DOI: 10.1002/ejoc.201300884

A Chiroptical Probe for Sensing Metal Ions in Water

Marco Caricato,[a] Nerea Jordana Leza,[a] Kinkini Roy,[b] Daniele Dondi,[a] Giuseppe Gattuso,[c] Linda S. Shimizu,[b] Douglas A. Vander Griend,[d] and Dario Pasini*[a,e]

Dedicated to the memory of Professor Christian G. Claessens[‡]

Keywords: Atropisomerism / Sensors / Chirality / Cations / Host-guest systems / Circular dichroism

We describe a novel Binol-containing macrocycle that behaves as a chiroptical probe for the detection of biologically relevant ions (Cu^{2+}, Zn^{2+}) under physiological conditions. The macrocycle synthesis is carried out with Binol-based synthons suitably derivatized in the 2,2′- and 3,3′-positions, by means of a room temperature esterification reaction as the cyclization procedure, followed by late-stage unmasking of the four carboxylic acid functionalities embedded within the macrocyclic framework. The recognition events, signaled using both UV and CD spectroscopy, are triggered by macrocyclic rearrangement induced by binding of the cations through coulombic interactions with the carboxylate anions in water at pH 7. The ample CD response ensures high chiroptical sensitivity and demonstrates the feasibility of chiroptical detection of biologically relevant cations by using a non-nitrogen-based ligand under aqueous conditions.

Introduction

The development of sensors that are capable of optical detection of biologically-relevant metal ions under physiological conditions is an area of active and current interest.[1] Chemical sensors are usually designed to respond to the recognition event by means of absorption, emission or electrochemical changes.[2,3] In the case of chiroptical sensors, Circular Dichroism (CD) spectroscopy is used as the signaling technique.[4,5] Not only can CD spectroscopy give useful information about the supramolecular conformation of the host:guest complex, but, in some instances, it can be used orthogonally to other spectroscopic techniques for sensing purposes.[5] This concept has been demonstrated recently in metallic nanoparticles, for which plasmonic CD responses have been used as a substitute for conventional UV/Vis absorption in the design of extremely sensitive sensors for DNA detection.[6] Porphyrin intercalators have also been used as inducers of strong chiroptical response for the sensing of biological molecules.[7]

Only a handful of chemical systems that are able to behave as chiroptical sensors for Cu^{2+} or Zn^{2+} in organic or mixed organic/aqueous solvent systems have been published to date, and these are typically based on acyclic nitrogen-based ligands.[8–17]

We have recently reported an efficient, direct esterification protocol for the preparation of several chiral macrocycles incorporating Binol (1,1′-bi-2-naphthol) units,[18–21] and we have shown their application in the chiroptical sensing of organic or anionic species.[22,23] Most reported macrocyclization procedures involve reductive aminations;[24] the introduction of bridging ester functionalities ensures chemical stability as well as supramolecular "silence", in terms of competition/interaction with other functionalities (FG in Figure 1) introduced within the macrocyclic framework, for sensing, recognition, or self-assembly[25–31] purposes.

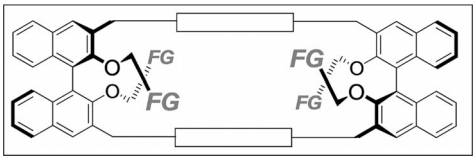


Figure 1. Schematic representation of a homochiral Binol-based macrocycle possessing D_2 molecular symmetry, with functional groups (FG) capable of recognition as structural moieties.

[a] Department of Chemistry, University of Pavia,
Viale Taramelli 10, 27100 Pavia, Italy
E-mail: dario.pasini@unipv.it
Homepage: www.unipv.it/labt
[b] Department of Chemistry and Biochemistry, University of South Carolina,
631 Sumter Street, Columbia, SC 29208, USA
[c] Department of Organic and Biological Chemistry, University of Messina,
Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy
[d] Department of Chemistry and Biochemistry, Calvin College, Grand Rapids, MI 49546-4403, USA
[e] INSTM Research Unit, Department of Chemistry, University of Pavia,
27100 Pavia, Italy
[‡] Professor Christian G. Claessens, a friend and a colleague who prematurely passed away on June 3, 2012, was Associate Professor of Organic Chemistry at Universidad Autónoma de Madrid.
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201300884.

In this paper we report on the synthesis of a homochiral, Binol-based macrocycle, bearing carboxylate functionalities within its framework, and investigate its ability to chiroptically sense biologically relevant metal cations at pH 7 in H_2O at 25 °C. The preorganization of the carboxylate functionalities in suitable positions of the macrocyclic backbone is essential to achieve supramolecular recognition, modifying the conformational properties and thus the isotropic and anisotropic optical response of the macrocyclic sensor: analyte ensemble.

