Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Strategy for the Synthesis of Multivalent Peptide-Based Nonsymmetric Dendrimers by Native Chemical Ligation

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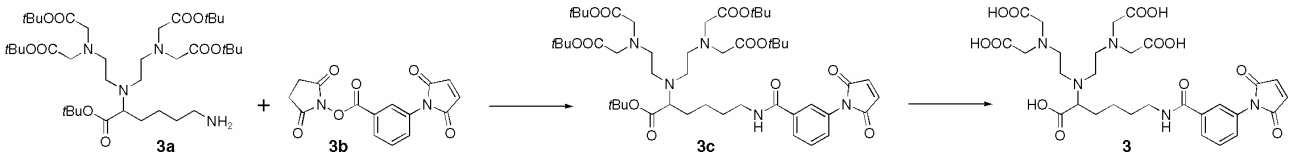
Solvents and Starting Materials. Unless stated otherwise, all reagents and solvents were purchased from commercial sources and used without any further purification.

Instrumentation. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Unity Inova 500 MHz spectrometer at 298 K. Reversed phase high pressure liquid chromatography (RP HPLC) was performed on a Varian ProStar HPLC system coupled to an UV-Vis detector probing at 214 nm using a VydacTM protein & peptide C18 column. The MALDI-TOF spectrum of **3c** was obtained at a Perspective Biosystems Voyager DE-pro MALDI-TOF spectrometer using a α-cyano-4-hydroxycinnamic acid as a matrix. Electrospray ionization mass spectrometry (ESI-MS) was performed on a Perkin Elmer PE SCIEX Turbo Ionspray.

Synthesis of the maleimide-functionalized DTPA synthon (3). *tert*-Butyl-6-amino-2-{{bis-{2-[bis-(*tert*-butoxycarbonylmethyl)amino]-ethyl}-amino}} hexanoate (**3a**)[1] and 3-maleimidobenzoyl *N*-hydroxysuccinimide ester (**3b**)[2] were synthesized according to literature procedures.



***tert*-Butyl-2-{{bis-{2-[bis-(*tert*-butoxycarbonylmethyl)amino]-ethyl}-amino}}-6-(1-maleimido-3-carbonyl-amino-phenyl) hexanoate (3c).** To a stirred solution of 1.00 g (1.34 mmol) of **3a** in 10 mL of DCM, 0.86 g (2.74 mmol) of **3b** was added. The reaction mixture was stirred for 1 hour under argon. ^{1}H NMR showed ~ 60% conversion to the desired product, while the starting materials were also still present. Elongation of the reaction time did not lead to a greater conversion, so the reaction was worked up. After removal of the DCM under reduced pressure, diethyl ether was added to precipitate unreacted **3b** from the reaction mixture. After filtration the solvent was removed *in vacuo*. The crude reaction mixture was purified by column chromatography on Silica using 9:1 *v/v* diethyl ether/*n*-hexane as an eluent (R_f = 0.20), rendering **3c** (0.60 g, 0.63 mmol, 47%) as a colorless solid. ^{1}H NMR (dmso-d_6, 500.62 MHz): δ (ppm) = 1.42 (s, 45H, C(CH_3)$_3$), 1.65 (m, 6H, C=ONHCH$_2$CH_2CH_2CH_2), 2.74 (m, 4H, NCH_2CH_2N), 3.30 (m, 1H, CH$_2$CHCOO*t*Bu), 3.40 (s,

8H, CH_2COO*t*Bu), 3.46 (m, 2H, C=ONHCH_2CH$_2$), 6.70 (t, 1H, *J* = 5.5 Hz, C=ON*H*CH$_2$), 6.85 (s, 2H, C*H*=C*H*), 7.44 (d, 1H, *J* = 8.0 Hz, H_{ar}), 7.49 (t, 1H, *J* = 8.0 Hz, H_{ar}), 7.79 (s, 1H, H_{ar}), 7.80 (d, 1H, *J* = 8.0 Hz, H_{ar}); ^{13}C NMR (dmso-d$_6$, 125.89 MHz): δ (ppm) = 23.6, 28.2, 28.3, 28.9, 29.4, 39.9, 50.2, 53.6, 56.0, 63.8, 80.9, 125.0, 126.4, 128.6, 129.2, 131.6, 134.3, 136.2, 166.3, 169.1, 170.7; MALDI-TOF MS calcd. for $C_{49}H_{77}N_5O_{13}$ ([M+H]$^+$): 944.16, found 944.46.

