTC GTCACAATAA AGATAGGGGG GCAATTAAAG GAAGCTCTAT TAGATACAGG AGCAGATGAT ACAGTATTAG AAGAAATGAA TTTGCCAGGA AGATGGAAAC CAAAAATGAT AGGGGGAATT GGAGGTTTTA TCAAAGTAAG ACAGTATGAT CAGATACTCA TAGAAATCTG CGGACATAAA GCTATAGGTA CAGTATTAGT AGGACCTACA CCTGTCAACA TAATTGGAAG AAATCTGTTG ACTCAGATTG GCTGCACTTT AAATTTT-3’) in KOD XL reactions buffer (2 μL) supplied by the manufacturer. Forty PCR cycles were run under the following conditions: denaturation for 1 min at 94 °C, annealing for 1 min at 60 °C, extension for 1 min at 72 °C, followed by a final extension step of 5 min at 72 °C. PCR products were analyzed on a 1.3% agarose gel stained with GelRed (Biotium, 10000× in H_2O) in 0.5×TBE buffer.

DNA–peptide conjugation experiment: Six reaction mixtures (+, C^{FPh}, 1, 2, 3, 4, 20 μL each) were prepared according to standard PEX procedure. All reaction mixtures contained DeepVent$_R^{TM}$(exo$^-$) DNA polymerase (New England BioLabs, 2 U μL$^-$, 4.5 μL), dGTP (4 mM, 0.15 μL) and dCTP (4 mM, 0.15 μL), **dC^{FPh}TP** (**5b**; 4 mM, 3 μL), primer (3 μM, 3 μL, Prim248 short: 3′-GGGTACG GCGGGTAC-5′), and 19-mer template (3 μM, 4.5 μL, Oligo1C: 5′-CCCGCCCATG CCGCCCATG-3') in DeepVent(exo$^-$) reaction buffer (2 μL) supplied by the manufacturer. Prim248 short was labeled by the use of [γ^{32}P]-ATP according to standard techniques. Reaction mixtures were incubated for 30 min at 60 °C in a thermal cycler and were stopped by addition of stop solution (12 μL, 80% [v/v] formamide, 20 mM EDTA, 0.025% [w/v] bromophenol blue, 0.025% [w/v] xylene cyanol) and heated 5 min at 95 °C. Samples 3 and 4 were purified by using the QIAquick nucleotide removal kit according to standard techniques and eluted with phosphate buffer (50 μL, 50 mM, pH 6.7). Then Ac-Ala-Lys-Ala-NH_2, **III** (0.7 M solution in 50 mM phosphate buffer, pH 6.7, 10.5 μL) and $NaBH_3CN$ (1.59 M solution in 50 mM phosphate buffer, pH 6.7, 1.5 μL) were added into each reaction mixture (1–4). Such prepared mixtures were stirred in the thermal cycler at 25 °C for 24 h/750 rpm. After this, reaction mixtures 2 and 4 were purified by NucAway^{TM} spin columns. In addition, reaction mixture 1 was not purified. Finally, all reaction mixtures (+, C^{FPh}, 1, 2, 3, 4) were separated by the use of a 12.5% denaturing PAGE and visualized by phosphorimaging.

Polyacrylamide gel electrophoresis: The PEX products containing loading buffer (12 μL, 80% [v/v] formamide, 20 mM EDTA, 0.025% [w/v] bromophenol blue, 0.025% [w/v] xylene cyanol) were subjected to vertical electrophoresis in 12.5% denaturing polyacrylamide gel containing 1×TBE buffer (pH 8) and urea (7 M) at 45 mA for about 50 min. Gels were dried (85 °C, 70 min) and the autoradiographs were visualized by using a phosphorimager (Typhoon 9410, Amersham Biosciences).

Acknowledgements

This work is a part of the research projects supported by the Academy of Sciences of the Czech Republic (Z40550506). It was specifically supported by the Czech Science Foundation (203/09/0317) and by Gilead Sciences, Inc. (Foster City, CA, USA).

[1] Reviews: a) H. Lönnberg, *Bioconjugate Chem.* **2009**, *20*, 1065–1094; b) E. Paredes, M. Evans, S. R. Das, *Methods* **2011**, *54*, 251–259; c) T. S. Zatsepin, D. A. Stetsenko, M. J. Gait, T. S. Oretskaya, *Bioconjugate Chem.* **2005**, *16*, 471–489; d) Y. Singh, P. Murat, E. Defrancq, *Chem. Soc. Rev.* **2010**, *39*, 2054–2070.

