Linking applicatory functions to the 3-position of pyrrole by click chemistry

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Abstract
A straight forward and reliable method was developed to tether recognition functions to a side chain in position 3 of pyrroles via triazole linkage. The products are precursors for functionalized polypyrroles, e. g. for coating magnetic nanoparticles or selective electrodes. A pyrrole with an azido function located at the terminus of a side chain in position 3 was submitted to copper-catalyzed Meldal-Sharpless click reaction with alkynes bearing biotin, nitrilotriacetic acid or a RGD-containing cyclopentapeptide. The latter presents a very versatile building block for the introduction of the RGD-moiety in a variety of potential substrates.

Keywords: Pyrroles, alkynes, azides, cycloadditions, biomolecules

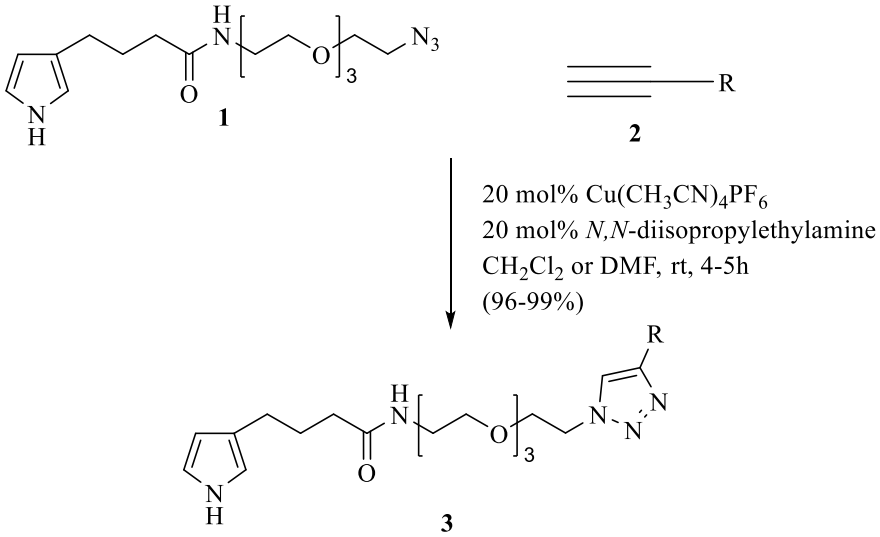
Introduction

Polypyrrole (PPy) is a conducting polymer,[1] which has found wide applications, such as in membranes[2] and in coating of electrodes[3-5] or of nanoparticles.[6-12] Amongst nanoparticles magnetic nanoparticles have attracted wide interest in nanotechnology and nanomedicine.[13,14] The scope of polypyrroles can be considerably extended when functions, such as reactive groups, biological recognition functions, ligands for metal complexation, or catalytic units are introduced.[15,16] As attachment points positions 1 and 3 of the pyrrole ring are preferred, because oxidative polymerizations of pyrroles to PPy run preferably via positions 2 and 5. In most cases, the functions are not directly attached to the pyrrole ring but are connected via linkers. This provides more freedom to the attached functions[17] and the effect of the substituents on the electron density in the pyrrole ring can be controlled by the type of linker. From the practical point of view it is advisable to introduce the function at a late stage of the synthetic sequence, i. e. to obtain a precursor which can accept the respective function in the last step. Following this

strategy, we recently developed pyrroles with azido or propargyl groups linked to position 1 of the pyrrole ring.[9] These derivatives can easily undergo Cu-catalyzed Meldal-Sharpless click reaction[18-20] with functions (glucose, cholesterol, biotin, nucleosides) equipped with an alkyne or azido moiety, respectively, resulting in 1,2,3-triazole linkages. A major advantage of this click-coupling method is the fact that many functional groups are tolerated thus avoiding protective group strategies. Since substituents in position 1 of the pyrrole generally somewhat hamper the oxidative polymerization to polypyrroles[21] and can give rise to branching[22] we seek to develop a way that allows to link interesting functions to position 3 of the pyrrole ring, thus enabling easier oxidative polymerization to polypyrroles. In this context it is worth mentioning that a DNA-sequence was linked to position 3 of a polypyrrole film by amide formation.[23]

Results and Discussion

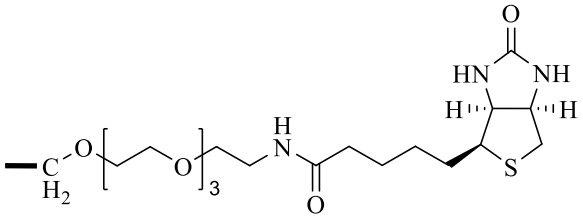
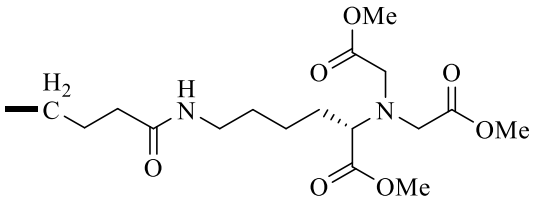
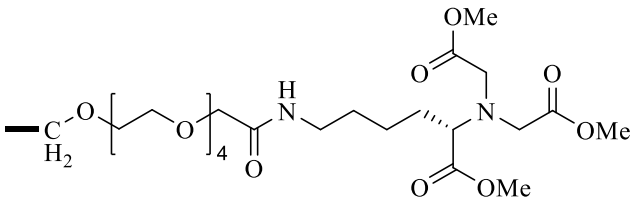
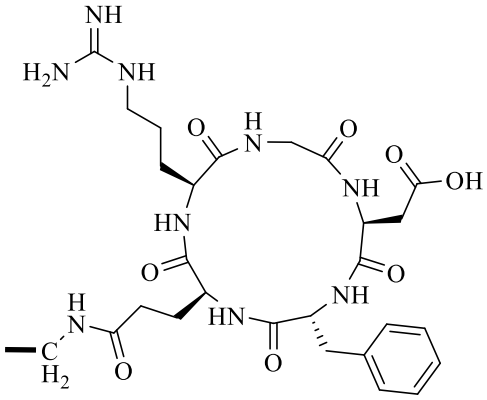
The 3-substituted azido-containing **1** fulfills all preconditions for the envisaged strategy. It carries a terminal azido group which is connected to position 3 via a polar oligo-ethylene glycol butyramide linker and does not decrease the electron density in the pyrrole ring. Thus it is a good candidate for both the Cu-catalyzed click reaction with alkynes and the subsequent oxidative polymerization to polypyrroles. As suitable coupling partners for the azido-functionalized pyrrole 1 we chose alkynes equipped with biotin (recognition of avidin or streptavidin),[24] nitrilotriacetic acid (complexing metal ions) and a RGD-containing cyclopentapeptide (medical application, *vide infra*) as interesting applicatory functions. The respective biotin-substituted alkyne **2a** is available by reaction of biotinylsuccinate[25] with

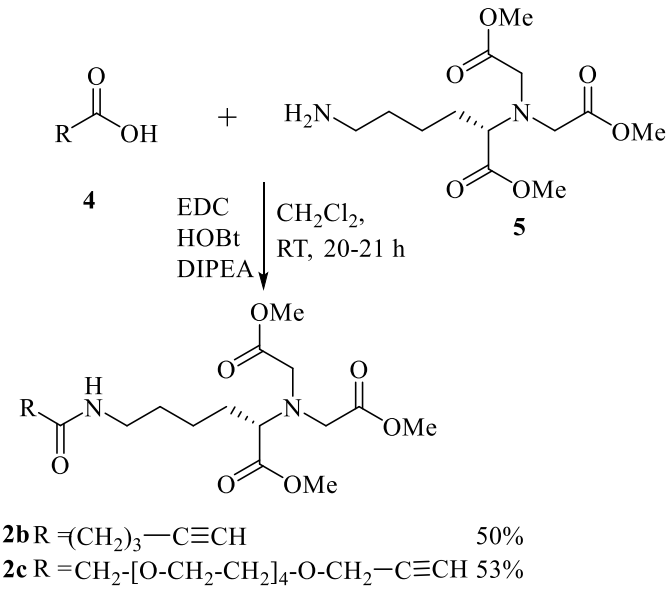


Scheme 1. Synthesis of functionalized pyrroles **3** by Cu-catalyzed click reaction of azidopyrrole **1** with alkynes **2.**

aminoethyltriethylenglycol propargylether in a straight forward one step reaction. The two unknown nitrilotriacetic acid derivatives **2b** and **2c** were obtained from the lysine derivative **5**[26] by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) assisted amide formation (Scheme 2).

