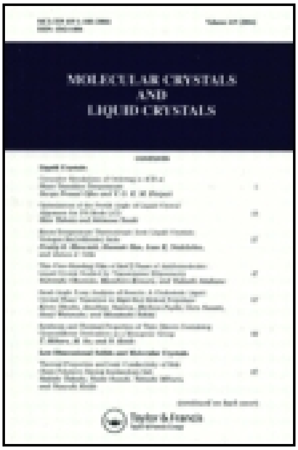


Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

Structural Study of Recognizable Adenine and Thymine Nucleobases Functionalized with Long Aliphatic Chains

J. Michas [a] , C. M. Paleos [a] , A. Skoulios [b] & P. Weber [b]

[a] NRC "Demokritos,", Aghia Paraskevi, Attiki, Greece

[b] Groupe des Matériaux Organiques, Institut de Physique et Chimie des Matériaux, 6, rue Boussingault, Strasbourg, France

Version of record first published: 04 Oct 2006.

To cite this article: J. Michas , C. M. Paleos , A. Skoulios & P. Weber (1994): Structural Study of Recognizable Adenine and Thymine Nucleobases Functionalized with Long Aliphatic Chains, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 239:1, 245-255

To link to this article: http://dx.doi.org/10.1080/10587259408047186

Mol. Cryst. Liq. Cryst., 1994, Vol. 239, pp. 245–255
Reprints available directly from the publisher
Photocopying permitted by license only

Printed in the United States of America

Structural Study of Recognizable Adenine and Thymine Nucleobases Functionalized with Long Aliphatic Chains

J. MICHAS and C. M. PALEOS*
NRC "Demokritos," Aghia Paraskevi, Attiki, Greece

and

A. SKOULIOS and P. WEBER
*Groupe des Matériaux Organiques, Institut de Physique et Chimie des Matériaux,
6, rue Boussingault, Strasbourg, France*

(Received April 14, 1993)

A series of complementary adenine and thymine alkyl derivatives were synthesized and characterized. Due to their amphiphilic-like chemical structure, these compounds, either in a pure state or in binary mixtures, form well developed lamellar structures from room temperature up to the clearing point. Hydrogen bonding between the recognizable moieties are responsible for the increased stability of the crystalline forms. The absence of stable liquid crystalline phases may also be attributed to the unfavorable shape of the alkylated nucleobases and of their complexes.

Keywords: molecular recognition, amphiphilic-like adenine and thymine nucleobases, hydrogen bonding

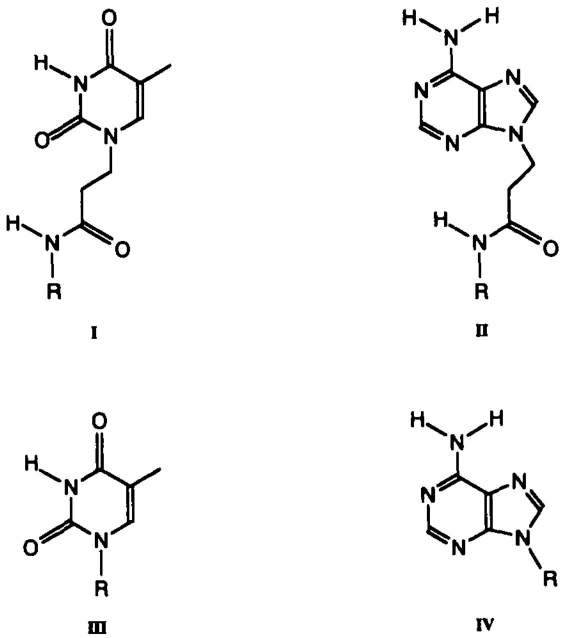
INTRODUCTION

Over the few past years, great interest has developed in the formation of hydrogen bonded complexes induced by the complementary character of the interacting molecules.[1-4] This type of complexation is favored either by the presence in the molecules of appropriate cavities[5-9] or by the existence of van der Waals stacking interactions.[10-11] However, complexation may still take place with molecules devoid of cavities, which yet are able to interact through hydrogen bond formation.[12-13] With the strategy of avoiding the presence of cavities in the molecules, a variety of crystalline solids[14-17] or liquid crystalline molecular and macromolecular materials[18-20] have thus been prepared.

