Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 3815–3820

TETRAHEDRON

A new class of protein mimics: preparation and electrophoretic properties of polycationic β-alanine-based dendrimers

Hak-Fun Chow,[a,*] Tony K.-K. Mong,[a] Yuk-Hang Chan[b] and Christopher H. K. Cheng[b]

[a]*Department of Chemistry, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR, People's Republic of China*
[b]*Department of Biochemistry, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR, People's Republic of China*

Received 27 September 2002; revised 12 December 2002; accepted 10 January 2003

Abstract—The synthesis and characterization of a series of polyammonium β-alanine containing dendrimers **4**–**6** were reported. Such polycationic species were shown to exhibit electrophoretic behavior similar to that of protein molecules such as lysozyme and aprotinin in acidic polyacrylamide gel electrophoresis. It was also found that their electromobility was directly related to their molecular size, and hence such biomimetic dendrimers are potential molecular weight markers for protein analysis.

1. Introduction

Dendritic polymers are a new class of polymeric compounds that are constructed by stepwise, controlled synthetic operations.[1] Due to their highly branched architecture, dendrimers are very different from traditional polymers in many aspects. Structurally, dendrimers will adopt a globular conformation once the molecular mass exceeds a critical value. As a result, they exhibit physical properties that are different from those of conventional, random coil long chain polymers. Synthetically, the judicious placement of various functional groups on a dendritic architecture can now be routinely executed to create functional dendrimers that can serve as molecular machines having light harvesting, catalytic, or electrochemical properties.[2] Furthermore, dendrimers can be prepared with a very narrow polydispersity and this enables us to correlate the observed properties to their physical dimensions in a well-defined manner. As a result of these attractive features, research efforts in dendrimer chemistry are expanding rapidly and we are beginning to see their applications in a number of material, medicinal and industrial arenas.

Among the various kinds of dendritic macromolecules, biomimetic dendrimers are of particular interest due to their structural resemblance to natural biomolecules. They are also considered as unique mimics for the exploration of the properties and functions of many biological molecules.[3] Several reports have already focused on the synthesis of amino acid-based and peptide-based dendrimers.[4] Recently we disclosed the synthesis of a series of Boc-protected poly(β-alanine) containing dendrimers (e.g. **1**–**3**) serving as artificial models for globular protein molecules.[5] These compounds were prepared by a stepwise, convergent synthetic procedure and were shown to have good structural homogeneity by a number of analytical methods. It was revealed that they formed non-specific yet discrete self-assembled aggregates even in polar solvents and that they exhibited unusually strong binding affinities towards protic solvents. The formation of such non-covalent aggregates was similar to the assembly of protein molecules into a quaternary structure. As an extension of this study, we wish to report herein the synthesis of the fully deprotected β-alanine-based dendrimers **4**–**6**. It is of interest to note that the structure of such fully deprotected species is similar to those of peptide biomolecules. In this case the various β-alanine residues are connected through amide/peptide linkages via the unnatural aromatic branching units. It is therefore anticipated that these fully deprotected, biomimetic dendrimers should possess solubility and electrophoretic properties similar to those of the native globular protein molecules. Under physiological conditions, these polyamino species should become protonated and behave as a class of ionic dendrimers.[6] Such polyionic species have been demonstrated to possess unusual micellar,[7] membrane transport,[8] ion exchange,[9] and polyelectrolyte properties.[10] Although polyacrylamide gel electrophoresis has been routinely used in the analysis of biomolecules such as proteins, oligonucleotides, DNA and RNA, this technique has rarely been used in the analysis of charged dendritic molecules. In fact, apart from polyamidoamine (PAMAM)[11] and polypropyleneimine dendrimers,[12] the electrophoretic properties of such polyionic species were not well documented. In this paper, we will examine the factors that contribute to the electrophoretic mobility of such polycationic species, and will also evaluate their

Keywords: dendrimers; β-alanine; PAGE; electrophoresis; molecular weight markers.

* Corresponding author. Tel.: +852-26096344; fax: +852-26035057; e-mail: hfchow@cuhk.edu.hk

0040–4020/03/$ - see front matter
doi:10.1016/S0040-4020(03)00431-9

potential to act as a new class of molecular weight markers for use in protein analysis by comparing their electrophoretic properties to that of natural occurring proteins.

