New Active Series of Growth Hormone Secretagogues

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New growth hormone secretagogue (GHS) analogues were synthesized and evaluated for growth hormone releasing activity. This series derived from EP-51389 is based on a gem-diamino structure. Compounds that exhibited higher in vivo GH-releasing potency than hexarelin in rat (subcutaneous administration) were then tested per os in beagle dogs and for their binding affinity to human pituitary GHS receptors and to *h*GHS−R 1a. Compound **7** (JMV 1843, H-Aib-(D)-Trp-(D)-gTrp-formyl) showed high potency in these tests and was selected for clinical studies.[1]

Introduction

Growth hormone (GH) is an important endocrine regulator of growth and anabolic processes.[2] The clinical use of recombinant human GH has demonstrated benefits in the therapy of GH-deficient children,[3] and it reverses some of the effects of aging in the elderly.[4] In recent years, the GH-releasing peptides (GHRPs) and peptidomimetics have received considerable attention as potential alternatives to the expensive recombinant human GH. During their studies on enkephalin analogues Bowers and co-workers discovered a series of peptides able to stimulate GH release from rat pituitary and opened a new avenue of research.[5] This new family of peptides[6] including GHRP-6, GHRP-1, GHRP-2, and hexarelin promotes the release of GH in man (Figure 1). This GH release mechanism was found to be different from the endogeneous growth hormone releasing hormone (GHRH)[7] and to be mediated through a G-protein-coupled receptor (GHS−R 1a).[8] The natural ligand of this receptor has been isolated and characterized very recently from rat stomach[9] and further identified in humans.[10] It is a 28 amino acid peptide in which serine 3 is *n*-octanoylated with the following sequence GSS-(octanoyl)FLSPEHQRVQQRKESKKPPAKLQPR. Several classes of small non-peptide secretagogues (benzolactam biphenyl tetrazoles,[11] camphor derivatives,[12] and 4-spiropiperidines[13]) have been described (Figure 2) that are able to release GH from the pituitary. Starting from the GHRP-6 sequence, various peptide molecules have been reported as potent GHRPs (Figure 3). Starting from hexarelin, we earlier described a tripeptide EP-51389, Aib-(D)-2-Me-Trp-(D)-2-Me-Trp-NH_2,[14] that is more potent than hexarelin in stimulating GH secretion in an infant rat model when injected subcutaneously, and it is orally active in dogs and horses. This 2-Me-Trp (Mrp) containing tripeptide was also proved to be subcutaneously more potent than hexarelin in an infant rat model and orally active in dogs and man.[15,16] In this paper, we report the identification of a new series of potent pseudotripeptide analogues that act as GH secretagogues.

Compound	Sequence
GHRP-6	His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2
GHRP-2	D-Ala-D-βNal-Ala-Trp-D-Phe-Lys-NH_2
GHRP-1	Ala-His-D-βNal-Ala-Trp-D-Phe-Lys-NH_2
hexarelin	His-D-Mrp-Ala-Trp-D-Phe-Lys-NH_2

Figure 1. Sequences of GHRP analogues.

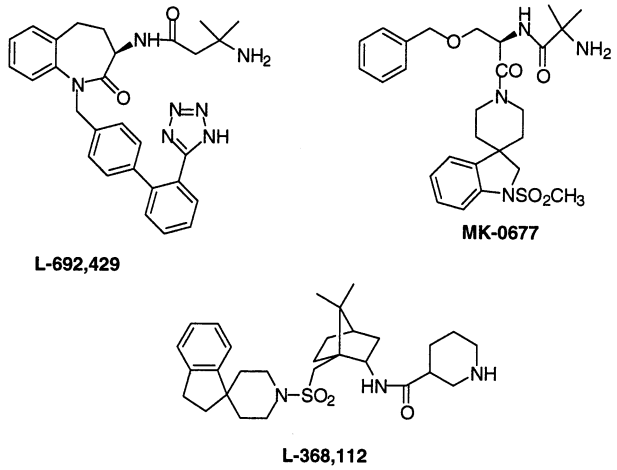

Figure 2. Structures of non-peptide GH secretagogues.

Our experience with bombesin analogues has shown that in this peptide, replacement of the C-terminus carboxamide residue Leu-NH_2 (or Met-NH_2) with the

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ipamorelin: Aib-His-D-βNal-D-Phe-Lys-NH_2
EP 51389: Aib-D-Mrp-D-Mrp-NH_2
L164,080: Aib-D-Trp-D-homoPhe-OEt

NN703:

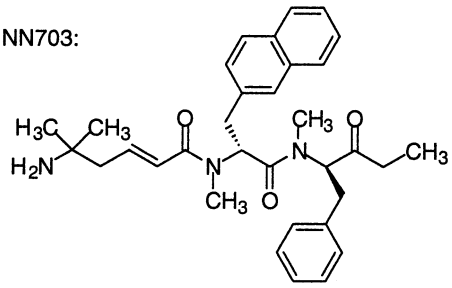
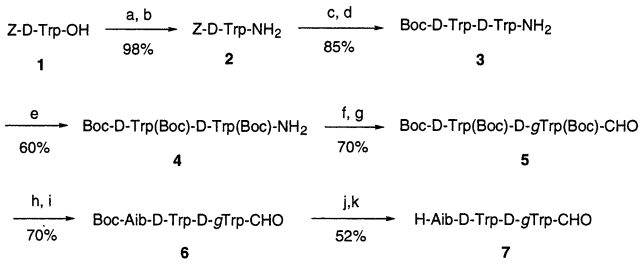


Figure 3. Structure of peptide or pseudo-peptide GH secretagogue containing an Aib moiety or an Aib substitute.

Scheme 1. Synthetic Pathway for JMV 1843 and Analogues[a]



[a] Reagents and conditions: (a) IBCF, NMM, DME, 0 °C; (b) NH_4OH; (c) H_2, Pd/C, EtOH, HCl; (d) BOP, NMM, DMF, Boc-(D)-Trp-OH; (e) Boc_2O, DMAP cat., anhydrous CH_3CN; (f) BTIB, pyridine, DMF/H_2O; (g) 2,4,5-trichlorophenylformate, DIEA, DMF; (h) TFA/anisole/thioanisole (8:1:1), 0 °C; (i) BOP, NMM, DMF, Boc-Aib-OH; (j) TFA/anisole/thioanisole (8:1:1), 0 °C; (k) RP preparative HPLC.

formyl gem C-terminus residue gLeu-For (or gMet-For) leads to very potent agonists (unpublished results of this laboratory). This strategy was also explored in the GHS analogues presented in this paper.

Chemistry

The preparation of **7** (JMV 1843, H-Aib-(D)-Trp-(D)-gTrp-formyl) and all parent analogues has been carried out in six steps in 10−20% overall yield from the commercially available *N*-*Z*-(D or L)-tryptophan, *N*-Boc-(D or L)-tryptophan, and *N*-Boc-aminoisobutyric acid as described in Scheme 1.

Dipeptide **3** was synthesized by standard procedures and then treated with di-*tert*-butyl dicarbonate (Boc_2O) in the presence of a catalytic amount of 4-(dimethylamino)pyridine (DMAP) to protect the two indole functions. Compound **4** was submitted to bis-trifluoroacetoxyiodobenzene (BTIB)[17] treatment in a mixture of DMF and water in the presence of pyridine to afford the pseudodipeptide, which was immediately acylated by 2,4,5-trichlorophenylformate (2,4,5-TCPF)[18] to yield **5**. All attempts to perform the Hoffman transposition without protection of the indole groups led to poor yields. Removing Boc protecting groups and coupling with Boc-Aib-OH in the presence of BOP[19] and NMM in DMF produced the N-protected desired compound **6**. Compound **7** was then obtained by treatment with a mixture of TFA/anisole/thioanisole (8:1:1) at 0 °C and purified by reversed-phase HPLC preparative chromatography.

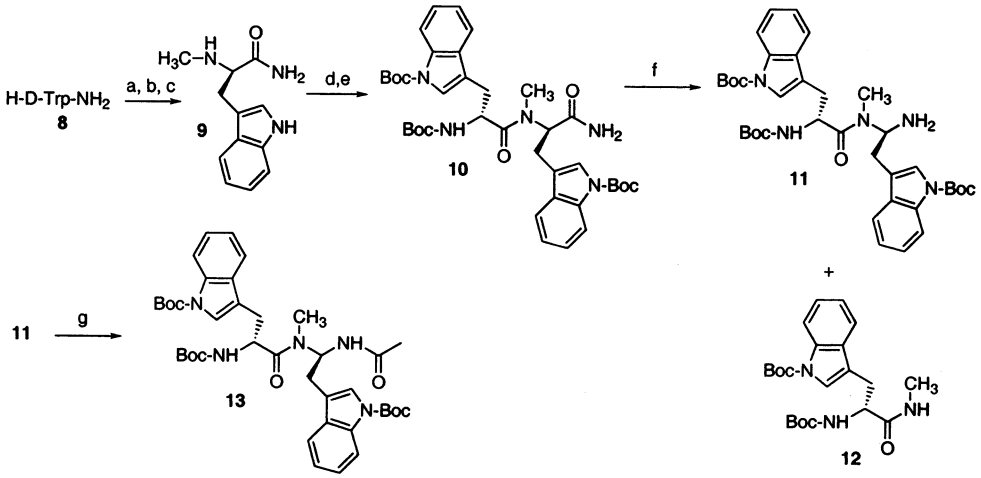
A structure−activity study of compound **7** was performed. The N-terminal amino group was methylated, and the C-terminal formyl group was replaced by an acetyl moiety. Methyl groups were also introduced on the amide bond between the two Trp residues or on the α carbon of the Trp residues. All attempts to introduce the methyl group between Trp and Aib residues were unsuccessful. N-methylation between both tryptophan residues was performed following Scheme 2. H-(D)-N(Me)Trp-amide **9** was obtained in three steps from **8** by the Fukuyama methylation method.[20] The coupling step to obtain the pseudodipeptide amide was then performed with HBTU,[21] and after coupling, the indole groups were protected as previously described to yield **10**. Compound **10** was then allowed to react with BTIB. At this stage we observed an instability of the gem-diamino compound **11** that led to a partial cleavage of the dipeptide at the *N*-methyl bond to yield **12** (Scheme 2). The gem-diamino dipeptide **11** was acylated with acetic anhydride to yield **13**, and compound **19** N(Me)-Aib-(D)-Trp-(D)-N(Me)-gTrp-acetyl was obtained as described for **7**. Compounds incorporating a methyl group at the C-terminus gem-diamino position were synthesized according to Scheme 3 by the Fukuyama procedure. The dipeptide amide **4** reacted with BTIB to yield the C-terminus gem-diamino compound in which amino group was trapped by *o*-nitrobenzenesulfonyl chloride to yield **14**. After N-methylation and obtention of **15**, the C-terminus amino group was deprotected in the presence of thioanisole and immediately acylated with acetic anhydride. After deprotection of **16**, Boc-N(Me)-Aib was introduced with DCC as a coupling reagent and the Boc group was removed to yield a mixture of diastereoisomers **17** and **18** corresponding to epimerization of the C-terminus gem-tryptophan residue that occurred during the synthesis. These diastereoisomers were separated by RP preparative HPLC and tested. A methyl group was also incorporated in the α position of the tryptophan residues. Preparation of the (*R*)-(Me)-Trp residue was performed following the Zhang and Fill procedure[22] in five steps and with an enantiomeric excess of 92%.

Results and Discussion

Because the aim of our search was the discovery of a new orally active drug, the first biological test of our compounds was performed in vivo. This was done by the sc route in infant rats at a dose of 300 μg/kg. This dose was selected on the basis of previous experiments that showed it elicited a near-maximal response with a large number of active GH secretagogues. Compounds that exhibited in this model a better biological activity (GH release) than hexarelin used as control were then tested for their activity in beagle dogs when they were given orally at a dose of 1 mg/kg. Only some of our compounds were tested for their ability to displace radiolabeled ghrelin from human pituitary membranes in order to determinate their IC_{50}.

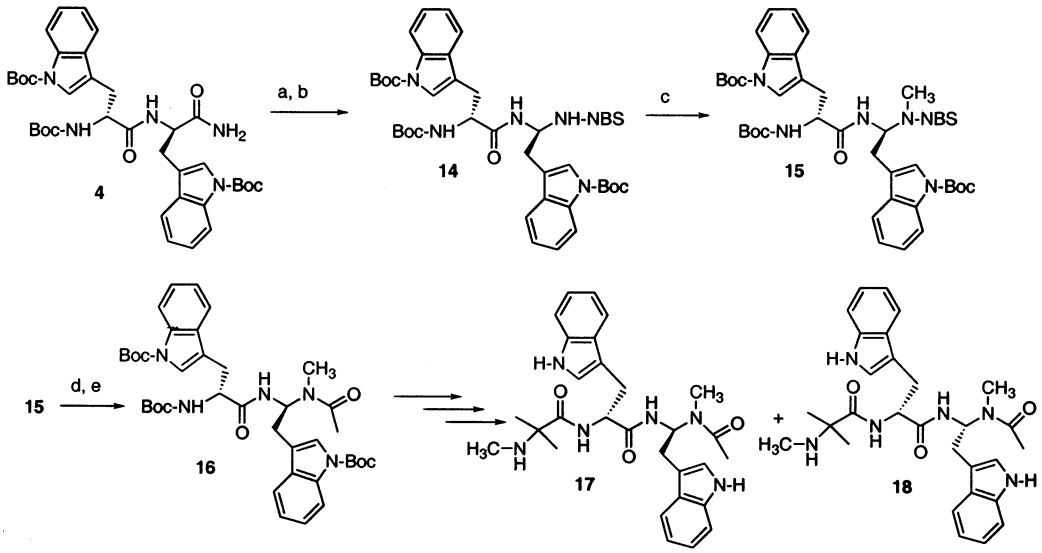
In Vivo Biological Evaluation in Rats. Compounds were administered by the sc route in infant rats at a dose of 300 μg/kg, and results are gathered in Table 1. Surprisingly, in the strict analogue of EP-51389 (compound **23**), no activity could be detected. Replacement of Mrp in EP-51389 by (D)-Trp led to the recovery of potency. The importance of the configuration of each residue was also studied. The presence of two tryp-

Scheme 2. N-Methylation between Both Trp Residues[a]



[a] Reagents and conditions: (a) *o*-NBSCI, DMF, Et_3N; (b) MeI, K_2CO_3, DMF; (c) PhSH, K_2CO_3, DMF, 84% over three steps, no purification needed; (d) Boc-(D)-TrpOH, NMM, HBTU, DMF, 56%; (e) Boc_2O, DMAP, ACN, 89%; (f) BTIB, Py, DMF; (g) Ac_2O, DMF, DIEA, 35−45% over last two steps.

