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# Synthesis of phosphoramidite monomers equipped with complementary bases for solid-phase DNA oligomerization

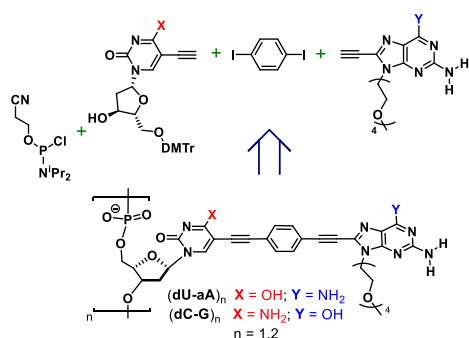
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Supporting Information Placeholder

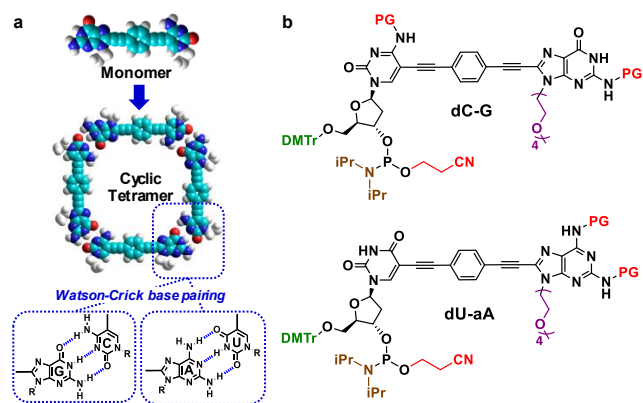


**ABSTRACT:** We describe the preparation of two monomers that bear complementary nucleobases at the edges (guanine-2'-deoxycytidine and 2-aminoadenine-2'-deoxyuridine) and that are conveniently protected and activated for solid-phase automated DNA synthesis. We report the optimized synthetic routes leading to the four nucleobase derivatives involved, their cross-coupling reactions into dinucleobase-containing monomers, as well as 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<sup>1</sup> or DNA-modifying enzymes, allured scientists from different fields to use it as a versatile building block in nanotechnology.<sup>2</sup> 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.<sup>3</sup> 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.<sup>4,5</sup>

In this context, our group has recently established a versatile strategy to prepare hydrogen-bonded macrocycles from modified lipophilic dinucleoside monomers,<sup>6</sup> 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<sup>7</sup> with extraordinary fidelity (Figure 1a), due to the record chelate cooperativities attained.<sup>8</sup>

More recently, we have demonstrated that this kind of macrocycles can undergo a nucleation-growth polymerization process that provides well-defined self-assembled nanotubes.<sup>9</sup>



**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.

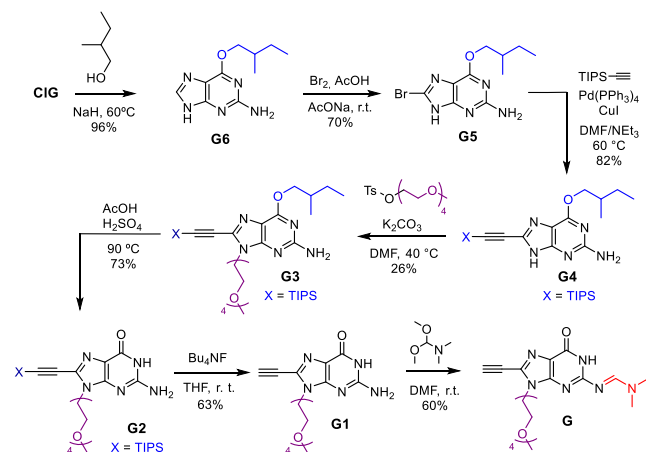
As a natural continuation of our research, we decided to transfer this supramolecular motif to real DNA, in order to modify the classic duplex assembly mode of this relevant biological polymer. We describe herein the preparation of dinucleobase-containing monomers that are functionalized to enhance water solubility in the final structures, and conveniently protected and activated for solid-phase automated DNA synthesis (Figure 1b). Particularly, we detail the optimized synthetic routes leading to the 4 nucleobase derivatives involved, their cross-coupling into dinucleobase-containing monomers, as well as 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 deoxycytidine (**dC**) and deoxyuridine (**dU**), carry an iodobenzene moiety at *C*-5. Besides, a tetraethyleneglycol 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<sup>10</sup> and then customize the purine *N*-9 or the 2'-deoxyribose moieties. We tried to pursue this convergent route as much as possible, but often resulted in tedious purifications, solubility issues, or low yields/reactivity, so we deviated from it whenever practical reasons demanded it, as explained below.

