NMR spectroscopic detection of chirality and enantiopurity in referenced systems without formation of diastereomers

Jan Labuta¹, Shinsuke Ishihara¹, Tomáš Šikorský², Zdeněk Futera³, Atsuomi Shundo¹,†, Lenka Hanyková⁴, Jaroslav V. Burda³, Katsuhiko Ariga¹,⁵ & Jonathan P. Hill¹,⁵

Enantiomeric excess of chiral compounds is a key parameter that determines their activity or therapeutic action. The current paradigm for rapid measurement of enantiomeric excess using NMR is based on the formation of diastereomeric complexes between the chiral analyte and a chiral resolving agent, leading to (at least) two species with no symmetry relationship. Here we report an effective method of enantiomeric excess determination using a symmetrical achiral molecule as the resolving agent, which is based on the complexation with analyte (in the fast exchange regime) without the formation of diastereomers. The use of N,N′-disubstituted oxoporphyrinogen as the resolving agent makes this novel method extremely versatile, and appropriate for various chiral analytes including carboxylic acids, esters, alcohols and protected amino acids using the same achiral molecule. The model of sensing mechanism exhibits a fundamental linear response between enantiomeric excess and the observed magnitude of induced chemical shift non-equivalence in the ¹H NMR spectra.

1 International Center for Materials Nanoarchitectonics (MANA), National Institute for Materials Science (NIMS), 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan. 2 CEITEC—Central European Institute of Technology, Masaryk University, CZ-62500 Brno, Czech Republic. 3 Charles University in Prague, Faculty of Mathematics and Physics, Department of Chemical Physics and Optics, Ke Karlovu 3, 121 16 Prague 2, Czech Republic. 4 Charles University in Prague, Faculty of Mathematics and Physics, Department of Macromolecular Physics, V Holešovičkách 2, 180 00 Prague 8, Czech Republic. 5 Japan Science and Technology Agency (JST), Core Research for Evolutional Science and Technology (CREST), 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan. † Present addresses: Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan (Z.F.); Department of Applied Chemistry, Faculty of Engineering, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan (A.S.). Correspondence and requests for materials should be addressed to J.H. (email: Jonathan.Hill@nims.go.jp).
Chirality plays a key role in many biological events but is also important in the development of new pharmaceuticals, for control of organic asymmetric reactions, and in several aspects of supramolecular science.

Therefore, the detection of chirality, including analysis of chiral purity and interactions between chiral species, is an intensive area of research. Various procedures for determining analyte chirality have been proposed and those of note are based on optical (for example ultraviolet/visible spectrophotometry (uv/Vis), circular dichroism (CD), fluorescence) and electrochemical methods involving a synthetic chiral host as a chirality detector. However, the use of an achiral host as a detector has only seldom been achieved, that is, through the formation of ternary diastereomeric complexes between chiral substrate molecules and achiral host or by the observation of excitation-coupled CD effects in porphyrin tweezer molecules where helicity is induced by complexation with a chiral guest.

Over several decades NMR spectroscopic methods, which depend on a chiral auxiliary such as chiral derivatization agents, have been extensively studied. NMR discrimination methods that do not use an auxiliary have also been developed although they are considered unusual as self-association between chiral analytes is required in the formation of diastereomeric dimers. Use of an achiral auxiliary in conjunction with 31P NMR spectroscopy has also been reported. However, in that case an achiral derivatization agent was used as a platform for covalent coupling of two chiral analyte molecules resulting in diastereomer formation and three well-separated NMR resonances could be observed. We have previously reported a method that facilitates the latter concept by using a solvating agent where a host–guest complex (formed via non-covalent interaction) is used to determine the chirality of an analyte. A solvating agent (CSA) have been extensively studied. NMR discrimination methods that do not use an auxiliary have also been developed although they are considered unusual as self-association between chiral analytes is required in the formation of diastereomeric dimers. Use of an achiral auxiliary in conjunction with 31P NMR spectroscopy has also been reported. However, in that case an achiral derivatization agent was used as a platform for covalent coupling of two chiral analyte molecules resulting in diastereomer formation and three well-separated NMR resonances could be observed. We have previously reported a method that facilitates the latter concept by using a solvating agent where a host–guest complex (formed via non-covalent interaction) is used to determine the chirality of an analyte.

In this work, we introduce a 1H NMR versatile prochiral solvating agent (pro-CSA) for determination of chirality and e.e. which does not rely on formation of diastereomeric pairing between prochiral host and chiral analyte; rather, a pair of enantiomeric complexes are formed. Detection of information about a chirality/e.e. without employing formation of diastereomers has not to our knowledge been reported and is an unusual and intriguing aspect of our pro-CSA host. The pro-CSA host molecule contains appropriate prochiral CH reporter groups from the binding site has an important role. Each CH reporter group exhibits different sensitivity (that is, magnitude of non-equivalence) which is capable of becoming chiral in a single desymmetrization step. It is known that when a prochiral compound (for example, an achiral primary carboxylic acid Ph(CH2)3CO2H) is placed in a chiral environment (for example, (+)/(−)(1R,2R)-1,2-diphenylethane-1,2-diamine) forming an acid-amine complex, the enantiotopic (prochiral) hydrogens of the acid exhibit chemical shift non-equivalence and thus are rendered diastereotopic. The pro-CSA, H, presented here contains enantiopure CH reporter groups as shown in Fig. 1a. When an enantiopure chiral guest (Fig. 1b) is added to a solution of host molecule H symmetrical splitting (sometimes with an associated up- or downfield shift) of the resonance due to the reporter groups can be observed (Fig. 1c). H complexes with a chiral guest by hydrogen bonding through pyrrolic NH groups located at its centre resulting in downfield shift of the NH proton resonance (Fig. 1c). Taking into account the host’s saddle shape and the relative rigidity of its macrocycle, the distance of the available CH reporter groups from the binding site has an important role. Each CH group exhibits different sensitivity (that is, magnitude of non-equivalence) to different chiral guests (Fig. 1c). As H has four such groups of practical use at different spatial positions (accessibility) and distances from the binding site there is a good chance that any chiral guest with affinity to bind to H will cause non-equivalence in selected or all of the CH reporter groups.

