Topochemical Synthesis of Triazole-linked Homobasic DNA

Atchutarao Pathigoolla, and Kana M. Sureshan*

School of Chemistry, Indian Institute of Science Education and Research Thiruvananthapuram, CET-Campus, Thiruvananthapuram-695016 (India).E-mail: kms@iisertvm.ac.in

Contents

1. Methods and materials
2. Origin of design
3. Synthesis of monomer **1**
4. Crystallization of compound **1**
5. Single crystal X-ray crystallographic analysis of **1**
6. Topochemical reaction of **1**
7. Kinetics of topochemical reaction
8. MALDI spectral analysis of polymer
9. NMR Characterization of triazole linked cytosine polymer
10. Uncatalyzed Huisgen cycloaddition reaction in solution
11. References

1. Methods and materials

All the chemicals were purchased from Sigma-Aldrich. TLC analyses were done using pre-coated TLC silica gel 60 F_{254} (Merck) plates. Melting points were recorded on a Stuart, SMP 30 melting point apparatus. NMR spectra were recorded on an Avance III-500 (Bruker) NMR spectrometer. IR spectra were recorded by preparing KBr pellets, using IR Prestige–21 (Shimadzu) spectrometer. DSC analyses were carried out using DSC Q20 differential scanning calorimeter, at a heating rate of 5 ºC/min. Powder X-ray diffraction spectra were recorded using an X'pertPRO (PANalytics) X-ray diffractometer. The PXRD experiments were done in a slow and continuous scan rate mode using Cu as the anode material (Kα1 = 1.540598Å). Axima CFRplus MALDI-TOF spectrometer (Shimadzu Biotech) was used to analyze molecular masses

of soluble fraction of polymer. Single crystal X-ray intensity data were collected on a Bruker KAPPA APEX-II diffractometer in omega and phi scan mode, MoKα = 0.71073 Å at 298 K.

2. Origin of design

Reported crystal structures of various cytosine derivatives were analyzed. N1-Substituted cytosine derivatives **A-J** (Chart S1)[1-10] showed very consistent crystal packing dictated by consistent N4H4…N3 and N4H4…O2 hydrogen bonds as shown in Fig. S1.

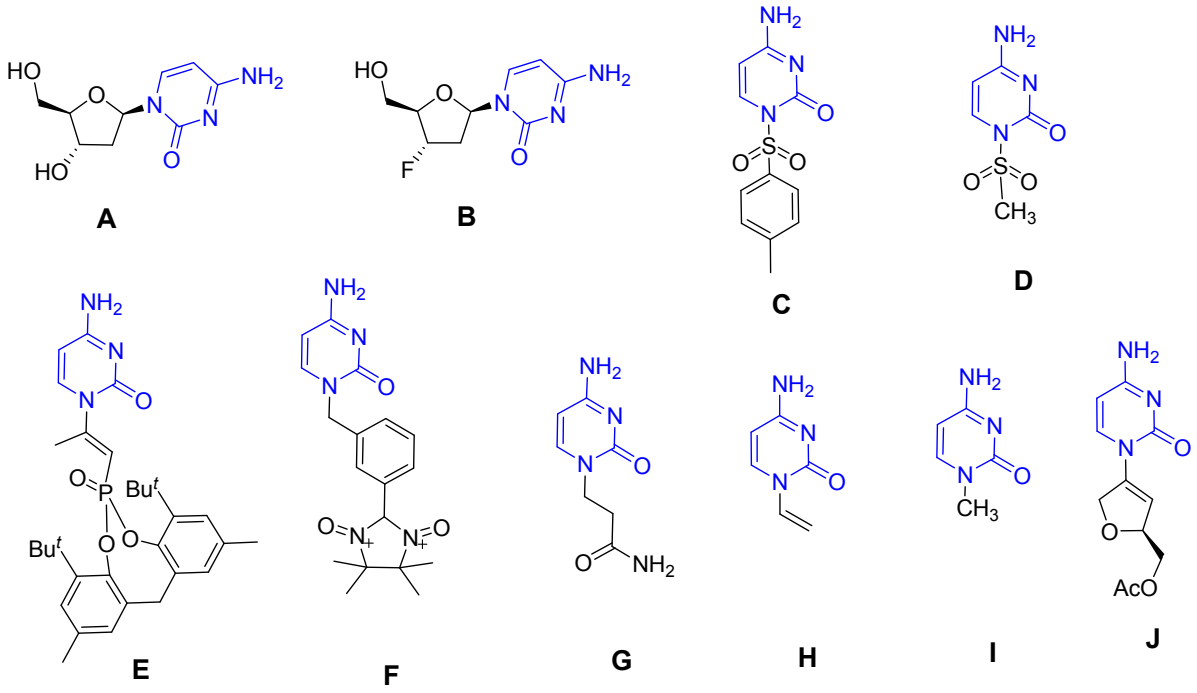


Chart S1: Representative cytosine derivatives having dimeric hydrogen bonding between N3 and N4H4 and N4H4…O2 in their crystal structure.

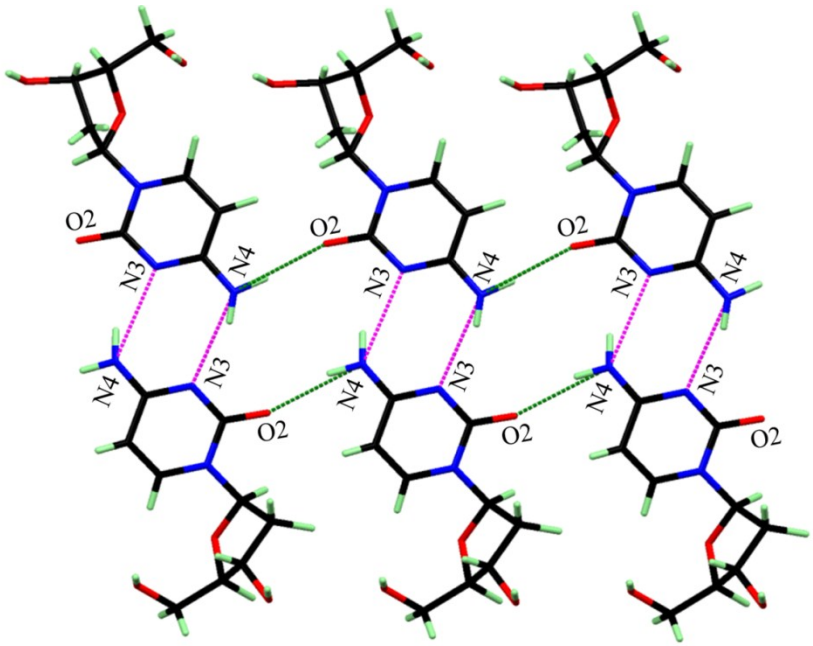
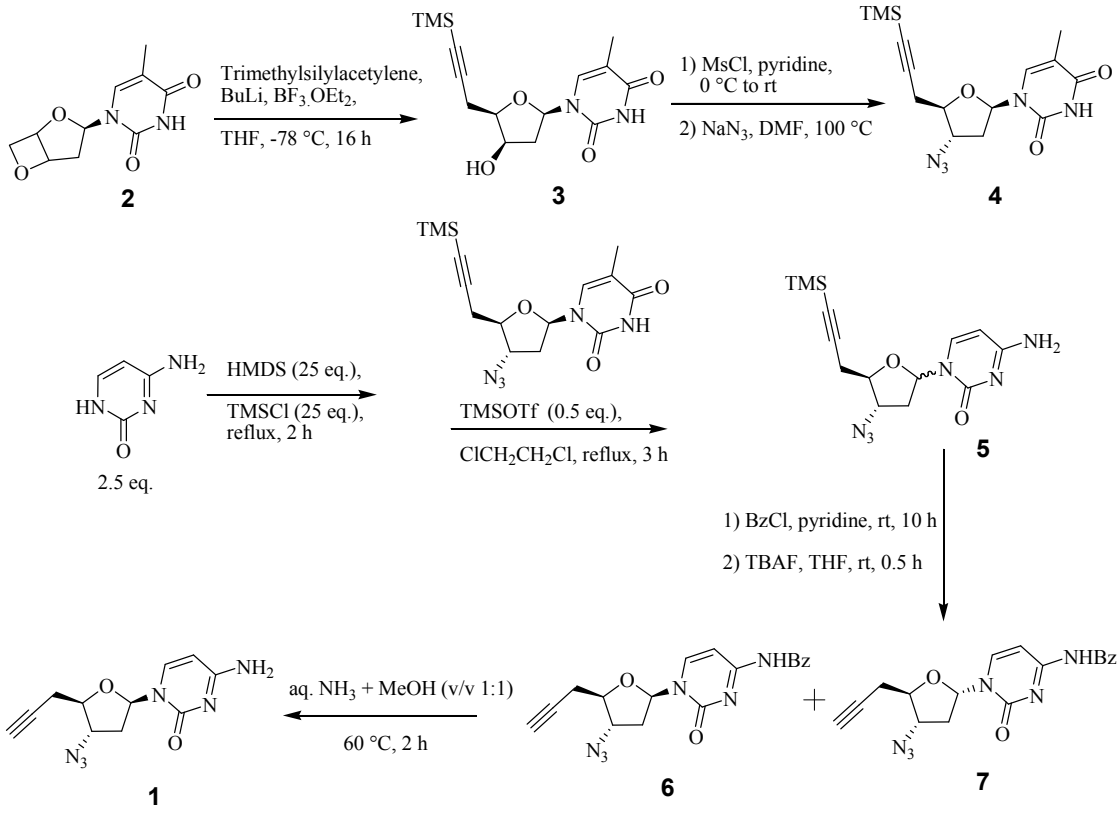


