RSC Advances

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Stereoselective synthesis of short benzyl malonyl peptides†

Cite this: *RSC Advances*, 2013, **3**, 13470

Received 17th April 2013,
Accepted 31st May 2013

DOI: 10.1039/c3ra41852a

www.rsc.org/advances

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A Rh-catalyzed addition reaction on non symmetric dehydro alanine retro-peptides is the key step in the reported three-step strategy for the diastereoselective synthesis of differently functionalized benzyl malonyl peptides (74% overall yield).

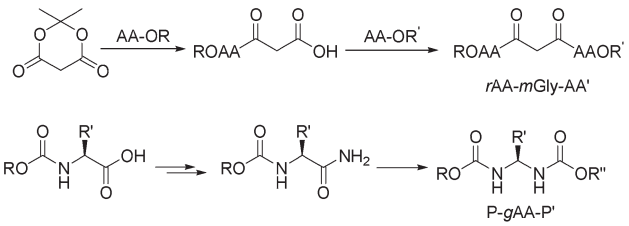
Introduction

Peptidomimetics have been designed to overcome the problems of pharmacokinetics[1] of the parent peptides and to be thus considered in different biological applications. In particular, retro-peptides are molecules that present the inversion of one or more peptide bonds of the backbone chain by the introduction of a malonyl residue and/or a *gem*-diaminic unit within the peptide chain.[2] These modifications increase the resistance to enzyme degradation,[3] without decreasing the receptor binding ability and biological response of native peptides.[4] Moreover, the inversion of the amide bond induces different conformational structures of peptidomimetics with respect to the native residue, by obtaining a rearrangement in stable β sheets.[5]

In the retro-peptide field, we reported the synthesis of non-symmetric disubstituted malonamides *r*AA-*m*Gly-AA[6] and of stable *N*,*N*′-protected *gem*-diaminic units P-*g*AA-P′ (Scheme 1) that can be successfully used in a tandem deprotection-coupling reaction to obtain more complex retro-peptide units.[7]

Continuing our studies, the interest was turned towards the possibility to modify the mimetic glycine residue of the *r*AA-*m*Gly-AA′ structures to obtain different alkyl malonyl units of kind *r*AA-*m*AA′′-AA′ and so introduce further modifications in the peptidomimetic backbone.

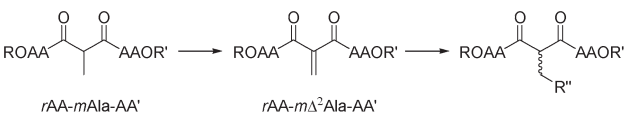
First of all, we attempted the classical reaction conditions to alkylate methylene active compounds using opportune halides (ethyl bromide, methyl iodide) in the presence of different organic or inorganic bases, such as tertiary (Et_3N) or secondary (piperidine)[8] amines or CaO,[9] on the symmetric *N*,*N*-Boc protected malonamide *r*Gly-*m*Gly-Gly. However, also by changing other reaction parameters (solvent, temperature, and/or times) the expected alkylated malonamide is not formed, probably due to the low electrophilicity of the reactive species coupled to the instability of the substrate under basic conditions.



Scheme 1 Synthesis of malonamidic and *gem*-diaminic units.

Then, a different synthetic strategy involving a rhodium-catalyzed conjugate addition to the double bond of malonyl dehydroalanines[10] *r*AA-*m*Δ^2Ala-AA′ (obtained from the corresponding *r*AA-*m*Ala-AA′) was considered (Scheme 2).

As is well known, in the last years rhodium was widely used as metal catalyst in various addition reactions to alkenes.[11] Recently, the synthesis of small peptides containing unnatural amino acid residues was reported by a rhodium-catalyzed 1,4-addition of aryl siloxanes or boronates to α,β-dehydroamino acid moieties.[12] Still always by the same group, several useful alanines were prepared using a rhodium-catalyzed conjugate addition of aryl boronic acids to dehydro derivatives.[13]



Scheme 2 Synthetic strategy for alkyl malonyl units.

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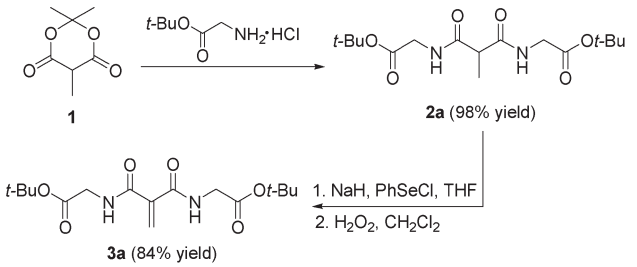
† Electronic supplementary information (ESI) available: General procedures, analytical and spectroscopic data, ^1H and ^{13}C NMR spectra of all new compounds. See DOI: 10.1039/c3ra41852a



Scheme 3 Synthesis of symmetric *N*,*N*′-Boc protected malonyl dehydroalanines *r*AA-*m*Δ^2Ala-AA′ **3a**.

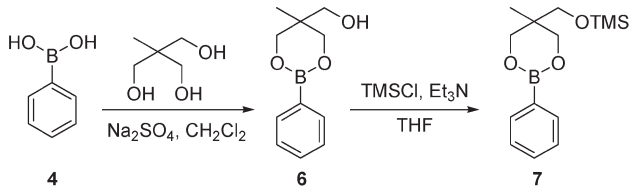
Results and discussion

The symmetric *N*,*N*′-Boc protected malonamide *r*Gly-*m*Ala-Gly **2a** was synthesized in good yields starting from **1** following the same methodology standardized on the Meldrum's acid.[6] Then, a selenoxide *syn* elimination was considered to obtain the corresponding *r*Gly(O-*t*-Bu)-*m*Δ^2Ala-Gly(O-*t*-Bu) **3a**, partially modifying the methodology reported in the literature for different active methylene compounds[14] (Scheme 3).

