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Original article

The influence of conformational restriction in the *C*-terminus of growth hormone secretagogues on their potency

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Abstract

In order to obtain more potent growth hormone secretagogues, a comparison of ipamorelin and **NN703** suggested the addition of a polar group at the *C*-terminus of **NN703**. A study was conducted using constrained amines for this purpose. Here, substituted 4-piperidinylamino- and 4-dimethylaminopiperidino-substitutents were found to give the most active compounds. A replacement of the 4-dimethylaminopiperidino-substituent with 4-hydroxypiperidino resulted in a series of compounds, which showed in vitro activity with EC_{50} values in the low nanomolar range, and favourable kinetic properties, such as 40% oral bioavailability. The most promising compound was also tested in a swine in vivo model, resulting in a growth hormone level with a C_{max} of over 40 ng mL^{-1}. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Growth hormone secretagogue; NN703; Peptidomimetic; Oral bioavailability; GH release

1. Introduction

Growth hormone secretagogues (GHSs) are compounds that are able to release growth hormone (GH) through the Ghrelin Pathway. This pathway was known through the eighties as the GHRP Pathway, reflecting the identification of GHRP-6 and related analogues. Through the last decade of the 20th century it was called the GHS Pathway, referring to the identification of small molecule analogues of the GHRP's, and finally after the identification of Ghrelin in 1999 [1], this system is now referred to as the GHrelin Pathway. Ghrelin is a 28-amino acid octanoylated peptide and is released from the stomach. It has a high affinity to the G-protein coupled GHS-1A receptor [2], and is able to release GH in vitro as well as in vivo. Despite the fact that Ghrelin and its corresponding receptor, were only identified in the late nineties a number of orally active growth hormone secretagogues [3,4] have been tested in clinical trials, for indications such as GH deficiency, catabolic states and sleep enhancement [5].

A number of different strategies have been used in the identification of orally active GHSs. One successful strategy has comprised the application of peptidomimetic methods on the pentapeptide ipamorelin [6] [7]. The orally active **NN703** [7,8] was developed by systematic reduction of molecular weight and removal of the hydrogen-donating amide bonds [9]. Extensive work has been done to improve **NN703** by *N*-terminus modifications [10], *C*-terminus modifications [11,12], or libraries with changes in the core of the tripeptide [12]. A recent publication suggests, that the *C*-terminal amide is necessary to obtain a reasonable in vivo activity [13] (Fig. 1).

A comparison of the starting lead compound—ipamorelin—and **NN703** shows, that an additional group with hydrogen-bonding capability at the *C*-ter-

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minus, such as an amine, could be beneficial for the in vivo activity. In the original approach [8] this additional pharmacophore was sacrificed in order to obtain good oral bioavailability. In this paper we want to show our attempts to identify the structural demands as well as an optimal positioning of this additional pharmacophoric group.

2. Chemistry

In our first approach, we used the working-hypothesis, that tertiary amines at the *C*-terminus would be a sufficient pharmacophoric group, if a basic hydrogen-bond acceptor was needed. Tertiary amines are not too polar to inhibit oral absorption of the compound. Therefore, we chose commercially available 1-methylpiperazine or 4-dimelthylaminopiperidine as conformationally of constrained amines for building blocks for the *C*-terminus. In order to have a greater variety constrained amines, we synthesised amines **4**, **13** and **14** starting from the BOC-protected amino acids isonipecotic acid (**1**), prolin (**5**) or nipecotic acid ethyl ester (**6**). In order to synthesise diamine **4**, acid **1** was reacted to amide **2**, which then was reduced with sodium borohydride–iodine to give the BOC-protected amine **3**. After removal of the protection group diamine **4** was isolated. For the synthesis of diamines **13** and **14**, a different strategy was chosen. Starting with the commercially available compounds **5** and **6**, the BOC-protected esters **7** and **8** were prepared. Reduction to the aldehydes **9** and **10** and reductive amination led to the BOC-protected amines **11** and **12**, which then upon deprotection gave the desired diamines **13** and **14** (Fig. 2).

The building blocks for the core *N*-methylated D-amino acids with aromatic side chains [8,14,15] were used. At the *N*-terminus, (2*E*)-5-aminoalk-2-enoic acids [8,10] or 3-aminomethylbenzoic acid [7,16–20] were used as dipeptidomimetics.

The tripeptides **15**–**41** were constructed via a BOC-strategy in solution as described in the literature [8,12] using EDAC and HOAt [21] as coupling reagents and trifluoroacetic acid as reagent for the deprotection of the BOC-groups.

3. Pharmacology

The in vitro-screening of the compounds was performed in rat pituitary cell assay [6,22], where the GH-releasing ability of the compounds were tested. The EC_{50} value was determined for each compound and is given as a mean value of at least two separate experiments. The efficacy was given as a fraction of the maximal stimulation obtained with GHRP-6 [6] as reference compound. The pharmacokinetic properties of compounds with sufficient potency in vitro were subsequently investigated in vivo in beagle dogs with special focus on oral bioavailability. Finally acute GH release was measured for compounds with promising pharmacokinetics in vivo in a single dose swine experiment.

4. Results and discussion

All the tripeptides **15**–**41** showed high or moderate activity as GHSs. The activity was, however, dependent on the positioning of the polar group at the *C*-terminus. Compounds **15**–**18** with a piperazine at the *C*-terminus showed moderate potency in the range of 39–310 nM. Steric bulk at the *N*-terminal amino acid resulted in more active compounds. When, however, an amine was placed one atom further away from the core of the tripeptide, as realised in compounds **19**–**24**, much more potent growth hormone secretagogues were obtained with EC_{50} between 2 and 12 nM. Thus, the extension can be done on both sides of the six-membered ring. It is noteworthy, that the heavily shielded primary amine in compounds **19**–**21** apparently had the same effect as a secondary amine in compounds **22**–**24**. The EC_{50}-values for these compounds were determined to be between 2.5 and12 nM. A further extension of the spacer between the peptide core and the amine as polar group with one carbon atom resulted in the GHS **25** with slightly reduced potency. For the investigation of a

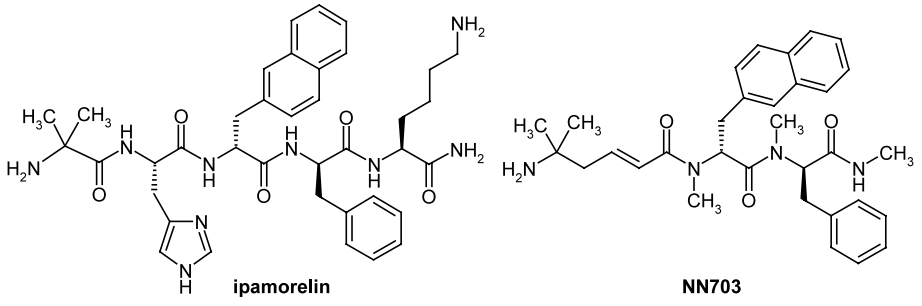


Fig. 1.

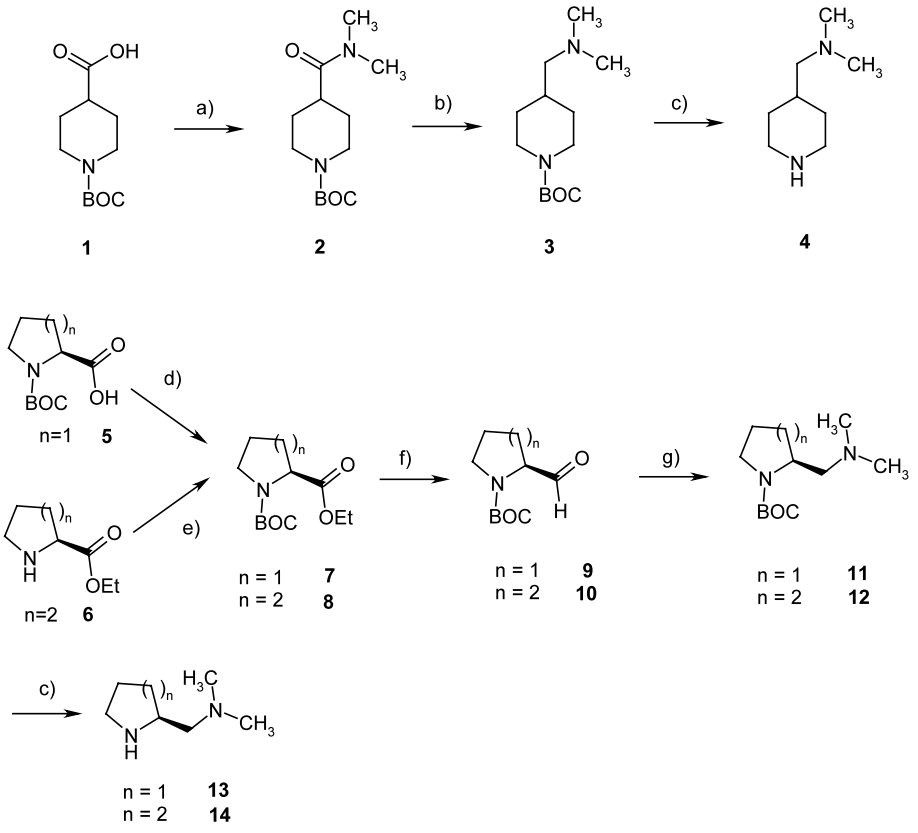
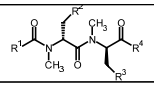


Fig. 2. (a) $HN(CH_3)_2$, HOAt, EDAC; (b) $NaBH_4/I_2$; (c) HCl/EtOAc; (d) EtOH, HOBt, EDAC, DMAP; (e) $(BOC)_2O$, NaOH; (f) DIBAL; (g) $HN(CH_3)_2$, $NaB(OAc)_3H$, HOAc.