Fig. S1: Consistent crystal packing of cytosine derivatives shown in chart S1. The N4-H4…N3 hydrogen bonds (pink dotted lines) make symmetric dimers and the N4-H4…O2 hydrogen bonds (green dotted lines) connects such dimers into a chain.

3. Synthesis of monomer 1

Compound **1** was synthesized, as per the Scheme S1, by following the reported procedures.[11]



Scheme S1: Synthesis of monomer **1**

4. Crystallization of monomer 1

The compound **1**was crystallized from a mixture of ethyl acetate and benzene (4:1v/v). The colorless needle type crystals thus obtained were used for single crystal XRD analysis and other studies described herein. The morphology of these crystals was intact even after heating at 100 ºC for 3 days.

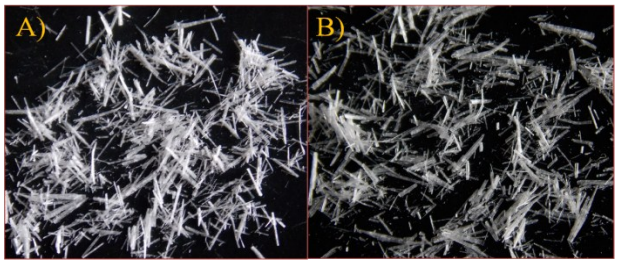


Fig. S2: (A) Crystals (approximate dimension 1-5 mm x 0.05-0.15 mm x 0.1-0.2 mm) of monomer **1** as prepared (B) crystals after heating at 100 ºC for 3 days.

5. Single crystal X-ray crystallographic analysis of 1

X-ray intensity data measurement of freshly grown crystals of **1** was carried out at 298K on a Bruker-KAPPA APEX II CCD diffractometer with graphite-monochromatized (MoK = 0.71073Å) radiation. The X-ray generator was operated at 50 kV and 30 mA. Data were collected with scan width of 0.3° at different settings of φ (0°, 90° and 180°) keeping the sample to detector distance fixed at 36 mm and the detector position (2θ) 25°. The X-ray data collection was monitored by SMART program (Bruker, 2003).[12] All the data were corrected for Lorentzian, polarization and absorption effects using SAINT and SADABS programs (Bruker, 2003). SHELX-97 was used for structure solution and full matrix least-squares refinement on *F*2.[13] All the hydrogen atoms were placed in geometrically idealized position (C-H = 0.99 Å for the methylene H-atoms and C-H = 1.0 Å for central sugar ring H-atoms) and constrained to ride on their parent atoms [U_{iso} (H) = 1.2U_{eq} (C)]. Molecular and packing diagrams were generated using ORTEP-3[14] and Mercury-3.[15] Geometrical calculations were performed using SHELXTL (Bruker, 2003) and PLATON.[16]

Crystal data of monomer (1)[11]: CCDC 1046487.$C_{11}H_{12}N_6O_2$, MW = 260.27, colorless needle type blocks, 0.25 x 0.15 x 0.1 mm^3, Triclinic, space group P1, a = 7.0000(7), b = 7.1645(8), c = 13.4574(16) Å, α = 94.095, β = 93.302, γ = 105.223, V = 647.52(12) $Å^3$, Z = 2, T = 296(2) K, $2\theta_{max}$ = 50.00°, D_{calc} (g cm^{-3}) = 1.335, F(000) = 272, μ (mm^{-1}) = 0.098, 9328 reflections collected, 4384 unique reflections [R(int) = 0.026], multi-scan absorption correction, T_{min} = 0.9903, T_{max} = 0.9760, number of parameters = 366, number of restraints = 45, GoF = 0.927, R_1 = 0.0603, wR_2 = 0.1639, R indices based on 4384 reflections with I >2s(I) (refinement on F2). $\Delta\rho_{max}$ = 0.33, $\Delta\rho_{min}$ = 0.003 ($eÅ^{-3}$).

