Synthesis and study of an acridine substituted Tröger's base: preferential binding of the (–)-isomer to B-DNA

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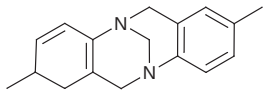
The acridine substituted Tröger's base 4 was prepared, resolved and the (−)-(7*R*,17*R*) enantiomer was shown to bind preferentially to calf-thymus B-DNA.

The development of molecular probes to study local conformations in DNA is of major interest; until now, only chiral metal complexes have been studied for enantioselective recognition of DNA conformations.[1] We report herein that combining the geometry and chirality of Tröger's base[2] (methanodibenzo-



Tröger's Base **1**

[1,5]diazocine) **1** with the DNA binding properties of acridines[3] leads to a new family of C_2-chiral DNA binding molecules, such as **4**. The geometry of the Tröger's base unit gives the molecules a helix shape which can be similar or opposite to the helicity of DNA (Fig. 1). A preliminary molecular modelling study[4] suggested that the binding of such probes to DNA should be enantioselective.

Compound **4** was prepared in two steps from monoacetylated proflavine **2** (Scheme 1).[5] Condensation of **2** with formaldehyde in TFA[6,7] gave the diacetamido derivative (±)-**3** (76%). Treatment of **3** under basic conditions (EtOH, NaOH) cleaved the two acetamido groups without affecting the methanodiazocine bridge, to give (±)-**4** (90%). The structure of **4** was supported by detailed 2D ^{1}H NMR studies.[8]

Resolution of (±)-**4** was achieved by crystallisation of its dibenzoyltartrate salts; (+)-**4** showed = $[\alpha]_D^{20}$ +4650 (*c* 9 × 10^{-3}, 95% EtOH). The enantiomeric excess (ee) was close to 80% (NMR).† Similarly, (+)-dibenzoyltartaric acid gave (−)-**4**. The circular dichroism (CD) spectra of both enantiomers are shown in Fig. 2.

The presence of the primary amino functions on the acridine rings of **4** turned out to be of major importance in the design of a molecule binding selectively to DNA. Solubility in water is considerably increased as compared to the parent unsubstituted molecule.[6] More important, the basicity is much higher (pK_a 5.5 and 6.8 *cf.* 3.2 for **1**)[9] and protonation occurs on the acridine ring nitrogens and not on the nitrogens of **1** (UV–VIS data). This protects **4** from racemization as is usually observed in acidic media for Tröger's base (such as **1**).[10] Indeed compound **4** did not racemize in 0.1 M HCl in DMF (20 °C, 40 days).

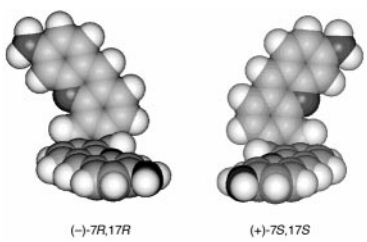
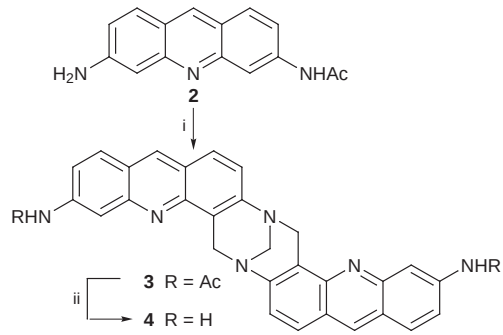


Fig. 1 Molecular modelling representation of the two enantiomers of **4**.



Scheme 1 *Reagents and conditions*: i, HCHO (1.5 equiv.), TFA, room temp., 4 h; EtOH–NaOH (10 M) (3 : 1), 85 °C, 6 h.

The absolute configuration of the enantiomers was assigned as follows. Enantiomer (+)-**4** displays a broad positive band of weak intensity at low energy (380–470 nm, $\Delta\varepsilon$ +26 M^{-1} cm^{-1}), and a strong bisignate system at high energy with maxima at 290 ($\Delta\varepsilon$ +218 M^{-1} cm^{-1}) and 250 nm ($\Delta\varepsilon$ −163 M^{-1} cm^{-1}). The low energy CD band corresponds to a broad UV absorption at 350–450 nm, whereas the bisignate pattern is centred on a strong UV band at 272 nm (ε 101400 M^{-1} cm^{-1}). There is evidence that each component of the bisignate system comprises at least two mutually overlapping bands. Given the two-fold symmetry of **4** and the nature of its chromophore, we assume that the bisignate pattern is due to the exciton coupling[11] of some long-axis polarised transitions of the aminoacridine units. Based on simple geometric arguments, we expect that in **4** the symmetric (*A*) coupling and the antisymmetric (*B*) coupling of these transitions in the two acridine units are polarised along the C_2 axis and perpendicularly to that axis, respectively. This picture together with the further assumption that the *A* coupling is at higher energy than the *B* coupling[12] leads us to assign the (*S*,*S*) configuration to (+)-**4**.

