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# Synthesis of Phosphoramidite Monomers Equipped with Complementary Bases for Solid-Phase DNA Oligomerization

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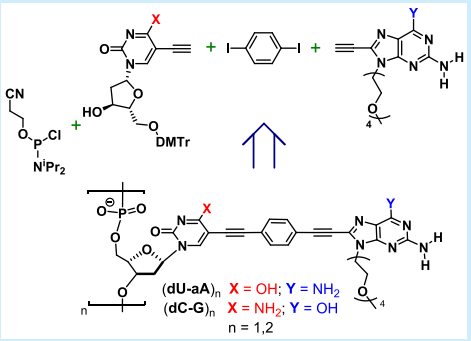
Supporting Information

**ABSTRACT:** We describe the preparation of two monomers that bear complementary nucleobases at the edges (guanine-2′-deoxycytidine and 2-aminoadenine-2′-deoxyuridine) and that are conveniently protected and activated for solid-phase automated DNA synthesis. We report the optimized synthetic routes leading to the four nucleobase derivatives involved, their cross-coupling reactions into dinucleobase-containing monomers, and their oligomerization in the DNA synthesizer.






DNA is a biopolymer with predictable and programmable interactions. This fact, together with the rapid development of tools for the synthesis of any arbitrary DNA sequence using automated phosphoramidite chemistry[1] or DNA-modifying enzymes, allured scientists from different fields to use it as a versatile building block in nanotechnology.[2] In parallel, a new frontier in organic chemistry has the goal of redesigning DNA by incorporation of non-natural bases or skeletons other than the ribose-phosphate in oligonucleotide sequences via automated DNA synthesis. A plethora of modified oligonucleotides has been developed to test noncanonical H-bonding pair motifs.[3] Artificial genetic sets are now available to provide a deeper insight into how DNA works and how far can we push DNA assembly away from Watson−Crick-bound duplexes.[4,5]

In this context, our group has recently established a versatile strategy to prepare hydrogen-bonded macrocycles from modified lipophilic dinucleoside monomers,[6] which comprise a $\pi$-conjugated *p*-diethynylbenzene unit substituted with complementary nucleobases at the edges. This rigid and linear linker structure, together with the 90° angle provided by Watson−Crick pairing, results in the formation of unstrained square-shaped H-bonded cyclic tetramers[7] with extraordinary fidelity (Figure 1a), due to the record chelate cooperativities attained.[8] More recently, we have demonstrated that this kind of macrocycles can undergo a nucleation−growth polymerization process that provides well-defined self-assembled nanotubes.[9]

As a natural continuation of our research, we decided to transfer this supramolecular motif to real DNA, in order to modify the classic duplex assembly mode of this relevant biological polymer. We describe herein the preparation of dinucleobase-containing monomers that are functionalized to enhance water solubility in the final structures, and conveniently protected and activated for solid-phase automated DNA synthesis (Figure 1b). Particularly, we detail the

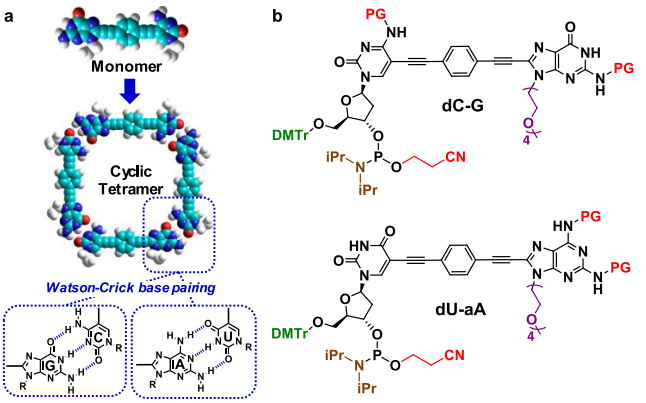



**Figure 1.** (a) Watson−Crick H-bonded cyclic tetramers self-assembled from monomers containing complementary nucleobases at the edges. (b) Chemical structure of target dinucleobase-containing monomers **dC-G** and **dU-aA** conveniently protected and activated as phosphoramidites.

