Synthesis of glutaryl-containing derivatives of GRGD and KRGD peptides*

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New derivatives of tetrapeptides GRGD (Gly-Arg(Pbf)-Gly-Asp(OMe)-OMe) and KRGD (Boc-Lys-Arg(Pbf)-Gly-Asp(OMe)-OMe) containing a glutaric acid fragment as a linker were synthesized. The linker provides the possibility for binding these derivatives to other biologically active molecules or nanoparticles.

Key words: GRGD, KRGD, peptides, amino acids, coupling agents, peptide synthesis.

The so-called vector molecules (antibodies, aptamers, peptides, *etc*.) are used in the design of drugs for the treatment of cancer to ensure their targeted delivery and retention in the tumor. The mechanism of accumulation of such compounds can be based, for example, on the interaction with receptor proteins expressed on the surface of tumor cells. Antibodies are the most efficient among vector molecules. However, their isolation, purification, and storage are complex and very expensive. During identification of the binding sites of various antibodies or polypeptides with antigens, short peptide sequences were identified exhibiting biological activity comparable to that of antibodies.[1—4] Thus, it was found that peptides containing the Arg-Gly-Asp amino acid sequence (RGD** motif) in their structure play a key role in molecular recognition during cell adhesion.[5] Peptides of this family specifically interact with transmembrane receptors, for example, integrins $\alpha_{IIb/IIIa}$, $\alpha_v\beta_3$, and $\alpha_v\beta_5$, which are involved in tumor metastasis and angiogenesis.[6—8] Therefore, the family of RGD peptides is considered as one of the most promising for use as vector molecules in the development of drugs for the diagnosis and treatment of cancer. The cyclic peptide Cilengitide (c(RGDf-N(Me)V)[9] is currently undergoing clinical trials as a potential antitumor drug for the treatment of patients with glioblastoma and unmethylated MGMT promoter status. Peptide derivatives of the RGD family are used in design of angiogenesis-imaging drugs containing in their composition isotopic,[10—15] fluorescent,[16—21] or MRI contrast[21—23] labels, cytostatic agents,[24—26] or agents for photodynamic therapy.[16,21,27,28] Such materials are synthesized based on both the linear derivatives of the RGD peptide and the cyclic peptides (c(RGDfK), c(RGDyK), c(RGDfV), c(RGDfE), c(RGDfC), *etc*.). The RGD peptide derivatives are either commercially available or can be obtained by solid-phase synthesis or by synthesis in solution. The solid-phase synthesis of pentapeptides, as a rule, is carried out according to the Fmoc strategy, starting from Gly[14,25,28—30] or L-Asp(OBu^t)[27,31,32] at the C-terminus. The linear derivatives of the RGD peptide are synthesized in solution using both the Fmoc and the Boc strategies. In this case, the synthesis begins from *C*-protected derivatives of L-Asp at the C-terminus[24,33—37] or from *N*-protected derivatives of L-Arg at the N-terminus.[27,38] For the covalent binding of cyclic peptides, for example, RGDyK or RGDfC, to biomolecules (or to the surface of nanoparticles), the ε-amino group of L-Lys of the RGDyK fragment or the thiol group of L-Cys of the RGDfC fragment, respectively, are used. In this case, the binding can be accomplished either directly[28] or through a linker (derivatives of polyethylene glycol,[10—13,15,27] tripeptides (GGG, DDD, SSS),[10,14] or maleimide cross-linkers,[20,21] ε-aminocaproic,[27,39] glutaric, or adipic acids[35,36]). The linear RGD peptide is modified at the α-amino group of L-Arg.[24]

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** In abbreviations of the peptide names c(RGDfK), c(RGDyK), c(RGDfV), c(RGDfE), and c(RGDfC), the letter c stands for cyclo- (cyclopeptide), all other symbols are the designation of amino acids in the single-letter system: R is L-Arg, L-arginine; G is Gly, glycine; D is L-Asp, L-aspartic acid; f is D-Phe, D-phenylalanine; y is D-Tyr, D-tyrosine; K is L-Lys, L-lysine; E is L-Glu, L-glutamic acid; C is L-Cys, L-cysteine; V is L-Val, L-valine; S is L-Ser, L-serine. The following protective group abbreviations were used: Pbf is 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; Mds is 4-methoxy-2,6-dimethylphenylsulfonyl; Mtr is 4-methoxy-2,3,6-trimethylphenylsulfonyl; Pmc is 2,2,5,7,8-pentamethylchroman-6-sulfonyl; Mis is 1,2-dimethylindole-3-sulfonyl.

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In the case of longer linear peptides, the ε-amino group of L-Lys is most often used.[15,23,28]

The purpose of the present work is to develop and optimize methods for the synthesis of glutaryl-containing derivatives of tetrapeptides GRGD (Gly-Arg(Pbf)-Gly-Asp(OMe)-OMe) и KRGD (Boc-Lys-Arg(Pbf)-Gly-Asp(OMe)-OMe), which are capable of conjugation with other biologically active molecules or nanoparticles without involvement in the synthesis of the guanidine group of Arg and the carboxyl groups of Asp and, therefore, are able to act as vector molecules in such constructs.

Results and Discussion

The synthesis was carried out using traditional methods of peptide synthesis in solution by sequential peptide chain extension, starting from the C-terminus and L-Asp(OMe)-OMe (**1**) (Scheme 1), similarly to the earlier developed[35,36] method for the preparation of linker-containing derivatives of RGD peptide. Protecting groups in the amino acids were chosen so that, in the molecules of the target peptides, the guanidine group of the Arg residue and the β-carboxyl group of Asp remained uninvolved in the formation of amide bonds, which is necessary to preserve the specificity of the peptide binding to $\alpha_v\beta_3$ integrins expressed on the surface of tumor cells.[10]

N-Protected dimethyl glycyl-L-aspartate (**7**) was obtained at the first stage (see Scheme 1). *N*-(Fluorenylmethoxycarbonyl)glycyl chloride (**2**) and *N*-(*tert*-butyloxycarbonyl)glycine (**3**) were used as *N*-protected glycine derivatives. Chloride **2** was synthesized in quantitative yield from Fmoc-Gly-OH treated with oxalyl chloride[36,40] and was further used for the synthesis of dipeptide **4** without additional purification. The coupling of Boc-Gly-OH (**3**) with Asp(OMe)-OMe (**1**) was carried out using various carbodiimide coupling agents (CMC, DCC, EDC·HCl) and uronium salts (PyBOP, TBTU) most often used in peptide synthesis.[41] The purity and yields of the isolated dipeptide **5** are given in Table 1, from which it follows that the use of TBTU as a coupling agent was most efficient.

For further coupling with Fmoc-Arg(Pbf)-OH (**8**), the amino groups of dipeptides Fmoc-Gly-Asp(OMe)-OMe (**4**) and Boc-Gly-Asp(OMe)-OMe (**5**) were deprotected (see Scheme 1).

In the case of dipeptide **4**, the Fmoc group was removed by standard procedures using piperidine (20%) in CH_2Cl_2 or MeOH.[36,42] However, these conditions led to the formation of dioxopiperazine **6** through the coupling of the glycine amino group with the α-carboxyl group of aspartic acid.[42] According to the ^{1}H NMR data, the yield of the target product was less than 10%.

To remove the Boc group, a solution of Boc-Gly-Asp(OMe)-OMe (**5**) in CH_2Cl_2 was treated with concentrated TFA (see Scheme 1). The target product was formed in pure form, impurities of the starting compound disappeared within 1 h after the beginning of the reaction (according to TLC). The isolated product **7** was used without additional purification.

Table 1. Optimization of the synthesis of Boc-Gly-Asp(OMe)-OMe (**5**)

Coupling agent	Yield*	Purity**
	%	
CMC	15	96.9
DCC	49	93.6
EDC	52	99.8
PyBOP	71	94.2
TBTU	81	99.1

* After purification by flash-column chromatography.
** HPLC data.

For the synthesis of the RGD derivative, we selected a *N*-protected L-arginine derivative of **8** containing N^{α}-Fmoc group, which in the next stage of the synthesis of tetrapeptide can be removed with organic bases, and N^{ω}-Pbf group, which is removed with TFA (see Scheme 1).

The protection of the guanidine group of Arg is necessary in order to prevent its elimination in basic media because of its high nucleophilicity. This can lead to the formation of ornithine and δ-lactam. At present, the most frequently used protection is the benzenesulfonyl-type electron-enriched groups, such as Mds, Mtr, Pmc, and Pbf.[43] The latter group is the most suitable because of its highest lability under acidic conditions. Not so long ago, Mis was proposed as a protective group,[43] which is even more labile under acidic conditions, but so far it has not been widely used and is not commercially available.

The protected tripeptide Fmoc-Arg(Pbf)-Gly-Asp(OMe)-OMe (**9**) was synthesized by coupling of dipeptide **7** with Fmoc-Arg(Pbf)-OH (**8**) using various coupling agents: CMC, EDC·HCl, PyBOP, TBTU, HBTU (Table 2). The best yields and product purity were achieved in the presence of uronium salts TBTU and HBTU. The Fmoc protective group in tripeptide **9** was removed by treatment with a 5 equiv. excess of piperidine in MeOH, which gave tripeptide **10** with a free amino group (see Scheme 1). The reaction was completed within 2 h (according to TLC).

