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A Versatile, Modular Platform for Multivalent Peptide Ligands Based on a Dendritic Wedge

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A general methodology for the synthesis of multifunctional AB_2, AB_3, AB_4, and AB_5 dendritic wedges is described based on an orthogonal protection strategy. Asymmetric polyamide dendrons that possess N-terminal cysteine residues at the periphery were quantitatively functionalized with C-terminal thioester peptides using native chemical ligation. Conjugation of biologically relevant groups at the focal point resulted in a series of related structures with a highly controlled valency (2–5) that can directly be used in a systematic study on the strength of multivalent interactions. Using our modular approach various ligands, functional groups and spacers can readily be combined in order to generate a toolbox for the development of smart biomaterials used in molecular medicine and imaging.

Introduction

Over the past two decades, multiple synthetic strategies have been developed to arrive at functional dendrimers. The ability of this type of macromolecule to present multiple groups at the surface, while maintaining a well-defined structure with low polydispersity makes them ideal vehicles for use in biomedical applications.[1] Inherent to their structure are opportunities to employ multivalent interactions to yield ligands with high affinity and specificity for biological targets. Beyond that, the preparation of well-defined multifunctional dendrimers – for example, by conjugation of therapeutic agents, targeting groups *and* radio- or fluorescent labels onto a single platform – is limited by the fact that most dendritic molecules possess uniformly protected functional groups in a spherical (or cylindrical) topology. This symmetry-related synthesis constraint is inherently contrary to generating molecular complexity within a single, well-defined molecular species.[2] Methodologies for the preparation of dendrimers with asymmetric functionalization have appeared recently: for example, by conjugation of functional units (e.g. biotin or chromophores) at the focal point of dendritic wedges;[3] by merging two separate wedges using click chemistry, native chemical ligation or Diels–Alder reactions;[4] or by a combination of convergent and divergent growth strategies.[5] While these desymmetrized dendrimers possess a highly controlled surface functionalization, they are limited by synthetic procedures based on either $1 \rightarrow 2$ branching or $1 \rightarrow 3$ branching. As a result the number of end groups increases exponentially with each generation.[6] For many biomedical applications, however, an arithmetic branching scheme is more attractive for elucidating and quantifying the fundamental aspects of multivalent ligand interactions for targeting. We present here a new type of bifunctional building block that enables the modular synthesis of multivalent peptide dendritic wedges with arithmetic control over the degree of branching (Figure 1).

The design of our biocompatible platform allows an optimal presentation of multiple functional groups and is inspired by Newkome's polyamide dendrimers.[7] In contrast to previously reported strategies, the number of ligands increases arithmetically, allowing a systematic study on the nature and strength of multivalent interactions. Two, three, four or five ligands can be presented at the periphery of a polyamide dendritic wedge, while another functional group is site-specifically introduced at the focal point. Poly(ethylene glycol) (PEG) units are used to append reactive cysteine residues to the dendritic core. Besides the fact that the length of this spacer can be easily tuned to inter-receptor distances, PEG-dendrimer hybrids are clinically approved and have shown to decrease toxicity and increase circulation times in vivo.[8] N-Terminal cysteine residues at the PEGylated wedge periphery were used for the introduction of multiple peptide-based ligands via their C-terminus using native chemical ligation, a highly efficient and specific coupling reaction.[9] Minor modifications will even allow the use of other ligation reactions such as oxime or "click"

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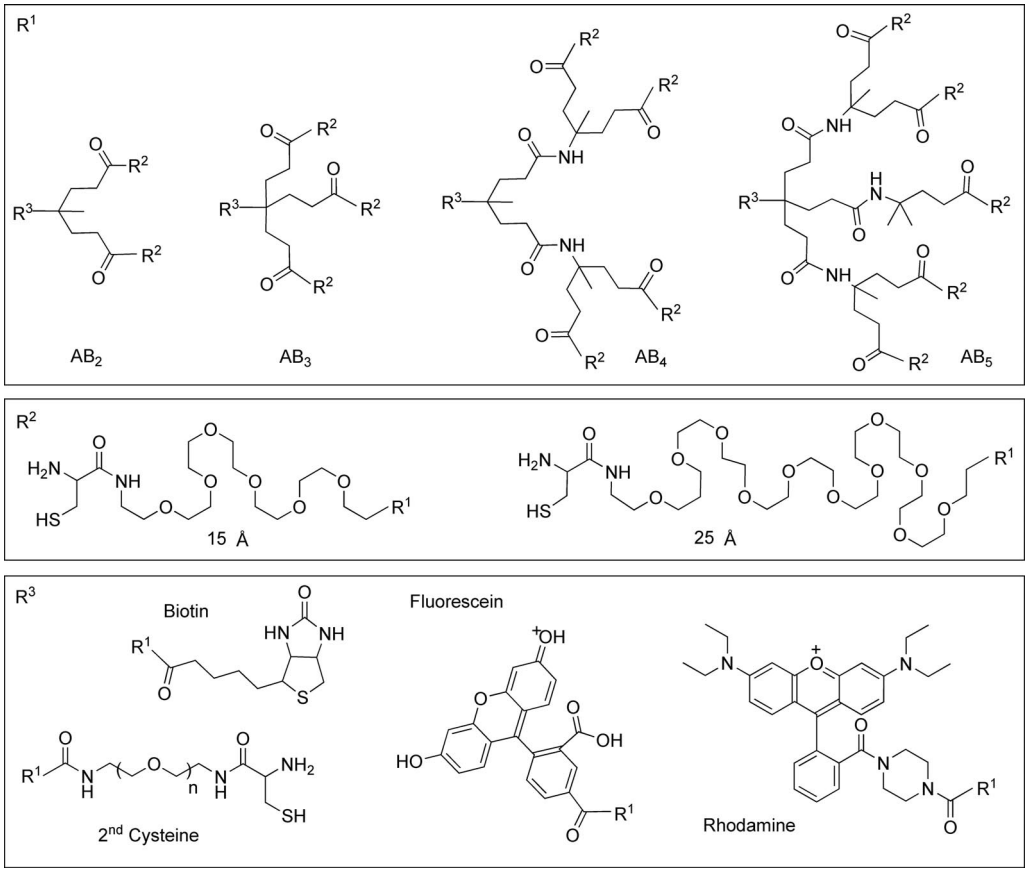


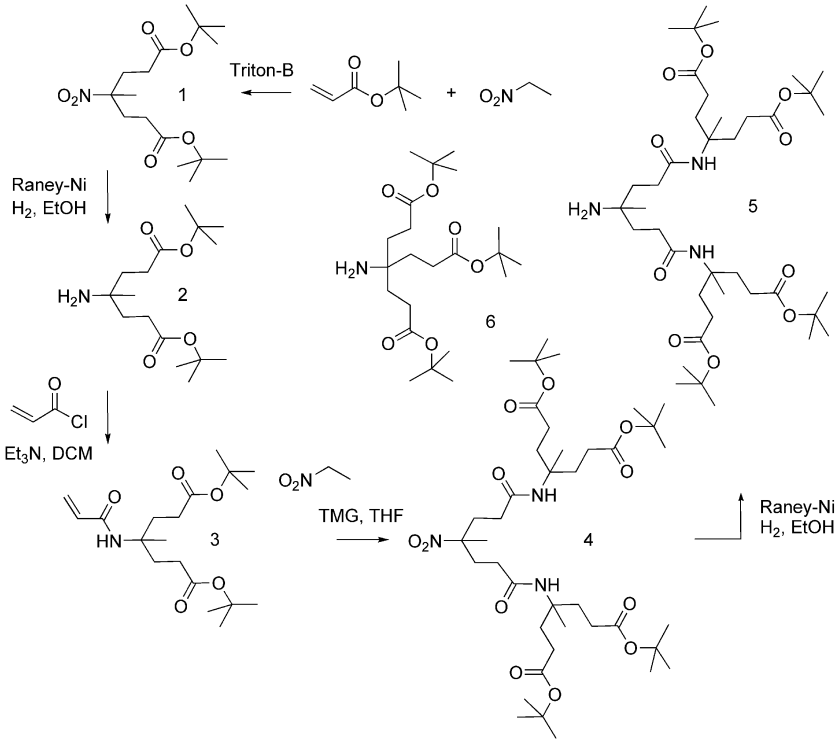
Figure 1. A modular approach towards multifunctional peptide wedges. Besides the valency (R^1) of the ligands conjugated to the periphery, also the linker length (R^2) and the functional group at the focal point (R^3) are easily varied.

chemistry. The related synthesis of the labeled pentameric peptide wedge (AB_5) has been published elsewhere since it uniquely mimics the multivalent architecture of peptides fused to the five pIII domains at the head of M13 bacteriophage. As a result, the affinity of phage-display-derived peptides can be significantly enhanced by restoring the multivalent binding character using the AB_5 dendrimer display.[10] In this work, we elaborate the complete synthetic route to biotin and fluorescein labeled multivalent peptide constructs (AB_2, AB_3, AB_4, and AB_5) for direct use in solid phase binding assays and fluorescence microscopy, respectively. The scope of the approach is demonstrated by presenting examples with a variety of targeting peptides.

