Varying solvent polarity to tune the enantioselective quenching of a calixarene host

Carol Lynam, Dermot Diamond*

National Centre for Sensor Research, Dublin City University, Dublin 9, Ireland.

Abstract: Calixarene **L1** has been designed to behave as a fluorescent molecular sensor capable of distinguishing between chiral amines on the basis of their size and chirality. Fluorescence quenching studies of calixarene **L1** in methanol demonstrated no enantiomeric selectivity for a short chain amino alcohol, phenylglycinol, while excellent selectivity was observed for a longer chain amino alcohol, phenylalaninol (PA). The effect of solvent on the fluorescent properties of this calixarene in the presence of PA has been studied, and demonstrates that varying solvent polarity allows the wavelength of enantiomer selectivity to be tuned from 227nm to 440nm. While enantiomeric selectivity is observed in methanol at 227nm, no discrimination is achieved in acetonitrile. Chiral discrimination is statistically possible with **L1** and PA in chloroform at 227nm, but it is not comparable with the extent of discrimination achieved in methanol. In chloroform a new emission band at a longer wavelength (440nm) is formed with R- PA in solution with **L1**, an effect that is not observed with the S-enantiomer. This new band in chloroform at 440nm allows very effective chiral discrimination and has been attributed to the presence of two different conformations of calixarene **L1**, which is reinforced by ¹H-NMR studies and molecular modelling studies.

Keywords: fluorescent calixarene, molecular sensor, chiral discrimination, solvent effect.

* To whom correspondence should be addressed; e-mail: dermot.diamond@dcu.ie

Introduction

Determination of enantiomeric composition is important due to both the role of chirality in many biological processes¹ and to increasing restrictions on the composition of pharmaceuticals, whose efficacy is dependent upon a chiral moiety^{2, 3}. Chiral molecules have identical physical and chemical properties, and differ only in their interactions with polarised light and other chiral entities. Our aim was to develop a molecular sensor, which could distinguish enantiomers of a chiral guest molecule. In order to fulfil its role, the molecular sensor must possess several essential features: (i) Functional groups must be present that bind reversibly with the target guest species, for example; hydrogen bonding groups or acidic/basic groups. (ii) These groups must be spatially pre-organised in order to provide the required selectivity. (iii) A quantifiable signal must be generated by the host–guest interaction⁴. Possible options for signalling include electrochemical (e.g. potentiometry, amperometry) and spectroscopic (e.g. fluorescence, absorbance) methods. (iv) The groups conferring recognition and signal transduction must be arranged in an interactive conformation, so that binding with a target species results in signal generation.

Calixarenes are cyclic oligomers that have an upper rim, defined by the para substituents of the phenolic rings and a lower rim defined by the phenolic hydroxy groups. Derivatisation of calixarenes at the upper and lower rim is desirable to introduce various types of functional groups, to increase their usefulness as new host molecules. Much of the analytical interest in calixarenes derives from their potential as selective and useful complexation agents, with the main area of interest to date being their use as molecular sensors⁵. This depends in part on the presence of appropriately sized cavities however for the more sophisticated recognition mechanisms it is also necessary that appropriate functional groups are present in a spatial arrangement that is complementary to binding sites in the guest molecule (i.e. shape-based recognition rather than size-based). The synthesis of calixarene **L1** (Figure 1) was previously described⁶. This molecular sensor incorporates a fluorescent naphthyl moiety, the necessary fluorophore for optical transduction, in proximity to hydrogen bonding and chiral centres in the host molecule in such a way that binding of the guest species results in quenching of the fluorescence.

