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Structure–Activity Analysis of the Growth Hormone Secretagogue GHRP-6 by α- and β-Amino γ-Lactam Positional Scanning

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Incorporation of amino lactams into biologically active peptides restricts conformational mobility and may enhance selectivity and increase potency. α- and β-amino γ-lactams (Agl and Bgl), in both *S* and *R* configurations, were introduced into the growth hormone secretagogue GHRP-6 using a Fmoc-compatible solid-phase protocol relying on *N*-alkylation with five- and six-membered cyclic sulfamidates, followed by lactam annulation under microwave heating. Using this protocol in conjunction with IRORI Kan™ techniques furnished eleven new GHRP-6 analogs, and their binding affinity IC_{50} values on both the growth hormone secretagogue receptor 1a (GHS-R1a) and CD36 receptors are herein reported. The results indicate that selectivity towards one receptor or the other can be modulated by lactam substitution, typically at the Ala[3] and the D-Phe[5] positions.

Key words: CD36, conformational constraint, cyclic sulfamidate, Freidinger–Veber lactam, GHRP-6, GHS-R1a, lactam, lactam scanning, peptide, peptidomimetic, solid-phase synthesis

Abbreviations: Agl, α-amino γ-lactam; Bgl, β-amino γ-lactam; BSA, *N,O*-bis(trimethylsilyl)-acetamide; CD36, cluster of differentiation 36; EDTA, ethylenediamine tetraacetate; EGTA, ethyleneglycol tetraacetate; FA, formic acid; GH, growth hormone; GHRP, growth hormone-releasing peptide; GHS-R1a, growth hormone secretagogue receptor 1a; HBTU, *O*-benzotriazole-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; RP-HPLC, reverse phase high performance liquid chromatography; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TES, triethyl silane; PBS, phosphate buffered saline.

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GHRP-6 (HwAWfK, Figure 1) is a synthetic hexapeptide member of the growth hormone-releasing peptides (GHRPs) family. GHRPs were derived from Met-enkephalin (1,2) and exert their GH secretagogue activity by a distinct pathway from that triggered by the growth hormone-releasing hormone (GHRH) (3). Their GH release-stimulating action is mediated by their interaction with the growth hormone secretagogue receptor 1a (GHS-R1a), a G-protein-coupled receptor located in pituitary and hypothalamic cells (4,5) that binds ghrelin as its natural ligand (6). GHRP-6 stimulates GH liberation from somatotrophs in a dose-dependant manner in different species including humans (2,7) and has clinical perspectives in the treatment of impaired GH secretion-related conditions like obesity (8), short stature (9), acromegaly (10) or aging (11). In this context, hexarelin (His-D-2-Me-Trp-Ala-Trp-D-Phe-Lys-NH_2) was developed, and displayed an increased GH-releasing activity following intravenous, subcutaneous, intranasal, and oral administration in humans (12).

Another important aspect of GHRPs pharmacological properties is their multiple effects on the cardiovascular system. For instance, GHRP-6 analogs are able to exert protective effects on ischemia/reperfusion injury of isolated rat heart (13) and have also been shown to display anti-atherosclerotic activity in apolipoprotein E-deficient mice (14). These effects have recently been shown to be mediated by their binding to CD36, a multiligand scavenger receptor expressed in cardiomyocytes and in the microvascular endothelial cells (15,16). The anti-atherosclerotic properties of GHRPs result from the overlapping of their binding domain on CD36 with that for oxidized low density lipoproteins (oxLDL). Therefore, by interacting with CD36, GHRPs decrease oxidized lipid internalization within macrophages and the formation of atherosclerotic lesions (17,18). Furthermore, CD36 features a distinct binding site for thrombospondin and by binding the scavenger receptor, GHRPs may convey neovascularisation inhibitory effects, suggesting their therapeutic potential in angiogenesis-related diseases such as age-related macular degeneration (19).

Considering that GHRP-6 exhibits dual affinity for both the GHS-R1a and the CD36 receptors, discovery of analogs selective for one

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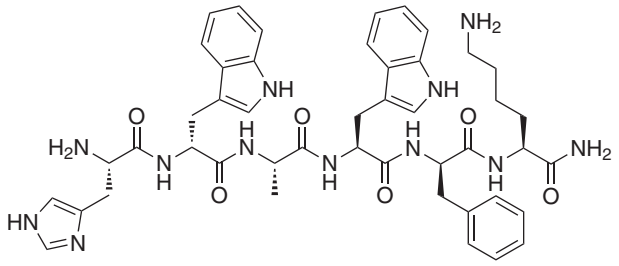


His-D-Trp-Ala-Trp- D-Phe-Lys-NH_2

Figure 1: Structure and sequence of GHRP-6.

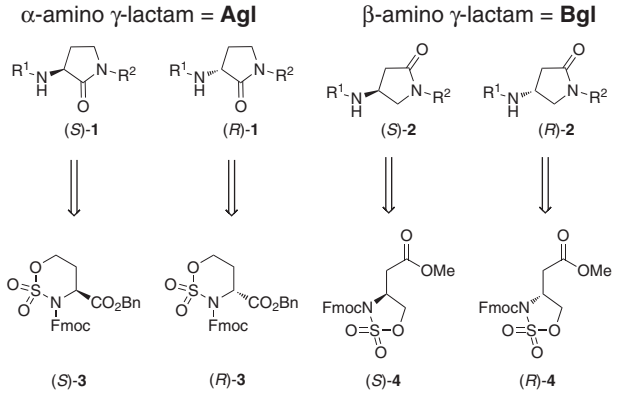


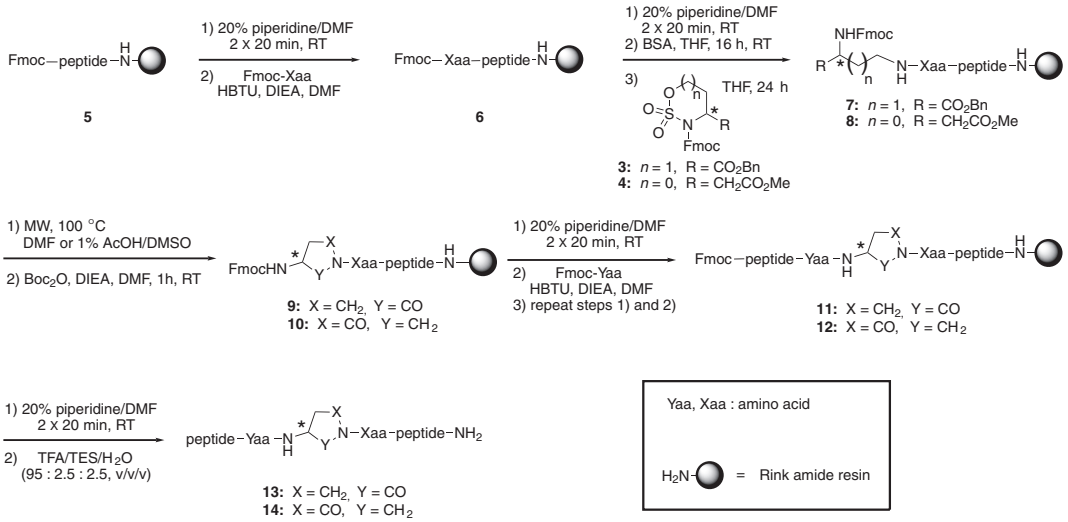
Figure 2: Three letter codes for amino lactam residues and cyclic sulfamidates **3** and **4**.

receptor is of significant interest to probe specific functions. In light of the large pharmacological profile displayed by CD36 and the fact that it represents a newly discovered target for GHRPs, selective binding partners for this receptor are attractive as drug leads, free of possible unwanted effects associated with action on GHS-R1a.