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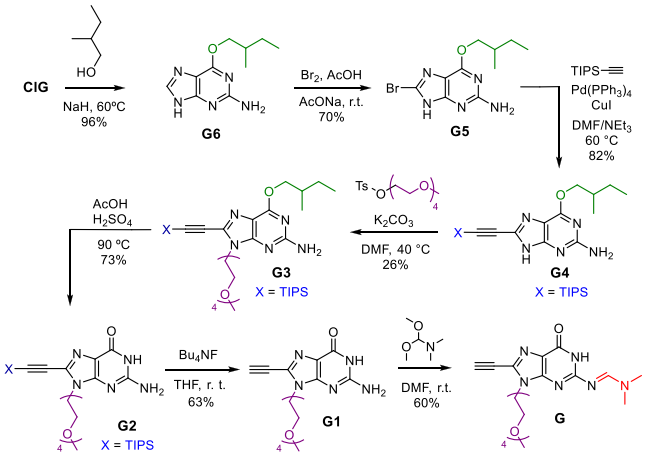
optimized synthetic routes leading to the four nucleobase derivatives involved, their cross-coupling into dinucleobase-containing monomers, and the corresponding first oligomers prepared in a DNA synthesizer.

The preparation of the mentioned derivatives requires the complementary functionalization of purines and pyrimidines to perform a final Sonogashira cross-coupling. This means that guanine (**G**) and 2-aminoadenine (**aA**) contain an ethynyl group at the *C*-8 position, while 2′-deoxycytidine (**dC**) and 2′-deoxyuridine (**dU**) carry an iodobenzene moiety at *C*-5. Besides, a tetraethylene glycol residue was introduced at position *N*-9 in the purines, to enhance solubility in water. Thus, for the preparation of these molecules, the most convergent synthetic pathway would first install the terminal ethynyl group[10] and then customize the purine *N*-9 or the 2′-deoxyribose moieties. We tried to pursue this convergent route as much as possible, but this often resulted in tedious purifications, solubility issues, or low yields/reactivity. As a result, we deviated from it whenever practical reasons demanded it, as explained below.

**Synthesis of Purines.** Purines carry solubilizing tetra(ethylene glycol) chains to increase aqueous solubility, while the exocyclic amines are blocked with diverse protecting groups.[11]

*Guanine.* Compound **G** was synthesized following three different routes from which we selected *Route 1*, as detailed below (Scheme 1), as the most efficient and convergent. It

**Scheme 1. Synthetic Route to G**

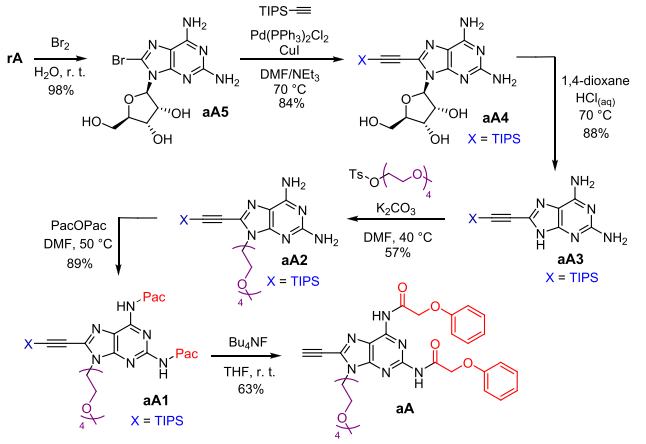



involves the preparation of the valuable ethynylated intermediate **G4**, to which any *N*-9 group, the position that provides functional versatility, could be attached. *Routes 2* and *3*, involving respectively a first alkylation reaction at *N*-9[12] or a depurination reaction, are described in the Supporting Information (SI).

The route starts from commercial 2-amino-6-chloropurine (**ClG**). An aromatic nucleophilic substitution reaction at *C*-6 with racemic 2-methyl-1-butanol in the presence of NaH[13] afforded **G6**. Bromination at *C*-8 leads to **G5**, which was then subjected to a cross-coupling Sonogashira reaction with tri(*iso*propyl)silylacetylene (TIPSA), to yield compound **G4**. The TIPS group was selected, since it offers enhanced solubility and robustness compared to the usual TMS. An alkylation reaction at *N*-9 with the tosylate of tetraethyleneglycol monomethyl ether produced compound **G3**. Only small amounts of the *N*-7 substituted product were obtained in this reaction, which was easily separated by column chromatography. Next, two protecting groups were removed, starting with the carbonyl protection at *C*-6, affording **G2**, and then with the silane protection of the ethynyl group, in the presence of $Bu_4NF$, generating guanine **G1**. Finally, protection of the amine group was set up with *N*,*N*-dimethylacetamide dimethyl acetal, to yield compound **G**.