2-{{bis-{2-[bis-(carboxymethyl)amino]-ethyl}-amino}}-6-(1-maleimido-3-carbonyl-amino-phenyl) hexanoic acid (3). 3c (0.53 g, 0.56 mmol) was dissolved in 1:1 *v/v* DCM/TFA and the solution was stirred overnight under Argon. Most of the solvent was removed under reduced pressure and the compound was redissolved in 1:1 *v/v* DCM/TFA. The solution was stirred for an additional hour to ensure complete conversion of the *tert*-butyl esters to the corresponding carboxylic acids. After removal of DCM under reduced pressure, the product was precipitated by the addition of diethyl ether. Filtration and subsequent washing with diethyl ether to remove the excess of TFA yielded **3** as a white solid (0.37 g, 0.56 mmol, 99%). ^{1}H NMR (dmso-d$_6$, 500.62 MHz): δ (ppm) = 1.53 (m, 4H, C=ONHCH$_2$C*H*$_2$C*H*$_2$CH$_2$), 1.89 (m, 2H, C=ONHCH$_2$CH$_2$CH$_2$C*H*$_2$), 3.03 (m, 4H, NC*H*$_2$CH$_2$NCH$_2$COOH), 3.27 (m, 2H, C=ONHC*H*$_2$), 3.32 (m, 4H, NCH$_2$C*H*$_2$NCH$_2$COOH), 3.54 (s, 8H, NC*H*$_2$COOH), 4.49 (t, 1H, *J* = 6.0 Hz, C*H*COOH), 7.22 (s, 2H, C*H*=C*H*), 7.50 (d, 1H, *J* = 8.0 Hz, H_{ar}), 7.58 (t, 1H, *J* = 8.0 Hz, H_{ar}), 7.80 (s, 1H, H_{ar}), 7.86 (d, 1H, *J* = 8.0 Hz, H_{ar}), 8.56 (d, 1H, *J* = 5.5 Hz, N*H*), 12.22 (bs, 5H, COO*H*); ^{13}C NMR (dmso-d$_6$, 125.89 MHz): δ (ppm) = 23.5, 26.5, 28.9, 40.1, 49.6, 50.5, 54.0, 63.9, 125.7, 126.1, 128.8, 129.4, 131.6, 134.7, 135.4, 165.2, 169.8, 172.6. ESI-MS calcd. for $C_{29}H_{37}N_5O_{13}$ ([M+H]$^+$): 664.24, found 664.3.

***t*Boc-mediated solid phase peptide synthesis (SPPS).** The poly(lysine) dendritic wedges **1** and **2** having a thioester functionality at their focal point and sulfhydryl groups along their periphery, and **6** with a thioproline residue at its focal point and cysteine residues along its periphery, as well as the thioester-functionalized RGDS peptide **7** were synthesized using the *in situ* neutralization/HBTU

activation procedure for *t*Boc chemistry on a *p*-methylbenzhydrylamine (MBHA) resin as previously described by Schnölzer *et al.*[3]

1st Generation DTPA-functionalized dendritic wedge (4). 10.6 mg (8.96×10^{-3} mmol) of **1** and 0.8 equivalents of **3** per sulfhydryl end group (18.1 mg, 0.027 mmol) were dissolved in 2 mL 0.1 M Tris (aq) (pH 6.98). The pH was adjusted to pH 6.5 by the addition of small aliquots of 0.5 M NaOH (aq). The reaction was monitored employing analytical RP HPLC using a C18 column for separation coupled to UV-Vis (λ_{probe} = 214 nm). Within 1 hour the reaction went to completion. The product was purified employing preparative RP HPLC over a C18 column (gradient: 20–40% 9:1 *v/v* MeCN/H_2O in H_2O, 0.1% TFA in 90 minutes). Freeze drying rendered 6.7 mg (1.75×10^{-3} mmol, 26%) of **4** as a fluffy white powder: ESI-MS calcd. for $C_{163}H_{230}N_{32}O_{65}S_5$ ($[M+H]^+$): 3838.1, found 3838.2 ± 0.5.