The bioactive conformation of GHRP-6 as GHS-R1a agonist has been suggested to adopt a turn motif based on molecular modeling (20). Preparation of GHRP-6 analogs bearing β-turn stabilizing peptide mimics may provide information about the structural features required to achieve activity and selectivity.

α-Amino γ-lactam (AgI, **1**, Figure 2), a member of the so-called Freidinger–Veber lactams, has been successfully utilized to constrain the backbone conformation of linear peptides to give type II and type II' β-turn mimics (21–23). β-Amino-γ-lactam (BgI, **2**, Figure 2) has been less frequently used to study peptide structure. Replacement of Asp^{11} by (*S*)-BgI in the fragment [6–13] of human growth hormone conserved insulin-potentiating activity and provided extended duration of action (24–27). In addition, BgI peptide analogs have exhibited CCK-A receptor agonism with high affinity and selectivity (28), as well as significant enhancement of the binding of [^{3}H]*N*-propylnorapomorphine to dopamine receptors (29). Conformational analysis of BgI peptides by NOESY-NMR studies in DMSO and molecular dynamics indicated that (*S*)-BgI may stabilize a type II′-turn-like conformation (27,30,31).

We have recently reported a general protocol for solid-phase synthesis of AgI and BgI-bridged peptides using, respectively, cyclic sulfamidate reagents **3** and **4** (Figure 2) (32). Employing this protocol (Scheme 1), ten lactam-bridged analogs of GHRP-6 were synthesized (peptides **15**–**19** using (*S*)-**3** and **20**–**24** using (*S*)-**4,** Table 3) (32). According to this protocol, standard conditions for solid-phase peptide synthesis with Fmoc protection were employed to synthesize the peptide chain **6** on Rink amide resin (33,34). When the appropriate residue for lactam formation was reached, the N-terminal Fmoc group was removed, and the free amine was reacted with *N,O*-bis(trimethylsilyl)acetamide (BSA) to limit bis-alkylation of the terminal amine with the respective sulfamidate **3** or **4**. Linear alkylated products were converted to the lactam by microwave-assisted annulation. Standard peptide synthesis was resumed on amino lactam peptides **9** and **10**. After final deprotection and cleavage with trifluoroacetic acid (TFA), ether precipitation gave the crude peptides, which were purified by preparative RP-HPLC to give the desired products. The binding affinity IC_{50} values of the GHRP-6



Scheme 1: General protocol for solid-phase synthesis of AgI and BgI lactam-bridged peptides.

(*S*)-amino lactam analogs on both GHS-R1a and CD36 were determined (32).

As GHRP-6 contains both L- and D-configured amino acids, a comprehensive lactam scan of the later peptide would involve the use of both (*S*)- and (*R*)-configured lactams. We now report the synthesis of eleven new GHRP-6 lactam analogs employing (*R*)-Agl (**25**–**29**) and (*R*)-Bgl residues (**30**–**35**) in an efficient split-and-mix approach using IRORI Kan™ techniques and cyclic sulfamidate reagents (*R*)-**3** and (*R*)-**4**. The respective (*R*)-amino lactam peptides have been tested for binding affinity with receptors CD36 and GHS-R1a and compared with their (*S*)-lactam counterparts in a summary of the biological data.

Materials and Methods

General methods

Sulfamidates (*R*)-**3** and (*R*)-**4** were synthesized, respectively, from D-methionine and D-aspartic acid as previously described (32). Rink amide resin SS (70–90 mesh, 0.7 mmol/g loading) was purchased from Advanced Chemtech™ (Louisville, Kentucky). Fmoc amino acids were purchased from Novabiochem® (EMD Bioscience Inc., San Diego, CA, USA) or GL Biochem Ltd. (Shangai, China). *O*-Benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate (HBTU) was purchased from GL Biochem (Shangai, China). Anhydrous tetrahydrofurane (THF) and dimethyl formamide (DMF) were obtained from a Seca Solvent Filtration System (GlassContour™ Laguna Beach, California). Diisopropylethylamine (DIEA; from Aldrich®, Saint-Louis, MO, USA) was distilled first from ninhydrin, then from calcium hydride. Peptide couplings and Fmoc removal were qualitatively monitored by Kaiser test (35). Reactions requiring anhydrous conditions were performed under an atmosphere of dry argon. Microwave-assisted reactions were preformed in a Biotage Initiator microwave apparatus (Biotage AB, Uppsala, Sweden) in Biotage 5 mL V-shaped glass reaction vessels containing a 10-mm triangular magnetic stirrer bar and sealed with a septum cap.[a] Alkylation and lactam formation reactions were monitored by LC-MS (UV: λ = 214 nm, 0–60% MeCN in H_2O, 0.1% formic acid (FA) in 4 min, 0.5 mL/min, Gemini™ 5u C18 110A, 50 × 4.6 mm, 5 μm). Analytical RP-HPLC analyses of crude and purified peptides were performed on a Gemini™ 5u C18 110A column (150 mm × 4.6 mm, 5 μm, column A; Phenomenex® Inc., Torrance, CA, USA) or a Synergi™ 4u Polar RP80A column (150 mm × 4.6 mm, 4 μm, column B; Phenomenex® Inc.) with a flow rate of 0.5 mL/min using a linear gradient of acetonitrile [0.1% formic acid (FA)] in water (0.1% FA). Retention times (t_R) from analytical RP-HPLC are reported in minutes; UV absorption was recorded at λ = 214 nm. Peptides were purified with a Gemini™ 5u C18 110A column (250 mm × 21.2 mm, 5 μm, column C; Phenomenex® Inc.) or a Synergi™ 4u Polar RP80A Axial Packed column (100 mm × 21.2 mm, 4 μm, column D; Phenomenex® Inc.) using a specified linear gradient of acetonitrile (0.1% FA), in water (0.1% FA), with a flow rate of 10.6 mL/min.

Combinatorial strategy using IRORI Kan™ reactors

With the goal to accelerate the process of lactam scanning, IRORI Kan™ reactors[b] were utilized in a combinatorial synthesis strategy to perform a (*R*)-lactam scan of GHRP-6 using 6-membered cyclic sulfamidate (*R*)-**3** and 5-membered cyclic sulfamidate (*R*)-**4**. The propylene mesh walled IRORI Kan™ reactors allow for reactions on confined resin beads. They have been used for the preparation of libraries of discreet compounds in solid-phase synthesis by a split-and-mix approach (36–40). In the context of this work, compound tracking was made possible using frequency tag microchips.