Scheme 3. N-Methylation at the gem-Diamino C-Terminus Amine[a]



[a] Reagents and conditions: (a) BTIB, Py, DMF; (b) *o*-NBSCI, DMF, Et_3N, 47% over two steps; (c) MeI, K_2CO_3, DMF, 60%; (d) PhSH, K_2CO_3, DMF; (e) Ac_2O, DMF, DIEA, 85% over last two steps.

tophan residues of D configuration (compound **7**) was required to recover the activity (see compounds **20**−**22**). Compound **7** exerted a higher activity than the parent molecule and was considered to be our new lead compound. It can be seen that MK-0677 was found to be less potent than hexarelin in the in vivo rat test.

To increase lipophilicity, enzyme stability, and perhaps the bioavailability of compound **7** and analogues, we decided to methylate amide bonds or the N-terminus amino function. These modifications led us to the second group of molecules, which are gathered in Table 1.

Introduction of one or two methyl groups on the amino function of Aib resulted in less potent compounds as shown by the results obtained with compounds **25** and **26**. Replacement of the formyl with an acetyl group also decreased the potency of compound **27**, while concomitant introduction of a methyl group and an acetyl moiety led to compound **28**, which exhibited activity similar to that of our lead compound **7**. A second methyl group on the Aib residue resulted in a total loss of activity (compound **29**). Introduction of a methyl group between Trp residues led to compound **30**, which is less potent than its analogue **27**, but the concomitant methylation of the N-terminal part yielded one of the most potent compounds, **19**. Alkylation of the α carbon of the first Trp residue with a methyl group yielded an inactive compound (**31**), while the same alkylation on the α carbon of the second Trp residue led to the analogue **32**, which is almost as potent as hexarelin. Replacement in this analogue of the formyl by an acetyl group and methylation of the N-terminus primary amine yielded compound **33** exhibiting the same activity as hexarelin, confirming that the concomitant introduction of an acetyl group at the C terminus and of a methyl group at the N terminus was favorable for biological activity. When a methyl group was introduced on the C-terminal amino function of the gem-diamino residue simultaneously with a methyl group on the primary amine of the Aib residue, a potent analogue (**17**) was obtained

Table 1. Biological Activity in Infant Rat (sc Injection)[a]

compound	sequence	compound/hexarelin GH release
solvent		0.01
hexarelin	His-(D)-Mrp-Ala-Trp-(D)-Phe-Lys-NH_2	1.0
ghrelin		0.65
MK-0677		0.31
EP-51389	H-Aib-(D)-Mrp-(D)-Mrp-NH_2	0.89
7	H-Aib-(D)-Trp-(D)-gTrp-formyl	1.31
20	H-Aib-(L)-Trp-(L)-gTrp-formyl	0.08
21	H-Aib-(L)-Trp-(D)-gTrp-formyl	0.07
22	H-Aib-(D)-Trp-(L)-gTrp-formyl	0.28
23	H-Aib-(D)-Mrp-(D)-gMrp-formyl	0.08
24	H-Aib-(D)-Mrp-(L)-gMrp-formyl	0.12
25	N(Me)-Aib-(D)-Trp-(D)-gTrp-formyl	0.64
26	$N(Me)_2$-Aib-(D)-Trp-(D)-gTrp-formyl	0.69
27	H-Aib-(D)-Trp-(D)-gTrp-acetyl	0.77
28	N(Me)-Aib-(D)-Trp-(D)-gTrp-acetyl	1.38
29	$N(Me)_2$-Aib-(D)-Trp-(D)-gTrp-acetyl	0.15
19	N(Me)-Aib-(D)-Trp-(D)-N(Me)-gTrp-acetyl	1.45
30	H-Aib-(D)-Trp-(D)-N(Me)-gTrp-acetyl	0.65
31	H-Aib-(*R*)-(Me)Trp-(D)-gTrp-formyl	0.12
32	H-Aib-(D)-Trp-(*R*)-(Me)-gTrp-formyl	0.91
33	N(Me)-Aib-(D)-Trp-(*R*)-(Me)-gTrp-acetyl	1.00
17	N(Me)-Aib-(D)-Trp-(D)-gTrp-N(Me)-acetyl	1.21
18	N(Me)-Aib-(D)-Trp-(L)-gTrp-N(Me)-acetyl	0.20
34	4-aminopiperidine-4-carbonyl-(D)-Trp-(D)-gTrp-formyl	0.95
35	4-aminopiperidine-4-carbonyl-(D)-Trp-(D)-gTrp-acetyl	0.46
36	isonipecotyl-(D)-Trp-(D)-gTrp-formyl	0.82
37	isonipecotyl-(D)-Trp-(D)-gTrp-acetyl	0.53
38	phenylsulfonyl-(D)-Trp-(D)-gTrp-formyl	0.14
39	isovaleryl-(D)-Trp-(D)-gTrp-acetyl	0.11
40	iovaleryl-(D)-Trp-(D)-gTrp-formyl	0.09
41	acetyl-(D)-Trp-(D)-gTrp-acetyl	0.13
42	N(Me)-(D)-Trp-(D)-gTrp-formyl, isomer A	0.09
43	N(Me)-(D)-Trp-(D)-gTrp-formyl, isomer B	0.09
44	methylsulfonyl-(D)-Trp-(D)-gTrp-formyl	0.09
45	H-Aib-(D)-Trp-(D)-gTrp-propionyl	0.69
46	N(Me)-Aib-(D)-Trp-(D)-gTrp-propionyl	0.93
47	H-Aib-(D)-Trp-(D)-gTrp-isovaleryl	0.24
48	N(Me)-Aib-(D)-Trp-(D)-gTrp-isovaleryl	0.10
49	H-Aib-(D)-Trp-(D)-gTrp-phenylacetyl	0.11
50	N(Me)-Aib-(D)-Trp-(D)-gTrp-phenylacetyl	0.16
51	H-Aib-(D)-Trp-(D)-gTrp-piperidine-2-carbonyl	1.34
52	N(Me)-Aib-(D)-Trp-(D)-gTrp-piperidine-2-carbonyl	0.85
53	H-Aib-(D)-Trp-(D)-gTrp-3-indoleacetyl	0.16
54	N(Me)-Aib-(D)-Trp-(D)-gTrp-3-indoleacetyl	0.17
55	H-Aib-(D)-Trp-(D)-gTrp-cyclohexane-3-propionyl	0.15
56	N(Me)-Aib-(D)-Trp-(D)-gTrp-cyclohexane-3-propionyl	0.16
57	H-Aib-(D)-Trp-(D)-gTrp-C(O)$NHCH_2CH_3$	1.40
58	H-Aib-(D)-Trp-(D)-gTrp-C(O)O CH_2CH_3	0.20

[a] Results are normalized as a ratio of GH release stimulated by 300 μg/kg of test compounds and GH release stimulated by 300 μg/kg of hexarelin.

that exhibited an activity greater than that of hexarelin. Its diastereoisomer **18** was found to be inactive.

A structure−activity relationship study was also performed on the N-terminal part of the molecule. In both compounds **7** and **28**, we replaced the Aib residue with a 4-aminopiperidine-4-carboxyl acid residue (compounds **34**, **35**), isonipecotyl (compounds **36**, **37**), phenylsulfonyl (compound **38**), isovaleryl (compounds **39**, **40**), acetyl (compound **41**), methyl (compounds **42**, **43**), or methylsulfonyl group (compound **44**). As shown in Table 1, replacement of the Aib residue in compound **7** by the 4-aminopiperidine-4-carboxyl group (compounds **34**) or the isonipecotyl moiety (compound **36**) resulted in less potent molecules than the parent compounds; nevertheless, these compounds were almost as potent as hexarelin. When, at the same time, the formyl group was replaced by the acetyl group, half of the biological activity was lost (compounds **35**, **37**). In all other compounds, attempts to replace the Aib moiety led to inactive compounds, showing the importance of the Aib residue or of a basic residue in the interaction with the receptors.

Modifications on the C-terminal part were then performed. The formyl and acetyl groups were replaced to change the steric hindrance and the lipophilicity of the molecules. Propionyl, isovaleryl, phenylacetyl, piperidine-2-carboxyl, 3-indoleacetyl, and cyclohexane-3-propionyl groups were introduced for this purpose, and the corresponding compounds were tested. Increase of the lipophilicity of the C-terminal part of compound **7** produced a loss in biological activity as shown in Table 1 (compounds **27**, **45**, **46**, and **49**). The concomitant introduction of a N(Me)-Aib with a propionyl group at the C terminus led to an increase of potency and to a compound as potent as hexarelin (compound **46**). This observation was not true in the case of isovaleryl (compounds **47**, **48**) and phenylacetyl (compounds **49**, **50**) groups. The presence of a piperidine-2-carbonyl

Table 2. Biological Activity in Beagle Dogs by Oral Administration at a Dose of 1 mg/kg

compound	GH peak (μg/L)	GH AUC$^{0\rightarrow180}$ (μg min L^{-1})
saline	0.4 ± 0.1	76.18 ± 1.28
MK-0677	5.4 ± 2.4	544.55 ± 166.74
7	5.5 ± 1.1	675.35 ± 94.47
28	1.1 ± 0.5	85.85 ± 18.49
51	0.5 ± 0.1	87.06 ± 15.36
19	5.5 ± 2.0	262.43 ± 27.87
57	0.4 ± 0.1	79.54 ± 1.7

group in this part of the molecule was well accepted by the receptors (compound **51**), leading to a potent compound that is even more active than compound **7**, suggesting a possible interaction between the secondary amine of the piperidyl group and a putative acid function in the receptor subsite. In this compound, introduction of a N(Me)-Aib led to a decrease in the potency but resulted in compound **52** almost as potent as hexarelin. Indolacetyl and cyclohexylpropionyl groups produced inactive compounds. An interesting activity was obtained by incorporation of a urea function on the C-terminal gem-diamine, leading to compound **57** which exhibited a strong in vivo activity in rat. This activity was surprisingly lost when the urea function was replaced by a carbamate moiety (compound **58**), although these two last compounds exhibited similar binding affinities in the receptor assays.

Oral Activity in Dog. The most potent compounds were tested for their activity in beagle dogs when they were given orally at a dose of 1 mg/kg. Results are gathered in Table 2. In this test, hexarelin at a dose of 1 mg/kg by oral route was completely ineffective in stimulating GH secretion in the dog. Two compounds **7** and **19** were found to be active when administered per os in dogs, while compounds **28** and **51**, which revealed good in vivo activity in rats when injected sc, were inactive in this model. Compound **7** had a more sustained GH response (peak, 5.5 ± 1.1 ng/mL; AUC_{0-180}, 675.35 ± 94.47; $n = 6$) than compound **19** (peak, 5.5 ± 2.0 ng/mL; AUC_{0-180}, 262.43 ± 27.87; $n = 4$), while other compounds (**28**, **51**, and **57**) and the vehicle did not induce a GH response. Compound **7** was found to be more active than MK-0677, hexarelin, or ghrelin. This result prompted us to choose this compound for further studies.

Binding to the Human Pituitary GHS Receptors and to the Cloned Human GHS−R 1a Receptors. Compounds exhibiting good in vivo activity in rats (sc injection) were further tested for their binding affinity to human GHS receptors. This study was performed in two distinct tests: (1) on autoptic human pituitary tissue membranes and (2) on the cloned *h*GHS−R 1a receptor transiently expressed in LLC PK-1 cells. Results are gathered in Table 3. In the first test, ^{125}I-Tyr^4-ghrelin was used as radiolabeled ligand. It was demonstrated to be a suitable ligand in this model as described in the experimental part. ^{125}I-His^9-ghrelin was used as a ligand in the second model in order to compare the binding affinities of our compounds with literature compounds.

Human Pituitary GHS Receptors. The ability of unlabeled ghrelin, hexarelin, MK-0677, and other compounds to compete with ^{125}I-Tyr^4-ghrelin on human pituitary membranes was measured. Ghrelin exhibited an IC_{50} of 9.8 ± 1.5 nM in this model, since hexarelin was found to be 2 times less potent and MK-0677 about 3 times less potent. Compounds **7**, **19**, **29**, **51**, **57**, and **58** exhibited almost the same affinity as hexarelin. Compounds **25**, **28**, and **48** were slighly less potent than hexarelin, while compounds **27**, **34**, **36**, **45**, and **48** were 10−100 times less potent than hexarelin. Compounds **35**, **37**, and **46** were not able to displace ^{125}I-Tyr^4-ghrelin from its binding sites. Some results were surprising. Although compounds **57** and **58** showed the same in vitro affinity data, compound **57** was able to elicit GH secretion while its close analogue **58** did not. This discrepancy could be explained by different metabolic stability and brain penetration properties or by differences of compounds in functional activity at the receptors. Compound **28**, which was found to be potent in releasing GH in the rat model, binds with a modest affinity to the human GHS receptor on pituitary membranes. Compounds **34** and **36**, which were in vivo as good as hexarelin, were revealed to be weak ligands for the human pituitary GHS receptor. The correlation between in vivo activity in rats and in vitro binding on human pituitary GHS receptor was satisfactory for other compounds.

