

Electronic Supplementary Information

Multivalency: Influence of the Residence Time and the Retraction Rate on Rupture Forces Measured by AFM

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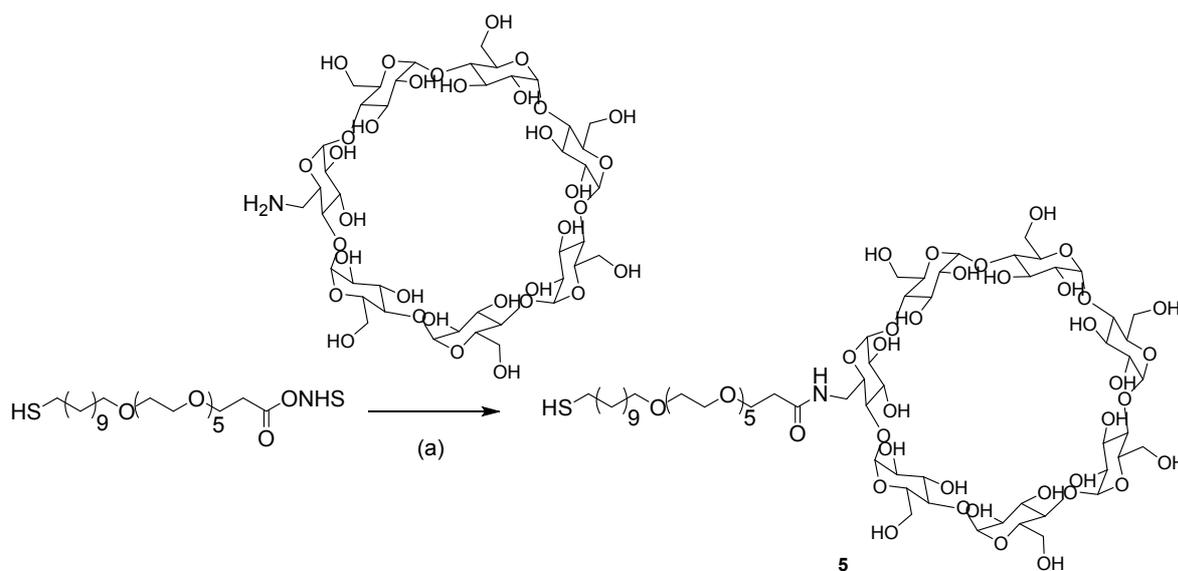
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General conditions.

All Fmoc amino acid derivatives and resins were purchased from Advanced ChemTech Europe (Brussels, Belgium), Bachem Biochimie SARL (Voisins-Les-Bretonneux, France) and France Biochem S.A. (Meudon, France). PyBOP was purchased from France Biochem and other reagents were obtained from either Aldrich (Saint Quentin Fallavier, France) or Acros (Noisy-Le-Grand, France). HS-C₁₁H₂₂-EG₆-COONHS was purchased from Prochimia Surfaces (ProChimia Surfaces, Sopot, Poland). N₃-EG₄-NHS was purchased from IRIS Biotech GMBH. RP-HPLC measurements were performed on a Waters system equipped with a Waters 600 controller and a Waters 2487 Dual Absorbance Detector. The purity of peptide derivatives was analyzed on an analytical column (Macherey-Nagel Nucleosil 120 Å 3 µm C18 particles, 30 x 4.6 mm) using the following solvent system: solvent A, water containing 0.09% TFA; solvent B, acetonitrile containing 0.09% TFA and 9.91% H₂O; a flow rate of 1.3 mL.min⁻¹ was employed with a linear gradient (5 to 100% B in 20 min). UV absorbances were monitored at 214 nm and 250 nm simultaneously. Preparative column (Delta-Pak™ 100 Å 15 µm C18 particles, 200 x 2.5 mm) were used to purify the crude peptides (when necessary) by using an identical solvent system at a flow rate of 22 mL.min⁻¹. ESI mass spectra were recorded on an Esquire 3000 (Bruker) spectrometer. The analyses were performed in positive mode for peptide derivatives using 50% aqueous acetonitrile as eluent. NMR spectra were recorded on BRUKER Avance 300 spectrometers. Chemical shifts are expressed in ppm and calculated taking the solvent peak as an internal reference; *J* values are given in Hz.

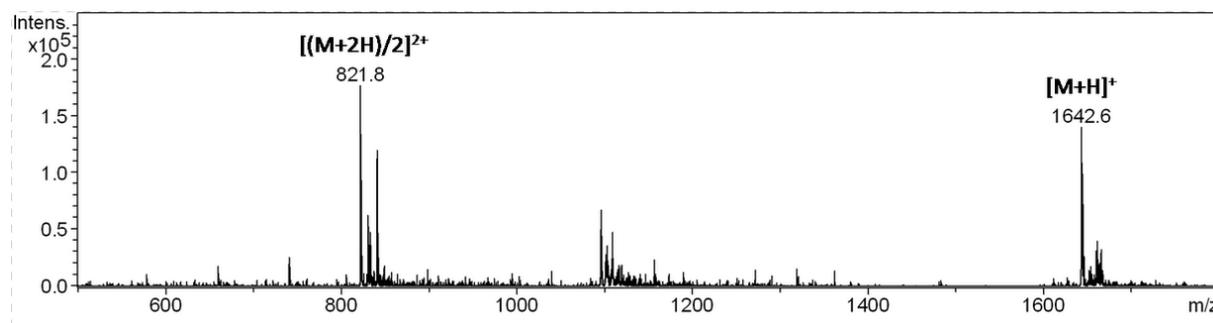


Scheme S1.

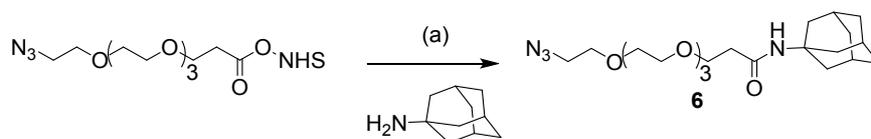
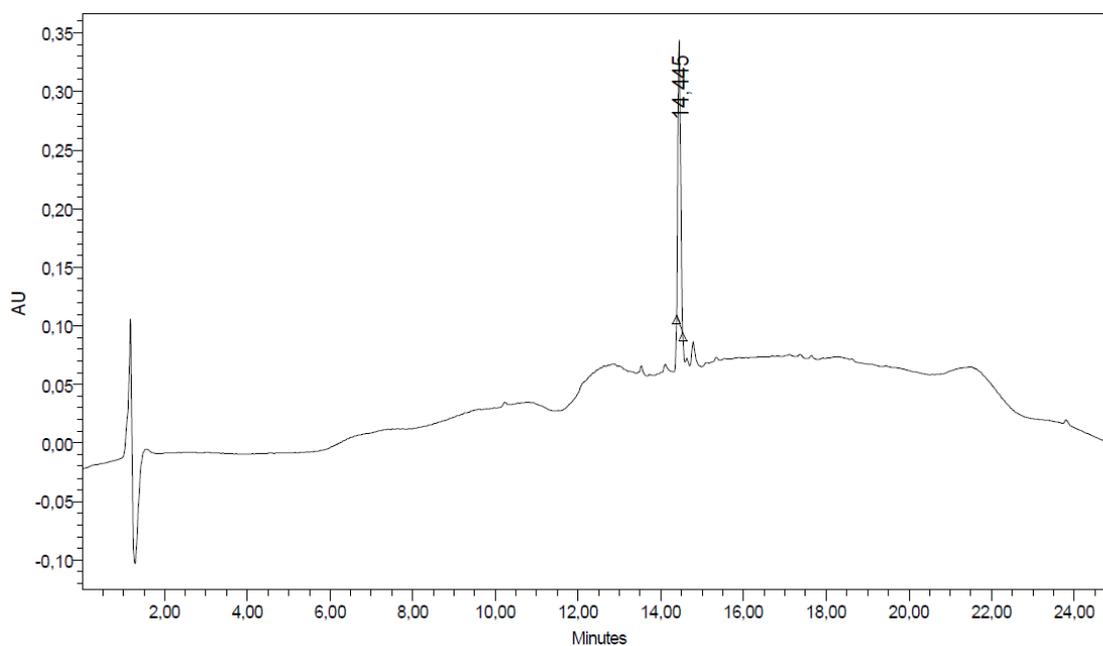
Synthesis of β -cyclodextrin alkanethiol **5**. *Reagents and conditions:* a) DIPEA, DMF, rt, 2h, 42%.

To a solution of commercially available HS-C₁₁H₂₂-EG₆-COONHS (50 mg, 0.08 mmol) in DMF (10 mL) was added DIPEA (18 μ L, 0.1 mmol) until the pH reach 8-9. A solution of 6-monodeoxy-6-monoamino- β -cyclodextrin (118 mg, 0.1 mmol) in DMF (5 mL) was added dropwise to the mixture and the medium was stirred at room temperature for 2h. After completion, the solvent was evaporated under reduced pressure and the crude material was purified by preparative RP-HPLC to yield HS-C₁₁H₂₂-EG₆-CD **5** (55 mg, 42%) as a white powder. **Mass spectrum** (ES-MS, positive mode) calc for C₆₇H₁₁₉NO₄₂S 1642.7, found m/z: 821.8 ([$(M+2H)/2$]²⁺, 100%), 1642.6 (80, [$M+H$]⁺).

