# Studies of hexaethylene glycol bridge as a non-nucleotide loop in oligonucleotide by fluorescence spectroscopy and molecular modelling

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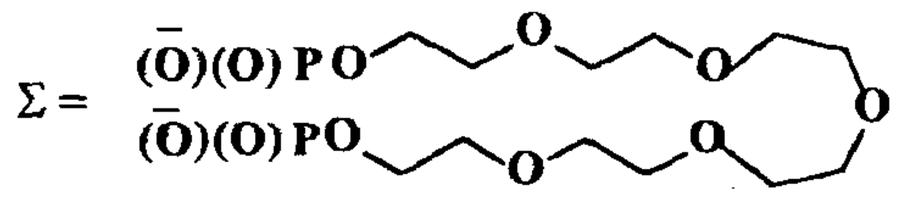
**Conformation of oligonucleotide containing hexaethylene glycol bridge with target having different length and stoichiometry are discussed.**

HEXAETHYLENE glycol bridge (–Σ–) as a non-nucleotide replacement for nucleotide loop is found to be more effective in stabilizing short helical double or triple-stranded oligonucleotide conformations. The results indicated that the complexes $dT_{10}$–Σ–$T_{10}$ form a triple helix with $dA_{10}$ and $A_{20}$, while with $dA_{30}$ they form a double helix. The results are also discussed in relation to stoichiometry of the hexaethylene glycol oligomer and the target.




**Scheme 1.**

There has been increasing interest in synthetic oligonucleotides which were designed to contain hairpin loops. Studies on the modification of these structure have led to the development of non-nucleotide linking groups which replace several nucleotides bridging a folded duplex or triplex structure[1–15]. Linking groups are potentially useful replacements of natural nucleotides as they can shorten the synthesis of a given structure by replacing several individual nucleotides which normally constitute a loop and confer resistance to degradation by nuclease which would ordinarily act on natural loop structures in biological applications[16–18]. Triple helix formation was first demonstrated by Felsenfeld *et al.*[19] with polynucleotides. It was then reported that a triple helix could be formed by short oligonucleotides bound to duplex DNA[20,21]. Since then a number of reports have dealt with triple helix forming oligonucleotides. The schematic representation of triple helix formation on a single stranded target was studied by Glovannangeli[3]. Here the loop joining the two homopyrimidine oligonucleotides consists of a hexamer of ethylene glycol linking the 3′ phosphate of the Hoogsteen strand to the 5′ phosphate of the oligonucleotide forming the Watson–Crick double helix. This complex has a much higher stability than the one obtained with two separate oligonucleotides, thereby opening the possibility of using these molecules to block translation of messenger RNAs or replication of single-stranded viral RNAs or DNAs.

Ethylene glycol oligomers have the advantage of good water solubility than corresponding hydrophobic chain analogues[22], and they can be easily derivatized in the same manner as nucleosides for incorporation in oligonucleotides. Hexaethylene glycol 'Σ' can serve as a replacement either for the single-stranded oligonucleotide loop characteristic of a hairpin structure or for an oligonucleotide segment connecting two pyrimidine oligonucleotide tracts of a triple helix. With the objective to get further information about the conformation of oligonucleotides containing hexaethylene glycol bridge with different target length and stoichiometry, we report here the formation of double or triple helixes by the hexaethylene glycol loop in oligonucleotides, with the help of ethidium bromide which serves as a convenient probe for double stranded and triple stranded oligonucleotides. Ethidium bromide binds to both the structures, but the affinity is greater for the double helix than the triple helix[23–25]. Application of computer molecular modelling [Modified molecular mechanics (MM2) force field within Hyperchem V5.01 (Hypercube, Waterloo, Ontario)] made it easy to study the conformation of hexaethylene glycol loop 'Σ' incorporated in the oligo-nucleotide forming the double helix.

