

Short malonyl dehydro peptides as potential scaffolds for peptidomimetics by an efficient Knoevenagel reaction

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Abstract Non-symmetric disubstituted malonamides *rAA-mGly-AA'*, obtained from Meldrum's acid, were considered as methylene active compounds and a Knoevenagel condensation methodology was developed involving piperidine and activated 4 Å molecular sieves as catalysts. The reaction is efficient, broad in scope, and easy to perform and allows access to *E/Z* mixtures of short malonyl dehydro peptides (MDHPs) *rAA-mΔ²AA''-AA'*, partially modified retro peptides characterized by an interesting combination of retro and dehydro modifications in the same structure.

Keywords Peptidomimetics · Amino acids · Malonamides · Knoevenagel reaction

Introduction

The structural manipulation of natural peptide backbone is widely applied to obtain peptidomimetic compounds that have potential applications in different fields, since the use of the same natural peptides is limited by their susceptibility to proteolysis and poor bioavailability. Recently, examples of modifications achieved through the introduction of small chemical molecule into the natural peptide are increasing (Qi et al. 2001; Bong and Ghadiri 2001; Ojida et al. 2005; Lawrence 2005). The introduction of a modification in a peptide chain, i.e. reversal of direction of the

amide bonds, increases the resistance to biodegradation, without decreasing the receptor binding ability and biological response of native peptides. Usually, a reverse amide bond $\psi[\text{NHCO}]$ allows to obtain peptidomimetics with different conformational structures than the native residue and that can be incorporated in stable β sheets. Retro peptide units can be obtained by the introduction of a *gem*-diamino moiety and/or a malonyl moiety into the peptide sequence (Goodman and Chorev 1979; Spatola 1983; Wiley and Rich 1993).

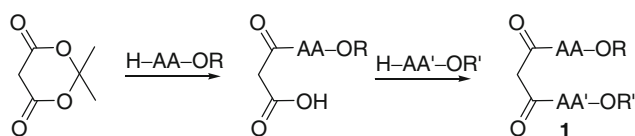
In this field, Meldrum's acid has been recently reported by us (Fioravanti et al. 2007) as a useful scaffold to obtain non-symmetric disubstituted malonamides *rAA-mGly-AA'* **1** as building blocks for the synthesis of retro peptides (Scheme 1).

Now our attention has turned towards the synthetic elaboration of **1** with the aim to introduce, into the malonamide backbone, the olefinic moiety, interesting potential site of further synthetic modifications, by a Knoevenagel condensation.

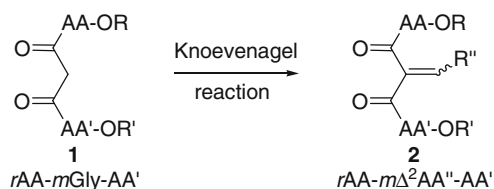
The Knoevenagel condensation is a very important reaction that directly provides the synthesis of different substituted alkenes (Reeves 1966; Jones 1967; Smith and March 2001; Li 2006). These last compounds are versatile starting materials to synthesize a great variety of small molecules and useful scaffolds in the construction of more complex compounds. During our studies, we have successfully tested several Knoevenagel conditions to synthesize different EWG-substituted alkenes by the condensation of aliphatic or aromatic aldehydes with suitable methylene active compounds. Recently, we reported a synthetic strategy involving an Al_2O_3 catalyzed Knoevenagel reaction (Texier-Boullet and Foucaud 1982) for the construction of a library of acrylonitriles carrying L- α -amino ester residues (Fioravanti et al. 2006), structural

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Scheme 1 Synthesis of malonyl peptides



Scheme 2 Synthesis of malonyl dehydro peptides

analogues of tyrphostins, protein tyrosine kinase (PTK) inhibitors (Levitzki and Gazit 1995).

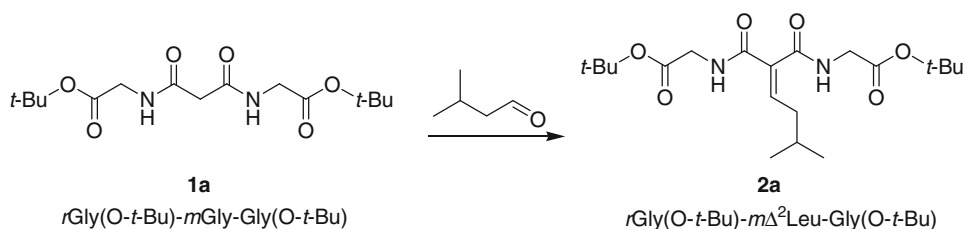
Starting from these last results, our attention was turned towards the synthetic elaboration of non-symmetric disubstituted malonamides *rAA-mGly-AA'* **1**, that can be regarded not only as building blocks for the synthesis of retro peptides but also as new potential methylene active compounds (Scheme 2).

To the best of our knowledge, only Ryabukhin and co-workers recently reported a TMSCl-promoted Knoevenagel reaction of symmetric malonamides with aromatic aldehydes. These reactions, carried out for 8–10 h at 100°C, worked for primary or secondary methylene active amides and failed with tertiary methylene active amides, due to the decreasing CH acidity and steric effects (Ryabukhin et al. 2007).

Results and discussion

With the aim to obtain the malonyl dehydro peptides (MDHPs) **2**, characterized by an interesting combination of retro and dehydro modifications in the same structure (Alemán et al. 1998; Alemán 2001), the symmetric *N,N*-Boc protected malonamide *rGly-mGly-Gly* **1a** was synthesized from Meldrum's acid by reported procedure and isovaleraldehyde was chosen as aldehyde to test the condensation reaction conditions (Scheme 3).

Scheme 3 Knoevenagel reaction on symmetric malonyl peptides



As reported in Table 1, the synthesis of **2a** failed using Al_2O_3 as catalyst. ^1H -NMR and ES Q-TOF analyses, performed on the crude mixture at regular times, showed only the presence of both reagents even after 8 h of stirring. A longer time of reaction (24 h) gave a complex mixture in which the target compound was not present. The classical Knoevenagel reaction conditions (Tietze and Beifuss 1993) at reflux of toluene in the presence of catalytic amount of piperidine (entry 2) were considered, but **2a** was obtained in low yields (25%) and the starting materials were recovered.

Finally, activated 4 Å molecular sieves were added at the reaction mixture and **2a** was obtained in nearly quantitative yield after 6 h.

The synthetic procedure was successfully applied to the construction of an 18 member library of different partially modified retro peptides **2b–s** by the combination of 10 *rAA-mGly-AA'* (**1**) and 8 different aldehydes (Scheme 4). The yields and the diastereomeric ratios are reported in Table 2.

As reported in Table 2, the reaction did not seem to suffer from steric hindrance or to depend on electronic effects, giving in all cases the expected *E* and *Z* malonyl dehydro peptides in good yields, using both aliphatic and aromatic aldehydes.

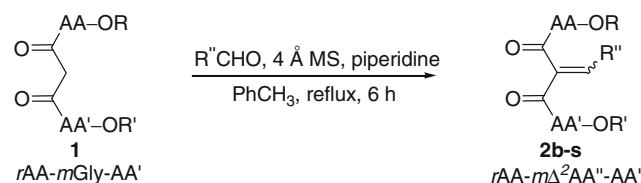
We would like to stress the high stereoselectivity obtained in the reaction performed on disubstituted malonamide *rPro(OMe)-mGly-Gly(OMe)* (entry 16), thus extending the applicability of the methodology here reported even starting from a malonamide carrying a tertiary amide moiety, with respect to the previously reported TMSCl-promoted Knoevenagel reaction (Ryabukhin et al. 2007).

