JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Ethynyl Side Chain Hydration during Synthesis and Workup of "Clickable" Oligonucleotides: Bypassing Acetyl Group Formation by Triisopropylsilyl Protection

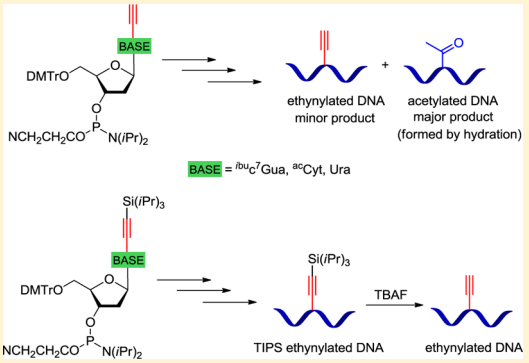
Sachin A. Ingale,[†,‡] Hui Mei,[†,‡] Peter Leonard,[†] and Frank Seela*[,†,‡]

[†]Laboratory of Bioorganic Chemistry and Chemical Biology, Center for Nanotechnology, Heisenbergstraße 11, 48149 Münster, Germany

[‡]Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie neuer Materialien, Universität Osnabrück, Barbarastraße 7, 49069 Osnabrück, Germany

Supporting Information

ABSTRACT: Clickable oligonucleotides with ethynyl residues in the 5-position of pyrimidines (^{eth}dC and ^{eth}dU) or the 7-position of 7-deazaguanine ($^{eth}c^7G_d$) are hydrated during solid-phase oligonucleotide synthesis and workup 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 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

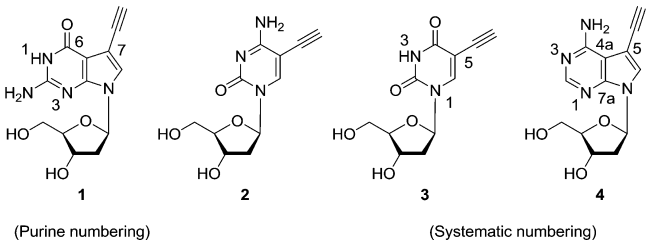


Figure 1. Structures of 7-ethynyl-7-deazapurine and 5-ethynylpyrimidine nucleosides.

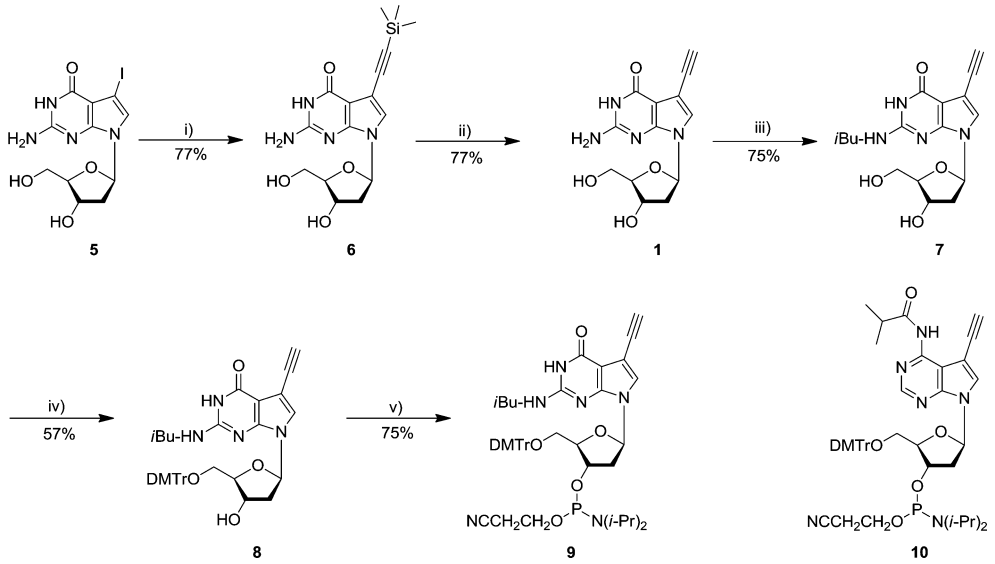
incorporated into DNA in the form of their triphosphates by a template-controlled polymerase chain reaction. By this means, the intracellular detection of cytosine was studied with the triphosphate of 5-ethynyl-2′-deoxycytidine (^{eth}dC) in genomic DNA.[4] The 7-ethynyl-7-deaza-2′-deoxyguanosine **1** ($^{eth}c^7G_d$) and 7-ethynyl-7-deaza-2′-deoxyadenosine **4** ($^{eth}c^7A_d$) were used for the metabolic labeling 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 the 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 a 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 workup procedure using oligonucleotide deprotection in concentrated