The reversible association of an oligonucleotide with a complementary sequence is of basic importance in biotechnology[26]. Studies of modified oligonucleotides have been conducted with the objective of gaining a further understanding and control of hybridization[27,28]. In exploring structural features that could prove useful as elements in organizing the oligonucleotide frame-work, we have prepared and examined a number of oligonucleotide derivatives with hexaethylene glycol bridge. The experiments were designed to study the formation of a double or triple helix using hexaethylene glycol as a non-nucleotide loop by thermal dissociation temperature and with the help of ethidium bromide intercalation. Hybridization was assayed by changes in $A_{260}$ as a function of the temperature. The interaction with the oligomer $dT_{10}$–Σ–$T_{10}$, forms a stable complex with a series of $dA_{10}$, $dA_{20}$ and $dA_{30}$. Thermal dissociation profiles and their fluorescence intensity with ethidium bromide are listed in Table 1. The $T_m$ $dT_{10}$–Σ–$T_{10}$ with $dA_{10}$, $dA_{20}$ and $dA_{30}$, leads to the question about the formation of duplex or triplex. Triple-stranded complexes bind ethidium less efficiently than related double stranded complexes[23–25]. It was observed that the decrease in fluorescence intensity indicates (Figure 1) that the complexes $dT_{10}$–Σ–$T_{10}$ with $dA_{10}$ and $dA_{20}$ may form a triple helix (I and II respectively). The increase in fluorescence intensity indicates that the complexes $dT_{10}$–Σ–$T_{10}$ with

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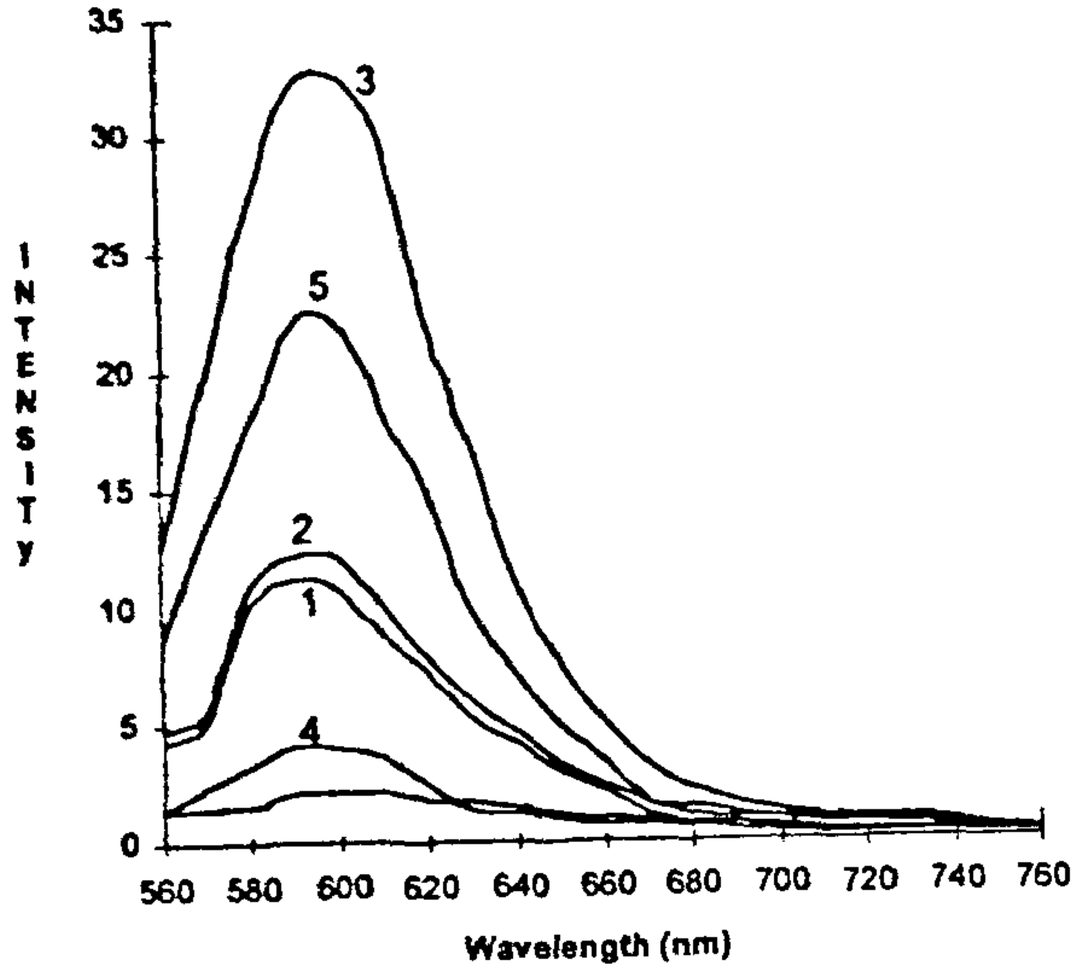
$dA_{40}$ form a double helix (III). The short targets represent minimal binding sites for the ligands and allow loops to bridge the pyrimidine domains without interference from the purine strand. The extended targets were designed to test specifically the interactions of a given loop/linker with the purine strand as its extends outward from the complex[29]. The possibility of structure (IV) and (V) has been excluded as per the fluorescence intensity results which clearly show the formation of the double helix (III) and the triple helix (II). Structure (III) can be well explained as the hexaethylene glycol can span the distance of four nucleotides in the target sequence forming the duplex structure[30]. The possible structures which may be formed according to the results obtained by fluorescence spectroscopy are represented in chart I.