The use of chiral aldehydes (entries 17 and 18) leads to the multifunctionalized malonyl dehydro peptides **2r** and **2s**, which can be regarded as the first examples of small partially modified retro peptide units where a glycosidic or an amino glycosidic moiety is linked to the malonamidic residue through a C=C double bond (Taylor 1998; Seitz et al. 2001; Davis 2002; Galonic et al. 2003). The selective ring opening reactions (Fioravanti et al. 2009) of the 1,3-dioxolane (entry 17) and of the 1,3-oxazolidine ring (entry 18), followed by the oxidation of the primary alcoholic functions, can provide new scaffolds carrying an α -hydroxy or an α -amino acid moiety.

All the diastereomeric MDHPs mixtures were separated by flash chromatography or by HPLC, and the (*E*)- and (*Z*)-**2c–p** were obtained as pure compounds.

Table 1 Optimization of Knoevenagel reaction conditions

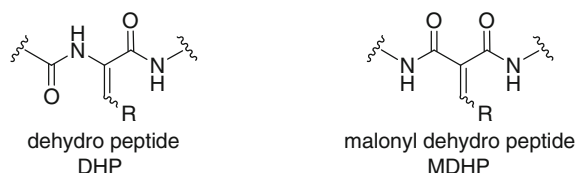
Entry	Catalyst	Solvent	T (°C)	Time (h)	Yield (%) ^a
1	Al ₂ O ₃	CH ₂ Cl ₂	rt	8	–
2	Piperidine	PhCH ₃	115	24	25
3	Piperidine 4 Å MS	PhCH ₃	115	6	95

^a After flash chromatography**Scheme 4** Standard Knoevenagel condensation

The *E/Z* isomerism of synthesized MDHP residues is analogous to that one present in the natural dehydro peptides (DHPs) containing one or more dehydro amino acids (DHAAs) in which the C α chirality is replaced by the double bond stereochemistry.

As known, natural dehydro peptides having antibiotic and phytotoxic activity, play an important role at the active site of some enzymes (Stammer 1982) and show interesting features, like the binding abilities towards metal ions (Świątek–Kozłowska et al. 2002), due to the isomerism of the double bond that determines the peptide conformations. The same isomerism (Fig. 1) present in MDHPs could exert a conformational influence when inserted into a peptidomimetic chain. The choice of the appropriate aldehyde in the synthesis of some reported MDHPs allows to introduce in the retro peptide backbone an unsaturated residue mimetic of natural amino acids. Thus, different (*E*)- and (*Z*)-MDHPs, namely malonyl dehydroleucine (**2b**, **c**, **i**, **j**, **l–q**), dehydroisoleucine (**2e**), dehydrotyrosine(OMe) (**2k**) and dehydrophenylalanine (**2f**, **h**) peptides were synthesized.

Noteworthy, the MDHP **2q** functionalized with the methyl L-prolinate was obtained with a very high diastereomeric purity (entry 16) and the *E* configuration of the major isomer was assigned by a NOESY experiment performed on the purified sample.

**Fig. 1** Natural and malonyl dehydro peptides

Finally, as a first attempt to test the reactivity of the synthesized malonyl peptides, **2a** was reacted with *N*-Boc L-cysteine methyl ester in the molar ratio **2a**/amino ester/NaH = 1:1.5:1.5 (Scheme 5).

After work-up, the analyses, performed on the crude mixture purified by a short silica gel column (hexane/ethyl acetate = 1:9), confirmed the synthesis of the expected diastereomeric Michael adduct **3** (dr = 1.3:1 by NMR spectra), suggesting thus the potential cysteine protease inhibitor activity of the MDHP double bonds like efficient electrophilic sites (Demarcus et al. 2001; Martina et al. 2005).

Conclusion

In conclusion, the Knoevenagel condensation reactions performed in the presence of 4 Å molecular sieves and a catalytic amount of piperidine led to the synthesis of short malonyl dehydro peptides starting from poorly reactive malonamides. The obtained MDHPs are characterized by an interesting combination of retro and dehydro modifications in the same structure and can be regarded as important synthetic targets not only for their potential biological applications, such as the corresponding *r*AA-*m*Gly-AA' units, like mimetic compounds (Dolle et al. 2008) of natural dehydro amino acid residues or like new optically active alkyl malonyl units by asymmetric hydrogenation (Kreuzfeld et al. 1998; Kreuzfeld et al. 1999; Dobler et al. 2002; Drexler et al. 2003; Au-Yeung et al. 2004; McFarland and Francis, 2005; Minnaard et al. 2007) for the construction of new peptidomimetic compounds (Davis 2003; Antos and Francis 2004, 2006; Joshi et al. 2004; Tilley and Francis 2006), but also for their use as scaffolds to synthesize more complex molecules by further functionalization of the double bond.

Experimental

IR spectra were recorded on a Perkin–Elmer 1600 FTIR spectrophotometer in CHCl₃ as the solvent. ¹H-NMR spectra were recorded at 200 and 300 MHz and ¹³C-NMR spectra at 50 and 75 MHz with Varian Gemini and Varian XL instruments. The NOESY experiment was performed at 400 MHz with a Bruker instrument. CDCl₃ was used as the solvent and CHCl₃ as the internal standard. HRMS and ES Q-TOF analyses were performed using a Micromass Q-TOF spectrometer equipped with an ESI source and a syringe pump. Anhydrous PhCH₃ was used as such. Type 4 Å molecular sieves (Merck, beads, diameter 1.7–2.4 mm) were activated by heating at 280°C, 2 h,

Table 2 Synthesis of MDHPs

Entry	AA-OR	AA'-OR	R''CHO	MDPH	Yield (%) ^a	Dr ^b
1	GlyOMe	Asp(OMe) ₂		2b	70	1.2/1 ^c
2	PheOMe	GlyOMe		2c	75	3/1
3	PheOMe	GlyOMe		2d	62	1.5/1
4	PheOMe	GlyOMe		2e	85	2/1
5	PheOMe	GlyOMe		2f	65	1.3/1
6	PheOMe	GlyOMe		2g	70	1.4/1
7	PheOMe	GlyO- <i>t</i> -Bu		2h	83	1.3/1
8	Phe-OMe	GlyO- <i>t</i> -Bu		2i	85	1.5/1
9	GlyOMe	β -AlaOMe		2j	60	2.5/1
10	GlyOMe	β -AlaOMe		2k	67	1.3/1
11	GlyOBz	β -AlaOMe		2l	83	2/1
12	AlaOMe	Asp(OMe) ₂		2m	60	1.5/1
13	AlaOMe	GlyOBz		2n	68	2/1
14	ValOMe	Asp(OMe) ₂		2o	85	1/1
15	PheOMe	MetOMe		2p	73	1/1
16	ProOMe	GlyOMe		2q	56	19/1
17	GlyO- <i>t</i> -Bu	GlyO- <i>t</i> -Bu		2r	78	–
18	GlyO- <i>t</i> -Bu	GlyO- <i>t</i> -Bu		2s	63	–