***h*GHS−R 1a Receptors.** Compounds were also tested for their affinity to the cloned *h*GHS−R 1a receptor. Binding affinities of human ghrelin and MK-0677 obtained from this model were in accordance with the literature.[23] The binding affinity of Tyr-Ala-hexarelin was found to be lower than of human pituitary membranes. Compounds **25**, **28**, and **19** exhibited a high binding affinity to the cloned *h*GHS−R 1a receptor (22.7 ± 7.4, 58 ± 20, and 29.5 ± 16.0 nM respectively). A comparison of these values with the ones obtained for compounds **7** and **27** (123 ± 25 and 228 ± 162 nM respectively) clearly indicated that N-methylation of the amino function of Aib was well accepted for the binding to the cloned *h*GHS−R 1a receptor. This observation was confirmed when comparing the IC_{50} obtained for compounds **45** versus **46** and **47** versus **48**. The obtained results also showed the importance of the steric hindrance of the gem-diamino acylating group: the binding affinity was reduced when the chain length was increased (non-N-methylated compounds **7**, **27**, **45**, and **47**; N-methylated compounds **25**, **28**, **46** and **48**). Dimethylation of the Aib amino function led to less potent compound **29**, while Aib replacement by 4-aminopiperidine-4-carboxyl or isonipecotyl moieties resulted in reduced binding affinity (compounds **34**, **36**, and **37**). Compounds **51**, **57**, and **58** presented an affinity similar to that of our lead compound **7**.

Results obtained in these two models led to several comments. The high observed binding affinities of ghrelin and MK-0677 toward the cloned *h*GHS−R 1a are in accordance with the literature because it was discovered with the radiolabeled ^{35}S-MK-0677 compound. The same receptor was used for the discovery of ghrelin. Affinity binding values of ghrelin and MK-0677 toward the human pituitary receptors were quite different. When comparing binding affinities of Tyr-Ala-hexarelin in both models, it appears that pituitary membranes also contain other receptor subtypes. These observations are well correlated with results reported in the literature. ^{35}S-MK-0677 and ^{125}I-His^9-ghrelin

Table 3. Binding Affinity of Ghrelin, MK-0677, Hexarelin, Tyr-Ala-hexarelin, and Other Compounds to the Human Pituitary GHS Receptors and to the Cloned Human GHS−R 1a Receptors

compound	sequence	IC_{50} (nM) for ^{125}I[Tyr^4]ghrelin human pituitary membranes	IC_{50} (nM) for ^{125}I[His^9]ghrelin *h*GHS−R 1a
Tyr-Ala-hexarelin	Tyr-Ala-His-(D)-Mrp-Ala-Trp-(D)-Phe-Lys-NH_2	12 ± 1	286 ± 70
hexarelin	His-(D)-Mrp-Ala-Trp-(D)-Phe-Lys-NH_2	18.0 ± 2.7	ND
ghrelin		9.8 ± 1.5	0.39 ± 0.10
MK-0677		32.0 ± 1.7	0.76 ± 0.10
7	H-Aib-(D)-Trp-(D)-gTrp-formyl	22.9 ± 1.3	123 ± 25
25	N(Me)-Aib-(D)-Trp-(D)-gTrp-formyl	39.0 ± 3.4	22.7 ± 7.4
27	H-Aib-(D)-Trp-(D)-gTrp-acetyl	3852 ± 290	228 ± 162
28	N(Me)-Aib-((D)-Trp-(D)-gTrp-acetyl	74.0 ± 5.4	58 ± 20
34	4-aminopiperidine-4-carbonyl-(D)-Trp-(D)-gTrp-formyl	1791 ± 171	337 ± 110
35	4-amino-piperidine-4-carbonyl-(D)-Trp-(D)-gTrp-acetyl	>10000	ND
36	isonipecotyl-(D)-Trp-(D)-gTrp-formyl	391 ± 40	1.155 ± 235
37	isonipecotyl-(D)-Trp-(D)-gTrp-acetyl	>10000	807 ± 154
45	H-Aib-(D)-Trp-(D)-gTrp-propionyl	185 ± 27	460 ± 11
47	H-Aib-(D)-Trp-(D)-gTrp-isovaleryl	59.8 ± 12	535 ± 140
51	H-Aib-(D)-Trp-(D)-gTrp-piperidine-2-carbonyl	21.5 ± 1.6	116 ± 35
46	N(Me)-Aib-(D)-Trp-(D)-gTrp-propionyl	>10000	167 ± 48
48	N(Me)-Aib-(D)-Trp-(D)-gTrp-isovaleryl	213 ± 30	397 ± 141
19	N(Me)-Aib-(D)-Trp-(D)-N(Me)-gTrp-acetyl	25.6 ± 1.8	29.5 ± 16.0
29	$N(Me)_2$-Aib-(D)-Trp-(D)-gTrp-acetyl	20.1 ± 1.5	440 ± 80
57	H-Aib-(D)-Trp-(D)-gTrp-C(O)$NHCH_2CH_3$	16.9 ± 2.8	162 ± 20
58	H-Aib-(D)-Trp-(D)-gTrp-C(O)OCH_2CH_3	16.2 ± 1.4	122 ± 1

have K_d values of about 10^{-10} M in various tissues and interact with few sites (about 10 fmol/mg of protein).[24] On the other hand, ^{125}I-Tyr^4-ghrelin or ^{125}I-Tyr-Ala-hexarelin revealed a larger number of different binding sites in various tissues (B_{max} = 1000−2000 fmol/mg of protein) and had K_d values of about 10^{-9} M.[25,26] Use of a photoactivable hexarelin derivative allowed identification of a 57 kDa protein that is different from *h*GHS−R 1a in pituitary membranes.[27] In this context, discrepancies observed for the binding affinities of our compounds with the cloned *h*GHS−R 1a and with the human pituitary GHS receptors also suggest the presence of receptor subtypes as already mentioned.[28−32] Actually, it is not known which receptor(s) is (are) involved in stimulation of GH release. It has been clearly shown that MK-0677, ghrelin, and JMV 1843 were able to elicit in vivo stimulation of GH release, but their specific target has still not been clearly identified.

Conclusion

We have synthesized a new class of GH secretagogues based on a gem-diaminotryptophan residue. Among these compounds, **7**, **17**, **19**, **28**, **51**, and **57** showed in vivo potencies higher than that of hexarelin in the rat (sc injection). Only two compounds (**7** and **28**) were found to be active per os in beagle dogs at 1 mg/kg. Compound **7** was found to exhibit high oral activity and specificity in human volunteers,[1] and additional clinical studies are in progress.

Experimental Section

1. In Vivo Experiments in the Rat. 1.1. Animals. Male 10-day-old Sprague−Dawley rats weighing about 25 g (Charles River, Calco, Italy) were used. Rats pups were received on the fifth day after birth and were housed in our facilities under controlled conditions (22 ± 2 °C, 65% humidity, and artificial light from 06.00 to 20.00 h). A standard dry diet and water were available ad libitum to the dams. One hour before the experiments, pups were separated from their respective dams and were divided randomly into groups of eight each. All the experiments were performed in accordance with the Italian Guidelines for the Use of Animals in Medical Research.

1.2. Methods. Pups were acutely challenged with solvent (DMSO, final dilution 1:300), hexarelin, or new peptides (300 μg/kg, sc) and killed by decapitation 15 min later. Trunk blood was collected and centrifuged immediately. Plasma samples were stored at −20 °C until assayed for the determination of plasma GH concentrations.

1.3. Growth Hormone Assay. GH concentrations in plasma were measured by radioimmunoassay (RIA) using materials kindly provided by the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health. Values are expressed in terms of NIDDK-rat-GHRP-2 standard (potency 2 IU/mg) as ng/mL plasma. The minimum detectable value of rat GH was about 1.0 ng/mL, and intra-assay variability was about 6%.

2. In Vivo Experiments in the Dog. 2.1. Animals. Experiments were performed in beagle dogs, a species that behaves like humans for many aspects of GH regulation and presents a GH secretory profile similar to that observed in humans.

Four to six young (age, 2−3 years; weight, 11−15 kg; male and female) well-trained beagle dogs were used in this study. Animals were fed normal dry food (Diete Standar, Charles River, Calco, Italy) once a day at 16.00 h with water available ad libitum. Animals were on a 12-h light and 12-h dark regimen, with lights on at 07.00 h. Before the study, the body weights of all dogs were stable and they had no observable disease. All the experimental procedures were in accordance with the protocol previously authorized by the Committee on Animal Care and Use of the University of Milan.

2.2. Acute Compounds Testing. After one baseline blood sample (−20 min) had been taken from the cephalic vein, dogs were administered, through an oral-gastric cannula in a randomized sequence, hexarelin or other compounds (1 mg/kg, per os) at time 0 and then samples were drawn at 15, 30, 60, 90, 120, and 180 min. All blood samples were collected in chilled tubes containing 1.2 mg of EDTA/mL of blood (Sigma Tau, Milan, Italy) and centrifuged. The plasma was then frozen at −20 °C until assayed.

2.3. GH RIA. Highly purified canine GH, used for iodination and as a standard, and monkey anticanine GH antiserum were obtained through the courtesy of Dr. A. F. Parlow (Pituitary Hormones and Antisera Center, Torrance, CA). The sensitivity of the assay was 0.4 ng/mL. The intra-assay coefficients of variation were 3.8% and 4.1% at concentrations of 12.5 and 3.1 ng/mL, respectively. To avoid possible interassay variation, all samples of a given experiment were assayed in a single RIA.

2.4. Statistical Analysis. GH was evaluated either in absolute mean values (ng/mL) ± SEM or as area under the GH response curve (AUC_{0-180} min; ng/mL × h) calculated by the trapezoid method. Since no difference in analytical levels between male and female dogs were observed, data were pooled.

3. In Vitro Determination of the Affinities on Human Pituitary Membranes. 3.1. Tissue Preparation. Pituitary glands were obtained at autopsy from seven subjects (four males and three females ranging from 39 to 64 years, median age 52 years) who died of trauma or neoplams and were submitted to autopsy for diagnostic purposes in the Department of Pathology, University of Turin. Tissue was removed 24−28 h after death with the approval of our hospital Ethical Committee and immediately stored at −35 °C for 1−2 months until processed for membrane preparation and binding studies. At histopathological examination, pituitary glands were all preserved from both an architectural and cytological point of view.

3.2. Binding Studies. GHS binding assay with tissue membranes (30 000 g fraction) was performed as previously described[25] using ^{125}I-Tyr^4-human ghrelin as the radioligand. ^{125}I-Tyr^4-ghrelin (specific activity: 2000 Ci/mmol) was radioiodinated as previously described[33] and proved to be a reliable probe for labeling ghrelin receptors in human hypothalamus and pituitary gland.[33,34] Pituitary membranes (corresponding to 100 μg of membrane protein) were incubated in triplicate at 20 °C for 120 min with constant shaking with approximately 0.5 nmol/L ^{125}I-Tyr^4-ghrelin in a final volume of 0.5 mL of assay buffer (50 mmol/L Tris, 2.5 mmol/L EGTA, 0.003% bacitracin, and 0.1% BSA, titrated with HCl to pH 7.4) in the absence and in the presence of increasing concentrations (from 1 nmol/L to 10 μmol/L) of various unlabeled competitors. Parallel incubations where 2 μmol/L unlabeled human ghrelin was also present were used to determine nonspecific binding, which was subtracted from total binding to yield specific binding values. In all assays, the binding reaction was terminated by adding ice-cold assay buffer followed by filtration through Whatman GF/B filters. The radioactivity remaining bound to the filters was measured by a Packard γ counter. The concentration of competitor required to inhibit radiotracer binding by 50% (IC_{50}) was calculated by iterative nonlinear curve-fitting with the Prism 3 program (GraphPad Software, Inc., San Diego, CA). Values are espressed as mean ± SE of three to four separate experiments.

4. In Vitro Determination of the Affinities on the Cloned Human GHS−R 1a. 4.1. Transient Transfection of LLC PK-1 Cells and Membrane Preparation. LLC PK-1 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS (v/v), glutamine (2 mM), and antibiotics (50 units/mL penicillin and 50 μg/mL streptomycin). The growth of cells was performed in a humidified incubator at 37 °C under an atmosphere of 5% CO_2 in air.

LLC PK-1 cells were transiently transfected using the electroporation method (Easyject Optima apparatus, Equibio). To this aim, 10×10^6 LLC PK-1 cells were washed twice in serum-free growing medium by centrifugation (50 g for 10 min) and transiently transfected with 0.5 μg of the pcDNA3 vector containing the cDNA for the human GHS−R 1a using the Optimix Kit according to the manufacturer's protocol (Equibio). Electroporation was carried out at room temperature according to the following parameters: single pulse, 250 V, 1500 μF, and infinite internal resistance (under these conditions, the pulse time was 22 ms). Transfected cells were immediately withdrawn and plated in 10 cm culture dishes containing complete growth medium without phenol red. After an overnight incubation, cells were washed with 10 mL of the same culture medium. Approximately 48 h post-transfection, cells were washed three times with phosphate-buffered saline, pH 6.95, and once with 10 mL of homogeneisation buffer (HB) containing 50 mM Tris (pH 7.3), 5 mM $MgCl_2$, 2.5 mM EDTA, and 30 μg/mL bacitracin, scrapped from the plate in 500 μL of HB, and frozen in liquid N_2. After thawing at 37 °C, cells were subjected to another cycle of freeze−thawing and then centrifuged at 10000*g* for 20 min at 4 °C. The membrane pellet was resuspended in a minimal volume of HB, aliquoted, and stored at −80 °C until use. Membrane protein concentrations were determined by the Bradford method using the Bio-Rad protein assay kit.

4.2. Receptor Binding Studies. Isolated plasma membranes from LLC PK-1 cells (10 μg of protein) were incubated in HB for 60 min at 25 °C (steady-state conditions) with 60 pM ^{125}I−His^9-ghrelin (Amersham) in the presence or absence of competing compounds. Nonspecific binding was defined using an excess (1 μM) of ghrelin. The binding reaction was stopped by addition of 4 mL of ice-cold HB followed by rapid filtration over Whatman GF/C filters presoaked with 0.5% polyethyleneimine to prevent excessive binding of radioligand to the filters. Filters were rinsed three times with 3 mL of ice-cold wash buffer (50 mM Tris (pH 7.3), 10 mM $MgCl_2$, 2.5 mM EDTA, and 0.015% (w/v) X-100 Triton), and the radioactivity bound to membranes was measured in a γ counter.

