Polyamine Analogues of 3β-[*N*-(*N*′,*N*′-Dimethylaminoethane)carbamoyl]-cholesterol (DC-Chol) as Agents for Gene Delivery**

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Abstract: Cationic liposomes are rapidly proving very effective at mediating the delivery of genes to cells in vitro. Moreover, the use of cationic liposomes for gene delivery in vivo is now under consideration. In previous work, we were able to demonstrate that cationic liposomes, formulated from 3β-[*N*-(*N*′,*N*′-dimethylaminoethane)carbamoyl]cholesterol (DC-Chol) and the neutral phospholipid, dioleoyl L-α-phosphatidylethanolamine (DOPE), were able to transfect the lungs of mice in vivo. However, it rapidly became apparent that substantial improvements in the gene delivery efficiency, by approximately two orders of magnitude, would be needed for human lung transfection to be possible. In the following paper we describe the synthesis of a range of polyamine analogues of DC-Chol, which were formulated into cationic liposomes with DOPE and evaluated for efficiency of gene delivery in vitro and in vivo in mice. We report that cationic liposomes formulated from DOPE and the novel pentamine N^{15}-cholesteryloxycarbonyl-3,7,12-triazapentadecane-1,15-diamine (CTAP) were 100 times more efficient than DC-Chol/DOPE liposomes at gene delivery in vivo (500 times more effective than DNA alone). Therefore, we believe that CTAP/DOPE cationic liposomes should have clinical applications in human gene therapy approaches to the treatment of lung disorders as well as to other clinical conditions.

Keywords: cations · drug delivery · gene technology · lipids · liposomes

Introduction

Genetic trait analysis will eventually be able to identify all the genetic loci which cause or contribute towards disease. With this information, a corrective gene or genes may be identified which, if introduced into the appropriate organs and cells of the body in vivo, should correct the basic pathophysiological defect of the disease. This is the basic concept of gene therapy. Such a simple approach should be capable of curing the disease, in contrast to most conventional pharmaceutical approaches, which typically treat symptoms only. However, introducing a potentially corrective gene or genes is not straightforward. Whilst naked DNA may be administered under certain circumstances, for the most part a delivery vehicle or vector is required to effect efficient gene delivery. Several physical, chemical and virus-based vector systems are known, but none are sufficiently efficacious for general use in human gene therapy. In spite of this, some vectors are showing some promise, in particular gene transfer systems based on cationic liposomes.[1]

Cationic liposomes are heterogeneous lipid vesicles, typically formed from either a single cationic amphiphile (sometimes known as a cytofectin) or more commonly from a combination of a cationic amphiphile and a neutral lipid. They mediate gene delivery by interacting electrostatically with negatively charged DNA sequences to form complexes which may enter cells by endocytosis[2] or phagocytosis[3] and then release DNA for expression in the cell nucleus.[1] We have shown that cationic liposomes formed from the cationic amphiphile 3β-[*N*-(*N*′,*N*′-dimethylaminoethane)carbamoyl]-cholesterol (DC-Chol, **1**) and the neutral phospholipid dioleoyl L-α-phosphatidylethanolamine (DOPE, **2**) were able to transfect the lungs of mice in vivo.[4] Since then, some preparatory human clinical trials have been performed with similar DC-Chol/DOPE cationic liposomes.[5] Both sets of experiments represent a proof in principle that gene therapy with cationic liposomes is possible. However, both sets of experiments also showed that DC-Chol/DOPE cationic liposomes are unlikely to be efficient enough at gene delivery for general use in human gene therapy. Moreover, it is difficult to make improvements in the absence of any understanding of

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[**] Cationic lipids for gene therapy, Part 1.

  0947-6539/98/0401-0137 $ 17.50+.25/0

cationic liposome structure – activity relationships. Therefore, in an attempt to understand some of the underlying chemical principles behind liposome-mediated gene delivery, we set out to make a systematic series of DC-Chol analogues which could be incorporated into cationic liposomes and evaluated for gene delivery. The following report outlines the synthetic routes taken and summarises our initial gene-delivery results in vitro and in vivo.

Results and Discussion

In order to make rational improvements to DC-Chol/DOPE liposomes a simple model for the association of **1** and **2** in the bilayer of a cationic liposome was devised (Figure 1). This was based upon the known behaviour of cholesterol in bilayer membranes[6] and a liposome model proposed by Felgner and co-workers.[7] Carbon atoms C1 to C9 of the oleoyl side chains

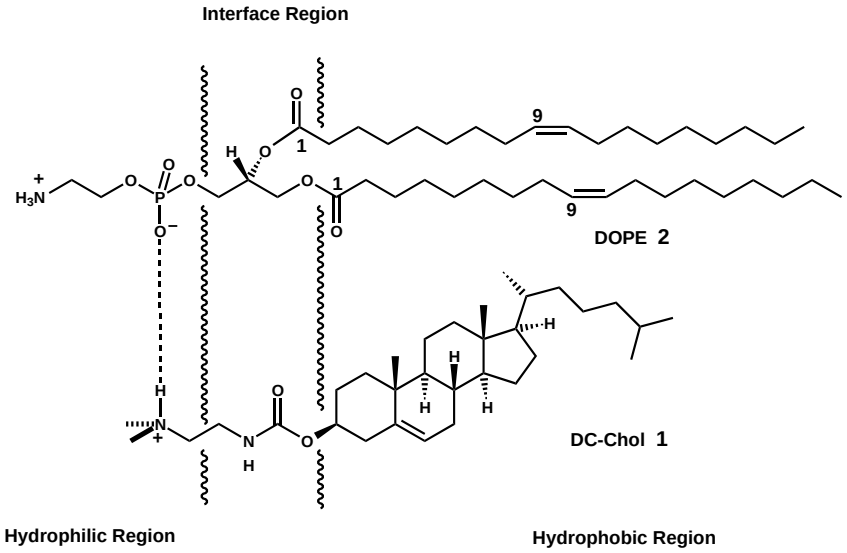

Figure 1. Putative alignment of DC-Chol **1** and DOPE **2** in cationic liposome bilayer.

of **2** pack against the four fused cholesterol rings of **1** so that the phosphate-ester group of **2** and the protonated tertiary amine functionality of **1** are aligned and neutralise each other. The positive charge of the liposome then derives from the protonated ethanolamine side-chain of **2**. On the basis of recent transfection experiments in mice and humans,[4,5] we anticipated that cationic liposomes formulated with polyamine analogues of either DC-Chol or DOPE, rather than **1** itself, would bind to DNA more tightly allowing for more efficient gene delivery. Since **2** is sensitive to oxidation of the oleoyl *cis* double bonds, we considered that it would be more appropriate to synthesise polyamine analogues of DC-Chol. The model (Figure 1) indicates that the methylene-group spacing between carbamoyl and the first amine functional group of a given DC-Chol polyamine analogue should be two or at most three in order to maintain charge complementation with **2**. Therefore, all our DC-Chol polyamine analogues were designed with this constraint in mind.

The syntheses of triamine analogues of DC-Chol were carried out as follows. Initially, amino alcohols **3** were *N*-protected with cholesteryl chloroformate, giving protected alcohols **4**, which were then oxidised by Swern-type oxidation[8] to give *N*-cholesteryloxycarbonylamino aldehydes **5** (Scheme 1). Typically, amino aldehydes can be quite unstable and prone to polymerisation, but the steric stabilising effect of the *N*-cholesteryl moiety resulted in crystalline compounds which could be stored for extended periods of time without any discernible decomposition. In parallel (Scheme 1), **3** were protected by smooth *N*-benzyloxycarbonylation,[9] converted into mesylates **6**[10] and then into *N*-benzyloxycarbonyl-protected amino azides **7**.[11] Finally, azides **7** were coupled to **5** by means of aza-Wittig methodology,[11,12] giving protected DC-Chol triamine analogues **8**, which were stored at this stage. In line with literature precedent[12] we found that aza-Wittig coupling reactions were more efficient with trimethylphosphine than with the customary triphenylphosphine. Also, the elimination of adventitious water with activated molecular sieves proved helpful in obtaining consistently high yields.[12] Prior to any gene delivery studies, protecting groups were removed by catalytic transfer hydrogenolysis to give triamine analogues **9** (Scheme 1) in 48 – 78 % overall yield.

The syntheses of tetramine analogues of DC-Chol were carried out in the following way. Initially, *N*-benzyloxycarbonyl-protected aminoalkyl bromides **10** were prepared by standard bromination of mesylates **6** (Scheme 2). Bromides **10** or mesylates **6,** as appropriate, were then used to mono-*N*-alkylate amino alcohols **3**, giving mono-*N*-benzyloxycarbonyl-protected diamino alcohols **11**, which were usually converted without purification into their crude *tert*-butyldiphenylsilyl ethers **12**. These crude ethers **12** were then treated directly with benzylchloroformate to give fully protected diamino ethers **13**, which were purified to homogeneity. Customarily, mono-*N*-alkylation of a primary amine is often considered difficult to control. Nevertheless, a combination of steric crowding in the reactants and mild reaction conditions have previously been shown to limit over-alkylation on N.[13] We found the same to be true here. Smooth fluoride-promoted desilylation of diamino ethers **13** then gave bona fide di-*N*-benzyloxycarbonyl-protected diamino alcohols **14**, which were efficiently converted into diamino azides **15** by mesylation followed by azidation (Scheme 2). Finally, protected DC-Chol tetramine analogues **16** were formed by coupling azides **15** to cholesterylamino aldehydes **5** by means of the the aza-Wittig procedure once more (Scheme 2). As for the preparation of **9** (Scheme 1), protecting groups were removed just prior to transfection studies by catalytic transfer hydrogenolysis, giving tetramine analogues **17** (Scheme 2) in 14 – 52 % overall yield. Gratifyingly, we found that the mono-*N*-alkylation procedure could be used equally well to prepare

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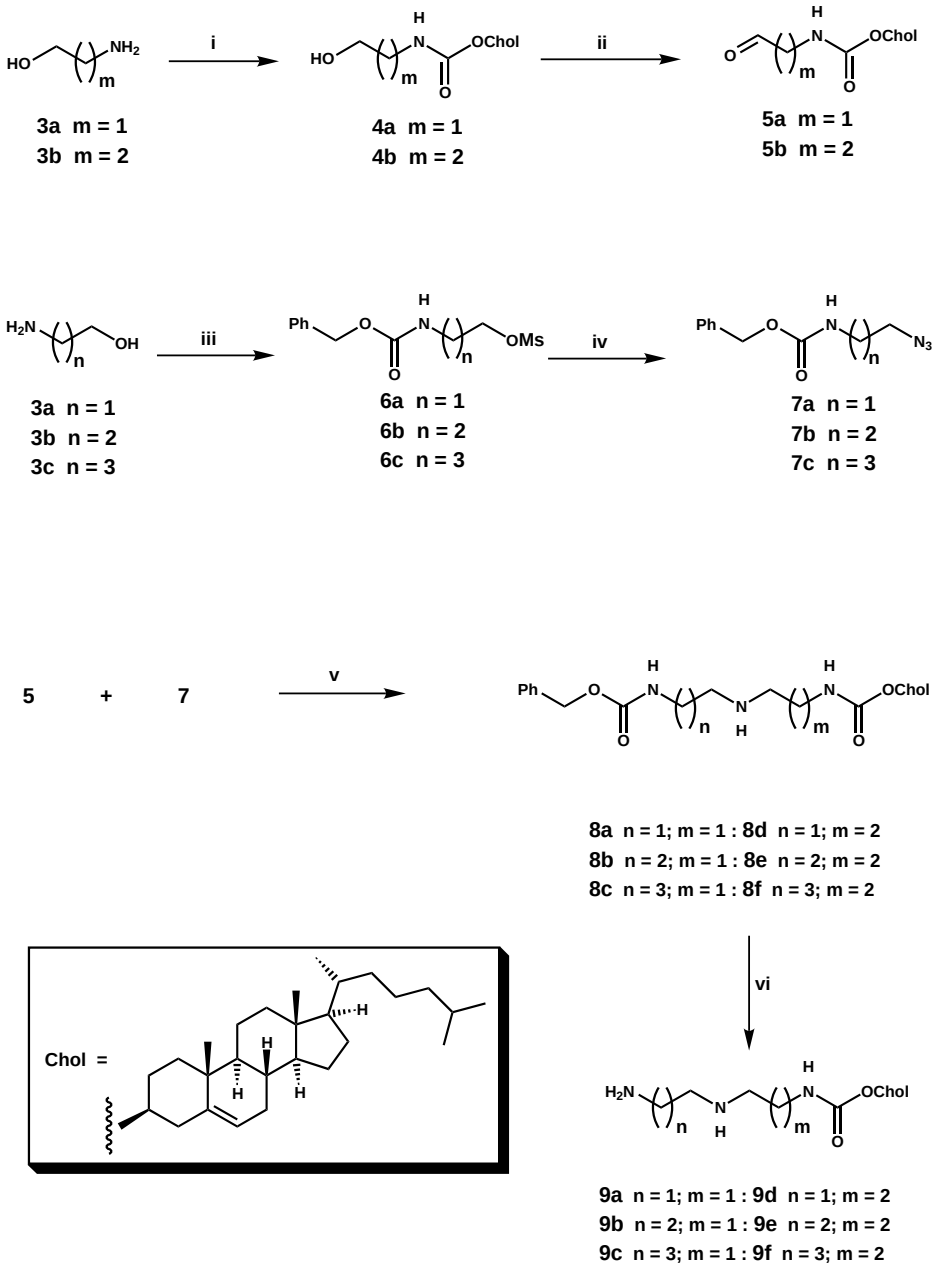
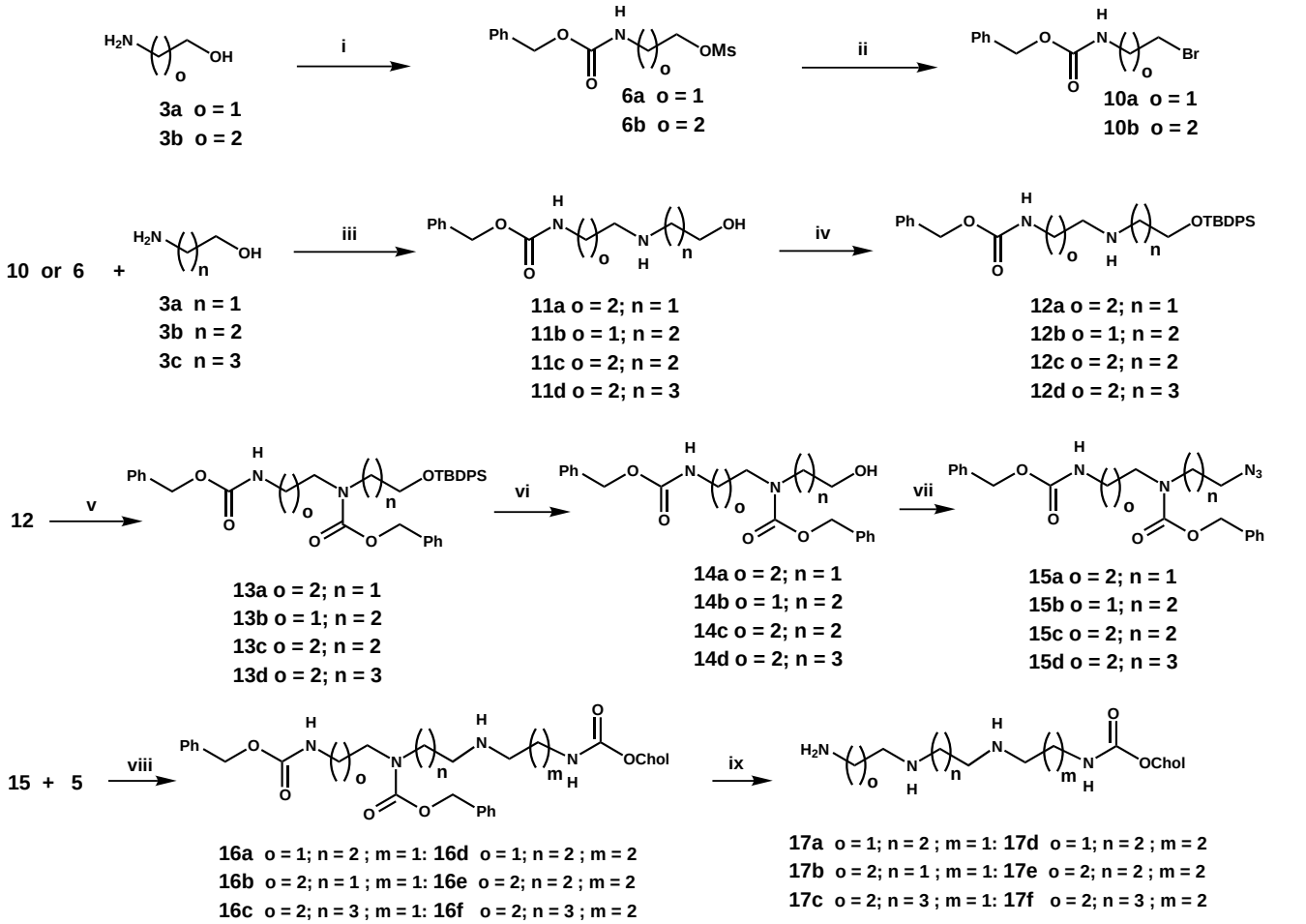


Scheme 1. Reagents: i) CH_2Cl_2, CholOC(O)Cl, 90–93%; ii) a) CH_2Cl_2, $(COCl)_2$, DMSO; b) **4** then *i*-Pr_2NEt, 90–95%; iii) a) CH_2Cl_2, $PhCH_2O$-C(O)Cl; b) CH_2Cl_2, Et_3N, CH_3SO_2Cl; iv) DMF, NaN_3, NaI, 2 stages (iii and iv) 68–87%; v) a) THF, **7**, 4 Å molecular sieves, PMe_3; b) **5** then EtOH, $NaBH_4$, 72–90%; vi) EtOH, *c*-C_6H_{10}, 10% Pd(C), 99%.

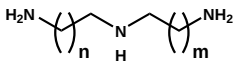
pentamine analogues of DC-Chol (Scheme 3). Firstly, *N*-cholesterylamino alcohol **4b** was converted into bromide **18**, which was then used to mono-*N*-alkylate amino alcohol **3c** giving a crude diamino alcohol product. This was immediately converted into a *tert*-butyldiphenylsilyl ether and *N*-protected by benzyloxycarbonylation giving homogeneous, fully protected diamino ether **19** after purification. Smooth desilylation of **19** resulted in a di-*N*-benzyloxycarbonyl-protected diamino alcohol **20**, which was then oxidised to diamino aldehyde **21**. Finally, several fully protected DC-Chol pentamine analogues **22** could be prepared by aza-Wittig coupling of **21** to di-*N*-benzyloxycarbonyl-protected diamino azides **15**. The free DC-Chol pentamine analogues **23** were prepared by hydrogenolysis of the protecting groups in the usual way (Scheme 3) in 18–42% overall yield.

The ability of cationic liposomes containing the different DC-Chol polyamine analogues to mediate gene delivery was analysed both in vitro and in vivo. Cationic liposomes were formulated by hydration of a dried lipid film containing a DC-Chol analogue and **2** in an appropriate molar ratio of 1:0, 1:1, 1:2 or 2:1, and vortex mixing.[14] Complexes of cationic liposomes and plasmid DNA were then prepared by adding appropriately diluted cationic liposome suspensions into equal volumes of aqueous plasmid DNA solutions at 30 °C and allowing the mixture to equilibrate to ambient temperature over 15 min.[14] In vitro studies were then performed

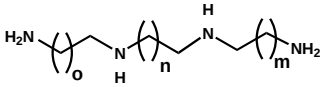


Scheme 2. Reagents: i) a) CH_2Cl_2, $PhCH_2OC(O)Cl$; b) CH_2Cl_2, Et_3N, CH_3SO_2Cl, 72–91%; ii) DMF, NaBr, 2 stages (i and ii) 68–87%; iii) $CHCl_3$ or DMF (only for **11c**), **3**, K_2CO_3, NaI (only for **11c**), **6** or **10**; iv) CH_2Cl_2, Et_3N, TBDPSCl, DMAP; v) CH_2Cl_2, $PhCH_2OC(O)Cl$, Et_3N, 3 stages (iii, iv and v) 58–82%; vi) THF, TBAF, 89–95%; vii) a) CH_2Cl_2, Et_3N, CH_3SO_2Cl; b) DMF, NaN_3, NaI, 80–96%; viii) a) THF, **15**, 4 Å molecular sieves, PMe_3; b) **5** then EtOH, $NaBH_4$, 54–80%; ix) EtOH, *c*-C_6H_{10}, 10% Pd(C), 99%.

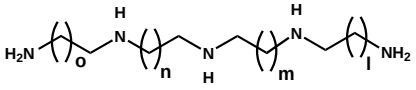
with immortalised cystic fibrosis airway epithelial (CFT1) cells followed by in vivo studies in which cationic liposome/plasmid DNA complexes were instilled intranasally into the lungs of female BALB/c mice.[14] Typically, cationic liposome gene delivery was first optimised in vitro so as to establish the best molar ratio of cationic liposome to plasmid DNA (DNA concentration was expressed as nucleotide concentration) as well as the best absolute quantities of both, as illustrated (Figure 2). This optimised combination was then tested in vivo. The in vitro results are shown (Figure 3). Six liposomes containing DC-Chol analogues conferred significant improvements on gene delivery efficiency over and above DC-Chol/DOPE liposomes formulated in a similar way. The analogues were **9a**, **9b**, **9e**, **9f**, **17a** and **23b**, the first four being triamines, the fifth a tetramine and the sixth a pentamine. With one exception (**9f**) these polyamine analogues contain inter-nitrogen methylene-group spacings not normally associated with the natural polyamines spermidine (**24**), spermine (**25**) and caldopentamine (**26**), upon which these structures are based. In vivo (Figure 4), the best DC-Chol analogues in liposomes were **17c**, **17f** and especially **23a**. Both **23a** and **17c** also contain unnatural methylene-group spacings. These in vivo results are a striking contrast to the in vitro data.