^a After flash chromatography^b Determined by ¹H-NMR and HPLC-UV spectra performed on the crude mixtures^c Not separated

under vacuum and Ar atmosphere. HPLC analyses were performed with an instrument Varian 9002 equipped with a Varian 9050 UV detector using an analytical column (3.9 × 300 mm, flow rate 1.3 ml/min). Eluents were

mixtures of HPLC grade hexane and ethyl acetate. NaH (55–65% suspension in mineral oil) was washed twice with pentane and dried under nitrogen. Compounds **1** were synthesized following reported procedures (Fioravanti et al. 2007).

di-*tert*-Butyl 2,2'-[(1,3-dioxopropane-1,3-diyl) diimino]diacetate (**1a**)

Pale yellow oil, 95%. IR: 3,419, 3,323, 1,736, 1,681 cm⁻¹. HRMS (ES Q-TOF) calcd for C₁₅H₂₆N₂NaO₆ (M + Na)⁺: 353.1689; found: 353.1701. ¹H-NMR δ 1.37 (s, 18 H), 3.25 (s, 2 H), 3.81–3.88 (m, 4 H), 7.72 (br, 2 H, NH). ¹³C-NMR δ 27.6 (six), 41.7 (two), 41.9, 81.5 (two), 167.3 (two), 168.3 (two).

Synthesis of malonyl dehydro peptides (**2a–s**). General procedure

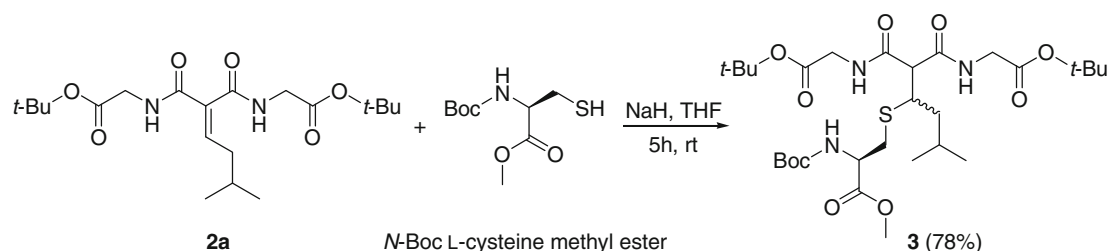
A solution of **1** (1 mmol) and aldehyde (3 mmol) in anhydrous toluene (10 ml) was added under Ar in a round-bottom flask containing previously activated 4 Å molecular sieves (5 g). A catalytic amount of piperidine (0.2 mmol) was added. The reactions were stirred at reflux and followed by ¹H-NMR analysis until disappearance of methylene proton signal of **1** (6 h). The crude mixtures were filtered under Ar through plugs of celite using diethyl ether as eluent. After solvent removal compounds **2** were obtained in the yields reported in Table 2. The pure diastereomers were obtained directly by flash chromatography of the crude or by HPLC-RI with a semipreparative column (7.8 × 300 mm, flow 3.5 ml/min), using as eluents different mixtures of hexane/ethyl acetate.

tert-Butyl 2-[2-(2-*tert*-butoxy-2-oxoethylcarbamoyl)-5-methylhex-2-enamido]acetate (**2a**)

Pale yellow oil, 95%. IR: 3,421, 3,332, 1,732, 1,678, 1,632 cm⁻¹. HRMS (ES Q-TOF) calcd for C₂₀H₃₄N₂NaO₆ (M + Na)⁺: 421.2315; found: 421.2319. ¹H-NMR δ 0.93 (d, *J* = 6.6 Hz, 6 H), 1.45 (s, 9 H), 1.47 (s, 9 H), 1.69–1.88 (m, 1 H), 2.33 (m, 2 H), 3.99 (dd, *J* = 5.3 Hz, *J* = 10.0 Hz, 4 H), 6.86 (br, 1 H, NH), 6.96 (t, *J* = 7.8 Hz, 1 H); 7.62 (br, 1 H, NH). ¹³C-NMR 22.3 (two), 27.9 (six), 33.8, 38.2, 42.0 (two), 82.1 (two), 129.1, 145.8, 167.2, 168.5, 169.2, 170.1.

Dimethyl (2*S*)-2-[2-(2-methoxy-2-oxoethylcarbamoyl)-5-methylhex-2-enamido]succinate (**2b**)

Orange oil, 70%. IR: 3,426, 3,332, 1,733, 1,685, 1,647 cm⁻¹. HRMS (ES Q-TOF) calcd for C₁₇H₂₆N₂NaO₈ (M + Na)⁺: 409.1587; found: 409.1583. ¹H-NMR δ 0.94 (major, d, *J* = 1.8 Hz, 6 H), 0.96 (minor, d, *J* = 1.8 Hz, 6



Scheme 5 Michael addition of a L-cysteine derivative

H), 1.73–1.87 (m, 2 H), 2.32 (major, t, $J = 7.6$ Hz, 2 H), 2.38 (minor, t, $J = 7.6$ Hz, 2 H), 2.83–3.14 (m, 4 H), 3.69 (major, s, 3 H), 3.70 (minor, s, 3 H), 3.75 (minor, s, 3 H), 3.76 (major, s, 3 H), 3.77 (major, s, 3 H), 3.78 (minor, s, 3 H), 4.08 (major, d, $J = 5.4$ Hz, 2 H), 4.14 (minor, d, $J = 5.4$ Hz, 2 H), 4.88–4.94 (major, m, 1 H), 4.95–5.00 (minor, m, 1 H), 6.94 (major, t, $J = 7.9$ Hz, 1 H), 7.05 (minor, t, $J = 7.7$ Hz, 1 H), 7.05–7.12 (m, 2 H, NH), 7.75–7.83 (m, 2 H, NH). $^{13}\text{C-NMR}$ δ 22.4, 28.4, 35.8 (minor), 36.0 (major), 38.2 (minor), 38.3 (major), 41.3 (major), 41.6 (minor), 48.7 (major), 49.0 (minor), 52.0 (major), 52.1 (minor), 52.2 (minor), 52.4 (major), 52.8 (minor), 52.9 (major), 131.2 (minor), 131.3 (major), 146.5 (major), 147.0 (minor), 164.3 (minor), 165.0 (major), 166.2 (minor), 166.5 (major), 169.9 (major), 170.0 (minor), 170.6 (major), 170.9 (minor), 171.0 (minor), 171.2 (major).

Methyl (2*S*)-2-[2-(2-methoxy-2-oxoethylcarbamoyl)-5-methylhex-2-enamido]-3-phenylpropanoate (**2c**)

