New aminocyclitols as modulators of glucosylceramide metabolism

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A series of 13 aminocyclitol derivatives belonging to two different families is described. Their configuration is governed by the regio- and stereocontrolled epoxide opening of a suitably protected conduritol-B epoxide. Studies on several glycosyl processing enzymes indicate that some of them are good inhibitors of glucosylceramide hydrolase. A rationale to account for preliminary structure–activity relationships is provided.

Introduction

Aminocyclitols are a group of natural compounds that are widely distributed throughout nature.[1] Several families of aminocyclitol analogues have been synthesized over the last decades and their biological properties have been thoroughly studied.[2] Among them, glycosidase inhibitors have gained momentum as pharmacological tools, as well as for their potential as new therapeutic agents.[3]

Over the course of our current research on new chemical entities as modulators of sphingolipid and glycosphingolipid (GSL) metabolism,[4-6] we have focussed on the regulation of ceramide (Cer) and glucosylceramide (GlcCer) levels owing to their relevance as biochemical targets for the development of new pharmacological tools with potential therapeutic applications.[7,8]

Along this line, in this work we report on the synthesis and glucosidase inhibitory activities of a series of new aminocyclitols derived from the regio- and sterocontrolled epoxide opening of a suitably protected (+)-conduritol-B epoxide 14[9] (see Scheme 1).

Results

Despite epoxide opening of substituted 1,2-epoxycyclohexanes being documented,[10] examples of regio- and stereocontrolled opening of cyclitol epoxides with nitrogen nucleophiles are scarce.[11] Although a *trans*-diaxial stereoselectivity is generally found, based on stereoelectronic effects,[12,13] the observed regioselectivity depends largely on conformational bias and can be modulated by a judicious choice of reaction conditions.

In a previous work, we accounted for an unexpected chelation-controlled C1 regioselective opening of epoxide 14 with nitrogen nucleophiles in the presence of Yb(OTf)$_3$.[14] Further to this, we have shown that Li salts behave similarly to promote epoxide opening through an intramolecular chelation process, which would require the participation of an apparently highly energetic "all-axial" conformation (Scheme 2).[15]
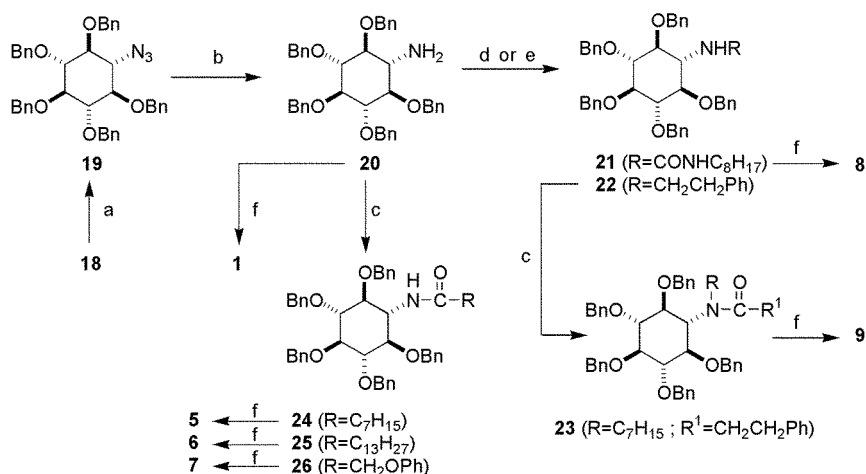
Thus, both aminolysis or azidolysis of epoxide 14 in the presence 2 N LiClO$_4$ cleanly afforded the corresponding C1 adducts 15–18, as shown in Scheme 2. The regiochemistry of the
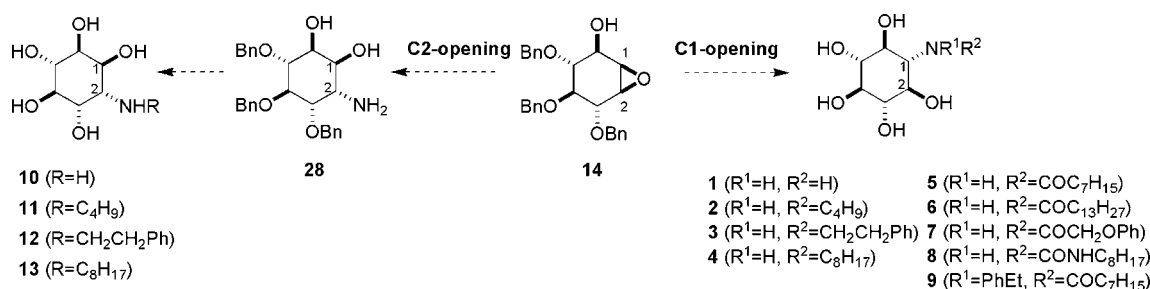

Scheme 1 Aminocyclitols described in this work.
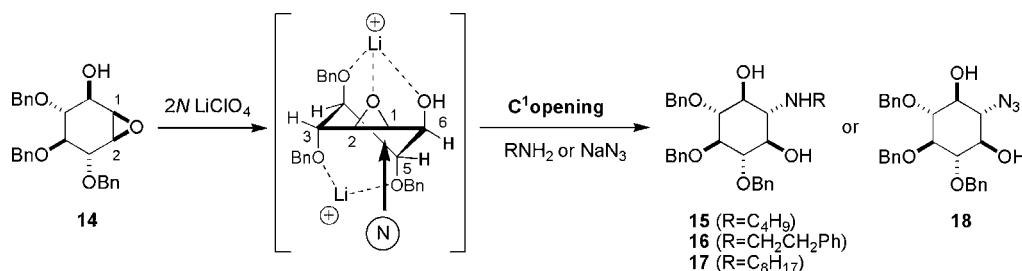
Scheme 2 Synthesis of cyclitol analogues by regioselective C1 opening of the starting epoxide.

Scheme 3 Synthesis of *N*-acylcyclitols **5–7**, **9** and ureidocyclitol **8** from azide **18**. *Reagents and conditions*: (a) BnCl, NaH, THF (95%); (b) LiAlH$_4$, THF (87%); (c) R^{1}COCl, Et$_3$N, CH$_2$Cl$_2$ (81–87%); (d) (i) COCl$_2$, toluene; (ii) octylamine, CH$_2$Cl$_2$ (67% overall); (e) PhCH$_2$CHO, NaBH$_3$CN, AcOH, MeOH (85%); (f) BCl$_3$, CH$_2$Cl$_2$ (quantitative).
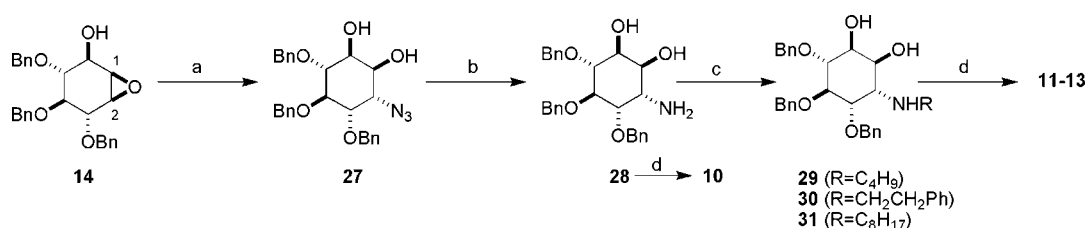
Scheme 4 Synthesis of aminocyclitols by regioselective C2 opening of epoxide **14**. *Reagents and conditions*: (a) NaN$_3$, NH$_4$Cl, MeOH–H$_2$O (80–81%); (b) LiAlH$_4$, THF (81%); (c) RCHO, NaBH$_3$CN, AcOH, MeOH (83–91%); (d) BCl$_3$, CH$_2$Cl$_2$ (quantitative).

epoxide opening under these conditions was corroborated by the simplification of the NMR pattern observed for these adducts due to symmetry considerations.

