yield as a white solid: IR ($CDCl_3$) 3444, 3267 (very weak) cm^{-1} (NH), 1739 cm^{-1} ($COOCH_2$), 1711 cm^{-1} (Boc); ^1H NMR ($CDCl_3$) δ 7.29 (s, 3 H, ArH), 5.15 (br s, 6 H, CH_2O), 4.93 (br d, 3 H, BocN*H*), 4.35 (m, 3 H, $C_\alpha H$), 1.7-1.5 (m, 9 H, CH, CH_2{*i*-Bu}), 1.43 (s, 27 H, *t*-Bu), 0.93 (d, 18 H, CH_3{*i*-Bu}).

TREN Trispeptide Derivative 4. Activation of Boc-L-leucine·H_2O as described and treatment of the active ester with $^1/_3$ equiv of tris(2-aminoethyl)amine afforded a glassy solid (67% yield) after flash chromatography purification (2.5-3% MeOH/CH_2Cl_2): FAB MS (3-nitrobenzyl alcohol) 786 $(M + H)^+$, 686 $(M + H\text{-Boc})^+$, 586 $(M + H\text{-}2Boc)^+$, 486 $(M + H\text{-}3Boc)^+$; IR ($CHCl_3$) 3440, 3332 (N-H), 1699, 1656 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 7.92 (br, 3 H, CH_2NH), 5.56 (br d, 3 H, BocN*H*), 4.45 (m, 3 H, $C_\alpha H$), 3.47, 2.93 (br split AB, 6 H, NCH_2CH_2NH), 2.56 (br, 6 H, NCH_2CH2NH), ~1.6 (br m, 9 H, CH and CH_2{*i*-Bu}), 1.41 (s, 27 H, *t*-Bu), 0.94 (m, CH_3{*i*-Bu}).

Single Chain Analogs. L-**PhCH$_2$NHCOCH(*i*-Bu)NHBoc (2).** Treatment of activated Boc-L-leucine with benzylamine afforded the product in 93% yield after purification by flash chromatography (1% MeOH/CH_2Cl_2) as a white solid: mp 76-9 °C; $[\alpha]^{32}_D = -28$ ($CHCl_3$, *c* 0.6); IR ($CHCl_3$) 3437 cm^{-1} (N-H), 1698, 1680 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 7.34-7.23 (m, 5 H, Ph), 6.51 (br, 1 H, CH_2NH), 4.82 (br, 1 H, BocN*H*), 4.43 (d, J = 5.7 Hz, 2 H, PhCH_2), 4.11 (m, 1 H, $C_\alpha H$), 1.41 (s, 9 H, *t*-Bu), 1.7-1.5 (m, 3 H, CH and CH_2{*i*-Bu}), 0.94 (m, 6 H, CH_3); $^{13}C\{^1H\}$ NMR (300 MHz, $CDCl_3$) δ 172.6 (*C*ON), 155.8 (*C*O-Boc), 138.1 (1-Ph), 127.5 (2-Ph), 128.6 (3-Ph), 127.3 (4-Ph), 80.0 (CMe_3), 53.2 (C_α), 43.3 ($ArCH_2$), 41.30 (CH_2{*i*-Bu}), 28.30 (CH_3{*t*-Bu}), 24.8 ($CHMe_2$), 22.9, 22.0 (CH_3{*i*-Bu}). Anal. Calcd for $C_{18}H_{28}N_2O_3$: C, 67.47; H, 8.81; N, 8.74. Found: C, 67.76; H, 8.78; N, 8.48.

L-**CH$_3$CH$_2$CH$_2$NHCOCH(*i*-Bu)NHBoc (5).** Activation of Boc-L-Leu·H_2O according to the general procedure and coupling with *n*-propylamine, followed by flash chromatography (ether/*n*-hexane 1:1) afforded the aliphatic single chain analog (87% yield) as a white crystalline material: mp 102-105 °C; $[a]^{30}_D = -28$ (MeOH, *c* 0.15); IR ($CHCl_3$) 3440 cm^{-1} (N-H), 1703 cm^{-1} (BocC=O), 1675 cm^{-1} (CONH); ^1H NMR ($CDCl_3$) δ 6.07 (br t, 1 H, CH_2NH), 4.84 (br d, 1 H, BocN*H*), 4.40 (m, 1 H, $C_\alpha H$), 3.21 (m, 2 H, CH_2N), 1.65 (m, 3 H, CH and CH_2{*i*-Bu}), 1.52 (m, 2 H, CH_2CH_2N), 1.44 (s, 9 H, *t*-Bu), 0.93 (m, 6 H, CH_3{*i*-Bu}), 0.92 (m, 3 H, CH_3{*n*-Pr}).

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Supplementary Material Available: Two tables providing full lists of the calculated conformations of the L-alanyl and the L-leucyl trispeptides (6 pages). Ordering information is given on any current masthead page.

Chiral Siderophore Analogs: Enterobactin

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Abstract: Two families of chiral enterobactin analogs, based on 1,3,5-tris(aminomethyl)benzene (TRAM) and on tris(2-aminoethyl)amine (TREN) as anchors and amino acids linking the anchor to catechol residues, have been prepared and their structures and iron(III) binding properties examined. The TRAM catechoylamides with L-leucyl (**5a**) and L-alanyl (**5b**) were found to adopt random conformations in protic solvents, while the corresponding TREN catechoylamides with L-leucyl (**8a**) and L-alanyl (**8b**) form H-bonded structures under analogous conditions. All ligands bind Fe^{3+} in a 1:1 stoichiometry, and most of them adopt preferentially Δ-cis configurations when L-amino acids are used, similar to enterobactin. The TREN derivative **8a** was shown to be the most efficient Fe^{3+} ion binder so far prepared, approaching enterobactin's binding constant within less than three orders of magnitude. The superiority of the TREN derivative **8a** is discussed in the light of experimental and theoretical (EFF calculations) results. Spectroscopic data include mainly the following: (i) NMR data of the protected ligands in relation to their H-bonded conformations and (ii) CD data of the Fe^{3+} complexes in relation to their optical purity.

Introduction

Biomimetic chemistry is a rapidly advancing area at the interface between chemistry and biology.[1] Its purpose is to identify the essential structural features that are responsible for the performance of natural compounds and to reproduce these features with the simplest possible synthetic molecules. Such molecules provide biological probes to elucidate biological processes and to reproduce specific functions of the biological machinery inside and outside living systems. In this article we describe synthetic enterobactin analogs that reproduce the essential structural features of natural enterobactin: its capability to adopt an organized conformation in the free state and to form complexes of high chiral purity that are stabilized by intramolecular H-bonding.

Enterobactin (**1**) (Figure 1) is a siderophore produced and excreted by bacteria in iron deficient media in order to bind and assimilate extracellular iron.[2-8] Enterobactin binds ferric ions with a very high formation constant ($\log K_f = 49$)[5] to give a charged octahedral triscatecholate complex with a Δ-cis configuration (Figure 1).[4] It is now well-established that after extracellular iron complexation the complex interacts with a specific receptor in the outer cell membrane and is then taken into the cell.[7] Recognition and membrane transport of the ferric complex by the ferric–enterobactin receptor has been shown to be stereoselective, such that enantioenterobactin which forms the Λ-cis complex lacks biological activity.[9]

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[‡] Department of Chemical Physics.

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(4) Raymond, K. N.; Mueller, G.; Matzanke, B. F. *Top. Curr. Chem.* **1984**, *123*, 49-102, and references therein.
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(6) Hider, R. C. *Structure and Bonding* **1984**, *58*, 25-87.
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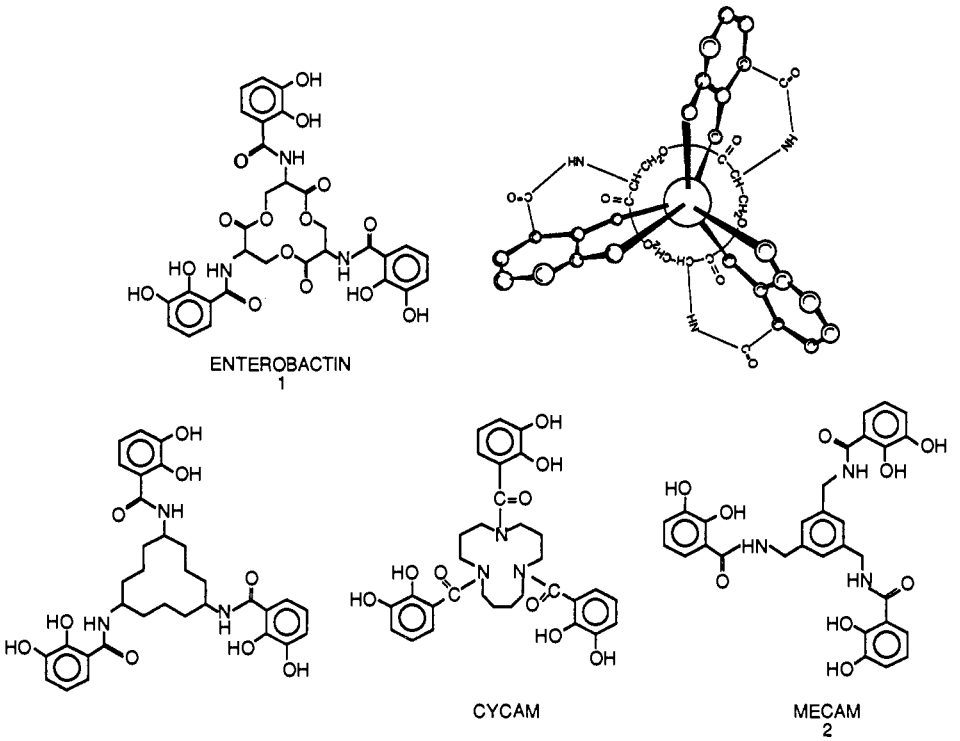

Figure 1. Enterobactin and its Δ-cis ferric complex (top) and synthetic enterobactin analogs (bottom). Carbocyclic derivative[10] (bottom left), CYCAM[4] (bottom middle) and MECAM[4] (bottom right)).