Finally, a rhodium-catalyzed addition to the C=C double bond was attempted on **3a** (1 mmol), by using commercial phenyl boronic acid **4** as alkylating agent, chloro(1,5-cyclooctadiene)rhodium(I) dimer {[Rh(COD)Cl]$_2$} as catalyst, 1,5-cyclooctadiene (COD) as ligand, and potassium hydroxide (1 mmol) as base.

The reactions were performed under different conditions (Table 1) and the best appeared using an equimolar ratio between the reagents, 2.5 mol% of Rh complex and working at reflux of THF for 2 h (entry 7). After work-up with water and purification through a short plug of silica gel using CH_2Cl_2 as eluent, the product **5a** was obtained in 69% yield with very high purity, as confirmed by NMR spectra.

With the aim of increasing the reaction yields, two different protected boronate **6**[15] and **7**[16] reagents were synthesized starting from **4** (Scheme 4) and used as alkylating agents under



Scheme 4 Synthesis of different protected aryl boronates.

the same conditions reported in entry 7 of Table 1. **5a** was obtained after 2 h with similar yields but in lower purity due to the presence of unidentified by-products.

Thus, different commercial *p*-substituted phenyl boronic acids **8–12** were considered to study the influence of a substituent in *para* position on the rhodium-catalyzed addition on **3a**. The results are reported in Table 2.

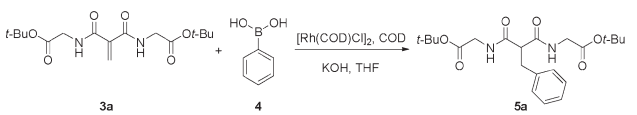
As expected, the presence of an electron donor substituent (entries 1 and 2) as well as a halogen atom (entries 3 and 4) favored the addition, while the electron withdrawing nitro group (entry 5) has the opposite effect and led to the formation of addition product in lower yields.

Then, the Rh-catalyzed addition reaction was extended to non symmetric malonyl dehydro peptides (MDHPs) **3b–f**, choosing commercially available **8** as alkylating agent to synthesize the different benzyl malonyl peptides **13b–f** and **13c′–f′** (Table 3).

In all cases, compounds **13** were obtained in good yields after fast purification on silica gel, but the reactions on **3c–f** performed under standardized conditions (80 °C) took place with low (dr = 6 : 4 for **3d–f**) or no (for **3c**) diastereoselectivity as determined by ^{1}H NMR spectra of crude mixtures. To improve the diastereoselectivity, these reactions were repeated at lower temperatures (50 °C and rt).

While working at room temperature only the reagent decomposition was observed (24 h), the addition reactions performed at 50 °C took place with high selectivity (dr ⩾ 9 : 1), as determined by ^{1}H NMR analyses performed on the crude mixtures, only if the phenylalanine residue was present in the starting MDPHs (entries 3–5), the stereoselectivity being controlled by electronic factors (entry 2).

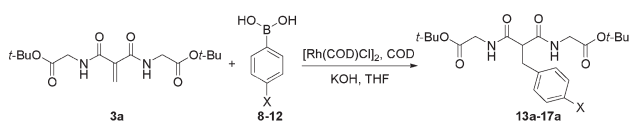
Table 1 Rhodium-catalyzed addition reaction on *r*Gly(O*t*-Bu)-*m*Δ^2Ala-Gly(O*t*-Bu) **3a**



Entry	**4** (eq.)	Catalyst (mol%)	COD (mol%)	*T*/°C	Time (h)	Yield (%)
1	16	2.5	10	100[a]	24	13
2	4	2.5	10	80	24	20
3	3	2.5	10	80	12	30
4	3	5.0	20	80	12	15
5	2	2.5	10	80	12	43
6	2	2.5	10	80	2	58
7	1	2.5	10	80	2	69

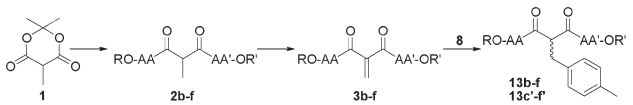
[a] Performed by using a mixture of dioxane/H_2O as solvent.

Table 2 Rh-catalyzed addition with different *p*-substituted phenyl boronic acids



Entry	Alkylating agent	X	Addition product	Yield (%)
1	**8**	Me	**13a**	90
2	**9**	OMe	**14a**	94
3	**10**	Br	**15a**	89
4	**11**	F	**16a**	88
5	**12**	NO_2	**17a**	57

Table 3 Synthesis of non symmetric benzyl malonyl peptides



Entry	AA-OR	AA′-OR′	2	Yield (%)	3	Yield (%)	13	Yield (%)	dr 50 °C
1	β-AlaOMe	GlyO*t*-Bu	**b**	96	**b**	83	**b**	75	—
2	L-LeuOMe	GlyOMe	**c**	94	**c**	84	**c,c′**	84	1 : 1
3	L-PheO*t*-Bu	GlyO*t*-Bu	**d**	95	**d**	78	**d,d′**	79	>9 : 1
4	L-PheOMe	β-AlaOMe	**e**	93	**e**	79	**e,e′**	82	>9 : 1
5	L-PheOMe	GlyOMe	**f**	92	**f**	77	**f,f′**[a]	80	>9 : 1

[a] Rh-addition was performed by using **9** as alkylating agent.

To gain further information, bidimensional NOESY experiments were performed on pure **13d** (Fig. 1). 2D NMR spectra clearly showed that the two aromatic systems of malonyl peptide are in *syn* position, due to the presence of dipolar couplings between the protons of the aromatic moieties. This permits to attribute the *S* configuration to the new chiral center.

Starting from these data, in Scheme 5 a catalytic cycle of the rhodium-catalyzed addition of aryl boronates to MDHPs **3a–f** is proposed.

