Multimeric Cyclic RGD Peptides as Potential Tools for Tumor Targeting: Solid-Phase Peptide Synthesis and Chemoselective Oxime Ligation

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Dedicated to Professor Dr. Peter Welzel on the occasion of his 65th birthday

Abstract: The $\alpha v\beta 3$ integrin receptor plays an important role in human metastasis and tumor-induced angiogenesis. Targeting this receptor may provide information about the receptor status of the tumor and enable specific therapeutic planning. Solid-phase peptide synthesis of multimeric *cyclo*(-RGDfE-)-peptides is described, which offer the possibility of enhanced integrin targeting due to polyvalency effects. These peptides contain an aminooxy group for versatile chemoselective oxime ligation. Conjugation with *para*-trimethylstannylbenzaldehyde results in a precursor for radioiododestannylation, which would allow them to be used as potential tools for targeting and imaging $\alpha v\beta 3$-expressing tumor cells. The conjugates were obtained in good yield without the need of a protection strategy and under mild conditions.

Keywords: chemoselective ligation · integrin · multimer · peptides · tumor targeting

Introduction

Cell–cell and cell–matrix interactions are involved in many physiological processes including embryogenesis, cell differentiation, hemostasis, wound healing, and immune response.[1–3] They are also fundamental to tumor invasion and formation of metastases[4] as well as to tumor-induced angiogenesis.[5–7] Integrins appear to be the major class of receptors through which cells attach to the extracellular matrix. In addition to these adhesive functions, integrins transduce messages by classical signaling pathways and may influence proliferation and apoptosis of tumor cells as well as activated endothelial cells.[5, 6, 8, 9] The tripetide sequence RGD (Arg-Gly-Asp) is a common cell-recognition motif for a wide variety of integrin receptors. The conformation of the RGD-containing loop and its flanking amino acids in proteins are mainly responsible for their different integrin affinity.[9–12] An integrin with a well-characterized involvement in angiogenesis[13, 14] and tumor invasiveness[15] is $\alpha v\beta 3$, a vitronectin receptor. This integrin is found on many cell types including certain tumor cells, activated endothelial cells and smooth-muscle cells, epithelial cells, platelets, and osteoclasts.[16] Cyclic peptides containing a conformationally restrained RGD sequence have been developed as ligands for the $\alpha v\beta 3$ integrin.[17] The first synthetic, highly active and selective antagonist of the $\alpha v\beta 3$ receptor, *cyclo*(-RGDfV-) was developed in our group.[12] Systematic derivatization of this peptide resulted in the *N*-alkylated cyclopeptide *cyclo*(-RGDf[*N*Me]V-),[18, 19] which has entered phase II clinical studies as an angiogenesis inhibitor (cilengitide, code EMD 121974, Merck KGaA). Furthermore, regression of primary tumors and eradication of micrometastases by combining a specific antiangiogenic therapy with our cyclopeptide and an immunotherapy with a tumor-specific antibody–interleukin 2 fusion protein have been reported.[20] In addition, the highly active and selective cyclic pentapeptides can be used for targeting and imaging $\alpha v\beta 3$-expressing tumor cells[15] as well as endothelial cells during neovascularization[4, 21] in research, diagnostics, and therapy. Since an exchange of valine in the cyclic pentapeptide for another amino acid has no significant influence on the activity and selectivity, this position is valuable for molecular and functional modifications.[22] *Cyclo*(-RGDfK-), which can easily be functionalized because of the ε-amino group on the lysine side chain was therefore used for tumor targeting[23] and imaging[24] as well as for stimulation of cell adhesion.[25, 26]

The aim of our work is to develop improved ligands for $\alpha v\beta 3$ integrin targeting. We decided to mimic naturally occurring examples to enhance the affinity of the receptor ligand interactions using polyvalency.[27–35] Thus we developed multimeric compounds in which the RGD sequence is locally enriched and can bind polyvalently, that is, simultaneously, to several integrins. The increased affinity of RGD ligands due to multivalent interactions was shown for example by Kok

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et al.[23] and Maheshwari et al.[36] To adapt the synthesis of our multimeric compounds to solid-phase peptide synthesis (SPPS) we used the MAP (multiple-antigen peptide) system developed by Tam et al.[37, 38] based on lysines as branching units. Multiple copies of a ligand can be bound to this simple, immunogenically inert branching core. The result is a large macromolecule with a unique three-dimensional configuration. For further elucidation of the influence of core on the immunogenicity of MAPs, Veprek et al.[39, 40] prepared a symmetrical core with β-Ala-Lys dipeptide as building unit. However, no significant improvement of immunological properties was detected.

For targeting it is necessary to use a radio label or a fluorescence label. We chose oxime ligation, a chemoselective reaction of an aldehyde group with an hydroxyl amino group. The high efficiency and selectivity of the aminooxy – aldehyde coupling reaction has been demonstrated successfully by attaching a variety of substances to proteins,[41–45] by chemical ligation of peptides,[46–50] by conjugation of peptides with carbohydrates[51–53] or oligonucleotides,[54] and by labeling of oligonucleotides and RNA.[55] The hydroxylamine functionality can easily be implemented as a *tert*-butyloxycarbonyl-protected aminooxy-functionalized amino acid building block. The oxime ligation represents an elegant way to link totally unprotected aminooxy-functionalized peptidic fragments to any marker molecule. The label can be incorporated site-specifically, that is, the conjugation does not interfere with the RGD binding motif. The oxime ligation is compatible with all standard amino-acid residues, with the exception of *N*-terminal cysteine. Moreover, the oxime bond is known to be reasonably stable both in vitro and in vivo. In order to introduce radioactivity, we used a trimethylstannyl precursor instead of a trimethylsilyl precursor, as suggested by Vaidyanathan et al.[56] The iododestannylation uses mild but high yielding conditions. To take advantage of these results, we decided to synthesize a trimethylstannylbenzaldehyde as the carbonyl partner for the chemoselective oxime formation.

The spatial arrangement and separations of the RGD moieties within one multimer for optimal cell targeting is not known. Thus we introduced aminohexanoic acid and a longer heptaethylene glycol moiety as spacer molecules. The use of ethylene glycol spacers is desirable due to the solubility problems of peptides containing long alkyl spacers.[57] Moreover, polyethylene glycol spacers are nontoxic and unreactive. The heptaethylene glycol spacer must be synthesized as a 9-fluorenylmethoxycarbonyl (Fmoc) -protected amino acid to make it available as a building block in SPPS. In order to use SPPS for coupling the RGD moiety to the branching core, we developed the pentapeptide *cyclo*(-Arg[Pbf]GlyAsp[*t*Bu]-D-PheGlu-). In this work glutaminic acid was chosen as fifth amino acid that offers a free carboxylic acid group for peptide coupling reactions.

The resulting general structure of our compounds is shown in Figure 1.

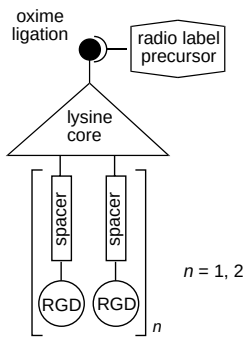
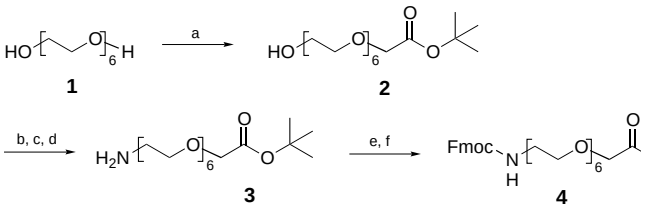


Figure 1. General structure of the target molecules.

Results and Discussion

Chemistry: A variety of conjugated *cyclo*(-RGDfE-) monomers and multimers were synthesized for radioiodination in order to use them as tracers for tumor targeting. The aminooxy group for the chemical ligation was introduced as *N*-β-(*N*-*tert*-butyloxycarbonylaminooxyacetyl)-L-diaminopropionic acid, and a lysine core was used as branching unit.

In order to prepare these compounds by SPPS we synthesized a heptaethylene glycol spacer with an amino acid superstructure, that is, a compound containing an Fmoc-protected amino group and a carboxyl group (Scheme 1). The Fmoc heptaethylene glycol amino acid **4** was synthesized in a similar manner to Boumrah et al.[57] in good yield without the need for purification by chromatography. A report on the same spacer was recently published by Dekker et al. whose synthesis is one step longer than ours.[58] Starting from hexaethylene glycol, we introduced the carboxyl moiety by treatment with diazoacetic acid *tert*-butylester in the presence of boron trifluoride etherate[59] with 82% yield. The resulting heptaethylene glycol acid *tert*-butylester was first activated with tosylchloride and then treated with sodium azide in dry DMF. The corresponding amine **3** was obtained by catalytic hydrogenation. Protection with an Fmoc group and final deprotection of the *tert*-butyl ester gave 20-(*N*-Fmoc)-amino-3,6,9,12,15,18-hexaoxaeicosanoic acid (Fmoc-Hegas) **4** in an overall yield of 33%.

