DNA Analogues

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Synthesis of Triazole-linked Homonucleoside Polymers through Topochemical Azide–Alkyne Cycloaddition**

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Abstract: *There is a great deal of interest in developing stable modified nucleic acids for application in diverse fields. Phosphate-modified DNA analogues, in which the phosphodiester group is replaced with a surrogate group, are attractive because of their high stability and resistance to nucleases. However, the scope of conventional solution or solid-phase DNA synthesis is limited for making DNA analogues with unnatural linkages. Other limitations associated with conventional synthesis include difficulty in making larger polymers, poor yield, incomplete reaction, and difficult purification. To circumvent these problems, a single-crystal-to-single-crystal (SCSC) synthesis of a 1,5-triazole-linked polymeric ssDNA analogue from a modified nucleoside through topochemical azide–alkyne cycloaddition (TAAC) is reported. This is the first solvent-free, catalyst-free synthesis of a DNA analogue that proceeds in quantitative yield and does not require any purification.*

Nucleic acids constitute a major class of biopolymers that form the basis of life.[1] Nucleic acids find application in therapeutics,[2a] drug delivery,[2b] biotechnology,[2c] chemical biology,[2d] biosensing,[2e] templated syntheses,[2f] supramolecular chemisty,[2g] charge transport,[2h] materials science,[2i–k] and nanotechnology.[2l,m] There is a great deal of interest in developing stable modified nucleic acids[3] with improved properties for application in various fields. Among the possible modifications, phosphate-modified analogues are attractive because of their high stability and resistance to nulceases.[4a] Peptide nucleic acids (PNA),[4b] in which the phosphodiester linkage is replaced by an amide linkage (Figure 1), are known to be functionally similar or even superior to DNA. The 1,2,3-triazole motif, which has similar polarity and topology to an amide linkage, is more stable under acidic, basic, and enzymatic conditions and is considered a stable functional mimic (bioisostere) of the amide linkage.[5a] Accordingly, there have been attempts to replace

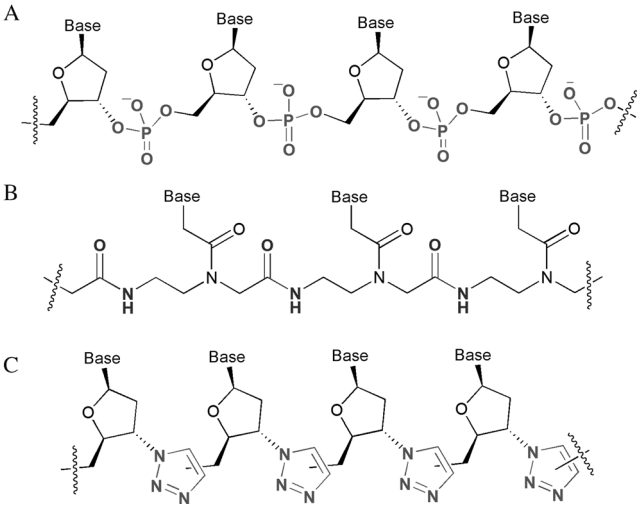

Figure 1. The structures of natural DNA (A), PNA (B), and triazolyl DNA (C). Base = nucleobase.

one or more phosphodiester linkages with 1,4-triazole units in synthetic DNA oligomers, and this linkage has been found to be a promising functional surrogate for the phosphodiester linkage in nucleic acids (Figure 1).[5b,c] However, the scope of solution or solid-phase synthesis of triazole-linked oligomers is limited owing to the difficulty in making larger polymers, poor yield,[5d] incomplete reaction, difficult purification, and degradation of oligonucleotides in presence of Cu^{I} catalysts.[5b] Herein, we report the first single-crystal-to-single-crystal (SCSC) synthesis of a triazole-linked polymeric DNA analogue through topochemical azide–alkyne cycloaddition (TAAC).

Topochemical reactions—reactions controlled by the geometry and proximity of the reactive partners in the crystal lattice—are attractive because they involve solvent-free and catalyst-free conditions, high yields, and stereo/regiospecificity.[6] Topochemical polymerization[7] enables the synthesis of highly ordered, stereoregular and homogeneous polymers.[8] However, owing to the inconvenience of designing crystals that fulfill the strict geometrical prerequisites, topochemical polymerization is limited to a small set of compounds.[6–9] Because of the possible attractive noncovalent forces such as dipole···π and π···π interactions between azide and alkyne motifs, they prefer to a adopt parallel orientation in close proximity in crystals.[10] They can then undergo topochemical azide–alkyne cycloaddition (TAAC) to form either 1,4-substituted or 1,5-substituted triazoles regiospecifically, depending on their orientation in the crystal lattice. We have recently introduced this strategy for the synthesis of

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triazole-linked polysaccharides.[10a,11] Following these lines, we envisioned that triazole-linked DNA analogues could be made through TAAC reaction of a nucleoside modified with azide and alkyne functional groups at the 3′ and 5′ positions.

