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A new lyotropic liquid crystalline phase formed in hydrocarbon solvents by a deoxyguanosine derivative through extensive hydrogen bonding

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Lipophilic deoxyguanosine **1** in pure hydrocarbons or in mixed $CHCl_3$–hydrocarbon solvents forms a new lyotropic liquid crystalline phase characterized by a two dimensional square packing. The structural elements of the phase are ribbon-like assembled species which are completely different from the columnar structures, based on G-quartets, which are the building blocks of the mesophases formed by deoxyguanosine oligonucleotides in water.

1. Introduction

The self-assembly and liquid crystal formation of guanosine mono- and oligo-nucleotides in water is well documented [1]. The process involves the formation of chiral columnar aggregates containing the G-quartet as the basic structure. The quartets are piled one on top of the other at the van der Waals distance in the inner part of the aggregate; the hydrophilic sugar-phosphate backbone runs externally, in contact with water. The structure is similar to the four-stranded helix formed by polyguanylic acid. The growth of these columnar aggregates is concentration and ion dependent: at the critical concentration they become long enough to induce mesophase formation. Cholesteric and hexagonal mesophases have been consistently observed (figure 1).

We have recently synthesized the lipophilic deoxyguanosine derivative **1** [2]. From NMR and ESI mass spectra, this molecule was found to be in a dimeric **2** or oligomeric ribbon-like form **3**, according to solvent and concentration [3].

Solvents which do not compete in hydrogen bonding and in which solute–solute interactions are favoured show fully bonded species even at relatively low concentrations. The presence of the ribbon-like structure **3** is in excellent agreement with the diffractogram displayed by the fibre obtained by slow evaporation of $CHCl_3$ solutions of **1** [3].

It should be noticed that assembled structure **3** is possible only if all the guanosines are disposed with the same of the two non-equivalent faces on the same side of the ribbon; the two faces of the ribbon are by no means equivalent.

2. Results and discussion

When derivative **1** was dissolved in the minimum amount of CH_2Cl_2 or $CHCl_3$ and hexane, heptane or hexadecane were added, a biphasic system or a compact birefringent gel-like phase was observed according to the solvent amount. The use of a chlorinated solvent is necessary due to the very low solubility of solid **1** in hydrocarbons. A typical texture of the compact gel-like phase is shown in figure 2, while biphasic systems show large isotropic fluid regions. When using hexadecane, it is possible to remove all the chlorinated solvent *in vacuo*. This operation, however, does not affect the gel-like phase: no crystallization or modification of the microscopic texture was observed even after several months.

The IR spectrum of the gel-like phase does not show signals corresponding to the free amino or imino groups. On the other hand, 2′,3′-didecanoyl-2′-deoxyinosine **4**, a closely related molecule which does not contain the exocylic 2-amino group, does not form gel-like phases in any of the solvents tested.

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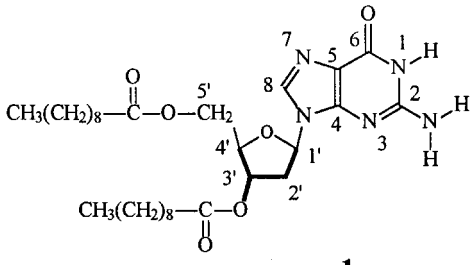

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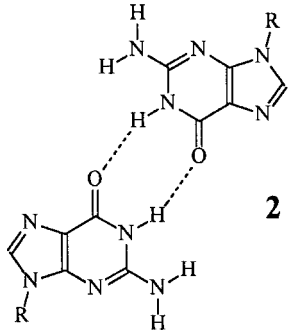