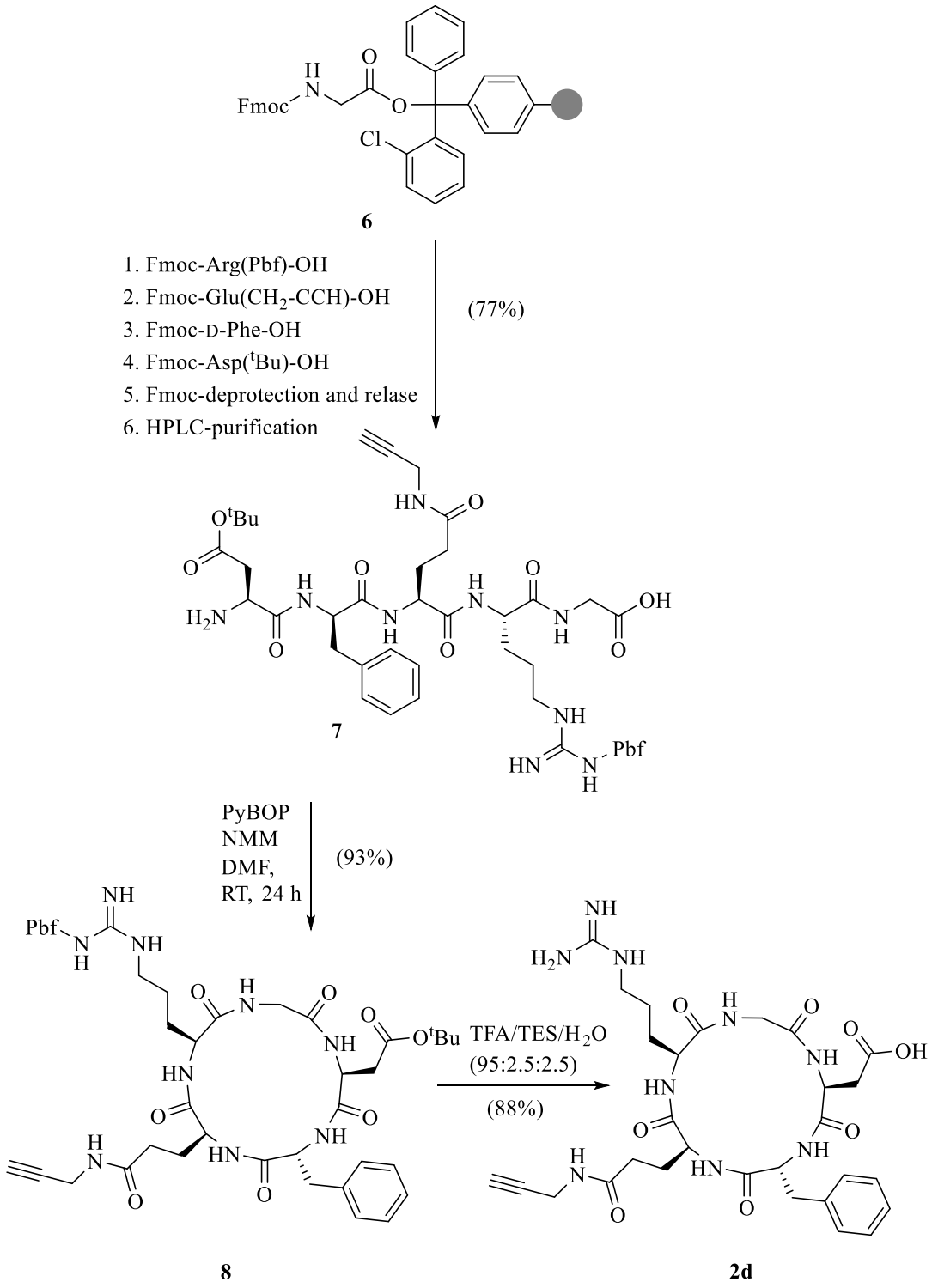
Table 1. 1,2,3-Triazoles **3** by *Meldal-Sharpless* click reaction

Product	R	Yield (%)
3a		96
3b		98
3c		99
3d		99



Scheme 2. Synthesis of alkyne-functionalized nitrilotriacetic esters **2b** and **2c.**

In order to obtain a suitable RGD-containing alkyne we designed a cyclo[Arg-Gly-Asp-D-Phe-Glu] wherein the properties of the RGD-sequence are not likely to be affected by the alkyne functionalization. Cyclic RGD-containing pentapeptides were applied as selective antagonists for the $\alpha_v\beta_3$ integrin receptor and have found wide applications.[27] For covalent linking of such cyclopeptides, modified or unmodified ε-amino lysine side chains were often used.[28-32] Recently Cu-catalyzed click reaction was performed at such a RGD-containing cyclopeptide wherein prior the lysine amino group was transferred into an azido group.[30] In another way the lysine amino group was acylated by propiolic acid and used to introduce an RGD-cyclopeptide into xylose by click chemistry.[32] Here, we chose the side chain of a glutamate in a cyclo[Arg-Gly-Asp-D-Phe-Glu] peptide as attachment point for an alkyne moiety (Scheme 3). The synthesis of the respective propargylamide **2d** started with Fmoc-protected glycine-loaded chlorotrityl resin **6**. The pentapeptide **7** was built up by solid phase peptide synthesis wherein Pbf-protected Arg was used in the first and *N*-propargylated Glu in the second coupling step. The pentapeptide **7** was obtained in 77 % after chromatographic purification. Macrolactamization was implemented by benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) activation providing high yield (93 %) of the cyclopentapeptide **8** which was treated with trifluoroacetic acid (TFA) giving rise to the deprotected product **2d**. This cyclopentapeptide **2d** was obtained in scales of hundred of mg. It represents a novel very versatile RGD-derivative which is an excellent candidate for Cu-catalyzed Meldal-Sharpless click reactions with a variety of azido-functionalized substrates.



Scheme 3. Synthesis of propargyl-functionalized RGD-containing cyclopentapeptide **2d.**

With all the starting materials **1** and **2** in hand we performed Cu-catalyzed Meldal-Sharpless click reaction using $Cu(CH_3CN)_4PF_6$ as catalyst. After the reaction, the copper catalyst was removed from the reaction mixture by Cuprisorb™ rendering the work up very easy. Pleasingly,

all products **3** were obtained in excellent yields (> 95%) (Scheme 1, Table 1). They were characterized by spectroscopic methods and are presently investigated in our laboratories for further application in the preparation of functionalized core-shell nanoparticles.

As a proof of principle for application of the triazolyl-substituted pyrroles **3** as components for the synthesis of functionalized polypyrroles, we submitted the biotin derivative **3a** to oxidative copolymerization (ammonium persulfate as oxidizing reagent) with unsubstituted pyrrole in the presence of magnetite nanoparticles **9** stabilized by a double layer of lauric acid (Scheme 4). The resulting polypyrrole-magnetite core shell nanoparticles **10** can easily isolated by magnetic separation with an external magnet and decantation leaving back polypyrrole which is not linked to the nanoparticles in the mother liquor. The incorporation of biotin moieties in the magnetic polypyrrole-magnetite core shell nanoparticles **10** is proved by the appearance of typical bands in the FTIR-spectrum (Figure 1). For comparison the FTIR-spectrum of the precursor magnetite nanoparticles **9** covered by a double layer of lauric acid is shown too (Fig. 1). The bands located at 616 cm^{-1} found in both spectra is specific for Fe-O bond in magnetite. The spectrum of **10** shows a band at 1642 cm^{-1} typical for the carbonyl group of the ureido moiety of biotin. A very broad intensive band situated at 3233 cm^{-1} can be assigned to NH stretching vibrations of the NH groups of biotin and to the CH stretching bands of the pyrrole rings. The adsorption band situated at 1547 cm^{-1} is ascribed to the collective vibration mode of intra-ring and inter-ring C=C/C–C of polypyrrole chains. At 1433 cm^{-1} appears the adsorption band specific for C-N bonds.