The efficiency of the quenching of a fluorescent species by a quenching species follows the Stern-Volmer relationship if the fluorophore and quencher concentrations are in the appropriate range: where I_0 is the fluorescence intensity in the absence of the quencher, I is the fluorescence intensity in the presence of quencher at a concentration $[Q]$, and K_{SV} is the Stern-Volmer constant. K_{SV} is a measure of quenching efficiency,⁷ and a large value for this parameter equates to a sensitive response. Ideally, the aim is to obtain a material that displays

a large K_{SV} for one enantiomer and zero for the other (no quenching effect). This material could subsequently be employed as an optical sensor.

$$I_0/I = 1 + K_{SV}[Q] \quad (1)$$

Fluorescence emission spectra are widely variable and depend on the chemical structure of the fluorophore and also the nature of solvent employed. In general, only fluorophores that are polar in nature will display a large sensitivity to solvent polarity, with non-polar molecules such as hydrocarbons, showing much less sensitivity to solvent polarity⁸. In previous work it has been shown that calixarenes^{9, 10, 11, 12} and more recently fluorescent calixarenes^{13, 14, 15} can discriminate between enantiomers. Here we present how solvent polarity has an effect not only on the extent of enantiomeric discrimination, but also on the wavelength at which discrimination occurs.

Experimental Section

Equipment and Materials

All fluorescence emission and quenching experiments were performed using a Perkin-Elmer Luminescence Spectrometer LS 50B (Beaconsfield, Buckinghamshire, UK), interfaced with a Pentium PC, which employs fluorescence data management software, FLWinlab. Post-run data processing was performed using Microsoft Excel '97 and 2000 after importing the spectra as ASCII files.

All fluorescence lifetime measurements were performed using an Edinburgh Analytical Instruments Single Photon Counter in a T-setting, which employs a nanosecond nitrogen flash-lamp nF900, with photo-multiplier detector, model S300 (-20°C to -30°C). Post-run data processing was performed using a Pentium PC, F900 data correlation program, version 3.13 and Microsoft Excel 2000 after importing the spectra as ASCII files.

All UV measurements were carried out using a Perkin-Elmer Lambda 900 UV/VIS/NIR spectrometer. The instrument was controlled via UV WinLab software and post-run data processing was performed using Microsoft Excel '97 and 2000 after importing the spectra as ASCII files.

Both enantiomers of phenylalaninol, (R)-(+)-phenylalaninol and (S)-(-)-phenylalaninol were of puriss grade (98% pure, the other 2% consisting of the other enantiomeric form), obtained from Fluka Biochemika (Gillingham, Dorset, UK). In addition as a control, this chiral amine was obtained of puriss grade from Sigma-Aldrich. The solvents

used (methanol, acetonitrile and chloroform - HPLC grade (fluorescence emission) and Spectrometric grade (lifetime measurements)) were obtained from Labscan (Stillorgan, Co. Dublin). The synthesis of calixarenes **L1**⁶ and **L2**¹⁶ have been described previously.

Preparation of solutions for Fluorescence Measurements

Solutions giving concentrations of the propranolol amide calix[4]arene **L1** ($0.7\text{-}\mu\text{mol dm}^{-3}$) and phenylalaninol in the range $1 - 25\text{-mmol dm}^{-3}$ in methanol were prepared as follows. A 0.1-mmol dm^{-3} stock solution of calixarene **L1** was prepared by dissolving 8.9 mg in 50mL of methanol. A 0.25-mol dm^{-3} stock solution of phenylalaninol was prepared by dissolving the required combination of the two enantiomers, totalling 0.945 g, in 25mL of methanol. Test solutions were prepared by taking 70 μ L of the calixarene stock solution in a 10mL volumetric flask, adding 0, 0.2, 0.4, 0.6, 0.8, and 1.0mL of phenylalaninol stock solution, and making up to the volume with methanol. The fluorescence emission of the solutions was measured at an excitation wavelength of 227nm, and the emission intensities were measured at 340nm.

Solutions giving concentrations of **L1** ($3.0\text{-}\mu\text{mol dm}^{-3}$) and phenylalaninol (in the range $1 - 20\text{-mmol dm}^{-3}$) in acetonitrile, and **L1** ($5.0\text{-}\mu\text{mol dm}^{-3}$) and phenylalaninol (in the range $1 - 44\text{-mmol dm}^{-3}$) in chloroform, were prepared as described for experiments in methanol. The fluorescence emission of these solutions was measured at an excitation wavelength of 285nm, and the emission intensities were measured at 340nm. Measurements were repeated a minimum of three times for each addition.