Tetrapeptide derivatives **12** and **14** were obtained by the coupling of tripeptide **10** with Fmoc-Gly-OH (**11**) or Boc-Lys(Fmoc)-OH (**13**) using TBTU, since this reagent showed the highest efficiency in the synthesis of peptide **9** (see Scheme 1). Tetrapeptides **12** and **14** were isolated in pure form by flash-column chromatography. The Fmoc group in both tetrapeptides was removed by treatment with 5 equiv. excess of piperidine in MeOH (2 h). If in the case of peptide **12** the removal of the Fmoc protection and the isolation of the target product **15** with the free amino group

Scheme 1

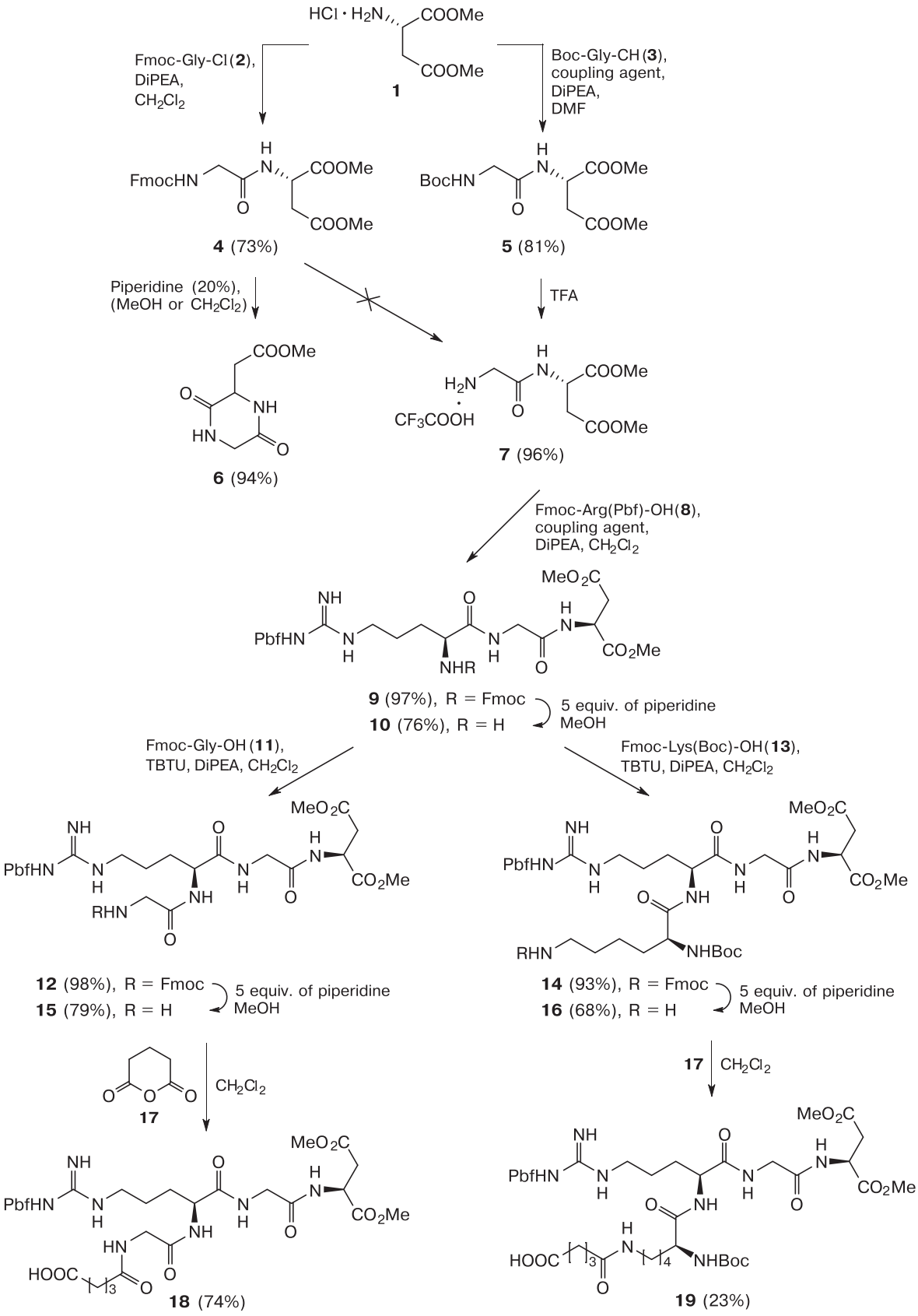
HCl · H2N
COOMe
COOMe
1
Fmoc-Gly-Cl (2),
DiPEA,
CH2Cl2
Boc-Gly-CH (3),
coupling agent,
DiPEA,
DMF
FmocHN
BocHN
4 (73%)
5 (81%)
Piperidine (20%),
(MeOH or CH2Cl2)
TFA
6 (94%)
7 (96%)
CF3COOH
Fmoc-Arg(Pbf)-OH (8),
coupling agent,
DiPEA, CH2Cl2
PbfHN
NHR
9 (97%), R = Fmoc
10 (76%), R = H
5 equiv. of piperidine
MeOH
Fmoc-Gly-OH (11),
TBTU, DiPEA, CH2Cl2
Fmoc-Lys(Boc)-OH (13),
TBTU, DiPEA, CH2Cl2
RHN
NHBoc
12 (98%), R = Fmoc
15 (79%), R = H
14 (93%), R = Fmoc
16 (68%), R = H
17
CH2Cl2
HOOC
18 (74%)
19 (23%)

Table 2. Optimization of the synthesis of Fmoc-Arg(Pbf)-Gly-Asp(OMe)-OMe (**9**)

Coupling agent	Yield*	Purity**
	%	
CMC	10	93.6
EDC	30	91.4
PyBOP	46	95.4
TBTU	96	96.5
HBTU	97	97.0

* After purification by flash-column chromatography.
** HPLC data.

proceeded quite smoothly, in the case of peptide **16** the reaction was accompanied by the formation of a large amount of unidentified byproducts. The presence of free amino groups in the molecules of the synthesized peptides **10**, **15**, and **16** allows their further conjugation with other biologically active molecules or nanoparticles containing carboxyl groups on the surface (for example, similarly to the works[44—46]).

The coupling of tetrapeptides **15** and **16** with glutaric anhydride (**17**) (see Scheme 1) gave protected tetrapeptides **18** and **19** containing glutaric acid residues with the free carboxyl group, which can be involved in the coupling with amino groups of other biomolecules or surface amino groups of nanoparticles (for example, similarly to the works[35,46—48]) in order to develop diagnostic or therapeutic agents for visualization and treatment of malignant neoplasms.

All the target compounds were purified by flash-column chromatography. The structure and purity of the obtained products were confirmed by ^{1}H NMR spectroscopy, high resolution mass spectrometry, elemental analysis, IR spectroscopy, and HPLC on normal and reverse stationary phases. The presence of a mixture of diastereomers of the RGD peptide derivatives cannot always be identified in the ^{1}H NMR spectra and in the HPLC chromatograms. However, it was shown earlier[36] that the diastereomers of the RGD peptide can differ in retention times when HPLC is performed on a chiral stationary phase (CSP). Therefore, we additionally evaluated the optical purity of key peptides **9**, **12**, and **14** by HPLC using a (S,S) Whelk-O1 column and detected the presence of only individual peaks in the chromatograms. This indirectly confirms that the peptides were obtained as individual stereoisomers. The specific optical rotation was measured for the target compounds.

In conclusion, we developed and optimized a method for the synthesis of new protected derivatives of tetrapeptides GRGD (Gly-Arg(Pbf)-Gly-Asp(OMe)-OMe) and KRGD (Boc-Lys-Arg(Pbf)-Gly-Asp(OMe)-OMe) containing a fragment of glutaric acid as a linker, which provides the possibility to bind these derivatives to other biologically active molecules or nanoparticles. The presence of protection of the guanidine group of Arg and the β-carboxyl group of Asp in the synthesized peptide derivatives allows their conjugation without involvement of these groups in the synthesis, which is necessary to preserve the biological activity of the resulting conjugates. Subsequently, the protective groups of these derivatives can be removed under mild conditions using traditional methods of peptide synthesis.