Nucleoside **1** (Figure 2A,B) was synthesized in six steps from the commercially available 3,5-anhydro-2-deoxy-β-D-threo-pentofuranosyl-1-thymine (see the Supporting Information) and crystallized from a 1:1 mixture of ethyl acetate and benzene. Single crystal X-ray analysis revealed that monomer **1** crystallizes in the orthorhombic $P2_12_12_1$ space group. The pentose ring adopts C3′-exo puckering ($_3$E). As expected, the azide and alkyne motifs of adjacent molecules are positioned at close proximity as a result of π···π interactions between them and the molecules are arranged linearly in a head-to-tail fashion in the *a* direction (Figure 2C,D). This linear arrangement is further supported by the π···π interaction and π···dipole interaction between the phenyl ring and the cytosine ring of adjacent molecules (d = 3.3 Å) along the *a* direction. The close proximity between the alkyne and azide motifs (d_{mean} = 3.33 Å) and their parallel alignment suggests that they could undergo a topochemical 1,3-dipolar cycloaddition reaction to form 1,5-substituted triazoles.

The crystals of the monomer (**1**; M.P = 147 °C) were stable under ambient conditions for several months. This suggests that despite the geometrical criteria for reaction being met, the crystals of **1** cannot undergo spontaneous topochemical polymerization reaction. However, compound **1** underwent azide–alkyne cycloaddition in its crystalline state upon heating above 80 °C, as evidenced from the ^{1}H NMR spectrum of a solution of the heated crystal dissolved in [D$_6$]DMSO. As expected, the solubility of the heated crystals decreased in common organic solvents. Crystals kept at 100 °C for 3 days were completely insoluble in any solvent, thus suggesting that the monomer underwent complete polymerization. However, a sample of crystals kept at 90 °C took 60 h for complete consumption of the monomer. The polymer obtained after keeping **1** at 90 °C for 60 h was partially soluble in DMSO and could be analyzed by NMR spectroscopy (Figure 3).

The polymerization reaction was also confirmed by using IR spectroscopy, differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD) and NMR spectroscopy. In the IR spectrum, **1** showed sharp signals at 2097 cm^{-1} and 2128 cm^{-1} corresponding to the azide and terminal alkyne functionalities, respectively, whereas these signals were almost absent in the IR spectrum of a sample kept at 90 °C for 60 h (Figure 3A). DSC analysis of **1** showed a melting point of 147 °C and further heating gave a broad exothermic peak as a result of uncontrolled polymerization in the molten state. Uncontrolled random polymerization in the molten stage was further confirmed by recording the ^{1}H NMR spectrum of a molten sample, which showed the presence of both 1,4- and 1,5-triazolyl linkages (see the Supporting Information). DSC analyses of crystals kept at 90 °C for different durations revealed the gradual polymerization of the monomer. Samples kept for longer duration showed lower melting points. A gradual depression in the melting point