In biological systems, the hydrogen bond pairing of the complementary nucleobases plays a decisive part, as well known. In DNA for instance, it develops through a cooperative template process, the zippering of the two complementary single strands.[21] In non-biological systems, the hydrogen bonding interaction of the nucleobases has been found to be significantly enhanced in the presence of non-hydrogen bonding solvents; such is the case in chloroform for example.[22-25] In aqueous media, on the other hand, stacking of the nucleobases has been found to predominate over hydrogen bonding of the molecules.[6]

Functionalization with long paraffin chains was expected, of course, to lower the melting temperature of the nucleobases, but also, and more importantly, to transform them in "amphiphilic" molecules. Due to the different physicochemical properties of the chains and the heterocyclic cores, the amphiphilic character of these compounds was expected to induce special characteristics liable to affect the hydrogen pairing to some extent. Specifically, it was expected to favor lamellar structures for the crystals at low temperature, corresponding to an alternating stacking of segregated paraffin and heterocyclic layers, and also to hopefully bring about thermotropic liquid crystalline structures at high temperature, below the ultimate melting transition.

With this in mind, we decided to synthesize alkylated adenine and thymine derivatives and to study their structural behavior as a function of temperature, both in the pure state and in the state of binary mixtures resulting from their molecular recognition through hydrogen bonding. In a preliminary paper,[26] we reported the synthesis and polymorphic behavior of compounds I and II (see Scheme I). The optical textures of these and of their binary mixtures, observed upon cooling from the melt, were interpreted as indicative of the presence of liquid crystals below the clearing point. Their structural analysis with x-ray diffraction remained, however, to be done; this is the object of the present paper, along with the synthesis and study of compounds III and IV.



SCHEME I

EXPERIMENTAL

Synthesis

Compounds I and II were synthesized through a series of steps,[27-29] starting from adenine and thymine as described previously.[26] As for compounds III and IV, they were synthesized using procedures analogous to those described by Browne *et al.*[30] In all these compounds (see Scheme I), radical R is a normal hexadecyl chain.

For the preparation of compound III, 0.003 mol of *n*-bromohexadecane and 0.011 potassium carbonate were added to a solution of 0.01 mol of thymine in dry dimethylsulfoxide. The dispersion was stirred at room temperature for one week. The insoluble material was filtered out, washed with water, and recrystallized twice from methanol. Analysis calculated for $C_{21}H_{38}N_2O_2$: C, 71.96; H, 10.93; N, 7.99. Found: C, 72.01; H, 11.08; N, 7.76.

For the preparation of compound IV, a suspension in dry dimethylformamide of 0.01 mol of adenine and 0.0105 mol of sodium hydride (60% dispersion in mineral oil) was stirred at room temperature under nitrogen for one hour. Added with 0.0105 mol of *n*-bromohexadecane, the suspension was further stirred at room temperature for one week. The insoluble material obtained was filtered out, washed with water, and recrystallized twice from ethanol. Analysis calculated for $C_{21}H_{37}N_5$: C, 70.16; H, 10.37; N, 19.47. Found: C, 70.31; H, 10.58; N, 19.46.

The thermal stability of all the compounds was analyzed using a Mettler TC10A instrument coupled with a TA processor and a Mettler M3 balance. It was found appropriate for the subsequent thermal studies planned in this work, especially for the rather lengthy x-ray experiments at high temperature. The samples withstood two-hour heating just below the melting temperature with less than 2% loss in weight.

Characterization Studies

Optical microscopy observations were performed employing a Leitz-Wetzlar polarizing microscope equipped with a Mettler FP 82 hot stage. Differential scanning calorimetry measurements were carried out with a Perkin-Elmer DSC-7 coupled with a TAC 7/DX controller (heating and cooling rates of 10°C/min). Small- and wide-angle x-ray diffraction patterns were recorded photographically using monochromatic $CuK_{\alpha 1}$ radiation and a Guinier focusing camera equipped with a custom-made heating sample-holder controlling the temperature within less than 1°C.

