A: mp >180 °C slow dec; TLC (system B) R_f 0.12; ¹H NMR (DMSO- d_6) δ 2.20 (m, 2 H, CH₂), 2.99 (s, 3 H, S-CH₃), 3.18 (m, 2 H, S-CH₂), 4.49 (br s, 3 H, C₉-H and CH), 6.66 (d, 2 H, C_{3',5'}-H), 7.05 (m, NH₂), 7.66 (d, 2 H, C_{2',6'}-H), 8.28 (distorted d, CONH), 8.65 (s, 1 H, C₇-H); ¹H NMR (DMSO- d_6 + DCl) δ 2.24 (m, 2 H, CH₂), 3.00 (s, 3 H, S-CH₃), 3.22 (m, 2 H, S-CH₂), 4.58 (m, 1 H, CH), 4.80 (s, 2 H, C₉-H), 6.64 (d, 2 H, C_{3',5'}-H), 7.56 (d, 2 H, C_{2',6'}-H), 8.84 (s, 1 H, C₇-H). Anal. (C₁₉H₂₁N₇O₆S-1.1H₂O) C, H, N, S.

Determination of Cofactor Activity in Permeabilized Cells. Murine leukemia L1210 cells were transplanted, maintained, and harvested as described.²⁹ TS activity was measured in suspensions of permeabilized cells³⁰ [(2-5) × 10⁷ cells/mL]. Partial permeabilization³¹ using dextran sulfate (500,000 MW, Pharmacia) was carried out as described.³² Cells were incubated

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- (32) Kalman, T. I.; Marinelli, E. R.; Xu, B.; Reddy, A. R. V.; Johnson, F.; Grollman, A. P. Inhibition of Cellular Thymidylate Synthesis by Cytotoxic Propenal Derivatives of Pyrimidine Bases and Deoxynucleosides. *Biochem. Pharmacol.* 1991, 42, 431-437.

with folate or analogs 8–12 at the indicated concentrations at 37 °C for 15 min in the presence of 0.2 mM NADPH, 1 mM serine (or 7.5 mM CH₂O), 200 mM 2-mercaptoethanol, 40 mM MgCl₂, 0.6 mM EDTA, 100 mM NaF and 80 mM Tris-acetate buffer, pH 7.4 [5-³H]dUMP (1 μ M) was added, and after an additional 30 min incubation, the reaction was terminated and the tritium released into water was measured as described.^{29,32}

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Inhibition of Tumor Cell Growth. Duplicate cultures of L1210 murine leukemia cells were exposed to increasing concentrations of test compounds in comparison with folic acid (PteGlu). After 48 h incubation at 37 °C in RPMI 1640 medium supplemented with 10% fetal calf serum, cell numbers were counted and concentrations corresponding to 50% inhibition of control growth (IC₅₀ values) were determined graphically. Viability was $\geq 90\%$ as determined by dye exclusion.

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Supplementary Material Available: Complete UV and IR data for compounds 8–12 and three tables of NMR chemical shift data for the free amino acids, amino acid esters 1–5, and folic acid analogues 8–12 before and after acidification of samples (5 pages). Ordering information is given on any current masthead page.

Cholesterol Lowering Bile Acid Binding Agents: Novel Lipophilic Polyamines

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A series of novel lipophilic polyamines was synthesized by the sodium cyanoborohydride-mediated reductive amination of various ketones and aldehydes with the polyamine tris(2-aminoethyl)amine. Two of these compounds, N,Nbis[2-(cyclododecylamino)ethyl]-N'-benzyl-1,2-ethanediamine trihydrochloride (36·3HCl) and N,N-bis[2-(cyclododecylmethylamino)ethyl]-N'/dimethyl-1,2-ethanediamine (23), are 29 and 24 times more potent than colestipol hydrochloride, respectively, for lowering animal serum cholesterol levels.

It is estimated that one-third of all deaths in industrial societies result from coronary artery diseases. The most compelling evidence for the positive relationship between high cholesterol levels and the incidence of coronary artery diseases comes from the results of the Lipid Research Clinics Coronary Primary Prevention Trial.¹ Due mainly to this evidence, the nonabsorbable, bile acid binding resins Colestid granules and Questran resin are recommended as first line therapy when patients cannot control their cholesterol levels through diet.² Colestid in combination with niacin has been reported to reverse the atherosclerotic process.^{3a,b} These resins bind to bile acids and cause a 3–10-fold increase in fecal bile acid excretion.⁴ This interruption in enterohepatic circulation causes increases in bile acid synthesis, HMG-CoA reductase activity, and hepatic low density lipoprotein (LDL) receptor-mediated uptake of LDL. It is the latter response that effectively lowers serum cholesterol levels.⁵ The search for more

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⁽²⁾ The Expert Panel. Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Arch. Intern. Med. 1988, 148, 36-69.

^{(3) (}a) Blankenhorn, D. H.; Nessim, S. A.; Johnson, R. L.; Sanmarco, M. E.; Azen, S. P.; Cashin-Hemphill, L. Beneficial Effects of Combined Colestipol-Niacin Therapy on Coronary Atherosclerosis and Coronary Venous Bypass Grafts. JAMA 1987, 257, 3233-3240. (b) Cashin-Hemphill, L.; Mack, W. J.; Pogoda, J. M.; Sanmarco, M. E.; Azen, S. P.; Blackenhorn, D. H. Beneficial Effects of Colestipol-Niacin on Coronary Atherosclerosis. JAMA 1990, 264, 3013-3017.

⁽⁴⁾ Packard, C. J.; Shepherd, J. The Hepatobiliary Axis and Lipoprotein Metabolism: Effects of Bile Acid Sequestrants and Ileal Bypass Surgery. J. Lipid Res. 1982, 23, 1081-1098.

Scheme I



potent agents with a similar mechanism of action is warranted.⁶ This report describes the synthesis of new lipophilic polyamines that bind bile acids and lower serum cholesterol levels in an animal model.

Chemistry

From an in vitro bile acid binding screen, 2a was identified as 5 times as potent as colestipol hydrochloride (CH), the active ingredient in Colestid. The lead compound 2a and other alkylpolyamines were synthesized by a modification of Borch's NaBH₃CN reductive amination procedure⁷ (Table I). We found 2 equiv of amine to ketone practical for the formation of mono adducts, for example 2b (54%). When 1 equiv of amine 1 was reacted with 2 equiv of cyclododecane, a statistical 1:2:1 ratio of 2b:3b:4b was formed. Further, treatment of amine 1 with 4 equiv of cyclododecanone afforded the tris adduct 4b (73%), with no trace of mono adduct 2b. Interestingly, these and many other analogues are freely soluble in hexane.

Hydrogenating the imine derived from hexadecyl aldehyde⁸ and 1 afforded 5a (Table II).⁹ Compound 5e was

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Table I. Synthesis of Polyamine Analogues from Ketones

10 (19%)

ketone + N(CH₂CH₂NH₂)₃ NaBH₃CN HOAc, MeOH



produced by reducing the intermediate imine with NaBH₄ in EtOH.¹⁰ We ascribe the low yields for compounds **5a** and **5f** (Table II) to the instability of the α -methylene

⁽¹⁰⁾ Billman, J. H.; Diesing, A. C. Reduction of Schiff Bases with Sodium Borohydride. J. Org. Chem. 1957, 22, 1068-1070.

Scheme II



aldehydes to the reaction conditions.

Amide derivatives of simple amines are readily available by a number of methods.¹¹ Benzoyl chloride when treated with amine 1 afforded none of the monoacylated material 7. Others also have shown that acid chlorides and polyamines do not yield primarily monoacylated products, yet less reactive acylating agents are preferred to maximize the yield of the mono adduct.¹² Benzoic anhydride and amine 1 formed the monoacylated compound 7 and diacylated 8 (Scheme I). Methyl benzoate and 1 also afforded 7 (Scheme VIII). Treatment of methyl octadecanoate on a steam bath with amine 1 yielded amide 9 and diamide 10 (Scheme I).

As compound 2b is active and can be prepared on large scale, it was chosen as a key intermediate to prepare additional analogues. The extension of the amine moiety involved selective Michael addition of 2 equiv of acrylonitrile to the primary amines of 2b to form 11a (Scheme II). The structure assigned is consistent with the symmetrical product as ascertained from the ¹³C NMR and not an adduct involving the hindered secondary amine. Reduction of the nitriles with LAH produced the hexamine

Table II. Synthesis of Polyamine Analogues from Aldehydes



12. In a similar fashion methyl acrylate and 2b produced the diester 11b. Following chemistry developed to produce starburst or arborol compounds,¹³ we treated 11b with

⁽¹¹⁾ Beckwith, A. L. J. Synthesis of Amides. In *The Chemistry of Amides*; Zabicky, J., Ed.; Interscience: New York, 1970; Chapter 2.

⁽¹²⁾ Jacobson, A. R.; Makris, A. N.; Sayre, L. M. Monoacylation of Symmetrical Diamines. J. Org. Chem. 1987, 52, 2592-2594.







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excess ethylenediamine or butylamine to yield amine extended analogues 13 and 14.