IRORI Kan™ experimental procedures for (R)-Agl and (R)-Bgl GHRP-6 analogs preparation

Ten IRORI Kan™ macroreactors containing frequency tag microchips with designated serial numbers were each charged with Rink amide resin SS (70–90 mesh, 300 mg, 0.7 mmol/g, 0.2 mmol). The Kan reactors were sealed, and peptide synthesis was preformed by placing the Kan reactors in a 250 mL glass bottle containing a stirrer bar. After each coupling step, the appropriate Kan was identified by scanning the microchip serial number using the SYNTHESIS MANAGER™ software[c], removed from the batch reaction vessel and transferred to a syringe tube for silylation and alkylation. Because of the incompatibility of the frequency tag and microwave conditions and to improve stirring, the resin was temporarily removed from the IRORI Kan™, transferred to a microwave reaction vessel for the microwave-assisted annulation for the specified time. The resin was then washed back into the IRORI Kan™ along with its corresponding frequency tag. The Kan containing the supported lactam-bridged peptide was recombined with the batch of Kan reactors and peptide synthesis was continued. When the scan was complete, the terminal Fmoc groups were removed, the IRORI Kans™ were then identified and separated, and the crude peptides were cleaved off the resin and purified by preparative RP-HPLC to give the desired peptides **25**–**35** as white fluffy solids (Table 2).

Resin swelling, Fmoc deprotection and washings

The ten Kan reactors were placed in a 250 mL bottle and suspended in DMF (100 mL) for 0.5 h in order for the resin to swell. The solvent was decanted, and the Kan reactors were suspended in a freshly prepared 20% piperidine in DMF solution (150 mL) and stirred gently for 20 min. The solution was decanted and the operation was repeated. For washings, the Kan reactors were sequentially stirred for 5 min in DMF (3 × 150 mL), MeOH (3 × 150 mL) and dichloromethane (DCM, 3 150 mL). Residual solvent was removed from the Kan reactors by centrifugation.

Amino acid coupling

Resin in Kan reactors was swollen as described above. In a 250 mL round-bottom flask, a solution of *N*-(Fmoc)amino acid (3 equiv.), HBTU (3 equiv.), and DIEA (6 equiv.) in DMF (150 mL for ten Kan reactors, 0.04 M in amino acid) was prepared, stirred for 10 min and then added to the bottle containing Kan reactors filled with swollen resin. The bottle was tightly sealed with a screw cap, and the reaction mixture was gently stirred at room temperature for 1 h, in the cases of Fmoc-Lys(Boc) and Fmoc-Ala couplings, and for

Table 1: Optimization of the alkylation reaction of peptidyl resin **36** with cyclic sulfamidate (*R*)-4

Entry	BSA (time)	(*R*)-**4** (time)	Alkylation temperature	DIEA (equiv.)	**37:38:39**[a]
1 (init.)	5 equiv. (12 h)	10 equiv. (48 h)	RT	_	53:47:0
2	5 equiv. (1 h)[b]	10 equiv. (2 h)	60 °C μW	_	52:48:0
3	5 equiv. (1 h)[b]	10 equiv. (1 h)	60 °C μW	_	55:45:0
4	3 equiv. (1 h)	10 equiv. (1 h)	RT	_	23:77:0
5	3 equiv. (1 h)[b]	10 equiv. (1 h)	60 °C μW	_	49:51:0
6	_	5 equiv. (1 h)	60 °C μW	_	57:43:0
7	_	5 equiv. (1 h)	60 °C μW	1 equiv.	80:20:0
8	_	5 equiv. (1 h)	60 °C μW	1.2 equiv.	52:27:21

μW, microwave irradiation; DIEA, diisopropylethylamine.
[a]Ratio determined by LC-MS analysis (UV: λ = 214 nm, 0–60% MeCN in H_2O, 0.1% FA over 4 min, 0.5 mL/min, Gemini^{TM} 5u C18 110A, 50 mm × 4.60 mm).
[b]Resin silylation performed in the microwave reaction vessel, washings with dry THF, and supernatant removal carried out by syringe under argon before alkylation.

Table 2: Yields and purities of (*R*)-lactam-bridged GHRP-6 peptides **25–35**

Peptide[a]	*n*°	Crude purity %[b]	Purity %[c]	Yield %[d]	HRMS *m/z* (calcd.)	HRMS *m/z* (obsd.)
(*R*)-Agl-D-Trp-Ala-Trp-D-Phe-Lys-NH_2	**25**	19	>93	14	819.4301	819.4308
His-**(*R*)-Agl**-Ala-Trp-D-Phe-Lys-NH_2	**26**	45	>99	12	770.4096	770.4097
His-D-Trp-**(*R*)-Agl**-Trp-D-Phe-Lys-NH_2	**27**	30	>98	4	443.2296	443.2301[e]
His-D-Trp-Ala-**(*R*)-Agl**-D-Phe-Lys-NH_2	**28**	61	>99	11	770.4096	770.4097
His-D-Trp-Ala-Trp-**(*R*)-Agl**-Lys-NH_2	**29**	55	>99	7	809.4205	809.4199
(*R*)-Bgl-D-Trp-Ala-Trp-D-Phe-Lys-NH_2	**30**	30	>97	5	819.4301	819.4307
Ac**(*R*)-Bgl**-D-Trp-Ala-Trp-D-Phe-Lys-NH_2	**31**	20	>99	7	861.4406	861.4413
His-**(*R*)-Bgl**-Ala-Trp-D-Phe-Lys-NH_2	**32**	31	>99	8	385.7085	385.7083[e]
His-D-Trp-**(*R*)-Bgl**-Trp-D-Phe-Lys-NH_2	**33**	22	>99	8	443.2296	443.2301
His-D-Trp-Ala-**(*R*)-Bgl**-D-Phe-Lys-NH_2	**34**	40	>99	5	770.4096	770.4095
His-D-Trp-Ala-Trp-**(*R*)-Bgl**-Lys-NH_2	**35**	60	>99	3	809.4205	809.4198

HRMS, high resolution mass spectrometry.
[a]Bold lettering indicates lactam residues.
[b]RP-HPLC purity at 214 nm of the crude peptide.
[c]RP-HPLC purity at 214 nm of the purified peptide.
[d]Yields after purification by RP-HPLC are based on Fmoc loading test for Rink resin.
[e]The dicharged cation $[M+2H]^{2+}$ was observed, theoretical mass was calculated accordingly.

180 min in the cases of Fmoc-D-Phe, Fmoc-Trp(Boc), Fmoc-D-Trp(Boc), and Fmoc-His(Trt) couplings. The resin in Kan reactors was then washed as described above.

Silylation and alkylation

For lactam synthesis, the appropriate Kan was taken out of the batch and transferred to a 12 mL plastic syringe tube. The resin was swollen in DMF (7 mL) for 0.5 h and treated twice with a freshly prepared 20% piperidine in DMF solution (7 mL), for 20 min. It was then washed sequentially with DMF (3 × 7 mL), MeOH (3 × 7 mL), and DCM (3 × 7 mL) and dried *in vacuo* for at least 180 min. The syringe tube containing the Kan was flushed with argon, and the resin was swollen in dry THF (7 mL) under argon, treated with BSA (*N,O*-bis(trimethylsilyl)-acetamide, 5 equiv.), and the tube was tightly sealed with stoppers and parafilm and shaken for 16 h. The solution was then filtered off under argon. A solution of sulfamidate (*R*)-**3** or (*R*)-**4** (5 equiv.) in dry THF (7 mL) was added to the Kan in the tube, which was tightly sealed with stoppers and parafilm. After shaking for 24 h, the solution was removed by suction, and the Kan was washed with dry THF (3 × 7 mL, under argon), MeOH (3 × 7 mL), and DCM (3 × 7 mL) and dried *in vacuo*.

