JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Parallel Stranded DNA Stabilized with Internal Sugar Cross-Links: Synthesis and Click Ligation of Oligonucleotides Containing 2′-Propargylated Isoguanosine

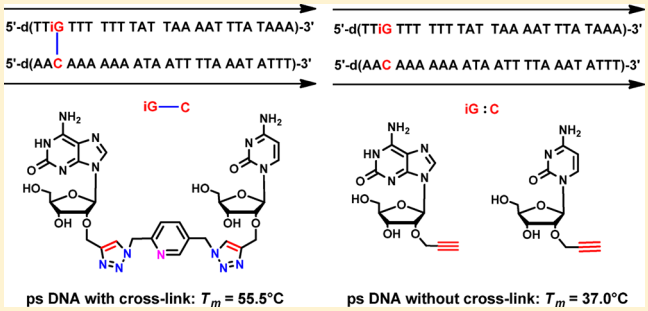
Suresh S. Pujari and Frank Seela*

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: Internal sugar cross-links were introduced for the first time into oligonucleotides with parallel chain orientation by click ligation. For this, the 2′- or 3′-position of the isoguanosine ribose moiety was functionalized with clickable propargyl residues, and the synthesis of propargylated cytosine building blocks was significantly improved. Phosphoramidites were prepared and employed in solid-phase synthesis. A series of oligo-2′-deoxyribonucleotides with parallel (ps) and antiparallel (aps) strand orientation were constructed containing isoguanine-cytosine, isoguanine-isocytosine, and adenine-thymine base pairs. Complementary oligonucleotides with propargylated sugar residues were ligated in a stepwise manner with a chelating bis-azide under copper catalysis. Cross-links were introduced within a base pair or in positions separated by two base pairs. From T_m stability studies it is evident that cross-linking stabilizes DNA with parallel strand orientation strongly (ΔT_m from +16 to +18.5 °C) with a similar increase as for aps DNA.



INTRODUCTION

The polymorphic nature of DNA[1] offers numerous opportunities to construct DNA-based nanostructures that find widespread applications from material science to DNA diagnostics and medicine.[2] As canonical duplex DNA is formed by Watson−Crick base pairing, it exhibits chains with antiparallel strand (aps) orientation. A new family of parallel stranded (ps) duplex DNA was realized by changing the Watson−Crick motif to the reverse mode and/or by replacing dG by 2′-deoxyisoguanosine ($isoG_d$) and dC by 2′-deoxy-5-methylisocytidine ($isoC_d$) (Figure 1).[3] Such parallel hybridization offers new opportunities for designing new oligonucleotide hybridization probes, antisense constructs, or nanodevices.[4] Advantages of the ps hybridization over naturally occurring aps hybrids include, e.g., the higher resistance of these molecules against nucleolytic enzymes or the application of the alternative hybridization system for diagnostic or nanoscopic purposes.[5] Despite having such favorable properties, dA-dT rich ps DNA is less stable than corresponding aps DNA.[6] However, stability of ps DNA was increased by the introduction of $isoG_d$-dC or $isoC_d$-dG base pairs.[3]

Different approaches for cross-linking of DNA have been reported in literature.[7] Recently, we developed and reported on the Cu(I) catalyzed azide−alkyne[8] "bis-click" reaction as an effective tool for cross-linking nucleosides or oligonucleotides and to construct macromolecular assemblies.[9] As an extension, to investigate its viability as well as to diversify the relevance of the developed bis-click protocol, the bis-click reaction was performed with sugar-modified DNA as well.[10] Further, a "stepwise click" protocol was employed using a large excess of bis-azide (alkyne/bis-azide, 1:15). To circumvent the use of excess azide, we recently reported on a chemoselective click reaction,[11] where equimolar amounts of bis-azide can be used

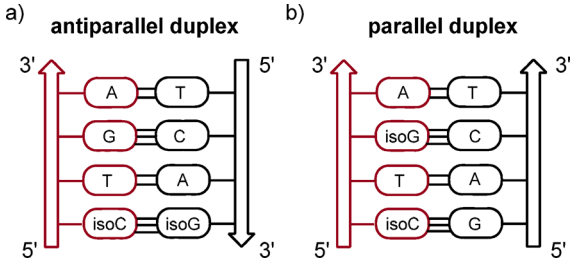


Figure 1. Schematic presentation of duplex DNA with (a) antiparallel strand orientation incorporating Watson−Crick dA-dT or $isoC_d$-$isoG_d$ base pairs and (b) parallel chain orientation with $isoG_d$-dC, $isoC_d$-dG, or dA-dT Donohue base pairs.

Received: June 12, 2013
Published: August 5, 2013

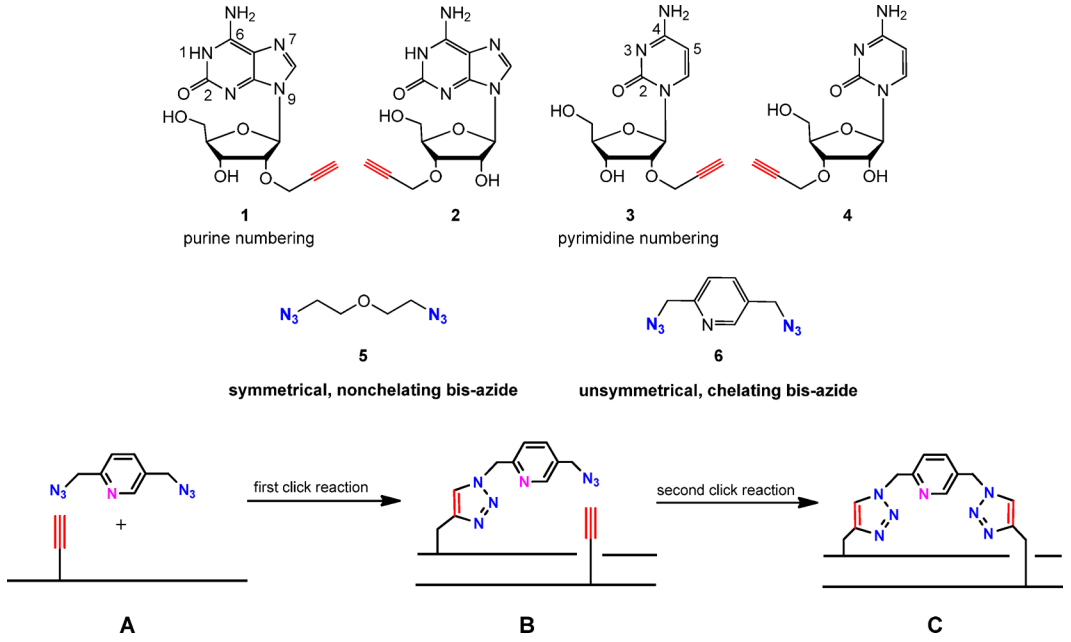
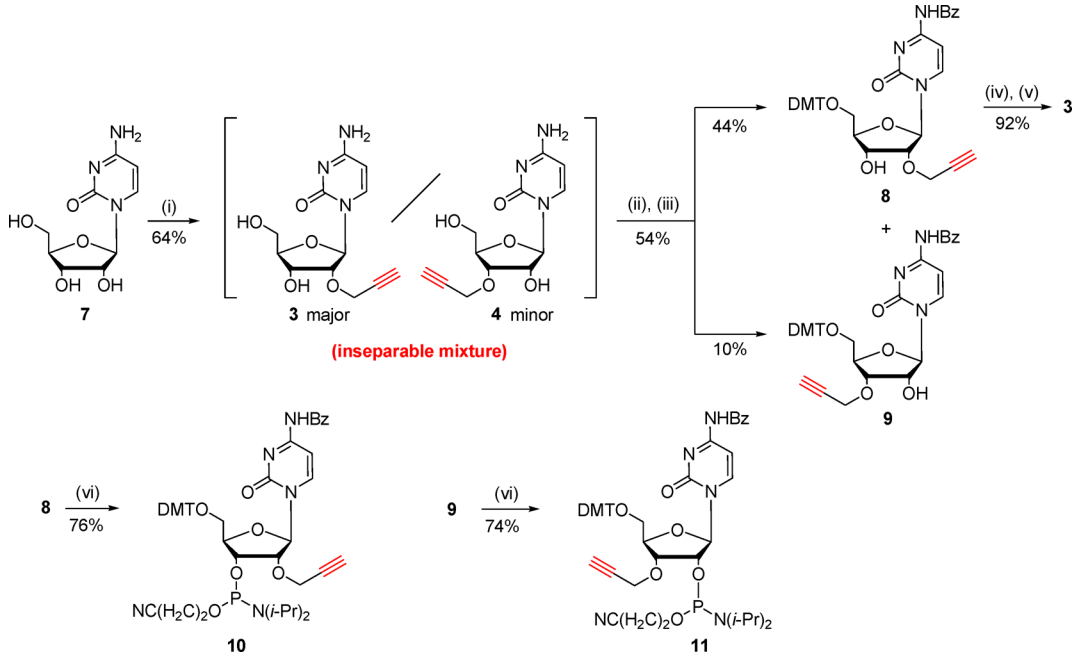


Figure 2. (Upper) Sugar-modified nucleobases and bifunctional azides used for cross-linking. (Lower) Schematic illustration (A–C) of the stepwise click reaction using bis-azide **6**.

Scheme 1. Synthesis of 2′- and 3′-*O*-Propargyl Derivatives of Cytidine[a]



[a]Reagents and conditions: (i) NaH, TBAI, DMF, propargyl bromide, 55 °C, 72 h; (ii) DMT-Cl, DMAP, pyridine, 3 h, rt; (iii) BzCl, TMSCl, pyridine, rt, 3 h; (iv) 28% aq NH_3, 60 °C, overnight; (v) AcOH, 1 h, rt; (vi) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, DIPEA, DCM, 1 h, rt.

to construct monofunctionalized oligonucleotides; though this protocol is limited to only chelating bis-azides.[11e] For illustration see Figure 2 (A–C).

Cross-links obtained by click chemistry have a favorable stabilizing effect on aps duplexes.[9−12] As DNA with parallel strand orientation shows a different helix structure than aps DNA, it was of utmost interest to perform cross-linking on ps DNA and to study the impact on ps duplex stability. Herein, we studied this phenomenon on 2′-*O*-propargylated DNA. In this regard, the new building blocks **1** and **2** were synthesized, while the synthesis of known building blocks **3** and **4**[13] was significantly improved (Figure 2). These compounds were further employed in oligonucleotide solid-phase synthesis, and the oligonucleotides were used for the construction of ps and aps DNA. Different bifunctional azides (**5**[14] and **6**;[15] Figure 2) and cross-linking protocols were verified on antiparallel stranded duplexes. The most promising protocol, cross-linking with chelating bis-azide **6**, was used to implement cross-links in duplexes with parallel chain orientation. The stability of the sugar cross-linked and noncross-linked ps duplexes was

dx.doi.org/10.1021/jo4012706 | J. Org. Chem. 2013, 78, 8545−8561

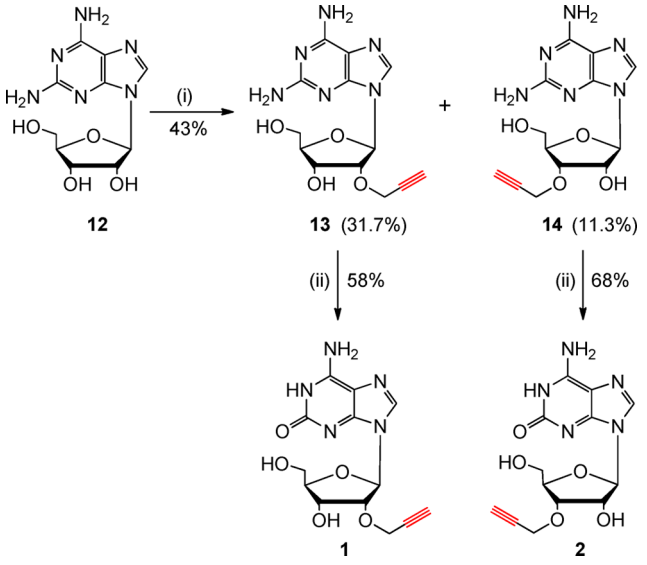
compared, and the influence of cross-links at various positions was studied.

RESULTS AND DISCUSSION

1. Synthesis of Monomers. Current methods describing the synthesis of propargylated nucleosides are often laborious and low-yielding.[13,16] Herein, we present an efficient synthetic protocol for the propargylation of the earlier described cytidine nucleosides **3** and **4** and apply this method to the preparation of the newly synthesized isoguanosine derivatives **1** and **2**. The propargylation of cytidine was performed in DMF using NaH and TBAI for 72 h at 55 °C giving a mixture of the 2′-*O*- and 3′-*O*-propargylated regioisomers **3** and **4** in 64% overall yield.[16b] From the ^{1}H NMR spectrum of the crude reaction mixture an isomeric ratio for the 2′-*O*- (**3**) to 3′-*O*-isomer (**4**) of 4.5:1 was detected (based on the integration of the C≡CH signal; Figure S6, Supporting Information). Thus, formation of the 2′-*O*-isomer (**3**) is favored. As the separation of these two regioisomers was difficult on that stage, the isomeric mixture was treated successively with DMT-chloride and benzoyl chloride,[17] yielding the protected compounds **8** and **9** (54% combined yield), which could be easily separated (Scheme 1). Compound **8** was deprotected with 28% aq NH_3 at 60 °C overnight and successively treated with acetic acid to give the propargylated nucleoside **3** in 92% yield. Phosphitylation of **8** and **9** gave the building blocks **10** and **11** in 76% and 74% yield, respectively (Scheme 1).

As described in literature, isoguanosine derivatives can be obtained from 2-aminoadenosine (**12**) by selective deamination of the 2-amino group.[18] Therefore, compound **12** was chosen as precursor for the synthesis of the propargylated isoguanosine derivatives **1** and **2**. Treatment of **12** with NaH and TBAI in DMF at 55 °C resulted in the formation of the regioisomers **13** and **14** in moderate overall yield (43%) due to the incomplete conversion of the starting material, which was recovered in 25% (Scheme 2). Different from the propargylated cytidine derivatives, the 2′-*O* and 3′-*O*-propargylated compounds **13** and **14** could be easily separated. From the ^{1}H NMR spectrum of the crude reaction mixture an isomeric ratio of 3.2:1, **13** to **14**, was determined (integration of the C≡CH signal; Figure S23, Supporting Information). Moreover, a 6−10 ppm downfield shift is observed in the ^{13}C NMR spectrum upon sugar propargylation at the particular carbon. In general, we can use this observation for determining the sugar propargylation sites.

Scheme 2. Synthesis of 2′- and 3′-*O*-Propargyl Isoguanosine Derivatives 1 and 2[a]



[a]Reagents and conditions: (i) NaH, TBAI, DMF, propargyl bromide, 55 °C, 48 h, 25% recovery of **12**; (ii) $NaNO_2$, AcOH, 50 °C, 20 min.

Next, compounds **13** and **14** were subjected to deamination with $NaNO_2$ in acetic acid (50 °C, 20 min) to give nucleosides **1** and **2** in 58% and 68% yield, respectively.[18] After protection of the amino group of **1** and **2** using *N*,*N*-dimethylacetamide dimethylacetal in MeOH at room temperature for 4 h, compounds **15** (79%) and **16** (89%) were obtained (Scheme 3). The 2-hydroxy group of compounds **15** and **16** was protected with *N*,*N*-diphenylcarbamoyl chloride in pyridine for 40 min at room temperature to afford compounds **17** (82%) and **18** (68%). Protection of the 5′-OH groups by DMT chloride under standard reaction conditions gave **19** and **20** (70% and 74%).[19] Subsequently, phosphitylation furnished the phosphoramidite building block **21** in good yield (84%) and the 3′-*O*-propargyl derivative **22** in moderate yield (37%) (Scheme 3).

