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A new and general method for the synthesis of tripeptide aldehydes based on the multi-component Ugi reaction

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ABSTRACT

Tripeptide aldehydes, such as *Z*-Leu-Leu-Leu-H (MG-132), are an important class of compounds due to their biological activity. A new, general method for the synthesis of tripeptide aldehydes based on the multi-component Ugi reaction was developed. A careful choice of isocyanides makes it possible to obtain tripeptide precursors whose functionalization led to target structures. This method can be used for the preparation of tripeptide aldehydes with non-natural amino acid side chains.

1. Introduction

Tripeptides and tripeptide mimetics are widely investigated due to their biological activity. Among them, an anti-inflammatory agent **1**,[1] an antibiotic **2**,[2] anti-parasitic agents,[3] and human rhinovirus 3C protease inhibitors[4] can be found (Fig. 1). Tripeptides with C-terminal aldehyde groups are reported as serine, cysteine, and aspartic inhibitors of proteasomes **3**,[5] human rhinovirus 3C protease **4**,[6] cathepsin K,[7] inhibitors,[8] and activators[9] of calpains. A special attention is paid to *N*-benzyloxycarbonyl-L-Leu-L-Leu-L-leucinal (**5**), a potent and selective inhibitor of 20S proteasome, which is often used as a reference in biomedical studies (Fig. 1).[5]

A diversity of tripeptide structures that can be obtained by the classical α-amino acid coupling methodology is limited by the availability of amino acids used for the synthesis. Another problem encountered in the synthesis of tripeptide aldehydes is a transformation of a precursor containing an equivalent of the C-terminal aldehyde group into the corresponding product.[2,7,10–12]

In most cases, *Z*-LLL-H and similar tripeptides were synthesized by the coupling of *Z*-leucine with a dipeptide containing C-terminal Weinreb amide group followed by reduction of the amide to the aldehyde group.[13] Peptide aldehydes were also obtained by solid phase synthesis starting from amino acetals. In this method, the final step relied on simultaneous hydrolysis of the acetal group and cleavage of the backbone amide linker to give the C-terminal peptide aldehydes.[14]

Two methods based on the application of multi-component reactions (MCR) for the synthesis of tripeptide mimetics were examined. The first method is based on the Passerini three-component reaction (P-3CR) and was used for the six-step chemoenzymatic synthesis of tripeptides and their *N*-alkylated analogues.[15] For the second method, the Ugi four-component reaction (U-4CR) was employed but its application was limited to residues containing glycine.[16] To the best of our knowledge, no other methods for tripeptide synthesis using multi-component reactions have been reported.

2. Results and discussion

The aim of our study was the development of a new and general strategy for the synthesis of tripeptide aldehydes, as depicted in Scheme 1. This strategy is based on: (a) formation of a tripeptide scaffold in the Ugi reaction, (b) deprotection of the amide function, and (c) formation of the C-terminal aldehyde group. Direct application of ammonia as a substrate for the Ugi reaction is often problematic.[17,18] Therefore, a careful choice of protective group for the amine function is required. Isocyanides possessing an aldehyde moiety cannot be used. This problem can be solved by the application of the isocyanides containing an equivalent of the aldehyde group (Y in Scheme 1).

2.1. Synthesis of isocyanides

The isocyanides required for the Ugi reaction were obtained according to modified literature procedures. Allyl isocyanide (**6a**) was obtained by the Hofmann method in 65% yield. Isocyanide **6b** was synthesized from glycine in five steps applying the method reported by Coffin et al.[19] Isocyanide **6c** was obtained according to Amato and Marcaccini[20] in two steps from amino acetaldehyde diethyl acetal. 2-Isocyanoethyl acetate (**6d**) was obtained from 2-aminoethanol by N-formylation, O-acylation, and final dehydration

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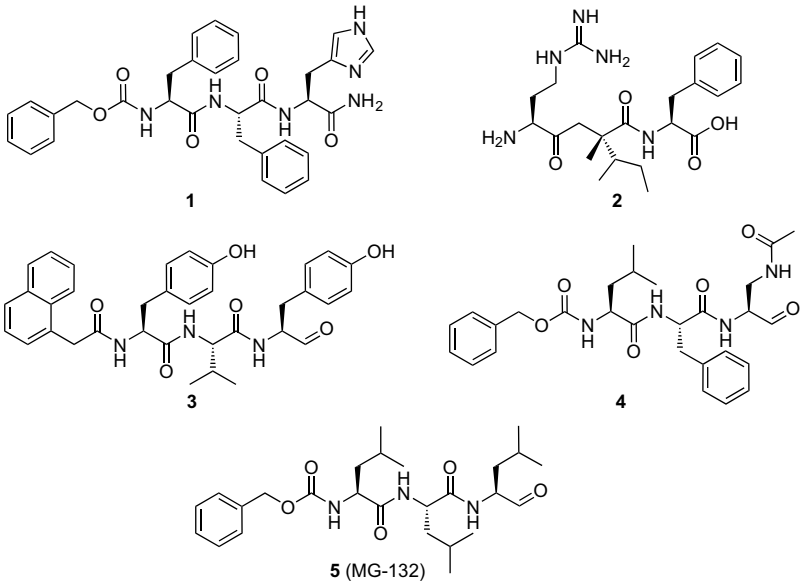
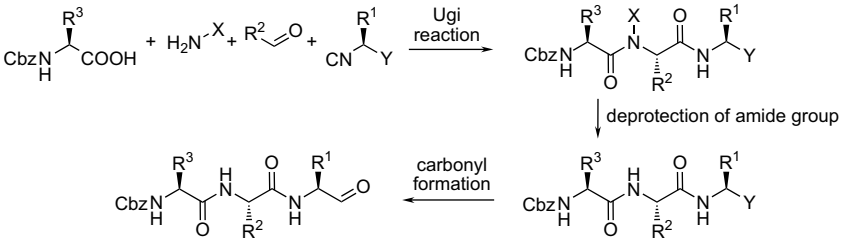


Figure 1. Bioactive tripeptides and tripeptide mimetics.



Scheme 1. The methodology of the synthesis of tripeptide aldehydes; R^1, R^3=H, alkyl; R^3=alkyl; X=*N*-amide bond protection; Y=aldehyde group equivalent.

of the formamide group. Ethyl isocyanoacetate (**6e**) was synthesized from glycine ethyl ester hydrochloride in two steps according to the method reported by Hartman and Weinstock.[21]

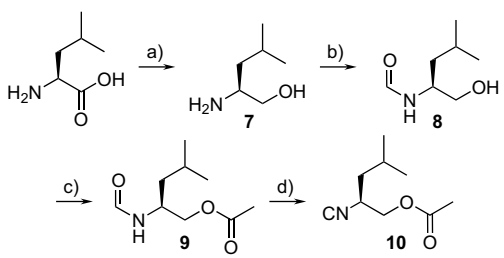
The synthesis of optically pure isocyanide **10** was more problematic. This compound was prepared from L-leucine in a four-step synthesis as shown in Scheme 2. Due to the possibility of racemization, five methods of dehydration of the formamide group were tested. Dehydration of compound **9** using triphenylphosphine gave isocyanide **10** possessing the highest optical purity (Table 1, entry 5).

2.2. Synthesis of tripeptide mimetics by Ugi reaction

In the first step of our studies on the direct synthesis of tripeptide **13**, a method based on using ammonia for the Ugi reaction was studied. Upon addition of ammonia to the reaction mixture, the expected product **13** was not obtained and only formation of byproducts was observed (Scheme 3). Because the direct synthesis of the unprotected tripeptide scaffold failed, application of primary amines for the Ugi reaction was investigated.

In the next step, *p*-methoxybenzyl amine (**14**) was used as an amine component for the Ugi reaction and four different synthetic routes were investigated for the synthesis of aldehyde **22** (Scheme 4).

Application of allyl isocyanide for the synthesis of compound **16** (Scheme 4) did not result in the formation of the expected product and the substrates were recovered as *p*-methoxybenzylammonium *N*-benzyloxycarbonylaminoacetate monohydrate in 55% yield. However, the model Ugi reaction of allyl isocyanide (**6a**) with cyclohexanone, benzyl amine, and acetic acid gave product **23** in 63%

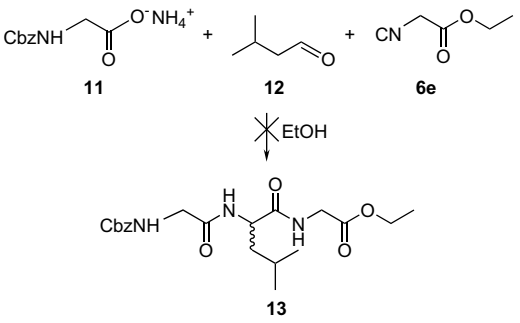


Scheme 2. Reagents and conditions: (a) $NaBH_4$, I_2, THF, reflux, 16 h, 54%; (b) HCOOEt, reflux, 4 h, 88%; (c) Ac_2O, pyridine, DMAP, DCM, rt, 2 h, 67%; (d) CCl_4, PPh_3, Et_3N, DCM, reflux, 3 h, 57%.

Table 1
Conditions for the dehydration of formamide **9** to isocyanide **10**

Entry	Conditions	Time [h]	Yield[a] [%]	$[\alpha]_D$[b]
1	Diphosgene, NMM, DCM, −40 °C	2.0	31	+1.30
2	Burgess reagent, DCM, rt	3.5	54	+5.08
3	$POCl_3$, TEA, THF, <5 °C	3.0	86	+5.05
4	$SOCl_2$, DMF, −50 to −30 °C	1.0	71	+4.59
5	PPh_3, CCl_4, TEA, DCM, reflux	3.0	57	+7.30

[a] Isolated overall yields.
[b] Optical rotatory power was measured in $CHCl_3$, at *c*=1 g of the isocyanide/100 mL at 25 °C.



Scheme 3. Ugi reaction with ammonia or ammonium salts.

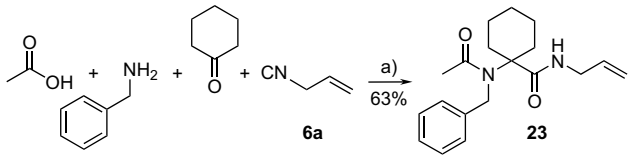
yield (Scheme 5). To our knowledge, this is the first example, which demonstrates such an influence of the carbonyl substrate on the course of the Ugi reaction.

Compund **17** with C-terminal Weinreb amide group was synthesized from isocyanide **6b** in 48% yield. Direct reduction of compound **17** using $LiAlH_4$ led to the formation of aldehyde **22** in 39% yield. Tripeptide mimetic **18** was obtained in the Ugi reaction using isocyanide **6c** in 67% yield. Compound **18** was easily transformed to aldehyde **22** by acetal group hydrolysis catalyzed by *p*-toluenesulfonic acid in 81% yield.

