Lipophilic Hexadentate Aluminum Complexes of New Phenolate-Derivatized Cyclohexanetriamine Ligands and Their Effect on the Peptide Transport System (PTS-1)

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The compounds $(RsalH_2)_3tachH_3$ (R = H, NO_2, OMe) have been synthesized by Schiff base condensation between *cis*-1,3,5-triaminocyclohexane and a substituted salicylaldehyde, followed by reduction with KBH_4. Reaction of these compounds with aluminum(III) salts gives uncharged hexacoordinate N_3O_3 complexes of type $Al(RsalH_2)_3$-tach. The complexes have been characterized by a combination of infrared, ^1H and $^{13}C\{^1H\}$ NMR, and mass spectroscopy. Structures of $Al(salH_2)_3tach$ and $Al(NO_2salH_2)_3tach$ have been determined by X-ray crystallography. $Al(salH_2)_3tach$: monoclinic, $P2_1/n$, a = 15.047(2) Å, b = 11.355(1) Å, c = 15.201(2) Å, β = 93.59(6)°, V = 2592(1) Å^3, Z = 4, R = 0.038, R_w = 0.052. $Al(NO_2salH_2)_3tach$: monoclinic, $P2_1/n$, a = 12.305(1) Å, b = 12.072(2) Å, c = 20.229(3) Å, β = 90.26(1)°, V = 3005(1) Å^3, Z = 4, R = 0.040, R_w = 0.047. The partition coefficients between 1-octanol and water for the complexes $Al(RsalH_2)_3tach$ have the values 33.0, 9.0, and 4.4 for the derivatives with R = H, NO_2, and OMe, respectively. We show that $(NO_2salH_2)_3tachH_3$ affects the action of aluminum on the peptide transport system (PTS-1).

Aluminum is toxic to the central nervous system.[2] The metal is involved in causing dialysis dementia in patients who are unable to eliminate aluminum because of renal dysfunction.[3,4] Other possible effects of aluminum on the central nervous system (CNS) may be related to Alzheimer's disease and amyotrophic lateral sclerosis.[5–7] In studies on laboratory animals the administration of aluminum leads to a progressive encephalomyelopathy with degeneration of cerebral nerve cells, brain stem demyelination, and the development of neurofibrillary tangles.[8–10]

One hypothesis regarding the possible etiology of dementia has suggested that a defective blood–brain barrier (BBB) allows toxins such as aluminum to gain access to the CNS.[11] Alternatively, aluminum might affect the function of the BBB by a direct action.[12] Impairment by aluminum of the usual regulation of the entry rate of normally nontoxic, behaviorally active substances such as amines and peptides might then lead to dysfunction of the CNS. Subsequently, it has been found that aluminum affects some of the membrane-like functions of the BBB, resulting in an increase in the rate of transmembrane diffusion and in selective changes in the saturable transport systems without disrupting the integrity of the membranes or altering CNS hemodynamics.[13] Aluminum can inhibit the peptide transport system (PTS-1) when its major ligands Tyr-MIF-1 and Met-enkephalin are used and also when peptides such as oxytocin and Try-W-MIF-1, which are less avidly transported by PTS-1, are used.[14] Compounds that complex aluminum and modify its interaction with the BBB therefore can change the neurological concentrations of this metal.

Tight binding of aluminum to a carrier ligand should affect its migration through the BBB. Ideally, the complex that is formed should be uncharged and lipophilic. Since the aluminum ion is a trivalent cation, and the coordination number of this ion is usually 6, stable uncharged complexes are likely to be formed with a trianionic hexadentate ligand. Several systems have been used as chelators for Al(III).[15,16] EDTA is a good chelator, but it is ineffective in clinical trials, possibly because it does not readily distribute into cells.[17–19] Desferrioxamine (DFO) is a hexadentate trihydroxamic acid Fe siderophore that is an effective chelator in most cases of aluminum accumulation.[20–23] Treatment failures have, however, been reported,[24] and this chelator cannot be administered orally. There is

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(1) (a) Department of Chemistry. (b) Veterans Affairs.
(2) Martin, R. B. *Acc. Chem. Res.* **1994**, *27*, 204.
(3) Alfrey, A. C.; Mishell, J. M.; Burks, J. *Trans.—Am. Soc. Artif. Intern. Organs* **1972**, *18*, 257.
(4) King, S. W.; Savory, J.; Wills, M. R. *CRC Crit. Rev. Clin. Lab. Sci.* **1981**, *14*, 1.
(5) Klatzo, I.; Wisniewski, H.; Streicher, E. *J. Neuropathol. Exp. Neurol.* **1965**, *24*, 187.
(6) Perl, D. P.; Gajdusek, D. C.; Garruto, R. M.; Yanagihara, R. T.; Gibbs, C. J. *Science* **1982**, *217*, 1053.
(7) Bowdler, N. C.; Beasley, D. S.; Fritze, E. C.; Goulette, A. M.; Hatton, J. D.; Hession, J.; Ostman, D. L.; Rugg, D. J.; Schmittdiel, C. J. *Pharmacol. Biochem. Behav.* **1979**, *10*, 505.
(8) Bugiani, O.; Ghetti, B. *Neurobiol. Aging* **1982**, *3*, 209.
(9) Ebrina, Y.; Okada, S.; Hamazaki, S.; Midorikaura, O. *Toxicol. Appl. Pharmcol.* **1984**, *75*, 211.
(10) Crapper, D. C.; Dalton, A. J. *Physiol. Behav.* **1973**, *10*, 935.
(11) Wisniewski, H. M.; Kozlowski, P. B. *Ann. N.Y. Acad. Sci.* **1982**, *396*, 119.
(12) Banks, W. A.; Kastin, A. J. *Lancet* **1983**, 1227.
(13) Banks, W. A.; Kastin, A. J. *Neurosci. Behav. Rev.* **1989**, *13*, 47.
(14) Banks, W. A.; Kastin, A. J. *Life Chem. Rep.* **1994**, *11*, 141. Banks, W. A.; Kastin, A. J.; Michals, E. A. *Peptides* **1987**, *8*, 899. Durham, D. A.; Banks, W. A.; Kastin, A. J. *Neuroendocrinology* **1991**, *53*, 447. Banks, W. A.; Kastin, A. J.; Ehrensing, C. A. *J. Neurosci. Res.* **1993**, *35*, 690.
(15) Yokel, R. A. *J. Toxicol. Environ. Health* **1994**, *41*, 131.
(16) Martell, A. E.; Motekaitis, R. J.; Smith, R. M. *Polyhedron* **1990**, *9*, 171.
(17) Delavalle, F.; Richalet, B.; Malvy, F.; Fries, D. *Nouv. Press. Med.* **1977**, *6*, 941.
(18) Adhemar, J. P.; Laederich, J.; Jaudon, M. C.; Masselot, J. P.; Galli, A.; Kleinknecht, D. *Lancet* **1980**, *33*, 1509.
(19) May, P. M.; Bulman, R. A. *Prog. Med. Chem.* **1983**, *20*, 225.
(20) Bergeron, R. J.; Pegram, J. J. *J. Org. Chem.* **1988**, *53*, 3131.
(21) Milne, F. J.; Sharf, B.; Bell, P. D.; Meyers, A. M. *Lancet* **1982**, 502.
(22) Ackrill, P.; Day, J. P.; Garstang, F. M.; Hodge, K. C.; Metcalfe, P. J.; Benzo, Z.; Hill, K.; Ralston, A. J.; Ball, J.; Denton, J. *Proc. Eur. Dial. Transplant Assoc.* **1982**, *19*, 203.

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therefore a need to develop better chelators for the *in vivo* binding of aluminum.

When choosing a chelator, one needs to consider several factors. Among these are the basicities of the donor atoms, the covalent character of the coordinate bonds, the ring sizes, and the preorganization of the ligands prior to complexation. This last effect is responsible for CDTA complexes being more stable than those of EDTA.[25,26] Since aluminum(III) forms stable phenolate[27] and hydroxypyridinone[28] complexes, we have selected the former as one of the sets of donor ligands. Nitrogen donor ligands are less common than oxygen donors in aluminum(III) complexes. Nevertheless examples of such complexes have been fully characterized.[29] Another set of hexadentate ligands that are of direct relevance to our work are chelates of the N_3O_3-type based on a cyclohexanetriamine backbone. Such a ligand is 1,3,5-triamino-1,3,5-trideoxy-*cis*-inositol, which gives high binding constants with aluminum(III). These tricationic complexes have sufficiently high binding constants that the hydrolysis of the aluminum complex is slow at 25 °C.[30] Similar N_4O_3 tripodal amine phenols have been used to synthesize complexes with Al^{3+}, but in this case they are cationic.[31]

The partitioning between octanol and water has been used to make a preliminary assessment of the aluminum chelation potential.[32] These values have been used to predict a compound's ability to be absorbed and to distribute out of the vascular compartment to intracellular sites of aluminum storage. This partitioning has been claimed to model the lipid solubility and therefore the brain capillary permeability of metal complexes.[33] It is considered possible that a highly lipophilic metal chelate complex may remain within the cell structure and redistribute within the organism to lipid tissues, such as the brain. In order to synthesize such complexes of aluminum(III), we have chosen a ligand system that has three phenolic residues appended to the *cis* form of 1,3,5-triaminocyclohexane.[34] The preorganization of the *cis* stereochemistry of the cyclohexane backbone is expected to facilitate complexation, and the presence of three phenolate groups will give lipophilic complexes with trivalent metal ions. The lipophilicity of the complexes formed with this ligand system can be modified by changing the substituent groups on the ligand periphery. In this paper we describe the synthesis and characterization of such ligands and aluminum complexes and measure their effects on the peptide transport system (PTS-1).