Debenzylation of **15–17** (Scheme 2) and **20** (obtained by *O*-benzylation and azide reduction of **18**, Scheme 3) afforded the corresponding aminocyclitols **1–4** (Scheme 1). Amide analogues **5–7**, **9** and ureido cyclitol **8** were obtained from **20**, as outlined in Scheme 3.

Treatment of epoxide **14** with sodium azide under acidic conditions (excess ammonium chloride in a methanol–water mixture) afforded the corresponding C2 regioadduct **27** with total regioselectivity. Standard azide reduction, reductive amination and *O*-benzyl deprotection led to aminocyclitols **11–13** in good overall yields (Scheme 4).

Compounds **1–13** have been tested as inhibitors of α-glucosidase (from commercial baker's yeast), β-glucosidase

(from commercial crude almond), GlcCer synthase (from rat liver microsomes), lysosomal glucocerbrosidase (from rat liver membranes) and recombinant GlcCer hydrolase (Imiglucerase, Cerezyme®, from Genzyme). All compounds were inactive when tested at 0.5 mM and 1 mM concentrations against GlcCer synthase and almond β-glucosidase, respectively, whereas **12** and **13** exhibited significant α-glucosidase inhibitory activity (IC$_{50}$ = 22 μM and 142 μM, respectively, Table 1).

Lineweaver–Burk plots of assays performed with α-glucosidase at different substrate and inhibitor concentrations showed that these compounds behave as non-competitive inhibitors, indicating that the substrate and inhibitor bind reversibly and independently at different enzyme sites. Replots of slopes *versus* the inhibitor concentration indicate that the K_i values for compounds **12** and **13** are 19 μM (Fig. 1) and 145 μM, respectively. Inhibitor **12** is the more active and also

Table 1 Enzyme inhibition for compounds **1–13**

Compound	α-Glucosidase (baker's yeast)		GCH (lysosomal)		Imiglucerase	
	IC$_{50}$/μM	K_i/μM	IC$_{50}$/μM	K_i/μM	IC$_{50}$/μM	K_i/μM
1	NI		NI		NI	
2	254	293	35%^{a}		15.0	2.7
3	47%^{a}		420	163	16.0	2.2
4	24%^{a}		352	136	15.7	2.4
5	NI		498	208	30%^{a}	
6	NI		NI		NI	
7	NI		412	168	NI	
8	NI		34%^{a}		1219	285
9	NI		350	43	12.0	0.9
10	30%^{a}		33%^{a}		NI	
11	900	877	NI		NI	
12	22	19	910	940	33%^{a}	
13	142	145	275	64	503	158
DNJ	ND	8.7^b	ND	ND	506^c	88

NI: No significant inhibition at 1 mM; ND: not determined.^{a} Inhibition at 1 mM. ^{b} See ref. 28. ^{c} See ref. 16.
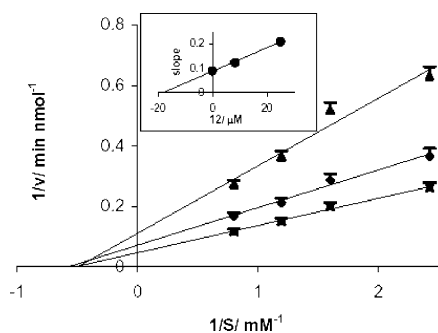
Fig. 1 Lineweaver–Burk plot for **12** with α-glucosidase (from baker's yeast).

highly specific, since its activity against GlcCer hydrolase is very low (33% inhibition at 1 mM).

Inhibitory activity against GlcCer hydrolase (both lysosomal and Imiglucerase) is shown in Table 1. IC_{50} values for the lysosomal enzyme are higher than those calculated for the purified enzyme, most likely due to the presence of other proteins in the lysosomal preparation that may interact with the compounds. Kinetic assays with active compounds were performed and K_i values were calculated (see Table 1 and Fig. 2). For comparative purposes, DNJ (deoxynojirimycin) was also tested.[16] Active compounds behaved as competitive inhibitors of GlcCer hydrolase, irrespective of the enzyme source used.
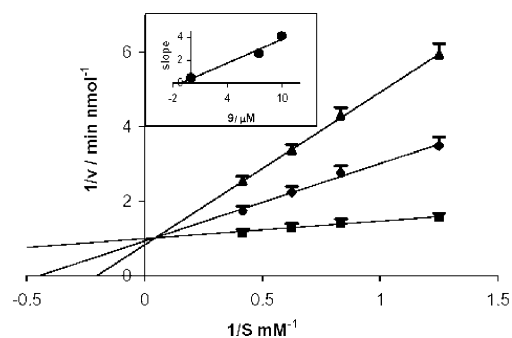

Fig. 2 Lineweaver–Burk plot for **9** with Imiglucerase.

As observed from Table 1, the only active aminocyclitols as GlcCer hydrolase inhibitors are those arising from regioselective C1 opening of the corresponding starting epoxides (compounds **2–4** and **9**, see Scheme 1). Nitrogen substitution also seems to be important for enzyme inhibition. Thus, only *N*-alkyl substituted aminocyclitols showed a remarkable inhibitory activity, whereas free amino (compound **1**), *N*-ureido (compound **8**) or *N*-acyl derivatives (compounds **5–7**) showed only marginal or no activity. Interestingly, the combination of both substitution patterns (*N*-acyl and *N*-alkyl) into a single molecule (compound **9**) afforded the most active compound in this study.

Discussion

The diverse range of competitive inhibition of GlcCer hydrolase displayed by the aminocyclitol derivatives described here indicates a differential interaction with the amino acid residues of the active site. To find a qualitative explanation for these results, we decided to compare the structure of the synthesized aminocyclitols with that of GlcCer, the enzyme substrate.[17] The starting hypothesis was that aminocyclitols are recognized as substrate mimics, the polyhydroxylated ring acting as a surrogate for the glucose moiety present in GlcCer and the exocyclic nitrogen playing the role of the anomeric oxygen. In this prediction, the *N*-alkyl chain would mimic the lipidic ceramide moiety present in the substrate.

