Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information (ESI)

Abbreviations

CCA = α-cyano-4-hydroxycinnamic acid; **DCM** = dichloromethane; **DIPEA** = N,N'-di-isopropyl ethylamine; **DMF** = *N,N*-dimethylformamide; **Dpa** = D/L-2,3-diaminopropionic acid; **HATU** = *O*-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate; **HBTU** = *O*-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronuim tetrafluoroborate; **HFIP** = 1,1,1,3,3,3-hexafluoro isopropanol; **HRMS** = high-resolution mass spectrometry; **NMP** = N-methyl-pyrrolidone; **RP-HPLC** = reversed-phase high performance liquid chromatography; **TFA** = trifluoroacetic acid.

General

Unless otherwise noted, starting materials were purchased from commercial sources and used without further purification. Solvents were dried under standard conditions. All reactions with moisture sensitive reagents were performed in dried glassware after a repeated cycle of evacuation and heating followed by flushing with nitrogen. All experiments under a protective atmosphere were carried out with nitrogen (purity 4.0 or 5.0) purchased from Linde or Messer Griesheim. The solvents used for column chromatography were distilled prior to use. Lyophilization from water was performed using deionised, distilled "MilliQ" (Millipore) (0.45 μm) filtered water. The Boc-protected Dpa was synthesized starting from the commercially available D/L-2,3-diaminopropionic in a slightly modified version of the procedure described by Sergheraert *et al.*,[1] and no attempt was performed to separate the two enantiomers. The partially dansylated dendrimer **2** was prepared as previously described.[2] All other compounds are new. Whenever possible, reactions were monitored by thin-layer chromatography (TLC) using TLC silica gel coated aluminium 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 a Bruker AMX 500 (500 MHz) spectrometer; the solvent signal was used for internal calibration. Mass spectra were recorded using a Varian MAT 711 Type CH5DF (FAB), a Bruker Reflex™ with delayed extraction source, and an Applied Biosystems Voyager System 4041 (MALDI-ToF). Melting points were recorded using a Büchi *510* (open capillaries, uncorrected values). RP-HPLC of the pentapeptide **1** was carried out using a Shimadzu HPLC *LC 8* System and a

Vydac *1520 TPC$_{18}$* column (300 A, 40 x 300 mm; *Eluents:* ***A*** = H_2O / 0.1 % TFA, ***B*** = H_2O / 80 % acetonitrile / 0.9 % TFA) and was used according to the following protocol: *flow rate:* 70 ml/min, gradient 20 --> 70 % ***B*** within 50 min). The purity of all other products was checked by an analytical RP-HPLC system consisting of a Machery-Nagel *Nucleosil*® column (C_{18}, 5 µm, 4 × 250 mm), a Knaur *HPLC pump 64*, and a Knaur *2501 UV Detector* (detection at $\lambda = 220$ nm) and was used according to the following protocol: *flow rate:* 1 ml/min; *eluent:* acetonitrile/water/TFA (49.98:49.97:0.05).

The provided MALDI-ToF mass spectra of dendrimers **3** and **4** (see Figs. ESI 4 and ESI 5) show fragment peaks which are usually observed with Boc-protected and/or dansylated dendrimers.[2] The Boc as well as the Pbf protecting groups can easily be cleaved off the molecules by traces of acid, heat and/or laser irradiation. The cleavage of the dansyl fluorescence tag by laser irradiation in the MALDI process was already described by Vögtle *et al.* who observed the cleavage of up to three dansyl of their dansylated poly(propylene amine) dendrimers.[3a] As dendrimer **3** carries six Boc-, three Pbf-, and three dansyl groups, numerous signals of partially deprotected or de-dansylated dendrimers can be observed in the corresponding MALDI-ToF mass spectra (see Fig. ESI 4). These signals do not represent impurities; they rather correspond to the intrinsic properties of the protected, dansylated dendrimer **3**. The same is true for the deprotected, water-soluble dendrimer **4** which carries three dansyl groups that can be cleaved by laser irradiation (the laser-wavelength of 337 nm corresponds coincidentally well with the absorption maximum of the dansyl fluorescent group at 340 nm[3a,b,c, 4]). Indeed, in the corresponding MALDI-ToF mass spectrum of dendrimer **4** a signal at 2438 (m/z) can be observed that corresponds to a dendrimer fragment where all three dansyl groups have been cleaved off the main dendrimer scaffold (ESI 5). The question if the observed dendrimer fragments are produced in the MALDI process or represent partially degraded dendrimers already present in the sample cannot finally be answered.