*2-Aminoadenine.* For the synthesis of compound **aA**, we decided to optimize a synthetic route that uses the ribose in 2-aminoadenosine (**rA**) as a *N*-9 protecting group, so that alkylation can also be performed at a later stage in the route, as shown in Scheme 2. Commercial 2-aminoadenosine was first

**Scheme 2. Synthetic Route to aA**




subjected to a halogenation reaction with bromine, yielding **aA5** after simple filtration and washing.[14] Then, we performed the direct Sonogashira reaction on **aA5** with TIPSA. Despite the low solubility of both reactants and products, **aA4** could be obtained in quite good yields. Subsequently, several conditions were studied to perform the depurination reaction[15] on **aA4**.[16−18] Glycosidic bond cleavage in a 1:1 1,4-dioxane/HCl mixture at 70 °C supplied **aA3** in good yields. Next, an alkylation reaction afforded hydrophilic *N*-9-substituted **aA2** as the only regioisomer. We then protected both exocyclic amine groups with 2-phenoxyacetyl anhydride, to generate **aA1**, followed by the deprotection of the 8-ethynyl group, to yield **aA**.

**Synthesis of Pyrimidines.** Pyrimidines were selected as the base that will carry the deoxyribose-phosphate backbone in the final oligonucleotides, due to the lower steric hindrance around the reactive 3′- and 5′-ribose positions. Hence, a synthetic route was optimized to provide **dC** and **dU**, protected with dimethoxytrityl (DMTr) at the 5′-position and bearing a *p*-phenylene central block and a free 3′-*OH* group, to install the phosphoramidite at a later stage. Slight modifications were introduced in the synthetic itinerary to these compounds regarding the order in which the central block and the DMTr group were incorporated (see also SI).

*2′-Deoxycytidine.* Commercial 2′-deoxycytidine **dCy** was first subjected to a previously described iodination reaction (Scheme 3), in the presence of *m*-chloroperbenzoic acid, to yield **dC5**.[19] The ethynyl group was then incorporated at the *C*-5 position by Sonogashira coupling with TMSA, to afford **dC4**,[20] which was then subjected to TMS deprotection in the presence of $K_2CO_3$, yielding intermediate **dC3**. From **dC3**, we considered whether to protect the 5′-*OH* function with



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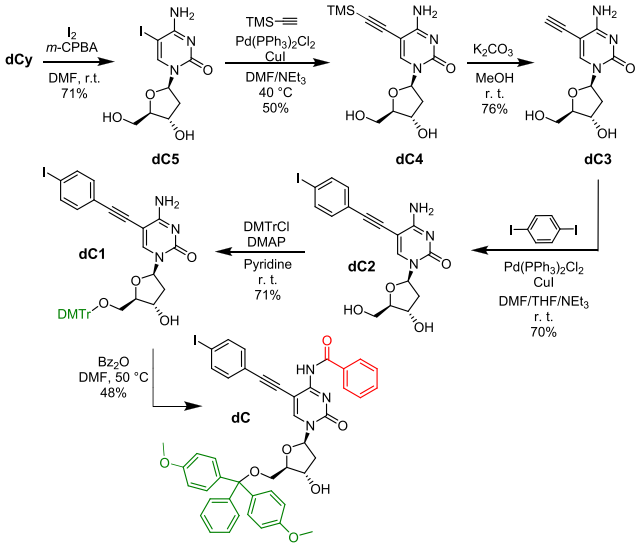
**Scheme 3. Synthetic Route to dC**