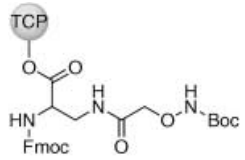


Scheme 1. Synthesis of Fmoc heptaethylene glycol amino acid **4**. a) Diazoacetic acid *tert*-butylester, $BF_3 \cdot OEt_2$, DCM; b) TsCl, Et_3N; c) NaN_3, DMF; d) H_2, Pd/C, EtOH, 34% (4 steps); e) FmocCl, 10% $NaHCO_3$, THF; f) 90% TFA/H_2O, 97% (2 steps).

The cyclic pentapeptide *cyclo*(-Arg[Pbf]GlyAsp[*t*Bu]-D-PheGlu-) was synthesized according to the method already developed in our group[22, 60] by a combined solid-phase – solution methodology. Starting from Gly in order to prevent racemization during cyclization later on, the linear peptide was synthesized by SPPS by using an Fmoc strategy[61] on tritylchloride polystyrene (TCP) resin[62, 63] in *N*-methyl-2-pyrrolidinon (NMP). The permanent protecting groups of the amino acid side chains were 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) for Arg, *tert*-butyl for Asp, and benzyl (Bzl) for Glu. Amino acids (2.5 equiv) were coupled stepwise with 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU, 2.5 equiv) and *N*-hydroxybenzotriazole (HOBt, 2.5 equiv) as coupling reagents and *N*,*N*-diisopropylethylamine (DIPEA, 7 equiv) as a base. In the coupling reaction of Fmoc-D-phenylalanine, collidine

(25 equiv) was used instead of DIPEA to prevent pyroglutamate formation. Cleavage from the resin occurred with dichloromethane (DCM)/acetic acid/2,2,2-trifluoroethanol (TFE) (3:1:1). Under these conditions the side chain protecting groups are not removed. Cyclization of the linear protected peptide was performed under high dilution in *N*,*N*-dimethylformamide with diphenylphosphoryl azide (DPPA)[64, 65] and $NaHCO_3$. The final benzyl deprotection of the Glu side chain by hydrogenation releases a partially deprotected cyclopeptide bearing a free carboxylic acid group. This is suitable for fragment coupling in solid-phase peptide synthesis.

The compounds containing the aminooxy linker for oxime ligation were synthesized by SPPS[66] by using the TCP resin[62, 63] and applying the Fmoc strategy[61] starting from the initiator unit *N*-α-Fmoc-*N*-β-(*N*-*tert*-butyloxycarbonylaminooxyacetyl)-L-diaminopropionic acid (DprAoa[Boc]) **5** as the site for chemoselective oxime formation.



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The monomers **6a–d** (Figure 2) with varying spacer lengths and diverse lipophilicity/hydrophilicity were synthesized in the same way as the multimeric compounds (Scheme 2) but without introducing a lysine branching unit.

Branching and spacer amino acids were coupled stepwise with TBTU (2.5 equiv) and HOBt (2.5 equiv) as coupling reagents and collidine (25 equiv) as a base. The cyclic pentapeptide *cyclo*-(-Arg[Pbf]GlyAsp[*t*Bu]-D-Phe-Glu-) is coupled as a fragment to the aminooxy-lysine spacer core by using 1.5 equiv cyclopeptide, 1.5 equiv *O*-(7-azabenzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU), 1.5 equiv 1-hydroxy-7-azabenzotriazole (HOAt) and 15 equiv collidine. Finally the peptides, except for the tetrameric RGD moieties, were cleaved from the resin with simultaneous deprotection by using a mixture of trifluoroacetic acid/water/triisopropylsilane (95:2.5:2.5). The tetramers were cleaved from the resin by using acetic acid and TFE in DCM and were purified by HPLC prior to deprotection by treatment with trifluoroacetic acid (TFA). HPLC chromatography yielded the pure precursors for oxime linkage.

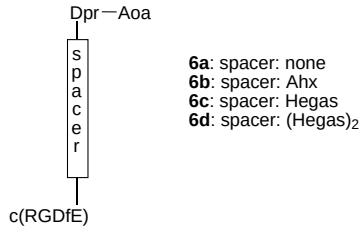
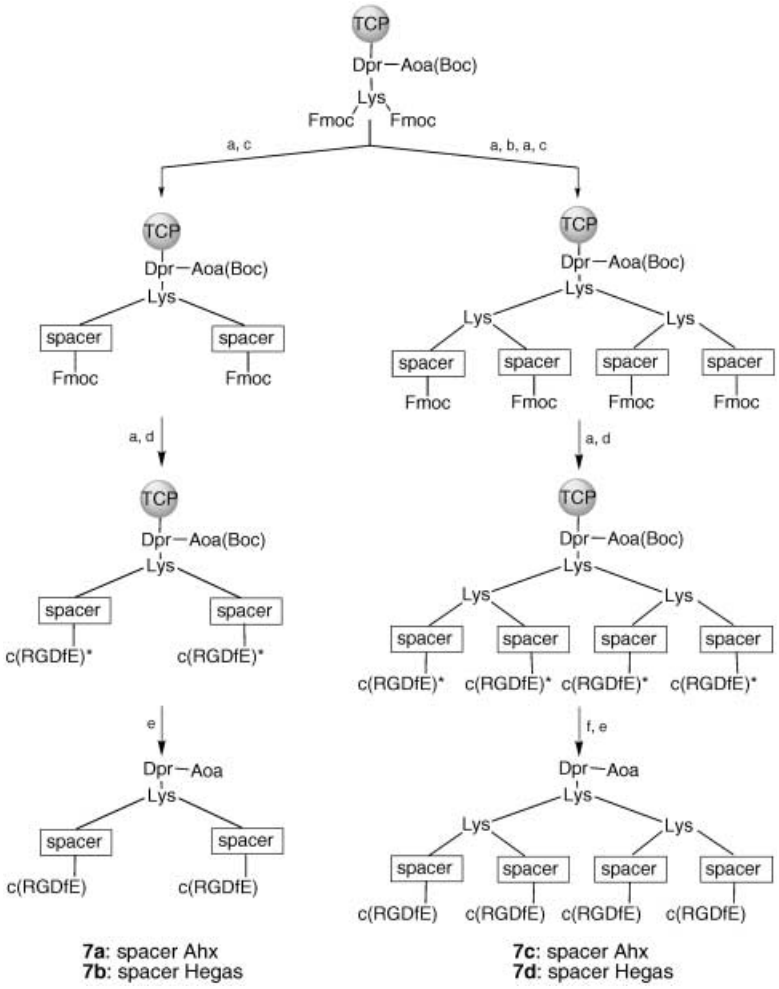
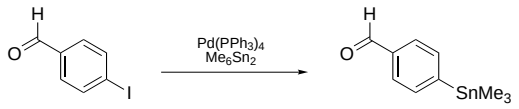


Figure 2. Monomeric compounds **6a–d**.

In addition to the trimethylstannylbenzaldehyde conjugates **12a–d** and **13a–d** for radioiodination, the analogue non-radioactive ^{127}I–benzaldehyde conjugates **10a–d** and **11a–d** were synthesized as a reference in the synthesis of the radiolabeled compounds and for receptor-affinity assays. The *para*-trimethylstannylbenzaldehyde (**9**) was synthesized by starting from the corresponding iodobenzaldehyde **8** with Stille coupling conditions (Scheme 3).



Scheme 2. Synthesis of the multimeric compounds **7a–d**. a) 20% piperidine in DMF; b) Fmoc-Lys(Fmoc), HATU, HOAt, collidine, NMP; c) Fmoc spacer amino acid (i.e. Fmoc-Ahx or Fmoc-Hegas), HATU, HOAt, collidine, NMP; d) *c*(-RGDfE-)* (i.e. *c*(-R[Pbf]GD[*t*Bu]fE-)), HATU, HOAt, collidine, NMP; e) 95% TFA/H_2O, TIPS; f) DCM/HOAc/TFE = 3:1:1; * indicates the protecting groups Pbf for Arg and *tert*-butyl for Asp.



8 **9**

Scheme 3. Synthesis of 4-trimethylstannylbenzaldehyde **9**.

The 4-iodobenzaldehyde **8** and the 4-trimethylstannylbenzaldehyde **9** react chemoselectively and quantitatively with the aminooxy-functionalized RGD moieties **6a–d**, **7a–d** without the need of a protection strategy and under mild conditions (Scheme 4).

Scheme 5 shows an example of a synthesized precursor for radioiodination, for example with the iodogen method.[67]

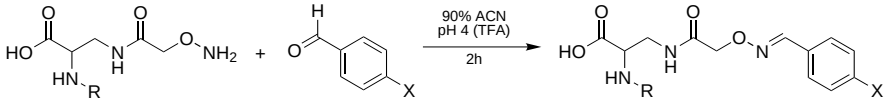
Biology: We tested the activities of the multimeric RGD compounds in inhibiting the interaction of ligands with isolated immobilized integrins. The ability of the RGD compounds to inhibit the binding of vitronectin to the isolated, immobilized $\alpha v\beta 3$ receptor was compared with that of the standard peptides GRGDSPK and *cyclo*(-RGDfV-). The results of the screening assays show the retaining integrin affinity (Table 1).