All synthesized compounds were characterized by ^{1}H NMR and ^{13}C NMR spectra as well as elemental analysis. DEPT-135 and ^{1}H−^{13}C gated-decoupled NMR spectra were used to assign the ^{13}C NMR signals. For details see Experimental Section (Tables 3 and 4; for spectra see Supporting Information).

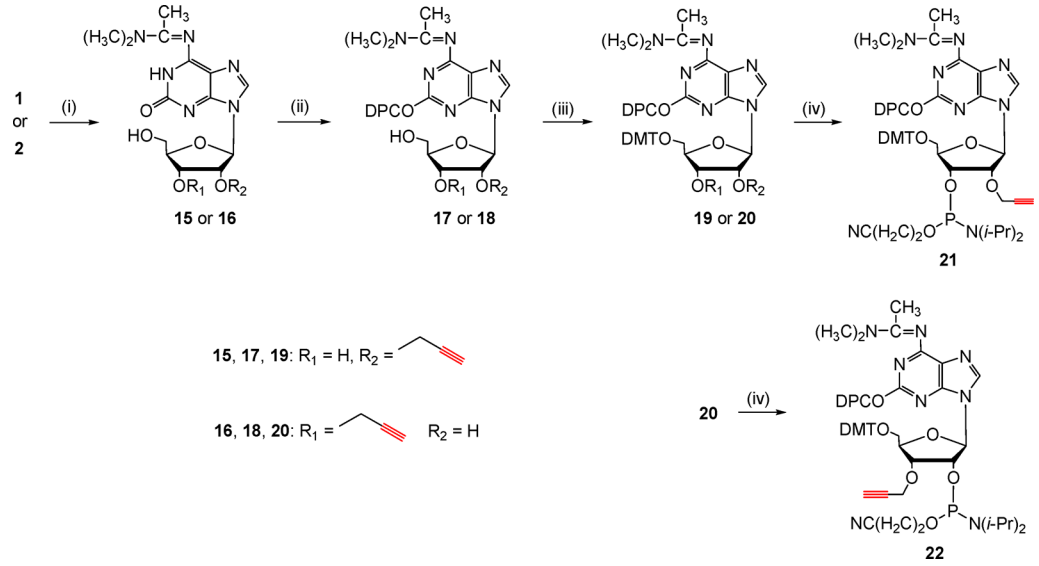
2. Synthesis and Cross-linking of Oligonucleotides. *2.1. Oligonucleotide Synthesis.* As in this study we wanted to focus on oligonucleotides with the native 3′-5′-phosphodiester linkage, we solely subjected 2′-*O*-propargylated phosphoramidite building blocks **10** and **21** to solid-phase oligonucleotide synthesis. Thus, a series of 12-mer and 25-mer oligonucleotides containing the propargylated nucleoside residues **1** and **3** at various positions were designed, which are able to form duplexes with either parallel or antiparallel chain orientation. The oligonucleotides were prepared on solid phase using the standard protocol of phosphoramidite chemistry. The coupling yields of the modified building blocks were always higher than 98%. The synthesized oligonucleotides were characterized by MALDI-TOF mass spectra (Tables 1 and 5).

2.2. Cross-Linking of 2′-O-Propargylated Oligonucleotides to Homodimers with Nonchelating and Chelating Bis-Azides. Two procedures for the "click" cross-linking of propargylated oligonucleotides were studied. The first method utilizes symmetrical nonchelating bis-azide **5**,[14] and the second makes use of the unsymmetrical bis-azide **6**[15] containing a chelating and a nonchelating azido group. Both procedures were performed in a stepwise manner ("stepwise click") with monofunctionalized azido compounds as intermediates.

First, the reaction was performed with ODN-**27** and bis-azide **5**. For the synthesis of monofunctionalized ODN-**33** bearing one azido group, a large excess of azide **5** (ODN-**27** to azide **5**, 1:15) was used (Scheme 4 and Experimental Section).[9] In a similar way, monofunctionalized ODNs 5′-d(TA**1*** GTC AAT ACT)-3′ (**32**), 5′-d(TAG GTC AAT A**3***T)-3′ (**34**), and 5′-d(AGT ATT GA**3*** CTA)-3′ (**35**) were synthesized (for detailed formulas see Figure S1, Supporting Information). A second click reaction was carried out next with the monofunctionalized ODN-**33** and propargylated ODN-**27** to afford the cross-linked homodimer ODN-**37**. For comparison, a number of propargylated oligonucleotides (**25**, **27**, **29**, **31**)

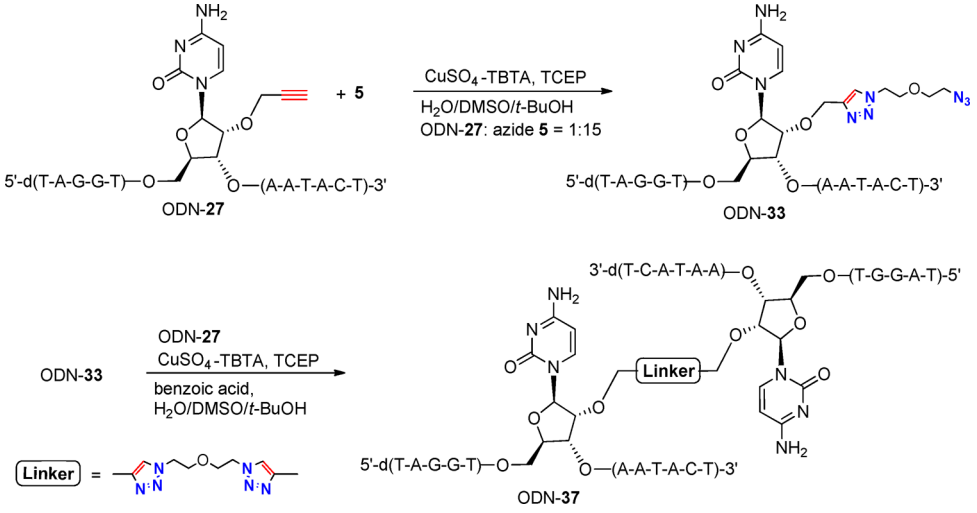
dx.doi.org/10.1021/jo4012706 | J. Org. Chem. 2013, 78, 8545−8561

Scheme 3. Synthesis of 2′- and 3′-*O*-Propargylated Isoguanosine Building Blocks[a]

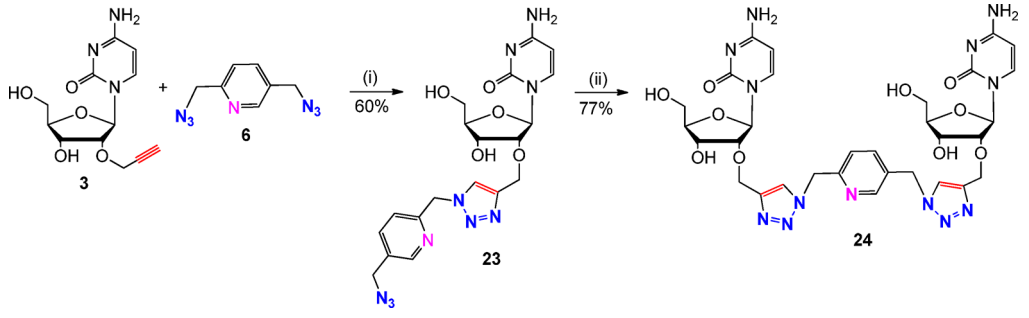


[a]Reagents and conditions: (i) *N*,*N*-dimethylacetamide dimethylacetal, MeOH, 4 h, rt; 79% for **15**; 89% for **16**. (ii) *N*,*N*-diphenylcarbamoyl chloride, pyridine, 30 min, rt; 82% for **17**; 68% **18**. (iii) DMT-Cl, pyridine, 90 min, rt; 70% for **19**; 74% for **20**. (iv) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, DIPEA, DCM, 2 h, rt; 84% for **21**; 37% for **22**.

Scheme 4. Stepwise Click Reaction of Propargylated DNA with Symmetrical Nonchelating Bis-azide 5



Scheme 5. Chelate-Assisted Chemoselective Click Reaction on Sugar Modified Nucleoside 3[a]

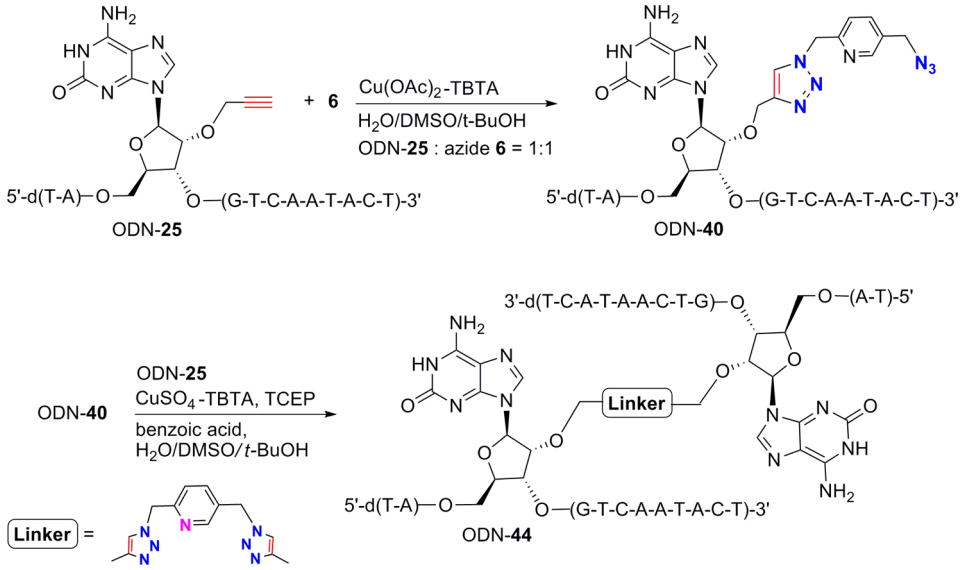


[a]Reagents and conditions: (i) $Cu(OAc)_2{\cdot}H_2O$, $MeOH/CH_2Cl_2$ (1:1, v/v), 8 h, rt; (ii) $Cu(OAc)_2{\cdot}H_2O$, sodium ascorbate, **3**, $MeOH/CH_2Cl_2$ (1:1, v/v), overnight, rt.

were cross-linked by the bis-click procedure with equimolar amounts of the oligonucleotide and bis-azide **5** (for details see Experimental Section). By this method, a number of interstrand cross-linked homodimeric oligonucleotides (ODNs **36**–**39**)

dx.doi.org/10.1021/jo4012706 | J. Org. Chem. 2013, 78, 8545–8561

Scheme 6. Stepwise and Second Click Reaction of Sugar-Modified DNA with Unsymmetrical Chelating Bis-azide 6



were constructed (Scheme 4 and Table 1). These ODNs are identical to the cross-linked oligonucleotide adducts obtained by the stepwise click procedure.

Next, the chemoselective stepwise click functionalization using chelating azide **6** was investigated on propargylated oligonucleotides. As the procedure consists of a stepwise protocol, formation of homodimers is suppressed. The chelating effect of the azide leads to the exclusive formation of monofunctionalized click adducts still carrying an azido functionality without using an excess of azide (oligonucleotide to azide ratio, 1:1).[11]

To prove the efficacy of the chemoselective click protocol, the reaction was initially studied on the propargylated nucleoside **3** using the bis-azide **6**. The first click was carried out in the presence of Cu(II)acetate in MeOH/CH_2Cl_2 (1:1) at room temperature without reducing agent, giving the monofunctionalized nucleoside **23** in 60% yield. Formation of a bifunctionalized click product was not observed. In the second step, compound **23** was used as azide precursor and was reacted with a second molecule of **3** in the presence of $Cu(OAc)_2{\cdot}H_2O$ and sodium ascorbate as a reducing agent to afford the cross-linked compound **24** in 77% yield (Scheme 5). Conclusively, we were able to show that the chemoselective click reaction can be carried out in good yield for sugar-modified nucleosides. Consequently, the chemoselective stepwise click reaction was utilized to cross-link propargylated oligonucleotides containing nucleosides **1** or **3** (Table 1).

The stepwise chemoselective click reaction was applied to propargylated ODN-**25**, which was reacted with bis-azide **6** in the presence of TBTA-$Cu(OAc)_2{\cdot}H_2O$ complex with $NaHCO_3$ as additive in H_2O/DMSO/*t*-BuOH (4:3:1) at room temperature (Scheme 6). After completion of the reaction (**8** h), the monofunctionalized ODN-**40** still carrying one azido group was obtained in good yield. Owing to this promising result, other monofunctionalized oligonucleotides, namely, 5′-d(TAG GT**23** AAT ACT)-3′ (**41**), 5′-d(TAG GTC AAT A**23**T)-3′ (**42**), and 5′-d(AGT ATT GA**23** CTA)-3′ (**43**), were synthesized in a similar way.

Next, monofunctionalized ODN-**40** still bearing a non-chelating azido group was used as a substrate for the second click reaction. For that, ODN-**25** was treated with a $CuSO_4$-TBTA complex. Then, the monofunctionalized ODN-**40** was added, and the reaction proceeded in the presence of TCEP and benzoic acid in H_2O/DMSO/*t*-BuOH at room temperature for 1 h (Scheme 6) to afford click adduct ODN-**44**. Accordingly, cross-linked ODNs **45**−**47** were synthesized in good yields (Table 1). The purity and molecular masses of the monofunctionlized and cross-linked oligonucleotides were approved by RP-18 HPLC and MALDI-TOF mass spectrometry (Tables 1 and 5).

Encouraged by the promising results of the stepwise chelate-assisted click reaction for oligonucleotides forming aps duplexes, we consequently used this protocol for the first time for the cross-linking of 2′-*O*-propargylated oligonucleotides, forming ps duplexes (see next section).

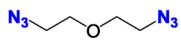
Earlier studies on sugar cross-linked homodimers hybridized with complementary oligonucleotides, thereby forming ligated duplexes, showed that cross-linking at the center of the duplex is slightly destabilizing, while for cross-linking at the termini of the helices stabilization was observed.[10] A similar trend is also found for the aps homodimeric duplexes presented in Table 1 with ΔT_m ranging from −6 °C to 2 °C compared to the noncross-linked duplexes.

2.3. Cross-Linking of Propargylated Oligonucleotides to Heterodimers with Parallel Chain Orientation. General Aspects. Parallel stranded duplex DNA was proposed by Pattabiraman already in 1986[20] and represents a unique DNA structure with both sugar-phosphate chains pointing to the same direction.[21] A series of manuscripts were published by our laboratory and by others reporting on the chemical synthesis and physical properties of ps DNA.[22,23] These studies demonstrated that the structural features and spectroscopic properties, enzymatic recognition as well as drug-binding properties of ps DNA are different from native DNA with antiparallel chains.[4d,24] However, up to now it is unknown whether the concept of cross-linking *via* sugar-modified oligonucleotides using click chemistry can be realized for parallel stranded duplexes in a similar way as observed for aps DNA despite their structural differences (grooves of almost identical size).