Table 1. Analytical Data (Found in Parentheses) for the Ligands and Complexes

compd	anal., % C	H	N	*m/e* (M^+)
$(salH_2)_3tachH_3 \cdot H_2O$	69.64 (68.95)	7.59 (7.58)	9.03 (8.72)	447
$Al(salH_2)_3tach \cdot H_2O$	66.23 (66.85)	6.60 (6.50)	8.58 (8.70)	471
$(NO_2salH_2)_3tachH_3 \cdot 3HCl \cdot 3H_2O$	43.46 (43.51)	5.28 (5.54)	11.27 (11.42)	583
$Al(NO_2salH_2)_3tach \cdot C_3H_6O$	54.21 (54.38)	5.02 (5.32)	12.65 (12.61)	607
$(MeOsalH_2)_3tachH_3$	67.01 (67.08)	7.33 (7.28)	7.74 (7.82)	539
$Al(MeOsalH_2)_3tach \cdot 2H_2O$	60.28 (60.49)	6.76 (6.64)	7.03 (7.03)	562

Experimental Section

All materials and solvents were standard reagent grade and used without further purification unless otherwise noted. Reagents were purchased from Aldrich Chemical Co. and used as supplied, with the exception of diethylene glycol (Eastman) and sodium triazide (Alfa). The $Al(NO_3)_3 \cdot 9H_2O$ (Aldrich) was >99% pure (metal basis) and used without further purification. Melting points, where appropriate, were obtained on a hot-stage apparatus. Infrared spectra were recorded as KBr pellets with a Mattson Cygnus 100 FT-IR spectrometer. Electronic spectra were recorded as acetonitrile solutions unless otherwise noted, with a Hewlett-Packard model 8451A diode array spectrophotometer. ^1H and ^{13}C NMR spectra were measured with a GE Omega 400 MHz spectrometer unless otherwise noted. Fast atom bombardment (FAB) mass spectra were obtained by means of a Kratos Concept 1H spectrometer with the samples introduced in a *m*-nitrobenzyl alcohol matrix. Elemental analyses were performed by Galbraith Inc., Knoxville, TN. Analytical and spectral data for the ligands and complexes are given in Tables 1−5.

1,3,5-*cis*-Tris(phenylsulfonyl)oxycyclohexane. 1,3,5-*cis*-cyclohexanetriol dihydrate (6.85 g, 40.7 mmol) was dissolved in freshly distilled pyridine (90 mL). This solution was maintained at 10 °C while benzenesulfonyl chloride (53 g, 0.3 mol) was added over a period of 3 h. The resulting mixture formed a thick off-white slurry. This slurry was added to a solution of water (175 mL), ethanol (350 mL), and concentrated HCl (140 mL), and the mixture was stirred for 30 min. The resulting white solid was filtered off and washed with ethanol. The product was purified by recrystallization by the addition of ethanol (1.5 L) to this solid, heating to reflux, and addition of sufficient dichloromethane to complete the dissolution. After cooling, the solution yielded the product as fine colorless needles.[35] Yield: 18.6 g, 35.6 mmol (87.4%). Mp: 189 °C dec. ^1H NMR (DMSO-d_6): δ 1.61 (q, 3H, ^3J(HH) = 12 Hz), δ 1.71 (m, 3H), δ 4.49 (m, 3H), δ 7.62 (t, 6H, ^3J(HH) = 8 Hz), δ 7.77 (d, 6H, ^3J(HH) = 8 Hz), δ 7.79 (t, 3H, ^3J(HH) = 8Hz).

1,3,5-*cis*-Triazidocyclohexane. 1,3,5-*cis*-Tris(phenylsulfonyl)oxycyclohexane (18.6 g, 35.6 mmol) was placed in a 250 mL flask equipped with a thermometer and magnetic stirrer. To this solid were added diethylene glycol (70 mL) and sodium azide (11.6 g, 178 mmol). After this mixture was stirred at 100 °C for 6 h, it turned a clear light brown color. After the solution had cooled to room temperature, it was poured into water (140 mL) and the mixture was stirred for a few minutes. To this mixture was added a solution of CH_2Cl_2 and THF (60 mL of 50/50 v/v) and the stirring continued for a further 15 min. This mixture was allowed to stand and the layers separated. The aqueous layer was extracted with a solution of CH_2Cl_2 and THF (2 × 50 mL of 50/50 v/v). The combined organic fractions were washed with water (1 × 50 mL portion), decolorized, and dried over $MgSO_4$. After removal of the solvent, a clear light tan oil was obtained. Yield: 5.8 g, 28 mmol (79%). ^1H NMR (DMSO): δ 1.28 (q, ^3J(HH) = 12 Hz), δ 2.16 (dt, ^3J(HH) = 11 Hz, 4 Hz), δ 3.53 (td, ^3J(HH) = 12 Hz, 4 Hz). *Caution!* Polyazides are potentially explosive.

(23) Malluche, H. H.; Smith, A. J.; Abreo, K.; Faugere, M.-C. *N. Engl. J. Med.* **1984**, *311*, 140.
(24) Russo, L. S.; Beale, G.; Sandroni, S.; Ballinger, W. E. *J. Neurol. Neurosurg. Psychiat.* **1992**, *55*, 697.
(25) Martell, A. E.; Hancock, R. D.; Motekaitis, R. J. *Coord. Chem. Rev.* **1994**, *133*, 39.
(26) Busch, D. L. *Chem. Rev.* **1993**, *93*, 847.
(27) For recent examples of aluminum(III) complexes having phenolate donor atoms see: Healy, M. D.; Barron, A. R. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 921. Healy, M. D.; Power, M. B.; Barron, A. R. *J. Coord. Chem.* **1990**, *21*, 363. Power, M. B.; Bott, S. G.; Clark, D. L.; Atwood, J. L.; Barron, A. R. *Organometallics* **1990**, *9*, 3086. Barron, A. R.; Dobbs, K. D.; Francl, M. M. *J. Am. Chem. Soc.* **1991**, *113*, 39. Healy, M. D.; Ziller, J. W.; Barron, A. R. *J. Am. Chem. Soc.* **1990**, *112*, 2949. Martell, A. E.; Motekaitis, R. J.; Smith, R. M. *Polyhedron* **1990**, *9*, 171. Evers, A.; Hancock, R. D.; Martell, A. E.; Motekaitis, R. J. *Inorg. Chem.* **1989**, *28*, 2189.
(28) Matsuba, A.; Nelson, W. O.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1988**, *27*, 1045. Finnegan, M. M.; Lutz, T. G.; Nelson, W. O.; Smith, A.; Orvig, C. *Inorg. Chem.* **1987**, *26*, 2171. Nelson, W. O.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1989**, *28*, 3153. Clevette, D. J.; Nelson, W. O.; Nordin, A.; Orvig, C.; Sjöberg, S. *Inorg. Chem.* **1989**, *28*, 2079. Yokel, R. A.; Datta, A. K.; Jackson, E. G. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 100. Clarke, E. T.; Martell, A. E. *Inorg. Chim. Acta* **1992**, *196*, 185. Clarke, E. T.; Martell, A. E. *Inorg. Chim. Acta* **1992**, *191*, 56.
(29) Healy, M. D.; Ziller, J. W.; Barron, A. R. *Organometallics* **1991**, *10*, 597.
(30) Hegetschweiler, K.; Ghisletta, M.; Fässler, T. F.; Nesper, R.; Schmalle, H. W.; Rihs, G. *Inorg. Chem.* **1993**, *32*, 2032.
(31) Liu, S.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1992**, *31*, 5400.
(32) Yokel, R. A.; Kostenbauder, H. B. *Toxicol. Appl. Pharmacol.* **1987**, *91*, 281.
(33) Levin, V. A. *J. Med. Chem.* **1980**, *23*, 682.
(34) For a preliminary communication see: Bollinger, J. E.; Roundhill, D. M. *Inorg. Chem.* **1994**, *33*, 1241.
(35) This procedure essentially follows that previously published: Fleischer, E. B.; Gebala, A. E.; Levey, A.; Tasker, P. A. *J. Org. Chem.* **1971**, *36*, 3042.

Table 2. Infrared Data (cm^{-1}) for the Ligands and Complexes

compd	ν(N−H)	δ(N−H)	δ(phenyl)	$\nu(NO_2)$
$(salH_2)_3tachH_3$	3263 s	1591 s	1608 m, 1471 s	
$Al(salH_2)_3tach$	3252 m	1570 w	1599 m, 1483 s	
$(NO_2salH_2)_3tachH_3 \cdot 3HCl$	2900−3100 b,w	1593 s	1622 m, 1496 s	1496 m, 1338 s
$Al(NO_2salH_2)_3tach$	3246 w	1578 m	1601 m, 1485 s	1482 s, 1332 s
$(MeOsalH_2)_3tachH_3$	3273 s	2939 m	1487 s	
$Al(MeOsalH_2)_3tach$	3236 m	2933 m	1491 s	

Table 3. UV/Visible Data: Maximum Absorbances for the Complexes

compd	λ_{max} (nm)	ϵ ($cm^{-1}\ M^{-1}$)
$Al(salH_2)_3tach$	284	1.6×10^4
	238	4.4×10^4
$Al(NO_2salH_2)_3tach$	360	4.59×10^4
	238	1.32×10^4
$Al(MeOsalH_2)_3tach$	306	8.7×10^3
	240	2.0×10^4

1,3,5-*cis*-Triaminocyclohexane Trihydrochloride (tach·3HCl). 1,3,5-*cis*-Triazidocyclohexane (5.8 g, 28 mmol) in freshly distilled THF (40 mL) was added to a rapidly stirred mixture of $LiAlH_4$ (4.7 g, 0.12 mol) in freshly distilled THF (40 mL) over a period of 2 h under nitrogen. After the addition was complete, the mixture was refluxed for 18 h. After cooling, water (5 mL) was added, followed by NaOH (5 mL of 15% aqueous solution) and water (15 mL). The resulting slurry was filtered through a Soxhlet thimble, and the filtrate was continuously extracted with a mixture of the supernatant and THF for 12 h. The solvent was removed under reduced pressure to give a nearly colorless oil. The crude product was taken up in EtOH (250 mL) and any insoluble material filtered out. To this stirred solution was added dropwise concentrated HCl (8 mL) to precipitate the compound as the trihydrochloride salt. The product was purified by dissolution in water (150 mL), followed by the filtered solution being made basic (pH 12) by addition of sodium hydroxide and the solvent being removed on a rotary evaporator. The pure compound was reprecipitated from an EtOH solution to yield a fine white powder. Yield: 4.84 g, 20.3 mmol (72%). ^{1}H NMR (DMSO-d_6): δ 1.48 (q, 3H, ^3J(HH) = 12 Hz), δ 2.31 (d, 3H, ^3J(HH) = 12 Hz), δ 3.21 (m, 3H), δ 8.52 (b s, 9H).

