New Dendrimer – Peptide Host – Guest Complexes: Towards Dendrimers as Peptide Carriers

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Adamantyl urea and adamantyl thiourea modified poly(propylene imine) dendrimers act as hosts for N-terminal tert*-butoxycarbonyl (Boc)-protected peptides and form chloroform-soluble complexes. Investigations with NMR spectroscopy show that the peptide is bound to the dendrimer by ionic interactions between the dendrimer outer shell tertiary amines and the C-terminal carboxylic acid of the peptide, and also through host-urea to peptide-amide hydrogen bonding. The hydrogen-bonding nature of the peptide – dendrimer interactions was further confirmed by using Fourier transform IR spectroscopy, for which the NH- and CO-stretch signals of the peptide amide moieties shift towards lower wavenumbers upon complexation with the dendrimer. Spatial analysis of the complexes with NOESY spectroscopy generally shows close proximity of the N-terminal Boc group of the peptide to the peripheral adamantyl groups on the dendrimer host. The influence of side-chain motif on interactions with the host is analyzed by using seven different* N*-Boc-protected tripeptides as guests for the dendrimer. Downfield shifts of up to 1.3 ppm were observed for the guest amide NH-proton signals. These shifts decreased with increasing 'bulkiness' of the amino acid side chains. Despite this, the dendrimer was capable of making multiple peptide – dendrimer complexes when presented with a library of seven peptides. The different peptides were all present in the host, which did not show specific preferences, and could be released under mild acidic conditions. These results show the general nature of the peptide – dendrimer interactions in the formation of either single- or multiple-peptide – dendrimer complexes.*

KEYWORDS:

dendrimers · host – guest systems · hydrogen bonding · peptides

Introduction

High-generation dendrimers with their cascade structures serve as interesting candidates for the formation of artificial macromolecular globular structures with multivalent outer shells and surfaces. Thus, there has been an immense interest in the use of dendrimers as hosts or carriers of a variety of guest molecules,[1] possible molecular vehic les in drug delivery,[2, 3] micelle mimics,[4] polyvalent catalysts,[5–9] or molecular sensors.[10, 11] In the early examples of host – guest chemistry involving dendritic hosts, the substrate was bound to the interior of the dendrimer (endoreceptor), either nonspecifically[12] or by well-defined multiple hydrogen-bonding interactions as found in the 'dendroclefts'.[13] However, in recent years application of the multivalent surfaces or outer shells in host – guest chemistry (exoreceptors) has gathered much attention, partly because the unimolecular multivalent structure gives rise to cooperative effects between the receptor sites and thus amplifies the function of the dendritic receptor compared to the respective monovalent receptor molecules.[14–16]

Recently, we reported the use of urea- and thiourea-modified poly(propylene imine) dendrimers (DAB-dendr-$(NHCONHAd)_{64}$; DAB = diaminobutane) as multivalent hosts for guest molecules that contain a urea – glycine 'tail' unit (Scheme 1 a). The guest interacts with the dendrimer host through urea – urea or urea – thiourea hydrogen bonding as well as through ionic interactions between the outer shell tertiary amines of the dendrimer and the carboxylic terminus of the guest.[17, 18] Instead of being limited to the use of the urea – glycine 'tail', we investigate herein the possibility of using urea- or thiourea-modified dendrimers as multivalent carriers of tripeptides (Scheme 1 b).

In addition to the use of dendrimers as potential carriers for peptides in chloroform, we are eager to study the influence of steric repulsion between peptide side chains and the dendritic host on the ability to form stable complexes between the peptide and the dendrimer. We can thus investigate the general nature of tripeptide binding and the ability of the dendrimer host to interact with peptide guests that have 'bulky' side chains.

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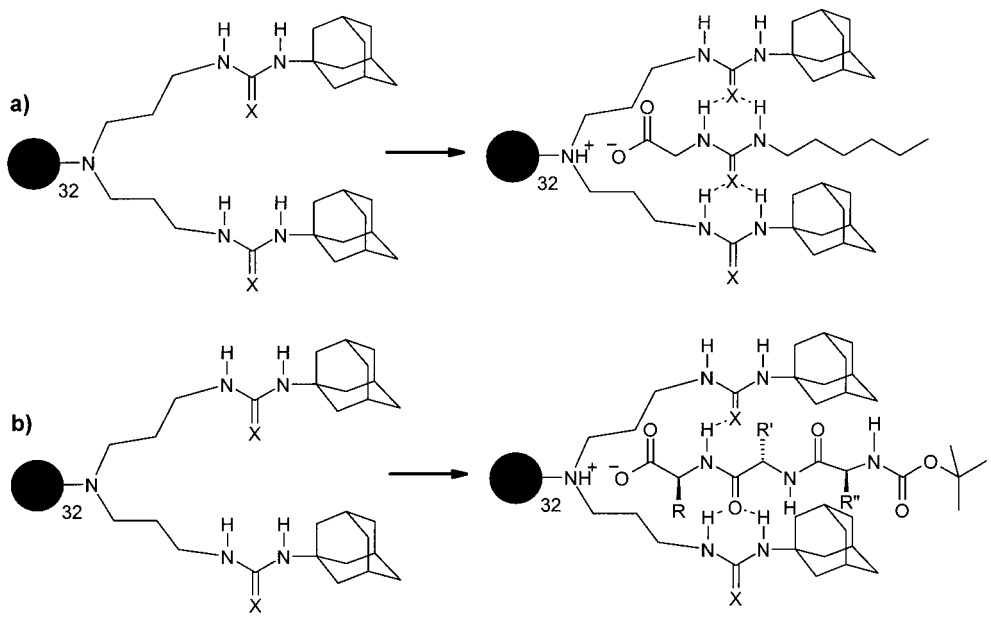