The radioiodination and biological evaluation of the radiolabeled compounds will be done in the Department of Nuclear Medicine of the Technische Universität München. In preliminary experiments, the in vivo stability of the oxime bond in our type of compound was evaluated in a tumor nude mouse model. Detailed studies will be published elsewhere.

We developed versatile compounds containing the aminooxy group for general ligation that can be used for fluorescence labeling, surface functionalization,[41, 68, 69] and cluster-

Table 1. Receptor-binding assay of RGD compounds **10a–d**, **11a–d**. The peptides GRGSPK ($IC_{50} = 1000$ nM) and *cyclo*(-RGDfV-) ($IC_{50} = 2.1$ nM) were used as references.

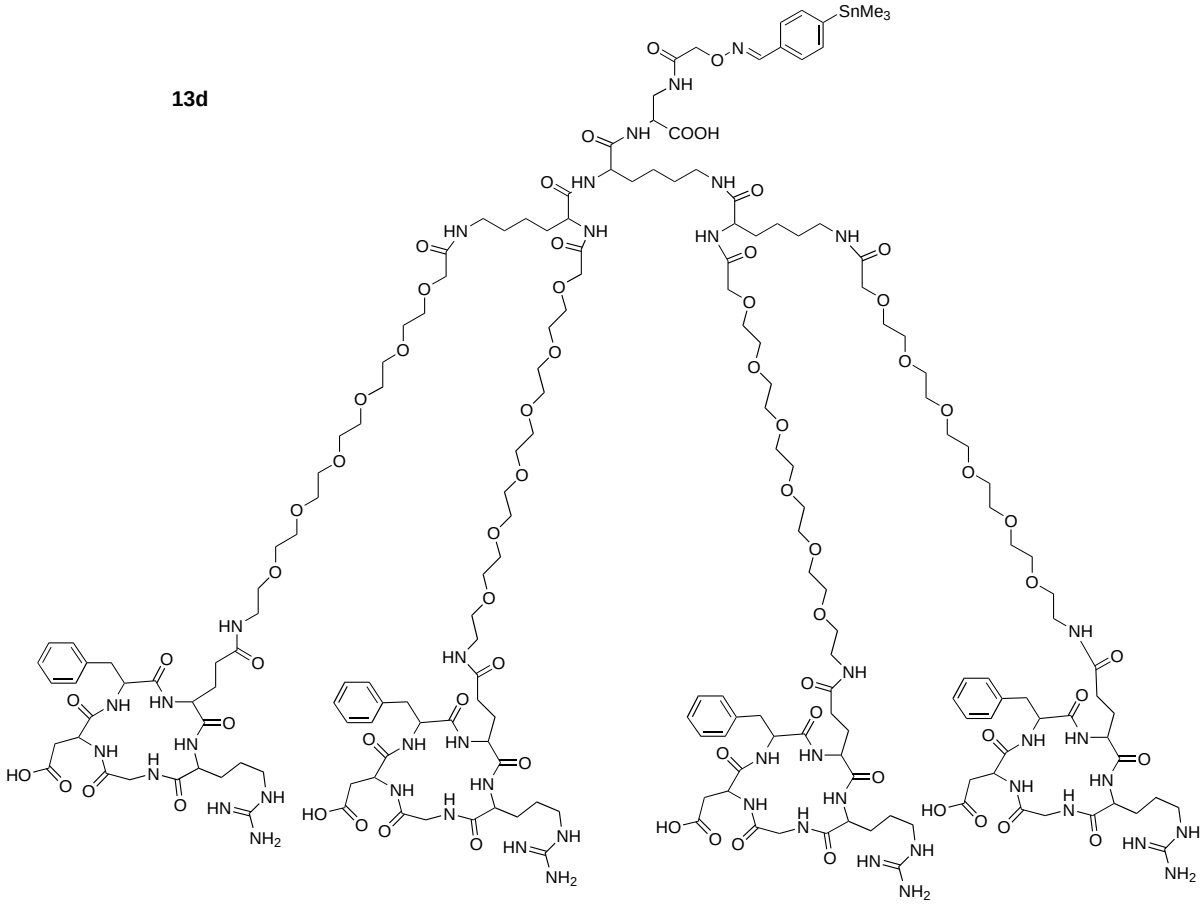
compound	**10a**	**10b**	**10c**	**10d**	**11a**	**11b**	**11c**	**11d**
IC_{50} [nM] on $\alpha v\beta 3$	10	10	20	7	0.9	3	5	0.2



6a-d, 7a-d **8** X = I, **9** X = $SnMe_3$ **10a-d**, **11a-d** X = I, **12a-d**, **13a-d** X = $SnMe_3$

Scheme 4. Chemoselective oxime ligation.

13d



Scheme 5. Tetrameric trimethylstannylbenzaldehyde conjugate with Hegas spacer **13d**.

ing studies of integrins.[36, 70] Furthermore, since lysine is commercially available with orthogonal protecting groups, it offers the possibility of synthesizing chimeric multimers containing further ligands besides RGD peptides.

Experimental Section

Chemistry

Materials and methods: All commercially available chemical reagents were used without further purification. The solvents DMF, DCM, MeOH, and Et_2O were distilled before use. Thin-layer chromatography (TLC) was performed on aluminium-backed plates Merck silica gel 60 F_{254}. Compounds were visualized by UV absorption at 254 nm, with 5% phosphomolybdic acid solution in 95% EtOH or with 3% ninhydrin solution in 97% EtOH. Analytical reversed-phase (RP) HPLC was performed on an Amersham Pharmacia Biotech instrument (system Äkta Basic 10F with autosampler A900, pump P-900, UV detector UV-900, software Unicorn) by using a YMC ODS-A C_{18} column (120 Å, 5 μm, 250 mm × 4.6 mm) with a flow rate of 1 mL min^{-1}. Semipreparative RP-HPLC separations were performed on a Beckman instrument (system Gold, pump 125, UV detector 166) by using an YMC ODS-A C_{18} column (120 Å, 5 μm, 250 mm × 20 mm) with a flow rate of 8 mL min^{-1}. Preparative RP-HPLC separations were performed on an Amersham Pharmacia Biotech instrument (system Äkta Basic 100F with pump P-900, UV detector UV-900, software Unicorn) by using a YMC ODS-A C_{18} column The eluent was a linear gradient from water (0.1% TFA) to acetonitrile (0.1% TFA) over 30 min. The retention time (R_t) of the analytical RP-HPLC is given in minutes with the gradient in percentage of acetonitrile. The detection was performed at 220 nm. Proton NMR spectra were obtained on a Bruker AC250 and DMX500 at 300 K. Chemical shifts are reported in ppm on the δ scale relative to the solvent signal used. ESI-MS spectra were performed on a LCQ Finnnigan and MALDI-TOF spectra on a Bruker Biflex III instrument.

*20-(*N*-Fmoc)-amino-3,6,9,12,15,18-hexaoxaicosanoic acid*: Three drops of freshly distilled boron trifluoride etherate ($BF_3 \cdot Et_2O$) were added to a mixture of hexaethylene glycol (30 g, 106.3 mmol) and ethyl diazoacetic acid *tert*-butylester (10 g, 70.3 mmol) in dry DCM (150 mL) under argon. After 15 min 3 further drops of $BF_3 \cdot Et_2O$ were added, and the resulting mixture was stirred for 60 min. The reaction mixture was diluted with ethyl acetate (450 mL) and washed with brine (1 × 200 mL and 4 × 50 mL), dried (Na_2SO_4), filtered, and concentrated to give **2** as orange oil with 18% di-*tert*-butyl ester as impurity. ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 4.02$ (s, 2H; OCH_2CO), 3.57–3.75 (m, 24H; CH_2 glycol), 1.47 (s, 9H; $C(CH_3)_3$); R_f ($CHCl_3$/MeOH 9:1) (**2**) = 0.6, (di-*t*Bu ester) = 0.7. The crude product was used in the next step without further purification.

This crude mixture was coevaporated with toluene (3 × 100 mL) and dissolved in dry DCM (200 mL), then triethylamine (29.4 mL, 210.9 mmol) and trimethylammonium chloride (1.3 g, 14.1 mmol) were added. Tosyl chloride (13.4 g, 70.3 mmol) in dry DCM (150 mL) was added dropwise over a period of 10 min at 0 °C. After removal of the ice bath, the mixture was stirred for 60 min, washed with H_2SO_4 (1N, 2 × 70 mL), saturated aqueous $NaHCO_3$ (1 × 70 mL), and brine (1 × 70 mL), dried (Na_2SO_4), filtered, and concentrated to give a dark yellow oil that was used in the next step without further purification.

The crude material was coevaporated with toluene (3 × 100 mL) and stirred overnight with NaN_3 (4.6 g, 70.3 mmol) in dry DMF (100 mL), and the solvent was removed in vacuo. The residue was taken up in ethyl acetate (300 mL), washed with brine (3 × 100 mL), dried (Na_2SO_4), filtered, and concentrated to give a yellow oil that was used in the next step without further purification. R_f ($CHCl_3$/MeOH 9:1) = 0.3.