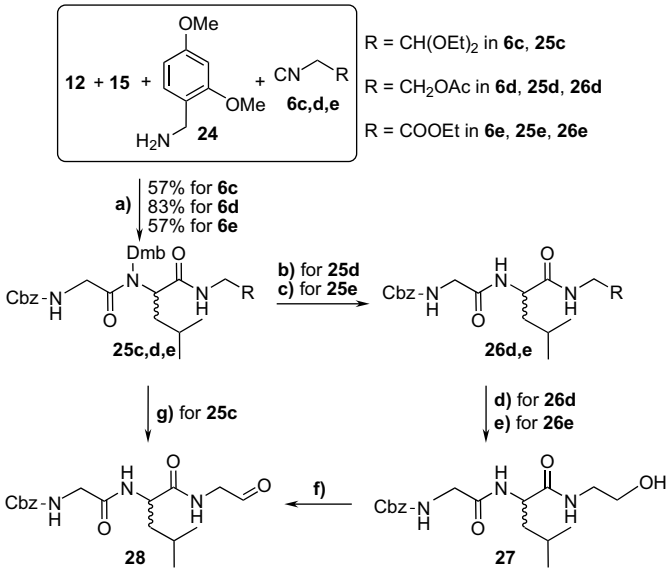
The Ugi reaction with 2-isocyanoethyl acetate (**6d**) led to compound **19** in 89% yield. Basic hydrolysis of **19** gave alcohol **21** in 96% yield. Analogous Ugi reaction with ethyl isocyanoacetate (**6e**) gave ester **20** in 71% yield. The ester group was reduced with $NaBH_4$ to give alcohol **21** in 93% yield. Then, the alcohol **21** was selectively oxidized to the aldehyde **22** using TEMPO reagent in 46% yield. The method with 2-isocyanoethyl acetate (**6d**) is the most convenient and efficient, although the improvement of the oxidation step yield is necessary.

2.3. Deprotection of the amide function and selective oxidation of hydroxyl group to aldehyde

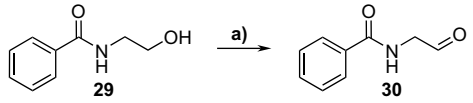
The next experiments were focused on deprotection of the amide group. Several methods for cleavage of the *p*-methoxybenzyl group (Pmb) from the amide function in compounds **19** and **20** were tested. Application of cerium ammonium nitrate (CAN)[22] or trifluoroacetic acid (TFA)[23] led to traces of the expected products. Reaction of compound **22** under similar conditions did not result in



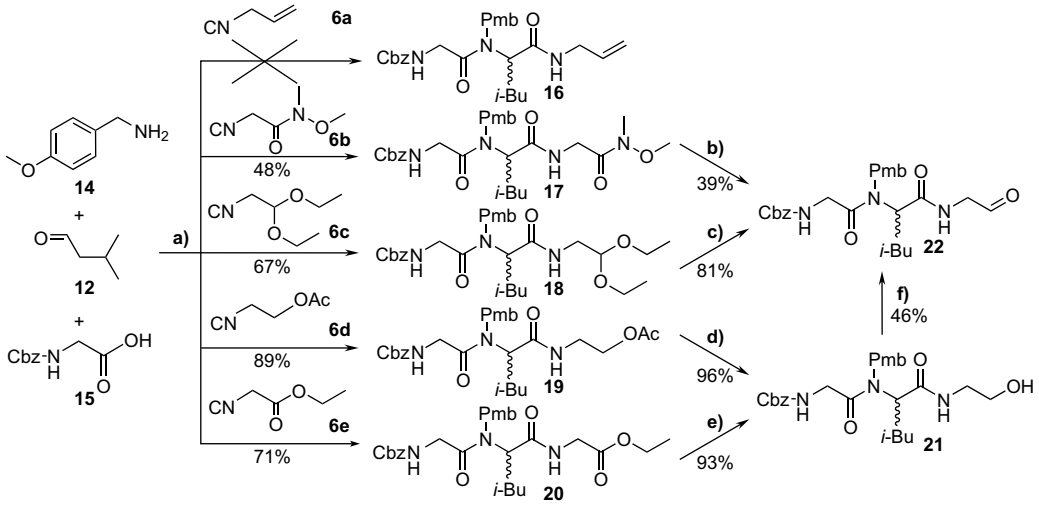
Scheme 5. Model Ugi reaction with allyl isocyanide: (a) dichloroethane, 40 °C, 10 h.



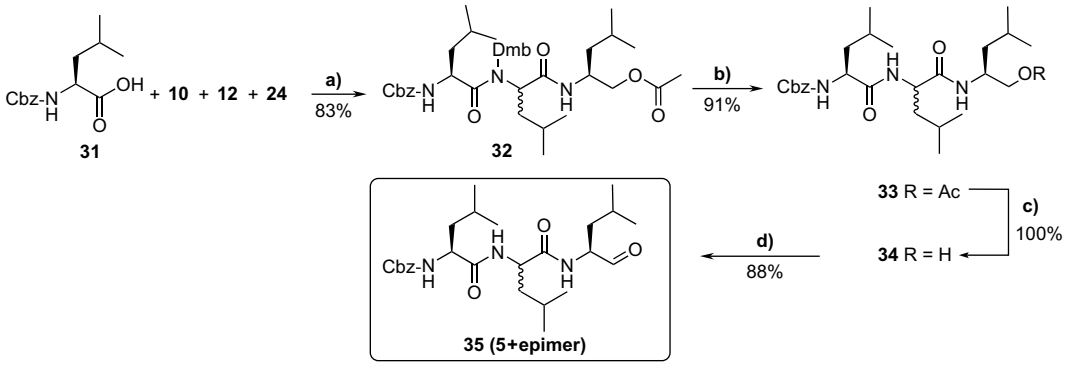
Scheme 6. Synthesis of deprotected aldehyde **28**. Reagents and conditions: (a) MeOH, rt, 48 h; (b) TFA, DCM, 50 °C, 1 h, 88%; (c) TFA, 30 min, rt, 76%; (d) $NaOH_{aq}$, MeOH, rt, 45 min, 88%; (e) $NaBH_4$, $CaCl_2$, LiCl, THF, 50 °C, 24 h, 50%; (f) Dess–Martin reagent, DCM, rt, 1 h, 93%; (g) TFA/water=19:1, rt, 1 h, 18%.



Scheme 7. Model reaction of selective oxidation of **29**. (a) Dess–Martin reagent, DCM, rt, 1.5 h, 85%.



Scheme 4. Synthesis of aldehyde **22** (Pmb=*p*-methoxybenzyl). (a) MeOH (EtOH), 48 h, rt; (b) LAH, THF, 10 min, −10 °C, 90 min, 0 °C; (c) *p*-TosH, acetone/water 2:1, 90 min, 50 °C; (d) $NaOH_{aq}$, MeOH, 30 min, rt; (e) $NaBH_4$, $CaCl_2$, LiCl, THF, 20 h, 35 °C; (f) TEMPO, NaClO, NaBr, water, DCM, 50 min, 0 °C.



Scheme 8. Reagents and conditions (Dmb=2,4-dimethoxybenzyl): (a) MeOH, rt, 48 h; (b) TFA, DCM, 50 °C, 1 h; (c) $NaOH_{aq}$, MeOH, rt, 30 min; (d) Dess–Martin reagent, DCM, rt, 1.5 h.

deprotection of the amide group. These results indicate that a more labile amide protecting group such as 2,4-dimethoxybenzyl (Dmb) should be used. The Ugi reactions with 2,4-dimethoxybenzyl amine (**24**) were performed and the products **25c**–**e** were obtained in 57%, 83%, and 57% yields, respectively (Scheme 6). The reactions of the tripeptides **25** with CAN did not lead to the formation of compounds **26**; the desired products were obtained by the treatment with TFA. Stirring of the compound **25e** in neat TFA at room temperature for 30 min gave the product **26e** in 76% yield. For the full conversion of compound **25d**, a 1 h heating in TFA diluted with DCM was required and thus the product **26d** was obtained in 88% yield. Next, the basic hydrolysis of acetate **26d** and the reduction of ester group in **26e** with $NaBH_4$ led to the alcohol **27** in 88% and 50% yield, respectively (Scheme 6).

Because the oxidation of compound **21** with TEMPO gave moderate yields, a model reaction was investigated. As a model substrate, *N*-(2-hydroxyethyl)benzamide (**29**) was used (Scheme 7). The methods using DCC, cyanuric chloride with DMSO, and Pyr–SO_3 with DMSO were not successful. Product **30** was obtained in 59% using the Swern method. Two other oxidations proved to be more efficient. Oxidation of alcohol **29** using TEMPO reagent or Dess–Martin periodinane resulted in formation of the aldehyde in 67% and 85% yields, respectively. It is important to note that no impurities were observed. Accordingly, the tripeptide alcohol **27** was oxidized to aldehyde **22** using Dess–Martin reagent. This resulted in an excellent 93% yield (Scheme 6).

Special attention was paid to the simultaneous one-step deprotection of two functional groups presented in the structure **25c**. Direct transformation of **25c** to the aldehyde **28** was especially noteworthy. The best result was obtained by the treatment of tripeptide **25c** with TFA/water mixture (19:1; v/v). Unfortunately, aldehyde **28** was isolated in low yield (18%).

2.4. Synthesis of *Z*-Leu-L/D-Leu-Leu-H

The presented synthetic methodology was used for the synthesis of *N*-benzyloxycarbonyl-L-Leu-L/D-Leu-L-leucinal (**35**). The Ugi reaction between isocyanide **10**, isovaleraldehyde (**12**), 2,4-dimethoxybenzyl amine (**24**), and *N*-benzyloxycarbonyl-L-leucine (**31**) gave the tripeptide mimetic **32** in 83% yield (Scheme 8). Compound **32** was obtained as a 49:51 mixture of two epimers according to the HPLC analysis. This indicates that the racemization of isocyanide **10** during the Ugi reaction does not occur. The following reactions did not change the diastereoisomeric ratios.

Deprotection of amide bond in compound **32** with trifluoroacetic acid led to the tripeptide **33** in 91% yield. Then, compound **33** was subjected to a basic hydrolysis and selective oxidation with Dess–Martin reagent to obtain the final aldehyde **34** in 66% overall yield (Scheme 8). Epimer *S*,*S*,*S*-**35** is known as a proteasome inhibitor MG-132 (**5**).