Scheme 1. *a) Proposed scheme for the complexation of a dendrimer host with a urea – glycine modified guest molecule; b) proposed scheme for the complexation of a dendrimer host with an N-terminal Boc-protected tripeptide. X = O, S*

Results and Discussion

Formation of peptide – dendrimer complexes

In this first study, chloroform was used as solvent because polar interactions are greatly stabilized by the hydrophobicity of this solvent, which thereby enhances the binding in this host – guest system. N-terminal *tert*-butoxycarbonyl (Boc)-protected tripeptide substrates were chosen as good guest candidates, rather than the *N*-acetyl- and *N*-benzyloxycarbonyl(Z)-protected peptides, because the more bulky Boc group decreases the ability of the peptide substrates to form insoluble aggregates, which is especially a problem in apolar solvents.[19] A soluble complex was formed when a suspension of the respective peptides in chloroform was exposed to the dendrimer.

IR spectroscopy was used to investigate the extent of hydrogen bonding between peptide and dendrimer. A shift towards lower wavenumbers was observed both in the NH-stretch region (Figure 1 a; approximately 100 cm^{-1}) and the CO-stretch region (Figure 1 b; approximately 20 cm^{-1}) for the complexed peptide compared to the free peptide (as the peptide-tri-*n*-octyl ammonium salt).[20] This indicates that hydrogen bonding takes place between the dendrimer host and the peptide. The red shift of the peptide CO-stretch vibrations becomes more clear when compared with the dendrimer thiourea CS-stretch vibration, which does not contribute to signals in this region (Figure 1 b). For the urea-modified poly(propylene imine) dendrimer, similar tendencies were observed.[21]

Upon complex formation between *N*-Boc-Gly-Gly-Gly-OH and the urea dendrimer, strong line-broadening in the ^{1}H NMR spectrum of the peptide as observed, indicative of increased rigidity of the peptide as it becomes part of a larger complex. In addition, we observed a downfield shift of the signals from the amide NH protons of the peptide of about 1.3 ppm compared to the free peptide (Figure 2).[22] These large downfield shifts indicate that pronounced hydrogen bonding between the dendrimer host and the encapsulated peptide is taking place. The relatively small changes observed for urea NH shifts of the dendrimer can be explained by the fact that the dendrimer ureas already form an intramolecular hydrogen-bond network in the uncomplexed state, which is not altered significantly by uptake of the peptide guests; this agrees well with earlier work.[17, 18]

When the thiourea-modified poly(propylene imine) dendrimer was used as host, similar behavior for the guest molecule NH shift was observed, with a slightly smaller change in amide NH shifts for the peptide guest ($\Delta\delta \approx 1.1$ ppm). In the thiourea host, a downfield shift of $\Delta\delta \approx 0.12$ ppm was observed for both thiourea NH-proton signals, which indicates a slight

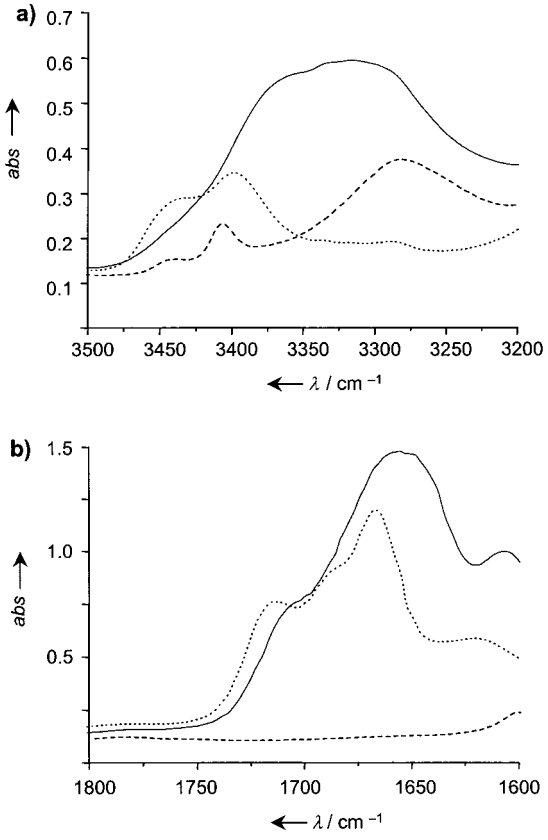

Figure 1. *Partial IR spectra of (a) the NH-stretch region and (b) the CO-stretch region for: (——) DAB-dendr-$(NHCSNHAd)_{64}$/Boc-Gly-Gly-Gly-OH complex, (••••) Boc-Gly-Gly-Gly-OH as a tri-*n*-octyl ammonium salt, and (- - - -) DAB-dendr-$(NHCSNHAd)_{64}$.*

