Convenient Solid-Phase Synthesis of Ureido-Pyrimidinone Modified Peptides


Keywords: Solid-phase synthesis / Modular approach / Peptides / Ureido-pyrimidinone / Supramolecular chemistry / Quadruple hydrogen bonding / Biomaterials

Peptides have been modified with quadruple hydrogen bonding ureido-pyrimidinone moieties to be applied in supramolecular architectures for biomedical applications. A convenient solid-phase synthesis method was developed to functionalize peptide sequences with ureido-pyrimidinone units. Two different ureido-pyrimidinone synthons were used: based on a carbonyldiimidazole-activated amine or on an isocyanate functionality. Oligopeptides were functionalized on the solid support using two coupling strategies: on the N-terminus or selectively on the ε-position of a C-terminal lysine. Several peptides were modified to show the generality of the approach, varying from cell adhesion sequences, to collagen binding peptides and cysteine derivatives which can be used for native chemical ligation.

Introduction

In recent research the importance of peptides in supramolecular architectures has been demonstrated. Many examples of oligo-peptide-based self-assembled aggregates have been disclosed in which hybrid conjugates are synthesized by the combination of peptide sequences with for example all kinds of polymers,[1,2] long alkyl chains or phospholipids.[3] These conjugates have great potential, especially in the biomedical field.

Various amphiphilic peptides have been studied by varying the length of the N-terminal alkyl tail to the GANPNAAG (Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly) sequence which is known for its preferred β-hairpin conformation.[4–6] These GANPNAAG-alkyl conjugates were entirely synthesized using solid-phase techniques. A polymer-peptide conjugate that formed spherical aggregates was also completely synthesized using solid-phase chemistry.[5] First an amine functionalized polystyrene polymer was coupled to a resin, after which the GANPNAAG peptide was built up.[5]

Polymer-peptide conjugates of poly(ethylene glycol)[7] or poly(n-butyl acrylate)[8] with strong β-sheet-forming peptides have been shown to assemble into tape structures. The peptide sequence was built up on a PEO-conjugated solid support[7] or directly on the resin after which the poly(n-butyl acrylate) was attached.[8]

Peptide nanotubes were made by non-covalent assembly using cyclic peptides, synthesized on the solid support.[9] The ion-channel properties of these tubes could be tuned. Also, charge transfer could be regulated using nanotubes forming cyclic peptides with 1,4,5,8-naphthalenetetracarboxylic acid diimide side chains.[10] The cyclic peptides were functionalized in solution or on the solid support. Other supramolecular architectures have been made based on small liquid-crystalline oligo-peptide derivatives. Oligo(glutamic acid) derivatives, synthesized in solution, have been studied with respect to their liquid-crystalline behaviour.[11] Furthermore, low molecular weight hydrogelators consisting of 1,3,5-cyclohexyl-tricarboxamidophenylalanine have been investigated.[12] The stability of the hydrogelators have been studied using various amino acid based substituents connected to the core.[13] In this case, solution phase chemistry was used to synthesize these hydrogelators.

Stupp et al. showed that functional threedimensional nanofibers are formed by peptide-based amphiphilic (PA) molecules, constructed of at least three important regions; a long alkyl tail, a linker region consisting of amino acids and the bioactive part.[14,15] Cysteine residues could be built in between the alkyl tail and bioactive part, which can be oxidized to form disulfide bonds resulting in polymerization of the supramolecular structure.[14] The peptide amphiphiles (PA) were entirely synthesized on the resin.

Recently, it is shown that functional materials can be prepared without tedious synthetic procedures but simply by assembly of supramolecular units.[16] Self-complementary quadruple hydrogen bonding 2-ureido-4(1H)-pyrimidinone (UPy) moieties were applied for this non-covalent, modular

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Convenient Solid-Phase Synthesis of Modified Peptides

Figure 1. A. UPy units dimerize through fourfold hydrogen bonding. UPy peptides B. Based on the GRGDS cell adhesion peptide sequence, modified by method 1, and C. Based on the HVWMQAPGGKG collagen binding peptide sequence, functionalized by method 2.

approach (Figure 1).[17–20] Supramolecular materials can be made using UPy-functionalized polymers in which the repeating units are held together by strong non-covalent hydrogen-bonding interactions ($K_F = 10^6–10^7 \text{ L mol}^{-1}$ in organic solvents). [17–20] These new materials show mechanical properties similar to conventional polymers, without losing their reversible nature. For introduction of bioactive components in this dynamic system, peptide sequences were modified with UPy moieties. The creation of a toolbox with different UPy-modified polymers and bioactive molecules allows for the off-the-shelf assembly of bioactive biomaterials for tissue engineering purposes.[16]

In this paper we present two methods to conveniently modify peptides with UPy units entirely performed on the solid support. Several peptide sequences were modified with a variety of UPy units (see examples in Figure 1; Table 1). Method 1 was used to functionalize peptides with UPy moieties on the N-terminus. Cell adhesion signals such as the GRGDS (Gly-Arg-Gly-Asp-Ser) peptides 1–4, and the synergistic PHSRN (Pro-His-Ser-Arg-Asn) signal 7 were synthesized according to this method.[21–24] As control sequences, the $\alpha$-enantiomer of GRGDS 5 and the scrambled GRDGS peptide 6 were made.[22] These N-terminal UPy-modified peptides can be used for cell adhesion experiments.[16]

A free N-terminal amine was necessary for the functioning of the peptides that were produced by method 2. The HVWMQAPGG (His-Val-Trp-Met-Gln-Ala-Pro-Gly-Gly) peptide 8, which has been found to bind to collagen type I using phage display techniques, was functionalized with UPy moieties (unpublished results). Also, the cysteine-containing peptides CGG (Cys-Gly-Gly) 9–10 were modified with UPy groups to be applied in native chemical ligation reactions[25] to ligate to thioester-containing bioactive molecules, such as peptides and proteins.[26] It is proposed that