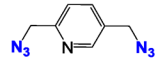
2.4. Synthesis of Heterodimeric Oligonucleotides with Parallel and Antiparallel Chain Orientation. For the

dx.doi.org/10.1021/jo4012706 | J. Org. Chem. 2013, 78, 8545−8561

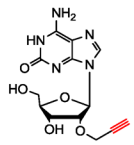
Table 1. T_m Values and MALDI-TOF Masses of Sugar Propargylated Duplexes and Cross-Linked Duplexes (Homodimers)[a]

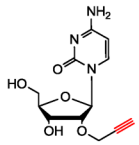


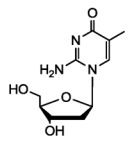
Duplex	M.W. calcd [b] M.W. found	T_m [°C]	Homo-cross-linked ODNs	M.W. calcd M.W. found	Duplex	T_m [°C]
5'-d(TA**1** GTC AAT ACT) (**25**) 3'-d(AT**iC** CAG TTA TGA) (**26**)	3698.4 3697.5	53	5'-d(TA**1** GTC AAT ACT) | (**36**) 5'-d(TA**1** GTC AAT ACT)	7553.1 7552.5	**26•36•26**	49
5'-d(TAG GT**3** AAT ACT) (**27**) 3'-d(ATC CAG TTATGA) (**28**)	3698.4 3697.3	46	5'-d(TAG GT**3** AAT ACT) | (**37**) 5'-d(TAG GT**3** AAT ACT)	7553.1 7553.6	**28•37•28**	41
5'-d(TAG GTC AAT A**3**T) (**29**) 3'-d(ATC CAG TTA TGA) (**28**)	3698.4 3697.4	50	5'-d(TAG GTC AAT A**3**T) | (**38**) 5'-d(TAG GTC AAT A**3**T)	7553.1 7554.4	**28•38•28**	52
5'-d(TAG GTC AAT ACT) (**30**) 3'-d(ATC **3**AG TTA TGA) (**31**)	3698.4 3697.1	49	3'-d(ATC **3**AG TTA TGA) | (**39**) 3'-d(ATC **3**AG TTA TGA)	7553.1 7553.7	**30•39•30**	44

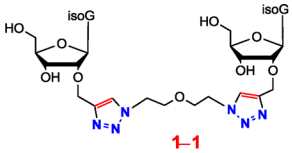


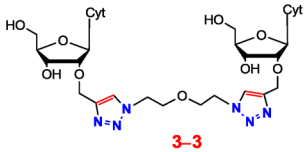
Duplex	M.W. calcd [b] M.W. found	T_m [°C]	Homo-cross-linked ODNs	M.W. calcd M.W. found	Duplex	T_m [°C]
5'-d(TA**1** GTC AAT ACT) (**25**) 3'-d(AT**iC** CAG TTA TGA) (**26**)	3698.4 3697.5	53	5'-d(TA**1** GTC AAT ACT) | (**44**) 5'-d(TA**1** GTC AAT ACT)	7586.1 7584.4	**26•44•26**	47
5'-d(TAG GT**3** AAT ACT) (**27**) 3'-d(ATC CAG TTATGA) (**28**)	3698.4 3697.3	46	5'-d(TAG GT**3** AAT ACT) | (**45**) 5'-d(TAG GT**3** AAT ACT)	7586.1 7585.1	**28•45•28**	41
5'-d(TAG GTC AAT A**3**T) (**29**) 3'-d(ATC CAG TTA TGA) (**28**)	3698.4 3697.4	50	5'-d(TAG GTC AA**3** ACT) | (**46**) 5'-d(TAG GTC AA**3** ACT)	7586.1 7585.4	**28•46•28**	51
5'-d(TAG GTC AAT ACT) (**30**) 3'-d(ATC **3**AG TTA TGA) (**31**)	3698.4 3697.1	49	3'-d(ATC **3**AG TTA TGA) | (**47**) 3'-d(ATC **3**AG TTA TGA)	7586.1 7585.2	**30•47•30**	48

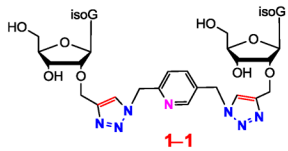


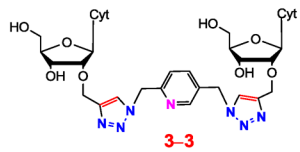












[a]Measured at 260 nm in a 1 M NaCl solution containing 100 mM $MgCl_2$ and 60 mM Na-cacodylate (pH 7.0) with 2.5 μM single-strand concentration of each strand in noncross-linked duplexes and 5 μM of cross-linked duplexes. The T_m values were determined from the melting curves using the software MELTWIN, version 3.0. [b]The molecular masses refer to the propargylated oligonucleotides. ODNs **36**–**39** were prepared by both stepwise and bis-click reaction using nonchelating azide **5**. ODNs **44**–**47** were prepared by stepwise click reactions using chelating bisazide **6**.

construction of cross-linked ps duplexes, oligonucleotides containing dA-dT sequence elements and 2′-*O*-propargyl modifications were used (**1**, **3**, **A***; for the structure of **A*** see Table 2), which are able to form duplexes with either parallel or antiparallel chain orientation (Table 2).

The stepwise chelate-assisted click reaction protocol was now applied to ODN-**50** (Table 2) containing 2′-*O*-propargylated

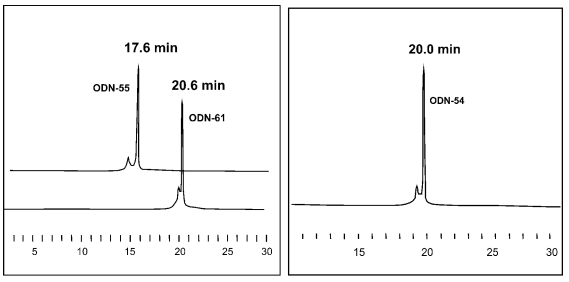
dx.doi.org/10.1021/jo4012706 | J. Org. Chem. 2013, 78, 8545–8561

isoguanosine (**1**) as described in Section 2.2. In the chelate-assisted first click reaction, ODN-**50** was reacted with bis-azide **6** in the presence of TBTA-$Cu(OAc)_2 \cdot H_2O$ complex at room temperature. After completion of the reaction (8 h), the monofunctionalized ODN-**55** was obtained in good yield. Accordingly, the monofunctionalized ODNs 3′-d(TT**1**^{az} TTT TTT TAT TAA AAT TTA TAA A)-5′ (ODN-**56**), 5′-d(AA**23** AAA AAA ATA ATT TTA AAT ATT T)-3′ (ODN-**57**), 5′-d(**A**^{az}AA AAA AAA ATA ATT TTA AAT ATT T)-3′ (ODN-**58**), and 5′-d(**A**^{az}AC AAA AAA ATA ATT TTA AAT ATT T)-3′ (ODN-**59**) were synthesized (for the structure of **A**^{az} see Table 2). For the second click reaction, monofunctionalized ODN-**55** still carrying the nonchelating azido group was reacted with complementary ODN-**60** ($CuSO_4$-TBTA complex, TCEP, benzoic acid, H_2O/DMSO/*t*-BuOH) to give the cross-linked ps duplex ODN-**61** (Table 2). It should be noted that by this protocol we were able to construct a sugar cross-link within the parallel base pair of 2′-*O*-propargylated isoguanosine (**1**) and cytidine (**3**) as a component of a duplex with parallel strand orientation. Similarly, other heterodimeric DNA constructs with ps strand orientation and internal sugar cross-links were synthesized (ODN-**52** and ODN-**54**). However, contrary to ODN-**61**, the 2′-*O*-propargyl modification of ODN-**52** and ODN-**54** was not part of a base pair. Instead, the cross-link was constructed between sugar residues separated by one dA-dT base pair (Table 2). For comparison, the reactions described above were also performed on ODNs with aps orientation. All monofunctionalized and cross-linked oligonucleotides were characterized by MALDI-TOF mass spectrometry (Table 5), ion-exchange HPLC, or gel electrophoresis (Figure 4) confirming the integrity of the DNA adducts.

The results clearly indicate that the chemoselective ligation protocol using bis-azide **6** can be used to construct heterodimeric DNA adducts with internal sugar cross-links at proximal (base pairs) and distant positions. We anticipate that the high local concentration of the duplex facilitates the reaction sites situated near the flexible end of the duplex to come together, leading to efficient cross-linking. This protocol can be applied as efficiently to duplexes with ps orientation as well as to duplexes with aps orientation, despite any differences in the helical structure of ps and aps DNA.

3. Characterization of Cross-Linked Oligonucleotide Adducts. Ion-exchange HPLC and denaturing gel electrophoresis (PAGE) were used to analyze monofunctionalized click adducts as well as heterodimeric DNA constructs with parallel strand orientation. Figure 3a shows the ion-exchange chromatography profile of the monofunctionalized 25-mer ODN-**55** with a retention time of 17.6 min. On the contrary, the heterodimeric ps adduct ODN-**61** obtained after cross-linking of monofunctionalized ODN-**55** and propargylated ODN-**60** shows a retention time of 20.6 min, confirming the formation of the interstrand cross-link within a base pair (mobility depends on the number of negative charges—phosphodiester anions—of the oligonucleotides). Similar to ODN-**61**, a retention time of 20.0 min was observed for ps adduct ODN-**54** with a cross-link of sugar residues at a distant position (Figure 3b).

Sugar cross-linking of ps oligonucleotide duplexes was also confirmed by denaturing PAGE.[10] As shown in Figure 4a, the cross-linked adducts ODN-**61** (lane 3) and ODN-**54** (lane 4) with parallel strand orientation migrate much more slowly than the 2′-*O*-propargylated starting material (lane 1, ODN-**50** and lane 2, ODN-**60**) and the corresponding monofunctionalized oligonucleotides still bearing one azido group (lane 5: ODN-**55**, lane 6: ODN-**57**, lane 7: ODN-**58**).



(a) (b)

Figure 3. Ion-exchange HPLC elution profiles of (a) monofunctionalized ODN-**55** and interstrand cross-linked ODN-**61** and (b) cross-linked ODN-**54**. Ion-exchange chromatography was performed on a 4 mm × 250 mm DNA Pac PA-100 column, using the following buffer system. Buffer A: 25 mM Tris-HCl, 10% MeCN, pH 7.0. Buffer B: 25 mM Tris-HCl, 1.0 M NaCl, and 10% MeCN, pH 7.0. Elution gradient: 0−30 min 20−80% B in A with a flow rate of 0.75 mL min^{-1}.

The cross-linked adducts ODN-**66** (lane 3) and ODN-**65** (lane 4) with antiparallel strand orientation (Figure 4b) show a comparable mobility as the corresponding parallel stranded cross-linked adduct ODN-**52** (lane 5), which proves the successful sugar cross-linking of ps duplexes.

4. Stability of Cross-Linked Duplexes. The structure of an aps Watson−Crick helix is significantly different from that of ps DNA. Theoretical studies have shown that the groove size of a dA-dT-rich Watson−Crick (aps) helix is 11 Å for the minor and 20 Å for the major groove, and 13 and 16 Å for the minor and major grooves, respectively, for the ps DNA.[25] The helix sense of ps DNA is right handed, the DNA has about 10.7 bp per turn, and the axial rise per base pair is 3.4 Å, leading to the height of one helix turn of 36.4 Å, compared to aps DNA with 10 bp per turn and a height of one helix turn of 34 Å. These findings were also supported by NMR experiments.[26] Moreover, it was shown that dA-dT-rich ps DNA is less stable than its aps counterpart.[25] Consequently, different protocols were developed to stabilize ps DNA by chemical modification. Our laboratory has introduced tridentate base pairs of dG·$isoC_d$ and dC·$isoG_d$, which stabilize ps DNA (Figure 5). 7-Halogenated 7-deaza-2′-deoxyisoguanosines, 7-substituted 8-aza-7-deaza-2′-deoxyisoguanosines as well as the G-clamp have been used for this purpose.[3d,27]Additionally, ps DNA was stabilized by incorporation of modified purine nucleosides in the reverse Watson−Crick base pair (Donohue pair) as it was reported for dA-dT base pairs in aps DNA (Figure 5).[28] A third possibility, which is the topic of this manuscript, is the cross-linking of ps DNA. However, it remains to be proven if ligation at "identical" sequence positions in aps and ps DNA can stabilize duplexes in a similar way. Due to the structural differences of ps and aps duplexes, structural modifications can induce more steric stress on ps DNA than on aps DNA. Such a negative phenomenon can obscure the favorable stability increase induced by cross-linking.

Table 2 summarizes the T_m data obtained form cross-linked and noncross-linked oligonucleotides synthesized in this study and compares the effect of cross-links in ps and aps DNA. The influence of the different helical geometries (groove size) on stability changes caused by cross-linking is demonstrated. The

dx.doi.org/10.1021/jo4012706 | J. Org. Chem. 2013, 78, 8545−8561

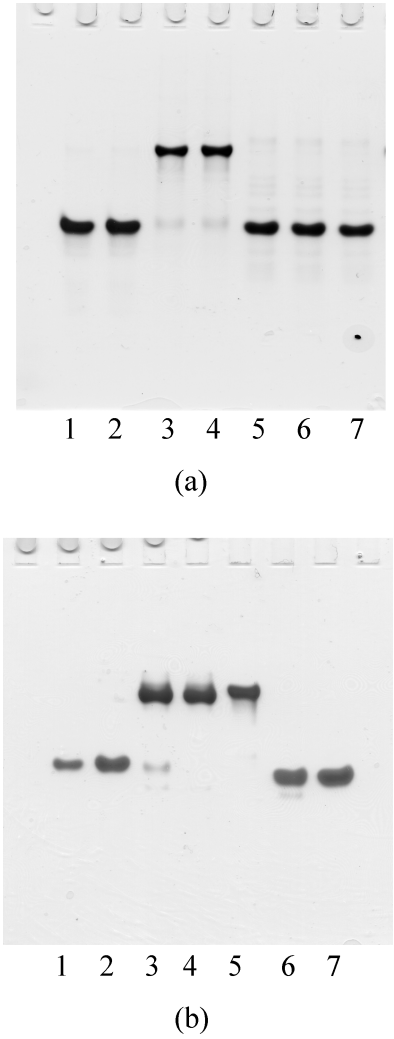


Figure 4. Denaturing PAGE analysis of monofunctionalized oligonucleotides and cross-linked click adducts on a 17% polyacrylamide gel. (a) Lane 1, ODN-**50**; 2, ODN-**60**; 3, cross-linked ps ODN-**61**; 4, cross-linked ps ODN-**54**; 5, monofunctionalized ODN-**55**; 6, monofunctionalized ODN-**57**; 7, monofunctionalized ODN-**58**. (b) Lane 1, monofunctionalized ODN-**56**; 2, monofunctionalized ODN-**59**; 3, cross-linked aps ODN-**66**; 4, cross-linked aps ODN-**65**; 5, cross-linked ps ODN-**52**; 6, ODN-**50**; 7, ODN-**51**.

cross-links were introduced between heterodimeric complementary oligonucleotides at the termini of duplexes (i) in a fully matching sequence with the cross-link in a two base pair distance (**52**), (ii) in a mismatched sequence with the cross-link in a two base pair distance (**54**), and (iii) in a fully matching sequence with the cross-link within a base pair (**61**).