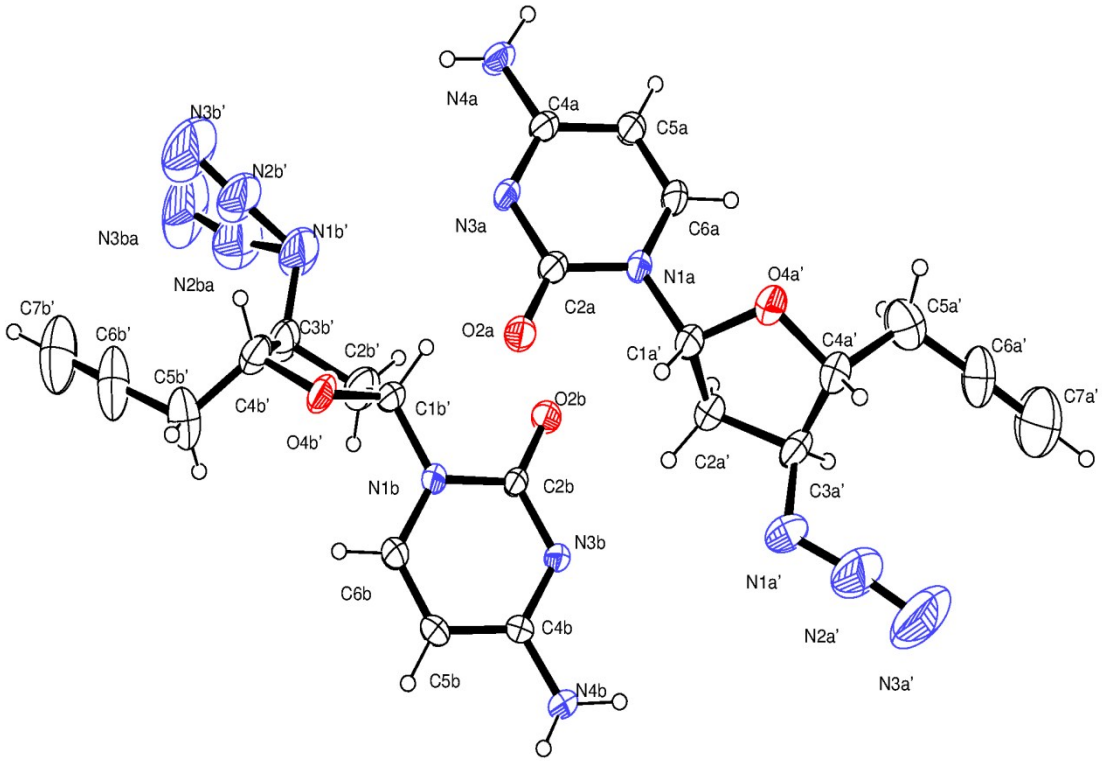


Fig. S3: ORTEP plot of monomer **1,** at 30% probability level.

6. Topochemical reaction of 1

The reactivities of the crystals of **1** at different temperatures were monitored using ^{1}H NMR spectroscopy. Seven clean test tubes were taken and 5 mg of crystals were placed in each of these test tubes. Seven different constant temperature baths of temperature 60 ºC, 70 ºC, 80 ºC, 90 ºC, 100 ºC, 110 ºC and 120 ºC were set up and each of these test tubes were placed in a particular constant temperature bath and heated for 6h. In all these cases, the crystals were intact and no melting was observed. After 6 h of heating, crystals in each of these test tubes were dissolved in DMSO-d6 and their ^{1}H NMR spectra were recorded. The crystals, heated at temperatures above 90 ºC showed new signals due to oligomers in their ^{1}H NMR spectra suggestive of the TAAC at these temperatures. Heating for longer time resulted in complete polymerization as evident from their insolubility in any of the common solvents. For instance, crystals of **1** kept at 100 ºC for 50 h was almost insoluble. The very minor

amount dissolved in DMSO-d6 showed broad peaks suggesting the presence of oligomers/polymers.

7. Kinetics of topochemical reaction

In a dry round bottom flask, freshly prepared crystalline sample of **1** (200 mg) was taken and heated at 100 °C. The ^{1}H NMR spectra of this sample were taken at regular intervals by withdrawing a small fraction and dissolving in DMSO-d6 (Fig.S4). After 5h, ^{1}H NMR spectrum has shown several new signals, importantly one broad peak at ~δ 6.25-6.35 and corresponding to anomeric proton of pentose and another broad peak at ~δ 8.1 corresponding to triazolic proton. After 10 h, TLC of the compound showed the complete disappearance of starting material and increase in the amount of higher oligomers. After 50 h of experiment, the solubility of material decreased and ^{1}H NMR spectrum of the dissolved fraction showed a broad spectrum suggesting that the monomer **1** underwent topochemical reaction to oligomers of different size (Fig.S4).

The proton signals corresponding H3′ shifted from 3.91 ppm to 5.28 ppm after the cycloaddition and the H1′ shifted from 6.10 ppm to 6.34 ppm. Also the signals due to alkynyl proton (3.01 ppm) vanished and triazolyl proton (8.1ppm) appeared after the reaction. With time, the proton signals corresponding to monomer gradually decreased and signals corresponding to oligomers/polymer increased as expected. The ratio of area of signal due to H-3′ in the product to the sum of areas of signals due to H-3′ in the monomer and product gives the fraction of reaction at any point of time. Fraction of reaction multiplied by 100 gives the percentage of reaction. The plot of percentage of reaction vs time showed a sigmoidal kinetics (Fig. S5), characteristic of a topochemical reaction.

$$Fraction\ of\ reaction\ \ at\ time\ 't' = \frac{peak\ area\ of\ H3'\ signal\ in\ polymer}{(peak\ area\ of\ H3'\ signal\ in\ polymer\ + peak\ area\ H3'\ signal\ i}$$

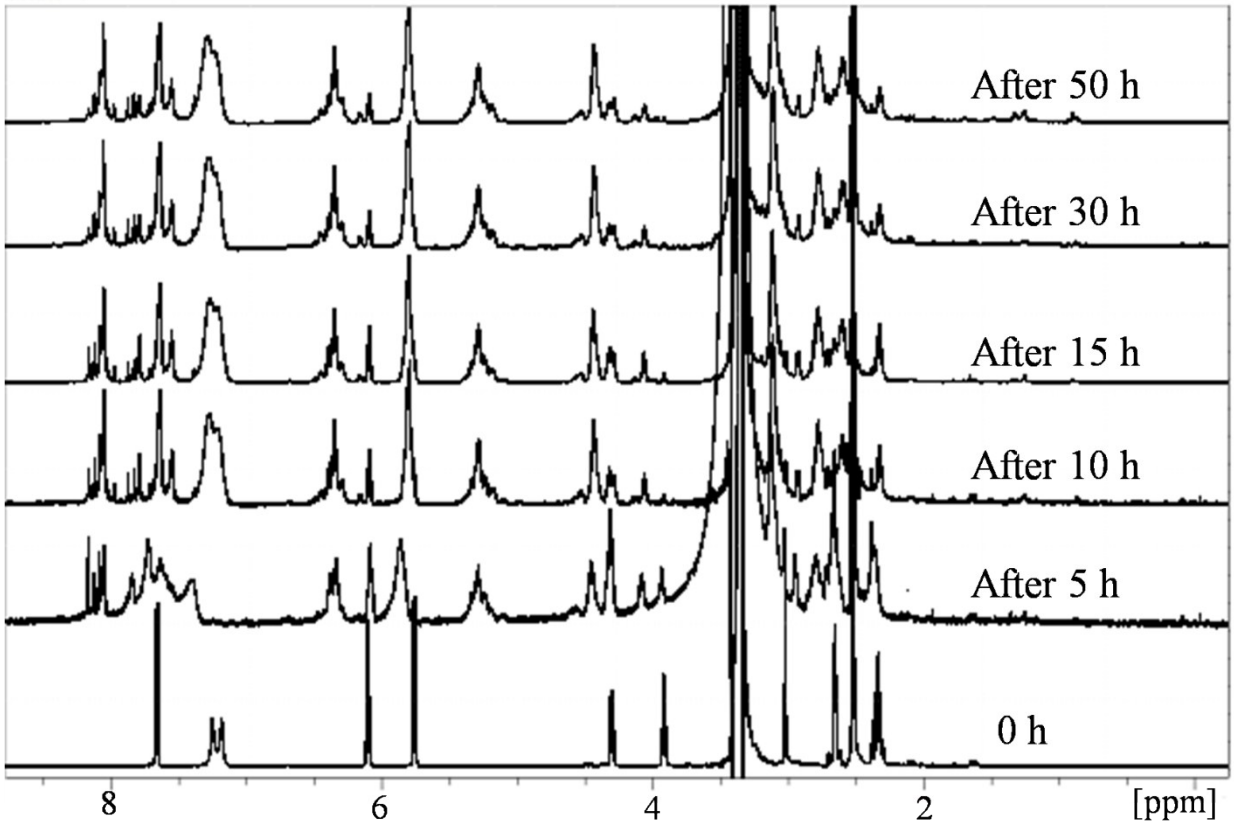


Fig. S4: ^{1}H NMR spectra of DMSO-d6 solution of crystals kept at 100 ºC for different durations.

