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Supplementary Material Available: ^{1}H, ^{13}C, and ^{31}P NMR spectra of **1**, **2**, and **3**, ^{1}H, ^{13}C, ^{15}N, and ^{31}P NMR spectra of 50% ^{13}C and 90+% ^{15}N enriched **1**, and experimental details for the synthesis of *R*- and *S*-**3** from D- and L-Asn (16 pages). Ordering information is given on any current masthead page.

A New Macrobicyclic (Cryptand) Siderophore Containing Three Endocyclic Hydroxamate Donor Groups

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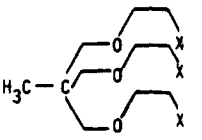
The natural hydroxamate siderophores contain bidentate hydroxamate donor groups in acyclic, exocyclic, and endocyclic arrangements.[1] Of these, the sexadentate endocyclic ligands, such as desferriferrioxamine E, have the highest affinities for iron(III), because of the involvement of the macrocyclic rings in coordination of the metal ion. The only synthetic endocyclic hydroxamate ligand previously reported is a diamino bishydroxamate macrocycle containing pendant carboxylate donors.[2] Up to the present time, no synthetic or natural endocyclic trishydroxamate cryptand has been reported, although the potential of such ligands for effective binding of trivalent metal ions has been pointed out.[3]

The orientation of oxygen donor groups in the hydroxamates places stringent demands on the polyatomic chains which link them together in a manner that places three pairs of oxygen donors symmetrically around a six-coordinate (octahedral) metal ion. Molecular models indicate that eight or more connecting atoms are needed to accomplish this effectively. It is the purpose of this paper to report the synthesis and properties of the first trishydroxamate cryptand. In this case the ligand has nine and 11 atoms between the bidentate hydroxamate units.

Cyanoethylation at 25–35 °C in *p*-dioxane of the 1,1,1-tris(hydroxymethyl)ethane gives the tricyano ether **1** in 83% yield. Hydrolysis of **1** with hydrogen chloride gas in methanol leads to the tricarboxylic acid trimethyl ester **2** (yield = 64%) (Calcd for $C_{17}H_{30}O_9$: C, 53.97; H, 7.94. Found: C, 53.63; H, 7.93. FAB MS $(M + H)^+ = 379$). Treatment of **2** with lithium aluminum hydride affords the triol **5** in 83% yield (Calcd for $C_{14}H_{30}O_6$: C, 57.12; H, 10.27. Found: C, 57.24; H, 10.20. FAB MS $(M + H)^+ = 295$). The tosylate **6**, prepared from tosyl chloride and **5**, was treated with *O*-benzylhydroxylamine in 1,2-dimethoxyethane to give the tris(*O*-benzylhydroxylamine) **7** (Calcd for $C_{35}H_{51}N_3O_6$: C, 68.96; H, 8.37; N, 6.90. Found: C, 68.68; H, 8.45; N, 6.69. FAB MS $(M + H)^+ = 610$, yield = 37%). The triacid chloride **4** was obtained by allowing **3**, which was prepared from its methyl ester, to react with oxalyl chloride in benzene, yield >95%. High dilution acylation of **7** with **4** in benzene gives the protected macrobicyclic trishydroxamate **8** (Calcd for $C_{49}H_{69}N_3O_{12}\cdot 4H_2O$: C, 61.06; N, 4.36; H, 8.00. Found: C, 60.95; N, 4.18; H, 7.10. FAB MS $(M + H)^+ = 893$) in 30% yield. The

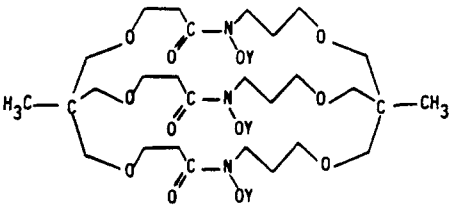


1 X = CN **5** X = CH_2OH

2 X = $COOCH_3$ **6** X = OTs

3 X = COOH **7** X = NHOBz

4 X = COCl



8 Y = Bz

9 Y = H

macrobicyclic trishydroxamate cryptand **9** was obtained from **8** by catalytic hydrogenation. This cryptand (1,13-dimethyl-3,11,15,23,26,34-hexaoxa-6,20,29-trioxo-7,19,30-tris(hydroxyaza)bicyclo[11.11.11]pentatricontane, H_3THX) was characterized by ^{1}H NMR, ^{13}C NMR, elemental analysis, and FAB MS (Calcd for $C_{28}H_{51}N_3O_{12}\cdot{}^{1}/_{2}H_2O$: C, 53.27; H, 8.40; N, 6.59. Found: C, 53.16; H, 8.19; N, 6.38. FAB MS $(M + H)^+ = 622$). This cryptand is very soluble in methanol and sparingly soluble in water. Its sodium salt is very water soluble.