24 n = 3; m = 2



25 o = 2; n = 3 ; m = 2



26 l, m, n, o = 2

None of the six best DC-Chol analogues in vitro worked well in vivo. Likewise the best DC-Chol analogues in vivo performed poorly in vitro. However, liposomes formed from

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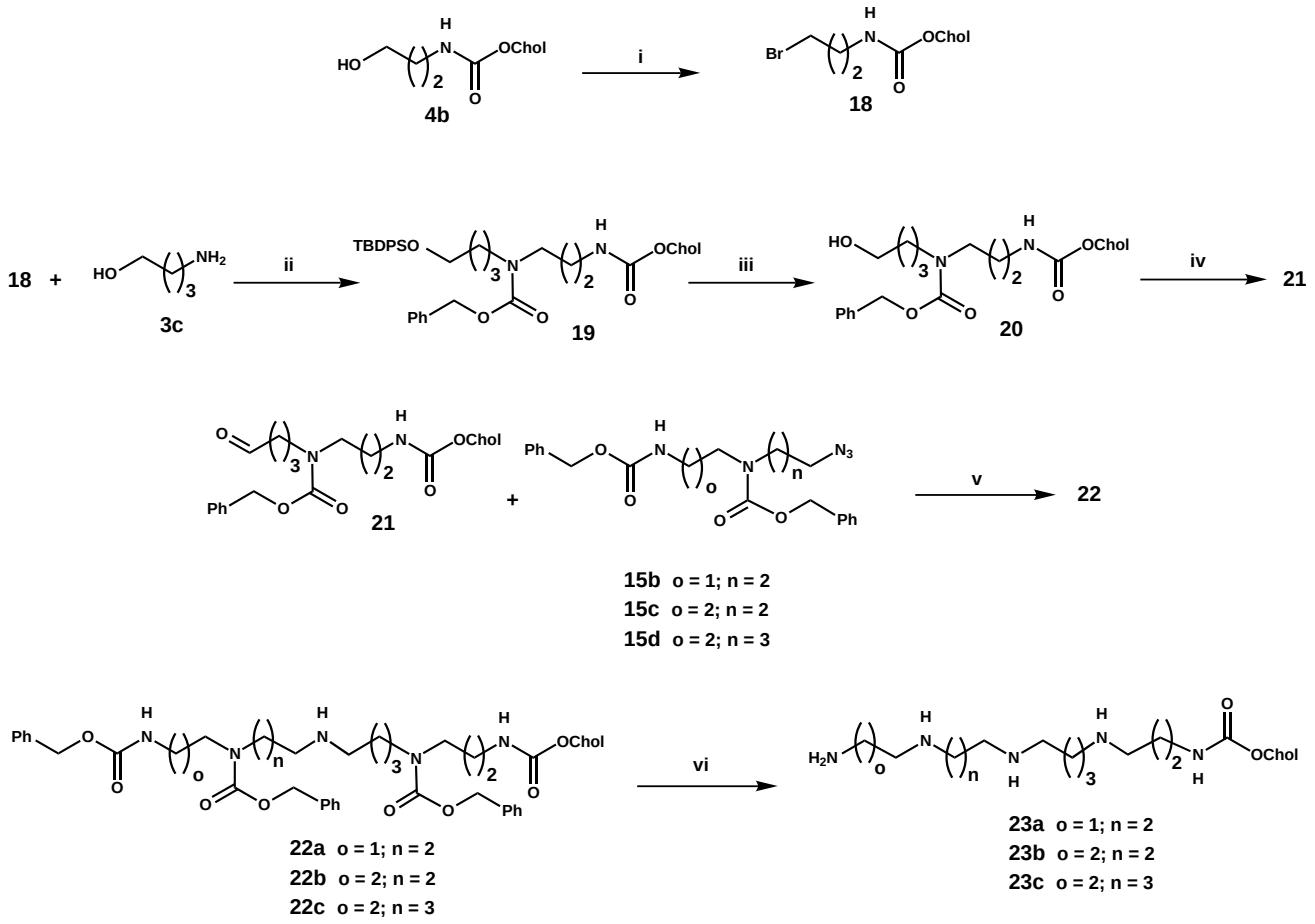
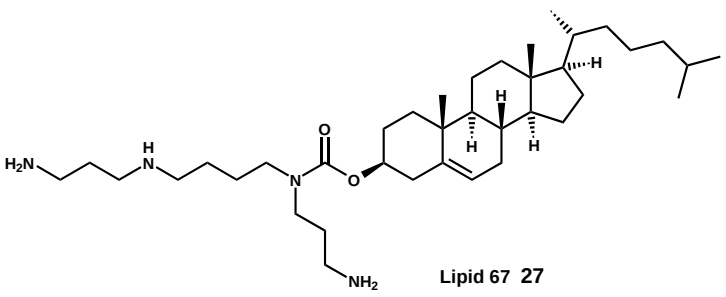
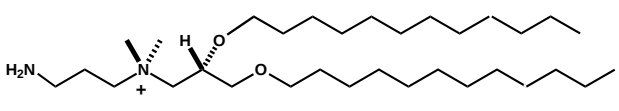


Scheme 3. Reagents: i) a) CH_2Cl_2, Et_3N, CH_3SO_2Cl; b) DMF, NaBr, 92%; ii) a) $CHCl_3$, **3c**, K_2CO_3, **18**; b) CH_2Cl_2, Et_3N, TBDPSCl, DMAP; c) CH_2Cl_2, $PhCH_2OC(O)Cl$, Et_3N, 78%; iii) THF, TBAF, 90%; iv) a) CH_2Cl_2, $(COCl)_2$, DMSO; b) **20** then *i*-Pr_2NEt, 94%; v) a) THF, **15**, 4 Å molecular sieves, PMe_3; b) **21** then EtOH, $NaBH_4$, 56–74%; vi) EtOH, *c*-C_6H_{10}, 10% Pd(C), 99%.

23a delivered genes about 100 times more effectively in mouse lung than those formed from **1**; that is, approximately 500 times better than plasmid DNA alone. Only liposomes containing one other cytofectin have been reported to function at this level of efficacy in vivo, namely those with lipid 67 (**27**), a T-shaped tetramine analogue of DC-Chol.[14] None of the other reported cationic liposomes appear to be close to this level of in vivo efficacy, with the possible exception of (±)-*N*-(3-aminopropyl)-*N*,*N*-dimethyl-2,3-*bis*-(dodecyloxy)-1-propanaminium bromide (GAP-DLRIE, **28**)-containing liposomes, which have been reported to work about 100 times better than plasmid DNA alone.[15] The in vivo efficacy of the other known cationic liposomes either has not been reported, or is generally rather poor.[1,16,17] Analogues **9f** and **17f** have been reported previously, either without details of synthesis and characterisation,[14] or else as impure mixtures known as SpdC and SpC respectively.[16] In the former case, in vitro and in vivo gene-delivery using liposomes containing these cytofectins was found to be comparable with our results reported here.[14] In the latter case, SpC was reported not to work well and to be relatively toxic.[16] Our data with analogue **17f** do not support these observations.





Conclusion

In conclusion, we have developed flexible synthetic routes to DC-Chol polyamine analogues; this has allowed us to identify analogues with optimised methylene-group spacing between amine functional groups for both in vitro and in vivo gene delivery. On the

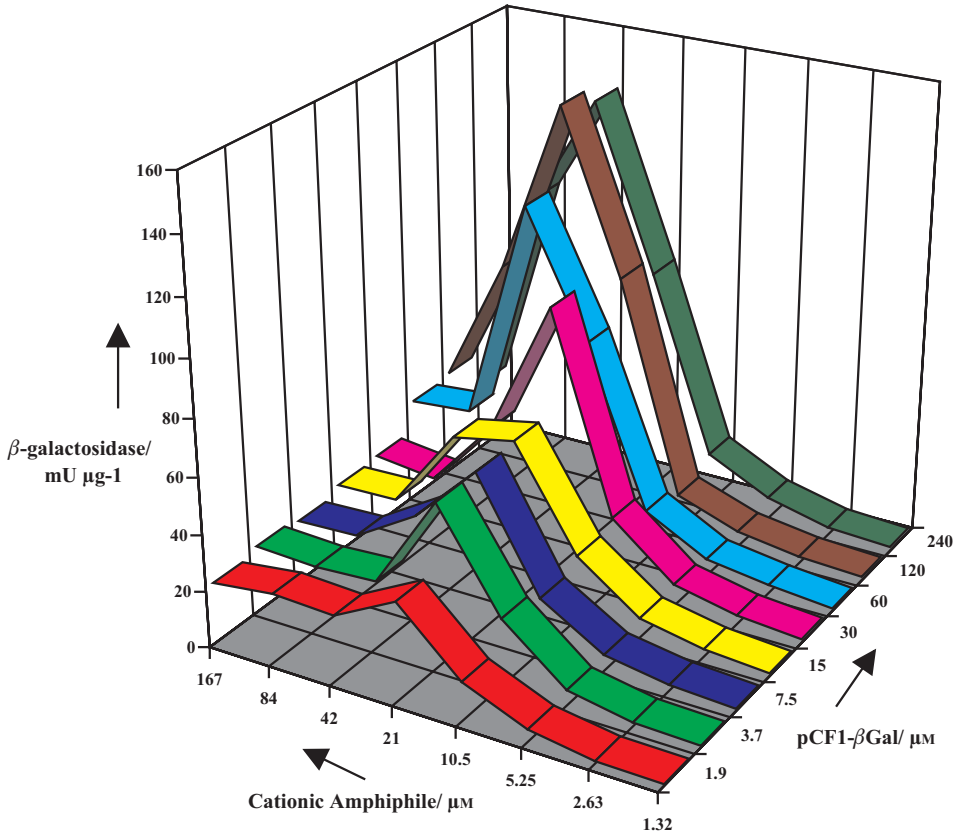


Figure 2. Example of in vitro optimisation of pCF1-βGal plasmid transfection of CFT1 cells with complexes of pCF1-βGal and cationic liposomes formulated from **9 f** and **2** (1:1) molar ratio. CFT1 cells were transfected with an array of different cationic liposome and plasmid ratios in a 96-well plate. The extent of transfection in each well was determined after 2 days by measuring the levels of β-galactosidase expression. Plasmid DNA concentration is expressed as the concentration of nucleotides assuming an average nucleotide formula weight of 330. Cationic liposome concentration is expressed in terms of the concentration of **9 f** alone.

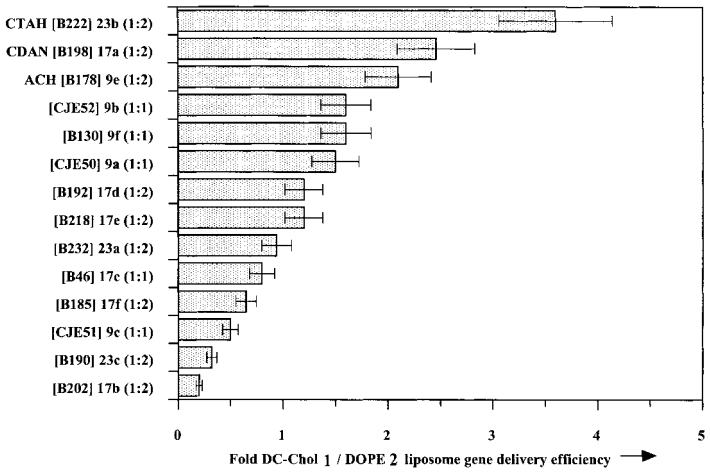


Figure 3. Rank order of DC-Chol polyamine analogues transfecting CFT1 cells in vitro with the pCF1-βGal plasmid. Gene-delivery activity is expressed as a proportion of the activity measured with standard liposomes containing **1** and **2**. The data shown are the averages of four separate experiments, each performed in triplicate. Ratios in curved brackets refer to the molar ratio of DC-Chol analogue:**2** used to formulate the liposomes. Numbers in square brackets refer to compound serial numbers. Where appropriate DC-Chol analogue name abbreviations are also included (see experimental section).

whole, cationic liposomes containing polyamines with unnatural methylene-group spacing appear to work better than those with natural spacing. Indeed, although the most effective DC-Chol polyamine analogues in vitro and in vivo are both pentamine analogues (**23b** and **23a** respectively), the methylene-group spacing almost appears to be a more critical factor in promoting efficient gene delivery than the absolute number of amine functional groups. We are not sure why this is. A possible reason is that liposomes containing such unnatural polyamines are able to interact with DNA more tightly than DC-Chol/DOPE cationic liposomes so as to promote efficient gene transfer across the outer cell membrane (as we anticipated in our original design process), but not so tightly that they are unable to release the DNA into the cell cytoplasm after transfer has taken place. In other words, by altering the methylene-group spacing of the liposome-associated polyamines, we may have been able in effect to tune the strength of the cationic-liposome/DNA interaction in order to find appropriate balance points at which in vitro and in vivo gene delivery are optimal. We are currently investigating this possibility further. What is certainly clear is that such a balance point must be different for in vitro and in vivo gene delivery; this would account for the apparent disparity between the performance of our cationic liposome formulations in vitro and in vivo. This disparity also suggests that whilst our in vitro studies may have assisted the process of formulating and

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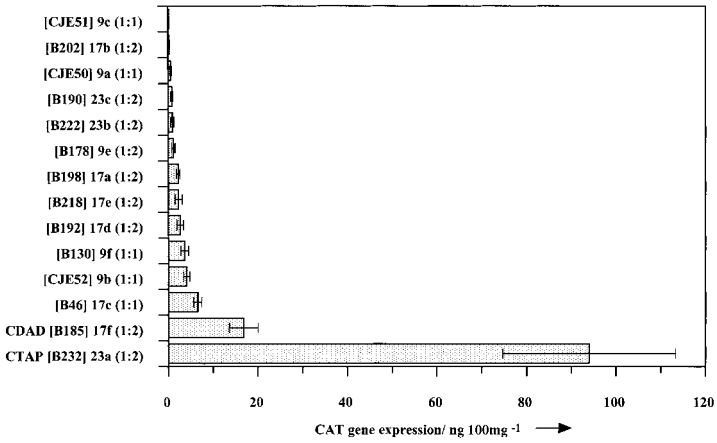

Figure 4. Rank order of DC-Chol polyamine analogues transfecting the lungs of female BALB/c mice with the pCF1-CAT plasmid. Mice were instilled with a solution of the plasmid (4 μm nucleotide concentration) and the optimal quantity and ratio of cationic liposome, in a total volume of 100 μL. Gene-delivery activity was determined as a function of chloramphenicol acetyl transferase activity in lung homogenates after 2 days. Data points were from separate experiments with each optimal formulation tested in 4 BALB/c mice. Ratios in curved brackets refer to the molar ratio of DC-Chol analogue:**2**. Numbers in square brackets refer to compound serial numbers. Where appropriate DC-Chol analogue name abbreviations are also included (see experimental section). The gene delivery efficiency of DC-Chol/DOPE liposomes (results not shown) is equivalent to the performance of liposomes containing DC-Chol analogue **9c**.[14]

optimising a cationic-liposome/DNA system for gene delivery, in vitro models which more closely reflect the in vivo environment will clearly be crucial if cationic liposome mediated gene delivery is to be efficiently optimised in the future.

Our best DC-Chol analogue in vivo, N^{15}-cholesteryloxycarbonyl-3,7,12-triazapentadecane-1,15-diamine (CTAP, **23a**) is a novel pentamine of a type not previously shown to transfect cells. The efficacy of gene delivery by cationic liposomes containing this compound easily reaches the level expected to be necessary for a cationic liposome to have a realistic chance of clinical use in the gene therapy of human lung disorders.[1,4,5,14] Therefore, the implicit goal of our work, to improve upon DC-Chol/DOPE cationic liposomes so as to derive a nonviral gene delivery system suitable for use in human gene therapy, is well on the way to being achieved.

Experimental Section

General: ^{1}H NMR spectra were recorded at ambient temperature (unless otherwise stated) on either Bruker AM500, Bruker DRX400, Bruker DRX300, or Jeol GX-270Q spectrometers, with residual nonisotopicaly labelled solvent (e.g., $CHCl_3$, $\delta_H = 7.26$) as an internal reference. ^{13}C NMR spectra were recorded on the same range of spectrometers at 125, 100, 75 and 68.5 MHz respectively, also with residual nonisotopically labelled solvent (e.g., $CHCl_3$, $\delta_C = 77.2$) as an internal reference. IR spectra were recorded on a Mattson 5000 FTIR spectrometer and mass spectra on a Micromass AutoSpecQ mass spectrometer. Where possible, elemental analyses were performed at the Imperial College Chemistry Department microanalytical laboratory. Melting points were recorded on a Reichert hot stage apparatus and are uncorrected. Chromatography refers to flash column chromatography, which was performed throughout on Merck Kieselgel 60 (230–400 mesh). Thin-layer chromatography was carried out on precoated Merck Kieselgel 60 F_{254} aluminium-supported plates; the plates were visualised after elution by either UV light (254 nm), iodine, 4,4′-bis(dimethylamino)benzhydrol in acetone, acidic ammonium molybdate(IV), aqueous potassium permanganate(VII), ethanolic vanillin or acidic methanolic 2,4-dinitrophenylhydrazine, as appropriate. Dichloromethane was distilled from phosphorus pentoxide before use. All other dry solvents and chemicals were purchased commercially from Aldrich (Poole, Dorset, UK).

Abbreviations: br: broad; Choc: cholesteryoxycarbonyl; Chol: cholesteryl; DMAP: *N*,*N*-dimethylaminopyridine; DMF: dimethyl formamide; DMSO: dimethyl sulfoxide; quin: quintet; TBAF: tetra-*n*-butylammonium fluoride; TBDPS: *tert*-butyldiphenylsilyl; THF: tetrahydrofuran; Z: phenylmethoxycarbonyl.

2-(Cholesteryloxycarbonyl)aminoethanol (**4a**): A solution of cholesteryl chloroformate (10.01 g, 22.28 mmol) in CH_2Cl_2 (100 mL) was added to a stirred solution of 2-aminoethanol (2.96 mL, 49.02 mmol, 2.2 equiv) in CH_2Cl_2 (145 mL) at 0 °C over a period of 15 min. After addition, the reaction mixture was allowed to warm to room temperature and stirred for 4 h. The reaction mixture was poured into saturated aqueous NH_4Cl (120 mL), the organic phase separated and the aqueous layer extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed with water (2 × 90 mL) and brine (130 mL), dried (Na_2SO_4) and the solvent evaporated under reduced pressure. The solid obtained was recrystallised (CH_2Cl_2/MeOH) to give **4a** as a white solid. Yield: 9.50 g (90%); m.p.: 168 °C; $R_f = 0.26$ (10% acetone/ether); IR (CH_2Cl_2): $\tilde{\nu}$ = 3353, 2942, 2870, 1693, 1674, 1562, 1467, 1382, 1264 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.35$ (d, $J = 5.0$ Hz, 1H, H6′), 5.29 (br s, 1H, ChocN*H*), 4.46 (m, 1H, H3′), 3.67 (m, 2H, H1), 3.30 (m, 2H, H2), 3.14 (s, 1H, O*H*), 2.32–2.23 (m, 2H, H4′), 2.01–1.77 (m, 5H, H2′, H7′, H8′), 1.58–1.01 (m, 21H, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 0.99 (s, 3H, H19′), 0.90 (d, $J = 6.5$ Hz, 3H, H21′), 0.84 (dd, J = 5.0, 2.0 Hz, 6H, H26′, H27′), 0.66 (s, 3H, H18′); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 157.01$ (NH*C*(O)O), 139.68 (C5′), 122.55 (C6′), 74.65 (C3′), 62.19 (C1), 56.65 (C14′), 56.15 (C17′), 49.96 (C9′), 43.35 (C2), 42.28 (C4′), 39.71 (C24′), 39.49 (C16′), 38.53 (C13′), 38.06 (C10′), 36.95 (C1′), 36.51 (C22′), 36.17 (C8′), 35.79 (C20′), 31.84 (C7′), 28.21 (C2′), 27.97 (C25′), 24.25 (C12′), 23.84 (C15′), 22.80 (C23′), 22.54 (C26′), 21.02 (C11′), 19.31 (C19′), 18.69 (C21′), 11.83 (C18′); MS (FAB$^+$): m/z = 496 $[M+Na]^+$, 474 $[M+H]^+$, 369 $[Chol]^+$, 255, 175, 145, 105, 95, 81, 43; HRMS (FAB$^+$) $C_{30}H_{52}NO_3$: $[M+H]^+$ calcd 474.3947, found 474.4020; $C_{30}H_{51}NO_3$ (473.4): calcd C 76.05, H 10.58, N 2.96; found C 75.94, H 10.48, N 3.00.