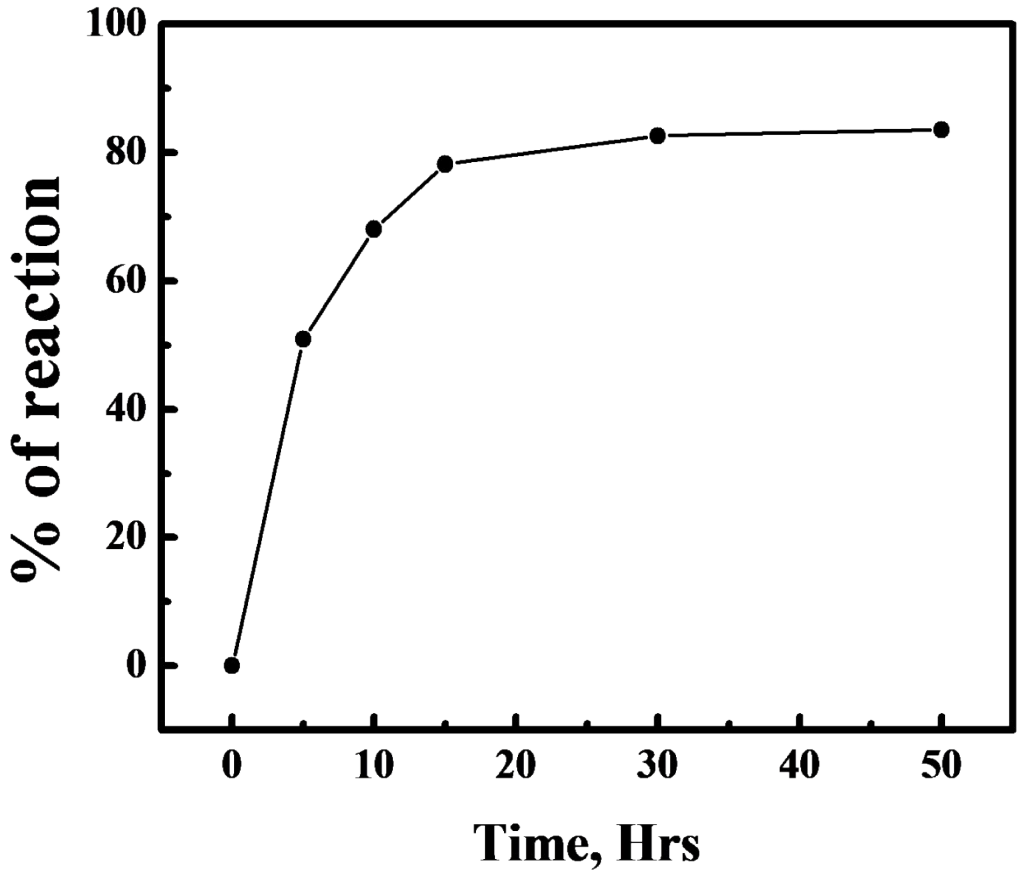


Fig. S5: Plot of % of reaction at 100 ºC with time

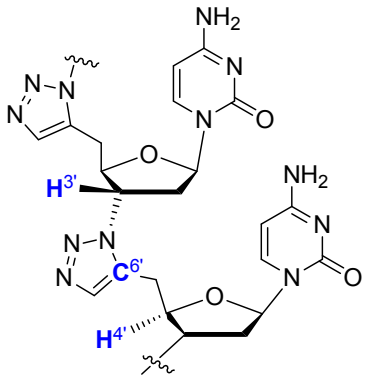
8. MALDI spectral analysis of polymer

The polymer obtained after heating the monomer **1** at 100 °C for 12 h was dissolved in a mixture of water and DMSO (1:1 v/v). The soluble fraction was seperated after centrifugation and analyzed by MALDI-TOF using dithranol as the matrix.

9. NMR Characterization of triazole linked cytosine polymer

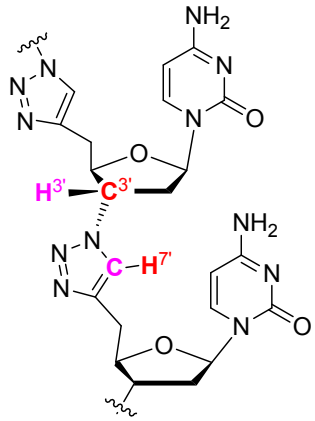
The monomer **1** was heated at 100 °C for 12 h and was analyzed using various NMR techniques (^{1}H, COSY, ^{13}C, DEPT, HMQC, HMBC and NOESY) to understand the linkage between the monomeric units. It is clear from ^{1}H NMR, ^{13}C NMR, DEPT and HMQC spectra that only one kind of triazole units are present in the oligomers. This is not surprising as the crystal structure of the monomer suggested only one type triazole (1,5-triazole) formation based on the orientation of the alkyne and azide units. Two theoretically possible structures for the oligomers are shown in Fig. S6; one having 1,4-linked triazoles and the other 1,5-linked triazoles. If 1,5-linked triazoles there will be cross peaks between the quarternary carbon (C6′) and H3′ in the HMBC spectrum. Also, C6′ will have cross peaks with H4′ of another sugar unit. HMBC spectrum clearly proved the 1,5-substituted triazole linkage between the nucleosides.The quarternary carbon (C6′) of the triazole showed cross peaks, arising from 3-bond connectivity, with both H3′ and H4′ (color-coded, Figure S6 and Figure S7). Also the absence of cross peaks

between tertiary carbon (C7′) of the triazole and H3′ and between C3′ and H7′ rule out 1,4-triazole linkage and also supports 1,5-triazole linkage between nucleosides.



1,5-linked triazoles

If this is the structure, there should be cross peaks between C6' & H3' and C6' & H4' in HMBC spectrum



1,4-linked triazoles

If this is the structure, there should be cross peaks between C7' & H3' and C3' & H7' in HMBC spectrum

Fig. S6: Two possible structures for the triazole-linked cytosine oligomer/polymer. Only the blue color coded coupling was observed in the HMBC spectrum (See Fig. S7).