NB. The synthesis of **4** was the first of the DTPA-functionalized dendritic wedges to be performed on a larger scale. The relatively low yield as compared to **5** may be attributed to losses during RP HPLC purification, since the Michael addition of **3** to **1** itself is a clean and fast reaction.

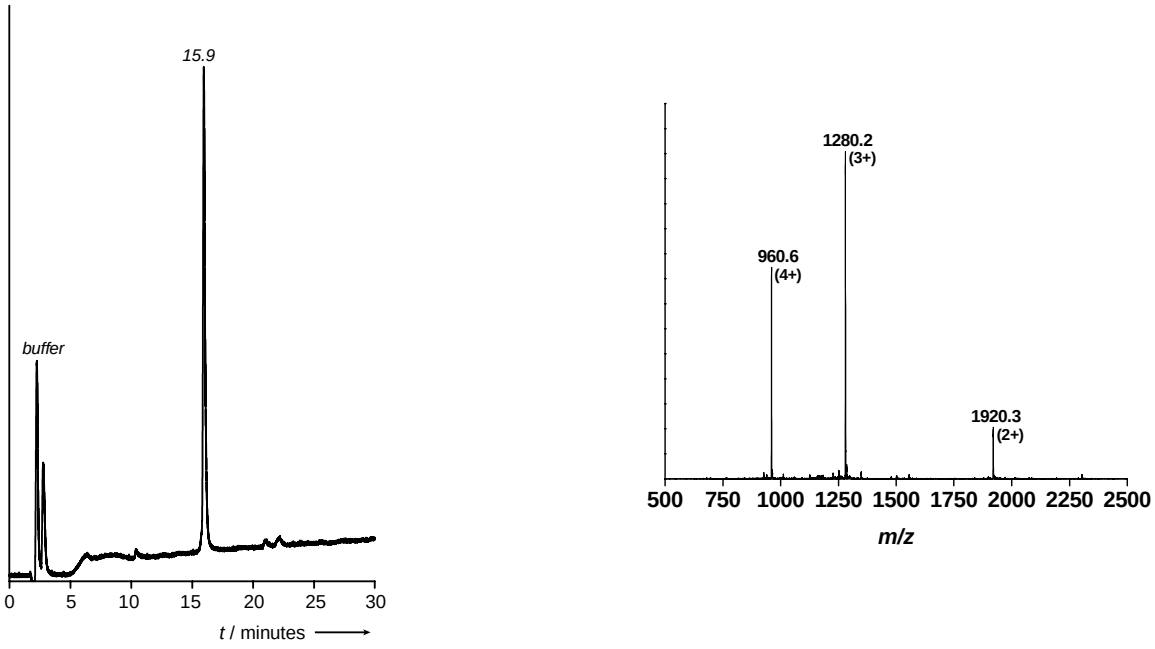


Figure 1. RP HPLC trace (gradient: 0–67% 9:1 *v/v* MeCN/H_2O in H_2O, 0.1% TFA in 30 minutes) (left) and the ESI-MS spectrum of **4** (right).

2nd Generation DTPA-functionalized dendritic wedge (5). 10.0 mg (4.88×10^{-3} mmol) of **2** and 0.8 equivalents of **3** per sulfhydryl end group (20.7 mg, 0.031 mmol) were dissolved in 2 mL 0.1 M Tris (aq) (pH 6.98). The pH was adjusted to pH 6.5 by the addition of small aliquots of 0.5 M NaOH (aq). The reaction was monitored employing analytical RP HPLC using a C18 column for separation coupled to UV-Vis (λ_{probe} = 214 nm). Within 1 hour the reaction went to completion. The product was purified employing preparative RP HPLC over a C18 column (gradient: 20–40% 9:1 *v/v* MeCN/H_2O in H_2O, 0.1% TFA in 90 minutes). Freeze drying rendered 22.8 mg (3.10×10^{-3} mmol, 80%) of **5** as a fluffy white powder: ESI-MS calcd. for $C_{315}H_{442}N_{60}O_{125}S_9$ ($[M+H]^+$): 7357.8, found 7358.4 ± 0.7.