This hypothesis is supported by the competitive nature of the inhibition and also by the extent of literature data on the

binding of this class of compounds, including X-ray structures of aminocyclitol derivatives bound to several glycosyl hydrolase enzymes.[18]

Our compounds define two structural families arising from either C1 or C2 opening of epoxycyclohexane **14** (Scheme 1). Except where a long chain alkyl substituent was present, products resulting from C2 opening were inactive (see compounds **10–13**, Table 1). In contrast, this effect is not observed for the C1 opening compounds. In this case, a similar inhibitory potency is obtained with different alkyl chain lengths (compounds **2–4**), the primary amine **1** remaining inactive. In this family, replacement of *N*-alkyl with *N*-acyl groups (compounds **5–8**) resulted in less active compounds, probably due to their minor proton acceptor ability as a result of the lower basicity of the amide nitrogen. In contrast, secondary amide **9** displays a strong inhibitory activity that can be attributed to its particular nitrogen substitution pattern. Interestingly, this observation indicates that, even in the absence of an amine nitrogen atom, good active site affinity can be achieved by a suitable arrangement of the lipidic moiety.

Based on the published X-ray structure of the enzyme[19,20] and according to the postulated binding model for GlcCer in the enzyme active site,[20] we have compared the three-dimensional structure of GluCer with those of representative molecules of each of the aminocyclitol families.[21] In this qualitative analysis we have found a close correlation between their inhibitory potency and their ability to superimpose with the glucose moiety, thus suggesting a sugar-mimicking role for the aminocyclitol scaffold. The best fit is observed for C1 adducts (Fig. 3 A, light blue color code) where close matches between, a) the CH–OH groups of aminocyclitol and the glucose moiety of GlcCer and b) the aminocyclitol nitrogen atom and the β-anomeric oxygen are found. The only structural mismatches are imposed by the presence of an aminocyclitol hydroxyl group in place of the glucoside hydroxymethyl group and the replacement of the pyranose oxygen by an aminocyclitol C(2)H–OH group.[22]
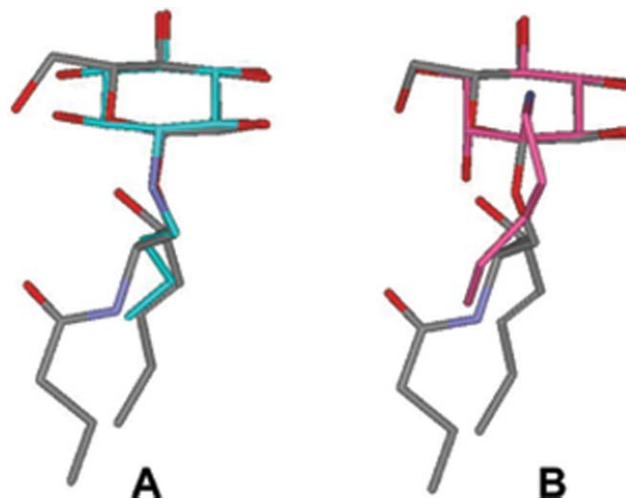

Fig. 3 Superimposition of the two regioisomeric aminocyclitol families (A, B, coloured bonds) with glucosylceramide (grey). **A**: C1-adducts; **B**: C2-adducts. Matching was done by first minimizing the structures on Chem3D, followed by manual docking using the "Molecular Overlay" tool provided with the DS Viewer Pro application from Accelrys.[23]

The C2 opening families have in common the two *trans*-diaxial substituents (the nitrogen group and the hydroxyl substituent) arising from the stereocontrolled opening of the starting epoxide. This generates an abrupt structural disparity with GlcCer, where all substituents are equatorial. Therefore, the ability of these compounds to mimic the substrate sugar moiety is lower than that for the corresponding C1 opening adducts. Thus, if we maintain the initial restriction that superimposes the aminocyclitol nitrogen atom to the GlcCer anomeric oxygen, important mismatches are found for the rest

of hydroxyl substituents. In contrast, when the hydroxyl groups are superimposed, the cyclitol amino group is "epimerized" with respect to the glucose anomeric group (see Fig. 3, B). These observations can explain why these compounds are, in general, less potent inhibitors. However, as a general trend, we found an increase in the inhibitory potency as the length (and hence the liphophilic character) of the nitrogen alkyl substituent increases, in agreement with the above mentioned contribution of the lipidic moiety to the substrate binding. In contrast, this tendency is not observed within the C1 adducts, which show similar K_i values irrespective of the nature of the N-alkyl substituent.

In summary, the ability of the aminocyclitol scaffold to superimpose the glucose moiety of the enzyme substrate appears to control the inhibitory potency of these compounds against GlcCer hydrolase. The aminocyclitol nitrogen substituent will be capable of correcting, to some extent, the hydroxyl mismatches when increasing its liphophilicity.

The design of selective competitive inhibitors of GlcCer hydrolase can be exploited for the development of chemical chaperones,[24] competitive inhibitors that promote the pharmacological rescue of a defective enzyme at subinhibitory concentrations. In this context, this approach represents a promising alternative for a more efficient therapy for Gaucher disease, a kind of sphingolipidosis caused by a deficiency of GluCer hydrolase, due, in part, to enzyme misfolding leading to its degradation during the control processes in the endoplasmic reticulum.

This rationale is being used for the optimisation of these families of inhibitors, currently underway in our group.

Experimental

General

Solvents were distilled prior to use and dried by standard methods.[25] Chemical shifts are reported in delta units (δ), parts per million (ppm) relative to the singlet at 7.24 ppm of CDCl$_3$ for ^{1}H and in ppm relative to the centre line of a triplet at 77.0 ppm of CDCl$_3$ for ^{13}C. $[a]_D^{25}$ Values are given in 10^{-1} deg cm^2 g^{-1}. Compound **14** (4,5,6-tri-O-benzyl-1,2-anhydro-*myo*-inositol) was obtained following literature procedures.[9]

Enzyme inhibition: enzyme assays

p-Nitrophenyl glucosides, 4-methylumbelliferyl-β-D-glucoside, ceramide and UDP-glucose were obtained from Sigma. [glucose-^{14}C(U)] UDP-glucose (303 mCi mmol^{-1}) was from New England Nuclear. α-Glucosidase (from baker's yeast, G5003) and β-glucosidase (from almonds, G0395), were purchased from Sigma. Imiglucerase (Cerezyme®) was kindly provided by Genzyme.

Commercial glucosidase solutions were prepared with the appropriate buffer and incubated in 96-well plates at 37 °C without (control) or with inhibitor (1 mM) for 5 min. After addition of the corresponding substrate solution, incubations were maintained during 3 min and stopped by the addition of 1 M Tris solution. The amount of *p*-nitrophenol formed was determined at 405 nm using a UV/VIS Lector Spectramax Plus (Molecular Devices Corporation) spectrophotometer. α-Glucosidase activity was determined with 1 mM *p*-nitrophenyl-α-D-glucopyranoside in 100 mM sodium phosphate buffer (pH 7.2). β-Glucosidase activity was determined with 1 mM *p*-nitrophenyl-β-D-glucopyranoside in 100 mM sodium acetate buffer (pH 5.0).

