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Ethynyl Side Chain Hydration During Synthesis and Work-up of "Clickable" Oligonucleotides: Bypassing Acetyl Group Formation by Triisopropylsilyl Protection

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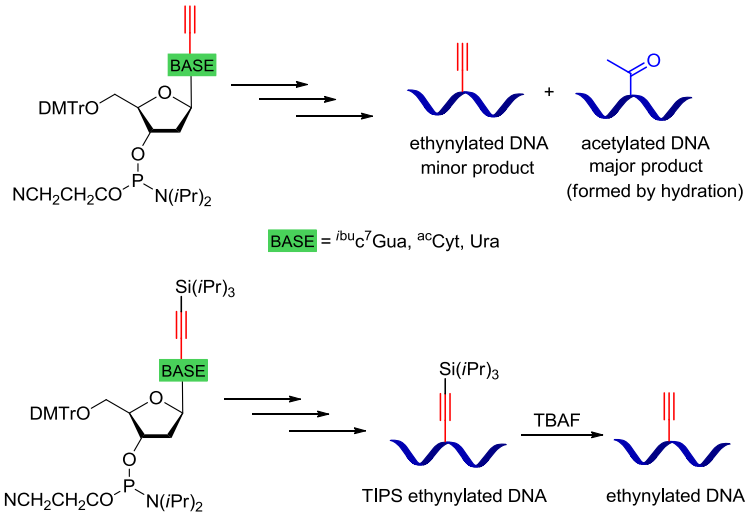


ABSTRACT

Clickable oligonucleotides with ethynyl residues in the 5-position of pyrimidines (^{eth}dC and ^{eth}dU) or the 7-position of 7-deazaguanine (^{eth}c^{7}G$_{d}$) are hydrated during solid-phase oligonucleotide synthesis and work-up conditions. The side products were identified as acetyl derivatives by MALDI-TOF mass spectra of oligonucleotides and by detection of modified nucleosides after enzymatic phosphodiester hydrolysis. Ethynyl → acetyl group conversion was also studied on ethynylated nucleosides under acidic and basic conditions. It could be shown that side chain conversion depends on the nucleobase structure. Triisopropylsilyl residues were introduced to protect ethynyl residues from hydration. Pure, acetyl group free oligonucleotides were isolated after desilylation in all cases.

INTRODUCTION

Alkynyl groups are currently used in nucleoside and oligonucleotide synthesis to make these compounds applicable for functionalization with different reporter groups by the copper promoted *Huisgen-Meldal-Sharpless* click chemistry.[1,2] As ethynyl groups are less space demanding than octadiynyl residues, they have been preferred over long side chain derivatives in enzyme assisted DNA synthesis catalyzed by polymerases.[3] Compounds **2**-**4** (Figure 1) were successfully incorporated into DNA in form of their triphosphates by a template controlled polymerase chain reaction. By this means, the intracellular detection of cytosine was studied with the triphosphate of 5-ethynyl-2'-deoxycytidine (^{eth}dC) in genomic DNA.[4] The 7-ethynyl-7-deaza-2'-deoxyguanosine **1** ($^{eth}c^7G_d$) and 7-ethynyl-7-deaza-2'-deoxyadenosine **4** ($^{eth}c^7A_d$) were used for the metabolic labelling of DNA *in vivo*.[5a] The triphosphate of 5-ethynyl-2'-deoxyuridine **3** (^{eth}dU) has found application in place of 5-bromo-2'-deoxyuridine in a cell proliferation assay.[5b]

The situation is different when phosphoramidite chemistry[6] is employed for the synthesis of oligonucleotides with alkynyl side chains on the nucleobases. While those with octadiynyl residues are commonly in use,[7] we noticed serious problems when phosphoramidites of ethynyl nucleosides were utilized.[8] Side products were formed, and they often became the major components instead of the target oligonucleotides. As chromatographic mobility of contaminated oligonucleotides is often the same as that of the ethynylated oligonucleotides, formation of the former can be easily overseen, and clean oligonucleotides are not accessible. We were able to circumvent this problem partially for the synthesis of 5-ethynyl-dU oligonucleotides, when short deprotection times were used.[8] Therefore, for functionalization of ethynyl side chains, the click reaction was performed on solid support or already functionalized phosphoramidites were used.[9] Thus, either ^{eth}dU oligonucleotides could not be obtained efficiently or were isolated after cumbersome purification in only moderate yield.

In this situation, we started a careful inspection of the side products formed during solid-phase synthesis and the work-up procedure using oligonucleotide deprotection in conc. aq. ammonia. This manuscript reports on the incorporation of nucleosides **1**-**4** (Figure 1) into oligonucleotides by using their phosphoramidites (**9**, **10**, **13** and **14**), evaluates the structures of the major side products and provides a protocol to circumvent these difficulties with phosphoramidites that are protected with triisopropylsilyl (TIPS) groups on the ethynyl side chains.

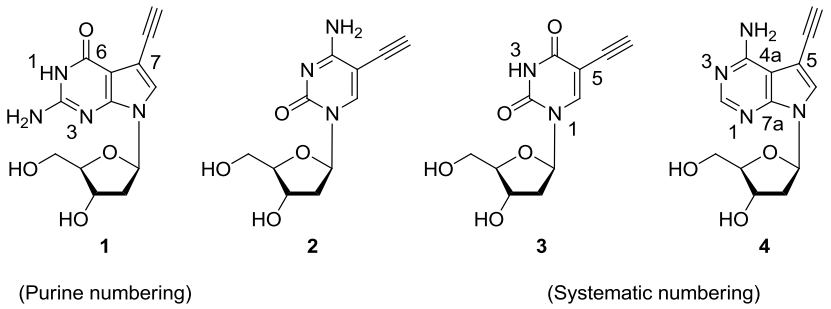


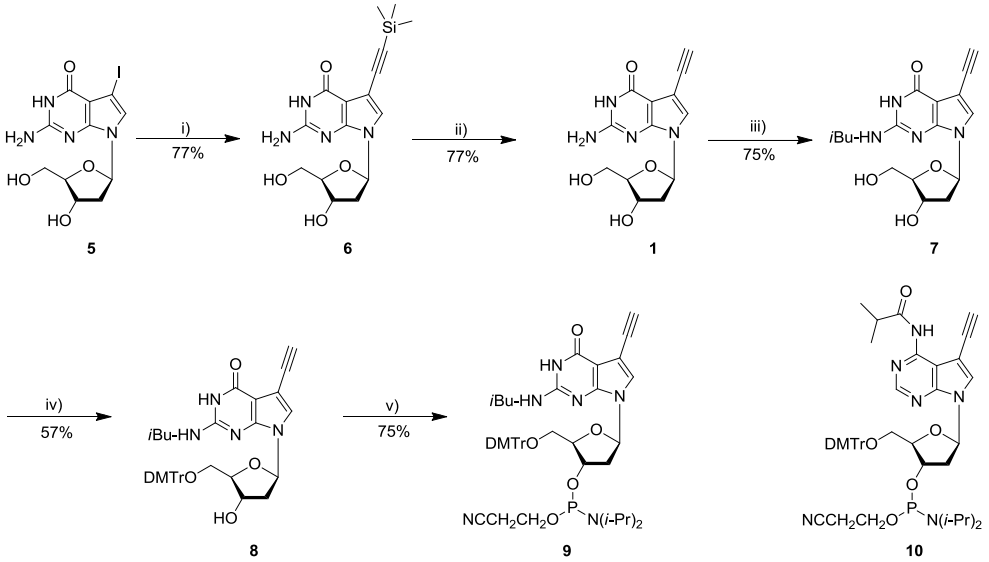
Figure 1. Structures of 7-ethynyl-7-deazapurine and 5-ethynylpyrimidine nucleosides.

RESULTS AND DISCUSSION

1. Synthesis of Phosphoramidites 9 and 13 with Ethynyl Side Chains. For this study, four different phosphoramidites based on the structures **1**-**4** were prepared. The building blocks **10**[8] and **14**[10] are known, while the syntheses of phosphoramidites **9** and **13** are described below. Recently, a two-step synthesis without isolation of the trimethylsilyl (TMS) intermediate **6** was reported for nucleoside **1** in small scale with low yield (19%).[5a] Now, the synthesis of nucleoside **1** was performed including the isolation of intermediate **6**. Toward this end, the palladium-catalyzed *Sonogashira* cross-coupling reaction was performed on nucleoside **5**[11] with trimethylsilylacetylene giving **6** in 77% yield. Deprotection in MeOH/K_2CO_3 gave nucleoside **1** in 77% yield. The overall yield over two steps was 59%. Then compound **1** was protected in the 2-position with an isobutyryl residue (→ **7**, 75%), converted to the 5'-*O*-

DMTr derivative **8** (57%) and was finally phosphitylated to afford **9** in 75% yield (Scheme 1). Phosphoramidite **10** was synthesized according to an already published procedure[8].

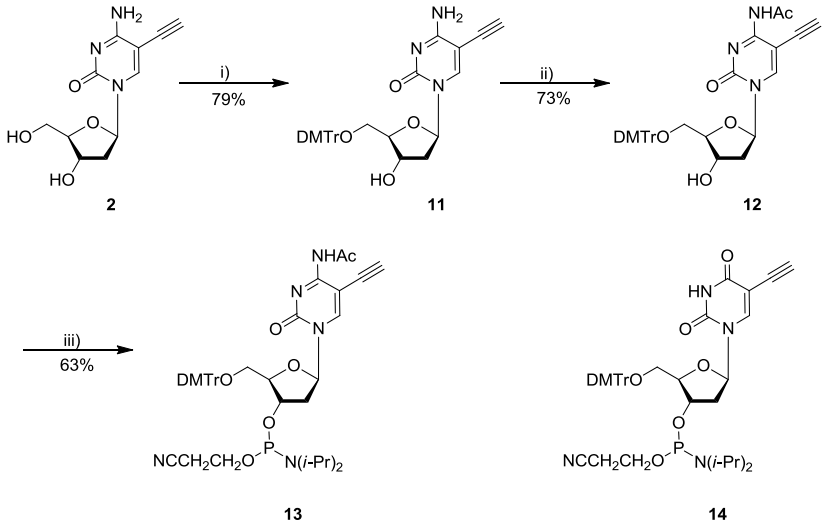
Scheme 1. Synthesis of the 7-Ethynyl-7-deazapurine Phosphoramidite 9[a]



[a]*Reagents and conditions*: i) trimethylsilylacetylene, [$Pd^0[P(Ph_3)_4]$], CuI, DMF, Et_3N, rt, 12 h; ii) K_2CO_3, MeOH, rt, 12 h; iii) *i*Bu_2O, TMSCl, anhydrous pyridine, rt, 3 h; iv) 4,4'-dimethoxytriphenylmethyl chloride, anhydrous pyridine, rt, 12 h; v) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $(i\text{-}Pr)_2NEt$, anhydrous CH_2Cl_2, rt.

Next, phosphoramidite **13** was prepared from 5-ethynyl-2'-deoxycytidine[12] (**2**). The 5'-DMTr residue was introduced under standard conditions to give compound **11** (79%). Acetylation of the 4-amino group gave **12** (73%). Final phosphitylation furnished phosphoramidite **13** in 63% yield (Scheme 2). Phosphoramidite **14** was prepared as described.[10] All compounds were characterized by elemental analyses or ESI-TOF mass spectra as well as by their ^1H and ^{13}C NMR spectra (see Supporting Information). The ^{13}C NMR chemical shifts are listed in the Experimental Section (Table 3) and were assigned by ^1H-^{13}C coupling constants (Tables S1 and S2, Supporting Information) and DEPT-135 NMR spectra.