The outstanding properties of enterobactin stimulated the preparation of a large variety of synthetic analogs.[4] Some of them, shown below (Figure 1), are C_3 symmetric molecules. MECAM **2**, prepared and studied by Raymond,[12,13] showed the highest Fe^{3+} binding constant among the synthetic analogs known up to now, reaching the value of log K_f = 43,[14] 6 orders of magnitude below the natural compound. It differs from enterobactin **1** by being nonchiral, thus forming racemic complexes, and by lacking any kind of preorganized structure, as proposed earlier for enterobactin.[15]

We have recently demonstrated that chiral C_3 symmetric trispeptides can form right handed propeller-type arrangements stabilized by interstrand H-bonds ("H-bond belts"),[16-18] analogous to those proposed for enterobactin.[15] Here we present triscatechoylamide ligands derived from such trispeptides by replacing the Boc protecting groups at the termini by 2,3-dihydroxybenzoates. These molecules are shown to provide chiral ligands that mimic the fundamental features of enterobactin[19] and exhibit the highest ferric ion binding efficiency of any enterobactin analog so far synthesized. By introducing systematic structural variations,

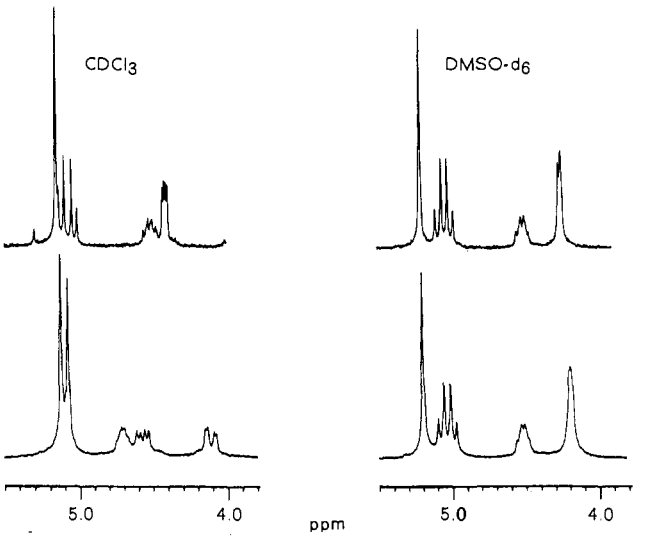

Figure 2. Part of the ^1H NMR trace of protected ligand **4a** (bottom) and single strand analog **3** (top) in $CDCl_3$ (left) and DMSO-d_6 (right).

the effect of specific intramolecular interactions on ion binding has been examined. The structures of the free ligands, their protected intermediates (precursors) and their metal complexes were studied by a combination of IR, NMR UV, and CD spectroscopy. Complementary information was obtained from the NMR studies of the diamagnetic Ga^{3+} complexes[20-23] and from

(10) Corey, E. J.; Hurt, S. D. *Tetrahedron Lett.* **1977**, 3923–3924.

(11) Abbreviations used: MECAM = 1,3,5-*N*,*N*',*N*''-tris(2,3-dihydroxybenzoyl)triaminomethylbenzene; NHS = *N*-hydroxysuccinimide; DCC = dicyclohexylcarbodiimide; THF = tetrahydrofuran; TRAM = 1,3,5-tris(aminomethyl)benzene (in the tables Ar is used as abbreviation); TREN = tris(2-aminoethyl)amine (in the tables Tr is used as abbreviation).

(12) Harris, W. R.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 6534–6541.

(13) Weitl, F. L.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 2728–2731.

(14) The latest binding constant for enterobactin was reported to be log K_f = +49[5], which is 3 orders of magnitude lower than earlier reported.[4] This difference is due to differences in the p*K* values of the free ligand, rather than to differences in the affinity of the deprotonated catecholate to Fe^{3+}. It is therefore concluded that the binding constant for MECAM is also 3 orders of magnitude lower than originally reported,[4] namely log K_f = +43 rather than +46.

(15) Shanzer, A.; Libman, J.; Lifson, S.; Felder, C. E. *J. Am. Chem. Soc.* **1986**, *108*, 7609–7619.

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(17) Tor, Y.; Libman, J.; Shanzer, A.; Felder, C. E.; Lifson, S. *J. Am. Chem. Soc.*, preceding paper in this issue.

(18) Tor, Y. Ph.D. Thesis, Feinberg Graduate School, The Weizmann Institute of Science, Rehovot, Israel, 1990.

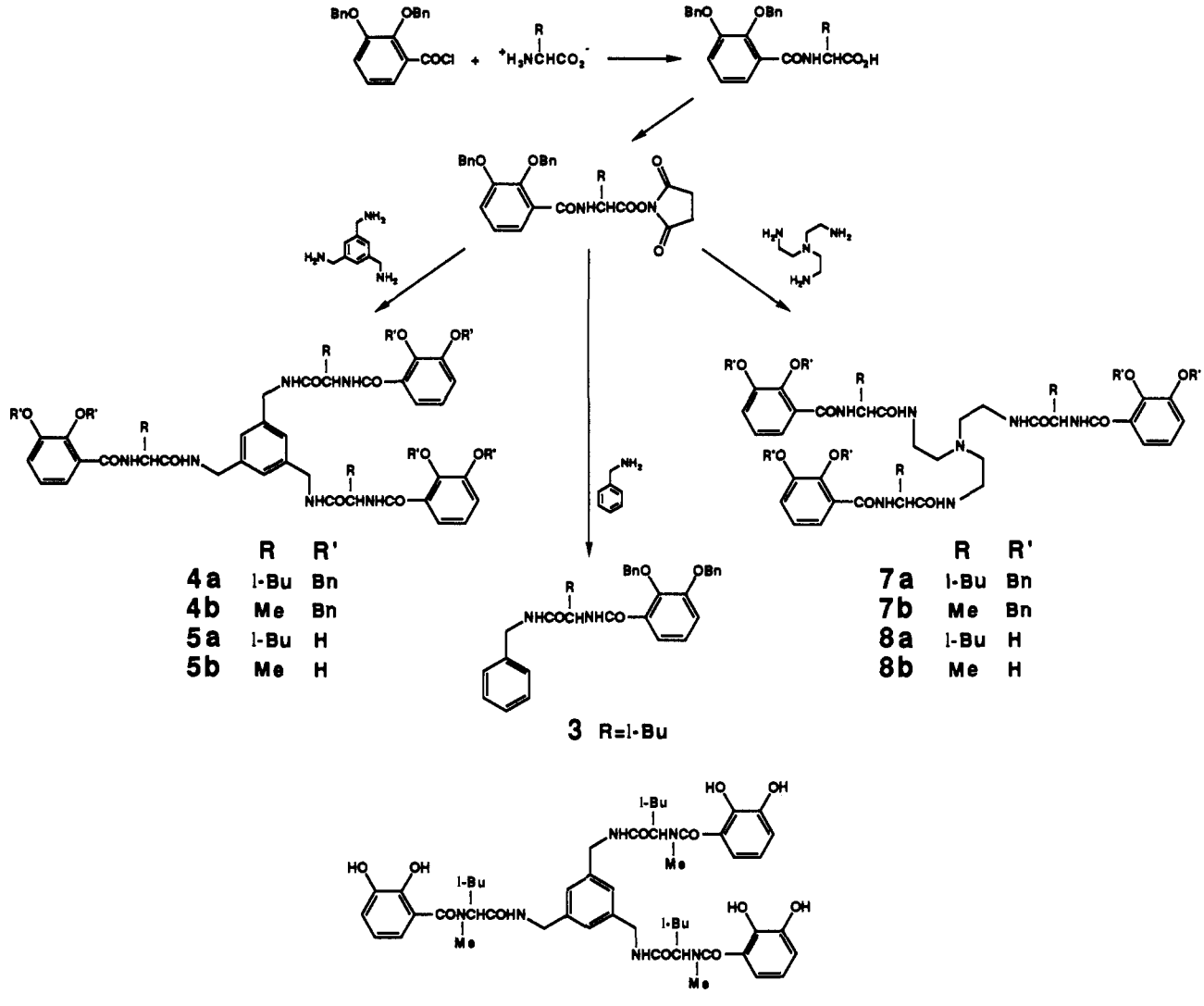
(19) Preliminary report of this work has been published, see: Tor, Y.; Libman, J.; Shanzer, A.; Lifson, S. *J. Am. Chem. Soc.* **1987**, *109*, 6517–6518.

(20) Diamagnetic Ga^{3+} (d^{10}) is amenable to high resolution ^1H NMR studies and structurally very close to paramagnetic Fe^{3+} (d^5). Both have the same charge and similar ionic radii in six-coordinated complexes (0.645 Å for Fe^{3+} compared to 0.620 Å for Ga^{3+}).[21] It was recently shown by Raymond that triscatecholate and trishydroxamate complexes of Fe^{3+} and Ga^{3+} have almost identical crystal structures.[22] Moreover, since neither have any crystal field stabilization and both have similar ionic radii, they are similar in their ligand exchange rates.[23]

(21) Shannon, R. D. *Acta Crystallogr. A* **1976**, *A32*, 751–767.

(22) Borgias, B. A.; Barclay, S. J.; Raymond, K. N. *J. Coord. Chem.* **1986**, *15*, 109–123.

Scheme I



optical (UV–vis) and circular dichroism (CD) spectroscopy of the ferric complexes.[24-26] The binding efficiencies of the chiral ligands relative to the nonchiral MECAM **2** was estimated by CD monitored competition experiments. These experimental data were supplemented by conformational calculations using the same empirical force field (EFF) calculations as in previous studies.[15,16,19,27]

Results and Discussion

Synthesis of Triscatechoylamide Ligands. The tripodal catechoylamide ligands have been prepared by N-acylation of the desired free amino acid with 2,3-bis(benzyloxy)benzoyl chloride[28] followed by activation (NHS/DCC) and coupling of three such strands with the desired trisamine anchor (or monoamine for single strand analogs) as shown in Scheme I. The free catechoylamides are obtained in high yields by catalytic hydrogenation of the protected benzyl derivatives. MECAM **2**[12,13] was also synthesized by condensation of 2,3-bis(benzyloxy)benzoyl chloride with 1,3,5-tris(aminomethyl)benzene (TRAM), and subsequent hydrogenation, and served as a reference molecule throughout this study.

Structure of the Protected TRAM Triscatechoylamide 4a. Since the low solubility of the triscatechoylamide ligands in apolar solvents prevents their full spectroscopic characterization, particular emphasis was placed on establishing the structural features of the protected ligand **4a**.

The ^{1}H NMR spectrum of **4a** in $CDCl_3$ reveals pronounced anisotropy ($\Delta\delta$ = 0.46 ppm)[29] of its anchor's diastereotopic ArCH_2NH protons (Figure 2). This anisotropy disappears in DMSO-d_6 and is replaced by an anisotropy ($\Delta\delta$ = 0.12) of one of the benzyloxy methylene groups. The ^{1}H NMR spectra of the single strand analog **3** in both $CDCl_3$ and DMSO-d_6 solvents resemble that of the triscatechoylamide **4a** in DMSO-d_6 (Figure 2). We suggest the following explanation to these observations.