ESI-MS analysis of HS-C₁₁H₂₂-EG₆-CD 5



RP-HPLC profile of HS-C₁₁H₂₂-EG₆-CD 5 (214 nm)



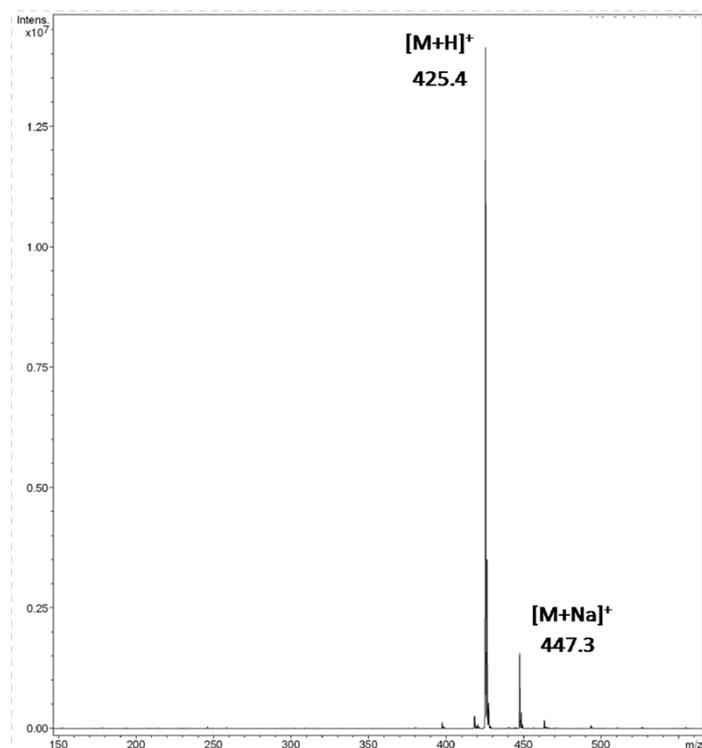
Scheme S2.

Synthesis of adamantyl azido fragment **6**. *Reagents and conditions:* a) DIPEA, DMF, rt, 2h, 89%.

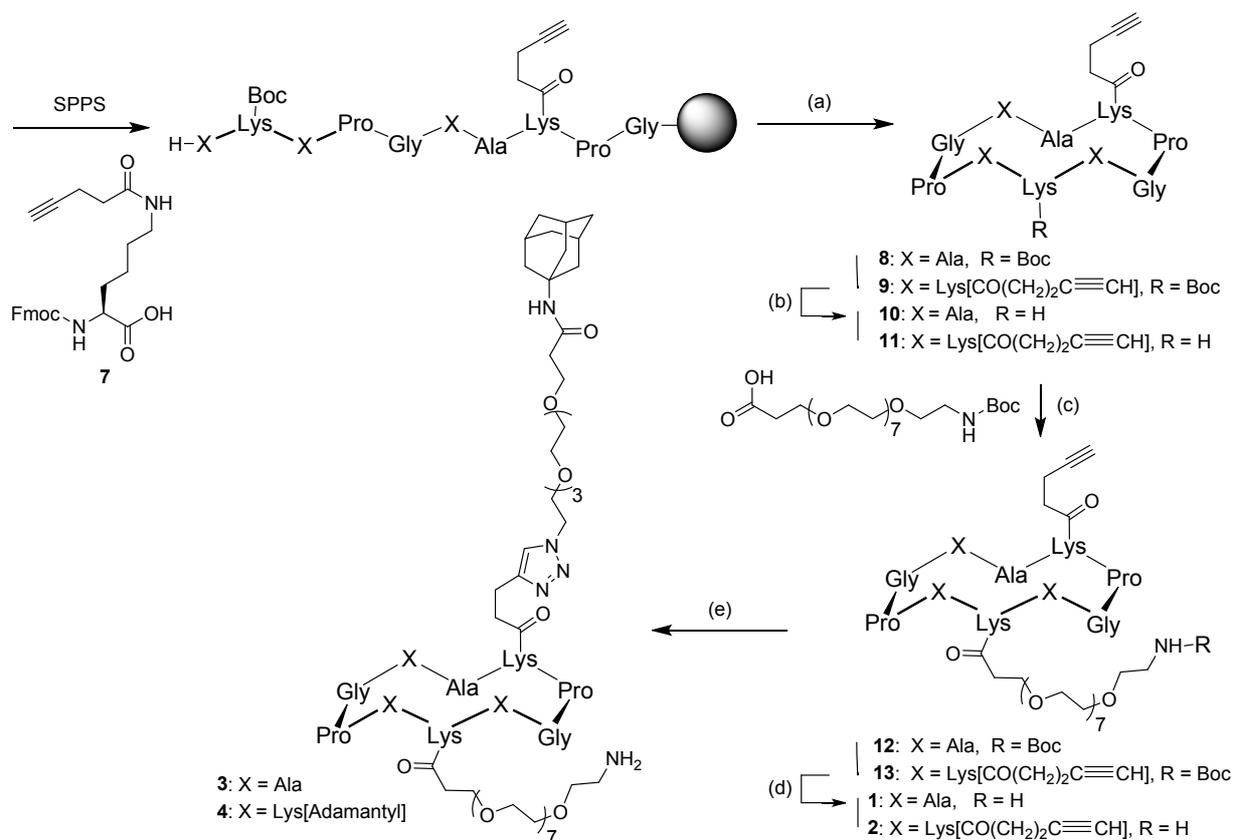
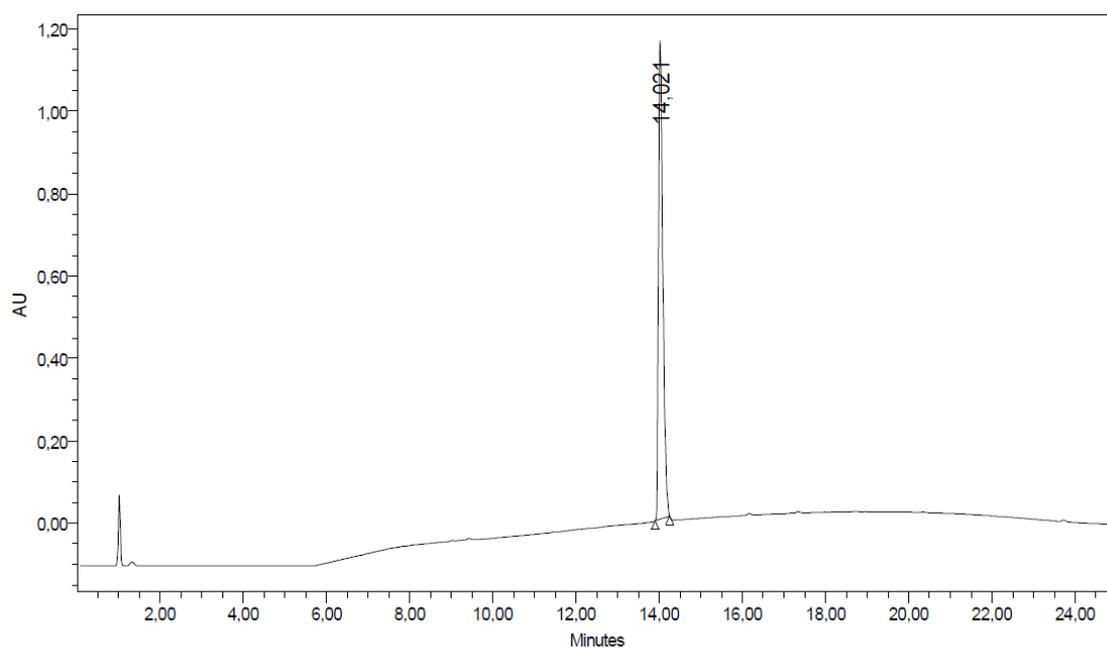
To a solution of 1-adamantylamine (17.5 mg, 116 μmol) in DMF (1 ml) was added a solution of N₃-EG₄-NHS (30 mg, 77 μmol) in DMF (30 μl). The pH of the resulting mixture was

adjusted to 8-9 by addition of DIPEA (42 μ L, 231 μ mol). After 2 h of stirring at room temperature, the crude material was purified by preparative RP-HPLC affording pure AD-EG₄-N₃ **6** as a colorless oil (29 mg, 89%). δ_{H} NMR (400 MHz, CDCl₃, Me₄Si) 6.21 (1H, br s, NH), 3.64 (16H, m, OCH₂), 3.37 (2H, m, COCH₂), 2.41 (2H, m, N₃CH₂), 2.05 (3H, m, CH), 1.98 (6H, m, CH₂) and 1.66 ppm (6H, m, CH₂). δ_{C} NMR (101 MHz, CDCl₃, Me₄Si) 171.67 (CO), 70.82 (OCH₂), 70.79 (OCH₂), 70.75 (OCH₂), 70.67 (OCH₂), 70.53 (OCH₂), 70.47 (OCH₂), 70.17 (OCH₂), 67.60 (OCH₂), 52.15 (N₃CH₂), 50.81 (C^{IV}), 41.62 (CH₂), 37.83 (COCH₂), 36.47 (CH₂) and 29.54 (CH) ppm. **Mass spectrum** (ES-MS, positive mode) calc for C₂₁H₃₆N₄O₅ 424.3, found m/z: 425.4 ([M+H]⁺, 100%), 447.3 (14, [M+Na]⁺).

ESI-MS analysis of AD-EG₄-N₃ **6**



RP-HPLC profile of AD-EG₄-N₃ 6 (214 nm)



Scheme S3.

Synthesis of adamantyl featuring peptidic scaffolds **3** and **4**. *Reagents and conditions*: (a) (i) TFA/DCM 1/99, rt, 20 min *3; (ii) PyBOP, DIPEA, DMF, rt, 1h, **8**: 98%, **9**: 98%; (b) TFA/DCM 1/1, rt, 2h, **10**: 97%, **11**: 97%; (c) PyBOP, DIPEA, DMF, rt, 1h, **12**: 93%, **13**: 88%; (d) TFA/DCM 1/1, rt, 2h, **1**: 98%, **2**: 97%; (e) CuAAC: **6**, CuSO₄, THPTA, AscNa, Phosphate buffer pH 7.4, DMF, 45°C, 3h, **3**: 72%, **4**: 68%.

General procedure for Solid-Phase Peptide Synthesis (SPPS).

Assembly of all protected peptides was carried out using the Fmoc/*t*-Bu strategy manually in a glass reaction vessel fitted with a sintered glass frit or automatically on a peptide synthesizer using 2-chlorotriylchloride[®]. Coupling reactions were performed manually by using 2 equiv of *N*-Fmoc-protected amino acid (relative to the resin loading) activated *in situ* with 2 equiv of PyBOP and 3-5 equiv of diisopropylethylamine (DIPEA) in DMF (10 mL/g resin) for 30 min except for the first coupling on 2-chlorotriylchloride[®]. Coupling reactions carried out on the synthesizer were performed twice. The coupling efficiency in manual synthesis was assessed by Kaiser and/or TNBS tests. *N*-Fmoc protecting groups were removed by treatment with a piperidine/DMF solution (1:4) for 10 min (10 mL/g resin). The process was repeated three times and the completeness of deprotection confirmed by the UV absorption of the piperidine washings at 299 nm. Synthetic linear peptides were recovered directly upon acid cleavage. Before cleavage, the resin was washed thoroughly with methylene chloride. The linear peptides were then released from the resin by treatments with a solution of acetic acid/trifluoroethanol/methylene chloride (1:1:8, 10 mL/mg resin, 2x30 min). Hexane (5-10 volumes) was added to the collected filtrates, and the crude peptides were isolated after evaporation as white solids. The residue was dissolved in the minimum of methylene chloride and diethyl ether was added to precipitate peptides. Then they were triturated and washed

three times with diethyl ether to obtain crude materials that were used in the next step without further purification.

