Preparation of Pseudo-Peptide Building Blocks with *retro*-Thioamide Bond Mediated *via* Thiocarbamoyl *Meldrum*'s Acid

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An easy and efficient synthesis of pseudo-tripeptide containing a thiomalonamide moiety was developed. Isothiocyanate derivatives of amino acids react smoothly with 2,2-dimethyl-1,3-dioxane-4,6-dione (*Meldrum*'s acid) to yield new thiocarbamoyl derivatives of *Meldrum*'s acids. Thermal decomposition of these new derivatives leads to thiocarbamoyl ketenes, which acylate amino acid esters to give pseudo-tripeptides.

Introduction. – It has been more than well-known that peptides play an essential role in virtually all biochemical processes. One can, at this point, mention the widespread use in medicine of long peptides derived from biotechnology such as, *e.g.*, well-known interferons, erythropoetin, insulin, *etc.*, to very short ones, like oxytocine, enkephalin, or tuftsine. But all peptides with long or short chains possess the same drawback, *i.e.*, their rapid enzymatic degradation in living organisms [1]. As a consequence, several modifications of the peptides backbone were undertaken. For example, an amide bond could be replaced by a *retro-inverso* hydroxyethylene [2], hydroxyethylene [3], ketomethylene [4], aminomethylene [5], *retro-inverso* [6] or thioamide moiety [7].

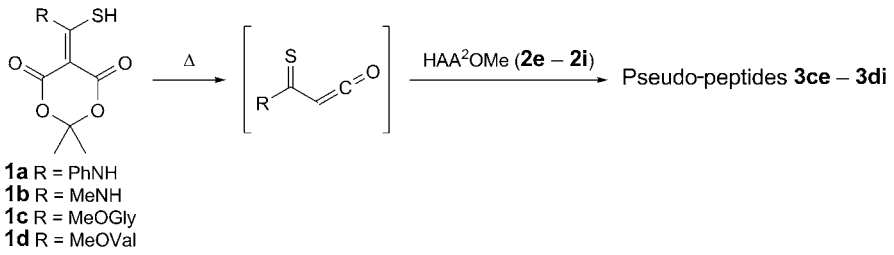
In our research, we focused on the possibility of obtaining a scaffold of *retro*-modified petides, which also contain a thioamide modification-introduced regioselectively.

One of the aproaches to *retro*-modification of peptide is based on the introduction of a malonodiamide moiety in the structure of the molecule. Implementation of this task requires the use of any malonic derivative. Most of the syntheses of malonodiamide type of *retro*-modified peptides start from mono- or diesters of malonic acid, which are incorporated into the peptide chain by the typical condensation procedures [8]. In another original aproach, *Meldrum*'s acid was used as an equivalent of malonic acid; such a strategy allows applying the acylating properties of 2,2-dimethyl-1,3-dioxane-4,6-dione (*Meldrum*'s acid) to facilitate preparation of the first amide bond, whereas the second amide bond was obtained by the classical method [9].

Recently, we have demonstrated that carbamoyl *Meldrum*'s acid derivatives in the presence of Me_3SiCl (TMSCl) may acylate efficiently also more basic nucleophiles as aliphatic secondary amines [10] in contrast to the work of *Pak* and co-workers [11]. In such a synthetic strategy, 5-[(aryl/alkylamino)hydroxymethylene]-2,2-dimethyl-1,3-dioxane-4,6-diones by thermal decomposition are a source of carbamoyl ketenes, which can acylate a broad spectrum of nucleophilic reagents.

Here, we propose that S analogs of carbamoyl *Meldrum*'s acid, **1**, could be used for the preparation of modified peptides containig a *retro*-thioamide bond. Realization of this goal requires, first, preparation of sufficiently stable compounds of type **1**, where R^1 is an amino acid moiety, second, generation of thiocarbamoyl ketenes from **1**, and finally acylation of *C*-protected amino acids with the new thiocarbamoyl ketenes (*Scheme 1*).

Scheme 1



Results and Discusion. – First, we decided to check if the 'non-peptide' S derivative 2,2-dimethyl-5-[(phenylamino)(sulfanyl)methylidene]-1,3-dioxane-4,6-dione (**1a**) would be a good source of thiocarbamoyl ketenes, and if the acylation of nucleophiles with this ketenes would be possible in the usual way.