Experimental

Dimethyl L-aspartate hydrochloride[49] and *N*-(*tert*-butyloxycarbonyl)glycine[50] were obtained according to the known procedures. The following agents were used: *N*-(fluorenylmethoxycarbonyl)glycine (Sigma—Aldrich, Germany), L-(N^{ω}-2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl-N^{α}-fluorenylmethoxycarbonyl)-L-arginine (Sigma—Aldrich, Germany), N^{ε}-fluorenylmethoxycarbonyl-N^{α}-*tert*-butyloxycarbonyl-L-lysine (Sigma—Aldrich, Germany), *N*-cyclohexyl-*N*′-{2-(*N*-methylmorpholino)ethyl}carbodiimide methyl *p*-toluenesulfonate (CMC, ICN Biomedical Inc., USA), *N*,*N*′-dicyclohexylcarbodiimide (DCC, Sigma—Aldrich, Germany), *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl, Alfa Aesar, UK), 1*H*-benzotriazol-1-yloxytri(pyrrolidinyl)phosphonium hexafluorophosphate (PyBOP, Alfa Aesar, UK), *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU, Alfa Aesar, UK), *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU, Alfa Aesar, UK), *N*,*N*-diisopropylethylamine (DIPEA, ICN Biomedical Inc., USA), glutaric anhydride (Sigma—Aldrich, Germany).

IR spectra were recorded on a Nicolet 6700 IR Fourier-transform spectrometer equipped with a diamond crystal Smart Orbit attenuated total reflection (ATR) accessory, the scanning region of ATR spectra was 400—4000 cm^{-1}, the number of scans was 64. NMR spectra were recorded on Bruker DRX400 (400 MHz, Germany) and Bruker Avance 500 (500 MHz, Germany) spectrometers in DMSO-d_6 at ~20 °C, using $SiMe_4$ as an internal standard. Optical rotation were measured on a Perkin—Elmer M341 polarimeter (USA) (expressed in (deg mL) (g dm)$^{-1}$, solution concentration in g (100 mL)$^{-1}$). Elemental analysis of the samples was carried out on a Perkin—Elmer PE 2400, series II automated CHN analyzer (USA). Mass spectra of compounds (HRMS) were recorded after chromatographic separation on a Bruker maXis impact HD instrument (Germany), using electrospray ionization (ESI) in the positive ion mode, an operating voltage on the needle 4.5 kV, carrier gas nitrogen, flow rate 4.5 L min^{-1}. Reversed-phase HPLC and chiral HPLC were performed on an Agilent 1100 chromatograph (USA) using Kromasil 100-5C18, Phenomenex Luna C-18, and (S,S) Whelk-O1 (CSP) columns, 250×4.6 mm, 5 μm, flow rate 0.8 mL min^{-1}. Normal-phase HPLC was carried out on a Knauer Smartline-1000 chromatograph (Germany) with a Reprosil 2 column, 250×4.6 mm, 5 μm, flow rate 1 mL min^{-1}. Detection at wavelengths of 220 or 254 nm. Mobile phases and retention times of compounds (τ, min) are indicated in specific procedures. Preparative HPLC was performed on a Shimadzu LC-20 chromatograph with a Reprosil-Pur-C18 column, 250×21.2 mm, 5 μm, flow rate 5 mL min^{-1}, detection at a wavelength of 220 nm. Silica gel 60 0.040—0.063 mm (Alfa Aesar, UK) was used for flash chromatographic

separation. Sorbfil plates (Imid Ltd., Russia) were used for TLC, peptides were visualized under UV light, in iodine vapors, and with a 0.2% solution of ninhydrin in acetone.

***N*-(Fluorenylmethoxycarbonyl)glycyl chloride (2).** Oxalyl chloride (0.513 mL, 6.072 mmol) and DMF (5 μL) was added to a pre-cooled to 0 °C suspension of Fmoc-Gly-OH (1.500 g, 5.060 mmol) in anhydrous benzene (15 mL). The reaction mixture was heated for 30 min to 50 °C (until the precipitate was completely dissolved) and stirring was continued for another 30 min at ~20 °C. The solvent was evaporated, the residue was dried *in vacuo* to obtain a light yellow crystalline powder acyl chloride **2** (similarly to the works[36,40]), which was used further in the synthesis of dipeptide **4** without additional purification. The yield was 1.566 g (98%), m.p. 101—108 °C.

Dimethyl *N*-(fluorenylmethoxycarbonyl)-glycyl-L-aspartate (4). A solution of dimethyl ester hydrochloride **1** (0.980 g, 4.960 mmol) and DIPEA (1.728 mL, 9.919 mmol) in CH_2Cl_2 (7 mL) was added to a pre-cooled to 0 °C solution of acyl chloride **2** (1.566 g, 4.960 mmol) in CH_2Cl_2 (7 mL) with stirring. The reaction mixture was allowed to stand for 24 h at ~20 °C, then sequentially washed with 5% aqueous HCl (3×10 mL), brine (1×10 mL), 5% aqueous Na_2CO_3 (3×10 mL), and brine until neutrality. The organic layer was dried with $MgSO_4$. The solvent was evaporated under reduced pressure. The product was dried *in vacuo*. The pure target product was obtained either by flash-column chromatography (benzene—EtOAc, gradient from 100 : 0 to 40 : 60), or by recrystallization from a mixture of hexane—EtOAc. The yield was 1.573 g (72%), a white crystalline powder, m.p. 127 °C, $[\alpha]_D^{20}$ +34.3 (*c* 1.04, $CHCl_3$). Found (%): C, 62.73; H, 2.71; N, 6.31. $C_{23}H_{24}N_2O_7$. Calculated (%): C, 62.72; H, 5.49; N, 6.36. HPLC (Kromasil 100-5C18, λ = 254 nm, MeCN—H_2O (7 : 3)), τ_R 5.0. R_f 0.66 (benzene—EtOAc (1 : 1)). ^{1}H NMR (500 MHz), δ: 8.37 (d, 1 H, NH (Asp), *J* = 7.9 Hz); 7.90 (d, 2 H arom. (Fmoc), *J* = 7.5 Hz); 7.71 (d, 2 H arom. (Fmoc), *J* = 7.5 Hz); 7.42 (t, 2 H arom. (Fmoc), *J* = 7.3 Hz); 7.33 (t, 2 H arom. (Fmoc), *J* = 7.3 Hz); 7.56 (t, 1 H, NH (Gly), *J* = 6.1 Hz); 4.67 (dt, 1 H, C_αH (Asp), J_1 = 7.3 Hz, J_2 = 6.9 Hz); 4.28 (d, 2 H, CH_2 (Fmoc), *J* = 7.0 Hz); 4.22 (t, 1 H, CH (Fmoc), *J* = 7.1 Hz); 3.64 (d, 2 H, CH_2 (Gly), *J* = 6.4 Hz); 3.62, 3.60 (both s, 3 H each, COOMe); 2.80 (dd, 1 H, CH_A, J_1 = 16.5 Hz, J_2 = 6.1 Hz); 2.71 (dd, 1 H, CH_B, J_1 = 16.5 Hz, J_2 = 6.9 Hz).

Dimethyl *N*-(*tert*-butyloxycarbonyl)glycyl-L-aspartate (5). DIPEA (2.98 mL, 18.27 mmol) and TBTU (2.013 g, 6.279 mmol) were added to a solution of Boc-Gly-OH (**3**) (1.00 g, 5.708 mmol) and dimethyl ester hydrochloride **1** (1.128 g, 5.708 mmol) in CH_2Cl_2 (20 mL). The resulting solution was stirred for 4 h. Then the reaction mixture was allowed to stand for 20 h at ~20 °C, diluted with CH_2Cl_2 (30 mL), and sequentially washed with 5% aqueous $NaHCO_3$ (4×20 mL), water (4×50 mL) to neutral pH, 5% aqueous citric acid (10 mL), water (2×10 mL) to neutral pH. The organic layer was dried with $MgSO_4$ and concentrated, the product was dried *in vacuo*. The yield was 1.266 g (81%), a colorless oil, $[\alpha]_D^{20}$ +45.4 (*c* 1.16, $CHCl_3$). Found (%): C, 49.12; H, 7.34; N, 8.58. $C_{13}H_{22}N_2O_7$. Calculated (%): C, 49.05; H, 6.97; N, 8.80. HPLC (Kromasil 100-5C18, MeCN—H_2O (6.3 : 3.7), τ_R 6.72. R_f 0.55 (benzene—EtOAc (1 : 1)). ^{1}H NMR (500 MHz), δ: 8.24 (d, 1 H, NH (Asp), *J* = 7.8 Hz); 6.98 (t, 1 H, NH (Gly), *J* = 6.0 Hz); 4.67 (dt, 1 H, C_αH (Asp), J_1 = 14.3 Hz, J_2 = 4.5 Hz); 3.62 (s, 3 H, MeO); 3.00 (s, 3 H, MeO); 3.54 (d, 2 H, CH_2 (Gly), *J* = 6.1 Hz); 2.79 (dd, 1 H, CH_B (Asp), J_1 = 16.6 Hz, J_2 = 6.1 Hz); 2.72 (dd, 1 H, CH_A (Asp), J_1 = 16.6 Hz, J_2 = 6.8 Hz); 1.38 (s, 9 H, Bu^t_3).

