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Original article

New highly potent dipeptidic growth hormone secretagogues with low molecular weight

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Abstract – Based on NN703, low molecular weight growth hormone secretagouges (GHSs) with a reduced number of hydrogen binding sites were designed by removal of the *C*-terminal amide group. The compounds were highly potent in combination with high efficacy in a rat pituitary cell assay, being characterized with EC_{50} values down to 0.8 nM. Selected compounds were tested in in vivo animal models. The oral bioavailability in dogs was 16–44%. Also, the ED_{50} values of the compounds were determined both in dog and swine. © 2000 Éditions scientifiques et médicales Elsevier SAS

growth hormone secretagogue / NN703 / peptidomimetic / oral bioavailability / GH release

1. Introduction

Growth hormone secretagouges (GHSs) [1, 2] have been a fast evolving area of medicinal chemistry and clinical research since Momany et al. discovered the hexapeptides GHRP-6 [3, 4] and GHRP-2 [5] that were able to release growth hormone from the pituitary gland. These findings gave the possibility to develop small, orally available drugs for growth hormone deficient patients. Most current methods of treatment use recombinant human growth hormone (hGH). A major drawback of this treatment is the way of administration. As a peptide of 191 amino acids the usual administration is by injection.

It is known that GHSs act via a different receptor [6, 7] than the known endogenous growth hormone releasing hormone (GHRH), but the exact mechanism of action is not known. Despite these uncertainties, a number of clinical candidates have been developed during the years, both of peptidic nature, such as hexarelin [8] or ipamorelin [9], of peptidomimetic nature, such as MK-0677 [10] or NN703 [11, 12], or of non-peptidic nature, such as benzolactams [13, 14].

Analogues of NN703 (*figure 1*) have been synthesized where variations have been made at the *N*-terminal [15] and at the *C*-terminal [16, 17]. In this paper, we present the synthesis of new NN703 analogues (*figure 1*) with further reduced molecular weight. The working hypothesis was, that a reduction of molecular weight would improve the oral activity.

2. Chemistry

The backbone of a peptide does not usually contribute to the binding of the peptide to a receptor. In a first attempt we therefore decided to remove the amide group at the *C*-terminal. The commercially available amine **1**, was reacted with BOC-protected D-*N*-(2-naphthyl)alanine [12, 18, 19] with 1-hydroxy-7-azabenzotriazole (HOAt) [20], *N*-(dimethylaminopropyl)-N′-ethylcarbodiimide (EDAC) and ethyldiisopropylamine as coupling reagent and deprotected with trifluoroacetic acid to give amine **2**. This was coupled under the same conditions as above to

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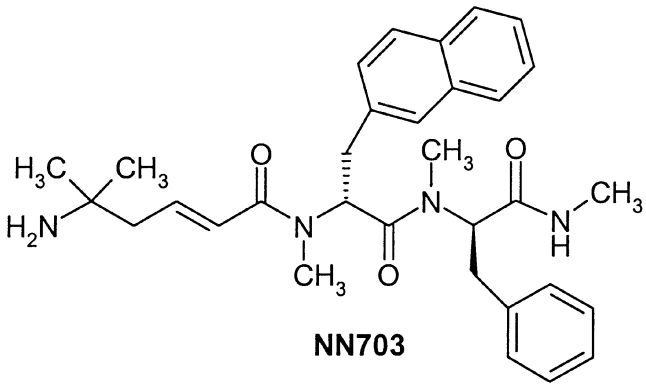

Figure 1. Structure of compound NN703.

BOC-protected (2*E*)-5-amino-5-methylhex-2-enoic acid [12]. The subsequent deprotection had to be carried out carefully at 0 °C with 50% trifluoroacetic acid in dichloromethane (*figure 2*). Prolonged reaction time under those acidic conditions usually cleaved the resulting compound **3** between the phenethyl and the naphthylalanine moieties [21].

Earlier results [10, 12, 22] suggested that a polar group at the *C*-terminal improved potency. We therefore decided to attach different polar groups such as alcohol, amides or sulfonamides at the aromatic ring. The 2-hydroxyethyl chain was attached to phenol **5**, which had been synthesized from acid **4**, via alkylation with ethyl bromoacetate. The reduction furnished the desired aminoalcohol **6** (*figure 3*).

The synthesis of the chain-homologue **11** was performed via phenol **10**, as it is outlined in *figure 4*, from the commercially available acid **7**, which was transferred into the corresponding methylamide **8**. The amide **8** was reduced with sodium borohydride/iodine and the resulting amine was BOC-protected to yield the benzyl ether **9**. The free phenol **10** was obtained by catalytic hydrogenation. Subsequent alkylation of phenyl **10** with 3-bromo-1-propanol followed by deprotection of the amino group with trifluoroacetic acid furnished the aminoalcohol **11**.

In order to prepare the analogues **15** and **16**, the key intermediate **14** had to be prepared (*figure 5*). The reaction of the known diamine **12** [23] with one equivalent of di-*tert*-butyl dicarbonate gave a mixture of di- and mono-protected amines **13** and **14**, which were easily separated on silica gel. The anilino group of **14** was

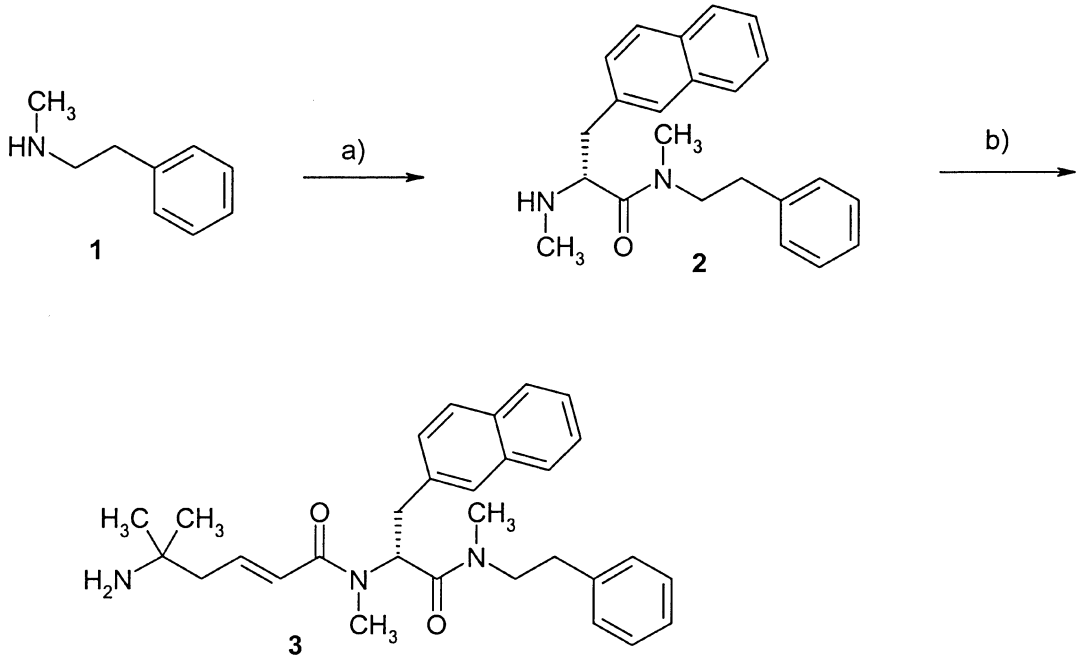

Figure 2. a) i BOC-*N*-Me-D-(2-naphthyl)alanine, EDAC, HOAt, DIPEA; ii 50% TFA in CH_2Cl_2, 0 °C; b) i BOC-5-methylhex-2-enoic acid, EDAC, HOAt, DIPEA; ii 50% TFA in CH_2Cl_2, 0 °C.

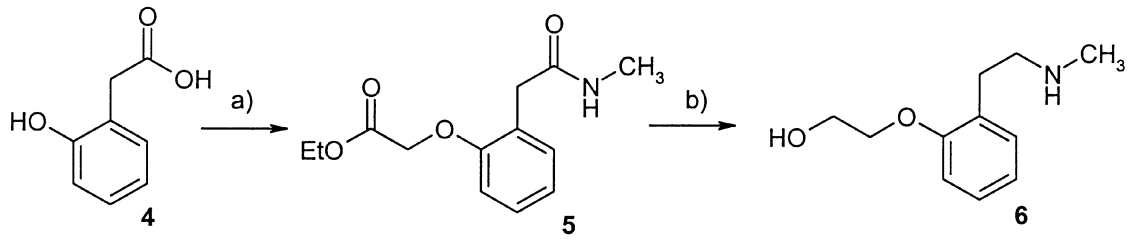

Figure 3. a) i H_2NCH_3, HOBT, EDAC; ii ethyl bromoacetate, K_2CO_3, KI, acetone; b) $NaBH_4/I_2$, THF.

reacted with FMOC-protected glycine using EDAC as coupling reagent or with methanesulfonyl chloride using triethylamine as base in dichloromethane at –78 °C. After deprotection of the amino group with trifluoroacetic acid, amines **15** and **16** were obtained.

The compounds **17**–**28** (*figure 6*) were synthesized either from known building blocks such as *N*-methyl-*N*-(2-(2-thienyl)ethyl)amine [24] or *N*-methyl-*N*-(3-phenylpropyl)amine [25] or the building blocks described in this paper with BOC-protected amino acids as described for the synthesis of **3**. The synthesis of those amino acids are described in the literature [12, 15]. When **27** was synthesized, the FMOC group was removed before the final BOC-deprotection step with triethanolamine as base [26].

Aiming for higher potency we decided to prepare conformationally constrained analogues of **3**. A cyclic constraint can be introduced either in the *C*-terminal part of the molecule or in the central part. Two examples were synthesized with cyclic constraints in the *C*-terminal (*figure 7*). One synthesis started with commercially available tetrahydroisoquinoline leading to compound **29**. The cyclopropane-derivative **33** was obtained as a diastereomeric mixture from racemic *trans*-2-phenylcyclopropylamine (**30**), afterwards this was formylated with formic acid and acetic anhydride to give **31**, which was subsequently reduced with sodium borohydride/iodine [27] to give **32**. A standard peptide-coupling deprotection sequence furnished the cyclopropyl derivative **33** (*figure 8*).

In order to achieve cyclization in the central part of the GHSs, we decided to prepare an amide to amide bridge. Since we believe that the activity of the GHSs is mainly achieved by the aromatic moieties and the amine and not by the amide bonds, a cyclization of this type should not influence the binding, other than restrict the number of possible conformations. The synthesis of the cyclic central moiety started with (2*R*)-2-amino-3-(2-naphthyl)propionic

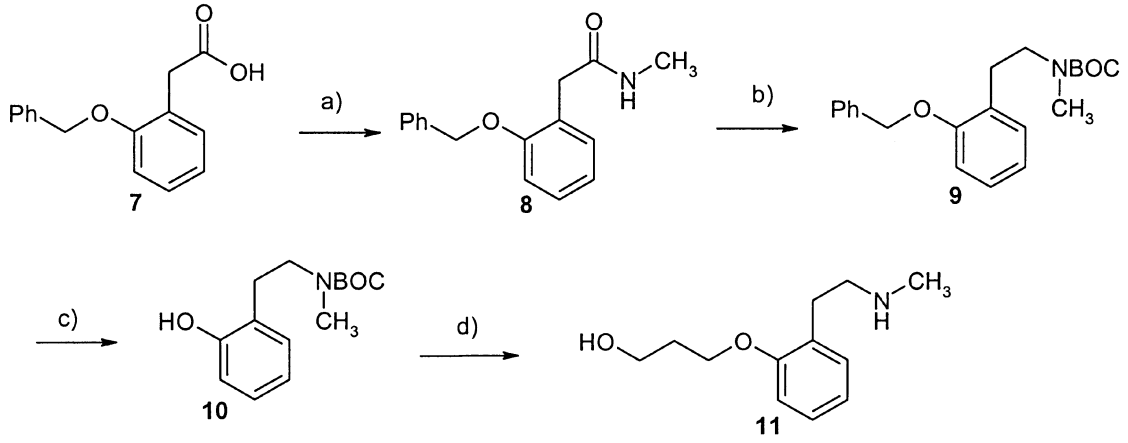

Figure 4. a) H_2NCH_3, HOBT, EDAC; b) i $NaBH_4/I_2$, THF; ii $(BOC)_2O$, NaOH; c) H_2 Pd/C; d) i 3-bromopropan-1-ol, K_2CO_3, CsCl, DMF; ii TFA, CH_2Cl_2.