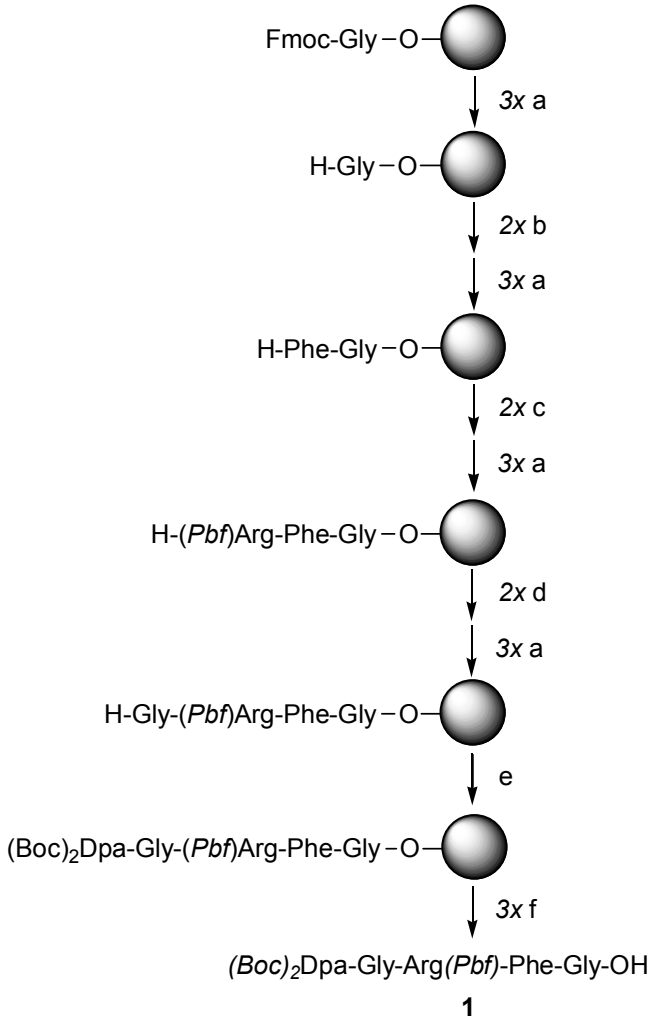
Solid Phase Peptide Synthesis

Synthesis of the C-terminal peptide part was performed utilizing a peptide synthesizer and commercial Fmoc-protected amino acids with a HBTU coupling strategy in the presence of DIPEA in NMP. Subsequent deprotection was achieved by repeated incubation with piperidine in DMF (1:5) (v:v) and was monitored by UV/VIS spectroscopy at 303 nm. For the side group protection of arginine the 2,2,4,6,7-pentamethyldihydrobenzo-furan-5-sulfonyl

(Pbf) group was chosen which was proved to be stable under the classical conditions of Sheppard solid phase peptide synthesis[5] and allowed the cleavage of the protected peptide from the resin. The final coupling of the *tert*-butyloxycarbonyl (Boc)-protected D/L-2,3-diaminopropionic acid, which was synthesized according to literature procedures,[1] was performed manually in a round bottom flask by activation with TATU in DMF in the presence of DIPEA. Cleavage of the protected pentapeptide **1** from the resin was finally achieved by reaction with a mixture of HFIP and dichloromethane in a 1:5 (v:v) ratio, three times at room temperature over a period of 60 minutes (scheme I). The purity of the peptide was subsequently checked by RP-HPLC.[6]

Preparation of (*Boc*)$_2$-Dpa-Gly-Arg(*Pbf*)-Phe-Gly-OH (1)

Solid phase peptide synthesis was performed with an ABI433A peptide synthesizer (*Applied Biosystems,* Foster City, USA) using a glycine-preloaded resin (*PepChem,* Tübingen, Germany) (435 mg, *loading* 0.51 mmol/g, 222 µmol). Coupling of the corresponding Fmoc-protected L-amino acid (*Novabiochem*, Läufelfingen, Switzerland) was performed with HBTU in NMP using a standard protocol under UV monitoring at 301 nm. Each coupling step was repeated two times. Fmoc deprotection was achieved by piperidine in NMP (1:5; v:v) and monitored by UV/VIS spectroscopy at 301 nm. After each complete coupling and deprotection step the resin was washed twice with methanol and DCM. All reactions were performed under a nitrogen atmosphere. For the final coupling of D/L-2,3-diaminopropionic acid (Dpa), the Boc-protected Dpa-precursor (135.79 mg, 0.4 mmol, 2 eq.) was dissolved in a 50 ml round bottom flask in DMF and activated manually by the addition of HATU (143 mg, 0.4 mmol, 2 eq.) at room temperature. After 2 min under vigorous stirring, the reaction mixture was added to a suspension of the peptide-loaded resin in DMF (50 ml) in the presence of DIPEA (152 µl, 0.8 mmol, 4 eq.). The reaction mixture was then stirred over night at room temperature. Cleavage of the protected pentapeptide **1** from the resin was achieved by three reaction cycles with HFIP/DCM (1:5/v:v) for 20 min and a final washing step with DCM. The filtrates were collected, the solvent evaporated *in vacuo*, and the peptide precipitated with diethyl ether. The procedure yielded the protected pentapeptide **1** (212 mg, 218 µmol, 98.2 %) as a colorless solid.



Scheme ESI 1 Solid phase synthesis of pentapeptide **1** on an *o*-chlorotrityl resin. *Reagents and conditions:* (a) Piperidine, NMP, 15 min; (b) Fmoc-L-Phe-OH (5 eq.), HBTU (5 eq.), DIPEA (10 eq.), NMP, 30 min; (c) Fmoc- L-Arg(Pbf)-OH (5 eq.), HBTU (DMF) (5 eq.), DIPEA (10 eq.), NMP, 30 min; (d) Fmoc-Gly-OH (5 eq.), HBTU (5 eq.), DIPEA (10 eq.), NMP, 30 min; (e) Boc_2-D/L-Dpa-OH (2 eq.), HATU (2 eq.), DIPEA (4 eq.), DMF, 20 h; (f) HFIP/CH_2Cl_2 (1/5), 20 min (98%).

$\boldsymbol{R}_\mathbf{f}$ = 0.19 (ethyl acetate/methanol (2:1/v:v)).