The Cat-NH's in **4a** may form two alternative H-bonds. Either they bind to the ether oxygen of the closest benzyloxy group forming a six-membered ring at each strand (Figure 3a), or they bind to the carbonyl oxygen of their adjacent strands forming a belt of H-bonds (Figure 3b). The second alternative seems to be preferred in $CDCl_3$ for trispeptide **4a**. The interstrand H-bonds immobilize the strands thus making the anchor's diastereotopic Ar-CH_2NH protons highly nonequivalent and leaving the benzyloxy groups free to rotate. The first alternative does precisely the opposite, fixing the 2-benzyloxy group and making their protons nonequivalent, while allowing free rotation of the strand as a whole, thus making the Ar-CH_2NH protons practically

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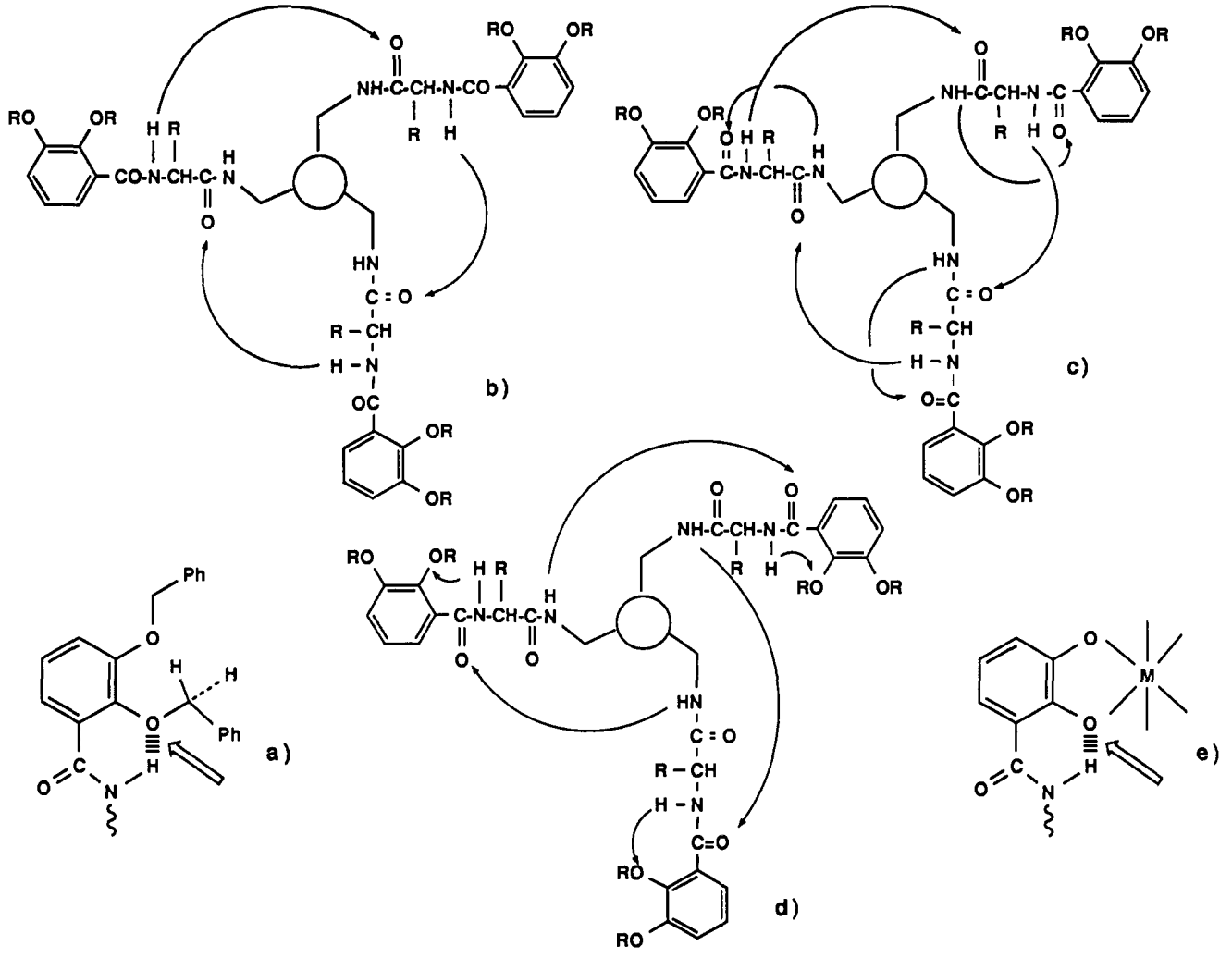

Figure 3. Alternative H-bonding patterns of triscatechoylamides: (a) intrastrand H-bonds Cat-NH····O-Bz, (b) interstrand H-bonds Cat-NH···O= C-TRAM, (c) mutually supporting intrastrand H-bonds Cat-C=O···HN-TRAM, and interstrand H-bonds Cat-NH···O=C-TRAM, (d) mutually supporting intrastrand H-bonds Cat-NH····OBz and interstrand H-bonds Cat-C=O···HN-TRAM or HN-TREN, and (e) intrastrand H-bonds Cat-NH····O-Cat in the metal complexes.

equivalent. This first alternative is obviously the only one available to the single strand analog **3**.[30]

The IR spectrum of the single stranded reference compound **3** in $CHCl_3$ shows two different NH absorptions. The first one at 3437 cm^{-1} indicates free NH, while the second one at 3361 cm^{-1} indicates H-bonded NH. The protected ligand **4a**, on the other hand, shows only low frequency NH absorption centered at 3354 cm^{-1} in dilute chloroform solution. This observation implies the presence of a fully H-bonded network in **4a** and consequently the presence of both interstrand and intrastrand H-bonds. The lack of NMR anisotropy of the benzyloxy protons Ph-CH_2O- together with NMR anisotropy of the diastereotopic Ar-CH_2NH- protons strongly suggest interstrand H-bonds between Cat-NH and TRAM-CO and consequently intrastrand H-bonds between the Cat-CO and TRAM-NH (Figure 3c). The occurrence of such intrastrand H-bonds in the triscatecholates but their absence in the corresponding parent trispeptides[16,17] is due to differences in the terminal carbonyl groups. The higher electron density of the amides in the triscatechoylamides, relative to the corresponding terminal carbamate carbonyls in the trispeptides,[16,17] enhances the tendency of the former to serve as H-acceptor in the intrastrand H-bonds.

The here observed H-bonds between the amide-NH and benzylated catecholate oxygen are believed to be reproduced in the ferric–catecholate complexes, particularly so, as the enhanced charge density of the catecholate oxygen in the complex should increase its proton acceptor properties.

L-Leucyl-triscatechoylamide 5a and Its Complexes. It was shown previously that the L-leucyl TRAM based trispeptide was the best choice for propeller-like conformations with right-handed H-bond belts.[16-18] Its triscatechoylamide derivative **5a** was therefore the first ligand examined. Addition of $Ga_2(SO_4)_3$ to a 10 mM DMSO-d_6 solution of **5a** resulted in the appearance of a second set of signals, most probably due to the formation of the neutral Ga^{3+} complex, which is kinetically stable on the NMR time scale. By adding $Ga_2(SO_4)_3$ followed by a weak base (C-D_3CO_2Na) a single but different set of signals was observed, which is attributed to the charged (triscatecholate-Ga)$^{3-}$. Both sets are characterized by a pronounced low field shift of the Cat-CON*H* proton and high field shifts of the catecholate aromatic protons, relative to that of free **5a** (Table I).

Similar studies were performed with MECAM **2**, and the data accumulated were compared to those reported for enterobactin **1**[31] (Table I). Some of the electrostatically induced differences

(29) The value in our previous communication (ref 19) was mistakenly reported as 0.56 rather than 0.46 ppm.

(30) The same type of H-bond, CatNH····OBz, has also been observed in the β-alanyl derivative, *N*-(2,3-dibenzyloxy)benzoyl-β-alanine methyl ester by X-ray diffraction. In the Leu derivative, *N*-(2,3-dibenzyloxy)benzoylleucine methyl ester, on the other hand, the H-bond of Cat-NH····OBz was broken and replaced by a H-bond of the type Cat-NH···O-COMe. The latter is reminiscent of the H-bond network suggested in free enterobactin **1**, where the Cat-NH has been reported to bind to the ether oxygens of the ring lactones.[15]

(31) Llinas, M.; Wilson, D. M.; Neilands, J. B. *Biochemistry* **1973**, *12*, 3836–3843.

Table I. ^{1}H NMR Data for the Free Ligands and Their Ga^{3+} Complexes[a]

proton	1[b]	5a[c]	2[c]
N*H*	9.06 (+2.66)	8.79 (+1.16) [+2.64][d] 8.57 (+0.13) [−0.15][e]	9.39 (+0.76) [+1.84]
catechol-*o* (6)	7.34 (−0.50)	7.42 (−0.42) [−0.30]	7.29 (−0.28) [−0.13]
catechol-*m* (5)	6.73 (−0.60)	6.67 (−0.33) [−0.62]	6.65 (−0.27) [−0.49]
catechol-*p* (4)	6.98 (−0.54)	6.91 (−0.34) [−0.39]	6.91 (−0.32) [−0.31]
$\Delta\delta/\Delta T$[f]	−4.7 (−1.3)	−5.0 (−2.4)[d] −6.1 (−6.2)[e]	

[a] Measured in DMSO-d_6. Plus sign indicates shift to lower field. [b] Data for enterobactin (1) taken from ref 31, for 45 mM at ca. 318 K. [c] The values in parentheses are for the induced shifts observed upon addition of $Ga_2(SO_4)_3$ to a 33 mM solution at 300 K. The values in brackets are for the induced shifts observed upon addition of $Ga_2(SO_4)_3$ and CD_3COONa to a 10 mM solution at 318 K. [d] Cα-N*H*. [e] CH_2NH. [f] Temperature coefficients (10^3 ppm/K). The values presented for **5a** are least mean square fits to data obtained in the 298–324 K range of a slow exchanging mixture of the ligand and its complex.

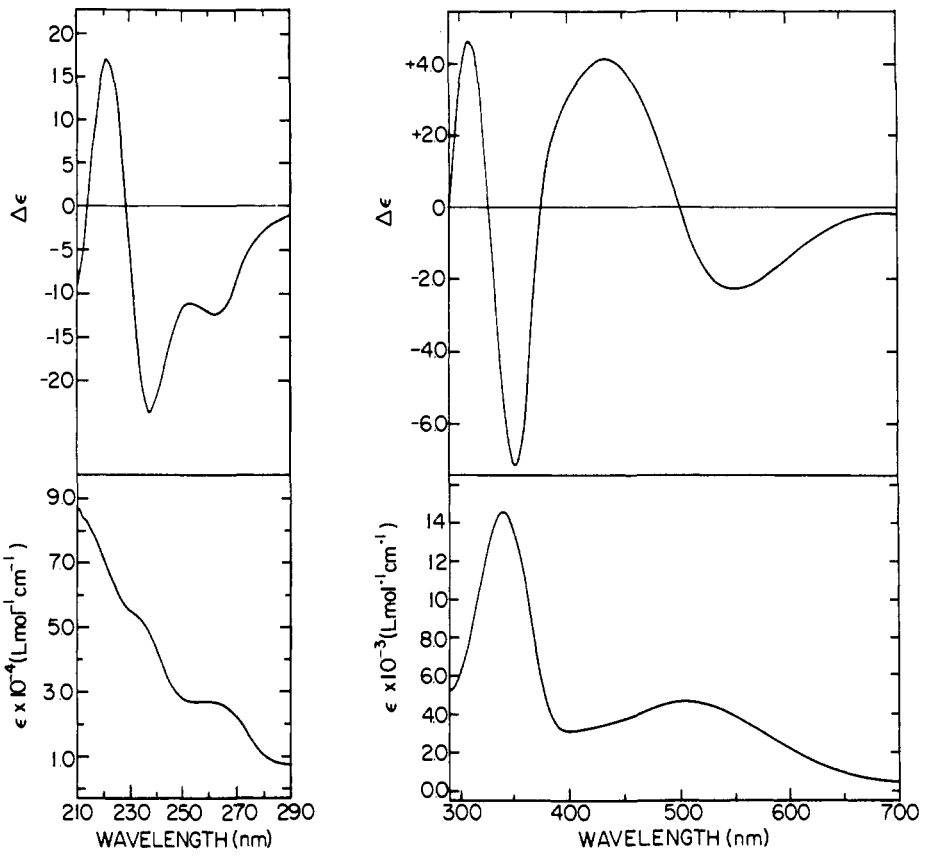

Figure 4. UV–vis and CD spectra of **5a**–Fe complex (10% MeOH in 0.1 M TRIS buffer, pH 8.97).