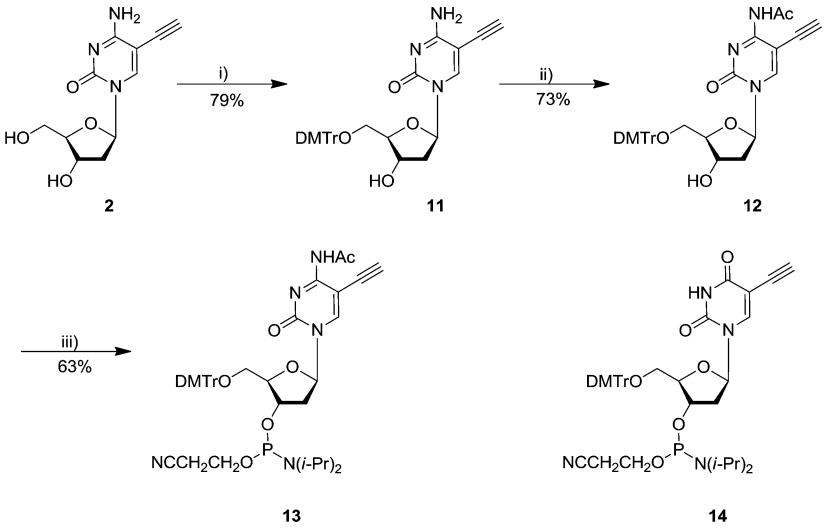
Received: August 14, 2013
Published: October 18, 2013

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, room temperature, 12 h; (ii) K_2CO_3, MeOH, room temperature, 12 h; (iii) iBu_2O, TMSCl, anhydrous pyridine, room temperature, 3 h; (iv) 4,4′-dimethoxytriphenylmethyl chloride, anhydrous pyridine, room temperature, 12 h; (v) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $(i\text{-}Pr)_2NEt$, anhydrous CH_2Cl_2, room temperature.

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



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

aqueous ammonia. This paper 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.

RESULTS AND DISCUSSION

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** on a 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]

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%).

dx.doi.org/10.1021/jo401780u | J. Org. Chem. 2013, 78, 11271−11282

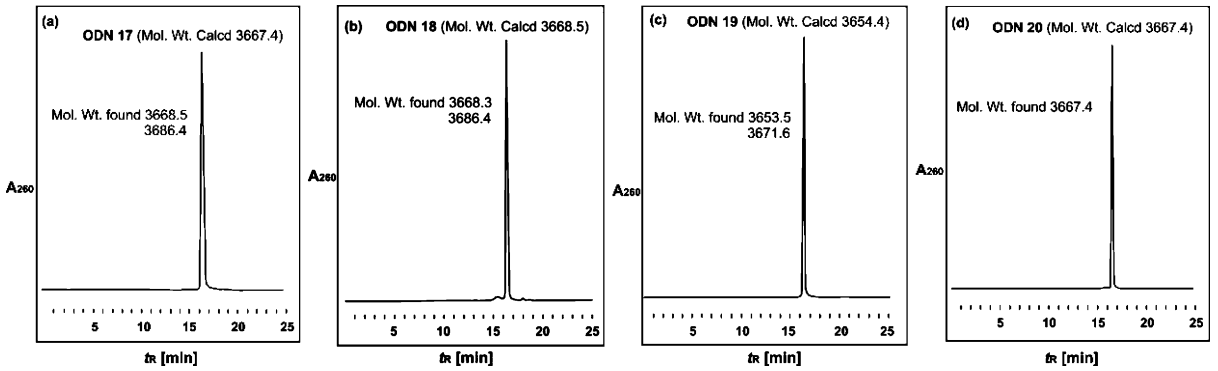


Figure 2. Reversed-phase HPLC elution profiles of purified oligonucleotides monitored at 260 nm using gradient system II: (a) 5′-d(AGT ATT **1**AC CTA) (ODN **17**); (b) 5′-d(TAG GT**2** AAT ACT) (ODN **18**); (c) 5′-d(AGT AT**3** GAC CTA) (ODN **19**); (d) 5′-d(TAG GTC **4**AT ACT) (ODN **20**).