3-(Cholesteryloxycarbonyl)aminopropanol (**4b**): This was prepared from **3b** in a similar fashion to **4a** on a 13.4 mmol scale and after recrystallisation (CH_2Cl_2/MeOH) gave **4b** as a white solid. Yield: 6.05 g (93%); m.p.: 182 °C; $R_f = 0.33$ (10% acetone/ether); IR (CH_2Cl_2): $\tilde{\nu}$ = 3351, 2936, 2904, 2868, 1726, 1537, 1467, 1380, 1266, 1037 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 5.33$ (d, $J = 3.5$ Hz, 1H, H6′), 5.20 (t, $J = 7.0$ Hz, 1H, ChocN*H*), 4.43 (m, 1H, H3′), 3.62 (q, $J = 5.5$ Hz, 2H, H1), 3.38 (m, 1H, O*H*), 3.26 (q, $J = 6.0$ Hz, 2H, H3), 2.40–2.17 (m, 2H, H4′), 1.99–1.78 (m, 5H, H2′, H7′, H8′), 1.65 (quin, $J = 6.0$ Hz, 2H, H2), 1.55–0.99 (m, 21H, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 0.96 (s, 3H, H19′), 0.87 (d, $J = 6.5$ Hz, 3H, H21′), 0.82 (dd, $J = 5.5$, 1.0 Hz, 6H, H26′, H27′), 0.64 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 157.19$ (NH*C*(O)O), 139.72 (C5′), 122.54 (C6′), 74.55 (C3′), 59.44 (C1), 56.69 (C14′), 56.20 (C17′), 50.01 (C9′), 42.31 (C4′), 39.75 (C3), 39.53 (C16′), 38.57 (C24′), 37.54 (C2), 37.00 (C1′), 36.55 (C22′), 36.21 (C8′), 35.81 (C20′), 32.62 (C7′), 28.23 (C2′), 28.16 (C25′), 24.29 (C12′), 23.88 (C15′), 22.82 (C23′), 22.57 (C26′), 21.06 (C11′), 19.34 (C19′), 18.74 (C21′), 11.87 (C18′); MS (FAB$^+$): m/z = 975 $[2M+H]^+$, 488 $[M+H]^+$, 444 $[M+H-CO_2]^+$, 369 $[Chol]^+$, 255, 145, 121, 95; HRMS (FAB$^+$) $C_{31}H_{54}NO_3$: $[M+H]^+$ calcd 488.4104, found 488.4055; $C_{31}H_{53}NO_3$ (487.4): calcd C 76.32, H 10.96, N 2.87; found C 76.36, H 10.81, N 2.89.

2-(Cholesteryloxycarbonyl)aminoethanal (**5a**): A solution of DMSO (2.84 mL, 40.03 mmol, 3 equiv) in CH_2Cl_2 (40 mL) was added through a cannula over 15 min to a stirred solution of ethanedioyl chloride (10.01 mL of a 2.0 M solution in CH_2Cl_2, 20.01 mmol, 1.5 equiv) in CH_2Cl_2 (40 mL) at −78 °C under a nitrogen atmosphere. The resulting mixture was then stirred for 10 min, and then a solution of **4a** (6.32 g, 13.34 mmol) in CH_2Cl_2

(120 mL) added dropwise through a cannula over 15 min. After a further 20 min, iPr$_2$NEt (6.97 mL, 40.03 mmol, 3 equiv) was slowly added and the solution allowed to warm to room temperature. The pale yellow solution was poured into water (50 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed with saturated aqueous NH_4Cl (100 mL), water (2 × 60 mL) and brine (100 mL) and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave a yellow oil, which on purification by chromatography (70% ether/petrol to 30% acetone/ether), gave **5a** as a white solid. Yield: 5.96 g (95%); m.p.: 230 °C (decomp.); R_f = 0.32 (ether); IR (CH_2Cl_2): $\tilde{\nu}$ = 3356, 2937, 2868, 1699, 1537, 1467, 1378, 1265, 1137 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.66 (s, 1H, C*H*O), 5.38 (m, 2H, ChocN*H*, H6′), 4.50 (m, 1H, H3′), 4.13 (d, J = 4.5 Hz, 2H, H2), 2.36–2.18 (m, 2H, H4′), 2.04–1.78 (m, 5H, H2′, H7′, H8′), 1.60–1.07 (m, 21H, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 1.03 (s, 3H, H19′), 0.92 (d, J = 6.5 Hz, 3H, H21′), 0.87 (dd, J = 5.5, 1.0 Hz, 6H, H26′, H27′), 0.69 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 196.85 (*C*HO), 156.00 (NH*C*(O)O), 139.64 (C5′), 122.68 (C6′), 75.12 (C3′), 56.71 (C14′), 56.18 (C17′), 51.62 (C2), 50.02 (C9′), 42.33 (C4′), 39.76 (C24′), 39.54 (C16′), 36.56 (C22′), 36.21 (C8′), 35.82 (C20′), 31.89 (C7′), 28.25 (C2′), 28.03 (C25′), 24.31 (C12′), 23.87 (C15′), 22.84 (C23′), 22.59 (C26′), 21.07 (C11′), 19.35 (C19′), 18.74 (C21′), 11.88 (C18′); MS (FAB$^+$): m/z = 369 [Chol]$^+$, 230, 159, 145, 119, 105, 95, 81, 69, 55; $C_{30}H_{49}NO_3$ (471.4): calcd C 76.37, H 10.48, N 2.97; found C 73.85, H 10.02, N 2.87.

3-(Cholesteryloxycarbonyl)aminopropanal (5b): This was synthesised from **4b** similarly to the preparation of **5a** on a 14.4 mmol scale followed by chromatography (ether) to give **5b** as a white solid. Yield: 6.29 g (90%); m.p.: 178 °C; R_f = 0.45 (80% ether/acetone); IR (CH_2Cl_2): $\tilde{\nu}$ = 3346, 2937, 2875, 1716, 1693, 1467, 1380, 1264 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 9.81 (s, 1H, C*H*O), 5.38 (d, J = 4.0 Hz, 1H, H6′), 4.99 (m, 1H, ChocN*H*), 4.48 (m, 1H, H3′), 3.46 (q, J = 6.0 Hz, 2H, H3), 2.71 (t, J = 6.0 Hz, 2H, H2), 2.38–2.21 (m, 2H, H4′), 2.04–1.78 (m, 5H, H2′, H7′, H8′), 1.63–1.04 (m, 21H, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 1.01 (s, 3H, H19′), 0.92 (d, J = 6.5 Hz, 3H, H21′), 0.87 (d, J = 6.5 Hz, 6H, H26′, H27′), 0.68 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 201.22 (*C*HO), 156.07 (NH*C*(O)O), 139.78 (C5′), 122.51 (C6′), 74.50 (C3′), 56.71 (C14′), 56.18 (C17′), 50.03 (C9′), 44.23 (C3), 42.33 (C4′), 39.76 (C24′), 39.53 (C16′), 38.55 (C13′), 38.06 (C10′), 36.99 (C1′), 36.56 (C22′), 36.21 (C8′), 35.80 (C20′), 34.36 (C2), 31.89 (C7′), 28.23 (C2′), 28.01 (C25′), 24.29 (C12′), 23.85 (C15′), 22.82 (C23′), 22.57 (C26′), 21.06 (C11′), 19.33 (C19′), 18.73 (C21′), 11.87 (C18′); MS (FAB$^+$): m/z = 486 [M+H]$^+$, 460, 369 [Chol]$^+$, 159, 147, 131, 109, 95, 55; $C_{31}H_{51}NO_3$ (485.4): calcd C 76.64, H 10.59, N 2.88; found C 76.82, H 10.46, N 2.98.

3-Aza-N^1-cholesteryloxycarbonyl-N^5-phenylmethoxycarbonylpentane-1,5-diamine (8a): Trimethylphosphine (2.39 mL of a 1M solution in THF, 2.39 mmol, 1.15 equiv) was slowly added to a stirred solution of 2-azido-*N*-(phenylmethoxycarbonyl)ethylamine **7a** (0.457 g, 2.08 mmol) and activated 4 Å molecular sieves (1.19 g) in THF (4.9 mL), under a nitrogen atmosphere, resulting in the liberation of nitrogen gas. After 30 min a solution of **5a** (1.09 g, 2.28 mmol, 1.1 equiv) in THF (4 mL) was added and stirring continued. After 1 h, the solvent was evaporated under a stream of nitrogen gas, the resultant residue redissolved in anhydrous EtOH (10.4 mL) and $NaBH_4$ (9.40 mL of a 0.5M solution in diglyme, 4.70 mmol, 2 equiv) added. Stirring was continued for a further 18 h, the reaction mixture filtered and the solvents removed under reduced pressure. The residue was partitioned between EtOAc (50 mL) and saturated $NaHCO_3$ (15 mL), the organic layer separated, the aqueous layer extracted with EtOAc (3 × 30 mL) and the combined organic layers washed with water (20 mL) and brine (20 mL) and dried (Na_2SO_4). Concentration under reduced pressure gave a pale yellow solid, which was further purified by chromatography (92:7:1 CH_2Cl_2/MeOH/NH_3), to give **8a** as a hygroscopic, white solid. Yield: 1.60 g (79%); R_f = 0.26 (92:7:1 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu}$ = 3340, 3091, 2939, 1695, 1537, 1463, 1377, 1263, 1112 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.42–7.33 (m, 5H, Ph), 5.49 (d, J = 4.5 Hz, 1H, ZN*H*), 5.43–5.41 (m, 1H, H6′), 5.26 (t, J = 4.5 Hz, 1H, ChocN*H*), 5.16 (s, 2H, CH_2Ph), 4.59–4.48 (m, 1H, H3′), 3.72 (t, J = 4.5 Hz, 1H, N*H*), 3.36–3.28 (m, 4H, H1, H5), 2.81–2.77 (m, 4H, H2, H4), 2.40–2.32 (m, 2H, H4′), 2.09–1.86 (m, 5H, H2′, H7′, H8′), 1.63–1.09 (m, 21H, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 1.05 (s, 3H, H19′), 0.98 (d, J = 6.5 Hz, 3H, H21′), 0.93 (d, J = 6.5 Hz, 6H, H26′, H27′), 0.74 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.78, 156.54 (NH*C*(O)O), 139.79 (C5′), 136.67 (C1″ of Ph), 128.48–128.03 (rest of Ph), 122.46 (C6′), 74.30 (C3′), 66.59 (Ph*C*H2), 56.69 (C14′), 56.20 (C17′), 50.00 (C9′), 48.73 (C4), 48.66 (C2), 42.32 (C4′), 40.70 (C1), 39.77 (C5′), 38.64 (C16′), 37.01 (C1′), 36.54 (C10′), 36.23 (C22′), 35.82 (C8′), 31.88 (C2′), 28.25 (C12′), 28.00 (C25′), 24.31 (C15′), 23.89 (C25′), 22.87 (C26′), 22.62 (C27′), 21.07 (C11′), 19.35 (C19′), 18.77 (C21′), 11.89 (C18′); MS (FAB$^+$): m/z = 651 [M+H]$^+$, 369 [Chol]$^+$, 282, 161, 147, 105, 91 [C_7H_7]$^+$, 69, 55; HRMS (FAB$^+$) $C_{40}H_{64}N_3O_4$: [M+H]$^+$ calcd 650.4897, found 650.4889.

3-Aza-N^1-cholesteryloxycarbonyl-N^6-phenylmethoxycarbonylhexane-1,6-diamine (8b): This was prepared with aldehyde **5a** and azide **7b** in a similar way to the preparation of **8a** on a 8.66 mmol scale and purified by chromatography (92:7:1 CH_2Cl_2/MeOH/NH_3) to give **8b** as a hygroscopic, white solid. Yield: 4.17 g (72%); R_f = 0.23 (92:7:1 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu}$ = 3342, 3089, 3035, 2939, 1697, 1529, 1378, 1259, 1110, 997 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.24–7.19 (m, 5H, Ph), 5.83 (br t, J = 5.5 Hz, 1H, ZN*H*), 5.51 (br t, J = 5.5 Hz, 1H, ChocN*H*), 5.28–5.25 (m, 1H, H6′), 4.99 (s, 2H, CH_2Ph), 4.45–4.35 (m, 1H, H3′), 3.16–3.13 (m, 5H, H1, H6, N*H*), 2.58–2.51 (m, 4H, H2, H4), 2.27–2.05 (m, 2H, H4′), 1.94–1.65 (m, 5H, H2′, H7′, H8′), 1.56–1.02 (m, 23H, H5, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 1.01 (s, 3H, H19′), 0.90 (s, 3H, H21′), 0.83 (d, J = 6.5 Hz, 6H, H26′, H27′), 0.68 (d, J = 6.5 Hz, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.68, 156.47 (NH*C*(O)O), 139.82 (C5′), 136.81 (C1″ of Ph), 128.44–127.96 (rest of Ph), 122.39 (C6′), 74.19 (C3′), 66.41 (Ph*C*H$_2$), 56.63 (C14′), 56.17 (C17′), 49.99 (C9′), 49.10 (C4), 46.73 (C2), 42.29 (C4′), 40.37 (C1), 39.75 (C6), 38.64 (C16′), 37.01 (C1′), 36.53 (C10′), 36.21 (C22′), 35.81 (C8′), 31.87 (C2′), 29.82 (C5), 28.24 (C12′), 27.99 (C25′), 24.29 (C15′), 23.88 (C25′), 22.87 (C26′), 22.61 (C27′), 21.06 (C11′), 19.34 (C19′), 18.76 (C21′), 11.88 (C18′); MS (FAB$^+$): m/z = 665 [M+H]$^+$, 621 [M+H − CO_2]$^+$ 369 [Chol]$^+$, 296, 161, 105, 91 [C_7H_7]$^+$, 69, 55; HRMS (FAB$^+$) $C_{41}H_{66}N_3O_4$: [M+H]$^+$ calcd 664.5047, found 664.5053.

3-Aza-N^1-cholesteryloxycarbonyl-N^7-phenylmethoxycarbonylheptane-1,7-diamine (8c): This was prepared with aldehyde **5a** and azide **7c** in a similar way to the preparation of **8a** on a 2.35 mmol scale and purified by chromatography (92:7:1 CH_2Cl_2/MeOH/NH_3) to give **8c** as a hygroscopic, white solid. Yield: 2.85 g (89%); R_f = 0.24 (92:7:1 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu}$ = 3346, 3035, 2927, 1699, 1532, 1530, 1459, 1378, 1139 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.23 (br s, 5H, Ph), 5.57 (br t, J = 5.5 Hz, 1H, ZN*H*), 5.49 (br t, J = 5.5 Hz, 1H, ChocN*H*), 5.29–5.26 (m, 1H, H6′), 4.99 (s, 2H, CH_2Ph), 4.43–4.38 (m, 1H, H3′), 3.54 (br t, J = 5.5 Hz, 1H, N*H*), 3.21–3.05 (m, 4H, H1′, H7′), 2.66–2.54 (m, 2H, H2), 2.53–2.49 (m, 2H, H4), 2.39–2.12 (m, 2H, H4′), 1.94–1.59 (m, 5H, H2′, H7′, H8′), 1.59–1.05 (m, 25H, H5, H6, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 0.90 (s, 3H, H19′), 0.84 (d, J = 6.5 Hz, 3H, H21′), 0.78 (d, J = 6.5 Hz, 6H, H26′, H27′), 0.59 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.59, 156.51 (NH*C*(O)O), 139.79 (C5′), 136.77 (C1″ of Ph), 128.59–127.98 (rest of Ph), 122.44 (C6′), 74.30 (C3′), 66.43 (Ph*C*H$_2$), 56.69 (C14′), 56.19 (C17′), 50.00 (C9′), 49.04 (C4), 48.93 (C2), 42.31 (C4′), 40.81 (C1′), 39.76 (C7), 38.63 (C16′), 37.01 (C1′), 36.53 (C10′), 36.22 (C22′), 35.81 (C8′), 31.87 (C2′), 29.72 (C5), 28.24 (C12′), 27.99 (C25′), 27.66 (C6), 24.30 (C15′), 23.88 (C23′), 22.86 (C26′), 22.61 (C27′), 21.06 (C11′), 19.34 (C19′), 18.76 (C21′), 11.88 (C18′); MS (FAB$^+$): m/z = 679 [M+H]$^+$, 635 [M+H − CO_2]$^+$, 369 [Chol]$^+$, 310, 165, 105, 91 [C_7H_7]$^+$, 69, 55; HRMS (FAB$^+$) $C_{42}H_{68}N_3O_4$: [M+H]$^+$ calcd 678.5210, found: 678.5201.

3-Aza-N^6-cholesteryloxycarbonyl-N^1-phenylmethoxycarbonylhexane-1,6-diamine (8d): This was prepared with aldehyde **5b** and azide **7a** in a similar fashion to **8a** on a 9.27 mmol scale and purified by chromatography (92:7:1 CH_2Cl_2/MeOH/NH_3) to give **8d** as a hygroscopic, white solid. Yield: 5.11 g (83%); R_f = 0.25 (92:7:1 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu}$ = 3343, 2937, 2902, 1698, 1525, 1467, 1373, 1255, 1105 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.36–7.30 (m, 5H, Ph), 5.48 (br s, 1H, ZN*H*), 5.37 (d, J = 3.5 Hz, 1H, H6′), 5.29 (br s, 1H, ChocN*H*), 5.10 (s, 2H, PhCH_2O), 4.49 (m, 1H, H3′), 3.29–3.22 (m, 4H, H1, H6), 2.72 (t, J = 5.5 Hz, 2H, H2), 2.65 (t, J = 6.5 Hz, 2H, H4), 2.37–2.27 (m, 2H, H4′), 2.03–1.81 (m, 5H, H2′, H7′, H8′), 1.65–1.04 (m, 24H, H3, H5, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 0.99 (s, 3H, H19′), 0.92 (d, J = 6.5 Hz, 3H, H21′), 0.87 (dd, J = 5.5, 1.0 Hz, 6H, H26′, H27′), 0.68 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.03, 156.76 (NH*C*(O)O), 140.21 (C5′), 137.06 (C1″ of Ph), 128.86–128.41 (rest of Ph), 122.82 (C6′), 74.61 (C3′), 66.96 (Ph*C*H$_2$), 57.06 (C14′), 56.52 (C17′), 50.36 (C9′), 49.40 (C6), 47.20 (C4), 42.69 (C4′), 40.12 (C2), 39.90 (C24′), 38.98 (C1), 37.36 (C5), 36.92 (C1′), 36.57 (C22′), 36.18 (C8′), 32.25 (C7′), 30.35 (C20′), 28.62 (C10′), 28.57 (C25′), 28.40 (C2′), 24.67 (C12′), 24.22 (C15′), 23.22 (C23′), 22.96 (C26′), 21.42 (C11′), 19.71 (C19′), 19.11 (C21′),

0947-6539/98/0401-0144 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 1

12.25 (C18′); MS (FAB$^+$): $m/z = 664$ $[M+H]^+$, 369 [Chol]$^+$, 269, 247, 175, 161, 91 $[C_7H_7]^+$, 69; HRMS (FAB$^+$) $C_{41}H_{66}N_3O_4$: $[M+H]^+$ calcd 664.5053, found 664.5005.

4-Aza-*N*^{1}-cholesteryloxycarbonyl-*N*^{7}-phenylmethoxycarbonylheptane-1,7-diamine (**8e**): This was prepared with aldehyde **5b** and azide **7b** in a similar fashion to **8a** on a 2.13 mmol scale and purified by chromatography (92:7:1 CH_2Cl_2/MeOH/NH_3) to give **8e** as a hygroscopic, white solid. Yield: 1.08 g (75%); $R_f = 0.28$ (92:7:1 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu}$ = 3342, 3091, 2942, 2868, 1691, 1532, 1467, 1378, 1261 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.36-7.28$ (m, 5H, Ph), 5.66 (br s, 1H, ZN*H*), 5.35 (m, 2H,ChocN*H*, H6′), 5.10 (s, 2H, PhCH_2O), 4.48 (m, 1H, H3′), 3.24 (m, 4H, H1, H7), 2.67–2.55 (m, 4H, H3, H5), 2.38–2.26 (m, 2H, H4′), 2.03–1.78 (m, 5H, H2′, H7′, H8′), 1.69–1.04 (m, 26H, H2, H4, H6, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 1.00 (s, 3H, H19′), 0.92 (d, $J = 6.5$ Hz, 3H, H21′), 0.87 (dd, $J = 5.5$, 1.0 Hz, 6H, H26′, H27′), 0.68 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.27, 156.04 (NH*C*(O)O), 139.57 (C5′), 136.48 (C1″ of Ph), 128.17–127.69 (rest of Ph), 122.10 (C6′), 73.88 (C3′), 66.17 (Ph*C*H$_2$), 56.39 (C14′), 55.86 (C17′), 49.70 (C9′), 47.07 (C3), 42.01 (C4′), 39.45 (C24′), 39.22 (C5), 38.96 (C1), 38.31 (C7), 36.69 (C22′), 36.25 (C8′), 35.90 (C2), 35.50 (C6), 31.59 (C7′), 29.39 (C20′), 27.93 (C10′), 27.70 (C2′), 23.99 (C12′), 23.55 (C15′), 22.53 (C23′), 22.27 (C26′), 20.75 (C11′), 19.02 (C19′), 18.44 (C21′), 11.57 (C18′); MS (FAB$^+$): $m/z = 678$ $[M+H]^+$, 369 [Chol]$^+$, 261, 247, 161, 121, 109, 91 $[C_7H_7]^+$, 69, 55; HRMS (FAB$^+$) $C_{42}H_{68}N_3O_4$: $[M+H]^+$ calcd 678.5210, found 678.5229.

