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γ-Glutamyl-dipeptides: easy tools to rapidly probe the stereoelectronic properties of the ionotropic glutamate receptor binding pocket.

Lucia Tamborini,[a] Veronica Nicosia,[a] Paola Conti,[a] Federica Dall'Oglio,[a] Carlo De Micheli,[a] Birgitte Nielsen,[b] Anders A. Jensen,[b] Darryl S. Pickering[b] and Andrea Pinto[a*]

[a]*Department of Pharmaceutical Sciences (DISFARM), University of Milan, Via Mangiagalli 25, 20133 Milan, Italy.*
[b]*Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen OE, Denmark*

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ABSTRACT

γ-Glutamyl-dipeptides, built by condensing the distal carboxylate of L-Glu (or D-Glu) onto a series of differently functionalized amino acids, were prepared and used as tools for rapidly probing the stereo-electronic properties of iGluRs, searching for subtype-selective ligands.

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1. Introduction

L-Glutamic acid (L-Glu, Figure 1) is the main excitatory neurotransmitter in the central nervous system (CNS), where it is involved in the modulation of many physiological processes such as learning, memory, and synaptic plasticity.[1] Once released into the synaptic cleft, L-Glu acts through both ionotropic and metabotropic L-Glu receptors (iGluRs and mGluRs, respectively). The iGluRs are divided into three classes based on sequence homology and ligand selectivity: *N*-methyl-D-aspartic acid (NMDA) receptors, (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) receptors, and kainic acid (KA) receptors. These classes of receptors are further subdivided into subtypes.[1-5] The action of L-Glu is then silenced by a specific reuptake transport system, the excitatory amino acid transporters (EAATs), which are localized both at the synaptic nerve terminals and at glial cells.

Starting from the structure of the endogenous ligand L-Glu, selectivity for iGluRs *vs* mGluRs can be achieved through conformational constraint, locking the ligand into a folded or extended conformation, respectively (*e.g.*, compound L-CCG-IV *vs* L-CCG-I, Figure1).[6] In contrast, when it comes to the design of ligands selective for a specific iGluR or, ideally, for an iGluR subtype, common strategies often rely on the increase of molecular complexity.[7] This strategy aims at exploiting the subtle differences among iGluRs in terms of stereo-electronic properties of the ligand binding domain (LBD) of the different receptor subunits (GluN1, GluN2A-C, GluN3; GluA1-4 and GluK1-5).

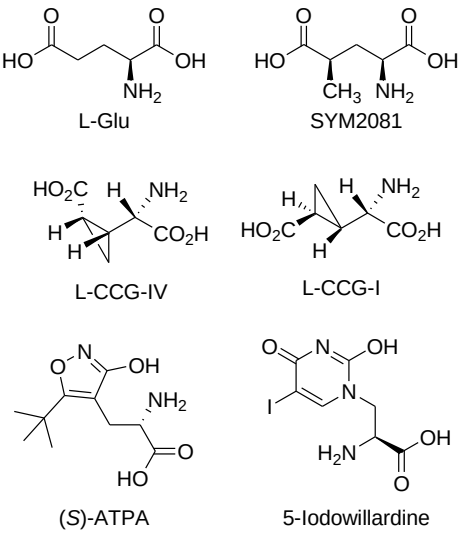


Figure 1. Structure of model compounds

* Corresponding author. Tel.: +39-02-50319323; e-mail: andrea.pinto@unimi.it

For instance, GluK1 selective agonists, such as the 5-*tert*-butyl derivative of AMPA, 2-amino-3-(5-*tert*-butyl-3-hydroxy-4-isoxazolyl) propionic acid (ATPA) and 5-iodowillardine (Figure 1), are sterically hindered by the side chain of Asp690 when docked into the GluK2 pocket and this is relieved by the presence of the smaller Ser706 at the equivalent position in GluK1.[7] Steric obstruction may also explain the reduced selectivity of ATPA for AMPARs. Steric occlusion partly explains also the higher selectivity of the agonist (2*S*,4*R*)-4-methylglutamate (SYM 2081, Figure 1) for kainate receptors over AMPA receptors.[8] In addition, homologation of the amino acidic chain is a strategy often pursued to turn agonists into antagonists, because it prevents the closure of the clamshell like LBD, thus leaving the channel pore closed.[9,10] Finally, it has to be noticed that, whereas AMPA and KA receptor ligands, analogously to the endogenous agonist L-Glu, usually are characterized by an *S*-configuration at the α-amino acid stereogenic center, NMDA receptors often exhibit a preference for *R*-configured amino acids, as in the case of the prototypical agonist NMDA.

On this ground, we envisaged that γ-glutamyl-dipeptides, built by condensing the distal carboxylate of L-Glu (or D-Glu) to a series of differently functionalized amino acids, could represent an easy strategy to synthesize ligands, featuring as ideal tools for rapidly probing the stereo-electronic properties of iGluRs, searching for subtype-selective antagonists (Figure 2). Indeed, γ-glutamyl-dipeptides are acidic amino acids characterized by a 6-atom spacer connecting the proximal and the distal acidic functions and by different side chains useful to confer an increasing degree of molecular complexity to the molecules, making possible the establishment of additional interaction/steric occlusion with the binding pocket.

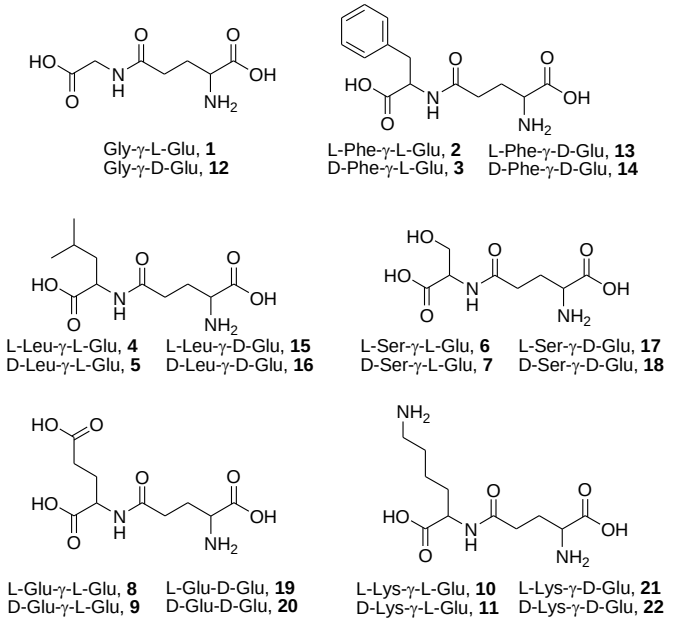
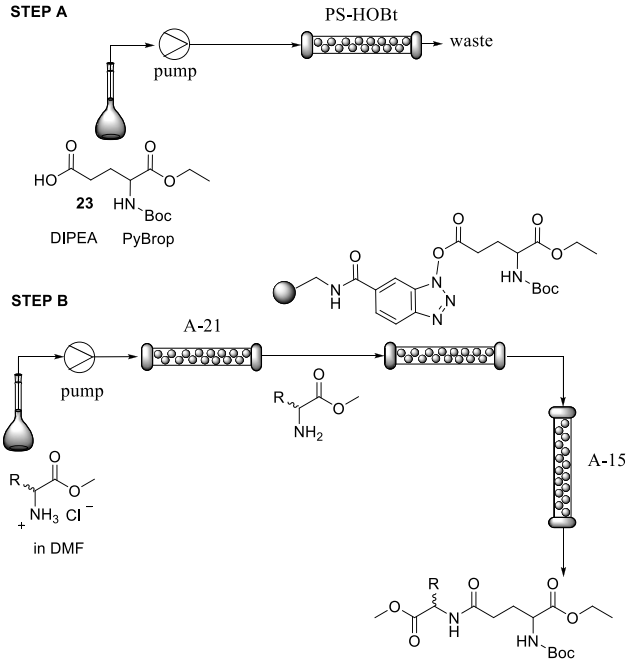