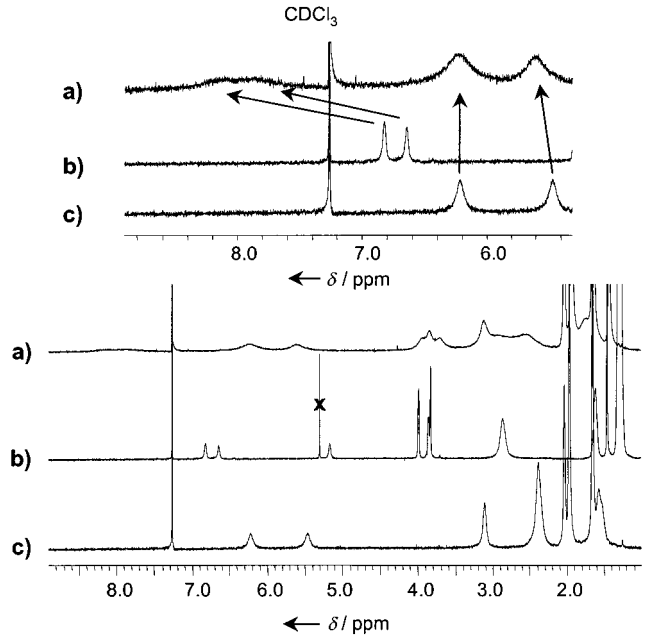

*Figure 2. Full and partial ^{1}H NMR spectra in $CDCl_3$ (25 °C) of: a) DAB-dendr-$(NHCONHAd)_{64}$/Boc-Gly-Gly-Gly-OH; b) Boc-Gly-Gly-Gly-OH as a tri-*n*-octyl ammonium salt; c) DAB-dendr-$(NHCONHAd)_{64}$.*

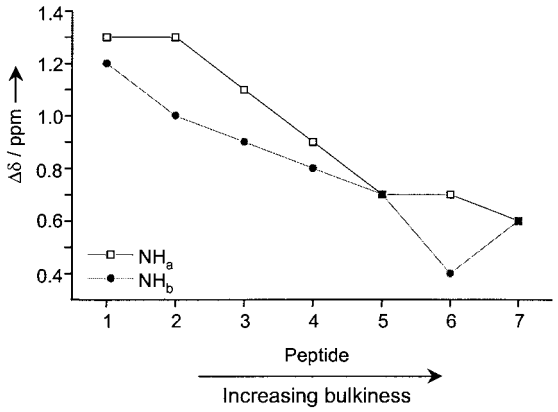

Figure 3. Change in peptide NH ^{1}H NMR shifts ($CDCl_3$, 25 °C) as a function of bulkiness of the peptide. 1: Boc-Gly-Gly-Gly-OH; 2: Boc-Ala-Gly-Gly-OH; 3: Boc-Gly-Gly-Ala-OH; 4: Boc-Phe-Phe-Gly-OH; 5: Boc-Ala-Ala-Ala-OH; 6: Boc-Gly-Val-Val-OH; 7: Boc-Phe-Phe-Phe-OH.

increase in hydrogen bonding upon uptake of the peptide guest. This agrees well with the fact that the thiourea dendrimer has less pronounced intramolecular hydrogen bonding compared to the urea dendrimer host.[18] In the aliphatic region, an additional signal was observed at approximately $\delta = 2.9$. This was assigned to the dendrimer outer shell CH_2N protons, which were shifted downfield because of protonation of the tertiary amines by the bound peptides, which creates multiple ionic interactions (Figure 2 a).

We determined the $\Delta\delta$(NH amide) between the free and complexed peptide for the seven peptides and observed that the $\Delta\delta$ of the amide NH protons (that is, hydrogen bonding) decreased with increasing bulkiness of the amino acid side chains. This indicates that the steric repulsion between the dendrimer and the peptide side chains decreases the hydrogen bonding of the peptide backbone with the dendrimer. Furthermore, bulkiness of the C-terminal amino acid is rather important in dendrimer – peptide hydrogen-bond formation (Figure 3).[23] A C-terminal glycine will make the peptide less sterically demanding, and thereby more able to get close to the dendrimer host to give denser packing around the dendrimer 'sphere'.