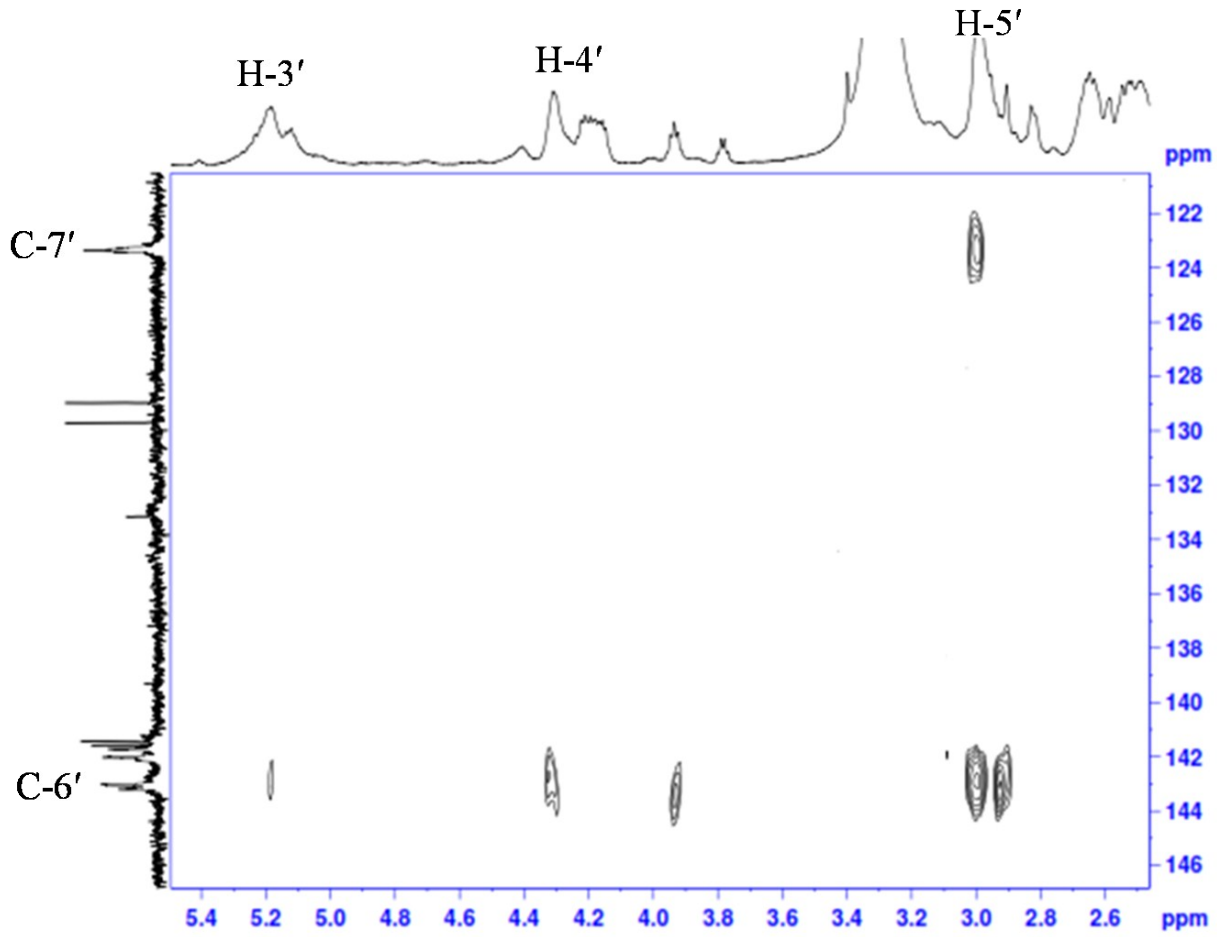


Fig. S7: HMBC spectrum of oligomers of **1**, showing the cross peaks due to 1,5-triazole-linkage