This crude oil was dissolved in EtOH/$CHCl_3$ (200:20 mL) and stirred overnight with 5% Pd/C catalyst (2 g) under a hydrogen atmosphere. The catalyst was removed by filtration over celite, and the filtrate was concentrated under reduced pressure. The residue was taken up in ethyl acetate (70 mL) and extracted with $KHSO_4$ (1N, 1 × 100 mL, 2 × 50 mL). The combined aqueous layers were made caustic with Na_2CO_3 and extracted with $CHCl_3$ (1 × 100 mL, 2 × 50 mL), dried (Na_2SO_4), filtered, and concentrated to give 20-amino-3,6,9,12,15,18-hexaoxaicosanoic acid *tert*-butylester (**3**) (10.8 g, 27.3 mmol, 39% over 4 steps) as a yellow oil. ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 4.01$ (s, 2H; OCH_2CO), 3.62–3.71 (m, 20H; 10 × CH_2), 3.51 (t, $J = 5.2$ Hz, 2H; $OCH_2CH_2NH_2$), 2.85 (t, $J = 5.2$ Hz, 2H; CH_2NH_2), 1.76 (br s, 2H; NH_2), 1.47 (s, 9H; $C(CH_3)_3$); R_f ($CHCl_3$/MeOH 9:1) = 0.1.

Amine **3** (10.8 g, 27.3 mmol) in THF (100 mL) at 0 °C was treated with sodium bicarbonate (10%, 70 mL) and 9-fluorenyl chloroformate (7.4 g, 28.7 mmol) in THF (100 mL), added dropwise over 10 min, and the resulting suspension was stirred at room temperature for 60 min. Volatiles were removed, and the residue was dissolved in ethyl acetate (250 mL), washed with brine, dried (Na_2SO_4), filtered, and concentrated to give a crude yellow oil that was used in the next step without further purification. R_f ($CHCl_3$/MeOH 9:1) = 0.6.

The crude product was treated with 90% TFA for 30 min at room temperature. After removal of the solvents under reduced pressure, the residue was taken up in ethyl acetate (300 mL) and washed with brine (2 × 70 mL) and saturated aqueous $NaHCO_3$ (6 × 70 mL) until the aqueous layer became caustic. The combined aqueous layers were treated with $KHSO_4$ (5.4 g), acidified with HCl (6N, approx. 20 mL) under vigorous stirring, and diluted with a mixture of DCM (150 mL) and Et_2O (300 mL). The whole procedure was repeated starting from washing with saturated aqueous $NaHCO_3$. Finally the organic phase was dried (Na_2SO_4), filtered, and concentrated to give 20-(*N*-Fmoc)-amino-3,6,9,12,15,18-hexaoxaeicosanoic acid (**4**) (15.0 g, 26.8 mmol, 98% over 2 steps) as a yellow oil. ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 9.72$ (br s, 1H; CO_2H), 7.73 (d, $J = 7.0$ Hz, 2H; Fmoc-*H*), 7.58 (d, $J = 7.0$ Hz, 2H; Fmoc-*H*), 7.17–7.35 (m, 4H; Fmoc-*H*), 5.54 (br s, 1H; N*H*), 4.34 (d, $J = 7.0$ Hz, 2H; Fmoc-*H*), 4.14 (t, $J = 7.0$ Hz, 1H; Fmoc-*H*), 4.13 (s, 2H; OCH_2CO_2H), 3.45–3.62 (m, 22H; 11CH_2), 3.24–3.37 (m, 2H; CH_2NH); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta =$ 172.4, 156.7, 143.9, 141.2, 127.6, 127.0, 125.0, 119.9, 77.2, 71.1, 70.2–70.5, 69.9, 68.7, 66.6, 47.2, 40.8; R_f ($CHCl_3$/MeOH 4:1) = 0.3; $R_t = 20.4$ min (10 → 90); M_w $C_{29}H_{39}NO_{10}$ calcd 561.3, found MS (ESI): m/z (%) = 1146.5 (11) $[2M+H+Na]^+$, 600.2 (22) $[M+K]^+$, 584.3 (50) $[M+Na]^+$, 562.3 (100) $[M+H]^+$, 340.3 $[M-Fmoc+H]^+$.

4-Trimethylstannylbenzaldehyde: A mixture of *p*-iodobenzaldehyde (0.40 g, 1.72 mmol), hexamethyldistannane (0.46 mL, 2.22 mmol), and tetrakis(triphenylphosphine) palladium (0.20 g, 0.17 mmol) in dry toluene (15 mL) was heated under reflux for 3 h. After removal of the solvent and the excess of hexamethyldistannane in vacuo, the residue was purified by column chromatography (hexane/ethyl acetate 19:1). Aldehyde **9** was obtained as a colorless oil (0.35 g, 1.29 mmol, 75%). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 9.95$ (s, 1H; C*H*O), 7.77 (d, $J = 7.9$ Hz, 2H; 3,5-Ar*H*), 7.65 (d, $J = 7.6$ Hz, 2H; 2,6-Ar*H*), 0.31 (s, 9H; $Sn(CH_3)_3$ [d, $J(^{117}Sn,H) = 53.4$ Hz, $^{117}Sn(CH_3)_3$, d, $J(^{119}Sn,H) = 55.9$ Hz, $^{119}Sn(CH_3)_3$]); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 192.40$, 152.14 (s, Sn-*C* [d, $J(^{117}Sn,C) = 403.9$ Hz, $^{117}Sn-C$, d $J(^{119}Sn,C) = 422.5$ Hz, $^{119}Sn-C$), 136.18 (s, Sn−*C* [d, $J = 35.3$ Hz, $^{117}Sn-C$, $^{119}Sn-C$]), 135.99, 128.42 (s, Sn−*C* [d, $J = 43.6$ Hz, $^{117}Sn-C$, $^{119}Sn-C$]), −9.63 (s, Sn−*C* [d, $J(^{117}Sn,C) = 347.9$ Hz, $^{117}Sn-C$, d, $J(^{119}Sn,C) = 348.1$ Hz, $^{119}Sn-C$]).

Peptide Synthesis

General procedures for solid phase peptide synthesis

General procedure I: Attachment of N*-Fmoc-amino acids to TCP resin*: After swelling with dry DCM (10 mL) for 20 min, the TCP resin (2.00 g, theoretical 0.96 mmol g^{-1}, 1.92 mmol) was treated with a solution of Fmoc-protected amino acids (1.2 equiv, 2.3 mmol) in dry DCM (10 mL) and DIPEA (980 μL, 3 equiv, 5.8 mmol) at room temperature for 2 h. MeOH (2 mL) and DIPEA (0.4 mL) were added to cap the free sites, and the reaction mixture was shaken for 15 min. The resin was washed with NMP (3 × 10 mL), DCM (5 × 10 mL), and MeOH (3 × 10 mL) and dried under vacuo to give resin-bound *N*-Fmoc-amino acids.

General procedure II. Fmoc deprotection: The Fmoc-protected resin was suspended in a solution of 20% piperidine in NMP (2 × 20 mL) and agitated for 5 and 15 min. The resin was washed with NMP (6 × 20 mL).

General procedure IIIa. Coupling with TBTU/HOBt: Fmoc-amino acid (2.5 equiv), TBTU (2.5 equiv), HOBt (2.5 equiv), and DIPEA (7 equiv (25 equiv *sym*-collidine instead of DIPEA in the case of Fmoc-D-phenylalanine)) were dissolved in NMP to give a 0.2 mmol L^{-1} solution which was added to the resin. The reaction mixture was shaken at room temperature for 90 min and washed with NMP (6 × 20 mL). The end of the coupling was controlled by the Kaiser test.[71, 72]

General procedure IIIb. Coupling with HATU/HOAt: Fmoc-amino acid (1.5 equiv), HATU (1.5 equiv), HOAt (1.5 equiv), and 2,4,6-collidine (15 equiv) were dissolved in NMP to give a 0.2 mmol L^{-1} solution, which was added to the resin. The reaction mixture was shaken at room temperature for 90 min and washed with NMP (6×20 mL). The end of the coupling was controlled by the Kaiser test.[71, 72]

General procedure IVa. Cleavage with acetic acid: The resin was washed with DCM (4×20 mL) and treated with a mixture of DCM, acetic acid, and trifluoroethanol (3:1:1; 20 mL) for 60 min and (2×20 mL) for 3×10 min. After removal of the resin by filtration, the filtrates were combined, and the solvent was removed in vacuo and lyophilized out of *tert*-BuOH/H_2O to yield the protected peptide as a yellow powder.

General procedure IVb. Cleavage with trifluoroacetic acid: The resin was washed with DCM (4×20 mL) and treated with a solution of TFA, H_2O, and triisopropylsilane (TIPS) (90:4.75:4.75; 20 mL) for 3×10 min. After removal of the resin by filtration, the filtrates were combined and stirred for another 2.5 h. The solvent was removed in vacuo and lyophilized out of *tert*-BuOH/H_2O to yield the product as a yellow powder.

