# A Surface-Modified Dendrimer Set for Potential Application as Drug Delivery Vehicles: Synthesis, In Vitro Toxicity, and Intracellular Localization

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**Abstract:** The synthesis, cytotoxicity, and behavior in cell culture of a new set of first- (G1) and second-generation (G2) dendrimers is reported. The surface functionality of these dendrimers has been varied to see whether structure/toxicity relations can be observed. The outermost functional groups are amines that are decorated either with protons, *tert*-butoxycarbonyl (Boc) or benzyloxycarbonyl (Cbz) protecting groups, Boc-protected or unprotected natural amino acid residues, ethylenediamine ligands, and/or dansyl fluorescence labels. The cytotoxicity was determined in vitro in concentration-dependent assays using the human MCF-7 breast cancer cell line. Cellular uptake and intracellular distribution was monitored by confocal fluorescence microscopy after internalization of the dansyl-labeled dendrimers by HeLa cells.

**Keywords:** cellular uptake · cytotoxicity · dendrimers · drug delivery · synthesis

## Introduction

Dendrimers have attracted considerable attention in the last two decades.[1,2] They combine a monodisperse nanoscale geometry with high endgroup density at their "surface," through which properties can be widely engineered.[3–5] Larger dendrimers contain dynamic inner cavities in which guest molecules can be trapped and released.[6–13] Some representatives possess charges in the periphery and, thus, are soluble in polar and protic solvents, sometimes even in water.[3,4,14–16] In the last few years, water-soluble dendrimers have gained more and more importance in biochemical and biomedical applications.[17,18] Especially the polyamidoamine (PAMAM) family is now being used extensively as transfection agents for DNA transfer into living cells,[19–23] while others are used as contrast agents for magnetic resonance imaging (MRI),[24,25] and in boron neutron capture therapy (BNCT) for cancer treatment.[26–29] Because of such applications these and related behavior of dendrimers in living organisms, as well as in cell culture, is being researched but little is known yet about their way into living cells, about their intracellular distribution, and aspects such as immunogenic response and cell toxicity.

In 1996 Roberts et al. investigated the biological behavior of PAMAM dendrimers of the third, fifth, and seventh generation in vitro and in vivo.[30] The authors studied the behavior of dendrimers in V79 cells and in Swiss–Webster mice for a number of biological properties, including in vitro and in vivo toxicity, immunogenicity, and biodistribution. No evidence for immunogenicity was found, but like Duncan et al.[31] a few years later, they found a concentration- and generation-dependent toxicity of the dendrimers. Especially the seventh-generation dendrimers exhibited a considerably larger toxicity than those of the fifth or third generation. The differences between the lower generations were not very pronounced, though an increase in toxicity with increasing generation could be concluded. In mice, the G3 dendrimer showed the highest accumulation in kidney tissue, while G5 and G7 preferentially localized in the pancreas. In 2000 Duncan and co-workers performed a broader, more systematic investigation on five different dendrimer types in vitro with three different cell lines (B16F19, CCRF, and HepG2), in vivo with Wistar rats, and with freshly pre-

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pared rat erythrocytes for haemolysis assays.[31] Dendrimers tested were the polycationic PAMAMs and two kinds of poly(propyleneimine) dendrimers with either a diaminobutane (DAB) or a diaminoethane (DAE) core, respectively. They also tested the polyanionic, carboxylate-terminated "half-generation" PAMAMs and DABs, as well as uncharged oligoethyleneoxide-terminated carbosilane (CSi-PEO) dendrimers. All investigations were carried out for various generations and concentrations. Furthermore, preliminary biodistribution studies with $^{125}$I-labeled PAMAM dendrimers in vivo were performed. The authors found that all cationic dendrimers were cytotoxic with the degree of toxicity depending on dendrimer-type, cell-type, and generation. They also caused haemolysis and changes in erythrocyte morphology. In contrast, dendrimers with carboxylate surfaces were neither haemolytic nor cytotoxic.[31] These examples show that polycationic species appear to be more problematic than polyanionic ones. Despite such advances, one still has not reached a substantial understanding of the inherent structure/toxicity relationships of dendrimers. It may well be that each dendrimer type and each generation within the types has its own behavior in biological systems and mechanism of toxicity. It is, thus, obviously necessary to more broadly explore the influence of different surface functionalizations of otherwise identical dendrimers on their in vitro and in vivo behavior. Such investigations are a prerequisite for considering these branched macromolecules as drug delivery systems, for example, as novel carriers for anticancer drugs.

In this context it would be desirable to learn how dendrimers distribute in cells. Such studies have already been performed, mainly with PAMAM dendrimers aimed for use as transfection or drug-delivery agents. Juliano and co-workers visualized the subcellular distribution of fluorescence-labeled dendrimer–oligonucleotide complexes by two-color fluorescence microscopy.[32] The authors found that the dendrimer–oligonucleotide complexes remained associated during the process of uptake into vesicular compartments and entry into the nucleus. A large majority of cells showed strong fluorescence intensity in the nuclei after one hour of incubation with dendrimers and oligonucleotides. Also cells treated with Oregon green 488-conjugated dendrimers alone showed nuclear fluorescence. It remained unclear in this study whether nuclear localization of the dendrimer–oligonucleotide complexes was just an intermediate stage of intracellular trafficking, or rather the final stage of intracellular distribution. The authors conclude from their nuclear fractionations that a substantial amount of dendrimer material was also located at other sites of the cell, presumably at the plasma membrane and at different intracellular membranes. Also, Baker et al. described the targeted uptake of folic acid-conjugated dendrimers carrying a fluorescein isothiocyanate (FITC) fluorescence tag and their intracellular recognition using confocal fluorescence microscopy.[33] After 24 h of incubation, a 'clumpy' cytoplasmatic staining of the cells could be observed, but the exact place of intracellular localization remained unclear. These investigations clearly demonstrate the necessity to get a better insight into the structure/action relationship of dendrimers in biological systems. More than ever it is now important to unravel the import pathways of the dendrimer into living cells to get an impression on how and where they act in living organisms.

Herein, we describe the synthesis of new sets of first- and second-generation (G1 and G2) polyamidoamine (yet not PAMAM) dendrimers, which differ only in surface motifs. As such were used a) quaternized amines (set A dendrimers), b) the natural, proteinogenic amino acids L-phenylalanine, L-methionine, and L-aspartic acid (set B dendrimers), c) diaminopropionic acid, a bidentate ligand, for example, for $Pt^{II}$-binding (set C dendrimers), as well as d) the 5-dimethylaminonaphthalene-1-sulfonyl (dansyl) motif[34] for partial or complete fluorescence labeling of the dendrimers (set D dendrimers). Some of the modified dendrimers carry chelating ligands for $Pt^{II}$ (L-methionine, diaminopropionic acid) and could thus be potentially used for binding cisplatin-like complexes.[35,36] As a fluorescence tag the dansyl group was chosen because of its high fluorescence intensity and polar nature, which should not detrimentally interfere with the dendrimers' required water solubility. Dendrimer sets A–C were used to start a systematic exploration of the influence of the surface functionalization on the dendrimer toxicity in cell culture, and first results of this endeavor will be reported here. Set D was employed in studies on cell uptake and intracellular distribution using confocal fluorescence microscopy. Initial conclusive findings will also be reported in this study.

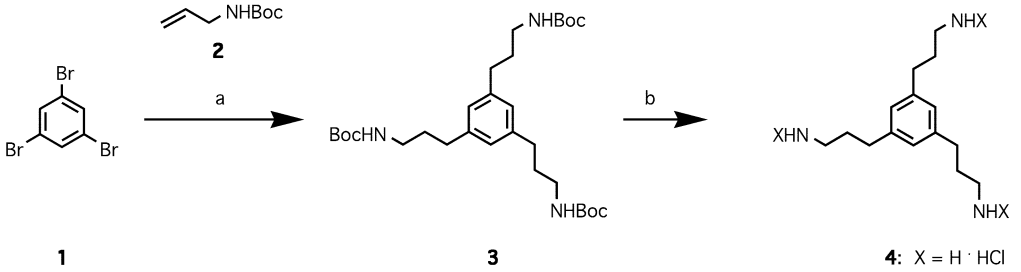
## Results and Discussion

**Synthesis and purification of building blocks and dendrimers**: Schemes 1–7 contain all syntheses performed. Scheme 1 describes the steps leading to core **4**, which was used as the center piece for all dendrimers. Scheme 2 shows the steps leading to *tert*-butyloxycarbonyl (Boc)-protected G1 (**7**) and G2 dendrimers (**9**), which together with core **4**, represent the parent set of all dendrimers reported (set A). Scheme 3 and Scheme 4 depict the sequences to the protected [**12a–c** (G0) and **20a–c** (G1)] and deprotected amino acid terminated dendrimers [**13a–c** (G0) and **21a–c** (G1)] (set B), and Scheme 3 and Scheme 5 the ones to the protected [**15** (G0) and **24** (G1)] and deprotected diaminopropionic acid terminated dendrimers [**16** (G0) and **25** (G1)] (set C) respectively. Finally, in Scheme 6 and Scheme 7 one can see the syntheses of the completely (**26**, **27**, **28**) and partially dansylated dendrimers (**33**–**36**) (set D). The two main reactions used are Suzuki–Miyaura cross-coupling[37–40] to attach the branches to the branching unit (phenyl) and standard peptide coupling reactions[41–43] for dendron and dendrimer assembly. Whenever possible, terminal amines were Boc protected during the conversions, as this protecting group is stable under the applied reaction conditions.[44–46] Purification of the protected dendrimers in most cases could easily be performed by standard column chromatography.

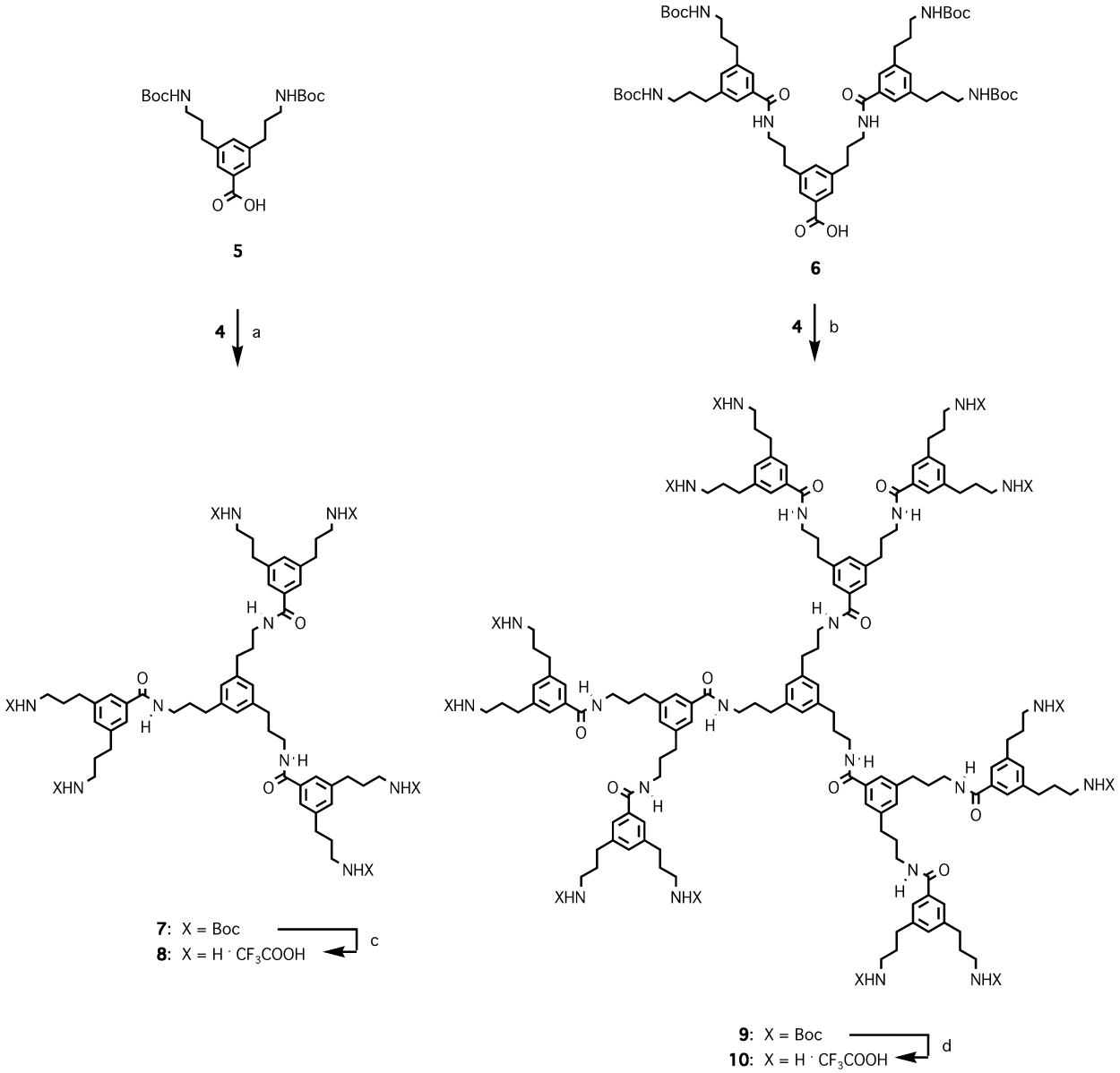
Although all synthetic steps are conventional, relatively easy to perform, and gave products in good yield and high purity, a few comments on synthetic issues seem nevertheless appropriate. For the triamine core molecule **4** 1,3,5-tri-

bromobenzene (**1**) and *tert*-butyl allylcarbamate **2** were used as starting components (Scheme 1). Their cross-coupling gave the Boc-protected core molecule **3** in 94% yield, which indicates a very high conversion per coupling step. Pure product **4** was obtained on a 10 g scale and precipitated from the reaction mixture as trishydrochloride. The known dendrons **5** and **6**[47] were attached to **4** with the EDC/HOBt strategy[43] in good overall yields of 75–78% to the corresponding set A G1 and G2 dendrimers **7** and **9**, respectively (Scheme 2).

The dendrimers of set B were obtained by starting from the Boc- and, in case of L-aspartic acid, Boc- and *t*Bu-pro-




Scheme 1. Synthesis of the triamine core molecule **4**. Reagents and conditions: a) 1. **2**, 9-BBN, toluene, room temperature, 12 h, 2. 1M KOH, **1**, $[Pd(PPh_3)_4]$, 60°C, 24 h (94%); b) **3**, aqueous HCl, THF, room temperature, 18 h (96%).

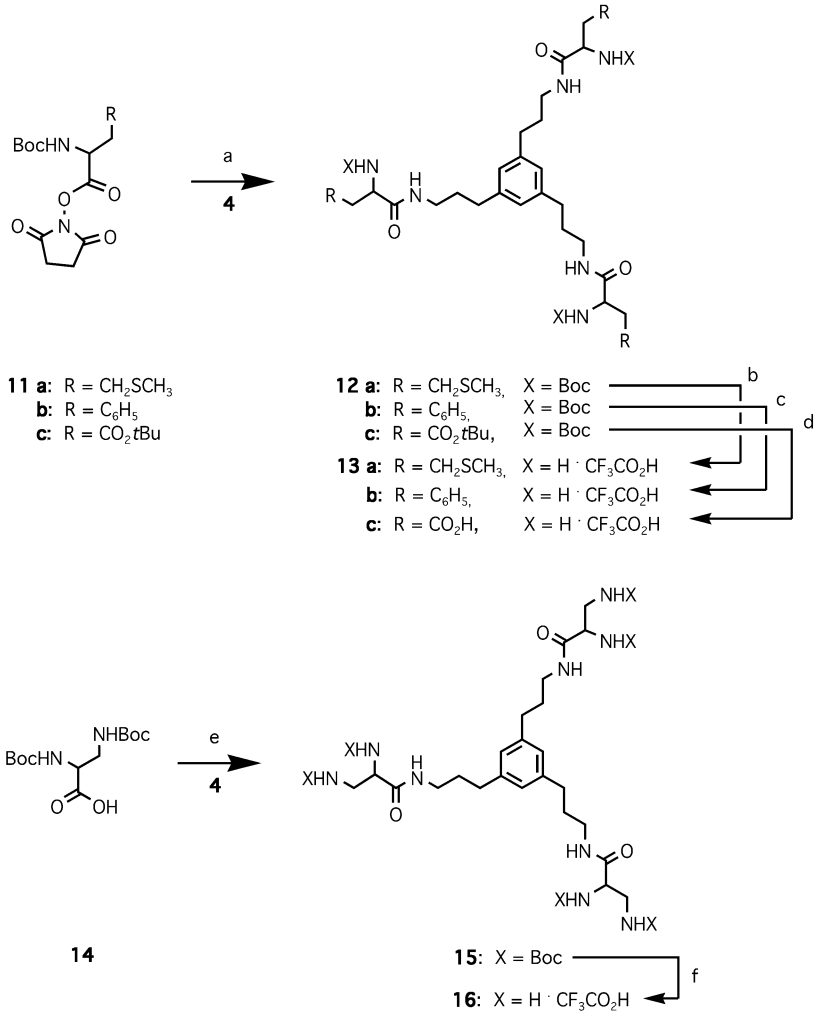



Scheme 2. Synthesis of the basic dendrimer set A. Reagents and conditions: a) 1. $CH_2Cl_2$, EDC, HOBt, −20°C/1 h, room temperature/2 h, 2) **4**, DIPEA, −30°C/1 h, room temperature 16 h (78%); b) 1. $CH_2Cl_2$, EDC, HOBt, −20°C/1 h, room temperature/2 h, 2. **4**, DIPEA, −30°C/1 h, room temperature 24 h (75%); c) $CH_2Cl_2$, $CF_3CO_2H$, room temperature, 1 h (98%); d) $CH_2Cl_2$, $CF_3CO_2H$, room temperature, 2 h (99%).

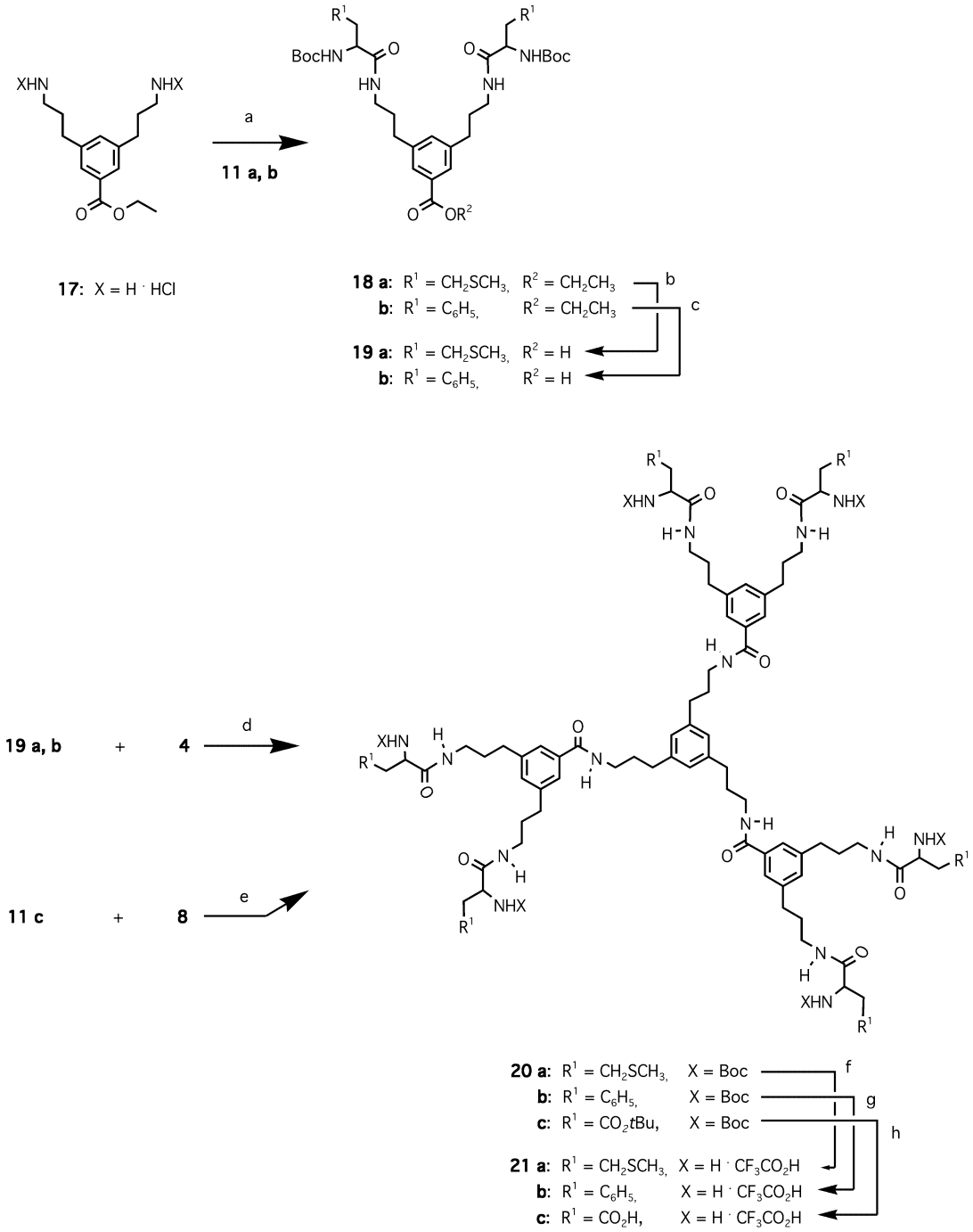
tected hydroxysuccinimide esters of L-phenylalanine, L-methionine, and L-aspartic acid. Isolated yields for the three G0 dendrimers **12a–c** were 80–89% (Scheme 3). The G1 dendrons **18a,b** (Scheme 4) were used to convergently access the corresponding L-methionine and L-phenylalanine G1 dendrimers **20a** and **20b**, respectively. In contrast to this mode of synthesis, the L-aspartic acid G1 dendrimer **20c** was built divergently by utilizing the Boc- and *t*Bu-protected L-aspartic acid hydroxysuccinimide ester **11c** and deprotected parent G1 dendrimer **8.** The reason for this change in strategy was difficulties in saponifying the G1 dendron of structure **18** with $R^1 = CO_2tBu$ and $R^2 = C_2H_5$ (not shown) at $R^2$ without cleaving the $R^1$ ester (Scheme 4). All reactions involving the oxidatively sensitive L-methionine were performed under nitrogen. The deprotections were carried out in the presence of the "cleavage cocktail" of ethanedithiol (EDT), thioanisole, and triisopropylsilane (TIPS) as nucleophilic scavengers to avoid undesired alkylation of the thioether. The MALDI-TOF mass spectra of the G1 dendrimer **20a** nevertheless showed signals of low intensity due to the incorporation of up to three oxygen atoms.

The non-proteinogenic D/L-diaminopropionic acid was used for the synthesis of dendrimers **15** (Scheme 3) and **24** (Scheme 5) of set C. They ought to exhibit even better chelating properties than their methionine analogs of set B. The synthetic route starts with the commercially available D/L-diaminopropionic acid, which was Boc-protected at both amine functionalities to **14** in a slightly modified version of the procedure described by Sergheraert et al.,[36] and no attempt was done to separate the two enantiomers. Compound **14** was activated with *O*-(1*H*-benzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU)[48] and reaction with core **4** gave the Boc-protected G0 (**15**) and G1 dendrimers (**24**) in 80 and 57% isolated yield, respectively (Scheme 3 and Scheme 5). The Boc-protected intermediates possessed poor solubility in dichloromethane, probably due to intramolecular hydrogen bonding. In all cases polar solvents such as DMF or methanol had to be added to render the diaminopropionic acid modified compounds completely soluble.




Scheme 3. Synthesis of the amino acid-terminated G0 dendrimers of set B and C. Reagents and conditions: a) **11a** + **4**, $CH_2Cl_2$, DIPEA, −10°C/1 h, room temperature, 18 h (85%); **11b** + **4**, $CH_2Cl_2$/DMF, DIPEA, 0°C/1 h, room temperature, 18 h (80%); **11c** + **4**, $CH_2Cl_2$, DIPEA, −10°C/1 h, room temperature, 18 h (89%); b) $CH_2Cl_2/CF_3CO_2H$/EDT/thioanisole/methanol/TIPS, r.t., 1 h (99%); c) $CH_2Cl_2$/methanol, $CF_3CO_2H$, room temperature, 1 h (99%); d) $CH_2Cl_2/CF_3CO_2H$, room temperature, 3 h (98%); e) 1. $CH_2Cl_2$/DMF, TBTU, DIPEA, −20°C/15 min, room temperature/30 min), 2. **4**, DIPEA, −20°C/30 min, room temperature/18 h (80%); f) $CH_2Cl_2$, $CF_3CO_2H$, room temperature, 1 h (100%).