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**Table 1. A convenient solid-phase synthesis approach to UPy-modified peptides.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Peptide sequence</th>
<th>UPy</th>
<th>Coupling method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GRGDS</td>
<td>11</td>
<td>N-terminus</td>
</tr>
<tr>
<td>2</td>
<td>GRGDS</td>
<td>12</td>
<td>N-terminus</td>
</tr>
<tr>
<td>3</td>
<td>GRGDS</td>
<td>13</td>
<td>N-terminus</td>
</tr>
<tr>
<td>4</td>
<td>GRGDS</td>
<td>13[a]</td>
<td>N-terminus</td>
</tr>
<tr>
<td>5</td>
<td>$\alpha$-GRGDS</td>
<td>11</td>
<td>N-terminus</td>
</tr>
<tr>
<td>6</td>
<td>GRDGS</td>
<td>11</td>
<td>N-terminus</td>
</tr>
<tr>
<td>7</td>
<td>PHSRN</td>
<td>13</td>
<td>N-terminus</td>
</tr>
<tr>
<td>8</td>
<td>HVWMQAPGGKG</td>
<td>13</td>
<td>Lys</td>
</tr>
<tr>
<td>9</td>
<td>CGGKG</td>
<td>11</td>
<td>Lys</td>
</tr>
<tr>
<td>10</td>
<td>CGGKG</td>
<td>13</td>
<td>Lys</td>
</tr>
</tbody>
</table>

[a] An additional pentylamide spacer was introduced.
these UPy peptides are able to assemble with UPy-functionalized polymers.\(^\text{[16]}\)

**Results and Discussion**

Two different UPy synthons were designed for this convenient solid-phase synthesis approach; based on a carbonyldiimidazole-activated amine precursor UPy\(^\text{[27]}\) \(11\)–\(12\) or on an isocyanate functionality\(^\text{[18]}\) \(13\) (Figure 2). The carbonyldiimidazole-activated amine precursor UPy was used with two different substituents at the \(\varepsilon\)-position of the pyrimidinone moiety; a methyl group\(^\text{[28]}\) \(11\) or an ethylpenty\(^\text{[27]}\) substituent \(12\). Both types of UPy synthons \(11\)–\(13\) were used in both coupling methods, that comprise of either N-terminal UPy functionalization of the peptide (method 1; see example Figure 1, B) or UPy coupling to the \(\varepsilon\)-position of the lysine next to the C-terminal glycine (method 2; see example Figure 1, C).

![Figure 2](image-url) The UPy synthons used for peptide modification.

Peptides (Table 1; \(1\)–\(10\)) were synthesized on the solid support using standard protecting and deprotecting Fmoc (fluorenlymethoxy carbonyl) chemistry\(^\text{[29]}\). The first method of functionalizing the peptide on the solid support was by the reaction of the N-terminal amine with any of the UPy synthons \(11\)–\(13\) (Table 1; \(1\)–\(7\)). The synthesis was performed according to Scheme 1, resulting in the UPy-modified GRGDS peptides \(1\)–\(4\) with different UPy moieties in the case of \(1\)–\(2\) and different alkyl spacers connecting the UPy group and the GRGDS peptide in the case of \(3\)–\(4\). First, the Fmoc-protecting group was removed from the N-terminal glycine using 20\% piperidine in dimethylformamide (DMF). Then the reaction of the free amine of the protected GRGDS peptide with the UPy synthon was performed. The reactions of the free amine with \(11\) (6 equiv.) and \(12\) (5 equiv.) were conducted in DMF at 50 °C for 16 h, resulting in the resin-bound protected \(1\) and \(2\), respectively. The free amine was treated with \(13\) (12 equiv.) in chloroform at ambient temperature for 16 h, yielding the protected compound \(3\). The excess of UPy synthon \(11\) was removed with acidic water, reactant \(12\) was washed away with DMF and compound \(13\) was removed by washing with chloroform. The UPy peptides \(1\), \(2\) and \(3\) were obtained in pure form after disconnection from the support and removal of the protecting groups with 95\% trifluoroacetic acid (TFA) and 5\% water, with reasonable yields of 72\%, 56\% and 55\%, respectively. For the synthesis of \(4\) an extra step was necessary to incorporate an additional pen-talamide spacer. Fmoc-protected aminoheptaneoic acid (4 equiv.) was coupled to the N-terminal free amine using 1-hydroxybenzotriazole (HOBt; 3.6 equiv.) and diisopropylcarbodiimide (DIPCII; 3.3 equiv.) as coupling reagents in DMF for 1 h at ambient temperature. The removal of the Fmoc group was again executed with 20\% piperidine in DMF for 20 min. The created amine was treated with \(13\) (10 equiv.) in chloroform for 16 h at ambient temperature, as described above. The excess of \(13\) was removed by washing with chloroform, yielding the protected \(4\) on the resin. The peptide was cleaved from the support using TFA as described above, resulting in pure \(4\) in a 57\% yield. The control UPy peptides were synthesized in a similar way as \(1\), obtaining pure \(5\) and pure \(6\) in yields of 75\% and 50\%, respectively. The synthesis of the synergistic UPy peptide \(7\) was performed according to the same method as used for \(3\). UPy peptide \(7\) was purified using preparative reversed phase liquid chromatography (RPLC) with a yield of 47\%.

In the second method the UPy synthon was coupled to the \(\varepsilon\)-amine of the lysine next to the C-terminal glycine to introduce an N-terminal amine (Table 1; \(8\)–\(10\)). A C-terminal glycine and lysine were only incorporated for synthetic reasons. The synthesis was performed according to Scheme 2, resulting in HVWMQAPGGK(-UPy-hexyl-urea) G \(8\). The \(\varepsilon\)-amine of the lysine was protected with the quasi-orthogonal protecting, 4-methyltrityl (Mtt) group. This Mtt group was selectively removed with 5\% TFA, 5\% triisopropylsilane (TIS) in dichloromethane (DCM) for 15 min. The resin was washed with 5\% diisopropylethylamine (DIPEA) in DCM. The UPy functionalization was performed using \(13\) (10 equiv.) in chloroform with DIPEA (1 equiv.) for 7 h at ambient temperature. The excess of \(13\) was removed with acidic water, resulting in the protected UPy peptide \(8\) on the resin. Then, the Fmoc group at the N-terminal histidine was removed with 20\% piperidine in DMF for 1 h. After that, the UPy-peptide was cleaved from the solid support and deprotected using 95\% TFA, 2.5\% water and 2.5\% TIS, as scavenger, for 4 h. The purity was estimated to be 40\%. Compound \(8\) was purified using preparative RPLC, resulting in 16\% of \(8\). The cysteine-containing peptides \(9\)–\(10\) were synthesized in a similar way as \(8\), using UPy synthon \(11\) for the synthesis of \(9\) and synthon \(13\) for the synthesis of \(10\). Pure peptides \(9\)–\(10\) were obtained after cleaving from the solid support with yields of 46\% and 52\%, respectively.