in chemical shifts observed in the chiral **5a** complexes are close to, others are smaller than, those reported for enterobactin **1**,[31] but all are larger than those observed in the achiral analogous MECAM **2** complexes. Since all three complexes contain the same metal ion and the same binding sites, the electrostatically induced shifts are determined mainly by their geometries.[32] The pronounced shift of the catechoylamide proton Cat-CONH to lower field upon Ga^{3+} complexation and the decrease in its temperature coefficient in **5a**, when compared to the free ligand (Table I), are both compatible with H-bonding to the 2-catecholate oxygen (Figure 3e). This type of H-bonding has been suggested to occur in Ga^{3+}-enterobactin[31] as well as in Fe^{3+}-enterobactin[15] based on NMR studies and force field calculations, respectively. Recent X-ray diffraction analyses on synthetic triscatecholates confirmed this possibility.[33]

The visible spectrum of **5a**–Fe complex showed a broad absorption band around 502 nm, which is characteristic of Fe^{3+} octahedrally coordinated to three catecholates,[25] and is assigned as a ligand-to-metal charge transfer (LMCT) band (Figure 4).[26] The corresponding CD spectrum in this region (Figure 4 and 1st row of Table II) showed two Cotton effects ($\Delta\epsilon$ = +4.2 at 432 nm, 0.0 at 502 nm, and −2.3 at 550 nm), that are close to those observed for the enterobactin–Fe complex ($\Delta\epsilon$ = +4.0 at 420 nm and −4.0 at 535 nm).[34] This demonstrates that the predominant absolute configuration of the **5a**–Fe complex is identical with that of the enterobactin–Fe complex, namely Δ-cis.[4,34]

Empirical force field (EFF) calculations support these observations. The lowest energy conformation of the ferric complex of ligand **5a** (Figure 5), reveals a relatively relaxed structure with a preferred Δ-cis absolute configuration around the metal ion. Calculated conformations of triscatecholates–ferric complexes derived from L-amino acids with the opposite absolute configuration (Λ-cis) were always of higher energy. The preference of the Δ-cis configuration in the complexes reflects the directionality of the H-bonds occurring in the "parent" trispeptides[16–18] which is similarly right-handed. The calculations also clearly showed the presence of strong H-bonds between the catechoyl amide Cat-CONH and the catecholate oxygens Cat-O$^-$. The short distance (1.50 Å) obtained by the EFF indicates a strong electrostatic interaction between the NH dipole and the negatively charged oxygen.

Performing a competition experiment (see Experimental Section) showed that 88% of the Cotton effect's intensity of **5a**–Fe

(32) Live, D.; Chan, S. I. *J. Am. Chem. Soc.* **1976**, *98*, 3769–3778.

Table II. Vis and CD Data, Preferred Absolute Configuration, and Relative Binding Efficiencies for Triscatecholate Ferric Complexes[a]

ligand[b]	Vis λ_{max} (ϵ)	CD λ_{ext} ($\Delta\epsilon$)			abs config	binding ratio[c]	K_L/K_M[d]
Ar(L-Leu), **5a**	502 (4650)	432 (+4.2)	502 (0.0)	550 (−2.3)	Δ-cis	7.3	50
Ar(L-Ala), **5b**	502 (4370)	435 (+3.3)	514 (0.0)	560 (−1.0)	Δ-cis		
Ar(D-Ala)	502 (4460)	435 (−3.3)	514 (0.0)	560 (+1.0)	Λ-cis	6.7	45
Ar(*N*-Me-L-Leu), **6**	496 (4790)	430 (−3.0)	486 (0.0)	540 (+2.1)	Λ-cis	0.3	0.1
Tr(L-Leu), **8a**	500 (5030)	428 (+3.8)	490 (0.0)	540 (−2.9)	Δ-cis	>50	>2500
Tr(L-Ala), **8b**	496 (5040)	430 (+3.5)	496 (0.0)	548 (−1.6)	Δ-cis	18	300
MECAM, **2**	496 (4700)					1.0	1
enterobactin, **1**[e]	494 (5330)	420 (+4.0)	435 (0.0)	535 (−4.0)	Δ-cis		10^6

[a] 0.1 mM ferric complex in 10–15% MeOH in Tris buffer 0.1 M pH 8.97. The λ_{max} and λ_{ext} values are given in nm. [b] The ligands are named according to the following code: Ar for 1,3,5-tris(aminomethyl)benzene derivatives and Tr for [tris(2-aminoethylamine)] derivatives. [c] The relative binding efficiency values are estimated from a CD monitored competition reaction between the chiral triscatecholate ligands and the nonchiral MECAM. [d] K_L/K_M is the ratio of the estimated binding efficiencies of the chiral ligand and MECAM. [e] Data are from ref 34.

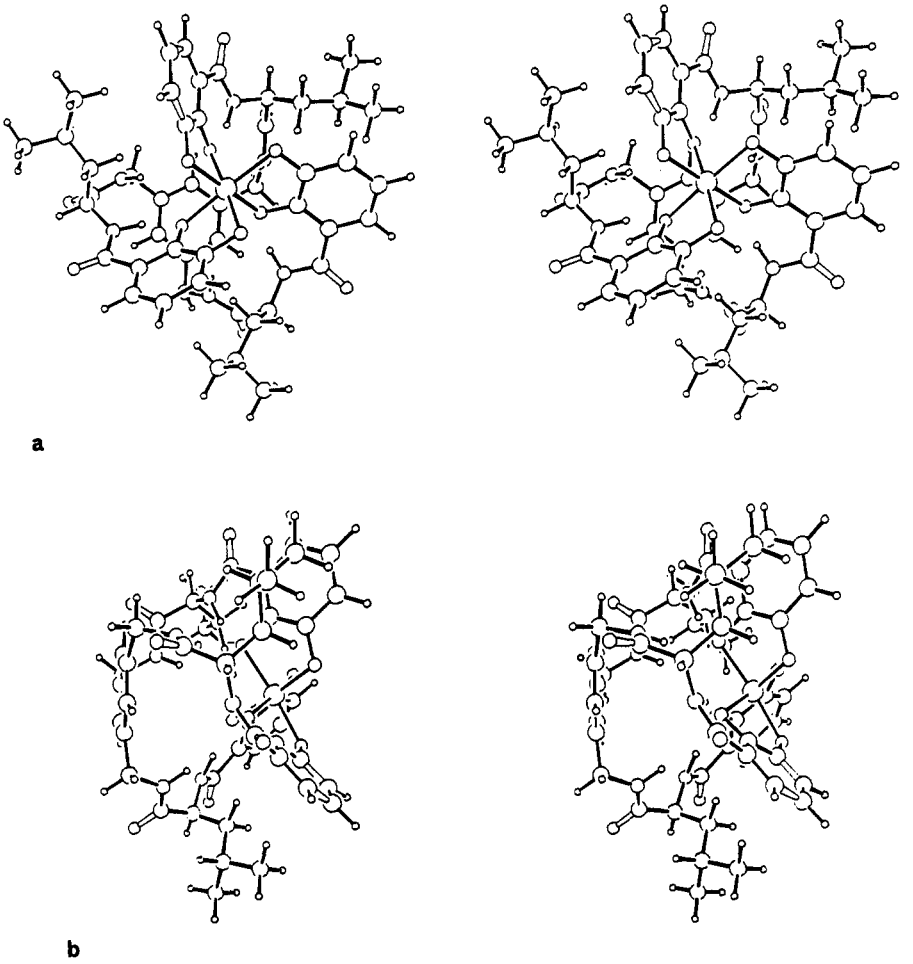

Figure 5. Calculated lowest energy conformation of **5a**–Fe in stereoviews: (a) along the symmetry axis and (b) perpendicular to the symmetry axis.

was retained at equimolar concentration of 1:1:1 of Fe^{3+}:**5a**: MECAM **2** (Table II, 1st row), indicating a factor of about 50 in binding efficiency of **5a** relative to MECAM **2**.

Other Triscatechoylamides and Their Metal Complexes. The triscatechoylamide ligand **5a** thus represents the first synthetic enterobactin analog that forms ferric ion complexes with the same absolute configuration, namely Δ-cis, as enterobactin and that favorably competes with MECAM **2**, the best synthetic triscatechoylamide hitherto available.[4] We next examined the effects of the nature of (i) the amino acid residues, (ii) the nature of the anchor, and (iii) the presence of the H-bonding network, on the ferric ion binding properties of such triscatechoylamides, with the purpose to find superior ion binders. In this search we were guided by our study of the parent trispeptides,[16-18] whose conformations had been shown to be affected by these parameters.

Following the above reasoning the Leu residue in the catechoylamides **4a** and **5a** was replaced by Ala to yield **4b** and **5b**. Also the amide group Cat-CONH in **5a** was methylated to provide the Cat-CONMe derivative **6**. Furthermore, the TRAM anchors of **4a**, **5a**, **4b**, and **5b** were substituted by the more flexible TREN anchor to provide **7a**, **8a**, **7b**, and **8b**, since TREN anchors had been found to yield conformations with H-bond belts even in polar media, while the H-bonding belts of trispeptides based on the TRAM anchors were broken in polar media.[16-18]

The synthesis of this series of binders was performed as illustrated in Scheme I, and their structures were examined by a combination of IR and NMR spectroscopy (Table III).