Results and Discussion

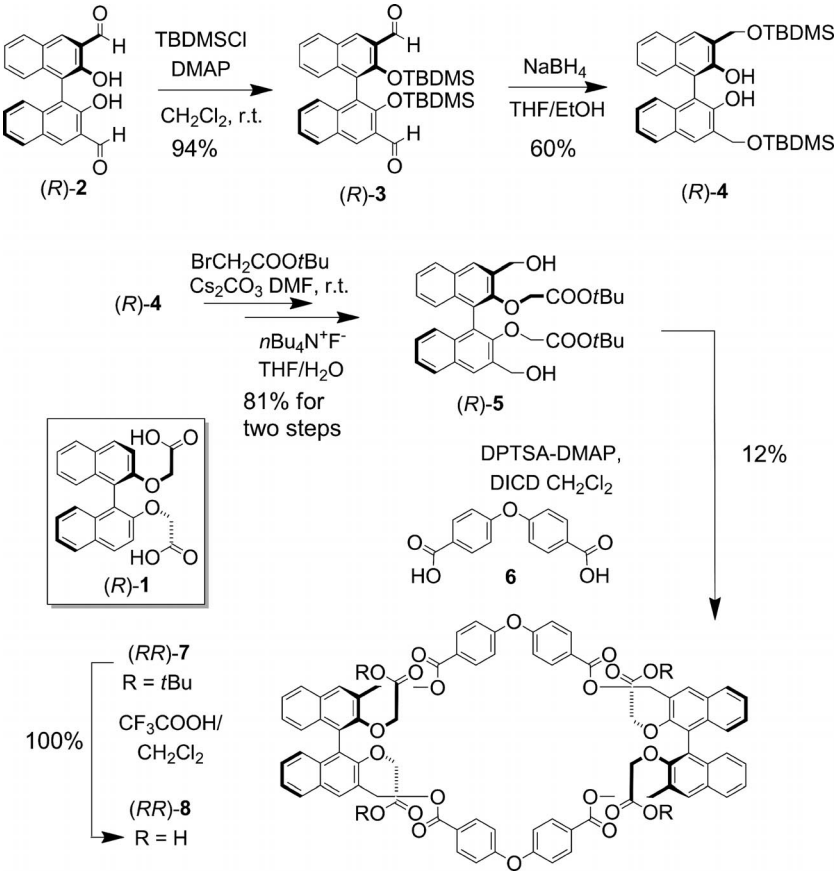
Synthesis of Precursors and Macrocycles

The synthesis of the key precursors and of the macrocycle is shown in Scheme 1. We have previously described[32–34] the synthesis of resolved, optically pure Binol derivatives bearing protected benzylic diols in the 3,3′-positions of the binaphthyl moieties, and carrying elaborated organic functionalities in the 2,2′-positions that are suitable for recognition purposes. The synthetic scheme develops through a chemoselective protection/deprotection approach that is capable of affording key compound (*R*)-**4** in good yields.

During the course of this work, we found that the synthesis of key compound (*R*)-**4** could be carried out with the same number of synthetic steps (Scheme 1, top), but with better yields and easier purification procedures than our published procedure.[32] In fact, by using our new protocol, excess TBDMSCl can be used without problems in the synthesis of dialdehyde (*R*)-**3**; during its reduction, a quantitative intramolecular migration[35] of the TBDMS groups from the 2,2′- to the 3,3′-positions occurs, to afford (*R*)-**4** in excellent yields. Subsequent alkylation under mildly basic conditions followed by deprotection afforded known precursor (*R*)-**5**. To define an internal, noncollapsible macrocyclic cavity,[36] we utilized commercially-available, aryl ether bridged dicarboxylic acid spacer **6**; the cyclization of elaborated precursor (*R*)-**5** afforded the homochiral macrocycle (*RR*)-**7** in 12% yield after purification by column chromatography, with large amounts of baseline oligomeric materials. Although the cyclization yield might seem low, we have previously reported[20] that alternative esterification conditions involving the use of activated esters, or ultrahigh dilution, in the coupling of aryl diacid and binaphthyl-containing benzylic diols structurally similar to (*R*)-**5**, resulted in worse yields. Deprotection of (*R*,*R*)-**7** with trifluoroacetic acid gave the tetracarboxylic acid macrocycle (*R*,*R*)-**8**, which was used without further purification.

Recognition Studies

Either undissociated carboxylic acids or carboxylate anions can be used as molecular recognition-active units.



Scheme 1. Synthesis of key precursors and macrocycles.

On one hand, preorganized, undissociated carboxylic acid functionalities have been elegantly employed as hydrogen-bonding tools for the stabilization of suitable organic guests.[37] On the other hand, it is well-known that carboxylate anions can function as ligands for transition metal or lanthanide ions in aqueous solution.[38] Calculations were initially performed on the fully protonated macrocycle **8** to verify its potential to bind neutral guests in organic solutions through hydrogen-bonding. Given that the macrocycle, owing to its flexibility, may be able to adopt several low-energy conformations, a first screening with semiempirical method PM3 was performed to locate local minima.[39] In fact, the lowest energy geometry (see Figure S1 in the Supporting Information) shows a hydrogen-bonding interaction that holds two of the four carboxylic groups in the center of the molecule. The open cavity could potentially include neutral guests such as adenine derivatives[37] to form host:guest complexes. Energy minimization of the 1:1 complex by molecular modeling (see Figure S2 in the Supporting Information) did not reveal the previously reported[37] efficient, optimal geometry, because only one of the carboxylic acids undergoes efficient hydrogen bonding with the guest, namely with the 6-adenine NH_2 hydrogen-bonding donor and the 1-adenine heterocyclic nitrogen acceptor. Indeed, ^{1}H NMR spectroscopic titrations of macrocycle (*R*,*R*)-**8** with ethyl-adenine-9-acetate in [D_8]tetrahydrofuran/[D_6]dimethyl sulfoxide ([D_8]THF/[D_6]DMSO, 9:1) or [D_8]THF/CD_3CN (9:1) did not suggest any detectable binding. Molecular modeling indicated that the complexation of metal ions (such as Zn^{2+}, Figure 2, bottom) should be feasible in the cavity of the dissociated, tetraanionic macrocycle **8**. The optimized geometry of the unbound tetraanionic macrocycle show a flexible structure (Figure 2, top) with a cavity that changes drastically upon complexation of the metal ion. The metal cation is located in the center of the macrocyclic cavity and is stabilized by two carboxylate anions attached to opposite Binol units. The change in conformation upon binding does not substantially modify the dihedral angles of the Binol units, which are 106 and 88° in the case of the 1:1 complex, and 110 and 89° in the case of the tetraanionic macrocycle.