We reacted the $C_{3\nu}$ symmetrical amine 4b with methyl acrylate to yield 15 (Scheme III). Treatment of 15 with ethylenediamine did not form 16 in high yield as expected; rather a mixture of products was obtained. Since ethylenediamine could form cross-linked compounds leading to a myriad of products, the reaction of 15 and butylamine was examined. Likewise, a number of products was again produced, and 17 could not be isolated. Careful analysis of the byproducts of this reaction revealed one compound was 4b. Simply heating 15 in MeOH at 70 °C for 24 h affords 4b (50%). The driving force for the retro-Michael reaction may be the relief of steric crowding. Compound 15 was reduced readily with LAH to yield 18. Polyamine 20 was constructed as outlined in Scheme IV. Amine 4b and acrylonitrile produced 19. The nitriles were reduced with RaNi to yield 20.¹⁴ LAH reduction also afforded 20





in good yield on small scale; however, upon scale-up the yield suffered. 15

Charged compounds are not readily absorbed from the GI tract unless there is a specific uptake mechanism operating.¹⁶ Exhaustive Eschweiler–Clark methylation of previous compounds afforded 22, 23 (Scheme V), and those in Table III.¹⁷ In slightly lower yield, the ethyl derivative 24 was produced employing Gribble's conditions.¹⁸ The less hindered amines of 21c could be selectively quarternized with MeI to afford the charged compound 25, consistent with ¹H NMR and ¹³C NMR analysis.

In order to assess the importance of the nitrogens for activity, a number of oxygen analogues were synthesized (Scheme VI). Reaction of ethanolamine and Na₂CO₃ with tosylate 26 produced 29 and the minor product 28. Reaction of the tosylate 26 with diethanolamine under similar conditions afforded 27. Alcohol 29 and acrylonitrile at room temperature yielded the mononitrile 31. Switching to CH₂Cl₂ as a solvent for the Michael reaction allowed for the formation of 30 in even higher yield. Attempts to use RaNi¹⁴ or LAH to reduce 30 resulted in a reverse Michael reaction affording 27 in 26% and 42% yield, respectively. The nitriles 30 and 31 were successfully reduced to the corresponding amines, 32 and 33, via catalytic hydrogenation using rhodium on alumina.²⁰ An additional

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⁽¹⁴⁾ Bergeron, R. J.; Garlich, J. R. Amines and Polyamines from Nitriles. Synthesis 1984, 782-784.

Scheme V



analogue was synthesized in which only the secondary amines of **3b** were replaced with oxygens. Reaction of **29** with phthalimide under Mitsunobu conditions yielded **34**.²¹ Removal of the protecting group with hydrazine produced **35** in 79% yield (Scheme VII).

The synthesis of **3b** is nonselective and requires a tedious chromatography step to separate it from mono and tris adducts. Much of the published work on the chemistry of polyamines involves modification of spermidine and its homologues.^{22,23} These syntheses rely on the selective functionalization of primary and secondary amines by blocking other reactive amines with the benzyl or the benzoyl protecting groups. Utilizing one of these groups to mono protect amine 1, followed by exposure of the adduct to reductive amination conditions, led to a selective synthesis of 3b (Scheme VIII). Deprotection of benzoyl amine 37 was achieved via basic hydrolysis (10% NaOH/MeOH).²⁴ While this reaction goes to completion on small scale (<1 g), on large scale a trace of starting material always remained. Complete deprotection of the benzyl-protected material 36 was achieved via catalytic hydrogenation using 10% palladium on carbon²⁵ (100%)

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Table IV. Formation of Polyamine Salts

base	acid	salt	yield, %
2b	HCl gasa	2b-4HCl	82
3b	$MeSO_{3}H^{b}$	3b•MeSO ₃ H	89
3b	10% HCl ^e	3b-2HCl	91
3b	10% HCl ^d	3b-3HCl	95
3b	10% HCl ^e	3b·4HCl	76
4b	10% HCl ^d	4b-3HCl	90
36	10% HCl ^d	36-3HCl	36
24	10% HCl ^d	24-3HC1	67

^aExcess. ^bOne equivalent. ^cTwo equivalents. ^dThree equivalents.

or transfer hydrogenation employing palladium black (97%).²⁶

Since analogue 3b is one of the most potent compounds, further derivatives were synthesized by functionalizing the primary amine. The alkyl derivatives produced by reductive amination are 38 and 39 (Scheme IX). The Michael reaction of acrylonitrile and 3b produced 40, which was reduced with rhodium on alumina, affording the amine extended substrate 41. Compounds 42 and 43, which are similar to 36, were synthesized from amine 5c (Scheme X).

Since many of the compounds in this series are thick oils, their crystalline salts (Table IV) are derivatives of interest. However, the polyamines undergo multiple protonation.

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(b) Velluz, L.; Amiard, G.; Heymes, R. Utilisation d'Intermediares N-benzyles en Synthese Peptidique. Bull. Soc. Chim. Fr. 1954, 1012-1015.

⁽²⁶⁾ ElAmin, B.; Ananthaacamiah, G. M.; Royer, G. P.; Means, G. E. Removal of Benzyl-type Protecting Groups from Peptides by Catalytic Transfer Hydrogenation with Formic Acid. J. Org. Chem. 1979, 44, 3442-3444.

Scheme VI

Scheme VII



Calculated pK_as employing experimentally-derived linear free energy relationships²⁷ are found in Figure 1. Sec-

ondary amines adjacent to the alkyl group are the most basic, and for 2b and 3b differ by only 0.46 pK_a unit. Smaller differences in pK_a values are observed at the second and third sites of protonation. At neutral pH, three of the sites are sufficiently basic to be fully protonated, whereas the fourth site ($pK_a = -0.30$) only may be protonated at extremely low pH.

^{(27) (}a) Perrin, D. D.; Dempsey, B.; Serjeant, E. P. *pK_a* Prediction for Organic Acids and Bases; Chapman and Hall: New York, 1981. (b) Clark, J.; Perrin, D. D. Prediction of the Strengths of Organic Bases. *Quart. Rev.* 1964, 18, 295-320.

Novel Lipophilic Polyamines



<u>4b</u>

Figure 1. Basicities of polyamines. The values are given in pK_a units, calculated according to procedures found in ref 27.



Figure 2. Serum VLDL + LDL cholesterol in cholesterol-fed Japanese quail treated with various doses of **4b** or **38**. Data are plotted as means \pm SEM; control groups contained 20 animals, treated groups contained 10 animals. * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

Biological Results and SAR Summary

The potencies of test compounds relative to colestipol hydrochloride (CH) were calculated using a common slope derived from several dose-response experiments for test compounds and CH. Representative examples of the dose-response effects on serum VLDL + LDL cholesterol are shown for two of the test compounds (Figure 2) and



Figure 3. Serum VLDL + LDL cholesterol in cholesterol-fed Japanese quail treated with several doses of colestipol hydrochloride (Colestid). Data are plotted as means \pm SEM; control group contained 20 animals, treated groups contained 10 animals. ** = p < 0.01; *** = p < 0.001.

for one of the CH dose-response experiments (Figure 3).²⁸ Control concentrations of serum VLDL + LDL varied from approximately 200 to 400 mg/dL in individual experiments. Therefore, potency estimates were always calculated using the CH and test compound responses determined within the same experiment.

For polyamine analogues (Tables I and II) a critical factor in terms of drug potency is the hydrophobicity of the appended alkyl group. The amine 1 or those analogues, for example 2c and 2d, with small rings are inactive. The larger ring compounds 2g, 2a, and 2b exhibit increased potencies of 5, 7, and 10 times that of CH, respectively, with increased ring size (12-, 15-, 16-membered). A cyclic alkyl group is not necessary for activity, as the alkyl chain derivative 5a is as potent as 2b. The addition of a second 12-membered ring to 2b afforded compound 3b, which is 13 times more potent than CH. The alkyl derivatives of 3b (38, 39, 36, and 36-3HCl) are 19-29 times more potent than CH (Table V).

The analogues without basic amines were not active. The acid-base interaction of bile acid to substrate must be of primary importance. With all the active compounds having the same number of amines, it is logical to us that

⁽²⁸⁾ See experimental section for a summary of the in vivo moderately hypercholesterolemic quail screen.

Scheme VIII



the lipophilic portion of the molecule accounts for the increased activity of the more potent compounds.

Experimental Section

Nearly all compounds which were submitted for biological evaluation were characterized by satisfactory elemental analysis for at least three elements (C, H, N), and corrections were made for water content as determined by Karl Fisher analysis. For the remainder, satisfactory high-resolution mass spectra were obtained. ¹H NMR spectra were recorded at 300 MHz. ¹³C NMR spectra were recorded at 75 MHz. These spectra are consistent with the assigned structures. Melting points in open capillaries are uncorrected. Unless specified, all Burdick and Jackson solvents and reagents purchased from Aldrich were used without further purification. THF and Et₂O were dried over molecular sieves.²⁹ All compounds were dried over MgSO₄. The solvent was removed on a rotovap under reduced pressure. Unless indicated otherwise, all products were obtained as liquids. Full experimental details for the preparation of many individual compounds have been described.³⁰

General Procedure for the Reductive Amination of Ketones with Polyamines. Preparation of Compounds 2, 3, 4

- (29) Burfield, D. R.; Gan, G.; Smithers, R. H. Molecular Sieves— Desiccants of Choice. J. Appl. Chem. Biotechnol. 1978, 28, 23-30.
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Scheme X



