Inorg. Chem. **2007**, *46*, 2485–2497

Toward Iron Sensors: Bioinspired Tripods Based on Fluorescent Phenol-oxazoline Coordination Sites

Raghavendra Kikkeri,[†] Hassan Traboulsi,[‡] Nicolas Humbert,[‡] Elzbieta Gumienna-Kontecka,[‡] Rina Arad-Yellin,[†] Galina Melman,[†] Mourad Elhabiri,[‡] Anne-Marie Albrecht-Gary,*[,‡] and Abraham Shanzer*[,†]

Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel, and the Laboratoire de Physico-Chimie Bioinorganique, ULP-CNRS, Institut de Chimie, Strasbourg, France

Received October 12, 2006

In the quest for fast throughput metal biosensors, it would be of interest to prepare fluorophoric ligands with surface-adhesive moieties. Biomimetic analogues to microbial siderophores possessing such ligands offer attractive model compounds and new opportunities to meet this challenge. The design, synthesis, and physicochemical characterization of biomimetic analogues of microbial siderophores from *Paracoccus denitrificans* and from the *Vibrio* genus are described. The (4*S*,5*S*)-2-(2-hydroxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazole-4-carbonyl group (**L^{a}**), noted here as an HPO unit, was selected for its potential dual properties, serving as a selective iron(III) binder and simultaneously as a fluorophore. Three tripodal symmetric analogues *cis*-**L^{b}**, *cis*-**L^{c}**, and *trans*-**L^{c}**, which mainly differ in the length of the spacers between the central carbon anchor and the ligating sites, were synthesized. These ferric-carriers were built from a tetrahedral carbon as an anchor, symmetrically extended by three converging iron-binding chains, each bearing a terminal HPO. The fourth chain could contain a surface-adhesive function (**L^{c}**). A combination of absorption and emission spectrophotometry, potentiometry, electrospray mass spectrometry, and electrochemistry was used to fully characterize the corresponding ferric complexes and to determine their stability. The quenching mechanism is consistent with an intramolecular static process and is more efficient for the analogue with longer arms. Detection limits in the low nanogram per milliliter range, comparable with the best chemosensors based on natural peptide siderophores, have been determined. These results clearly demonstrate that these tris(phenol-oxazoline) ligands in a tripodal arrangement firmly bind iron(III). Due to their fluorescent properties, the coordination event can be easily monitored, while the fourth arm is available for surface-adhesive moieties. The tripodal system is therefore an ideal candidate for integration with solid-state materials for the development of chip-based devices and analytical methodologies.

Introduction

Fluorescent materials are now routinely used for a wide variety of sensing applications,[1−4] and there is considerable current interest in the development of sensors dedicated to biologically relevant metal ions. Among them, iron is a metal of widespread interest which, due to its low water solubility at neutral pH, is poorly bioavailable. Under iron deficient conditions, microorganisms secrete low molecular weight iron-carriers, siderophores that are capable of solubilizing external iron and transferring the siderophore−iron complex into the cytoplasm. Iron acquisition from the environment is essential in growth and proliferation of microorganisms, as it is for the survival of all living organisms. Iron is crucial for a wide variety of vital cell functions, ranging from oxygen metabolism and electron-transfer processes to DNA and RNA synthesis.[5−11] Just as a shortage of iron is problematic, so is a surplus of iron, which induces oxidative damage, leading

* To whom correspondence should be addressed. E-mail: amalbre@chimie.u-strasbg.fr (A.-M.A.-G.); abraham.shanzer@weizmann.ac.il (A.S.).
† The Weizmann Institute of Science.
‡ Institut de Chimie.

(1) Ceroni, P.; Vicinelli, V.; Maestri, M.; Balzani, V.; Lee, S.-K.; Heyst, J. V.; Gorka, M.; Vögtle, F. *J. Organomet. Chem.* **2004**, *689*, 4375−4383.
(2) Prodi, L. *New J. Chem.* **2005**, *29*, 20−31.
(3) Callan, J. F.; Silva, A. P. D.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551−8588.
(4) Bergamini, G.; Ceroni, P.; Balzani, V.; Cornelissen, L.; Heyst, J. V.; Lee, S.-K.; Vögtle, F. *J. Mater. Chem.* **2005**, *15*, 2959−2964.

10.1021/ic061952u CCC: $37.00
Published on Web 02/28/2007

to organ malfunction and mortality.[12,13] Meticulous control and quantitative monitoring of iron levels are therefore major environmental[14−18] and therapeutic targets.[19−22]

The development of iron biosensors requires the following two major features: (i) a selective binding of the targeted metal ion, and (ii) the incorporation of a "signaling" element to report the formation or the dissociation events. Over the years, we were able to couple iron binding or its release with a signaling component in order to elicit either chemical,[23] electrochemical,[24,25] or spectrophotometric signals.[26−32] We attached covalently either fluorescent groups or surface-adhesive functions[24,33,34] to ferrichrome analogues[35] at auxiliary sites that do not alter iron binding or receptor recognition. To develop future analytical tools, with fast throughput as in plate-reader technology, it would be desirable to separate between the optical signaling of metal coordination and the surface-adhesive moiety. It occurred to us that, like in the siderophores of the pyoverdin family,[36−40] the iron(III) binding site could be fused with a fluorescent probe into a single functionality (Figure 1).

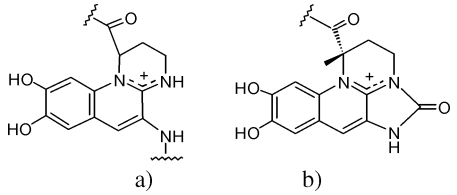


Figure 1. Fluorescent bidentate 2,3-diamino-6,7-dihydroxy-quinoline binding units (a) of pyoverdin *PaA*[40] and (b) of azotobactin *δ*.[42]

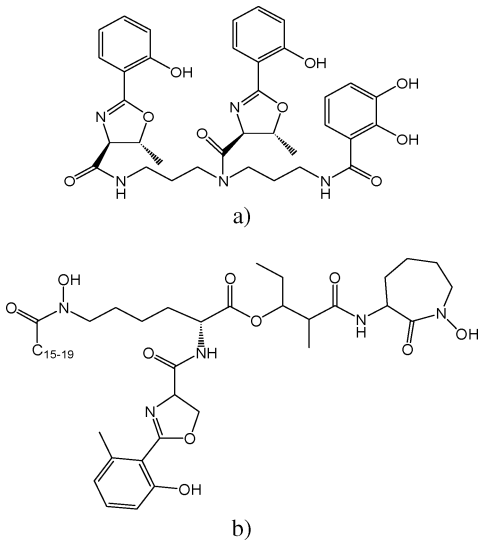


Figure 2. Chemical structure (a) of L-vulnibactin and of (b) mycobactin P.

The hydroxy-phenyl-oxazoline (HPO) pattern has been identified in various bacterial siderophores as a component of a composite ligand, in combination with catechols as in L-parabactin[43] (*Paracoccus denitrificans*) and in L-vulnibactin (*Vibrio vulnificus*)[44,45] (Figure 2a) and with hydroxamates as in mycobactin P (*Mycobacterium paratuberculosis*)[46−48] (Figure 2b).[41]

(5) Winkelmann, G.; van der Helm, D.; Neilands, J. B. *Iron Transport in Microbes, Plants, and Animals*; VCH: Weinheim, Germany, 1987.
(6) Neilands, J. B. *Struct. Bonding* **1984**, *58*, 1−24.
(7) Matzanke, B. F.; Müller-Matzanke, G.; Raymond, K. N. *Iron Carriers and Iron Proteins*; VCH Publishers: New York, 1989; Vol. 5.
(8) Liu, X.; Theil, E. C. *Acc. Chem. Res.* **2005**, *38*, 167−175.
(9) Tezcan, F. A.; Kaiser, J. T.; Mustafi, D.; Walton, M. Y.; Howard, J. B.; Rees, D. C. *Science* **2005**, *309*, 1377−1380.
(10) Grinberg, A. V.; Hannemann, F.; Schiffler, B.; Muller, J.; Heinemann, U.; Bernhardt, R. *Proteins* **2000**, *40*, 590−612.
(11) Videira, A.; Duarte, M. *Biochim. Biophys. Acta-Bioenerg.* **2002**, *1555*, 187−191.
(12) Lieu, P. T.; Heiskala, M.; Peterson, P. A.; Yang, Y. *Mol. Aspects Med.* **2001**, *22*, 1−87.
(13) Crichton, R. R. *Inorganic Biochemistry of Iron Metabolism: From Molecular Mechanisms to Clinical Consequences*, 2nd ed.; John Wiley & Sons: Chichester, 2001.
(14) Murphy, T. P.; Lean, D. R. S.; Nalewajko, C. *Science* **1976**, *192*, 900−902.
(15) Matsumiya, H.; Iki, N.; Miyano, S. *Talanta* **2004**, *62*, 337−342.
(16) Wells, M. L.; Price, N. M.; Bruland, K. W. *Mar. Chem.* **1995**, *48*, 157−182.
(17) Behrenfeld, M. J.; Bale, A. J.; Kolber, Z. S.; Aiken, J.; Falkowski, P. G. *Nature* **1996**, *383*, 508−511.
(18) Martin, J. H.; Fitzwater, S. E. *Nature* **1988**, *331*, 341−343.
(19) *Bioinorganic Chemistry*; Theil, E. C., Raymond, K. N., Bertini, I., Gray, H. B., Lippard, S. J., Valentine J. S., Eds.; University Science Books: Mill Valley, 1993; pp 1−35.
(20) Price, N. M.; Morel, F. M. M. *Metal Ions in Biological Systems*; Sigel, A., Sigel H., Eds.; Dekker: New York, 1998; Vol. 35, pp 1−36.
(21) Brittenham, G. M.; Badman, D. G. *Blood* **2003**, *101*, 15−19.
(22) Beutler, E; Hoffbrand, A. V.; Cook, J. D. *Hematology (Am. Soc. Hematol. Educ. Program*) **2003**, 40−61.
(23) Dayan, I.; Libman, J.; Agi, Y.; Shanzer, A. *Inorg. Chem.* **1993**, *32*, 1467−1475.
(24) Rubinstein, I.; Steinberg, S.; Tor, Y.; Shanzer, A.; Sagiv, J. *Nature* **1988**, *332*, 426−429.
(25) Steinberg, S.; Tor, Y.; Shanzer, A.; Rubinstein, I. *Thin Fims*; Ulman, A., Ed.; Academic Press: San Diego, 1995; Vol. 20, pp 183−205.
(26) Weizman, H.; Ardon, O.; Mester, B. *J. Am. Chem. Soc.* **1996**, *118*, 12368−12375.
(27) Ardon, O.; Weizman, H.; Libman, J.; Shanzer, A.; Chen, Y.; Hadar, Y. *Microbiology* **1997**, *143*, 3625−3631.
(28) Weizman, H.; Libman, J.; Shanzer, A. *J. Am. Chem. Soc.* **1998**, *120*, 2188−2189.
(29) Nudelman, R.; Ardon, O.; Hadar, Y.; Chen, Y.; Libman, J.; Shanzer, A. *J. Med. Chem.* **1998**, *41*, 1671−1678.
(30) Ardon, O.; Nudelman, R.; Caris, C.; Libman, J.; Shanzer, A.; Chen, Y.; Hadar, Y. *J. Bacteriol.* **1998**, *180*, 2021−2026.
(31) Kornreich-Leshem, H.; Ziv, C.; Gumienna-Kontecka, E.; Arad-Yellin, R.; Chen, Y.; Elhabiri, M.; Albrecht-Gary, A. M.; Hadar, Y.; Shanzer, A. *J. Am. Chem. Soc.* **2005**, *127*, 1137−1145.
(32) Palanché, T.; Marmolle, F.; Abdallah, M. A.; Shanzer, A.; Albrecht-Gary, A. M. *J. Biol. Inorg. Chem.* **1999**, *4*, 188−198.
(33) Gafni, Y.; Weizman, H.; Libman, J.; Shanzer, A.; Rubinstein, I. *Chem. Eur. J.* **1996**, *2*, 759−766.
(34) Raghavendra, K.; Linda, P.; Arad-Yellin, R.; Shanzer, A. Unpublished results.
(35) Shanzer, A.; Libman, J.; Lazar, R.; Tor, Y. *Pure Appl. Chem.* **1989**, *61*, 1529−1534.
(36) Barelmann, I.; Fernández, D. U.; Budzikiewicz, H.; Meyer, J. M. *Biometals* **2003**, *16*, 263−270.
(37) Meyer, J.-M.; Geoffroy, V. A. *Iron Transport in Bacteria*; Crosa, J. H., Mey, A. R., Payne S. M., Eds.; American Society for Microbiology: Washington, DC, 2004; pp 451−468.
(38) Albrecht-Gary, A. M.; Crumbliss, A. L. *Metal Ions in Biological Systems*; Sigel, A., Sigel, H., Eds.; Marcel Dekker: New York, 1998, Vol. 35, pp 239−327.
(39) Albrecht-Gary, A. M.; Crumbliss, A. L. *Scientific Bridges for 2000 and Beyond*, TEC and DOC eds.; Académie des Sciences, Institut de France: Paris, 1999; pp 73−89.
(40) Albrecht-Gary, A. M.; Blanc, S.; Rochel, N.; Ocaktan, A. Z.; Abdallah, M. A. *Inorg. Chem.* **1994**, *33*, 6391−6402.
(41) See Figures S1 and S2.
(42) Palanché, T.; Blanc, S.; Hennard, C.; Abdallah, M. A.; Albrecht-Gary, A. M. *Inorg. Chem.* **2004**; *43*, 1137−1152.
(43) (a) Peterson, T.; Neilands, J. B. *Tetrahedron Lett.* **1979**, 4805−4807. (b) Peterson, T.; Falk, K. E.; Leong, S. A.; Klein, M. P.; Neilands, J. B. *J. Am. Chem. Soc.* **1980**, *102*, 7715−7718.
(44) Simpson, L. M.; Oliver, J. D. *Infect. Immun.* **1983**, *41*, 644−649.
(45) Okujo, N.; Saito, M.; Yamamoto, S.; Yoshida, T.; Miyoshi, S.; Shinoda, S. *Biometals* **1994**, *7*, 109−116.
(46) Xu, Y.; Miller, M. J. *J. Org. Chem.* **1998**, *63*, 4314−4322.
(47) Hu, J.; Miller, M. J. *J. Am. Chem. Soc.* **1997**, *119*, 3462−3468.