3. Conclusions

A new and general methodology for the multi-component synthesis of tripeptide aldehydes was developed. Application of the Ugi reaction allows the preparation of the tripeptide scaffold in one step when chiral acid and isocyanide are used. Use of (2,4-dimethoxy)benzyl amine as a substrate for the Ugi reaction enables easy deprotection of the amide function in the next step of synthesis. When isocyanide contains a carbonyl group equivalent, the product of the Ugi reaction is easily converted into a tripeptide with C-terminal aldehyde group. Due to the diversity of available aldehydes the presented method is applicable for the preparation of tripeptides with non-natural amino acid side chains.

4. Experimental

4.1. General

NMR spectra were measured with a Varian 200 GEMINI and Varian 400 GEMINI spectrometers, with TMS used as an internal standard. TLCs were performed with silica gel 60 (230–400 mesh, Merck) and silica gel 60 PF_{254} (Merck). CHN analysis was performed on a Perkin Elmer 240 Elemental Analyzer. MS spectra were recorded on an API-365 (SCIEX) apparatus. IR spectra were recorded on a Perkin Elmer FT-IR Spectrum 2000 spectrometer. Optical rotations were measured with a JASCO DIP-360 polarimeter. HPLC experiments were carried out on KROMASIL 100 C-18 column, eluent methanol/water 8:2 (v/v), flow: 1 mL/min, λ=230 nm.

4.2. Synthesis of isocyanides

*4.2.1. 3-Isocyanoprop-1-ene (allyl isocyanide) (**6a**)*

A mixture of allylamine (11.4 g, 152 mmol), chloroform (11.7 g, 146 mmol), triethylbenzylammonium chloride (0.2 g, 0.9 mmol), and DCM (30 mL) was added dropwise for 20 min to an aqueous solution of sodium hydroxide (60 mL, 50%) heated to 45 °C. The reaction mixture was intensively stirred for additional 3 h at 45 °C. Then, the reaction mixture was cooled down to room temperature and extracted with DCM (2×50 mL). The combined organic layers were washed with water and fractionally distilled to obtain product **6a** as an offensively smelling, colorless oil (6.6 g, 99 mmol). Yield: 65%; bp 90 °C at atmosphere pressure (lit.[24] 98 °C). The product was directly used for the subsequent reactions without further purification.

*4.2.2. Preparation of 2-isocyano-N-methoxy-N-methylacetamide (**6b**)*

4.2.2.1. N-(tert-Butoxycarbonyl)aminoacetic acid. To a solution of sodium hydroxide (0.572 g, 14.3 mmol) in water (15 mL), glycine (0.96 g, 13.0 mmol) and *tert*-butyl alcohol (10 mL) were added. Then, *tert*-butoxycarbonyl anhydride (2.90 g, 13.0 mmol) was added for 20 min at room temperature. After 16 h, the reaction mixture was washed with hexane (2×40 mL). The combined organic layers were extracted with saturated sodium bicarbonate solution (2×40 mL). The aqueous layers were combined and acidified with 10% solution of potassium bisulfate up to pH=2 and extracted with diethyl ether (4×40 mL). The organic layers were combined, dried (Na_2SO_4), and evaporated to leave a thick yellow oil. The oil was dissolved in ethyl acetate and hexane was added. After evaporation and vacuum drying, the residue crystallized as white crystals. Yield: 83%, 1.9 g (10.8 mmol); mp 84–86 °C (lit.[19] 87–88 °C). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.46 (s, 9H, $C(CH_3)_3$), 3.95 (s, 2H, CH_2), 6.84 (s, 1H, NH), 10.24 (s, 1H, OH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.6, 42.6, 80.8, 156.3, 175.1. Spectroscopic data were consistent with literature data.[19]

4.2.2.2. tert-Butyl-(2-(methoxy(methyl)amino)-2-oxoethyl)-carbamate. To a solution of *N*,*O*-dimethylhydroxylamine hydrochloride (0.585 g, 6.0 mmol) in DCM (30 mL) at 0 °C, triethylamine (0.92 mL, 6.6 mmol) and *N*-(*tert*-butoxycarbonyl)aminoacetic acid (0.876 g, 5.0 mmol) were added. After 30 min, dicyclohexylcarbodiimide (1.15 g, 5.5 mmol) and 4-(dimethylamino)pyridine (20 mg) were added. The reaction mixture was stirred at 0 °C for 2 h and then at room temperature for 16 h. The precipitated crystals of DCU and triethylamine hydrochloride were filtered off. During concentration of the filtrate, white crystals of product were precipitated. Yield: 82%, 0.90 g (4.1 mmol); mp 95–98 °C (lit.[25] 102–103 °C). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.46 (s, 9H, $(CCH_3)_3$), 3.21 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 4.08 (d, J=4.8 Hz, 2H, CH_2), 5.30 (br s, 1H, NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.7, 32.7, 42.0, 61.7, 79.9, 156.1, 175.0; IR (film in $CHCl_3$) ν_{max}: 3315, 2913, 2847, 1617, 1571, 1532, 1434, 1310, 1241, 1068, 1044 cm^{-1}. Spectroscopic data were consistent with literature data.[25]

4.2.2.3. 2-Formamido-N-methoxy-N-methylacetamide. A solution of *tert*-butyl-(2-(methoxy(methyl)amino)-2-oxoethyl)carbamate (0.785 g, 3.6 mmol) in formic acid (10 mL) was refluxed for 1.5 h. Excess of formic acid was evaporated. The residue was dissolved in ethyl formate (5 mL), and triethylamine (1.05 mL, 7.56 mmol) was added. The mixture was refluxed for 16 h, then evaporated, diluted with water (20 mL), and extracted with DCM (4×20 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated. Yield: 53%, 0.278 g (1.9 mmol) of a colorless oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.24 (s, 3H, CH_3), 3.74 (s, 3H, CH_3), 4.25 (d, J=4.4 Hz, 2H, CH_2), 6.70 (br s, 1H, NH), 8.28 (s, 1H, CHO). Spectroscopic data were consistent with literature data.[25]

*4.2.2.4. 2-Isocyano-N-methoxy-N-methylacetamide (**6b**).* To a solution of 2-formamido-*N*-methoxy-*N*-methylacetamide (0.278 g, 1.9 mmol) and triethylamine (0.80 mL, 5.70 mmol) in DCM (15 mL) cooled to −50 °C, phosphorous oxychloride (0.26 mL, 2.85 mmol) was added dropwise in 30 min. The reaction mixture was allowed to reach room temperature and it was stirred for additional 2 h. Then, the reaction mixture was cooled to 0 °C, diluted with water (10 mL), and an aqueous solution of sodium bicarbonate was added (20 mL, 1 M). The aqueous layer was extracted with DCM (3×20 mL), combined organic layers were dried ($MgSO_4$), the solvent was evaporated, and product was purified by flash chromatography (silica gel, 35–70 mesh, 5.9 g, hexane/EtOAc, 75:25, v/v). Yield: 53%, 130 mg (1.01 mmol) of creamy crystals; mp 77–79 °C (lit.[26] 82 °C); R_f=0.26 (hexane/EtOAC, 5:5, v/v). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.24 (s, 3H, CH_3), 3.73 (s, 3H, CH_3), 4.40 (s, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 32.7, 44.0, 62.5, 160.9, 164.0; IR (film in $CHCl_3$) ν_{max}: 3336, 2994, 2957, 2163, 1682, 1467, 1406, 1328, 1200, 1011, 961, 916, 615, 556 cm^{-1}. Spectroscopic data were consistent with literature data.[25]

*4.2.3. Preparation of 1,1-diethoxy-2-isocyanoethane (**6c**)*

4.2.3.1. N-(2,2-Diethoxyethyl)formamide. A mixture of 2,2-diethoxyethanamine (10 mL, 69 mmol) and ethyl formate (6.7 mL, 83 mmol) was heated to 85 °C for 48 h. Then, the reaction mixture was concentrated and the product was distilled (80 °C, 0.4 mmHg) (lit.[20] 110–111, 0.5 mmHg). Yield: 83%, 9.21 g (57 mmol) of a colorless oil. ^{1}H NMR ($CDCl_3$, 200 MHz) (for two observed rotamers): major rotamer: δ 1.16 (t, J=7.1 Hz, 6H), 3.38 (t, J=5.4 Hz, 2H), 3.44–3.90 (m, 4H), 4.47 (t, J=5.4 Hz, 1H), 6.18 (br s, 1H), 8.14 (s, 1H); minor rotamer: δ 3.25 (dd, J=5.5, 6.5 Hz, 2H), 4.39 (t, J=5.2 Hz, 1H), 7.99 (d, J=13.5 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): major rotamer: δ 40.7, 63.1, 100.7, 161.5; minor rotamer: δ 44.9, 63.6, 101.9, 165.2. Anal. Calcd for $C_7H_{13}NO_2$: C, 58.72; H, 9.15; N, 9.78. Found: C, 58.75; H, 9.10; 9.75. Spectroscopic data were consistent with literature data.[20]

*4.2.3.2. 1,1-Diethoxy-2-isocyanoethane (**6c**).* A mixture of *N*-(2,2-diethoxyethyl)formamide (5.0 g, 31 mmol), triethylamine (4.3 mL, 31 mmol), triphenylphosphine (9.18 g, 33 mmol), and carbon tetrachloride (3.2 mL, 33 mmol) in DCM (30 mL) was refluxed for 3.5 h. Then, the reaction mixture was cooled to 5 °C for 15 min. The precipitated solid was filtered off through the bed of Celite and washed with diethyl ether (20 mL). Filtrate was concentrated and product was purified by distillation (45 °C, 0.5 mmHg) (lit.[20] 60–61, 1 mmHg). Yield: 79%, 3.52 g (25.4 mmol) of a colorless oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.20 (t, J=7.2 Hz, 6H), 3.46 (d, J=5.6 Hz, 2H), 3.50–3.90 (m, 4H), 4.67 (t, J=5.4 Hz, 1H); ^{13}C NMR (50 MHz, CDCl3) δ 15.3, 44.7, 44.8, 45.0, 63.4, 99.7. Spectroscopic data were consistent with literature data.[20]

*4.2.4. Acetic acid 2-isocyanoethyl ester (**6d**)*

2-Aminoethanol (10 mL, 167 mmol) was refluxed with ethyl formate (30 mL, 37 mmol) for 3 h. The reaction mixture was cooled to room temperature and evaporated to obtain crude *N*-(2-hydroxyethyl)formamide (15 g). Then, acetic anhydride (30 mL, 32 mmol) was added and the reaction mixture was refluxed for additional 5 h, cooled, and evaporated to obtain crude 2-formamidoethyl acetate as a colorless oil (21 g). A portion of 2-formamidoethyl acetate (3 g, 23 mmol) and Et_3N (16 mL, 115 mmol) were dissolved in DCM (30 mL) and cooled to −60 °C. Phosphorous oxychloride (2.5 mL, 27.6 mmol) was added dropwise for 30 min. The reaction mixture was allowed to reach the room temperature, stirred for additional 2 h, and then poured into water with ice. The layers were separated and the aqueous layer was extracted with DCM (3×30 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and evaporated. The product was purified by column chromatography (silica gel, 60 mesh, 50 g, DCM). Overall yield: 50%, 1.35 g (11.9 mmol) of a yellow oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.08 (s, 3H, CH_3), 3.63 (t, 2H, CH_2O), 4.21 (qu, 2H, CH_2N); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.8, 41.0, 61.4, 97.4, 170.7; IR (film) ν_{max}: 2960 (CH_3), 2150 (N≡C), 1740 (C=O), 1225 (CH_2–O) cm^{-1}.