M.p. 121-123 °C.

^{1}H NMR (500 MHz, CD_3OD): δ = 1.41 (m, br, 2 H, $CH_{2,\,Arg}$), 1.47 (s, 18 H $C(CH_3)_{3,\,Boc}$), 1.49 (s, 6 H, $C(CH_3)_{2,\,Pbf}$), 1.66 (m, 2 H, $CH_{2,\,Arg}$), 2.12 (s, 3 H, $CH_{3,\,Pbf}$), 2.55 (s, 3 H, $CH_{3,\,Pbf}$), 2.61 (s, 3 H, $CH_{3,\,Pbf}$), 2.99 (m, 1 H, CH_2), 3.04 (s, 2 H, $CH_{2,\,Pbf}$), 3.08 (m, 1 H, CH_2), 3.14 (m, 2 H, CH_2), 3.30 (m, 1 H, CH_2), 3.48 (m, 1 H, CH_2), 3.88 (m, 2 H, $CH_{2,\,Gly}$), 3.97 (m, 2 H, $CH_{2,\,Gly}$), 4.16 (m, 1 H, CH_{Arg}), 4.24 (m, 1 H, CH_{Dpa}), 4.70 (m, 1 H, CH_{Phe}), 7.19 (m, 2 H, ArH_{Phe}), 7.28 (m, 3 H, ArH_{Phe}).

^{13}C NMR (126 MHz, CD_3OD): δ = 12.81, 18.70, 19.88, 26.71, 29.02 (C*C*$H_{3,\ Boc}$), 29.94, 38.68, 41.82, 42.22, 43.02, 44.28, 55.37, 56.25, 57.36, 80.86 (CH), 81.45 (CH), 87.96 (CH), 118.74 (Ar*C*), 126.34 (Ar*C*), 128.05 (Ar*C*), 129.77 (Ar*C*), 130.64 (Ar*C*), 133.82 (Ar*C*), 134.71 (Ar*C*), 138.96 (Ar*C*), 139.71 (Ar*C*), 158.39 (CON_{Boc}), 159.07 (CON_{Boc}), 160.19 (CN_{Arg}), 173.11 (CO), 173.51 (CO), 173.81 (CO), 174.11 (CO), 174.31 (CO).

MS (FAB+, MNBA/DMSO): *m/z* (%): 996 (12.7) $[M+Na]^+$, 975 (70.1) $[M+H]^+$; monoisotopic mass calcd. for $\mathbf{C_{45}H_{68}N_9O_{13}S^+}$: 974.5, found: 974.6.

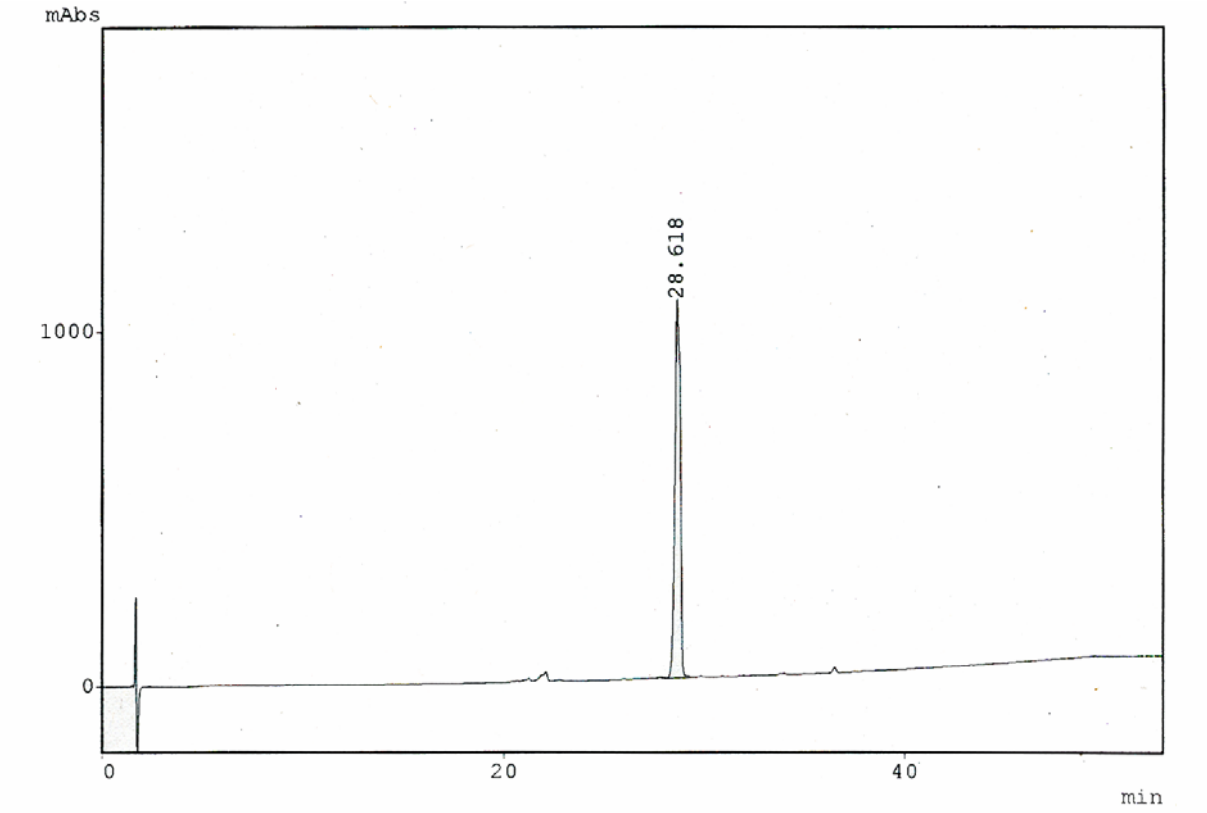


Figure ESI 1 Analytical RP-HPLC trace of the synthetic, Boc- and Pbf-protected pentapeptide **1** (e*luent:* linear gradient of acetonitrile (16-56 %) in water during a period of 50 min.; *purity:* 98 %)