In the first experiments, we heated in boiling $PhCH_2Me$ 1 equiv. of **1a** in the presence of 2 equiv. of aniline. The process was stopped when TLC analysis showed disappearance of all starting *Meldrum*'s derivative **1a**. From the reaction mixture, we isolated 3-anilino-*N*-phenyl-3-thioxopropanamide (**3aa**) in 79% yield (*Table*, *Entry 1*). In the next very similar experiment, we used *p*-toluidine as a trapping nucleophile. The result of this experiment was surprising, as we isolated from the mixture an inseparable mixture of two very similar thiomalonamides **3ab** and **3xb** (*Entry 2*).

The unexpected formation of a second product **3xb** may occur following two paths: first, by the vinylic substitution on the starting carbamoyl *Meldrum*'s acid derivative, followed by decomposition to thiocarbamoyl ketene and its reaction with the nucleophil, or, by a second path, which involves transamidation of the initially formed thioamide **3ab** (*Scheme 2*).

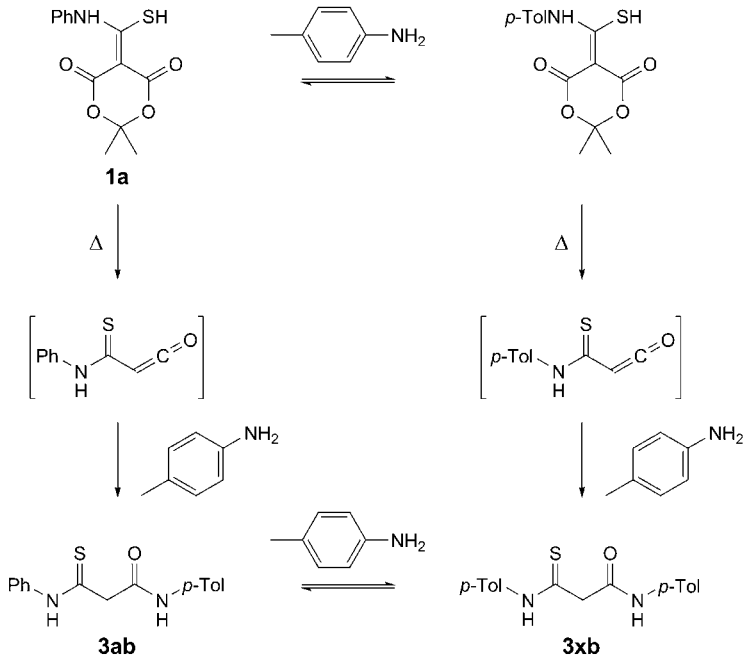
To decide which of the paths leads to the formation of thioamide **3xb**, we performed two experiments. In the first one, we heated in boiling $PhCH_2Me$, 1 equiv. of **3aa**, which was previously prepared in an independent way, with 2 equiv. of *p*-toluidine (4-methylaniline) for 1 h. The ^{1}H-NMR spectra of this mixture indicated formation of *N*-(4-methylphenyl)-3-[(4-methylphenyl)amino]-3-thioxopropanamide (**3xb**). The ratio of thiomalonoamides **3ab** and **3xb** was estimated on the basis of integration of the malonic CH_2 group and indicated that **3xb**, from classical transamidation, arose only in 9% yield, while, in the reaction of **1a** with toluidine, **3xb** was formed with 16% yield within only 35 min. In the second experiment, we heated **1a** with a 15-fold excess of *p*-toluidine in CH_2Cl_2 as a low boiling solvent, in order to ensure a non thermolytic condition, to check if the vinylic substitution would be the predominant reaction.