Microwave-assisted annulation

The Kan was opened, the resin was separated from the corresponding radio frequency tag and equally split into two 5 mL V-shaped microwave glass vessels fitted with a triangular stirring bar. After DMF (2 mL) addition, both vessels were sealed and heated under microwave irradiation at 100 °C (pressure 1 bar) for 75 min or as specified. The two batches of resin were then combined back in an IRORI Kan with their radio frequency tag and transferred to a 12 mL plastic filtration tube with polyethylene frit, where they were washed sequentially with DMF (3 × 7 mL), MeOH (3 × 7 mL), and DCM (3 × 7 mL) and dried *in vacuo*.

Table 3: IC_{50} Binding values for GHS-R1a and CD36 receptors for AgI and BgI GHRP-6 analogs

	Compound	Peptide	IC_{50} binding GHS-R1a	IC_{50} binding CD36
1	GHRP-6	His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2	6.08×10^{-9} M	
2	Hexarelin	His-D-2-Me-Trp-Ala-Trp-D-Phe-Lys-NH_2		3.33×10^{-6} M
3	**15**[a]	**(*S*)-AgI**-D-Trp-Ala-Trp-D-Phe-Lys-NH_2	4.09×10^{-8} M	1.45×10^{-5} M
4	**25**	**(*R*)-AgI**-D-Trp-Ala-Trp-D-Phe-Lys-NH_2	3.46×10^{-6} M	$>>10^{-5}$ M
5	**20**[a]	**(*S*)-BgI**-D-Trp-Ala-Trp-D-Phe-Lys-NH_2	3.71×10^{-7} M	$>>10^{-5}$ M
6	**30**	**(*R*)-BgI**-D-Trp-Ala-Trp-D-Phe-Lys-NH_2	1.01×10^{-6} M	1.37×10^{-5} M
7	**31**	Ac**(*R*)-BgI**-D-Trp-Ala-Trp-D-Phe-Lys-NH_2	2.75×10^{-6} M	$>>10^{-5}$ M
8	**16**[a]	His-**(*S*)-AgI**-Ala-Trp-D-Phe-Lys-NH_2	2.86×10^{-5} M	1.34×10^{-5} M
9	**26**	His-**(*R*)-AgI**-Ala-Trp-D-Phe-Lys-NH_2	$>>10^{-5}$ M	$>>10^{-5}$ M
10	**21**[a]	His-**(*S*)-BgI**-Ala-Trp-D-Phe-Lys-NH_2	$>>10^{-5}$ M	$>>10^{-5}$ M
11	**32**	His-**(*R*)-BgI**-Ala-Trp-D-Phe-Lys-NH_2	$>>10^{-5}$ M	$>>10^{-5}$ M
12	**17**[a]	His-D-Trp-**(*S*)-AgI**-Trp-D-Phe-Lys-NH_2	2.39×10^{-6} M	7.45×10^{-6} M
13	**27**	His-D-Trp-**(*R*)-AgI**-Trp-D-Phe-Lys-NH_2	2.82×10^{-6} M	$>>10^{-5}$ M
14	**22**[a]	His-D-Trp-**(*S*)-BgI**-Trp-D-Phe-Lys-NH_2	6.54×10^{-7} M	$>>10^{-5}$ M
15	**33**	His-D-Trp-**(*R*)-BgI**-Trp-D-Phe-Lys-NH_2	3.39×10^{-6} M	2.11×10^{-5} M
16	**18**[a]	His-D-Trp-Ala-**(*S*)-AgI**-D-Phe-Lys-NH_2	$>>10^{-5}$ M	$>>10^{-5}$ M
17	**28**	His-D-Trp-Ala-**(*R*)-AgI**-D-Phe-Lys-NH_2	$>>10^{-5}$ M	$>>10^{-5}$ M
18	**23**[a]	His-D-Trp-Ala-**(*S*)-BgI**-D-Phe-Lys-NH_2	$>> 10^{-5}$ M	$>> 10^{-5}$ M
19	**34**	His-D-Trp-Ala-**(*R*)-BgI**-D-Phe-Lys-NH_2	$>> 10^{-5}$ M	$>> 10^{-5}$ M
20	**19**[a]	His-D-Trp-Ala-Trp-**(*S*)-AgI**-Lys-NH_2	3.10×10^{-6} M	2.14×10^{-5} M
21	**29**	His-D-Trp-Ala-Trp-**(*R*)-AgI**-Lys-NH_2	$>>10^{-5}$ M	$>>10^{-5}$ M
22	**24**[a]	His-D-Trp-Ala-Trp-**(*S*)-BgI**-Lys-NH_2	2.45×10^{-5} M	2.65×10^{-5} M
23	**35**	His-D-Trp-Ala-Trp-**(*R*)-BgI**-Lys-NH_2	$>>10^{-5}$ M	9.40×10^{-5} M

[a]Reference (32).

Optimized alkylation and lactamization conditions for (*R*)-BgI peptides 32 and 33 (*vide infra*)

After Fmoc deprotection, as described above, the resin was taken out of its Kan, separated from the corresponding radio frequency tag microchip and split into two 5 mL microwave glass vessels. A solution of cyclic sulfamidate (*R*)-**4** (5 equiv.) and DIEA (1 equiv.) in THF (2 mL) was added, the reactors were sealed and irradiated under microwave at 60 °C for 1.5 h. The two batches of resin were then combined into a 12 mL syringe tube, washed with DMF (3 × 7 mL), MeOH (3 × 7 mL), DCM (3 × 7 mL), and with three cycles comprising washing with 2% AcOH in DMSO (7 mL) for 30 min and washing with MeCN (7 mL) for 5 min. The resin was split back into two 5 mL V-shaped microwave glass vessels fitted with a triangular stirring bar, treated with a DMSO/H_2O/AcOH 75:23:2 mixture (2 mL), and the vessels were sealed and irradiated under microwave at 100 °C for 3 h. The resin was then washed from the microwave vessels into a 12 mL plastic filtration tube fitted with a polyethylene frit and washed sequentially with DMF (3 × 7 mL), MeOH (3 × 7 mL), and DCM (3 × 7 mL) and dried *in vacuo*.

Resin capping and peptide completion

The resin in a 12 mL plastic filtration tube was swollen in DMF, suspended in a solution of di-*tert*-butyl dicarbonate (5 equiv.) in DMF (7 mL), treated with DIEA (10 equiv.), shaken for 1 h, filtered and washed sequentially with DMF (3 × 7 mL), MeOH (3 × 7 mL) and DCM (3 × 7 mL). The resin was then transferred back to a Kan with the corresponding frequency tag microchip. The peptide synthesis was then completed, and the N-terminal Fmoc group removed as described above.