Methyl 2-(3,6-dioxopiperazin-2-yl)acetate (6). Dipeptide **4** (0.533 g, 1.210 mmol) was dissolved in 20% piperidine solution in CH_2Cl_2 (6 mL) with magnetical stirring. The stirring was continued for 4 h at ~20 °C. A white precipitate was formed during the reaction, which further was collected by filtration and dried. The yield was 0.212 g (94%), a white crystalline powder, m.p. 199—201 °C (decomp.). Found (%): C, 44.85; H, 5.64; N, 14.81. $C_7H_{10}N_2O_4$. Calculated (%): C, 45.16; H, 5.41; N, 15.05. HPLC (Kromasil 100-5C18, λ = 254 nm, MeCN—H_2O (6.3 : 3.7)), τ_R 6.2. R_f 0.85 (benzene—EtOAc (1 : 1)). ^{1}H NMR (500 MHz), δ: 8.12 (s, 1 H, NH (Asp)); 8.12, 8.06 (both s, 1 H each, NH); 4.15 (ddd, 1 H, C_αH (Asp), J_1 = 5.6 Hz, J_2 = 5.2 Hz, J_3 = 1.2 Hz); 3.79 (ddd, 1 H, CH_A (Gly), J_1 = 17.3 Hz, J_2 = 1.5 Hz, J_3 = 1.5 Hz); 3.71 (ddd, 1 H, CH_B (Gly), J_1 = 17.3 Hz, J_2 = 2.35 Hz, J_3 = 0.8 Hz); 3.60 (s, 3 H, COOMe); 2.78 (dd, 1 H, CH_A(Asp), J_1 = 16.8 Hz, J_2 = 5.2 Hz); 2.71 (dd, 1 H, CH_B(Asp), J_1 = 16.8 Hz, J_2 = 5.3 Hz).

Dimethyl glycyl-L-aspartate trifluoroacetate (7). TFA (4 mL) was added to a solution of dipeptide **5** (0.180 g, 0.566 mmol) in CH_2Cl_2 (2 mL) and the mixture was allowed to stand for 4 h at ~20 °C. The solvent was evaporated. The residue was dried *in vacuo*. The yield was 0.189 g (98%), an oil. Found (%): C, 35.45; H, 4.32; F, 18.02; N, 7.64. $C_8H_{14}N_2O_5 \cdot 1.1\ CF_3CO_2H$. Calculated (%): C, 35.65; H, 4.43; F, 18.24; N, 8.15. HPLC (Phenomenex Luna C18(2), MeOH—H_2O—TFA (6 : 4 : 0.1)), τ_R 3.0. R_f 0.56 ($CHCl_3$—MeOH (3 : 1)). ^{1}H NMR (400 MHz), δ: 8.90 (d, 1 H, NH (Asp), *J* = 7.7 Hz); 8.01 (br.s, 1 H, NH(Gly)); 4.73 (ddd, 1 H, C_αH (Asp), J_1 = 7.4 Hz; J_2 = 6.4 Hz, J_3 = 5.9 Hz); 3.65 (s, 3 H, MeO); 3.62 (s, 3 H, MeO); 3.60 (m, 2 H, CH_2 (Gly)); 2.85 (dd, 1 H, CH_B (Asp), J_1 = 16.8 Hz, J_2 = 5.8 Hz); 2.79 (dd, 1 H, CH_A (Asp), J_1 = 16.8 Hz, J_2 = 6.7 Hz); 1.38 (s, 9 H, Bu^t_3).

Dimethyl L-(*N*$^\omega$-2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl-*N*$^\alpha$-fluorenylmethoxycarbonyl)arginyl-glycyl-L-aspartate (9). HBTU (0.115 g, 0.304 mmol) and DIPEA (0.20 mL, 1.15 mmol) were added to a solution of dipeptide trifluoroacetate **7** (Gly-Asp(OMe)$_2 \cdot 1.1\ CF_3CO_2H$) (0.193 g, 0.562 mmol) and Fmoc-Arg(Pbf)-OH (**8**) (0.197 g, 0.304 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred for 20 h at ~20 °C, diluted with CH_2Cl_2 (15 mL), and sequentially washed with 5% aqueous citric acid (3×15 mL), water (3×15 mL), 5% aqueous $NaHCO_3$ (3×15 mL), water (4×15 mL) to neutral pH. The organic layer was dried with $MgSO_4$, the product was purified by flash-column chromatography in the $CHCl_3$—MeOH solvent system (gradient from 100 : 0 to 95 : 5). The yield was 0.250 g (97%), m.p. 100—102 °C, $[\alpha]_D^{20}$ +8.0 (*c* 1.01, MeOH). Found (%): C, 58.37; H, 6.34; N, 10.45. $C_{42}H_{52}N_6O_{11}S \cdot 0.1\ CHCl_3$. Calculated (%): C, 58.74; H, 6.10; N, 9.76. HPLC (Kromasil 100-5C18, MeCN—H_2O (7 : 3)), τ_R 7.9; ((S,S) Whelk-O1, MeCN—H_2O (7 : 3), λ = 220 and 254 nm), τ_R 15.26. R_f 0.67 ($CHCl_3$—MeOH (9 : 1)). IR, ν/cm^{-1}: 3319 (NH); 2930, 2865 (CH_2, CH); 1737, 1728, 1710 (C=O (COOMe)); 1665, 1620 (C=O (CO—NH (amide 1)); NH—CO—O, C—C arom.); 1546, 1536 (N—C=O (amide 2), C—C arom.); 1440, 1407, 1369 (C—N (amide 3), R—SO_2—N); 1227 (C—H); 1166, 1089, 1031, 993 (C—O (COOMe), R—SO_2—N, N—H, C—C arom.); 851, 807, 782, 760, 740 (C—H, N—H, S—O); 660, 641, 620, 566, 506. ^{1}H NMR (400 MHz), δ: 8.25 (d, 1 H, NH (Asp), *J* = 7.9 Hz); 8.21 (t, 1 H, NH (Gly), *J* = 5.6 Hz); 7.89 (d, 2 H, Ar (Fmoc), *J* = 7.9 Hz); 7.72 (t, 2 H, Ar (Fmoc), *J* = 7.0 Hz); 7.58 (d, 1 H, NH (Arg),

J = 7.6 Hz); 7.41 (t, 2 H, Ar (Fmoc), *J* = 7.5 Hz); 7.31 (t, 2 H, Ar (Fmoc), *J* = 7.5 Hz); 7.05, 6.67, 6.39 (all br.s, 3 H, NH (Arg)); 4.67 (dt, 1 H, $C_\alpha H$ (Asp), J_1 = 7.6 Hz, J_2 = 6.5 Hz); 4.35—4.17 (m, 3 H, CH, CH_2 (Fmoc)); 3.97 (m, 1 H, $C_\alpha H$ (Arg)); 3.72 (d, 2 H, CH_2 (Gly), *J* = 6.0 Hz); 3.61 (s, 3 H, MeO); 3.57 (s, 3 H, MeO); 3.03 (m, 2 H, $C(5)H_2$ (Arg)); 2.94 (br.s, 2 H, CH_2 (Pbf)); 2.78 (dd, 1 H, CH_B (Asp), J_1 = 16.4 Hz, J_2 = 6.2 Hz); 2.69 (dd, 1 H, CH_A (Asp), J_1 = 16.4 Hz, J_2 = 7.0 Hz); 2.48 (s, 3 H, Me (Pbf)); 2.42 (s, 3 H, Me (Pbf)); 2.00 (s, 3 H, Me (Pbf)); 1.70—1.30 (m, 4 H, $C(3)H_2$, $C(4)H_2$ (Arg)); 1.39 (s, 6 H, 2 Me (Pbf)).

Dimethyl (*N*$^{\omega}$-2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-L-arginyl-glycyl-L-aspartate (10). Tetrapeptide **9** (0.416 g, 0.490 mmol) was dissolved in MeOH (5 mL) with magnetical stirring, followed by the addition of piperidine (0.24 mL, 2.45 mmol). The stirring was continued for 2 h at ~20 °C. The reaction mixture was concentrated, washed with hexane, and dried in a vacuum cabinet. The target compound was isolated by flash-column chromatography (eluent $CHCl_3$—MeOH, gradient from 100 : 0 to 20 : 80). The yield was 0.236 g (76%), a foamed oil, m.p. 101—107 °C, $[\alpha]_D^{20}$ +16.1 (*c* 1.02, MeOH). Found (%): C, 49.81; H, 6.34, N, 12.39. $C_{27}H_{42}N_6O_9S \cdot 0.3CHCl_3$. Calculated (%): C, 49.49; H, 6.44; N, 12.68. HPLC (Kromasil 100-5C18, MeOH—0.1%CF_3COOH (7 : 3)), τ_R = 5.0. R_f 0.62 ($CHCl_3$—MeOH (1 : 1)). IR, ν/cm^{-1}: 3319 (NH); 2931, 2860 (CH_2, CH); 1738, 1728 (C=O (COOMe)); 1687, 1659, 1620 (C=O (CO—NH (amide 1), C—C arom.); 1547, 1536 (N—C=O (amide 2), C—C arom.); 1440, 1408, 1369 (C—N (amide 3), R—SO_2—N); 1220 (C—H); 1158, 1089, 993 (C—O (COOMe), R—SO_2—N, N—H, C—C arom.); 851, 808, 782, 733 (C—H, N—H, S—O); 659, 641, 618, 565, 507. ^{1}H NMR (400 MHz), δ: 8.60 (br.s, 1 H, NH (Gly)); 8.53 (d, 1 H, NH (Asp), *J* = 7.7 Hz); 8.10—7.10 (br.s, 2 H, NH_2 (Arg), *J* = 7.6 Hz); 6.88, 6.45 (both br.s, 3 H, NH (Arg)); 4.68 (m, 1 H, $C_\alpha H$ (Asp)); 3.80—3.70 (m, 1 H, $C_\alpha H$ (Arg)); 3.80 (d, 2 H, CH_2 (Gly), *J* = 4.9 Hz); 3.62 (s, 3 H, MeO); 3.61 (s, 3 H, MeO); 3.09—3.00 (m, 2 H, $C(5)H_2$ (Arg)); 3.02—2.93 (m, 2 H, CH_2 (Pbf)); 2.82 (dd, 1 H, CH_B (Asp), J_1 = 16.5 Hz, J_2 = 6.1 Hz); 2.72 (dd, 1 H, CH_A (Asp), J_1 = 16.6 Hz, J_2 = 6.8 Hz); 2.48 (s, 3 H, Me (Pbf)); 2.42 (s, 3 H, Me (Pbf)); 2.01 (s, 3 H, Me (Pbf)); 1.70—1.60 (m, 2 H, $C(3)H_2$); 1.60—1.50 (m, 2 H, $C(4)H_2$ (Arg)); 1.41 (s, 6 H, 2 Me (Pbf)).