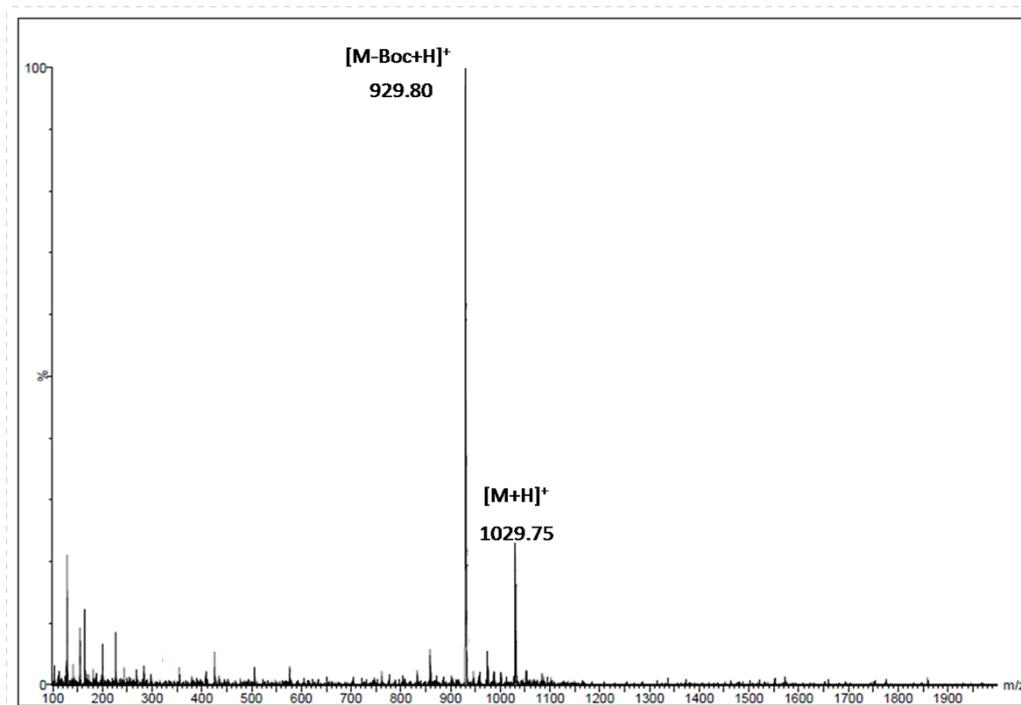
General procedure for cyclization reactions.

All linear peptides (0.5 mM) were dissolved in DMF and the pH values were adjusted to 8-9 by addition of DIPEA. PyBOP (1 equiv) was added and the solution stirred at room temperature for 1 h. Solvent was removed under reduced pressure and the residue dissolved in the minimum of methylene chloride. Diethyl ether was added to precipitate peptides. Then they were triturated and washed three times with diethyl ether to obtain crude materials that were used in the next step without further purification.

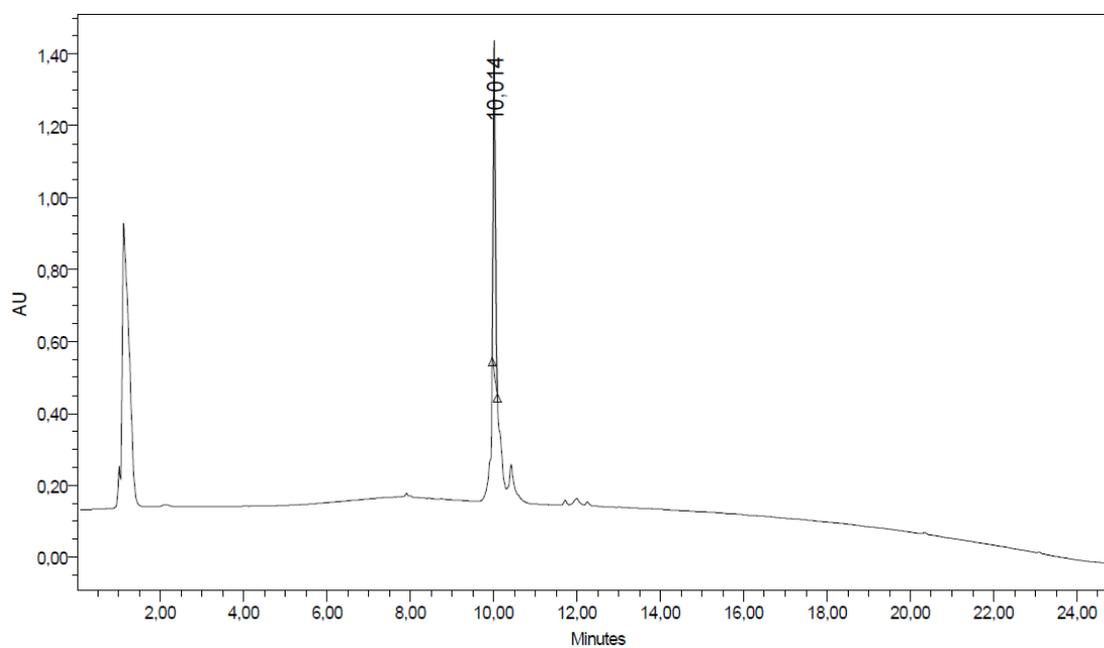
Cyclodecapeptide 8.

Applying general procedures, linear decapeptide H-Ala-Lys(*N*-Boc)-Ala-Pro-Gly-Ala-Ala-Lys(*N*-4-Pentynoic acid)-Pro-Gly-OH (117 mg, 112 μmol) was assembled using modified amino acid Fmoc-Lys[*N*-4-Pentynoic acid]-OH **7** with 95% yield. After cleavage from the resin, the cyclization afforded cyclodecapeptide **8** as a white powder (114 mg, 98%). **Mass spectrum** (ES-MS, positive mode) calc for C₄₈H₇₈N₁₂O₁₃ 1029.19, found m/z: 929.80 ([M-Boc+H]⁺, 100%), 1029.75 (22, [M+H]⁺).

ESI-MS analysis of crude peptide 8



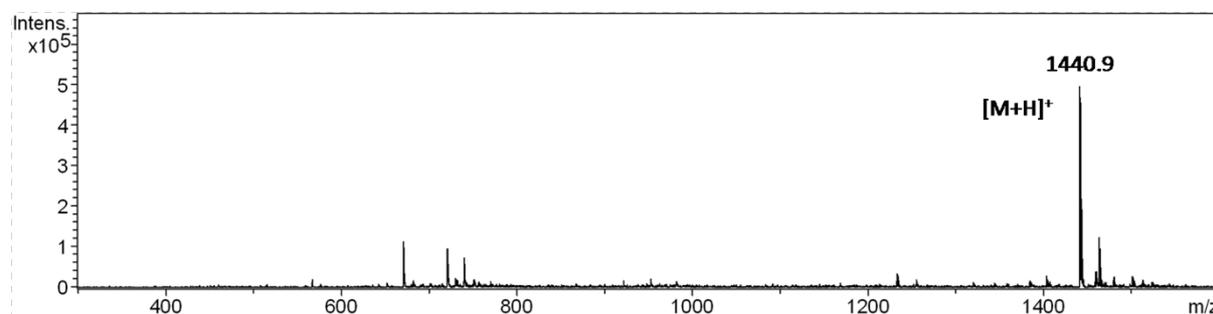
RP-HPLC profile of crude peptide 8 (214 nm)



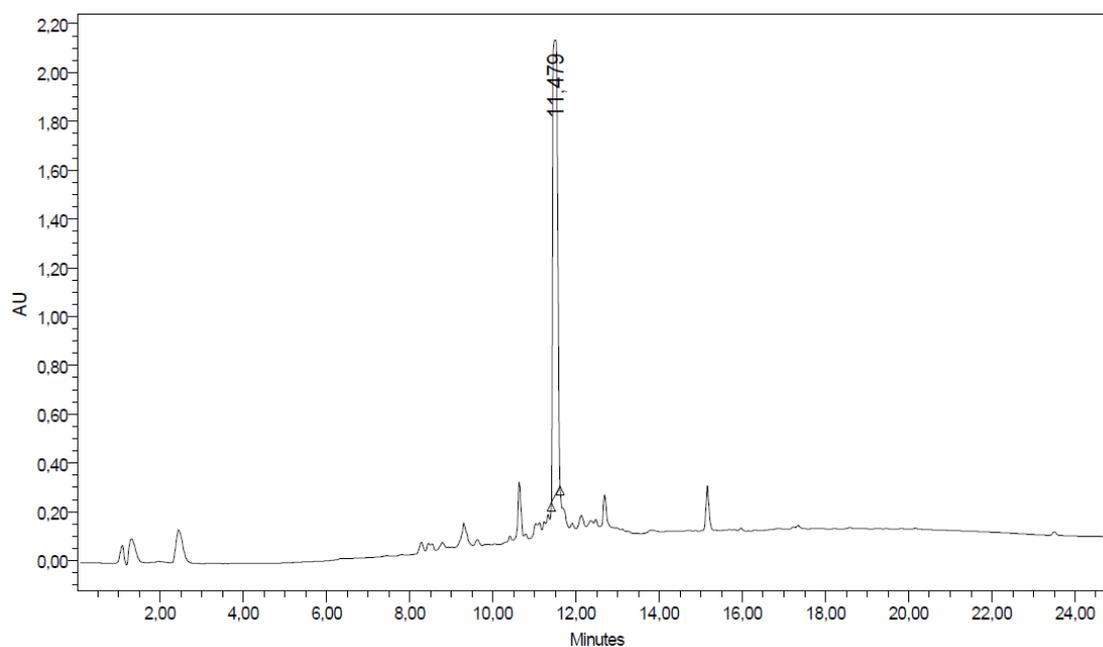
Cyclodecapeptide 9.