General procedure V. Cyclization of the linear pentapeptide: The linear pentapeptide was dissolved in high dilution ($c = 5 \times 10^{-3}$ M) in DMF. After addition of DPPA (3 equiv) and $NaHCO_3$ (5 equiv) as solid base, the suspension was stirred for 48 h at room temperature. The solid base was removed by filtration and, after the solution had been concentrated in vacuo, the cyclopeptide was precipitated and washed several times with H_2O.

General procedure VI. Bzl deprotection: The cyclic pentapeptide (1 mmol) was dissolved in *N*,*N*-dimethylacetamide (30 mL) and stirred for 4 h with 5% Pd/C catalyst (0.5–1 g) under a hydrogen atmosphere. After removal of the solvent in vacuo, the residue was taken up in MeOH (50 mL) and filtered over celite, and the filtrate was concentrated to precipitate the peptide with Et_2O.

General procedure VII. Pbf and OtBu deprotection with trifluoroacetic acid (TFA): The protected peptide was dissolved in TFA/H_2O (95:5). After 3 h, the solvent was removed under reduced pressure, and the residue was lyophilized out of *t*BuOH/H_2O to yield the product as white powder.

General procedure VIII. Conjugation with benzaldehyde: Peptide (1 mmol) was dissolved in ACN/H_2O (1 mL, 90%) and TFA was added until the pH reached 4. After addition of benzaldehyde (3 mmol) the mixture was stirred for 2 h, and the solvent was removed in vacuo. The residue was washed with Et_2O (4×10mL) and lyophilization out of *t*BuOH/H_2O yielded the product as a white powder.

Cyclo*(-R[Pbf]GD[tBu]fE-)*: The procedure is similar to the general procedures for I, II, IIIa, IVa, V, and VI (4.22 g, 4.62 mmol, 79%, white powder). $R_t = 22.2$ min ($5 \rightarrow 90$%), M_w $C_{43}H_{60}N_8O_{12}S$ calcd 912.4, found MS (ESI): *m*/*z* (%) = 951.3 (9) $[M+K]^+$, 935.3 (14) $[M+Na]^+$, 913.3 (100) $[M+H]^+$, 857.4 (32) $[M-\text{isobuten}+H]^+$, 476.3 (14) $[(M-K+H)/2]^{2+}$, 448.4 (15) $[(M-\text{isobuten}+K+H)/2]^{2+}$.

Monomeric compounds without spacer: The monomeric compound **6a** was synthesized in a similar way to the general procedures I, II, IIIb, IVa, and VII as a white powder (41 mg, 0.05 mmol, 55%) after purification by semipreparative HPLC ($10 \rightarrow 50$). ^{1}H NMR (500 MHz, DMSO): $\delta = 8.30$ (dd, $J = 4.2/7.5$ Hz, 1H; H^N-Gly), 8.06–8.00 (m, 3H; $H^N{}_\alpha$-Dpr, H^N-Glu, H^N-Asp), 7.96–7.94 (m, 2H; H^N-D-Phe, $H^N{}_\beta$-Dpr), 7.77 (d, $J = 7.9$ Hz, 1H; $H^N{}_\alpha$-Arg), 7.44–7.43 (m, 1H; $H^N{}_\varepsilon$-Arg), 7.27–7.14 (m, 5H; $^{D-Phe}$C–*H*), 6.78 (br s, 2H; NH_2–Arg), 4.62 (m, 1H; H^α-Asp), 4.48 (q, $J = 7.1$ Hz, 1H; H^α-D-Phe), 4.33 (m, 1H; H^α-Dpr), 4.14–4.03 (m, 5H; H^α-Arg, CH_2-Aoa, H^α-Gly, H^α-Glu), 3.53–3.38 (m, 2H; H^β-Dpr), 3.24 (dd, $J = 15.5/4.2$ Hz, 1H; H^α-Gly), 3.10–3.07 (m, 2H; H^δ-Arg), 2.96 (dd, $J = 13.6/7.1$ Hz, 1H; H^β-D-Phe), 2.79 (dd, $J = 13.6/6.5$ Hz, 1H; H^β-D-Phe), 2.68 (dd, $J = 16.2/8.6$ Hz, 1H; H^β-Asp), 2.35 (dd, $J = 15.9/5.6$ Hz, 1H; H^β-Asp), 2.04–2.01 (m, 2H; H^γ-Glu), 1.85–1.72 (m, 2H; H^β-Glu, H^β-Arg), 1.70–1.61 (m, 1H; H^β-Glu), 1.51–1.32 (m, 3H; H^β-Arg, H^γ-Arg); $R_t = 9.8$ min ($5 \rightarrow 80$%); M_w $C_{31}H_{45}N_{11}O_{12}$ calcd 763.3, found MS (ESI): *m*/*z* (%) = 764.4 (100) $[M+H]^+$.

The conjugation of **6a** (1.5 mg, 1.7×10^{-3} mmol) with 4-iodobenzaldehyde (0.5 mg, 2.1×10^{-3} mmol) according to the general procedure VIII yielded **10a** as a white powder (1.8 mg, 1.6×10^{-3} mmol, 94%). $R_t = 19.0$ min ($5 \rightarrow 80$%), M_w $C_{38}H_{48}IN_{11}O_{12}$ calcd 977.3, found MS (ESI): *m*/*z* (%) = 978.3 (100) $[M+H]^+$.

The conjugation of **6a** (3.0 mg, 3.4×10^{-3} mmol) with 4-trimethylstannylbenzaldehyde (2.7 mg, 10.2×10^{-3} mmol) yielded **12a** as a white powder (1.2 mg, 1.1×10^{-3} mmol, 83%). $R_t = 22.5$ min ($5 \rightarrow 80$%), M_w $C_{41}H_{57}N_{11}O_{12}Sn$ calcd 1015.3, found MS (ESI): *m*/*z* (%) = 1016.3 (100) $[M+H]^+$.

Monomeric compounds with aminohexanoic acid spacer: The monomeric compound **6b** was synthesized in a similar manner to the general procedures I, II, IIIa, IIIb, IVa, and VII and yielded a white powder (44 mg, 0.04 mmol, 42%) after purification by semipreparative HPLC ($10 \rightarrow 50$). ^{1}H NMR (500 MHz, DMSO): $\delta = 8.30$–8.28 (m, 1H; H^N-Gly), 8.07–7.96 (m, 5H; $H^N{}_\alpha$-Dpr, H^N-Glu, $H^N{}_\beta$-Dpr, H^N-D-Phe, H^N-Asp), 7.75 (d, $J = 8.3$ Hz, 1H; $H^N{}_\alpha$-Arg), 7.69 (t, $J = 5.5$ Hz, 1H; H^N-Ahx), 7.46–7.45 (m, 1H; $H^N{}_\varepsilon$-Arg), 7.26–7.10 (m, 5H; $^{D-Phe}$C-*H*), 6.82 (br s, 2H; NH_2-Arg), 4.65–4.60 (m, 1H; H^α-Asp), 4.45 (q, $J = 7.1$ Hz, 1H; H^α-D-Phe), 4.36–4.31 (m, 1H; H^α-Dpr), 4.16–4.12 (m, 3H; H^α-Arg, CH_2-Aoa), 4.05 (dd, $J = 15.2/7.7$ Hz, 1H; H^α-Gly), 4.01–3.96 (m, 1H; H^α-Glu), 3.51–3.32 (m, 2H; H^β-Dpr), 3.24 (dd, $J = 15.2/3.9$ Hz, 1H; H^α-Gly), 3.10–3.07 (m, 2H; H^δ-Arg), 3.00–2.93 (m, 3H; H^ε-Ahx, H^β-D-Phe), 2.78 (dd, $J = 13.4/6.3$ Hz, 1H; H^β-D-Phe), 2.69 (dd, $J = 15.8/8.5$ Hz, 1H; H^β-Asp), 2.35 (dd, $J = 15.8/5.7$ Hz, 1H; H^β-Asp), 2.10 (t, $J = 7.4$ Hz, 2H; H^α-Ahx), 2.01–1.90 (m, 2H; H^γ-Glu), 1.83–1.62 (m, 3H; H^β-Glu, H^β-Arg), 1.51–1.33 (m, 7H; H^β-Arg, H^β-Ahx, H^γ-Arg, H^δ-Ahx), 1.26–1.20 (m, 2H; H^γ-Ahx); $R_t = 12.9$ min ($5 \rightarrow 60$%); M_w $C_{37}H_{56}N_{12}O_{13}$ calcd 876.4, found MS (ESI): *m*/*z* (%) = 877.5 (100) $[M+H]^+$.

The conjugation of **6b** (1.9 mg, 1.9×10^{-3} mmol) with 4-iodobenzaldehyde (1.3 mg, 5.7×10^{-3} mmol) according to the general procedure VIII yielded **10b** as a white powder (1.2 mg, 1.9×10^{-3} mmol, 99%). $R_t = 18.9$ min ($5 \rightarrow 80$%), M_w $C_{44}H_{59}IN_{12}O_{13}$ calcd 1090.3, found MS (ESI): *m*/*z* (%) = 1091.4 (100) $[M+H]^+$.