[2] Reviews: a) B. Sacca, C. Niemeyer, *Chem. Soc. Rev.* **2011**, *40*, 5910–5921; b) C. Niemeyer, *Angew. Chem.* **2010**, *122*, 1220–1238; *Angew. Chem. Int. Ed.* **2010**, *49*, 1200–1216; recent articles: c) M. Kitaoka, Y. Tsuruda, Y. Tanaka, M. Goto, M. Mitsumori, K. Hayashi, Y. Hiraishi, K. Miyawaki, S. Noji, N. Kamiya, *Chem. Eur. J.* **2011**, *17*, 5387–5392; d) J. H. Lee, N. Y. Wong, L. H. Tan, Z. Wang, Y. Lu, *J. Am. Chem. Soc.* **2010**, *132*, 8906–8908.

[3] Selected recent examples: a) D. A. Stetsenko, M. J. Gait, *Bioconjugate Chem.* **2001**, *12*, 576–586; b) G. P. Miller, E. T. Kool, *J. Org. Chem.* **2004**, *69*, 2404–2410; c) J. D′Onofrio, D. Montesarchio, L. De Napoli, G. Di Fabio, *Org. Lett.* **2005**, *7*, 4927–4930; d) G. Godeau, C. Staedel, P. Barthelemy, *J. Med. Chem.* **2008**, *51*, 4374–4376; e) G. Pourceau, A. Meyer, J. J. Vasseur, F. Morvan, *J. Org. Chem.* **2009**, *74*, 6837–6842; f) J. G. Aaronson, L. J. Klein, A. A. Momose, A. M. O'Brien, A. W. Shaw, T. J. Tucker, Y. Yuan, D. M. Tellers, *Bioconjugate Chem.* **2011**, *22*, 1723–1728.

[4] Review: a) P. M. E. Gramlich, C. T. Wirges, A. Manetto, T. Carell, *Angew. Chem.* **2008**, *120*, 8478–8487; *Angew. Chem. Int. Ed.* **2008**, *47*, 8350–8358; recent examples: b) J. Gierlich, K. Gutsmiedl, P. M. E. Gramlich, A. Schmidt, G. A. Burley, T. Carell, *Chem. Eur. J.* **2007**, *13*, 9486–9494; c) P. M. E. Gramlich, S. Warncke, J. Gierlich, T. Carell, *Angew. Chem.* **2008**, *120*, 3491–3493; *Angew. Chem. Int. Ed.* **2008**, *47*, 3442–3444; d) F. Seela, V. R. Sirivolu, P. Chittepu, *Bioconjugate Chem.* **2008**, *19*, 211–224; e) F. Seela, V. R. Sirivolu, *Org. Biomol. Chem.* **2008**, *6*, 1674–1687; f) G. A. Burley, J. Gierlich, M. R. Mofid, H. Nir, S. Tal, Y. Eichen, T. Carell, *J. Am. Chem. Soc.* **2006**, *128*, 1398–1399.

[5] K. Gutsmiedl, D. Fazio, T. Carell, *Chem. Eur. J.* **2010**, *16*, 6877–6883.

[6] a) S. H. Weisbrod, A. Marx, *Chem. Commun.* **2007**, 1828–1830; b) A. Baccaro, S. H. Weisbrod, A. Marx, *Synthesis* **2007**, 1949–1954.

[7] a) V. Borsenberger, S. Howorka, *Nucleic Acids Res.* **2009**, *37*, 1477–1485; b) V. Borsenberger, M. Kukwikila, S. Howorka, *Org. Biomol. Chem.* **2009**, *7*, 3826–3835; c) J. Schoch, M. Wiessler, A. Jäschke, *J. Am. Chem. Soc.* **2010**, *132*, 8846–8847.

[8] H. Rohde, J. Schmalisch, Z. Harpaz, F. Diezman, O. Seitz, *ChemBioChem* **2011**, *12*, 1396–1400; b) H. Eberhard, F. Diezmann, O. Seitz, *Angew. Chem.* **2011**, *123*, 4232–4236; *Angew. Chem. Int. Ed.* **2011**, *50*, 4146–4150.

[9] A. Okamoto, K. Tainaka, I. Saito, *Tetrahedron Lett.* **2002**, *43*, 4581–4583.