Table. *Reactions of Thiocarbamoyl* Meldrum*'s Acid with Nucleophiles*

Entry	**1**	R^1	**2**	H–Nu	Product **3**	Solvent[a])	Time [h]	Yield [%]
1[b])	**a**	PhNH	**a**	H–NHPh	**3aa**	*A*	0.6	79
2[b])	**a**	PhNH	**b**	H–NH–*p*-Tol	**3ab** + **3xb**	*A*	0.6	–[c])
3	**b**	MeNH	**b**	H–NH–*p*-Tol	**3bb**	*A*	1	100
4	**b**	MeNH	**c**	H–NH–*t*-Bu	**3bc**	*A*	1	99
5	**b**	MeNH	**d**	H–NH–Bn	**3bd**	*A*	1	75
6	**c**	MeOGly	**b**	H–NH–*p*-Tol	**3cb**	*B*	4.1	68
7	**c**	MeOGly	**e**	H–GlyOMe	**3ce**	*B*	6	50
8	**c**	MeOGly	**f**	H–AlaOMe	**3cf**	*B*	6.5	62
9	**c**	MeOGly	**f**	H–AlaOMe	**3cf**	*C*	1.1	58
10	**c**	MeOGly	**g**	H–ValOMe	**3cg**	*B*	5.5	49
11	**c**	MeOGly	**h**	H–LeuOMe	**3ch**	*B*	5.5	71
12	**d**	MeOVal	**e**	H–GlyOMe	**3de**	*B*	7	50
13[d])	**c**	MeOGly	**f**	H–AlaOMe	**3cf**	*B*	6.5	51
14	**c**	MeOGly	**i**	H–NHCH$_2$CH$_2$NHAc	**3ci**	*B*	18	33
15[d])	**c**	MeOGly	**i**	H–NHCH$_2$CH$_2$NHAc	**3ci**	*B*	18	70
16[d])	**d**	MeOVal	**i**	H–NHCH$_2$CH$_2$NHAc	**3di**	*B*	22.5	73

[a]) Solvent: *A*, PhCH$_2$Me, *B*, ClCH$_2$CH$_2$Cl, *C*, toluene. [b]) 2 Equiv. of **2** were used. [c]) Mixture of two products. [d]) TMSCl (2 equiv.) was used.

Scheme 2



However, we observed only unreacted starting material in the mixture. These observations strongly indicate that **3xb** is formed *via* both reaction paths, but higher yields of transamidation product are obtained from the reaction where the starting material was **1a**, and suggests that vinylic substitution at **1a** is a faster reaction than classical transamidation in already formed thiomalonamide.

The complications in the experiment with **1a** are connected with the properties of aromatic amines as moderately good leaving groups. Hence, in the next experiments, we used 2,2-dimethyl-5-[(methylamino)(sulfanyl)methylidene]-1,3-dioxane-4,6-dione (**1b**; *Entries 3–5*). Application of the methylamino derivative allowed obtaining thiomalonoamides with good yields without transamidation products; these experiments confirmed that thiocarbamoyl ketenes can be generated from **1** and trapped by nucleophiles.

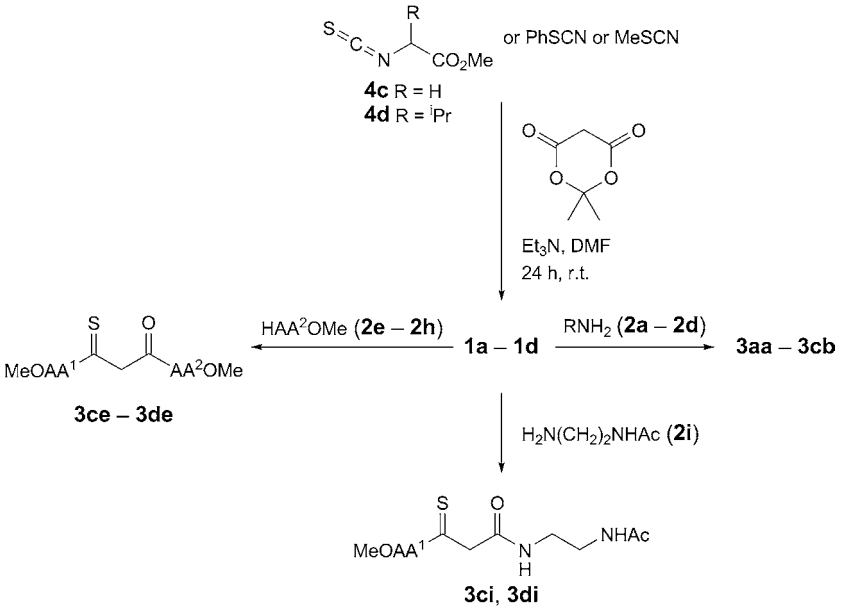
In the next step, we synthesized compounds **1** with substitutent R^1 other than alkyl or aryl group, *e.g.*, to obtain the Gly and Val derivatives **1c** and **1d**, which, after thermolysis in the presence of a nucleophile, would be equivalents of *retro*-thioamide dipeptides GlyΨ(NHCS)Gly or ValΨ(NHCS)Gly, respectively [12]. For our purposes, we synthesised isothiocyanato derivatives of acetic and isovaleric acid **4c** and **4d**, respectively, by the method described in [13]. These compounds were used for the preparation of *Meldrum*'s acid derivatives by a modification of the procedure introduced by *Pak* and co-workers [11]. We obtained new *Meldrum*'s acid derivatives **1c** and **1d**, however, it should be stressed that these new derivatives were much less stable than ordinary *Meldrum*'s acid derivatives and decomposed when trying to purify or standing at room temperature. Hence, for our reactions we have used freshly prepared crude compounds **1c** and **1d** (*Scheme 3*).