Figure 2. Structure of target compounds

2. Results and discussion

2.1. Chemistry

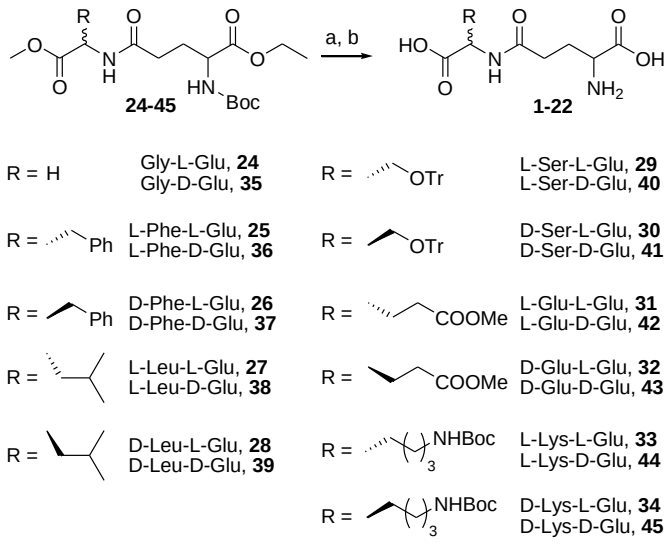
L-Glu (or D-Glu) was used as the starting material for the synthesis of the suitably protected amino acid **23** (Scheme 1), obtained in good yield and high purity, following a literature procedure.[11] On the other hand, each amino acid used in the coupling reaction was protected at the carboxylic function using thionyl chloride in MeOH, yielding the corresponding methyl ester in quantitative yield as a hydrochloride salt. The amino acids Lys, Glu and Ser were further protected at the side chain functional groups.[12] The coupling reaction was performed exploiting the use of a flow chemistry reactor in combination with an immobilized coupling reagent (polymer supported (PS)-HOBt) and scavengers.[13] PS-HOBt was packed into an Omnifit® glass column, then, after a washing/swelling step with DMF, a solution of the carboxylic acid **23**, DIPEA and the phoshponium coupling reagent bromo-*tris*-pyrrolidino phosphonium hexafluorophosphate (PyBroP) in DMF was fluxed through it at 100 μL/min (Scheme 1, step A). The column was then washed with DMF, leaving an activated amino ester covalently attached to the polymer support. The column was then connected with two prewashed columns of Amberlyst A-21 and Amberlyst A-15 linked in series (Scheme 1, step B). A solution of the second amino acid methyl ester as hydrochloride salt in DMF was fluxed through the column containing A-21 to liberate the free amine, which was then fluxed into the column containing the activated ester, where the coupling took place. The exiting flow stream was finally directed into the column filled with A-15 to perform an *in-line* purification, by scavenging any unreacted amine.



Scheme 1. In flow coupling reaction

The resulting solution was then evaporated to obtain the protected dipeptides **24-45** in high yields (75-90%), in high purities (> 95%) without any aqueous work up or column chromatography and with a significant reduction of time compared to traditional batch synthesis (20 min *vs* 2 h). The final compounds **1-22** were obtained after deprotection of intermediates **24-45** (Scheme 2). These steps were easily carried out in batch, through the hydrolysis of the esters with 1 N NaOH in MeOH and removing the Boc protecting groups in acidic conditions using a 30% trifluoroacetic acid solution in CH_2Cl_2 (Scheme 2). These acidic conditions allowed also the removal of the trityl protecting group in intermediates **29**, **30**, **40** and **41**. In the case of compounds **21** and **22**, which possess the distal amino function of Lys, the acidic ion

exchange resin Amberlite IR-120 was used to liberate the distal amine from the trifluoroacetate salt.



Scheme 2. Reagents and conditions: a) 1 N NaOH, MeOH, b) 30% TFA, CH_2Cl_2

3. Pharmacology

All dipeptides were preliminarily submitted to binding assays at native iGluRs, using rat brain synaptic membranes from male Sprague–Dawley rats.

Table 1. Receptor binding affinities at native rat iGluRs[a]

Compd	[^{3}H]AMPA K_i (μM)	[^{3}H]KAIN K_i (μM)	[^{3}H]CGP39653 K_i (μM)
1	> 100	4.1[5.39 ± 0.05]	5.4 [5.29 ± 0.07]
2	> 100	10 [5.00 ± 0.03]	40 [4.42 ± 0.10]
3	> 100	> 100	> 100
4	> 100	15 [4.81 ± 0.02]	44 [4.39 ± 0.11]
5	> 100	> 100	> 100
6	> 100	4.0 [5.40 ± 0.04]	5.8 [5.27± 0.10]
7	> 100	42 [4.38 ± 0.07]	21 [4.73± 0.10]
8	8.4 [5.08 ± 0.07]	0.74 [6.15 ± 0.10]	0.92 [6.04± 0.03]
9	> 100	24 [4.62 ± 0.03]	9.6 [5.04 ± 0.08]
10	> 100	9.8 [5.06 ± 0.14]	22 [4.73 ± 0.14]
11	> 100	> 100	> 100
12	> 100	> 100	7.2 [5.15 ± 0.05]
13	> 100	> 100	21 [4.67 ± 0.03]
14	> 100	> 100	> 100
15	> 100	> 100	≥ 100
16	>100	> 100	> 100
17	> 100	> 100	6.8 [5.18 ± 0.06]
18	≥ 100	25 [4.60 ± 0.03]	33 [4.51 ± 0.09]
19	> 100	> 100	3.3 [5.48 ± 0.01]
20	> 100	> 100	85 [4.07 ± 0.01]
21	> 100	> 100	72 [4.14 ± 0.02]
22	> 100	> 100	> 100

[a] Data are given as mean [mean pKi ± SEM] of three to four independent experiments each conducted in triplicate.

These binding data show that several compounds characterized by the presence of the L-Glu moiety displayed moderate affinity ($IC_{50} \leq 10$ μM) for native KARs (*i.e.*, compounds **1**, **2**, **6**, **8** and **10**). Unfortunately, the compounds lack KAR selectivity because they also exhibited affinity towards NMDA receptors. Indeed, the highest-affinity hit **8** and the compounds **1** and **6** displayed high affinity also for NMDARs (K_i = 0.9, 5.4 and 5.8 μM, respectively), The ability of the endogenous dipeptide γ-L-Glu-L-Glu and few other dipeptides or tripeptides (such as glutathione) to bind with micromolar affinity to ionotropic glutamate receptors was previously observed.[14] Interestingly, several derivatives within the D-Glu series (*i.e.*, compounds **12**, **13**, **17** and **19**) behaved as selective NMDA receptor ligands exhibiting low micromolar binding affinities (K_i = 7.2, 21, 6.8 and 3.3 μM, respectively) at these receptors.

