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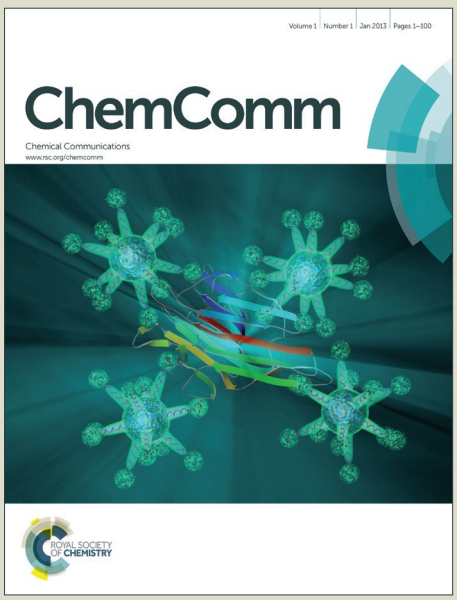

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Topochemical Synthesis of Triazole-linked Homobasic DNA

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Triazolyl-DNA (^{TL}DNA), DNA-analog wherein phosphodiester units are replaced by triazole motifs, is of great interest. We have synthesized ^{TL}DNA oligomers by adopting Topochemical Azide-Alkyne Cycloaddition (TAAC) reaction. A nucleoside decorated with azide and alkyne crystallized with proximal placement of azide and alkyne units of adjacent molecules underwent TAAC reaction to ^{TL}DNA oligomers.

There is great interest in natural and modified nucleic acids as they find applications in many areas of science such as therapeutics, nanoscience, biotechnology, chemical biology, investigation of life's origin, etc.[1] Many modified nucleic acids such as sugar-modified,[2] backbone-modified[3] and base-modified[4] nucleic acid analogs have been reported. 1,2,3-triazole motif has been emerged as a novel promising backbone modification in nucleic acids.[5] While the conventional solid-phase synthesis and solution-phase synthesis are impressive for the preparation of smaller oligonucleotides with a few triazole modifications, these methods are not suitable for the synthesis of longer oligomers with several triazolic modification due to poor yield, poor solubility, usage of copper catalysts, requirement of excess of reagents, etc.[5] Topochemical azide-alkyne cycloaddition (TAAC) reaction, a new entrant to the category of topochemical reactions,[6] offers catalyst-free, solvent-free synthesis of triazoles.[7] Proximally placed azide and alkyne, in a crystal lattice, can have attractive interactions such as dipole-dipole, dipole-π and π-π interactions between them, which can place them in parallel arrangement.[7,8] Upon activation, they can undergo cycloaddition to 1,4-substituted triazole or 1,5-substituted triazole depending on their relative orientation. We have recently achieved the synthesis of a stereoregular crystalline triazolylpolysaccharide[7a-b] and a triazolylpolynucleoside having a protected base with unnatural α-anomeric linkage using TAAC reaction.[7d] We herein report for the first time, a catalyst-free, solvent-free and protecting group-free synthesis of triazole linked DNA analog with natural β-glycosidic linkage through TAAC reaction.

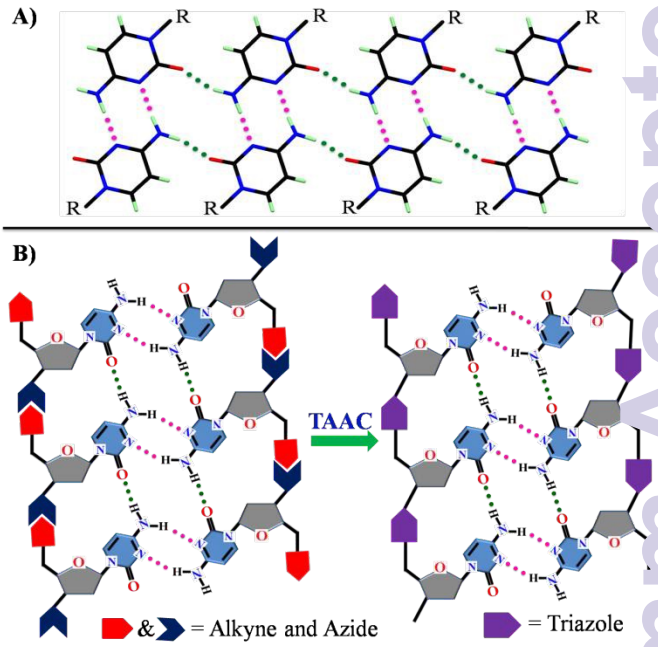

Fig. 1 A) The self-assembly pattern in the crystal structures of cytosine derivatives $N^4H...N^3$ hydrogen bonds are shown in magenta and $N^4H...O^2$ hydrogen bonds in green dotted lines. B) Proposed topochemical synthesis of a triazole-linked DNA analog.