At this point, the origin of chemical shift non-equivalence of CH reporter groups can be elucidated in terms of the symmetry of H and disruption thereof upon interaction with a chiral guest. The term ‘prochirality’ is frequently used to describe systems where replacement of a single ligand leads to a stereogenic centre. The equivalent of this in non-covalently interacting systems would be the formation of diastereotropic nuclei from initially enantiotopic nuclei due to an external factor as illustrated above in the case of acid-amine complexes where the methylene carbon of Ph(CH2)3CO2H can be considered a stereogenic centre. However, the CH reporter groups of H do not possess the property that any of its carbon atoms could be converted to a stereogenic centre. For simplicity let us consider the complexation of H with 1a where the CH reporter groups of interest are at the β positions of the N-alkylated pyrrole group (β-H; Fig. 2a,b). In the structure of H, two prochiral planes are present (Fig. 2a). More specifically, two onefold improper rotations S (mirror planes σ) which are identical with prochiral planes, can be identified. These mirror planes make group A and B of H interconvertible and hence enantiotopic (magnetically equivalent with isochronous NMR resonances). Note that H also possesses a proper symmetry axis C2 which might suggest that groups A and B are homotopic hence isochronous even in a chiral environment. However, groups A and B (as defined in Fig. 2a) cannot be interconverted using C2. Therefore, in general, β-H nuclei are required to be identified as groups A and B so that they are interconvertible by improper rotation S (including plane σ or centre of symmetry i) and not various chiral guest molecules G, including carboxylic acids and esters, amino acid esters, N-protected amino acids, and some terpenoids such as menthol and camphor. Experimental and theoretical methods (NMR, UV/Vis, CD, quantum mechanical (QM) calculations and classical molecular dynamics (MD) simulations) are used to elucidate the chirality and/or e.e. sensing properties of the system presented here.

**Results**

**Symmetry and origin of chemical shift non-equivalence.** The e.e. discrimination method presented here relies fundamentally on the concept of prochirality, defined as ‘the geometric property of an achiral object (or spatial arrangement of points or atoms) which is capable of becoming chiral in a single desymmetrization step’. It is known that when a prochiral compound (for example, an achiral primary carboxylic acid Ph(CH2)3CO2H) is placed in a chiral environment (for example, (+)/(−)(1R,2R)-1,2-diphenylethane-1,2-diamine) forming an acid-amine complex, the enantiotopic (prochiral) hydrogens of the acid exhibit chemical shift non-equivalence and thus are rendered diastereotopic. The pro-CSA, H, presented here contains enantiopure CH reporter groups as shown in Fig. 1a. When an enantiopure chiral guest (Fig. 1b) is added to a solution of host molecule H symmetrical splitting (sometimes with an associated up- or downfield shift) of the resonance due to the reporter groups can be observed (Fig. 1c). H complexes with a chiral guest by hydrogen bonding through pyrrolic NH groups located at its centre resulting in downfield shift of the NH proton resonance (Fig. 1c). Taking into account the host’s saddle shape and the relative rigidity of its macrocycle, the distance of the available CH reporter groups from the binding site has an important role. Each CH group exhibits different sensitivity (that is, magnitude of non-equivalence) to different chiral guests (Fig. 1c). As H has four such groups of practical use at different spatial positions (accessibility) and distances from the binding site there is a good chance that any chiral guest with affinity to bind to H will cause non-equivalence in selected or all of the CH reporter groups.

At this point, the origin of chemical shift non-equivalence of CH reporter groups can be elucidated in terms of the symmetry of H and disruption thereof upon interaction with a chiral guest. The term ‘prochirality’ is frequently used to describe systems where replacement of a single ligand leads to a stereogenic centre. The equivalent of this in non-covalently interacting systems would be the formation of diastereotropic nuclei from initially enantiotopic nuclei due to an external factor as illustrated above in the case of acid-amine complexes where the methylene carbon of Ph(CH2)3CO2H can be considered a stereogenic centre. However, the CH reporter groups of H do not possess the property that any of its carbon atoms could be converted to a stereogenic centre. For simplicity let us consider the complexation of H with 1a where the CH reporter groups of interest are at the β positions of the N-alkylated pyrrole group (β-H; Fig. 2a,b). In the structure of H, two prochiral planes are present (Fig. 2a). More specifically, two onefold improper rotations S (mirror planes σ) which are identical with prochiral planes, can be identified. These mirror planes make group A and B of H interconvertible and hence enantiotopic (magnetically equivalent with isochronous NMR resonances). Note that H also possesses a proper symmetry axis C2 which might suggest that groups A and B are homotopic hence isochronous even in a chiral environment. However, groups A and B (as defined in Fig. 2a) cannot be interconverted using C2. Therefore, in general, β-H nuclei are required to be identified as groups A and B so that they are interconvertible by improper rotation S (including plane σ or centre of symmetry i) and not various chiral guest molecules G, including carboxylic acids and esters, amino acid esters, N-protected amino acids, and some terpenoids such as menthol and camphor. Experimental and theoretical methods (NMR, UV/Vis, CD, quantum mechanical (QM) calculations and classical molecular dynamics (MD) simulations) are used to elucidate the chirality and/or e.e. sensing properties of the system presented here.
by operation of proper symmetry axes \( C_n \). In that case, groups A and B are enantiotopic.