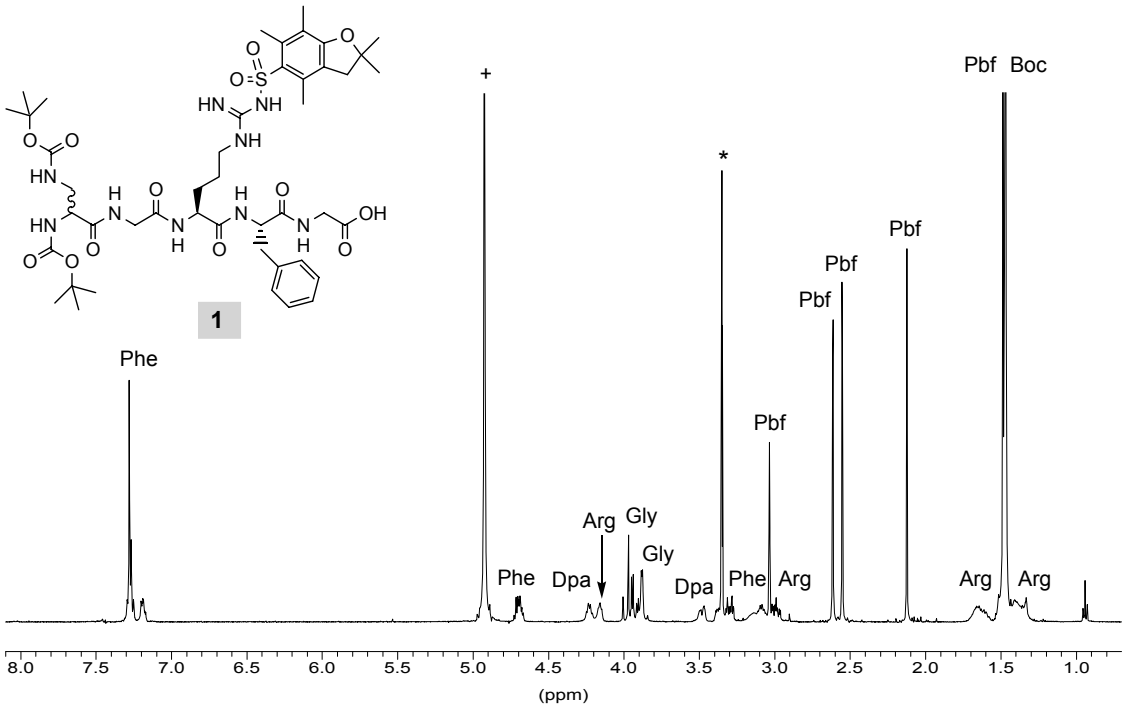


Figure ESI 2 ^{1}H NMR (500 MHz) of the protected pentapeptide **1** in [D_4] methanol. (*) = methanol, (+) = H_2O.

Preparation of 1,3,5-Tris{3-[(*Boc*)$_2$-Dpa-Gly-Arg(*Pbf*)-Phe-Gly-]-5-[(5-dimethylamino-naphthalene-1-sulfonylamino)propyl]-*N*-propylbenzamide}benzene (3)

To a solution of peptide **1** (124 mg, 127 µmol) in dry DMF the coupling reagent HATU (53.2 mg, 140 µmol) and DIPEA (70.3 µl, 53.4 mg, 413 µmol) were added at 0 °C and stirred for additional 5 min. After complete formation of the active ester (TLC) the reaction mixture was cooled down to – 20 °C and a solution of the partially dansylated dendrimer **2** (42.5 mg, 26.5 µmol) in a small amount of dry DMF was added drop wise under vigorous stirring. The solution was then allowed to warm up to room temperature slowly and stirred for additional 12 hours in the dark. After complete reaction (TLC-monitored) the reaction mixture was washed once with a saturated sodium carbonate solution, and once with brine. The organic phase was dried with magnesium sulfate and the solvent removed *in vacuo*. Column chromatography (silica gel, DCM cont. 7 % methanol as eluent) gave the dansylated peptide dendrimer **3** (82.1 mg, 18.3 µmol, 69.1 %) as a light yellow, fluorescent oil which could be lyophilized from dioxane.

R_f = 0.16 (DCM/methanol (9:1/v:v)).

M.p. 152-153 °C.

