Tetrahedron Vol. 46, No. 8, pp. 2725-2736, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00

SYNTHESIS AND CHARACTERIZATION OF A NEW MACROBICYCLIC (CRYPTAND) SIDEROPHORE CONTAINING THREE ENDOCYCLIC HYDROXAMATE DONOR GROUPS

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(*Received in USA* 11 *October* 1989)

Abstract. The first trishydroxamate cryptand ligand was synthesized by tripodal coupling of a *tris* acid chloride and a *tris* *O*-benzylhydroxylamine. The spectrophotometric properties of its Fe(III) complex were investigated and compared with those of desferriferrioxamine B. Its 1:1 Ga(III) complex was also prepared and characterized.

Introduction

New ligands for the effective and selective complexation of iron(III), gallium(III), indium(III), and gadolinium(III) are of considerable interest because of their potential applications for the treatment of iron overload disease (Cooley's anemia),[1-6] the formation of Ga(III) and In(III) radiopharmaceuticals for the therapeutic and diagnostic imaging of organs and tumors in the human body,[7-9] and the formation of Mn(II), Fe(III), and Gd(III) complexes as paramagnetic NMR contrast agents.[10] The natural and synthetic siderophores containing catecholate and hydroxamate donor groups have been of interest in this connection because of their high affinity for the ferric ion and for other trivalent metal ions. Many synthetic ligands containing catechol donor groups, including some macrocyclic and macrobicyclic ligands, have been reported and found to have high affinities for iron(III).[5,6,11-16]

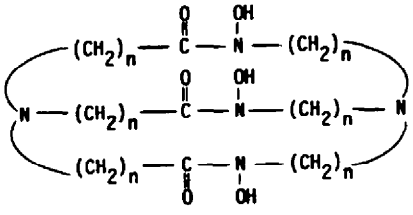
The natural hydroxamate siderophores contain bidentate hydroxamate donor groups in acyclic, exocyclic, and endocyclic arrangements.[17] Of these, the sexadentate endocyclic ligands, such as desferriferrioxamine E,[18,19] have the highest affinities for iron(III), because of the involvement of the macrocyclic rings in coordination of the metal ion. The only synthetic endocyclic hydroxamate ligand previously reported is a diamino bishydroxamate macrocycle containing pendant carboxylate donors.[20] 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.[2]

Although the synthesis of many types of macrobicyclic (cryptand) ligands has been explored extensively in the past decade,[13,15,21,22] the preparation of endocyclic trishydroxamates presents special problems. The structure of the bidentate hydroxamate functional donor group places stringent demands on the connecting polyatomic groups.[20] Also

the hydroxamate group is chemically much more delicate than the other functional groups frequently employed, such as ether, amino and catecholate groups. The design and synthesis of a macrobicyclic trishydroxamate ligand therefore presents a challenge to the coordination chemist. We describe here the design and synthesis of the first trishydroxamate cryptand as well as preliminary coordination chemistry studies of its Fe(III) and Ga(III) complexes.

Ligand Design

The first suggestion of a trishydroxamate cryptand was proposed in 1981[2] and is indicated by 1. From space-filling molecular models and comparison with the naturally occurring macrocyclic trishydroxamate ferrioxamine E, 2, it appears that connecting chains of nine or more atoms are needed between the hydroxamate functional groups, which translates to $n = 4$ or 5 in 1.



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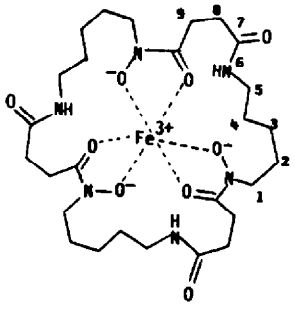

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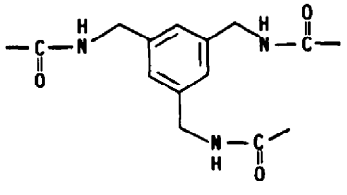
However, an aliphatic chain of more than three carbons ($-CH_2-CH_2-CH_2-$) is not considered suitable because of synthetic problems. It was therefore decided that the first modification of the original design indicated by 1 is to find a suitable bridgehead or cap to connect the three hydroxamate chains. The aromatic caps or spacers such as 3 and 4:



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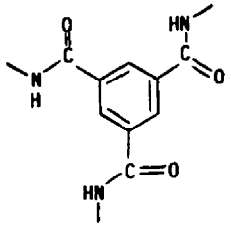

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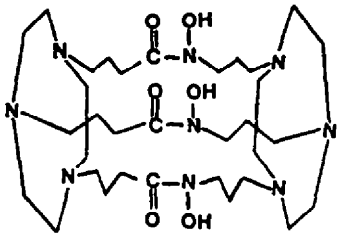
which have been widely used by Vögtle,[13] would form a somewhat rigid structure, making the secondary hydroxamate functional group less flexible, and would also lower water solubility. An aliphatic nine membered ring 1,4,7-triazacyclononane (9N3) was selected as spacers for our second design, illustrated by 5.



