Convergent synthesis of D-(−)-quinic and shikimic acid-containing dendrimers as potential C-lectin ligands by sulfide ligation of unprotected fragments

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The preparation of D-(−)-quinic and (−)-shikimic acid-derived dendrimers with valencies of 4, 8 and 16, respectively, as potential C-lectin ligands is reported. D-(−)-Quinic and shikimic acids were branched to an (*S-tert*-butylthio-L-cysteine)-containing tripeptide on solid phase to furnish compounds **1** and **3**. These intermediates were reduced upon treatment with tri-*n*-butylphosphine and linked to *N*-chloroacetylated L-lysinyl cores *via* a nucleophilic substitution performed in aqueous DMF.

Introduction

Lectins (carbohydrate-binding proteins) play essential roles in numerous biological events including clearance from the circulatory system and intracellular routing of glycoproteins, cell–cell adhesion and immune defence.[1] However, the affinity of lectins for carbohydrates, although highly specific, is generally weak. High-affinity complexes can be obtained when a multivalent sugar ligand interacts with clustered receptors on the lectins, a property known as the cluster effect.[2] Among the synthetic structures which can mimic natural oligosaccharide counterparts, glycodendrimers are especially well adapted since they satisfy the multivalency criteria and can be fully characterised in terms of shape, size and carbohydrate content.[3] Such constructions might be used as antibody, drug or molecule carriers. Within the scope of a synthetic vaccine programme, we have planned to deliver antigens to antigen presenting cells (APCs), such as dendritic cells or macrophages by targeting dendrimers at their mannose receptors.[4] A similar approach relying upon an antigen linked to lysine-based mannoside clusters had been envisaged by Koning and co-workers[5] and met with substantial success. In this paper, the synthesis of potential dendrimeric ligands of APCs mannose receptors is presented (Scheme 1). These dendrimers are formed by modified *N*-chloroacetylated L-lysinyl cores, easily amenable to a ligation with peptide antigens. In this study, we postulated that D-(−)-quinic and shikimic acids could replace D-mannose as the glycosyl vector:[6] In fact, the mannose receptor of APCs belongs to one of the C-type superfamily lectins.[7] For these lectins, preponderant interactions involve a calcium ion coordination by glutamic and asparagine residues in the carbohydrate-recognition-domain and by two vicinal hydroxy groups of the ligand (3- and 4-OH for D-mannose), provided they are in a *trans* di-equatorial relationship,[8] as illustrated in the X-ray structure of the rat mannose-binding protein complexed with an oligosaccharide.[9] Thus, mannose receptors are able to bind not only D-mannose but also *N*-acetyl-D-glucosamine or L-fucose excluding D-galactose and related sugar-containing molecules. Adopting a glycomimetic approach, we hypothesized that non-carbohydrate compounds such as D-(−)-quinic or (−)-shikimic acid might be considered as potential mannose receptor ligands since they possess a conveniently arranged vicinal diol (at the C-4 and C-5 position). Equally, they might offer a greater stability than mannose in a biological environment since the pyranose ring is replaced by a cyclo-hexane or cyclohexene. Finally, the presence of a carboxylic acid group renders functionalisation more easy than in the mannose case, particularly when using a solid-phase strategy. Shikimic acid **8** and the tetra-*O*-acetylated quinic acid derivative **9** have been condensed to peptidyl resins **7a** or **7b** to give compounds **1** and **3**, after acidic cleavage. These intermediates were deprotected and assembled with the preformed dendrimeric cores *via* a sulfide linkage at the ultimate stage of the synthesis. This strategy affords greater flexibility, minimises and simplifies the purification procedure and avoids side-reactions which might have occurred during deprotection steps (Scheme 1).

Results and discussion

Synthesis of the L-lysinyl cores

We decided to synthesise dendrimers based on L-lysine trees, in view of their biocompatibility and, in particular, their lack of intrinsic immunogenicity.[10] The use of such poly-L-lysine scaffolds has been extended more recently to the preparation of glycodendrimers, mainly by Roy *et al*.[11] However, the reported syntheses have been modified for our purpose: the ε-amino group of the first lysinyl residue was not incorporated into the scaffold, in order to permit an ulterior linkage with fluorescent labels or with peptide antigens (Scheme 2). The syntheses have been performed on solid support and monitored by ninhydrin[12] and 2,4,6-trinitrobenzenesulfonic acid (TNBS)[13] tests. Boc-β-alanine (0.25 equiv.) has been anchored to a 4-methylbenzydrylamine resin (MBHA) **11** using *N*-[1*H*-benzotriazol-1-yl(dimethylamino)methylene]-*N*-methylmethan-aminium hexafluorophosphate *N*-oxide (HBTU)–HOBt as acylating agents[14] in order to adjust the loading at 0.1 mmol g^{-1}. The unchanged amino groups were then capped by acetylation. After acidic deprotection of the β-alanine amino group, the peptidyl resin was coupled to a lysine, which was chain protected with a permanent 2-chlorobenzyloxycarbonyl group. A second lysine was introduced as its $N^{\alpha},N^{\varepsilon}$-di-Boc derivative and was deprotected by TFA treatment to furnish peptidyl resin **12**. One third of **12** was allowed to react with chloroacetic anhydride, obtained from chloroacetic acid and diisopropylcarbodiimide. *N*-Chloroacetylated wedges of the trees will allow the ligation between the dendritic cores and D-(−)-quinic and (−)-shikimic acid derivatives. Compound **13** was obtained following cleavage from the resin by HF–anisole treatment and
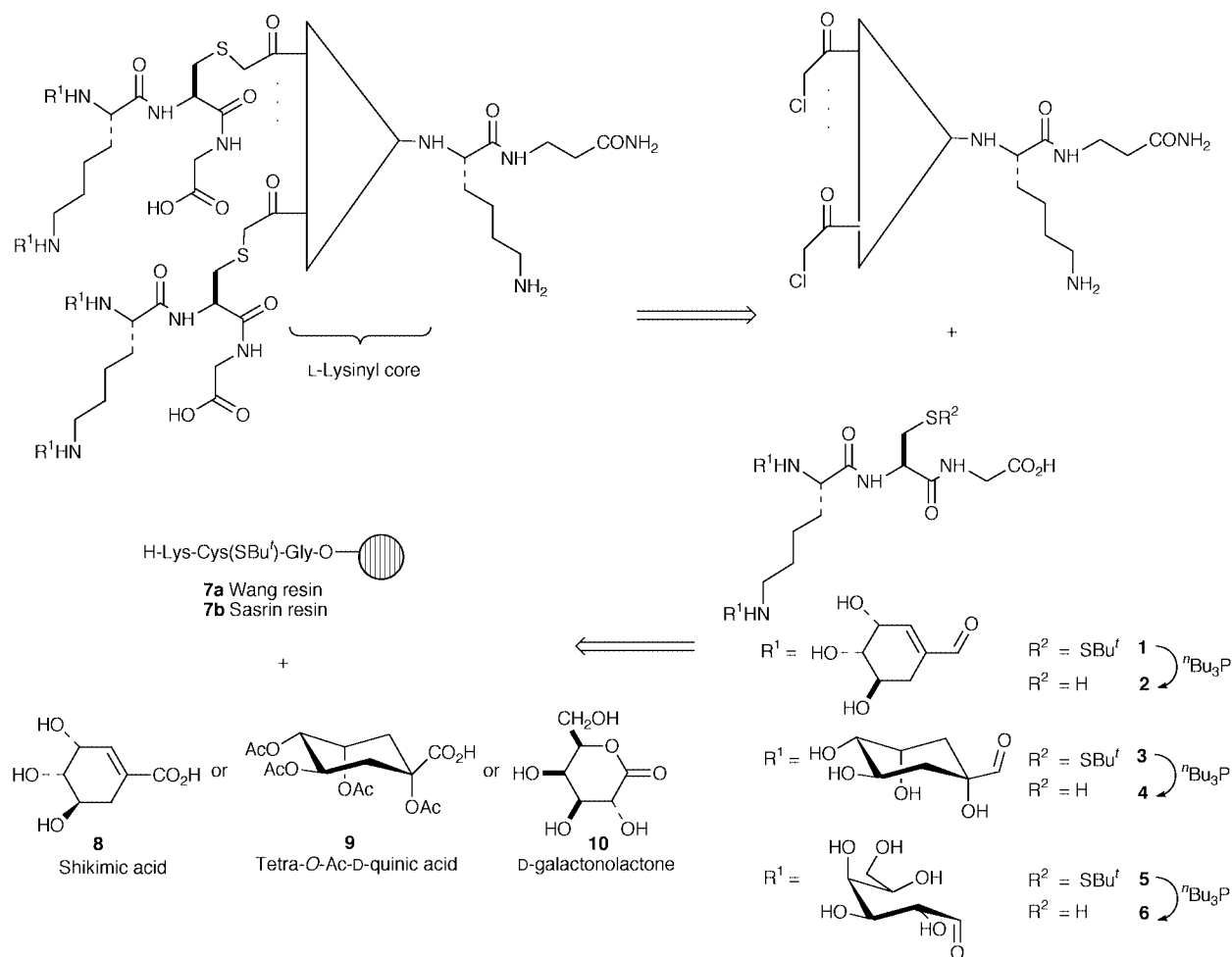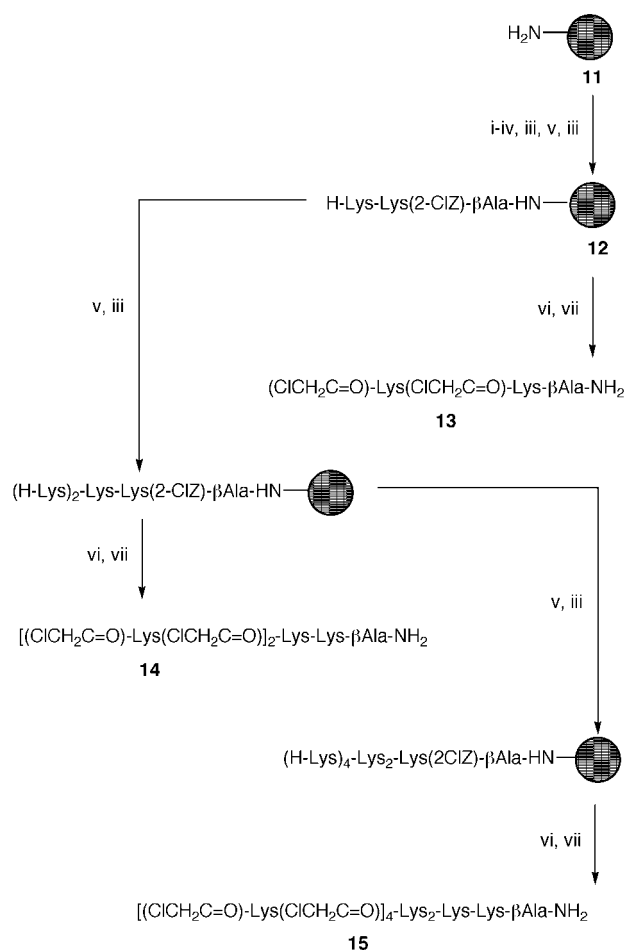
Scheme 1