Scheme 2. Synthesis of the 5-Ethynyl Pyrimidine Phosphoramidite 13[a]



[a]*Reagents and conditions:* i) 4,4'-dimethoxytriphenylmethyl chloride, anhydrous pyridine, rt, 16 h; ii) acetic anhydride, DMF, rt, 15 h; iii) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $(i\text{-}Pr)_2NEt$, anhydrous CH_2Cl_2, rt.

2. Syntheses of Oligonucleotides Using Ethynylated Phosphoramidites 9, 10, 13 and 14.

Following monomer synthesis, oligonucleotides (ODNs) were prepared on solid-phase using the phosphoramidites **9**, **10**, **13** and **14** together with standard building blocks. Nucleosides **1-4** were incorporated into central positions of the oligonucleotides 5'-d(TAGGTCAATACT) (**15**) or 3'-d(ATCCAGTTATGA) (**16**), thereby replacing particular dG, dC, dT or dA residues (Table 1). After solid-phase synthesis, the oligonucleotides were cleaved from the solid support and deprotected in conc. aq. ammonia at 55°C for 16 h. The oligonucleotides were purified by reversed-phase HPLC (RP-18), detritylated with 2.5% dichloroacetic acid in dichloromethane and again purified by HPLC. Materials of the single peaks were isolated in all cases (Figure 2). Subsequently, the molecular masses of the single peak contents were determined by MALDI-TOF mass spectrometry. Unexpectedly, the mass spectra of the isolated oligonucleotides **17**-**19** containing nucleosides **1-3** showed two masses (Figure 2a-c). The lower masses in the MALDI-TOF spectra correspond to the calculated values for the ethynylated oligonucleotides (3668.5 for ODN **17**; 3668.3 for ODN **18**, 3653.5 for ODN **19**),

while the higher masses indicate the presence of "side products" (3686.4 for ODN **17**; 3686.4 for ODN **18**; 3671.6 for ODN **19**) (Table 1, Experimental Section and Figures S1-S4, Supporting Information). The mass difference between the expected product masses and the masses for the side product amounts to ~18 mass units. In all cases (ODNs **17**-**19**), the "side product" masses gave the major mass peaks, and the expected ethynylated oligonucleotides were only the minor components. Unfortunately, the mobility on reversed-phase HPLC of the expected oligonucleotide and side products are the same, and the product content could only be verified by their mass differences (Figure 2). Oligonucleotide **20** incorporating the 2'-deoxyadenosine derivative **4** was an exception. It showed a single HPLC peak with the calculated mass and without contamination (Figure 2d).

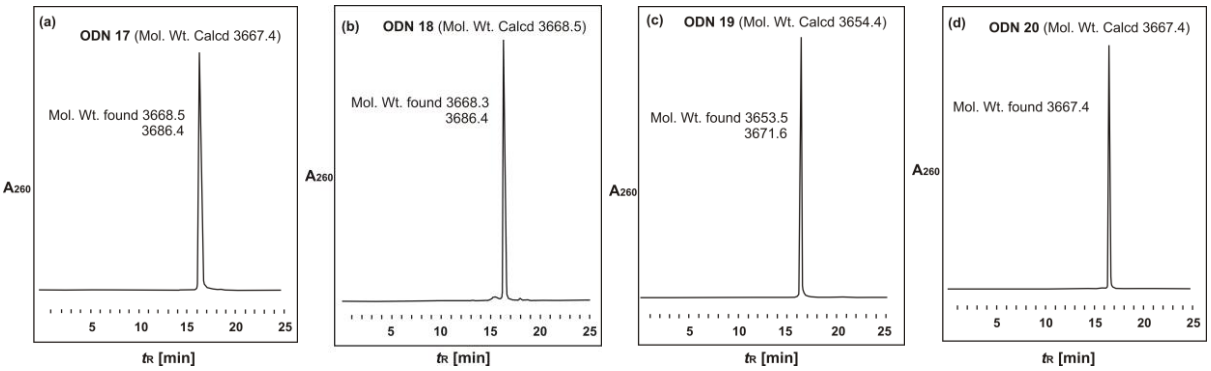


Figure 2. Reversed-phase HPLC elution profiles of purified oligonucleotides (a) 5'-d(AGT ATT **1**AC CTA) ODN **17**; (b) 5'-d(TAG GT**2** AAT ACT) ODN **18**; (c) 5'-d(AGT AT**3** GAC CTA) ODN **19** and (d) 5'-d(TAG GTC **4**AT ACT) ODN **20**. Monitored at 260 nm using gradient system II.

The presence of side products was further evidenced by enzymatic hydrolysis of the synthesized oligonucleotides. Toward this end, the nucleoside composition of ethynylated ODNs **17**-**20** containing **1**-**4** was determined by tandem enzymatic hydrolysis with snake venom phosphodiesterase and alkaline phosphatase in 0.1 M Tris-HCl buffer (pH 8.3) at 37 °C (for details, see Experimental Section). The mixtures obtained from the digest were analyzed by reversed-phase HPLC (RP-18, at 260 nm) showing the peaks for the canonical

nucleosides and the ethynylated nucleosides **1**-**4** (Figure 3). For oligonucleotides incorporating nucleosides **1**-**3**, additional peaks were found indicating formation of side products (Figure 3a-c). The ratio of ethynyl to side product was roughly 1:3 for ODN **17** and **18**. In case of ODN-**19**, two side products were formed. Only the digest of oligonucleotide **20** containing **4** gave a clean pattern without showing side product formation and thereby confirming the mass spectrometric data.

Peak heights corresponding to the ethynylated nucleosides and side products are small compared to those of the canonical nucleosides. This is a result of single incorporations of the modified residues and their low 260 nm extinction coefficients (see Table 2, Experimental Section, for UV-spectra see Figure S6, Supporting information). According to their mass spectra, side products were probably formed by hydration as they contain 18 mass units more than the ethynyl compounds. However, it was to examine whether these reactions occurred during oligonucleotide synthesis (iodine oxidation in water and/or detritylation with trichloroacetic acid) and/or work-up (conc. aq. ammonia treatment). Therefore, additional information on the behaviour of monomeric nucleosides **1-3** under acidic and alkaline conditions was collected.

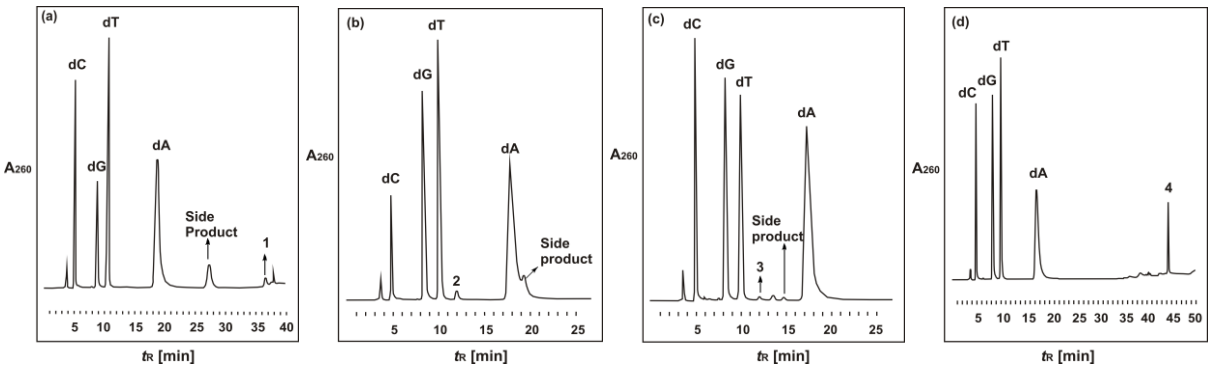
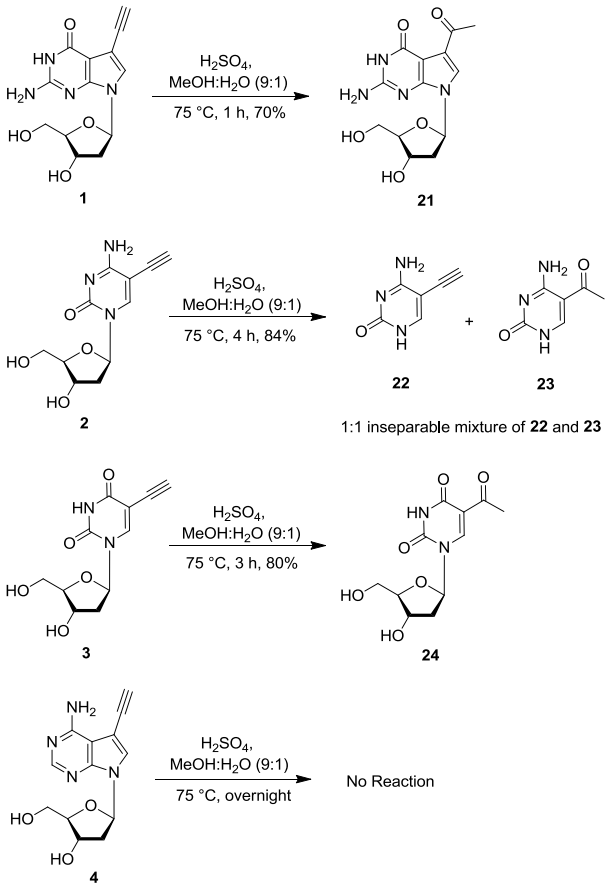


Figure 3. HPLC profiles of the enzymatic hydrolysis products of (a) ODN **17**; (b) ODN **18**; (c) ODN **19**; (d) ODN **20**. Monitored at 260 nm using gradient system III and IV.

3. Acid-Catalyzed Hydration of Ethynyl Modified Nucleosides 1-4. It is expected that side product formation occurs under acidic or oxidative aqueous conditions during solid-phase oligonucleotide synthesis of compounds **1**-**3**. Consequently, the nucleosides were subjected to acid treatment. Hydration of 5-ethynyl-2'-deoxyuridine was already reported by Walker[13] using 0.1 M aq. H_2SO_4 (7 days at rt) and the compound was also prepared by Mertes[14] by glycosylation of the acetylated nucleobase. Water addition follows the Markovnikov rule and yields acetyl compounds.[15] According to these observations and the molecular weight increase of the oligonucleotides (corresponding to one water molecule), the acid catalyzed hydration reaction of nucleosides **1**-**4** in an MeOH/H_2O (9:1 mixture) containing H_2SO_4 was studied first. Only nucleosides **1** and **3** provided new nucleosides (**21** and **24**) which were isolated in 70-80% yield. The reaction product of **3** was already described as **24**,[13] while the hydration product of **1** was assigned to **21** on the basis of mass spectra, and ^{1}H NMR and ^{13}C NMR chemical shifts. The signals of a methyl group appeared in the ^{1}H NMR spectrum of **21**, and the side chain gave ^{13}C NMR signals similar to those of nucleoside **24** (Table 3, Experimental Section). For the conversion of the ethynylated 7-deaza-2'-deoxyguanosine (**1**), only a catalytic amount of acid (0.1 eq. of H_2SO_4 relative to alkyne) was required to yield the 7-acetyl nucleoside **21**, while the conversion of the dU analog **3** to the acetyl compound **24** required 1 eq. of H_2SO_4. In case of ^{eth}dC (**2**), the situation is different. Here, acid treatment led to cleavage of the N-glycosylic bond and formation of a 1:1 mixture of 5-ethynylcytosine[16] (**22**) and 5-acetylcytosine[17] (**23**) in 84% overall yield. Both components were identified by NMR spectra and mass data. Treatment of 7-deaza-2'-deoxyadenosine derivative **4** with H_2SO_4 did not yield any product. So, the reactivity of the ethynyl side chains towards H_2SO_4 in MeOH/H_2O strongly depends on the nucleobase structure. 7-Deazaguanine nucleoside **1** is the most sensitive nucleoside towards water addition, while the ^{eth}dC **2** is the most sensitive nucleoside towards acid-catalyzed glycosylic bond hydrolysis. The 7-deazaadenosine analog **4** was stable under these conditions.