First, we performed a reaction of 1 equiv. of **1c** with 1 equiv. of *p*-toluidine. The reaction was carried out in boiling $PhCH_2Me$ up to complete decomposition of **1c**, and we obtained a dark-brown mixture with a lot of tar. TLC Analysis indicated the presence of a complex mixture of compounds. We repeated this experiment with $ClCH_2CH_2Cl$ as a lower-boiling solvent, which increased the reaction time but allowed us to obtain the required thiomalonoamide **3cb** in 68% yield (*Entry 6*).

Encouraged by the results of this experiment, we conducted a series of reactions to obtain the desired pseudo-peptides with a thiomalonoamido residue. We reacted in boiling $ClCH_2CH_2Cl$ 1 equiv. of **1c** or **1d** in the presence of amino acid hydrochloride. From the mixtures, we isolated pseudo-tripeptides in good yields (*Entries 6–12*). We also performed one experiment in toluene as a higher-boiling solvent to check if it would be possible to reduce the time required for completion of the reaction (*Entry 9*). Indeed, the reaction time was shortened; however, the yield was slightly lower. For this reason, in the remaining experiments we used only the less harsh conditions.

The prepared pseudo-peptides **3** are terminated with carboxylic groups on both ends. For practical application, pseudo-peptide building blocks terminated with an amino group on one side would be more useful [14]. For this purpose, we used $NH_2CH_2CH_2NHAc$ as a trap for thiocarbamoyl ketenes. However, from the first experiment, carried out with **1d** and the *N*-(2-aminoethyl)acetamide (**2i**), we obtained the pseudo-tripeptide in low yield (*Entry 14*). This case, again, confirmed the previous observation [10] that less basic nucleophiles, *i.e.*, esters of amino acids, provide better yield than more basic ones, *e.g.*, derivatives of ethylenediamine.

Scheme 3



Based on our previous experiences with acylation of amines by ketenes generated from *Meldrum*'s acid derivatives, we used TMSCl for a solution of this problem. From the reaction mixture formed by heating **2i** with **1c** or **1d** in $ClCH_2CH_2Cl$ in the presence of 2 equiv. of TMSCl ,we isolated *retro*-thiotripeptides **3ci** and **3di**, respectively, in high yields. Additionally, we repeated the reaction of **1c** with alanine methyl ester hydrochloride in the presence of TMSCl, and, in this case, in accordance with our predictions, we did not observe an increase of yield (*Entry 13*).

In summary, we developed a new one pot method for the preparation of *retro*-thioamide-modified peptides, based on thermolysis of new *Meldrum*'s acid derivatives. It should be mentioned that our method allows two concurrent modifications, *i.e.*, the regioselective introduction of a thioamide and a *retro*-amide moiety.

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Experimental Part

General. All of the org. solvents used in this study were dried on appropiate drying agents and distilled prior to use. Commercially available reagents were from *Sigma–Aldrich.* Commercially unavailable reagents were prepared according to literature procedures: *2,2-dimethyl-5-[(phenylamino)(sulfanyl)methylidene]1,3-dioxane-4,6-dione* (**1a**): [15], amino acid isothiocyanates: [13]. TLC: *Merck Kieselgel 60* F_{254}. Flash column chromatography (CC): *Zeochem ZEOprep 60/40-63*. M.p: *Warsztat Elektromechaniczny W-wa*; uncorrected. NMR Spectra: *Varian Unity Plus 500* (^{1}H: 500 and ^{13}C: 125 MHz), *Varian Gemini 200* (^{1}H: 200 and ^{13}C: 50 MHz); chemical shifts (δ) in ppm rel. to internal Me_4Si; coupling constants *J* in Hz. HR-ESI-MS: *MicroMas Quattro LCT* mass spectrometer.