subsequent purification by reversed-phase high-performance liquid chromatography (RP-HPLC) in 52% overall yield. Second- and third-generation cores **14** and **15** were obtained by repeating coupling steps with N^α,N^ϵ-di-Boc-L-lysine from peptidyl resin **12** in 42% and 20% overall yield.

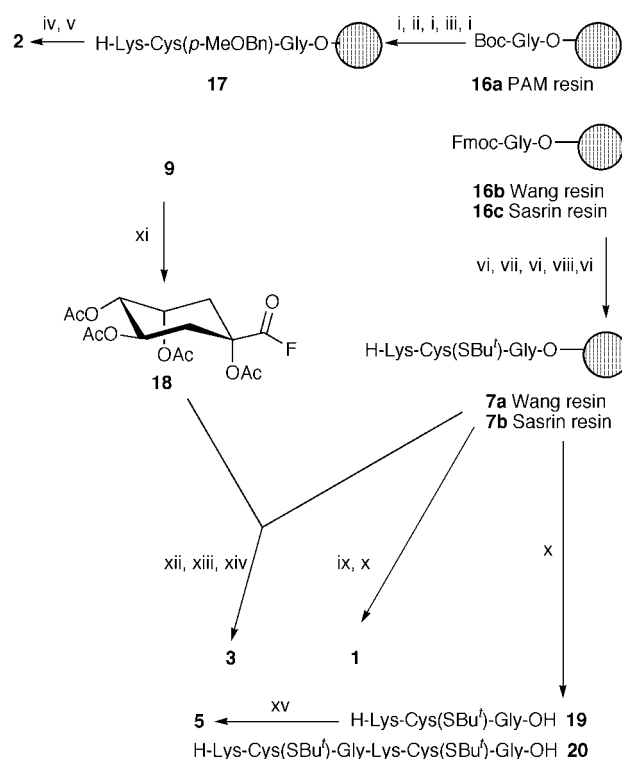### Functionalisation of D-(−)-quinic and shikimic acids

For their attachment to the lysine trees, commercially available D-(−)-quinic and shikimic acids should be functionalised by a thiol group. For this purpose, they were coupled to a cysteinyl-containing tripeptide on a solid support. We first devised a route leading to compounds such as **2**, ready for coupling with the L-lysinyl cores. Compound **2** was indeed obtained from Boc-glycyl PAM resin **16a** using the Boc/benzyl strategy [15] and HBTU–HOBt as acylating agents (Scheme 3). Peptidyl resin **16a** was deprotected upon treatment with 50% TFA in DCM, coupled with Boc-L-Cys(p-MeC$_6$H$_4$CH$_2$)-OH and further submitted to TFA deprotection. At this stage, an N^α,N^ϵ-di-Boc-L-lysine residue was added to peptidyl resin to double the vector valency and equally to increase more rapidly the dendrimer size. After removal of the *tert*-butyloxycarbonyl protecting groups, peptidyl resin **17** was actually coupled at both amino termini with unprotected shikimic acid, preactivated for 30 seconds with HBTU–HOBt–DIPEA (1:1:3 equivalents) in DMF. Compound **2** was obtained after release from the resin by HF–thiocresol–p-thiocresol treatment, which was followed by RP-HPLC purification in 42% yield. The shikimic moieties were perfectly stable under the strong acidic cleavage conditions applied. In particular, no isomerisation of the double bond of the natural isomer to the C-1/C-6 positions occurred.[16] Such stability is of singular importance since purification by chrom-

atographic means and characterisation of the final dendrimers would not be compatible with the presence of partially isomer-ised shikimic acid residues. Compound **2** could have been used directly in sulfide ligation experiments but it appeared to be slightly unstable during storage. We postulated that the observed decomposition was occasioned by the free sulfhydryl (thiol) groups. We sought to obtain **2** in an *S*-protected form to alleviate this problem. A new synthesis based upon Fmoc chemistry [17] was developed starting with Fmoc-glycine *p*-benzyloxy-benzyl ester resin (Fmoc-Gly-O-Wang resin), **16b**. L-Cysteine was introduced to the deprotected peptidyl resin as its *S-tert*-butylthio derivative. This protecting group [18] survives both the synthesis and cleavage conditions while its removal can be easily performed by reduction with trialkylphosphines. Such an approach was preferred to the direct transformation of **2** into the corresponding disulfide as one oxidation step is saved. Pep-tidyl resin **7a** was obtained after coupling with N^α,N^ϵ-di-Fmoc-L-lysine and treatment with piperidine. Acylation of **7a** with unprotected shikimic acid followed by cleavage from the resin (TFA–Me$_2$S–H$_2$O 95:2.5:2.5), and RP-HPLC purification, furnished stable compound **1** in 14% overall yield. The same strategy was applied for the preparation of compound **3**, the quinic acid analog of **1**, from Fmoc-glycine *o*-methoxy-*p*-(benzyloxy)benzyl ester resin (Fmoc-Gly-O-Sasrin®),[19] **16c**. However, the coupling of unprotected D-(−)-quinic acid with peptidyl resin **7b** proved to be abortive whatever the activation used and led to the immediate formation of a known bicyclic γ-lactone,[20] coming from esterification of the C-1 carboxy group with the C-5 hydroxy group. Preliminary protection of the hydroxy groups was thus required to prevent quinic acid from undergoing intramolecular cyclisation. D-(−)-Quinic acid has been peracetylated upon treatment with acetic anhydride in

Scheme 2 *Reagents and conditions*: i, 0.25 equiv./NH$_2$ Boc-β-Ala-OH, HBTU–HOBt–DIPEA (0.25:0.25:0.75 equiv.)/NH$_2$, DMF, 1 h; ii, Ac$_2$O–DIPEA–DCM (5:10:85), 10 min; iii, TFA–DCM (50:50), 15 min; then DIPEA–DCM (5:95); iv, 4 equiv. Boc-L-Lys[(2-Cl)Z]-OH, HBTU–HOBt–DIPEA (4:4:12 equiv.), DMF, 45 min; v, 4 equiv./NH$_2$, Boc-L-Lys(Boc)-OH, HBTU–HOBt–DIPEA (4:4:12 equiv.)/NH$_2$, DMF, 45 min; vi, 8 equiv./NH$_2$ ClCH$_2$CO$_2$H, 4 equiv. DCC, DMF, 30 min; vii, HF–anisole (90:10), 0 °C, 1 h.

Scheme 3 *Reagents and conditions*: i, TFA–DCM (50:50), 20 min; then DIPEA–DCM (5:95), 3 × 1 min; ii, 4 equiv. Boc-L-Cys(*p*-MeHC$_8$H$_4$CH$_2$)-OH, HBTU–HOBt–DIPEA (4:4:12 equiv.), DMF, 45 min; iii, 4 equiv. Boc-L-Ly(Boc)-OH, HBTU–HOBt–DIPEA (4:4:12 equiv.), DMF, 45 min; iv, 1 equiv./NH$_2$ **8**, HBTU–HOBt–DIPEA (1:1:3 equiv.)/NH$_2$, DMF, 45 min; v, HF–*p*-cresol–*p*-thiocresol (10:0.75:0.25) (v/w/w), 0 °C, 1.5 h; vi, piperidine–NMP (20:80), 20 min; vii, 2 equiv. Fmoc-L-Cys(SBu^t)-OH, HBTU–DIPEA (2:3 equiv.) NMP, 40 min; viii, 2 equiv. Fmoc-L-Lys(Fmoc)-OH, HBTU–DIPEA (2:3; equiv.) NMP, 40 min; ix, 2 equiv./NH$_2$ **8**, HBTU–DIPEA (2:3 equiv./NH$_2$, NMP, 1 h; x, TFA–H$_2$O–Me$_2$S (95:2.5:2.5), rt, 2 h; xi, 8 equiv. cyanuric fluoride, 1 equiv. pyridine, DCM, reflux, 2 h; xii, 2 equiv./NH$_2$ **18**, 3 equiv./NH$_2$ DIPEA, NMP, 1 h; xiii, TFA–^{i}Pr$_3$SiH–DCM (2.5:2.5:95), 4 × 5 min; xiv, MeONa, MeOH, rt, 3 h; xv, 4 + 2 equiv. **10**, 3.5 equiv. DIPEA, MeOH, reflux 48 h.