The presence of a chiral guest leads to magnetic non-equivalence of group A and B as it affects each group of protons in a different manner as can be illustrated from MD simulations by the preference of certain orientations of guest occupation (that is, conformations) over others as shown in Fig. 2c,d. A chiral environment breaks mirror symmetry and the initially enantiotopic groups A and B become diastereotopic with distinguishable anisochronous NMR resonances (Fig. 2b). Note that group A and B are contained in the same molecule so that no actual diastereomers are present. This is also the reason why at any arbitrary e.e. the split peaks have the identical intensities. The second-order quartet (which exhibits roofing) appears as a consequence of the close structural connection between HA and HB protons, which have a vicinal scalar \( J \)-coupling constant. From QM calculations it can be identified which group of protons will experience upfield or downfield shifts (see Supplementary Fig. S4). QM results for the \( H \cdot (R)\)-1a complex reveal that group A is shifted relatively upfield compared with group B and vice versa due to symmetry of the complex \( H \cdot (S)\)-1a.

**NMR spectral properties and binding model.** Hydrogen bonding between H and chiral guest appears to be weak so that a relatively high concentration of chiral guest is required to obtain well-resolved spectral lines (Fig. 1c). Interestingly, if the concentration of chiral guest is kept constant and e.e. is varied then the magnitude of splitting \( \Delta \delta \) of spectral lines varies linearly with e.e. (Fig. 3a–d, for other guests see Supplementary Figs S5–S14). The linear dependence of \( \Delta \delta \) on e.e. is a fundamental property of pro-CSA molecules as will be shown later. When determining the true resonance frequency of the N-alkylated pyrrolic \( \beta \)-H, N-H pyrrolic \( \beta \)-H and hemiquinonoid ortho-H spin systems it must be taken into consideration that these spins are second-order strongly coupled systems, and the fitting procedure should be carried out with appropriate care (see \( ^1H \) NMR spectra fitting in Supplementary Methods). The characteristic roofed shape of the split N-alkylated pyrrolic \( \beta \)-H resonances and the cross-peaks between them in \( ^1H–^1H \) COSY spectrum (Fig. 3e) clearly indicates that both peaks originate from the same host molecule. Similar cross-peaks were observed in N-H pyrrolic \( \beta \)-H and hemiquinonoid ortho-H in, for example, \( H \cdot (–)\)-4 complex (see \( ^1H \) NMR spectra fitting in Supplementary Methods). As e.e. tends towards 0% (that is, racemic mixture), a single set of sharp well-resolved resonances remain. At 0% e.e. these converge to a centred singlet for \( H \cdot 1a, H \cdot 2a \) and a doubllet for \( H \cdot 4 \) (Fig. 3a–c). The lack (at 0% e.e.) of two sets of peaks, corresponding to stable complexes of \( H \cdot (R)\)-enantiomer and \( H \cdot (S)\)-enantiomer, indicates fast exchange of chiral guests at the host’s binding site.

The above-mentioned features helped us to elucidate the origin of the linear relationship between magnitude of splitting \( \Delta \delta \) and e.e., as well as a model of the host–guest binding mechanism. The mechanism of binding should be considered as competitive involving binding of water molecules W and the chiral guest \( G^* \) to the host molecule H, as titration of a chloroform solution of W yields a not insignificant response (and high binding constant) to traces of water (Fig. 4a, for \( ^1H \) NMR spectra see Supplementary Fig. S15). Moreover, water is ubiquitous and is difficult to remove from chloroform, which also explains why a relatively high concentration of \( G^* \) is required to obtain wellresolved split NMR spectra. Some examples of titrations of H with W are shown in Fig. 4b–e (for other guests and other types of titrations see Supplementary Figs S16–S25). For the two types of titration shown it can be observed that the chemical shift of pyrrolic NH resonance (\( \delta_{\text{NH}} \)) increases monotonically while splitting of N-substituted pyrrolic \( \beta \)-H (\( \Delta \delta \)) does not necessarily increase monotonically (compare Fig. 4b,c). These observations suggest that the NH group reflects only the overall binding of guests \( (G^* \text{ or } W) \) while the pyrrolic \( \beta \)-H protons are sensitive not only to overall binding but also to the nature of chiral guest and its e.e. Therefore, binding isomers are required to be constructed (and fitted) simultaneously for both \( \delta_{\text{NH}} \) and \( \Delta \delta \), as they each partially express the same characteristic (binding strength).