The highly fluorescent dansyl group was chosen for the fluorescence-tagged dendrimers of set D.[34] To achieve the fully labeled series, the parent dendrimers **4**, **8**, and **10** were treated with an 1.5–3-fold excess of dansyl chloride per amine functionality (Scheme 6). The respective highly fluorescent dendrimers **26**, **27**, and **28** were isolated in yields of 65–92%. In contrast to the procedures recently described by Vögtle et al.[34g,h,i] for dansylation of poly(propylene amine) and polylysine dendrimers, the reactions proceeded fast and were in most cases already finished after one hour at room temperature (TLC monitoring). This finding is consistent with the results of Bartzatt, who described dansylation of primary and secondary amines in aqueous $Na_2CO_3$ buffer to be complete within one hour.[34d] The degree of dansylation was quantified by NMR spectroscopy and found to be beyond 95%. In accordance with this the MALDI-TOF mass spectra showed clean

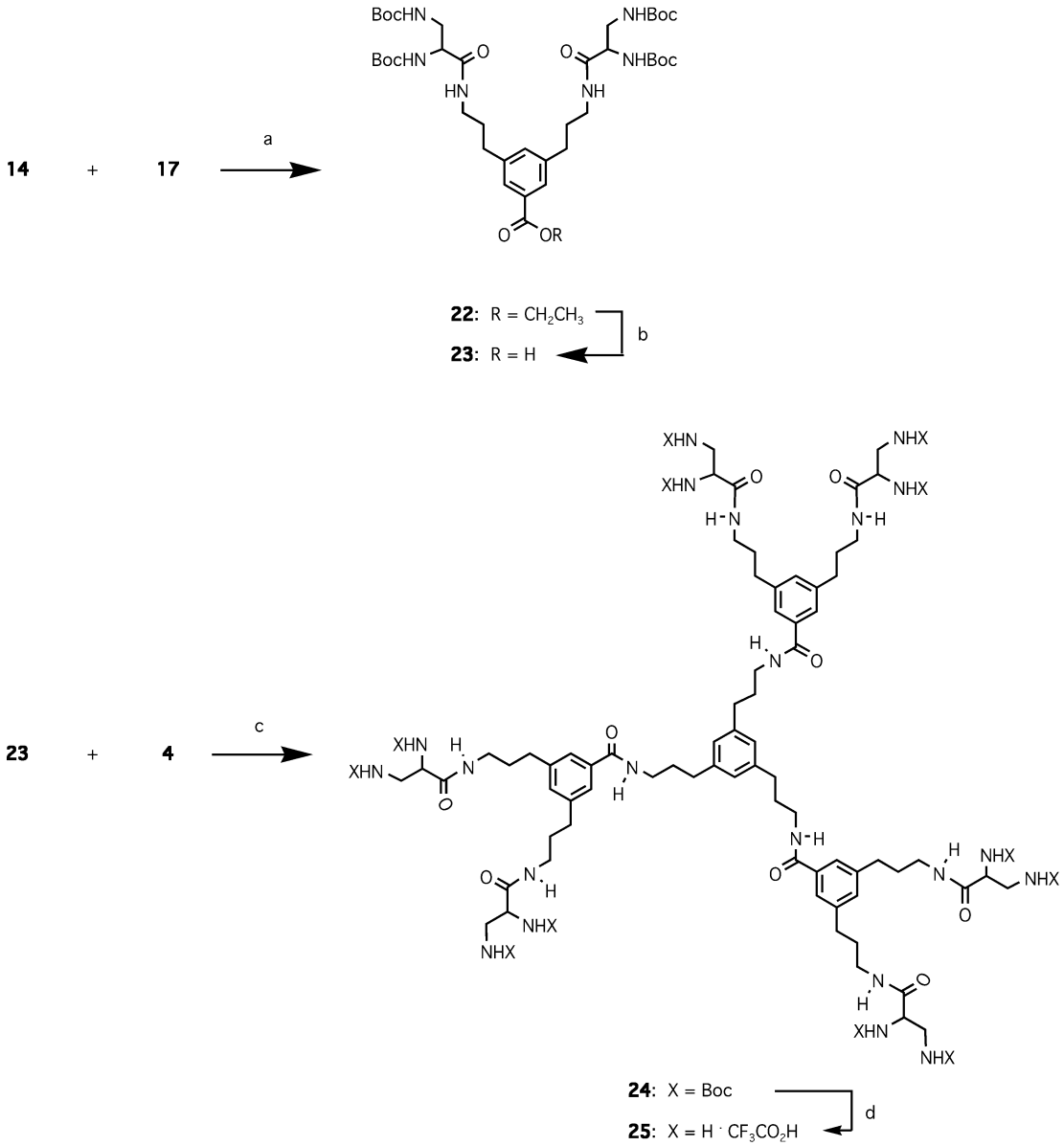



Scheme 4. Synthesis of the amino acid terminated G1 dendrimers of set B. Reagents and conditions: a) **11a + 17**, $CH_2Cl_2$, DIPEA, −10 °C/1 h, room temperature/18 h (79 %); **11b + 17**, $CH_2Cl_2$/DMF, DIPEA, room temperature/18 h (82 %); b) 1 M KOH, methanol/water/THF, 40 °C, 12 h (80 %); c) 1 M KOH, methanol/water/THF, 50 °C, 12 h (92 %); d) 1. **19a**, [**19b**], $CH_2Cl_2$/DMF, EDC, HOBt, −20 °C [5 °C]/1 h, room temperature 2 h , 2. **4**, DIPEA, −30 °C [0 °C]/1 h, room temperature 18 h (**20a**: 54 %, **20b**: 48 %); e) $CH_2Cl_2$/DMF, DIPEA, −10 °C/1 h, room temperature 18 h (71 %); f) $CH_2Cl_2$/$CF_3CO_2H$/EDT/thioanisole/methanol/TIPS, room temperature, 1 h (97 %); g) $CH_2Cl_2$, $CF_3CO_2H$, room temperature, 1 h (99 %); h) $CH_2Cl_2$, $CF_3CO_2H$, room temperature, 3 h (100 %).

molecular ion peaks. Dendrimers **26**–**28** were well soluble in dichloromethane/methanol mixtures, but exhibited poor solubility in water. Compounds **27** and **28** tended to aggregate in water and in cell culture media (with **28** forming the larger aggregates under comparable conditions), whereas for the small G0 dendrimer **26** no aggregation was observed. Despite these properties dendrimers **26** and **27** showed cell uptake (see below).

In addition, dendrimers **33**–**36** carrying a second functional group besides the fluorescence label were synthesized (Scheme 7). The synthetic strategy applied differs from the direct sulfonamide derivatization used by Vögtle et al. for their labeling of light-harvesting dendrimers with different chromophoric groups.[34c] The orthogonally protected branching unit **30** served as the key compound for the synthetic route leading to dendrimers **33**–**36**. The corresponding

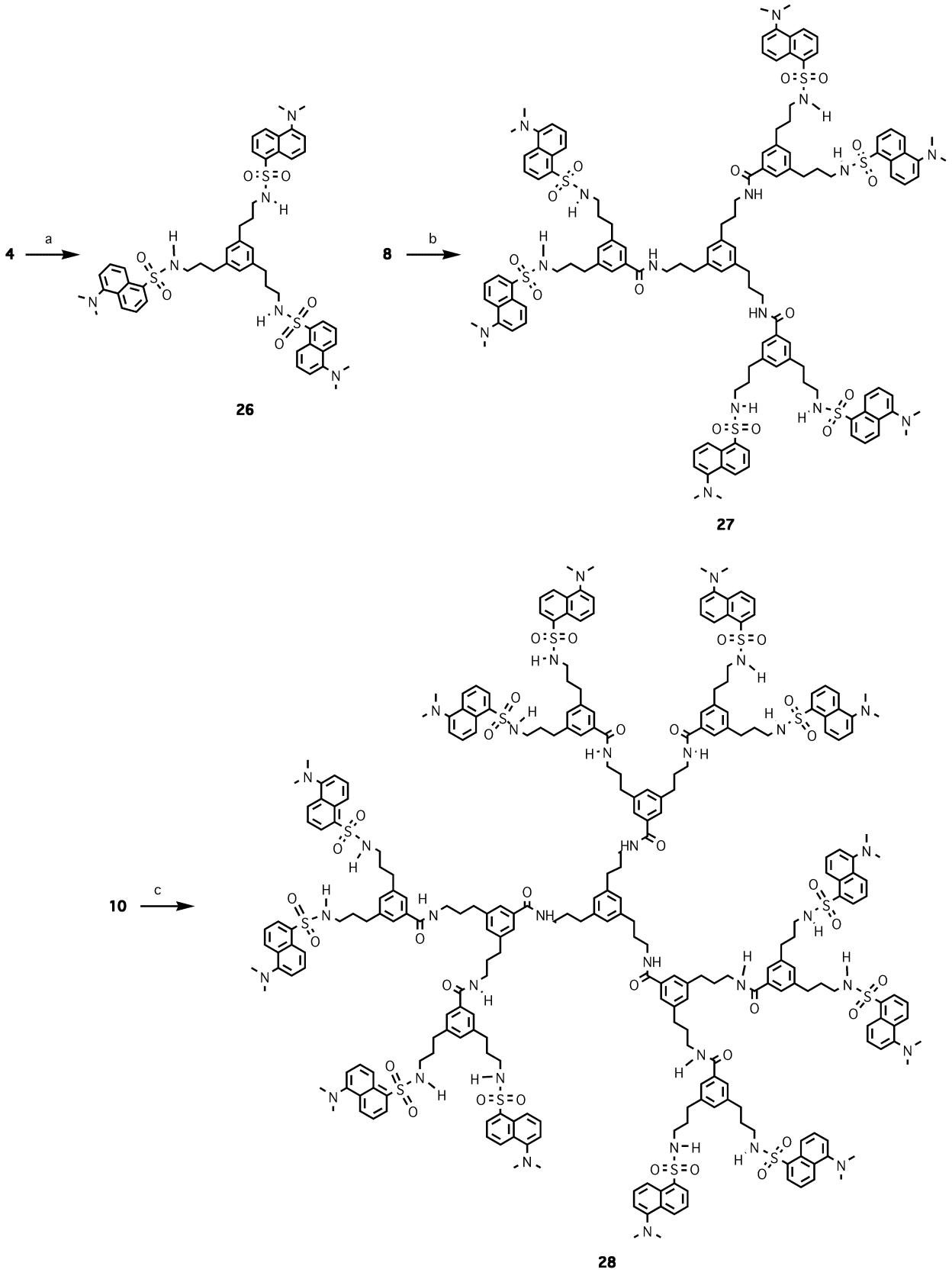



Scheme 5. Synthesis of the diaminopropionic acid terminated G1 dendrimer of set C. Reagents and conditions: a) 1. **14**, $CH_2Cl_2$/DMF, TBTU, DIPEA, −20 °C/30 min, room temperature/15 min, 2. **17**, DIPEA, −20 °C/30 min, room temperature/12 h (90 %); b) 1 M KOH, methanol/THF, 50 °C, 12 h (87 %); c) 1. **23**, $CH_2Cl_2$/DMF, TBTU, DIPEA, −10 °C/15 min, room temperature/30 min, 2. **4**, DIPEA, 0 °C/15 min, room temperature/18 h (57 %); d) $CH_2Cl_2$, $CF_3COOH$, room temperature, 1 h (99 %).

G1-dendrimer **31** was isolated in 63 % yield. Its partial deprotection to **32** and subsequent dansylation gave the highly fluorescent dendrimer **33** in 99 % yield.

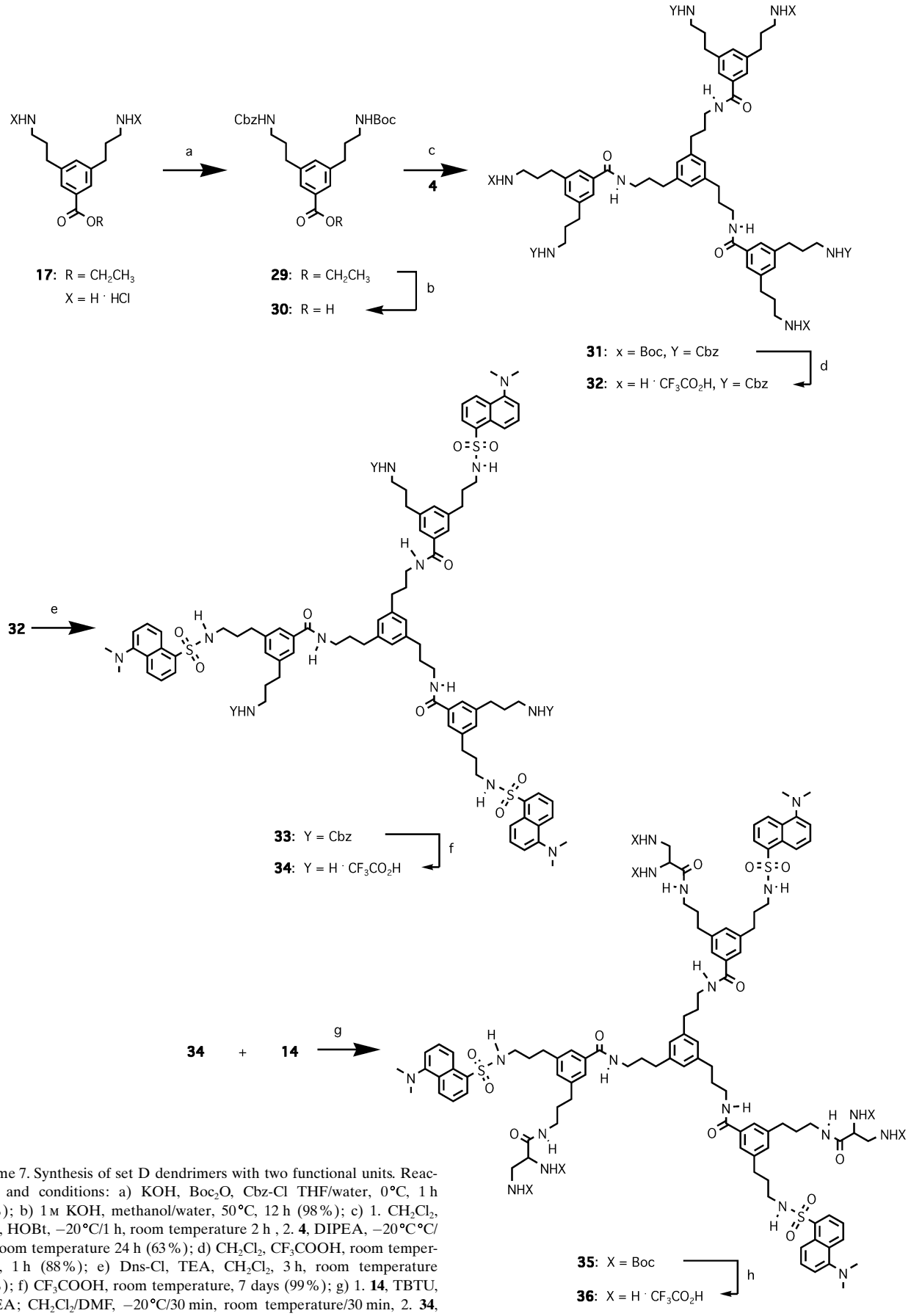
Deprotection of its remaining three benzyloxycarbonyl (Cbz) protecting groups proved to be difficult, but could finally be realized by reaction with neat trifluoroacetic acid over a period of seven days. Reaction of the dansylated and deprotected dendrimer **34** with the Boc-protected diaminopropionic acid **14** gave dendrimer **35** in 33 % yield. Its quantitative deprotection finally resulted in dendrimer **36**, which carried both the diaminopropionic acid chelating ligands as well as the dansyl imaging functions.

In all cases, the purity of the dendrimers was checked by analytical RP-HPLC in their protected form and found to be above 98 %. Figure 1 (see p. 1175) shows the elution curves of four typical cases (dendrimers **10**, **20c**, **24**, and **35**). All compounds were characterized by their fully assigned $^1H$ and $^{13}C$ NMR spectra and molecular ion peaks in the mass spectra. The composition of those compounds that did not give correct data from elemental analysis was proven by high-resolution EI, FAB, or isotopically resolved MALDI-TOF mass spectrometry (see Experimental Section). Figure 1 shows the enlargened molecular ion regions of molecular ion peaks of the MALDI-TOF MS spectra of dendrimers **10**, **20c**, **24**, and **35**. The $^1H$ NMR spectra of the deprotected dendrimers **10** and **36** illustrate the achieved purity (Figure 2; see p. 1177). All deprotections were quantified by high-field $^1H$ NMR integration and found to be vir-




Scheme 6. Synthesis of completely dansylated set D dendrimers. Reagents and conditions: a) Dns-Cl, TEA, $CH_2Cl_2$, 1 h, room temperature (92%); b) Dns-Cl, TEA, $CH_2Cl_2$, 6 h, room temperature (74%); c) Dns-Cl, TEA, $CH_2Cl_2$, 12 h, room temperature (65%).




Scheme 7. Synthesis of set D dendrimers with two functional units. Reactions and conditions: a) KOH, $Boc_2O$, Cbz-Cl THF/water, 0°C, 1 h (35%); b) 1 M KOH, methanol/water, 50°C, 12 h (98%); c) 1. $CH_2Cl_2$, EDC, HOBt, −20°C/1 h, room temperature 2 h , 2. **4**, DIPEA, −20°C°C/1 h, room temperature 24 h (63%); d) $CH_2Cl_2$, $CF_3COOH$, room temperature, 1 h (88%); e) Dns-Cl, TEA, $CH_2Cl_2$, 3 h, room temperature (99%); f) $CF_3COOH$, room temperature, 7 days (99%); g) 1. **14**, TBTU, DIPEA; $CH_2Cl_2$/DMF, −20°C/30 min, room temperature/30 min, 2. **34**, DIPEA, −20°C/1 h, room temperature/24 h (33%); h) $CH_2Cl_2$, $CF_3COOH$, room temperature, 1 h (97%).

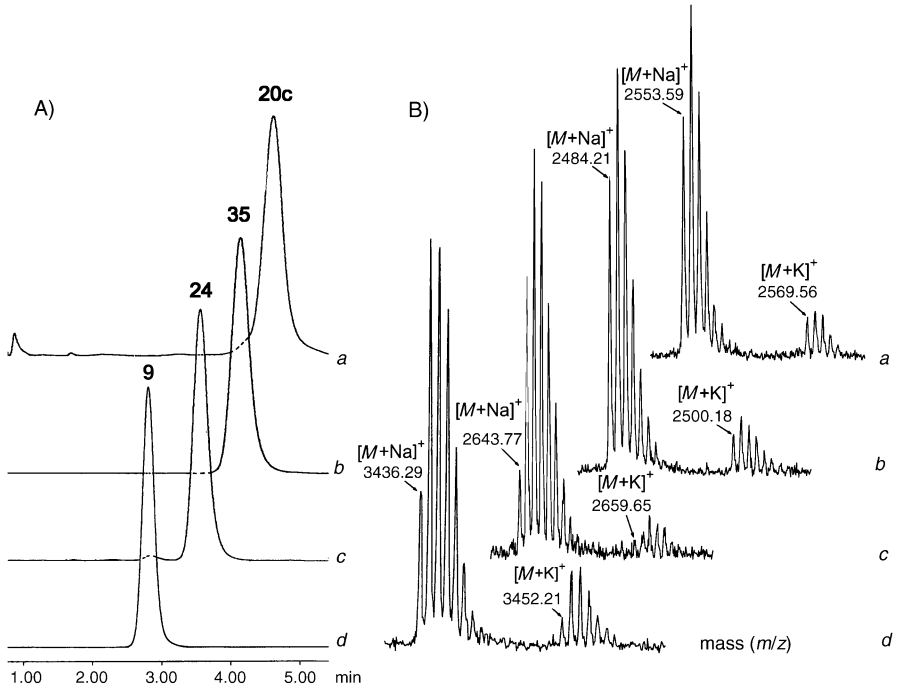



Figure 1. A) Analytical HPLC traces of the Boc-protected dendrimers **9**, **20c**, **24**, and **35**. Purity of the compounds was determined by UV detection (254 nm) and subsequent integration. Aa) dendrimer **20c**; eluent: methanol/$H_2O$ (9:1/v:v), flow rate: 1 mL min$^{-1}$; purity: 97.2% (aggregate: 2.6%). Substance eluting after 0.89 min (*) (2.6%) appeared within the void volume of the used column (for details see Experimental Section); it is consequently likely to be an artefact or an aggregate of high molar mass. Ab) dendrimer **35**; eluent: methanol/$H_2O$ (9:1/v:v), flow rate: 1 mL min$^{-1}$; purity: 100%. (Ac) dendrimer **24**; eluent: methanol/$H_2O$ (9:1/v:v), flow rate: 1 mL min$^{-1}$; purity: 98.3%. (Ad) dendrimer **9**; eluent: methanol/$H_2O$ (9:1/v:v), flow rate: 1 mL min$^{-1}$; purity: 100%. B) MALDI-TOF-MS spectra of dendrimers **9**, **20c**, **24**, and **35**; dithranol was used as a matrix (for details see Experimental Section). From the whole spectra only the enlargend parts of the isotopically resolved $[M+K]^+$ and $[M+Na]^+$ signals are depicted. In no case signals of higher mass were observed. Ba) dendrimer **20c**; Bb) dendrimer **35**; Bc) dendrimer **24**; Bd) dendrimer **9**.

tually quantitative with the conversion **33** to **34** being the only exception. Traces of remaining Cbz (500 MHz NMR) were still found even after repeated deprotection attempts.

The deprotected dendrimers of sets A–D were fully soluble in water and in buffered cell culture media as judged by visual inspection, and could immediately been examined in cell culture. The only exception was the mixed dansylated dendrimer **36**, which turned out to be highly water-soluble but precipitated with phosphate in phosphate buffered saline (PBS) buffer at a concentration of 1 μM.

Ammonium salts of most primary amines have p$K_a$ values of 9–10. At pH 7 most of the amines are thus protonated. It is reasonable to assume that most of the amines on the dendrimer basically behave like independent primary amines and, thus, are also protonated, though their degree of protonation was not determined. Standard PAMAMs are only partially protonated at physiological pH due to the surprisingly low p$K_a$ values of their terminal (p$K_a$ = 6.9[49]) and internal amines (p$K_a$ = 3.9[49]).[19] In the following the unprotected dendrimers are therefore somewhat unspecifically referred to as being polycationic and no correlation to total numbers of charges is attempted.

**In vitro cytotoxicity in MCF-7 cell culture**: All dendrimers were examined in MCF-7 cell culture at concentrations of 1–20 μM over a period of nine days (Figures 3–8). The exact conditions are given in the Experimental Section. Despite their charge differences all dendrimers, by visual inspection, gave molecularly dispersed solutions in the cell experiments. The only two exceptions were compounds **28** and **36**, whose behavior in buffered cell media was already described above. The completely dansylated dendrimers **26** and **27** of set D possessed only low solubility in water, but caused no extensive aggregate formation in fetal calf serum (FCS) containing cell culture media. Only the dansylated G2 dendrimer **28** exhibited a strong tendency to aggregate even in cell culture media and was thus not used in the subsequent cell uptake studies. Dendrimers **26**, **27**, and **36** were mixed with cell culture media by low-energy ultrasonification prior to addition to the cultured cells. This always resulted in clear solutions for the cytotoxicity and cell uptake experiments as judged by visual inspection. Only for dendrimers **27** and **36** fluorescence microscopy proved the formation of smaller aggregates. At 37 °C, however, at which all experiments were performed, they never formed aggregates which could have been precipitated by centrifugation. Mainly the same procedure was performed for all Boc-protected dendrimers of sets A–C, except that only two concentrations were tested (5 and 10 μM) because of the expected lower water solubility. It has to be noted, however, that at none of the tested concentrations precipitation of the dendrimers was observed. Control experiments with similar concentrations of the sodium salts of the used counterions (chloride and trifluoroacetate) were performed and showed no effect in MCF7 cell culture, which assures that all cytotoxic effects were caused by the dendrimers.

The water-soluble dendrimers of set A exhibited a concentration- and generation-specific cytotoxicity (Figure 3b; see p. 1178). While core **4** was inactive at every concentration tested, the G1 dendrimer **8** showed a concentration-dependent reduction of cell proliferation at 10 μM. The maximum effect was reached for each concentration at the end of the test (Figure 3b: after 220 h at 20 μM: $T/C$ = 15%). The antiproliferative effect of its G2 analogue **10** was much higher than for **8**. At concentrations of 5–10 μM no surviving cells were observed. Concentrations from 0.5–3 μM were then chosen to examine the concentration-dependent cytotoxic effects of this dendrimer. Its absolute cytotoxicity was the highest observed for all dendrimers examined; even a

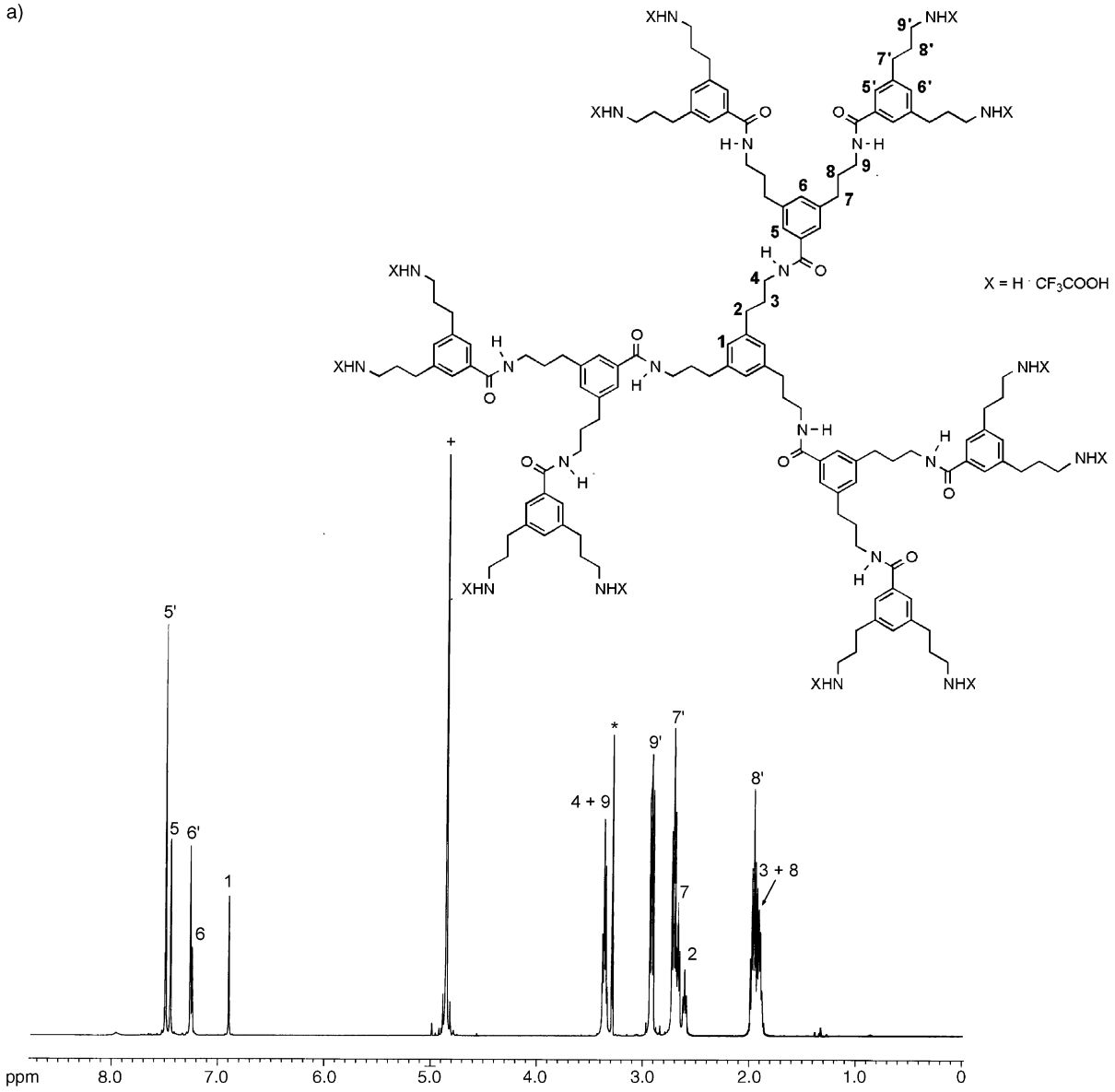



Figure 2. $^1$H NMR spectra (500 MHz) of the two deprotected dendrimers **10** (a) and **36** (b) in [$D_4$]methanol; protons 9 and 11 of dendrimer **36** show diastereotopic coupling and are therefore split into two distinct signals. In both spectra [$D_4$]methanol is marked by (*), and remaining $H_2O$ from lyophilization is assigned by (+); the signal of proton 17 was reduced in size, as indicated by (≈).