2. Results and discussion

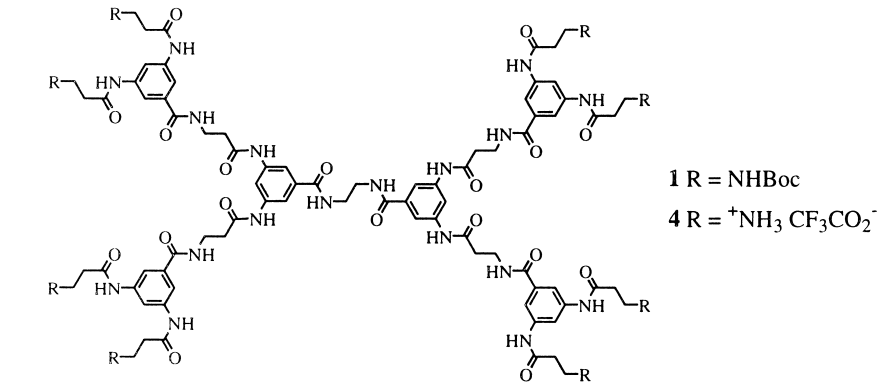
2.1. Synthesis

The polycationic dendritic species **4**–**6** of the second to fourth generation were prepared from their corresponding electrically neutral Boc-protected species **1**–**3**.[5] Initially hydrochloric acid (1.2 M in ethanol) was used to remove the Boc groups. Unfortunately, some of the partially Boc-deprotected products precipitated from the reaction mixture and hence complete removal of the Boc groups was not possible at room temperature. At a higher temperature (~50°C) the precipitate could be redissolved in the solvent but this led to partial hydrolysis of the amide bonds in the dendrimers. Finally, a 60% solution of trifluoroacetic acid (TFA) in dichloromethane could be used to smoothly remove the Boc groups at room temperature (Scheme 1). The progress of this reaction was monitored at regular time intervals by scrutinizing the disappearance of the ^{1}H NMR resonance signals due to the carbamate N–H and *t*-butyl protons of the starting material. The time required for the complete deprotection of the Boc groups was about 12 h. The resulting trifluoroacetate salts **4**–**6** were obtained as highly hygroscopic, glassy solids.

2.2. Characterization

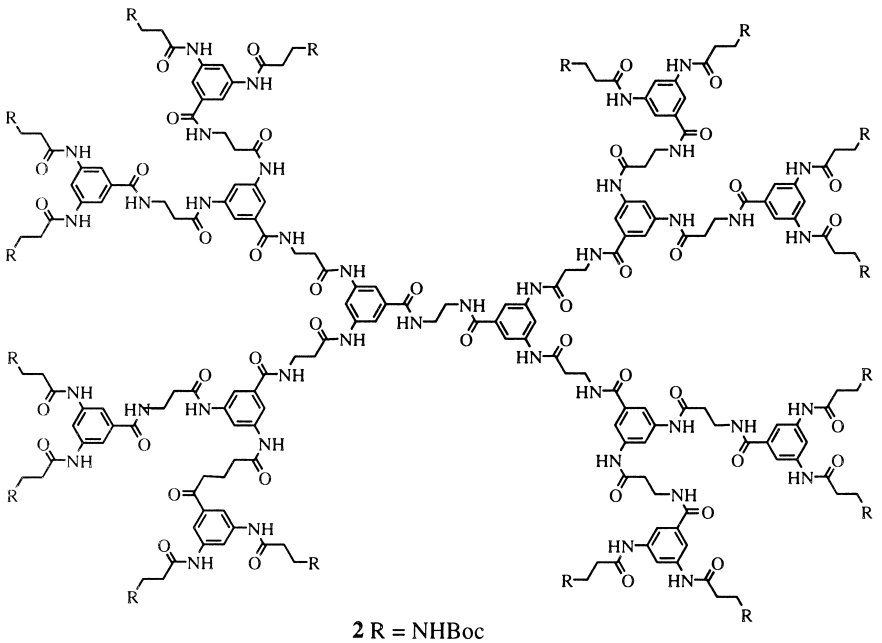
2.2.1. ^{1}H NMR spectroscopy. Due to the presence of a large number of polar amide and amine functionalities, the polyammonium salts **4**–**6** tended to form hydrates and were highly hygroscopic. As a result, the samples had to be placed under vacuum (0.1 mm Hg) for 24 h to remove the trapped solvents. Despite such operations, a significant amount of water was still trapped inside the dendritic matrix and hence satisfactory elemental analysis could not be obtained for these compounds.

The structural identities and purities of the products were examined by ^{1}H NMR spectroscopy. Upon complete removal of the Boc-groups, the *t*-butyl proton (at ~δ 1.38) and the carbamate N–H at ~δ 6.85 signals due to the Boc-moieties disappeared in the NMR spectra of target compounds **4**–**6** (Fig. 1). As a result, only two sets of N–H signals, one due to the anilide moieties (~δ 10.2) and the other due to the β-alanine amides (~δ 8.5) were observed.



