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Original article

Synthesis and in vitro characterization of new growth hormone secretagogues derived from ipamorelin with dipeptidomimetic *N*-terminals

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Abstract – The structural requirements for *N*-terminal features for the minimal structure of growth hormone secretagogues derived from ipamorelin are investigated. It is found, that incorporation of nonpolar peptidomimetic amino acids at the *N*-terminal can replace the Aib-His moiety and lead to compounds with high in vitro potency with respect to their growth hormone secretagogue properties. New unnatural amino acids with double bonds, ether-linkages, and 1,3-phenylene-moieties in the backbone proved to be valuable dipeptidomimetics. Using them, growth hormone secretagogues with high potencies were obtained. © Elsevier, Paris

growth hormone secretagogue / ipamorelin / peptidomimetic / constrained amino acid / GHRP-6 / GHRP-2 / MK677

1. Introduction

Human growth hormone (hGH) is a pleotropic hormone which interacts with most tissues in the human body. Growth hormone deficiency may not only result in short stature in children, but may also lead to decreased muscle mass, increased fat mass and unfavourable alteration in blood lipid concentrations [1–3]. These numerous effects of hGH deficiency make it desirable to find easy and inexpensive methods of treatment. The only established method at the present time is the treatment with hGH itself, a peptide with 191 amino acids. However, due to the size and metabolic instability of hGH, oral administration, the preferred way of delivery, is not feasible.

A way to circumvent this challenge could be to control the hGH secretion, since most patients, who suffer from growth hormone deficiency, synthesize hGH in the pituitary but their ability for hGH secretion is hampered. Two hypothalamic hormones are known to control the release of hGH: growth hormone releasing hormone (GHRH), a peptide of 44 amino acids, stimulates the synthesis and release of hGH, and somatostatin (SRIF), a peptide of 14 amino acids which inhibits the release of hGH [4].

Growth hormone secretagogues (GHSs) [5, 6] are another class of compounds, which have been known since C. Y. Bowers discovered small peptides [7, 8], with the ability to release hGH. It is known that GHSs act through a distinct mechanism, which is different from GHRH or SRIF. It is believed that GHSs elicit their effect at the hypothalamic as well as the pituitary level. Although the endogenous ligand is to our knowledge still unknown, a group at Merck has recently cloned a receptor [9, 10] to which MK677 [11, 12] binds. MK677 is a derivatized dipeptide, which is currently used in clinical studies as an orally active GHS.

The low molecular weight of GHSs, compared to those of GHRH, SRIF and hGH, make them more attractive as orally active drugs against growth hormone deficiency and other hGH related disorders. A number of highly potent compounds have been discovered. Among these are the classical GHRP-6 [7, 8] and GHRP-2 [13], both hexapeptides, discovered by C. Y. Bowers and coworkers. The pentapeptide ipamorelin [14] discovered by

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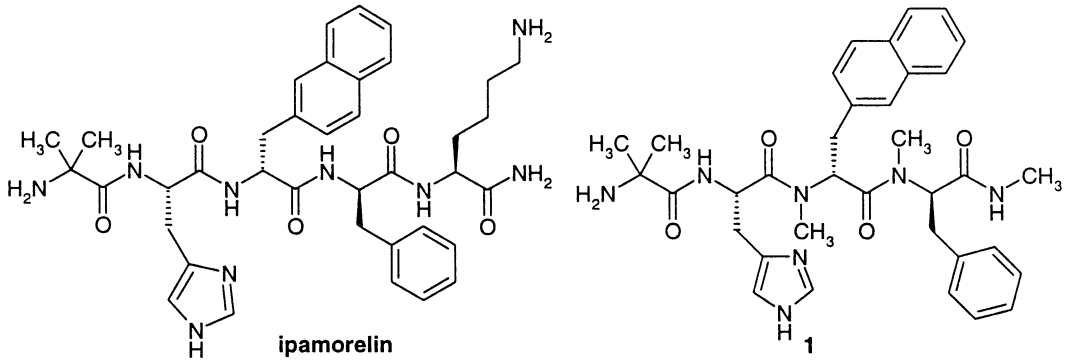


Figure 1. Short peptide growth hormone secretagogues.

Novo Nordisk and hexarelin from Europeptide [15] are more recent examples of short peptides, having GHS-properties. Even lower molecular weights can be achieved by peptidomimetic GHSs, as it has been demonstrated by two different compounds, found by Merck, L-692,429 [16, 17] and MK677 [11].

As reported earlier, an approach to reduce the polarity and molecular size of ipamorelin [18, 19] led to the tetrapeptide **1** *(figure 1)* [18]. Even though there is a decrease in both molecular weight and polarity, the GHS activity is maintained.

In this paper, novel peptidomimetic amino acids at the *N*-terminal of **1** are presented.

Previously the Aib-His moiety at the *N*-terminal of GHSs, has been replaced by peptidomimetics such as (2*E*)-5-methyl-5-aminohex-2-enoic acid (AMH) [19] or 3-aminomethylbenzoic acid (3-AMB) [18] and the thiophene analogues [20]. Isonipecotic acid has also been used extensively [21, 22].

2. Chemistry

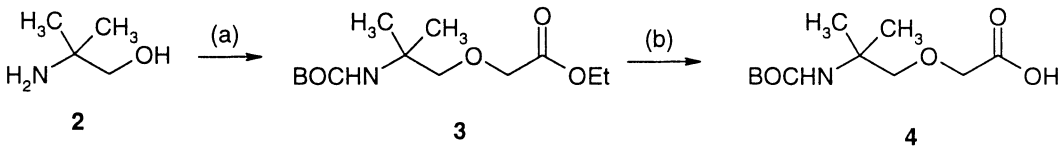
We reasoned that the success of the known dipeptidomimetic amino acids at the *N*-terminal of growth hormone secretagogues is explained by a combination of the distance between the amide carbonyl group and the amino group, conformational constraint of the double bond and the geminal methyl groups in the case of AMH, the aromatic rings in the case of 3-AMB and the heterocyclic analogues, or by the ring in the case of isonipecotic acid.

The first series of compounds should clarify the role of the double bond in AMH. A replacement of the double bond by an ether linkage gives the peptidomimetic more rotational freedom in this area. The syntheses were straightforward, as it is exemplified for **4** in scheme 1. The commercially available amino alcohol **2** was BOC-protected and allowed to react under dirhodium tetraacetate catalysis with ethyl diazoacetate [23, 24]. The resulting ester **3** was saponified to give the amino acid **4** which was used as a dipeptidomimetic.

Accordingly, the amino acids **5**, **6**, **7**, and **8** were prepared from the BOC-protected forms of commercially available amino alcohols *(figure 2)*.

The successful use of isonipecotic acid as the *N*-terminal amino acid of small growth hormone secretagogues inspired us to synthesize a number of analogues with altered degrees of conformational freedom, or varied distances between the amide carbonyl and the amino moieties.

The BOC-protected amino acids **10** [25, 26], **11**, and **12** *(figure 3)* were prepared from (2-pyridinyl)acetic



Scheme 1. Reagents (a) i $(BOC)_2O$, NaOH ii $[Rh_2(OAc)_4]$, $N_2CHCOOEt$; (b) LiOH.

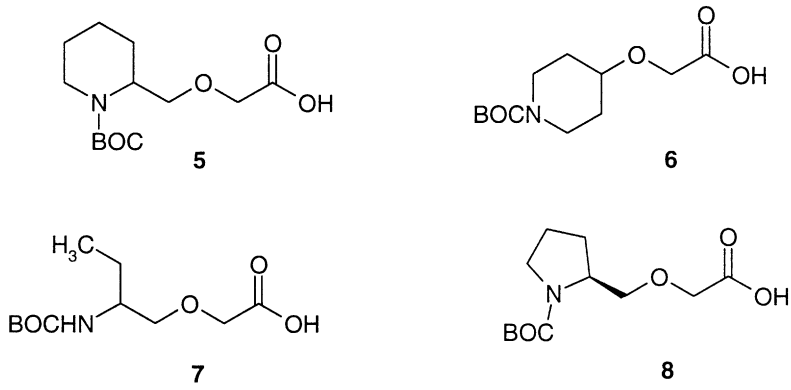
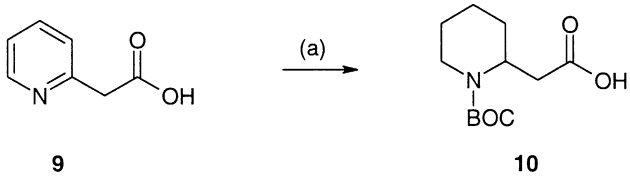


Figure 2. Ether-peptidomimetic amino acids.

acid [9], (3-pyridinyl)acetic acid, and 2-methylpyridine-3-carboxylic acid by hydrogenation with a palladium catalyst.

A number of substituted acrylic acids have been synthesized as analogues of AMH [19]. The acids **16** and **21** are constrained both by the double bond and the presence of a ring. Acid **16** was synthesized by BOC-protection of 4-piperidone (**13**) to give ketone **14**. A Wittig reaction with (carbethoxymethylene)triphenylphosphorane and subsequent saponification furnished acid **16**. In order to synthesize acid **21,** acid **17** was BOC-protected and successively reduced to alcohol **18**. A Swern-oxidation gave the aldehyde **19**. Wittig-Horner reaction then saponification with aqueous lithium hydroxide led to the desired peptidomimetic **21**.



Scheme 2. Reagents (a) i H_2/Rh ii $(BOC)_2O$, NaOH.