Analysis of Cambridge Structural Database (CSD version 5.35) revealed that many cytosine derivatives (Supplementary Information) form $N^4H...N^3$ hydrogen bonded symmetric dimers, which are connected in perpendicular direction through $N^4H...O^2$ hydrogen bonds forming anti-parallel chains in their crystals (Fig. 1A). Consistent with this fact, the crystal structure of 5′-ethynyl-3′-azido-2′,3′,5′-tri-deoxycytosine (**1**) also show similar packing in which the azide and alkyne units of adjacent molecules in a chain are placed at proximity.

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anticipated that crystals of **1** can undergo TAAC reaction upon activation to yield triazole-linked DNA analogs (Fig. 1B).

We have synthesized the modified nucleoside **1** as reported[9] and crystallized from a mixture of ethyl acetate and benzene (4:1 v/v). Single crystal X-ray analysis revealed that we could crystallize **1** in the same crystal form as reported, with two symmetrically independent molecules in the asymmetric unit. Pseudocentrosymmetric N-H...N hydrogen bonds between the two conformers forms a supramolecular dimer along '*b*' direction and these dimers are connected through N-H...O hydrogen bonding in perpendicular ('*a*' direction), forming a supramolecular chain (Fig. 2B). This assembly places both azide and alkyne at proximity in parallel arrangement, a transition state-like arrangement for their cycloaddition (Fig. 2C). Furthermore, CH…N hydrogen bonds between C(5')H_2 and two nitrogen atoms of azide unit (N2' and N3') stabilize this orientation. The average distance between the azide and alkyne units is 4.22 Å-4.27 Å and is well within the range for topochemical cycloaddition reactions.[6a] It is clear that the distance and their parallel geometry are suitable for the formation of 1,5-substituted triazole-linked DNA oligomers under topochemical control.

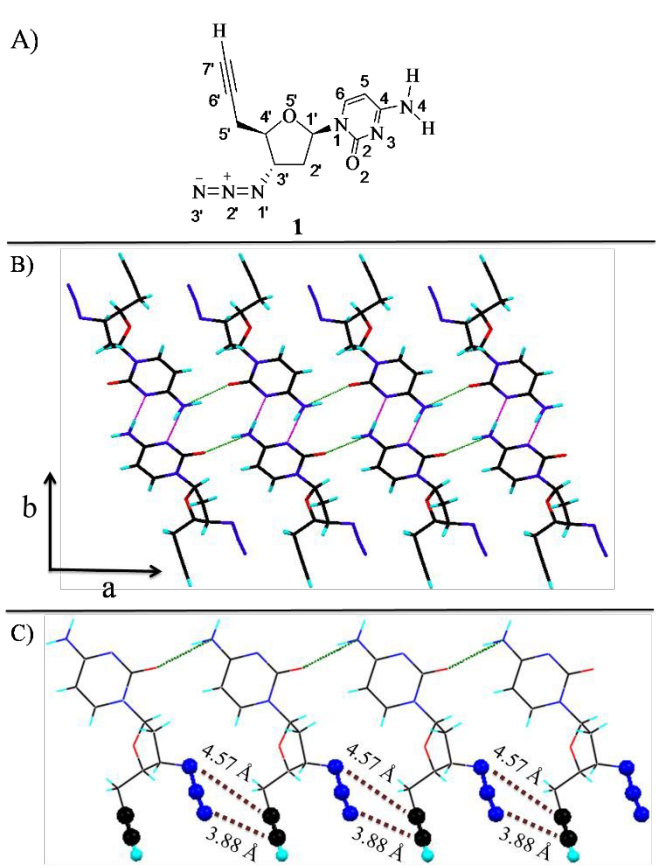

Fig. 2 A) Structure of **1**. B) Crystal packing of **1** in 'ab' plane through the $N^4H...N^3$ hydrogen bonding along 'b' direction (pink dotted lines) and $N^4H...O^2$ along 'a' direction (green dotted lines). C) Parallel orientation of azide and alkyne motifs of neighbouring nucleosides in a chain.