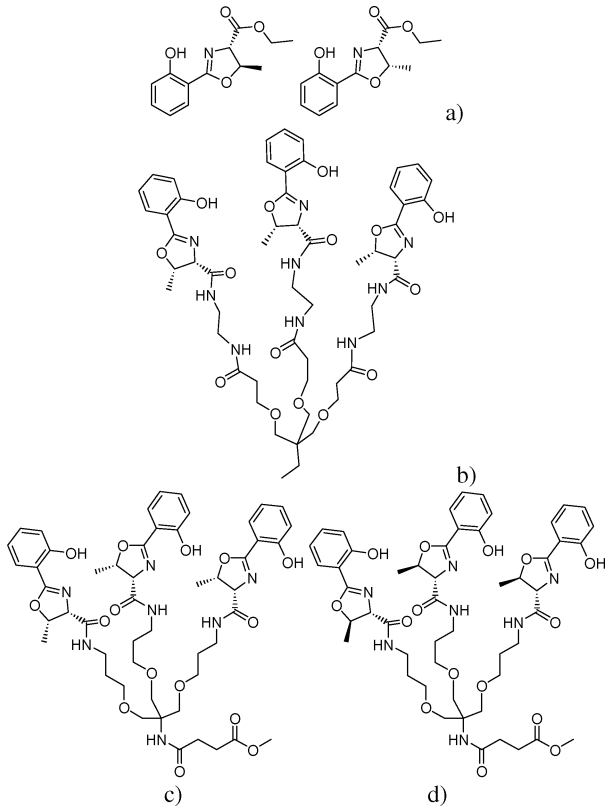


Figure 3. Chemical structures of *cis*- and *trans*-HPO and their tripodal derivatives: (a) *cis*- and *trans*-**L**^{a}, ethyl (4*S*,5*S*)-2-(2-hydroxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazole-4-carboxylate group (noted HPO); (b) *cis*-**L**^{b}; (c) *cis*-**L**^{c}; (d) *trans*-**L**^{c}.

Following the same concept, we have selected phenol-oxazoline building blocks serving simultaneously as efficient iron binders and fluorescent markers (Figure 3).

In this paper, we describe a new class of biomimetic iron(III) ligands based on HPO. Phenol-oxazoline ligands can be prepared in two geometrical isomers (*cis*[49] and *trans*[50]), depending on the mode of cyclization and in a number of optical isomers. We have prepared both *cis* and *trans* isomers. The C_3 symmetric compounds (*cis*-**L**^{b} and *cis*- and *trans*-**L**^{c}), anchored on a quaternary carbon, and extended by three converging arms bearing the HPO coordination sites, could be fitted with an optional surface-adhesive unit being attached as a fourth chain. **L**^{b} and **L**^{c} differ in the length of the spacers between the quaternary carbon and the binding sites. Since, within experimental errors, there is no significant difference in their physicochemical properties, we mainly focused our attention on the *cis* derivatives.

We will present the synthesis of the monomers *cis*- and *trans*-**L**^{a}, which are intermediates in the synthetic route of the tripods *cis*-**L**^{b} and *cis*- and *trans*-**L**^{c} (Figure 3). **L**^{a}, which possesses an ester group instead of an amide functionality in **L**^{b} and **L**^{c}, was used to compare the acido-basic and coordination behavior for *cis* and *trans* isomers. We will also examine the corresponding metal binding affinity and their absorption and emission spectrophotometric properties. The synthetic strategy for the preparation of these bioinspired fluorescent ligands will be described and their signaling ability discussed in terms of photophysics and analysis. Finally, preliminary results showing the relevance of geometrical isomers in bacterial receptor recognition will be discussed.

Experimental Section

General Information. All the reagents used for the synthesis were purchased from Sigma and were used without further purification. Benzyl-protected L-threnonine was purchased from NovaBioChem (Laufelfingen, Switzerland). All solvents (HPLC grade) for synthesis and purification were obtained from Labscan (Dublin, Ireland). Dichloromethane was dried over basic alumina; THF was distilled over Na/benzophenone under argon atmosphere. Flash chromatography was performed using Merck 0.04−0.63 mm mesh silica gel. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-250 MHz spectrometer. Ligands **L**^{a}, **L**^{b}, and **L**^{c} were characterized by electrospray mass spectrometry (ESMS) performed with a LCZ-4000 instrument (Micromass, Manchester, U.K.).

For solubility reasons, the physicochemical properties of ligands **L**^{a}, **L**^{b}, and **L**^{c} and their ferric complexes were examined in a mixture methanol/water (80/20 by weight). Distilled water was further purified by passing through a mixed bed of ion-exchanger (Bioblock Scientific R3-83002, M3-83006) and activated carbon (Bioblock Scientific ORC-83005). Both distilled water and spectroscopic grade methanol (Merck, Uvasol, p.a.) were deoxygenated using CO_2- and O_2-free argon prior to use (Sigma Oxiclear cartridge). All the stock solutions were prepared by weighting solid products using an AG 245 Mettler Toledo analytical balance (precision 0.01 mg). The complete dissolution of the ligands was obtained with the help of ultrasonic bath. The ionic strength was adjusted to 0.1 M with sodium perchlorate (Merck p.a.). ***Caution!*** *Perchlorate salts combined with organic ligands are potentially explosive and should be handled in small quantity and with the necessary precautions.*[51] Iron(III) perchlorate stock solutions (Fluka) were prepared in the same solvent mixture (methanol/water 80/20 by weight) at acidic pH (pH $\approx$ 1.5) immediately before use and were back-titrated with thorium nitrate (Merck) in excess of standardized Na_2H_2EDTA solution (Merck, Titriplex III, 10^{-3} M) and xylenol orange (Merck) as an indicator.[52] The glassware used was rinsed after each experiment with a hydrochloric acid solution to remove all traces of iron. The free hydrogen ion concentrations were measured with a combined glass electrode (Metrohm 6.0234.500, Long Life) filled with 0.1 M NaCl (Fluka, p.a.) in MeOH/H_2O (80/20 by weight). Potential differences were given by a Tacussel Isis 20.000 millivoltmeter. Standardization of the millivoltmeter and verification of the linearity (2.00 < pH < 13.23) of the electrode were performed according to classical methods. The 10^{-2} M perchloric acid (Merck) (pH 2.00), 2×10^{-2} M oxalic acid (R. P.)/10^{-2} M ammonium oxalate (R. P.) (pH 3.13), 2×10^{-2} M succinic acid (R. P.)/10^{-2} M lithium methylate (Fluka purum) (pH 6.01), and 1.7×10^{-1} M sodium hydroxide (BDH, AnalR) (pH 13.23) buffers used for standardization were prepared according to published procedures in methanol/water solvent (80/20 by

(48) Vergne, A. F.; Walz, A. J.; Miller, M. J. *Nat. Prod. Rep.* **2000**, *17*, 99−116.
(49) Ait-Haddou, H.; Hoarau, O.; Cramailere, D.; Pezet, F.; Daran, J.-C.; Balavoine, G. G. A. *Chem. Eur. J.* **2004**, *10*, 699−707.
(50) Reddy, L. R.; Saravanan, P.; Corey, E. J. A. *J. Am. Chem. Soc.* **2004**, *126*, 6230−6231.
(51) (a) Wolsey, W. C. *J. Chem. Educ.* **1978**, *55*, A355−A337. (b) Raymond, K. N. *Chem. Eng. News* **1983**, *61*, 4.
(52) *Méthodes d'analyses complexométriques avec les Titriplex*; Merck: Darmstadt, 1990; p 45.

weight).[53] The experiments were carried out at (25.0 ± 0.2) °C maintained with the help of Haake FJ thermostats.

Synthetic Procedures. Synthesis of (2*S*,3*R*)-3-Hydroxy-2-[(2-benzyloxybenzoyl)amino]butanoic Acid (1). (i) Methyl 2-(benzyloxy)benzoate. Anhydrous potassium carbonate (8.6 g, 65 mmol) was added to 100 mL of acetone solution containing methyl salicylate (2.5 g, 16.4 mmol) and benzyl bromide (2.2 mL, 19.6 mmol). The reaction mixture was refluxed for 12 h. Potassium carbonate was filtered out, and the solvent was evaporated. The residue was dissolved in ethyl acetate (100 mL) washed with 1 N NaOH, water, and brine, and dried over sodium sulfate. Evaporation of solvent afforded the title compound, 3.2 g (81%). ^{1}H NMR ($CDCl_3$): 3.9 (s, 3H, **CH_3**−O−CO); 5.2 (s, 2H, O−**CH_2**−C_6H_5); 7.1 (q, J = 7.5 Hz, 2H, Ar); 7.35 (m, 6H Ar); 7.8 (d, J = 7.5 Hz, 1H, Ar).

(ii) 2-(Benzyloxy)benzoic Acid. Methyl 2-(benzyloxy)benzoate (2.5 g, 10 mmol) was dissolved in methanol (90 mL), 1 N NaOH was added, and the reaction was stirred overnight. The solution was acidified with 1 N HCl, and the solvent was evaporated. The residue was dissolved in ethyl acetate, washed with water and brine, dried over magnesium sulfate, and evaporated. Flash chromatography with $CHCl_3$/ CH_3OH (6%) as eluent afforded the title compound, 1.51 g (61%). ^{1}H NMR ($CDCl_3$): 5.3 (s, 2H, O−**CH_2**−C_6H_5); 7.1 (q, J = 7.5 Hz, 2H, Ar); 7.34 (m, 6H, Ar); 8.2 (d, J = 7.5 Hz, 1H, Ar).

(iii) Benzyl (2*S*,3*R*)-3-Hydroxy-2-[(2-benzyloxybenzoyl)amino]-butanoate. Benzyl (2*S*,3*R*)-2-amino-3-hydroxybutanoate (L-threonine benzyl ester) (63 mg, 0.3 mmol) was dissolved in dichloromethane (10 mL), a few drops of triethylamine were added, and the solution was stirred for 5 min. Water (5 mL) was added, and layers were separated. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated. 2-Benzyloxybenzoic acid (100 mg, 0.3 mmol) and L-threonine benzyl ester were dissolved in dichloromethane (10 mL), cooled to 0 °C, diisopropyl carbodiimide (45 μL, 0.35 mmol) and HOBt (4 mg, 0.03 mmol) were added, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with dichloromethane (10 mL), washed with water, saturated sodium bicarbonate solution, 5% citric acid aqueous solution, water, and brine, dried over sodium sulfate, filtered, and concentrated. Flash chromatography with $CHCl_3$/CH_3OH (2%) as eluent afforded 100 mg (64%) of the title compound. ^{1}H NMR ($CDCl_3$): 1.0 (d, J = 6.4 Hz, 3H, −**CH_3**(γ)); 4.2 (dq, J_1 = 6.4 Hz, J_2 = 8.3 Hz, 1H, −CH_3(γ)−**CH**(β)−CH(α)); 4.7 (dd, J_1 = 2.5 Hz, J_2 = 8.3 Hz, 1H, −CH_3−CH(β)−**CH**(α)); 5.1 (s, 4H, −O−**CH_2**−C_6H_5); 7.1 (m, 2H, Ar); 7.35 (m, 8H, Ar); 7.45 (m, 3H, Ar); 8.2 (dd, J_1 = 1.7 Hz, J_2 = 7.7 Hz, 1H, Ar); 8.5 (br, 1H, −**NH**).

(iv) Compound 1. Benzyl (2*S*,3*R*)-3-hydroxy-2-[(2-benzyloxybenzoyl)amino]butanoate (100 mg, 0.24 mmol) was dissolved in methanol (10 mL), and 6 mL of 1 N NaOH was added. The reaction mixture was stirred for 2 h at room temperature. Once the reaction was completed, methanol was evaporated, and the aqueous solution was acidified with 1 N HCl up to pH = 5. The aqueous phase was extracted with ethyl acetate several times, and the organic layers were collected, dried with sodium sulfate, and evaporated to obtain the title compound 60 mg (76%). ^{1}H NMR ($CDCl_3$): 1.2 (d, J = 6.4 Hz, 3H, −**CH_3**(γ)); 4.3(dq, J_1 = 6.4 Hz, J_2 = 8.3 Hz, 1H, −CH_3(γ)−**CH**(β)−CH(α)); 4.6 (dd, J_1 = 2.5 Hz, J_2 = 8.3 Hz, 1H, −CH_3−CH(β)−**CH**(α)); 5.1 (s, 2H, −O−**CH_2**−C_6H_5); 7.1 (m, 2H, Ar); 7.35 (m, 6H, Ar); 8.2 (d, J = 8 Hz, 1H, Ar).

Synthesis of Pentachlorophenyl (4*S*,5*R*)-2-(2-Hydroxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazole-4-carboxylate (2). (i) Benzyl (4*S*,5*R*)-2-(2-Benzyloxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazole-4-carboxylate. Benzyl (2*S*,3*R*)-3-hydroxy-2-[(2-benzyloxybenzoyl)-amino] butanoate (1 g, 2.38 mmol) was dissolved in toluene (60 mL), and p-toluenesulfonic acid (45 mg, 0.24 mmol) was added. The reaction mixture was refluxed for 12 h with dean stark receiver. The solvent was evaporated, and yellow oily residue was further purified by flash chromatography with $CHCl_3$/CH_3OH (1−2%) as eluent to afford the title compound, 450 mg (47%). ^{1}H NMR ($CDCl_3$): 1.56 (d, J = 6.3 Hz, 3H, −**CH_3(γ)**); 4.5(d, 1H, J = 7 Hz, CH_3(γ)−CH(β)−**CH(α)**); 4.9 (m, 1H, J_1 = 6.3 Hz, J_2 = 7 Hz, CH_3(γ)−**CH(β)**−CH(α)); 5.1 (s, 4H, O−**CH_2**−C_6H_5); 7.0 (m, 2H, Ar); 7.35 (m, 11H, Ar); 7.7 (d, J = 7.9 Hz, 1H, Ar).

(ii) (4*S*,5*R*)-2-(2-Hydroxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazole-4-carboxylic Acid. Benzyl (4*S*,5*R*)-2-(2-benzyloxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazole-4-carboxylate (400 mg, 1.4 mmol) was dissolved in absolute ethanol (30 mL), and 10% Pd/C (120 mg) was added. Hydrogenolysis was carried out at room temperature for 10 h at 1 atm. The catalyst was filtered out, and the evaporation of the solvent afforded the title compound, 200 mg (90%). ^{1}H NMR ($CDCl_3$): 1.5 (d, J = 6.4 Hz, 3H, −**CH_3**(γ)); 4.5-(d, 1H, J = 7 Hz, CH_3(γ)−CH(β)−**CH**(α)); 4.9 (m, 1H, J_1 = 6.3 Hz, J_2 = 7 Hz, CH_3(γ)−**CH**(β)−CH(α)); 6.8 (dt, J = 8.1 Hz, 1H, Ar); 6.9 (d, J = 7.4 Hz, 1H, Ar); 7.4 (t, J = 7.4 Hz, 1H, Ar); 7.6 (d, J = 8.1 Hz, 1H, Ar).