geometric change of the spacer, the amines **26** and **27**, with a substituent attached at the 3-position of the piperidine were synthesised. The number of atoms between the peptide core and the *C*-terminal amine in these compounds is the same as in the very active GHSs **19**–**24**. The compounds were, however, less potent with EC_{50} of 25 and 18 nM. Similar results were found with the five-membered ring spacer in compound **28** or **29**. From these data it was concluded that *C*-termini as in **19**–**24** had the best geometrical constitution. From these results with the 4-dimethylaminopiperidine used in **23** and **24** it was decided to use 4-hydroxypiperidine at the *C*-terminus instead. This was expected to improve the oral bioavailability of the compounds, and the strategy seemed to be very successful: The alcohol **30** showed with an EC_{50} of 3 nM even better potency than the corresponding dimethylamine **23**. Similarly, the alcohol **41** had the same activity as the dimethylamine **24**. The thorough change and permutation of the two core and the *N*-terminal amino acids resulted in a number of highly active GHSs. The replacement of the phenyl group with a 2-thienyl-group resulted in the highly active compounds **31**–**34**. Also the 4-fluoro-substitution of the phenyl group was acceptable in a highly active GHS, as it was demonstrated with compound **35**. Here, however, a sharp drop in the activity was observed after a slight change in the *N*-terminal amino acid, giving rise to compound **36**. This sharp change in the activity was dependent on the other building blocks in the tripeptides as demonstrated by the comparison of the D-(2-naphthyl)alanine containing compound **40** and its close analogue **30**. Compounds **37** and **38** with a biphenylylalanine in the core were, however, equipotent to each other despite the change in the *N*-terminal amino acid. A change from the gem-dimethyl-feature at the *N*-terminus to a cyclobutyl-moiety was tolerated with both naphthyl and biphenylyl residues as it was demonstrated with the compounds **39** and **41**. Most of the amino alcohols **30**–**41** were full GHSs, showing efficacies comparable to that of the reference compound GHRP-6. In this series compounds **33**, **34**, **36**, **38**, and **40** with a 5-amino-3,5-dimethylhex-2-enoyl moiety as *N*-terminus were, however, only partial agonists with efficacies between 46 and 75% compared with the GH-release obtained by GHRP-6. The same effect of this particular *N*-terminus could be observed for the diamines. Also here the corresponding compounds **16** and **20** were rather ineffective GHSs despite their high potency. The efficacy was independent of the tested *C*-termini, except for the (*S*)-2-((dimethylamino)methyl)pyrrolidin-1-yl-moiety which reduced the efficacy in compound **28** to 75% compared with the

Table 1
In vitro screening of growth hormone secrstagogues **15**–**41**



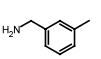
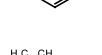
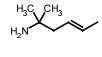
entry	R^1	R^2	R^3	R^4	EC_{50} [nM]	efficacy[a)]
15					135	120
16					39	65
17					46	95
18					310	75
19					2.5	100
20					3.3	90
21					2	75
22					2.5	110
23					12	100
24					6	100
25					21	85
26					25	95
27					18	105
28					31	75
29					22	85

Table 1 (*Continued*)

entry	R^1	R^2	R^3	R^4	EC_{50} [nM]	efficacy[a)]
30					3	85
31					17	95
32					4	105
33					11	60
34					8	85
35					16	100
36					60	70
37					4.5	95
38					5	75
39					2.5	100
40					9.5	45
41					2	75
42					8	105

[a] % of maximal stimulation of GH-release obtained by GHRP-6.

corresponding compounds with different *C*-termini **15**, **19**, **22** or **23**, which efficacies lie in a range from 100 to 120% (Table 1).

The pharmacokinetic properties of the most promising compounds from the in vitro-screening were tested in a beagle dog model. In contrast to compounds **19**, **23**, and **30**, compounds **22** and **39** showed surprisingly long plasma half lives. Poor oral bioavailability was expected when the polar group at the *C*-terminus was an amine-moiety, we did not expect good oral bioavailability, because two amine groups would result in a doubly charged species. Indeed, we found, that the diamines **19**, **22**, and **23** were not orally absorbed and further screening of diamines was omitted. Instead amino alcohols were used because better oral bioavailability might be accomplished by substitution of the amine with a hydroxyl group. This hypothesis was explored with the compounds **30** and **39**. Even though the result of amino alcohol **30** was rather disappointing, the amino alcohol **39** was orally absorbed very readily (Table 2).

In order to study the influence of the ring-restriction at the *C*-terminus on the pharmacodynamic and

Table 2
Pharmacokinetic screening results in beagle dogs

Entry	V_z [a] (l kg^{-1})	CL [b] (ml min^{-1} kg^{-1})	$t_{1/2}$ [c] (min)	f_{po} [d] (%)
19	0.71	57	8.4	0
22	0.40	5	53.4	0
23	0.37	32	7.8	0
30	0.85	35	16.8	0
39	8.35	27	180	40
42	6.57	13	354	1

[a] Volume of distribution (i.v.).
[b] Clearance (i.v.).
[c] Plasma half life (i.v.).
[d] Oral bioavailability.

pharmacokinetic properties of the compounds, we synthesised the open-chain analogue of compound **30**, the aminoalcohol **42**. In both molecules, a hydroxyl group is placed on a three-carbon spacer at the *C*-terminal amide bond. As it can be seen from Table 1, aminoalcohol **42** was similarly potent as compound **30**, while higher efficacy was observed for compound **42**. The compounds were, however, very different with respect to pharmacokinetics. The volume of distribution for compound **42** was with 6.57 l kg^{-1} much higher than for compound **30** 0.85 l kg^{-1}) and accordingly the plasma half life was almost one order of magnitude higher for compound **42**. The oral bioavailability of the open-chain analogue **42** was very poor and similar to that of the ring-restricted compound **30**.

The pharmacokinetic data in combination with the results form the functional in vitro-screening, clearly suggested to test compound **39** in vivo for GH release. A swine model was chosen, because the resemblance to humans with respect to physiology in general and endocrinology in particular is better than for other common laboratory animals such as rat or dog [6–8]. A dose-dependent GH secretion was observed, when compound **39** was administered orally. As it is shown in Fig. 3, at a dose of 5000 nmol kg^{-1} the maximal concentration of GH, C_{max}, was found to be over 40 ng mL^{-1}.

5. Conclusion

The strategy to determine the best position of an additional amine-pharmacophore of compounds similar to **NN703**, and a subsequent change to a hydroxyl group proved to be successful. Substituted 4-piperidinylamino-substitutents and 4-dimethylaminoperidino-substitutents were the most suitable *C*-termini, resulting in the most active compounds in vitro (compounds **19**–**24**). None of those compounds were orally bioavailable, probably as a result of the *C*-terminal amino group. The change to a hydroxyl group, which was accomplished by the incorporation of a 4-hydroxypiperidino-moiety gave, however, in one instance the orally absorbed compound **39**, which also showed very promising in vivo efficacy in a swine model.

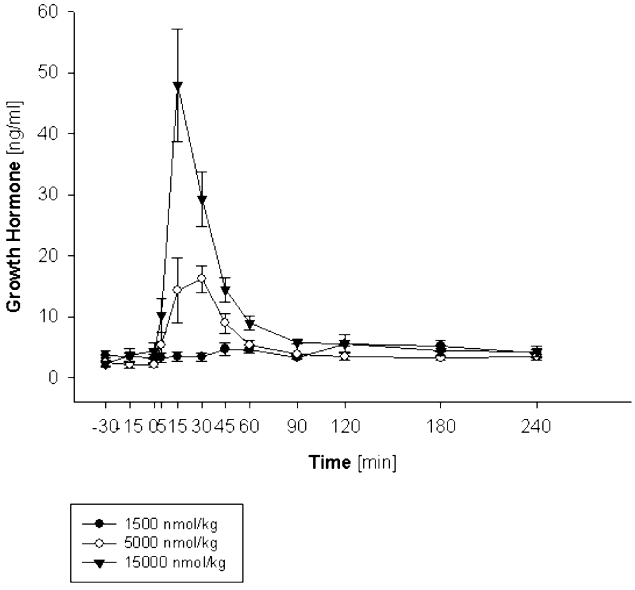


Fig. 3. Growth hormone response to increasing doses of compound **39**. Pigs were dosed p.o. at time 0. Data are mean ± SEM.

6. Experimental

6.1. Pharmacology

6.1.1. In vitro studies

The in vitro studies were performed as described in the literature [6,22].

6.1.2. Pharmacokinetic studies in dogs

The oral bioavailability of each compound was studied in a single male beagle dog, except for NN703 which was studied in two male and two female dogs. The dogs were fasted overnight prior to dosing. Diet was withheld for at least 3 h postdosing. A 1-week washout period separated oral (p.o.) and intravenous (i.v.) dosing. The compounds were administered in a vehicle of citrate–phosphate buffer, pH 5.0. For p.o. administration the dogs received a dose of 2.5 mg kg^{-1} of body weight via gavage. For i.v. administration the dogs received a dose of 0.5 mg kg^{-1} of body weight as a bolus in a hind leg vein. EDTA blood samples were drawn from a front leg vein at intervals up to 6 h after dosing. Blood samples were placed on an ice–water bath immediately after sampling. Plasma was separated by centrifugation and stored frozen pending analysis. An HPLC assay with UV detection and solid phase extraction was developed for each compound. Analytical C8 columns and disposable C3 extraction columns were used. The oral bioavailability was calculated as the total area under the plasma concentration versus time curve following p.o. administration divided by the area following i.v. administration, appropriately corrected for dose.