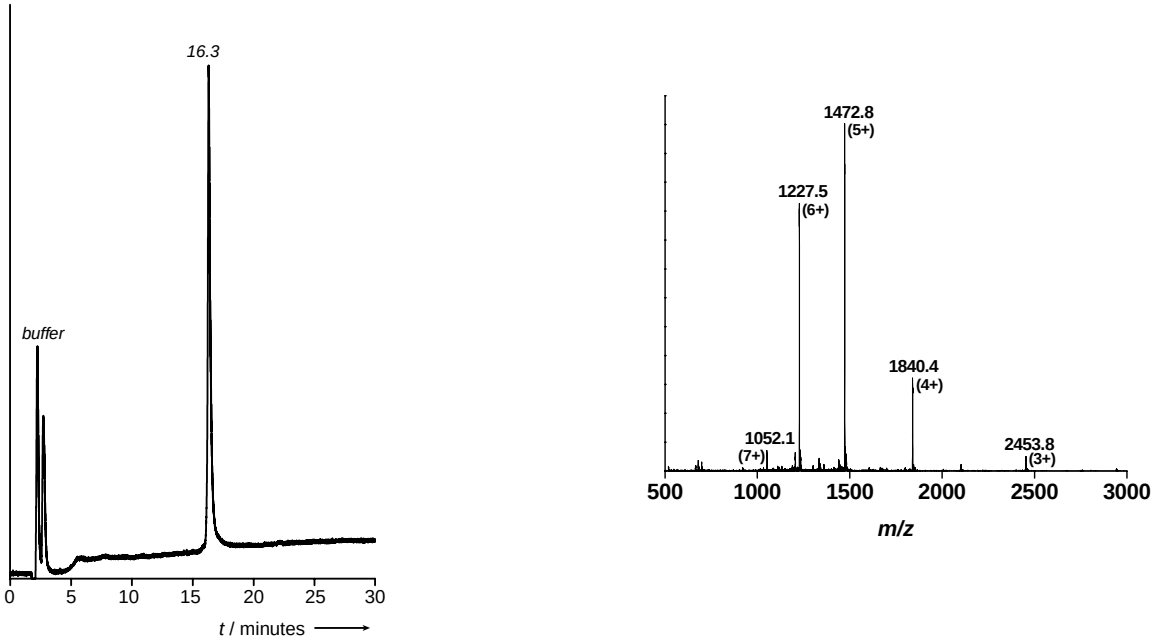


Figure 2. RP HPLC trace (gradient: 0–67% 9:1 *v/v* MeCN/H_2O in H_2O, 0.1% TFA in 30 minutes) (left) and the ESI-MS spectrum of **5** (right).

1st Generation RGDS-functionalized dendritic wedge (8). 54.6 mg (0.052 mmol) of **6** and 184.8 mg (0.218 mmol, 1.05 equivalents per cysteine residue) of **7** were dissolved in 5 mL of 6 M Guanidine in 0.07 M Tris (aq) (pH 8). To this solution 50 µL (1 *v*-%) of thiophenol were added. The pH was adjusted to pH ~7 by the addition of small aliquots of 0.5 M NaOH (aq). The reaction was continued for 1 hour at 37 ˚C. The reaction mixture was filtered and the product was purified

employing preparative RP HPLC over a C18 column (gradient: 5–25% 9:1 *v/v* MeCN/H_2O in H_2O, 0.1% TFA in 90 minutes). Freeze drying rendered 61.4 mg (0.017 mmol, 33%) of **8** as a fluffy white powder. ESI-MS calcd. for $C_{132}H_{220}N_{54}O_{53}S_5$ ($[M+H]^+$): 3571.8, found 3571.5 ± 0.4.