At first, the influence of the clickable sugar modification on duplex stability is compared (Table 2) and leads to the following conclusions: (i) as reported before,[6] the 25-mer ps duplexes are less stable than the corresponding aps duplexes, which results from the thermodynamically less stable dA-dT Donohue base pairs compared to the Watson−Crick pairs; (ii) the introduction of propargylated residues (**1**, **3**, **A***) leads to a slight destabilization of the ps duplexes compared to the unmodified parent duplex **48**·**49**, independent of the position of the propargylated residue or of a matching or mismatching situation; and (iii) the additional functionalization of the side chains by bis-azide **6** generating an azido-modified side chain

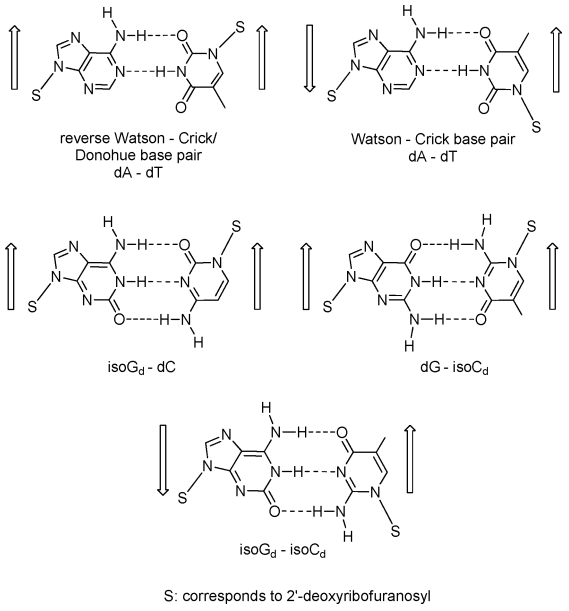


Figure 5. Base pair motifs of ps and aps DNA; arrows indicate chain orientation.

stabilizes ps and aps duplexes slightly more than the shorter propargyl chain.

Different from the noncross-linked duplexes, cross-linked DNA generated with bis-azide **6** and propargylated sugar residues leads to a strong stabilization of ps DNA. In addition, a destabilized mismatched ps duplex is as efficiently stabilized by cross-linking as a fully matching duplex. From Table 2, it is apparent that the cross-links stabilize both ps and aps duplexes significantly. The increase in T_m is similar in both cases, showing that the cross-links are well accepted in ps and aps duplexes.

CONCLUSION AND OUTLOOK

In this work, we demonstrated that complementary oligonucleotides with propargylated sugar residues and parallel strand orientation can be ligated efficiently in a stepwise manner with chelating bis-azides as it was observed for antiparallel duplexes. For this, a series of propargylated homodimeric and heterodimeric duplexes were prepared. Corresponding phosphoramidites of propargylated isoG$_d$ were prepared, and the synthesis of propargylated dC was improved. From the above findings, it is apparent that the cross-links stabilize both ps and aps duplexes significantly. The increase in T_m is similar in both cases, clearly indicating that the cross-links are well accommodated in ps DNA leading to the strong stabilization. This was rather unexpected as the different helix geometries of ps and aps DNA can induce more steric stress on ps DNA than on aps DNA. The application of the method expands the repertoire of construction tools for larger assemblies and nanodevices based on DNA including walkers, tweezers, and nanomachines.[29]

EXPERIMENTAL SECTION

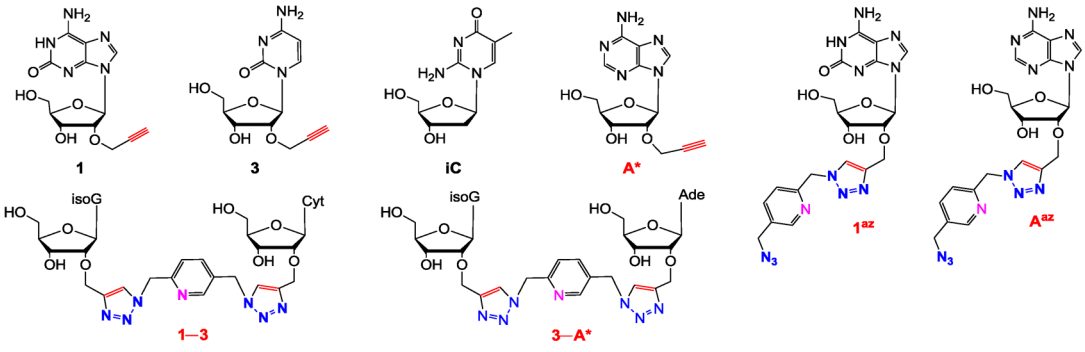
General Methods and Materials. All chemicals and solvents were of laboratory grade as obtained from commercial suppliers and were used without further purification. Thin-layer chromatography (TLC) was performed on TLC aluminum sheets covered with silica gel 60 F254 (0.2 mm). Flash column

dx.doi.org/10.1021/jo4012706 | J. Org. Chem. 2013, 78, 8545−8561

Table 2. T_m Values of Sugar-Modified and Sugar Cross-Linked ps and aps Duplexes[a]

ps Duplexes	T_m [°C]	ΔT_m[b] [°C]	ΔT_m[c] [°C]
5'-d(TTT TTT TTT TAT TAA AAT TTA TAA A)-3' (**48**) 5'-d(AAA AAA AAA ATA ATT TTA AAT ATT T)-3' (**49**)	42.0	--	--
5'-d(T T **1** TTT TTT TAT TAA AAT TTA TAA A)-3' (**50**) 5'-d(**A***AC AAA AAA ATA ATT TTA AAT ATT T)-3' (**51**)	40.0	+16.0	-2.0
5'-d(TT**1** TTT TTT TAT TAA AAT TTA TAA A)-3' 5'-d(**A***AC AAA AAA ATA ATT TTA AAT ATT T)-3' (**52**)	56.0		+14.0
5'-d(T T **1** TTT TTT TAT TAA AAT TTA TAA A)-3' (**50**) 5'-d(**A***A**A** AAA AAA ATA ATT TTA AAT ATT T)-3' (**53**)	35.5	+18.5	-6.5
5'-d(TT**1** TTT TTT TAT TAA AAT TTA TAA A)-3' 5'-d(**A***A**A** AAA AAA ATA ATT TTA AAT ATT T)-3' (**54**)	54.0		+12.0
5'-d(TT**1**[az] TTT TTT TAT TAA AAT TTA TAA A)-3' (**55**) 5'-d(AA**3** AAA AAA ATA ATT TTA AAT ATT T)-3' (**60**)	38.0	-	-4.0
5'-d(TT**1** TTT TTT TAT TAA AAT TTA TAA A)-3' (**50**) 5'-d(AA**3** AAA AAA ATA ATT TTA AAT ATT T)-3' (**60**)	37.0	+18.5	-5.0
5'-d(TT**1** TTT TTT TAT TAA AAT TTA TAA A)-3' 5'-d(AA**3** AAA AAA ATA ATT TTA AAT ATT T)-3' (**61**)	55.5		+13.5

aps Duplexes	T_m [°C]	ΔT_m[b] [°C]	ΔT_m[d] [°C]
5'-d(TTT TTT TTT TAT TAA AAT TTA TAA A)-3' (**48**) 3'-d(AAA AAA AAA ATA ATT TTA AAT ATT T)-5' (**62**)	56.0	--	--
3'-d(TT **1** TTT TTT TAT TAA AAT TTA TAA A)-5' (**63**) 5'-d(**A***A**iC** AAA AAA ATA ATT TTA AAT ATT T)-3' (**64**)	59.0	+11.0	+3.0
3'-d(TT **1** TTT TTT TAT TAA AAT TTA TAA A)-5' 5'-d(**A***A**iC** AAA AAA ATA ATT TTA AAT ATT T)-3' (**65**)	70.0		+14.0
3'-d(T T**1** TTT TTT TAT TAA AAT TTA TAA A)-5' (**63**) 5'-d(**A***A**A** AAA AAA ATA ATT TTA AAT ATT T)-3' (**53**)	52.0	+15.0	-4.0
3'-d(TT**1** TTT TTT TAT TAA AAT TTA TAA A)-5' 5'-d(**A***A**A** AAA AAA ATA ATT TTA AAT ATT T)-3' (**66**)	67.0		+11.0
5'-d(T T**1** TTT TTT TAT TAA AAT TTA TAA A)-3' (**50**) 3'-d(AA**iC** AAA AAA ATA ATT TTA AAT ATT T)-5' (**67**)	54.5	-	-1.5
5'-d(TT**1**[az] TTT TTT TAT TAA AAT TTA TAA A)-3' (**55**) 3'-d(AA**iC** AAA AAA ATA ATT TTA AAT ATT T)-5' (**67**)	55.0	+0.5	-1.0



dx.doi.org/10.1021/jo4012706 | J. Org. Chem. 2013, 78, 8545−8561

Table 2. continued

[a]Measured at 260 nm in 0.1 M NaCl, 10 mM $MgCl_2$, 10 mM Na-cacodylate (pH 7.0) with 5 μM + 5 μM single-strand concentration for noncross-linked oligonucleotides and 1 μM for cross-linked oligonucleotides. [b]ΔT_m was calculated as T_m cross-linked duplex − T_m propargylated duplex. [c]ΔT_m was calculated as T_m modified duplex − T_m unmodified duplex using **48**·**49** as comparison. [d]ΔT_m was calculated as T_m modified duplex − T_m unmodified duplex using **48**·**62** as comparison. Monofunctionalized and cross-linked ODNs were prepared by stepwise click reactions.

Table 3. ^{13}C NMR Table for Cytidine Derivatives[a]

	C(2)[b]	C(4)[b]	C(5)[b]	C(6)[b]	C≡C	CH_2	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C=O	OMe	pyridynyl linker	triazole
7[c]	155.3	165.4	93.9	141.3			89.0	73.8	69.3	83.9	60.5				
3	154.6	165.2	94.1	141.3	80.1, 77.3	56.8	87.1	80.4	68.0	84.3	60.2				
8	154.2	163.2	96.2	144.5	80.9, 80.2	57.2	88.5[d]	82.1	67.8	86.0[d]	61.7	167.4	55.0		
9	154.3	163.1	96.0	144.5	79.9, 77.7	56.7	91.3	71.9	80.0	86.1	61.1	167.2	56.1		
23	154.9	165.6	93.8	141.1		50.6, 54.1	87.3	81.2	67.9	84.0	60.0			155.1, 149.2, 137.4, 130.9, 122.1	144.2, 124.8
24	155.0	165.6	93.9	141.0		50.0, 54.0	87.3	81.2	67.9	84.0	60.0			155.1, 149.1, 131.2, 122.2, 122.2	144.4, 144.2, 124.8, 124.2

[a]Measured in DMSO-d_6 at 298 K. [b]Pyrimidine numbering. [c]Reference 30a. [d]Tentative.

Table 4. ^{13}C NMR Table for Purine-2,6-diamine and Isoguanine Nucleosides[a]

	C(2)[b,c]	C(4)[b,c]	C(5)[b]	C(6)[b,c]	C(8)[b]	C≡C	CH_2	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	CH=N	NMe_2	=CMe	C=O
12[e]	156.3	151.4	113.6	160.1	136.3			87.1	73.2	70.7	85.5	61.8				
13	156.2	151.4	113.4	160.1	135.8	79.7, 77.6	56.9	86.2	79.8	68.9	84.8	61.6				
14	156.3	151.4	113.6	160.0	136.1	80.5, 77.6	57.2	86.9	72.7	77.2	83.3	61.6				
isoG[f]	158.3	—[d]	109.8	155.9	138.4			87.8	73.0	70.9	85.6	61.7				
1	—[d]	—[d]	109.2	155.3	137.9	79.1, 77.3	56.6	86.6	79.5	68.8	85.4	61.4				
2	—[d]	—[d]	109.6	155.6	138.4	80.4, 77.7	57.2	87.7	72.5	77.3	83.9	61.7				
15	156.2	153.6	113.1	156.9	140.2	79.1, 77.5	56.8	86.7	79.7	68.9	85.5	61.6	163.9	—[d], 38.0	18.8	
16	156.2	153.7	113.4	156.9	140.5	80.4, 77.7	57.2	87.6	72.2	77.3	83.9	61.8	164.0	—[d], 38.1	18.8	
17	160.6	151.4	123.7	155.3	141.7	77.5, 79.5	56.8	86.4	79.8	68.7	84.9	61.2	162.3	37.6, 38.2	17.2	151.7
18	160.8	151.5	124.0	155.3	141.9	80.3, 77.3	57.2	87.3	77.3	61.3	83.6	61.3	162.4	37.7, 38.3	17.3	151.8
19	160.8	151.5	123.8	158.0	141.9	79.6, 77.7	57.2	85.7	79.8	69.0	84.8	63.6	162.5	37.7, 38.4	17.3	151.9
20	160.8	151.5	123.9	158.0	141.9	80.1, 77.5	57.0	87.1	72.0	76.7	85.7	63.1	162.4	37.7, 38.3	17.2	151.9

[a]Measured in DMSO-d_6 at 298 K. [b]Purine numbering. [c]Tentative. [d]Not detected. [e]Reference 30b. [f]Reference 30c.

chromatography (FC) was on silica gel 60 (40−60 μM) at 0.4 bar. UV spectra were recorded on a spectrophotometer; λ_{max} in nm, ε in dm^3 mol^{-1} cm^{-1}. 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 J values are given in Hz; δ values are 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. The ^{13}C NMR signals were assigned on the basis of DEPT-135 and ^{1}H−^{13}C gated-decoupled NMR spectra (for coupling constants see Tables S1 and S2, Supporting Information). Reversed-phase HPLC was carried out on a 250 mm × 4 mm RP-18 LiChrospher 100 column with a HPLC pump connected with a variable wavelength monitor, a controller, and an integrator. Gradients used for HPLC chromatography: A = MeCN; B = 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN, 95:5. Conditions: (I) 3 min 15% A in B, 12 min 15−50% A in B, and 5 min 50−10% A in B, flow rate 0.7 mL min^{-1}; (II) 0−25 min 0−20% A in B, flow rate 0.7 mL min^{-1}. Melting curves were measured with a UV−vis spectrophotometer equipped with a thermoelectrical controller with a heating rate of 1 °C/min. The T_m values were determined from the melting curves using the software MELTWIN, version 3.0. (J. A. Mc Dowell, 1996). ESI-TOF mass spectra of the nucleosides were measured with 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 (Tables 1 and 5).

One-Pot Synthesis of 4-(Benzoylamino)-1-[5-*O*-(4,4′-dimethoxytrityl)-2-*O*-(propargyl)-β-D-ribofuranosyl]-2*H*-pyrimidin-2-one (8) and 4-(Benzoylamino)-1-[5-*O*-(4,4′-dimethoxytrityl)-3-*O*-(propargyl)-β-D-ribofuranosyl]-2*H*-pyrimidin-2-one (9). Cytidine **7** (1.0 g, 4.11 mmol) was dissolved in hot anhydrous DMF (25 mL) under an argon atmosphere. The solution was cooled to 5 °C, and NaH (0.13 g, 3.3 mmol, 60% dispersion in mineral oil) was subsequently added, followed by the addition of tetrabutylammoniumiodide (TBAI) (0.68 g, 1.85 mmol) and propargyl bromide (0.49 g, 4.11 mmol). The reaction mixture was allowed to stir for 3 days at 55 °C. After completion of the reaction (TLC monitoring), evaporation of the solvent under reduced pressure gave a suspension, which was absorbed on silica gel. FC (silica gel, CH_2Cl_2/MeOH, 9:1) gave a chromatographically inseparable mixture of 2′-*O*-propargylcytidine (**3**) and 3′-*O*-propargylcytidine (**4**) as colorless solid (0.74 g, 64%). TLC (silica gel, CH_2Cl_2/MeOH, 9:1) R_f 0.6.