*4.2.5. Synthesis of (2S)-2-isocyano-4-methylpentyl acetate (**10**)*

*4.2.5.1. (2S)-2-Amino-4-methylpentan-1-ol (L-(S)-leucinol) (**7**).* To a suspension of sodium borohydride (2.9 g, 76.0 mmol) in THF (60 mL), L-leucine (5.0 g, 38.1 mmol) was added under nitrogen atmosphere. The reaction mixture was cooled to 0 °C and a solution of iodine (9.67 g, 38.1 mmol) in THF (20 mL) was added for 30 min. Then, the reaction mixture was stirred until the gas bubbles

stopped evolving and was refluxed for 16 h, cooled to room temperature and methanol was added until the mixture changed to clear. After 30 min, the volatiles were evaporated. The residue was dissolved in aqueous solution of potassium hydroxide (100 mL, 20%), stirred for 3 h, and extracted with DCM (3×50 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated. The residual dense oil was distilled under reduced pressure (40 °C, 0.6 mmHg). Yield: 54%, 2.41 g (20.6 mmol) of a colorless oil; R_f=0.42 ($CHCl_3$/MeOH/NH_4OH, 10:2:0.25, v/v/v). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.89 (t, J=6.4 Hz, 6H), 1.17 (t, J=7.0 Hz, 2H), 1.64–1.70 (m, 1H), 2.10 (br s, 3H), 2.82–2.96 (m, 1H), 3.21 (dd, J=10.6, 7.8 Hz, 1H), 3.55 (dd, J=10.6, 3.8 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.5, 23.7, 25.0, 44.0, 50.9, 67.4; $[\alpha]_D^{23}$ +2.78 (*c* 1.87, EtOH) (lit.[27] +4.2 (*c* 0.9, EtOH)).

*4.2.5.2. N-[(1S)-1-(Hydroxymethyl)-3-methylbutyl]formamide (**8**).* A solution of L-(*S*)-leucinol (2.4 g, 20.5 mmol) in ethyl formate (15 mL, 185 mmol) was refluxed for 4 h. Then, the reaction mixture was cooled, volatiles were evaporated, and residue was distilled under reduced pressure (132–136 °C, 0.3 mmHg) Yield: 88%, 2.39 g (16.5 mmol) of slightly yellow oil; R_f=0.69 ($CHCl_3$/MeOH/NH_4OH, 10:2:0.25, v/v/v). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.93 (d, J=6.6 Hz, 6H), 1.20–1.48 (m, 2H), 1.50–1.66 (m, 1H), 3.40–3.78 (m, 3H), 4.12 (br s, 1H), 6.11 (br s, 1H), 8.18 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.4, 23.4, 25.2, 40.4, 49.2, 65.8, 162.3; IR (film) ν_{max}: 3280 (O–H), 2958 (CH_3), 1660 (C=O) cm^{-1}; $[\alpha]_D^{25}$ −49.9 (*c* 1.0, EtOH).

*4.2.5.3. (2S)-2-Formamido-4-methylpentyl acetate (**9**).* To a solution of *N*-[(*S*)-1-(hydroxymethyl)-3-methylbutyl]formamide (1.45 g, 10.3 mmol) in DCM (25 mL), pyridine (1.5 mL, 18.5 mmol), acetic anhydride (4.6 mL, 50 mmol), and *N*,*N*-dimethylaminopyridine (20 mg) were added under nitrogen atmosphere. The reaction mixture was stirred for 2 h at room temperature, washed with an aqueous solution of copper sulfate (2×10 mL, 10%), a saturated aqueous solution of sodium bicarbonate (2×10 mL) and water (2×10 mL). The organic layer was dried ($MgSO_4$) and the solvent was evaporated to leave a product that was used without further purification. Yield: 67%, 1.31 g (7.0 mmol) of a colorless oil; R_f=0.50 (hexane/EtOAct, 5:5, v/v). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.80–1.00 (m, 6H), 1.28–1.48 (m, 2H), 1.50–1.75 (m, 1H), 3.70–3.60 (m, 2H), 3.61–3.72 (m, 1H), 6.76 (br s, 1H), 8.15 (s, 1H); IR (film) ν_{max}: 2960 (CH_3), 1740 (NC=O), 1660 (OC=O), 1242 (CH_2–O) cm^{-1}; $[\alpha]_D^{25}$ −36.41 (*c* 1.0, $CHCl_3$).

*4.2.5.4. (2S)-2-Isocyano-4-methylpentyl acetate (**10**).* To a solution of (*S*)-2-formamido-4-methylpentyl acetate (700 mg, 3.74 mmol) in DCM (20 mL), triphenylphosphine (1.12 g, 4.25 mmol), carbon tetrachloride (286 μl, 4.00 mmol), and triethylamine (521 μL, 3.74 mmol) were added. The reaction mixture was refluxed for 3 h, cooled to room temperature, and washed with water (2×10 mL). The organic layer was dried (Na_2SO_4), the solvent was evaporated, and the product was purified by column chromatography (silica gel, 70–230 mesh, 15 g, DCM). Yield: 57%, 360 mg (2.13 mmol) yellow oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.90 (dd, J=8.8, 6.6 Hz, 6H), 1.14–1.34 (m, 1H), 1.50–1.71 (m, 1H), 1.71–1.91 (s, 1H), 2.06 (s, 3H), 3.70–3.88 (m, 1H), 3.94–4.20 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.9, 21.4, 23.2, 24.9, 40.3, 52.3, 65.6, 170.7; IR (film) ν_{max}: 2960 (CH_3), 2142 (N≡C), 1745 (C=O), 1235 (CH_2–O) cm^{-1}. Anal. Calcd for $C_9H_{15}NO_2$: C, 63.86; H, 8.94; N, 8.28. Found: C, 63.91; H, 8.82; N, 8.24; $[\alpha]_D^{23}$ +7.30 (c 1.0, $CHCl_3$).

4.3. Model reaction of the selective oxidation

*4.3.1. N-(2-Hydroxyethyl)benzamide (**29**)*

To a solution of 2-aminoethanol (3.00 g, 49.1 mmol) in ethyl acetate (50 mL), benzoyl chloride (7.51 g, 53.4 mmol) and a solution of sodium bicarbonate (10 g, 120 mmol) in water (50 mL) were added. The reaction mixture was stirred for 4 h at room temperature. The layers were separated, the aqueous layer was extracted with ethyl acetate (2×30 mL). Organic layers were combined, dried ($MgSO_4$), and solvent was evaporated. The residual colorless oil was diluted with ethyl acetate and product was precipitated with diethyl ether. Yield 71%, 5.77 g (35.0 mmol) of white crystals; mp 60–62 °C (lit.[28] 52–56 °C); R_f=0.27 ($CHCl_3$/MeOH=95:5). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.63 (t, J=4.7 Hz, 2H, $NHCH_2$), 3.83 (t, J=5.1 Hz, 2H, CH_2O), 3.87 (s, 1H, OH), 7.20 (br s, 1H, NH), 7.35–7.70 (m, 3H, ArH), 7.81 (d, J=7.4 Hz, 2H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 43.1, 62.2, 127.3, 128.8, 131.9, 134.3, 169.0; IR (film) ν_{max}: 3307 (O–H), 1630 (C=O), 1535 (C–H) cm^{-1}.

*4.3.2. N-(2-Oxoethyl)benzamide (**30**)*

To a suspension of Dess–Martin reagent (127.2 mg, 0.3 mmol) in DCM (2 mL). *N*-(2-Hydroxyethyl)benzamide (**29**) (33 mg, 0.2 mmol) was added. The reaction mixture was stirred for 1.5 h at room temperature and passed over a short pad of basic alumina (2.5 g) and eluted with DCM. The solvent was evaporated and the product was purified by column chromatography (silica gel, 230–400 mesh, 1 g, hexane/EtOAc, 8:2, v/v). Yield 85%, 27.6 mg (0.17 mmol) of a colorless oil; R_f=0.30 ($CHCl_3$/MeOH=95:5). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.50–3.65 (m, 2H, $NHCH_2$), 7.16 (br s, 1H, NH), 7.40–7.75 (m, 3H, ArH), 7.84 (d, J=7.4 Hz, 2H, ArH), 9.75 (m, 1H, CHO); ^{13}C NMR (50 MHz, $CDCl_3$) δ 47.2, 127.9, 128.6, 131.8, 133.9, 168.1, 197.2; IR (film) ν_{max}: 3330 (N–H), 1724 (HC=O), 1645 (NC=O), 1540 (C–H) cm^{-1}.

4.4. Synthesis of tripeptides

4.4.1. General procedure A for the Ugi reaction

A 1 M solution of an amine (1 equiv) and an aldehyde (1 equiv) in methanol (or ethanol if ethyl ester or diethyl acetal was used) was stirred for 15 min at room temperature. Then, an acid (1 equiv) was added. After additional 15 min, an isocyanide (1 equiv) was added. The reaction mixture was stirred at room temperature for 2 days. The solvent was evaporated off and the product was purified by gradient flash column chromatography (silica gel, 70–230 mesh, hexane/EtOAc mixture).

