ACCOUNTS of chemical research

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Chiral Sensing by Nonchiral Tetrapyrroles

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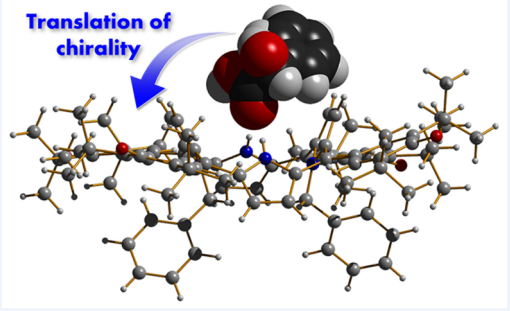
CONSPECTUS: Enantiomeric excess (ee) is a measure of the purity of an enantiomer of a chiral compound with respect to the presence of the complementary enantiomer. It is an important aspect of chemistry, especially in the fields of pharmaceuticals and asymmetric catalysis. Existing methods for determination of enantiomeric excesses using nuclear magnetic resonance (NMR) spectroscopy mostly rely on special chiral reagents (auxiliaries) that form two or more diastereomeric complexes with a chiral compound. As a result of this, the NMR spectrum of each enantiomer is different, allowing the determination of enantiomeric excess.



In this Account, we describe a molecular design process that has allowed us to prepare prochiral solvating agents for NMR determination of ee of a wide variety of analyte types. At the outset of this work, we initially encountered the phenomenon of NMR peak splitting in the oxoporphyrinogen (**OxP**) host component of a supramolecular host−guest complex, where the extent of the splitting is apparently proportional to the guests' ee. Upon closer examination of the mechanism of action, it was found that several complicating factors, including prototropic tautomerism, macrocyclic inversion (ring-flipping), and 1:2 host−guest stoichiometry, obstruct potential applications of **OxP** as a chiral solvating agent. By considering the molecular conformation of the **OxP** host, a saddle-shaped calix[4]pyrrole, we moved to study the tetraphenylporphyrin (**TPP**) dication since it has a similar form, and it was found that it could also be used to probe ee. However, although **TPP** does not suffer from disadvantageous tautomeric processes, it is still subject to macrocyclic inversion and has the additional serious disadvantage of operating for ee sensing only at depressed temperatures. The intrinsic disadvantages of the **OxP** and **TPP** systems were finally overcome by covalently modifying the **OxP** chromophore by regioselective N-alkylation at one face of the molecule. This procedure yields a host **Bz_2OxP** that undergoes 1:1 host−guest interactions, cannot be protonated (and so does not suffer drawbacks due to tautomeric processes), and can interact solely through hydrogen bonding with a much wider range of analyte types, including acids, esters, amines (including amino acid derivatives), and ketones, for the determination of their ee at room temperature.

Chiral sensing, in this case, can be understood by considering the breakdown of the host's symmetry when it interacts with a chiral guest under fast exchange. Furthermore, chirality discrimination (i.e., which is the major enantiomer in a sample) can be performed by addition of a small amount of one of the known enantiomers. Adaptation of a symmetrical molecule for ee sensing presents certain intrinsic advantages, including identical binding constants of each enantiomer. Our results indicate that other symmetrical molecules might also be useful as NMR probes of enantiopurity. These systems could provide insights into important chirality principles such as majority rule, intermolecular chirality transfer, and asymmetric reactions. The **Bz_2OxP** system is also of note from the point of view that it does not rely on the formation of diastereomers.

1. INTRODUCTION

A molecule is chiral if it cannot be superimposed onto its mirror image. Therefore, it has two distinguishable forms with the same chemical composition. In chemistry, these two forms of molecule are usually referred to as enantiomers.[1] In nature, chirality is largely due to molecular chirality[2] (i.e., amino acids except glycine, carbohydrates), although helical structures also possess chirality.[3] Asymmetry has also become an important feature in manmade materials,[4] especially pharmaceuticals,[5] where absolute determination of a material's chiral properties is essential to make a precise characterization of its activity, including any hazards. The often-quoted example of this is the pharmaceutically active chiral compound thalidomide, whose

Received: January 6, 2015
Published: March 3, 2015

DOI: 10.1021/acs.accounts.5b00005
Acc. Chem. Res. 2015, 48, 521−529

enantiomers act as a sedative ((*R*)-enantiomer) and a teratogen ((*S*)-enantiomer).[6] Thalidomide also racemizes *in vivo*, actually precluding its originally intended use even if it were to be administered as its pure (*R*)-form.

Certain parameters can be used to describe the chirality of a molecule, including its absolute configuration (i.e., spatial arrangement of atoms) and its related optical rotatory dispersion. In addition, in mixtures of enantiomers of the same chiral compound, the relative proportions of the enantiomers present is denoted by the enantiomeric excess (ee), which is defined as ee = ([R] − [S])/([R] + [S]), where [R] and [S] are concentrations of (*R*)- and (*S*)-enantiomer, respectively. While absolute configuration is the primary parameter for the structure of a chiral compound, its ee can be considered a measure of its purity. Values of ee range from −1 to 1 and are usually reported in percent. An equimolar mixture of enantiomers (a racemic mixture) possesses an ee of 0%, whereas either pure enantiomer has an absolute ee value equal to 100%. The determination of ee has become an increasingly important subject given the increasing use of asymmetric compounds in pharmaceuticals, and this is largely due to the differing activities between pairs of enantiomers and the requirement that their properties be well-documented prior to use. Additionally, there are other aspects of ee, including the origin of homochirality in natural systems[7] and the monitoring of asymmetric reactions,[8] whose studies remain challenging.