The 1:1 Fe(III) complex of the cryptand was prepared by combining Fe(III) chloride with a slight excess of the neutral (acid) form of the ligand to form a 2.0×10^{-4} molar solution in 2:8 v/v methanol–water and gradually increasing the pH to the desired neutral value. The absorbance spectra of the cryptate (Figure 1) compare well with those of the Fe(III) chelate of desferriferrioxamine B, DFB, reported by Anderegg et al.[4] At pH 4.0 the Fe(III) cryptate has a molar absorbance of 2700 at λ_{max} 423 nm (see Figure 1), while that of Fe^{III}-DFB is 2640 (λ_{max} 440 nm) at pH 4.[4] The absorbance shifts with pH are also similar for the cryptate and DFB complexes, with an isosbestic point at 480 nm for the cryptand and 481 for the DFB complex. The close similarity in the magnitudes of the molar absorbances of the iron(III) complexes of DFB and the cryptand provide assurance that all three bidentate donor groups of the latter are coordinated to the Fe(III) center. The observed isosbestic point indicates the conversion of one pure Fe(III) complex to another as the pH increases. It is suggested that the reaction corresponds to a monoprotonated complex $FeHTHX^+$, having two coordinated hydroxamate groups, and one protonated, non-coordinated hydroxamate group, which is converted at higher pH to the octahedral Fe(III) cryptate FeTHX, with three coordinated hydroxamate groups arranged in an octahedral fashion around the metal ion. At pH 4.4 and above there is little further increase in absorbance, and one therefore concludes the cryptate to be fully formed, with a molar absorbance of 2750 at λ_{max} = 430 nm.

Because the Ga(III) ionic radius is only slightly smaller than that of Fe(III), the new cryptand would also be expected to complex Ga(III) strongly in an octahedral fashion. The 1:1 Ga(III) complex was prepared by the reaction of molar equivalents of $Ga(OH)_4^-$ and the ligand in aqueous solution at pH 8.9. This is above the pH at which Ga(III) precipitates as $Ga(OH)_3$. The white solid which separated was characterized by elemental analysis and mass spectra (Calcd for $C_{28}H_{48}N_3O_{12}Ga\cdot 2H_2O$: C,

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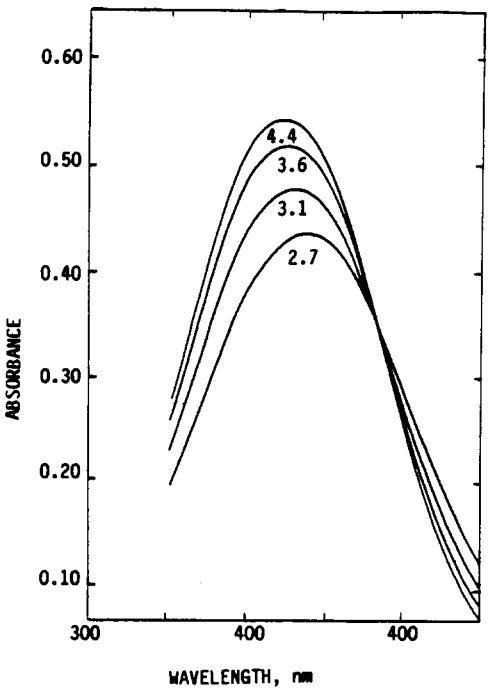

Figure 1. UV–vis spectra of Fe^{III}-H_3THX solutions measured at –log [H^+] values indicated. T_{FeL} = 2.0 × 10^{-4} M, t = 25.0 °C in CH_3OH–H_2O, 1:4 (v/v).

46.38; H, 7.18; N, 5.80. Found: C, 46.48; H, 7.22; N, 5.48. FAB MS $(M + H)^+$ = 688, yield = 85%).