Peptide cleavage

Each Kan was shaken in a separate 12 mL syringe tube containing TFA/H_2O/TES (7 mL, 95/2.5/2.5, v/v/v) for 2 h. The liquid was filtered off, the resin was washed with TFA (2 × 8 mL), and the combined filtrate was concentrated *in vacuo*. The resulting residue was dissolved in a minimum volume of TFA (~1 mL), transferred to a centrifuge tube and precipitated by the addition of ice-cold diethyl ether (40 mL). The suspension was then centrifuged, and the diethyl ether was carefully decanted from the tube. The precipitate was resuspended in cold diethyl ether, and the process was repeated twice. The resulting white solid was dissolved in water (10 mL) and freeze-dried to give a white fluffy solid that was purified by preparative RP-HPLC using the specified conditions.

(R)-AgI-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 (25)

(*R*)-AgI-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 (**25**) was prepared using the split-and-mix process as described above in IRORI Kans™. Microwave-assisted annulation gave the desired lactam-bridged peptide TFA salt (97 mg) in 19% crude purity as determined by analytical RP-HPLC (column A, 2–40% MeCN in H_2O, 0.1% FA, 60 min gradient). Final lactam peptide purification was carried out by preparative RP-HPLC (column C, 2–40% MeCN in H_2O, 0.1% FA, 60 min gradient) to give the desired FA salt product (8 mg, 14%) as a white fluffy solid. The purified product was analyzed by analytical RP-HPLC (column A) using both MeCN t_R 11.39 (0–60% MeCN in H_2O, 0.1% FA, 30 min gradient) and MeOH t_R 14.50 (0–60% MeOH in H_2O, 0.1% FA, 30 min gradient) and revealed >93% purity. HRMS calcd. m/z for $C_{44}H_{55}O_6N_{10}$ $[M+H]^+$ 819.4301, found 819.4308.

His-(R)-Agl-Ala-Trp-D-Phe-Lys-NH$_2$ (26)

His-(*R*)-Agl-Ala-Trp-D-Phe-Lys-NH_2 (**26**) was prepared as described above in an IRORI Kan^{TM}. Microwave-assisted annulation gave the desired lactam-bridged peptide TFA salt (178 mg) in 45% crude purity as determined by analytical RP-HPLC (column A, 0–20% MeCN in H_2O, 0.1% FA, 20 min gradient). Final purification was carried out by preparative RP-HPLC (column C, 0–20% MeCN in H_2O, 0.1% FA, 25 min gradient) to give the desired FA salt product (19 mg, 12%) as a white fluffy solid. The purified product was analyzed by analytical RP-HPLC (column A) using both MeCN t_R 8.80 (0–60% MeCN in H_2O, 0.1% FA, 30 min gradient) and MeOH t_R 10.03 (0–60% MeOH in H_2O, 0.1% FA, 30 min gradient) and revealed >99% purity. HRMS calcd. m/z for $C_{39}H_{52}O_6N_{11}$ $[M+H]^+$ 770.4096, found 770.4097.

His-D-Trp-(R)-Agl-Trp-D-Phe-Lys-NH$_2$ (27)

His-D-Trp-(*R*)-Agl-Trp-D-Phe-Lys-NH_2 (**27**) was prepared as described above in an IRORI Kan^{TM}. Microwave-assisted annulation gave the desired lactam-bridged peptide TFA salt (144 mg) in 30% crude purity as determined by analytical RP-HPLC (column A, 0–40% MeCN in H_2O, 0.1% FA, 15 min gradient). Purification was carried out by preparative RP-HPLC (column C, 0–40% MeCN in H_2O, 0.1% FA, 60 min gradient) to give the desired FA salt product (7 mg, 4%) as a white fluffy solid. The purified product was analyzed by analytical RP-HPLC (column A) using both MeCN t_R 10.60 (0–60% MeCN in H_2O, 0.1% FA, 30 min gradient) and MeOH t_R 13.77 (0–60% MeOH in H_2O, 0.1% FA, 15 min gradient) and revealed >98% purity. HRMS calcd. m/z for $C_{47}H_{58}O_6N_{12}$ $[M+2H]^{2+}$ 443.2296, found 443.2301.

His-D-Trp-Ala-(R)-Agl-D-Phe-Lys-NH$_2$ (28)

His-D-Trp-Ala-(*R*)-Agl-D-Phe-Lys-NH_2 (**28**) was prepared as described above in an IRORI Kan^{TM}. Microwave-assisted annulation gave the desired lactam-bridged peptide TFA salt (132 mg) in 61% crude purity as determined by analytical RP-HPLC (column A, 0–20% MeCN in H_2O, 0.1% FA, 35 min gradient). Purification was carried out by preparative RP-HPLC (column C, 0–20% MeCN in H_2O, 0.1% FA, 20 min gradient) to give the desired FA salt product (17 mg, 11%) as a white fluffy solid. The purified product was analyzed by analytical RP-HPLC (column A) using both MeCN t_R 8.68 (0–60% MeCN in H_2O, 0.1% FA, 30 min gradient) and MeOH t_R 9.77 (0–60% MeOH in H_2O, 0.1% FA, 30 min gradient) and revealed >99% purity. HRMS calcd. m/z for $C_{39}H_{52}O_6N_{11}$ $[M+H]^+$ 770.4096, found 770.4097.

His-D-Trp-Ala-Trp-(R)-Agl-Lys-NH$_2$ (29)

His-D-Trp-Ala-Trp-(*R*)-Agl-Lys-NH_2 (**29**) was prepared as described above in an IRORI Kan^{TM}. Microwave-assisted annulation over 90 min gave the desired lactam-bridged peptide TFA salt (101 mg) in 55% crude purity as determined by analytical RP-HPLC (column A, 0–20% MeCN in H_2O, 0.1% FA, 15 min gradient). Purification was carried out by preparative RP-HPLC (column C, 0–20% MeCN in H_2O, 0.1% FA, 20 min gradient) to give the desired FA salt product (11 mg, 7%) as a white fluffy solid. The purified product was analyzed by analytical RP-HPLC (column A) using both MeCN t_R 8.83 (0–60% MeCN in H_2O, 0.1% FA, 30 min gradient) and MeOH t_R 10.21 (0–60% MeOH in H_2O, 0.1% FA, 30 min gradient) and revealed >99% purity. HRMS calcd. m/z for $C_{41}H_{53}O_6N_{12}$ $[M+H]^+$ 809.4205, found 809.4199.

(R)-Bgl-D-Trp-Ala-Trp-D-Phe-Lys-NH$_2$ (30)

(*R*)-Bgl-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 (**30**) was prepared in an IRORI Kan^{TM} as described above. Microwave-assisted annulation over 180 min gave the desired lactam peptide TFA salt **30** (78 mg) in 30% crude purity as determined by analytical RP-HPLC (column A, 0–30% MeCN in H_2O, 0.1% FA, 20 min gradient). After final Fmoc deprotection, and before cleavage, half of the resin was kept for preparation of compound **31** (*vide infra*). Purification was carried out by preparative RP-HPLC (column C, 10–25% MeCN in H_2O, 0.1% FA, 50 min gradient, flow rate: 15 mL/min) to give the desired FA salt **30** (4.8 mg, 5%) as a white fluffy solid. The purified product was analyzed by analytical RP-HPLC (column A) using both MeCN t_R 15.50 (0–60% MeCN in H_2O, 0.1% FA, 25 min gradient) and MeOH t_R 13.50 (0–60% MeOH in H_2O, 0.1% FA, 25 min gradient) and revealed >97% purity. HRMS calcd. m/z for $C_{44}H_{55}O_6N_{10}$ $[M+H]^+$ 819.4301, found 819.4307.

