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Selectivity in molecular recognition of steroids, alkanes and alicyclic substrates in aqueous media†

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A new, chiral, and enantiomerically pure receptor has been prepared. The molecule binds to cyclohexanoid and alicyclic alkanes. Binding to stereoisomeric menthols, diastereomeric cyclohexane derivatives, and steroids has been studied. The use of the molecule as a chiral shift reagent is demonstrated.

INTRODUCTION

Investigations of new synthetic receptors for neutral organic molecules often involve hydrogen bond based systems for enhancing solute interactions.[2] The pioneering work of Tabushi, Koga, Whitlock, and Murakami demonstrated the promise of water soluble cyclophanes as hosts for lipophilic substrates in aqueous media.[3] Investigators following these leads have focused predominantly on aromatic guests.[4,5] In this paper we will not discuss aromatic substrates, but instead we will dwell on less symmetrical and more stereochemically rich targets. We will discuss some of the issues and our recent results dealing with binding alkanes and alicyclic molecules.[6]

Nature provides many examples of selective binding of alicyclic substrates[7] (Fig 1). The enantiomers of carvone may be distinguished by their characteristic odours. Honey bees can be trained to discriminate between the enantiomers of 4-methylhexanoic acid. Only one enantiomer of 4-methyl 3-heptanone is an alarm pheromone for the ant *Atta texana*. Mammalian hormone receptors are an important example of alicyclic binding molecules. Steroids, prostaglandins, and other lipid components are frequently used to carry information throughout an organism.

Selective alkane binding sites might be used in new separation technologies and for hormone delivery and transport. Such synthetic receptors could also be used in analytical applications. In addition, shape selective alicyclic binding sites should be useful for solubilization of reactants and reagents and could be incorporated into new strategies for reaction control and catalysis as applied to alicyclic reactants.

We identify two distinct types of selectivity that can be observed with synthetic receptors for alicyclic systems (Fig 2). The first, called here 'molecular selectivity', is the more commonly observed phenomenon. Two completely different molecules, or two diastereomers, or enantiomeric substrates, can show different affinities for a receptor. This type of selectivity is the source of the specific actions of the pheromones and hormones shown in Figure 1. A second type of selectivity we call 'structural selectivity'. When a receptor and substrate combine to form only a single structural isomer of the complex, 'structural selectivity' is fully realized. Most often, however water based synthetic receptors for alicyclic substrates are not so well controlled. If the substrate is larger than the receptor, then structurally isomeric complexes may form when more than one site is available for binding. Site selectivity in synthetic receptor systems is a subcategory of structural selectivity. Even when a receptor encompasses a substrate, and only one binding site is available, structural selectivity may be poor. Figure 2 illustrates several structurally isomeric complexes that differ in the relative positioning of functional groups on the receptor and substrate. Control of molecular and structural selectivity is an important challenge in the area of alicyclic molecular recognition.

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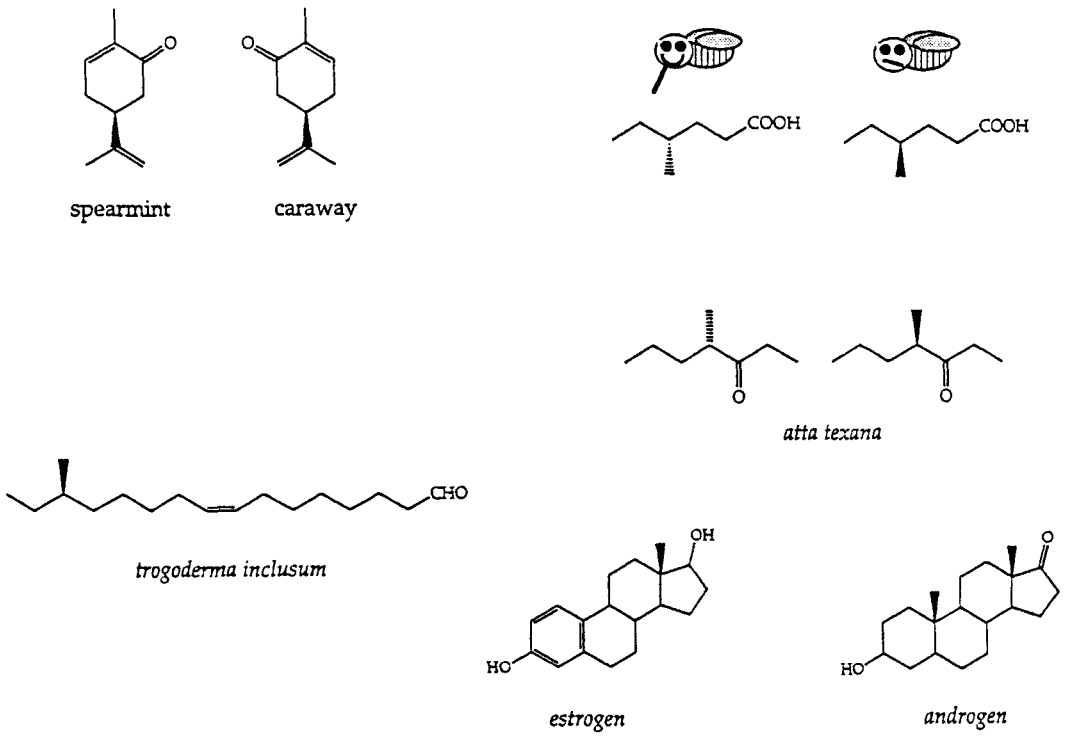


Figure 1 Examples of alkanes that are bound selectively in nature.