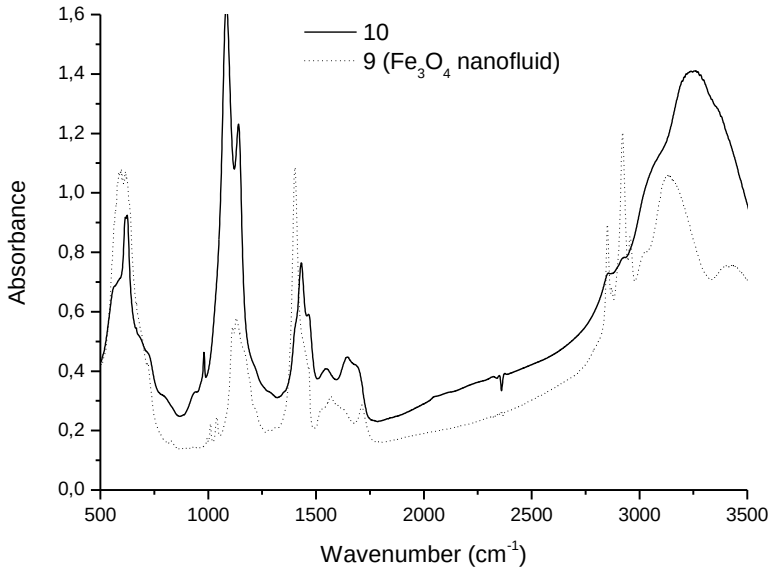
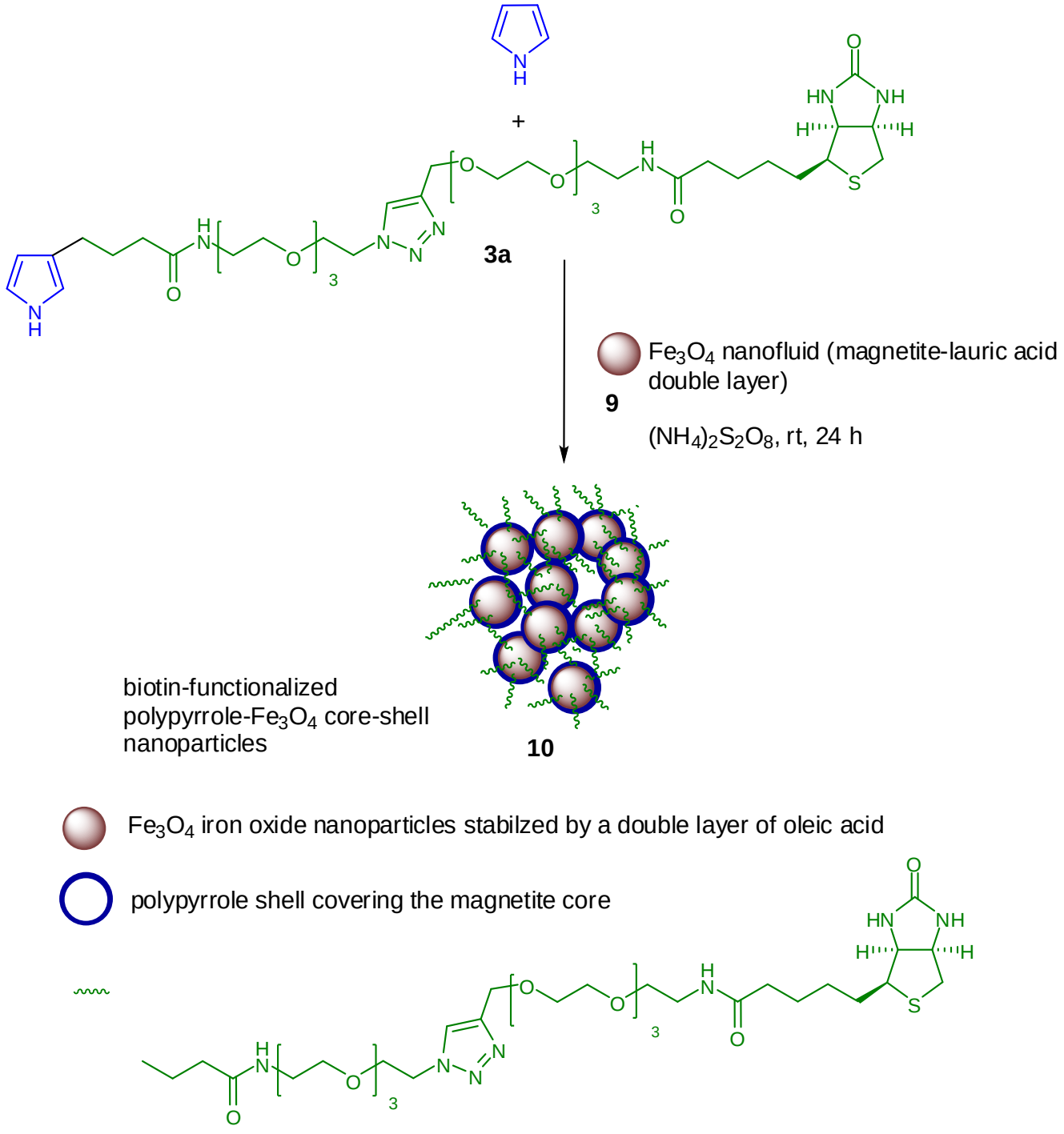


Figure 1. FTIR-Spectra (in KBr) of biotin-functionalized polypyrrole-magnetite nanoparticles **10** and the precursor magnetite nanoparticles **9** stabilized by a double layer of lauric acid.



Scheme 4. Synthesis of biotin-functionalized polypyrrole-magnetite core-shell nanoparticles **10.**

Conclusions

In summary, novel pyrroles were developed which contain interesting applicatory functions (biotin, nitrilotriacetic acid, RGD-containing cyclopentapeptide) tethered to a substituent in position 3 via 1,2,3-triazole linkage. These pyrroles represent promising precursor for functionalized polypyrroles useful for magnetic core-shell nanoparticles or as electrode coatings. The pyrroles were obtained in a straight forward way by Cu-catalyzed Meldal-Sharpless click reaction of a pyrrole with an azido group tethered to position 3 via a linker and alkyne moieties equipped with the respective functions. Amongst the latter the alkyne -containing RGD-cyclopentapeptide represents a versatile building block for potential tethering the RGD-sequence to various targets. The biotin-triazole-pyrrole conjugate was applied in the preparation of new magnetic polypyrrole-magnetite core-shell nanoparticles equipped with biotin as recognition function for avidin or streptavidin.

Experimental Section

General. Chemicals were purchased from Aldrich and Acros. Silica gel 60 (0.04-0.063 mm, Acros) was used for preparative column chromatography. Melting points were determined on a Boetius hotstage apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 or 300 and 125 or 75 MHz, respectively, on a Bruker AV-500 or Bruker AV-300 with TMS as an internal standard. High resolution mass spectra (ESI) were measured with a Thermo Finnigan LTQ-FT-ICR-MS with MeOH as a solvent. FTIR spectra were measured with a Jasco FT/IR-4200-Spectrometer.

1,2,3-Triazoles 3. General procedure. A solution of azidopyrrole **1**[33] (200 μmol) and the alkyne **2** (200 μmol) in CH_2Cl_2 (10 ml) was degassed by argon. $Cu(CH_3CN)_4PF_6$ (15 mg, 40.μmol) and *N*-ethyldiisopropylamine (DIPEA) (7 μl, 40.μmol) were added and the mixture was stirred at rt until the reaction went to completion (TLC, about 4-5 h). Cuprisorb^{TM} was added and stirring was continued for 60 min. After filtration the filtrate was concentrated under vacuum by a rotary evaporator leaving behind the product.

4-[(2-[2-(2-[2-(Biotinylamino)ethoxy]ethoxy)ethoxy]ethoxy)methyl]-1-[(2-[2-(2-[4-(1*H*-pyrrol-3-yl)-butanoylamino]ethoxy)ethoxy]ethoxy)ethyl]-1*H*-1,2,3-triazole (3a). Reaction time 5 h. Highly viscous dark brown oil; yield: 156 mg (96 %), R_f 0.14 (CH_2Cl_2/MeOH/HCOOH, 90:10:1). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.38 (m, 2H, CH_2-C$\underline{H}_2$-CH_2), 1.63 (m, 4H, CH_{biotin}-C$\underline{H}_2$-C$\underline{H}_2$), 1.85 (m, 2H, CH_2-C$\underline{H}_2$-CH_2), 2.16 (t, 4H, *J* 7.4, C_{ar}-C$\underline{H}_2$-CH_2, CH_2-C$\underline{H}_2$-CO), 2.47 (t, 2H, *J* 7.4, CH_2-C$\underline{H}_2$-CO), 2.68 (d, 1H, *J* 12.7, CH_{biotin}-C$\underline{H}_2$-S), 2.84 (dd, 1H, J_1 4.8, J_2 12.7, CH_{biotin}-C$\underline{H}_2$-S), 3.08 (m, 1H, C$\underline{H}_{biotin}$-CH_2-CH_2), 3.37 (m, 4H, 2xNH-C$\underline{H}_2$-CH_2-O), 3.50 (m, 4H, 2xNH-CH_2-C$\underline{H}_2$-O), 3.54-3.66 (m, 20H, 5xO-C$\underline{H}_2$-C$\underline{H}_2$-O), 3.81 (t, 2H, *J* 5.1, C$\underline{H}_2$-CH_2-$N_{triazole}$), 4.25 (dd, 1H, J_1 4.5, J_2 7.1, NH_{biotin}-C$\underline{H}$-CH-S), 4.44 (dd, 1H, J_1