Inspection of Table III allows us to discern three types of conformations of protected triscatechoylamides. The first one, expressed by the Leu derivative **4a**, is characterized by bonded NH and anisotropic benzyl protons, ArCH_2NH, and isotropic benzyloxy protons, PhCH_2O. This is in agreement with interstrand H-bonds and intrastrand H-bonds as discussed above and depicted in Figure 3c. The second type is realized by the TREN derivatives **7a** and **7b**, which like **4a** also contain merely bonded NH but have both diastereotopic NCH_2CH_2 and PhCH_2O protons appear as

Table III. IR and ^{1}H NMR Data of Protected and Free Triscatechoylamides in $CDCl_3$ and CD_3OD

compd	IR ν (cm^{-1}) NH $CHCl_3$	^{1}H NMR δ (ppm) $PhCH_2O$ $CDCl_3$	CH_2NH $CDCl_3$	CH_2NH CD_3OD
3	3437, 3361	5.16 (br s), 5.12, 5.04 (ABq)	4.43 (m)	
4a	3354	5.12 (s), 5.08 (s)	4.56, 4.11 (ABq)	
4b	3435w, 3356	5.12 (s), 5.10, 5.01 (ABq)	4.41, 4.47 (ABq)	
5a	3435, 3345 3322			4.33 (s)
5b				4.35 (s)
7a	3344	5.07 (s), 5.16, 5.07 (ABq)	3.48, 2.95 (ABq)	
7b	3345	5.10 (s), 5.18, 5.04 (ABq)	3.48, 2.95 (ABq)	
8a	3435, 3257 3277			3.38, 3.21 (ABq)
8b				3.39, 3.28 (ABq)

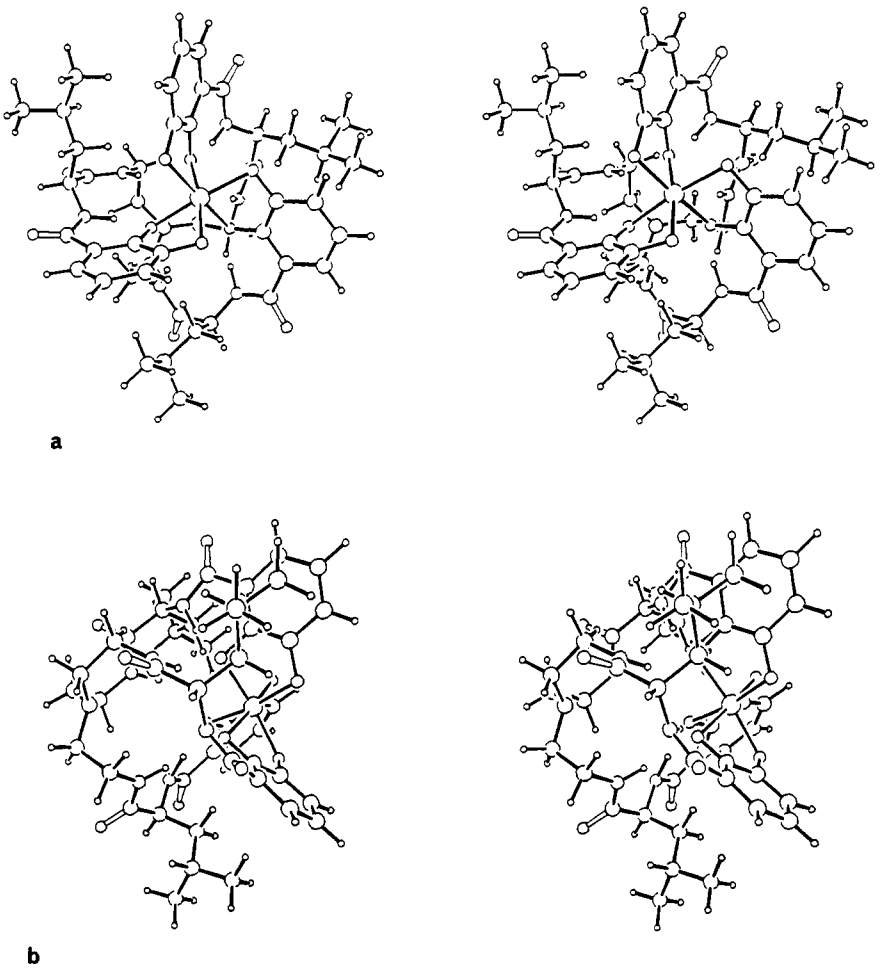

Figure 6. Calculated lowest energy conformation of the ferric complex **8a**–Fe in stereoviews: (a) along the symmetry axis and (b) perpendicular to the symmetry axis.

AB quartet. These observations are in agreement with intrastrand H-bonds to the benzyloxy groups and inter-strand H-bonds as shown in Figure 3d. The third type is found in the Ala derivative **4b**, which is similar to the second type (Figure 3d), except for some residual IR frequency of free NH bonds, indicating some broken H-bonds.

The restriction of conformational freedom was observed to be stronger in the TREN-based free ligands than in the TRAM ones. While the TRAM based free ligands **5a** and **5b** failed to show different NMR signals for their diastereotopic $-CH_2NH$ protons in the polar solvent CD_3OD (Table III), the TREN based ligands **8a** and **8b** showed AB quartets for these protons even in this solvent. The chemical shift differences were larger for the Leu derivative **8a** (0.17 ppm) than for the Ala derivative **8b** (0.11 ppm). This behavior resembles that of the related trispeptides,[16-18] where the H-bonding networks of the TRAM derivatives were broken but of those of the TREN derivatives were retained in CD_3OD solution, as indicated by the NMR spectra of the respective compounds. This observation demonstrates the presence of a stronger H-bond network in the TREN than in the TRAM derivatives. The favorable H-bond network in the former indicates that the TREN is less strained when H-bonded. This may be attributed to two factors: (i) the strands may be better oriented when emerging from the amino- rather than phenyl based anchor and (ii) the presence of an additional methylene group on each strand of the TREN anchor imparts one degree of freedom more to each strand and may thereby allow better adjustment of the molecule to the constraints imposed by H-bonding.

All binders, **5a**, **5b**, **8a**, and **8b**, were shown to bind ferric ions in a 1:1 stoichiometry and to adopt preferentially Δ-cis configurations when L-amino acids residues were employed but Λ-cis configurations when D-amino acids were used. Empirical force

Table IV. UV and CD Data for Free Triscatechoylamides **5a** and **8a**[a]

ligand[b]	UV λ_{max} ($\epsilon \times 10^{-4}$)			CD λ_{ext} ($\Delta\epsilon$)	
Ar(L-Leu), **5a**	221 (9.3)	253 (2.3)	334 (1.4)	250 (+4.8)	325 (+8.2)
Tr(L-Leu), **8a**	222 (8.9)	253 (2.4)	335 (1.5)	250 (+6.8)	325 (+8.0)

[a] 0.1 mM free ligand in 10% MeOH in TRIS buffer 0.1 M pH 8.97. [b] The ligands are named according to the following code: Ar for TRAM derivatives, Tr for TREN derivatives.

field calculations confirmed these observations, yielding always a Δ-cis configuration in the lowest energy conformation for L-amino acid derivatives. A stereoview of the calculated ferric complex of the TREN-L-leucyl derivative **8a** is presented in Figure 6.

The relationship that links the L-configuration of the amino acid residue with the Δ-cis configuration of the complex was violated by the N-methylated L-Leu derivative **6**, whose ferric complex adopted preferentially the Λ-cis configuration. Molecular models suggest that the N-methylated complex **6** is highly distorted with the N–Me groups pointing outwards from the coordination center. Binder **6** lacks the capability to form H-bonds with the catecholate anions O^-. Such H-bonds with the adjacent amides (which characterize the other binders[15,19,33]) stabilize the catecholate complex and seem to support the linkage between the L-configuration of the amino acid and the Δ-cis configuration of the complex, by making the L-Δ diastereoisomer energetically preferred over the alternative L-Λ isomer. The calculated structures (Figures 5 and 6) also indicate that the amino acids' side chains point into the groove between the tripodal arms in close proximity to the catecholate rings. This computational result was confirmed experimentally by the high field shift of the methyl protons in the Ala derivative **7b** upon binding (Figure 7). It also suggests that hydrophobic interactions may play a role in stabilizing these complexes.

Independent information on the chiral purity of the triscatecholate complexes was obtained by measuring the ^1H NMR spectra of their Ga^{3+} complexes in DMSO-d_6. While both Leu derivatives **5a** and **8a** as well as the Ala derivative **8b** gave rise to a single set of signals when binding Ga^{3+}, indicating the predominance of one stereoisomer, the Ala derivative **5b** showed two sets of signals (although of unequal intensities), indicating the presence of two isomeric complexes, namely Δ-cis and Λ-cis isomers (Figure 7). Tables II, IV, and V summarize the electronic and CD spectral data obtained for the free triscatechoylamide ligands and their ferric complexes. The free ligands exhibit three main absorptions in the UV region which are located around 220, 250, and 335 nm (Table IV) and can be attributed to the 2,3-dihydroxybenzamide chromophore.[24] The CD spectra show small Cotton effects, with extremes approximately located around the absorption maxima. Upon ferric complexation the absorptions are shifted to longer wavelengths and the CD spectra are significantly changed (Figure 4 and Table V). The most intense band around 230 nm gives rise to two strong Cotton effects in the CD spectra, which indicate exciton coupling of the aromatic chromophores due to their proximity and mutual right-handed orientation (see Figure 4 and Table V). The second aromatic transition around 260 nm gives rise to a negative Cotton effect (in the Δ-cis complexes), while the weakest transition in the UV region (around 340 nm) shows again a double Cotton effect. In addition, all ferric catecholates show a charge-transfer band[25,26] around 500 nm which gives rise to two Cotton effects of opposite absolute sign around 430 and 550 nm. The dichroic values for the Leu derivatives **5a**–Fe and **8a**–Fe are higher than for the Ala derivatives **5b**–Fe and **8b**–Fe, suggesting, but not proving, higher chiral preference.

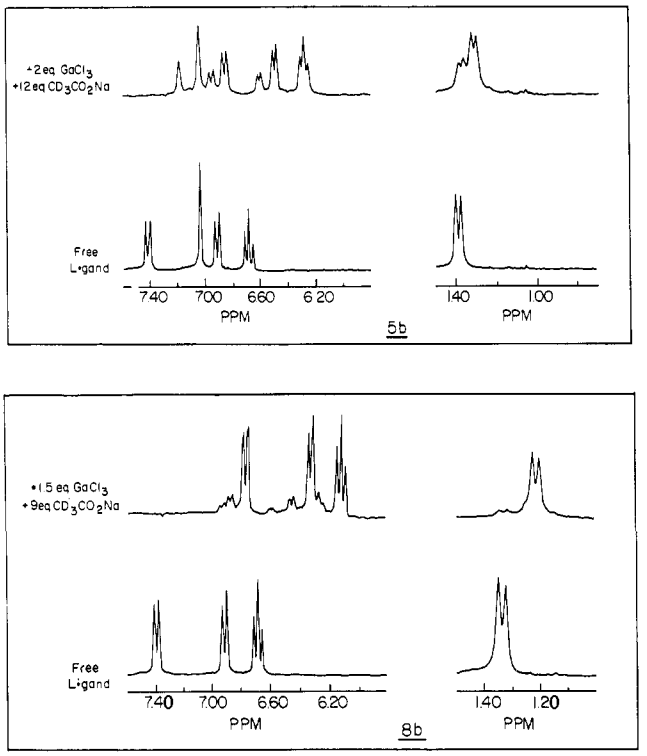

Figure 7. Parts of the ^1H NMR trace of free triscatechoylamides **5b** (upper frame, bottom trace) and **8b** (lower frame, bottom trace) and their Ga^{3+} complexes Ga-**5b** (upper frame, top trace) and Ga-**8b** (lower frame, top trace) in DMSO-d_6.

The ferric complex of the *N*-Me-L-Leu derivative **6** deviated from this pattern and failed to show the split CD bands centered around 230 and 330 nm (Table V). The deviations of the N–Me derivative **6**–Fe can be attributed to lack of coplanarity between the catecholate ring and amide linkage, which is not any more engaged in Cat-NH···O-Cat H-bonding. This deviation is in accord with the preferential formation of the Λ-cis rather than the Δ-cis configuration of the **6**–Fe complex, as discussed above.