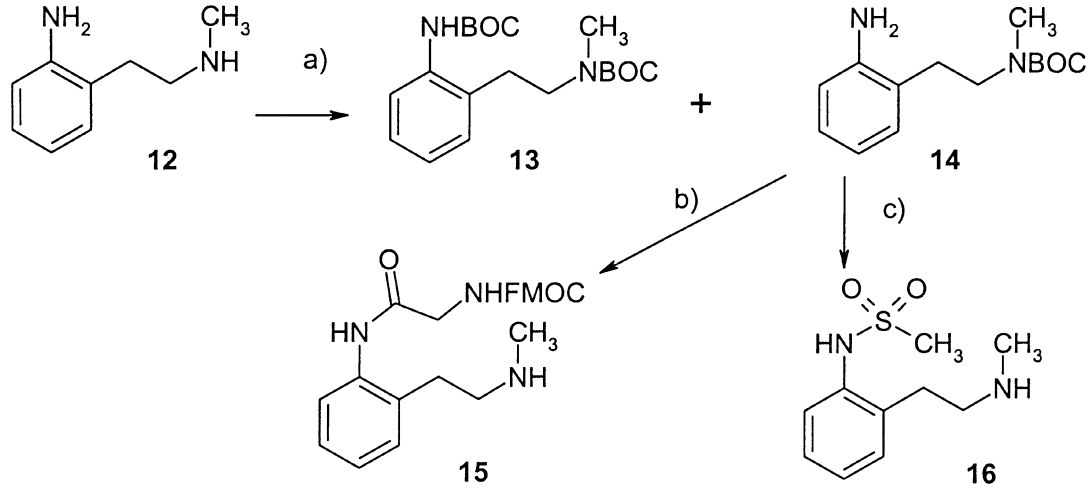

Figure 5. a) $(BOC)_2O$, NaOH, THF, H_2O; b) i $FMOCHNCH_2COOH$, EDAC; ii HCl, EtOAc; c) i CH_3SO_2Cl, NEt_3, CH_2Cl_2, –78 °C; ii TFA, CH_2Cl_2.

acid, which was subjected to an esterification and subsequently *N*-alkylated [28] to give ester **34** (*figure 9*). Upon deprotection of the amino group, a ring formation occurred after neutralization yielding piperazinone **35**. Protection of the secondary amine followed by alkylation under strongly basic conditions (potassium hydroxide in DMSO) furnished the intermediate **36**. The GHSs **37** and **38** were prepared from **36** by coupling of the known BOC-protected amino acids [12, 15] and subsequent deprotection.

As has been shown in the benzolactam series of GHSs [13], an (*R*)-hydroxypropyl group as substitutent on the free amine can be advantageous in achieving highly potent compounds [29]. We therefore synthesized one example (**39**) of this type, by reductive alkylation of **3** with the silyl protected 2-hydroxypropanal [30]. After removal of the protection group with tetrabutylammonium fluoride (TBAF), the aminoalcohol **39** was isolated (*figure 10*).

3. Pharmacology

The primary test in our screening was a determination of the EC_{50} values in a rat pituitary cell assay. The efficacy of the compounds was compared to the maximal stimulation obtained with GHRP-6 [9]. Compounds, showing high potency and efficacy in the primary screening, were tested in vivo. An oral bioavailability of at least 20% in beagle dogs was a minimum requirement for further development of the compounds. The GH release ability was also tested in swine, since this species has better resemblance to humans with respect to physiology in general and endocrinology in particular, than other common laboratory animals such as rat or dog [12].

4. Results and discussion

The in vitro data of *table I* show that several potent GHSs have been found within this series of compounds. In compound **3**, which is structurally closest to the clinical candidate NN703, the number of groups which are able to form hydrogen bonds was decreased. This could eventually improve the oral availability and result in an improved oral activity. Compound **3** was found to have an EC_{50} of 32 nM. Even though this is a loss of potency of nearly one order of magnitude, we were excited to see this result. Both the exchange of the benzene moiety to a thiophene (**17**) and an additional methyl group at the *N*-terminal (**21**) gave compounds with higher potency. The combination of those exchanges gave compound **18**, the most potent compound in this series. Also, an exchange of the naphthalene with a biphenyl-moiety, as shown in compound **24**, enhanced the activity to a compound almost equipotent to NN703. The elongation of the spacer for the benzene moiety with one methylene group in compound **19** resulted in a significant loss of activity. Similarly, the change of the structure of the *N*-terminal from a propendiyl spacer to a phenylene spacer, which is realized in compound **20**, led to a compound with only weak GHS activity. This change

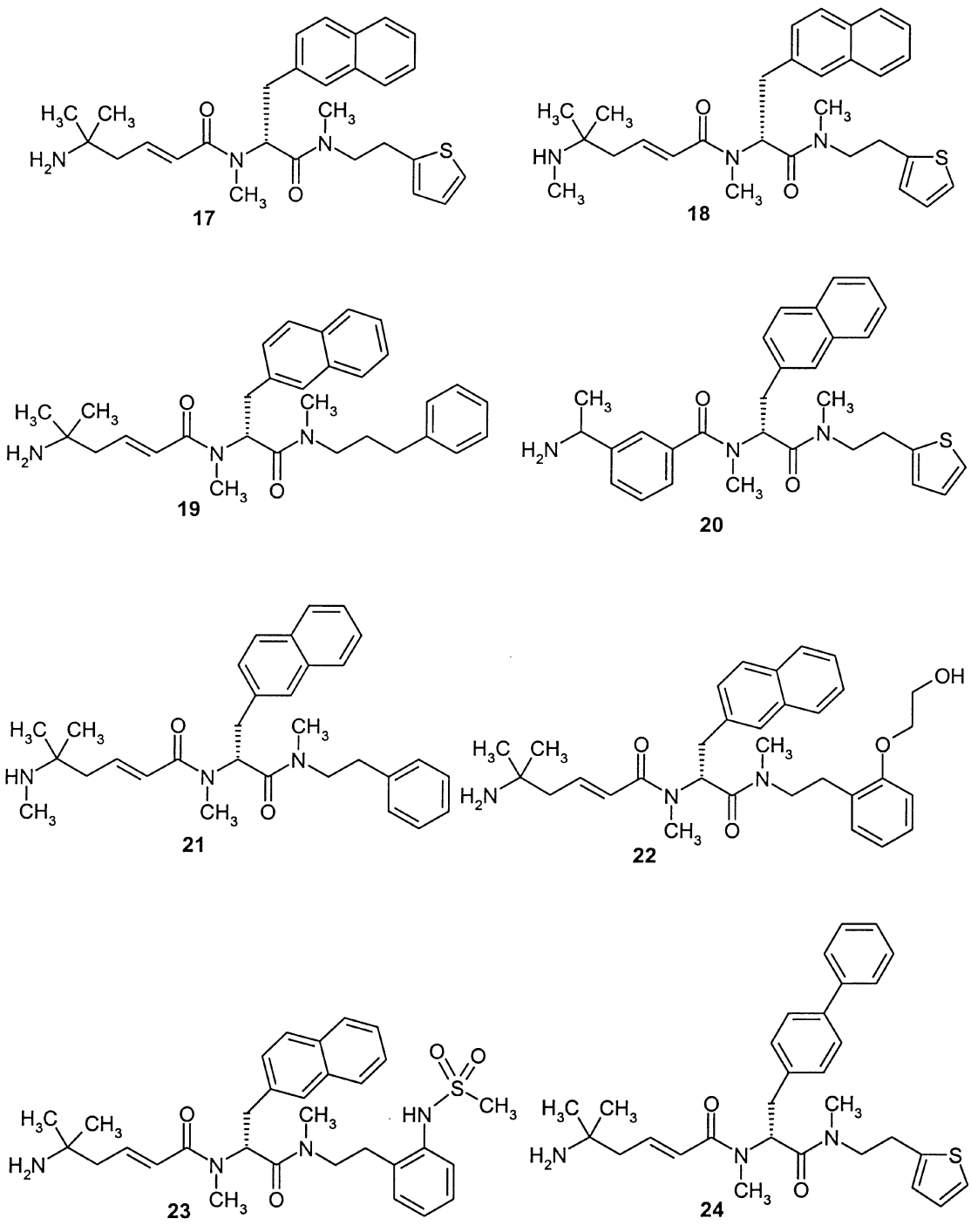

Figure 6. Structures of compounds **17**–**28**.

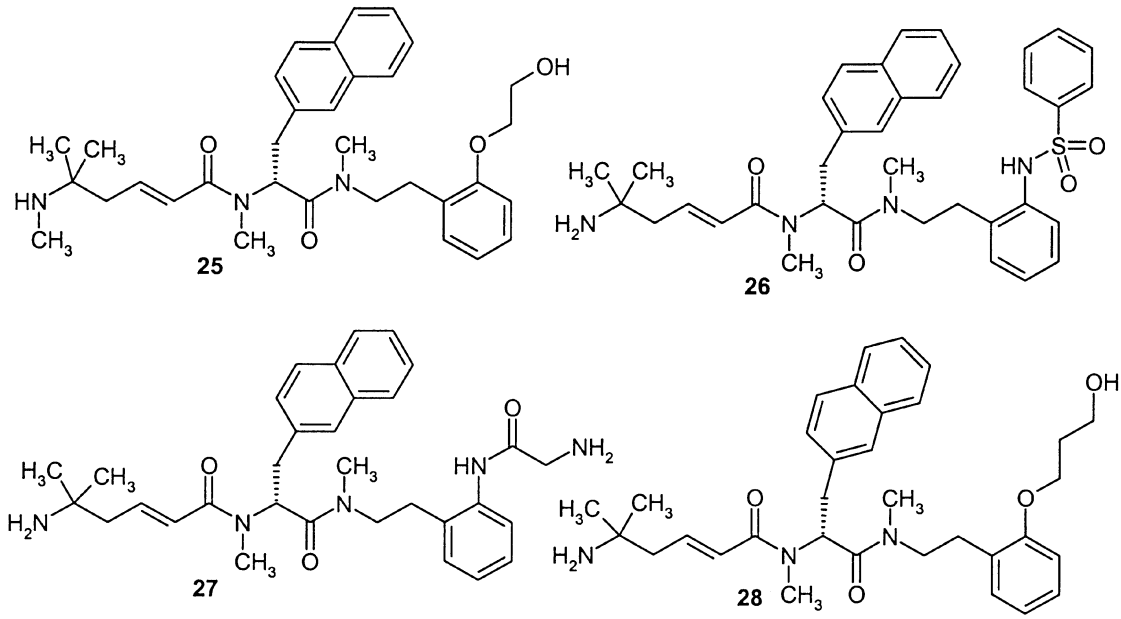

Figure 6. Continued.

was perfectly tolerated in NN703 [15]. Our attempts to achieve higher activity by reduction of conformational freedom by cyclization failed. The different types of cyclization at the *C*-terminal, as represented by compounds **29** and **33**, led to compounds that showed significantly lower GH release. Similar results were obtained when the cyclization was performed in the central part of the molecules. The piperazinone **37** proved to be the most potent of the cyclized analogues in this series, which is almost equipotent to our initial compound **3**. A simple change of the *N*-terminal amino acid resulted in compound **38** with much lower activity. An additional hydroxyl group located at the *C*-terminal seems to be beneficial when it is positioned properly. It is known from similar compounds [12], that such as a hydroxyl group can enhance the GH releasing activity. The enhanced activity of **22**, when compared to **3**, could be due to an additional binding interaction of the GHS with the receptor. This hypothesis is supported by the fact that an elongation with only one methylene group destroys this effect completely, as is shown with compound **28**. The rather poor activity of **39** suggests that the binding site for the hydroxyl group is not in the vicinity of the *N*-terminal, as there is a binding site for a hydroxyl group in the benzolactam GHSs [29]. The additional amine moiety in **27** on the other hand, did not have a great influence on the activity. The diamine **27** is equipotent to **3**, which may be due to the position of the amino group. Since we expected a decreased oral bioavailability for diamines compared to monoamines, we did not try to optimize the activity of this class of compounds. A comparison of this type of GHSs with MK-0677 suggested that an addition of a sulfonamide group in the *ortho*-position of the benzene-ring could be beneficial for the activity as GHS. The impact on activity of the sulfonamide group was, however, limited; a slight enhancement in potency of the sulfonamide **23** compared to compound **3** was observed. This effect is completely diminished when a more bulky group is attached to the sulfonamide, as demonstrated in compound **26**.