**Synthesis of purines.** Purines carry solubilizing tetra(ethyleneglycol) chains to increase aqueous solubility, while the exocyclic amines are blocked with diverse protecting groups.<sup>11</sup>

**Guanine.** Compound **G** was synthesized following 3 different routes from which we selected *Route 1*, as detailed below (Scheme 1), as the most efficient and convergent. It 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<sup>12</sup> or a depurination reaction, are described in the S.I.

**Scheme 1. Synthetic route to G.**

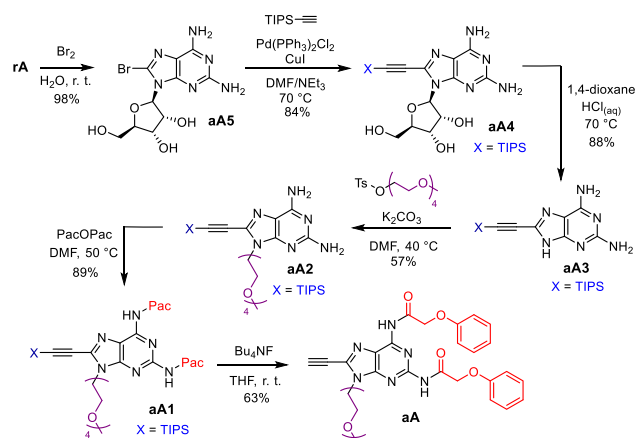


The route starts from commercial 2-amino-6-chloropurine (**ClG**). An aromatic nucleophilic substitution reaction at *C*-6 with racemic 2-methyl-1-butanol in the presence of NaH,<sup>13</sup> afforded **G6**. Bromination at *C*-8 leads to **G5**, which was then subjected to a cross-coupling Sonogashira reaction with tri(*isopropyl*)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<sub>4</sub>NF, generating guanine **G1**. Finally, the 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 subjected to a halogenation reaction with bromine, yielding **aA5** after simple filtration and washing.<sup>14</sup> 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<sup>15</sup> on **aA4**.<sup>16-18</sup> 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**.

**Scheme 2. Synthetic route to aA.**

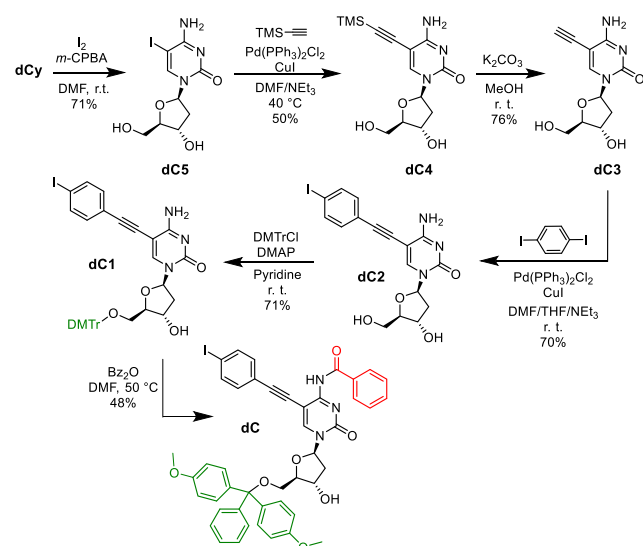


**Synthesis of pyrimidines.** Pyrimidines were selected as the base that will carry the deoxyribose-phosphate backbone in the final oligonucleotides, due to the lower steric hindrance around the reactive 3'- and 5'-ribose positions. Hence, a synthetic route was optimized to provide **dC** and **dU**, 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 S.I.).

**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**.<sup>19</sup> The ethynyl group was then incorporated at the *C*-5 position by Sonogashira coupling with TMSA, to afford **dC4**,<sup>20</sup> which was then subjected to TMS deprotection in the presence of K<sub>2</sub>CO<sub>3</sub>, yielding intermediate **dC3**. From **dC3**, we

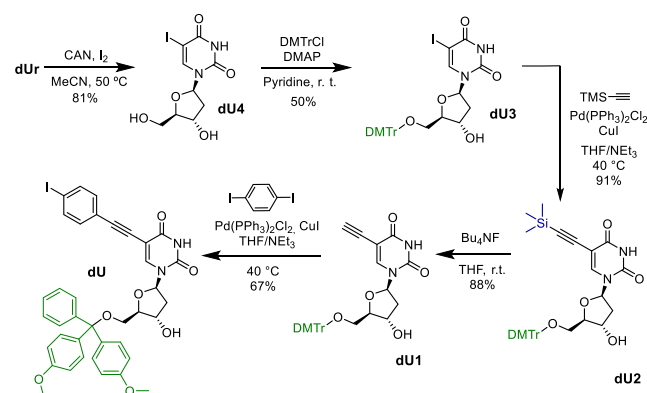
considered whether to protect the 5'-OH function with DMTrCl or to install first the phenylene spacer. The latter was a better option in terms of overall yields (70%). Hence, the Pd-catalysed reaction of **dC3** with an excess of *p*-diiodobenzene (to maximize the monocoupling reaction) provided **dC2**. Subsequently, different conditions were tested for the protection of the 5'-OH with the bulky DMTr group.<sup>21-23</sup> 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 S.I.). 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 S.I.).