Binding isomers for \( \delta_{\text{NH}} \) and \( \Delta \delta \) can be constructed based on a 1:1 host:ligand (where ligand can be chiral guest \( G^* \) or water W) competitive binding model (Fig. 5a) as described by the equilibrium equations 1.

\[
\begin{align*}
H + G^* &\overset{K_{HG}}{\rightleftharpoons} H \cdot G^* \\
H + W &\overset{K_{HW}}{\rightleftharpoons} H \cdot W
\end{align*}
\]

where \( K_{HG} \) and \( K_{HW} \) are the equilibrium binding constants of formation of \( H \cdot G^* \) and \( H \cdot W \) complexes, respectively. In the derivation of binding isomers we assume fast exchange between

---

**Figure 1 | Structures of host and guests and their NMR properties.**

(a) Structure of achiral host \( \text{N}_{21,15,19,22,23-} \text{bis}((4-bromobenzyl)-3,5-	ext{di}-t-	ext{butyl}-4-\text{oxocyclohexadien}-2,5-	ext{ylidene})\text{porphyrinogen}, \ H \). Symbols denote the peak assignments in all \( ^1H \) NMR spectra in this study. (b) Structure of chiral guests used; ibuprofen \( 1a \), 2-phenylpropionic acid \( 1b \), N-boc-2-phenylglycine \( 1c \), N-boc-phenylalanine \( 1d \), leucic acid \( 1e \), 2-phenoxypropionic acid methyl ester \( 2a \), valine methyl ester \( 2b \), atropine (hyoscyamine) \( 2c \), menthol \( 3 \) and camphor \( 4 \) (boc = tert-butoxycarbonyl). (c) Typical \( ^1H \) NMR spectra of chloroform-d solution of neat \( H \) (ca. 0.7 mM) and \( H \) (ca. 0.7 mM) with ca. 400 equiv. of selected chiral guests. Key: Green square (pyrrolic NH); enantiotopic CH reporter groups: orange square and circle (hemiquinonoid ortho-H), green circle (N-H pyrrolic \( \beta \)-H) and red circle (N-alkylated pyrrolic \( \beta \)-H). Hash symbol denotes residual chloriform (CHCl\(_3\)). Full \( H \) spectrum assignment is provided in Supplementary Figs S1–S3.
the three states of host \( H \cdot G' \), \( H \) and \( H \cdot W \) (Fig. 5a) and that binding of \((R)\)- or \((S)\)-enantiomer to the host is statistical due to the symmetry of \( H \). Then \( \delta_{NH} \) and \( \Delta \delta \) can be expressed in the form of equations 2 and 3, respectively (for the full derivation see Methods).

\[
\delta_{NH} = p_{HG} \times NH \delta_{HG} + p_{H} \times NH \delta_{H} + p_{HW} \times NH \delta_{HW}
\]

(2)

\[
\Delta \delta = \Delta \delta_{\text{max}} \times \text{e.e.} \times p_{HG}
\]

(3)

where \( p_{HG} \), \( p_{HW} \) and \( p_{H} \) (as defined by equation 5 in Methods) are populations of all the host forms, \( H \) bound to \( G' \) or \( W \) and free \( H \), respectively. Simultaneous fitting of the binding isotherms, \( \delta_{NH} \) and \( \Delta \delta \), with experimental data yields characteristic values, in particular \( K_{HG} \), \( \Delta \delta_{\text{max}} \) and \( \Delta \delta_{\text{max}} \) (Table 1), for each pair of host and chiral guest. These values reveal that \( \Delta \delta_{\text{max}} \) is independent of \( K_{HG} \) and seems to be strongly related to the dynamic structural conformation of each host-guest pair. The value of \( \Delta \delta_{\text{max}} \) (or \( \Delta \delta \) at particular concentration) for different.

**Figure 2** | Effect of guest complexation on symmetry of the host molecule. (a) Scheme of symmetry of host molecule and corresponding shape of NMR spectra. Dashed lines denote axes of \( S_i \) symmetry elements (mirror planes) also identical to prochiral planes. (b) Disruption of host’s symmetry upon complexation with chiral guest and resulting splitting of NMR spectral pattern. (c) Schematic plot of reference system connected with host and orientation of guest within it using \( \phi \) and \( \theta \) coordinates used in the MD simulations. (d) Plot of probability density function \( \mu(\phi) \) as obtained from MD simulations (for \( H \cdot (R)-1a \) and \( H \cdot (S)-1a \) complexes) for phenyl centroid (PhC). Solid red or blue lines show average \( \mu(\phi) \) over \( 5^\circ \). Light red or blue background denotes standard variance of \( \mu(\phi) \). Supplementary Movie 1 depicts MD of the spontaneous release of \((S)-1a\) from the binding site with subsequent binding of \((R)-1a\) (video length in real time is 4.4 ns).

**Figure 3** | \(^1H\) NMR data showing dependency of chemical shift non-equivalency on e.e. (a,b,c) Spectra of \( H \) (0.68 mM, chloroform-\( d \)) in the presence of \( 1a \) (460 equiv.), \( 2a \) (370 equiv.) and \( 4 \) (490 equiv.) at 25 °C, respectively. Hash symbol denotes carbon-13 satellite from chloroform-\( d \) solvent. All spectra were fitted (grey = original spectrum, red = fit) and true resonance frequencies as obtained by fit are indicated by lines under each spectrum. Spectra for \( 1a \) and \( 2a \) were taken as AB second-order strongly coupled quartet. Left part of spectrum (orange circle and square) for guest \( 4 \) was fitted as two independent EF and E’F’ second-order spin systems and right part of spectrum (green circle) as CDX strongly coupled quartet of doublets (for details see \(^1H\) NMR spectra fitting in Supplementary Methods). (d) Plot of dependency of \( \Delta \delta \) on the e.e. value of host’s splitted resonance (denoted by arrows in a-c) showing linear relationship (for all \( R^2 > 0.997 \)). (e) Homonuclear \(^1H\)-\(^1H\) COSY spectrum of N-alkylated pyrrolic \( \beta\)-H resonances of \( H \) (1.42 mM, chloroform-\( d \)) with \((S)-1a\) (200 equiv.) at 25 °C.
combined concentration (in equiv.) of both enantiomers. Parameters see Table 1. (concentrations were evaluated although water concentration did not vary significantly from initial value during titration. For binding constants and other estimations using density functional theory can be obtained) chiral guests studied have a low equilibrium binding constant so that direct confirmation of 1:1 binding stoichiometry using Job’s plot is not feasible as variation in δNH and Δδ was virtually unobservable when construction of a Job’s plot was attempted. However, a competitive 1:1 binding model is supported by the fact that all titration experiments for various chiral guests, QM calculations and MD simulations show good agreement with the suggested model.