The conjugation of **6b** (3.7 mg, 3.8×10^{-3} mmol) with 4-trimethylstannylbenzaldehyde (3.1 mg, 11.4×10^{-3} mmol) yielded **12b** as a white powder (1.3 mg, 1.1×10^{-3} mmol, 28%). $R_t = 22.4$ min ($5 \rightarrow 80$%), M_w $C_{47}H_{68}N_{12}O_{13}Sn$ calcd 1128.4, found MS (ESI): *m*/*z* (%) = 1129.4 (100) $[M+H]^+$.

Monomeric compounds with Hegas spacer: The monomeric compound **6c** was synthesized in a similar manner to the general procedures I, II, IIIa, IIIb, IVa, and VII as a white powder (57 mg, 0.04 mmol, 44%) after purification by semipreparative HPLC ($10 \rightarrow 50$). ^{1}H NMR (500 MHz, DMSO): $\delta = 8.30$ (dd, $J = 4.2/7.5$ Hz, 1H; H^N-Gly), 8.06–8.04 (m, 2H; $H^N{}_\alpha$-Dpr, H^N-Glu), 8.00–7.97 (m, 2H; H^N-D-Phe, H^N-Asp), 7.92 (d, $J = 7.8$ Hz, 1H; $H^N{}_\beta$-Dpr), 7.78–7.73 (m, 2H; H^N-Hegas, $H^N{}_\alpha$-Arg), 7.44–7.43 (m, 1H; $H^N{}_\varepsilon$-Arg), 7.26–7.13 (m, 5H; $^{D-Phe}$C-*H*), 6.78 (br s, 2H; NH_2-Arg), 4.64–4.60 (m, 1H; H^α-Asp), 4.45 (q, $J = 7.2$ Hz, 1H; H^α-D-Phe), 4.40–4.36 (m, 1H; H^α-Dpr), 4.16–4.11 (m, 1H; H^α-Arg), 4.07–4.02 (m, 3H; CH_2-Aoa, H^α-Gly), 4.00–3.96 (m, 1H; H^α-Glu), 3.90 (s, 2H; OCH_2CO-Hegas), 3.60–3.38 (m, 24H; H^β-Dpr, CH_2-Hegas), 3.24 (dd, $J = 15.2/3.9$ Hz, 1H; H^α-Gly), 3.19–3.16 (m, 2H; CH_2NH-Hegas), 3.10–3.06 (m, 2H; H^δ-Arg), 2.95 (dd, $J = 13.4/7.6$ Hz, 1H; H^β-D-Phe), 2.79 (dd, $J = 13.4/6.3$ Hz, 1H; H^β-D-Phe), 2.69 (dd, $J = 16.3/8.7$ Hz, 1H; H^β-Asp), 2.35 (dd, $J = 16.2/5.7$ Hz, 1H; H^β-Asp), 2.02–1.90 (m, 2H; H^γ-Glu), 1.83–1.61 (m, 3H; H^β-Glu, H^β-Arg), 1.51–1.32 (m, 3H; H^β-Arg, H^γ-Arg); $R_t = 14.5$ min ($5 \rightarrow 60$%); M_w $C_{45}H_{72}N_{12}O_{19}$ calcd 1084.5, found MS (ESI): *m*/*z* (%) = 1085.6 (100) $[M+H]^+$.

The conjugation of **6c** (1.8 mg, 1.5×10^{-3} mmol) with 4-iodobenzaldehyde (1.0 mg, 4.5×10^{-3} mmol) according to general procedure VIII yielded **10c** as a white powder (1.5 mg, 1.1×10^{-3} mmol, 72%). $R_t = 18.2$ min ($5 \rightarrow 80$%), M_w $C_{52}H_{75}IN_{12}O_{19}$ calcd 1298.4, found MS (ESI): *m*/*z* (%) = 1299.5 (100) $[M+H]^+$.

The conjugation of **6c** (3.5 mg, 3.0×10^{-3} mmol) with 4-trimethylstannylbenzaldehyde (2.4 mg, 9.0×10^{-3} mmol) yielded **12c** as a white powder (1.5 mg, 1.0×10^{-3} mmol, 35%). $R_t = 22.6$ min ($5 \rightarrow 80$%), M_w $C_{55}H_{84}N_{12}O_{19}Sn$ calcd 1336.5, found MS (ESI): *m*/*z* (%) = 1337.5 (100) $[M+H]^+$.

Monomeric compounds with (Hegas)$_2$ spacer: The monomeric compound **6d** was synthesized in a similar manner to general procedures I, II, IIIa, IIIb, IVa, and VII as a white powder (45 mg, 0.03 mmol, 27%) after purification by semipreparative HPLC ($20 \rightarrow 50$). ^{1}H NMR (500 MHz, DMSO): $\delta = 8.30$ (dd, $J = 3.8/7.4$ Hz, 1H; H^N-Gly), 8.10–8.09 (m, 1H; $H^N{}_\alpha$-Dpr), 8.05 (d, $J = 7.2$ Hz, 1H; H^N-Glu), 8.00–7.97 (m, 2H; H^N-D-Phe, H^N-Asp), 7.92 (d, $J = 7.9$ Hz, 1H; $H^N{}_\beta$-Dpr), 7.78–7.73 (m, 2H; H^N-Hegas, $H^N{}_\alpha$-

Arg), 7.64–7.62 (m, 1H; H^N-Hegas), 7.44–7.42 (m, 1H; $H^N{}_\varepsilon$-Arg), 7.26–7.13 (m, 5H; $^{D-Phe}$C-*H*), 6.77 (brs, 2H; NH_2-Arg), 4.65–4.60 (m, 1H; H^α-Asp), 4.45 (q, J = 7.2 Hz, 1H; H^α-D-Phe), 4.41–4.37 (m, 1H; H^α-Dpr), 4.16–4.12 (m, 3H; H^α-Arg, CH_2-Aoa), 4.05 (dd, J = 15.2/7.7 Hz, 1H; H^α-Gly), 4.00–3.96 (m, 1H; H^α-Glu), 3.90 (s, 2H; OCH_2CO-Hegas), 3.86 (s, 2H; OCH_2CO-Hegas), 3.59–3.37 (m, 46H; H^β-Dpr, CH$_2$-Hegas), 3.27–3.22 (m, 3H; CH_2NH-Hegas, H^α-Gly), 3.19–3.15 (m, 2H; CH_2NH-Hegas), 3.10–3.06 (m, 2H; H^δ-Arg), 2.95 (dd, J = 13.4/7.7 Hz, 1H; H^β-D-Phe), 2.79 (dd, J = 13.4/6.3 Hz, 1H; H^β-D-Phe), 2.69 (dd, J = 16.2/8.6 Hz, 1H; H^β-Asp), 2.38–2.33 (m, 1H; H^β-Asp), 2.02–1.90 (m, 2H; H^γ-Glu), 1.85–1.61 (m, 3H; H^β-Glu, H^β-Arg), 1.51–1.32 (m, 3H; H^β-Arg, H^γ-Arg); R_t = 16.2 min (5 → 60%); M_w $C_{59}H_{99}N_{13}O_{26}$ calcd 1405.7, found MS (ESI): m/z (%) = 1406.8 (100) $[M+H]^+$.

The conjugation of **6d** (3.0 mg, 2.0×10^{-3} mmol) with 4-iodobenzaldehyde (1.4 mg, 6.0 mmol) according to general procedure VIII yielded **10d** as a white powder (1.8 mg, 1.0×10^{-3} mmol, 53%). R_t = 18.7 min (5 → 80%), M_w $C_{66}H_{102}IN_{13}O_{26}$ calcd 1619.6, found MS (ESI): m/z (%) = 1620.6 (100) $[M+H]^+$.

The conjugation of **6d** (6.0 mg, 3.9×10^{-3} mmol) with 4-trimethylstannylbenzaldehyde (3.1 mg, 11.7×10^{-3} mmol) yielded **12d** as a white powder (5.0 mg, 2.8×10^{-3} mmol, 71%). R_t = 22.5 min (5 → 80%), M_w $C_{69}H_{111}N_{13}O_{26}Sn$ calcd 1657.7, found MS (ESI): m/z (%) = 1658.7 (100) $[M+H]^+$.