Spin – lattice relaxation (T1) and spin – spin relaxation (T2) measurements were carried out to see whether the mobility of the peptide guest was altered by complexation with the dendrimer host. The α-CH protons in the peptide backbone were chosen as the 'measuring point'. The host – guest complexes where formed in $CDCl_3$ (at a complex concentration of 0.4 mM). The peptide-tri-*n*-octyl ammonium salt was dissolved in $CDCl_3$ as a reference. The general tendency was that, upon complexation, the T1 values of the backbone α-protons increased compared to those of the unbound state. This is an indication of restricted motion in the complexed state, in agreement with both the line broadening observed in the one-dimensional spectra of the complexes and observations made on previously studied guest systems.[17] We found no direct correlation between T1 relaxation times and the ability to form stable complexes between the peptide and dendrimer.[24]

Spatial interactions between the peptide guest and the dendrimer host in the complexes were investigated by NOESY spectroscopy. To avoid artifacts caused by spin diffusion (fast relaxation), NOESY experiments were carried out with a range of mixing times (100, 250, 400, and 800 ms). The 100 ms mixing time was appropriate in the rigid system. In the NOESY spectrum of Boc-Gly-Gly-Gly-OH with the urea dendrimer host, NOE interactions were observed between the N-terminal Boc groups (*tert*-butyl protons) on the peptide and the peripheral adamantyl groups of the dendrimer, which indicates close proximity between these groups (Figure 4). At the 100 ms mixing time, no NOE interactions were observed between α protons in the protein backbone and the methylene protons of the dendrimer. However, phenylalanine-containing peptides showed NOE interactions between the side-chain phenyl protons of the peptide and the adamantyl protons of the dendrimer.

Release of the peptides from the dendrimer host

To determine the combination of peptides that bound to the dendrimer host when it was presented to a mixture of the seven peptides, we searched for a mild method to release the bound peptides from the host without cleavage of the acid-sensitive Boc group. 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) has been used as a mild acidic cleavage reagent for preparation of acid-sensitive Boc-peptides on the solid phase.[25] HFIP has, in addition to its mild acidity, a low boiling point (58 °C),[26] which facilitates its removal by evaporation. Methanol was used as solvent because its hydrogen-bonding properties mean that it breaks down the host – guest hydrogen bonding. In model experiments with Boc-Gly-Gly-Gly-OH and a methanol/HFIP (9:1) cleavage mixture, ^{1}H NMR analysis showed complete recovery of

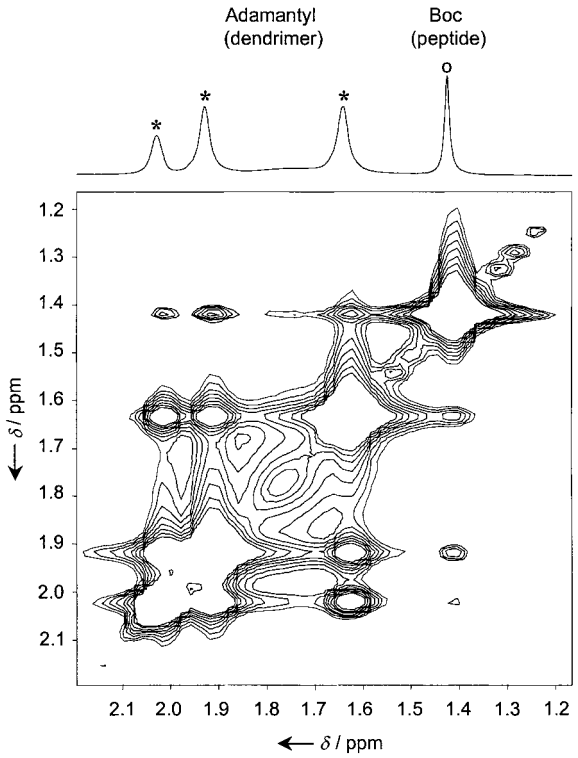

Figure 4. *Partial ^{1}H NOESY spectrum ($CDCl_3$, 25 °C) of the DAB-dendr-(NHCON-HAd)$_{64}$/Boc-Gly-Gly-Gly-OH complex, which shows NOE interactions between adamantyl groups of the dendrimer (*) and the* tert*-butyl group of the peptide (o).*

the protected peptide without cleavage of the Boc group since the protected peptide was released in a quantitative yield from the dendrimer.

Formation of multiple-peptide – dendrimer complexes

To investigate the ability of the dendrimer to act as a host for peptides with different side-chain motifs, and thereby the generality of the formation of peptide – dendrimer complexes, the dendrimer was mixed together with four equivalents of each of the seven peptides (Figure 5). Biobeads filtration was used to remove loosely bound or adhered peptides from the dendrimer. Elution through a silica gel column with the methanol/HFIP (9:1) cleavage mixture released the *N*-Boc protected peptides from the dendrimer host. HPLC was used to analyze the amount of the peptides present in the cleavage mixture, which was quantified by comparison to HPLC standard curves for each peptide. Analysis of the cleavage mixture showed that all the different free peptides were present in 48 – 81 % isolated yield. Thus, it can be concluded that the dendrimer host is able to bind all the different peptides, regardless of side-chain motifs.

In contradiction to the NMR spectroscopy $\Delta\delta$ measurements, we observed that the more bulky peptides were present in the dendrimer host in larger amounts than the less bulky peptides. An explanation for this could be the greater solubility of the more hydrophobic bulky peptides compared to the less bulky ones in a hydrophobic solvent like chloroform. The more soluble bulky peptides will thus be present in solution in a higher concentration. This points towards the general problem in comparing peptides with different side-chain motifs, namely that the different peptides have different properties, for example, hydrophobicity and acidity.

