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Synthesis of 1,3,5-*cis*,*cis*-Triaminocyclohexane *N*-Pyridyl Derivatives as Potential Antitumor Agents

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Received July 25, 2002

Iron deprivation has been previously proven to be a promising strategy in treating tumor cells. A series of *cis*,*cis*-1,3,5-triaminocyclohexane *N*-pyridyl derivatives as iron-depleting antitumor agents were prepared. Cytotoxic activity of these derivatives was evaluated in the HeLa cancer cell line. Among the tested derivatives, *N*-ethyl-*N*,*N*′,*N*″-tris(2-pyridylmethyl)-*cis*,*cis*-1,3,5-triaminocyclohexane (**17**) exhibited potent cytotoxicity against this cancer cell line. On the basis of the structure of **17**, a bifunctional iron chelator **24** was designed and prepared. Bifunctional agent **24** possessing a maleimide linker that is functional for conjugation to thiolated monoclonal antibodies is a promising lead compound for development of antitumor conjugates for antibody-targeted therapies.

Introduction

Iron deprivation has been developed as a therapeutic strategy of malignant tumor cells.[1] In this tumor therapy, iron chelators,[2] gallium nitrate,[3] or monoclonal antibodies (mAbs) against the transferrin receptor[4] have been employed to produce cellular iron depletion. Numerous studies have shown that these iron depletion agents exhibit antiproliferative activity and induce apoptosis in tumor cells.[1] In particular, iron chelators such as desferrioxamine[2a] and pyridoxyl isonicotonyl hydrazide derivatives[2b] are known to possess potent efficacy in treatment of cancer cells including hepatoma xenografts, leukemia, neuroblastoma, and lymphoma. Recently, we have prepared *N*,*N*′,*N*″-tris(2-pyridylmethyl)-*cis*,*cis*-1,3,5-triaminocyclohexane (tachpyr, **1)** as a novel iron chelator and investigated its potential as an anti-cancer drug.[5] Cell culture experiments have shown that **1** exhibits significant antiproliferative activity and induces a cytotoxic effect by apoptotic cell death.[6a,b] Moreover, tachpyr has been shown to be indifferent to activation of the tumor suppressor *p*53 for its antiproliferative activity or cytotoxic effect.[6b] This finding is of significant importance in that tumor cell lines without *p*53 are substantially less responsive to numerous chemotherapeutic agents than tumor cell lines with *p*53, which is true of over 50% of human cancers.[7] Therefore, **1** possesses great promise not only as a single antitumor agent, but for use in combination with *p*53-targeted antibodies or other chemotherapeutic agents.

This potential of **1** has led us to design tachpyr analogues **7**, **11**, and **17** and to investigate their ability to act as cytotoxic agents. Tachpyr, being a small molecule, has a limited biological half-life. Accordingly, we sought to offset this limitation by creating a bifunctional tachpyr analogue that could be linked to other molecules or proteins, potentially monoclonal antibodies, specifically to produce a conjugate that might then be selectively targeted to tumor cells. On the basis of the observed cytotoxicity of the tachpyr analogues described herein, we have proceeded to the design and preparation of the bifunctional tachpyr **24** possessing a maleimide

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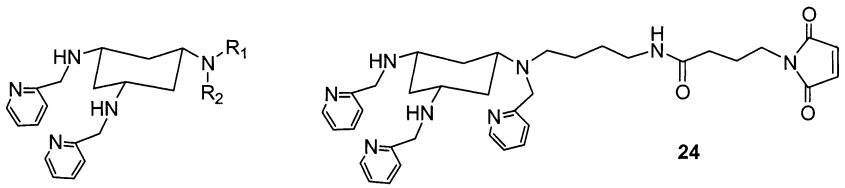
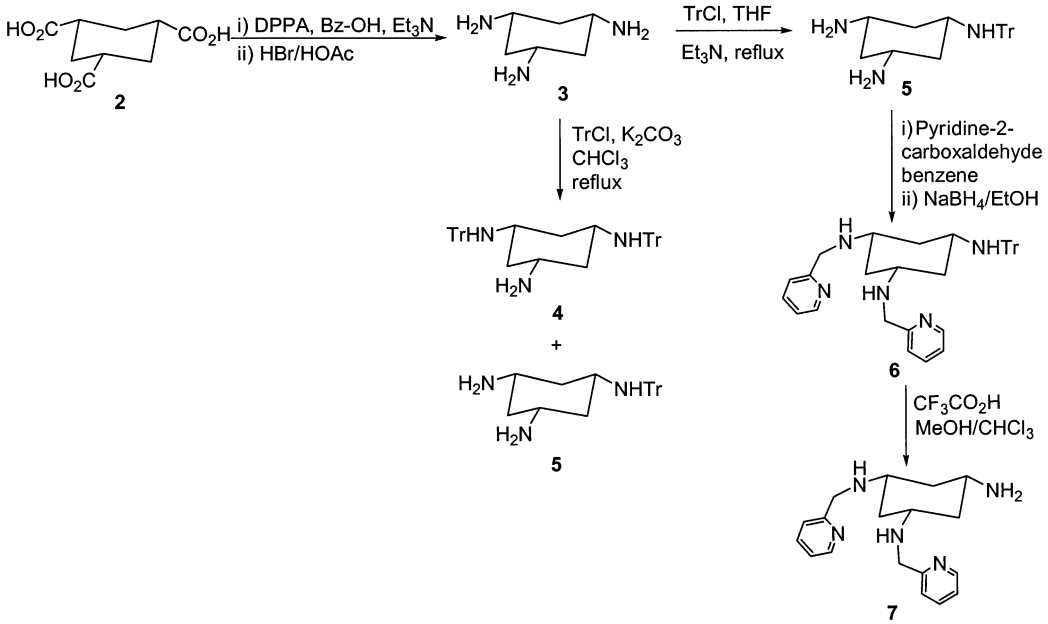
10.1021/jo0204911 CCC: $22.00
Published on Web 10/23/2002

SCHEME 1



1, $R_1 = CH_2Pyr$; $R_2 = H$
7, R_1, $R_2 = H$
11, $R_1 = CH_2CH_2CH_3$; $R_2 = H$
17, $R_1 = CH_2Pyr$; $R_2 = CH_2CH_3$
21, $R_1 = CH_2Pyr$; $R_2 = (CH_2)_4NH_2$

SCHEME 2



moiety. Thus, routine coupling of **24** to thiolated monoclonal antibodies (mAbs) via Michael addition of thiol to maleimide is expected to afford a variety of antitumor conjugates for the targeted delivery of tachpyr cytotoxicity. Herein, we describe the synthesis of the tach *N*-pyridyl derivatives **7**, **11**, and **17** and the bifunctional tachpyr **24** either for use as single antitumor agents or for use in antibody-targeted therapies.