6.1.3. Oral dose–response to compound *39* in pigs

6.1.3.1. Animals. Female Danish slaughter pigs were ordered with a weight range between 35 and 40 kg life weight at time of delivery. The pigs had at least 2 weeks to acclimatise to their new environment before they entered the experiment.

6.1.3.2. Surgery. Pigs were fasted for 24 h before surgery, but had free access to water. On the day of

surgery the animals were anaesthetised with a mixture of: Zoletil, Xylazin, Ketaminol and Methadon. One mL per 15 kg BW of this mixture was given i.m. to induce anaesthesia within 5 min after injection. Atropin (0.05 mg kg^{-1}) i.m. was given with the above mixture. Once the animal had lost consciousness an ear vein catheter was inserted to gain venous access. A standard dose of antibiotics (Novocilin) was given i.v. at that time. The flank and the neck of the animal was then carefully clipped and shaved outside the operating theatre. The shaved areas were thoroughly disinfected with chlorhexidin and iodine solution and all surgical procedures were performed under strictly aseptic conditions. The animal was positioned on the operating table and an endotracheal tube was inserted. Total relaxation of the pig was induced by administration of a 5–10 mL Propofol (5 mg kg^{-1}) (Rapinovet) bolus. After intubation anaesthesia was maintained by 1–1.5% isoflurane in 2 l min^{-1} 100% oxygen. The pigs were kept on spontaneous respiration. All vital data were noted on the anaesthesia protocol. The pigs were also connected to a saline drip infusion (500 mL h^{-1}) to stabilise their cardio–vascular system. A laparotomy was performed with the pig in left recumbency. The stomach was gently retracted and a purse–string suture was placed in the muscular part of the stomach wall 5–6 cm from the pyloric end. A hole was made with a diatherimic needle and a silicone catheter was inserted into the gastric cavity. The catheter was held in place by two silicone cuffs, one inside and one outside the stomach wall. The wound was closed using standard techniques and the catheter was exteriorised at the back of the animal. The pig was then placed in dorsal recumbency and a paramedian skin incision was made over the jugular furrow. The jugular vein and carotid artery were dissected and fitted on 20 cm with a silicone catheter. The catheters were tunnelled to the back of the neck and the wound was closed using standard techniques.

6.1.3.3. Post surgical care. All animals received a standard 4 days course of antibiotics (Novocilin). Analgesia (Fenadyne, 0.5 mL per 10 kg BW, i.v.) was provided for 3 days after surgery. All blood sampling catheters are flushed daily with 10 mL of saline for the first 3 days and thereafter very third.

6.1.3.4. Experimental procedures. The pigs were dosed orally at 09:00 h after an overnight fast. Compound **39**, as an acetate salt, was freshly dissolved in sterile water to the needed concentration and dosed as 20 mL bolus. The stomach catheters were flushed with 150 mL water for injection. Blood samples (3.5 mL) were taken at −30, −15, 0, 5, 15, 30, 45, 60, 90, 120, 180 and 240 min relative to the dosing of the animals. The blood was transferred to tubes containing EDTA, placed on ice and centrifuged at least once every 60 min (3000 rpm, 10 min, 4 °C). Plasma was frozen at −20 °C until analysis. The pigs were dosed with increasing doses (1500, 5000, 15 000 nmol kg^{-1} BW) of compound **39** with a 48 h wash-out period between doses. The plasma samples were analysed for growth hormone by radioimmuno assay, fully validated for porcine plasma.

6.2. Chemistry

Amino acids were purchased from Synthetech or Bachem. The MS analyses were performed on a PE Sciex API 100 LC–MS System using a Waters® 3 × 150 mm 3.5 m C-18 Symmetry column and positive ionspray with a flow rate of 20 mL min^{-1}. The column was eluted with a linear gradient of 5–90% acetonitrile, 85–0% water and 10% trifluoroacetic acid (0.1%)–water in 15 min at a flow rate of 1 mL min^{-1}. NMR spectra were obtained at 400 MHz on a Bruker instrument.

6.2.1. HPLC-methods

Two different elution conditions were used.

6.2.1.1. Method A1. The RP–HPLC analysis was performed using UV detections at 214, 254, 276, and 301 nm on a Vydac 218TP54 4.6 × 250 mm 5 m C-18 silica column (The Seperations Group, Hesperia), which was eluted at 1 mL min^{-1} at 42 °C. The column was equilibrated with 5% acetonitrile in a buffer consisting of 0.1 M ammonium sulphate, which was adjusted to pH 2.5 with 4 M sulphuric acid. After injection the sample was eluted by a gradient of 5–60% acetonitrile in the same buffer during 50 min.

6.2.1.2. Method B1. The RP–HPLC analysis was performed using UV detections at 214, 254, 276, and 301 nm on a Vydac 218TP54 4.6 × 250 mm 5 m C-18 silica column (The Seperations Group, Hesperia), which was eluted at 1 mL min^{-1} at 42 °C. The column was equilibrated with 5% acetonitrile–0.1% TFA–water and eluted by a gradient of 5% acetonitrile–0.1% TFA–water to 60% acetonitrile–0.1% TFA–water during 50 min.

6.2.2. Protocols

6.2.2.1. 4-(Dimethylcarbamoyl)piperidine-1-carboxylic acid tert-butyl ester (2). 1-(*tert*-Butoxycarbonyl)piperidine-4-carboxylic acid (**1**) (8.0 g, 35 mmol) was dissolved in dichloromethane (70 mL) and *N*,*N*-dimethylformamide (35 mL). 1-Hydroxy-7-azabenzotriazole (4.75 g, 35 mmol) was added. The solution was cooled to 0 °C. *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (6.69 g, 35 mmol) was added. The reaction mixture was stirred for 20 min at

0 °C. A 5.6 M solution of dimethylamine in ethanol (37 mL, 209 mmol) was added. The reaction mixture was stirred for 3 days, while it was warming up to room temperature (r.t.). It was diluted with ethyl acetate (400 mL) and washed with a 10% aqueous solution of sodium hydrogen sulphate (400 mL). The aqueous phase was extracted with ethyl acetate (2 × 200 mL). The combined organic layers were washed with a saturated aqueous solution of sodium hydrogen carbonate (300 mL) and dried over magnesium sulphate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (300 g), using dichloromethane–methanol 20:1 as eluent, to give 4.56 g of amide **2**.

^{1}H-NMR ($CDCl_3$): δ 1.47 (s, 9H); 1.70 (m, 4H); 2.60–2.90 (m, 3H); 2.96 (s, 3H); 3.08 (s, 3H); 4.17 (m, 2H).

*6.2.2.2. 4-((Dimethylamino)methyl)piperidine-1-carboxylic acid tert-butyl ester (**3**).* At 0 °C a solution of amide **2** (4.56 g, 18 mmol) in tetrahyrofuran (80 mL) was added dropwise to a suspension of sodium borohydride (1.61 g, 43 mmol) in tetrahydrofuran (80 mL). The reaction mixture was stirred for 20 min at 0 °C. A solution of iodine 4.51 g, 18 mmol) in tetrahydrofuran (80 mL) was added dropwise at 0 °C. The reaction mixture was heated to reflux for 16 h. It was cooled to 4 °C. Methanol (200 mL) was added dropwise. The solvent was removed in vacuo. The residue was dissolved in a 20% aqueous solution of sodium hydroxide (200 mL) and *tert*-butyl methyl ether (150 mL). The phases were separated. The aqueous phase was extracted with *tert*-butyl methyl ether (3 × 100 mL). The combined organic layers were dried over magnesium sulphate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (100 g), using dichloromethane–methanol–25% aqueous ammonia (100:10:1) as eluent, to give 4.07 g of amine **3**.

^{1}H-NMR ($CDCl_3$): δ 1.22 (m, 2H); 1.44 (s, 9H); 1.85 (d, 2H); 2.09 (m, 1H); 2.61 (s, 6H); 2.65 (m, 2H); 2.78 (t, 2H); 4.05 (d, 2H).

*6.2.2.3. N,N-Dimethyl-N-((piperidin-4-yl)methyl)amine (**4**).* A 3 M solution of hydrogen chloride in ethyl acetate (120 mL, 360 mmol) was added to a solution of amine **3** (2.0 g, 14 mmol) in ethyl acetate (50 mL). The reaction mixture was stirred for 30 min at r.t. The solvent was removed in vacuo to give 2.3 g of the crude dihydrochloride salt of diamine **4**, which was used without purification for the next step.

^{1}H-NMR ($CDCl_3$, selected values): δ 1.48 (m, 2H); 1.92 (s, 6H); 3.22 (d, 2H).

*6.2.2.4. (2S)-Pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-ethyl ester (**7**).* *N-tert*-Butoxycarbonylprolin (24.38 g, 113 mmol) was dissolved in dichloromethane (60 mL). Ethanol (7.9 mL, 135 mmol) and 1-hydroxybenzotriazole (15.25 g, 113 mmol) and 4-dimethylaminopyridine (1.52 g, 12.5 mmol) were added. The solution was cooled to 0 °C. *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (23.88 g, 113 mmol) was added. The reaction mixture was stirred for 16 h, while it was warming up to r.t.. Ethyl acetate (400 mL) was added. It was washed with a 10% aqueous solution of sodium hydrogen sulphate (300 mL). The aqueous phase was extracted with ethyl acetate (3 × 200 mL). The combined organic layers were washed with a saturated aqueous solution of sodium hydrogen carbonate (300 mL) and dried over magnesium sulphate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (400 g), using ethyl acetate (1:4) as eluent, to give 17.11 g of ester **7**.

^{1}H-NMR ($CDCl_3$): δ 1.28 (m, 3H); 1.43 and 1.46 (both s, together 9H); 2.95 (m, 3H); 2.22 (m, 1H); 3.50 (m, 2H); 4.18 and 4.30 (m and dd, together 3H).