chiral guests is, in general, not predictable (only rough estimations using density functional theory can be obtained) and without having a sample of pure enantiomer of analyte (or its known e.e. form) in hand it cannot be determined. Thus, construction of a linear calibration curve as seen in Fig. 3d seems to be a limiting factor for all pro-CSA.

The case of the H · (R)-1a complex was examined in detail using density functional theory (DFT) methods. Three possible conformations of host–guest complexes (Supplementary Data 1–3) and chemical shifts of N-alkylated pyrrolic β-H (for predicted value of Δδmax see Table 1) were calculated (see Supplementary Fig. S4). From data contained in Table 1 it can be seen that all the chiral guests studied have a low equilibrium binding constant so that direct confirmation of 1:1 binding stoichiometry using Job’s plot is not feasible as variation in δNH and Δδ was virtually unobservable when construction of a Job’s plot was attempted. However, a competitive 1:1 binding model is supported by the fact that all titration experiments for various chiral guests, QM calculations and MD simulations show good agreement with the suggested model.

Figure 4 | 1H NMR titration experiments supporting the competitive binding model. (a) Titration of H (0.41 mM, chloroform-d) with water W at 25 °C. (b) Titration of H (0.65 mM, chloroform-d) with (−)-3 at 25 °C. (c) Titration of H (0.74 mM, chloroform-d) with (−)-3 at 25 °C. Before titration (with (−)-3) 150 equiv. of (+)-3 was added to the solution. Bottom panel shows dependency of Δδ on e.e. during the titration. Grey curves were fitted according to a competitive binding model (see Fig. 5) using isotherms expressed in equations 2 and 3. During titrations chiral guest and water concentrations were evaluated although water concentration did not vary significantly from initial value during titration. For binding constants and other parameters see Table 1. (d,e) Spectra taken during titration shown in b,c, respectively (grey = original spectrum, red = fit). Double dagger (‡) in c,e denotes combined concentration (in equiv.) of both enantiomers.

Figure 5 | Competitive binding model and its schematic 1H NMR spectral manifestation. (a) Partial (meso-substituents removed) structural model of host, H, showing its three forms and (at right) an image illustrating the situation in solution. Equilibrium binding constants and populations are indicated. Protons of special interest (Hα, Hβ and Hγ) are also highlighted. (b) Schematic description of pyrrolic NH resonance (Hα) behaviour. (c) Schematic description of N-alkylated pyrrolic β-H resonances (Hα and Hb). Arrows indicate the true resonance frequency of spectral patterns.
Equation 3 has some interesting consequences. It indicates a linear dependence on e.e., as well as on population of the H·G’ complex. The apparent universal linearity of ΔH/Δe.e. plots indicates in turn that a calibration curve can always be constructed from a single measured point obtained from a sample of enantiopure analyte or a sample of independently measured e.e. (considering that a second point required for construction of a calibration curve has zero chemical shift non-equivalence ΔH = 0 for the racemate where e.e. = 0). Also, the value of ΔH can be obtained by measurement only as a non-negative value, hence, only the absolute value of e.e. can be determined and enantiomeric identity remains unknown (for example, measurement gives e.e. = 80% but the excess enantiomeric form is not known). This principal indistinguishableness originates from the symmetry between enantiomeric complexes H·(R)-G’ and H·(S)-G’ and is present for all pro-CSA. It can be overcome only when a small amount of known enantiomer of guest is added. ΔH is either increased when the same enantiomer (as that in excess in solution) is added or decreased when the other enantiomer is added36 (an example can be seen in Fig. 4b,d compared with Fig. 4c,e).

In order to investigate the transfer of chiral information from guest to host molecule the uv/Vis and CD spectra were recorded for most of the H·G’ pairs (Supplementary Figs S26–S32). However, CD signal induced in the structure of H upon binding of G’ is very weak (compared with other systems for example, Mizuno et al.14) and can be detected only when large quantities of G’ are added to solution. This is due to the rigidity of the host’s macrocyclic structure.

Temperature effects on the efficiency of e.e. sensing were explored using variable temperature (VT) 1H NMR measurements (Supplementary Fig. S33). It can be seen that chemical shift non-equivalency ΔH is decreasing with decreasing temperature. This behaviour is rather unusual since the opposite (increasing chemical shift non-equivalency) should be observed with decreasing temperature. However, this is a consequence of the competitive binding model where at depressed temperature water binds to the host more strongly than the chiral guest leading to a decrease of overall ΔH value (which is reflected in equation 3 as a decrease of population of H·G’ complex P_HGC). VT-1H NMR measurements also show that ambient temperature is optimal for e.e. analysis as it gives ΔC close to the maximum values, which is practical from the point-of-view of applications.
also improve our understanding of important chirality principles such as majority rule, chirality transfer between molecules and asymmetric reactions.