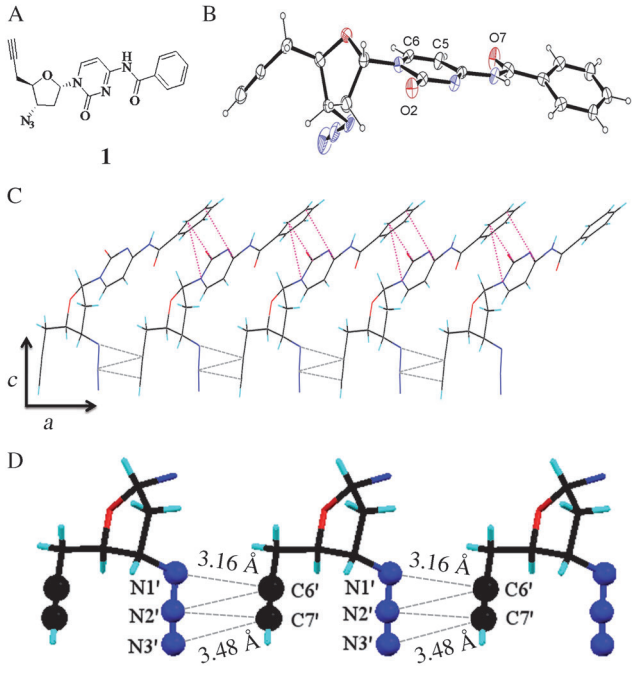

Figure 2. A) The structure of the monomer (**1**). B) An ORTEP diagram of **1**. C) Packing along the *ac* plane with π···π interaction between the azide and alkyne groups in the *a* direction. The pink dotted lines represent π···π interactions between aromatic rings in the *a* direction and the gray dashed lines represent interactions between the azide and alkyne groups. D) A close-up view of the interactions between the azide and alkyne groups.

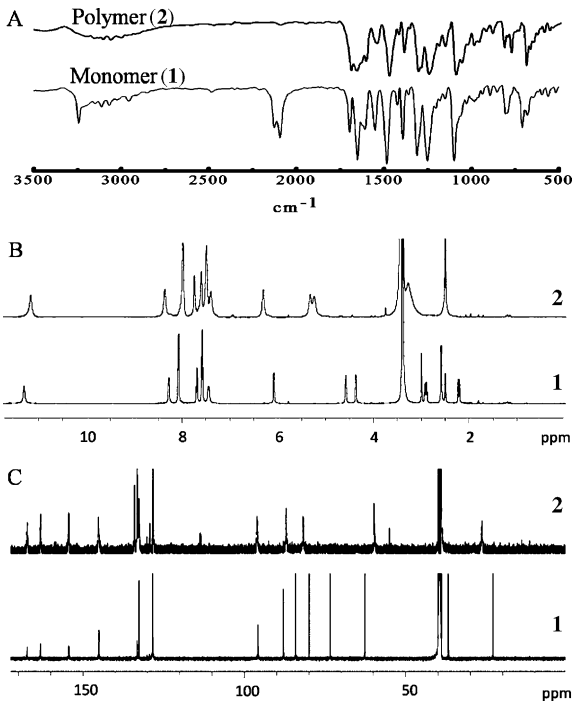

Figure 3. A) A comparison of IR spectra of the monomer (**1**) and polymer (**2**), showing the absence of azide signal for the polymer. The ^{1}H NMR spectra (B) and ^{13}C NMR spectra (C) of **1** and **2** show clear and well resolved signals for all of the carbon and hydrogen atoms of the repeating unit.

proportionate to the exposure time was observed, as anticipated based on the formation of small amounts of oligomers in the crystals of monomer with time. The ^{1}H and ^{13}C NMR spectra (in [D_6]DMSO) of a solution of the crystals of monomer **1** kept at 90 °C for 60 h suggested its smooth polymerization under these conditions. The signals were clear with distinct peaks for each proton and carbon atom of the repeating unit, thus suggesting that the polymer (**2**) is highly homogeneous, pure, and stereoregular in nature (Figure 3). Furthermore, the polymeric product showed high thermal stability as shown by thermogravimetric analysis (see the Supporting Information).

The fact that the polymerization occurs at a temperature much below the melting point and the morphology of the crystals remains the same and provides a regiospecific polymer suggests that the polymerization is a topochemical reaction. The topochemical nature of the polymerization was also substantiated by recording the PXRD spectra at regular intervals of a sample of **1** kept at 90 °C. The sample maintained its crystalline nature throughout the reaction as evidenced from PXRD (see the Supporting Information). As the morphology of the crystals was intact even after polymerization, we solved the single-crystal X-ray structure of the polymer (Figure 4). It should be noted that the reliability of the data was very good with an R-factor of 4.34, thus suggesting that the polymers formed are homogeneous and monodisperse. To our knowledge, such high resolution structures are not known for DNA or its analogues. While the space group of the polymer ($P2_12_12_1$) was same as that of the monomer, slight changes in the unit-cell parameters were observed. While *a* (7.0231 Å in **1**, 5.810 Å in **2**) decreased considerably, *b* (11.402 Å in **1**, 11.566 Å in **2**) and *c* (22.047 Å in **1**, 24.503 Å in **2**) increased slightly, thereby leading to an overall reduction in cell volume (ca. 6.7 %) and a concomitant 7.22 % increase in density. The pentose sugar ring slightly changed its conformation to $^{4}_{3}T$ from $_{3}E$. The monomer units are connected through a 1,5-triazolyl moiety in the *a* direction to form an infinite polymer chain. The polymer is isotactic with the shape of a comb (Figure 4C).