There are several means to determine the ee of a substance (analyte), including by using high-performance liquid chromatography (HPLC) on chiral media[9] or by applying nuclear magnetic resonance (NMR) spectroscopy in conjunction with a suitable chiral solvating reagent[10] or chiral derivatizing reagent.[11] It has been considered that NMR spectroscopic methods cannot be used directly to sense chirality or determine ee except in the presence of a chiral reagent or, for example, because of self-association behavior of a chiral analyte where diastereomeric species can be detected and quantified in solution by measurement of the relative intensities of the relevant peaks in the NMR spectrum. In contrast, in our work,[12−16] we have studied the possibility of using NMR spectroscopy to access information regarding molecular chirality by applying an *achiral* reagent. We use the term pro-chiral solvating agent (pro-CSA) for such reagents since they operate under conditions of fast exchange between a free form and bound to chiral analyte similarly to standard chiral solvating agents (CSA). Pro-chirality of the reagent means that upon formation of noncovalent host−guest complexes the chiral information is transferred from chiral guest to the originally achiral host, which becomes chiral. We have found that an achiral host molecule can be used to probe ee without formation of diastereomers by observing the degree of splitting of the relevant NMR peaks of the achiral host. It also proved to be possible to use this phenomenon to develop a protocol for rapid estimation of the ee of a wide range of differently functional compounds. In this Account, we describe the scientific process leading to the development of these novel pro-chiral solvating agents (based on tetrapyrrole macrocycles) and also discuss the inherent advantages and disadvantages of their use for measuring enantiomeric excesses.

2. PORPHYRINS FOR CHIRAL SENSING

Porphyrins comprise a significant class of compounds with important catalytic and photochemical properties. Among these, some synthetic porphyrins have been proposed to be sensors of chirality due to their strong optical absorptions, which enable the application of circular dichroism (CD) spectrophotometry. Important contributions in this field have come from Inoue,[17] Borovkov,[18] Nakanishi,[19] and Berova.[20,21] It is apparent from those studies that a site for molecular recognition should be present within the structure of any prospective host molecule. We chose the molecule 5,10,15,20-*tetrakis*(3,5-di-*t*-butyl-4-oxocyclohexa-2,5-dienylidene)-porphyrinogen[22] (**OxP**) as a starting point since it possesses the requisite guest binding site(s) and is incidentally highly colored (we were also interested in any colorimetric responses[23]). Also, apart from the large body of work that has been published regarding the use of porphyrins for analyses of chiral compounds,[24] reports of their use for the same purpose in conjunction with NMR spectroscopy are rare.

3. TRANSFER OF CHIRAL INFORMATION IN THE OXOPORPHYRINOGEN SYSTEM

The chemical and X-ray crystal structures of **OxP**[25] are shown in Figure 1a,b, illustrating the saddle-like form of the macrocycle. Guest species interact with **OxP** through hydrogen bonding at the pyrrolic NH groups. Initially, we investigated the spectroscopic properties of **OxP** in the presence of mandelic acid.[12] In NMR spectra, we observed that addition of an excess (8.8 equiv) of mandelic acid (**MA**) racemate into a solution of

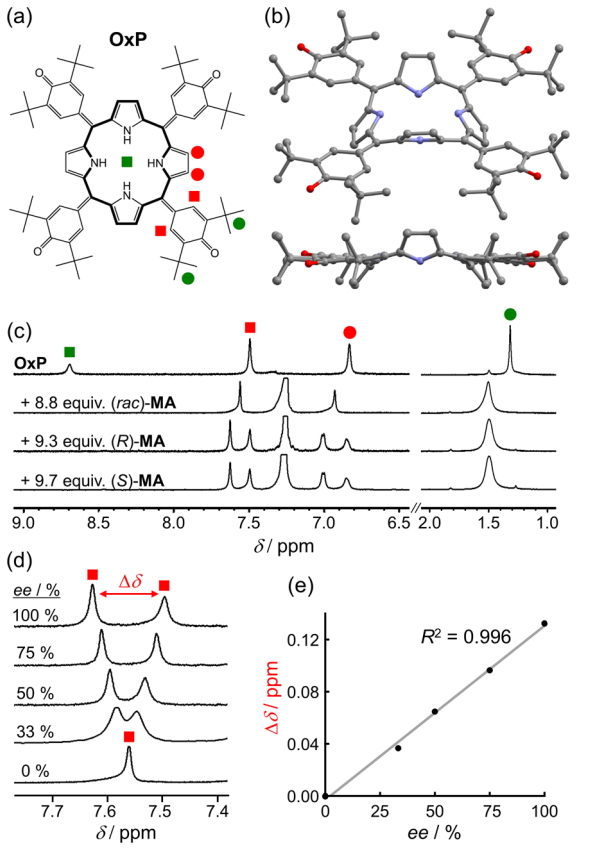


Figure 1. (a) Chemical structure of **OxP**. (b) X-ray structure of **OxP**.[25] (c) ^{1}H NMR spectra of **OxP** (∼1.4 mM in CD_2Cl_2 at 25 °C) with various enantiomeric compositions of **MA** (as indicated). (d) Partial ^{1}H NMR spectra of **OxP** (quinonoid protons) in the presence of **MA** with different ee values. (e) Correlation between chemical shift difference Δδ and ee values. Adapted from ref 12. Copyright 2009 American Chemical Society.