Glucocerebrosidase: lysosomal glucocerebrosidase activity in rat liver membrane suspensions[16] was determined with 1 mM 4-methylumbelliferyl-β-D-glucopyranoside in McIlvaine buffer (100 mM sodium citrate and 200 mM sodium phosphate buffer, pH 5.2). Imiglucerase activity was determined with 2.4 mM 4-methylumbelliferyl-β-D-glucopyranoside in the presence of 0.25% (w/v) sodium taurocholate and 0.1% (v/v) Triton X-100

in McIlvaine buffer (pH 5.2). Enzyme solutions were incubated at 37 °C without (control) or with inhibitor (1 mM) during 30 min and, after addition of corresponding substrate solution, incubations were maintained at 37 °C for 30 min (lysosomal glucocerebrosidase) or 10 min (Imiglucerase). Enzymatic reactions were stopped by the addition of 100 mM glycine–NaOH buffer (pH 10.6). The amount of 4-methylumbelliferone formed was determined using a 1420 VICTOR^2 Multilabel Counter (Wallac) fluorometer at 355 nm (excitation) and 460 nm (emission).

Glucosylceramide synthase: liver microsomes were obtained from Sprage-Dawley rats as described.[26] Microsomal protein was incubated with 0.12 mM of *N*-octanoyl-D-sphingosine–BSA complex and 1.6 mM NADP in 50 mM Tris–HCl buffer (pH 7.4) with or without (control) 0.5 mM inhibitor for 10 min at 37 °C. After addition of 0.04 mM [^{14}C]UDP-glucose (2 mCi mmol^{-1}) incubations were prolonged for 35 min and stopped by the addition of 0.5 ml CHCl$_3$: CH$_3$OH (2 : 1). The [^{14}C]-glucosyl-*N*-octanoyl-D-sphingosine formed was extracted[27] and quantified using a Wallac 1410 (Pharmacia) liquid scintillation counter.

Inhibition parameters. The IC$_{50}$ values were determined by plotting percent activity *versus* log [*I*], using at least five different inhibitor concentrations. Type of inhibition and K_i values for more active inhibitors were determined by Lineweaver–Burk or Dixon plots of assays performed with different concentrations of inhibitor and substrate.

Synthesis

(1S,2S,3S,4S,5R,6R)-3-Azido-4,5,6-tris-benzyloxycyclohexane-1,2-diol 27. To a solution of the epoxide **14** (95 mg, 0.22 mmol) in a 8 : 1 methanol–water mixture (4.5 cm^3) was added portionwise NaN$_3$ (162 mg, 2.5 mmol) and NH$_4$Cl (59 mg, 14.1 mmol). After stirring at 80 °C for 20 h, the reaction mixture was cooled to rt, quenched with water (10 cm^3) and extracted with diethyl ether (3 × 10 cm^3). The organic layers were washed with brine (10 cm^3), dried and concentrated under a reduced pressure. The crude product was purified by flash chromatography using hexane–ethyl acetate (2 : 1) as eluent to afford **27** in 81% yield as a white solid. $[a]_D^{25}$ +16.7 (*c* 1.00, CHCl$_3$). ν_{max}(film)/cm^{-1} 3452, 3031, 2907, 2109, 1497, 1454, 1362. ^{13}C NMR: 60.2, 70.7, 72.9, 73.1, 75.7, 77.9, 82.3, 82.9, 127.8, 127.9, 128.0, 128.1, 128.5, 128.6, 137.4, 138.0. ^{1}H NMR: 2.75 (m, 1H), 3.4–3.6 (m, 2H), 3.71–3.85 (m, 2H), 3.87–3.92 (m, 1H), 4.50–5.0 (m, 6H), 7.2–7.4(m, 6H). $[a]_D^{25}$ +15.5 (*c* 1.45, CHCl$_3$). EIHRMS, calc. for C$_{27}$H$_{29}$N$_3$O$_5$: 475.2107; found: 475.2123.

Reaction of epoxide 14 with amines in the presence of lithium perchlorate: synthesis of amino alcohols 15–17. A solution of epoxide **14** (300 mg, 0.7 mmol) in CH$_3$CN (10 cm^3) was added dropwise under an argon atmosphere over lithium perchlorate (2 g, 19 mmol) at rt. A solution of the corresponding amine (5 mmol) in CH$_3$CN (10 cm^3) was next added and the reaction mixture stirred at 80 °C under argon. After 18 h, the reaction mixture was cooled to rt, quenched with water (10 cm^3), extracted with dichloromethane (3 × 20 cm^3) and dried. Filtration and evaporation afforded crude compounds, which were purified as indicated below.

(1R,2s,3S,4R,5r,6S)-4,5,6-Trisbenzyloxy-2-butylaminocyclohexane-1,3-diol 15. (92%) The compound was purified by flash chromatography using a 9 : 1 mixture of dichloromethane–methanol. Oil; ν_{max}(film)/cm^{-1} 3432, 3027, 2924, 2912, 1495 1456. δ_H (200 MHz, CDCl$_3$, Me$_4$Si) 0.92 (3H, t, *J* = 7.2 Hz, C*H*$_3$), 1.19–1.43 (4H, m, 2 × C*H*$_2$), 2.86 (1H, t, $J_{2-1} = J_{2-3}$ 10.8, 2-H), 3.18 (2H, m, NC*H*$_2$), 3.47–3.50 (3H, m, 4-H, 5-H and 6-H), 3.81 (2H, t, $J_{1-2} = J_{6-1}$ 10.8, 1-H and 3-H), 4.6–5.0 (6H, m, 3 × OC*H*$_2$Ph), 7.2–7.4 (15H, m, 3 × Ph). δ_C (50.3 MHz, CDCl$_3$, Me$_4$Si) 13.9 (q), 20.3 (t), 32.4 (t) (CH$_3$CH$_2$CH$_2$), 44.1 (t, NCH$_2$), 61.2 (d, C2), 71.0 (d, C1, C3), 75.1 (OCH$_2$Ph), 75.7 (OCH$_2$Ph),

81.2 (d, C5), 84.3 (d, C4, C6), 127.5–128.4 (*C*HAr), 138.3–138.8 (*C*Ar). Found: C, 73.81; H, 8.02; N, 2.67%. $C_{31}H_{39}NO_5$ requires C, 73.63; H, 7.77; N, 2.77%.