4-Aza-*N*^{1}-cholesteryloxycarbonyl-*N*^{8}-phenylmethoxycarbonyloctane-1,8-diamine (**8f**): This was prepared with aldehyde **5b** and azide **7c** in a similar fashion to **8a** on a 9.27 mmol scale and purified by chromatography (92:7:1 CH_2Cl_2/MeOH/NH_3) to give **8f** as a hygroscopic, white solid. Yield: 5.78 g (90%); $R_f = 0.22$ (92:7:1 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu}$ = 3333, 3033, 2938, 2905, 1699, 1537, 1456, 1378, 1259, 1139 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.36-7.29$ (m, 5H, Ph), 5.44 (br s, 1H, ZN*H*), 5.37 (d, $J = 3.0$ Hz, 1H, H6′), 5.09 (br m, 3H, PhCH_2O, ChocN*H*), 4.48 (m, 1H, H3′), 3.21–3.19 (m, 4H, H1, H8), 2.67–2.58 (m, 4H, H3, H5), 2.37–2.26 (m, 2H, H4′), 2.03–1.82 (m, 5H, H2′, H7′, H8′), 1.64 (quin, $J = 6.5$ Hz, 2H, H2), 1.59–1.04 (m, 26H, H4, H6, H7, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 1.00 (s, 3H, H19′), 0.92 (d, $J = 6.5$ Hz, 3H, H21′), 0.87 (dd, $J = 5.5$, 1.0 Hz, 6H, H26′, H27′), 0.68 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.51, 156.34 (NH*C*(O)O), 139.89 (C5′), 136.77 (C1″ of Ph), 128.49–128.05 (rest of Ph), 122.43 (C6′), 74.15 (C3′), 66.51 (Ph*C*H$_2$), 56.70 (C14′), 56.16 (C17′), 50.02 (C9′), 49.36 (C1), 42.33 (C4′), 39.76 (C24′), 39.54 (C3), 38.64 (C8), 37.02 (C5), 36.57 (C22′), 36.21 (C8′), 35.82 (C2), 31.90 (C7′), 29.72 (C20′), 28.24 (C10′), 28.02 (C2′), 27.80 (C 6), 27.31 (C7), 24.31 (C12′), 23.86 (C15′), 22.85 (C23′), 22.59 (C26′), 21.06 (C11′), 19.36 (C19′), 18.74 (C21′), 11.88 (C18′); MS (FAB$^+$): $m/z = 692$ $[M+H]^+$, 539, 369 [Chol]$^+$, 280, 235, 161, 147, 119, 91 $[C_7H_7]^+$, 67, 55, 41; HRMS (FAB$^+$) $C_{43}H_{70}N_3O_4$: $[M+H]^+$ calcd 692.5366, found 692.5379.

3-Aza-*N*^{1}-cholesteryloxycarbonylpentane-1,5-diamine (**9a**): A flask containing **8a** (1.03 g, 1.59 mmol) was thoroughly flushed with nitrogen before adding 10% palladium on charcoal (84.4 mg, 79.3 μmol, 0.05 equiv). The mixture was again flushed with nitrogen, and EtOH (8.0 mL) was slowly added with stirring. Cyclohexene (3.21 mL, 31.7 mmol, 20 equiv) was added to this suspension and the mixture brought to a gentle reflux for 1.5 h. The solution was allowed to cool to room temperature and filtered through a pad of Celite®, and the filter cake washed several times with portions of 10% Et_3N/EtOH (100 mL). The solvents were removed under reduced pressure to give a white solid, which was redissolved in CH_2Cl_2 and refiltered and the solvent again removed to give **9a** as a hygroscopic, white solid. Yield: 0.807 g (99%); IR (CH_2Cl_2): $\tilde{\nu}$ = 3357–3200, 2932, 2859, 1699, 1545, 1510, 1467, 1381, 1222, 1120, 1033 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 5.66$ (br s, 2H, ChocN*H*), 5.20–5.17 (m, 1H, H6′), 4.37–4.27 (m, 1H, H3′), 3.47 (m, 3H, NH, NH_2), 3.20–3.01 (m, 2H, H1), 2.72–2.51 (m, 6H, H2, H4, H5), 2.21–2.07 (m, 2H, H4′), 1.99–1.63 (m, 5H, H2′, H7′, H8′), 1.33–0.95 (m, 21H, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 0.83 (s, 3H, H19′), 0.74 (d, $J = 6.5$ Hz, 3H, H21′), 0.69 (d, $J = 6.5$ Hz, 6H, H26′, H27′), 0.51 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.45 (NH*C*(O)O), 139.77 (C5′), 122.38 (C6′), 74.14 (C3′), 56.64 (C14′), 56.13 (C17′), 49.97 (C9′), 48.85 (C4), 42.26 (C4′), 40.89 (C2), 39.71 (C16′), 39.48 (C24′), 38.59 (C1), 36.98 (C1′), 36.50 (C22′), 36.16 (C5), 35.77 (C20′), 31.83 (C8′), 28.19 (C12′), 27.94 (C25′), 24.25 (C15′), 23.83 (C23′), 22.80 (C26′), 22.55 (C27′), 21.01 (C11′), 19.31 (C19′), 18.70 (C21′), 11.83 (C18′); MS (FAB$^+$): $m/z = 516$ $[M+H]^+$, 471 $[M-CO_2]^+$, 369 [Chol]$^+$, 130 [M–OChol]$^+$, 69, 55; HRMS (FAB$^+$) $C_{32}H_{58}N_3O_2$: $[M+H]^+$ calcd 516.4529, found 516.4511.

3-Aza-*N*^{1}-cholesteryloxycarbonylhexane-1,6-diamine (**9b**): This was prepared from **8b** in a similar manner to **9a** on a 4.23 mmol scale to give **9b** as a hygroscopic, white solid. Yield: 2.23 g (99%); IR (CH_2Cl_2): $\tilde{\nu}$ = 3349–3210, 2937, 2851, 1515, 1460, 1381, 1221, 1120, 1037 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 5.75$ (br t, $J = 5.5$ Hz, 1H, ChocN*H*), 5.30–5.28 (m, 1H, H6′), 4.42–4.33 (m, 1H, H3′), 3.20–3.13 (m, 2H, H1), 2.73–2.59 (m, 6H, H2, H4, H6), 2.29–2.17 (m, 2H, H4′), 1.96–1.72 (m, 5H, H2, H7′, H8′), 1.58–1.01 (m, 23H, H5, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 0.93 (s, 3H, H19′), 0.84 (d, $J = 6.5$ Hz, 3H, H21′), 0.79 (d, $J = 6.5$ Hz, 6H, H26′, H27′), 0.61 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.33 (NH*C*(O)O), 139.72 (C5′), 122.26 (C6′), 73.95 (C3′), 56.58 (C14′), 56.07 (C17′), 49.91 (C9′), 49.13 (C4), 42.19 (C4′), 40.44 (C2), 39.65 (C16′), 39.42 (C24′), 38.55 (C1), 36.93 (C1′), 36.43 (C22′), 36.10 (C6), 35.70 (C20′), 31.77 (C8′), 29.62 (C5), 28.14 (C12′), 27.87 (C25′), 24.19 (C15′), 23.78 (C23′), 22.75 (C26′), 22.50 (C27′), 20.96 (C11′), 19.25 (C19′), 18.64 (C21′), 11.77 (C18′); MS (FAB$^+$): $m/z = 530$ $[M+H]^+$, 485 $[M-CO_2]^+$, 369 [Chol]$^+$, 144 [M–OChol]$^+$, 69, 55; HRMS (FAB$^+$) $C_{33}H_{60}N_3O_2$: $[M+H]^+$ calcd 530.4686, found 530.4675.

3-Aza-*N*^{1}-cholesteryloxycarbonylheptane-1,7-diamine (**9c**): This was prepared from **8c** in a similar manner to **9a** on a 1.63 mmol scale to give **9c** as a hygroscopic, white solid. Yield: 0.874 g (99%); IR (CH_2Cl_2): $\tilde{\nu}$ = 3371–3260, 2937, 1517, 1444, 1377, 1226, 1120 1037, 956 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 5.37$ (br s, 2H, ChocN*H*), 5.31–5.29 (m, 1H, H6′), 4.43–4.37 (m, 1H, H3′), 3.60 (br t, $J = 5.5$ Hz, 1H, N*H*), 3.21 (br t, $J = 5.5$ Hz, 2H, NH_2), 2.68–2.55 (m, 2H, H1), 2.54–2.10 (m, 8H, H2, H4′, H4, H7), 1.96–1.61 (m, 5H, H2′, H7′, H8′), 1.47–1.00 (m, 25H, H5, H6, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 0.94 (s, 3H, H19′), 0.84 (d, $J = 6.5$ Hz, 3H, H21′), 0.79 (d, $J = 6.5$ Hz, 6H, H 26′, H27′), 0.61 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.40 (NH*C*(O)O), 139.80 (C5′), 122.44 (C6′), 74.33 (C3′), 56.69 (C14′), 56.16 (C17′), 50.02 (C9′), 48.91 (C4), 42.31 (C4′), 41.79 (C2), 39.75 (C16′), 39.52 (C24′), 38.61 (C1), 37.01 (C1′), 36.56 (C22′), 36.20 (C7), 35.80 (C20′), 31.89 (C8′), 29.70 (C5), 28.23 (C12′), 28.00 (C25′), 27.43 (C6), 24.29 (C15′), 23.85 (C23′), 22.83 (C26′), 22.57 (C27′), 21.05 (C11′), 19.34 (C19′), 18.73 (C21′), 11.87 (C18′); MS (FAB$^+$): $m/z = 544$ $[M+H]^+$, 499 $[M-CO_2]^+$, 369 [Chol]$^+$, 158 [M–OChol]$^+$, 69, 55; HRMS (FAB$^+$) $C_{34}H_{62}N_3O_2$: $[M+H]^+$ calcd 544.4842, found 544.4837.

3-Aza-*N*^{6}-cholesteryloxycarbonylhexane-1,6-diamine (**9d**): This was prepared from **8d** in a similar manner to **9a** on a 0.82 mmol scale to give **9d** as a pale yellow solid. Yield: 435 mg (99%); $R_f = 0.51$ (75:22:3 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu}$ = 3346–3107, 2930, 2869, 1697, 1540, 1467, 1379, 1244, 1120, 1033 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.73$ (br s, 1H, ChocN*H*), 5.29 (m, 1H, H6′), 4.39 (m, 1H, H3′), 3.68–3.36 (br m, 3H, H3, NH_2), 3.14 (m, 2H, H6), 2.74 (m, 4H, H1, H2), 2.62 (t, $J = 5.5$ Hz, 2H, H4), 2.32–2.19 (m, 2H, H4′), 1.96–1.75 (m, 5H, H2′, H7′, H8′), 1.70–1.00 (m, 23H, H5, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–25′), 0.93 (s, 3H, H19′), 0.84 (d, $J = 6.5$ Hz, 3H, H21′), 0.79 (dd, $J = 5.5$, 1.0 Hz, 6H, H26′, H27′), 0.60 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.36 (NH*C*(O)O), 139.81 (C5′), 122.46 (C6′), 73.98 (C3′), 56.64 (C14′), 56.13 (C17′), 49.97 (C9′), 47.18 (C6), 42.25 (C4′), 41.17 (C2), 39.70 (C16′), 39.47 (C24′), 38.60 (C4), 36.97 (C1′), 36.49 (C22′), 36.15 (C8′), 35.75 (C20′), 31.83 (C7′), 29.62 (C5), 28.18 (C2′), 27.93 (C25′), 24.24 (C12′), 23.82 (C15′), 22.79 (C23′), 22.54 (C26′), 21.01 (C11′), 19.30 (C19′), 18.69 (C21′), 11.82 (C18′); MS (FAB$^+$): $m/z = 530$ $[M+H]^+$, 487, 369 [Chol]$^+$, 247, 186, 161, 147, 121, 105, 77, 57; HRMS (FAB$^+$) $C_{33}H_{60}N_3O_2$: $[M+H]^+$ calcd 530.4686, found 530.4765.

4-Aza-*N*^{1}-cholesteryloxycarbonylheptane-1,7-diamine (**ACH**, **[B178]**, **9e**): This was prepared from **8e** in a similar manner to **9a** on a 5.31 mmol scale to give **9e** as a hygroscopic, white solid. Yield: 2.86 g (99%); $R_f = 0.49$ (75:22:3 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu}$ = 3347, 2937, 2905, 2868, 1698, 1534, 1467, 1379, 1264 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.76$ (br s, 1H, ChocN*H*), 5.22 (m, 1H, H6′), 4.33 (m, 1H, H3′), 3.08 (m, 2H, H1), 2.65 (t, $J = 6.5$ Hz, 2H, H7), 2.53 (t, $J = 6.5$ Hz, 2H, H3), 2.41 (m, 2H, H5), 2.24–2.12 (m, 2H, H4′), 1.90–1.69 (m, 5H, H2′, H7′, H8′), 1.55–0.93 (m, 28H, H2, H4, H6, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′, NH_2), 0.88 (s, 3H, H19′), 0.79 (d, $J = 6.5$ Hz, 3H, H21′), 0.73 (dd, $J = 6.0$, 0.5 Hz, 6H, H26′, H27′), 0.55 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.29 (NH*C*(O)O), 139.77 (C5′), 122.24 (C6′), 73.85 (C3′), 56.59 (C14′), 56.09 (C17′), 49.92 (C9′), 47.61 (C1), 47.52 (C3), 42.20 (C4′), 40.22 (C5), 39.66 (C16′), 39.42 (C24′), 38.57 (C7), 36.94 (C2), 36.44 (C22′), 36.11

(C8′), 35.71 (C20′), 33.04 (C7′), 31.78 (C6), 28.14 (C2′), 27.87 (C25′), 24.19 (C12′), 23.78 (C15′), 22.74 (C23′), 22.49 (C26′), 20.96 (C11′), 19.25 (C19′), 18.65 (C21′), 11.77 (C18′); MS (FAB$^+$): $m/z = 544$ $[M+H]^+$, 369 $[Chol]^+$, 273, 186, 145, 119, 95, 43; HRMS (FAB$^+$) $C_{34}H_{62}N_3O_2$: $[M+H]^+$ calcd 544.4842, found 544.4885.

4-Aza-*N*^{1}-cholesteryloxycarbonyloctane-1,8-diamine (9 f): This was prepared from **8 f** in a similar fashion to **9 a** on a 2.10 mmol scale to give **9 f** as a hygroscopic, white solid. Yield: 1.16 g (99 %); $R_f = 0.44$ (75:22:3 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu} = 3452-3345$, 2937, 1694, 1531, 1468, 1380, 1256, 1135 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.71$ (br s, 1H, ChocN*H*), 5.30 (m, 1H, H6′), 4.40 (m, 1H, H3′), 3.27 (br s, 3H, H4, NH_2), 3.17 (m, 2H, H1), 2.69–2.58 (m, 6H, H3, H5, H8), 2.25–2.16 (m, 2H, H4′), 1.92–1.80 (m, 5H, H2′, H7′, H8′), 1.64–1.06 (m, 27H, H2, H6, H7, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 0.94 (s, 3H, H19′), 0.85 (d, $J = 6.5$ Hz, 3H, H21′), 0.80 (d, $J = 6.5$ Hz, 6H, H26′, H27′), 0.61 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.40$ (NH*C*(O)O), 139.83 (C5′), 122.35 (C6′), 74.04 (C3′), 56.64 (C14′), 56.12 (C17′), 49.97 (C9′), 49.28 (C1), 47.17 (C3), 42.26 (C4′), 39.71 (C16′), 39.48 (C24′), 38.61 (C5), 36.98 (C1′), 36.51 (C22′), 36.16 (C8′), 35.77 (C20′), 31.84 (C7′), 29.36 (C6), 28.19 (C2′), 27.95 (C25′), 27.06 (C7), 24.25 (C12′), 23.83 (C15′), 22.80 (C23′), 22.55 (C26′), 21.01 (C11′), 19.32 (C19′), 18.69 (C21′), 11.83 (C18′); MS (FAB$^+$): $m/z = 558$ $[M+H]^+$, 539, 369 $[Chol]^+$, 145, 121, 95, 69; HRMS (FAB$^+$) $C_{35}H_{64}N_3O_2$: $[M+H]^+$ calcd 558.4999, found 558.5022.

7-(*N*-Phenylmethoxycarbonyl)amino-4-azaheptanol (11 c): A solution of 3-bromo-*N*-(phenylmethoxycarbonyl)propanamine **10 b** (3.80 g, 13.97 mmol) in DMF (10 mL plus 5 mL wash) was added to a mixture of NaI (420 mg, 2.80 mmol, 0.2 equiv), K_2CO_3 (3.87 g, 28.0 mmol, 2 equiv) and 3-aminopropanol **3 b** (4.27 mL, 56.0 mmol, 4 equiv) in dry DMF (50 mL) under a nitrogen atmosphere. The suspension was stirred at room temperature for 48 h, after which the solvent was removed in vacuo and the viscous oil resuspended in CH_2Cl_2 (40 mL). The suspension was filtered through a short pad of Celite and the filter bed washed with CH_2Cl_2 (100 mL). Removal of the solvent gave a pale yellow oil, which was purified by chromatography (92:7:1 CH_2Cl_2/MeOH/NH_3 to 75:22:3 CH_2Cl_2/MeOH/NH_3) to give **11 c** as a colourless oil. Yield: 3.00 g (81 %); $R_f = 0.29$ (75:22:3 CH_2Cl_2/MeOH/NH_3); IR (neat, KBr): $\tilde{\nu} = 3458-3212$, 3058, 2935, 2825, 1696, 1541, 1473, 1456, 1374 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.35$ (m, 5H, Ph), 5.77 (br s, 1H, ZN*H*), 5.09 (s, 2H, PhCH_2O), 3.72 (t, $J = 5.5$ Hz, 2H, H1), 3.22–3.16 (m, 4H, H7, H4, O*H*), 2.75 (t, $J = 6.0$ Hz, 2H, H3), 2.61 (t, $J = 7.0$ Hz, 2H, H5), 1.71–1.60 (m, 4H, H2, H6); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.70$ (NH*C*(O)O), 136.78 (C1″ of Ph), 128.44–127.96 (rest of Ph), 66.43 (Ph*C*H$_2$), 62.75 (C1), 48.70 (C3), 47.03 (C5), 39.10 (C7), 31.22 (C2), 29.81 (C6); MS (FAB$^+$): $m/z = 267$ $[M+H]^+$, 221 $[M - C_2H_5O]^+$, 154, 136, 120, 107, 91 $[C_7H_7]^+$, 69; HRMS (FAB$^+$) $C_{14}H_{23}N_2O_3$: $[M+H]^+$ calcd 267.1709, found 267.1724.

4-Aza-7-*t*-butyldiphenylsilyloxy-*N*^{1}-phenylmethoxycarbonylheptanamine (12 c): TBDPSCl (858 μL, 3.30 mmol, 1.5 equiv) was added dropwise to a mixture of **11 c** (583 mg, 2.20 mmol), Et_3N (613 μL, 4.40 mmol, 2 equiv) and DMAP (30 mg, 0.22 mmol, 0.1 equiv) in CH_2Cl_2 (20 mL), under a nitrogen atmosphere. The solution was then stirred at room temperature for 5 h, after which the reaction mixture was poured into saturated $NaHCO_3$ (20 mL) and the aqueous layer extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with water (2 × 40 mL) and brine (50 mL) and dried (Na_2SO_4). Concentration under reduced pressure gave a yellow oil, which was purified by chromatography (97:2.7:0.3 CH_2Cl_2/MeOH/NH_3 to 92:7:1 CH_2Cl_2/MeOH/NH_3) to give **12 c** as a colourless liquid. Yield: 1.02 g (93 %); $R_f = 0.42$ (92:7:1 CH_2Cl_2/MeOH/NH_3); IR (neat, KBr): $\tilde{\nu} = 3531-3267$, 3069, 2930, 2878, 1700, 1530, 1471, 1390, 1260, 1112, 1071, 821 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.73-7.70$ (m, 4H, H3″, H5″ of Ph_2Si), 7.46–7.34 (m, 11H, rest of Ph), 5.71 (br s, 1H, ZN*H*), 5.13 (s, 2H, PhCH_2), 3.79 (t, $J = 6.0$ Hz, 2H, H7), 3.30 (m, 2H, H1), 2.75 (t, $J = 7.0$ Hz, 2H, H5), 2.71 (t, $J = 6.5$ Hz, 2H, H3), 1.78 (quin, $J = 6.5$ Hz, 2H, H6), 1.67 (quin, $J = 6.5$ Hz, 2H, H2), 1.63 (br s, 1H, H4), 1.11 (s, 9H, Me of *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.56$ (NH*C*(O)O), 136.93–127.71 (Ph), 66.48 (Ph*C*H$_2$), 62.55 (C7), 47.99 (C5), 47.11 (C3), 40.11 (C1), 32.68 (C6), 29.69 (C2), 26.96 (Me of *t*-Bu), 19.25 (Me_3C); MS (FAB$^+$): $m/z = 505$ $[M+H]^+$, 447 $[M+H - t\text{-Bu}]^+$, 221, 197, 183, 135, 121, 105, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 57, 44; HRMS (FAB$^+$) $C_{30}H_{41}N_2O_3Si$: $[M+H]^+$ calcd 505.2886, found 505.2903.