DOI: 10.1021/acs.accounts.5b00005
Acc. Chem. Res. 2015, 48, 521−529

OxP leads to downfield shifts of peaks due to the quinonoid alkene protons, *t*-butyl protons, and pyrrolic β-proton and disappearance of the peak due to pyrrolic NH (Figure 1c) caused by formation of an H-bonded complex of **OxP·MA**. In contrast, in the case of 100% (*R*)-enantiomer, quinonoid and pyrrolic β-proton resonances in **OxP** are split into two peaks and shifted downfield (Figure 1c). The extent of splitting did not change even after a large excess (~30 equiv) of **MA** had been added, indicating that the two peaks do not correspond to those of the H-bonded complex and free **OxP**. Similar observations were made for pure (*S*)-enantiomer (Figure 1c). Thus, guest chirality causes the unusual peak splitting. This was the primary observation of the peak-splitting phenomenon based on the chirality of a guest in the **OxP** system. Subsequently, we applied a variety of nonracemic mixtures of mandelic acid in this system, revealing that peak separations are dependent on the ee (Figure 1d) of the analyte. Surprisingly, plots of chemical shift differences $\Delta\delta$ for the split peaks against the respective ee values gave a linear correlation (coefficient of $r^2 = 0.996$; Figure 1e). This indicates that information regarding guest chirality is transferred to the host and manifests itself as splitting of the NMR signals of the achiral host. At the time, this was the first demonstration of the utilization of an achiral host for detection of ee by observing the magnitude of splitting of NMR resonances of the host. This differs substantially from the case for chiral hosts (shift reagents), where the ee is determined from the ratio of areas of resonances due to diastereomeric host–guest complexes.[26] Since **OxP** is a highly colored species, it is also possible to assess physical changes using spectroscopic techniques, including electronic absorption (UV–vis) and circular dichroism (CD). When excesses of (*R*)- or (*rac*)-**MA** were added to CH_2Cl_2 solutions of **OxP**, color changes of the solutions from purple to red were observed (Figure 2a). UV–vis spectra of these solutions were identical (Figure 2a, red-lined spectrum) whereas CD spectra of the same solutions showed substantial ICD signals depending on the chirality of the guest (Figure 2b). UV–vis titration studies using (*R*)-**MA** at room temperature revealed a gradual intensification of a new absorption band at 789 nm with an accompanying decrease in intensity and splitting of the band originally due to free **OxP**. Curve fitting of a plot of the absorbance change at 789 nm against (*R*)-**MA** concentrations based on a 1:2 stoichiometry (Figure 2c) leads to an excellent correlation coefficient ($r^2 >$ 0.999; Figure 2d) with values of $K_1 = 1.3 \times 10^3$ M^{-1} and $K_2 = 5.3 \times 10^3$ M^{-1} for the first and second apparent binding constants, respectively, which indicates a substantial cooperativity α of binding due to $\alpha = 4K_2/K_1 = 16.3 \gg 1$. Note that the UV–vis spectrum of the complex of **OxP**·(*R*)-**MA** (Figure 2a) is different from that found for hydrogen-bonding **OxP**·anion complexes.[23] Thus, formation of the complex should involve a different mechanism of binding, related to the acidity of the guest since identical spectra are obtained in the presence of organic acids such as methanesulfonic acid. Splitting of the Soret band in the UV–vis spectra of **OxP** complexes with acids strongly indicates symmetry breaking from D_{2d} to D_2, leading to our assignment of the changes as being due to prototropic tautomerization of **OxP** to a porphodimethene structure bearing phenol and hemiquinone substituents in a 5,15-type structural configuration. Existence of the protonated porphodimethene form (Figure 3a,b) suggests that the host–guest complex must be based partly on electrostatic interactions between anions of **MA** and cationic moieties of the protonated form (tautomer) of **OxP**. This is a similar situation to what has previously been observed for complexes of tetraphenylporphyrin dications and other organic anions.[27]

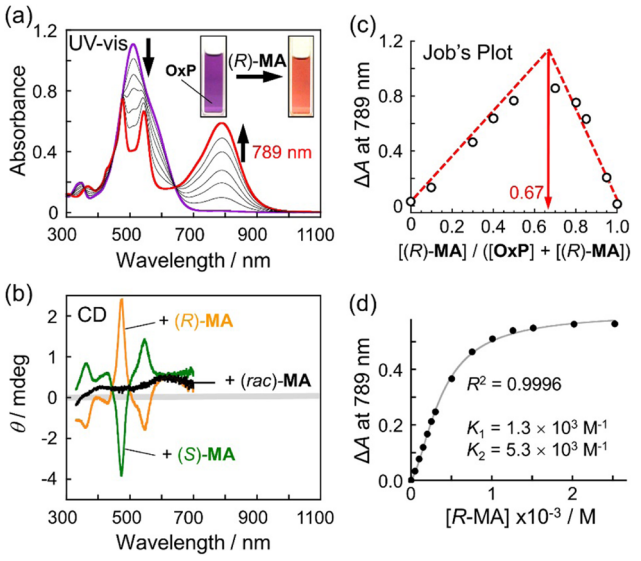


Figure 2. (a) UV–vis spectral changes observed during titration of **OxP** (10^{-5} M in CH_2Cl_2) with (*R*)-**MA**. (b) CD spectra of **OxP** (10^{-5} M in CH_2Cl_2) in the presence of excess (*R*)-, (*S*)-, or (*rac*)-**MA**. (c) Job's plot for complexation of **OxP** and (*R*)-**MA** at constant total concentration (10^{-3} M) showing 1:2 **OxP·MA** stoichiometry. (d) Profile of absorbance change at 789 nm against concentration of (*R*)-**MA** obtained from data in panel (a). Gray line shows fitted 1:2 binding isotherm. Adapted from ref 12. Copyright 2009 American Chemical Society.

4. COMPLICATIONS IN THE OXOPORPHYRINOGEN SYSTEM

Prototropic tautomerism is a complicating factor, and it obstructs rationalization of the mechanism of ee sensing in **OxP**.[12] Matters are further obscured by the possibility of macrocyclic inversion between saddle conformations caused by a ring-flipping process.[14] Protonation causes changes in the structure of **OxP** and di-*tert*-butylated oxocyclohexadienylidene groups at its *meso*-positions interconvert reversibly with their phenol forms (Figure 3b). **OxP** has D_{2d} symmetry until protonation with 2 equiv of mandelic acid **MA** ($pK_a = 3.41$), which leads to its adoption of D_2 symmetry and concomitant chirality (Figure 3b) so that $\mathbf{OxPH_2}^{2+}$ exists as dynamically interchangeable enantiomers, (+)-$\mathbf{OxPH_2}^{2+}$ and (−)-$\mathbf{OxPH_2}^{2+}$. Both ring-flip and tautomerism mediate interchange between these enantiomers (Figure 3c). This interconversion between enantiomers resembles a reported chiral memory system.[27]