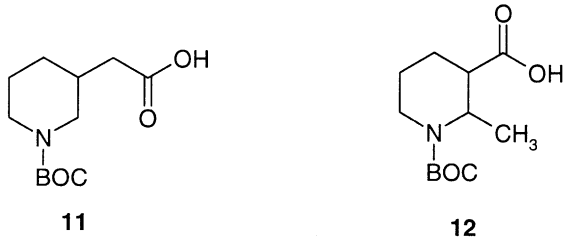
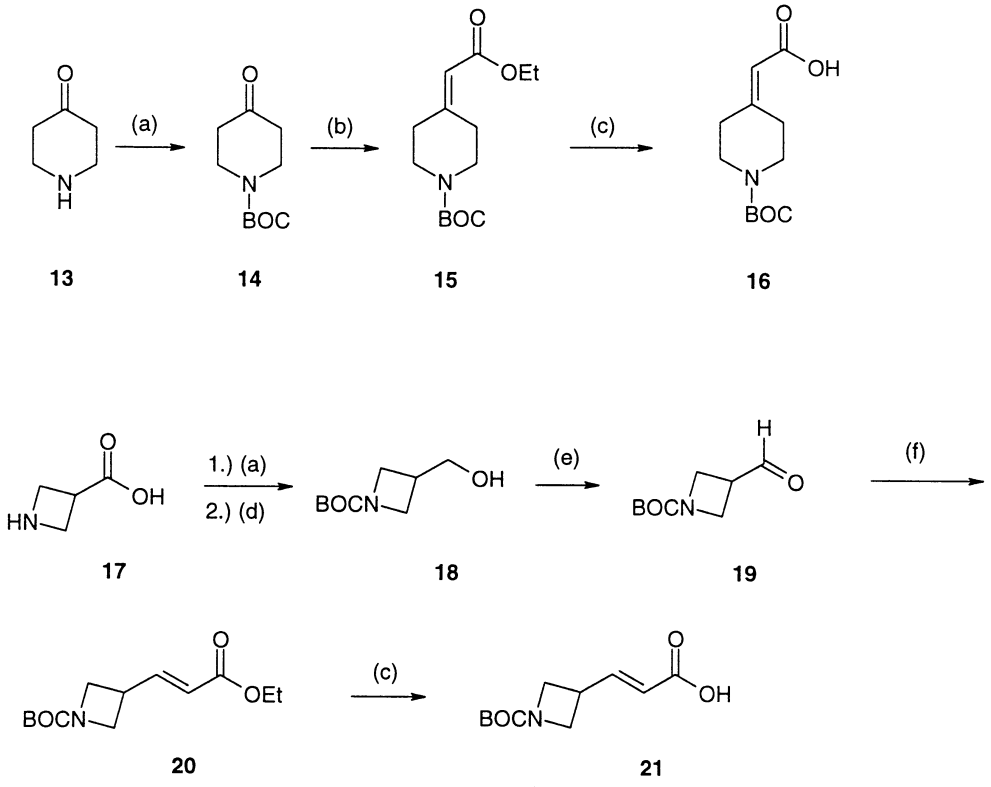


Figure 3. Cyclic constrained peptidomimetic amino acids.

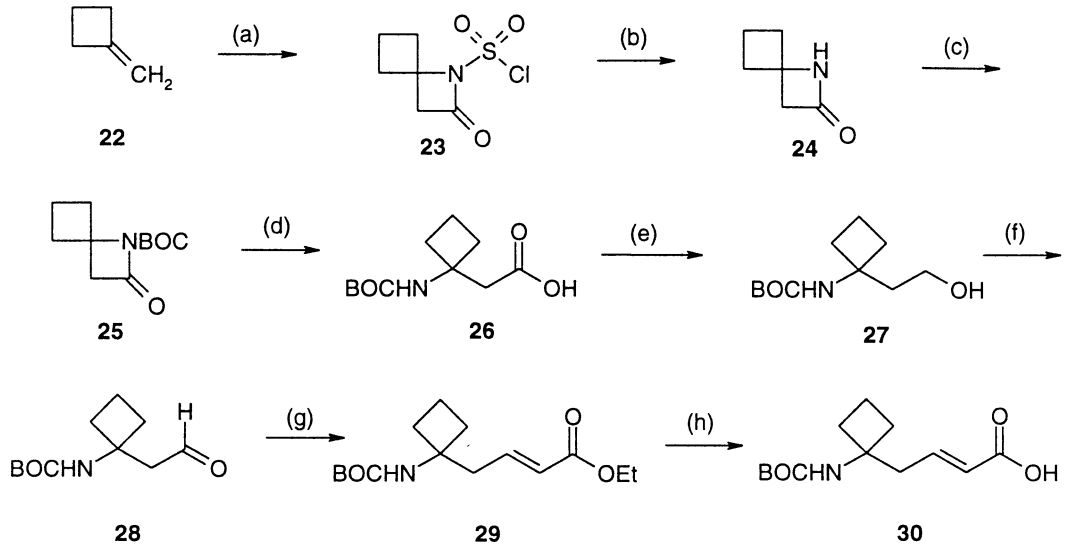
The effect of increasing the steric bulk around the amino moiety of the *N*-terminal was investigated by the synthesis of compound **30** in which the geminal methyl groups are tied together in a four-membered ring. Acid **30** was synthesized from alkene **22**, starting with a [2 + 2] cycloaddition, which furnished the spiro-compound **23**. *N*-deprotection to the lactam **24**, followed by BOC-protection to *N*-BOC-lactam **25** [27] and ring opening gave acid **26**. The aldehyde **28** was obtained by reduction of acid **26** to alcohol **27** and subsequent Swern oxidation. Wittig-Horner olefination produced the ester **29**, which was saponified to acid **30**.

The successful use of 3-AMB [18, 20] at the *N*-terminal of GHSs derived from ipamorelin suggests more bulky non-polar spacers are accepted by the biological system. Acids **33** and **36** were synthesized in order to elucidate whether a non-polar moiety as a side chain of the double-bond spacer, such as an aromatic or an aliphatic group, increases the potency. The synthesis of intermediate **33** starts with aldehyde **31** [19]. A Wittig reaction with triphenylphosphinebenzylcarbomethoxy methylene [28] furnishes ester **32**. Ester **35** is synthesized from the commercially available ketone **34**, which was protected at the amino group with BOC and subsequently reacted with triethyl phosphonoacetate. The esters **32** and **35** were saponified with lithium hydroxide.

A reduction of hydrogen bonding sites at the *N*-terminal of GHSs of type **1** can be achieved by alkylation of the amino group. This reduction of the number of hydrogen bonding sites is interesting because it could potentially improve the oral bioavailability. As



Scheme 3. Reagents (a) $(BOC)_2O$, NaOH (b) $Ph_3P{=}CHCOOEt$, toluene, reflux (c) LiOH (d) i $ClC(=O)OEt$, NEt_3 ii $LiBH_4$ (e) DMSO, $(COCl)_2$, NEt_3 (f) $(EtO)_2P(=O)CH_2COOEt$, KO*t*-Bu.



Scheme 4. Reagents (a) $OCNSO_2Cl$ (b) Na_2SO_3 (c) $(BOC)_2O$, NEt_3 (d) NaOH (e) i $ClC(=O)Oet$, NEt_3 ii $LiBH_4$ (f) DMSO, $(COCl)_2$, NEt_3 (g) $(EtO)_2P(=O)CH_2COOEt$, KO*t*-Bu (h) LiOH.

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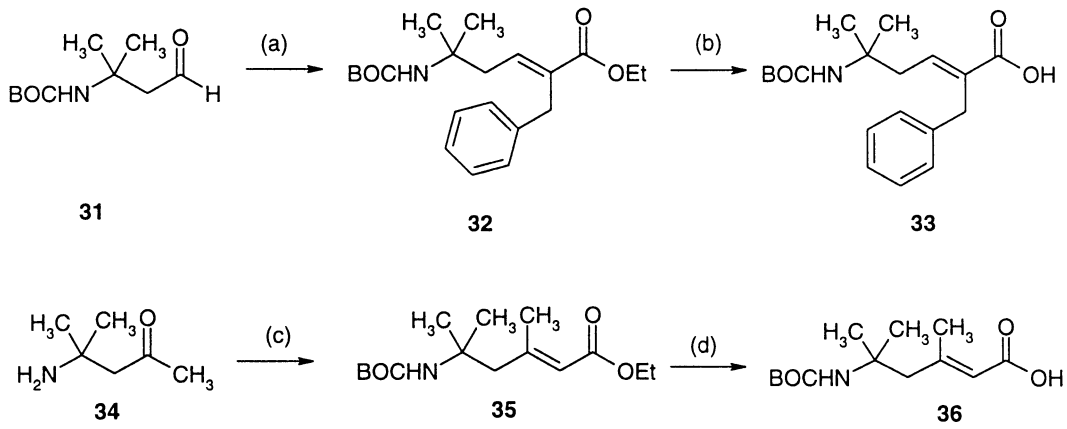


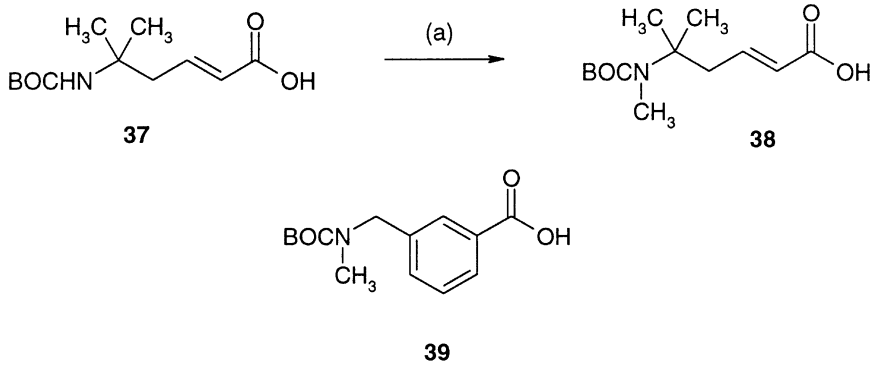
Scheme 5. Reagents (a) $(Ph_3P)_3C(Bn)COOEt$, toluene, reflux (b) KOH, MeOH, H_2O (c) i $(BOC)_2O$, NaOH ii $(EtO)_2P(=O)CH_2COOEt$, KO*t*-Bu (d) LiOH.

the results [21, 22] of isonipecotic acid as the *N*-terminal of GHSs show, one hydrogen at the *N*-terminal amino group is sufficient to obtain high activity. Therefore the *N*-methylated derivatives from AMH [19] and 3-AMB [18], **38** and **39** [29] were prepared. The synthesis of intermediate **38** was done by methylation of *N*-BOC-amino acid **37** in tetrahydrofuran with sodium hydride as base.

The tripeptides that were used for screening for GHS-activity were synthesized from the known dipeptide **40** [19].