Conclusion

In the present paper, we show that poly(propylene imine) dendrimers modified with adamantyl urea and thiourea end groups can be used as hosts or carriers for a variety of *N*-Boc-protected peptides in chloroform. The peptides can be released under mild acidic conditions to yield the intact peptide, even when the peptide is protected with an acid-labile group at the N terminus. IR spectroscopy and various NMR spectroscopy techniques show that the peptide is bound to the dendrimer by hydrogen bonding as well as ionic interactions. Despite indications of weaker hydrogen bonding between the den-

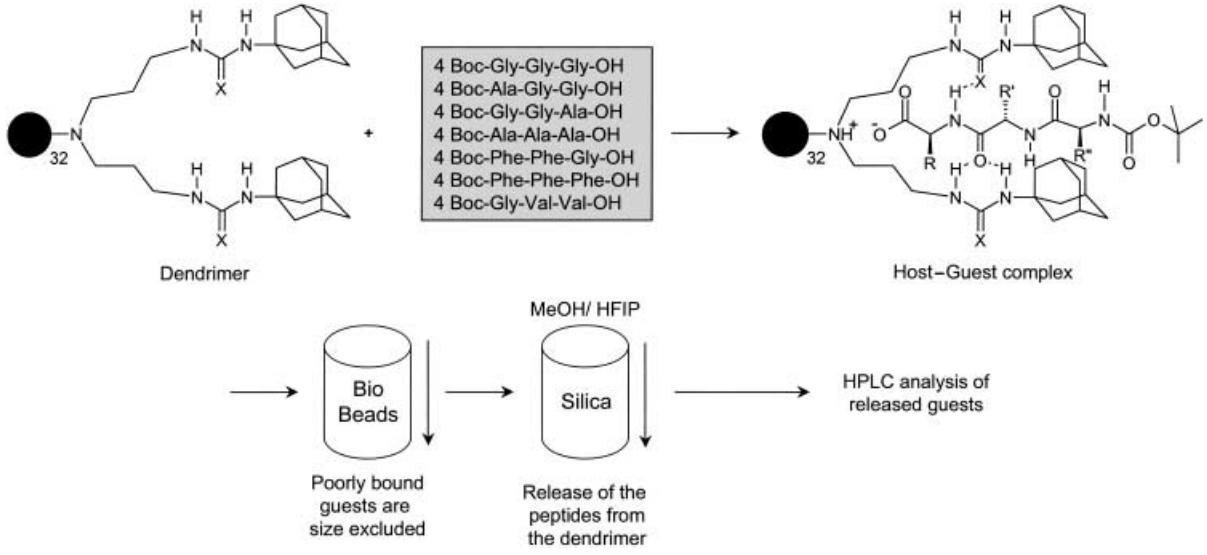

Figure 5. *Formation of multiple-peptide – dendrimer complexes.*

drimer carrier and peptides with bulky side chains, these peptides were complexed to the dendrimer as well as peptides with a less bulky sequence. Thus, this general host – guest binding motif is promising for the development of, for example, drug delivery containers with the ability to bind several different peptides at once. Our first focus hereafter will be on the use of this supramolecular approach in aqueous media.

Experimental Section

General:

The solvents and reagents were of analytical reagent grade. Chloroform was dried over molecular sieves (3 Å) prior to use. Boc-Gly-Gly-Gly-OH, Boc-Phe-Phe-Gly-OH, and H-Phe-Phe-Phe-OH were purchased from Bachem, unprotected dipeptides from Sigma, and N-protected amino acids and chlorotrityl resin were purchased from Nova Biochem. All amino acids were of the L configuration. Solid-phase syntheses were carried out manually by using polypropylene syringes with polyethylene filters. Melting points were measured on a Büchi B-540 apparatus and are uncorrected. IR analysis was performed on a Perkin Elmer Spectrum One FT-IR instrument by using a solution cell (1.0 mm, F-05NT, NaCl windows) or the attenuated reflection (ATR) technique. All routine ^{1}H and ^{13}C NMR spectroscopy measurements were recorded on a Varian Inova 500, a Mercury Vx 400, or a Gemini 300 instrument unless indicated otherwise; all chemical shifts (δ) are reported in ppm downfield of tetramethylsilane.

NMR spectroscopy experiments: Spin – lattice relaxation times (T1) were determined with an inversion – recovery pulse sequence and spin – spin relaxation times (T2) were determined by using the Carr – Purcell Meiboom – Gill pulse sequence. Both T1 and T2 relaxation experiments were evaluated by exponential fitting of the experimental data with the Varian VNMR software. The NOESY experiment was performed with a short mixing time (100 ms) to minimize artifacts caused by spin diffusion, which were found to be prevalent at higher mixing times.