^{1}H NMR (500 MHz, CD_2Cl_2/CD_3OD): δ = 1.35 (m, br, 6 H, $CH_{2,\,Arg}$), 1.41 (s, 27 H $C(CH_3)_{3,\,Boc}$), 1.43 (s, 27 H $C(CH_3)_{3,\,Boc}$), 1.45 (s, 18 H, $C(CH_3)_{2,\,Pbf}$), 1.63 (m, 6 H, CH_2), 1.70 (m, 6 H, $CH_{2,\,Dendr.}$), 1.80 (m, 6 H, $CH_{2,\,Dendr.}$), 1.94 (m, 6 H, $CH_{2,\,Dendr.}$), 2.09 (s, 9 H, $CH_{3,\,Pbf}$), 2.51 (s, 9 H, $CH_{3,\,Pbf}$), 2.54 (m, 6 H, $CH_2Ar_{Dendr.}$), 2.57 (s, 9 H, $CH_{3,\,Pbf}$), 2.62 (m, 6 H, $CH_2Ar_{Dendr.}$), 2.84 (m, 6 H, $CH_2Ar_{Dendr.}$), 2.87 (m, 6 H + 18 H, CH_2 + $CH_{2,Dns}$), 2.99 (s, 18 H, $NCH_{3,\,Pbf}$), 3.16 (m, 6 H, CH_2), 3.28 (m, 3 H, CH_2N) 3.38 (m, 6 H + 6 H, $CHCH_{2,\,Dpa}$ + CH_2N), 3.44 (m, 6 H, CH_2), 3.84 (m, 6 H, $CH_{2,\,Gly}$), 3.87 (m, 6 H, $CH_{2,\,Gly}$), 4.15 (m, 3 H, CH_{Arg}), 4.15 (m, 3 H, CH_{Dpa}), 4.51 (m, 3 H, CH_{Phe}), 6.92 (s, 3 H, Ar*H*), 7.04 (s, 3 H, Ar*H*), 7.11 (d, ^3J (H,H) = 5.8 Hz, 3 H, ArH_{Dns}), 7.23 (m, 15 H, ArH_{Phe}), 7.34 (s, 3 H, Ar*H*), 7.42 (s, 3 H, Ar*H*), 7.52 (t, ^3J (H,H) = 8.0 Hz, 3 H, ArH_{Dns}), 7.56 (t, ^3J (H,H) = 8.2 Hz, 3 H, ArH_{Dns}), 8.17 (d, ^3J (H,H) = 7.2 Hz, 3 H, ArH_{Dns}), 8.34 (d, ^3J (H,H) = 8.5 Hz, 3 H, ArH_{Dns}).

^{13}C NMR (126 MHz, CD_2Cl_2/CD_3OD): δ = 12.50, 18.16, 18.60, 19.41, 28.43, 28.58, 30.15, 31.00, 31.26, 31.48, 31.64, 32.69, 32.92, 33.64, 36.94, 39.10, 40.21, 42.07, 42.64, 43.19, 43.32, 43.53, 43.71, 45.57, 55.07, 56.13, 56.34, 115.72, 119,47, 123.72, 125.29, 125.34, 125.46, 126.70, 127.23, 128.66, 128.94, 129.55, 129.61, 129.72, 130.14, 130.37, 130.67, 132.30, 132.78, 133.51, 135.09, 135.74, 137.77, 137.91, 138.74, 142.38, 142.44, 142.71, 152.43, 157.04, 159.16, 163.78, 168.45, 169.41, 170.47, 171.71, 172.88, 173.64.

MS (MALDI-ToF, dithranol): *m/z:* 4508 $[M+K]^+$, 4492 $[M+Na]^+$, 4470 $[M+H]^+$, 4218 $[M-C_{13}H_{17}O_3S+H]^+$, 3702, 2889;
monoisotopic mass calcd. for $\mathbf{C_{225}H_{39}N_{39}NaO_{45}S_6^+}$: 4492.13, found: 4492.10.