2 μM concentration lowered the viable cell mass under the amount at the beginning of the test (Figure 3b).

The same experiments were also carried out with set B dendrimers. Figure 4 (see p. 1179) depicts the results of the deprotected G0 and G1 dendrimers **13a–c** and **21a–c**, and Figure 5 those of their protected analogues **12a–c** and **20a–c**, respectively. The cationic **13a** and the zwitterionic **13c** did not show significant antiproliferative effects even at 20 μM (Figure 4), while the cationic **13b** exhibited a pronounced cytotoxicity increase with increasing concentration (Figure 4a). It should be noted that in this case the highest activity was observed after an incubation time of 70–100 h, which was lowered during the course of incubation. This can be interpreted to mean that the cells become resistant to this compound. The same trend was observed with the corresponding G1 dendrimers. Whereas **21c** did not show any antiproliferative effects even at 20 μM, **21a** was slightly antiproliferative at higher concentrations, and **21b** exhibited the highest level of cytotoxicity of this series without any appearance of resistance (Figure 4b).

The protected set B dendrimers **12a–c** and **20a–c** (Figure 5; see p. 1180) showed a somewhat unexpected behavior in that most, but not all, of them were not antiproliferative. **12a** and **12b** turned out to be cytotoxic. While **12b** showed concentration dependency, no difference between the two concentrations tested (5 and 10 μM) was observed for **12a**. This might indicate that the available amount of **12a** in solution is not higher than 5 μM. The reason for this unexpected behavior of the smaller protected dendrimers is unclear. All other protected dendrimers showed no effect on cell proliferation.

For the Boc-protected set C dendrimers **15** and **24** no antiproliferative effect was observed. A rather unexpected and potentially important result was found for the unprotected

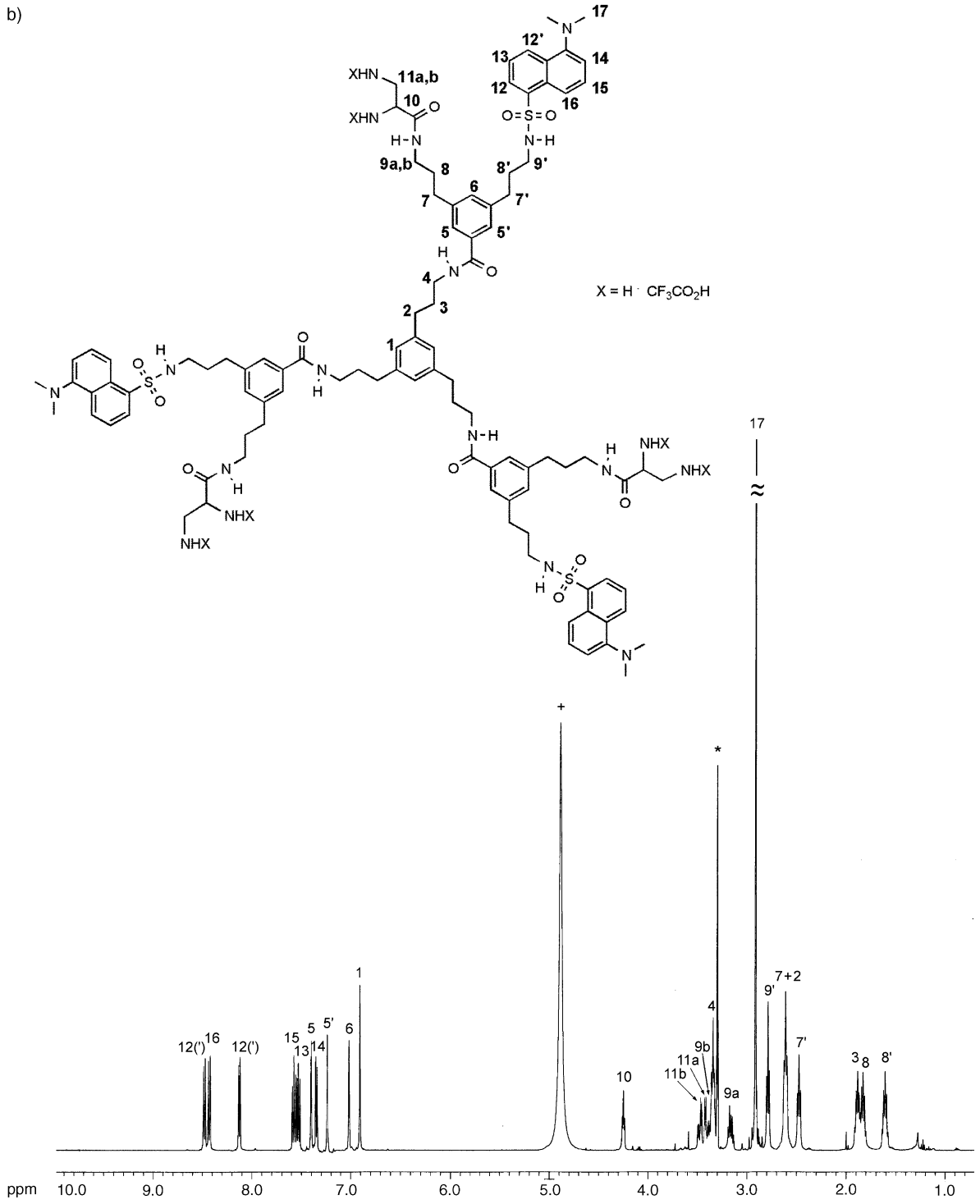



Figure 2. (Continued).

dendrimers **16** and **25**. Despite their positive charges both compounds had no effect on proliferation at all concentrations tested (Figure 6; see p. 1180).

The set D dendrimers **26**–**28** showed no cytotoxicity at all concentrations tested (Figure 7; see p. 1181). In contrast, the partially dansylated dendrimers **34** and **36** were both cytotoxic at higher concentrations (10 and 20 μM) (Figure 8). **34** reached even cytocidal effects at 20 μM. Dendrimer **36**, which carries both diaminopropionic acid and dansyl substituents, can be considered a hybrid of the fully dansylated G1 dendrimer **27** and dendrimer **25**, which is fully covered with diaminopropionic acid groups. In this sense, the fact that only **36** shows toxicity is somewhat surprising.

The findings regarding the deprotected representatives of sets A and B correlate well with the observations by Roberts et al.[30] and Duncan et al.,[31] who also found polycationic dendrimers to be cytotoxic depending on their generation

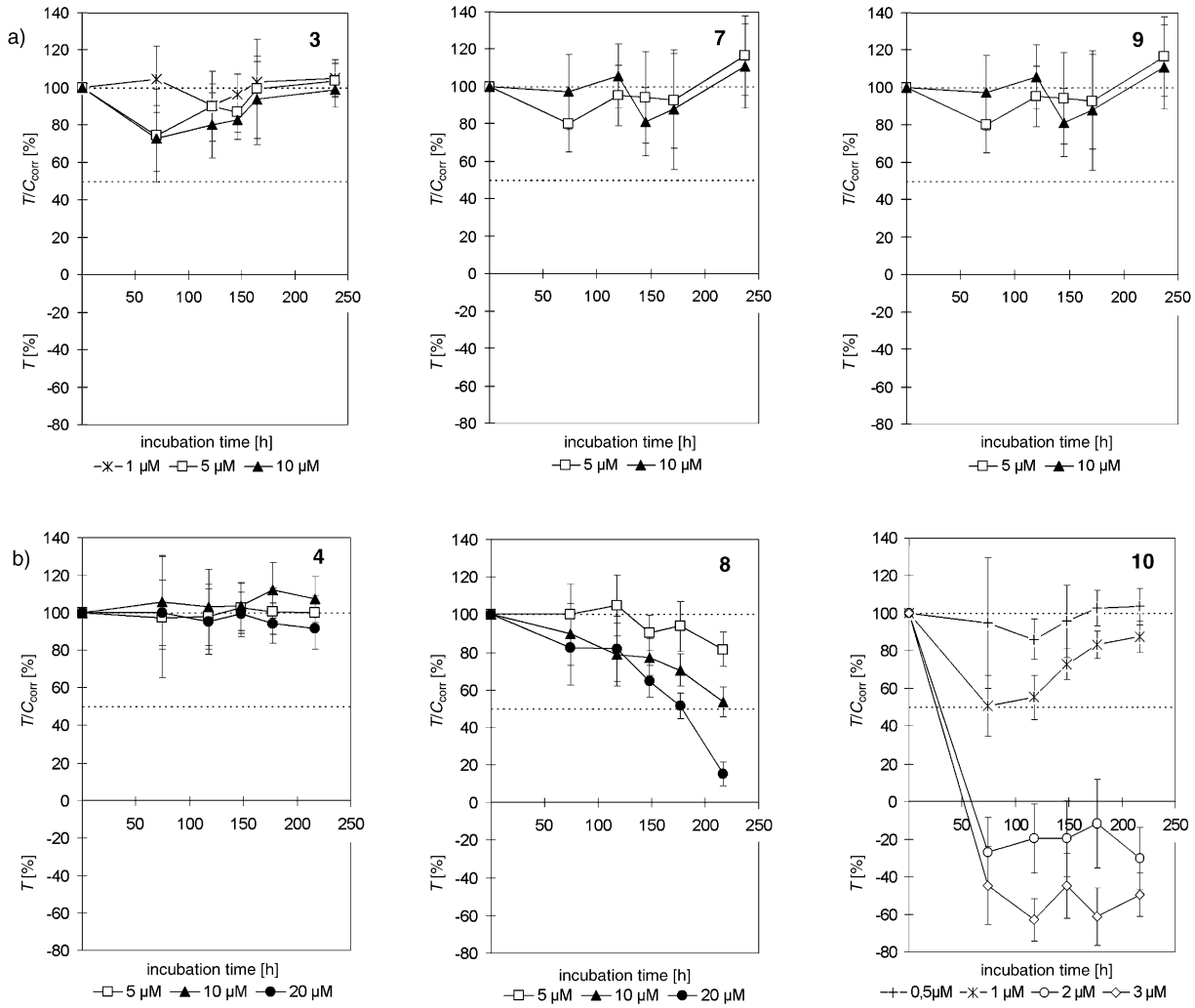



Figure 3. In vitro cytotoxicity of core molecule **4** and of the basic dendrimers **8** and **10**. Comparison of the a) Boc-protected and the b) deprotected forms. For detailed information on the investigation of the $T/C_{corr}$[%] and $T$[%] values see Experimental Section.

number and concentration in cell culture media. Since cytotoxic behavior of polycationic compounds was not only reported for dendrimers, but especially also for positively charged polyelectrolytes like poly(ethyleneimine) (PEI), poly(L-lysine hydro bromide) (PLL), poly(diallyl-dimethylammonium chloride) (DAD-MAC), diethylaminoethyl (DEAE)-dextran, and chitosans,[50,51] it is conceivable that toxicity correlates with the presence of positive charges, though a detailed molecular picture of the toxic mechanism is not yet available. The cytotoxic effect of polycations is sometimes explained by means of charge density and flexibility of the macromolecules.[50,52] Ryser proposed that the three-dimensional structure of compounds is important in their biological response on cell membranes.[53] Branched molecules were found to be more efficient in neutralizing the cell surface charge than polymers with linear or globular structures, and rather rigid molecules like dendrimers were less toxic than linear or branched polymers.[50] Fischer et al. concluded from their experiments that membrane leakage occured first in cells exposed to cationic polymers and is followed by a decrease in metabolic activity.[50] In contrast to these findings it is somewhat surprising that the completely ethylenediamine-modified dendrimers of set C exhibited no cytotoxic effects at all concentrations tested (Figure 6). It is not yet clear whether the bidendate nature of the terminal diaminopropionic amide substituents with its different protonation, metal ion complexation, and hydrogen bonding behavior is responsible for this finding. While the completely dansylated dendrimers of set D did not exhibit antiproliferative effects, the observed cytotoxicity of the mixed dansylated (**34** and **36**) and phenylalanine dendrimers (**13b** and **21b**) suggests that 'tenside effects' (disruption of cell membranes by interaction with negatively charged groups and hydrophobic parts) are likely to be responsible for the observed cell death. Also, induction of apoptosis by the added dendrimers cannot be completely excluded, though indications for such a mechanism have not yet been reported.[50] It is unlikely that details of the interior structure of the dendrimers, in particular the phenyl branching groups, are responsible for the observed toxicity, because in the analogous studies under identical conditions the Boc-protected dendrimers **3**, **7**, and **9** (Figure 3) did not show cytotoxicity. It should be

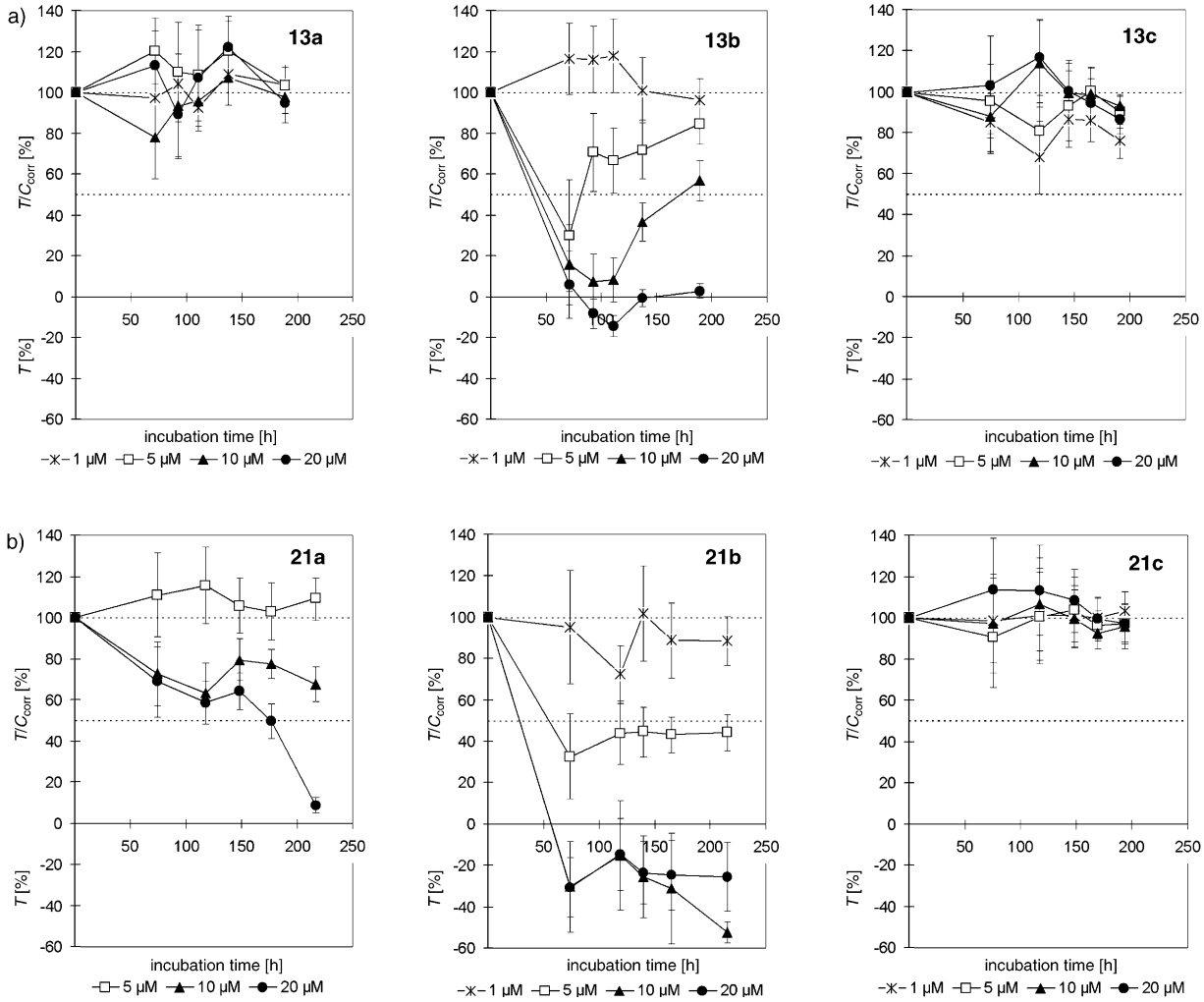



Figure 4. In vitro cytotoxicity of the a) amino acid-terminated G0 dendrimers **13a**, **13b**, **13c** , and b) of the corresponding G1 dendrimers **21a**, **21b**, **21c**.

noted, however, that these compounds have a lower solubility in cell culture media and, thus, their bioavailability may be reduced.

Nevertheless, the polycationic nature of compounds cannot fully explain the observed cytotoxicity in every case. The results of dendrimers **13b**, **21b**, **16**, **25**, **34**, and **36** show that other structural factors may also play a role. A correlation of cytotoxicity data with structural motifs such as the hydrophobic or ionic groups is tempting but does not seem to be appropriate at the present state. An even more broad study would be required.

**Cellular uptake and localization**: To study cellular uptake and intracellular distribution of the dansylated dendrimers, human HeLa cells were incubated for 20 h with dendrimers **26**, **27**, **34**, and **36** at a final concentration of 5 μM. Then, the cells were fixed and, for better orientation within the cell, immunostained with an antibody raised against the membrane forms of the lamina-associated polypeptide 2 (LAP-2), which are enriched at the inner membrane of the nuclear envelope and are partially also present in the endoplasmic reticulum.[54] Integral membrane proteins of the nuclear envelope interact with lamins and chromosomes, and binding is modulated by mitotic phosphorylation. The cells were then analyzed by confocal fluorescence microscopy. Figure 9 and Figure 10 (see p. 1181 and 1182, respectively) present typical confocal images of such experiments. Similar experiments with human MCF-7 cells and mouse fibroblast cells (NIH 3T3) performed under the same conditions resulted in the same distribution pattern (not shown).

All experiments were reproducible, and the observed intracellular patterns were representative for the cells of each single experiment. At least 30–40 % of all cells exhibited a specific intracellular fluorescence, and 90 % of the fluorescence-positive cases showed practically the same distibution patterns as presented in Figure 9 and Figure 10.

Figure 9 shows the differential interference contrast (DIC) and fluorescence images of the cells incubated with dendrimer **26**, obtained by confocal fluorescence microscopy. Already in the DIC image, large intracellular aggregates with a granular structure were identified, (red arrows in Figure 9a). Upon excitation (364 nm), these aggregates exhibited a strong green-blue fluorescence and could, therefore, easily be identified and located within cells (dendrimer fluorescence encoded in blue in Figure 9c and Figure 10). Compared with the fluorescence pattern of Cy2-immunolabeled

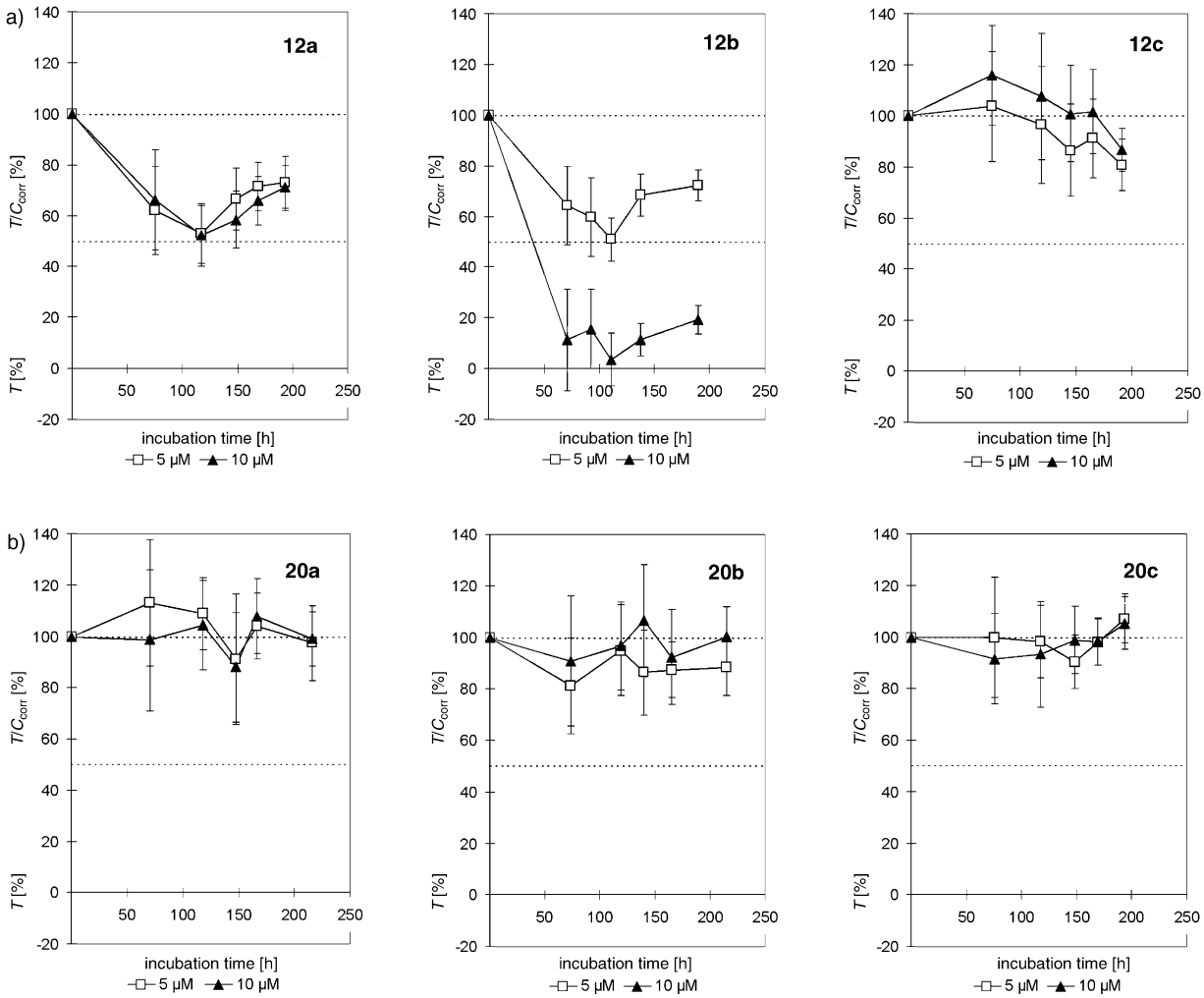



Figure 5. In vitro cytotoxicity of a) the Boc-protected amino acid-terminated G0 dendrimers **12a**, **12b**, **12c**, and b) of the corresponding G1 dendrimers **20a**, **20b**, **22c**.

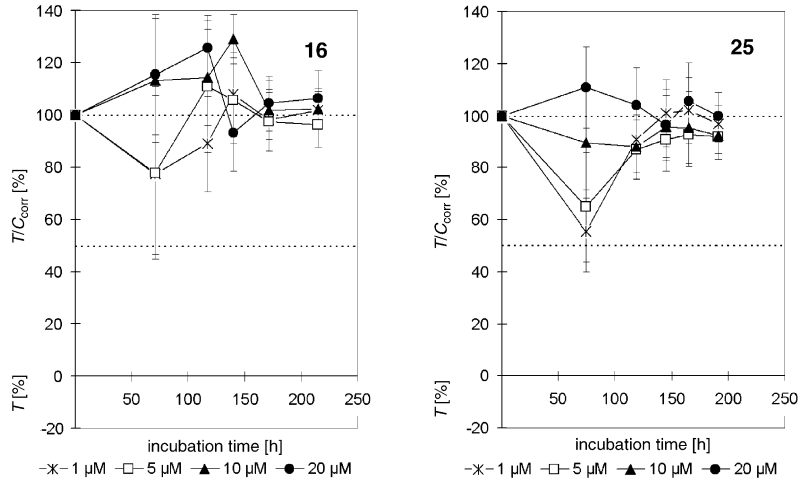



Figure 6. In vitro cytotoxicity of the diaminopropionic acid G0 and G1 dendrimers **16** and **25**.

LAP-2, the distribution of the aggregated dendrimers was distinguishable from the nuclear envelope staining (Figure 9c). The granular structures were typically located near the cell nucleus. Between one and three aggregates were normally observed per cell, and their diameters ranged from 2 to 4 μm. For dendrimer **27** the size of the formed aggregates was larger, and they appeared more granular (Figure 10a). The rather long incubation time prior to observation in comparison with typical cellular trafficking time-scales of dendrimers[33,55–57] suggests that a stable situation had been reached. The water-soluble dendrimer **34**, on the other hand, showed a more punctated distribution pattern, with a diameter of the granules of approximately 0.5–1 μm on average. This pattern could be of endosomal or lysosomal origin, since distribution and size agree well with that of compartments formed by fused endo- or lysosomes (Figure 10b). The intracellular distribution pattern of dendrimer **36**, finally, resembled that of **26**

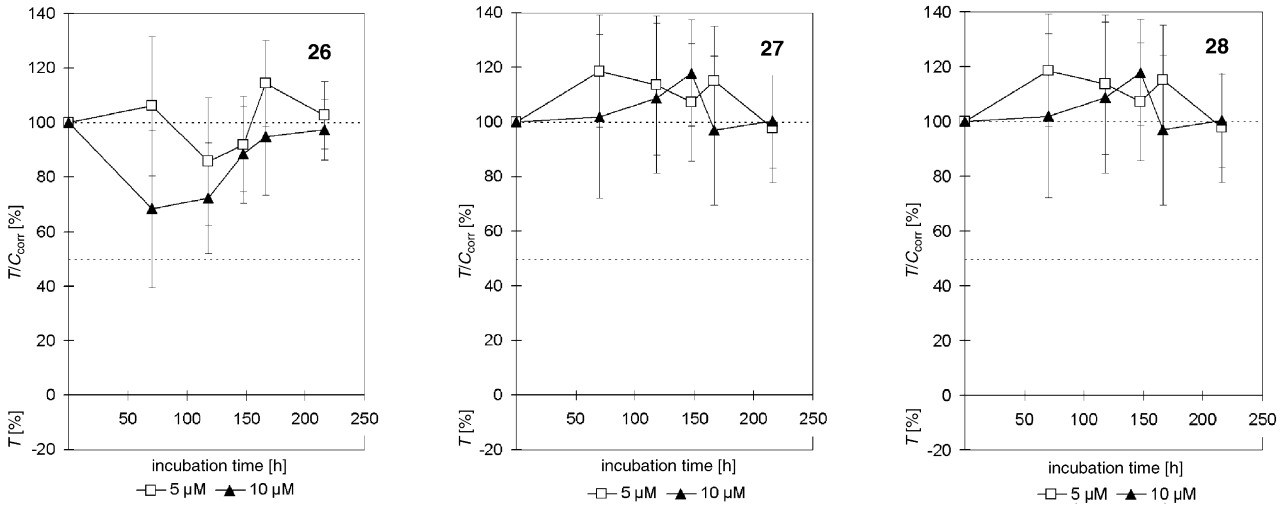



Figure 7. In vitro cytotoxicity of the dansylated dendrimers **26**, **27**, and **28**.