This solid was repeatedly co-evaporated with pyridine (3 × 10 mL) and suspended in pyridine (20 mL). The suspension was treated with dimethoxytrityl chloride (1.73 g, 5.12 mmol), triethylamine (0.52 g, 5.12 mmol), and 4-(dimethylamino)-pyridine (0.13 g, 1.07 mmol) and stirred at room temperature for 4.5 h. After consumption of the starting material (TLC

dx.doi.org/10.1021/jo4012706 | J. Org. Chem. 2013, 78, 8545−8561

monitoring, CH_2Cl_2/acetone, 8:2), trimethylsilyl chloride (1.39 g, 12.81 mmol) was added, and stirring was continued at room temperature for 30 min. Then, benzoyl chloride (1.80 g, 12.81 mmol) was added, and stirring was continued for another 3 h. The reaction mixture was cooled in an ice-bath, and 5 mL of water was added. After 5 min, 10 mL of 28−30% aq ammonia was added, and stirring was continued at rt for another 30 min. The reaction mixture was evaporated to dryness. The remaining residue was dissolved in dichloromethane (100 mL) and extracted with 5% aq $NaHCO_3$ (50 mL). The aqueous layer was washed with dichloromethane (2 × 100 mL). The organic layer was collected, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was co-evaporated with toluene (3 × 10 mL) and subjected to FC (silica gel, column 15 cm × 3 cm, CH_2Cl_2/acetone, 80:20). From the fast migrating zone compound **8** (0.77 g, 44%) was isolated as yellowish foam, and from the slower migrating zone compound **9** (0.18 g, 10%) was isolated as a colorless foam.

4-(Benzoylamino)-1-[5-*O*-(4,4′-dimethoxytrityl)-2-*O*-(propargyl)-β-D-ribofuranosyl]-2*H*-pyrimidin-2-one (8). TLC (silica gel, CH_2Cl_2/acetone, 80:20) R_f 0.4. λ_{max} (MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 23 500), 235 (32 600), 304 (11 600). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.49 (t, J = 2.4 Hz, 1H, C≡CH), 3.76 (s, 6H, 2 × OCH_3), 4.05−4.08 (m, 2H, C3′-H, C4′-H), 4.33−4.40 (m, 1H, C2′-H), 4.49 (d, J = 2.1 Hz, 2H, CH_2), 5.34 (d, J = 7.2 Hz, 1H, C3′-OH), 5.89 (d, J = 1.5 Hz, 1H, C1′-H), 6.88−6.94 (m, 4H, Ar-H), 7.14 (d, J = 7.5 Hz, 1H, C5-H), 7.24−7.43 (m, 9H, Ar-H), 7.49−7.54 (m, 2H, Ar-H), 7.60−7.65 (m, 1H, Ar-H), 7.98−8.01 (m, 2H, Ar-H), 8.38 (d, J = 7.5 Hz, 1H, C6-H), 11.33 (br s, 1H, NH). Anal. Calcd for $C_{40}H_{37}N_3O_8$ (687.7): C 69.86, H 5.42, N 6.11. Found: C 70.00, H 5.49, N 6.10.

4-(Benzoylamino)-1-[5-*O*-(4,4′-dimethoxytrityl)-3-*O*-(propargyl)-β-D-ribofuranosyl]-2*H*-pyrimidin-2-one (9). TLC (silica gel, CH_2Cl_2/acetone, 80:20) R_f 0.1. λ_{max} (MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 24 300), 236 (32 400), 304 (11 300). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.52 (t, J = 2.4 Hz, 1H, C≡CH), 3.76 (s, 6H, 2 × OCH_3), 4.17−4.19 (m, 2H, C5′-H, C4′-H), 4.24 (d, J = 2.4 Hz, 1H, C3′-H), 4.32−4.41 (m, 3H, CH_2, C2′-H), 5.77−5.79 (m, 2H, C2′-OH, C1′-H), 6.92−6.65 (m, 4H, Ar-H), 7.14 (d, J = 7.2 Hz, 1H, C5-H), 7.24−7.44 (m, 9H, Ar-H), 7.49−7.54 (m, 2H, Ar-H), 7.60−7.65 (m, 1H, Ar-H), 7.99−8.02 (m, 2H, Ar-H), 8.46 (d, J = 7.2 Hz, 1H, C6-H), 11.29 (s, 1H, NH). Anal. Calcd for $C_{40}H_{37}N_3O_8$ (687.7): C 69.86, H 5.42, N 6.11. Found: C 70.00, H 5.60, N 6.01.

4-(Benzoylamino)-1-[5-*O*-(4,4′-dimethoxytrityl)-2-*O*-(propargyl)-β-D-ribofuranosyl]-2*H*-pyrimidin-2-one 3-*O*-(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (10). Compound **8** (0.58 g, 0.84 mmol) was dissolved in dry CH_2Cl_2 (15 mL), (i-Pr)$_2$NEt (0.457 g, 594 μL, 3.54 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (0.398 g, 375 μL, 1.68 mmol) were added, and the solution was stirred for 1 h at rt. After completion of the reaction (TLC monitoring), the reaction mixture was diluted with CH_2Cl_2 (30 mL), poured into 5% aq $NaHCO_3$ (50 mL), and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated. The residual foam was applied to FC (silica gel, CH_2Cl_2/acetone, 90:10). Evaporation of the main zone afforded **10** (0.57 g, 76%) as a light yellow foam. TLC (silica gel, CH_2Cl_2/acetone, 80:20) R_f 0.8. ^{31}P NMR ($CDCl_3$, 121.5 MHz): 150.33, 150.43.

4-(Benzoylamino)-1-[5-*O*-(4,4′-dimethoxytrityl)-3-*O*-(propargyl)-β-D-ribofuranosyl]-2*H*-pyrimidin-2-one 2-*O*-(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (11). As described for **10**. Compound **9** (0.2 g, 0.29 mmol) in dry CH_2Cl_2 (10 mL), (i-Pr)$_2$NEt (0.157 g, 204 μL, 1.22 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (0.137 g, 129 μL, 0.58 mmol). FC (silica gel, CH_2Cl_2/acetone, 90:10). Evaporation of the main zone afforded **11** (0.192 g, 74%) as a colorless foam. TLC (silica gel, CH_2Cl_2/acetone, 80:20) R_f 0.7. ^{31}P NMR ($CDCl_3$, 121.5 MHz): 149.7.

4-Amino-1-[(2-*O*-propargyl)-β-D-ribofuranosyl]-2*H*-pyrimidin-2-one (3). Compound **8** (0.4 g, 0.58 mmol) was dissolved in 28% aq ammonia (50 mL) and stirred at 60 °C overnight in an autoclave. The solvent was evapoated, 85% aq AcOH (10 mL) was added, and the solution was stirred at rt for 1 h. After completion of the reaction, the solvent was evaporated, and the residue was co-evaporated with ethanol and subjected to FC (silica gel, CH_2Cl_2/MeOH, 85:15) to give **3** (0.15 g, 92%) as a colorless solid. TLC (silica gel, CH_2Cl_2/MeOH, 80:20) R_f 0.3. λ_{max} (MeOH)/nm 272 (ε/dm^3 mol^{-1} cm^{-1} 9 800). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.42 (t, J = 2.4 Hz, 1H, C≡CH), 3.53−3.57 (m, 1H, C5′-H), 3.65−3.69 (m, 1H, C5′-H), 3.82−3.83 (m, 1H, C4′-H), 3.94 (t, J = 2.4 Hz, 1H, C3′-H), 4.04−4.11 (m, 1H, C2′-H), 4.32 (s, 2H, CH_2), 5.10−5.17 (m, 2H, C5′-OH, C3′-OH), 5.71 (d, J = 7.2 Hz, 1H, C1′-H), 5.84 (d, J = 3.9 Hz, 1H, C5-H), 7.20 (br s, 2H, NH_2), 7.88 (s, 1H, C6-H). ESI-TOF m/z calcd for $C_{12}H_{15}N_3O_5$ [M + Na$^+$] 304.0910, found 304.0904.

Propargylation of 9-[β-D-Ribofuranosyl]purin-2,6-diamine 12. Compound **12** (2.0 g, 7.08 mmol) was dissolved in hot anhydrous DMF (100 mL) under argon atmosphere. The solution was cooled to 5 °C, and NaH (0.23 g, 5.73 mmol, 60% dispersion in mineral oil) was subsequently added, followed by the addition of TBAI (1.18 g, 3.19 mmol) and propargyl bromide (1.05 g, 788 μL, 8.85 mmol). The reaction mixture was allowed to stir for 3 days at 55 °C. Evaporation of the solvent under reduced pressure resulted in a suspension, which was absorbed on silica. FC (CH_2Cl_2/MeOH, 95:5) afforded compound **13** (colorless foam, 31.7%) from fast migrating zone and compound **14** (colorless solid, 11.3%) from the slower migrating zone; 25% of the starting material **12** was recovered.

9-[(2-*O*-Propargyl)-β-D-ribofuranosyl]purin-2,6-diamine (13). TLC (silica gel, CH_2Cl_2/MeOH, 90:10) R_f 0.6. λ_{max} (MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 8 400), 280 (10 900). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.38 (t, J = 2.4 Hz, 1H, C≡CH), 3.54−3.57 (m, 1H, C5′-H), 3.65−3.59 (m, 1H, C5′-H), 3.92−3.95 (m, 1H, C4′-H), 4.19 (d, J = 2.4 Hz, 1H, CH_2), 4.23 (d, J = 2.4 Hz, 1H, CH_2), 4.28−4.32 (m, 1H, C3′-H), 4.51−4.55 (m, 1H, C2′-H), 5.28 (d, J = 5.1 Hz, 1H, C3′-OH), 5.45−5.49 (m, 1H, C5′-OH), 5.75 (s, 2H, 2-NH_2), 5.85 (d, J = 6.6 Hz, 1H, C1′-H), 6.79 (br s, 2H, 6-NH_2), 7.94 (s, 1H, C8-H). Anal. Calcd for $C_{13}H_{16}N_6O_4$ (320.3): C 48.75, H 5.03, N 26.24. Found: C 48.62, H 5.10, N 26.09.

9-[(3-*O*-Propargyl)-β-D-ribofuranosyl]purin-2,6-diamine (14). TLC (silica gel, CH_2Cl_2/MeOH, 90:10) R_f 0.5. λ_{max} (MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 9 800), 281 (11 400). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.47 (t, J = 2.4 Hz, 1H, C≡CH), 3.49−3.57 (m, 1H, C5′-H), 3.61−3.68 (m, 1H, C5′-H), 4.01−4.04 (m, 1H, C4′-H), 4.09 −4.14 (m, 1H, C3′-H), 4.35 (d, J = 2.4 Hz, 1H, CH_2), 4.38 (d, J = 2.4 Hz, 1H, CH_2), 4.65−4.71 (m, 1H, C2′-H), 5.54−5.58 (m, 2H, C2′-OH, C5′-OH), 5.72 (d, J = 6.9 Hz, 1H, C1′-H), 5.76 (br s, 2H, 2-NH_2),

dx.doi.org/10.1021/jo4012706 | J. Org. Chem. 2013, 78, 8545−8561

6.81 (br s, 2H, 6-NH_2), 7.91 (s, 1H, C8-H). Anal. Calcd for $C_{13}H_{16}N_6O_4$ (320.3): C 48.75, H 5.03, N 26.24. Found: C 48.93, H 5.15, N 26.10.

6-Amino-1,9-dihydro-9-[(2-*O*-propargyl)-β-D-ribofuranosyl]-2*H*-purin-2-one (1). The diamino nucleoside **13** (1.12 g, 3.49 mmol) was suspended in water (32 mL) at 50 °C, and $NaNO_2$ (0.93 g, 13.49 mmol) in H_2O (7 mL) was introduced. Then, acetic acid (1.45 g, 1.38 mL, 24.23 mmol) was added dropwise to the solution at 50 °C over 2 min. The resulting clear solution was stirred for 20 min at 50 °C. After completion of the reaction (TLC monitoring), the reaction mixture was diluted with water (15 mL) and cooled in an ice-bath, and conc aq ammonia was added dropwise until the pH reached 8. Then, the solution was evaporated and the remaining residue was redissolved in 100 mL of water. The pH of the solution was adjusted to 5−6 and adsorbed on RP-18 silica gel (mesh 40−63 μM, 50 g). Inorganic salts were removed with water (2 × 10 mL), and the nucleoside was eluted with methanol (2 × 50 mL). The combined methanol portions were evaporated to obtain nucleoside **1** as a pale yellow solid (0.652 g, 58%). TLC (silica gel, *i*-PrOH/H_2O/NH_3, 70:20:10) R_f 0.8. λ_{max} (MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 6000), 298 (8 900). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.35 (t, J = 2.4 Hz, 1H, C≡CH), 3.49−3.52 (m, 1H, C5′-H), 3.59−3.64 (m, 1H, C5′-H), 3.94−3.95 (m, 1H, C4′-H), 4.17 (d, J = 2.1 Hz, 1H, CH_2), 4.22 (d, J = 2.4 Hz, 1H, CH_2), 4.26−4.27 (m, 1H, C3′-H), 4.53 (t, J = 5.4 Hz, 1H, C2′-H), 5.28 (d, J = 4.8 Hz, 1H, C3′-OH), 5.76 (d, J = 6.9 Hz, 1H, C1′-H), 7.91 (s, 1H, C8-H), 10.67 (br s, 1H, NH). Anal. Calcd for $C_{13}H_{15}N_5O_5$ (321.3): C 48.60, H 4.71, N 21.80. Found: C 48.46, H 4.95, N 21.81. ESI-TOF m/z calcd for $C_{13}H_{15}N_5O_5$ [M + Na$^+$] 344.0971, found 344.0969.

6-{[1-(Dimethylamino)ethylidene]amino}-1,9-dihydro-9-[(2-*O*-propargyl)-β-D-ribofuranosyl]-2*H*-purin-2-one (15). To a suspension of compound **1** (0.700 g, 2.18 mmol) in methanol (40 mL) was added *N*,*N*-dimethylacetamide dimethyl acetal (0.78 g, 859 μL, 5.85 mmol), and the resulting mixture was stirred at rt for 3 h (TLC monitoring). The solvent was evaporated, the residue was co-evaporated with methanol and subjected to FC (silica gel, CH_2Cl_2/MeOH, 90:10) to obtain **15** (0.67 g, 79%) as a colorless solid. TLC (silica gel, CH_2Cl_2/MeOH, 30:20) R_f 0.8. λ_{max} (MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 10 300), 336 (18 300). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.13 (s, 3H, CH_3), 3.09 (s, 3H, CH_3), 3.16 (s, 3H, CH_3), 3.38 (t, J = 2.4 Hz, 1H, C≡CH), 3.48−3.55 (m, 1H, C5′-H), 3.60−3.65 (m, 1H, C5′-H), 3.95−3.96 (m, 1H, C4′-H), 4.20 (d, J = 2.4 Hz, 1H, CH_2), 4.24 (d, J = 2.4 Hz, 1H, CH_2), 4.27−4.31 (m, 1H, C3′-H), 4.56−4.60 (m, 1H, C2′-H), 5.29 (d, J = 5.1 Hz, 1H, C3′-OH), 5.71−5.78 (m, 2H, C1′-H, C5′-OH), 8.01 (s, 1H, C8-H), 11.03 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{22}N_6O_5$ (390.4): C 52.30, H 5.68, N 21.53. Found: C 52.15, H 5.70, N 21.50.