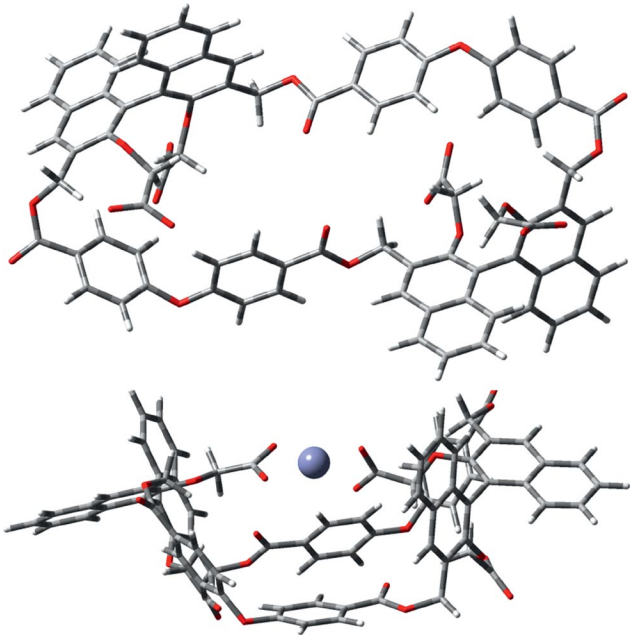


Figure 2. Optimized geometries (method PM3) for macrocycle **8** (top), and for the 1:1 complex with Zn^{2+} (bottom).

Initial screening experiments were conducted by using ^{1}H NMR spectroscopic analysis (in [D_6]DMSO) on a series of transition-metal salts that are soluble in DMSO and with widely different characteristics [$Cd(ClO_4)_2$, $Ag(NO_3)_2$, $Zn(NO_3)_2$], but all capable of forming stable diacetate coordination compounds. The triethyl ammonium salt of the macrocycle (obtained by treatment of the macrocyclic tetracarboxylic acid with an excess of Et_3N), which is soluble in DMSO, was used for this preliminary screening.

Detectable shifts for the proton resonances of the binaphthyl units were observed only in the case of Zn^{2+} salt, with saturation occurring at one equivalent (Figure S3). This result confirmed the potential of macrocycle **8** in terms of transition-metal ion recognition, and prompted us to examine its behavior under physiological conditions.

Macrocycle (*R*,*R*)-**8**, in its tetraanionic form, is poorly soluble in buffered H_2O at neutral pH at concentrations needed for NMR titrations (10^{-4} M). Control compound (*R*)-**1**, in contrast, did show sufficient solubility, but the NMR titration with Zn^{2+} indicated no resonance shift for any of the proton signals of the host (Figure S4). At the concentrations required for optical detection (10^{-5} M), both macrocycle (*R*,*R*)-**8** and the control compound (*R*)-**1** showed good solubility in phosphate buffer (50 mM, pH 7) aqueous solution. The UV spectra (Figures S6 and S7) of control compound (*R*)-**1** under these conditions show a maximum absorbance around 230 nm, to which is associated CD activity (Figure 3, top, 0 equiv. guest added curve). The CD band is a bisignate exciton couplet signal typical of binaphthyl compounds. In addition to this band, macrocycle (*R*,*R*)-**8** shows CD-induced activity (Figure 3, bottom, 0 equiv. guest added curve) associated with the different UV/Vis bands of other chromophores (i.e., the aryl ether spacer, Figure S6 and S7) between 250 and 300 nm.

No changes were detected with the control compound (*R*)-**1** in the presence of either Zn^{2+} or Cu^{2+}, either by UV/Vis or by CD spectroscopy. These data support the NMR titration results and indicate that intermolecular recognition of the cations by carboxylates belonging to different molecules (or the formation of a coordination polymer) does not occur over the concentration range of 2 μM to 2 mM. In contrast, in the presence of either Zn^{2+} or Cu^{2+}, large changes could be detected in the UV/Vis and CD titrations with macrocycle (*R*,*R*)-**8** in phosphate buffer at pH 7 (Figure 3, bottom and Figure S5). It is evident that the complexation of the metal ions in the cavity of the macrocycle brings about a conformational rearrangement of CD active (the binaphthyl) and induced CD active (the spacer) chromophores.