Deep orange oil, 75%. IR: 3,426, 3,335, 1,742, 1,678, 1,631 cm^{-1} . HRMS (ES Q-TOF) calcd for $\text{C}_{21}\text{H}_{28}\text{N}_2\text{NaO}_6$ ($\text{M} + \text{Na}$) $^+$: 427.1845; found: 427.1856. Flash chromatography on silica gel (eluent: hexane/ethyl acetate 65:35). Major: $^1\text{H-NMR}$ δ 0.83 (d, $J = 6.6$ Hz, 6 H), 1.64–1.81 (m, 1 H), 2.27 (t, $J = 7.4$ Hz, 2 H), 3.08–3.34 (m, 2 H), 3.75 (s, 3 H), 3.80 (s, 3 H), 4.04–4.12 (m, 2 H), 4.83–5.02 (m, 1 H), 6.58 (br, 1 H, NH), 6.95 (t, $J = 7.8$ Hz, 1 H), 7.10–7.14 (m, 5 H), 7.62 (br, 1 H, NH). $^{13}\text{C-NMR}$ δ 22.3 (two), 28.2, 37.2, 38.1, 41.3, 52.2 (two), 53.5, 127.0, 128.4 (two), 129.1 (two), 131.1, 135.7, 146.0, 164.6, 166.5, 169.8, 171.6. Minor: $^1\text{H-NMR}$ δ 0.87 (d, $J = 6.5$ Hz, 6 H), 1.56–1.78 (m, 1 H), 2.28 (t, $J = 6.9$ Hz, 2 H), 2.96–3.22 (m, 2 H), 3.68 (s, 3 H), 3.72 (s, 3 H), 4.02–4.13 (m, 2 H), 4.73–4.88 (m, 1 H), 6.78 (t, $J = 7.7$ Hz, 1 H), 6.88 (br, 1 H, NH), 7.04–7.25 (m, 5 H), 7.33 (br, 1 H, NH). $^{13}\text{C-NMR}$ δ 22.3 (two), 28.2, 37.4, 38.2, 43.1, 52.8 (two), 54.1, 127.0, 128.4 (two), 129.2 (two), 131.0, 135.8, 146.0, 164.8, 165.9, 169.8, 171.6.

Methyl (2*S*)-2-[2-(2-methoxy-2-oxoethylcarbamoyl)hept-2-enamido]-3-phenylpropanoate (**2d**)

Deep orange oil, 62%. IR: 3,415, 3,342, 1,736, 1,672, 1,631 cm^{-1} . HRMS (ES Q-TOF) calcd for $\text{C}_{21}\text{H}_{28}\text{N}_2\text{NaO}_6$ ($\text{M} + \text{Na}$) $^+$: 427.1845; found: 427.1840. Flash chromatography on silica gel (eluent: hexane/ethyl acetate 35:65). Major: $^1\text{H-NMR}$ δ 0.84 (t, $J = 6.6$ Hz, 3 H), 1.05–1.36 (m, 4 H), 1.85–2.08 (m, 2 H), 2.85–3.18 (m, 2 H), 3.62 (s, 3 H), 3.65 (s, 3 H), 4.05–4.32 (m, 2 H), 4.63–4.89 (m, 1 H), 6.58 (t, $J = 7.8$ Hz, 1 H), 6.80 (br, 1 NH), 7.20–7.25 (m, 5 H), 7.48 (br, 1 NH). $^{13}\text{C-NMR}$ δ 22.3, 34.1, 35.4 (two), 37.6, 42.1, 52.1 (two), 53.5, 126.8, 128.3 (two), 129.0 (two), 131.3, 135.8, 145.6, 167.0, 167.3, 169.8, 171.5. Minor: $^1\text{H-NMR}$ δ 0.81 (t, $J = 6.6$ Hz, 3 H), 1.06–1.30 (m, 4 H), 1.88–2.07 (m, 2 H), 2.85–3.09 (m, 2 H), 3.60 (s, 3 H), 3.69 (s, 3 H), 4.12–4.29 (m, 2 H), 4.70–4.85 (m, 1 H), 6.77 (t, $J = 7.8$ Hz, 1 H), 7.01–7.19 (m, 5 H), 7.40 (br, 1 NH), 7.56 (br, 1 NH). $^{13}\text{C-NMR}$ δ 22.4, 34.2, 36.4 (two), 38.0, 41.9, 51.9 (two), 53.5, 126.3, 128.3 (two), 128.8 (two), 131.8, 135.7, 146.7, 166.9, 167.8, 170.1, 171.9.

Methyl (2*S*)-2-[2-(2-methoxy-2-oxoethylcarbamoyl)-4-methylhex-2-enamido]-3-phenylpropanoate (**2e**)

Deep orange oil, 85%. IR: 3,412, 3,342, 1,742, 1,672, 1,637 cm^{-1} . HRMS (ES Q-TOF) calcd for $\text{C}_{21}\text{H}_{28}\text{N}_2\text{NaO}_6$ ($\text{M} + \text{Na}$) $^+$: 427.1845; found: 427.1853. Flash chromatography on silica gel (eluent: hexane/ethyl acetate 65:35). Major: $^1\text{H-NMR}$ δ 0.91 (t, $J = 6.5$ Hz, 3 H), 1.03 (d, $J = 6.5$ Hz, 3 H), 1.22–1.49 (m, 2 H), 2.67–2.90 (m, 1 H), 3.02–3.38 (m, 2 H), 3.76 (s, 3 H), 3.79 (s, 3 H), 4.04–4.21 (m, 2 H), 4.78–4.99 (m, 1 H), 6.68 (d, $J = 8.4$ Hz, 1 H), 6.88 (br, 1 H, NH), 7.10–7.46 (m, 5 H), 7.58 (br, 1 H, NH). $^{13}\text{C-NMR}$ δ 21.9, 22.2, 28.3, 32.7, 38.0, 41.2, 52.2 (two), 53.5, 126.9, 128.3 (two), 129.2 (two), 131.3, 135.6, 145.9, 164.6, 166.5, 169.8, 171.9. Minor: $^1\text{H-NMR}$ δ 0.72 (t, $J = 6.5$ Hz, 3 H), 1.05 (d, $J = 6.5$ Hz, 3 H), 1.21–1.48 (m, 2 H), 2.35–2.51 (m, 1 H), 3.02–3.38 (m, 2 H), 3.71 (s, 3 H),

3.78 (s, 3 H), 4.04–4.23 (m, 2 H), 4.80–5.00 (m, 1 H), 6.59 (d, $J = 8.4$ Hz, 1 H), 7.10–7.44 (m, 5 H), 7.57 (br, 1 H, NH), 7.79 (br, 1 H, NH). ^{13}C -NMR δ 21.9, 22.2, 28.3, 32.4, 38.0, 41.2, 52.4 (two), 53.5, 126.9, 128.3 (two), 129.2 (two), 131.1, 135.6, 146.2, 164.6, 166.5, 170.0, 171.6.

Methyl (2*S*)-2-[2-(2-methoxy-2-oxoethylcarbamoyl)-3-phenylprop-2-enamido]-3-phenylpropanoate (**2f**)

Deep orange oil, 65%. IR: 3,413, 3,331, 1,743, 1,672, 1,645 cm^{-1} HRMS (ES Q-TOF) calcd for $\text{C}_{23}\text{H}_{24}\text{N}_2\text{NaO}_6$ ($\text{M} + \text{Na}$) $^+$: 447.1532; found: 447.1528. Flash chromatography on silica gel (eluent: hexane/ethyl acetate 60:40). Major: ^1H -NMR δ 2.95–3.21 (m, 2 H), 3.60 (s, 6 H), 3.87–3.96 (m, 2 H), 4.71–4.88 (m, 1 H), 6.29 (br, 2 H, NH), 7.09–7.41 (m, 10 H), 7.71 (s, 1 H). ^{13}C -NMR δ 37.2, 41.6, 52.3 (two), 53.5, 127.0 (four), 128.6 (two), 128.9 (two), 129.4 (two), 129.8 (two), 133.1, 141.0, 163.6, 167.5, 169.7, 171.5. Minor: ^1H -NMR δ 3.05–3.15 (m, 2 H), 3.65 (s, 6 H), 3.94 (d, $J = 5.5$ Hz, 2 H), 4.73–4.85 (m, 1 H), 6.48 (br, 2 H, NH), 7.09–7.43 (m, 10 H), 7.75 (s, 1 H). ^{13}C -NMR δ 37.8, 41.3, 52.1 (two), 53.3, 126.9 (four), 128.4 (two), 129.2 (two), 129.5 (two), 129.8 (two), 135.4, 140.9, 163.2, 167.7, 169.3, 171.1.