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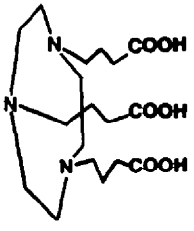
Although the molecular model of 5 fits nicely around the Fe(III) ion, this design seems to be impractical because the starting material 9N3 is too expensive, and the two subunits 6 and 7 are difficult to synthesize and to purify. Also, molecule with three tertiary amine arranged in a small 9-membered ring would trap a proton strongly thus making chromatographic purification difficult.



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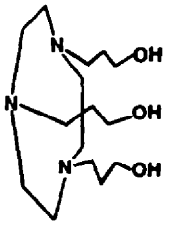

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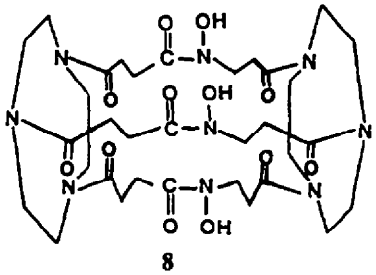
In order to at least partially avoid the above problems the following design, 8, has also been considered. However, this structure places the hydroxamate groups in β-elimination positions, which may render the molecule vulnerable. Another design was set up by employing the subunit indicated by 9.



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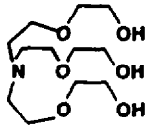


9

This is a known chemically stable compound.[23] In the present work it was prepared from inexpensive starting materials and purified by high vacuum distillation (4 μ, 174-176°C). The literature procedure, which in our hands gave a poor yield (11%) was improved to 84% by modification of the published procedure.[24] It can easily be prepared in 100 g amounts. However thus far it has not been possible to obtain its tritosylate derivative in pure

form. Also, whenever a chromatographic purification was employed, it was complicated by the tertiary amino group.

Most of the aliphatic cryptands described in the literature contain tertiary amino bridgeheads primarily for synthetic reasons. Generally the third macrocyclic ring was inserted by means of the high dilution reaction of an acid chloride with a secondary amino group of a macrocycle. Removal of the carbonyl oxygen of the resulting amide by reduction then produced the tertiary amino bridgehead. During the course of this synthetic program it became obvious that stepwise build-up of cryptands with endocyclic hydroxamate groups would be difficult, or at best awkward, because of the chemical sensitivity of this group, which required that it would need to be protected with special reagents. For that reason it was decided to prepared the trishydroxamate cryptand by a tripodal, single-step high-dilution condensation, and for this type of synthesis the tertiary amino bridgehead was not necessary. Therefore a substituted carbon bridgehead was selected, leading to a proposed cryptand structure indicated by formula **10** (see Scheme). Detailed procedures for the synthesis of **10** are given in the Experimental.