5. Chemistry. Ascending TLC was performed on precoated plates of silica gel 60 F_{254} (Merck). Peptide derivatives were located with charring reagent or ninhydrine. Column chromatography was performed with silica gel Kieselguhr Merck G, 0.05−0.2 mm. HPLC purifications were run on a Waters 4000 preparative apparatus on a C18 Deltapak column (100 mm × 40 mm, 15 μm, 100 Å), with UV detection at 214 nm, at a flow rate of 50 mL/min of a mixture of solutions A (water with 0.1% TFA) and B (acetonitrile with 0.1% TFA in gradient mode). Analytical HPLC chromatograms were performed on a Beckman Gold apparatus composed of the 126 solvent module, the 168 detector, and the 32 Karat software. Runs were performed on a Deltapak Waters C18 column (150 mm × 3.9 mm, 5 μm) at a flow rate of 1 mL/min from solution A to solution B in a 50 min gradient (conditions A) or on a Symmetry Shield C18 column (50 mm × 4.6 mm, 3.5 μm) at a flow rate of 1 mL/min from solution A to solution B in a 15 min gradient (conditions B). Mass spectrum analyses were recorded on a Platform II (Micromass, Manchester, U.K.) quadrupole mass spectrometer fitted with an electrospray interface. The (L)- and (D)-amino acids and derivatives were from Senn Chemicals, Neosystem, and Advanced Chemtech. All reagents were of analytical grade.

The following abbreviations were used: BOP (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; DME, ethylene glycol dimethyl ether; DMF, dimethylformamide; NMM, *N*-methylmorpholine; BTIB, bis(trifluoroacetoxy)-iodobenzene; 2,4,5-TCP, 2,4,5-trichlorophenylformate; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate. Other abbreviations used were those recommended by IUPAC-IUB Commission (*Eur. J. Biochem.* **1984**, *138*, 9−37).

All final compounds were purified by reversed-phase HPLC. The purity was assessed by analytical reversed-phase C18 HPLC, and the structure was confirmed by MS (electrospray) and ^{1}H NMR. In some compounds, the presence of two isomers could be detected by ^{1}H NMR (for example, because of the presence of *cis*- and *trans*-formylamide conformations). In the NMR description, these isomers were called A and B. Compounds (D)- and (L)-Mrp, EP-51389, hexarelin, Tyr-Ala-hexarelin, and MK-0677 were supplied by Europeptides (Argenteuil, France).

Boc-N(Me)-Aib-OH. Boc-Aib-OH (5 g, 24.6 mmol) was dissolved in THF (200 mL). Then, NaH (1.8 g, 78 mmol) and ICH_3 (12.3 mL, 203 mmol) were successively added at 0 °C. After 24 h, the solvent was removed in vacuo. The mixture was diluted in ether (150 mL) and water (150 mL). The aqueous layer was acidified at pH 1. Then the residue was dissolved in ethyl acetate (100 mL) and washed with aqueous sodium thiosulfate (200 mL, 1 M), aqueous potassium hydrogen sulfate (200 mL, 1 M), and saturated aqueous sodium chloride (200 mL). The organic layer was dried over sodium sulfate and filtered and the solvent removed in vacuo to afford Boc-N(Me)-Aib-OH as a white foam in 81% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.45 (s, 9H, Boc), 1.48 (s, 6H, Aib), 2.93 (s, 3H, NMe). MS (ES), *m/z*: 118.45 [M + H − Boc]$^+$, 162.20 [M

+ H − isobutene]$^+$, 218.27 [M + H]$^+$, 240.24 [M + Na]$^+$, 435.05 [2M + H]$^+$, 457.15 [2M + Na]$^+$, 673.75 [3M + H]$^+$.

Boc-N(Me)-Aib-OBzl. Boc-N(Me)-Aib-OH (1 g, 4.6 mmol) was dissolved in acetonitrile (50 mL). Then, DBU (690 μL, 4.63 mmol) and BzlBr (521 μL, 4.4 mmol) were successively added to 60 °C. After 30 min, the solvent was removed in vacuo. The residue was dissolved in ethyl acetate (100 mL) and washed with saturated aqueous sodium hydrogen carbonate (200 mL), aqueous potassium hydrogen sulfate (200 mL, 1 M), and saturated aqueous sodium chloride (200 mL). The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo to afford Boc-N(Me)-Aib-OBzl as an oil in 85% yield. R_f = 0.35 (ethyl acetate/hexane,1:9). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.43 (s, 9H, Boc), 1.47 (s, 6H, Aib), 2.93 (s, 3H, NMe), 5.16 (s, 2H, CH_2 (Bzl)), 7.38 (m, 5H, Bzl). MS (ES), *m*/*z*: 208.16 [M + H − Boc]$^+$, 252.12 [M + H − isobutene]$^+$, 308.33 [M + H]$^+$, 330.27 [M + Na]$^+$, 637.42 [2M + Na]$^+$.

N(Me)-Aib-OBzl. Boc-N(Me)-Aib-OBzl (1.2 g, 3.9 mmol) was dissolved in trifluoroacetic acid (20 mL) for 30 min at 0 °C. The solvent were removed in vacuo to give TFA, N(Me)-Aib-OBzl as a white foam (yield: 98%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.64 (s, 6H, Aib), 2.69 (s, 3H, NMe), 5.26 (s, 2H, CH_2 (Bzl)), 7.39 (m, 5H, Bzl). MS (ES), *m*/*z*: 208.10 [M + H]$^+$.

***N,N*-(diMe)-Aib-OBzl.** TFA, N(Me)-Aib-OBzl (1.47 g, 4.6 mmol) was dissolved in water (1.2 mL). Then, formic acid (868 μL, 23 mmol) and 37% formaldehyde (1.21 mL, 16.1 mmol) were successively added, and the mixture was placed under reflux. After 24 h, the mixture was diluted in ether (5 mL) and water (5 mL). The aqueous layer was neutralized at pH 7. Then the residue was dissolved in ethyl acetate (10 mL) and washed with saturated aqueous sodium chloride (20 mL). The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo to afford *N*,*N*-(diMe)-Aib-OBzl as an oil. R_f = 0.41 (ethyl acetate/pyridine/acetic acid/water, 40:20:5:10). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.34 (s, 6H, Aib), 2.31 and 2.32 (2s, 6H, 2Me), 5.21 (s, 2H, CH_2 (Bzl)), 7.39 (m, 5H, Bzl). MS (ES), *m*/*z*: 222.34 [M + H]$^+$.

***N,N*-(diMe)-Aib-OH.** *N*,*N*-(diMe)-Aib-OBzl (4.6 mmol) was dissolved in ethanol (100 mL), then palladium on activated charcoal (10%) (0.1 equiv) was added to the stirred mixture. The solution was bubbled under hydrogen for 24 h. When the reaction went to completion, the palladium was filtered on Celite. The solvent was removed in vacuo. *N*,*N*-(diMe)-Aib-OH was precipitated in a solution of ether/hexane as a white foam: 41% yield starting from TFA, N(Me)-Aib-OBzl. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.42 (s, 6H, Aib), 2.7 (s, 6H, 2Me). MS (ES), *m*/*z*: 132.44 [M + H]$^+$.

***Z*-(D)-Trp-NH_2 (2).** *Z*-(D)-Trp-OH, **1** (8.9 g, 26 mmol), was dissolveld in DME (25 mL) and stored in an ice/water bath at 0 °C. NMM (3.5 mL, 31 mmol), IBCF (4.1 mL, 31 mmol), and an ammonia solution 28% (8.9 mL, 130 mmol) were successively added. The mixture was diluted with water (100 mL), and compound **2** was precipitated. It was filtered and dried in vacuo to afford 8.58 g (98%) of a white foam. R_f = 0.46 (chloroform/methanol/acetic acid, 180:10:5). ^{1}H NMR (250 MHz, DMSO-d_6): δ 2.9 (dd, 1H, H_β, $J_{\beta\beta'}$ = 14.5 Hz, $J_{\beta\alpha}$ = 9.8 Hz), 3.1 (dd, 1H, $H_{\beta'}$, $J_{\beta'\beta}$ = 14.5 Hz, $J_{\beta'\alpha}$ = 4.3 Hz), 4.2 (sextuplet, 1H, H_α), 4.95 (s, 2H, CH_2 (*Z*)), 6.9−7.4 (m, 11H), 7.5 (s, 1H, H^2), 7.65 (d, 1H, *J* = 7.7 Hz), 10.8 (s, 1H, N^1H). MS (ES), *m*/*z*: 338 [M + H]$^+$, 360 [M + Na]$^+$, 675 [2M + H]$^+$, 697 [2M + Na]$^+$.

Boc-(D)-Trp-(D)-Trp-NH_2 (3). 2 (3 g, 8.9 mmol) was dissolved in DMF (100 mL). HCl, 36% (845 μL, 9.8 mmol), water (2 mL), and palladium on activated charcoal (10%) (95 mg) were added to the stirred mixture. The solution was bubbled under hydrogen for 24 h. When the reaction went to completion, the palladium was filtered on Celite. The solvent was removed in vacuo to afford HCl, H-(D)-Trp-NH_2 as a colorless oil. In 10 mL of DMF, HCl, H-(D)-Trp-NH_2 (8.9 mmol), Boc-(D)-Trp-OH (2.98 g, 9.8 mmol), NMM (2.26 mL, 20.3 mmol), and BOP (4.33 g, 9. 8 mmol) were successively added. After 1 h, the mixture was diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium hydrogen carbonate (200 mL), aqueous potassium hydrogen sulfate (200 mL, 1 M), and saturated aqueous sodium chloride (100 mL). The organic layer was dried over sodium sulfate and filtered and the solvent was removed in vacuo to afford 4.35 g (85%) of **3** as a white foam. R_f = 0.48 (chloroform/methanol/acetic acid, 85:10:5). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.28 (s, 9H, Boc), 2.75−3.36 (m, 4H, 2 $(CH_2)_\beta$), 4.14 (m, 1H, CH_α), 4.52 (m, 1H, CH_α), 6.83−7.84 (m, 14H, 2 indoles (10H), NH_2, NH (urethane), and NH (amide)), 10.82 (d, 1H, *J* = 2 Hz, N^1H), 10.85 (d, 1H, *J* = 2 Hz, N^1H). MS (ES), *m*/*z*: 490 [M + H]$^+$, 512 [M + Na]$^+$, 979 [2M + H]$^+$.

Boc-(D)-(N^iBoc)-Trp-(D)-(N^iBoc)-Trp-NH_2 (4). 3 (3 g, 6.13 mmol) was dissolved in acetonitrile (25 mL). To this solution, di-*tert*-butyl dicarbonate (3.4 g, 15.3 mmol) and 4-(dimethylamino)pyridine (150 mg, 1.2 mmol) were successively added. After 1 h, the mixture was diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium hydrogen carbonate (200 mL), aqueous potassium hydrogen sulfate (200 mL, 1 M), and saturated aqueous sodium chloride (200 mL). The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel, eluting with ethyl acetate/hexane, 5:5, to afford 2.53 g (60%) of **4** as a white foam. R_f = 0.23 (ethyl acetate/hexane, 5:5). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.25 (s, 9H, Boc), 1.58 (s, 9H, Boc), 1.61 (s, 9H, Boc), 2.75−3.4 (m, 4H, 2 $(CH_2)_\beta$), 4.2 (m, 1H, CH_α), 4.6 (m, 1H, CH_α), 7.06−8 (m, 14H, 2 indoles (10H), NH (urethane), NH and NH_2 (amides)). MS (ES), *m*/*z*: 690 [M + H]$^+$, 712 [M + Na]$^+$, 1379 [2M + H]$^+$, 1401 [2M + Na]$^+$.

Boc-(D)-(N^iBoc)-Trp-(D)-(N^iBoc)-gTrp-H (4′). 4 (3 g, 4.3 mmol) was dissolved in a mixture of DMF/water (18 mL:7 mL). Then, pyridine (772 μL, 9.5 mmol) and bis(trifluoroacetoxy)-iodobenzene (2.1 g, 4.7 mmol) were added. After 1 h, the mixture was diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium hydrogen carbonate (200 mL), aqueous potassium hydrogen sulfate (200 mL, 1 M), and aqueous saturated sodium chloride (200 mL). The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo. **4′** was used immediately in the acylation reaction. R_f = 0.14 (ethyl acetate/hexane, 7:3). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.29 (s, 9H, Boc), 1.61 (s, 18H, 2 Boc), 2.13 (s, 2H, NH_2 (amine)), 3.1−2.8 (m, 4H, 2 $(CH_2)_\beta$), 4.2 (m, 1H, CH_α), 4.85 (m, 1H, CH_α), 6.9−8 (m, 12H, 2 indoles (10H), NH (urethane), NH (amide)). MS (ES), *m*/*z*: 662 [M + H]$^+$, 684 [M + Na]$^+$.

Boc-(D)-(N^iBoc)-Trp-(D)-(N^iBoc)-gTrp-CHO (5). 4′ (2.84 g, 4.3 mmol) was dissolved in DMF (20 mL). Then, *N*,*N*-diisopropylethylamine (815 μL, 4.7 mmol) and 2,4,5-trichlorophenylformate (1.08 g, 4.7 mmol) were added. After 30 min, the mixture was diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium hydrogen carbonate (200 mL), aqueous potassium hydrogen sulfate (200 mL, 1 M), and saturated aqueous sodium chloride (200 mL). The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel, eluting with ethyl acetate/hexane (5:5) to afford 2.07 g of **5** (70% for both steps) as a white foam. R_f = 0.27 (ethyl acetate/hexane, 5:5). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.28 (s, 9H, Boc), 1.6 (s, 9H, Boc), 1.61 (s, 9H, Boc), 2.75−3.1 (m, 4H, 2 $(CH_2)_\beta$), 4.25 (m, 1H, $(CH)\alpha_A$ and $(CH)\alpha_B$), 5.39 (m, 0.4H, $(CH)\alpha'_B$), 5.72 (m, 0.6H, $(CH)\alpha'_A$), 6.95−8.55 (m, 14H, 2 indoles (10H), NH (urethane), 2 NH (amides), CHO (formyl)). MS (ES), *m*/*z*: 690 [M + H]$^+$, 712 [M + Na]$^+$, 1379 [2M + H]$^+$.