1,3,5-*cis*-Tris(salicylaldimino)cyclohexane ($(sal)_3tachH_3$). tach·3HCl (2.0 g, 8.4 mmol) was dissolved in water (50 mL), and sodium hydroxide pellets were added (1.0 g). The solvent was removed under reduced pressure on a rotary evaporator. To the resulting residue was added absolute ethanol (20 mL). The mixture was sonicated for 5 min, allowed to stand at 5 °C for 1 h, and filtered. Salicylaldehyde (4.5 g, 37 mmol) was added to the supernatant, this mixture was refluxed for 20 min and cooled to 5 °C, and the yellow solid was filtered off and washed with cold ethanol. If discoloration occurred, the solid was refluxed in water (75 mL) for 15 min. The reaction mixture was cooled and filtered to yield the product as a yellow microcrystalline powder.[36] Yield: 3.3 g, 7.4 mmol (88%). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.72 (q, 3H, ^3J(HH) = 12 Hz), δ 2.04 (m, 3H), δ 3.70 (m, 3H), δ 6.89 (t, 6H, ^3J(HH) = 8 Hz), δ 7.33 (t, 3H, ^3J(HH) = 6 Hz), δ 7.45 (d, 3H, ^3J(HH) = 8 Hz), δ 8.67 (s, 3H), δ 13.42 (s, 3H).

1,3,5-*cis*-Tris((2-hydroxybenzyl)amino)cyclohexane ($(salH_2)_3tachH_3$). $(sal)_3$tach (3.3 g, 7.4 mmol) was added to a mixture of borax (1.4 g, 7.0 mmol) in absolute ethanol (200 mL) under nitrogen. KBH_4 (1.5 g, 28 mmol) was then slowly added with stirring. The mixture was stirred for 12 h at ambient temperature and refluxed for 3 h, after which the solution became clear and light brown. Water (20 mL) was stirred into the solution and the mixture filtered. To the supernatant was added ammonium chloride (12 g) in water (80 mL) to give a white precipitate. The product was purified by recrystallization from a mixture of water and THF. Yield: 2.4 g, 5.4 mmol (73%).

(36) This procedure is similar to the published method: Rudman, D. A.; Huffman, J. C.; Childers, R. F.; Streib, W. E.; Wentworth, R. A. D. *Inorg. Chem.* **1975**, *14*, 747. The general abbreviation $Al(RsalH_2)_3$-tach refers to the complexes $Al(salH_2)_3$tach (R = H), $Al(NO_2salH_2)_3$-tach (R = NO_2), and $Al(MeOsalH_2)_3$tach (R = OMe).

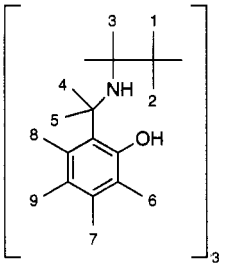
1,3,5-*cis*-Tris(nitrosalicylaldimino)cyclohexane ($(NO_2sal)_3tachH_3$). The preparation of this compound was similar to that of $(sal)_3tachH_3$. The compound tach·3HCl (2.00 g, 8.4 mmol) was neutralized and dissolved in absolute ethanol. To this solution was added nitrosalicylaldehyde (4.9 g, 29 mmol) along with sufficient ethanol to increase the solution volume to 100 mL. This mixture was refluxed for 3 h, during which the mixture thickened and a color change from yellow to green-yellow was observed. The mixture was cooled to 5 °C and filtered. The solid was rinsed with ethanol (3 × 10 mL) to yield the product as a yellow powder. Yield: 4.2 g, 7.3 mmol (87%). Mp: 305 °C dec. IR (KBr pellet): ν_{max} (cm^{-1}) 3097 w, 3055 w, 1670 s, 1604 s, 1327 s. ^{1}H NMR (DMSO-d_6): δ 1.92 (q, 3H, ^3J(HH) = 12 Hz), δ 2.28 (m, 3H), δ 3.93 (m, 3H), δ 6.82 (d, 3H, ^3J(HH) = 10 Hz), δ 8.11 (dd, 3H, ^3J(HH) = 9 Hz, ^5J(HH) = 3 Hz), δ 8.50 (d, 3H, ^5J(HH) = 3 Hz), δ 8.87 (s, 3H).

1,3,5-*cis*-Tris((2-hydroxy-5-nitrobenzyl)amino)cyclohexane ($(NO_2salH_2)_3tachH_3$). The preparation of this compound was similar to that of $(salH_2)_3tachH_3$ except now $(NO_2sal)_3$tach (4.2 g, 7.3 mmol) was used in a solution volume of 200 mL. The mixture was refluxed after the addition of borax and KBH_4 for a total of 6 h. Upon heating, the mixture became clear orange, followed by the formation of a yellow precipitate. After the mixture cooled to ambient temperature, water (50 mL) was added and the solution stirred for 0.5 h and filtered. To the supernatant was added ammonium chloride (14 g) in water (80 mL), causing the formation of a yellow precipitate. The mixture was refrigerated and filtered, and the solid was washed with water (2 × 10 mL) and then ethanol (2 × 10 mL). The crude yield was 3.4 g, 6.3 mmol (86%). This compound was purified *via* the hydrochloride salt. The crude product was suspended in a mixture of water (60 mL) and ethanol (60 mL). To this mixture was added concentrated HCl (20 mL) dropwise. Recrystallization from this same solvent system yielded the product as a fine crystalline powder.

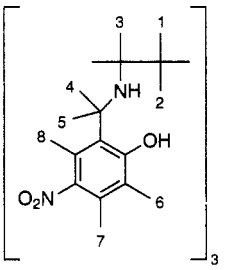
1,3,5-*cis*-Tris(methoxysalicylaldimino)cyclohexane ($(MeOsal)_3tachH_3$). The preparation of this compound was similar to that of $(sal)_3tachH_3$. The compound tach·3HCl (2.00 g, 8.4 mmol) was neutralized by dissolution in water, followed by passage of the solution through a Dowex anion exchange column (1.5 cm × 15 cm) in its hydroxy form. The solvent was removed on a rotary evaporator and the resulting amine dissolved in absolute ethanol (100 mL). To this solution was added methoxysalicylaldehyde (4.6 g, 30 mmol) and the mixture stirred until a yellow precipitate formed. This mixture was refluxed for 1 h, cooled to 5 °C, and filtered. The solid was rinsed with ethanol (3 × 10 mL) to yield the product as a yellow powder. Yield: 3.9 g, 7.4 mmol (88%). IR (KBr pellet): ν_{max} (cm^{-1}) 2937 w, 2831 w, 1639 s, 1587 s, 1491 s, 1462 m, 1276 s. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.75 (q, 3H, ^3J(HH) = 12 Hz), δ 1.98 (m, 3H), δ 3.67 (m, 3H), δ 3.68 (s, 9H), δ 6.80 (d, 3H, ^3J(HH) = 9 Hz), δ 6.93 (dd, 3 H, ^3J(HH) = 9 Hz, ^5J(HH) = 3 Hz), δ 7.04 (d, 3H, ^5J(HH) = 3 Hz), δ 8.61 (s, 3H), δ 12.77 (s, 3H).

1,3,5-*cis*-Tris((2-hydroxy-5-methoxybenzyl)amino)cyclohexane ($(MeOsalH_2)_3tachH_3$). $(MeOsal)_3$tach (3.9 g, 7.4 mmol) was dissolved in absolute ethanol (150 mL) to give a saturated solution. To this mixture was added borax (1.4 g, 7.0 mmol), and after 15 min of stirring, KBH_4 (1.5 g, 28 mmol) was added slowly. The stirring was continued for 30 min under nitrogen. The mixture was refluxed for 1 h and then stirred at ambient temperature for a further 12 h. The suspension became colorless. The mixture was decanted from the borax, and water (20 mL) was added dropwise under nitrogen. Ammonium acetate (12 g) in water (80 mL) was added to the reaction mixture. The resultant

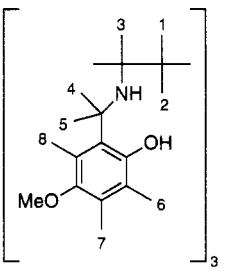
Table 4. ^{1}H NMR (DMSO-d_6) Assignments (δ) for the Ligands and the Aluminum Complexes



$(salH_2)_3tachH_3$	$(salH_2)_3tachH_3$·3HCl	$Al(salH_2)_3tach$	assgn	$(salH_2)_3tachH_3$	$(salH_2)_3tachH_3$·3HCl	$Al(salH_2)_3tach$	assgn
0.89 (q, 3H)[e]	1.77 (q, 3H)[a]	1.63 (b d, 3H)[g]	*H*1	6.67 (q, 6H)[d]			*H*6 + *H*8
2.19 (b d, 3H)[f]	2.75 (b d, 3H)[b]	2.48 (b d, 3H)[g]	*H*2		7.45 (d, 3H)[d]	6.91 (t, 3H)[d]	*H*8
2.42 (b s, 3H)	3.23 (b s, 3H)	2.92 (b s, 3H)	*H*3		7.21 (t, 3H)[c]	6.95 (d, 3H)[c]	*H*7
		3.36 (b d, 3H)[h]	*H*4	7.03 (t, 6H)[d]			*H*7 + *H*9
3.84 (s, 6H)	4.07 (s, 6H)		*H*4 + *H*5		6.82 (t, 3H)[d]	6.38 (t, 3H)[d]	*H*9
		3.92 (b t, 3H)[f]	*H*5	3−4 (broad)	9.52 (b s, 6H)	4.19 (b d, 3H)[e]	N*H*
	6.97 (d, 3H)[c]	6.08 (d, 3H)[c]	*H*6		10.30 (s, 3H)		O*H*



$(NO_2salH_2)_3tachH_3$	$(NO_2salH_2)_3$-$tachH_3$·3HCl	$Al(NO_2salH_2)_3tach$	assgn	$(NO_2salH_2)_3tachH_3$	$(NO_2salH_2)_3$-$tachH_3$·3HCl	$Al(NO_2salH_2)_3tach$	assgn
1.13 (m, 3H)	1.80 (m, 3H)	1.83 (b d, 3H)[i]	*H*1	6.54 (d, 3H)[l]	7.18 (d, 3H)[l]	6.21 (d, 3H)[l]	*H*6
2.30 (m, 3H)	2.74 (m, 3H)	2.56 (b d, 3H)[i]	*H*2	7.91 (d, 3H)[l]	8.18 (d, 3H)[l]	7.99 (d, 3H)[l]	*H*7
2.75 (m, 3H)	3.33 (m, 3H)	3.03 (b s, 3H)	*H*3	8.04 (s, 3H)	8.47 (s, 3H)	7.94 (s, 3H)	*H*8
		3.34 (b d, 3H)[j]	*H*4	4−6 (broad)	9.65 (b s, 6H)	5.31 (b d, 3H)[k]	N*H*
3.95 (m, 6H)	4.20 (s, 6H)		*H*4 + *H*5		12.10 (b s, 3H)		O*H*
		4.13 (t, 3H)[j]	*H*5				