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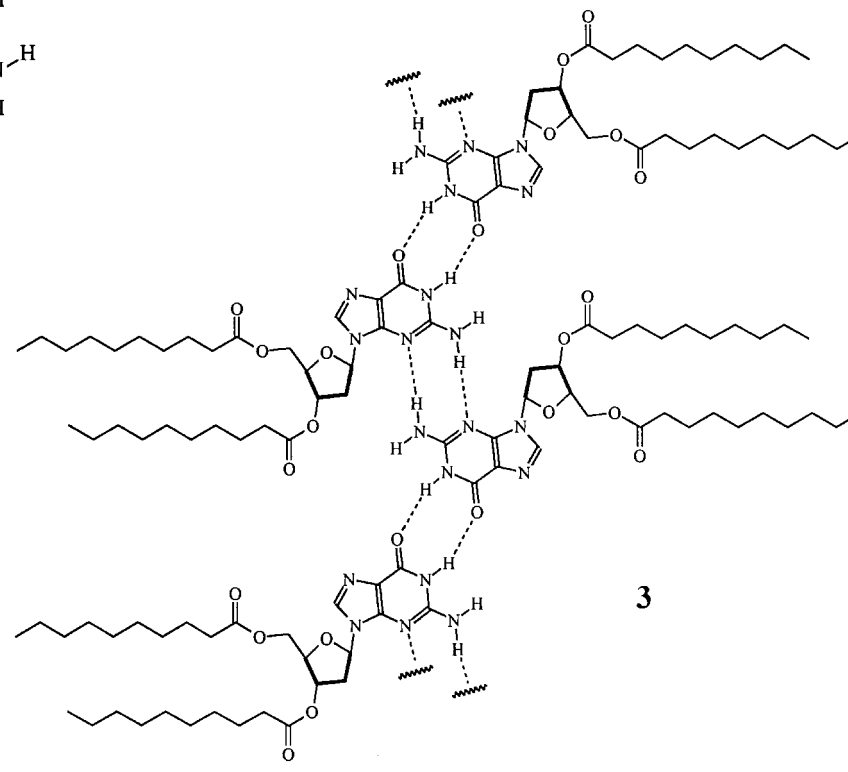

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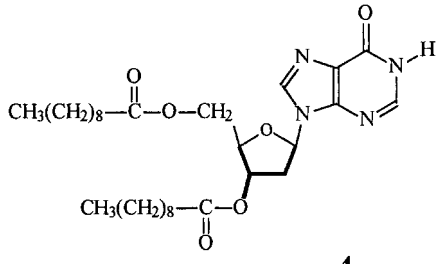

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The molecular mass of the hydrocarbon used as a solvent does not seem to affect the texture of the phase. In hexadecane, the biphasic system is first observed at *c.* 1% w/w and the pure gel-like phase is present between 8 and 10% w/w. Above 10% w/w, the gel-like phase appears to coexist with the solid. In no cases could the fibrillar structures typical of many gels be observed [4]. Microscopic observation seems to be in favour of a liquid crystalline phase rather than of an anisotropic gel. The very narrow range of existence of this phase should be underlined.

The structure of the gel-like phase was derived from X-ray diffraction experiments. At first, derivative **1** was analysed as a function of the hexadecane concentration, in the absence of a third component (i.e. all the chlorinated solvent was removed). The X-ray diffraction profile obtained from a sample in hexadecane (10% concentration) is shown in figure 3: the low angle region, $s < (10\,\text{Å})^{-1}$, is characterized by a series of narrow Bragg reflections, while a diffuse band is observed in the high angle region, $s > (10\,\text{Å})^{-1}$. This pattern clearly indicates that the structure combines short range disorder with long range order. Moreover, the low angle higher order diffractions are not easily detectable, even if very long exposure times were used in the X-ray diffraction experiments. This is a typical result for liquid crystalline samples (both thermotropic and lyotropic), because they are intrinsically disordered. The X-ray diffraction pattern thus confirms the indication from optical microscopy that the gel-like phase is a liquid crystalline state.

The identification of the structure is therefore based on the analysis of the spacing ratios of the few reflections observed in the X-ray low angle diffraction profile [5, 6].