(1*R*,2*s*,3*S*,4*R*,5*r*,6*S*)-4,5,6-Tris-benzyloxy-2-phenethylamino-cyclohexane-1,3-diol 16. (91%) Purified by flash chromatography (silica gel treated with 3% triethylamine) using a 1 : 1 mixture of hexanes and ethyl acetate. Oil v_{max}(film)/cm^{-1} 3575, 3421, 3087, 2923, 2856, 1953, 1879, 1741, 1439, 1454. δ_H (200 MHz, CDCl$_3$, Me$_4$Si) 2.59 (1H, t, $J_{2-1} = J_{2-3}$ 10.8, 2-H), 2.86 (2H, m, NC*H*$_2$), 3.23–3.43 (5H, m,C*H*$_2$Ph, 4-H, 5-H and 6-H), 3.83 (2H, t, $J_{1-2} = J_{6-1}$ 10.8, 1-H and 3H), 4.71–4.79 (6H, m, 3 × OC*H*$_2$Ph), 7.2–7.4 (20H, m, 3 × Ph). δ_C (50.3 MHz, CDCl$_3$, Me$_4$Si) 36.9 (t, *C*H$_2$Ph), 46.1 (t, N*C*H$_2$), 61.4 (d, C2), 71.5 (d, C1, C3), 75.3 (O*C*H$_2$Ph), 75.7 (O*C*H$_2$Ph), 82.8 (d, C5), 84.2 (d, C4, C6), 126.1–128.6 (*C*HAr), 138.3, 138.5, 139.7 (*C*Ar). Found: C, 76.21; H, 7.42; N, 2.57%. $C_{36}H_{41}NO_5$ requires C, 76.16; H, 7.28; N, 2.47%.

(1*R*,2*s*,3*S*,4*R*,5*r*,6*S*)-4,5,6-Tris-benzyloxy-2-octylaminocyclohexane-1,3-diol 17. (90%) Purified by flash chromatography using a 9 : 1 mixture of dichloromethane–methanol. Oil; v_{max}(film)/cm^{-1} 3414, 3316, 3105, 3067, 3032, 2964, 2929, 2856, 1490, 1456. δ_H (200 MHz, CDCl$_3$, Me$_4$Si) 0.92–1.43 (15H, m, C*H*$_3$, 7 × C*H*$_2$), 2.48 (2H, m, NC*H*$_2$), 2.87, (1H, t, $J_{2-1} = J_{2-3}$ 10.5, 2-H), 3.17 (2H, m, NC*H*$_2$), 3.47–3.50 (3H, 4-H, 5-H and 6-H), 3.78 (2H, t, $J_{1-2} = J_{6-1}$ 10.4, 1-H and 3-H), 4.6–5.0 (6H, m, 3 × OC*H*$_2$Ph), 7.2–7.4 (15H, m, 3 × Ph). δ_C (50.3 MHz, CDCl$_3$, Me$_4$Si) 14.0 (q), 22.6 (t), 26.3 (t), 26.5 (t), 28.9 (t), 29.0 (t), 31.6 (t) (*C*H$_3$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 49.7 (t, N *C*H$_2$), 61.2 (d, C2), 69.6 (d, C1, C3), 75.3–75.7 (O*C*H$_2$Ph), 82.4 (d, C5), 84.3 (d, C4, C6), 127.7–128.5 (*C*HAr), 138.3–138.5(*C*Ar). Found: C, 75.23; H, 8.65; N, 2.47%. $C_{36}H_{49}NO_5$ requires C, 75.10; H, 8.58; N, 2.43%.

(1*R*,2*s*,3*S*,4*R*,5*r*,6*S*)-2-Azido-4,5,6-trisbenzyloxy-1,3-cyclohexanol 18. A solution of sodium azide (1.0 g, 15.4 mmol) and lithium perchlorate (6.9 g, 64.8 mmol) was added to a solution of epoxide **14** (650 mg, 1.5 mmol) in anhydrous acetonitrile (35 cm^3) and stirred at 80 °C under an atmosphere of argon. After 18 h, the reaction mixture was cooled to rt, quenched with water (15 cm^3) and extracted with diethyl ether (3 × 30 cm^3). The combined organic layers were washed with brine (25 cm^3), dried and concentrated under a reduced pressure. The crude azidoalcohol was purified by filtration through a plug of silica and elution with a 1 : 1 mixture of hexane and ethyl acetate to afford **18** in 92% yield as an oil. v_{max}(film)/cm^{-1} 3350 (br s), 2109. δ_H (200 MHz, CDCl$_3$, Me$_4$Si) 2.78 (1H, br s, 2-H), 3.46 (5H, m, 1-H, 3-H, 4-H, 5-H and 6-H), 4.67–4.99 (6H, m, 3 × OC*H*$_2$Ph), 7.20–7.35 (15H, m, 3 × Ph). δ_C (50.3 MHz, CDCl$_3$, Me$_4$Si) 53.5 (d, C2), 65.9 (d, C1 and C3), 73.0 (O*C*H$_2$Ph), 75.7 (O*C*H$_2$Ph), 82.4 (d, C5), 82.9 (d, C4 and C6), 127.8–128.7 (*C*HAr), 138.1–138.2 (*C*Ar).

Reduction of azides 19 and 27: synthesis of 20 and 28. A solution of the starting azide (2.0 mmol) in anhydrous tetrahydrofuran (30 cm^3) was added to a cooled solution (0 °C) of lithium aluminium hydride (45 mg, 1.2 mmol) in anhydrous tetrahydrofuran (30 cm^3) under argon. The reaction mixture was stirred for 30 min at rt. Ethyl acetate (20 cm^3) and water (20 cm^3) were slowly added and the mixture was extracted with diethyl ether (3 × 50 cm^3). The combined organic layers were washed with brine (50 cm^3), dried and concentrated under a reduced pressure. The crude product was purified by flash chromatography on deactivated silica gel (3% triethylamine) using dichloromethane–methanol (12 : 1) as eluent to afford the final compounds.

(1*s*,2*R*,3*S*,4*r*,5*R*,6*S*)-2,3,4,5,6-Pentakis-benzyloxycyclohexylamine 20. (87% from **19**); oil. v_{max}(film)/cm^{-1} 3331, 3062, 3031, 2907, 1580, 1456, 1359. δ_H (300 MHz, CDCl$_3$, Me$_4$Si) 2.97 (1H, t, $J_{1-2} = J_{2-3} = 9.9$, 2-H), 3.35 (2H, m), 3.62 (3H, m),

4.68–5.01 (m, 10H), 7.24–7.40 (20H, m). δ_C (75 MHz, CDCl$_3$, Me$_4$Si) 66.8 (d), 75.8 (2 × d), 81.0 (2 × t), 82.4 (t), 83.2 (2 × t), 127.7–128.5 (25 × d), 138.2–138.3 (5 × s). Found: C, 78.35; H, 7.02; N, 2.37%. $C_{41}H_{43}NO_5$ requires C, 78.19; H, 6.88; N, 2.22%.