$(MeOsalH_2)_3tachH_3$	$(MeOsalH_2)_3$-$tachH_3$·3HCl	$Al(MeOsalH_2)_3tach$	assgn	$(MeOsalH_2)_3tachH_3$	$(MeOsalH_2)_3$-$tachH_3$·3HCl	$Al(MeOsalH_2)_3tach$	assgn
0.83 (q, 3H)[m]	1.78 (q, 3H)[m]	1.60 (b d, 3H)[p]	*H*1	6.56 (d, 3H)[o]	6.79 (d, 3H)[o]	6.00 (d, 3H)[o]	*H*6
2.15 (b d, 3H)[q]	2.77 (b d, 3H)[n]	2.19 (b d, 3H)[p]	*H*2	6.60 (d, 3H)[o]	6.88 (d, 3H)[o]	6.56 (d, 3H)[o]	*H*7
2.36 (m, 3H)	3.21 (m, 3H)	2.91 (b s, 3H)	*H*3	6.65 (s, 3H)	7.16 (s, 3H)	6.56 (s, 3H)	*H*8
		3.37 (b d, 3H)[m]	*H*4		9.62 (b s, 6H)	4.04 (m, 3H)	N*H*
3.78 (s, 6H)	4.04 (s, 6H)		*H*4 + *H*5		9.78 (s, 3H)		O*H*
		3.84 (b t, 3H)[n]	*H*5	3.61 (s, 9H)	3.67 (s, 9H)	3.59 (s, 9H)	O*CH*$_3$

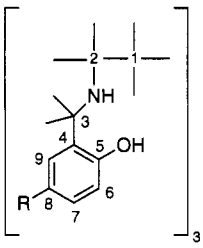
[a] ^3J(HH) = 12 Hz. [b] ^3J(HH) = 9 Hz. [c] ^3J(HH) = 8 Hz. [d] ^3J(HH) = 7 Hz. [e] ^3J(HH) = 11 Hz. [f] ^3J(HH) = 10 Hz. [g] ^2J(HH) = 15 Hz. [h] ^2J(HH) = 12 Hz. [i] ^2J(HH) = 15 Hz. [j] ^2J(HH) = 12 Hz. [k] ^3J(HH) = 10 Hz. [l] ^3J(HH) = 9 Hz. [m] ^2J(HH) = 12 Hz. [n] ^3J(HH) = 10 Hz. [o] ^3J(HH) = 8 Hz. [p] ^2J(HH) = 14 Hz. [q] ^3J(HH) = 11 Hz.

mixture was cooled to 5 °C and filtered to yield the product as a cream-colored powder. Yield: 3.5 g, 6.5 mmol (88%).

Synthesis of Aluminum Complexes. These syntheses were carried out on a small scale (<100 mg) in 100 mL round-bottomed or Schlenk flasks. The reactions were carried out under dry nitrogen. Aluminum salts were stored and weighed in a drybox before use. All metal complexes were found to have decomposition points greater than 280 °C.

Al(salH$_2$)$_3$tach. ($salH_2)_3$tach (0.1 g, 0.22 mmol) was added to dry methanol (50 mL), and the mixture was stirred until the maximum quantity of solute had dissolved. $Al(NO_3)_3$·9H_2O (0.083 g, 0.22 mmol) was dissolved separately in methanol (5 mL) and the solution allowed to stand over molecular sieves (3 Å type) for 15 min. The solution of aluminum nitrate was then added dropwise to the stirred solution of the ligand, forming a nearly colorless solution of the complex. After 15 min, diisopropylethylamine (1 mL) was added dropwise to the solution and the mixture refluxed for 12 h. After refluxing, the solvent was removed under reduced pressure and the residue heated at 100 °C and 0.3 atm for 12 h to remove excess amine. The residue was dissolved in a mixture of dichloromethane and methanol (4 mL of 90/

Table 5. $^{13}C\{^1H\}$ NMR (DMSO-d_6) Assignments (δ) for the Ligands and the Aluminum Complexes



$(salH_2)_3tachH_3$ (L1)	Al(L1)	assgn	$(salH_2)_3tachH_3$ (L1)	Al(L1)	assgn
38.8	30.9	*C*1	118.8	119.3	*C*6
52.8	53.2	*C*2	128.2	128.1	*C*7
48.4	53.1	*C*3	115.9	114.6	*C*8
124.6	126.9	*C*4	128.7	129.1	*C*9
158.2	164.7	*C*5			

$(NO_2salH_2)_3$-tachH$_3$ (L2)	Al(L2)	assgn	$(NO_2salH_2)_3$-tachH$_3$ (L2)	Al(L2)	assgn
30.3	29.5	*C*1	116.2	125.0	*C*6
52.6	52.5	*C*2	127.1	126.4	*C*7
42.4	51.4	*C*3	139.6	136.8	*C*8
119.7	119.0	*C*4	128.5	127.1	*C*9
163.1	173.5	*C*5			

$(MeOsalH_2)_3$-tachH$_3$ (L3)	Al(L3)	assgn	$(MeOsalH_2)_3$-tachH$_3$ (L3)	Al(L3)	assgn
38.9	31.1	*C*1	116.2	119.0	*C*6
48.3	53.2	*C*2	113.1	114.4	*C*7
52.9	53.3	*C*3	151.7	149.3	*C*8
125.5	126.8	*C*4	114.4	114.1	*C*9
152.1	158.5	*C*5	55.7	55.9	OCH_3

10 v/v) and purified by flash chromatography (silica gel, 325 mesh, 15 cm × 1.5 cm column, $R_f = 0.35$) in this mixed solvent. After TLC analysis, the solvent was removed to yield the product as a nearly colorless powder. Yield: 0.085 g, 0.18 mmol (82%).

Al(NO_2salH_2)$_3$tach. This preparation was similar to that of Al($salH_2$)$_3$tach using (NO_2salH_2)$_3$tach (0.22 mmol) and $Al(NO_3)_3 \cdot 9H_2O$ (0.083 g, 0.22 mmol). Addition of diisopropylethylamine (1.5 mL) caused the solution to form a thick yellow flocculate. After the mixture was refluxed for 15 h, a yellow precipitate formed. The precipitate was filtered out as previously described to give the product as a fine yellow-orange powder. Yield: 0.11 g, 0.19 mmol (88%).

Al($MeOsalH_2$)$_3$tach. The synthesis followed a procedure similar to that for Al($salH_2$)$_3$tach using ($MeOsalH_2$)$_3$tach (0.22 mmol) and $Al(NO_3)_3 \cdot 9H_2O$ (0.083 g, 0.22 mmol). Addition of the metal salt to the mixture of the ligand in ethanol caused dissolution and a color change to light yellow. Purification and subsequent removal of solvent yielded the product as a light yellow powder. Yield: 0.07g, 0.12 mmol (55%).

Alternate Method. An alternate synthetic method for the complexes used the same metal and ligand proportions except that anhydrous aluminum chloride was used and dissolved in 2−4 mL of solvent without the use of molecular sieves. The solvents used were the same as described in the previous method except that diethylamine (1 mL) was used in place of diisopropylethylamine. Mixtures were refluxed for 12 h under nitrogen. Purfication of the crude product was identical to that described previously.

Partition Coefficients. Octanol:water partition coefficients were determined for the complexes by dissolving a small amount (i.e., the tip of a microspatula) of material as completely as possible in 10 mL of octanol in a 25 mL screw-cap vial. To this vial was added deionized water (10 mL). The mixture was stirred for 18 h at a rate that avoided emulsification. The mixture was allowed to stand overnight; it was then poured into a 60 mL separation funnel, whereupon the two layers separated. For analysis, the layers were centrifuged or filtered through a Gooche crucible to remove any particulates. The samples were diluted as necessary such that the primary absorption in their UV/visible spectra was <0.5. The corrected absorption values were compared directly between octanol and water layers and expressed as the given ratios.

X-ray Crystallographic Methods. Colorless crystals of Al($salH_2$)$_3$-tach were obtained from the slow diffusion of water into dimethyl sulfoxide solutions of the complex, and amber crystals of Al(NO_2-$salH_2$)$_3$tach from the slow diffusion of ethanol into dimethylformamide solutions of the complex. The crystals were cut to size, coated with a thin film of epoxy cement, and affixed to thin glass fibers. General procedures for crystal orientation, unit cell determination, and data collection on the CAD-4 diffractometer have been published.[37] Details

Table 6. Summary of Crystallographic Data

complex	Al($salH_2$)$_3$tach·3.5H_2O	Al(NO_2salH_2)$_3$tach·C_2H_5OH
formula	$C_{27}H_{37}N_3O_{6.5}Al$	$C_{29}H_{33}N_6O_{10}Al$
fw	535.10	652.60
cryst size, mm	0.53 × 0.20 × 0.56	0.2 × 0.33 × 0.36
cryst system	monoclinic	monoclinic
space group	$P2_1/n$	$P2_1/n$
a, Å	15.047(2)	12.305(1)
b, Å	11.355(1)	12.072(2)
c, Å	15.201(2)	20.229(3)
β, deg	93.59(6)	90.26(1)
V, Å^3	2592(1)	3005(1)
Z	4	4
ϱ_{calc}, g cm^{-3}	1.39	1.44
μ, cm^{-1}	1.24	1.29
range of transm factors	0.9493−0.9988	
temp, K	293	295
radtn	Mo Kα (graphite monochromated, λ = 0.710 73 Å)	
scan type	$\omega/2\theta$	$\omega/2\theta$
scan range, deg	0.80 + 0.34(tan θ)	0.80 + 0.20tan(θ)
2θ range, deg	1.0−46.0	0.5−25.0
tot. no. of refls	3983	5839
no. of unique refls	3569	5280
R_{int}	0.016	0.027
no. of obs data	2604 ($I \geq 3\sigma(I)$)	3105 ($I \geq 2\sigma(I)$)
no. of param	363	431
$(\Delta/\sigma)_{max}$ in last cycle	0.03	0.01
R [a]	0.038	0.040
R_w [b]	0.052	0.047
GOF[c]	1.81	1.32
$\Delta\varrho$ in final ΔF map, e Å^{-3}	0.48 to −0.08	0.18 to −0.08

[a] $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w(|F_o|)^2]^{1/2}$ with $w = 1/(\sigma_F)^2$; $\sigma_F = \sigma(F^2)/2F$; $\sigma(F^2) = [(\sigma_I)^2 + (0.04F^2)^2]^{1/2}$. [c] GOF = $[\Sigma w(|F_o| - |F_c|)^2/(N_o - N_v)]^{1/2}$ where N_o and N_v are, respectively, the number of observations and variables.

specific to the present work are presented in Table 6. The monoclinic cell indicated by the CAD-4 software was confirmed by the observation of 2/*m* diffraction symmetry. The space group was uniquely determined by the systematic absences observed in the final data set. The data were corrected for Lorentz and polarization effects and, for Al(NO_2-salH_2)$_3$tach, a linear 11.4% decay in the intensity monitors. No absorption correction was deemed necessary. For Al(salH_2)$_3$tach, only statistical fluctuations were observed in the intensity monitors. An absorption correction was carried out with ψ scans on four reflections with χ near 90°. The position of the metal atom was obtained from an origin-removed Patterson function and the remainder of the structure developed through successive cycles of full-matrix, least-squares refinement followed by $\Delta\rho$ syntheses. In the late stages of the refinement, most hydrogen atoms were visible in the difference map. Those hydrogens attached to N(1)−N(3) were refined, and the remainder were placed in calculated positions (C−H = 0.95 Å) with isotropic thermal parameters 20% larger than those of the attached carbon atoms and updated periodically.