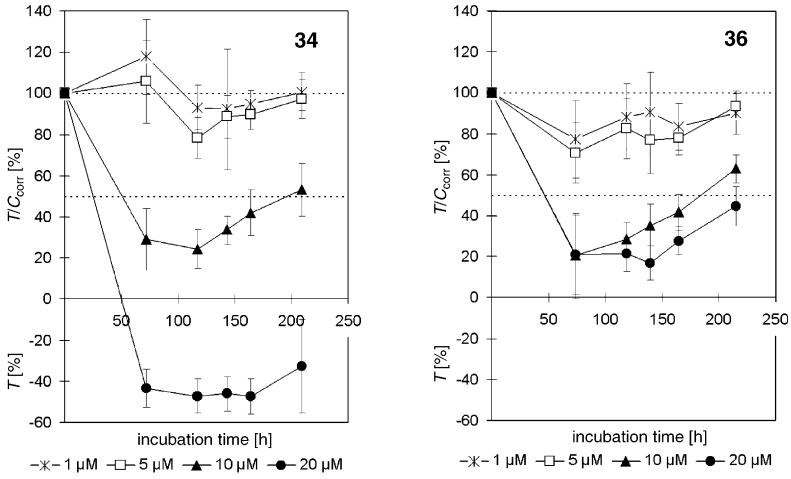



Figure 8. In vitro cytotoxicity of the mixed dendrimers **34** and **36** with two different surface moieties.

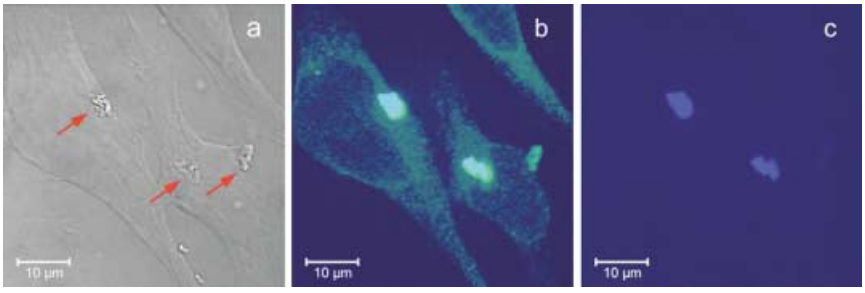



Figure 9. Confocal fluorescence microscopy images. HeLa cells were incubated with a final concentration of 5 µM of dendrimer **26** for 20 h at 37 °C, washed, fixed and immunostained for the nuclear envelope marker LAP-2 (membrane isoforms) which was visualized with a Cy2-labeled secondary antibody. Dendrimer localization is indicated by red arrows. a) DIC image; b) overlay of LAP-2 staining (green) and dendrimer fluorescence (blue); c) Dendrimer fluorescence (blue channel).

and **27**. One to three large aggregates near the cell nucleus were typically observed (Figure 10c).

A similar punctated intracellular distribution pattern has been described previously for other dendrimers. Polyproline-based dendrimers of Giralt and co-workers exhibited a more or less vesicular distribution in normal rat kidney (NRK) cells after one hour of incubation. In this case, endocytosis was suggested to be the mechanism of cellular uptake.[55] Juliano et al. also found a slightly "clumpy" cellular distribution of Oregon green 488-conjugated PAMAM dendrimer/oligonucleotide complexes in HeLa cells. Dendrimers were not only found inside the nucleus but also at other sites in the cell, presumably at the plasma membrane and in endomembrane compartments.[32,58] As shown in Figure 9 and Figure 10, none of the dendrimers investigated in this study displays a localization inside of the nucleus. Instead, they are rather always found directly next to the nucleus. This could suggest a localization within or close to the Golgi apparatus. An example for such a distribution was given by Merlin et al. who found a sequestration of daunorubicin in the Golgi vesicles in drug-resistent MCF-7 cells.[59,60] Tanke, Reedijk and co-workers report a punctated staining of a cytoplasmatic region, when human osteosarcoma (U2-OS) cells were incubated with fluorophore-labeled platinum complexes.[61] Co-localization experiments with a Golgi apparatus-selective stain also indicated the involvement of Golgi-vesicles in the intracellular processing. A related early endosomal or lysosomal localisation also seems to be likely for dendrimer **34** because of its ob-

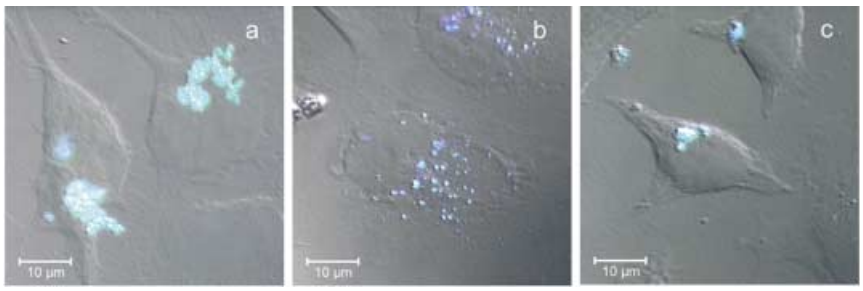



Figure 10. Confocal fluorescence microscopy images; overlay of DIC-image, and dendrimer fluorescence. HeLa cells were incubated with dendrimers at a final concentration of 5 µM (see text for details). a) dendrimer **27**; b) dendrimer **34**; c) dendrimer **36**.

served distribution pattern (Figure 10), but this has not been proven yet.

To finally answer the question of intracellular localization of the dendrimers reported here after uptake by the cell, further kinetic and co-localization studies have to be performed. Initial kinetic studies indicate that cellular uptake of the dendrimers is a rather fast process and probably finishes after 4–5 h (data not shown). If the dendrimers are taken up by endocytosis, they should initially be localized in clathrin-coated vesicles which subsequently fuse to larger endosomes and then lysosomes. Some of the dendrimers might also be directed towards the Golgi apparatus. Therefore, the exact intracellular localization of the dendrimers needs to be characterized in more detail, for example by co-localization with known markers for the different intracellular compartments.[62]

## Conclusion

With a series of simple and optimized steps a set of low generation polyamidoamine dendrimers was synthesized, many of which are highly water-soluble. Convergent and divergent strategies were applied in order to make accessible a bouquet of differently surface decorated representatives at the least synthetic effort. The surfaces of the dendrimers were decorated with natural amino acid and ethylenediamine moieties, fluorescence labels, and simple amino-protecting groups either as the only substituents or in combinations.

In vitro cytotoxicity experiments were performed with all these dendrimers using the human breast cancer cell line MCF-7. These experiments proved that surface-functionalization grossly influences dendrimer toxicity. Based on the data obtained a broader structure/toxicity correlation could not be established yet. A few interesting conclusions can nevertheless be drawn: The internal structure of the presented dendrimers does not seem to play a profound role, despite the common view that the interior of low-generation dendrimers is accessible. The surface decoration, however, is crucial for toxicity. Most of the examined noncharged dendrimers (e.g., the protected and dansylated ones) are nontoxic though they are clearly bioavailable as was proved by cell uptake experiments, and all completely diamino propionic acid decorated dendrimers are also nontoxic. Especially this last case shows that positive charges on a dendrimer surface do not automatically cause cell toxicity as one may have been inclined to conclude both from literature results and those obtained in the present work. It is not clear yet whether the bidendate nature of ethylenediamine plays a role here, for example by chelating metal ions.

Confocal fluorescence microscopy studies revealed that all dansylated dendrimers are internalized by HeLa cells and remain intracellularly present over a 20-hour incubation period. Further kinetic studies will address the velocity of the cellular uptake and the intracellular trafficking to their final destination.

Hopefully, the results of such experiments, in combination with studies on metabolic degradation and the biodistribution pattern in animals will finally enable the design of optimized and nontoxic drug-carrier systems.

## Experimental Section

**Abbreviations**: 9-BBN: 9-borabicyclononane; Boc: *tert*-butyloxycarbonyl; Cbz: benzyloxycarbonyl; CCA: α-cyano-4-hydroxy-cinnamonic acid; DCM: dichloromethane; DIPEA: N-ethyl-diisoropylamine; DMF: *N*,*N*-dimethylformamide; DMSO: dimethyl sulfoxide; Dns: 5-dimethylaminonaphthalene-1-sulfonyl; Dpa: diaminopropionic acid; EDC: N-(3-dimethylaminopropyl)-N′-ethyl-carbodiimide hydrochloride; EDTA: ethylenediamine tetraacetate; FCS: fetal calf serum; HOBt: hydroxy-benzotriazole hydrate; HRMS: high-resolution mass spectrometry; MNBA: *m*-nitrobenzyl alcohol; PBS: phosphate-buffered saline; RP-HPLC: reversed-phase high-performance liquid chromatography; TBTU: *O*-(1*H*-benzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; THF: tetrahydrofuran.

**Synthesis and purification of the dendrimers: general remarks**: All starting materials were purchased from commercial sources and used without further purification. Solvents were dried under standard conditions. Lyophilization from water was performed by using deionized, destilled, ′MilliQ′ (Millipore) (0.45 µm) filtered water. The following compounds were prepared according to literature procedures: *N*-(*tert*-butyloxycarbonyl)allylamine (**2**),[63] **5**,[47c] **6**,[47b,c] **14**,[36] and **17**.[47b] Compounds **29** and **30** are known,[47b] but were prepared according to a different procedure.[64] Therefore their analytical and spectral data are not given. All other compounds are new. Whenever possible, reactions were monitored by thin-layer chromatography (TLC) using TLC silica gel coated aluminum plates $60F_{254}$ (Merck). Compounds were detected by UV light (254 nm or 366 nm) and/or by treatment with a solution of ninhydrine in ethanol followed by heating. $^{1}H$ and $^{13}C$ NMR spectra were recorded using Bruker AC 500 (500 MHz) and AB 250 (250 MHz) instruments; the solvent signal was used for internal calibration. Mass spectra were recorded by using a Varian MAT 711 and CH6 (EI) or Type CH5DF (FAB), and a Bruker Reflex with delayed extraction source (MALDI-TOF). Elemental analyses were performed by using a Perkin-Elmer EA 240. Because of the polarity of the prepared compounds, it was generally difficult to obtain correct data from elemental analysis. This was specifically so for the Boc-protected and dansylated dendrimers, and for some of the free carboxylic acids, for which the carbon values obtained differed from the calculated ones by up to 1%. Analytical RP-HPLC was carried out using an HPLC System consisting of a Gynkotek UVD 340 S Diode Array Detector, a Gynkotek Mod. 480 Pump, and a Knaur Eurosphere column ($C_{18}$, 100–5 µm, 4 × 120 mm).

For clarity reasons a simplified nomenclature has been added in front of each compound name in the Experimental Section. For example, in ***(Boc-N)$_3$3 D0*** the text in parentheses (here ***Boc-N***) defines the functional endgroup, the subscript number, here ($_3$), is number of those groups, the regular sized number (***3***) gives the total number of modifiable endgroups, and finally the letter ***D*** plus a number (here **0**) stands for a dendrimer of the corresponding specific generation.

***(Boc-N)$_3$3 D0***: **1,3,5-tris-[(*tert*-butyloxycarbonylamino)propyl]benzene (3)**: 9-BBN (15.4 g, 126 mmol) was added at 0 °C to a solution of *tert*-butyl allylcarbamate **2** (16.5 g, 105 mmol) in dry toluene. The reaction mixture was allowed to warm up to room temperature and stirred for an additional 12 h. Then 1,3,5-tribromobenzene (**1**, 7.87 g, 25.0 mmol) and a 1 M solution of KOH in water (225 mL) were added. After degassing the reaction mixture, [Pd(PPh$_3$)$_4$] (1.16 g, 1.00 mmol) was added. The reaction mixture was degassed repeatedly thereafter and stirred at 50 °C for 24 h. After complete reaction (TLC) the layers were separated, the organic phase was washed with brine once, and the aqueous phase was extracted with diethyl ether. The combined organic layers were dried over magnesium sulfate and the solvent was removed in vacuo. Column chromatography (silica gel, hexane/ethyl acetate (3:1/v:v)) and subsequent recristallization from hexane/ethyl acetate (4:1/v:v) yielded the Boc-protected core (12.9 g, 23.5 mmol; 94.0 %) as a colorless solid. $R_f$ = 0.30 (hexane/ethyl acetate = 2:1/v:v); m.p. 89 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.41 (s, 27 H; $CH_3$), 1.75 (q, $^3J$(H,H) = 7.4 Hz, 6 H; $CH_2$), 2.54 (t, $^3J$(H,H) = 7.6 Hz, 6 H; $CH_2$Ar), 3.08 (m, 6 H; $CH_2$N), 4.65 (s, br, 3 H; NH), 6.78 ppm (s, 3 H; Ar*H*); $^{13}$C NMR (63 MHz, $CDCl_3$): $\delta$ = 28.38 ($CCH_3$), 31.58 ($CH_2$), 32.85 ($CH_2$Ar), 40.07 ($CH_2$N), 78.97 ($CCH_3$), 126.10 (Ar*C*), 141.67 (Ar*C*), 155.95 ppm (CO); MS (EI, 80 eV, 180 °C); *m/z* (%): 549 (3.2) $[M]^+$, 449 (49.8) $[M-C_5H_8O_2]^+$, 263 (100.0); elemental analysis calcd (%) for $C_{30}H_{51}N_3O_6$ (549.75): C 65.54, H 9.35, N 7.64; found: C 65.47, H 9.06, N 7.51.

***(N)$_3$3 D0*: 1,3,5-tris(propylamine)benzene trishydrochloride (4)**: The Boc-protected core **3** (8.80 g, 16.0 mmol) was dissolved in THF and stirred with a 25 % HCl solution (28.0 mL, 192 mmol) for 12 h at room temperature. Part of the pure product already precipitated from the reaction mixture, for the other part the solvent was removed in vacuo, the residue dissolved in ethanol and precipitated with diethyl ether. The procedure yielded the trishydrochloride (5.49 g, 15.3 mmol; 95.6 %) as a colorless solid. M.p. 290 °C (decomp); $^1$H NMR (500 MHz, [D$_4$]methanol): $\delta$ = 2.04 (quin, $^3J$(H,H) = 7.6 Hz, 6 H; $CH_2$), 2.74 (t, $^3J$(H,H) = 7.6 Hz, 6 H; $CH_2$Ar), 2.99 (t, $^3J$(H,H) = 7.8 Hz, 6 H; $CH_2$N), 7.05 ppm (s, 3 H; Ar*H*); $^{13}$C NMR (63 MHz, [D$_4$]methanol): $\delta$ = 30.16 ($CH_2$), 33.34 ($CH_2$Ar), 40.35 ($CH_2$N), 127.55 (Ar*C*), 142.45 ppm (Ar*C*); MS (EI, 80 eV, 200 °C): *m/z* (%): 249 (15.0) $[M]^+$, 219 (100.0) $[M-CH_2NH_2]^+$; HRMS: *m/z*: monoisotopic mass calcd for $C_{15}H_{27}N_3^+$: 249.22050, found: 249.22221.

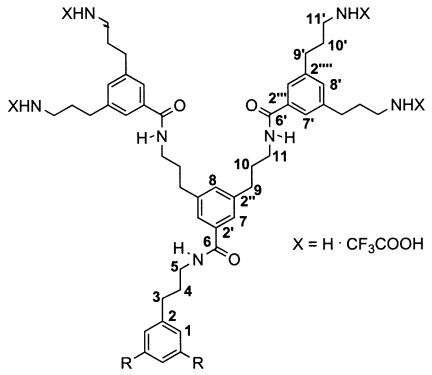
***(Boc-N)$_6$6 D1*: 1,3,5-tris-{3,5-bis[(*tert*-butyloxycarbonylamino)propyl]-*N*-propylbenzamide}benzene (7)**: The free acid **5** (1.83 g, 4.20 mmol) was dissolved in dry dichloromethane, HOBt (674 mg, 4.40 mmol) was added, and the solution was stirred for 15 min. Afterwards the reaction mixture was cooled down to −20 °C, EDC (882 mg, 4.60 mmol) was added, and the mixture was subsequently stirred for additional 2 h. During that time the reaction mixture wass allowed to warm up to room temperature slowly. After complete reaction (TLC), the mixture was cooled down to −30 °C, and DIPEA (2.01 mL, 1.53 g, 11.8 mmol) was added. The trisamine core **4** (359 mg, 1.00 mmol) was dissolved in a small amount of dry methanol and slowly added to the reaction mixture under vigorous stirring. The solution was stirred for an additional 16 h, and during that time allowed to warm up to room temperature slowly. After complete reaction the solution was washed twice with sodium hydrogencarbonate solution and once with brine. The organic layer was dried over magnesium sulfate, the solvent removed in vacuo, and the crude product purified by column chromatography (silica gel, dichloromethane containing 2 % methanol as eluent) to afford the G1 dendrimer (1.18 g, 784 μmol, 78.4 %) as a colorless foam. $R_f$ = 0.37 (dichloromethane/methanol = 19:1/v:v); m.p. 89 °C; $^1$H NMR (250 MHz, $CDCl_3$): $\delta$ = 1.40 (s, 54 H; $CH_3$), 1.72 (m, 12 H; $CH_2$), 1.94 (m, 6 H; $CH_2$), 2.55 (m, 6 H + 12 H; $CH_2$Ar), 3.05 (m, 12 H; $CH_2$N), 3.41 (m, 6 H; $CH_2$N), 4.78 (m, br, 6 H; NH), 7.85 (s, 3 H; Ar*H*), 7.02 (s, br, 3 H; NH), 7.03 (s, 3 H; Ar*H*), 7.37 ppm (s, 6 H; Ar*H*); $^{13}$C NMR (63 MHz, $CDCl_3$): $\delta$ = 28.41 ($CCH_3$), 30.85 ($CH_2$), 31.35 ($CH_2$), 32.48 ($CH_2$), 33.16 ($CH_2$), 39.58 ($CH_2$), 48.77 ($CH_2$), 79.10 ($CCH_3$), 124.79 (Ar*C*), 126.28 (Ar*C*), 131.45 (Ar*C*), 134.91 (Ar*C*), 141.71 (Ar*C*), 141.78 (Ar*C*), 156.09 ($CON_{Boc}$), 167.75 ppm (CON); MS (FAB+, MNBA/DCM/DMSO): *m/z* (%): 1505 (17.8) $[M+H]^+$, 1504 (17.8) $[M]^+$, 1404 (57.1) $[M-C_5H_9O_2]^+$, 1304 (22.8) $[M-2(C_5H_9O_2)]^+$, 1204 (23.3) $[M-3(C_5H_9O_2)]^+$, 1104 (22.8) $[M-4(C_5H_9O_2)]^+$, 1004 (21.5) $[M-5(C_5H_9O_2)]^+$, 904 (100) $[M-6(C_5H_9O_2)]^+$; MS (MALDI-TOF, dithranol): *m/z*: 1543 $[M+K]^+$, 1527 $[M+Na]^+$; monoisotopic mass calcd for $C_{84}H_{129}N_9NaO_{15}^+$: 1526.95, found: 1526.94.

***(N)$_6$6 D1*: 1,3,5-tris[3,5-bis(3-aminopropyl)-*N*-propylbenzamide]benzene hexatrifluoroacetate (8)**: The Boc-protected G1 dendrimer **7** (336 mg, 223 μmol) was dissolved in a small amount of dichloromethane (10 mL). Trifluoroacetic acid (2 mL) was added, and the reaction mixture was stirred for 1 h at room temperature. After complete reaction (TLC) the solvent was removed in vacuo to give the deprotected dendrimer (348 mg, 219 mmol; 98.2 %) as a colorless oil which could be lyophilized from water. M.p. 97 °C; $^1$H NMR (250 MHz, [D$_4$]methanol): $\delta$ = 2.02 (m, 6 H + 12 H; $CH_2$), 2.69 (t, $^3J$(H,H) = 7.4 Hz, 6 H; $CH_2$Ar), 2.79 (t, $^3J$(H,H) = 7.8 Hz, 12 H; $CH_2$Ar), 2.99 (t, $^3J$(H,H) = 7.5 Hz, 12 H; $CH_2$N), 3.44 (t, $^3J$(H,H) = 7.1 Hz, 6 H; $CH_2$N), 6.97 (s, 3 H; Ar*H*), 7.33 (s, 3 H; Ar*H*), 7.57 ppm (s, 6 H; Ar*H*); $^{13}$C NMR (63 MHz, [D$_4$] methanol): $\delta$ = 30.12 ($CH_2$), 32.21 ($CH_2$), 33.28 ($CH_2$), 34.33 ($CH_2$), 40.26 ($CH_2$), 40.87 ($CH_2$), 126.30 (Ar*C*), 127.26 (Ar*C*), 132.66 (Ar*C*), 136.58 (Ar*C*), 142.71 (Ar*C*), 143.23 (Ar*C*), 170.24 ppm (CON); MS (FAB+, DMSO/2-nitrophenol): *m/z* (%): 927 (3.3) $[M+Na]^+$, 905 (15.6) $[M+H]^+$, 219 (100) $[C_{13}H_{19}N_2O]^+$; MS (MALDI-TOF, CCA): *m/z*: monoisotopic mass calcd for $C_{54}H_{82}N_9O_3^+$: 904.65, found: 904.85 $[M+H]^+$.

***(Boc-N)$_{12}$12 D2*: 1,3,5-Tris(3,5-bis[3,5-bis[(*tert*-butyloxycarbonylamino)propyl]-*N*-propylbenzamide}-*N*-propylbenzamide)benzene (9)**: The G2 acid **4** (830 mg, 800 μmol) was dissolved in dry dichloromethane. HOBt (130 mg, 851 μmol) was added, and the reaction mixture was stirred for 15 min at room temperature. The mixture was cooled down to −20 °C and EDC (171 mg, 890 μmol) was added. The solution was stirred for an additional 2 h and allowed to warm up to room temperature. After complete reaction (TLC) the mixture was cooled down to −30 °C and (381 μL, 290 mg, 2.24 mmol) DIPEA was added. The trisamine core **4** (65.0 mg, 180 μmol) was dissolved in a small amount of dry methanol and slowly added to the reaction mixture under vigorous stirring. The solution was stirred for an additional 12 h and during that time allowed to warm up to room temperature. Afterwards the mixture was washed twice with sodium hydrogencarbonate solution and once with brine. The organic layer was dried over magnesium sulfate, and the solvent removed in vacuo. The crude product was purified by column chromatography (silica gel, dichloromethane containing 4 % methanol as eluent) to afford (526 mg, 154 μmol; 85.6 %) of the G2 dendrimer **9** as a colorless foam. $R_f$ = 0.19 (dichloromethane/methanol = 19:1/v:v); m.p. 106 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.38 (s, 108 H; $CH_3$), 1.69 (quin, $^3J$(H,H) = 7.4 Hz, 24 H; $CH_2$), 1.82 (m, 12 H; $CH_2$), 1.86 (m, 6 H; $CH_2$), 2.52 (m, 24 H + 12 H + 6 H; $CH_2$Ar), 3.29 (m, 24 H; $CH_2$N), 3.31 (m, 12 H; $CH_2$N), 3.37 (m, 6 H; $CH_2$N), 4.94 (s, br, 12 H; NH), 6.83 (s, 3 H; Ar*H*), 7.00 (s, 3 H; Ar*H*), 7.03 (s, 6 H; Ar*H*), 7.28 (s, br, 6 H + 3 H; NH), 7.31 (s, 6 H; Ar*H*), 7.43 ppm (s, 12 H; Ar*H*); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 28.43 ($CCH_3$), 29.68 ($CH_2$), 30.74 ($CH_2$), 31.33 ($CH_2$), 32.51 ($CH_2$), 32.85 ($CH_2$), 33.37 ($CH_2$), 39.32 ($CH_2$), 39.66 ($CH_2$), 53.40 ($CH_2$), 79.06 ($CCH_3$), 124.91 (Ar*C*), 126.33 (Ar*C*), 131.38 (Ar*C*), 131.63 (Ar*C*), 134.73 (Ar*C*), 134.83 (Ar*C*), 141.75 (Ar*C*), 141.84 (Ar*C*), 141.95 (Ar*C*) 142.04 (Ar*C*), 156.16 ($CON_{Boc}$), 167.86 (CON), 167.96 ppm (CON); MS (MALDI-TOF, IAA): *m/z*: 3452 $[M+K]^+$, 3436 $[M+Na]^+$; monoisotopic mass calcd for $C_{192}H_{285}N_{21}NaO_{33}^+$: 2436.12, found: 2436.29.

***(N)$_{12}$12 D2*: 1,3,5-Tris{3,5-bis[3,5-bis(3-aminopropyl)-*N*-propylbenzamide]-*N*-propylbenzamide}benzene dodecatrifluoroacetate (10)**: The Boc-protected G2 dendrimer **9** (180 mg, 54.1 μmol) was dissolved in dichloromethane (8 mL). Trifluoroacetic acid (2 mL) was added, and the reaction mixture was stirred for 2 h at room temperature. After complete reaction (TLC) the solvent was removed in vacuo to give the deprotected dendrimer (192 mg, 53.6 μmol, 99.1 %) as a colorless oil which could be lyophilized from water. M.p. 110–112 °C; $^1$H NMR (500 MHz, [D$_4$]methanol): $\delta$ = 1.97 (m, 18 H; *H*-4 + *H*-10), 2.02 (m, 24 H; *H*-10′) 2.67 (t, $^3J$(H,H) = 7.4 Hz, 6 H; *H*-3), 2.73 (t, $^3J$(H,H) = 7.6 Hz, 12 H; *H*-9), 2.77 (t, $^3J$(H,H) = 7.8 Hz, 24 H; *H*-9′), 2.98 (t, $^3J$(H,H) = 7.7 Hz, 24 H; *H*-11′), 3.43 (m, 18 H; *H*-11 + *H*-5), 6.96 (s, 3 H; *H*-1), 7.30 (s, 3 H; *H*-8), 7.32 (s, 6 H; *H*-8′), 7.50 (s, 6 H; *H*-8), 7.55 ppm (s, 12 H; *H*-7′); $^{13}$C NMR (126 MHz, [D$_4$]methanol): $\delta$ = 30.11 (*C*-10′), 31.95 (*C*-10), 32.04 (*C*-4), 33.28 (*C*-9′),




34.19 (*C*-9), 34.35 (*C*-3), 40.27 (*C*-11′), 40.72 (*C*-11), 40.86 (*C*-5), 126.02 (*C*-7), 126.30 (*C*-7′), 127.33 (*C*-1), 132.69 (*C*-8′), 132.91 (*C*-8), 136.01 (*C*-2–2′′′′), 136.50 (*C*-2–2′′′′), 142.72 (*C*-2–2′′′′), 143.25 (*C*-2–2′′′′), 143.70 (*C*-2–2′′′′), 170.22 (*C*-6,6′), 170.48 ppm (*C*-6′,6); MS (MALDI-TOF; CCA): *m/z*: 2252 $[M+K]^+$, 2236 $[M+Na]^+$, 2214 $[M+H]^+$; monoisotopic mass calcd for $C_{132}H_{190}N_{21}O_9^+$: 2213.51, found: 2213.55.