The rhodium complex **I** was formed *in situ* and it coordinates the C=C double bond of **3**. If the MDPH contains the phenylalanine residue, probably the rhodium coordinates also the aromatic ring leading to the formation of the intermediate **II** and determining the observed high diastereoselectivity. In fact, in **II** the attack of the nucleophile on the double bond can occur only on the same side of the benzyl residue of phenylalanine.

Conclusions

In conclusion, a new three-step synthetic strategy for the synthesis of differently functionalized benzyl malonyl peptides (74% overall yield) was developed, the key step being a Rh-catalyzed addition reaction performed on non symmetric dehydro alanine retro-peptides. The target compounds were obtained in very high diastereomeric purity probably due to the formation of a stable coordination complex between the phenylalanine aromatic residue and the organometallic reagent. Furthermore, the synthesized short retro-peptide units are enriched by a mimetic residue of phenylalanine or *p*-substituted analogs, in which the aromatic ring substituent is a potential interesting modification able to modulate the retro-peptide polarity.

Experimental section

General remarks

All the commercial available reagents and the anhydrous solvents were purchased from *Aldrich* and used without further purification. The reactions were monitored by ^{1}H NMR spectroscopy. Flash chromatography was performed on Merck silica gel (60, particle size: 0.040–0.063 mm). ^{1}H NMR

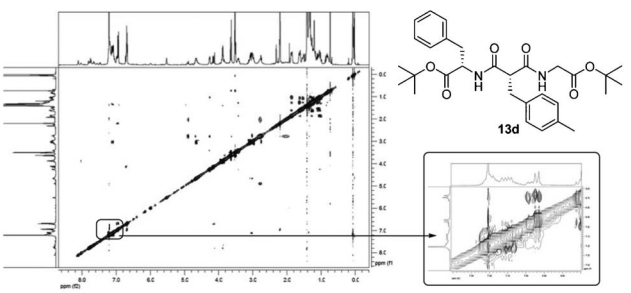
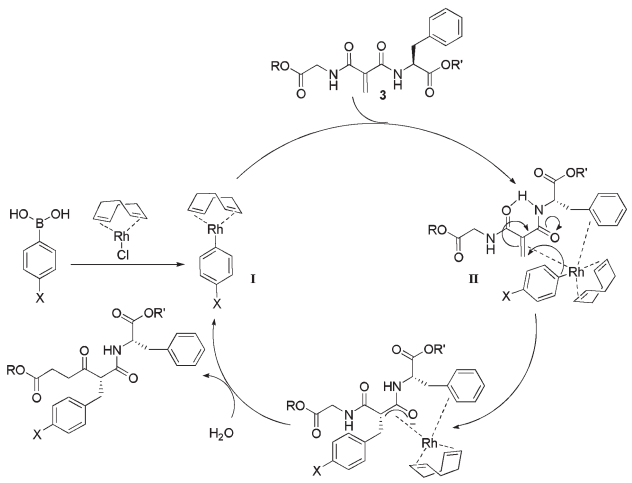


Fig. 1 NOESY spectrum of **13d**. Spatial correlations between two aromatic systems are evidenced. The existence of these cross peaks is possible only if the two rings face the same plane.



Scheme 5 Proposed catalytic cycle of the rhodium-catalyzed addition of aryl boronates.

and ^{13}C NMR spectra were recorded on a VARIAN XL-300 spectrometer at room temperature. $CDCl_3$ was used as the solvent and $CHCl_3$ as the internal standard. 2D NMR spectra were recorded by a Bruker Avance III 400 MHz NMR spectrometer and used to assist in structure elucidation.[17] FT-IR spectra were recorded on a Perkin-Elmer 1600 spectrophotometer in $CHCl_3$ as the solvent. HRMS analyses were performed using a Micromass Q-TOF Micro quadrupole-time of flight (TOF) mass spectrometer equipped with an ESI source and a syringe pump. The experiments were conducted in the positive ion mode.

General procedure for the synthesis of methyl malonyl peptides

Starting from the methyl Meldum's acid, the same reported synthetic procedure for the synthesis of malonyl peptides[6] was followed.

Di-*tert*-butyl 2,2′-[(2-methylmalonyl)bis(azanediyl)]diacetate (2a). Yield 98%. White solid mp 125.5–126.2 °C. Purified by flash chromatography on silica gel (eluent: hexane/ethyl acetate = 45 : 55). ν_{max} cm^{-1} 3424, 3330, 1731, 1672. ^1H NMR ($CDCl_3$): δ 1.47 (s, 18H), 1.51 (d, J = 7.3 Hz, 3H), 3.21 (q, J = 7.2 Hz, 1H), 3.93 (d, J = 5.2 Hz, 4H), 7.00 (br, 2H). ^{13}C NMR ($CDCl_3$): δ 16.2, 27.8 (6C), 41.9 (2C), 47.3, 81.7 (2C), 168.5 (2C), 171.4 (2C). HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{16}H_{29}N_2O_6$ 345.2026, found 345.2021.

Methyl 3-{3-[(2-*tert*-butoxy-2-oxoethyl)amino]-2-methyl-3-oxopropanamido}propanoate (2b). Yield 96%. White solid, mp 123.2–124.7 °C. Purified by flash chromatography on silica gel (eluent: hexane/ethyl acetate = 5 : 95). ν_{max} cm^{-1} 3425, 3336, 1732, 1673. ^1H NMR ($CDCl_3$): δ 1.47 (s, 9H), 1.63 (d, J = 7.2 Hz, 3H), 2.55 (t, J = 6.5 Hz, 2H), 3.12 (q, J = 7.2 Hz, 1H), 3.49–3.55 (m, 2H), 3.70 (s, 3H), 3.91 (d, J = 6.5 Hz, 2H), 7.02 (br, 2H). ^{13}C NMR ($CDCl_3$) δ 75.5 MHz): 16.6, 27.9 (3C), 33.6, 35.1, 42.0, 48.0, 51.7, 82.1, 168.5, 171.3, 171.4, 172.5. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{14}H_{25}N_2O_6$ 317.1713, found 317.1720.