**Methods**

**Materials.** Chloroform-d (Cambridge Isotope Ltd.) for $^1$H NMR spectroscopy was mostly used in all reactions. The specific preparations of residual water were removed using 4 Å molecular sieves (Wako Chem. Co.). Before use molecular sieves were activated at 200 °C in vacuum. (R)-ibuprofen (Nacalai Tesque), (S)-ibuprofen (TCI Co. Ltd.), (R)-ibuprofen (TCl Co. Ltd.), (R)-2-phenylpropionic acid (Wako Chem. Co.), (S)-2-phenylpropionic acid (TCl Co. Ltd.), N-boc-i-phenylglycine (TCl Co. Ltd.), N-boc-o-phenylglycine (TCl Co. Ltd.), L-leucic acid (TCI Co. Ltd.), DL-leucic acid (TCI Co. Ltd.), (–)-hyoscyamine (TCI Co. Ltd.), (±)-hyoscyamine (TCl Co. Ltd.), (±)-menthol (Sigma-Aldrich Co. LLC), (–)-camphor (TCl Co. Ltd.), (+)-camphor (Wako Chem. Co.) were used as received.

**Synthesis.** (R)-2-phenylpropionic acid methyl ester and (S)-2-phenylpropionic acid methyl ester were synthesized from (R)-2-phenylpropionic acid and (S)-2-phenylpropionic acid (both Wako Chem. Co.), respectively, through esterification of the corresponding carboxylic acids. 2-Phenylpropionic acid (1.0 g), iodoethane (5 equiv) and potassium carbonate (10 equiv.) were stirred in refluxing acetone for 2 h then acetone was removed under reduced pressure. Crude product was passed through a silica gel column (eluents: dichloromethane, DCM) to yield the final product as a colourless liquid.

D-valine methyl ester and L-valine methyl ester were synthesized from D-valine methyl ester hydrochloride and L-valine methyl ester hydrochloride (both TCI Co. Ltd.), respectively, through neutralization of the corresponding hydrochloride salts. The hydrochlorides were dissolved in pure water and neutralized with an excess of potassium carbonate solution (pH = 12) then the aqueous solution was extracted three times using DCM. DCM fractions were combined then dried over potassium carbonate solution (pH ≤ 5 M, subscript 't' denotes total concentration) and subsequently titrated into water (pH ≤ 5 M) from 500–1,000 equiv. of the host molecule $H$.

**General spectrometric methods.** $^1$H NMR spectra were obtained at 25 °C (unless stated otherwise) using an AL300BX spectrometer (JEOL) operating at 300.4 MHz. When variable temperature measurements (VT-$^1$H NMR) were performed the appropriate JEOL temperature control unit (temperature fluctuation c.a. 0.5 °C) was used. Before the experiment, host $H$ was dissolved in chloroform-d (usually ca. 0.6 mM) in an NMR tube and subsequently ligand (chiral guest or water) was added/injected directly into the NMR tube. Molar ratio of ligand and host was carefully determined by integration of the corresponding peaks in spectra. All spectra were referenced to tetramethylsilane. Number of scans typically used for (ca. 0.6 mM of host molecule $H$): 16–32 scans for samples up to 500 equiv. of guest $G$; 32–128 scans for samples containing from 500–1,000 equiv. of $G$; 128–512 scans for samples containing in excess of 1,000 equiv. of $G$.

Electro-nuclear experiments and CD spectra were measured using a 1.820 CD spectrophotometer (JASCO) at room temperature (ca. 25 °C). When titration experiments were performed a chloroform-d solution of host $H$ (ca. $7 \times 10^{-3}$ M) was prepared and 0.17 ml of this solution was placed in a 1-mm pathlength quartz cell. In order to maintain host concentration constant chloroform-d stock solutions of host $H$ with various chiral guests were prepared ([H], ca. $3 \times 10^{-5}$ M, [G]), up to ca. 1.5 M, subscript 't' denotes total concentration) and subsequently titrated into the host solution by using a syringe (0–600 µL). Then UV/Vis and CD spectra were recorded simultaneously.

**Derivation of competitive binding model.** From equilibrium equations 1, the equilibrium binding constants and mass balance equations can then be expressed as equations 4.

$$K_{HGC} = [HG^+] / ([H][G'])$$

$$K_{HGW} = [HW] / ([H][W])$$

$$K_{GWC} = [G^+] / ([G][W])$$

$$[G'] = [G^+] + [HG']$$

$$[W] = [W] + [HW]$$

$$[H] = [H] + [HG'] + [HW]$$

(4)

where $[H]$, $[G']$, and $[W]$ denote total concentrations of $H$, $G'$ and $W$, respectively. $[H]$, $[G']$ and $[W]$ denote concentrations of free $H$, $G'$ and $W$, respectively. $[HG']$ and $[HW]$ are concentrations of the corresponding complexed forms. A solution (especially values of $[HG']$, $[H]$ and $[HW])$ can be obtained using either numerical Newton iterative methods or rather complex but exact analytic solutions (see Supplementary Methods). Then populations of all the host forms (one free and two bound) can be expressed in the form of equations 5.

$$P_{HG} = \frac{[HG^+]}{[H][G']} \quad P_H = \frac{[H]}{[H]} \quad P_{HW} = \frac{[HW]}{[H][W]}$$

(5)

Because of fast exchange between the three states of host $H$–$G'$, $H$ and $H$–$W$ (Fig. 5a) the resulting chemical shift of pyrrolic NH, $\delta_{HG}$, takes the form of a population averaged chemical shift of the respective states (Fig. 5b) and can be expressed as equation 2.