Fitting with a 1:1 binding isotherm could not be carried out satisfactorily. To adequately delineate the thermo-

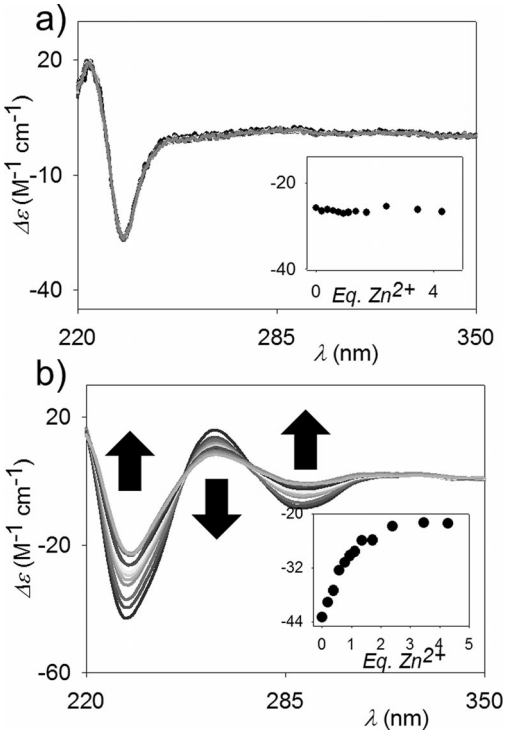


Figure 3. CD titrations of control compound (*R*)-**1** (2.6×10^{-5} M; Panel a) and of macrocycle (*R*,*R*)-**8** (3.2×10^{-5} M; Panel b) with $ZnCl_2$ in 10 mM phosphate buffer at pH 7. Guest concentration: see inset (for inset, values at ca. 230 nm, are plotted vs. added guest equivalents).

dynamic association constants and the stoichiometry of the supramolecular complexes, the entire set of wavelengths were modeled simultaneously by using Sivvu, a nonlinear least-squares regression program for performing equilibrium-restricted factor analysis.[40] The data sets from both the UV and CD titrations could be used. The 1:1 binding stoichiometry was confirmed to be the predominant equilibrium in solution, with binding constants calculated from the CD data ($\log K_a$ = 5.4 for Cu^{2+} and 5.2 for Zn^{2+}) essentially matching those obtained from the UV data ($\log K_a$ = 5.5 for Cu^{2+} and 4.6 for Zn^{2+}). The model for the best fit of both UV and CD titration data needed to include both 1:2 and 2:1 macrocycle/ligand complex stoichiometries (Table S1 and S2). The presence of variable 2:1 or 1:2 macrocycle/metal ion stoichiometries can be rationalized on the basis of clustering effects, probably induced by the hydrophobicity of the receptor; this consideration is substantiated by the peculiar solubility behavior of the tetraanionic macrocycle (*R*,*R*)-**8** in buffered aqueous media. Job plots showed a maximum at 0.5 in the case of Cu^{2+}, and at 0.65 for Zn^{2+}, which again reinforced the argument of the presence of multiple macrocycle/zinc stoichiometries in both systems (Figure S8).[41] Phosphate buffer is not ideal in our studies because the solubility of Cu^{2+} and Zn^{2+} phosphate salts in H_2O is known to be low.[42] Using one of the Good's buffers[43] (PIPES 10 mM, pH 7), UV titrations with Cu^{2+} and Zn^{2+} did confirm the above binding constants and preferred stoichiometries, despite the sparingly soluble nature of macrocycle (*R*,*R*)-**8**, which required that experiments be carried out at a macrocycle concentration of 1.3×10^{-5} M.

Conclusions

We have reported the chirality sensing under physiological conditions of transition-metal ions by using a suitably designed Binol-based macrocycle of novel conception. The synthesis of the key macrocycle was achieved through the use of elaborated binaphthyl synthons, in which chemically efficient differentiation between the 2,2′- and 3,3′-position of the binaphthyl skeleton allows the insertion of carboxylate functionalities in the internal cavity of the cyclic covalent structure. As supported by molecular modeling,[44] the rigid macrocyclic framework possesses enough flexibility to enable the presentation of two carboxylates attached to opposite binaphthyl units to the guests. By comparison with a model compound, we demonstrate that the macrocyclic framework is needed to ensure the required preorganization of the binding functionalities for the described chiroptical sensing purposes. Given the high absorption coefficients of macrocycle **8** in the buffered aqueous system described, we estimate a detection limit in the micromolar range for both Cu^{2+} and Zn^{2+} ions.