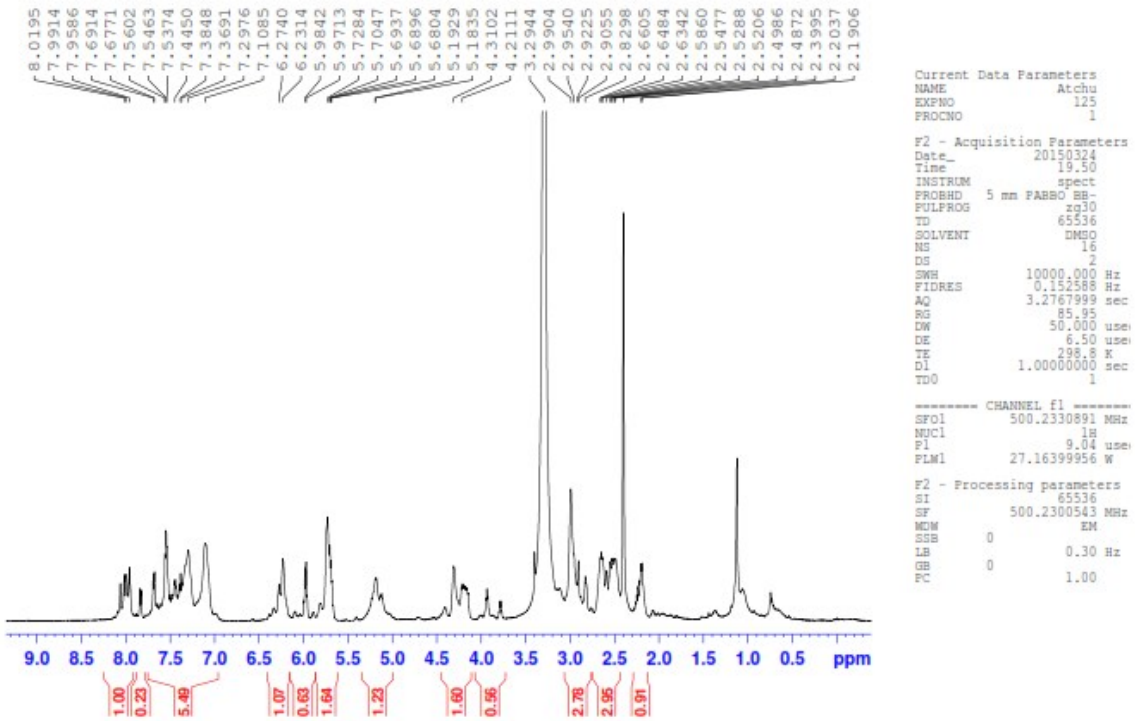


Fig. S8: ^{1}H NMR spectrum of polymer of **1**

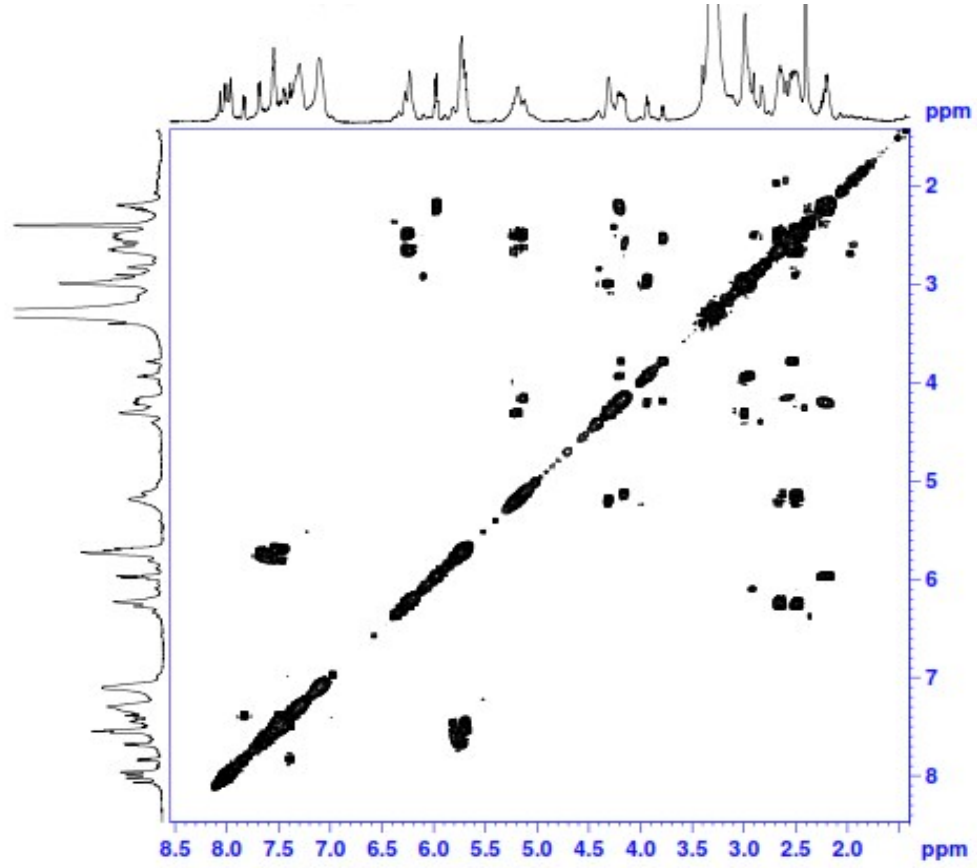
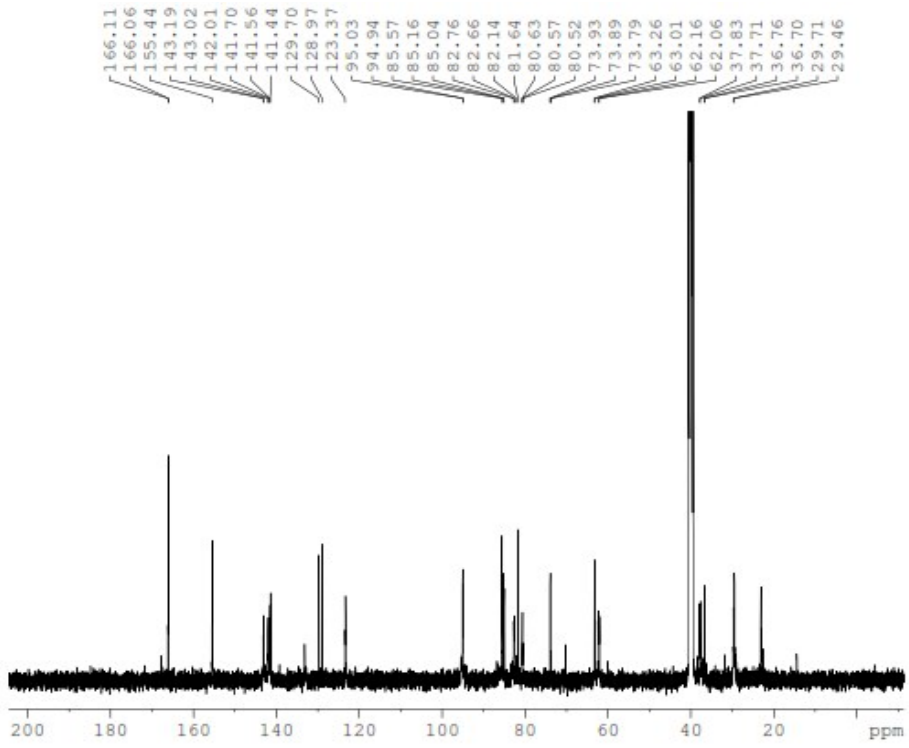
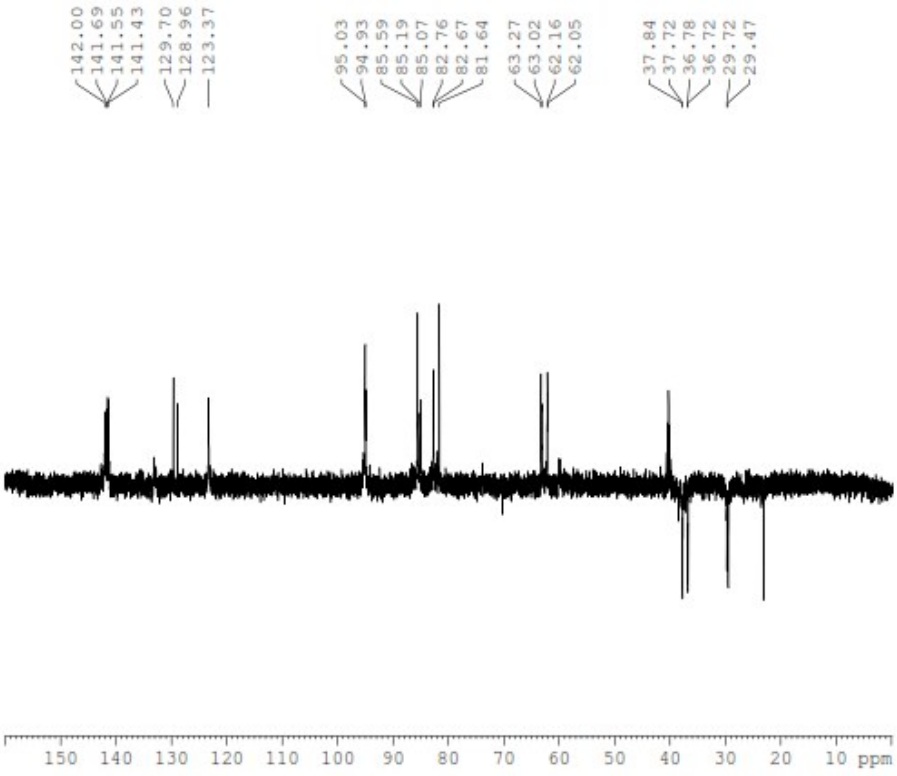


Fig. S9: COSY NMR spectrum of polymer of **1**




```
Current Data Parameters
NAME                  Atchu
EXPNO                   126
PROCNO                    1

F2 - Acquisition Parameters
Date_               20150324
Time                   19.53
INSTRUM                spect
PROBHD   5 mm PABBO BB-
PULPROG             zgpg30
TD                   65536
SOLVENT                DMSO
NS                      8804
DS                         4
SWH              29761.904 Hz
FIDRES            0.454131 Hz
AQ                1.1010048 sec
RG                   191.08
DW                  16.800 usec
DE                   6.50 usec
TE                  299.0 K
D1               2.00000000 sec
D11              0.03000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1            125.7955112 MHz
NUC1                    13C
P1                     8.65 usec
PLW1          120.50000000 W

======== CHANNEL f2 ========
SFO2            500.2320009 MHz
NUC2                    1H
CPDPRG[2             waltz16
PCPD2                 80.00 usec
PLW2             27.16399956 W
PLW12             0.34685999 W
PLW13             0.22199000 W

F2 - Processing parameters
SI                      32768
SF              125.7829340 MHz
WDW                        EM
SSB          0
LB                     1.00 Hz
GB           0
PC                     1.40
```

Fig. S10: ^{13}C NMR spectrum of polymer of **1**




```
Current Data Parameters
NAME                  Atchu
EXPNO                   131
PROCNO                    1

F2 - Acquisition Parameters
Date_               20150325
Time                   11.20
INSTRUM                spect
PROBHD   5 mm PABBO BB-
PULPROG          deptsp135
TD                   65536
SOLVENT                DMSO
NS                      1084
DS                         4
SWH              20161.291 Hz
FIDRES            0.307637 Hz
AQ                1.6252928 sec
RG                   191.08
DW                  24.800 usec
DE                   6.50 usec
TE                  301.5 K
CNST2           145.0000000
D1               2.00000000 sec
D2               0.00344828 sec
D12              0.00002000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1            125.7929956 MHz
NUC1                    13C
P1                     8.65 usec
P13                 2000.00 usec
PLW0        0 W
PLW1          120.50000000 W
SPNAM[5]     Crp60comp.4
SPOAL5                0.500
SPOFFS5     0 Hz
SPW5           13.77600002 W

======== CHANNEL f2 ========
SFO2            500.2315998 MHz
NUC2                    1H
CPDPRG[2             waltz16
P3                     9.04 usec
P4                    18.08 usec
PCPD2                 80.00 usec
PLW2             27.16399956 W
PLW12             0.34685999 W

F2 - Processing parameters
SI                      32768
SF              125.7829340 MHz
WDW                        EM
SSB          0
LB                     1.00 Hz
GB           0
PC                     1.40
```