NaBH₃CN AcOH, MeOH



Table V. Lipophilic Polyamines Which Are More Potent Than Colestipol Hydrochloride (CH) (Potency of CH = 1)

no.	structure location ^a	doses tested, mg/kg BW per day	rel potency to CH
36-3HC1	SVIII	50	29
23	sv	50	24
40	SIX	50	21
39	SIX	50	21
38	SIX	25, 50	19
36	SVIII	25, 50	18
3b	TI	10, 25, 50, 100, 150	13
42	SX	50	13
3b-3HCl	TI	50	13
3b-4HCl	TI	50	12
37	SVIII	50	11
2g	TI	50	10
24-3HCl	\mathbf{SV}	100	8
11b	SII	50, 150	7
2a	TI	100, 150, 300	7
21c	TIII	150	7
3a	TI	150	7
4b	TI	25, 50, 100	6
5 a	TII	100	6
8	SI	25, 50, 100	5
2b	TI	100, 300	5
22	sv	150	5
13	SII	100	5
15	SIII	50	4
12	SII	150	4
4c	TI	150	3
32	SVI	150	2
5d	TH	100	2
25	SV	150	2
lla	SII	150	2

 $^{a}T = Table; S = Scheme.$

(Table I), 36-39, 42, and 43. N,N-Bis(2-aminoethyl)-N'cyclododecyl-1,2-ethanediamine (2b). According to a modified procedure of Borch, amine 1 (8.2 g, 56 mmol), MeOH (100 mL), AcOH (26 mL, 0.45 mol), and cyclododecanone (5.1 g, 28 mmol) were combined and stirred at room temperature for 0.5 h. NaBH₃CN (1.85 g, 28 mmol) was added, and the reaction was continued at room temperature for 24 h. The mixture was concentrated in vacuo and 20% NaOH was added, pH = 12. The aqueous portion was extracted with $CHCl_3$ (3 × 100 mL). The organic fractions were combined, dried, and concentrated in vacuo. The crude material was chromatographed on SiO₂ (300 g) and eluted with CHCl₃/MeOH/NH₄OH (10:4:1) to yield 6.9 g of material contaminated with solvent (for higher boiling compounds solvent was removed by heating to 100 °C in a Kugelrohr oven at 0.1 mm). Bulb-to-bulb distillation of the crude material in a Kugelrohr apparatus afforded 2b (4.74 g, 54%): bp 180-200 °C (0.08 mm); [†]H NMR (CDCl₃) δ 1.22–1.60 (m, 27 H, CH₂, NH, NH₂), 2.47–2.70 (m, 13 H, NHCH, NCH₂CH₂); ¹³C NMR (CDCl₃) ppm 57.9, 55.3, 54.9, 45.5; 40.0, 29.4, 25.0, 24.5, 23.1, 23.0, 20.9; IR (liquid) 3363, 3283, 2932, 1658, 1470, 1446, 1347 cm⁻¹; MS for $C_{18}H_{40}N_4$, m/z (relative intensity) 313 (MH⁺, 1), 282 (42), 278 (16),

209 (10), 196 (22), 116 (100), 99 (76), 87 (63). Anal. (C₁₈H₄₀N₄) C, H, N.

General Procedure for the Reductive Amination of Aldehydes with Polyamines. Preparation of Compounds 5a-f and 6c. N,N-Bis(2-aminoethyl)-N'-benzyl-1,2-ethanediamine (5c) and N,N-Bis(2-aminoethyl)-N',N'-dibenzyl-1,2-ethanediamine (6c). Amine 1 (13.0 mL, 86.9 mmol), toluene (470 mL), and benzaldehyde (5.0 g, 47.1 mmol) were combined and stirred over molecular sieves for 96 h. The reaction mixture was filtered and concentrated in vacuo. The residue was dissolved in absolute EtOH (150 mL) and hydrogenated over 10% palladium on carbon (1.0 g) at 50 psi overnight. The reaction mixture was filtered through a Solka-Floc pad, the pad was washed with Et₂O, and the filtrate was concentrated in vacuo. The residue was chromatographed, eluting with CHCl₃/MeOH/NH₄OH (10:4:1). The appropriate fractions were combined and concentrated in vacuo to afford the mono adduct 5c (6.2 g, 56%) and a small amount of the bis adduct 6c (1.1 g, 7%). Data for 5c: ¹H NMR (CDCl₃) δ 2.03 (s, 5 H, NH, NH₂), 2.48 (t, 4 H, J = 6.1 Hz, CH₂NH₂), 2.59 $(t, 2 H, J = 5.8 Hz, NHCH_2), 2.67-2.75 (m, 6 H, NCH_2), 3.79 (s,$ 2 H, PhCH₂), 7.25-7.33 (m, 5 H, arom); ¹³C NMR (CDCl₃) ppm 140.0, 128.1, 127.9, 126.7, 57.0, 53.9, 53.6, 46.5, 39.3; IR (liquid) 3358, 3286, 2942, 2824, 1584, 1494, 1464, 746, 700 cm⁻¹; MS for $C_{13}H_{24}N_4$, m/z (relative intensity) 206 (37), 189 (15), 175 (8), 148 (5), 134 (16), 116 (60), 99 (100). Anal. (C₁₃H₂₄N₄) C, H, N. Data for 6c: ¹³C NMR (CDCl₃) ppm 140.2, 128.5, 128.2, 127.0, 57.4, 54.3, 54.0, 46.9, 39.7. Anal. (C₂₀H₃₀N₄) C, H, N.

N', N'-Bis(2-aminoethyl)-N-(2-aminoethyl)octadecanamide (9) and N,N-Bis[2-(octadecanoylamino)ethyl]-1,2-ethanediamine (10). The amine 1 (2.92 g, 20 mmol) and methyl octadecanoate (2.98 g, 10 mmol) were heated on a steam bath, under nitrogen, for 24 h. The resulting solid was heated in H₂O and was then filtered to remove excess amine. The solid was chromatographed on SiO₂ (40 g), eluting first with MeOH/CHCl₃, followed by CHCl₃/MeOH/NH₄OH (10:4:1). The diamide 10 (0.66 g, 19%) eluted first followed by the amide 9 (1.89 g, 46%). Data for 9: ¹H NMR (CDCl₃) δ 0.88 (t, 3 H, J = 6.7 Hz, CH₃), 1.25 (s, 28 H, CH₂), 1.61 (br s, 6 H, O=CCH₂CH₂, NH, NH₂), 2.16 (t, 2 H, J = 7.2 Hz, O=CCH₂), 2.52-2.61 (m, 6 H, CH₂N(CH₂)CH₂), 2.78 (t, 4 H, CH_2NH_2), 3.31 (dd, 2 H, J = 5 Hz, J = 6 Hz, $NHCH_2$), 7.10-7.18 (m, 1 H, O=CNH); IR (mineral oil mull) 3306, 2907, 1643, 1551, 1471 cm⁻¹; MS for $C_{24}H_{52}N_4O$, m/z (relative intensity) 382 (100), 353 (10), 310 (41), 116 (22). Data for 10: ¹H NMR $(CDCl_3) \delta 0.88 (t, 6 H, J = 6.7 Hz), 1.25 (s, 56 H, CH_2), 1.55-1.68$ (m, 4 H, O=CCH₂CH₂), 2.07 (br s, 2 H, NH₂), 2.52 (t, 2 H, J =6.0 Hz, $CH_2CH_2NH_2$), 2.58 (t, 4 H, J = 6.0 Hz, $NHCH_2CH_2$), 2.73 $(t, 2 H, J = 6 Hz, NH_2CH_2), 3.26-3.87 (m, 4 H, NHCH_2), 6.71-6.80$ (m, 2 H, NH); IR (mineral oil mull) 3297, 3079, 2919, 1637, 1557, 1467, 1367 cm⁻¹; MS for $C_{42}H_{86}N_4O$, m/z (relative intensity) 677 (3), 649 (40), 648 (85), 620 (9), 382 (100), 353 (24), 310 (69).

3,3'-[[[2-(Cyclododecylamino)ethyl]imino]bis(2,1ethanediylimino)]bis[propanenitrile] (11a). Alkylamine 2b (5.0 g, 16 mmol) and MeOH (16 mL) were cooled in an ice bath. Acrylonitrile (2.1 mL, 1.7 g, 32 mmol) was added at 0.3 mL/min. After 3 h the reaction was complete, and the contents of the flask were concentrated in vacuo to 7.22 g. This was chromatographed on SiO_2 (200 g), eluting first with MeOH/CHCl₃, followed by CHCl₃/MeOH/NH₄OH (10:4:1) to provide a pure fraction of 11a (4.12 g, 62%) followed by a less pure fraction (2.0 g). Data for pure fraction: ¹H NMR (CDCl₃) δ 1.20–1.60 (m, 22 H, CH₂), 1.72 (br s, 3 H, NH), 2.50–2.75 (m, 17 H), 2.93 (t, 4 H, J = 6.6 Hz, NCH₂CH₂CN); ¹³C NMR (CDCl₃) ppm 118.7 (s), 55.1 (d), 54.4, 54.0, 46.7, 45.0, 29.0, 24.8, 24.2, 22.8, 22.8, 20.7, 18.5; IR (neat) 3306, 2934, 2905, 2247, 1470, 1132, 1061 cm⁻¹; MS for C₂₄H₄₆N₆, m/z (relative intensity) 335 (100), 278 (27), 265 (27), 222 (51), 196 (49), 152 (37), 140 (68); chemical ionization (NH₃) MS for C₂₄H₄₆N₆, (relative intensity) 419 (MH⁺, 100). Anal. (C₂₄H₄₆N₆) C, H, N.