As reported in table 1, the peak reciprocal spacings are in the ratio $1:\sqrt{2}:\sqrt{4}$, indicative of a two dimensional square packing. Within this space group, the equation which defines the spacing of the reflections is in fact [7, 8]:

$$s_{hk} = (1/a)[(h^2 + k^2)]^{1/2} \qquad (1)$$

where a is the unit cell dimension and h, k are the Miller indices of the reflection. The corresponding unit cell is 32.4 Å. The absence of extra low angle peaks indicates

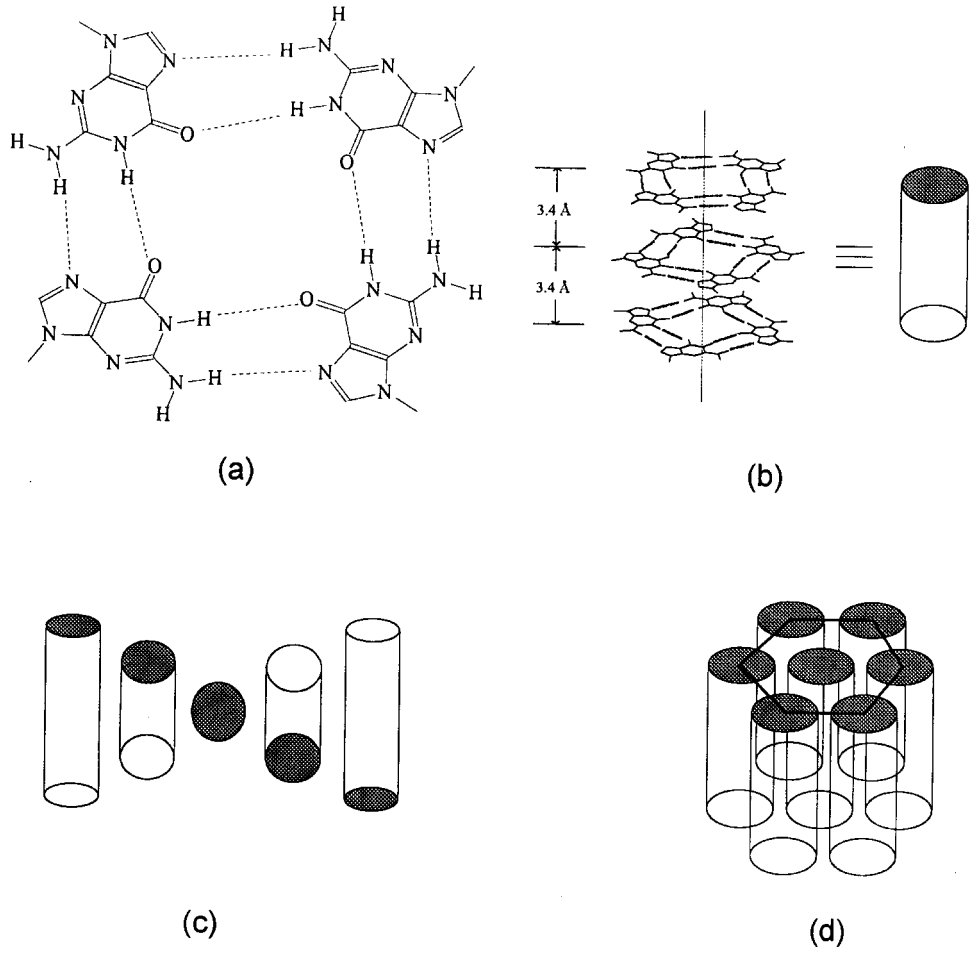


Figure 1. The cyclic Hoogsteen H-bonded quartet (*a*) formed by four guanylic residues and the self-assembly process leading to cylindrical objects (*b*) and eventually to cholesteric (*c*) and hexagonal (*d*) mesophases.

the absence of a long range three dimensional order, i.e. no correlation exists in the direction perpendicular to the two dimensional square cell. Moreover, the high angle region does not show the typical 3.4 Å reflection always present for the columnar phases based on G-quartets [9, 10]. As reported before, a very diffuse band centred at about $s = (4.4\,\text{Å})^{-1}$ is observed instead.