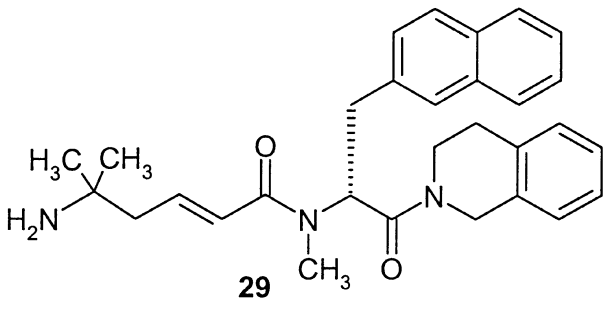

Figure 7. Structure of compound **29**.

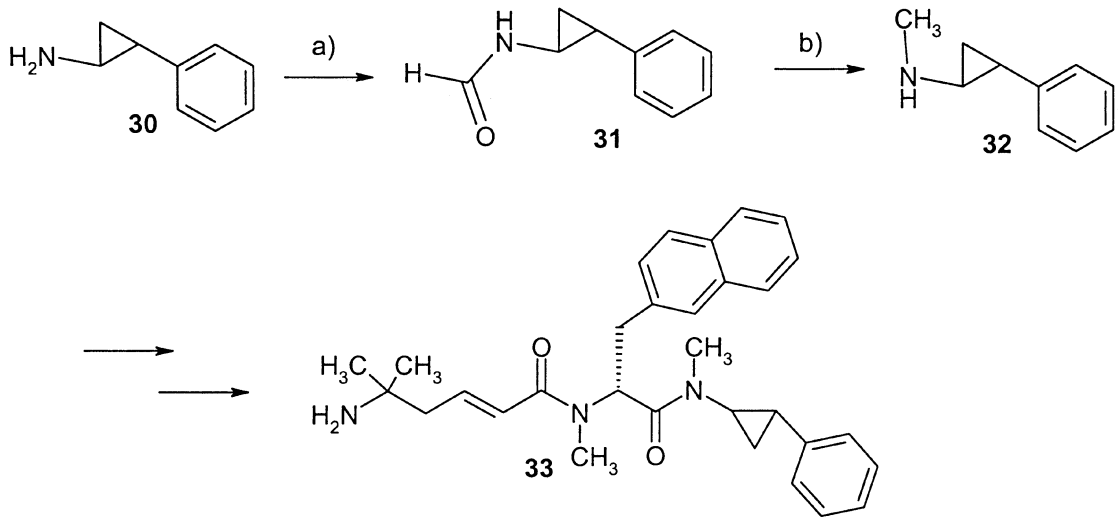

Figure 8. a) HCOOH, Ac_2O; b) $NaBH_4/I_2$.

The most promising compounds **17**, **18**, **22**, **23** and **25** were chosen for in vivo experiments. The results are shown in *table II*. We expected very good oral availability since the molecules were designed to be of low molecular mass and to bear only a very limited number of hydrogen-bonding elements. The oral bioavailability in dogs of all five compounds was relatively high. The growth hormone release induced by the compounds in dogs, however, is much lower than would be suggested from the in vitro data, when compared to NN703. Alcohol **22**, for example, is equipotent to NN703 with a slightly higher efficacy in the in vitro rat pituitary cell assay, and has the same oral availability. The amount of GH, which is released in the dog is significantly lower than that released by NN703 at the same dose. One explanation could be that the compounds had a short plasma elimination half-life of approximately 1 h and therefore a short action of duration and an overall low exposure of the compounds. A second hypothesis is that more than one receptor [6, 7] is involved in GH release, and that various GHSs act differently at these subtypes.

Similar observations were made when the compounds were tested in swine after p.o. administration. Only compound **18** induced a growth hormone secretion comparable to NN703. All other compounds showed much weaker activity at 50 nmol/kg than the reference compound.

5. Conclusion

We have found dipeptides which are highly potent GHSs. The compounds show equipotency with the starting lead compound NN703 in the in vitro rat pituitary cell assay. The most active compounds in this assay are those with either a (2-thienyl)ethyl or a hydroxyethoxy substituted ethylphenyl group at the *C*-terminal. As predicted from empirical rules [31], the oral bioavailability of those compounds is high. The high activity as GHS of the thiophene containing compound **18**, when given orally to swine, shows that dipeptides with even lower molecular weights than NN703 can be efficient GHSs. In general, however, the growth hormone release compared to NN703 was not as high as predicted from the efficacy in the in vitro rat pituitary cell assay. The *C*-terminal amide bond of NN703 seems to have a major influence on the plasma elimination half-life of NNC703, since compounds lacking this amide bond show significantly shorter half-lives. The poor in vivo efficacy of the compounds is most likely due to overall low exposure of the compounds in vivo.

6. Experimental protocols

6.1. Pharmacology

The in vitro studies were performed as described in the literature [9, 32].

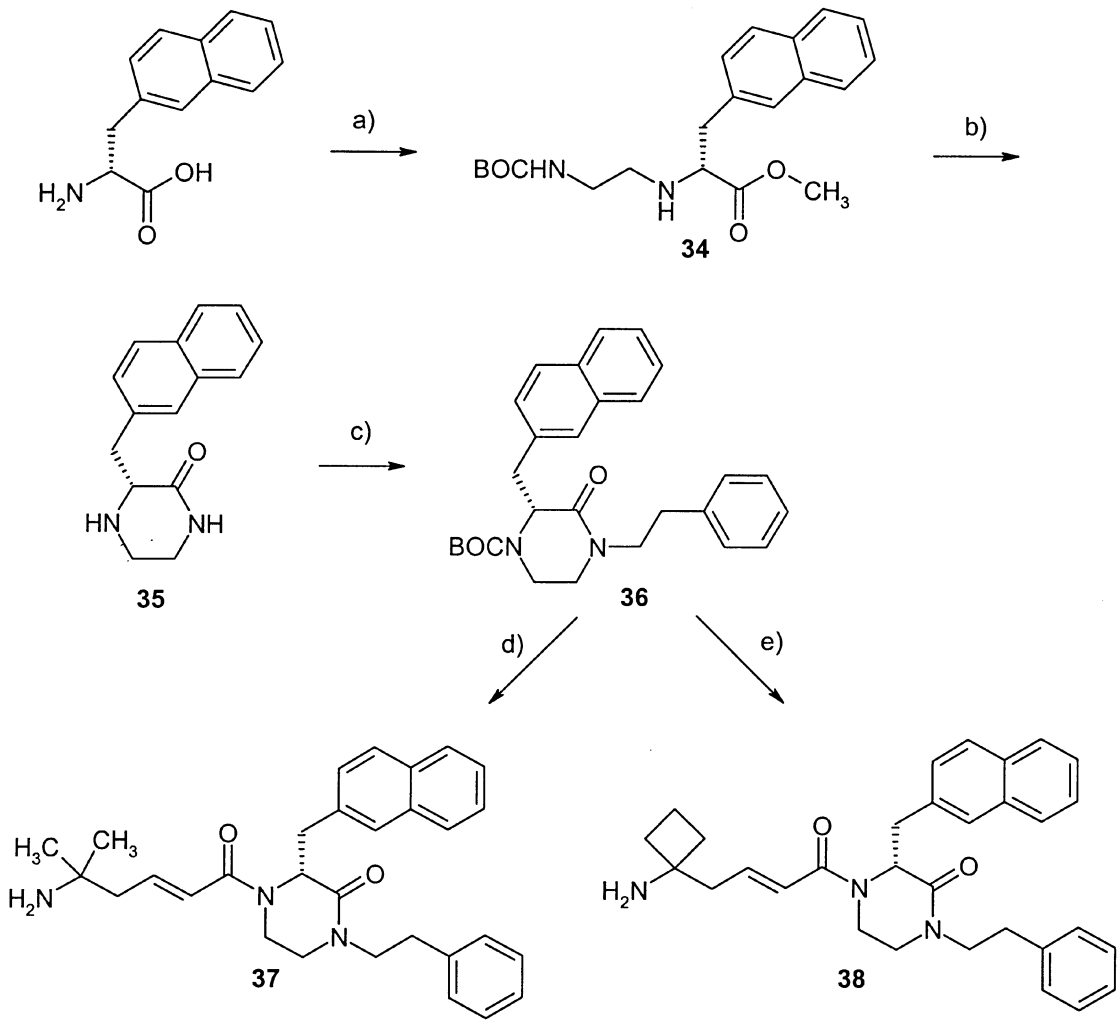

Figure 9. a) i CH_3OH, $SOCl_2$; ii $BOCHNCH_2CHO$, $NaCNBH_3$, mole sieves; b) i TFA; ii $NaHCO_3$; c) i NaOH, H_2O, $(BOC)_2O$, THF; ii KOH, DMSO $PhCH_2CH_2Br$; d) i TFA, CH_2Cl_2; ii (2*E*)-5-*tert*-butoxycarbonylamino-5-methylhex-2-enoic acid, HOAT, EDAC, DIPEA; iii TFA, CH_2Cl_2; e) i TFA, CH_2Cl_2; ii (2*E*)-4-(1-(*tert*-butoxycarbonylamino)cyclobutyl)but-2-enoic acid, HOAT, EDAC, DIPEA; iii TFA, CH_2Cl_2.

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 post-dosing. A one-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 of body weight via gavage. For i.v. administration the dogs received a dose of 0.5 mg/kg 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

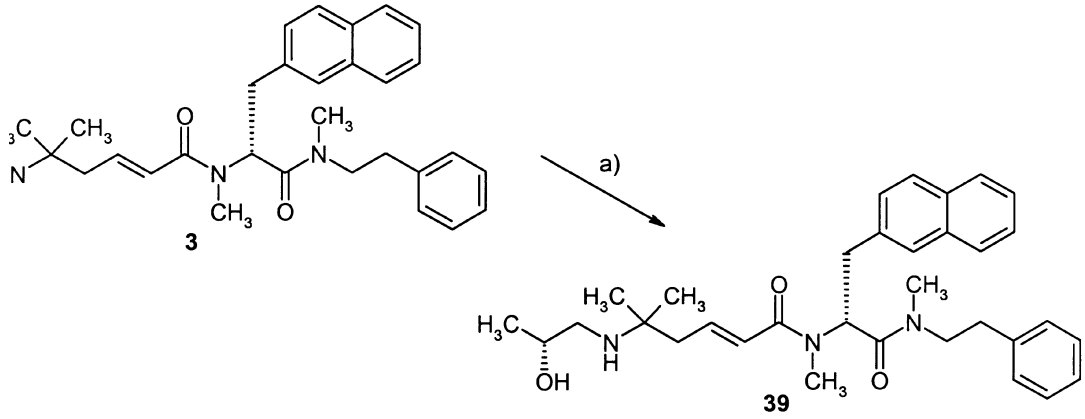

Figure 10. a) i (*R*)-(*tert*-butyldimethylsilyloxy)propanal, $NaCNBH_3$, HOAc; ii TBAF, THF.

versus time curve following p.o. administration divided by the area following i.v. administration, appropriately corrected for dose.

For the in vivo characterisation in conscious swine, four female 30–40 kg Danish slaughter swine of the breed Landrace Yorkshire cross were used for each GH secretagogue. The swine were housed at least 1 week prior to experiments. Prior to experiments it was tested that the swine used had similar basal GH levels. Indwelling jugular catheters were inserted and fixed under general haloghane anaesthesia at day 0. The test compounds were administered as 50 nmol/kg i.v. bolus injections with at least 48 h intervals between compounds. Each group of swine was used to test a maximum of five different compounds. The test compounds were dissolved in phosphate/citrate buffer diluted in saline containing 0.5% porcine serum albumin. Blood samples were drawn from the jugularis catheter at frequent intervals from 1 h prior to stimulation until 3 h poststimulation.

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. 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. NMR spectra were obtained at 400 MHz on a Bruker instrument.

Table I. Initial screening, in vitro rat-pituitary cell assay (n = 2).

Entry	NN703	**3**	**17**	**18**	**19**	**20**	**21**
EC_{50} [nM]	3 ± 1	32 ± 9	7.5 ± 0.5	0.8[b] ± 0.1	175 ± 125	143 ± 58	3 ± 3
E_{max}[a]	110 ± 7	90 ± 5	135 ± 2	115[b] ± 20	65 ± 12	100 ± 19	105 ± 25
Entry	**22**	**23**	**24**	**25**	**26**	**27**	**28**
EC_{50} [nM]	5 ± 4	18[b] ± 9	5 ± 4	5[b] ± 3	80 ± 1	27 ± 14	40 ± 10
E_{max}[a]	120 ± 22	115[b] ± 14	105 ± 26	150[b] ± 9	80 ± 5	145 ± 27	88 ± 12
Entry	**29**	**33**	**37**	**38**	**39**		
EC_{50} [nM]	60[b] ± 26	165 ± 20	45 ± 5	140 ± 140	750 ± 50		
E_{max}[a]	75[b] ± 12	80 ± 17	80 ± 8	90 ± 18	110 ± 143		

[a] [% of GHRP-6]; [b] n = 3.