The interaction between complexes $dT_{10}$–Σ–$T_{10}$ (2 equiv.) with $dA_{30}$ (1 equiv.) (Figure 2 *a*), showed




1. dTTTTTTTTTT-Σ-TTTTTTTTTT (1 equiv.) + dAAAAAAAAAA (1 equiv.)
2. dTTTTTTTTTT-Σ-TTTTTTTTTT (1 equiv.) + dAAAAAAAAAAAAAAAAAAAA (1 equiv.)
3. dTTTTTTTTTTTTTTTTTTTT-Σ-TTTTTTTTTT (1 equiv.) + dAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (1 equiv.)
4. dTTTTTTTTTT-Σ-TTTTTTTTTT (2 equiv.) + dAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (1 equiv.)
5. *d*GATCCGATTGTG-Σ-CACAATCG

**Figure 1.** Fluorescent intensity with 8 μM ethidium bromide in 0.1 M NaCl, 0.01 M Tris HCl buffer (pH = 7.0). Conc. of oligonucleotide is 10 μM. The bottom line is base line with ethidium bromide, no nucleotides.

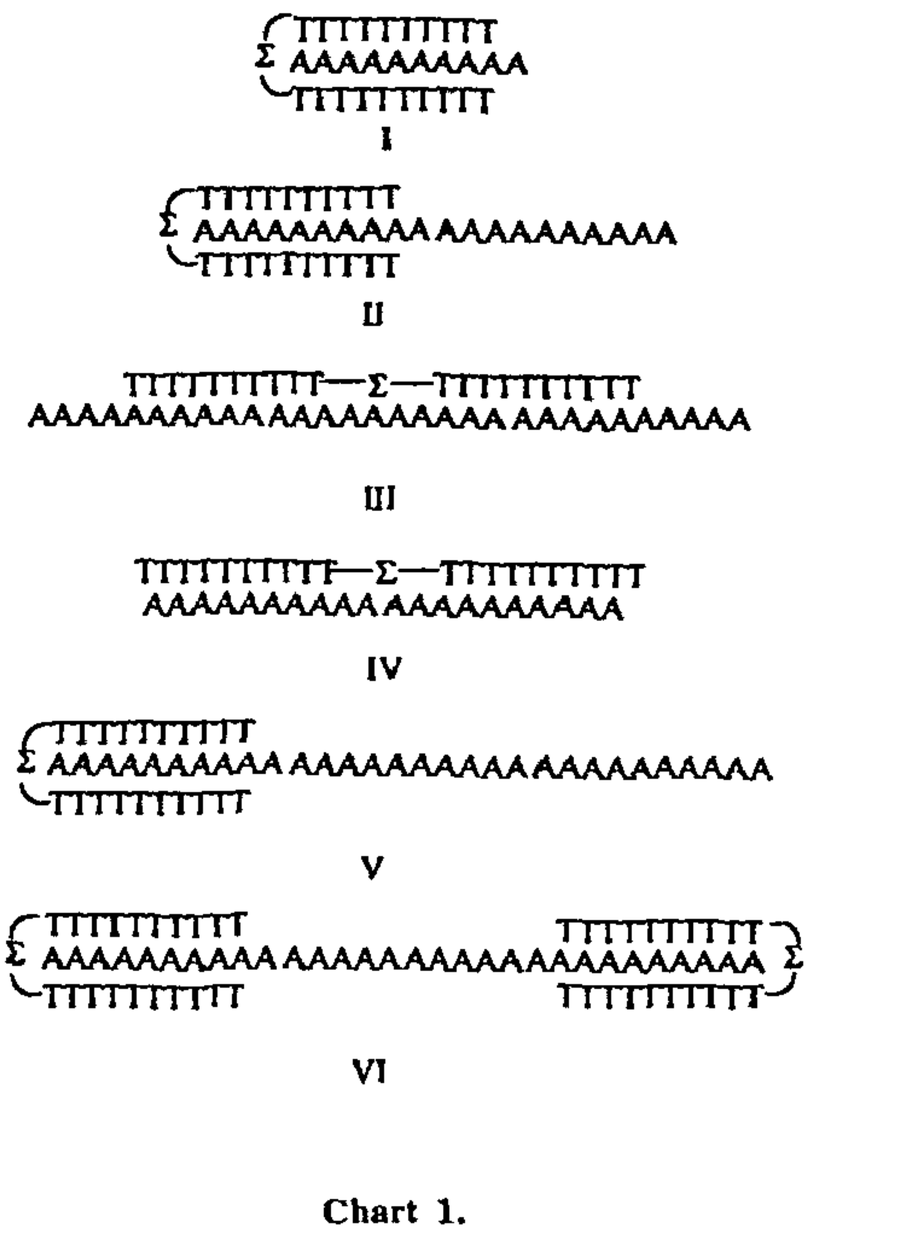



**Chart 1.**

**Table 1.** Thermal dissociation of oligomer complexes

| Expt. no | Oligonucleotide | $T_m$°C (a) No salt | $T_m$°C (a) 0.1 M NaCl | $T_m$°C (a) 1 M NaCl | Fluorescent intensity (b) |