3,3'-[[[2-(Cyclododecylamino)ethyl]imino]bis(2,1ethanediylimino)]bis[propanamine] (12). A solution of dinitrile 11a (2.92 g, 6.9 mmol) and THF (5 mL) was added to a cooled (0 °C) mixture of LAH (1.06 g, 28 mmol) and THF (25 mL). The reaction was warmed to room temperature and stirred for 6 h. Saturated aqueous NH₄Cl (20 mL) was used to quench the reaction and 20% NaOH was used to bring the mixture to pH = 12. The aluminum salts were removed by filtration, and the filtrate was extracted with CHCl₃ (3 × 150 mL). The organic layers were combined, dried, and concentrated to provide 12 (3.3 g, 110%): ¹H NMR (CDCl₃) δ 1.20–1.70 (m, 34 H, CH₂, NH, NH₂), 2.50–2.90 (m, 21 H, NCH₂, NCH); ¹³C NMR (CDCl₃) ppm 55.1, 54.9, 54.4, 47.8, 45.6, 40.4, 33.9, 29.2, 25.0, 24.4, 22.9, 22.8, 20.7; IR (neat heated) 3286, 2930, 1634, 1598, 1470, 1446, 1125 cm⁻¹; chemical ionization (isobutylene) MS for C₂₄H₅₄N₆, m/z (relative intensity) 427 (MH⁺, 100).

Dimethyl 3,3'-[[[2-(Cyclododecylamino)ethyl]imino]bis-(2,1-ethanediylimino)]bis[propanoate] (11b). At 0 °C alkylamine 2b (3.12 g, 10 mmol), MeOH (10 mL), and methyl acrylate (1.8 mL, 1.72 g, 20 mmol) were combined. After 1 h the reaction had warmed to room temperature, and the reaction was continued for an additional 5 h at room temperature. Concentration of the reaction in vacuo left an oil which was chromatographed on SiO_2 (150 g) and eluted with MeOH/CHCl₃, followed by CHCl₃/ MeOH/NH₄OH (10:4:1). Combination of the appropriate fractions yielded 11b (3.29 g, 68%): ¹H NMR (CDCl₃) δ 1.20–1.60 (m, 22 H, CH₂), 2.13 (br s, 3 H, NH), 2.40–2.74 (m, 17 H, NCH₂, $O = CCH_2$, 2.88 (t, 4 H, J = 7 Hz, $NCH_2CH_2C = O$), 3.68 (s, 6 H, CH₃); ¹³C NMR (CDCl₃) ppm 173.1 (s), 55.2 (q), 54.6, 54.2, 51.4, 47.3, 45.1, 45.0, 34.3, 29.0, 25.0, 24.4, 22.9, 22.85, 20.7; IR (neat) 3306, 2934, 1739, 1663, 1470, 1443, 1438, 1196, 1177 cm⁻¹; MS for C₂₆H₅₂N₄O₄, m/z (relative intensity) 485 (MH⁺, 2), 453 (13), 411 (9), 368 (100), 288 (63), 276 (40), 173 (67). Anal. (C₂₆H₅₂N₄O₄) C, H, N. Corrected for 1.9% H₂O found by Karl Fisher analysis.

16-Amino-N-(2-aminoethyl)-7-[2-(cyclododecylamino)ethyl]-13-oxo-4,7,10,14-tetraazahexadecanamide (13). Ethylenediamine (49.4 g, 0.824 mol) was added to a solution of the diester 11b (5.0 g, 10.3 mmol) in CH_3OH (105 mL). The reaction was stirred at room temperature for 4 days. The solution was concentrated in vacuo, and solvent traces were removed under vacuum (0.1 mm), producing a green oil, 13 (4.39 g, 88.5%): ¹H NMR (CDCl₃) δ 1.35 (s, 18 H, CH₂), 1.48–1.55 (m, 4 H, NCHCH₂), 1.61 (s, 7 H, NH and NH₂), 2.35-2.45 (m, 4 H, O=CCH₂), 2.59 (s, 4 H, NHCH₂CH₂C=O and NHCHCH₂), 2.67 (s, 6 H, NCH₂), 2.75–2.89 (m, 10 H, CH_2NH_2 and $NHCH_2CH_2N$), 3.25–3.38 (m, 4 H, O=CNHCH₂), 7.80 (s, 2 H, O=CNH); ¹³C NMR (CDCl₃) ppm 172.8, 55.1, 54.7, 54.1, 47.2, 45.7, 45.0, 41.9, 41.4, 35.8, 29.2, 24.9, 24.3, 22.9, 22.8, 20.7; IR (liquid) 3291, 3058, 2932, 2860, 1651, 1554, 1470, 1445 cm⁻¹; MS for $C_{28}H_{60}N_8O_2$, m/z (relative intensity) 541 (MH+, 58), 540 (4), 481 (6), 408 (11), 396 (9), 210 (14), 97 (46), 85 (38), 56 (57), 44 (100). Anal. $(C_{28}H_{60}N_8O_2)$ C, H, N.

N-Butyl-7-[2-(cyclododecylamino)ethyl]-13-oxo-4,7,10,14tetraazaoctadecanamide (14). N-Butylamine (37.7 g, 0.515 mol) was added to a solution of the ester 13 (4.8 g, 10.3 mmol) in methanol (65 mL). The reaction was stirred at room temperature for 4 days. The solution was concentrated in vacuo, producing a green oil. This was chromatographed, and solvent traces were removed under vacuum (0.1 mm) with stirring, affording 14 (36 g, 61.9%): ¹H NMR (CDCl₃) δ 0.87 (t, 6 H, J = 7.3 Hz, CH₃), 1.28-1.38 (m, 22 H, CH₃CH₂ and NCHCH₂CH₂), 1.41-1.49 (m, 8 H, CH₃CH₂CH₂ and NCHCH₂), 1.85 (br s, 3 H, NH), 2.30 (t, 4 H, J = 6.0 Hz, O=CCH₂), 2.51-2.63 (m, 13 H, NCH₂, NHC-H₂CH₂N, and NHCH), 2.80 (t, 4 H, J = 6.0 Hz, O=CCH₂CH₂N), 3.14-3.21 (m, 4 H, O=CNHCH₂), 7.40-7.52 (m, 2 H, O=CNH); ¹³C NMR (CDCl₃) ppm 172.5, 55.3, 54.9, 54.3, 47.3, 45.8, 45.3, 38.9, 35.8, 31.7, 29.3, 25.1, 24.5, 23.0, 23.0, 20.8, 20.2, 13.8; IR (liquid) 3296, 3076, 2950, 2933, 2862, 1647, 1556, 1469, 1127 cm⁻¹; MS for $C_{32}H_{66}N_6O_2$, m/z (relative intensity) 565 (MH⁺, 1), 409 (100), 397 (12), 370 (52), 358 (27), 278 (11), 214 (58), 157 (57). Anal. ($C_{32}H_{66}N_6O_2$) C, H, N.

Methyl 4,10-Dicyclododecyl-7-[2-[cyclododecyl(3-methoxy-3-oxopropyl)amino]ethyl]-13-oxo-14-oxo-4,7,10-triazapentadecanoate (15). The amine 4b (9.8 g, 15 mmol) MeOH (100 mL), and methyl acrylate (4.0 mL, 45 mmol) were combined and heated at 60 °C. After 20 h, additional methyl acrylate (4.0 mL) was added followed by 4 mL more after another 2 h. The reaction was continued for an additional 18 h, cooled, and then concentrated in vacuo. The material was triturated with EtOAc and filtered. The white solid was dried in vacuo to yield 15 (10.8 g, 80%): mp 84-87 °C; ¹H NMR (CDCl₃) § 1.15-1.58 (m, 66 H, CH_2), 2.39 (t, 6 H, J = 6.8 Hz, $CH_2C=0$), 2.42-2.62 (m, 15 H, NCH, NCH₂), 2.73 (t, 6 H, J = 6.8 Hz, NCH₂CH₂C=O), 3.66 (s, 9 H, OCH₃); ¹³C NMR (CDCl₃) ppm 173.1, 55.8, 54.9, 51.2, 48.6, 46.7, 35.0, 25.7, 24.1, 23.9, 23.0, 22.3, 22.1; IR (mineral oil mull) 2948, 1743, 1469, 1438, 1260, 1203, 1114, 1025 cm⁻¹; MS for $C_{54}H_{102}N_4O_6$ by FAB, m/z (relative intensity) 903 (M⁺, 100), 620 (24), 454 (27), 325 (21), 296 (39), 282 (87), 130 (66). Anal. (C₅₄H₁₀₂N₄O₆) C, H, N.

Tris[2-[2-cyclododecyl(3-hydroxypropyl)amino]ethyl]amine (18). LAH (2.73 g, 72 mmol) and THF (60 mL) were cooled to -78 °C. The triester 15 (5.42 g, 6 mmol) dissolved in THF (60 mL) was added slowly to the mixture. The reaction was warmed to room temperature and continued for 17 h. The reaction was carefully quenched with H₂O (3 mL), followed by 20% NaOH (3 mL) and H_2O (9 mL). The mixture was filtered through Celite to remove the white aluminum salts, and the cake was washed with Et₂O. The filtrate was concentrated in vacuo and the residue was lyophilized to yield 18 (4.51 g, 92%), as a white solid: mp 91-93 °C; ¹H NMR (CDCl₃) δ 1.15-1.55 (m, 66 H, CH₂), 1.55-1.72 (m, 6 H, OCH₂CH₂), 2.50-2.78 (m, 21 H, NCH, NCH₂), 3.70-3.85 (m, 6 H, OCH₂), 5.24 (s, 3 H, OH); ¹³C NMR (CDCl₃) ppm 63.6, 54.4, 54.3, 50.2, 47.2, 28.7, 25.4, 24.0, 23.9, 23.3, 22.6, 22.2; IR (mineral oil mull) 3379 (OH), 2968, 2855, 2811, 1469, 1448, 1440, 1268, 1247, 1110, 1077, 1070, 917, 711 cm⁻¹; FAB-MS for C₅₁- $H_{102}N_4O_3$, m/z (relative intensity) 819 (MH⁺, 47), 564 (15), 398 (7), 297 (7), 268 (63), 254 (100), 102 (69). Anal. $(C_{51}H_{102}N_4O_3)$ C. H. N.