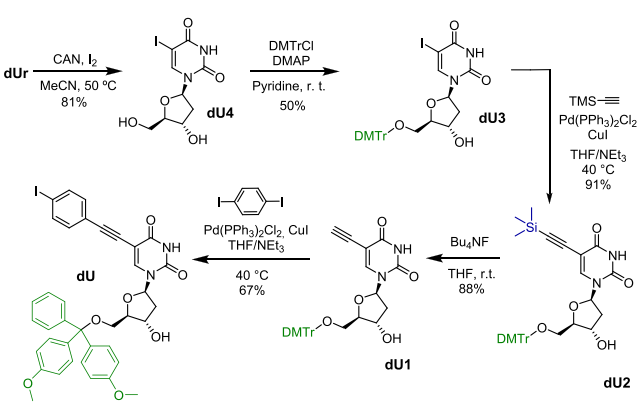



DMTrCl or to install first the phenylene spacer. The latter was a better option in terms of overall yields (70%). Hence, the Pd-catalyzed reaction of **dC3** with an excess of *p*-diiodobenzene (to maximize the monocoupling reaction) provided **dC2**. Subsequently, different conditions were tested for the protection of the 5′-*OH* with the bulky DMTr group.[21−23] The best results were achieved using DMTrCl/DMAP/py in the presence of 3 Å molecular sieves, which led to **dC1** in reasonable yields. Then, we dealt with the incorporation of the 4-amine protecting group. A resilient and relatively bulky benzoyl group was chosen for this purpose, as previous trials with the more labile acetyl moiety led to the loss of this protecting group in subsequent steps (see SI). The reaction between benzoic anhydride and **dC1** proved to be selective for the amine without the need for protecting the 3′-*OH* function, yielding **dC**. An alternative approach would involve the protection of the 3′-*OH* as a TBDMS group, before the amine protection (see SI).

*2′-Deoxyuridine.* These analogs (Scheme 4) do not require nucleobase protecting groups and are more soluble and usually easier to purify than their dC analogs.

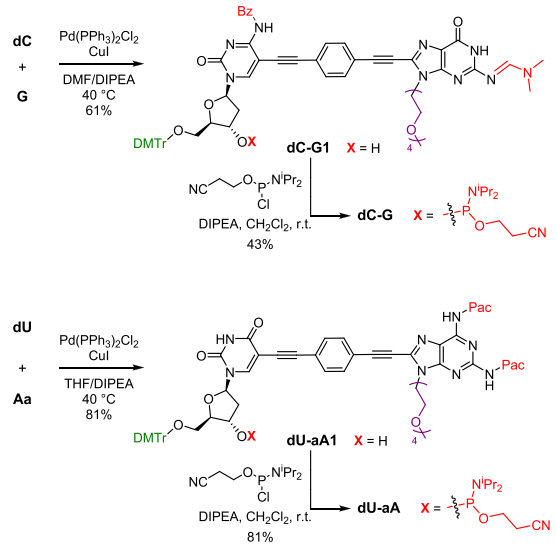
We started with the iodination of commercial 2′-deoxyuridine (**dUr**) following a reported procedure.[24] The next reaction, in contrast to the **dC** route, included incorporation of the bulky DMTr substituent at the 5′-*O* position, as described in the literature,[25] to provide **dU3**.[25d] Cross-coupling with TMSA and subsequent TMS-cleavage protocols were then performed to afford **dU2** and **dU1**, in that order. This last compound was finally subjected to a Sonogashira reaction with excess 1,4-diiodobenzene, giving **dU** in satisfactory yields.

**Scheme 4. Synthetic Route to dU**




**Monomer Synthesis.** The hydrophilic **dC-G** and **dU-aA** monomers targeted for DNA solid-phase synthesis were prepared in two additional steps. The first one consists of a Pd-catalyzed Sonogashira coupling between the purine (**G**, **aA**) and pyrimidine (**dC**, **dU**) fragments previously prepared (Scheme 5). In general, the bulkier anhydrous NEt($^i$Pr)$_2$ (DIPEA) base was used in these reactions to prevent the deprotection of the base-sensitive protecting groups. Besides, a slight excess of the 8-ethynylpurines was slowly incorporated dropwise during the Pd-catalyzed coupling reaction, to minimize the formation of homocoupled side products. In the last step, we installed a reactive phosphoramidite at the 3′-*O* position of the pyrimidines, in order to perform the oligomerization afterwards by automated means. Such reaction is carried out under rigorous anhydrous conditions with 2-cyanoethyl-*N*,*N*-di*iso*propylchlorophosphoramidite, to obtain **dC-G** and **dU-aA**. The introduction of the phosphoramidite results in the appearance of two different diastereoisomers, which can be observed in the $^1$H and $^{31}$P NMR spectra (see SI).