(1*S*,2*S*,3*S*,4*S*,5*R*,6*R*)-3-Amino-4,5,6-trisbenzyloxycyclohexane-1,2-diol 28. (81% from **27**); oil. $[a]_D^{25}$ +10.7 (*c* 1.00, CHCl$_3$). v_{max}(film)/cm^{-1} 3423, 3316, 3153, 3102, 3084, 2925, 2886, 2856, 1499, 1469. δ_H (200 MHz, CDCl$_3$, Me$_4$Si) 3.53 (t, $J = 3.7$, 1H, H3), 3.74 (t, $J = 9.9$, 1H, H5), 3.92 (m, 3H, H4, H5, H1), 4.06 (dd, $J = 3.7, 10.5$, 1H, H2), 4.6–5 (m, 6H, 3 × OCH$_2$Ph), 7.23–7.41 (m, 15 H, 3 × Ph). δ_C (50.3 MHz, CDCl$_3$, Me$_4$Si) 51.3 (d, C3), 71.0 (d, C4), 72.1 (O*C*H$_2$Ph), 72.7 (O*C*H$_2$Ph), 74.9 (O*C*H$_2$Ph), 75.1 (O*C*H$_2$Ph), 80.3 (d, C1), 80.8 (d, C5), 81.2 (d, C6), 127.7–128.6 (*C*H$_{Ar}$), 138.3–138.5 (*C*$_{Ar}$). Found: C, 72.25; H, 7.12; N, 3.37%. $C_{27}H_{31}NO_5$ requires C, 72.14; H, 6.95; N, 3.12%.

Reductive amination: synthesis of 22, 29–31. A solution of the starting amino alcohol **20** or **28** (0.05 mmol) in methanol (2 cm^3) under an atmosphere of argon was treated successively with sodium cyanoborohydride (4.2 mg, 0.108 mmol), acetic acid (3.5 µL) and the aldehyde (0.05 mmol). After stirring for 18 h at rt, the mixture was quenched with water (2 cm^3) and extracted with diethyl ether (3 × 20 cm^3). The combined organic layers were washed with brine, dried and concentrated under a reduced pressure to afford crude amino alcohols, which were purified by flash chromatography using a mixture of dichloromethane–methanol (12 : 1).

(1*s*,2*R*,3*S*,4*r*,5*R*,6*S*)-2,3,4,5,6-Pentakis-benzyloxy-*N*-phenethylcyclohexylamine 22. (85% from **20**), oil. v_{max}(film)/cm^{-1} 3057, 3027, 2957, 1480, 1456. δ_H (500 MHz, CDCl$_3$, Me$_4$Si) 2.93 (3H, m), 3.15 (3H, m), 3.55–3.68 (5H, m), 4.81–5.00 (m, 10H), 7.12 (2H, d, $J = 5.5$), 7.24–7.40 (28H, m). δ_C (125 MHz, CDCl$_3$, Me$_4$Si) 37.0 (t), 65.9 (d), 75.2 (2 × d), 76.0 (d), 76.1 (2 × d), 79.9 (2 × t), 82.8 (t), 84.8 (2 × t), 85.4 (t), 126.2 (2 × d), 127.7–128.9 (28 × d), 138.6–138.9 (5 × s), 140.6 (s).

(1*S*,2*S*,3*R*,4*R*,5*S*,6*S*)-3,4,5-Tris-benzyloxy-6-butylaminocyclohexane-1,2-diol 29. (90% from **28**), oil. $[a]_D^{25}$ +34.3 (*c* 1.00, CHCl$_3$). v_{max}(film)/cm^{-1} 3356, 3031, 2909, 1496, 1454, 1359. δ_H (300 MHz, CDCl$_3$, Me$_4$Si) 0.88 (3H, t, 7.2, J C*H*$_3$), 1.25–1.44 (4H, m, 2 × C*H*$_2$), 2.40–2.60 (2H, m, C*H*$_2$), 3.19 (t, 1H, $J = 4.2$, C*H*NHR), 3.69 (t, 1H, $J = 8.7$, C*H*OH), 3.85–4.05 (4H, m, CH), 4.50–5.00 (6H, m, OC*H*$_2$Ph), 7.2–7.4 (15H, m, Ar). δ_C (75 MHz, CDCl$_3$, Me$_4$Si) 13.9 (q), 20.3 (t), 32.4 (t), 48.1 (t), 58.2 (d), 69.1 (2 × d), 71.0 (2 × d), 72.4 (2 × d), 74.9 (d), 75.1 (d), 79.6 (t), 81.2 (t), 127.5–128.6 (12 × d), 138.4 (s), 138.7 (s). Found: C, 73.81; H, 7.98; N, 2.59%. $C_{31}H_{39}NO_5$ requires C, 73.63; H, 7.77; N, 2.77%.

(1*S*,2*S*,3*R*,4*R*,5*S*,6*S*)-3,4,5-Tris-benzyloxy-6-phenethylaminocyclohexane-1,2-diol 30. (91% from **28**). $[a]_D^{25}$ +21.4 (*c* 0.8, CHCl$_3$). v_{max}(film)/cm^{-1} 3356, 3042, 2919, 1506, 1437, 1423. δ_H (300 MHz, CDCl$_3$, Me$_4$Si) 0.88 (3H, t, $J = 7.2$, C*H*$_3$), 1.25–1.44 (4H, m, 2 × C*H*$_2$), 2.40–2.60 (2H, m, C*H*$_2$), 3.19 (t, 1H, $J = 4.2$, C*H*NHR), 3.69 (t, 1H, $J = 8.7$, C*H*OH), 3.85–4.05 (4H, m, CH), 4.50–5.00 (6H, m, OC*H*$_2$Ph), 7.2–7.4 (15H, m, Ar). δ_C (75 MHz, CDCl$_3$, Me$_4$Si) 13.9 (q), 20.3 (t), 32.4 (t), 48.1 (t), 58.2 (d), 69.1 (2 × d), 71.0 (2 × d), 72.4 (2 × d), 74.9 (d), 75.1 (d), 79.6 (t), 81.2 (t), 127.5–128.6 (12 × d), 138.4 (s), 138.7 (s). Found: C, 76.11; H, 7.28; N, 2.65%. $C_{35}H_{39}NO_5$ requires C, 75.92; H, 7.10; N, 2.53%.