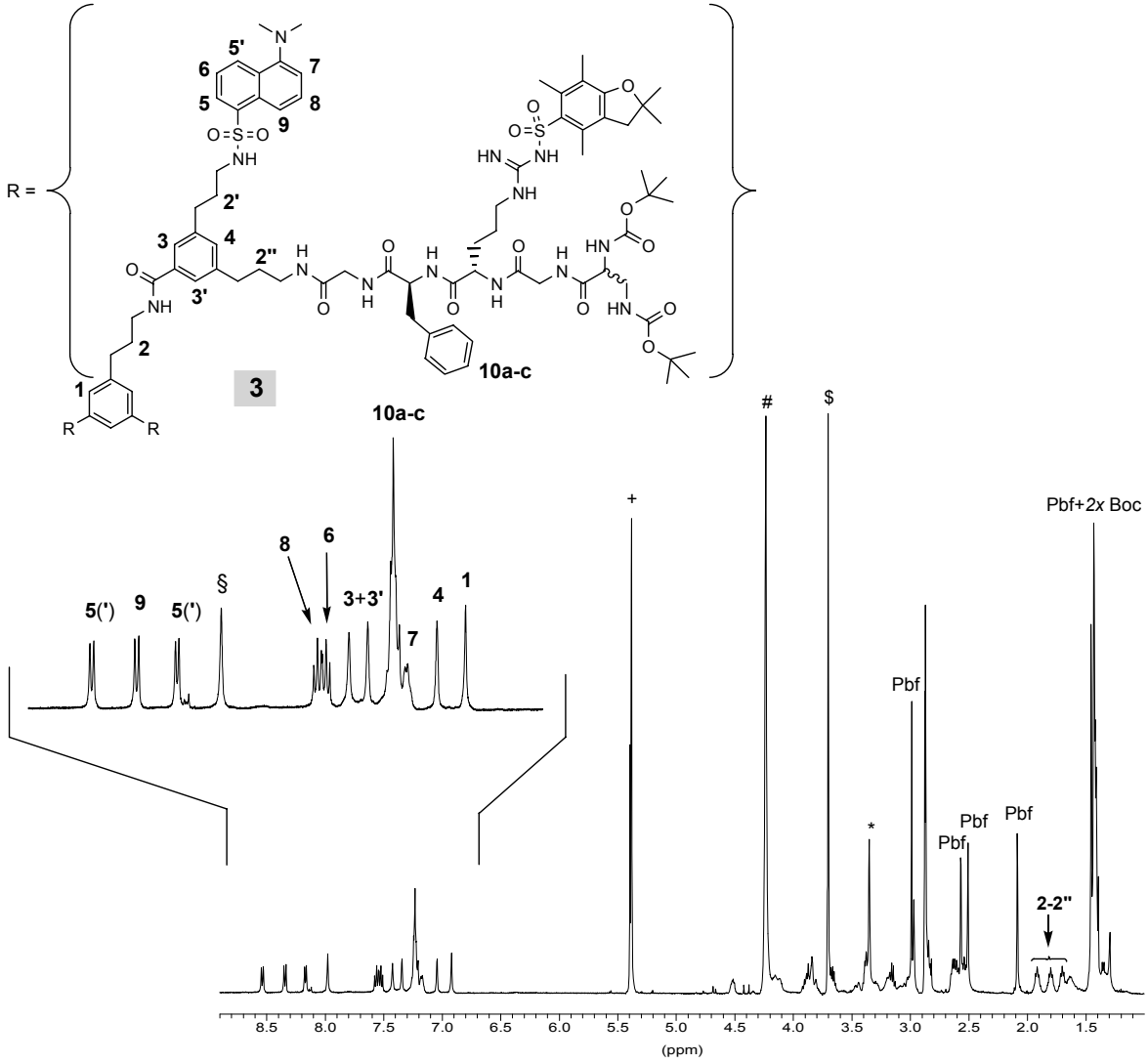


Figure ESI 3 ^{1}H NMR (500MHz) of the protected, partially dansylated peptide dendrimer **3** with a terminal D/L-2,3-diaminopropionic acid in a solvent mixture of [D_2] DCM und [D_4] methanol as obtained after column chromatography over silica gel (DCM with 7 % Methanol as eluent). (*) = methanol; (+) = DCM; ($) = dioxane; (#) = H_2O; (§) = DMF.

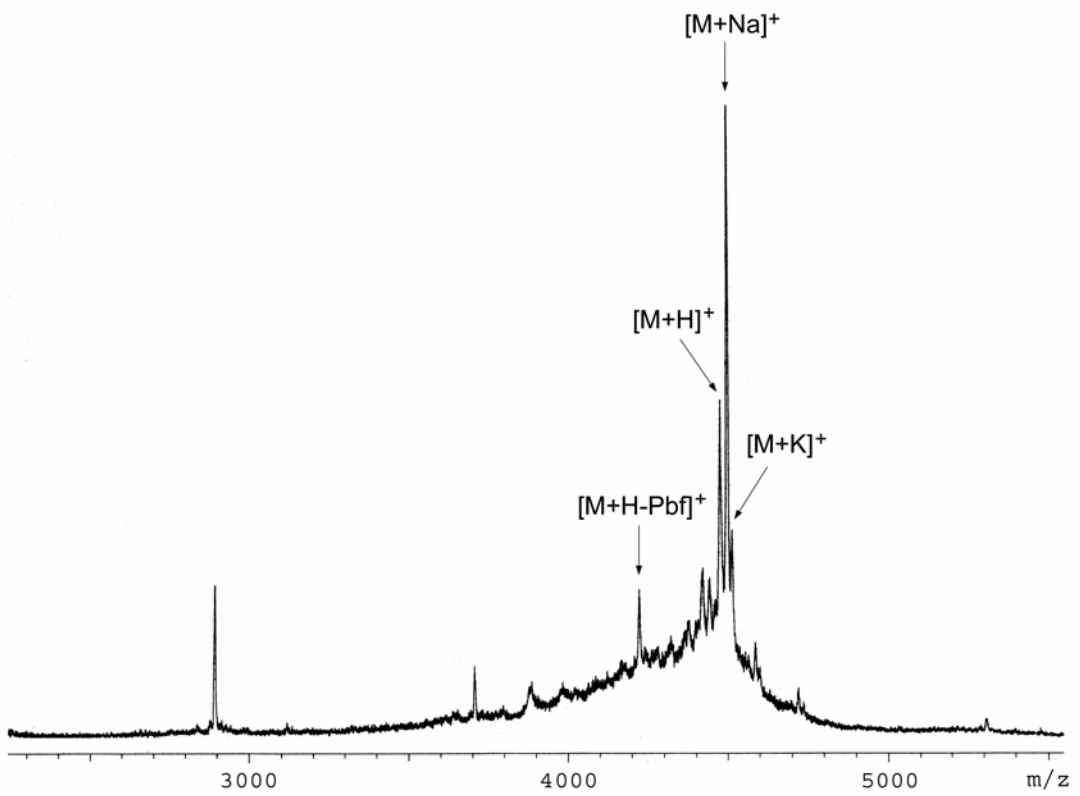


Figure ESI 4 MALDI-ToF mass spectrum of the protected, partially dansylated peptide dendrimer **3** with terminal D/L-2,3-diaminopropionic acid (linear mode; *matrix:* dithranol).