As the symmetry is two dimensional, we analysed these data considering the possible occurrence of a columnar phase, i.e. a phase in which the structure elements are elongated aggregates (rods), each parallel to the other and infinite in length, embedded in the solvent. In such a structure, the projection on the plane perpendicular to the rod axis defines the two dimensional lattice, the symmetry of which belongs to one of the two dimensional square space groups. According to the International Tables of X-ray Crystallography [7], there are different possibilities for packing the aggregates in a two dimensional square lattice. Considering for example the *p*4 two dimensional square space group (N.10 of the International Tables of Crystallography, but the same conclusions can be obtained by considering the other two dimensional square space groups), it is possible to pack one rod per unit cell in position a (co-ordinates 0, 0) of symmetry (*4*) or two rods per unit cell in positions c (co-ordinates 1/2, 0; 0, 1/2) of symmetry (*2*). In the latter case, a further condition ($h + k = 2n$, n integer) limits the possible reflections; therefore the spacing ratios of the three observed Bragg peaks should read as $\sqrt{2}$: $\sqrt{4}$: $\sqrt{8}$ and the unit cell becomes 45.8 Å. The two possible packings are represented in figure 4: it should be noticed that the section of the aggregates in the two dimensional lattice plane should respect the symmetry of the space group; therefore, in the first case the rod projection is sketched as a square and in the second as an ellipsoid.

Information on the guanosine arrangement inside the aggregates can be obtained from the analysis of the cross-sectional area of the rods. By analogy with the more usual type II (inverse, water-in-oil) lipid or surfactant hexagonal phases [5, 6], we hypothesize that the guanine residues are clustered in regions from which

Figure 2. An optical micrograph of the LC phase formed in $CHCl_3$/heptane observed in polarized light with crossed polarizers. The bar corresponds to 10 μm.

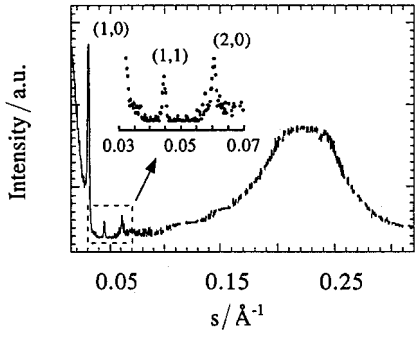


Figure 3. X-ray diffraction profile of the gel-like phase observed at 10% w/w in hexadecane ($T = 25$°C).

the hydrocarbon chains are excluded, and that the didecanoyl chains are completely dissolved in the solvent. Therefore, the cross-sectional area of the core of the rods (S_{core}) can be calculated by using the following equation:

$$N S_{core} = a^2 c_{v,gua} \qquad (2)$$

Table 1. Indexing of the experimental X-ray low angle diffraction pattern observed for the gel-like phase in pure hexadecane. The $p4$ two dimensional space group (N.10 of the International Tables of Cystallography [7]) Whickoff position a has been considered. s (in $Å^{-1}$, see definition in the text) is the reciprocal spacing of the reflection with h, k Miller indices; obs refers to the observed spacings, while clc refers to those calculated for the selected space group.

Concentration	s_{obs}	h, k	s_{clc}
10% w/w, $a = 32.4$ Å	0.0309	1, 0	0.0309
	0.0438	1, 1	0.0436
	0.0615	2, 0	0.0617
9% w/w, $a = 35.1$ Å	0.0285	1, 0	0.0285
	0.0404	1, 1	0.0403
	0.0570	2, 0	0.0570
8% w/w, $a = 36.1$ Å	0.0276	1, 0	0.0309
	0.0392	1, 1	0.0436
	0.0554	2, 0	0.0617

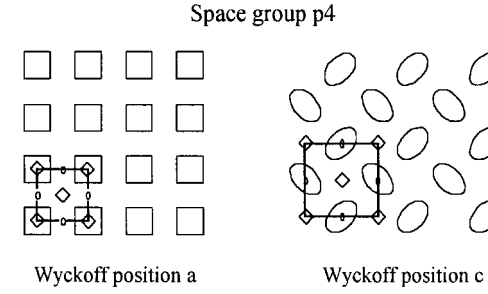


Figure 4. Representation of the columnar 2-D square packing described in the text for the gel-like phase. The heavy black lines mark the unit cell; some of the symmetry elements are also shown. The section of the columns is represented as required by the point symmetry of the special positions where the rods are centred. The Wyckoff positions a and c are defined according to the International Tables of Crystallography (space group $p4$, N.10) [7].