Experimental Section

General: All commercially available reagents and solvents were used as received. THF (Na, benzophenone) and CH_2Cl_2 (CaH_2) were dried and distilled before use. Compounds PTSA-DMAP,[20] (*R*)-**1**,[45] (*R*)-**2**,[32] and (*R*)-**5**[32] were obtained by previously published procedures. Analytical thin-layer chromatography was performed on chromophore loaded, commercially available silica gel plates. Flash chromatography was carried out by using silica gel (pore size 60 Å, 230–400 Mesh). ^1H and ^{13}C NMR spectra were recorded from solutions in $CDCl_3$ on a 200, 300, or 500 MHz spectrometer by using the solvent residual proton signal or tetramethylsilane as the internal standard. The UV/Vis spectroscopic studies were recorded with commercially available spectrophotometers. Mass spectra were recorded with an electrospray ionization instrument. Optical rotations were measured with a polarimeter with a sodium lamp (λ = 589 nm) and are reported as follows: $[a]_{25}^{D}$ [c = g (100 mL)$^{-1}$, solvent]. CD spectroscopy was performed with a spectropolarimeter; spectra were recorded at 25 °C at a scanning speed of 50 nm min^{-1} and were background corrected.

Compound (*R*)-3: Et_3N (2.04 mL, 14.6 mmol, 5 equiv.) was added under nitrogen to a solution of (*R*)-**2** (1.00 g, 2.92 mmol, 1 equiv.) and DMAP (0.071 g, 0.585 mmol, 0.2 equiv.) in anhydrous CH_2Cl_2 (100 mL). After 10 min stirring, TBDMSCl (2.21 g, 14.62 mmol, 5 equiv.) was added and the solution was stirred for an additional 4 h. The reaction mixture was then quenched by the addition of H_2O (100 mL), extracted with CH_2Cl_2 (3 × 100 mL), and dried (Na_2SO_4). The solution was filtered and concentrated in vacuo and the crude product was purified by column chromatography (hexanes/CH_2Cl_2, 8:2) to give (*R*)-**3** (1.57 g, 94%) as a yellow solid. $[a]_D^{25}$ = +136 (c = 0.001, CH_2Cl_2). ^1H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 10.55 (s, 2 H, aldehyde), 8.56 (s, 2 H, binaphthyl), 8.02 (d, J = 7.4 Hz, 2 H, binaphthyl), 7.38 (m, 4 H, binaphthyl), 7.21

(d, J = 7.4 Hz, 2 H, binaphthyl), 0.82 (s, 18 H, tBu), –0.05 (s, 6 H, -SiCH_3), –1.10 (s, 6 H, -SiCH_3) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ = 190.5 (CH aldehyde), 152.2 (Cq), 137.6 (Cq), 130.8 (CH), 130.3 (CH), 128.9 (CH), 128.6 (Cq), 128.3 (Cq), 126.5 (CH), 124.8 (CH), 122.7 (Cq), 25.3 (CH_3 tBu), 18.1 (Cq-Si), –4.50 (-SiCH$_3$), –4.65 (-SiCH$_3$) ppm. $C_{34}H_{42}O_4Si_2$ (570.88): calcd. C 71.5, H 7.4; found C 71.4, H 7.4.

Compound (*R*)-4: $NaBH_4$ (34 mg, 0.432 mmol, 6 equiv.) was added to a solution of (*R*)-**3** (41 mg, 0.0719 mmol, 1 equiv.) in EtOH/THF (4:1, 8 mL). The solution was stirred for 4 h, then the reaction mixture was concentrated in vacuo and the crude product was treated with H_2O (20 mL), extracted with CH_2Cl_2 (3 × 20 mL), and dried (Na_2SO_4). The solution was filtered and concentrated in vacuo to obtain compound (*R*)-**4** (25 mg, 60%), which was used without further purification. ^{1}H NMR spectrum was identical to that of the compound prepared by using the published route.[32]

Compound (*R*)-5: Prepared by using the published route.[32]

Macrocycle (*R*,*R*)-7: Compound (*R*)-**5** (300 mg, 0.523 mmol, 1 equiv.) and PTSA-DMAP (324 mg, 1.05 mmol, 2 equiv.) were suspended in CH_2Cl_2 (14 mL) under N_2, and a solution of diacid **6** (135 mg, 0.523 mmol, 1 equiv.) in CH_2Cl_2 (14 mL) was added. After 15 min stirring, DICD (198 mg, 1.57 mmol, 3 equiv.) was added and the solution was stirred under N_2 for a further 15 h at room temperature. The reaction mixture was quenched by the addition of H_2O (30 mL), extracted with CH_2Cl_2 (3 × 25 mL), and dried (Na_2SO_4). The solution was filtered and concentrated in vacuo and the crude product was purified by column chromatography (CH_2Cl_2/EtOAc, 98:2) to give macrocycle (*R*,*R*)-**7** (49 mg, 12%) as a white solid. $[\alpha]_D^{25}$ = +79 (c = 0.001, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 8.15 (s, 4 H, binaphthyl), 8.10 (d, J = 7.9 Hz, 8 H, phenyl), 7.93 (d, J = 7.7 Hz, 4 H, binaphthyl), 7.44 (t, J = 7.7 Hz, 4 H, binaphthyl), 7.28 (t, J = 7.7 Hz, 4 H, binaphthyl), 7.10 (d, J = 7.7 Hz, 4 H, binaphthyl), 6.99 (d, J = 7.9 Hz, 8 H, phenyl), 5.71 (s, 8 H, OCH_2-COO), 4.21 (AB system, 8 H, BinCH_2O-), 1.07 (s, 36 H, t-But) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ = 167.7 (Cq), 165.6 (Cq), 160.2 (Cq), 154.7 (Cq), 134.2 (Cq), 132.8 (CH), 131.9 (2CH), 130.4 (Cq), 129.2 (Cq), 128.3 (CH), 127.3 (CH), 125.7 (Cq), 125.4 (CH), 125.3 (CH), 124.2 (Cq), 118.5 (2CH), 81.2 (Cq tBu), 70.9 (CH_2), 63.6 (CH_2), 27.6 (CH_3) ppm. MS(ESI): m/z (%) = 1615.3 (100) [M + Na]$^+$. $C_{96}H_{88}O_{22}$ (1593.74): calcd. C 72.4, H 5.6; found C 72.7, H 5.7.