Scheme 3. Acid-Catalyzed Hydration Reactions of Ethynyl Modified Nucleosides 1-4



4. Stability of Nucleosides 1-4 in Concentrated Aqueous Ammonia. Next, the effect of conc. aq. ammonia on the ethynylated nucleosides **1-4** was investigated at elevated temperature (55 °C, 16 h). These conditions correspond to those used for oligonucleotide deprotection. First, the reactions were performed on analytical scale and were monitored by TLC. The crude mixtures of nucleosides **1** and **4** did not show any additional spot, while those of compounds **2** and **3** showed new products migrating faster than the starting materials. The formation of these products was further evidenced by HPLC analysis shown in Figure 4.

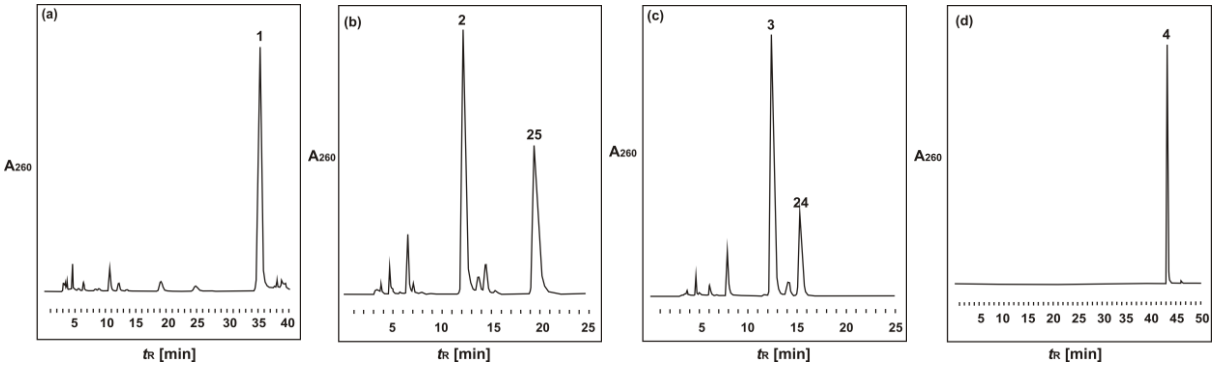
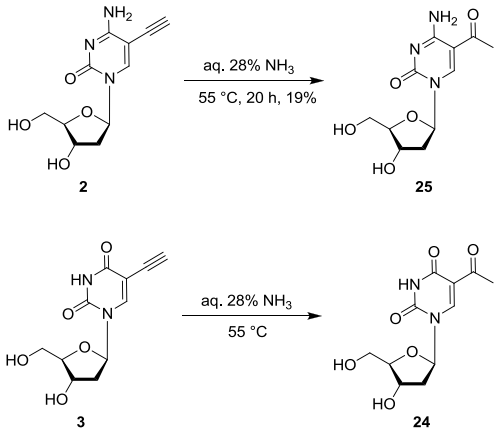


Figure 4. Reversed-phase HPLC elution profiles of the crude reaction mixtures of nucleosides **1**-**4** treated with conc. aq. ammonia at elevated temperature (55 °C for 16 h). (a) $^{eth}c^{7}G_{d}$ **1**; (b) ^{eth}dC **2**; (c) ^{eth}dU **3** and (d) $^{eth}c^{7}A_{d}$ **4**. Monitored at 260 nm using gradient system III and IV.

The ratio of the ethynyl nucleoside **2** and side product **25** (after 16 h treatment with conc. aq. ammonia at 55 °C) was approximately 2:1, and for nucleoside **3** and side product **24** a ratio of 4:1 was observed by HPLC. The mobility of the side product formed by ^{eth}dU (**3**), which was obtained by ammonia treatment, was identical to that of side product **24** obtained from **3** by acid treatment (Scheme 4, Figure S7, Supporting Information). This confirmed that hydration of the side chain of **3** (→ **24**) occurs under acidic as well as alkaline conditions. For identification of the side product of ^{eth}dC, compound **2** was treated with conc. aq. ammonia at 55 °C for 20 h (Scheme 4). The product was isolated and identified as 5-acetyl-dC on the basis of its mass and the typical ^{1}H and ^{13}C NMR chemical shifts of the acetyl side chain (see Experimental Section and Table 3). As hydration of a triple bond is uncommon under alkaline conditions, we searched in the literature for similar observations. Indeed, the silylated arabinofuranosyl-5-ethynylcytosine nucleoside was partially converted to the acetyl compound (5%) upon treatment with potassium carbonate in methanol.[18]

Scheme 4. Base-Catalyzed Hydration Reaction on Ethynyl Modified Nucleosides 2 and 3



5. Comparison of Side Products Obtained from the Enzymatic Hydrolysis of Oligonucleotides with Those Obtained by Synthesis. For the identification of side products formed during oligonucleotide synthesis, ODNs **17**-**20** were digested by tandem enzymatic hydrolysis using snake venom phosphodiesterase and alkaline phosphatase. The enzymatic hydrolysis mixtures of these oligonucleotides were co-injected together with the ethynylated nucleosides **1**-**3** and the newly synthesized acetylated compounds **21**, **24** and **25** (Figure 5). Figures 5a-c clearly indicate that the co-injected nucleosides match the hydrolysis products (compare Figures 3 and 5). A minor side product whose signal appeared between the peaks for nucleosides **3** and **24** was not identified. These results prove that the side products formed during oligonucleotide synthesis and work-up are the acetyl nucleosides **21**, **24** and **25**. As expected, the HPLC profile of the enzymatic hydrolysis of ODN **20** (containing nucleoside **4**) showed the presence of ethynyl nucleoside **4** exclusively without any detectable amounts of side products (Figure 5d). We noted that side product formation, occurring during oligonucleotide synthesis, takes place on protected ethynyl nucleosides (except for **3**). Side products are deprotected under work-up conditions. While we are able to correlate side product formation to the process of deprotection under alkaline conditions, we cannot provide evidence at what stage of oligonucleotide synthesis hydration takes place.

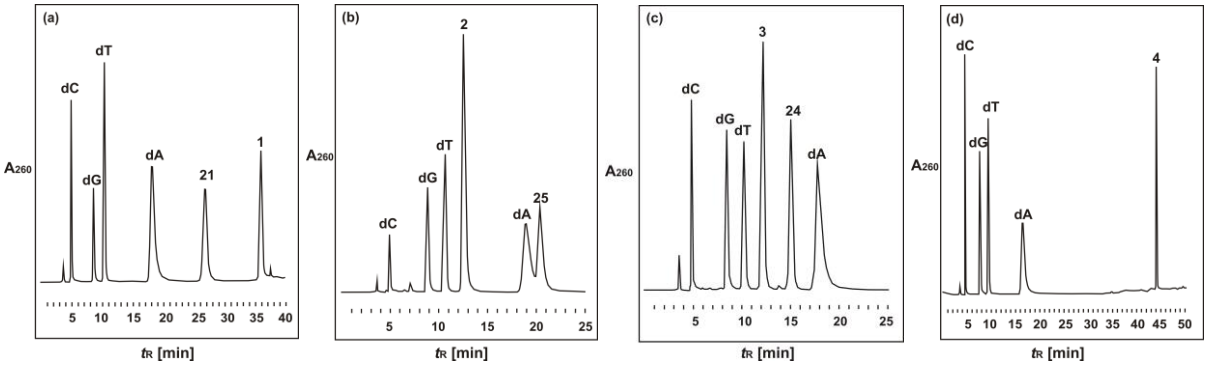
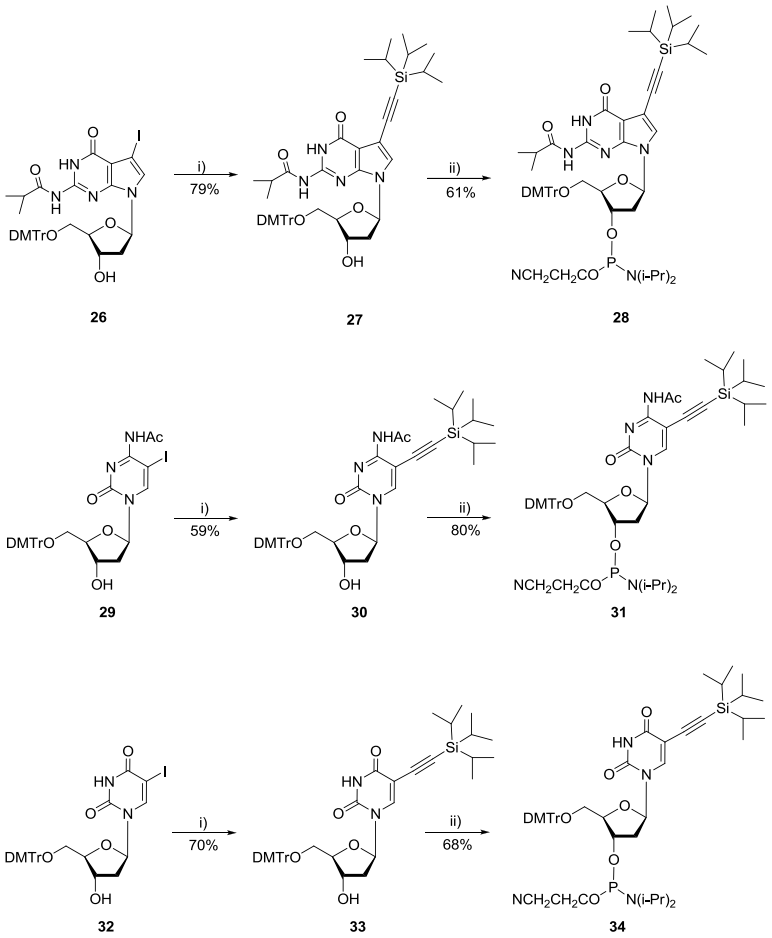


Figure 5. HPLC profiles of mixtures of the enzymatic hydrolysis products of oligonucleotides, ethynyl modified nucleosides and acetyl modified nucleosides. (a) ODN **17** + nucleosides **1** and **21**; (b) ODN **18** + nucleosides **2** and **25**; (c) ODN **19** + nucleosides **3** and **24** and (c) ODN **20** + nucleoside **4**. Monitored at 260 nm using gradient system III and IV.