Boc-Aib-(D)-Trp-(D)-gTrp-CHO (6). 5 (1.98 g, 2.9 mmol) was dissolved in a mixture of trifluoroacetic acid (16 mL), anisole (2 mL), and thioanisole (2 mL) for 30 min at 0 °C. The solvents were removed in vacuo, the residue was stirred in ether, and the precipitated TFA salt was filtered. TFA, H-(D)-Trp-(D)-gTrp-CHO (2.9 mmol), Boc-Aib-OH (700 mg, 2.9 mmol), NMM (2.4 mL, 12.2 mmol), and BOP (1.53 g, 3.5 mmol) were successively added in 10 mL of DMF. After 1 h, the mixture was diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium hydrogen carbonate (200 mL),

aqueous potassium hydrogen sulfate (200 mL, 1 M), and saturated aqueous sodium chloride (200 mL). The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel, eluting with ethyl acetate to afford 1.16 g of **6** as a white foam (70%). $R_f = 0.26$ (chloroform/methanol/acetic acid, 180:10:5). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.21 (s, 6H, 2 CH_3(Aib)), 1.31 (s, 9H, Boc), 2.98−3.12 (m, 4H, 2 $(CH_2)_\beta$), 4.47 (m, 1H, $(CH)\alpha_A$ and $(CH)\alpha_B$), 5.2 (m, 0.4H, $(CH)\alpha'_B$), 5.7 (m, 0.6H, $(CH)\alpha'_A$), 6.95−8.37 (m, 15H, 2 indoles (10H), 3 NH (amides), 1 NH (urethane), CHO (formyl)), 10.89 (m, 2H, 2 N^1H (indoles)). MS (ES) *m/z* 575 $[M + H]^+$, 597 $[M + Na]^+$, 1149 $[2M + H]^+$, 1171 $[2M + Na]^+$.

TFA, H-Aib-(D)-Trp-(D)-gTrp-CHO (7). 6 (1 g, 1.7 mmol) was dissolved in a mixture of trifluoroacetic acid (8 mL), anisole (1 mL), and thioanisole (1 mL) for 30 min at 0 °C. The solvents were removed in vacuo, the residue was stirred in ether, and the precipitated TFA, H-Aib-(D)-Trp-(D)-gTrp-CHO was filtered. **7** was purified by preparative HPLC and obtained in 52% yield. ^{1}H NMR (400 MHz, DMSO-d_6) + correlation ^{1}H−^{1}H: δ 1.21 (s, 3H, CH_3 (Aib)), 1.43 (s, 3H, CH_3 (Aib)), 2.97 (m, 2H, $(CH_2)_\beta$), 3.1 (m, 2H, $(CH_2)_{\beta'}$), 4.62 (m, 1H, $(CH)\alpha_A$ and $(CH)\alpha_B$), 5.32 (q, 0.4H, $(CH)\alpha'_B$), 5.71 (q, 0.6H, $(CH)\alpha'_A$), 7.3 (m, 4H, H_5 and H_6 (2 indoles)), 7.06−7.2 (4d, 2H, H_{2A} and H_{2B} (2 indoles)), 7.3 (m, 2H, H_4 or H_7 (2 indoles)), 7.6−7.8 (4d, 2H, H_{4A} and H_{4B} or H_{7A} and H_{7B}), 7.97 (s, 3H, NH_2 (Aib) and C*H*O (formyl)), 8.2 (d, 0.4H, NH_{1B} (diamino)), 8.3 (m,1H, NH_A and NH_B), 8.5 (d, 0.6H, NH_{1A} (diamino)), 8.69 (d, 0.6H, NH_{2A} (diamino)), 8.96 (d, 0.4H, NH_{2B} (diamino)), 10.8 (s, 0.6H, N^1H_{1A} (indole)), 10.82 (s, 0.4H, N^1H_{1B} (indole)), 10.86 (s, 0.6H, N^1H_{2A} (indole)), 10.91 (s, 0,4H, N^1H_{2B} (indole)). MS (ES), *m/z*: 475 $[M + H]^+$, 949 $[2M + H]^+$. HPLC t_R: 16.26 min (conditions A).

N(Me)-(D)-Trp-NH_2 (9). H-(D)-Trp-NH_2 (2.49 g, 12.3 mmol) was dissolved in DMF (25 mL). Et_3N (2.6 mL, 18.84 mmol) and *o*-NBSCl (2.85 g, 12.9 mmol) were successively added. After 15 min, the mixture was diluted with ethyl acetate (300 mL) and washed with saturated aqueous sodium hydrogen carbonate, aqueous potassium hydrogen sulfate (1 M), and saturated aqueous sodium chloride. The organic layer was dried over sodium sulfate and filtered and the solvent was removed in vacuo to afford 4.30 g of *o*-NBS-(D)-Trp-NH_2 as an orange foam. An amount of 4.26 g of *o*-NBS-(D)-Trp-NH_2 was dissolved in DMF (25 mL). K_2CO_3 (2.20 g, 15.9 mmol) and MeI (1.53 mL, 24.5 mmol) were successively added. After 1 h, the mixture was diluted with ethyl acetate (300 mL) and washed with saturated aqueous sodium hydrogen carbonate, aqueous potassium hydrogen sulfate (1 M), and saturated aqueous sodium chloride. The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo to afford 4.10 g of *o*-NBS-N(Me)-(D)-Trp-NH_2 as a yellow foam. An amount of 4.10 g of *o*-NBS-N(Me)-(D)-Trp-NH_2 was dissolved in DMF (30 mL). K_2CO_3 (3.39 g, 24.5 mmol) and PhSH (1.51 mL, 14.7 mmol) were successively added. After 1 h, the mixture was diluted with ethyl acetate (300 mL) and washed with aqueous potassium hydrogen sulfate and the organic layer was discarded. The aqueous layer was then treated with NaOH pellets until pH 10 was attained and washed with 300 mL of ethyl acetate. The organic layer was washed with saturated aqueous sodium chloride, dried over sodium sulfate, and filtered and the solvent was removed in vacuo to afford 2.24 g (10.31 mmol, 84% over three steps) of N(Me)-(D)-Trp-NH_2 as a colorless oil. $R_f = 0.19$ (ethyl acetate/pyridine/acetic acid/water, 80:20:5:10). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.15 (s, 3H, $N(CH_3)$), 2.75 (dd, 1H, $H_{\beta'}$, $J_{\beta'\beta} = 14.7$ Hz, $J_{\beta'\alpha} = 7.8$ Hz), 2.95 (dd, 1H, H_β, $J_{\beta\beta'} = 14.4$ Hz, $J_{\beta\alpha} = 5.7$ Hz), 3.08 (dd, 1H, H_α, $J_{\alpha\beta'} = 14.4$ Hz, $J_{\alpha\beta} = 5.7$ Hz), 6.90−6.98 (m, 2H, 1H indole and 1H amide), 7,02 (t, 1H, $J = 7.2$ Hz, 1H indole), 7.10 (s, 1H, H indole), 7.22 (s, 1H, 1 NH amide), 7.25 (d, 1H, $J = 8.0$ Hz, H indole), 7.54 (d, 1H, $J = 7.8$ Hz, 1H indole), 10.75 (s, 1H, N^1H). MS (ES), *m/z*: 218.1 $[M + H]^+$.

Boc-(D)-(N^iBoc)-Trp-N(Me)-(D)-(N^iBoc)-Trp-NH_2 (10). N(Me)-(D)-Trp-NH_2 (2.23 g, 10.3 mmol) and Boc-(D)-Trp-OH (3.29 g, 10.8 mmol) were dissolved in 30 mL of DMF. NMM (2.84 mL, 25.8 mmol) and HBTU (3.91 g, 10.3 mmol) were successively added. After 1 h, the mixture was diluted with ethyl acetate and washed with saturated aqueous sodium hydrogen carbonate, aqueous potassium hydrogen sulfate (1 M), and saturated aqueous sodium chloride. The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel, eluting with ethyl acetate/hexane (9:1) to afford 2.92 g (5.80 mmol, 54%) of Boc-(D)-Trp-N(Me)-(D)-Trp-NH_2 as a white foam. Boc-(D)-Trp-N(Me)-(D)-Trp-NH_2 (2.81 g, 5.60 mmol) was dissolved in acetonitrile (25 mL). To this solution, di-*tert*-butyl dicarbonate (2.4 g, 11.2 mmol) and 4-(dimethylamino)pyridine (164 mg, 1.3 mmol) were successively added. After 3 h, the mixture was diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium hydrogen carbonate, aqueous potassium hydrogen sulfate, and saturated aqueous sodium chloride. The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel, eluting with ethyl acetate/hexane, 5:5, to afford 3.51 g (5.0 mmol, 89%) of **10** as a white foam. $R_f = 0.44$ (ethyl acetate/hexane, 7:3). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.15−1.70 (m, 27H, 3 × Boc), 2.60−3.40 (m, 7H, 2 $(CH_2)_\beta$ + N−CH_3), 4.46 (m, 1/2H, CH_α), 4.60 (m, 1/2H, CH_α), 5.00 (m, 1/2H, $CH_{\alpha'}$), 5.20 (m, 1/2H, $CH_{\alpha'}$), 6.80−8.08 (m, 13H, 2 indoles (10H), NH (urethane), 2 NH_2 (amides)). MS (ES), *m/z*: 648.4 $[M + H - 56]^+$, 704.3 $[M + H]^+$, 726.7 $[M + Na]^+$.

Boc-(D)-(N^iBoc)-Trp-N(Me)-(D)-(N^iBoc)-gTrp-C(O)CH_3 (13). Compound **13** was synthesized as described for compound **5** starting from **10** (500 mg, 0.71 mmol). The gem-diamino compound was acetylated with acetic anhydride (88 μL, 0.92 mmol) in the presence of *N*,*N*-diisopropylethylamine (158 μL, 0.92 mmol). The usual workup was performed and the residue was purified by flash chromatography on silica gel, eluting with ethyl acetate/hexane, 5:5, to afford 340 mg (48%) of Boc-(D)-(N^iBoc)-Trp-N(Me)-(D)-(N^iBoc)-gTrp-C(O)CH_3 as a white solid. ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.20−1.71 (m, 27H, 3 × Boc), 1.79 (2s, 2.1H, acetyl), 1.81 (s, 0.9H, acetyl), 2.75−3.15 (m, 7H, 2 $(CH_2)_\beta$ + N−CH_3), 4.61 (m, 0.7H, CH_α), 5.05 (m, 0.3H, CH_α), 5,91 (m, 0.3H, $CH_{\alpha'}$), 6.21 (m, 0.7H, $CH_{\alpha'}$), 6.90−8.1 (m, 11H, 2 indoles (10H), 1NH (urethane), 8.41 (d, 0.7H, 1 NH (amide)), 8.85 (d, 0.3H, 1 NH (amide)). MS (ES), *m/z*: 717.8 $[M + H]^+$, 739.8 $[M + Na]^+$.

Boc-(D)-(N^iBoc)-Trp-(D)-(N^iBoc)-gTrp-*o*-NBS (14). 14 was synthesized starting from **4** (500 mg, 0.72 mmol) to generate **4′**. The gem-diamino dipeptide was used without purification and was reacted with *o*-NBSCl (290 mg, 1.08 mmol) in the presence of NEt_3 (354 μL, 2.5 mmol) in 10 mL of DCM for 1 h. The mixture was diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium hydrogen carbonate (100 mL), aqueous potassium hydrogen sulfate (100 mL, 1 M), and saturated aqueous sodium chloride (50 mL). The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel, eluting with ethyl acetate/hexane, 3:7, to afford 320 mg (52%) of **14** as a white solid. $R_f = 0.39$ (ethyl acetate/hexane, 3:7). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.27 (1s, 9H, Boc), 1.60 (1s, 18H, 2 × Boc), 2.73 (m, 2H, $CH_{2\beta}$), 3.03 (m, 2H, $CH_{2\beta'}$), 4.14 (m, 1H, CH_α), 5.44 (m, 1H, $CH_{\alpha'}$), 6.93 (d, 1H, NH urethane), 7.19−7.73 (m, 12H, arom), 7.91 (d, 1H, arom), 8.03 (d, 1H, arom), 8.55 (d, 1H, NH amide), 8.70 (d, 1H, NH amide). MS (ES), *m/z*: 589.07 $[M + H - (56 + NO_2 - C_6H_4 - SO_2NH_2)]^+$, 645.26 $[M + H - (NO_2 - C_6H_4 - SO_2NH_2)]^+$, 847.5 $[M + H]^+$, 869.7 $[M + Na]^+$.

Boc-(D)-(N^iBoc)-Trp-(D)-(N^iBoc)-gTrp-N(Me)-*o*-NBS (15). An amount of 310 mg (0.37 mmol) of **14** was dissolved in dry acetonitrile (2 mL), and the mixture was stored under an argon atmosphere. Both 202 mg (1.58 mmol) of potassium carbonate and 228 μL (3.7 mmol) of ICH_3 were added to the solution at room temperature. The reaction mixture was brought to 35 °C for 3 h and was monitored by TLC. After conversion was complete, the mixture was diltuted with DCM (10 mL) and water (10 mL). The aqueous layer was separated and extracted with DCM (3 × 5 mL), and the combined organic layers were washed with brine (10 mL), dried over sodium sulfate, and

concentrated to give a crude product that was purified by flash chromatography on silica gel, eluting with ethyl acetate/hexane, 3:7, to afford 200 mg (65%) of **15**. R_f = 0.41 (ethyl acetate/hexane, 3:7). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.25 (1s, 9H, Boc), 1.59 (1s, 18H, 2 × Boc), 2.60−2.78 (m, 2H, $CH_{2\beta}$), 2.86 (2s, 3H, N−Me), 3.00−3.29 (m, 2H, $CH_{2\beta'}$), 4.21 (m, 1H, CH_α), 6.12 (m, 1H, $CH_{\alpha'}$), 6.98 (2d, 1H, NH urethane), 7.15−8.10 (m, 14H, arom), 9.10 (2d, 1H, NH amide). MS (ES), *m*/*z*: 589.2 [M + H − (56 + NO_2 − C_6H_4 − SO_2NH − Me)]$^+$, 645.4 [M + H − (NO_2 − C_6H_4 − SO_2NH − Me)]$^+$, 861.0 [M + H]$^+$, 883.6 [M + Na]$^+$.