2,2-Dimethyl-5-[(methylamino)(sulfanyl)methylidene]-1,3-dioxane-4,6-dione (**1b**). To a soln. of *Meldrum*'s acid (0.72 g, 5 mmol) in dry DMF (2 ml) in a glass ampoule, Et_3N (1.4 ml, 10 mmol) was added. Methyl isothiocyanate (0.365 g, 5 mmol) was added, and the ampoule was sealed. The ampoule was placed in the bath for 24 h at 40°. The mixture was poured into ice-cold aq. 2M HCl (15 ml). The precipitate was filtered and washed with cold H_2O. The precipitate was dissolved in AcOEt (30 ml) and dried ($MgSO_4$). The solvent was removed under reduced pressure. Crystallization from AcOEt/hexane gave 0.400 g (37%) of **1b**. M.p. 99–101°. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.72 (*s*, 6 H); 3.16 (*d*, *J* = 5.0, 3 H); 11.32 (br. *s*, 1 H); 13.40 (br. *s*, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 26.5; 32.1; 82.8; 104.7; 163.4; 170.8; 182.1. HR-ESI-MS: 240.0331 ($[M+Na]^+$, $C_8H_{11}NNaO_4S^+$; calc. 240.0305).

Methyl N-*[(2,2-Dimethyl-4,6-dioxo-1,3-dioxan-5-ylidene)(sulfanyl)methyl]glycinate*; (**1c**). To a soln. of *Meldrum*'s acid (0.72 g, 5 mmol) in dry DMF (2 ml) in a glass ampoule, Et_3N (1.4 ml, 10 mmol) was added. *Methyl 2-(isothiocyanato)acetate* [13] (**4**; 0.655 g, 5 mmol) was added, and the ampoule was sealed and kept for 24 h at r.t. The mixture was poured into ice-cold aq. 2M HCl (15 ml). The precipitate was filtered and washed thoroughly with cold H_2O. The precipitate was dried in vaccum desicator over P_4O_{10} and used without further purification. Yield: 1.16 g (88%). Yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.77 (*s*, 6 H); 3.84 (*s*, 3 H); 4.36 (*d*, *J* = 5.2, 2 H); 11.61 (br. *s*, 1 H); 16.25 (br. *s*, 1 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 26.2; 46.5; 53.1; 83.9; 105.0; 163.6; 168.2; 172.9; 184.5. HR-ESI-MS: 274.0395 ($[M-H]^-$, $C_{10}H_{12}NO_6S^-$; calc. 274.0384).

Methyl N-*[(2,2-Dimethyl-4,6-dioxo-1,3-dioxan-5-ylidene)(sulfanyl)methyl]valinate* (**1d**). To a soln. of *Meldrum*'s acid (0.72 g, 5 mmol) in dry DMF (2 ml) in a glass ampoule, Et_3N (1.4 ml, 10 mmol) was added. *Methyl 2-(isothiocyanato)-3-methylbutanoate* [13] (0.865 g, 5 mmol) was added, and the ampoule was sealed and kept for 24 h at r.t. The mixture was poured into ice-cold aq. 2M HCl (15 ml). The mixture was extracted with AcOEt (2 × 30 ml), and the org. layer was washed with brine (2 × 30 ml) and sat. aq. $NaHCO_3$ (2 × 30 ml). The alkaline layer was acidified with 2M HCl and extracted with AcOEt (2 × 30 ml), and the org. layer was dried ($MgSO_4$). After filtration, the solvents were removed under reduced pressure. The obtained yellow oil was used without further purification. Yield: 0.72 g (47%). ^{1}H-NMR (500 MHz, $CDCl_3$): 0.99 (*d*, *J* = 6.7, 3 H); 1.02 (*d*, *J* = 7.0, 3 H); 1.71 (*s*, 6 H); 2.30–2.37 (*m*, 1 H); 3.73 (*s*, 3 H); 4.60 (*dd*, *J* = 7.9, 4.8, 2 H); 11.62 (*d*, *J* = 7.9, 1 H); 15.93 (br. *s*, 1 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 18.3; 19.6; 26.1; 31.3; 52.7; 63.1; 83.7; 104.7; 163.1; 170.3; 183.9.

Preparation of N,N'*-Disubstituted Thiomalonamides or Pseudo-Peptides,* **3aa**–**3dg**, *with* retro-*Thioamide Bond. General Procedure.* A soln. of **1** (2 mmol) and the corresponding amine or amino acid hydrochloride **2** (2 mmol) in an anh. solvent ($PhCH_2Me$ (*A*), $ClCH_2CH_2Cl$ (*B*), or toluene (*C*); 10 ml) was stirred and heated to reflux for the time specified in the *Table*. After completion of the reaction, the solvent was removed under vacuum, and the residue was purified.