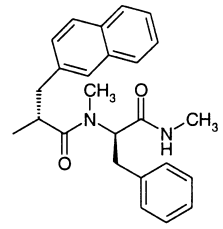
The peptides **42** to **62** *(table I)* were prepared from the known dipeptide **40** and a dipeptidomimetic amino acid which were either synthesized as described above, purchased commercially, or in the case of 3-aminoethylbenzoic acid [29], synthesized according to a literature procedure. The *N*-BOC-protected amino acids were activated with 1-hydroxy-7-azabenzotriazole (HOAt) [30] and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDAC) and coupled to amine **40** [19], as is shown in scheme 7 for compound **42**. In the final deprotection step the cleavage of the product between the naphthylalanine and phenylalanine moieties was observed as side reaction. However, when the reaction was run at 0 °C and stopped by addition of sodium hydrogen carbonate immediately after the TLC-control showed completion, satisfactory yields of all peptides were obtained.

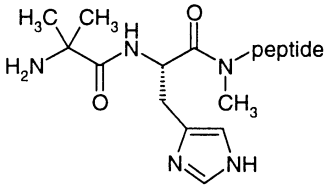
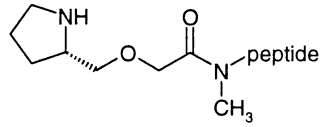
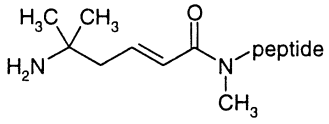
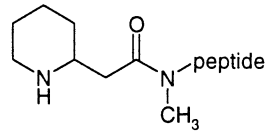
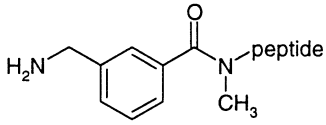
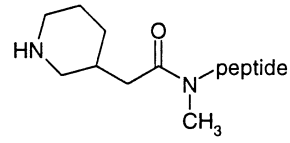
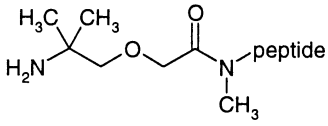
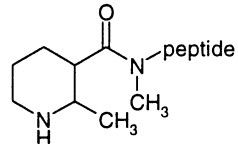
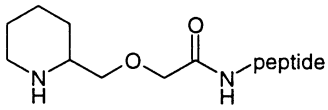
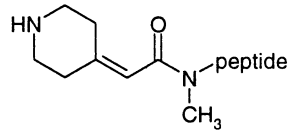
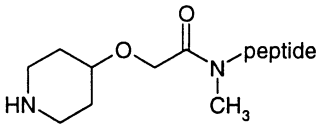
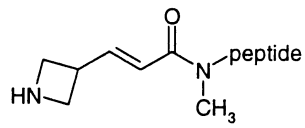
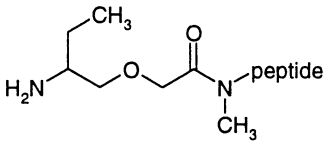
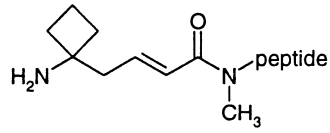
The (2*R*)-hydroxypropyl group, which was originally found to improve the potency of compounds of the benzolactam-type [31], has been introduced into compound **64**. It was synthesized from tripeptide **41** [19], as outlined in scheme 8 by reductive alkylation with (2*R*)-2-(*tert*-butyldimethylsilyloxy)propanal [32] to *N*-alkylamine **63** and subsequent removal of the silyl-protection group with tetrabutylammonium fluoride (TBAF).



Scheme 6. Reagents (a) CH_3I, NaH.

Table I. Synthesized tripeptides.

peptide = 

1		**47**	
41		**48a/b**	
42		**49a/b**	
43		**50a/b**	
44		**51**	
45		**52**	
46		**53**	

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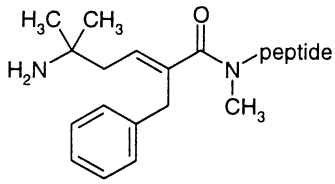
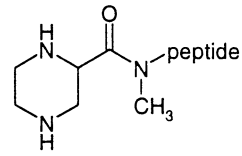
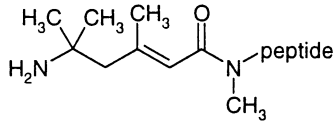
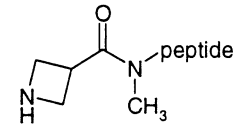
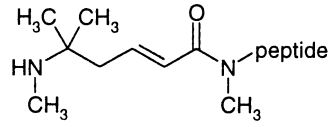
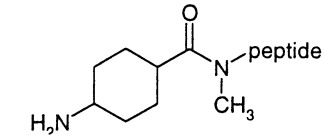
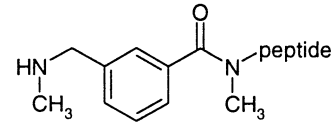
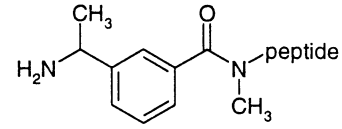
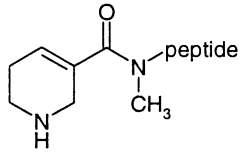
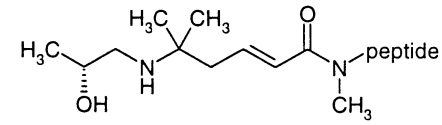
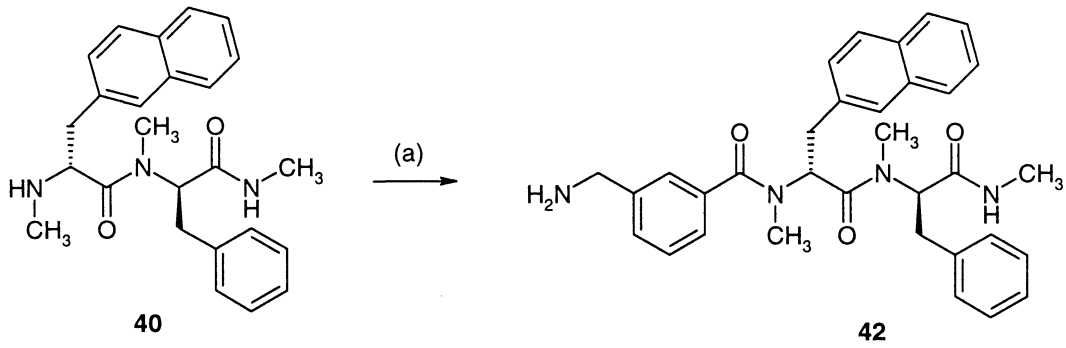
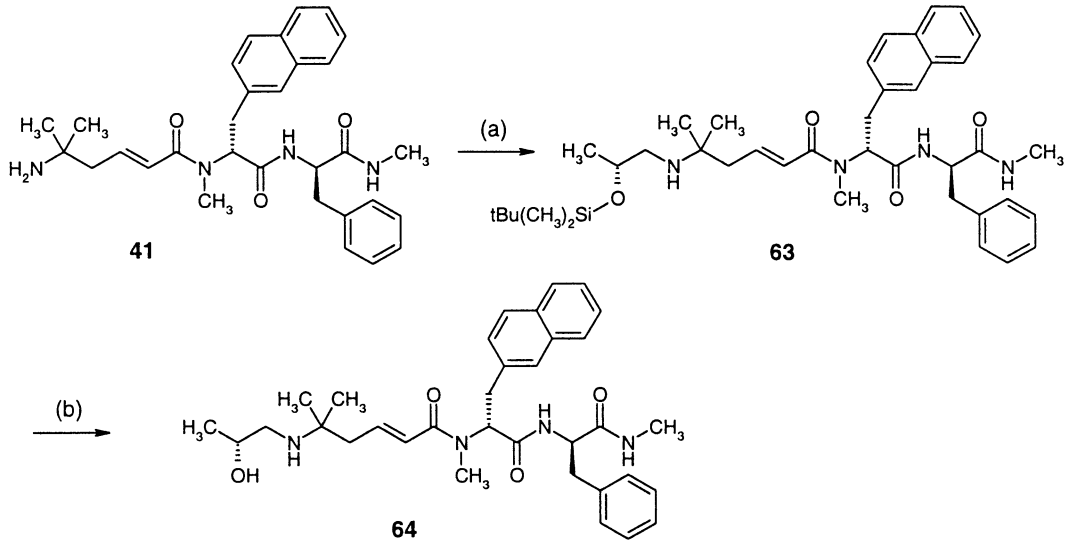
54		**59a/b**	
55		**60**	
56		**61**	
57		**62**	
58		**64**	



Scheme 7. Reagents (a) *N*-BOC-3-AMB, HOAt, EDAC, N(*i*-Pr)$_2$Et ii TFA, CH_2Cl_2.



Scheme 8. Reagents (a) (2R)-2-(*tert*-butylmethylsilanyloxy)propanol, HOAc, $NaCNBH_4$ (b) TBAF

3. Pharmacology

The EC_{50} values of the peptides were determined in a rat pituitary assay. The efficacy was compared to maximal stimulation with GHRP-6 [14, 33].

4. Results and discussion

As the in vitro screening results in *table II* suggest, highly potent compounds can be obtained by replacement of an Aib-His moiety with a peptidomimetic amino acid at the *N*-terminal of GHSs. Only amino acids with four atoms between the amide carbonyl moiety and the amino group give highly potent compounds. Almost equipotent compounds to **1** are **41**, **53**, **55**, and **56**. Compounds derived from *N*-terminal amino acids that are too short to serve as a dipeptidomimetic, as in **48a**, **48b**, **49a**, **49b**, **50**, **58**, **59a**, **59b**, or **60** do not exhibit such high potencies. Compound **45**, with five atoms between the amide carbonyl moiety and the amino group in the *N*-terminal amino acid also shows decreased activity. Reduction of the conformational freedom in the backbone of the peptidomimetic by a double bond, as in **41**, **53**, **55**, and **56,** or a 1,3-phenylene ring as in **42** and **62,** does not enhance the activity, as a comparison with the ether analogues **43**, **46**, and **47** that are almost equipotent to **41**, **53**, **55**, and **56** shows. A cyclohexane moiety in the backbone of **61** decreases the potency. Attempts to enhance the potency by ring formation from the amino group to the backbone of the peptidomimetic were realized by the synthesis of different types of six-membered rings as in **44**, **45**, **49**, and **51**. All six membered rings are unfavourable. Even more, the four membered ring in **52** diminishes the activity almost completely. The pyrrolidinyl ether analogue **47**, with a five membered ring however, is equipotent to **46**. The incorporation of a non-polar group in the proximity of the *N*-terminal amino group, such as a methyl or a methylene group gave compounds with similar activity, as it can be observed in the pairs of **41**/**53**, **41**/**55**, **42**/**62**, and **42**/**57**. A slight decrease of activity is observed when a polar hydroxyl moiety is incorporated, as demonstrated in **64**. The rather poor activity of **54** might be explained by the size of the benzyl group giving sterical problems.