***(Boc-Met)$_3$3D0*: 1,3,5-tris-[L-(3-methylsulfanyl-1-propylcarbamoylpropyl)carbamic acid *tert*-butyl ester]benzene (12a)**: The hydroxysuccinimide ester **11a** (3.74 g, 10.8 mmol) was dissolved in dry dichloromethane under a nitrogen atmosphere, and the solution was cooled down to −10 °C. Afterwards DIPEA (1.57 mL, 1.16 g, 9.00 mmol) and a solution of core **4** (1.08 g, 3.00 mmol) in dry methanol was added dropwise. The reaction mixture was stirred for an additional 12 h, and during that time allowed to warm up to room temperature. After complete reaction the mixture was washed twice with sodium hydrogencarbonate solution and once with brine, and dried over magnesium sulfate. The solvent was removed under reduced pressure, and the crude product was subsequently purified by column chromatography (silica gel, dichloromethane containing 2% methanol as eluent) under a nitrogen atmosphere. The procedure yielded **12a** (2.40 g, 2.54 mmol, 84.7%) as a colorless foam. $R_f$ = 0.32 (dichloromethane/methanol = 19:1/v:v); m.p. 162 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.41 (s, 27H; $CCH_3$), 1.80 (m, 6H; $CH_2$), 1.98 (m, 3H; $CHCH_2$), 2.03 (m, 3H; $CHCH_2$), 2.08 (s, 9H; $SCH_3$), 2.55 (m, 6H + 6H; $CH_2Ar$ + $CH_2S$), 3.17 (m, 6H; $CH_2N$), 4.34 (m, br, 3H; CH), 5.80 (s, br, 3H; NH), 6.77 (s, 3H; Ar*H*), 7.17 ppm (s, br, 3H; NH); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 15.31 ($SCH_3$), 28.36 ($CCH_3$), 30.26 ($CH_2S$), 30.51 ($CH_2$), 31.98 ($CHCH_2$), 32.28 ($CH_2Ar$), 38.34 ($CH_2N$), 53.61 (CH), 79.91 ($CCH_3$), 126.45 (Ar*C*), 141.24 (Ar*C*), 155.90 ($CON_{Boc}$), 171.98 ppm (CON); MS (FAB+, MNBA/ethanol): *m/z* (%): 965 (1.5) $[M+Na]^+$, 943 (2.4) $[M+H]^+$, 57 (100) $[C_4H_9]^+$; elemental analysis calcd (%) for $C_{45}H_{78}N_6O_9S_3$ (943.3): C 57.29, H 8.33, N 8.91, S 10.20; found: C 57.24, H 8.29, N 8.81, S 10.26.

***(Boc-Phe)$_3$3D0*: 1,3,5-tris-[L-(2-phenyl-1-propylcarbamoylethyl)-carbamic acid *tert*-butyl ester]benzene (12b)**: The hydroxysuccinimide ester **11b** (3.91 g, 10.8 mmol) was dissolved in dry dichloromethane under a nitrogen atmosphere and cooled down to 0 °C. Subsequently DIPEA (1.57 mL, 1.16 g, 9.00 mmol) and a solution of **4** (1.08 g, 3.00 mmol) in dry methanol were added dropwise. The reaction mixture was stirred for additional 12 h, and during that time allowed to warm up to room temperature. After complete reaction the mixture was washed twice with sodium hydrogencarbonate solution and once with brine, dried over magnesium sulfate, and the solvent was evaporated in vacuo. Column chromatography (silica gel; dichloromethane containing 3% methanol as eluent) afforded **12b** (2.37 g, 2.39 mmol, 79.7%) as a colorless solid. $R_f$ = 0.46 (dichloromethane/methanol = 19:1/v:v); m.p. 190 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.34 (s, 27H; $CH_3$), 1.65 (m, 6H; $CH_2$), 2.39 (t, $^3J$(H,H) = 6.4 Hz, 6H; $CH_2Ar$), 3.02 (m, 6H; $CH_2N$), 3.02 (m, 6H + 3H; $CH_2N$ + $CHCH_2$), 3.10 (m, 3H; $CHCH_2$), 4.44 (m, br, 3H; CH), 5.64 (s, br, 3H; NH), 6.67 (s, 3H; Ar*H*), 6.81 (s, br, 3H; NH), 7.19 ppm (m, 15H; $ArH_{phe}$); $^{13}$C NMR (127 MHz, $CDCl_3$): $\delta$ = 28.31 ($CCH_3$), 30.45 ($CH_2$), 32.25 ($CH_2Ar$), 38.29 ($CHCH_2$), 38.95 ($CH_2N$), 55.42 (CH), 79.84 ($CCH_3$), 126.31 (Ar*C*), 126.65 ($ArC_{phe}$), 128.40 ($ArC_{phe}$), 129.35 ($ArC_{phe}$), 137.05 ($ArC_{phe}$), 141.05 (Ar*C*), 155.52 ($CON_{Boc}$), 171.69 ppm (CON); MS (FAB+, MNBA/methanol/DMSO): *m/z* (%): 1013 (0.3) $[M+Na]^+$, 991 (0.9) $[M+H]^+$, 120 (100) $[C_8H_{10}N]^+$; elemental analysis calcd (%) for $C_{57}H_{78}N_6O_9$ (991.3): C 69.06, H 7.93, N 8.48; found: C 69.10, H 7.83, N 8.29.

***(Boc/tBu-Asp)$_3$3D0*: 1,3,5-tris-(L-3-*tert*-butoxycarbonylamino-*N*-propylsuccinamic acid *tert*-butyl ester)benzene (12c)**: The hydroxysuccinimide ester **11c** (2.67 g, 6.90 mmol) was dissolved in dry dichloromethane and cooled down to −10 °C. DIPEA (1.02 mL, 775 mg, 6.00 mmol) and a solution of core **4** (718 mg, 2.00 mmol) in dry methanol were added dropwise. The reaction mixture was stirred for additional 12 h, and during that time allowed to warm up to room temperature. After complete reaction the mixture was washed twice with sodium hydrogencarbonate solution and once with brine, dried over magnesium sulfate, and the solvent was evaporated in vacuo. Column chromatography (silica gel, dichloromethane containing 3% methanol as eluent) afforded pure **12c** (1.88 g, 1.77 mmol; 88.5%) as a colorless foam. $R_f$ = 0.39 (dichloromethane/methanol = 19:1/v:v); m.p. 80 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.42 (s, 27H; $CH_3$), 1.43 (s, 27H; $CH_3$), 1.79 (quin, $^3J$(H,H) = 7.2 Hz, 6H; $CH_2$), 2.55 (t, $^3J$(H,H) = 7.4 Hz, 6H; $CH_2Ar$), 2.62 (dd, $^2J$(H,H) = 16.7 Hz, $^3J$(H,H) = 6.7 Hz, 3H; $CHCH_2$), 2.80 (dd, $^2J$(H,H) = 16.7 Hz, $^3J$(H,H) = 5.0 Hz, 3H; $CHCH_2$), 3.19 (q, $^3J$(H,H) = 6.6 Hz, 6H; $CH_2NH$), 4.42 (m, br, 3H; CH), 5.79 (s, br, 3H; CHN*H*), 6.71 (s, br, 3H; CONH), 6.78 ppm (s, 3H; Ar*H*); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 28.02 ($CCH_3$), 28.32 ($CCH_3$), 30.81 ($CH_2$), 32.56 ($CH_2Ar$), 37.49 ($CHCH_2$), 38.75 ($CH_2N$), 50.85 (CH), 80.26 ($CCH_3$), 81.45 ($CCH_3$), 126.31 (Ar*C*), 141.42 (Ar*C*), 155.57 ($CON_{Boc}$), 170.93 ($CO t_{Bu}$), 171.09 ppm (CON); MS (FAB+, MNBA/DCM): *m/z* (%): 1064 (0.4) $[M+H]^+$, 964 (2.3) $[M-C_5H_8O_2]^+$, 57 (100) $[C_4H_9]^+$; elemental analysis calcd (%) for $C_{54}H_{90}N_6O_{15}$ (1063.3): C 61.00, H 8.53, N 7.90; found: C 60.84, H 8.22, N 7.73.

***(Met)$_3$3D0*: 1,3,5-tris(L-2-amino-4-methylsulfanyl-*N*-propylbutyramide)-benzene tristrifluoroacetate (13a)**: Under a nitrogen atmosphere dendrimer **12a** (755 mg, 800 μmol) was dissolved in a ′cleavage cocktail′ mixture of dichloromethane/trifluoroacetic acid/ethanedithiol/thioanisole/methanol/triisopropylsilane (50:37.5:5:5:2:0.5/v:v), and stirred for 1 h at room temperature. After complete reaction (TLC) the ′cleavage cocktail′ mixture was removed in vacuo to give the deprotected methionine G0 dendrimer **13a** (778 mg, 790 μmol; 98.8%) as a colorless oil, which could be lyophilized from water. M.p. 84–86 °C; $^1$H NMR (500 MHz, [D$_4$]methanol): $\delta$ = 1.87 (quin, $^3J$(H,H) = 7.4 Hz, 6H; $CH_2$), 2.16 (s, 9H; $SCH_3$), 2.17 (m, 6H; $CHCH_2$), 2.62 (m, 6H; $SCH_2$), 2.65 (m, 6H; $CH_2Ar$), 3.25 (m, 3H; $CH_2N$), 3.34 (m, 3H; $CH_2N$), 3.99 (t, $^3J$(H,H) = 6.6 Hz, 3H; CH), 6.92 ppm (s, 3H; Ar*H*); $^{13}$C NMR (127 MHz, [D$_4$]methanol): $\delta$ = 15.16 ($SCH_3$), 29.93 ($SCH_2$), 32.10 ($CH_2$), 32.19 ($CHCH_2$), 34.10 ($CH_2Ar$), 40.37 ($CH_2NH$), 53.87 (CH), 127.28 (ArC), 143.06 (ArC), 169.68 ppm (CON); MS (EI, 80 eV, 290 °C): *m/z* (%): 642 (6.5) $[M]^+$, 539 (88.2) $[M-C_4H_9NS]^+$, 350 (100) $[M-C_{12}H_{26}N_3OS_2]^+$; HRMS: *m/z*: monoisotopic mass calcd for $C_{27}H_{48}N_6O_3S_2^+$: 568.32366 $[M-C_3H_6S]^+$, found: 568.32294.

***(Phe)$_3$3D0*: 1,3,5-Tris(L-2-amino-3-phenyl-*N*-propylpropionamide)benzene tristrifluoroacetate (13b)**: The L-phenylalanine G0 dendrimer **12b** (991 mg, 1.00 mmol) was dissolved in dichloromethane/methanol = 19:1/v:v (40 mL). Trifluoroacetic acid (5 mL) was added, and the reaction mixture was stirred at room temperature for 1 h. After complete reaction (TLC) the solvent was removed in vacuo to afford the deprotected L-phenylalanine dendrimer **13b** (1.02 g, 987 μmol; 98.7%) as a colorless solid. M.p. 115–117 °C; $^1$H NMR (500 MHz, [D$_4$]methanol): $\delta$ = 1.72 (quin, $^3J$(H,H) = 7.5 Hz, 6H; $CH_2$), 2.49 (t, $^3J$(H,H) = 7.6 Hz, 6H; $CH_2Ar$), 3.15 (m, 3H; $CH_2N$), 3.16 (m, 3H; $CH_2N$), 3.26 (m, 6H; $CHCH_2$), 4.09 (t, $^3J$(H,H) = 7.5 Hz, 3H; CH), 6.81 (s, 3H; Ar*H*), 7.32 (m, 9H; $ArH_{phe}$), 7.35 ppm (m, 6H; $ArH_{phe}$); $^{13}$C NMR (127 MHz, [D$_4$]methanol): $\delta$ = 32.16 ($CH_2$), 34.22 ($CH_2Ar$), 39.02 ($CH_2N$), 40.54 ($CHCH_2$), 56.15 (CH), 127.48 (Ar*C*), 129.08 ($ArC_{phe}$), 130.33 ($ArC_{phe}$), 130.80 ($ArC_{phe}$), 136.02 ($ArC_{phe}$), 143.21 (Ar*C*), 169.73 ppm (CON); MS (EI, 80 eV, 150–180 °C): *m/z* (%): 690 (2.8) $[M]^+$, 513 (59.3) $[M-C_{10}H_{13}N_2O]^+$, 44 (100) $[C_3H_8]^+$; HRMS: *m/z*: monoisotopic mass calcd for $C_{42}H_{54}N_6O_3$: 690.42622, found: 690.42572.

***(Asp)$_3$3D0*: 1,3,5-Tris(L-3-amino-*N*-propylsuccinamic acid)benzene tristrifluoroacetate (13c)**: Trifluoroacetic acid (4 mL) was added to a solution of **12c** (106 mg, 100 μmol) in dichloromethane (10 mL), and the reaction mixture was stirred for 2 h at room temperature. After complete reaction (TLC) the solvent was removed in vacuo to yield the deprotected aspartic acid G0 dendrimer (92 mg, 98 μmol; 98%) as a colorless oil,

which could be lyophilized from water. M.p. 106–109 °C; $^1$H NMR (500 MHz, [D$_4$]methanol): $\delta$ = 1.86 (quin, $^3J$(H,H) = 7.3 Hz, 6H; $CH_2$), 2.64 (t, $^3J$(H,H) = 7.6 Hz, 6H; $CH_2$Ar), 2.87 (dd, $^2J$(H,H) = 17.5 Hz, $^3J$(H,H) = 7.8 Hz, 3H; CHC$H_2$), 2.94 (dd, $^2J$(H,H) = 17.6 Hz, $^3J$(H,H) = 5.2 Hz, 3H; CHC$H_2$), 3.23 (m, 3H; C$H_2$N), 3.31 (m, 3H; C$H_2$N), 4.19 (m, 3H; CH), 6.92 ppm (s, 3H; Ar*H*); $^{13}$C NMR (63 MHz, [D$_4$]methanol): $\delta$ = 31.92 ($CH_2$), 33.85 ($CH_2$), 36.41 ($CH_2$), 40.27 ($CH_2$), 51.22 (CH), 127.39 (Ar*C*), 143.00 (Ar*C*), 169.19 (CON), 173.12 ppm (COO); MS (FAB-, MNBA/methanol): *m*/*z* (%): 593 (2.3) [*M*−H]$^-$, 113 (89.9) [$C_4H_5N_2O_2$]$^-$; monoisotopic mass calcd for $C_{27}H_{41}N_6O_9^-$: 593.3, found: 593.3 [*M*−H]$^-$.

***(Boc-Dpa)$_3$3D0*: 1,3,5-tris[(2-*tert*-butoxycarbonylamino-1-propylcarbamoylethyl)carbamic acid *tert*-butyl ester]benzene (15)**: A solution of **14** (4.11 g, 13.5 mmol) in a mixture of dry dichloromethane/DMF (14:1/v:v) was cooled down to - 20 °C. DIPEA (2.30 mL, 1.74 g, 13.5 mmol) and a solution of TBTU (4.43 g, 14.1 mmol) in DMF were added slowly and allowed to warm to room temperature. The reaction mixture was stirred at room temperature for additional 30 min, cooled down to −20 °C again, followed by the dropwise addition of DIPEA (3.80 mL, 2.91 g; 22.5 mmol) and a solution of the trishydrochloride core **4** (1.08 g, 3.00 mmol) in dry methanol. The mixture was stirred for additional 12 h and during that time allowed to warm to room temperature. After complete reaction (TLC), the mixture was washed twice with sodium hydrogencarbonate solution, once with brine, and dried over magnesium sulfate. The solvent was removed under reduced pressure and column chromatography (silica gel, dichloromethane containing 4% methanol as eluent) afforded the desired product (2.66 g, 2.40 mmol, 80.0%) as a colorless foam. $R_f$ = 0.21 (dichloromethane/methanol = 19:1/v:v); m.p. 113 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.38 (s, 27H; $CH_3$), 1.40 (s, 27H; $CH_3$), 1.79 (m, 6H; $CH_2$), 2.54 (t, $^3J$(H,H) = 7.0 Hz, 6H; $CH_2$Ar), 3.14 (m, 6H; $CH_2$N), 3.44 (m, 6H; CHC$H_2$), 4.26 (m, br, 3H; CH), 5.56 (s, br, 3H; $NH_{Boc}$), 6.05 (s, br, 3H; $NH_{Boc}$), 6.79 (s, 3H; Ar*H*), 7.08 ppm (s, br, 3H; CONH); $^{13}$C NMR (127 MHz, $CDCl_3$): $\delta$ = 28.30 (C$CH_3$), 30.39 ($CH_2$), 32.08 ($CH_2$Ar), 38.14 ($CH_2$N), 42.56 (CH$CH_2$), 55.60 (CH), 77.25 (C$CH_3$), 79.66 (C$CH_3$), 126.52 (Ar*C*), 141.14 (Ar*C*), 156.10 ($CON_{Boc}$), 157.05 ($CON_{Boc}$), 170.75 ppm (CON); MS (FAB+, MNBA/DCM/methanol): *m*/*z* (%): 1131 (0.9) [*M*+Na]$^+$, 1009 (4.8) [*M*−$C_5H_8O_2$+H]$^+$, 609 (2.2) [*M*−5($C_5H_8O_2$)+H]$^+$, 508 (21.2) [*M*−6($C_5H_8O_2$)]$^+$, 57 (100) [$C_4H_9$]$^+$; elemental analysis calcd (%) for $C_{54}H_{93}N_9O_{15}$ (1108.4): C 58.52, H 8.46, N 11.37; found: C 58.31, H 8.23, N 11.33.

***(Dpa)$_3$D0*: 1,3,5-tris(2,3-diamino-*N*-propylpropionamide)benzene hexatrifluoroacetate (16)**: Trifluoroacetic acid (5 mL) was added to a solution of the Boc-protected diaminopropionic acid G0 dendrimer **15** (554 mg, 500 μmol) in dichloromethane (10 mL), and the mixture was stirred for 1 h at room temperature. After complete reaction (TLC) the solvent and the excess of trifluoroacetic acid were removed in vacuo. Lyophilization from water yielded deprotected **16** (595 mg, 499 μmol; 99.8%) as a colorless solid. M.p. 101–103 °C; $^1$H NMR (500 MHz, [D$_4$]methanol): $\delta$ = 1.89 (quin, $^3J$(H,H) = 7.4 Hz, 6H; $CH_2$), 2.66 (t, $^3J$(H,H) = 7.7 Hz, 6H; $CH_2$Ar), 3.25 (m, 3H; $CH_2$N), 3.38 (m, 3H; CHC$H_2$), 3.40 (m, 3H; $CH_2$N), 3.47 (dd, $^2J$(H,H) = 13.7 Hz, $^3J$(H,H) = 5.5 Hz, 3H; CHC$H_2$), 4.19 (t, $^3J$(H,H) = 6.0 Hz, 3H; CH), 6.94 ppm (s, 3H; Ar*H*); $^{13}$C NMR (63 MHz, [D$_4$]methanol): $\delta$ = 31.77 (s, $CH_2$), 33.97 (s, $CH_2$Ar), 40.65 (s, $CH_2$N), 41.13 (s, $CHCH_2$), 52.23 (s, CH), 118.03 (q, $^1J$(C,F) = 294.4 Hz, *C*$F_3CO_2$H), 127.34 (s, Ar*C*), 142.97 (s, Ar*C*), 163.35 (q, $^2J$(C,F) = 34.2 Hz, $CF_3CO_2$H), 166.99 ppm (s, CON); MS (FAB+, MNBA/DMSO): *m*/*z* (%): 640 (1.1) [*M*+Cs]$^+$, 508 (3.2) [*M*+H]$^+$; elemental analysis calcd (%) for $C_{36}F_{18}H_{51}N_9O_{15}$ (1191.8): C 36.28, H 4.31, N 10.58; found: C 35.89, H 4.38, N 10.70.

**3,5-Bis[L-(3-methylsulfanyl-1-propylcarbamoylpropyl)carbamic acid *tert*-butyl ester]benzoic acid ethyl ester (18a)**: The Boc-L-methionine hydroxysuccinimide ester **11a** (1.59 g, 4.60 mmol) was dissolved in dry dichloromethane under a nitrogen atmosphere, and cooled down to −10 °C. DIPEA (680 μL, 517 mg; 4.00 mmol) and the G1 hydrochloride **17** (675 mg, 2.00 mmol) dissolved in dry methanol were added dropwise. The reaction mixture was allowed to warm up to room temperature while being stirred for additional 12 h. After complete reaction (TLC) the organic layer was washed twice with sodium hydrogencarbonate solution and once with brine. The organic phase was subsequently dried over magnesium sulfate, and the solvent was removed in vacuo. The crude product was purified by column chromatography (silica gel, dichloromethane containing 2–3% methanol as eluent) to afford the desired product (1.15 g, 1.58 mmol, 79.0%) as a colorless foam. $R_f$ = 0.09 (dichloromethane/methanol = 49:1/v:v); m.p. 75 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.34 (t, $^3J$(H,H) = 7.1 Hz, 3H; $CH_2CH_3$), 1.37 (s, 18 H $CCH_3$), 1.81 (quin, $^3J$(H,H) = 7.0 Hz, 4H; $CH_2$), 1.92 (m, 2H; CHC$H_2$), 2.02 (m, 2H; CHC$H_2$), 2.05 (s, 6H; $SCH_3$), 2.51 (t, $^3J$(H,H) = 7.3 Hz, 4H; $CH_2$Ar), 2.61 (m, 4H; $SCH_2$), 3.15 (m, 4H; $CH_2$N), 4.29 (m, br, 2H; CH), 4.31 (q, $^3J$(H,H) = 7.1 Hz, 2H; C$H_2$$CH_3$), 5.69 (d, br, $^3J$(H,H) = 6.1 Hz, 2H; CHN*H*), 7.05 (s, br, 2H; CONH), 7.14 (s, 1H; Ar*H*), 7.62 ppm (s, 1H; Ar*H*); $^{13}$C NMR (63 MHz, $CDCl_3$): $\delta$ = 14.26 ($CH_2CH_3$), 15.24 ($SCH_3$), 28.28 (C$CH_3$), 30.23 ($CH_2$), 30.44 ($CH_2$), 32.00 ($CH_2$), 32.11 ($CH_2$), 38.16 ($CH_2$), 53.62 (CH), 60.83 ($CH_2CH_3$), 79.83 (C$CH_3$), 127.16 (Ar*C*), 130.78 (Ar*C*), 133.60 (Ar*C*), 141.42 (Ar*C*), 155.89 ($CON_{Boc}$), 166.63 (CON), 172.09 ppm (COO); MS (FAB+, MNBA/DCM): *m*/*z* (%): 727 (10.3) [*M*+H]$^+$, 627 (22.2) [*M*−$C_5H_9O_2$+H]$^+$, 527 (62.0) [*M*−2($C_5H_9O_2$)+H]$^+$, 57 (100) [$C_4H_9$]$^+$; elemental analysis calcd (%) for $C_{35}H_{58}N_4O_8S_2$ (727.0): C 57.82, H 8.04, N 7.71; found: C 57.54, H 7.94, N 7.60.

**3,5-Bis[L-(2-phenyl-1-propylcarbamoylethyl)carbamic acid *tert*-butyl ester]benzoic acid ethyl ester (18b)**: A solution of the Boc-L-phenylalanine hydroxysuccinimide ester **11b** (5.22 g, 14.4 mmol) in dry dichloromethane was cooled down to 5 °C. DIPEA (2.09 mL, 1.55 g, 12.0 mmol) and the bishydrochloride dendron **17** (2.02 g, 6.00 mmol), dissolved in dry methanol, were added slowly. The reaction mixture was allowed to warm up to room temperature slowly and was stirred for an additional 12 h. After complete reaction (TLC) the organic layer was washed twice with sodium hydrogencarbonate solution and once with brine. The organic phase was subsequently dried over magnesium sulfate. The solvent was evaporated under reduced pressure, and column chromatography (silica gel, dichloromethane containing 2% methanol as eluent) afforded the Boc-L-phenylalanine dendron (3.72 g, 4.90 mmol; 81.7%) as a colorless solid. $R_f$ = 0.59 (dichloromethane/methanol = 19:1/v:v); m.p. 121 °C; $^1$H NMR (250 MHz, $CDCl_3$): $\delta$ = 1.35 (s, 18H; $CCH_3$), 1.36 (t, $^3J$(H,H) = 7.3 Hz, 3H; $CH_2CH_3$), 1.69 (m, 4H; $CH_2$), 2.47 (t, $^3J$(H,H) = 7.0 Hz, 4H; $CH_2$Ar), 3.03 (m, 4H; $CH_2$N), 3.05 (m, 4H; CHC$H_2$), 4.40 (m, br, 2H; CH), 4.33 (q, $^3J$(H,H) = 7.1 Hz, 2H; C$H_2$$CH_3$), 5.61 (d, br, $^3J$(H,H) = 5.9 Hz, 2H; CHN*H*), 6.68 (s, br, 2H; CONH), 7.05 (s, 1H; Ar*H*), 7.19 (m, 10H; Ar$H_{Phe}$), 7.60 ppm (s, 2H; Ar*H*); $^{13}$C NMR (63 MHz, $CDCl_3$): $\delta$ = 14.33 ($CH_2CH_3$), 28.31 (C$CH_3$), 30.42 ($CH_2$), 32.11 ($CH_2$), 38.25 ($CH_2$), 38.82 ($CH_2$), 52.00 (CH), 60.87 ($CH_2CH_3$), 79.98 (C$CH_3$), 126.78 (Ar*C*), 127.16 (Ar*C*), 128.51 (Ar*C*), 129.34 (Ar*C*), 130.84 (Ar*C*), 133.57 (Ar*C*), 136.98 (Ar*C*), 141.47 (Ar*C*), 155.61 ($CON_{Boc}$), 166.69 (CON), 171.64 ppm (COO); MS (FAB+, MNBA/DCM): *m*/*z* (%): 781 (0.2) [*M*+Na]$^+$, 759 (5.0) [*M*+H]$^+$, 659 (11.2) [*M*−$C_5H_9O_2$+H]$^+$, 559 (35.8) [*M*−2($C_5H_9O_2$)+H]$^+$, 120 (100) [$C_8H_{10}$N]$^+$, 57 (70.1) [$C_4H_9$]$^+$; elemental analysis calcd (%) for $C_{43}H_{58}N_4O_8$ (758.9): C 68.05, H 7.70, N 7.38; found: C 67.79, H 7.66, N 7.31.