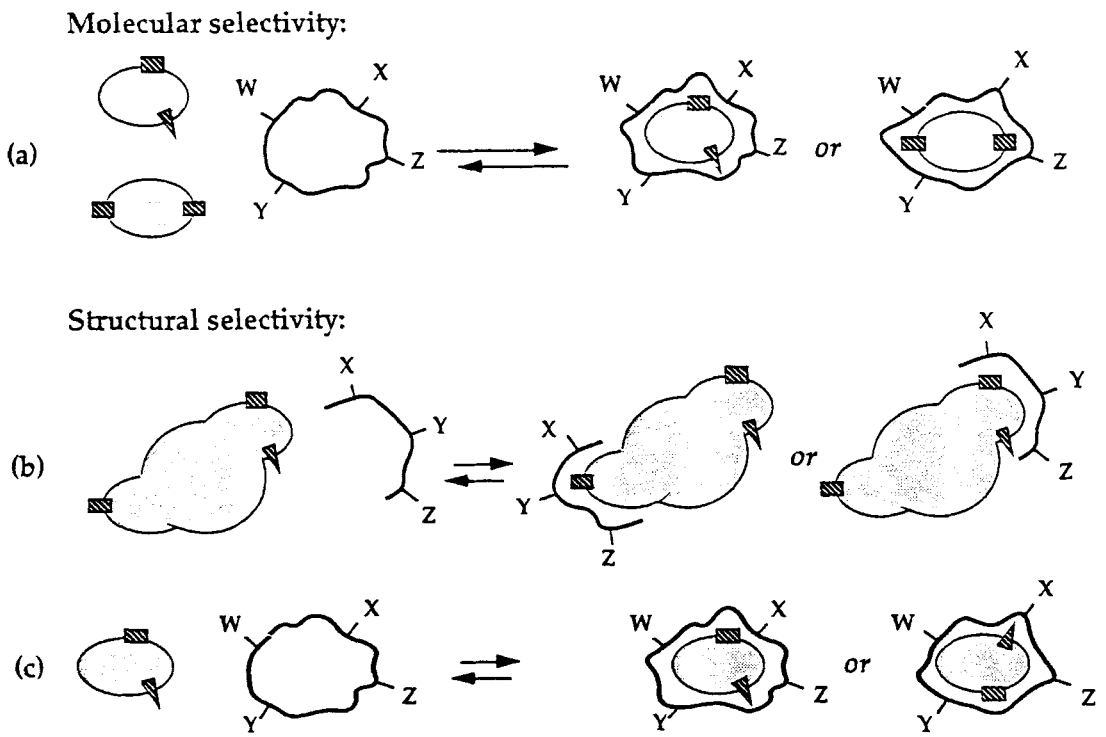


Figure 2 (a) Molecular selectivity. This is present when two molecules show different affinities for the same receptor. Structural selectivity may include case (b) where a receptor binds to only one site on a substrate, or case (c) where the receptor–substrate complex has only one of several possible structures.

RESULTS AND DISCUSSION

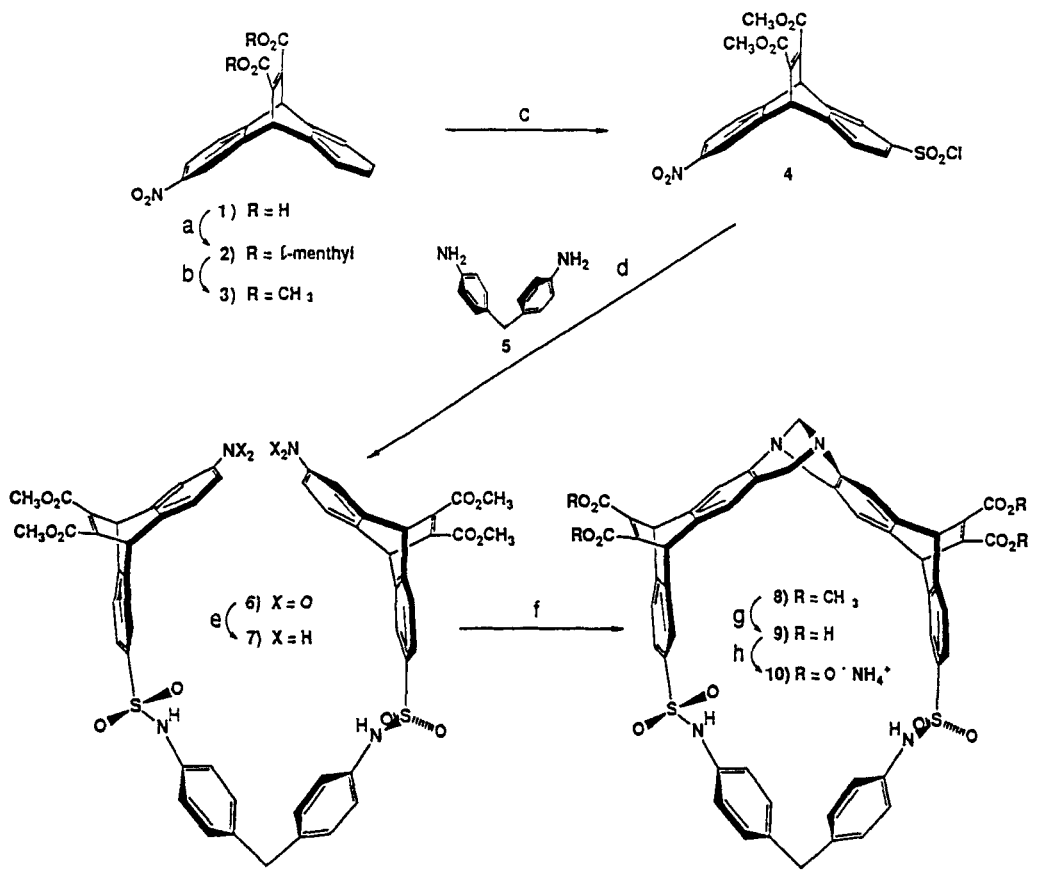
We recently prepared the rigid water soluble cyclophane **10** (Scheme 1) in enantiomerically pure form.[6] This molecule was designed to be complementary to cyclohexanoid substrates. Molecular modelling results indicate that the molecule is able to enclose a cyclohexanoid ring that has no axial substituents, but that axial substituents are unlikely to fit into this binding site and should affect binding interactions.