5. Conclusion

It has been shown that the *N*-terminal dipeptide moiety of growth hormone secretagogues of type **1** can be replaced by dipeptidomimetics. The most active compounds have been obtained with a double bond in the backbone of the dipeptidomimetic amino acid, ethers and 1,3-phenylene groups however can give compounds of similar potency.

Table II. In vitro growth hormone secretagogue activity.

Entry	Range of EC_{50}[a] [nM]	Entry	Range of EC_{50}[a] [nM]
1	2.5–5	**51**	320–380 [b]
41	6–38	**52**	> 10 000 [b]
42	30–50 [c]	**53**	10–10
43	5–20	**54**	3–100 [c]
44	70–140 [c]	**55**	115
45	220–300 [b]	**56**	3–10
46	6–30	**57**	8–18
47	12–20	**58**	> 450–> 850 [b]
48a	> 600–> 1 200 [b]	**59a**	400–600 [b]
48b	> 400–> 2 200 [b]	**59b**	8–2 000 [c]
49a	30–40 [b]	**60**	20–120 [c]
49b	60–90 [b]	**61**	10–200 [c]
50a	> 300–> 2 200 [b]	**62**	11–15
50b	> 3 000–> 4 200 [b]	**64**	40–100 [c]

[a] Rat pituitary cell assay; [b] significant $P < 0.05$ compared to **1**; [c] tendancy to be less potent than **1**.

6. Experimental

6.1. Methods

For the in vitro studies, male albino Sprague-Dawley rats (250 +/– 25 g) were used. The rats were purchased from Møllegaard, Lille Skensved, Denmark. The rats were housed in group cages (4–8 animals/cage) and placed in rooms with a 12 h light cycle. The room temperature varied from 19–24 °C and the humidity from 30–60%.

All media were obtained from Gibco, trypsin from Worthington, BSA, DNase, T_3 and dexamethazone from Sigma.

Pituitary glands were aseptically removed from Sprague-Dawley male rats and cultures of pituitary cells were prepared according to Heiman [33]. Briefly, the rats were decapitated and the pituitaries dissected. The neuro-intermediate lobes were removed and the remaining tissue was immediately placed in ice-cold isolation buffer (Gey's medium supplemented with 0.25% D-glucose, 2% non-essential amino acids and 1% BSA, pH 7.3). The tissue was cut into small pieces and transferred to isolation buffer supplemented with 3.8 mg/mL of trypsin and 330 μg/mL of DNase. This mixture was incubated at 70 rotations/min for 35 min at 37 °C in a 95/5% atmosphere of O_2/CO_2. The tissue was then washed three times in the above buffer. Using a standard Pasteur pipette, the tissue was then aspirated into single cells. After dispersion, cells were filtered through a nylon filter (160 μm) to remove undigested tissue. The cell suspension was washed three times with isolation buffer supplemented with trypsin inhibitor (0.75 mg/mL) and finally resuspended in culture medium; DMEM supplemented with 25 mM HEPES, 4 mM glutamine, 0.075% sodium bicarbonate, 0.1% non-essential amino acid, 2.5% FCS, 3% horse serum, 10% fresh rat serum, 1 nM T_3 and 40 μg/L dexamethazone, pH 7.3, to a density of 2×10^5 cells/mL. The cells were seeded into microtitre plates (Nunc, Denmark), 200 μL /well, and cultured for 3 d at 37 °C and 8% CO_2.

Following the culture period the cells were washed twice with stimulation buffer (HBSS supplemented with 1% BSA, 0.25% D-glucose and 25 mM HEPES, pH 7.3) and preincubated for 1 h at 37 °C and 5% CO_2. The buffer was exchanged with 90 μL of stimulation buffer (37 °C). Ten μL test compound solution was added and the plates were incubated for 15 min at 37 °C and 5% CO_2. The medium was decanted and analysed for GH content in a rat GH SPA (Scintillation Proximity) test system. Each concentration of the compound was measured in triple determinations.

6.2. Chemistry

Amino acids were purchased from Synthetech. HRMS was performed at Odense University using a Varian M311 apparatus. PDMS spectra were run on a Bioion 1 100 (Upsala, Sweden). NMR spectra were obtained at 400 MHz on a Bruker instrument.

The RP-HPLC analysis was performed using UV detections at 214, 254, 276, and 301 nm on a Vydac 218TP54 4.6 mm × 250 mm 5m C-18 silica column (The Seperations Group, Hesperia), which was eluted at 1 mL/min at 42 °C.

Two different elution conditions were used:

Method A1: 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% to 60% acetonitrile in the same buffer for 50 min.

Method B1: 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.

6.2.1. (2-tert-Butoxycarbonylamino-2-methylpropoxy)-acetic acid ethyl ester (**3**)

A solution of 2-*tert*-butoxycarbonylamino-2-methylpropanol (**2**, 5.0 g, 26.46 mmol) and rhodium(II)acetate (90 mg) in 500 mL of dichloroethane was heated to 80 °C. Then ethyldiazoacetate (4.0 g, 34.78 mmol) was slowly added over a period of 1 h, and the mixture was stirred at reflux for 3 h. Another 90 mg of rhodium(II) acetate was added and the mixture was refluxed for another 5 h. The mixture was cooled overnight and 500 mL of saturated sodium bicarbonate was added, the yellow organic layer was isolated and washed twice with saturated sodium bicarbonate (2 × 200 mL). The organic layer was dried over magnesium sulfate and concentrated in vacuo.

6.2.2. (2-tert-Butoxycarbonylamino-2-methylpropoxy)-acetic acid (**4**)

The yellow oil of **3** was taken up in 200 mL of 1 M lithium hydroxide in methanol:water (3:1) and stirred overnight. The solvent was removed in vacuo to a minimum and water was added (pH > 9) and the mixture was washed with ether. Hydrochloric acid (1 M) was then added until pH < 4 and the mixture was extracted with ethyl acetate, dried over magnesium sulfate and concentrated in vacuo to give 2.5 g (38%) of acid **4** as a clear oil.

^{1}H-NMR ($CDCl_3$) δ 1.3 (s, 6H); 1.45 (s, 9H); 3.5 (s, 2H); 4.15 (s, 2H); 9.9 (b, 1H).

6.2.3. ((1-(-tert-Butoxycarbonyl)piperidin-2-yl)methoxy)-acetic acid (**5**)

((1-(-*tert*-Butoxycarbonyl)piperidin-2-yl)methoxy) acetic acid (**5**) was synthesized analogously to acid **4** from *N*-(*tert*-butoxycarbonyl)-2-hydroxymethylpiperidine.

^{1}H-NMR ($CDCl_3$): δ 1.45 (s, 9 H); 1.55 (m, 2 H); 1.85 (m, 2 H); 3.10 (m, 2 H); 3.60 (m, 1 H); 3.80 (M, 2 H); 4.15 (s, 2 H).

6.2.4. (1-(tert-Butoxycarbonyl)piperidin-4-yloxy)acetic acid (**6**)

(1-(*tert*-Butoxycarbonyl)piperidin-4-yloxy)acetic acid (**6**) was synthesized analogously to acid **4** from 4-hydroxypiperidine-1-carboxylic acid *tert*-butyl ester.

^{1}H-NMR ($CDCl_3$): δ 1.45 (s,)H); 1.55 (m, 2 H); 1.90 (m, 2 H); 3.10 (m, 2 H); 3.60 (m, 1 H); 3.80 (m, 2 H); 4.20 (s, 2 H).

6.2.5. (2-(tert-Butoxycarbonylamino)butoxy)acetic acid (**7**)

(2-(*tert*-Butoxycarbonylamino)butoxy)acetic acid (**7**) was synthesized analogously to acid **4** from (1-(hydroxymethyl)propyl)carbamic acid *tert*-butyl ester.

^{1}H-NMR ($CDCl_3$): δ 0.95 (t, 3 H); 1.45 (s, 9 H); 1.60 (m, 3 H); 3.55 (m, 2 H); 4.10 (s, 2H).

6.2.6. (((2S)-1-(tert-butoxycarbonyl)pyrrolidin-2-yl)-methoxy)acetic acid (**8**)

(((2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl)methoxy)acetic acid (**8**) was synthesized analogously to acid **4** from (*S*)-*N*-*tert*-butoxycarbonylprolinol.

^{1}H-NMR ($CDCl_3$): δ 1.45 (s, 9 H); 1.90 (m, 4 H); 3.55 (t, 2 H); 3.60 (m, 3 H); 4.10 (s, 2 H); 10.60 (s, 1 H).

6.2.7. 1-tert-Butoxycarbonylpiperidine-2-carboxylic acid (**10**)

A suspension of (2-pyridyl)acetic acid (**9**, 0.5 g, 3.02 mmol) and rhodium on aluminumoxide (0.4 g) in ethanol (50 mL) [34] was charged with hydrogen (60 psi) and shaken for 20 h. The reaction mixture was filtered through a filter paper and the ethanol was removed in vacuo. The remaining oil was dissolved in tetrahydrofuran (20 mL) and an aqueous 1 N sodium hydroxide solution. A solution of di-(*tert*-butoxy) dicarbonate (0.474 g, 2.17 mmol) in tetrahydrofuran (10 mL) was added dropwise. The reaction mixture was stirred for 2 d. The tetrahydrofuran was removed in vacuo. The residue was acidified with a saturated aqueous solution of potassium hydrogen phosphate. It was extracted with diethyl ether (2 × 20 mL). The organic layer was washed with brine (20 mL) and dried over magnesium sulfate. The solvent was removed in vacuo to give 207 mg of crude aicd **10**, which was used for the further syntheses.

^{1}H-NMR ($CDCl_3$): δ 1.40 (s, 9 H); 1.60 - 1.70 (m, 6 H); 2.60 (m, 2 H); 2.80 (m, 1H); 4.0 (dd, 1 H); 4.70 (m, 1 H).