In the past, application of the exciton chirality method to **1** itself has lead to a wrong assignment of its absolute configuration,[13] because the direction of polarisation of the considered transitions have not been correctly established. For this reason we found it desirable to verify the consistency of our

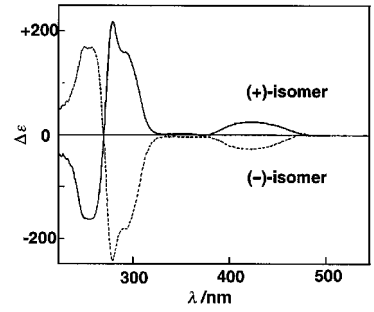


Fig. 2 Circular dichroism spectra of (+)-**4** (solid line) and (−)-**4** (dotted line) in ethanol (10^{-4} M). For (+)-**4**: $[\alpha]_D^{20}$ +4200 (*c* 1.6 × 10^{-3}, ethanol) and for (−)-**4**: $[\alpha]_D^{20}$ −4800 (*c* 1.5 × 10^{-3}, ethanol).

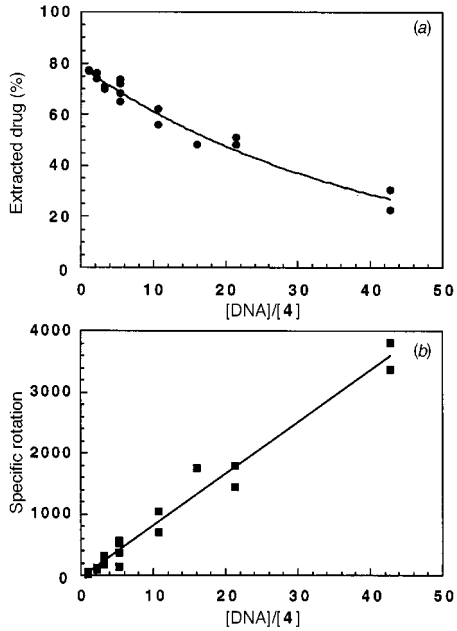


Fig. 3 (*a*) Percent of **4** extracted in butanol as a function of DNA(bp) : **4** ratio. The concentration was calculated from the absorbance measured at 390 nm (ε = 18 800 M^{-1} cm^{-1} in butanol); (*b*) Optical activity of the butanol layer as a function of the DNA(bp) : **4** ratio. The specific rotation ($[\alpha]_D^{20}$) was recorded in 95% ethanol, the concentration being calculated from the absorbance at 390 nm (ε = 20 000 M^{-1} cm^{-1} in 95% ethanol).

assumptions regarding the exciton mechanism in **4**. This was done by semi-empirical calculations[14] of the electronic transitions in **4** and in the corresponding monomeric fragment. The calculated absorption spectrum of the monomer shows two strong transitions at 325 and 310 nm, which are polarised along the long axis. The corresponding transition levels in the dimer are each split into two levels which are almost degenerate and orthogonally polarised (314/314 nm and 283/282 nm); in each pair, one of the components is parallel and the other perpendicular to the C_2 axis. These features are in line with the exciton mechanism and account for the splitting of each component of the bisignate couplet in the experimental CD spectrum.

The UV–VIS spectrum of (±)-**4** was recorded in the presence of increasing concentrations of calf thymus DNA in phosphate buffered solutions (pH 7). Large changes of the spectrum were observed, characterised by the appearance of a new broad band centred at 467 nm, whose intensity increased when the DNA to drug ratio *r* was increased in the range 0–10. These experiments demonstrate that **4** interacts with DNA. The variations observed in the presence of increasing amounts of DNA are similar to those occurring when the spectrum of **4** was recorded at acidic pHs. The simplest interpretation is that the interaction with DNA is accompanied by protonation of the two acridines. The existence of acidic domains surrounding the DNA molecule has already been proposed by Lamm *et al.*[15] Comparison of the CD spectra of **4** in the presence of calf thymus DNA and in 3 M HCl solution leads to the same conclusion.

Liquid–liquid partition[16] of (±)-**4** between an aqueous solution of DNA and butanol revealed that the binding of **4** to calf thymus DNA is enantioselective. Solutions containing various ratios of (±)-**4** and calf thymus DNA in phosphate buffered aqueous solution (pH 7) were mixed with butanol, in which **4** is soluble and DNA is not. After vigorous stirring, the two phases were separated and analysed by CD spectroscopy. The butanol layers gave spectra showing a broad positive band centred at 420 nm, similar to that of the (+)-enantiomer recorded independently under the same conditions. The intensity of this band increased with the DNA : **4** ratio *r*. The spectra of the aqueous phases were similar to those of solutions of (−)-**4** added to various concentrations of calf thymus DNA in phosphate buffer (pH 7). These results indicate that (+)-**4** and (−)-**4** distribute differently between the DNA-containing aqueous phase and butanol, and that (−)-**4** preferentially associates to calf thymus DNA. The enantioselectivity was evaluated by determining the quantities and ees of **4** extracted in the butanol layer (from the UV absorbance and rotation, respectively). The results are reported in Fig. 3(*a*) and 3(*b*) for DNA : drug ratios *r* varying in the range 1–40. As expected, when the DNA : **4** ratio increased, the quantity of **4** extracted in the butanol phase decreased, while the corresponding ee increased (*e.g.* 30% ee when 50% of **4** was extracted and 70% ee when only 20% of **4** was extracted).