All calculations were performed with the MolEN[38] suite of programs on a VAX station 3100 computer. Atomic scattering factors include the real and imaginary parts of the corrections for the effects of anomalous dispersion.[39] Crystallographic details are summarized in Table 6, positional parameters are given in Tables 7 and 8, and selected bond distances are given in Table 9.

***In Vivo* Aluminum Chelate Studies.** The procedures used for the *in vivo* animal studies with these new compounds follow closely those that have been used previously in these laboratories and described elsewhere.[40] The solutions used for intraperitoneal (i.p.) injections were prepared as follows. An acidified saline control solution (solution 1) was prepared by the addition of hydrochloric acid (0.5 mL of 0.2 M) to normal saline solution (100 mL) in order to buffer the solution at pH ~3. An aluminum control solution (solution 2) was prepared by the dissolution of $AlCl_3$·$6H_2O$ (8.9g) in normal saline solution (100 mL) to give a final solution that is 0.37 *m* in aluminum chloride.

The solutions used for subcutaneous (s.c.) injections were prepared as follows. A mixed control solution containing saline and DMSO (solution 3) was prepared by the addition of DMSO (4.6 mL) to normal saline solution (95 mL) to give a final solution that has 5% by weight of DMSO. Ligand solutions (solution 4) for the three compounds (RsalH_2)$_3$tachH_3 (R = H, NO_2, OMe) were prepared in the 5% DMSO/saline (w/w) mixed control solution. (salH_2)$_3$tach (0.028 g, 0.060 mmol) was added to the 5% DMSO solution (100 mL) along with HCl (1 mL of 0.2 M). This procedure yielded a colorless solution (0.60 mM) with a pH ~5. (NO_2salH_2)$_3$tach·3HCl (0.042 g, 0.060 mmol) was added to the 5% DMSO/saline solution (100 mL), which provided a light yellow solution (0.60 mM) with a pH ~5. (MeOsalH_2)$_3$tach (0.033 g, 0.060 mmol) was added to the 5% DMSO solution (100 mL) to which was then added HCl (1 mL of 0.2 M). This procedure yielded a colorless solution (0.60 mM) with a pH ~6.

The mice used in this study were male ICR mice purchased from Charles River Laboratories, Wilmington, MA. All mice weighed 17−20 g. To preserve consistency, the given procedures were conducted simultaneously in three groups as a single experiment. The three groups, saline control, aluminum control, and aluminum/chelator combination, consisted of six or seven mice apiece. Experiments were repeated three or four times for a total of about 20 mice per group. Mice in the experimental group and the aluminum control group received an i.p. injection of aluminum (100 mg/kg) in normal saline (0.2 mL) (solution 2). This dose increases blood levels of aluminum to about 10 times above baseline to levels seen in dialysis dementia.[41] The saline control group received an acidified saline solution (0.2 mL) (solution 1). Immediately afterward, mice in all groups received a s.c. injection over the thoracic spine. The experimental group received a sample of the ligand dissolved in the solution containing 5% DMSO in the saline control solution (0.2 mL of 0.6 mM) (solution 4). The other two groups received only the pure solution of 5% DMSO in the saline solution (0.2 mL) (solution 3).

A subsequent experiment, testing the action of the chelator independently, was conducted in a fashion identical to those described above. It consisted of the three previously described groups with an additonal fourth group. The added group received a s.c. injection of the (NO_2salH_2)$_3$tach solution (solution 4) and i.p. acidified saline control solution.

After about 35 min, the mice were anesthetized with urethane (40% in saline, 0.2 mL) by i.p. injection. The scalp was then exposed and a hole 3.0−3.5 mm deep was made into the left lateral ventricle of the brain 1.0 mm lateral and 1.0 mm posterior to the bregma. This was accomplished with a 26-gauge needle sheathed with polyethylene tubing to cover all but the terminal 3.5 mm.[42] After a maximum of 65 min following the first i.p. and s.c. injections, an intracerebroventricular injection (i.c.v.) of 1 μL lactated Ringer's solution containing 25 × 10^3 cpm radioactive peptide (^{125}I labeled Tyr-MIF-1) into this ventricle was made using a 1 μL Hamiltonian syringe (Hamilton Co., Reno, NV).

Mice were decapitated 20 min after i.c.v. injection and their brains removed, excluding the pineal and pituitary. The brains were placed individually into 10 × 75 mm test tubes and centrifuged for 5 min at 2500*g* to seat them at the base of the tubes. Each brain was then

Table 7. Positional Parameters (Esd's) for Al(salH_2)$_3$tach

atom	*x*	*y*	*z*	B_{eq},[a] Å^2
Al	0.02796(5)	0.19982(6)	−0.18398(5)	2.01(1)
O1	0.1249(1)	0.2965(2)	−0.1567(1)	2.43(4)
O2	0.0026(1)	0.1750(2)	−0.0699(1)	2.48(4)
O3	−0.0531(1)	0.3209(2)	−0.2032(1)	2.56(4)
N1	0.0572(1)	0.2126(2)	−0.3183(1)	2.38(4)
N2	0.1166(1)	0.0527(2)	−0.1735(1)	2.41(4)
N3	−0.0727(1)	0.0782(2)	−0.2219(1)	2.23(4)
C1	0.1292(2)	0.4013(2)	−0.1975(2)	2.31(5)
C2	0.1257(2)	0.5068(3)	−0.1515(2)	2.84(6)
C3	0.1336(2)	0.6129(3)	−0.1957(2)	3.54(6)
C4	0.1443(2)	0.6153(3)	−0.2849(2)	3.64(7)
C5	0.1448(2)	0.5103(3)	−0.3311(2)	3.17(6)
C6	0.1362(2)	0.4029(2)	−0.2884(2)	2.47(5)
C7	0.1366(2)	0.2873(2)	−0.3350(2)	2.83(6)
C8	0.0676(2)	0.1675(2)	−0.0059(2)	2.48(5)
C9	0.0747(2)	0.2470(3)	0.0638(2)	3.19(6)
C10	0.1420(2)	0.2342(3)	0.1296(2)	3.88(7)
C11	0.2033(2)	0.1453(3)	0.1268(2)	4.01(7)
C12	0.1982(2)	0.0675(3)	0.0565(2)	3.54(6)
C13	0.1310(2)	0.0779(2)	−0.0102(2)	2.73(5)
C14	0.1203(2)	−0.0061(2)	−0.0852(2)	2.98(6)
C15	−0.1299(2)	0.3168(2)	−0.1619(2)	2.44(5)
C16	−0.1542(2)	0.4058(3)	−0.1053(2)	3.47(6)
C17	−0.2342(2)	0.3986(3)	−0.0651(2)	4.12(7)
C18	−0.2904(2)	0.3041(3)	−0.0811(2)	3.56(6)
C19	−0.2673(2)	0.2162(3)	−0.1377(2)	3.04(6)
C20	−0.1873(2)	0.2225(2)	−0.1786(2)	2.37(5)
C21	−0.1615(2)	0.1329(2)	−0.2450(2)	2.74(6)
C22	0.0599(2)	0.1009(3)	−0.3709(2)	2.78(6)
C23	0.1323(2)	0.0173(3)	−0.3330(2)	3.18(6)
C24	0.1109(2)	−0.0381(2)	−0.2462(2)	2.84(6)
C25	0.0201(2)	−0.0960(2)	−0.2537(2)	2.94(6)
C26	−0.0539(2)	−0.0146(2)	−0.2883(2)	2.59(5)
C27	−0.0305(2)	0.0403(3)	−0.3756(2)	3.05(6)
O1s	−0.2230(1)	0.1952(2)	0.4635(2)	4.82(5)
O2s	−0.0734(2)	0.5141(3)	−0.3056(3)	14.0(1)
O3s	0.0611(2)	0.6866(3)	−0.5252(2)	12.2(1)
O4s	−0.0640(4)	0.5389(6)	−0.4605(3)	7.4(2)
H1n	0.010(2)	0.257(2)	−0.345(2)	2.8(5)*
H2n	0.168(1)	0.085(2)	−0.173(1)	2.0(5)*
H3n	−0.075(1)	0.041(2)	−0.175(1)	2.5(5)*
H1sa	−0.271(2)	0.203(3)	0.422(2)	6.6(9)*
H1sb	−0.241(3)	0.213(5)	0.512(3)	14(2)*

[a] Starred values are for atoms refined isotropically. Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as $B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}a^*_i a^*_j \mathbf{a}_i\cdot\mathbf{a}_j$.

(37) Mague, J. T.; Lloyd, C. L. *Organometallics* **1988**, *7*, 983.
(38) *MolEN: An Interactive Structure Solution Procedure*; Enraf-Nonius: Delft, The Netherlands, 1990.
(39) Cromer, D. T.; Waber, J. T. *International Tables for X-ray Crystallography*; The Kynoch Press: Birmingham, England, 1974: Vol. IV, Table 2.2B. Cromer, D. T. *Ibid.*, Table 3.2.1.
(40) Banks, W. A.; Kastin, A. J. *Methods Enzymol.* **1989**, *168*, 652.
(41) Kim, Y. S.; Lee, M. H.; Wisniewski, H. M. *Brain Res.* **1986**, *377*, 286.
(42) Noble, E. P.; Wurtman, A. J.; Axelrod, J. *Life Sci.* **1967**, *6*, 281.