(iii) Pentachlorophenyl (4*S*,5*R*)-2-(2-Hydroxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazole-4-carboxylate (2). (4*S*,5*R*)-2-(2-Hydroxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazole-4-carboxylic acid (200 mg, 0.9 mmol) was dissolved in 5 mL of THF and diluted with 30 mL of dichloromethane, and then pentachlorophenol (240 mg, 0.9 mmol), *N*-hydroxybenzotriazole (HOBt) (12 mg, 0.09 mmol), and finally, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (248 mg, 1.3 mmol) were added. The reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated and dissolved in minimum ethyl acetate. The precipitated urea was filtered out, and the residue was concentrated. Flash chromatography with hexane/chloroform (80/20−25) as eluent afforded 94 mg (23%) of compound **2**. ^{1}H NMR ($CDCl_3$): 1.7 (d, J = 6.4 Hz, 3H, −**CH_3**(γ)); 4.9(d, 1H, J = 7 Hz, CH_3(γ)−CH(β)−**CH**(α)); 5.25 (q, 1H, J_1 = 6.3 Hz, J_2 = 7 Hz, CH_3(γ)−**CH**(β)−CH(α)); 6.9 (t, J = 8.1 Hz, 1H, Ar); 7.0 (d, J = 7.4 Hz, 1H, Ar); 7.4 (t, J = 7.4 Hz, 1H, Ar); 7.7 (d, J = 8.1 Hz, 1H, Ar).

Synthesis of 1,1,1-Tris{{2-[(*tert*-butoxycarbonyl)amino]ethoxy}-methyl}propane (3). (i) 1,1,1-Tris[(2-cyanoethoxy)methyl]propane. A 40% sodium hydroxide solution (1 mL) was added by portions to a 1,1,1-tris(hydroxymethyl)propane (2 g, 16.6 mmol) and acrylonitrile (4.5 g, 84.9 mmol) mixture at such a rate that the temperature is maintained at 30 °C. The reaction mixture was stirred for 24 h at room temperature, followed by neutralization with 1 N HCl solution. The aqueous solution was extracted with ethyl acetate several times. The combined organic phase was washed with water and brine, dried with sodium sulfate, and concentrated under reduced pressure. Flash chromatography with $CHCl_3$/CH_3OH (13%) as eluent afforded the title compound, 3.2 g (66%) as an oily product. ^{1}H NMR ($CDCl_3$): 0.8 (t, J = 7.5 Hz, 3H, **CH_3**−CH_2−C−CH_2−O); 1.3 (q, J = 7.5 Hz, 2H, CH_3−**CH_2**−C−CH_2−O); 2.6 (t, J = 6.25 Hz, 6H, CH_2−**CH_2**−CN); 3.4 (s, 6H, CH_2−C−**CH_2**−O); 3.7 (t, J = 6.25 Hz, 6H, **CH_2**−CH_2−CN).

(ii) 1,1,1-Tris[(2-carboxy-ethoxy)methyl]propane. 1,1,1-Tris-[(2-cyanoethoxy)methyl]propane (2 g, 6.8 mmol) was dissolved in 5 mL of 36% concentrated HCl and refluxed for 4 h. The reaction mixture was allowed to cool down, and ethyl acetate (30 mL) was

(53) (a) Alfenaar, M.; De Ligny, C. L. *Recl. Trav. Chim. Pays-Bas* **1967**, *86*, 1185−1190. (b) Gelsema, W. J.; De Ligny, C. L.; Remijnse, A. G.; Blijleven, H. A. *Recl. Trav. Chim. Pays-Bas* **1966**, *85*, 647−660.

added. The organic layer was washed with water several times. Flash column chromatography with $CHCl_3/CH_3OH$ (6%) as eluent afforded the title compound, 560 mg (12%). ^1H NMR ($CDCl_3$): 0.9 (t, $J = 7.5$ Hz, 3H, **CH_3**$-CH_2-C-$**CH_2**$-O$); 1.3 (q, $J = 7.5$ Hz, 2H, CH_3-**CH_2**$-C-CH_2-O$); 2.6 (t, $J = 6.25$ Hz, 6H, CH_2-**CH_2**$-COOH$); 3.3 (s, 6H, $-CH_2-C-$**CH_2**$-O$); 3.6 (t, $J = 6.25$ Hz, 6H, **CH_2**$-CH_2-COOH$).

(iii) Compound 3. 1,1,1-Tris(2-carboxy-ethoxymethyl)propane (500 mg, 1.4 mmol) and 2-(*N*-*tert*-butoxycarbonyl)amino-ethylamine (0.75 g, 4.62 mmol) were dissolved in dry dichloromethane, and the reaction mixture was cooled to 0 °C. HOBt (62 mg, 0.46 mmol) and DIC (0.71 mL, 4.6 mmol) were added, and the reaction mixture was stirred overnight at room temperature. The urea was filtered out, and the solution was concentrated. Flash column chromatography with $CHCl_3$/MeOH (5%) as eluent afforded the compound (**3**), 360 mg (35%). ^1H NMR ($CDCl_3$): 0.8 (t, $J = 7.5$ Hz, 3H, **CH_3**$-CH_2-C-$**CH_2**$-O$); 1.3 (q, $J = 7.5$ Hz, 2H, CH_3-**CH_2**$-C-CH_2-O$); 1.4 (s, 27H, **Boc**); 2.4 (t, $J = 6.25$ Hz, 6H, CH_2-**CH_2**$-CONH$); 3.3 (m, 12H, $-CH_2-C-$**CH_2**$-O$, $CONH-$**CH_2**$-CH_2-NHBoc$); 3.4 (m, 6H, $CONH-CH_2-$**CH_2**$-NHBoc$); 3.6 (t, $J = 6.25$ Hz, 6H, $-O-$**CH_2**$-CH_2-CONH$).

Synthesis of Methyl 4-{*N*-Tris{{3-[(*tert*-butoxycarbonyl)-amino]propoxy}methyl}methylamino}-4-oxobutanoate (4). (i) *N*-{Tris[(2-cyanoethoxy)methyl]}methylamine. A 40% sodium hydroxide solution (1 mL) was added by portions to a tris-(hydroxymethyl)methylamine (2 g, 16.5 mmol) and acrylonitrile (4.5 g, 84.9 mmol) mixture at a rate that the temperature of the reaction is maintained at 30 °C. The reaction mixture was stirred overnight at room temperature, and then neutralized with 1 N HCl solution. Aqueous solution was extracted with ethyl acetate several times, and the organic layers were collected, washed with water and brine, dried over sodium sulfate, and concentrated. Flash chromatography with $CHCl_3/CH_3OH$ (13%) as eluent afforded the title compound, 2.8 g (64%) as an oily product. ^1H NMR ($CDCl_3$): 2.0 (t, $J = 6$ Hz, 6H, CH_2-**CH_2**$-CN$); 3.4 (s, 6H, NH_2-C-**CH_2**$-O$); 3.7 (t, $J = 6$ Hz, 6H, **CH_2**$-CH_2-CN$).

(ii) 4-{*N*-{Tris[2-(cyanoethoxy)methyl]}methylamino}-4-oxobutanoic Acid. *N*-{Tris[(2-cyanoethoxy)methyl]} methylamine (2 g, 7.1 mmol) was dissolved in dichloromethane (20 mL) and treated with succinic anhydride (0.7 g, 7 mmol). Triethylamine was added to the reaction mixture, and stirring was continued overnight at room temperature. The solvent was removed by evaporation, and the residue was used as such for the next step.

(iii) Methyl 4-{*N*-{Tris[2-(cyanoethoxy)methyl]}methyl amino}-4-oxobutanoate. The above residue **ii** (1 g, 2.63 mmol) was dissolved in methanol (50 mL), 10 drops of concentrated sulfuric acid were added, and the reaction mixture was refluxed overnight. The solvent was removed by evaporation. The residue was dissolved in ethyl acetate, washed with 1 N $NaHCO_3$ and water, dried over $MgSO_4$, and concentrated. Flash column chromatography with $CHCl_3/CH_3OH$ (6%) as eluent afforded the title compound, 0.57 g (55%). ^1H NMR ($CDCl_3$): 2.5 (q, $J = 6$ Hz, 2H, $-CH_2-$**CH_2**$-CONH$); 2.6 (t, $J = 6$ Hz, 8H, $-$**CH_2**$-CH_2-CONH$, CH_2-**CH_2**$-CN$); 3.7 (t, $J = 6$ Hz, 6H, **CH_2**$-CH_2-CN$); 3.7 (s, 3H, **CH_3**$-O-CO-CH_2-CH_2-CONH$); 3.8 (s, 6H, $C-$**CH_2**$-O-$); 5.8 (br, 3H, $-$**NH**).

(iv) Compound 4. The synthesis was done following the procedure of S. Caddick et al.[54] Compound **iii** (300 mg, 0.8 mmol) was dissolved in methanol. Nickel chloride (330 mg, 2.54 mmol), sodium borohydride (670 mg, 17.8 mmol), and di-*tert*-butyl carbonate (1.11 g, 5.1 mmol) were added. The reaction mixture was stirred for 36 h at room temperature. The solvent was removed by evaporation. The residue was dissolved in a mixture of ethyl acetate (25 mL) and 1 N $NaHCO_3$ (25 mL), the mixture was filtered, the layers were separated, and the aqueous layer was extracted 2−3 times with ethyl acetate. The organic fractions were combined, dried over $MgSO_4$, and concentrated. Flash chromatography with $CHCl_3/CH_3OH$ (3−4%) as eluent afforded title compound **4**, 0.45 g (67%). ^1H NMR ($CDCl_3$): 1.4 (s, 27H, **Boc**); 1.7 (m, 6H, $-CH_2-$**CH_2**$-CH_2-NHBoc$); 2.6 (m, 4H, CH_3OCO-**$(CH_2)_2$**$-CONH$); 3.2 (m, 6H, $-CH_2-CH_2-$**CH_2**$-NHBoc$); 3.5 (t, $J = 6$ Hz, 6H, $O-$**CH_2**$-CH_2-CH_2-NHBoc$); 3.7 (s, 3H, **CH_3**$-O-CO-CH_2-CH_2-CONH-$); 3.7 (s, 6H, $-$**CH_2**$-O-CH_2$); 4.9 (s, 3H, $-$**NH**); 6.6 (br, 1H, $-$**NH**).

Synthesis of Ethyl (4*S*,5*S*)-2-(2-Hydroxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazole-4-carboxylate (*cis*-L^a). (i) Ethyl (2*S*,3*R*)-3-Hydroxy-2-[(2-benzyloxybenzoyl)amino] Butanoate. (2*S*,3*R*)-3-Hydroxy-2-[(2-benzyloxybenzoyl)amino]butanoic acid (**1**) (1 g, 3 mmol) was dissolved in ethanol (50 mL), and 10 drops of concentrated sulfuric acid were added. The reaction mixture was refluxed overnight. The solvent was evaporated; the residue was dissolved in a ethyl acetate (50 mL) and washed with water several times. The organic layer was dried over $MgSO_4$ and concentrated. Flash chromatography with $CHCl_3/CH_3OH$ (2−3%) as eluent afforded the title compound, 0.64 g (59%). ^1H NMR ($CDCl_3$): 1.0 (d, $J = 6.4$ Hz, 3H, $-$**$CH_3(\gamma)$**); 1.2 (t, $J = 7$ Hz, 3H, **CH_3**$-CH_2-O-CO-$); 4.15 (q, $J = 7$ Hz, 2H, CH_3-**CH_2**$-O-CO-$); 4.2 (dq, $J_1 = 6.4$ Hz, $J_2 = 8.3$ Hz, 1H, $-CH_3(\gamma)-$**$CH(\beta)$**$-CH(\alpha)$); 4.7 (dd, $J_1 = 2.5$ Hz, $J_2 = 8.3$ Hz, 1H, $-CH_3-CH(\beta)-$**$CH(\alpha)$**); 5.1 (s, 2H, $-O-$**CH_2**$-C_6H_5$); 7.1 (m, 2H, Ar); 7.35 (m, 6H, Ar); 8.2 (d, $J = 8$ Hz, 1H, Ar); 8.5 (br, 1H, $-$**NH**).

(ii) Ethyl (4*S*,5*S*)-2-(2-Benzyloxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazole-4-carboxylate. Ethyl (2*S*,3*R*)-3-hydroxy-2-[(2-benzyloxybenzoyl)amino] butanoate **i** (100 mg, 0.28 mmol) was dissolved in dichloromethane (20 mL), and thionyl chloride (170 μL, 1.5 mmol) was added. The reaction mixture was stirred at room temperature for 16 h. The anhydrous sodium carbonate was added until the solution turned basic. The precipitate was filtered out, and the organic phase was concentrated. The residue was purified by flash chromatography with $CHCl_3/CH_3OH$ (1−2%) as eluent to afford the title compound, 60 mg (63%). ^1H NMR ($CDCl_3$): 1.25 (t, $J = 7$ Hz, 3H, **CH_3**$-CH_2-O-CO-$); 1.4 (d, $J = 6.4$ Hz, 3H, $-$**$CH_3(\gamma)$**); 4.15 (q, $J = 7$ Hz, 2H, CH_3-**CH_2**$-O-CO-$); 5.1 (m, 4H, $O-$**CH_2**$-C_6H_5$, $CH_3(\gamma)-$**$CH(\beta)$**$-CH(\alpha)$); 7.0 (m, 2H, Ar); 7.35 (m, 6H, Ar); 7.7 (d, $J = 7.9$ Hz, 1H, Ar).