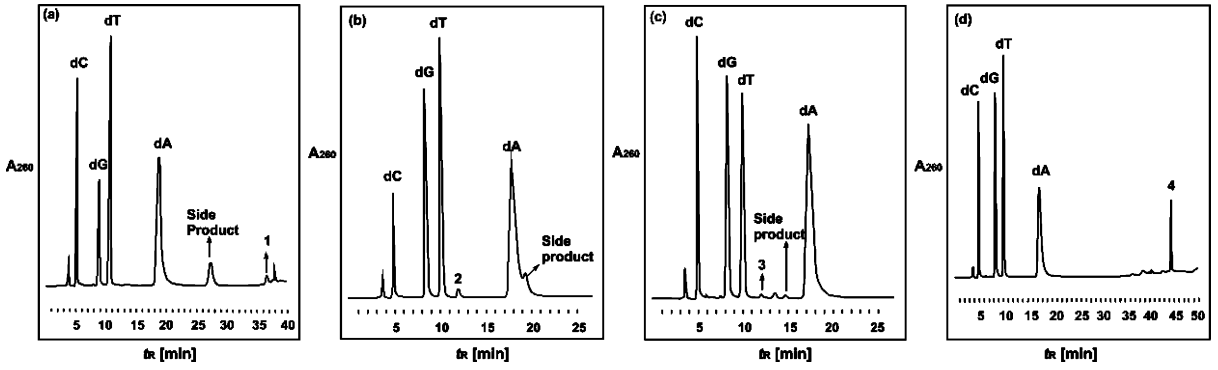


Figure 3. HPLC profiles of the enzymatic hydrolysis products monitored at 260 nm using gradient systems III and IV: (a) ODN **17**; (b) ODN **18**; (c) ODN **19**; (d) ODN **20**.

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 ^{1}H and ^{13}C NMR spectra (see the Supporting Information). The ^{13}C NMR chemical shifts are given in the Experimental Section (Table 3) and were assigned by ^{1}H−^{13}C coupling constants (Tables S1 and S2, Supporting Information) and DEPT-135 NMR spectra.

Syntheses of Oligonucleotides Using Ethynylated Phosphoramidites 9, 10, 13, and 14. Following monomer synthesis, oligonucleotides (ODNs) were prepared on a 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 concentrated aqueous 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 in the Experimental Section and Figures S1−S4 in the 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 mobilities 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).

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 the 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 and Figure S5 in the Supporting Information). 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 ODNs **17** and

dx.doi.org/10.1021/jo401780u | J. Org. Chem. 2013, 78, 11271−11282

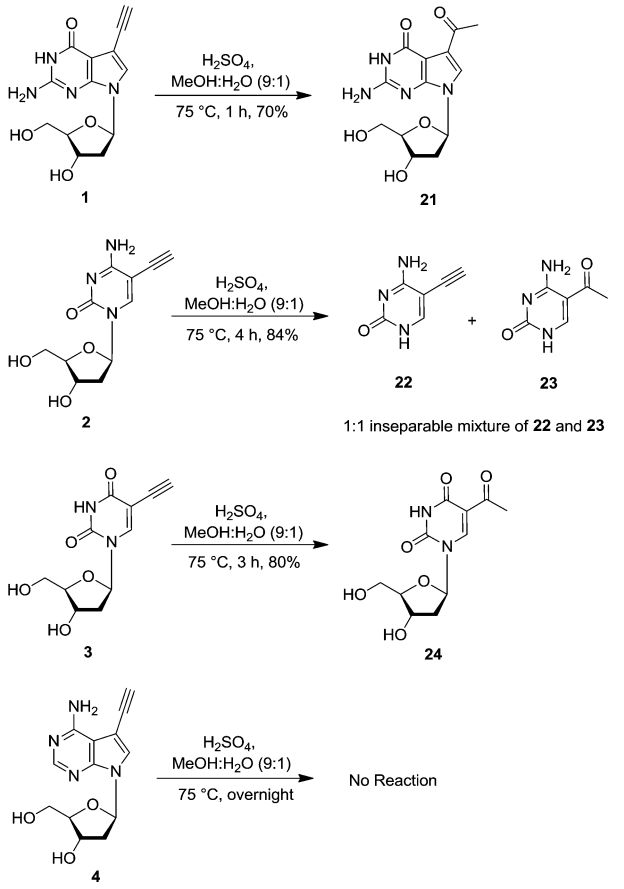
18. In the case of ODN-19, two side products were formed. Only the digest of oligonucleotide 20, containing 4, gave a clean pattern without showing side product formation, thereby confirming the mass spectrometric data.

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 in the Experimental Section; for UV spectra see Figure S6 in the 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 necessary to examine whether these reactions occurred during oligonucleotide synthesis (iodine oxidation in water and/or detritylation with trichloroacetic acid) and/or workup (concentrated aqueous ammonia treatment). Therefore, additional information on the behavior of monomeric nucleosides 1−3 under acidic and alkaline conditions was collected.