*4.4.2. {[(4-Methoxybenzyl)-(1-{[(methoxymethylcarbamoyl)-methyl]-carbamoyl}-3-methylbutyl) carbamoyl]-methyl}-carbamic acid benzyl ester (**17**). General procedure A*

Yield: 48%, 130 mg (0.24 mmol) of yellow oil; R_f=0.27 ($CHCl_3$/MeOH, 95:5, v/v). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (dd, J=6.3, 11.7 Hz, 6H), 1.26 (s, 1H), 1.42–1.58 (m, 2H), 1.86 (t, J=7.4 Hz, 1H), 2.05 (d, J=3.3 Hz, 1H), 3.19 (s, 3H), 3.69 (s, 3H), 3.78 (s, 3H), 3.99–4.10 (m, 2H), 4.52 (s, 2H), 5.07 (t, J=7.0 Hz, 1H), 5.09 (s, 2H), 5.81 (br s, 1H), 6.84 (d, J=8.6 Hz, 2H), 6.92 (br s, 1H), 7.11 (d, J=8.4 Hz, 2H), 7.27–7.39 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.4, 22.7, 25.1, 37.0, 40.1, 43.4, 47.5, 55.2, 56.4, 61.4, 66.8, 114.2, 127.4, 127.9, 128.0, 128.2, 128.4, 136.4, 156.1, 158.9, 169.4, 170.5, 170.6; IR (film in $CHCl_3$) ν_{max}: 3329, 2958, 1722, 1655, 1513, 1442, 1249, 1177, 1030, 807, 754, 698 cm^{-1}; HRMS calcd for $C_{28}H_{38}N_4O_7Na$ $[M+Na]^+$: 565.2633; found: 565.2643.

*4.4.3. {[(1-(2,2-Diethoxyethylcarbamoyl)-3-methylbutyl)-(4-methoxybenzyl)-carbamoyl]-methyl}-carbamic acid benzyl ester (**18**). General procedure A*

The product after purification by column chromatography was recrystallized from a mixture of acetone and water. Yield: 67%, 372 mg (0.67 mmol) of white crystals; mp 92–93 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (dd, J=6.4, 8.4 Hz, 6H), 1.18 (dt, J=2.7, 6.8 Hz, 6H), 1.40–1.56 (m, 2H), 1.78–1.93 (m, 1H), 3.18–3.30 (m, 1H), 3.30–3.40 (m, 1H), 3.42–3.55 (m, 2H), 3.57–3.71 (m, 2H), 3.78 (s, 3H), 3.96 (d, J=4.4 Hz, 2H), 4.44 (t, J=5.4 Hz, 1H), 4.51 (s, 2H), 4.96 (t,

J=6.8 Hz, 1H), 5.08 (s, 2H), 5.74 (t, J=4.3 Hz, 1H), 6.54 (t, J=5.2 Hz, 1H), 6.85 (d, J=8.4 Hz, 2H), 7.08 (d, J=8.4 Hz, 2H), 7.20–7.40 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.2, 22.3, 22.7, 25.0, 37.1, 41.6, 43.3, 47.5, 55.2, 56.6, 62.5, 62.6, 66.8, 100.3, 114.2, 127.4, 127.9, 128.1, 128.4, 136.2, 156.1, 159.0, 170.4; IR (film in $CHCl_3$) ν_{max}: 3328, 2957, 1723, 1648, 1514, 1455, 1249, 1176, 1128, 1058, 755, 698 cm^{-1}. Anal. Calcd for $C_{30}H_{43}N_3O_7$: C, 64.61; H, 7.77; N, 7.53. Found: C, 64.55; H, 7.42; N, 7.58.

4.4.4. {[(4-Methoxybenzyl)-(3-methyl-1-propylcarbamoylbutyl)-carbamoyl]methyl}carbamic acid benzyl ester (**19**). General procedure A

Yield: 89%, 471 mg (0.89 mmol) of a colorless oil; R_f=0.36 (hexane/EtOAc, 4:6, v/v). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (dd, J=6.4, 8.4 Hz, 6H), 1.40–1.52 (m, 2H), 1.82–1.93 (m, 1H), 2.03 (s, 3H), 3.41 (q, J=5.5 Hz, 2H), 3.78 (s, 3H), 3.98 (dd, J=4.8, 12.0 Hz, 2H), 4.02–4.10 (m, 1H), 4.10–4.18 (m, 1H), 4.51 (s, 2H), 4.92 (t, J=4.8 Hz, 1H), 5.09 (s, 2H), 5.71 (br s, 1H), 6.70 (br s, 1H), 6.86 (d, J=8.4 Hz, 2H), 7.09 (d, J=8.4 Hz, 2H), 7.28–7.38 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.8, 22.3, 22.6, 25.1, 36.9, 38.5, 43.3, 47.6, 55.2, 56.9, 62.8, 66.9, 114.3, 127.4, 128.0, 128.1, 128.5, 136.2, 156.2, 159.0, 170.4, 170.8, 171.1; IR (film in $CHCl_3$) ν_{max}: 3329, 2957, 1726, 1650, 1514, 1455, 1368, 1249, 1177, 1051, 754, 698 cm^{-1}; HRMS calcd for $C_{28}H_{37}N_3O_7Na$ $[M+Na]^+$: 550.2524; found: 550.2502.

4.4.5. {2-[(2-Benzyloxycarbonylaminoacetyl)-(4-methoxybenzyl)-amino]-4-methylpentanoylamino}acetic acid ethyl ester (**20**). General procedure A

Yield: 71%, 750 mg (1.42 mmol) of a colorless oil; R_f=0.46 (hexane/EtOAc, 5:5, v/v). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.78 (t, J=5.7 Hz, 6H), 1.19 (t, J=7.1 Hz, 3H), 1.28–1.58 (m, 2H), 1.60–1.94 (m, 1H), 3.72 (s, 3H), 3.83 (t, J=5.8 Hz, 2H), 3.93 (br s, 2H), 4.11 (q, J=7.2 Hz, 2H), 4.44 (s, 2H), 4.97 (br s, 1H), 5.03 (s, 2H), 5.64 (br s, 1H), 6.78 (d, J=7.8 Hz, 2H), 6.87 (br s, 1H), 7.04 (d, J=7.4 Hz, 2H), 7.10–7.40 (m, 5H); IR (film in $CHCl_3$) ν_{max}: 3330, 2958, 1724, 1649, 1514, 1465, 1418, 1249, 1200, 1178, 1030, 755, 698 cm^{-1}. Anal. Calcd for $C_{28}H_{37}N_3O_7$: C, 63.74; H, 7.07; N, 7.96. Found: C, 63.62; H, 7.46; N, 7.57.

4.4.6. Synthesis of compound **16**. General procedure A

The product **16** was not obtained. The substrates were recovered as *p*-methoxybenzylammonium *N*-benzyloxycarbonylaminoacetate monohydrate; mp 134–137 °C. ^{1}H NMR (CD_3OD, 400 MHz) δ 3.73 (s, 2H), 3.79 (s, 3H), 4.02 (s, 2H), 5.07 (s, 2H), 6.96 (d, J=8.4 Hz, 2H), 7.20–7.40 (m, 7H); ^{13}C NMR (CD_3OD, 100 MHz) δ 44.7, 45.5, 56.6, 68.4, 116.3, 127.2, 129.7, 129.8, 130.3, 132.4, 139.1, 159.7, 162.7, 176.5. Anal. Calcd for $C_{18}H_{22}N_2O_5+H_2O$: C, 59.33; H, 6.64; N, 7.69. Found: C, 59.57; H, 6.51; 7.60.

4.4.7. {[(1-(2-Hydroxyethylcarbamoyl)-3-methylbutyl)-(4-methoxybenzyl)-carbamoyl]-methyl}-carbamic acid benzyl ester (**21**)

4.4.7.1. Method 1. To a solution of **19** (196 mg, 0.38 mmol) in methanol (2 mL), an aqueous solution of sodium hydroxide (375 μL, 1.50 mmol, 4 M) was added. After 30 min, the reaction mixture was diluted with EtOAc (10 mL) and washed with an aqueous solution of hydrochloric acid (10 mL, 1 M). Layers were separated and the aqueous layer was extracted with EtOAc (10 mL). The combined organic layers were dried ($MgSO_4$) and solvent was evaporated. Product was used to the next step without further purification. Yield: 96%, 177 mg (0.36 mmol) of a colorless oil; R_f=0.13 (hexane/EtOAc, 4:6, v/v). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (dd, J=6.2, 12.0 Hz, 6H), 1.42–1.55 (m, 2H), 1.83–1.95 (m, 1H), 3.20–3.32 (m, 1H), 3.33–3.43 (m, 1H), 3.60–3.66 (m, 2H), 3.79 (s, 3H), 3.83 (dd, J=5.2, 16.8 Hz, 1H), 3.99 (dd, J=5.2, =17.2 Hz, 1H), 4.50 (d, J=17.2 Hz, 1H), 4.57 (d, J=17.2 Hz, 1H), 4.70 (s, 1H), 4.96 (t, J=7.0 Hz, 1H), 5.09 (d, J=2.4 Hz, 2H), 5.75 (br s, 1H), 6.81 (br s, 1H), 6.87 (d, J=8.8 Hz, 2H), 7.11 (d, J=8.4 Hz, 2H), 7.28–7.38 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.4, 22.6, 25.1, 37.0, 42.4, 43.3, 47.9, 55.3, 56.9, 61.8, 67.1, 114.4, 127.5, 128.0, 128.1, 128.2, 128.5, 136.1, 156.8, 159.1, 171.1, 171.2; IR (film in $CHCl_3$) ν_{max}: 3329, 2957, 1726, 1650, 1514, 1455, 1368, 1249, 1177, 1051, 753, 698 cm^{-1}.

4.4.7.2. Method 2. To a solution of **20** (150 mg, 0.28 mmol) in THF (2 mL), calcium chloride (62 mg, 0.56 mmol), lithium chloride (24 mg, 0.56 mmol), and sodium borohydride (53 mg, 1.4 mmol) were added. The reaction mixture was heated to 35 °C for 20 h, cooled to room temperature, and methanol (5 mL) was added. Then, the reaction mixture was filtered and filtrate was evaporated to dryness. The residue was dissolved in DCM (5 mL), washed with an aqueous solution of hydrochloric acid (10 mL, 1 M), dried ($MgSO_4$), and solvent was evaporated. Product was purified by column chromatography (silica gel, 70–325 mesh, 2.5 g, hexane/EtOAc, 7:3, v/v). Yield: 93%, 126 mg (0.26 mmol) of a colorless oil; R_f=0.23 ($CHCl_3$/MeOH, 95:5, v/v). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (dd, J=6.2, 11.4 Hz, 6H), 1.45–1.56 (m, 2H), 1.84–1.95 (m, 1H), 3.20–3.32 (m, 1H), 3.34–3.43 (m, 1H), 3.60–3.65 (m, 2H), 3.79 (s, 3H), 3.83 (dd, J=5.3, 16.8 Hz, 1H), 3.99 (dd, J=5.1, 17.0 Hz, 1H), 4.50 (d, J=17.2 Hz, 1H), 4.57 (d, J=17.2 Hz, 1H), 4.70 (s, 1H), 4.96 (t, J=7.1 Hz, 1H), 5.09 (d, J=2.2 Hz, 2H), 5.78 (t, J=4.7 Hz, 1H), 6.80 (m, 1H), 6.87 (d, J=8.4 Hz, 2H), 7.12 (d, J=8.4 Hz, 2H), 7.29–7.38 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.4, 22.6, 25.1, 37.0, 42.2, 43.3, 47.9, 55.3, 56.9, 61.8, 67.1, 114.4, 127.5, 128.0, 128.1, 128.2, 128.5, 136.1, 156.8, 159.1, 171.1, 171.2; IR (film in $CHCl_3$) ν_{max}: 3322, 2956, 1719, 1648, 1514, 1456, 1351, 1249, 1176, 1053, 754, 698 cm^{-1}; HRMS calcd for $C_{26}H_{35}N_3O_6Na$ $[M+Na]^+$: 508.2418; found: 508.2414.