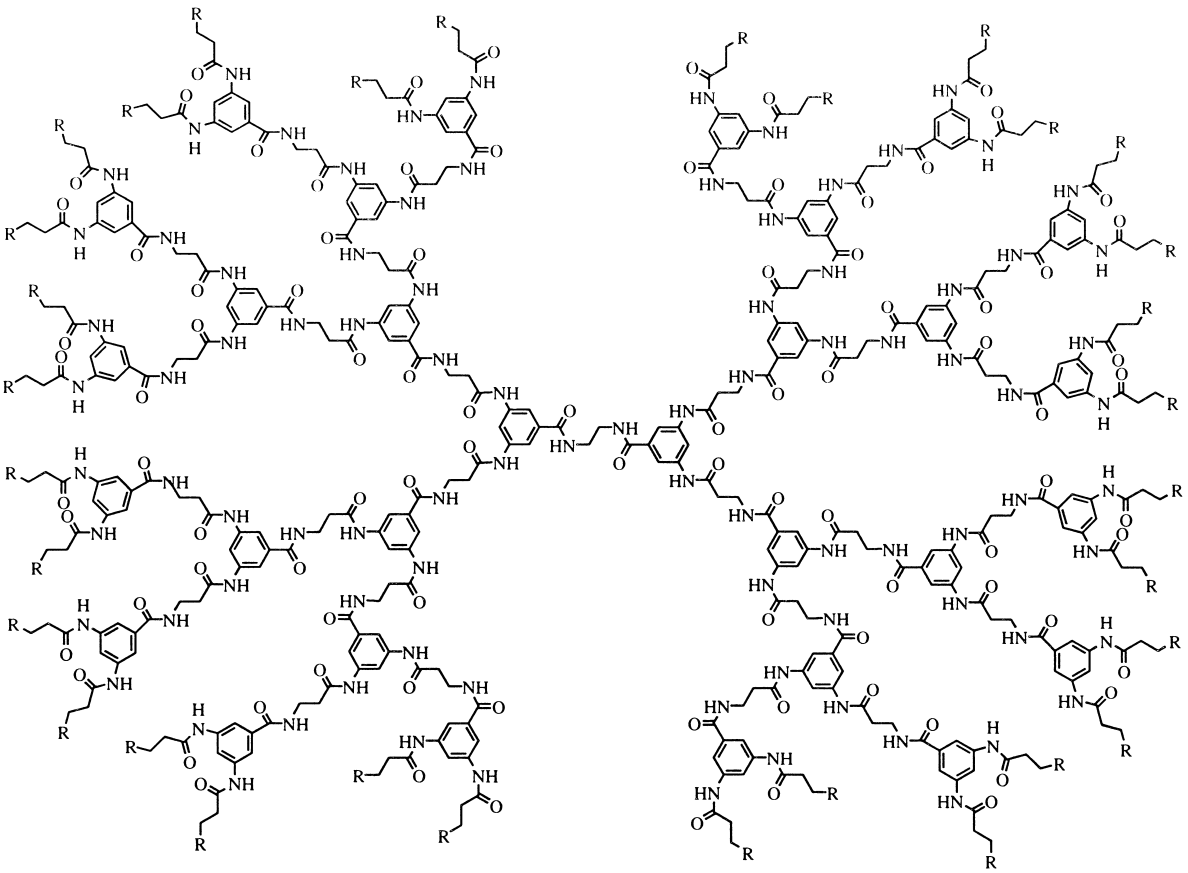
1 R = NHBoc

4 R = $^+NH_3\ CF_3CO_2^-$



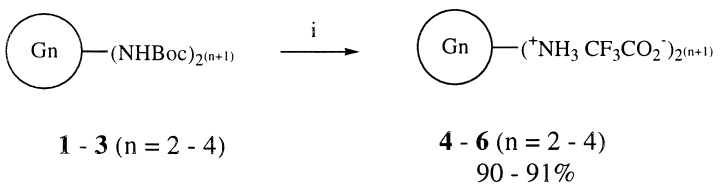
2 R = NHBoc

5 R = $^+NH_3\ CF_3CO_2^-$



3 R = NHBoc

6 R = $^{+}NH_3\ CF_3CO_2^{-}$



1 - **3** (n = 2 - 4)

4 - **6** (n = 2 - 4)
90 - 91%

Scheme 1. (i) TFA, CH_2Cl_2.