3,3',3''-[Nitrilotris[2,1-ethanediy](cyclododecylamino)]]tris[propanenitrile] (19). Amine 4b (10.0 g, 15.5 mmol) and MeOH (100 mL) were heated to 60 °C. Acrylonitrile (10.0 mL, 155.2 mmol) was added to the reaction slowly. After 40 h, an additional portion of acrylonitrile (10.0 mL) was added. After an additional 144 h the reaction was concentrated in vacuo. The solid was slurried in EtOAc (125 mL) and filtered to yield the trisnitrile 19 (11.48 g, 92%): mp 107-109 °C; ¹H NMR (CDCl₃) δ 1.10-1.70 (m, 66 H, CH₂), 2.38 (t, 6 H, J = 6.6 Hz, CH₂CN), 2.46-2.64 (m, 15 H, NCH, NCH₂), 2.73 (t, 6 H, J = 6.6 Hz, CH₂CH₂CN); ¹³C NMR (CDCl₃) ppm 119.2, 55.8, 55.3, 48.8, 47.0, 26.0, 24.0, 23.8, 23.0, 22.2, 22.1, 19.0; IR (mineral oil mull) 2971, 2249, 1470, 1444, 1111, 1004, 994, 722, 710 cm⁻¹; MS for C₅₁H₉₃N₇, m/z (relative intensity) 555 (40), 554 (100), 501 (29), 448 (38), 263 (30), 97 (84). Anal. (C₅₁H₉₃N₇) C, H, N.

Tris[(2-cyclododecyl(3-aminopropyl)amino)ethyl]amine (20). A Parr bottle was charged with trisnitrile 19 (3.5 g, 4.3 mmol), THF (100 mL), NaOH (130 mg), and RaNi (a 10-mL heavy slurry). The hydrogenation reaction was conducted under 40 psi of hydrogen for 48 h. The contents of the flask were filtered through Celite, and the flask was successively washed with THF, EtOH, CHCl₃, and CHCl₃/MeOH/NH₄OH. Upon addition of NH₄OH the solution turned from a clear green to a pale blue and a precipitate formed. The material was filtered and the blue color was removed. The concentrated product (4.17 g) was chromatographed on SiO₂ (120 g), eluting with CHCl₃/MeOH/NH₄OH (20:4:1). The purified material 20 (2.76 g, 79%) was isolated from like fractions. Analytically pure material was prepared by dissolving the product in hexane, filtering, and concentrating the material in vacuo to 2.62 g of 20, a white solid: mp 105-107 °C; ¹H NMR (CDCl₃) δ 1.20–1.45 (m, 66 H, CH₂), 1.40–1.60 (m, 6 H, $NH_2CH_2CH_2$), 2.38–2.52 (m, 18 H, NCH₂), 2.55–2.66 (m, 3 H, NCH), 2.74 (t, 6 H, J = 7 Hz, NH_2CH_2); ¹³C NMR (CDCl₃) ppm 55.9, 54.3, 48.4, 48.3, 40.4, 33.1, 25.6, 24.1, 23.9, 23.1, 22.5, 22.2; IR (liquid) 3373, 3291, 2931, 2849, 1469, 1445 cm⁻¹. Anal. $(C_{51}H_{105}N_7)$ C, H, N.

General Procedure for the Methylation of Polyamines. Preparation of Compounds 21a-c (Table III), 22, and 23. N-Cyclododecyl-N-methyl-N',N'-bis[2-(dimethylamino)ethyl]-1,2-ethanediamine (21c). The alkylamine 2b (5.0 g, 16 mmol) was cooled to 0 °C and formic acid (6.2 g, 128 mmol, 95%) was added, followed by formaldehyde (9.1 g, 112 mmol, 36% aqueous). The reaction was heated to 80 °C with vigorous evolution of gas. After the reaction was continued overnight, the material was concentrated in vacuo. The residue was brought to pH 12 with 10% NaOH. The aqueous portion was extracted with $CHCl_3$ (3 × 50 mL), dried, and concentrated to 6.0 g. Bulb-to-bulb distillation of the liquid afforded pure 21c (5.29 g, 86%): bp 160-180 °C (0.1 mm); ¹H NMR (CDCl₃) δ 1.22-1.52 (m, 22 H, CH₂), 2.24 (s, 15 H, NCH₃), 2.32-2.66 (m, 13 H, NCH, NCH₂); ¹³C NMR (CDCl₃) ppm 57.6 (d), 57.4, 53.9, 52.9, 51.3, 45.7 (q), 37.9 (q), 25.3, 23.7, 23.6, 23.4, 22.4, 22.3; IR (neat) 2938, 2907, 1691, 1469, 1265, 1123, 1042 cm⁻¹; MS for $C_{23}H_{50}N_4$, m/z (relative intensity) 324 (52), 224 (58), 223 (42), 210 (56), 172 (100), 160 (14), 129 (22), 72 (82). Anal. (C₂₃H₅₀N₄) C, H, N.

N-Cyclododecyl-N',N'-bis[2-(diethylamino)ethyl]-Nethyl-1,2-ethanediamine (24). Amine 2b (5.0 g, 16.0 mmol) was dissolved in acetic acid (212 mL) and the solution was heated to 50-55 °C. NaBH₄ (pellets, 15.1 g, 0.40 mol) was added in 1-g portions over a period of about 2 h. The reaction was stirred at 50-55 °C for 42 h. The reaction was cooled in an ice bath and quenched with H_2O (390 mL). The solution was adjusted to pH 12 with NaOH pellets, H_2O was added to dissolved the excess, and the solution was extracted with $CHCl_3$ (7 × 180 mL). The organic layers were combined, dried, filtered, and concentrated in vacuo. The residue was chromatographed, eluting with CHCl₃/CH₃OH/NH₄OH (40:40:1). The appropriate fractions were combined and concentrated in vacuo, yielding 24 (3.69 g, 50.9%). as an orange oil: ¹H NMR (CDCl₃) δ 0.97–1.06 (m, 15 H, CH₃), 1.30-1.50 (m, 22 H, CH₂), 2.41-2.60 (m, 23 H, NCH₂CH₃); ¹³C NMR (CDCl₃) ppm 55.5, 54.5, 53.2, 50.9, 48.0, 47.3, 44.6, 25.8, 24.0, 23.8, 23.3, 22.4, 15.0, 11.5; IR (neat) 2968, 2931, 2907, 2850, 1469, 1446, 1383, 1347, 1205 cm⁻¹; MS for $C_{28}H_{60}N_4$, m/z (relative intensity) 367 (17), 366 (74), 238 (49), 228 (100), 214 (18), 157 (27), 100 (97). Anal. (C₂₈H₆₀N₄) C, H, N.

2,2'-[[2-(Cyclododecylmethylamino)ethyl]imino]bis[*N*,-*N*,*N*-trimethylethanaminium] Diiodide (25). Alkylamine 21c (1.0 g, 2.6 mmol) and CH₃CN (250 mL) were combined. MeI (740 mg, 5.2 mmol) in CH₃CN (1 mL) was added to the reaction. After 14 h a white solid was present in the flask. The contents of the flask were concentrated in vacuo to yield 1.67 g (96%) of 25: ¹H NMR (CDCl₃) δ 1.01–1.30 (m, 22 H, CH₂), 2.03 (s, 3 H, NCH₃), 2.30–2.42 (m, 2 H, CH₃NCH₂), 2.50–2.60 (m, 2 H, CH₃NCH₂), 2.72–2.86 (m, 1 H, CH₃NCH), 3.03 (t, 4 H, J = 7 Hz, NCH₂), 3.24 (s, 18 H, N⁺(CH₃)₃), 3.72 (t, 4 H, J = 7 Hz, N⁺CH₂); ¹³C NMR (CDCl₃) pp 61.6, 58.0 (d), 52.8 (q), 51.3, 50.6, 46.7, 37.5 (q), 24.9, 23.5, 23.4, 23.0, 22.2; IR (mineral oil mull) 3475, 2922, 2856, 1468, 1377, 947, 918 cm⁻¹; MS for C₂₅H₅₆N₄I₂, *m/z* (relative intensity) 667 (MH⁺, 1), 539 (85), 397 (24), 338 (22), 224 (85), 58 (100). Anal. (C₂₅H₅₆N₄I₂) C, H, N.