6. Synthesis of Triisopropylsilyl Protected Phophoramidites 28, 31 and 34. From the results described above, it is obvious that the ethynylated nucleosides **1**-**3** are not stable, neither during oligonucleotide synthesis nor under work-up conditions or both. Although solid-phase oligonucleotide synthesis is performed in anhydrous acetonitrile (10 ppm water content), water is present during iodine oxidation of the phosphorous (III) to phosphorous (V) residues. We noticed that the extent of side chain modification increases with the number of coupling steps and ethynyl group transformation is more pronounced when the modified base is incorporated near the 3'-end of the oligonucleotide (data not shown). Another source for ethynyl group hydration is aq. ammonia treatment. Therefore, we silylated the ethynyl group by a triisopropylsilyl (TIPS) residue. This bulky and hydrophobic protecting group is sufficiently stable during oligonucleotide synthesis and under work-up conditions. A similar protection strategy was used by Gramlich *et al* for the protection of the terminal triple bond of octadiynyl-dC utilized in sequential click reactions.[2e] Earlier experiments using the trimethylsilyl group failed as this group got lost during oligonucleotide synthesis.[10] The 5- or 7-TIPS-ethynylated phosphoramidite building blocks **28**, **31** and **34** were synthesized from the

5- or 7-iodinated nucleosides **26**[19], **29** and **32**[20] by the palladium catalyzed *Sonogashira* cross-coupling reaction with triisopropylsilylacetylene. Triisopropylsilyl ethynyl nucleosides **27**, **30** and **33** were obtained in 59% to 79% yield. Phosphitylation under standard conditions furnished the phosphoramidites **28**, **31** and **34** (61-80% yield) (Scheme 5).

Scheme 5. Synthesis of Phosphoramidite Building Blocks 28, 31 and 34[a]



[a]*Reagents and conditions*: i) triisopropylsilylacetylene, [Pd^0[$P(Ph_3)_4$], CuI, DMF, Et_3N, rt, 12 h; ii) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $(i\text{-}Pr)_2NEt$, anhydrous CH_2Cl_2, rt.

*6.1 Synthesis of Oligonucleotides with TIPS-Phosphoramidites **28**, **31** and **34**.* The phosphoramidites **28**, **31** and **34** were used together with unmodified building blocks in the synthesis of TIPS ethynyl oligonucleotides by solid-phase synthesis. Oligonucleotides were characterized by MALDI-TOF mass spectrometry (Table 1, Figure S8, Supporting

Information), and the masses matched the calculated values for ODNs **35**-**37** (Figure 6). No cleavage of the TIPS group or hydration of the ethynyl side chain was detected by MALDI-TOF, even after deprotection of the oligonucleotides in concentrated aq. ammonia at elevated temperature. Only the dC derivative was an exception when rigorous conditions were used. Partial cleavage (~ 20%) of the TIPS group was observed during deprotection with conc. aq. ammonia at elevated temperature. This problem could be overcome by application of mild deprotection conditions and the use of 4-*tert*-butylphenoxyacetyl-protected canonical phosphoramidites together with 4-*tert*-butylphenoxyacetic anhydride as capping reagent (see Experimental Section). Subsequently, deprotection of the oligonucleotides was performed with conc. aq. ammonia at rt, thus preventing cleavage of the TIPS group.

Finally, the TIPS groups of the oligonucleotides **35**-**37** were removed with tetrabutylammonium fluoride (TBAF) in CH_3CN/DMF (4:1) at 45 °C for 16 h (Scheme 6). After completion of the reaction, the oligonucleotides were precipitated by adding sodium acetate buffer and isopropanol (for details see Experimental Section). The deprotected oligonucleotides were purified by reversed-phase HPLC. Figure 6 shows the HPLC profiles of TIPS protected oligonucleotides and oligonucleotides with deprotected intact ethynyl groups.

Scheme 6. Deprotection of the TIPS Group from Oligonucleotides (35-37)

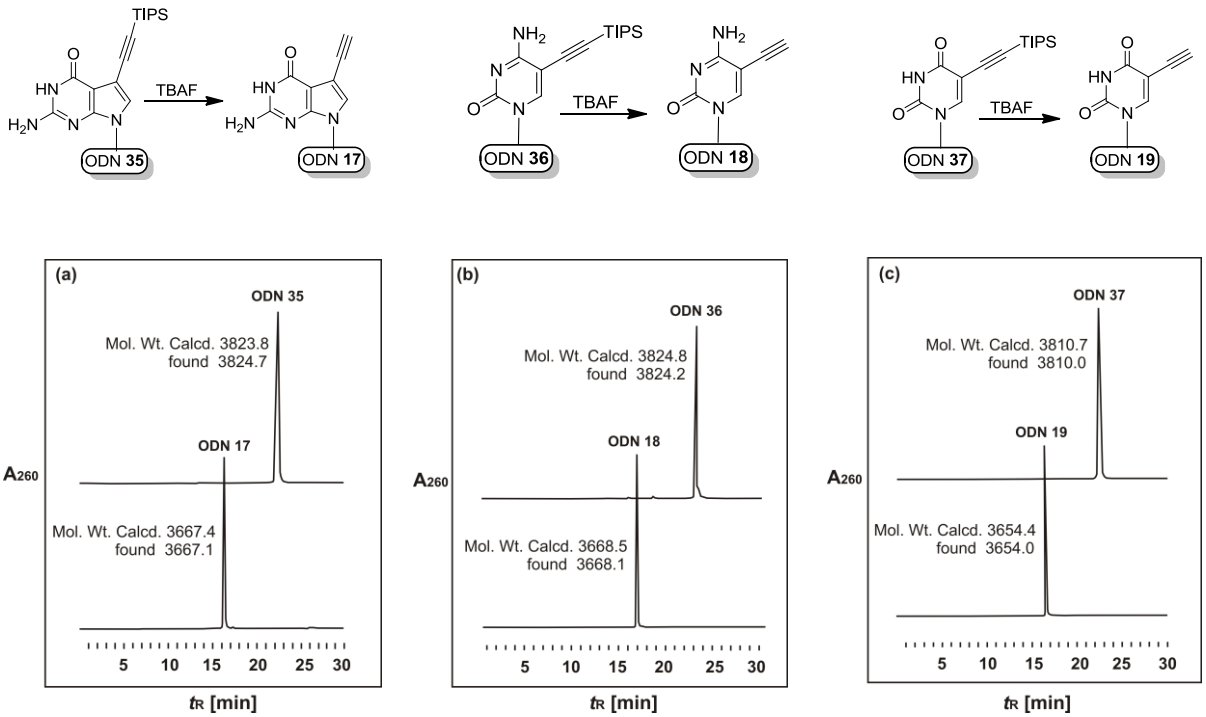


Figure 6. Reversed-phase HPLC elution profiles of purified ethynylated and silylated oligonucleotides. (a) ODN **17** and ODN **35**; (b) ODN **18** and ODN **36**; (c) ODN **19** and ODN **37**. Monitored at 260 nm using gradient system II.

The TIPS ethynyl oligonucleotides (**35**-**37**) can be easily distinguished from the ethynylated oligonucleotides (**17**-**19**) due to their different HPLC mobility (Figure 6). The purity of ethynyl modified oligonucleotides (prepared after deprotection of TIPS group) was confirmed by enzymatic hydrolysis and MALDI-TOF spectra (Figure S9 and S10, Supporting Information). This method was also applied for the synthesis of ODN **38** containing two consecutive 7-TIPS ethynyl-7-deazaguanine residues, which after deprotection gave the pure ethynylated ODN **39** (Table 1).

As discussed above, protection of ethynyl groups with TIPS residues prevents hydration of the side chain and results in the formation of noncontaminated oligonucleotides.

CONCLUSION AND OUTLOOK

Oligonucleotides with ethynyl groups in the 5-position of dC or dU or 7-position of 7-deaza-dG are in part hydrated during their synthesis and work-up. The major side products were identified as acetyl derivatives formed by Markovnikov hydration of ethynylated nucleobase side chains. To confirm the structures of the hydration products, the corresponding acetylated nucleosides were prepared from ethynylated nucleosides by acid or base treatment. The efficacy of hydration depended strongly on the structure of the nucleobase moieties. Only the 7-ethynyl-7-deazaadenine base was stable under the conditions of oligonucleotide synthesis and deprotection, while 7-ethynyl-7-deazaguanine was extremely labile. These side reactions were circumvented by triisopropylsilyl protection of the ethynyl groups. Pure oligonucleotides were isolated after removal of the TIPS residues with tetrabutylammonium fluoride. By use of TIPS protected phosphoramidites, clean ethynylated oligonucleotides are now accessible which are ready to be used in the post-synthetic modification by the *Huisgen-Meldal-Sharpless* "click" chemistry.

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. Thin-layer chromatography (TLC) was performed on TLC aluminium sheets covered with silica gel 60 F254 (0.2 mm). Flash column chromatography (FC): silica gel 60 (40-60 μM, for flash chromatography) at 0.4 bar. UV-spectra were recorded on a spectrophotometer: λ_{max} (ε) in nm, ε in $dm^3\ mol^{-1}\ cm^{-1}$. NMR spectra were measured at 300.15 MHz for ^1H, 75.48 MHz for ^{13}C and 121.52 MHz for ^{31}P. The ^{13}C NMR signals were assigned on the basis of DEPT-135 and ^1H-^{13}C gated-decoupled NMR spectra (for coupling constants see Tables S1-2, Supporting Information). The *J* values are given in Hz; δ values in ppm relative to Me_4Si as

internal standard. For NMR spectra recorded in DMSO-d_6, the chemical shift of the solvent peak was set to 2.50 ppm for ^{1}H NMR and 39.50 ppm for ^{13}C NMR. Reversed-phase HPLC was carried out on a 4 × 250 mm RP-18 (10 μm) LiChrospher 100 column with a HPLC pump connected with a variable wavelength monitor, a controller and an integrator. ESI-TOF mass spectra of nucleosides were recorded on a Micro-TOF spectrometer. Molecular masses of oligonucleotides were determined by MALDI-TOF mass spectrometry in the linear positive mode with 3-hydroxypicolinic acid (3-HPA) as a matrix.

Synthesis, Purification, and Characterization of Oligonucleotides. The oligonucleotides were synthesized on an automated DNA synthesizer on a 1 μmol scale employing standard phosphoramidites as well as the phosphoramidites **9**, **10**, **13**, **14**, **28**, **31** and **34**. After cleavage from the solid support, the oligonucleotides were deprotected in concentrated aqueous ammonia solution for 16 h at 55 °C. ODN **36** was prepared by solid-phase synthesis using 4-*tert*-butylphenoxyacetyl protected canonical phosphoramidites as well as phosphoramidite **31**. In addition, the capping reagent 4-*tert*-butylphenoxyacetic anhydride instead of acetic anhydride was used. After cleavage from the solid support, ODN **36** was deprotected in concentrated aqueous ammonia solution for 16 h at room temperature. The purification of the "trityl-on" oligonucleotides was carried out on reversed-phase HPLC using the following 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 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 (ODNs **17**-**20**, **39**) or 0-20 min 0-25% A in B; 20-25 min, 25% A in B; flow rate 0.8 mL/min (ODNs **35**-**37**). 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. Extinction coefficients ε_{260} of the unmodified nucleosides (H_2O): dA 15400, dG 11700, dT 8800, dC 7300.