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 aqueous H_2SO_4 (7 days at room temperature), 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 MeOH/H_2O (9/1 mixture) containing H_2SO_4 was studied first (Scheme 3). 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 in the Experimental Section). For the conversion of the ethynylated 7-deaza-2′-deoxyguanosine (1), only a catalytic amount of acid (0.1 equiv of H_2SO_4 relative to alkyne) was required to yield the 7-acetyl nucleoside 21, while the conversion of the dU analogue 3 to the acetyl compound 24 required 1 equiv of H_2SO_4. In the 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. Therefore, the reactivity of the ethynyl side chains toward H_2SO_4 in MeOH/H_2O strongly depends on the nucleobase structure. The 7-deazaguanine nucleoside 1 is the most sensitive nucleoside toward water addition, while the ^{eth}dC 2 is the most sensitive nucleoside toward acid-catalyzed glycosylic bond hydrolysis. The 7-deaza-2′-deoxyadenosine analogue 4 was stable under these conditions.

Stability of Nucleosides 1−4 in Concentrated Aqueous Ammonia. Next, the effect of concentrated aqueous ammonia on the ethynylated nucleosides 1−4 was investigated

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



at elevated temperature (55 °C, 16 h). These conditions correspond to those used for oligonucleotide deprotection. First, the reactions were performed on an 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.

The ratio of the ethynyl nucleoside 2 and side product 25 (after 16 h treatment with concentrated aqueous 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 with that of side product 24 obtained from 3 by acid treatment (Scheme 4 and Figure S7 in the 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 concentrated aqueous 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 the 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]

dx.doi.org/10.1021/jo401780u | J. Org. Chem. 2013, 78, 11271−11282

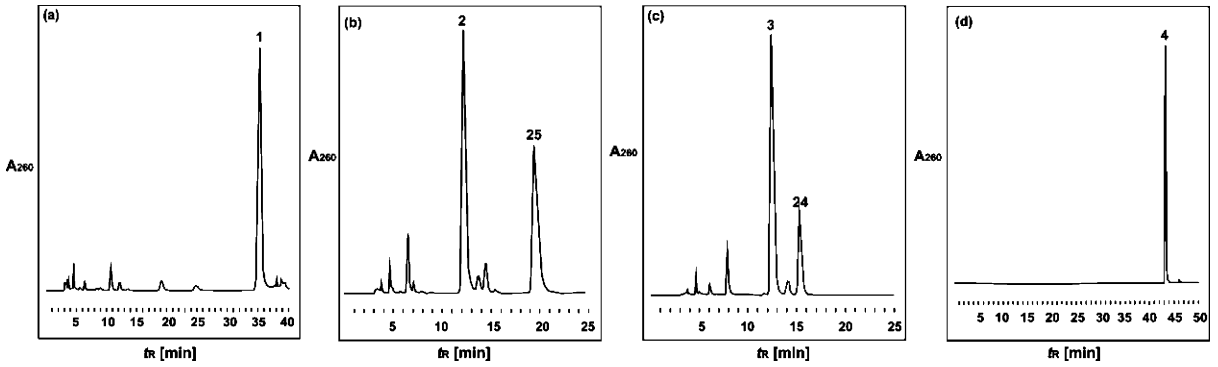
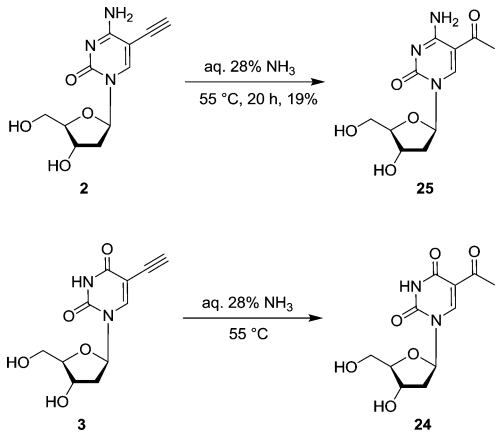


Figure 4. Reversed-phase HPLC elution profiles of the crude reaction mixtures of nucleosides **1**–**4** treated with concentrated aqueous ammonia at elevated temperature (55 °C for 16 h), monitored at 260 nm using gradient systems III and IV: (a) $^{eth}c^7G_d$ (**1**); (b) ^{eth}dC (**2**); (c) ^{eth}dU (**3**); (d) $^{eth}c^7A_d$ (**4**).

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



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 coinjected together with the ethynylated nucleosides **1**–**3** and the newly synthesized acetylated compounds **21**, **24**, and **25** (Figure 5). Figure 5a–c clearly indicates that the coinjected 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 workup 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 workup 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.