In parallel, since it is known that an increase in molecular complexity of the aspartate/glutamate skeleton may generate molecules that act as blockers of the EAATs,[15] we have also tested the new derivatives **1-11** as potential inhibitors of the EAAT subtypes 1, 2 and 3 (EAAT1-3). In detail, the inhibition of the compounds at human EAAT1-3 stably expressed in HEK293 cells was determined in a [^{3}H]-D-Aspartate uptake assay performed as previously described using L-Glu and D,L-TBOA as reference ligands.[16] Compound **6** displayed weak inhibitory activity at all the three transporters at high micromolar concentrations, while the other derivatives proved to be inactive up to concentrations of 300 μM or 1000 μM.

4. Conclusion

In the present work, we have developed an easy route to rapidly generate a small library of ligands able to interact, with different binding affinities and degrees of selectivity, with KA and NMDA receptors. The length of the amino acidic skeleton obtained by coupling the distal carboxylate of D- or L-Glu, to an α-amino acid seems to be appropriate to fit the iGluR binding site, whereas additional natural and non-natural amino acids remain to be investigated, in order to maximize the receptor affinity and possibly increase the selectivity profile.

5. Experimental

5.1 Materials and methods

All reagents were purchased from Sigma. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Mercury 300 (300 MHz) spectrometer. Chemical shifts (δ) are expressed in ppm downfield from tetramethylsilane, and coupling constants (*J*) are expressed in Hz. Optical rotation determinations were carried out using a Jasco P-1010 spectropolarimeter, coupled with a Haake N3-B thermostat. TLC analyses were performed on commercial silica gel 60 F_{254} aluminum sheets; spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution or ninhydrin. MS analyses were performed on a Varian 320-MS triple quadrupole mass spectrometer with ESI source. Microanalyses (C, H, N) of new compounds were within ±0.4% of theoretical values. The continuous-flow peptide synthesis was performed using a R2+/R4 flow reactor, commercially available from Vapourtec equipped with Omnifit glass column.

5.2 Binding assays

Affinities for native NMDA, AMPA, and KA receptors in rat cortical synaptosomes were determined using 2 nM

[^{3}H]CGP39653, 5 nM [^{3}H]AMPA, and 5 nM [^{3}H]KA, respectively, with minor modifications as previously described.[17]

5.3. General procedures

5.3.1 General procedure for peptide coupling.

0.5 g of PS-HOBt was packed (loading 1.0 mmol/g) into a glass column (Ø: 6.6 mm; h: 100 mm) and DMF was fluxed for 15 minutes (flow rate: 200 µL/min). PyBroP (559 mg, 1.2 mmol) and DIPEA (261 µL; 1.5 mmol) were added to a 0.5 M solution of **23** (275 mg; 1.0 mmol in 2.0 mL of DMF) and the resulting solution was fluxed into the flow reactor at 100 µL/min. 0.5 g of Amberlyst A-21 was packed into a glass column (Ø: 6.6 mm; h: 100) and DMF was fluxed for 10 minutes (flow rate: 200 µL/min). 0.5 g of Amberlyst A-15 were packed into a third glass column (Ø: 6.6 mm; h: 100 mm) and DMF was fluxed for 10 minutes (flow rate: 200 µL/min). The column containing A-21 was connected in series with the column packed with PS-HOBt and with the column containing A-15. A 100 psi backpressure regulator was connected. A 0.2 M solution of the amino acid methyl ester hydrochloride (0.5 mmol in 2.5 mL of DMF) was injected into the reactor. The flow rate was set in order to obtain a residence time of 20 minutes.

5.3.2 General procedure for dipeptide deprotection.

1) The protected dipeptide (0.5 mmol) was dissolved in MeOH (1.5 mL) and 1N NaOH (1.5 mL, 1.5 mmol) was added. The mixture was stirred at rt for 3 h and the disappearance of the starting material was monitored by TLC (CH_2Cl_2/MeOH 9/1 + 1% AcOH). After evaporation of MeOH, the residue was diluted with distilled H_2O (5 mL) and washed with CH_2Cl_2 (2 × 5 mL). After that, the aqueous phase was made acidic (pH = 2) with 2N aqueous HCl and then extracted with EtOAc (3 × 5 mL). The organic phase was dried over anhydrous Na_2SO_4 and, after evaporation of the solvent, the intermediate was obtained as a white solid.

2) The intermediate obtained from the previous step (0.5 mmol) was treated with a 30% solution of TFA in CH_2Cl_2 (1.25 mL) at 0 °C. The solution was stirred at rt for 2 h and the reaction was followed by TLC (CH_2Cl_2/MeOH 9/1 + 1% AcOH). The volatiles were removed under reduced pressure. The obtained solid was dissolved in water (1 mL) and lyophilized to give the final dipeptide.

5.4 (*S*)-Ethyl 2-(*tert*-butoxycarbonylamino)-5-(2-methoxy-2-oxoethylamino)-5-oxopentanoate (24)

Yield: 80%; purple oil; R_f (cyclohexane/EtOAc 1/1): 0.41; $[\alpha]_D^{20}$: + 11.6 (c: 1.00 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): 1.25 (t, *J* = 7.0, 3H); 1.45 (s, 9H); 1.80–2.00 (m, 1H); 2.10–2.25 (m, 1H); 2.30–2.38 (m, 2H); 3.75 (s, 3H); 4.05 (dd, *J* = 5.5, 9.4, 2H); 4.20 (q, *J* = 7.0, 2H); 4.25–4.40 (m, 1H); 5.35 (bd, *J* = 7.1, 1H); 6.70 (bd, *J* = 5.5, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 14.4, 28.5, 29.4, 32.5, 41.5, 52.6, 53.1, 61.8, 80.3, 156.2, 170.7, 172.5, 172.6. Anal. calcd for $C_{15}H_{26}N_2O_7$: C, 52.01; H, 7.57; N, 8.09; found: C, 52.30; H, 7.68; N, 8.17.

5.5 (*S*)-Ethyl 2-(*tert*-butoxycarbonylamino)-5-((*S*)-1-methoxy-1-oxo-3-phenylpropan-2-ylamino)-5-oxopentanoate (25)

Yield: 76%; yellow oil; R_f (cyclohexane/EtOAc 6/4): 0.3; $[\alpha]_D^{20}$: + 37.5 (c: 1.00 in $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): 1.25 (t, *J* = 7.0, 3H); 1.45 (s, 9H); 1.85–2.00 (m, 1H); 2.10–2.20 (m, 1H); 2.20–2.30 (m, 2H); 3.10 (dd, *J* = 6.8, 14.1, 1H); 3.15 (dd, *J* = 5.4, 14.1, 1H); 3.75 (s, 3H); 4.20 (q, *J* = 7.0, 2H); 4.35–4.45 (m, 1H); 4.80–4.90 (m, 1H); 5.20 (bd, *J* = 6.9, 1H); 6.35 (bd, *J* = 5.8, 1H); 7.10–7.15 (m, 2H); 7.20–7.30 (m, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): 14.4, 28.5, 29.7, 32.6, 38.0, 52.6, 53.1, 53.8, 61.8, 80.4, 127.3, 128.8, 129.5, 136.3, 156.2, 172.1, 172.4, 172.5. Anal. calcd for $C_{22}H_{32}N_2O_7$: C, 60.54; H, 7.39; N, 6.42; found: C, 60.21; H, 7.22; N, 6.54.