**Scheme 5. Synthetic Route to Monomers dC-G and dU-aA**




**Oligomerization of the Dinucleobase-Containing Monomers.** Finally, we tested the reactivity of compounds **dU-aA** and **dC-G** in the DNA synthesizer, using only slightly modified conditions with respect to the standard ones, and optimized a protocol for the purification of these oligomers, which are more lipophilic than regular DNA (see SI). Our objective at this point was to prove their incorporation in oligonucleotide strands, not only individually but also consecutively. For this reason, and as a proof of concept, we targeted four different short oligonucleotides in which we varied the number of modified **dU-aA** and **dC-G** monomers introduced: 1 or 2 (Figure 2).

We decided to use CPG coated with thymidine (**T**), since it offered additional possibilities for quantification by UV−vis absorbance, in which the molar extinction coefficient of the



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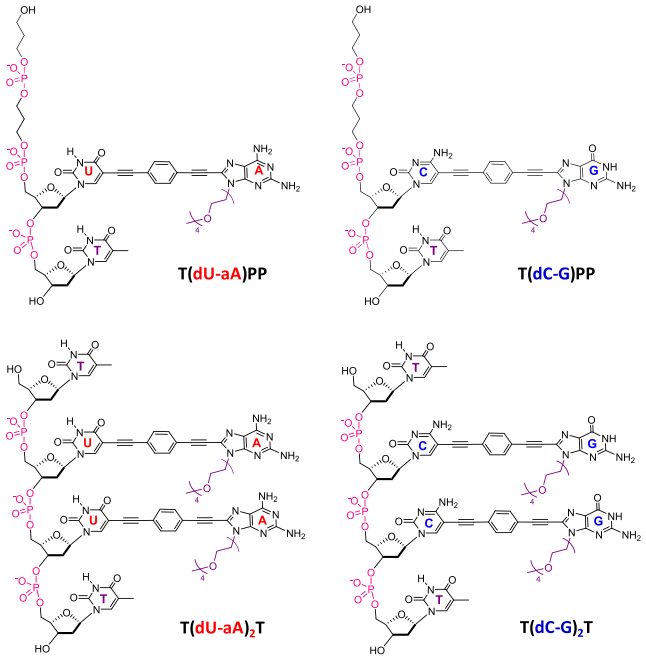


**Figure 2.** Schematic representation of the four oligonucleotides prepared, bearing monomers **dU-aA** or **dC-G**.

monomers at 355 nm (36294 ± 500 $M^{-1}cm^{-1}$ for **dU-aA**; 47135 ± 750 $M^{-1}$ $cm^{-1}$ for **dC-G**) was employed. At the 5′-ends we introduced phosphate groups carrying a propylene chain (**P**) or thymines (**T**), which provide a higher number of ionic groups that enabled us to use standard DNA methodologies to purify our compounds (more details can be found in the SI). The final oligonucleotides, which are perfectly soluble in water below $10^{-3}$ M, were characterized by UV−vis spectroscopy and MALDI-TOF spectrometry (see sections 1.6−1.7 in the SI).

In summary, the optimized synthetic routes leading to two pairs of self-complementary nucleobases (**G**, **dC** and **aA**, **dU**) have been described and are complemented with additional routes described in the SI. These valuable novel synthetic intermediates were, in a subsequent step, cross-coupled through Sonogashira reactions and activated as phosphoramidites, leading to **dC-G** and **dU-aA**. Finally, we explored the reactivity of these modified nucleotides in solid-phase automated DNA synthesis, coupling them to short oligonucleotide sequences and testing consecutive couplings. The overall yields obtained are considered high enough to approach in the future the synthesis of longer modified oligonucleotides, with the aim to explore their supramolecular influence on DNA self-assembly.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.9b03801.