6.2.8. (1-(tert-Butoxycarbonyl)piperidin-3-yl)acetic acid (**11**)

(1-(*tert*-Butoxycarbonyl)piperidin-3-yl)acetic acid (**11**) was synthesized analogously to acid **10** from (pyridin-3-yl)acetic acid.

^{1}H-NMR ($CDCl_3$): δ 1.25 (m, 1 H); 1.45 (s, 9 H); 1.50 (m, 1 H); 1.65 (m, 1 H); 1.90 (m, 1 H); 2.00 (m, 1 H); 2.23 and 2.33 (both ABX, together 2 H); 2.50–2.80 (br, 1 H); 2.90 (br, 1 H); 3.80 (m, 1 H); 4.00 (br, 1 H).

6.2.9. (1-(tert-Butoxycarbonyl)-2-methylpiperidin-3-yl)-acetic acid (**12**)

(1-(*tert*-Butoxycarbonyl)-2-methylpiperidin-3-yl)acetic acid (**12**) was synthesized analogously to acid **10** from 2-methylpyridine-3-carboxylic acid.

^{1}H-NMR (DMSO-d_6): δ 1.00 (d, 3 H); 1.27 (m, 1 H); 1.65 (m, 3 H); 2.75 (br, 1 H); 3.35 (m, 1 H); 3.78 (m, 1 H); 4.55 (m, 1 H); 12.40 (br, 1 H).

6.2.10. 4-Oxopiperidine-1-carboxylic acid tert-butyl ester (**14**)

Piperidin-4-one hydrochloride (**13**, 10.0 g; 74.3 mmol) was dissolved in tetrahydrofuran (100 mL) and an aqueous solution of sodium hydroxide (74 mL; 74.3 mmol; 1 N) was added. Di-*tert*-butyl dicarbonate (19.5 g; 89.2 mmol) was dissolved in tetrahydrofuran (50 mL) and added dropwise. The reaction mixture was stirred for 12 h at room temperature and evaporated in vacuo. The residue was extracted with ethyl acetate (3 × 100 mL). The combined organic phases were washed with a 10% aqueous solution of sodium hydrogen sulfate (100 mL), dried over magnesium sulfate and evaporated. The residue was crystallized from heptane and dried in vacuo to afford 10.9 g of ester **14**.

^{1}H-NMR ($CDCl_3$) δ 1.50 (s, 9H); 2.44 (t, 4H); 3.71 (t, 4 H).

6.2.11. 4-Carboxymethylenepiperidine-1-carboxylic acid tert-butyl ester (**16**)

Ester **14** (8.0 g; 40.2 mmol) was dissolved in toluene (80 mL). Carboethoxymethylene triphenylphosphorane (17.5 g; 50.2 mmol) was added and the reaction mixture was heated for 12 h at reflux. The reaction mixture was evaporated in vacuo and the residue was chromatographed on silica (120 g) using diethyl ether/heptane (1:1) as eluent to afford 9.5 g (35.7 mmol) of 4-ethoxycarbonylmethylenepiperidine-1-carboxylic acid *tert*-butyl ester (**15**), which was dissolved in 1,4-dioxane and cooled to 0 °C. Lithium hydroxide (2.73 g; 114 mmol) was dissolved in water (20 mL) and added. The reaction mixture was stirred for 12 h at room temperature. Ethyl acetate (200 mL) and water (100 mL) were added. A 10% aqueous sodium hydrogen sulfate solution was added until pH 2. The organic phase was washed with water (100 mL) and dried over magnesium sulfate. The solvent was removed in vacuo to afford 5.49 g of ester **16**.

^{1}H-NMR ($CDCl_3$) δ 1.47 (s, 9H); 2.31 (t, 2H), 2.94 (t, 2H); 3.50 (dt, 4H); 5.75 (s, 1H); 10.75 (s, 1H).

6.2.12. 3-Hydroxymethylazetidine-1-carboxylic acid tert-butyl ester (**18**)

Azetidine-3-carboxylic acid (**17**, 10.0 g; 98.9 mmol) was dissolved in tetrahydrofuran (120 mL) and water (20 mL). An aqueous 1 N solution of sodium hydroxide (10 mL) was added. Di-*tert* butyl dicarbonate (25.9 g; 118.7 mmol) was dissolved in tetrahydrofuran (80 mL) and added dropwise to the reaction mixture. The reaction mixture was stirred for 12 h at room temperature and evaporated in vacuo. To the residue was added water (100 mL) and an aqueous 1 N solution of sodium hydroxide (100 mL). The aqueous phase was extracted with diethyl ether (2 × 100 mL). The aqueous phase was acidified with an aqueous 1 N solution of sodium hydrogensulfate until pH 2. Diethyl ether (200 mL) was added and the organic phase was dried over magnesium sulfate. The solvent was removed in vacuo to afford 20 g of 3-carboxyazetidine-1-carboxylic acid *tert*-butyl ester. A portion of 1-carboxyazetidine-1-carboxylic acid *tert*-butyl ester (5.0 g; 24.8 mmol) was dissolved in anhydrous tetrahydrofuran. Triethylamine (4.1 mL; 29.8 mmol) was added and the reaction mixture was cooled to 0 °C. Ethyl chloroformate (2.4 mL; 24.8 mmol) was added and the reaction mixture was stirred for 40 min at 0 °C. The reaction mixture was filtered and the filter cake was washed with anhydrous tetrahydrofuran (30 mL). The combined filtrates were cooled to 0 °C and a 2.0 M solution of lithium borohydride in tetrahydrofuran (31 mL; 62.1 mmol) was added dropwise to the reaction mixture. The reaction mixture was warmed to room temperature and stirred for 12 h. The mixture was cooled to 0 °C and methanol (10 mL) was added dropwise. An aqueous 10% solution of sodium hydrogencarbonate (100 mL) was added and the mixture was extracted with ethyl acetate (4 × 100 mL). The combined organic phases were washed with a saturated aqueous solution of sodium hydrogencarbonate (100 mL) and dried over magnesium sulfate. The solvent was removed in vacuo to afford 3.43 g of alcohol **18**.

^{1}H-NMR ($CDCl_3$) δ 1.43 (s, 9H); 2.7 (p, 1H); 3.63–3.70 (m, 2H), 3.74 (d, 1H); 3.88 (d, 1H); 3.9-4.0 (m, 2H).

6.2.13. 3-Formylazetidine-1-carboxylic acid tert-butyl ester (**19**)

Oxalyl chloride (2.1 mL; 24.0 mmol) was dissolved in dichloromethane (30 mL) and cooled to –78 °C. Dimethylsulfoxide (2.3 mL; 32.0 mmol) was added. A solution of alcohol **18** (3.0 g; 16.0 mmol) in dichloromethane

(20 mL) was added dropwise. Triethylamine (11.1 mL; 80.1 mmol) was added and the reaction mixture was warmed to room temperature. Dichloromethane (200 mL) and 1 N hydrochloric acid (200 mL) were added. The aqueous phase was extracted with dichloromethane (100 mL). The combined organic phases were washed with a saturated aqueous solution of sodium hydrogen carbonate (100 mL), dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica (90 cm) using ethyl acetate/heptane (4:1) as eluent to afford 1.11 g of aldehyde **19**.

^{1}H-NMR ($CDCl_3$) δ 1.43 (s, 9H); 3.37 (p, 1H); 4.05-4.15 (m, 4H); 9.82 (s, 1H).

6.2.14. 3-((E)-2-Ethoxycarbonylvinyl)azetidine-1-carboxylic acid tert-butylester (**20**)

Triethyl phosphonoacetate (1.9 mL; 9.72 mmol) was dissolved in tetrahydrofuran (30 mL). Potassium *tert*-butoxide (1.1 g; 9.72 mmol) was added portionwise. Aldehyde **19** (1.0 g; 5.40 mmol) was dissolved in tetrahydrofuran (6 mL) and added to the reaction mixture. The reaction mixture was stirred for 1 h at room temperature. Ethyl acetate (100 mL) and 1 N hydrochloric acid (100 mL) were added and the phases were separated. The aqueous phase was extracted with ethyl acetate (2 × 50 mL) and the combined organic phases were washed with a saturated aqueous solution of sodium hydrogen carbonate (100 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 afford 1.0 g of diester **20**.

^{1}H-NMR ($CDCl_3$) δ 1.24 (t, 3H); 1.48 (s, 9H); 3.22–3.32 (m, 1H); 3.75 (dd, 2H); 4.08 (t, 2H); 4.15 (q, 2H); 5.8 (d, 1H); 7.02 (dd, 1H).

6.2.15. 3-((E)-2-Carboxyvinyl)azetidine-1-carboxylic acid tert-butyl ester (**21**)

Diester **20** (0.95 g; 3.72 mmol) was dissolved in 1,4-dioxane (15 mL). Lithium hydroxide (98 mg; 4.1 mmol) and water (10 mL) were added. The reaction mixture was stirred for 12 h at room temperature. Water (70 mL) was added and the reaction mixture was washed with *tert*-butyl methyl ether (70 mL). The phases were separated. The aqueous phase was adjusted to pH 2 with an aqueous 10% solution of sodium hydrogensulfate and extracted with *tert*-butyl methylether (3 × 70 mL). These extracts were combined and dried over magnesium sulfate. The solvent was removed in vacuo to afford 0.76 g of acid **21**.

^{1}H-NMR ($CDCl_3$) δ 1.43 (s, 9H); 3.31–3.42 (m, 1H); 3.84 (dd, 2H); 4.16 (t, 2H); 5.88 (d, 1H); 7.18 (dd, 1H).

6.2.16. 1-Aza-spiro[3.3]heptan-2-one (**24**) *[27]*

Methylenecyclobutane (**22**, 40.0g, 0.587 mol) was dissolved in diethylether (250 mL). At –40 °C chlorosulfonylisocyanate (26 mL, 0.294 mol) was added dropwise. The reaction mixture was warmed to 10 °C, An exothermic reaction was observed, and precipitation was formed. The reaction mixture was cooled to –20 °C. It was stirred for 16 h, while it was warming up to room temperature. A saturated aqueous solution of sodium sulfite (100 mL) was added dropwise. The reaction mixture was stirred vigorously for 1 h. Another saturated aqueous solution of sodium sulfite (100 mL) was added dropwise. Solid sodium hydrogen carbonate was added, until pH 7. Dichloromethane (500 mL) was added. The phases were separated. The organic layer was dried over magnesium sulfate. The solvent was removed in vacuo, to give 23.59 g of the bicyclic lactam **24**.