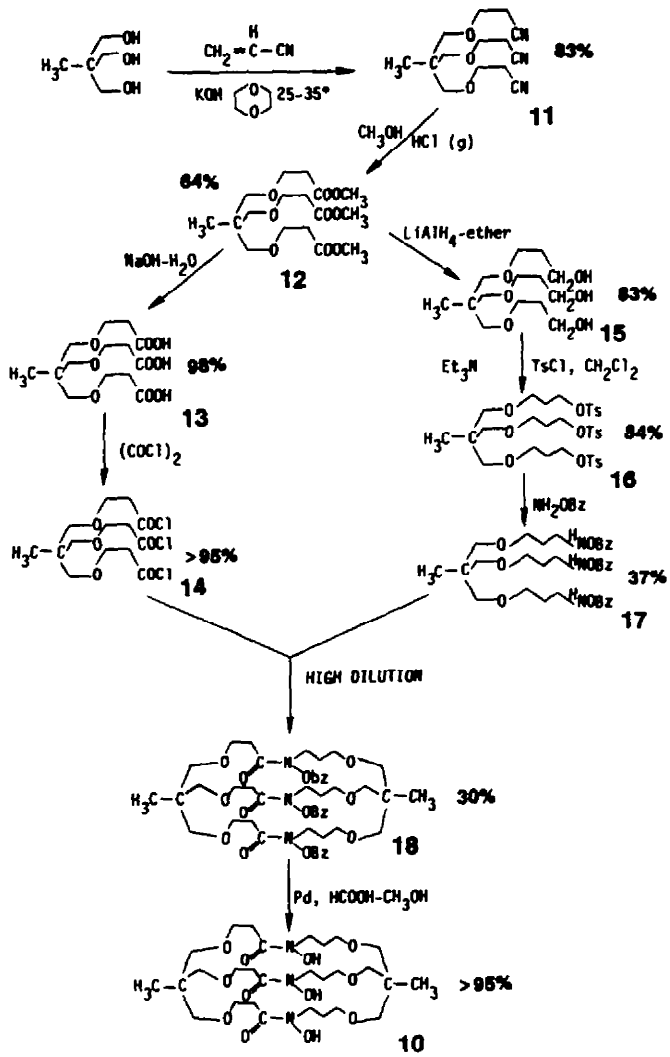
Results and Discussion

Abe et al.[25] described a procedure for the synthesis of the tricyano compound **11**, involving distillation at 201°C/0.0005 mm Hg, and obtained a 69% yield. In the present work, the more detailed procedure described in the Experimental, and purification of the product with alumina by flash chromatography, gave a higher yield (83%). Abe et al.[25] also published an outline for their synthesis of the tricarboxylic acid, **13**, by direct hydrolysis of the tricyano compound. In our hands this procedure in some cases resulted in β-elimination, to form the dicarboxylic acid as by-product. In other cases the hydrolysis was not complete, and the mono cyano dicarboxylic acid formed. Although both impurities may be removed by conversion to their sodium salts followed by cation-exchange chromatography, the procedure is tedious and inconvenient for large scale preparation. In the present work it was found that combining hydrolysis and esterification of the cyano compound in one operation is easier to control, and the trimethyl ester obtained is easy to purify by alumina flash chromatography. In the hydrolysis of the trimethyl ester in dilute NaOH solution, there are nearly no side reactions, and the yield of the tricarboxylic acid **13** is practically quantitative.

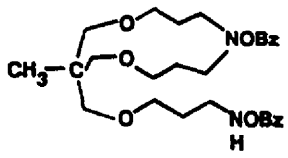
Two methods were investigated for preparation of the triol **15**: BH_3-THF reduction of the tricarboxylic acid and $LiAlH_4$ reduction of the trimethyl ester. The latter produced pure triol with higher yield.

The tritosylate, **16**, is not stable above room temperature. It was isolated and washed at dry ice bath temperature and was prepared immediately before use.

The reactions for the preparation of the tris-*O*-benzylhydroxylamine **17** from the tosylate precursor **16** is similar to that employed for the preparation of the bishydroxamate macrocycles described previously.[20] A nearly quantitative yield for that bis(benzyloxy)-



amino compound was obtained. However, when similar conditions were employed to this tris-(benzyloxy)amino compound the yield was less than 5%.It was then found that the main product formed is **19**. This shows that intramolecular alkylation competes strongly with the intermolecular reaction. After increasing the molar ratio of *O*-benzylhydroxylamine to the tris tosylate to 25:1 an acceptable yield of 37% was obtained.



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Fe(III) Complexes of the trishydroxamate cryptand, 10

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 x 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) compares well with those of the

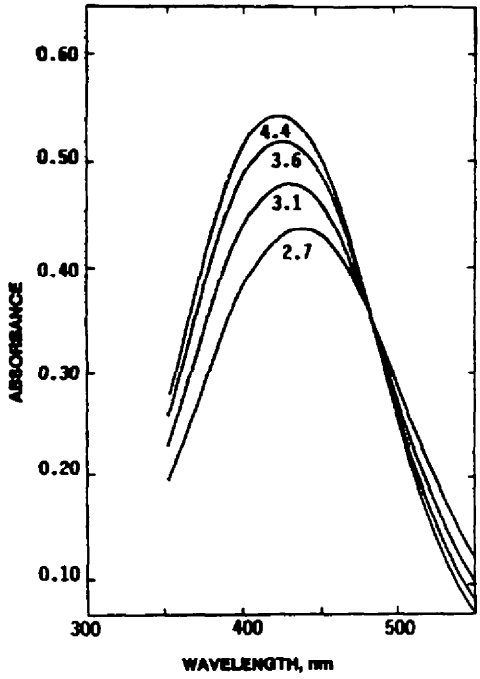

Figure 1. UV-visible spectra of Fe(III)-H_3THX solutions measured at -log [H^+] values indicated. T_{FeL} = 2.0 x 10^{-4} M, t = 25.0°C in CH_3OH-H_2O, 2:8 (v/v).

Fe(III) chelate of desferriferrioxamine-B, DFB, reported by Anderegg et al.[26] At pH 4.0 the Fe(III) cryptate has a molar absorbance of 2700 at λ_{max} 423 nm while that of Fe(III)-DFB is 2640 (λ_{max} 440nm) at pH 4.[26] 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 initial reaction corresponds to formation of 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.

In view of the fact that structures of the self-assembled trishydroxamates of Fe(III) are well known,[26] it was decided to attempt molecular mechanics calculations to provide information on the possible conformation of the Fe(III) cryptand of **10**. Preliminary calculations carried by Hancock[27] gave a low-energy conformer illustrated by Figure 2. Further calculations on this complex are continuing, and work on the preparation of crystals suitable for X-ray structure determination is in progress.