Results and Discussion

An important requirement for the realization of our synthetic strategy is the use of robust and distinct orthogonal protecting groups that allow functionalization in the presence of other reactive groups. Poly(ethylene glycol) (PEG) units were used to link acid-labile trityl-protected cysteine residues to the periphery, while an orthogonal base-labile fluorenylmethoxylcarbonyl (Fmoc) group at the focal point allowed us to manipulate the material so as to incorporate a variety of anchoring groups. The branched core of the dimeric structure (AB_2) was built up via a Michael addition of *tert*-butyl acrylate to nitroethane in the presence of Triton-B (Scheme 1). Catalytic reduction of the nitro group using Raney nickel and H_2 in ethanol afforded **2**. This AB_2 synthon is a key building block and can be used as a starting point for the synthesis of AB_4 dendron **5** and the AB_5 dendron.

Therefore **2** was treated with acryloyl chloride followed byanother Michael addition to nitroethane and a catalytic reduction step, producing the aminotetraester **5** in good overall yield. Aminotriester **6** is commercially available and was used directly for further synthesis. With these three basic core structures in hand, the following series of transformations were performed on each molecule to arrive at the labeled multivalent peptide dendrons. Protection of the free amine at the focal point with an Fmoc-group was followed by removal of the *tert*-butyl protecting groups at the periphery using formic acid. The polyacid was activated to tetrafluorophenyl esters, which have a unique ^{1}H NMR chemical shift that confirms a complete reaction. Conjugation of the tetrafluorophenol activated wedge with cysteine-functionalized PEG **12** resulted in an orthogonally protected dendritic structure. After removal of the Fmoc protecting group, various labels can be coupled to the focal point using uronium-based HOAt-mediated condensation. In this manner, both 6-(biotinylamino)caproic acid and 5-carboxyfluorescein were successfully coupled via HATU activation to



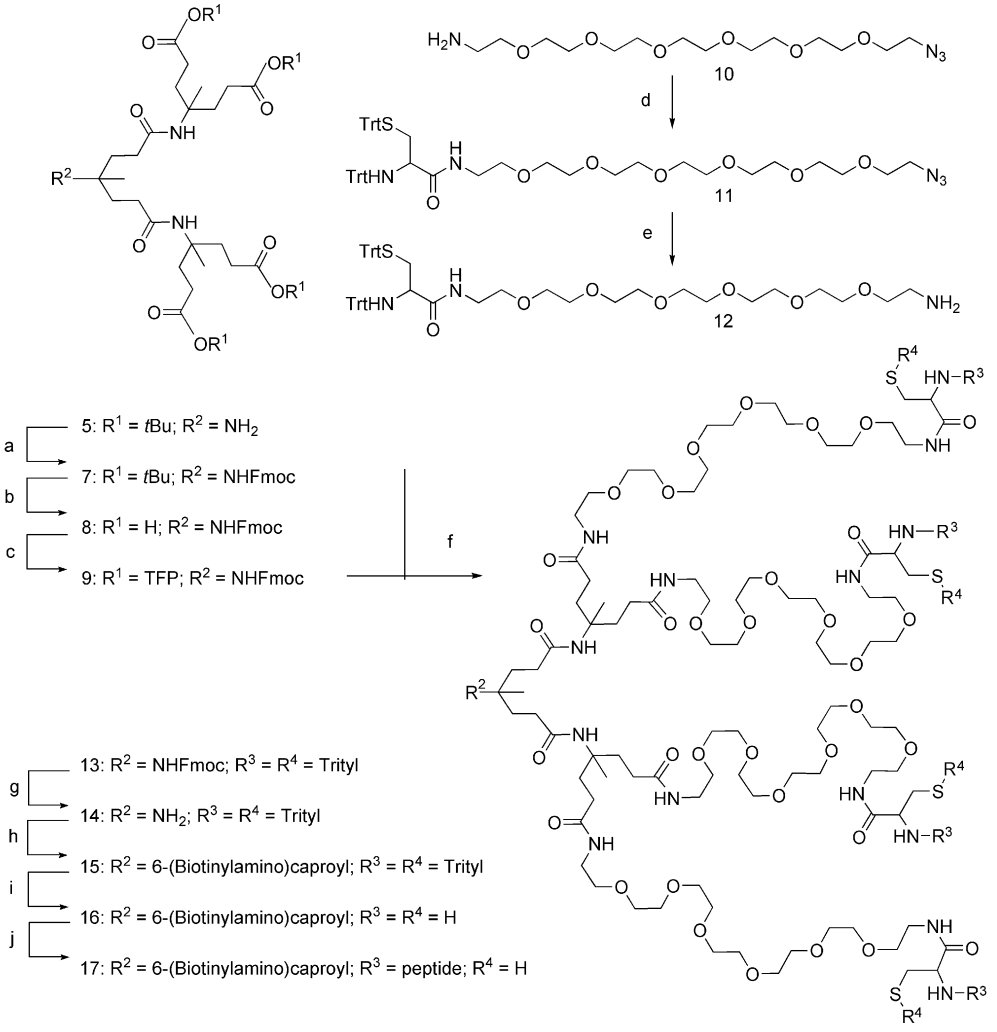
Scheme 1. Synthesis and structure of AB_2 (**2**), AB_3 (**6**) and AB_4 (**5**) polyamide dendritic core molecules.

all wedges. After deprotection of the trityl groups present on the cysteines, reverse phase HPLC purification was used to obtain the desired compounds in high purity. Figures S7–S12 (see Supporting Information) show the LC-MS characterization of Cys_2-Biotin (**16a**), Cys_3-Biotin (**16b**) and Cys_4-Biotin (**16c**) as well as Cys_2-Fluorescein (**16d**), Cys_3-Fluorescein (**16e**) and Cys_4-Fluorescein (**16f**). All compounds show a single sharp peak in the chromatogram indicating >95% purity and their masses correspond to the calculated molecular weights. As an example, the complete synthesis route for the preparation of a biotin-labeled tetrameric wedge functionalized with peptides is outlined in Scheme 2.

To assess the general applicability of our approach, different peptides with various lengths and sequences were coupled to the labeled AB_2, AB_3, AB_4, and AB_5 constructs. Thereto, a variety of biomedically relevant targeting peptides were selected from the literature and prepared with a C-terminal thioester using Fmoc-mediated solid phase peptide synthesis on a safety-catch resin.[11] Peptides containing the amino acid sequence Gly-Arg-Gly-Asp-Ser (GRGDS), which mimics the integrin binding motif of adhesive proteins in the extracellular matrix (e.g. fibronectin) mediate adhesion and spreading of cells.[12] Synthetic multivalent molecules displaying these small peptide fragments are therefore of high interest for tumor targeting, imaging angiogenesis or the development of new biomaterials.[13] Native chemical ligation of an Ac-GRGDS-containing thioester peptide to the biotin-labeled AB_4 dendritic wedge showed full conversion after 16 h. LC-MS analysis was used to characterize the crude ligation mixture (Figure 2, **a**). Cell-penetrating peptides (RQIKIWFQNRRMKWKK) originating from the *Drosophila* transcription factor Antennapedia spontaneously internalize exogeneous macromolecules.[14] Conjugation of three of these 16-amino-acid peptides to the fluorescein-labeled AB_3 wedge makes it a valuable construct for the development of new therapeutic approaches (Figure 2, **b**). A biotin-labeled dimeric wedge functionalized with the type 1 collagen-binding domain of bone sialoprotein (NGVFKYRPRYFLYKHAYFYPPLKRFPVQ)[15] was also prepared and characterized (Figure 2, **c**). This result shows that even these long, 28-amino-acid peptides can be efficiently coupled to these dendritic structures and might be useful for the initiation of bone mineralization after joint injury. Finally, using the methodologies above, we synthesized the AB_5 dendron with collagen type I binding peptides (HVWMQAPGGG) and a fluorescein label at the focal point.[10] The examples illustrate that our approach is indeed quite versatile allowing conjugation of a variety of peptides to a series of AB_x dendritic wedges.