|---|---|---|---|---|---|
| 1 | dTTTTTTTTTT–Σ–dTTTTTTTTTT (1 equiv.) + dAAAAAAAAAA (1 equiv.) | 13 | 30.5 | 48 | 11.2 |
| 2 | dTTTTTTTTTT–Σ–dTTTTTTTTTT (1 equiv.) + dAAAAAAAAAAAAAAAAAAAA (1 equiv.) | 13 | 30.5 | 49 | 12.2 |
| 3 | dTTTTTTTTTT–Σ–dTTTTTTTTTT (1 equiv.) + dAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (1 equiv.) | 18 | 34 | 52 | 32.5 |
| 4 | dTTTTTTTTTT–Σ–dTTTTTTTTTT (2 equiv.) + dAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (1 equiv.) | 18 | 34 | 44 and 53 | 3.4 |
| 5 | dTTTTTTTTTT–Σ–dTTTTTTTTTT (3 equiv.) + dAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (1 equiv.) | 18 | 34 | 45 and 54 | – |
| 6 | dGATCCGATTGTG–Σ–dCACAATCG | 67 | 77 | 78 | 21.9 |

(a) 10 mM Tris phosphate buffer, pH = 7. The total nucleotide concentration was ~ 0.2 $A_{260}$ units/ml. (b) Fluorescence intensity with 8 μm ethidium bromide in 0.1 M NaCl, 0.01 M Tris HCl buffer (pH = 7.0). Concentration of oligonucleotide is 10 μM.