Macrocycle (*R*,*R*)-8: CF_3COOH (0.3 mL) was added to a solution of macrocycle (*R*,*R*)-**7** (20 mg, 0.012 mmol, 1 equiv.) in CH_2Cl_2 (4 mL). After stirring for 6 h, full conversion of the starting material could be verified by TLC analysis (hexane/EtOAc, 6:4). The reaction mixture was then quenched by the addition of H_2O (15 mL), extracted with EtOAc (3 × 10 mL) and dried (Na_2SO_4). The solution was filtered and concentrated in vacuo to remove the excess solvents and CF_3COOH to give pure macrocycle (*R*,*R*)-**8** (17 mg, 100%), which was used without further purification. $[\alpha]_D^{25}$ = +89 (c = 0.003, CH_3COCH_3). ^{1}H NMR ($CDCl_3$, 500 MHz, 25 °C): δ = 8.16 (s, 4 H, 4-H), 7.88–7.85 (m, 12 H, 8-H, 2′-H), 7.42 (t, J = 7.7 Hz, 4 H, 7-H), 7.27 (dd, J = 8.5, 7.7 Hz, 4 H, 6-H), 7.08 (d, J = 8.5 Hz, 4 H, 5-H), 6.85 (d, J = 7.9 Hz, 8 H, 3′-H), 5.55 and 5.47 (AB system, J_{AB} = 9.9 Hz, 8 H, ArCH_2), 4.88 (br. s, 4 H, OH), 4.17 and 3.80 (AB system, J_{AB} = 15.8 Hz, 8 H, OCH_2). ^{1}H NMR ($CDCl_3$, 125 MHz, 25 °C): δ = 171.2, 165.8, 160.5, 153.5, 134.0, 131.9 (×2), 130.7, 128.6, 127.8, 126.0, 125.3, 124.1, 118.7, 69.9, 63.5 ppm.

General Procedure for the UV/Vis or CD Titration and Job Plots Experiments: Distilled, deionized H_2O was used to prepare phosphate buffer at pH 7 with $Na_2HPO_4{\cdot}2H_2O$ and NaH_2PO_4. An analytical balance (with a precision of 10^{-4} g) was used to weigh the samples for the stock solutions. Aliquots of these stock solutions were then taken by using high-precision pipettes to prepare the cuvette samples for spectrophotometric analyses.

Titration Experiments: To a stock solution of the macrocycle (*R*,*R*)-**8** (solution A) in phosphate buffer, were added several aliquots of a cation (solution B, either $ZnCl_2$ or $CuCl_2$). Solution B was made by diluting the cation at higher concentration in solution A, to maintain constant macrocycle concentration.

Job Plots Experiments: A solution of (*R*,*R*)-**8** (1 μM) was prepared in 10 mM phosphate buffer (30 mL), as was a solution of each cation (1 μM, 10 mL). The CD spectrum of the solution of (*R*,*R*)-**8** (2 mL) was recorded from 220 to 350 nm in a 1 cm quartz cuvette. The CD spectra of the following mixtures [mL] of (*R*,*R*)-**8** with cations were recorded: 1.7:0.3, 1.3:0.7, 1:1, 0.7:1.3, 0.3:1.7. Plotting $\Delta\varepsilon \times$ [**8**] against [**8**]/([**8**] + [cation]) as described previously[41] produced the Job plot.

^{1}H NMR Complexation Experiments on (*R*)-1: All spectra were recorded at 500 MHz and at 25 °C. Samples were prepared by adding to a 0.5 mL solution of (*R*)-**1** (5 mM in a 10 mM pH 7.6 phosphate buffer in D_2O), successive aliquots of a stock solution composed of $Zn(NO_3)_2$ (62.5 mM) and (*R*)-**1** (5 mM) dissolved in the same 10 mM, pH 7.0 phosphate buffer in D_2O, to a final volume of 0.9 mL. Eight values of δ_{obs} for the H-4 resonances were collected by keeping the [host] to [guest] ratio in the 1:0.25–1:10 interval, but no detectable complexation-induced shift of the resonances was detected.

Supporting Information (see footnote on the first page of this article): Additional NMR, UV and CD spectra, titration profiles, and computational details.

Acknowledgments

This work was financially supported by the Ministero dell'Università e della Ricerca (MIUR) (PRIN2009A5Y3N9), the CINECA Supercomputer Center (ISCRA NANOCHIR project HP10CKIGGH), the US National Science Foundation (NSF) (CHE-0911527 and CHE-1012298), and the American Chemical Society Petroleum Research Fund (49503-UR3). D. P. wishes to thank the Royal Society of Chemistry for a "Journal Grant for International Authors" supporting the part of this research carried out in Linda Shimizu's group at the University of South Carolina, USA.