where $c_{v,gua}$ is the volume concentration of the deoxyguanosine residue and N is the number of rods per unit cell ($N = 1$ and 2 for positions a and c discussed above, respectively. Therefore, the cross-sectional area of the rod turns out to be the same for both packing arrangements). Considering the molecular volume of the derivative **1** ($V_{mol} = 955$ Å^3) and that of the deoxyguanosine residue ($V_{gua} = 415$ Å^3), and assuming that the solvent and the lipophilic derivative have similar specific volumes, from the nominal sample composition ($c = 10\%$ w/w), a volume concentration $c_{v,gua}$ of 0.04 has been calculated (see analogous calculations for the lyotropic lipid system reported by Luzzati [5] or by some of us [6]). The value of S_{core} obtained by solving equation (2) was 46 ± 2 Å^2, clearly incompatible with the cross-section of about 450 Å^2 calculated from the

radius of 12.5 Å of the G-quartet structure forming the columnar aggregates in the case of hydrophilic guanosine derivatives [8, 10] (see figure 1). By contrast, the observed area is comparable to the average cross-section of the ribbon-like aggregates made of guanosines in the extended H-bonded structure **3**. From molecular graphics, the estimated area of the cross-section of the ribbon-like aggregate is in fact 47 Å^2.

X-ray data were then obtained at different hexadecane concentrations over the narrow range of existence of the gel-like phase. At a concentration of 9% w/w, the X-ray diffraction profile shows the same sequence of peaks observed at the higher concentration (see table 1), as well as the diffuse band at about $s = (4.4\,\text{Å})^{-1}$. A two-dimensional square unit cell of 35.1 Å (*cf.* 32.4 Å at 10%) was obtained. Decreasing the concentration to 8% w/w, the X-ray diffraction profile maintains its features (see again table 1) and the two dimensional square unit cell increases to 36.1 Å. Any further reduction of the concentration does not change the X-ray diffraction profile or the measured unit cell: in agreement with the microscopic observations, this result indicates that the biphasic region has been reached (i.e. the two dimensional phase is in an excess of solvent or of an isotropic phase). At the two concentrations of 8 and 9%, and using equation (2), cross-sectional areas $S_{core} = 48 \pm 4$ Å^2 and $S_{core} = 45 \pm 4$ Å^2 were calculated, respectively (see table 2). The values obtained confirm that sample dilution increases only the lateral axis-to-axis distance between the aggregates, which behave like rigid objects with a constant cross-section.

Diffraction data for the gel-like phase thus appear consistent with a structure formed by ribbon-like elements of finite width and thickness and indefinite length packed in a two dimensional square lattice. The ribbons contain the guanine residues, in the extended hydrogen-bonded configuration **3**, while the didecanoyl chains, together with the hydrocarbon solvent in which they are dissolved, fill the lateral gap between the ribbons. The fact that the inosine derivative **4** does not form a gel-like phase underlines the importance of the extended hydrogen-bonded ribbons for the formation of the phase.

Table 2. Structural parameters observed for the two dimensional square phase in some of the different conditions investigated. *C* is the nominal concentration in % w/w of the derivative **1** in the mixture.

Solvent	*C*/%, w/w	Unit cell/Å
Hexadecane	8	36.1 ± 0.5
Hexadecane	9	35.1 ± 0.5
Hexadecane	10	32.4 ± 0.5
$CHCl_3$/hexadecane (42% w/w)	9	36.0 ± 1.0
$CHCl_3$/hexadecane (73% w/w)	11	35.7 ± 1.0

X-ray diffraction data also give information about the conformation of both the hydrocarbon chains of derivative **1** and the solvent molecules. The diffuse band observed at $(4.4\,\text{Å})^{-1}$ is in fact characteristic of liquid paraffins [5], and indicates a disordered (liquid-like, type a) organization inside the hydrocarbon region. Due to the expected miscibility, the 4.4 Å band reflects the mean separation of the paraffinic chains (which include both hexadecane and didecanoyl residues).