Preparation of 1,3,5-Tris{3-(*H*-Dpa-Gly-Arg-Phe-Gly]-5-[(5-dimethylamino-naphthalene-1-sulfonylamino)propyl]-*N*-propylbenzamide}benzene nonatrifluoroacetate (4)

The protected peptide dendrimer **3** (25.0 mg, 5.57 µmol) was dissolved in aqueous TFA (95 %) and stirred at room temperature for 3 h. After complete reaction (TLC) the solvent was removed under reduced pressure and the deprotected dendrimer repeatedly redissolved in methanol/water (1:1/v:v). Finally the solvent was removed *in vacuo* to yield the desired deprotected peptide dendrimer **4** (23.0 mg, 5.55 µmol, 99.6 %) as a light yellow, fluorescent oil, which could be lyophilised from acetonitrile/water (3:1/v:v).

M.p. 152-154 °C.

^{1}H NMR (500 MHz, CD_3CN/D_2O): δ = 1.23 (m, br, 6 H, $CH_{2,\,Arg}$), 1.33 (m, br, 6 H, $CH_{2,\,Arg}$), 1.56 (m, 6 H, $CH_{2,\,Dend.}$), 1.60 (m, 6 H, $CH_{2,\,Dendr.}$), 1.75 (m, 6 H, $CH_{2,\,Dendr.}$), 2.40 (t, ^3J (H,H) = 7.5 Hz, 6 H, $CH_2Ar_{Dendr.}$), 2.45 (t, ^3J (H,H) = 7.7 Hz, 6 H, $CH_2Ar_{Dendr.}$), 2.50 (t, ^3J (H,H) = 7.3 Hz, 6 H, $CH_2Ar_{Dendr.}$), 2.77 (t, ^3J (H,H) = 6.9 Hz, 6 H, $CH_2N_{Dendr.}$), 2.89 (m,

3 H, C$H_{2,\ Phe}$), 2.95 (t, ^{3}J (H,H) = 6.9 Hz, 6 H, CH_2N_{Arg}), 3.01 (m, 6 H, $CH_2N_{Dendr.}$), 3.05 (m, 3 H, C$H_{2,\ Phe}$), 3.23 (t, ^{3}J (H,H) = 5.7 Hz, 6 H, $CH_2N_{Dendr.}$), 3.32 (s, 18 H, NCH_3), 3.45 (d, 6 H, $CH_{2,\ Dpa}$), 3.93 (t, ^{3}J (H,H) = 5.7 Hz, 6 H, $CH_{2,\ Gly}$), 4.22 (6 H, $CH_{2,\ Gly}$, hidden by the solvent signal), 4.14 (m, 3 H, CH_{Arg}), 4.33 (t, ^{3}J (H,H) = 5.8 Hz, 3 H, CH_{Dpa}), 4.49 (t, ^{3}J (H,H) = 7.5 Hz, 3 H, CH_{Phe}), 6.83 (s, 3 H, Ar*H*), 6.99 (s, 3 H, Ar*H*), 7.09 (s, 3 H, Ar*H*), 7.13 (m, 15 H, ArH_{Phe}), 7.27 (s, 3 H, Ar*H*), 7.75 (m, 6 H, ArH_{Dns}), 8.18 (d, ^{3}J (H,H) = 7.4 Hz, 3 H, ArH_{Dns}), 8.32 (d, ^{3}J (H,H) = 8.7 Hz, 3 H, ArH_{Dns}), 8.73 (d, ^{3}J (H,H) = 8.8 Hz, 3 H, ArH_{Dns}).

^{13}C NMR (126 MHz, CD_3CN/D_2O): δ = 25.10, 29.23, 31.19, 31.29, 31.48, 32.69, 33.03, 33.7, 37.82, 39.64, 40.34, 41.46, 42.99, 43.28, 43.45, 47.7, 45.57, 51.42, 54.35, 55.28, 115.94, 119.52, 124.58, 124.91, 125.58, 126.34, 126.98, 127.07, 127.15, 128.10, 128.79, 129.36, 129.72, 130.11, 131.99, 135.23, 137.51, 137.58, 139.71, 139.78, 142.87, 143.11, 143.44, 157.64, 167.40, 169.85, 171.15, 171.21, 173.55, 173.63.

MS (MALDI-ToF, CCA): *m/z:* 3204 (not yet assigned), 3136 $[M+Na]^+$, 3114 $[M+H]^+$, 3099 (not yet assigned), 2438 $[M-3x(C_{12}H_{11}NO_2S)+Na]^+$, 1557 $[M+2H]^{2+}$;
monoisotopic mass calcd. for $\mathbf{C_{156}H_{213}N_{39}O_{24}S_3^+}$: 3113.59, found: 3113.65.