**3,5-Bis[L-(3-methylsulfanyl-1-propylcarbamoylpropyl)carbamic acid *tert*-butyl ester]benzoic acid (19a)**: The L-methionine G1 dendron **18a** (2.18 g, 3.00 mmol) was dissolved in methanol/water/THF (3:1:1/v:v) under a nitrogen atmosphere, a 1 M KOH solution (24.0 mL, 24.0 mmol) was added, and the reaction mixture was subsequently heated to 40 °C for 12 h. After complete reaction (TLC), acetic acid was added to give pH 5, and the product subsequently extracted with dichloromethane. The united organic layers were dried over magnesium sulfate and the solvent was removed in vacuo. Chromatographic workup (silica gel, dichloromethane containing 4% methanol as eluent) gave the desired product (1.68 g, 2.40 mmol; 80.0%) as a colorless foam. $R_f$ = 0.11 (dichloromethane/methanol = 19:1/v:v); m.p. 89 °C; $^1$H NMR (250 MHz, [D$_4$]methanol): $\delta$ = 1.48 (s, 18H; $CCH_3$), 1.88 (m, 4H; $CH_2$), 1.91 (m, 2H; CHC$H_2$), 2.03 (m, 2H; CHC$H_2$), 2.08 (s, 6H; $SCH_3$), 2.53 (m, 4H; $SCH_2$), 2.67 (t, $^3J$(H,H) = 7.8 Hz, 4H; $CH_2$Ar), 3.22 (m, 4H; $CH_2$N), 4.14 (t, $^3J$(H,H) = 6.7 Hz, 2H; CH), 7.30 (s, 1H; Ar*H*), 7.69 (s, 2H; Ar*H*), 8.05 ppm (m, br, 2H; CONH); $^{13}$C NMR (63 MHz, [D$_4$]methanol): $\delta$ = 15.39 ($SCH_3$), 28.71 (C$CH_3$), 31.15 ($CH_2$), 31.15 ($CH_2$), 31.85 ($CH_2$), 32.95 ($CH_2$), 33.59 ($CH_2$), 55.12 (CH), 80.68 (C$CH_3$), 128.31 (Ar*C*), 131.98 (Ar*C*), 134.37 (Ar*C*), 143.22 (Ar*C*), 157.51 ($CON_{Boc}$), 169.98 (CON), 174.43 ppm (COO); MS (FAB+, MNBA/DCM): *m*/*z* (%): 699 (4.8) [*M*+H]$^+$, 599 (7.9) [*M*−$C_5H_9O_2$+H]$^+$, 499 (23.5) [*M*−2($C_5H_9O_2$)+H]$^+$, 57 (100) [$C_4H_9$]$^+$; monoisotopic mass calcd for $C_{33}H_{55}N_4O_8S_2^+$: 699.4, found: 699.5 [*M*+H]$^+$.

**3,5-Bis[L-(2-phenyl-1-propylcarbamoylethyl)carbamic acid tert-butyl ester]benzoic acid (19b)**: A 1 M KOH solution (13.6 mL, 13.6 mmol) was added to a solution of the phenylalanine G1 dendron **18b** (1.29 g, 1.70 mmol) in methanol/water/THF (3:1:1), and the reaction mixture was stirred at 50 °C for 12 h. After complete saponification (TLC), acetic acid was added to give pH 5. The product was subsequently extracted with dichloromethane, and the organic phases were dried with magnesium sulfate. Evaporation of the solvent and subsequent chromatographic purification (silica gel, dichloromethane containing 4% methanol) afforded the G1 acid **19b** (1.15 g, 1.57 mmol, 92.4%) as a colorless foam. $R_f$ = 0.25 (dichloromethane/methanol = 19:1/v:v); m.p. 152 °C; $^1$H NMR (250 MHz, [D$_4$]methanol): $\delta$ = 1.41 (s, 18H; $CCH_3$), 1.75 (quin, $^3J$(H,H) = 7.3 Hz, 4H; $CH_2$), 2.59 (t, $^3J$(H,H) = 7.7 Hz, 4H; $CH_2$Ar), 2.90 (m, 2H; CHC$H_2$), 3.06 (m, 2H; CHC$H_2$), 3.19 (m, 4H; $CH_2$N), 4.32 (t, $^3J$(H,H) = 7.3 Hz, 2H; CH), 7.25 (s, 1H; Ar$H$), 7.28 (m, 10H; Ar$H_{phe}$), 7.70 (s, 2H; Ar$H$), 8.00 ppm (m, br, CONH); $^{13}$C NMR (63 MHz, [D$_4$]methanol): $\delta$ = 28.67 ($CCH_3$), 31.88 ($CH_2$), 33.65 ($CH_2$), 39.52 ($CH_2$), 39.84 ($CH_2$), 57.62 (CH), 80.66 ($CCH_3$), 127.75 (Ar$C$), 128.37 (Ar$C$), 129.44 (Ar$C$), 130.39 (Ar$C$), 132.11 (Ar$C$), 134.46 (Ar$C$), 138.55 (Ar$C$), 143.46 (Ar$C$), 157.46 ($CON_{Boc}$), 170.09 (CON), 174.17 ppm (COO); MS (FAB+, MNBA/DCM): $m/z$ (%): 753 (6.9) $[M+Na]^+$, 731 (14.2) $[M+H]^+$, 631 (19.6) $[M-C_5H_9O_2+H]^+$, 531 (67.8) $[M-2(C_5H_9O_2)+H]^+$, 120 (100) $[C_8H_{10}N]^+$, 57 (38.2) $[C_4H_9]^+$; monoisotopic mass calcd for $C_{41}H_{54}N_4NaO_8^+$: 753.4, found: 753.5 $[M+Na]^+$.

***(Boc-Met)$_6$6D1*: 1,3,5-tris-{3,5-bis[L-(3-methylsulfanyl-1-propylcarbamoylpropyl)carbamic acid *tert*-butyl ester]-*N*-propylbenzamide}benzene (20a)**: The G1 acid **19b** (1.10 g, 1.58 mmol) was dissolved in dry dichloromethane under a nitrogen atmosphere. HOBt (246 mg, 1.61 mmol) was added, and the mixture was stirred at room temperature for 15 min. Afterwards the reaction mixture was cooled down to −20 °C, EDC (323 mg, 1.68 mmol) was added, and the flask was allowed to warm up to room temperature while being stirred for an additional 2 h. After the active ester had formed (TLC), the reaction was cooled down to −30 °C, DIPEA (726 μL, 552 mg, 4.30 mmol), and the trishydrochloride core **4** (123 mg, 340 μmol), dissolved in dry methanol (5 mL), were added dropwise. The reaction mixture was stirred for additional 18 h, and during that time was allowed to warm up to room temperature slowly. After complete reaction (TLC), the mixture was washed twice with sodium hydrogencarbonate solution and once with brine, and the organic layer was dried over magnesium sulfate. The solvent was evaporated under reduced pressure and the crude product purified by column chromatography (silica gel, dichloromethane containing 2% methanol as eluent) to afford the desired product (421 mg, 184 μmol, 54.1%) as a colorless foam. $R_f$ = 0.21 (dichloromethane/methanol = 19:1/v:v); m.p. 171 °C; $^1$H NMR (500 MHz, [D$_4$]methanol): $\delta$ = 1.39 (s, 54H; $CCH_3$), 1.80 (m, 12H; $CH_2$), 1.83 (m, 6H; CHC$H_2$), 1.92 (m, 6H; $CH_2$), 1.97 (m, 6H; CHC$H_2$), 2.05 (s, 18H; $SCH_3$), 2.49 (m, 12H; $SCH_2$), 2.63 (m, 6H + 12H; $CH_2$Ar + $CH_2$S), 3.17 (m, 12H; $CH_2$N), 3.38 (m, 6H; $CH_2$N), 4.18 (m, br, 6H; CH), 6.95 (s, 3H; Ar$H$), 7.21 (s, 3H; Ar$H$), 7.46 ppm (s, 6H; Ar$H$); $^{13}$C NMR (63 MHz, [D$_4$]methanol): $\delta$ = 15.51 ($SCH_3$), 28.71 ($CCH_3$), 30.94 ($CH_2$), 31.34 ($CH_2$), 31.65 ($CH_2$), 32.84 ($CH_2$), 33.32 ($CH_2$), 33.95 ($CH_2$), 39.37 ($CH_2$), 40.48 ($CH_2$), 54.79 (CH), 80.66 ($CCH_3$), 125.75 (Ar$C$), 126.96 (Ar$C$), 132.53 (Ar$C$), 135.56 (Ar$C$), 142.67 (Ar$C$), 142.74 (Ar$C$), 157.09 ($CON_{Boc}$), 169.86 (CON), 173.90 ppm (CON); MS (MALDI-TOF, dithranol): $m/z$: 2330 $[M+K]^+$, 2313 $[M+Na]^+$; monoisotopic mass calcd for $C_{114}H_{183}N_{15}NaO_{21}S_6^+$: 2313.19, found: 2313.55.

***(Boc-Phe)$_6$6D1*: 1,3,5-tris-{3,5-bis[L-(2-phenyl-1-propylcarbamoylethyl)-carbamic acid *tert*-butyl ester]-*N*-propylbenzamide}benzene (20b)**: HOBt (297 mg, 2.20 mmol) was added to a solution of the G1 acid **19b** (1.53 mg, 2.10 mmol) in a mixture of dry dichloromethane/DMF (19:1/v:v), and the reaction mixture was stirred for 15 min at room temperature. The flask was cooled down to 0 °C, EDC (441 mg, 2.30 mmol) was added, and the solution was stirred for additional 2 h while warming up to room temperature. When the formation of the active ester was complete (TLC), the reaction mixture was cooled down to 5 °C again, and DIPEA (1.03 mL, 763 mg, 5.90 mmol) and the trishydrochloride core **4** (179 mg, 500 μmol), dissolved in dry methanol (5 mL), were added slowly. The reaction mixture was allowed to warm up to room temperature slowly while being stirred for additional 18 h. After complete reaction (TLC) the solution was washed twice with sodium hydrogencarbonate solution and once with brine, and the organic layer was dried over magnesium sulfate. Evaporation of the solvent under reduced pressure and subsequent purification by column chromatography (silica gel, dichloromethane containing 3% methanol as eluent) gave the Boc-protected phenylalanine-G1-dendrimer **20b** (575 mg, 241 μmol, 48.2%) as a colorless foam. $R_f$ = 0.32 (dichloromethane/methanol = 19:1/v:v); m.p. 173 °C; $^1$H NMR (500 MHz, $CDCl_3$/[D$_4$]methanol): $\delta$ = 1.37 (s, 54H; $CCH_3$), 1.72 (quin, br, 12H; $CH_2$), 1.96 (quin, $^3J$(H,H) = 7.3 Hz, 6H; $CH_2$), 2.51 (t, br, 12H; $CH_2$Ar), 2.66 (t, $^3J$(H,H) = 7.5 Hz, 6H; $CH_2$Ar), 2.96 (m, 6H; CHC$H_2$), 3.03 (m, 6H; CHC$H_2$), 3.11 (m, 12H; $CH_2$N), 3.42 (m, 6H; $CH_2$N), 4.31 (m, br, 6H; CH), 6.92 (s, 3H; Ar$H$), 7.06 (s, 3H; Ar$H$), 7.19 (m, 18H; Ar$H_{phe}$), 7.25 (m, 12H; Ar$H_{phe}$), 7.38 ppm (s, 6H; Ar$H$); $^{13}$C NMR (127 MHz, [D$_4$]methanol): $\delta$ = 28.50 ($CCH_3$), 30.78 ($CH_2$), 31.30 ($CH_2$), 32.86 ($CH_2$), 33.61 ($CH_2$), 38.94 ($CH_2$), 39.21 ($CH_2$), 40.15 ($CH_2$), 56.47 (CH), 80.44 ($CCH_3$), 125.39 (Ar$C$), 126.68(Ar$C$), 127.20 (Ar$C$), 128.86 (Ar$C$), 129.43 (Ar$C$), 129.70 (Ar$C$), 132.18 (Ar$C$), 135.16 (Ar$C$), 137.32 (Ar$C$), 142.27 (Ar$C$), 156.41 ($CON_{Boc}$), 169.40 (CON), 172.90 ppm (CON); MS (MALDI-TOF, dithranol): $m/z$: 2425 $[M+K]^+$, 2409 $[M+Na]^+$; monoisotopic mass calcd for $C_{114}H_{183}N_{15}NaO_{21}S_6^+$: 2409.36, found: 2409.36.

***(Boc/tBu-Asp)$_6$6D1*: 1,3,5-tris-[3,5-bis(L-3-*tert*-butoxycarbonylamino-*N*-propylsuccinamic acid *tert*-butyl ester)-*N*-propylbenzamide]benzene (20c)**: A solution of the L-aspartic acid hydroxysuccinimide ester **11c** (522 mg, 1.35 mmol) in dry dichloromethane was cooled down to −10 °C. The G1 dendrimer **8** (238 mg, 150 μmol), dissolved in dry methanol (3 mL), and DIPEA (153 μL, 116 mg, 900 μmol) were added dropwise. The solution was stirred at −10 °C for 1 h, and was afterwards allowed to warm up to room temperature slowly while being stirred for an additional 18 h. After complete reaction (TLC) the mixture was washed twice with sodium hydrogencarbonate solution and once with brine. The organic phase was dried over magnesium sulfate, the solvent subsequently evaporated in vacuo, and the crude product was purified by column chromatography (silica gel, dichloromethane containing 3% methanol as eluent) to afford pure **20c** (269 mg, 106 μmol; 70.7%) as a colorless foam. $R_f$ = 0.15 (dichloromethane/methanol = 19:1/v:v); m.p. 107 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.39 (s, 54H; CC$H_3$), 1.41 (s, 54H; CC$H_3$), 1.75 (quin, $^3J$(H,H) = 7.0 Hz, 12H; $CH_2$), 1.95 (quin, $^3J$(H,H) = 7.2 Hz, 6H; $CH_2$), 2.55 (t, $^3J$(H,H) = 7.2 Hz, 12H; $CH_2$Ar), 2.64 (t, $^3J$(H,H) = 7.7 Hz, 6H; $CH_2$Ar), 2.44 (dd, $^2J$(H,H) = 16.8 Hz, $^3J$(H,H) = 6.3 Hz, 6H; CHC$H_2$), 2.79 (dd, $^2J$(H,H) = 16.6 Hz, $^3J$(H,H) = 5.2 Hz, 6H; CHC$H_2$), 3.13 (m, 6H; C$H_2$N), 3.19 (m, 6H; C$H_2$N), 3.42 (m, 6H; $CH_2$N), 4.42 (m, br, 6H; CH), 5.80 (d, br, $^3J$(H,H) = 7.7 Hz, 6H; CHN$H$), 6.75 (t, br, $^3J$(H,H) = 5.6 Hz, 6H; CONH), 6.90 (s, 3H; Ar$H$), 7.05 (s, 3H; Ar$H$), 7.17 (t, br, 3H; CONH), 7.37 ppm (s, 6H; Ar$H$); $^{13}$C NMR (127 MHz, $CDCl_3$): $\delta$ = 28.02 ($CCH_3$), 28.33 ($CCH_3$), 30.64 ($CH_2$), 30.78 ($CH_2$), 32.28 ($CH_2$), 33.07 ($CH_2$), 37.54 ($CH_2$), 38.43 ($CH_2$), 39.61 ($CH_2$), 51.01 (CH), 80.23 ($CCH_3$), 81.55 ($CCH_3$), 125.07 (Ar$C$), 126.34 (Ar$C$), 131.56 (Ar$C$), 134.86 (Ar$C$), 141.51 (Ar$C$), 141.69 (Ar$C$), 155.59 ($CON_{Boc}$), 167.95 (CON), 170.97 ($CO_{tBu}$), 171.06 ppm (CON); MS (MALDI-TOF, dithranol): $m/z$: 2570 $[M+K]^+$, 2554 $[M+Na]^+$; monoisotopic mass calcd for $C_{132}H_{207}N_{15}NaO_{33}^+$: 2553.49, found: 2553.59 .

***(Met)$_6$6D1*: 1,3,5-tris-[3,5-bis(L-2-amino-4-methylsulfanyl-*N*-propylbutyramide)-*N*-propylbenzamide]benzene hexatrifluoroacetate (21a)**: The Boc-protected G1 dendrimer **20a** (150 mg, 65.4 μmol) was dissolved in a 'cleavage cocktail' mixture (10 mL) containing dichloromethane/trifluoroacetic acid/ethanedithiol/thioanisole/methanol/triisopropylsilane (50:37.5:5:5:2:0.5/v:v) under a nitrogen atmosphere, and stirred for 1 h at room temperature. After complete deprotection (TLC), the 'cleavage cocktail' mixture was removed by repeated evaporation under reduced pressure by using methanol as solvent. The residue was dried under high vacuum, and lyophilization from water afforded the desired product (150 mg, 63.1 μmol, 96.5%) as a colorless solid. M.p. 91–92 °C; $^1$H NMR (500 MHz, [D$_4$]methanol): $\delta$ = 1.91 (quin, $^3J$(H,H) = 7.4 Hz, 12H; $CH_2$), 1.97 (quin, $^3J$(H,H) = 7.7 Hz, 6H; $CH_2$), 2.14 (s, 18H; $SCH_3$), 2.13 (m, 6H; CHC$H_2$), 2.17 (m, 6H; CHC$H_2$), 2.61 (m, 12H; $CH_2$S), 2.69 (m, 6H; $CH_2$Ar), 2.72 (m, 12H; $CH_2$Ar), 3.28 (m, 6H; C$H_2$N), 3.33 (m, 6H; C$H_2$N), 3.43 (t, $^3J$(H,H) = 7.1 Hz, 6H; $CH_2$N), 4.00 (t, $^3J$(H,H) = 6.6 Hz, 6H; CH), 6.97 (s, 3H; Ar$H$), 7.28 (s, 3H; Ar$H$), 7.51 ppm (s, 6H; Ar$H$); $^{13}$C NMR (127 MHz, [D$_4$]methanol): $\delta$ = 15.47 ($SCH_3$), 30.25 ($SCH_2$), 32.16 ($CH_2$), 32.41 ($CH_2$), 32.46 ($CHCH_2$), 34.22 ($CH_2$Ar), 34.61 ($CH_2$Ar), 40.55 ($CH_2$N), 41.12 ($CH_2$N), 54.08 (CH), 126.37 (Ar$C$), 127.61 (Ar$C$), 133.13 (Ar$C$), 136.49 (Ar$C$), 143.51 (Ar$C$), 143.75 (Ar$C$), 170.04

(CON), 170.77 ppm (CON); MS (MALDI-TOF, CCA): *m/z*: monoisotopic mass calcd for $C_{84}H_{136}N_{15}O_9S_6^+$: 1690.90, found: 1690.91 $[M+H]^+$.

***(Phe)$_6$G1D1*: 1,3,5-tris-[3,5-bis(L-2-amino-3-phenyl-*N*-propylpropionamide)-*N*-propylbenzamide]benzene hexatrifluoroacetate (21b)**: Trifluoroacetic acid (1.5 mL) was added to a solution of the Boc-protected G1 dendrimer **20b** (24 mg, 10 μmol) in dichloromethane (4 mL), and the reaction mixture was stirred for 1 h at room temperature. When cleavage of the Boc protecting group was complete (TLC), the solvent was removed in vacuo to yield the deprotected G1 L-phenylalanine dendrimer (25 mg, 9.9 μmol, 99%) after lyophilization from water. M.p. 126–127°C; $^1$H NMR (500 MHz, [D$_4$]methanol): $\delta=1.74$ (quin, $^3J$(H,H)=7.4 Hz, 12H; $CH_2$), 1.96 (quin, $^3J$(H,H)=7.3 Hz, 6H; $CH_2$), 2.56 (t, $^3J$(H,H)=7.6 Hz, 12H; $CH_2$Ar), 2.69 (t, $^3J$(H,H)=7.5 Hz, 6H; $CH_2$Ar), 3.13 (m, 12H; CHC$H_2$), 3.16 (m, 6H; $CH_2$N), 3.27 (m, 6H; $CH_2$N), 3.43 (t, $^3J$(H,H)=7.1 Hz, 6H; $CH_2$N), 4.08 (t, $^3J$(H,H)=7.5 Hz, 6H; CH), 6.97 (s, 3H; Ar*H*), 7.16 (s, 3H; Ar*H*), 7.29 (m, 18H; Ar$H_{phe}$), 7.35 (m, 12H; Ar$H_{phe}$), 7.45 ppm (s, 6H; Ar*H*); $^{13}$C NMR (127 MHz, [D$_4$]methanol): $\delta=31.95$ ($CH_2$), 32.49 ($CH_2$), 34.08 (*C*$H_2$Ar), 34.63 (*C*$H_2$Ar), 39.05 (CH*C*$H_2$), 40.44 ($CH_2$N), 41.13 ($CH_2$N), 56.18 (CH), 126.32 (Ar*C*), 127.61 (Ar*C*), 129.14 (Ar$C_{phe}$), 130.37 (Ar$C_{phe}$), 130.80 (Ar$C_{phe}$), 133.08 (Ar*C*), 136.01 (Ar$C_{phe}$), 136.42 (Ar*C*), 143.54 (Ar*C*), 143.68 (Ar*C*), 169.75 (CON), 170.80 ppm (CON); MS (MALDI-TOF, CCA): *m/z*: 1825 $[M+K]^+$, 1809 $[M+Na]^+$, 1787 $[M+H]^+$; monoisotopic mass calcd for $C_{108}H_{136}N_{15}O_9^+$: 1787.06, found: 1787.21.

***(Asp)$_6$G1D1*: 1,3,5-tris[3,5-bis(L-3-amino-*N*-propylsuccinamic acid)-*N*-propylbenzamide]benzene hexatrifluoroacetate (21c)**: Trifluoroacetic acid (2 mL) was added to a solution of dendrimer **20c** (101 mg, 40.0 μmol) in dichloromethane (2 mL), and the solution was stirred at room temperature for 1 h. When the deprotection was complete (TLC), the solvent was repeatedly removed in vacuo. Lyophilization from water afforded the deprotected L-aspartic acid dendrimer (91 mg, 40 μmol, 100%) as a colorless solid. M.p. 155–156°C; $^1$H NMR (500 MHz, [D$_4$]methanol): $\delta=1.89$ (quin, $^3J$(H,H)=7.2 Hz, 12H; $CH_2$), 1.97 (quin, $^3J$(H,H)=7.3 Hz, 6H; $CH_2$), 2.71 (m, 18H; $CH_2$Ar), 2.93 (dd, $^2J$(H,H)=17.8 Hz, $^3J$(H,H)=7.9 Hz, 6H; CHC$H_2$), 3.00 (dd, $^2J$(H,H)=17.7 Hz, $^3J$(H,H)=5.0 Hz, 6H; CHC$H_2$), 3.26 (m, 6H; $CH_2$N), 3.32 (m, 6H; $CH_2$N), 3.38 (t, $^3J$(H,H)=7.1 Hz, 6H; $CH_2$N), 4.17 (m, 6H; CH), 6.92 (s, 3H; Ar*H*), 7.23 (s, 3H; Ar*H*), 7.45 ppm (s, 6H; Ar*H*); $^{13}$C NMR (127 MHz, [D$_4$]methanol): $\delta=$ 32.08 ($CH_2$), 32.38 ($CH_2$), 34.07 (*C*$H_2$Ar), 34.65 (*C*$H_2$Ar), 36.52 (CH*C*$H_2$), 40.54 ($CH_2$N), 41.22 ($CH_2$N), 51.51 (CH), 126.37 (Ar*C*), 127.62 (Ar*C*), 133.26 (Ar*C*), 136.41 (Ar*C*), 143.52 (Ar*C*), 143.77 (Ar*C*), 169.41 (CON), 170.83 (CON), 173.02 ppm (COO); MS (MALDI-TOF, CCA): *m/z*: 1594.9; monoisotopic mass calcd for $C_{78}H_{112}N_{15}O_{21}^+$: 1594.82, found: 1594.85 $[M+H]^+$.