Dimeric compounds with aminohexanoic acid spacer: The dimeric compound **7a** was synthesized in a similar manner to the general procedures I, II, IIIa, IIIb, IVa, and VII as a white powder (66 mg, 0.04 mmol, 31%) after purification by semipreparative HPLC (20 → 50). ^{1}H NMR (500 MHz, DMSO): δ = 8.31–8.29 (m, 2H; H^N-Gly), 8.16 (d, J = 5.7 Hz, 1H; $H^N{}_\alpha$-Lys) 8.07–8.05 (m, 3H; $H^N{}_\alpha$-Dpr, H^N-Glu), 8.00–7.97 (m, 4H; H^N-D-Phe, H^N-Asp), 7.90–7.89 (m, 1H; $H^N{}_\beta$-Dpr), 7.74–7.69 (m, 5H; $H^N{}_\alpha$-Arg, H^N-Ahx, $H^N{}_\varepsilon$-Lys), 7.46–7.45 (m, 2H; $H^N{}_\varepsilon$-Arg), 7.25–7.14 (m, 10H; $^{D-Phe}$C-*H*), 6.81 (brs, 4H; NH_2-Arg), 4.63–4.60 (m, 2H; H^α-Asp), 4.46–4.41 (m, 2H; H^α-D-Phe), 4.32–4.29 (m, 2H; H^α-Lys, H^ε-Lys), 4.21–3.97 (m, 9H; H^α-Dpr, H^α-Arg, CH_2-Aoa, H^α-Gly, H^α-Glu), 3.51–3.38 (m, 2H; H^β-Dpr), 3.24 (d, 2H; J = 14.6 Hz, H^α-Gly), 3.09–2.94 (m, 11H; H^ε-Lys, H^δ-Arg, H^ε-Ahx, H^β-D-Phe), 2.80–2.77 (m, 2H; H^β-D-Phe), 2.71–2.66 (m, 2H; H^β-Asp), 2.38–2.34 (m, 2H; H^β-Asp), 2.11–2.10 (m, 4H; H^α-Ahx), 2.02–1.93 (m, 6H; H^γ-Glu, H^β-Lys), 1.78–1.62 (m, 6H; H^β-Glu, H^β-Arg), 1.46–1.21 (m, 22H; H^β-Arg, H^β-Ahx, H^γ-Arg, H^δ-Ahx, H^δ-Lys, H^γ-Ahx, H^γ-Lys); R_t = 12.3 min (5 → 90%); M_w $C_{75}H_{113}N_{23}O_{23}$ calcd 1703.8, found MS (ESI): m/z (%) = 1704.8 (22) $[M+H]^+$, 853.4 (100) $[(M+2H)/2]^{2+}$.

The conjugation of **7a** (1.0 mg, 0.6×10^{-3} mmol) with 4-iodobenzaldehyde (0.4 mg, 1.8×10^{-3} mmol) according to the general procedure VIII yielded **11a** as a white powder (1.1 mg, 0.6×10^{-3} mmol, 97%). R_t = 16.5 min (5 → 90%), M_w $C_{82}H_{116}IN_{23}O_{23}$ calcd 1917.8, found MS (ESI): m/z (%) = 1918.7 (5) $[M+H]^+$, 960.5 (100) $[(M+2H)/2]^{2+}$.

The conjugation of **7a** (1.7 mg, 1.0×10^{-3} mmol) with 4-trimethylstannylbenzaldehyde (0.8 mg, 3.0×10^{-3} mmol) yielded **13a** as a white powder (1.6 mg, 0.8×10^{-3} mmol, 83%). R_t = 18.8 min (5 → 90%), M_w $C_{85}H_{125}N_{23}O_{23}Sn$ calcd 1955.8, found MS (ESI): m/z (%) = 1956.9 (6) $[M+H]^+$, 979.2 (100) $[(M+2H)/2]^{2+}$.

Dimeric compounds with Hegas spacer: The dimeric compound **7b** was synthesized in a similar manner to the general procedures I, II, IIIa, IIIb, IVb as a white powder (53.3 mg, 0.03 mmol, 23%) after purification by semipreparative HPLC (20 → 50). R_t = 12.9 min (5 → 90%); M_w $C_{91}H_{145}N_{23}O_{35}$ calcd 2120.0, found MS (ESI): m/z (%) = 1061.5 (100) $[(M+2H)/2]^{2+}$; MS (MALDI-TOF): m/z = 2120.7 $[M+H]^+$.

The conjugation of **7b** (1.0 mg, 0.5×10^{-3} mmol) with 4-iodobenzaldehyde (0.3 mg, 1.4×10^{-3} mmol) according to general procedure VIII yielded **11b** as a white powder (1.1 mg, 0.5×10^{-3} mmol, 96%). ^{1}H NMR (500 MHz, DMSO): δ = 8.31–8.29 (m, 3H; H^N-Gly, $H^N{}_\alpha$-Lys) 8.08–8.04 (m, 3H; $H^N{}_\alpha$-Dpr, H^N-Glu), 8.00–7.97 (m, 4H; H^N-D-Phe, H^N-Asp), 7.90–7.89 (m, 1H; $H^N{}_\beta$-Dpr), 7.77–7.73 (m, 3H; H^N-Hegas, $H^N{}_\alpha$-Arg), 7.64–7.60 (m, 2H; $H^N{}_\varepsilon$-Lys H^N-Hegas), 7.45–7.44 (m, 2H; $H^N{}_\varepsilon$-Arg), 7.25–7.13 (m, 10H; $^{D-Phe}$C-*H*), 6.79 (brs, 4H; NH_2-Arg), 4.65–4.60 (m, 2H; H^α-Asp), 4.47–4.43 (m, 2H; H^α-D-Phe), 4.34–4.28 (m, 3H; H^α-Lys, H^ε-Lys, H^α-Dpr), 4.16–4.12 (m, 2H; H^α-Arg) 4.07–3.96 (m, 6H; CH_2-Aoa, H^α-Gly, H^α-Glu), 3.91 (s, 2H; OCH_2CO-Hegas), 3.84 (s, 2H; OCH_2CO-Hegas), 3.55–3.49 (m, 46H; H^β-Dpr, CH$_2$-Hegas), 3.39–3.37 (m, 2H; CH_2NH-Hegas), 3.27–3.22 (m, 2H; H^α-Gly), 3.18–3.16 (m, 2H; CH_2NH-Hegas), 3.09–3.04 (m, 5H; H^δ-Arg, H^ε-Lys), 2.95 (dd, J = 14.4/7.1 Hz, 2H; H^β-D-Phe), 2.79 (dd, J = 13.7/6.4 Hz, 2H; H^β-D-Phe), 2.69 (dd, J = 16.3/8.7 Hz, 2H; H^β-Asp), 2.35 (dd, J = 16.3/5.4 Hz, 2H; H^β-Asp), 2.02–1.90 (m, 4H; H^γ-Glu), 1.83–1.63 (m, 7H; H^β-Glu, H^β-Arg, H^β-Lys), 1.57–1.36 (m, 9H; H^β-Lys, H^β-Arg, H^γ-Arg, H^δ-Lys), 1.26–1.22 (m, 2H; H^γ-Lys); R_t = 16.9 min (5 → 90%); M_w $C_{98}H_{148}IN_{23}O_{35}$ calcd 2334.0, found MS (ESI): m/z (%) = 1179.0 (8) $[(M+Na+H)/2]^{2+}$, 1168.7 (100) $[(M+2H)/2]^{2+}$, 792.2 (13) $[(M+2Na+H)/3]^{3+}$, 786.9 (10) $[(M+Na+2H)/3]^{3+}$, 779.5 (7) $[(M+3H)/3]^{3+}$.

The conjugation of **7b** (2.0 mg, 0.9×10^{-3} mmol) with 4-trimethylstannylbenzaldehyde (0.8 mg, 2.8×10^{-3} mmol) according to the general procedure VIII yielded **13b** as a white powder (2.2 mg, 0.9×10^{-3} mmol, 99%). R_t = 19.0 min (5 → 90%); M_w $C_{101}H_{157}N_{23}O_{35}Sn$ calcd 2372.0, found MS (ESI): m/z (%) = 1197.6 (12) $[(M+Na+H)/2]^{2+}$, 1187.2 (100) $[(M+2H)/2]^{2+}$, 804.7 (18) $[(M+2Na+H)/3]^{3+}$, 799.2 (17) $[(M+Na+2H)/3]^{3+}$.

Tetrameric compounds with amino hexanoic acid spacer: The tetrameric compound **7c** was synthesized in a similar manner to the general procedures I, II, IIIa, IIIb, IVb, and VII with additional preparative HPLC (60 → 100) purification between steps IVb and VII. After purification by semipreparative HPLC, (20 → 50) **7c** was yielded as a white powder (73 mg, 0.02 mmol, 18%). ^{1}H NMR (500 MHz, DMSO): δ = 8.32–8.30 (m, 4H; H^N-Gly), 8.12 (d, J = 7.1 Hz, 3H; $H^N{}_\alpha$-Lys), 8.07–8.05 (m, 5H; $H^N{}_\alpha$-Dpr, H^N-Glu), 8.00–7.98 (m, 8H; H^N-D-Phe, H^N-Asp), 7.88–7.80 (m, 4H; $H^N{}_\beta$-Dpr, $H^N{}_\varepsilon$-Lys), 7.73–7.68 (m, 8H; $H^N{}_\alpha$-Arg, H^N-Ahx), 7.45–7.44 (m, 4H; $H^N{}_\varepsilon$-Arg), 7.25–7.13 (m, 20H; $^{D-Phe}$C-*H*), 6.81 (brs, 8H; NH_2-Arg), 4.62 (dd, J = 8.6 Hz, 4H; H^α-Asp), 4.45 (dd, J = 7.2 Hz, 4H; H^α-D-Phe), 4.33–4.29 (m, 3H; H^α-Lys), 4.23–3.97 (m, 8H; H^ε-Lys, H^α-Dpr, H^α-Arg) 4.07–3.96 (m, 10H; H^α-Gly, CH_2-Aoa, H^α-Glu), 3.51–3.39 (m, 2H; H^β-Dpr), 3.25 (dd, J = 15.0/3.6 Hz, 4H; H^α-Gly), 3.08 (m, 8H; H^δ-Arg),2.98–2.93 (m, 15H; H^ε-Ahx, H^ε-Lys, H^β-D-Phe), 2.79 (dd, J = 6.4 Hz, 4H; H^β-D-Phe), 2.69 (dd, J = 8.2 Hz, 4H; H^β-Asp), 2.38–2.34 (m, 4H; H^β-Asp), 2.13–1.89 (m, 22H; H^α-Ahx, H^γ-Glu, H^β-Lys), 1.83–1.62 (m, 12H; H^β-Glu, H^β-Arg), 1.47–1.35 (m, 34H; H^β-Arg, H^β-Ahx, H^γ-Arg, H^δ-Ahx, H^δ-Lys), 1.22–1.20 (m, 14H; H^γ-Ahx, H^γ-Lys); R_t = 13.0 min (5 → 90%); M_w $C_{151}H_{227}N_{45}O_{43}$ calcd 3358.7, found MS (ESI): m/z (%) = 1680.7 (11) $[(M+2H)/2]^{2+}$, 1121.2 (56) $[(M+3H)/3]^{3+}$, 841.4 (100) $[(M+4H)/]^{4+}$; MS (MALDI-TOF): m/z = 3359.5 $[M+H]^+$.