The crystals of nucleoside **1** were stable at room temperature for several months. The crystals did not melt at all and decomposed in the solid state at around 200 °C. In order to check its reactivity, the crystals were heated at different temperatures and monitored using ^{1}H NMR spectroscopy after dissolving the heated crystals in DMSO-d6. Crystals of **1** underwent cycloaddition reaction at temperatures above 90 °C to give oligomers of different lengths as was evident from ^{1}H NMR spectra. The kinetics of the reaction at 100 °C was probed using ^{1}H NMR spectroscopy. Crystals were heated at a constant temperature of 100 °C and small fractions were withdrawn at different time and analyzed using ^{1}H NMR after dissolving in DMSO-d6. As time progressed, the intensity of the signals of monomer decreased with concomitant increase in intensity of the signals due to oligomeric products. A plot of % of reaction against time revealed that the reaction followed a sigmoidal kinetics as expected of a topochemical reaction (Supplementary Information). At 50 h, the crystals were completely converted to polymer, which was insoluble in common solvents such $CHCl_3$, DCM, EtOAc, EtOH, THF, MeCN, MeOH, DMF and H_2O and was sparingly soluble in hot DMSO.

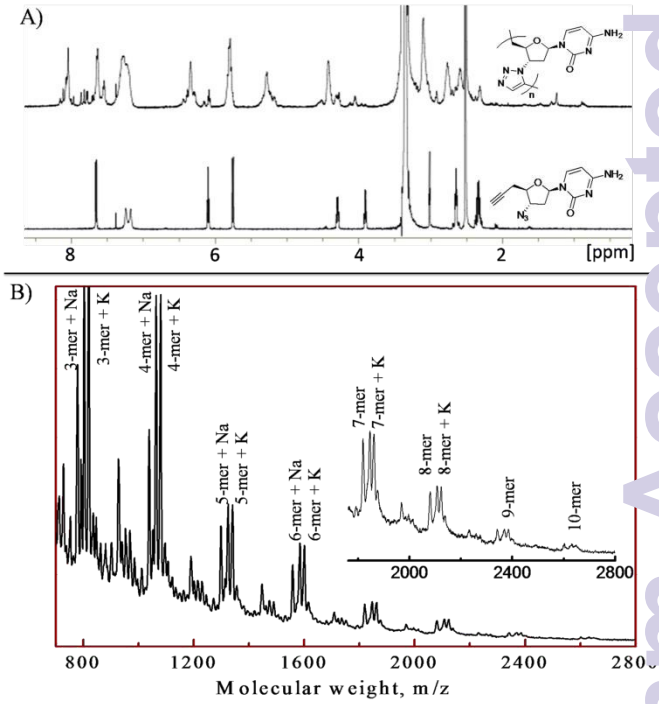

Fig. 3 A) Comparison of ^{1}H NMR spectra of monomer **1** and its topochemically formed polymer/oligomer. B) MALDI spectrum of the soluble fraction of the crystals of **1** kept at 100 °C for 50 h, showing the presence of trimer to decamer. The low intensities of the higher oligomers could be due to their lower solubilities.

The ^{1}H NMR spectrum of the soluble fraction (probably lower oligomers) of the heated sample was clear and distinct peaks corresponding to each protons of the repeating unit of the polymer/oligomer could be seen, as in the NMR spectrum of a typical regular polymer (Fig. 3A). This suggests that the oligomer has only one kind of triazolyl linkages. The broad signals in the ^{1}H NMR spectrum of the polymer suggest the presence of oligomers of different lengths in the soluble fraction. Clearly a new broad signal centered at ~8.1 ppm characteristic of triazolyl proton could be observed. Further

have confirmed the structure of the polymer using various NMR techniques such as ^{1}H, ^{13}C, COSY, DEPT, HMQC, HMBC and NOESY. Only 1,5-triazolyl linkage was observed between the nucleoside units as anticipated from the orientation of azide and alkyne motifs in the crystal structure of **1** (See ESI).