*6.2.2.5. (3R)-Piperidine-1,3-dicarboxylic acid 1-tert-butyl ester 3-ethyl ester (**8**).* (*R*)-Ethyl nipetcotate tartrate (10.0 g, 32.5 mmol) were suspended in tetrahydrofuran (90 mL). An 1 N solution of sodium hydroxide in water (98 mL, 98 mmol) was added. A solution of *di-tert*-butyl dicarbonate (7.10 g, 32.5 mmol) in tetrahydrofuran (90 mL) was added. The reaction mixture was stirred for 16 h at r.t. Ethyl acetate (400 mL) was added. The reaction mixture was washed with a 10% aqueous solution of sodium hydrogen sulphate (400 mL). The aqueous solution was extracted with ethyl acetate (2 × 200 mL). The combined organic layers were washed with a saturated aqueous solution of sodium hydrogen carbonate (200 mL) and dried over magnesium sulphate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (90 g), using ethyl acetate–heptane 1:4 as eluent, to give 4.13 g of ester **8**.

^{1}H-NMR ($CDCl_3$): δ 1.27 (t, 3H); 1.48 (s, 9H); 1.54 (m, 1H); 1.62 (m, 1H); 1.73 (m, 2H); 2.05 (m, 1H); 2.45 (m, 1H); 2.81 (m, 1H); 2.98 (br, 1H); 3.93 (m, 1H); 4.14 (q, 1H).

*6.2.2.6. N-t-Butyloxycarbonyl-(S)-prolinal (**9**).* At −78 °C, a 1.2 M solution of diisobutylaluminum hydride (31.7 mL, 38 mmol) in toluene was added dropwise to a solution of ester **7** (4.02 g, 16.5 mmol) in diethyl ether (15 mL). The reaction mixture was stirred for 3 h at −78 °C. Water (9.9 mL) was added dropwise. The reaction mixture was warmed to r.t. The mixture was filtered through a plug of celite. The celite was washed with *tert*-butyl methyl ether (3 × 100 mL). The combined organic layers were dried over magnesium sulphate. The solvent was removed in vacuo, to give 2.34

g of crude aldehyde **9**, which was used for the next step without further purification.

^{1}H-NMR ($CDCl_3$): δ 1.42 and 1.47 (both s, together 9H); 1.70–2.20 (m, 4H); 3.20–4.30 (m, 3H); 9.45 and 9.55 (both s, together 1H).

*6.2.2.7. (3R)-3-Formylpiperidine-1-carboxylic acid tert-butyl ester (**10**).* A 1.2 M solution of diisobutylaluminum hydride in toluene (30.8 mL, 36.9 mmol) was added at −78 °C to a solution of ester **8** (4.13 g, 16.1 mmol) in diethyl ether (30 mL). The reaction mixture was stirred for 2.5 h at −78 °C. Water (9.6 mL) was added dropwise. The reaction mixture was warmed to r.t. The precipitation was removed by filtration through a plug of celite. The celite was washed with *tert*-butyl methyl ether (3 × 100 mL). The liquids were combined and dried over magnesium sulphate. The solvent was removed in vacuo, to give 1.94 g of crude aldehyde **10**, which was used for the next step without further purification.

^{1}H-NMR ($CDCl_3$): δ 1.45 (s, 9H); 1.67 (m, 2H); 1.95 (m, 1H); 2.43 (m, 1H); 3.10 (m, 1H); 3.32 (dd, 1H); 3.52 (d, 1H); 3.66 (m, 1H); 3.95 (m, 1H); 9.69 (s, 1H).

*6.2.2.8. (2S)-2-((Dimethylamino)methyl)pyrrolidine-1-carboxylic acid tert-butyl ester (**11**).* Crude aldehyde **9** (2.34 g, 11.7 mmol) was dissolved in dichloromethane (90 mL). A 5.6 M solution of dimethylamine in ethanol (4.19 mL, 23.5 mmol) was added. Mol sieves (0.4 nm, 10.0 g) was added. Sodium triacetoxyborohydride (7.47 g, 35.2 mmol) and glacial acetic acid (1.34 mL, 23.5 mmol) were added successively. The reaction mixture was stirred for 3 days. It was filtered through a plug of celite. The celite was washed with methanol (150 mL). An 1 N aqueous solution of sodium hydroxide (150 mL) and *tert*-butyl methyl ether (150 mL) were added. The phases were separated. The aqueous phase was extracted with *tert*-butyl methyl ether (3 × 100 mL). The combined organic layers were dried over magnesium sulphate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (90 g), using dichloromethane–methanol–25% aqueous ammonia (100:10:1) as eluent, to give 1.29 g of amine **11**.

^{1}H-NMR ($CDCl_3$): δ 1.48 (s, 9H); 1.90 (m, 4H); 2.15 and 2.23 (AB, 2H); 2.26 (s, 6H); 3.31 (br, 2H); 3.85 (br, 1H).

*6.2.2.9. (3S)-3-(Dimethylaminomethyl)piperidine-1-carboxylic acid tert-butyl ester (**12**).* A solution of crude aldehyde **10** (1.94 g, 9.1 mmol) in dichloromethane (80 mL) was prepared. A 5.6 M solution of dimethylamine in ethanol (3.2 mL, 18.2 mmol) and molsieves were added successively. Sodium triacetoxyborohydride (5.78 g, 27.3 mmol) was added to this mixture. Acetic acid (1.04 mL, 18.2 mmol) was added. The reaction mixture was stirred for 16 h at r.t. An 1 N aqueous solution of sodium hydroxide (70 mL) and *tert*-butyl methyl ether (70 mL) were added. The phases were separated. The aqueous solution was extracted with *tert*-butyl methyl ether (3 × 70 mL). The combined organic layers were dried over magnesium sulphate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (40 g), using dichloromethane–methanol–25% aqueous ammonia (100:10:1) as eluent, to give 866 mg of amine **12**.

^{1}H-NMR ($CDCl_3$): δ 1.10 (m, 1H); 1.45 (s, 9H), 1.45 (m, 1H); 1.64 (m, 2H); 1.85 (m, 1H); 2.10 (m, 2H); 2.20 (s, 6H); 2.50 (br, 1H); 2.79 (m, 1H); 3.95 (m, 2H).

*6.2.2.10. N-Dimethyl-N-(((2S)-pyrrolidin-2-yl)methyl)-amine (**13**).* A 2.7 M solution of hydrogen chloride in ethyl acetate (75 mL, 202 mmol) was given to a solution of amine **11** (1.29 g, 5.65 mmol) in ethyl acetate (30 mL). The reaction mixture was stirred for 30 min at r.t. The solvent was removed in vacuo to give 1.36 g of the crude dihydrochorlide salt of diamine **13**, which was used for the next step without further purification.

^{1}H-NMR ($CDCl_3$): δ 1.90 (m, 2H); 2.17 (m, 1H); 2.40 (m, 1H); 2.90 (m, 2H); 3.14 (s, 6H); 3.55 (m, 2H); 4.35 (m, 1H).

*6.2.2.11. N,N-Dimethyl-N-(((3R)-piperidin-3-yl)methyl)amine (**14**).* Amine **12** (1.25 g, 5.15 mmol) was dissolved in ethyl acetate (30 mL). A 2.7 M solution of hydrogen chloride in ethyl acetate (75 mL, 203 mmol) was added. The reaction mixture was stirred for 45 min at r.t. The solvent was removed in vacuo to give 976 mg of the crude dihydrochloride salt of diamine **14**, which was used for the next step without further purification.

^{1}H-NMR (CD_3OD): δ 1.42 (m, 1H); 1.86 (m, 1H); 2.00 (m, 2H); 2.38 (m, 1H); 2.85 (t, 1H); 2.95 (s, 6H); 2.98 (m, 1H); 3.16 (m, 2H); 3.42 (m, 1H); 3.53 (m, 1H).

*6.2.2.12. (2E)-5-Amino-5-methylhex-2-enoic acid N-((1R)-1-{N-[(1R)-1-benzyl-2-(4-methylpiperazin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide (**15**).* Two hundred and ninety-eight mg of compound **15** were prepared as described for compound **26** using *N*-methylpiperazine, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-phenylpropionic acid, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino-3-(2-naphthyl))propionic acid, and (2*E*)-5-(butoxycarbonylamino)-5-methylhex-2-enoic acid.

^{1}H-NMR ($CDCl_3$; selected values) δ 1.24 (s, 6H); 1.65 (s, 3H); 2.35 (s, 3H); 2.80 (s, 3H); 5.68 (dd, 1H); 5.78 (dd, 1H); 6.18 (dd, 1H); 6.95(m, 1H); 7.15–7.80 (m, 12H).

HPLC: $T_{ret} = 25.03$ min (100%, 254 nm, A1); $T_{ret} = 27.50$ min (100%, 254 nm, B1).

MS (ES): $m/z = 598.4$; calc. for ($[M + H]^+$: 598.4).

*6.2.2.13. (2E)-5-Amino-3,5-dimethylhex-2-enoic acid N-((1R)1-{N-[(1R)1-benzyl-2-(4-methylpiperazin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide (**16**).* One hundred and thirty mg of compound **16** were prepared as described for compound **26** using *N*-methylpiperazine, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-phenylpropionic acid, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino-3-(2-naphthyl))propionic acid, and (2*E*)-5-(butoxycarbonylamino)-3,5-methylhex-2-enoic acid.

^{1}H-NMR ($CDCl_3$; selected values) δ 1.18 (s, 6H); 1.68 (s, 3H); 1.95 (s, 3H); 2.30 (s, 3H); 2.85 (s, 3H); 3.40 (dd, 1H); 3.54–3.75 (m, 2H); 5.68–5.85 (m, 3H); 7.15–7.80 (m, 12H).