Methyl (2*S*)-2-[3-(furan-2-yl)-2-[(2-methoxy-2-oxoethyl)carbamoyl]acrylamido]-3-phenylpropanoate (**2g**)

Deep orange oil, 70%. IR: 3,419, 3,323, 1,736, 1,681, 1,645 cm^{-1} HRMS (ES Q-TOF) calcd for $\text{C}_{21}\text{H}_{22}\text{N}_2\text{NaO}_7$ ($\text{M} + \text{Na}$) $^+$: 437.1325; found: 437.1329. Flash chromatography on silica gel (eluent: hexane/ethyl acetate 60:40). Major: ^1H -NMR δ 2.92–3.47 (m, 2 H), 3.68 (s, 6 H), 4.17 (d, $J = 5.5$ Hz, 2 H), 4.69–4.82 (m, 1 H), 6.42–6.50 (m, 1 H), 6.81–6.89 (m, 2 H), 7.12–7.36 (m, 5 H), 7.50 (s, 1 H), 7.65 (br, 2 H, NH). ^{13}C -NMR δ 37.6, 41.2, 51.9 (two), 54.0, 112.3, 117.5, 124.7, 126.1 (two), 128.2 (two), 129.1, 136.0, 145.3, 149.1, 152.3, 163.5, 167.2, 169.7, 171.3. Minor: ^1H -NMR δ 2.94–3.28 (m, 2 H), 3.68 (s, 3 H), 3.71 (s, 3 H), 3.93 (d, $J = 5.5$ Hz, 2 H), 4.90–5.04 (m, 1 H), 6.27–6.32 (m, 1 H), 6.53–6.61 (m, 2 H), 6.99–7.28 (m, 5 H), 7.39 (s, 1 H), 7.53 (br, 2 H, NH). ^{13}C -NMR δ 37.1, 41.5, 53.3 (two), 53.9, 112.1, 117.3, 124.5, 126.0 (two), 128.4 (two), 129.0, 136.2, 145.2, 148.8, 153.1, 164.1, 167.1, 169.5, 171.7.

Methyl (2*S*)-2-[2-(2-*tert*-butoxy-2-oxoethylcarbamoyl)-3-phenylacrylamido]-3-phenylpropanoate (**2h**)

Deep orange oil, 83%. IR: 3,412, 3,342, 1,736, 1,672, 1,642 cm^{-1} HRMS (ES Q-TOF) calcd for $\text{C}_{26}\text{H}_{30}\text{N}_2\text{NaO}_6$ ($\text{M} + \text{Na}$) $^+$: 489.2002; found: 489.1996. HPLC-RI with a

semi-preparative column (eluent hexane/ethyl acetate 70:30). Major: ^1H -NMR δ 1.48 (s, 9 H), 2.96–3.00 (m, 2 H), 3.64 (s, 3 H), 3.98–4.02 (m, 2 H), 4.85–4.94 (m, 1 H), 7.15–7.43 (m, 10 H), 7.70 (br, 2 H, NH), 7.88 (s, 1 H, CH). ^{13}C -NMR δ 27.8 (three), 37.7, 42.4, 52.1, 53.4, 82.2, 127.1 (two), 127.2 (two), 128.4 (four), 128.7, 129.0, 129.3, 133.1, 136.1, 140.3, 167.4, 168.2, 171.2, 171.5. Minor: ^1H -NMR δ 1.43 (s, 9 H), 3.13–3.22 (m, 2 H), 3.74 (s, 3 H), 3.87–3.94 (m, 2 H), 4.83–4.94 (m, 1 H), 7.16–7.45 (m, 10 H), 7.83 (s, 1 H), 7.95 (br, 2 H, NH). ^{13}C -NMR δ 27.7 (three), 37.6, 42.4, 52.0, 53.5, 82.2, 127.2 (two), 127.5 (two), 128.3 (four), 128.8, 129.0, 129.4, 134.2, 136.3, 139.1, 167.6, 168.4, 171.0, 171.9.

Methyl (2*S*)-2-[2-(2-*tert*-butoxy-2-oxoethylcarbamoyl)-5-methylhex-2-enamido]-3-phenylpropanoate (**2i**)

Deep orange oil, 85%. IR: 3,422, 3,333, 1,739, 1,678, 1,641 cm^{-1} . HRMS (ES Q-TOF) calcd for $\text{C}_{24}\text{H}_{34}\text{N}_2\text{NaO}_6$ ($\text{M} + \text{Na}$) $^+$: 469.2315; found: 469.2309. HPLC-RI with a semi-preparative column (eluent hexane/ethyl acetate 70:30). Major: ^1H -NMR δ 0.83 (d, $J = 6.2$ Hz, 6 H), 1.47 (s, 9 H), 1.64–1.90 (m, 1 H), 2.00–2.19 (m, 2 H), 2.99–3.26 (m, 2 H), 3.71 (s, 3 H), 3.89–4.07 (m, 2 H), 4.80–5.00 (m, 1 H), 6.79 (br, 1 H, NH), 6.90 (t, $J = 7.7$ Hz, 1 H), 7.08–7.32 (m, 5 H), 7.52 (br, 1 H, NH). ^{13}C -NMR δ 22.4 (two), 27.7 (three), 28.1, 37.2, 38.2, 41.3, 52.2, 53.4, 82.1, 127.1, 128.4 (two), 129.1 (two), 131.1, 135.7, 146.0, 164.6, 166.6, 169.9, 171.2. Minor: ^1H -NMR δ 0.90 (d, $J = 6.2$ Hz, 6 H), 1.48 (s, 9 H), 1.69–1.89 (m, 1 H), 1.96–2.11 (m, 2 H), 2.98–3.22 (m, 2 H), 3.63 (s, 3 H), 3.68–3.95 (m, 2 H), 4.69–4.92 (m, 1 H), 6.77 (br, 1 H, 1 NH), 6.84 (t, $J = 7.7$ Hz, 1 H), 6.99–7.27 (m, 5 H), 7.50 (br, 1 H, NH). ^{13}C -NMR δ 22.4 (two), 27.6 (three), 28.1, 37.2, 38.2, 41.3, 52.2, 53.4, 82.1, 127.3, 128.3 (two), 128.9 (two), 131.3, 135.7, 145.8, 164.6, 166.6, 170.0, 171.3.