Experimental procedures, $^{1}H$ NMR, $^{13}C$ NMR and HRMS characterization data, $^{1}H$ and $^{13}C$ NMR spectra for all novel compounds, and MALDI-TOF and UV−vis spectra of the oligonucleotides; alternative routes for the synthesis of **G** and **dC** (PDF)

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### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES


(1) Ellington, A.; Pollard, J. D. Synthesis and purification of oligonucleotides. *Current Protocols in Molecular Biology*; John Wiley & Sons, Inc.: 2001.

(2) (a) Seeman, N. C. Nanomaterials Based on DNA. *Annu. Rev. Biochem.* **2010**, *79*, 65−87. (b) Special issue on DNA nanotechnology. *Nat. Nanotechnol.* *6*, **2011**.

(3) (a) Carell, T.; Brandmayr, C.; Hienzsch, A.; Müller, M.; Pearson, D.; Reiter, V.; Thoma, I.; Thumbs, P.; Wagner, M. Structure and Function of Noncanonical Nucleobases. *Angew. Chem., Int. Ed.* **2012**, *51*, 7110−7131. (b) Benner, S. A. Understanding Nucleic Acids Using Synthetic Chemistry. *Acc. Chem. Res.* **2004**, *37*, 784−797.

(4) (a) Kool, E. T.; Morales, J. C.; Guckian, K. M. Mimicking the Structure and Function of DNA: Insights into DNA Stability and Replication. *Angew. Chem., Int. Ed.* **2000**, *39*, 990−1009. (b) Winnacker, M.; Kool, E. T. Artificial Genetic Sets Composed of Size-Expanded Base Pairs. *Angew. Chem., Int. Ed.* **2013**, *52*, 12498−12508. (c) Mohapatra, B.; Pratibha; Verma, S. Directed adenine functionalization for creating complex architectures for material and biological applications. *Chem. Commun.* **2017**, *53*, 4748−4758.

(5) Hirao, I.; Kimoto, M.; Yamashige, R. Natural versus Artificial Creation of Base Pairs in DNA: Origin of Nucleobases from the Perspectves of Unnatural Base Pair Studies. *Acc. Chem. Res.* **2012**, *45*, 2055−2065.

(6) (a) Montoro-García, C.; Camacho-García, J.; López-Pérez, A. M.; Bilbao, N.; Romero-Pérez, S.; Mayoral, M. J.; González-Rodríguez, D. High-Fidelity Noncovalent Synthesis of Hydrogen-Bonded Macrocyclic Assemblies. *Angew. Chem., Int. Ed.* **2015**, *54*, 6780−6784. (b) Romero-Pérez, S.; Camacho-García, J.; Montoro-García, C.; López-Pérez, A. M.; Sanz, A.; Mayoral, M. J.; González-Rodríguez, D. G-Arylated Hydrogen-Bonded Cyclic Tetramer Assemblies with Remarkable Thermodynamic and Kinetic Stability. *Org. Lett.* **2015**, *17*, 2664−2667. (c) Montoro-García, C.; Camacho-García, J.; López-Pérez, A. M.; Mayoral, M. J.; Bilbao, N.; González-Rodríguez, D. Role of the Symmetry of Multipoint Hydrogen Bonding on Chelate Cooperativity in Supramolecular Macrocyclization Processes. *Angew. Chem., Int. Ed.* **2016**, *55*, 223−227. (d) Montoro-García, C.; Mayoral, M. J.; Chamorro, R.; González-Rodríguez, D. How Large Can we Build a Cyclic Assembly? Impact of Ring Size on Chelate Cooperativity in Noncovalent Macrocyclizations. *Angew. Chem., Int. Ed.* **2017**, *56*, 15649−15653. (e) Mayoral, M. J.; Serrano-Molina, D.; Camacho-García, J.; Magdalena-Estirado, E.; Blanco-Lomas, M.; Fadaei, E.; González-Rodríguez, D. Understanding