3-Aza-1-*t*-butyldiphenylsilyloxy-*N*^{3,6}-di(phenylmethoxycarbonyl)hexan-6-amine (13 a): 2-Amino ethanol **3 a** (7.86 mL, 130 mmol, 10 equiv) was added to [3-(phenylmethoxycarbonyl)amino]propane-1-methanesulfonate (**6 b**, 3.74 g, 13.0 mmol) under a nitrogen atmosphere. The mixture was stirred vigorously for 5 h, after which the resulting mixture was suspended in $CHCl_3$ (40 mL) and K_2CO_3 (3.60 g, 26.1 mmol, 2 equiv) added. Stirring was continued for 30 min, after which the reaction was filtered through a short pad of Celite and the filter bed washed with 10 % MeOH/$CHCl_3$ (100 mL). The filtrate was washed with water (3 × 40 mL) and dried (Na_2SO_4), and the solvents removed to give **11 a** as a pale yellow oil. To this was added Et_3N (3.63 mL, 26.1 mmol, 2 equiv), DMAP (79.6 mg, 0.652 mmol, 0.05 equiv) and CH_2Cl_2 (26.1 mL), under a nitrogen atmosphere. TBDPSCl (4.07 mL, 15.6 mmol, 1.2 equiv) was added slowly and the solution stirred for 5 h, after which the reaction mixture was poured into saturated $NaHCO_3$ (20 mL) and the aqueous layer extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with water (2 × 40 mL) and brine (50 mL) and dried (Na_2SO_4). Concentration under reduced pressure gave **12 a** as a yellow oil, which was redissolved in CH_2Cl_2 (30 mL) and Et_3N (3.63 mL, 26.1 mmol, 2 equiv). The reaction was cooled to 0 °C, the mixture stirred and a solution of phenylmethoxycarbonyl chloride (2.05 mL, 14.3 mmol, 1.1 equiv) in CH_2Cl_2 (35 mL) was slowly introduced over 15 min. On warming to room temperature, stirring was continued for 4 h and the reaction mixture poured into saturated aqueous NH_4Cl (50 mL). The organic layer was removed, the aqueous layer extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic layers washed with water (2 × 50 mL). Drying (Na_2SO_4), followed by removal of the solvents yielded a yellow oil, which was purified by chromatography (20 %–50 % ether/petrol) to give **13 a** as a colourless oil. Yield: 2.93 g (82 %); $R_f = 0.25$ (50 % ether/petrol); IR (neat, KBr): $\tilde{\nu} = 3335$, 2955, 2931, 2848, 1701, 1525, 1455, 1248, 1137 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 7.75$ (d, $J = 5.5$ Hz, 4H, H3″, H5″ of Ph_2Si), 7.46–7.31 (m, 16H, rest of Ph), 5.99 (br s, 1H, ZN*H*), 5.17 (s, 2H, PhCH_2O), 5.11 (s, 2H, PhCH_2O), 3.92–3.81 (m, 2H, H1), 3.49–3.43 (m, 4H, H2, H4), 3.24–3.20 (m, 2H, H6), 1.74 (m, 2H, H5), 1.16 (s, 9H, Me of *t*-Bu); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 156.62$, 155.51 (NH*C*(O)O), 137.15–127.65 (Ph), 67.20, 67.10 (Ph*C*H$_2$), 62.19 (C1), 48.77 (C2), 45.42 (C4), 37.82 (C6), 28.09 (C5), 26.94 (Me of *t*-Bu), 19.17 (Me_3C); MS (FAB$^+$): $m/z = 625$ $[M+H]^+$, 581 $[M+H - CO_2]^+$, 624 $[M - t\text{-Bu}]$, 503, 197, 154, 136, 121, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 69, 55; HRMS (FAB$^+$) $C_{37}H_{45}N_2O_5Si$: $[M+H]^+$ calcd 625.3098, found 625.3094.

3-Aza-6-*t*-butyldiphenylsilyloxy-*N*^{1,3}-di(phenylmethoxycarbonyl)hexan-amine (13 b): This was prepared from mesylate **6 a** (or bromide **10 a**) and 3-aminopropanol **3 b** in a similar fashion to **13 a** on a 38.80 mmol scale; intermediates **11 b** and **12 b** were not isolated and characterised. After chromatography (20–75 % ether/petrol), **13 b** was obtained as a viscous, colourless liquid. Yield: 14.59 g (58 %); $R_f = 0.35$ (50 % ether/petrol); IR (neat, KBr): $\tilde{\nu} = 3335$, 2955, 2931, 2848, 1701, 1525, 1455, 1248, 1137 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.69$ (d, $J = 6.5$ Hz, 4H, H3″, H5″ of Ph_2Si), 7.49–7.34 (m, 16H, rest of Ph), 5.51 (br s, 1H, ZN*H*), 5.14 (s, 2H, PhCH_2O), 5.10 (s, 2H, PhCH_2O), 3.70 (m, 2H, H6), 3.47–3.44 (m, 6H, H1, H2, H4), 1.80 (m, 2H, H5), 1.10 (s, 9H, Me of *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 157.46$, 157.17 (NH*C*(O)O), 137.15–127.98 (Ph), 67.70, 67.04 (Ph*C*H$_2$), 61.93 (C6), 47.71 (C4), 45.49 (C2), 40.51 (C1), 32.16 (C5), 27.43 (Me of *t*-Bu), 19.71 (Me_3C); MS (FAB$^+$): $m/z = 625$ $[M+H]^+$, 581 $[M+H - CO_2]^+$, 567, 491, 305, 268, 197, 135, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$; HRMS (FAB$^+$) $C_{37}H_{45}N_2O_5Si$: $[M+H]^+$ calcd 625.3098, found 625.3094.

4-Aza-7-*t*-butyldiphenylsilyloxy-*N*^{1,4}-di(phenylmethoxycarbonyl)heptan-amine (13 c): A solution of phenylmethoxycarbonyl chloride (0.30 mL, 2.18 mmol, 1.1 equiv) in CH_2Cl_2 (5 mL) was added to a stirred solution of **12 c** (1.27 g, 1.98 mmol) and Et_3N (0.41 mL, 2.97 mmol, 1.5 equiv) in CH_2Cl_2 (8.0 mL) at 0 °C, over a period of 15 min. The reaction was allowed to warm to room temperature, stirring was continued for 4 h, after which the reaction mixture was poured into saturated aqueous NH_4Cl (10 mL). The organic layer was removed, the aqueous layer extracted with CH_2Cl_2 (2 × 10 mL) and the combined organic layers washed with water (2 × 10 mL). Drying (Na_2SO_4) and removal of the solvents yielded a yellow oil, which after chromatography (50–100 % ether/petrol) gave **13 c** as a colourless liquid. Yield: 1.12 g (88 %); $R_f = 0.31$ (50 % ether/petrol); IR (neat, KBr): $\tilde{\nu} = 3345$, 2931, 2847, 1700, 1519, 1455, 1222, 1136 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.73$ (d, $J = 6.0$ Hz, 4H, H3″, H5″ of Ph_2Si), 7.52–7.37 (m, 16H, rest of Ph), 5.66 (br s, 1H, ZN*H*), 5.18 (s, 4H, PhCH_2), 3.75 (m, 2H, H7), 3.43 (m, 4H, H3, H5), 3.23 (m, 2H, H1), 1.85–1.81 (m, 2H, H6), 1.76 (m, 2H, H2), 1.14 (s, 9H, Me of *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.95$, 156.92 (NH*C*(O)O), 136.81–127.83 (Ph), 67.23, 66.54 (Ph*C*H$_2$), 61.41 (C7),

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44.47 (C5), 44.11 (C3), 37.69 (C1), 31.70 (C6), 28.10 (C2), 26.97 (Me of *t*-Bu), 19.29 (Me_3C); MS (FAB^+): $m/z = 1277$ $[2M+H]^+$, 639 $[M+H]^+$, 581 $[M - t\text{-Bu}]^+$, 561, 505, 383, 268, 181, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$; HRMS (FAB^+) $C_{38}H_{47}N_2O_5Si$: $[M+H]^+$, 639.3254, found 639.3268.

4-Aza-8-*t*-butyldiphenylsilyloxy-*N*^{1,4}-di(phenylmethoxycarbonyl)octan-1-amine (**13d**): This was prepared from mesylate **6b** (or bromide **10b**) and 4-aminobutanol **3c** in a similar fashion to **13a** on a 38.8 mmol scale; intermediates **11d** and **12d** were not isolated and characterised. After chromatography (20–80% ether/petrol), **13d** was obtained as a viscous, colourless oil. Yield: 503 mg (77%); $R_f = 0.65$ (ether); IR (neat, KBr): $\tilde{\nu}$= 3336, 3071, 3022, 2931, 2846, 1698, 1519, 1473, 1426, 1361, 1221, 1110 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.69$ (d, $J = 6.5$ Hz, 4H, H3″, H5″ of Ph_2Si), 7.45–7.31 (m, 16H, rest of Ph), 5.77 (br s, 1H, ZN*H*), 5.14 (m, 4H, PhCH_2O), 3.68 (m, 2H, H8), 3.36–3.18 (m, 6H, H1, H3, H5), 1.72–1.54 (m, 6H, H2, H6, H7), 1.08 (s, 9H, Me of *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.92, 156.61 (NHC(O)O), 136.81–127.75 (Ph), 67.22, 66.54 (PhCH_2), 63.54 (C8), 46.79 (C3), 44.13 (C5), 37.71 (C1), 29.86 (C2), 28.17 (C7), 26.98 (C6), 25.17 (Me of *t*-Bu), 19.30 (Me_3C); MS (FAB^+): $m/z = 653$ $[M+H]^+$, 609, 197, 154, 135, 107, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 57; HRMS (FAB^+) $C_{39}H_{49}N_2O_5Si$: $[M+H]^+$ calcd 653.3411, found 653.3429.

6-Amino-3-aza-*N*^{3,6}-di(phenylmethoxycarbonyl)hexanol (**14a**): TBAF (3.46 mL of a 1M solution in THF, 3.46 mmol, 1.1 equiv) was added to a solution of **13a** (1.97 g, 3.15 mmol) in THF (31.5 mL) and the resulting solution stirred for 2 h. The reaction was poured into a mixture of ether (100 mL) and saturated aqueous $NaHCO_3$ (50 mL), the organic phase separated and the aqueous layer extracted with ether (2×50 mL). The combined organic layers were washed with water (2×100 mL) and brine (100 mL) and dried (Na_2SO_4). The solvents were removed under reduced pressure to give a pale yellow oil, which was further purified by chromatography (ether to 5% acetone/ether) to yield **14a** as a colourless oil. Yield: 1.11 g (92%); $R_f = 0.50$ (5% acetone/ether); IR (neat, KBr): $\tilde{\nu}$= 3345, 3089, 3065, 3033, 2948, 1695, 1681, 1536, 1455, 1366, 1139, 1027 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.33$ (m, 10H, Ph), 5.87–5.28 (br m, 1H, ZN*H*), 5.11–5.07 (m, 4H, PhCH_2O), 4.15 (br s, 1H, O*H*), 3.70 (m, 2H, H1), 3.37 (m, 4H, H2, H4), 3.14 (m, 2H, H6), 1.72 (m, 2H, H5); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.73$ (NHC(O)O), 136.70 (C1″ of Ph), 129.23–128.06 (rest of Ph), 67.33, 66.56 (PhCH_2), 61.16 (C1), 50.41 (C2), 45.68 (C4), 38.25 (C6), 28.85 (C5); MS (FAB^+): $m/z = 387$ $[M+H]^+$, 343 $[M+H - CO_2]^+$, 167, 150, 121, 105, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$; HRMS (FAB^+) $C_{21}H_{27}N_2O_5$: $[M+H]^+$ calcd 387.1920, found 387.1937.

6-Amino-4-aza-*N*^{4,6}-di(phenylmethoxycarbonyl)hexanol (**14b**): This was prepared from **13b** in similar fashion to **14a** on a 12.2 mmol scale and after chromatography (ether to 20% acetone/ether) gave **14b** as a viscous, colourless oil. Yield: 4.18 g (89%); $R_f = 0.41$ (75% ether/acetone); IR (CH_2Cl_2): $\tilde{\nu}$= 3646–3396, 3054, 2950, 1679, 1670, 1519, 1452, 1247, 1139 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.36$–7.32 (m, 10H, Ph), 5.68–5.41 (br m, 1H, ZN*H*), 5.14 (s, 2H, PhCH_2O), 5.09 (s, 2H, PhCH_2O), 3.58 (m, 2H, H1), 3.45–3.39 (m, 6H, H3, H5, H6), 2.70–2.59 (br s, 1H, O*H*), 1.70 (m, 2H, H2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 157.10$, 156.77 (NHC(O)O), 136.45, 136.30 (C1″ of Ph), 128.57–127.92 (rest of Ph), 67.56, 66.68 (PhCH_2), 59.50 (C1), 47.00 (C3), 44.38 (C5), 39.73 (C6), 30.56 (C2); MS (FAB^+): $m/z = 387$ $[M+H]^+$, 343, 279, 242, 167, 150, 133, 120, 105, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 69, 55, 41; HRMS (FAB^+) $C_{21}H_{27}N_2O_5$: $[M+H]^+$ calcd 387.1920, found 387.1914.

7-Amino-4-aza-*N*^{4,7}-di(phenylmethoxycarbonyl)heptanol (**14c**): This was prepared from **13c** in a similar manner to the preparation of **14a** on a 3.44 mmol scale and purified by chromatography (ether to 20% acetone/ether) to give **14c** as a colourless liquid. Yield: 1.24 g (90%); $R_f = 0.36$ (75% ether/acetone); IR (neat, KBr): $\tilde{\nu}$= 3373, 2945, 1698, 1681, 1536, 1532, 1455, 1367, 1253, 1145, 1059 cm^{-1}; ^1H NMR (500 MHz, C_7D_8, 100 °C): $\delta = 7.15$–7.02 (m, 10H, Ph), 4.95 (s, 4H, PhCH_2O), 3.25 (m, 2H, H1), 3.08 (t, $J = 7.0$ Hz, 2H, H3), 2.98 (t, $J = 7.0$ Hz, 2H, H5), 2.86 (q, $J = 6.5$ Hz, 2H, H7), 1.43–1.37 (m, 4H, H2, H6); ^{13}C NMR (125 MHz, C_7D_8, 100 °C): δ = 157.00, 157.65 (NHC(O)O), 137.77 (C1″ of Ph), 129.34–127.95 (rest of Ph), 67.68, 66.86 (PhCH_2), 59.86 (C1), 45.36 (C3), 44.71 (C5), 39.13 (C7), 32.28 (C2), 29.55 (C6); MS (FAB^+): $m/z = 401$ $[M+H]^+$, 357, 265, 154, 136, 107, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 69, 55; HRMS (FAB^+) $C_{22}H_{29}N_2O_5$: $[M+H]^+$ calcd 401.2076, found 401.2087.

8-Amino-5-aza-*N*^{5,8}-di(phenylmethoxycarbonyl)octanol (**14d**): This was prepared from **13d** in a similar manner to **14a** on a 0.47 mmol scale and purified by chromatography (ether to 20% acetone/ether) to give **14d** as a viscous, colourless oil. Yield: 188 mg (95%); $R_f = 0.38$ (ether); IR (neat, KBr): $\tilde{\nu}$= 3341, 3033, 2945, 1698, 1681, 1531, 1455, 1365, 1139 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$): $\delta = 7.40$–7.31 (m, 10H, Ph), 5.72 (br s, 1H, ZN*H*), 5.13–5.10 (m, 4H, PhCH_2O), 3.63 (m, 2H, H1), 3.35–3.16 (m, 6H, H4, H6, H8), 2.09 (br s, 1H, O*H*), 1.74–1.50 (m, 6H, H2, H3, H7); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.49$, 156.02 (NHC(O)O), 136.53, 136.38 (C1″ of Ph), 128.34–127.64 (rest of Ph), 67.04, 66.35 (PhCH_2), 62.04 (C1), 46.56 (C6), 44.03 (C4), 37.56 (C8), 29.57 (C7), 27.93 (C2), 24.85 (C3); MS (FAB^+): $m/z = 415$ $[M+H]^+$, 371 $[M+H - CO_2]^+$, 290, 279, 150, 120, 105, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 69; HRMS (FAB^+) $C_{23}H_{31}N_2O_5$: $[M+H]^+$ calcd 415.2233, found 415.2200.

4-Aza-6-azido-*N*^{1,4}-di(phenylmethoxycarbonyl)hexanamine (**15a**): Methanesulfonyl chloride (0.87 mL, 11.25 mmol, 2.5 equiv) was added dropwise to a stirred solution of **14a** (1.74 g, 4.51 mmol) and Et_3N (1.88 mL, 13.5 mmol, 3 equiv) in CH_2Cl_2 (40 mL) at 0 °C under a nitrogen atmosphere. After addition, the solution was allowed to warm to room temperature and stirring continued for 30 min. The reaction was quenched with ice (0.25 g) and poured into a mixture of ether (100 mL) and saturated NH_4Cl (50 mL), and the organic layer was separated off. The aqueous layer was extracted with ether (2×100 mL) and the combined organic layers washed with water (2×100 mL) and brine (100 mL) and dried (Na_2SO_4). Removal of the solvents gave a pale yellow oil, to which was added NaN_3 (1.46 g, 22.5 mmol, 5 equiv), NaI (675 mg, 4.51 mmol) and DMF (30 mL) under a nitrogen atmosphere. The suspension was carefully heated to 80 °C for 2 h, allowed to cool and the solvent removed in vacuo. The viscous residue was redissolved in ether (200 mL), washed with water (2×50 mL), brine (50 mL) and dried (Na_2SO_4), and the solvent removed. Chromatography (50–100% ether/petrol) gave **15a** as a colourless liquid. Yield: 1.78 g (96%); $R_f = 0.58$ (75% ether/acetone); IR (CH_2Cl_2): $\tilde{\nu}$= 3630–3346, 2946, 2102, 1698, 1526, 1455, 1364, 1296, 1143 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.36$–7.31 (m, 10H, Ph), 5.71 (br s, 1H, ZN*H*), 5.16 (s, 2H, PhCH_2O), 5.11 (s, 2H, PhCH_2O), 3.38 (m, 6H, H3, H5, H6), 3.17 (m, 2H, H1), 1.73 (quin, $J = 6.5$ Hz, 2H, H2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.54$, 156.08 (NHC(O)O), 136.82 (C1″ of Ph), 128.67–127.99 (rest of Ph), 67.53, 66.55 (PhCH_2), 49.94 (C6), 46.26 (C5), 45.37 (C3), 37.78 (C1), 28.20 (C2); MS (FAB^+): $m/z = 412$ $[M+H]^+$, 368, 165, 152, 120, 105, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 69, 55; HRMS (FAB^+) $C_{21}H_{26}N_5O_4$: $[M+H]^+$ calcd 412.2023, found 412.1985.

3-Aza-6-azido-*N*^{1,3}-di(phenylmethoxycarbonyl)hexanamine (**15b**): This was prepared from **14b** in the same way as **15a** on a 10.4 mmol scale. After purification by chromatography (50–100% ether/petrol), **15b** was obtained as a colourless liquid. Yield: 3.82 g (89%); $R_f = 0.64$ (75% ether/acetone); IR (neat, KBr): $\tilde{\nu}$= 3355, 2931, 2096, 1651, 1524, 1455, 1247, 1139 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.35$ (m, 10H, Ph), 5.60 (br s, 1H, ZN*H*), 5.13 (s, 2H, PhCH_2O), 5.09 (s, 2H, PhCH_2O), 3.38–3.25 (m, 8H, H1, H2, H4, H6), 1.81 (m, 2H, H5); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.65 (NHC(O)O), 136.48 (C1″ of Ph), 128.61–127.96 (rest of Ph), 67.42, 66.69 (PhCH_2), 48.85 (C6), 47.26 (C4), 45.27 (C2), 39.88 (C1), 28.02 (C5); MS (FAB^+): $m/z = 412$ $[M+H]^+$, 368, 165, 152, 120, 105, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 69, 51; HRMS (FAB^+) $C_{21}H_{26}N_5O_4$: $[M+H]^+$ calcd 412.2023, found 412.2004.

4-Aza-7-azido-*N*^{1,4}-di(phenylmethoxycarbonyl)heptanamine (**15c**): This was prepared with **14c** in a similar manner to the synthesis of **15a** on a 5.75 mmol scale and purified by chromatography (5–25% ether/acetone) to give **15c** as a colourless liquid. Yield: 2.16 g (88%); $R_f = 0.57$ (75% ether/acetone); IR (neat, KBr): $\tilde{\nu}$= 3344, 3034, 2941, 2097, 1642, 1529, 1424, 1358, 1294, 1252, 1138 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.36$ (m, 10H, Ph), 5.70 (br s, 1H, ZN*H*), 5.15 (s, 2H, PhCH_2O), 5.10 (s, 2H, PhCH_2O), 3.31 (m, 6H, H3, H5, H7), 3.17 (m, 2H, H1), 1.79 (m, 2H, H6), 1.70 (t, J = 6.5 Hz, 2H, H2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.54$ (NHC(O)O), 136.80, 136.54 (C1″ of Ph), 128.63–127.96 (rest of Ph), 67.36, 66.54 (PhCH_2), 48.91 (C7), 44.59 (C5), 44.31 (C3), 37.76 (C1), 28.97 (C6), 27.97 (C2); MS (FAB^+): $m/z = 426$ $[M+H]^+$, 167, 150, 133, 120, 113, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 69, 55; HRMS (FAB^+) $C_{22}H_{28}N_5O_4$: $[M+H]^+$ calcd 426.2141, found 426.2150.

4-Aza-8-azido-*N*^{1,4}-di(phenylmethoxycarbonyl)octanamine (**15d**): This was prepared from **14d** in a similar manner to the synthesis **15a** on a 4.50 mmol scale and purified by chromatography (ether to 30% acetone/ether) to give **15d** as a colourless liquid. Yield: 523 mg (80%); $R_f = 0.64$ (75% ether/acetone); IR (CH_2Cl_2): $\tilde{\nu}$= 3415, 3340, 3124, 2922, 2069, 1718, 1698, 1525,

1475, 1253, 1139, 1025 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.36 - 7.30$ (m, 10H, Ph), 5.61 (br s, 1H, ZN*H*), 5.13 (s, 4H, PhCH_2O), 3.31 – 3.18 (m, 8H, H1, H3, H5, H8), 1.74 (m, 2H, H2), 1.67 (m, 4H, H6, H7); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.21$, 155.70 (NH*C*(O)O), 136.49, 136.27 (C1″ of Ph), 128.85 – 127.54 (rest of Ph), 66.87, 66.08 (Ph*C*H$_2$), 50.65 (C8), 45.87 (C3), 43.83 (C5), 37.37 (C1), 27.73 (C7), 25.74 (C6); MS (FAB$^+$): $m/z = 879$ $[2M+H]^+$, 440 $[M+H]^+$, 369, 332, 306, 261, 160, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 57; HRMS (FAB$^+$) $C_{23}H_{30}N_5O_4$: $[M+H]^+$ calcd 440.2298, found 440.2342.