Results and Discussion

As shown in Scheme 1, the tachpyr analogues as iron chelators have three structural features, i.e., the lipophilic cyclohexane ring, the 2-pyridyl unit, and the cyclohexyl-based amines. In preparing the desired tachpyr analogues **7**, **11**, and **17**, selective partial protection of the primary amines in *cis*,*cis*-1,3,5-triaminocyclohexane (tach, **3**) is the key step. We employed the sterically bulky trityl chloride to partially protect either one or two of the primary amines in **3**. The trityl group is an attractive amine protecting group that is known to be selective for primary amines over secondary amines and has the advantages of easy removal and enhanced liphophilicity.[8] Since trityl-protected amines are considerably lipophilic, separation of product(s) can be efficiently achieved via routine silica gel chromatography. Deprotection of tritylated amines in acidic media is also quite straightforward, and the simple workup neatly produces deprotected amines.

As the starting material for trityl protection, tach **3** was prepared from commercially available carboxylic acid **2** according to a procedure published previously (Scheme 2).[5] When an equimolar amount of **3** and trityl chloride was refluxed in chloroform in the presence of K_2CO_3, the reaction provided both diprotected tach **4** and monoprotected tach **5** with yields of 43% and 20%, respectively. Interestingly, reaction of **3** with trityl chloride in the absence of base still provided both **4** and **5** albeit with increased yields of 52% and 28%, respectively. However, when an equimolar amount of **3** and trityl chloride in THF was refluxed in the presence of Et_3N as a base, the reaction selectively provided only monoprotected **5**. Synthesis of bis(2-pyridylmethyl)tach **7** is shown in Scheme 2. Thus, monoprotected **5** was reacted with pyridine-2-carboxaldehyde to afford the corresponding imine, which was subsequently reduced to **6** with $NaBH_4$. Removal of the trityl group in **6** by treatment with CF_3CO_2H afforded **7** in 83% yield. The synthesis of *N*-propylated-*N*′,*N*″-bis-(2-pyridylmethyl)tach **11** is shown in Scheme 3. The *N*,*N*′-bis(trityl)tach **4** was reacted with propionyl chloride to

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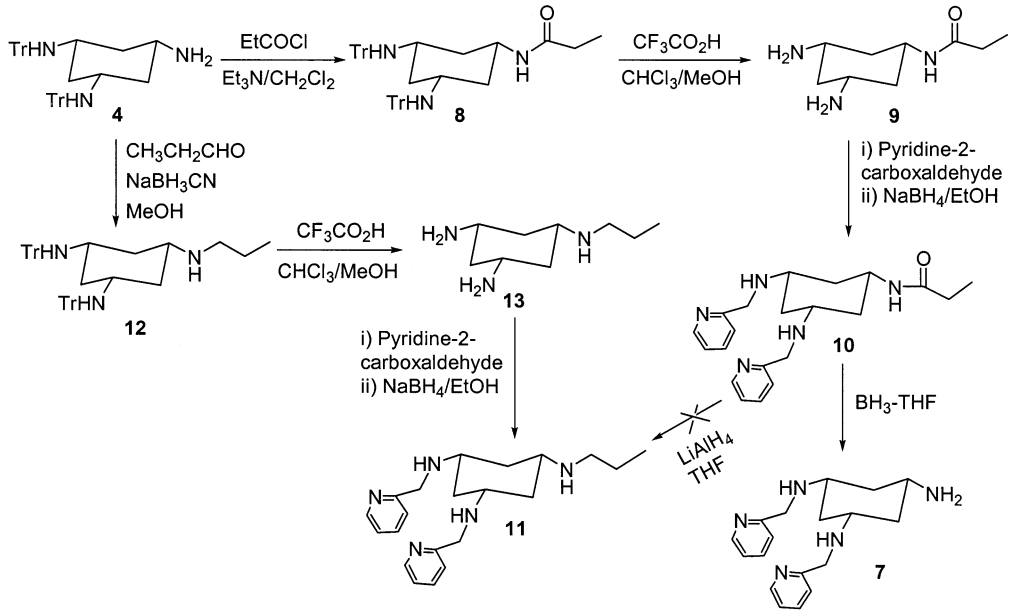
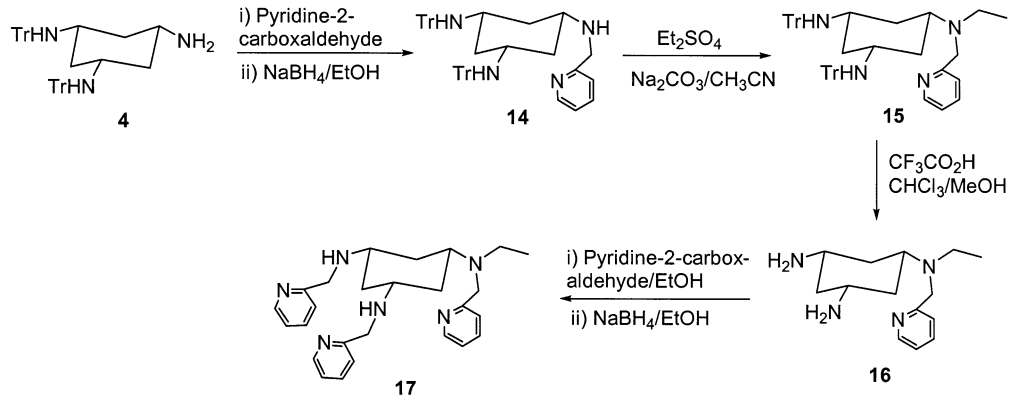
SCHEME 3



SCHEME 4



afford amide **8** in 51% yield. Removal of the trityl groups in **8** followed by reaction of the deprotected amines with pyridine-2-carboxaldehyde and subsequent reduction of the imines with $NaBH_4$ produced **10**. Reduction of **10** with $LiAlH_4$ provided the desired amine **11** along with a mixture of decomposition products as determined by mass spectral analysis. However, purification of **11** via column chromatography turned out to be quite difficult. Attempted reduction of **10** with BH_3 in THF also failed to provide the desired amine **11**. Interestingly, this particular reduction procedure provided only the amide chain cleavage product **7** in 88% yield. To avoid these undesirable complications, **4** was first alkylated to **12** using the modified Borch reductive amination[9] with propionaldehyde and $NaBH_3CN$ and was then deprotected in $CF_3CO_2H/MeOH/CHCl_3$. Condensation of amine **13** with pyridine-2-carboxaldehyde and subsequent reduction with $NaBH_4$ successfully provided **11** in 58% yield. Starting with the key intermediate **4**, we were able to synthesize the tachpyr analogue **17** having an ethyl group (Scheme 4). Condensation of **4** with pyridine-2-carboxaldehyde followed by reduction with $NaBH_4$ provided **14**, which was subsequently reacted with ethyl sulfate in CH_3CN at room temperature to afford **15** in 80% yield. Deprotection of the trityl groups in **15** followed by reaction with pyridine-2-carboxaldehyde and subsequent treatment with $NaBH_4$ provided compound **17** in 86% yield.