Boc-(D)-(N^{i}Boc)-Trp-(D)-(N^{i}Boc)-gTrp-N(Me)-C(O)CH$_3$ (16A and 16B). 15 (200 mg, 0.23 mmol) was dissolved in DMF (3 mL). K_2CO_3 (151 mg, 1.10 mmol) and PhSH (112 μL, 1.10 mmol) were successively added. After 4 h, the mixture was diluted with ethyl acetate (50 mL) and washed with aqueous potassium hydrogen sulfate (20 mL) and brine (20 mL). The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo. The residue was dissolved in DMF (3 mL). Acetic anhydride (140 μL, 1.48 mmol) and DIEA (370 μL, 2.15 mmol) were added, and the reaction was monitored by TLC. After completion, the mixture was diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium hydrogen carbonate (100 mL), aqueous potassium hydrogen sulfate (100 mL, 1 M), and saturated aqueous sodium chloride (50 mL). The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel, eluting with ethyl acetate/hexane, 5:5, to afford 80 mg (48% for two steps) of **16A** and **16B**, which were not separated at this step. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.15 (2s, 9H, Boc), 1.35 (2s, 9H, Boc), 1.46 (s, 9H, Boc), 1.85 (2s, 3H, acetyl), 2.50−2.80 (m, 5H, $CH_{2\beta}$ + N(Me)), 2.85−3.05 (m, 2H, $CH_{2\beta'}$), 4.13 (m, 1H, CH_α), 5.58 (m, 1H, $CH_{\alpha'}$), 6.80 (2d, 1H, NH urethane), 7.10−8.00 (m, 10H, arom), 8.40 (d, 1/2H, NH amide), 8.75 (d, 1/2H, NH amide). MS (ES), *m*/*z*: 718.5 [M + H]$^+$, 740.6 [M + Na]$^+$.

N(Me)-Aib-(D)-Trp-(D)-gTrp-N(Me)-C(O)CH$_3$ (17) and N-(Me)-Aib-(D)-Trp-(L)-gTrp-N(Me)-C(O)CH$_3$ (18). 16A and **16B** were deprotected as described for compound **7**, and the deprotected compounds were allowed to couple with Boc-N(Me)-Aib-OH as described for compound **25**. The final compounds were deprotected and after a classical workup were purified by preparative HPLC. Both compounds exhibited the same ^{1}H NMR and mass spectra, suggesting an epimerization during the synthesis. The D configuration was attributed to the active compound **17**. ^{1}H NMR (360 MHz, DMSO-d_6): δ 1.20 and 1.45 (2s, 6H, 2CH_3 (Aib)), 2.25 (s, 3H, N-Me(Aib)), 2.70 (s, 3H, N(Me), 2.80−3.25 (2m, 4H, $CH_{2\beta}$ + $CH_{2\beta'}$), 4.75 (m, 1H, CH_α), 5.71 (m, 1H, $CH_{\alpha'}$), 6.90−7.80 (m, 10H, arom), 8.38 (d, 1H, NH amide), 8.70 (m, 1H, NH amine), 9.10 (d, 1H, NH amide), 10.80 and 10.91 (2s, 2N^{1}H). MS (ES), *m*/*z*: 517.5 [M + H]$^+$, 539.0 [M + Na]$^+$. HPLC t_R: 6.79 min (conditions B). **18**, HPLC t_R: 7.20 min (conditions B).

N(Me)-Aib-(D)-Trp-N(Me)-(D)-gTrp-acetyl (19). 19 was synthesized from TFA, H-(D)-Trp-N(Me)-(D)-gTrp-acetyl as described for compound **25**. ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.32−1.53 (m, 6H, CH_3 (Aib)), 1.83 (m, 3H, C(O)CH_3), 2.32−2.4 (m, 3H, N(CH_3)), 2.6−3.2 (m, 7H, 2(CH_2)$_\beta$ and N(CH3)), 4.94 (m, 1H, (CH)$_\alpha$), 5.27 (m, 1H, (CH)$_\alpha$), 6.01 (m, 1H, (CH)$_{\alpha'}$), 6.26 (m, 1H, (CH)$_{\alpha'}$), 6.59−8.72 (m, 13H, 10H (2 indoles), 2NH (amides) and 1 NH amine), 10.64−10.94 (m, 2H, 2N^{1}H). MS (ES), *m*/*z*: 516.77 [M + H]$^+$. HPLC t_R: 11.59 min (conditions A).

TFA, H-Aib-(D)-Mrp-(D)-gMrp-CHO (23). ^{1}H NMR (400 MHz, DMSO-d_6) + correlation ^{1}H−^{1}H: δ 1.19 (s, 2H, $(CH_3)_{1A}$ (Aib)), 1.23 (s, 1H, $(CH_3)_{1B}$ (Aib)), 1.41 (s, 2H, $(CH_3)_{2A}$ (Aib)), 1.44 (s, 2H, $(CH_3)_{2B}$ (Aib)), 2.33−2.35 (4s, 6H, 2 CH_3 (indoles)), 2.93 (m, 2H, $(CH_2)_\beta$), 3.02 (m, 2H, $(CH_2)_{\beta'}$), 4.65 (m, 0.6H, (CH)α_A), 4.71 (m, 0.4H, (CH)α_B), 5.2 (m, 0.4H, (CH)α'_B), 5.6 (m, 0.6H, (CH)α'_A), 6.95 (m, 4H, H_5 and H_6 (2 indoles)), 7.19 (m, 2H, H_4 or H_7 (2 indoles)), 7.6 (m, 2H, H_4 or H_7 (2 indoles)), 7.9 (s, 1H, C*H*O (formyl)), 7.95 (s, 2H, NH_2 (Aib)), 8.05 (d, 0.4H, NH_{1B} (diamino)), 8.3 (m,1H, NH_A and NH_B), 8.35 (m, 0.6H, NH_{1A} (diamino)), 8.4 (d, 0.6H, NH_{2A} (diamino)), 8.75 (d, 0.4H, NH_{2B} (diamino)), 10.69 (s, 0.6H, N$^1H_{1A}$ (indole)), 10.71 (s, 0.4H, N$^1H_{1B}$ (indole)), 10.80 (s, 0.6H, N$^1H_{2A}$ (indole)), 10.92 (s, 0.4H, N$^1H_{2B}$ (indole)). MS (ES), *m*/*z*: 503.1 [M + H]$^+$. HPLC t_R: 21.08 min (conditions A).

N(Me)-Aib-(D)-Trp-(D)-gTrp-CHO (25). The titled compound was synthesized from compound **5** but with coupling of Boc-N(Me)-Aib-OH. After coupling and classical workup, it was purified by flash chromatography on silica gel, eluting with ethyl acetate/methanol (9:1) to afford 180 mg (53%) of Boc-N(Me)-Aib-(D)-Trp-(D)-gTrp-CHO as a white foam. This compound was deprotected as described earlier and precipitated in ether to yield TFA, N(Me)-Aib-(D)-Trp-(D)-gTrp-CHO (**25**), which was filtered. The product was purified by preparative HPLC (39 mg, 21%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.19 (s, 3H, CH_3 (Aib)), 1.42 (s, 3H, CH_3 (Aib)), 2.26 (s, 3H, NCH_3), 3.12 (m, 4H, 2 $(CH_2)_\beta$), 4.66 (m, 1H, (CH)$_\alpha$), 5.32 and 5.7 (m, 1H, (CH)$_\alpha$), 6.9−7.8 (m, 10H, 2 indoles), 8 (m, 1H, CHO (formyl)), 8.2−9 (m, 4H, 3 NH (amides) and NH (amine)), 10.87 (m, 2H, 2 N^{1}H (indoles)). MS (ES), *m*/*z*: 489.29 [M + H]$^+$. HPLC t_R: 17.09 min (conditions A).

N(Me)$_2$-Aib-(D)-Trp-(D)-gTrp-CHO (26). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.2 (s, 3H, CH_3 (Aib)), 1.39 (s, 3H, CH_3 (Aib)), 2.29 (m, 3H, NCH_3), 2.99−3.33 (m, 4H, 2 $(CH_2)_\beta$), 4.68 (m, 1H, (CH)$_\alpha$), 5.3 and 5.69 (m, 1H, (CH)$_{\alpha'}$), 6.97−7.72 (m, 10H, 2 indoles), 7.97 (2s, 1H, formyl), 8.2−9.47 (m, 3H, 3 NH (amides)), 10.85 (m, 2H, 2 NH (indoles)). MS (ES), *m*/*z*: 503.45 [M + H]$^+$. HPLC t_R: 6.93 min (conditions B).

H-Aib-(D)-Trp-(D)-gTrp-C(O)CH$_3$ (27). 27 was synthesized from **4′**, but the amine function was acetylated instead of formylated. The synthetic pathway after acetylation was the same as for compound **7**. Compound **27** was purified by preparative HPLC (80 mg, 31%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.22 (s, 3H, CH_3 (Aib)), 1.44 (s, 3H, CH_3 (Aib)), 1.8 (s, 3H, C(O)CH_3), 3.06 (m, 4H, 2 $(CH_2)_\beta$), 4.6 (m, 1H, (CH)$_\alpha$), 5.6 (m, 1H, (CH)$_\alpha$), 6.9−7.8 (m, 10H, 2 indoles), 7.99 (s, 2H, NH_2 (Aib)), 8.2−8.6 (m, 3H, 3 NH (amides)), 10.83 (s, 2H, 2 N^{1}H (indoles)). MS (ES), *m*/*z*: 489.32 [M + H]$^+$. HPLC t_R: 16.76 min (conditions A).

N(Me)-Aib-(D)-Trp-(D)-gTrp-C(O)CH$_3$ (28). 28 was synthesized as the previous compound but by coupling Boc-N(Me)-Aib-OH instead of Boc-Aib-OH on the dipeptide. Compound **28** was purified by preparative HPLC (220 mg, 40%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.17 (s, 3H, CH_3 (Aib)), 1.4 (s, 3H, CH_3 (Aib)), 1.78 (s, 3H, C(O)CH_3), 2.23 (s, 3H, NCH_3), 3.15 (m, 4H, 2 $(CH_2)_\beta$), 4.7 (m, 1H, (CH)$_\alpha$), 5.55 (m, 1H, (CH)$_\alpha$), 6.9−7.9 (m, 10H, 2 indoles), 8.2−8.8 (s, 4H, NH (amine) and 3 NH (amides)), 10.8 (s, 2H, 2 N^{1}H (indoles)). MS (ES), *m*/*z*: 503.19 [M + H]$^+$. HPLC t_R: 16.78 min (conditions A).

N(Me)$_2$-Aib-(D)-Trp-(D)-gTrp-C(O)CH$_3$ (29). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.22 (s, 3H, CH_3 (Aib)), 1.4 (s, 3H, CH_3 (Aib)), 1.8 (s, 3H, acetyl), 2.28 (d, 3H, NCH_3), 2.96−3.22 (m, 4H, 2 $(CH_2)_\beta$), 4.7 (m, 1H, (CH)$_\alpha$), 5.60 (m, 1H, (CH)$_{\alpha'}$), 6.98−7.75 (m, 10H, 2 indoles), 8.2−9.47 (m, 3H, 3 NH (amides)), 10.84 (m, 2H, 2 NH (indoles)). MS (ES), *m*/*z*: 517.34 [M + H]$^+$. HPLC t_R: 7.07 min (conditions B).

Aib-(D)-Trp-N(Me)-(D)-gTrp-acetyl (30). 30 was synthesized from TFA, H-(D)-Trp-N(Me)-(D)-gTrp-acetyl as described for compound **7**. ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.35−1.53 (m, 6H, CH_3 (Aib)), 1.82 (m, 3H, C(O)CH_3), 2.6−3.2 (m, 7H, 2(CH_2)$_\beta$ and N(CH_3)), 4.94 (m, 1H, (CH)$_\alpha$), 5.27 (m, 1H, (CH)$_\alpha$), 6.01 (m, 1H, (CH)$_{\alpha'}$), 6.26 (m, 1H, (CH)$_{\alpha'}$), 6.59−8.64 (m, 14H, 10H (2 indoles), 2 NH (amides) and 2 NH_2 amine), 10.64−10.92 (m, 2H, 2 N^{1}H). MS (ES), *m*/*z*: 502.80 [M + H]$^+$, 1004.76 [2M + H]$^+$. HPLC t_R: 11.99 min (conditions A).

H-Aib-(*R*)-(Me)Trp-(D)-gTrp-CHO (31). (*R*)-(Me)-Trp was synthesized as reported,[18] and the titled compound was prepared following the procedure described for compound **7**. MS (ES), *m*/*z*: 489.37 [M + H]$^+$, 977.56 [2M + H]$^+$. HPLC t_R: 7.35 min (conditions B).

H-Aib-(D)-Trp-(*R*)-(Me)-gTrp-CHO (32). 32 was prepared following the procedure described for compound **7**. MS (ES), *m*/*z*: 489.24 [M + H]$^+$, 511.45 [M + Na]$^+$. HPLC t_R: 7.38 min (conditions B).

N(Me)-Aib-(D)-Trp-(*R*)-(Me)-gTrp-C(O)CH_3 (33). 33 was prepared following the procedure described for compound **28**. MS (ES), *m/z*: 517.34 $[M + H]^+$. HPLC t_R: 7.76 min (conditions B).

4-Aminopiperidine-4-carbonyl-(D)-Trp-(D)-gTrp-CHO (34). 34 was synthesized by coupling of Boc-(N^4Boc)Pip-OH on TFA, H-(D)-Trp-(D)-gTrp-CHO as described earlier. Compound **34** was purified by preparative HPLC (127 mg, 37%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.81 (m, 2H, CH_2 (Pip)), 2.30 (m, 2H, CH_2 (Pip)), 3.1 (m, 8H, 2 $(CH_2)_\beta$ and 2 CH_2 (Pip)), 4.68 (m, 1H, $(CH)_\alpha$), 5.30 and 5.73 (2m, 1H, $(CH)_\alpha$), 6.9−7.7 (m, 10H, 2 indoles), 7.98 (2s, 1H, CHO (formyl)), 8.2−9.2 (m, 6H, NH_2 and NH (Pip) and 3 NH (amides)), 10.9 (m, 2H, 2 N^1H (indoles)).MS (ES), *m/z*: 516.37 $[M + H]^+$, 538.27 $[M + Na]^+$. HPLC t_R: 13.78 min (conditions A).