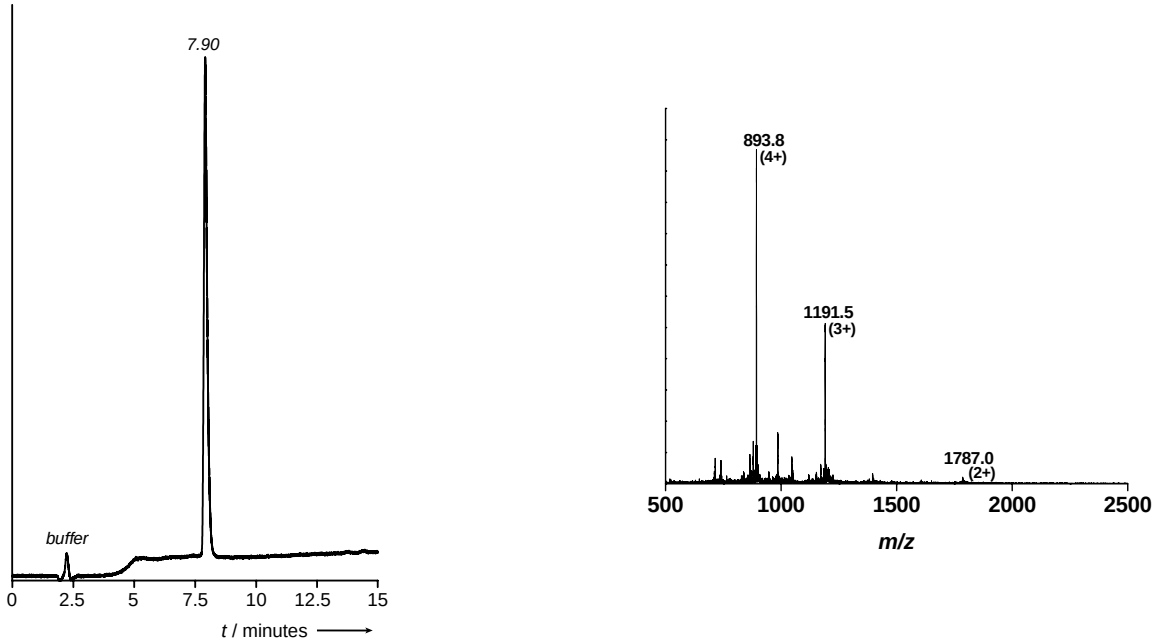


Figure 3. RP HPLC trace (gradient: 0–67% 9:1 *v/v* MeCN/H_2O in H_2O, 0.1% TFA in 15 minutes) (left) and the ESI-MS spectrum of **8** (right).

Ligation of two dendritic wedges on an analytical scale:

Synthesis of the RGDS-functionalized dendritic wedge (9). The thioproline residue of **8** was converted to a cysteine residue by dissolving it in 0.2 M solution of methoxylamine.HCl in 6 M Guanidine in 0.07 M Tris (aq) (pH ~4) at RT, such that the concentration of **8** was 10 mg/mL. The reaction was monitored employing analytical RP HPLC using a C18 column for separation coupled to UV-Vis (λ_{probe} = 214 nm) and analyzed by ESI-MS. Full conversion was reached after 2 hours. The reaction mixture was used as such for the ligation reaction with the DTPA-functionalized dendritic wedges. ESI-MS calcd. for $C_{131}H_{220}N_{54}O_{53}S_5$ ($[M+H]^+$): 3559.8, found 3557.3 ± 0.3.