The 7*R*,17*R* configuration of (−)-**4** is consistent with the best fit of this enantiomer to right-handed B-DNA,[17] either through intercalation or by interaction in the groove. Work is in progress to determine the mode of interaction.

Notes and references

† All new compounds have been fully characterised by UV–VIS spectroscopy, FAB MS, ^{1}H NMR and elemental analysis.

1 B. Norden, P. Lincoln, B. Akerman and E. Tuite, in *Metal Ions in Biological Systems*, eds. A. Sigel and H. Sigel, Marcel Dekker, New York, 1996, vol. 33, ch. 7, pp. 205–252; C. M. Dupureur and J. K. Barton, *Inorg. Chem.*, 1997, **36**, 33 and references cited therein; S. Satyanarayana, J. C. Dabrowiak and J. B. Chaires, *Biochemistry*, 1993, **32**, 2573; A. D. Baker, R. J. Morgan and T. C. Strekas, *J. Am. Chem. Soc.*, 1991, **113**, 1411.

2 For a review on Tröger's base, see: F. Vögtle, *Fascinating Molecules in Organic Chemistry*, J. Wiley, New York, 1992, pp. 237–249.

3 R. B. Silverman, *The organic chemistry of drug design and drug action*, Academic Press, San Diego, 1992, pp. 239–241.

4 Y. Coppel, Ce. Coulombeau, C. Coulombeau, J. Lhomme, M.-L Dheu-Andries and P. Vatton, *J. Biomol. Struct. Dyn.*, 1995, **12**, 637.

5 A. Wardani and J. Lhomme, *Tetrahedron Lett.*, 1993, **34**, 6411.

6 A. Tatibouët, M. Demeunynck, H. Salez, R. Arnaud, J. Lhomme and C. Courseille, *Bull. Soc. Chim. Fr.*, 1997, **134**, 495.

7 H. Salez, A. Wardani, M. Demeunynck, A. Tatibouët and J. Lhomme, *Tetrahedron Lett.*, 1995, **36**, 1271; A. Tatibouët, M. Demeunynck and J. Lhomme, *Synth. Commun.*, 1996, **26**, 4375.

8 M. Demeunynck, C. Fontaine and J. Lhomme, *Magn. Reson. Chem.*, 1998, in the press.

9 B. M. Wepster, *Recl. Trav. Chim. Pays. Bas.*, 1953,**72**, 661.

10 A. Greenberg, N. Molinaro and M. Lang, *J. Org. Chem.*, 1984, **49**, 1127.

11 N. Harada and K. Nakanishi, *Circular Dichroic Spectroscopy*, University Science Books, Mill Valley, California, 1983. See also: K. Nakanishi, N. Berova and R. W. Woody, *Circular Dichroism, Principles and Applications*, VCH, Weinhem, 1994

12 This situation, which prevails in the exciton coupling of most C_2 systems, is supported in this specific case by examination of Dreiding models.

13 S. F. Mason, G. W. Vane, K. Schofield, R. J. Wells and J. S. Whitehust, *J. Chem. Soc. (B)*, 1967, 553; S. H. Wilen, J. Z. Qi and P. G. Williard, *J. Org. Chem.*, 1991, **56**, 485.

14 The absorption wavelengths and the transition moments were calculated by means of a CNDO/S program parametrized to reproduce the properties of the molecules in solution: C. Andraud, T. Brotin, C. Garcia, F. Pellé, P. Goldner, B. Bigot and A. Collet, *J. Am. Chem. Soc.*, 1994, **116**, 2094; T. Brotin, C. Andraud, I. Ledoux, S. Brasselet, J. Zyss, M. Perrin, A. Thozet and A. Collet, *Chem. Mater.*, 1996, **8**, 890.

15 G. Lamm and G. Pack, *Proc. Natl. Acad. Sci. U.S.A.*, 1990, **87**, 9033.

16 S. S. Peacock, D. M. Walba, F. C. A. Gaeta, R. C. Helgeson and D. J. Cram, *J. Am. Chem. Soc.*, 1980, **102**, 2043.

17 Right-handed twist is also observed for other DNA intercalating drugs such as anthracyclines; for a review, see: J. W. Lown, *Chem. Soc. Rev.*, 1993, 165.

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