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, during oligonucleotide synthesis, under workup 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 phosphorus(III) to phosphorus(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

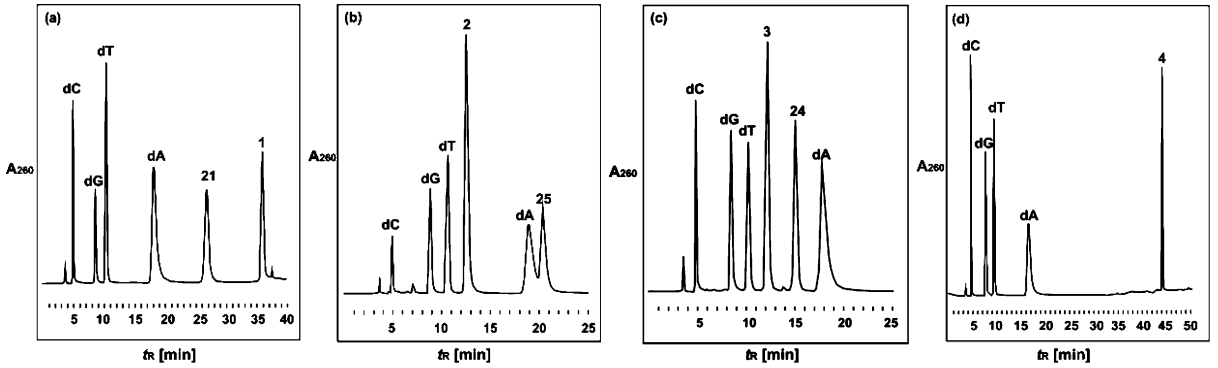
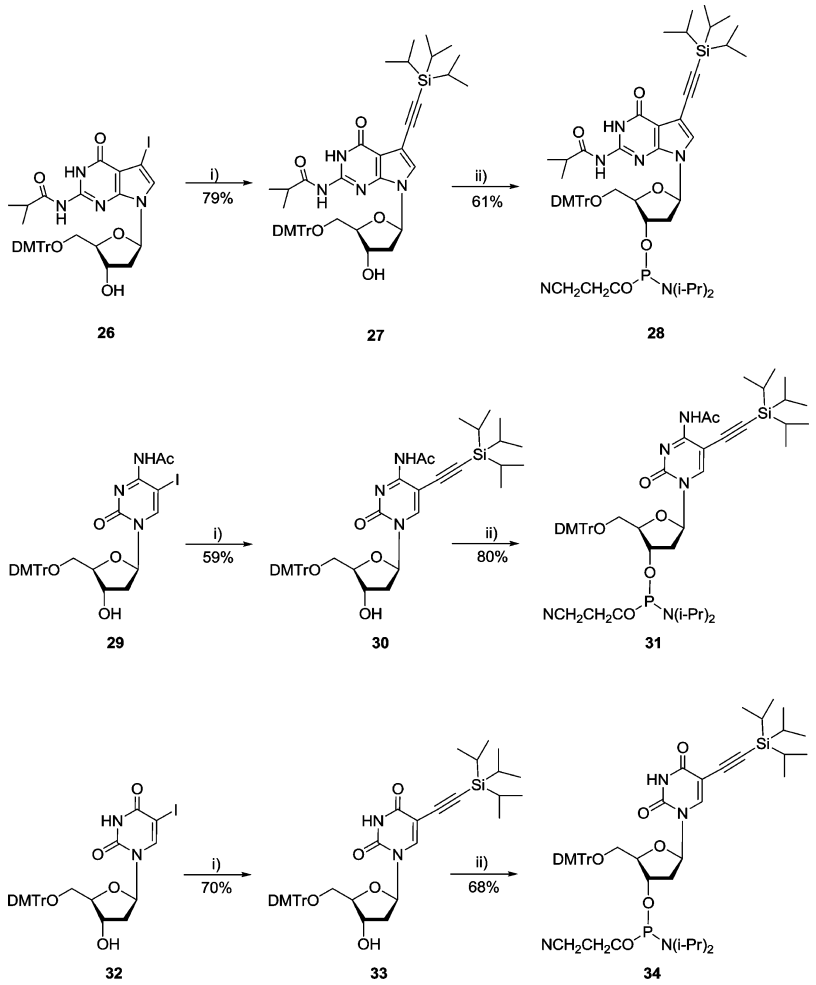


Figure 5. HPLC profiles of mixtures of the enzymatic hydrolysis products of oligonucleotides, ethynyl modified nucleosides, and acetyl modified nucleosides, monitored at 260 nm using gradient system III and IV: (a) ODN **17** + nucleosides **1** and **21**; (b) ODN **18** + nucleosides **2** and **25**;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

dx.doi.org/10.1021/jo401780u | J. Org. Chem. 2013, 78, 11271–11282

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, room temperature, 12 h; (ii) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $(i\text{-}Pr)_2NEt$, anhydrous CH_2Cl_2, room temperature.