Methyl (2*S*)-2-[3-(2-methoxy-2-oxoethylamino)-2-methyl-3-oxopropanamido]-4-methylpentanoate (2c). Yield 94%. White solid, mp 64.0–65.6 °C. Purified by flash chromatography on silica gel (eluent: hexane/ethyl acetate = 30 : 70). ν_{max} cm^{-1} 3424, 3342, 1742, 1672. ^1H NMR ($CDCl_3$): δ (*diastereomer I*) 0.92 (d, J = 6.1, 6H), 1.49 (d, J = 1.5 Hz, 3H), 1.54–1.68 (m, 3H), 3.21 (q, J = 7.3 Hz, 1H), 3.73 (s, 3H), 3.76 (s, 3H), 4.03 (d, J = 5.3 Hz, 2H), 4.54–4.60 (m, 1H), 6.89 (br, 2H); (*diastereomer II*) 0.94 (d, J = 6.1, 6H), 1.51 (d, J = 1.5 Hz, 3H), 1.54–1.68 (m, 3H), 3.22 (q, J = 7.3 Hz, 1H), 3.73 (s, 3H), 3.77 (s, 3H), 4.05 (d, J = 5.5 Hz, 2H), 4.54–4.60 (m, 1H), 7.10 (br, 2H). ^{13}C NMR ($CDCl_3$): δ (*diastereomer I*) 16.2, 21.3, 22.5 (2C), 40.3, 40.9, 47.2, 50.7 (2C), 51.9, 169.8, 171.2, 171.8, 172.9; (*diastereomer II*) 16.3, 21.4, 24.5 (2C), 40.4, 41.0, 47.2, 50.7 (2C), 51.9, 169.9, 171.3, 171.8, 173.0. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{14}H_{25}N_2O_6$ 317.1713, found 317.1720.

***tert*-Butyl (2*S*)-2-{3-[(2-*tert*-butoxy-2-oxoethyl)amino]-2-methyl-3-oxopropanamido}-3-phenylpropanoate (2d).** Yield 95%. Yellow oil. Purified by flash chromatography on silica gel (eluent: hexane/ethyl acetate = 20 : 80). ν_{max} cm^{-1} 3423, 3335, 1731, 1675. ^1H NMR ($CDCl_3$): δ (*diastereomer I*) 1.20 (d, J = 7.0 Hz, 3H), 1.42 (s, 18H), 3.02–3.29 (m, 3H), 3.90 (d, J = 5.2 Hz, 2H), 4.72–4.79 (m, 1H), 7.18–7.38 (m, 5H), 7.40 (br, 1H), 7.50 (br, 1H); (*diastereomer II*) 1.20 (d, J = 7.0 Hz, 3H), 1.48 (s, 18H), 3.02–3.29 (m, 3H), 3.90 (d, J = 5.2 Hz, 2H), 4.72–4.79 (m, 1H), 7.18–7.38 (m, 5H), 7.40 (br, 1H), 7.50 (br, 1H). ^{13}C NMR ($CDCl_3$): δ (*diastereomer I*) 16.4, 27.7 (6C), 37.6, 41.8, 47.5, 53.7, 81.8, 82.0, 126.6, 128.1 (2C), 129.3 (2C), 136.1, 168.5, 170.1, 170.6, 171.0; (*diastereomer II*) 16.4, 27.8 (6C), 37.7, 41.9, 47.6, 53.7, 81.9, 82.0, 126.7, 128.1 (2C), 129.3 (2C), 136.1, 168.5, 170.1, 170.8, 171.1. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{23}H_{35}N_2O_6$ 435.2495, found 435.2493.

Methyl (2*S*)-2-{3-[(3-methoxy-3-oxopropyl)amino]-2-methyl-3-oxopropanamido}-3-phenylpropanoate (2e). Yield 93%. Yellow oil. Purified by flash chromatography on silica gel (eluent: hexane/ethyl acetate = 40 : 60). ν_{max} cm^{-1} 3422, 3334, 1733, 1674. ^1H NMR ($CDCl_3$): δ (*diastereomer I*) 1.36–1.42 (m, 3H), 2.55 (t, J = 6.1 Hz, 2H), 3.02–3.19 (m, 3H), 3.45–3.59 (m, 2H), 3.71 (s, 3H), 3.75 (s, 3H), 4.79–4.88 (m, 1H), 7.05 (br, 2H), 7.14–7.34 (m, 5H); (*diastereomer II*) 1.36–1.42 (m, 3H), 2.55 (t, J = 6.1 Hz, 2H), 3.02–3.19 (m, 3H), 3.45–3.59 (m, 2H), 3.72 (s, 3H), 3.75 (s, 3H), 4.79–4.88 (m, 1H), 7.14–7.34 (m, 5H), 7.73 (br, 2H). ^{13}C NMR ($CDCl_3$): δ (*diastereomer I*) 16.6, 33.6, 35.0, 37.7, 48.1, 51.8, 53.2 (2C), 127.1, 128.5 (2C), 129.1 (2C), 135.8, 170.9, 171.0, 171.6 172.5; (*diastereomer II*) 16.5, 33.6, 35.0, 37.6, 48.2, 52.3, 53.3 (2 C), 127.1, 128.5 (2C), 129.2 (2C), 135.8, 170.8, 170.9, 171.6, 172.5. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{18}H_{25}N_2O_6$ 365.1713, found 365.1710.