Cytotoxicity of the derivatives **7**, **11**, and **17** was evaluated using the HeLa cell line (Figure 1).[10] Among the tested derivatives, both **7** and **11** exhibited somewhat reduced cytotoxicity as compared to tachpyr **1**. Thus, replacement of a pyridyl group in tachpyr **1** by either a propyl group (**11**) or hydrogen (**7**) resulted in a significant decrease in cytotoxicity. However, introduction of an ethyl group (**17**) into tachpyr **1** seemed to have little effect on cytotoxicity. The novel tachpyr analogue **17** exhibited reasonably comparable cytotoxicity to tachpyr **1**. This was a gratifying result in that we were very aware that tachpyr derivatives wherein all three amines alkylated to tertiary amines by either methyl, ethyl, or propyl groups essentially eliminated biological activity.[11] Thus, up until this point it was unclear whether monoderiva-

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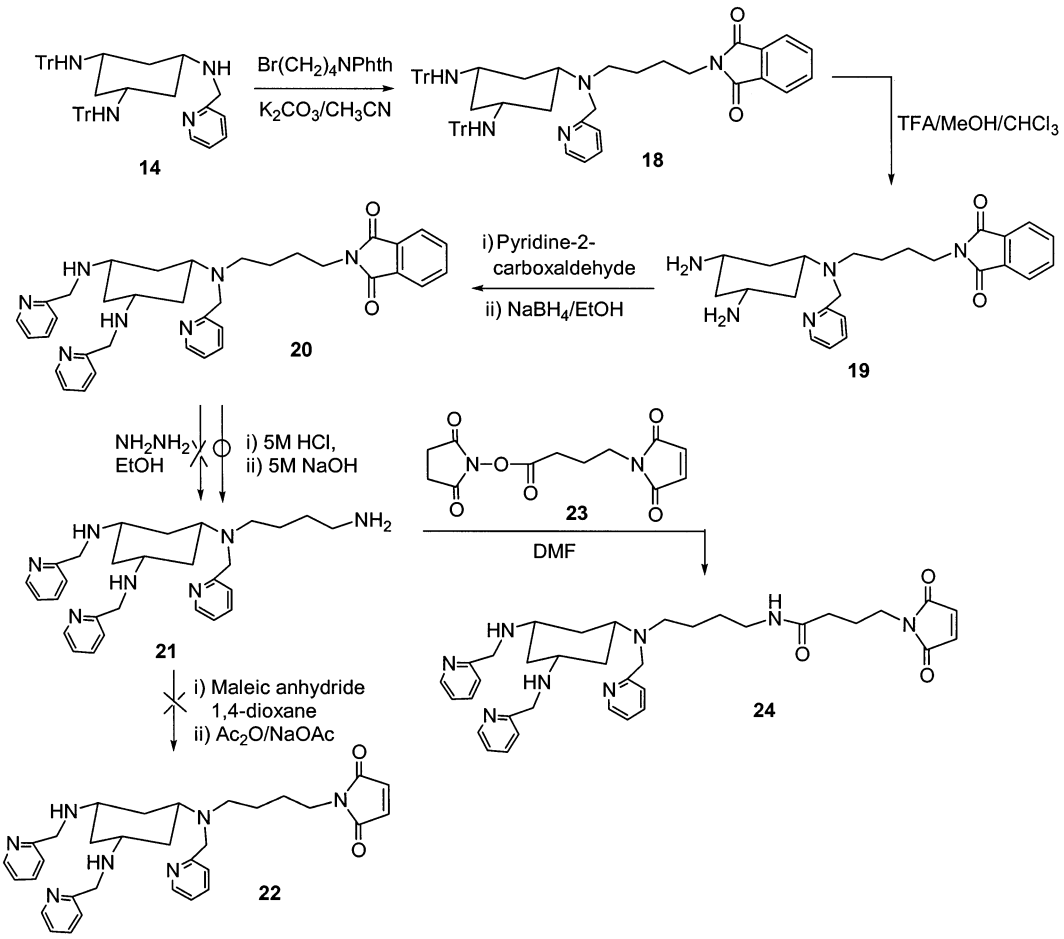
SCHEME 5



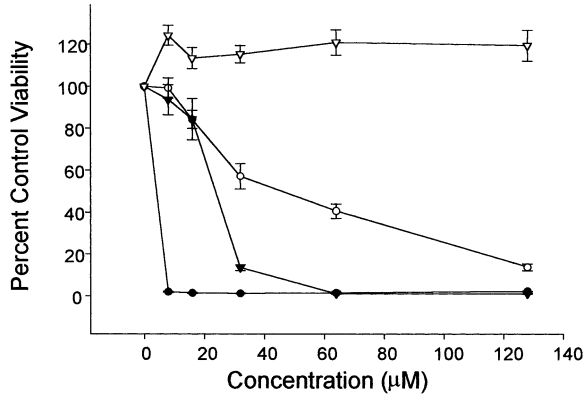


FIGURE 1. Effects of chelators **7** (○), **11** (▽), **17** (▼), and tachpyr **1** (●) on cell viability. Cells were incubated for 72 h with chelators at various concentrations and viability assessed as described in the Experimental Section. Each point represents the mean and standard error of octuplicate cultures.

tization of an amine of tachpyr **1** would in fact be tolerated. The slight decrease in cytotoxicity was felt to be completely acceptable in light of the goal of a selectively targetable analogue, by which such a property one might reasonably expect to then compensate for a mild loss of activity as observed at this point.