We investigated the strength of acid–base interactions, including temperature dependency, in the host–guest complex $\mathbf{OxPH_2}^{2+}\cdot\mathbf{2MA}^{1-}$ by titrating in conjunction with ^{1}H NMR spectroscopy (Figure 4). $\mathbf{OxPH_2}^{2+}\cdot\mathbf{2MA}^{1-}$ also involves hydrogen bonding between the NH groups of **OxP** and carboxylate groups of mandelic acid. Tautomerism and ring-flip exhibit interesting and nontrivial dependencies on temperature and the respective concentrations of host **OxP**, guest **MA**, and water (**W**). In the presence of 6.1 equiv of (*R*)-**MA**, all **OxP** molecules are effectively doubly protonated over the temperature range studied, and free or monoprotonated forms were not observable. The dynamic behavior of **OxP** leads to several notable spectral features. At the temperatures studied, fast

DOI: 10.1021/acs.accounts.5b00005
Acc. Chem. Res. 2015, 48, 521–529

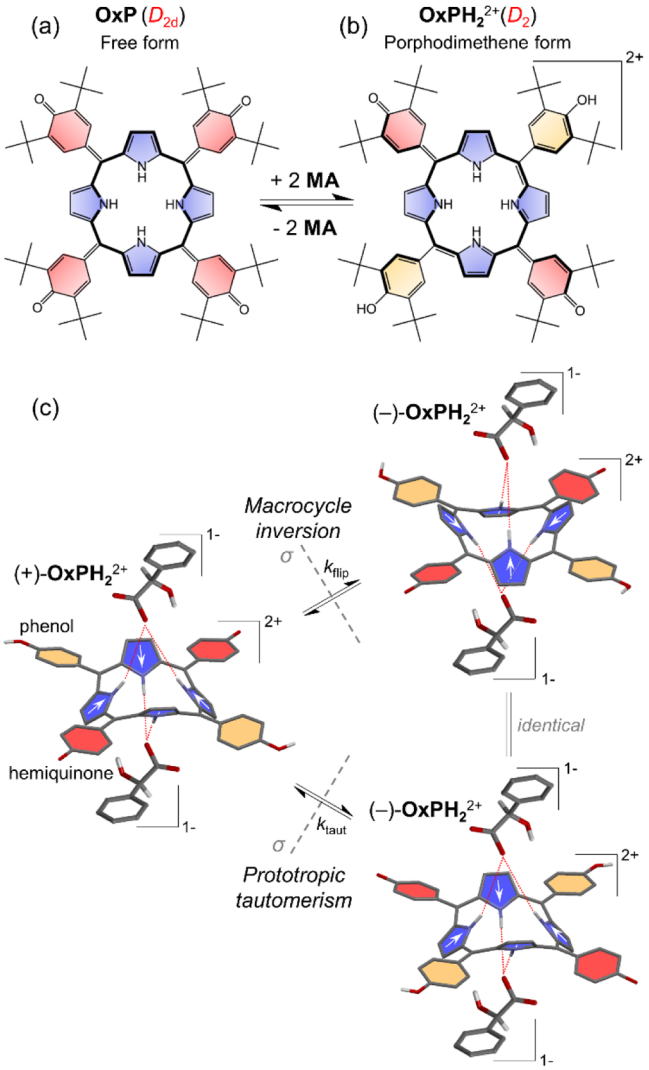


Figure 3. 1:2 host−guest complexation (diprotonation) process in **OxP**·**MA** system and related reduction in symmetry. (a) Free **OxP**. (b) Porphodimethene of **OxP**. (c) Interconversion processes between (+)-$OxPH_2^{2+}$ and (−)-$OxPH_2^{2+}$ enantiomers: macrocyclic inversion (ring-flip) and prototropic tautomerism. Note that (−)-$OxPH_2^{2+}$ structures on the right side are identical. Reprinted from ref 14. Copyright 2014 American Chemical Society.

exchange occurs of the **MA** counterions (with **MA** in bulk) bound at the two binding sites of **OxP**, with **OxP** maintained in a diprotonated state. From DFT calculations, phenol groups of diprotonated **OxP** are predominantly at opposing 5,15-positions (i.e., trans, as shown in Figure 3b) of the macrocycle. There is a difference of 3.5 kcal/mol (at 25 °C) in the free energies of the cis (5,10-isomer) and trans conformations, indicating prevalence of the trans form, with only 0.3% in the cis state (the cis form is considered to be a less stable intermediate). Exponential dependencies of the rate of prototropic tautomerism k_{taut} on temperature (for various **MA** concentrations) are shown in Figure 5a (top), whereas Figure 5b shows the dependencies of k_{taut} on **MA** concentration (at different temperatures). k_{taut} increases with increasing mandelic acid concentration [**MA**] toward saturation, indicating that **MA** anions actively participate as proton carriers in the tautomeric processes of **OxP**. Interestingly, at low temperatures (−56 °C), conversion between the enantiomers (+)-$OxPH_2^{2+}$ and (−)-$OxPH_2^{2+}$ is suspended due to the arrest of both tautomerism and ring-flipping. Under these conditions, the proton NMR spectrum can be assigned as that of two stable diastereomers formed by complexation of (*R*)-**MA** with (+)-$OxPH_2^{2+}$ and (−)-$OxPH_2^{2+}$. Thus, the chiral acid guest can be seen to be operating as a chiral solvating agent capable of differentiating the two enantiomers of $OxPH_2^{2+}$. The spectrum reveals that they are similarly abundant.

This investigation of dynamic processes of **OxP** essentially amounts to our use of the chiral guest as a probe of prototropic tautomerism and macrocylic inversion. Ultimately, the chirality of the guest permitted the observation of the two enantiomers of the **OxP** macrocycle through the formation of diastereomers, which had not been previously achieved. However, despite the interesting scientific aspects of **OxP** hosting of chiral acid guests, we finally had to conclude that complicating factors in the **OxP** system, including tautomeric processes, unsymmetrical substitution, and ambiguous peak assignments, made determination of an unequivocal mechanism for ee inconvenient.