UPy peptides \(1\)–\(10\) were characterized with NMR, infrared (IR) spectroscopy, RPLC and mass spectrometry (MS). Unfortunately, the IR vibrations, characteristic for the UPy moiety (between approximately 1700 and 1520 cm\(^{-1}\)), are undistinguishable from the strong amide I (at 1646 cm\(^{-1}\))
Scheme 1. Synthesis of the UPy-modified cell adhesion peptides 1, 2, 3, 4 using N-terminal coupling. i = 20% piperidine, DMF, 20 min; 1. ii = 11 (6 equiv.), DMF, 16 h, 50 °C; iii = 95% TFA, 5% H₂O, 4 h; γ = 72%. 2. ii = 12 (5 equiv.), DMF, 16 h, 50 °C; iii = 95% TFA, 5% H₂O, 6 h; γ = 58%. 3. ii = 13 (12 equiv.), CHCl₃, 16 h, 21 °C; iii = 95% TFA, 5% H₂O, 6 h; γ = 55%. 4. ii = Fmoc-C₅H₁₀-COOH (4 equiv.), HOBT (3.6 equiv.), DIPCID (3.3 equiv.), DMF, 1 h, 21 °C; iii = 20% piperidine, DMF, 20 min; iv = 13 (10 equiv.), CHCl₃, 16 h, 21 °C; v = 95% TFA, 5% H₂O, 5 h; γ = 57%. Fmoc = 9-fluorenlymethoxycarbonyl, OrBu = tert-butoxy, tBu = tert-butyl, Pmc = 2,2,5,7,8-pentamethylenrom-6-sulfonyl.
and amide II (at 1539 cm$^{-1}$) vibrations present in the peptides. $^1$H NMR in D$_2$O/[D$_3$]ACN clearly shows the alkylidene protons of the UPy unit for 1–10, at approximately 5.98 ppm, demonstrating the presence of the UPy moiety. Using RPLC-MS only one (major) peak was found in the chromatograms for all UPy peptides 1–10 corresponding to the correct molecular mass (see also Supporting Information).
Conclusions

Several biologically relevant peptides have been modified with UPy moieties using solid-phase peptide synthesis employing two different UPy synthons; based on a carbonyl-dimidazole-activated amine or on an isocyanate group. The peptides were functionalized on the N-terminus or on the ε-amine of the lysine next to the C-terminal glycine, resulting in N-terminal or C-terminal UPy-functionalized peptides. The synthesized GRGDS and PHSRN peptides could for instance be used in order to make bioactive UPy-containing polymers.[16] The collagen binding peptide might be applied for deposition of collagen on a particular place in a scaffold. Native chemical ligation can be used to modify proteins with UPy groups using the cysteine-containing UPy peptides.

The approach to modification of peptides with ureido-pyrimidinone moieties is very promising because in this way easy functionalization of several peptides is possible, which may be used for biomedical applications, such as biomaterials for tissue engineering. The major advantage of this easy functionalization method is the possibility, to not only change the amount of bioactive peptide in the biomaterial, but also the composition of the scaffolds. The composition of the UPy-modified polymeric scaffold might be regulated using peptides with different functions. Another manner of variation of the composition is the usage of peptides with various binding affinity to the UPy polymer by introducing for example alkyl spacers or urea groups in between the UPy unit and the peptide. In this way, it is proposed to get control over the dynamics of binding of the UPy peptides to UPy polymeric materials.

Experimental Section

General Materials: 2-Amino-4-hydroxy-6-methylpyrimidine was purchased from Aldrich. 1,6-Diisocyanatohexane was obtained from Fluka. The Fmoc-protected amino acids and the Wang resin for solid-phase peptide synthesis (SPPS) were obtained from Bachem. 6-|9H-Fluoren-9-ylmethoxycarbonyl]amino|hexanoic acid was purchased from Sigma-Aldrich. Potassium hexafluorophosphate was obtained from Acros. Commercial products were used without further purification. All solvents purchased from Acros Chimica or Sigma–Aldrich were of p.a. quality. Deuterated solvents were obtained from Cambridge Isotope Laboratories. Water was always demineralized prior to use.

General Methods: The syntheses of the UPy synths were performed under argon. Chloroform was dried with 4 Å zeolites. The presence or absence of primary free amines during solid-phase peptide synthesis (SPPS) was determined with the Kaiser test. All reactions (couplings, deprotections, cleavages) on the resin were performed while shaking the resin suspension at 21 °C. 1H NMR and 13C NMR spectra were recorded with a Varian Gemini (300 MHz for 1H NMR, 75 MHz for 13C NMR) or Varian Mercury (400 MHz for 1H NMR, 100 MHz for 13C NMR) spectrometer at 298 K. 19F NMR (with potassium hexafluorophosphate as internal standard) was measured with a Varian Mercury (377 MHz for 19F NMR) spectrometer at 298 K. 2D 1H,1H-COSY spectra were recorded with a Varian Mercury (400 MHz) or Varian Unity Inova (500 MHz) spectrometer at 298 K. Chemical shifts (δ) are given in ppm values relative to tetramethylsilane (TMS) or relative to the solvent residual peak. Infrared (IR) spectra were recorded with a Perkin–Elmer Spectrum One FT-IR spectrometer with a Universal ATR Sampling Accessory for solids. Melting points were determined with a Büchi Melting Point B-540 apparatus. Analytical reversed-phase liquid chromatography (RPLC) was performed with a Shimadzu FCV-10 AL VP with a Shimadzu SCL-10A VP system controller, Shimadzu LC-10AD VP liquid chromatography pumps [with an Alltima C18 5u (150 mm × 3.2 mm) reversed phase column and gradients of water/acetonitrile, supplemented with 0.2% trifluoroacetic acid], a Shimadzu DGU-14A degasser and a Shimadzu SPD-M10A VP diode array detector. Preparative reversed-phase liquid chromatography (prep-RPLC) was performed with a system consisting of the following components: Shimadzu SCL-10A VP system controller with Shimadzu LC-8A preparative liquid chromatography pumps [with an Alltima C18 5u (150 × 10 mm) semi-preparative reversed-phase column and gradients of water/acetonitrile, supplemented with 0.2% trifluoroacetic acid], a Shimadzu SIL-10AD VP auto injector, a Shimadzu FRC-10A fraction collector and a Shimadzu SPD-10A VP UV/Vis detector. Reversed-phase liquid chromatography–mass spectrometry (RPLCMS) was performed with a system consisting of the following components: Shimadzu SCL-10A VP system controller with Shimadzu LC-10AD VP liquid chromatography pumps [with an Alltima C18 3u (50 mm × 2.1 mm) reversed-phase column and gradients of water/acetonitrile supplemented with 0.1% formic acid], a Shimadzu DGU-14A degasser, a Thermo Finnigan surveyor Autosampler, a Thermo Finnigan surveyor PDA detector and a Finnigan LCQ Deca XP Max.