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Long-Chain Polystyrene-Grafted Polyethylene Film Matrix: A New Support for Solid-Phase Peptide Synthesis[1]

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Current methods for the linear scheme of solid-phase synthesis, producing a single peptide analogue per synthesis, are widely based on the original methodology[2] employing divinylbenzene cross-linked polystyrene beads. We report here preliminary investigations on a new support, namely a polystyrene-grafted polyethylene film, that is particularly well-suited for peptide synthesis in a parallel fashion, permitting the simultaneous production of

70
Boc-Ala-Asp(OBzl)-Lys(2Cl-Z)-Ala-Asp(OBzl)-

76
Val-Asp(OBzl)-Val-Leu-Thr(Bzl)-Lys(2Cl-Z)-

84
Ala-Lys(2Cl-Z)-Ser(Bzl)-Gln-OCH_2-C_6H_4-CH_2-CO-

NH-CH_2-polystyrene-grafted polyethylene film

Figure 1. Protection scheme for the solid-phase assembly of [Asp^{76}]-hPTH fragment (70–84) on 440 wt % polystyrene-grafted polyethylene film.

a multitude of peptide analogues.[3] The pendant long-chain linear polystyrene graft, having a molecular weight on the order of 10^6, may be more accessible for chemical reactions than the polystyrene chains embedded in the traditionally used beads where the molecular weight between cross-links is on the order of 10^4. Both the coupling efficiency during the synthesis and the yield and purity of the final products were comparable to those normally obtained by using the traditional polystyrene bead matrices. The grafted film was prepared by γ-irradiation of polyethylene film placed in a methanolic solution of styrene monomer.[4] The presence of methanol during the grafting process serves the purpose of reducing the swelling of the polystyrene-grafted polyethylene and hence reducing the mobility of the growing polystyrene chains. This effect tends to slow down the diffusion-controlled chain-termination processes and is essential to obtain very long-chain grafts and a high level of chain initiation. In 1972 Tregear reported the use of polystyrene that was 10 wt % radiation-grafted onto Kel F particles for solid-phase synthesis.[5] The polystyrene–Kel F graft support was handled in the same way as the traditional polystyrene beads, in contrast with the new support which can be treated or manipulated as sheets, which can be readily separated from one another.

Preparation of the Matrix. A low-density non-cross-linked polyethylene sheet (0.29 g, 54 μm thickness, 55 cm^2) was γ-irradiated in a sealed ampoule containing approximately 20 mL of thoroughly degassed 30% (v/v) styrene in methanol solution. After irradiation at a dose rate of 400 Gy/h in a ^{60}Co source for 10 h at room temperature the ampoule was left overnight. The film was then extracted in a Soxhlet apparatus with dichloromethane for 96 h and dried to afford a polyethylene film grafted with 1.28 g of polystyrene, i.e., 440 wt % grafting. The weight-average molecular weight (M_w) of the extracted styrene homopolymer, which is also formed during irradiation, was determined by size exclusion chromatography (SEC) to be ca. 2 × 10^6 g/mol, and the ratio of weight-average to number-average molecular weight (M_w/M_n) to be 3. As the styrene homopolymer and the polystyrene grafts are formed under conditions that differ only in the initiation step, these numbers are taken as a measure of the molecular weight characteristics of the polystyrene grafts. Further support for this interpretation was obtained by molecular weight characterization of both the original polyethylene film and the grafted film. For the original film the values M_w = 4 × 10^4 g/mol and M_w/M_n = 5 are obtained by high temperature SEC in 1,2,4-trichlorobenzene. The grafted film is soluble in hot xylene, and SEC was run in this solvent at 90 °C to obtain M_w = 6 × 10^6 g/mol and M_w/M_n = 2, which are similar to the values for the styrene homopolymer by product. The molecular weight of both the polyethylene and the polystyrene-grafted polyethylene were estimated relative to polystyrene standards. The solubility

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(1) This work was presented in part at the 20th European Peptide Symposium, Tübingen, West Germany, September 4–10, 1988. Patent protection has been applied for.

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(3) In the parallel synthesis of multiple peptides, each peptide analogue is synthesized on a labeled sheet of grafted film. The common steps of deprotection, neutralization, washings, and coupling of identical amino acids are performed in a single reaction vessel, while the coupling of different amino acids are carried out in separate reaction vessels. Some recent examples of similar approaches include the following: (a) Geysen, H. M.; Meloen, R. H.; Barteling, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 3998. (b) Houghten, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5131.

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