Table 1. Molecular Masses of Oligonucleotides Measured by MALDI-TOF Mass Spectrometry[a]

Oligonucleotides	Molecular Weight	
	Calc.	**Found**
5'-d(AGT ATT **1**AC CTA) (**17**)	3667.4	3668.5, 3686.4
5'-d(TAG GT**2** AAT ACT) (**18**)	3668.5	3668.3, 3686.4
5'-d(AGT AT**3** GAC CTA) (**19**)	3654.4	3653.5, 3671.6
5'-d(TAG GTC **4**AT ACT) (**20**)	3667.4	3667.4
5'-d(AGT ATT $\mathbf{1}^{\mathbf{TIPSE}}$AC CTA) (**35**)	3823.8	3824.7
5'-d(TAG GT$\mathbf{2}^{\mathbf{TIPSE}}$ AAT ACT) (**36**)	3824.8	3824.2
5'-d(AGT AT$\mathbf{3}^{\mathbf{TIPSE}}$ GAC CTA) (**37**)	3810.7	3810.0
5'-d(AGT ATT **1**AC CTA) (**17**) after deprotection	3667.4	3667.1
5'-d(TAG GT**2** AAT ACT) (**18**) after deprotection	3668.5	3668.1
5'-d(AGT AT**3** GAC CTA) (**19**) after deprotection	3654.4	3654.0
5'-d(TA$\mathbf{1}^{\mathbf{TIPSE}}$ $\mathbf{1}^{\mathbf{TIPSE}}$TC AAT ACT) (**38**)	4003.1	4002.5
5'-d(TA**1** **1**TC AAT ACT) (**39**) after deprotection	3690.5	3689.5

[a] Measured in the positive linear mode. TIPSE = triisopropylsilylethynyl.

Tandem Enzymatic Hydrolysis of Oligonucleotides. The enzymatic hydrolysis of oligonucleotides **17**-**20** was performed using snake-venom phosphodiesterase (EC 3.1.15.1, *Crotallus adamanteus*) and alkaline phosphatase (EC 3.1.3.1, *Escherichia coli*) in 0.1 M Tris HCl buffer (pH 8.5) at 37 °C. The resulting mixtures were analyzed by reversed-phase HPLC (RP-18). The enzymatic digestion products were analyzed by reversed-phase HPLC using gradient *III* (ODNs **17**, **20)**: 25 min 100% B, 25–60 min 0–40% A in B and gradient *IV* (ODNs **18**, **19**): 25 min 100% B; flow rate 0.7 mL/min. (A) MeCN, (B) 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN, 95:5.

Table 2. UV Maxima and Extinction Coefficients of Ethynyl and Acetyl Nucleosides[a,b]

Comp.	λ_{260} (nm), ε (dm^3 $mol^{-1}cm^{-1}$)	λ_{max} (nm), ε (dm^3 $mol^{-1}cm^{-1}$)
7-Ethynyl-c^7G_d (**1**)	7400	270, 9000
7-Acetyl-c^7G_d (**21**)	6600	307, 5800
5-Ethynyl-dC (**2**)	3600	292, 8100
5-Acetyl-dC (**25**)	4600	285, 8900
5-Ethynyl-dU (**3**)	3800	287, 10300
5-Acetyl-dU (**24**)	4900	282, 12700
7-Ethynyl-c^7A_d (**4**)	7400	277, 12100

[a] Data were determined in water. [b] For the corresponding UV-spectra see Figure S6, Supporting Information.

Conversion of Ethynyl Nucleosides to Acetyl Nucleosides. Ethynyl modified nucleosides (**1**-**4**, 5 mg each) were dissolved in of 28% aq. ammonia (1 mL), and the reaction mixture was heated at 55 °C in a closed vessel for 16 h. The solvent was removed, and the residue was dissolved in water (200 µM). The formation of the new product was monitored by TLC (CH_2Cl_2/MeOH, 90:10) and HPLC. For HPLC elution profiles see Figure 4.

Table 3. ^{13}C-NMR Chemical Shifts of 7-Deazapurine and Pyrimidine Derivatives[a]

	C2[b] C2[c] C2[d]	C6[b] C4[c] C6[d]	C5[b] C4a[c] C5[d]	C7[b] C5[c] -	C8[b] C6[c] -	C4[b] C7a[c] C4[d]	C≡C	CH/CH_2/CH_3/OCH_3	C=O	C1'	C2'	C3'	C4'	C5'
1	153.2	157.8	99.5	97.9	122.7	150.3	80.7, 78.1	-	-	82.3	-[f]	70.9	87.2	61.8
6	153.2	157.7	100.1	98.5	123.1	150.3	99.2, 93.6	-/-/0.15/-	-	82.3	-[f]	70.9	87.2	61.8
7	147.7	155.8	103.5	98.5	125.0	147.2	81.6, 77.2	34.8/-/18.9/-	180.1	82.7	-[f]	70.8	87.4	61.7
8	147.7	155.9	103.9	98.7	124.9	147.3	81.6, 77.0	34.8/-/18.9/55.1	180.2	82.8	-[f]	70.5	85.6	64.1
21	153.2	158.2	96.9	120.5	123.4	152.1	-	-/-/30.3/-	193.4	82.6	-[f]	70.9	87.3	61.7
27	147.8	155.7	103.8	99.6	124.5	147.2	100.1, 90.7	34.8/-/10.9,18.9/55.0	180.1	82.8	-[f]	70.7	85.7	64.1
2	153.4[e]	145.4	88.8	-	-	164.2[e]	85.9, 75.9	-	-	85.4	40.9	69.9	87.5	60.9
11	153.2[e]	144.7	89.1	-	-	164.2[e]	85.9, 75.3	-/-/-/55.0	-	85.8[e]	41.0	70.6	85.8[e]	63.7
12	152.3[e]	147.2	92.4	-	-	161.0[e]	87.2, 74.5	-/-/25.0/55.0	-	87.1	40.9	70.3	86.4	63.5
22 + **23**	154.9 154.5	147.2 153.0	87.3 102.1	-	-	164.9 163.6	85.5, 75.8 -	-/-/-/- -/-/26.0/-	- 195.7	-	-	-	-	-
25	152.9[e]	150.2	103.1	-	-	163.0[e]	-	-/-/26.2/-	195.9	86.3	41.4	69.1	87.7	60.2
29	152.9[e]	149.5	86.9	-	-	161.6[e]	-	-/-/24.5/55.0	-	86.4	40.9	70.4	85.9	61.1
30	152.1[e]	146.4	97.7	-	-	160.9[e]	97.4, 93.3	-/-/10.6, 18.3, 24.9/54.9	-	87.3	40.7	70.3	86.5	63.4
3[g]	149.5	144.5	97.5	-	-	161.6	83.6, 76.4	-	-	84.7	-[f]	70.9	87.5	61.8
24	149.9	146.5	112.1	-	-	161.6	-	-/-/30.2/-	193.4	85.5	-[f]	70.2	87.9	61.0
33	148.7	144.1	98.3[e]	-	-	160.9	98.3[e], 93.3	-/-/10.1,17.8/54.4	-	84.8	-[f]	69.9	85.3	63.1

[a] Measured in DMSO-d_6 at 298 K. [b] Purine numbering for 7-deazapurine derivatives. [c] Systematic numbering for 7-deazapurine derivatives. [d] Systematic numbering for pyridmidine derivatives. [e] Tentative. [f] Superimposed by DMSO. [g] Reference 21.

2-Amino-7-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-3,7-dihydro-5-(trimethylsilylethynyl)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (6). To a suspension of **5**[11] (2.0 g, 5.10 mmol) and CuI (0.194 g, 1.02 mmol) in anhydrous DMF (20 mL) was added successively [$Pd(PPh_3)_4$] (0.589 g, 0.51 mmol), anhydrous Et_3N (1.239 g, 12.2 mmol) and trimethylsilylacetylene (5.01 g, 51.0 mmol). The reaction mixture was stirred under inert atmosphere and allowed to proceed until the starting material was consumed (TLC monitoring). The solvent was evaporated and the residue was adsorbed on silica gel and subjected to FC (silica gel, column 15 x 4 cm, CH_2Cl_2/MeOH, 90:10) to give the product **6** (1.42 g, 77%) as a yellowish foam. TLC (CH_2Cl_2/MeOH, 90:10) R_f 0.40. UV λ_{max} (MeOH)/nm 243 (ε/dm^3 mol^{-1} cm^{-1} 24800), 276 (11500), 294 (11200). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 0.18 (s, 9H, 3 x CH_3), 2.03-2.10 (m, 1H, H_α-2'), 2.27-2.29 (m, 1H, H_β-2'), 3.44-3.53 (m, 2H, 2 x H-5'), 3.74-3.75 (m, 1H, H-4'), 4.26-4.27 (m, 1H, H-3'), 4.92 (t, J = 5.4 Hz, 1H, HO-5'), 5.21 (d, J = 3.6 Hz, 1H, HO-3'), 6.26 (dd, J = 6.0, 5.7 Hz, 1H, H-1'), 6.36 (s, 2H, NH_2), 7.31 (s, 1H, H-8), 10.45 (s, 1H, NH). Anal. Calcd. for $C_{16}H_{22}N_4O_4Si$ (362.46): C, 53.02; H, 6.12; N, 15.46. Found: C, 53.02; H, 6.00; N, 15.30.

2-Amino-7-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-5-ethynyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (1). To a solution of **6** (1.2 g, 3.31 mmol) in MeOH (50 mL) was added K_2CO_3 (1.464 g, 10.6 mmol). After stirring for 12 h at room temperature, the suspension was filtered, adsorbed on silica gel and applied to FC (silica gel, column 15 x 3 cm, CH_2Cl_2/MeOH, 90:10) to give the product **1** (0.740 g, 77%) as a reddish solid. TLC (CH_2Cl_2/MeOH, 90:10) R_f 0.35. UV λ_{max} (H_2O)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 7400), 270 (9000). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 2.04-2.11 (m, 1H, H_α-2'), 2.27-2.36 (m, 1H, H_β-2'), 3.45-3.54 (m, 2H, 2 x H-5'), 3.74-3.77 (m, 1H, H-4'), 3.88 (s, 1H, C≡CH), 4.27-4.28 (m, 1H, H-3'), 4.93 (t, J = 5.4 Hz, 1H, HO-5'), 5.22 (d, J = 3.9 Hz, 1H, HO-3'), 6.27 (dd, J = 6.0, 5.7 Hz, 1H, H-1'), 6.35 (s, 2H, NH_2), 7.30 (s, 1H, H-8), 10.50 (s, 1H, NH). Anal. Calcd for

$C_{13}H_{14}N_4O_4$ (290.27): C, 53.79; H, 4.86; N, 19.30. Found: C, 53.65; H, 5.00; N, 19.21. The obtained NMR data correspond to earlier reported literature values.[5a]

7-(2-Deoxy-*β*-D-*erythro*-pentofuranosyl)-5-ethynyl-3,7-dihydro-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (7). Compound **1** (0.500 g, 1.72 mmol) was dried by repeated co-evaporation with anhydrous pyridine (3 x 8 mL) and dissolved in anhydrous pyridine (12 mL). Then, trimethylsilyl chloride (0.936 g, 8.61 mmol) was added to the solution. The reaction mixture was stirred for 15 min at rt, then isobutyric anhydride (1.362 g, 8.61 mmol) was added and the solution was stirred for additional 3 h at rt. Then, the reaction mixture was cooled in an ice-bath, H_2O (1.5 mL) and subsequently (5 min later) 28-30% aq. NH_3 solution (1 mL) were added, and stirring was continued for 30 min at room temperature. The solvent was evaporated to near dryness, co-evaporated with toluene (3 x 10 mL), and the residue was purified by FC (silica gel, column 15 x 4 cm, CH_2Cl_2/MeOH, 90:10) to give **7** (0.467 g, 75%) as a colorless solid. TLC (CH_2Cl_2/MeOH, 90:10) R_f 0.60. UV λ_{max} (MeOH)/nm 235 (ε/dm^3 mol^{-1} cm^{-1} 17600), 280 (14700). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 1.10, 1.12 (2s, 6H, 2 x CH_3), 2.11-2.18 (m, 1H, H_α-2'), 2.33-2.42 (m, 1H, H_β-2'), 2.69-2.78 (m, 1H, CH), 3.49-3.54 (m, 2H, 2 x H-5'), 3.78-3.81 (m, 1H, H-4'), 4.00 (s, 1H, C≡CH), 4.31-3.32 (m, 1H, H-3'), 4.96 (t, J = 5.1 Hz, 1H, HO-5'), 5.26 (d, J = 3.3 Hz, 1H, HO-3'), 6.37 (dd, J = 6.0, 8.1 Hz, 1H, H-1'), 7.62 (s, 1H, H-8), 11.57 (s, 1H, NH), 11.82 (s, 1H, NH). Anal. Calcd for $C_{17}H_{20}N_4O_5$ (360.36): C, 56.66; H, 5.59; N, 15.55. Found: C, 56.42; H, 5.72; N, 15.49.