Table II. In vivo data of selected compounds.

Entry	NN703[d]	**17**	**18**	**22**	**23**	**25**
$f_{p.o.}$ [%][a]	44	16	20	44	25	21
$t_{½}$ [h][b]	3.2	0.9	1.1	0.9	0.3	1.9
Volume of distribution [l/kg][b]	10.5	0.47	1.25	3.58	1.85	3.45
Total clearance [L/(h kg)][b]	2.40	0.36	0.79	2.75	3.81	1.24
Maximal GH release in dog [ng/mL][b]	64	23	10	14	22	4
Maximal GH release in swine [ng/mL][c]	25[e]	2	30	10	13	15

[a] In beagle dogs; [b] in beagle dogs after i.v. administration at 0.5 mg/kg; [c] at 50 nmol/kg compound in swine after p.o. administration; [d] NN703 was tested in two male and two female beagle dogs (results are therefore listed as means of four), all other compounds were tested in one male beagle dog; [e] extrapolation from a dose–response curve (Hill plot) using 1, 13, 30, 100, 300, 1 000 and 10 000 nmol/kg.

6.2.1. HPLC-methods

Three different elution conditions were used.

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 5m C-18 silica column (The Seperations Group, Hesperia), which was eluted at 1 mL/min at 42 °C. The column was equilibrated with 5% acetonitrile in a buffer consisting of 0.1 M ammonium sulfate, which was adjusted to pH 2.5 with 4 M sulfuric acid. After injection the sample was eluted by a gradient of 5–60% acetonitrile in the same buffer for 50 min.

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 5m C-18 silica column (The Seperations Group, Hesperia), which was eluted at 1 mL/min 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 for 50 min.

Method H8:

The RP-HPLC analysis was performed using UV detections at 214 and 254 nm on a 218TP54 4.6 × 150 mm C-18 silica column, which was eluted at 1 mL/min at 42 °C. The column was equilibrated with 5% acetonitrile, 85% water and 10% of a solution of 0.5% trifluoroacetic acid in water and eluted by a linear gradient from 5% acetonitrile, 85% water and 10% of a solution of 0.5% trifluoroacetic acid to 90% acetonitrile and 10% of a solution of 0.5% trifluoroacetic acid over 15 min.

6.2.2. Protocols

6.2.2.1. (2R)-2-(Methylamino)-3-(2-naphthyl)propionic acid N-methyl-N-phenethylamide **2**

(2*R*)-2-(*N*-*tert*-Butoxycarbonyl-*N*-methylamino)-3-(2-naphthyl)propionic acid (1.40 g, 4.3 mmol) was dissolved in *N*,*N*-dimethylformamide (5 mL) and dichloromethane (5 mL). Hydroxy-7-azabenzotriazole (0.59 g, 4.3 mmol) was added as a solid. The solution was cooled to 0 °C. *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (0.99 g, 5.2 mmol) was added. The solution was stirred for 20 min at 0 °C. *N*-Methyl-*N*-phenethylamine (**1**, 0.86 mL, 6.0 mmol) was added. The solution was stirred for 16 h, while it was warming up to room temperature. It was diluted with water (300 mL) and ethyl acetate (150 mL). Ten percent sodium hydrogen sulfate solution (80 mL) was added. The phases were separated. The aqueous phase was extracted with ethyl acetate (4 × 50 mL). The combined organic layers were washed with saturated sodium hydrogen carbonate solution (200 mL) and dried over magensium sulfate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (90 g), using ethyl acetate/heptane 1:1 as eluent to give 1.89 g (100%) of *N*-methyl-*N*-((1*R*)-1-(*N*-methyl-*N*-phenethylcarbamoyl)-2-(2-naphthyl)ethyl)carbamic acid *tert*-butylester. ^{1}H-NMR ($CDCl_3$): δ 1.01, 1.09, 1.25 and 1.30 (all s, together 9 H); 2.60–3.85 (m, 12 H); 4.75, 5.03, 5.31 and 5.37 (all dd, together 1 H); 7.00–7.85 (m, 12 H).

N-Methyl-*N*-((1*R*)-1-(*N*-methyl-*N*-phenethylcarbamoyl)-2-(2-naphthyl)ethyl)carbamic acid *tert*-butylester (1.84 g, 4.12 mmol) was dissolved in dichloromethane (6 mL). The solution was cooled to 0 °C. Trifluoroacetic acid (6 mL) was added. The solution was stirred for 10 min at 0 °C. The

solvent was removed in vacuo at 20 °C. The residue was dissolved in dichloromethane (100 mL) and the solvent was removed in vacuo. This latter procedure was repeated twice. The crude product was purified by flash chromatography on silica (70 g), using dichloromethane/methanol/25% aqueous ammonia (100:10:1) as eluent, to give 350 mg (25%) of amine **2**. ^{1}H-NMR ($CDCl_3$): δ 1.72 (br, 1 H); 2.12, 2.30, 2.44 and 2.87 (all s, together 6 H); 2.58, 2.76, 2.91, 2.98, 3.09, 3.25, 3.50, 3.61 and 3.73 (all m, together 7 H); 6.90–7.85 (m, 12 H).

6.2.2.2. (2R)-2-(N-((2E)-5-Amino-5-methylhex-2-enoyl)-N-methylamino)-3-(2-naphthyl)propionic acid N-methyl-N-phenethylamide **3**

(2*E*)-5-(*tert*-Butoxycarbonylamino)-5-methylhex-3-enoic acid (913 mg, 3.75 mmol) was dissolved in dichloromethane (10 mL). Hydroxy-7-azabenzotriazole (511 mg, 3.75 mmol) was added as a solid. The solution was cooled to 0 °C. *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (719 mg, 3.75 mmol) was added. The solution was stirred for 10 min at 0 °C. Amine **2** (1.30 g, 3.75 mmol) was dissolved in dichloromethane (10 mL) and *N*,*N*-dimethylformamide (10 mL) and added to the reaction mixture. Ethyldiisopropylamine (0.64 mL, 3.75 mmol) was added. The reaction mixture was stirred for 16 h while it was warming to room temperature. The solution was diluted with ethyl acetate (150 mL). Ten percent aqueous sodium hydrogen sulfate solution (50 mL) was added. The phases were separated, and the aqueous phase was extracted with ethyl acetate (4 × 50 mL). The combined organic layers were washed with saturated sodium hydrogen carbonate solution (200 mL) and dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (90 g), using ethyl acetate/heptane 1:1 as eluent, to give 1.76 g (82%) of (3*E*)-1,1-dimethyl-4-(*N*-methyl-*N*-((1*R*)-1-(*N*-methyl-*N*-phenethylcarbamoyl)-2-(2-naphthyl)ethyl)carbamoyl)but-3-enylcarbamic acid *tert*-butylester. ^{1}H-NMR ($CDCl_3$): δ 1.14, 1.17, 1.23 and 1.26 (all s, together 6 H); 1.38 and 1.41 (both s, together 9H); 2.40–3.10, 3.30–3.60 and 3.92 (all m, together 8 H); 2.78, 2.89 and 3.03 (all s, together 6 H); 4.28 and 4.40 (both br, together 1 H); 5.78 and 5.85 (both dd, together 1 H); 6.15 and 6.23 (both d, together 1 H); 6.70 and 6.80 (both m, together 1 H); 7.00–7.85 (m, 12 H).

(3*E*)-1,1-Dimethyl-4-(*N*-methyl-*N*-((1*R*)-1-(*N*-methyl-*N*-phenethylcarbamoyl)-2-(2-naphthyl)ethyl)carbamoyl)-but-3-enylcarbamic acid *tert*-butylester (528 mg, 0.85 mmol) was dissolved in dichloromethane (10 mL). Trifluoroacetic acid (10 mL) was added. The solution was stirred at room temperature for 50 min. Dichloromethane (100 mL) was added. A saturated aqueous solution of sodium hydrogen carbonate (70 mL) was added. Solid sodium hydrogen carbonate was added until pH 7 was obtained. The phases were separated. The aqueous phase was extracted with dichloromethane (3 × 70 mL). The combined organic layers were dried over magnesium sulfate. 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 1.08 g (75%) of amine **3** at 20 °C. HPLC: R_t 33.92 min; purity: 95% (254 nm; A1). R_t 35.67 min; purity: 88% (214 nm; B1). NMR ($CDCl_3$, selected values, free base): δ 1.04, 1.05, 1.11 and 1.12 (all s, together 6 H); 5.78 and 5.87 (both dd, together 1 H); 6.14 and 6.23 (both d, together 1 H); 6.78 and 6.87 (both dt, together 1 H). MS: calc. for $[M + H]^+$: 472; found 472.1.

6.2.2.3. Ethyl 2-(2-((N-methyl-carbamoyl)methyl)phenoxy)acetate **5**

(2-Hydroxyphenyl)acetic acid (**4**, 9.89 g, 63.7 mmol) and 1-hydroxybenzotriazole hydrate (8.61 g, 63.7 mmol) were dissolved in *N*,*N*-dimethylformamide (50 mL) and dichloromethane (200 mL). The solution was cooled to 0 °C. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (8.67 g, 63.7 mmol) was added. The reaction mixture was stirred for 30 min at 0 °C. An 8.0 M solution of methyl amine (39 mL, 318 mmol) was added. The reaction mixture was stirred for 16 h while it was slowly warming up to room temperature. It was diluted with ethyl acetate (600 mL) and washed with a 10% aqueous solution of sodium hydrogen sulfate (2 × 300 mL). The combined aqueous phases were extracted with ethyl acetate. The combined organic layers were washed with saturated sodium hydrogen carbonate solution (300 mL) and dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (180 g), using ethyl acetate/heptane (1:1) as eluent to give 4.90 g (47%) of 2-(2-hydroxyphenyl)-*N*-methylacetamide. M.p.: 105–106 °C (ethyl acetate/heptane). ^{1}H-NMR ($CDCl_3$): δ 2.82 (d, 3 H); 3.56 (s, 2 H); 6.20 (br, 1 H); 6.83 (m, 1 H); 7.00 (m, 2 H); 7.18 (m, 1 H); 9.85 (s, 1 H).

Potassium carbonate (2.81 g, 20.34 mmol) was given to a solution of 2-(2-hydroxyphenyl)-*N*-methylacetamide (3.36 g, 20.34 mmol) in acetone (150 mL). Ethyl bromoacetate (2.13 mL, 19.32 mmol) and potassium iodide (166 mg, 1.02 mmol) were added successively. The reaction mixture was heated to reflux for 6 h. It was left at room temperature for 16 h. The solid was filtered off. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (80 g), using

ethyl acetate/dichloromethane (1:1) as eluent, to give 5.00 g (98%) of ether **5**. ^{1}H-NMR ($CDCl_3$): δ 1.33 (t, 3 H); 2.74 (d, 3 H); 3.61 (s, 2 H); 4.30 (q, 2 H); 4.70 (s, 2 H); 6.68 (br, 1 H); 6.76 (d, 1 H); 6.98 (t, 1 H); 7.24 (t, 1 H); 7.32 (d, 1 H).

6.2.2.4. 2-(2-(2-(Methylamino)ethyl)phenoxy)ethanol **6**

At 0 °C, a solution of ethyl ether **5** (5.00 g, 19.9 mmol) in tetrahydrofuran (75 mL) was added dropwise to a suspension of sodium borohydride (2.26 g, 59.7 mmol) in tetrahydrofuran (75 mL). A solution of iodine (5.05 g, 19.9 mmol) in tetrahydrofuran (150 mL) was added dropwise. The solution was warmed to room temperature and heated to reflux for 16 h. It was cooled to 0 °C. Methanol (150 mL) was added dropwise. The solvent was removed in vacuo. The solid residue was dissolved in 20% aqueous sodium hydroxide solution/*tert*-butyl methyl ether (150 mL/150 mL). The phases were separated. The aqueous phase was extracted with *tert*-butyl methyl ether (3 × 150 mL). The combined organic layers were dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (80 g), using dichloromethane/methanol/10% aqueous ammonia (first: 100:10:1, then 70:30:3) as eluent, to give 1.12 g (29%) of alcohol **6**. ^{1}H-NMR ($CDCl_3$): δ 2.40 (s, 3 H); 2.82 (m, 2 H); 2.92 (m, 2 H); 3.05 (br, 2 H); 3.94 (m, 2 H); 4.10 (m, 2 H); 6.87 (d, 1 H); 6.92 (t, 1 H); 7.17 (m, 2 H).