5.1, J_2 7.1, NH biotin-CH-CH$_2$-S), 4.47 (t, 2H, J 5.1, CH$_2$-CH$_2$-N triazole), 4.62 (s, 2H, O-CH$_2$-C triazole), 5.79 (s, 1H, CO-NH), 5.98 (dd, 1H, J_1 2.5, J_2 4.1, CH Py), 6.30 (t, 1H, J 5.4, CO-NH), 6.48 (s, 1H, CO-NH), 6.52 (m, 1H, CH Py), 6.64 (dd, 1H, J_1 2.5, J_2 4.7, CH Py), 6.83 (t, 1H, J 5.5, CO-NH), 7.69 (s, 1H, CH triazole), 8.84 (s, br, 1H, NH Py). ^{13}C-NMR (CDCl$_3$, 125 MHz): δ 25.6 (CH$_2$-CH$_2$-CH$_2$), 26.4 (CH$_2$-CH$_2$-CH$_2$), 27.1 (CH$_2$-CH$_2$-CO), 28.1 (CH biotin-CH$_2$-CH$_2$), 28.3 (CH biotin-CH$_2$-CH$_2$), 35.9 (CH$_2$-CH$_2$-CO), 36.1 (C$_{ar}$-CH$_2$-CH$_2$), 39.1 (NH-CH$_2$-CH$_2$-O), 39.2 (NH-CH$_2$-CH$_2$-O), 40.5 (CH biotin-CH$_2$-S), 50.2 (CH$_2$-CH$_2$-N triazole), 55.7 (CH biotin-CH$_2$-CH$_2$), 60.3 (NH biotin-CH-CH$_2$-S), 61.8 (NH biotin-CH-CH-S), 64.5 (O-CH$_2$-C triazole), 69.4 (O-CH$_2$-CH$_2$-O), 69.6 (O-CH$_2$-CH$_2$-O), 69.9 (2xO-CH$_2$-CH$_2$-O), 70.1 (O-CH$_2$-CH$_2$-O), 70.2 (O-CH$_2$-CH$_2$-O), 70.4 (2xO-CH$_2$-CH$_2$-O), 70.5 (5xO-CH$_2$-CH$_2$-O), 108.2 (CH Py), 115.3 (CH Py), 117.8 (CH Py), 122.9 (C$_{q,\ Py}$), 124.0 (CH triazole), 144.7 (C$_{q,\ triazole}$), 164.1 (C=O), 173.5 (2xC=O).

FTIR (cm^{-1}): 3294 ν (NH & triazole), 2924 ν_{as} (CH$_2$), 2859 ν_s (CH$_2$), 1698 ν (C=O biotin), 1648 ν (C=O), 1550 ν (C=C triazole), 1455 δ (CH$_2$), 1093 ν_{as} (C-O-C TEG), 843 γ (CH triazole), 730 δ (CH Py). HRMS (ESI): *m/z* [M+H]$^+$ calcd for C$_{37}$H$_{63}$N$_8$O$_{10}$S: 811.4382; found: 811.4384.

***N*$_\alpha$, *N*$_\alpha$-Bis(2-methoxycarbonylmethyl)-*N*$_\varepsilon$-[([1-(2-[2-(2-[4-(1*H*-pyrrol-3-yl)-butanoylamino]ethoxy)ethoxy]ethoxy)ethyl]-1*H*-1,2,3-triazol-4-yl)butanoylamino]-L-lysinmethylester (3b).** Reaction time 4 h. Brown oil; yield: 147 mg (98 %), R$_f$ 0.25 (CH$_2$Cl$_2$/MeOH, 9:1).

^{1}H-NMR (CDCl$_3$, 500 MHz): δ 1.47 (m, 4H, 2xCH$_2$-CH$_2$-CH$_2$), 1.65 (m, 2H, CH-CH$_2$-CH$_2$), 1.87 (m, 2H, CH$_2$-CH$_2$-CH$_2$), 1.94 (m, 2H, CH$_2$-CH$_2$-CO), 2.16 (t, 2H, J 7.6, C$_{ar}$-CH$_2$-CH$_2$), 2.20 (t, 2H, J 7.1, CH$_2$-CH$_2$-CO), 2.47 (t, 2H, J 7.2, CH$_2$-CH$_2$-CH$_2$), 2.69 (t, 2H, J 7.1, CH$_2$-CH$_2$-C triazole), 3.19 (m, 2H, NH-CH$_2$-CH$_2$), 3.38 (m, 3H, NH-CH$_2$-CH$_2$, CH), 3.49 (m, 2H, NH-CH$_2$-CH$_2$-O), 3.53-3.69 (m, 21H, 2xO-CH$_2$-CH$_2$-O, 2xCH$_2$-COOCH$_3$, 3xOCH$_3$), 3.80 (t, 2H, J 4.9, CH$_2$-CH$_2$-N triazole), 4.43 (t, 2H, J 4.9, CH$_2$-CH$_2$-N triazole), 5.99 (m, 1H, CH Py), 6.19 (t, 1H, J 3.8, CO-NH), 6.36 (t, 1H, J 4.9, CO-NH), 6.52 (m, 1H, CH Py), 6.65 (m, 1H, CH Py), 7.44 (s, 1H, CH triazole), 8.74 (s, br, 1H, NH Py).

^{13}C-NMR (CDCl$_3$, 125 MHz): δ 23.0 (CH$_2$-CH$_2$-CH$_2$), 24.9 (CH$_2$-CH$_2$-C triazole), 25.7 (CH$_2$-CH$_2$-CH$_2$), 26.4 (CH$_2$-CH$_2$-CH$_2$), 27.1 (CH$_2$-CH$_2$-CO), 28.7 (CH$_2$-CH$_2$-CH$_2$), 29.8 (C$_\alpha$H-CH$_2$-CH$_2$), 35.6 (CH$_2$-CH$_2$-CO), 36.1 (C$_{ar}$-CH$_2$-CH$_2$), 39.2 (2xNH-CH$_2$-CH$_2$), 50.1 (CH$_2$-CH$_2$-N triazole), 51.5 (OCH$_3$), 51.7 (2xOCH$_3$), 52.6 (2xCH$_2$-COOCH$_3$), 64.4 (C$_\alpha$H), 69.5 (O-CH$_2$-CH$_2$-O), 69.9 (O-CH$_2$-CH$_2$-O), 70.2 (O-CH$_2$-CH$_2$-O), 70.4 (O-CH$_2$-CH$_2$-O), 70.5 (2xO-CH$_2$-CH$_2$-O), 108.3 (CH Py), 115.3 (CH Py), 117.8 (CH Py), 122.2 (CH triazole), 122.9 (C$_{q,\ Py}$), 147.3 (C$_{q,\ triazole}$), 171.9 (2xC=O), 172.9 (C=O), 173.1 (C=O), 173.4 (C=O).

FTIR (cm^{-1}): 3305 ν (NH & triazole), 2927 ν_{as} (CH$_2$), 2865 ν_s (CH$_2$), 1733 ν (C=O ester), 1647 ν (C=O), 1537 ν (C=C triazole), 1435 δ (CH$_2$), 1202, 1143 ν (C-O ester), 1107 ν_{as} (C-O-C TEG), 844 γ (CH triazole), 729 δ (CH Py).

HRMS (ESI): *m/z* [M+H]$^+$ calcd for C$_{35}$H$_{57}$N$_7$O$_{11}$: 752.4189; found: 752.4187.

***N*$_\alpha$, *N*$_\alpha$-Bis(2-methoxycarbonylmethyl)-*N*$_\varepsilon$-[([1-(2-[2-(2-[4-(1*H*-pyrrol-3-yl)-butanoylamino]ethoxy)ethoxy]ethoxy)ethyl]-1*H*-1,2,3-triazol-4-yl)-2-(2-[2-(2-[2-methoxyethoxy]ethoxy)**

ethoxy]ethoxy)acetamido]-L-lysine methyl ester (3c). Reaction time 4 h. Dark brown oil; yield 184 mg (99 %), R_f 0.27 (CH_2Cl_2/MeOH, 9:1).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.42 (m, 4H, 2xCH_2-C$\underline{H}_2$-CH_2), 1.64 (m, 2H, CH-C$\underline{H}_2$-CH_2), 1.83 (m, 2H, CH_2-C$\underline{H}_2$-CH_2), 2.13 (t, 2H, *J* 7.5, C_{ar}-C$\underline{H}_2$-CH_2), 2.44 (t, 2H, *J* 7.2, CH_2-C$\underline{H}_2$-CO), 3.20 (m, 2H, NH-C$\underline{H}_2$-CH_2), 3.36 (m, 3H, NH-C$\underline{H}_2$-CH_2, C$\underline{H}$), 3.45-3.69 (m, 39H, 13xO-C$\underline{H}_2$-CH_2-O, 2xC$\underline{H}_2$-$COOCH_3$, 3xOC$\underline{H}_3$), 3.79 (t, 2H, *J* 5.0, C$\underline{H}_2$-CH_2-N $_{triazole}$), 3.90 (s, 2H, O-C$\underline{H}_2$-CONH), 4.44 (t, 2H, *J* 5.0, CH_2-C$\underline{H}_2$-N $_{triazole}$), 4.59 (s, 2H, O-C$\underline{H}_2$-C $_{triazole}$), 5.96 (m, 1H, C$\underline{H}$ $_{Py}$), 6.13 (m, br, 1H, CO-N$\underline{H}$), 6.49 (m, 1H, C$\underline{H}$ $_{Py}$), 6.62 (m, 1H, C$\underline{H}$ $_{Py}$), 6.92 (m, br, 1H, CO-N$\underline{H}$), 7.65 (s, 1H, C$\underline{H}$ $_{triazole}$), 8.73 (s, br, 1H, N$\underline{H}$ $_{Py}$).