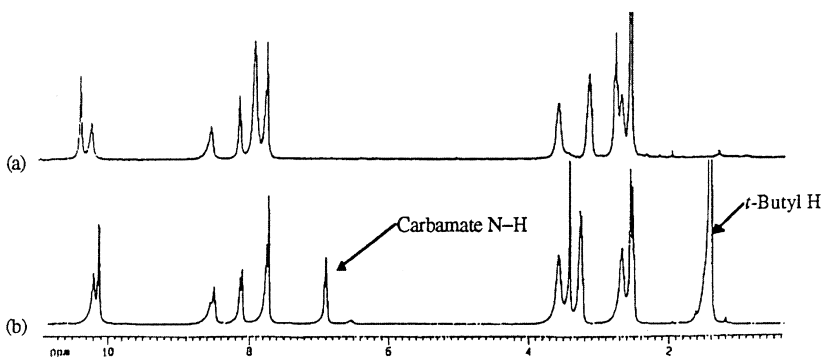


Figure 1. The ^{1}H NMR spectra of (a) G4 polyammonium dendrimer **6** and (b) Boc-protected G4 poly(β-alanine) dendrimer **3**.

Furthermore, the anilide N–H protons attached to the surface aromatic ring were downfield shifted (δ 10.1→δ 10.3) with respect to those belonging to the Boc-protected series of compounds, possibly due to their closer proximity to the cationic ammonium center. On the other hand, the chemical shift value of the interior anilide N–H protons remained essentially unchanged (δ 10.18) in both the Boc-protected and Boc-deprotected series. Finally, the ^{1}H NMR

signal of the ammonium $^{+}NH_3$ protons appeared as a broad singlet at ~δ 7.9 for all the target dendrimers.

2.2.2. ^{13}C NMR spectroscopy. The ^{13}C NMR resonance signals of the various functional entities of the poly-ammonium dendrimers were located in distinct spectral regions. The presence of the trifluoroacetate counterions was confirmed by the presence of two ^{13}C quartets at δ 117 ($^{1}J_{CF}$=300 Hz) and 159 ($^{2}J_{CF}$=40 Hz). Two types of C=O groups could be identified; one originated from the β-alanine moiety and the other due to the aromatic branches. The former gave rise to ^{13}C signals clustered at around δ 167 while the latter located at δ 169. In the aliphatic region, the tertiary (δ 77) and primary carbon signals (δ 28) of the original *t*-butyl groups had disappeared. The ^{13}C signals of the aromatic brancher were located in two separate subregions; one at around δ 113 and the other at δ 136–139. The aliphatic ^{13}C signals due to the β-alanines located on the surface and the interior could also be differentiated, and were scattered between δ 33–37. Similar to the Boc-protected dendrimers **1**–**3**, the ^{13}C signal of the core ethylene unit was obscured by the ^{13}C signals of d^6-DMSO solvent. Its presence was revealed by hetero-nuclear magnetic correlation resonance (HMQC) spectroscopy.

2.2.3. Mass spectrometry. Satisfactory mass spectroscopic data were obtained for the G2–G4 dendrimers **4**–**6**. The molecular ion in the form of $[M-n\text{TFA}+\text{H}]^{+}$ was always found, where *n* denotes the number of TFA molecules associated with the dendritic species. Hence, The G2 octaammonium dendrimer salt **4** gave a molecular ion peak at *m*/*z* 1718, which was due to the mono-protonated form of the corresponding octaamine free base. The corresponding mass peaks for the G3 and G4 dendrimers were at *m*/*z* 3926 and 8345, respectively.

2.3. Electrophoretic properties

Some protein or peptide molecules, due to the presence of exposed charged groups at the two termini and on the side chains, can migrate under the influence of an electric field. Being similar in three-dimensional structure to native proteins, our completely deprotected poly-ammonium dendrimer salts **4**–**6** should also acquire such electrophoretic properties under physiological or acidic conditions.

Electrophoresis experiments were conducted on polyacrylamide gel in an acetic acid buffer solution. Polyacrylamide gel was employed as the supporting medium because the porosity of the polymeric network can be adjusted by varying the amount of the acrylamide monomer and *N*,*N*-methylene bisacrylamide crosslinker. Generally, 5–25% was suitable for the electrophoretic analysis of most protein species. For the analysis of our deprotected dendrimers, due to their smaller size (2–8 kD), a higher acrylamide concentration (~40%) was used. The gel was divided into the stacking and resolving portions. The stacking gel served to concentrate the sample into a thin zone before entering the resolving gel where the dendrimer samples **4**–**6** and two protein standards (aprotinin 7 kD and lysozyme 14 kD) should exhibit different mobilities.