RESULTS AND DISCUSSION

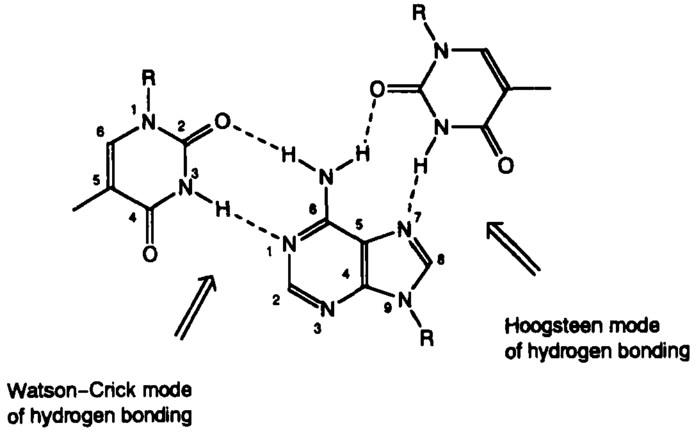
A common characteristic of all the compounds considered in the present work is that they carry the aliphatic chain at the 9 and 1 position of the adenine and thymine molecules respectively, that is, at positions remote from the sites involved in the hydrogen bonding molecular recognition processes. Two types of aliphatic substituent were used. The first is an alkyl chain with an amide group (compounds I and II), while the second is a simple normal hexadecyl chain (compounds III and IV).

The presence of an amide group in the molecules is, of course, susceptible of affecting the organizational features of the nucleobase amphiphiles through a possibility of extra hydrogen bonding interactions of the amide groups with themselves on one hand, as well as with the heterocyclic aromatic cores on the other. Furthermore, the adenine derivatives with a primary amino group available for hydrogen bonding and with more than one hydrogen bond acceptor can recognize two molecules of thymine, as shown in Scheme II. This is why complexes with 1:1 and 1:2 molar ratios were prepared.

Pure Compounds

The thermal polymorphism of compounds I–IV was analyzed using DSC (see Table I). A typical example of thermogram registered is shown in Figure 1. The difference between the amide-containing compounds I and II and the amide-free compounds III and IV is twofold. First, the latter show only one, while the former show two phase transitions upon heating. Second, the amide-free derivatives melt at temperatures significantly lower than the amide-containing ones; hydrogen bonding of the amide groups raises, therefore, the clearing point as expected. All these phase transitions (except for the weak one of compound II) showed a strong hysteresis upon cooling (about 30°C for I; 25°C for II; 40°C for III; and 50°C for IV), indicative of the nucleation process generally observed in first order transitions with crystalline phases.

The thermal behavior of the samples was then examined by optical microscopy. Upon heating, all the transitions detected by DSC could also be observed optically at the right temperatures. For compounds I and II, inserted as a powder between the glass plates, the grains of the material heated above the first transition temperature were softening, becoming easy to spread by gentle pressure on the cover slip into a thin, translucent film displaying an inextricable birefringent texture; one is tempted to interpret this observation as indicating that the paraffin chains dispose in this temperature range of some mobility and fluidity. Above the second transition temperature, the material melted completely, turning into an isotropic liquid. For



SCHEME II

TABLE I

Transition temperatures (T), enthalpies (ΔH) and entropies (ΔS) of pure compounds I–IV, as determined by DSC upon heating samples recrystallized from the melt (molar quantities were calculated per mole of adenine or thymine derivative)

Compound	T/°C	ΔH/kJ.mol^{-1}	DS/J.mol^{-1}K^{-1}
I	131 175	28.7 28.1	71.0 62.7
II	123 170	02.1 38.4	04.8 86.7
III	120	37.4	95.0
IV	122	66.1	167.5