acetic acid in the presence of a catalytic amount of perchloric acid to furnish **9** in 86% yield. This intermediate was transformed into the corresponding acid fluoride **18** with cyanuric fluoride in DCM at reflux.[21] Subsequently, the fluoride was further condensed with the peptidyl resin **7b** in DCM in the presence of DIPEA. This activation is one of the most convenient for the coupling of sterically hindered carboxylic acids.[22] Release from the resin was carried out by treatment with 2.5% TFA in DCM using triisopropylsilane as carbocation scavenger. After lyophilisation, the crude residue was deprotected with sodium methoxide in MeOH and purified by RP-HPLC to furnish **3** in 59% overall yield. The coupling constants between protons 3-H/4-H and 4-H/5-H of both quinic acid residues in **3**, determined by ^{1}H NMR, were 2.9 Hz and 9.6 Hz, respectively. These values are consistent with axial–equatorial and axial–axial couplings and with a chair-like conformation of the cyclohexanes having an equatorial amide group. Similarly, the coupling constants between protons 3-H/4-H and 4-H/5-H of both shikimic acid residues in **1**, determined by ^{1}H NMR, were 4.6 Hz and 9.2 Hz, respectively. These values are compatible with a pseudo-equatorial orientation of hydroxy groups at the C-4 and C-5 positions of the shikimic acids, and support the structural analogy made between D-(−)-quinic and shikimic acids and D-mannose. Having biological assays in prospect, we need to prepare *a priori* structures unrecognised by the mannose receptor. Such molecules will indeed be helpful in allowing us to discriminate between spe-

cific and non-specific interactions. However, a good candidate should share a close structural similarity with the D-(−)-quinic and shikimic acid-derived dendrimers, *i.e.* all constructs must eventually be formed of an L-lysinyl scaffold branched through the same tripeptide to polyhydroxylated molecules, which must be linked to the peptide *via* an amide bond. In fact, the unrecognised dendrimer could be formed from a polyhydroxylated compound whose stereochemistry is related to D-galactose. Since quinic and shikimic acid isomers are not commercially available, we designed a synthesis from D-galactonolactone. Tripeptide **19** was obtained from peptidyl resin **7a** after acidic treatment (TFA–Me$_2$S–H$_2$O 95:2.5:2.5), in 60% yield together with a dimer **20** (4%). Finally, intermediate **19** was condensed with commercially available D-galactonolactone **10**[23] in refluxing MeOH to give **5** in 69% yield.[24]

Synthesis of the hyper-branched L-lysinyl dendrimers

Having prepared the different building blocks, their final assembly was undertaken *via* a straightforward two-step procedure. In fact, all glycodendrimer syntheses based upon the nucleophilic substitution of chloroacetyl groups so far reported have been achieved using per-acetylated glycosides in dry organic solvents.[11,25] Besides, only a few papers have related the synthesis of glycodendrimers without using protecting groups[23,26] although one chemical and purification step, performed on sophisticated molecules, is saved by adopting such a strategy. Furthermore, the deprotection step is sometimes

troublesome (*e.g.*, partial deprotection and O→N migration have been encountered during deacetylation).[27] Protecting groups might also alter the growth of dendrimers, especially during attempts to obtain densely packed constructs.[26b] In preliminary experiments, we noticed that the reduction and the substitution could not be performed in one pot. In fact, every

nucleophile present in the reaction mixture was able to compete with the free thiols for the substitution of the chloroacetyl groups, including the liberated *tert*-butyl mercaptan, the reducing agent [either tributylphosphine or the less nucleophile tris(carboxyethyl)phosphine], and even the amino group of the L-lysinyl core when the experiment was conducted at insufficiently low pH. These results sharply contrasted with some reported procedures in which substitution of *N*-chloroacetylated lysine trees by cysteine-containing peptides was performed in the presence of a large excess of trialkylphosphines.[28] These competitive reactions proved less efficient than the desired substitution yet made purification procedures difficult and resulted in a loss of overall yield. In our approach, the disulfide bond of compounds **1**, **3** and **5** was first reduced by treatment with tri-*n*-butylphosphine, probably the best reducing reagent,[29] in a mixture of degassed ^{n}PrOH–H$_2$O 1:1 to give intermediates **2**, **4** and **6** (Scheme 1). The use of propan-1-ol as co-solvent gave better results than other recommended solvents such as trifluoroethanol.[30] At the end of the reaction, the mixture was carefully evaporated to dryness to remove any *tert*-butyl mercaptan formed. Extraction of the crude residue was avoided by preferential use of the minimum amount of phosphine though this resulted in a somewhat prolonged reaction time (24 h). Intermediates **2**, **4** and **6** (1.5 equiv. per chloroacetyl group to be substituted) were actually treated, without further purification, with L-lysinyl cores **13**, **14** and **15** in a degassed mixture of DMF–H$_2$O (90:10) in the presence of potassium carbonate. The use of DMF is known to increase the thiol nucleophilicity and to limit disulfide bonding, forming dimers.[31] After 72–96 h, reactions were essentially complete as revealed by the RP-HPLC profiles of the crude mixtures (see Fig. 1*a* for one example). Dendrimers **21**–**27** were obtained after RP-HPLC purification and further characterized by analytical capillary zone electrophoresis (CZE) and electrospray ionization mass spectra (ESI-MS) as illustrated for compound **26** in Fig. 1*b*, 1*c* and Fig. 2. Loss of galactosyl chains in dendrimer **27**, through ring closure to lactone, was observed during its purification in an acidic buffer, which prompted us to switch to a phosphate buffer system of a neutral pH.
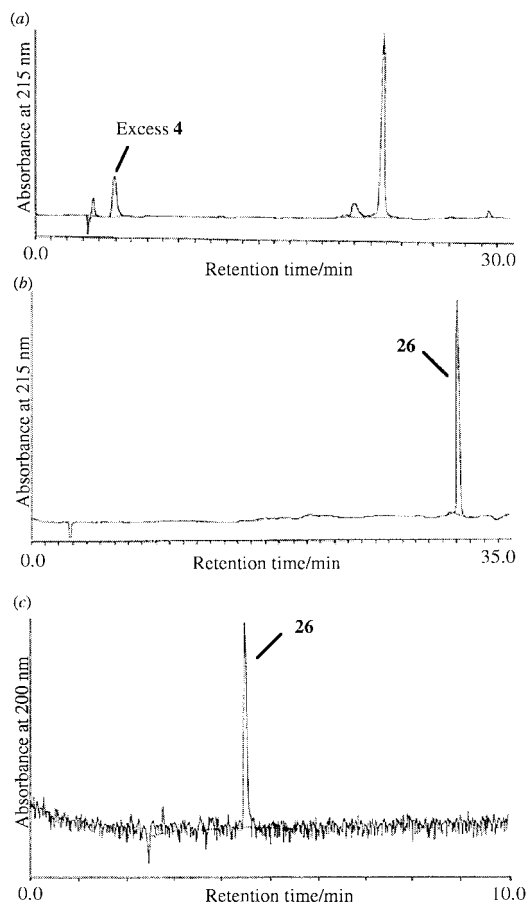

Fig. 1 (*a*) RP-HPLC chromatogram of a crude mixture of compound **26** after lyophilisation. (*b*) RP-HPLC chromatogram of purified compound **26**. Chromatographic conditions: Beckman ultrapore C8 (300 Å, 5 µm, 4.6 × 25 mm). Flow rate 1 mL min^{-1}, rt. Buffer A: 0.05% aq. TFA. Buffer B: 0.05% TFA in CH$_3$CN–H$_2$O (80:20). Gradient 0–30% B over 30 min (*a*); 0% B for 5 min then 0–25% B over 30 min (*b*). (*c*) CZE profile of compound **26** (see Experimental section for conditions).

Conclusions

The substitution of *N*-chloroacetylated L-lysinyl cores has been extended to free polyhydroxylated molecules, resulting in the synthesis of potential C-lectin ligand dendrimers having a valency as high as 16. Their labelling or their linkage to antigens
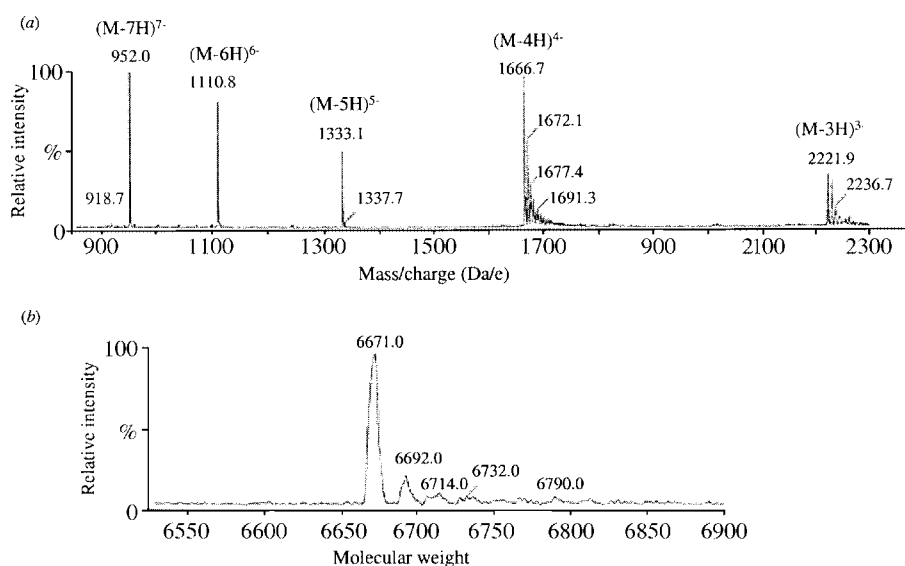

Fig. 2 (*a*) Negative ESI-MS of compound **26**; flow rate 3 µL min^{-1} at a concentration of 5 pmol mL^{-1} in 1% NH$_3$ in CH$_3$CN–H$_2$O (50:50). (*b*) ESI-MS true mass scale of compound **26**.