Methyl (2*S*)-2-(3-(2-methoxy-2-oxoethylamino)-2-methyl-3-oxopropanamido)-3-phenylpropanoate (2f). Yield 92%. Yellow oil. Purified by flash chromatography on silica gel (eluent: hexane/ethyl acetate = 25 : 75). ν_{max} cm^{-1} 3420, 3335, 1733, 1675. ^1H NMR ($CDCl_3$): δ (*diastereomer I*) 1.25 (d, J = 7.1 Hz, 3H), 3.02–3.17 (m, 2H), 3.34 (q, J = 7.1 Hz, 1H), 3.74 (s, 3H), 3.76 (s, 3H), 4.01 (d, J = 6.2 Hz, 2H), 4.79–4.88 (m, 1H), 6.83 (br, 2H), 7.08–7.31 (m, 5H); (*diastereomer II*) 1.27 (d, J = 7.1 Hz, 3H), 3.02–3.17 (m, 2H), 3.34 (q, J = 7.1 Hz, 1H), 3.74 (s, 3H), 3.76 (s, 3H), 4.01 (d, J = 6.2 Hz, 2H), 4.79–4.88 (m, 1H), 6.96 (br, 2H), 7.08–7.31 (m, 5H). ^{13}C NMR ($CDCl_3$): δ (*diastereomer I*) 16.4, 37.6, 41.2, 47.9, 51.3, 51.4, 52.4, 127.1, 128.5 (2C), 129.2 (2C), 135.7, 169.3, 169.9, 170.9, 171.3; (*diastereomer II*) 16.5, 37.7, 41.2, 48.0, 51.4, 51.5, 53.3, 127.7, 128.5 (2C), 130.2 (2C), 136.3, 169.4, 170.7, 171.1, 171.6. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{17}H_{23}N_2O_6$ 351.1556, found 351.1559.

General procedure for the synthesis of dehydro malonyl peptides

Methyl malonyl peptide (1.0 mmol) was added slowly to a stirred suspension of NaH (0.036 g, 1.5 mmol) in 40 mL anhydrous THF at 0 °C. After 1 h, a solution of PhSeCl (0.766 g, 4.0 mmol) in 5 mL anhydrous THF was added dropwise. After 24 h, the solutions were diluted with diethyl ether, washed with saturated $NaHCO_3$ and saturated NaCl, and dried over Na_2SO_4. The solvents were evaporated under reduced pressure. The crude mixtures were dissolved in 20 mL CH_2Cl_2 and stirred with 40% H_2O_2 (0.3 mL, 4 mmol) for 3 h at 0 °C. Then, the mixtures were washed with saturated $NaHCO_3$ and saturated NaCl and dried over Na_2SO_4. After solvent evapora-

tion, the expected dehydro malonyl peptides were obtained as pure compounds and used without further purification.

Di-*tert*-butyl 2,2′-[(2-methylenemalonyl)bis(azanediyl)] diacetate (3a). Yield 84%. Yellow oil. ν_{max} cm^{-1} 3423, 3334, 1731, 1679, 1632. ^1H NMR ($CDCl_3$): δ 1.47 (s, 18H), 4.00 (d, J = 5.1 Hz, 4H), 6.44 (s, 2 H), 7.77 (br, 2H). ^{13}C NMR ($CDCl_3$): δ 27.9 (6C), 42.1 (2C), 82.2 (2C), 128.6, 129.1, 168.5 (2C), 170.0 (2C). HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{16}H_{27}N_2O_6$ 343.1869, found 343.1865.

Methyl 3-[2-(2-*tert*-butoxy-2-oxoethylcarbamoyl)acrylamido] propanoate (3b). Yield 83%. Yellow oil. ν_{max} cm^{-1} 3425, 3336, 1735, 1678, 1633. ^1H NMR ($CDCl_3$): δ 1.41 (s, 9H), 2.54 (t, J = 6.2 Hz, 2H), 3.53 (q, J = 6.2 Hz, 2H), 3.63 (s, 3H), 3.92 (d, J = 5.5 Hz, 2H), 6.29 (s, 1H), 6.35 (s, 1H), 7.90 (br, 1H), 8.06 (br, 1H). ^{13}C NMR ($CDCl_3$): δ 27.8 (3C), 33.4, 35.1, 42.1, 51.6, 82.1, 136.1, 137.0, 165.1, 165.2, 168.4, 172.4. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{14}H_{23}N_2O_6$ 315.1556, found 315.1560.

Methyl (2*S*)-2-[(2-{[(2-methoxy-2-oxoethyl)amino]carbonyl} acryloyl)amino]-4-methylpentanoate (3c). Yield 84%. Yellow oil. ν_{max} cm^{-1} 3422, 3330, 1742, 1674,1631. ^1H NMR ($CDCl_3$). δ 0.75–0.98 (m, 6H), 1.50–1.71 (m, 3H), 3.70 (s, 3H), 3.72 (s, 3H), 4.06 (d, J = 5.0 Hz, 2H), 4.50–4.63 (m, 1H), 6.41 (s, 1H), 6.43 (s, 1H), 7.94–7.97 (m, 1H), 8.05 (br, 1H). ^{13}C NMR ($CDCl_3$): δ 21.7, 22.7, 24.8, 40.9, 41.3, 51.1, 52.2, 52.3, 135.7, 136.5, 164.8, 165.5, 169.8, 172.9. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{14}H_{23}N_2O_6$ 315.1556, found 315.1550.

***tert*-Butyl (2*S*)-2-[2-(2-*tert*-butoxy-2-oxoethylcarbamoyl)acrylamido]-3-phenylpropanoate (3d).** Yield 78%. Orange oil. ν_{max} cm^{-1} 3427, 3336, 1739, 1678, 1630. ^1H NMR ($CDCl_3$): δ 1.41 (s, 9H), 1.48 (s, 9H), 3.10–3.21 (m, 2H), 4.00 (d, J = 5.4 Hz, 2H), 4.76–4.81 (m, 1H), 6.26 (s, 1H), 6.45 (s, 1H), 7.16–7.29 (m, 5H), 7.59 (br, 1H), 8.01 (br, 1H). ^{13}C NMR ($CDCl_3$): δ 27.8 (3C), 27.9 (3C), 37.7, 42.2, 54.0, 82.2, 82.4, 126.9, 128.3 (2C), 129.4 (2C), 129.8, 136.0, 137.0, 164.5, 164.8, 168.4, 170.0. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{23}H_{33}N_2O_6$ 433.2339, found 433.2333.