To test this hypothesis the affinity of receptor **10** toward *cis*- and *trans*-4-*tert*-butylcyclohexanol was evaluated. A mixture of the two stereisomers (0.32×10^{-3} M *cis*, 0.68×10^{-3} M *trans*) in D_2O at pH 6.8 (phosphate buffer) was prepared. Throughout the titration (298 K, host varied from 0–1.7 mM) the ionic strength was maintained ($I = 0.34$) by the use of KCl. Observed chemical shift changes for the two *t*-butyl groups are plotted in Figure 3. Host **10** binds to *trans*-4-*tert*-butylcyclohexanol more strongly than to *cis*-4-*tert*-butylcyclohexanol. [$K_{cis} = 6300\ M^{-1}$, $K_{trans} = 43{,}000\ M^{-1}$]. For the stronger binding host, chemical shift changes induced by added host follow an approximately hyperbolic function. For the weaker binding guest, sigmoidal changes are observed.

The sigmoidal response curve for the more weakly bound substrate in this three component mixture arises because the first increments of added receptor are occupied immediately by the more strongly bound substrate. Later, when the more strongly bound substrate is nearly consumed, added receptor begins to affect the weak binding substrate. This experiment illustrates a potentially useful approach to shifting and modifying response curves.

The binding of these diastereomeric guests is primarily influenced by two factors. First, the shapes of the guests and the cavity – steric and electronic aspects of the receptor/substrate interactions – is important. Second, and equally important in controlling this selectivity, is the relative solvation energies of the guest surfaces that are involved in complexation.

Molecular modelling indicates that the axial substituent in the *cis* isomer cannot be accommodated within the macrocyclic host – the molecule is just too tall for the available space. As that is so, then for the complex, the average position of the host should be moved more toward the *tert*-butyl group in the *cis*



Scheme 1.

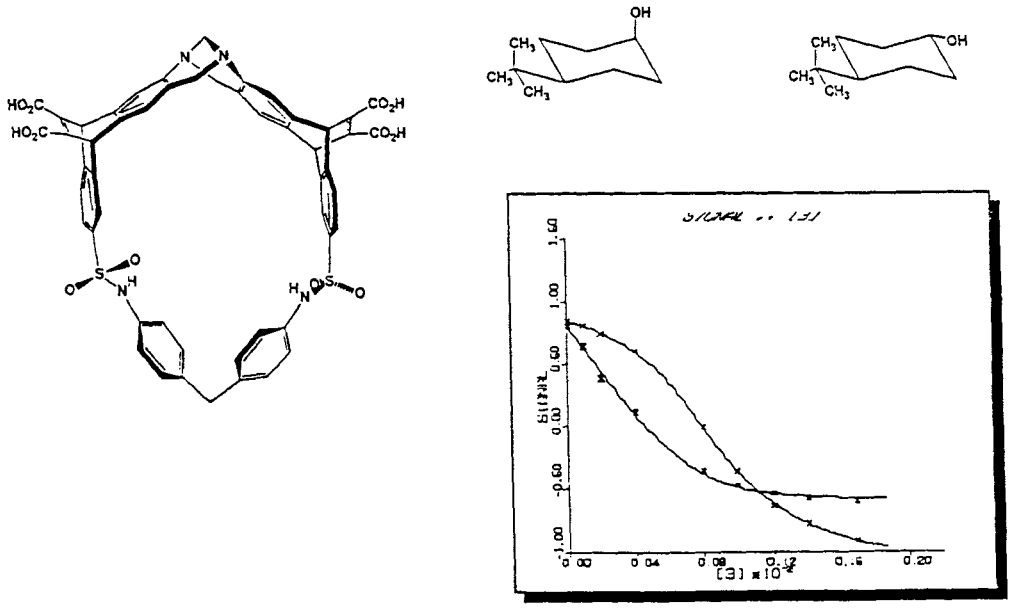


Figure 3 Diastereomeric cyclohexane derivatives showing differing affinities for synthetic receptor **10**.