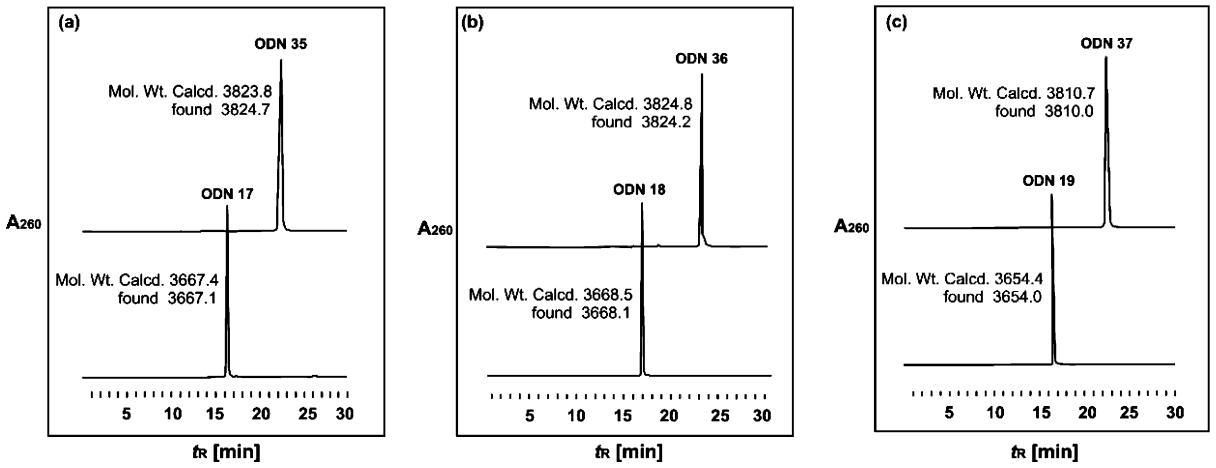
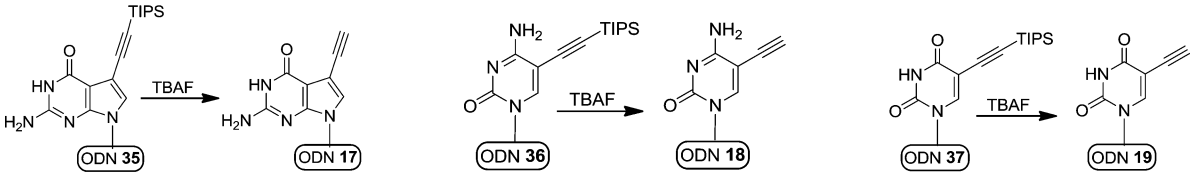


Figure 6. Reversed-phase HPLC elution profiles of purified ethynylated and silylated oligonucleotides, monitored at 260 nm using gradient system II: (a) ODN **17** and ODN **35**; (b) ODN **18** and ODN **36**; (c) ODN **19** and ODN **37**.

group hydration is aqueous 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 workup 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**

dx.doi.org/10.1021/jo401780u | J. Org. Chem. 2013, 78, 11271−11282

Scheme 6. Deprotection of the TIPS Group from Oligonucleotides 35−37



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. Triisopropylsilylethynyl nucleosides **27**, **30**, and **33** were obtained in 59−79% yield. Phosphitylation under standard conditions furnished the phosphoramidites **28**, **31**, and **34** (61−80% yield) (Scheme 5).

*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 and Figure S8 in the 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 aqueous 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 concentrated aqueous 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 the Experimental Section). Subsequently, deprotection of the oligonucleotides was performed with concentrated aqueous ammonia at room temperature, 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 isopropyl alcohol (for details see the 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.

The TIPS ethynyl oligonucleotides (**35**−**37**) can be easily distinguished from the ethynylated oligonucleotides (**17**−**19**) due to their different HPLC mobilities (Figure 6). The purity of ethynyl-modified oligonucleotides (prepared after deprotection of the TIPS group) was confirmed by enzymatic hydrolysis and MALDI-TOF spectra (Figures S9 and S10 in the 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 workup. 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 postsynthetic 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 aluminum 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. 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 (Table 1). UV spectra were recorded on a

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

	mol wt	
oligonucleotide	calcd	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 **1**^{TIPSE}AC CTA) (**35**)	3823.8	3824.7
5′-d(TAG GT**2**^{TIPSE} AAT ACT) (**36**)	3824.8	3824.2
5′-d(AGT AT**3**^{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**1**^{TIPSE} **1**^{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.

dx.doi.org/10.1021/jo401780u | J. Org. Chem. 2013, 78, 11271−11282

spectrophotometer: λ_{max} (ε) in nm, ε in dm^3 mol^{-1} cm^{-1} (Table 2). NMR spectra were measured at 300.15 MHz for ^{1}H, 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 ^{1}H−^{13}C gated-decoupled NMR spectra (Table 3; for coupling constants see Tables S1 and S2 in the Supporting Information). The J values are given in Hz; δ values are given 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.