Solid-Phase Peptide Synthesis: The peptides (GRGDS, p-GRGDS, GRDGDS, PHSRN, HZWQMAGPGGGK and CGGKG) were synthesized according to conventional SPPS techniques using standard Fmoc-coupling chemistry[29] on a Wang (p-alkoxybenzyl alcohol; 200–400 mesh) resin. The loading of the Wang resin was performed according to the next procedure (Table 2). The resin was washed 3 times with dichloromethane (DCM), after which the resin was suspended in DMF. The protected amino acid (2 equiv.), 1-hydroxybenzotriazole (4 equiv., HOBt), disopropylcarbodiimide (2 equiv., DIPCDI) and 4(dimethylamino)pyridine (2 equiv., DMAP) were added to this suspension. The reaction mixture was shaken for 24 h at 21 °C. The resin was filtered and washed twice successively with DCM, DMF and 2-propanol, three times with DCM and three times with 2-propanol. The resin was dried in vacuo and weighed. The remaining hydroxy end groups were capped with benzyl chloride (0.34 mL/g resin) and pyridine (0.28 mL/g resin) in DMF for 30 min at 21 °C. The resin was filtered and washed twice successively with DCM, DMF and 2-propanol, three times with DCM and three times with diethyl ether. The resin was dried in vacuo and weighed. The loading of the resin was determined gravimetrically (in mmol amino acid per g resin).

The Fmoc removal and amino acid coupling cycles were performed at 21 °C according to the following procedure (Table 2). The Fmoc-protection groups were removed with 20% piperidine in dimethylformamide (DMF) for 20 min. The resin was washed 3 times with DMF for 5 min. The protected (if necessary) amino acids (3 equiv.) were dissolved in DMF. As coupling reagents HOBt (3.6 equiv.) and disopropylcarbodiimide (3.3 equiv., DIPCDI) in DMF were used. The amino acids were coupled for 30–60 min. The resin was washed 3 times with DMF for 5 min. The cycle was repeated until the last amino acid was coupled. In between the deprotection and coupling steps the presence or absence, respectively, of primary free amines on the resin was determined with the Kaiser test.[30] After coupling of the last amino acid, the resin was washed subsequently,

with DMF, DCM and diethyl ether. The resin with protected peptide sequence was used for the following reactions to obtain the UPy-modified peptides 1–10.

**UPy-GRGDS (1):** The following protected peptide sequence on the resin was used for the reaction with 1,1′-carbonyldimidazole-activated\(^{27}\) methylisocytosine (11): 0.84 g Fmoc-Gly-Arg(Pmc)-Gly-Asp(OBu)-Ser(Bu) resin (0.36 mmol peptide). First, the Fmoc group at the N-terminus was removed with 20% piperidine in DMF for 20 min at 21 °C. The resin was washed with DMF. The reaction of the free amine of the protected GRGDS peptide with 11 (6 equiv., 2.1 mmol, 0.46 g) was performed in dry DMF (mol. sieves) under argon for 16 h at 50 °C resulting in the protected UPy-GRGDS (1) on the resin. The resin was filtered and washed subsequently with DMF, DCM, methanol, water, acidic water (to wash the excess of 11 away), water, methanol, DCM. The peptide was deprotected and cleaved from the support with 95% trifluoroacetic acid (TFA) and 5% water for 4 h. The suspension was filtered and the peptide was precipitated in (cold) diethyl ether, spun down and washed three times with diethyl ether. Subsequently, the product was lyophilized at least three times from water with 10–20% acetonitrile, which resulted in a white fluffy powder. Yield: 56%, 63 mg, 0.087 mmol. \(^1\)H NMR (D\(_2\)O/[D\(_3\)]ACN): \(\delta = 5.99\) (s, 1 H, C=CH, UPy), 4.78 (t, 1 H, NH-CH-CH-CH(OH), Asp), 4.48 (t, 1 H, NH-CH-CH-CH(OH), Ser), 4.32 (t, 1 H, NH-CH-CH-CH(OH)-NH-(C=NH)-NH2, Arg), 3.98–3.84 [s, 2 H, NH-CH2, Gly(1)]; s, 2 H, NH-CH2, Gly(2); m, 2 H, NH-CH-CH-CH(OH), Ser, 3.15 [t, 2 H, NH-CH-CH-CH2-CH2-NH-(C=NH)-NH2, Arg], 2.90–2.78 (m, 2 H, NH-CH-CH-CH(OH), Asp), 2.23 (s, 3 H, CH3, UPy), 1.85–1.61 [m, 3 H, 4 NH, NH-CH-CH-CH2-CH2-NH-(C=NH)-NH2, Arg] ppm. The assignment of the \(^1\)H NMR spectrum is confirmed by 2D \(^1\)H,\(^1\)H COSY spectroscopy. \(^{13}\)C NMR (D\(_2\)O/[D\(_3\)]ACN): \(\delta = 173.3\) (CH2), 172.9, 172.0, 171.1 (2 CH2), 170.2, 159.8, 155.9, 155.4, 150.2, 104.4, 60.3, 54.2, 52.7, 49.0, 41.9, 41.7, 39.7, 34.6, 27.0, 23.5, 19.7 ppm. \(^{19}\)F NMR (D\(_2\)O/[D\(_3\)]ACN), with potassium hexafluorophosphate as internal standard showed that the sample contained less than 0.1 wt.-% TFA. IR (ATR): \(\nu = 3287, 3072, 2935, 1703, 1646\) (amide I), 1539 (amide II), 1411, 1232, 1180, 1135, 1076, 1045 cm\(^{-1}\). RPLC-MS (purity > 99%): one peak in chromatogram with \(m/z:\) calcd. 641.3 g/mol, obsd. \([M + H]^+ = 642.2\) g/mol and \([M + H]^2+ = 321.7\) g/mol.