The UV–vis and CD spectra of the Fe–enterobactin complex **1**–Fe are close to those of the Fe^{3+} complexes of the synthetic analogs containing L-amino acids, **5a**–Fe, **5b**–Fe, **8a**–Fe, and **8b**–Fe, but not identical. As far as the CD pattern is concerned, the only difference resides in the transition around 340 nm, which gives rise to a double Cotton effect in the synthetic complexes but a single Cotton effect in the natural enterobactin. All the other Cotton effects are identical in their absolute signs and similar in their positions, although the dichroic values are larger in the natural **1**–Fe. The differences in the 340-nm transition, which is attributed to a ligand based n-π^* transition,[36] suggest subtle

Table V. UV and CD Data of Triscatecholate Ferric Complexes[a]

ligand[b]	UV λ_{max} ($\epsilon \times 10^{-4}$)			CD λ_{ext} ($\Delta\epsilon$)						
Ar(L-Leu), **5a**	230 (5.5)	261 (2.7)	340 (1.5)	221 (+17)	228 (0)	237 (−24)	262 (−12)	309 (+4.6)	328 (0)	352 (−7.0)
Ar(L-Ala), **5b**	229 (5.1)	260 (2.6)	338 (1.5)	223 (+6.7)	228 (0)	237 (−16)	259 (−12)	310 (+5.5)	332 (0)	350 (−4.5)
Ar(D-Ala)	230 (4.8)	259 (2.5)	339 (1.5)	223 (−6.7)	228 (0)	237 (+15)	260 (+12)	310 (−5.5)	332 (0)	350 (−4.0)
Ar(*N*-Me-L-Leu), **6**			310 (1.5)	213 (−23)	229 (0)		245 (+5.5)	296 (−2.5)		
Tr(L-Leu), **8a**	231 (5.9)	262 (2.6)	340 (1.5)	223 (+14)	228 (0)	237 (−25)	265 (−10)	308 (+6.4)	327 (0)	350 (−10.3)
Tr(L-Ala), **8b**	229 (5.8)	260 (2.7)	340 (1.6)	223 (+15)	229 (0)	236 (−22)	263 (−11)	307 (+8.0)	327 (0)	344 (−9.2)
MECAM, **2**	229 (6.0)	258 (3.3)	334 (1.4)							
enterbactin[c]			346 (1.9)	220 (+48)	230 (0)	240 (−70)	275 (−16)		320 (+13)	

[a] 0.1 mM Ferric complex in 10–15% MeOH in TRIS buffer 0.1 M pH 8.97. λ_{max} and λ_{ext} values are given in nm. [b] The ligands are named as indicated in Table IV. [c] Data are as from ref 34.

differences in the orientation of the chromophores or diminished conjugation between the catechoylamide amide and the catechol ring in the synthetic complexes. The smaller dichroic values for some of the remaining Cotton effects indicate either (i) lower isomeric purity of the synthetic analogs or (ii) diminished exciton coupling due to larger distances or deviations from perfect octahedral orientation (i.e., weaker binding) or both.

The relative iron binding efficiencies of the ligands **5b**, **6**, **8a**, and **8b** were estimated by CD monitored competition experiments with nonchiral MECAM **2** (see Experimental Section). The results of these experiments are summarized in the two last columns of Table II. Examination of the values obtained leads to the following conclusions: 1. All the chiral catechoylamide derivatives synthesized hitherto, except the N-methylated **6**, show higher binding efficiency than the nonchiral MECAM **2**. Substituting the amide proton in CONH by a methyl group reduces significantly the binding efficiency and inverts the absolute configuration of the resulting Fe^{3+} complex. 2. The leucyl derivative is the strongest binder among the TRAM based α-amino acid derivatives. 3. The aliphatic TREN based catechoylamides bind Fe^{3+} more efficiently than the TRAM based ligands. 4. The leucyl derivative of the TREN based catechoylamide **8a** forms the most stable ferric complex synthesized hitherto, approaching the binding efficiency of enterobactin within less than three orders of magnitude.

In order to examine whether the enhanced binding efficiencies of the chiral triscatechoylamides for Fe^{3+} is related to enhanced selectivity, competition experiments between Fe^{3+} and Al^{3+} were performed. One equivalent of Al^{3+} was added to methanolic solutions of the ferric complexes of **5a**, **8a**, and MECAM **2**, and the mixtures were allowed to equilibrate for 3 h. The amount of Fe^{3+} displaced by Al^{3+} was found to be 15%, 8%, and 5% for MECAM **2**, **5a**, and **8a**, respectively, as estimated from the decrease in the ligand-to-metal charge-transfer transitions (LMCT) of the ferric triscatecholates around 500 nm. These semiquantitative results suggest that the strongest Fe^{3+} binder, **8a**, is also the most selective one.

Summary and Conclusions

Extending two families of C_3 symmetric trispeptides with catecholate binding sites has been shown to provide binders that form Fe^{3+} complexes of preferential Δ-cis configuration when L-amino acids are used. These complexes are stabilized by intramolecular H-bonds from the catechoylamides CONH to the catecholate oxygens O^-, as in enterobactin. When the possibility of this type of H-bond is eliminated by replacing the amide proton CONH by an amide *N*-methyl CONMe (ligand **6**), the chiral preference of the complex is inverted from the Δ-cis to the Λ-cis configuration. All these ligands, with the exception of the methylated derivative **6**, were found to be efficient Fe^{3+} binders with the TREN derivative **8a** being the most efficient of all, approaching enterobactin's binding efficiency within less than three orders of magnitude.

The superiority of the TREN triscatechoylamide **8a** as Fe^{3+} binder in comparison to its Ala analog **8b** and particularly the TRAM triscatecholates **5a** and **5b** could derive from reduced strain in the complex, reduced entropy loss upon binding, or a combination of both. The accurate determination of the contribution of each, enthalpy and entropy, by detailed thermodynamic studies is beyond the scope of this work. Force field calculations failed to reveal pronounced differences in the strain between the complexes of the TRAM and TREN based ligands. Although various approaches to estimate strain energies were pursued, no definite answer could be reached, since the strain is spread over a great number of degrees of freedom. On the other hand, experimental data on the two families of compounds revealed the presence of conformationally restricted structures in the TREN based triscatechoylamides, and particularly the Leu derivative **8a**, in polar solvents where complexation is being performed, while the TRAM based ligands were found to adopt random conformations under identical conditions. It may thus be concluded, that the restricted conformational freedom of the TREN derivative **8a**, which reduces the entropy loss upon binding, might significantly contribute to its higher binding affinity.

Experimental Section

General. Unless otherwise stated, all NMR spectra were recorded on a 270-MHz Bruker WH-270 instrument. Chemical shifts are reported in ppm relative to internal Me_4Si. Infrared spectra were measured using a Nicolet MX-1 FTIR spectrophotometer. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. Electronic (UV–vis) and circular dichroism (CD) spectra were measured using a Hewlett-Packard Model 8450A spectrophotometer and a JASCO J-500C spectropolarimeter. Elemental analyses were performed by the Microanalysis Laboratory at the Hebrew University, Jerusalem, Israel. Low resolution FAB MS were performed at the University of Texas at Austin, using a Finnigan-MAT TSQ-70 instrument. Melting points are uncorrected.

Synthesis. *N*-[2,3-Bis(benzyloxy)benzoyl]-L-leucine. A suspension of L-leucine (1.0 g, 7.6 mmol) in 5 mL of water was cooled to 0 °C and treated with sodium hydroxide (0.64 g, 16 mmol, in 5 mL of water). 2,3-Bis(benzyloxy)benzoyl chloride[28] (2.77 g, 7.5 mmol, in 20 mL of THF) was added dropwise. Stirring was continued for 1 h at 0 °C and for 2 h at room temperature. The resulting mixture was cooled and acidified to pH 1.5–2 (10% HCl), the THF was evaporated, and the residue was extracted with ethyl acetate (200 mL). The ethyl acetate was washed twice with water, dried (Na_2SO_4), and evaporated. Purification by flash chromatography (0–10% $MeOH/CH_2Cl_2$) afforded 2.92 g (87% yield) of a viscous oil: ^1H NMR (CD_3OD) δ 7.16–7.58 (m, 13 H, aromatic), 5.19 (s, 2 H, PhCH_2O), 5.14 (s, 2 H, PhCH_2O), 4.54 (m, 1 H, $C_\alpha H$), 1.59 (m, 1 H, $CHMe_2$), 1.55, 1.39 (sp ABq, 2 H, CH_2[i-Bu]), 0.84 (m, 6 H, CH_3).

***N*-[2,3-Bis(benzyloxy)benzoyl]-L-alanine.** Treating L-alanine with the acyl halide according to the same procedure afforded the product (69% yield) after recrystallization from ethyl acetate/*n*-hexane, mp 121–123 °C: $[\alpha]^{30}_D$ = +11 (MeOH, *c* 0.2); IR ($CHCl_3$) 3351, 3350, 3208 cm^{-1} (N–H, O–H) 1760, 1722, 1653 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 8.54 (br d, *J* = 6.2 Hz, 1 H, NH), 7.74 (dxd, 1 H, aromatic), 7.50–7.13 (m, 12 H, aromatic), 5.17 (s, 2 H, PhCH_2O), 5.13 (s, 2H, PhCH_2O), 4.54 (m, 1 H, $C_\alpha H$), 1.19 (d, *J* = 7.2 Hz, CH_3). The D-alanine derivative was prepared according to the same procedure in 50% yield: mp 126–7 °C; $[\alpha]^{30}_D$ = −12.5 (MeOH, *c* 0.2).

***N*-[2,3-Bis(benzyloxy)benzoyl]-*N*-methyl-L-leucine.** Reacting *N*-methyl-L-leucine with the acyl halide according to the same procedure afforded the product (77% yield) after purification by chromatography (0–5% $MeOH/CHCl_3$) as a viscous oil. The ^1H NMR ($CDCl_3$) spectrum of this product showed the presence of several species in slow exchange (at ambient temperature). The signals of the dominant one are given: δ 7.40–7.30 (m, aromatic), 5.13 (br s, PhCH_2O), 2.75 (br s, NMe), 0.88 (br, CH_3[i-Bu]).

General Procedure for the Coupling of the Acylated Amino Acids with Amines. To a cold THF solution of amino acid (1.0 equiv) and *N*-hydroxysuccinimide (1.1 equiv) was added DCC (1.1 equiv), followed by a catalytic amount of DMAP (0.1 equiv). The reaction mixture was kept at 0–4 °C for 8–12 h. The dicyclohexylurea (DCU) was filtered and washed with THF, and the active ester solution was allowed to react with TRAM or TREN (0.33 equiv) at room temperature for ca. 24 h. The THF was evaporated, and the residue was dissolved in chloroform, washed twice with water, dried ($MgSO_4$ or Na_2SO_4), and evaporated to dryness. Purification of the products was performed by flash chromatography using mixtures of methanol in dichloromethane as eluents. The corresponding single chain analogue was prepared according to the same procedure by replacing the trifunctional amines by benzylamine.