(iii) Compound *cis*-L^a. The benzyl protected compound **ii** (50 mg, 0.14 mmol) was dissolved in absolute ethanol (15 mL), and 10% Pd/C (15 mg) was added. Hydrogenolysis was carried out at room temperature for 10 h at 1 atm. The catalyst was filtered out, and the evaporation of the solvent afforded the desired final compound *cis*-**L^a**, 20 mg (55%). ^1H NMR ($CDCl_3/CD_3OD$): 1.2 (t, $J = 7$ Hz, 3H, **CH_3**$-CH_2-O-CO-$); 1.4 (d, $J = 6.4$ Hz, 3H, $-$**$CH_3(\gamma)$**); 4.15 (q, $J = 7$ Hz, 2H, CH_3-**CH_2**$-O-CO-$); 5.1 (m, 2H, $-CH_3(\gamma)-$**$CH(\beta)$**$-CH(\alpha)$); 6.8 (dt, $J = 8.1$ Hz, 1H, Ar); 6.9 (d, $J = 7.4$ Hz, 1H, Ar); 7.4 (t, $J = 7.4$ Hz, 1H, Ar); 7.6 (d, $J = 8.1$ Hz, 1H, Ar). ^{13}C NMR ($CDCl_3$): 13.2 (CH_3); 15.9 (CH_3); 59.3 (CH_2); 70.2 (CH); 77.3 (CH); 110.0 (C); 116.0 (CH); 118.0 (CH); 128.0 (CH); 133 (CH); 160.1 (CH); 167.5 (C); 169.6 (CO). ESI-MS: 250 $[M + H]^+$; 272 $[M + Na]^+$.

Synthesis of Ethyl (4*S*,5*R*)-2-(2-Hydroxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazole-4-carboxylate (*trans*-L^a). Pentachlorophenyl (4*S*,5*R*)-2-(2-hydroxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazole -4-carboxylate **2** (200 mg, 0.5 mmol) was dissolved in ethanol (10

(54) Caddick, S.; de K. Haynes, A. K.; Judd, D. B.; Williams, M. R. V. *Tetrahedron Lett.* **2000**, *41*, 3513−3516.

mL) and 5−6 drops of triethylamine. This mixture was stirred overnight at room temperature. The solvent was evaporated and purified by flash chromatography with $CHCl_3/CH_3OH$ (5−6%) as eluent to afford 54 mg (41%) of compound *trans*-**L**[a]. ^{1}H NMR ($CDCl_3$): 1.2 (t, J = 7 Hz, 3H, **CH$_3$**−CH_2−O−CO−); 1.6 (d, J = 6.4 Hz, 3H, −**CH$_3$**(γ)); 4.15 (q, J = 7 Hz, 2H, CH_3−**CH$_2$**−O−CO−); 4.4(d, 1H, J = 7 Hz, CH_3(γ)-CH(β)-**CH**(α)); 4.9 (m, 1H, J_1 = 6.3 Hz, J_2 = 7 Hz, CH_3(γ)-**CH**(β)-CH(α)); 6.8 (dt, J = 8.1 Hz, 1H, Ar); 6.9 (d, J = 7.4 Hz, 1H, Ar); 7.4 (t, J = 7.4 Hz, 1H, Ar); 7.6 (d, J = 8.1 Hz, 1H, Ar). ESI-MS: 250 $[M + H]^+$; 272 $[M + Na]^+$.

Synthesis of Methyl 4-{*N*-Tris{{3-{[(4*S*,5*S*)-2-(2-hydroxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazol-4-yl]carbamido}propoxy}methyl}methylamino}-4-oxobutanoate (*cis*-L[c]). (i) Methyl 4-{*N*-Tris{{3-{(2*S*,3*R*)-3-hydroxy-2-[(2-benzyloxy benzoyl)amino]butyrylamino}propoxy}methyl} methyl amino}-4-oxobutanoate. Compound **3** (100 mg, 0.14 mmol) was dissolved in dichloromethane (10 mL) and TFA (3 mL). The mixture was stirred at room temperature for an hour. The solvent was removed by evaporation. The residue was dissolved in THF (20 mL), and the (2*S*,3*R*)-3-hydroxy-2-[(2-benzyloxybenzoyl)amino]butanoic acid (**1**) (170 mg, 0.5 mmol) was added. Triethylamine was added up to pH = 8, the reaction mixture was cooled to 0 °C, and HOBt (5 mg, 0.05 mmol) and DIC (88 μL, 0.64 mmol) were added. The stirring was continued overnight at room temperature. The solvent was evaporated. Flash chromatography with $CHCl_3/CH_3OH$ (7%) as eluent afforded the title compound 60 mg (26%). ^{1}H NMR ($CDCl_3$): 1.1 (d, J = 6.4 Hz, 9H, −**CH$_3$(γ)**); 1.7 (m, 6H, −CH_2−**CH$_2$**−CH_2−NHCO); 2.5 (m, 4H, CH_3−O−CO−**CH$_2$**−**CH$_2$**−CONH); 3.3 (m, J = 6 Hz, 6H, −CH_2−CH_2−**CH$_2$**−NHCO); 3.4 (t, J = 6 Hz, 6H, O−**CH$_2$**−CH_2−CH_2−NHCO); 3.5 (s, 3H, **CH$_3$**−O−CO−CH_2−CH_2−CONH); 3.6 (s, 6H, −**CH$_2$**−O−CH_2−CH_2−CH_2−NHCO); 4.3 (dq, J_1 = 6.4 Hz, J_2 = 8.3 Hz, 3H, CH_3(γ)−**CH(β)**−CH(α)); 4.5 (dd, J_1 = 2.4 Hz, J_2 = 8.3 Hz, 3H, −CH_3−CH(β)−**CH(α)**); 5.1 (s, 6H, −O−**CH$_2$**−C_6H_5); 7.1 (m, 7H, Ar); 7.4 (m, 17H, Ar); 7.6 (dd, J_1 = 1.7 Hz, J_2 = 7.9 Hz, 3H, Ar); 8.8 (br, 3H, **NH**).

(ii) Methyl 4-{*N*-Tris{{3-{[(4*S*,5*S*)-2-(2-benzyloxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazol-4-yl]carbamido}propoxy}methyl}-methylamino}-4-oxobutanoate. Compound **i** (50 mg, 0.03 mmol) was dissolved in dichloromethane (20 mL), and thionyl chloride (35 μL, 0.3 mmol) was added. The mixture was stirred at room temperature for 16 h. The anhydrous sodium carbonate was added until the solution turned to be basic. The precipitate was filtered out, and the organic phase was concentrated. The residue was purified by flash chromatography with $CHCl_3/CH_3OH$ (4−5%) as eluent to afford the title compound, 30 mg (62%). ^{1}H NMR ($CDCl_3$): 1.3 (d, J = 6.4 Hz, 9H, −**CH$_3$(γ)**); 1.5 (m, 6H, −CH_2−**CH$_2$**−CH_2−NHCO); 2.4 (m, 4H, CH_3−O−CO−**CH$_2$**−**CH$_2$**−CONH); 3.4 (m, 12H, −O−**CH$_2$**−CH_2−**CH$_2$**−NHCO); 3.6 (s, 9H, −**CH$_2$**−O−CH_2−CH_2−CH_2−NHCO, **CH$_3$**−O−CO−CH_2−CH_2−NHCO); 4.8 (d, J = 8.3 Hz, 3H, −CH_3−CH(β)−**CH(α)**); 5.1 (m, 9H, O−**CH$_2$**−C_6H_5, CH_3(γ)−**CH(β)**−CH(α)) 7.0 (m, 7H, Ar); 7.35 (m, 17H, Ar); 7.7 (d, J = 7.9 Hz, 3H, Ar).

(iii) Compound *cis*-L[c]. *N*-{Tris{3-{2-[2-(2-benzyloxy-phenyl)-5*S*-methyl-4,5-dihydrooxazol-4*S*-yl]carbamido}propyloxymethyl}-methyl-4-methoxycarbonylpropionamide **ii** (30 mg, 0.188 mmol) was dissolved in absolute ethanol (15 mL), and 10% Pd/C (15 mg) was added. Hydrogenolysis was carried at room temperature for 10 h at 1 atm. The catalyst was filtered out, and the evaporation of the solvent afforded the desired final compound **L**[c], 15 mg (65%). $[\alpha]_D^{27}$ −71.8 (*c* 0.85, $CHCl_3$). ^{1}H NMR ($CDCl_3/CD_3OD$): 1.3 (d, J = 6.4 Hz, 9H, −**CH$_3$**(γ)); 1.5 (m, 6H, −CH_2−**CH$_2$**−CH_2−NHCO); 2.3 (m, 4H, CH_3−O−CO−**CH$_2$**−**CH$_2$**−CONH); 3.34 (m, 12H, −O−**CH$_2$**−CH_2−**CH$_2$**−NHCO); 3.6 (s, 9H, −**CH$_2$**−O−CH_2−CH_2−CH_2−NHCO, **CH$_3$**−O−CO−CH_2−CH_2−CONH); 4.9 (d, J = 8.3 Hz, 3H, −CH_3−CH(β)−**CH**(α)); 5.1(m, J_1 = 6.4 Hz, J_2 = 8.3 Hz, 3H, −CH_3(γ)−**CH**(β)−CH(α)); 6.5 (br, 1H, −**NH**); 6.8 (br, 3H, −**NH**); 6.7 (t, J = 7.9 Hz, 3H, Ar); 6.9 (d, J = 8.4 Hz, 3H, Ar); 7.4 (t, J = 8.4 Hz, 3H, Ar); 7.6 (d, J = 7.9 Hz, 3H, Ar). ^{13}C NMR ($CDCl_3$):15.9 (CH_3), 29.4 (CH_2), 36.6 (CH_2), 41.96 (CH_2), 51.5 (CH_2), 59.6 (CH_2), 68.6 (CH_2), 69.5 (C), 70.9 (CH), 76.5 (CH), 110.1 (C), 116.8 (CH), 119.0 (CH), 128.4 (CH), 134.2 (CH), 159.7 (CH), 167.6 (C), 168.6 (CO). ESI-MS: 1016 $[M + H]^+$; 1038 $[M + Na]^+$.

Synthesis of Methyl 4-{*N*-Tris{{3-{[(4*S*,5*R*)-2-(2-hydroxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazol-4-yl]carbamido}propoxy}methyl}methylamino}-4-oxobutanoate (*trans*-L[c]). Compound **4** (50 mg, 0.07 mmnol) was dissolved in dichloromethane (10 mL) and TFA (3 mL). The mixture was stirred at room temperature for 1 h. The solvent was removed by evaporation. The residue was dissolved in THF (20 mL) and pentachlorophenyl (4*S*,5*R*)-2-(2-hydroxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazole-4-carboxylate (**2**) (164 mg, 0.35 mmol) followed by addition of triethylamine up to pH = 8. The reaction mixture was stirred overnight at room temperature. The solvent was evaporated. Flash chromatography with $CHCl_3/CH_3OH$ (4−5%) as eluent afforded the title compound, 60 mg (90%). $[\alpha]_D^{27}$ −22.1 (*c* 0.78, $CHCl_3$). ^{1}H NMR ($CDCl_3/CD_3OD$): 1.6 (d, J = 6.3 Hz, 9H, −**CH$_3$(γ)**); 1.7 (m, 6H, −CH_2−**CH$_2$**−CH_2−NHCO); 2.3 (m, 4H, CH_3−O−CO−**CH$_2$**−**CH$_2$**−CONH); 3.34 (m, 12H, −O−**CH$_2$**−CH_2−**CH$_2$**−NHCO); 3.6 (s, 9H, C−**CH$_2$**−O−CH_2−CH_2−CH_2−NHCO, **CH$_3$**−O−CO−CH_2−CH_2−CONH); 4.3 (d, J = 7.6 Hz, 3H, −CH_3−CH(β)−**CH(α)**); 4.9 (m, J_1 = 6.3 Hz, J_2 = 7.6 Hz, 3H, −CH_3(γ)−**CH(β)**−CH(α)); 6.5 (br, 1H, −**NH**); 6.8 (br, 3H, −**NH**); 6.7 (t, J = 7.9 Hz, 3H, Ar); 6.9 (d, J = 8.4 Hz, 3H, Ar); 7.4 (t, J = 8.4 Hz, 3H, Ar); 7.6 (d, J = 7.9 Hz, 3H, Ar). ^{13}C NMR ($CDCl_3$):21.5 (CH_3), 29.2 (CH_2), 36.6 (CH_2), 41.96 (CH_2), 51.5 (CH_2), 59.6 (CH_2), 68.6 (CH_2), 69.5 (C), 73.9 (CH), 79.6 (CH), 110.1 (C), 116.8 (CH), 119.0 (CH), 128.4 (CH), 134.2 (CH), 159.7 (CH), 167.6 (C), 168.6 (CO). ESI-MS: 1016 $[M + H]^+$; 1038 $[M + Na]^+$.

Synthesis of 1,1,1-Tris{{2-{{[(4*S*,5*S*)-2-(2-hydroxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazol-4-yl]carbamido}ethylaminocarbonyl}ethoxy}methyl}propane (*cis*-L[b]). (i) 1,1,1-Tris{{2-{{(2*S*,3*R*)-3-hydroxy-2-[(2-benzyloxybenzoyl)]butyrylamino}ethylaminocarbonyl}ethoxy}methyl}propane. The compound was prepared by using the procedure described for methyl 4-{*N*-tris{{3-{(2*S*,3*R*)-3-hydroxy-2-[(2-benzyloxybenzoyl)amino]butyrylamino}propoxy}methyl}methylamino}-4-oxobutanoate. Flash chromatography with $CHCl_3/CH_3OH$ (6%) as eluent afforded the desired compound, 156 mg (21%). ^{1}H NMR ($CDCl_3$): 0.8 (t, J = 7.5 Hz, 3H, **CH$_3$**−CH_2−C−CH_2−O); 1.0 (d, J = 6.4 Hz, 9H, **CH$_3$(γ)**); 1.3 (q, J = 7.5 Hz, 2H, CH_3−**CH$_2$**−C−CH_2−O); 2.5 (t, J = 6.2 Hz, 6H, CONH−CH_2−**CH$_2$**−NHCO); 3.2 (m, 18H, −C−**CH$_2$**−O−CH_2−**CH$_2$**−CONH−**CH$_2$**−CH_2−NHCO); 3.6 (m, 6H, −C−CH_2−O−**CH$_2$**−CH_2−CONH); 4.38 (q, J_1 = 6.4 Hz, J_2 = 8.35 Hz, 3H, CH_3(γ)−**CH(β)**−CH(α)); 4.46 (dd, J = 8.35 Hz, 3H, −CH_3−CH(β)−**CH(α)**); 5.2 (s, 6H, O−**CH$_2$**−C_6H_5); 7.06 (m, 7H, Ar); 7.4 (m, 17H, Ar); 8.0 (dd, J = 7.4 Hz, 3H, Ar); 8.68 (b, 3H, **NH**).