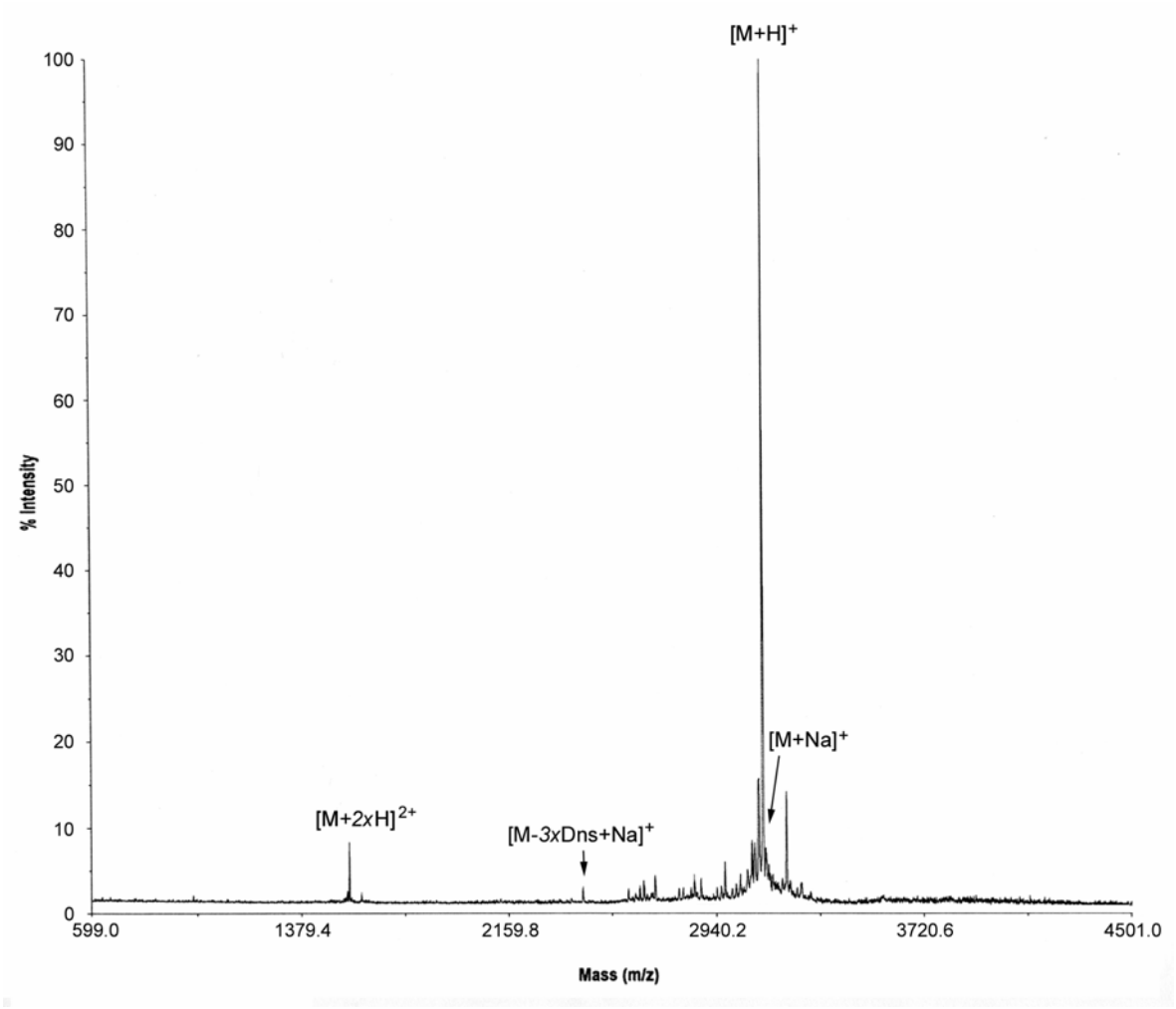


Figure ESI 5 MALDI-ToF mass spectrum of the deprotected, partially dansylated peptide dendrimer **4** with terminal D/L-2,3-diaminopropionic acid (reflector mode; *matrix:* CCA).

References

[1] (a) C. Sergheraert, P. Mäes, A. Tartar, *J. Chem. Soc., Perkin Trans. I,* 1986, 1061-1064;
(b) no attempt was performed to seperate the two enantiomers of the Boc-D/L-diaminopropionic acid.

[2] S. Fuchs, T. Kapp, H. Otto, T. Schöneberg, P. Franke, R. Gust, A. D. Schlüter, *Chem. Eur. J.,* 2004, **5**, 1167-1192.

[3] (a) F. Vögtle, S. Gestermann, C. Kauffmann, P. Ceroni, V. Vicinelli, L. De Cola, V. Balzani, *J. Am. Chem. Soc.,* 1999, **121**, 12161-12166 (S1-8); (b) V. Balzani, P. Ceroni, S. Gestermann, C. Kauffmann, M. Gorka, F. Vögtle, *Chem. Commun.,* 2000, 853-854; (c) V. Balzani, P. Ceroni, S. Gestermann, M. Gorka, C. Kauffmann, F. Vögtle, *J. Chem. Soc., Dalton. Trans.,* 2000, 3765-3771.

[4] C. M. Cardona, J. Alvarez, A. E. Kaifer, T. D. McCarley, S. Pandey, G. A. Baker, N. J. Bonzagni, F. V. Bright, *J. Am. Chem. Soc.,* 2000, **122**, 6139-6144.

[5] (a) E. Atherton, R. C. Sheppard, J. D. Wade, *J. Chem. Soc., Chem. Commun.,* 1983, 1060-1062; (b) E. Atherton, C. L. Logan, R. C. Sheppard, *J. Chem. Soc., Perkin Trans. 1,* 1981, 538-546.

[6] Eluted with a linear gradient of 0 - 100 % acetonitrile in water within 45 min from a Vydac *1520 TPC$_{18}$* C-18 column.