Conclusions

The importance of peptide-mediated targeting and/or trafficking of biomolecules in living systems is becoming increasingly apparent. Mechanistic investigations into these complex interactions in vitro and in vivo have been limited by the lack of suitable multifunctional molecular probes, in particular when these interactions involve multivalent ligands. We have addressed this deficiency through the development of a polyamide dendritic platform for the presenta-



Scheme 2. Chemical synthesis of a biotin-labeled tetrameric dendritic wedge functionalized with peptide ligands to cellular targets. a) 9-Fluorenylmethyl succinimidyl carbonate, DIPEA, DCM. b) HCO_2H. c) TFP, PPTS, DCC, DCM. d) Trt-C(Trt)-OSu, Et_3N, DCM. e) PPh_3,10% H_2O in THF. f) Trt-C(Trt)-PEG-NH_2, DIPEA, DCM. g) 20% Piperidine in DCM. h) 6-(Biotinylamino)caproic acid, HATU, DIPEA, DMF. i) TFA, triisopropylsilane, H_2O. j) Peptide thioester, thiophenol, benzyl mercaptan, 6 M guanidine, 70 mM Tris, pH 8.0.

tion of peptide (or protein) ligands with controlled valency (AB_x: x = 2 to 5), spacer length, primary sequence along with suitable labels for many biological investigations, including binding assays via introduction of biotin moieties or fluorescence microscopy via fluorescein dyes. The synthesis presented here relies on an orthogonal protecting group strategy that was inspired by what is used in routine solid phase synthesis of linear peptides. With it comes access to a toolbox of AB_2, AB_3, AB_4, and AB_5 wedges that enables a systematic study on the role of valency in determining the strength of multivalent interactions in chemical biology. As quantitative functionalization of the periphery with peptides ranging from 5–28 amino acids was readily achieved using native chemical ligation, this approach is also expected to provide access to multivalent protein architectures via ligation of recombinant protein thioesters – although, our approach readily lends itself to other efficient bioorthogonal ligation chemistries. The molecular-level information gleaned by probes is considered a crucial first step for the further development of systems used in peptide-targeted drug delivery, molecular imaging and material science.

Experimental Section

General: Unless otherwise stated, all solvents used were reagent grade and all chemicals were used as supplied. ^1H and ^{13}C NMR spectra were acquired on a Varian 400-MR spectrometer at 400 and 100 MHz, respectively, in deuterated solvents containing 0.5% (v/v) TMS; chemical shifts are listed in ppm relative to TMS as an internal standard. ESI-MS spectra were recorded on an Applied Biosystems Single Quadrupole Electrospray Ionization Mass Spectrometer API-150EX in positive mode. Reversed-phase HPLC was performed on a Shimadzu LC-8A HPLC system by using a VY-DAC protein & peptide C18 column.

Di-*tert*-Butyl 4-Methyl-4-nitroheptanedioate (1): A mixture of nitroethane (1.46 g, 19.4 mmol) and *tert*-butyl acrylate (10.0 g, 78.0 mmol) in EtOH (30 mL) was cooled in an ice bath before the dropwise addition of Triton-B (2 mL). The mixture was allowed to gradually warm to room temp. over 16 h. An additional portion of

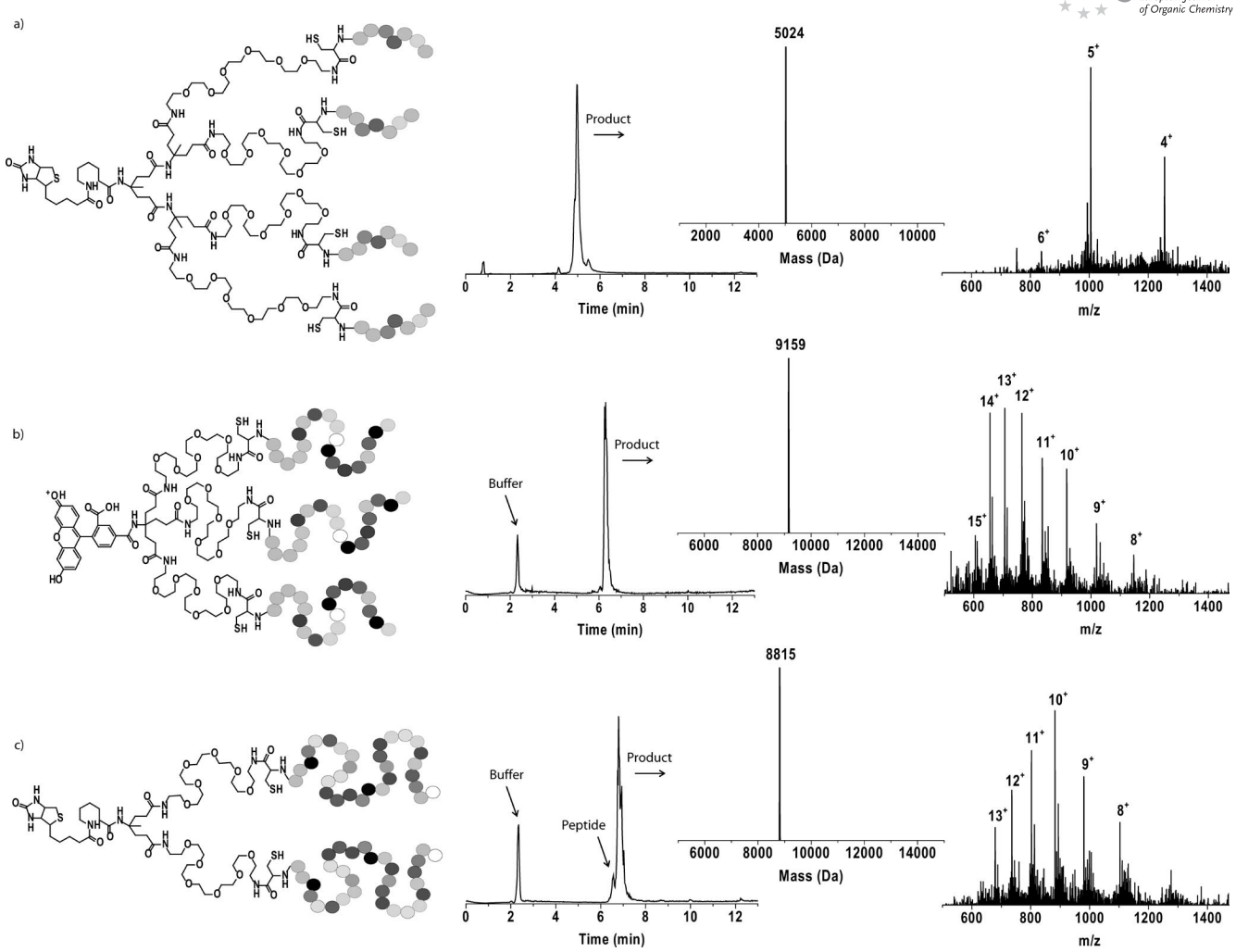


Figure 2. LC-MS characterization of crude labeled multivalent peptide constructs. a) Biotin-labeled tetrameric wedge conjugated with cell adhesion peptides (calcd. mass: 5023 Da). b) Fluorescein-labeled trimeric wedge conjugated with cell-penetrating peptides (calcd. mass: 9158 Da). c) Biotin-labeled dimeric wedge conjugated with peptides corresponding to the type 1 collagen-binding domain of bone silaoprotein (calcd. mass: 8815 Da).

Triton-B (2 mL) was added to the reaction mixture, which was then stirred at room temp. for a total of 48 h. After removing the volatiles in vacuo, the product was recovered after recrystallization from EtOH/H_2O as a colorless solid (5.78 g, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.35–2.07 (m, 8 H, CH_2CH_2), 1.53 (s, 3 H, CH_3), 1.44 [s, 18 H, C(CH_3)$_3$] ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.3, 89.9, 81.0, 34.3, 30.1, 28.0, 21.7 ppm. IR: $\tilde{\nu}$ = 2979, 2934, 1728, 1539, 1454, 1367, 1308, 1152, 947, 847, 759 cm^{-1}. EI-MS: calcd. $C^{16}H_{29}NO_6^+$: *m*/*z* 331.20; found *m*/*z* 258, 220, 202, 173, 155, 127, 109, 57, 41.