4-Aminopiperidine-4-carbonyl-(D)-Trp-(D)-gTrp-C(O)CH_3 (35). 35 was purified by preparative HPLC (135 mg, 42%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.79 (m, 2H, CH_2 (Pip)), 1.81 (s, 3H, C(O)CH_3), 2.3 (m, 2H, CH_2 (Pip)), 3.1 (m, 8H, 2 $(CH_2)_\beta$ and 2 CH_2 (Pip)), 4.7 (m, 1H, $(CH)_\alpha$), 5.6 (m, 1H, $(CH)_{\alpha'}$), 6.9−7.8 (m, 10H, 2 indoles), 8.2−9 (m, 6H, NH_2 and NH (Pip) and 3 NH (amides)), 10.85 (m, 2H, 2 N^1H (indoles)). MS (ES), *m/z*: 530.39 $[M + H]^+$, 552.41 $[M + Na]^+$. HPLC t_R: 14.02 min (conditions A).

Isonipecotyl-(D)-Trp-(D)-gTrp-CHO (36). 36 was synthesized as described for compound **34** from TFA, H-(D)-Trp-(D)-gTrp-CHO (250 mg, 0.33 mmol) and Fmoc-isonipecotic-OH (144 mg, 0.32 mmol). The Fmoc group was removed in a mixture of DMF (8 mL) and piperidine (2 mL) in 30 min. The solvents were removed in vacuo, the residue was stirred in ether, and the precipitated isonipecotyl-(D)-Trp-(D)-gTrp-CHO was recovered by filtration. Compound **36** was purified by preparative HPLC (81 mg, 33%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.65 (m, 4H, 2 CH_2 (Iso)), 2.4 (m, 1H, CH (Iso)), 2.7−3.3 (m, 8H, 2 $(CH_2)_\beta$ and 2 CH_2 (Iso)), 4.6 (m, 1H, $(CH)_\alpha$), 5.3 and 5.7 (2m, 1H, $(CH)_\alpha$), 6.9−7.7 (m, 10H, 2 indoles), 7.97 (2s, 1H, CHO (formyl)), 8−8.8 (m, 4H, NH (Iso) and 3 NH (amides)), 10.9 (m, 2H, 2 N^1H (indoles). MS (ES), *m/z*: 501.36 $[M + H]^+$. HPLC t_R: 15.34 min (conditions A).

Isonipecotyl-(D)-Trp-(D)-gTrp-C(O)CH_3 (37). 37 was synthesized as described for compound **35** from TFA, H-(D)-Trp-(D)-gTrp-C(O)CH_3 (250 mg, 0.33 mmol) and Fmoc-isonipecotic-OH (141 mg, 0.4 mmol). The Fmoc group was removed as described for compound **36**. The solvents were removed in vacuo, the residue was stirred in ether, and the precipitated isonipecotyl-(D)-Trp-(D)-gTrp-C(O)CH_3 was filtered. Compound **37** was purified by preparative HPLC (65 mg, 26%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.66 (m, 4H, 2 CH_2 (Iso)), 1.79 (s, 3H, C(O)CH_3), 2.7−3.3 (m, 8H, 2 $(CH_2)_\beta$ and 2 CH_2 (Iso)), 4.54 (m, 1H, $(CH)_\alpha$), 5.59 (m, 1H, $(CH)_\alpha$), 6.9−7.7 (m, 10H, 2 indoles), 8−8.6 (m, 4H, NH (Iso) and 3 NH (amides)), 10.82 (m, 2H, 2 N^1H (indoles)). MS (ES), *m/z*: 515.44 $[M + H]^+$. HPLC t_R: 15.32 min (conditions A).

Ph-SO_2-(D)-Trp-(D)-gTrp-CHO (38). 38 was synthesized as described for compound **7** starting from TFA, H-(D)-Trp-(D)-gTrp-CHO (100 mg, 0.14 mmol) and $PhSO_2Cl$ (20 μL, 0.154 mmol). Compound Ph-SO_2-(D)-Trp-(D)-gTrp-CHO (77 mg, 73%) was purified by preparative HPLC. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.85 (m, 4H, 2 $(CH_2)_\beta$), 4 (m, 1H, $(CH)_\alpha$), 5 and 5.5 (2m, 1H, $(CH)_\alpha$), 6.9−7.6 (m, 15H, 2 indoles (10H) and benzyl (5H)), 7.9 (s, 1H, CHO), 8−8.7 (m, 3H, NH (sulfonamide) and 2 NH (amides)), 10.98 (m, 2H, 2 N^1H). MS (ES), *m/z*: 530.21 $[M + H]^+$. HPLC t_R: 20.19 min (conditions A).

Isovaleryl-(D)-Trp-(D)-gTrp-C(O)CH_3 (39). 39 was synthesized as described for compound **7** starting from TFA, H-(D)-Trp-(D)-gTrp-C(O)CH_3 (100 mg, 0.19 mmol), isovaleric acid (25 μL, 0.23 mmol), BOP (101 mg, 0.23 mmol), and NMM (48 μL, 0.43 mmol). Isovaleryl-(D)-Trp-(D)-gTrp-C(O)CH_3 (49 mg, 52%) was purified by preparative HPLC. ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.71 and 0.77 (2d, 6H, 2 CH_3), 1.78 (s, 3H, CH_3), 1.9 (m, 3H, CH and CH_2), 3.0 (m, 4H, 2 $(CH_2)_\beta$), 4.6 (m, 1H, $(CH)_\alpha$), 5.6 (m, 1H, $(CH)_\alpha$), 6.9−7.2 (m, 6H), 7.3 (m, 2H), 7.6 (m, 2H), 8.1 (m, 1H, NH (amide)), 8.2 (m, 2H, 2 NH (amides)), 10.81 (m, 2H, 2 N^1H). MS (ES), *m/z*: 488.41 $[M + H]^+$, 975.91 $[2M + H]^+$. HPLC t_R: 19.99 min (conditions A).

Isovaleryl-(D)-Trp-(D)-gTrp-CHO (40). 40 was synthesized as described for compound **7** starting from TFA, H-(D)-Trp-(D)-gTrp-CHO (100 mg, 0.20 mmol), isovaleric acid (26 μL, 0.24 mmol), BOP (105 mg, 0.23 mmol), and NMM (50 μL, 0.45 mmol). Isovaleryl-(D)-Trp-(D)-gTrp-CHO (40 mg, 43%) was purified by preparative HPLC. ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.71 and 0.77 (2d, 6H, 2 CH_3), 1.86 (m, 3H, CH and CH_2), 3 (m, 4H, 2 $(CH_2)_\beta$), 4.6 (m, 1H, $(CH)_\alpha$), 5.3 and 5.7 (2m, 1H, $(CH)_\alpha$), 6.9−7.2 (m, 6H), 7.3 (m, 2H), 7.6 (m, 2H), 7.9 (m, 1H, CHO), 7.8−8.7 (m, 3H, 3 NH (amides)), 10.9 (m, 2H, 2 N^1H). MS (ES), *m/z*: 474.39 $[M + H]^+$, 496.39 $[M + Na]^+$, 967.64 $[2M + H]^+$, 989.17 $[2M + Na]^+$. HPLC t_R: 16.26 min (conditions A).

CH_3C(O)-(D)-Trp-(D)-gTrp-C(O)CH_3 (41). In 5 mL of DMF, TFA, H-(D)-Trp-(D)-gTrp-C(O)CH_3 (100 mg, 0.19 mmol), DIEA (36 μL, 0.21 mmol), and acetic anhydride (28 μL, 0.21 mmol) were successively added. After 1 h, the mixture was diluted with ethyl acetate (25 mL) and washed with saturated aqueous sodium hydrogen carbonate (50 mL), aqueous potassium hydrogen sulfate (50 mL, 1 M), and saturated aqueous sodium chloride (50 mL). The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo. The product **41** was purified by preparative HPLC (47 mg, 46%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.77 (s, 3H, C(O)CH_3), 1.79 (s, 3H, C(O)CH_3), 2.81−3.12 (m, 4H, 2 $(CH_2)_\beta$), 4.5 (m, 1H, $(CH)_\alpha$), 5.63 (m, 1H, $(CH)_\alpha$), 6.98−7.15 (m, 6H), 7.25 (m, 2H), 7.62 (m, 2H), 8.0 (d, 1H, NH (amide)), 8.16 (m, 1H, NH (amide)), 8.30 (d, 1H, NH (amide)), 10.81 (s, 1H, 1 N^1H), 10.83 (s, 1H, 1 N^1H). MS (ES), *m/z*: 446.75 $[M + H]^+$, 467.86 $[M + Na]^+$. HPLC t_R: 11.99 min (conditions A).

N(Me)-(D)-Trp-(D)-gTrp-CHO A and B (42, 43). Boc-N(Me)-(D)-(N^iBoc)Trp-(D)-(N^iBoc)-gTrp-formyl (295 mg, 0.42 mmol) was dissolved in a mixture of trifluoroacetic acid (8 mL), anisole (1 mL), and thioanisole (1 mL) for 1 h at 0°C. The solvents were removed in vacuo, the residue was stirred in ether, and the precipitated TFA, N(Me)-(D)-Trp-(D)-gTrp-formyl was filtered. Analytical HPLC chromatograms showed the presence of two equivalent peaks that possessed the same MS spectrum and were attributed to two isomers. Compounds TFA, N(Me)-(D)-Trp-(D)-gTrp-formyl A (52 mg, 24%) and TFA, N(Me)-(D)-Trp-(D)-gTrp-formyl B (60 mg, 28%) were separated by preparative HPLC.

Compound 42. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.1 (m, 3H, NCH_3), 3.1 (m, 4H, 2 $(CH_2)_\beta$), 3.89 (m, 1H, CH_α), 5.42 and 5.82 (m, 1H, CH_α), 6.9−7.7 (m, 10H, 2 indoles), 8 (s, 1H, CHO), 8.3−9.3 (m, 3H, 2 NH (amides) and NH (amine)), 10.9 (m, 1H, N^1H), 11.05 (m, 1H, N^1H). MS (ES), *m/z*: 404.5 $[M + H]^+$. HPLC t_R: 113.30 min (conditions A).

Compound 43. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.49 (m, 3H, NCH_3), 2.8 (m, 2H, $(CH_2)_\beta$), 3.1 (m, 2H, $(CH_2)_\beta$), 3.9 (m, 1H, CH_α), 5.2 and 5.65 (m, 1H, CH_α), 6.8−7.6 (m, 10H, 2 indoles), 7.9 (s, 1H, CHO), 8.2−9.3 (m, 3H, 2 NH (amides) and NH (amine)), 10.85 (m, 1H, N^1H), 11 (m, 1H, N^1H). MS (ES), *m/z*: 404.44 $[M + H]^+$. HPLC t_R: 15.69 min (conditions A).

Me-SO_2-(D)-Trp-(D)-gTrp-CHO (44). 44 was synthesized as described for compound **7** starting from H-(D)-Trp-(D)-gTrp-CHO (100 mg, 0.14 mmol) and $MeSO_2Cl$ (12 μL, 0.154 mmol). Compound Me-SO_2-(D)-Trp-(D)-gTrp-CHO was purified by preparative HPLC (46 mg, 51%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.26 and 2.31 (2s, 3H, CH_3), 3 (m, 4H, 2 $(CH_2)_\beta$), 4.05 (m, 1H, $(CH)_\alpha$), 5.3 and 5.7 (2m, 1H, $(CH)_\alpha$), 6.9−7.7 (m, 11H, 2 indoles (10H) and NH (sulfonamide)), 8 (s, 1H, CHO), 8.1−8.9 (m, 2H, 2 NH (amides)), 10.98 (m, 2H, 2 N^1H). MS (ES), *m/z*: 468.23 $[M + H]^+$, 490.31 $[M + Na]^+$. HPLC t_R: 117.59 min (conditions A).

H-Aib-(D)-Trp-(D)-gTrp-propionyl (45). 45 was synthesized as described for compound **7** starting from Boc-(D)-(N^i-Boc)-Trp-(D)-(N^iBoc)-gTrp-H (**4′**) (958 mg, 1.45 mmol), NMM (351 μL, 3.16 mmol), propionic acid (130 μL, 1.74 mmol), and BOP (770 mg, 1.74 mmol) to afford 250 mg (85%) of Boc-Aib-(D)-Trp-(D)-gTrp-propionyl as a foam. Boc-Aib-(D)-Trp-(D)-gTrp-propionyl (250 mg, 1.18 mmol) was dissolved in a mixture of

trifluoroacetic acid (8 mL), anisole (1 mL), and thioanisole (1 mL) for 30 min at 0 °C. The solvents were removed in vacuo, the residue was stirred in ether, and the precipitated TFA, H-Aib-(D)-Trp-(D)-gTrp-propionyl was filtered. Compound **45** was purified by preparative HPLC (80 mg, 31%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.96 (t, 3H, CH_3), 1.23 and 1.44 (2s, 6H, 2 CH_3 (Aib)), 2.1 (q, 2H, CH_2), 3 (m, 4H, 2 $(CH_2)_\beta$), 4.6 (m, 1H, $(CH)_\alpha$), 5.6 (m, 1H, $(CH)_\alpha$), 6.9−7.2 (m, 6H), 7.3 (m, 2H), 7.7 (2d, 2H), 7.9 (s, 2H, NH_2), 8.2 (d, 1H, NH (amide)), 8.3 (d, 1H, NH (amide)), 8.5 (d, 1H, NH (amide)), 10.83 (m, 2H, 2 N^{1}H). MS (ES), *m*/*z*: 503.4 $[M + H]^+$. HPLC t_R: 16.26 min (conditions A).

N(Me)-Aib-(D)-Trp-(D)-gTrp-propionyl (46). 46 was purified by preparative HPLC (92 mg, 50%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.96 (t, 3H, CH_3), 1.19 and 1.42 (2s, 6H, 2 CH_3 (Aib)), 2.07 (q, 2H, CH_2), 2.58 (s, 3H, $N(CH_3)$), 3 (m, 4H, 2 $(CH_2)_\beta$), 4.65 (m, 1H, $(CH)_\alpha$), 5.6 (m, 1H, $(CH)_\alpha$), 6.9−7.2 (m, 6H), 7.3 (m, 2H), 7.7 (2d, 2H), 8.2 (d, 1H, NH (amide)), 8.3 (d, 1H, NH (amide)), 8.55 (d, 1H, NH (amide)), 8.65 (m, 1H, NH (amine)), 10.83 (m, 2H, 2 N^{1}H). MS (ES), *m*/*z*: 517.38 $[M + H]^+$, 539.45 $[M + Na]^+$. HPLC t_R: 12.12 min (conditions A).