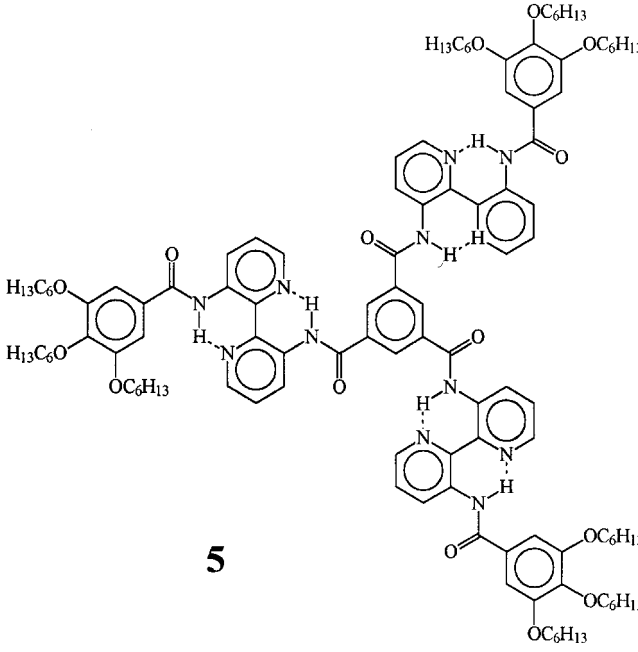
One point should be stressed: the section of the ribbons does not respect the group symmetry, and therefore an orientational disorder of the guanine aggregate has to be invoked. Orientational disorder is characteristic of liquid crystalline columnar phases: in lyotropic systems, the structural elements are considered to rotate freely about their long axes and translate in a direction perpendicular to the two dimensional cell in order to respect the group symmetry [5, 6, 11]. In the present case, if the free rotation of the ribbons inside the liquid hydrocarbon moiety is possible, the model packing with one rod per unit cell (position a) fits the experimental data perfectly.

However, because of the didecanoyl chains, the free rotation could be difficult due to internal friction (viscosity). Therefore, an orientational 'static' disorder should be evoked. Simulation shows, in fact, that in a two dimensional square lattice with two rods per unit cell (position c), orientational 'static' disorder is enough to obtain the peak sequence detected here, extinctions being observed only for larger h, k values†. It should be noticed that orientational disorder induces a large decrease of the peak intensities as a function of s, as observed experimentally. Therefore, within the hypothesis of a restricted rotation, one has to choose this second two dimensional square lattice.

According to optical microscopy observations, derivative **1** also forms the gel-like phase in a number of possible solvents and solvent mixtures. X-ray diffraction experiments were therefore performed to characterize the structure in hexadecane in the presence of chloroform. X-ray diffraction profiles and lattice parameters identical to those observed in hexadecane confirmed that chlorinated solvents do not affect the structure of the gel-like phase. In particular, in table 2, the structural parameters obtained at two different chloroform concentrations are compared with data obtained using pure hexadecane. In the ternary mixture, the two dimensional cell appears almost constant (within the experimental error), indicating that the system is biphasic; no extra peaks occur. The high solubility of solid **1** in chlorinated solvents seems to favour the formation of an isotropic phase in equilibrium with the two dimensional square

†Computation was performed with the public domain NIH Image software.

phase. It must be stressed that derivative **1** is not hygroscopic and does not behave as a surfactant. The present liquid crystalline phase has little in common with the phases formed by soaps, water and hydrocarbons [12]. On the other hand, the present phase is very different from the lyotropic phases N_c and D_{h0} formed in hydrocarbon solvents by disc-shaped compounds containing the 3,3′-diacylamino-2,2′-bipyridine unit **5** [13].