5. DEVELOPMENT OF PROCHIRAL SOLVATING AGENTS

Although the **OxP** system presented a rather difficult introduction to chiral sensing in these compounds, it allowed us to develop prochiral solvating agents that may be of some practical application. The first step in the development entailed consideration of the structural components that should be requirements for sensing of ee by saddle-shaped tetrapyrroles. For **OxP**, we considered that the presence of hydrogen bonding sites, i.e., pyrrolic NHs, is indispensable, although protonation-driven tautomerization is not a desirable feature since it involves large variation in the structure of the host. Since these tautomeric processes are driven by the presence of electronegative atoms at the molecule's periphery, we dispensed with these and continued our investigation by assessing the utility of dications of simple tetraalkyl- and tetraphenylporphyrins. Thus, *tetrakis*(*t*-butyl)porphyrin (**T*t*BuP**[28]) and tetraphenylporphyrin (**TPP**[29]) were prepared and purified according to literature procedures and then studied for their use as probes of ee.[13] The use of these molecules effectively removes any possibilities involving tautomeric processes and consequent variations in molecular symmetry.

In solution, there are no apparent interactions between **TPP** and an excess of a chiral carboxylic acid (2-phenoxypropionic acid, **PA**) at 25 °C. However, on cooling (to −32.5 °C, the optimum temperature for **TPP**), distinct variations occur in the ^{1}H NMR spectrum of the solution (Figure 6a) as a result of the double protonation of **TPP**, which is corroborated by changes in the UV−vis spectrum (Figure 6b). Additionally, solutions of **TPP** and **PA** exhibited a minimal monosignated Cotton effect in CD spectra also on cooling (Figure 6c). The latter suggests a small macrocyclic distortion on chiral guest binding. Regardless, the Cotton effect is so weak that it is unlikely to be connected with the significant nonequivalence of the NMR chemical shifts of the pyrrolic *β*-H of **TPP**. In solutions of **TPP** with (*R*)-**PA** at −32.5 °C, *ortho*-phenyl proton and pyrrole proton resonances are split into two sets of symmetrical signals (Figure 6d). Extent of the splitting ($\Delta\delta$) depends on the ee of the chiral carboxylic acid counteranion/guest. Similarly to that for **OxP**, plots of $\Delta\delta$ against ee reveal the linear relationship, $\Delta\delta = \Delta\delta_{max} \times$ ee (Figure 6e), where $\Delta\delta_{max}$ is characteristic of a particular acid (for a derivation of this expression, see ref 13). Again, in

DOI: 10.1021/acs.accounts.5b00005
Acc. Chem. Res. 2015, 48, 521−529

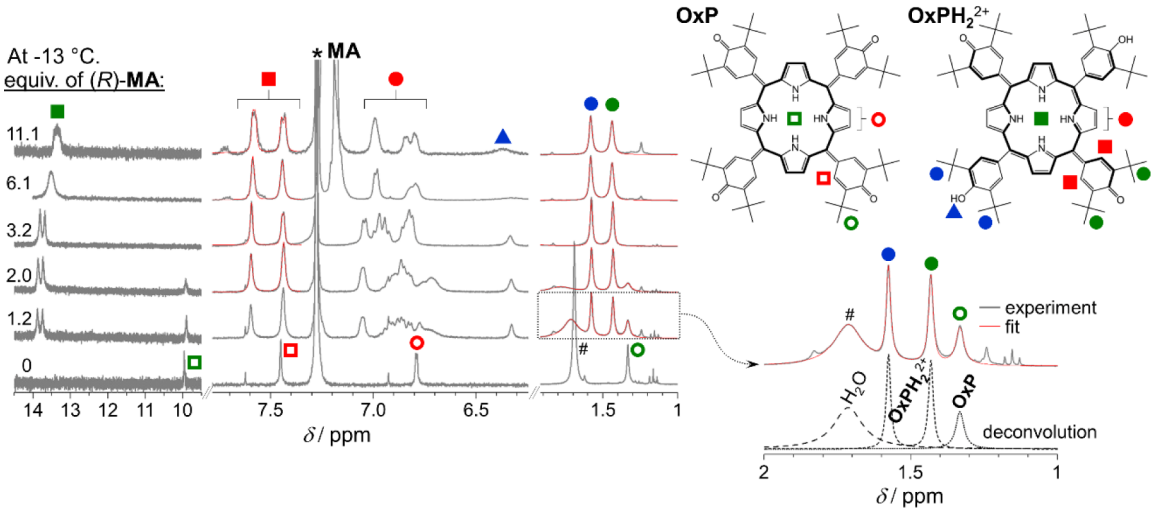


Figure 4. ^{1}H NMR spectra (gray, measured; red, fit) at constant temperature (−13 °C) during titration of **OxP** (1.5 mM, chloroform-*d*, ca. 23 equiv water) with (*R*)-**MA** (number of equivalents is indicated at each spectrum). Asterisk (*) and hash mark (#) denote residual chloroform and water, respectively. Spectral assignment is shown at the right. Expansion illustrating spectral deconvolution. Reprinted from ref 14. Copyright 2014 American Chemical Society.

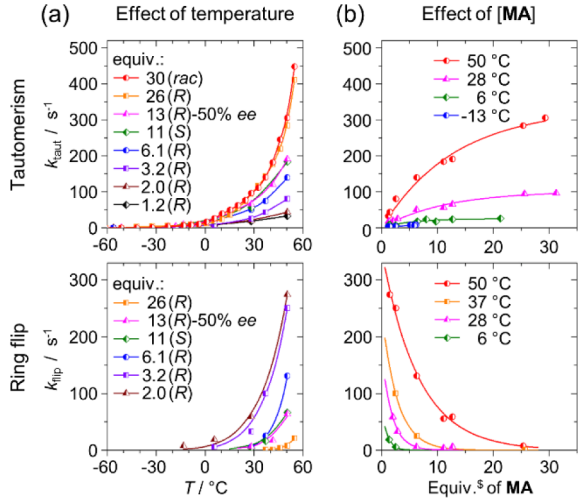


Figure 5. Dependencies of tautomerism rate k_{taut} (top panel) and ring-flip rate k_{flip} (bottom panel) values on various parameters, (a) temperature and (b) concentration of **MA**, as obtained from ^{1}H NMR measurements and fitting procedures. Reprinted from ref 14. Copyright 2014 American Chemical Society.

common with **OxP**, the mechanism of chiral sensing leading to a linear relationship between $\Delta\delta$ and ee at −32.5 °C is due to ion pair formation stabilizing a 1:2 host−guest complex[27] (Figure 7). Fast exchange of guest counteranions occurs at the two binding sites with the host effectively remaining in a doubly protonated state on the NMR time scale.