Dimethyl *N*-fluorenylmethoxycarbonyl-glycyl-(*N*$^{\omega}$-2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-L-arginyl-glycyl-L-aspartate (12). DIPEA (0.267 mL, 1.532 mmol) and TBTU (0.246 g, 0.766 mmol) were added to a solution of tripeptide **10** (0.480 g, 0.766 mmol) and Fmoc-Gly-OH (**11**) (0.228 g, 0.766 mmol) in CH_2Cl_2 (25 mL) with stirring. The reaction mixture was allowed to stand for 20 h at ~20 °C. Then the reaction mixture was diluted with CH_2Cl_2 (15 mL) and sequentially washed with 5% aqueous $NaHCO_3$ (4×20 mL), water (3×25 mL) to neutral pH, 5% aqueous citric acid (5 mL), and water (3×10 mL) to neutral pH. The organic layer was dried with $MgSO_4$ and concentrated, the product was dried *in vacuo* and purified by flash-column chromatography in the $CHCl_3$—MeOH solvent system (gradient from 100 : 0 to 85 : 15). The yield was 0.698 g (98%), a foamed oil, m.p. 125 °C, $[\alpha]_D^{20}$ −2.1; (*c* 1.54, MeOH). Found (%): C, 57.22; H, 6.05, N, 10.34; S, 3.47. $C_{44}H_{55}N_7O_{12}S \cdot H_2O$. Calculated (%): C, 57.19; H, 6.22; N, 10.61; S, 3.47. HPLC (Kromasil 100-5C18, MeCN—H_2O (6 : 4)), τ_R 11.96; (Phenomenex LunaC-18, MeCN—H_2O (7 : 3)), τ_R 6.18; ((S,S) Whelk-O1, MeOH), τ_R 11.35; (Reprosil 2, hexane—Pr^{i}OH—MeOH (3.0 : 0.7 : 0.3)), τ_R 15.05. R_f 0.46 ($CHCl_3$—MeOH (7 : 1)). IR, ν/cm^{-1}: 3321 (NH); 2949, 2870 (CH_2, CH); 1730 (C=O (COOMe)); 1656, 1616 (C=O (CO—NH (amide 1), NH—CO—O, C—C arom.); 1540 (N—C=O (amide 2), C—C arom.); 1438, 1407, 1369 (C—N (amide 3), R—SO_2—N); 1224 (C—H); 1157, 1089, 1049, 1031, 994 (C—O (COOMe), R—SO_2—N, N—H, C—C arom.); 852, 807, 783, 759, 741 (C—H, N—H, S—O); 660, 641, 620, 566, 507. ^{1}H NMR (400 MHz), δ: 8.30 (d, 1 H, NH (Asp), *J* = 8.0 Hz); 8.25 (t, 1 H, NH (Gly-1), *J* = 11.4 Hz); 8.03 (d, 1 H, NH (Arg), *J* = 7.7 Hz); 7.89 (d, 2 H, Ar (Fmoc), *J* = 7.5 Hz); 7.70 (t, 2 H, Ar (Fmoc), *J* = 7.6 Hz); 7.48 (t, 1 H, NH (Gly-2), *J* = 6.0 Hz); 7.41 (t, 2 H, Ar (Fmoc), *J* = 7.4 Hz); 7.32 (t, 2 H, Ar (Fmoc), *J* = 7.4 Hz); 7.00, 6.65, 6.35 (all br.s, 3 H, NH(Arg)); 4.65 (dt, 1 H, $C_\alpha H$ (Asp), J_1 = 14.1 Hz, J_2 = 14.2 Hz); 4.30 (m, 3 H, Ar (Fmoc)); 4.22 (dt, 1 H, $C_\alpha H$ (Arg), J_1 = 15.6 Hz, J_2 = 14.1 Hz); 3.72 (t, 2 H, CH_2 (Gly-1), *J* = 6.1 Hz); 3.66 (d, 2 H, CH_2 (Gly-2), *J* = 6.1 Hz); 3.61 (s, 3 H, MeO); 3.59 (s, 3 H, MeO); 3.02 (dt, 2 H, $C(5)H_2$ (Arg), J_1 = 12.3 Hz, J_2 = 11.9 Hz); 2.95 (s, 2 H, CH_2 (Pbf)); 2.78 (dd, 1 H, CH_A (Asp), J_1 = 16.6 Hz, J_2 = 6.1 Hz); 2.72 (dd, 1 H, CH_B (Asp), J_1 = 16.4 Hz, J_2 = 6.7 Hz); 2.47 (s, 3 H, Me (Pbf)); 2.42 (s, 3H, Me (Pbf)); 2.00 (s, 3 H, Me (Pbf)); 1.70—1.60 (m, 2 H, $C(3)H_2$); 1.50—1.40 (m, 2 H, $C(4)H_2$ (Arg)); 1.39 (s, 6 H, 2 Me (Pbf)). HRMS, found: *m/z* 906.3698 $[M + H]^+$, calculated for $C_{44}H_{56}N_7O_{12}S$: 906.3708; found: *m/z* 928.3514 $[M + Na]^+$, calculated for $C_{44}H_{55}N_7NaO_{12}S$: 928.3527.

Dimethyl *N*$^{\varepsilon}$-fluorenylmethoxycarbonyl-*N*$^{\alpha}$-*tert*-butyloxycarbonyl-L-lysyl-(*N*$^{\omega}$-2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-L-arginyl-glycyl-L-aspartate (14). DIPEA (0.272 mL, 1.56 mmol) and TBTU (0.251 g, 0.781 mmol) were added to a solution of tripeptide **10** (0.445 g, 0.710 mmol) and Boc-Lys(Fmoc)-OH (**13**) (0.333 g, 0.710 mmol) in CH_2Cl_2 (25 mL) with stirring, the stirring was continued for 16 h at ~20 °C. The reaction mixture was diluted with CH_2Cl_2 (25 mL) and sequentially washed with 5% aqueous $NaHCO_3$ (15 mL), water (3×15 mL) to neutral pH, 5% aqueous citric acid (5 mL), and water (3×10 mL) to neutral pH. The organic layer was dried with $MgSO_4$ and concentrated. The product was dried *in vacuo* and purified by flash-column chromatography in the $CHCl_3$—MeOH solvent system (gradient from 100 : 0 to 85 : 15). The yield was 0.681 g (93%), a foamed oil, m.p. 106 °C, $[\alpha]_D^{20}$ −4.1 (*c* 2.86, MeOH). Found (%): C, 58.90; H, 6.77; N, 10.79; S, 2.93. $C_{53}H_{72}N_8O_{14}S \cdot 0.2H_2O$. Calculated (%): C, 58.90; H, 6.75; N, 10.37; S, 2.97. HPLC (Kromasil 100-5C18, MeCN—H_2O (7 : 3)), τ_R 11.64; ((S,S) Whelk-O1, MeOH), τ_R 12.19; (Reprosil 2, hexane—Pr^{i}OH—MeOH (3.0 : 0.7 : 0.3)), τ_R 10.05. R_f 0.67 ($CHCl_3$—MeOH (1 : 1)). IR, ν/cm^{-1}: 3319 (NH); 2935, 2865 (CH_2, CH); 1738 (C=O (COOMe)); 1685, 1658, 1616 (C=O (CO—NH (amide 1), NH—CO—O, C—C arom.); 1536, 1518 (N—C=O (amide 2), C—C arom.); 1439, 1408, 1367 (C—N (amide 3), R—SO_2—N); 1242 (C—H); 1161, 1089, 1049, 1019, 994 (C—O (COOMe), R—SO_2—N, N—H, C—C arom.); 852, 806, 782, 759, 741 (C—H, N—H, S—O); 660, 641, 620, 564, 507. ^{1}H NMR (400 MHz), δ: 8.32 (d, 1 H, NH (Asp), *J* = 5.2 Hz); 8.21 (t, 1 H, NH (Gly), *J* = 5.6 Hz); 7.85 (d, 1 H, NH (Arg), *J* = 7.8 Hz); 7.68 (d, 2 H, Ar (Fmoc), *J* = 7.5 Hz); 7.40 (t, 2 H, Ar (Fmoc), *J* = 7.6 Hz); 7.32 (t, 2 H, Ar (Fmoc), *J* = 7.4 Hz); 6.90 (d, NH_ε (Lys), *J* = 8.4 Hz); 6.79, 6.65, 6.35 (all br.s, 3 H, NH (Arg)); 4.65 (dt, 1 H, $C_\alpha H$ (Asp), J_1 = 11.4 Hz, J_2 = 6.7 Hz);