HPLC experiments: The following solvents were used for HPLC: 0.1 % TFA in H_2O (A); 0.1 % TFA in CH_3CN (B). HPLC analysis was performed on a Waters system (600E pump and 996 PDA detector) on a Symmetry 300 C4 5 μm column. Gradient: initial 100 % A; at 25 min 100 % B; held at 100 % B until 30 min; at 31 min 100 % A; held at 100 % A until 40 min. High-resolution mass spectrometry (HRMS) was performed in the positive fast atom bombardment (FAB^+) mode with *meta*-nitrobenzyl alcohol (*m*-NBA), poly(ethylene glycol) (PEG)-600, and PEG-700 as the matrix.

IR and NMR spectroscopy solutions:

Peptide (as the tri-*n*-octyl ammonium salt): The peptide (60.5 μmol) was suspended in dry chloroform (5 ml). Tri-*n*-octylamine (26.4 μL, 60.5 μmol) was added to dissolve the peptide. The suspension was heated gently and sonicated for 5 min to create a clear solution. The peptide NH- and CO-stretch regions were investigated by IR spectroscopy (3200 – 3500 cm^{-1} and 1600 – 1800 cm^{-1}, respectively).

(DAB-dendr-$(NHCONHAd)_{64}$): The fifth generation adamantyl urea or thiourea modified poly(propylene imine) dendrimer[27] (0.8 μmol) was dissolved in dry chloroform (2 ml). The dendrimer NH- and CO-stretch regions were investigated by IR spectroscopy (3200 – 3500 cm^{-1} and 1600 – 1800 cm^{-1}, respectively).

DAB-dendr-$(NHCONHAd)_{64}$ – peptide complex: The peptide (24.2 μmol) and fifth generation adamantyl urea or thiourea modified poly(propylene imine) dendrimer (0.8 μmol) were suspended in dry chloroform (2 ml). The suspension was sonicated and heated gently until a clear solution was obtained, which indicated the formation of the dendrimer – peptide complex. The NH- and CO-stretch regions were investigated by IR spectroscopy (3200 – 3500 cm^{-1} and 1600 – 1800 cm^{-1}, respectively).

NMR solutions: These were made in a similar way to the solutions above with deuterated chloroform as the solvent.

Synthesis:

Boc-Phe-Phe-Phe-OH[28] (**1**):

H-Phe-Phe-Phe-OH (0.500 g, 1.09 mmol) was suspended in tetrahydrofuran (THF; 17 mL) and water (5 ml). Di-*tert*-butyl-dicarbonate (0.260 g, 1.14 mmol) was added followed by 1 M aqueous NaOH (1.1 mL) to create a clear solution. The mixture was stirred for 3 h at RT. THF was removed in vacuo, water (10 mL) was added, and the aqueous layer was acidified with 10 % aqueous citric acid (8 ml). The aqueous layer was subsequently extracted with ethyl acetate (2 × 15 ml), and the combined organic layers were dried (Na_2SO_4) and evaporated in vacuo. Residual di-*tert*-butyl-dicarbonate was removed by suspension of the residue in boiling diethyl ether followed by removal of the supernatant. Yield: 0.440 g (72 %); mp: 187 – 188 °C; ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 1.26$ (s, 9 H, *t*Bu), 2.54 – 3.10 (m, 6 H, CH_2-benzyl), 4.09 (br s, q, 1 H, C_α), 4.45 (q, $J = 5.5$ Hz, 1 H, C_α), 4.59 (m, 1 H, C_α), 6.85 (d, $J = 9.23$ Hz, 1 H, NH-Boc), 7.16 – 7.26 (m, 15 H, aromatic), 7.91 (d, $J = 7.97$ Hz, 1 H, NH-amide), 8.37 (d, $J = 7.14$ Hz, 1 H, NH-amide); ^{13}C NMR (75 MHz, [D_6]DMSO): $\delta = 28.3$, 36.9, 37.8, 38.0, 38.5, 53.5, 53.7, 56.0, 78.3, 126.3, 126.5, 126.7, 128.2, 128.4, 129.3, 129.6, 137.6, 137.7, 138.3, 155.2, 171.1, 171.5, 172.9; HRMS (FAB^+): calcd: 560.2761 [*M*H$^+$] (monoisotopic); found: 560.2758.

Boc-Ala-Gly-Gly-OH[29] (**2**): H-Ala-Gly-Gly-OH (0.50 g, 2.46 mmol) was treated with di-*tert*-butyl-dicarbonate (0.59 g, 2.58 mmol) and 1 M NaOH (2.5 mL) in THF (20 mL) and water (6 mL) in a procedure analogous to that for **1**. Yield: 0.260 g (35 %); mp: 170 – 172 °C; ^{1}H NMR (400 MHz, D_2O): $\delta = 1.20$ (d, $J = 7.0$ Hz, 3 H, CH_3 (Ala)), 1.27 (s, 9 H, *t*Bu), 3.83 (s, 2 H, C_α (Gly)), 3.87 (s, 2 H, C_α (Gly)), 3.92 (q, $J = 7.7$ Hz, 1 H, C_α (Ala)); ^{13}C NMR (125 MHz, D_2O): $\delta = 16.8$, 27.7, 41.2, 41.4, 42.4, 43.5, 51.1, 81.9, 157.8, 172.1, 173.5, 177.1; HRMS (FAB^+): calcd: 304.1509 [*M*H]$^+$ (monoisotopic); found: 304.1509.