**UPy*-GRGDS (2):** The following protected peptide sequence on the resin was used for the reaction with 1,1′-carbonyldimidazole-activated\(^{27}\) ethylpentyl-isocytosine (12): 0.47 g Fmoc-Gly-Arg(Pmc)-Gly-Asp(OBu)-Ser(Bu) resin (0.16 mmol peptide). First, the Fmoc group at the N-terminus was removed with 20% piperidine in DMF for 20 min at 21 °C. The resin was washed with DMF. The reaction of the free amine of the protected GRGDS peptide with 12 (5 equiv., 0.82 mmol, 0.25 g) was performed in dry DMF (mol. sieves) under argon atmosphere for 16 h at 50 °C resulting in the protected UPy*-GRGDS 2 on the resin. The resin was filtered and washed subsequently with DMF, DCM, chloroform and diethyl ether. The peptide was deprotected and cleaved from the support with 95% trifluoroacetic acid (TFA) and 5% water for 6 h. The resin was filtered and the peptide was precipitated in (cold) diethyl ether, spun down and washed three times with diethyl ether. Subsequently, the product was lyophilized at least three times from water with 10–20% acetonitrile, which resulted in a white fluffy powder. Yield: 56%, 63 mg, 0.087 mmol. \(^1\)H NMR (D\(_2\)O/[D\(_3\)]ACN): \(\delta = 5.99\) (s, 1 H, C=CH, UPy), 4.78 (t, 1 H, NH-CH-CH-CH(OH), Asp), 4.48 (t, 1 H, NH-CH-CH-CH(OH), Ser), 4.32 (t, 1 H, NH-CH-CH-CH(OH)-NH-(C=NH)-NH2, Arg), 3.98–3.84 [s, 2 H, NH-CH2, Gly(1)]; s, 2 H, NH-CH2, Gly(2); m, 2 H, NH-CH-CH-CH(OH), Ser, 3.15 [t, 2 H, NH-CH-CH-CH2-CH2-NH-(C=NH)-NH2, Arg], 2.90–2.78 (m, 2 H, NH-CH-CH-CH(OH), Asp), 2.23 (s, 3 H, CH3, UPy), 1.85–1.61 [m, 3 H, 4 NH, NH-CH-CH-CH2-CH2-NH-(C=NH)-NH2, Arg] ppm. The assignment of the \(^1\)H NMR spectrum is confirmed by 2D \(^1\)H,\(^1\)H COSY spectroscopy. \(^{13}\)C NMR (D\(_2\)O/[D\(_3\)]ACN): \(\delta = 174.2\) (CH2), 173.7, 172.6, 171.9, 171.6, 171.0, 159.1, 158.1, 151.2, 156.7, 166.5, 104.2, 61.1, 59.6, 53.1, 52.9, 50.3, 49.7, 42.6, 42.5, 40.5, 35.8, 32.6, 28.7, 26.5, 24.3, 22.0, 13.2, 10.9 ppm. \(^{19}\)F NMR (D\(_2\)O/[D\(_3\)]ACN), with potassium hexafluorophosphate as internal standard showed that the sample contained less than 0.5 wt.-% TFA. IR (ATR): \(\nu = 3287, 3072, 2935, 1703, 1646\) (amide I), 1539 (amide II), 1411, 1232, 1180, 1135, 1076, 1045 cm\(^{-1}\). RPLC-MS (purity > 99%): one peak in chromatogram with \(m/z:\) calcd. 725.3 g/mol, obsd. \([M + H]^+ = 726.3\) g/mol and \([M + H]^2+ = 363.7\) g/mol.

**UPy-hexyl-urea-GRGDS (3):** The following protected peptide sequence on the resin was used for the reaction with 2-(6-isocyanato-hexylaminocarbonylaminoo)-6-methyl-1(H)-pyrimidinone\(^{38}\) (13): 0.44 g Fmoc-Gly-Arg(Pmc)-Gly-Asp(OBu)-Ser(Bu) resin (0.15 mmol peptide). First, the Fmoc group at the N-terminus was removed with 20% piperidine in DMF for 20 min at 21 °C. The resin was washed with DMF and then with chloroform. Before the addition of 13 to the resin, the suspension of 13 (12 equiv.,
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UPy-hexyl-urea-pentyl-GRGDS (4): The following protected peptide sequence on the resin was used for the reaction with 6-[9H-fluoren-9-ylmethoxycarbonyl]amino]hexanoic acid and then with 2-(6-isocyanatohexylaminocarbonylamino)-6-methyl-4(1H)-pyrimidinone (13): 0.32 g Fmoc-Gly-Arg(Pmc)-Gly-Ser(tBu) resin (0.26 mmol peptide). First, the Fmoc group at the N-terminus was removed with 20% piperidine in DMF for 20 min at 21 °C. The resin was washed and dried with DMF. Subsequently, the product was lyophilized at least three times from water with 10–20% acetonitrile, which resulted in a white fluffy powder. Yield: 75%, 190 mg, 0.30 mmol. 1H NMR (D2O/[D3]ACN): δ = 173.5, 173.1, 172.8, 172.1, 169.4, 164.3, 162.8, 159.4, 153.1, 151.1, 104.5, 60.6, 54.4, 52.7, 49.2, 43.4, 42.6, 41.9, 40.0, 39.3, 39.0, 34.9, 28.7, 28.1, 25.3 (2×2), 23.8, 20.1 ppm. The assignment of the 1H NMR spectrum is confirmed by 2D 1H,1H COSY spectroscopy. 13C NMR (D2O/[D3]ACN): δ = 173.5, 173.1, 172.8, 172.1, 169.4, 164.3, 162.8, 159.4, 153.1, 151.1, 104.5, 60.6, 54.4, 52.7, 49.2, 43.4, 42.6, 41.9, 40.0, 39.3, 39.0, 34.9, 28.7, 28.1, 25.3 (2×2), 23.8, 20.1 ppm. 19F NMR (D2O/[D3]ACN), with potassium hexafluorophosphate as internal standard showed that the sample contained less than 1 wt.% TFA. IR (ATR): v = 3253, 3073, 2940, 1646 (amide I), 1549, 1439, 1419, 1374, 1338, 1284, 1136, 1079, 1046 cm⁻¹. RPLC-MS (purity > 98%): one peak in chromatogram with m/z: calcld. 874.4 g/mol, obsd. [M + H]⁺ = 874.3 g/mol and [M + H]²⁺ = 493.7 g/mol.