5.6 (*S*)-Ethyl 2-(tert-butoxycarbonylamino)-5-((*R*)-1-methoxy-1-oxo-3-phenylpropan-2-ylamino)-5-oxopentanoate (26)

Yield: 88%; yellow oil; R_f (cyclohexane/EtOAc 6/4): 0.22; $[\alpha]_D^{20}$: – 20.3 (c: 1.00 in $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): 1.25 (t, *J* = 7.0, 3H); 1.45 (s, 9H); 1.80–1.90 (m, 1H); 2.10–2.30 (m, 3H); 3.06 (dd, *J* = 6.8, 14.1, 1H); 3.17 (dd, *J* = 5.4, 14.1, 1H); 3.75 (s, 3H); 4.20 (q, *J* = 7.0, 2H); 4.35–4.45 (m, 1H); 4.80–4.90 (m, 1H); 5.30 (bd, *J* = 6.8, 1H); 6.75 (bd, *J* = 5.9, 1H); 7.10–7.20 (m, 2H); 7.20–7.30 (m, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): 14.4, 28.5, 29.7, 32.6, 38.0, 52.6, 53.1, 53.8, 61.8, 80.4, 127.3, 128.8, 129.5, 136.3, 156.2, 172.1, 172.4, 172.5. Anal. calcd for $C_{22}H_{32}N_2O_7$: C, 60.54; H, 7.39; N, 6.42; found: C, 60.30; H, 7.28; N, 6.55.

5.7 (*S*)-Ethyl 2-(*tert*-butoxycarbonylamino)-5-((*S*)-1-methoxy-4-methyl-1-oxopentan-2-ylamino)-5-oxopentanoate (27)

Yield: 82%; yellow oil; R_f (cyclohexane/EtOAc 6/4): 0.30; $[\alpha]_D^{20}$: + 5.5 (c: 1.10 in $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): 0.94 (d, *J* = 6.3, 6H); 1.25 (t, *J* = 7.1, 3H); 1.45 (s, 9H); 1.50–1.70 (m, 3H); 1.80–1.90 (m, 1H); 2.10–2.25 (m, 1H); 2.28–2.36 (m, 2H); 3.75 (s, 3H); 4.20 (q, *J* = 7.1, 2H); 4.20–4.30 (m, 1H); 4.55–4.65 (m, 1H); 5.25 (bd, *J* = 7.7, 1H); 6.35 (bd, *J* = 6.6, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): 14.4, 22.1, 23.1, 25.1, 28.5, 29.3, 32.6, 41.7, 51.0, 52.5, 53.2, 61.8, 80.3, 156.1, 171.9, 172.5, 173.8. Anal. calcd for $C_{19}H_{34}N_2O_7$: C, 56.70; H, 8.51; N, 6.96; found: C, 57.01; H, 8.65; N, 7.17.

5.8 (*S*)-Ethyl 2-(*tert*-butoxycarbonylamino)-5-((*R*)-1-methoxy-4-methyl-1-oxopentan-2-ylamino)-5-oxopentanoate (28)

Yield: 75%; yellow oil; R_f (cyclohexane/EtOAc 6/4): 0.20; $[\alpha]_D^{20}$: + 12.7 (c: 1.55 in $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): 0.94 (d, *J* = 6.0, 6H); 1.25 (t, *J* = 7.0, 3H); 1.45 (s, 9H); 1.50–1.70 (m, 3H); 1.80–1.90 (m, 1H); 2.10–2.25 (m, 1H); 2.28–2.36 (m, 2H); 3.75 (s, 3H); 4.20 (q, *J* = 7.0, 2H); 4.35–4.45 (m, 1H); 4.50–4.60 (m, 1H); 5.32 (bd, *J* = 7.1, 1H); 6.90 (bd, *J* = 6.8, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): 14.4, 22.1, 23.1, 25.1, 28.5, 29.9, 32.7, 41.4, 51.3, 52.5, 53.1, 61.8, 80.5, 156.3, 172.3, 172.6, 173.8. Anal. calcd for $C_{19}H_{34}N_2O_7$: C, 56.70; H, 8.51; N, 6.96; found: C, 56.96; H, 8.67; N, 7.12.

5.9 (*S*)-Ethyl 2-(*tert*-butoxycarbonylamino)-5-((*S*)-1-methoxy-1-oxo-3-(trityloxy)propan-2-ylamino)-5-oxopentanoate (29)

Yield: 75%; Solid state: pale yellow oil; R_f (cyclohexane/EtOAc 7/3): 0.21; $[\alpha]_D^{20}$: – 21.0 (c: 1.00 in $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): 1.25 (t, *J* = 7.0, 3H); 1.45 (s, 9H); 1.85–2.00 (m, 1H); 2.10–2.20 (m, 1H); 2.30 (m, 2H); 3.35 (dd, *J* = 3.3, 9.4, 1H); 3.60 (dd, *J* = 3.3, 9.4, 1H); 3.75 (s, 3H); 4.15 (q, *J* = 7.0, 2H); 4.20–4.30 (m, 1H); 4.70 (ddd, *J* = 3.3, 3.3, 8.0, 1H); 5.24 (bd, *J* = 8.0, 1H); 6.55 (bd, *J* = 8.3, 1H); 7.20–7.40 (m, 15H); ^{13}C-NMR (75 MHz, $CDCl_3$): 14.4, 28.5, 28.9, 32.6, 52.6, 52.9, 60.6, 61.7, 63.8, 80.7, 86.9, 127.5, 128.1, 128.7, 143.6, 155.8, 171.2, 171.8, 172.5. Anal. calcd for $C_{35}H_{42}N_2O_8$: C, 67.94; H, 6.84; N, 4.53; found: C, 67.70; H, 6.67; N, 4.68.

5.10 (*S*)-Ethyl 2-(*tert*-butoxycarbonylamino)-5-((*R*)-1-methoxy-1-oxo-3-(trityloxy)propan-2-ylamino)-5-oxopentanoate (30)

Yield: 77%; Solid state: yellow oil; R_f (cyclohexane/EtOAc 1/1): 0.63; $[\alpha]_D^{20}$: – 1.0 (c: 1.00 in $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): 1.25 (t, *J* = 7.0, 3H); 1.45 (s, 9H); 1.80–2.00 (m, 1H); 2.10–2.40 (m, 3H); 3.35 (dd, *J* = 3.2, 9.4, 1H); 3.60 (dd, *J* = 3.2, 9.4, 1H); 3.75 (s, 3H); 4.20 (q, *J* = 7.0, 2H); 4.35–4.45 (m, 1H); 4.72 (ddd, *J* = 3.2, 3.2, 7.9, 1H); 5.35 (bd, *J* = 7.9, 1H); 6.86 (bd, *J* = 7.9, 1H); 7.20–7.40 (m, 15H); ^{13}C-NMR (75 MHz, $CDCl_3$): 14.4, 28.5, 29.0, 32.5, 52.9, 53.1, 59.4, 61.8, 63.1, 80.6, 86.9, 127.5, 128.1, 128.9, 143.6, 156.1, 171.2, 171.2, 172.5. Anal. calcd for $C_{35}H_{42}N_2O_8$: C, 67.94; H, 6.84; N, 4.53; found: C, 68.24; H, 7.00; N, 4.40.