Ac(R)-Bgl-D-Trp-Ala-Trp-D-Phe-Lys-NH$_2$ (31)

H_2N-(*R*)-Bgl-D-Trp(Boc)-Ala-Trp(Boc)-D-Phe-Lys(Boc)-NH-Rink (prepared in the synthesis of lactam peptide **30**) was acetylated using a solution of acetic anhydride (5 equiv.) and DIEA (5 equiv.) in DMF (4 mL) over 1.5 h. After washing, cleavage, and precipitation as described above, the desired lactam peptide TFA salt (68 mg) was obtained in 20% crude purity as determined by analytical RP-HPLC (column A, 0–30% MeCN in H_2O, 0.1% FA, 20 min gradient). Purification was carried out by preparative RP-HPLC (column C, 20–40% MeCN in H_2O, 0.1% FA, 40 min gradient, flow rate: 15 mL/min) to give the desired FA salt (7 mg, 7%) as a white fluffy solid. The purified product was analyzed by analytical RP-HPLC (column A) using both MeCN t_R 14.42 (0–80% MeCN in H_2O, 0.1% FA, 25 min gradient) and MeOH t_R 19.42 (0–90% MeOH in H_2O, 0.1% FA, 25 min gradient) and revealed >99% purity. HRMS calcd. m/z for $C_{46}H_{57}O_7N_{10}$ $[M+H]^+$ 861.4406, found 861.4413.

His-(R)-Bgl-Ala-Trp-D-Phe-Lys-NH$_2$ (32)

His-(*R*)-Bgl-Ala-Trp-D-Phe-Lys-NH_2 (**32**) was prepared in an IRORI Kan^{TM} using the optimized alkylation and lactamization conditions (*vide infra*), to give the desired lactam peptide TFA salt (112 mg) in 31% crude purity as determined by analytical RP-HPLC (column B, 0–80% MeCN in H_2O, 0.1% FA, 30 min gradient). Purification was carried out by preparative RP-HPLC (column D, 10–40% MeCN in H_2O, 0.1% FA, 30 min gradient) to give the desired FA salt product (16 mg, 8%) as a white fluffy solid. The purified product was analyzed by analytical RP-HPLC (column B) using both MeCN t_R 14.26 (0–60% MeCN in H_2O, 0.1% FA, 25 min gradient) and MeOH t_R 15.91 (0–60% MeOH in H_2O, 0.1% FA, 30 min gradient) and revealed >99% purity. HRMS calcd. m/z for $C_{39}H_{53}O_6N_{11}$ $[M+2H]^{2+}$ 385.7085, found 385.7083.

His-D-Trp-(R)-Bgl-Trp-D-Phe-Lys-NH_2 (33)

His-D-Trp-(*R*)-Bgl-Trp-D-Phe-Lys-NH_2 (**33**) was prepared in an IRORI Kan™ using the optimized alkylation and lactamization conditions (*vide infra*), to give the desired lactam peptide TFA salt (120 mg) in 22% crude purity as determined by analytical RP-HPLC (column B, 15–40% MeCN in H_2O, 0.1% FA, 30 min gradient). Purification was carried out by preparative RP-HPLC (column D, 8–30% MeCN in H_2O, 0.1% FA, 30 min gradient) to give the desired FA salt (14 mg, 8%) as a white fluffy solid. The purified product was analyzed by analytical RP-HPLC (column A) using both MeCN t_R 17.57 (0–60% MeCN in H_2O, 0.1% FA, 25 min gradient) and MeOH t_R 21.38 (0–80% MeOH in H_2O, 0.1% FA, 25 min gradient) and revealed >99% purity. HRMS calcd. m/z for $C_{47}H_{58}O_6N_{12}$ $[M+2H]^{2+}$ 443.2296, found 443.2301.

His-D-Trp-Ala-(R)-Bgl-D-Phe-Lys-NH_2 (34)

His-D-Trp-Ala-(*R*)-Bgl-D-Phe-Lys-NH_2 (**34**) was prepared in an IRORI Kan™ as described above. Microwave-assisted annulation over 180 min gave the desired lactam peptide TFA salt (180 mg) in 40% crude purity as determined by analytical RP-HPLC (column A, 0–30% MeCN in H_2O, 0.1% FA, 25 min gradient). Purification was carried out by preparative RP-HPLC (column C, 10–25% MeCN in H_2O, 0.1% FA, 20 min gradient) to give the desired FA salt product (9.6 mg, 5%) as a white fluffy solid. The purified product was analyzed by analytical RP-HPLC (column A) using both MeCN t_R 12.13 (0–60% MeCN in H_2O, 0.1% FA, 20 min gradient) and MeOH t_R 14.32 (0–60% MeOH in H_2O, 0.1% FA, 20 min gradient) and revealed >99% purity. HRMS calcd. m/z for $C_{39}H_{52}O_6N_{11}$ $[M+H]^+$ 770.4096, found 770.4095.

His-D-Trp-Ala-Trp-(R)-Bgl-Lys-NH_2 (35)

His-D-Trp-Ala-Trp-(*R*)-Bgl-Lys-NH_2 (**35**) was prepared in an IRORI Kan™ as described above. Microwave-assisted annulation over 180 min gave the desired lactam peptide TFA salt (67 mg) in 60% crude purity as determined by analytical RP-HPLC (column A, 0–30% MeCN in H_2O, 0.1% FA, 25 min gradient). Purification was carried out by preparative RP-HPLC (column C, 0–30% MeCN in H_2O, 0.1% FA, 25 min gradient) to give the desired FA salt (5.4 mg, 3%) as a white fluffy solid. The purified product was analyzed by analytical RP-HPLC (column A) using both MeCN t_R 12.05 (0–60% MeCN in H_2O, 0.1% FA, 20 min gradient) and MeOH t_R 14.11 (0–60% MeOH in H_2O, 0.1% FA, 20 min gradient) and revealed >99% purity. HRMS calcd. m/z for $C_{41}H_{53}O_6N_{12}$ $[M+H]^+$ 809.4205, found 809.4198.

Membrane preparation for GHS-R1a receptor

Transfection LLC-PK1 cells were seeded at 1.5×10^6 cells/10-cm Petri dishes and grown for 24 h in DMEM high-glucose (4.5 g/L) with 10% fetal bovine serum supplemented with penicillin (10 000 Units/mL) and streptomycin (10 000 μg/mL), and cultured at 37 °C, with 5% CO_2. Four to 5 h after replacement of the medium, transfection was performed using $Ca_3(PO_4)_2$ calcium phosphate. The DNA solution consisted of 40 μg of DNA in a volume of 500 μL in which 500 μL of 2 mM Tris–HCl pH 8.0, 0.2 mM EDTA pH 8.0 containing 500 mM $CaCl_2$ was added to a final volume of 1 mL, followed by 1 mL of 50 mM Hepes, 280 mM NaCl, 1.5 mM Na_2HPO_4 pH to 7.1 (HBSS) by alternating one drop/two air bubbles. The transfection mix reaction was incubated at RT for 30 min. After the incubation period, 1 mL of the mix was added to each plate and distributed evenly for incubation. The media was replaced with standard DMEM-high glucose media for another 24 h, and cells were collected for membrane preparation.