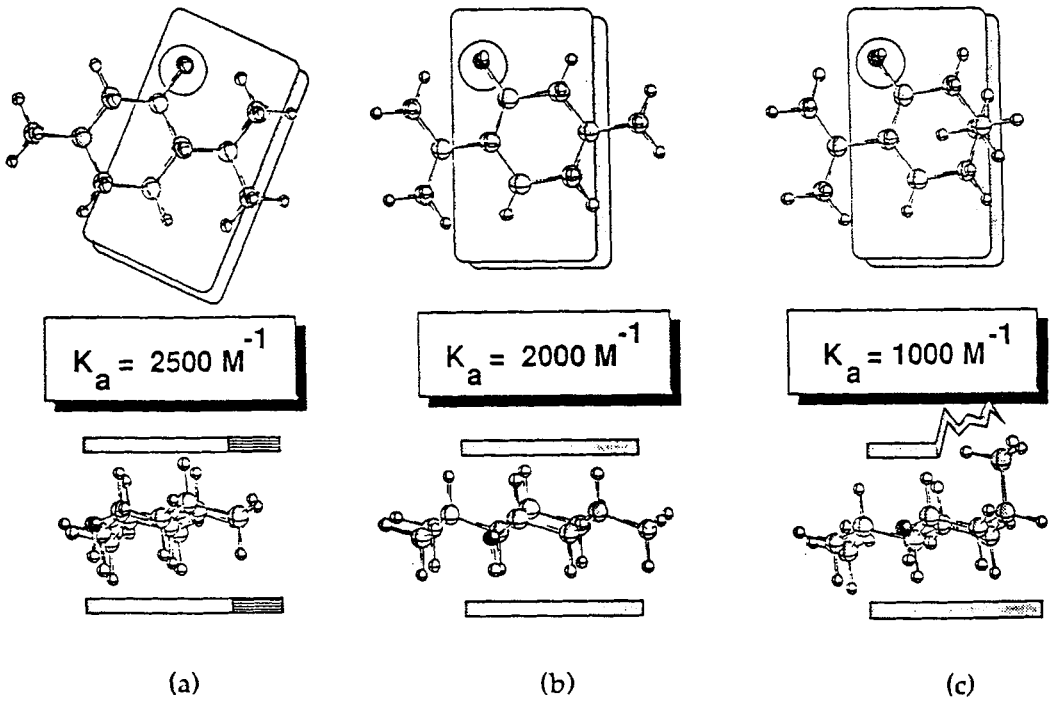


Figure 4 (a) (+)-Menthol, (b) (−)-menthol, and (c) (+)-isomenthol showing differing affinities for receptor **10**. The grey boxes represent the possible position of the host on the guest. The axial group in isomenthol should inhibit binding.

isomer (to avoid the too-tall hydroxy group) and less toward the *tert*-butyl group in the *trans* isomer. In fact, limiting chemical shifts for the *tert*-butyl groups in the two complexes supports this hypothesis. For the more strongly bound substrate, the *tert*-butyl group is moved upfield by 1.4 ppm when the complex forms. For the less strongly bound substrate, the upfield shift is 2.0 ppm.

To further evaluate this aspect of shape selectivity, the binding of isomeric menthols was investigated. Two enantiomers of menthol were tested, and (−)-menthol was found to bind better than (+)-menthol with this receptor. The differences were small (Fig 4) but significant. The binding of iso-menthol, which bears an axial methyl group rather than an equatorial methyl group, was also quantified. Here again it was

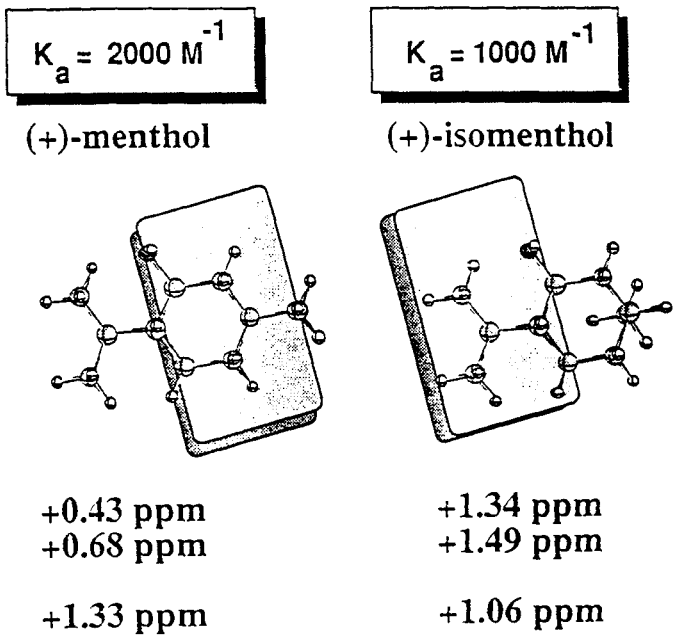


Figure 5 Chemical shift changes due to binding that are observed for (a) the isopropyl methyl groups, and (b) the ring methyl group, in menthol and isomenthol.