6.2.2.5. 2-(2-Benzyloxyphenyl)-N-methylacetamide **8**

2-(2-Benzyloxyphenyl)acetic acid (**7**, 15.0 g, 62 mmol) was dissolved in dichloromethane (270 mL) and *N*,*N*-dimethylformamide (70 mL). 1-Hydroxybenzotriazole (8.37 g, 62 mmol) was added. The solution was cooled to 0 °C. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (11.89 g, 62 mmol) was added. The reaction mixture was stirred for 20 min. An 8.0 M solution of methylamine in ethanol (38.8 mL, 310 mmol) was added. The solution was stirred for 16 h while it was warming up to room temperature. It was diluted with ethyl acetate (500 mL) and washed with a 10% aqueous solution of sodium hydrogen sulfate solution (500 mL). The aqueous phase was extracted with ethyl acetate (2 × 300 mL). The combined organic layers were washed with a saturated aqueous solution of sodium hydrogen carbonate (400 mL) and dried over magnesium sulfate. The solvent was removed in vacuo. The remaining crystals were washed with a mixture of ethyl acetate/heptane 1:4 (100 mL). They were dried in vacuo. They were dissolved in ethyl acetate. The solution was washed with a saturated aqueous solution of sodium hydrogen carbonate (2 × 500 mL) and dried over magnesium sulfate. The solvent was removed in vacuo to give 10.24 g (65%) of amide **8**. ^{1}H-NMR (DMSO-d_6): δ 2.57 and 2.58 (both s, together 3 H); 3.44 (s, 2 H); 5.60 and 5.61 (both s, together 2 H); 6.89 (t, 1 H); 7.03 (d, 1 H); 7.19 (m, 2 H); 7.30 (m, 1 H); 7.38 (m, 2 H); 7.45 (m, 2 H); 7.69 (br, 1 H).

6.2.2.6. N-(2-(2-Benzyloxyphenyl)-ethyl)-N-methylcarbamic acid tert-butyl ester **9**

At 0 °C, a solution of amide **8** (9.39 g, 36.8 mmol) in tetrahydrofuran (150 mL) was added dropwise to a suspension of sodium borohydride (1.67 g, 44.12 mmol) in tetrahydrofuran (100 mL). After the addition was finished, a solution of iodine (4.67 g, 18.39 mmol) in tetrahydrofuran (200 mL) was added dropwise. The solution was warmed to reflux for 16 h. It was cooled to 0 °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 (200 mL). The phases were separated. The aqueous phase was extracted with *tert*-butyl methyl ether (3 × 75 mL). The combined organic layers were dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (400 g), using dichloromethane/methanol/25% aqueous ammonia as eluent (100:10:1), to give 4.73 g (38%) of *N*-(2-(2-benzyloxyphenyl)ethyl)-*N*-methylamine. ^{1}H-NMR ($CDCl_3$): δ 2.40 (s, 3 H); 2.70 (br, 1 H); 2.87 (m, 4 H); 5.07 (s, 2 H); 6.89 (m, 2 H); 7.18 (m, 2 H); 7.35 (m, 5 H).

A solution of di-*tert*-butyl dicarbonate (3.80 g, 17.4 mmol) in tetrahydrofuran (8.7 mL) was added dropwise to a solution of *N*-(2-(2-benzyloxyphenyl)ethyl)-*N*-methylamine (3.82 g, 15.8 mmol) in tetrahydrofuran (8.7 mL) and a 1 N aqueous sodium hydroxide solution (17.4 mL, 17.4 mmol). The reaction mixture was stirred for 16 h at room temperature. It was diluted with ethyl acetate (200 mL) and water (200 mL). The phases were separated. The aqueous phase was extracted with ethyl acetate (200 mL). The combined organic layers were washed with a saturated aqueous solution of sodium hydrogen carbonate (400 mL) and dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (225 g), using ethyl acetate/heptane 1:4 as eluent, to give 4.73 g (88%) of carbamate **9**. ^{1}H-NMR ($CDCl_3$): δ 1.32 and 1.42 (both br, together 9 H); 2.74 and 2.86 (both br, together 5 H); 3.43 (t, 2 H); 5.08 (s, 2 H); 6.89 (m, 2 H); 7.17 (br, 2 H); 7,40 (m, 5 H).

6.2.2.7. N-(2-(2-Hydroxyphenyl)-ethyl)-N-methylcarbamic acid tert-*butyl ester* **10**

Carbamate **9** (4.66 g, 13.65 mmol) was dissolved in ethanol (35.6 mL) and was hydrogenated at room pressure in the presence of 10% palladium on activated carbon for 16 h. The reaction mixture was filtered through a plug of celite. The celite was washed with ethyl acetate (50 mL). The liquid phases were collected. The solvents were removed in vacuo. The crude product was purified by flash chromatography on silica (300 g), using ethyl acetate/heptane (1:2) as eluent, to give 2.84 g (83%) of phenol **10**. ^{1}H-NMR ($CDCl_3$): δ 1.45 (s, 9 H); 2.86 (t, 2 H); 2.90 (s, 3 H); 3.34 (br, 2 H); 6.81 (t, 1 H); 6.87 (br, 1 H), 7.03 (d, 1 H), 7.12 (t, 1 H).

6.2.2.8. 3-[2-(2-Methylamino-ethyl)phenoxy]propan-1-ol **11**

Phenol **10** (702 mg, 2.79 mmol) was dissolved in *N*,*N*-dimethylformamide (6 mL). Potassium carbonate (1.93 g, 13.97 mmol) and caesium chloride (24 mg, 0.14 mmol) were added. 3-Bromo-1-propanol (0.28 mL, 3.07 mmol) was added. The reaction mixture was stirred at 80 °C for 16 h. It was cooled to room temperature and diluted with ethyl acetate (75 mL) and water (75 mL). The phases were separated. The aqueous phase was extracted with ethyl acetate (2 × 50 mL). The combined organic layers were washed with a 10% aqueous solution of sodium hydrogen sulfate solution (70 mL) and a saturated aqueous solution of sodium hydrogen carbonate (70 mL). They were dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (80 g), using ethyl acetate/heptane (1:1) as eluent, to give 606 mg (70%) of {2-[2-(3-hydroxypropoxy)phenyl]ethyl}-*N*-methylcarbamic acid *tert*-butyl ester. ^{1}H-NMR ($CDCl_3$): δ 1.39 (br, 9 H); 2.06 (m, 2 H); 2.82 (br, 5 H); 3.45 (br, 2 H); 3.90 (br, 2 H); 4.14 (t, 2 H); 6.85 (m, 2 H); 7.09 (br, 1 H); 7.17 (t, 1 H).

{2-[2-(3-Hydroxypropoxy)phenyl]ethyl}-*N*-methylcarbamic acid *tert*-butyl ester (0.587 g, 1.90 mmol) was dissolved in dichloromethane (5 mL). The solution was cooled to 0 °C. Trifluoroacetic acid (5 mL) was added. The reaction mixture was stirred for 30 min at 0 °C. Dichloromethane (50 mL) was added. A saturated aqueous solution of sodium hydrogen carbonate (50 mL) was added dropwise. Solid sodium hydrogen carbonate was added, until pH 7 was obtained. The phases were separated. The aqueous solution was extracted with dichloromethane (3 × 70 mL). The aqueous phase was made basic to pH 14 with a 20% aqueous sodium hydroxide solution. It was extracted with *tert*-butyl methyl ether (3 × 100 mL). The *tert*-butyl methyl ether extracts were combined and dried over magnesium sulfate. The solvent was removed in vacuo to give 227 mg (57%) of crude aminoalcohol **11**. The crude product was used in the next step without further purification. ^{1}H-NMR ($CDCl_3$): δ 1.19 (s, 1 H); 2.03 (m, 2 H); 2.25 (br, 1 H); 2.39 (s, 3 H); 2.83 (m, 4 H); 3.87 (m, 2 H); 4.10 (m, 2 H); 5.90 (m, 2 H); 7.15 (m, 2 H).

*6.2.2.9. {2-[2-(N-(*tert-*Butoxycarbonyl)-N-methylamino)ethyl]-phenyl}carbamic acid* tert-*butyl ester* **13** *and N-(2-(2-amino-phenyl)ethyl)-N-methylcarbamic acid* tert-*butyl ester* **14**

A solution of di-*tert*butoxy dicarboxylate (7.78 g, 36 mmol) in tetrahydrofuran (18 mL) was added to a solution of amine **12** (4.87 g, 32 mmol) [23] in tetrahydrofuran (18 mL) and a 1 N aqueous sodium hydroxide solution (36 mL, 36 mmol). The reaction mixture was stirred for 16 h at room temperature. It was diluted with ethyl acetate (400 mL) and water (300 mL). The phases were separated. The aqueous phase was extracted with ethyl acetate (4 × 100 mL). The combined organic layers were washed with a saturated aqueous solution of sodium hydrogen carbonate (300 mL) and dried over magnesium sulfate. The solvent was removed in vacuo. Flash chromatography on silica (60 g), using ethyl acetate/heptane 1:2 gave 4.95 g (44%) of dicarbamate **13** and 3.08 g (38%) of monocarbamate **14**.

13: ^{1}H-NMR ($CDCl_3$): δ 1.45 (br, 9 H); 1.54 (s, 9 H); 2.78 (t, 2 H); 2.88 (s, 3 H); 3.30 (br, 2 H); 6.98 (br, 1 H); 7.08 (br, 1 H); 7.20 (t, 1 H); 7.60–8.10 (br, 2 H).

14: ^{1}H-NMR ($CDCl_3$): δ 1.47 (s, 9 H); 2.75 (t, 2 H); 2.90 (s, 3 H); 3.35 (br, 2 H); 3.71 (br, 1 H); 4.23 (br, 1 H); 6.68 (m, 2 H); 7.00 (d, 1 H); 7.05 (t, 1 H).

MS: calc. for $[M + H]^+$: 152; found: 151.2.

6.2.2.10. {[2-(2-(Methylamino)ethyl)phenylcarbamoyl]-methyl}carbamic acid 9H-((fluoren-9-yl)methyl) ester **15**

2-(((9-Fluorenyl)methoxycarbonyl)amino)acetic acid (2.49 g, 8.36 mmol) was suspended in dichloromethane (40 mL). The suspension was cooled to 0 °C. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (802 mg, 4.19 mmol) was added. The reaction mixture was stirred for 30 min at 0 °C. A solution of amine **14** (698 mg, 2.79 mmol) in dichloromethane (15 mL) was added. The reaction mixture was stirred for 16 h while it was warming up to room temperature. It was diluted with dichloromethane (100 mL) and washed with brine (100 mL). The aqueous solution was extracted with dichloromethane (2 × 30 mL). The combined organic layers were dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (80 g), using ethyl acetate/

heptane (1:1) as eluent, to give 1.41 g (96%) of ({2-[2-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)ethyl]phenylcarbamoyl}methyl)carbamic acid ((9-fluorenyl)methyl) ester. ^{1}H-NMR ($CDCl_3$, selected values): δ 1.50 (br, 9 H); 2.80 (m, 2 H); 2.98 (br, 3 H); 3.22 (m, 2 H); 4.25 (m, 3 H); 4.40 (m, 2 H); 6.32 (br, 1 H); 9.20 (br, 1 H).

({2-[2-(*N*-(*tert*-Butoxycarbonyl)-*N*-methylamino)ethyl]phenylcarbamoyl}methyl)carbamic acid ((9-fluorenyl)methyl) ester (1.342 g, 2.53 mmol) was dissolved in 3.0 M hydrogen chloride in ethyl acetate (10 mL). The reaction mixture was stirred for 2 h at room temperature. Diethyl ether (40 mL) was added. The precipitation was filtered off and dried in vacuo to give 857 mg (73%) of crude amine **15** as hydrochloride, which was used for the next step without purification. ^{1}H-NMR (DMSO-d_6, selected values): δ 2.99 (br, 4 H); 9.05 (br, 2 H); 9.68 (br, 1 H).

6.2.2.11. N-(2-(2-(Methylamino)-ethyl)phenyl)methanesulfonamide **16**

A solution of amine **14** (723 mg, 2.9 mmol) and triethylamine (0.48 mL, 3.5 mmol) in dichloromethane (10 mL) was cooled to –78 °C. A solution of methanesulfonyl chloride (0.22 mL, 2.9 mmol) in dichloromethane (2 mL) was added dropwise. The reaction mixture was stirred for 16 h while it was warming up to room temperature. It was diluted with ethyl acetate (50 mL) and washed with 10% aqueous sodium hydrogen sulfate solution (150 mL). The aqueous phase was extracted with ethyl acetate (3 × 80 mL). The combined organic layers were washed with saturated aqueous sodium hydrogen carbonate solution (150 mL) and dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (60 g), using ethyl acetate/heptane (1:1) as eluent, to give 870 mg (92%) of *N*-(2-(2-(methylsulfonylamino)phenyl)-ethyl)-*N*-methylcarbamic acid *tert*-butyl ester. ^{1}H-NMR ($CDCl_3$) δ 1.50 (s, 9 H); 2.87 (m, 2 H); 2.91 (s, 3 H); 3.02 (s, 3 H); 3.30 (br, 2 H); 7.05–7.30 (m, 3 H); 7.57 (br, 1 H); 8.65 (br, 1 H).