Similarly, a $\Delta$ binding isotherm (describing chemical shift non-equivalence) can be constructed under the following assumptions. For reasons of symmetry, we assume that the binding of (R)- or (S)-enantiomers to the host is statistical. When a chiral guest $G'$ binds to the host the probability that the site is occupied by (R)- or (S)-enantiomer can be determined as a function of $e$ by equations 6.

$$p_e(e) = \frac{[G^+]}{[G^+] + [G']} = 1 - e$$

(6)

where $[G^+]$ and $[G']$ are total concentrations of (R)- and (S)-enantiomer, respectively. As mentioned above, the $^1$H–$^1$H COSY spectrum of the pyrrolic $\beta$-H resonance indicates that both split peaks must be at the same pyrrolic ring.

Moreover, mirror symmetry of the host’s binding site and the intrinsic saddle shape of the host’s macrocycle causes a preference for certain mutual host–guest conformations (see MD study, Figs. 2c,d) and therefore does not permit isotropic rotation of guest at the binding site. Thus, when chiral guest is present, an asymmetric shielding effect at (for example) pyrrolic $\beta$-H positions occurs. In this situation, the chemical shift of respective pyrrolic $\beta$-H is given by equations 7.

$$\delta_{HG} = \delta_{HG} + \delta_{HG}e(e) + \delta_{HG}e(e)$$

(7)

$$\delta_{HG} = \delta_{HG} + \delta_{HG}e(e) + \delta_{HG}e(e)$$

(8)

where $\delta_{HG}$ and $\delta_{HG}$ are chemical shifts of protons $H$ and $H$, respectively. As mentioned above, the $^1$H–$^1$H COSY spectrum of the pyrrolic $\beta$-H resonance indicates that both split peaks must be at the same pyrrolic ring.

The same derivation of binding isotherm applied to $^1$H–$^1$H COSY spectrum of the pyrrolic $\beta$-H also applies to hemiquinonoid resonance $\delta_{HG}$ of pyrrolic $\beta$-H with (R)- or (S)-enantiomers implies that $\delta_{HG}$ and $\delta_{HG}$ represent the same quantity for $H$, $H$, $H$, and $H$, respectively. For convenience we let introduce $\Delta\delta_{HG} = \delta_{HG} - \delta_{HG}$ as a measure of the maximum chemical shift difference between $H$ and $H$, protons as a characteristic value for each host–guest combination. When the competitive exchange of $G'$ with water $W$ is taken into account the overall chemical shift $\delta_{HG}$ and $\delta_{HG}$ of protons $H$ and $H$, respectively, will be described as population weighted chemical shifts (Fig. 5c) of respective type of host equations by 8.

$$\Delta\delta_{HG} = \delta_{HG} - \delta_{HG}$$

(8)

where $\delta_{HG}$ and $\delta_{HG}$ are chemical shifts of N-alkylated pyrrolic $\beta$-H corresponding to free host $H$ and host complexed to water $W$, respectively.

References

1. Hutt, A. J. in Introduction to the Principles of Drug Design and Action (ed. Smith, H. J.) Chapter 5 (CRC Press, Taylor & Francis, 2006).

2. Comprehesive organic catalysis, Vols 1 to 3 (eds Jacobsen, E. N., Pfaltz, A. & Yamamoto, H.) (Springer, 1999).

3. Heitbaum, M., Glorius, F. & Escher, I. Asymmetric heterogeneous catalysis. Angew. Chem. Int. Ed. 45, 4732–4762 (2006).

4. Yoon, T. P. & Jacobsen, E. N. Privileged chiral catalysts. Science 299, 1691–1693 (2003).

5. Kondo, T., Oyama, K. & Yoshiida, K. Chiral molecular recognition on formation of a metalloalloxan: a supramolecular metal complex pigment from blue flowers of Salvia patens. Angew. Chem. Int. Ed. 40, 894–897 (2001).

6. Ishi-i, T., Crego-Calama, M., Timmerman, P., Reinholdt, D. N. & Shinkai, S. Elaborate formation of a dynamic hydrogen-bonded assembly based on the chiral memory concept. J. Am. Chem. Soc. 124, 14631–14641 (2002).

7. Maeda, K., Morino, K., Okamoto, Y., Sato, T. & Yashima, E. Mechanism of helix induction on a stereoregular poly((carboxyethyl)acetylene) with chiral amine and memory of the macromolecular helicity assisted by interaction with achiral amines. J. Am. Chem. Soc. 126, 4329–4342 (2004).

8. Scarso, A., Shivanuk, A., Hayashida, O. & Rebek, J. Jr. Asymmetric environments in encapsulation complexes. J. Am. Chem. Soc. 125, 6239–6243 (2003).
11. Kubo, Y., Maeda, S., Tokita, S. & Kubo, M. Colorimetric chiral recognition by a molecular sensor. Nature 382, 522–524 (1996).