(1*S*,2*S*,3*R*,4*R*,5*S*,6*S*)-3,4,5-Tris-benzyloxy-6-octylamino-cyclohexane-1,2-diol 31. (83% from **28**); oil. $[a]_D^{25}$ +24.8 (*c* 1.00, CHCl$_3$). v_{max}(film)/cm^{-1} 3306, 3029, 2909, 1485, 1444, 1359, 1345. δ_H (300 MHz, CDCl$_3$, Me$_4$Si) 0.88 (3H, t, $J = 7.2$, C*H*$_3$), 1.25–1.44 (8H, m, 2 × C*H*$_2$), 2.38–2.60 (2H, m, C*H*$_2$), 3.23 (t, 1H, $J = 4.6$, C*H*NHR), 3.67 (t, 1H, $J = 8.6$, C*H*OH), 3.78–4.10 (4H, m, CH), 4.50–4.98 (6H, m, OC*H*$_2$Ph), 7.2–7.4 (15H, m, Ar). δ_C (75 MHz, CDCl$_3$, Me$_4$Si) 14.1 (q), 20.4 (t),

25.9 (t), 28.6 (t), 29.4 (t), 29.5 (t), 32.3 (t), 48.1 (t), 58.2 (d), 68.9 (2 × d), 71.1 (2 × d), 72.4 (2 × d), 75.0 (d), 75.2 (d), 79.6 (t), 81.3 (t), 127.5–128.6 (12 × d), 138.3 (s), 138.7 (s). Found: C, 75.01; H, 8.64; N, 2.77%. $C_{35}H_{47}NO_5$ requires C, 74.83; H, 8.43; N, 2.49%.

***N*-Acylation: synthesis of 23–26.** To a solution of the corresponding amine (0.060 mmol) and triethylamine (25 µl) in anhydrous dichloromethane (1 cm³) were added 1.2 eq. per mol of the corresponding acyl chloride. After stirring for 18 h at rt the solution was washed with water (1 cm³). The organic layer was dried and the solvent removed under a reduced pressure. The residue was purified by column chromatography (hexane–ethyl acetate, 10 : 1).

(1′*s*,2′*R*,3′*S*,4′*r*,5′*R*,6′*S*)-*N*-Phenethyl-*N*-(2,3,4,5,6-pentakis-benzyloxycyclohexyl)octanamide 23. (82% from **22**). v_{max}(film)/cm⁻¹ 3039, 3020, 2957, 2918, 1684, 1521, 1477, 1389, 1332. δ_H (500 MHz, CDCl₃, Me₄Si) 0.88 (3H, t, $J = 6.5$, CH_3), 1.20–1.40 (10H, m, CH_2), 1.6–1.7 (m, 2H), 2.19 (2H, t, $J = 8.0$), 2.76 (2H, t, $J = 9.5$, NHCH₂CH_2Ar), 2.97 (2H, m), 3.42 (2H, m), 3.51 (2H, t, $J = 10.5$), 3.69 (1H, t, $J = 9.5$), 4.60 (1H, d, $J = 10.0$), 4.67 (1H, t, $J = 8.0$), 4.81–5.02 (9H, m), 7.11 (2H, d, $J = 5.0$), 7.24–7.40 (28 H, m). δ_C (125 MHz, CDCl₃, Me₄Si) 14.3 (q), 22.8 (t), 24.5 (t), 24.9 (t), 29.1 (t), 29.9 (t), 34.2 (t), 35.6 (t), 37.0 (t), 43.5 (t), 54.5 (d), 69.5 (d), 70.4 (d), 75.8 (d), 76.2 (d), 78.6 (d), 82.9 (t), 84.7 (t), 126.2 (d), 127.2–129.8 (29 × d), 138.4–138.7 (5 × s), 140.6 (d), 179.9 (s). Found: C, 76.01; H, 7.39; N, 3.47%. $C_{49}H_{58}N_2O_6$ requires C, 76.33; H, 7.58; N, 3.63%.

(1′*s*,2′*R*,3′*S*,4′*r*,5′*R*,6′*S*)-*N*-(2,3,4,5,6-Pentakis-benzyloxy-cyclohexyl)octanamide 24. (85% from **20**); oil. v_{max}(film)/cm⁻¹ 3269, 3089, 3065, 3030, 2925, 2854, 1644, 1456, 1497, 1365, 1213. δ_H (300 MHz, CDCl₃, Me₄Si) 0.88 (3H, t, $J = 7.2$, CH₃), 1.15–1.35 (10H, m, CH₂), 1.99 (2H, t, $J = 7.2$, CH_2CONHR), 3.60–3.68 (4H, m, CH), 3.88 (2H, m, CH), 4.62–4.95 (10H, m, OCH_2Ph), 5.43 (1H, d, $J = 6.9$, NH), 7.2–7.4 (25H, m, Ar). δ_C (75 MHz, CDCl₃, Me₄Si) 14.1 (q), 22.6 (t), 25.56 (t), 29.3 (t), 29.4 (t), 29.7 (t), 31.1 (t), 55.5 (d), 74.9 (2 × d), 75.6 (2 × d), 75.7 (d), 78.9 (2 × t), 83.7 (t), 84.0 (2 × t), 127.6–128.4 (20 × d), 138.48 (5 × s), 173.49 (s). Found: C, 77.71; H, 7.82; N, 2.07%. $C_{49}H_{57}NO_6$ requires C, 77.85; H, 7.60; N, 1.85%.

(1′*s*,2′*R*,3′*S*,4′*r*,5′*R*,6′*S*)-*N*-(2,3,4,5,6-Pentakis-benzyloxy-cyclohexyl)tetradecanamide 25. (87% from **20**); oil. v_{max}(film)/cm⁻¹ 3209, 3078, 3029, 2917, 2854, 1654, 1457, 1483, 1345. δ_H (300 MHz, CDCl₃, Me₄Si) 0.88 (3H, t, $J = 7.2$, CH₃), 1.15–1.35 (26H, m, CH₂), 1.99 (2H, t, $J = 6.9$, CH_2CONHR), 3.61–3.68 (4H, m, CH), 3.88 (2H, m, CH), 4.57–5.00 (10H, m, OCH_2Ph), 5.69 (1H, d, $J = 6.3$, NH), 7.2–7.4 (25H, m, Ar). δ_C (75 MHz, CDCl₃, Me₄Si) 14.3 (q), 23.1 (t), 25.6 (t), 28.9 (t), 29.1 (2 × t), 29.3 (2 × t), 29.4 (2 × t), 29.5 (t), 29.6 (2 × t), 29.9 (t), 31.1 (t), 55.4 (d), 74.9 (2 × d), 75.6 (2 × d), 75.8 (d), 78.9 (2 × t), 83.6 (t), 84.2 (2 × t), 127.6–128.4 (20 × d), 138.7 (5 × s), 173.49 (s). Found: C, 78.51; H, 8.02; N, 1.75%. $C_{55}H_{69}NO_6$ requires C, 78.63; H, 8.28; N, 1.67%.

(1′*s*,2′*R*,3′*S*,4′*r*,5′*R*,6′*S*)-*N*-(2,3,4,5,6-Pentakis-benzyloxy-cyclohexyl)phenoxyacetamide 26. (81% from **20**); oil. v_{max}(film)/cm⁻¹ 3250, 3029, 2927, 1654, 1459, 1501, 1365. δ_H (300 MHz, CDCl₃, Me₄Si) 3.63–3.68 (3H, m, CH), 3.82–3.87 (2H, m, CH), 4.62–4.95 (11H, m, CHN, OCH_2Ph), 6.59 (1H, d, $J = 6.6$, NH), 6.80 (2H, dd, $J = 1.0$, $J = 7.8$, CH), 7.01 (1H, t, $J = 7.6$, CH), 7.2–7.4 (25H, m, Ar). δ_C (75 MHz, CDCl₃, Me₄Si) 55.2 (d), 67.0 (t), 75.1 (2 × d), 75.6 (2 × d), 75.7 (d), 78.9 (2 × t), 82.6 (t), 84.0 (2 × t), 114.5 (2 × d), 122.0 (s), 127.6–128.4 (20 × d), 129.7 (2 × d), 138.2 (5 × s), 156.8 (s), 168.4 (s). Found: C, 77.21; H, 6.62; N, 1.97%. $C_{49}H_{49}NO_7$ requires C, 77.04; H, 6.47; N, 1.83%.