(ii) 1,1,1-Tris{{2-{{[(4*S*,5*S*)-2-(2-benzyloxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazo l-4-yl]carbamido}ethylaminocarbonyl}ethoxy}methyl}propane. The compound was prepared following the procedure described for methyl 4-{*N*-*tris*{{3-{[(4*R*,5*R*)-2-(2-benzyloxyphenyl)-5-methyl-4,5-dihydro-1,3-oxazol-4 -yl]carbamido}propoxy}methyl}methylamino}-4-oxobutanoate. Flash chromatog-

raphy with $CHCl_3/CH_3OH$ (4%) as eluent afforded the desired compound, 73 mg (56%). ^1H NMR ($CDCl_3$): 0.8 (t, $J = 7.5$ Hz, 3H, **CH_3**$-CH_2-C-CH_2-O$); 1.28 (q, $J = 7.5$ Hz, 2H, CH_3-**CH_2**$-C-CH_2-O$); 1.34 (d, $J = 6.4$ Hz, 9H, **$CH_3(\gamma)$**); 2.36 (t, $J = 6.2$ Hz, 6H, $CONH-CH_2-$**CH_2**$-NHCO$); 3.2 (m, 18H, CH_3-CH_2-C-**CH_2**$-O-CH_2-$**CH_2**$-CONH-$**CH_2**$-CH_2-NHCO-$); 3.6 (m, 6H, $CH_3-CH_2-C-CH_2-O-$**CH_2**$-CH_2-CONH$); 4.95 (d, $J = 8.35$ Hz, 3H, $-CH_3-CH(\beta)-$**$CH(\alpha)$**); 5.2 (m, 9H, $O-CH_2-C_6H_5$, $CH_3(\gamma)-$**$CH(\beta)$**$-CH(\alpha)$); 7.0 (m, 7H, Ar); 7.35 (m, 17H, Ar); 7.8 (t, $J = 7.9$ Hz, 3H, Ar).

(iii) Compound *cis*-L^b. The compound was prepared using the procedure described for compound ***cis*-L^c**. Preparative TLC with $CHCl_3/CH_3OH$ (6%) as eluent afforded the final desired compound ***cis*-L^b**, 8 mg (30%). $[\alpha]_D^{27}$ $^-$44.4 (*c* 0.61, $CHCl_3$). ^1H NMR ($CDCl_3$): 0.8 (t, $J = 7.5$ Hz, 3H, **CH_3**$-CH_2-C-CH_2-O$); 1.3 (q, $J = 7.5$ Hz, 2H, CH_3-**CH_2**$-C-CH_2-O$); 1.4 (d, $J = 6.4$ Hz, 9H, **$CH_3(\gamma)$**); 2.6 (t, $J = 6.2$ Hz, 6H, $CONH-CH_2-$**CH_2**$-NHCO$); 3.25 (m, 18H, CH_3-CH_2-C-**CH_2**$-O-CH_2-$**CH_2**$-CONH-$**CH_2**$-CH_2-NHCO-$); 3.6 (t, $J = 6.2$ Hz, 6H, $CH_3-CH_2-C-CH_2-O-$**CH_2**$-CH_2-CONH$); 4.9 (d, $J = 8.35$ Hz, 3H, $-CH_3-CH(\beta)-$**$CH(\alpha)$**); 5.2 (m, 3H, $CH_3(\gamma)-$**$CH(\beta)$**$-CH(\alpha)$); 6.4 (br, 3H, $-$**NH**); 6.9 (t, $J = 7.9$ Hz, 3H, Ar); 7.0 (d, $J = 8.37$ Hz, 3H, Ar); 7.1 (br, 3H, $-$**NH**), 7.4 (t, $J = 8.4$ Hz, 3H, Ar); 7.7 (d, $J = 7.9$ Hz, 3H, Ar). ESI-MS: 1086 $[M + H]^+$; 1108 $[M + Na]^+$.

Electrospray Mass Spectrometric Measurements. Electrospray mass spectra of ferric complexes with monomer *cis*-**L^a** and tripods *cis*-**L^b** and *cis*-**L^c** were obtained with an ion-trap instrument (Bruker Esquire 3000plus, Bruker Daltonic, Bremen, Germany) equipped with an Agilent electrospray (ESI) ion source (Agilent Headquarters, Palo Alto, CA). The solutions of ligands *cis*-**L^a** (2.78×10^{-5} M) with 0.33 equiv of iron(III), and *cis*-**L^b** (1.66×10^{-5} M) or *cis*-**L^c** (4.68×10^{-5} M) with 0.5 equiv of iron(III), prepared in MeOH/H_2O solvent (80/20 by weight), were continuously introduced into the mass spectrometer source with a syringe pump (Cole-Parmer Instrument Company, Vernon Hills, IL) at a flow rate of 3.33 μL/min. For electrospray ionization, the drying gas was heated at 350 °C (**L^a**) or 150 °C (**L^b** and **L^c**). Its flow was set at 5 L/min, with 43 psi (**L^a**) or 18 psi (**L^b** and **L^c**) nebulizer pressure. The capillary and skimmer voltage were set at 4000 and 40 V, respectively. The capillary exit was adjusted at 250 V (**L^a**) or 280 V (**L^b** and **L^c**). Scanning was performed from $m/z = 200$ to 2000, and no fragmentation process was observed.

Protonated Species of Monomer L^a. A solution of **L^a** ([*cis*-**L^a**]$_{tot}$ $= 9.23 \times 10^{-5}$ M or [*trans*-**L^a**]$_{tot}$ $= 1.20 \times 10^{-4}$ M) was prepared by quantitative dissolution of solid sample in methanol/water solvent (80/20 by weight). An aliquot of 40 mL was introduced into a jacketed cell (Metrohm 6.1414.150). The solution was continuously deoxygenated by bubbling with oxygen-free argon. The titration of *cis*-**L^a** ($2.06 < pH < 10.81$) or *trans*-**L^a** ($2.38 < pH < 10.86$) was carried out by addition of known volumes of sodium hydroxide solution (BDH, AnalR). Special care was taken to ensure that complete equilibration was attained. Absorption spectra versus pH were recorded using a Varian CARY 50 spectrophotometer fitted with Hellma optical fibers (Hellma, 041.002-UV) and an immersion probe made of quartz suprazil (Hellma, 661.500-QX).

Protonated Species of Tripods L^b and L^c. Potentiometric titrations of tripods *cis*-**L^c** (1.41×10^{-3} M) and *trans*-**L^c** (1.64×10^{-3} M) were performed with an automatic titrator system DMS 716 Titrino (Metrohm) and a combined glass electrode (Metrohm 6.0234.100, Long Life). The combined glass electrode was calibrated as a hydrogen concentration probe by titrating known amounts of perchloric acid ($\approx 10^{-1}$ M) with CO_2-free sodium hydroxide solutions ($\approx 10^{-1}$ M).[55] A stream of argon, presaturated with water vapor, was passed over the surface of the solution.

Ferric Complexes with Monomer L^a. Aliquots of 40 mL of *cis*-**L^a** (7.85×10^{-5} M) or of 20 mL of *trans*-**L^a** (8.00×10^{-5} M) were introduced into a jacketed cell. An adequate volume of ferric stock solution (1.24×10^{-2} M) was added in order to obtain a [**L^a**]$_{tot}$/[Fe(III)]$_{tot}$ ratio equal to 3. The titrations of the ferric complexes of *cis*-**L^a** ($2.04 < pH < 9.31$) or *trans*-**L^a** ($2.32 < pH < 9.54$) were carried out by addition of a sodium hydroxide solution. Special care was taken to ensure that complete equilibration was attained. Absorption spectra versus pH were recorded using a CARY 50 spectrophotometer fitted as previously described.

Ferric Complexes with Tripod *cis*-L^b. An aliquot of 5 mL of ligand *cis*-**L^b** (1.33×10^{-5} M) was mixed with 54 μL of iron(III) solution (1.23×10^{-3} M) in order to obtain a [*cis*-**L^b**]$_{tot}$/[Fe(III)]$_{tot}$ ratio equal to 1. An aliquot of 4 mL of this solution was introduced into a 2 cm optical cell (Hellma). The titration of the ferric complexes of *cis*-**L^b** ($3.37 < pH < 9.57$) was carried out by addition of sodium hydroxide. Special care was taken to ensure that complete equilibration was attained. Absorption spectra were recorded on a KONTRON 941 spectrophotometer.

Ferric Complexes with Tripods L^c. An aliquot of 2 mL of ligand **L^c** ([*cis*-**L^c**]$_{tot}$ $= 4.68 \times 10^{-5}$ M or [*trans*-**L^c**]$_{tot}$ $= 4.45 \times 10^{-5}$ M) was mixed in a 1 cm optical cell (Hellma) with ferric stock solution (2.06×10^{-3} M) in order to obtain a [**L^c**]$_{tot}$/[Fe(III)]$_{tot}$ ratio equal to 1. The titrations of the ferric complexes of *cis*-**L^c** ($1.87 < pH < 10.67$) or *trans*-**L^c** ($2.78 < pH < 10.83$) were carried out by addition of sodium hydroxide. Special care was taken to ensure that complete equilibration was attained. Absorption spectra were recorded using a CARY 300 spectrophotometer.

Fluorescence Titrations. Solutions of *cis*-**L^b** (1.1×10^{-5} M) and *cis*-**L^c** (8.85×10^{-6} M) were prepared by dilution of stock solutions in methanol/water (80/20 by weight). The pH was maintained at 5.80 ± 0.05 by the use of 0.05 M sodium acetate (Prolabo) and perchloric acid (Prolabo, normapur, 70% min). The protonation constant of acetic acid is equal to 6.34 in this solvent.[56] The luminescence titrations were carried out on samples with an absorbance smaller than 0.05 at wavelengths $\geq \lambda_{exc}$ in order to avoid any errors due to the inner filter effect or reabsorption. Aliquots (2 mL) of *cis*-**L^b** or *cis*-**L^c** were introduced into a 1 cm cell (Hellma), and the titrations of the tripodal ligands were carried out by addition of microvolumes of the ferric stock solution with an Eppendorf microburette. The [Fe(III)]$_{tot}$/[**L**]$_{tot}$ ratio was varied from 0 to 3.0. The kinetics of complex formation is fast enough to ensure that all the samples did reach equilibrium under our experimental conditions. Fluorescence emission spectra were recorded on a Perkin-Elmer LS-50B spectrofluorimeter. For *cis*-**L^b** and *cis*-**L^c**, the following parameters were used: *cis*-**L^b** $\lambda_{exc} = 340 \pm 1$ nm, $\lambda_{em} = 412 \pm 1$ nm, excitation and emission slits 4 nm; *cis*-**L^c** $\lambda_{exc} = 340 \pm 1$ nm, $\lambda_{em} = 408 \pm 1$ nm, excitation and emission slits 12 nm. The source was a pulsed xenon flash lamp with a pulse width at half peak height < 10 μs and power equivalent to 20 kW.

Refinement of the Data. The spectrophotometric data were processed with the Specfit program, which adjusts the stability constants and the corresponding molar extinction coefficients (M^{-1} cm^{-1}) of the species at equilibrium. Specfit[57−60] uses factor analysis

(55) Gans, P.; O'Sullivan, B. *Talanta* **2000**, *51*, 33−37.
(56) Rorabacher, D. B.; MacKellar, W. J.; Shu, F. R.; Bonavita, M. *Anal. Chem.* **1971**, *43*, 561−573.
(57) Gampp, H.; Maeder, M.; Meyer, C. J.; Zuberbühler, A. D. *Talanta* **1985**, *32*, 95−101.
(58) Rossoti, F. J. C.; Rossoti, H. S.; Whewell, R. J. *J. Inorg. Nucl. Chem.* **1971**, *33*, 2051−2065.

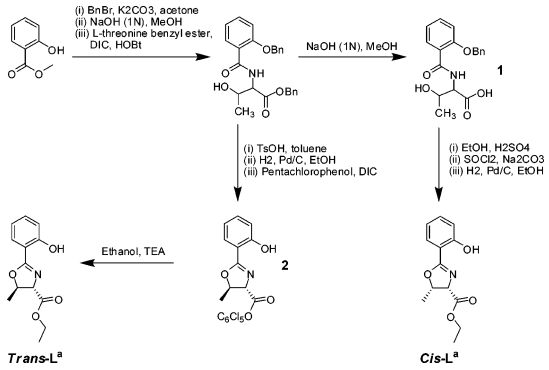
to reduce the absorbance matrix and to extract the eigenvalues prior to the multiwavelength fit of the reduced data set according to the Marquardt algorithm.[61,62] Distribution curves of the various species were calculated using the Haltafall program.[63] Origin 5.0 was used to process the analytical results.[64] The potentiometric data (about 140 points collected over the pH range 2.6−12.0) were refined[65,66] with Hyperquad 2000. For the sake of simplicity, charges are omitted in all the chemical equilibria.

Electrochemistry. Cyclic voltammetry of the ferric complexes with *cis*-**L**^{a} was performed on a Voltalab 50 potentiostat/galvanostat (Radiometer Analytical) controlled by the Voltamaster 4 electrochemical software at different scan rates and at room temperature (23(1) °C). A conventional three-electrode cell (5 mL) was employed in our experiments with a glassy carbon disk ($s = 1$ cm^2) as a working electrode, a Pt wire as a counter electrode, and KCl (3.5 M)/Ag/AgCl reference electrode (+205 mV vs NHE).[67] Redox potentials were determined from oxidation and reduction potentials. (*cis*-**L**^{a})$_3$Fe complexes (1.34×10^{-3} M) were prepared by mixing an acidic $Fe(ClO_4)_3$ solution with ligand *cis*-**L**^{a} dissolved in a mixed solvent (80% DMF/20% water; v/v; $NaClO_4$, 1 M). The apparent free hydrogen ion concentration was measured with a combined glass electrode (Metrohm 6.0234.500, Long Life) and a Tacussel Isis 20.000 millivoltmeter. The Ag/AgCl reference electrode was filled with NaCl (0.01 M, Fluka, p.a.) and $NaClO_4$ (0.09 M, Fluka, p.a.). Standardization of the millivoltmeter and verification of the linearity ($3.00 < pH < 9.00$) of the electrode were performed using commercial buffers (Merck, Titrisol) according to classical methods.[68] All the solutions were deoxygenated with argon for 30 min (Sigma Oxiclear cartridge).

Results and Discussion

Synthesis. 2-Oxazolines have found numerous uses in synthetic organic chemistry[69] during the more than 100 years since their discovery. This heterocycle has served as protecting group,[70] coordinating ligand,[71,72] as well as activating moiety. The well-defined reactivity of chiral oxazolines has given rise to many efficient approaches for asymmetric synthesis including their use as ligands in asymmetric catalysis.[73−75] In this paper, we describe a new class of biomimetic iron(III) ligands based on HPO.