7-[2-Deoxy-5-*O*-(4,4'-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-5-ethynyl-3,7-dihydro-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (8). Compound **7** (0.390 g, 1.08 mmol) was dried by repeated co-evaporation with anhydrous pyridine (3 x 8 mL). The residue was dissolved in anhydrous pyridine (12 mL) and stirred with 4,4'-dimethoxytrityl chloride (0.706 g, 1.84 mmol) at room temperature for 5 h. The solution was poured into 5%

aq. $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 x 50 mL). The combined extracts were dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by FC (silica gel, column 15 x 3 cm, CH_2Cl_2/acetone, 80:20) to give the product **8** (0.41 g, 57%) as a colorless foam. TLC (CH_2Cl_2/acetone, 80:20) R_f 0.44. UV λ_{max} (MeOH)/nm 234 (ε/dm^3 mol^{-1}, cm^{-1} 41300), 281 (19600). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 1.10, 1.12 (2s, 6H, 2 x CH_3), 2.19-2.26 (m, 1H, H$_\alpha$-2'), 2.46-2.55 (m, 1H, H$_\beta$-C2'), 2.71-2.80 (m, 1H, CH), 3.07-3.19 (m, 2H, 2 x H-5'), 3.72 (s, 6H, 2 x OCH_3), 3.90-3.91 (m, 1H, H-4'), 4.02 (s, 1H, C≡CH), 4.33-4.34 (m, 1H, H-3'), 5.32 (d, J = 3.9 Hz, 1H, HO-3'), 6.38 (t, J = 6.3 Hz, 1H, H-1'), 6.82-6.86 (m, 4H, Ar-H), 7.17-7.37 (m, 9H, Ar-H), 7.48 (s, 1H, H-8), 11.61 (s, 1H, NH), 11.86 (s, 1H, NH). Anal. Calcd for $C_{38}H_{38}N_4O_7$ (662.73): C, 68.87; H, 5.78; N, 8.45. Found: C, 68.93; H, 5.87; N, 8.32.

7-[2-Deoxy-5-*O*-(4,4'-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-5-ethynyl-3,7-dihydro-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 3'-(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (9). To a solution of **8** (0.1 g, 0.15 mmol) in dry CH_2Cl_2 (10 mL), (i-Pr)$_2$NEt (0.049 g, 0.38 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (0.064 g, 0.27 mmol) were added. After stirring for 45 min at rt, the solution was diluted with CH_2Cl_2 (30 mL) and extracted with 5% aq. $NaHCO_3$ solution (20 mL), the combined organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by FC (silica gel, column 10 x 2 cm, CH_2Cl_2/acetone, 95:15) affording product **9** (0.098 g, 75%) as a colorless foam. TLC (CH_2Cl_2/acetone, 85:15) R_f 0.75. ^{31}P NMR ($CDCl_3$, 121 MHz) (δ, ppm): 148.1, 147.4. ESI-TOF m/z calcd for $C_{47}H_{55}N_6O_8P$ $[M + Na]^+$ 885.3711, found 885.3688.

1-(2-Deoxy-*β*-D-*erythro*-pentofuranosyl)-5-ethynylcytosine (2). To a solution of 5-trimethylsilylethynyl-2'-deoxycytidine[9c] (4.0 g, 12.4 mmol) in MeOH (50 mL) was added K_2CO_3 (0.060 g, 0.43 mmol). The reaction mixture was stirred at room temperature for 1 h

(TLC monitoring). Then, the solvent was evaporated and the remaining residue was purified by FC (silica gel, column 20 x 4 cm, CH_2Cl_2/MeOH, 80:20). Evaporation of the solvent gave nucleoside **2** (2.65 g, 85%) as amorphous solid. TLC (CH_2Cl_2/MeOH, 80:20) R_f 0.40. UV λ_{max} (H_2O)/nm 260 (ε/ dm^3 $mol^{-1}cm^{-1}$ 3600), 292 (8100). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 1.94-2.03 (m, 1H, H_α-2'), 2.12-2.19 (m, 1H, H_β-2'), 3.53-3.65 (m, 2H, H-5'), 3.78-3.81 (m, 1H, H-4'), 4.19-4.22 (m, 1H, H-3'), 4.34 (s, 1H, C≡CH), 5.10 (t, J = 5.1 Hz, 1H, 5'-OH), 5.22 (d, J = 4.2 Hz, 1H, 3'-OH), 6.09 (t, J = 6.3 Hz, 1H, H-1'), 6.83 (s, 1H, NH_a), 7.71 (s, 1H, NH_b), 8.26 (s, 1H, H-6). The obtained NMR data correspond to earlier reported literature values.[9c,12]

1-[2-Deoxy-5-*O*-(4,4'-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-5-ethynylcytosine (11). Compound **2** (2.5 g, 9.95 mmol) was dried by repeated co-evaporation with anhydrous pyridine (2 x 10 mL) before dissolving in anhydrous pyridine (20 mL). Then 4,4'-dimethoxytrityl chloride (4.4 g, 12.99 mmol) was added and the solution was stirred at room temperature for 5h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and extracted with 5% aq. $NaHCO_3$ solution (100 mL), the organic layer was dried over Na_2SO_4, and then concentrated. Purification by FC (silica gel, column 15 x 3 cm, CH_2Cl_2/acetone, 90:10) gave **11** as colorless foam (4.36 g, 79%). TLC (CH_2Cl_2/MeOH, 90:10) R_f 0.5. λ_{max} (MeOH)/nm 235 (ε/ dm^3 $mol^{-1}cm^{-1}$ 34600), 283 (8000). ^{1}H NMR (DMSO-d_6, 300 MHz): 2.08-2.15 (m, 1H, H_α-2'), 2.21-2.24 (m, 1H, H_β-2'), 3.12-3.25 (m, 2H, H-5'), 3.74 (s, 6H, 2 x OCH_3), 3.93-3.94 (m, 1H, H-4'), 4.20-4.22 (m, 2H, H-3', C≡CH), 5.30 (d, J = 4.2 Hz, 1H, 3'-OH), 6.11 (t, J = 6.6 Hz, 1H, H-1'), 6.87-6.90 (m, 5H, 4 x Ar-H, 1 x NH_a), 7.19-7.41 (m, 9H, Ar-H), 7.77 (s, 1H, NH_b), 7.95 (s, 1H, H-6). ESI-TOF m/z calcd for $C_{32}H_{31}N_3O_6$ $[M + Na]^+$ 576.2105, found 576.2108.

N^4-Acetyl-1-[2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-5-ethynylcytosine (**12**). To a solution of compound **11** (4.3 g, 7.77 mmol) in *N*,*N*-dimethylformamide (20 mL) was added acetic anhydride (900 µL, 9.41 mmol) and the reaction mixture was stirred at room temperature for 24 h. Then, another portion of acetic anhydride (200 µL, 2.09 mmol) was added, and the mixture was stirred for additional 12 h (TLC monitoring). After evaporation of DMF under reduced pressure, the residue was applied to FC (silica gel, column 15 x 4 cm, CH_2Cl_2/acetone, 80:20). After evaporation of the solvent from the main zone, compound **12** was isolated as colorless foam (3.38 g, 73%). TLC (CH_2Cl_2/acetone, 80:20) R_f 0.53. λ_{max} (MeOH)/nm 235 (ε/ dm^3 $mol^{-1}cm^{-1}$ 38500), 282 (6700). ^{1}H NMR (DMSO-d_6, 300 MHz): 2.15-2.24 (m, 1H, H_α-2'), 2.30 (s, 3H, CH_3), 2.37-2.41 (m, 1H, H_β-2'), 3.17-3.27 (m, 2H, 2 x H-5'), 3.73 (s, 6H, 2 x OCH_3), 4.00 (bs, 1H, H-4'), 4.23 (bs, 1H, H-4'), 4.36 (s, 1H, C≡CH), 5.35 (d, J = 4.2 Hz, 1H, 3'-OH), 6.05 (t, J = 6.3 Hz, 1H, H-1'), 6.87-6.90 (m, 4H, Ar-H), 7.19-7.39 (m, 9H, Ar-H), 8.26 (s, 1H, H-6), 9.36 (s, 1H, NH). ESI-TOF m/z calcd for $C_{34}H_{33}N_3O_7$ $[M + Na]^+$ 618.2211, found 618.2196.

N^4-Acetyl-1-[2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-5-ethynyl cytosine 3'-(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite) (13). A stirred solution of **12** (1.0 g, 1.67 mmol) in anhydrous CH_2Cl_2 (15 mL) was treated with (i-Pr)$_2$NEt (400 µL, 2.4 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylphosphoramido chloridite (520 µL, 2.4 mmol). The reaction mixture was stirred for 15 min (TLC monitoring) at room temperature, then the solution was diluted with CH_2Cl_2 (30 mL) and extracted with 5% aq. $NaHCO_3$ solution (20 mL). The organic layer was dried over Na_2SO_4, concentrated and purified by FC (silica gel, column 10 x 4 cm, CH_2Cl_2/acetone, 80:20). Evaporation of the solvent from the main zone gave **13** (0.850 g, 63%) as colorless foam. TLC (CH_2Cl_2/acetone, 80:20) R_f 0.7. ^{31}P NMR ($CDCl_3$, 121 MHz) (δ, ppm): 149.2, 148.6. ESI-TOF m/z calcd for $C_{43}H_{50}N_5O_8P$ $[M + Na]^+$ 818.3289, found 818.3281.

5-Acetyl-2-amino-7-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (21). To a solution of compound **1** (0.073 g, 0.25 mmol) in MeOH (9 mL) was added water (1 mL) and H_2SO_4 (0.025 mmol). The reaction mixture was stirred for 1 h at 75 °C. The reaction mixture was brought to pH 7 by the use of a strongly basic ion-exchange resin. The resin was filtered-off and washed with MeOH (20 mL). The filtrate was evaporated and the remaining residue was purified by FC (silica gel, column 10 x 2 cm, CH_2Cl_2/MeOH, 80:20) to give the product **21** (0.054 g, 70%) as a colorless powder. TLC (CH_2Cl_2/MeOH, 75:25) R_f 0.26. λ_{max} (H_2O)/nm 260 (ε/dm^3 mol^{-1}, cm^{-1} 6600), 307 (5800). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 2.12-2.15 (m, 1H, H_α-2'), 2.29-2.38 (m, 1H, H_β-2'), 2.62 (s, 3H, CH_3), 3.47-3.53 (m, 2H, 2 x H-5'), 3.76-3.80 (m, 1H, H-4'), 4.29-4.30 (m, 1H, H-3'), 4.94 (t, J = 5.4 Hz, 1H, HO-5'), 5.23 (d, J = 3.6 Hz, 1H, HO-3'), 6.32 (dd, J = 6.0, 5.7 Hz, 1H, H-1'), 6.43 (s, 2H, NH_2), 7.63 (s, 1H, H-8), 10.61 (s, 1H, NH). ESI-TOF m/z calcd for $C_{13}H_{16}N_4O_5$ [M + Na]$^+$ 331.1013, found 331.1008.