At 0 °C, trifluoroacetic acid (6 mL) was added to a solution of *N*-(2-(2-(methylsulfonylamino)phenyl)ethyl)-*N*-methylcarbamic acid *tert*-butyl ester (870 mg, 2.6 mmol) in dichloromethane (6 mL). The reaction mixture was stirred for 50 min. Dichloromethane (25 mL) was added. A saturated aqueous solution of sodium hydrogen carbonate (35 mL) was added. Solid sodium hydrogen carbonate was added, until pH 7 was obtained. The phases were separated. The aqueous phase was extracted with dichloromethane (3 × 50 mL). The combined organic layers were dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (60 g), using dichloromethane/methanol/25% aqueous ammonia (100:10:1) as eluent to give 308 mg (53%) of amine **16**. ^{1}H-NMR ($CDCl_3$) δ 2.51 (s, 3 H); 2.84 (m, 2 H); 2.94 (m, 2 H); 3.00 (s, 3 H); 5.70–6.70 (br, 1 H); 7.03 (m, 1 H); 7.12 (d, 1 H); 7.22 (t, 1 H); 7.53 (d, 1 H).

6.2.2.12. (2E)-5-Amino-5-methyl-N-methyl-N-((1R)-1-(N-methyl-N-(2-(2-thienyl)-ethyl)carbamoyl)-2-(2-naphthyl)ethyl)hex-2-enamide **17**

Compound **17** (478 mg) (46% over 4 steps) was synthesized analogously to compound **3**, starting with *N*-methyl-*N*-(2-(2-thienyl)ethyl)amine [24] instead of amine **1**. HPLC: R_t = 33.48 min; purity: 91% (254 nm; A1). R_t = 35.13 min; purity: 86% (214 nm; B1). ^{1}H-NMR ($CDCl_3$, selected values): δ 1.04, 1.05, 1.11 and 1.11 (all s, together 6 H); 2.80, 2.90, 3.04 and 3.07 (all s, together 6 H); 5.83 and 5.88 (both dd, together 1 H); 6.14 and 6.25 (both d, together 1 H). MS: calc. for $[M + H]^+$: 478; found: 478.2. For biological testing, the title compound was dissolved in 0.5 M acetic acid (25 mL) and lyophilized.

6.2.2.13. (2E)-5-Methyl-5-(methylamino)-N-methyl-N-((1R)-1-(N-methyl-N-(2-(2-thienyl)-ethyl)carbamoyl)-2-(2-naphthyl)ethyl)hex-2-enamide **18**

Compound **18** (164 mg) (30% over 4 steps) was synthesized analogously to compound **3** using *N*-methyl-*N*-(2-(2-thienyl)ethyl)amine [24] instead of amine **1** and (2*E*)-5-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-5-methylhex-2-enoic acid instead of (2*E*)-5-(*tert*-butoxycarbonylamino)-5-methylhex-2-enoic acid. HPLC: R_t = 33.27 min; purity: 89% (254 nm; A1). R_t = 35.28 min; purity: 94% (214 nm; B1). ^{1}H-NMR ($CDCl_3$, selected values): δ 1.00 and 1.08 (both s, together 6 H); 2.23 and 2.30 (both s, together 3 H); 2.80, 2.89, 3.04 and 3.07 (all s, together 6 H); 5.85 (m, 1 H); 6.13 and 6.25 (both d, together 1 H). MS: calc. for $[M + H]^+$: 492; found: 492.0. For biological testing, it was transformed into the acetate by lyophilization from 0.5 N acetic acid (25 mL).

6.2.2.14. (2E)-5-Amino-5-methylhex-2-enoic acid N-methyl-N-((1R)-1-(N-methyl-N-(3-phenylpropyl)carbamoyl)-2-(2-naphthyl)ethyl)amide **19**

Compound **19** (436 mg) (20% over 4 steps) was prepared analogously to compound **3**. *N*-Methyl-*N*-(3-phenylpropyl)amine [25] was used instead of amine **1**. HPLC: R_t = 36.62 min; purity: 96% (254 nm; A1). R_t = 38.93 min; purity: 92% (214 nm; B1). ^{1}H-NMR ($CDCl_3$, selected values): δ 1.10 and 1.11 (both s, together 6 H); 2.85, 3.13, 3.15, and 3.50 (all s, together 6 H); 5.89 and 5.97 (both dd, together 1 H); 6.23 and 6.24 (both d, together 1 H). MS: calc. for $[M + H]^+$: 486; found: 486.4.

For biological testing, the title compound was transferred into its acetate salt by lyophilization from 0.5 M aqueous acetic acid (50 mL).

6.2.2.15. (2R)-2-(N-(3-(1-Aminoethyl)-benzoyl)-N-methylamino)-N-methyl-3-(2-naphthyl)-N-(2-(2-thienyl)ethyl)propionamide **20**

Compound **20** (293 mg) (50% over 4 steps) was synthesized analogously to compound **3**. *N*-Methyl-*N*-(2-(2-thienyl)ethyl)amine was used instead of amine **1**. (3-1-(*tert*-Butoxycarbonylamino)ethyl)benzoic acid was used instead of (2*E*)-5-(*tert*-butoxycarbonylamino)-5-methylhex-2-enoic acid. HPLC: R_t = 34.30; purity: 99% (254 nm; A1). R_t = 36.85; purity: 88% (214 nm; B1). ^{1}H-NMR ($CDCl_3$, selected peaks): δ 1.15 and 1.27 (both d, together 3 H); 2.87 (s, 3 H); 3.00 and 3.03 (both s, together 3 H); 5.90 and 6.00 (both dd, together 1 H). MS: calc. for $[M + H]^+$: 500; found: 500.0. For biological testing, the compound was dissolved in 0.5 M acetic acid (50 mL) and lyophilized.

6.2.2.16. (2E)-5-Methyl-N-methyl-5-(methylamino)-N-((1R)-1-(N-methyl-N-phenethyl-carbamoyl)-2-(2-naphthyl)ethyl)hex-2-enamide **21**

Compound **21** (80 mg) (39% over 2 steps) was synthesized analogously to compound **3**. (2*E*)-5-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-5-methylhex-2-enoic acid was used instead of (2*E*)-5-(*tert*-butoxycarbonylamino)-5-methylhex-2-enoic acid. HPLC: R_t = 34.30 min; purity: 97% (254 nm; A1). R_t = 36.28 min; purity: 99% (214 nm; B1). ^{1}H-NMR ($CDCl_3$, selected values): δ 1.00 and 1.06 (both s, together 6 H); 2.25 and 2.31 (both s, together 3 H); 2.76, 2.87 and 3.05 (all s, together 6 H); 5.77 and 5.85 (t and dd, together 1 H); 6.14 and 6.23 (both d, together 1 H); 6.78 (m, 1 H); 7.00–7.90 (m, 12 H). MS: calc. for $[M + H]^+$: 486; found 486.0. For biological testing, the title compound was transferred into its acetate salt by lyophilization with 0.5 M acetic acid (50 mL).

6.2.2.17. (2R)-2-(N-((2E)-5-Amino-5-methylhex-2-enoyl)-N-methylamino)-N-(2-(2-(2-hydroxyethoxy)-phenyl)ethyl)-N-methyl-3-(2-naphthyl)propionamide **22**

Compound **22** (218 mg) (44% over 4 steps) was synthesized analogously to compound **3**. Amine **6** was used instead of amine **1**. HPLC: R_t = 32.75 min; purity: 96% (254 nm; A1). R_t = 33.82 min; purity: 96% (214 nm; B1). ^{1}H-NMR ($CDCl_3$, selected values): δ 1.04 and 1.12 (both s, together 6 H); 2.93, 2.99, 3.02 and 3.07 (all s, together 6 H); 5.68 and 5.87 (both dd, together 1 H); 6.05 and 6.25 (both d, together 1 H). MS: calc. for $[M + H]^+$: 532; found: 532.2. For biological testing, the title compound was transferred into its acetate salt by lyophilization with 0.5 M acetic acid (50 mL).

6.2.2.18. (2R)-2-(N-((2E)-5-Amino-5-methylhex-2-enoyl)-N-methylamino)-N-methyl-3-(2-naphthyl)-N-(2-(2-methylsulfonylaminophenyl)ethyl)propionamide **23**

Compound **23** (82 mg) (12% over 4 steps) was prepared analogously to compound **3**. Amine **16** was used instead of amine **1**. HPLC: R_t = 32.08 min; purity: 94% (254 nm; A1). R_t = 32.53 min; purity: 92% (214 nm; B1). ^{1}H-NMR ($CDCl_3$, selected values) δ 1.08 and 1.15 (both s, together 1 H); 2.93 and 2.95 (both s, together 3 H); 2.99 and 3.05 (both s, together 3 H); 3.12 and 3.13 (both s, together 3 H); 5.57 and 5.88 (t and dd, together 1 H); 6.18 and 6.30 (both d, together 1 H). MS: calc. for $[M + H]^+$: 565; found: 565.0. For biological testing, the title compound was transferred into its acetate salt by lyophilization with 0.5 M acetic acid (40 mL).

6.2.2.19. (2E)-5-Amino-N-((1R)-2-(biphenyl-4-yl)-1-(N-methyl-N-(2-(2-thienyl)ethyl)-carbamoyl)ethyl)-5-methyl-N-methylhex-2-enamide **24**

Compound **24** (152 mg) (39% over 4 steps) was synthesized analogously to compound **3**. (2*R*)-2-(*N*-(*tert*-Butoxycarbonyl)-*N*-methylamino)-3-(biphenyl-4-yl)propionic acid was used instead of (2*R*)-2-(*N*-(*tert*-buoxycarbonyl)-*N*-methylamino)-3-(2-naphthyl)propionic acid. HPLC R_t = 37.87 min; purity: 96% (254 nm; A1). R_t = 38.52 min; purity: 92% (214 nm; B1). ^{1}H-NMR ($CDCl_3$, selected values): δ 1.09 and 1.22 (both s, together 6 H); 2.21 and 2.27 (both d, together 2 H); 2.85, 2.90, 3.07 and 3.08 (all s, together 6 H); 5.78 (m, 1 H); 6.20 and 6.26 (both d, together 1 H); 6.65–6.95, 7.09 and 7.20–7.60 (all m, together 13 H). MS: calc. for $[M + H]^+$: 504; found: 504.0. For biological testing, the title compound was transferred into its acetate salt by lyophilization with 0.5 M acetic acid (40 mL).

6.2.2.20. (2E)-N-((1R)-1-(N-(2-(2-(2-Hydroxyethoxy)-phenyl)ethyl)-N-methylcarbamoyl)-2-(2-naphthyl)ethyl)-N-methyl-5-methyl-5-(methylamino)hex-2-enamide **25**

Compound **25** (118 mg) (24% over 4 steps) was prepared analogously to compound **3**. Amine **6** was used instead of amine **1** and (2*E*)-5-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)-5-methylhex-2-enoic acid was used instead of (2*E*)-5-(*tert*-butoxycarbonylamino)-5-methylhex-2-enoic acid. HPLC: R_t = 33.47 min; purity: 92% (254 nm; A1). R_t = 34.25 min; purity: 97% (214 nm; B1). ^{1}H-NMR ($CDCl_3$, selected values): δ 1.02 and 1.10 (both s, together 6 H); 2.27 and 2.32 (both s, together 3 H); 2.91, 2.98, 3.02 and 3.06 (all s, together 6 H); 5.65 and 5.86

(both dd, together 1 H); 6.10 and 6.25 (both d, together 1 H); 6.55–7.90 (m, 12 H). MS: calc. for $[M + H]^+$: 546; found: 546.0. For biological testing, the title compound was transferred into its acetate salt by lyophilization with 0.5 M acetic acid (40 mL).