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 23.2 (CH_2-$\underline{C}H_2$-CH_2), 26.3 (CH_2-$\underline{C}H_2$-CO), 27.0 (CH_2-$\underline{C}H_2$-CH_2), 29.2 (CH_2-$\underline{C}H_2$-CH_2), 30.0 (C_αH-$\underline{C}H_2$-CH_2), 36.0 (C_{ar}-$\underline{C}H_2$-CH_2), 38.6 (NH-$\underline{C}H_2$-CH_2), 39.1 (NH-$\underline{C}H_2$-CH_2), 50.1 (CH_2-$\underline{C}H_2$-N $_{triazole}$), 51.4 (O$\underline{C}H_3$), 51.6 (2xO$\underline{C}H_3$), 52.3 (2x$\underline{C}H_2$-$COOCH_3$), 64.5 (O-$\underline{C}H_2$-C $_{triazole}$), 64.6 ($\underline{C}_\alpha$H), 69.3 ($\underline{C}H_2$-CH_2-N $_{triazole}$), 69.6 (O-$\underline{C}H_2$-CH_2-O), 69.8 (O-$\underline{C}H_2$-CH_2-O), 70.1 (O-$\underline{C}H_2$-CH_2-O, O-$\underline{C}H_2$-CO), 70.3 (2xO-$\underline{C}H_2$-CH_2-O), 70.5 (7xO-$\underline{C}H_2$-CH_2-O), 70.9 (O-$\underline{C}H_2$-CH_2-O), 108.1 ($\underline{C}$H $_{Py}$), 115.2 ($\underline{C}$H $_{Py}$), 117.7 ($\underline{C}$H $_{Py}$), 122.8 ($\underline{C}_{q,\ Py}$), 123.8 ($\underline{C}$H $_{triazole}$), 144.8 ($\underline{C}_{q,\ triazole}$), 169.8 ($\underline{C}$=O), 171.7 (2x$\underline{C}$=O), 172.9 ($\underline{C}$=O), 173.3 ($\underline{C}$=O).

FTIR (cm^{-1}): 3337 ν (NH & triazole), 2922 ν_{as} (CH_2), 2866 ν_s (CH_2), 1732 ν (C=O $_{ester}$), 1659 ν (C=O), 1535 ν (C=C $_{triazole}$), 1435 δ (CH_2), 1202, 1139 ν (C-O $_{ester}$), 1099 ν_{as} (C-O-C $_{TEG}$), 843 γ (CH $_{triazole}$), 729 δ (CH $_{Py}$).

HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{42}H_{71}N_7O_{16}Na$: 952.4850; found: 952.4849.

***cyclo*-(L-Argininyl-glycinyl-L-aspartyl-D-phenylalaninyl-N_γ-[([1-(2-[2-(2-[4-(1*H*-pyrrol-3-yl)-butanoylamino]ethoxy)ethoxy]ethoxy)ethyl]-1*H*-1,2,3-triazol-4-yl)methylamino]-L-glutamic acid) (3d).** Reaction time 5 h. Brown oil; yield: 197 mg (99 %)

^{1}H-NMR (DMF-d_7, 500 MHz): δ 1.58 (m, 2H, C_αH-CH_2-C$\underline{H}_{2,\ Arg}$), 1.76 (m, 3H, C_αH-C$\underline{H}_2$-$CH_{2,\ Arg}$, CH_2-C$\underline{H}_2$-CH_2), 1.83 (d, 1H, *J* 5.1, C_αH-C$\underline{H}_2$-$CH_{2,\ Arg}$), 1.96 (m, 2H, C_αH-C$\underline{H}_2$-$CH_{2,\ Glu}$), 2.15 (t, 4H, *J* 7.4, C_{ar}-C$\underline{H}_2$-CH_2, CH_2-C$\underline{H}_2$-CO $_{Glu}$), 2.39 (m, 4H, CH_2-C$\underline{H}_2$-CO, C_αH-C$\underline{H}_2$-CO $_{Asp}$), 2.83 (s, 1H, C_αH-C$\underline{H}_2$-Ph $_{Phe}$), 3.01 (dd, 1H, J_1 6.8, J_2 13.2, C_αH-C$\underline{H}_2$-Ph $_{Phe}$), 3.27 (m, 5H, NH-C$\underline{H}_2$-CH_2-O, CH_2-C$\underline{H}_2$-NH $_{Arg}$, 1xC$\underline{H}_{2,\ Gly}$), 3.42-3.64 (m, 10H, 5xO-C$\underline{H}_2$-CH_2), 3.85 (s, 2H, C$\underline{H}_2$-CH_2-N $_{triazole}$), 4.20 (m, 3H, C_αH $_{Glu}$, C_αH $_{Phe}$, 1xC$\underline{H}_{2,\ Gly}$), 4.38 (s, 2H, NH-C$\underline{H}_2$-C $_{triazole}$), 4.53 (s, 2H, CH_2-C$\underline{H}_2$-N $_{triazole}$), 4.63 (d, 1H, *J* 5.0, C_αH $_{Arg}$), 4.79 (m, 1H, C_αH $_{Asp}$), 5.88 (s, 1H, C$\underline{H}$ $_{Py}$), 6.54 (s, 1H, C$\underline{H}$ $_{Py}$), 6.65 (s, 1H, C$\underline{H}$ $_{Py}$), 7.17 (m, 5H, 5xC$\underline{H}_{ar,\ Phe}$), 7.44 (m, br, 2H, 2xN$\underline{H}$), 7.65 (m, br, 1H, N$\underline{H}$), 7.80 (s, 1H, N$\underline{H}$), 7.87 (s, 1H, C$\underline{H}$ $_{triazole}$), 8.14 (d, 1H, *J* 6.3, N$\underline{H}$), 8.18 (m, br, 2H, 2xN$\underline{H}$), 8.36 (s, br, 2H, 2xN$\underline{H}$), 10.43 (s, br, 1H, COO$\underline{H}$).

^{13}C-NMR (DMF-d_7, 125 MHz): δ 26.1 (C_αH-CH_2-$\underline{C}H_{2,\ Arg}$), 27.1 (CH_2-$\underline{C}H_2$-CH_2), 28.1 (CH_2-$\underline{C}H_2$-CO), 28.4 (C_αH-$\underline{C}H_2$-$CH_{2,\ Glu}$), 28.5 (C_αH-$\underline{C}H_2$-$CH_{2,\ Arg}$), 32.7 (CH_2-$\underline{C}H_2$-CO $_{Glu}$), 35.2 (NH-$\underline{C}H_2$-C $_{triazole}$), 36.1 (C_αH-$\underline{C}H_2$-CO $_{Asp}$, C_{ar}-$\underline{C}H_2$-CH_2), 37.8 (C_αH-$\underline{C}H_2$-Ph $_{Phe}$), 39.4 (NH-$\underline{C}H_2$-CH_2-O), 41.4 (CH_2-$\underline{C}H_2$-NH $_{Arg}$), 44.0 ($\underline{C}H_{2,\ Gly}$), 50.4 (CH_2-$\underline{C}H_2$-N $_{triazole}$), 53.1 ($\underline{C}_\alpha$H $_{Asp}$), 54.7 ($\underline{C}_\alpha$H $_{Glu}$), 55.1 ($\underline{C}_\alpha$H $_{Arg}$), 55.4 ($\underline{C}_\alpha$H $_{Phe}$), 69.7 ($\underline{C}H_2$-CH_2-N $_{triazole}$), 70.2 (O-$\underline{C}H_2$-CH_2-O), 70.3 (O-$\underline{C}H_2$-CH_2-O), 70.6 (O-$\underline{C}H_2$-CH_2-O), 70.7 (O-$\underline{C}H_2$-CH_2-O), 70.8 (NH-CH_2-$\underline{C}H_2$-O), 108.1 ($\underline{C}$H $_{Py}$), 115.6 ($\underline{C}$H $_{Py}$), 118.1 ($\underline{C}$H $_{Py}$), 123.1 ($\underline{C}_{q,\ Py}$), 126.7 ($\underline{C}$H $_{triazole}$), 126.9 ($\underline{C}H_{ar}$), 128.8

(2x<u>C</u>H$_{ar}$), 129.9 (2x<u>C</u>H$_{ar}$), 138.4 (<u>C</u>$_{q, ar}$), 145.9 (<u>C</u>$_{q, triazole}$), 158.3 (<u>C</u>$_q$=NH), 170.4 (<u>C</u>=O), 171.3 (<u>C</u>=O), 171.9 (<u>C</u>=O), 172.3 (<u>C</u>=O), 172.4 (<u>C</u>=O), 173.1 (<u>C</u>=O), 173.3 (<u>C</u>=O), 173.5 (<u>C</u>=O).