21 R =

22 R =

23 R =

24 R =

is made easier owing to the presence of a unique amino group on these structures. In preliminary measurements, the binding affinity of these constructs for concanavalin A has been determined by Enzyme Linked Lectin Assay as described by Pagé *et al.*[11e] This lectin does not constitute a good model, since formation of high-affinity complexes requires a greater interaction than simple binding with two *trans* di-equatorial vicinal

Table 1 Inhibition of binding of yeast mannan to concanavalin A by *p*-nitrophenyl α-D-mannopyranoside and dendrimers **25**, **26** and **27**

Inhibitor	Con A $IC_{50}/\mu M$
Man-α-OC$_6$H$_4$NO$_2$-*p*	110
25	40
26	130
27	420

OH groups as with mannose receptors:[32] higher branched dendrimers **25** and **26** have, however, shown similar or higher affinity than *p*-nitrophenyl α-D-mannopyranoside used as a reference.

Surprisingly, weak affinity was also observed for dendrimer **27** (Table 1). To further investigate their activity, the dendrimers will next be labelled with fluorescein isothiocyanate and evaluated with dendritic cells' mannose receptor.
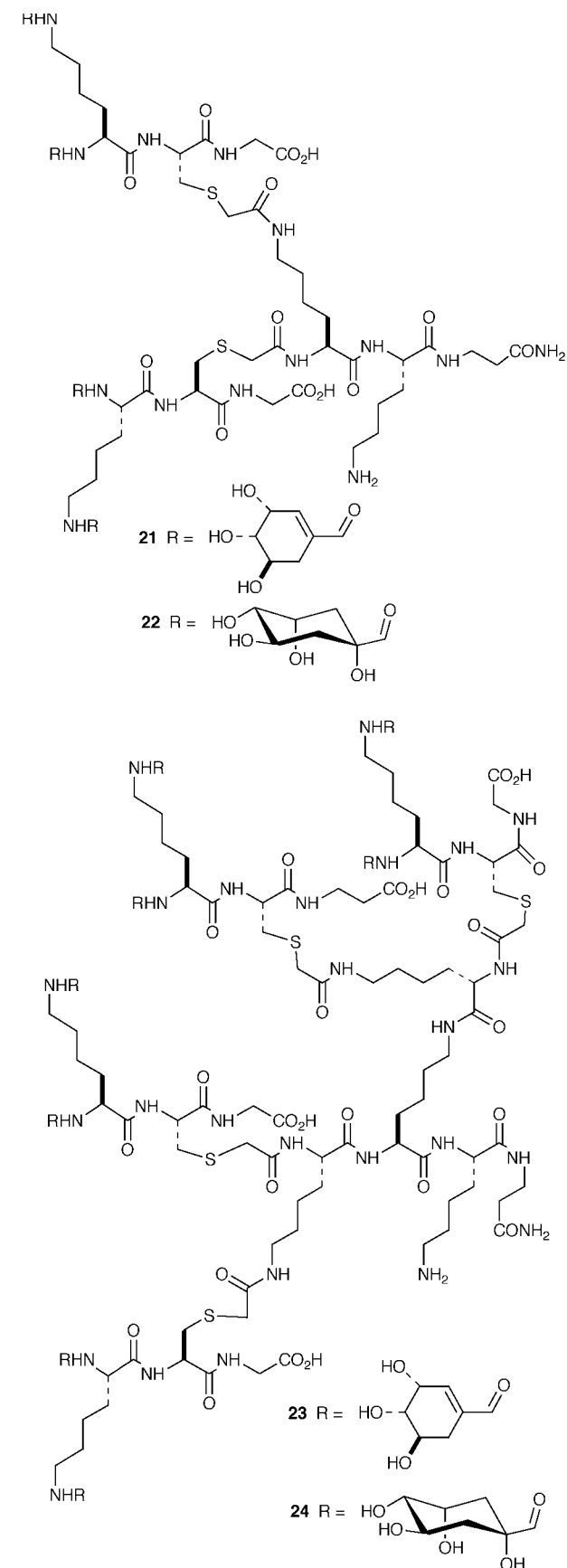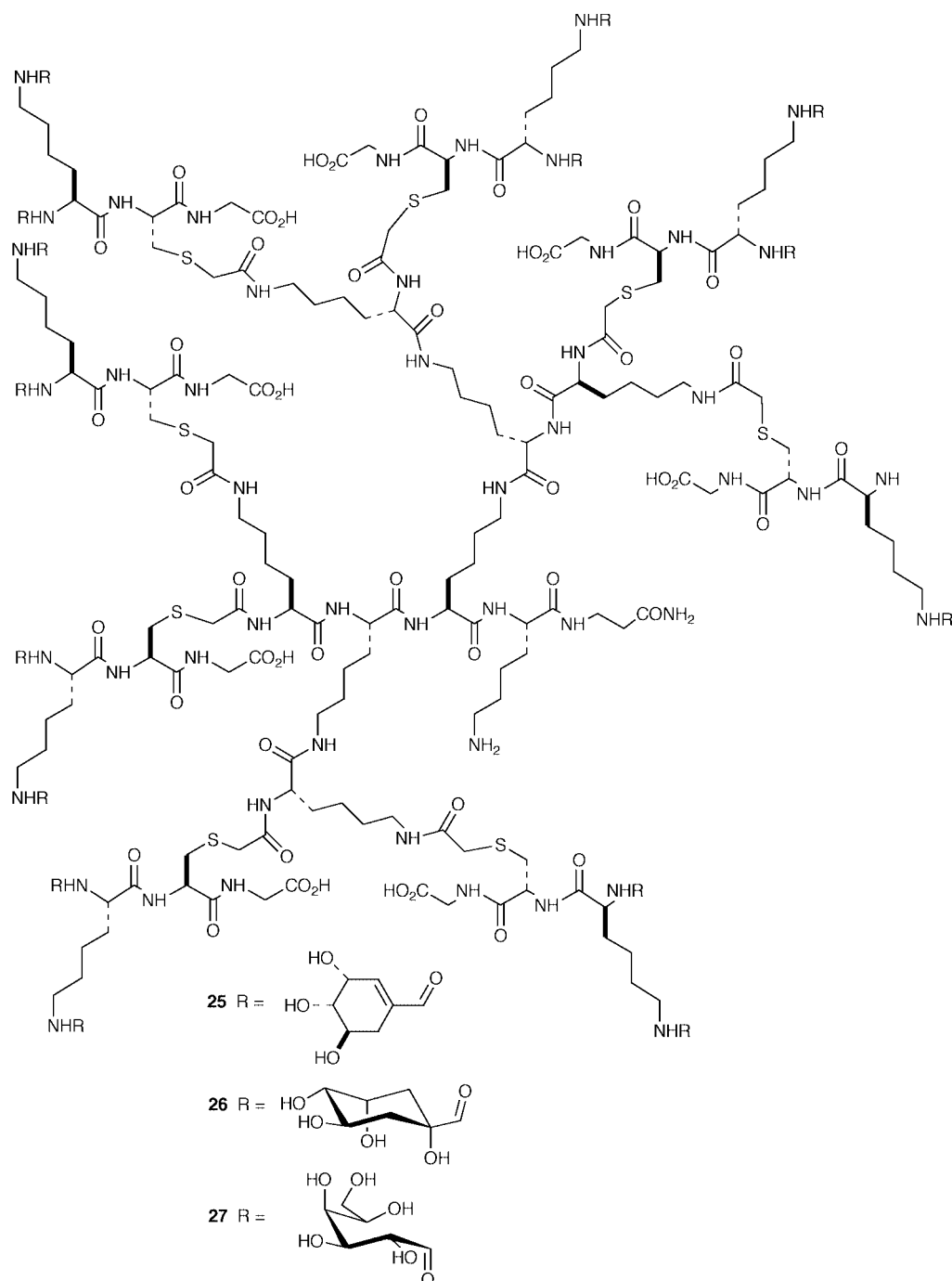
Experimental

Materials and methods

Optical rotations were measured with a Perkin-Elmer 241 polarimeter; $[a]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. Analytical and semi-preparative reversed-phase high-performance liquid chromatography (RP-HPLC) separations were performed on Shimadzu LC-6A and LC-4A systems on a Beckman ultrapore C-8 (300 Å; 5 μm; 4.6 × 25 mm), or a Hypersil hyperprep C-18 (300 Å; 8 μm; 15 × 500 mm), column at a flow rate of 1 or 3 mL min^{-1} with detection at 215 or 230 nm. Solvent system A: 0.05% TFA in water; solvent system B: 0.05% TFA in 80% acetonitrile–20% water; solvent system C: 0.05% TFA in 60% acetonitrile–40% water; solvent system D: phosphate buffer 50 mmol, pH 6.95; solvent system E: 50% phosphate buffer 50 mmol, pH 6.95–50% acetonitrile. Time-of-flight–plasma desorption mass spectrometry (TOF-PDMS) spectra were recorded upon a Bio-Ion 20 Plasma Desorption Mass Spectrometer (Uppsala, Sweden), and ESI-MS spectra on a Micromass Quatro II Electrospray Mass Spectrometer. Compounds were verified for homogeneity by analytical Capillary Zone Electrophoresis in a 75 μm × 500 mm fused silica capillary, with a 28 mA current and a 30 kV field in an Applied Biosystems Model 270A-HT system (Foster City, USA). Separations were performed at 30 °C using a 100 mM sodium borate migration buffer at pH 9.2. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX 300 or DRX 600 spectrometers. Chemical shifts are given in ppm and referenced to internal TMS or sodium 3-(trimethylsilyl)-[2,2,3,3-d_4]-propionate (TMSP), when the spectra were recorded in H$_2$O–D$_2$O (90:10). For the assignment of signals ^{1}H, ^{1}H–^{1}H correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), nuclear Overhauser effect (NOE), ^{13}C and ^{1}H–^{13}C heteronuclear single-quantum correlation (HSQC) spectroscopy experiments were used. *J*-Values are given in Hz.