Applying general procedures, suitable linear decapeptide H-Lys(*N*-4-Pentynoic acid)-Lys(*N*-Boc)-Lys(*N*-4-Pentynoic acid)-Pro-Gly-Lys(*N*-4-Pentynoic acid)-Ala-Lys(*N*-4-Pentynoic acid)-Pro-Gly-OH (249 mg, 0.17 mmol) was assembled using modified amino acid Fmoc-Lys[*N*-4-Pentynoic acid]-OH **7** with 94% yield. After cleavage from the resin, the cyclization afforded cyclodecapeptide **9** as a white powder (241 mg, 98%). **Mass spectrum** (ES-MS, positive mode) calc for C₇₂H₁₀₉N₁₅O₁₆ 1439.82, found m/z: 1440.9 ([M+H]⁺, 100%).

ESI-MS analysis of crude peptide **9**



RP-HPLC profile of crude peptide **9** (214 nm)



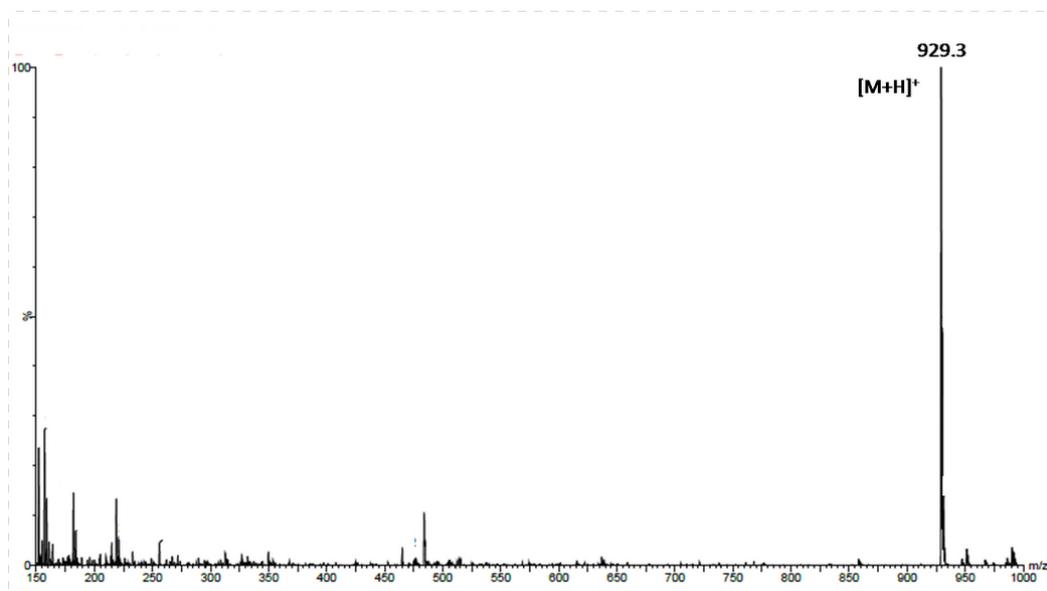
General procedure A for Boc-protecting group removal.

Cyclodecapeptide derivatives featuring an amine group protected by a tert-butoxycarbonyl (Boc) were dissolved in a TFA/CH₂Cl₂ (1/1) solution. The reaction mixtures were stirred for 2 h at room temperature. The residue were concentrated under reduced pressure, triturated and washed several times with Et₂O to afford the deprotected cyclodecapeptides. When necessary, the crude was subjected to preparative RP-HPLC with a step gradient of ACN in water containing 0.1% TFA to afford pure cyclodecapeptides derivatives.

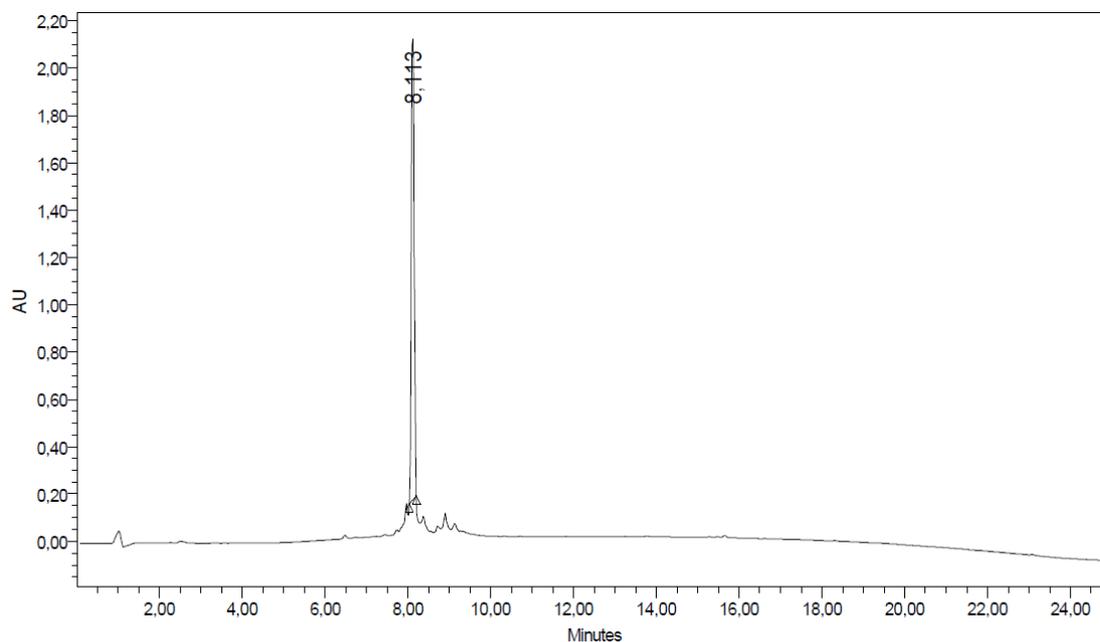
Cyclodecapeptide 10.

General procedure A was applied to peptide **8** (114 mg, 110 μmol) in TFA/CH₂Cl₂ (1/1) solution (12 mL) to afford peptide **10** as a white powder (100 mg, 97%). **Mass spectrum** (ESI-MS, positive mode) calc for C₄₃H₆₇N₁₂O₁₁ 928.07, found m/z: 929.3 ([M+H]⁺, 100%).

ESI-MS analysis of crude peptide 10



RP-HPLC profile of crude peptide 10 (214 nm)



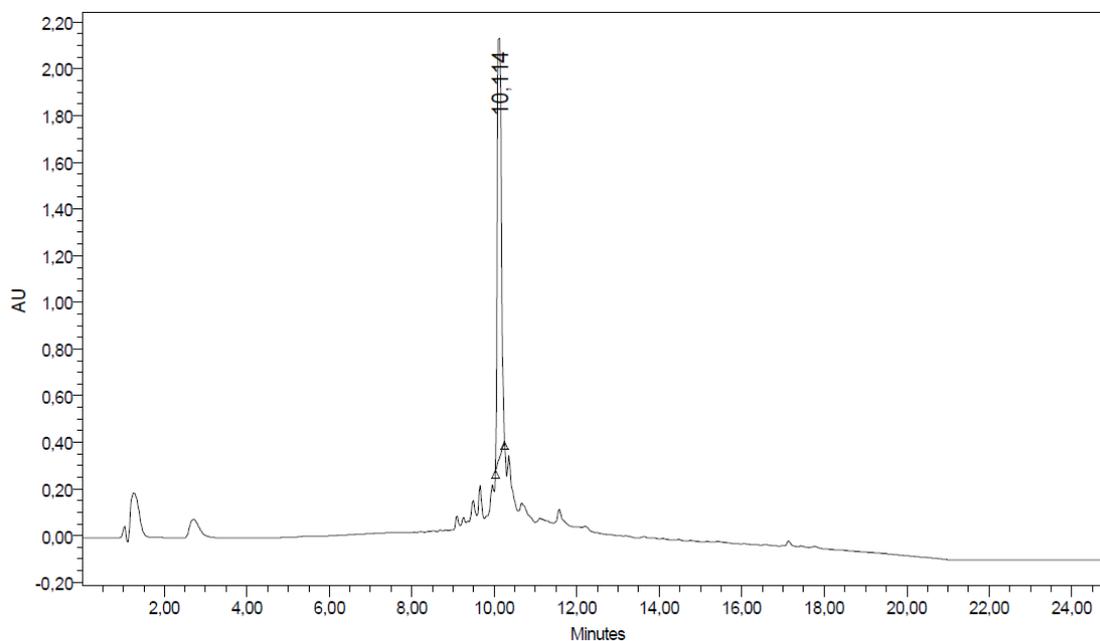
Cyclodecapeptide 11.

General procedure **A** was applied to peptide **9** (240 mg, 17 μ mol) in TFA/CH₂Cl₂ (1/1) solution (20 mL) to afford peptide **11** as a white powder (217 mg, 97%). **Mass spectrum** (ESI-MS, positive mode) calc for C₆₇H₁₀₀N₁₅O₁₄ 1339.82, found m/z: 1340.8 ([M+H]⁺, 100%).

