Determination of the Absolute Configuration of Monosaccharides by a Colour Change in a Chiral Cholesteric Liquid Crystal System

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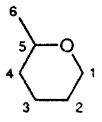
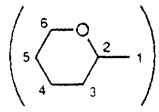
Cholesterylboronic acid complexes of monosaccharides alter the colour of a composite chiral cholesteric liquid crystal membrane, the direction of the colour change is indicative of the absolute configuration of the monosaccharide.

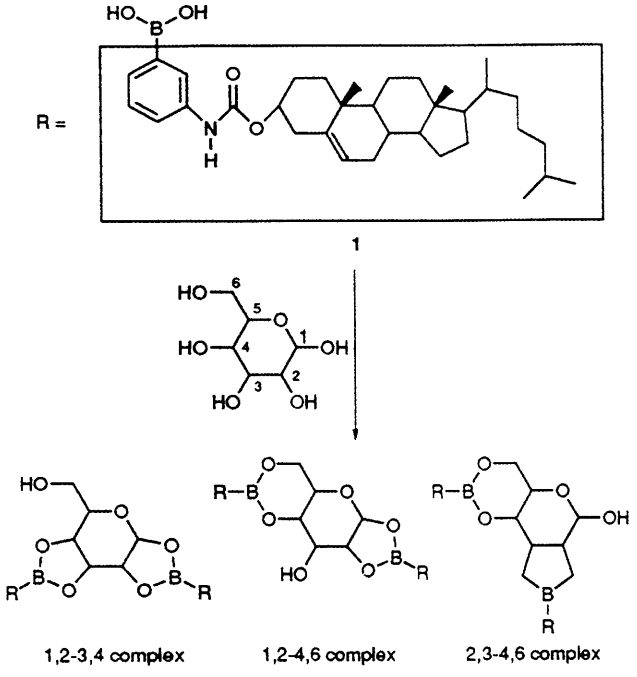
Systems able to directly convert chiral interactions into readable outputs are scarce, often relying on indirect measurements of association constants, membrane transport rates, etc.[1]

With 'chromogenic' crown ethers[2,3] colour has been used to detect metal ion binding. This concept has been successfully coupled with chiral recognition by Kaneda *et al.*[4] who has shown that chiral point interactions in chiral azophenolic acerands can be directly 'read' as a colour change. This concept has also been investigated in steroidal crown ethers and cholesteric liquid crystal systems.[5–9] For example, our previous work with steroidal crown ethers[5] has shown that such interactions occurring in a chiral environment[6–8] can also be detected by colour changes.[5] When chiral ammonium ions (particularly, those of α-amino acid esters) are added to a ternary blend of cholesterol chloride, cholesterol nanoate, and steroidal crown ether, the helical pitch of the mixed cholesteric liquid crystal is altered resulting in a visible colour change. The direction of the induced shift is indicative of the absolute configuration of the added ammonium ions.[5] In this communication, we address a novel method for the chiral recognition of monosaccharides in a cholesteric liquid crystal system with the aim of expanding the applicability and scope of this method and to generalise the design of such detectors for use in chiral liquid crystal systems.

We found previously that the steroidal unit of the 'detective' must be rigidly linked to the moiety that interacts with the chiral 'suspect'. It is known that the phenylboronic acid moiety

Table 1 Saccharide diols used to form cyclic-boronate esters and type of ring formed

	General numbering			Fructose numbering		
		Five-membered ring				Six-membered ring
	Saccharide	1,2-Diol	2,3-Diol	3,4-Diol	4,5-Diol	4,6-Diol
I	D-Fucose	*cis*		*cis*		
	L-Arabinose	*cis*		*cis*		
	L-Fucose	*cis*		*cis*		
	D-Arabinose	*cis*		*cis*		
	D-Fructose		(*cis*)		(*cis*)	
II	D-Glucose	*cis*				*trans*
	D-Allose		*cis*			*trans*
	D-Xylose	*cis*		*trans*		
	L-Glucose	*cis*				*trans*
	D-Mannose		*cis*			*trans*
III	D-Galactose	*cis*				*cis*
	L-Galactose	*cis*				*cis*
	D-Talose	*cis* (~70%)	*cis* (~30%)			*cis*
	2-Deoxy-D-galactose					*cis*



serves as a versatile receptor for saccharides and offers itself as an obvious first choice, since it can be used to detect the chirality of saccharides.[10]