Methyl 3-[2-(2-methoxy-2-oxoethylcarbamoyl)-5-methylhex-2-enamido]propanoate (**2j**)

Yellow oil, 67%. IR: 3,426, 3,358, 1,742, 1,678, 1,636 cm^{-1} . HRMS (ES Q-TOF) calcd for $\text{C}_{15}\text{H}_{24}\text{N}_2\text{NaO}_6$ ($\text{M} + \text{Na}$) $^+$: 351.1532; found: 351.1538. Flash chromatography on silica gel (eluent: hexane/ethyl acetate 40:60). Major: ^1H -NMR δ 0.93 (d, $J = 6.2$ Hz, 6 H), 1.51–1.77 (m, 1 H), 2.02–2.31 (m, 2 H), 2.47–2.65 (m, 2 H), 3.39–3.58 (m, 2 H), 3.66 (s, 3 H), 3.72 (s, 3 H), 3.98–4.10 (m, 2 H), 6.82 (t, $J = 7.7$ Hz, 1 H), 6.90 (br, 1 H, NH), 7.83 (br, 1 H, NH). ^{13}C -NMR δ 22.3 (two), 28.3, 33.6, 35.1, 38.2, 41.3, 51.7 (two), 132.3, 143.8, 165.0, 166.6, 169.3, 172.5. Minor: ^1H -NMR δ 0.93 (d, $J = 6.2$ Hz, 6 H), 1.49–1.53 (m, 1 H), 2.01–2.33 (m, 2 H), 2.47–2.72 (m, 2 H), 3.43–3.61 (m, 2 H), 3.70 (s, 3 H), 3.78 (s, 3 H), 3.98–4.15 (m, 2

H), 6.80 (t, $J = 7.7$ Hz, 1 H), 7.66 (br, 1 H, NH), 7.80 (br, 1 H, NH). ^{13}C -NMR δ 21.3 (two), 28.5, 33.4, 35.1, 38.2, 41.5, 51.8 (two), 131.8, 143.8, 165.0, 166.7, 169.3, 172.4.

Methyl 3-[2-(2-methoxy-2-oxoethylcarbamoyl)-3-(4-methoxyphenyl)acrylamido]propanoate (**2k**)

Yellow oil, 67%. IR: 3,430, 3,351, 1,735, 1,673, 1,639 cm^{-1} . HRMS (ES Q-TOF) calcd for $\text{C}_{18}\text{H}_{22}\text{N}_2\text{NaO}_7$ ($\text{M} + \text{Na}$) $^{+}$: 401.1325; found: 401.1329. Flash chromatography on silica gel using (hexane/ethyl acetate 50:50). Major: ^1H -NMR δ 2.48–2.52 (m, 2 H), 3.46–3.59 (m, 2 H), 3.67 (s, 3 H), 3.75 (s, 3 H), 3.80 (s, 3 H), 3.97–4.20 (m, 2 H), 7.19 (br, 1 H, NH), 7.25–7.42 (m, 4 H), 7.75 (s, 1 H), 7.92 (br, 1 H, NH). ^{13}C -NMR δ 32.9, 35.0, 41.3, 51.5, 52.2, 55.2, 114.1 (two), 126.6, 127.4 (two), 128.2, 131.1, 140.4, 168.7, 169.8, 172.2 (two). Minor: ^1H -NMR δ 2.48–2.52 (m, 2 H), 3.46–3.59 (m, 2 H), 3.67 (s, 3 H), 3.75 (s, 3 H), 3.80 (s, 3 H), 3.97–4.20 (m, 2 H), 7.25–7.42 (m, 4 H), 7.59 (s, 1 H), 7.92 (br, 1 H, NH), 8.11 (br, 1 H, NH). ^{13}C -NMR δ 33.5, 35.7, 41.5, 51.6, 52.3, 55.2, 114.1 (two), 125.7, 127.4 (two), 128.2, 131.5, 140.0, 168.1, 169.6, 172.0 (two).

Methyl 3-{2-[2-(benzyloxy)-2-oxoethylcarbamoyl]-5-methylhex-2-enamido}propanoate (**2l**)

Yellow oil, 83%. IR: 3,436, 3,342, 1,741, 1,678, 1,637 cm^{-1} . HRMS (ES Q-TOF) calcd for $\text{C}_{21}\text{H}_{28}\text{N}_2\text{NaO}_6$ ($\text{M} + \text{Na}$) $^{+}$: 427.1845; found: 427.1849. Flash chromatography on silica gel (eluent: hexane/ethyl acetate 65:35). Major: ^1H -NMR δ 0.90 (d, $J = 6.6$ Hz, 6 H), 1.19–1.28 (m, 2 H), 1.35–1.46 (m, 1 H), 2.29 (t, $J = 7.2$ Hz, 2 H), 3.27–3.48 (m, 2 H), 3.70 (s, 3 H), 4.02–4.17 (m, 2 H), 5.15 (s, 2 H), 6.93 (t, $J = 7.6$ Hz, 1 H), 7.32–7.34 (m, 5 H), 7.67–7.78 (m, 2 H, NH). ^{13}C -NMR δ 22.3 (two), 28.3, 33.6, 35.3, 38.2, 41.4, 51.7, 67.0, 128.2, 128.5 (two), 128.8 (two), 131.8, 135.9, 146.5, 165.0, 166.6, 169.3, 172.4. Minor: ^1H -NMR δ 0.90 (d, $J = 6.6$ Hz, 6 H), 1.20–1.29 (m, 2 H), 1.35–1.46 (m, 1 H), 2.29 (t, $J = 7.2$ Hz, 2 H), 3.27–3.48 (m, 2 H), 3.73 (s, 3 H), 4.02–4.21 (m, 2 H), 5.17 (s, 2 H), 6.87 (t, $J = 7.6$ Hz, 1 H), 7.09–7.17 (m, 1 H, NH), 7.32–7.34 (m, 5 H), 7.73–7.78 (m, 1 H, NH). ^{13}C -NMR δ 22.1 (two), 28.3, 33.5, 35.0, 38.1, 41.6, 51.8, 67.2, 128.2, 128.5 (two), 128.8 (two), 131.2, 135.9, 146.5, 164.9, 166.9, 169.3, 172.6.

Dimethyl (2*S*)-2-{2-[(1*S*)-1-methoxy-1-oxopropan-2-ylcarbamoyl]-5-methylhex-2-enamido}succinate (**2m**)

Yellow oil, 60%. IR: 3,426, 3,332, 1,746, 1,674, 1,637 cm^{-1} . HRMS (ES Q-TOF) calcd for $\text{C}_{18}\text{H}_{28}\text{N}_2\text{NaO}_8$ ($\text{M} + \text{Na}$) $^{+}$: 423.1743; found: 423.1751. Flash chromatography on silica gel (eluent: hexane/ethyl acetate 55:45).

Major: ^1H -NMR δ 0.92 (d, $J = 6.6$ Hz, 6 H), 1.41 (d, $J = 7.2$ Hz, 3 H), 1.72–1.81 (m, 1 H), 2.25–2.33 (m, 2 H), 2.80–2.91 (m, 2 H), 3.66 (s, 3 H), 3.71 (s, 3 H), 3.75 (s, 3 H), 4.51–4.65 (m, 1 H), 5.58–5.75 (m, 1 H), 6.98 (t, $J = 7.9$ Hz, 1 H), 7.18 (d, $J = 7.8$ Hz, 1 H, NH), 7.86 (d, $J = 7.9$ Hz, 1 H, NH). ^{13}C -NMR δ 17.7, 22.2 (two), 28.3, 35.6, 38.1, 48.2, 48.4, 51.9, 52.3, 52.7, 131.4, 146.2, 164.6, 165.6, 170.9, 171.0, 173.0. Minor: ^1H -NMR δ 0.92 (d, $J = 6.6$ Hz, 6 H), 1.44 (d, $J = 7.2$ Hz, 3 H), 1.72–1.81 (m, 1 H), 2.25–2.33 (m, 2 H), 2.95–3.10 (m, 2 H), 3.67 (s, 3 H), 3.73 (s, 3 H), 3.74 (s, 3 H), 4.85–4.97 (m, 1 H), 5.56–5.64 (m, 1 H), 6.90 (t, $J = 7.8$ Hz, 1 H), 7.02 (d, $J = 7.8$ Hz, 1 H, NH), 7.85 (d, $J = 7.0$ Hz, 1 H, NH). ^{13}C -NMR δ 17.5, 22.3 (two), 28.3, 35.2, 38.4, 48.4, 48.9, 52.0, 52.4, 52.8, 131.2, 146.4, 164.0, 164.2, 170.6, 170.9, 172.9.