Di-*tert*-butyl 4-Amino-4-methylheptanedioate (2): A mixture of **1** (5.50 g, 16.6 mmol) and Raney nickel (2 cm^3) in EtOH (100 mL) was purged with argon for 30 min before hydrogenation in a Parr reactor (p_{H2} = 70 psi). ***Caution:*** *Raney nickel catalyst is pyrophoric!* After 8 h, the reaction mixture was carefully filtered through Celite under an argon atmosphere. After removal of the volatiles in vacuo, the title compound was dried with P_2O_5 yielding a colorless low melting solid (5.00 g, 100%) which was subsequently stored at –20 °C until needed. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.27 (t, *J* = 8.2 Hz, 4 H, CH_2CO), 1.65 (t, *J* = 8.2 Hz, 4 H, NCCH_2), 1.44 [s, 18 H, C(CH_3)$_3$], 1.02 (s, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 173.3, 80.2, 50.7, 37.5, 30.5, 28.0, 27.2 ppm. IR: $\tilde{\nu}$ = 2977, 2932, 1724, 1607, 1456, 1421, 1392, 1366, 1306, 1255, 1147, 952, 848, 757 cm^{-1}. ESI-MS: calcd. $C_{16}H_{32}NO_4^+$: *m*/*z* 302.23; found *m*/*z* 302.18.

Di-*tert*-butyl 4-Acryloylamino-4-methylheptanedioate (3): A solution of **2** (5.00 g, 16.6 mmol) and Et_3N (4.20 g, 41.5 mmol) in DCM (50 mL) was cooled in an ice bath before adding a solution of acryloyl chloride (1.50 g, 16.6 mmol) in DCM (20 mL) dropwise over 30 min via an addition funnel. The reaction mixture was allowed to gradually warm to room temp. overnight. After 16 h, the volatiles were removed in vacuo and the mixture dissolved in EtOAc (100 mL). The organic layer was washed with water (3 × 50 mL), satd. $NaHCO_3$ (3 × 50 mL) and brine (50 mL) before drying over Na_2SO_4. After concentration, the crude mixture was purified by flash chromatography using 2:1 hexane/EtOAc eluent. The product was isolated as a colorless solid (4.43 g, 75%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.20 (d, *J* = 16.8 Hz, 1 H, *H*CC), 6.07 (s, 1 H, N*H*), 6.00 (dd, J_1 = 16.8, J_2 = 9.8 Hz, 1 H, C*H*CO), 5.56 (d, *J* = 9.9 Hz, 1 H, *H*CC), 2.29 (t, *J* = 8.0 Hz, 4 H, CH_2CO), 2.09 (m, 2 H, NCCH_2), 1.94 (m, 2 H, NCCH_2), 1.43 [s, 18 H, C(CH_3)$_3$], 1.35 (s, 3H. CH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 173.3, 164.9, 131.9, 125.7, 80.7, 55.5, 33.5, 30.3, 28.1, 23.6 ppm. IR: $\tilde{\nu}$ = 3311, 3063, 2975, 2936, 1721, 1658, 1620, 1539, 1459, 1367,

1304, 1249, 1149, 1115, 991, 955, 849, 810 cm^{-1}. ESI-MS: calcd. $C_{19}H_{34}NO_5^+$: *m*/*z* 336.24; found *m*/*z* 336.20.

(*t*BuO)$_4$-NO$_2$ (4): A solution of nitroethane (362 mg, 4.82 mmol), acrylamide **3** (3.60 g, 10.13 mmol) and *N*,*N*,*N*′,*N*′-tetramethylguanidine (TMG, 500 μL) in THF (20 mL) was heated at 50 °C for 48 h, during which the reaction progress was monitored by THF-SEC. After removal of the volatiles in vacuo, the residue was dissolved in ethyl acetate (150 mL) and the organic layer washed with water (4 × 50 mL), 1.0 M HCl (4 × 50 mL), satd. $NaHCO_3$ (4 × 50 mL) and brine (50 mL). The organic layer was dried with Na_2SO_4 and passed through a short plug of silica before concentrating. The crude reaction mixture was purified by flash chromatography using a gradient elution of 1:1 to 2:3 hexane/EtOAc. The product was obtained as a colorless solid (3.37 g, 89%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.92 (s, 2 H, N*H*), 2.3–2.0 (m, 20 H, CH_2), 1.88 (m, 4 H, NO_2CCH_2), 1.51 (s, 3 H, NO_2CCH_3), 1.44 [s, 36 H, C(CH_3)$_3$], 1.28 (s, 6 H, NHCCH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 173.2, 170.3, 90.4, 80.7, 55.5, 34.7, 33.3, 31.6, 30.3, 28.1, 23.6, 21.8 ppm. IR: $\tilde{\nu}$ = 3332, 2979, 2937, 1729, 1655, 1539, 1453, 1392, 1368, 1305, 1256, 1153, 953, 847, 758 cm^{-1}. ESI-MS: calcd. $C_{40}H_{72}N_3O_{12}^+$: *m*/*z* 786.50; found *m*/*z* 786.33.

(tBuO)$_4$-NH$_2$ (5): A mixture of (*t*BuO)$_4$-NO$_2$ **4** (1 mmol) and Raney nickel (0.25 cm^3) in EtOH (50 mL) was purged with argon for 30 min before hydrogenation in a Parr reactor (p_{H2} 70 psi). After 8–16 h, the reaction mixture was carefully filtered through Celite under an argon atmosphere. After removal of the volatiles in vacuo, the title compounds were dried with P_2O_5 yielding a colorless oil (0.74 g, 98%), which were subsequently stored –20 °C until needed. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.61 (s, 2 H, NH_2), 2.33 (t, *J* = 7.7 Hz, 4 H, CH_2CON), 2.22 (t, *J* = 7.9 Hz, 8 H, CH_2COO), 2.08 (m, 4 H, NH_2CCH_2), 1.85 (m, 8 H, NHCCH_2), 1.42 [s, 36 H, C(CH_3)$_3$], 1.24 (s, 6 H, NHCCH_3), 1.23 (s, 3 H, NH_2CCH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 173.4, 172.6, 80.6, 55.4, 36.1, 33.3, 33.2, 31.7, 30.4, 28.1, 25.3, 23.6 ppm. IR: $\tilde{\nu}$ = 3285, 2977, 2933, 1726, 1648, 1541, 1453, 1391, 1366, 1304, 1254, 1146, 1113, 1037, 951, 921, 847, 758 cm^{-1}. MALDI-TOF-MS: calcd. $C_{40}H_{74}N_3O_{10}^+$: *m*/*z* 756.53; found *m*/*z* 756.68.

(tBuO)$_3$-NH$_2$ (6): Commercially available.

General Procedure for the Preparation of (*t*BuO)$_n$-NHFmoc Wedges: A mixture of (*t*BuO)$_n$-NH$_2$ (1 mmol) and DIPEA (3.3 mmol) in DCM (2 mL) was cooled in an ice-bath before the addition of 9-fluorenylmethyl succinimidyl carbonate (1.1 mmol). After 30 min, the reaction was removed from the ice bath and the reaction warmed to room temp. After 4 h, the crude reaction mixture was concentrated, redissolved in EtOAc (100 mL) and washed with satd. $NaHCO_3$ (3 × 100 mL). After drying over $MgSO_4$, filtration and concentration, the crude product was purified by flash chromatography using a gradient elution of chloroform to chloroform containing 2–5% MeOH. The title compounds were obtained as colorless solids; yields 80–100%.

(*t*BuO)$_2$-NHFmoc (7a): Yield of 80% from **2** (2.00 g, 6.63 mmol). This material could also be recrystallized from pentane/Et_2O. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.76 (d, *J* = 7.3 Hz, 2 H, CH_{ar}), 7.59 (d, *J* = 6.6 Hz, 2 H, CH_{ar}), 7.39 (t, *J* = 7.0 Hz, 2 H, CH_{ar}), 7.31 (t, *J* = 7.5 Hz, 2 H, CH_{ar}), 4.96 (s, 1 H, N*H*), 4.37 (br., 2 H, CH_2O), 4.19 (t, *J* = 6.6 Hz, 1 H, C*H*CH$_2$O), 2.23 (br., 4 H, CH_2CO), 2.02 (br., 2 H, NCCH_2), 1.87 (br., 2 H, NCCH_2), 1.44 [s, 18 H, C(CH_3)$_3$], 1.24 (s, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 172.9, 154.5, 144.0, 141.3, 127.6, 127.0, 125.0, 119.9, 80.5, 65.9, 54.4, 47.4, 33.6, 30.2, 28.1, 23.8 ppm. IR: $\tilde{\nu}$ = 3330, 3049, 2977, 2940, 1725, 1696, 1531, 1452, 1421, 1366, 1308, 1259, 1229, 1210, 1144, 1092, 1060, 934, 922, 848, 760, 740 cm^{-1}. MALDI-TOF-MS: calcd. $C_{31}H_{41}NO_6Na^+$: *m*/*z* 546.29; found *m*/*z* 546.45.