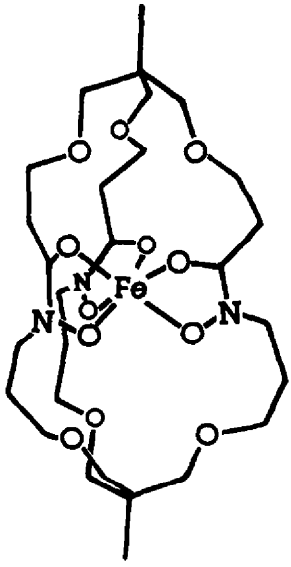

Figure 2. Calculated low-energy conformer of Fe(III)-trishydroxamate cryptate

The Ga(III) Complex of 10

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.

Experimental

Materials and Methods. 1,1,1-Tris(hydroxymethyl)ethane, acrylonitrile, lithium aluminum hydride, *p*-toluenesulfonyl chloride, triethylamine, *O*-benzylhydroxylamine hydrochloride, oxalyl chloride, palladium(II) chloride were purchased from Aldrich Chemical Company and were used without further purification. *p*-Dioxane and ethylene glycol dimethyl ether, were

refluxed with $LiAlH_4$ and redistilled. The benzene for the high dilution reaction was refluxed with sodium until its 1% benzophenone solution became deep blue in color, the distillate was collected under dry N_2 atmosphere. The pyridine and triethylamine were purified with conventional methods. Benzene, formic acid (88%) and methanol were purchased from Fisher Scientific and were used without further purification.

The proton and carbon-13 NMR spectra were recorded with a Varian XL-200 NMR spectrometer operating at 200 MHz, and the chemical shifts are reported in ppm relative to tetramethylsilane. The mass spectra were obtained with a VG analytical 70S high resolution double focusing magnetic sector spectrometer, with attached VG analytical 11/250J data system. The uv-visible spectral determinations were made with a Perkin Elmer Model 553 fast-scan spectrophotometer equipped with 1.000 ± 0.001 cm matched cells at 25.0 ± 0.1°C. The pH values of the solutions used in Fe(III) complexes experiments were measured with a Beckman Research pH meter fitted with a Sargent Welch miniature combination glass electrode and was calibrated with strong acid and base at 25.0°C and μ = 0.100 M (KCl) so that all pH values are directly expressed as -log [H^+]. The C, H, N analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee.

1,1,1-Tris(cyanoethoxymethyl)ethane, 11.[25] 1,1,1,-Tris(hydroxymethyl)ethane, 24 g, 0.2 mole, was added to 25 ml of pure p-dioxane containing 1.2 g of 40% KOH aqueous solution. To this suspension, 40 ml, (32.2 g, 0.202 mole) of acrylonitrile was added dropwise with vigorous stirring. The temperature of the reaction mixtures was maintained at 20-25°C with a water bath. About midway in the addition, a sudden increase in the reaction rate was observed from the evolution of a large amount of heat. At this time, crushed ice was added to keep the temperature of the reaction mixture below 35-40°C. After the addition was finished, the reaction mixture was kept at 25° for 20 hr.

To the above reaction mixture, 3.8 ml of 2.5 M HCl was added. The insoluble material was removed by filtration and the solvent was removed by distillation. The pale yellow oil obtained was dissolved in 40 ml of methylene chloride and dried with anhydrous $MgSO_4$ for 2 hr. After the drying agent and solvent were removed, 60 g of pale yellow oil was obtained.

26g of the above oil was loaded on the top of 90 g of neutral alumina column (from Fisher Scientific A-950, Brockman Activity 1, 80-200 mesh). The absorbent was placed in a 150 ml sinter glass funnel (D/H=65x50 mm) and was pre-washed with 2x40 ml of benzene (Fisher Scientific B-245-4). Four to six portions of 40 ml benzene was used to elute the product, and 19.3 of colorless liquid was obtained. ^1H NMR (in $CDCl_3$): 0.99 (s, 3H, methyl); 2.6 (t, 6H, $-CH_2-CN$); 3.37 (s, 6H, $-C-(CH_2-O)_3$); 3.66 (t, 6H, $-O-CH_2-$). Yield = 83%