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

compd	λ_{260} (nm)	λ_{max} (nm), ε (dm^3 mol^{-1}cm^{-1})
7-Ethynyl-c^{7}G$_d$ (**1**)	7400	270, 9000
7-Acetyl-c^{7}G$_d$ (**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^{7}A$_d$ (**4**)	7400	277, 12100

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

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, in M^{-1} cm^{-1}): dA, 15400; dG, 11700; dT, 8800; dC, 7300.

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 the following gradients: gradient III (ODNs **17** and **20**), 25 min 100% B, 25−60 min 0−40% A in B; gradient IV (ODNs **18** and **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).

Conversion of Ethynyl Nucleosides to Acetyl Nucleosides. Ethynyl-modified nucleosides (**1**−**4**, 5 mg each) were dissolved in 28% aqueous 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.

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

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	147.2	87.3			164.9	85.5, 75.8	−/−/−/−						
	154.5	153.0	102.1			163.6		−/−/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]Ref 21.

dx.doi.org/10.1021/jo401780u | J. Org. Chem. 2013, 78, 11271−11282

mixture was stirred under an inert atmosphere and the reaction 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 × 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 × CH_3), 2.03−2.10 (m, 1H, H$_\alpha$-2′), 2.27−2.29 (m, 1H, H$_\beta$-2′), 3.44−3.53 (m, 2H, 2 × 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 it was stirred for 12 h at room temperature, the suspension was filtered, adsorbed on silica gel, and applied to FC (silica gel, column 15 × 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$_\alpha$-2′), 2.27−2.36 (m, 1H, H$_\beta$-2′), 3.45−3.54 (m, 2H, 2 × 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 literature values reported earlier.[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 coevaporation with anhydrous pyridine (3 × 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 room temperature, and then isobutyric anhydride (1.362 g, 8.61 mmol) was added and the solution was stirred for an additional 3 h at room temperature. Then, the reaction mixture was cooled in an ice bath, H_2O (1.5 mL) and subsequently (5 min later) 28−30% aqueous NH_3 solution (1 mL) were added, and stirring was continued for 30 min at room temperature. The solvent was evaporated to near dryness and coevaporated with toluene (3 × 10 mL), and the residue was purified by FC (silica gel, column 15 × 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 × CH_3), 2.11−2.18 (m, 1H, H$_\alpha$-2′), 2.33−2.42 (m, 1H, H$_\beta$-2′), 2.69−2.78 (m, 1H, CH), 3.49−3.54 (m, 2H, 2 × 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 coevaporation with anhydrous pyridine (3 × 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% aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 × 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 × 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 × 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 × H-5′), 3.72 (s, 6H, 2 × 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) were added (*i*-Pr)$_2$NEt (0.049 g, 0.38 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (0.064 g, 0.27 mmol). After it was stirred for 45 min at room temperature, the solution was diluted with CH_2Cl_2 (30 mL) and extracted with 5% aqueous $NaHCO_3$ solution (20 mL); the combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by FC (silica gel, column 10 × 2 cm, CH_2Cl_2/acetone, 95/5), 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 × 4 cm, CH_2Cl_2/MeOH, 80/20). Evaporation of the solvent gave nucleoside **2** (2.65 g, 85%) as an 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$_\alpha$-2′), 2.12−2.19 (m, 1H, H$_\beta$-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 literature values reported earlier.[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 coevaporation with anhydrous pyridine (2 × 10 mL) before it was dissolved 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 5 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and extracted with 5% aqueous $NaHCO_3$ solution (100 mL), and the organic layer was dried over Na_2SO_4 and then concentrated. Purification by FC (silica gel, column 15 × 3 cm, CH_2Cl_2/acetone, 90/10) gave **11** as a colorless foam (4.36 g, 79%). TLC (CH_2Cl_2/MeOH, 90:10): R_f 0.5. UV: 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$_\alpha$-2′), 2.21−2.24 (m, 1H, H$_\beta$-2′), 3.12−3.25 (m, 2H, H-5′), 3.74 (s, 6H, 2 × 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 × Ar-H, 1 × 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 an additional 12 h (TLC monitoring). After evaporation of DMF under reduced pressure, the residue was applied to FC (silica gel, column 15 × 4 cm, CH_2Cl_2/acetone, 80/20). After evaporation of the solvent from the main zone, compound **12** was isolated as a colorless foam (3.38 g, 73%). TLC (CH_2Cl_2/acetone, 80/20): R_f 0.53. UV: 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,