^{1}H-NMR ($CDCl_3$): δ 1.75 (m, 2 H); 2.26 (m, 2 H); 2.39 (m, 2 H); 2.96 (s, 2 H); 6.55 (br, 1 H).

6.2.17. 2-Oxo-1-azaspiro[3.3]heptane-1-carboxylic acid tert-butylester (**25**) *[27]*

A solution of di-*tert*-butyl dicarbonate (55.7 g, 0.211 mol) in dichloromethane (100 mL) was added dropwise to a solution of **24**, triethylamine (36 mL, 0.255 mol), and 4-dimethylaminopyridine (2.6 g, 0.021 mol) in dichloromethane (100 mL). The reaction mixture was stirred for 16 h at room temperature. It was washed with a 10% aqueous solution of ammonium chloride (100 mL), water(100 mL) and a saturated aqueous solution of sodium hydrogen carbonate (100 mL). The organic layer was dried over magnesium sulfate. The solvent was removed in vacuo to give 48.24 g of crude *N*-BOC-lactam **25**, which was used for the next step without purification.

^{1}H-NMR ($CDCl_3$): δ 1.55 (s, 9 H); 1.78 (m, 1 H); 1.92 (m, 1 H); 2.18 (m, 2 H); 2.90 (m, 2 H); 3.04 (s, 1 H).

6.2.18. (1-(tert-Butoxycarbonylamino)cyclobutyl) acetic acid (**26**)

A 1 N aqueous solution of lithium hydroxide (227 mL, 227 mmol) was added to a solution of *N*-BOC-lactam **25** (48 g, 0.227 mmol) in tetrahydrofuran (200 mL). The reaction mixture was stirred for 2 h. Diethyl ether (200 mL) and water (200 mL) were added. The mixture was stirred for 16 h. The organic layer was isolated. The aqueous phase was extracted with diethyl ether (200 mL). The aqueous phase was acidified with a 10% aqueous solution of sodium hydrogen sulfate until pH 3. The formed precipitation was filtered off, washed with water, and dried in vacuo, to give 38.84 g of acid **26**.

^{1}H-NMR ($CDCl_3$): δ 1.45 (s, 9 H); 1.85 (m, 1 H); 1.95 (m, 1 H); 2.25 (m, 4 H); 2.87 (m, 2 H); 5.15 and 6.20 (both br, together 1 H).

6.2.19. 2E-4-(1-(tert-Butoxycarbonylamino)cyclobutyl)-but-2-enoic acid (**30**)

Acid **30** was synthesized starting with acid **26** analogously to the synthesis of intermediate **21** starting with acid **17**.

^{1}H-NMR ($CDCl_3$): δ 1.43 (s, 9 H); 1.84 (m, 1 H); 1.95 (m, 1 H); 2.10 (m, 2 H); 2.20 (m, 2 H); 2.70 (m, 2 H); 4.75 (br, 0.5 H); 5.90 (m, 1 H); 6.35 (br, 0.5 H); 6.95 (m, 1 H).

6.2.20. (-2-Benzyl-5-tert-butoxycarbonylamino-5-methyl-hex-2-enoic acid ethyl ester (**32**)

3-(*tert*-Butoxycarbonylaminomethyl)-3-methylbutanal [19] (**31**, 1.7 g, 8.5 mmol) was dissolved in toluene (30 mL). 3-Phenyl-2-(triphenylphosporanylidene)proionic acid ethyl ester [35] (4.4 g, 10.1 mmol) was added. The solution was heated to reflux for 4.5 h. The solvent was removed in vacuo. The residue was treated with ethyl acetate/heptane (30 mL /100 mL). The remaining solid was filtered off. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silcia (600 g) with ethyl acetate heptane (1:2) to give 1.2 g of ester **32**.

^{1}H-NMR ($CDCl_3$): δ 1.22 (t, 3 H); 1.28 (s, 6 H); 1.45 (s, 9H); 2.73 (d, 2 H); 3.71 (s, 2 H); 4.15 (q, 2 H); 4.49 (br, 1 H); 6.95 (t, 1 H); 7.10–7.30 (m, 3 H).

6.2.21. (-2-Benzyl-5-tert-butoxycarbonylamino-5-methyl-hex-2-enoic acid (**33**)

Ester **32** (600 mg, 1.66 mmol) was dissolved in methanol (6 mL). A solution of potassium hydroxide (120 mg, 2.16 mmol) in water/methanol (2.5 mL, 10.0 mL) was added. The solution was stirred over night at room temperature. The solvent was removed in vacuo. The residue was dissolved in water (10 mL) and acidified to pH 1 with 1 N hydrochloric acid. The aqueous phase was extracted with dichloromethane (4 × 15 mL). The combined organic layers were dried over magnesium sulfate. The solvent was removed in vacuo and the residue was dissolved in a 10% solution of potassium hydroxide in methanol/water (5 mL /5 mL). The solution was heated to reflux for 1.5 h. The solvent was removed in vacuo. The residue was dissolved in water (5 mL). The aqueous phase was extracted with *tert*-butyl methyl ether. The aqueous phase was acidified with 10% sodium hydrogen sulfate solution to pH 1. It was extracted with *tert*-butyl methyl ether (3 × 20 mL). The combined organic layers were dried over magnesium sulfate. The solvent was removed in vacuo to give 83 mg of crude acid **33** which was used for the further synthesis.

^{1}H-NMR (DMSO d_6): δ 1.18 (s, 6 H); 1.36 (s, 9 H); 2.63 (d, 2 H);3.60 (s, 2 H); 6.65 (br, 1 H); 6.85 (t, 1 H); 7.10–7.30 (m, 5 H); 12. 25 (br, 1 H).

6.2.22. (-5-tert-Butoxycarbonylamino-3,5-dimethylhex-2-enoic acid ethyl ester (**35**)

Diacetonamine hydrogen oxalate (**34**, 30.0 g; 146 mmol) was suspended in tetrahydrofuran (400 mL). An aqueous solution of sodium hydroxide (1 N; 146 mL) was added. Di-*tert*-butyl dicarbonate (38.3 g; 175 mmol) was dissolved in tetrahydrofuran (100 mL) and added dropwise to the reaction mixture. The reaction mixture was stirred for 2 h at room temperature. Sodium hydroxide (1 N; 146 mL) was added and the reaction mixture was stirred for 12 h at room temperature. Water (200 mL) and ethyl acetate (200 mL) were added. The aqueous phase was extracted with ethyl acetate (4 × 200 mL). The combined organic phases were dried over magnesium sulfate, and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica (200 g), using ethyl acetate/heptane (1:3) as eluent, to afford 28.4 g of (1,1-dimethyl-3-oxobutyl)carbamic acid *tert*-butyl ester.

Triethyl phosphonoacetate (4.7 g; 20.9 mmol) was dissolved in tetrahydrofuran (36 mL). Potassium *tert*-butoxide (2.3 g; 20.9 mmol) was added and the reaction mixture was stirred for 40 min at room temperature. (1,1-Dimethyl-3-oxobutyl)carbamic acid *tert*-butyl ester (2.5 g; 11.6 mmol) was dissolved in tetrahydrofuran (15 mL) and added dropwise to the reaction mixture which was heated to reflux for 12 h. Ethyl acetate (100 mL) and hydrochloric acid (1 N; 100 mL) were added and the phases were separated. The aqueous phase was extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with an aqueous solution of sodium hydrogen carbonate (saturated; 100 mL), dried (magnesium sulfate) and evaporated in vacuo. The residue was purified by flash chromatography on silica (120 g) using ethyl acetate/heptane (1:2) as eluent to afford 2.0 g of ester **35**.

^{1}H-NMR ($CDCl_3$) δ 1.25 (t, 3H); 1.30 (s, 6H); 1.44 (s, 9H); 2.21 (s, 3H); 2.58 (s, 2H); 4.14 (q, 2H); 4.48 (s, 1H); 5.65 (s, 1H).

6.2.23. (-5-tert-Butoxycarbonylamino-3,5-dimethylhex-2-enoic acid (**36**)

Ester **35** (1.95 g; 6.83 mmol) was dissolved in 1,4-dioxane (25 mL) and water (15 mL). Lithium hydroxide (0.18 g; 7.52 mmol) was added and the reaction mixture was stirred for 12 h at room temperature. Water (150 mL)

and *tert*-butyl methyl ether (150 mL) were added. The aqueous phase was diluted with a 10% aqueous solution of sodium hydrogensulfate until pH 2.5 and extracted with *tert*-butyl methyl ether (3 × 100 mL). The combined organic phases were dried over magnesium sulfate and evaporated in vacuo. The residue was recrystallized from heptane (20 mL) to afford 0.6 g of (-5-*tert*-Butoxycarbonylamino-3,5-dimethylhex-2-enoic acid (**36**).

^{1}H-NMR ($CDCl_3$) δ 1.29 (s, 6H); 1.44 (s, 9H); 2.23 (s, 3H); 2.62 (s, 2H); 4.45 (s, 1H); 5.66 (s, 1H).

6.2.24. (-5-(N-(tert-Butoxycarbonyl)-N-methylamino)-5-methylhex-2-enoic acid (**38**)

(-5-(*tert*-Butoxycarbonylamino)-5-methylhex-2-enoic acid **37** [19] (5.00 g, 20.6 mmol) was dissolved in tetrahydrofuran (70 mL). Methyliodide (10.3 mL, 164 mmol) was added and the solution was cooled to 0 °C. Sodium hydride (60% dispersion in mineral oil, 2.07 g, 61.6 mmol) was added in portions and the solution was stirred at room temperature for 4 d. First ethyl acetate (70 mL) and then water (60 mL) were added dropwise and the solvent was removed in vacuo. The crude product was dissolved in water (40 mL) and diethyl ether (40 mL). The organic phase was washed with a saturated aqueous solution of sodium hydrogen carbonate (30 mL). The aqueous phases were mixed and a 5% aqueous solution of citric acid was added until pH 3. The aqueous phase was extracted with ethyl acetate (4 × 50 mL). The organic phase was washed with water (2 × 40 mL), a 5% aqueous solution of sodium thiosulfate (40 mL), and water (40 mL) and dried over magnesium sulfate. The solvent was removed in vacuo. The residue was dissolved in ethyl acetate (45 mL) and washed with an aqueous 10% aqueous solution of sodium hdyrogen sulfate (3 × 30 mL) and dried over magnesium sulfate. The solvent was removed in vacuo, to give 4.00 g of acid **38**.