1,1,1-Tris(carboxyethoxymethyl)ethane trimethyl ester, 12. 1,1,1-Tris(cyanoethoxymethyl)ethane, 24 g (0.085 mole) was dissolved in 280 ml of methyl alcohol and the solution was saturated with hydrogen chloride gas. No attempt was made to cool the solution during this time. The saturation was complete in about 20-30 min, the reaction mixture was then heated to reflux for an additional hour, and then was allowed to stand at room temperature for 18 hr. The ammonium chloride produced was removed by filtration and the methanol was mainly removed by evaporation. To the residue obtained, 180 ml of benzene and 2-3 drops of concentration H_2SO_4 were added and the reaction mixture was brought to reflux and distilled azoetropically until no water was produced. After the benzene was removed, 33.3 g of colorless oil was obtained. The 33 g of crude product was loaded on 180 g of neutral alumina and was eluted with benzene (the same procedure that was employed with tricyano compound). From four 80 ml of eluant (benzene solution), 21 g of pure product was obtained, yield = 64%. ^1H NMR (in $CDCl_3$): 0.88 (s, 3H, methyl); 2.6 (t, 6H, $-CH_2COO-$); 3.2 (s, 6H, $-C-(CH_2-O-)_3$); 3.67 (t, 6H, $-O-CH_2$); 3.69 (s, 9H, $-COOCH_3$). Anal. calcd. for $C_{17}H_{30}O_9$: C, 53.97; H, 7.94. Found: C, 53.63; H, 7.93. FAB M.S. $(M+H)^+$ = 379.

1,1,1-Tris(carboxyethoxymethyl)ethane, 13. To 17 g (0.045 mole) of the trimethyl ester, 12, 270 g of 10% NaOH aqueous solution was added. The reaction mixture was stirred at room temperature for 15 hr, and then heated in a 80°C bath for 24 hr until a clear aqueous solution was obtained. It was carefully neutralized with 56 ml of concentrated hydrochloric acid to pH = 1. After the water was removed by evaporation, 4x80 ml of ether was used to extract the product. The ether solution was dried with anhydrous MgSO4 for 16 hr. 14.8 g of

pure product was obtained, yield = 98%. ^{1}H NMR (in D_2O): 0.90 (s, 3H, methyl); 2.47 (t, 6H, -$\underline{C}H_2$COOH); 3.38 (s, 6H, -C-(CH_2-)$_3$); 3.73 (t, 6H, -O-CH_2-). ^{13}C NMR (in acetone-d_6): 16.0 (methyl); 35.7 (-$\underline{C}H_2$-COOH); 42.0 (-$\underline{C}$-CH_2-)$_3$); 68.0 (-O-$\underline{C}H_2$-CH_2-); 74.5 (-C-($\underline{C}H_2$-O-)$_3$); 173.8 (-COOH).

1,1,1-Tris(chlorocarbonylethoxymethyl)ethane, 14.[20] To 2.6 g (7.5 mmole) of the tricarboxylic acid, **13**, 15 ml of dry benzene was added. Then 4 ml of oxalyl chloride (46 mmole) and one drop of pure pyridine were added, and the reaction mixture was stirred at room temperature for 24 hr. The benzene and excess oxalyl chloride was removed by distillation under vacuum and protected with dry Ar gas in a 30°C bath. Two 10 ml batches of dry benzene were added to the residue and removed by vacuum distillation. Then 20 ml of dry benzene was added to the product, and a sticky insoluble material appeared on the wall of the flask. The clear benzene solution containing the product was separated by a syringe and the solvent was removed by vacuum distillation at 30°C/1 mm Hg. The product was dried under these conditions for 30 min, yield > 95%. ^{1}H NMR (in $CDCl_3$): 0.90 (s, 3H, methyl); 3.10 (t, 6H, -CH_2-COCl); 3.27 (s, 6H, -C(-CH_2-O-)$_3$); 3.73 (t, 6H, -O-$\underline{C}H_2$-CH_2). ^{13}C NMR (in $CDCl_3$): 17.5 (methyl); 41.1 (-$\underline{C}$(-CH_2-O-)$_3$); 47.5 (-$\underline{C}H_2$-COCl); 66.2 (-O-$\underline{C}H_2$-CH_2-); 73.5 (-C(-CH_2-O-)$_3$); 172.2 (-COCl).

1,1,1-Tris(3-hydroxypropoxymethyl)ethane, 15.[28] To 5.3 g of powdered $LiAlH_4$, 320 ml of dry ethyl ether (from Mallinckrodt 0848-1) was added and the mixture was stirred at room temperature for 15 min, while the system was covered with dry N_2 gas. When the oil bath temperature reached 30°C, a solution of 20 g(0.053 mole) of 1,1,1-tris(carboxyethoxymethyl)ethane trimethyl ester, **12**, in 120 ml dry ethyl ether was added dropwise at such a rate that the solvent refluxes gently. The reaction mixture was stirred by a motor driven stirrer. After 70-80 ml of ester-ether solution was added, another 1.5 g of $LiAlH_4$ was quickly added to the reaction mixture. The addition was complete in about one hour, the mixture was then stirred at room temperature for another hour.