Procedure for Fluorescence Measurements of di-functionalised R-propranolol amide calix[4]arene in Methanol

Solutions giving concentrations of the R-propranolol diamide calix[4]arene **L2** ($0.7\text{-}\mu\text{mol dm}^{-3}$) and phenylalaninol in the range $1 - 20\text{-mmol dm}^{-3}$ in methanol were prepared as follows. A 0.1-mmol dm^{-3} stock solution of calixarene **L2** and 0.25-mol dm^{-3} stock solutions of enantiomers of phenylalaninol were prepared as previously described. Test solutions were prepared by taking 70 μ L of the calixarene stock solution in a 10mL volumetric flask, adding 0 - 0.8mL of phenylalaninol stock solution, and making up to the volume with methanol. The fluorescence emission of the solutions was measured at an excitation wavelength of 227nm, and fluorescence intensities were measured at 340nm. Measurements were repeated a minimum of three times for each addition.

Preparation of solutions for Fluorescence Lifetime Measurements

Solutions giving absorbance readings at 300nm of 0.474 of the calix[4]arene **L1** and therefore a concentration of $56.1\text{-}\mu\text{mol dm}^{-3}$ in methanol were prepared. Extinction coefficient ($\epsilon_{300\text{nm}}$) of **L1** is $8449\text{ dm}^3\text{ mol}^{-1}\text{ cm}^{-1}$. Each sample was spiked with the R-enantiomer of phenylalaninol and the consequent fluorescence lifetime was measured. The concentration range of R-phenylalaninol examined after an addition of 0, 10, 20, 30 and 40 μL of the 1.0-mol dm^{-3} stock solution of R-phenylalaninol was $4\text{-}16\text{-mmol dm}^{-3}$. Solutions giving absorbance readings of 0.453 of **L1** and therefore a concentration of $53.6\text{-}\mu\text{mol dm}^{-3}$ in methanol were prepared and measured, and were spiked with the S-enantiomer of phenylalaninol in the same general procedure as in the case of the R-enantiomer.

Solutions giving absorbance readings of 0.570 of **L1** and therefore a concentration of $44.9\text{-}\mu\text{mol dm}^{-3}$ in chloroform were prepared. Each sample was spiked with the R-enantiomer of phenylalaninol and the consequent fluorescence lifetime was measured. The concentration range of phenylalaninol examined after an addition of 0 - 1000 μL of a 1.0-mol dm^{-3} stock solution of R-phenylalaninol was $40\text{ - }400\text{-mmol dm}^{-3}$. Solutions of **L1** spiked with S-phenylalaninol were prepared in the same general manner as in the case of the R-enantiomer of PA.

Results

Excitation and Emission Spectra

The design and synthesis of p-allyl calix[4]arene-tetra-S-propranolol amide **L1** is reported elsewhere⁶. The excitation and emission spectra of **L1** at a concentration of $0.7\text{-}\mu\text{mol dm}^{-3}$ in methanol are shown in Figure 2, in the absence and presence of racemic phenylalaninol. The maximum of the excitation spectrum is at 227 nm (Figure 2a), and the maximum of the emission spectrum obtained using an excitation wavelength of 227 nm is at 338 nm (Figure 2b). Considering that phenylalaninol and methanol do not absorb in these regions, 227nm is a suitable excitation wavelength for the following experiments in methanol.

The excitation and emission spectra of calix[4]arene **L1** at a concentration of $3.0\text{-}\mu\text{mol dm}^{-3}$ in acetonitrile are shown in Figure 3, in the absence and presence of racemic phenylalaninol. There are maxima in the excitation spectrum at 220nm, 235nm and 285nm (Figure 3a), and the maximum of the emission spectrum obtained using an excitation wavelength of 285nm is at 339 nm (Figure 3b). The guest species PA and solvent do not

absorb in the region of the spectrum higher than 280nm, hence 285nm was chosen as the excitation wavelength for following experiments in acetonitrile.

The excitation and emission spectra of calix[4]arene **L1** at a concentration of 5.0- $\mu\text{mol dm}^{-3}$ in chloroform are shown in Figure 4, in the absence and presence of racemic phenylalaninol. The maxima of the excitation spectrum are at 285, 338 and 400 nm (Figure 4a), and the maximum of the emission spectrum obtained using an excitation wavelength of 285 nm is at 338 nm (Figure 4b). Given that the guest species PA and solvent do not absorb in this region, 285nm was used as the excitation wavelength for following experiments in chloroform.