Preparation of the chloroacetyl poly-L-lysinyl cores

Each lysinyl core was synthesised at a 0.5 mmol scale starting with 15 g of MBHA resin (initial substitution, 0.4 mmol g^{-1}) (Senn chemicals), using the Boc/benzyl solid-phase peptide-synthesis strategy. The coupling steps were performed using a 4-fold excess of amino acid per amine to be treated *via* HBTU–HOBt–DIPEA activation in DMF and monitored by the TNBS and ninhydrin tests: typically, HBTU dissolved in DMF (4 equiv., 0.5 mmol mL^{-1}), was added to a mixture of the amino acid (4 equiv., 0.5 mmol mL^{-1}), HOBt (4 equiv.) and DIPEA (8 equiv.) in DMF. After stirring for 1 min, the mixture was added to the peptidyl resin (1 equiv.) swollen in DMF containing DIPEA (4 equiv.), and mechanically shaken for 45 min. Following filtration, the peptidyl resin was washed successively with

25 R =

26 R =

27 R =

DMF (3 × 2 min) and DCM (3 × 2 min). Cleavage of the Boc protecting groups was achieved by treatment with TFA–DCM (50:50; 1 × 2 min, 1 × 20 min), followed by washing with DCM (2 × 2 min), neutralisation with DIPEA–DCM (5:95; 3 × 1 min), and washing with DCM (2 × 1 min). 0.25 Equivalents of Boc-β-Ala-OH was first anchored to the resin followed by a capping of the unchanged amino groups with Ac₂O–DIPEA– DCM (5:10:85) for 10 min followed by washing with DCM (3 × 1 min), to diminish the loading. Every coupling was followed by acetylation (10 min). Chain protection of the first lysinyl residue introduced was secured by a 2-chlorobenzyl-oxycarbonyl [(2-Cl)Z] group. The second lysinyl residue was added as its Boc-L-Lys(Boc)-OH derivative. At this stage, one third of the resin was deprotected and acylated using an 8-fold excess of preformed chloroacetic anhydride, prepared *via* DCC activation to provide the first level carrier core. The second- and third-level cores were obtained by repeating the last two steps. The cores were cleaved from the resin and deprotected by HF–anisole (10:1; 11 mL per g of peptidyl resin), for 1 h at 0 °C, precipitated in cold *tert*-butyl methyl ether, centrifuged, dis-

solved in water and lyophilised. Finally, the crude peptides were purified by semi-preparative RP-HPLC [gradient: 100:0 to 50:50 (A:B) in 120 min], to furnish core **13** (160 mg, 52%); *m/z* (TOF-PDMS) 498 (M + H)$^+$; core **14** (215 mg, 42%); *m/z* (TOF-PDMS) 928 (M + Na)$^+$, 907 (M + H)$^+$; core **15** (182 mg, 20%); *m/z* (TOF-PDMS) 1726 (M + H)$^+$.

(1s$_n$,3R,4s$_n$,5R)-1,3,4,5-Tetraacetoxycyclohexanecarboxylic acid 9[33,34]

To a suspension of D-(−)-quinic acid (4 g, 21 mmol) in a 2:1 mixture of acetic acid–acetic anhydride (30 mL) was added one drop of perchloric acid at room temperature. As the reaction temperature increased to 50–60 °C, the mixture became clear. The solution was stirred for a further 12 h, diluted with chloroform and then extracted succesively with saturated aq. NaHCO₃ and water. The organic layer was dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was finally precipitated by addition of pentane to give **9** (6.45 g, 86%); δ_H(300 MHz; CDCl₃) 1.87 (1 H, dd, $J_{5,6}$ 10.2 and $J_{6,6'}$

13.6, 6-H), 1.96, 1.98, 2.03 and 2.08 (4 × 3 H, 4 s, 4 × CH$_3$), 1.98–2.03 (1 H, m, 6-H′), 2.36 (1 H, dd, $J_{2,2'}$ 15.9 and $J_{2,3}$ 3.3, 2-H), 2.50 (1 H, dd, $J_{2,2'}$ 15.9 and $J_{2',3}$ 3.2, 2-H′), 4.97 (1 H, dd, $J_{3,4}$ 3.4 and $J_{4,5}$ 9.4, 4-H), 5.31 (1 H, ddd, $J_{4,5}$ 9.4, $J_{5,6}$ 10.2 and $J_{5,6'}$ 4.2, 5-H), 5.50 (1 H, m, 3-H); δ_C(81.3 MHz; CDCl$_3$) 21.0, 21.1, 21.2 and 21.4 (4 × CH$_3$), 32.0 and 36.3 (2- and 6-C), 66.7, 67.7 and 71.3 (3-, 4- and 5-C), 78.8 (1-C), 170.1–170.5 (4 × CO$_2$Me), 173.1 (CO$_2$H); m/z (TOF-PDMS) 1102 (M + Na)$^+$, 1080 (M + H)$^+$, 1022 (M + H − ′Bu)$^+$.

(1s$_n$,3R,4s$_n$,5R)-1,3,4,5-Tetraacetoxycyclohexanecarbonyl fluoride 18

To a solution of D-(−)-1,3,4,5-tetraacetoxycyclohexane-1-carboxylic acid (500 mg, 1.39 mmol) in CH$_2$Cl$_2$ (6 mL) containing pyridine (112 µL, 1.39 mmol) was added dropwise cyanuric fluoride (937 µL, 11.10 mmol). The reaction mixture was held at reflux under N$_2$ for 2 h. The mixture, from which a white precipitate had settled, was filtered through Celite and extracted with water. Removal of the solvent from the organic layer after drying with Na$_2$SO$_4$ provided an oil, which was further dried under vacuum for several hours. The crude compound 18 was characterised without purification: δ_H(300 MHz; CDCl$_3$) 1.97–2.05 (1 H, m, 6-H), 2.03, 2.06, 2.08 and 2.16 (4 × 3 H, 4 s, 4 × CH$_3$), 2.45 (1 H, dd, $J_{2,2'}$ 15.4 and $J_{2,3}$ 3.7, 2-H), 2.50–2.66 (2 H, m, 2-H′ and 6-H′), 5.08 (1 H, dd, $J_{3,4}$ 3.5 and $J_{4,5}$ 9.1, 4-H), 5.38 (1 H, ddd, $J_{4,5}$ 9.1, $J_{5,6'}$ 9.1 and $J_{5,6}$ 5.1, 5-H), 5.56 (1 H, m, 3-H); δ_C(81.3 MHz; CDCl$_3$) 20.4, 20.6, 20.8 and 20.9 (4 × CH$_3$), 31.9 and 35.4 (2- and 6-C), 66.0, 68.2 and 70.4 (3-, 4- and 5-C), 76.7 ($J_{C,F}$ 53.2, 1-C), 160.3 ($J_{C,F}$ 373.4, COF), 169.6, 169.7, 169.8 and 169.8 (4 × CO$_2$Me).

N^α,N^ε-Bis-[(1s$_n$,3R,4s$_n$,5R)-1,3,4,5-tetrahydroxycyclohexane-carbonyl]-L-lysyl-[S-(tert-butylthio)]-L-cysteinyl-glycine 3