4.4.8. ({(4-Methoxybenzyl)-[3-methyl-1-(2-oxoethylcarbamoyl)-butyl]-carbamoyl}-methyl)-carbamic acid benzyl ester (**22**)

4.4.8.1. Method 1. To a solution of alcohol **21** (63 mg, 0.13 mmol) in DCM (3 mL), water (1 mL), 2,2,6,6-tetramethyl (1.4 mg, 0.013 mmol), and sodium bromide (1.3 mg, 0.013 mmol) were added. The reaction mixture was cooled to 0 °C and an aqueous solution of sodium hypochlorite (0.5 mL, 0.2 M solution saturated with sodium bisulfate) was added dropwise for 30 min. After additional 20 min, the reaction mixture was diluted with DCM (10 mL) and water (10 mL). The layers were separated and the aqueous layer was extracted with DCM (10 mL). The organic layers were combined, washed with an aqueous solution of hydrochloric acid (10 mL, 1 M) containing potassium iodide (166 mg, 1 mmol), an aqueous solution of sodium thiosulfate (10 mL, 10%) and water, and dried ($MgSO_4$). The solvent was evaporated and the product was purified by column chromatography (silica gel, 70–325 mesh, 1 g, hexane/EtOAc, 8:2, v/v). Yield: 46%, 28 mg (0.06 mmol) of a colorless oil; R_f=0.38 (hexane/EtOAc, 4:6, v/v). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.60–1.05 (m, 6H), 1.30–1.64 (m, 2H), 1.68–2.00 (m, 1H), 3.78 (s, 3H), 4.02 (d, J=3.6 Hz, 4H), 4.51 (s, 2H), 5.07 (br s, 1H), 5.09 (s, 2H), 5.65–5.90 (m, 1H), 6.85 (d, J=8.4 Hz, 2H), 7.05 (br s, 1H), 7.10 (d, J=8.4 Hz, 2H), 7.34 (s, 5H), 9.54 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.8, 23.0, 25.5, 37.3, 43.8, 48.1, 50.3, 55.6, 56.9, 67.4, 114.7, 127.9, 128.3, 128.5, 128.8, 136.6, 156.6, 159.4, 171.3, 194.6.

4.4.8.2. Method 2. To a solution of **17** (54.2 mg, 0.1 mmol) in THF (3 mL) cooled to −50 °C, lithium aluminum hydride (4.8 mg, 0.125 mmol) was added. The reaction mixture was stirred for 10 min at −10 °C and for 90 min at 0 °C. Then, an aqueous solution of sodium bisulfate (0.15 mL, 0.15 mmol, 1 M) and water (10 mL) were added. The layers were separated and the aqueous layer was extracted with diethyl ether (10 mL). The combined organic layers were washed with an aqueous solution of hydrochloride acid

(2×15 mL, 1 M), aqueous saturated solution of sodium bisulfate (15 mL), brine (15 mL) and were dried ($MgSO_4$). The solvent was evaporated and the product was purified by column chromatography (silica gel, 70–325 mesh, 6.6 g, hexane/EtOAc, 8:2, v/v). Yield: 39%, 19 mg (0.039 mmol) of a colorless oil; R_f=0.36 (hexane/EtOAc, 4:6, v/v). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.70–0.95 (m, 6H), 1.42–1.54 (m, 2H), 1.82–1.94 (m, 1H), 3.70–3.82 (m, 2H), 3.79 (s, 3H), 3.94–4.15 (m, 2H), 4.52 (s, 2H), 5.09 (br s, 1H), 5.10 (s, 2H), 5.73 (br s, 1H), 6.86 (d, J=8.1 Hz, 2H), 7.01 (br s, 1H), 7.11 (d, J=8.4 Hz, 2H), 7.24–7.39 (m, 5H), 9.56 (s, 1H); IR (film in $CHCl_3$) ν_{max}: 3330, 2958, 1710, 1653, 1514, 1456, 1420, 1350, 1249, 1177, 1031, 803, 754, 698 cm^{-1}.

4.4.8.3. Method 3. To a solution of **18** (114 mg, 0.204 mmol) in acetone (2 mL), water (1 mL) and *p*-toluenesulfonic acid (41 mg, 0.214 mmol) were added. The reaction mixture was stirred for 1.5 h at 50 °C. Then, the reaction mixture was cooled to room temperature, diluted with EtOAc (10 mL), and washed with an aqueous saturated solution of sodium bicarbonate (15 mL) and brine (15 mL). Organic layer was dried ($MgSO_4$) and solvent was evaporated. Product was purified by column chromatography (silica gel, 35–70 mesh, 2.0 g, hexane/EtOAc, 7:3, v/v). Yield: 81%, 80 mg (0.17 mmol) of a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (dd, J=6.4, 8.0 Hz, 6H), 1.38–1.65 (m, 2H), 1.78–1.95 (m, 1H), 3.79 (s, 3H), 3.90–4.20 (m, 4H), 4.51 (d, J=3.6 Hz, 2H), 5.00 (br s, 1H), 5.10 (s, 2H), 5.71 (br s, 1H), 6.85 (d, J=8.4 Hz, 2H), 6.97 (br s, 1H), 7.10 (d, J=8.4 Hz, 2H), 7.20–7.40 (m, 5H), 9.56 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.4, 22.6, 25.1, 36.8, 43.4, 47.8, 50.0, 55.3, 56.6, 67.0, 114.3, 127.5, 128.0, 128.1, 128.5, 136.3, 156.3, 159.1, 170.9, 194.3; IR (film in $CHCl_3$) ν_{max}: 3331, 2957, 1712, 1649, 1514, 1455, 1350, 1249, 1177, 1035, 755, 698 cm^{-1}; HRMS calcd for $C_{27}H_{37}N_3O_7Na$ [M+MeOH+Na]$^+$: 538.2524; found: 538.2548.

*4.4.9. 1-(Acetyl-benzylamino)cyclohexane carboxylic acid allylamide (**23**)*

To a mixture of benzyl amine (111.3 mg, 1.04 mmol) and cyclohexanone (103.3 mg, 1.05 mmol) in dichloroethane (1 mL), acetic acid (62.2 mg, 1.04 mmol) was added. After 15 min, allyl isocyanide (78.4 mg, 1.17 mmol) was added. The reaction mixture was heated to 40 °C for 10 h and then solvent was evaporated. Product was purified by column chromatography (silica gel, 70–230 mesh, 7.2 g, hexane/EtOAc, 7:3, v/v). Yield: 63%, 209 mg (0.66 mmol) of a colorless oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.25–1.40 (m, 1H), 1.45–1.85 (m, 7H), 2.18 (s, 3H), 2.54 (d, J=9 Hz, 2H), 3.96 (t, J=5.4 Hz, 2H), 4.73 (s, 2H), 5.15–5.40 (m, 2H), 5.80–6.05 (m, 1H), 6.59 (br s, 1H), 7.25–7.55 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.3, 24.5, 25.7, 33.3, 42.5, 49.2, 66.5, 67.4, 116.5, 126.2, 127.5, 129.1, 134.8, 138.8, 173.6. Anal. Calcd for $C_{19}H_{26}N_2O_2$: C, 72.58; H, 8.33N, 8.91. Found: C, 72.46; H, 8.29; N, 8.98.

*4.4.10. Acetic acid 2-{2-[(2-benzyloxycarbonylaminoacetyl)-(2,4-dimethoxybenzyl)-amino]-4-methylpentanoylamino}-ethyl ester (**25d**). General procedure A*

Yield: 83%, 926 mg (1.66 mmol) of a colorless oil; R_f=0.38 ($CHCl_3$/MeOH, 95:5, v/v). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (t, J=6.5 Hz, 6H), 1.50 (qu, J=6.5 Hz, 1H), 1.51–1.63 (m, 1H), 1.86–1.96 (m, 1H), 2.03 (s, 3H), 3.35 (q, J=5.6 Hz, 2H), 3.79 (s, 3H), 3.83 (s, 3H), 4.05 (t, J=5.4 Hz, 2H), 4.12 (d, J=7.2 Hz, 2H), 4.41 (s, 2H), 4.64 (br s, 1H), 5.12 (s, 2H), 5.70 (br s, 1H), 6.45 (s, 2H), 6.63 (br s, 1H), 6.99 (d, J=9.0 Hz, 1H), 7.29–7.38 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.8, 22.5, 22.6, 25.1, 36.7, 38.4, 43.1, 44.7, 55.2, 55.4, 57.5, 62.9, 66.8, 98.6, 104.0, 115.8, 128.0, 128.1, 128.4, 128.9, 136.4, 156.2, 158.0, 160.8, 170.3, 170.6, 171.0; IR (film in $CHCl_3$) ν_{max}: 3330, 2957, 1726, 1651, 1615, 1589, 1508, 1456, 1368, 1231, 1210, 1158, 1046, 755, 698 cm^{-1}; HRMS calcd for $C_{29}H_{39}N_3O_8Na$ [M+Na]$^+$: 580.2629; found: 580.2606.