N-*Phenyl-3-(phenylamino)-3-thioxopropanamide* (**3aa**). Purification by crystallization from AcOEt/hexane. M.p. 141–144°. ^{1}H-NMR (200 MHz, (D_6)acetone): 4.08 (*s*, 2 H); 7.14–7.81 (*m*, 10 H); 8.51 (*s*, 1 H); 11.20 (*s*, 1 H). ^{13}C-NMR (50 MHz, (D_6)acetone): 55.2; 119.4; 123.1; 123.7; 126.4; 128.8; 129.0; 139.2; 139.7; 165.8; 194.9. HR-ESI-MS: 293.0731 ($[M+Na]^+$, $C_{15}H_{14}N_2NaOS^+$; calc. 293.0725).

3-(Methylamino)-N-*(4-methylphenyl)-3-thioxopropanamide* (**3bb**). Purification by crystallization from AcOEt/hexane. M.p. 127–129°. ^{1}H-NMR (500 MHz, $CDCl_3$): 2.34 (*s*, 3 H); 3.20 (*d*, *J* = 4.8, 3 H); 3.95 (*s*, 2 H); 7.14 (*d*, *J* = 8.3, 2 H); 7.40 (*d*, *J* = 8.3, 2 H); 9.07 (*s*, 1 H); 9.79 (*s*, 1 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 21.2; 33.4; 53.0; 120.7; 129.8; 134.7; 135.1; 166.3; 196.0. HR-ESI-MS: 245.0730 ($[M+Na]^+$, $C_{11}H_{14}N_2NaOS^+$; calc. 245.0725).

N-*(*tert*-Butyl)-3-(methylamino)-3-thioxopropanamide* (**3bc**). Purification by CC (AcOEt/hexane 1:1). M.p. 115–118°. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.38 (*s*, 9 H); 3.20 (*d*, *J* = 4.8, 3 H); 6.51 (*s*, 1 H); 9.97 (*s*, 1 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 28.8; 29.9; 33.2; 52.3; 167.9; 196.4. HR-ESI-MS: 211.0877 ($[M+Na]^+$, $C_8H_{16}N_2NaOS^+$; calc. 211.0881).

N-*Benzyl-3-(methylamino)-3-thioxopropanamide* (**3bd**). Purification by crystallization from AcOEt/hexane. M.p. 94–96°. ^{1}H-NMR (500 MHz, $CDCl_3$): 3.19 (*d*, *J* = 4.8, 3 H); 3.77 (*s*, 2 H); 4.42 (*d*, *J* = 5.8, 2 H); 7.15 (*s*, 1 H); 7.26–7.37 (*m*, 5 H); 9.80 (*s*, 1 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 33.3; 44.1; 51.8; 127.9; 128.0; 129.1; 137.4; 168.3; 195.9. HR-ESI-MS: 245.0721 ($[M+Na]^+$, $C_{11}H_{14}N_2NaOS^+$; calc. 245.0725).

Methyl N-*{3-[(4-Methylphenyl)amino]-3-oxopropanethioyl}glycinate* (**3cb**). Purification by CC (AcOEt/hexane 2:3). M.p. 105–107°. ^{1}H-NMR (500 MHz, $CDCl_3$): 2.32 (*s*, 3 H); 3.80 (*s*, 3 H); 3.97 (*s*, 2 H); 4.43 (*d*, *J* = 4.8, 2 H); 7.12 (*d*, *J* = 7.81, 2 H); 7.40 (*d*, *J* = 7.81, 2 H); 8.69 (*s*, 1 H); 9.86 (*s*, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 21.4; 47.8; 53.1; 53.2; 120.9; 129.9; 135.0; 135.2; 166.2; 169.1; 197.2. HR-ESI-MS: 303.0789 ($[M+Na]^+$, $C_{13}H_{16}N_2NaO_3S^+$; calc. 303.0779).

Dimethyl 2,2′-[(1-Oxo-3-thioxopropane-1,3-diyl)diimino]diacetate (**3ce**). Purification by CC (AcOEt/hexane 7:2). Orange oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 3.76 (*s*, 3 H); 3.79 (*s*, 3 H); 3.86 (*s*, 2 H); 4.07 (*d*, *J* = 5.5, 2 H); 4.43 (*d*, *J* = 4.8, 2 H); 7.28 (*s*, 1 H); 9.92 (*s*, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 41.3; 47.2; 50.7; 52.5; 168.1; 168.6; 169.8; 196.0. HR-ESI-MS: 285.0518 ($[M+Na]^+$, $C_9H_{14}N_2NaO_5S^+$; calc. 285.0521).