Scheme 1. Synthetic Route Employed for Compounds **1** and *cis*- and *trans*-**L**^{a}



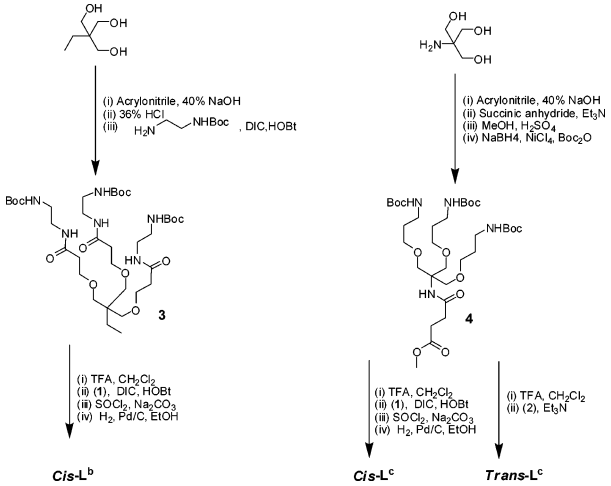
The preparation of optically pure (*S*)-2-(2′-hydroxyphenyl)-4,5-dihydrooxazole-4-carboxylic acid was first reported by Maurer and Miller.[76] 2-Benzyloxybenzoic acid was coupled with methyl ester of L-serine using dicyclohexylcarbodiimide (DCC), and cyclization of the resulting product was achieved by using $SOCl_2$. With a similar strategy, compound **1** was synthesized by attaching a hydroxyl amino-acid, L-threonine, to salicylic acid. An intramolecular dehydrative cyclization with $SOCl_2/Na_2CO_3$,[77,78] followed by debenzylation, provided the HPO-type ligand *cis*-**L**^{a} (Scheme 1), while the formation of the *trans* derivative (**2**) was afforded under mild acidic conditions (Scheme 1).[50] Two distinct geometrical isomers (*cis*[49] and *trans*[50]) can be obtained, depending on the mode of cyclization. In this paper, we will deal with both the *cis* and *trans* isomers derived from the natural L-threonine.

Three target molecules of tripodal structure were prepared. Two different anchoring segments (**3** and **4**) were utilized to couple with precursors **1** and **2**. The first one (**3**) was prepared from 1,1,1-tris(hydroxymethyl)propane, while the second one (**4**) was prepared from tris(hydroxymethyl)-aminomethane (Scheme 2). The anchoring part (**4**) possesses an amine group, which can be elongated with any appropriate fragment for surface binding. In the case of **L**^{c}, monomethyl succinate ester was used to obtain the fourth functional terminal chain.

The synthesis of *cis*-**L**^{b} and *cis*-**L**^{c} was completed by intramolecular dehydrative cyclization followed by dehydrogenolysis and resulted in *cis*-phenol-oxazoline-type ligands **L**^{b} and **L**^{c} (Scheme 2). The *trans* derivative was obtained from direct coupling of compounds **2** and **4** to yield *trans*-**L**^{c}. The tripodal ligands are based on a C_3 symmetrical carbon anchor and possess a binding cavity for metals with an octahedral geometry. Receptor *cis*-**L**^{b} differs from *cis*-**L**^{c} in the chain length between the carbon anchor and the

(59) Gampp, H.; Maeder, M.; Meyer, C. J.; Zuberbühler, A. D. *Talanta* **1985**, *32*, 257−264.
(60) Gampp, H.; Maeder, M.; Meyer, C. J.; Zuberbühler, A. D. *Talanta* **1986**, *33*, 943−951.
(61) Marquardt, D. W. *J. Soc. Ind. Appl. Math.* **1963**, *11*, 431−441.
(62) Maeder, M.; Zuberbühler, A. D. *Anal. Chem.* **1990**, *62*, 2220−2224.
(63) Ingri, N.; Kakolowicz, W.; Sillen, L. G.; Warnqvist, B. *Talanta* **1967**, *14*, 1261−1286.
(64) *Microcal Origin, 1997*; Microcal Software, Inc.: Northampton, MA.
(65) (a) Gans, P.; Sabatini, A.; Vacca, A. *HYPERQUAD2000, Version 2.1 (NT)*; Leeds, U.K., and Florence, Italy, 2000. (b) Gans, P.; Sabatini, A.; Vacca, A. *Talanta* **1996**, *43*, 1739−1753.
(66) Gans, P. *Data Fitting in the Chemical Sciences*; John Wiley & Sons: Chichester, 1992.
(67) Sawyer, D. T.; Sobkowiak, A.; Roberts, J. L., Jr. *Electrochemistry for Chemists*, 2nd Ed.; Wiley: New York, 1995; p 192.
(68) Martell, A. E.; Motekaitis, R. J. *Determination and Use of Stability Constants*; VCH: Weiheim, Germany, 1988; Chapter 1.
(69) Gant, T. G.; Meyers, A. I. *Tetrahedron* **1994**, *50*, 2297−2360.
(70) Meyers, A. I.; Temple, D. L.; Haidukewych, D.; Mihelich, E. D. *J. Org. Chem.* **1974**, *39*, 2787−2793.
(71) Berg, D. J.; Zhou, C.; Barclay, T.; Fei, X.; Feng, S.; Ogilvie, K. A.; Gossage, R. A.; Twamley, B.; Wood, M. *Can. J. Chem.* **2005**, *83*, 449−459.
(72) (a) Hahn, F. E.; McMurry, T. J.; Hugi, A.; Raymond, K. N. *J. Am. Chem. Soc.* **1990**, *112*, 1854−1860. (b) Bergeron, R. J.; Liu, C. Z.; McManis, J. S.; Xia, M. X. B.; Algee, S. E.; Wiegand, J. *J. Med. Chem.* **1994**, *37*, 1411−1417. (c) Brittenham, G. M. *Alcohol* **2003**, *30*, 151−158.
(73) Zhou, J.; Tang, Y. *Chem. Soc. Rev.* **2005**, *34*, 664−676.
(74) McManus, H. A.; Guiry, P. J. *Chem. Rev.* **2004**, *104*, 4151−4202.
(75) (a) Gómes, M.; Muller, G.; Rocamora, M. *Coord. Chem. Rev.* **1999**, *193*−*195*, 769−835. (b) Braunstein, P.; Naud, F. *Angew. Chem., Int. Ed.* **2001**, *40*, 680−699.
(76) Maurer, P. J.; Miller, M. J. *J. Am. Chem. Soc.* **1983**, *105*, 240−245.
(77) Johnson, W. S.; Schubert, E. N. *J. Am. Chem. Soc.* **1950**, *72*, 2187−2190.
(78) Makino, K.; Okamoto, N.; Hara, O.; Hamada., Y. *Tetrahedron: Asymmetry* **2001**, *12*, 1757−1762.

Scheme 2. Synthetic Route Employed for Anchoring Fragments **3** and **4** and for Compounds *cis*-**L**^{b} and *cis*- and *trans*-**L**^{c}



binding units, while receptors *cis*-**L**^{c} and *trans*-**L**^{c} present different geometrical isomers of the oxazoline ring. It is noteworthy that the coordination of bidentate moieties, such as N,O in 2-(2′-hydroxyphenyl-oxazoline) derivatives, to an octahedral metal could result in two optical isomers (Δ and Λ)[79] and two geometric ones (facial and meridional).[80] The anchoring of ligating groups controls their orientation and restricts the number of isomers favoring the *fac* isomer. Moreover, it has also been shown in analogous systems that the amide-containing spacers induce interstrand hydrogen bonds thus minimizing random coiling and stabilizing specific conformations.[81] All these features together could result in strong preorganization of the HPO binding sites in **L**^{b} and **L**^{c}.

Thermodynamics and Spectrophotometry. Ligand Properties. In order to evaluate the competition between protons and iron, it was necessary to determine first the acido-basic properties of the ligands considered in this work. Both tripods **L**^{b} and **L**^{c} possess three 2-(2′-hydroxylphenyl)-oxazoline binding units and therefore six protonation sites. The statistical treatment[57−60] of the spectrophotometric and potentiometric data of *cis*-**L**^{a} between pH 2.06 and 10.91 and *trans*-**L**^{a} between pH 2.38 and 10.86 led to two successive protonation constants (Table 1).

Under basic conditions, deprotonated ligand **L**^{a} is characterized by a main absorption band centered at 311 nm with a shoulder at about 332 nm. The longer wavelength bands at $\lambda > 300$ nm have been attributed to a $\pi-\pi^*$ state with charge-transfer character,[82,83] while the band centered at 245 nm concerns the oxazoline chromophore. The first protonation led to the concomitant hypsochromic shift and blending of these two absorption band to 306 nm, while the transitions lying at higher energies remain unchanged in agreement with the protonation of the phenolate unit. The second protonation induced bathochromic shifts (about 15 nm) of the $\pi-\pi^*$ and oxazoline transitions, respectively, and evidenced the protonation of the imine function (Figure S3 for *cis*-**L**^{a} and Figure S4 for *trans*-**L**^{a}). Furthermore, no difference was observed between the acido-basic properties of monomers *cis*- and *trans*-**L**^{a}. In spite of different experimental conditions, the protonation constants of **L**^{a} are in good agreement with those of 2-hydroxyphenylbenzoxazole,[84] 2-(2′-hydroxyphenyl) pyridine,[85] 8-hydroxyquinoline,[86] and 2-(2′-hydroxyphenyl) oxazole,[83] which possess analogous functionalities (Table S1).

Table 1. Protonation Constants of the HPO Derivatives[a]

monomer	phenol log K_1^H	oxazoline log K_2^H
cis-**L**^{a}	9.83(5)	2.6(1)
trans-**L**^{a}	9.34(3)	3.0(1)

	phenol			oxazoline		
tripod	log K_1^H	log K_2^H	log K_3^H	log K_4^H	log K_5^H	log K_6^H
cis-**L**^{c}	13.0[b]	12.5(1)	12.0(4)	3.0(3)	2.5[b]	2.0[b]
trans-**L**^{c}	13.0[b]	12.5[b]	12.0(1)	2.4(1)	1.9[b]	1.4[b]

[a] Solvent = methanol/water (80/20 by weight); $I = 0.1$ M ($NaClO_4$); $T = (25.0 \pm 0.2)$ °C. The reported errors are given as 3σ. [b] Estimated value.

Potentiometric titrations of *cis*-**L**^{c} yielded three constants for the six protonation sites of the tripod (Table 1, Figure S5). As was previously observed for **L**^{a}, *trans*-**L**^{c} exhibited acido-basic properties similar to those of *cis*-**L**^{c} (Table 1, Figure S6). The previous attribution of the protonation sites could be held for **L**^{b} and **L**^{c}, the higher constants concerning the phenolate units, while the lower ones correspond to the imine functions. Our data for *cis*-L^{c} suggested statistical interactions between the three arms (Table 1). The protonation constants, which could not be determined under our experimental conditions, were calculated according to a statistical model[87] with $\Delta \log K = 0.48$ for three independent sites.

Characterization of the Ferric Complexes. The use of electrospray mass spectrometry (ESMS) to characterize the stoichiometry of metallic complexes in solution is well documented,[88,89] and has been employed by us for this purpose on previous occasions.[90−93] ESMS spectra of ferric complexes with ligand **L**^{a} showed two major complexes,

(79) (a) York, R. J.; Bonds, W. D., Jr.; Costoradis, B. P.; Archer, R. D. *Inorg. Chem.* **1969**, *8*, 789−795. (b) Godbole, M. D.; Puig, M. P.; Tanase, S.; Kooijman, H.; Spek, A. L.; Bouwman, E. *Inorg. Chim. Acta* **2006**, doi: 10.1016/j.ica.2006.10.015.
(80) (a) Piper, T. S. *J. Am. Chem. Soc.* **1961**, *83*, 3908−3909. (b) Fay, R. C.; Piper, T. S. Inorg. *Chem.* **1964**, *3*, 348−356. (c) Gordon, J. G.; Holm, R. H. *J. Am. Chem. Soc.* ***1970***, *92*, 5319−5332. (d) Girgis, A. Y.; Fay, R. C. *J. Am. Chem. Soc.* **1970**, *92*, 7061−7072.
(81) Dayan, I.; Libman, J.; Agi, Y.; Shanzer, A. *Inorg. Chem.* **1993**, *32*, 1467−1475.
(82) (a) Keck, J.; Kramer, H. E. A.; Port, H.; Hirsch, T.; Fischer, P.; Rytz, G. *J. Phys. Chem.* **1996**, 100, 14468−14475. (b) Guallar, V.; Moreno, M.; Lluch, J. M.; Amat-Guerri, F.; Douhal, A. *J. Phys. Chem.* **1996**, *100*, 19789−19794.
(83) LeGourriérec, D.; Kharlanov, V. A.; Brown, R. G.; Rettig, W. *J. Photochem. Photobiol., A* **2000**, *130*, 101−111.
(84) Holzbecher, Z. *Collect. Czech. Chem. Commun.* **1959**, *24*, 3915−3919.
(85) Albert, A.; Reese, C.; TomLinson, A. *Br. J. Exp. Pathol.* **1956**, *37*, 500−511.
(86) Takata, S.; Kyuno, E.; Tsuchiya, R. *Bull. Chem. Soc. Jpn.* **1968**, *41*, 2416−2418.
(87) Perlmutter-Hayman, B. *Acc. Chem. Res.* **1986**, *19*, 90−96.
(88) Piguet, C.; Bernardinelli, G.; Hopfgartner, G. *Chem. Rev.* **1997**, *97*, 2005−2062.
(89) (a) Hopfgartner, G.; Piguet, C.; Henion, J. D.; Williams, A. F. *Helv. Chim. Acta* **1993**, 76, 1759−1766. (b) Hopfgartner, G.; Piguet, C.; Henion, J. D. *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 748−756.

Table 2. Successive Stability Constants of the Ferric Complexes Formed with $\mathbf{L^a}$[a]

equilibria	log $K \pm 3\sigma$ *cis*-$\mathbf{L^a}$	*trans*-$\mathbf{L^a}$
$L^a + Fe(III) \overset{K_{LFe}}{\rightleftharpoons} \mathbf{L^aFe}$	13.0(2)	12.6(2)
$\mathbf{L^a} + \mathbf{L^aFe(III)} \overset{K_{L2Fe}}{\rightleftharpoons} \mathbf{(L^a)_2Fe}$	11.8(5)	9.9(4)
$L^a + (L^a)_2Fe \overset{K_{L3Fe}}{\rightleftharpoons} (L^a)_3Fe$	7.6(7)	8.3(5)

[a] Solvent: methanol/water (80/20 by weight); $I = 0.1$ M ($NaC1O_4$); $T = (25.0 \pm 0.2)$°C.