2-(Cyclododecyloxy)-1-[[(4-methylphenyl)sulfonyl]oxy]ethane (26). Cyclododecanone (109.4 g, 0.6 mol) was treated with ethylene glycol and TsOH in refluxing toluene, with azeotropic removal of the water, to yield the corresponding ketal (overall yield 72%; first crop from Et_2O /hexane provided 54.7 g, mp 70-71 °C, second crop 42.3 g, mp 68-70 °C). AlCl₃ (10.7 g, 80 mmol) was added to a flask cooled in an ice bath, followed by Et₂O (25 mL). After 30 min of stirring, the mixture became a light gray solution. A slurry of LAH (0.76 g, 20 mmol) in Et₂O (25 mL) was added dropwise to the mixture. After the reaction was stirred for 30 min, we added the ketal (9.0 g, 40 mmol), dissolved in Et₂O (80 mL), to the reaction. The reaction was heated at reflux for 3 h. The cooled reaction was quenched carefully with H₂O until the gas evolution subsided. Aqueous 10% H₂SO₄ (50 mL) was then added cautiously. Additional H_2O was added and the aqueous layer was extracted with Et_2O (2 × 100 mL). The organic portions were combined, dried, and concentrated in vacuo to 8.44 g of [[2-(cyclododecyloxy)ethyl]imino]ethanol (92%):³¹ ¹H NMR (CDCl₃) δ 1.20–1.70 (m, 22 H, CH₂), 2.19 (t, 1 H, OH), 3.40–3.50 (m, 1 H, OCH), 3.51–3.60 (m, 2 H, OCH₂), 3.64–3.72 (m, 2 H, CH₂OH). The alcohol (8.44 g, 37 mmol) and pyridine (100 mL) were cooled to 0 °C. Solid toluenesulfonyl chloride (14.1 g, 74 mmol) was added to the flask and swirled until all the solid had dissolved. The reaction was placed in the freezer for 1 week. The reaction was poured into ice water (800 mL). The tosylate at first oiled out of solution and then solidified. The solid was filtered, washed with water, and dried to yield 26 (13.11 g, 93%): ¹H NMR (CDCl₃) δ 1.20–1.60 (m, 22 H, CH₂), 2.44 (s, CH₃), 3.37 (m, 1 H, OCH), 3.61 (t, 2 H, J = 5 Hz, OCH₂), 4.14 (t, 2 H, J = 5 Hz), 7.32 (d, 2 H, J = 8 Hz, arom), 7.80 (d, 2 H, J = 8 Hz, arom).

2,2'-[[2-(Cyclododecyloxy)ethyl]imino]bis[ethanol] (27). Tosylate 26 (18.5 g, 48.4 mmol) was added to a solution of diethanolamine (11.4 g, 108 mmol) in CH₃CN (380 mL). The solution was refluxed in the presence of Na_2CO_3 (11.4 g) for 20 h. The reaction mixture was concentrated in vacuo. The residue was dissolved in H₂O (200 mL) and extracted with CHCl₃ (4 \times 200 mL). The organic layers were combined, dried with $MgSO_4$, filtered, and concentrated in vacuo. The residue was chromatographed, eluting with $CHCl_3/CH_3OH$ (20:1). The appropriate fractions were combined and concentrated in vacuo, producing a pale yellow oil. Solvent traces were removed under high vacuum to yield 27 (12.08 g, 79.1%): ¹H NMR (CDCl₃) δ 1.32-1.36 (m, 18 H, CH₂), 1.46–1.67 (m, 4 H, CH₂CHOCH₂), 2.73 (t, 2 H, J =5.1 Hz, NCH_2CH_2OCH), 2.78 (t, 4 H, J = 5.1 Hz, NCH_2CH_2OH), 3.43-3.46 (m, 1 H, CH₂OCH), 3.50 (t, 2 H, J = 5.1 Hz, NCH₂CH₂OCH), 3.59 (t, 4 H, J = 5.1 Hz, NCH₂CH₂OH); ¹³C NMR (CDCl₃) ppm 78.0, 67.0, 59.8, 56.9, 54.2, 28.4, 24.7, 24.3, 22.9, 22.8, 20.4; IR (liquid) 3399, 2939, 2863, 2850, 1471, 1446, 1101, 1086, 1044 cm⁻¹; MS for $C_{18}H_{37}NO_3$, m/z (relative intensity) 315 $(M^+, 0.2), 284 (29), 132 (8), 118 (100), 102 (10)$

2-[Bis[2-(cyclododecyloxy)ethyl]amino]ethanol (29). In a similar manner to the preparation of **27**, ethanolamine (2.3 g, 20.9 mmol), tosylate **26** (16.0 g, 41.8 mmol), and Na₂CO₃ (4.8 g) in CH₃CN (160 mL) afforded crude material which was chromatographed, eluting with CHCl₃/CH₃OH (40:1). The appropriate fractions were combined and concentrated in vacuo to yield a yellow oil, **29** (5.32 g, 52.8%): ¹H NMR (CDCl₃) δ 1.33–1.62 (m, 44 H, CH₂), 2.72–2.80 (m, 6 H, NCH₂), 3.38–3.42 (m, 2 H, OCH), 3.50 (t, 4 H, J = 5.9 Hz, OCH₂), 3.55 (t, 2 H, J = 5.1 Hz, CH₂OH); ¹³C NMR (CDCl₃) ppm 77.6, 67.0, 59.5, 56.7, 54.6, 28.5, 24.7, 24.3, 23.0, 22.9, 20.5; IR (liquid) 3454, 2936, 2863, 1471, 1446, 1104, 1088, 1052, 755 cm⁻¹; HI RES MS for C₃₀H₅₉NO₃ calcd 480.4416, found 480.4412, m/z (relative intensity) 481 (M⁺, 1), 450 (32), 438 (1), 298 (24), 284 (100), 268 (5), 252 (4), 240 (1), 224 (1), 132 (7), 118 (32), 102 (36). Anal. (C₃₀H₅₉NO₃) C, H, N.

3,3'-[[[2-(Cyclododecyloxy)ethyl]imino]bis(2,1ethanediyloxy)]bis[propanenitrile] (30). Alcohol 27 (8.7 g, 27.6 mmol) was dissolved in tert-butyl alcohol (140 mL). Sodium methoxide (0.17 g) was added, and the solution was stirred at room temperature. After the sodium methoxide had dissolved, acrylonitrile (3.6 mL, 55.2 mmol) was added, and the reaction was stirred at room temperature for 42 h. The solution was concentrated in vacuo, producing an orange oil which was chromatographed, eluting with CHCl₃/CH₃OH (20:1). The appropriate fractions were combined and concentrated in vacuo, yielding the product 30, as a pale yellow oil (5.91 g, 50.7%): ¹H NMR (CDCl₃) δ 1.33 (s, 18 H, CH₂), 1.47–1.60 (m, 4 H, OCHCH₂), 2.63 (t, 4 H, J = 6.3 Hz, CH_2CN), 2.75–2.84 (m, 6 H, NCH_2), 3.39–3.41 (m, 1 H, OCHCH₂CH₂), 3.47-3.61 (m, 6 H, OCH₂CH₂N), 3.68 (t, 4 $H, J = 6.3 Hz, OCH_2CH_2CN); {}^{13}C NMR (CDCl_3) ppm 117.9, 69.9,$ 66.7, 65.5, 54.9, 54.4, 28.6, 24.6, 24.2, 23.0, 22.9, 20.5, 18.8; IR (liquid) 2934, 2864, 2251, 1471, 1446, 1415, 1120, 756 cm⁻¹; HI RES MŠ (FAB) for $C_{24}H_{43}N_3O_3$ calcd 422.3382 found 422.3400, m/z(relative intensity) 422 (MH⁺, 100), 421 (7), 369 (18), 351 (15), 337 (34), 256 (13), 238 (37), 224 (87), 185 (15), 127 (34), 55 (75). Anal. $(C_{24}H_{43}N_3O_3)$ C, H, N.

3-[2-[Bis[2-(cyclododecyloxy)ethyl]amino]ethoxy]propanenitrile (31). In a similar manner to the preparation of 30, alcohol 29 (13.2 g, 27.4 mmol), NaOH (0.26 g), and acrylonitrile

⁽³¹⁾ For the preparation of a similar alcohol, see: Daignault, R. A.; Eliel, E. L. Cyclohexyloxyethanol. Organic Synthesis; Wiley: New York, 1973; Collect. Vol. V, pp 303-306.

(1.8 mL, 27.4 mmol) dissolved in tert-butyl alcohol (140 mL) afforded, after concentrating in vacuo, an orange oil. The oil was chromatographed, eluting first with CHCl₃ and then with $CHCl_3/CH_3OH$ (30:1). The appropriate fractions were combined and concentrated in vacuo to yield a yellow oil, 31 (8.55 g, 58.3%): ¹H NMR (CDCl₃) δ 1.33–1.61 (m, 44 H, CH₂), 2.60 (t, 2 H, J = 6.5 Hz, CH₂CN), 2.74-2.83 (m, 6 H, NCH₂), 3.37-3.41 (m, 2 H, OCH), 3.47-3.59 (m, 6 H, OCH₂CH₂N), 3.68 (t, 2 H, J = 6.5 Hz, OCH₂CH₂CN); ¹³C NMR (CDCl₃) ppm 117.9, 77.5, 70.0, 67.0, 66.9, 65.6, 59.5, 56.7, 55.0, 54.6, 54.5, 28.7, 28.5, 24.7, 24.3, 24.2, 23.0, 22.9, 20.6, 20.5, 18.7; IR (liquid) 3457, 2932, 2863, 2252, 1471, 1446, 1105, 1070 cm⁻¹; HI RES MS for C₃₃H₆₂N₂O₃ calcd 534.4760, found 534.4752, m/z (relative intensity) 451 (3), 450 (12), 351 (12), 338 (22), 337 (100), 284 (8), 268 (4), 171 (35), 155 (38). For testing, 2.9 g of this material was rechromatographed, eluting with $CHCl_3/CH_3OH$ (30:1) to yield 2.0 g of analytically pure material. Anal. (C₃₃H₆₂N₂O₃) C, H, N. ¹H and ¹³C NMR spectral data are identical to the data above.