[10] W. Hirose, K. Sato, A. Matsuda, *Angew. Chem.* **2010**, *122*, 8570–8572; *Angew. Chem. Int. Ed.* **2010**, *49*, 8392–8394.

[11] C. Dohno, A. Okamoto, I. Saito, *J. Am. Chem. Soc.* **2005**, *127*, 16681–16684.

[12] a) A. Kittaka, T. Kuze, T. Asakura, K. Ito, T. Miyasaka, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3207–3210; b) A. Kittaka, C. Horii, T. Kuze, T. Asakura, K. Ito, K. T. Nakamura, T. Miyasaka, J. Inoue, *Synlett* **1999**, 869–872; c) A. Kittaka, H. Takayama, M. Kurihara, C. Horii, H. Tanaka, T. Miyasaka, J. Inoue, *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 669–672; d) T. Sugiyama, A. Kittaka, M. Tomioka, Y. Ida, R. Kuroda, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2847–2851.

[13] C. T. Wirges, J. Timper, M. Fischler, A. S. Sologubenko, J. Mayer, U. Simon, T. Carell, *Angew. Chem.* **2008**, *121*, 225–229; *Angew. Chem. Int. Ed.* **2008**, *48*, 219–223.

[14] T. Angelov, A. Guainazzi, O. D. Schärer, *Org. Lett.* **2009**, *11*, 661–664.

[15] Selected examples: a) T. S. Zatsepin, D. A. Stetsenko, A. A. Arzumanov, E. A. Romanova, M. J. Gait, T. S. Oretskaya, *Bioconjugate Chem.* **2002**, *13*, 822–830; b) J. M. Tilquin, M. Dechamps, E. Sonveaux, *Bioconjugate Chem.* **2001**, *12*, 451–457; c) I. A. Kozlov, P. C. Melnyk, K. E. Stromsborg, M. S. Chee, D. L. Barker, C. Zhao, *Biopolymers* **2004**, *73*, 621–630; d) X. Li, Z.-Y. J. Zhan, R. Knipe, D. G. Lynn, *J. Am. Chem. Soc.* **2002**, *124*, 746–747; e) J. Haralambidis, L. Lagniton, G. W. Tregear, *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1005–1010; f) Z. Skrzypczynski, S. Wayland, *Bioconjugate Chem.* **2003**, *14*, 642–652; g) O. Y. Wong, A. E. Mulcrone, S. K. Silverman, *Angew. Chem.* **2011**, *123*, 11883–11888; *Angew. Chem. Int. Ed.* **2011**, *50*, 11679–11684.

[16] V. Raindlová, R. Pohl, M. Šanda, M. Hocek, *Angew. Chem.* **2010**, *122*, 1082–1084; *Angew. Chem. Int. Ed.* **2010**, *49*, 1064–1066.

[17] Review: a) M. Hocek, M. Fojta, *Org. Biomol. Chem.* **2008**, *6*, 2233–2241; recent examples from our laboratory: b) P. Brázdilová, M. Vrábel, R. Pohl, H. Pivoňková, L. Havran, M. Hocek, M. Fojta, *Chem. Eur. J.* **2007**, *13*, 9527–9533; c) H. Cahová, L. Havran, P. Brázdilová, H. Pivoňková, R. Pohl, M. Fojta, M. Hocek, *Angew. Chem.* **2008**, *120*, 2089–2092; *Angew. Chem. Int. Ed.* **2008**, *47*, 2059–2062; d) M. Vrábel, P. Horáková, H. Pivoňková, L. Kalachova, H. Černocká, H. Cahová, R. Pohl, P. Šebest, L. Havran, M. Hocek, M. Fojta, *Chem. Eur. J.* **2009**, *15*, 1144–1154; e) J. Riedl, P. Horáková, P. Šebest, R. Pohl, L. Havran, M. Fojta, M. Hocek, *Eur. J. Org. Chem.* **2009**, 3519–3525; f) H. Macíčková-Cahová, M. Hocek, *Nucleic Acids Res.* **2009**, *37*, 7612–7622; g) S. Ikonen, H. Macíčková-Cahová, R. Pohl, M. Šanda, M. Hocek, *Org. Biomol. Chem.* **2010**, *8*, 1194–1201; h) H. Macíčková-Cahová, R. Pohl, P. Horáková, L. Havran, J. Špaček, M. Fojta, M. Hocek, *Chem. Eur. J.* **2011**, *17*, 5833–5841; i) H. Macíčková-Cahová, R. Pohl, M. Hocek, *ChemBioChem* **2011**, *12*, 431–438; j) P. Kielkowski, R. Pohl, M. Hocek, *J. Org. Chem.* **2011**, *76*, 3457–3462; k) P. Kielkowski, H. Macíčková-Cahová, R. Pohl, M. Hocek, *Angew. Chem.* **2011**, *123*, 8886–8889;

Angew. Chem. Int. Ed. **2011**, *50*, 8727–8730; l) J. Balintová, R. Pohl, P. Horáková, P. Vidláková, L. Havran, M. Fojta, M. Hocek, *Chem. Eur. J.* **2011**, *17*, 14063–14073.