Linear Response range

It is important to use a concentration of the calixarene within the linear range in order to ensure that no self-quenching occurs and therefore that no alternative self-quenching mechanisms are present. The linear response range of fluorescence intensity of calixarene **L1** in methanol was determined to be between 0.1 and 0.7- $\mu\text{mol dm}^{-3}$. A concentration of 0.7- $\mu\text{mol dm}^{-3}$ was chosen for **L1** for subsequent experiments to examine the effects of phenylalaninol (PA), and hence any quenching observed can be related to the effect of the target species on the ligand.

The linear response range of fluorescence intensity of calixarene **L1** in acetonitrile was determined to be between 0.1 and 4.0 $\mu\text{mol dm}^{-3}$. A concentration of 3.0- $\mu\text{mol dm}^{-3}$ was chosen for subsequent experiments in acetonitrile. The linear response range of fluorescence intensity of calixarene **L1** in chloroform was determined to be between 0.1 and 6.0 $\mu\text{mol dm}^{-3}$ hence a concentration of 5.0- $\mu\text{mol dm}^{-3}$ was chosen for **L1** for following experiments in chloroform.

Variation of Stern-Volmer plot with enantiomeric composition in methanol

The Stern-Volmer plot of **L1** in methanol was found to be linear over the range 0-25 mmol dm^{-3} of racemic phenylalaninol (PA). Figure 5 illustrates the Stern-Volmer plots for the quenching of the fluorescence of calixarene **L1** (0.7 $\mu\text{mol dm}^{-3}$ in methanol), upon addition of 0% S-PA: 100% R-PA, 50% S-PA: 50% R-PA (racemic mix) and 100% S-PA: 0% R-PA respectively, at a concentration range of 0 – 25mmol dm^{-3} . The Stern-Volmer constant K_{SV} , is a measure of the quenching efficiency and a large value for this parameter indicates a sensitive response. The values for the Stern-Volmer constants (K_{SV}) are 0.17, 0.12 and 0.09 after the addition of 0 % S-PA: 100% R-PA, 50% S-PA: 50% R-PA and 100% S-PA: 0% R-PA respectively and the (K_{SV}) ratio [100% R/100% (S)] is 1.9. Because the Stern-Volmer

plots show such a large difference in the K_{SV} values of each enantiomer, it can be concluded that in methanol, calix[4]arene **L1** exhibits significant ability to discriminate between the enantiomers of phenylalaninol.

Previous results show that it is possible to discriminate between the enantiomers of chiral amines with appropriately arranged hydrogen bonding groups, chiral centres and quenching sites using suitably functionalised calix[4]arenes^{14, 15}. The position of the chiral centre relative to the hydrogen bonding groups and the aryl group in the guest molecule appears to be vitally important, illustrated by the fact that the calix[4]arene host **L1** does not discriminate between enantiomers of phenylglycinol without an extra methylene spacer between the chiral centre and phenyl ring, while excellent discrimination is observed with phenylalaninol, with a methylene spacer⁶. The importance of the aryl group in the transduction process (quenching) was convincingly demonstrated by preceding results using cyclohexylethylamine as guest¹³. **L1** encompasses four appended chains with chiral units in the S-configuration. For this reason it was envisaged that **L1** would preferentially interact with R-enantiomers of the guest species through hydrogen bonding and π - π overlap. Fluorescence quenching studies confirm this proposed preferential association, with the R-enantiomer of phenylalaninol giving larger Stern-Volmer constants than the S-enantiomer.

Variation of Stern-Volmer plot of a partially functionalised calix[4]arene with enantiomeric composition

As a control to test the postulation that enantiomeric discrimination is due to the three-dimensional cavity of the lower calixarene rim, fluorescence-quenching studies were carried out on the p-allyl calix[4]arene- di-R-propranolol amide **L2** (Figure 1) in the presence of both enantiomers of phenylalaninol in methanol. As can be seen from Figure 6 practically no difference is discerned in the Stern-Volmer plots for **L2** with each enantiomer, and in some cases the error bars for both series overlap. Consequently there is no difference in the K_{SV} constants for each enantiomer of phenylalaninol in the presence of **L2** in methanol. This therefore signifies that the partially substituted equivalent of the aforementioned calixarene **L1** does not possess the ability to discriminate between the enantiomers of phenylalaninol. This confirms the previous suggestion that discrimination is achieved due to an effect of the 3-D distribution of pendent groups at the calixarene lower rim.