With vigorous stirring, 6.8 g of water, 6.8 g 15% NaOH aqueous solution and 20.4 g of water were carefully added to the reaction mixture in succession in an ice-water bath.[28b] After the addition was complete the mixture was stirred in an ice-water bath for 15 min. The reaction mixture was then filtered with suction and the lithium aluminum hydroxide was washed thoroughly with ethyl ether. The last washing was obtained from refluxing 250 ml of ether with the lithium aluminum hydroxide for 2-3 hr. The filtrate and washings were combined and evaporated under vacuum. A colorless oil was obtained. It was dissolved in about 150 ml of CH_2Cl_2 and dried with anhydrous $MgSO_4$ for 16 hr. After the solvent and drying agent were removed 12.8 g of colorless oil was obtained, yield = 83%. ^{1}H NMR (in $CDCl_3$): 0.92 (s, 3H, -CH_3); 1.82 (quintet, 6H, -CH_2-$\underline{C}H_2$-CH_2-); 3.32 (s, 6H, -C(-$\underline{C}H_2$-O-)$_3$); 3.4 (b, 3H, -OH); 3.60 (t, 6H, -$\underline{C}H_2$-OH); 3.78 (t, 6H, -O-$\underline{C}H_2$-CH_2-CH_2-). ^{13}C NMR (in D_2O): 15.5 (-CH_3); 29.5 (-CH_2-$\underline{C}H_2$-CH_2-); 38.7 (-C(-CH_2-O-)$_3$); 57.3 (-$\underline{C}H_2$-OH); 67.0 (-O-$\underline{C}H_2$-CH_2-); 71.7 (-C(-$\underline{C}H_2$-O-)$_3$). Anal. calcd. for $C_{14}H_{30}O_6$: C, 57.12; H, 10.27. Found: C, 57.24; H, 10.20. FAB M. S. $(M+H)^+$ = 295.

1,1,1,-Tris(4-toluenesulfonyloxypropoxymethyl)ethane, 16. A solution of 1,1,1-tris(3-hydroxypropoxymethyl)ethane (2.84 g, 0.0097 mole), triethylamine (3.08 g, 0.0306 mole) and methylene chloride (2.8 ml) was added dropwise to a solution of 4-toluenesulfonyl chloride (5.8 g, 0.0306 mole) in 6.6 ml of methylene chloride in 40 min in an ice-water bath. The reaction mixture was placed in a refrigerator for 22 hr. The triethylammonium chloride was removed by filtration and was washed with methylene chloride. The filtrate and washing were combined (about 35 ml) and was washed with 2x20 ml cold 2 M HCl; 20 ml 2% Na_2CO_3 and 20 ml of saturated NaCl solution. The methylene chloride phase was separated and filtered and dried with anhydrous Na_2SO_4 for 30 min. After the solvent and drying agent were removed 8 g of colorless oil was obtained. This oily product was dissolved in 5 ml of absolute ethyl alcohol to form a clear pink solution. Methyl alcohol (10 ml) was added to the ethanol solution and the mixture was cooled in a dry ice bath, and a sticky product separated out. The clear ethanol-methanol solution was removed by decantation and was discarded. The sticky product was washed with 3x5 ml of methyl alcohol at dry ice bath temperature, and was dried at 25°C 1 mm Hg for 20 min. 6.1 g product was obtained, yield = 84%. This product had a good ^{1}H NMR and was used immediately for the preparation of compound **17**. ^{1}H NMR (in

$CDCl_3$): 0.73 (s, 3H, CH_3-C-); 1.85 (quintet, 6H, -CH_2-$\underline{CH_2}$-CH_2-); 2.44 (s, 9H, CH_3- of tolyl); 3.1 (s, 6H, -C(-CH_2-O-)$_3$); 3.36 (t, 6H, -O-$\underline{CH_2}$-CH_2-); 4.10 (t, 6H, -CH_2-OTs); 7.34 (d, 6H, arom); 7.07 (d, 6H, arom).