Methyl N-*{3-[(2-Methoxy-2-oxoethyl)amino]-3-thioxopropanoyl}alaninate* (**3cf**). Purification by CC (AcOEt/hexane 2:1). M.p. 116–120°. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.45 (*d*, *J* = 7.3, 3 H); 3.73 (*s*, 3 H); 3.78 (*s*, 3 H); 3.84 (*s*, 2 H); 4.42 (*d*, *J* = 4.9, 2 H); 4.53–4.59 (*m*, 1 H); 7.20 (*d*, *J* = 6.3, 1 H); 9.99 (*s*, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 18.4; 47.8; 48.9; 51.4; 53.1; 168.0; 169.2; 173.5; 196.7. HR-ESI-MS: 299.0689 ($[M+Na]^+$, $C_{10}H_{16}N_2NaO_5S^+$; calc. 299.0678).

Methyl N-*{3-[(2-Methoxy-2-oxoethyl)amino]-3-thioxopropanoyl}valinate* (**3cg**). Purification by CC (AcOEt/hexane 5:4). Orange oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.92 (*d*, *J* = 3.9, 3 H); 0.95 (*d*, *J* = 3.9, 3 H); 2.14–2.20 (*m*, 1 H); 3.75 (*s*, 3 H); 3.79 (*s*, 3 H); 3.86 (*s*, 2 H); 4.46 (*d*, *J* = 4.9, 2 H); 4.49–4.56 (*m*, 1 H); 7.07 (*d*, *J* = 8.4, 1 H); 7.28 (*s*, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 17.7; 18.8; 31.0; 47.2; 51.0; 52.1; 52.4; 57.4; 167.7; 168.5; 171.8; 196.2. HR-ESI-MS: 327.0999 ($[M+Na]^+$, $C_{12}H_{20}N_2NaO_5S^+$; calc. 327.0991).

Methyl N-*{3-[(2-Methoxy-2-oxoethyl)amino]-3-thioxopropanoyl}leucinate* (**3ch**). Purification by CC (AcOEt/hexane 5:4). Yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.92–0.94 (*m*, 6 H); 1.58–1.70 (*m*, 3 H); 3.74 (*s*, 3 H); 3.79 (*s*, 3 H); 3.84 (*s*, 2 H); 4.38–4.48 (*m*, 2 H); 4.57–4.61 (*m*, 1 H); 7.05 (*d*, *J* = 7.8, 1 H); 9.97 (*s*, 1 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 22.1; 23.0; 25.1; 41.3; 47.6; 51.2; 51.4; 52.7; 52.8; 168.0; 168.9; 173.3; 196.5. HR-ESI-MS: 341.1140 ($[M+Na]^+$, $C_{13}H_{22}N_2NaO_5S^+$; calc. 341.1147).

Methyl N-*{3-[(2-Methoxy-2-oxoethyl)amino]-3-oxopropanethioyl}valinate* (**3de**). Purification by CC (AcOEt/hexane 5:4). Yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.95 (*d*, *J* = 6.8, 3 H); 1.00 (*d*, *J* = 6.8, 3 H); 2.27–2.35 (*m*, 1 H); 3.72 (*s*, 3 H); 3.85 (*d*, *J* = 6.3, 2 H); 4.03 (*d*, *J* = 5.4, 2 H); 4.95–4.98 (*m*, 1 H); 7.38 (*s*, 1 H); 9.95 (*d*, *J* = 7.32, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 18.9; 19.2; 31.2; 41.8; 51.1; 52.7; 52.9; 64.0; 169.1; 170.4; 171.2; 196.6. HR-ESI-MS: 327.1002 ($[M+Na]^+$, $C_{12}H_{20}N_2NaO_5S^+$; calc. 327.0991).

Methyl N-*(3-{[2-(Acetylamino)ethyl]amino}-3-oxopropanethioyl)glycinate* (**3ci**). Purification by CC (AcOEt/hexane 5:4). M.p. 130–134°. ^{1}H-NMR (500 MHz, $CDCl_3$): 2.04 (*s*, 3 H); 3.44 (*s*, 4 H); 3.81 (*s*, 3 H); 3.82 (*s*, 2 H); 4.46 (*d*, *J* = 4.8, 2 H); 6.89 (*s*, 1 H); 7.48 (*s*, 1 H); 10.10 (*s*, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 22.5; 39.0; 39.4; 47.1; 51.7; 52.5; 168.9; 169.3; 172.5; 197.9. HR-ESI-MS: 298.0861 ($[M+Na]^+$, $C_{10}H_{17}N_3NaO_4S^+$; calc. 298.0836).