[1] C. Lodeiro, J. L. Capelo, J. C. Mejuto, E. Oliveira, H. M. Santos, B. Pedras, C. Nuñez, *Chem. Soc. Rev.* **2010**, *39*, 2948–2976.
[2] A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, *Chem. Rev.* **1997**, *97*, 1515–1566.
[3] P. D. Beer, P. A. Gale, G. Z. Chen, *Coord. Chem. Rev.* **1999**, *186*, 3–36.
[4] G. A. Hembury, V. V. Borovkov, Y. Inoue, *Chem. Rev.* **2008**, *108*, 1–73.
[5] N. Berova, L. Di Bari, G. Pescitelli, *Chem. Soc. Rev.* **2007**, *36*, 914–931.
[6] A. D'Urso, A. Mammana, M. Balaz, A. E. Holmes, N. Berova, R. Lauceri, R. Purrello, *J. Am. Chem. Soc.* **2009**, *131*, 2046–2047.
[7] Z. T. Li, Z. N. Zhu, W. J. Liu, Y. L. Zhou, B. Han, Y. Gao, Z. Y. Tang, *J. Am. Chem. Soc.* **2012**, *134*, 3322–3325.
[8] R. Kramer, *Angew. Chem.* **1998**, *110*, 804; *Angew. Chem. Int. Ed.* **1998**, *37*, 772–773.
[9] A. E. Holmes, D. Das, J. W. Canary, *J. Am. Chem. Soc.* **2007**, *129*, 1506–1507.