12. Zhu, L. & Anslyn, E. V. Facile quantification of enantiomeric excess and concentration with indicator-displacement assays: an example in the analyses of α-hydroxycids. J. Am. Chem. Soc. 126, 3676–3677 (2004).

13. Tsukahara, K. et al. Visual enantiomeric recognition using chiral phenolphthalein derivatives. Org. Lett. 3, 4071–4073 (2001).

14. Mizuno, Y., Aida, T. & Yamaguchi, K. Chirality-memory molecule: crystallographic and spectroscopic studies on dynamic molecular recognition events by fully substituted chiral porphyrins. J. Am. Chem. Soc. 122, 5278–5285 (2000).

15. Nieto, S., Lynch, V. M., Anslyn, E. V., Kim, H. & Chin, J. High-throughput screening of identity, enantiomeric excess, and concentration using MLCT transitions in CD spectroscopy. J. Am. Chem. Soc. 130, 9232–9233 (2008).

16. James, T. D., Sandanayake, K. R. A. S. & Shinkai, S. Chiral discrimination of monosaccharides using a fluorescent molecular sensor. Nature 374, 345–347 (1995).

17. Matsuura, M. et al. High-throughput screening by using a blue-fluorescent antibody sensor. Angew. Chem. Int. Ed. 42, 5984–5987 (2003).

18. Mei, X. & Wolf, C. Enantioselective sensing of chiral barboxylic acids. J. Am. Chem. Soc. 126, 14736–14737 (2004).

19. Yu, S. & Pu, L. Pseudoenantiomeric fluorescent sensors in a chiral assay. J. Am. Chem. Soc. 132, 17698–17700 (2010).

20. Mierta, G. et al. Electrochemical method for the determination of enantiomeric excess of binol using redox-active boronic acids as chiral sensors. J. Am. Chem. Soc. 132, 8903–8905 (2010).

21. Rekharsky, M. V. et al. Chiral recognition in cucurbituril cavities. J. Am. Chem. Soc. 128, 14871–14880 (2006).

22. Borovkov, V. V., Lintuluoto, J. M. & Inoue, Y. Supramolecular chirogenesis in zinc porphyrins: mechanism, role of guest structure, and application for the absolute configuration determination. J. Am. Chem. Soc. 123, 2979–2989 (2001).

23. Kubo, Y. et al. Chirality-transfer control using a heterotopic zinc(II) porphyrin dimer. J. Am. Chem. Soc. 123, 12700–12701 (2001).

24. Li, X., Tanasova, M., Vasileiou, C. & Borhan, B. Fluorinated porphyrin tweezers: a powerful reporter of absolute configuration for erythro and three diols, amino alcohols, and diamines. J. Am. Chem. Soc. 130, 1885–1893 (2008).

25. Hwang, X. et al. Absolute configurational assignments of secondary amines by CD-sensitive dimeric zinc porphyrin host. J. Am. Chem. Soc. 124, 10320–10335 (2002).

26. Dale, J. A., Dull, D. L. & Mosher, H. S. α-Methoxy-α-trifluoromethylphenylacetic acid, a versatile reagent for the determination of enantiomeric composition of alcohols and amines. J. Org. Chem. 34, 2543–2549 (1969).

27. Parker, D. NMR determination of enantiomeric purity. Chem. Rev. 91, 1441–1457 (1991).

28. Wenzel, T. J. & Chisholm, C. D. Assignment of absolute configuration using chiral reagents and NMR spectroscopy. Chirality 23, 190–214 (2011).

29. Duddeck, H. & Gömez, E. D. Chiral recognition of ethers by NMR spectroscopy. Chirality 21, 51–62 (2009).

30. Schwenningen, R. et al. Metal complexes of a biconcave porphyrin with D2 structure - versatile chiral shift agents. Chem. Eur. J. 7, 2676–2686 (2001).

31. Williams, T., Pitcher, R. G., Bommer, P., Gutzwiller, J. & USokókó, M. Diastereomeric solute-solute interactions of enantiomers in achiral solvents. Nonequivalence of the nuclear magnetic resonance spectra of racemic and optically active dhidroxyquinine. J. Am. Chem. Soc. 91, 1871–1872 (1969).

32. Dobashi, A., Saito, N., Motoyama, Y. & Haras, S. Self-induced nonequivalence in the association of α- and 1-amino acid derivatives. J. Am. Chem. Soc. 108, 307–308 (1986).

33. Luchinat, C. & Roelens, S. Enantiomeric purity determination of 1,2-diols through NMR spectroscopy without chiral auxiliaries. J. Am. Chem. Soc. 108, 4873–4878 (1986).

34. Feringa, B. L., Smaardijk, A. & Wynberg, H. Simple 31P NMR method for the determination of enantiomeric purity of alcohols not requiring chiral auxiliary compounds. J. Am. Chem. Soc. 107, 4798–4799 (1985).

35. Shundo, A., Labuta, J., Hill, J. P., Ishihara, S. & Ariga, K. Nuclear magnetic resonance signaling of molecular chiral information using an achiral reagent. J. Am. Chem. Soc. 131, 9494–9495 (2009).

36. Labuta, J. et al. Chirality sensing by nonchiral porphines. Chem. Eur. J. 17, 3558–3561 (2011).

37. Merlet, D., Loewenstein, A., Smadja, W., Courtieu, J. & Lesot, P. Quantitative description of the facial discrimination of molecules containing a prochiral group by NMR in a chiral liquid crystal. J. Am. Chem. Soc. 120, 963–969 (1998).