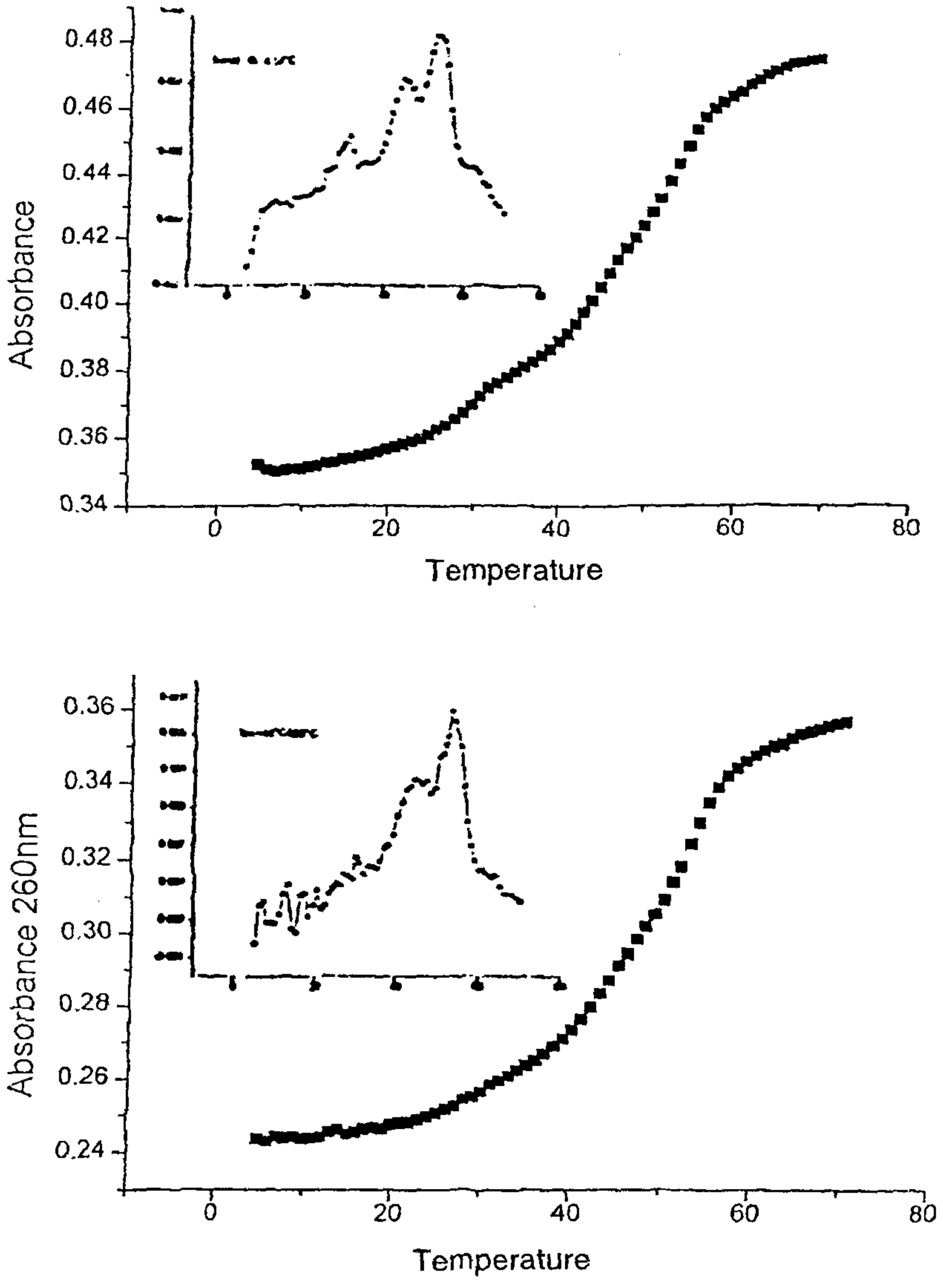



**Figure 2.** *a*, $T_m$ curve for d(TTTTTTTTTT–Σ–TTTTTTTTTT) (2 equiv.) with $dA_{10}$ (1 equiv.): *b*, $T_m$ curve for d(TTTTTTTTTT–Σ–TTTTTTTTTT) (3 equiv.) with $dA_{30}$ (1 equiv.) 1.0 M NaCl Phosphate pH = 7.0.

marked decrease in the fluorescence intensity, which indicates the formation of a triple helix (VI). The melting curve showed two transitions, differing in $T_m$ by ~9°C. The melting curve obtained in low salt concentration (0–0.1 M NaCl) showed a single transition, and at 1 M salt concentration two transitions were observed. The association curve obtained by cooling the solution of the oligomer, slowly from 80°C to 0°C demonstrated that the transitions are fully reversible. The result suggested that the appearance of the second transition indicates triple-stranded complexes. The oligomer dTGTG–Σ–CACA forms a double helix as is visualized with the help of a computer molecular modelling technique[31] (Figure 3 *a* and *b*). Here the hexaethylene glycol bridge is clearly seen on the top with six oxygen atoms which are shown in red incorporated between guanosine and cytidine. The presence of nitrogen molecules, shown in dark navy blue, conclude the presence of intermolecular hydrogen bonding of Watson–Crick duplex structure.

In conclusion, hexaethylene glycol bride 'Σ' can span the distance between the 3′ terminal of one oligonucleotide and 5′ terminal of the other, while forming