4.28 (m, 3 H, Ar (Fmoc)); 4.20 (dt, 1 H, $C_\alpha H$ (Arg), J_1 = 13.9 Hz, J_2 = 7.0 Hz); 3,87 (m, 1 H, $C_\alpha H$ (Lys)); 3.72 (d, 2 H, CH_2 (Gly), J = 5.7 Hz); 3.61 (s, 3 H, MeO); 3.58 (s, 3 H, MeO); 3.02 (dt, 2 H, C(5)H_2 (Arg), J_1 = 12.3 Hz, J_2 = 5.5 Hz); 2.95 (s, 2 H, CH_2 (Pbf)); 2.92—2.94 (m, 2 H, C(6)H_2 (Lys)); 2.78 (dd, 1 H, CH_A (Asp), J_1 = 16.6 Hz, J_2 = 6.3 Hz); 2.72 (dd, 1 H, CH_B (Asp), J_1 = 16.6 Hz, J_2 = 6.8 Hz); 2.47 (s, 3 H, Me (Pbf)); 2.41 (s, 3 H, Me (Pbf)); 2.00 (s, 3 H, Me (Pbf)); 1.70—1.60 (m, 2 H, C(3)H_2); 1.50—1.40 (m, 2 H, C(4)H_2 (Arg)); 1.50—1.40 (m, 6 H, C(5)H_2 + + C(4)H_2 + C(3)H_2 (Lys)); 1.40 (s, 6 H, 2 Me (Pbf); 1.35 (s, 9 H, 3 Me (Boc)). HRMS, found: *m/z* 1077.4956 $[M + H]^+$, calculated for $C_{53}H_{73}N_8O_{14}S$: 1077.4967; found: *m/z* 1099.4779 $[M + Na]^+$, calculated for $C_{53}H_{72}N_8NaO_{14}S$: 1099.4786.

Dimethyl glycyl-(N^ω-2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-L-arginyl-glycyl-L-aspartate (15). Piperidine (0.294 mL, 2.98 mmol) was added to a solution of tetrapeptide **12** (0.539 g, 0.595 mmol) in MeOH (5 mL). The reaction mixture was allowed to stand for 2 h at ~20 °C and concentrated, the product was dried *in vacuo* and purified by flash-column chromatography in the $CHCl_3$—MeOH solvent system (gradient from 95 : 5 to 20 : 80), as well as by preparative HPLC (eluent MeOH—0.2%AcOH (65 : 35)). The yield was 0.321 g (79%), a foamed oil, m.p. 126 °C, $[\alpha]_D^{20}$ +4.5 (*c* 1.67, MeOH). Found (%): C, 47.64; H, 6.40; N, 13.15; S, 4.28. $C_{29}H_{45}N_7O_{10}S \cdot 2H_2O \cdot CH_3COOH$. Calculated (%): C, 47.74; H, 6.85; N, 12.57; S, 4.11. HPLC (Kromasil 100-5C18, MeOH—0.1%CF_3COOH (3 : 7)), τ_R 3.51. R_f 0.30 ($CHCl_3$—MeOH (3 : 1)). IR, ν/cm^{-1}: 3308 (NH); 2940, 2870 (CH_2, CH); 1737 (C=O (CO—NH (amide 1), C—C arom.); 1658, 1618 (C=O (COOMe)); 1537 (N—C=O (amide 2), C—C arom.); 1438, 1406, 1369 (C—N (amide 3), R—SO_2—N); 1278, 1235, 1202 (C—H); 1165, 1089, 1033, 993 (C—O (COOMe), R—SO_2—N, N—H, C—C arom.); 852, 807, 783 (C—H, N—H, S—O); 659, 641, 618, 566, 507. ^{1}H NMR (400 MHz), δ: 8.30 (d, 1 H, NH (Asp), J = 8.0 Hz); 8.25 (t, 1 H, NH (Gly-1), J = 11.4 Hz); 8.03 (d, 1 H, NH (Arg), J = 7.7 Hz); 7.89 (d, 3 H, NH(Arg)); 4.65 (dt, 1 H, $C_\alpha H$ (Asp), J_1 = 14.1 Hz, J_2 = 14.2 Hz); 4.22 (dt, 1 H, $C_\alpha H$ (Arg), J_1 = 15.6 Hz, J_2 = 14.1 Hz); 3.72 (t, 2 H, CH_2 (Gly-1), J = 6.1 Hz); 3.66 (d, 2 H, CH_2 (Gly-2), J = 6.1 Hz); 3.61 (s, 3 H, MeO); 3.59 (s, 3 H, MeO); 3.02 (dt, 2 H, C(5)H_2 (Arg), J_1 = 12.3 Hz, J_2 = 11.9 Hz); 2.95 (s, 2 H, CH_2 (Pbf)); 2.78 (dd, 1 H, CH_A (Asp), J_1 = 16.6 Hz, J_2 = 6.1 Hz); 2.72 (dd, 1 H, CH_B (Asp), J_1 = 16.4 Hz, J_2 = 6.7 Hz); 2.47 (s, 3 H, Me (Pbf)); 2.42 (s, 3 H, Me (Pbf)); 2.00 (s, 3 H, Me (Pbf)); 1.70—1.60 (m, 2 H, C(3)H_2); 1.50—1.40 (m, 2 H, C(4)H_2 (Arg)); 1.39 (s, 6 H, 2 Me (Pbf)). HRMS, found: *m/z* 684.3022 $[M + H]^+$. Calculated for $C_{29}H_{46}N_7O_{10}S$: 684.3027.

Dimethyl N^α-*tert*-butyloxycarbonyl-L-lysyl-(N^ω-2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-L-arginyl-glycyl-L-aspartate (16) was obtained similarly to compound **15**. The product was purified by flash-column chromatography in the $CHCl_3$—MeOH solvent system (gradient from 100 : 0 to 85 : 15) and preparative HPLC (eluent MeOH—0.2%AcOH (35 : 65)). The yield was 0.198 g (68%), a foamed oil, m.p. 111 °C, $[\alpha]_D^{20}$ −6.6 (*c* 0.45, MeOH). Found (%): C, 52.44; H, 7.35, N, 10.70. $C_{38}H_{62}N_8O_{12}S \cdot MeCOOH$. Calculated (%):C, 52.50; H, 7.27; N, 12.25; S, 3.50. HPLC (Kromasil 100-5C18, MeOH—0.15%CF_3COOH (75 : 25)), τ_R 6.41. R_f 0.38 ($CHCl_3$—MeOH (1 : 1)). IR, ν/cm^{-1}: 3302 (NH); 2938, 2870 (CH_2, CH); 1738 (C=O (COOMe)); 1685, 1657 (C=O (CO—NH (amide 1), NH—CO—O, C—C arom.); 1545 (N—C=O (amide 2), C—C arom.); 1439, 1408, 1367 (C—N (amide 3), R—SO_2—N); 1243 (C—H); 1163, 1090, 995 (C—O (COOMe), R—SO_2—N, N—H, C—C arom.); 852, 807, 783, 734 (C—H, N—H, S—O); 659, 641, 620, 567, 507. ^{1}H NMR (400 MHz), δ: 8.32 (d, 1 H, NH (Asp), J = 5.2 Hz); 8.21 (t, 1 H, NH (Gly), J = 5.6 Hz); 7.85 (d, 1 H, NH (Arg), J = 7.8 Hz); 6.9 (d, 1 H, NH_ε (Lys), J = 8.4 Hz); 6.85 (d, 1 H, NH_α (Lys), J = 7.7 Hz); 6.79, 6.65, 6.35, (all br.s, 3 H, NH (Arg)); 4.65 (dt, 1 H, $C_\alpha H$ (Asp), J_1 = 11.4 Hz, J_2 = 6.7 Hz); 4.20 (dt, 1 H, $C_\alpha H$ (Arg), J_1 = 13.9 Hz, J_2 = 7.0 Hz); 3.87 (m, 1 H, $C_\alpha H$ (Lys)); 3.72 (d, 2 H, CH_2 (Gly), J = 5.7 Hz); 3.61 (s, 3 H, MeO); 3.58 (s, 3 H, MeO); 3.02 (dt, 2 H, C(5)H_2 (Arg), J_1 = 12.3 Hz, J_2 = 5.5 Hz); 2.95 (s, 2 H, CH_2 (Pbf)); 2.92—2.94 (m, 2 H, C(6)H_2 (Lys)); 2.78 (dd, 1 H, CH_A (Asp), J_1 = 16.6 Hz, J_2 = 6.3 Hz); 2.72 (dd, 1 H, CH_B (Asp), J_1 = 16.6 Hz, J_2 = 6.8 Hz); 2.47 (s, 3 H, Me (Pbf)); 2.41 (s, 3 H, Me (Pbf)); 2.00 (s, 3 H, Me (Pbf)); 1.70—1.60 (m, 2 H, C(3)H_2); 1.50—1.40 (m, 2 H, C(4)H_2 (Arg)); 1.50—1.40 (m, 6 H, C(5)H_2 + C(4)H_2 + C(3)H_2 (Lys)); 1.40 (s, 6 H, 2 Me (Pbf); 1.35 (s, 9 H, 3 Me (Boc)). HRMS, found: *m/z* 855.4275 $[M + H]^+$. Calculated for $C_{38}H_{63}N_8O_{12}S$: 855.4286.