The peptide was deprotected and cleaved from the support with 95% trifluoroacetic acid (TFA) and 5% water for 4 h. The suspension was filtered and washed with diethyl ether, spun down and washed three times with diethyl ether. Subsequently, the product was lyophilized at least three times from water with 10–20% acetonitrile, which resulted in a white fluffy powder. Yield: 57%, 56 mg, 0.062 mmol. 1H NMR (D2O/[D3]ACN): δ = 5.78 (s, 1 H, C=CH, UPy), 4.02 (t, 1 H, NH-CH-CH₂-COOH, Asp), 4.54 (t, 1 H, NH-CH₂-CH₂-OH, Ser), 3.38 (t, 1 H, NH-CH₂-CH₂-OH, Asp), 2.6 (m, 4 H, NH-CH₂-CH₂-OH, Asp); m, 2 H, NH-CH₂-CH₂-OH, Gly; s, 2 H, NH-CH₂-Gly(tBu). The assignment of the 1H NMR spectrum is confirmed by 2D 1H,1H COSY spectroscopy. 13C NMR (D2O/[D3]ACN): δ = 174.1, 173.2, 172.3, 171.4, 171.2 (2×2), 170.5, 159.3, 156.3, 155.4, 151.1, 104.8, 60.7, 49.5, 53.2, 49.4, 42.1 (2×2), 40.1, 35.0, 27.5, 23.9, 19.8 ppm. 19F NMR (D2O/[D3]ACN), with potassium hexafluorophosphate as internal standard showed that the sample contained less than 0.1 wt.% TFA. IR (ATR): v = 3266, 3065, 2938, 2863, 1708, 1646 (amide I), 1547 (amide II), 1439, 1419, 1374, 1338, 1284, 1136, 1079, 1046 cm⁻¹. RPLC-MS (purity > 98%): one peak in chromatogram with m/z: calcld. 864.6 g/mol, obsd. [M + H]⁺ = 874.4 g/mol and [M + H]²⁺ = 493.7 g/mol.

UPy-hexyl-urea-pentyl-GRGDS (5): The following protected peptide sequence on the resin was used for the reaction with 1,1-carboxyldimidezole-activated277 methylisotocytosine (11): 1.0 g Fmoc-Gly-Arg(Pmc)-Gly-Asp(tBu)-Ser(tBu) resin (0.40 mmol peptide). First, the Fmoc group at the N-terminus was removed with 20% piperidine in DMF for 20 min at 21 °C. The resin was washed with DMF. Subsequently, the product was lyophilized at least three times from water with 10–20% acetonitrile, which resulted in a white fluffy powder. Yield: 75%, 190 mg, 0.30 mmol. 1H NMR (D2O/[D3]ACN): δ = 173.5, 173.1, 172.8, 172.1, 169.4, 164.3, 162.8, 159.4, 153.1, 151.1, 104.5, 60.6, 54.4, 52.7, 49.2, 43.4, 42.6, 41.9, 40.0, 39.3, 39.0, 34.9, 28.7, 28.1, 25.3 (2×2), 23.8, 20.1 ppm. The assignment of the 1H NMR spectrum is confirmed by 2D 1H,1H COSY spectroscopy. 13C NMR (D2O/[D3]ACN): δ = 173.5, 173.1, 172.8, 172.1, 169.4, 164.3, 162.8, 159.4, 153.1, 151.1, 104.5, 60.6, 54.4, 52.7, 49.2, 43.4, 42.6, 41.9, 40.0, 39.3, 39.0, 34.9, 28.7, 28.1, 25.3 (2×2), 23.8, 20.1 ppm. 19F NMR (D2O/[D3]ACN), with potassium hexafluorophosphate as internal standard showed that the sample contained less than 1 wt.% TFA. IR (ATR): v = 3253, 3073, 2940, 1646 (amide I), 1547 (amide II), 1439, 1419, 1374, 1338, 1284, 1136, 1079, 1046 cm⁻¹. RPLC-MS (purity > 98%): one peak in chromatogram with m/z: calcld. 864.6 g/mol, obsd. [M + H]⁺ = 874.4 g/mol and [M + H]²⁺ = 493.7 g/mol.
The reaction of the free amine of the protected GRDGS peptide with 11 (6 equiv., 1.6 mmol, 0.34 g) was performed in dry DMF (mol. sieves), by first heating the mixture for 5 min and then by shaking the mixture for 16 h at 21 °C resulting in the protected UPy-GRDGS 6 on the resin. The resin was filtered and washed subsequently with DMF, DCM, methanol, water, acidic water (to wash the excess of 11 away), water, methanol, DCM. The peptide was deprotected and cleaved from the support with 95% trifluoroacetic acid (TFA) and 5% water for 5 h. The suspension was filtered and the peptide was precipitated in (cold) diethyl ether, spun down and washed three times with diethyl ether. Subsequently, the product was lyophilized at least three times from water with 10–20% acetonitrile, which resulted in a white fluffy powder. Yield: 50%, 82 mg, 0.13 mmol. 1H NMR (D2O/[D3]ACN): δ = 5.99 (s, 1 H, C=CH, UPy), 4.70–4.57 (t, 1 H, NH-CH2-CH2-COOH, Asp), 4.47 (t, 1 H, NH-CH2-CH2-OH, Ser), 4.32 [t, 1 H, NH-CH2-CH2-NH-(C=N)-NH2, Arg], 4.00–3.82 [s, 2 H, NH-CH2, Gly(1)], 3.82 [s, 2 H, NH-CH2, Gly(2)], 3.15 [t, 2 H, NH-CH2-CH2-NH-(C=N)-NH2, Arg], 2.98–2.79 (m, 2 H, NH-CH2-CH2-COOH, Asp), 2.24 (s, 3 H, CH3, UPy), 1.86–1.56 [3 m, 4 H, NH-CH2-CH2-NH-(C=N)-NH2, Arg] ppm. The assignment of the 1H NMR spectrum is confirmed by 2D 1H,1H COSY spectroscopy. 13C NMR (D2O/[D3]ACN): δ = 173.2, 172.8, 172.3, 171.7, 171.1, 170.2, 166.5, 159.6, 156.1, 155.3, 150.8, 147.4, 115.8, 104.7, 60.6, 54.2, 52.9, 49.6, 42.2, 41.9, 40.0, 34.6, 27.3, 23.8, 19.9 ppm. 19F NMR (D2O/[D3]ACN), with potassium hexafluorophosphate as internal standard showed that the sample contained less than 0.1 wt.-% TFA. IR (ATR): ν = 3278, 2939, 1661 (amide I), 1594, 1540 (amide II), 1441, 1364, 1316, 1252, 1202, 1133, 1082 cm⁻¹. RPLC-MS (purity > 99%): one peak in chromatogram with m/z: calcld. 402.4 g/mol, obsd. [M + H]⁺ = 402.3 g/mol and [M + H]²⁺ = 201.7 g/mol.