6.2.2.21. (2E)-5-Amino-5-methylhex-2-enoic acid N-((1R)-1-{N-[2-(2-(benzenesulfonylamino)phenyl)ethyl]-N-methylcarbamoyl}-2-(2-naphthyl)ethyl)-N-methylamide **26**

Compound **26** (252 mg) (33% over 6 steps) was prepared analogously to compound **3**. The amine used was *N*-[2-(2-(methylamino)ethyl)phenyl]benzenesulfonamide, which was prepared analogously to amine **16**, using benzensulfonyl chloride instead of methansulfonyl chloride. HPLC R_t = 37.87 min; purity: 87% (254 nm; A1). R_t = 40.23 min; purity: 91% (214 nm; B1). ^{1}H-NMR ($CDCl_3$, selected values): δ 1.09 and 1.12 (both s, together 6 H); 2.84 and 2.86 (both s, together 3 H); 3.15 and 3.20 (both s, together 3 H); 5.65 and 5.87 (t and dd, together 1 H); 6.22 and 6.32 (both d, together 1 H). MS: calc. for $[M + H]^+$: 627; found: 627.2. For biological testing, the title compound was transferred into its acetate salt by lyophilization with 0.5 M acetic acid (40 mL).

6.2.2.22. 2-Amino-N-(2-(2-(N-((2R)-2-(N-((2E)-5-amino-5-methylhex-2-enoyl)-N-methylamino)-3-(2-naphthyl)-propionyl)-N-methylamino)ethyl)phenyl)acetamide **27**

(2*R*)-2-(*N*-(*tert*-Butoxycarbonyl)-*N*-methylamino)-3-(2-naphthyl)propionic acid (590 mg, 1.79 mmol) and 1-hydroxy-7-azabenzotriazole (243 mg, 1.79 mmol) were dissolved in dichloromethane (12 mL) and *N*,*N*-dimethylformamide (6 mL). The solution was cooled to 0 °C. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (343 mg, 1.79 mmol) was added. The solution was stirred for 25 min at 0 °C. The hydrochloride of amine **15** (834 mg, 1.79 mmol) and ethyldiisopropylamine (0.62 mL, 3.58 mmol) were added successively. The reaction mixture was stirred for 16 h while it was warming up to room temperature. It was diluted with ethyl acetate (30 mL) and washed with 10% aqueous sodium hydrogen sulfate solution (30 mL). The aqueous phase was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with a saturated aqueous solution of sodium hydrogen carbonate (30 mL) and dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (60 g), using ethyl acetate/heptane (1:1) as eluent, to give 1.29 g (98%) of *N*-{(1*R*)-1-[*N*-(2-{2-[2-((fluoren-9-ylmethoxycarbonyl)-amino)acetylamino]phenyl}ethyl)-*N*-methylcarbamoyl]-2-(2-naphthyl)ethyl}-*N*-methylcarbamic acid *tert*-butyl ester. ^{1}H-NMR ($CDCl_3$, selected values): δ (1.25 and 1.30, both br, together 9 H); 2.85, 3.03 and 3.04 (all s, together 6 H); 5.08 and 5.45 (both t, together 1 H); 9.31 and 9.45 (both s, together 1 H).

N-{(1*R*)-1-[*N*-(2-{2-[2-((Fluoren-9-ylmethoxycarbonyl)-amino)acetylamino]phenyl}ethyl)-*N*-methylcarbamoyl]-2-(2-naphthyl)ethyl}-*N*-methylcarbamic acid *tert*-butyl ester (1.18 g, 1.59 mmol) was dissolved in 3.0 M hydrogen chloride in ethyl acetate (8 mL). The reaction mixture was stirred for 2.25 h at room temperature. The solvent was removed in vacuo. The residue was washed with diethyl ether (3 × 20 mL) and dried in vacuo to give crude [(2-{2-[*N*-methyl-*N*-((2*R*)-2-methylamino-3-(2-naphthyl)-propionyl)amino]ethyl}phenylcarbamoyl)methyl]carbamic acid (fluoren-9-yl)methyl ester as hydrochloride, which was used for the next step without purification. ^{1}H-NMR (DMSO-d_6, selected values): δ 4.41 and 4.67 (both m, together 1 H).

(2*E*)-5-(*tert*-Butoxycarbonylamino)-5-methylhex-2-enoic acid (200 mg, 0.82 mmol) and 1-hydroxy-7-azabenzotriazole (112 mg, 0.82 mmol) were dissolved in dichloromethane (4 mL) and *N*,*N*-dimethylformamide (2 mL). The solution was cooled to 0 °C. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (157 mg, 0.82 mmol) was added. The reaction mixture was stirred for 20 min at 0 °C. The hydrochloride of crude [(2-{2-[*N*-methyl-*N*-((2*R*)-2-methylamino-3-(2-naphthyl)propionyl)amino]ethyl}phenylcarbamoyl)methyl]-carbamic acid (fluoren-9-yl)methyl ester (553 mg, 0.82 mmol) and ethyldiisopropylamine (0.28 mL, 1.64 mmol) were added successively. The reaction mixture was stirred for 16 h, while it was warming up to room temperature. It was diluted with ethyl acetate (50 mL) and washed with 10% aqueous sodium hydrogen sulfate solution (50 mL). The aqueous phase was extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with a saturated aqueous solution of sodium hydrogen carbonate (50 mL) and dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (40 g), using ethyl acetate/heptane (2:1) as eluent, to give 365 mg (58% over 2 steps) of (3*E*)-4-(*N*-((1*R*)-1-(*N*-(2-(2-((((9H-9-fluorenyl)-methoxycarbonyl)amino)acetylamino)phenyl)ethyl)-*N*-*methylcarbmoyl)-2-(2-naphthyl)ethyl)-*N*-methylcarbamoyl)-1,1-dimethylbut-3-enylcarbamic acid *tert*-butyl ester. ^{1}H-NMR ($CDCl_3$, selected values): δ 5.51 and 5.94 (t and dd, together 1 H); 6.13 and 6.25 (both d, together 1 H); 6.25 (br, 1 H); 6.75 and 6.83 (both m, together 1 H).

At room temperature tris(2-aminoethyl)amine (2.99 mL, 19.8 mmol) was added to a solution of (3*E*)-4-(*N*-((1*R*)-1-(*N*-(2-(2-((((9-fluorenyl)methoxycarbonyl)-amino)acetylamino)phenyl)ethyl)-*N*-methylcarbmoyl)-2-(2-naphthyl)ethyl)-*N*-methylcarbamoyl)-1,1-dimethylbut-3-enylcarbamic acid *tert*-butyl ester (346 mg, 0.40 mmol) in dichloromethane (2.8 mL). The reaction mixture was stirred for 1.2 h at room temperature. It was diluted with dichloromethane (40 mL) and washed with brine (50 mL). The aqueous phase was extracted with dichloromethane (3 × 20 mL). The combined organic layers were washed with a buffer of sodium dihyrogen phosphate and dipotassium hydrogen phosphate (pH 6.4, 3 × 30 mL) and successively with brine (20 mL). They were dried over magnesium sulfate. 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 185 mg (72%) of (3*E*)-4-(*N*-((1*R*)-1-(*N*-(2-(2-(2-aminoacetyl-amino)phenyl)ethyl)-*N*-methylcarbamoyl)-2-(2-naphthyl)-ethyl)-*N*-methylcarbamoyl)-1,1-dimethylbut-3-enylcarbamic acid *tert*-butyl ester. ^{1}H-NMR ($CDCl_3$, selected values): δ 1.14, 1.15, 1.23, and 1.24 (all s, together 6 H); 1.39 and 1.40 (both s, together 9 H); 2.90, 2.94, 3.02 and 3.10 (all s, together 6 H); 5.64 and 5.90 (t and dd, together 1 H); 6.12 and 6.26 (both d, together 1 H); 6.63 and 6.82 (both m, together 1 H); 9.42 and 9.53 (both br, together 1 H).

(3*E*)-4-(*N*-((1*R*)-1-(*N*-(2-(2-(2-Aminoacetylamino)phenyl)ethyl)-*N*-methylcarbamoyl)-2-(2-naphthyl)ethyl)-*N*-methylcarbamoyl)-1,1-dimethylbut-3-enylcarbamic acid *tert*-butyl ester (175 mg, 0.27 mmol) was dissolved in dichloromethane (2 mL). The solution was cooled to 0 °C. Trifluoroacetic acid (2 mL) was added. The reaction mixture was stirred for 25 min at 0 °C. Dichloromethane (20 mL) and ethanol (20 mL) were added successively. The solvent was removed in vacuo without warming. The residue was dissolved in dichlromethane (40 mL) and the solvent was removed in vacuo. The last procedure was repeated. The crude product was purified by flash chromatography on silica (15 g), using dichloromethane/methanol/25% aqueous ammonia /(first: 100:10:1, then: 100:20:2) as eluent, to give 128 mg (87%) of compound **27**. HPLC: R_t = 7.15 min; purity: 89% (214 nm; H8). ^{1}H-NMR ($CDCl_3$, selected values): δ 1.03 and 1.12 (both s, together 6 H); 2.92, 2,94, 3.01 and 3.12 (all s, together 6 H); 5.62 and 5.90 (t and dd, together 1 H); 6.10 and 6.25 (both d, together 1 H); 6.70 and 6.89 (both m, together 1 H); 9.48 and 9.52 both br, together 1 H). For biological testing, the title compound was transferred into its acetate salt by lyophilization with 0.5 M acetic acid (40 mL).

6.2.2.23. (2R)-2-(N-((2E)-5-Amino-5-methylhex-2-enoyl)-N-methylamino)-N-(2-(2-(3-hydroxypropoxy)-phenyl)ethyl)-N-methyl-3-(2-naphthyl)propionamide **28**

Compound **28** (64 mg) (17% over 4 steps) was prepared analogously to compound **3**. Amine **11** was used instead of amine **1**. HPLC: R_t = 32.57 min; purity: 95% (254 nm; A1). R_t = 34.60 min; purity: 100% (254 nm; B1). ^{1}H-NMR ($CDCl_3$, selected values): δ 0.98, 0.99, 1.10 and 1.11 (all s, together 6 H); 2.82, 2.85, 2.91 and 3.03 (all s, together 6 H); 5.47 and 5.84 (both dd, together 1 H); 5.95 and 6.19 (both d, together 1 H); 6.55 (m, 1 H). MS: calc. for $[M + H]^+$: 546; found: 546.0. For biological testing, the title compound was transferred into its acetate salt by lyophilization with 0.5 M actic acid (40 mL).

6.2.2.24. (2E)-5-Amino-5-methylhex-2-enoic acid N-((1R)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-1-((2-naphthyl)methyl)-2-oxoethyl)-N-methylamide **29**

Compound **29** (234 mg) (14% over 4 steps) was prepared analogously to compound **3**. Commercially available 1,2,3,4-tetrahydroqunoline was used as amine instead of amine **1**. HPLC: R_t = 33.48 min; purity: 96% (254 nm, A1). ^{1}H-NMR ($CDCl_3$, selected values): δ 1.08 and 1.10 (both s, together 6 H); 2.20 and 2.25 (both d, together 2 H); 2.96 and 3.08 (both s, together 3 H); 5.98 (m, 1 H); 6.06 and 6.23 (both d, together 1 H); 6.70–7.80 (m, 12 H). MS: calc. for $[M + H]^+$: 470; found: 470.0. For biological testing, the title compound was transferred into its acetate salt by lyophilization with 0.5 M acetic acid (50 mL).

6.2.2.25. N-((1R,2S*)-2-phenylcyclopropyl)formamide* **31**

Commercially available *trans*-2-phenylcyclopropylamine hydrochloride (**30**, 5.00 g, 29.47 mmol) was dissolved in ethyl acetate (100 mL) and a 1 N aqueous solution of sodium hydroxide (100 mL). The phases were separated. The aqueous phase was extracted with ethyl acetate (80 mL). The combined organic layers were dried over magnesium sulfate. The solvent was removed. The residue was dissolved in formic acid (34 mL). The solution was cooled to 0 °C. Acetic anhydride (13 mL) was added dropwise. The reaction mixture was stirred for 16 h while it was warming up to room temperature. It was cooled to 0 °C. Water (13 mL) was added dropwise. The solvents were removed in vacuo. The residue was dissolved in ethyl acetate (150 mL) and washed with brine (2 × 100 mL). The organic phase was dried over magnesium sulfate. The solvent was removed to give 4.40 g (93%) of crude **31**. The crude product was used for the next step without further purification. ^{1}H-NMR ($CDCl_3$): δ 1.30 (m, 2 H); 2.15 (m, 1 H); 2.90 (m, 1 H); 5.90 and

6.10 (both br, together 1 H); 7.00–7.40 (m, 5 H); 8.20 and 8.32 (s and d, together 1 H).