***N*^{1}-Cholesteryloxycarbonyl-3,7-diaza-*N*^{7,9}-di(phenylmethoxycarbonyl)nonane-1,9-diamine (16a)**: This was prepared from **15b** and **5a** in a similar manner to the preparation of **8a** on a 4.25 mmol scale and purified by chromatography (96:3.5:0.5 CH_2Cl_2/MeOH/NH_3 to 92:7:1 CH_2Cl_2/MeOH/NH_3) to give **16a** as a hygroscopic, white solid. Yield: 1.93 g (54 %); $R_f = 0.29$ (92:7:1 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu} = 3423 - 3311$, 3035, 2956, 2867, 1718, 1700, 1681, 1526, 1467, 1378, 1139 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.32$ (m, 10H, Ph), 5.70 – 5.50 (br m, 2H, ChocN*H*, ZN*H*), 5.35 (m, 1H, H6′), 5.10 (s, 2H, PhCH_2O), 5.07 (s, 2H, PhCH_2O), 4.47 (m, 1H, H3′), 3.34 – 3.16 (m, 8H, H1, H6, H8, H9), 2.65 – 2.50 (m, 4H, H2, H4), 2.33 – 2.18 (m, 2H, H4′), 2.03 – 1.81 (m, 5H, H2′, H7′, H8′), 1.69 – 1.08 (m, 24H, H3, H5, H1′, H9′, H11′, H12′, H14′ – H17′, H20′, H22′ – H25′), 0.99 (s, 3H, H19′), 0.92 (d, $J = 6.5$ Hz, 3H, H21′), 0.87 (dd, $J = 5.5$, 1.0 Hz, 6H, H26′, H27′), 0.68 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.45$ (NH*C*(O)O), 139.87 (C5′), 136.54 (C1″ of Ph), 128.56 – 127.94 (rest of Ph), 122.44 (C6′), 74.29 (C3′), 67.35 (Ph*C*H$_2$), 56.71 (C14′), 56.20 (C17′), 50.04 (C9′), 49.01 (C1), 47.48 – 46.66 (C2, C6, C8, C9), 45.74 (C4), 42.34 (C4′), 39.78 (C16′), 39.55 (C24′), 38.64 (C5), 36.56 (C22′), 36.23 (C8′), 35.82 (C20′), 31.90 (C7′), 28.25 (C2′), 28.02 (C25′), 24.31 (C12′), 23.88 (C15′), 22.86 (C23′), 22.61 (C26′), 21.08 (C11′), 19.35 (C19′), 18.77 (C21′), 11.90 (C18′); MS (FAB$^+$): $m/z = 841$ $[M+H]^+$, 644, 369 [Chol]$^+$, 147, 121, 91 $[C_7H_7]^+$, 43; HRMS (FAB$^+$) $C_{51}H_{77}N_4O_6$: $[M+H]^+$ calcd 841.5843, found 841.5884.

***N*^{1}-Cholesteryloxycarbonyl-3,6-diaza-*N*^{6,9}-di(phenylmethoxycarbonyl)nonane-1,9-diamine (16b)**: This was prepared from **15a** and **5a** in a similar fashion to **8a** on a 2.10 mmol scale and purified by chromatography (96:3.5:0.5 CH_2Cl_2/MeOH/NH_3 to 92:7:1 CH_2Cl_2/MeOH/NH_3) to give **16b** as a hygroscopic, white solid. Yield: 1.12 g (64 %); $R_f = 0.30$ (92:7:1 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu} = 3332$, 2948, 2867, 1689, 1531, 1455, 1366, 1139 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.33$ (m, 10H, Ph), 5.80 (m, 2H, ChocN*H*, ZN*H*), 5.36 (m, 1H, H6′), 5.11 (s, 2H, PhCH_2O), 5.08 (s, 2H, PhCH_2O), 4.47 (m, 1H, H3′), 3.32 (m, 4H, H5, H7), 3.15 (m, 4H, H1, H9), 2.74 (m, 4H, H2, H4), 2.33 – 2.19 (m, 2H, H4′), 2.04 – 1.82 (m, 5H, H2′, H7′, H8′), 1.72 – 1.06 (m, 24H, H3, H8, H1′, H9′, H11′, H12′, H14′ – H17′, H20′, H22′ – H25′), 1.00 (s, 3H, H19′), 0.93 (d, $J = 6.5$ Hz, 3H, H21′), 0.87 (dd, $J = 5.5$, 1.0 Hz, 6H, H26′, H27′), 0.68 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.59$ (NH*C*(O)O), 139.82 (C5′), 136.74 (C1″ of Ph), 128.60 – 127.98 (rest of Ph), 122.49 (C6′), 74.82 (C3′), 67.35, 66.52 (Ph*C*H$_2$), 56.72 (C14′), 56.21 (C17′), 50.03 (C9′), 48.82 (C1), 47.61 – 46.55 (C2, C5, C7, C9), 45.05 (C4), 42.34 (C4′), 39.78 (C16′), 39.55 (C24′), 38.63 (C8), 36.56 (C22′), 36.23 (C8′), 35.83 (C20′), 31.90 (C7′), 28.25 (C2′), 28.01 (C25′), 24.31 (C12′), 23.89 (C15′), 22.86 (C23′), 22.61 (C26′), 21.08 (C11′), 19.35 (C19′), 18.77 (C21′), 11.90 (C18′); MS (FAB$^+$): $m/z = 841$ $[M+H]^+$, 644, 369 [Chol]$^+$, 147, 121, 91 $[C_7H_7]^+$, 43; HRMS (FAB$^+$) $C_{51}H_{77}N_4O_6$: $[M+H]^+$ calcd 841.5843, found 841.5750.

***N*^{1}-Cholesteryloxycarbonyl-3,8-diaza-*N*^{8,11}-di(phenylmethoxycarbonyl)undecane-1,11-diamine (16c)**: This was prepared from **15d** and **5a** in a similar fashion to **8a** on a 1.08 mmol scale and purified by chromatography (96:3.5:0.5 CH_2Cl_2/MeOH/NH_3 to 92:7:1 CH_2Cl_2/MeOH/NH_3) to give **16c** as a hygroscopic, white solid. Yield: 752 mg (80 %); $R_f = 0.35$ (92:7:1 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu} = 3332$, 3062, 3033, 2948, 2867, 1699, 1537, 1455, 1378, 1254, 1145 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.35$ (m, 10H, Ph), 5.71 (br s, 1H, ZN*H*), 5.38 (m, 1H, H6′), 5.13 (s, 2H, PhCH_2O), 5.10 (s, 2H, PhCH_2O), 4.95 (br s, 1H, ChocN*H*), 4.51 (m, 1H, H3′), 3.35 – 3.19 (m, 8H, H1, H7, H9, H11), 2.67 – 2.63 (m, 4H, H2, H4), 2.38 – 2.28 (m, 2H, H4′), 2.04 – 1.79 (m, 5H, H2′, H7′, H8′), 1.71 (t, $J = 6.0$ Hz, 2H, H10), 1.62 – 1.05 (m, 26H, H3, H5, H6, H1′, H9′, H11′, H12′, H14′ – H17′, H20′, H22′ – H25′), 1.01 (s, 3H, H19′), 0.93 (d, $J = 6.5$ Hz, 3H, H21′), 0.88 (d, $J = 6.5$ Hz, 6H, H26′, H27′), 0.69 (s, 3H, H18′); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 156.29$ (NH*C*(O)O), 139.78 (C5′), 136.66 (C1″ of Ph), 128.40 – 127.77 (rest of Ph), 122.40 (C6′), 74.23 (C3′), 67.13, 66.43 (Ph*C*H$_2$), 56.63 (C14′), 56.08 (C17′), 49.96 (C9′), 48.91 (C1), 47.25 – 46.66 (C7, C9), 44.11 (C2), 42.25 (C4′), 40.45 (C4), 39.64 (C24′), 39.46 (C11), 38.54 (C16′), 37.61 (C10), 36.49 (C22′), 36.13 (C8′), 35.73 (C20′), 31.82 (C7′), 28.16 (C2′), 27.94 (C25′), 27.16 (C6), 24.22 (C12′), 23.77 (C15′), 22.76 (C23′), 22.50 (C26′), 20.98 (C11′), 19.26 (C19′), 18.66 (C21′), 11.78 (C18′); MS (FAB$^+$): $m/z = 869$ $[M+H]^+$, 426, 369 [Chol]$^+$, 161, 147, 133, 121, 105, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 55; HRMS (FAB$^+$) $C_{53}H_{81}N_4O_6$: $[M+H]^+$ calcd 869.6156, found 869.6096.

***N*^{10}-Cholesteryloxycarbonyl-3,7-diaza-*N*^{1,3}-di(phenylmethoxycarbonyl)decane-1,10-diamine (16d)**: This was prepared from **15b** and **5b** in a similar fashion to **8a** on a 3.65 mmol scale and purified by chromatography (96:3.5:0.5 CH_2Cl_2/MeOH/NH_3 to 92:7:1 CH_2Cl_2/MeOH/NH_3) to give **16d** as a hygroscopic, white solid. Yield: 2.15 g (69 %); $R_f = 0.34$ (92:7:1 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu} = 3333$, 2944, 2867, 1698, 1531, 1467, 1368, 1139 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.33$ (m, 10H, Ph), 5.97 – 5.61 (m, 2H, ChocN*H*, ZN*H*), 5.37 (m, 1H, H6′), 5.12 (s, 2H, PhCH_2O), 5.08 (s, 2H, PhCH_2O), 4.51 (m, 1H, H3′), 3.37 (m, 6H, H1, H2, H4), 3.20 (m, 2H, H10), 2.55 (m, 4H, H6, H8), 2.31 – 2.22 (m, 2H, H4′), 1.96 – 1.82 (m, 5H, H2′, H7′, H8′), 1.71 – 1.08 (m, 26H, H5, H7, H9, H1′, H9′, H11′, H12′, H14′ – H17′, H20′, H22′ – H25′), 1.00 (s, 3H, H19′), 0.95 (d, $J = 6.5$ Hz, 3H, H21′), 0.90 (dd, $J = 5.5$, 1.0 Hz, 6H, H26′, H27′), 0.70 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.64$, 156.34 (NH*C*(O)O), 139.86 (C5′), 136.60 (C1″ of Ph), 128.51 – 127.85 (rest of Ph), 122.36 (C6′), 74.01 (C3′), 67.22, 66.50 (Ph*C*H$_2$), 56.69 (C14′), 56.18 (C17′), 50.01 (C9′), 46.55 (C10), 47.34 – 46.22 (C2, C4, C6, C8), 45.81 (C1), 42.31 (C4′), 39.86 (C16′), 39.77 (C24′), 39.54 (C5), 38.68 (C9), 36.53 (C22′), 36.23 (C8′), 35.81 (C20′), 31.88 (C7′), 28.26 (C2′), 27.99 (C25′), 24.31 (C12′), 23.88 (C15′), 22.88 (C23′), 22.62 (C26′), 21.07 (C11′), 19.34 (C19′), 18.78 (C21′), 11.90 (C18′); MS (FAB$^+$): $m/z = 855$ $[M+H]^+$, 398, 369 [Chol]$^+$, 154, 121, 105, 91 $[C_7H_7]^+$, 57; HRMS (FAB$^+$) $C_{52}H_{79}N_4O_6$: $[M+H]^+$ calcd 855.6000, found 855.6009.

***N*^{1}-Cholesteryloxycarbonyl-4,8-diaza-*N*^{8,11}-di(phenylmethoxycarbonyl)undecane-1,11-diamine (16e)**: This was prepared from **15c** and **5b** in a similar fashion to **8a** on a 2.35 mmol scale and purifed by chromatography (96:3.5:0.5 CH_2Cl_2/MeOH/NH_3 to 92:7:1 CH_2Cl_2/MeOH/NH_3) to give **16e** as a hygroscopic, white solid. Yield: 1.53 g (75 %); $R_f = 0.28$ (92:7:1 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu} = 3334$, 2901, 2867, 1698, 1532, 1467, 1455, 1373, 1137 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.33$ (m, 10H, Ph), 5.80 – 5.50 (br m, 2H, ChocN*H*, ZN*H*), 5.37 (m, 1H, H6′), 5.12 (s, 2H, PhCH_2O), 5.08 (s, 2H, PhCH_2O), 4.49 (m, 1H, H3′), 3.31 (br m, 4H, H7, H9), 3.17 (br m, 4H, H1, H11), 2.56 (m, 4H, H3, H5), 2.37 – 2.26 (m, 2H, H4′), 2.04 – 1.82 (m, 5H, H2′, H7′, H8′), 1.72 – 1.07 (m, 28H, H2, H4, H6, H10, H1′, H9′, H11′, H12′, H14′ – H17′, H20′, H22′ – H25′), 1.00 (s, 3H, H19′), 0.93 (d, $J = 6.5$ Hz, 3H, H21′), 0.88 (dd, $J = 5.5$, 1.0 Hz, 6H, H26′, H27′), 0.69 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.53$, 156.36 (NH*C*(O)O), 139.86 (C5′), 136.64 (C1″ of Ph), 128.56 – 127.89 (rest of Ph), 122.42 (C6′), 74.09 (C3′), 67.23, 66.45 (Ph*C*H$_2$), 56.69 (C14′), 56.17 (C17′), 50.02 (C9′), 47.52 – 46.36 (C1, C7, C9, C11), 44.93 (C3), 44.35 (C5), 42.32 (C4′), 39.76 (C16′), 39.54 (C24′), 38.67 (C2), 37.02 (C6), 36.56 (C22′), 36.22 (C8′), 35.81 (C20′), 31.89 (C7′), 28.98 (C10), 28.25 (C2′), 28.01 (C25′), 24.31 (C12′), 23.87 (C15′), 22.87 (C23′), 22.61 (C26′), 21.07 (C11′), 19.34 (C19′), 18.77 (C21′), 11.89 (C18′); MS (FAB$^+$): $m/z = 869$ $[M+H]^+$, 369 [Chol]$^+$, 161, 145, 121, 105, 91 $[C_7H_7]^+$, 69, 55; HRMS (FAB$^+$) $C_{53}H_{81}N_4O_6$: $[M+H]^+$ calcd 869.6156, found 869.6154.

***N*^{1}-Cholesteryloxycarbonyl-4,9-diaza-*N*^{9,12}-di(phenylmethoxycarbonyl)dodecane-1,12-diamine (16f)**: This was prepared from **15d** and **5b** analogously to **8a** on a 2.90 mmol scale and purified by chromatography (96:3.5:0.5 CH_2Cl_2/MeOH/NH_3 to 92:7:1 CH_2Cl_2/MeOH/NH_3) to give **16f** as a hygroscopic, white solid. Yield: 1.78 g (71 %); $R_f = 0.25$ (92:7:1 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu} = 3567 - 3349$, 3037, 2942, 1681, 1531, 1455, 1378, 1255, 1139 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.33 - 7.30$ (m, 10H, Ph), 6.08 (br s, 1H, ZN*H*), 5.77 (br s, 1H, ChocN*H*), 5.37 (m, 1H, H6′), 5.12 (s, 2H, PhCH_2O), 5.08 (s, 2H, PhCH_2O), 4.49 (m, 1H, H3′), 3.25 (m, 6H, H3, H5, H12), 3.11 (m, 2H, H1), 2.61 – 2.52 (m, 4H, H8, H10), 2.34 – 2.19 (m, 2H, H4′), 2.06 – 1.86 (m, 5H, H2′, H7′, H8′), 1.66 – 1.13 (m, 30H, H2, H6, H7, H9, H11, H1′, H9′, H11′, H12′, H14′ – H17′, H20′, H22′ – H25′), 1.01 (s, 3H, H19′), 0.95 (d, $J = 6.0$ Hz, 3H, H21′), 0.91 (d, $J = 6.5$ Hz, 6H, H26′, H27′), 0.71 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.62$, 156.27, 155.96 (NH*C*(O)O), 139.88 (C5′), 136.89, 136.72 (C1″ of Ph), 128.49 – 127.73 (rest of Ph), 122.30 (C6′), 74.01 (C3′), 67.03, 66.29 (Ph*C*H$_2$), 56.66 (C14′), 56.15 (C17′), 49.98 (C9′), 49.27 (C1), 47.53 – 46.34 (C3, C5, C12), 42.28 (C4′), 39.74 (C24′), 39.52 (C10), 38.63 (C8), 37.01 (C11), 36.51 (C22′), 36.21 (C8′), 35.80 (C20′), 31.58 (C7′), 29.48 (C2), 28.22 (C2′), 27.98 (C25′), 27.01 (C7), 24.29 (C12′), 23.87 (C15′), 22.88 (C23′), 22.62 (C26′), 21.05 (C11′), 19.34 (C19′), 18.77 (C21′), 11.88 (C18′); MS (FAB$^+$): $m/z = 883$

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$[M+H]^+$, 515, 369 $[Chol]^+$, 261, 221, 133, 121, 105, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 55; HRMS (FAB^+) $C_{54}H_{83}N_4O_6$: $[M+H]^+$ calcd 883.6313, found 883.6341.

***N*¹-Cholesteryloxycarbonyl-3,7-diazanonane-1,9-diamine (CDAN, [B198], 17a)**: This was prepared from **16a** in a similar manner to the synthesis of **9a** on a 1.50 mmol scale to give **17a** as a hygroscopic, white solid. Yield: 850 mg (99%); IR (CH_2Cl_2): $\tilde{\nu}$ = 3584–3245, 2937, 2868, 1695, 1538, 1469, 1379, 1251, 1133, 1014 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.82 (br s, 1H, ChocN*H*), 5.23 (m, 1H, H6′), 4.33 (m, 1H, H3′), 3.54–2.55 (m, 16H, H1–H4, H6–H9, NH_2), 2.21–2.09 (m, 2H, H4′), 1.97–1.73 (m, 5H, H2′, H7′, H8′), 1.55–0.99 (m, 23H, H5, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 0.88 (s, 3H, H19′), 0.78 (d, J = 6.0 Hz, 3H, H21′), 0.74 (d, J = 6.5 Hz, 6H, H26′, H27′), 0.55 (s, 3H, H18′); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.38 (NH*C*(O)O), 139.70 (C5′), 122.30 (C6′), 73.99 (C3′), 56.56 (C14′), 56.05 (C17′), 51.91 (C1), 49.88 (C9′), 47.95 (C2), 42.18 (C4′), 41.12 (C8), 39.63 (C16′), 39.40 (C24′), 38.53 (C2), 36.90 (C1′), 36.42 (C22′), 36.08 (C8′), 35.69 (C20′), 31.75 (C7′), 29.62 (C5), 28.12 (C2′), 27.86 (C25′), 24.17 (C12′), 23.75 (C15′), 22.73 (C23′), 22.48 (C26′), 20.94 (C11′), 19.24 (C19′), 18.62 (C21′), 11.76 (C18′); MS (FAB^+): m/z = 573 $[M+H]^+$, 544, 513, 369 $[Chol]^+$, 215, 175, 147, 121, 95, 69, 55; HRMS (FAB^+) $C_{35}H_{65}N_4O_2$: $[M+H]^+$ calcd 573.5108, found 573.5139.

***N*¹-Cholesteryloxycarbonyl-3,6-diazanonane-1,9-diamine (17b)**: This was prepared from **16b** in a similar fashion to **9a** on a 1.01 mmol scale to give **17b** as a hygroscopic, white solid. Yield: 56 mg (99%); IR (CH_2Cl_2): $\tilde{\nu}$ = 3439–3294, 2938, 2868, 1698, 1530, 1467, 1379, 1253, 1135, 1032 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 5.55 (br s, 1H, ChocN*H*), 5.33 (m, 1H, H6′), 4.43 (m, 1H, H3′), 3.63–2.99 (m, 10H, H1, H2, H4, H5, H9), 2.77 (m, 2H, H7), 2.36–2.18 (m, 2H, H4′), 2.00–1.79 (m, 5H, H2′, H7′, H8′), 1.65–1.05 (m, 27H, H3, H6, H8, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–25′, NH_2), 0.98 (s, 3H, H19′), 0.89 (d, J = 5.5 Hz, 3H, H21′), 0.84 (d, J = 6.5 Hz, 6H, H26′, H27′), 0.65 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.76 (NH*C*(O)O), 139.82 (C5′), 122.43 (C6′), 74.37 (C3′), 56.70 (C14′), 56.19 (C17′), 51.48 (C9′), 50.03 (C1), 48.80 (C2), 44.69 (C4), 42.31 (C7), 40.43 (C4′), 39.76 (C16′), 39.51 (C24′), 38.62 (C9), 37.02 (C1′), 36.55 (C22′), 36.20 (C8′), 35.79 (C20′), 31.88 (C7′), 28.21 (C2′), 27.97 (C25′), 24.27 (C12′), 23.86 (C15′), 22.80 (C23′), 22.55 (C26′), 21.05 (C11′), 19.33 (C19′), 18.72 (C21′), 11.86 (C18′); MS (FAB^+): m/z = 573 $[M+H]^+$, 369 $[Chol]^+$, 215, 161, 147, 121, 105, 95, 81, 69, 55; HRMS (FAB^+) $C_{35}H_{65}N_4O_2$: $[M+H]^+$ calcd 573.5108, found 573.5136.