Dimethyl *N*-carboxybutanoyl-glycyl-(N^ω-2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-L-arginyl-glycyl-L-aspartate (18). Glutaric anhydride **17** (0.119 g, 0.168 mmol) was added to a solution of tetrapeptide **15** (0.115 g, 0.168 mmol) in $CHCl_3$ (3 mL) with magnetical stirring. The reaction mixture was allowed to stand for 16 h at ~20 °C, followed by the evaporation of volatile components. The product was dried *in vacuo*. The target compound was isolated by flash-column chromatography (eluent $CHCl_3$—MeOH, gradient from 100 : 0 to 0 : 100). The yield was 0.099 g (74%), a crystallized oil, m.p. 100 °C, $[\alpha]_D^{20}$ −1.5 (*c* 3.22, MeOH). Found (%): C, 51.05; H, 6.31; N, 11.94; S, 3.93. $C_{34}H_{51}N_7O_{13}S$. Calculated (%): C, 51.18; H, 6.44; N, 12.29; S, 4.02. HPLC (Kromasil 100-5C18, MeOH—0.5%AcOH, gradient from 3 : 7 to 9 : 1), τ_R 17.86. R_f 0.40 ($CHCl_3$—MeOH (2 : 1)). IR, ν/cm^{-1}: 3309 (NH); 2928, 2856 (CH_2, CH); 1736 (C=O (COOMe)); 1643 (C=O (CO—NH (amide 1), C—C arom.); 1537 (N—C=O (amide 2), C—C arom.); 1438, 1408, 1370 (C—N (amide 3), R—SO_2—N); 1222 (C—H); 1156, 1090, 1026, 994 (C—O (COOMe), R—SO_2—N, N—H, C—C arom.); 851, 808, 782, 733 (C—H, N—H, S—O); 659, 640, 618, 565, 506. ^{1}H NMR (400 MHz), δ: 8.28 (d, 1 H, NH (Asp), J = 7.7 Hz); 8.24 (t, 1 H, NH (Gly-1), J = 5.8 Hz); 8.04 (d, 1 H, NH (Arg), J = 8.8 Hz); 8.02 (t, 1 H, NH (Gly-2), J = 5.6 Hz); 7.05, 6.67, 6.39 (all br.s, 3 H, NH (Arg)); 4.66 (dt, 1 H, $C_\alpha H$ (Asp), J_1 = 13.6 Hz, J_2 = 6.8 Hz); 4.23 (dt, 1 H, $C_\alpha H$ (Arg), J_1 = 13.2 Hz, J_2 = 7.7 Hz); 3.71 (m, 4 H, 2 CH_2 (Gly-1, Gly-2)); 3.61 (s, 3 H, MeO); 3.57 (s, 3 H, MeO); 3.03 (m, 2 H, C(5)H_2 (Arg)); 2.51 (br.s, 2 H, CH_2 (Pbf)); 2.71 (dd, 1 H, CH_B (Asp), J_1 = 16.7 Hz, J_2 = 7.0 Hz); 2.79 (dd, 1 H, CH_A (Asp), J_1 = 16.5 Hz, J_2 = 6.2 Hz); 2.48 (s, 3 H, Me (Pbf)); 2.40 (s, 3 H, Me (Pbf)); 2.20 (t, 2 H, CH_2 (glutaric acid), J = 7.4 Hz); 2.16 (t, 2 H, CH_2 (glutaric acid), J = 7.6 Hz); 2.00 (s, 3 H, Me (Pbf)); 1.70 (m, 2 H, CH_2 (glutaric acid); 1.60—1.40 (m, 4 H, C(3)H_2, C(4)H_2 (Arg)); 1.41 (s, 6 H, 2 Me (Pbf)). HRMS, found: *m/z* 798.3333 $[M + H]^+$. Calculated for $C_{34}H_{52}N_7O_{13}S$: 798.3344.

Dimethyl N^ε-carboxybutanoyl-N^α-*tert*-butyloxycarbonyl-L-lysyl-(N^ω-2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-arginyl-glycyl-L-aspartate (19) was obtained similarly to compound **18**. The product was purified by flash-column chromatography in the $CHCl_3$—MeOH solvent system (gradient from 100 : 0 to 75 : 25) and preparative HPLC (eluent MeOH—0.2%AcOH (65 : 35)). The yield was 0.051 g (23%), a crystallized

oil, m.p. 99 °C, $[\alpha]_D^{20}$ –3.1 (*c* 0.25, MeOH). Found (%): C, 50.42; H, 7.39; N, 11.52; S, 3.37. $C_{43}H_{68}N_8O_{15}S \cdot 2H_2O \cdot CH_3COOH$. Calculated (%):C, 50.74; H, 7.19; N, 10.52; S, 3.01. HPLC (Kromasil 100-5C18, MeOH—0.15%CF_3COOH (65 : 35)), τ_R 10.11. R_f 0.40 ($CHCl_3$—MeOH (2 : 1)). IR, ν/cm^{-1}: 3297 (NH); 2973, 2935, 2860 (CH_2, CH); 1738 (C=O (COOMe)); 1659 (C=O (CO—NH (amide 1), NH—CO—O, C—C arom.); 1537 (N—C=O (amide 2), C—C arom.); 1439, 1403, 1392, 1367 (C—N (amide 3), R—SO_2—N); 1242, 1203 (C—H); 1162, 1090, 995 (C—O (COOMe), R—SO_2—N, N—H, C—C arom.); 852, 803, 782, 733, 722 (C—H, N—H, S—O); 658, 641, 618, 566, 507. ^{1}H NMR (400 MHz), δ: 8.27 (dt, 1 H, NH (Asp), J = 5.2 Hz); 8.27 (t, 1 H, NH (Asp), J = 3.5 Hz); 8.25 (t, 1 H, NH (Gly), J = 5.4 Hz); 7.85 (d, 1 H, NH (Arg), J = 7.7 Hz); 7.75 (t, 1 H, NH_ε (Lys), J = 6.1 Hz); 6.85 (d, 1 H, NH_α (Lys), J = 7.7 Hz); 4.65 (dt, 1 H, $C_\alpha H$ (Asp), J_1 = 13.3 Hz, J_2 = 7.5 Hz); 4.20 (dt, 1 H, $C_\alpha H$ (Arg), J_1 = 13.9 Hz, J_2 = 7.0 Hz); 3,87 (m, 1 H, $C_\alpha H$ (Lys)); 3.72 (d, 2 H, CH_2 (Gly), J = 4.9 Hz); 3.61 (s, 3 H, MeO); 3.58 (s, 3 H, MeO); 3.02 (dt, 2 H, C(5)H_2 (Arg), J_1 = 11.6 Hz, J_2 = 6.0 Hz); 2.95 (s, 2 H, CH_2 (Pbf)); 2.92—2.94 (m, 2 H, C(6)H_2 (Lys)); 2.78 (dd, 1 H, CH_A (Asp), J_1 = 16.6 Hz, J_2 = 6.3 Hz); 2.72 (dd, 1 H, CH_B (Asp), J_1 = 16.6 Hz, J_2 = 6.8 Hz); 2.47 (s, 3 H, Me (Pbf)); 2.41 (s, 3 H, Me (Pbf)); 2.00 (s, 3 H, Me (Pbf)); 1.65—1.75 (m, 6 H, 3 CH_2 (glutaric acid)); 1.70—1.60 (m, 2 H, C(3)H_2); 1.50—1.40 (m, 2 H, C(4)H_2 (Arg)); 1.45—1.40 (m, 6 H, C(5)H_2 + C(4)H_2 + C(3)H_2 (Lys)); 1.35 (s, 6 H, 2 Me (Pbf); 1.32 (s, 9 H, 3 Me(Boc)). HRMS, found: *m/z* 969.4598 $[M + H]^+$, calculated for $C_{43}H_{69}N_8O_{15}S$: 969.4603; found: *m/z* 991.4415 $[M + Na]^+$, calculated for $C_{43}H_{68}N_8NaO_{15}S$: 991.4423.