1,1,1-Tris(O-benzylhydroxylaminopropoxymethyl)ethane, 17. A solution of 6.1 g (0.0081 mole) of the tritosyl ester **16** in 15 ml of ethylene glycol dimethyl ether was added dropwise to a solution of 25.4 g (0.206 mole) *O*-benzylhydroxylamine in 30 ml of ethylene glycol dimethyl ether with stirring at room temperature. The reaction mixture was heated in a 80-90°C bath for 18 hr. The solvent was removed by evaporation. To the residue 50 ml of methylene chloride was added, and the insoluble white crystalline material (*O*-benzylhydroxylamine toluenesulfonic acid salt) was removed by filtration. The filtrate and washings were combined and evaporated, about 30 g of colorless oil remained. This oil material was dissolved in 60 ml of CH_2Cl_2, and to this solution 27 ml of 6 M HCl was added slowly in an ice-water bath. The white precipitate, which is the *O*-benzylhydroxylamine hydrochloride salt, was removed by filtration, and was thoroughly washed with 5x20 ml of CH_2Cl_2. The filtrate which contained two phases was combined with the washings and the aqueous phase was discarded. The CH_2Cl_2 phase of about 100 ml was washed with 2x25 ml 2.5 M HCl, and several times with 50 ml 2% Na_2CO_3 until the pH of the washing became > 9. The CH_2Cl_2 solution was filtered and dried with anhydrous Na_2SO_4 for 20 hr. After the solvent and drying agent were removed, 6 g of colorless oil was obtained. This crude product was loaded on the top of 80 g silica gel column (Merck, grade 60, 230-400 mesh, 60 Å, from Aldrich Co. 22,719-6) which was pre-equilibrated with 2x200 ml of benzene. The size of the column was DxH 68x50 mm. Benzene, and benzene-ethyl acetate solutions were used successively to elute the impurities. $CHCl_3$:CH_3OH 90:10 was then used to elute out the product. From this eluant 1.85 g of colorless pure product was obtained, yield = 37%. ^{1}H NMR (in $CDCl_3$): 0.88 (s, 3H, -CH_3); 1.76 (quintet, 6H, -CH_2-$\underline{CH_2}$-CH_2-); 3.02 (t, 6H, -$\underline{CH_2}$-NH-O); 3.20 (s, 6H, -C(-CH_2-O-)$_3$); 3.42 (t, 6H, -O-$\underline{CH_2}$-CH_2-); 4.70 (s, 6H, -CH_2- of benzyl); 7.30-7.37 (m, 15H, arom). ^{13}C NMR (in $CDCl_3$): 17.7 (-CH_3); 27.4 (-CH_2-$\underline{CH_2}$-CH_2-); 40.9 (-$\underline{C}$(-CH_2-O-)$_3$); 49.9 (-$\underline{CH_2}$-NH-O-); 69.9 (-O-$\underline{CH_2}$-CH_2-); 73.7 (-C(-CH_2-O-)$_3$); 76.2 (-CH_2- of benzyl); 127.9; 128.5 and 138.2 (arom). Anal. calcd. for $C_{33}H_{51}N_3O_6$: C, 68.96; H, 8.37; N, 6.90. Found: C, 68.68; H, 8.45; N, 6.69. FAB M.S. $(M+H)^+$ = 610.

1,13-Dimethyl-3,11,15,23,26,34-hexaoxa-6,20,29-trioxo-7,19,30-trisbenzyloxyazabicyclo[11,11,11]pentatriacontane, 18, (High dilution method). A 2.95 g (7.55 mmole) amount of the triacyl chloride **14** was dissolved in 250 ml of dry benzene, and 4.6 g (7.55mmole) of the tris(benzyloxy)amine, **17**, was dissolved in 250 ml of dry benzene. These two solutions were added to 2.2 L of dry benzene to which 12.3 ml (12 g, 0.151 mole) of dry pyridine had been added. The reaction mixture was kept at room temperature and the system was protected with dry Ar. The addition was accomplished in 14 hr with vigorous stirring. After the addition the reaction mixture was allowed to stand at room temperature for 12 hr. The pyridine salt was filtered out, and the benzene solvent was removed by evaporation at 40-45°C. After vacuum drying at 40°C, 5 mm Hg for 30 min, 6.7 g of sticky yellow oil was obtained. This yellow oil was loaded on the top of 80 g of silica gel (Merck, grade 60, 230-400 mesh, from Aldrich Co., 22, 719-6) (column size: DxH 65x50 mm). The absorbent was pre-equilibrated with 2x200 ml of benzene (Fisher Scientific, B-245-4). The column was eluted with benzene, then benzene ethyl acetate solutions to remove the impurities. The product began to come off the column when it was eluted with $CHCl_3$:CH_3OH 98:2. After two successive chromatographic purifications, 2 g of pure product was obtained, yield = 30%. ^{1}H NMR (in $CDCl_3$): 0.89-0.92 (m, 6H, -CH_3); 1.86-1.89 (b, 6H, -CH_2-$\underline{CH_2}$-CH_2-); 2.5-2.7 (b, 6H, -CH_2-CO-); 3.2-3.5 (m & b, 18H, -C(-CH_2-O-)$_3$ & -NOBz-CH_2); 3.68 (t, 12H, -O-$\underline{CH_2}$-CH_2-); 4.86 (s, 6H, -CH_2- of benzyl); 7.34-7.37 (m, 15H, arom). ^{13}C NMR (in $CDCl_3$): 17.8 (-CH_3); 27.5-27.8 (-CH_2-$\underline{CH_2}$-CH_2-); 33.1 (-$\underline{CH_2}$CO-); 41.0-41.5 (-C(-CH_2-O-)$_3$); 67.6-67.8 (-O-$\underline{CH_2}$-CH_2-); 68-69 (-CH_2-NOBz-); 73.8-74.5 (-C(-$\underline{CH_2}$-O-)$_3$); 76.6 (-CH_2- of benzyl); 128.6-129.5 & 134.8-135.5 (arom); 172.8-173.0 (-CO-NOBz). Anal. calcd. for $C_{49}H_{69}N_3O_{12}$.$4H_2O$: C, 61.06; H, 8.00; N, 4.36. Found: C, 60.95; H, 7.1; N, 4.18. FAB M.S. $(M+H)^+$ = 893.