6-{[1-(Dimethylamino)ethylidene]amino}-9-[(2-*O*-propargyl)-β-D-ribofuranosyl]purin-2-yl-diphenylcarbamate (17). To a solution of compound **15** (0.8 g, 2.05 mmol) in anhydrous pyridine (17 mL) were added *N*,*N*-diphenylcarbamoyl chloride (0.711 g, 3.06 mmol) and (*i*-Pr)$_2$EtN (0.396 g, 515 μL, 3.07 mmol). The mixture was stirred at rt for 40 min, poured in 5% aq $NaHCO_3$ (20 mL), and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was separated, dried over Na_2SO_4, and evaporated, and the residue was applied to FC (silica gel, CH_2Cl_2/MeOH, 96:4) to yield **17** (0.986 g, 82%) as colorless foam. TLC (silica gel, CH_2Cl_2/MeOH, 90:10) R_f 0.5. λ_{max} (MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 12 700), 310 (30 300). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.11 (s, 3H, CH_3), 3.12−3.14 (m, 3H, CH_3), 3.32 (t, J = 2.4 Hz, 1H, C≡CH), 3.55−3.61 (m, 1H, C5′-H), 3.63−3.68 (m, 1H, C5′-H), 3.98−4.01 (m, 1H, C4′-H), 4.22 (d, J = 2.4 Hz, 1H, CH_2), 4.26 (d, J = 2.4 Hz, 1H, CH_2), 4.31−4.34 (m, 1H, C3′-H), 4.59−4.63 (m, 1H, C2′-H), 5.21 (t, J = 5.7 Hz, 1H, C5′-OH), 5.39 (d, J = 5.4 Hz, 1H, C3′-OH), 6.00 (d, J = 6.3 Hz, 1H, C1′-H), 7.27−7.34 (m, 2H, Ar-H), 7.42−7.46 (m, 8H, Ar-H), 8.45 (s, 1H, C8-H). Anal. Calcd for $C_{30}H_{31}N_7O_6$ (585.6): C 61.53, H 5.34, N 16.74. Found: C 61.60, H 5.40, N 16.73.

6-{[1-(Dimethylamino)ethylidene]amino}-9-[5-*O*-(4,4′-dimethoxytrityl)-2-*O*-(propargyl)-β-D-ribofuranosyl)]-purin-2-yl-diphenylcarbamate (19). To a solution of compound **17** (0.35 g, 0.59 mmol) in anhydrous pyridine (600 μL) was added 4,4′-dimethoxytrityl chloride (0.303 g, 0.89 mmol). The mixture was stirred at rt for 90 min. After completion of the reaction (TLC monitoring), 10 mL of CH_2Cl_2 was added and washed with 5% aq $NaHCO_3$ (5 mL). The organic layer was dried over Na_2SO_4, evaporated to dryness, and co-evaporated with toulene (3 × 5 mL), and the residue was applied to FC (silica gel, CH_2Cl_2/acetone, 90:10) to obtain compound **19** (0.367 g, 70%) as a light yellow foam. TLC (silica gel, CH_2Cl_2/acetone, 4:1) R_f 0.6. λ_{max} (MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 15 100), 308 (29 700). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.09 (s, 3H, CH_3), 3.12 (d, J = 3.6 Hz, 6H, CH_3), 3.12−3.20 (m, 2H, C5′-H), 3.43 (t, J = 2.1 Hz, 1H, C≡CH), 3.69 (s, 6H, 2 × OCH_3), 4.06−4.07 (m, 1H, C4′-H), 4.29 (d, J = 2.4 Hz, 1H, CH_2), 4.33 (d, J = 2.4 Hz, 1H, CH_2), 4.41−4.43 (m, 1H, C3′-H), 4.67 (t, J = 5.1 Hz, 1H, C2′-H), 5.44 (d, J = 6.0 Hz, 1H, C3′-OH), 6.04 (d, J = 5.4 Hz, 1H, C1′-H), 6.82 (d, J = 8.4 Hz, 4H, Ar-H), 7.18−7.38 (m, 11H, Ar-H), 7.39−7.44 (m, 8H, Ar-H), 8.28 (s, 1H, C8-H). Anal. Calcd for $C_{51}H_{49}N_7O_8$ (888.0): C 68.98, H 5.56, N 11.04. Found: C 68.83, H 5.60, N 10.92.

6-{[1-(Dimethylamino)ethylidene]amino}-9-[5-*O*-(4,4′-dimethoxytrityl)-2-*O*-(propargyl)-β-D-ribofuranosyl)]-purin-2-yl-diphenylcarbamate 3-*O*-(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (21). To a solution of compound **19** (0.45 g, 0.51 mmol) in dry CH_2Cl_2 (10 mL) were added (*i*-Pr)$_2$NEt (0.114 g, 150 μL, 0.87 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (0.159 g, 150 μL, 0.66 mmol), and the reaction mixture was stirred for 2 h at rt. After completion of the reaction (TLC monitoring), the reaction mixture was diluted with CH_2Cl_2 (30 mL), poured into 5% $NaHCO_3$ solution (30 mL), and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated. The residual foam was applied to FC (silica gel, eluted with CH_2Cl_2/acetone, 85:15). Evaporation of the main zone afforded **21** (0.466 g, 84%) as a colorless foam. TLC (silica gel, CH_2Cl_2/acetone, 85:15) R_f 0.7. ^{31}P NMR ($CDCl_3$, 121.5 MHz): 150.51, 150.53. ESI-TOF m/z calcd for $C_{60}H_{66}N_9O_9P$ [M + H$^+$] 1088.4800, found 1088.4794.

6-Amino-1,9-dihydro-9-[(3-*O*-propargyl)-β-D-ribofuranosyl]-2*H*-purin-2-one (2). The ribonucleoside **14** (0.190 g, 0.59 mmol) was suspended in 6 mL of water at 50 °C, and $NaNO_2$ (0.158 g, 2.29 mmol) in 1 mL of H_2O was added. Then acetic acid (0.247 g, 235 μL, 4.11 mmol) was added dropwise to the solution at 50 °C over 2 min. The resulting clear solution was stirred for 20 min at 50 °C. After completion of the reaction (TLC monitoring), the reaction mixture was diluted with water (3 mL), and cooled in an ice-bath, and aq ammonia was added dropwise until the pH reached 8. At this pH, a

dx.doi.org/10.1021/jo4012706 | J. Org. Chem. 2013, 78, 8545−8561

precipitate was formed that was then separated from the mother liquor by filtration, washed with water (3 × 5 mL), and dried under reduced pressure to obtain nucleoside **2** as colorless solid (0.130 g, 68%). TLC (silica gel, *i*-PrOH/H_2O/NH_3, 70:20:10) R_f 0.7. λ_{max} (MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 4900), 248 (8200), 298 (8900). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.47 (s, 1H, C≡CH), 3.50−3.54 (m, 1H, C5′-H), 3.62−3.66 (m, 1H, C5′-H), 4.07 (s, 2H, C2′-H, C4′-H), 4.31−4.43 (m, 2H, CH_2), 4.69 (br s, 1H, C3′-H), 5.59 (d, J = 5.7 Hz, 1H, C2′-OH), 5.62 (d, J = 6.9 Hz, 1H, C5′-OH), 5.92 (d, J = 8.7 Hz, 1H, C1′-H), 7.95 (s, 1H, C8-H), 10.86 (br s, 1H, NH). Anal. Calcd for $C_{13}H_{15}N_5O_5$ (321.3): C 48.60, H 4.71, N 21.80. Found: C 48.48, H 4.80, N 21.95. ESI-TOF m/z calcd for $C_{13}H_{15}N_5O_5$ [M + Na$^+$] 344.0971, found 344.0977.

6-{[1-(Dimethylamino)ethylidene]amino}-1,9-dihydro-9-[(3-*O*-propargyl)-β-D-ribofuranosyl]-2*H*-purin-2-one (16). As described for **15**. Compound **2** (0.102 g, 0.32 mmol) in methanol (5 mL) was treated with *N*,*N*-dimethylacetamide dimethyl acetal (0.114 g, 125 μL, 0.86 mmol), and the mixture was stirred at rt for 3 h. FC (silica gel, CH_2Cl_2/MeOH, 88:12) gave **16** (0.110 g, 89%) as colorless solid. TLC (silica gel, CH_2Cl_2/MeOH, 30:20) R_f 0.7. λ_{max} (MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 7 900), 337 (21 400), 248 (8 900). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.14 (s, 3H, CH_3), 3.09 (s, 3H, CH_3), 3.16 (s, 3H, CH_3), 3.47 (t, J = 2.4 Hz, 1H, C≡CH), 3.55−3.58 (m, 1H, C5′-H), 3.62−3.67 (m, 1H, C5′-H), 4.05−4.09 (m, 2H, C4′-H, C3′-H), 4.35 (d, J = 2.4 Hz, 1H, CH_2), 4.38 (d, J = 2.4 Hz, 1H, CH_2), 4.72−4.78 (m, 1H, C2′-H), 5.58 (d, J = 6.6 Hz, 1H, C2′-OH), 5.65 (d, J = 7.2 Hz, 1H, C5′-OH), 5.84−5.88 (m, 1H, C1′-H), 7.98 (s, 1H, C8-H), 11.04 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{22}N_6O_5$ (390.4): C 52.30, H 5.68, N 21.53. Found: C 52.28, H 5.60, N 21.30.

6-{[1-(Dimethylamino)ethylidene]amino}-9-[(3-*O*-propargyl)-β-D-ribofuranosyl]purin-2-yl-diphenylcarbamate (18). As described for **17**. Compound **16** (0.240 g, 0.61 mmol) in anhydrous pyridine (5 mL), *N*,*N*-diphenylcarbamoyl chloride (0.213 g, 0.92 mmol), (*i*-Pr)$_2$EtN (0.119 g, 154 μL, 0.92 mmol). FC (silica gel, CH_2Cl_2/MeOH, 95:5). Compound **18** was isolated as colorless foam (245 mg, 68%). TLC (silica gel, CH_2Cl_2/MeOH, 90:10) R_f 0.7. λ_{max} (MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 9 600), 310 (24 000), 234 (28 100). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.11 (s, 3H, CH_3), 3.13 (2s, 6H, 2 × CH_3), 3.48 (t, J = 2.4 Hz, 1H, C≡CH), 3.55−3.62 (m, 1H, C5′-H), 3.65−3.72 (m, 1H, C5′-H), 4.08−4.10 (m, 1H, C4′-H), 4.14−4.16 (m, 1H, C3′-H), 4.35 (d, J = 2.4 Hz, 1H, CH_2), 4.38 (d, J = 2.4 Hz, 1H, CH_2), 4.69−4.75 (m, 1H, C2′-H), 5.26 (t, J = 5.7 Hz, 1H, C5′-OH), 5.68 (d, J = 5.4 Hz, 1H, C2′-OH), 5.85 (d, J = 6.3 Hz, 1H, C1′-H), 7.27−7.34 (m, 2H, Ar-H), 7.43−7.44 (m, 8H, Ar-H), 8.43 (s, 1H, C8-H). Anal. Calcd for $C_{30}H_{31}N_7O_6$ (585.6): C 61.53, H 5.34, N 16.74. Found: C 61.38, H 5.40, N 16.81.

6-{[1-(Dimethylamino)ethylidene]amino}-9-[5-*O*-(4,4′-dimethoxytrityl)-3-*O*-(propargyl)-β-D-ribofuranosyl)]-purin-2-yl-diphenylcarbamate (20). As described for **19**. To compound **18** (0.150 g, 0.25 mmol) in anhydrous pyridine (500 μL) was added 4,4′-dimethoxytrityl chloride (0.130 g, 0.38 mmol), and the solution was stirred for 1 h at rt. FC (silica gel, CH_2Cl_2/acetone, 90:10) to obtain compound **20** (0.169 g, 74%) as a light yellow foam. TLC (silica gel, CH_2Cl_2/acetone, 40:10) R_f 0.4. λ_{max} (MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 16 400), 309 (26 100). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.09 (s, 3H, CH_3), 3.12 (2s, 6H, 2 × CH_3), 3.18−3.20 (m, 1H, C5′-H), 3.24−3.29 (m, 1H, C5′-H), 3.48 (t, J = 2.1 Hz, 1H, C≡CH), 3.70 (s, 6H, 2 × OCH_3), 4.10−4.15 (m, 1H, C4′-H), 4.32−4.36 (m, 3H, CH_2, C3′-H), 4.80−4.86 (m, 1H, C2′-H), 5.76 (d, J = 5.7 Hz, 1H, C2′-OH), 5.87 (d, J = 5.4 Hz, 1H, C1′-H), 6.81−6.85 (m, 4H, Ar-H), 7.19−7.32 (m, 12H, Ar-H), 7.35−7.44 (m, 7H, Ar-H), 8.30 (s, 1H, C8-H). Anal. Calcd for $C_{51}H_{49}N_7O_8$ (888.0): C 68.98, H 5.56, N 11.04. Found: C 69.45, H 5.82, N 10.65.

6-{[1-(Dimethylamino)ethylidene]amino}-9-[5-*O*-(4,4′-dimethoxytrityl)-3-*O*-(propargyl)-β-D-ribofuranosyl)]-purin-2-yl-diphenylcarbamate 2-*O*-(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (22). As described for **21**. To compound **20** (0.1 g, 0.11 mmol) in dry CH_2Cl_2 (5 mL) were added (*i*-Pr)$_2$NEt (0.027 g, 35 μL, 0.21 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (0.037 g, 35 μL, 0.15 mmol). FC (silica gel, eluted with CH_2Cl_2/acetone, 85:15). Evaporation of the main zone afforded **22** (45 mg, 37%) as a colorless foam. TLC (silica gel, CH_2Cl_2/acetone, 90:10) R_f 0.4. ^{31}P NMR ($CDCl_3$, 121.5 MHz): 150.79, 151.54. ESI-TOF m/z calcd for $C_{60}H_{66}N_9O_9P$ [M + H$^+$] 1088.4800, found 1088.4794.

4-Amino-1-{[2-[6-[1-(5-azidomethyl)pyridin-2-yl]-methyl]methyl-1*H*-1,2,3-triazol-4-yl]-β-D-ribofuranosyl}-2*H*-pyrimidin-2-one (23). Nucleoside **3** (0.1 g, 0.35 mmol) was dissolved in CH_3OH/CH_2Cl_2 (1:1, v/v, 2 mL), 2,5-bis(azidomethyl)pyridine **6** (0.067 g, 0.35 mmol) was added, and subsequently an aqueous solution of Cu(OAc)$_2$·H_2O (89 μL, 0.4 M in H_2O, 0.035 mmol) was added. The reaction mixture was stirred in the dark at room temperature for 8 h. After completion of the reaction (TLC monitoring), the solvent was evaporated, and the residue was applied to FC (silica gel, column 8 cm × 3 cm, CH_2Cl_2/MeOH, 85:15) to give **23** (0.1 g, 60%) as pale yellow solid. TLC (CH_2Cl_2/MeOH, 80:20) R_f 0.2. λ_{max} (MeOH)/nm 265 (ε/dm^3 mol^{-1} cm^{-1} 11 800). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.52−3.55 (m, 1H, C5′-H), 3.65−3.69 (m, 1H, C5′-H), 3.80−3.83 (m, 1H, C4′-H), 3.89 (t, J = 4.2 Hz, 1H, C3′-H), 4.04−4.11 (m, 1H, C2′-H), 4.51 (s, 2H, CH_2), 4.69−4.78 (m, 2H, CH_2), 5.10 (br s, 2H, C5′-OH, C3′-OH), 5.66−5.69 (m, 3H, C1′-H, CH_2), 5.88 (d, J = 3.6 Hz, 1H, C4-H), 7.17 (br d, 2H, NH_2), 7.28 (d, J = 8.10 Hz, 1H, Ar-H), 7.80−7.88 (m, 2H, C5-H, Ar-H), 8.15 (s, 1H, triazole-H), 8.53 (d, J = 1.8 Hz, 1H, Ar-H). ESI-TOF m/z calcd for $C_{19}H_{22}N_{10}O_5$ [M + Na$^+$] 493.1673, found 493.1667.