UPLC-MS: t_R 2.45 min; m/z 995.51; (Column UPLC® *BEH C18* 2.1 x 50 mm, 1.7 μm; gradient: acetonitril : water, 05 : 95 => 50 : 05; flow: 0.6 ml/min).

FTIR (cm^{-1}): 3302 ν (NH & triazole), 2926 ν_{as} (CH$_2$), 2870 ν_s (CH$_2$), 1652 ν (C=O), 1541 ν (C=C$_{triazole}$), 1096 ν_{as} (C-O-C$_{TEG}$), 844 γ (CH$_{triazole}$).

HRMS (ESI): *m/z* [M+ H]$^+$ calcd for $C_{45}H_{67}N_{14}O_{12}$: 995.5057; found: 995.5058

***N*α, *N*α-Bis(2-methoxycarbonylmethyl)-*N*ε-(5-hexinamido)-L-lysine trimethyl ester (2b).** EDC (365 mg, 2.35 mmol), 1-hydroxybenzotriazol (HOBt) (366 mg, 2.72 mmol) and DIPEA (257 mg, 1.99 mmol) were added to a solution of 5-hexynoic acid **4a** (203 mg, 1.81 mmol) and lysine derivative **5** (605 mg, 1.99 mmol) in CH_2Cl_2 (18 ml) under argon. After stirring for 20 h the reaction mixture was diluted with CH_2Cl_2 (18 ml) and washed with 3 M aqeous HCl (2 x 20ml) and water (2 x 25ml). The organic layer was dried over $MgSO_4$ and the solvent was removed under vacuum by a rotary evaporator. The remainder was purified by column chromatography providing 359 mg (0.90 mmol, 50 %) of the product **2b** as yellow oil.

R_f 0.36 (CH_2Cl_2/MeOH, 95:5).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.46 (m, 4H, C$_α$H-C<u>H</u>$_2$-C<u>H</u>$_2$), 1.65 (m, 2H, CH$_2$-C<u>H</u>$_2$-CH$_2$), 1.81 (m, 2H, CH$_2$-C<u>H</u>$_2$-CH$_2$), 1.93 (t, 1H, *J* 2.6, CH$_2$-C≡C<u>H</u>), 2.20 (m, 2H, C<u>H</u>$_2$-C≡CH), 2.82 (t, 2H, *J* 7.4, CH$_2$-C<u>H</u>$_2$-CONH), 3.20 (m, 2H, CH$_2$-C<u>H</u>$_2$-NH$_2$), 3.39 (t, 1H, *J* 7.6, C$_α$<u>H</u>), 3.58 (s, 4H, 2xC<u>H</u>$_2$-COOCH$_3$), 3.65 (s, 9H, 3xOC<u>H</u>$_3$), 6.06 (s, br, 1H, N<u>H</u>).

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 18.0 (<u>C</u>H$_2$-C≡CH), 22.9 (CH$_2$-<u>C</u>H$_2$-CH$_2$), 24.4 (CH$_2$-<u>C</u>H$_2$-CH$_2$), 28.5 (CH$_2$-<u>C</u>H$_2$-CH$_2$), 29.7 (C$_α$H-<u>C</u>H$_2$-CH$_2$), 35.1 (CH$_2$-<u>C</u>H$_2$-CONH), 39.2 (CH$_2$-<u>C</u>H$_2$-NH), 51.5 (O<u>C</u>H$_3$), 51.8 (2xO<u>C</u>H$_3$), 52.6 (2x<u>C</u>H$_2$-COOCH$_3$), 64.3 (<u>C</u>$_α$H), 69.1 (CH$_2$-C≡<u>C</u>H), 83.7 (<u>C</u>$_q$, CH$_2$-<u>C</u>≡CH), 171.9 (2x<u>C</u>=O), 172.6 (<u>C</u>=O), 173.2 (<u>C</u>=O).

FTIR (cm^{-1}): 3293 ν (NH & CH: C≡C<u>H</u>), 2951 ν_{as} (CH$_2$), 2865 ν_s (CH$_2$), 2114 ν (C≡C), 1730 ν (C=O$_{ester}$), 1648 ν (C=O), 1536 ν (N-C=O), 1434 δ (CH$_2$), 1200, 1153 ν (C-O$_{ester}$).

HRMS (ESI): *m/z* [M+ Na]$^+$ calcd for $C_{19}H_{30}N_2O_7Na$: 421.1945; found: 421.1945.

***N*α, *N*α-Bis(2-methoxycarbonylmethyl)-*N*ε-(2-[2-(2-[2-(2-propargyloxyethoxy)-ethoxy] ethoxy)ethoxy]acetamido)-L-lysine trimethyl ester (2c)**. The glycolic acid derivative **4b** (406 mg, 1.40 mmol) and the lysine derivative **5** (469 mg, 1.54 mmol) were reacted in CH_2Cl_2 (14 ml) with EDC (283 mg, 1.82 mmol), HOBt (284 mg, 2.10 mmol) and DIPEA (199 mg, 1.54 mmol) as shown for the preparation of **2b** resulting in 426 mg (0.74 mmol, 53 %) of the product **2c** as yellow oil. R_f 0.42 (CH_2Cl_2/MeOH, 9:1).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.29 (m, 1H, C$_α$H-C<u>H</u>$_2$-CH$_2$), 1.45 (m, 3H, C$_α$H-C<u>H</u>$_2$-C<u>H</u>$_2$), 1.64 (m, 2H, CH$_2$-C<u>H</u>$_2$-CH$_2$), 2.40 (t, 1H, *J* 2.4, CH$_2$-C≡C<u>H</u>), 3.21 (dd, 2H, *J*$_1$ 7.0, *J*$_2$ 13.4, CH$_2$-C<u>H</u>$_2$-NH$_2$), 3.35 (t, 1H, *J* 7.6, C$_α$<u>H</u>), 3.55-3.70 (m, 29H, 4xO-C<u>H</u>$_2$-C<u>H</u>$_2$-O, 2xC<u>H</u>$_2$-COOCH$_3$, 3xOC<u>H</u>$_3$), 3.91 (s, 2H, O-C<u>H</u>$_2$-CO), 4.14 (d, 2H, *J* 2.4, C<u>H</u>$_2$-C≡CH), 6.94 (t, 1H, *J* 5.7, CO-N<u>H</u>).

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 23.3 (CH$_2$-<u>C</u>H$_2$-CH$_2$), 29.3 (CH$_2$-<u>C</u>H$_2$-CH$_2$), 30.1 (C$_α$H-<u>C</u>H$_2$-CH$_2$), 38.6 (CH$_2$-<u>C</u>H$_2$-NH), 51.4 (O<u>C</u>H$_3$), 51.6 (2xO<u>C</u>H$_3$), 52.4 (2x<u>C</u>H$_2$-COOCH$_3$), 58.4 (<u>C</u>H$_2$-C≡CH), 64.7 (<u>C</u>$_α$H), 69.1 (O-<u>C</u>H$_2$-CO), 70.2 (O-<u>C</u>H$_2$-CH$_2$-O), 70.4 (2xO-<u>C</u>H$_2$-CH$_2$-O), 70.6

(4xO-$\underline{C}H_2$-CH_2-O), 71.0 (O-$\underline{C}H_2$-CH_2-O), 74.6 (CH_2-C≡$\underline{C}$H), 79.7 (C_q, CH_2-$\underline{C}$≡CH), 169.8 ($\underline{C}$=O), 171.7 (2x$\underline{C}$=O), 173.0 ($\underline{C}$=O).

FTIR (cm^{-1}): 3351 ν (NH), 3264 ν (CH: C≡C$\underline{H}$), 2950 ν_{as} (CH_2), 2866 ν_s (CH_2), 2113 ν (C≡C), 1731 ν (C=O $_{ester}$), 1669 ν (C=O), 1531 ν (N-C=O), 1435 δ (CH_2), 1200, 1143 ν (C-O $_{ester}$), 1099 ν_{as} (C-O-C $_{TEG}$). HRMS (ESI): *m/z* [M+ Na]$^+$ calcd for $C_{26}H_{44}N_2O_{12}Na$: 599.2786; found: 599.2786.