Methyl (2*S*)-2-[2-(3-methoxy-3-oxopropylcarbamoyl)acrylamido]-3-phenylpropanoate (3e). Yield 79%. Orange oil. IR ($CHCl_3$) ν_{max} cm^{-1} 3426, 3334, 1736, 1677, 1631. ^1H NMR ($CDCl_3$) δ: 2.57 (t, J = 6.3 Hz, 2H), 3.07–3.23 (m, 2H), 3.57 (q, J = 6.3 Hz, 2H), 3.69 (s, 3H), 3.72 (s, 3H), 4.80–4.88 (m, 1H), 6.24 (s, 1H), 6.29 (s, 1H), 7.10–7.29 (m, 5H), 7.87 (br, 1H), 7.95 (br, 1H). ^{13}C NMR ($CDCl_3$): δ 33.4, 35.0, 37.5, 51.7, 52.2, 53.6, 127.0, 128.4 (2C), 129.0 (2C), 135.8, 136.4, 137.0, 164.9, 165.2, 171.4, 172.3. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{18}H_{23}N_2O_6$ 363.1556, found 363.1550.

Methyl (2*S*)-2-[2-(2-methoxy-2-oxoethylcarbamoyl)acrylamido]-3-phenylpropanoate (3f). Yield 77%. Yellow oil. ν_{max} cm^{-1} 3428, 3332, 1738, 1678, 1631. ^1H NMR ($CDCl_3$): δ 3.02–3.25 (m, 2H), 3.74 (s, 3H), 3.77 (s, 3H), 4.10 (d, J = 4.6 Hz, 2H), 4.83–4.92 (m, 1H), 6.25 (s, 1H), 6.48 (s, 1H), 7.08–7.29 (m, 5H), 7.97 (br, 2H). ^{13}C NMR ($CDCl_3$): δ 33.7, 37.5, 52.2, 53.6, 63.6, 127.0, 128.4 (2C), 129.0 (2C), 130.2, 135.8, 136.5, 164.5, 165.9, 171.3, 171.8. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{17}H_{21}N_2O_6$ 349.1400, found 349.1398.

General procedure for the synthesis of alkyl malonyl peptides

An oven dried flask was charged with malonyl dehydro peptide (1 mmol), $[Rh(COD)Cl]_2$ (2.5 mol%), cyclooctadiene (10 mol%), KOH (1 mmol), an appropriate *p*-substituted phenyl boronic acid (1 mmol) or (**6**) or (**7**), and tetrahydrofuran (4 mL), evacuated and backfilled with argon and stirred at 80 °C or 50 °C for 2 h. The reaction mixture was diluted with ethyl acetate, washed with a saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and the solvent was evaporated. The residue was purified by column chromatography to afford the desired compound.

Di-*tert*-butyl 2,2′-{[2-(benzyl)malonyl]bis(azanediyl)}diacetate (5a). Yield 89%. Dark yellow oil. ν_{max} cm^{-1} 3424, 3337, 1735, 1678. ^1H NMR ($CDCl_3$): δ 1.45 (s, 18H), 3.55–3.70 (m, 3H), 3.84–3.97 (m, 4H), 6.81–6.86 (m, 5H), 7.15–7.20 (br, 1H), 8.08 (br, 1H). ^{13}C NMR ($CDCl_3$): δ 27.9 (6C), 33.7, 41.9 (2C), 53.5, 82.6 (2C), 115.3, 119.8 (2C), 129.4 (2C), 156.4, 166.2, 167.9, 169.4, 170.0. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{22}H_{33}N_2O_6$ 421.2339, found 421.2330.

Di-*tert*-butyl 2,2′-{[2-(4-methylbenzyl)malonyl]bis(azanediyl)}diacetate (13a). Yield 90%. Dark yellow oil. ν_{max} cm^{-1} 3422, 3333, 1734, 1679. ^1H NMR ($CDCl_3$): δ 1.44 (s, 18H), 2.28 (s, 3H), 3.16–3.27 (m, 3H), 3.73–3.97 (m, 4H), 6.92 (br, 2H), 7.05–7.10 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 20.9, 27.9 (6C), 37.7, 42.1 (2C), 56.5, 82.2 (2C), 128.6, 129.1 (2C), 134.8 (2C), 136.2, 168.4 (2C), 170.0 (2C). HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{23}H_{35}N_2O_6$ 435.2495, found 435.2498.

Di-*tert*-butyl 2,2′-{[2-(4-methoxybenzyl)malonyl]bis(azanediyl)}diacetate (14a). Yield 94%. Dark yellow oil. ν_{max} cm^{-1} 3423, 3336, 1736, 1677. ^1H NMR ($CDCl_3$): δ 1.45 (s, 18H), 3.52–3.56 (m, 3H), 3.73 (s, 3H), 3.86–4.03 (m, 4H), 6.74–6.75 (m, 4H), 8.03 (br, 2H). ^{13}C NMR ($CDCl_3$): δ 27.9 (6C), 33.8, 41.9 (2C), 53.6, 55.7, 82.6 (2C), 114.5, 114.7, 116.0 (2C), 120.6 (2C), 166.1, 168.0, 169.5, 170.0. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{23}H_{35}N_2O_7$ 451.2444, found 451.2450.