The conjugation of **7c** (1.0 mg, 0.3×10^{-3} mmol) with 4-iodobenzaldehyde (0.2 mg, 0.9×10^{-3} mmol) according to general procedure VIII yielded **11c** as a white powder (0.8 mg, 0.2×10^{-3} mmol, 77%). R_t = 15.5 min (5 → 90%); M_w $C_{158}H_{230}IN_{45}O_{43}$ calcd 3572.6, found MS (ESI): m/z (%) = 1205.0 (5) $[(M+2Na+H)/3]^{3+}$, 1192.5 (14) $[(M+3H)/]^{3+}$, 904.2 (12) $[(M+2Na+2H)/4]^{4+}$, 894.9 (100) $[(M+4H)/4]^{4+}$, 723.7 (8) $[(M+2Na+3H)/5]^{5+}$.

The conjugation of **7c** (3.3 mg, 1.0×10^{-3} mmol) with 4-trimethylstannylbenzaldehyde (0.8 mg, 3.0×10^{-3} mmol) according to general procedure VIII yielded **13c** as a white powder (3.2 mg, 0.9×10^{-3} mmol, 90%). R_t = 17.0 min (5 → 90%); M_w $C_{161}H_{239}N_{45}O_{43}Sn$ calcd 3610.7, found MS (ESI): m/z (%) = 1205.0 (7) $[(M+3H)/3]^{3+}$, 913.5 (9) $[(M+2Na+2H)/4]^{4+}$, 904.0 (100) $[(M+4H)/4]^{4+}$, 730.7 (8) $[(M+2Na+3H)/5]^{5+}$.

Tetrameric compounds with Hegas spacer: The tetrameric compound **7d** was synthesized in a similar manner to the general procedures I, II, IIIa, IIIb, IVb, and VII with additional preparative HPLC (60 → 100) purification between steps IVb and VII (Protected: 117 mg, 17%, pale yellow powder, R_t = 22.6 min (30 → 100%)). After final purification by semipreparative HPLC (20 → 50) **7d** was yielded as a white powder (43 mg, 0.01 mmol, 8%). ^{1}H NMR (500 MHz, DMSO): δ = 8.31–8.30 (m, 4H; H^N-Gly), 8.14 (d, 3H; J = 8.3 Hz, $H^N{}_\alpha$-Lys), 8.08–8.06 (m, 5H; $H^N{}_\alpha$-Dpr, H^N-Glu), 7.99–7.98 (m, 8H; H^N-D-Phe, H^N-Asp), 7.90–7.89 (m, 1H; $H^N{}_\beta$-Dpr), 7.82–7.73 (m, 6H; H^N-Hegas, $H^N{}_\alpha$-Arg), 7.65–7.59 (m, 5H; $H^N{}_\varepsilon$-Lys H^N-Hegas), 7.54–7.49 (m, 4H; $H^N{}_\varepsilon$-Arg), 7.25–7.13 (m, 20H; $^{D-Phe}$C-*H*), 6.89 (brs, 8H; NH_2-Arg), 4.65–4.60 (m, 4H; H^α-Asp), 4.47–4.43 (m, 4H; H^α-D-Phe), 4.33–4.12 (m, 11H; H^α-Lys, H^α-Dpr, H^ε-Lys, H^α-Arg), 4.07–3.96 (m, 10H; CH_2-Aoa, H^α-Gly, H^α-Glu), 3.91 (s, 4H; OCH_2CO-Hegas), 3.84 (s, 4H; OCH_2CO-Hegas), 3.54–3.39 (m, 94H; H^β-Dpr, CH$_2$-Hegas, CH_2NH-Hegas), 3.25 (d, 4H; J = 13.5 Hz, H^α-Gly), 3.18–3.17 (m, 4H; CH_2NH-Hegas), 3.09–3.04 (m, 11H; H^δ-Arg, H^ε-Lys), 3.00–2.92 (m, 4H; H^β-D-Phe), 2.80–2.76 (m, 4H; H^β-D-Phe), 2.69 (dd, 4H; J = 16.2/8.9 Hz, H^β-Asp), 2.35 (dd, 4H; J = 16.2/5.7 Hz, H^β-Asp), 2.00–1.90 (m, 8H; H^γ-Glu), 1.82–1.72 (m, 8H; H^β-Glu, H^β-Arg), 1.67–1.64 (m, 8H; H^β-Glu, H^β-Lys), 1.48–1.22 (m, 32H; H^β-Lys, H^β-Arg, H^γ-Arg, H^δ-Lys, H^γ-Lys); R_t =

13.4 min (5 → 90 %); M_w $C_{183}H_{291}N_{45}O_{67}$ calcd 4191.1, MS (MALDI-TOF): $m/z = 4191.9$ $[M+H]^+$.

The conjugation of **7d** (1.1 mg, 0.3×10^{-3} mmol) with 4-iodobenzaldehyde (0.2 mg, 0.8×10^{-3} mmol) according to general procedure VIII yielded **11d** as a white powder (1.1 mg, 0.3×10^{-3} mmol, 99 %). $R_t = 14.9$ min (5 → 90 %); M_w $C_{190}H_{294}IN_{45}O_{67}$ calcd 4405.0, found MS (ESI): m/z (%) = 1470.0 (12) $[(M+3H)/3]^{3+}$, 1113.6 (7) $[(M+2Na+2H)/4]^{4+}$, 1103.1 (100) $[(M+4H)/4]^{4+}$, 894.9 (14) $[(M+3Na+2H)/5]^{5+}$, 890.3 (14) $[(M+2Na+3H)/5]^{5+}$.

The conjugation of **7d** (3.8 mg, 0.9×10^{-3} mmol) with 4-trimethylstannylbenzaldehyde (0.7 mg, 2.7×10^{-3} mmol) according to general procedure VIII yielded **13d** as a white powder (3.9 mg, 0.9×10^{-3} mmol, 95 %). $R_t = 17.3$ min (5 → 90 %); M_w $C_{193}H_{303}N_{45}O_{67}Sn$ calcd 4443.1, found MS (ESI): m/z (%) = 1482.7 (15) $[(M+3H)/3]^{3+}$, 1121.9 (11) $[(M+2Na+2H)/4]^{4+}$, 1118.0 (7) $[(M+Na+3H)/4]^{4+}$, 1112.4 (100) $[(M+4H)/4]^{4+}$, 897.5 (18) $[(M+2Na+3H)/5]^{5+}$, 894.4 (8) $[(M+Na+4H)/5]^{5+}$.

Biology

Isolated integrin–ligand binding assays: The production of recombinant human $\alpha v\beta 3$ has been described elsewhere.[73] The inhibitory activity of the compounds described here was tested in ligand binding inhibition assays,[19] by using immobilized integrin as the target, and biotinylated human serum vitronectin as ligand. Purified vitronectin (1 mg mL^{-1}; pH 8.2) was biotinylated with *N*-hydroxysuccinimidobiotin (100 μg mL^{-1}; 1 h, 20 °C) before dialysis into phosphate buffer saline. In brief, 96-well ELISA plates were coated with 1 μg mL^{-1} integrin. After blocking residual sites on the plate with bovine serum albumin, biotinylated ligands (1 μg mL^{-1}) were added in the presence or absence of serial dilutions of inhibitor, and after incubation and washing, bound biotin was detected with a peroxidase-coupled antibiotin antibody and *N*,*N*,*N*′,*N*′-tetramethylbenzene substrate. IC_{50}, the concentration of inhibitor needed to inhibit ligand binding in the absence of inhibitor by 50 %, was established by curve fitting, and the values presented are usually the mean of three or more such independent determinations. External standards of linear GRGDSPK and *cyclo*-(-RGDfV-) were routinely included to monitor the dynamic range and the stability of the assay. Intra-assay variation, the relationship between IC_{50} values of standard inhibitors and the test substances, was typically less than ±5 %, and interassay variation in absolute IC_{50} was typically a factor of less than ±2.

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