(*t*BuO)$_3$-NHFmoc (7b): Yield of 89% from **6** (2.00 g, 4.81 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.76 (d, *J* = 7.6 Hz, 2 H, CH_{ar}), 7.59 (d, *J* = 7.2 Hz, 2 H, CH_{ar}), 7.39 (t, *J* = 7.3 Hz, 2 H, CH_{ar}), 7.32 (t, *J* = 7.2 Hz, 2 H, CH_{ar}), 5.02 (s, 1 H, N*H*), 4.38 (d, *J* = 7.1 Hz, 2 H, CH_2O), 4.18 (t, *J* = 7.2 Hz, 1 H, C*H*CH$_2$O), 2.31–1.18 (m, 6 H, CH_2CO), 2.00–1.85 (m, 6 H, NCCH_2), 1.43 [s, 27 H, C(CH_3)$_3$] ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 172.7, 154.2, 144.0, 141.3, 127.7, 127.1, 125.0, 120.0, 80.7, 65.9, 56.5, 47.3, 30.1, 29.7, 28.1 ppm. IR: $\tilde{\nu}$ = 3356, 2977, 2935, 1724, 1524, 1451, 1392, 1367, 1314, 1244, 1148, 1082, 948, 847, 758, 739 cm^{-1}. MALDI-TOF-MS: calcd. $C_{37}H_{51}NO_8Na^+$: *m*/*z* 660.35; found *m*/*z* 660.23.

(*t*BuO)$_4$-NHFmoc (7c): Yield of 100% from **5** (920 mg, 1.21 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.76 (d, *J* = 7.7 Hz, 2 H, CH_{ar}), 7.62 (d, *J* = 7.7 Hz, 2 H, CH_{ar}), 7.39 (t, *J* = 7.1 Hz, 2 H, CH_{ar}), 7.31 (t, *J* = 7.6 Hz, 2 H, CH_{ar}), 5.91 (s, 2 H, N*H*), 5.75 (s, 1 H, N*H*), 4.37 (d, *J* = 6.6 Hz, 2 H. CH_2O), 4.19 (t, *J* = 6.6 Hz, 1 H, C*H*CH$_2$O), 2.3–1.8 (m, 24 H, CH_2), 1.42 [s, 36 H, C(CH_3)$_3$], 1.26 (overlapping s, 6H + 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 173.2, 172.6, 155.0, 144.1, 141.3, 127.6, 127.0, 125.2, 119.9, 80.6, 80.5, 65.9, 55.3, 54.6, 47.3, 34.6, 33.3, 32.0, 30.3, 28.0, 27.8, 24.2, 23.7 ppm. IR: $\tilde{\nu}$ = 3330, 3065, 2977, 2935, 1724, 1652, 1531, 1451, 1423, 1392, 1367, 1304, 1249, 1147, 1113, 1085, 947, 846, 757, 740 cm^{-1}. MALDI-TOF-MS: calcd. $C_{55}H_{83}N_3O_{12}Na^+$: *m*/*z* 999.60; found *m*/*z* 1000.45.

General Procedure for the Synthesis of (HO)$_n$-NHFmoc Wedges: The title compounds were prepared from (*t*BuO)$_n$-NHFmoc (1 mmol) after 8–16 h stirring at room temp. in formic acid (10 mL). After removal of formic acid in vacuo, colorless solids were obtained; yields 95–100%.

(HO)$_2$-NHFmoc (8a): Yield of 100% from **7a** (2.52 g, 4.81 mmol). ^{1}H NMR [D$_6$]DMSO, 400 MHz): δ = 7.86 (d, *J* = 7.7 Hz, 2 H, CH_{ar}), 7.69 (d, *J* = 7.7 Hz, 2 H, CH_{ar}), 7.39 (t, *J* = 7.3 Hz, 2 H, CH_{ar}), 7.30 (t, *J* = 7.0 Hz, 2 H, CH_{ar}), 7.00 (s, 1 H, N*H*), 4.20 (br., 2 H, CH_2O), 4.18 (overlapping t, 1 H, C*H*CH$_2$O), 2.13 (br., 4 H, CH_2CO), 1.95 (br., 2 H, NCCH_2), 1.68 (br., 2 H, NCCH_2), 1.08 (s, 3 H, CH_3) ppm. ^{13}C NMR [D$_6$]DMSO, 100 MHz): δ = 175.1, 154.8, 144.4, 141.2, 128.1, 127.5, 125.7, 120.5, 65.2, 53.9, 47.2, 33.5, 29.1, 23.7 ppm. IR: $\tilde{\nu}$ = 3356, 3200, 3063–2900, 1719, 1693, 1536, 1450, 1413, 1343, 1292, 1264, 1233, 1212, 1183, 1171, 1129, 1093, 960, 947, 817, 756, 737 cm^{-1}. MALDI-TOF-MS: calcd. $C_{23}H_{25}NO_6Na^+$: *m*/*z* 434.17; found *m*/*z* 434.31.

(HO)$_3$-NHFmoc (8b): Yield of 95% from **7b** (2.34 g, 3.67 mmol). ^{1}H NMR [D$_6$]DMSO, 400 MHz): δ = 7.90 (d, *J* = 7.3 Hz, 2 H, CH_{ar}), 7.72 (d, *J* = 7.7 Hz, 2 H, CH_{ar}), 7.41 (t, *J* = 7.3 Hz, 2 H, CH_{ar}), 7.32 (t, *J* = 7.3 Hz, 2 H, CH_{ar}), 7.02 (s, 1 H, N*H*), 4.26–4.16 (overlapping signals, 2 H, 1 H, C*H*CH_2O), 2.18–2.08 (m, 6 H, CH_2CO), 1.85–1.76 (m, 6 H, NCCH_2) ppm. ^{13}C NMR [D$_6$]DMSO, 100 MHz): δ = 174.5, 154.2, 144.0, 140.8, 127.7, 127.1, 125.4, 120.1, 64.9, 55.8, 46.829.2, 28.1 ppm. IR: $\tilde{\nu}$ = 3348, 2962, 1742, 1701, 1542, 1451, 1406, 1292, 1246, 1178, 1092, 954, 826, 741 cm^{-1}. MALDI-TOF-MS: calcd. $C_{25}H_{27}NO_8Na^+$: *m*/*z* 492.16; found *m*/*z* 492.09.

(HO)$_4$-NHFmoc (8c): Yield of 100% from **7c** (1.18 g, 1.21 mmol). ^{1}H NMR [D$_6$]DMSO, 400 MHz): δ = 12.00 (s, 4 H, O*H*), 7.86 (d, *J* = 7.3 Hz, 2 H, CH_{ar}), 7.70 (d, *J* = 7.7 Hz, 2 H, CH_{ar}), 7.39 (t, *J* = 7.3 Hz, 2 H, CH_{ar}), 7.31 (t, *J* = 7.3 Hz, 2 H, CH_{ar}), 7.23 (s, 2 H, N*H*), 6.99 (s, 1 H, N*H*), 4.17 (br., 2 H, 1 H, C*H*CH_2O), 2.11 (m, 8 H, CH_2CO), 2.01 (m, 8 H, NCCH_2), 1.82 (m, 2 H, CH_2), 1.69

(m, 4 H, 2 H, CH_2), 1.24 (s, 3 H, CH_3), 1.08 (s, 6 H, CH_3) ppm. ^{13}C NMR [D$_6$]DMSO, 100 MHz): δ = 175.1, 172.6, 154.9, 144.4, 141.1, 128.0, 127.5, 125.8, 120.5, 79.6, 54.4, 47.2, 34.6, 33.3, 31.2, 29.1, 23.9, 23.8 ppm. IR: $\tilde{\nu}$ = 3331, 2964, 1703, 1633, 1535, 1450, 1414, 1384, 1258, 1216, 1185, 796, 756, 740 cm^{-1}. MALDI-TOF-MS: calcd. $C_{39}H_{51}N_3O_{12}Na^+$: *m*/*z* 776.35; found *m*/*z* 776.25.