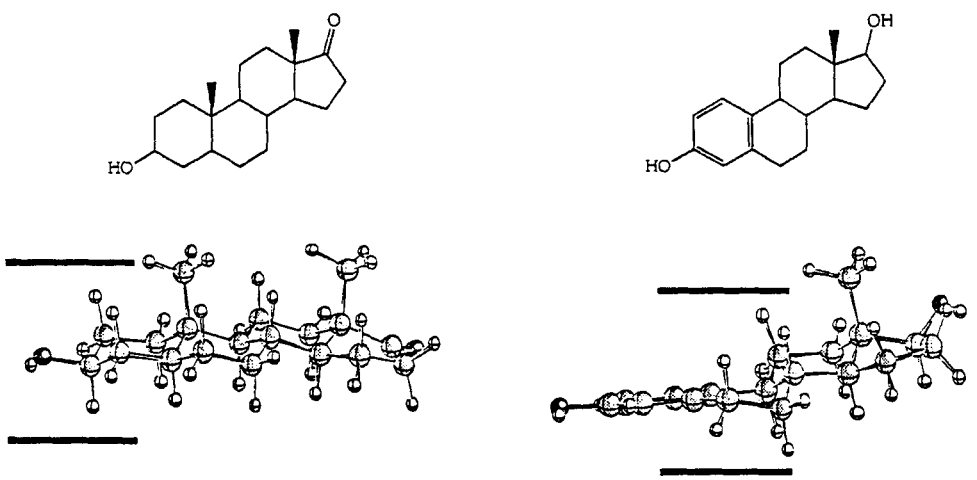


Figure 6 A comparison of the shapes of androsterone (left) and oestradiol (right). The horizontal bars approximate the limiting vertical inside dimension of the synthetic receptor.

observed that the cyclohexanoid guest bearing an axial substituent is less well accommodated in this cavity. The limiting chemical shifts for the isopropyl methyl groups and for the ring methyl group in (+)-menthol and in (+)-isomenthol were determined (Fig 5). The changes in shift for these two diastereomers indicate that here, as in the *tert*-butylcyclohexanol case, the axial substituent has caused the footprint of the host to shift toward the other end of the molecule – in this case toward the isopropyl group.

These data suggested that the synthetic receptor **10** is sufficiently well organized to exclude cyclohexanoid rings bearing axial substituents. If that is so, then receptor **10** might show molecular and structural selectivity towards steroids. The normal androstane steroid nucleus, exemplified here by androsterone (Figure 6) bears two axial methyl groups. These methyl groups should inhibit binding of this type of molecule in the synthetic receptor. The distance between the methyl groups (4.7 Å) is too short for the host to rest between these groups. In contrast, the oestrogen oestradiol has an aromatic A-ring and lacks the 19-methyl group present in androsterone. This molecule should be able to fit more effectively within the synthetic receptor. Because more lipophilic surface contact will be made with oestradiol than with

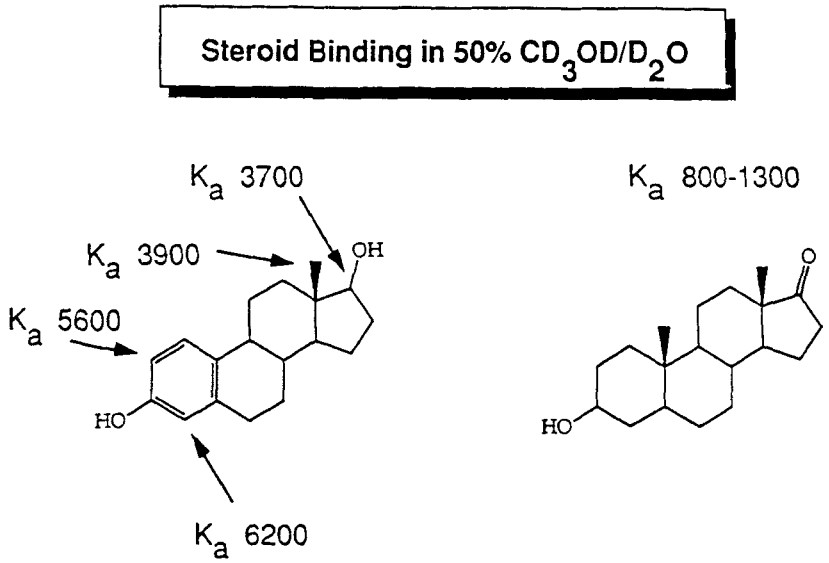


Figure 7 Steroid binding in 50% CD_3OD/D_2O. The exact result depends on the proton that is observed (see text), but there is no doubt that oestradiol is bound more strongly than androsterone.

androsterone, binding may be stronger for oestrogen than for the androgen.[9]

The solubility of common steroids in water is extremely low. This makes it very difficult to use NMR to determine accurate association constants for steroids in water.[9] For this reason we have carried out our binding studies in deuterated methanol–water mixtures. The effects of added methanol on the binding of (−)-menthol are very large. In phosphate buffer at pH 6.8 the association of receptor **10** with (−)-menthol has an equilibrium constant of 2500 M^{-1}. When the buffer solution contains 9% methyl alcohol, the association constant drops to 1500 M^{-1}. In the presence of 30% methyl alcohol the association constant is 700 M^{-1}. Finally, at 50% methyl alcohol the association constant for (−)-menthol is only 100 M^{-1}. There is thus a 25-fold difference in association of menthol and this host in water as compared with 50% methanol–water.