**Ethyl-3,5-bis-[(2-*tert*-butoxycarbonylamino-2-propylcarbamoylethyl)carbamic acid *tert*-butyl ester]benzoate (22)**: A solution of the Boc-protected diaminopropionic acid **14** (3.96 g, 13.0 mmol) in a solvent mixture of dry dichloromethane/DMF (29:1/v:v) was cooled down to −20°C. Under vigorous stirring TBTU (4.34 g, 13.5 mmol), dissolved in DMF, and DIPEA (2.21 mL, 1.68 g, 13.0 mmol) were added slowly. The reaction mixture was stirred at −20°C for additional 30 min, and was then allowed to warm up to room temperature. After complete formation of the active ester (TLC), the reaction mixture was cooled down to −20°C again. The G1 hydrochloride **17** (1.69 g, 5.00 mmol) was dissolved in a small amount of dry methanol, and, together with DIPEA (3.91 mL, 2.97 g, 23.0 mmol), was added to the reaction mixture slowly. The solution was stirred for an additional 30 min at −20°C, and was then allowed to warm up to room temperature slowly. After being stirred for additional 12 h the solution was washed twice with sodium hydrogencarbonate solution, and once with brine. The organic phase was dried over magnesium sulfate, the solvent removed in vacuo, and the crude product was purified by column chromatography (silica gel, dichloromethane containing 3% methanol as eluent) to give the desired product (3.79 g, 4.52 mmol, 90.4%) as a colorless solid. $R_f=0.31$ (dichloromethane/methanol=19:1/v:v); m.p. 147°C; $^1$H NMR (250 MHz, [D$_7$]DMF): $\delta=1.40$ (t, $^3J$(H,H)=7.2 Hz, 3H; $CH_2CH_3$), 1.43 (s, 18H; *C*$CH_3$), 1.45 (s, 18H; *C*$CH_3$), 1.86 (quin, $^3J$(H,H)=7.3 Hz, 4H; $CH_2$), 2.75 (t, $^3J$(H,H)=8.2 Hz, 4H; $CH_2$Ar), 3.27 (m, 4H; $CH_2$N), 3.46 (m, 4H; CHC$H_2$), 4.25 (m, 2H; CH), 4.39 (q, $^3J$(H,H)=7.3 Hz, 2H; C$H_2$CH$_3$), 6.73 (d, br, $^3J$(H,H)=7.3 Hz, 2H; CHN*H*), 6.86 (t, br, 2H; $NH_{Boc}$), 7.45 (s, 1H; Ar*H*), 7.74 (s, 2H; Ar*H*), 8.14 ppm (t, br, 2H; CON*H*); $^{13}$C NMR (127 MHz, [D$_7$]DMF): $\delta=14.52$ ($CH_2CH_3$), 28.44 (C$CH_3$), 31.88 ($CH_2$), 33.16 ($CH_2$), 39.15 ($CH_2$), 42.98 ($CH_2$), 56.37 (CH), 61.30 (*C*$H_2$CH$_3$), 78.74 (*C*$CH_3$), 78.99 (*C*$CH_3$), 127.39 (Ar*C*), 131.19 (Ar*C*), 134.25 (Ar*C*), 143.47 (Ar*C*), 156.28 ($CON_{Boc}$), 157.08 ($CON_{Boc}$), 166.89 (COO), 171.04 ppm (CON); MS (FAB+, MNBA/DCM): *m/z* (%): 859 (1.8) $[M+Na]^+$, 837 (1.1) $[M+H]^+$, 737 (6.1) $[M-C_5H_9O_2+H]^+$, 637 (2.0) $[M-2(C_5H_9O_2)+H]^+$, 537 (3.9) $[M-3(C_5H_9O_2)+H]^+$, 438 (15.2) $[M-4(C_5H_9O_2)]^+$, 57 (100) $[C_4H_9]^+$; elemental analysis calcd (%) for $C_{41}H_{68}N_6O_{12}$ (837.0): C 58.83, H 8.19, N 10.04; found: C 58.62, H 7.96, N 10.05.

**3,5-Bis-[(2-*tert*-butoxycarbonylamino-2-propylcarbamoylethyl)carbamic acid *tert*-butyl ester]benzoic acid (23)**: KOH (2.08 g, 37.0 mmol) was added to a solution of the G1 ester **22** (3.87 g, 4.60 mmol) in methanol/THF/water (3:1:1), and the reaction mixture was stirred for 12 h at 50°C. After complete reaction (TLC) acetic acid was added to give pH 5, and the desired product was subsequently extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate, and the solvent was evaporated in vacuo. Chromatographic separation (silica gel, dichloromethane containing 5% methanol as eluent) gave the G1 acid (3.22 g, 3.98 mmol, 86.5%) as a colorless solid. $R_f=0.38$ (dichloromethane/methanol=9:1/v:v); m.p. 118°C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta=$ 1.37 (s, 18H; *C*$CH_3$), 1.40 (s, 18H; *C*$CH_3$), 1.80 (quar, br, 4H; $CH_2$), 2.60 (t, br, 4H; $CH_2$Ar), 3.16 (m, 4H; $CH_2$N), 3.42 (m, 2H; CHC$H_2$), 3.47 (m, 2H; CHC$H_2$), 4.32 (m, 2H; CH), 5.61 (s, br, 2H; NH), 6.13 (d, br, 2H; NH), 7.19 (s, 1H; Ar*H*), 7.30 (s, br, 2H; NH), 7.65 ppm (s, 2H; Ar*H*); $^{13}$C NMR (127 MHz, $CDCl_3$): $\delta=28.30$ (C$CH_3$), 30.33 ($CH_2$), 32.09 ($CH_2$), 38.14 ($CH_2$), 42.48 ($CH_2$), 55.41 (CH), 79.75 (*C*$CH_3$), 80.14 (*C*$CH_3$), 127.66 (Ar*C*), 130.14 (Ar*C*), 134.25 (Ar*C*), 141.51 (Ar*C*), 156.14 ($CON_{Boc}$), 157.07 ($CON_{Boc}$), 169.53 (COO), 170.96 ppm (COO); MS (FAB+, MNBA/DCM/methanol): *m/z* (%): 831 (1.3) $[M+Na]^+$, 809 (0.1) $[M+H]^+$, 709 (1.5) $[M-C_5H_9O_2+H]^+$, 609 (0.8) $[M-2(C_5H_9O_2)+H]^+$, 509 (1.7) $[M-3(C_5H_9O_2)+H]^+$, 409 (20.8) $[M-4(C_5H_9O_2)+H]^+$, 57 (100) $[C_4H_9]^+$; elemental analysis calcd (%) for $C_{39}H_{64}N_6O_{12}$ (837.0): C 57.90, H 7.97, N 10.39; found: C 57.64, H 7.96, N 10.28.

***(Boc-Dpa)$_6$G1D1*: 1,3,5-tris{3,5-bis-[(2-*tert*-butoxycarbonylamino-2-propylcarbamoylethyl)carbamic acid *tert*-butyl ester]-*N*-propylbenzamide}benzene (24)**: The G1 acid **23** (1.46 g, 1.80 mmol) were dissolved in a solvent mixture of dry dichloromethane/DMF (14:1/v:v), and cooled down to −10°C. TBTU (607 mg, 1.90 mmol), dissolved in a small amount of DMF, and DIPEA (306 μL, 233 mg, 1.80 mmol) were added slowly under vigorous stirring. The reaction mixture was allowed to warm up to room temperature, and stirred at room temperature for additional 30 min. After complete formation of the active ester (TLC), the reaction mixture was cooled down to 0°C, and the trishydrochloride core **4** (144 mg, 400 μmol), dissolved in a small amount of dry methanol, and DIPEA (510 μL, 388 mg, 3.00 mmol) were added dropwise. The mixture was allowed to warm up to room temperature slowly, and was then stirred for additional 18 h. After the reaction was finished (TLC), the solution was washed once with sodium hydrogencarbonate solution, and once with brine. The organic phase was dried with magnesium sulfate. After evaporation of the solvent under reduced pressure, the crude product was purified by column chromatography (silica gel, dichloromethane containing 5% methanol as eluent) to yield the G1 dendrimer (601 mg, 229 μmol; 57.3%) as a colorless solid. $R_f=0.23$ (dichloromethane/methanol=9:1/v:v); m.p. 141°C; $^1$H NMR (500 MHz, [D$_4$]methanol): $\delta=1.44$ (s, 54H; *C*$CH_3$), 1.47 (s, 54H; *C*$CH_3$), 1.86 (quin, $^3J$(H,H)=7.1 Hz, 12H; $CH_2$), 1.97 (quin, $^3J$(H,H)=7.2 Hz, 6H; $CH_2$), 2.68 (m, 18H; $CH_2$Ar), 3.22 (m, 12H; $CH_2$N), 3.32 (m, 6H; CHC$H_2$), 3.41 (m, 6H; CHC$H_2$), 3.43 (m, 6H; $CH_2$N), 4.20 (m, br, 6H; CH), 6.96 (s, 3H; Ar*H*), 7.26 (s, 3H; Ar*H*), 7.48 ppm (s, 3H; Ar*H*); $^{13}$C NMR (127 MHz, [D$_4$]methanol): $\delta=29.08$ (C$CH_3$), 32.23 ($CH_2$), 32.43 ($CH_2$), 33.98 ($CH_2$Ar), 34.61 ($CH_2$Ar), 40.04 ($CH_2$N), 41.07 ($CH_2$N), 43.52 (CHC$H_2$), 57.21 (CH), 80.77 (*C*$CH_3$), 81.10 (*C*$CH_3$), 126.44 (Ar*C*), 127.64 (Ar*C*), 133.36 (Ar*C*), 136.31 (Ar*C*), 143.49 (Ar*C*), 143.73 (Ar*C*), 157.98 ($CON_{Boc}$), 158.96 ($CON_{Boc}$), 170.71 (CON), 173.25 ppm (CON); MS (MALDI-TOF, dithranol): *m/z*: 2660 $[M+K]^+$, 2644 $[M+Na]^+$; monoisotopic mass calcd for $C_{132}H_{213}N_{21}NaO_{33}^+$: 2643.55, found: 2643.77 $[M+Na]^+$.

***(Dpa)$_6$G1D1*: 1,3,5-tris[3,5-bis-(2,3-diamino-*N*-propyl-propionamide)-*N*-propylbenzamide]benzene dodecatrifluoroacetate (25)**: Trifluoroacetic acid (5 mL) was added to a vigorously stirred solution of dendrimer **24** (105 mg, 40.0 μmol) in dichloromethane (5 mL). The reaction mixture

was stirred for 1 h at room temperature. When deprotection was complete (TLC), the solvent was removed in vacuo and the desired product could be lyophilized from water. The procedure yielded the deprotected G1 dendrimer (110 mg, 39.4 μmol, 98.5 %) as a colorless solid. M.p. 119–121 °C; $^1$H NMR (500 MHz, [D$_4$]methanol): $\delta$ = 1.92 (m, 18H; $CH_2$), 2.68 (t, $^3J$(H,H) = 7.4 Hz, 6H; $CH_2$Ar), 2.73 (t, $^3J$(H,H) = 7.6 Hz, 12H; $CH_2$Ar), 3.25 (m, 6H; $CH_2$N), 3.43 (m, 12H; $CH_2$N), 3.45 (dd, $^2J$(H,H) = 13.9 Hz, $^3J$(H,H) = 6.3 Hz, 6H; $CHCH_2$), 3.52 (dd, $^2J$(H,H) = 13.9 Hz, $^3J$(H,H) = 5.4 Hz, 6H; $CHCH_2$), 4.29 (t, $^3J$(H,H) = 5.8 Hz, 6H; CH), 6.96 (s, 3H; Ar*H*), 7.29 (s, 3H; Ar*H*), 7.51 ppm (s, 3H; Ar*H*); $^{13}$C NMR (127 MHz, [D$_4$]methanol): $\delta$ = 31.86 (s, $CH_2$), 32.37 (s, $CH_2$), 34.15 (s, $CH_2$Ar), 34.61 (s, $CH_2$Ar), 40.85 (s, $CH_2$N), 41.13 (s, $CH_2$N), 41.47 (s, *C*$H_2$CH), 52.55 (s, CH), 118.36 (q, $^1J$(C,F) = 292.5 Hz, *C*$F_3CO_2$H), 126.36 (s, Ar*C*), 127.61 (s, Ar*C*), 133.21 (s, Ar*C*), 136.43 (s, Ar*C*), 143.51 (s, Ar*C*), 143.73 (s, Ar*C*), 163.49 (q, $^2J$(C,F) = 35.4 Hz, $CF_3$*C*$O_2$H), 167.48 (s, CON), 170.84 ppm (s, CON); MS (MALDI-TOF, CCA): *m/z*: 1459 [*M*+K]$^+$, 1443 [*M*+Na]$^+$, 1421 [*M*+H]$^+$; monoisotopic mass calcd for $C_{72}H_{118}N_{21}O_9^+$: 1420.94, found: 1420.93.

***(Dns)$_3$3 D0*: 1,3,5-tris-(5-dimethylaminonaphthalene-1-sulfonic acid propylamide)benzene (26)**: Dry triethylamine (2.00 mL , 1.46 g, 14.4 mmol) and the trishydrochloride core **4** (431 mg, 1.20 mmol), dissolved in a small amount of dry methanol, were added dropwise at room temperature to a vigorously stirred solution of 5-dimethylamino-naphthalene-1-sulfonyl chloride (dansyl chloride) (1.46 g, 5.40 mmol) in dry dichloromethane. The reaction was stirred for 1 h in the dark and was continuously checked by TLC. After complete reaction the organic layer was washed once with brine, once with a saturated sodium carbonate solution, and once again with brine. Column chromatography (silica gel, dichloromethane containing 1 % methanol as eluent) gave the dansylated G0 dendrimer (1.04 g, 1.10 mmol, 91.7 %) as a bright yellow-greenish solid. $R_f$ = 0.46 (dichloromethane/methanol = 49:1/v:v); m.p. 96 °C; $^1$H NMR (500 MHz, $CDCl_3$, 30 °C): $\delta$ = 1.57 (quin, $^3J$(H,H) = 7.1 Hz, 6H; $CH_2$), 2.31 (t, $^3J$(H,H) = 7.5 Hz, 6H; $CH_2$Ar), 2.84 (q, $^3J$(H,H) = 6.6 Hz, 6H; $CH_2$N), 2.85 (s, 18H; $NCH_3$), 4.97 (t, br, $^3J$(H,*N*H) = 5.4 Hz, 3H; NH), 6.40 (s, 3H; Ar*H*), 7.14 (d, $^3J$(H,H) = 7.6 Hz, 3H; Ar$H_{dns}$), 7.45 (t, $^3J$(H,H) = 7.9 Hz, 3H; Ar$H_{dns}$), 7.50 (t, $^3J$(H,H) = 8.1 Hz, 3H; Ar$H_{dns}$), 8.19 (d, $^3J$(H,H) = 7.6 Hz, 3H; Ar$H_{dns}$), 8.30 (d, $^3J$(H,H) = 8.7 Hz, 3H; Ar$H_{dns}$), 8.51 ppm (d, $^3J$(H,H) = 8.4 Hz, 3H; Ar$H_{dns}$); $^{13}$C NMR (127 MHz, CDCl3): $\delta$ = 31.03 ($CH_2$), 32.34 ($CH_2$Ar), 42.64 ($CH_2$N), 45.44 ($NCH_3$), 115.28 (Ar$C_{dns}$), 119.01 (Ar$C_{dns}$), 123.30 (Ar$C_{dns}$), 126.11 (Ar*C*), 128.35 (Ar$C_{dns}$), 129.57 (Ar$C_{dns}$), 129.64 (Ar$C_{dns}$), 129.77 (Ar$C_{dns}$), 130.28 (Ar$C_{dns}$), 134.93 (Ar$C_{dns}$), 141.14 (Ar*C*), 151.70 ppm (Ar$C_{dns}$); MS (EI, 80 eV, 320 °C); *m/z* (%): 948 (2.0) [*M*]$^+$, 714 (1.4) [*M*−$C_{12}H_{12}NO_2S$]$^+$, 171 (100) [$C_{12}H_{12}N$]$^+$, 64 (38.3) [$SO_2$]$^+$; HRMS: *m/z*: monoisotopic mass calcd for $C_{51}H_{60}N_6O_6S_3^+$: 948.37365, found: 948.37652.

***(Dns)$_6$6 D1*: 1,3,5-Tris{3,5-bis-[3-(5-dimethylaminonaphthalene-1-sulfonylamino)propyl]-*N*-propylbenzamide}benzene (27)**: A solution of dry triethylamine (1.26 mL, 923 mg, 9.12 mmol) and the G1 dendrimer **8** (477 mg, 300 μmol) in dry methanol were added slowly to a vigorously stirred solution of dansyl chloride (1.17 g, 4.32 mmol) in dry dichloromethane at room temperature. The reaction mixture was continuously monitored by TLC and stirred in the dark for 6 h. After complete reaction the solution was washed once with brine, once with a saturated sodium carbonate solution, and once again with brine. The organic phase was dried over magnesium sulfate, the solvent removed in vacuo, and the crude product purified by column chromatography (silica gel, dichloromethane containing 2–3 % methanol as eluent). The procedure yielded the dansylated G1 dendrimer as a bright yellow-greenish oil (512 mg, 222 μmol; 74.0 %) that could be lyophilized from dioxane. $R_f$ = 0.50 (dichloromethane/methanol = 19:1/v:v); m.p. 114–117 °C; $^1$H NMR (500 MHz, $CDCl_3$, 50 °C): $\delta$ = 1.61 (quin, $^3J$(H,H) = 6.8 Hz, 12H; $CH_2$), 1.83 (quin, $^3J$(H,H) = 6.8 Hz, 6H; $CH_2$), 2.42 (t, $^3J$(H,H) = 7.2 Hz, 12H; $CH_2$Ar), 2.53 (t, $^3J$(H,H) = 6.9 Hz, 6H; $CH_2$Ar), 2.80 (q, $^3J$(H,H) = 6.3 Hz, 12H; $CH_2$N), 2.84 (s, 36H; $NCH_3$), 3.32 (q, $^3J$(H,H) = 5.9 Hz, 12H; $CH_2$N), 5.52 (s, br, 6H; $SO_2$NH), 6.75 (s, 3H; Ar*H*), 6.77 (t, br, $^3J$(H,*N*H) = 5.4 Hz, 3H; CONH), 6.82 (s, 3H; Ar*H*), 7.12 (d, $^3J$(H,H) = 7.3 Hz, 6H; Ar$H_{dns}$), 7.26 (s, 6H; Ar*H*), 7.42 (m, 12H; Ar$H_{dns}$), 8.14 (d, $^3J$(H,H) = 7.2 Hz, 6H; Ar$H_{dns}$), 8.33 (d, $^3J$(H,H) = 8.7 Hz, 6H; Ar$H_{dns}$), 8.50 ppm (d, $^3J$(H,H) = 8.5 Hz, 6H; Ar$H_{dns}$); $^{13}$C NMR (127 MHz, $CDCl_3$): $\delta$ = 30.56 ($CH_2$), 30.77 ($CH_2$), 32.09 ($CH_2$Ar), 33.26 ($CH_2$Ar), 39.78 ($CH_2$N), 42.34 ($CH_2$N), 45.51 ($NCH_3$), 115.50 (Ar$C_{dns}$), 119.62 (Ar$C_{dns}$), 123.47 (Ar$C_{dns}$), 125.00 (Ar*C*), 126.15 (Ar*C*), 128.26 (Ar$C_{dns}$), 129.33 (Ar$C_{dns}$), 129.38 (Ar$C_{dns}$), 129.57 (Ar$C_{dns}$), 129.99 (Ar$C_{dns}$), 131.42 (Ar*C*), 134.64 (Ar*C*), 135.07 (Ar$C_{dns}$), 141.45 (Ar*C*), 141.93 (Ar*C*), 151.51 (Ar$C_{dns}$), 167.81 ppm (CON); MS (MALDI-TOF, dithranol): *m/z*: 2341 [*M*+K]$^+$, 2325 [*M*+Na]$^+$, 2303 [*M*+H]$^+$; monoisotopic mass calcd for $C_{126}H_{148}N_{15}O_{15}S_6^+$ : 2302.96, found: 2303.08.

***(Dns)$_{12}$12 D2*: 1,3,5-tris-(3,5-bis{3,5-bis[3-(5-dimethylaminonaphthalene-1-sulfonylamino)propyl]-*N*-propylbenzamide}-*N*-propylbenzamide)benzene (28)**: A solution of dry triethylamine (166 μL, 121 mg, 1.20 mmol) and the deprotected G2 dendrimer **10** (72 mg, 20 μmol) in dry methanol were added dropwise to a vigorously stirred solution of dansyl chloride (194 mg, 720 μmol) in dry dichloromethane at room temperature. While the solution was stirred in the dark for an additional 12 h the reaction was continuously monitored by TLC. After complete reaction the solution was washed once with brine, once with a saturated sodium carbonate solution, and once again with brine. The organic phase was dried over magnesium sulfate, the solvent removed in vacuo, and the crude product purified by column chromatography (silica gel, dichloromethane containing 2–4 % methanol as eluent). The procedure afforded the desired G2 dendrimer (65 mg, 13 μmol; 65 %) as a bright yellow-greenish oil which could be lyophilized from dioxane. $R_f$ = 0.44 (dichloromethane/methanol = 19:1/v:v); m.p. 132–134 °C; $^1$H NMR (500 MHz, [D$_4$]methanol, 30 °C): $\delta$ = 1.63 (quin, $^3J$(H,H) = 7.0 Hz, 24H; $CH_2$), 1.80 (m, 6H; $CH_2$), 1.86 (m, 12H; $CH_2$), 2.45 (m, 24 H + 6H; $CH_2$Ar), 2.46 (t, $^3J$(H,H) = 6.9 Hz, 12H; $CH_2$Ar), 2.59 (m, 24H; $CH_2$N), 2.92 (s, 72H; $NCH_3$), 3.31 (m, 6H; + 12H; $CH_2$N), 6.75 (s, 3H; Ar*H*), 6.78 (s, 6H; Ar*H*), 7.12 (s, 3H; Ar*H*), 7.26 (s, 6H; Ar*H*), 7.29 (s, 12H; Ar*H*), 7.38 (d, $^3J$(H,H) = 5.5 Hz, 12H; Ar$H_{dns}$), 7.49 (m, 24H; Ar$H_{dns}$), 8.13 (d, $^3J$(H,H) = 7.3 Hz, 12H; Ar$H_{dns}$), 8.41 (d, $^3J$(H,H) = 8.0 Hz, 12H; Ar$H_{dns}$), 8.55 ppm (d, $^3J$(H,H) = 8.7 Hz, 12H; Ar$H_{dns}$); $^{13}$C NMR (127 MHz, [D$_4$]methanol, 30 °C): $\delta$ = 30.04 ($CH_2$), 30.61 ($CH_2$), 31.18 ($CH_2$), 32.51 ($CH_2$Ar), 33.34 ($CH_2$Ar), 33.62 ($CH_2$Ar), 39.87 ($CH_2$N), 40.15 ($CH_2$N), 42.50 ($CH_2$N), 46.02 ($NCH_3$), 116.46 (Ar$C_{dns}$), 123.50 (Ar$C_{dns}$), 124.31 (Ar$C_{dns}$), 125.27 (Ar*C*), 125.38 (Ar*C*), 128.49 (Ar$C_{dns}$), 129.55 (Ar$C_{dns}$), 129.70 (Ar$C_{dns}$), 129.98 (Ar$C_{dns}$), 130.03 (Ar$C_{dns}$), 132.08 (Ar*C*), 132.19 (Ar*C*), 134.85 (Ar*C*), 134.91 (Ar*C*), 135.88 (Ar$C_{dns}$), 140.23 (Ar*C*), 142.06 (Ar*C*), 142.26 (Ar*C*), 142.56 (Ar*C*), 152.53 (Ar$C_{dns}$), 169.06 (CON), 169.09 ppm (CON); MS (MALDI-TOF, dithranol): *m/z*: 5048 [*M*+K]$^+$, 5032 [*M*+Na]$^+$, 5010 [*M*+H]$^+$; monoisotopic mass calcd for $C_{276}H_{322}N_{33}O_{33}S_{12}^+$: 5010.12, found: 5010.21 [*M*+H]$^+$.

**Ethyl-3-[3-(3-Benzyloxycarbonylaminopropyl)-5-(3-*tert*-butoxycarbonylamino)propyl]benzoate (29)**: Dendron **17** (12.0 g, 35.6 mmol) was suspended in 1000 mL THF. An aqueous 1 M KOH solution (300 mL, 300 mmol) was added, and the mixture was cooled down to 0 °C. A solution of di-*tert*-butyl dicarbonate (31.0 g, 142 mmol) and benzyl chloroformate (6.07 g, 35.6 mmol) in THF was added slowly, and the reaction mixture was stirred for 1 h. After complete reaction (TLC) the layers were separated, the organic phase was washed with brine once, and the aqueous layer was extracted with diethyl ether. The combined organic phases were dried over magnesium sulfate, and the solvent was removed in vacuo. Chromatographic separation (silica gel, dichloromethane containing 1 % methanol as eluent) yielded the mixed-protected dendron **29** (6.13 g, 12.3 mmol, 34.6 %) as a colorless oil. $R_f$ = 0.71 (dichloromethane/methanol = 19:1/v:v).

**3-(3-Benzyloxycarbonylaminopropyl)-5-[3-(*tert*-butoxycarbonylamino)-propyl]benzoic acid (30)**: A solution of the mixed-protected dendron **29** (6.00 g, 12.0 mmol) and KOH (3.08 g, 77.0 mmol) in methanol/water (3:1/v:v) was stirred at 50 °C for 14 h. When the reaction was finished (TLC), acetic acid was added to give pH 5. The product was extracted with dichloromethane, and the combined organic layers dried over magnesium sulfate. After evaporation of the solvent under reduced pressure the mixed-protected acid (5.50 g, 11.7 mmol, 97.5 %) was received as a colorless solid. $R_f$ = 0.35 (dichloromethane/methanol = 19:1/v:v); m.p. 128 °C.