5,5′-[Pyridine-2,5-diyl-bis(methylene-1*H*-1,2,3-triazol)]-bis[1-(β-D-ribofuranosyl)-4-amino-2*H*-pyrimidin-2-one] (24). Monofunctionalized nucleoside **23** (0.050 g, 0.11 mmol) was dissolved in CH_3OH/CH_2Cl_2 (1:1, v/v, 2 mL), nucleoside **3** (0.030 g, 0.11 mmol) was added, and subsequently an aqueous solution of Cu(OAc)$_2$·H_2O (26 μL, 0.4 M in H_2O, 0.011 mmol) and freshly prepared sodium ascorbate (21 μL, 1 M in H_2O, 0.022 mmol) was added. The reaction mixture was stirred in the dark at room temperature overnight. After completion of the reaction (TLC monitoring), the solvent was evaporated, and the residue was applied to FC (silica gel, column 8 cm × 3 cm, CH_2Cl_2/MeOH, 70:30 with 1% NEt_3) to give **24** (0.061 g, 77%) as colorless solid. TLC (CH_2Cl_2/MeOH, 50:50 with 2% NEt_3) R_f 0.2. λ_{max} (MeOH)/nm 270 (ε/dm^3 mol^{-1} cm^{-1} 16 800). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.53−3.57 (m, 2H, 2 × C5′-H), 3.66−3.70 (m, 2H, 2 × C5′-H), 3.81−3.84 (m, 2H, 2 × C4′-H), 3.87−3.92 (m, 2H, 2 × C3′-H), 4.05−4.07 (m, 2H, 2 × C2′-H), 4.69−4.79 (m, 4H, 2 × CH_2), 5.10 (br s, 4H, 2 × C5′-OH, 2 × C3′-OH), 5.63 (s, 2H, 2 × C1′-H), 5.69 (t, J = 3.6 Hz, 4H, 2 × CH_2), 5.88 (t, J = 3.9 Hz, 2 × C4-H), 7.20 (br s, 4H, 2 × NH_2),

dx.doi.org/10.1021/jo4012706 | J. Org. Chem. 2013, 78, 8545−8561

7.28 (d, J = 8.10 Hz, 1H, Ar-H), 7.72−7.76 (m, 2H, C5-H, Ar-H), 7.86−7.89 (m, 2H, Ar-H), 8.14 (s, J = 6.3 Hz, 2H, 2 × triazole-H), 8.56 (d, J = 1.8 Hz, 1H, Ar-H). ESI-TOF m/z calcd for $C_{31}H_{37}N_{13}O_{10}$ [M + Na^+] 774.2684, found 774.2679.

Synthesis, Purification, and Characterization of Oligonucleotides. The syntheses of oligonucleotides **25**−**31**, **48**−**51**, **53**, **60**, **62**−**64**, and **67** were performed on a DNA synthesizer at a 1 μmol scale (trityl-on mode) using the phosphoramidites **10**, **21** and the phosphoramidite of 2′-*O*-propargyladenosine,[10] as well as the standard phosphoramidite building blocks following the synthesis protocol for 3′-*O*-(2-cyanoethyl)phosphoramidites. After cleavage from the solid support, the oligonucleotides were deprotected in 28% aqueous ammonia solution for 18 h at 60 °C. The purification of the "trityl-on" oligonucleotides was carried out on reversed-phase HPLC (RP-18 column; gradient system I). The purified "trityl-on" oligonucleotides were treated with 2.5% of $Cl_2CHCOOH$/CH_2Cl_2 for 5 min at 0 °C to remove the dimethoxytrityl residues. The detritylated oligomers were purified by reversed-phase HPLC (gradient II). The oligomers were desalted on a short column using distilled water for elution of salt, while the oligonucleotides were eluted with H_2O/MeOH (2:3). Then, the solvent was evaporated using a Speed Vac evaporator to yield colorless solids that were frozen at −24 °C. The molecular masses of the oligonucleotides were determined by MALDI-TOF mass spectrometry in the linear positive mode (Table 5). Extinction coefficients ε_{260} (H_2O) of nucleosides are dA, 15400; dG, 11700; dT, 8800; dC, 7300; **1**, 6000 (MeOH); **3**, 8200 (MeOH); **A***, 14800 (MeOH); $isoC_d$, 6100 (MeOH).

Synthesis of Azido-Modified Oligonucleotides Using the Nonchelating Azide 5 ("First Click"). To the single-stranded oligonucleotide **25**, **27**, **29**, or **31** (5.0 A_{260} units, 50 μmol) were added a mixture of a $CuSO_4$-TBTA ligand complex (50 μL of 20 mmol solution of $CuSO_4$ in H_2O/*t*-BuOH/DMSO (1:1:3) and 50 μL of 20 mmol solution of TBTA in *t*-BuOH/DMSO (1:3), tris(carboxyethyl)phosphine (TCEP; 50 μL of 20 mmol solution in H_2O), sodium bicarbonate ($NaHCO_3$, 50 μL of 100 mmol solution in H_2O),[9] the bis-azide **5** (37.5 μL of 20 mmol stock solution in THF/H_2O, 1:1), and 30 μL of DMSO, and then the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated in a Speed Vac, dissolved in 0.2 mL of bidistilled water, and centrifuged for 20 min at 14,000 rpm. The supernatant was collected and further purified by reversed-phase HPLC, using gradient I to give about 50−60% (2.5 A_{260} units) isolated yield of the azido-modified oligonucleotides **32**−**35**. The molecular masses of the azidomethylpyridine oligonucleotides of ODNs **32**−**35** were determined by MALDI-TOF mass spectrometry (Table 5).

Synthesis of Azido-Modified Oligonucleotides Using the Chelating Azide 6 ("First Click"). To the single-stranded oligonucleotides **25**, **27**, **29**, or **31** (3.0 A_{260} units, 30 μmol) or ODNs **50**, **51**, **53**, **60**, or **63** (3.0 A_{260} units, 30 μmol) were added a mixture of a $Cu(OAc)_2$-TBTA ligand complex (30 μL of 20 mmol solution of $Cu(OAc)_2$ in H_2O/*t*-BuOH/DMSO (4:1:3) and 20 μL of 20 mmol solution of TBTA in H_2O/*t*-BuOH/DMSO (4:1:3), 2,5-bis(azidomethyl)pyridine **6** (3 μL of a 20 mM stock solution in dioxane/H_2O, 1:1), sodium bicarbonate (20 μL of a 200 mM aq solution), and 30 μL of DMSO, and the reaction was stirred at room temperature for 8 h. The reaction mixture was concentrated in a Speed-Vac, dissolved in 200 μL of bidistilled water, and centrifuged for 20 min at 14,000 rpm. The supernatant solution was collected and further purified by reversed-phase HPLC (gradient II) to give about 70−75% isolated yield of the azidomethylpyridine oligonucleotides **40**−**43** and **55**−**59**. The molecular masses of the azidomethylpyridine oligonucleotides were determined by MALDI-TOF mass spectrometry (Table 5).

Cross-Linking of Azido-Modified Oligonucleotides Prepared with Nonchelating Azide 5 ("Second Click"). To the single-stranded oligonucleotides **25**, **27**, **29**, or **31** (2.0 A_{260} units, 20 μmol) were added a mixture of a $CuSO_4$-TBTA ligand complex (20 μL of 20 mmol solution of $CuSO_4$ in H_2O/*t*-BuOH/DMSO (1:1:3) and 20 μL of 20 mmol solution of TBTA in *t*-BuOH/DMSO (1:3), tris(carboxyethyl)phosphine (TCEP; 20 μL of 20 mmol solution in H_2O), sodium bicarbonate ($NaHCO_3$, 50 μL of 100 mmol solution in H_2O),[9] monofunctionalized ODNs **32**−**35** (1.5 A_{260} units, 15 μmol), and 30 μL of DMSO, and then the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated in a Speed Vac, dissolved in 0.2 mL of bidistilled water, and centrifuged for 20 min at 14,000 rpm. The supernatant was collected and further purified by reversed-phase HPLC, using gradient I to give about 50−60% (2.5 A_{260} units) of the cross-linked oligonucleotides **36**−**39**. The molecular masses of the cross-linked oligonucleotides were determined by MALDI-TOF mass spectrometry (Table 1).

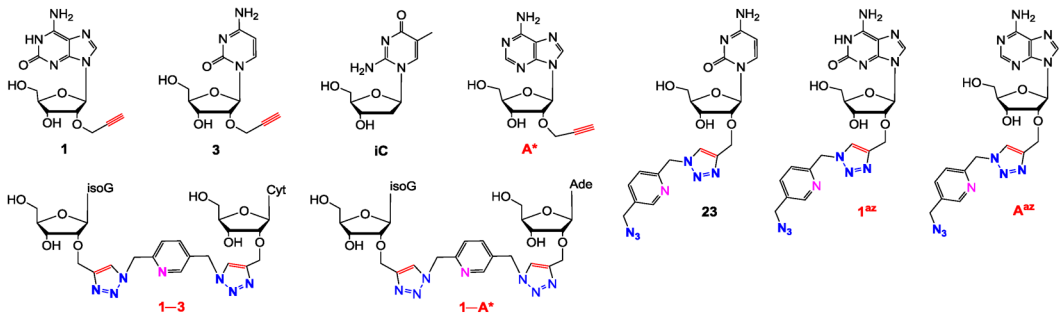
Cross-Linking of Azido-Modified Oligonucleotides Prepared with the Chelating Azide 6 Affording Homodimers ODNs 44−47 ("Second Click"). To the propargylated oligonucleotides **25**, **27**, **29**, or **31** (1.5 A_{260} units, 15 μmol) were added a mixture of the TBTA-$CuSO_4$ ligand complex (30 μL of a 20 mM stock solution in H_2O/DMSO/*t*-BuOH, 4:3:1 for TBTA; 20 μL of a 20 mM stock solution in H_2O/DMSO/*t*-BuOH, 4:3:1 for $CuSO_4$), tris-(carboxyethyl)phosphine (TCEP; 20 μL of a 20 mM stock solution in water), the azidomethylpyridine-labeled oligonucleotides **40**−**43** (2 A_{260} units, 20 μmol), benzoic acid (10 μL of a 100 mM stock solution in DMSO),[31] and 30 μL of DMSO, and the reaction was stirred at room temperature for 1 h. Then, sodium bicarbonate (100 μL of a 200 mM aq solution) was used to neutralize the excess of benzoic acid. The reaction mixture was concentrated in a Speed-Vac, dissolved in 200 μL bidistilled water, and centrifuged for 20 min at 14,000 rpm. The supernatant was collected and further purified by reversed-phase HPLC (gradient II) to give about 50−55% isolated yield of the cross-linked oligonucleotides **44**−**47**. The molecular masses of the cross-linked oligonucleotides were determined by MALDI-TOF mass spectrometry (Table 1).

Cross-Linking of Azido-Modified Oligonucleotides Prepared with the Chelating Azide 6 Affording Heterodimeric Click Adducts ODNs 52, 54, 61, 65, and 66 ("Second Click"). To a propargylated oligonucleotides **51**, **53**, **60**, or **64** (1.5 A_{260} units, 15 μmol) were added a mixture of the TBTA-$CuSO_4$ ligand complex (30 μL of a 20 mM stock solution in H_2O/DMSO/*t*-BuOH, 4:3:1 for TBTA; 20 μL of a 20 mM stock solution in H_2O/DMSO/*t*-BuOH, 4:3:1 for $CuSO_4$), tris(carboxyethyl)phosphine (TCEP; 20 μL of a 20 mM stock solution in water), the azidomethylpyridine-labeled oligonucleotides **55**−**59** (2 A_{260} units, 20 μmol), benzoic acid (10 μL of a 100 mM stock solution in DMSO),[31] and 30 μL of DMSO, and the reaction was stirred at room temperature for 1 h. Then, sodium bicarbonate (100 μL of a 200 mM aq solution) was used to neutralize the excess of benzoic acid. The reaction mixture was concentrated in a Speed-Vac, dissolved in 200 μL bidistilled water, and centrifuged for 20 min at 14,000

dx.doi.org/10.1021/jo4012706 | J. Org. Chem. 2013, 78, 8545−8561

Table 5. Molecular Masses of Oligonucleotides Measured by MALDI-TOF Mass Spectrometry[a]

Oligonucleotides	Mol. Wt. (Calc.)	Mol. Wt. (Found)
Propargylated oligonucleotides		
5'-d(TT**1** TTT TTT TAT TAA AAT TTA TAA A)-3' (**50**)	7703.0	7702.1
5'-d(**A***AA AAA AAA ATA ATT TTA AAT ATT T)-3' (**53**)	7741.1	7739.9
5'-d(AA**3** AAA AAA ATA ATT TTA AAT ATT T)-3' (**60**)	7717.1	7716.7
3'-d(TT**1** TTT TTT TAT TAA AAT TTA TAA A)-5' (**63**)	7703.0	7704.1
Azidoether-modified oligonucleotides		
5'-d(TA**1*** GTC AAT ACT)-3' (**32**)	3854.6	3854.7
5'-d(TAG GT**3*** AAT ACT)-3' (**33**)	3854.6	3855.0
5'-d(TAG GTC AAT A**3***T)-3' (**34**)	3854.6	3856.0
5'-d(AGT ATT GA**3*** CTA)-3' (**35**)	3854.6	3854.0
Azidomethylpyridine-modified oligonucleotides		
5'-d(TA**1**^{az} GTC AAT ACT) (**40**)	3887.6	3887.6
5'-d(TAG GT**23** AAT ACT) (**41**)	3887.6	3888.5
5'-d(TAG GTC AAT A**23**T) (**42**)	3887.6	3888.3
3'-d(ATC **23**AG TTA TGA) (**43**)	3887.6	3886.1
5'-d(TT**1**^{az} TTT TTT TAT TAA AAT TTA TAA A)-3' (**55**)	7892.2	7891.0
3'-d(TT**1**^{az} TTT TTT TAT TAA AAT TTA TAA A)-5' (**56**)	7892.2	7891.5
5'-d(AA**23** AAA AAA ATA ATT TTA AAT ATT T)-3' (**57**)	7906.3	7908.7
5'-d(**A**^{az}AA AAA AAA ATA ATT TTA AAT ATT T)-3' (**58**)	7930.3	7929.6
Cross-linked oligonucleotides		
5'-d(TT**1** TTT TTT TAT TAA AAT TTA TAA A)-3' 5'-d(**A***AA AAA AAA ATA ATT TTA AAT ATT T)-3' (**54**)	15633.3	15635.3
5'-d(TT**1** TTT TTT TAT TAA AAT TTA TAA A)-3' 5'-d(AA**3** AAA AAA ATA ATT TTA AAT ATT T)-3' (**61**)	15609.3	15612.4
3'-d(TT**1** TTT TTT TAT TAA AAT TTA TAA A)-5' 5'-d(**A***AA AAA AAA ATA ATT TTA AAT ATT T)-3' (**66**)	15633.3	15635.6



[a]Measured in the linear positive mode.

rpm. The supernatant was collected and further purified by reversed-phase HPLC (gradient II) to give the cross-linked heterodimeric oligonucleotides **52**, **54**, **61**, **65**, and **66** (50–60% yield). The molecular masses of all cross-linked oligonucleotides were determined by MALDI-TOF mass spectrometry (Table 5).