DOI: 10.1021/acs.orglett.9b03801
*Org. Lett.* XXXX, XXX, XXX−XXX

Complex Supramolecular Landscapes: Non-covalent Macrocyclization Equilibria Examined by Fluorescence Resonance Energy Transfer. *Chem. Sci.* **2018**, *9*, 7809−7821.
(7) Bilbao, N.; Destoop, I.; De Feyter, S.; González-Rodríguez, D. Two-Dimensional Nanoporous Networks Formed by Liquid-to-Solid Transfer of Hydrogen-Bonded Macrocycles Built from DNA Bases. *Angew. Chem., Int. Ed.* **2016**, *55*, 659−663.
(8) (a) Mayoral, M. J.; Bilbao, N.; González-Rodríguez, D. Hydrogen-bonded Macrocyclic Supramolecular Systems in Solution and onto Surfaces. *ChemistryOpen* **2016**, *5*, 10−32. (b) Aparicio, F.; Mayoral, M. J.; Montoro-García, C.; González-Rodríguez, D. Guidelines for the Assembly of Hydrogen-bonded Macrocycles. *Chem. Commun.* **2019**, *55*, 7277−7299.
(9) Vázquez-González, V.; Mayoral, M. J.; Chamorro, R.; Hendrix, M. M. R. M.; Voets, I. K.; González-Rodríguez, D. Noncovalent Synthesis of Self-assembled Nanotubes through Decoupled Hierarchical Cooperative Processes. *J. Am. Chem. Soc.* **2019**, *141*, 16432−16438.
(10) (a) Chinchilla, R.; Nájera, C. Recent advances in Sonogashira reactions. *Chem. Soc. Rev.* **2011**, *40*, 5084−5121. (b) Agrofoglio, L. A.; Gillaizeau, I.; Saito, Y. Palladium-Assisted Routes to Nucleosides. *Chem. Rev.* **2003**, *103*, 1875−1916. (c) Bielski, R.; Witczak, Z. J. Strategies for Coupling Molecular Units if Subsequent Decoupling Is Required. *Chem. Rev.* **2013**, *113*, 2205−2243.
(11) Wei, X. Coupling activators for the oligonucleotide synthesis via phosphoramidite approach. *Tetrahedron* **2013**, *69*, 3615−3637.
(12) (a) Bilbao, N.; Vázquez-González, V.; Aranda, M. T.; González-Rodríguez, D. Synthesis of 5-/8-Halogenated or Ethynylated Lipophilic Nucleobases as Potential Synthetic Intermediates for Supramolecular Chemistry. *Eur. J. Org. Chem.* **2015**, *2015*, 7160−7175. (b) Camacho-García, J.; Montoro-García, C.; López-Pérez, A. M.; Bilbao, N.; Romero-Pérez, S.; González-Rodríguez, D. Synthesis and Complementary Self-association of Novel Lipophilic $\pi$-Conjugated Nucleoside Oligomers. *Org. Biomol. Chem.* **2015**, *13*, 4506−4513.
(13) Sun, F. P.; Darbre, T. Synthesis and Irradiation of Vitamin-B12-Derived Complexes Incorporating Peripheral G·C Base Pairs. *Helv. Chim. Acta* **2002**, *85*, 3002−3018.
(14) Kaloudis, P.; D'Angelantonio, M.; Guerra, M.; Gimisis, T.; Mulazzani, Q. G.; Chatgilialoglu, C. One-electron reduction of 8-bromo-2-aminoadenosine: Chemical radiation and DFT studies of the mechanism. *J. Phys. Chem. B* **2008**, *112*, 5209−5217.
(15) (a) Garrett, E. R.; Mehta, P. J. Solvolysis of adenine nucleosides. I. Effects of sugars and adenine substituents on acid solvolyses. *J. Am. Chem. Soc.* **1972**, *94*, 8532−8541. (b) Rios, A. C.; Yu, H. T.; Tor, Y. Hydrolytic fitness of N-glycosyl bonds: comparing the deglycosylation kinetics of modified, alternative, and native nucleosides. *J. Phys. Org. Chem.* **2015**, *28*, 173−180.
(16) Kaiya, T.; Tanaka, H.; Kohda, K. Formation of 2'-deoxy-2-nitroadenosines by reaction of 2'-deoxyadenosines with copper(II) Nitrate/acetic anhydride. *Nucleosides, Nucleotides Nucleic Acids* **2002**, *21*, 427−433.