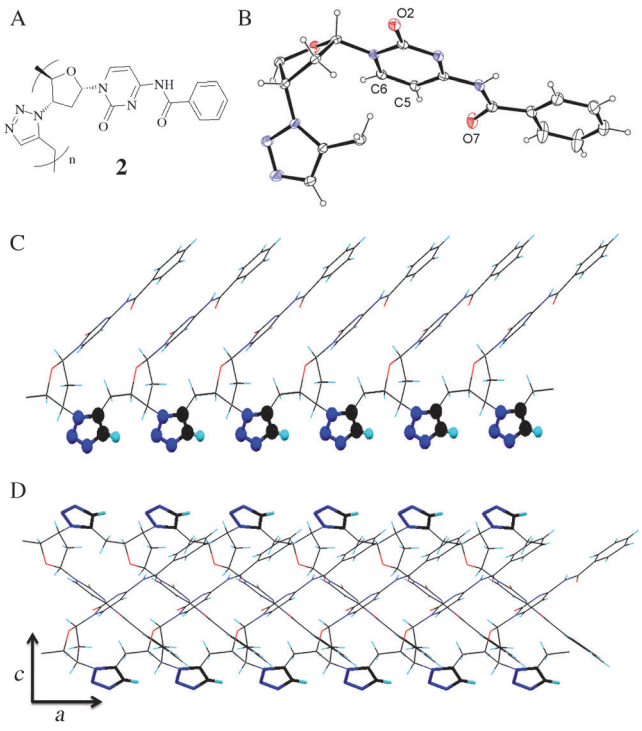

Figure 4. A) The structure of the polymer (**2**). B) An ORTEP diagram of **2**. C) The conformation of a single polymer chain. D) Packing along the *ac* plane. The triazole-linked polymer aligned in the *a* direction.

A comparison of the crystal structures of the monomer (**1**) and the polymer (**2**) revealed that major positional changes after polymerization are in the *a* direction (see the Supporting Information) owing to the formation of covalent linkages at the expense of noncovalent interactions in this direction. Interestingly, the π···π stacking between *N*-benzoylcytosine moieties is conserved even after polymerization. Also the hydrogen bond between the amide and O2 is intact even after polymerization. The adjacent polymeric chains are connected through CH···O hydrogen bonds (C5–H5···O7, C6–H6···O7) in the *b* direction to form a zig-zag arrangement. Such noncovalent zig-zag chains are packed in the *c* direction through interdigitated *N*-benzoylcytosine units of adjacent chains.

Although solid-state structures of protein-complexed ssDNA oligomers are known, to our knowledge, crystal structures of isolated ssDNA or its analogues are not known. The present report is the first crystal structure of an ssDNA analogue. While natural polynucleotides have six-bond periodicity in their backbone, our 1,5-triazole-linked DNA analogue has only five-bond periodicity. This restricts the degrees of freedom. Moreover, the flexible phosphodiester linkage in natural nucleic acids is replaced by a rigid triazole ring. As a result of these conformational constraints, the nucleobases are stacked on one side of the polymer. The stacking between adjacent bases is interesting and is partially responsible for the isotactic conformation of the polymer. Small isosequential ssDNA oligomers are known to adopt base stacked conformations in solution.[12]

Conventional solid-phase synthesis is not suitable for the synthesis of large nucleic acid polymers[13] or polymers with unnatural linkages. By adopting topochemical polymerization of an appropriately substituted nucleoside with complementary reacting motifs, namely alkyne and azide groups, we were able to synthesize a highly homogeneous, enzyme stable, crystalline DNA analogue regiospecifically in a crystalline state in quantitative yield without using solvents and catalysts. This is the first successful synthesis of a DNA analogue (1,5-triazolyl DNA) by topochemical polymerization. While the head-to-tail arrangement of monomers in the crystal lattice facilitates polymer formation, the parallel orientation of the alkyne and azide motifs in the crystal lattice dictates that the reaction is regiospecific to give the 1,5-triazole-linked polymer. Although DNA is proposed to have important applications in various fields, its anionic nature and the presence of coordinated (H-bonded) water molecules and counterions pose difficulties for some of these applications. Given that the triazole ring is an uncharged phosphate surrogate, triazolyl DNA analogues would be ideal substitutes for natural DNA for such applications. The π-stacked ssDNA analogues with

a rigid triazole backbone might also have potential for use in single-molecule electronic applications.

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