HPLC: $T_{ret} = 25.70$ min (99%, 254 nm, A1); $T_{ret} =$ 28.27 min (93%, 254 nm, B1).

MS (ES): $m/z = 612.4$; calc. for ($[M + H]^+$: 612.4).

*6.2.2.14. (2E)-4-(1-Aminocyclobutyl)but-2-enoic acid N-((1R)1-{N-[(1R)1-benzyl-2-(4-methylpiperazin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide (**17**).* One hundred and eighty mg of compound **17** were prepared as described for compound **26** using *N*-methylpiperazine, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-phenylpropionic acid, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino-3-(2-naphthyl))propionic acid, and (2*E*)-4-(1-(butoxycarbonylamino)cyclobutyl)but-2-enoic acid.

^{1}H-NMR ($CDCl_3$; selected peaks) δ 1.62 (s, 3H); 2.35 (s, 3H); 2.80 (s, 3H); 5.70 (dd, 1H); 5.80 (dd, 1H); 6.22 (d, 1H); 6.98 (m, 1H); 7.15–7.80 (m, 12H).

HPLC: $T_{ret} = 25.88$ min (99%, 254 nm, A1); $T_{ret} =$ 28.65 min (99%, 254 nm, B1).

MS (ES): $m/z = 610.4$; calc. for ($[M + H]^+$: 610.4).

*6.2.2.15. 3-Aminomethyl-N-((1R)-1-{N-[(1R)-1-benzyl-2-(4-methylpiperazin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylbenzamide (**18**).* Three hundred and thirty mg of compound **18** was prepared as described for example **26** using *N*-methylpiperazine, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-phenylpropionic acid, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino-3-(2-naphthyl))propionic acid, and 3-((*tert*-butoxycarbonylamino)-methyl)benzoic acid.

^{1}H-NMR ($CDCl_3$; selected values) δ 3.30 (m, 1H); 3.50 (dd, 1H); 3.75 (m, 1H); 3.95 (s, 2H); 5.78 (t, 1H); 3.88 (m, 1H); 7.00–7.80 (16H).

HPLC: $T_{ret} = 24.55$ min (95%, 254 nm, A1); $T_{ret} =$ 26.52 min (93%, 254 nm, B1).

MS (ES): $m/z = 606.4$; calc. for ($[M + H]^+$: 606.3).

*6.2.2.16. (2E)-5-Amino-5-methylhex-2-enoic acid N-methyl-N-((1R)-1-{N-methyl-N-[(1R)-2-phenyl-1-((2,2,6,6-tetramethylpiperidin-4-yl)carbamoyl)ethyl]carbamoyl}-2-(2-naphthyl)ethyl)amide (**19**).* Three hundred and seventy-eight mg of compound **19** were prepared as described for compound **26** using 4-amino-2,2,6,6-tetramethylpiperidin, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-phenylpropionic acid, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino-3-(2-naphthyl))-propionic acid, and (2*E*)-5-(butoxycarbonylamino)-5-methylhex-2-enoic acid.

^{1}H-NMR ($CDCl_3$; selected values) δ 1.25 (s, 6H); 1.40 (two s, 6H); 1.52 (two s, 6H); 2.92 (s, 3H); 3.02 (two s, 3H); 5.10 (dd, 1H); 5.50 (dd, 1H); 6.15 (d, 1H); 6.75 (m, 1H); 7.00–8.00 (m, 12H).

HPLC: $T_{ret} = 29.27$ min (98%, 254 nm, A1); $T_{ret} =$ 31.67 min (98%, 254 nm, B1).

MS (ES): $m/z = 654.8$; calc. for ($[M + H]^+$: 654.4).

*6.2.2.17. (2E)-5-Amino-3,5-dimethylhex-2-enoic acid N-methyl-N-((1R)-1-{N-methyl-N-[(1R)-2-phenyl-1-((2,2,6,6-tetramethylpiperidin-4-yl)carbamoyl)ethyl]carbamoyl}-2-(2-naphthyl)ethyl)amide (**20**).* Three hundred and twenty-seven mg of compound **20** were prepared as described for compound **26** using 4-amino-2,2,6,6-tetramethylpiperidin, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-phenylpropionic acid, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino-3-(2-naphthyl))-propionic acid, and (2*E*)-5-(butoxycarbonylamino)-3,5-methylhex-2-enoic acid.

^{1}H-NMR ($CDCl_3$; selected values) δ 3.92–4.30 (m, 1H); 5.05–5.88 (m, 3H); 7.00–7.80 (m, 12H).

HPLC: $T_{ret} = 29.80$ min (96%, 254 nm, A1); $T_{ret} =$ 32.43 min (93%, 254 nm, B1).

MS (ES): $m/z = 668.4$; calc. for ($[M + H]^+$: 668.4).

*6.2.2.18. 3-Aminomethyl-N-methyl-N-((1R)1-{N-methyl-N-[(1R)-2-phenyl-1-((2,2,6,6-tetramethylpiperidin-4-yl)carbamoyl)ethyl]carbamoyl}-2-(2-naphthyl)ethyl)-benzamide (**21**).* Three hundred and seventy two mg of compound **21** were prepared as described for compound **26** using 4-amino-2,2,6,6-tetramethylpiperidin, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-phenylpropionic acid, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino-3-(2-naphthyl))propionic acid, and 3-((*tert*-butoxycarbonylamino)methyl)benzoic acid.

^{1}H-NMR ($CDCl_3$; selected values) δ 3.60–3.85 (m, 2H); 3.90–4.30 (m, 1H); 5.25–5.95 (m, 2H); 6.70–7.90 (m, 16H).

HPLC: $T_{ret} = 29.27$ min (93%, 254 nm, A1); $T_{ret} =$ 31.55 min (93%, 254 nm, B1).

MS (ES): $m/z = 662.4$; calc. for ($[M + H]^+$: 662.4).

*6.2.2.19. (2E)-5-Amino-5-methylhex-2-enoic acid N-methyl-N-[(1R)-1-(N-methyl-N-{(1R)-1-[N-methyl-N-(1-methylpiperidin-4-yl)carbamoyl]-2-phenylethyl}carbamoyl)-2-(2-naphthyl)ethyl]amide (**22**).* Two hundred and ten mg of compound **22** were prepared as described for compound **26** using 1-methyl-4-(methylamino)-piperidine, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-phenylpropionic acid, (2*R*)-2-(*N*-(*tert*-bu-

toxycarbonyl) - *N* - methylamino - 3 - (2 - naphthyl))propionic acid, and (2*E*)-5-(butoxycarbonylamino)-5-methylhex-2-enoic acid.

^{1}H-NMR ($CDCl_3$; selected values) δ 5.50–6.08 (m, 2H); 6.20–6.70 (m, 2H); 7.10–7.85 (m, 12H).

*6.2.2.20. (2E)-5-Amino-5-methylhex-2-enoic acid N-((1R)-1-{N-[(1R)-1-benzyl-2-(4-(dimethylamino)piperidin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide (**23**).* Two hundred and fourteen mg of compound **23** were prepared as described for compound **26** using 4-(dimethylamino)piperidine hydrochloride salt, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-phenylpropionic acid, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl) - *N* - methylamino - 3 - (2 - naphthyl))propionic acid, and (2*E*)-5-(butoxycarbonylamino)-5-methylhex-2-enoic acid.

^{1}H-NMR: ($CDCl_3$; selected values) δ 1.40 (s, 6H); 2.00 (s, 6H); 4.42–4.85 (2H); 5.45–5.90 (m, 2H); 6.28 (dd, 1H); 6.85(m, 1H); 7.10–7.85(m, 12H).

MS (ES): $m/z = 626.2$; calc. for ($[M + H]^+$: 626.4).

*6.2.2.21. (2E)-4-(1-Aminocyclobutyl)but-2-enoic acid N-((1R)-1-{N-[(1R)-1-benzyl-2-(4-(dimethylamino)piperidin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide (**24**).* Two hundred and forty-nine mg of compound **24** were prepared as described for compound **26**, using 4-*N*,*N*-dimethylpiperazine, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-phenylpropionic acid, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl) - *N* - methylamino - 3 - (2 - naphthyl))propionic acid, and (2*E*)-4-(1-(butoxycarbonylamino)cyclobutyl)but-2-enoic acid.

^{1}H-NMR ($CDCl_3$; selected peaks) δ 1.90 (s, 3H); 2.38 (s, 3H); 2.45 and 2.47 (two s, 3H) 2.78 and 2.80 (two s, 3H); 6.32 (dd, 1H); 6.90 (m, 1H); 7.15–7.84 (m, 12H).

MS (ES): $m/z = 638.4$; calc. for ($[M + H]^+$: 638.4).

*6.2.2.22. (2E)-5-Amino-5-methylhex-2-enoic acid N-((1R)-1-{N-[(1R)-1-benzyl-2-(4-((dimethylamino)methyl)piperidin1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide (**25**).* Five mg of compound **25** were prepared as described for compound **26**, using diamine **4**, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-phenylpropionic acid, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-(2-naphthyl)propionic acid, and (2*E*)-5-(*tert*-butoxycarbonylamino)-5-methylhex-2-enoic acid.

^{1}H-NMR ($CDCl_3$, selected values): δ 1.20 (s, 6H); 2.28, 2.32, 2.41, 2.49, 2.56, 2.57, 2.82, and 2.83 (all s, together 12H); 5.58, 5.78, and, 5.92 (m, m, and dd, together 2H); 6.16 and 6.19 (both d, together 1H); 7.00 (m, 1H).

HPLC: $T_{ret} = 39.23$ min (94%, 254 nm, A1); $T_{ret} =$ 41.55 min (95%, 254 nm, B1).