ESI-MS analysis of crude peptide 11



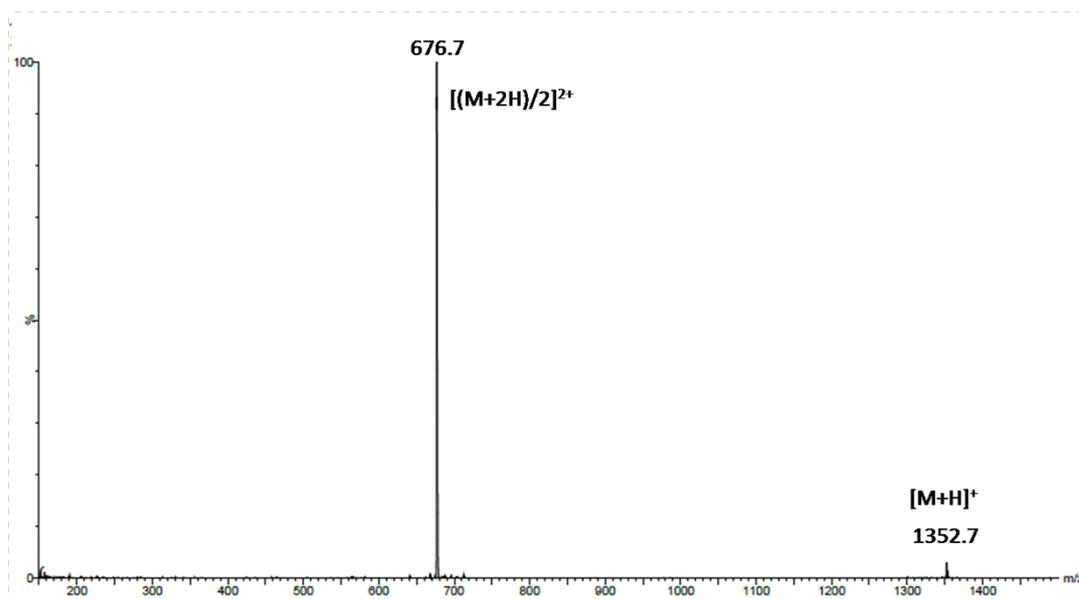
RP-HPLC profile of crude peptide 11 (214 nm)



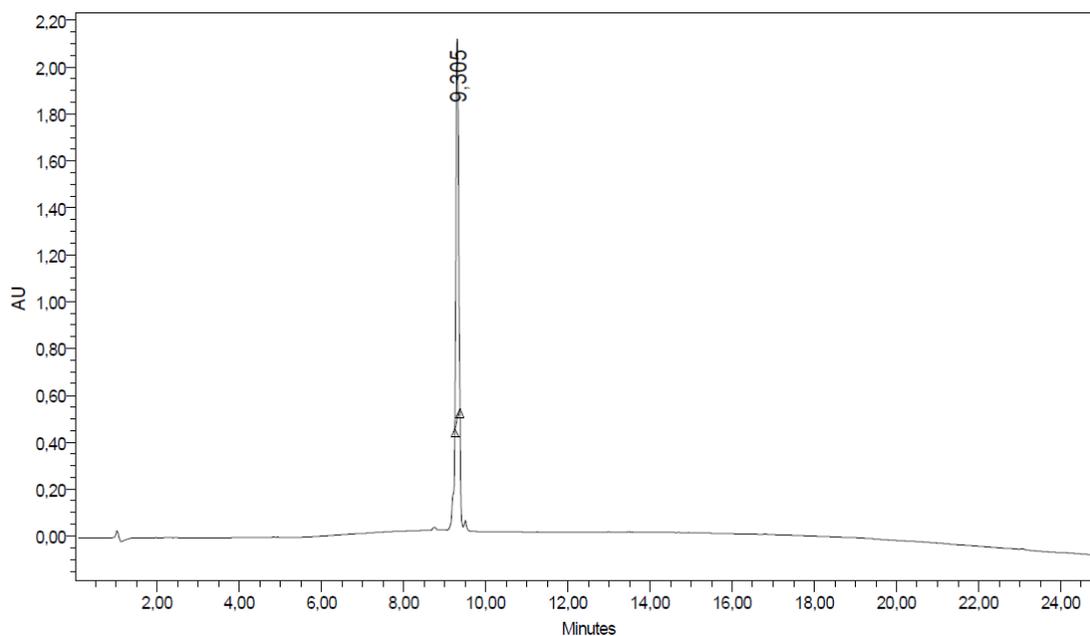
Compound 1.

General procedure **A** was applied to peptide **12** (36 mg, 25 μmol) in TFA/ CH_2Cl_2 (1/1) solution (5 mL) to afford peptide **1** as a white powder (33 mg, 98%). **Mass spectrum** (ESI-MS, positive mode) calc for $\text{C}_{62}\text{H}_{105}\text{N}_{13}\text{O}_{20}$ 1351.76, found m/z : 676.7 ($[(\text{M}+2\text{H})/2]^{2+}$, 100%), 1352.7 (3, $[\text{M}+\text{H}]^+$).

ESI-MS analysis of crude compound 1



RP-HPLC profile of crude compound 1 (214 nm)

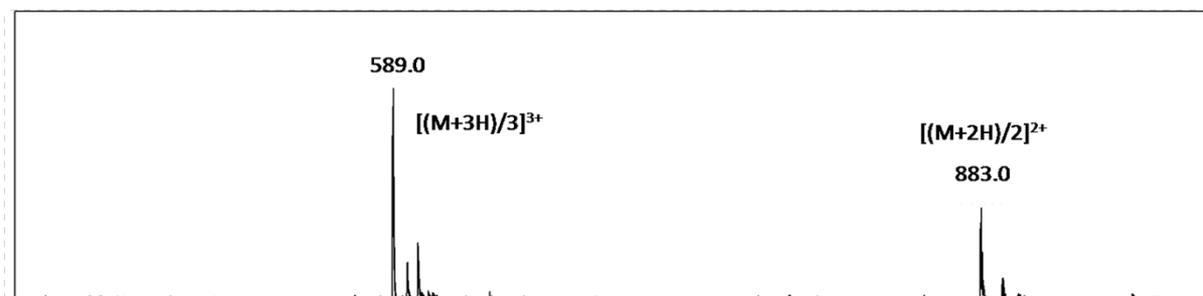


Compound 2.

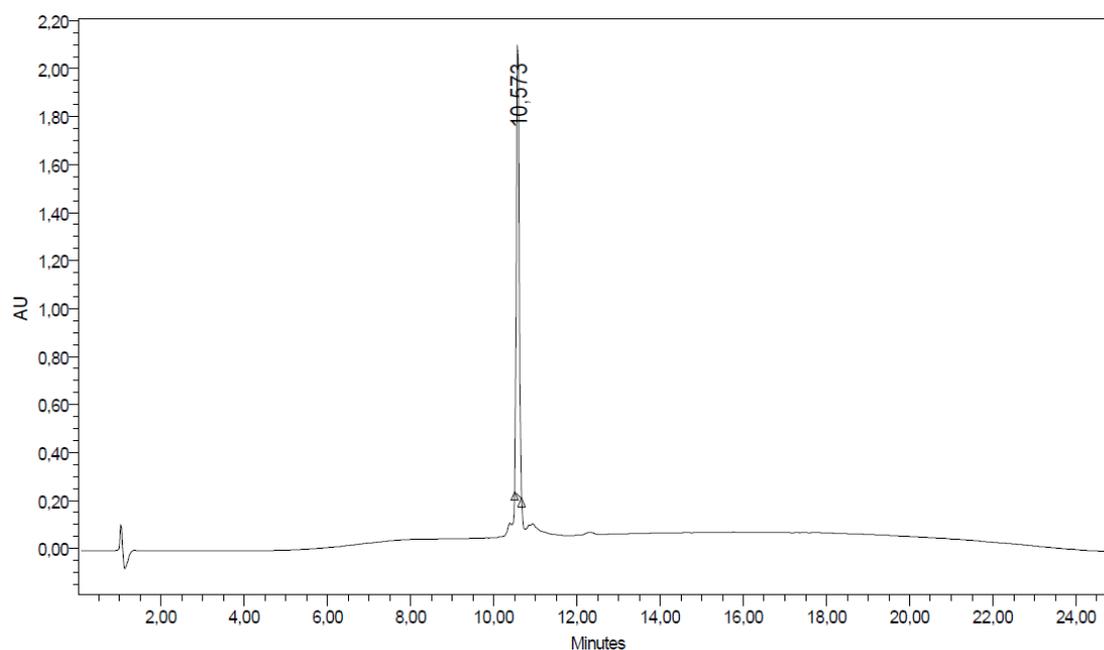
General procedure **A** was applied to peptide **13** (14 mg, 7.5 μ mol) in TFA/ CH_2Cl_2 (1/1) solution (3 mL) to afford peptide **2** as a white powder (13 mg, 97%). **Mass spectrum** (ES-

MS, positive mode) calc for $C_{86}H_{138}N_{16}O_{23}$ 1763.01, found m/z: 589.0 ($[(M+3H)/3]^{3+}$, 100%), 883.0 (44, $[(M+2H)/2]^{2+}$).

ESI-MS analysis of crude compound 2



RP-HPLC profile of crude compound 2 (214 nm)



General procedure B for HOOC-EG₈-NHBoc coupling.

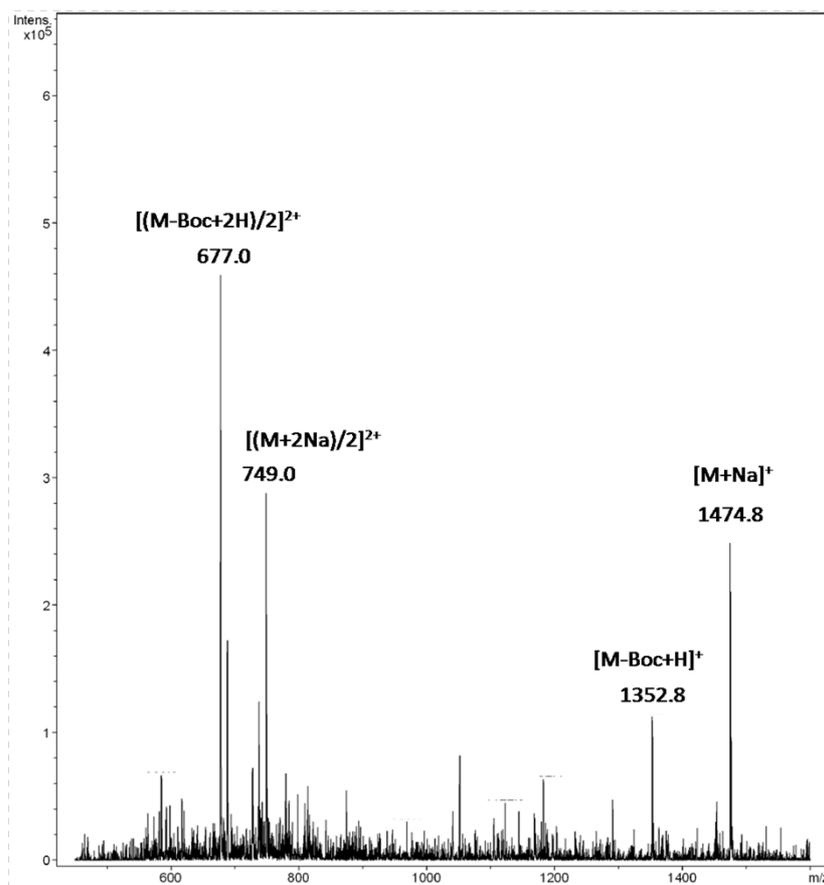
To a solution of amino featuring cyclodecapeptide (1 equiv) and HOOC-EG₈-NHBoc (2 equiv) in DMF was added PyBOP (2 equiv). The pH of the solution was adjusted to 8-9 by addition of DIPEA. The resulting mixture was stirred for 1h at room temperature and

concentrated under reduced pressure. The residue was triturated and washed in Et₂O several times to afford cyclodecapeptides **12** or **13**.