Treatment of cholesterol chloroformate with *m*-aminophenylboronic acid in the presence of pyridine under reflux gave the desired compound **1** in 51% yield. The product was identified by MS, ^{1}H NMR and elemental analysis.†

Solvent extraction of saccharides was carried out at 25 °C using solid–liquid ($CDCl_3$) extraction. As much by serendipity as by design the cholesterol boronic acid was a much more efficient saccharide extractor than that boronic acid previously used;[10] complete extraction was observed for all monosaccharides. Selection within the saccharides similar to that previously observed was seen in the rate of extraction, the less favourable saccharides taking longer to be completely extracted. However, after 48 h all the saccharides investigated were completely extracted.

Table 2 Shifts in the reflectance maxima caused by added 2:1 complex: **1**/saccharide

Group	2:1 Complex **1**/saccharide	Induced shift[a] relative to compound **1** Mol% 2.4[b]	Mol% 1.2[c]
I	D-Fucose		123 ± 13
	L-Arabinose		27 ± 8
	L-Fucose	−164 ± 12	−95 ± 6
	D-Arabinose	−82 ± 10	−45 ± 6
	D-Fructose	−37 ± 11	−23 ± 11
II	D-Glucose	91 ± 10	42 ± 12
	D-Allose	69 ± 10	19 ± 10
	D-Xylose		34 ± 15
	L-Glucose	−71 ± 10	−61 ± 6
	D-Mannose	−3 ± 10	5 ± 6
III	D-Galactose	−44 ± 10	−33 ± 11
	L-Galactose	−17 ± 10	−21 ± 7
	D-Talose	3 ± 11	
Blank	2-Deoxy-D-galactose	6 ± 15	

[a] Shifts are the average of five repeats, errors given are the maximum deviation from the mean. [b] At 2.4 mol% compound **1** has a base reflectance of 625 ± 10. [c] At 1.2 mol% compound **1** has a base reflectance of 650 ± 6.

Characterisation of the extracted saccharides could be achieved employing just ^{1}H NMR spectroscopy ($CDCl_3$). The molar ratio of the extracted species could be estimated conveniently by the integral intensities of selected ^{1}H resonances of the monosaccharide *vs.* either the phenyl or the alkenyl proton of compound **1**. In the case of the D-fucose complex the anomeric proton of the monosaccharide at δ 5.9, and the alkenyl proton of compound **1** at δ 5.4 were employed. In general all the saccharides form a 1:2 complex with compound **1**. Two main structural classes exist for the extracted monosaccharides, either, two five-membered rings are formed (1,2-3,4 complex), or, a five- and a six-membered ring are formed (1,2-4,6 and 2,3-4,6 complexes: Table 1).

trans-Six-membered rings are less stable than the five-membered rings.[11] From Table 1 it can be seen that this

† M.p. 220 °C (decomp.); ^{1}H NMR ($CDCl_3$) δ 0.6–2.6 (43 H, m), 4.4–4.8 (1 H, m), 5.4 (1 H, d, *J* 4 Hz), 6.8 (1 H, s), 7.2–8.0 (4 H, m); satisfactory elemental analyses were obtained MS (SIMS, NPOE/NBA) No M + 1 was detected, however the M + 1 after protection of the boronic acid with propane-1,3-diol was obtained; 590 (M + 1, For: $C_{37}H_{56}O_4NB$).