General Procedure for the Synthesis of (TFP)$_n$-NHFmoc Wedges: To a mixture of (HO)$_n$-NHFmoc (1 mmol), 2,3,5,6-tetrafluorophenol (TFP, $n \times 1.25$ mmol) and PPTS ($n \times 0.1$ mmol) in DCM ([R-CO$_2$H] = 0.15–0.25 M) was added DCC ($n \times 1.25$ mmol) in small portions over 30 min. After 16 h, the reaction mixture was filtered through a short plug of Celite to remove the urea byproduct. The crude reaction mixture was then purified by flash chromatography using a gradient elution of chloroform with up to 15% Et$_2$O. The title compounds were obtained as colorless solids; yields 76–94%.

(TFP)$_2$-NHFmoc (9a): Yield of 94% from **8a** (412 mg, 1 mmol). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 7.77 (d, *J* = 7.3 Hz, 2 H, CH_{ar}), 7.61 (d, *J* = 7.7 Hz, 2 H, CH_{ar}), 7.40 (t, *J* = 7.5 Hz, 2 H, CH_{ar}), 7.33 (t, *J* = 7.5 Hz, 2 H, CH_{ar}), 7.00 (m, 2 H, *H*CCF), 4.65 (s, 1 H, N*H*), 4.54 (br., 2 H, CH_2O), 4.20 (t, *J* = 5.5 Hz, 1 H, C*H*CH$_2$O), 2.62 (br., 4 H, CH_2CO), 2.14 (br., 2 H, NCCH_2), 2.02 (br., 2 H, NCCH_2), 1.24 (s, 3 H, CH_3) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz): δ = 169.2, 154.4, 146.0 [ddt, $J_{F,C}$ = 249 Hz], 143.8, 141.4, 140.6 (ddd, $J_{F,C}$ = 248 Hz), 129.6 (dt), 127.7, 127.1, 124.8, 120.0, 103.3 (t, $J_{F,C}$ = 46 Hz), 65.6, 54.2, 47.4, 33.2, 28.2, 24.0 ppm. ^{19}F NMR (CDCl$_3$, 376 MHz): δ_F = –142.0, –156.1 ppm. IR: $\tilde{\nu}$ = 3405, 3074, 2936, 1785, 1709, 1645, 1522, 1487, 1451, 1416, 1379, 1301, 1271, 1230, 1178, 1086, 1065, 953, 839, 759, 740, 716 cm^{-1}. MALDI-TOF-MS: calcd. $C_{35}H_{25}F_8NO_6Na^+$: *m*/*z* 730.15; found *m*/*z* 730.38.

(TFP)$_3$-NHFmoc (9b): Yield of 80% from **8b** (520 mg, 1.11 mmol). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 7.79 (d, *J* = 7.3 Hz, 2 H, CH_{ar}), 7.59 (d, *J* = 6.6 Hz, 2 H, CH_{ar}), 7.41 (t, *J* = 7.2 Hz, 2 H, CH_{ar}), 7.33 (t, *J* = 7.3 Hz, 2 H, CH_{ar}), 7.01 (m, 3 H, *H*CCF), 4.65–4.53 (m, 3 H, N*H*, CH_2O), 4.20 (t, *J* = 6.6 Hz, 1 H, C*H*CH$_2$O), 2.70–2.55 (m, 6 H, CH_2CO), 2.32–2.20 (m, 6 H, NCCH_2) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz): δ = 169.1, 154.2, 146.1 (ddt, $J_{F,C}$ = 256 Hz), 143.8, 141.7, 140.7 (ddd, $J_{F,C}$ = 43 Hz), 129.6 (m), 127.9, 127.2, 124.7, 120.1, 103.8 (t, $J_{F,C}$ = 248 Hz), 65.9, 56.7, 54.9, 53.0, 47.5, 29.9, 27.5 ppm. ^{19}F NMR (CDCl$_3$, 376 MHz): δ = –142.0, –156.1 ppm. IR: $\tilde{\nu}$ = 3395, 3081, 2949, 1783, 1708, 1645, 1521, 1487, 1451, 1271, 1178, 1094, 1065, 954, 840, 760, 741, 716 cm^{-1}. MALDI-TOF-MS: calcd. $C_{43}H_{27}F_{12}NO_8Na^+$: *m*/*z* 936.14; found *m*/*z* 936.11.

(TFP)$_4$-NHFmoc (9c): Yield of 76% from **8c** (400 mg, 0.66 mmol). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 7.73 (d, *J* = 7.2 Hz, 2 H, CH_{ar}), 7.58 (d, *J* = 7.2 Hz, 2 H, CH_{ar}), 7.37 (t, *J* = 7.4 Hz, 2 H, CH_{ar}), 7.28 (t, *J* = 7.4 Hz, 2 H, CH_{ar}), 6.98 (m, 4 H, *H*CCF), 5.59 (s, 2 H, N*H*), 5.44 (s, 1 H, N*H*), 4.36 (br., 2 H, CH_2O), 4.18 (t, *J* = 6.8 Hz, 1 H, C*H*CH$_2$O), 2.68 (t, *J* = 7.6 Hz, 8 H, CH_2CO), 2.48 (m, 4 H, CH_2), 2.18 (br., 4 H, CH_2), 2.08 (m, 4 H, CH_2), 1.96 (br., 4 H, CH_2), 1.30 (s, 6 H, CH_3), 1.25 (s, 3 H, CH_3) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz): δ = 172.9, 169.4, 155.1, 146.0 (ddt, $J_{F,C}$ = 249 Hz), 143.8, 141.3, 140.4 (ddd, $J_{F,C}$ = 248 Hz), 129.5 (dt), 127.7, 127.0, 124.9, 120.0, 103.2 (t, $J_{F,C}$ = 46 Hz), 66.0, 55.2, 54.8, 47.3, 34.7, 33.0, 32.0, 28.3, 24.5, 23.8 ppm. ^{19}F NMR (CDCl$_3$, 376 MHz): δ = –142.0, –156.1 ppm. IR: $\tilde{\nu}$ = 3399, 3282, 3070, 2935, 1786, 1703, 1660, 1521, 1487, 1452, 1386, 1305, 1267, 1178, 1092, 1064, 954, 840, 760, 739, 716 cm^{-1}. MALDI-TOF-MS: calcd. $C_{63}H_{51}F_{16}N_3O_{12}Na^+$: *m*/*z* 1368.32; found *m*/*z* 1368.22.

Trt-C(Trt)-PEG-N$_3$ (11): A mixture of azido-PEG-amine **10** (Polypure, Norway, 446 mg, 1.27 mmol), Trt-C(Trt)-OSu (938 mg, 1.33 mmol) and Et$_3$N (386 mg, 3.81 mmol) in DCM (5 mL) was stirred at room temp. for 16 h and then loaded onto a column for purification via flash chromatography using an eluent of 2% MeOH in DCM. The product was recovered as a colorless glass (1.10 g, 92%). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 7.37–7.34 (m, 6 H, CH_{ar}), 7.20–7.12 (m, 24 H, CH_{ar}), 6.40 (t, *J* = 3.7 Hz, 1 H, N*H*), 3.71–2.90 (m, 29 H, CH_2, C*H*), 2.79 (d, *J* = 5.5 Hz, 1 H, N*H*Trt), 2.22 (m, 2 H, CH_2S) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz): δ = 172.7, 145.5, 144.6, 129.7, 128.9, 127.9, 127.8, 126.7, 126.6, 71.5, 70.70, 70.67, 70.63, 70.55, 70.53, 70.46, 70.19, 70.02, 69.7, 66.7, 56.5, 38.8, 36.7 ppm. IR: $\tilde{\nu}$ = 3312, 3084, 3056, 3031, 2867, 2100, 1669, 1594, 1520, 1489, 1445, 1347, 1284, 1246, 1103, 1032, 884, 851, 744, 701 cm^{-1}. MALDI-TOF-MS: $C_{55}H_{64}N_5O_7S^+$: *m*/*z* 938.44; found *m*/*z* 938.48.