Variation of Stern-Volmer plot with enantiomeric composition in acetonitrile

The Stern-Volmer plot of **L1** in acetonitrile ($3.0 \mu\text{mol dm}^{-3}$) was found not to be linear over the range $0\text{--}40 \text{ mmol dm}^{-3}$ of racemic phenylalaninol. Since the Stern-Volmer plots (not

shown) upon addition of 100% (R)- and 100% (S)-phenylalaninol respectively, show such similarities in the behaviour of each enantiomer at a concentration range of 0 – 40 mmol dm⁻³, it can be concluded that **L1** does not exhibit significant ability to discriminate between the enantiomers of phenylalaninol in acetonitrile.

Fluorescence emission in Acetonitrile

Figure 7 (a) shows the emission of **L1** in acetonitrile after the addition of the phenylalaninol guest, which results in an initial large decrease in fluorescence intensity in the calixarene spectrum followed by only minor changes subsequent to further aliquots of guest addition. This trend was observed for both enantiomers of phenylalaninol. This may be due to the formation of a 1:1 complex between the host and guest upon the addition of the guest to the calixarene solution; however the exact mechanism of quenching is not clear at present. In view of the fact that similar effects were observed for both enantiomers of phenylalaninol, it can be concluded that **L1** does not possess the ability to discriminate between the enantiomers of phenylalaninol in acetonitrile.

Variation of Stern-Volmer plot with enantiomeric composition in chloroform

The Stern-Volmer plot of **L1** in chloroform (5.0-μmol dm⁻³) was found to be linear over the range 0 - 40-mmol dm⁻³ of racemic phenylalaninol. The Stern-Volmer plots upon the addition of 100% (R): 0% (S) and 100% (S): 0% (R)-phenylalaninol at a concentration range of 0 – 40 mmol dm⁻³, yield K_{SV} values of 0.014 and 0.011 for the R- and S-enantiomers of phenylalaninol respectively, i.e. a K_{SV} ratio of 1.25. Although the slopes for each enantiomer in the presence of **L1** are statistically different, these results taken at an emission wavelength of 338nm show great similarities in the behaviour of each enantiomer in the presence of **L1**, when compared to the methanol solutions previously discussed. Hence it can be concluded that the ability of **L1**, to discriminate enantiomers of phenylalaninol at 338nm in chloroform, is greatly reduced compared to methanol.

Fluorescence emission in Chloroform

Preliminary studies of the absorption spectra of ligand **L1** in chloroform show that the addition of guest phenylalaninol produces a simple additive effect to the calixarene absorption spectrum. However when the excitation spectrum of **L1** is examined in the absence and presence of phenylalaninol (Figure 4a), differences are noted. When the emission spectrum of calixarene **L1** in chloroform is examined in the presence of R- and S-phenylalaninol variations can clearly be seen (see Figure 7b and c). A decrease in fluorescence intensity is noted at 338nm after addition of both enantiomers of PA, however in the presence of R-

phenylalaninol the weak emission band of **L1** at 430nm is greatly enhanced. This enhancement is not observed however for **L1** in the presence of S-phenylalaninol.

Figure 8 shows that by monitoring the fluorescence intensity of **L1** in the presence of each enantiomer of phenylalaninol (at an emission wavelength of 430nm), a clear distinction between the effects of the R- and S-isomers of phenylalaninol can be observed, i.e. an increase in fluorescence, which is in contrast to the results previously obtained. When methanol is employed as solvent, the enantiomeric discrimination can be observed at 340nm as a decrease in the fluorescence intensity with a corresponding increase in guest concentration. In the case of chloroform as solvent, if the fluorescence intensity is monitored at a wavelength of 430nm, the enantiomeric discrimination can be observed as an increase in the fluorescence intensity with a corresponding increase in guest concentration. By monitoring at this wavelength (430nm) it can clearly be discerned, which enantiomer is in solution with calixarene **L1**.