H-Aib-(D)-Trp-(D)-gTrp-isovaleryl (47). 47 was synthesized from **4′** as described for **45**, by substituting propionic acid with isovaleric acid. ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.83 (m, 6H, 2 CH_3), 1.22 and 1.43 (2s, 6H, 2 CH_3 (Aib)), 1.9 (m, 3H, CH and CH_2), 3 (m, 4H, 2 $(CH_2)_\beta$), 4.6 (m, 1H, $(CH)_\alpha$), 5.6 (m, 1H, $(CH)_\alpha$), 6.9−7.2 (m, 6H), 7.3 (m, 2H), 7.7 (2d, 2H), 7.9 (s, 2H, NH_2), 8.2 (d, 1H, NH (amide)), 8.3 (d, 1H, NH (amide)), 8.6 (d, 1H, NH (amide)), 10.82 (m, 2H, 2 N^{1}H). MS (ES), *m*/*z*: 531.48 $[M + H]^+$. HPLC t_R: 18.41 min (conditions A).

N(Me)-Aib-(D)-Trp-(D)-gTrp-isovaleryl (48). 48 was synthesized from TFA, H-(D)-Trp-(D)-gTrp-isovaleryl as described for the previous title compound. ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.83 (m, 6H, 2 CH_3), 1.19 and 1.41 (2s, 6H, 2 CH_3 (Aib)), 1.94 (m, 3H, CH and CH_2), 2.25 (m, 3H, NCH_3), 2.99−3.21 (m, 4H, 2 $(CH_2)_\beta$), 4.67 (m, 1H, $(CH)_\alpha$), 5.64 (m, 1H, $(CH)_\alpha$), 7−7.07 (m, 4H), 7.15−7.18 (m, 2H), 7.3−7.36 (m, 2H), 7.66 and 7.73 (2d, 2H), 8.21 (d, 1H, NH (amide)), 8.3 (d, 1H, NH (amide)), 8.58 (d, 1H, NH (amide)), 8.6 (m, 1H, NH (amine)), 10.83 (m, 2H, 2 N^{1}H). MS (ES), *m*/*z*: 545 $[M + H]^+$. HPLC t_R: 18.34 min (conditions A).

H-Aib-(D)-Trp-(D)-gTrp-phenylacetyl (49). 49 was synthesized from **4′** as described for **45**, by substituting propionic acid with phenylacetic acid. ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.22 and 1.42 (2s, 6H, 2 CH_3 (Aib)), 3 (m, 4H, 2 $(CH_2)_\beta$), 3.43 (s, 2H, CH_2), 4.6 (m, 1H, $(CH)_\alpha$), 5.6 (m, 1H, $(CH)_\alpha$), 6.9−7.4 (m, 13H), 7.7 (2d, 2H), 7.9 (s, 2H, NH_2), 8.3 (d, 1H, NH (amide)), 8.5 (d, 1H, NH (amide)), 8.7 (d, 1H, NH (amide)), 10.83 (m, 2H, 2 N^{1}H). MS (ES), *m*/*z*: 565.40 $[M + H]^+$. HPLC t_R: 119.56 min (conditions A).

N(Me)-Aib-(D)-Trp-(D)-gTrp-phenylacetyl (50). 50 was synthesized from TFA, H-(D)-Trp-(D)-gTrp-phenylacetyl as described for compound **46**. ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.19 and 1.4 (2s, 6H, 2 CH_3 (Aib)), 2.24 (m, 3H, NCH_3), 2.91−3.2 (m, 4H, 2 $(CH_2)_\beta$), 4.66 (m, 1H, $(CH)_\alpha$), 5.63 (m, 1H, $(CH)_\alpha$), 6.99−7.37 (m, 13H), 7.66 and 7.74 (2d, 2H), 8.29 (d, 1H, NH (amide)), 8.52 (d, 1H, NH (amide)), 8.6 (m, 2H, NH (amide) and NH (amine)), 10.88 (m, 2H, 2 N^{1}H). MS (ES), *m*/*z*: 565.40 $[M + H]^+$. HPLC t_R: 19.23 min (conditions A).

H-Aib-(D)-Trp-(D)-gTrp-isonipecotyl (51). 51 was synthesized from **4′** as described for compound **45**, by substituting propionic acid for Fmoc-isonipecotic acid. ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.22 and 1.43 (2s, 6H, 2 CH_3 (Aib)), 1.7 (m, 4H, 2 CH_2 (Iso)), 2.4 (m, 1H, CH (Iso)), 2.8−3.3 (m, 8H, 2 CH_2 (Iso) and 2 $(CH_2)_\beta$), 4.6 (m, 1H, $(CH)_\alpha$), 5.6 (m, 1H, $(CH)_{\alpha'}$), 6.9−7.2 (m, 6H), 7.3 (2d, 2H), 7.7 (2d, 2H), 7.9 (s, 2H, NH_2), 8.3 (m, 2H, 2 NH (amides)), 8.7 (d, 1H, NH (amide)), 10.82 (m, 2H, 2 N^{1}H). MS (ES), *m*/*z*: 558.44 $[M + H]^+$. HPLC t_R: 13.51 min (conditions A).

N(Me)-Aib-(D)-Trp-(D)-gTrp-isonipecotyl (52). 52 was synthesized from TFA, H-(D)-Trp-(D)-gTrp-isonipecotyl as described for compound **46**. ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.20 and 1.41 (2s, 6H, 2 CH_3 (Aib)), 1.69 (m, 4H, 2 CH_2 (Iso)), 2.25 (m, 3H, NCH_3), 2.4 (m, 1H, CH (Iso)), 2.85−3.34 (m, 8H, 2 CH_2 (Iso) and 2 $(CH_2)_\beta$), 4.66 (m, 1H, $(CH)_\alpha$), 5.64 (m, 1H, $(CH)_{\alpha'}$), 6.98−7.18 (m, 6H), 7.3 (2d, 2H), 7.7 (2d, 2H), 8.34 and 8.64 (2m, 2H and 3H, 3 NH (amides) and 2 NH (amines)), 10.85 (m, 2H, 2 N^{1}H). MS (ES), *m*/*z*: 571.91 $[M + H]^+$. HPLC t_R: 9.11 min (conditions A).

H-Aib-(D)-Trp-(D)-gTrp-3-indolylacetyl (53). 53 was synthesized from **4′** as described for compound **45**, by substituting propionic acid for 3-indolylacetic acid. ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.18 and 1.42 (2s, 6H, 2 CH_3 (Aib)), 3 (m, 4H, 2 $(CH_2)_\beta$), 3.53 (s, 2H, CH_2), 4.6 (m, 1H, $(CH)_\alpha$), 5.7 (m, 1H, $(CH)_\alpha$), 6.9−7.8 (m, 15H), 7.9 (s, 2H, NH_2), 8.3 (m, 2H, 2 NH (amides)), 8.6 (d, 1H, NH (amide)), 10.8 (m, 1H, N^{1}H), 10.85 (s, 2H, 2 N^{1}H). MS (ES), *m*/*z*: 604.51 $[M + H]^+$. HPLC t_R: 19.50 min (conditions A).

N(Me)-Aib-(D)-Trp-(D)-gTrp-3-indolylacetyl (54). 54 was synthesized from TFA, H-(D)-Trp-(D)-gTrp-3-indolylacetyl as described for compound **46**. ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.06 and 1.39 (2s, 6H, 2 CH_3 (Aib)), 2.23 (m, 3H, NCH_3), 2.92−3.2 (m, 4H, 2 $(CH_2)_\beta$), 3.53 (s, 2H, CH_2), 4.67 (m, 1H, $(CH)_\alpha$), 5.68 (m, 1H, $(CH)_\alpha$), 6.59−7.15 (m, 9H), 7.31−7.35 (m, 3H), 7.43 (d, 1H), 7.63 and 7.72 (2d, 2H), 8.32 (m, 2H, NH (amine) and NH (amide)), 8.6 (m, 2H, 2 NH (amides)), 8.6 (d, 1H, NH (amide)), 10.81 (d, 1H, N^{1}H), 10.85 (s, 2H, 2 N^{1}H). MS (ES), *m*/*z*: 618 $[M + H]^+$, 640 $[M + Na]^+$. HPLC t_R: 19.20 min (conditions A).

H-Aib-(D)-Trp-(D)-gTrp-cyclohexyl-3-propionyl (55). 55 was synthesized from **4′** as described for compound **45**, by substituting propionic acid for cyclohexyl-3-propionic acid. ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.83 (m, 2H, CH_2), 1.22 and 1.44 (2s, 6H, 2 CH_3 (Aib)), 1−1.7 (m, 11H, 5 CH_2 and CH), 2 (t, 2H, CH_2), 3 (m, 4H, 2 $(CH_2)_\beta$), 4.6 (m, 1H, $(CH)_\alpha$), 5.6 (m, 1H, $(CH)_\alpha$), 6.9−7.2 (m, 6H), 7.3 (m, 2H), 7.7 (2d, 2H), 7.9 (s, 2H, NH_2), 8.1 (d, 1H, NH (amide)), 8.3 (m, 1H, NH (amide)), 8.5 (d, 1H, NH (amide)), 10.82 (m, 2H, 2 N^{1}H). MS (ES), *m*/*z*: 585.65 $[M + H]^+$. HPLC t_R: 23.47 min (conditions A).

N(Me)-Aib-(D)-Trp-(D)-gTrp-cyclohexyl-3-propionyl (56). 56 was synthesized from TFA, H-(D)-Trp-(D)-gTrp-cyclohexyl-3-propionyl as described for compound **46**. ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.8 (m, 2H, CH_2), 1.09 (m, 4H, 2 CH_2), 1.19 (s, 3H, CH_3 (Aib)), 1.3−1.4 (m, 2H, CH_2), 1.42 (s, 3H, CH_3 (Aib)), 1.61−1.7 (m, 5H, 2 CH_2 and CH), 2.06 (m, 2H, CH_2), 2.25 (s, 3H, NCH_3), 3−3.2 (m, 4H, 2 $(CH_2)_\beta$), 4.66 (m, 1H, $(CH)_\alpha$), 5.62 (m, 1H, $(CH)_\alpha$), 7−7.16 (m, 6H), 7.31−7.36 (m, 2H), 7.64 and 7.73 (2d, 2H), 8.2 (d, 1H, NH (amine)), 8.3 (d, 1H, NH (amide)), 8.5 (d, H, NH (amide)), 8.67 (d, 1H, NH (amide)), 10.83 (m, 2H, 2 N^{1}H). MS (ES), *m*/*z*: 599 $[M + H]^+$. HPLC t_R: 12.58 min (conditions B).

H-Aib-(D)-Trp-(D)-gTrp-C(O)-NHCH$_2$CH$_3$ (57). 57 was synthesized by coupling Boc-(D)-(N^{i}Boc)-Trp-(D)-(N^{i}Boc)-gTrp-H (**4**) with ethyl isocyanate, under usual deprotection conditions, and coupling to Aib residue as described for compound **7**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.95 (t, 3H, −C(O)−NHCH$_2$C*H*$_3$), 1.05 and 1.10 (2s, 6H, 2 CH_3 (Aib)), 2.88−3.18 (m, 6H, 2($CH_{2\beta}$) and −C(O)−NHC*H*$_2$CH$_3$), 4.55 (m, 1H, $(CH)_{\alpha1}$), 5.45 (m, 1H, $(CH)_{\alpha2}$), 6.08 (t, 1H, −C(O)−N*H*CH$_2$CH$_3$), 6.25 (d, 1H, −N*H*−C(O)−NHCH$_2$CH$_3$), 6.90−7.60 (10H, 2 indoles), 8.00 (sl, 1H, NH amide), 8.35 (d, 1H, NH amide), 10.75 (s, 1H, 1N^{1}H), 10.80 (s, 1H, 1 N^{1}H). MS (ES), *m*/*z*: 430.1 $[M + H - CH_3CH_2NH(CO)NH_2]^+$, 518.4 $[M + H]^+$, 540.3 $[M + Na]^+$. HPLC t_R: 7.25 min (conditions B).

H-Aib-(D)-Trp-(D)-gTrp-C(O)-OCH$_2$CH$_3$ (58). 58 was synthesized by coupling gem-diamino Boc-(D)-(N^{i}Boc)-Trp-(D)-(N^i-Boc)-gTrp-H (**4**) with ethyl chloroformate (ClC(O)OCH$_2$CH$_3$) in the presence of Et_3N and under the usual deprotection conditions and coupling to Aib as described previously. ^{1}H NMR (360 MHz, DMSO-d_6): δ 1.12 (t, 3H, −C(O)−OCH$_2$C*H*$_3$), 1.21 and 1.41 (2s, 6H, 2 CH_3 (Aib)), 2.99 (d, 1H, $H_{\beta1}$, $J_{\beta1\beta'1}$ = 13.5 Hz), 3.04 (m, 2H, $CH_{2\beta2}$), 3.15 (dd, 1H, $H_{\beta'1}$, $J_{\beta'1\ \beta1}$ = 14.5 Hz, $J_{\beta'1\alpha1}$ = 3.5 Hz), 3.95 (q, 2H, −C(O)−OC*H*$_2$CH$_3$), 4.63 (m, 1H, $(CH)_{\alpha1}$), 5.45 (m, 1H, $(CH)_{\alpha2}$), 6.95−7.15 (m, 4H, H indole), 7.12 (s, 1H, H indole), 7.18 (s, 1H, H indole), 7.30−7.40 (m, 2H, H indole), 7.55 (m, 1H, −N*H*−C(O)−OCH$_2$CH$_3$), 7.65 (d, 1H, H indole), 7.71 (d, 1H, H indole), 8.25 (d, 1H, NH amide), 8.60 (d, 1H, NH amide), 10.80 (s, 1H, 1N^{1}H), 10.82 (s, 1H, 1

N^1H). MS (ES), *m*/*z*: 430.3 $[M + H - CH_3CH_2O(CO)NH_2]^+$, 519.1 $[M + H]^+$, 541.6 $[M + Na]^+$. HPLC t_R: 7.22 min (conditions B).

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