Table 3. Stability and Protonation Constants of Ferric Complexes with Tripods $\mathbf{L^b}$ and $\mathbf{L^c}$[a]

equilibria	*cis*-$\mathbf{L^b}$	*cis*-$\mathbf{L^c}$	*trans*-$\mathbf{L^c}$
$L + Fe \overset{K_{LFe}}{\rightleftharpoons} LFe$	36.0(1.1)	34.3(1.1)	33.7(1.1)
$LFe + H \overset{K_{LFeH}}{\rightleftharpoons} LHFe$	5.5(4)	7.1(4)	6.8(4)

[a] Solvent: methanol/water (80/20 by weight); $I = 0.1$ M ($NaC1O_4$); $T = (25.0 \pm 0.2)$ °C.[98]

$(\mathbf{L^a})_2$Fe and $(\mathbf{L^a})_3$Fe. These results are in line with the X-ray structure[94] obtained for tris[2-(2-oxazolin-2-yl)phenolato]-iron(III). Probably due to a weak ESMS response, the monochelate species $\mathbf{L^a}$Fe was not observed. For tripods $\mathbf{L^b}$ and $\mathbf{L^c}$, the respective spectra clearly evidenced the formation of the corresponding monoferric complexes $\mathbf{L^b}$Fe and $\mathbf{L^c}$Fe. The ionization of the ferric complexes was mainly obtained by the addition of protons and sodium ions. The pseudo-molecular ions of the different observed species are collected in Table S2 and are in excellent agreement with the simulated monoisotopic masses.

Stability and Absorption Spectra of the Ferric Complexes. To quantify the interactions between the phenol-oxazoline ligands ($\mathbf{L^a}$, $\mathbf{L^b}$, and $\mathbf{L^c}$) and iron(III), we have carried out versus pH spectrophotometric titrations of the ligands in the presence of 0.33 equiv of iron(III) for monomer $\mathbf{L^a}$ and 1.0 equiv for tripods $\mathbf{L^b}$ and $\mathbf{L^c}$.

For monomer $\mathbf{L^a}$, the experimental data showed, in agreement with ESMS, the formation of three monoferric complexes with one, two, and three ligands, respectively. The respective stability constants related to the successive binding of the three phenol-oxazoline units around the ferric cation are reported in Table 2.

The pH variations between 2 and 10 result in the appearance of ligand-to-metal charge-transfer bands LMCT which undergo strong hypsochromic shift from one to three ligands (Figure 4 and Figure S7).

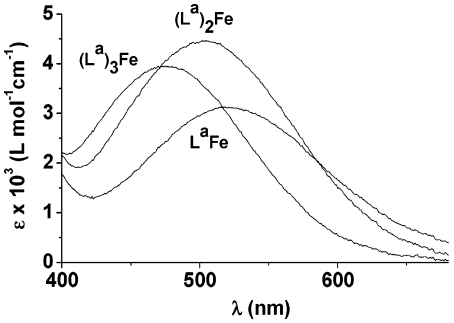


Figure 4. Electronic spectra of ferric complexes with *trans*-$\mathbf{L^a}$: solvent = methanol/water (80/20 by weight); $I = 0.1$ M; $T = (25.0 \pm 0.2)$ °C.

Similar shifts have been reported for catecholates[95] and for a large variety of (N,O)-bidentate ligands.[38,39] The electronic spectra display the phenolate-to-iron(III) CT bands in the visible region, the imine function of the oxazoline group being unable to produce charge transfer. Previous studies have demonstrated that the absorptivity of the LMCT band is additive for successive phenolate binding, amounting to $1-2 \times 10^3$ M^{-1} cm^{-1} per phenolate ligand.[96,97] The data obtained for $\mathbf{L^a}$ show the same trend in the extinction coefficients, when an additional phenol-oxazoline is bound to the $\mathbf{L^a}$Fe complex, while a "ceiling effect"[97] is observed for the $(\mathbf{L^a})_3$Fe complexes (Figure 4).

For tripods $\mathbf{L^b}$ and $\mathbf{L^c}$, the thermodynamic constants are given in Table 3. The stability constants were calculated from conditional ones at pH 2.0, due to the high affinity of these ligands for iron(III) (Table S3 and Figures S8−S10).

With about 2 orders of magnitude of difference, *cis*-$\mathbf{L^b}$ reveals to be a stronger binder for iron(III) than *cis*-$\mathbf{L^c}$, as reflected by the loss of an arm under more acidic conditions for $\mathbf{L^b}$ (Table 3). This effect can be explained by the length of the spacers between the carbon anchor and the binding units. Longer arms of about 40% in $\mathbf{L^b}$ compared to $\mathbf{L^c}$ allow the ligand to more easily self-organize around the metallic center and to fulfill the octahedral coordination requirements of iron(III) (Table 3, Figures 5 and S11).

Importantly, our results also indicate that geometrical and stereochemical factors do not play a significant role in the stability of the ferric complexes, as evidenced by similar binding constants measured within experimental errors for both the *cis* and *trans* isomers of $\mathbf{L^c}$. As an example, the electronic spectra of the *cis*-$\mathbf{L^c}$ ferric complex and of its monoprotonated analogue are given in Figure 6 (Figure S12).

As for the monomer $\mathbf{L^a}$, the number of bidentate phenol-oxazoline arms surrounding the ferric cation in $\mathbf{L^b}$ and $\mathbf{L^c}$ depends on the acidity of the medium. Upon protonation of one bidentate unit and its subsequent dissociation, the LMCT absorption band experiences a bathochromic shift of about

(90) Blanc, S.; Yakirevitch, P.; Leize, E.; Meyer, M.; Libman, J.; Van Dorsselaer, A.; Albrecht-Gary, A. M.; Shanzer, A. *J. Am. Chem. Soc.* **1997**, *119*, 4934−4944.
(91) Fatin-Rouge, N.; Blanc, S.; Leize, E.; Van Dorsselaer, A.; Baret, P.; Pierre, J. L.; Albrecht-Gary, A. M. *Inorg. Chem.* **2000**, *39*, 5771−5778.
(92) Fatin-Rouge, N.; Blanc, S.; Pfeil, A.; Rigault, A.; Albrecht-Gary, A. M.; Lehn, J. M. *Helv. Chim. Acta* **2001**, *84*, 1694−1711.
(93) Hamacek, J.; Blanc, S.; Elhabiri, M.; Leize, E.; Van Dorsselaer, A.; Piguet, C.; Albrecht-Gary, A. M. *J. Am. Chem. Soc.* **2003**, *125*, 1541−1550.
(94) Kooijman, H.; Spek, A. L.; Hoogenraad, M.; Bouwman, E.; Haasnoot, J. G.; Reedijk, J. *Acta. Crystallogr., Sect. C* **2002**, *C58*, m390−m390.
(95) Anderson, B. F.; Buckingham, D. A.; Robertson, G. B.; Webb, J.; Murray, K. S.; Clark, P. E. *Nature (London)* **1976**, *262*, 722−724.
(96) (a) Gaber, B. P.; Miskowski, V.; Spiro, T. G. *J. Am. Chem. Soc.* **1974**, *96*, 6868−6873. (b) Ackerman, G. A.; Hesse, D. *Z. Anorg. Allg. Chem.* **1970**, *375*, 77−86.
(97) Ainscough, E. W.; Brodie, A. M.; Plowman, J. E.; Brown, K. L.; Addison, A. W.; Gainsford, A. R. *Inorg. Chem.* **1980**, *19*, 3655−3663.
(98) The protonation constants have been determined for $\mathbf{L^c}$. For $\mathbf{L^b}$, the corresponding values have been supposed to be equal.

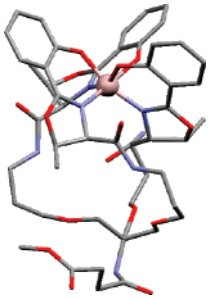


Figure 5. Modeled structure of the ferric complex with *cis*-$\mathbf{L^c}$ (Molecular Mechanics MM+).

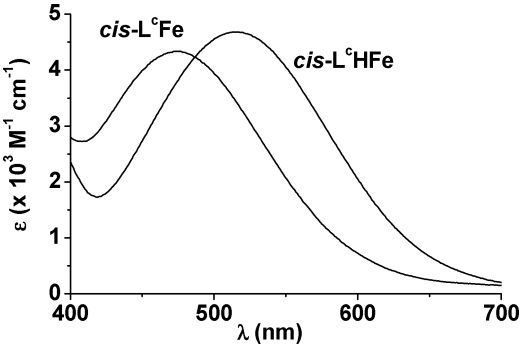


Figure 6. Electronic spectra of ferric complexes with *cis*-$\mathbf{L^c}$: solvent = methanol/water (80/20 by weight); I = 0.1 M; T = (25.0 ± 0.2) °C.

Table 4. Spectrophotometric Data for Phenol-oxazoline Ferric Complexes[a]

ligand	λ_{max} (nm)	$\epsilon(\lambda_{max})$ (10^3 M^{-1} cm^{-1})	solvent
phenol-oxazoline[94]	461	4.1	CH_3OH
trans-$\mathbf{L^a}$	475	4.0(4)	CH_3OH/H_2O (80/20 w/w)
cis-$\mathbf{L^b}$	477	3.1(3)	
cis-$\mathbf{L^c}$	475	4.3(4)	
trans-$\mathbf{L^c}$	469	5.2(5)	

[a] Experimental errors: λ_{max} = ±2 nm.

40 nm (Figure 6).[96,97] This red shift of about 1700 cm^{-1} for tripods $\mathbf{L^b}$ and $\mathbf{L^c}$ is in agreement with the values found (≈2000 cm^{-1}) for the successive and complete dissociation of various bidentate units containing phenolate groups. We present in Table 4 the spectrophotometric characterization of the ferric phenol-oxazoline compounds examined in this work. Taking into account experimental errors and solvent effects, all these data, which are related to the binding of iron(III) by three phenol-oxazoline units, are in good agreement.

In order to discuss the stability of the ferric complexes with $\mathbf{L^a}$, $\mathbf{L^b}$, and $\mathbf{L^c}$, respectively, we present in Table 5 the corresponding pFe values ($[L]_{tot} = 10^{-5}$ M, $[Fe(III)]_{tot} = 10^{-6}$ M, pH = 7.4).[99−105] Under these conditions, the comparison

Table 5. pFe^{III} Values with Various Synthetic and Biological Chelators[a]

ligand	binding site	pFe[a]	solvent
enterobactin[100,106]	3 CAT	35.6	H_2O
TRENCAMS	3 CAT	29.6	
O-TRENSOX[101−102,107]	3 HQ	29.5	
ferrichrome[103−104]	3 HOX	25.2	
TRENPYPOLS[105]	3 PPY	23.6	
N-TRENSOX[102,108]	3 HQ	22.2	
cis-$\mathbf{L^a}$	3 HPO	16.0	CH_3OH/H_3O (80/20 *w*/*w*
trans-$\mathbf{L^a}$		15.5	
cis-$\mathbf{L^b}$		21.8	
cis-$\mathbf{L^c}$		20.1	
trans-$\mathbf{L^c}$		19.4	

[a] Abbreviations used: catecholate = CAT; 2-phenolate-oxazoline = HPO; 2-phenolate-pyridine = PPY; 8-hydroxyquinolilne = HQ; hydroxamate = HOX.

of the pFe values shows that the catecholate, hydroxamate and hydroxyquinoline-type ligands have stronger affinities for iron(III) than our HPO derivatives (Table 5).

The comparison between $\mathbf{L^a}$ and $\mathbf{L^b}$ (or $\mathbf{L^c}$) shows that the tripodal structure induces a stabilization of the ferric complexes of about 4−6 orders of magnitude compared to monomer $\mathbf{L^a}$, which could be explained by a preorganization of the binding cavity, chelating effect, and a limited number of geometrical isomers (Figure 5). It is noteworthy that all the systems form five-membered rings upon coordination of the organic scaffold to the central core iron(III), while TRENPYPOLS, $\mathbf{L^a}$, $\mathbf{L^b}$, and $\mathbf{L^c}$ lead to six-membered rings and lower pFe values. The affinities for iron(III) of L-agrobactin and L-parabactin (Figure S2) were reported to be orders of magnitude higher than that of the trishydroxamate siderophore ferrichrome. Due to the presence of two catechol groups, the stability of ferric L-agrobactin and L-parabactin are comparable in magnitude to the triscatecholate siderophore enterobactin.[106,109−111]

Electrochemistry. A preliminary electrochemical study on the monomer $\mathbf{L^a}$ was performed in order to investigate the redox chemistry of Fe−HPO complexes.[112] The results thus obtained demonstrated that pH > 9 and stoichiometric conditions are necessary prerequisites for obtaining quasireversible voltammograms ($\Delta E \approx 115$ mV, Figure S13).

(99) Harris, W. R.; Carrano, C. J.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *100*, 2213−2214.
(100) Harris, W. R.; Carrano, C. J.; Cooper, S. R.; Sofen, S. R.; Avdeef, A. E.; McArdle, J. V.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 6097−6104.
(101) Albrecht-Gary, A. M.; Blanc, S.; Biaso, F.; Thomas, F.; Baret, P.; Gellon, G.; Pierre, J. L.; Serratrice, G. *Eur. J. Inorg. Chem.* **2003**, 2596−2605.
(102) Gerard, C.; Chehhal, H.; Hugel, R. P. *Polyhedron* **1994**, *13*, 591−597.
(103) Wong, G. B.; Kappel, M. J.; Raymond, K. N.; Matzanke, B.; Winkelmann, G. *J. Am. Chem. Soc.* **1983**, *105*, 810−815.
(104) (a) Emery, T. F. *Biochemistry* **1966**, *5*, 3694−3701. (b) Anderegg, G.; L'Eplattenier, F.; Schwarzenbach, G. *Helv. Chim. Acta* **1963**, *46*, 1409−1422.
(105) Baret, P.; Béguin, C.; Gellon, G.; Pierre, J. L.; Serratrice, G.; Thomas, F.; Lauljère, J. P.; Saint-Aman, E. *Eur. J. Inorg. Chem.* **2000**, 1219−1227.
(106) Loomis, L. D.; Raymond, K. N. *Inorg. Chem.* **1991**, *30*, 906−911.
(107) Serratrice, G.; Boukhalfa, H.; Béguin, C.; Baret, P.; Caris, C.; Pierre, J. L. *Inorg. Chem.* **1997**, *36*, 3898−3910.
(108) Boukhalfa, H. Ph.D. Thesis, Université Josef Fournier, Grenoble, France, 1990.
(109) Ong, S. A.; Peterson, T.; Neilands, J. B. *J. Biol. Chem.* **1979**, *254*, 1860−1865.
(110) Miyasaka, T.; Nagao, Y.; Fujita, E.; Sakurai, H.; Ishizu, K. *J. Chem. Soc., Perkin Trans 2* **1987**, 1543−1549.
(111) Neilands, J. B.; Peterson, T.; Leong, S. A. In *Inorganic Chemistry in Biology and Medicine*; Martell, A. E., Ed.; American Chemical Society: Washington, DC, 1980; pp 263−278.
(112) Robinson, J. P.; McArdle, J. V. *J. Inorg. Nucl. Chem.* **1981**, *43*, 1951−1953.