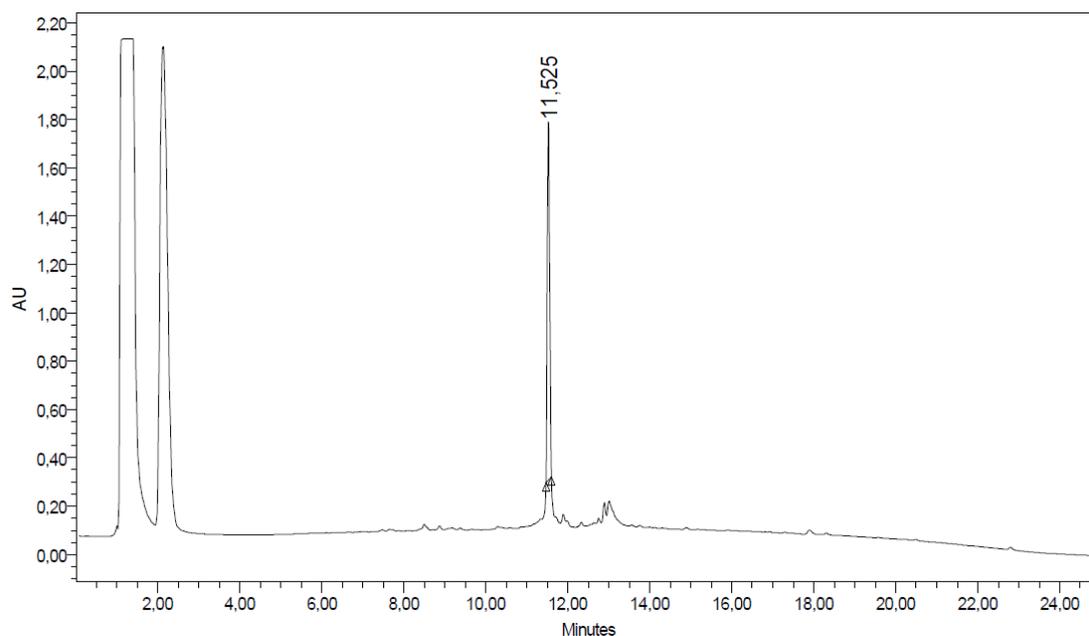
Compound 12.

General procedure **B** was applied to peptide **10** (25 mg, 27 μmol), HOOC-EG₈-NHBoc (29 mg, 54 μmol) and PyBOP (28 mg, 54 μmol) in DMF (3 mL) to afford peptide **12** as a white powder (36 mg, 93%). **Mass spectrum** (ES-MS, positive mode) calc for C₆₇H₁₁₃N₁₃O₂₂ 1451.81, found m/z: 677.0 ([M-Boc+2H]/2)²⁺, 100%), 749.0 (61, [(M+2Na)/2]²⁺), 1474.8 (54, [M+Na]⁺), 1352.8 (24, [M-Boc+H]⁺).

ESI-MS analysis of crude compound 12



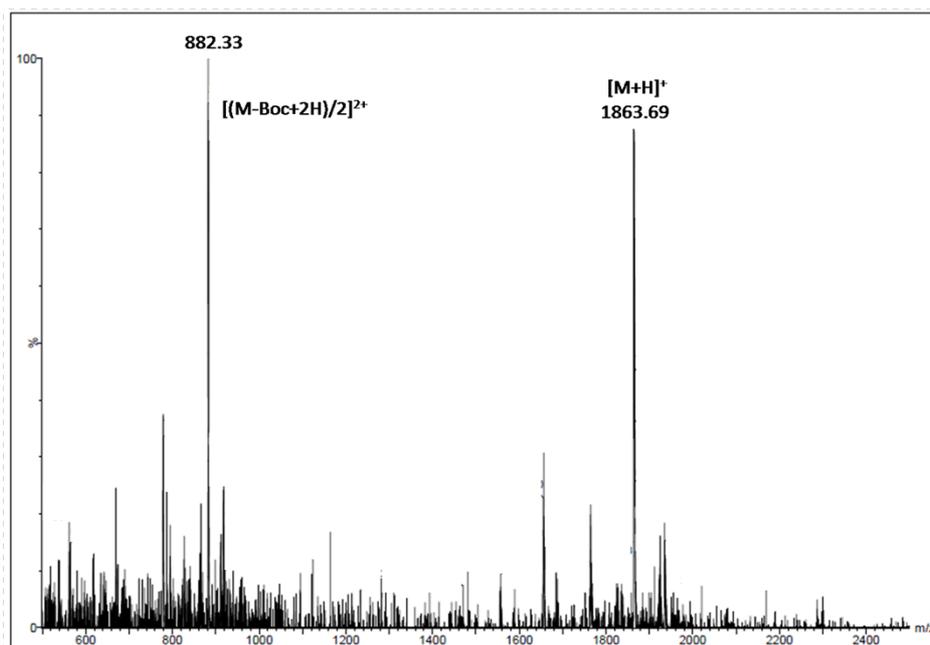
RP-HPLC profile of crude compound 12 (214 nm)



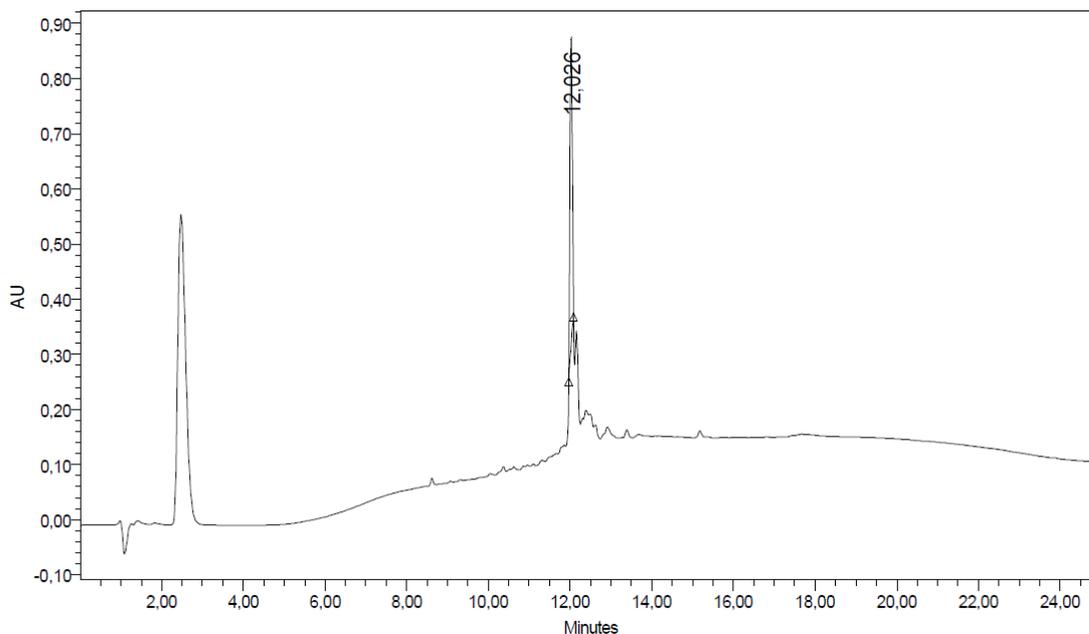
Compound 13.

General procedure **B** was applied to peptide **11** (25 mg, 19 μmol), HOOC-EG₈-NHBoc (20 mg, 37 μmol) and PyBOP (19 mg, 37 μmol) in DMF (3 mL) to afford peptide **13** as a white powder (24 mg, 88%). **Mass spectrum** (ES-MS, positive mode) calc for C₉₁H₁₄₆N₁₆O₂₅ 1863.06, found m/z: 882.33 ($[(M-\text{Boc}+2\text{H})/2]^{2+}$, 100%), 1863.69 (88, $[M+\text{H}]^+$).

ESI-MS analysis of crude compound 13



RP-HPLC profile of crude compound 13 (214 nm)



General procedure C for CuAAc.

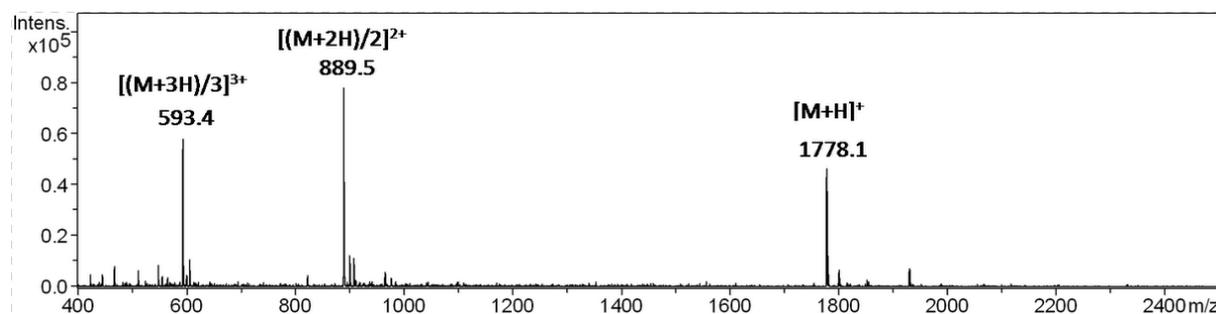
To a solution of alkyne featuring cyclodecapeptide **1** or **2** (1 equiv) and adamantyl azide fragment AD-EG₄-N₃ **6** (respectively 2 or 6 equiv) in degassed DMF under nitrogen atmosphere, was added a solution of CuSO₄ (1 equiv) and THPTA (3 equiv) in degassed phosphate buffer (100 mM, pH 7.4). A solution of sodium ascorbate (10 equiv) in degassed phosphate buffer was added to the medium. The resulting mixture was heated at 45°C for 2h30. After completion, the crude was directly subjected to preparative RP-HPLC with a step gradient of ACN in water containing 0.1% TFA to afford pure cyclodecapeptides derivatives **3** or **4**.