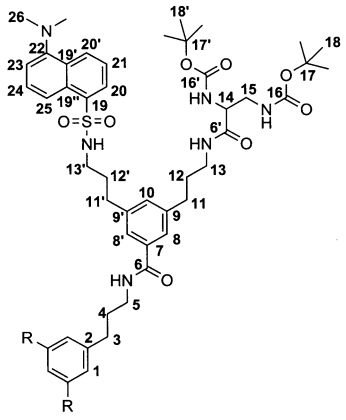
***(Cbz-N)$_3$(Boc-N)$_3$6 D1*: 1,3,5-tris-{3-[3-(benzyloxycarbonylamino)propyl]-5-[3-(*tert*-butyloxycarbonylamino)propyl]-*N*-propylbenzamide}benzene (31)**: HOBt (1.63 g, 12.1 mmol) was added to a solution of the mixed-protected G1 acid **30** (5.41 g, 11.5 mmol) in dry dichloromethane under a nitrogen atmosphere, and the suspension was stirred at room temperature for 20 min. Thereafter, the mixture was cooled down to −20 °C, EDC (2.43 g, 12.7 mmol) was added, and the reaction was allowed to warm up to room temperature slowly while being stirred for ad-

ditional 2 h. After complete formation of the active ester (TLC), the reaction mixture was cooled down to −20 °C again. A solution of the trishydrochloride core **4** (918 mg, 2.56 mmol) and DIPEA (7.6 mL, 5.6 g, 43 mmol) in dry methanol was added dropwise while the solution was stirred at −20 °C for 1 h. The reaction was allowed to warm up to room temperature slowly and was stirred for additional 24 h. After complete reaction (TLC) the solution was washed twice with sodium hydrogencarbonate solution, and once with brine. The organic phase was dried over magnesium sulfate, and the solvent removed in vacuo. Column chromatography (silica gel, dichloromethane containing 3% methanol as eluent) afforded the mixed-protected G1 dendrimer (2,61 g, 1.62 mmol, 63.3%) as a colorless solid. $R_f = 0.43$ (dichloromethane/methanol = 19:1/v:v); m.p. 66 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 1.40$ (s, 27H; $CCH_3$), 1.69 (m, 6H; $CH_2$), 1.74 (m, 6H; $CH_2$), 1.89 (quin, $^3J$(H,H) = 6.8 Hz, 6H; $CH_2$), 2.54 (m, 12H; $CH_2$Ar), 2.58 (t, $^3J$(H,H) = 7.2 Hz, 6H; $CH_2$Ar), 3.02 (m, 6H; $CH_2$N), 3.10 (m, 6H; $CH_2$N), 3.37 (m, 6H; $CH_2$N), 4.72 (s, br, 3H; NH), 5.03 (s, 6H; $CH_2Ar_{Cbz}$), 5.17 (s, br, 3H; NH), 6.83 (s, 3H; Ar*H*), 7.02 (s, 3H; Ar*H*), 7.11 (s, br, 3H; NH), 7.29 (m, 15H; Ar$H_{Cbz}$), 7.36 (s, 3H; Ar*H*), 7.38 ppm (s, 3H; Ar*H*); $^{13}$C NMR (127 MHz, $CDCl_3$): $\delta =$ 28.41 ($CCH_3$), 30.70 ($CH_2$), 31.12 ($CH_2$), 31.31 ($CH_2$), 32.44 ($CH_2$Ar), 33.13 ($CH_2$Ar), 39.63 ($CH_2$N + $CH_2$N), 40.15 ($CH_2$N), 66.55 ($CH_2Ar_{Cbz}$), 79.17 ($CCH_3$), 124.82 (Ar*C*), 124.90 (Ar*C*), 126.27 (Ar*C*), 127.97 (Ar$C_{Cbz}$), 128.02 (Ar$C_{Cbz}$), 128.45 (Ar$C_{Cbz}$), 131.56 (Ar*C*), 134.74 (Ar*C*), 136.59 (Ar$C_{Cbz}$), 141.68 (Ar*C*), 141.75 (Ar*C*), 141.82 (Ar*C*), 156.10 ($CON_{Boc}$), 156.55 ($CON_{Cbz}$), 167.86 ppm (CON); MS (MALDI-TOF, dithranol): *m/z*: 1645 $[M+K]^+$, 1629 $[M+Na]^+$; monoisotopic mass calcd for $C_{93}H_{123}N_9NaO_{15}^+$: 1628.90, found: 1628.99.

***(Cbz-N)$_3$(N)$_3$6D1*: 1,3,5-tris{3-[3-(benzyloxycarbonylamino)propyl]-5-(aminopropyl)-*N*-propylbenzamide}benzene tristrifluoroacetate (32)**: Trifluoroacetic acid (25 mL) was added to a solution of the mixed-protected G1 dendrimer **31** (2.41 g, 1.50 mmol) in dichloromethane (100 mL), and the reaction mixture was stirred for 1 h at room temperature. After complete reaction (TLC) the solvent was repeatedly removed in vacuo to afford the partially deprotected dendrimer **32** (2.09 g, 1.35 mmol, 90.0%) as a colorless oil. $^1$H NMR (500 MHz, [D$_4$]methanol): $\delta = 1.81$ (quin, $^3J$(H,H) = 7.6 Hz, 6H; $CH_2$), 1.91 (quin, $^3J$(H,H) = 7.1 Hz, 6H; $CH_2$), 1.96 (quin, $^3J$(H,H) = 7.4 Hz, 6H; $CH_2$), 2.64 (m, 12H; $CH_2$Ar), 2.71 (t, $^3J$(H,H) = 7.6 Hz, 6H; $CH_2$Ar), 2.91 (t, $^3J$(H,H) = 7.7 Hz, 6H; $CH_2$N), 3.10 (t, $^3J$(H,H) = 6.7 Hz, 6H; $CH_2$N), 3.37 (t, $^3J$(H,H) = 7.1 Hz, 6H; $CH_2$N), 5.05 (s, 6H; $CH_2Ar_{Cbz}$), 6.91 (s, 3H; Ar*H*), 7.22 (s, 3H; Ar*H*), 7.29 (m, 15H; Ar$H_{Cbz}$), 7.44 (s, 3H; Ar*H*), 7.48 ppm (s, 3H; Ar*H*); $^{13}$C NMR (127 MHz, [D$_4$]methanol): $\delta = 30.38$ ($CH_2$), 32.38 ($CH_2$), 32.69 ($CH_2$), 33.55 ($CH_2$Ar), 33.92 ($CH_2$Ar), 34.61 ($CH_2$Ar), 40.51 ($CH_2$N), 41.11 ($CH_2$N), 41.37 ($CH_2$N), 67.66 ($CH_2Ar_{Cbz}$), 126.38 (Ar*C*), 126.56 (Ar*C*), 127.61 (Ar*C*), 128.96 (Ar*C*), 129.26 (Ar*C*), 129.77 (Ar*C*), 133.11 (Ar*C*), 136.67 (Ar*C*), 138.74 (Ar*C*), 142.64 (Ar*C*), 143.52 (Ar*C*), 144.13 (Ar*C*), 159.22 ($CON_{Cbz}$), 170.60 ppm (CON); MS (MALDI-TOF, dithranol): *m/z*: monoisotopic mass calcd for $C_{78}H_{100}N_9O_9^+$: 1306.76, found: 1306.76 $[M+H]^+$.

***(Cbz-N)$_3$(Dns)$_3$6D1*: 1,3,5-tris-{3-[3-(benzyloxycarbonylamino)propyl]-5-(5-dimethylaminonaphthalene-1-sulfonic acid propylamide)}-*N*-propylbenzamide}benzene (33)**: A solution of the G1 trifluoroacetate **32** (2.09 g, 1.35 mmol) and dry triethylamine (2.82 mL, 2.05 g, 20.3 mmol) in dry methanol (20 mL) was added dropwise to a vigorously stirred solution of dansyl chloride (2.18 g, 8.10 mmol) in dry dichloromethane (250 mL). The reaction was monitored by TLC. After complete reaction, the mixture was washed once with brine, once with a saturated sodium carbonate solution, and once again with brine. The organic layer was dried over magnesium sulfate, and the solvent was removed in vacuo. Chromatographic purification (silica gel, dichloromethane containing 4% methanol as eluent) afforded the partially dansylated G1 dendrimer (2.68 g, 1.34 mmol; 99.3%) as a bright yellow solid. $R_f = 0.38$ (dichloromethane/methanol = 19:1/v:v); m.p. 98–101 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta =$ 1.61 (quin, $^3J$(H,H) = 6.9 Hz, 6H; $CH_2$), 1.67 (quin, $^3J$(H,H) = 7.3 Hz, 6H; $CH_2$), 1.82 (quin, $^3J$(H,H) = 6.8 Hz, 6H; $CH_2$), 2.47 (m, 12H; $CH_2$Ar), 2.52 (t, $^3J$(H,H) = 7.1 Hz, 6H; $CH_2$Ar), 2.77 (q, $^3J$(H,H) = 6.2 Hz, 6H; $CH_2$N), 2.85 (s, 18H; $NCH_3$), 3.06 (q, $^3J$(H,H) = 6.7 Hz, 6H; $CH_2$N), 3.31 (q, $^3J$(H,H) = 6.0 Hz, 6H; $CH_2$N), 5.02 (s, 6H; $CH_2Ar_{Cbz}$), 5.15 (t, br, 3H; NH), 5.82 (s, br, 3H; NH), 6.80 (s, 3H; Ar*H*), 6.86 (s, 3H; Ar*H*), 7.00 (s, br, 3H; NH), 7.14 (d, $^3J$(H,H) = 7.3 Hz, 3H; Ar$H_{dns}$), 7.26 (m, 15H; Ar$H_{Cbz}$), 7.28 (s, 3H; Ar*H*), 7.36 (s, 3H; Ar*H*), 7.42 (m, 6H; Ar$H_{dns}$), 8.13 (d, $^3J$(H,H) = 7.3 Hz, 3H; Ar$H_{dns}$), 8.36 (d, $^3J$(H,H) = 8.7 Hz, 3H; Ar$H_{dns}$), 8.52 ppm (d, $^3J$(H,H) = 8.5 Hz, 3H; Ar$H_{dns}$); $^{13}$C NMR (127 MHz, $CDCl_3$): $\delta = 30.72$ ($CH_2$), 30.83 ($CH_2$), 31.19 ($CH_2$), 32.12 ($CH_2$Ar), 32.55 ($CH_2$Ar), 33.31 ($CH_2$Ar), 39.80 ($CH_2$N), 40.36 ($CH_2$N), 42.28 ($CH_2$N), 45.60 ($NCH_3$), 66.65 ($CH_2Ar_{Cbz}$), 115.59 (Ar$C_{dns}$), 119.40 (Ar$C_{dns}$), 123.50 (Ar$C_{dns}$), 123.58 (Ar$C_{dns}$), 124.93 (Ar*C*), 125.21 (Ar*C*), 126.31 (Ar*C*), 128.05 (Ar$C_{Cbz}$), 128.11 (Ar$C_{Cbz}$), 128.30 (Ar$C_{dns}$), 128.56 (Ar$C_{Cbz}$), 129.46 (Ar$C_{dns}$), 130.16 (Ar$C_{dns}$), 131.53 (Ar*C*), 134.94 (Ar*C*), 135.23 (Ar$C_{dns}$), 136.70 (Ar$C_{Cbz}$), 141.42 (Ar*C*), 141.90 (Ar*C*), 142.00 (Ar*C*), 151.65 (Ar$C_{dns}$), 156.69 ($CON_{Cbz}$), 167.90 ppm (CON); MS (MALDI-TOF, dithranol): *m/z*: 2028 $[M+Na]^+$, 2006 $[M+H]^+$; monoisotopic mass calcd for $C_{114}H_{133}N_{12}O_{15}S_3^+$: 2005.92, found: 2005.94.

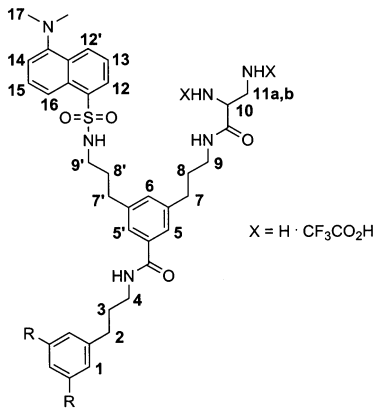
***(N)$_3$(Dns)$_3$6D1*: 1,3,5-tris{3-(aminopropyl)-5-(5-dimethylaminonaphthalene-1-sulfonic acid propylamide)-*N*-propylbenzamide}benzene (34)**: A solution of the mixed dansylated dendrimer **33** (401 mg, 200 μmol) in trifluoroacetic acid (8 mL) was stirred for seven days at room temperature. The reaction was continuously monitored by TLC. After complete reaction the solvent was repeatedly removed in vacuo to afford the partially deprotected dendrimer **34** as a yellow-greenish solid (379 mg, 198 μmol, 99.0%) after lyophilization from water. M.p. 119–120 °C; $^1$H NMR (500 MHz, [D$_4$]methanol): $\delta = 1.68$ (quin, $^3J$(H,H) = 7.1 Hz, 3H; $CH_2$), 1.96 (m, 12H; $CH_2$), 2.56 (t, $^3J$(H,H) = 7.4 Hz, 6H; $CH_2$Ar), 2.66 (t, $^3J$(H,H) = 7.6 Hz, 6H; $CH_2$Ar), 2.71 (t, $^3J$(H,H) = 7.7 Hz, 6H; $CH_2$Ar), 2.84 (t, $^3J$(H,H) = 6.7 Hz, 6H; $CH_2$N), 2.87 (s, 18H; $NCH_3$), 2.95 (t, $^3J$(H,H) = 7.7 Hz, 6H; $CH_2$N), 3.40 (t, $^3J$(H,H) = 5.6 Hz, 6H; $CH_2$N), 6.96 (s, 3H; Ar*H*), 7.09 (s, 3H; Ar*H*), 7.27 (d, $^3J$(H,H) = 6.9 Hz, 3H; Ar$H_{dns}$), 7.34 (s, 3H; Ar*H*), 7.49 (s, 3H; Ar*H*), 5.53 (t, $^3J$(H,H) = 7.9 Hz, 3H; Ar$H_{dns}$), 7.58 (t, $^3J$(H,H) = 8.1 Hz, 3H; Ar$H_{dns}$), 8.14 (d, $^3J$(H,H) = 7.3 Hz, 3H; Ar$H_{dns}$), 8.39 (d, $^3J$(H,H) = 8.8 Hz, 3H; Ar$H_{dns}$), 8.53 ppm (d, $^3J$(H,H) = 8.5 Hz, 3H; Ar$H_{dns}$); $^{13}$C NMR (127 MHz, [D$_4$]methanol): $\delta =$ 30.39 ($CH_2$), 32.39 ($CH_2$), 32.42 ($CH_2$), 33.54 ($CH_2$Ar), 33.55 ($CH_2$Ar), 34.63 ($CH_2$Ar), 40.53 ($CH_2$N), 41.12 ($CH_2$N), 43.37 ($CH_2$N), 46.13 ($NCH_3$), 116.81 (Ar$C_{dns}$), 120.90 (Ar$C_{dns}$), 124.66 (Ar$C_{dns}$), 126.37 (Ar*C*), 126.54 (Ar*C*), 127.63 (Ar*C*), 128.28 (Ar$C_{dns}$), 129.46 (Ar$C_{dns}$), 130.42 (Ar$C_{dns}$), 131.37 (Ar$C_{dns}$), 133.07 (Ar*C*), 136.50 (Ar$C_{dns}$), 137.32 (Ar$C_{dns}$), 142.61 (Ar*C*), 142.99 (Ar*C*), 143.55 (Ar*C*), 143.81 (Ar*C*), 153.25 (Ar$C_{dns}$), 170.57 ppm (CON); MS (FAB+, MNBA/methanol): *m/z* (%):1604 (1.2) $[M+H]^+$, 170 (42.5) $[C_{12}H_{12}N]^+$; MS (MALDI-TOF, dithranol): *m/z*: monoisotopic mass calcd for $C_{90}H_{115}N_{12}O_9S_3^+$: 1603.81, found: 1603.81.




***(Boc-Dpa)$_3$(Dns)$_3$6D1*: 1,3,5-tris{3-[(2-*tert*-butoxycarbonylamino-2-propylcarbamoylethyl)carbamic acid *tert*-butyl ester]-5-(5-dimethylaminonaphthalene-1-sulfonic acid propylamide)-*N*-propylbenzamide}benzene (35)**: A solution of the Boc-protected diaminopropionic acid **14** (274 mg, 900 μmol) in dry dichloromethane/DMF (18:2/v:v) was cooled down to −20 °C. DIPEA (153 μL, 116 mg, 900 μmol), and TBTU (303 mg, 945 μmol), dissolved in dry DMF (8 mL), were added slowly under vigorous stirring. The reaction mixture was allowed to warm up to room temperature and stirred for an additional 30 min. After complete formation of the active ester (TLC), the solution was cooled down to −20 °C again. DIPEA (204 μL, 155 mg, 1.20 mmol) and the deprotected dansylated

dendrimer **34** (195 mg, 100 μmol), dissolved in a small amount of methanol (2 mL), were added dropwise to the vigorously stirred solution. The reaction mixture was stirred at −20 °C for one hour, and was then allowed to warm up to room temperature slowly. Afterwards the solution was stirred at room temperature for an additional 24 h. When the reaction was complete (TLC), the solution was washed twice with a saturated sodium carbonate solution, and once with brine. The organic phase was dried over magnesium sulfate, the solvent removed in vacuo, and the crude product purified by column chromatography (silica gel, dichloromethane containing 4% methanol as eluent). Lyophilization from dioxane gave the mixed dansylated and diaminopropionic acid functionalized dendrimer **35** (81 mg, 33 μmol; 33%) as a brilliant yellow-greenish solid. $R_f = 0.16$ (dichloromethane/methanol = 19:1/v:v); m.p. 122–123 °C; $^1$H NMR (500 MHz, [D$_4$]methanol): $\delta = 1.42$ (s, 27H; *H*-18,18′), 1.44 (s, 27H; *H*-18′,18), 1.61 (quin, $^3J$(H,H) = 7.0 Hz, 6H; *H*-12′), 1.78 (quin, br, 6H; *H*-12), 1.92 (quin, $^3J$(H,H) = 6.8 Hz, 6H; *H*-4), 2.46 (t, br, 6H; *H*-11′), 2.58 (t, br, 6H; *H*-11), 2.64 (t, br, 6H; *H*-3), 2.84 (t, $^3J$(H,H) = 6.5 Hz, 6H; *H*-13′), 2.96 (s, 18H; *H*-26), 3.19 (m, 6H; *H*-13), 3.30 (m, 3H; *H*-15a), 3.38 (m, 3H; *H*-15b), 3.40 (m, 6H; *H*-13), 4.17 (t, $^3J$(H,H) = 5.9 Hz, 3H; *H*-14), 6.93 (s, 3H; *H*-1), 6.97 (s, 3H; *H*-10), 7.26 (s, 3H; *H*-8,8′), 7.40 (m, 6H; *H*-8′,8 + *H*-23), 7.60 (m, 6H; *H*-21 + *H*-24), 8.18 (d, $^3J$(H,H) = 7.3 Hz, 3H; *H*-20,20′), 8.51 ppm (m, 6H; *H*-25 + *H*-20′,20); $^{13}$C NMR (127 MHz, [D$_4$]methanol): $\delta = 29.04$ (*C*-18,18′), 29.06 (*C*-18′,18), 32.24 (*C*-12), 32.35 (*C*-4), 32.45 (*C*-12′), 33.65 (*C*-11′), 33.97 (*C*-11), 34.62 (*C*-3), 40.11 (*C*-13′), 41.08 (*C*-5), 43.53 (*C*-13 + *C*-15), 46.50 (*C*-26), 57.26 (*C*-14), 80.81 (*C*-17,17′), 81.14 (*C*-17′,17), 117.42 (*C*-23), 120.29 (*C*-25), 125.32 (*C*-21), 126.35 (*C*-8′), 126.36 (*C*-8), 127.66 (*C*-1), 129.37 (*C*-24), 130.65 (*C*-20,20′), 131.23 (*C*-20′,20), 133.14 (*C*-10), 136.23 (*C*-19,19′,19″), 137.78 (*C*-19,19′,19″), 137.89 (*C*-19,19′,19″), 143.46 (*C*-9,9′), 143.52 (*C*-9′,9), 143.70 (*C*-7), 151.80 (*C*-22), 158.02 (*C*-16,16′), 158.98 (*C*-16′,16), 170.64 (*C*-6), 173.25 ppm (*C*-6′); MS (MALDI-TOF, dithranol): *m/z*: 2500 $[M+K]^+$, 2484 $[M+Na]^+$; monoisotopic mass calcd for $C_{129}H_{180}N_{18}NaO_{24}S_3^+$: 2484.25, found: 2484.21.




***(Dpa)$_3$(Dns)$_3$6D1*: 1,3,5-tris-[3-(2,3-diamino-*N*-propylpropionamide)-5-(5-dimethylaminonaphthalene-1-sulfonic acid propylamide)-*N*-propylbenzamide]benzene (36)**: Trifluoroacetic acid (2 mL) was added to a solution of dendrimer **35** (37.0 mg, 15.0 μmol) in dichloromethane (3 mL), and the solution was stirred for 1 h at room temperature. After complete reaction (TLC) the solvent was repeatedly removed in vacuo. Lyophilization from water yielded the deprotected mixed dansylated and diaminopropionic acid modified dendrimer **36** as a brilliant yellow-greenish solid (37.0 mg, 14.5 μmol; 96.7%). M.p. 110–112 °C; $^1$H NMR (500 MHz, [D$_4$]methanol): $\delta = 1.66$ (quin, $^3J$(H,H) = 7.1 Hz, 6H; *H*-8′), 1.88 (m, 6H; *H*-8), 1.94 (m, 6H; *H*-3), 2.53 (t, $^3J$(H,H) = 7.4 Hz, 6H; *H*-7′), 2.66 (m, 12H; *H*-7 + *H*-2), 2.84 (t, $^3J$(H,H) = 6.7 Hz, 6H; *H*-9′), 2.96 (s, 18H; *H*-17), 3.21 (m, 3H; *H*-9a), 3.39 (m, 6H; *H*-4), 3.41 (m, 3H; *H*-9b), 3.46 (dd, $^2J$(H,H) = 14.0 Hz, $^3J$(H,H) = 6.2 Hz, 3H; *H*-11a), 3.53 (dd, $^2J$(H,H) = 13.9 Hz, $^3J$(H,H) = 5.4 Hz, 3H; *H*-11b), 4.30 (t, $^3J$(H,H) = 5.6 Hz, 3H; *H*-10), 6.96 (s, 3H; *H*-1), 7.07 (s, 3H; *H*-6), 7.29 (s, 3H; *H*-5′), 7.40 (d, $^3J$(H,H) = 7.6 Hz, 3H; *H*-14), 7.45 (s, 3H; *H*-5), 7.58 (t, $^3J$(H,H) = 7.9 Hz, 3H; *H*-13), 7.63 (t, $^3J$(H,H) = 8.2 Hz, 3H; *H*-15), 8.18 (d, $^3J$(H,H) = 7.3 Hz, 3H; *H*-12,12′), 8.48 (d, $^3J$(H,H) = 8.7 Hz, 3H; *H*-16), 8.53 ppm (d, $^3J$(H,H) = 8.5 Hz, 3H; *H*-12′,12); $^{13}$C NMR (127 MHz, [D$_4$]methanol): $\delta = 31.86$ ($CH_2$), 32.31 ($CH_2$), 32.45 ($CH_2$), 33.59 ($CH_2$Ar), 34.11 ($CH_2$Ar), 34.61 ($CH_2$Ar), 40.87 ($CH_2$N), 41.10 ($CH_2$N), 41.28 ($CH_2$N), 43.42 (*C*H$_2$CH), 46.35 (NCH$_3$), 52.42 (CH), 117.29 (Ar$C_{dns}$), 121.80 (Ar$C_{dns}$), 125.09 (Ar$C_{dns}$), 126.24 (Ar*C*), 126.41 (Ar*C*), 127.65 (Ar*C*), 129.40 (Ar$C_{dns}$), 130.59 (Ar*C*), 130.86 (Ar$C_{dns}$), 131.22 (Ar$C_{dns}$), 133.14 (Ar*C*), 136.28 (Ar$C_{dns}$), 137.03 (Ar$C_{dns}$), 137.53 (Ar$C_{dns}$), 143.54 (Ar*C*), 143.57 (Ar*C*), 143.58 (Ar*C*), 151.91 (Ar$C_{dns}$), 166.95 (CON), 170.73 ppm (CON); MS (MALDI-TOF, CCA): *m/z*: 1900 $[M+K]^+$, 1884 $[M+Na]^+$, 1862 $[M+H]^+$; monoisotopic mass calcd for $C_{99}H_{133}N_{18}O_{12}S_3^+$: 1861.95, found: 1862.02.

**Cell culture**: The human MCF-7 breast cancer cell line was obtained from the American Culture Collection (ATCC, Rockville, Md.). The MCF-7 cells were maintained in MEM Eaglés medium containing L-glutamine, supplemented with $NaHCO_3$ (2.2 g L$^{-1}$), sodium pyrovate (110 mg L$^{-1}$), gentamycin (50 mg L$^{-1}$), and 10% fetal calf serum (FCS) using 75 cm$^2$ culture flasks in a water-saturated atmosphere (95% air/5% $CO_2$) at 37 °C. The cells were serially passaged weekly following trypsinization using 0,05% trypsin/0,02% ethylenediaminetetraacetic acid (EDTA). Mycoplasma contamination was routinely monitored, and only mycoplasma-free cultures were used.

Human HeLa-cells were cultured at 37 °C in a humidified atmosphere with 5% $CO_2$ in RPMI 1640 containing 10% fetal bovine serum, 100 μg mL$^{-1}$ penicillin, 100 μg mL$^{-1}$ streptomycin, and additional glutamine and nonessential amino acids with the following final concentrations: 4 mM glutamine, 1 mM alanine, 1.5 mM aspartic acid, 1.2 mM asparagine, 1.2 mM glutamic acid, 1.2 mM glycine, 1.2 mM proline, and 1.3 mM serine.

**In vitro chemosensitivity assay**: The in vitro testing of the dendrimers on the cytotoxic activity was carried out on exponentially dividing MCF-7 cells according to a previously published microtiter assay.[65–67] Briefly, in 96-well microtiter assay plates (Nunc), 100 μL of a cell suspension at 7000 cells mL$^{-1}$ culture medium were plated into each well and incubated at 37 °C for 2–3 days in a humidified atmosphere (5% $CO_2$). By addition of an adequate volume of a stock solution of the respective compound (solvent: DMF) to the medium the desired test concentration was obtained. For each test concentration and for the control, which contained the corresponding amount of DMF, 16 wells were used. After the proper incubation time the medium was removed, the cells were fixed with a glutaraldehyde solution and stored at 4 °C. Cell biomass was determined by a crystal violet staining technique. The influence of the dendrimers on cell growth, was obtained by corrected *T*/*C* values according to Equations (1) and (2).

$$\text{Cytostatic effect}: \; T/C_{corr}\,[\%] = [(T-C_0)/(C-C_0)] \times 100 \quad (1)$$

$$\text{Cytocidal effect}: \; T\,[\%] = [(T-C_0)/C_0] \times 100 \quad (2)$$

In Equations (1) and (2), *T* (test) and *C* (control) are the optical densities at 578 nm of the crystal violet extract of the cell lawn in the wells (i.e. the chromatin-bound crystal violet extracted with ethanol 70%), and $C_0$ is the density of the cell extract immediately before treatment.Equation (2) allows the automatic estimation of the optical density of the crystal violet extract in the wells of a Flashscan S19 microplatereader (Analytikjena, Jena, Germany).

**Fluorescence microscopy**: The day before exposition to the dendrimers, HeLa cells were seeded in 12-well plates containing a 12 mm glass cover slip in such a density that the next day about 60% confluency was obtained. Dendrimers were added to the cells in a concentration of 5 μM. 20 h after adding the dendrimer, cells were washed in PBS, fixed with 4% paraformaldehyde and probed with the rabbit polyclonal antibody R1[68] raised against the membrane isoforms of LAP-2 and a Cy2-conjugated goat anti-rabbit IgG antibody as secondary antibody following standard procedures.[69] The fluorescence images of the cells were obtained with the Zeiss laser-scanning microscope LSM 510 and a plan-neofluar 63×1.25 oil-immersion objective. The dyes were excited at 364 nm (dansyl-labelled dentrimers) or 488 nm (Cy2) and fluorescent light was collected by a photomultiplier after passage through a 505–530 nm BP filter. Differential interference contrast (DIC) images were taken in parallel.

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