Di-*tert*-butyl 2,2′-{[2-(4-bromobenzyl)malonyl]bis(azanediyl)}diacetate (15a). Yield 89%. Dark yellow oil. ν_{max} cm^{-1} 3425, 3334, 1737, 1679. ^1H NMR ($CDCl_3$): δ 1.44 (s, 18H), 3.52–3.69 (m, 3H), 3.85–3.96 (m, 4H), 6.68–6.72 (m, 2H), 7.22–7.25 (m, 2H), 8.07 (br, 2H). ^{13}C NMR ($CDCl_3$): δ 27.9 (6C), 33.7, 41.9 (2C), 53.6, 82.7 (2C), 111.5, 117.3 (2C), 132.1 (2C), 155.8, 166.2, 167.9, 169.5, 170.0. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{22}H_{32}BrN_2O_6$ 499.1444, found 499.1446.

Di-*tert*-butyl 2,2′-{[2-(4-fluorobenzyl)malonyl]bis(azanediyl)}diacetate (16a). Yield 88%. Dark yellow oil. ν_{max} cm^{-1} 3421, 3335, 1734, 1676. ^1H NMR ($CDCl_3$): δ 1.44 (s, 18H), 3.65–3.77 (m, 3H), 3.87–4.02 (m, 4H), 6.86–6.90 (m, 2H), 8.06–8.10 (m, 2H), 8.40 (br, 2H). ^{13}C NMR ($CDCl_3$). δ 27.9 (6C), 33.8, 41.9 (2C), 53.6, 82.6 (2C), 115.5, 115.8 (2C), 116.1 (2C), 152.5, 166.1, 168.0, 170.0, 170.3. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{22}H_{32}FN_2O_6$ 439.2244, found 439.2239.

Di-*tert*-butyl 2,2′-{[2-(4-nitrobenzyl)malonyl]bis(azanediyl)} diacetate (17a). Yield 57%. Orange oil. ν_{max} cm^{-1} 3423, 3335, 1735, 1680, 1540. ^1H NMR ($CDCl_3$): δ 1.44 (s, 18H), 3.65–3.77 (m, 3H), 3.87–4.02 (m, 4H), 6.86–6.90 (m, 2H), 8.06–8.11 (m, 2H), 8.77 (br, 2H). ^{13}C NMR ($CDCl_3$): δ 27.9 (6C), 33.6, 41.9 (2C), 53.6, 82.8 (2C), 115.0, 115.6 (2C), 126.0 (2C), 140.6, 166.3,

167.9, 169.5, 170.0. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{22}H_{32}N_3O_8$ 466.2189, found 466.2187.

Methyl 3-{3-[(2-*tert*-butoxy-2-oxoethyl)amino]-2-(4-methylbenzyl)-3-oxopropanamido}propanoate (13b). Yield 75%. Dark yellow oil. ν_{max} cm^{-1} 3424, 3336, 1736, 1679. ^1H NMR ($CDCl_3$): δ 1.44 (s, 9H), 2.22 (s, 3H), 2.50–2.58 (m, 2H), 3.06–3.09 (m, 2H), 3.45–3.47 (m, 2H), 3.54 (s, 3H), 3.71–3.74 (m, 1H), 3.89–3.95 (m, 2H), 6.69–6.72 (m, 2H), 6.93–6.99 (m, 2H), 8.01 (br, 1H), 8.10 (br, 1H). ^{13}C NMR ($CDCl_3$): δ 20.3, 27.9 (3C), 33.4, 33.7, 35.0, 40.7, 51.8, 53.5, 82.5, 115.1, 128.4 (2C), 129.7 (2C), 133.8, 165.9, 166.2, 167.9, 172.2. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{21}H_{31}N_2O_6$ 407.2182, found 407.2183.

Methyl (2*S*)-2-[3-(2-methoxy-2-oxoethylamino)-2-(4-methylbenzyl)-3-oxopropanamido]-4-methylpentanoate (13c,c′). Yield 84%. Dark yellow oil. ν_{max} cm^{-1} 3425; 3337; 1739; 1679. ^1H NMR ($CDCl_3$): δ (*diastereomer I*) 0.84–1.00 (m, 6H), 1.19–1.32 (m, 1H), 1.54–1.75 (m, 2H), 2.26 (s, 3H), 3.09–3.18 (m, 2H), 3.73 (s, 6H), 3.95–4.01 (d, J = 10.7 Hz, 2H), 4.06–4.13 (m, 1H), 4.43–4.66 (m, 1H), 6.69–6.76 (m, 2H), 6.97–7.06 (m, 2H), 8.12 (br, 2H); (*diastereomer II*) 0.84–1.00 (m, 6H), 1.19–1.32 (m, 1H), 1.54–1.75 (m, 2H), 2.35 (s, 3H), 3.09–3.18 (m, 2H), 3.76 (s, 6H), 3.95–4.01 (d, J = 10.7 Hz, 2H), 4.06–4.13 (m, 1H), 4.43–4.66 (m, 1H), 6.69–6.76 (m, 2H), 6.97–7.06 (m, 2H), 8.22 (br, 2H). ^{13}C NMR ($CDCl_3$): δ (*diastereomer I*) 20.4, 21.6 (2C), 24.8, 36.9, 41.0, 41.3, 51.0, 52.2 (2C), 53.6, 128.3 (2C), 133.0 (2C), 140.6, 142.9, 169.0 (2C), 172.5 (2C); (*diastereomer II*) 20.7, 22.7 (2C), 24.9, 36.9, 41.1, 41.3, 51.3, 52.4 (2C), 53.8, 129.9 (2C), 133.9 (2C), 140.6, 142.9, 169.7 (2C), 173.2 (2C). HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{21}H_{31}N_2O_6$ 407.2182, found 407.2191.