Compound 3.

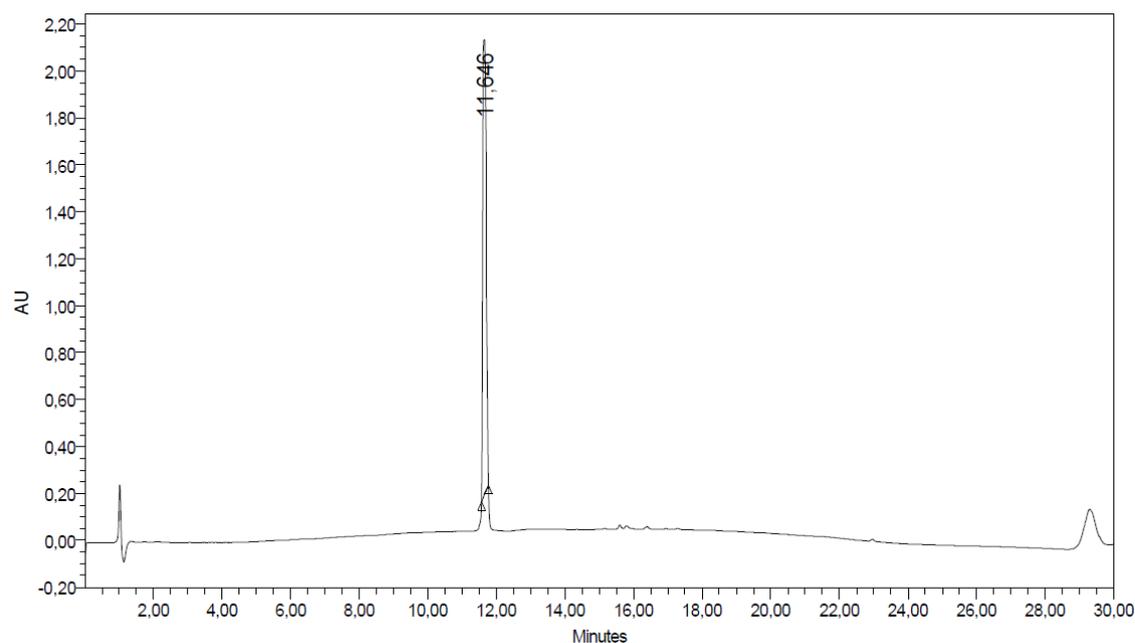
General procedure C was applied to peptide **1** (19 mg, 14 μmol) and azide fragment AD-EG₄-N₃ **6** (12 mg, 28 μmol) **2** in DMF (400 μL) and phosphate buffer (600 μL) to afford peptide **3** as a white powder (19 mg, 72%). **Mass spectrum** (ES-MS, positive mode) calc for

$C_{83}H_{141}N_{17}O_{25}$ 1776.03, found m/z : 889.5 ($[(M+2H)/2]^{2+}$, 100%), 593.4 (75, $[(M+3H)/3]^{3+}$), 1778.1 (63, $[M+H]^+$).

ESI-MS analysis of compound 3



RP-HPLC profile of compound 3 (214 nm)

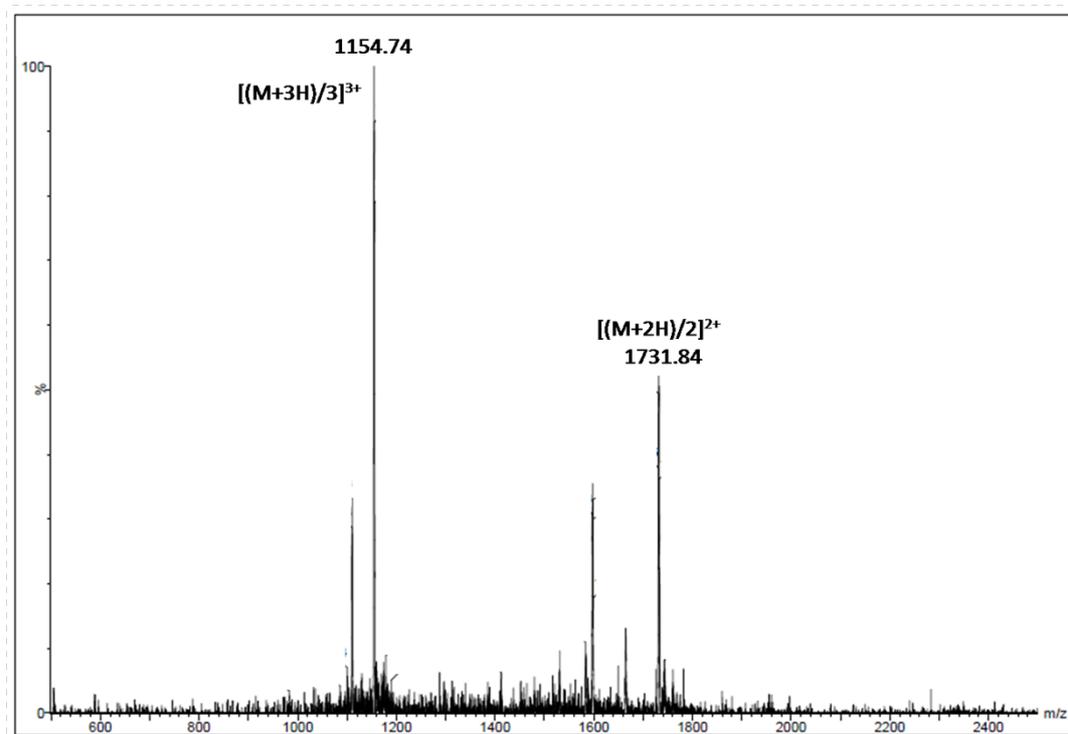


Compound 4.

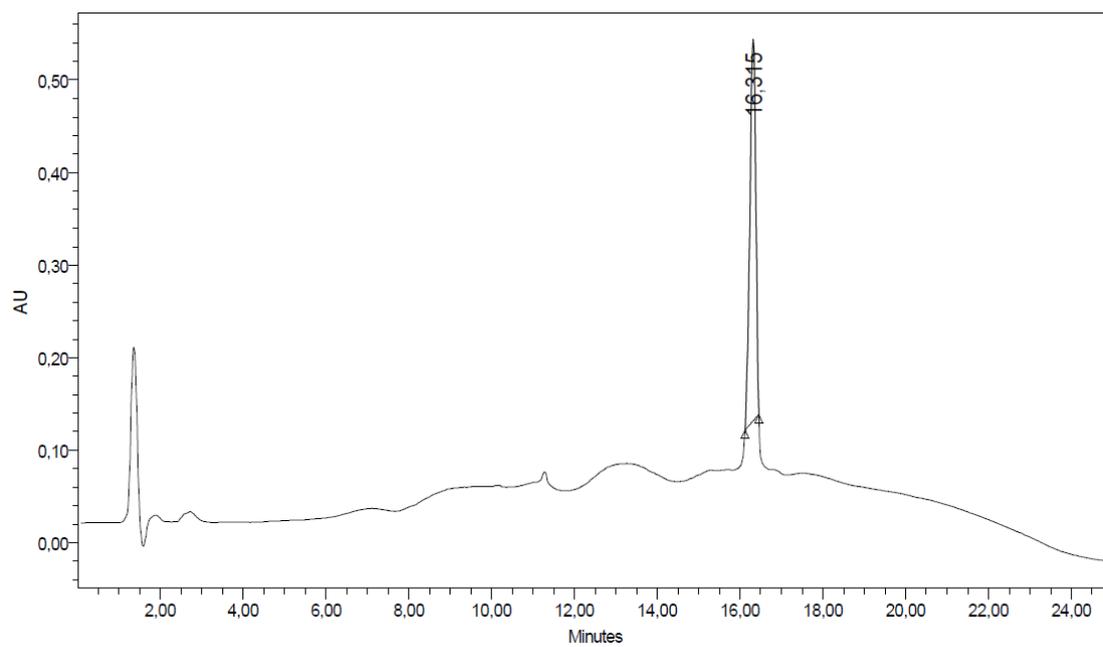
General procedure C was applied to peptide 2 (1.3 mg, 0.7 μ mol) and azide fragment AD-EG₄-N₃ 6 (1.8 mg, 4.2 μ mol) in DMF (40 μ L) and phosphate buffer (100 μ L) to afford peptide 4 as a white powder (1.7 mg, 68%). **Mass spectrum** (ES-MS, positive mode) calc for

$C_{170}H_{284}N_{32}O_{43}$ 3461.4, found m/z : 1154.74 ($[(M+3H)/3]^{3+}$, 100%), 1731.84 (53, $[(M+2H)/2]^{2+}$).