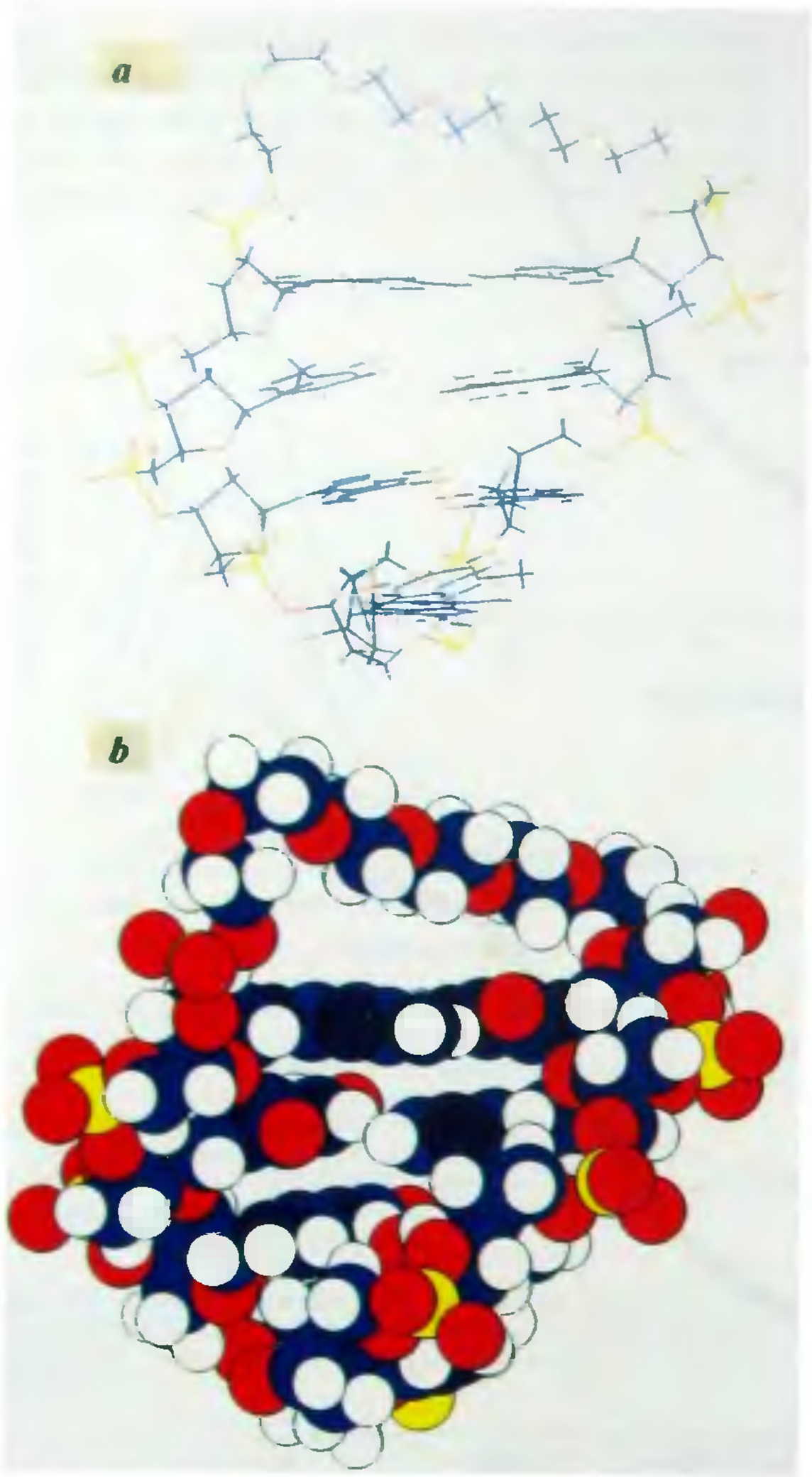



**Figure 3.** MM2-minimized structure for the hairpin TGTG–Σ–CACA. Hexaethylene glycol bridge is shown at the top of the structure (oxygen atom is shown in red, hydrogen in white, carbon in blue, nitrogen in darkblue and phosphorous as yellow).

a double helix as shown by molecular modelling studies and hence may facilitate the formation of a triple helix as further confirmed by ethidium bromide intercalation studies. Thus, the results of the fluorescence intensity obtained with ethidium bromide in oligonucleotide complexes gives precise information about duplex or triplex formation.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31. The hairpin structure was calculated with a modified molecular mechanics (MM2) force field within Hyperchem V5.1 (Hypercube, Waterloo, Ontario).


ACKNOWLEDGEMENTS. Financial assistance from the Council of Scientific and Industrial Research, New Delhi is gratefully acknowledged. We are thankful to Dr K. N. Ganesh and Dr A. A. Natu, National Chemical Laboratory, Pune for encouraging discussion. We thank Dr Xiang of North-Western University, USA for help in computer molecular modelling.

Received 20 July 1998; revised accepted 24 November 1998