6.2.2.26. N-Methyl-N-((1R,2S*)-2-phenylcyclopropyl)amine* **32**

A solution of crude **31** (3.00 g, 18.61 mmol) in tetrahydrofuran (41 mL) was added dropwise to a mixture of sodium borohydride (0.84 g, 22.33 mmol) in tetrahydrofuran (70 mL). A solution of iodine (2.36 g, 9.31 mmol) in tetrahydrofuran (80 mL) was added dropwise. After the addition was finished, the reaction mixture was heated to reflux for 16 h. It was cooled to 0 °C. Methanol (160 mL) was added dropwise. The solvents were removed in vacuo. The residue was dissolved in a 20% aqueous solution of sodium hydroxide (250 mL) and *tert*-butyl methyl ether (150 mL). The phases were separated. The aqueous phase was extracted with *tert*-butyl methyl ether (2 × 100 mL). The combined organic layers were dried over magnesium sulfate. The solvent was removed. The crude product was purified by flash chromatography on silica (80 g), using first ethyl acetate/heptane/triethylamine (10:10:1) and then dichloromethane/methanol/25% aqueous ammonia (100:10:1) as eluent, to give 1.05 g (38%) of **32**. ^{1}H-NMR ($CDCl_3$): δ 0.95 (m, 1 H); 1.08 (m, 1 H); 1.77 (br, 1 H); 1.90 (m, 1 H); 2.33 (m, 1 H); 2.50 (s, 3 H); 7.00–7.30 (m, 5 H).

6.2.2.27. (2E)-5-Amino-5-methylhex-2-enoic acid N-methyl-N-((1R)-1-(N-methyl-N-((1R,2S*)-2-phenylcyclopropyl)carbamoyl)-2-(2-naphthyl)ethyl)amide* **33**

Compound **33** (193 mg) (23% over 4 steps) was prepared as a mixture of diastereoisomeres analogously to compound **3**. The diasetereosiomeres were inseparable on HPLC. Amine **32** was used instead of amine **1**. HPLC: Rt = 36.15 min; purity: 100% (254 nm; A1). Rt = 38.33 min; purity: 100% (214 nm; B1). ^{1}H-NMR ($CDCl_3$, selected values): δ 0.80–1.40 (m, 2 H); 1.11 and 1.11 (both s, together 6 H); 2.22 (d, 2 H); 2.87, 2.95, 3.14 and 3.19 (all s, together 6 H); 5.85 and 6.25 (both m, together 2 H); 6.70–7.80 (m, together 12 H). MS: calc. for $[M + H]^+$: 484; found: 484.2. For biological testing, the title compound was transferred into its acetate salt by lyophilization with 0.5 M acetic acid (50 mL).

6.2.2.28. (2R)-2-((2-(tert-Butoxycarbonylamino)-ethyl)amino)-3-(2-naphthyl)propionic acid methylester **34**

(2*R*)-2-Amino-3-(2-naphthyl)propionic acid (5.0 g, 23 mmol) was added to methanol (150 mL) and thionyl chloride (2.0 mL; 23 mmol) was added dropwise and the mixture was stirred overnight and then refluxed for 2.5 h. The solvent was removed in vacuo and the residue was dissolved in a mixture of methanol (95 mL) and acetic acid (5 mL). (2-Oxoethyl)carbamic acid *tert*-butyl ester [28] (3.4 g, 23 mmol), sodium cyanoborohydride (1.9 g, 31 mmol) and molecular sieves (50 g, Fluka, 3 Å) were added and the mixture was left overnight. The mixture was filtered and the filtrate was added to water (200 mL) and extracted with dichloromethane (3 × 100 mL). The combined organic phases were dried (magnesium sulfate), and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica (80 g) to afford 3.55 g (41%) of ester **34**. ^{1}H-NMR: ($CDCl_3$): δ 1.39 (s, 9H); 2.56 (m, 1H); 2.75 (m, 1H); 3.09 (m, 3H); 3.59 (m, 1H); 3.65 (s, 3H); 7.28–7.81 (7 arom. H).

6.2.2.29. (3R)-3-((2-Naphthyl)methyl)piperazin-2-one **35**

Ester **34** (3.4 g, 9.1 mmol) was stirred for 1 h in a mixture of trifluoroacetic acid (5 mL) and dichloromethane (5 mL). The volatiles were removed in vacuo and the residue was dissolved in a mixture of water (40 mL) and methanol (100 mL). Sodium hydrogencarbonate (2.3 g) was added and the mixture was stirred overnight. The solvent was removed in vacuo and the residue was dissolved in water (40 mL) and extracted with ethyl acetate (10 × 50 mL). The combined organic phases were dried (magnesium sulfate) and the solvent was removed in vacuo to afford 1.96 g (90%) of piperazinone **35**. ^{1}H-NMR: ($CDCl_3$; selected peaks for major rotamer): δ 2.95 (m, 1H); 3.05 (m, 2H); 3.24 (m, 1H); 3.39 (m, 1H); 3.59 (dd, 1H); 3.72 (dd, 1H).

6.2.2.30. (2R)-2-(2-Naphthyl)methyl-3-oxo-4-phenethylpiperazine-1-carboxylic acid tert-butyl ester **36**

Piperazinone **35** (1.9 g; 7.9 mmol) and di-*tert*-butyl dicarbonate (2.1 g; 9.5 mmol) was suspended in a mixture of tetrahydrofuran (20 mL) and aqueous sodium hydroxide (1 M, 8 mL) and stirred overnight. The solvent was removed in vacuo and water (30 mL) was added. The aqueous phase was extracted with ethyl acetate (2 × 50 mL). The combined organic phases were dried (magnesium sulfate) and the solvent was removed in vacuo. The residue was dissolved in a mixture of dimethylsulfoxide (15 mL) and potassium hydroxide (1.3 g). (2-bromoethyl)benzene (2.2 g, 11 mmol) was added and the mixture was stirred for 1 h. Water (30 mL) and dichloromethane (60 mL) were added. The organic phase was washed with water (5 × 10 mL) and the solvent was removed in vacuo. The residue was chromatographed on silica (60 g) using ethyl acetate/heptane 1:2 as eluent to afford 1.25 g (36%) of piperazone **36**. ^{1}H-NMR ($CDCl_3$; selected peaks for major rotamer): δ 1.15 (s, 9H); 2.76 (t, 2H); 3.39 (t, 3H).

6.2.2.31. (3R)-4-((2E)-5-Amino-5-methylhex-2-enoyl)-3-((2-naphthyl)methyl)-1-phenethylpiperazin-2-one **37**

Piperazone **36** (1.2 g; 2.7 mmol) was dissolved in a mixture of trifluoroacetic acid (5 mL) and dichloromethane (5 mL) and stirred for 15 min. Dichloromethane (30 mL) and a saturated aqueous solution of sodium hydrogencarbonate was added to pH 8. The mixture was extracted with dichloromethane (3 × 10 mL) and the combined aqueous phases were dried (magnesium sulfate) and the solvent was removed in vacuo. Part of the residue (400 mg 1.2 mmol) was added to a mixture of (2*E*)-5-(*tert*-butoxycarbonylamino)-5-methylhex-2-enoic acid (282 mg; 1.2 mmol); 1-hydroxy-7-azabenzotriazole (158 mg; 1.2 mmol), EDAC (245 mg; 1.3 mmol) and ethyldiisopropylamine (150 mg; 1.2 mmol) and stirred overnight. Dichloromethane (50 mL) was added and the mixture was washed with 10% solution of aqueous sodium hydrogensulfate (50 mL); a saturated aqueous solution of sodium hydrogencarbonate (50 mL) and water (50 mL). The organic phase was dried (magnesium sulfate) and the solvent removed in vacuo. The residue was purified by flash chromatography on silica (20 g) and the residue was dissolved in a mixture of trifluoroacetic acid (2 mL) and dichloromethane (2 mL) and stirred for 5 min. Dichloromethane and a saturated aqueous solution of sodium hydrogencarbonate was added until pH 8 was obtained. The mixture was extracted with dichloromethane (2 × 10 mL). The organic phase was dried (magnesium sulfate) and the solvent was removed in vacuo to afford 310 mg (66% over 3 steps) of compound **37**. ^{1}H-NMR: ($CDCl_3$; selected peaks for major rotamer): δ 0.99 (s, 6H); 4.51 (dd, 1H); 5.61 (d, 1H); 6.56 (m, 1H). HPLC: R_t = 32.47 min, purity: 99% (214 nm; A1). MS: calc. for $[M + H]^+$: 470; found: 470.5.

6.2.2.32. (3R)-4-(4-(1-Aminocyclobutyl)but-2-enoyl)-3-((2-naphthyl)methyl)-1-phenethylpiperazin-2-one **38**

Compound **38** (250 mg) (46% over 3 steps) was prepared analogously to compound **37**. (2*E*)-4-(1-(*tert*-Butoxycarbonylamino)cyclobutyl)but-2-enoic acid was used instead of (2*E*)-5-(*tert*-butoxycarbonylamino)-5-methylhex-2-enoic aicd. HPLC: R_t = 33.45 min; purity: 97% (214 nm; A1). ^{1}H-NMR ($CDCl_3$, selected peaks): δ 3.90 (m, 1 H); 4.51 (d, 1 H); 4.63 (dd, 1 H); 5.65 (d, 1 H); 6.48 (td, 1 H); 7.00–7.90 (m, 12 H). MS: calc. for $[M + H]^+$: 482; found: 481.9.

6.2.2.33. (2R)-2-(N-((2E)-5-((2R)-2-Hydroxypropylamino)-5-methylhex-2-enoyl)-N-methylamino)-N-methyl-3-(2-naphthyl)-N-phenethylpropionamide **39**

Compound **3** (179 mg, 0.38 mmol) was dissolved in methanol (10 mL). Glacial acetic acid (0.30 mL, 5.30 mmol) and mol. sieves (3 Å, 5.0 g) were added successively. (2*R*)-2-(*tert*-Butyldimethylsilyloxy)propanal [30] (500 mg, 2.66 mmol) was dissolved in methanol (3 mL) and added to the reaction mixture. Sodium cyanoborohydride (95 mg, 1.51 mmol) was added as a solid. The reaction mixture was stirred for 3 h at room temperature. Another portion of sodium cyanoborohydride (95 mg, 1.51 mmol) was added. The reaction mixture was stirred 16 h at room temperature. The mol. sieves was filtered off through a plug of celite, which was washed with methanol (30 mL). The solvent was removed in vacuo. The residue was dissolved in water/1 N sodium hydroxide solution (50 mL/50 mL). The solution was extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (60 g) using ethyl acetate/heptane/triethylamine (10:10:1) as eluent to give 160 mg (65%) of (2*R*)-2-(*N*-((2*E*)-5-((2*R*)-2-(*tert*-butoxydimethylsilyloxy)-propylamino)-5-methylhex-2-enoyl)-*N*-methylamino)-*N*-methyl-3-(2-naphthyl)-*N*-phenethylpropionamide. ^{1}H-NMR ($CDCl_3$, selected values): δ = 5.80 and 5.86 (t and dd, together 1 H); 6.14 and 6.23 (both d, together 1 H); 6.85 (m, 1 H).

(2*R*)-2-(*N*-((2*E*)-5-((2*R*)-2-(*tert*-Butoxydimethylsilyloxy)-propylamino)-5-methylhex-2-enoyl)-*N*-methylamino)-*N*-methyl-3-(2-naphthyl)-*N*-phenethylpropionamide (135 mg, 0.21 mmol) was dissolved in tetrahydrofuran (2 mL). A 1.1 M solution of tetrabutylammonium fluoride in tetrahydrofuran (0.42 mL, 0.46 mmol) was added. The reaction mixture was stirred for 1 h at room temperature. The solution was diluted with ethyl acetate (50 mL). It was extracted with saturated sodium hydrogen carbonate solution (3 × 20 mL). The combined organic layers were dried over magnesium sulfate. The solvents were removed in vacuo. The residue was purified on silica (20 g), using dichloromethane/methanol/25% aqueous ammonia (100:10:1) as eluent to give 24 mg of the crude product. The residue was purified by HPLC-chromatography on a 25 × 250 mm 10m C18 silica column at 40 °C with a gradient of 30.0–43.5% acetonitrile in a 0.1 M ammonium sulfate buffer, which was adjusted to pH 2.5 with 4 M sulfuric acid. The peptide containing fractions were collected, diluted with 3 volumes of water and applied to a Sep-Pak® C18 cartridge which was equilibrated with 0.1% trifluoroacetic acid. The peptide was eluted from the Sep-Pak® cartridge with 70% acetonitrile in a 0.1% trifluoroacetic acid solution in water. The product was lyophilized to give 10.7 mg (10%) of compound **39** as trifluoroacetate. HPLC: R_t = 35.13; purity: 88% (254 nm; A1). R_t = 37.08; purity: 77% (214 nm; B1). MS: calc. for $[M + H]^+$: 530; found: 530.8.

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