*4.4.11. Acetic acid 2-[2-(2-benzyloxycarbonylaminoacetylamino)-4-methylpentanoylamino]-ethyl ester (**26d**)*

To a solution of **25d** (569 mg, 1.02 mmol) in DCM (5 mL), trifluoroacetic acid (2 mL) was added. The reaction mixture was stirred for 1 h at 50 °C. Then, the reaction mixture was cooled in ice-water bath, diluted with DCM (5 mL), and neutralized with an aqueous saturated solution of sodium bicarbonate until violet color disappeared. Layers were separated and the aqueous layer was extracted with DCM (2×15 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), dried ($MgSO_4$), and evaporated. Product was purified by column chromatography (silica gel, 70–230 mesh, 8 g, $CHCl_3$/MeOH, 95:5, v/v). Yield: 88%, 367 mg (0.90 mmol) of a colorless oil; R_f=0.29 ($CHCl_3$/MeOH, 95:5, v/v). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, J=6.8 Hz, 6H), 1.45–1.55 (m, 1H), 1.55–1.72 (m, 2H), 2.05 (s, 3H), 3.40–3.55 (m, 2H), 3.84–3.92 (m, 2H), 4.06–4.20 (m, 2H), 4.43 (dt, J=6.05, 8.5 Hz, 1H), 5.12 (s, 2H), 5.77 (br s, 1H), 6.89 (br s, 1H), 6.91 (br s, 1H), 7.30–7.37 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 20.6, 20.8, 22.0, 22.7, 24.7, 38.7, 40.1, 44.5, 51.6, 60.4, 62.7, 67.2, 128.1, 128.3, 128.5, 136.1, 156.7, 169.3, 171.2, 172.0; HRMS calcd for $C_{20}H_{29}N_3O_6Na$ [M+Na]$^+$: 430.1949; found: 430.1959.

*4.4.12. {[1-(2-Hydroxyethylcarbamoyl)-3-methylbutylcarbamoyl]-methyl}-carbamic acid benzyl ester (**27**). Method 1—hydrolysis of acetyl ester (**26d**)*

To a solution of **26d** (367 mg, 0.9 mmol) in methanol (5 mL), an aqueous solution of sodium hydroxide (0.56 mL, 2.25 mmol, 4 M) was added. The reaction mixture was stirred for 45 min at room temperature and the solvent was evaporated. The residue was dissolved in an aqueous solution of hydrochloric acid (10 mL, 1 M) and extracted with EtOAc (3×10 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated. The product was purified by column chromatography (silica gel, 70–230 mesh, 5 g, $CHCl_3$/MeOH, 95:5, v/v). Yield: 85%, 278 mg (0.76 mmol) of a colorless oil; R_f=0.23 ($CHCl_3$/MeOH, 98:2, v/v). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89 (dd, J=5.9, 9.6 Hz, 6H), 1.48–1.70 (m, 3H), 3.16–3.28 (m, 2H), 3.38–3.50 (m, 2H), 3.54–3.68 (m, 2H), 3.85 (dq, J=5.3, 17.5 Hz, 2H), 4.46–4.58 (m, 1H), 5.08 (s, 2H), 6.11 (br s, 1H), 7.28–7.37 (m, 5H), 7.40 (t, J=5.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.2, 23.1, 41.3, 42.5, 44.6 52.3, 61.6, 67.5, 128.3, 128.6, 128.8, 136.3, 157.3, 170.2, 173.3; HRMS calcd for $C_{18}H_{27}N_3O_5Na$ [M+Na]$^+$: 388.1843; found: 388.1825.

*4.4.13. {2-[(2-Benzyloxycarbonylaminoacetyl)-(2,4-dimethoxybenzyl)-amino]-4-methylpentanoylamino}-acetic acid ethyl ester (**25e**). General procedure A*

Yield: 57%, 301 mg (0.57 mmol) as colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (d, J=6.2 Hz, 6H), 1.25 (t, J=7.2 Hz, 3H), 1.46–1.60 (m, 2H), 1.85–1.97 (m, 1H), 3.79 (s, 3H), 3.81 (s, 3H), 3.84 (d, J=5.5 Hz, 2H), 3.98 (d, J=5.3 Hz, 1H), 4.17 (q, J=7.2 Hz, 2H), 4.42 (s, 2H), 4.74 (t, J=6.8 Hz, 1H), 5.11 (s, 2H), 5.80 (br s, 1H), 6.43 (s, 2H), 6.85 (t, J=5.0 Hz, 1H), 7.01 (d, J=9.0 Hz, 1H), 7.27–7.38 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.5, 25.0, 36.7, 41.2, 43.1, 36.7, 41.2, 43.1, 44.7, 55.2, 55.3, 57.1, 61.3, 66.8, 67.0, 98.6, 103.9, 115.8, 127.9, 128.0, 128.1, 128.4, 128.4, 128.9, 136.4, 156.2, 158.0, 160.8, 169.5, 170.4, 170.8; IR (film in $CHCl_3$) ν_{max}: 3333, 2958, 1724, 1651, 1615, 1589, 1508, 1455, 1289, 1260, 1209, 1158, 1119, 1045 cm^{-1}; HRMS calcd for $C_{29}H_{39}N_3O_8Na$ [M+Na]$^+$: 580.2629; found: 580.2602.

*4.4.14. [2-(2-Benzyloxycarbonylaminoacetylamino)-4-methylpentanoylamino]-acetic acid ethyl ester (**26e**)*

A solution of **25e** (214 mg, 0.38 mmol) in trifluoroacetic acid (1 mL, 13.4 mmol) was stirred at room temperature for 30 min. Then, the reaction mixture was diluted with DCM (10 mL) and neutralized with an aqueous saturated solution of sodium bicarbonate until violet color disappeared. Layers were separated and

the aqueous layer was extracted with DCM (2×10 mL). The combined organic layers were washed with water (10 mL) and brine (10 mL), dried ($MgSO_4$), and evaporated. Product was purified by column chromatography (silica gel, 70–325 mesh, 2.6 g, hexane/EtOAc, 8:2, v/v). Yield: 76%, 117 mg (0.29 mmol) of a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89 (dd, J=5.9, 9.9 Hz, 6H), 1.24 (t, J=7.2 Hz, 3H), 1.47–1.57 (m, 1H), 1.57–1.72 (m, 2H), 3.90 (d, J=4.6 Hz, 2H), 3.92–3.97 (m, 2H), 4.15 (q, J=7.2 Hz, 2H), 4.57–4.66 (m, 1H), 5.09 (s, 2H), 6.00 (t, J=5.0 Hz, 1H), 7.26–7.36 (m, 6H), 7.41 (t, J=5.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 20.6, 21.9, 22.7, 24.6, 41.0, 44.3, 51.5, 61.4, 67.0, 127.9, 128.1, 127.4, 136.2, 156.7, 169.6, 169.7, 172.3; IR (film in $CHCl_3$) ν_{max}: 3300, 3069, 2958, 1731, 1657, 1537, 1455, 1353, 1240, 1204, 1157, 1047, 753, 698 cm^{-1}; HRMS calcd for $C_{20}H_{29}N_3O_6Na$ [M+Na]$^+$: 430.1949; found: 430.1927.

4.4.15. {[1-(2-Hydroxyethylcarbamoyl)-3-methylbutylcarbamoyl]-methyl}-carbamic acid benzyl ester (**27**). Method 2—reduction of ester (**26e**)

To a solution of **26e** (41 mg, 0.1 mmol) in THF (5 mL), sodium borohydride (19 mg, 0.5 mmol), calcium chloride (22 mg, 0.2 mmol), and lithium chloride (8.4 mg, 0.2 mmol) were added. The reaction mixture was stirred for 24 h at 50 °C. Then, the reaction mixture was cooled to room temperature and methanol (2 mL) was added. White solid was filtered off and washed with methanol. Filtrate was evaporated, diluted with DCM (10 mL), and washed with an aqueous solution of hydrochloric acid (10 mL, 1 M). The aqueous layer was extracted with DCM (5 mL). The combined organic layers were washed with brine (10 mL), dried ($MgSO_4$), and solvent was evaporated. Product was purified by column chromatography (silica gel, 70–230 mesh, 0.7 g, $CHCl_3$/MeOH, 98:2, v/v). Yield: 50%, 17 mg (0.05 mmol). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.88 (s, 6H), 1.40–1.70 (m, 3H), 3.15–3.32 (m, 2H), 3.34–3.52 (m, 2H), 3.54–3.70 (m, 2H), 3.75–4.00 (m, 2H), 4.40–4.60 (m, 1H), 5.08 (s, 2H), 6.05 (br s, 1H), 7.18–7.50 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.3, 23.2, 41.3, 42.6, 44.9 52.3, 61.7, 67.7, 128.4, 128.6, 128.9, 136.3, 157.4, 170.2, 173.2; IR (film in $CHCl_3$) ν_{max}: 3299, 3069, 2957, 1709, 1651, 1541, 1455, 1255, 1155, 1052, 754, 697 cm^{-1}.

4.4.16. {[3-Methyl-1-(2-oxoethylcarbamoyl)-butylcarbamoyl]-methyl}-carbamic acid benzyl ester (**28**). Method 1—oxidation of **27**

To a suspension of alcohol **27** (36.5 mg, 0.10 mmol) in DCM (2 mL), the Dess–Martin reagent (63.6 mg, 0.15 mmol) was added. The reaction mixture was stirred for 1 h at room temperature and then passed through a column with basic alumina (2.1 g) ($CHCl_3$/MeOH, 95:5, v/v). Yield: 93%, 33.8 mg (0.093 mmol) of a colorless oil; R_f=0.27 ($CHCl_3$/MeOH, 95:5, v/v). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.78–1.00 (m, 6H), 1.42–1.75 (m, 3H), 3.60–3.96 (m, 3H), 4.00–4.25 (m, 1H), 4.40–4.70 (m, 1H), 5.10 (s, 2H), 5.75–6.05 (m, 1H), 7.13 (br s, 1H), 7.26–7.38 (m, 5H), 9.53 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.9, 22.9, 24.7, 39.9, 40.8, 41.6, 42.0, 44.3, 50.0, 51.6, 67.3, 128.1, 128.3, 128.5, 136.1, 156.8, 172.5, 196.7; IR (film in $CHCl_3$) ν_{max}: 3300, 3068, 2958, 1710, 1653, 1538, 1455, 1247, 1155, 1049, 755, 697 cm^{-1}. HRMS calcd for $C_{18}H_{25}N_3O_5Na$ [M+Na]$^+$: 386.1686; found: 386.1705.