Table 8. Positional Parameters (Esd's) for $Al(NO_2salH_2)_3tach$

atom	x	y	z	B,[a] Å^2
Al	0.14165(6)	0.20318(7)	0.21652(4)	2.04(1)
O1	0.2260(1)	0.2320(2)	0.29066(8)	2.48(4)
O2	0.2313(1)	0.0940(2)	0.18396(8)	2.52(4)
O3	0.2106(1)	0.3078(2)	0.16667(9)	2.49(4)
O4	0.1399(2)	0.2800(2)	0.5918(1)	5.67(7)
O5	0.557(3))	0.1223(3)	0.5739(1)	9.05(9)
O6	0.4419(2)	0.0986(2)	-0.0954(1)	4.78(6)
O7	0.2733(2)	0.0882(3)	-0.1220(1)	6.08(7)
O8	0.0693(2)	0.6790(2)	-0.0281(1)	5.94(6)
O9	-0.0287(3)	0.7207(2)	0.0560(1)	7.27(7)
N1	0.0523(2)	0.0844(2)	0.2645(1)	2.24(5)
N2	0.0302(2)	0.1771(2)	0.1369(1)	2.25(5)
N3	0.0340(2)	0.3246(2)	0.2534(1)	2.43(5)
N4	0.1136(2)	0.2041(3)	0.5549(1)	4.56(7)
N5	0.3453(2)	0.0937(2)	-0.0802(1)	3.58(6)
N6	0.0374(2)	0.6614(3)	0.0284(1)	4.78(7)
C1	0.1979(2)	0.2253(2)	0.3538(1)	2.25(5)
C2	0.2350(2)	0.3046(3)	0.3992(1)	2.89(6)
C3	0.2064(2)	0.2974(3)	0.4647(1)	3.15(6)
C4	0.1405(2)	0.2125(3)	0.4852(1)	3.02(6)
C5	0.1020(2)	0.1330(3)	0.4416(1)	3.02(6)
C6	0.1308(2)	0.1390(2)	0.3757(1)	2.34(6)
C7	0.1027(2)	0.0481(3)	0.3285(1)	2.71(6)
C8	0.2597(2)	0.0941(2)	0.1209(1)	2.30(6)
C9	0.1781(2)	0.0923(2)	0.0715(1)	2.40(6)
C10	0.2071(2)	0.0934(3)	0.0057(1))	2.68(6)
C11	0.3159(2)	0.0950(2)	-0.0114(1)	2.70(6)
C12	0.3971(2)	0.0960(3)	0.0363(1)	2.86(6)
C13	0.3688(2)	0.0957(3)	0.1020(1)	2.80(6)
C14	0.0628(2)	0.0837(3)	0.0932(1)	2.64(6)
C15	0.1671(2)	0.3906(2)	0.1334(1)	2.41(6)
C16	0.0887(2)	0.4585(2)	0.1628(1)	2.64(6)
C17	0.0455(3)	0.5464(3)	0.1278(2)	3.44(7)
C18	0.0812(3)	0.5669(3)	0.0642(1)	3.49(7)
C19	0.1563(3)	0.5002(3)	0.0338(1)	3.60(7)
C20	0.1993(2)	0.4119(3)	0.0681(1)	3.12(6)
C21	0.0634(2)	0.4410(3)	0.2346(1)	2.89(6)
C22	-0.0693(2)	0.0929(3)	0.2673(1)	2.76(6)
C23	-0.1080(2)	0.2024(3)	0.2961(1)	2.92(6)
C24	-0.0856(2)	0.3011(3)	0.2515(1)	2.77(6)
C25	-0.1255(2)	0.2825(3)	0.1809(1)	2.93(6)
C26	-0.0889(2)	0.1722(3)	0.1519(1)	2.74(6)
C27	-0.1153(2)	0.0766(3)	0.1982(1)	2.91(6)
O1s	0.9181(2)	0.1443(2)	0.7781(1)	3.73(5)
C1s	0.9728(3)	0.2274(3)	0.7407(2)	4.29(8)
C2s	1.0884(3)	0.2334(4)	0.7599(2)	5.4(1)
H1n	0.062(2)	0.027(2)	0.240(1)	2.2(5)*
H2n	0.038(2)	0.233(2)	0.115(1)	3.2(6)*
H3n	0.051(2)	0.321(2)	0.295(1)	3.0(6)*

[a] Starred values are for atoms refined isotropically. Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as: $B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}a^*_i a^*_j \mathbf{a}_i\cdot\mathbf{a}_j$.

Table 9. Selected Crystallographic Data

	bond distances, Å	
	$Al(salH_2)_3tach$	$Al(NO_2salH_2)_3tach$
Al−N1	2.120(2)	2.054(3)
Al−N2	2.137(2)	2.133(3)
Al−N3	2.104(2)	2.114(3)
Al−O1	1.852(2)	1.853(2)
Al−O2	1.821(2)	1.842(2)
Al−O3	1.849(2)	1.828(2)

	average bond angles, deg[a]	
complex	*trans* N−Al−O	O−Al−O
$Al(salH_2)_3tach$	174.4(0.6)	95.3(0.3)
$Al(NO_2salH_2)_3tach$	175.0(2.3)	93.1(2.1)

[a] Numbers in parentheses indicate the standard deviation (σ_{n-1}) of the averaged angles.

counted for 3 min with a γ-counter (Micromedic 10/200, Horsham, PA) to determine the level of residual, or nontransported, peptide in the brain. Results are presented as means with their standard errors. Groups were compared for statistically significant differences by analysis of variance (ANOVA) followed by Duncan's multiple range test.

Results and Discussion

The phenolate-derivatized cyclohexanetriamine ligands were synthesized by the procedure shown in Scheme 1. This route was chosen from 1,3,5-*cis*-cyclohexanetriol because we found it to be the most reliable route for synthesizing 1,3,5-*cis*-triaminocyclohexane. An alternate one-step literature procedure to this triamine from phloroglucinol was initially followed, but we found that the yields of product we obtained were consistently very low.[43] Schiff base condensation between 1,3,5-*cis*-traminocyclohexane and either salicylaldehyde or a substituted salicylaldehyde gives the unsaturated imine $(Rsal)_3tachH_3$. The saturated C−N derivative $(RsalH_2)_3tachH_3$ then is obtained by reduction of the imine with potassium borohydride. The final conversion step to give saturated C−N bonds has been carried out because unsaturated C=N bonds are considered to be a cleavage point for biodegradation under *in vivo* conditions.

Each intermediate in Scheme 1 has been isolated and characterized by ^{1}H NMR spectroscopy. The reduced products $(salH_2)_3tachH_3$, $(NO_2salH_2)_3tachH_3$, and $(MeOsalH_2)_3tachH_3$ have been characterized by a combination of microanalytical and spectroscopic techniques. The FAB mass spectra of these compounds show the presence of a parent peak for the molecular ion M^+. The compounds show bands due to ν(NH) in the 3000−3300 cm^{-1} range and to δ(NH) in the 1590−1600 cm^{-1} range. The compound $(NO_2salH_2)_3tachH_3$ shows additional bands due to $\nu(NO_2)$ at 1496 and 1338 cm^{-1}, and the compounds also show bands due to an aliphatic ν(CH) at 2939 cm^{-1}.[44]

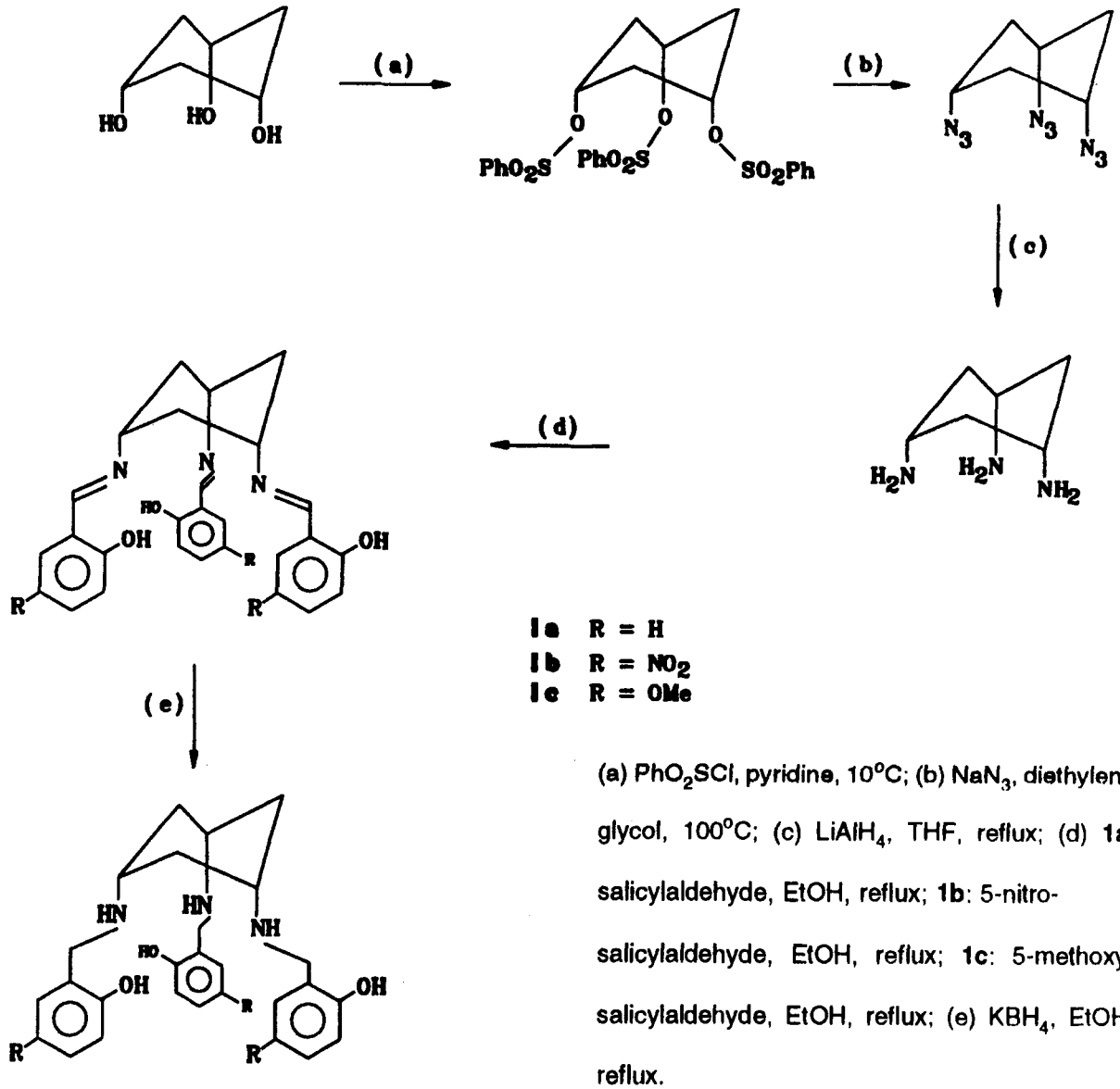
The ^{1}H NMR spectra of the ligands show resonances characteristic of substituted cyclohexane and phenyl rings and of the appended O*H* and N*H* functional groups. The nine cyclohexane ring backbone hydrogens are observed as three sets of resonances due to the H1, H2, and H3 protons. The peaks are broad, and their assignments have been made on the basis of spin multiplicity and chemical shift values. The methylene protons in both the free amine and its hydrochloride salt are observed as an equivalent pair (H4 + H5) because of free rotation about the appended C−C and C−N single bonds. The resonances of the phenyl protons are observed in the δ 6.54−8.04 range. In some cases, individual resonances can be assigned, but in other cases, overlapping makes this impossible to accomplish with any degree of reliability. The spectrum of $(MeOsalH_2)_3tachH_3$ shows an additional resonance at δ 3.61 for the methoxy protons. In the unprotonated compounds the resonances due to the N*H* and O*H* protons are not always observed. After protonation with HCl, however, these resonances can be identified in the salts $(salH_2)_3tachH_3$·3HCl, $(NO_2sal)_3tachH_3$·3HCl, and $(MeOsal)_3tachH_3$·3HCl. The positions of the N*H* resonances range from δ 9.52 to 9.65, and those for the O*H* resonances range from δ 9.78 to 12.10.