Reducing the complexity of the host tetrapyrrole molecule achieved effectively by replacing the quinonoid groups of **OxP** with simple phenyl groups while maintaining the saddle conformation of the macrocycle (since the porphyrin dication coincidentally has an almost identical form to **OxP**[30]) allowed us to develop a model mechanism for the host−guest interactions in this system and rationalize the linear relationship between the degree of splitting of NMR peaks and enantiomeric excess. However, we had also inadvertently introduced a disadvantage for the application of this method: the inconvenience of measuring NMR spectra at depressed temperatures. Regardless of this, both **OxP** and porphyrin dication systems suffer from the disadvantage of being applicable only for analyses of chiral carboxylic acids. For this reason, we sought a class of compounds whose structures could ameliorate the disadvantages of the **OxP** and **TPP** systems, namely, compounds that (1) do not require protonation to operate as a host, (2) have no potential for intramolecular tautomerism, (3) operate at room temperature, (4) interact at 1:1 stoichiometry (to avoid diastereomer formation), (5) interact with a larger range of analytes, and (6) have split resonances in their proton NMR spectra whose $\Delta\delta$ is proportional to the ee of the sample. We finally located a type of compound that fulfils this list of demands by reconsidering the **OxP** system. The **OxP** molecule is unique among the calix[4]pyrroles in that it can be regioselectively alkylated at its central nitrogen atoms.[31] N-alkylation occurs at N_{21} and N_{23}, leading to a single di-N-substituted product with no accompanying change in the molecule's morphology, i.e., N_{21},N_{23}-dibenzyl-5,10,15,20-*tetrakis*(3,5-di-*t*-butyl-4-oxocyclohexa-2,5-dienylidene)porphyrinogen, **Bz_2OxP**, possesses a saddle conformation. It also fulfils several of the design prerequisites stated above; it has a single binding site for interaction with a hydrogen-bond acceptor and N-alkylation restricts tautomerism. We also found that **Bz_2OxP** resists protonation, whereas its activity as an NMR reporter of ee by splitting of the relevant peaks is maintained even at room temperature. Finally, since this molecular host relies only on hydrogen bonding for its interaction with chiral analytes, it is useful for the analysis of a much wider range of analyte types, including amines, amino acid derivatives, alcohols, and ketones.

Bz_2OxP-type compounds are the primary example of what we refer to as prochiral solvating agents (or pro-CSAs, Figure 8a).[15,16] When an enantiopure chiral guest (for examples, see Figure 8b) is introduced to a solution of **Bz_2OxP**, symmetrical splitting (occasionally with a concurrent up- or downfield shift) of certain peaks (of the reporter groups) in its NMR spectrum is observed (Figure 8c). **Bz_2OxP** interacts with chiral guests through hydrogen bonding at its pyrrolic NH groups, resulting in a downfield shift of the NH proton resonance (Figure 8c). Regarding the saddle-shaped form of the host and the rigidity

DOI: 10.1021/acs.accounts.5b00005
Acc. Chem. Res. 2015, 48, 521−529

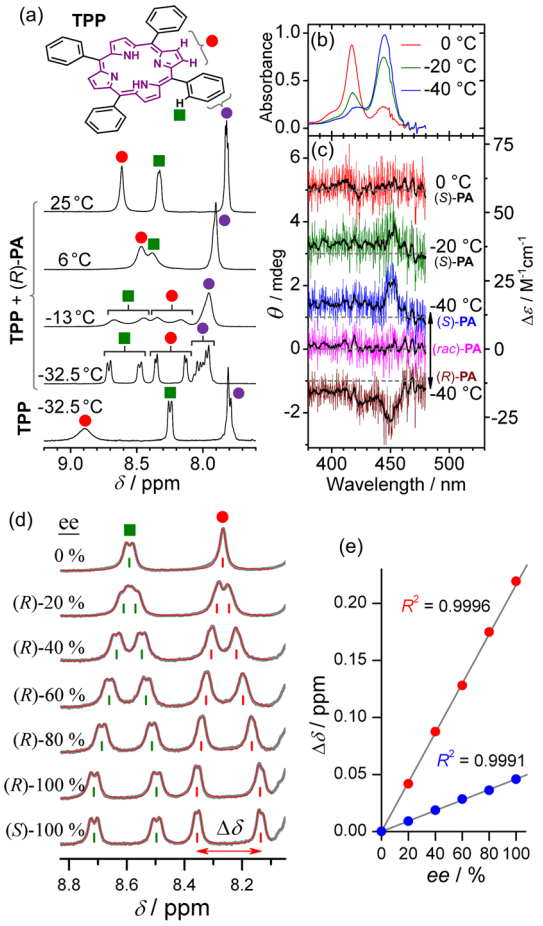


Figure 6. (a) Variations in the ^{1}H NMR spectra of **TPP** (14.7 mM, $CDCl_3$) in the presence of 8 equiv of (*R*)-**PA** during cooling to −32.5 °C. Spectrum of doubly protonated **TPP** (−32.5 °C, **TPP** + (*R*)-**PA**) is distinct from that of free **TPP** (−32.5 °C, **TPP**). (b) UV−vis spectra of **TPP** (0.025 mM, $CDCl_3$, 1 mm cell) in the presence of excess (500 equiv) (*S*)-**PA** upon cooling to −40 °C. Absorption maximum band (445 nm) of porphine dication increases in intensity on cooling. (c) CD spectra of **TPP** (0.025 mM, $CDCl_3$, 1 mm cell) in the presence of excess (500 equiv) (*S*)-**PA** during cooling (top three curves) and in the presence of excess (500 equiv) (*S*)-, (*rac*)-, and (*R*)-**PA** at −40 °C (bottom three curves). CD curves are vertically shifted for clarity (dashed line denotes onset). (d) ^{1}H NMR spectra at −32.5 °C of the **TPP** resonances used to determine $\Delta\delta$. All spectra were fitted (gray, original spectrum; red, fit), and true resonance frequencies, as obtained by fit, are indicated by green or red lines at each spectrum. (e) Plots of dependency of $\Delta\delta$ on the ee value of porphine β-H resonances for **TPP** in the presence of **PA** (red) and **T*t*BuP** in the presence of ibuprofen (blue) illustrating the linear relationship. Reprinted with permission from ref 13. Copyright 2011 Wiley-VCH.