MS (ES): $m/z = 640.4$; calc. for ($[M + H]^+$: 640.4).

*6.2.2.23. (2E)-5-Amino-5-methylhex-2-enoic acid N-((1R)-1-{N-[(1R)-1-benzyl-2-((3S)-3-(dimethylaminomethyl)piperidin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide (**26**).* At 0 °C *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (870 mg, 4.54 mmol) was added to a solution (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-phenylpropionic acid (1.27 g, 4.54 mmol) and 1-hydroxy-7-azabenzotriazole (617 mg, 4.54 mmol) in dichloromethane (20 mL) and *N*,*N*-dimethylformamide (10 mL). The reaction mixture was stirred for 20 min at 0 °C. A solution of the crude dihydrochloride salt of diamine **14** (976 mg, 4.54 mmol) in dichloromethane (20 mL) and *N*,*N*-dimethylformamide (10 mL) and ethyldiisopropylamine (3.9 ml, 22.7 mmol) were added successively. The reaction mixture was stirred for 3 days, while it was warming up to r.t. Ethyl acetate (300 ml) was added. The solution was washed with a saturated aqueous solution of sodium hydrogen carbonate (300 ml). The aqueous phase was extracted with ethyl acetate (2 × 200 ml). The combined organic layers were dried over magnesium sulphate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (90 g), using dichloromethane–methanol–25% aqueous ammonia (100:10:1) as eluent, to give 1.69 g of *N*-[(1*R*)-1-benzyl-2-((3*S*)-3-(dimethylaminomethyl)piperidin-1-yl)-2-oxoethyl]-*N*-methylcarbamic acid *tert*-butyl ester.

^{1}H-NMR ($CDCl_3$, selected values): δ 1.20, 1.24, 1.31, and 1.32 (all s, together 9H); 2.12, 2.13, and 2.18 (all s, together 6H); 2.81 (m, 3H); 4.97 and 5.30 (both m, together 1H); 7.05–7.35 (m, 5H).

At 0 °C, trifluoroacetic acid (25 ml) was added to a solution of *N*-[(1*R*)-1-benzyl-2-((3*S*)-3-(dimethylaminomethyl)piperidin-1-yl)-2-oxoethyl]-*N*-methylcarbamic acid *tert*-butyl ester (1.69 g, 4.2 mmol) in dichloromethane (25 ml). The reaction mixture was stirred for 30 min at 0 °C. The solvent was removed in vacuo. The residue was dissolved in dichloromethane (100 ml) and the solvent was removed in vacuo. The latter procedure was repeated two times. The crude product was purified by flash chromatography on silica (90 g), using dichloromethane–methanol–25% aqueous ammonia (100:10:1) as eluent, to give 1.15 g of (2*R*)-1-((3*S*)-3-((dimethylamino)methyl)piperidin-1-yl)-2-methylamino-3-phenylpropan-1-one.

^{1}H-NMR ($CDCl_3$, selected values): δ 0.38, 1.11, 1.37, and 1.65 (all m, together 4H); 2.11, 2.19, 2.25, and 2.31 (all s, together 9H); 4.37 and 4.53 (both m, together 1H); 7.10–7.35 (m, 5H).

At 0 °C *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (379 mg, 1.98 mmol) was added to a solution of (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-(2-naphthyl)propionic acid (651 mg, 1.98 mmol) and 1-hydroxy-7-azabenzotriazole (269 mg, 1.98 mmol) in dichloromethane (10 ml) and

N,*N*-dimethylformamide (5 ml). The reaction mixture was stirred for 20 min at 0 °C. A solution of (2*R*)-1-((3*S*)-3-((dimethylamino)methyl)piperidin-1-yl)-2-methylamino-3-phenylpropan-1-one (600 mg, 1.98 mmol) in dichloromethane (10 ml) and ethyldiisopropylamine (0.51 ml, 2.97 mmol) were added successively. The reaction mixture was stirred for 3 days, while it was warming up to r.t. Ethyl acetate (100 ml) was added. The solution was washed with a saturated aqueous solution of sodium hydrogen carbonate (100 ml). The aqueous phase was extracted with ethyl acetate (3 × 50 ml). The combined organic layers were dried over magnesium sulphate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (90 g), using dichloromethane–methanol–25% aqueous ammonia (100:10:1) as eluent, to give 1.18 g of *N*-((1*R*)-1-{*N*-[(1*R*)-1-benzyl-2-((3*S*)-3-((dimethylamino)methyl)piperidin-1-yl)-2-oxoethyl]-*N*-methylcarbamoyl}-2-(2-naphthyl)ethyl)-*N*-methylcarbamic acid *tert*-butyl ester.

^{1}H-NMR ($CDCl_3$, selected values): δ 0.45 and 0.71 (both m, together 1H); 1.03, 1.05, 1.15, 1.20, 1.28, 1.36, and 1.42 (all s, together 9H); 2.12, 2.15, 2.21, 2.26, 2.29, 2.85 (all s, together 6H); 5.05, 5.44, 5.58, 5.71, 5.85, and 6.00 (all s, together 2H); 7.10–7.80 (m, 12H).

At 0 °C, trifluoroacetic acid (20 ml) was added to a solution of *N*-((1*R*)-1-{*N*-[(1*R*)-1-benzyl-2-((3*S*)-3-((dimethylamino)methyl)piperidin-1-yl)-2-oxoethyl]-*N*-methylcarbamoyl}-2-(2-naphthyl)ethyl)-*N*-methylcarbamic acid *tert*-butyl ester (1.18 g, 1.92 mmol) in dichloromethane (20 ml). The reaction mixture was stirred for 50 min at 0 °C. The solvent was removed in vacuo. The residue was dissolved in dichloromethane (80 ml) and the solvent was removed in vacuo. The latter procedure was repeated two times. The crude product was purified by flash chromatography on silica (40 g), using dichloromethane–methanol–25% aqueous ammonia (100:10:1) as eluent, to give 788 mg of (2*R*)-*N*-[(1*R*)-1-benzyl-2-((3*S*)-3-((dimethylamino)methyl)-piperidin-1-yl)-2-oxoethyl]-*N*-methyl-2-(methylamino)-3-(2-naphthyl)propionamide.

^{1}H-NMR ($CDCl_3$, selected values): δ 2.01 and 2.25 (both s, together 9H); 3.72 (m, 2H); 3.95 and 4.27 (both m, together 1H); 5.77, 5.86, and 6.03 (t, m, and dd, together 1H); 7.10 and 7.85 (m, 12H).

At 0 °C, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (105 mg, 0.55 mol) was added to a solution of (2*E*)-5-(*tert*-butoxycarbonylamino)-5-methylhex-2-enoic (136 mg, 0.55 mmol) and 1-hydroxy-7-azabenzotriazole (74 mg, 0.55 mmol) in dichloromethane (5 ml). The reaction mixture was stirred for 20 min at 0 °C. A solution of (2*R*)-N-[(1*R*)-1-benzyl-2-((3*S*)-3-((dimethylamino)methyl)piperidin-1-yl)-2-oxoethyl]-*N*-methyl-2-(methylamino)-3-(2-naphthyl)propionamide (281 mg, 0.55 mmol) in dichloromethane (5 ml) and *N*,*N*-dimethylformamide (5 ml) and ethyldiisopropylamine (0.094 ml, 0.55 mmol) were added successively. The reaction mixture was stirred for 16 h, while it was warming upto r.t. It was diluted with ethyl acetate (70 ml) and washed with a saturated aqueous solution of sodium hydrogen carbonate (70 ml). The aqueous phase was extracted with ethyl acetate (3 × 50 ml). The combined organic layers were dried over magnesium sulphate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (40 g), using dichloromethane–methanol–25% aqueous ammonia (100:10:1) as eluent, to give 398 mg of {(3*E*)-4-[*N*-((1*R*)-1-{*N*-[(1*R*)-1-benzyl-2-((3*S*)-3-((dimethylamino)methyl)piperidin-1-yl)-2-oxoethyl]-*N*-methylcarbamoyl}-2-(2-naphthyl)ethyl)-*N*-methylcarbamoyl]-1,1-dimethylbut-3-enyl}carbamic acid *tert*-butyl ester.

^{1}H-NMR ($CDCl_3$, selected values): δ 1.44 (s, 9H); 5.58, 5.75, and 5.86 (all m, 2H); 6.09 and 6.17 (both d, together 1H); 6.84 (m, 1H); 7.10–7.80 (m, 12H).

At 0 °C, trifluoroacetic acid (7 ml) was added to a solution of {(3*E*)-4-[*N*-((1*R*)-1-{*N*-[(1*R*)-1-benzyl-2-((3*S*)-3-((dimethylamino)methyl)piperidin-1-yl)-2-oxoethyl]-*N*-methylcarbamoyl}-2-(2-naphthyl)ethyl)-*N*-methylcarbamoyl]-1,1-dimethylbut-3-enyl}carbamic acid *tert*-butyl ester (398 mg, 0.54 mmol) in dichloromethane (7 ml). The reaction mixture was stirred for 40 min at 0 °C. The solvent was removed in vacuo. The residue was dissolved in dichloromethane (20 ml) and the solvent was removed in vacuo. The latter procedure was repeated two times. The crude product was purified by flash chromatography on silica (40 g), using dichloromethane–methanol–25% aqueous ammonia (100:10:1) as eluent, to give 150 mg of compound **26**.

^{1}H-NMR ($CDCl_3$, selected values): δ 1.08, 1.12, 1.14, and 1.15 (all s, together 6H), 5.46, 5.59, 5.75, and 5.94 (all m, together 2H); 6.15 (m, 1H); 6.93 (m, 1H).