Cross-Linking of Homodimeric Oligonucleotides by the Bis-click Reaction Using Azide 5. To the single-stranded oligonucleotide **25**, **27**, **29**, or **31** (5.0 A_{260} units, 50

dx.doi.org/10.1021/jo4012706 | J. Org. Chem. 2013, 78, 8545–8561

μmol) were added a mixture of a $CuSO_4$-TBTA ligand complex (50 μL of 20 mmol solution of $CuSO_4$ in H_2O/*t*-BuOH/DMSO (1:1:3) and 50 μL of 20 mmol solution of TBTA in *t*-BuOH/DMSO (1:3), tris(carboxyethyl)phosphine (TCEP; 50 μL of 20 mmol solution in H_2O), sodium bicarbonate ($NaHCO_3$, 50 μL of 100 mmol solution in H_2O),[9] the bis-azide **5** (2.5 μL of 20 mmol stock solution in THF/H_2O, 1:1), and 30 μL of DMSO, and then the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated in a Speed Vac, dissolved in 0.2 mL of bidistilled water, and centrifuged for 20 min at 14,000 rpm. The supernatant was collected and further purified by reversed-phase HPLC, using gradient I to give about 50−60% (2.5 A_{260} units) of the cross-linked oligonucleotides **36**−**39**. The molecular masses of the cross-linked oligonucleotides obtained by the bis-click reaction correspond to those of the cross-linked oligonucleotides obtained by the stepwise procedure (Table 1).

Denaturing Polyacrylamide Gel Electrophoresis (PAGE). PAGE was performed on oligonucleotides **50**, **51**, **55**−**60** and cross-linked oligonucleotide adducts **52**, **54**, **61**, **65**, and **66**. PAGE analysis was carried out on a 17% polyacrylamide gel (acrylamide/bisacrylamide 19:1 with 7 M urea; 10 cm × 10 cm in 0.1 M tris-borate-EDTA (TBE) buffer containing 20 mM $MgCl_2$, pH 8.4). A 30 min prerun was performed in TBE buffer at room temperature. From a stock solution of the respective oligonucleotide (40 A_{260} units in 200 μL), a 2 μL portion was added to 5 μL of distilled water. Then, 10 μL of gel loading buffer (TBE) containing formamide (1:1) was added, and the oligonucleotide solution was loaded onto the gel. Electrophoresis was performed at rt for 4.5 h at a constant field strength of 12 V/cm. The gel was stained with 0.02% methylene blue for 20 min, incubated in water for 1 h to remove excess dye and scanned.

Ion-Exchange Chromatography. Ion-exchange chromatography was performed on a 4 mm × 250 mm DNA PA-100 column with a precolumn using a HPLC apparatus. Elution profiles were recorded at 260 nm. The azidomethylpyridine-labeled oligonucleotide ODN-**55** and cross-linked oligonucleotides ODN-**54**, ODN-**61** (0.1 A_{260} units each) were dissolved in 100 μL of water and then directly injected into the apparatus. The compounds were eluted using the following gradient. Buffer A: 25 mM Tris-HCl, 10% MeCN, pH 7.0. Buffer B: 25 mM Tris-HCl, 1.0 M NaCl, and 10% MeCN, pH 7.0. Elution gradient: 0−30 min 20−80% B in A with a flow rate of 0.75 mL min^{-1}.

■ ASSOCIATED CONTENT

Supporting Information

Coupling constants of nucleosides, structures of monofunctionalized oligonucleotides, HPLC profiles of selected oligonucleotides, melting curves of selected duplexes, ^1H, ^{13}C NMR, DEPT-135, and $^1H-^{13}C$ gated-decoupled NMR spectra of the nucleoside derivatives and click conjugates. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: frank.seela@uni-osnabrueck.de.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We would like to thank Dr. S. Budow and Dr. P. Leonard for helpful discussions and support while preparing the manuscript. We also thank Mr. H. Mei for measuring the NMR spectra and Mr. Nhat Quang Tran for the oligonucleotide syntheses. We would like to thank Dr. M. Letzel, Organisch-chemisches Institut, Universität Münster, Germany, for the measurement of the MALDI spectra. Financial support by ChemBiotech, Münster, Germany, is highly appreciated.

■ REFERENCES

(1) (a) *DNA Topology and Its Biological Effects*; Cozzarelli, N. R., Wang, J. C., Eds.; Cold Spring Harbor Laboratory Press: New York, 1990. (b) *DNA Structure and Function*; Sinden, R. R., Ed.; Academic Press: New York, 1994.

(2) (a) Lee, J. B.; Campolongo, M. J.; Kahn, J. S.; Roh, Y. H.; Hartman, M. R.; Luo, D. *Nanoscale* **2010**, *2*, 188−197. (b) Maiti, P. K.; Pascal, T. A.; Vaidehi, N.; Goddard, W. A. *J. Nanosci. Nanotechnol.* **2007**, *7*, 1712−1720. (c) Mahenthirlingam, E.; Bischof, J.; Byrne, S. K.; Radomski, C.; Davies, J. E.; Av-Gay, Y.; Vandamme, P. *J. Clin. Microbiol.* **2000**, *38*, 3165−3173. (d) Kwon, Y.-W.; Lee, C. H.; Choi, D.-H.; Jin, J.-I. *J. Mater. Chem.* **2009**, *19*, 1353−1380.

(3) (a) Seela, F.; Wei, C. *Helv. Chim. Acta* **1999**, *82*, 726−745. (b) Seela, F.; Peng, X.; Li, H. *J. Am. Chem. Soc.* **2005**, *127*, 7739−7751. (c) Geinguenaud, F.; Mondragon-Sanchez, J. A.; Liquier, J.; Shchyolkina, A. K.; Klement, R.; Arndt-Jovin, D. J.; Jovin, T. M.; Taillandier, E. *Spectrochim. Acta, Part A* **2005**, *61*, 579−587. (d) Shchyolkina, A. K.; Borisova, O. F.; Livshits, M. A.; Jovin, T. M. *Mol. Biol.* **2003**, *37*, 223−231. (e) Sugiyama, H.; Ikeda, S.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 9994−9995.

(4) (a) Rippe, K.; Fritsch, V.; Westhof, E.; Jovin, T. M. *EMBO J.* **1992**, *11*, 3777−3786. (b) Marfurt, J.; Leumann, C. *Angew. Chem., Int. Ed.* **1998**, *37*, 175−177. (c) Soyfer, V.; Potaman, V. N. *Triple-Helical Nucleic Acids*; Springer-Verlag: New York, 1996. (d) Fritzsche, H.; Akhebat, A.; Taillandier, E.; Rippe, K.; Jovin, T. M. *Nucleic Acids Res.* **1993**, *21*, 5085−5091.

(5) (a) Cory, J. G.; Suhadolnik, R. J. *Biochemistry* **1965**, *4*, 1729−1732. (b) Förtsch, I.; Fritzsche, H.; Birch-Hirschfeld, E.; Evertsz, E.; Klement, R.; Jovin, T. M.; Zimmer, C. *Biopolymers* **1995**, *38*, 209−220.

(6) van de Sande, J. H.; Ramsing, N. B.; Germann, M. W.; Elhorst, W.; Kalisch, B. W.; von Kitzing, E.; Pon, R. T.; Clegg, R. C.; Jovin, T. M. *Science* **1988**, *241*, 551−557.

(7) (a) Xu, X.; Muller, J. G.; Ye, Y.; Burrows, C. J. *J. Am. Chem. Soc.* **2008**, *130*, 703−709. (b) Kiviniemi, A.; Virta, P.; Lönnberg, H. *Bioconjugate Chem.* **2010**, *21*, 1890−1901. (c) Noll, D. M.; Mason, T. M.; Miller, P. S. *Chem. Rev.* **2006**, *106*, 277−301 and references therein. (d) Li, H.; Broughton-Head, V. J.; Peng, G.; Powers, V. E. C.; Ovens, M. J.; Fox, K. R.; Brown, T. *Bioconjugate Chem.* **2006**, *17*, 1561−1567. (e) Yoshimura, Y.; Ito, Y.; Fujimoto, K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1299−1301. (f) Hong, I. S.; Greenberg, M. M. *J. Am. Chem. Soc.* **2005**, *127*, 10510−10511.

(8) (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. (d) Meldal, M.; Tornøe, C. V. *Chem. Rev.* **2008**, *108*, 2952−3015. (e) Fischler, M.; Sologubenko, A.; Mayer, J.; Clever, G.; Burley, G.; Gierlich, J.; Carell, T.; Simon, U. *Chem. Commun.* **2008**, 169−171. (f) Seela, F.; Sirivolu, V. R.; Chittepu, P. *Bioconjugate Chem.* **2008**, *19*, 211−224.

(9) Pujari, S. S.; Xiong, H.; Seela, F. *J. Org. Chem.* **2010**, *75*, 8693−8696.

(10) Pujari, S. S.; Seela, F. *J. Org. Chem.* **2012**, *77*, 4460−4465.

(11) (a) Brotherton, W. S.; Michaels, H. A.; Simmons, J. T.; Clark, R. J.; Dalal, N. S.; Zhu, L. *Org. Lett.* **2009**, *11*, 4954−4957. (b) Kuang, G.-C.; Michaels, H. A.; Simmons, J. T.; Clark, R. J.; Zhu, L. *J. Org. Chem.* **2010**, *75*, 6540−6548. (c) Kuang, G.-C.; Guha, P. M.; Brotherton, W.

S.; Simmons, J. T.; Stankee, L. A.; Nguyen, B. T.; Clark, R. J.; Zhu, L. *J. Am. Chem. Soc.* **2011**, *133*, 13984−14001. (d) Uttamapinant, C.; Tangpeerachaikul, A.; Grecian, S.; Clarke, S.; Singh, U.; Slade, P.; Gee, K. R.; Ting, A. Y. *Angew. Chem., Int. Ed.* **2012**, *51*, 5852−5856. (e) Ingale, S. A.; Seela, F. *J. Org. Chem.* **2013**, *78*, 3394−3399.

(12) Alefelder, S.; Sigurdsson, S. Th. *Bioorg. Med. Chem.* **2000**, *8*, 269−273.

(13) (a) Grøtli, M.; Douglas, M.; Eritja, R.; Sproat, B. S. *Tetrahedron* **1998**, *54*, 5899−5914. (b) 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. (c) Srivastava, S. C.; Raza, S. K. Propargyl Modified Nucleosides and Nucleotides, US005744595A, 1998.

(14) Thomas, J. R.; Liu, X.; Hergenrother, P. J. *J. Am. Chem. Soc.* **2005**, *127*, 12434−12435.

(15) Yuan, Z.; Kuang, G.-C.; Clark, R. J.; Zhu, L. *Org. Lett.* **2012**, *14*, 2590−2593.

(16) (a) Jawalekar, A. M.; Meeuwenoord, N.; Cremers, J. (Sjef) G. O.; Overkleeft, H. S.; van der Marel, G. A.; Rutjes, F. P. J. T.; van Delft, F. L. *J. Org. Chem.* **2008**, *73*, 287−290. (b) Jawalekar, A. M.; Op de Beeck, M.; van Delft, F. L.; Madder, A. *Chem. Commun.* **2011**, *47*, 2796−2798. (c) Anisuzzaman, A. K. M.; Alam, F.; Soloway, A. H. *Polyhedron* **1990**, *9*, 891−892. (d) Zatesepin, T. S.; Romanova, E. A.; Oretskaya, T. S. *Russ. Chem. Rev.* **2002**, *71*, 513−534. (e) Wojtczak, B. A.; Andrysiak, A.; Grüner, B.; Lesnikowski, Z. J. *Chem.—Eur. J.* **2008**, *14*, 10675−10682. (f) Egli, M.; Minasov, G.; Tereshko, V.; Pallan, P. S.; Teplova, M.; Inamati, G. B.; Lesnik, E. A.; Owens, S. R.; Ross, B. S.; Prakash, T. P.; Manoharan, M. *Biochemistry* **2005**, *44*, 9045−9057.

(17) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316−1319.

(18) (a) Seela, F.; Gabler, B. *Helv. Chim. Acta* **1994**, *77*, 622−630. (b) Jurczyk, S. C.; Kodra, J. T.; Park, J.-H.; Benner, S. A.; Battersby, T. R. *Helv. Chim. Acta* **1999**, *82*, 1005−1015. (c) Davoll, J. *J. Am. Chem. Soc.* **1951**, *73*, 3174−3176.

(19) Seela, F.; Wei, C. *Helv. Chim. Acta* **1997**, *80*, 73−85.

(20) Pattabiraman, N. *Biopolymers* **1986**, *25*, 1603−1606.

(21) (a) Phillips, K.; Dauter, Z.; Murchie, A. I. H.; Lilley, D. M. J.; Luisi, B. *J. Mol. Biol.* **1997**, *273*, 171−182. (b) Parvathy, V. R.; Bhaumik, S. R.; Chary, K. V. R.; Govil, G.; Liu, K.; Howard, F. B.; Miles, H. T. *Nucleic Acids Res.* **2002**, *30*, 1500−1511.

(22) Seela, F.; He, Y. In *Modified Nucleosides, Synthesis and Applications*; Loakes, D., Ed.; Transworld Research Network: Kerala, 2002; pp 57−85.

(23) (a) Otto, C.; Thomas, G. A.; Rippe, K.; Jovin, T. M.; Peticolas, W. L. *Biochemistry* **1991**, *30*, 3062−3069. (b) Germann, M. W.; Zhou, N.; van de Sande, J. H.; Vogel, H. J. In *Methods in Enzymology*; Thomas, L. J., Ed.; Academic Press: New York, 1995; Vol. *261*, pp 207−225. (c) Rippe, K.; Jovin, T. M. *Methods Enzymol.* **1992**, *211*, 199−220.

(24) Jain, A. K.; Bhattacharya, S. *Bioconjugate Chem.* **2010**, *21*, 1389−1403.

(25) Cubero, E.; Luque, F. J.; Orozco, M. *J. Am. Chem. Soc.* **2001**, *123*, 12018−12025.

(26) (a) Yang, X.-L.; Sugiyama, H.; Ikeda, S.; Saito, I.; Wang, A. H.-J. *Biophys. J.* **1998**, *75*, 1163−1171. (b) Chen, X.; Kierzek, R.; Turner, D. H. *J. Am. Chem. Soc.* **2001**, *123*, 1267−1274.

(27) Ming, X.; Ding, P.; Leonard, P.; Budow, S.; Seela, F. *Org. Biomol. Chem.* **2012**, *10*, 1861−1869.

(28) Seela, F.; Wei, C.; Becher, G.; Zulauf, M.; Leonard, P. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 289−292.

(29) (a) Yan, H.; Zhang, X.; Shen, Z.; Seeman, N. C. *Nature* **2002**, *415*, 62−65. (b) Niemeyer, C. M.; Adler, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 3779−3783. (c) Muller, B. K.; Reuter, A.; Simmel, F. C; Lamb, D. C. *Nano Lett.* **2006**, *6*, 2814−2820. (d) Shin, J.-S.; Pierce, N. A. *J. Am. Chem. Soc.* **2004**, *126*, 10834−10835. (e) Wilner, O. I.; Willner, I. *Chem. Rev.* **2012**, *112*, 2528−2556.

(30) (a) Kozerski, L.; Sierzputowska-Gracz, H.; Krzyżosiak, W.; Bratek Wiewiórowska, M.; Jaskólski, M.; Wiewiórowski, M. *Nucleic Acids Res.* **1984**, *12*, 2205−2223. (b) Robins, M. J.; Hansske, F.; Bernier, S. E. *Can. J. Chem.* **1981**, *59*, 3360−3364. (c) Seela, F.; Fröhlich, T. *Helv. Chim. Acta* **1994**, *77*, 399−408.

(31) Shao, C.; Wang, X.; Xu, J.; Zhao, J.; Zhang, Q.; Hu, Y. *J. Org. Chem.* **2010**, *75*, 7002−7005.