An explanation for this increase in intensity of the emission band at 430nm is attributed to the presence of two emitting species in solution, which we have assigned as two different conformations of the calixarene **L1**. The ^1H -NMR spectrum suggests that the calixarene does not exist as a symmetrical cone in CDCl_3 solution, but rather a distorted cone, which alters confirmation to bind with metal ions in solution⁶. Energy minimised molecular modelling shows that the appended chains of this ligand can be flexible (see Figure 9)⁶. The modelling studies and ^1H -NMR spectrum strengthen the argument that two conformations of the calixarene are present in chloroform solution. In the case of **L1**, there is a relatively well-defined 3-D chiral space through which the enantiomers must pass in order to facilitate quenching. Clearly, if this is the case, then the R-phenylalaninol must be better predisposed to passing through the chiral space than the S-enantiomer. Upon addition of R-phenylalaninol, the conformation of **L1** changes to accommodate and associate with the guest. This changes the equilibrium of conformations present in solution, which seems to generate more of the conformer which emits at 430nm, and less of the form whose emission is observed at 340nm. This would explain the intensity enhancement of the band at 430nm. The fact that the band at 340nm decreases in the presence of the S-enantiomer of phenylalaninol may be attributed to weak complex formation between S- phenylalaninol and **L1**.

Variation of fluorescence lifetimes with enantiomeric composition

Quenching that results from diffusive encounters between the fluorophore and quencher during the lifetime of the excited state is termed “dynamic quenching”. Quenching can also occur as a result of the formation of a non-fluorescent complex between the fluorophore and the quencher i.e. “static quenching”. The fluorescence intensity decrease is linearly dependent

on the concentration of the quencher, a case that is identical for both static and dynamic quenching. In general, mechanisms of quenching can be distinguished by their differing dependence on temperature and viscosity, or preferably by lifetime measurements.

Employing an excitation wavelength of 307nm and scanning at an emission wavelength of 340nm, values of 9.4 and 4.6 ns were measured for the lifetime of **L1** in methanol, and 6.6 and 1.6 ns for the lifetime of **L1** in chloroform, both in the absence and presence of R-PA (see Table 1). This would suggest that two fluorescing species are present in solution, and that addition of R-PA does not affect the lifetime of **L1**. Since spectroscopic grade solvents were used the second value was not attributed to an impurity. The two lifetimes may be due to different conformations of the calixarene, as the array of naphthalene groups can be differently positioned in one conformer over the other, possibly altering the electronic state of the molecule. It is interesting to note that these and any following lifetime measurements were obtained in both oxygenated and degassed solutions, and that no difference was discerned between values acquired. This implies that molecular oxygen has no effect on the fluorescence spectra of these solutions, failing to contribute to the quenching process.

The lifetime of the excited state of **L1** was also measured in the absence and presence of the S-enantiomer of phenylalaninol. Two values of 9.2 and 4.6 ns were measured in methanol, and 6.6 and 1.6 ns in chloroform. The results illustrate that the presence of this guest enantiomer in solution does not perturb the excited state lifetime of the calixarene, confirming that both enantiomers of phenylalaninol form ground state complexes with calixarene **L1** in methanol and chloroform, but clearly by different degrees as confirmed by the emission quenching data. The fluorescence lifetime data confirms that the mechanism of quenching of the fluorescence of **L1** by both enantiomers of phenylalaninol is static. Hence an excited state complex is not accountable for the increase in intensity of the emission band at 440nm. This reinforces the argument that there is a change in equilibrium of two calixarene conformations in the presence of R-phenylalaninol in chloroform solution.

At present we cannot distinguish which conformation or rotamer is responsible for the emission bands at 340nm and 440nm. Fluorescence emission and lifetime measurements of metal complexes of **L1** in chloroform may help to discern whether a more rigid cone conformation induced by metal ion complexation is responsible for one of the emission bands. It is clear however, that enantiomeric discrimination is possible with calixarene **L1** in chloroform solution if the emission intensity is monitored at 440nm. Since the intensity of **L1** in chloroform changes very little when in solution of S-PA (Figure 7b), and undergoes a four-fold increase when in the presence of R-PA (Figure 7c) (highest concentration) it is immediately obvious which enantiomer one has in solution with this calixarene. It is clear that

the enantiomeric composition of PA can be determined from the slope of the Stern-Volmer plots. However, a faster and less tedious method that involves only a single fluorescence measurement of the sample at 440nm in chloroform could be employed.