1,13-Dimethyl-3,11,15,23,26,34-hexaoxa-6,20,29-trioxo-7,19,30-trishydroxyazabicyclo[11,11,11]pentatriacontane, 10, (H_3THX).[29] 0.2 g of the tribenzylhydroxamate cryptand **18** was dissolved in 10 ml of 4.4% HCOOH-CH_3OH solution. This solution was added to 10 ml of 4.4% HCOOH-CH_3OH solution

containing 200 mg of freshly prepared palladium black catalyst.[30] The mixture was stirred at room temperature under Ar atmosphere for 6 hr. The product was isolated by filtering off the catalyst and washing with additional 15-20 ml of methyl alcohol. The filtrate and washings were combined and evaporated at 40-45°C. The residue obtained was vacuum dried at 30°C, 0.1 mm Hg for 3 hr, yield > 95%. ^{1}H NMR (in CD_3OD): 0.88-0.94 (m, 6H, $-CH_3$); 1.9 (b, 6H, $-CH_2-\underline{CH_2}-CH_2-$); 2.7 (b, 6H, $-CH_2CO-$); 3.3-3.7 (m, 18H, $-C(-CH_2-O-)_3$ & $-\underline{CH_2}-NOH-$); 3.7 (b, 12H, $-O-\underline{CH_2}-CH_2-$). ^{13}C NMR (in CD_3OD): 17.9 ($-CH_3$); 27.7 ($-CH_2-\underline{CH_2}-CH_2-$); 33.8 ($-\underline{CH_2}-CO-$); 41.6 ($-\underline{C}(-CH_2-O-)_3$); 68.3 ($-O-\underline{CH_2}-CH_2-$); 70.0 ($-CH_2-NOH-$); 75.1-75.6 ($-C(-\underline{CH_2}-O-)_3$); 173.4 ($-\underline{C}O-NOH$). Anal. calcd. for $C_{28}H_{51}N_3O_{12}.1/2H_2O$: C, 53.27; H, 8.40, N, 6.59. Found: C, 53.16; H, 8.19; N, 6.38. FAB M.S. $(M+H)^+$ = 622.

1,13-Dimethyl-3,11,15,23,26,34-hexaoxa-6,20,29-trioxo-7,19,30-trishydroxyazabicyclo[11,11,11]pentatriacontanato-iron(III) complex, Fe(III)-THX. To 1 ml of 2 x 10^{-3} M $FeCl_3$ in 0.1 M HCl aqueous solution 1 mL 2.5 x 10^{-3} M H_3THX-methanol solution and 1 mL methanol were added. KOH 0.1 M aqueous solution was used to adjust the pH of the solution and the total volume of the solution was diluted to 10 ml in a volumetric flask with water. The absorbance spectra of the solutions was determined at pH = 2.7, 3.1, 3.6., 4.4, and 7.2, yielding A_{max} (maximum absorbance) values of 0.441, 0.486, 0.527, 0.552, 0.550, respectively. The absorbance does not change after standing at room temperature for 24 hr.

1,13-Dimethyl-3,11,15,23,26,34-hexaoxa-6,20,29-trioxo-7,19,30-trishydroxyazabicyclo[11,11,11]pentatriacontanato-gallium(III) complex, Ga(III)-THX. To 0.030 g (0.048 mmole) of H_3THX, 0.144 ml of 1 M KOH was added to give a pH 9.5 K_3THX solution. To 0.10 ml 0.47 M $GaCl_3$ solution (~1 M HCl), 0.188 ml of 1 M KOH was added to form a pH 11.5 $Ga(OH)_4^-$ solution. The above two solutions were mixed and 1 M HCl was added to adjust the pH of the reaction mixture to 9.0. The reaction mixture was stirred at room temperature for 24 hr. The white precipitate was separated by filtration (pH of the filtrate, 8.9) and washed with distilled water. After vacuum drying at room temperature over P_2O_5 for 24 hr 28 mg of product was obtained, yield = 85%. Anal. calcd. for $C_{28}H_{48}N_3O_{12}Ga.2H_2O$: C, 46.38; H, 7.18; N, 5.80. Found: C, 46.48; H, 7.22; N, 5.48. FAB M.S. $(M+H)^+$ = 688.

Acknowledgement: This work was supported by the National Cancer Institute, U. S. Public Health Service, under Contract No. CA-42925. The authors thank Dr. T. R. Sharp for fast atom bombardment mass spectral analyses.

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