Fig. S11: DEPT NMR spectrum of polymer of **1**

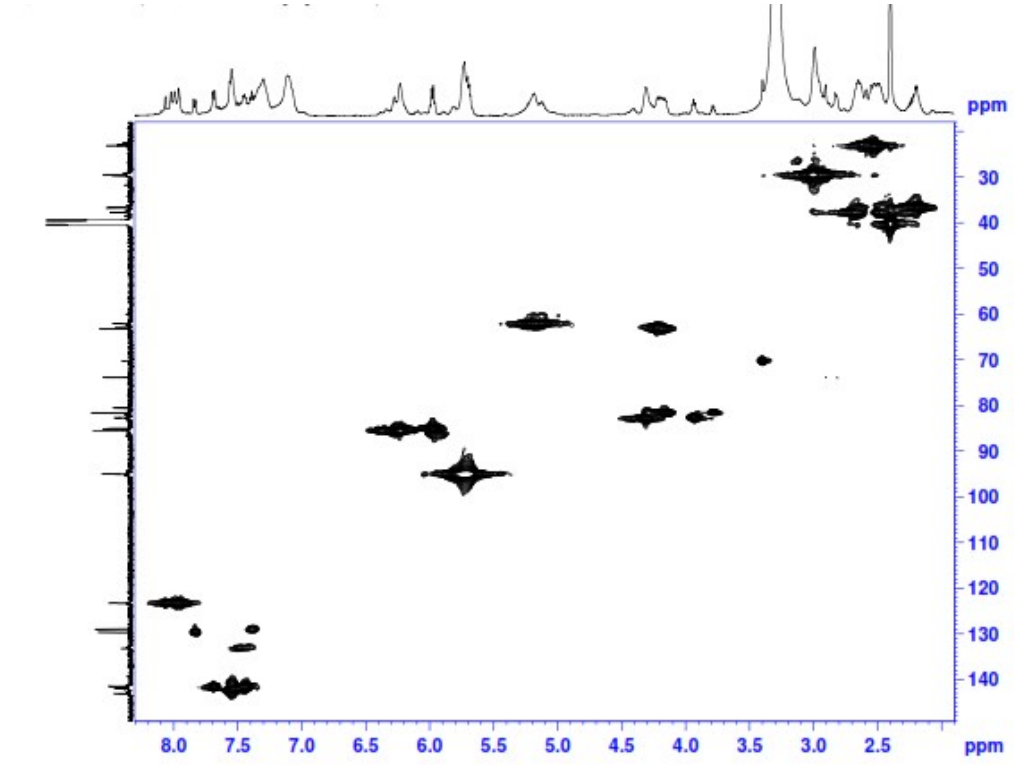


Fig. S12: HMQC NMR spectrum of polymer of **1**

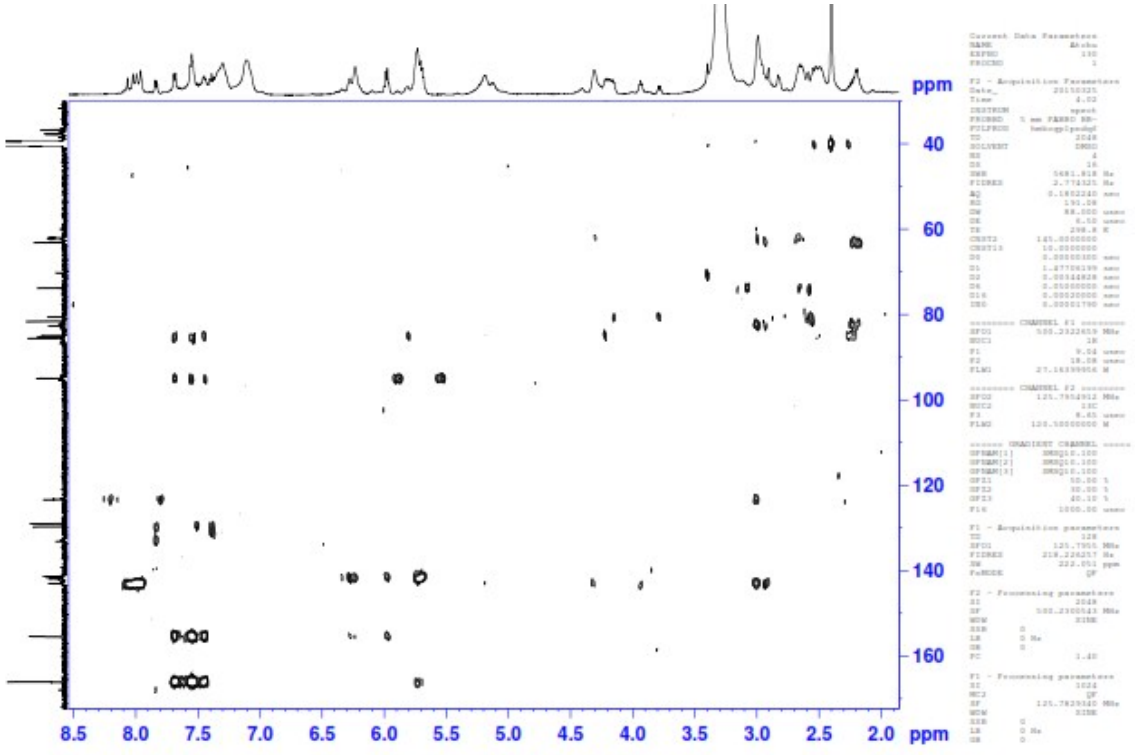


Fig. S13: HMBC NMR spectrum of polymer of **1**

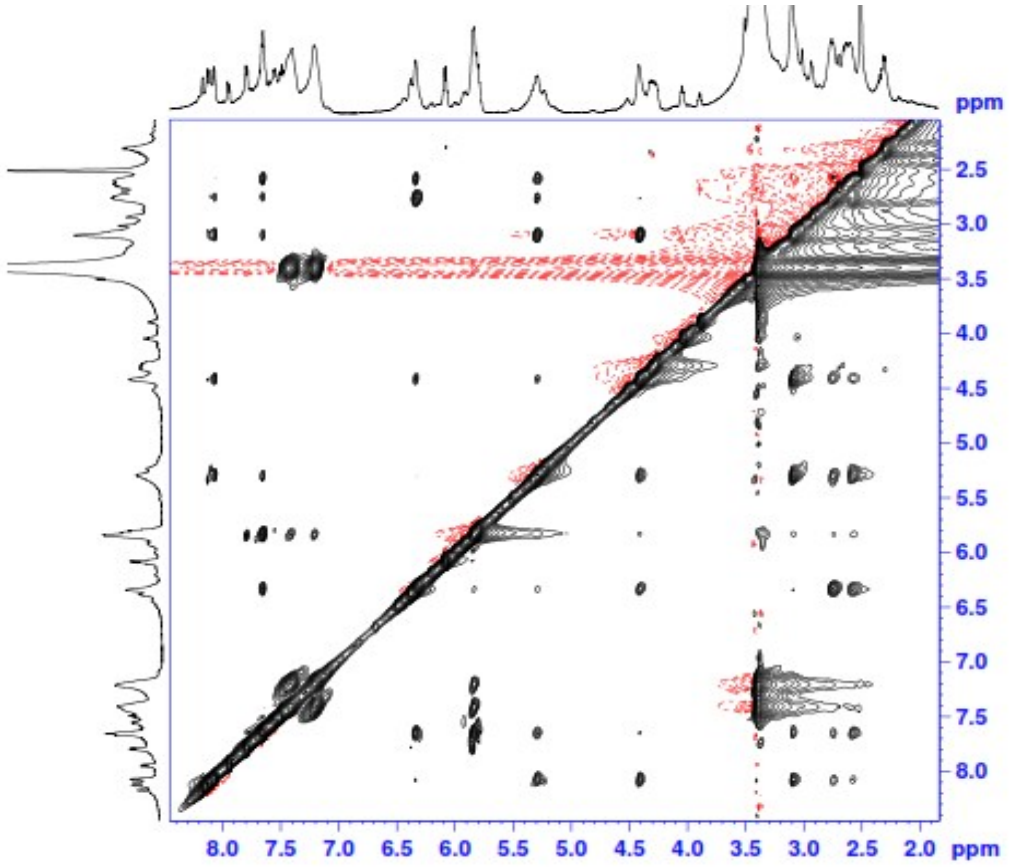


Fig. S14: NOESY spectrum of polymer of **1**

10. Uncatalyzed Huisgen cycloaddition reaction in solution

A solution of monomer **1** in DMSO-d6 was heated at 130 °C for 48 h and its ^{1}H NMR spectrum was recorded (Fig. S15). It is clear, from the NMR spectrum, that in solution, the cycloaddition leads to a complex mixture of products. This could be due to uncontrolled cycloaddition leading to oligomers having both the possible linkages (1,4- and 1,5- triazoles at random positions). This study also supports that crystal packing and thus the topochemical control dictated the reaction in the solid state.

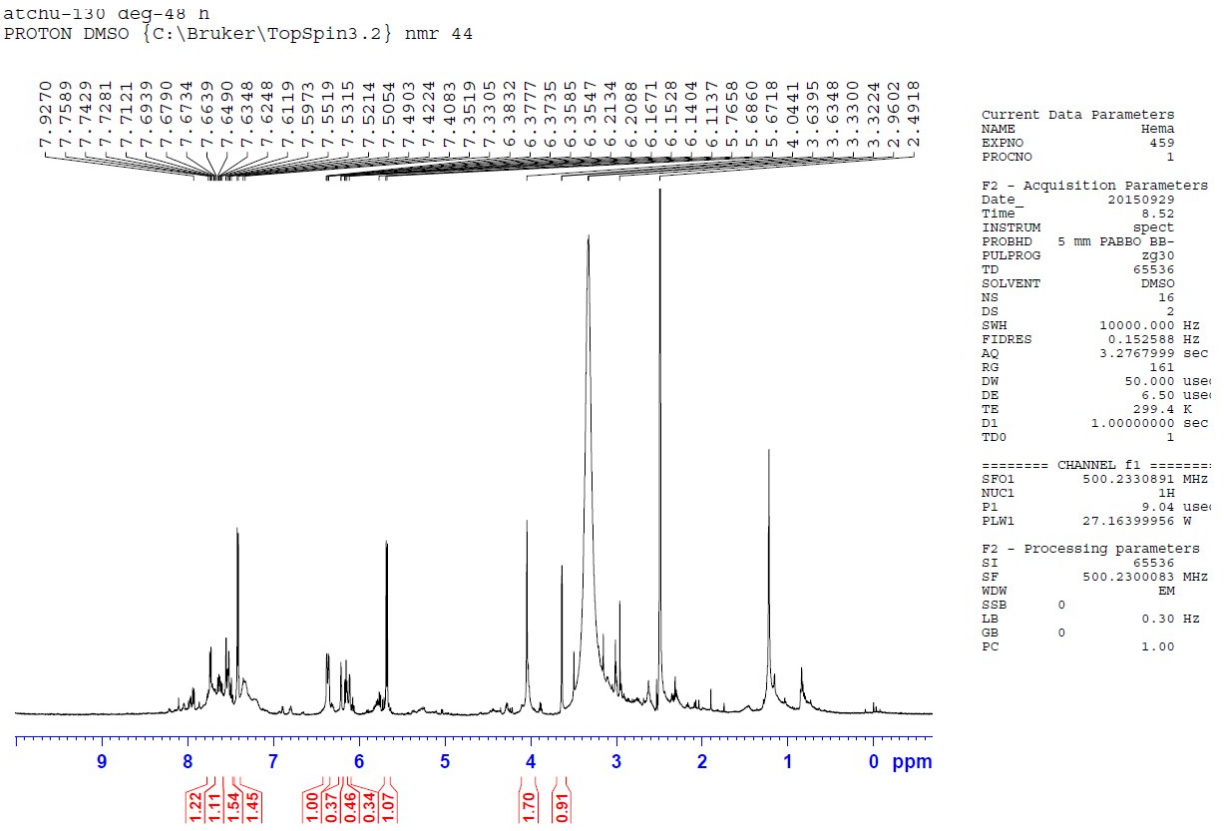


Fig. S15: ^{1}H NMR spectrum of the heated solution of monomer **1** in DMSO-d6

11. References

(1) D. W. Young and H. R. Wilson, *Acta Crystallogr. Sect. B: Struct. Crystallogr. Cryst. Chem.,* 1975, **31**, 961.

(2) H. L. De Winter, N. M. Blaton, O. M. Peeters, C. J. DeRanter, A. VanAerschot and P. Herdewijn, *Acta Crystallogr. Sect. C: Cryst. Struct. Commun*., 1991, **47**, 832.

(3) A. Visnjevac, M. Luic, R. Kobetic, D. Genbarovski and B. Zinic, *Polyhedron*, 2009, **28**, 1057.

(4) A. Visnjevac, N. Biliskov and B. Zinic, *Polyhedron,* 2009, **28**, 3101.