An Fmoc solid-phase peptide-synthetic strategy was adopted for the preparation of this compound. The synthesis was performed on a 2 mmol scale starting with Fmoc-Gly-Sasrin® ester resin (substitution, 0.8 mmol g^{-1}) (Bachem, Switzerland), 2 equiv. of amino acids, HBTU–DIPEA 2:3 equiv. in N-methyl-2-pyrrolidone (NMP) and by monitoring the acylation and the deprotection reactions during chain assembly using TNBS and ninhydrin tests. Peptidyl resin 7b was acylated with quinic acid derivative 18 (2 equiv. per amino group), with DIPEA (3 equiv. per amino group), in NMP at room temperature for 1 h and then washed successively with NMP (3 × 2 min), and then DCM (3 × 2 min). This reaction was repeated using 1 equiv. per amino group of both reagents. The compound was cleaved from the resin upon treatment with CH$_2$Cl$_2$–TFA–triisopropylsilane (95:2:3; 4 × 20 mL) for 4 × 5 min. The solution was concentrated under reduced pressure, the residue dissolved in water and lyophilised to furnish a crude compound (1.40 g), which was then dissolved in MeOH (25 mL). Methanolic 1 M sodium methoxide was added dropwise to raise the apparent pH ≈ 9. The reaction mixture was allowed to stand at room temperature and was monitored by RP-HPLC. After 3 h, the mixture was concentrated under reduced pressure. A sample of the residue (100 mg) was purified by semi-preparative RP-HPLC [gradient: 100:0 to 90:10 in 10 min, then 90:10 to 50:50 (A:B) in 70 min] to yield compound 3 (41 mg, 59%) following lyophilisation; $[a]_D^{25}$ −73 (c 0.49, H$_2$O); δ_H(300 MHz; H$_2$O–D$_2$O 90:10) 1.12 (3 × 3 H, s, 3 × CH$_3$), 1.15–1.22 (2 H, m, 2 × lysyl γ-H), 1.34 (2 H, quintet, $J_{γ,δ}$ 7.2 and $J_{δ,ε}$ 7.2, 2 × lysyl δ-H), 1.57–1.90 (10 H, m, 2 × lysyl β-H, 4 × 2-H and 4 × 6-H), 2.83 (1 H, dd, $J_{α,β}$ 8.6 and $J_{β,β'}$ 14, cysteinyl β-H), 3.02 (3 H, m, cysteinyl β-H′ and 2 × lysyl ε-H), 3.33 (2 H, dd, $J_{3,4}$ 2.9 and $J_{4,5}$ 9.6, 2 × 4-H), 3.79 (2 H, d, $J_{α,NH}$ 5.8, 2 × glycyl α-H), 3.80–3.90 (2 H, m, 2 × 5-H), 4.01 (2 H, br s, 2 × 3-H), 4.11 (1 H, dt, $J_{α,NH}$ 7.0 and $J_{α,β}$ 7.2, lysyl α-H), 4.54 (1 H, ddd, $J_{α,β'}$ 6.0, $J_{α,NH}$ 7.6 and $J_{α,β}$ 8.6, cysteinyl α-H), 8.02 (1 H, t, $J_{ε,NH}$ 5.9, lysyl ^{ε}NH), 8.11 (1 H, d, $J_{α,NH}$ 7.0, lysyl ^{α}NH), 8.21 (1 H, t, $J_{α,NH}$ 5.8, glycyl NH),

8.35 (1 H, d, $J_{α,NH}$ 7.6, cysteinyl NH); δ_C(81.3 MHz; H$_2$O–D$_2$O 90:10) 22.7 (lysyl γ-C), 28.3 (lysyl δ-C), 29.4 [C(CH$_3$)$_3$], 30.9 (lysyl β-C), 37.5 (2 × 2-C), 39.4 (lysyl ε-C), 40.7 (cysteinyl β-C and 2 × 6-C), 41.8 (glycyl α-C), 48.6 [C(CH$_3$)$_3$], 53.1 (cysteinyl α-C), 54.4 (lysyl α-C), 66.7 and 66.8 (2 × 3-C), 70.8 (2 × 5-C), 75.4 (2 × 4-C), 77.2 (2 × 1-C), 172.5, 173.4, 174.4, 177.1 and 177.4 (5 × C=O); m/z (ESI-MS) 741 (M − H)$^−$.

General procedure for solid-phase synthesis of compound 1 and tripeptide 19

An Fmoc solid-phase peptide-synthetic strategy was adopted for the preparation of these compounds. The syntheses were performed on a 2 mmol scale starting with Fmoc-Gly-O-Wang resin ester (substitution, 0.8 mmol g^{-1}) (Novabiochem, Switzerland), with 4 or 2 equiv. of amino acids for the preparation of 1 and 19, respectively and HBTU–DIPEA as activating system in NMP, and by monitoring the acylation and the deprotection reactions during chain assembly using TNBS and ninhydrin tests. Single-coupling protocol was performed. Shikimic acid (2 equiv. per amino group) was preactivated with HBTU–DIPEA (1:1 equiv. per amino group) for 30 s in NMP and then added to peptidyl resin 7a in NMP containing DIPEA (1 equiv. per amino group). After 40 min, the solvent was removed by filtration, and the resin was washed successively with NMP (3 × 2 min), and DCM (3 × 2 min). After drying over P$_2$O$_5$ the compounds were released from the resin upon treatment with TFA–H$_2$O–Me$_2$S (95:2.5:2.5; 25 mL) at room temperature for 2 h. The solutions were concentrated under reduced pressure, and the residue were solubilised in water and lyophilised to furnish the crude compounds.

N^α,N^ε-Bis-[(3R,4S,5R)-3,4,5-trihydroxycyclohexenecarb-onyl]-L-lysyl-[S-(tert-butylthio)]-L-cysteinyl-glycine 1.

1.64 g of a pale yellow powder was obtained after lyophilisation. A sample of the residue (107 mg) was purified by semi-preparative RP-HPLC [gradient: 100:0 to 50:50 (A/B), 110 min], yielding 15 mg (14%), of pure compound 1; $[a]_D^{25}$ −117 (c 0.19, H$_2$O); δ_H(300 MHz; H$_2$O–D$_2$O 90:10) 1.16 [3 × 3 H, 3 s, C(CH$_3$)$_3$], 1.14–1.35 (2 H, m, 2 × lysyl γ-H), 1.37–1.44 (2 H, m, 2 × lysyl δ-H), 1.62–1.75 (2 H, m, 2 × lysyl β-H), 2.00–2.11 (2 H, m, 2 × 6-H), 2.59 (1 H, dd, J 11.3 and J 17.4, 6-H), 2.60 (1 H, dd, J 11.1 and J 17.0, 6-H), 2.90 (1 H, dd, $J_{α,β}$ 7.0 and $J_{β,β'}$ 14.0, cysteinyl β-H), 3.04–3.16 (2 H, m, 2 × lysyl ε-H), 3.09 (1 H, dd, $J_{α,β}$ 4.9 and $J_{β,β'}$ 14, cysteinyl β-H), 3.58 and 3.59 (2 H, 2 dd, $J_{3,4}$ 4.6 and $J_{4,5}$ 9.2, 2 × 4-H), 3.73–3.76 (2 H, m, 2 × glycyl α-H), 3.83–3.88 (2 H, m, 2 × 5-H), 4.17 (1 H, m, lysyl α-H), 4.25–4.29 (2 H, m, 2 × 3-H), 4.52 (1 H, m, cysteinyl α-H), 6.22 and 6.28 (2 H, 2 br d, $J_{2,3}$ 4.0, 2 × 2-H), 7.86 (1 H, t, $J_{ε,NH}$ 5.4, lysyl ^{ε}NH), 8.02 (1 H, t, $J_{α,NH}$ 5.6, glycyl NH), 8.06 (1 H, d, $J_{α,NH}$ 6.6, lysyl ^{α}NH), 8.32 (1 H, d, $J_{α,NH}$ 7.7, cysteinyl NH); δ_C(81.3 MHz; H$_2$O–D$_2$O 90:10) 22.9 (lysyl γ-C), 28.3 (lysyl δ-C), 29.4 [C(CH$_3$)$_3$], 30.7 (lysyl β-C), 31.7 and 31.5 (2 × 6-C), 39.8 (lysyl ε-C), 40.6 (cysteinyl β-C), 41.8 (glycyl α-C), 48.7 [C(CH$_3$)$_3$], 53.3 (cysteinyl α-C), 54.9 (lysyl α-C), 66.3 and 66.8 (2 × 4-C and 2 × 5-C), 71.9 and 72.0 (2 × 3-C), 131.1 and 132.1 (2 × 1-C), 133.1 and 133.7 (2 × 2-C), 170.5, 171.0, 172.7, 173.4 and 174.6 (5 × C=O); m/z (TOF-PDMS) 729 (M + Na)$^+$.

L-Lysyl-[S-(tert-butylthio)]-L-cysteinyl-glycine bis(trifluoroacetate) salt 19.

1.20 g of a pale yellow powder was obtained after lyophilisation. A sample of the residue was purified by semi-preparative RP-HPLC [gradient: 100:0 to 85:15 (A/B) for 15 min, then 85:15 to 45:55 (A/B) for 100 min] to furnish compound 19 (120 mg, 60%), and a dimeric compound 20 (15 mg, 4%); δ_H(300 MHz; H$_2$O–D$_2$O 90:10) 1.23 [3 × 3 H, 3 s, C(CH$_3$)$_3$], 1.25–1.32 (2 H, m, 2 × lysyl γ-H), 1.59 (2 H, quintet, $J_{γ,δ}$ 7.6 and $J_{δ,ε}$ 7.6, 2 × lysyl δ-H), 1.79–1.87 (2 H, m, 2 × lysyl β-H), 2.89 (2 H, br s, 2 × lysyl ε-H), 2.96 (1 H, dd, $J_{α,β}$ 7.2 and $J_{β,β'}$ 14.0, cysteinyl β-H), 3.09 (1 H, dd, $J_{α,β}$ 5.6 and $J_{β,β'}$ 14.0, cysteinyl β-H), 3.88 (2 H, br s, 2 × glycyl α-H), 3.96 (1 H, t, $J_{α,β}$

6.4, lysyl α-H), 4.60–4.64 (1 H, m, 2 × cysteinyl α-H), 8.45 (1 H, t, $J_{\alpha,NH}$ 5.6, glycyl NH), 8.80 (1 H, d, $J_{\alpha,NH}$ 7.2, cysteinyl NH); δ_C(81.3 MHz; H$_2$O–D$_2$O 90:10) 21.4 (lysyl γ-C), 26.8 (lysyl δ-C), 29.4 [C(CH$_3$)$_3$], 30.8 (lysyl β-C), 39.5 (lysyl ε-C), 40.7 (cysteinyl β-C), 42.1 (glycyl α-C), 48.8 [C(CH$_3$)$_3$], 53.3 and 53.5 (cysteinyl α-C and lysyl α-C), 170.0, 172.7 and 173.8 (3 × C=O); m/z (TOF-PDMS) 416 (M + Na)$^+$, 395 (M + H)$^+$.