3,3'-[[[2-(Cyclododecyloxy)ethyl]imino]bis(2,1ethanediyloxy)]bis[propanamine] (32). Bisnitrile 30 (7.0 g, 16.6 mmol) was dissolved in 10% NH4OH/EtOH (95%) (150 mL) and hydrogenated over 5% Rh/alumina (1.4 g) at 35 psi for 24 h. The reaction mixture was filtered through a Celite pad, and the pad was washed with ethanol. The filtrate was concentrated in vacuo, producing a pale yellow oil. The oil was chromatographed, eluting with CHCl₃/CH₃OH/NH₄OH (20:8:1). The appropriate fractions were combined and concentrated in vacuo, producing a pale yellow oil. The oil was taken up in hexane and filtered. The filtrate was concentrated in vacuo. Solvent traces were removed under vacuum (0.1 mm) to yield 32 (3.75 g, 52.6%): ¹H NMR (CDCl₃) δ 1.33–1.59 (m, 22 H, CH₂), 1.73 (quintet, 4 H, J = 6.4 Hz, $CH_2CH_2NH_2$), 2.18 (s, 4 H, NH_2), 2.76–2.84 (m, 10 H, NCH₂ and CH₂NH₂), 3.35-3.41 (m, 1 H, OCHCH₂), 3.48-3.54 (m, 10 H, OCH₂); ¹³C NMR (CDCl₃) ppm 77.3, 69.1, 67.1, 55.0, 54.4, 39.4, 32.6, 28.7, 24.7, 23.0, 22.9, 20.5; IR (liquid) 3360, 2936, 2862, 1594, 1471, 1446, 1348, 1335, 1112 cm⁻¹; HI RES MS for $C_{24}H_{51}N_3O_3$ calcd 429.3930, found 429.3926, m/z (relative intensity) 355 (2), 342 (10), 341 (42), 286 (24), 268 (6), 246 (6), 232 (37), 177 (11), 114 (55), 97 (61), 88 (30), 56 (100). Anal. (C24-H₅₁N₃O₃) C, H, N. Corrected for 1.47% H₂O found by Karl Fisher analysis

3-[2-[Bis[2-(cyclododecyloxy)ethyl]amino]ethoxy]propanamine (33). In a manner similar to the preparation of 32, nitrile 31 (5.0 g, 9.35 mmol) in 10% NH₄OH/95% EtOH (80 mL) was hydrogenated over 5% Rh/alumina (0.50 g) at 35 psi for 22 h. Upon workup, the oil was chromatographed, eluting first with CHCl₃/CH₃OH (20:1) and then CHCl₃/CH₃OH/NH₄OH (20:8:1). The appropriate fractions were combined and concentrated in vacuo to yield a yellow oil. The oil was rechromatographed, eluting with 10% methanol/acetone. The appropriate fractions were combined and concentrated in vacuo to yield an orange oil, 33 (1.5 g, 30%): ¹H NMR (CDCl₃) δ 1.33-1.36 (m, 36 H, CH₂), 1.47-1.61 (m, 8 H, OCHCH₂), 1.74 (quintet, 2 H, J = 6.3 Hz, CH₂CH₂NH₂), 2.10 (s, 2 H, NH₂), 2.74-2.85 (m, 8 H, NCH₂ and CH2NH2), 3.37-3.41 (m, 2 H, CHOCH2), 3.49-3.55 (m, 8 H, OCH₂); ¹³C NMR (CDCl₃) ppm 77.4, 69.2, 69.1, 66.7, 54.8, 54.5, 39.5, 32.6, 28.7, 24.6, 24.2, 23.0, 22.9, 20.6; IR (liquid) 3419, 2936, 2863, 1470, 1446, 1337, 1105 cm⁻¹; HI RES MS for C₃₃H₆₆N₂O₃ calcd 538.5073, found 538.5064, m/z (relative intensity) 538 (M⁺, 2), 537 (3), 464 (7), 450 (46), 355 (19), 341 (100), 296 (10), 286 (24), 266 (20), 252 (9). Anal. $(C_{33}H_{66}N_2O_3)$ C, H, N. Corrected for 0.75% H₂O found by Karl Fisher analysis.

2-[Bis[2-(cyclododecyloxy)ethyl]amino]-1-phthalimidoethane (34). Phthalimide (2.8 g, 19.1 mmol) was added to a solution of the alcohol 29 (9.2 g, 19.1 mmol) in THF (100 mL). Diethyl azodicarboxylate (3.0 mL, 19.1 mmol) was added, and the solution was cooled to 0 °C. Triphenylphosphine (5.0 g, 19.1 mmol) was added portionwise. The solution was stirred at 0 °C for approximately 10 min. The solution was then allowed to warm to room temperature and was stirred for 22 h. The solution was concentrated in vacuo, producing a green oil. The oil was dissolved in H₂O (110 mL), adjusted to pH 12 with 20% NaOH, and extracted with CHCl₃ (4 × 110 mL). The organic layers were combined, dried with MgSO₄, filtered, and concentrated in vacuo. The residue was chromatographed, eluting with 2% CH₃OH/ CH₂Cl₂. The appropriate fractions were combined and concentrated in vacuo, producing a green oil. Solvent traces were removed under vacuum (0.1 mm) with heating to yield **34** (8.47 g, 72.6%): ¹H NMR (CDCl₃) δ 1.32 (s, 36 H, CH₂), 1.41–1.58 (m, 8 H, OCHCH₂), 2.76 (t, 4 H, J = 6.4 Hz, NCH₂CH₂O), 2.85 (t, 2 H, J = 6.6 Hz, NCH₂CH₂NC=O), 3.31–3.33 (m, 2 H, OCH), 3.43 (t, 4 H, J = 6.4 Hz, NCH₂CH₂O), 3.76 (t, 2 H, J = 6.6 Hz, CH₂NC=O), 7.69–7.73 (m, 2 H, arom), 7.81–7.85 (m, 2 H, arom); ¹³C NMR (CDCl₃) ppm 168.2, 133.6, 132.1, 123.0, 77.3, 66.9, 54.5, 52.7, 36.3, 28.5, 24.7, 24.3, 23.0, 22.9, 20.5; IR (liquid) 2936, 2862, 1775, 1716, 1615, 1469, 1445, 1395, 719 cm⁻¹; HI RES MS for C₃₈H₆₂N₂O₄ calcd 611.4788, found 611.4797, *m/z* (relative intensity) 611 (MH⁺, 51), 610 (12), 450 (13), 427 (25), 413 (24), 203 (18), 174 (82), 160 (9), 97 (21), 83 (47), 69 (61), 55 (100). Anal. (C₃₈H₆₂N₂O₄) C, H, N. Corrected for 0.80% CH₂Cl₂.

N,N-Bis[2-(cyclododecyloxy)ethyl]-1,2-ethanediamine (35). Hydrazine monohydrate (4.8 mL, 98.0 mmol) was added to a solution of the imide 34 (6.0 g, 9.8 mmol) in absolute ethanol (260 mL). The solution was heated at reflux for 22 h. After 22 h, the reaction mixture was allowed to cool to room temperature and was concentrated in vacuo. The residue was dissolved in H₂O (200 mL), adjusted to pH 12 with 20% NaOH, and extracted with $\rm CHCl_3$ (5 \times 200 mL). The organic layers were combined, dried, filtered, and concentrated in vacuo, producing a pale green oil. The oil was chromatographed, eluting with EtOAc/CH₃OH. The appropriate fractions were combined and concentrated in vacuo. The residue was taken up in hexane and filtered. The filtrate was concentrated in vacuo, and solvent traces were removed under vacuum (0.1 mm) to yield 35 as a colorless oil (3.71 g, 78.7%): ¹H NMR (CDCl₃) δ 1.35-1.40 (m, 36 H, CH₂), 1.49-1.61 (m, 8 H, OCHCH₂), 2.59 (t, 2 H, J = 5.7 Hz, NCH₂CH₂NH₂), 2.68–2.73 $(m, 2 H, CH_2NH_2), 2.70 (t, 4 H, J = 6.4 Hz, NCH_2CH_2O), 3.37-3.41$ (m, 2 H, OCH), 3.49 (t, 4 H, J = 6.4 Hz, OCH₂); ¹³C NMR (CDCl₃) ppm 77.4, 67.0, 58.4, 54.8, 40.0, 28.7, 24.7, 24.2, 23.0, 22.9, 20.5; IR (liquid) 3372, 2935, 2863, 1678, 1588, 1470, 1446, 1103, 1076 cm⁻¹; MS for $C_{30}H_{60}N_2O_2$, m/z (relative intensity) 481 (MH⁺, 100), 480 (M⁺, 9), 464 (5), 450 (23), 297 (12), 283 (6), 252 (8), 97 (34), 83 (34), 69 (46), 55 (69). Anal. $(C_{30}H_{60}N_2O_2)$ C, H, N.