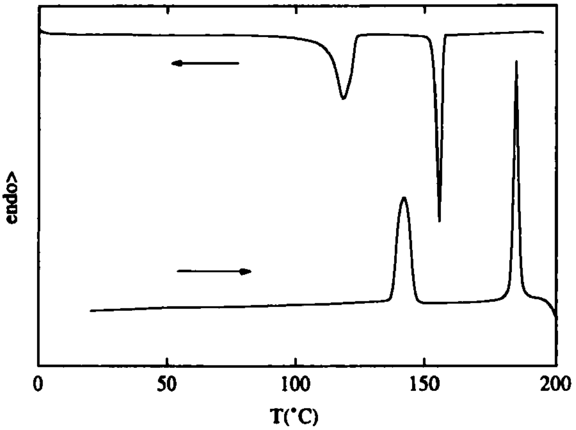
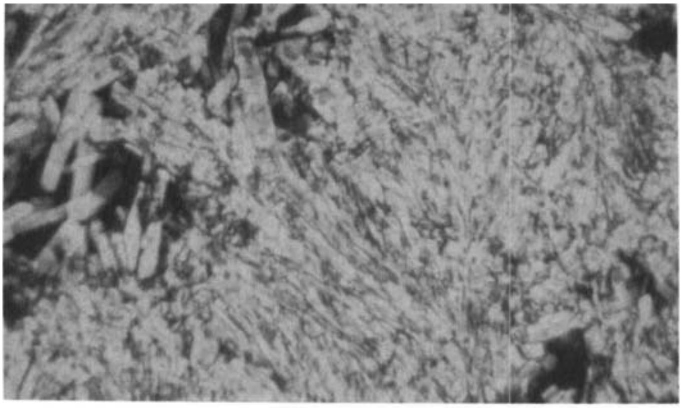


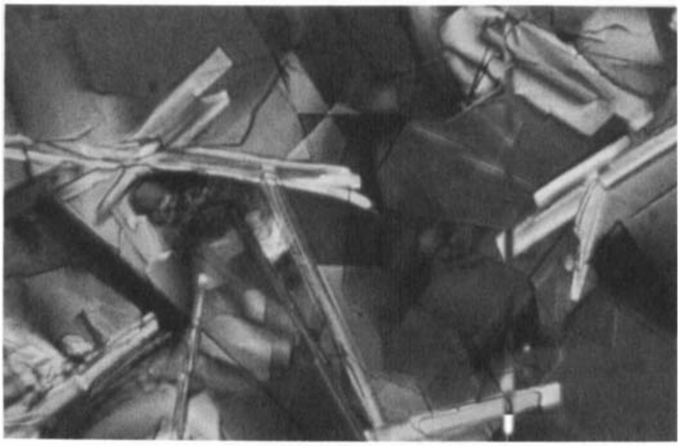
FIGURE 1 DSC thermogram of amide-containing thymine derivative I, obtained upon heating and cooling with a rate of ± 10 K/min.

compounds III and IV, the melting occurred in one step, directly from the powder into the isotropic liquid. To get better developed textures, the usual procedure, consisting in cooling the samples slowly from the melt, was then applied. The optical textures observed for compounds III and IV were clearly characteristic of crystals, whereas, for the amide-containing compounds I and II, they could be taken (as already stated in Reference 26) as indicative of mesomorphic phases (see Figure 2).

To check up the crystalline nature of the low temperature phases and, more interestingly, the possibly mesomorphic nature of the high temperature phases of compounds I and II, x-ray diffraction was used systematically. Quite disappointingly, however, the recorded x-ray patterns invariably contained a large number



(a)



(b)

FIGURE 2 Typical optical textures obtained by polarizing microscopy upon cooling from the melt: (a) compound I at about 140°C and (b) compound IV at room temperature (crossed polars, magnification 180×). See Color Plate VI.

of reflections in the small-angle as well as in the wide-angle region; they thus clearly indicated that the structure of all the compounds was not mesomorphic but periodic in three-dimensions, that is, crystalline throughout the whole range of temperature explored, up to the final melting point.