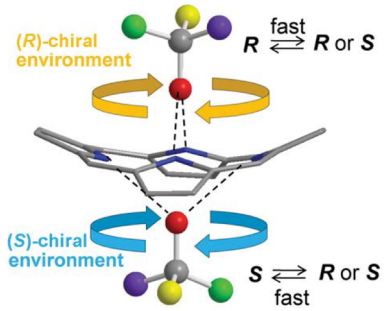


Figure 7. Schematic illustration of translation of chiral information from analyte to achiral host. Saddle-shaped porphyrin dication binds two chiral guests in fast exchange equilibrium, with each face of the macrocycle acting independently. Reprinted with permission from ref 11. Copyright 2011 Wiley-VCH.

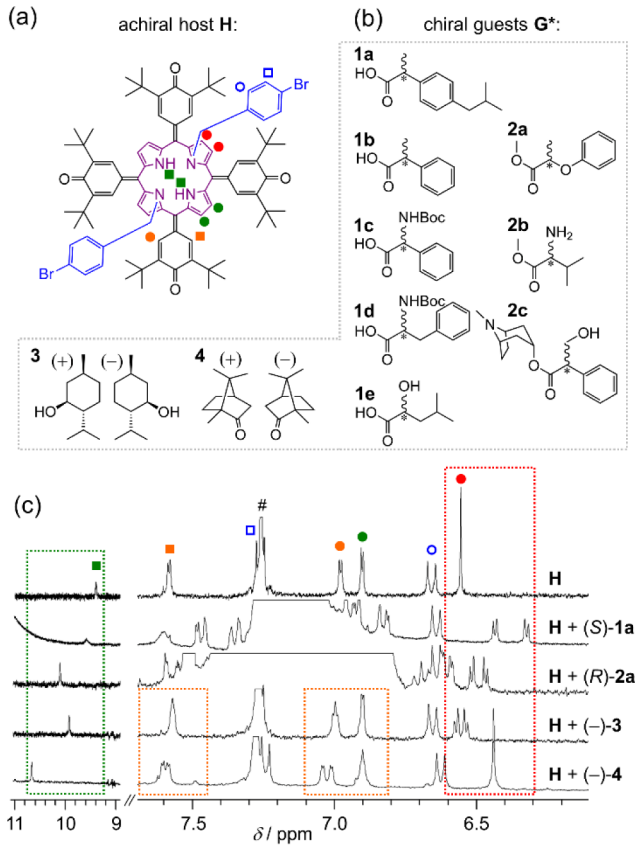


Figure 8. (a) Structure of achiral host (**H**) **Bz$_2$OxP**-type compound. (b) Structures of chiral guests. (c) ^{1}H NMR spectra of chloroform-*d* solution of neat **H** (ca. 0.7 mM) and **H** (ca. 0.7 mM) with ca. 400 equiv of selected chiral guests. Key: Green square, pyrrolic NH; enantiotopic CH reporter groups: orange square and circle, hemiquinonoid *ortho*-H; green circle, N−H pyrrolic β-H; and red circle, *N*-alkylated pyrrolic β-H. Hash mark denotes residual chloroform. Reprinted with permission from ref 15. Copyright 2013 Macmillan Academic Publishers.

of its macrocycle, the distance between the methine reporting groups and the binding site is important. Each methine group available for sensing has different sensitivities (i.e., magnitude of peak splitting) for different chiral guests (Figure 8c). Since there are four such groups of practical use in **Bz$_2$OxP** at various relative spatial positions and at different distances from its binding site, it is highly likely that the presence of chiral guests with some affinity to bind to **Bz$_2$OxP** (i.e., contains an electronegative atom for H-bonding) will lead to observation of peak splitting in some of the methine reporter groups.

The presence of a chiral species that interacts with **Bz$_2$OxP** leads to nonequivalence of proton groups A and B (Figure 9a,b) due to each group being affected in a different way, as indicated [molecular dynamics (MD) simulations] by the preference for some orientations of complexation (conformations) over others (Figure 9c,d). Mirror symmetry of the molecule is broken in the presence of chiral species, and groups A and B, which are initially enantiotopic, become diastereotopic with nonidentical anisochronous NMR chemical shifts (Figure

DOI: 10.1021/acs.accounts.5b00005
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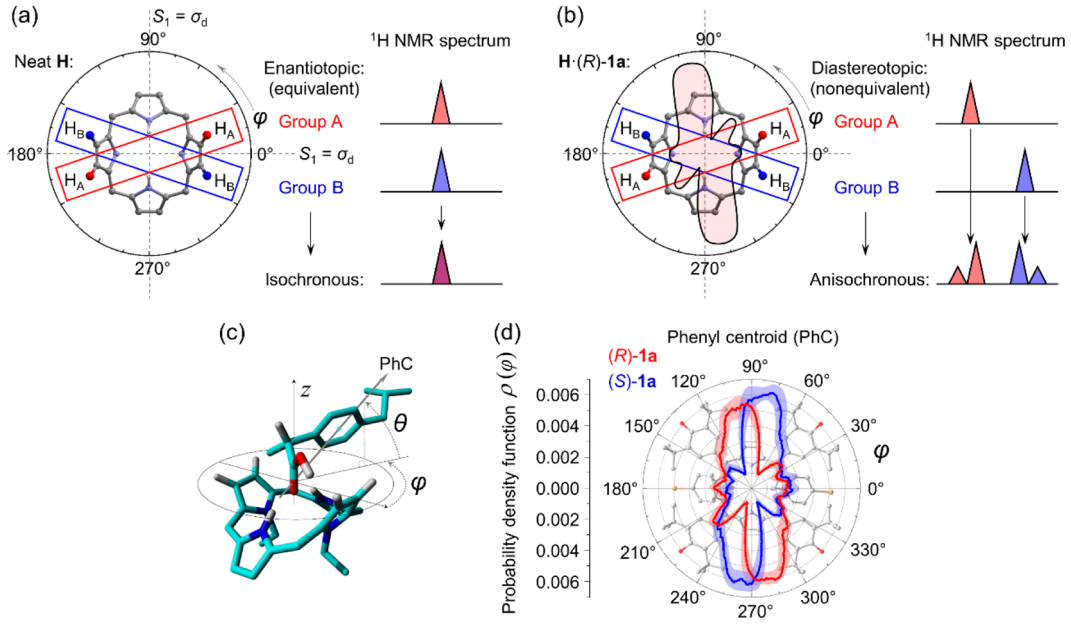