[18] Examples of polymerase incorporations of modified dNTPs from other groups: a) T. Gourlain, A. Sidorov, N. Mignet, S. J. Thorpe, S. E. Lee, J. A. Grasby, D. M. Williams, *Nucleic Acids Res.* **2001**, *29*, 1898–1905; b) H. A. Held, S. A. Benner, *Nucleic Acids Res.* **2002**, *30*, 3857–3869; c) S. Jäger, G. Rasched, H. Kornreich-Leshem, M. Engeser, O. Thum, M. Famulok, *J. Am. Chem. Soc.* **2005**, *127*, 15071–15082; d) M. Kuwahara, J. Nagashima, M. Hasegawa, T. Tamura, R. Kitagata, K. Hanawa, S. Hososhima, T. Kasamatsu, H. Ozaki, H. Sawai, *Nucleic Acids Res.* **2006**, *34*, 5383–5394; e) A. Shoji, T. Hasegawa, M. Kuwahara, H. Ozaki, H. Sawai, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 776–779; f) S. Obeid, M. Yulikow, G. Jeschke, A. Marx, *Angew. Chem.* **2008**, *120*, 6886–6890; *Angew. Chem. Int. Ed.* **2008**, *47*, 6782–6785; g) B. Holzberger, A. Marx, *Bioorg. Med. Chem.* **2009**, *17*, 3653–3658; h) A. Baccaro, A. Marx, *Chem. Eur. J.* **2010**, *16*, 218–226; i) N. Ramsay, A.-S. Jemth, A. Brown, N. Crampton, P. Dear, P. Holliger, *J. Am. Chem. Soc.* **2010**, *132*, 5096–5104; j) Y. Wang, B. A. Tkachenko, P. R. Schreiner, A. Marx, *Org. Biomol. Chem.* **2011**, *9*, 7482–7490.

[19] Examples: a) I. Huc, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106–2110; b) M. Hochgurtel, H. Kroth, D. Piecha, M. W. Hofmann, C. Nicolau, S. Krause, O. Schaaf, G. Sonnenmoser, A. V. Eliseev, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 3382–3387; c) S. Zameo, B. Vauzaeilles, J.-M. Beau, *Eur. J. Org. Chem.* **2006**, 5441–5444.

[20] P. M. Pojer, *Aust. J. Chem.* **1979**, *32*, 201–204.

[21] A. F. Abdel-Magid, C. Maryanoff, R. Shas, *J. Org. Chem.* **1996**, *61*, 3849–3862.

[22] Y. Chen, P. Vogel, M. Šanda, *J. Org. Chem.* **1994**, *59*, 2487–2496.

[23] G. Gribble, C. Nutaitis, *Org. Prep. Proced. Int.* **1985**, *17*, 317–384.

[24] Leading recent references for affinity cross-linking of nucleic acids with proteins: a) M. Winnacker, V. Welzmiller, R. Strasser, T. Carell, *ChemBioChem* **2010**, *11*, 1345–1349; b) M. Winnacker, S. Breeger, R. Strasser, T. Carell, *ChemBioChem* **2009**, *10*, 109–118; c) S. Kuboe, M. Yoda, A. Ogata, Y. Kitade, Y. Tomari, Y. Ueno, *Chem. Commun.* **2010**, *46*, 7367–7369; d) K. Lu, W. Ye, L. B. Collins, X. Chen, A. Gold, L. M. Ball, J. A. Swenberg, *J. Am. Chem. Soc.* **2010**, *132*, 3388–3399; e) R. L. Loeber, E. D. Michaelson-Richie, S. G. Condreanu, D. C. Liebler, C. R. Campbell, N. Y. Tretyakova, *Chem. Res. Toxicol.* **2009**, *22*, 1151–1162.

Received: October 17, 2011
Published online: February 15, 2012