5.11 (*S*)-Dimethyl 2-((*S*)-4-(*tert*-butoxycarbonylamino)-5-ethoxy-5-oxopentanamido)pentanedioate (31)

Yield: 83%; crystallized from diisopropyl ether as white crystals; mp: 95-97 °C; R_f (cyclohexane/EtOAc 4/6): 0.37; $[\alpha]_D^{20}$: + 21.4 (c: 1.00 in $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): 1.25 (t, *J* = 7.1, 3H); 1.45 (s, 9H); 1.90–2.10 (m, 2H); 2.15–2.25 (m, 2H); 2.30–2.40 (m, 2H); 2.40–2.50 (m, 2H); 3.65 (s, 3H); 3.75 (s, 3H); 4.20 (q, *J* = 7.1, 2H); 4.20–4.30 (m, 1H); 4.60 (ddd, *J* = 5.0, 7.6, 7.6, 1H); 5.25 (bd, *J* = 7.6, 1H); 6.60 (bd, *J* = 7.6, 1H). Anal. calcd for $C_{19}H_{32}N_2O_9$ C, 52.77; H, 7.46; N, 6.48; found: C, 52.96; H, 7.70; N, 6.25.

5.12 (*R*)-Dimethyl 2-((*S*)-4-(*tert*-butoxycarbonylamino)-5-ethoxy-5-oxopentanamido)pentanedioate (32)

Yield: 73%; crystallized from diisopropyl ether as white crystals; mp: 75-77 °C; R_f (cyclohexane/EtOAc 1/1): 0.19; $[\alpha]_D^{20}$: – 6.5 (c: 1.00 in $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): 1.25 (t, *J* = 7.0, 3H); 1.45 (s, 9H); 1.85–2.00 (m, 1H); 2.00–2.10 (m, 1H); 2.15–2.25 (m, 2H); 2.30–2.40 (m, 2H); 2.40–2.50 (m, 2H); 3.65 (s, 3H); 3.75 (s, 3H); 4.20 (q, *J* = 7.0, 2H); 4.35–4.45 (m, 1H); 4.60 (ddd, *J* = 5.0, 7.6, 7.6, 1H); 5.31 (bd, *J* = 7.6, 1H); 6.75 (bd, *J* = 7.6, 1H). Anal. calcd for $C_{19}H_{32}N_2O_9$ C, 52.77; H, 7.46; N, 6.48; found: C, 53.05; H, 7.70; N, 6.30.

5.13 (*S*)-Methyl 6-(*tert*-butoxycarbonylamino)-2-((*S*)-4-(*tert*-butoxycarbonylamino)-5-ethoxy-5-oxopentanamido)hexanoate (33)

Yield: 79%; yellow oil; R_f (cyclohexane/EtOAc 1/1): 0.33; $[\alpha]_D^{20}$: + 8.0 (c: 0.50 in $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): 1.25 (t, *J* = 7.0, 3H); 1.45 (s, 18H); 1.30–1.50 (m, 1H); 1.65–1.95 (m, 6H); 2.10–2.25 (m, 1H); 2.30–2.40 (m, 2H); 3.05–3.15 (m, 2H); 3.75 (s, 3H); 4.20 (q, *J* = 7.0, 2H); 4.20–4.30 (m, 1H); 4.55 (ddd, *J* = 5.0, 7.7, 7.7, 1H); 4.65 (bs., 1 H); 5.25 (bd, *J* = 7.7, 1H); 6.50 (bd, *J* = 7.7, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): 14.4, 22.7, 28.5, 28.6, 29.6, 29.8, 31.9, 32.6, 40.3, 52.4, 52.5, 53.1, 61.8, 79.3, 80.4, 156.2, 156.3, 172.3, 172.6, 173.1. Anal. calcd for $C_{24}H_{43}N_3O_9$ C, 55.69; H, 8.37; N, 8.12; found: C, 55.91; H, 8.56; N, 7.90.

5.14 (*R*)-Methyl 6-(*tert*-butoxycarbonylamino)-2-((*S*)-4-(*tert*-butoxycarbonylamino)-5-ethoxy-5-oxopentanamido)hexanoate (34)

Yield: 87%; crystallized from diisopropyl ether as white crystals; mp: 75 °C; R_f (cyclohexane/EtOAc 1/1): 0.40; $[\alpha]_D^{20}$: + 5.3 (c: 0.50 in $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): 1.25 (t, *J* = 7.1, 3H); 1.45 (s, 18H); 1.35–1.50 (m, 1H); 1.65–1.95 (m, 6H); 2.10–2.25 (m, 1H); 2.30–2.40 (m, 2H); 3.05–3.15 (m, 2H); 3.75 (s, 3H); 4.20 (q, *J* = 7.1, 2H); 4.35–4.45 (m, 1H); 4.55 (ddd, *J* = 5.0, 7.4, 7.4, 1H); 4.65 (bs., 1 H); 5.30 (bd., *J* = 6.6, 1H); 6.90 (bd., *J* = 6.6, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): 14.4, 22.7, 28.5, 28.6, 29.5, 29.8, 31.9, 32.6, 40.3, 52.4, 52.5, 53.1, 61.8, 79.3, 80.4, 156.2, 156.3, 172.3, 172.5, 173.1. Anal. calcd for $C_{24}H_{43}N_3O_9$ C, 55.69; H, 8.37; N, 8.12; found: C, 55.99; H, 8.50; N, 7.95.

5.15 (*R*)-Ethyl 2-(*tert*-butoxycarbonylamino)-5-(2-methoxy-2-oxoethylamino)-5-oxopentanoate (35)

$[\alpha]_D^{20}$: – 11.3 (c: 1.00 in $CHCl_3$). Anal. calcd for $C_{15}H_{26}N_2O_7$: C, 52.01; H, 7.57; N, 8.09; found: C, 52.27; H, 7.70; N, 8.22.

5.16 (*R*)-Ethyl 2-(*tert*-butoxycarbonylamino)-5-((*S*)-1-methoxy-1-oxo-3-phenylpropan-2-ylamino)-5-oxopentanoate (36)

$[\alpha]_D^{20}$: + 19.7 (c: 1.00 in $CHCl_3$). Anal. calcd for $C_{22}H_{32}N_2O_7$: C, 60.54; H, 7.39; N, 6.42; found: C, 60.28; H, 7.23; N, 6.60.

5.17 (*R*)-Ethyl 2-(*tert*-butoxycarbonylamino)-5-((*R*)-1-methoxy-1-oxo-3-phenylpropan-2-ylamino)-5-oxopentanoate (37)

$[\alpha]_D^{20}$: – 37.0 (c: 1.00 in $CHCl_3$). Anal. calcd for $C_{22}H_{32}N_2O_7$: C, 60.54; H, 7.39; N, 6.42; found: C, 60.30; H, 7.20; N, 6.61.