HPLC: $T_{ret} = 27.55$ min (72%, 254 nm, A1); $T_{ret} = 30.23$ min (70%, 254 nm, B1).

MS (ES): $m/z = 640.4$; calc. for ($[M + H]^+$: 640.4).

For biological testing, the title compound was transferred into its acetate salt, by lyophilisation of a solution of 150 mg of compound **26** in 0.5 M acetic acid (40 mL).

*6.2.2.24. (2E)-4-(1-Aminocyclobutyl)but-2-enoic acid N-((1R)-1-{N-[(1R)-1-benzyl-2-((3S)-3-(dimethylaminomethyl)piperidin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide (**27**).* One hundred and eighty mg of compound **27** were prepared as described for compound **26**, using diamine **14**, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-phenylpropionic acid, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-(2-naphthyl)propionic acid, and (2*E*)-4-(1-(*tert*-butoxycarbonylamino)cyclobutyl)but-2-enoic acid.

^{1}H-NMR ($CDCl_3$, selected values): δ 0.40 and 0.74 (both m, together 2H); 3.73 and 4.22 (both m, together 2H); 5.57, 5.77, and 5.91 (all m, together 2H); 6.15 and 6.24 (both d, together 1H); 6.85 and 6.96 (both m, together 1H); 7.22, 7.92, and 7.74 (all m, together 12H).

HPLC: $T_{ret} = 28.03$ min (90%, 254 nm, A1); $T_{ret} =$ 29.92 min (89%, 254 nm, B1).

MS (ES): $m/z = 652.4$; calc. for ($[M + H]^+$: 652.4).

6.2.2.25. (2E)-5-Amino-5-methylhex-2-enoic acid N-((1R)-1-{N-[(1R)-1-benzyl-2-((2S)-2-((dimethylamino)-methyl)pyrrolidin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide ***(28)***. Two hundred and eighty five mg of compound **28** were prepared as described for compound **26**, using diamine **13**, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-phenylpropionic acid (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-(2-naphthyl)propionic acid, and (2*E*)-5-(*tert*-butoxycarbonylamino)-5-methylhex-2-enoic acid.

^{1}H-NMR ($CDCl_3$, selected values): δ 0.55 (m, 1H); 1.11, 1.12, and 1.17 (all s, together 6H); 2.25 (s, 6H); 2.45 (s, 3H); 2.85 (s, 3H); 4.02 (m, 1H); 5.48 (dd, 1H); 5.80 and 5.93 (m, and dd, together 1H); 6.10 and 6.18 (both d, together 1H); 6.87 and 7.00 (both m, together 1H); 7.10–7.90 (m, 12H).

HPLC: $T_{ret} = 27.97$ min (96%, 254 nm, A1); $T_{ret} =$ 27.80 min (95%, 254 nm, B1).

MS (ES): $m/z = 626.4$; calc. for ($[M + H]^+$: 626.4).

6.2.2.26. N-((1R)-1-{N-[(1R)-1-Benzyl-2-((2S)-2-((dimethylamino)methyl)pyrrolidin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methyl-3-((methylamino)methyl)benzamide ***(29)***. Four hundred and thirty-six mg of compound **29** were prepared as described for compound **26**, using diamine **13**, (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-phenylpropionic acid (2*R*)-2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-3-(2-naphthyl)propionic acid, and 3-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)benzoic acid.

^{1}H-NMR ($CDCl_3$, selected values): δ 0.87 (m, 1H); 1.22 (m, 1H); 1.45 (m, 1H); 1.67 (m, 1); 4.09 (m, 1H); 5.53 and 5.90 (dd and m, together 2H); 6.80–7.90 (m, 16H).

HPLC: $T_{ret} = 28.43$ min (89%, 254 nm, A1); $T_{ret} =$ 30.63 min (85%, 254 nm, B1).

MS (ES): $m/z = 648.4$; calc. for ($[M + H]^+$: 648.4).

6.2.2.27. (2E)-5-Amino-5-methylhex-2-enoic acid N-((1R)-1-{N-[(1R)-1-benzyl-2-(4-hydroxypiperidin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide ***(30)***. One hundred and fifty-six mg of compound **30** was prepared as described for compound **26** using 4-hydroxypiperidine, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-phenylpropionic acid, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(2-naphthyl)propionic acid, and (2*E*)-5-*tert*-butoxycarbonylamino-5-methylhex-2-enoic acid as starting materials.

^{1}H-NMR (CD_3OD, two sets of signals, selected values for the major rotamere): δ 1.38 (s, 6H); 2.52 (s 3H); 2.99 (s, 3H); 5.87 (m, 1H); 6.38 (d, 1H).

HPLC: $T_{ret} = 29.88$ min (95%, 254 nm, A1); $T_{ret} =$ 31.41 min (92%, 254 nm, B1).

MS (ES): $m/z = 599.4$; calc. for ($[M + H]^+$: 599.4).

6.2.2.28. (2E)-5-Amino-5-methylhex-2-enoic acid N-((1R)-1-{N-[(2R)-2-(4-hydroxypiperidin-1-yl)-2-oxo-1-((2-thienyl)methyl)ethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide ***(31)***. Two hundred and seventeen mg of compound **31** were prepared as described for compound **26** using 4-hydroxypiperidine, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(2-thienyl)-propionic acid, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(2-naphthyl)propionic acid, and (2*E*)-5-*tert*-butoxycarbonylamino-5-methylhex-2-enoic acid as starting materials.

^{1}H-NMR ($CDCl_3$, two sets of signals, selected values): δ 1.20 (s, 6H); 2.60 and 2.65 (both s, together 3H); 2.85 (s, 3H); 5.60 (m, 1H); 5.95 (m, 1H); 6.25 (d, 1H); 6.85 (m, 2H); 7.00 (m, 1H); 7.10–7.90 (m, 8H).

HPLC: $T_{ret} = 29.07$ min (96%, 254 nm, A1); $T_{ret} =$ 30.81 min (91%, 254 nm, B1).

MS (ES): $m/z = 605.4$; calc. for ($[M + H]^+$: 605.3).

6.2.2.29. (2E)-5-Amino-5-methylhex-2-enoic acid N-((1R)-2-(biphenyl-4-yl)-1-{N-[(2R)-2-(4-hydroxypiperidin-1-yl)-2-oxo-1-((2-thienyl)methyl)ethyl]-N-methylcarbamoyl}ethyl)-N-methylamide ***(32)***. Two hundred and seventy nine mg of compound **32** were prepared as described for compound **26** using 4-hydroxypiperidine, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(2-thienyl)propionic acid and (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(biphenyl-4-yl)propionic acid and (2*E*)-5-*tert*-butoxycarbonylamino-5-methylhex-2-enoic acid as starting materials.

^{1}H-NMR ($CDCl_3$, two sets of signals, selected values): δ 1.20 (s, 6H); 2.60 and 2.62 (both s, together 3H); 2.85 and 2.87 (both s, together 3H); 5.65 (m, 1H); 5.85 (m, 1H); 6.22 and 6.23 (both dd, together 1H); 6.75–7.65 (m, 13H).

HPLC: $T_{ret} = 32.20$ min (97%, 254 nm, A1); $T_{ret} =$ 34.09 min (95%, 254 nm. B1).

MS (ES): $m/z = 631.2$; calc. for ($[M + H]^+$: 631.3).

6.2.2.30. (2E)-5-Amino-3,5-dimethylhex-2-enoic acid N-((1R)-1-{N-[(2R)-2-(4-hydroxypiperidin-1-yl)-2-oxo-1-((2-thienyl)methyl)ethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide ***(33)***. Two hundred and eight mg of compound **33** were prepared as described for compound **26** using 4-hydroxypiperidine, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(2-thienyl)-

propionic acid and (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(2-naphthyl)propionic acid and (2*E*)-5-*tert*-butoxycarbonylamino-3,5-dimethylhex-2-enoic acid as starting materials.

^{1}H-NMR ($CDCl_3$, two sets of signals, selected values): δ 1.20 (s, 6H); 1.95 and 1.97 (both s, together 3H); 2.55 and 2.60 (both s, together 3H); 3.87 (s, 3H); 5.60 (m, 1H); 5.80 (s, 1H); 5.95 (m, 1H); 6.70–7,90 (m, 10H).

HPLC: $T_{ret} = 29.76$ min (96%, 254 nm, A1); $T_{ret} =$ 31.62 min (95%, 254 nm., B1).

MS (ES): $m/z = 619.4$; calc. for ($[M + H]^+$: 619.3).

*6.2.2.31. (2E)-5-Amino-3,5-dimethylhex-2-enoic acid N-((1R)-2-(biphenyl-4-yl)-1-{N-[(1R)-2-(4-hydroxypiperidin-1-yl)-2-oxo-1-((2-thienyl)methyl)ethyl]-N-methylcarbamoyl}ethyl)-N-methylamide (**34**).* Two hundred and twenty-three mg of compound **34** were prepared as described for compound **26** using 4-hydroxypiperidine, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(2-thienyl)propionic acid and (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(biphenyl-4-yl) propionic acid and (2*E*)-5-*tert*-butoxycarbonylamino-3,5-dimethylhex-2-enoic acid as starting materials.

^{1}H-NMR ($CDCl_3$, two sets of signals, selected values): δ 1.15 (s, 6H); 1.92 and 1.95 (both s, together 3H); 2.50 and 2.55 (both s, together 3H); 2.87 and 2.90 (both s, together 3H); 5.70 (m, 1H); 5.75 (s, 1H); 5.85 (m, 1H); 6.80–7.60 (m, 12H).

HPLC: $T_{ret} = 32.89$ min (100%, 254 nm, A1); $T_{ret} =$ 34.82 min (98%, 254 nm, B1).