The result prompted us to design a bifunctional tachpyr based on the structure of **17**. We were particularly interested in the preparation of a bifunctional tachpyr possessing a pendent maleimide group, which would readily undergo Michael addition with a nucleophilic thiol group. We felt that this group would provide adequate orthogonal nature to the functional groups of tachpyr while permitting reasonably efficient conjugation to proteins. Thus, the bifunctional tachpyr might be conjugated to a variety of thiolated mAbs via linkage of the maleimide and an introduced thiol group to afford antitumor conjugates for targeted therapy.[12] Our strategy in the construction of the desired bifunctional tachpyr **24** was to introduce a side chain having a pendant primary amino group to the tachpyr moiety, which could be converted to an aminoalkylmaleimide. The synthetic strategy for **24** incorporating a maleimide group is outlined in Scheme 5. As a starting material, compound **14** was reacted with 4-bromobutylphthalimide to provide **18** in 81% yield. Deprotection of the trityl groups in **18** and subsequent reactions with pyridine-2-carboxaldehyde and $NaBH_4$ provided **20** in 73% yield. Removal of the phthalimide group in **20** by treating with hydrazine hydrate failed to cleanly provide the deprotected amine **21**. However, hydrolysis of **20** by refluxing in 5M HCl completely removed the phthalimide group providing

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amine **21** in 75% yield. Our initial effort to synthesize *N*-aminoalkylmaleimide **22** by reaction of **21** directly with maleic anhydride and subsequent dehydrative cyclization was unsuccessful. The reaction provided chromatographically inseparable multiple products no doubt due to the presence of the competing secondary amines in **21**. We then tried selective reaction of the primary amine in **21** with the succinimidyl active ester reagent **23**[13] to introduce a formed maleimide group. To our satisfaction, treatment of **21** with **23** at room-temperature resulted in almost complete transformation to the desired product **24** as evidenced by both HPLC and NMR analysis. Purification by silica gel flash column provided the conjugate product **24** in 88% yield.

The synthetic techniques to the tachpyr derivatives (**7**, **11**, **17**, **21**, and **24**) reported herein might be directly applied to preparation of numerous metal chelating agents as potential anti-cancer drugs having substituents other than pyridyl groups that might then lead to extensive structure−activity relationship studies. Additionally, entry to numerous mixed donor chelating agents based on the *cis*,*cis*-1,3,5-triaminocyclohexane platform is now clearly available permitting detailed studies of their fundamental coordination chemistry. Bifunctional tachpyr **24** possesses significant potential for use in targeted therapies. Preparation and biological evaluation of antibody conjugates of **24** are being actively pursued and will be published in due course.

Experimental Section

General Methods. ^{1}H, ^{13}C, and APT NMR spectra were obtained at 300 MHz in $CDCl_3$ solution. Fast atom bombardment mass spectra (FAB-MS) were obtained in the positive-ion detection mode. Elemental microanalysis was performed by Galbraith Laboratories, Knoxville, TN.

***N,N′*-Bis-trityl-*cis,cis*-1,3,5-triaminocyclohexane (4) and *N*-Trityl-*cis,cis*-1,3,5-triaminocyclohexane (5).** To a solution of tach·3HBr (9.30 g, 25.2 mmol)[5] in H_2O (150 mL) was added NaOH (3.02 g, 75 mmol). The resulting solution was concentrated in vacuo, and EtOH (60 mL) was added into the residue. The resulting mixture was stirred for 5 min and put into a freezer for 1 h. The mixture was filtered, and the filtrate was concentrated in vacuo. The residue was dissolved into $CHCl_3$ and filtered, and the filtrate was concentrated in vacuo to provide the free amine **3** (3.0 g, 94%).

Method 1. To a mixture of **3** (1.20 g, 9.3 mmol) and K_2CO_3 (990 mg, 9.3 mmol) in $CHCl_3$ (30 mL) was added a solution of TrCl (2.59 g, 9.3 mmol) in $CHCl_3$ (20 mL) over 0.5 h. The resulting mixture was refluxed for 2 h, at which time the reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was purified via column chromatography on silica gel eluting with 10% $CH_3OH-CH_2Cl_2$. Pure **4** (1.79 g, 43%) was thereby obtained as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.86 (q, 2 H), 1.12−1.20 (m, 4 H), 1.70 (d, J = 4.2 Hz, 2 H), 2.12−2.30 (m, 5 H), 7.22−7.45 (m, 18 H), 7.64 (d, J = 8.4 Hz, 12 H); ^{13}C NMR ($CDCl_3$) δ 42.8 (t), 44.1 (t), 47.7 (d), 49.3 (d), 71.0 (s), 126.1 (d), 127.6 (d), 128.5 (d), 146.8 (s); HRMS (positive-ion FAB) calcd for $C_{42}H_{43}N_3$ [M + H]$^+$ m/z 614.3535, found [M + H]$^+$ m/z 614.3536.

Further elution with $CHCl_3$/MeOH/NH_4OH at 12:4:1 provided pure **5** (690 mg, 20%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.83−1.0 (m, 4 H), 1.42 (d, J = 6.3 Hz, 2 H), 1.86−2.60 (m, 8 H), 7.20−7.39 (m, 9 H), 7.75 (d, J = 7.4 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 44.66 (t), 44.72 (t), 47.72 (d), 49.4 (d), 71.3 (s), 126.2 (d), 127.8 (d), 128.6 (d), 147.0 (s); HRMS (positive-ion FAB) calcd for $C_{33}H_{29}N_3$ [M + H]$^+$ m/z 372.2440, found [M + H]$^+$ m/z 372.2440.

Method 2. To a solution of **3** (1.20 g, 9.3 mmol) in $CHCl_3$ (30 mL) was added a solution of TrCl (2.59 g, 9.3 mmol) in $CHCl_3$ (20 mL) over 1.5 h. The resulting mixture was stirred for 0.5 h and refluxed for 2 h, at which time the reaction mixture was cooled to room temperature and concentrated in vacuo. The same chromatographic purification procedure employed in method 1 provided **4** and **5** in 52% and 28% isolated yields, respectively.

***N*-Trityl-*cis,cis*-1,3,5-triaminocyclohexane (5).** To a solution of **3** (1.20 g, 9.3 mmol) in THF (70 mL) were sequentially added Et_3N (1.43 mL, 10.23 mmol) and TrCl (2.59 g, 9.3 mmol). The resulting mixture was refluxed for 4 h, at which time the reaction mixture was cooled to room temperature and concentrated in vacuo. The same chromatographic purification procedure employed in method 1 provided pure **5** (1.1 g, 32%).

General Procedure for the Reaction of Amines (4, 5, 13, 16, and 19) with Pyridine-2-carboxaldehyde and Subsequent Reduction of Imines with $NaBH_4$. To a solution of amines (1 mmol) in benzene (10 mL) was added pyridine-2-carboxaldehyde (2 mmol). The resulting mixture was refluxed using a Dean−Stark trap for 7 h. The reaction mixture was cooled to room temperature and evaporated to dryness to provide pure imine as determined by NMR. The obtained imine was dissolved in EtOH (15 mL) and reacted with $NaBH_4$ (2 mmol). The resulting mixture was stirred at room temperature for 24 h and filtered, and the filtrate was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (15 mL), filtered, and dried ($MgSO_4$), and the filtrate was concentrated in vacuo.