In order to prove the formation of oligomers, MALDI spectral analysis was done. It is worthy to note that most of the solid did not dissolve suggesting that higher oligomers/polymers are formed in large quantities than lower oligomers. MALDI Mass spectral analysis of soluble fraction of the polymer of **1** has shown the presence of trimer to decamer (10-mer). The low intensity signals of the higher oligomers may presumably be due to their lower solubilities (Fig. 3B).

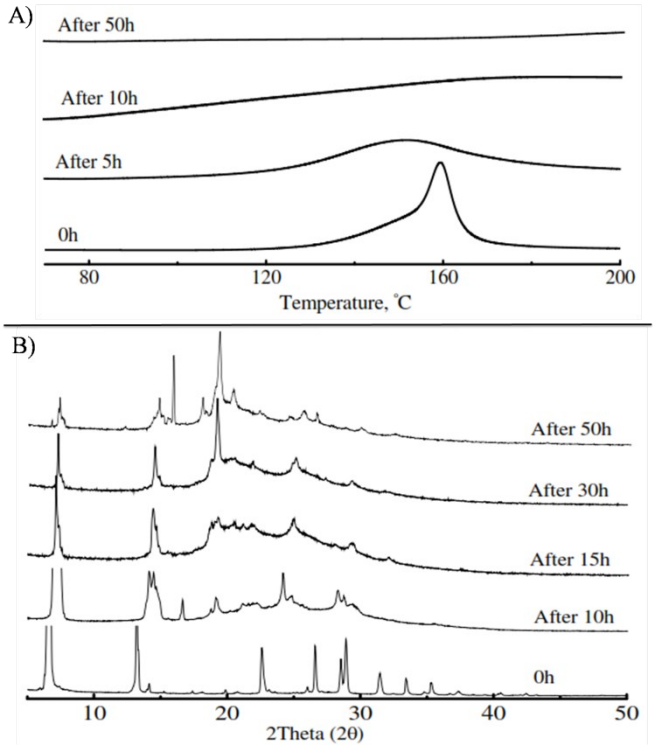

Fig. 4 A) Overlay of DSC spectra of crystals of **1** kept at 100 °C for different durations showing the gradual decrease of exothermic peak with time. B) Overlay of P-XRD spectra of crystals of **1** kept at 100 °C for different durations showing the gradual conversion of monomer to polymer without losing crystallinity

DSC analysis of crystals of **1** showed a broad exothermic peak centered at 159 °C due to the uncontrolled azide-alkyne cycloaddition reaction. The topochemical reaction at 100 °C was also followed by DSC. The crystals of **1** were heated at 100 °C and the DSC analyses were done at regular intervals by withdrawing 2 mg of sample each time (Fig. 4A). As the time progressed, the height of the broad exothermic peak (at ~150 °C) gradually decreased suggesting the gradual consumption of azide and alkyne as a result of topochemical reaction at 100 °C. The topochemical reaction of monomer **1** was also followed by PXRD. PXRD spectra of crystals of **1** kept at 100 °C were recorded at regular intervals (Fig. 4B). As anticipated, the crystallinity was maintained throughout the course of the reaction. As the reaction progressed, the peaks due to the monomer disappeared gradually with concomitant appearance of new peaks due to oligomers/polymers.

In conclusion, we have reported the first synthesis of triazolyl-DNA oligomers in their natural β-glycosidic configuration through topochemical reaction. A deoxycytidine derivative decorated with complementary reacting motifs (CRMs) *viz.* azide and alkyne crystallized in such a way that the CRMs of adjacent molecules are proximally disposed for their topochemical reaction within the crystal lattice. While the self-recognition of the nucleobase ensured the proper head-to-tail assembly of the monomers in the crystals, the weak attractive interaction between azide and alkyne kept these complimentary reacting motifs in transition-state like parallel arrangement. Upon thermal activation, the crystals underwent TAAC reaction to yield 1,5-triazole-linked DNA analogs. While this study shows the proof-of-concept for the topochemical synthesis of homobasic-DNA analogs, there is enough potential to extend this methodology elsewhere.

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