HVMQAPGGK(-urea-hexyl-UPy)G (8): The following protected peptide sequence on the resin was used for the reaction with 2-(6-isocyanatohexylaminocarboxylylamino)-6-methyl-4(1H)-pyrimidinone(11) (13): 0.27 g Fmoc-His(Trt)-Val-Trp(Boc)-Met-Gln(Trt)-Ala-Pro-Gly-Gly-Lys(Mtt) resin (0.062 mmol peptide). First, the Mtt-protection group was selectively removed from the Lys using 5% TFA, 5% TIS and 90% DCM for 15 min. The resin was filtered and washed twice with DCM and four times with 5% disopropylmethylethylamine (DIEPA) in DCM. Before the addition of 13 to the resin, the suspension of 13 (10 equiv., 0.61 mmol, 0.18 g) in dry chloroform (mol. sieves) was heated and filtered. The clear filtrate was added to the resin. The reaction of the free amine of the protected Fmoc-HVMQAPGGK peptide with 13 was performed in the presence of DIEPA (1 equiv.) for 7 h at 21 °C, while shaking the solution. This resulted in the protected Fmoc-HVMQAPGGK(-urea-hexyl-UPy)G (8) on the resin. The resin was filtered and washed subsequently with DCM, methanol, water, acidic water (to remove the excess of 13), water, methanol, DCM and DMF. Then, the Fmoc group at the N-terminus was removed with 20% piperidine in DMF for 1 h at 21 °C. The resin was washed with DCM and then with chloroform. Before the addition of 13 to the resin, the suspension of 13 (11 equiv., 2.0 mmol, 0.58 g) in dry chloroform (mol. sieves) was heated and filtered. The clear filtrate was added to the resin. The reaction of the free amine of the protected PHSRN peptide with 13 was performed for 16 h at 21 °C, while shaking the solution. This resulted in the protected UPy-PHSRN 7 on the resin. The resin was filtered and washed subsequently with chloroform, DMF, DCM, chloroform and diethyl ether. The peptide was deprotected and cleaved from the support with 95% trifluoroacetic acid (TFA), 2.5% triisopropylsilane (TIS) and 2.5% water for 4 h. The resin was filtered and the peptide was precipitated in (cold) diethyl ether, spun down and washed three times with diethyl ether. Subsequently, the product was lyophilized at least three times from water with 10–20% acetonitrile which resulted in a white fluffy powder. The peptide was purified using preparative reversed phase liquid chromatography (RPLC). Yield: 82%, 74 mg, 0.051 mmol (after prep-RPLC; 16%). 1H NMR (D2O/[D3]ACN): the spectrum is too complicated. The alkyldiene proton is visible at 5.99 (s, C=CH, UPy) ppm. 19F NMR (D2O/[D3]ACN), with potassium hexafluorophosphate as internal standard showed that the sample contained less than 0.1 wt.-% TFA. IR (ATR): ν = 3278, 3072, 2941, 1649 (amide I), 1529 (amide II), 1412, 1340, 1225, 1316, 1079, 1046 cm⁻¹. RPLC-MS (purity > 96%): one peak in chromatogram with m/z: calcld. 458.1 g/mol, obsd. [M + H]⁺ = 457.9 g/mol and [M + H]²⁺ = 229.0 g/mol.
twice with DCM and four times with 5% diisopropylethylamine (DPEA) in DCM. The reaction of the free amine of the protected Fmoc-CGGKG peptide with 11 (9 equiv., 0.73 mmol, 0.16 g) was performed in dry DMF (mol. sieves), by first heating the mixture for 5 min and then by shaking the mixture for 8 h at 21 °C in the presence of DPEA (1 equiv.). This resulted in the protected Fmoc-CGGK(-UPy)G 9 on the resin. The resin was filtered and washed subsequently with DCM, methanol, water, acidic water (to remove the excess of 11), water, methanol, DCM and DMF. Then, the Fmoc group at the N-terminus was removed by heating and filtered. The clear filtrate was added to the resin. The suspension of 6-(2-ethylpentyl)isocytosine (12) (CDI-activated ethylpentyl-isocytosine): 0.21 g Fmoc-Cys(Trt)-Gly-Gly-Lys(Mtt)-Gly resin (9 equiv., 0.73 mmol, 0.16 g) was dissolved in chloroform. CDI (4.4 mmol, 0.71 g) was added to the resin, the suspension of 6-(2-ethylpentyl)-2-(1-imidazolylcarbonylamino)-4(1H)-pyrimidinone (18) was heated and filtered. The remaining organic layer was evaporated in vacuo.

The reaction mixture was stirred for 3 h at 21 °C. The entire mixture was extracted with brine for three times and filtered. The clear filtrate was added to the resin. The reaction of the free amine of the protected Fmoc-CGGKG peptide with 13 was performed in the presence of DPEA (1 equiv.) for 8 h at 21 °C, while shaking the solution. This resulted in the protected Fmoc-CGGK(-urea-hexyl-UPy)G (10) on the resin. The resin was filtered and washed subsequently with DCM, methanol, water, acidic water (to remove the excess of 13), water, methanol, DCM and DMF. Then, the Fmoc group at the N-terminus was removed by heating 5% piperidine in DMF for 2 h at 21 °C. The resin was washed with DMF and then with DCM. The peptide was deprotected and cleaved from the support with 95% trifluoroacetic acid (TFA), 2.5% triisopropylsilane (TIS) and 2.5% water for 4 h. The nitrile which resulted in a white fluffy powder. Yield: 46%, 22 mg, 0.038 mmol. 

CH3SH, Cys; t, 1 H, NH=CH—CH2—CH2—CH2—NH—(C=O)—NH, Lys), 4.08–3.92 [m, 6 H, NH2—CH2—Gly(t)]; NH2—CH2—Gly(2); NH2—CH2—Gly(3); 3.28–3.13 [m, 6 H, CH2—NH—(C=O)—NH—H; m, 2 H, NH2—CH2—CH2—SH, Cys], 2.29 (s, 3 H, CH3, UPy), 1.88–1.40 (m, 14 H, CH2—CH2—CH2—CH2 ppm. The assignment of the 1H NMR spectrum is confirmed by 2D 1H,1H COSY spectroscopy. 13C NMR (D2O/[D3]ACN): δ = 173.1, 173.1, 172.7, 172.2, 170.2, 170.1, 167.8, 165.5, 159.5, 153.7, 151.9, 104.5, 53.9, 52.8, 42.0, 41.8, 40.2, 39.1, 38.9, 30.4, 28.9, 28.4, 28.2 (2×), 25.3, 24.2, 21.8 ppm. 19F NMR (D2O/[D3]ACN), with potassium hexafluoroacetate as internal standard, showed that the sample contained less than 1 wt-% TFA. IR (ATR): ν = 3287, 3040, 2935, 2856, 1656 (amide I), 1558, 1527 (amide II), 1442, 1415, 1379, 1247, 1201, 1135, 1028 cm−1. RPLC-MS (purity > 95%): one peak in chromatogram with m/z: calculated. 713.3 g/mol, obsd. [M – M + H+] = 4145.5 g/mol (S-S dimer), [M + H]3+ = 714.3 g/mol and [M + H]2+ = 457.7 g/mol.