[10] J. M. Castagnetto, J. W. Canary, *Chem. Commun.* **1998**, 203–204.
[11] A. Moletti, C. Coluccini, D. Pasini, A. Taglietti, *Dalton Trans.* **2007**, *16*, 1588–1592.
[12] M. Boiocchi, M. Bonizzoni, A. Moletti, D. Pasini, A. Taglietti, *New J. Chem.* **2007**, *31*, 352–356.
[13] P. Carney, S. Lopez, A. Mickley, K. Grniberg, W. Zhang, Z. Dai, *Chirality* **2011**, *23*, 916–920.
[14] B. K. Kanungo, M. Baral, R. K. Bera, S. K. Sahoo, *Monatsh. Chem.* **2010**, *141*, 157–168.
[15] A. Bencini, C. Coluccini, A. Garau, C. Giorgi, V. Lippolis, L. Messori, D. Pasini, S. Puccioni, *Chem. Commun.* **2012**, *48*, 10428–10430.
[16] C. Bazzicalupi, A. Bencini, S. Puccioni, B. Valtancoli, P. Gratteri, A. Garau, V. Lippolis, *Chem. Commun.* **2012**, *48*, 139–141.
[17] E. M. Nolan, J. Jaworski, M. E. Racine, M. Sheng, S. J. Lippard, *Inorg. Chem.* **2006**, *45*, 9748–9757.
[18] C. Coluccini, A. Mazzanti, D. Pasini, *Org. Biomol. Chem.* **2010**, *8*, 1807–1815.
[19] S. Colombo, C. Coluccini, M. Caricato, C. Gargiulli, G. Gattuso, D. Pasini, *Tetrahedron* **2010**, *66*, 4206–4211.
[20] C. Coluccini, D. Dondi, M. Caricato, A. Taglietti, M. Boiocchi, D. Pasini, *Org. Biomol. Chem.* **2010**, *8*, 1640–1649.
[21] M. Caricato, N. J. Leza, C. Gargiulli, G. Gattuso, D. Dondi, D. Pasini, *Beilstein J. Org. Chem.* **2012**, *8*, 967–976.
[22] M. Caricato, C. Coluccini, D. Dondi, D. A. Vander Griend, D. Pasini, *Org. Biomol. Chem.* **2010**, *8*, 3272–3280.
[23] M. Caricato, A. Olmo, C. Gargiulli, G. Gattuso, D. Pasini, *Tetrahedron* **2012**, *68*, 7861–7866.
[24] Z.-B. Li, J. Lin, L. Pu, *Angew. Chem.* **2005**, *117*, 1718; *Angew. Chem. Int. Ed.* **2005**, *44*, 1690–1693.
[25] D. Pasini, M. Ricci, *Curr. Org. Synth.* **2007**, *4*, 59–80.
[26] D. T. Bong, T. D. Clark, J. R. Granja, M. R. Ghadiri, *Angew. Chem.* **2001**, *113*, 1016; *Angew. Chem. Int. Ed.* **2001**, *40*, 988–1011.
[27] C. Grave, A. D. Schlüter, *Eur. J. Org. Chem.* **2002**, 3075–3098.
[28] Y. Yamaguchi, Z. Yoshida, *Chem. Eur. J.* **2003**, *9*, 5430–5440.
[29] D. Zhao, J. S. Moore, *Chem. Commun.* **2003**, 807–818.
[30] S. Höger, *Chem. Eur. J.* **2004**, *10*, 1320–1329.
[31] W. Zhang, J. S. Moore, *Angew. Chem.* **2006**, *118*, 4524; *Angew. Chem. Int. Ed.* **2006**, *45*, 4416–4439.
[32] C. Coluccini, A. Castelluccio, D. Pasini, *J. Org. Chem.* **2008**, *73*, 4237–4240.
[33] M. Asakawa, P. R. Ashton, W. Hayes, H. M. Janssen, E. W. Meijer, S. Menzer, D. Pasini, J. F. Stoddart, A. J. P. White, D. J. Williams, *J. Am. Chem. Soc.* **1998**, *120*, 920–931.
[34] M. Asakawa, P. R. Ashton, S. E. Boyd, C. L. Brown, S. Menzer, D. Pasini, J. F. Stoddart, M. S. Tolley, A. J. P. White, D. J. Williams, P. G. Wyatt, *Chem. Eur. J.* **1997**, *3*, 463–481.
[35] P. R. Ashton, S. E. Boyd, G. Gattuso, E. Y. Hartwell, R. Königer, N. Spencer, J. F. Stoddart, *J. Org. Chem.* **1995**, *60*, 3898–3903.
[36] L. S. Shimizu, A. D. Hughes, M. D. Smith, M. J. Davis, P. Zhang, H.-C. zur Loye, K. D. Shimizu, *J. Am. Chem. Soc.* **2003**, *125*, 14972–14973.
[37] J. M. Lavin, K. D. Shimizu, *Chem. Commun.* **2007**, 228–230.
[38] R. D. Hancock, D. L. Melton, J. M. Harrington, F. C. McDonald, R. T. Gephart, L. L. Boone, S. B. Jones, N. E. Dean, J. R. Whitehead, G. M. Cockrell, *Coord. Chem. Rev.* **2007**, *251*, 1678–1689.
[39] These minima were submitted to cycles of molecular dynamics and further optimizations. This method proved useful to explore the reasonable space of conformers. Lower energy minima were optimized by using DFT methods, with the B3LYP functional at the 6–31G(d,p) level of theory and LANL2DZ as the basis set for the metal ions. Polarization on hydrogen atoms was introduced because hydrogen bonds can play an active role in determining the macrocycle structure.
[40] Sivvu: D. A. Vander Griend, M. J. DeVries, Calvin College, **2005** (www.calvin.edu/~dav4/Sivvu.htm).
[41] S. R. Beeren, J. K. M. Sanders, *J. Am. Chem. Soc.* **2011**, *133*, 3804–3807.
[42] The K_{sp} (25 °C) for $Zn_3(PO_4)_2$ and for $Cu_3(PO_4)_2$ are 9×10^{-33} and 1.4×10^{-37}, respectively, so that in a 10 mM phosphate buffer at pH 7, the theoretical concentration of free Cu^{2+} and Zn^{2+} can be calculated as 3.3×10^{-6} and 8.2×10^{-8} M, respectively. Our titrations were not conducted in a pure water environment. Inclusion of the K_{sp} equilibria into the Sivvu models was not feasible. According to the K_{sp}, and given the high association constants in binding with the host, precipitation would occur for almost all the amount of added metal ions above one equivalent with respect to the macrocycle concentration. We cannot rule out the possibility that some precipitation might have occurred during the titrations. In blank experiments, the turbidity of 10^{-4} M solutions of Zn^{2+} and Cu^{2+} in 10 mM phosphate buffer at pH 7 resulted in a perturbed baseline in the UV/Vis measurements. We did not observe any such turbidity in our solutions during the titration.
[43] a) Y. Baudry, G. Bollot, V. Gorteau, S. Litvinchuk, J. Mareda, M. Nishihara, D. Pasini, F. Perret, D. Ronan, N. Sakai, M. R. Shah, A. Som, N. Sorde, P. Talukdar, D.-H. Tran, S. Matile, *Adv. Funct. Mater.* **2006**, *16*, 169–179. The solubility of macrocycle **8** in HEPES buffer (10 mM, pH 7.6) was found to be even lower than that in PIPES buffer. Both buffers absorb consistently below 250 nm b) A. Pacini, M. Caricato, S. Ferrari, D. Capsoni, A. Martínez de Ilarduya, S. Muñoz-Guerra, D. Pasini, *J. Polym. Sci., Part A* **2012**, *50*, 4790–4799.
[44] Numerous crystallization experiments were attempted on the macrocyclic precursor **7**, on the deprotected macrocycle **8** and on 1:1 complexes with Cu^{2+} and Zn^{2+}. Unfortunately, we were not able to obtain single crystals that were suitable for X-ray crystallographic analysis.
[45] a) R. H. Wang, L. J. Xu, X. S. Li, Y. M. Li, Q. Shi, Z. Y. Zhou, M. C. Hong, A. S. C. Chan, *Eur. J. Inorg. Chem.* **2004**, 1595–1599; b) A. K. Sharma, M. Caricato, E. Quartarone, S. Edizer, A. Giacometti Schieroni, R. Mendichi, D. Pasini, *Polym. Bull.* **2012**, *69*, 911–923.

Received: June 16, 2013
Published Online: August 5, 2013