***N*¹-Cholesteryloxycarbonyl-3,8-diazaundecane-1,11-diamine (17c)**: This was prepared from **16c** in a similar fashion to **9a** on a 0.18 mmol scale to give **17c** as a hygroscopic, white solid. Yield: 107 mg (99%); IR (CH_2Cl_2): $\tilde{\nu}$ = 3396, 2936, 2867, 1701, 1476, 1256, 1197 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 5.31 (d, J = 5.0 Hz, 1H, H6′), 5.23 (br m, 1H, ChocN*H*), 4.42 (m, 1H, H3′), 3.16 (m, 2H, H1), 2.73–2.55 (m, 10H, H2, H4, H7, H9, H11), 2.32–2.16 (m, 2H, H4′), 1.96–1.77 (m, 5H, H2′, H7′, H8′), 1.59 (quin, J = 7.0 Hz, 2H, H10), 1.56–1.01 (m, 29H, H3, H5, H6, H8, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–25′, NH_2), 0.94 (s, 3H, H19′), 0.84 (d, J = 6.5 Hz, 3H, H21′), 0.79 (dd, J = 5.5, 1.5 Hz, 6H, H26′, H27′), 0.60 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.39 (NH*C*(O)O), 139.88 (C5′), 122.45 (C6′), 74.26 (C3′), 56.70 (C14′), 56.15 (C17′), 50.03 (C9′), 49.28 (C9), 48.96 (C7), 42.32 (C1), 40.43 (C4′), 39.75 (C16′), 39.52 (C24′), 38.61 (C4), 37.01 (C1′), 36.19 (C8′), 35.80 (C20′), 31.90 (C7′), 28.22 (C2′), 28.01 (C25′), 27.85 (C5), 24.29 (C12′), 23.84 (C15′), 22.82 (C23′), 22.57 (C26′), 21.05 (C11′), 19.34 (C19′), 18.72 (C21′), 11.86 (C18′); MS (FAB^+): m/z = 601 $[M+H]^+$, 544, 525, 369 $[Chol]^+$, 159, 145, 121, 105, 95, 81, 69, 57; HRMS (FAB^+) $C_{37}H_{69}N_4O_2$: $[M+H]^+$ calcd 601.5421, found 601.5394.

***N*¹⁰-Cholesteryloxycarbonyl-3,7-diazadecane-1,10-diamine (17d)**: This was prepared from **16d** in a similar fashion to **9a** on a 2.14 mmol scale, to give **17d** as a hygroscopic, white solid. Yield: 1.24 g (99%); IR (CH_2Cl_2): $\tilde{\nu}$ = 3334, 2904, 2871, 1698, 1534, 1467, 1379, 1254 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.87 (br s, 1H, ChocN*H*), 5.16 (m, 1H, H6′), 4.26 (m, 1H, H3′), 3.02 (m, 2H, H10), 2.62 (m, 2H, H1), 2.51–2.46 (m, 8H, H2, H4, H6, H8), 2.18–1.92 (m, 2H, H4′), 1.83–0.86 (m, 34H, H3, H5, H7, H9, H1′, H2′, H7′–H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′, NH_2), 0.81 (s, 3H, H19′), 0.72 (d, J = 6.5 Hz, 3H, H21′), 0.66 (dd, J = 5.0, 1.5 Hz, 6H, H26′, H27′), 0.48 (s, 3H, H18′); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.20 (NH*C*(O)O), 139.68 (C5′), 122.15 (C6′), 73.68 (C3′), 56.49 (C14′), 55.98 (C17′), 52.24 (C10), 49.82 (C9′), 48.06 (C8), 42.11 (C4′), 41.40 (C6), 39.56 (C16′), 39.34 (C24′), 38.51 (C2), 36.35 (C22′), 36.02 (C8′), 35.63 (C20′), 31.68 (C7′), 29.91 (C5), 28.07 (C2′), 27.80 (C25′), 24.11 (C12′), 23.69 (C15′), 22.69 (C23′), 22.44 (C26′), 20.88 (C11′), 19.19 (C19′), 18.57 (C21′), 11.70 (C18′); MS (FAB^+): m/z = 587 $[M+H]^+$, 369 $[Chol]^+$, 161, 149, 131, 121, 105, 95, 81, 55; HRMS (FAB^+) $C_{36}H_{67}N_4O_2$: $[M+H]^+$ calcd 587.5264, found 587.5302.

***N*¹-Cholesteryloxycarbonyl-4,8-diazaundecane-1,11-diamine (17e)**: This was prepared from **16e** in a similar fashion to **9a** on a 1.53 mmol scale to give **17e** as a hygroscopic, white solid. Yield: 909 mg (99%); IR (CH_2Cl_2): $\tilde{\nu}$ = 3347–3294, 2937, 2868, 1698, 1533, 1467, 1379, 1250, 1132, 1037 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 5.75 (br s, 1H, ChocN*H*), 5.22 (m, 1H, H6′), 4.33 (m, 1H, H3′), 3.09 (m, 2H, H1), 2.65 (t, J = 6.5 Hz, 2H, H3), 2.58–2.48 (m, 8H, H5, H7, H9, H11), 2.28–2.12 (m, 2H, H4′), 1.96–1.69 (m, 5H, H2′, H7′, H8′), 1.57–0.92 (m, 31H, H2, H4, H6, H8, H10, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′, NH_2), 0.87 (s, 3H, H19′), 0.78 (d, J = 6.5 Hz, 3H, H21′), 0.73 (dd, J = 5.5, 1.5 Hz, 6H, H26′, H27′), 0.54 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.28 (NH*C*(O)O), 139.78 (C5′), 122.24 (C6′), 73.84 (C3′), 56.58 (C14′), 56.07 (C17′), 49.92 (C9′), 48.32 (C1), 48.21 (C3), 47.76 (C5), 42.31 (C9), 42.20 (C4′), 39.65 (C16′), 39.42 (C24′), 38.57 (C8), 36.94 (C1′), 36.44 (C22′), 36.10 (C8′), 35.70 (C20′), 31.78 (C7′), 29.75 (C2), 28.14 (C2′), 27.88 (C25′), 24.19 (C12′), 23.76 (C15′), 22.75 (C23′), 22.50 (C26′), 20.96 (C11′), 19.26 (C19′), 18.65 (C21′), 11.77 (C18′); MS (FAB^+): m/z = 601 $[M+H]^+$, 369 $[Chol]^+$, 273, 255, 229, 215; HRMS (FAB^+) $C_{37}H_{69}N_4O_2$: $[M+H]^+$ calcd 601.5421, found 601.5449.

***N*¹-Cholesteryloxycarbonyl-4,9-diazadodecane-1,12-diamine (CDAD, [B185], 17f)**: This was prepared from **16f** in a similar fashion to **9a** on a 1.64 mmol scale to give **17f** as a hygroscopic, white solid. Yield: 990 mg (99%); IR (CH_2Cl_2): $\tilde{\nu}$ = 3349, 2937, 2868, 1697, 1468, 1378, 1253 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.70 (br s, 1H, ChocN*H*), 5.27 (m, 1H, H6′), 4.38 (m, 1H, H3′), 3.28–3.14 (m, 6H, H1, H4, H9, NH_2), 2.71 (t, J = 6.5 Hz, 2H, H12), 2.63–2.52 (m, 8H, H3, H5, H8, H10), 2.33-2.17 (m, 2H, H4′), 1.93–1.85 (m, 5H, H2′, H7′, H8′), 1.77–1.07 (m, 29H, H2, H6, H7, H11, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 1.03 (s, 3H, H19′), 0.97 (d, J = 6.0 Hz, 3H, H21′), 0.77 (dd, J = 5.0, 1.5 Hz, 6H, H26′, H27′), 0.59 (s, 3H, H18′); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.25 (NH*C*(O)O), 139.77 (C5′), 122.26 (C6′), 73.87 (C3′), 56.57 (C14′), 56.04 (C17′), 49.90 (C1), 49.60 (C9′), 49.47 (C3), 47.40 (C10), 42.19 (C4′), 39.63 (C16′), 39.40 (C24′), 38.55 (C8), 36.92 (C5), 36.44 (C22′), 36.08 (C8′), 35.69 (C20′), 31.77 (C7′), 28.13 (C2′), 27.87 (C25′), 27.53 (C2), 24.18 (C16), 23.74 (C12′), 22.73 (C23′), 22.48 (C26′), 20.94 (C11′), 19.25 (C19′), 18.62 (C21′), 11.76 (C18′); MS (FAB^+): m/z = 615 $[M+H]^+$, 539, 369 $[Chol]^+$, 161, 147, 129, 105, 81, 69, 57; HRMS (FAB^+) $C_{38}H_{71}N_4O_2$: $[M+H]^+$ calcd 615.5577, found 615.5626.

3-Bromo-*N*-(cholesteryloxycarbonyl)propanamine (18): Methanesulfonyl chloride (4.76 mL, 61.5 mmol, 2.5 equiv) was added dropwise to a solution of **4b** (12.0 g, 24.6 mmol) and Et_3N (10.30 mL, 73.8 mmol, 3 equiv) in CH_2Cl_2 (120 mL) at 0 °C under a nitrogen atmosphere. After addition, the solution was allowed to warm to room temperature and stirring continued for 30 min. The reaction was quenched with ice (1 g) and poured into a mixture of ether (200 mL) and saturated NH_4Cl (100 mL) and the organic layer separated. The aqueous layer was extracted with ether (2 × 150 mL), the combined organic layers washed with water (2 × 100 mL) and brine (100 mL) and dried (Na_2SO_4). Removal of the solvents gave a white solid, to which was added NaBr (12.66 g, 123 mmol, 5 equiv) and DMF (120 mL), under a nitrogen atmosphere. The suspension was carefully heated to 80 °C for 2 h, allowed to cool and the solvent removed in vacuo. The viscous residue was redissolved in ether (300 mL), washed with water (2 × 100 mL) and brine (100 mL), dried (Na_2SO_4) and the solvent removed. Chromatography (50–100% ether/petrol) gave **18** as a white crystalline solid. Yield: 12.5 g (92%); m.p.: 131 °C; R_f = 0.67 (ether); IR (CH_2Cl_2): $\tilde{\nu}$ = 3333, 2904, 2822, 1684, 1540, 1467, 1380, 1264, 1135 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$): δ = 5.35 (d, J = 5.0 Hz, 1H, H6′), 4.88 (br s, 1H, ChocN*H*), 4.49 (m, 1H, H3′), 3.42 (t, J = 6.5 Hz, 2H, H3), 3.30 (q, J = 6.0 Hz, 2H, H1), 2.33–2.21 (m, 2H, H4′), 2.07–1.81 (m, 7H, H2, H2′, H7′, H8′), 1.60–1.02 (m, 21H, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 0.99 (s, 3H, H19′), 0.89 (d, J = 6.5 Hz, 3H, H21′), 0.84 (dd, J = 6.0, 1.0 Hz, 6H, H26′, H27′), 0.66 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.23 (NH*C*(O)O), 139.75 (C5′), 122.55 (C6′), 74.45 (C3′), 56.70 (C14′), 56.18 (C17′), 50.02 (C9′), 44.53 (C1), 42.33 (C4′), 39.76 (C24′), 39.54 (C16′), 38.58 (C13′), 37.01 (C1′), 36.57 (C22′), 36.21 (C8′), 35.82 (C3), 31.90 (C7′), 30.70 (C2), 28.25 (C2′), 28.02 (C25′), 24.31 (C12′), 23.87 (C15′), 22.84 (C23′), 22.59 (C26′), 21.07 (C11′), 19.36 (C19′), 18.74 (C21′), 11.88 (C18′); MS (FAB^+): m/z = 369 $[Chol]^+$, 255, 159, 145, 133, 119, 105, 91, 81, 69, 55; $C_{31}H_{52}BrNO_2$ (550.3): calcd C 67.60, H 9.52, N 2.54; found: C 67.62, H 9.57, N 2.57.

4-Aza-8-*t*-butyldiphenylsilyloxy-*N*¹-cholesteryloxycarbonyl-*N*⁴-phenylmethoxycarbonyloctanamine (19): This was prepared from **18** and **3c** in a

similar manner to **13a** on a 1.81 mmol scale and purified by chromatography (25–80% ether/petrol) to give **19** as a viscous, colourless oil. Yield: 1.48 g (78%); $R_f = 0.30$ (50% ether/petrol); IR (neat, KBr): $\tilde{\nu} = 3351, 3070, 2936, 2905, 2867, 1699, 1509, 1468, 1381, 1266, 1247, 1112, 737, 701\ cm^{-1}$; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.69$ (d, $J = 6.5$ Hz, 4H, H3″, H5″ of Ph_2Si), 7.46–7.35 (m, 11H, rest of Ph), 5.56 (br s, 1H, ChocN*H*), 5.41 (d, $J = 5.0$ Hz, 1H, H6′), 5.16 (s, 2H, PhCH_2O), 4.54 (m, 1H, H3′), 3.68 (m, 2H, H8), 3.37–3.16 (m, 6H, H1, H3, H5), 2.38–2.27 (m, 2H, H4′), 2.08–1.82 (m, 5H, H2′, H7′, H8′), 1.78–1.13 (m, 27H, H2, H6, H7, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 1.09 (s, 9H, Me of *t*-Bu), 1.06 (s, 3H, H19′), 0.97 (d, $J = 6.5$ Hz, 3H, H21′), 0.92 (d, $J = 6.5$ Hz, 3H, H26′), 0.91 (d, $J = 6.5$ Hz, 3H, H27′), 0.73 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.86$, 156.32 (NH*C*(O)O), 140.05 (C5′), 136.81–127.70 (Ph), 122.38 (C6′), 74.16 (C3′), 67.12 (Ph*C*H_2), 63.50 (C8), 56.76 (C14′), 56.21 (C17′), 50.09 (C9′), 47.02–46.56 (C3, C5), 44.13 (C1), 42.38 (C4′), 39.82 (C16′), 39.59 (C24′), 38.67 (C2), 37.48 (C1′), 36.62 (C10′), 36.26 (C22′), 35.86 (C20′), 31.96 (C8′), 29.84 (C7), 28.31 (C6), 28.26 (C12′), 26.94 (Me of *t*-Bu), 24.36 (C15′), 23.91 (C23′), 22.91 (C26′), 22.66 (C27′), 21.12 (C11′), 19.41 (C19′), 19.25 (Me_3C), 18.81 (C21′), 11.94 (C18′); MS (FAB$^+$): $m/z = 931$ $[M+H]^+$, 887 $[M+H-CO_2]^+$, 797 $[M+H-Z]^+$, 614, 519, 369 [Chol]$^+$, 197, 161, 135, 91 $[C_7H_7]^+$; HRMS (FAB$^+$) $C_{59}H_{87}N_2O_5Si$: $[M+H]^+$ calcd 931.6615, found 931.6639.

8-(*N*-Cholesteryloxycarbonyl)amino-5-aza-*N*^{5}-phenylmethoxycarbonyloctanol (**20**): This was prepared from **19** in a similar way to the synthesis of **14a** on a 1.02 mmol scale and after chromatography (ether to 35% acetone/ether) gave **20** as a waxy solid. Yield: 644 mg (90%); $R_f = 0.51$ (75% ether/acetone); IR (CH_2Cl_2): $\tilde{\nu} = 3422, 3353, 3065, 2943, 2906, 2868, 1699, 1525, 1468, 1380, 1167\ cm^{-1}$; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.32-7.30$ (m, 5H, Ph), 5.62 (br s, 1H, ChocN*H*), 5.34 (m, 1H, H6′), 5.10 (s, 2H, PhCH_2O), 4.46 (m, 1H, H3′), 3.56 (m, 2H, H1), 3.23–3.15 (m, 4H, H4, H6), 3.10 (m, 2H, H8), 2.30–2.18 (m, 2H, H4′), 2.02–1.81 (m, 5H, H2′, H7′, H8′), 1.69 (m, 2H, H7), 1.57–1.05 (m, 26H, H2, H3, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′, O*H*), 0.99 (s, 3H, H19′), 0.90 (d, $J = 6.5$ Hz, 3H, H21′), 0.85 (d, $J = 6.5$ Hz, 6H, H26′, H27′), 0.67 (s, 3H,H18′); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.74$, 156.35 (NH*C*(O)O), 139.88 (C5′), 136.70 (C1″ of Ph), 128.54–127.70 (rest of Ph), 122.40 (C6′), 74.18 (C3′), 67.16 (Ph*C*H_2), 62.00 (C1), 56.69 (C14′), 56.16 (C17′), 50.01 (C9′), 47.32 (C6), 46.55 (C4), 44.32 (C8), 42.31 (C4′), 39.75 (C16′), 39.54 (C24′), 38.61 (C7), 37.01 (C1′), 36.55 (C10′), 36.21 (C22′), 35.82 (C20′), 31.88 (C8′), 29.74 (C2), 28.26 (C3), 28.20 (C12′), 28.01 (C25′), 24.31 (C15′), 23.87 (C23′), 22.87 (C26′), 22.62 (C27′), 21.07 (C11′), 19.36 (C19′), 18.76 (C21′), 11.89 (C18′); MS (FAB$^+$): $m/z = 693$ $[M+H]^+$, 649 $[M+H-CO_2]^+$, 626, 603, 559, 369 [Chol]$^+$, 325, 281, 133, 121, 105, 91 $[C_7H_7]^+$, 81 $[C_6H_9]^+$, 69, 55; HRMS (FAB$^+$) $C_{43}H_{69}N_2O_5$: $[M+H]^+$ calcd 693.5206, found 693.5240.

8-(*N*-Cholesteryloxycarbonyl)amino-5-(*N*-phenylmethoxycarbonyl)azaoctanal (**21**): This was prepared from **20** in a similar fashion to **5a** on a 7.65 mmol scale and after purification by chromatography (50% ether/petrol 1% NH_3) gave **21** as a waxy solid. Yield: 4.96 g (94%); m.p.: 230°C (decomp.); $R_f = 0.28$ (ether); IR (CH_2Cl_2): $\tilde{\nu} = 3431, 3348, 2944, 1698, 1527, 1455, 1380, 1253, 1172\ cm^{-1}$; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 9.72-9.62$ (m, 1H, C*H*O), 7.33 (m, 5H, Ph), 5.51 (br s, 1H, ChocN*H*), 5.35 (m, 1H, H6′), 5.11 (s, 2H, PhCH_2O), 4.45 (m, 1H, H3′), 3.26 (m, 4H, H4, H6), 3.11 (m, 2H, H8), 2.45–2.25 (m, 4H, H2, H4′), 2.02–1.82 (m, 7H, H3, H2′, H7′, H8′), 1.68 (m, 2H, H7), 1.53–1.06 (m, 21H, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 0.99 (s, 3H, H19′), 0.90 (d, $J = 6.5$ Hz, 3H, H21′), 0.85 (d, $J = 6.5$ Hz, 6H, H26′, H27′), 0.66 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 201.09$ (*C*HO), 156.23 (NH*C*(O)O), 139.90 (C5′), 136.54 (C1″ of Ph), 128.58–127.95 (rest of Ph), 122.39 (C6′), 74.14 (C3′), 67.26 (Ph*C*H_2), 56.69 (C14′), 56.16 (C17′), 50.01 (C9′), 47.36 (C4), 46.33 (C6), 44.25 (C2), 42.31 (C4′), 40.79 (C8), 39.76 (C16′), 39.53 (C24′), 38.61 (C3), 37.02 (C1′), 36.56 (C22′), 36.21 (C8′), 35.81 (C20′), 31.89 (C7′), 28.26 (C2′), 28.20 (C10′), 28.01 (C25′), 24.31 (C15′), 23.86 (C12′), 22.87 (C23′), 22.61 (C26′), 21.06 (C11′), 19.36 (C19′), 18.76 (C21′), 11.89 (C18′); MS (FAB$^+$): $m/z = 369$ [Chol]$^+$, 159, 129, 117, 105, 95, 91 $[C_7H_7]^+$, 79, 69, 55; $C_{43}H_{66}N_2O_5$ (690.5): calcd C 74.73, H 9.63, N 4.06; found: C 74.54, H 9.40, N 3.95.