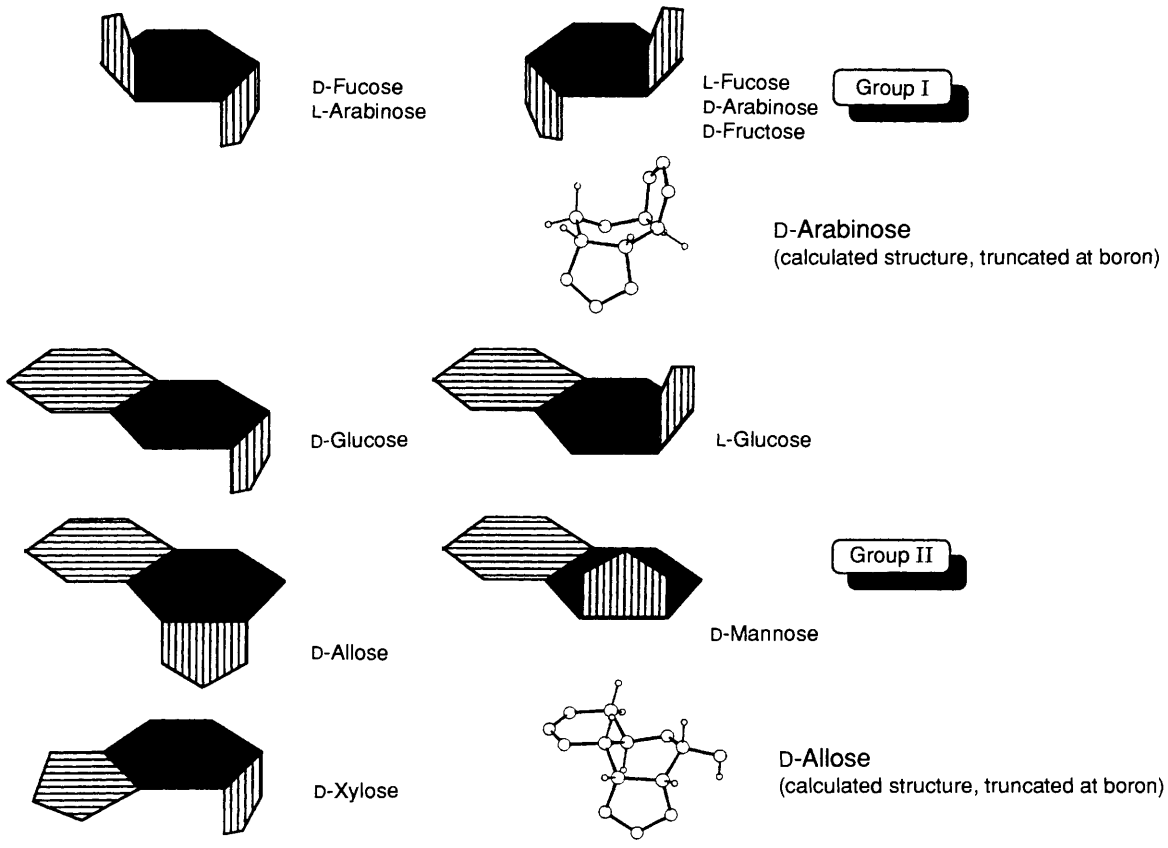


Fig. 1 Structure of the 2:1 complex (**1**/monosaccharide) with fixed conformations, and two representative examples of calculated structures for comparison

chiaroscuro analysis falls short of describing the actual situation. Some systems that could have formed perfectly good five-membered rings opt for the six-membered ring alternative, why do these systems prefer the six-membered ring? With galactose, mannose and talose the ring is a *cis*- rather than a *trans*-six-membered one, and it is known from decalin that the *cis*-fused six-membered ring system is conformationally flexible whereas the *trans* system is fixed, this conformational lability may provide enough advantageous entropic energy to tip the balance in favour of the six-membered ring. With allose a *trans*-six-membered ring is formed, but this may not be an inherent preference for the six-membered ring, rather a preference for the 2,3-five-membered ring over the 1,2-five-membered ring. In a 1:2 complex once the 2,3-five ring has formed the only choice remaining is the formation of a *trans*-six-membered ring.

The induced shifts produced by the extracted monosaccharides in a cholesteryl chloride, nanoate composite liquid crystal‡ are given in Table 2. Analysis of Tables 1 and 2 shows that saccharides with similar structural features induce shifts in the same sense (Fig. 1). Within the saccharides extracted three such structure/shift group relationships exist, the first group includes saccharides with two (*cis*)-five-membered boronate ester rings (Group I), when the first ring