Boc-Ala-Ala-Ala-OH[30] (**3**): H-Ala-Ala-OH (0.50 g, 3.12 mmol) was suspended in THF (17 mL) and water (8 mL). Boc-alanine-*N*-hydroxy-succinimide-ester (Boc-Ala-Osu; 0.89 g, 3.09 mmol) was added, followed by addition of 1 M aqueous NaOH (3.2 mL). The mixture was stirred for 3 h, which slowly created a clear solution. THF was removed in vacuo. The aqueous layer was acidified with 10 % aqueous citric acid (12 mL) and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were dried (Na_2SO_4) and evaporated in vacuo to afford crude Boc-Ala-Ala-Ala-OH as a sticky solid. Impurities were removed by heating the compound in boiling ethyl acetate (15 mL) and filtration of the warm suspension. Cooling of the filtrate on ice crystallized the product to yield 0.30 g (29 %) as a white powder. Mp: 193 – 194 °C (Ref. [30]: 183 – 184 °C); ^{1}H NMR (300 MHz, D_2O): $\delta = 1.20$ (d, $J = 6.6$ Hz, 3 H, CH_3 (Ala)), 1.26 – 1.32 (m, 15 H, *t*Bu (9 H), CH_3 (Ala) (6 H)), 3.93 (q, $J = 7.14$ Hz, 1 H, C_α (Ala)), 4.21 (m, 2 H, C_α (Ala)); ^{13}C NMR (75 MHz, D_2O): $\delta = 16.4 – 16.7$, 27.7, 29.8, 48.9 – 50.5, 81.7, 157.6, 174.7, 176.0, 176.8; HRMS (FAB^+): calcd: 332.1822 [*M*H$^+$] (monoisotopic); found: 332.1812.

Boc-Gly-Gly-Ala-OH (**4**): In a syringe equipped with a filter, 2-chlorotrityl chloride derivatized polystyrene resin (0.600 g, 0.5 mmol; loading: 0.83 mmol g^{-1}) was made to swell in dry dichloromethane (DCM; 5 ml). 9-fluorenylmethoxycarbonyl (Fmoc)-Ala-OH (0.187 g,

0.600 mmol) was added, followed by diisopropylethylamine (DIEA; 0.413 mL, 2.400 mmol). The suspension obtained was shaken for 90 min at RT. The supernatant was removed by suction and then a dichloromethane/methanol/DIEA (17:2:1) mixture (2×5 ml, 2×15 min) was added to cap the residual chlorotrityl groups on the resin. The resin was washed with DCM (10 volumes) and dimethylformamide (DMF; 5 volumes) then shrunk by MeOH (2 volumes). The new loading of the derivatized resin was determined by Fmoc quantification as follows: 2.9 mg (2.4 mmol theoretical loading) were suspended in 20% piperidine (pip) in DMF and shaken for 15 min. UV absorption (A_{290}) was measured by using 20% pip in DMF as a reference and gave a measured loading of 0.541 mmol g^{-1}. The N-terminal Fmoc protecting group was removed by 20% pip in DMF ($3 + 17$ min). The resin was washed with DCM (10 volumes) and DMF (5 volumes). The Kaiser test gave a positive result, which indicated the presence of free primary amine.[31] Fmoc-Gly-OH (0.60 g, 2.00 mmol), 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU; 0.72 g, 1.9 mmol), and *N*-hydroxybenzotriazole (HOBt; 0.27 g, 2.00 mmol) were dissolved in a DMF/DCM (1:1) mixture (5 mL; DCM prevents the resin shrinking upon addition of aromatic reactants), and DIEA (0.67 mL, 3.9 mmol) was added. After 5 min of preactivation the mixture was added to the resin in the filter syringe. The mixture was shaken for 1 h at RT. then washed with DCM (10 volumes) and DMF (5 volumes). The Kaiser test now gave a negative result. Fmoc-deprotection followed by washing (as above) gave a positive Kaiser test result. Boc-Gly-OH (0.35 g, 2.00 mmol), HBTU (0.72 g, 1.9 mmol), and HOBt (0.27 g, 2.00 mmol) were dissolved in a DMF/DCM (1:1) mixture (5 mL) and DIEA (0.67 mL, 3.9 mmol) was added. After 5 min of preactivation the mixture was added to the resin in the filter syringe. The mixture was shaken for 1 h at RT. and washed with DCM (10 volumes) and DMF (5 volumes). The Kaiser test gave a negative result. The peptide was cleaved from the resin by using 20% HFIP in DCM followed by evaporation in vacuo and crystallization from diethyl ether.[32] Yield: 0.070 g (71%); mp: 97–98 °C; ^{1}H NMR (300MHz, D_2O): $\delta = 1.26$–1.28 (m, 12H, *t*Bu (9H), CH_3 (3H)), 3.66 (s, 2H, C_α (Gly)), 3.82 (s, 2H, C_α (Gly)), 4.23 (q, $J = 7.2$ Hz, 1H, C_α (Ala)); ^{13}C NMR (75 MHz, D_2O): $\delta = 15.8$, 27.1, 41.7, 43.1, 48.4, 81.4, 157.9, 170.5, 172.8, 176.2; HRMS (FAB^+): calcd: 304.1509 [MH^+] (monoisotopic); found: 304.1513.