Membrane preparation

The experiment was carried out at 4 °C unless specified. Cells were washed two times with PBS and with the homogenization buffer (HB) consisting of 50 mM Tris, 5 mM $MgCl_2$, 2.5 mM EDTA, 30 μg/mL bacitracin at pH 7.3 and were scraped in Eppendorf tubes. Cells were lyzed with two cycles of freeze/thawing using liquid nitrogen and were then centrifuged at 4 °C for 20 min at 10 000 × *g* to collect the membranes. The membranes were resuspended in a small volume of HB and aliquoted for storage at −80 °C.

GHS-R1a receptor binding assay

The competitive binding assay consists of 200 μL HB, 100 μL ^{125}I-Ghrelin (40,000 cpm), 100 μL competitive ligand (from 10^{-12} to 10^{-5} M), and 100 μL of GHS-R1a transiently transfected in LLC-PK1 cells as source of binding sites (10 μg protein/tube). The non-specific binding was determined by excess of competitive ligand at 10^{-5} M. The reaction was carried out at RT for 1 h. After the incubation period, the separation of bound from free fraction was performed by filtration over a GF/C filter presoaked with 0.5% polyethyleneimine, and the filters were washed with 4 mL of HB consisting of 50 mM Tris, 10 mM $MgCl_2$, 2.5 mM EDTA, and 3 mL of wash buffer consisting of 50 mM Tris–10 mM $MgCl_2$, 20 mM EDTA, 0.015% Triton X100 (pH 7.3) and were then collected for radioactivity counting using a gamma scintillation counter (LKB Wallac 1277, Turku Finland).

Membrane preparation for CD36

Animal use was in accordance with the Institutional Animal Ethics Committee and the Canadian Council on Animal Care guidelines for the use of experimental animals. Sprague Dawley (275–350 g) rats were anaesthetized with sodium pentobarbital, and their hearts were promptly removed in ice-cold saline, and the cardiac membranes were prepared according to Harigaya and Schwartz (41).

Competitive covalent CD36 binding assay using photoactivatable [^{125}I]-Tyr-Bpa-Ala-Hexarelin as radioligand

The radioiodination procedure of the photoactivatable ligand and the receptor binding assays were performed as previously described by Ong *et al.* (42). Briefly, the rat cardiac membranes (200 μg) as source of CD36 were incubated in the dark, in 525 μL of 50 mM Tris–HCl pH 7.4 containing 2 mM EGTA (Buffer A) in the presence of a fixed concentration of [^{125}I]-Tyr-Bpa-Ala-Hexarelin (750 000 cpm) in Buffer B (50 mM Tris–HCl pH 7.4 containing 2 mM EGTA and 0.05% Bacitracin) and with increasing concentrations of competitive ligands (ranging from 0.1 to 50 μM). Non-specific binding

was defined as binding not displaced by 50 μM peptide. After an incubation period of 60 min at 22 °C, membranes were submitted to UV irradiation at 365 nm for 15 min at 4 °C. After centrifugation at 12 000 g for 15 min, the pellets were resuspended in 100 μL of sample buffer consisting of 62 mM Tris–HCl, pH 6.8, 2% SDS, 10% glycerol, 15% 2-mercapto-ethanol, and 0.05% bromophenol blue, and boiled for 5 min, prior to being subjected to electrophoresis on 7.5% SDS-PAGE. The SDS-PAGE gels were fixed, colored in Coomassie Brilliant Blue R-250, dried, exposed to a storage phosphor intensifying screen (Amersham Biosciences), and analysed by using a Typhoon PhosphorImager (Amersham Biosciences) and IMAGEQUANT 5.0 software[d] to establish competition curves. Protein bands corresponding to the specifically labeled protein of 87 kDa were quantified by densitometry analysis.

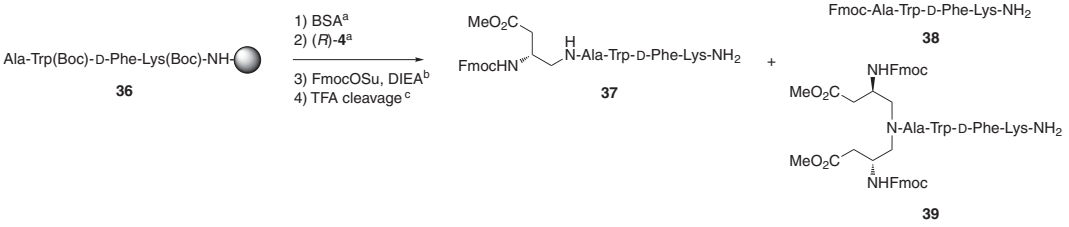
Results and discussion

Optimized protocol

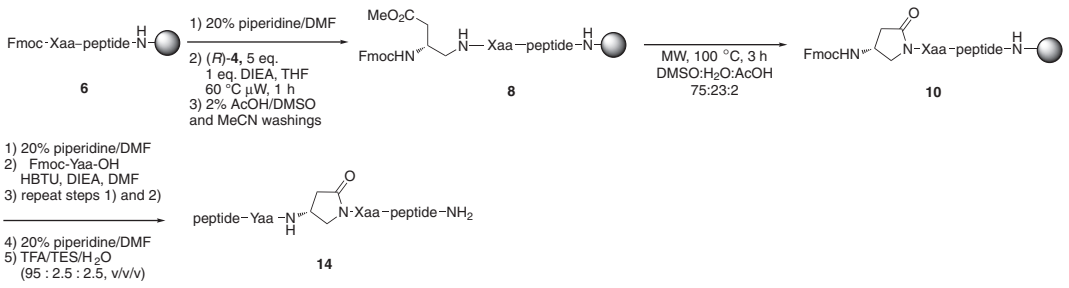
Using our standard protocol, lactam peptides **25**–**31** and **34**–**35** have been prepared in moderate to good crude purity and isolated in acceptable yields (Table 2). However, the syntheses of peptides **32** and **33** were problematic, giving product in low crude purity (≈5%), both from IRORI Kan^{TM} and syringe tubes. This led us to retool our method for difficult Bgl synthesis cases, beginning by monitoring the alkylation step protocol using LC-MS. Considering that quantification depended on the presence of a chromophore on starting material and product, direct cleavage of small resin sample was deemed biased, because it afforded alkylation product bearing a UV active Fmoc group and starting peptide without Fmoc group. A Fmoc reprotection step was thus performed before cleavage by treating the resin aliquot with a solution of *N*-(9-fluorenyl-methoxycarbonyloxy) succinimide (Fmoc-OSu) and DIEA (Scheme 2). Optimization of the alkylation conditions involved varying the stoichiometry of BSA and (*R*)-**4**, the temperature, the reaction time, and the use of base as additive. Alkylation at the Ala residue of GHRP-6 with (*R*)-**4** was used for the optimization experiments (peptide **36**, Scheme 2; Table 1).