Methyl (2*S*)-2-{2-[2-(benzyloxy)-2-oxoethylcarbamoyl]-5-methylhex-2-enamido}propanoate (**2n**)

Orange oil, 68%. IR: 3,435, 3,339, 1,741, 1,678, 1,637 cm^{-1} . HRMS (ES Q-TOF) calcd for $\text{C}_{21}\text{H}_{28}\text{N}_2\text{NaO}_6$ ($\text{M} + \text{Na}$) $^{+}$: 427.1845; found: 427.1839. Flash chromatography on silica gel (eluent: hexane/ethyl acetate 60:40). Major: ^1H -NMR δ 0.90 (d, $J = 6.6$ Hz, 6 H), 1.49 (d, $J = 4.6$ Hz, 3 H), 1.88–1.97 (m, 1 H), 2.30 (t, $J = 7.2$ Hz, 2 H), 3.70 (s, 3 H), 4.02–4.17 (m, 2 H), 4.46–4.65 (m, 1 H), 5.15 (s, 2 H), 7.06 (t, $J = 7.6$ Hz, 1 H), 7.32–7.34 (m, 5 H), 7.67–7.78 (m, 2 H, NH). ^{13}C -NMR δ 17.7, 22.9 (two), 24.8, 33.7, 41.3, 42.3, 48.2, 52.3, 52.7, 67.1, 128.2, 128.4 (two), 128.5 (two), 135.1, 166.8, 167.6, 169.4, 172.9. Minor: ^1H -NMR δ 0.90 (d, $J = 6.6$ Hz, 6 H), 1.49 (d, $J = 4.6$ Hz, 3 H), 1.88–1.97 (m, 1 H), 2.30 (t, $J = 7.2$ Hz, 2 H), 3.73 (s, 3 H), 4.02–4.17 (m, 2 H), 4.46–4.65 (m, 1 H), 5.17 (s, 2 H), 6.90 (t, $J = 7.6$ Hz, 1 H), 7.12 (d, $J = 7.5$ Hz, 1 H, NH), 7.32–7.34 (m, 5 H), 7.73–7.78 (m, 1 H, NH). ^{13}C -NMR δ 18.3, 22.9 (two), 25.5, 33.7, 41.3, 42.3, 47.9, 52.3, 52.5, 68.0, 128.2, 128.4 (two), 128.5 (two), 135.0, 166.8, 167.8, 170.0, 173.6.

Dimethyl (*R*)-2-[[2-(1*S*)-1-methoxy-3-methyl-1-oxobutan-2-ylcarbamoyl]-5-methylhex-2-enamido}succinate (**2o**)

Deep orange oil, 85%. IR: 3,448, 3,354, 1,736, 1,672, 1,637 cm^{-1} . HRMS (ES Q-TOF) calcd for $\text{C}_{20}\text{H}_{32}\text{N}_2\text{NaO}_8$ ($\text{M} + \text{Na}$) $^{+}$: 451.2056; found: 451.2051. Flash chromatography on silica gel (eluent: hexane/ethyl acetate 65:35). Major: ^1H -NMR δ 0.86–0.98 (m, 12 H), 1.72–1.86 (m, 1 H), 2.30 (t, $J = 7.2$ Hz, 2 H), 2.78–3.14 (m, 3 H), 3.68 (s, 3 H), 3.72 (s, 3 H), 3.74 (s, 3 H), 4.47–4.55 (m, 1 H), 4.76–4.98 (m, 1 H), 7.01 (t, $J = 8.0$ Hz, 1 H), 7.15 (d, $J = 8.0$ Hz, 1 H, NH), 7.85 (d, $J = 8.0$ Hz, 1 H, NH). ^{13}C -NMR δ 18.3

(two), 25.6 (two), 29.7, 31.4, 37.3, 38.9, 49.5, 53.2, 53.6 (two), 56.2, 133.2, 145.2, 168.4 (two), 174.3 (two), 179.1. Minor: $^1\text{H-NMR}$ δ 0.86–0.98 (m, 12 H), 1.72–1.86 (m, 1 H), 2.30 (t, $J = 7.2$ Hz, 2 H), 2.78–3.14 (m, 3 H), 3.66 (s, 3 H), 3.72 (s, 3 H), 3.77 (s, 3 H), 4.58–4.66 (m, 1 H), 5.64–5.74 (m, 1 H), 6.85–6.92 (m, 1 H, NH), 6.93 (t, $J = 7.9$ Hz, 1 H), 7.72 (d, $J = 8.0$ Hz, 1 H, NH). $^{13}\text{C-NMR}$ δ 18.5 (two), 25.9 (two), 29.7, 31.4, 37.3, 39.1, 49.5, 53.3, 53.9 (two), 56.2, 133.4, 145.5, 168.4 (two), 175.2 (two), 178.9.

Methyl (2*S*)-2-{2-[(1*S*)-1-methoxy-1-oxo-3-phenylpropan-2-ylcarbamoyl]-5-methylhex-2-enamido}-4-(methylthio)butanoate (**2p**)

Deep orange oil, 73%. IR: 3,418, 3,330, 1,736, 1,678, 1,632 cm^{-1} . HRMS (ES Q-TOF) calcd for $\text{C}_{24}\text{H}_{34}\text{N}_2\text{NaO}_6\text{S}$ ($\text{M} + \text{Na}$) $^+$: 501.2035; found: 501.2041. Flash chromatography on silica gel (eluent: hexane/ethyl acetate 35:65). Major: $^1\text{H-NMR}$ δ 0.88 (d, $J = 6.2$ Hz, 6 H), 1.57–1.75 (m, 1 H), 1.92–2.41 (m, 4 H), 2.05 (s, 3 H), 2.45–2.51 (m, 2 H), 3.00–3.28 (m, 2 H), 3.67 (s, 3 H), 3.70 (s, 3 H), 4.59–4.91 (m, 1 H), 5.49–5.69 (m, 1 H), 6.86 (t, $J = 7.8$ Hz, 1 H), 7.05–7.27 (m, 5 H), 7.55 (d, $J = 7.5$ Hz, 1 H, NH), 7.77 (d, $J = 7.8$ Hz, 1 H, NH). $^{13}\text{C-NMR}$ δ 15.2, 22.1, 22.2, 28.0, 29.9 (two), 37.0, 37.5, 51.6, 52.1, 52.2, 53.5, 127.1, 128.3 (two), 128.5 (two), 131.2, 135.6, 146.2, 164.3, 166.1, 171.6, 171.9. Minor: $^1\text{H-NMR}$ δ 0.90 (d, $J = 6.2$ Hz, 6 H), 1.57–1.75 (m, 1 H), 1.92–2.29 (m, 4 H), 2.05 (s, 3 H), 2.45–2.51 (m, 2 H), 3.00–3.28 (m, 2 H), 3.71 (s, 3 H), 3.73 (s, 3 H), 4.59–4.91 (m, 1 H), 5.44–5.63 (m, 1 H), 6.77 (t, $J = 7.7$ Hz, 1 H), 6.92 (d, $J = 7.6$ Hz, 1 H, NH), 7.05–7.27 (m, 5 H), 7.40 (d, $J = 7.8$ Hz, 1 H, NH). $^{13}\text{C-NMR}$ δ 15.2, 22.1, 22.2, 28.1, 31.2 (two), 37.8, 38.8, 51.8, 52.2, 52.3, 53.8, 126.9, 128.8 (two), 129.0 (two), 131.2, 135.9, 146.2, 164.5, 166.5, 171.7, 172.0.