5-Ethynylcytosine (22) and 5-Acetylcytosine (23). As described for **21**, using compound **2**[12] (0.063 g, 0.25 mmol) in MeOH (9 mL), water (1 mL) with H_2SO_4 (0.25 mmol). FC (silica gel, column 10 x 2 cm, CH_2Cl_2/MeOH 85:15) gave an inseparable mixture of products **22** and **23** (1:1) (0.030 g, 84%) as a colorless powder. TLC (CH_2Cl_2/MeOH, 85:15) R_f 0.34. ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 2.35 (s, 3H, CH_3), 4.28 (s, 1H, C≡CH), 6.69, 7.85, 8.39 (3br s, 4H, 2 x NH_2), 7.74, 8.45 (2s, 1H, 2 x H-6), 11.33 (br s, 2H, 2 x NH). ESI-TOF m/z calcd for **22** $C_6H_5N_3O$ [M + Na]$^+$ 158.0325, found 158.0331 and for **23** $C_6H_7N_3O_2$ [M + Na]$^+$ 176.0430, found 176.0438. The obtained NMR data correspond to earlier reported literature values.[16-17]

5-Acetyl-1-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)uracil (24). As described for **21** compound **3**[16a] (0.063 g, 0.25 mmol) in MeOH (9 mL) and water (1 mL) with H_2SO_4 (0.25 mmol) and FC (silica gel, column 10 x 2 cm, CH_2Cl_2/MeOH, 85:15) to give the product **24** (0.054 g, 80%) as a colorless powder. TLC (CH_2Cl_2/MeOH, 85:15) R_f 0.36. UV λ_{max} (H_2O)/nm 260 (ε/ dm^3 $mol^{-1}cm^{-1}$ 4900), 282 (12700). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 2.11-2.22 (m, 2H, H-2'), 2.43 (s, 3H, CH_3), 3.52-3.58 (m, 2H, H-5'), 3.83-3.87 (m, 1H, H-4'), 4.21-4.23 (m, 1H, H-3'), 5.04 (t, J = 4.5 Hz, 1H, 5'-OH), 5.27 (d, J = 4.2 Hz, 1H, 3'-OH), 6.11 (t, J = 6.3 Hz, 1H, H-1'), 8.64 (s, 1H, H6), 11.62 (s, 1H, NH). The obtained NMR data correspond to earlier reported literature values.[13,14]

5-Acetyl-1-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)cytosine (25). Compound **2**[12] (0.150 g, 0.60 mmol) was dissolved in 28% aqueous NH_3 (200 mL) and stirred at 55 °C for 20 h in an autoclave. The resulting solution was concentrated, and the residue was applied to FC (silica gel, column 15 x 3 cm, CH_2Cl_2/MeOH, 90:10) to give **25** as a colorless solid (0.03 mg, 19%). TLC (CH_2Cl_2/MeOH, 90:10) R_f 0.40. UV λ_{max} (H_2O)/nm 260 (ε/ dm^3 $mol^{-1}cm^{-1}$ 4600), 285 (8900). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 2.08-2.16 (m, 1H, H_α-2'), 2.24-2.31 (m, 1H, H_β-2'), 2.36 (s, 3H, CH_3), 3.59-3.73 (m, 2H, H-5'), 3.86-3.87 (m, 1H, H-4'), 4.24-4.26 (m, 1H, H-3'), 5.24-5.28 (m, 2H, 5'-OH and 3'-OH), 6.10 (t, J = 5.7 Hz, 1H, H-1'), 7.94 (s, 1H, NH_a), 8.33 (s, 1H, NH_b), 9.06 (s, 1H, H-6). ESI-TOF m/z calcd for $C_{11}H_{15}N_3O_5$ $[M + Na]^+$ 292.0904, found 292.0903.

7-[2-Deoxy-5-*O*-(4,4'-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-5-(triisopropylsilylethynyl)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (27). To a suspension of **26** (0.400 g, 0.52 mmol) and CuI (0.020 g, 0.10 mmol) in anhydrous DMF (5 mL) was added successively $[Pd(PPh_3)_4]$ (0.090 g, 0.08 mmol), anhydrous Et_3N (0.131 g, 1.30 mmol) and triisopropylsilylacetylene (0.382 g, 2.10 mmol). The reaction mixture was

stirred under inert atmosphere overnight. The solvent was evaporated, and the residue was purified by FC (silica gel, column 15 x 4 cm, CH_2Cl_2/acetone, 90:10) to give the product **27** (0.340 g, 79%) as a light yellow foam. TLC (CH_2Cl_2/acetone, 90:10) R_f 0.31. λ_{max} (MeOH)/nm 283 (ε/dm^3 mol^{-1} cm^{-1} 22400). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 1.09-1.11 (m, 27H, 8 x CH_3, 3 x CH), 2.18-2.21 (m, 1H, H_α-2'), 2.53-2.55 (m, 1H, H_β-C2'), 2.72-2.76 (m, 1H, CH), 3.05-3.19 (m, 2H, 2 x H-5'), 3.69, 3.70 (2s, 6H, 2 x OCH_3), 3.91 (bs, 1H, H-4'), 4.32 (bs, 1H, H-3'), 5.30 (d, J = 3.9 Hz, 1H, HO-3'), 6.37 (t, J = 6.3 Hz, 1H, H-1'), 6.80-6.84 (m, 4H, Ar-H), 7.13-7.37 (m, 9H, Ar-H), 7.45 (s, 1H, H-8), 11.56 (s, 1H, NH), 11.79 (s, 1H, NH). ESI-TOF m/z calcd for $C_{47}H_{58}N_4O_7Si$ $[M + Na]^+$ 841.3967, found 841.3956.

7-[2-Deoxy-5-*O*-(4,4'-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-3,7-dihydro-2-(iso-butyrylamino)-5-(triisopropylsilylethynyl)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 3'-(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (28). To a solution of **27** (0.2 g, 0.24 mmol) in dry CH_2Cl_2 (10 mL), (i-Pr)$_2$NEt (0.075 g, 0.58 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (0.099 g, 0.42 mmol) were added. After stirring for 45 min at rt, the solution was diluted with CH_2Cl_2 (30 mL) and extracted with 5% aq. $NaHCO_3$ solution (20 mL), the combined organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by FC (silica gel, column 10 x 2 cm, CH_2Cl_2/acetone, 95:5) to give the product **28** (0.152 g, 61%) as a colorless foam. TLC (CH_2Cl_2/acetone, 95:5) R_f 0.50. ^{31}P NMR ($CDCl_3$, 121 MHz) (δ, ppm): 148.0, 147.4. ESI-TOF m/z calcd for $C_{56}H_{75}N_6O_8PSi$ $[M + Na]^+$ 1041.5045, found 1041.5032.

N^4-Acetyl-1-[2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-5-iodocytosine (**29**). To a solution of 1-[2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-*β*-D-*erythro*-

pentofuranosyl]-5-iodocytosine[22] (2.93 g, 4.46 mmol) in *N*,*N*-dimethylformamide (20 mL) was added acetic anhydride (1.05 mL, 11.15 mmol), and the reaction mixture was stirred at room temperature for 24 h. After evaporation of the solvent under reduced pressure, the residue was applied to FC (silica gel, column 15 x 4 cm, CH_2Cl_2/acetone, 80:20). After evaporation of the solvent from the main zone, compound **29** was isolated as colorless foam (2.69 g, 86%). TLC (CH_2Cl_2/MeOH 90:10) R_f 0.63. UV λ_{max} (MeOH)/nm 321.0 (ε/ dm^3 $mol^{-1}cm^{-1}$ 11200). ^{1}H NMR (DMSO-d_6, 300 MHz): 2.14-2.23 (m, 1H, H_α-2'), 2.23 (s, 3H, CH_3), 2.33-2.37 (m, 1H, H_β-2'), 3.17-3.26 (m, 2H, H-5'), 3.74 (s, 6H, 2 x OCH_3), 3.98-3.99 (m, 1H, H-4'), 4.19-4.20 (m, 1H, H-3'), 5.33 (d, *J* = 4.5 Hz, 1H, 3'-OH), 6.06 (t, *J* = 6.4 Hz, 1H, H-1'), 6.89-6.91 (m, 4H, Ar-H), 7.20-7.41 (m, 9H, Ar-H), 8.28 (s, 1H, H-C6), 9.43 (s, 1H, NH). ESI-TOF *m/z* calcd. for $C_{32}H_{32}IN_3O_7$ [M + Na^+] 720.1177, found 720.1156.

N^4-Acetyl-1-[2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-5-(triisopropylsilylethynyl)cytosine (**30**). To a solution of **29** (0.558 g, 0.80 mmol) in anhydrous DMF (10 mL), CuI (0.031 g, 0.16 mmol), [$Pd(PPh_3)_4$] (0.139 g, 0.12 mmol), anh. Et_3N (0.3 mL, 2.16 mmol) and triisopropylsilylacetylene (448 µL, 2.0 mmol) were added. The mixture was stirred at room temperature under N_2 overnight. The solvent was removed and the remaining residue was purified by FC (silica gel, column 20 x 4 cm, CH_2Cl_2/acetone, 90:10). Evaporation of the solvent from the main zone gave **30** (0.354 g, 59%) as slightly yellow amorphous solid. TLC (CH_2Cl_2/acetone, 80:20) R_f 0.30. UV λ_{max} (MeOH)/nm 318 (ε/ dm^3 $mol^{-1}cm^{-1}$ 13500). ^{1}H NMR (DMSO-d_6, 300 MHz): 0.95-0.96 (m, 21H, 8 x CH_3, 3 x CH), 2.11-2.17 (m, 1H, H_α-2'), 2.33-2.39 (m, 4H, CH_3, H_β-2'), 3.07-3.25 (m, 2H, H-5'), 3.71 (s, 6H, 2 x OCH_3), 4.00-4.01 (m, 1H, H-4'), 4.12-4.13 (m, 1H, H-3'), 5.31 (d, *J* = 4.5 Hz, 1H, 3'-OH), 6.03 (t, *J* = 6.6 Hz, 1H, H-1'), 6.84-6.87 (m, 4H, Ar-H), 7.16-7.39 (m, 9H, Ar-H), 8.20 (s, 1H, H-6), 9.08 (s, 1H, NH). ESI-TOF *m/z* calcd. for $C_{43}H_{53}N_3O_7Si$ [M + Na^+] 774.3545, found 774.3526.