(17) Rodenko, B.; Wanner, M. J.; Alkhaldi, A. A. M.; Ebiloma, G. U.; Barnes, R. L.; Kaiser, M.; Brun, R.; McCulloch, R.; Koomen, G.; de Koning, H. P. Targeting the Parasite's DNA with Methyltriazenyl Purine Analogs Is a Safe, Selective, and Efficacious Antitrypanosomal Strategy. *Antimicrob. Agents Chemother.* **2015**, *59*, 6708−6716.
(18) Rodriguez, A. A.; Cedillo, I.; Mowery, B. P.; Gaus, H. J.; Krishnamoorthy, S. S.; McPherson, A. K. Formation of the $N^2$-acetyl-2,6-diaminopurine oligonucleotide impurity caused by acetyl capping. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 3243−3246.
(19) Münzel, M.; Globisch, D.; Trindler, C.; Carell, T. Efficient Synthesis of 5-Hydroxymethylcytosine Containing DNA. *Org. Lett.* **2010**, *12*, 5671−5673.
(20) Andersen, N. K.; Dossing, H.; Jensen, F.; Vester, B.; Nielsen, P. Duplex and Triplex Formation of Mixed Pyrimidine Oligonucleotides with Stacking of Phenyl-triazole Moieties in the Major Groove. *J. Org. Chem.* **2011**, *76*, 6177−6187.
(21) (a) Andersen, N. K.; Dossing, H.; Jensen, F.; Vester, B.; Nielsen, P. Duplex and Triplex Formation of Mixed Pyrimidine Oligonucleotides with Stacking of Phenyl-triazole Moieties in the Major Groove. *J. Org. Chem.* **2011**, *76*, 6177−6187. (b) Seela, F.; Budow, S.; Leonard, P. Oligonucleotides forming an i-motif: the pH-dependent assembly of individual strands and branched structures containing 2′-deoxy-5-propynylcytidine. *Org. Biomol. Chem.* **2007**, *5*, 1858−1872. (c) Song, Z.; Zhang, Q. Design, Synthesis, and Incorporation of Fluorous 5-Methylcytosines into Oligonucleotides. *J. Org. Chem.* **2011**, *76*, 10263−10268.
(22) Roychowdhury, A.; Illangkoon, H.; Hendrickson, C. L.; Benner, S. A. 2′-Deoxycytidines Carrying Amino and Thiol Functionality: Synthesis and Incorporation by Vent (Exo$^-$) Polymerase. *Org. Lett.* **2004**, *6*, 489−492.
(23) (a) Dohno, C.; Shibata, T.; Okazaki, M.; Makishi, S.; Nakatani, K. Amphiphilic DNA Duplex Stabilized by a Hydrophobic Zipper. *Eur. J. Org. Chem.* **2012**, *2012*, 5317−5323. (b) Pujari, S. S.; Seela, F. Parallel Stranded DNA Stabilized with Internal Sugar Cross-Links: Synthesis and Click Ligation of Oligonucleotides Containing 2′-Propargylated Isoguanosine. *J. Org. Chem.* **2013**, *78*, 8545−8561.
(24) Asakura, J.; Robins, M. J. Cerium(IV)-mediated halogenation at C-5 of uracil derivatives. *J. Org. Chem.* **1990**, *55*, 4928−4933.
(25) (a) Börjesson, K.; Wiberg, J.; El-Sagheer, A. H.; Ljungdahl, T.; Mårtensson, J.; Brown, T.; Nordén, B.; Albinsson, B. Functionalized Nanostructures: Redox-Active Porphyrin Anchors for Supramolecular DNA Assemblies. *ACS Nano* **2010**, *4*, 5037−5046. (b) Tashiro, R.; Iwamoto, M.; Morinaga, H.; Emura, T.; Hidaka, K.; Endo, M.; Sugiyama, H. Linking two DNA duplexes with a rigid linker for DNA nanotechnology. *Nucleic Acids Res.* **2015**, *43*, 6692−6700. (c) Gaballah, S. T.; Netzel, T. L. Synthesis of 5-(Pyridinyl and Pyridiniumyl)-20-Deoxyuridine Nucleosides: Reversible Electron Traps for DNA. *Nucleosides, Nucleotides Nucleic Acids* **2002**, *21*, 681−694. (d) Segal, M.; Yavin, E.; Kafri, P.; Shav-Tal, Y.; Fischer, B. Detection of mRNA of the Cyclin D1 Breast Cancer Marker by a Novel Duplex-DNA Probe. *J. Med. Chem.* **2013**, *56*, 4860−4869.


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