Figure 9. Effect of guest complexation on symmetry of the host. (a) Scheme of symmetry of host molecule and corresponding shape of NMR spectra. Dashed lines denote axes of S_1 symmetry elements (mirror planes) also identical to prochiral planes. (b) Disruption of host's symmetry upon complexation with 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 polar coordinates (φ, θ) used in MD simulations. (d) Plot of probability density function $\rho(\varphi)$ as obtained from MD simulations (for **H**·(*R*)-**1a** and **H**·(*S*)-**1a** complexes) for phenyl centroid (PhC) direction. Solid red or blue lines show average $\rho(\varphi)$ over 5°. Light red or blue background denotes standard variance of $\rho(\varphi)$. Reprinted with permission from ref 15. Copyright 2013 Macmillan Academic Publishers.

9b). It is noteworthy that groups A and B are positioned in the molecule so that no diastereomers are formed. This feature is the cause of the identical intensities of peaks observed at any arbitrary ee. The two second-order doublets (with roofing) are a consequence of the close proximity of H_A and H_B protons, which exhibit a vicinal scalar ^3J-coupling. Quantum mechanical (QM) calculations were used to identify protons that experience, respectively, up- or downfield shifts in the presence of a chiral guest. For the **Bz$_2$OxP**·(*R*)-ibuprofen complex, QM results revealed that group A is shifted upfield relative to group B ((and vice versa for **Bz$_2$OxP**·(*S*)-ibuprofen complex due to its symmetry).

For practical uses, pro-CSA **Bz$_2$OxP** offers some interesting properties. If the ee of an analyte is required to be repetitively determined, for instance, in analyses for pharmaceutical development, then one time construction of a calibration curve using pure enantiomers is possible. This facilitates subsequent rapid ee analyses involving a very simple protocol. Thus, certain host−guest pairs possess characteristic values of $\Delta\delta$ under specific conditions. However, in the case of new chiral analytes that might undergo aggregative processes such as dimerization, a calibration should be generally performed using a greater number of points obtained from known ee references. Aggregation affects the binding model, causing departure from linearity, although we have not observed any such behavior even for carboxylic acid dimerization so far, indicating that aggregation may be neglected. Because the pro-CSA method is based on splitting of resonances, it is inherently less sensitive than traditional CSAs, which are based on NMR integration. The pro-CSA reagents are also appropriate as a rapid probe of ee for use in various racemization or enantioenrichment reactions (e.g., for study of the Soai reaction[32]). Our method should be applicable, in fact, in any case where initial purification (by chiral HPLC or crystallization) of a chiral analyte is required. In situ monitoring of variation of ee without a chiral probe (i.e., using **Bz$_2$OxP**) is also possible and represents a significant advantage of this system since the outcome of, for instance, enantiomer enrichment processes might be biased by the presence of a chiral probe.[33−35] Additionally, for conventional CSA, each type of analyte requires a particular CSA reagent (e.g., the analyte−CSA pairs: carboxylic acids−chiral amines, aminoalcohols−carboxylic acids). Pro-CSA **Bz$_2$OxP** is very versatile since it provides acceptable chemical shift nonequivalence for a large variety of differently functional analytes, which is due to interaction of **Bz$_2$OxP** with the analyte through H-bonding. Additionally, as a result of the fact that chiral information is transferred from the analyte to **Bz$_2$OxP** and then read out from its NMR resonances, synthetic variation of **Bz$_2$OxP** (at its methine reporter groups, e.g., to C−F groups) will allow chiral information to be retrieved from ^{19}F NMR spectra where other resonances due to analyte and solvent do not cause interference. Finally, this system is also of note from the point of view that it does not rely on the formation of diastereomers. This is perhaps counterintuitive given the prevailing opinion that formation of diastereomers is required in the NMR spectroscopic analysis of ee.

6. SUMMARY AND OUTLOOK

In summary, we have performed a molecular design process leading to preparation of prochiral solvating agents (pro-CSA's) based on saddle-shaped tetrapyrrole macrocycles for room temperature NMR determination of ee in a wide range of analyte types, including acids, esters, amines (including amino acid derivatives), and ketones. Currently, we are developing other derivatives of the **Bz$_2$OxP** type in attempts to extend its applicability. These efforts include preparing water-soluble derivatives for analysis of aqueous systems and synthesis of

DOI: 10.1021/acs.accounts.5b00005
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molecules containing different NMR active nuclei for ee determinations using other NMR channels (in order to avoid issues involving peak overlapping). Furthermore, we may be able to prepare **OxP** derivatives with better guest binding strengths in order to improve the protocols for ee determination, whereas in other work, we are preparing chiral derivatives to determine if there are any perhaps unexpected benefits in introducing chirality to the **Bz_2OxP** host. These investigations have helped us to open up previously unknown synthetic pathways in the OxP system, which will contribute to our future investigations of these molecular species.

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Funding

This work was in part supported by World Premier International Research Center (WPI) Initiative on Materials Nanoarchitectonics, MEXT, Japan, and JST, CREST.

Notes

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