Methyl (*E*)-(2*S*)-1-[2-(2-methoxy-2-oxoethylcarbamoyl)-5-methylhex-2-enoyl]pyrrolidine-2-carboxylate (**2q**)

Yellow oil, 56%. IR: 3,422, 3,334, 1,735, 1,659, 1,640 cm^{-1} . HRMS (ES Q-TOF) calcd for $\text{C}_{17}\text{H}_{26}\text{N}_2\text{NaO}_6$ ($\text{M} + \text{Na}$) $^+$: 377.1689; found: 377.1695. $^1\text{H-NMR}$ δ 0.92 (dd, $J = 3.3, 6.6$ Hz, 6 H), 1.69–1.85 (m, 1 H), 1.87–2.15 (m, 6 H), 2.22–2.41 (m, 1 H), 3.30–3.45 (m, 1 H), 3.70 (s, 3 H), 3.76 (s, 3 H), 3.85 (dd, $J = 5.0, 17.8$ Hz, 1 H), 4.30 (dd, $J = 6.6, 17.8$ Hz, 1 H), 4.61–4.66 (m, 1 H), 6.97 (t, $J = 7.9$ Hz, 1 H), 7.48 (br, 1 H, NH). $^{13}\text{C-NMR}$ δ 22.4 (two), 24.7, 28.1, 29.3, 38.4, 41.3, 48.0, 52.1, 52.6, 58.3, 132.3, 143.0, 163.1, 166.7, 170.1, 173.1.

tert-Butyl (*R*)-[2-(2-*tert*-butoxy-2-oxoethylcarbamoyl)-3-(2,2-dimethyl-1,3-dioxolan-4-yl)acrylamido]acetate (**2r**)

Pale yellow oil, 78%. IR: 3,428, 3,334, 1,735, 1,682, 1,648 cm^{-1} . HRMS (ES Q-TOF) calcd for $\text{C}_{21}\text{H}_{34}\text{N}_2\text{NaO}_8$ ($\text{M} + \text{Na}$) $^+$: 465.2213; found: 465.2207. $^1\text{H-NMR}$ δ 1.28–1.52 (m, 24 H), 3.69–4.42 (m, 6 H), 5.14–5.29 (m, 1 H), 6.87 (d, $J = 7.3$ Hz, 1 H), 7.34 (br, 1 H, NH), 7.93 (br, 1 H, NH). $^{13}\text{C-NMR}$ δ 24.9 (two), 28.0 (six), 42.2, 42.3, 69.6, 82.3 (two), 82.5, 110.1, 132.2, 145.3, 164.6 (two), 168.3 (two).

tert-Butyl (*R*)-5-[3-(2-*tert*-butoxy-2-oxoethylamino)-2-(2-*tert*-butoxy-2-oxoethylcarbamoyl)-3-oxoprop-1-enyl]-2,2-dimethyloxazolidine-3-carboxylate (**2s**)

Yellow oil, 63%. IR: 3,430, 3,338, 1,737, 1,645, 1,643 cm^{-1} . HRMS (ES Q-TOF) calcd for $\text{C}_{26}\text{H}_{43}\text{N}_3\text{NaO}_9$ ($\text{M} + \text{Na}$) $^+$: 564.2897; found: 564.2882. $^1\text{H-NMR}$ δ 1.18–1.59 (m, 33 H), 3.68–3.85 (m, 2 H), 3.91–4.08 (m, 1 H), 4.10 (dd, $J = 6.0, 9.4$ Hz, 2 H), 4.22 (dd, $J = 6.9, 17.3$ Hz, 2 H), 6.92 (d, $J = 11.3$ Hz, 1 H), 8.41 (br, 1 H, NH), 9.72 (br, 1 H, NH). $^{13}\text{C-NMR}$ δ 24.5, 27.3, 27.9 (three), 28.0 (three), 28.3 (three), 42.2, 42.4, 55.1, 67.6, 81.6, 81.7 (two), 94.3, 131.4, 140.6, 153.1, 163.3 (two), 166.7, 168.4.

tert-Butyl (6*RS*,9*R*)-5-(2-*tert*-butoxy-2-oxoethylcarbamoyl)-6-isobutyl-9-(methoxycarbonyl)-13,13-dimethyl-4,11-dioxo-12-oxa-7-thia-3,10-diazatetradecanoate (**3**)

N-tert-(Butoxycarbonyl)-L-cysteine methyl ester (1.5 mmol) was added under Ar to a stirred suspension of NaH (1.5 mmol) in dry THF (2.5 ml). After 5 min. a THF solution (0.5 ml) of **2a** (1 mmol) was added. After stirring at room temperature (5 h), the solvent was removed in vacuo and the residue dissolved in EtOAc (10 ml) and washed with saturated ammonium chloride solution (2 $\times$ 10 ml) and with H_2O (2 $\times$ 10 ml) then dried on MgSO_4 . The solvent was removed in vacuo and the crude mixture was purified by a short silica gel column (hexane/ethyl acetate = 1:9) to afford diastereomeric **3** as a deep orange oil (78%).

IR: 3,424, 3,318, 1,737, 1,713, 1,678 cm^{-1} . HRMS (ES Q-TOF) calcd for $\text{C}_{29}\text{H}_{51}\text{N}_3\text{NaO}_{10}\text{S}$ ($\text{M} + \text{Na}$) $^+$: 656.3193; found: 656.3201. $^1\text{H-NMR}$ δ 0.88 (major, d, $J = 6.8$ Hz, 6 H), 0.90 (minor, d, $J = 6.8$ Hz, 6 H), 1.42 (major, s, 18 H), 1.43 (minor, s, 18 H), 1.44 (s, 18 H), 1.77–1.94 (m, 4 H), 2.23–2.35 (m, 2 H), 2.89–3.32 (m, 6 H), 3.44–3.53 (m, 2 H), 3.72 (minor, s, 3 H), 3.73 (major, s, 3

H), 3.86–4.02 (m, 8 H), 4.38–4.67 (m, 2 H), 6.95 (major, br, 1 NH), 7.21 (minor, br, 1 H, NH), 7.47 (minor, t, $J = 5.5$ Hz, 1 H, NH), 7.52 (major, t, $J = 5.2$ Hz, 1 H, NH), 7.67 (br, 2 H, NH). ^{13}C -NMR δ 20.9 (major, two), 21.0 (minor, two), 23.4 (minor), 23.5 (major), 28.0 (twelve), 28.2 (major, three), 28.3 (minor, three), 29.6 (two), 30.3 (two), 35.1 (major), 35.2 (minor), 42.0 (two), 42.1 (minor), 42.2 (major), 52.5 (two), 59.5 (two), 60.2 (two), 82.0 (major), 82.1 (minor), 82.2 (two), 82.3 (two), 155.1 (two), 168.2 (minor), 168.3 (major), 168.4 (two), 169.2 (two), 171.1 (major), 171.2 (minor), 171.4 (two).

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