***β-tert*-Butyl-L-aspartyl-D-phenylalaninyl-N_γ-(2-propinylamino)-L-glutamyl-N_ω-2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl-L-argininyl-glycine (Asp(O^{t}Bu)-D-Phe-Glu(CH_2-C≡CH)-Arg(Pbf)-Gly) (7).** Fmoc-Gly-loaded 2-chlorotrityl resin (2.10 g, 1.14 mmol, loading 0.543 mmol*g^{-1}) was shaken in *N,N*-dimethylformamide (DMF) (10 ml) in a reversed frit filter funnel for 30 min. After removal of the DMF the resin was sequentially washed with CH_2Cl_2/MeOH/DIPEA (17:2:1) (3x6 ml), with CH_2Cl_2 (3x6 ml), with DMF (3x6 ml) and with CH_2Cl_2 (3x6 ml) and then treated with a piperidine/DMF-solution (1:4, 2x6 ml) under shaking for 10 min. The resin was washed with DMF (3x6 ml), CH_2Cl_2 (3x6 ml) and DMF (3x6 ml) and then combined with a solution of Fmoc-L-Arg(Pbf)-OH (1.48 g, 2.28 mmol, 2.0 eq.), HBTU (822 mg, 2.17 mmol, 1.9 eq.), HOBt (310 mg, 2.28 mol, 2.0 eq.) and *N*-methylmorpholine (NMM) (500 µl, 4.56 mmol, ρ 0.920 g*cm^{-3}, 4.0 eq.) in CH_2Cl_2 (3 ml) and DMF (12 ml). After shaking at rt for 1 h the solution was removed and the resin washed with DMF (3x6 ml), CH_2Cl_2 (3x6 ml) and (3x6 ml). For capping the resin was shaken with 30 % acetic anhydride in pyridine (6 ml) for 10 min and subsequently washed with 30 % acetic anhydride in pyridine (6 ml), DMF (3x6 ml), CH_2Cl_2 (3x6 ml) and DMF (3x6 ml). Using the same procedures, the following amino acids were coupled in the subsequent steps: Fmoc-L-Glu(CH_2-C≡CH)-OH (927 mg, 2.28 mmol, 2.0 eq.), Fmoc-D-Phe-OH (884 mg, 2.28 mmol, 2.0 eq.), Fmoc-L-Asp(O^{t}Bu)-OH (938 mg, 2.28 mmol, 2.0 eq.) affording the Fmoc- Asp(O^{t}Bu)-D-Phe-Glu(CH_2-C≡CH)-Arg(Pbf)-Gly sequence at the resin. After deprotection by treatment with piperidine/DMF (1:4, 2x6 ml) and washing with DMF (3x6 ml), CH_2Cl_2 (3x6 ml) and DMF (3x6 ml) the pentapaeptide was deliberated by shaking with a 1 % solution of TFA in CH_2Cl_2 (4x8 ml) for 3 min. The washed solution was poured into a 10% solution of pyridine in methanol (40 ml), in which also were given all solutions obtained by further washing of the resin with CH_2Cl_2 (3x6 ml), methanol (3x6 ml), CH_2Cl_2 (3x6 ml) and methanol (3x6 ml). All solvents were removed under vacuum by a rotary evaporator. The residue was purified by semi-preparative HPLC providing 885 mg (77%) of the pentapeptide **7** as a colorless solid. mp 180-182 °C (partial decomposition).

^{1}H-NMR (DMF-d_7, 300 MHz): δ 1.39 (s, 9H, C($C\underline{H}_3$)$_3$), 1.42 (d, 6H, C($C\underline{H}_3$)$_2$), 1.57 (m, 2H, C_αH-CH_2-$C\underline{H}_{2,\ Arg}$), 1.73 (m, 1H, C_αH-$C\underline{H}_2$-$CH_{2,\ Arg}$), 1.89 (m, 2H, C_αH-$C\underline{H}_2$-$CH_{2,\ Glu}$, C_αH-$C\underline{H}_2$-$CH_{2,\ Arg}$), 2.10 (m, 6H, $C_{q,\ ar}$-$C\underline{H}_3$, C_αH-$C\underline{H}_2$-$C\underline{H}_{2,\ Glu}$), 2.50 (s, 3H, $C_{q,\ ar}$-$C\underline{H}_3$), 2.57 (s, 3H, $C_{q,\ ar}$-$C\underline{H}_3$), 2.74 (m, 2H, C_αH-$C\underline{H}_2$-Ph $_{Phe}$), 3.00 (m, 4H, C_αH-$C\underline{H}_2$-CO $_{Asp}$, CH_2-C≡C$\underline{H}$, C_q-$C\underline{H}_2$-$C_{q,\ ar}$), 3.16 (m, 3H, C_αH-$C\underline{H}_2$-CO $_{Asp}$, CH_2-$C\underline{H}_2$-NH $_{Arg}$), 3.63 (m, 1H, $C\underline{H}_{2,\ Gly}$), 3.83 (m, 1H, $C\underline{H}_{2,\ Gly}$), 3.95 (dd, 2H, J_1 2.2, J_2 5.2, $C\underline{H}_2$-C≡CH), 4.36 (m, 3H, $C_\alpha\underline{H}$ $_{Arg}$, $C_\alpha\underline{H}$ $_{Glu}$, $C_\alpha\underline{H}$ $_{Phe}$), 4.67 (m, 1H, $C_\alpha\underline{H}$ $_{Asp}$), 6.76 (s, br, 2H, 2xCO-N$\underline{H}$), 7.10 (s, br, 1H, CO-N$\underline{H}$), 7.25 (m, 5H, 5x$C\underline{H}_{ar,\ Phe}$), 7.79 (s,

br, 1H, CO-NH), 8.16 (d, 1H, *J* 7.7, CO-NH), 8.34 (t, 2H, *J* 5.3, 2xCO-NH), 8.79 (d, 1H, *J* 6.5, CO-NH), 8.97 (s, br, 1H, C_q=NH).

^{13}C-NMR (DMF-d_7, 75 MHz): δ 12.2 ($C_{q,\,ar}$-CH_3), 17.9 ($C_{q,\,ar}$-CH_3), 19.1 ($C_{q,\,ar}$-CH_3), 26.0 (CH_2-CH_2-$CH_{2,\,Arg}$), 27.5 (C_αH-CH_2-$CH_{2,\,Arg}$), 27.7 (3xC(CH_3)$_3$), 28.3 (2xC(CH_3)$_2$), 28.5 (CH_2-C≡CH), 29.3 (C_αH-CH_2-$CH_{2,\,Glu}$), 32.1 (CH_2-CH_2-CO $_{Glu}$), 37.4 (C_αH-CH_2-Ph), 38.0 (C_αH-CH_2-CO $_{Asp}$), 40.6 (CH_2-CH_2-NH $_{Arg}$), 42.7 ($CH_{2,\,Gly}$), 43.0 (C_q-CH_2-$C_{q,\,ar}$), 50.3 (C_αH $_{Arg}$), 53.2 (C_αH $_{Phe}$), 54.3 (C_αH $_{Glu}$), 55.9 (C_αH $_{Asp}$), 72.3 (CH_2-C≡CH), 81.1 (C_q, CH_2-C≡CH), 81.8 (C(CH_3)$_3$), 86.9 (C(CH_3)$_2$), 117.1 ($C_{q,\,ar}$-CH_3), 125.1 ($C_{q,\,ar}$-CH_3), 127.1 (CH_{ar}), 129.7 (2xCH_{ar}), 129.7 (2xCH_{ar}), 132.3 ($C_{q,\,ar}$-CH_3), 134.7 ($C_{q,\,ar}$), 137.5 ($C_{q,\,ar}$), 138.2 ($C_{q,\,ar}$), 157.2 (C_q=NH), 158.3 ($C_{q,\,ar}$-O-C(CH_3)$_2$-CH_2), 164.8 (C=O), 169.8 (C=O), 170.3 (C=O), 172.2 (C=O), 172.4 (C=O), 172.6 (2xC=O).

Semi-preparative HPLC-MS: t_R 9.5 min; *m/z* 968, (column: Luna-Phenyl-Hexyl 21.2 x 250 mm, 10 µm; isocratic: methanol : water, 65 : 35;flow: 22.0 ml/min).

HRMS (ESI): *m/z* [M+ H]$^+$ calcd for $C_{46}H_{66}N_9O_{12}S$: 968.4546; found: 968.4544.

***cyclo*-(*N*$_\omega$-2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl-L-argininyl-glycinyl-*β*-*tert*-butyl-L-aspartyl-D-phenylalaninyl-*N*$_\gamma$-(2-propinylamino)-L-glutamic acid) *cyclo*-(Arg(Pbf)-Gly-Asp(O^{t}Bu)-D-Phe-Glu(CH$_2$-C≡CH))(8).** PyBOP (169 mg, 0.325 mmol) and NMM (61 µl, 0.550 mmol, ρ 0.92 g cm^{-3}) were added to a mixture of the pentapeptide **7** (242 mg, 0.250 mmol) and dry DMF (250 ml) under argon. After stirring for 24 h the solvent was removed under vacuum by a rotary evaporator and the remainder was purified by column chromatography giving rise to the product **8 (**222 mg, 93 %) as yellow solid. mp. 215−216 °C (partial decomposition), R_f 0.23 (CH_2Cl_2/MeOH, 9:1).