5

The phases formed by **5** resemble those observed in the case of water-soluble guanosine nucleotides which form columnar chiral nematics and hexagonal structures. Guanosine **1** does not behave like the water soluble homologues, but shows a very different self-assembly pattern which is the origin of the new phase observed.

To our knowledge, there are no reports in the literature of liquid crystalline phases similar to the one described in the present paper.

3. Conclusions

Derivative **1** in pure hydrocarbons or mixed $CHCl_3$–hydrocarbon solvents forms a gel-like phase. X-ray diffraction of the gel-like phase in hydrocarbon solvents shows a liquid crystalline order with a two dimensional square packing. The calculated cross-sectional area of the elongated aggregates, which are the building structure of the phase, is in good agreement with the extended hydrogen-bonded model deduced from a spectroscopic study [3]. This new phase is completely different from the G-quartet-based columnar liquid crystals formed in water by guanosine nucleotides. In fact the water soluble derivatives self-assemble to give columnar structures based on G-quartets and their assembled species are the basis of the cholesteric and hexagonal mesophases observed. The lipophilic derivative, instead, self-assembles to give ribbon-like aggregates which are completely different from the aggregates observed in water. This structural diversity is the key to the new phase described in the present report.

4. Experimental

Synthesis

Preparation of 3′,5′-didecanoyl-2′-deoxyguanosine **1** has been described elsewhere [3]. 3′,5′-Didecanoyl-2′-deoxyinosine **4** was prepared as follows. 2′-Deoxyinosine (Aldrich) (0.252 g, 1 mmol) was co-evaporated *in vacuo* three times with dry pyridine (5 ml) and then suspended in dry pyridine (10 ml). To the vigorously stirred suspension *n*-decanoyl chloride (0.50 ml, 2.4 mmol) was added dropwise. The mixture was allowed to react for 1 h, when dry MeOH (0.5 ml) was added and stirring was continued for 15 min. Solvents were removed under reduced pressure. The residue was co-evaporated twice with 10 ml portions of toluene and dissolved in CH_2Cl_2 (40 ml). The resulting solution was washed with 5% $NaHCO_3$ (20 ml) and then with water (20 ml), and the organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by chromatography over silica gel (CH_2Cl_2: MeOH 97:3 v/v), affording the title compound in 71% yield as a white solid. ^{1}H NMR (200 MHz) δ ($CDCl_3$): 0.86 (m, 6H, CH_3 s), 1.22 (m, 24H, CH_2 s), 1.61 (m, 4H, $COCH_2C\underline{H}_2$ s), 2.34 (m, 4H, $COC\underline{H}_2CH_2$ s), 2.60 and 2.82 (m, m, 2H, H2′ and H2″), 4.34 (m, 3H, H5′, H5″ and H4′), 5.39 (m, 1H, H3′), 6.38 (m, 1H, H1′), 8.02 (s, 1H, Ar-H), 8.13 (s, 1H, Ar-H), 13.15 (bs, 1H, NH) ppm.

Preparation of the LC phase

The liquid crystalline phase was prepared by dissolving compound **1** (in a typical experiment, 30 mg) in the chlorinated solvent CH_2Cl_2 or $CHCl_3$ (0.4 ml) and adding the relevant amount of hydrocarbon solvent hexane, heptane or hexadecane (0.35–4 ml). When the hydrocarbon solvent was hexadecane, the chlorinated solvent was then removed under vacuum. The absence (< 2%) of residual chlorinated solvent was checked by GC.

X-ray diffraction

Low angle X-ray diffraction experiments were performed using a 3.5 kW Philips PW1830 X-ray generator equipped with a Guinier-type focusing camera operating in vacuum: a bent quartz crystal monochromator was used to select the Cu-K_α radiation ($\lambda = 1.54$ Å). The

samples were mounted in vacuum-tight cells with thin mica windows. In order to reduce the spottiness arising from possible macroscopic monodomains, the cells were continuously rotated during exposure. The sample cell temperature was controlled with an accuracy of 0.5°C by using a circulating thermostat. The diffraction patterns were recorded on a stack of four Kodak DEF-392 films.

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