Preparation of 3b by the Removal of a Benzyl Group. N,N-Bis[2-(cyclododecylamino)ethyl]-1,2-ethanediamine (3b). Palladium black (14.0 g) was added to the reaction vessel and cooled to 0 °C. A 4.4% HCO₂H/CH₃OH solution (500 mL) was added via an addition funnel. A solution of the amine 36 in the HCO_2H/CH_3OH solution (100 mL) was added to the reaction mixture via an addition funnel. The reaction mixture was then stirred at room temperature for 1 h (until vigorous evolution of CO had ceased). The reaction was heated at 55 °C with stirring for 3.5 h. The reaction mixture was allowed to cool to room temperature and was filtered. The filtrate was concentrated in vacuo, producing a pale yellow oil. The oil was taken up in H_2O (125 mL), adjusted to pH 12 with 20% NaOH, and extracted with $CHCl_3$ (5 × 125 mL). The organic layers were combined, dried, filtered, and concentrated in vacuo to yield a pale green oil. The oil was dissolved in hexane and filtered. The filtrate was concentrated in vacuo to yield 3b (11.5 g, 97.3%): ¹H NMR (CDCl₃) δ 1.34 (s, 36 H, CH₂), 1.49-1.54 (m, 8 H, NHCHCH₂), 2.51 (t, 2 H, J = 6.1 Hz, NCH₂CH₂NH₂), 2.59–2.67 (m, 10 H, NCH₂CH₂NH and NHCH), 2.76 (t, 2 H, J = 6.1 Hz, CH₂NH₂); ¹³C NMR (CDCl₃) ppm 57.7, 55.2, 54.7, 45.3, 39.9, 29.1, 25.0, 24.4, 22.9, 22.8, 20.7; IR (liquid) 3349, 2932, 2903, 2862, 1682, 1470, 1445, 753 cm⁻¹; MS for $C_{30}H_{62}N_4$, m/z (relative intensity) 478 (M⁺, 1) 448 (6), 294 (3), 282 (100), 270 (42), 253 (10), 239 (2), 210 (11), 196 (28), 182 (3). Anal. $(C_{30}H_{62}N_4)$ C, H, N. Corrected for 0.46% H₂O found by Karl Fisher analysis.

3-[[2-[Bis[2-(cyclododecylamino)ethyl]amino]ethyl]amino]propanenitrile (40). Polyamine 3b (10.0 g, 20.9 mmol) was dissolved in methanol, and the solution was cooled to 0 °C. Acrylonitrile (1.4 mL, 20.9 mmol) was added and the solution was stirred for 30 min at 0 °C. The reaction was then stirred at room temperature for 5 h. The solution was concentrated in vacuo, producing a yellow oil. The oil was chromatographed, eluting with CHCl₃/CH₃OH and then with CHCl₃/CH₃OH/NH₄OH (10:4:1). The appropriate fractions were combined and concentrated in vacuo, and solvent traces were removed under vacuum (0.1 mm) to yield 40 (8.21 g, 73.9%), as a yellow oil: ¹H NMR (CDCl₃) δ 1.25-1.45 (m, 36 H, CH₂), 1.48-1.55 (m, 8 H, HNCHCH₂), 2.50 (t, 2 H, J = 6.8 Hz, CH₂CN), 2.55-2.75 (m, 14 H, HNCH₂CH₂N and HNCH), 2.93 (t, 2 H, J = 6.1 Hz, HNCH₂CH₂CH); ¹³C NMR (CDCl₃) ppm 118.5, 55.2, 54.8, 54.2, 47.0, 45.3, 29.2, 25.0, 24.5, 22.9, 22.8, 20.7, 18.6; IR (liquid) 3296, 2933, 2903, 2248, 1470, 1446 cm⁻¹; HI RES MS for C₃₃H₆₆N₅ calcd 532.5318, found 532.5352, m/z (relative intensity) 532 (MH⁺, 100), 531 (6), 448 (5), 347 (8), 335 (9), 210 (13), 196 (21), 109 (14), 44 (23). Anal. (C₃₃H₆₅N₅) C, H, N.

N-[2-[Bis[2-(cyclododecylamino)ethyl]amino]ethyl]-1,3propanediamine (41). In a manner similar to the preparation of 32, nitrile 40 (5.0 g, 9.4 mmol) in NH₄OH/95% EtOH (9:1, 100 mL) was hydrogenated over 5% Rh/alumina for 22 h. Upon workup the oil was chromatographed, eluting with CHCl₃/ CH₃OH/NH₄OH (20:8:1). The resulting material was taken up in hexane and filtered. The filtrate was concentrated in vacuo, and solvent traces were removed under vacuum (0.1 mm).

In Vivo Screen. Male Japanese quail were reared at Miles Quail Farm, Gobles, MI, from a colony of animals originally derived at The Upjohn Co. Birds, 4–6 weeks of age, were randomly distributed into groups of 10 quail each. They were housed individually in 10-cage units and fed a commercial diet (Purina Game Bird Layena, Ralston Purina Co., St. Louis, MO) mixed with 0.5% cholesterol and 1% peanut oil. Test compounds were mixed for 20 min into 2.4 kg of the diet using a Hobart A-200 mixer. Experimental groups contained from 8 to 10 animals. Control groups (n = 17-20 animals) received diet alone, and positive control groups (n = 8-10 animals) received diet mixed with colestipol hydrochloride (1000 mg/kg per day). After 2 weeks, each bird was bled from the right jugular vein, and serum samples were obtained after low-speed centrifugation.

 β - and α -lipoproteins (VLDL + LDL and HDL, respectively) were isolated from individual serum samples by precipitation using PEG-8000 in 0.2 M glycine buffer, pH 9. Serum (300 μ L) was mixed with 300 μ L of solution A (20 g of PEG-8000 + 100 mL of glycine buffer), and after 10 min at room temperature the samples were centrifuged for 45 min at 2000g at 4 °C. The α -lipoprotein supernatant was decanted, and the β -lipoprotein pellet was dissolved in 300 μ L of solution B (10 mL of Triton X-100 + 1 L of Milli Q water). Cholesterol concentrations in the α - and β -lipoprotein fractions were measured using a Demand autoanalyzer (Cooper Biomedical, Diagnostics Division, Freehold, NJ) and Demand enzymatic reagents.

Statistical Methods. Cholesterol values were transformed to logarithms to achieve more homogeneous within-group variances. The mean response for each test compound was calculated using the transformed data. Treated:control ratios (T/C) of antilogs of the log means were then calculated and analyzed. In order to compare the test compounds to CH, which was also run in each test at 1000 mg/kg, a relative potency value was calculated.³² To do this we used the linear relationship that exists between T/C and log (dose). Using the linear portions of dose response data from seven experiments for CH and nine experiments (data for only eight are presented in Table V) for different test compounds, there was no lack of homogeneity detected between the individual slopes (p = 0.31) so a common slope of -0.79 (SE = 0.08) was computed.

Since this was a screening environment, many of the compounds were tested at only one dose. Therefore, to be able to rank and compare the various compounds, the relative potency was computed as the ratio of the doses of the compound and colestipol hydrochloride (CH) that produce a T/C = 0.5 using the equation log (dose of CH or drug to produce a T/C = 0.5) = $(T/C - 0.5)/(-0.79) + \log$ (dose tested) and the relative potency = $10^{(\log dose of CH)-\log(dose of drug)}$.

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Registry No. 1, 4097-89-6; 2a, 112647-36-6; 2b, 129323-67-7; 2b.4HCl, 139200-93-4; 2c, 129323-91-7; 2d, 129323-75-7; 2e, 139200-90-1; 2f, 129323-64-4; 2g, 129323-87-1; 3a, 129323-73-5; 3b, 129323-68-8; 3b-MeSO₂H, 139200-94-5; 3b-2HCl, 139200-95-6; 3b-3HCl, 139200-96-7; 3b-4HCl, 139200-97-8; 3d, 129323-76-8; 4b, 129323-69-9; 4b-3HCl, 139200-98-9; 4c, 129323-74-6; 5a, 129323-65-5; 5b, 129323-81-5; 5c, 129323-79-1; 5d, 129323-95-1; 5e, 139200-91-2; 5f, 129323-88-2; 6c, 129323-80-4; 8, 129323-85-9; 9, 129323-94-0; 10, 129345-65-9; 11a, 129345-63-7; 11b, 129323-70-2; 12, 129323-92-8; 13, 129323-89-3; 14, 139201-11-9; 15, 139201-08-4; 18, 139201-12-0; 19, 139201-13-1; 20, 139201-14-2; 21a, 139200-92-3; 21b, 129323-71-3; 21c, 129323-93-9; 22, 139201-07-3; 23, 139201-01-7; 24, 139201-10-8; 24-3HCl, 139201-00-6; 25, 129345-64-8; 26, 139201-15-3; 26 (alcohol), 32399-56-7; 27, 129323-72-4; 29, 139201-16-4; 30, 139201-17-5; 31, 139201-18-6; 32, 139201-09-5; 33, 139201-19-7; 34, 139201-20-0; 35, 139201-21-1; 36, 139201-04-0; 36-3HCl, 139200-99-0; 37, 139201-06-2; 38, 139201-03-9; 39, 139201-02-8; 40, 139242-69-6; 41, 139242-70-9; 42, 139201-05-1; cyclopentadecanone, 502-72-7; cyclododecanone, 830-13-7; cyclohexanone, 108-94-1; 1,3-dioxaspiro[4.5]decane, 177-10-6; methyl eicosanoate, 1120-28-1; octadecanamide, 124-26-5; cyclo-hexadecanone, 2550-52-9; heptadecanal, 629-90-3; 2-trifluoromethyl-benzeneacetaldehyde, 30934-62-4; benzeneacetaldehyde, 100-52-7; 4-phenyl-benzeneacetaldehyde, 61502-90-7; 3,4-bis-(phenylmethoxy)benzeneacetaldehyde, 95301-32-9; nonanal, 124-19-6; methyl octadecanoate, 112-61-8; acrylonitrile, 107-13-1; methyl acrylate, 96-33-3; ethylenediamine, 107-15-3; cyclododecanone ketal, 650-06-6; diethanolamine, 111-42-2; ethanolamine, 141-43-5; phthalimide, 85-41-6; cholesterol, 57-88-5.

⁽³²⁾ Finney, D. J. Statistical Method in Biological Assay; Charles Griffin and Co.: London, 1964; pp 125–127.