^{1}H-NMR (DMF-d_7, 300 MHz): δ 1.37 (s, 9H, C(CH_3)$_3$), 1.44 (s, 6H, C(CH_3)$_2$), 1.72 (m, 4H, C_αH-CH_2-C$H_{2,\,Arg}$, C_αH-CH_2-$CH_{2,\,Glu}$), 1.91 (m, 2H, C_αH-CH_2-$CH_{2,\,Arg}$), 2.06 (s, 3H, $C_{q,\,ar}$-CH_3), 2.16 (dd, 2H, *J*$_1$ 8.7, *J*$_2$ 15.7, CH_2-CH_2-CO $_{Glu}$), 2.42 (dd, 1H, *J*$_1$ 6.0, *J*$_2$ 15.7, C_αH-CH_2-CO $_{Asp}$), 2.52 (s, 3H, $C_{q,\,ar}$-CH_3), 2.59 (s, 3H, $C_{q,\,ar}$-CH_3), 2.70 (m, 1H, C_αH-CH_2-CO $_{Asp}$), 2.90 (m, 1H, C_αH-CH_2-Ph $_{Phe}$), 3.01 (m, 2H, C$H_{2,\,Gly}$), 3.06 (m, 4H, C_αH-CH_2-Ph $_{Phe}$, CH_2-C≡CH, C_q-CH_2-$C_{q,\,ar}$), 3.19 (m, 2H, CH_2-CH_2-NH $_{Arg}$), 3.97 (dd, 2H, *J*$_1$ 2.2, *J*$_2$ 5.7, CH_2-C≡CH), 4.21 (m, 2H, C_αH $_{Glu}$, C_αH $_{Phe}$), 4.74 (m, 2H, C_αH $_{Arg}$, C_αH $_{Asp}$), 6.77 (m, br, 3H, 3xNH), 7.26 (m, 5H, 5xC$H_{ar,\,Phe}$), 8.23 (m, 5H, 5xNH), 8.37 (dd, 2H, *J*$_1$ 7.8, *J*$_2$ 11.3, 2xNH).

^{13}C-NMR (DMF-d_7, 75 MHz): δ 12.4 ($C_{q,\,ar}$-CH_3), 18.1 ($C_{q,\,ar}$-CH_3), 19.3 ($C_{q,\,ar}$-CH_3), 26.6 (C_αH-CH_2-$CH_{2,\,Arg}$), 26.7 (C_αH-CH_2-$CH_{2,\,Glu}$), 27.9 (3xC(CH_3)$_3$), 28.4 (C_αH-CH_2-$CH_{2,\,Arg}$), 28.5 (2xC(CH_3)$_2$), 28.7 (CH_2-C≡CH), 32.5 (CH_2-CH_2-CO $_{Glu}$), 37.2 (C_αH-CH_2-CO $_{Asp}$), 37.8 (C_αH-CH_2-Ph $_{Phe}$), 40.9 (CH_2-CH_2-NH $_{Arg}$), 43.3 ($CH_{2,\,Gly}$), 46.6 (C_q-CH_2-$C_{q,\,ar}$), 50.1 (C_αH $_{Asp}$), 53.3 (C_αH $_{Glu}$), 55.2 (C_αH $_{Arg}$), 55.3 (C_αH $_{Phe}$), 72.5 (CH_2-C≡CH), 80.6 (C_q, CH_2-C≡CH), 81.6 (C(CH_3)$_3$), 87.0 (C(CH_3)$_2$), 117.2 ($C_{q,\,ar}$-CH_3), 125.2 ($C_{q,\,ar}$-CH_3), 127.0 (CH_{ar}), 128.8 (2xCH_{ar}), 129.9 (2xCH_{ar}), 132.5 ($C_{q,\,ar}$-CH_3), 135.3 ($C_{q,\,ar}$), 138.3 ($C_{q,\,ar}$), 138.4 ($C_{q,\,ar}$), 157.3 ($C_{q,\,ar}$-O-C(CH_3)$_2$-CH_2), 158.5 (C_q=NH), 170.1 (C=O), 170.3 (C=O), 171.3 (C=O), 171.9 (C=O), 172.0 (C=O), 172.4 (C=O), 172.7 (C=O).

HRMS (ESI): *m/z* [M+ H]$^+$ calcd for $C_{46}H_{64}N_9O_{11}S$: 950.4446; found: 950.4452.

***cyclo*-(L-Argininyl-glycinyl-L-aspartyl-D-phenylalaninyl-N_γ-(2-propinylamino)-L-glutamic acid) *cyclo*-(Arg-Gly-Asp-D-Phe-Glu(CH_2-C≡CH)) (2d).** The cyclic pentapeptide **8** (342 mg, 0.360 mmol) was combined with a solution of TFA/triethylsilane/water (95:2.5:2.5) (6 ml) under stirring and ice cooling. The mixture was stirred under ice cooling for 30 min and then at rt for 2 h. CH_2Cl_2 (20 ml) was added and the solvents were distilled off under vacuum by a rotary evaporator. The remainder was dissolved in TFA (2 ml), combined with ice-cooled diethyl ether (8 ml) to precipitate the product, centrifuged, and the supernatant was removed by decantation. This treatment was repeated twice. The colorless solid product was dried by lyophilization providing the cyclic peptide **2d** (203 mg, 88 %) as colorless solid. mp 219−221 °C (partial decomposition).

^{1}H-NMR (DMF-d_7, 500 MHz): δ 1.61 (m, 3H, C_αH-C$\underline{H}_2$-C$\underline{H}_{2,\ Arg}$, C_αH-C$\underline{H}_2$-$CH_{2,\ Glu}$), 1.85 (m, 1H, C_αH-C$\underline{H}_2$-$CH_{2,\ Arg}$), 1.98 (m, 2H, C_αH-C$\underline{H}_2$-$CH_{2,\ Glu}$), 2.15 (m, 2H, CH_2-C$\underline{H}_2$-CO $_{Glu}$), 2.48 (dd, 1H, J_1 4.6, J_2 16.2, C_αH-C$\underline{H}_2$-CO $_{Asp}$), 2.88 (m, 2H, C_αH-C$\underline{H}_2$-CO $_{Asp}$, C_αH-C$\underline{H}_2$-Ph $_{Phe}$), 3.06 (m, 2H, C_αH-C$\underline{H}_2$-Ph $_{Phe}$, CH_2-C≡C$\underline{H}$), 3.35 (m, 3H, CH_2-C$\underline{H}_2$-NH $_{Arg}$, 1xC$\underline{H}_{2,\ Gly}$), 3.96 (s, 2H, C$\underline{H}_2$-C≡CH), 4.23 (m, 3H, $C_\alpha\underline{H}$ $_{Glu}$, $C_\alpha\underline{H}$ $_{Phe}$, 1xC$\underline{H}_{2,\ Gly}$), 4.67 (dd, 1H, J_1 6.5, J_2 13.5, $C_\alpha\underline{H}$ $_{Arg}$), 4.83 (dd, 1H, J_1 7.9, J_2 13.2, $C_\alpha\underline{H}$ $_{Asp}$), 7.23 (m, 5H, 5xC$\underline{H}_{ar,\ Phe}$), 7.42 (m, br, 1H, N$\underline{H}$), 7.65 (m, br, 2H, 2xN$\underline{H}$), 7.99 (s, 2H, 2xN$\underline{H}$), 8.05 (d, 1H, J 8.3, N$\underline{H}$), 8.15 (d, 1H, J 7.6, N$\underline{H}$), 8.23 (m, 2H, 2xN$\underline{H}$), 8.35 (m, 2H, N$\underline{H}$, COO$\underline{H}$).

^{13}C-NMR (DMF-d_7, 125 MHz): δ 26.0 (C_αH-CH_2-$\underline{C}H_{2,\ Arg}$), 28.3 (C_αH-$\underline{C}H_2$-$CH_{2,\ Glu}$), 28.5 (C_αH-$\underline{C}H_2$-$CH_{2,\ Arg}$), 28.6 ($\underline{C}H_2$-C≡CH), 32.5 (CH_2-$\underline{C}H_2$-CO $_{Glu}$), 36.6 (C_αH-$\underline{C}H_2$-CO $_{Asp}$), 37.9 (C_αH-$\underline{C}H_2$-Ph $_{Phe}$), 41.4 (CH_2-$\underline{C}H_2$-NH $_{Arg}$), 44.0 ($\underline{C}H_{2,\ Gly}$), 50.0 ($\underline{C}_\alpha$H $_{Asp}$), 53.0 ($\underline{C}_\alpha$H $_{Glu}$), 55.2 ($\underline{C}_\alpha$H $_{Arg}$), 55.4 ($\underline{C}_\alpha$H $_{Phe}$), 72.5 (CH_2-C≡$\underline{C}$H), 81.5 (C_q, CH_2-$\underline{C}$≡CH), 126.9 ($\underline{C}H_{ar}$), 128.8 (2x$\underline{C}H_{ar}$), 129.9 (2x$\underline{C}H_{ar}$), 138.4 ($\underline{C}_{q,\ ar}$), 158.2 ($\underline{C}_q$=NH), 170.3 ($\underline{C}$=O), 171.3 ($\underline{C}$=O), 171.9 ($\underline{C}$=O), 172.1 ($\underline{C}$=O), 172.3 ($\underline{C}$=O), 172.5 ($\underline{C}$=O), 173.0 ($\underline{C}$=O).

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{29}H_{40}N_9O_8$: 642.3000; found: 642.3002

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