dx.doi.org/10.1021/jo401780u | J. Org. Chem. 2013, 78, 11271−11282

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 × H-5′), 3.73 (s, 6H, 2 × 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-ethynylcytosine 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 $(i\text{-Pr})_2$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, and then the solution was diluted with CH_2Cl_2 (30 mL) and extracted with 5% aqueous $NaHCO_3$ solution (20 mL). The organic layer was dried over Na_2SO_4, concentrated, and purified by FC (silica gel, column 10 × 4 cm, CH_2Cl_2/acetone, 80/20). Evaporation of the solvent from the main zone gave **13** (0.850 g, 63%) as a 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) were 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 × 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 × 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). The procedure was as described for **21**, using compound **2**[12] (0.063 g, 0.25 mmol) in MeOH (9 mL) and water (1 mL) with H_2SO_4 (0.25 mmol). FC (silica gel, column 10 × 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 × NH_2), 7.74, 8.45 (2s, 2H, 2 × H-6), 11.33 (br s, 2H, 2 × NH). ESI-TOF: m/z calcd for **22** $C_6H_5N_3O$ $[M + Na]^+$ 158.0325, found 158.0331; m/z calcd for **23** $C_6H_7N_3O_2$ $[M + Na]^+$ 176.0430, found 176.0438. The obtained NMR data correspond to literature values reported earlier.[16,17]

5-Acetyl-1-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)uracil (24). The procedure was as described for **21**, using 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 × 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 literature values reported earlier.[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 × 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) were 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 an inert atmosphere overnight. The solvent was evaporated, and the residue was purified by FC (silica gel, column 15 × 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. UV: 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 × CH_3, 3 × 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 × H-5′), 3.69, 3.70 (2s, 6H, 2 × 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-(isobutyrylamino)-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) were added $(i\text{-Pr})_2$NEt (0.075 g, 0.58 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (0.099 g, 0.42 mmol). After it was stirred for 45 min at room temperature, the solution was diluted with CH_2Cl_2 (30 mL) and extracted with 5% aqueous $NaHCO_3$ solution (20 mL); the combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by FC (silica gel, column 10 × 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 × 4 cm, CH_2Cl_2/acetone, 80/20). After evaporation of the solvent from the main zone, compound **29** was isolated as a 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 × 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) were added CuI (0.031 g, 0.16 mmol), $[Pd(PPh_3)_4]$ (0.139 g, 0.12 mmol), anhydrous Et_3N (0.3 mL, 2.16 mmol), and triisopropylsilylacetylene (448 μL, 2.0 mmol). 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 × 4 cm, CH_2Cl_2/acetone, 90/10). Evaporation of the solvent from the main zone gave **30** (0.354 g, 59%) as a 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, 6 × CH_3, 3 × 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 × 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*-Pr)$_2$EtN (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 room temperature. Then the solution was diluted with CH_2Cl_2 (30 mL) and extracted with 5% aqueous $NaHCO_3$ solution (20 mL). The organic layer was dried over Na_2SO_4 and concentrated. FC (silica gel, column 10 × 4 cm, CH_2Cl_2/acetone, 90/10) and evaporation of the solvent from the main zone gave **31** (0.374 g, 80%) as a 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) were added 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). 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 × 4 cm, CH_2Cl_2/acetone, 90/10). Evaporation of the solvent from the main zone gave **33** (0.380 g, 70%) as a white foam. TLC (CH_2Cl_2/acetone, 85/15): R_f 0.35. UV: λ_{max} (MeOH)/nm 235 (ε/dm^3 mol^{-1}cm^{-1} 27110), 277 (9316), 287 (11000), 295 (11280). ^{1}H NMR (DMSO-d_6, 300 MHz): 0.69−1.28 (m, 21H, 8 × CH_3, 3 × CH), 2.16−2.21 (m, 2H, H-2′), 3.11−3.18 (m, 2H, H-5′), 3.71 (s, 6H, 2 × 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, and then the solution was diluted with CH_2Cl_2 (50 mL) and extracted with 5% aqueous $NaHCO_3$ solution (30 mL). The organic layer was dried over Na_2SO_4 and concentrated. FC (silica gel, column 10 × 4 cm, CH_2Cl_2/acetone, 90/10) and evaporation of the solvent from the main zone gave **34** (0.131 g, 68%) as a 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 incubating 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.

■ ASSOCIATED CONTENT

Supporting Information

Figures and tables giving ^{1}H−^{13}C coupling constants, HPLC profiles, and ^{1}H, ^{13}C, 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.

■ AUTHOR INFORMATION

Corresponding Author

*F.S.: tel, +49 (0) 251 53406 500; fax, +49 (0) 251 53406 857; e-mail, Frank.Seela@uni-osnabrueck.de; web, www.seela.net.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. S. Budow for helpful discussions and support while preparing the paper and Prof. Dr. Helmut Quast for critical reading of the paper. We also thank Mr. S. S. Pujari for measuring the NMR spectra and Mr. Nhat Quang Tran for the oligonucleotide syntheses. We thank Dr. M. Letzel, Organisch-Chemisches Institut, Universität Münster, Münster, Germany, for the measurement of the MALDI spectra. Financial support by ChemBiotech, Münster, Germany, is highly appreciated.

■ 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. U.S.A.* **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.