Trt-C(Trt)-PEG-NH$_2$ (12): Trt-C(Trt)-PEG-N$_3$ **11** (940 mg, 1.00 mmol) was dissolved in THF (18 mL) before the addition of H$_2$O (2 mL), followed by PPh$_3$ (328 mg, 1.25 mmol) in small portions over 30 min. The reaction mixture was stirred at room temp. for 6 h, after which the IR band at 2100 cm^{-1} for the azide had completely disappeared. The reaction mixture was concentrated in vacuo before purification by flash chromatography using a multistage eluent of 2% MeOH in DCM to 10% MeOH in DCM to 10% MeOH in DCM containing 5% isopropylamine. The desired compound was obtained as a colorless glass (900 mg, 99%). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 7.37–7.34 (m, 6 H, CH_{ar}), 7.20–7.12 (m, 24 H, CH_{ar}), 6.43 (t, *J* = 3.7 Hz, 1 H, N*H*), 3.71–2.80 (m, 29 H, CH_2, C*H*), 2.78 (d, *J* = 5.5 Hz, 1 H, N*H*Trt), 2.25 (m, 2 H, CH_2S), 1.8 (br. s, 2 H, NH_2) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz): δ = 172.7, 145.5, 144.6, 129.7, 128.9, 127.9, 127.8, 126.7, 126.6, 73.2, 71.5, 70.56, 70.53, 70.50, 70.46, 70.28, 70.18, 69.68, 66.7, 56.5, 41.8, 38.8, 36.7 ppm. IR: $\tilde{\nu}$ = 3306, 3084, 3056, 3030, 2867, 1667, 1595, 1520, 1489, 1445, 1347, 1291, 1245, 1100, 1033, 940, 902, 848, 744, 701 cm^{-1}. MALDI-TOF-MS: calcd. $C_{55}H_{66}N_3O_7S^+$: *m*/*z* 912.45; found *m*/*z* 912.46.

General Procedure for the Synthesis of [Trt-C(Trt)-PEG-NH]$_n$-NHFmoc Wedges: A solution of (TFP)$_n$-NHFmoc (1 mmol), Trt-C(Trt)-PEG-NH$_2$ ($n \times 1.05$ mmol) and DIPEA ($n \times 3.15$ mmol) in DCM ([TFP-activated ester] = 0.25 M) was stirred at room temp. for up to 4 h and subsequently purified by flash chromatography using a gradient elution of 5% to 10% MeOH in DCM. The products were obtained as a colorless glass; yields 73–98%.

[Trt-C(Trt)-PEG-NH]$_2$-NHFmoc (13a): Yield of 98% from **9a** (150 mg, 214 μmol). ^{1}H NMR (CDCl$_3$, 400 MHz): see Figure S1 ppm. ^{13}C NMR (CDCl$_3$, 100 MHz): δ = 173.1, 172.8, 154.5, 145.5, 144.6, 144.1, 141.3, 129.7, 128.9, 127.9, 127.8, 127.6, 127.0, 126.7, 126.6, 125.1, 119.9, 71.5, 70.5, 70.4, 70.2, 70.1, 69.8, 69.7, 66.7, 65.7, 56.5, 54.5, 47.4, 39.3, 38.8, 36.7, 34.4, 31.4 ppm. IR: $\tilde{\nu}$ = 3315, 3056, 2869, 1722, 1652, 1595, 1526, 1489, 1446, 1348, 1246, 1085, 1033, 940, 903, 849, 742, 699 cm^{-1}. MALDI-TOF-MS: calcd. $C_{133}H_{151}N_7O_{18}S_2Na^+$: *m*/*z* 2222.06; found *m*/*z* 2222.15.

[Trt-C(Trt)-PEG-NH]$_3$-NHFmoc (13b): Yield of 73% from **9b** (200 mg, 220 μmol). ^{1}H NMR (CDCl$_3$, 400 MHz): see Figure S2. ^{13}C NMR (CDCl$_3$, 100 MHz): δ = 173.0, 172.8, 145.8, 144.9, 144.2, 141.5, 129.8, 129.1, 128.0, 127.8, 127.7, 127.1, 126.9, 126.8, 125.4, 120.1, 71.6, 70.7, 70.3, 70.2, 69.8, 69.7, 66.8, 57.0, 56.5, 47.4, 39.4, 38.9, 36.9, 31.2, 30.7 ppm. IR: $\tilde{\nu}$ = 3309, 3054, 2869, 1721, 1652, 1523, 1489, 1446, 1348, 1244, 1097, 1033, 941, 902, 851, 743, 702 cm^{-1}. MALDI-TOF-MS: calcd. $C_{190}H_{216}N_{10}O_{26}S_3Na^+$: *m*/*z* 3175.05; found *m*/*z* 3173.83.

[Trt-C(Trt)-PEG-NH]$_4$-NHFmoc (13c): Yield of 75% from **9c** (150 mg, 110 μmol). ^{1}H NMR (CDCl$_3$, 400 MHz): see Figure S3. ^{13}C NMR (CDCl$_3$, 100 MHz): δ = 173.4, 172.8, 145.5, 144.6, 144.1, 140.1, 129.6, 128.9, 127.9, 127.8, 127.1, 126.7, 126.6, 119.9, 71.5,

70.5, 70.1, 69.7, 66.7, 56.5, 55.3, 50.4, 39.3, 38.8, 37.6, 36.7, 34.3, 31.1, 23.7 ppm. IR: $\tilde{\nu}$ = 3311, 3058, 2873, 1656, 1595, 1541, 1490, 1447, 1348, 1249, 1220, 1106, 1033, 905, 772, 744, 698 cm^{-1}. MALDI-TOF-MS: calcd. $C_{259}H_{303}N_{15}O_{36}S_4Na^+$: *m/z* 4353.60; found *m/z* 4352.45.

General Procedure for the Synthesis of [Trt-C(Trt)-PEG-NH]$_n$-NH$_2$ Wedges: A solution of [Trt-C(Trt)-PEG-NH]$_n$-NHFmoc (1 mmol) in 20% (v/v) piperidine/DCM (10 mL) was stirred at room temp. for 1 h. The reaction mixture was diluted with DCM (50 mL) before washing with a 1.0 M phosphate buffer containing 1.0 M NaCl at pH 5.5 (5 × 50 mL). After drying and concentration, the crude product mixture was purified by flash chromatography using 3% MeOH in DCM, followed by 10% MeOH in DCM containing up to 5% isopropylamine to elute the title compounds; yields 95–100%.

[Trt-C(Trt)-PEG-NH]$_2$-NH$_2$ (14a): Yield of 95% from **13a** (440 mg, 200 μmol). ^{1}H NMR ($CDCl_3$, 400 MHz): see Figure S4. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 173.2, 172.7, 145.5, 144.6, 129.7, 128.9, 127.9, 127.8, 126.7, 126.6, 71.5, 70.5, 70.4, 70.2, 70.1, 69.8, 69.7, 66.7, 56.5, 53.4, 51.5, 39.3, 38.8, 37.5, 36.7, 31.3, 27.2 ppm. IR: $\tilde{\nu}$ = 3312, 3057, 3031, 2869, 1652, 1595, 1532, 1489, 1446, 1348, 1287, 1246, 1184, 1098, 1032, 922, 904, 848, 743, 699 cm^{-1}. MALDI-TOF-MS: calcd. $C_{118}H_{141}N_7O_{16}S_2Na^+$: *m/z* 1999.99; found *m/z* 2000.03.

[Trt-C(Trt)-PEG-NH]$_3$-NH$_2$ (14b): Yield of 100% from **13b** (150 mg, 48 μmol). ^{1}H NMR ($CDCl_3$, 400 MHz): see Figure S5. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 173.3, 172.8, 145.5, 144.6, 129.6, 129.0, 128.9, 128.2, 127.9, 127.8, 126.7, 126.6, 125.3, 71.5, 70.5, 70.4, 70.3, 70.1, 69.9, 69.6, 69.4, 66.7, 58.3, 56.5, 39.3, 38.8, 36.7, 32.0, 30.3, 29.8 ppm. IR: $\tilde{\nu}$ = 3412, 3055, 3021, 2926, 2052, 1650, 1525, 1490, 1447, 1397, 1349, 1247, 1222, 1102, 1033, 940, 745, 703 cm^{-1}. MALDI-TOF-MS: calcd. $C_{175}H_{207}N_{10}O_{24}S_3^+$: *m/z* 2930.75; found *m/z* 2929.99.