The small-angle reflections registered were found to be sharp and equidistant, indicating for the crystals a well developed lamellar structure with large stacking periods, of the order of the molecular lengths as estimated by molecular modeling (PC MODEL software, kindly provided by K. Stelliou, University of Montreal) (see Table II). Of course, this type of ordering is due to the amphiphilic character of the materials, the molecules of which were indeed designed to contain two distinct parts, one heterocyclic and one aliphatic, able to segregate in space and hence to fit in superposed layers. For compounds II and III the lamellar period, comprised between one and two molecular lengths, may easily be taken as suggesting a double-layered structure for the lamellae. For compounds I and IV, on the other hand, the lamellar period is difficult to interpret safely, as it is very close to one molecular length; it may very well be analyzed in terms of single layers of upright molecules interdigitated in a head-to-tail arrangement, as well as in terms of double layers of molecules strongly tilted with respect to the normal.

The textures of compounds I and II, taken as indicative of mesomorphic phases, are at variance with these x-ray observations. They may, however, be easily understood in a number of different ways. Either monotropic mesophases do actually occur upon cooling from the melt, far below the clearing point, just before the crystal is formed; however, these are too unstable to be detected by x-ray diffraction or DSC, although they are able enough to lend the crystalline phases some features of their textures (paramorphosis). Or the three-dimensional positional correlations of the lamellae in the crystal are weak enough to let the material behave (from the standpoint of the geometrical constraints responsible for the formation of focal-conic textures) more like a smectic than like a usual crystal. Or else the high temperature phase, at least that of compound I which shows two phase transitions of equal strength, is indeed an ordered smectic phase very much like those defined as true crystals. Or finally the system between the two transition temperatures is not homogeneous but consists of the mixture of two coexisting phases, one liquid and one crystalline, finely interdispersed; this may be understood as an indication of the possible presence at low temperature of two polymorphic modifications melting at different temperatures.

TABLE II

Lamellar spacings (d) and molecular lengths (L) of compounds I–IV, determined at temperatures T

Compound	T/°C	d/A°	L/A°
I	120 160	29.5 32.7	28
II	100 130	42.1 46.5	29
III	30	44.3	22
IV	30	22.4	24

Mixtures

The 1:1 molar and 2:1 molar complexes of thymine (I or III) and adenine (II or IV) derivatives were prepared following the standard method of slow evaporation of chloroform solutions. This method is known[6] to enhance recognition interactions through hydrogen bonding. It is important to note immediately that, in the case of adenine and thymine nucleobases, cross recognition is by far stronger than self-recognition.[25] Samples were dried and kept over phosphorus pentoxide before use.

The thermal and structural polymorphic behavior of these mixtures was examined using DSC. Inspection of Table III as compared with Table I suggests the following comments. First, the transition temperatures measured for the mixtures, together with the corresponding enthalpies and entropies, are different from those previously presented for the pure compounds. This may be taken as clear evidence that complexation through molecular cross-recognition does actually occur to a large extent. Second, the number of the phase transitions detected is larger with mixtures than with pure compounds. This indicates either that the thermal polymorphism of the systems is richer or, much more reasonably as will be seen below, that mixtures contain more than one type of complex.

Third, the presence of a significant exothermic peak in the thermograms of III–IV mixtures at low temperature, immediately following an isoenergetic endothermic one (Figure 3), suggests that prime crystallization from the melt (taking place after a huge supercooling down to 27°C, with a cooling rate of −10 K/min)

TABLE III

Transition temperatures (T), enthalpies (ΔH) and entropies (ΔS) of 1:1 and 1:2 molar binary mixtures of compounds I–IV, as determined by DSC upon heating samples recrystallized from the melt (molar quantities refer to number average molecular weights of mixtures)

Compound	T/°C	ΔH/kJ.mol^{-1}	ΔS/J.mol^{-1}K^{-1}
I-II (1:1)	105	06.6	17.4
	121	01.3	03.2
	155	27.9	65.2
I-II (2:1)	106	05.4	14.4
	119	01.3	03.3
	136	08.9	21.8
	154	27.0	63.3
III-IV (1:1)	35	14.2	45.9
	42	-14.1	-44.8
	90	22.9	63.1
	103	06.7	18.7
III-IV (2:1)	39	32.6	104.5
	48	-32.7	-101.8
	82	06.4	017.9
	93	31.2	085.2