(1*s*,2*S*,3*R*,4*r*,5*S*,6*R*)-1-Azido-2,3,4,5,6-pentakis-benzyolxy-cyclohexane 19. A solution of alcohol **18** (1.4 g, 3,0 mmol) in

anhydrous THF (20 cm³) was added dropwise over an ice-cooled suspension of sodium hydride (60% dispersion in mineral oil, 270 mg, 11.25 mmol), previously washed with hexane (3 × 10 cm³) in tetrahydrofurane (25 cm³) under an atmosphere of argon. Stirring was continued until complete gas evolution and benzyl chloride (0.45 cm³, 3.79 mmol) was next added. The reaction mixture was stirred at rt for 24 h, quenched with water (10 cm³) and extracted with diethyl ether (3 × 25 cm³). The combined organic layers were washed with brine (20 cm³), dried and concentrated. The resulting oil was purified by flash chromatography using a mixture of hexane–ethyl acetate (4 : 1) as eluent to afford azide **19** (95%); oil. v_{max}(film)/cm⁻¹ 3031, 2909, 2107, 1496, 1454, 1359. δ_H (300 MHz, CDCl₃, Me₄Si) 3.34–3.38 (2 H, m, 2 × CH), 3.46–3.58 (4 H, m, 4 × CH), 4.84–4.90 (10H, m, 5 × OCH_2Ph), 7.2–7.4 (25 H, m, Ar). δ_C (75 MHz, CDCl₃, Me₄Si) 66.9 (d), 75.9 (3 × d), 81.0 (2 × t), 82.4 (t), 83.1 (2× t), 127.7–128.4 (20 × d), 137.7 (2× s), 138.1 (3× s).

(1*s*,2*S*,3*R*,4*r*,5*S*,6*R*)-1-Octyl-3-(2,3,4,5,6-pentakis-benzyloxy-cyclohexyl)urea 21. To an ice-cooled solution of **20** (50 mg, 0.077 mmol) in anhydrous dichloromethane (3 cm³) were added 60 µl (0.144 mmol) of a 20% solution of phosgene in toluene. After 1 h stirring at rt, the reaction mixture was quenched with aqueous NaOH 0.1 N (2 cm³) and the organic layer washed with brine and dried. Removal of the solvent under a reduced pressure afforded a residue which was treated with octylamine (15 µl, 0.09 mmol) in anhydrous dichloromethane (3 cm³). After stirring for 36 h at rt, the reaction mixture was quenched with water (2 cm³). The organic layer was washed with brine, dried and the solvent was removed under a reduced pressure. The resulting crude mixture was purified by column chromatography (hexane–ethyl acetate, 1 : 1) to give urea **21** (40 mg, 67%) as an oil. v_{max}(film)/cm⁻¹ 3028, 2980, 2910, 2865, 1638, 1456, 1407, 1356. δ_H (300 MHz, CDCl₃, Me₄Si) 0.83 (3H, t, $J = 7.1$), 1.14–1.40 (12H, m, CH₂), 2.95 (2H, q, $J = 6.6$, NHCH_2), 3.38 (1H, m. CHNHCO), 3.50–3–60 (m, 5H, CH), 4.4–5.00 (10H, m, 5 × OCH_2Ph), 7.2 × 7.4 (25H, m, Ar). δ_C (75 MHz, CDCl₃, Me₄Si) 14.1 (q), 22.7 (t), 26.8 (t), 29.1 (t), 29.2 (t), 29.6 (t), 29.8 (t), 31.1 (t), 40.4 (d), 75.3 (d), 75.5 (2 × d), 75.8 (d), 77.19 (2 × d), 80.4 (2 × t), 82.6 (t), 83.7 (2 × t), 127.6–128.4 (20 × d), 137.8 (2 × s), 138.48 (3 × s), 148.3 (s). Found: C, 76.21; H, 7.62; N, 3.57%. $C_{49}H_{58}N_2O_6$ requires C, 76.33; H, 7.58; N, 3.63%.

Synthesis of amino alcohols 1–13 by debenzylation with boron trichloride[29]. A solution of 0.05 mmol of the benzylated amino alcohol in dichloromethane (2 cm³) under an atmosphere of nitrogen at −78 °C, was treated with 2.5 eq. per benzyl group of a 1 M solution of boron trichloride in heptane. After stirring for 2 h at −78 °C, the reaction mixture was allowed to warm to 0 °C and stirred for an additional 24 h. The mixture was cooled to −78 °C, quenched with methanol (1 cm³) and evaporated under a reduced pressure. The resulting residue was taken up in a 1 : 1 MeOH–H₂O mixture, filtered through a small pad of charcoal and lyophilised to afford the required amino alcohols in quantitative yield. HRMS. **1**: m/z calcd. for $C_6H_{13}NO_5$ (M + 1): 180.0872; found: 180.0866. **2**: m/z calcd. for $C_{10}H_{21}NO_5$ (M + 1): 236.1498; found: 236.1507. **3**: m/z calcd. for $C_{14}H_{21}NO_5$ (M + 1): 284.1498; found: 284.1503. **4**: m/z calcd. for $C_{14}H_{29}NO_5$ (M + 1): 292.2124; found: 292.2119. **5**: m/z calcd. for $C_{14}H_{27}NO_6$ (M + 1): 306.1916; found: 306.1905. **6**: m/z calcd. for $C_{20}H_{39}NO_6$ (M + 1): 390.2855; found: 390.2867. **7**: m/z calcd. for $C_{14}H_{19}NO_7$ (M + 1): 314.1239; found: 314.1233. **8**: m/z calcd. for $C_{15}H_{30}N_2O_6$ (M + 1): 335.2182; found: 335.2193. **9**: m/z calcd. for $C_{22}H_{35}NO_6$ (M + 1): 410.2542; found: 410.2553; **10**: m/z calcd. for $C_6H_{13}NO_5$ (M + 1): 180.0872; found: 180.0867. **11**: m/z calcd. for $C_{10}H_{21}NO_5$ (M + 1): 236.1498; found: 236.1504. **12**: m/z calcd. for $C_{14}H_{21}NO_5$ (M + 1): 284.1498; found: 284.1487. **13**: m/z calcd. for $C_{14}H_{29}NO_5$ (M + 1): 292.2124; found: 292.2137.

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