***N*^{15}-Cholesteryloxycarbonyl-3,7,12-triaza-*N*^{1,3,12}-tri(phenylmethoxycarbonyl)pentadecane-1,15-diamine** (**22a**): This was prepared from **15b** and **21** in an analogous way to the synthesis of **8a** on a 2.91 mmol scale and purified by chromatography (96:3.5:0.5 CH_2Cl_2/MeOH/NH_3 to 92:7:1 CH_2Cl_2/MeOH/NH_3) to give **22a** as a white, hygroscopic solid. Yield: 2.28 g (74%); $R_f = 0.33$ (92:7:1 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu} = 3630-3346, 3064, 2942, 1693, 1531, 1455, 1366, 1253, 1139\ cm^{-1}$; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.32-7.28$ (m, 15H, Ph), 6.05–5.75 (br m, 2H, ChocN*H*, ZN*H*), 5.37 (m, 1H, H6′), 5.23 (s, 2H, PhCH_2O), 5.09 (s, 2H, PhCH_2O), 5.06 (s, 2H, PhCH_2O), 4.49 (m, 1H, H3′), 3.32–3.12 (m, 12H, H1, H2, H4, H11, H13, H15), 2.50 (m, 4H, H6, H8), 2.34–2.20 (m, 2H, H4′), 2.04–1.86 (m, 5H, H2′, H7′, H8′), 1.68 (m, 4H, H5, H14), 1.56 (m, 4H, H9, H10), 1.53–1.07 (m, 22H, H7, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 1.00 (s, 3H, H19′), 0.94 (d, $J = 6.0$ Hz, 3H, H21′), 0.89 (d, $J = 6.5$ Hz, 6H, H26′, H27′), 0.69 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.61$, 156.27, 155.95 (NH*C*(O)O), 139.90 (C5′), 136.75, 136.62 (C1″ of Ph), 128.49–127.76 (rest of Ph), 122.32 (C6′), 74.03 (C3′), 67.14, 67.04, 66.44 (Ph*C*H_2), 56.67 (C14′), 56.15 (C17′), 49.99 (C9′), 49.42 (C15), 46.87–46.34 (C1, C2, C4, C11, C13), 42.29 (C4′), 39.76 (C24′), 39.53 (C6), 38.64 (C8), 37.02 (C5), 36.53 (C22′), 36.21 (C8′), 35.80 (C20′), 31.86 (C7′), 28.23 (C2′), 27.99 (C25′), 27.13 (C9), 24.31 (C12′), 23.87 (C15′), 22.90 (C23′), 22.64 (C26′), 21.07 (C11′), 19.35 (C19′), 18.78 (C21′), 11.90 (C18′); MS (FAB$^+$): $m/z = 1060$ $[M+H]^+$, 369 [Chol]$^+$, 147, 121, 105, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 67, 57; HRMS (FAB$^+$) $C_{64}H_{94}N_5O_8$: $[M+H]^+$, 1060.7102, found: 1060.7083.

***N*^{16}-Cholesteryloxycarbonyl-4,8,13-triaza-*N*^{1,4,13}-tri(phenylmethoxycarbonyl)hexadecane-1,16-diamine** (**22b**): This was prepared from **15c** and **21** in a similar fashion to **8a** on a 2.89 mmol scale and after chromatography (96:3.5:0.5 CH_2Cl_2/MeOH/NH_3 to 92:7:1 CH_2Cl_2/MeOH/NH_3) gave **22b** as a white, hygroscopic solid. Yield: 1.32 g (71%); $R_f = 0.27$ (92:7:1 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu} = 3325, 3033, 2942, 2867, 1699, 1527, 1475, 1455, 1368, 1252, 1139, 1083\ cm^{-1}$; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.33$ (m, 15H, Ph), 5.78–5.53 (br m, 2H, ChocN*H*, ZN*H*), 5.36 (d, $J = 5.0$ Hz, 1H, H6′), 5.12 (s, 2H, PhCH_2O), 5.11 (s, 2H, PhCH_2O), 5.08 (s, 2H, PhCH_2O), 4.48 (m, 1H, H3′), 3.30–3.14 (m, 12H, H1, H3, H5, H12, H14, H16), 2.52 (m, 4H, H8, H10), 2.32–2.18 (m, 2H, H4′), 2.03–1.83 (m, 5H, H2′, H7′, H8′), 1.78–1.06 (m, 32H, H2, H6, H7, H9, H11, H15, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 1.01 (s, 3H, H19′), 0.93 (d, $J = 6.5$ Hz, 3H, H21′), 0.87 (dd, $J = 5.5$, 1.0 Hz, 6H, H26′, H27′), 0.68 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.50$, 156.27 (NH*C*(O)O), 139.97 (C5′), 136.67 (C1″ of Ph), 128.56–127.84 (rest of Ph), 122.37 (C6′), 74.15 (C3′), 67.21, 67.13, 66.48 (Ph*C*H_2), 56.71 (C14′), 56.18 (C17′), 50.04 (C9′), 49.50 (C1), 47.31–46.46 (C3, C5, C12, C14, C16), 44.25 (C10), 42.33 (C4′), 39.77 (C16′), 39.54 (C24′), 38.63 (C8), 37.04 (C11), 36.58 (C22′), 36.22 (C8′), 35.81 (C20′), 31.91 (C7′), 28.24 (C2′), 28.02 (C25′), 27.22 (C2), 24.31 (C12′), 23.86 (C15′), 22.87 (C23′), 22.61 (C26′), 21.08 (C11′), 19.36 (C19′), 18.77 (C21′), 11.90 (C18′); MS (FAB$^+$): $m/z = 1074$ $[M+H]^+$, 706, 369 [Chol]$^+$, 154, 136, 121, 107, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$ 69, 43; HRMS (FAB$^+$) $C_{65}H_{96}N_5O_8$: $[M+H]^+$ calcd 1074.7256, found 1074.7279.

***N*^{1}-Cholesteryloxycarbonyl-4,9,14-triaza-*N*^{4,14,17}-tri(phenylmethoxycarbonyl)heptadecane-1,17-diamine** (**22c**): This was prepared from **15d** and **21** analogously to **8a** on a 2.28 mmol scale and purified by chromatography (92:7:1 CH_2Cl_2/MeOH/NH_3) to give **22c** as a hygroscopic, white solid. Yield: 1.40 g (56%); $R_f = 0.25$ (92:7:1 CH_2Cl_2/MeOH/NH_3); IR (CH_2Cl_2): $\tilde{\nu} = 3649-3384, 3069, 2933, 1679, 1655, 1483, 1455, 1366, 1139\ cm^{-1}$; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.34$ (m, 15H, Ph), 5.88 (br s, 1H, ZN*H*), 5.64 (br s, 1H, ChocN*H*), 5.39 (m, 1H, H6′), 5.14 (s, 2H, PhCH_2O), 5.13 (s, 2H, PhCH_2O), 5.10 (s, 2H, PhCH_2O), 4.50 (m, 1H, H3′), 3.38–3.16 (m, 12H, H1, H3, H5, H13, H15, H17), 2.55 (m, 4H, H8, H10), 2.35–2.23 (m, 2H, H4′), 2.06–1.84 (m, 5H, H2′, H7′, H8′), 1.70 (m, 4H, H2, H16), 1.57–1.10 (m, 30H, H6, H7, H9, H11, H12, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 1.02 (s, 3H, H19′), 0.94 (d, $J = 6.5$ Hz, 3H, H21′), 0.90 (d, $J = 6.5$ Hz, 6H, H26′, H27′), 0.70 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.53$, 156.28 (NH*C*(O)O), 139.93 (C5′), 136.75 (C1″ of Ph), 128.52–127.75 (rest of Ph), 122.34 (C6′), 74.09 (C3′), 67.09, 66.41 (Ph*C*H_2), 56.69 (C14′), 56.16 (C17′), 50.02 (C9′), 49.55 (C1), 46.63–46.12 (C3, C5, C13), 44.23 (C15), 42.31 (C4′), 39.76 (C24′), 39.54 (C17), 38.63, 37.03 (C8, C10), 36.56 (C22′), 36.21 (C8′), 35.80 (C20′), 31.89 (C7′), 28.22 (C2′), 28.01 (C25′), 27.29, 26.44 (C7, C11), 24.31 (C12′), 23.86 (C15′), 22.89 (C23′), 22.63 (C26′), 21.07 (C11′), 19.36 (C19′), 18.77 (C21′), 11.90 (C18′); MS (FAB$^+$): $m/z = 1088$ $[M+H]^+$, 369 [Chol]$^+$, 147, 133, 121, 105, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 57; HRMS (FAB$^+$) $C_{66}H_{98}N_5O_8$: $[M+H]^+$ calcd 1088.7415, found 1088.7404.

***N*^{15}-Cholesteryloxycarbonyl-3,7,12-triazapentadecane-1,15-diamine (CTAP, [B232], 23a)**: This was prepared from **22a** in the same way as the preparation of **9a** on a 1.47 mmol scale, to give **23a** as a hygroscopic, white solid. Yield: 957 mg (99%); IR (CH_2Cl_2): $\tilde{\nu} = 3568-3295, 2937, 1690, 1537, 1467, 1380, 1130, 1019\ cm^{-1}$; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.76$ (br s, 1H, ChocN*H*), 5.22 (m, 1H, H6′), 4.32 (m, 1H, H3′), 3.21 (m, 2H, H15), 2.65 (t, $J = 5.5$ Hz, 2H, H13), 2.56–2.45 (m, 12H, H1, H2, H4, H6, H8, H11), 2.18–

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2.05 (m, 2H, H4′), 1.97–1.67 (m, 10H, H3, H7, H12, H2′, H7′, H8′, NH_2), 1.59–0.91 (m, 29H, H5, H9, H10, H14, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 0.86 (s, 3H, H19′), 0.77 (d, J = 6.5 Hz, 3H, H21′), 0.72 (dd, J = 6.0, 1.0 Hz, 6H, H26′, H27′), 0.53 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.24 (NH*C*(O)O), 139.77 (C5′), 122.21 (C6′), 73.81 (C3′), 56.57 (C14′), 56.05 (C17′), 49.91 (C9′), 49.67 (C15), 48.20 (C13), 42.19 (C4′), 39.64 (C16′), 39.40 (C24′), 38.56 (C2), 36.92 (C1′), 36.43 (C22′), 36.08 (C8′), 35.68 (C20′), 31.77 (C7′), 28.12 (C2′), 27.86 (C25′), 27.69 (C14), 27.63 (C5), 24.17 (C12′), 23.74 (C15′), 22.73 (C23′), 22.48 (C26′), 20.94 (C11′), 19.25 (C19′), 18.63 (C21′), 11.76 (C18′); MS (FAB$^+$): m/z = 658 $[M+H]^+$, 539, 369 $[Chol]^+$, 147, 133, 121, 109, 95, 84, 69, 57; HRMS (FAB$^+$) $C_{40}H_{76}N_5O_2$: $[M+H]^+$ calcd 658.5999, found 658.6056.

N^{16}-Cholesteryloxycarbonyl-4,8,13-triazahexadecane-1,16-diamine (**CTAH, [B222]**, **23b**): This was prepared from **22b** in a similar fashion to **9a** on a 0.90 mmol scale to give **23b** as a hygroscopic, white solid. Yield: 598 mg (99%); IR (CH_2Cl_2): $\tilde{\nu}$ = 3344, 2936, 2855, 1700, 1536, 1468, 1379, 1265, 1122, 1028 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.69 (br s, 1H, ChocN*H*), 5.22 (d, J = 4.0 Hz, 1H, H6′), 4.33 (m, 1H, H3′), 3.20 (m, 2H, H1), 2.63 (t, J = 6.5 Hz, 2H, H3), 2.55–2.35 (m, 12H, H5, H8, H10, H12, H14, H16), 2.23–2.08 (m, 7H, H4, H9, H13, H4′, NH_2), 1.90–1.63 (m, 5H, H2′, H7′, H8′), 1.57–0.90 (m, 31H, H2, H6, H7, H11, H15, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′), 0.87 (s, 3H, H19′), 0.78 (d, J = 6.5 Hz, 3H, H21′), 0.73 (d, J = 6.5 Hz, 6H, H26′, H27′), 0.55 (s, 3H, H18′); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.27 (NH*C*(O)O), 139.79 (C5′), 122.24 (C6′), 73.86 (C3′), 56.58 (C14′), 56.06 (C17′), 49.92 (C9′), 49.62 (C1), 49.72 (C3), 49.62 (C12), 47.52 (C14), 42.20 (C4′), 39.65 (C16′), 39.42 (C24′), 38.56 (C8), 36.93 (C1′), 36.45 (C22′), 36.09 (C8′), 35.69 (C20′), 31.78 (C7′), 28.14 (C2′), 27.88 (C25′), 27.74 (C2), 24.19 (C12′), 23.74 (C15′), 22.75 (C23′), 22.49 (C26′), 20.95 (C11′), 19.26 (C19′), 18.64 (C21′), 11.77 (C18′); MS (FAB$^+$): m/z = 672 $[M+H]^+$, 584, 570, 539, 369 $[Chol]^+$, 133, 121, 105, 95, 84, 69, 55; HRMS (FAB$^+$) $C_{41}H_{78}N_5O_2$: $[M+H]^+$ calcd 672.6156, found 672.6205.

N^1-Cholesteryloxycarbonyl-4,9,14-triazaheptadecane-1,17-diamine (**23c**): This was prepared from **22c** in a similar fashion to **9a** on a 1.21 mmol scale to give **23c** as a hygroscopic, white solid. Yield: 821 mg (99%); IR (CH_2Cl_2): $\tilde{\nu}$ = 3357, 2936, 2845, 1697, 1469, 1433 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.69 (br s, 1H, ChocN*H*), 5.20 (m, 1H, H6′), 4.31 (m, 1H, H3′), 3.06 (m, 2H, H1), 2.64–2.45 (m, 14H, H3, H5, H8, H10, H13, H15, H17), 2.33–2.13 (m, 2H, H4′), 2.11–1.84 (m, 5H, H2′, H7′, H8′), 1.79–1.01 (m, 38H, H2, H4, H6, H7, H9, H11, H12, H14, H16, H1′, H9′, H11′, H12′, H14′–H17′, H20′, H22′–H25′, NH_2), 0.98 (s, 3H, H19′), 0.77 (d, J = 6.5 Hz, 3H, H21′), 0.71 (dd, J = 5.0, 2.0 Hz, 6H, H26′, H27′), 0.53 (s, 3H, H18′); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.01 (NH*C*(O)O), 139.54 (C5′), 121.99 (C6′), 73.59 (C3′), 56.33 (C14′), 55.80 (C17′), 52.29 (C1), 49.66 (C9′), 49.35 (C3), 41.95 (C4′), 39.39 (C16′), 39.16 (C24′), 36.68 (C22′), 36.20 (C8′), 35.84 (C20′), 31.53 (C7′), 27.88 (C2′), 27.63 (C25′), 27.47 (C2), 27.43 (C16), 23.93 (C12′), 23.49 (C15′), 22.49 (C23′), 22.24 (C26′), 20.70 (C11′), 19.01 (C19′), 18.39 (C21′), 11.52 (C18′); MS (FAB$^+$): m/z = 686 $[M+H]^+$, 558, 539, 369 $[Chol]^+$, 173, 147, 121, 105, 95, 84, 69, 57; HRMS (FAB$^+$) $C_{42}H_{80}N_5O_2$: $[M+H]^+$ calcd 686.6312, found 686.6364.

In vitro and in vivo testing of cationic liposomes

For in vitro and in vivo tests, a dried lipid film containing the given polyamine DC-Chol analogue and **2** (in a 1:0, 1:1, 1:2 or 2:1 molar ratio), was hydrated for 10 min in sterile pyrogen-free water and then the liposomes were produced by 2 min vortex mixing. Average diameter was between 200–400 nm.[14] Cationic liposomes containing **1** and **2** were formulated as described elsewhere.[4,18] Cationic liposome/plasmid DNA complexes were then prepared as follows. Both the cationic liposome suspensions and the DNA (either pCF1-βGal plasmid expressing β-galactosidase or pCF1-CAT expressing chloramphenicol acetyl transferase)[14] solutions were separately preincubated for 5 min at 30°C before being diluted to the appropriate final concentrations and then combined. Usually, cationic liposome suspensions were added to an approximately equal volume of plasmid DNA solutions. Complexes were allowed to equilibrate for a minimum of 15 min at ambient temperature and used within 2 h of preparation. In vitro and in vivo gene-delivery assays were then performed as described previously using CFT1 cells and female BALB/c mice, respectively.[14]

Received: August 29, 1997 [F808]

Acknowledgements: This work was supported by the MRC through the provision of a Cell Engineering/LINK award, by the BBSRC and by the Royal Society.

[1] a) X. Gao, L. Huang, *Gene Ther.* **1995**, *2*, 710–722, and references therein; b) A. D. Miller, R. G. Cooper, C. J. Etheridge, L. Stewart, in *Microspheres, Microcapsules and Liposomes* (Ed.: R. Arshady), Wiley, **1998**, in press, and references therein; c) P. L. Felgner, *Hum. Gene Ther.* **1996**, *7*, 1791–1793.

[2] a) H. Farhood, N. Serbina, L. Huang, *Biochim. Biophys. Acta* **1995**, *1235*, 289–295; b) J. Zabner, A. J. Fasbender, T. Moninger, K. A. Poellinger, M. J. Welsh, *J. Biol. Chem.* **1995**, *270*, 18997–19007.

[3] H. Mitsui, L. G. Johnson, S. H. Randell, R. C. Boucher, *J. Biol. Chem.* **1997**, *272*, 1117–1126.

[4] E. W. F. W. Alton , P. G. Middleton , N. J. Caplen, S. N. Smith, D. M. Steel, F. M. Munkonge, P. K. Jeffery, D. M. Geddes, S. L. Hart, R. Williamson, K. I. Fasold, A. D. Miller, P. Dickinson, B. J. Stevenson, G. McLachlan, J. R. Dorin, D. J. Porteous, *Nat. Genet.* **1993**, *5*, 135–142.

[5] a) N. J. Caplen, E. W. F. W. Alton, P. G. Middleton, J. R. Dorin, B. J. Stevenson, X. Gao, S. R. Durham, P. K. Jeffery, M. E. Hodson, C. Coutelle, L. Huang, D. J. Porteous, R. Williamson, D. M. Geddes, *Nat. Med.* **1995**, *1*, 39–46; b) G. J. Nabel, E. G. Nabel, Z.-Y. Yang, B. A. Fox, G. E. Plautz, X. Gao, L. Huang, S. Shu, D. Gordon, A. E. Chang, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 11307–11311.

[6] *Liposomes: A Practical Approach* (Ed.: R. R. C. New), IRL, Oxford, **1990**.

[7] J. H. Felgner, R. Kumar, C. N. Sridhar, C. J. Wheeler, Y. J. Tsai, R. Border, P. Ramsey, M. Martin, P. L. Felgner, *J. Biol. Chem.* **1994**, *269*, 2550–2561.

[8] K. Omura, D. Swern, *Tetrahedron* **1978**, *34*, 1651–1660.

[9] a) F. Schneider, K. Bernauer, *Helv. Chim. Acta* **1974**, *57*, 434–440; b) A. K. Mallams, J. B. Morton, P. Reichert, *J. Chem. Soc. Perkin Trans. 1* **1981**, 2186–2208; c) C. A. Townsend, A. Basak, *Tetrahedron* **1991**, *47*, 2591–2602.

[10] a) H. H. Lee, B. D. Palmer, B. C. Baguley, M. Chin, W. D. McFadyen, G. Wickham, D. Thorsbourne-Palmer, L. P. G. Wakelin, W. A. Denny, *J. Med. Chem.* **1992**, *35*, 2983–2987; b) R. T. Lee, K. G. Rice, N. B. N. Rao, Y. Ichikawa, T. Barthel, V. Piskarev, Y. C. Lee, *Biochemistry* **1989**, *28*, 8351–8358.

[11] a) H. Staudinger, J. Meyer, *Helv. Chim. Acta* **1919**, *2*, 635–646; b) E. Fabiano, B. T. Golding, M. M. Sadeghi, *Synthesis* **1987**, 190–192; c) B. T. Golding, M. C. O'Sullivan, L. L. Smith, *Tetrahedron Lett.* **1988**, *29*, 6651–6654; d) Y. G. Gololobov, L. F. Kasukhin, *Tetrahedron* **1992**, *48*, 1353–1406; e) A. W. Johnson, W. C. Kaska, K. A. O. Starzewski, D. A. Nixon, *Ylides and Imines of Phosphorus* (Ed.: A. W. Johnson), Wiley, New York, **1993**, ch. 13, p. 403–483.

[12] S. Knapp, J. J. Hale, M. Bastos, A. Molina, K. Y. Chen, *J. Org. Chem.* **1992**, *57*, 6239–6256.

[13] a) J. E. Baldwin, R. M. Adlington, A. S. Elend, M. L. Smith, *Tetrahedron* **1995**, *51*, 11581–11594; b) R. M. Moriarty, S. M. Tuladhar, L. Guo, S. Wehrli, *Tetrahedron Lett.* **1994**, *35*, 8103–8106.

[14] E. R. Lee, J. Marshall, C. S. Siegel, C. Jiang, N. S. Yew, M. R. Nichols, J. B. Nietupski, R. J. Ziegler, M. B. Lane, K. X. Wang, N. C. Wan, R. K. Scheule, D. J. Harris, A. E. Smith, S. H. Cheng, *Hum. Gene Ther.* **1996**, *7*, 1701–1717.

[15] C. J. Wheeler, P. L. Felgner, Y. J. Tsai, J. Marshall, L. Sukhu, S. G. Doh, J. Hartikka, J. Nietupski, M. Manthorpe, M. Nichols, M. Plewe, X. Liang, J. Norman, A. Smith, S. H. Cheng, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 11454–11459.

[16] J. K. Guy-Caffey, V. Bodepudi, J. S. Bishop, K. Jayaraman, N. Chaudhary, *J. Biol. Chem.* **1995**, *270*, 31391–31396.

[17] a) J.-P. Vigneron, N. Oudrhiri, M. Fauquet, L. Vergely, J.-C. Bradley, M. Basseville, P. Lehn, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 9682–9686; b) J. G. Lewis, K.-Y. Lin, A. Kothavale, W. M. Flanagan, M. D. Matteucci, B. De Prince, R. A. Mook, Jr., R. W. Hendren, R. W. Wagner, *ibid.* **1996**, *93*, 3176–3181; c) N. Oudrhiri, J.-P. Vigneron, M. Peuchmaur, T. Leclerc, J.-M. Lehn, P. Lehn, *ibid.* **1997**, *94*, 1651-1656; d) D. Moradpour, J. I. Schauer, V. R. Zurawski, Jr., J. R. Wands, R. H. Boutin, *Biochem. Biophys. Res. Commun.* **1996**, *221*, 82–88.

[18] X. Gao, L. Huang, *Biochem. Biophys. Res. Commun.* **1991**, *179*, 280–285.