Boc-Gly-Val-Val-OH (**5**): H-Val-Val-OH (0.50 g, 2.3 mmol) was suspended in THF (4 mL). Aqueous NaOH (0.092 g per 4 ml) was added to create a clear solution. *N*-Boc-Gly-OSu (0.61 g, 2.25 mmol) was added and the mixture was stirred overnight at RT. $KHSO_4$ (1 M, 5 mL) was added and the polar layer was extracted with ethyl acetate (3×10 mL). The organic layers were washed with brine (30 mL), dried (Na_2SO_4), and evaporated in vacuo. The residue was triturated 3 times from diethyl ether to give the product as a white crystalline solid. Yield: 0.760 g (90%); mp: 60–62 °C; ^{1}H NMR (500MHz, [D_6]DMSO): $\delta = 0.84$–0.90 (m, 12H, CH_3 (Val)), 1.37 (s, 9H, *t*Bu), 1.94 (sept, $J = 6.4$ Hz, 1H, CH (Val)), 2.03 (sept, $J = 6.8$ Hz, 1H, CH (Val)), 3.55 (d, $J = 6.0$ Hz, CH_2 (Gly)), 4.07 (dd, $J = 6.0$, 2.1 Hz, C_α (Val)), 4.34 (dd, $J = 6.4$, 2.1 Hz, C_α (Val)), 7.01 (br t, 1H, NH (Boc)), 7.50 (d, $J = 9.0$ Hz, NH (amide)), 8.02 (d, $J = 8.1$ Hz, NH (amide)); ^{13}C NMR (125 MHz, [D_6]DMSO): $\delta = 15.2$, 18.9, 19.3, 20.2, 21.9, 29.3, 30.7, 32.3, 44.5, 56.1, 57.9, 58.5, 60.9, 79.3, 170.2, 172.3, 173.9; HRMS (FAB^+): calcd: 374.2291 [MH^+] (monoisotopic); found: 374.2303.

Formation and cleavage of multiple-peptide–dendrimer complexes: Boc-Gly-Gly-Gly-OH (3.5 mg, 12.1 μmol), Boc-Ala-Gly-Gly-Gly-OH (3.7mg, 12.1 μmol), Boc-Gly-Gly-Ala-OH (3.7 mg, 12.1 μmol), Boc-Ala-Ala-Ala-OH (4.0 mg, 12.1 μmol), Boc-Gly-Val-Val-OH (4.5 mg, 12.1 μmol), Boc-Phe-Phe-Gly-OH (5.7 mg, 12.1 μmol), and Boc-Phe-Phe-Phe-OH (6.8 mg, 12.1 μmol) were suspended in dry chloroform (5 mL). DAB-dendr-$(NHCONHAd)_{64}$ (56.0 mg, 3.0 μmol) was added and the suspension was allowed to stand for 16 h at RT. to form a clear solution. The mixture was filtered through a Gelman Acrodisc 13 (0.45 μm) filter, and eluted through a biobeads S-X3 column with two void volumes of dichloromethane. The collected fractions, which contained the peptide–dendrimer complexes, were evaporated in vacuo. The residue was taken up in a small volume of dichloromethane (2 mL) and put on a dry silica plug. The silica was eluted with a methanol/HFIP (9:1) mixture (20 mL) and the collected mixture of free peptides was isolated by evaporation. The residue was dissolved in methanol (10 mL) and the composition of the mixture was analyzed by reversed-phase HPLC, with detection at 215 nm and 254 nm. Cleavage yields of peptides from the dendrimer: Boc-Gly-Gly-Gly-OH, 1.97 mg (56%); Boc-Ala-Gly-Gly-OH, 2.53 mg (68%); Boc-Gly-Gly-Ala-OH, 2.73 mg (74%); Boc-Ala-Ala-Ala-OH, 3.11 mg (78%); Boc-Gly-Val-Val-OH 3.65 mg (81%); Boc-Phe-Phe-Gly-OH 2.72 mg (48%); Boc-Phe-Phe-Phe-OH 5.13 mg (75%).

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[20] The peptides were investigated as their tri-*n*-octyl ammonium salts to increase solubility and in addition to prevent aggregation of the peptides.
[21] Partial IR spectra of the urea dendrimer–peptide complex can be found in the Supporting Information.

[22] Amide and carbamate NH-protons of the Boc-triglycine-tri-*n*-octyl ammonium salt were thoroughly assigned by H, H COSY NMR spectroscopy. We assume that there is no 'cross-over' of the NH signals when complexation to the dendrimer takes place.
[23] A relevant table can be found in the Supporting Information.
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