### Scheme 3. Synthetic route to dC.



**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.

### Scheme 4. Synthetic route to dU.

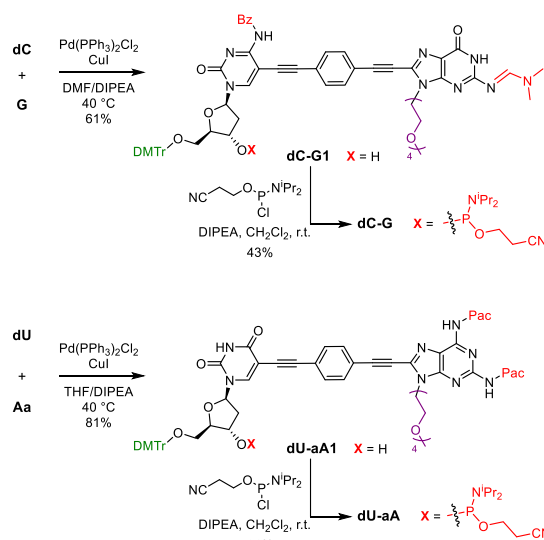


We started with the iodination of commercial 2'-deoxyuridine (**dUr**) following a reported procedure.<sup>24</sup> The next reaction, in contrast to the **dC** route, consisted in the incorporation of the bulky DMTr substituent at the 5'-O position, as described in the

literature,<sup>25</sup> to provide **dU3**.<sup>25d</sup> 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.

**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-catalysed Sonogashira coupling between the purine (**G**, **aA**) and pyrimidine (**dC**, **dU**) fragments previously prepared (Scheme 5). In general, the bulkier anhydrous NET(*i*Pr)<sub>2</sub> (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*-diisopropylchlorophosphoramidite, 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 <sup>1</sup>H and <sup>31</sup>P NMR spectra (see S.I.).

### Scheme 5. Synthetic route to monomers dC-G and dU-aA.

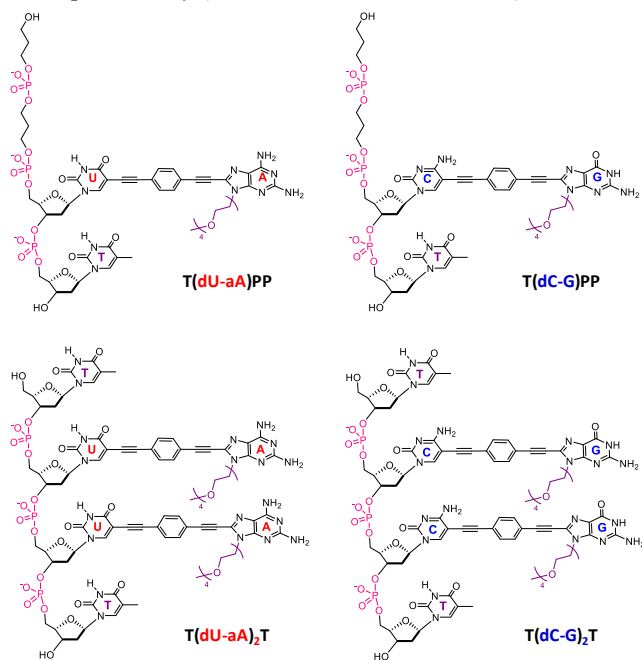


### Oligomerization of the dinucleobase-containing monomers.

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 S.I.). 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 4 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 us additional possibilities for quantification by UV-Vis absorbance, in which the molar extinction coefficient of the monomers at 355 nm ( $36294 \pm 500 \text{ M}^{-1}\text{cm}^{-1}$  for **dU-aA**; 47135

$\pm 750 \text{ M}^{-1}\text{cm}^{-1}$  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 at the S. I.). The final oligonucleotides, which are perfectly soluble in water below  $10^{-3} \text{ M}$ , were characterized by UV-vis spectroscopy and MALDI-TOF spectrometry (see Sections 1.6-1.7 at the S.I.).



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

In short, the optimized synthetic routes leading to 2 pairs of self-complementary nucleobases (**G**, **dC** and **aA**, **dU**) have been described and are complemented with additional routes described in the S.I. 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 on the ACS Publications website.

PDF file including experimental procedures,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and HRMS characterization data,  $^1\text{H}$  and  $^{13}\text{C}$  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** are also detailed.

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## Author Contributions

All authors have given approval to the final version of the manuscript.

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