[Trt-C(Trt)-PEG-NH]$_4$-NH$_2$ (14c): Yield of 100% from **13c** (200 mg, 46 μmol). ^{1}H NMR ($CDCl_3$, 400 MHz): see Figure S6. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 173.3, 172.7, 145.5, 144.6, 129.7, 128.8, 127.9, 127.8, 126.7, 126.6, 71.5, 70.5, 70.4, 70.3, 70.1, 69.8, 69.7, 69.5, 66.7, 56.5, 53.4, 39.3, 38.8, 36.7, 30.5, 27.9 ppm. IR: $\tilde{\nu}$ = 3306, 3055, 3025, 2887, 1651, 1530, 1489, 1446, 1363, 1303, 1246, 1225, 1099, 1032, 938, 743, 698 cm^{-1}. MALDI-TOF-MS: calcd. $C_{224}H_{293}N_{15}O_{34}S_4Na^+$: *m/z* 4108.33; found *m/z* 4107.85.

General Procedure for the Synthesis of Biotin- and Fluorescein-Labeled [Cys-PEG-NH]$_n$-NHR Wedges: A solution of [Trt-C(Trt)-PEG-NH]$_n$-NH$_2$ (10 μmol) in DMF (500 μL) was added to a solution of 6-(biotinylamino)caproic acid (30 μmol) or alternatively 5-carboxyfluorescein (Novabiochem, 30 μmol) and DIPEA (90 μmol) in DMF (500 μL). This mixture was cooled in an ice bath before the addition of HATU (30 μmol) as a solid. The reaction mixture warmed to room temp. gradually. After 12 h, the reaction mixture was diluted with DCM (50 mL) and the organic layer washed with a 1:4 dilution of satd. $NaHCO_3$ (4 × 50 mL) and then H_2O (50 mL). At this stage, MALDI-TOF MS was used to determine extent of reaction. In all cases, greater than 75% conversion was observed. After drying and concentration, the reaction mixture was immediately subjected to trityl deprotection as follows. An ice bath cooled solution (5 mL) of 95% TFA-containing 2.5% (v/v) of triisopropylsilane and H_2O was used to dissolve the crude [Trt-C(Trt)-PEG-NH]$_n$-NH-R labeled wedges (10 μmol). After 30 min of stirring at 4 °C, the volatiles were removed in vacuo and the residue taken up into H_2O (20 mL). The aqueous layer was washed with Et_2O (4 × 20 mL) before freeze drying. The labeled dendrons were purified by RP-HPLC using gradient of 15% to 45% ACN in H_2O, each containing 0.1% TFA, to give the biotinylated compounds as colorless solids or the fluorescein labeled compounds as yellow solids.

[Cys-PEG-NH]$_2$-Biotin (16a): Yield of 48% from **14a** over two steps. ESI-MS: calcd. $C_{58}H_{110}N_{10}O_{19}S_3$: *m/z* 1347.77; found *m/z* 1347.0 (Figure S7).

[Cys-PEG-NH]$_3$-Biotin (16b): Yield of 26% from **14b** over two steps. ESI-MS: calcd. $C_{77}H_{147}N_{13}O_{27}S_4$: *m/z* 1815.34; found *m/z* 1814.2 (Figure S8).

[Cys-PEG-NH]$_4$-Biotin (16c): Yield of 21% from **14c** over two steps. ESI-MS: calcd. $C_{108}H_{206}N_{18}O_{37}S_5$: *m/z* 2509.25; found *m/z* 2508.7 (Figure S9).

[Cys-PEG-NH]$_2$-Fluorescein (16d): Yield of 52% from **14a** over two steps. ESI-MS: calcd. $C_{64}H_{97}N_7O_{21}S_2$: *m/z* 1364.64; found *m/z* 1364.8 (Figure S10).

[Cys-PEG-NH]$_3$-Fluorescein (16e): Yield of 38% from **14b** over two steps. ESI-MS: calcd. $C_{83}H_{134}N_{10}O_{29}S_3$: *m/z* 1832.23; found *m/z* 1832.8 (Figure S11).

[Cys-PEG-NH]$_4$- Fluorescein (16f): Yield of 42% from **14c** over two steps. ESI-MS: calcd. $C_{114}H_{193}N_{15}O_{39}S_4$: *m/z* 2526.13; found *m/z* 2526.4 (Figure S12).

Solid-Phase Peptide Synthesis of Peptide Thioesters: For the preparation of three peptides with a C-terminal thioester using Fmoc chemistry the procedure of Ingenito et al.[11] was followed. 4-Sulfamylbutyryl AM resin (1 g, 1.1 mmol, Novabiochem) was suspended in CH_2Cl_2 (10 mL) before the addition of 9.0 equiv. of DIPEA (1.65 mL) and 3.0 equiv. of Fmoc-Gly-OH (0.98 g). The mixture was stirred for 10 min at room temperature followed by cooling to –20 °C. After 20 min, 3.0 equiv. of PyBOP (1.72 g) were added as a solid and the mixture was stirred overnight. The resin was then washed with CH_2Cl_2, DMF, MeOH and dried in vacuo. The extent of incorporation was quantified by the Fmoc release UV assay and showed a loading of 0.68 mmol/g. Peptide assembly on the Fmoc-Gly-Sulfamylbutyryl resin was performed using an automated peptide synthesizer (Prelude, Protein technologies) following the standard Fmoc peptide synthesis protocol (100 μmol scale). Each amino acid was allowed to couple for two times 20 min and the N-terminus was acetylated using a mixture of pyridine, acetic anhydride and DMF (1:1:3). Alkylation of the peptide-acylsulfonamide resin was performed by swelling the resin in THF (2 mL) before the addition of 1 M TMS-CH_2N_2 (50:50, v/v, hexane/THF). After stirring for 2 h, the resin was washed with THF and DMF. Ethyl-3-mercaptopropionate (40 equiv., 1 M in DMF) and sodium thiophenate (0.4 equiv.) were added and stirred for 24 h. The resin was filtered and washed with DMF. The filtrate containing the peptide was collected and dried in vacuo. The cleaved, protected peptides were subsequently treated with 96% TFA, 2% H_2O and 2% triisopropylsilane and stirred for 2 h. Cold diethyl ether was added and the mixture was stored for 2 h at –30 °C. The precipitate formed after centrifugation was dissolved in water and lyophilized. Purification was done by preparative RP-HPLC using a gradient of acetonitrile in water (both containing 0.1% TFA).

Ac-GRGDSGG-SR: Gradient RP-HPLC: 10–20% in 20 min. ESI-MS: Calcd. *m/z* 762.6; found *m/z* 762.3 (Figure S13).

Ac-RQIKIWFQNRRMKWKKGGG-SR: Gradient RP-HPLC: 15–30% in 45 min. ESI-MS: Calcd. 2576.0 *m/z*; found *m/z* 2575.6 (Figure S14).

Ac-NGVFKYRPRYFLYKHAYFPPLKRFPVQGG-SR: Gradient RP-HPLC: 15–35% in 45 min. ESI-MS: Calcd. *m/z* 3868.4; found *m/z* 3867.7, and 3979.4 (TFA adduct) (Figure S15).

Multivalent Peptides via Native Chemical Ligation: For these reactions, 1.10–1.25 equiv. of peptide per N-terminal cysteine on the dendritic wedge were used. Thiophenol was employed as the transthioesterification catalyst with benzyl mercaptan as the in situ reducing agent. Both thiols were added to solutions of the pentameric dendritic wedge in the ligation buffer (6 M guanidine, 70 mM Tris, pH 8.0) before adding the peptide thioesters, also in the ligation buffer. The addition of thiols brought the overall pH to 7.6–7.8. Reaction mixtures were kept at 37 °C overnight and characterized by LC-MS analysis (see Figures S15–17 and 2).

(Ac-GRGDSGGC)$_4$-Biotin: ESI-MS: Calcd. *m*/*z* 5023; found *m*/*z* 5024.

(Ac-RQIKIWFQNRRMKWKKGGGC)$_3$-Fluorescein: ESI-MS: Calcd. *m*/*z* 9158; found *m*/*z* 9159.

(Ac-NGVFKYRPRYFLYKHAYFYPPLKRFPVQGGC)$_2$-Biotin: ESI-MS: Calcd. *m*/*z* 8815; found *m*/*z* 8815.

Supporting Information (see also the footnote on the first page of this article): ^{1}H NMR spectra of compounds **13** and **14** and additional LC-MS characterization of the dendritic wedges and the peptides.

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