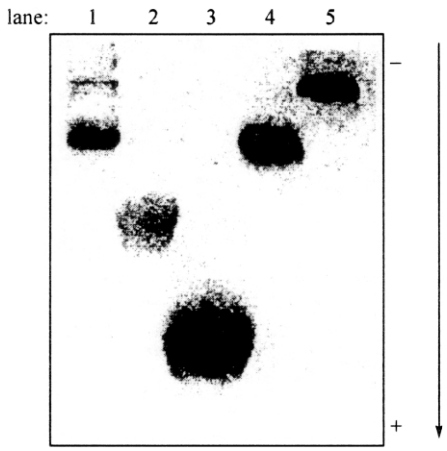


Figure 2. Acidic polyacrylamide gel electrophoresis of protein standards and polycationic dendrimers [lane 1=G4 dendrimer **6** (8 kD); 2=G3 dendrimer **5** (4 kD); 3=G2 dendrimer (2 kD); 4=aprotinin (7 kD); 5=lysozyme (14 kD)].

The electrophoretic behavior of the cationic poly(β-alanine) dendrimers **4**–**6** and the two protein standards in acidic 40% polyacrylamide gel is shown in Figure 2. Lanes 1–3 correspond to the polycationic G4 **6** (8 kD), G3 **5** (4 kD) and G2 dendrimers **4** (2 kD), respectively. Lanes 4 and 5 are aprotinin (7 kD) and lysozyme (14 kD), respectively. The synthetic poly(β-alanine) dendrimers exhibited electrophoretic mobility similar to that of native proteins and could also be stained with a common protein staining dye (Commassie Blue R-250). The dendrimers appeared as narrow bands on the electrophoretogram further confirming their structural homogeneity. In terms of migratory distances, the largest homologue G4 dendrimer **6** in lane 1 was retarded by the polyacrylamide gel network to the greatest extent relative to the smaller dendrimers **4** and **5**. Thus the electrophoretic mobility was in an ascending order of **6**, **5** and **4**. This was consistent with that observed for lysozyme and aprotinin, in which case lysozyme with the largest molecular weight had the smallest mobility while aprotinin had an almost identical mobility as G4 dendrimer **6** due to their nearly similar molecular weights. It was clear that the sieving effect of the gel was the most crucial factor in controlling the mobilities of these charged species. Although the three dendritic species had similar mass to charge ratios, their electrophoretic mobilities were very different. However, it should be noted that the migratory zones of G2 **4** and G3 dendrimer **5** were somewhat diffuse in appearance. This was presumably due to passive diffusion of these smaller size entities during the experiment. Hence the fully deprotected poly(β-alanine) dendrimers possessed similar electrophoretic characteristics to that of natural protein molecules, and may conceivably be employed as molecular weight markers in protein analysis. The advantage of using such artificial markers is that their molecular weight and size can be synthetically fine tuned by varying the nature of the branching units and the amino acid arms. Hence, a range of molecular weight markers can be made available for analysis. Furthermore, they can be synthesized in large quantities at relatively low cost.

The effect of the porosity of the polyacrylamide gel on the electrophoretic mobility of the dendrimers is shown in

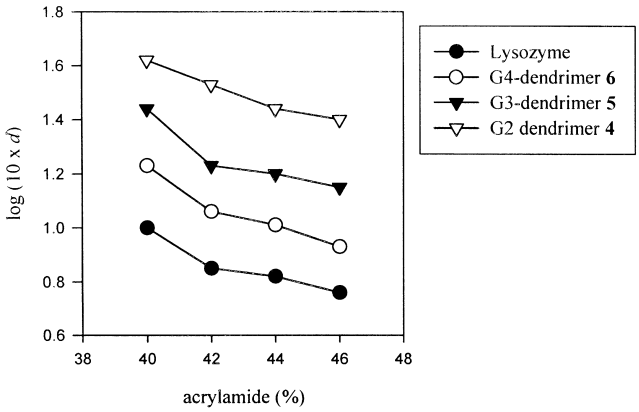


Figure 3. A plot of log(10×*d*) vs polyacrylamide gel concentration for G2–G4 dendrimers (**4**–**6**) and lysozyme.