In the $^{13}C\{^1H\}$ NMR spectra of $(salH_2)_3tachH_3$, $(NO_2salH_2)_3tachH_3$, and $(MeOsalH_2)_3tachH_3$, the resonances for the aliphatic *C*H and *C*H$_2$ carbons are grouped in the δ 30−53 range, whereas those for the aromatic carbons are in the δ 110−165 range. As expected, the position of *C*8 shows the greatest sensitivity to the nature of the substituent R, changing from δ 115.9 to δ 139.6 to δ 151.7 as R varies from H to NO_2 to OMe, respectively.

Aluminum(III) Complexes. The aluminum complexes of $(RsalH_2)_3tach$ (R = H, NO_2, OMe) have been synthesized by

(43) Wentworth, R. A. D.; Felten, J. J. *J. Am. Chem. Soc.* **1968**, 90, 621.
(44) Bellamy, L. J. *The Infra-red Spectra of Complex Molecules*; Wiley: New York, 1958.

Scheme 1



1a R = H
1b R = NO_2
1c R = OMe

(a) PhO_2SCl, pyridine, 10°C; (b) NaN_3, diethylene glycol, 100°C; (c) $LiAlH_4$, THF, reflux; (d) **1a**: salicylaldehyde, EtOH, reflux; **1b**: 5-nitro-salicylaldehyde, EtOH, reflux; **1c**: 5-methoxy-salicylaldehyde, EtOH, reflux; (e) KBH_4, EtOH, reflux.

the treatment of aluminum nitrate with the ligands in a 1:1 ratio (eq 1). The complexes are obtained in high yield (~90%) as

$$Al(NO_3)_3 + (RsalH_2)_3tachH_3 \rightarrow Al(RsalH_2)_3tach + 3HNO_3 \quad (1)$$

powders that vary in color from colorless to yellow-orange. The infrared spectra (Table 2) are similar to those obtained for the protonated ligands, except that the values for ν(NH) and δ(NH) are shifted to somewhat lower energies.

The ^1H NMR of the aluminum complexes (Table 4) show all the expected resonances. In general, the cyclohexyl resonances more closely correspond to those found for the hydrochloride salts than those for the free amines, in agreement with the metal ion being complexed to the amine nitrogens. Further support for nitrogen coordination comes from the observed large upfield shifts (4–5 ppm) in δ(NH). As expected for an uncharged phenolate complex, the O*H* resonances are absent in the spectra of aluminum complexes. For the Al(III) complexes, the chelation eliminates the free rotation that causes *H*4 and *H*5 to become equivalent; therefore these individual protons are observed as an AB pair of broad doublets. The $^{13}C\{^1H\}$ NMR spectra of the Al(III) complexes show all the expected resonances, with downfield shifts for *C*3 and *C*5 being observed as compared to those of the free ligand.

The absorption spectra of the aluminum complexes all show two peaks in the UV region of the spectrum, both of which have the large extinction coefficients expected for intraligand $\pi-\pi^*$ transitions (Table 3). The compound $Al(NO_2salH_2)_3$-tach shows the long wavelength at 360 nm rather than in the 280–300 nm range because of the presence of a chromophore due to the nitro group. An overlay of the spectra of the three complexes is shown in Figure 1.

The X-ray structures of $Al(salH_2)_3$tach and $Al(NO_2salH_2)_3$-tach have been solved. We decided to solve the two crystal structures in order to be certain that the large difference in the partition coefficients between $Al(salH_2)_3$tach and $Al(NO_2salH_2)_3$-tach is not due to any major structural difference between the two complexes. Both complexes crystallize in a monoclinic $P2_1/n$ space group. The selected bond distances and angles collected in Table 9 show only small differences between the complexes. The *trans* N–Al–O angles are only slightly less than 180°, and the N–Al–N angles are slightly less than 90°. For $Al(salH_2)_3$tach the aluminum–nitrogen bond distances are 2.120(2), 2.137(2), and 2.104(2) Å for Al–N1, Al–N2, and Al–N3, respectively. For $Al(NO_2salH_2)_3$tach these respective distances are 2.054(3), 2.133(3), and 2.114(3) Å. Although aluminum(III) complexes with such N_3O_3 hexadentate ligands are rare, comparative Al–N distances can be obtained from the

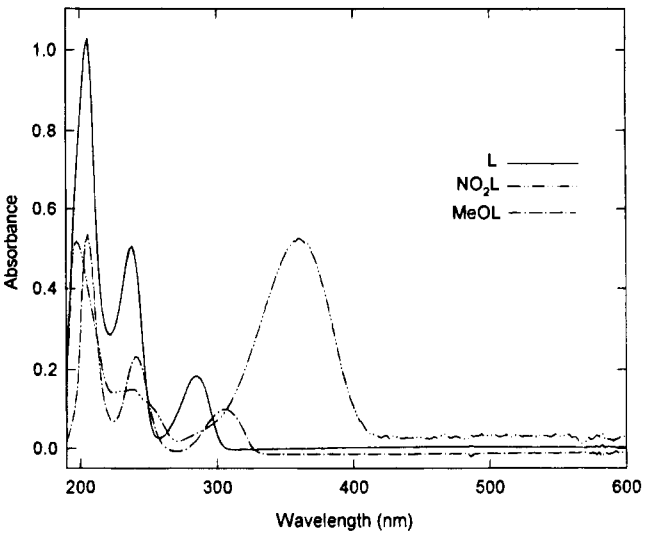


Figure 1. Overlay of the absorption spectra of $Al(RsalH_2)_3tach$ (R = H (L), NO_2 (NO_2L), OMe (MeOL)).

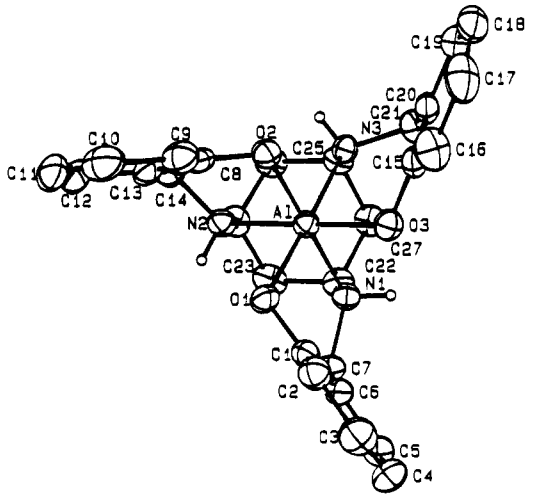


Figure 2. ORTEP representation of $Al(salH_2)_3tach$.

few Schiff-base organoaluminum complexes that have been crystallographically characterized.[45,46] In these SALEN complexes the Al−N distances are very close to 2.0 Å, which makes these distances in our complexes somewhat longer than anticipated.[47] For $Al(salH_2)_3tach$ the aluminum−oxygen bond distances are 1.852(2), 1.821(2), and 1.849(2) Å for Al−O1, Al−O2, and Al−O3, respectively. For $Al(NO_2salH_2)_3tach$ these respective distances are 1.853(2), 1.842(2), and 1.828(2) Å. These distances are very close to those obtained with the aluminum SALEN complexes. These structural comparisons suggest that the tridentate N_3 chelate group is slightly larger than the optimal size for coordinating the aluminum ion. This is supported by the slightly elongated Al−N bonds and the N−Al−N angles averaging to less than 90° in both structures. ORTEP representations of the molecules are shown in Figures 2 and 3. The structure of $Al(salH_2)_3tach$ has the aluminum coordinated in an octahedral environment with the ligand acting as a hexadentate. This configuration results in a network of skewed six-membered rings with each oxygen moiety being *trans* to the amine of a neighboring pendant arm. The structure

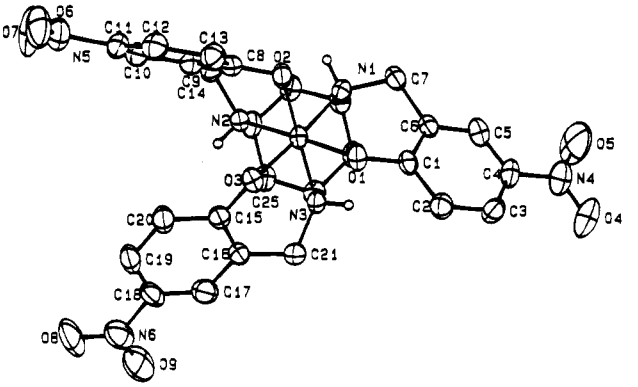


Figure 3. ORTEP representation of $Al(NO_2salH_2)_3tach$.

has a "pinwheel" configuration of approximately C_3 symmetry. By contrast, the structure of $Al(NO_2salH_2)_3tach$ has considerably less symmetry. For this structure, two of the pendants assume a propeller-like conformation while the third does not, reflecting an inversion of one six-membered ring as compared to the other two. Each crystal incorporates solvent in the lattice. The waters or ethanol weakly hydrogen-bond to one of the coordinated oxygens, and no inner sphere coordination to the aluminum center occurs. The observation of equivalence in the three pendants but two separate resonances for the individual pendant methylene hydrogens H4 and H5 in the ^1H NMR spectrum argues in favor of these structures being semirigid.