^{1}H-NMR ($CDCl_3$): δ 1.38 (s, 6 H), 1.45 (s, 9H); 2.80 (d, 2 H); 2.85 (s, 3 H); 5.88 (d, 1 H); 7.01 (q, 1 H).

6.2.25. 3-Aminomethyl-N-methyl-N-((1R)-1-(N-methyl-N-((1R)-1-(methylcarbamoyl)-2-phenylethyl)carbamoyl)-2-(2-naphthyl)ethyl)benzamide (**42**)

Tripeptide **42** was synthesized from dipeptide **40** analogously to tripeptide **43**. (*tert*-Butoxycarbonylamino)methylbenzoic acid (**39**) was used instead of acid **4**.

MS: 535.7 $[M]^+$; HPLC: 31.20 min (A1), 36.35 min (B1).

*6.2.26. (2R)-2-[N-Methyl-N-((2-amino-2-methylpropoxy)acetyl)amino]-N-methyl-N-((1R)-1-(methylcarbamoyl)-2-phenylethyl)-3-(2-naphthyl)propionamide (***43***)*

A solution of acid **4** (184 mg, 0.74 mmol), 1-hydroxy-7-azobenzotriazole (101 mg, 0.74 mmol) and 1-ethyl-3-dimethylaminopropyl carbodiimide hydrochloride (157 mg, 0.82 mmol) in 9 mL of dichloromethane and *N*, *N*-dimethylformamide (1 mL) was stirred for 15 min. Then, dipeptide **40** (300 mg, 0.74 mmol) and diisopropylethylamine (96 mg, 0.74 mmol) in dichloromethane (1 mL) were added and allowed to stir for 16 h. The mixture was washed with saturated sodium bicarbonate, dried over magnesium sulfate and concentrated in vacuo. The crude product was purified by flash chromatography on silica (50 g) with ethyl acetate to give 360 mg (76%) of 2-[1-methyl-2-(2-(*tert*-butoxycarbonyl)amino-(2-methylpropoxy))acetylamino]-*N*-(1-methyl-1-((1-methylcarbamoyl)-2-(2-phenylethyl)-3-(2-naphthyl))propionamide. The obtained mixture was taken up in a mixture of trifluoro acetic acid and dichloromethane (1 mL/1 mL) and stirred at 0 °C for 5 min. Then, a saturated aqueous solution of sodium bicarbonate was slowly added to the cooled solution and the organic layer was separated, washed with a saturated aqueous solution of sodium bicarbonate (10 mL), dried over magnesium sulfate and concentrated in vacuo to give 185 mg (47% from **4**) of tripeptide **43** as an oil. The obtained oil was dissolved in 0.1 N acetic acid (50 mL) and lyophilized to give an amorphous white powder.

^{1}H-NMR ($CDCl_3$, free amine) δ 0.9 (d, 3H); 1.05 (d, 3H); 2.35 (s, 3H); 2.75 (s, 3H); 2.8 (s, 3H); 2.9 (s, 2H); 3.0 (s,2H); 3.0–2.7 (m, 2 H); 3.25 (m, 2H); 3.7 (t, 1H); 5.1 (dd, 1H); 5.8 (t, 1H); 7.8–6.9 (m, 12H).

HPLC: 30.65 min (A1).

6.2.27. (2R)-N-Methyl-N-((1R)-1-(methylcarbamoyl)-2-phenylethyl)-2-(N-methyl-N-{[(2-piperidinyl)methoxy]acetyl}amino)-3-(2-naphthyl)propinamide (**44**)

Tripeptide **44** was synthesized analogously to tripeptide **43**, using acid **5** as starting material instead of acid **4**. The diastereoisomers were inseparable on HPLC.

^{1}H-NMR ($CDCl_3$, selected peaks): δ 5.30 (m 1 H); 5.75 (t, 1 H); 7.00–7.80 (m, 12 H).

MS: 559.5 $[M + 1]^+$; HPLC: 32.00 min (A1).

6.2.28. (2R)-N-Methyl-N-((1R)-1-(methylcarbamoyl)-2-phenylethyl)-2-(methyl[{piperidin-4-yloxy}acetyl]amino)-3-(2-naphthyl)propionamide (**45**)

Tripeptide **45** was synthesized analogously to tripeptide **43** using acid **6** as starting material instead of acid **4**.

^{1}H-NMR ($CDCl_3$, selected peaks): δ 3.75 (q, 2 H); 5.50 (m, 1 H); 5.80 (t, 1 H); 7.00–7.80 (m, 12 H).

MS: 544.5 $[M]^+$; HPLC: 28.90 min (A1).

6.2.29. (2R)-2-(N-[(2R)-2-(N-[{2-Aminobutoxy}acetyl]-N-methylamino)-3-(2-naphthyl)propionyl]-N-methyl-amino)-N-methyl-3-phenylpropionamide (**46**)

Tripeptide **46** was synthesized analogously to tripeptide **43** using acid **7** as starting material instead of acid **4**. The diastereoisomers were inseparable on HPLC.

^{1}H-NMR (DMSO-d_6, selected peaks): δ 0.60–0.80 (m, 3 H); 2.65 (d, 3 H); 2.75 (s, 3 H); 5.35 (dd, 1 H); 5.65 (dd, 1 H), 7.10–7.90 (m, 12 H).

MS: 533.0 $[M + 1]^+$; HPLC: 31.10 min (A1).

6.2.30. (2R)-N-Methyl-2-(N-methyl-N-((2R)-2-(N-methyl-N-((((2S)-pyrrolidin-2-yl)methoxy)acetyl)amino)-3-(2-naphthyl)propionyl)amino)-3-phenylpropionamide (**47**)

Tripeptide **47** was synthesized analogously to tripeptide **43** using acid **8** as starting material instead of acid **4**.

^{1}H-NMR (DMSO-d_6, selected peaks): δ 0.75 (m, 1 H); 1.35 (m, 1 H); 1.60 (m, 1 H); 1.70 (m, 1 H); 2.65 (d, 3 H); 2.75 (d, 3 H), 3.95 (d, 2 H); 5.35 (dd, 1 H); 5.55 (dd, 1 H).

MS: 544.8 $[M + 1]^+$; HPLC: 31.10 min (A1).

6.2.31. (2R)-N-Methyl-2-(N-methyl-((2R)-2-(N-methyl-N-((piperidin-2-yl)acetyl)amino)-3-(2-naphthyl)propionyl)amino)-3-phenylpropionamide (**48a** and **48b**)

Tripeptides **48a** and **48b** were synthesized analogously to tripeptide **43** using acid **10** as starting material instead of acid **4**. The diastereoisomers were separated by HPLC-chromatography.

48a: MS: 529.2 $[M + 1]^+$; HPLC: 32.20 min (A1), 32.77 min (B1).

48b: MS: 529.4 $[M + 1]^+$; HPLC: 31.80 min (A1), 33.43 min (B1).

6.2.32. (2R)-N-Methyl-2-(N-methyl-((2R)-2-(N-methyl-N-((piperidin-3-yl)acetyl)amino)-3-(2-naphthyl)propionyl)amino)-3-phenylpropionamide (**49a** and **49b**)

Tripeptides **49a** and **49b** were synthesized analogously to tripeptide **43** using acid **11** as starting material instead of acid **4**. The diastereoisomers were separated by HPLC-chromatography.

49a: MS: 529.4 $[M + 1]^+$; HPLC: 30.75 min (A1), 35.60 min (B1).

49b: MS: 529.1 $[M + 1]^+$; HPLC: 29.92 min (A1), 34.83 min (B1).

6.2.33. (2R)-N-Methyl-2-(N-methyl((2R)-2-(N-methyl-N-((2-methylpiperidin-3-yl)carbonyl)amino)-3-(2-naphthyl)-propionyl)amino)-3-phenylpropionamide (**50a** and **50b**)

Tripeptides **50a** and **50b** were synthesized analogously to tripeptide **43** using acid **12** as starting material instead of acid **4**. The diastereoisomers were separated by HPLC-chromatography.

50a: MS: 529.0 $[M + 1]^+$; HPLC: 30.73 min (A1), 32.17 min (B1).

50b: MS: 528.6 $[M + 1]^+$; HPLC: 31.08 min (A1), 32.38 min (B1).

6.2.34. (2R)-N-Methyl-N-((1R)-1-(methylcarbamoyl)-2-phenylethyl)-2-[N-methyl-N-(piperidin-4-ylideneacetyl) amino]-3-(2-naphthyl)propionamide (**51**)

Tripeptide **51** was synthesized analogously to tripeptide **43** using acid **16** as starting material instead of acid **4**.

^{1}H-NMR ($CDCl_3$, selected peaks): δ 5.18; 5.38; 5.58; 5.90 (all dd, 2 H); 5.49, 5.52 (both s, 1H)

MS: 527.4 $[M + 1]^+$; HPLC: 28.62 min (A1).

6.2.35. (-3-(Azetidin-3-yl)-N-methyl-N-{(1R)-1-[N-methyl-N-((1R)-1-(methylcarbamoyl)-2-phenylethyl)carbamoyl]-2-(naphthyl)ethyl}acrylamide (**52**)

Tripeptide **52** was synthesized analogously to tripeptide **43** using acid **21** as starting material instead of acid **4**.

^{1}H-NMR ($CDCl_3$, selected peaks): δ 2.25, 2.26, 2.28 (all s, 3 H); 5.12, 5.31, 5.59, 5.88 (all dd, 2H); 6.00 (dd, 1H); 6.91 (m, 1H).

MS: 513.2 $[M + 1]^+$; HPLC: 29.40 min (A1).

6.2.36. (4-(1-Aminocyclobutyl)but-2-enoic acid N-methyl-N-((1R)-1-(N-methyl-N-((1R)-1-(methylcarbamoyl)-2-phenylethyl)carbamoyl)-2-(2-naphthyl)ethyl)amide (**53**)

Tripeptide **53** was synthesized analogously to tripeptide **43** using acid **30** as starting material instead of acid **4**.