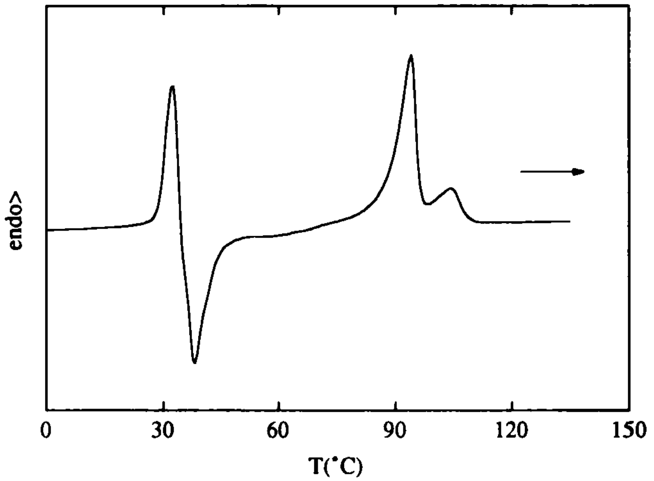


FIGURE 3 DSC thermogram of 1:1 molar binary mixture of amide-free compounds III and IV upon heating at 10 K/min.

produces metastable crystals; this is presumably due to the molecular geometry of the complexed molecules in the melt, which is not necessarily adequate for the immediate formation of stable crystals. The absence of such a phenomenon with mixtures of amide-containing I and II compounds suggests that the extra hydrogen bonding offered by the amide groups improves the lateral register in a way that facilitates the nucleation process as well as the overall crystallization of the system. Another evidence of the constraints imposed by the hydrogen bonding of the molecules in the melt upon the crystallization of the mixtures may be found in the small values of the enthalpies measured as compared with those observed with pure compounds whose hydrogen bonding is much weaker in the isotropic melt.

Finally, comparison of the 2:1 and 1:1 molar mixtures suggests that complexation through molecular recognition is not unique: it does not lead to homogeneous systems. In the case of I–II (2:1) mixture, the phase transitions observed are exactly the same as those found in the case of I–II (1:1) mixture, with an extra transition at 136°C, similar to that of pure compound I. This suggests, as will be further supported by x-ray diffraction, that the 2:1 complex (see Scheme II) is not formed. Assuming that only the 1:1 complex is formed, half of compound I (molar concentration 1/3) should remain uncomplexed and the enthalpy at the extra transition should be reduced to one third of the nominal value, as found experimentally. In the case of III–IV mixtures, both 1:1 and 2:1 complexes are presumably formed, as implied by the enthalpy inversion of the two high temperature transitions (see Table III).

Optical microscope observations as a function of temperature showed the mixtures to be heterogeneous, formed of crystals melting at different temperatures. X-ray diffraction investigations confirmed these results. At low temperatures, the patterns registered showed the presence of two lamellar structures, disappearing stepwise upon heating (Table IV). It is of interest to note that the relative intensities of the Bragg reflections at lamellar spacings d_1 and d_2 for the III–IV mixtures

TABLE IV

Lamellar spacings (d) of 1:1 and 1:2 molar binary mixtures of compounds I–IV, determined at temperatures T

Compound	T/°C	d_1/A°	d_2/A°
I-II(1:1)	110	41.9	36.5
	142	43.3	
I-II(2:1)	115	43.3	28.5
	134	43.3	28.5
	146	43.3	33.5
III-IV(1:1)	90	23.5	24.1
	105	23.4	
III-IV(2:1)	83	24.1	22.4

undergo the same inversion with the molar concentration as observed with DSC, supporting the view that the 1:1 and 2:1 complexes are formed simultaneously in the mixture.

CONCLUDING REMARKS

Adenine and thymine nucleobases substituted with long paraffin chains, taken in the pure state as well as in binary mixtures, are found to organize in the solid state at low temperature, forming lamellar structures as expected for alkylated systems, amphiphilic in nature to some extent. This type of ordering is maintained up to the final melting of the material, suggesting strong hydrogen bonding and lateral interactions of the molecules. The absence of clear-cut mesomorphic phases may well be due to the excessive stability of the crystals, but more likely to the very shape of the molecules and their complexes, which is not necessarily adapted to a suitable packing of the molecular species into liquid crystalline phases. Further work with different substituents and molecular geometries is in progress in view of producing stable liquid crystals and thus of getting an opportunity to evaluate the specific role of amphiphilic character and lateral molecular register in molecular recognition through hydrogen bonding.