L1 has been designed to have hydrogen bonding sites and the chiral centre, separated from the naphthyl groups by an ether group. The guest molecule, PA (Figure 1), contains a group capable of hydrogen bonding (primary amine) as part of a chiral centre attached to an aryl group via a methylene spacer. This arrangement could facilitate simultaneous π - π interactions between the naphthyl groups on **L1** and the PA aryl group and hydrogen bonding between the hydroxyl group on **L1** and the PA amine. However, the presence of this arrangement of structural features on its own does not explain the enantiomeric selectivity displayed by **L1**, as **L2** does not exhibit this behaviour, with just two appended chains.

In order to facilitate enantiomeric discrimination it is generally accepted that a number of points of attachment is desirable via hydrogen bonding^{17, 18}. Molecular modelling calculations suggest that up to four hydrogen bonds may occur between the (R)-enantiomer of phenylalaninol and the calixarene host **L1**¹⁹. Energy minimised structures of the calix[4]arene **L1** (see Figure 9) suggest that the four (S)-propranolol amide groups present a well-defined 3D chiral space into which the (R)-enantiomer of phenylalaninol is preferentially bound, resulting in enhanced quenching compared to the (S)-enantiomer.

Conclusion

L1 was found to successfully discriminate between enantiomers of phenylalaninol in methanol at 338nm. Fluorescence lifetime studies provide further information on the mechanism of quenching, and it was established that static quenching was the cause of the decrease in fluorescence intensity at 338nm of **L1** in the presence of PA.

Chiral discrimination of PA is not possible with **L1** in acetonitrile. There appears to be a 1:1 host: guest association between **L1** and PA in this solvent. Since acetonitrile is a polar non-protic solvent, there is no hydrogen bonding between the solvent and the calixarene. This implies that the guest does not need to disrupt any host-solvent associations when spiked into the calixarene solution and therefore immediately associates with the host.

Chiral discrimination is statistically possible with **L1** and PA at 338nm in chloroform, but is not comparable to that observed in methanol. When the emission intensity of **L1** in chloroform is monitored at 440nm in the presence of PA, huge differences are seen with respect to the two enantiomers. Practically no change in emission is observed at 440nm with the S –enantiomer, whereas a dramatic increase in fluorescence intensity is observed when the R- enantiomer is added. Fluorescence lifetime experiments have established a static

quenching mechanism between **L1** and R-PA, thus ruling out exciplex formation, and show two fluorescing species in solution. These have been attributed to two different calixarene conformations in chloroform. When one consults energy minimised structures of **L1** (see Figure 9) it is clear that the calixarene is quite flexible and may exist in conformations other than a rigid cone in solution. This theory is supported by ^1H -NMR spectra⁶.

Figure 1: *Molecular structures of L1, L2 and PA.*

(a)

(b)

Figure 2: Excitation and emission spectra of calix[4]arene **L1** at a concentration of $0.7\text{-}\mu\text{mol dm}^{-3}$ in methanol. (a) Excitation spectrum of the calixarene in the absence (i) and presence (ii) of 25-mmol dm^{-3} phenylalaninol at an emission wavelength of 338nm . (b) Emission spectra of the calixarene in the absence (i) and presence (ii) of 25-mmol dm^{-3} phenylalaninol at an excitation wavelength of 227nm .

(a)

(b)

Figure 3: Excitation and emission spectra of calix[4]arene **LI** at a concentration of $3.0\text{-}\mu\text{mol dm}^{-3}$ in acetonitrile. (a) Excitation spectrum of the calixarene in the absence and presence of phenylalaninol (PA) (20-mmol dm^{-3}) at an emission wavelength of 339nm . (b) Emission spectra of the calixarene in the absence and presence of phenylalaninol (PA) (20-mmol dm^{-3}) at an excitation wavelength of 285nm .