L,L,L-1,3,5-C_6H_3[CH_2NHCOCH(i-Bu)NHCOC_6H_3(OBzl)$_2$]$_3$ (4a). *N*-[2,3-Bis(benzyloxy)benzoyl]-L-leucine (2.46 gr, 5.5 mmol) was activated with NHS (0.75 g, 6.5 mml), DCC (1.28 gr, 6.2 mmol), and DMAP (40 mg, 0.33 mmol) in THF according to the general procedure. Half of the filtered active ester solution was treated with 1,3,5-tris(aminomethyl)benzene (0.15 g, 0.9 mmol) for 46 h at room temperature. Usual workup and flash chromatography (2.5% $MeOH/CH_2Cl_2$) affords 0.85 g (65% yield) of a waxy solid: mp 75–6 °C; IR ($CHCl_3$) 3354 cm^{-1} (N–H), 1680, 1647 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 8.44 (d, *J* = 7.3 Hz, 3 H, $C_\alpha NH$), 7.65–7.10 (m, 42 H, CH_2NH and aromatic), 5.12 (s,

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6 H, PhCH_2O), 5.08 (s, 6 H, PhCH_2O), 4.71 (m, 3 H, C$_\alpha H$), 4.56, 4.11 (dxAB, ^2J = 15.6 Hz, $^3J_1$ = 7.3 Hz, $^3J_2$ = 4.5 Hz, 6 H, PhCH_2NH), 1.53 (br m, 6 H, CH$_2${i-Bu}), 1.31 (m, 3 H, CH{i-Bu}), 0.77 (m, 18 H, CH$_3${i-Bu}).

L,L,L-**1,3,5-C$_6$H$_3$[CH$_2$NHCOCH(i-Bu)NHCOC$_6$H$_3$(OH)$_2$]$_3$ (5a).** The protected ligand **4a** (0.47 g, 0.32 mmol) in ethanol–methanol (150 mL, 1:1) was added to a suspension of 10% Pd/C (0.47 g) in ethanol (15 mL), and the mixture was hydrogenated for 1.5 h at atmospheric pressure. The catalyst was filtered, and the solvent was evaporated. Purification on a short reversed phase column (RP-18, loading with methanol, elution with acetonitrile). The product (0.27 g, 92% yield) was precipitated from ethyl acetate by ether and *n*-hexane as a white powder: mp 132–135 °C; FAB MS (glycerol) 913 $(M + H)^+$; IR ($CHCl_3$) 3435, 3345, 3322 cm^{-1} (N–H), 1678, 1639 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6) δ 8.76 (d, J = 7.6 Hz, 3 H, C$_\alpha$N*H*), 8.56 (br t, 3 H, CH$_2$N*H*), 7.42 (d, J = 8 Hz, 3 H, cat-6), 6.92 (d, J = 7.7 Hz, 3 H, cat-4), 6.68 (t[dxd], $J \sim$ 7.8 Hz, 3 H, cat-5), 7.02 (s, 3 H, ArH), 4.55 (br m, 3 H, C$_\alpha H$), 4.23 (br d, 6 H, ArCH_2NH), 1.75 (m, 3 H, CH{i-Bu}), 1.61 (br m, 6 H, CH$_2${i-Bu}), 0.89 (m, 18 H, CH$_3${i-Bu}).

L,L,L-**1,3,5-C$_6$H$_3$[CH$_2$NHCOCH(Me)NHCOC$_6$H$_3$(OBzl)$_2$]$_3$ (4b).** *N*-[2,3-Bis(benzyloxy)benzoyl]-L-alanine (1.22 g, 3 mmol) was activated with NHS (0.4 g, 3.5 mmol), DCC (0.7 g, 3.4 mmol), and DMAP (15 mg, 0.12 mmol) in THF. Half of the active ester solution was treated with 1,3,5-tris(aminomethyl)benzene (83 mg, 0.5 mmol, in 2 mL of THF) for 24 h at room temperature. Usual workup and flash chromatography (2.5% MeOH/CH_2Cl_2) afforded 0.53 g (79% yield) of an off-white solid, mp 192–194 °C; FAB MS, 1327 $(M + H)^+$, 1349 $(M + Na)^+$; IR ($CHCl_3$) 3435 (w), 3356 cm^{-1} (NH), 1680, 1647 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$) δ 8.39 (d, J = 6.8 Hz, C$_\alpha$N*H*), 7.60–7.06 (m, 39 H, aromatic), 7.04 (s, 3 H, ArH), 5.12 (s, 6 H, PhCH_2O), 5.10, 5.01 (ABq, 6 H, PhCH_2O), 4.55 (m, 3 H, C$_\alpha H$), 4.41, 4.17 (sp ABq, 6 H, CH_2NH), 1.17 (d, J = 7 Hz, 9 H, CH$_3$).

L,L,L-**1,3,5-C$_6$H$_3$[CH$_2$NHCOCH(Me)NHCOC$_6$H$_3$(OH)$_2$]$_3$ (5b).** A suspension of 10% Pd/C (0.2 g) and the protected ligand **4b** (0.25 g, 0.19 mmol) in EtOH–MeOH (200 mL, 1:1) was hydrogenated at atmospheric pressure for 1.5 h. The catalyst was filtered, and the solvent was evaporated. Further purification on a short reversed phase column (Merck RP-18, elution with CH_3CN) afforded creamy-white powder (0.12 g, 80% yield): mp 178–180 °C (phase transition at 155 °C); FAB-MS (negative ion) 785 $(M - H)^-$, (positive ion) 787 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 8.86 (d, J = 7 Hz, 3 H, C$_\alpha$N*H*), 8.54 (br t, 3 H, CH$_2$N*H*) 7.41 (d, J = 7.8 Hz, 3 H, cat-6), 6.91 (d, J = 7.2 Hz, 3 H, cat-4), 6.68 (d, J = 7.9 Hz, 3 H, cat-5), 7.03 (s, 3 H, ArH), 4.52 (m, 3 H, C$_\alpha H$), 4.25 (m, 6 H, CH_2NH), 1.38 (d, J = 7 Hz, CH$_3$).

PhCH$_2$NHCOCH(i-Bu)NHCOC$_6$H$_3$(OBzl)$_2$. *N*-[2,3-Bis(benzyloxy)-benzoyl]-L-leucine NHS active ester (0.1 mmol, prepared as described above for **4a**) was treated with benzylamine (10 mg, 0.093 mmol) at room temperature for 24 h. Usual workup and flash chromatography (50–80% ether/*n*-hexane) afforded 47 mg (84% yield) of a glassy material: IR ($CHCl_3$) 3437, 3361 cm^{-1} (N–H), 1677, 1656 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 8.33 (br d, J = 7.4 Hz, 3 H, C$_\alpha$N*H*), 7.72, 7.46–7.15 (m, 13 H, aromatic), 6.67 (br t, 3 H, CH$_2$N*H*), 5.16 (br s, 6 H, PhCH_2O), 5.12, 5.04 (ABq, J = 10.3 Hz, 6 H, PhCH_2O), 4.52 (m, 3 H, C$_\alpha H$), 4.43 (m, 6 H, PhCH_2N), ~1.6 (m, 3 H, CH and CH$_2${i-Bu}), 0.82 (m, 6 H, CH$_3${i-Bu}).

L,L,L-**N[CH$_2$CH$_2$NHCOCH(i-Bu)NMeCOC$_6$H$_3$(OH)$_2$]$_3$ (6).** The active ester solution (1.8 mmol in 8 mL of THF, prepared as described for **4a**) was treated with TREN (50 mg, 0.3 mmol) for 48 h at room temperature. Usual workup and flash chromatography (1–2% MeOH/CH_2Cl_2) provided 0.24 g (54%) of protected catechoylamide [IR ($CHCl_3$) 3354 cm^{-1} (N–H), 1678 and 1630 cm^{-1} (C=O)]. This material (0.18 g, 0.12 mmol) was dissolved in a 1:1 mixture of methanol and ethanol and hydrogenated under atmospheric pressure in the presence of 0.18 g of 10% Pd/C for 2.5 h. The material was purified on a RP column to yield 0.105 g of pure catechoylamide **6**.

L,L,L-**N[CH$_2$CH$_2$NHCOCH(i-Bu)NHCOC$_6$H$_3$(OH)$_2$]$_3$ (8a).** The active ester solution (2.75 mmol in 30 mL of THF, prepared as described for **4a**) was treated with TREN (135 mg, 0.9 mmol) for 46 h at room temperature. Usual workup and flash chromatography (2% MeOH/CH_2Cl_2) afforded 0.7 g (54% yield) of a glassy solid: IR ($CHCl_3$) 3344 cm^{-1} (N–H), 1670 (sh), 1647 cm^{-1} (NC=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.381 (d, J = 8 Hz, 3 H, C$_\alpha$N*H*), 7.974 (br t, 3 H, CH$_2$N*H*), 7.49 (d, J = 7.2 Hz, 3 H, cat-5), 7.380 (m, 12 H, Ph), 7.305 (d, J = 8.1 Hz, 3 H, cat-3), 7.266 (m, 18 H, Ph), 7.129 (br t, 3 H, cat-4), 5.190 (br s, 6 H, PhCH_2O), 5.064, 4.981 (ABq, J = 10.3 Hz, PhCH_2O), 3.093 (br, 6 H, NCH$_2$CH_2NH), 2.466 (br t, 6 H, NCH_2CH$_2$NH), 1.497 (m, 3 H, CH{i-Bu}), 1.369 (m, 6 H, CH$_2${i-Bu}), 0.790, 0.749 (two d, J = 6.3 Hz, 18 H, CH$_3${i-Bu}).

A suspension of 10% Pd/C (0.4 g) and the protected ligand **7a** (0.38 g, 0.265 mmol) in methanol–ethanol (50–20 mL) was hydrogenated at atmospheric pressure for 2 h. The catalyst was filtered, and the solvent was evaporated. Short reversed phase column chromatography (RP-18, elution with methanol) afforded 0.205 g (86% yield) of a white powder (mp 123–7 °C) after precipitation from ethyl acetate (3 mL, contains 1 drop of methanol) by ether and *n*-hexane; FAB MS (methanol/glycerol), 894 $(M + H)^+$; IR ($CHCl_3$, ~0.2 mM) 3435, 3357, 3277 cm^{-1} (N–H), 1675 (sh), 1662, 1641 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 318 K) δ 12.0 (br, 3 H, 2-O*H*), 9.12 (br, 3 H, 3-O*H*), 8.62 (d, J = 8 Hz, 3 H, C$_\alpha$N*H*), 7.94 (br, 3 H, CH$_2$N*H*), 7.40 (d, J = 7.6 Hz, 3 H, cat-6), 6.91 (d, J = 7.8 Hz, 3 H, cat-4), 6.68 (t[dxd], J = 7.9 Hz, 3 H, cat-5), 4.51 (br m, 3 H, C$_\alpha H$), 3.14 (br, 6 H, NCH$_2$CH_2NH), ~2.5 (NCH_2CH$_2$NH, overlap with solvent), 1.59 (br m, 9 H, CH and CH$_2${i-Bu}), 0.87 (br t, 18 H, CH$_3${i-Bu}).

L,L,L-**N[CH$_2$CH$_2$NHCOCH(Me)NHCOC$_6$H$_3$(OH)$_2$]$_3$ (8b).** The active ester (20 mL, 1.5 mmol, prepared as described for **4b**) was treated with TREN (73 mg, 0.5 mmol, in 3 mL of THF) for 24 h at room temperature. Usual workup and flash chromatography (2.5–3% MeOH/CH_2Cl_2) afforded 0.49 g (75% yield) of a viscous oil: IR ($CHCl_3$) 3345 cm^{-1} (N–H), 1670 (sh), 1646 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 8.45 (d, J = 6.7 Hz, 3 H, C$_\alpha$N*H*), 7.82 (br t, 3 H, CH$_2$N*H*), 7.55–7.28 (m, 39 H, aromatic) 5.10 (s, 6 H, PhCH_2O), 5.18, 5.04 (ABq, 6 H, PhCH_2O), 4.61 (m, 3 H, C$_\alpha H$), 3.48, 2.95 (br split ABq, 6 H, CH_2NH) 2.70, 2.42 (br split ABq, 6 H, NCH_2), 1.19 (d, J = 6.9 Hz, 9 H, CH$_3$).