Figure 3. In this experiment, the percentage of acrylamide used in the preparation of the gel was varied from 40 to 46%, therefore the porosity of the supporting medium was progressively decreased. As expected, both the dendrimers and lysozyme were retarded to some extent leading to a decrease in mobility (measured in terms of the migratory distance *d*). The response of the synthesized poly(β-alanine) dendrimers to porosity variation was parallel to lysozyme. However, the response of mobility to the gel concentration did not follow a linear relationship and so the retardation coefficient[13] (expressed as the slope of the semi-log plot of migratory distance *d* against gel concentration), an empirical parameter that could be used to estimate the molecular weight of proteins, could not be determined. Hence, in this respect, our artificial dendritic peptides behaved somewhat differently from native proteins under electrophoretic conditions. Whether such a subtle difference was due to the hyperbranched nature of our dendritic species requires further investigations.

3. Conclusions

The synthesis, characterization and electrophoretic property of a series of polyammonium β-alanine-based dendrimers **4**–**6** were reported. It was found that these synthesized dendritic peptide mimics exhibited electrophoretic mobilities similar to those of native protein standards of comparable size. Furthermore, the mobility was sensitive to the change in porosity of the supporting medium. These results suggested that such chemically synthesized artificial peptide mimics may be used as size markers for protein analysis. The electrophoretogram also provided an alternative means to determine the structural homogeneity of such polyionic dendrimer species.

4. Experimental

4.1. Synthesis

General. ^{1}H (300 MHz) and ^{13}C NMR (75.5 MHz) spectra were acquired on a Brüker Avance DPX spectrometer and were recorded in d^{6}-DMSO unless otherwise stated. Mass spectrometry was achieved either by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) technique or by liquid secondary ionization mass spectrometry (L-SIMS) method on a Brüker APEX 47E FTMS model. The reported molecular mass (*m*/*z*) values were monoisotopic mass.

General procedure for the deprotection of poly(β-alanine) dendrimers **1**–**3**. The Boc-protected dendrimer (**1**–**3**) was added to a stirred solution of TFA in dichloromethane (60% v/v, 6.0 mL) at 25°C for ~12 h. The excess TFA and solvent were removed in vacuo at 35°C to give the ammonium salt (**4**–**6**). The product was dried under reduced pressure (0.1 mm Hg) for 24 h.

4.1.1. Octaammonium G2-dendrimer salt (4). Starting from the Boc-protected G2-dendrimer **1** (0.40 g, 0.16 mmol), the final product **4** was obtained as a white glass (0.38 g, 91%), mp>250°C. ^{1}H NMR: 2.63 (t, 8H, *J*=7 Hz, interior CH_2CH_2CO), 2.71 (t, 16H, *J*=7 Hz, surface CH_2CH_2CO), 3.09 (q, 16H, *J*=7 Hz, surface CH_2CH_2CO), 3.38 (br s, 4H, core CH_2), 3.50–3.60 (m, 8H, interior CH_2CH_2CO), 7.70 (s, 8H, surface ArH), 7.73 (s, 4H, interior ArH), 7.85 (br s, 24H, $^{+}NH_3$), 8.05–8.16 (m, 6H, surface and interior ArH), 8.49 (t, 4H, *J*=7 Hz, ArCONH), 8.56 (t, 2H, core ArCONH), 10.18 (s, 4H, interior CONHAr), 10.33 (s, 8H, surface CONHAr); ^{13}C NMR: 33.4, 35.1, 36.1, 36.3, 112.9, 113.6, 116.9 (q, $^{1}J_{CF}$= 300 Hz), 136.1, 139.3, 139.5, 158.5 (q, $^{2}J_{CF}$=40 Hz), 166.7, 166.9, 168.7, 169.8. MS (L-SIMS): *m*/*z* 1717.8 ($M-8CF_3CO_2H+H^{+}$).

4.1.2. Hexadecaammonium G-3 dendrimer salt (5). Starting from the Boc-protected G3-dendrimer **2** (0.40 g, 0.07 mmol), the target compound **5** was obtained as a white glassy solid (0.36 g, 90%). ^{1}H NMR: 2.63 (t, 24H, *J*=7 Hz, interior CH_2CH_2CO), 2.71 (t, 32H, *J*=7 Hz, surface CH_2CH_2CO), 3.09 (q, 32H, *J*=7 Hz, surface CH_2CH_2CO), 3.38 (br s, 4H, core CH_2), 3.50–3.60 (m, 24H, interior CH_2CH_2CO), 7.70 (s, 16H, surface ArH), 7.72 (s, 12H, interior ArH), 7.86 (br s, 48H, $^{+}NH_3$), 8.00–8.14 (m, 14H, surface and interior ArH), 8.40–8.60 (m, 14H, ArCONH), 10.18 (s, 12H, interior CONHAr), 10.33 (s, 16H, surface CONHAr); ^{13}C NMR: 33.5, 35.1, 36.1, 36.3, 112.9, 113.6, 116.1 (q, $^{1}J_{CF}$=300 Hz), 136.1, 139.3, 139.5, 158.6 (q, $^{2}J_{CF}$=40 Hz), 166.7, 166.9, 168.7, 169.8. MS (MALDI-TOF): *m*/*z* 3925.7 ($M-16CF_3CO_2H+H^{+}$).