(a)

(b)

Figure 4: Excitation and emission spectra of calix[4]arene **L1** at a concentration of $5.0\text{-}\mu\text{mol dm}^{-3}$ in chloroform. (a) Excitation spectrum of the calixarene in the absence and presence of phenylalaninol (PA) (20-mmol dm^{-3}) at an emission wavelength of 440nm . (b) Emission spectra of the calixarene in the absence and presence of phenylalaninol (20-mmol dm^{-3}) at an excitation wavelength of 285nm .

Figure 5: Stern-Volmer plots for the quenching of *S*-propranolol calixarene **L1** ($0.7 \mu\text{mol dm}^{-3}$ in methanol) upon addition of (a) 0% *S*-PA: 100% *R*-PA, (b) 50% *S*-PA: 50% *R*-PA and (c) 100% *S*-PA: 0% *R*-PA in methanol. Standard deviations are shown as error bars ($n=3$), which may be masked by symbols. Measurements were taken at an excitation wavelength of 227nm and an emission wavelength of 338nm.

Figure 6: Stern-Volmer plots for the quenching of di-*R*-propranolol calixarene **L2** ($0.7 \mu\text{mol dm}^{-3}$ in methanol) upon addition of 100% (*R*)- (a (●)) and 100% (*S*)- (b (■)) phenylalaninol in methanol. Standard deviations are shown as error bars ($n=3$), which may be masked by

symbols. Measurements were taken at an excitation wavelength of 227nm and an emission wavelength of 338nm.

(a)

(b)

(c)

Figure 7: (a) Fluorescence emission spectra of calixarene **L1** alone ($3.0 \mu\text{mol dm}^{-3}$) in acetonitrile (dotted line) and in the presence of varying amounts of R-phenylalaninol ($0 - 20 \text{ mmol dm}^{-3}$ – uppermost-lowest plain lines respectively). Measurements were taken at an excitation wavelength of 285nm. (b) Fluorescence emission spectra of calixarene **L1** alone ($5.0 \mu\text{mol dm}^{-3}$) in chloroform (dotted line) and in the presence of varying amounts of S-phenylalaninol ($4-40 \text{ mmol dm}^{-3}$ – uppermost-lowest plain lines respectively). Measurements were taken at an excitation wavelength of 285nm. (c) Fluorescence emission spectra of calixarene **L1** alone ($5.0 \mu\text{mol dm}^{-3}$) in chloroform (dotted line) and in the presence of varying amounts of S-phenylalaninol ($4-40 \text{ mmol dm}^{-3}$ –uppermost-lowest plain lines respectively). Measurements were taken at an excitation wavelength of 285nm. For (a), (b) and (c) PA concentration increases as fluorescence intensity decreases.

Figure 8: Fluorescence intensity of calixarene **L1** emission band at λ -emission = 430nm with increasing phenylalaninol concentration, calixarene concentration $5.0 \mu\text{mol dm}^{-3}$ in the case of each enantiomer.

Solution	τ_1 and τ_2 / Nanoseconds	
	In Methanol	In Chloroform
Calixarene L1 alone	9.4, 4.6 (± 0.1)	6.7, 1.6 (± 0.1)
L1 + R-PA	9.4, 4.6 (± 0.2)	6.6, 1.6 (± 0.1)
Calixarene L1 alone	9.3, 4.4 ($\pm 0.$)	6.7, 1.6 (± 0.1)
L1 + S-PA	9.2, 4.6 (± 0.2)	6.6, 1.6 (± 0.1)

Table 1: Fluorescence lifetimes of calixarene **L1** in the absence and presence of R-phenylalaninol and S-phenylalaninol, in methanol and chloroform.

Figure 9: Side-on view of **L1** and PA (left of picture) and **L2** and PA (right of picture), showing how the guest may be included in the calixarene cavity, and also the extent of steric crowding involved. The modelling calculations were carried out using Spartan [20] SGI Version 5.1.1. The models shown correspond to the conformer found with the lowest energy.

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20 Spartan, Wavefunction Inc., 18401 Von Karman Ave., Suite 370, Irvine, CA 92612, U.S.A. <http://www.wavefun.com/>.