***N,N′*-Bis(2-pyridylmethyl)-*N″*-trityl-*cis,cis*-1,3,5-triaminocyclohexane (6).** Pure **6** (477 mg, 86%) was thereby obtained as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.64−0.91 (m, 3 H), 1.28 (d, J = 4.8 Hz, 2 H), 1.90 (d, J = 6.1 Hz, 1 H), 2.02−2.43 (m, 6 H), 3.56 (s, 4 H), 6.97−7.15 (m, 13 H), 7.35−7.51 (m, 8 H), 8.38 (d, J = 3.8 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 39.8 (t), 41.9 (t), 49.3 (t), 52.1 (t), 53.8 (t), 71.2 (s), 121.8 (d), 122.2 (d), 126.1 (d), 127.7 (d), 128.6 (d), 136.3 (d), 147.0 (d), 149.1 (d), 159.4 (d); HRMS (positive-ion FAB) calcd for $C_{45}H_{39}N_5$ [M + H]$^+$ m/z 554.3283, found [M + H]$^+$ m/z 554.3312.

***N*-(2-Pyridylmethyl)-*N′,N″*-bis-trityl-*cis,cis*-1,3,5-triaminocyclohexane (14).** Pure **14** (656 mg, 93%) was thereby obtained as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.35−0.58 (m, 6 H), 0.64−0.80 (m, 3 H), 0.88−1.64 (m, 7 H), 1.84−2.08 (m, 4 H), 3.15 (s, 2 H), 6.92 (t, J = 3.8 Hz, 1 H), 7.0−7.20 (m, 20 H), 7.42 (d, J = 8.8 Hz, 12 H), 8.32 (d, J = 3.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 41.4 (t), 45.5 (t), 49.4 (d), 51.8 (t), 53.6 (d), 71.1 (s), 121.6 (d), 121.9 (d), 126.0 (d), 127.9 (d), 128.6 (d), 136.0 (d), 147.0 (s), 149.0 (d), 159.7 (s); HRMS (positive-ion FAB) calcd for $C_{48}H_{48}N_4$ [M + H]$^+$ m/z 705.3957, found [M + H]$^+$ m/z 705.3945.

***N*-Ethyl-*N,N′,N″*-tris(2-pyridylmethyl)-*cis,cis*-1,3,5-triaminocyclohexane (17).** Pure **17** (379 mg, 88%) was thereby obtained as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.03 (t, J = 9.0 MHz, 3 H), 1.17−1.29 (m, 4 H), 2.18 (d, J = 4.5 Hz, 2 H), 2.26 (d, J = 5.8 Hz, 1 H), 2.55−2.70 (m, 6 H), 3.79 (s, 2 H), 3.95 (s, 4 H), 7.10−7.18 (m, 2 H), 7.30 (d, J = 6.7 Hz, 2 H), 7.54 (d, J = 5.4 Hz, 1 H), 7.64 (tt, J = 9.8 Hz, 4 H), 8.49 (d, J = 2.7 Hz, 1 H), 8.55 (d, J = 3.2 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 14.3 (q), 35.6 (t), 40.4 (t), 44.9 (t), 52.5 (t), 54.2 (t), 56.2 (t), 56.4 (t), 121.4 (d), 121.8 (d), 122.2 (d), 136.2 (d), 136.4 (d), 148.6 (d), 149.1 (d), 159.7 (s), 162.0 (s); HRMS (positive-ion FAB) calcd for $C_{26}H_{34}N_6$ M_r^+ m/z 431.2923, found M_r^+ m/z 431.2932.

***N,N′*-Bis(2-pyridylmethyl)-*N″*-propyl-*cis,cis*-1,3,5-triaminocyclohexane (11).** The residue was purified via flash column chromatograpy eluting with triethylamine, methanol, and CH_2Cl_2 at 3:15:100 to provide **11** (205 mg, 58%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 9.2 Hz, 3 H), 0.98−1.16 (m, 4 H), 1.38−1.57 (m, 2 H), 2.20−2.28 (m, 4 H), 2.50−2.61 (m, 8 H), 3.91 (s, 4 H), 7.12 (t, J = 6.0 Hz, 2 H), 7.26 (d,

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J = 7.5 Hz, 2 H), 7.61 (t, J = 8.2 Hz, 2 H), 8.51 (d, J = 6.0 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 11.7 (q), 23.1 (t), 39.8 (t), 40.2 (t), 48.6 (t), 52.4 (t), 53.7 (d), 53.8 (d), 121.8 (d), 122.3 (d), 136.4 (d), 149.2 (d), 159.7 (s); HRMS (positive-ion FAB) calcd for $C_{33}H_{31}N_5$ [M + H]$^+$ m/z 354.2657, found [M + H]$^+$ m/z 354.2658.

***N*-(4-Phthaloylbutyl)-*N*,*N*′,*N*″-tris(2-pyridylmethyl)-*cis*,*cis*-1,3,5-triaminocyclohexane (20).** Pure **20** (537 mg, 89%) was thereby obtained as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.95−1.50 (m, 6 H), 1.80−2.04 (3 H), 2.20−2.42 (m, 6 H), 3.08 (t, J = 7.1 Hz, 2 H), 3.58 (s, 2 H), 3.75 (s, 4 H), 4.40 (s, 2 H), 6.90−7.50 (m, 13 H), 8.19 (d, J = 4.6 Hz, 2 H), 8.32 (d, J = 5.3 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 25.6 (t), 26.8 (t), 35.1 (t), 39.3 (t), 49.9 (t), 51.8 (t), 54.0 (t), 53.4 (d), 56.3 (d), 63.3 (t), 121.4 (d), 121.6 (d), 122.0 (d), 122.2 (d), 129.8 (d), 130.1 (d), 135.7 (s), 136.2 (d), 136.3 (d), 138.6 (s), 148.1 (d), 148.7 (d), 159.1 (s), 161.0 (s), 169.0 (s); HRMS (positive-ion FAB) calcd for $C_{36}H_{41}N_7O_2$ [M + H]$^+$ m/z 604.3486, found [M + H]$^+$ m/z 604.3477.