Table 6. Redox Potentials of Fe−Siderophore Complexes[a]

iron complex	$E_{1/2}$ (mV/NHE) conditions	log β L_3Fe^{III}	log β L_3Fe^{II}
$(8HQ)_3Fe$[113]	−251[a]	38.0	22.13
$(AHA)_3Fe$[114]	−293(3)[b]	28.3	17.4
$(NMeAHA)_3Fe$[114]	−348(3)[c]	29.4	11.1
(*cis*-**L**[a])$_3$Fe	−375(5)[e]	32.4[d]	13[d]

[a] Dioxane/H_2O (50:50 by volume). [b] AHA = acetohydroxamic acid, H_2O, pH 7.0, 1 M $NaClO_4$. [c] NMeAHA = *N*-methylacetohydroxamic acid, H_2O, basic pH. [d] CH_3OH/H_2O (80:20 by weight), 0.1 M $NaClO_4$. [e] DMF/H_2O (80:20 by volume), pH* 10.5, 1 M $NaClO_4$. [f] Dioxane/H_2O.

For the sake of comparison, we present some data from the literature[113,114] on trischelate iron(III) complexes based on 8-hydroxyquinoline and hydroxamates (Table 6).

If we take into account the stability constant (log β_{L3Fe} = 32.4(5)) that we determined in CH_3OH/H_2O (80/20 by weight) and the redox potential (−375 mV/NHE; 9.4 < pH < 10.8) which was measured in DMF/H_2O (80/20 by v/v), we could approximate the stability constant of the ferrous trisHPO complex at log β_{L3Fe} = 13(1). The drastic change in the solvent system was due to the poor solubility of the HPO species at higher concentration (4×10^{-3} M) for electrochemical measurements.

These results indicate, if we compare them with literature data for 8-hydroxyquinoline,[113] that the trisHPO ferric chelates are of about 4−5 orders of magnitude less stable than the tris(8-hydroxyquinoline) corresponding species and similar in stability to trishydroxamic analogues.[114] If we compare the ferrous complexes, HPO provides a higher Fe(III)/Fe(II) selectivity than 8-hydroxyquinoline[113] and is comparable in this respect to *N*-methylacetohydroxamic acid.[114]

Fluorescence Properties. The absorption and emission spectra of the synthetic HPO-type *cis*-**L**[b] and *cis*-**L**[c] are given in Figure S14. Upon excitation into the ligand transitions, a maximum of emission at about 410 nm was observed (Figure S14). This fluorescence emission originates from an excited-state intramolecular proton transfer (ESIPT). The phenol-oxazoline ligands are indeed characterized by a strong six-membered intramolecular hydrogen bond that may be photoinduced to undergo proton transfer in the excited state.[82,83] Since our tripodal ligands **L**[b] and **L**[c] are bearing three phenol-oxazoline fluorescent probes (absolute quantum yields ≈ 1−3%), which act as iron(III) binding units, it was worthy to examine in detail the emission properties of the corresponding ferric species.

The fluorescence intensities were measured as a function of *x*, where *x* is defined as the molar fraction of iron(III) versus ligand concentrations. For the sake of comparison with our previous work,[32] we have chosen to work at pH 5.8. Using the stability constants given in Table 3, we could calculate that, under our experimental conditions, the ferric complex is totally formed ((*cis*-**L**[b])Fe = 67%, (*cis*-**L**[b]H)Fe = 33%; (*cis*-**L**[c])Fe = 6%, (*cis*-**L**[c]H)Fe = 94%; Figure S15). Typical fluorescence emission spectra, that decrease with increasing iron(III) concentrations, are shown in Figure 7.

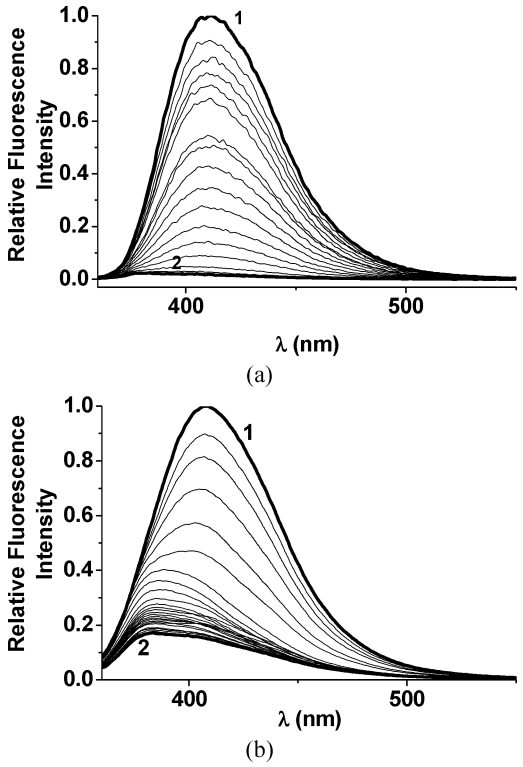


Figure 7. Spectrofluorimetric titrations of ligands (a) *cis*-**L**[b] and (b) *cis*-**L**[c] by iron(III): solvent = methanol/water (80/20 by weight); I = 0.05 M (sodium acetate, pH = 5.8); T = (25.0 ± 0.2) °C. (a) $[cis\text{-}\mathbf{L}^{b}]_{tot} = 1.1 \times 10^{-5}$ M; λ_{exc}= 340 nm; excitation and emission slit widths = 4 nm; spectra: (1) $x = [Fe(III)]_{tot}/[L^{b}]_{tot} = 0.0$, (2) $x = [Fe(III)]_{tot}/[cis\text{-}\mathbf{L}^{b}]_{tot} = 1.8$. (b) $[cis\text{-}\mathbf{L}^{c}]_{tot} = 8.85 \times 10^{-6}$ M; λ_{exc}= 340 nm; excitation and emission slit width = 12 nm; spectra: (1) $x = [Fe(III)]_{tot}/[cis\text{-}\mathbf{L}^{c}]_{tot} = 0.0$, (2) $x = [Fe(III)]_{tot}/[cis\text{-}\mathbf{L}^{c}]_{tot} = 2.8$.

An efficient quenching of the ligand-centered emission is observed for *cis*-**L**[b], while a residual emission shifted to lower wavelength is still observed for *cis*-**L**[c] even if the molar fraction of iron(III) added is significantly higher than 1.0 (Figure 7). No further decrease in the fluorescence intensity emission could be observed when an excess of iron(III) was added to the solution. Therefore, the mechanism of the observed fluorescence quenching was exclusively attributed to a static process ($F_o/F = 1 + K_{LFe}[Fe(III)]_{tot}$), the intermolecular collisions ($K_{SV} \approx 10\ M^{-1}$) and reabsorption of the emitted light by the iron complex being negligible under our experimental conditions.[115] The minimum fluorescence intensity value reached in the presence of an excess of iron(III) is about zero for *cis*-**L**[b], but non-negligible (≈20%) for *cis*-**L**[c]. In the latter case, the intramolecular quenching process is less efficient, and could be explained by the dissociation at pH 5.8 of a protonated arm in (*cis*-**L**[c]H)Fe. The protonated nonbound phenol-oxazoline moiety is responsible of the blue-shifted residual emission (Figure 7b).

(113) (a) Seo, E. T.; Riechel, T. L; Sawyer, D. T. *Inorg. Chem.* **1977**, *16*, 734−738. (b) Tomkinson, J.; Williams, R. *J. Chem. Soc.* **1958**, 2010−2018.

(114) (a) Wawrousek, E. F.; McArdle, J. V. *J. Inorg. Biochem.* **1982**, *17*, 169−183. (b) Spasojevic, I.; Armstrong, S. K.; Brickman, T. J.; Crumbliss, A. L. *Inorg. Chem.* **1999**, *38*, 449−454. (c) Wirgau, J. I.; Spasojevic, I.; Boukhalfa, H.; Batinic-Haberle, I. L; Crumliss, A. L. *Inorg. Chem.* **2002**, *41*, 1464−1473.

(115) Fraiji, L. K.; Hayes, D. M.; Werner, T. C. *J. Chem. Educ.* **1992**, *69*, 424−428.

Table 7. Analytical Parameters (Detection Limit DL and Quantification Limit QL) Related to Determination of Iron(III) Traces with Various Fluorescent Ligands[a]

ligand	solvent	slope *p*	DL[a] (ng mL^{-1})	QL[a] (ng mL^{-1})
azotobactin δ (2×10^{-6} M)[32,42]	water (pH = 4.4)	1.068(8)	0.5	1.5
anthryl-DFC[32] (4×10^{-6} M)	methanol/water (80/20 by weight, pH = 5.8)	1.16(1)	0.6	2.1
cis-**L**^{b} (1.1×10^{-5} M)	methanol/water (80/20 by weight, pH = 5.8)	0.81(1)	0.5	1.6
cis-**L**^{c} ($8.85\ 10^{-6}$ M)	methanol/water (80/20 by weight, pH = 5.8)	0.79(1)	2.9	9.5

[a] $-\mathrm{DL} = 3(S_B/\sqrt{N})$; $\mathrm{XL} = 10(S_B/\sqrt{N})$ with $N = 200$; S_B: standard deviation of the blank signal (signal at zero analyte).[117] The limits of detection and quantification reported are expressed as mass values.

According to a static quenching mechanism, a linear plot of F/F_o versus the molar ratio of iron(III) leads to a slope *p*,[116] which is expected to be close to 1.0 (Figure S16). By contrast with azotobactin δ and ferrichrome analogues[42] and due to the presence of bipodal ferric monoprotonated complexes, as minor fluorescent species, significant deviations are observed with weaker slopes[64] **(**Table 7), which decrease the sensitivity of the detection.

Nevertheless, the tripodal ligand with longer arms **L**^{b} reveals to be a sensitive fluorescent marker for iron(III) traces, which is very similar to the trishydroxamate derivative anthryl-DFC bearing an additional fluorescent probe in methanol/water. Even if in the case of azotobactin δ the fluorophoric binding site is very efficient in water, the remarkable advantage of the phenol-oxazoline tripod **L**^{b} with suitable spacers is to possess a carbon anchor free for surface-adhesive substituents. Moreover, the interference due to other metals could be neglected since a significant and high selectivity for iron(III) (pFe = 17.6) over divalent cations such as Cu(II) and Zn(II) (pCu $\approx$ pZn $\approx$ 8) was measured at pH 6.35.[118]

Conclusions

We have demonstrated the feasibility of fused iron binder and fluorophore to sense iron by fluorescent quenching. The synthesized phenol-oxazoline unit may serve as a chromophoric ligand. The importance of extended, flexible spacers versus a short and tight one has been clearly demonstrated in the fluorescent quenching experiments with the tripodal sensors *cis*-**L**^{b} and *cis*-**L**^{c}. Complete quenching with the extended tripod and incomplete and blue-shifted quenching with the rigid system may result from a change in the binding mode and subsequent size of the iron binding cavity. Because these two ferric complexes possess different protonation constants ($\Delta \log K_{\mathrm{LHFe}} \approx 1.6$) and comparable affinities, the use of shorter spacers will lead to dissociation of one binding strand from the metallic center at higher pH for *cis*-**L**^{b} than *cis*-**L**^{c}.

In spite of similar binding constants for the *trans*- and *cis*-**L**^{c} isomers, preliminary screening experiments with *Paracoccus denitrificans* revealed that the *trans*-**L**^{c} isomer is superior in inducing growth promotion in comparison to the *cis*-**L**^{c} isomer. These results indicate that geometrical as well as stereochemical factors play a considerable role in receptor recognition.[119] Further investigation is currently in progress.

Detection limits have been found to be in the low nanogram per milliliter range, comparable with the best chemosensors based on natural peptide siderophores. Last but not least, the moderate binding affinities of the tripodal HPO ligands to ferric ions guarantee a favorable exchange to competing chelators under mild conditions, a prerequisite for reversibility. It may be anticipated that similar chelators substituted with surface-adhesive functionality will reach analytical methodology as selective coats in plate-readers with amplified fluorescence resulting from multiple chromophoric groups and reaching high sensitivity.

Acknowledgment. This work has been supported by the Centre National de la Recherche Scientifique (UMR 7177), the Mordechai Glakson Fund, the G. M. J. Schmidt Minerva Center on Supramolecular Architectures, and the Helen and Martin Kimmel Center for Molecular Design. E.G.-K. thanks the European Community for granting her a Marie Curie Intra-European Fellowship within the 6th European Community Framework Programme. The authors express their thankfulness to Mrs. Raymonde Baltenweck-Guyot and Mrs. Rachel Lazar for their skillful assistance in this work. A.S. holds the Siegfried and Irma Ullman Professorial Chair.

Supporting Information Available: Figures showing spectral data obtained for the spectrophotometric titrations, solution speciation diagrams, and electronic spectra of protonated species of the free ligands and of the ferric complexes. Figures showing the chemical structure of pyoverdin *PaA*, azotobactin δ, parabactin, vulnibactin, mycobactin. Tables showing the stability constants of ferric complexes formed with **L**^{a}, the protonation constants of phenolate-type ligands, and the ESMS data. This material is available free of charge via the Internet at http://pubs.acs.org.

IC061952U

(116) Miller, J. N. *Analyst* **1991**, *116*, 3−14.
(117) Ingle, J. D.; Wilson, Jr, A. L. *Anal. Chem.* **1976**, *48*, 1641−1642.
(118) Traboulsi, A.; Humbert, N.; Elhabiri, M.; Shanzer, A.; Albrecht-Gary, A. M. Unpublished results.
(119) Bergeron, R. J.; Weimar, W. R.; Dionis, J. B. *J. Bacter.* **1988**, *170*, 3711−3717.