4.1.3. G-4 Dendrimer salt (6). Starting from the Boc-protected G4-dendrimer **3** (0.40 g, 0.03 mmol), the target compound **6** was obtained as a white glassy solid (0.32 g, 90%). ^{1}H NMR: 2.62 (t, 56H, *J*=7 Hz, interior CH_2CH_2CO), 2.71 (t, 64H, *J*=7 Hz, surface CH_2CH_2CO), 3.08 (q, 64H, *J*=7 Hz, surface CH_2CH_2CO), 3.40–3.60 (m, 60H, interior CH_2CH_2CO and core CH_2), 7.70 (s, 32H, surface ArH), 7.72 (s, 28H, interior ArH), 7.88 (br s, 96H, $^{+}NH_3$), 8.00–8.14 (m, 30H, surface and interior ArH), 8.40–8.60 (m, 30H, ArCONH), 10.19 (s, 28H, interior CONHAr), 10.34 (s, 32H, surface CONHAr); ^{13}C NMR: 33.5, 35.1, 36.2, 36.3, 113.0, 113.6, 116.7 (q, $^{1}J_{CF}$=300 Hz), 136.1, 139.3, 139.5, 158.7 (q, $^{2}J_{CF}$=40 Hz), 166.7, 166.9, 168.7, 169.8. MS (MALDI-TOF): *m*/*z* 8345 ($M-32CF_3CO_2H+H^{+}$).

4.2. Polyacrylamide gel electrophoresis

4.2.1. Instrumentation. Gel electrophoresis experiments

were conducted on a Bio-Rad Mini-Protean II electrophoresis unit.

4.2.2. Preparation of acidic polyacrylamide gel system. Our electrophoretic system was a modified version of the acidic gel system developed by Bonner.[14] The gel was divided into stacking and resolving portions and was prepared by photocatalytic polymerization of an appropriate amount of acrylamide and bisacrylamide initiated by daylight exposure. The stacking gel was prepared by the polymerization of a solution of acrylamide (60% w/v, 0.70 mL) and bisacrylamide (2.5% w/v, 0.40 mL) in a mixture of glacial acetic acid (0.35 mL), aqueous ammonia solution (35%, 0.02 mL), *N*,*N*-tetramethylethylene diamine (TMEDA) (0.03 mL) and riboflavin (0.004% solution, 1.0 mL) and then diluted with water to a final volume of 4.97 mL. The 40% resolving gel was prepared from the polymerization of a solution of acrylamide (60% w/w, 4.00 mL) and bisacrylamide (2.5% w/w, 0.48 mL) in a mixture of glacial acetic acid (0.35 mL), aqueous ammonia solution (35%, 0.02 mL), TMEDA (0.03 mL) and riboflavin (0.004% solution, 0.20 mL) and then diluted with water to a final volume of 5.73 mL. The dimensions of the resolving and stacking gels were 8.5×5.5×0.075 and 8.5×1.5×0.0075 cm^3, respectively. By varying the amount of acrylamide monomer, 42, 44 and 46% polyacrylamide gels were also prepared.

4.2.3. Sample analysis. The deprotected polycationic dendrimers **4**–**6**, lysozyme (14 kD) and aprotinin (7 kD) standards (each at a concentration of 10 mg/mL) were dissolved in the sample buffer that was prepared by mixing glacial acetic acid (0.6 mL), aqueous ammonia solution (35%, 0.03 mL) and urea (3.6 g) and then diluted to a final volume of 10 mL with distilled water. For each sample, an 1.5–2.5 μL aliquot was transferred into a sample well with an automatic pipette. The ionic buffer was prepared by dissolving glacial acetic acid (60 mL) and glycine (7.5 g) in water to a final volume of 1000 mL. The samples were then subjected to electrophoretic analysis at 4 mA constant current for 12 h. After the run, the stacking gel was peeled off and the separated samples in the resolving gel were visualized by staining with a Coomassie Blue staining solution (0.11% Coomassie Blue R-250 in ethanol/formaldehyde/water=4/3/1) for 12 h, followed by 12 h background destaining with a destaining solution (ethanol/formaldehyde/water=4/3/1). The migrating distance *d* in cm was measured from the cutting edge of the resolving gel to the middle of the stained sample zone. In addition to using a 40% acrylamide gel, acrylamide gels of 42, 44 and 46% were also employed in this study.