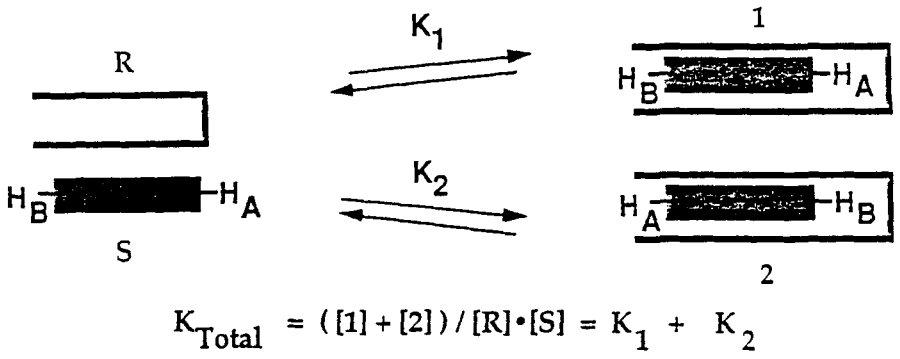
The binding of receptor **10** with androsterone and with oestradiol was measured in deuterated 50% methanol–water. Both steroids bound well to this host. The association constant for oestradiol and receptor **10** in 50% methanol–water is approximately 5000 M^{-1} and the association constant for oestradiol is approximately 5 times higher than the association constant of androsterone. There is an interesting feature to these experiments. Different protons on the steroid nucleus can be observed during the titration experiment and different association constants are calculated for the host–guest association event depending upon which proton is observed. What information is provided by this observation?

The most obvious and least arguable conclusion is that the binding of oestradiol to this synthetic receptor does not conform to the simple host–guest binding model wherein a pair of solutes associate to afford a single structurally unique complex. There are two models that might be proposed to explain why different protons give different association constants. First, if binding is not structurally selective, then association constants could vary from proton to proton. A receptor (R in Fig 8) and a substrate (S in Fig 8) might associate to give structurally isomeric complexes, these complexes might differ in standard free energy. Because two protons (A and B in Fig 8) will have different environments in the two complexes, they may have different chemical shifts. As an extreme example, consider the case where one complex has no effect on proton A, and the other complex has no effect on proton B. It is possible to calculate the chemical shifts of protons A and B in this system if the two association constants are known and if the effects of binding on chemical shift are known. In eqns 1 and 2 below, $\Delta\delta_{A,1}$ is the effect on proton A due to formation of complex 1 (Fig 7), $\Delta\delta_{A,2}$ is the effect on proton A due to formation of complex 2, $\Delta\delta_{B,1}$ is the effect on proton B due to formation of complex 1, and $\Delta\delta_{B,2}$ is the effect on proton B due to formation of complex 2. In the extreme example just posited, $\Delta\delta_{B,1}$ and $\Delta\delta_{A,2}$ are equal to zero.

$$\delta_{A,obs} = \delta_{A,free} + \chi_1 \cdot [\Delta\delta_{A,1} + (K_2/K_1) \cdot \Delta\delta_{A,2} \quad (1)$$

$$\delta_{B,obs} = \delta_{B,free} + \chi_2 \cdot [\Delta\delta_{B,2} + (K_1/K_2) \cdot \Delta\delta_{B,1} \quad (2)$$

Formation of structurally isomeric complexes cannot



$$K_{Total} = ([1] + [2]) / [R] \cdot [S] = K_1 + K_2$$

Figure 8 Formation of structurally isomeric complexes. They may form with unequal probabilities.

be detected *if only* 1:1 complexes are present (Fig 8). All protons will fit a 1:1 binding isotherm based on the sum of the individual 1:1 association constants. However, there is another complication in these binding experiments. Two receptors might associate with the same substrate. This type of higher order aggregation can cause different protons to give different association constants depending on the magnitude of chemical shift change caused by the binding events.

The data in these experiments are not sufficient to allow a detailed analysis of structurally diverse binding to be pursued further but other experiments are now underway to shed more light on this phenomenon. We expect that NMR data can be used to probe site selectivity in molecular recognition, and to evaluate structural selectivity in complexation whenever both 1:1 and 2:1 complexes are present.

CONCLUSIONS

Receptor **10** is a chiral and enantiomerically pure water soluble cyclophane and shows shape selectivity in binding alicyclic substrates. The data support the idea that the receptor is able to bind cyclohexanoid substrates very well but the presence of an axial substituent significantly inhibits binding. The macrocycle is promising as a model for steroid receptors and shows some ability to bind selectively to steroids based on shape considerations. The macrocycle can also distinguish between enantiomers of menthol.

This nascent program of alkane molecular recognition has led us to consider some of the issues that will confront us and other workers as this field develops further. When branched and alicyclic alkanes of low symmetry are bound, structurally isomeric complexes may form. The probability and standard free energy associated with these complexes is variable, and one of the challenges in the field will be to make receptors that form only one molecular complex rather than a family of complexes. The binding of steroids also will be enriched by the possibilities of site selectivity – binding sites for the A-ring or for the D-ring of the androstane nucleus can be envisioned.

One practical application for this chiral receptor has already been demonstrated. The receptor **10** is a very effective chiral shift reagent for alkanes. For example, a sample of citronellol, when combined with 20 mol% of the receptor, is clearly revealed to be a racemic mixture (Fig 9). There are two important advantages to the use of a chiral shift reagent of this type. First, the reagent will bind specifically to lipophilic portions of the molecule. The usual chiral shift reagents require Lewis basic sites and these sites must be not too far from the stereogenic unit of interest. Receptor **10** is a chiral shift reagent that will work for molecules that have stereogenic units remote from any Lewis base site. The second advantage of this type of reagent is that the molecule is unlikely to induce the bothersome line broadening that is often found with lanthanide shift reagents.