General Procedure for Deprotection of Trityl Groups in 6, 15, and 18. CF_3CO_2H (2 mL) was slowly added to a mixture of **6**, **15**, or **18** (1 mmol) in $CHCl_3$ (1 mL) and CH_3OH (1 mL) at −5 °C. The resulting mixture was warmed to room temperature and stirred for 48 h, at which time the mixture was evaporated to dryness. H_2O (10 mL) was added into the residue, and the resulting mixture was extracted with $CHCl_3$ (2 × 30 mL) to remove the triphenylmethane. The aqueous solution was adjusted to pH 7 with 5 M NaOH, washed with $CHCl_3$ (10 mL), adjusted to pH 13, and extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo.

***N*,*N*′-Bis(2-pyridylmethyl)-*cis*,*cis*-1,3,5-triaminocyclohexane (7).** Pure 7 (259 mg, 83%) was thereby obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.95−1.20 (m, 6 H), 1.92 (s, 2 H), 2.02−2.20 (m, 3 H), 2.50−2.78 (m, 2 H), 3.87 (s, 4 H), 7.08 (t, J = 6.0 Hz, 2 H), 7.24 (t, J = 7.6 Hz, 2 H), 7.54 (t, J = 7.6 Hz, 2 H), 8.48 (d, J = 6.0 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 39.8 (t), 43.5 (t), 47.6 (d), 52.3 (t), 53.6 (d), 121.7 (d), 122.2 (d), 136.3 (d), 149.1 (d), 159.6 (s); HRMS (positive-ion FAB) calcd for $C_{18}H_{25}N_5$ [M + H]$^+$ m/z 312.2188, found [M + H]$^+$ m/z 312.2179.

***N*-Ethyl-*N*,*N*′*N*″-tris(2-pyridylmethyl)-*cis*,*cis*-1,3,5-triaminocyclohexane (16).** Pure **16** (209 mg, 84%) was thereby obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.85−1.13 (m, 8 H), 1.97 (d, J = 6.6 Hz, 4 H), 2.54−2.77 (m, 6 H), 3.77 (s, 2 H), 7.12 (t, J = 6.3 Hz, 1 H), 7.55 (d, J = 3.8 Hz, 1 H), 7.64 (t, J = 6.6 Hz, 1 H), 8.49 (d, J = 2.5 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.2 (q), 38.5 (t), 44.9 (t), 46.8 (t), 48.1 (d), 56.1 (t), 56.4 (d), 121.5 (d), 122.2 (d), 136.2 (d), 148.6 (d), 162.0 (s); HRMS (positive-ion FAB) calcd for $C_{14}H_{24}N_4$ [M + H]$^+$ m/z 249.2079, found [M + H]$^+$ m/z 249.2076.

***N*-(4-Phthaloylbutyl)-*N*-(2-pyridylmethyl)-*cis*,*cis*-1,3,5-triaminocyclohexane (19).** Pure **19** (236 mg, 56%) was thereby obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.58 (dd, J = 10.3 Hz, 1 H), 0.79 (dd, J = 10.3 Hz, 2 H), 1.0−1.18 (m, 5 H), 1.32−1.42 (m, 2 H), 1.65 (d, J = 11.3 Hz, 3 H), 2.20−2.43 (m, 5 H), 3.35 (t, J = 7.2 Hz, 2 H), 3.52 (s, 2 H), 6.75 (t, J = 3.1 Hz, 1 H), 7.23 (t, J = 7.2 Hz), 7.25−7.43 (m, 3 H), 7.52 (d, J = 9.3 Hz, 2 H), 8.15 (d, J = 4.1 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.6 (t), 25.8 (t), 37.4 (t), 37.7 (t), 45.9 (t), 47.7 (d), 49.7 (t), 56.2 (d), 56.4 (t), 121.2 (d), 121.9 (d), 122.7 (d), 131.6 (s), 133.4 (d), 136.1 (d), 148.1 (d), 161.3 (s), 167.9 (s); HRMS (positive-ion FAB) calcd for $C_{24}H_{31}N_5O_2$ [M + H]$^+$ m/z 422.2556, found [M + H]$^+$ m/z 422.2549.

General Procedure for Deprotection of Trityl Groups in Compounds 8 and 12. CF_3CO_2H (4 mL) was slowly added to a mixture of **8** or **12** (2 mmol) in $CHCl_3$ (2 mL) and CH_3OH (2 mL) at −5 °C. The resulting mixture was warmed to room temperature and stirred for 48 h, at which time the mixture was evaporated to dryness. H_2O (10 mL) was added to the residue, and the resulting mixture was extracted with $CHCl_3$ (2 × 30 mL) to remove the triphenylmethane. The aqueous solution was adjusted to pH 7 with 5 M NaOH, washed with $CHCl_3$ (10 mL), and adjusted to pH 13 with 5 M NaOH. The solvent was evaporated under high vacuum, and the residue was dissolved in ethanol (20 mL), sonicated for 10 min, and concentrated in vacuo. The residue was dissolved in $CHCl_3$ (150 mL), dried ($MgSO_4$), and filtered, and the filtrate was concentrated in vacuo.

***N*,*N*′-Bis(2-pyridyl)-*N*″-propionyl-*cis*,*cis*-1,3,5-triaminocyclohexane (9).** Pure **9** (297 mg, 80%) was thereby obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.95 (dd, J = 10.8 Hz, 4 H), 1.15 (t, J = 8.4 Hz, 3 H), 1.56 (s, 4 H), 1.97−2.28 (m, 4 H), 2.78−2.96 (m, 2 H), 3.90−4.00 (m, 1 H), 5.88 (d, J = 4.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 9.78 (q), 29.6 (t), 42.2 (t), 43.9 (t), 45.1 (t), 45.4 (d), 47.2 (t), 172.7 (s); HRMS (positive-ion FAB) calcd for $C_9H_{19}N_3O$ [M + H]$^+$ m/z 186.1606, found [M + H]$^+$ m/z 186.1602.