Acknowledgements

We thank the Research Grants Council of the Hong Kong SAR (CUHK4273/00P), the Chinese University of Hong Kong (Lee Hysan and Endowment Research Funds), and United College (Student Campus Work Schemes) for their financial support.

References

1. (a) Tomalia, D. A.; Naylor, A. M.; Goddard, W. A., III *Angew. Chem. Int. Ed.* **1990**, *29*, 138–175. (b) Newkome, G. R.; Moorefield, C. N.; Vögtle, F. *Dendrimers and Dendrons. Concepts, Syntheses, Applications*; Wiley-VCH: Weinheim, 2001.
2. (a) Chow, H.-F.; Mong, T. K.-K.; Nongrum, M. F.; Wan, C.-W. *Tetrahedron* **1998**, *54*, 8543–8660. (b) Bosman, A. W.; Janssen, H. M.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1665–1688. (c) Fischer, M.; Vögtle, F. *Angew. Chem. Int. Ed.* **1999**, *38*, 884–905.
3. (a) Smith, D. K.; Diederich, F. *Chem. Eur. J.* **1998**, *4*, 1353–1361. (b) Hecht, S.; Fréchet, J. M. J. *Angew. Chem. Int. Ed.* **2001**, *40*, 74–91.
4. (a) Posnett, D. N.; McGrath, H.; Tam, J. P. *J. Biol. Chem.* **1988**, *263*, 1719–1725. (b) Chapman, T. M.; Hillyer, G. L.; Mahan, E. J.; Shaffer, K. A. *J. Am. Chem. Soc.* **1994**, *116*, 11195–11196. (c) Mulders, S. J. E.; Brouwer, A. J.; Liskamp, R. M. J. *Tetrahedron Lett.* **1997**, *38*, 3085–3088. (d) Prévôté, D.; Le Roy-Gourvennec, S.; Caminade, A.-M.; Masson, S.; Majoral, J.-P. *Synthesis* **1997**, 1199–1207. (e) Kayser, B.; Altman, J.; Beck, W. *Chem. Eur. J.* **1999**, *5*, 754–758. (f) Kim, Y.; Zeng, F.; Zimmerman, S. C. *Chem. Eur. J.* **1999**, *5*, 2133–2138.
5. Mong, T. K.-K.; Niu, A.; Chow, H.-F.; Wu, C.; Li, L.; Chen, R. *Chem. Eur. J.* **2001**, *7*, 686–699.
6. Engel, R. In *Ionic Dendrimers and Related Materials. Advances in Dendritic Macromolecules*; Newkome, G. R., Ed.; JAI: Stamford, 1995; Vol. 2, pp 73–99.
7. (a) Newkome, G. R.; Moorefield, C. N.; Baker, G. R.; Saunders, M. J.; Grossman, S. H. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1178–1180. (b) Hawker, C. J.; Wooley, K. L.; Fréchet, J. M. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1287–1297.
8. Sakai, N.; Matile, S. *Tetrahedron Lett.* **1997**, *38*, 2613–2616.
9. Cherestes, A.; Engel, R. *Polymer* **1994**, *35*, 3343–3344.
10. (a) Koper, G. J. M.; van Genderen, M. H. P.; Elissen-Román, C.; Baars, M. W. P. L.; Meijer, E. W.; Borkovec, M. *J. Am. Chem. Soc.* **1997**, *119*, 6512–6521. (b) Kabanov, V. A.; Zezin, A. B.; Rogacheva, V. B.; Gulyaeva, Z. G.; Zansochova, M. F.; Joosten, J. G. H.; Brackman, J. *Macromolecules* **1998**, *31*, 5142–5144.
11. (a) Brothers, H. M., II; Piehler, L. T.; Tomalia, D. A. *J. Chromatogr. A* **1998**, *814*, 233–246. (b) Ebber, A.; Vaher, M.; Peterson, J.; Lopp, M. J. *J. Chromatogr. A* **2002**, *949*, 351–358.
12. Stöckigt, D.; Lohmer, G.; Belder, D. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 521–526.
13. Bollag, D. M.; Rozycki, M. D.; Edelstein, S. J. *Protein Methods*; 2nd ed., Wiley-Liss: New York, 1996; pp 169–170.
14. Bonner, W. M.; West, M. H. P.; Wu, R. S. *Electrophoresis* **1984**, *5*, 133–138.