N^ε-{L-Lysyl-[S-(*tert*-butylthio)]-L-cysteinyl-glycinyl}-L-lysyl-[S-(*tert*-butylthio)]-L-cysteinyl-glycine tris(trifluoroacetate) salt 20 (dimeric structure).

δ_H(600 MHz; H$_2$O–D$_2$O 90:10) 1.34 [3 × 3 H, 3 s, C(CH$_3$)$_3$], 1.36–1.46 (4 H, m, 4 × lysyl γ-H), 1.55 (2 H, quintet, $J_{\gamma,\delta}$ 7.5 and $J_{\delta,\varepsilon}$ 7.5, 2 × lysyl δ-H), 1.70 (2 H, quintet, $J_{\gamma,\delta}$ 7.5 and $J_{\delta,\varepsilon}$ 7.9, 2 × lysyl δ-H), 1.88–1.98 (4 H, m, 4 × lysyl β-H), 3.00 (2 H, br s, 2 × lysyl ε-H), 3.05–3.13 (2 H, m, 2 × cysteinyl β-H), 3.19–3.22 (4 H, m, 2 × cysteinyl β-H and 2 × lysyl ε-H), 3.91 (2 H, d, $J_{\alpha,NH}$ 5.8, 2 × glycyl α-H), 3.98 (2 H, d, $J_{\alpha,NH}$ 5.8, 2 × glycyl α-H), 4.03–4.07 (2 H, m, 2 × lysyl α-H), 4.60–4.64 (2 H, m, 2 × cysteinyl α-H), 7.55 (br s, 4 × $^\alpha$NH$_2$ and 2 × $^\varepsilon$NH$_2$), 7.81 (1H, t, $J_{\alpha,NH}$ 5.9, lysyl $^\varepsilon$NH), 8.46 (1 H, t, $J_{\alpha,NH}$ 5.8, glycyl NH), 8.61 (1 H, t, $J_{\alpha,NH}$ 5.7, glycyl NH), 8.84 (1 H, d, $J_{\alpha,NH}$ 7.4, cysteinyl NH), 9.02 (1 H, d, $J_{\alpha,NH}$ 7.6, cysteinyl NH); m/z (TOF-PDMS) 772 (M + H)$^+$.

N^α,N^ε-Bis-[(2R,3S,4S,5R)-2,3,4,5,6-Pentahydroxycyclohexanecarbonyl]-L-lysyl-[S-(*tert*-butylthio)]-L-cysteinyl-glycine 5

To a solution of **19** (140 mg, 0.22 mmol) in MeOH (5 mL) were added DIPEA (137 μL, 0.79 mmol) and D-galactonolactone (160 mg, 0.90 mmol). Following 24 h at reflux, a further amount of D-galactonolactone (80 mg, 0.45 mmol), was added to the reaction mixture and the reflux was maintained for 24 h. After completion of the reaction, monitored by RP-HPLC, the solvent was evaporated off under reduced pressure and the residue was purified by RP-HPLC [gradient: 100:0 to 80:20 (A:B), 40 min], to furnish **5** (116 mg, 69%), following lyophilisation; $[a]_D^{25}$ −5.7 (c 0.77, H$_2$O); δ_H(300 MHz; H$_2$O–D$_2$O 90:10) 1.17 [3 × 3 H, 3 s, C(CH$_3$)$_3$], 1.24–1.28 (2 H, m, 2 × lysyl γ-H), 1.30–1.48 (2 H, m, 2 × lysyl δ-H), 1.63–1.79 (2 H, m, 2 × lysyl β-H), 2.85 (1 H, dd, $J_{\alpha,\beta}$ 9.0 and $J_{\beta,\beta'}$ 14.0, cysteinyl β-H), 3.07 (1 H, $J_{\alpha,\beta'}$ 4.8 and $J_{\beta,\beta'}$ 14, cysteinyl β-H′), 3.11–3.16 (2 H, m, 2 × lysyl ε-H), 3.50–3.57 (6 H, m, 2 × 2-H and 4 × 6-H), 3.75–3.85 (6 H, m, 2 × glycyl α-H, 2 × 3-H and 2 × 5-H), 4.21 (1 H, dt, $J_{\alpha,\beta}$ 5.7 and $J_{\alpha,NH}$ 6.9, lysyl α-H), 4.27 and 4.35 (2 H, 2 br d, $J_{3,4}$ 1.1, 2 × 4-H), 4.58 (1 H, ddt, $J_{\alpha,NH}$ 7.8, $J_{\alpha,\beta}$ 9.0 and $J_{\alpha,\beta'}$ 4.8, cysteinyl α-H), 7.96 (1 H, t, $J_{\varepsilon,NH}$ 6.0, lysyl $^\varepsilon$NH), 8.12 (1 H, t, $J_{\alpha,NH}$ 6.1, glycyl NH), 8.15 (1 H, d, $J_{\alpha,NH}$ 6.9, lysyl $^\alpha$NH), 8.16 (1 H, $J_{\alpha,NH}$ 7.8, cysteinyl NH); δ_C(81.3 MHz; H$_2$O–D$_2$O 90:10) 22.7 (lysyl γ-C), 28.4 (lysyl δ-C), 29.4 [C(CH$_3$)$_3$], 30.9 (lysyl β-C), 40.5 (lysyl ε-C), 40.5 (cysteinyl β-C), 41.9 (glycyl α-C), 48.7 [C(CH$_3$)$_3$], 53.1 (cysteinyl α-C), 54.5 (lysyl α-C), 63.7 (2 × 6-C), 69.7 and 69.8 (2 × 2-C), 70.4, 71.2, 71.3, 71.5 and 71.8 (2 × 3-C, 2 × 4-C and 2 × 5-C), 172.7, 173.7, 174.6, 175.8 and 176.7 (5 × C=O); m/z (TOF-PDMS) 772 (M + Na)$^+$.

General procedure for the formation of sulfides

To a solution of compound **1**, **3** or **5** (1.5 equiv. per chloracetyl group to be substituted in the second step), in a mixture of degassed ^{n}PrOH–H$_2$O 50:50 (1 mL), was introduced ^{n}Bu$_3$P (1 equiv.). Each mixture was stirred at room temperature under N$_2$ for 24 h. The solvent was evaporated off under reduced pressure and the residues further dried over P$_2$O$_5$ under vacuum for 15 min. To the crude, reduced compounds dissolved in a mixture of degassed DMF–H$_2$O (90:10; 500 μL) were added L-lysine core **13**, **14** or **15** (1–5 μmol, 1 equiv.), and the pH adjusted to a value of 8–8.5 (paper) by adding solid K$_2$CO$_3$. Each mixture was again stirred at room temperature for 72–96 h and monitored by RP-HPLC. On completion, each mixture was diluted in H$_2$O, lyophilised and purified by RP-HPLC to furnish compounds **21**–**27**.

Owing to the repetitive structure of dendrimers only selected NMR data are reported.

Tetramer 21 (3.39 mg, 61%) was obtained after semi-preparative RP-HPLC [gradient: 100:0 to 70:30 (A/C), 70 min], as a white powder; δ_H(600 MHz; H$_2$O–D$_2$O 90:10) 1.31–1.46 (8 H, m, 8 × lysyl γ-H), 1.43–1.60 (6 H, m, 6 × lysyl δ-H), 1.64 (2 H, m, 2 × lysyl δ-H), 1.68–1.89 (8 H, m, 8 × lysyl β-H), 2.17–2.23 (4 H, m, 4 × 6-H), 2.46 and 2.51 (2 × 1 H, 2 dt, $J_{\alpha,\alpha'}$ 15.4 and $J_{\alpha,\beta}$ 6.2, 2 × alanyl α-H), 2.70–2.80 (4 H, m, 4 × 6-H′), 2.89–2.95 (2 H, m, 2 × cysteinyl β-H), 2.96–3.01 (2 H, m, 2 × lysyl ε-H), 3.06 (2 H, dd, $J_{\alpha,\beta}$ 5.3 and $J_{\beta,\beta'}$ 14.9, 2 × cysteinyl β-H′), 3.21 (2 H, m, 2 × lysyl ε-H),), 3.25 (2 H, m, 2 × lysyl ε-H), 3.27 (1 H, d, J 16.5, CHH), 3.30 (1 H, d, J 16.5, CHH), 3.33 (2 H, s, CH$_2$), 3.41–3.50 (2 × 1 H, 2 ddt, $J_{\alpha,\beta}$ 6.2, $J_{\beta,\beta'}$ 12.6 and $J_{\beta,NH}$ 6.2, 2 × β-alanyl β-H), 3.71–3.76 (4 H, m, 4 × 4-H), 3.95 (4 H, d, $J_{\alpha,NH}$ 6.3, 4 × glycyl α-H), 4.00–4.05 (4 H, m, 4 × 5-H), 4.25 (2 H, dt, $J_{\alpha,\beta}$ 5.6 and $J_{\alpha,NH}$ 6.3, 2 × lysyl α-H), 4.32–4.38 (4 H, m, 4 × lysyl α-H), 4.42–4.44 (4 H, m, 4 × 3-H), 4.58–4.62 (2 H, m, 2 × cysteinyl α-H), 6.38 and 6.44 (2 × 2 H, 2 br d, $J_{2,3}$ 4.0, 2 × 2 × 2-H), 6.90 (1 H, s, NHH), 7.54 (br s, $^\varepsilon$NH$_2$), 7.60 (1 H, s, NHH), 8.07 (2 H, t, $J_{\varepsilon,NH}$ 6.8, 2 × lysyl $^\varepsilon$NH), 8.15 (1 H, t, $J_{\alpha,NH}$ 6.2, β-alanyl NH), 8.24 (1 H, t, $J_{\varepsilon,NH}$ 6.3, lysyl $^\varepsilon$NH), 8.25–8.27 (2 H, m, 2 × lysyl $^\alpha$NH), 8.31 and 8.32 (2 × 1 H, 2 t, $J_{\alpha,NH}$ 6.3, 2 × glycyl NH), 8.42 (1 H, d, $J_{\alpha,NH}$ 6.8, lysyl $^\alpha$NH), 8.47 (1 H, d, $J_{\alpha,NH}$ 6.3, lysyl $^\alpha$NH), 8.61 (2 H, d, $J_{\alpha,NH}$ 7.6, 2 × cysteinyl NH); m/z (ESI-MS) 1660.4 (M − H)$^-$, 829.9 (M − 2H)$^{2-}$.