5.18 (*R*)-Ethyl 2-(*tert*-butoxycarbonylamino)-5-((*S*)-1-methoxy-4-methyl-1-oxopentan-2-ylamino)-5-oxopentanoate (38)

$[\alpha]_D^{20}$: – 13.1 (c: 1.10 in $CHCl_3$). Anal. calcd for $C_{19}H_{34}N_2O_7$: C, 56.70; H, 8.51; N, 6.96; found: C, 56.93; H, 8.68; N, 7.15.

5.19 (*R*)-Ethyl 2-(*tert*-butoxycarbonylamino)-5-((*R*)-1-methoxy-4-methyl-1-oxopentan-2-ylamino)-5-oxopentanoate (39)

$[\alpha]_D^{20}$: – 5.3 (c: 1.00 in $CHCl_3$). Anal. calcd for $C_{19}H_{34}N_2O_7$: C, 56.70; H, 8.51; N, 6.96; found: C, 57.00; H, 8.71; N, 7.20.

5.20 (*R*)-Ethyl 2-(*tert*-butoxycarbonylamino)-5-((*S*)-1-methoxy-1-oxo-3-(trityloxy)propan-2-ylamino)-5-oxopentanoate (40)

$[\alpha]_D^{20}$: + 1.3 (c: 1.00 in $CHCl_3$). Anal. calcd for $C_{35}H_{42}N_2O_8$: C, 67.94; H, 6.84; N, 4.53; found: C, 68.15; H, 7.04; N, 4.38.

5.21 (*R*)-Ethyl 2-(*tert*-butoxycarbonylamino)-5-((*R*)-1-methoxy-1-oxo-3-(trityloxy)propan-2-ylamino)-5-oxopentanoate (41)

$[\alpha]_D^{20}$: + 20.6 (c: 1.00 in $CHCl_3$). Anal. calcd for $C_{35}H_{42}N_2O_8$: C, 67.94; H, 6.84; N, 4.53; found: C, 68.20; H, 6.98; N, 4.32.

5.22 (*S*)-Dimethyl 2-((*R*)-4-(*tert*-butoxycarbonylamino)-5-ethoxy-5-oxopentanamido)pentanedioate (42)

$[\alpha]_D^{20}$: + 6.9 (c: 1.00 in $CHCl_3$). Anal. calcd for $C_{19}H_{32}N_2O_9$ C, 52.77; H, 7.46; N, 6.48; found: C, 53.00; H, 7.70; N, 6.32.

5.23 (*R*)-Dimethyl 2-((*R*)-4-(*tert*-butoxycarbonylamino)-5-ethoxy-5-oxopentanamido)pentanedioate (43)

$[\alpha]_D^{20}$: – 21.0 (c: 0.95 in $CHCl_3$). Anal. calcd for $C_{19}H_{32}N_2O_9$ C, 52.77; H, 7.46; N, 6.48; found: C, 52.94; H, 7.65; N, 6.34.

5.24 (*S*)-Methyl 6-(*tert*-butoxycarbonylamino)-2-((*R*)-4-(*tert*-butoxycarbonylamino)-5-ethoxy-5-oxopentanamido)heptanoate (44)

$[\alpha]_D^{20}$: – 5.0 (c: 0.50 in $CHCl_3$). Anal. calcd for $C_{24}H_{43}N_3O_9$ C, 55.69; H, 8.37; N, 8.12; found: C, 55.84; H, 8.49; N, 8.00.

5.25 (*R*)-Methyl 6-(*tert*-butoxycarbonylamino)-2-((*R*)-4-(*tert*-butoxycarbonylamino)-5-ethoxy-5-oxopentanamido)heptanoate (45)

$[\alpha]_D^{20}$: – 8.0 (c: 0.50 in $CHCl_3$). Anal. calcd for $C_{24}H_{43}N_3O_9$ C, 55.69; H, 8.37; N, 8.12; found: C, 55.90; H, 8.52; N, 7.95.

5.26 (*S*)-2-Amino-5-(carboxymethylamino)-5-oxopentanoic acid (1)

Yield: 31%; R_f (*n*-butanol/H_2O/AcOH 4:2:1): 0.18; mp: dec. T > 159 °C; $[\alpha]_D^{20}$: + 10.0 (c: 0.30 in H_2O); lit.[18] $[\alpha]_D^{20}$: + 11.1 (c: 1.00 in H_2O); ^{1}H-NMR (300 MHz, D_2O): 2.00–2.08 (m, 2H); 2.35 (ddd, *J* = 1.4, 8.5, 8.5, 2H); 3.70 (dd, *J* = 6.3, 6.3, 1H); 3.80 (s, 2H); ^{13}C-NMR (75 MHz, D_2O): 26.1, 31.2, 41.5, 53.6, 173.3, 174.0, 175.2; $[M+H]^+$: 205.0. Anal. calcd for $C_7H_{12}N_2O_5$ C, 41.18; H, 5.92; N, 13.72; found: C, 41.00; H, 5.80; N, 13.89.

5.27 (*S*)-2-Amino-5-((*S*)-1-carboxy-2-phenylethylamino)-5-oxopentanoic acid (2)

Yield: 80%; R_f (*n*-butanol/H_2O/AcOH 4:2:1): 0.49; $[\alpha]_D^{20}$: + 18.2 (c: 0.25 in H_2O); mp: T > 186-189 °C; ^{1}H-NMR (300 MHz, D_2O): 1.80–1.90 (m, 2H); 2.25 (m, 2H); 2.85 (dd, *J* = 9.3, 14.0, 1H); 3.10 (dd, *J* = 5.3, 14.0, 1H); 3.60 (dd, *J* = 6.5, 6.5, 1H); 4.55 (dd, *J* = 5.3, 9.3 1H); 7.10–7.30 (m, 5H); ^{13}C-NMR (75 MHz, D_2O): 26.1, 31.3, 36.8, 53.2, 54.3, 127.3, 128.8, 129.3, 136.8, 172.7, 174.2, 175.3; $[M+H]^+$: 295.0. Anal. calcd for $C_{14}H_{18}N_2O_5$ C, 57.13; H, 6.16; N, 9.52; found: C, 56.90; H, 6.28; N, 9.40.

5.28 (*S*)-2-Amino-5-((*R*)-1-carboxy-2-phenylethylamino)-5-oxopentanoic acid (3)

Yield: 89%; R_f (CH_2Cl_2/MeOH 9:1 + 1% AcOH): 0.62; $[\alpha]_D^{20}$: + 3.7 (c: 0.30 in H_2O); mp: 150 °C; ^{1}H-NMR (300 MHz, D_2O): 1.80–2.00 (m, 2H); 2.28 (ddd, *J* = 3.0, 8.2, 8.2, 2H); 2.82 (dd, *J* = 9.7, 14.1, 1H); 3.12 (dd, *J* = 5.3, 14.1, 1H); 3.60 (dd, *J* = 3.0, 6.5, 1H); 4.55 (dd, *J* = 5.3, 9.7, 1H); 7.10–7.25 (m, 5H); ^{13}C-NMR (75 MHz, D_2O): 25.7, 30.4, 36.8, 52.2, 54.0, 127.2, 128.8, 129.4, 136.8, 171.8, 174.0, 175.1; $[M+H]^+$: 295.0. Anal. calcd for $C_{14}H_{18}N_2O_5$ C, 57.13; H, 6.16; N, 9.52; found: C, 57.32; H, 5.98; N, 9.78.