ESI-MS analysis of compound 4



RP-HPLC profile of compound 4 (214 nm)



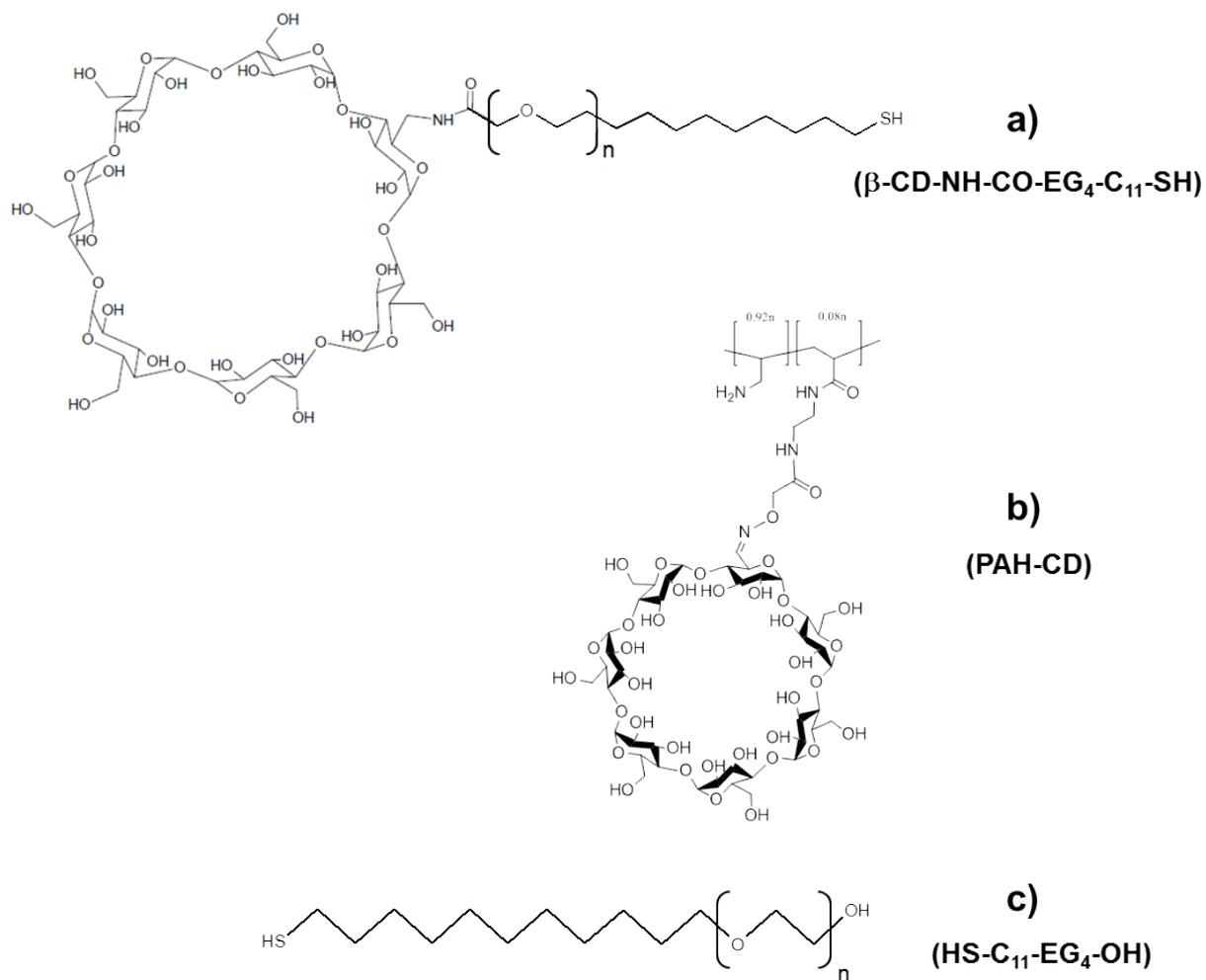


Figure S1.

Chemical structure of the monolayers compounds. a) corresponds to the β -CD thiol or HS-C₁₁H₂₂-EG₄-CONH-CD, b) corresponds to the formula of the PAH-CD and c) is chemical structure of the dilution thiol or HS-C₁₁H₂₂-EG₄-OH.

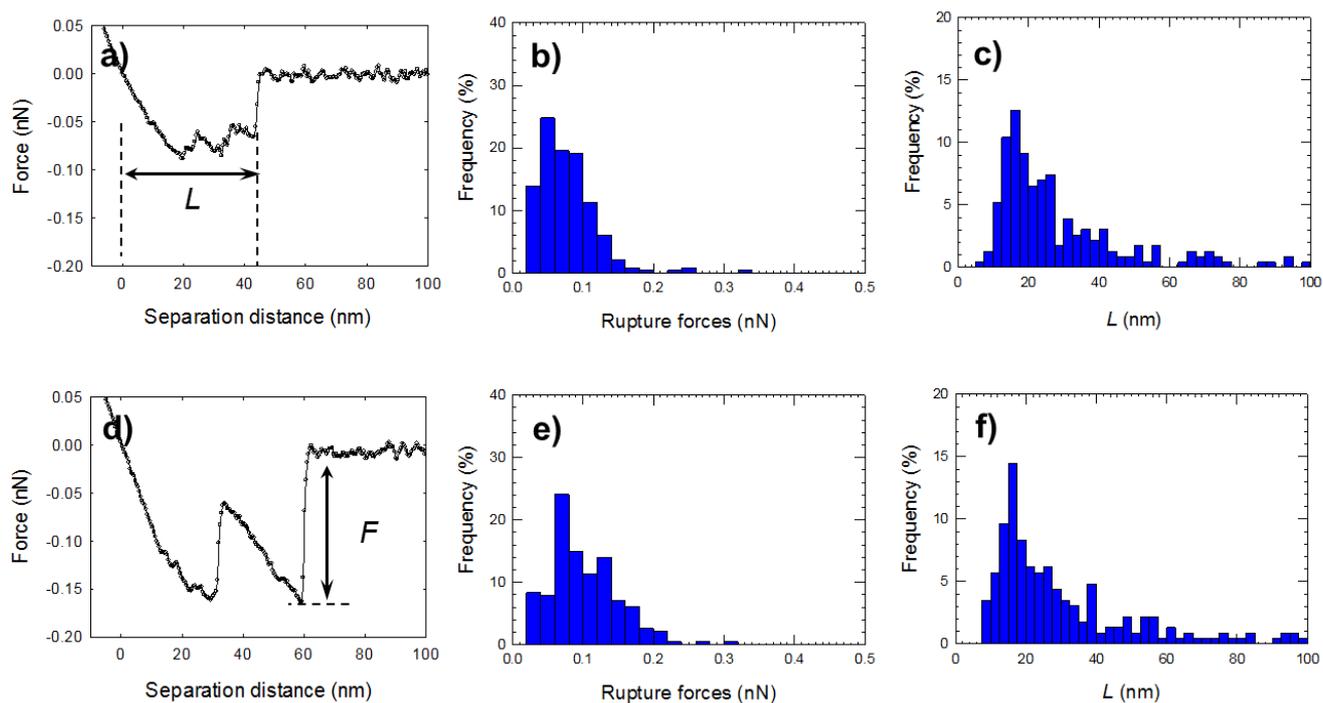


Figure S2.

a, b, c) Typical retraction force curve and the corresponding statistical distributions of the rupture forces and ruptures distance for experiments performed by Force Spectroscopy with the scaffold 1AD grafted to the AFM-tip and over a SAM-CD(NP) sample. d, e, f) Typical retraction force curve and the corresponding statistical distributions of the rupture forces and ruptures distance for experiments performed by Force Spectroscopy with the scaffold 4AD grafted to the AFM-tip and over a SAM-CD(NP) sample.

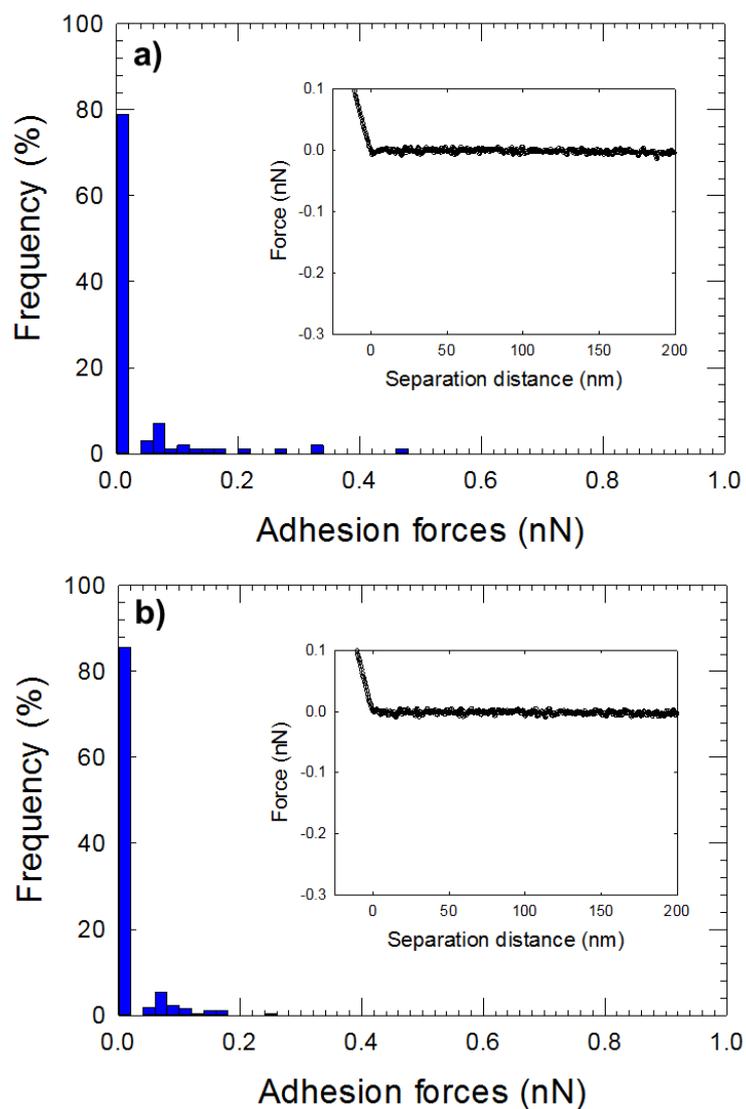


Figure S3.

a) Statistical distribution of the adhesion forces distances and a representative retraction force curve as inset carried out by Single Molecule Force Spectroscopy onto the thiolated PEG-OH monolayer over $5\mu\text{m}\times 5\mu\text{m}$ surface area in aqueous medium at 37°C with the monovalent adamantyl scaffold grafted to the AFM-tip. b) Statistical distribution of the adhesion forces distances and a representative retraction force curve as inset carried out by Single Molecule Force Spectroscopy onto the thiolated PEG-OH monolayer over $5\mu\text{m}\times 5\mu\text{m}$ surface area in aqueous medium at 37°C with the tetravalent adamantyl scaffold grafted to the AFM-tip. All

these force measurements were performed with an approach rate of 1000 nm/s, a loading force of 250 pN, a retraction rate of 1000 nm/s and a residence time of 10s.