4.4.17. {[[1-(2,2-Diethoxyethylcarbamoyl)-3-methylbutyl]-(2,4-dimethoxybenzyl)-carbamoyl]-methyl}-carbamic acid benzyl ester (**25c**). General procedure A

Crude product after column chromatography was diluted in the mixture of hexane/EtOAc (1:1; v/v) (50 mL) and passed over a bed of neutral aluminum oxide. Yield: 57%, 322 mg (0.57 mmol) of a colorless oil; R_f=0.44 (hexane/EtOAc, 5:5, v/v). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (dd, J=1.3, 6.42 Hz, 6H), 1.18 (dt, J=4.3, 7.1 Hz, 6H), 1.46–1.64 (m, 2H), 1.93 (qu, J=7.0 Hz, 1H), 3.12–3.26 (m, 2H), 3.44–3.54 (m, 2H), 3.65 (dq, J=7.0, 9.4 Hz, 2H), 3.79 (s, 3H), 3.82 (s, 3H), 4.13–4.20 (m, 2H), 4.32–4.48 (m, 3H), 4.57 (t, J=7.0 Hz, 1H), 5.12 (s, 2H), 5.74 (br s, 1H), 6.39–6.46 (m, 3H), 7.00 (d, J=9.0 Hz, 1H), 7.28–7.38 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.2, 15.3, 22.5, 22.6, 25.2, 36.8, 41.9, 43.2, 45.0, 55.4, 57.2, 62.9, 62.9, 66.8, 98.7, 100.8, 104.0, 115.7, 128.0, 128.1, 128.5, 129.3, 136.4, 156.1, 157.1, 160.9, 169.7, 170.5; IR (film in $CHCl_3$) ν_{max}: 3331, 2957, 1723, 1651, 1615, 1589, 1508, 1455, 1259, 1210, 1130, 1057, 773, 698 cm^{-1}; HRMS calcd for $C_{31}H_{45}N_3O_8Na$ [M+Na]$^+$: 610.3099; found: 610.3070.

4.4.18. {[3-Methyl-1-(2-oxoethylcarbamoyl)-butylcarbamoyl]-methyl}-carbamic acid benzyl ester (**28**). Method 2—deprotection and hydrolysis of acetal (**25c**)

Compound **25c** (90 mg, 0.204 mmol) was dissolved in a mixture of trifluoroacetic acid (950 μL) and water (50 μL). The reaction mixture was stirred for 1 h at room temperature. Then it was diluted with DCM (5 mL) and neutralized with an aqueous saturated solution of sodium bicarbonate until violet color disappeared. The layers were separated and the aqueous layer was extracted with DCM (10 mL). The combined organic layers were washed with water (10 mL) and brine (10 mL), dried ($MgSO_4$), and solvent was evaporated. The product was purified by column chromatography (silica gel, 70–325 mesh, 1.3 g, $CHCl_3$/MeOH, 9:1, v/v). Yield: 18%, 13 mg (0.035 mmol) of a colorless oil; R_f=0.36 ($CHCl_3$/MeOH, 9:1, v/v). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85–0.97 (m, 6H), 1.50–1.74 (m, 2H), 1.84–1.96 (m, 1H), 3.84–3.94 (m, 2H), 4.04–4.12 (m, 2H), 4.56 (dt, J=5.5, 8.7 Hz, 1H), 5.10 (s, 2H), 5.70 (br s, 1H), 6.83 (d, J=8.4 Hz, 1H), 7.16 (br s, 1H), 7.29–7.38 (m, 5H), 9.55 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.9, 22.8, 24.7, 40.8, 44.6, 50.0, 51.6, 67.3, 128.1, 128.3, 128.6, 136.0, 139.5, 172.5, 196.6; IR (film in $CHCl_3$) ν_{max}: 3312, 3066, 2956, 1712, 1658, 1509, 1455, 1298, 1205, 1037, 754, 698 cm^{-1}.

4.4.19. Acetic acid 2-{2-[(2-benzyloxycarbonylamino-4-methyl-pentanoyl)-(2,4-dimethoxybenzyl)-amino]-4-methylpentanoyl-amino}-4-methylpentyl ester (**32**). General procedure A with (2S)-2-isocyano-4-methylpentyl acetate (**10**)

Yield: 83%, 551 mg (0.82 mmol) of colorless oil; a mixture of diastereoisomers R_{fI}=0.30, R_{fII}=0.38 ($CHCl_3$/MeOH, 95:5, v/v). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.70–1.05 (m, 18H), 1.10–1.35 (m, 3H), 1.35–1.70 (m, 6H), 1.85–2.05 (m, 3H), 3.78 (s, 6H), 3.87–4.05 (m, 1H), 4.05–4.25 (m, 2H), 4.26–4.45 (m, 1H), 4.46–4.70 (m, 1H), 4.73–4.95 (m, 1H), 5.08 (s, 2H), 6.35–6.52 (m, 2H), 6.70–6.80 (m, 1H), 6.98–7.20 (m, 2H), 7.34 (s, 5H); IR (film in $CHCl_3$) ν_{max}: 3311, 3065, 2957, 1738, 1725, 1639, 1529, 1508, 1466, 1367, 1257, 1210, 1158, 1117, 1041, 754, 698 cm^{-1}; HRMS calcd for $C_{37}H_{55}N_3O_8Na$ [M+Na]$^+$: 692.3881; found: 692.3874; retention time of epimers: t_{RI}=10.29 min, t_{RII}=11.38 min.

4.4.20. Acetic acid 2-[2-(2-benzyloxycarbonylamino-4-methyl-pentanoylamino)-4-methylpentanoylamino]-4-methylpentyl ester (**33**)

To a solution of **32** (352 mg, 0.53 mmol) in DCM (2 mL) trifluoroacetic acid was added (0.5 mL, 6.7 mmol). The reaction mixture was stirred at 50 °C for 1 h 45 min, and then cooled to room temperature, diluted with DCM (5 mL), and neutralized with an aqueous saturated solution of sodium bicarbonate until violet color disappeared. Layers were separated and the aqueous layer was extracted with DCM (3×10 mL). The combined organic layers were washed with brine (15 mL), dried ($MgSO_4$), and solvent was evaporated. Product was purified by column chromatography (silica gel, 70–230 mesh, 5.2 g, hexane/EtOAc, 8:2, v/v). Yield: 91%, 249 mg (0.48 mmol) of a colorless oil; R_f=0.29 ($CHCl_3$/MeOH, 95:5, v/v). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.80–1.14 (m, 18H), 1.20–1.40 (m, 2H), 1.41–1.75 (m, 7H), 2.04 (d, J=2.8 Hz, 3H), 3.90–4.30 (m, 4H), 4.31–4.52 (m, 1H), 5.09 (s, 2H), 5.30–5.50 (m, 1H), 6.45–6.70 (m, 2H), 7.33 (s, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.3, 22.4, 22.6, 23.4, 25.0, 40.8, 41.0, 42.1, 46.8, 47.8, 52.3, 66.5, 67.5, 128.3, 128.6, 128.9, 136.7, 170.9,

171.6, 172.5; IR (film in $CHCl_3$) ν_{max}: 3283, 3070, 2957, 1745, 1704, 1640, 1548, 1455, 1368, 1239, 1042, 755, 696 cm^{-1}; HRMS calcd for $C_{37}H_{55}N_3O_8Na$ $[M+Na]^+$: 542.3201; found: 542.3176.

4.4.21. {1-[1-(1-Hydroxymethyl-3-methylbutylcarbamoyl)-3-methylbutylcarbamoyl]-3-methylbutyl}-carbamic acid benzyl ester (**34**)

To a solution of **33** (208.9 mg, 0.4 mmol) in MeOH (5 mL) an aqueous solution of sodium hydroxide (0.25 mL, 1.0 mmol, 4 M) was added. The reaction mixture was stirred at room temperature for 30 min, and then solvent was evaporated. The residue was dissolved in an aqueous solution of hydrochloric acid (5 mL, 1 M) and extracted with EtOAc (3×10 mL). The organic layers were combined, dried (Na_2SO_4), and the solvent was evaporated off to leave the product, which was used without further purification. Yield: 100%, 192.5 mg (0.4 mmol) as colorless oil; R_f=0.19 ($CHCl_3$/MeOH, 95:5, v/v). ^1H NMR ($CDCl_3$, 200 MHz) δ 0.78–1.05 (m, 18H), 1.15–1.42 (m, 3H), 1.43–1.80 (m, 6H), 2.93 (br s, 1H), 3.30–3.70 (m, 2H), 3.90–4.08 (m, 1H), 4.10–4.28 (m, 1H), 4.30–4.55 (m, 1H), 5.09 (s, 2H), 5.40–5.58 (m, 1H), 5.60–5.73 (m, 1H), 6.65–6.70 (m, 1H), 7.25–7.45 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.3, 22.5, 23.2, 23.4, 25.1, 25.2, 40.2, 40.6, 40.9, 41.5, 50.5, 52.3, 54.3, 66.0, 67.6, 128.4, 128.7, 128.9, 136.3, 172.4, 173.0; IR (film in $CHCl_3$) ν_{max}: 3288, 3069, 2956, 1701, 1641, 1545, 1468, 1368, 1260, 1043, 696 cm^{-1}; HRMS calcd for $C_{26}H_{43}N_3O_5Na$ $[M+Na]^+$: 500.3095; found: 500.3113.

4.4.22. {1-[1-(1-Formyl-3-methylbutylcarbamoyl)-3-methylbutylcarbamoyl]-3-methylbutyl}-carbamic acid benzyl ester (**35**)

To a solution of alcohol **34** (50.7 mg, 0.106 mmol) in DCM (2 mL), the Dess–Martin reagent (85 mg, 0.20 mmol) was added. The reaction mixture was stirred at room temperature for 1.5 h. An aqueous solution of sodium hydroxide (2 mL, 5%) was added and the mixture was stirred for additional 10 min. Then, water (10 mL) was added, layers were separated, and the aqueous layer was extracted with DCM (4×10 mL). The combined organic layers were dried ($MgSO_4$) and solvent was evaporated. Product was purified by column chromatography (silica gel, 70–230 mesh, 1 g, hexane/EtOAc, 7:3, v/v). Yield: 88%, 44 mg (0.093 mmol) of a colorless oil; R_f=0.48 ($CHCl_3$/MeOH, 9:1, v/v). ^1H NMR ($CDCl_3$, 200 MHz) δ 0.80–1.20 (m, 18H), 1.40–1.90 (m, 9H), 4.10–4.40 (m, 1H), 4.42–4.55 (m, 1H), 4.56–4.78 (m, 1H), 5.15 (s, 2H), 5.64–5.94 (m, 1H), 6.98–7.32 (m, 2H), 7.38 (s, 5H), 9.57 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.1, 22.4, 22.5, 23.0, 23.2, 23.4, 25.0, 25.1, 37.7, 40.1, 41.0, 41.7, 51.8, 52.0, 53.9, 57.6, 67.4, 128.2, 128.5, 128.9, 136.4, 156.6, 172.5, 173.0, 199.9; IR (film in $CHCl_3$) ν_{max}: 3289, 3068, 2957, 1736, 1700, 1645, 1538, 1468, 1386, 1262, 1238, 1120, 1045, 696 cm^{-1}; HRMS calcd for $C_{26}H_{41}N_3O_5Na$ $[M+Na]^+$: 498.2938; found: 498.2936.

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