Methyl N-*(3-{[2-(Acetylamino)ethyl]amino}-3-oxopropanethioyl)valinate* (**3di**). Purification by CC (AcOEt/hexane 5:4). Oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.03 (*d*, *J* = 6.8, 3 H); 1.07 (*d*, *J* = 6.8, 3 H); 2.00 (*s*, 3 H); 2.33–2.40 (*m*, 1 H); 3.33–3.49 (*m*, 4 H); 3.78 (*s*, 3 H); 3.80 (*s*, 2 H); 4.96–4.98 (*m*, 1 H); 6.71 (*s*, 1 H); 7.48 (*s*, 1 H); 10.07 (*d*, *J* = 6.84, 1 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 18.8; 19.2; 23.4; 30.8; 39.7; 40.1; 51.7; 52.6; 64.0; 169.3; 171.4; 171.9; 197.1. HR-ESI-MS: 340.1302 ($[M+Na]^+$, $C_{13}H_{23}N_3NaO_4S^+$; calc. 340.1307).

REFERENCES

[1] A. Giannis, T. Kolter, *Angew. Chem., Int. Ed.* **1993**, *32*, 1244.

[2] S. A. Boyd, A. K. L. Fung, W. R. Baker, R. A. Mantei, Y.-L. Armiger, H. H. Stein, J. Cohen, D. A. Egan, J. L. Barlow, V. Klinghofer, K. M. Verburg, D. L. Martin, G. A. Young, J. S. Polakowski, D. J. Hoffman, K. W. Garren, T. J. Perun, H. D. Kleinert, *J. Med. Chem.* **1992**, *35*, 1735.

[3] M. W. Holladay, F. G. Salituro, D. H. Rich, *J. Med. Chem.* **1987**, *30*, 374; J. S. Tung, D. L. Davis, J. P. Anderson, D. E. Walker, S. Mamo, N. Jewett, R. K. Hom, S. Sinha, E. D. Thorsett, V. John, *J. Med. Chem.* **2002**, *45*, 259.

[4] P. S. Dragovich, T. J. Prins, R. Zhou, S. A. Fuhrman, A. K. Patick, D. A. Matthews, C. E. Ford, J. W. Meador III, R. A. Ferre, S. T. Worland, *J. Med. Chem.* **1999**, *42*, 1203.
[5] L. Quartara, G. Fabbri, R. Ricci, R. Patacchini, V. Pestellini, C. A. Maggi, V. Pavone, A. Giachetti, F. Arcamone, *J. Med. Chem.* **1994**, *37*, 3630.
[6] M. Chorev, M. Goodman, *Acc. Chem. Res.* **1993**, *26*, 266.
[7] J. H. Miwa, A. K. Patel, N. Vivatrat, S. M. Popek, A. M. Meyer, *Org. Lett.* **2001**, *3*, 3373.
[8] P. Weyermann, P. B. Dervan, *J. Am. Chem. Soc.* **2002**, *124*, 6872; M. Cushman, J. Jurayj, J. D. Moyer, *J. Org. Chem.* **1990**, *55*, 3186; M. Heinze, G. Brezesinski, B. Dobner, A. Langner, *Bioconjugate Chem.* **2010**, *21*, 696; G.-H. Chu, M. Gu, J. A. Cassel, S. Belanger, T. M. Graczyk, R. N. DeHaven, N. Conway-James, M. Koblish, P. J. Little, D. L. DeHaven-Hudkins, R. E. Dolle, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1951.
[9] S. Fioravanti, A. Morreale, L. Pellacani, F. Ramadori, P. A. Tardella, *Synlett* **2007**, 2759.
[10] K. Janikowska, S. Makowiec, *Synth. Commun.* **2012**, *42*, 975.
[11] H. K. Lee, J. P. Lee, G. H. Lee, C. S. Pak, *Synlett* **1996**, 1209.
[12] M. D. Fletcher, M. M. Campbell, *Chem. Rev.* **1998**, *98*, 763.
[13] P. Kindt, W. Dölling, M. Augustin, *Monatsh. Chem.* **1989**, *120*, 871.
[14] G. Guichard, F. Connan, R. Graff, M. Ostankovitch, S. Muller, J.-G. Guillet, J. Choppin, J.-P. Briand, *J. Med. Chem.* **1996**, *39*, 2030.
[15] A. Basheer, Z. Rappoport, *J. Phys. Org. Chem.* **2008**, *21*, 483.

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