Finally, we note that there has been no published discussion of the fact that when NMR is used to measure association constants, results can differ depending on which proton is observed. One reason for this silence may be that until now most studies have focused on relatively symmetrical guests and hosts. Also, when binding is weak and a great many structurally isomeric complexes are present, then 2:1 complexation may be unlikely. As less symmetrical yet more effective hosts are developed (in order to obtain better molecular discrimination) and less symmetrical guests are investigated, it is to be expected that structurally isomeric complexes will be observed and that site selectivity will eventually be achieved. The detailed information that is available in NMR data obtained by observing the chemical shift changes for protons at different sites on a substrate can be used to illuminate new aspects of structural selectivity in

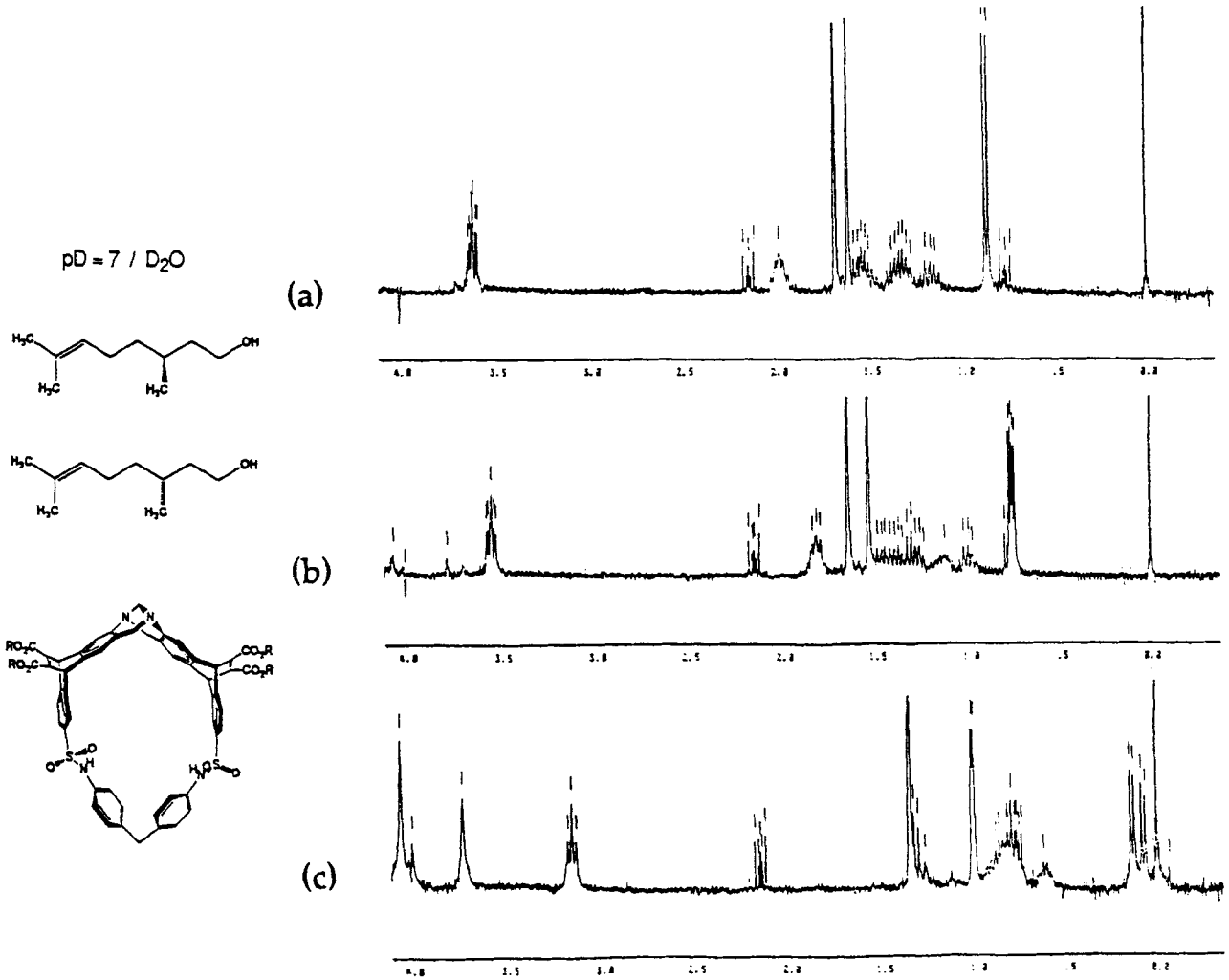


Figure 9 Receptor **10** as an effective chiral shift reagent. (a) NMR of racemic citronellol (1 mM) in D_2O. (b) Same sample containing 20 mol% receptor **10**. (c) Same sample containing 30 mol% receptor **10**.

molecular recognition when both 1:1 and 2:1 complexes are present.