A suspension of 10% Pd/C (0.2 g) and the protected ligand **7b** (0.24 g, 0.21 mmol) in EtOH–MeOH (50 mL, 1:1) was hydrogenated at atmospheric pressure for 3 h. The catalyst was filtered off and the solvent was evaporated. Purification on a short reversed phase column (Merck RP-18) using MeOH as an eluent and precipitation with acetonitrile afforded 117 mg (73% yield) of a creamy powdered material: ^{1}H NMR (DMSO-d_6) δ 12.0 (s, 3 H, 2-O*H*), 9.26 (s, 3 H, 3-O*H*), 8.80 (br, 3 H, C$_\alpha$N*H*) 7.97 (br t, 3 H, CH$_2$N*H*), 7.40 (d, J = 8.0 Hz, 3 H, cat-6), 6.92 (d, J = 7.7 Hz, 3 H, cat-4), 6.69 (t, J = 8.0 Hz, 3 H, cat-5), 4.49 (m, 3 H, C$_\alpha H$), 3.13 (br, 6 H, CH_2NH), 2.5 (overlap with solvent, NCH_2). Note: The D-enantiomer was prepared according to the same procedures from D-Ala.

MECAM 2. 1,3,5-Tris(aminoethyl)benzene (0.165 g, 1 mmol) in THF containing triethylamine (0.5 mL, 6.8 mmol) was cooled to 0 °C and treated with 2,3-bis(benzyloxy)benzoyl chloride[28] (3 mmol). Stirring was continued for 1 h at 0 °C and 1 h at room temperature. The THF was evaporated, and the residue was dissolved in dichloromethane. The dichloromethane was washed with 1 N HCl, 2 N sodium bicarbonate and water, dried (Na_2SO_4), and evaporated. Flash chromatography (1% MeOH/CH_2Cl_2) afforded a viscous syrup (0.71 g, 64% yield). Anal. Calcd for $C_{72}H_{63}N_3O_9$: C, 77.61; H, 5.70; N, 3.77. Found: C, 77.90; H, 5.49; N, 4.04.

A suspension of 10% Pd/C (0.6 g) and protected MECAM (0.6 g, 0.54 mmol) in methanol–ethanol (150 mL, 1:1) was hydrogenated at atmospheric pressure for 1.5 h. The catalyst was filtered, and the solvent was evaporated. The residue was dissolved in ethyl acetate, and the product was precipitated with ether and *n*-hexane, affording a white (slightly pink) material (0.276 g, 89% yield): mp 125–130 °C (lit. 123–5 °C,[35] 130–5 °C[12]). ^{1}H NMR (DMSO-d_6) δ 9.27 (br t, 3 H, N*H*), 7.28 (d, J = 7.5 Hz, 3 H, cat-6), 7.20 (s, 3 H, ArH), 6.91 (d, J = 7.3 Hz, 3 H, cat-4), 6.65 (t, $J \sim$ 7.8 Hz, cat-5), 4.46 (br d, 6 H, CH$_2$N).

Metal Complexation

Experimental Method. Stock solutions of the triscatechoylamide ligands (1–2 mM) were prepared by dissolving a weighted amount into volumetric flasks. The solutions were kept in the dark at 0–4 °C. An aqueous stock solution of 1.0×10^{-2} M Fe(ClO$_4$)$_3$·9H$_2$O containing 9×10^{-3} M $HClO_4$ was used as the ferric ion source. The general complexation procedure was the following: Into a 5-mL volumetric flask was added 250 μL of 2 mM methanolic ligand solution followed by 50 μL of Fe^{3+} solution. Tris buffer (0.1 M, pH 8.97) was then added up to 5 mL, thus giving a final concentration of 0.1 mM ferric complex in 5% MeOH/Tris. In some cases, where the ratio of the MeOH/Tris had to be kept constant (such as competition experiments), an additional amount of MeOH was added to the desired percentage. In other cases, when different solvents were examined, the complex was generated in a similar manner and 50 μL of 0.1 M KOH was added as a base, followed by the desired solvent. The pH of aqueous solutions made in this way was found to be 10.2.

Competition Experiments

Due to the preferential formation of either the Δ-cis or Λ-cis ferric complexes of our chiral ligands, the relative binding efficiencies could be estimated by competition experiments with the

nonchiral MECAM, measuring the Cotton effects in the presence and absence of MECAM. The ternary system was prepared by mixing equivalent amounts of the methanolic solution of the chiral ligand and of the methanolic solution of MECAM, followed by addition of 1 equiv of Fe^{3+}. The Tris buffer was then added, and the UV and CD spectra were measured after several hours of equilibration at room temperature and compared to the pure chiral complex solution containing 10% MeOH. Identical results were obtained after 24 h. The binary system was prepared in the same way without MECAM. The distribution of Fe^{3+} between the chiral ligand and MECAM was obtained from the difference between the intensities of the Cotton effect in the ternary and binary systems as follows: Let the intensities of the Cotton effects in the binary and ternary systems be I_{bin} and I_{ter}, respectively. The ratio between the concentrations of the chiral and achiral complexes in the ternary system is then $I_{tern}/(I_{bin} - I_{tern})$. The accuracy in estimating the binding ratio depends mainly on the accuracy in reading the difference $I_{bin} - I_{tern}$. The smaller the difference, the larger the error. We found our readings reproducible to within 2% and therefore could attribute to ligand **8a** only a lower limit (see Table II).

On the basis of these measurements, the ratio K_L/K_M is obtained as follows: Let $c = c_L = c_M = c_{Fe}$ be the equimolar initial concentration of the corresponding species, let xc and yc be the concentrations of the chiral ligand and MECAM metal complexes, respectively, and $(1 - x)c$, $(1 - y)c$, and $(1 - x - y)c$ be the concentrations of the free species, respectively. Then by the mass action law

$$K_L c = \frac{x}{(1 - x)(1 - x - y)} \quad (1)$$

$$K_M c = \frac{y}{(1 - y)(1 - x - y)} \quad (2)$$

The ratio K_L/K_M is seen to depend on x and y only

$$\frac{K_L}{K_M} = \frac{x}{1 - x} \cdot \frac{1 - y}{y} \quad (3)$$

From eq 1 y is obtained as a function of x

$$y = 1 - x - \frac{x}{(1 - x)K_L c} = (1 - x)\left[1 - \frac{x}{(1 - x)^2 K_L c}\right] \quad (4)$$

In all our ligands $K_L c$ is very large, so $x/(1 - x)^2 K_L c$ is negligibly small, and eq 4 is reduced to

$$y = 1 - x \quad (5)$$

Therefore

$$K_L/K_M = \frac{x^2}{(1 - x)^2} - \frac{(1 - d)^2}{d^2} \quad (6)$$

where $d = 1 - x$ is proportional to the measured difference between the intensities of ternary (I_{tern}) and binary (I_{bin}) systems.

When the ratio K_L/K_M is very large, d is too small to be measurable. In such cases one may add more MECAM to the ternary system. Letting $m = c_L/c_M$, it is easy to show that

$$K_L/K_M = (1 - d)(m - d)/d^2 \quad (7)$$

It follows that d_{min}, the smallest measurable d, is proportional to $\sqrt{m}$.

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Registry No. 2, 69146-59-4; **2** (protected), 90734-96-6; **2** (Fe^{3+} complex), 141979-30-8; **2** (Ga^{3+} complex), 141902-59-2; **3**, 110374-76-0; **4a**, 110374-73-7; **4b**, 141849-39-0; **5a**, 110374-74-8; **5a** (Fe^{3+} complex), 141879-31-4; **5a** (Ga^{3+} complex), 141879-36-9; L,L,L-**5b**, 141849-41-4; L,L,L-**5b** (Fe^{3+} complex), 141879-32-5; D,D,D-**5b**, 141901-29-3; D,D,D-**5b** (Fe^{3+} complex), 141977-94-8; **6**, 141849-35-6; **6** (Fe^{3+} complex), 141879-33-6; **7a**, 141879-30-3; **7b**, 141849-40-3; **8a**, 141849-42-5; **8a** (Fe^{3+} complex), 141879-34-7; **8b**, 141849-43-6; **8b** (Fe^{3+} complex), 141879-35-8; TRAM, 77372-56-6; TREN, 4097-89-6; H-Leu-OH, 61-90-5; H-Ala-OH, 56-41-7; H-D-Ala-OH, 338-69-2; H-MeLeu-OH, 3060-46-6; 2,3-$(BnO)_2C_6H_3CO$-Leu-OH, 141849-36-7; 2,3-$(BnO)_2C_6H_3CO$-Ala-OH, 123198-04-9; 2,3-$(BnO)_2C_6H_3CO$-D-Ala-OH, 141849-37-8; 2,3-$(BnO)_2C_6H_3CO$-MeLeu-OH, 141849-38-9; 2,3-$(BnO)_2C_6H_3COCl$, 69146-58-3; $BnNH_2$, 100-46-9.

Rhodium(I)- and Iridium(I)-Catalyzed Hydroboration Reactions: Scope and Synthetic Applications

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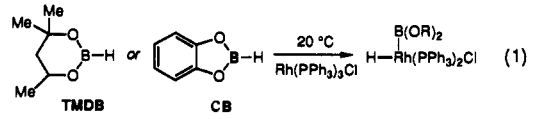
Abstract: A study of the rhodium(I)- and iridium(I)-catalyzed hydroboration of olefins with catecholborane is described. Applications to organic synthesis were one focus of this investigation. The scope of the reaction was defined, and issues of stereoselection were addressed. The rhodium-catalyzed hydroboration of several classes of allylic alcohols was found to be highly diastereoselective, preferentially affording the isomer complementary to that furnished by the uncatalyzed variant of the reaction (9-BBN). The first two general approaches to effecting a directed olefin hydroboration were developed. Both phosphinites and amides proved capable of delivering the transition metal reagent.

Introduction

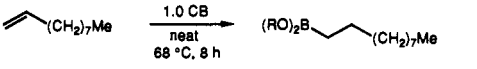
The foundation for the present investigation was laid in 1975 by the observation that $Rh(PPh_3)_3Cl$ (Wilkinson's catalyst) undergoes oxidative addition when treated with either 4,4,6-trimethyl-1,3,2-dioxaborinane (TMDB) or catecholborane (1,3,2-benzodioxaborole, CB) (eq 1).[2] The structure of the TMDB–



(1)

Wilkinson's catalyst adduct was characterized by Kono and Ito.

At the time of this report, the capacity of catecholborane[3,4] and TMDB[5] to hydroborate olefins had been demonstrated, and it had been established that the reactions required elevated temperatures (eq 2). Furthermore, Wilkinson's catalyst[6] was known to catalyze



(2)

(1) (a) NSF Predoctoral Fellow. (b) American Cancer Society Postdoctoral Fellow.

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