^{1}H-NMR ($CDCl_3$, selected peaks for major rotamere): δ 2.26 (s, 3 H); 2.98 (s, 3 H); 3.00 (s, 3 H); 5.15 (dd, 1 H); 5.57 (dd, 1 H); 6.11 (d, 1 H).

HPLC: 32.20 min (A1).

6.2.37. (-5-Amino-2-benzyl-5-methylhex-2-enoic acid N-methyl-N-((1R)-1-(N-methyl-N-((1R)-1-methylcarbamoyl-2-phenylethyl)carbamoyl)-2-(2-naphthyl)ethyl)amide (**54**)

Tripeptide **54** was synthesized analogously to tripeptide **43** using acid **33** as starting material instead of acid **4**.

^{1}H-NMR ($CDCl_3$, selected peaks for major rotamere): δ 0.89 (s, 3 H); 0.92 (s, 3 H); 2.38 (d, 3 H); 2.45 (s, 3 H); 2.95 (s, 3 H); 5.21 (m, 1 H); 5.45 (m, 1 H).

MS: 618.2 $[M + 1]^+$; HPLC: 37.53 min (A1).

6.2.38. (-5-Amino-3,5-dimethylhex-2-enoic acid N-methyl-N-((1R)-1-(N-methyl-N-((1R)-1-methylcarbamoyl -2-phenylethyl)carbamoyl)-2-(2-naphthyl)ethyl)amide (**55**)

Tripeptide **55** was synthesized analogously to tripeptide **43** using acid **36** as starting material instead of acid **4**.

^{1}H-NMR ($CDCl_3$, selected peaks for major rotamere) δ 1.18 (s, 3 H); 1.19 (s, 3 H); 1.67 (s, 3 H); 2.75 (d, 3 H); 2.76 (s, 3 H); 2.95 (s, 3 H); 5.52 (dd, 1 H), 5.62 (s, 1 H); 5.86 (dd, 1H).

MS: 542.8 $[M + 1]^+$; HPLC: 31.78 min (A1).

6.2.39. (-5-Methyl-5-methylaminohex-2-enoic acid N-methyl-N-((1R)-1-(N-methyl-N-((1R)-1-(methylcarbamoyl) -2-phenylethyl)carbamoyl)-2-(2-naphthyl)ethyl)amide (**56**)

Tripeptide **56** was synthesized analogously to tripeptide **43** using acid **38** as starting material instead of acid **4**.

^{1}H-NMR ($CDCl_3$, selected peaks for major rotamere) δ 1.25 (s, 3 H); 1.30 (s, 3 H); 2.28 (d, 3 H); 2.52 (s, 3 H); 2.72 (s, 3 H); 2.99 (d, 3 H); 5.69 (dd, 1 H), 5.81 (dd, 1 H); 6.13 (d, 1 H).

MS: 544.4 $[M + 1]^+$; HPLC: 31.30 min (A1).

6.2.40. N-Methyl-N-((1R)-1-(N-methyl-N-((1R)-1-(methyl-carbamoyl)-2-phenylethyl)carbamoyl)-2-(2-naphthyl)ethyl)-3-((N-methylamino)methyl)benzamide (**57**)

Tripeptide **57** was synthesized analogously to tripeptide **43** using acid **39** as starting material instead of acid **4**.

MS: 551.1 $[M + 1]^+$; HPLC: 31.37 min (A1), 33.25 min (B1).

6.2.41. 1,2,5,6-tetrahydropyridine-3-carboxylic acid N-methyl-N-((1R)-1-(N-methyl-N-((1R)-1-(methylcarbamoyl)-2-phenylethyl)carbamoyl)-2-(2-naphthyl)ethyl) amide (**58**)

Tripeptide **58** was synthesized analogously to tripeptide **43** using *N*-BOC-protected guvacine [36] as starting material instead of acid **4**.

MS: 512.2 $[M + 1]^+$; HPLC: 29.67 min (A1), 31.07 min (B1).

6.2.42. Piperazine-2-carboxylic acid N-methyl-N-((1R)-1-(N-methyl-N-((1R)-1-(methylcarbamoyl)-2-phenyl-ethyl)-carbamoyl)-2-(2-naphthyl)ethyl)amide (**59a** and **59b**)

Tripeptides **59a** and **59b** were synthesized analogously to tripeptide **43** using bis-*N*-BOC-protected piperazine-2-carboxylic acid [36] as starting material instead of acid **4**. The diastereisomers were separated by HPLC.

59a: MS: 516.7 $[M + 1]^+$; HPLC: 26.82 min (A1).
59b: MS: 516.7 $[M + 1]^+$; HPLC: 25.43 min (A1).

6.2.43. Azetidine-3-carboxylic acid N-methyl-N-{(1R)-1-[N-methyl-N-((1R)-1-methylcarbamoyl-2-phenylethyl)-carbamoyl]-2-(2-naphtyl)ethyl}amide (**60**)

Tripeptide **60** was synthesized analogously to tripeptide **43** using *N*-BOC-protected azetidine-3-carboxylic acid [36] as starting material instead of acid **4**.

^{1}H-NMR (DMSO-d_6, selected peaks): δ 4.73 and 5.35 (both dd, together 1 H); 5.16 and 5.63 (both dd, together 1 H); 7.00–7.90 (m, 12 H).

MS: 487 $[M + 1]^+$; HPLC: 28.40 min (A1).

6.2.44. 4-Aminocyclohexanecarboxylic acid N-methyl-N-((1R)-1-[N-methyl-N-{(1R)-1-methylcarbamoyl-2-phenyl-ethyl}carbamoyl]-2-(2-naphtyl)ethyl)amide (**61**)

Tripeptide **61** was synthesized analogously to tripeptide **43** using *N*-BOC-protected 4-aminocyclohexane-carboxylic acid [36] as starting material instead of acid **4**.

^{1}H-NMR (DMSO-d_6, selected values): δ 1.20 – 1.50 (m, 8 H); 2.05 (s, 3 H); 2.65 (d, 3 H); 2.70 (s, 3 H); 2.50–3.30 (m, 6 H); 5.40 (m, 1 H); 5.60 (m, 1 H); 7.10–7.80 (m, 12 H).

MS: 529 $[M + 1]^+$; HPLC: 30.20 min (A1).

6.2.45. 3-(1-Aminoethyl)benzoic acid N-methyl-N-((1R)-1-(N-methyl-N-((1R)-1-(methylcarbamoyl)-2-phenyl-ethyl)carbamoyl)-2-(2-naphthyl)ethyl)amide (**62**)

Tripeptide **62** was synthesized analogously to tripeptide **43** using *N*-BOC-protected 3-aminoethylbenzoic acid [29] as starting material instead of acid **4**. The diastereoisomeres were not separable by HPLC.

MS: 551.3 $[M + 1]^+$; HPLC: 31.80 min (A1), 33.93 min (B1).

6.2.46. (2R)-2-(N-((2R)-2-(N-((-5-((2R)-2-(tert-Butyl-dimethylsilyloxy)propylamino)-5-methylhex-2-enoyl)-N-methylamino)-3-(2-naphthyl)propionyl)-N-methylamino) -N-methyl-3-phenylpropionamide (**63**)

Amine **41** [19] was dissolved in methanol (20 mL) and glacial acetic acid (0.48 mL, 8.4 mmol). 3 Å mol sieves (9 g) and a solution of (2*R*)-2-(*tert*-butyldimethyl-silyloxy)propanal [32] (1.33 g, 7.06 mmol) in methanol (10 mL) were added successively. Sodium cyanoborohydride (220 mg, 3.53 mmol) was added as a solid. The reaction mixture was stirred at room temperature for 45 min before a second batch of sodium cyanoborohydride (220 mg, 3.53 mmol) was added. The reaction mixture was stirred for 16 h at room temperature. The mol sieves were filtered off through a plug of celite. The celite was washed with methanol (200 mL). The solvents of the combined filtrates were removed in vacuo. The

residue was dissolved in 1 N sodium hydroxide solution (50 mL) and *tert*-butyl methyl ether (50 mL). The phases were separated. The aqueous phase was extracted with *tert*-butyl methyl 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 (35 g), using ethyl acetate/heptane/triethylamine (20:10:1) as eluent, to give 99 mg of silyl ether **63**.

^{1}H-NMR ($CDCl_3$, selected values): δ 0.04, 0.05, and 0.10 (all s, together 6 H); 0.85 and 0.91 (both s, together 9 H); 3.86 (m, 1 H); 6.04 and 6.08 (both d, together 1 H); 6.89 (m, 1 H).

MS: 701.2 $[M + 1]^+$.

6.2.47. (2R)-2-(N-((2R)-2-(N-((-5-((2R)-2-Hydroxypropylamino)-5-methylhex-2-enoyl)-N-methylamino)-3-(2-naphthyl)propionyl)-N-methylamino)-N-methyl-3-phenylpropionamide (**64**)

Silyl ether **63** (99 mg, 0.14 mmol) was dissolved in tetrahydrofuran (2 mL). Tetra-*n*-butylammonium fluoride (0.18 mL of a 1.1 M solution in tetrahydrofuran, 0.2 mmol) was added. The solution was stirred for 3 h, before another portion of tetra-*n*-butylammonium fluoride (0.23 mL of a 1.1 M solution in tetrahydorfuran, 0.25 mmol) was added. The reaction mixture was stirred for 2.5 h at room temperature and diluted with ethyl acetate (50 mL). It was extracted with 10% sodium carbonate solution (30 mL). The aqueous phase was extracted with ethyl acetate (20 mL). The organic layers were combined and dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was purified by flash-chromatography on silica (30 g), using dichloromethane/methanol/25% aqueous ammonia (100:10:1) as eluent, to give 28 mg of hydroxypropylamine **64**.

^{1}H-NMR ($CDCl_3$, selected values): δ 3.65 and 3.72 (both m, together 1 H); 5.15 and 5.30 (both dd, together 1 H); 5.60 and 5.90 (both dd, together 1 H); 6.03 and 6.05 (both d, together 1 H); 6.78 (m, 1 H).

MS: 587.2 $[M + 1]^+$; HPLC: 27.47 min (A1), 27.12 min (B1).

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