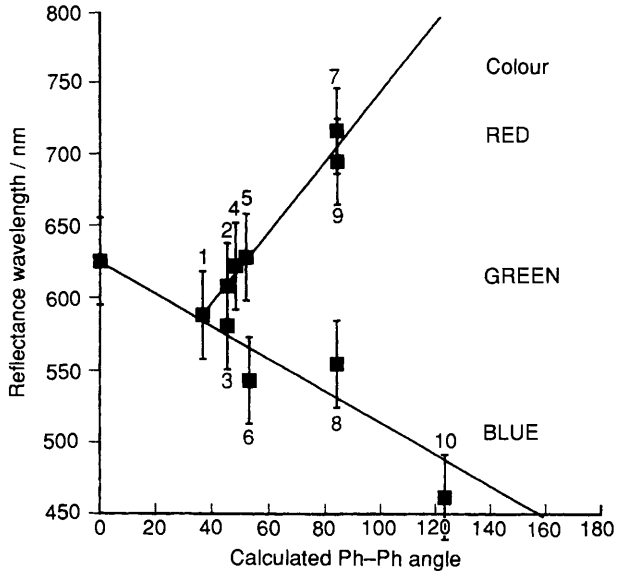


Fig. 2 Plots of reflectance wavelength *vs.* calculated Ph–Ph dihedral angle for the 2:1 complex (**1**/monosaccharide) at 2.4 mol%. 1, D-fructose; 2, L-galactose; 3, D-galactose; 4, D-mannose; 5, L-arabinose; 6, D-arabinose; 7, D-glucose; 8, L-glucose; 9, D-allose; 10, L-fucose.

(following normal saccharide numbering) is down with respect to the saccharide plane and the second is up a red shift is induced (D-fucose, L-arabinose). Conversely, when the first ring is up and the second is down, a blue shift is induced (D-fructose, D-arabinose, L-fucose). The next structure/shift sub-group contains saccharides with one *cis*-five-membered ring and either a *trans*-five- or a *trans*-six-membered *trans* ring (Group II). When the first ring (*cis*-five-membered) is down a red shift is induced (D-glucose, D-allose, D-xylose), and

‡ A solution containing cholesteryl nanoate (1.2×10^{-5} mol), cholesteryl chloride (0.8×10^{-5} mol) and the 2:1 boronic acid saccharide complex (5.2×10^{-7} mol) in chloroform was prepared. An aliquot (200 μl) was spread on a quartz plate and mixed with minute glass beads having a uniform diameter (10 ± 0.2 μmol dm^{-3}). The sample was dried and then sandwiched between another quartz plate. The thickness of the sample prepared is then regulated by the glass beads. The wavelength of maximum reflection ($\lambda_R = nP$, where n is the mean index of reflection and P is the helical pitch of the cholesteric mesophase) was measured spectrophotometrically at 27 °C. For details of the measurement method see: P. J. Shannon, *Macromolecules*, 1984, **17**, 1873.,

conversely when this ring is up a blue or no shift is induced (L-glucose, D-mannose). The final structure/shift group is the most anomalous, the saccharide contains both a *cis*-five-membered ring and *cis*-six-membered ring (Group III). When the first ring is either up or down a blue shift is induced (D-galactose, L-galactose), D-talose induces no shift. This anomalous behaviour may be a result of the conformational lability of the *cis*-six-membered ring, or, as explained below the behaviour may not be anomalous if a threshold must be overcome before a shift in the pitch occurs.

From the above qualitative structural analysis and the result that the 1 : 1 complex of 2-deoxy-D-galactose causes no shift, the spatial disposition of two cholesteryl boronic acid moieties is the driving force for the change in the cholesteric pitch. Structural analysis of the complexes utilising a molecular orbital calculation§ reveals a quantitative relationship between the magnitude and direction of the induced shift and the angle between the phenyl planes of the two boronic acid moieties (Fig. 2). For the correlation depicted in Fig. 2, one apparent anomaly needs to be clarified. Why are blue shift additives 'effective' at all angles, but those compounds inducing a red shift have a threshold value of about 40° (D-fructose)? This can be easily explained by the inherent twist of the support liquid crystal; complexes that add to this inherent twist cause blue shifts, and those that subtract cause red shifts, with D-fructose (40°) acting as the effective zero or threshold.

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§ Energy minimizations of the **1**/saccharide complexes were conducted using a semiempirical molecular orbital calculation method with full-geometry optimization (MOPAC version 6.0, AM1 Hamiltonian).[12] The imput structures for the complexes were established using the molecular modelling system (MOL-GRAPH version 3.0, Daikin Ind. Ltd). These calculations were preformed on the engineering workstation system (SUN 4/2 and IRIS 4D/35G). The illustrations in Fig. 1 were made by the ORTEPC[13] for the optimized structures.