MS (ES): $m/z = 645.4$; calc. for ($[M + H]^+$: 645.3).

*6.2.2.32. (2E)-5-Amino-5-methylhex-2-enoic acid N-((1R)-1-{N-[(1R)-1-(4-fluorobenzyl)-2-(4-hydroxypiperidin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide (**35**).* One hundred and eighty mg of compound **35** were prepared as described for compound **26** using 4-hydroxypiperidine, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(4-fluorophenyl)propionic acid and (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(2-naphthyl)propionic acid and (2*E*)-5-*tert*-butoxycarbonylamino-5-methylhex-2-enoic acid as starting materials.

^{1}H-NMR (CD_3OD, trifluoroacetate salt, two sets of signals, selected values): δ 1.00 (m, 1H); 1.22, 1.24, and 1.35 (all s, together 6H); 2.70, 2.90, and 2.97 (all s, together 6H); 5.65 and 5.75 (both dd, together 1H); 5.90 (m, 1H); 6.40 and 6.65 (both m, together 2H); 6.85–7.90 (m, 11H).

HPLC: $T_{ret} = 30.27$ min (99%, 254 nm, A1); $T_{ret} =$ 31.60 min (99%, 254 nm, B1).

MS (ES): $m/z = 617.4$; calc. for ($[M + H]^+$: 617.3).

*6.2.2.33. (2E)-5-Amino-3,5-dimethylhex-2-enoic acid N-((1R)-1-{N-[(1R)-1-(4-fluorobenzyl)-2-(4-hydroxypiperidin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide (**36**).* One hundred and thirty-five mg of compound **36** were prepared as described for compound **26** using 4-hydroxypiperidine, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(4-fluorophenyl)propionic acid and (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(2-naphthyl)propionic acid and (2*E*)-5-*tert*-butoxycarbonylamino-3,5-dimethylhex-2-enoic acid as starting materials.

^{1}H-NMR (CD_3OD, trifluoroacetate salt, two sets of signals, selected values): δ 1.25, 1.30, 1.35, and 1.40 (all s, together 6H); 2.50, 2.95, 3.00, 3.30 (all s, together 9H); 5.70 (m, 1H); 5.80 (m, 1H); 5.95 (m, 1H); 6.90–7.90 (m, 12H).

HPLC: $T_{ret} = 30.98$ min (86%, 254 nm, A1); $T_{ret} =$ 32.38 min (100%, 254 nm, B1).

MS (ES): $m/z = 631.4$; calc. for ($[M + H]^+$: 631.4).

*6.2.2.34. (2E)-5-Amino-5-methylhex-2-enoic acid N-((1R)-1-{N-[(1R)-1-benzyl-2-(4-hydroxypiperidin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(biphenyl-4-yl)ethyl)-N-methylamide (**37**).* Compound **37** (1.20 g) were prepared as described for compound **26** using 4-hydroxypiperidine, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-phenylpropionic acid and (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(biphenyl-4-yl)propionic acid and (2*E*)-5-*tert*-Butoxycarbonylamino-5-methylhex-2-enoic acid as starting materials.

^{1}H-NMR ($CDCl_3$, two sets of signals, selected values): δ 0.75 and 0.95 (both m, together 1H); 1.05 and 1.25 (both s, together 6H); 2.30 and 2.20 (m, 2H); 2.35, 2.40, 2.85 and 2.88 (all s, together 6H); 2.70 (m, 1H); 2.75–3.35 (m, 8H); 3.45 and 3.65 (both m, together 1H); 3.85 and 4.00 (both m, together 1H); 5.70 (m, 1H); 5.80 (m, 1H); 6.15 (m, 1H); 7.00 (m, 1H); 7.10–7.65 (m, 14H).

HPLC: $T_{ret} = 32.65$ min (93%, 254 nm, A1); $T_{ret} =$ 34.02 min (92%, 254 nm, B1).

MS (ES): $m/z = 625.4$; calc. for ($[M + H]^+$: 625.4).

*6.2.2.35. (2E)-5-Amino-3,5-dimethylhex-2-enoic acid N-((1R)-1-{N-[(1R)-1-benzyl-2-(4-hydroxypiperidin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(biphenyl-4-yl)ethyl)-N-methylamide (**38**).* Two hundred and twenty five mg of compound **38** were prepared as described for compound **26**, using 4-hydroxypiperidine, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-phenylpropionic acid, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(biphenyl-4-yl)propionic acid and (2*E*)-5-*tert*-butoxycarbonylamino-3,5-dimethylhex-2-enoic acid as starting materials.

^{1}H-NMR ($CDCl_3$, two sets of signals, selected values): δ 0.70 and 0.95 (both m, together 1H); 1.00–1.80 (m, 4H); 2.30 (m, 4H); 2.35, 2.40, 2.85 and 2.87 (all s, together 6H); 2.60–4.10 (m, 6H); 5.72 (m, 1H); 5.80 (m, 1H); 6.15 (dd, 1H); 7.00 (m, 1H); 7.15–7.5 (m, 10H).

HPLC: $T_{ret} = 33.29$ min (99%, 254 nm, A1); $T_{ret} =$ 36.40 min (97%, 254 nm, B1).

MS (ES): $m/z = 639.4$; calc. for $(M + H]^+$: 639.4).

*6.2.2.36. (2E)-4-(1-Aminocyclobutyl)but-2-enoic acid N-((1R)-1-{N-[(1R)-1-benzyl-2-(4-hydroxypiperidin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(biphenyl-4-yl)ethyl)-N-methylamide (**39**).* Compound **39** (1.4 g) were prepared as described for compound **26**, but using 4-hydroxypiperidine, (2*R*)-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-phenylpropionic acid, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(biphenyl-4-yl)propionic acid, and (2*E*)-4-(1-(*tert*-butoxycarbonylamino)cyclobutyl)but-2-enoic acid as starting materials.

HPLC: $T_{ret} = 33.58$ min (99%, 254 nm, A1); $T_{ret} =$ 34.95 min (99%, 254 nm, B1).

MS (ES): $m/z = 637.4$; calc. for $([M + H]^+$: 637.4).

Compound **39** was transferred into its acetate salt by lyophilisation of a solution of compound **39** in an 0.5 M aqueous solution of acetic acid.

^{1}H-NMR (DMSO-d_6, selected values): δ 2.37, 2.40, 2.75, and 2.80 (all s, together 6H); 5.60 (m, 2H); 6.30 (m, 1H); 6.70 (m, 1H); 7.10–7.70 (m, 14H).

$C_{39}H_{48}N_4O_4 \cdot C_2H_4O_2 \cdot H_2O$ (636.84·60.05·18.02) Calc. for: C, 68.88; H, 7.61; N, 7.84. Found: C, 68.86; H, 7.48; N, 7.81%.

*6.2.2.37. (2E)-5-Amino-3,5-dimethylhex-2-enoic acid N-((1R)-1-{N-[(1R)-1-benzyl-2-(4-hydroxypiperidin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide (**40**).* One hundred and fifty-two mg of compound **40** were prepared as described for compound **26**, using 4-hydroxypiperidine, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-phenylpropionic acid and (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(2-naphthyl)propionic acid and (2*E*)-5-*tert*-butoxycarbonylamino-3,5-dimethylhex-2-enoic acid as starting materials.

^{1}H-NMR (CD_3OD, two sets of signals, selected values of the major rotamer): δ 1.39 (s, 6H); 2.40 (s, 3H);); 2.45 (s, 3H); 3.00 (s, 3H); 5.91 (m, 1H).

HPLC: $T_{ret} = 30.57$ min (94%, 254 nm, A1); $T_{ret} =$ 32.14 min (77%, 254 nm, B1).

MS (ES): $m/z = 613.4$; calc. for $([M + H]^+$: 613.4).

*6.2.2.38. (2E)-4-(1-Aminocyclobutyl)but-2-enoic acid N-((1R)-1-{N-[(1R)-1-benzyl-2-(4-hydroxypiperidin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide (**41**).* Four hundred and four mg of compound **41** were prepared as described for compound **26**, using 4-hydroxypiperidine, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-phenylpropionic acid and (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(2-naphthyl)propionic acid and (2*E*)-4-(1-(*tert*-butoxycarbonylamino)cyclobutyl)but-2-enoic acid as starting materials.

^{1}H-NMR (CD_3OD, two sets of signals, selected values of the major rotamer) δ 1.48 (s, 6H); 2.44 (s, 3H); 2.94 (s, 3H); 5.78 (m, 1H); 5.94 (d, 1H).

HPLC: $T_{ret} = 30.82$ min (95%, 254 nm, A1); $T_{ret} =$ 32.40 min (88%, 254 nm, B1).

MS (ES): $m/z = 611.4$; calc. for $([M + H]^+$: 611.4).

*6.2.2.39. (2E)-5-Amino-5-methylhex-2-enoic acid N-((1R)-1-(((1R)-1-((2S)-2-hydroxypropylcarbamoyl)-2-phenylethyl)-methylcarbamoyl)-2-(2-naphthyl)ethyl)-N-methylamide (**42**).* One hundred and five mg of compound **42** were prepared as described for compound **26**, using 3-aminopropanol, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-phenylpropionic acid, (2*R*)-2-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(2-naphthyl)propionic acid, and (2*E*)-5-*tert*-butoxycarbonylamino-5-methylhex-2-enoic acid as starting materials.

^{1}H-NMR ($CDCl_3$, selected values) δ 3.90 (m, 1H); 5.55 (dd, 1H); 5.58 (d, 1H).

HPLC: $T_{ret} = 29.03$ min (87%, 254 nm, A1); $T_{ret} =$ 32.25 min (81%, 254 nm, B1).

MS (ES): $m/z = 573.5$; calc. for $([M + H]^+$: 573.4).

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