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References

1. L. D. Field, J. B. Delehanty, Y. C. Chen, I. L. Medintz, *Acc. Chem. Res.*, 2015, **48**, 1380.
2. Y. L. Janin, *Amino Acids*, 2003, **25**, 1.
3. A. K. Mitra, V. Agrahari, A. Mandal, K. Cholkar, C. Natarajan, S. Shah, M. Joseph, H. M. Trinh, R. Vaishya, X. Yang, Y. Hao, V. Khurana, D. Pal, *J. Control. Release*, 2015, **219**, 248.
4. A. N. Zakutskii, N. I. Chalisova, T. F. Subbotina, *Russ. J. Bioorg. Chem.*, 2008, **34**, 135.
5. M. D. Pierschbacher, E. Ruoslahti, *Nature*, 1984, **309**, 30.
6. R. Silva, G. D'Amico, D. K. M. Hodivala, L. E. Reynolds, *Arterioscler. Thromb. Vasc. Biol.*, 2008, **28**, 1703.
7. C. Höltke, A. Faust, *Rep. Med. Imag.*, 2017, **10**, 17.
8. S. L. Bellis, *Biomaterials*, 2011, **32**, 4205.
9. M. A. Dechantsreiter, E. Planker, B. Mathä, E. Lohof, G. Hölzemann, A. Jonczyk, S. L. Goodman, H. J. Kessler, *J. Med. Chem.*, 1999, **42**, 3033.
10. S. Liu, *Bioconjug. Chem.*, 2015, **26**, 1413.
11. D. Xu, Z.-Q. Zhao, S.-T. Chen, Y. Yang, W. Fang, S. Liu, *Nucl. Med. Biol.*, 2017, **48**, 1.
12. Z.-Q. Zhao, S. Ji, X.-Y. Li, W. Fang, S. Liu, *Appl. Radiat. Isotopes*, 2019, **148**, 168.
13. Y. Shao, W. Liang, F. Kang, W. Yang, X. Ma, G. Li, S. Zong, K. Chen, J. Wang, *Amino Acids*, 2014, **46**, 2355.
14. X. Chen, R. Park, A. H. Shahinian, J. R. Bading, P. S. Conti, *Nucl. Med. Biol.*, 2004, **31**, 11.
15. M. G. Rakhimov, A. Ya. Maruk, A. B. Bruskin, *Russ. Chem. Bull.*, 2016, **65**, 363.
16. C. G. Alves, R. Lima-Sousa, D. de Melo-Diogo, R. O. Louro, I. J. Correia, *Int. J. Pharm.*, 2018, **542**, 164.
17. Y. Zheng, S. Ji, A. Czerwinski, F. Valenzuela, M. Pennington, S. Liu, *Bioconjugate Chem.*, 2014, **25**, 1925.
18. Y. Wu, W. Cai, X. Chen, *Mol. Imaging Biol.*, 2006, **8**, 226.
19. R. Tang, J. Xue, B. Xu, D. Shen, G. P. Sudlow, S. Achilefu, *ACS Nano*, 2015, **9**, 220.
20. B. R. Smith, Z. Cheng, A. De, A. L. Koh, R. Sinclair, S. S. Gambhir, *Nano Lett.*, 2008, **8**, 2599.
21. K. Chen, J. Xie, H. Xu, D. Behera, M. H. Michalski, S. Biswal, A. Wang, X. Chen, *Biomaterials*, 2009, **30**, 6912.
22. X. Huang, Y. Yuan, W. Ruan, L. Liu, M. Liu, S. Chen, X. Zhou, *J. Nanobiotechnol.*, 2018, **16**, 30.
23. Y. Yang, J. Zhou, K. Yu, *Amino Acids*, 2014, **46**, 449.
24. A. Nortcliffe, I. N. Fleming, N. P. Botting, D. O'Hagan, *Tetrahedron*, 2014, **70**, 8343.
25. R. Colombo, M. Mingozzi, L. Belvisi, D. Arosio, U. Piarulli, N. Carenini, P. Perego, N. Zaffaroni, M. De Cesare, V. Castiglioni, E. Scanziani, C. Gennari, *J. Med. Chem.*, 2012, **55**, 10460.
26. X. Wang, X. Qiao, Y. Shang, S. Zhang, Y. Li, H. He, S. Chen, *Amino Acids*, 2017, **49**, 931.
27. M. Boisbrun, R. Vanderesse, P. Engrand, A. Olié, S. Hupont, J.-B. Regnouf-de-Vains, C. Frochot, *Tetrahedron*, 2008, **64**, 3494.
28. C. Frochot, B. Di Stasio, R. Vanderesse, M.-J. Belgy, M. Dodeller, F. Guillemin, M.-L. Viriot, M. Barberi-Heyo, *Bioorg. Chem.*, 2007, **35**, 205.
29. H. Vilac, P. M. T. Ferreira, N. M. Micaelo, *Tetrahedron*, 2014, **70**, 5420.
30. K. Yamada, I. Nagashima, M. Hachisu, I. Matsuo, H. Shimizu, *Tetrahedron Lett.*, 2012, **53**, 1066.
31. Y. Liu, L. Zhang, J. Wan, Y. Li, Y. Xu, Y. Pan, *Tetrahedron*, 2008, **64**, 10728.
32. C. Koutsas, Y. Sarigiannis, G. Stavropoulos, M. Liakopoulou-Kyriakides, *Protein Peptide Lett.*, 2007, **14**, 1014.
33. D. J. Welsh, D. K. Smith, *Org. Biomol. Chem.*, 2011, **9**, 4795.
34. O. V. Mel´nik, V. P. Martinovich, V. P. Golubovich, *Russ. J. Bioorg. Chem.*, 2006, **32**, 122.
35. A. M. Demin, A. Y. Vigorov, I. A. Nizova, M. A. Uimin, N. N. Shchegoleva, A. E. Ermakov, V. P. Krasnov, *Mendeleev Commun.*, 2014, **24**, 20.
36. A. Yu. Vigirov, A. M. Demin, I. A. Nizova, V. P. Krasnov, *Russ. J. Bioorg. Chem.*, 2014, **40**, 142.
37. E. G. Stepanenko, Yu. L. Sebyakin, *Russ. J. Bioorg. Chem.*, 2004, **30**, 111.
38. M. Abo-Ghalia, S. Abd El-Rahman, A. El-Kafrawy, A. Kalomuch, *Amino Acids*, 2003, **24**, 405.
39. S. Bahareh, Z. Afshin, S. Soraya, M. Reza, K. Farzad, *Arch. Pharm. Chem. Life Sci.*, 2019, e1800379.

40. L. A. Carpino, B. J. Cohen, K. E. Stephens, Jr., S. Y. S.-A. J.-H. Tien, D. C. Langridge, *J. Org. Chem.*, 1986, **51**, 3732.
41. E. Valeur, M. Bradley, *Chem. Soc. Rev.*, 2009, **38**, 606.
42. *Fmoc Solid Phase Peptide Synthesis. A Practical Approach*, Eds W. C. Chan, P. D. White, Oxford University Press, New York, 2000, 346 pp.
43. A. Isidro, D. Latassa, M. Giraud, M. Alvarez, F. Albericio, *Org. Biomol.Chem.*, 2009, **7**, 2565.
44. A. M. Demin, A. G. Pershina, A. S. Minin, A. V. Mekhaev, V. V. Ivanov, S. P. Lezhava, A. A. Zakharova, I. V. Byzov, M. A. Uimin, V. P. Krasnov, L. M. Ogorodova, *Langmuir*, 2018, **34**, 3449.
45. A. M. Demin, A. V. Mekhaev, A. A. Esin, D. K. Kuznetsov, P. S. Zelenovskiy, V. Y. Shur, V. P. Krasnov, *Appl. Surf. Sci.*, 2018, **440**, 1196.
46. A. M. Demin, M. S. Valova, A. G. Pershina, V. P. Krasnova, *Russ. Chem. Bull.*, 2019, **68**, 1088.
47. A. M. Demin, A. G. Pershina, V. V. Ivanov, K. V. Nevskaya, O. B. Shevelev, A. S. Minin, I. V. Byzov, A. E. Sazonov, V. P. Krasnov, L. M. Ogorodova, *Inter. J. Nanomed.*, 2016, **11**, 4451.
48. A. M. Demin, A. G. Pershina, K. V. Nevskaya, L. V. Efimova, N. N. Shchegoleva, M. A. Uimin, D. K. Kuznetsov, V. Ya. Shur, V. P. Krasnov, L. M. Ogorodova, *RSC Adv.*, 2016, **6**, 60196.
49. M. Brenner, W. Huber, *Helv. Chim. Acta.*, 1953, **36**, 1109.
50. V. F. Pozdnev, *Chem. Nat. Compd.*, 1974, **10**, 782.

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