References

1. D. J. Cram, *Angew. Chem. Int. Ed.*, **27**, 1009 (1988).
2. J. M. Lehn, *Angew. Chem. Int. Ed.*, **27**, 90 (1990).
3. A. Raevskii, *Russian Chem. Rev.*, **59**, 375 (1990).

4. A. A. Hamilton, *J. Chem. Educ.*, **67**, 821 (1990).
5. J. Rebek Jr., *Acc. Chem. Res.*, **23**, 399 (1990).
6. J. Rebek Jr., *Angew. Chem. Int. Eng. Ed.*, **29**, 245 (1990).
7. S. C. Zimmerman, W. Wu and Z. Zeng, *J. Am. Chem. Soc.*, **113**, 196 (1991).
8. A. Hamilton and D. V. Engen, *J. Am. Chem. Soc.*, **109**, 5035 (1987).
9. K. S. Jeong, T. Tjivikua, A. Muehldorf, G. Deslongchamps, M. Famulok and J. Rebek Jr., *J. Am. Chem. Soc.*, **113**, 201 (1991).
10. J. Rebek Jr., B. Askew, P. Ballester, C. Buhr, S. Jones, D. Nemeth and K. Williams, *J. Am. Chem. Soc.*, **109**, 5033 (1987).
11. J. Rebek Jr., B. Askew, P. Ballester, C. Buhr, A. Costero, S. Jones and K. Williams, *J. Am. Chem. Soc.*, **109**, 6866 (1987).
12. M. C. Etter, *Acc. Chem. Res.*, **23**, 120 (1990).
13. M. C. Etter and S. M. Reutzel, *J. Am. Soc.*, **113**, 2586 (1986).
14. M. C. Etter, Z. U. Urbanczyk-Lipokowska, M. Zia-Ebrahimi and T. W. Panunto, *J. Am. Chem. Soc.*, **112**, 8415 (1990).
15. H. Iwahasi, H. Sugeta and Y. Kuogoku, *Biochemistry*, **21**, 631 (1982).
16. C. T. Seto and G. M. Whitesides, *J. Am. Chem. Soc.*, **115**, 905 (1993).
17. F. G. Tellado, S. G. Geib, S. Goswami and A. D. Hamilton, *J. Am. Chem. Soc.*, **113**, 9265 (1991).
18. M. J. Brienne, J. Gabart, J. M. Lehn and I. Stibor, *J. Chem. Soc., Chem. Commun.*, 1868 (1989).
19. J. M. Lehn, M. Pascal, A. Decian and J. Fischer, *J. Chem. Soc., Chem. Commun.*, 479 (1990).
20. C. Fouquey, J. M. Lehn and A. M. Levelut, *Adv. Materials*, **2**, 254 (1990).
21. J. S. Lindsay, *New J. Chem.*, **15**, 153 (1991).
22. L. Katz and S. Penman, *J. Mol. Biol.*, **15**, 220 (1966).
23. R. M. Hamlin Jr., R. C. Lord and A. Rich, *Science*, **148**, 1734 (1965).
24. J. Pitha, R. N. Jones and P. Pithova, *Can. J. Chem.*, **44**, 1045 (1966).
25. Y. Kyogoku, R. C. Lord and A. Rich, *J. Am. Chem. Soc.*, **89**, 496 (1967).
26. J. Michas and C. M. Paleos, *Liq. Cryst.*, **11**, 773 (1992).
27. H. Ahlers, H. Ringsdorf, H. Rosemeyer and F. Seela, *Coll. and Polym. Sci.*, **268**, 132 (1990).
28. E. P. Lira and C. W. Huffman, *J. Org. Chem.*, **31**, 2188 (1965).
29. C. G. Overberger and Y. Inaki, *J. Polym. Sci. Chem. Ed.*, **17**, 1739 (1979).
30. D. T. Browne, J. Eisinger and N. J. Leonard, *J. Am. Chem. Soc.*, **90**, 7302 (1968).