***N*-Propyl-*cis*,*cis*-1,3,5-triaminocyclohexane (13).** Pure **13** (303 mg, 88%) was thereby obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.62−0.80 (m, 5 H), 0.82−1.42 (m, 8 H), 1.90 (t, J = 12.0 Hz, 3 H), 2.36−2.52 (m, 3 H), 2.55−2.72 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 11.7 (q), 23.4 (t), 43.3 (t), 47.0 (t), 47.7 (d), 49.0 (t), 53.9 (d); HRMS (positive-ion FAB) calcd for $C_9H_{21}N_3$ [M + H]$^+$ m/z 172.1814, found [M + H]$^+$ m/z 172.1812

***N*-Propionyl-*N*′,*N*″-bistrityl-*cis*,*cis*-1,3,5-triaminocyclohexane (8).** To a solution of **4** (1.76 g, 2.88 mmol) and Et_3N (0.4 mL, 2.88 mmol) in CH_2Cl_2 (30 mL) at −20 °C was slowly added propionyl chloride (0.25 mL, 2.88 mL). The resulting mixture was stirred for 10 min at the same temperature and purified on a silica gel column sequentially eluting with hexane and 20% EtOAc in hexane. Pure **8** (980 mg, 51%) was thereby obtained as colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.76 (dd, J = 10.4 Hz, 4 H), 0.91−1.50 (m, 7 H), 1.78−2.30 (m, 6 H), 7.24−7.41 (m, 18 H), 7.53 (d, J = 8.4 Hz, 12 H); ^{13}C NMR ($CDCl_3$) δ 10.1 (q), 30.0 (t), 41.3 (t), 43.9 (t), 45.5 (d), 49.4 (d), 71.2 (s), 126.3 (d), 127.9 (d), 128.7 (d), 147.0 (s), 172.2 (s). Anal. Calcd for $C_{47}H_{49}N_3O$: C, 84.02; H, 7.35. Found: C, 84.18; H, 7.23.

***N*,*N*′-Bis(2-pyridylmethyl)-*N*″-propionyl-*cis*,*cis*-1,3,5-triaminocyclohexane (10).** To a solution of **9** (223 mg, 1.21 mmol) in EtOH (20 mL) was added pyridine-2-carboxaldehyde (258 mg, 2.42 mmol). The resulting mixture was refluxed for 4 h. The reaction mixture was cooled to room temperature and evaporated to dryness to provide pure imine as determined by NMR. The residue was dissolved in EtOH (20 mL) and was reacted with $NaBH_4$ (183 mg, 4.84 mmol). The resulting mixture was stirred at room temperature for 24 h and filtered, and the filtrate was evaporated to dryness. The residue was dissolved in CH_2Cl_2 (10 mL), dried ($MgSO_4$), and filtered. The filtrate was concentrated in vacuo to provide compound **10** (415 mg, 93%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.88−1.02 (m, 7 H), 1.65−1.82 (m, 2 H), 1.97−2.10 (m, 5 H), 2.43−2.55 (m, 2 H), 3.75 (s, 4 H), 6.12 (d, J = 4.2 Hz, 1 H), 6.98 (t, J = 5.6 Hz, 1 H), 7.10 (d, J = 4.5 Hz, 1 H), 7.46 (t, J = 7.5 Hz, 1 H), 8.36 (d, J = 3.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 9.6 (q), 29.3 (t), 39.3 (t), 39.5 (t), 44.9 (d), 51.9 (t), 53.0 (d), 121.6 (d), 121.9 (d), 136.1 (d), 148.8 (d), 159.1 (s), 172.7 (s). HRMS (positive-ion FAB) calcd for $C_{20}H_{29}N_5O$ [M + H]$^+$ m/z 368.2450, found [M + H]$^+$ m/z 368.2447.

$LiAlH_4$ Reduction of 10. To a solution of **10** (210 mg, 0.57 mmol) in THF (6 mL) at 0 °C was added $LiAlH_4$ (43 mg, 1.13 mmol). The resulting mixture was stirred for 0.5 h and warmed to room temperature. The resulting mixture was refluxed for 18 h and quenched with MeOH (1 mL) at 0 °C. The resulting mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified via column chromatography on silica gel eluting with Et_3N/MeOH/CH_2Cl_2 at 3:15:100 to provide pure **11** (12 mg, 6%) as a slightly brown oil.

Borane Reduction of 10. To a solution of **10** (190 mg, 0.52 mmol) in THF (5 mL) at 0 °C was added 1 M BH_3−THF (1 mL, 1.04 mmol) over 20 min. The resulting mixture was stirred for 2 h at 0 °C and warmed to room temperature. The resulting mixture was refluxed for 18 h, cooled to room temperature, and evaporated to dryness. HCl (6 M, 1 mL) was added into

the residue, and the resulting solution was refluxed for 1 h and evaporated to dryness. NaOH (5 M) was added to the mixture adjusting pH to 13. The resulting solution was evaporated, and the residue was extracted with $CHCl_3$ (2 × 30 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to provide **7** (140 mg, 88%) as a colorless oil.

***N*-Propyl-*N′*,*N″*-bis-trityl-*cis*,*cis*-1,3,5-triaminocyclohexane (12).** To a solution of **4** (612 mg, 1 mmol) in MeOH (25 mL) at room temperature were added propionaldehyde (46 mg, 0.8 mmol) and $NaBH_3CN$ (63 mg, 1 mmol). The resulting mixture was stirred for 14 h. The mixture was dissolved into water (75 mL), the pH was adjusted to pH 10 with 0.5 N NaOH, and the solution was extracted with $CHCl_3$ (2 × 100 mL). The combined organic layers was dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified via silica gel column chromatograpy eluting with 3% MeOH in CH_2Cl_2 to provide **12** (438 mg, 68%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.46−0.60 (m, 2 H), 0.81−0.91 (m, 4 H), 1.15−1.56 (m, 6 H), 1.74 (t, J = 10.7 Hz, 1 H), 2.00−2.38 (m, 6 H), 7.24−7.38 (m, 18 H), 7.62 (d, J = 8.6 Hz, 12 H); ^{13}C NMR ($CDCl_3$) δ 11.5 (q), 22.5 (t), 41.2 (t), 45.5 (t), 48.4 (t), 49.5 (d), 53.8 (d), 71.2 (s), 126.1 (d), 127.7 (d), 128.6 (d), 147.1 (s); HRMS (positive-ion FAB) calcd for $C_{47}H_{49}N_3$ [M + H]$^+$ m/z 656.4037, found [M + H]$^+$ m/z 656.4005.

***N*-Ethyl-*N*-(2-pyridylmethyl)-*N′*,*N″*-bistrityl-*cis*,*cis*-1,3,5-triaminocyclohexane (15).** To a mixture of **14** (170 mg, 0.24 mmol) and Na_2CO_3 (26 mg, 0.24 mmol) in CH_3CN (2 mL) was added diethyl sulfate (37 mg, 0.24 mmol). The resulting mixture was stirred at room temperature for 24 h and filtered, and the filtrate was evaporated in vacuo. The residue was purified on basic alumina eluting with 15% EtOAc−hexane and 25% EtOAc−hexane to provide **15** (240 mg, 80%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.35−0.60 (m, 5 H), 0.81−1.04 (m, 3 H), 1.15−1.63 (m, 4 H), 1.80−2.10 (m, 4 H), 3.08 (s, 2 H), 6.95 (t, J = 5.4 Hz, 1 H), 7.03−7.18 (m, 20 H), 7.42 (d, J = 8.8 Hz, 12 H), 8.30 (d, J = 3.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 13.9 (q), 37.4 (t), 44.7 (t), 46.1 (t), 50.1 (t), 55.6 (d), 56.2 (d), 71.2 (s), 121.2 (d), 122.1 (d), 126.2 (d), 127.7 (d), 128.6 (d), 136.0 (d), 147.2 (s), 148.4 (s), 162.5 (s); positive-ion FAB calcd for $C_{52}H_{52}N_4$ [M + H]$^+$ m/z 733.01, found [M + H]$^+$ m/z 733.42.