5.29 (*S*)-2-Amino-5-((*S*)-1-carboxy-3-methylbutylamino)-5-oxopentanoic acid (4)

Yield: 100%; R_f (*n*-butanol/H_2O/AcOH 4:2:1): 0.56; $[\alpha]_D^{20}$: – 15.0 (c: 0.25 in H_2O); lit.[19] $[\alpha]_D^{20}$: – 13.5 (c: 1.00 in H_2O); mp: 187 °C; ^{1}H-NMR (300 MHz, D_2O): 0.70 (d, *J* = 5.8, 3H); 0.80 (d, *J* = 5.8, 3H); 1.45–1.55 (m, 3H); 1.95–2.05 (m, 2H); 2.30–2.40 (m, 2H); 3.72 (dd, *J* = 6.2, 6.2, 1H); 4.20 (dd, *J* = 6.4, 6.4, 1H); ^{13}C-NMR (75 MHz, D_2O): 20.7, 22.3, 24.6, 26.2, 31.3, 39.5, 51.9, 53.5, 173.1, 174.7, 177.0; $[M+H]^+$: 261.0. Anal. calcd for $C_{11}H_{20}N_2O_5$ C, 50.76; H, 7.74; N, 10.76; found: C, 50.45; H, 7.60; N, 10.50.

5.30 (*S*)-2-Amino-5-((*R*)-1-carboxy-3-methylbutylamino)-5-oxopentanoic acid (5)

Yield: 86%; R_f (*n*-butanol/H_2O/AcOH 4:2:1): 0.56; $[\alpha]_D^{20}$: + 35.0 (c: 0.25 in H_2O); mp: 100-102 °C; ^{1}H-NMR (300 MHz, D_2O): 0.70 (d, *J* = 6.2, 3H); 0.80 (d, *J* = 6.2, 3H); 1.45–1.55 (m, 3H); 1.95–2.10 (m, 2H); 2.35 (ddd, *J* = 1.8, 8.8, 8.8, 2H); 3.80 (dd, *J* = 6.4, 6.4, 1H); 4.22 (dd, *J* = 7.0, 7.0, 1H); ^{13}C-NMR (75 MHz, D_2O): 20.6, 22.3, 24.5, 25.9, 30.9, 39.4, 51.7, 53.0, 172.5, 174.5, 176.9; $[M+H]^+$: 261. Anal. calcd for $C_{11}H_{20}N_2O_5$ C, 50.76; H, 7.74; N, 10.76; found: C, 50.98; H, 7.90; N, 10.53.

5.31 (*S*)-2-Amino-5-((*S*)-1-carboxy-2-hydroxyethylamino)-5-oxopentanoic acid (6)

Yield: 40%; R_f (*n*-butanol/H_2O/AcOH 4:2:1): 0.24; mp: dec. T > 164 °C; $[\alpha]_D^{20}$: + 11.6 (c: 0.35 in H_2O); ^{1}H-NMR (300 MHz, D_2O): 2.00–2.10 (m, 2H); 2.40 (ddd, *J* = 3.5, 6.2, 6.2, 2H); 3.70–3.85 (m, 3H); 4.38 (dd, *J* = 4.1, 4.1, 1H); ^{13}C-NMR (75 MHz, D_2O): 26.5, 31.9, 54.5, 57.3, 62.1, 174.3, 174.6, 176.4; $[M+H]^+$: 235.0. $[\alpha]_D^{20}$: – 13 (c: 0.35 in H_2O). Anal. calcd for $C_8H_{14}N_2O_6$ C, 41.03; H, 6.03; N, 11.96; found: C, 41.30; H, 6.22; N, 11.78.

5.32 (*S*)-2-Amino-5-((*R*)-1-carboxy-2-hydroxyethylamino)-5-oxopentanoic acid (7)

Yield: 40%; R_f (*n*-butanol/H_2O/AcOH 4:2:1): 0.26; mp: dec. T > 156 °C; $[\alpha]_D^{20}$: +12.3 (c: 0.40 in H_2O); ^{1}H-NMR (300 MHz, D_2O): 2.00-2.10 (m, 2H); 2.40 (ddd, *J* = 3.5, 6.2, 6.2, 2H); 3.70-3.85 (m, 3H); 4.38 (dd, *J* = 4.1, 4.1, 1H); ^{13}C-NMR (75 MHz, D_2O): 25.9, 31.0, 53.2, 55.1, 61.2, 172.9, 173.8, 174.6; $[M+H]^+$: 235.0. $[\alpha]_D^{20}$: – 13 (c: 0.35 in H_2O). Anal. calcd for $C_8H_{14}N_2O_6$ C, 41.03; H, 6.03; N, 11.96; found: C, 41.28; H, 6.20; N, 11.80.

5.33 (*S*)-2-((*S*)-4-Amino-4-carboxybutanamido)pentanedioic acid (8)

Yield: 59%, R_f (*n*-butanol/H_2O/AcOH 4:2:1): 0.29; $[\alpha]_D^{20}$: – 2.5 (c: 0.50 in H_2O); $[\alpha]_D^{20}$: + 3.2 (c: 0.50 in 0.5 N HCl); lit.[20] $[\alpha]_D^{20}$: + 3.4 (c: 1.00 in 0.5 N HCl); mp: 188-190 °C; ^{1}H-NMR (300 MHz, D_2O): 1.80–1.95 (m, 1H); 2.00–2.15 (m, 3H); 2.30–2.45 (m, 4H); 3.80 (dd, *J* = 6.3, 6.3, 1H); 4.25 (dd, *J* = 5.0, 9.1, 1H); ^{13}C-NMR (75 MHz, D_2O): 25.9, 26.0, 30.3, 31.3, 52.5, 53.5, 173.1, 174.7, 175.5, 177.3; $[M+H]^+$: 277.0. Anal. calcd for $C_{10}H_{16}N_2O_7$ C, 43.48; H, 5.84; N, 10.14; found: C, 43.70; H, 6.00; N, 9.98.

5.34 (*S*)-2-((*R*)-4-Amino-4-carboxybutanamido)pentanedioic acid (9)

Yield: 44%; R_f (*n*-butanol/H_2O/AcOH 4:2:1): 0.29; $[\alpha]_D^{20}$: + 15.2 (c: 0.25 in H_2O); mp: 103-105 °C; ^{1}H-NMR (300 MHz, D_2O): 1.80–1.95 (m, 1H); 2.00–2.10 (m, 3H); 2.30–2.40 (m, 4H); 3.80 (dd, *J* = 6.3, 6.3, 1H); 4.25 (dd, *J* = 5.0, 9.1, 1H); ^{13}C-NMR (75 MHz, D_2O): 25.9, 26.0, 30.2, 31.0, 52.4, 53.3, 172.9, 174.6, 175.5, 177.3; $[M+H]^+$: 277.0. Anal. calcd for $C_{10}H_{16}N_2O_7$ C, 43.48; H, 5.84; N, 10.14; found: C, 43.75; H, 6.06; N, 9.90.

5.35 (*S*)-6-Amino-2-((*S*)-4-amino-4-carboxybutanamido)hexanoic acid (10)

The residue was dissolved in a few drops of bidistilled water and purified on a column packed with Amberlite IR120. The resin was washed with bidistilled H_2O until the pH was neutral and then the product was released from the resin with aqueous 1N NH_3.The fractions containing the dipeptide were collected and lyophilized to give the desired product.