***tert*-Butyl (2*S*)-2-[3-(2-*tert*-butoxy-2-oxoethylamino)-2-(4-methylbenzyl)-3-oxopropanamido]-3-phenylpropanoate (13d,d′).** Yield 79%. Brown oil. ν_{max} cm^{-1} 3423, 3334, 1737, 1676. ^1H NMR ($CDCl_3$): δ 1.46 (s, 18H), 2.25 (s, 3H), 3.01–3.15 (m, 4H), 3.44–3.51 (m, 1H), 3.89–3.96 (m, 2H), 4.67–4.76 (m, 1H), 6.73–6.78 (m, 2H), 6.96–7.02 (m, 2H), 7.13–7.25 (m, 5H), 8.08 (br, 2H) [(*S*,*S*) major]; 1.45 (s, 18H), 2.31 (s, 3H), 3.01–3.15 (m, 4H), 3.44–3.51 (m, 1H), 3.89–3.96 (m, 2H), 4.67–4.76 (m, 1H), 6.73–6.78 (m, 2H), 6.96–7.02 (m, 2H), 7.13–7.25 (m, 5H), 8.16 (br, 2H) [(*S*,*R*) minor]. ^{13}C NMR ($CDCl_3$): δ 20.4, 27.9 (6 C), 33.8, 37.9, 41.9, 53.7, 54.2, 82.5 (2 C), 115.0, 115.1 (2 C), 126.9, 128.3 (2 C), 129.5 (2 C), 129.8 (2 C), 130.7, 135.8, 167.7, 167.9, 169.3, 169.6 [(*S*,*S*) major]; 20.4, 27.8 (6C), 33.8, 37.9, 42.0, 53.8, 54.2, 82.5 (2C), 114.9, 115.1 (2C), 126.9, 128.3 (2C), 129.4 (2C), 129.7 (2C), 130.6, 135.8, 167.7, 167.9, 169.3, 169.7 [(*S*,*R*) minor]. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{30}H_{41}N_2O_6$ 525.2965, found 525.2958.

Methyl (2*S*)-2-{3-[(3-methoxy-3-oxopropyl)amino]-2-(4-methylbenzyl)-3-oxopropanamido}-3-phenylpropanoate (13e,e′). Yield 82%. Brown oil. ν_{max} cm^{-1} 3422, 3334, 1735, 1678. ^1H NMR ($CDCl_3$): δ 2.25 (s, 3H), 2.43–2.60 (m, 2H), 3.02–3.19 (m, 4H), 3.63–3.77 (m, 3H), 3.68 (s, 6H), 4.69–4.92 (m, 1H), 6.71–6.75 (m, 2H), 6.98–7.03 (m, 2H), 7.08–7.25 (m, 5H), 8.08 (br, 2H) [(*S*,*S*) major]; 2.35 (s, 3H), 2.43–2.60 (m, 2H), 3.02–3.19 (m, 4H), 3.63–3.77 (m, 3H), 3.69 (s, 6H), 4.69–4.92 (m, 1H), 6.71–6.75 (m, 2H), 6.98–7.03 (m, 2H), 7.08–7.25 (m, 5H), 8.16 (br, 2H) [(*S*,*R*) minor]. ^{13}C NMR ($CDCl_3$): δ 20.4, 33.5, 35.0, 37.6, 38.7, 51.9 (2C), 53.5, 68.0, 114.5, 115.1 (2C), 127.2, 128.6 (2C), 129.2 (2C), 129.9 (2C), 133.8, 135.8, 165.4 (2C), 172.8 (2C) [(*S*,*S*) major]; 21.6, 33.2, 35.0, 37.7, 38.5, 51.7 (2C), 53.7, 68.1, 114.3, 115.1 (2C), 127.0, 128.5 (2C), 129.1 (2C), 129.9 (2C), 133.8, 135.8, 165.8 (2C), 172.6 (2C) [(*S*,*R*) minor]. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{25}H_{31}N_2O_6$ 455.2182, found 455.2187.

Methyl (2*S*)-2-(3-(2-methoxy-2-oxoethylamino)-2-(4-methoxybenzyl)-3-oxopropanamido)-3-phenylpropanoate (13f,f′). Yield 80%. Yellow oil. ν_{max} cm^{-1} 3425, 3335, 1734, 1676. ^1H NMR ($CDCl_3$): δ 2.86–3.28 (m, 4H), 3.72 (s, 3H), 3.74 (s, 6H), 3.85–3.91 (m, 1H), 3.97–3.99 (m, 2H), 4.72–4.83 (m, 1H), 6.74–7.30 (m, 9H), 8.01 (br, 2H) [(*S*,*S*) major]; 2.86–3.28 (m, 4H), 3.71 (s, 3H), 3.74 (s, 6H), 3.85–3.91 (m, 1H), 4.01–4.03 (m, 2H), 4.72–4.83 (m, 1H), 6.74–7.30 (m, 9H), 8.17 (br, 2H) [(*S*,*R*) minor]. ^{13}C NMR ($CDCl_3$): δ 37.2, 37.8, 41.2, 52.3 (2C), 53.4, 55.1, 56.6, 113.9 (2C), 120.6, 127.0, 128.4 (2C), 129.1 (2C), 129.7 (2C), 135.4, 135.8, 169.4, 169.7, 169.9, 171.3 [(*S*,*S*) major]; 37.3, 37.7, 41.2, 52.2 (2C), 53.2, 55.1, 56.9, 114.0 (2C), 120.7, 127.0, 128.5 (2C), 129.2 (2C), 129.9 (2C), 135.4, 135.8, 169.5, 169.7, 169.9, 171.3 [(*S*,*R*) minor]. HRMS (ESI Q-TOF) m/z $[M + H]^+$ calcd for $C_{24}H_{29}N_2O_7$ 457.1975, found 457.1981.

Acknowledgements

Università degli Studi di Roma ''La Sapienza'' is gratefully acknowledged for financial support. We thank Professor C. G. Frost of the University of Bath for preliminary helpful advice.

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