(5) K. C. K. Swamy, E. Balaraman and N. S. Kumar, *Tetrahedron,* 2006, **62**, 10152.

(6) D. Shiomi, M. Nozaki, T. Ise, K. Sato, T. Takui, *J. Phys. Chem. B,* 2004, **108**, 16606.

(7) S. Fujita, A. Takenaka and Y. Sasada, *Acta Crystallogr. Sect. C: Cryst. Struct. Commun.,* 1984, **40**, 1467.

(8) D. Pfeiffer, L. Kutschabsky and S. Hoffmann, *Acta Crystallogr. Sect. C: Cryst. Struct. Commun.,* 1982, **11**, 1635.

(9) M. Rossi and T. J. Kistenmacher, *Acta Crystallogr. Sect. B: Struct. Crystallogr. Cryst. Chem.,* 1977, **33**, 3962.

(10) D. C. Swenson, V. Nair and S. Bera, *Acta Crystallogr. Sect. C: Cryst. Struct. Commun.,* 2000, **56**, e600.

(11) T. Fujino, N. Tsunaka, M. Guillot-Nieckowski, W. Nakanishi, T. Iwamoto, E. Nakamura and H. Isobe, *Tetrahedron Lett.,* 2010, **51**, 2036.

(12) Bruker (**2003**). *SADABS* (Version 2.05), *SMART* (Version 5.631), *SAINT* (Version 6.45) and *SHELXTL* (Version 6.14). Bruker AXS Inc., Madison, Wisconsin, USA.G. M. Sheldrick, *Acta Crystallogr*. **2008**, *A64*, 112.
(13) G. M. Sheldrick, *Acta Crystallogr*., 2008, **A64**, 112.
(14) L. J. Farrugia, *J. Appl. Cryst.,* 1997, **30**, 565.
(15) F. Macrae, P. R. Edgington, P. McCabe, E. Pidcock, G. P. Shields, R. Taylor, M. Towler and J. van de Streek, *J. Appl. Crystallogr*., 2006, **39**, 453.
(16) A. L. Spek, PLATON - a multipurpose crystallographic tool, Utrecht University, The Netherlands, *J. Appl. Cryst.,* 2003, **36**, 7.