2-(1-Imidazolylcarboxyleno)-6-methyl-4(1H)-pyrimidinone (11) (CDI-activated methyl-isocytosine): 2-Amino-4-hydroxy-6-methylpyrimidine was activated with 1,1-carboxyldimidazole (CDI) in a similar way as described before.27,28 2-Amino-4-hydroxy-6-methylpyrimidine (4.0 mmol, 0.84 g) was suspended in DMSO and stirred for 1 h at 80 °C. The reaction mixture was cooled down and acetone was added. The product precipitated from the solution and was filtered off. The precipitate was washed with acetone. The product was obtained as a white powdered and was dried in vacuo at 30 °C for 1 h. Yield: 98%, 0.86 g, 3.9 mmol; m.p. 258 °C. Remark: this compound is difficult to characterize in solution due to its low solubility in most solvents, also in DME. 1H NMR ([D3]ACN): δ = 8.3 (s, 1 H, N=CH=N), 7.77 [s, 1 H, (C=O)—N=CH=CH-], 7.20 [s, 1 H, (C=O)—NH—C=O], 6.77 (2×, 2 H, -NH,), 6.06 (1 H, 2, Gly(1); NH—C=O), 2.49 (s, 3 H, CH3) ppm. 13C NMR ([D3]ACN): δ = 162.3, 160.1, 157.6, 153.2, 135.4, 121.8, 115.5, 105.4, 21.6 ppm. IR (ATR): ν = 3175, 3075, 2548, 1701, 1645, 1601, 1508, 1479, 1375, 1334, 1319, 1276, 1233, 1224, 1190, 1169, 1090, 1065, 1026, 983 cm−1.

6-(2-Ethylpentyl)-2-(1-Imidazolylcarboxyleno)-4(1H)-pyrimidinone (12) (CDI-activated ethylpentyl-isocytosine): 6-(2-Ethylpentyl)isocytosine was synthesized by ring closure of the corresponding β-keto ester with guanidine carbonate in ethanol as described before.31,34 Subsequently, the 6-(2-ethylpentyl)isocytosine was activated with 1,1-carboxyldimidazole (CDI) in a similar manner as shown before.27 6-(2-ethylpentyl)isocytosine (2.0 mmol, 0.42 g) was dissolved in chloroform. CDI (4.4 mmol, 0.71 g) was added to this clear solution. The reaction mixture was stirred for 3 h at 21 °C. The entire mixture was extracted with brine for three times and filtered. The water layers were combined and extracted with chloroform. The combined chloroform layers were dried with Na2SO4 and the remaining organic layer was evaporated in vacuo which resulted in a light yellow powder. Yield: 85%, 0.52 g, 1.7 mmol; m.p. 132 °C. 1H NMR (CDCl3): δ = 12.69 (s, 2 H, -NH2), 8.77 [s, 1 H, N=CH=CH-], 7.61 [s, 1 H, (C=O)—N=CH=CH-], 7.04 [s, 1 H, (C=O)—NH—C=O], 5.80 [s, 1 H, (C=O)—NH=CH-], 2.52 [m, 1 H, (CH2)n2CHC], 1.74 (m, 4 H, CH2), 1.30 (m, 4 H, CH2), 0.95 (t, 3 H, CH3), 0.92 (t, 3 H, CH3) ppm. 13C NMR (CDCl3): δ = 161.2, 159.4, 157.5, 156.9, 137.8, 128.3, 117.5, 103.9, 45.3, 32.7, 29.3, 26.6, 22.5, 13.8, 11.6 ppm. IR (ATR): ν = 3149, 2959, 2931, 2860, 2690, 1915, 1704, 1690, 1626, 1600, 1466, 1418, 1373, 1310, 1277, 1221, 1174, 1092, 1067, 1022, 1004, 984 cm−1.

2-(6-Isocyanatoethylamino)carboxyleno)-6-methyl-4(1H)-pyrimidinone (13) (UPy-ethyl-isocyanate synthone): The synthesis was performed in a similar way as described before.18 2-Amino-4-hydroxy-6-methylpyrimidine (0.23 mol, 29.1 g) was dissolved in 1,6-diisocy-
anatohexane (1.6 mol, 272.3 g) and heated at 100 °C for 16 h. The reaction mixture was cooled and pentane was added. The resulting precipitate was filtered and thoroughly washed with pentane. The product was dried at 50 °C in vacuo, yielding a white powder. Yield: 98%, 66.8 g, 0.23 mol; m.p. 185 °C. 1H NMR (CDCl3): δ = 13.14 (s, 1 H, CH2-C-NH2), 11.87 [s, 1 H, CH2-NH-(C=O)-NH2], 10.19 [t, 1 H, CH2-NH-(C=O)-NH2], 5.82 (s, 1 H, CH=CH2), 3.27 [m, 4 H, NH-(C=O)-NH-CH2 + CH2-NCO], 2.23 (s, 3 H, CH3), 1.61 (m, 4 H, N-CH2-CH2), 1.41 (m, 4 H, CH2-CH2-CH2-CH2-CH2-CH2-CH2-NCO). 13C NMR (CDCl3): δ = 173.1, 156.7, 154.8, 148.4, 106.8, 43.0, 39.9, 31.3, 29.4, 26.3, 26.2, 19.0 ppm. IR (ATR): ν = 2931, 2262 (NCO stretch), 1698 (UPy), 1667 (UPy), 1577 (UPy), 1414, 1205, 1159, 1068, 993, 904, 840, 645 cm–1. C13H19N5O3 (293.32): calcd. C 53.2, H 6.5, N 23.9; found C 53.2, H 6.2, N 24.0.

Supporting Information (see also the footnote on the first page of this article): RPLC-MS chromatograms are shown for all peptides synthesized.

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