***N*-(4-Phthaloylbutyl)-*N*,*N′*,*N″*-tris(2-pyridylmethyl)-*cis*,*cis*-1,3,5-triaminocyclohexane (18).** To a solution of **14** (2.03 g, 2.88 mmol) in CH_3CN (10 mL) were added K_2CO_3 (796 mg, 5.76 mmol) and *N*-(4-bromobutyl)phthalimide (813 mg, 2.88 mmol). The resulting mixture was refluxed for 36 h, cooled to room temperature, and filtered. The filtrate was concentrated in vacuo, and the residue was purified on silica gel column sequentially eluting with 20% EtOAc−hexane and 50% hexanes−EtOAc to provide **18** (2.11 g, 81%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.03−1.75 (m, 6 H), 1.90−2.25 (m, 3 H), 3.22 (s, 2 H), 3.50 (t, J = 3.6, 2 H), 4.01−4.14 (m, 2 H), 7.03−7.28 (m, 40 H), 7.50−7.78 (m, 38 H), 8.38 (d, J = 3.6 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.6 (t), 25.9 (t), 36.8 (t), 37.7 (t), 46.3 (t), 49.5 (t), 50.0 (d), 56.2 (t), 56.5 (d), 71.2 (t), 121.2 (d), 121.9 (d), 123.1 (d), 126.2 (d), 127.7 (d), 128.6 (d), 132.0 (s), 133.8 (d), 136.1 (d), 147.1 (s), 148.3 (d), 162.1 (s), 168.2 (s); HRMS (positive-ion FAB) calcd for $C_{60}H_{59}N_5O_2$ [M + H]$^+$ m/z 904.4951, found [M + H]$^+$ m/z 904.4566.

***N*-(4-Aminobutyl)-*N*,*N′*,*N″*-tris(2-pyridylmethyl)-*cis*,*cis*-1,3,5-triaminocyclohexane (21).** Compound **20** (350 mg, 0.58 mmol) was dissolved in 5 M HCl (5 mL), and the resulting mixture was refluxed for 5 h, cooled to 5 °C, and filtered. The filtrate was adjusted to pH 7 with 5 M NaOH and washed with $CHCl_3$ (10 mL). The aqueous solution was adjusted to pH 13 with 5 M NaOH. The resulting solution was extracted with $CHCl_3$ (30 mL), dried ($MgSO_4$), and filtered. The filtrate was concentrated in vacuo to provide **21** (206 mg, 75%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.94−1.40 (m, 6 H), 1.75−2.25 (m, 8 H), 2.40−2.64 (m, 6 H), 3.75 (s, 2 H), 3.92 (s, 4 H), 7.11−7.16 (m, 3 H), 7.41−7.62 (m, 4 H), 8.45−8.56 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 26.2 (t), 31.3 (t), 35.5 (t), 40.3 (t), 41.9 (t), 50.6 (t), 52.5 (t), 54.5 (d), 56.6 (d), 56.8 (t), 121.6 (t), 121.9 (d), 122.3 (d), 136.3 (d), 136.4 (d), 148.6 (d), 149.2 (d), 159.7 (s), 161.8 (s); HRMS (positive-ion FAB) calcd for $C_{28}H_{39}N_7$ [M + H]$^+$ m/z 474.3345, found [M + H]$^+$ m/z 474.3330.

Compound 24. To a solution of amine **21** (31.7 mg, 0.067 mmol) in anhydrous DMF (2 mL) was added **23**[13] (18.8 mg, 0.067 mmol). The resulting mixture was stirred for 18 h at room temperature, and solvent was removed via high vacuum pump at room temperature. The residue was purified via flash chromatograpy eluting with triethylamine, methanol, and CH_2Cl_2 at 1:38:460 to provide **24** (38 mg, 88%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.90−1.41 (m, 10 H), 1.70−1.87 (m, 2 H), 1.97−2.20 (m, 4 H), 2.38−2.50 (m, 6 H), 3.05 (q, J = 5.2 Hz, 2 H), 0.3.41 (t, J = 5.3 Hz, 2 H), 3.64 (s, 2 H), 3.82 (s, 4 H), 5.90−5.95 (m, 1 H), 6.55 (s, 2 H), 6.98−7.05 (m, 3 H), 7.16 (d, J = 6.9 Hz, 2 H), 7.35 (d, J = 6.9 Hz, 1 H), 7.49−7.54 (m, 3 H), 8.35 (d, J = 4.6 Hz, 1 H), 8.41 (d, J = 4.6 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 24.9 (t), 25.9 (t), 27.3 (t), 33.6 (t), 35.3 (t), 37.1 (t), 39.3 (t), 39.9 (t), 50.3 (t), 52.2 (t), 54.2 (t), 56.6 (t), 56.7 (t), 121.7 (t), 122.0 (d), 122.4 (d), 122.5 (d), 134.1 (d), 136.5 (d), 136.6 (d), 148.7 (d), 149.2 (d), 159.2 (d), 161.5 (s), 170.9 (s), 171.6 (s); HRMS (positive-ion FAB) calcd for $C_{36}H_{48}N_8O_3$ [M + H]$^+$ m/z 638.3801, found [M + H]$^+$ m/z 638.3771.

Cytotoxicity Assay. HeLa cells were obtained from the American Type Culture Collection and cultured at 37 °C in a humidified 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium with high glucose (4.5 g/L) (DMEM) supplemented with 10% fetal bovine serum (FBS). Cells were seeded at 2000 cells/well in 96-well microtiter plates (Costar, Cambridge, MA) and allowed to attach overnight. The next day, chelators were added at the indicated concentrations. After 72 h, viability was measured using an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye reduction assay, as previously described.[10]

Acknowledgment. We thank the structural mass spectra group (Dr. L. Pannell, NIDDK, Bethesda, MD) for obtaining HRMS spectra of all the compounds. This work was supported in part by Grant No. DK 57781 (S.V.T) from the National Institutes of Health.

Supporting Information Available: Copies of the ^{1}H and ^{13}C NMR spectra for all prepared compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0204911