N^4-Acetyl-1-[2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-5-(triisopropylsilylethynyl)cytosine 3'-(2-Cyanoethyl)-*N,N*-diisopropylphosphoramidite (**31**). A stirred solution of **30** (0.371 g, 0.49 mmol) in anhydrous CH_2Cl_2 (10 mL) was treated with $(i\text{-Pr})_2EtN$ (145 µL, 0.85 mmol) and 2-cyanoethyl-*N,N*-diisopropylphosphoramido chloridite (145 µL, 0.65 mmol). Stirring was continued for 15 min (TLC monitoring) at rt. Then the solution was diluted with CH_2Cl_2 (30 mL) and extracted with 5% aq. $NaHCO_3$ solution (20 mL). The organic layer was dried over Na_2SO_4 and concentrated. FC (silica gel, column 10 x 4 cm, CH_2Cl_2/acetone, 90:10) and evaporation of the solvent from the main zone gave **31** (0.374 g, 80%) as colorless foam. TLC (CH_2Cl_2/acetone, 90:10) R_f 0.63. ^{31}P NMR ($CDCl_3$, 121 MHz) (δ, ppm): 149.2, 148.5. ESI-TOF *m/z* calcd. for $C_{52}H_{70}N_5O_8PSi$ $[M + Na^+]$ 974.4623, found 974.4602.

1-(2-Deoxy-5-*O*-(4,4'-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl)-5-(triisopropylsilylethynyl)uracil (33). To a solution of **32** (0.5 g, 0.76 mmol) in anhydrous DMF (10 mL), CuI (0.029 g, 0.015 mmol), $[Pd(PPh_3)_4]$ (0.088 g, 0.076 mmol), anhydrous Et_3N (0.193 g, 1.90 mmol) and triisopropylsilylacetylene (0.486 g, 2.67 mmol) were added. The mixture was stirred at room temperature under N_2 overnight. The solvent was evaporated and the residue was purified by FC (silica gel, column 20 x 4 cm, CH_2Cl_2/acetone, 90:10). Evaporation of the solvent from the main zone gave **33** (0.380 g, 70%) as white foam. TLC (CH_2Cl_2/acetone, 85:15) R_f 0.35. λ_{max} (MeOH)/nm 235 (ε/ dm^3 $mol^{-1}cm^{-1}$ 27110), 277 (9316), 287 (11000), 295 (11280). ^1H NMR (DMSO-d_6, 300 MHz): 0.69-1.28 (m, 21H, 8 x CH_3, 3 x CH), 2.16-2.21 (m, 2H, H-2'), 3.11-3.18 (m, 2H, H-5'), 3.71 (s, 6H, 2 x OCH_3), 3.89 (bs, 1H, H-4'), 4.14 (bs, 1H, H-3'), 5.29 (d, J = 4.2 Hz, 1H, 3'-OH), 6.07 (t, J = 6.3 Hz, 1H, H-1'), 6.84-6.87 (m, 4H, Ar-H), 7.18-7.39 (m, 9H, Ar-H), 7.83 (s, 1H, H-6), 11.66 (s, 1H, NH). ESI-TOF m/z calcd for $C_{41}H_{50}N_2O_7Si$ $[M + Na]^+$ 733.3279, found 733.3277.

1-(2-Deoxy-5-*O*-(4,4'-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl)-5-(triisopropylsilylethynyl)uracil 3'-(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (**34**). A stirred solution of **33** (0.150 g, 0.21 mmol) in anhydrous CH_2Cl_2 (10 mL) was treated with (*i*-Pr)$_2$EtN (0.045 g, 0.35 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylphosphoramido chloridite (0.064 g, 0.27 mmol). The reaction mixture was stirred for 15 min (TLC monitoring) at room temperature, then the solution was diluted with CH_2Cl_2 (50 mL) and extracted with 5% aq. $NaHCO_3$ solution (30 mL). The organic layer was dried over Na_2SO_4 and concentrated. FC (silica gel, column 10 x 4 cm, CH_2Cl_2/acetone, 90:10) and evaporation of the solvent from the main zone gave **34** (0.131 g, 68%) as colorless foam. TLC (CH_2Cl_2/acetone, 90:10) R_f 0.66. ^{31}P NMR ($CDCl_3$, 121 MHz) (δ, ppm): 149.2, 148.6. ESI-TOF m/z calcd for $C_{50}H_{67}N_4O_8PSi$ $[M + Na]^+$ 933.4358, found 933.4337.

Genral Procedure for the Deprotection of the TIPS group. Triisoproylsilylethynyl modified oligonucleotides (10 A_{260} unit) were dissolved in CH_3CN/DMF (4:1, 150 µL) and tetrabutylammonium fluoride (10 µL). The resulting reaction mixture was stirred at 45 °C for 16 h. Deprotected oligonucleotides were precipitated by adding 3 M NaOAc buffer, pH 5.2 (20 µL) and 2-propanol (600 µL), and incubation at 0 °C for 24 h. After centrifugation at 14000 rpm for 30 min, the solvent was decanted and the remaining residue was washed with 75% ethanol (150 µL). The resulting oligonucleotide was dried and purified by reversed-phase HPLC using gradient *II*.

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Supporting Information. ^{1}H-^{13}C coupling constants, HPLC profiles, copies of ^{1}H, ^{13}C NMR, DEPT-135, and ^{1}H-^{13}C gated-decoupled NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

(1) (a) Huisgen, R.; Szeimies, G.; Möbius, L. *Chem. Ber.* **1967**, *100*, 2494-2507. (b) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057-3064. (c) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596-2599.

(2) (a) Amblard, F.; Cho, J. H.; Schinazi, R. F. *Chem. Rev.* **2009**, *109*, 4207–4220. (b) Kappe, C. O.; Van der Eycken, E. *Chem. Soc. Rev.* **2010**, *39*, 1280–1290. (c) El-Sagheer, A. H.; Brown, T. *Chem. Soc. Rev.* **2010**, *39*, 1388–1405. (d) Ustinov, A. V.; Stepanova, I. A.; Dubnyakova, V. V.; Zatsepin, T. S.; Nozhevnikova, E. V.; Korshun, V. A. *Russ. J. Bioorg. Chem.* **2010**, *36*, 401-445. (e) Gramlich, P. M. E.; Warncke, S.; Gierlich, J.; Carell, T. *Angew. Chem. Int. Ed.* **2008**, *47*, 3442-3444. (f) Seela, F.; Ingale, S. A. *J. Org. Chem.* **2010**, *75*, 284–295. (g) Ingale, S. A.; Pujari, S. S.; Sirivolu, V. R.; Ding, P.; Xiong, H.; Mei, H.; Seela, F. *J. Org. Chem.* **2012**, *77*, 188−199. (h) Mei, H.; Ingale, S. A.; Seela, F. *Tetrahedron* **2013**, *69*, 4731-4742. (i) Ingale, S. A.; Seela, F. *J. Org. Chem.* **2013**, *78*, 3394-3399. (j) Yamada, T.; Peng, C. G.; Matsuda, S.; Addepalli, H.; Jayaprakash, K. N.; Alam, Md. R.; Mills, K.; Maier, M. A.; Charisse, K.; Sekine, M.; Manoharan, M.; Rajeev, K. G. *J. Org. Chem.* **2011**, *76*, 1198-1211.

(3) (a) Macíčková-Cahová, H.; Pohl, R.; Hocek, M. *ChemBioChem* **2011**, *12*, 431-438. (b) Salic, A.; Mitchison T. J. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 2415-2420. (c) Ménová, P.; Raindlová, V.; Hocek, M. *Bioconjugate Chem.* **2013**, *24*, 1081-1093.

(4) Guan, L.; van der Heijden, G. W.; Bortvin, A.; Greenberg, M. M. *ChemBioChem* **2011**, *12*, 2184 –2190.

(5) (a) Neef, A. B.; Samain, F.; Luedtke, N. W. *ChemBioChem* **2012**, *13*, 1750-1753. (b) Qu, D.; Wang, G.; Wang, Z.; Zhou, L.; Chi, W.; Cong, S.; Ren, X.; Liang, P.; Zhang, B. *Anal. Biochem.* **2011**, *417*, 112–121.

(6) Beaucage, S. L.; Caruthers M. H. *Tetrahedron Lett.* **1981**, *22*, 1859-1862.

(7) (a) Seela, F.; Sirivolu, V. R.; Chittepu, P. *Bioconjugate Chem.* **2008**, *19*, 211-224. (b) Seela, F.; Sirivolu, V. R. *Helv. Chim. Acta* **2007**, *90*, 535-552. (c) Gierlich, J.; Burley, G. A.; Gramlich, P. M. E.; Hammond, D. M.; Carell, T. *Org. Lett.* **2006**, *8*, 3639-3642.

(8) Ding, P.; Wunnicke, D.; Steinhoff, H.-J.; Seela, F. *Chem. Eur. J.* **2010**, *16*, 14385-14396.

(9) (a) Jakobsen, U.; Shelke, S. A.; Vogel, S.; Sigurdsson, S. Th. *J. Am. Chem. Soc.* **2010**, *132*, 10424–10428. (b) Kaura, M.; Kumar, P.; Hrdlicka, P. J. *Org. Biomol. Chem.* **2012**, *10*, 8575–8578. (c) Andersen, N. K.; Døssing, H.; Jensen, F.; Vester, B.; Nielsen, P. *J. Org. Chem.* **2011**, *76*, 6177-6187

(10) Graham, D.; Parkinson, J. A.; Brown, T. *J. Chem. Soc. Perkin Trans 1*, **1998**, 1131-1138.

(11) Ramzaeva, N.; Seela, F. *Helv. Chim. Acta* **1995**, *78*, 1083-1090.

(12) Barr, P. J.; Jones, A. S.; Serafinowski, P.; Walker, R. T. *J. Chem. Soc. Perkin Trans. 1*, **1978**, 1263-1267.

(13) Barr, P. J.; Chananont, P.; Hamor, T. A.; Jones, A. S.; O'leary, M. K.; Walker, R. T. *Tetrahedron*, **1980**, *36*, 1269-1273.

(14) Kampf, A.; Pillar, C. J.; Woodford, W. J.; Mertes, M. P. *J. Med. Chem.* **1976**, *19*, 909-915.

(15) Mizushima, E.; Sato, K.; Hayashi, T.; Tanaka, M. *Angew. Chem. Int. Ed.* **2002**, *41*, 4563-4565.

(16) (a) Jones, A. S.; Serafinowski, P.; Walker, R. T. *Tetrahedron Lett.* **1977**, *28*, 2459-2460. (b) Kielkowski, P.; Pohl, R.; Hocek, M. *J. Org. Chem.* **2011**, *76*, 3457-3462.

(17) Birari, D. R.; Ghagare, M. G.; Kazi, M. A.; Bagul, S. M.; Ghotekar, B. K.; Toche, R. B.; Jachak, M. N. *Org. Prep. Proced. Int.* **2009**, *41*, 515–532.

(18) Bobek, M.; Kavai, I.; Sharma, R. A.; Grill, S.; Dutschman, G.; Cheng, Y.-C. *J. Med. Chem.* **1987**, *30*, 2154-2157.

(19) Ramzaeva, N.; Seela, F. *Helv. Chim. Acta* **1996**, *79*, 1549-1558.

(20) Hudson, R. H. E.; Ghorbani-Choghamarani, A. *Synlett* **2007**, *6*, 870–873.

(21) Borsenberger, V.; Kukwikila, M.; Howorka, S. *Org. Biomol. Chem.* **2009**, *7*, 3826–3835.

(22) Roychowdhury, A.; Illangkoon, H.; Hendrickson, C. L.; Benner, S. A. *Org. Lett.* **2004**, *6*, 489-492.