The partition coefficients for the distribution of $Al(RsalH_2)_3$-tach (R = H, NO_2, OMe) between 1-octanol have values of 33.0, 9.0, and 4.4 for the derivatives with R = NO_2 and OMe, respectively. Such values are representative of highly lipophilic aluminum(III) complexes.[32]

From these data on the complexes, it is clear that we have available a group of uncharged lipophilic aluminum(III) complexes that have significant solubility and stability in aqueous solution. This raises the possibility that these ligand systems could potentially affect the biological actions of aluminum. One of the most profound and easily measurable biological effects of aluminum is its action on the peptide transport system (PTS-1). Inhibition by aluminum of PTS-1, responsible for the saturable component of the brain to blood transport of Tyr-MIF-1 and methionine enkephalin,[48] has been well characterized.[49] Although the mechanism by which this inhibition occurs has not been elucidated, it is well established that in a dose-dependent manner aluminum blocks PTS-1 as assessed with I-Tyr-MIF-1. PTS-1 is almost completely blocked at the 100 mg/kg dose of aluminum, a dose that increases blood levels about 10-fold over naturally occurring levels and duplicates levels seen in disease states such as dialysis dementia.[41] To study these effects, Tyr-MIF-1 radiolabeled with ^{125}I at the tyrosine residue is injected directly through the cortex into the cerebral lateral ventricle of an anesthetized mouse. After a specific time interval, the mouse is decapitated and the entire brain removed. The radioactivity remaining in the brain is counted in a γ-counter and has been shown to represent I-Tyr-MIF-1 not transported out of the brain.

The effect of aluminum on the transport system over the 20 min time period is determined by the inclusion of a 0.2 mL i.p. injection of 89 mg/mL of aluminum chloride hexahydrate in normal saline. It has been previously shown that the most dramatic effect of aluminum upon the transport of Tyr-MIF-1 occurs 60−90 min after i.p. injection in experiments involving a 10 min incubation period.[49] This general time frame has been

(45) Dzugan, S. J.; Goedken, V. L. *Inorg. Chem.* **1986**, *25*, 2858.
(46) Gurian, P. L.; Cheatham, L. K.; Ziller, J. W.; Barron, A. R. *J. Chem. Soc., Dalton Trans.* **1991**, 1449.
(47) Although the nitrogen donor groups in the SALEN ligand are not entirely analogous to those in our ligand system, our conclusion is justified on the basis that the imine nitrogens in the SALEN ligand should be less basic, and therefore on acid−base considerations alone, the Al−N bonds should be shorter and not longer in our complexes.
(48) Banks, W. A.; Kastin, A. J. *Am. J. Physiol.* **1990**, *259*, E1−E10.
(49) Banks, W. A.; Kastin, A. J.; Fasold, M. B. *J. Pharmacol. Exp. Ther.* **1988**, *244*, 579.

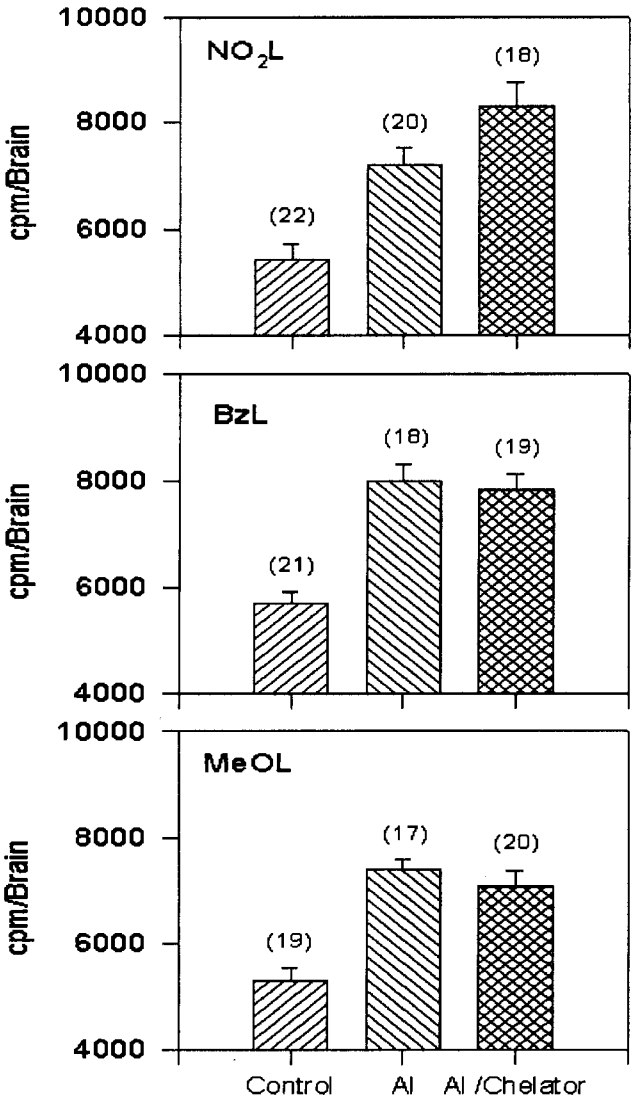


Figure 4. Bar graph for the *in vivo* studies: NO_2L = $(NO_2salH_2)_3tachH_3$; BzL = $(salH_2)_3tachH_3$; MeOL = $(MeOsalH_2)_3tachH_3$. Range test analysis for the groups (1) control, (2) Al control, (3) Al/chelate and the three ligands are as follows. NO_2L: (1)−(2) = 0.001, (2)-(3) = 0.035, (1)−(3) = 0.000. BzL: (1)−(2) = 0.000, (2)−(3) = 0.651, (1)−(3) = 0.000. MeOL: (1)−(2) = 0.000, (2)−(3) = 0.366, (1)−(3) = 0.000. The numbers in parentheses indicate number of animals per group.

adhered to for the present experiments such that the entire 20 min incubation period falls between 60 and 90 min after the injection of aluminum. After analysis of the brains from control mice, it has been found that the amount of peptide not transported averages about 5500 cpm/brain. After treatment with aluminum, this increases to about 7400 cpm/brain. In all cases, this represented a statistically significant increase (see Figure 4). However, this increase is less than that typically seen and suggests that inhibition of PTS-1 by aluminum in the current study is less than maximal.

To examine the effect of chelating agents on the effect of aluminum, 0.06 M chelating agent has been included in the s.c. injection of 5% DMSO/saline. This dose is calculated to achieve a plasma concentration 10 times that of aluminum.[41] Figure 4 shows the mean total amount of remaining activity in the brains of each experimental group in cpm/brain, with standard errors and number of animals per group. The results show that for all three groups there is a marked increase in remaining counts from the saline control to the aluminum control, as expected. ANOVA shows that, in all cases, statistically significant differences occur among the experimental groups: $(NO_2salH_2)_3tachH_3$, $F(2,57) = 16.0$, $p < 0.001$; $(salH_2)_3tachH_3$, $F(2,56) = 20.8$, $p < 0.001$; $(MeOsalH_2)_3tachH_3$, $F(2,54) = 21.5$, $p < 0.001$. The range test showed that, in all groups, treatment with aluminum in the absence of chelator significantly ($p < 0.05$) increased the amount of radioactivity retained by the brain, indicating inhibition of PTS-1. In the case of $(NO_2salH_2)_3tachH_3$, but not $(salH_2)_3tachH_3$ or $(MeOsalH_2)_3tachH_3$, the chelator significantly ($p < 0.05$) enhances the effect of aluminum. None of the chelators are observed to significantly decrease the inhibitory effect of aluminum. To examine whether these observations can reflect an independent action of the chelating agent, an experiment has been performed with a s.c. injection of $(NO_2salH)_3tachH_3$ without the i.p. injection of aluminum. The results of this experiment show no significant differences between this experimental group and the control.

These results suggest that the $(NO_2salH_2)_3tachH_3$ chelator is capable of binding aluminum from the circulation and that the resulting complex may enhance delivery of biologically active aluminum to its site of action. Because it is not known whether the effect of aluminum occurs in the blood or brain side of the BBB, speculation on the mechanism of its enhancement by this ligand is difficult. The nature of the interaction of metal and $(NO_2salH_2)_3tachH_3$ *in vivo* is also speculative. The active $(NO_2salH_2)_3tachH_3$ aluminum complex has an octanol:water partition coefficient that is intermediate between those of the other two complexes with the inactive ligands. The compound also has a much lower solubility. It is possible, as a result, that this ligand aids in the delivery of aluminum through or into the membranes composing the BBB and does so more efficiently or rapidly than its less lipophilic analogs. This model requires that the complex itself be biologically active or that, after such delivery, the aluminum is released into its active form.[50] Because of the high molecular weight of $(NO_2salH_2)_3tachH_3$ and $Al(NO_2salH_2)_3tach$, it does not appear likely that either molecule will cross the BBB.[51]

Thus the results show that one of the new chelators ($(NO_2salH_2)_3tachH_3$) significantly affects the action of aluminum on the PTS-1 transport system, although neither the structural nor the solution data pinpoint the precise reason for its observed activity. Although $(NO_2salH_2)_3tachH_3$ does not appear to be appropriate for use as an aluminum-sequestering agent to counter the toxic properties of this metal, it may prove useful in the study of these effects on the BBB.

Acknowledgment. We thank the Center for Bioenvironmental Research and the Veterans Affairs Medical Center for financial support.

Supplementary Material Available: Tables of anisotropic and general displacement parameters, bond distances, and bond angles (13 pages). Ordering information is given on any current masthead page.

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(50) The formation of charged species other than simple 1:1 complexes cannot be entirely ruled out in these experiments. Because any discrete multiligated metal complex would have a resultant charge, these species would not, however, be expected to readily cross membrane barriers. They might in some way enhance the presence of the active aluminum at the site of action and act to prevent clearance of aluminum by the kidney from the bloodstream, thereby effectively enhancing blood levels of the metal. It is unlikely that the nitrated ligand, although observed to form apparent cross-linked gels in vitro, is combining with aluminum to form a physical barrier to peptide migration.

(51) Green, M. A.; Welch, M. J.; Mathias, C. J.; Fox, K. A. A.; Knabb, R. M.; Huffman, J. C. *J. Nucl. Med.* **1985**, *26*, 170.