Tetramer 22 (5.98 mg, 66%) was obtained after semi-preparative RP-HPLC [gradient: 100:0 to 75:25 (A/C), 25 min; then isocratic] as a white powder; δ_H(300 MHz; H$_2$O–D$_2$O 90:10) 1.22–1.28 (8 H, m, 8 × lysyl γ-H), 1.34–1.43 (6 H, m, 6 × lysyl δ-H), 1.52 (2 H, m, 2 × lysyl δ-H), 1.57–1.64 (8 H, m, 8 × lysyl β-H), 1.65–1.99 (16 H, m, 8 × 2-H and 8 × 6-H), 2.33 (2 H, t, $J_{\alpha,\beta}$ 6.3, 2 × β-alanyl α-H), 2.69–2.84 (4 H, m, 2 × cysteinyl β-H and 2 × lysyl ε-H), 2.92 (2 H, dd, $J_{\alpha,\beta}$ 5.3 and $J_{\beta,\beta'}$ 14, 1 2 × cysteinyl β-H), 2.98–3.11 (6 H, m, 6 × lysyl ε-H), 3.15 and 3.19 (2 × 2 H, 2 s, 2 × CH$_2$), 3.23 and 3.34 (2 × 1 H, 2 ddt, $J_{\alpha,\beta}$ 6.3, $J_{\beta,\beta'}$ 13.0 and $J_{\beta,NH}$ 5.7, 2 × β-alanyl β-H), 3.38 (4 H, dd, $J_{3,4}$ 3.1 and $J_{4,5}$ 9.7, 4 × 4-H), 3.76 and 3.77 (4 H, 2 t, $J_{\alpha,NH}$ 6.3, 2 × glycyl α-H), 3.87–3.96 (4 H, m, 4 × 5-H), 4.06 (4 H, br s, 4 × 3-H), 4.10–4.16 (4 H, m, 4 × lysyl α-H), 4.60–4.64 (2 H, m, 2 × cysteinyl α-H), 6.73 and 7.42 (2 × 1 H, 2 s, NHH and NHH), 7.96 (1 H, t, $J_{\alpha,NH}$ 5.7, β-alanyl NH), 8.07 (1 H, t, $J_{\alpha,NH}$ 6.3, lysyl $^\varepsilon$NH), 8.10 (1 H, t, $J_{\alpha,NH}$ 6.9, lysyl $^\varepsilon$NH), 8.11 (2 H, t, $J_{\alpha,NH}$ 6.3, 2 × glycyl NH), 8.12 (1 H, t, $J_{\varepsilon,NH}$ 6.8, lysyl $^\varepsilon$NH), 8.13 (2 H, d, $J_{\alpha,NH}$ 6.8, 2 × lysyl $^\alpha$NH), 8.25 (1 H, d, $J_{\alpha,NH}$ 6.7, lysyl $^\alpha$NH), 8.29 (1 H, d, $J_{\alpha,NH}$ 6.3, lysyl $^\alpha$NH), 8.39 (2 H, d, $J_{\alpha,NH}$ 7.6, 2 × cysteinyl NH; m/z (ESI-MS) 1732.5 (M − H)$^-$, 865.6 (M − 2H)$^{2-}$.

Octamer 23 (2.25 mg, 42%), was obtained after semi-preparative RP-HPLC [gradient: 100:0 to 80:20 (A/C), 35 min; then isocratic] as a white powder; ESI-MS: Found: 3235.0. Calc. 3235.6, m/z 1616.4 (M − 2H)$^{2-}$, 1077.3 (M − 3H)$^{3-}$, 807.7 (M − 4H)$^{4-}$.

Octamer 24 (5.75 mg, 56%), was obtained after semi-preparative RP-HPLC [gradient: 100:0 to 75:25 (A/C), 25 min; then isocratic] as a white powder; ESI-MS: Found: 3379.0. Calc.: M, 3379.7; m/z 1688.3 (M − 2H)$^{2-}$, 1125.3 (M − 3H)$^{3-}$, 843.8 (M − 4H)$^{4-}$.

16-mer 25 (3.15 mg, 43%) was obtained after semi-preparative RP-HPLC [gradient: 100:0 to 75:25 (A/C), 40 min; then isocratic], as a white powder; ESI-MS: Found: 6382.0. Calc.: M, 6383.0; m/z 1594.5 (M − 4H)$^{4-}$, 1275.5 (M − 5H)$^{5-}$, 1062.8 (M − 6H)$^{6-}$, 910.8 (M − 7H)$^{7-}$.

16-mer 26 (7.03 mg, 61%) was obtained after semi-preparative RP-HPLC [gradient: 100:0 to 0:20 (A/C), 35 min; then isocratic] as a white powder; ESI-MS: Found: 6671.0. Calc. for M, 6671.2; m/z 2221.9 (M − 3H)$^{3-}$, 1666.7 (M − 4H)$^{4-}$, 1333.1 (M − 5H)$^{5-}$, 1110.8 (M − 6H)$^{6-}$, 952.0 (M − 7H)$^{7-}$.

16-mer **27** (3.90 mg, 53%) was obtained after semi-preparative RP-HPLC [gradient: 100:0 to 80:20 (D:E), 40 min; then isocratic], followed by desalting as a white powder; δ_H(600 MHz; H$_2$O–D$_2$O 90:10) 1.26–1.43 (32 H, m, 32 × lysyl γ-H), 1.44–1.62 (30 H, m, 30 × lysyl δ-H), 1.65–1.94 (34 H, m, 2 × lysyl δ-H and 32 × lysyl β-H), 2.48 (2 H, t, $J_{\alpha,\beta}$ 6.3, 2 × alanyl α-H), 2.89–3.01 (10 H, m, 8 × cysteinyl β-H and 2 × lysyl ε-H), 3.11 (8 H, $J_{\alpha,\beta'}$ 4.0 and $J_{\beta,\beta'}$ 13.5, 8 × cysteinyl β-H′), 3.17–3.22 and 3.24–3.32 (30 H, m, 30 × lysyl ε-H), 3.30 (8 H, s, 4 CH$_2$), 3.33 (4 H, d, J 15.2, 4 × CHH), 3.37 (4 H, d, J 15.2, 4 × CHH), 3.40–3.43 and 3.49–3.57 (2 × 1 H, m, 2 β-alanyl β-H), 3.65–3.73 (56 H, m, 8 glycyl α-H, 16 × 2-H and 32 × 6-H), 3.83 (4 H, br d, $J_{\alpha,NH}$ 6, × 4 glycyl α-H), 3.86 (4 H, d, $J_{\alpha,NH}$ 5.9, 4 × glycyl α-H), 3.96–3.99 (16 H, m, 16 × 5-H), 4.01 (16 H, br dd, $J_{2,3}$ 9.5 and $J_{3,4}$ 3.3, 16 3-H), 4.18–4.41 (16 H, m, 16 × lysyl α-H), 4.43 and 4.53 (2 × 8 H, 2 br s, 16 × 4-H), 4.58–4.64 (8 H, m, 8 × cysteinyl α-H), 6.88 (1 H, s, NHH), 7.58 (1 H, s, NHH), 7.95–8.00 (9 H, m, 8 × glycyl NH and lysyl $^\varepsilon$NH), 8.03 (1 H, t, $J_{\alpha,NH}$ 6.2, β-alanyl NH), 8.10–8.15 (9 H, m, lysyl $^\alpha$NH and 8 × lysyl $^\varepsilon$NH), 8.18 (1 H, t, $J_{\alpha,NH}$ 6.2, lysyl $^\varepsilon$NH), 8.19–8.23 (4 H, m, 4 × lysyl $^\varepsilon$NH), 8.25–8.29 (10 H, m, 2 × lysyl $^\alpha$NH and 8 × cysteinyl NH), 8.30 (1 H, d, $J_{\alpha,NH}$ 6.4, lysyl $^\alpha$NH), 8.32 (8 H, br d, $J_{\alpha,NH}$ 6.0, 8 × lysyl $^\alpha$NH), 8.40 (2 H, br d, $J_{\alpha,NH}$ 6.3, 2 × lysyl $^\alpha$NH), 8.43 (2 H, br s, 2 × lysyl $^\alpha$NH); ESI-MS: Found 6732.5. Calc. 6733.0; Found: 6555.0. Calc. for (M − C$_6$H$_{10}$O$_6$): 6554.9; m/z 2243.3 (M − 3H)$^{3-}$, 1682.9 (M − 4H)$^{4-}$.

Acknowledgements

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