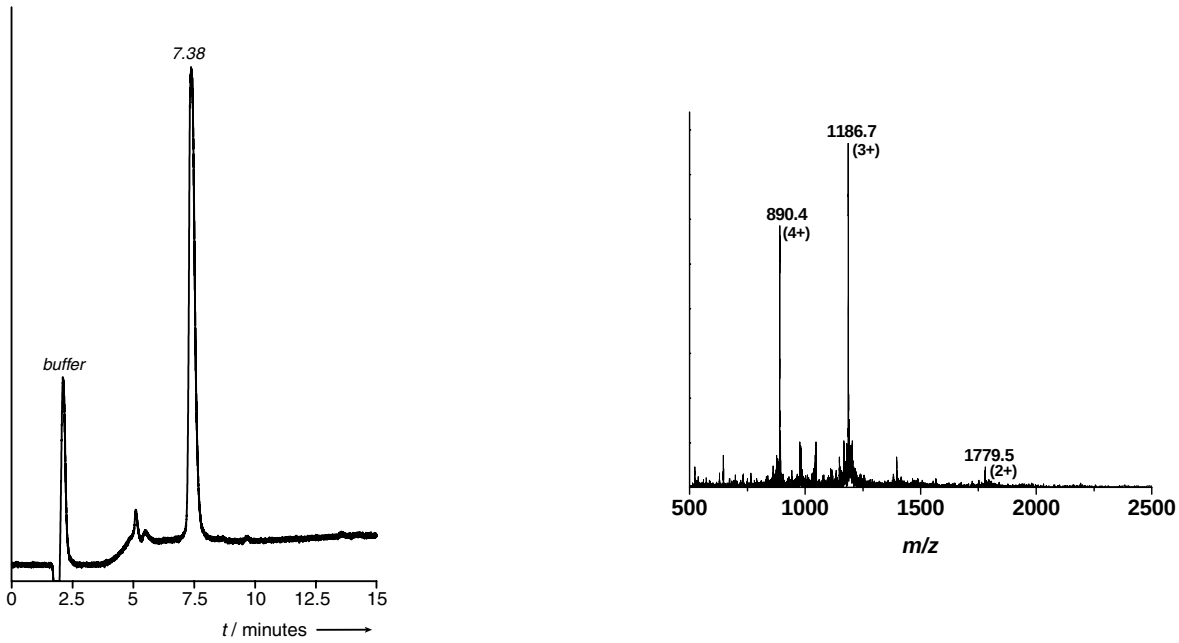


Figure 4. RP HPLC trace of the crude reaction mixture of the conversion of the thioproline residue of **8** to a cysteine by reaction with methoxylamine to give **9** (gradient: 0–67% 9:1 *v/v* MeCN/H_2O in H_2O, 0.1% TFA in 15 minutes) (left) and the ESI-MS spectrum of **9** (right).

Ligation of 8 to 4. For this 2.8 mg (7.8×10^{-4} mmol) of **8** was first converted to **9** as described above. This solution was added to 3.0 mg (7.8×10^{-4} mmol) of **4**. The pH was adjusted to pH ~7 by adding small aliquots of 0.5 M NaOH (aq). The reaction was monitored employing analytical RP HPLC using a C18 column for separation coupled to UV-Vis (λ_{probe} = 214 nm). After 1 hour the ligation product **10** was formed as confirmed by ESI-MS.

Ligation of 8 to 5. For this 1.5 mg (4.2×10^{-4} mmol) of **8** was first converted to **9** as described above. This solution was added to 3.0 mg (4.1×10^{-4} mmol) of **5**. The pH was adjusted to pH ~7 by adding small aliquots of 0.5 M NaOH (aq). The reaction was monitored employing analytical RP HPLC using a C18 column for separation coupled to UV-Vis (λ_{probe} = 214 nm). After 1 hour the ligation product **11** was formed as confirmed by ESI-MS.

[1] P. L. Anelli, F. Fedeli, O. Gazzotti, L. Lattuada, G. Lux, F. Rebasti, *Bioconjugate Chem.*, 1999, **10**, 137-140.

[2] T. Kitagawa, T. Aikawa, *J. Biochem.*, 1976, **79**, 233-236.

[3] M. Schnölzer, P. Alewood, A. Jones, D. Alewood, S. B. H. Kent, *Int. J. Peptide Protein Res.*, 1992, **40**, 180-193.