Yield: 79%, R_f (*n*-butanol/H_2O/AcOH 4:2:1): 0.14, $[\alpha]_D^{20}$: + 4.0 (c: 0.20 in H_2O); lit.[21] $[\alpha]_D^{20}$: + 3.5 (c: 1.00 in H_2O); mp: dec. T > 215°C; ^{1}H-NMR (300 MHz, D_2O): 1.25–1.35 (m, 2H); 1.50–1.60 (m, 3H); 1.60–1.70 (m, 1H); 1.95–2.05 (m, 2H); 2.30–2.40 (m, 2H); 2.86 (ddd, *J* = 7.6, 7.6, 7.6, 2H); 3.62 (dd, *J* = 6.5, 6.5, 1H); 4.00 (dd, *J* = 5.3, 8.5, 1H); ^{13}C-NMR (75 MHz, D_2O): 22.3, 26.5, 26.5, 31.0, 31.9, 39.4, 54.4, 55.2, 174.2, 174.4, 179.2; $[M+H]^+$:

276.0. Anal. calcd for $C_{11}H_{21}N_3O_5$ C, 47.99; H, 7.69; N, 15.26; found: C, 47.70; H, 7.48; N, 15.46.

5.36 (*R*)-6-Amino-2-((*S*)-4-amino-4-carboxybutanamido)hexanoic acid (11)

Yield: 65%; R_f (*n*-butanol/H_2O/AcOH 4:2:1): 0.14; $[\alpha]_D^{20}$: − 4.5 (c: 0.35 in H_2O); mp: dec. T > 205 °C; ^{1}H-NMR (300 MHz, D_2O): 1.20–1.40 (m, 2H); 1.50–1.60 (m, 2H); 1.60–1.75 (m, 2H); 1.95–2.05 (m, 2H); 2.25–2.35 (m, 2H); 2.86 (ddd, *J* = 7.3, 7.3, 7.3, 2H); 3.66 (dd, *J* =5.9, 5.9, 1H); 4.02 (dd, *J* = 5.0, 8.2, 1H); ^{13}C-NMR (75 MHz, D_2O): 22.3, 26.4, 26.5, 31.0, 31.2, 39.4, 54.3, 55.2, 174.2, 174.9, 179.2; $[M+H]^+$: 276.0. Anal. calcd for $C_{11}H_{21}N_3O_5$ C, 47.99; H, 7.69; N, 15.26; found: C, 47.75; H, 7.50; N, 15.50.

5.37 (*R*)-2-Amino-5-(carboxymethylamino)-5-oxopentanoic acid (12)

$[\alpha]_D^{20}$: − 10.0 (c: 0.30 in H_2O). Anal. calcd for $C_7H_{12}N_2O_5$ C, 41.18; H, 5.92; N, 13.72; found: C, 40.98; H, 5.81; N, 13.93.

5.38 (*R*)-2-Amino-5-((*S*)-1-carboxy-2-phenylethylamino)-5-oxopentanoic acid (13)

$[\alpha]_D^{20}$: − 5.0 (c: 0.25 in H_2O). Anal. calcd for $C_{14}H_{18}N_2O_5$ C, 57.13; H, 6.16; N, 9.52; found: C, 57.38; H, 6.30; N, 9.40.

5.39 (*R*)-2-Amino-5-((*R*)-1-carboxy-2-phenylethylamino)-5-oxopentanoic acid (14)

$[\alpha]_D^{20}$: − 17.1 (c: 0.30 in H_2O). Anal. calcd for $C_{14}H_{18}N_2O_5$ C, 57.13; H, 6.16; N, 9.52; found: C, 57.30; H, 6.00; N, 9.80.

5.40 (*R*)-2-Amino-5-((*S*)-1-carboxy-3-methylbutylamino)-5-oxopentanoic acid (15)

$[\alpha]_D^{20}$: – 33.0 (c: 0.25 in H_2O). Anal. calcd for $C_{11}H_{20}N_2O_5$ C, 50.76; H, 7.74; N, 10.76; found: C, 50.93; H, 7.90; N, 10.50.

5.41 (*R*)-2-Amino-5-((*R*)-1-carboxy-3-methylbutylamino)-5-oxopentanoic acid (16)

$[\alpha]_D^{20}$: + 15.6 (c: 0.25 in H_2O). Anal. calcd for $C_{11}H_{20}N_2O_5$ C, 50.76; H, 7.74; N, 10.76; found: C, 50.50; H, 7.58; N, 10.87.

5.42 (*R*)-2-Amino-5-((*S*)-1-carboxy-2-hydroxyethylamino)-5-oxopentanoic acid (17)

$[\alpha]_D^{20}$: – 12.3 (c: 0.40 in H_2O). Anal. calcd for $C_8H_{14}N_2O_6$ C, 41.03; H, 6.03; N, 11.96; found: C, 40.80; H, 5.80; N, 12.10.

5.43 (*R*)-2-Amino-5-((*R*)-1-carboxy-2-hydroxyethylamino)-5-oxopentanoic acid (18)

$[\alpha]_D^{20}$: – 13.0 (c: 0.35 in H_2O). Anal. calcd for $C_8H_{14}N_2O_6$ C, 41.03; H, 6.03; N, 11.96; found: C, 40.85; H, 5.82; N, 12.16.

5.44 (*R*)-2-((*S*)-4-Amino-4-carboxybutanamido)pentanedioic acid (19)

$[\alpha]_D^{20}$: – 16.0 (c: 0.25 in H_2O). Anal. calcd for $C_{10}H_{16}N_2O_7$ C, 43.48; H, 5.84; N, 10.14; found: C, 43.69; H, 5.98; N, 9.97.

5.45 (*R*)-2-((*R*)-4-amino-4-carboxybutanamido)pentanedioic acid (20)

$[\alpha]_D^{20}$: + 3.0 (c: 0.50 in H_2O). Anal. calcd for $C_{10}H_{16}N_2O_7$ C, 43.48; H, 5.84; N, 10.14; found: C, 43.80; H, 6.00; N, 9.93.

5.46 (*S*)-6-Amino-2-((*R*)-4-amino-4-carboxybutanamido)hexanoic acid (21)

The residue was dissolved in a few drops of bidistilled water and purified on a column packed with Amberlite IR120. The resin was washed with bidistilled H_2O until the pH was neutral and then the product was released from the resin with aqueous 1 N NH_3.The fractions containing the dipeptide were collected and lyophilized to give the desired product.

$[\alpha]_D^{20}$: + 4.0 (c: 0.35 in H_2O). Anal. calcd for $C_{11}H_{21}N_3O_5$ C, 47.99; H, 7.69; N, 15.26; found: C, 48.23; H, 7.90; N, 15.00.

5.47 (*R*)-6-Amino-2-((*R*)-4-amino-4-carboxybutanamido)hexanoic acid (22)

$[\alpha]_D^{20}$: − 4.0 (c: 0.20 in H_2O). Anal. calcd for $C_{11}H_{21}N_3O_5$ C, 47.99; H, 7.69; N, 15.26; found: C, 48.18; H, 7.93; N, 14.98.

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