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REFERENCES

1 Adrian, J.C., Jr.; Wilcox, C.S.; *J. Am. Chem. Soc.* **1992**, *114*, 1398–1403.

2 (a) Bell, T.W.; Liu, J.; *J. Amer. Chem. Soc.* **1988**, *110*, 3673. (b) Adrian, J.C., Jr.; Wilcox, C.S.; *J. Amer. Chem. Soc.* **1989**, *111*, 8054. (c) Liu, R.; Sanderson, P.E.; Still, W.C.; *J. Org. Chem.* **1990**, 55, 5184. (d) Seto, C.T.; Whitesides, G.M.; *J. Amer. Chem. Soc.* **1990**, 112, 6409. (e) Kelly, T.R.; Maguire, M.P.; *J. Amer. Chem. Soc.* **1987**, *109*, 6549. (f) Chapman, K.T.; Still, W.C.; *J. Amer. Chem. Soc.* **1989**, *111*, 3075. (g) Neder, K.M.; Whitlock, H.W., Jr.; *J. Amer. Chem. Soc.* **1990**, *112*, 9412. (h) Aoyama, Y.; Tanaka, Y.; Toy, H.; Ogoshi, H.; *J. Amer. Chem. Soc.* **1988**, *110*, 634. (i) Hegde, V.; Madhukar, J.D.; Thummel, R.P.; *J. Amer. Chem. Soc.* **1990**, *112*, 4549. (j) Doig, A.J.; Williams, D.H.; *J. Amer. Chem. Soc.* **1992**, *114*, 338. (k) Hamilton, A.D.; Van Engen, D.; *J. Amer. Chem. Soc.* **1987**, *109*, 5035. (l) Chang, S.K.; Fan, E.; Van Engen, D.; Hamilton, A.D.; *J. Amer. Chem. Soc.* **1991**, *3*, 1318. (m) Hamilton, A.D.; in *Advances in Supramolecular Chemistry*, **1991**, Vol. 1 (Gokel, G., ed.), Jai Press, Greenwich. (n) Osterberg, C.E.; Arif, A.M.; Richmond, T.G.; *J. Amer. Chem. Soc.* **1988**, *110*, 6903. (o) Nowick, J.S.; Chen, J.S.; *J. Amer. Chem. Soc.* **1992**, *114*, 1107. (p) Huang, C.Y.; Cabell, L.A.; Lynch, V.; Anslyn, E.V.; *J. Amer. Chem. Soc.* **1992**, *114*, 1900. (q) Sheridan, R.E.; Whitlock, H.W.; *J. Amer. Chem. Soc.* **1986**, *108*, 7210–7211. (r) Gellman, S.H.; Adams, B.R.; *Tetrahedron Lett.* **1989**, *30*, 3381. (s) Dado, G.P.; Gellman, S.H.; *J. Amer. Chem. Soc.* **1992**, *114*, 3138. (t) Etter, M.; *Acc. Chem. Res.* **1990**, *23*, 120–126. (u) Rebek, J., Jr.; *Acc. Chem. Res.* **1990**, *23*, 399. (v) Rebek, J., Jr.; *Science*, **1987**, *235*, 1478–1484.

3 (a) Tabushi, I.; Kimura, Y.; *Tetrahedron Lett.* **1976**, 3327–3330. (b) Odashima, K.; Koga, K.; in *Cyclophanes*, **1983**, Vol. II (Kheen, P.M. and Rosenfeld, S.M., eds.), Academic Press, New York. (c)

Jarvi, E.T.; Whitlock, H.W., Jr.; *J. Amer. Chem. Soc.* **1980**, *102*, 657–662. (d) Murakami, Y.; *Top. Curr. Chem.* **1983**, *115*, 103–151.

4 (a) Diederich, F.; Dick, K.; *J. Amer. Chem. Soc.* **1984**, *106*, 8024–8036. (b) Wilcox, C.S.; Cowart, M.D.; *Tetrahedron Lett.* **1986**, *27*, 5563–5566. (c) Cowart, M.D.; Suchoeiki, I.; Bukownik, R.R.; Wilcox, C.S.; *J. Amer. Chem. Soc.* **1988**, *110*, 6204–6210. (d) Petti, M.A.; Shepodd, T.J.; Barrans, R.E., Jr.; Dougherty, D.A.; *J. Amer. Chem. Soc.* **1988**, *110*, 6825.

5 Diederich, F.; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 362–386.

6 Webb, T.H.; Suh, H.; Wilcox, C.S.; *J. Amer. Chem. Soc.* **1991**, *113*, 8554–8555.

7 Silverstein, R.M.; In *Semiochemistry Flavors and Pheromones* (Acree, T.E. and Soderlund, D.M., eds.), Walter de Gruyter, New York, **1985**, pp. 121–139.

8 Steroid binding in macrocyclic cyclophanes has been investigated by three groups: (a) Carcanague, D.R.; Diederich, F.; *Angew. Chem. Intl. Ed. Engl.* **1990**, *29*, 769. (b) Murakami, Y.; Hayashida, O.; Ito, T.; Hisaeda, Y.; *Chem. Lett.* **1992**, 497. (c) Kikuchi, Y.; Kobayashi, K.; Aoyama, Y.; *J. Amer. Chem. Soc.* **1992**, *114*, 1351.

9 Wilcox, C.S.; in *Frontiers of Supramolecular Organic Chemistry and Photochemistry* **1990**, (Schneider, H.-J. and Dürr, H., eds.), VCH, Weinheim.