The silylation time could be reduced to 1 h (entry 2) and the alkylation time to 1 h (entry 3) by performing the alkylation at 60 °C under microwave irradiation without affecting the conversion to secondary amine **37**. Conversion to the latter was however halved by conducting the reaction at room temperature (entry 4). The silylation step could be removed without any significant change in the ratio of non-alkylated and mono alkylated product **38**:**37** (around 1:1) without bis-alkylation product **39** (entry 6). We considered that the sulfamic acid, formed by the opening of the cyclic sulfamidate, protonated the terminal primary amines preventing alkylation leading to only 50% conversion. Employment of DIEA (1 equiv., entry 7) as base confirmed our hypothesis and gave 80% conversion to **37**. However, increasing the amount of base to 1.2 equiv. gave rise to a noticeable amount of bis-alkylation product **39** (21%, entry 8), indicating that excess base should be avoided. In these studies, ACS grade THF worked as effectively as dry THF.

The alkylated peptidyl resin from conditions described in entry 7 of Table 2 was subjected to lactam formation in 1% AcOH/DMSO at 100 °C under microwave for 3 h (32). LC-MS analysis of a cleaved sample indicated the presence of the expected lactam compound along with the corresponding Fmoc-deprotected lactam, accounting, respectively, for 75% and 16% of the mixture. A plausible explanation would be the presence of DIEA trapped on the resin as an ammonium sulfamate salt. Thermal decomposition of the sulfamate during microwave irradiation would liberate free DIEA accountable for Fmoc deprotection at 100 °C. Improvements in lactam formation yields were obtained by washing the resin beforehand with three



Scheme 2: Alkylation reaction of peptide **36** with cyclic sulfamidate (*R*)-**4**. [a]see table 2 for condition; [b]with *N*-(9-fluorenyl-methoxycarbonyloxy)succinimide (Fmoc-OSu, 0.04M in DCM, 1 mL), DIEA (3 drops), 1 h, RT; [c]TFA/H2O/TES 95/2.5/2.5, 2h, RT.



Scheme 3: Optimized protocol for the synthesis of Bgl-peptides.

cycles of 2% AcOH in DMSO (7 mL) before lactamization, and by performing the microwave irradiation at 100 °C for 3h in a DMSO/H_2O/AcOH 75:23:2 mixture. Addition of water to the lactamization mixture proved to enhance conversion to the lactam compounds by 5–10%.

Using this optimized protocol (Scheme 3) gave peptides **32** and **33,** respectively, in 35% and 21% crude purity; both being isolated in 8% yield after purification on preparative RP-HPLC.

Biology

The affinity of AgI- and BgI-containing GHRP-6 analogs **25**–**35** for the ghrelin receptor was assessed in a competition-binding study using radiolabelled ghrelin [1–28] as radioligand and a GHS-R1a transfected cell membrane as source of binding site (43). The affinity of the GHRP-6 amino lactam peptides for the receptor CD36 has been assessed by covalent competition binding study using a radiolabelled photoactivatable hexarelin derivative as radioligand and rat cardiac membranes as source of CD36 receptor as previously reported (15). GHRP-6 lactam analogs were used as competitive ligands in the range concentrations from 10^{-12} to 10^{-6} M and IC_{50} values of competition binding curves were calculated by iterative nonlinear curve-fitting program[e].

Lactam analogs generally lost affinity for both the GHS-R1a and CD36 receptors. The (*S*)-AgI and (*R*)-BgI peptides exhibited generally better affinity for the CD36 receptor than their enantiomeric counterparts. In the case of the GHS-R1a receptor, the (*S*)-lactam peptide displayed better affinities.

At His^1, the (*S*)-AgI and (*S*)-BgI peptides **15** and **20** (entries 3 and 5) displayed a 10- to 100-fold loss of affinity towards the ghrelin receptor (32), as observed with previous analogs featuring substitutions at this position (44); however, (*R*)-AgI^1, (*R*)-BgI^1, and Ac(*R*)-BgI^1 peptides (peptides **25**, **30** and **31**) caused a drop of affinity of 2 to 6×10^3 for the GHS-R1a receptor. (*S*)-AgI^1 and (*R*)-BgI^1 peptides **15** and **30** conserved some affinity for the CD36 receptor.

Replacement of the Trp residues by AgI and BgI of both configurations was particularly detrimental to the binding affinity at the GHS-R1a receptor (entries 8–11 and 16–19) likely because of the importance of the indolic side chains for interaction with the ghrelin receptor. The same trend was observed for binding to CD36 receptor with the exception of (*S*)-AgI^2 **16** (entries 8) that exhibited a fourfold reduced affinity compared with hexarelin.

Similarly, substitution of $D\text{-}Phe^5$ by (*S*)-AgI (**19**), (*R*)-AgI (**29**), and (*S*)-BgI (**24**) (entries 20–22) all caused significant loss of affinity for the GHS-R1a receptor, yet, only a factor of 10 loss in binding to the CD36, receptor compared with hexarelin for **19** and **24**. The residue at position 5 could play a role for selectivity towards CD36. (*R*)-BgI peptide **35** lost affinity for both receptors.

(*S*)-AgI^3 peptide **17** (32) and (*R*)-BgI^3 peptide **33** only lost, respectively, five and sixfold affinity for CD36 yet nearly 500-fold affinity for the GHS-R1a receptor. Because both AgI and BgI may induce turn structures, binding of these analogs are consistent with the hypothesis of a β-turn about residues 2–4 of GHRP-6, which may account for binding and differentiating the CD36 receptor from the GHS-1Ra receptor (45).

Conclusion

A (*R*)-AgI and (*R*)-BgI positional scan of GHRP-6 was efficiently accomplished using IRORI Kan™ techniques and cyclic sulfamidates (*R*)-**3** and (*R*)-**4** to produce eleven new GHRP-6 analogs in good overall yields and crude purities. Improvements in the synthesis of BgI were made by microwave heating in the presence of a base during the alkylation step and introduction of a mild acid wash before microwave-assisted annulation in a DMSO/H_2O/AcOH 75:23:2. The new compounds were evaluated for their binding affinity towards the GHS-R1a and the CD36 receptors. Results emphasized the importance of Trp residues for affinity especially with the ghrelin receptor. The study yielded compounds selective for the CD36 receptor relative to GHS-R1a, typically with substitution of Ala^3 and $D\text{-}Phe^5$ with both AgI and BgI. In the structure–affinity relationship contingent on stereochemistry and sequence, changes in selectivity were observed using AgI and BgI residues, suggesting that they may induce complementary turn structures. AgI and BgI lactam scanning has thus identified new leads for selective GHRP-6 mimics as well as a better understanding of the importance of side chains and conformation on affinity and selectivity.

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Notes

[a]This vessel and stirring bar size and shape combination provided the best stirring efficiency and yields as well as avoided resin grinding. Efficient stirring was also insured by loading the reactors with a maximum of 150 mg of resin when 70–90 mesh resin was used. If 100–200 mesh resin is used, the reactor can be loaded with up to 300 mg of resin.
[b]http://www.nexusbio.com/Products/CombiChem/irori_cc_kan_reactors.php.
[c]SYNTHESIS MANAGER™ Nexus Biosystems, Inc., Poway, CA, USA.
[d]IMAGEQUANT 5.0, GE Healthcare, Piscataway, NJ, USA.
[e]GRAPHPAD PRISM 3, GraphPad Software, Inc., La Jolla, CA, USA.