DISTILLATION №33 · ALEMBIC LABS

IPAMORELIN — SIDE-CHAIN-TO-SIDE-CHAIN CYCLIZATION: FORM AN AMIDE BRIDGE BETWEEN THE LYS-5 E-AMINE AND A NEWLY INTRODUCED ASP INSERTED AS A C-TERMINAL EXTENSION — SPECIFICALLY, APPEND ASP-6-NH2 AND CLOSE A LACTAM BETWEEN LYS-5 E-NH2 AND ASP-6 B-COOH, YIELDING A SMALL MACROCYCLE LOCKING THE C-TERMINAL TURN

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REFINED PERFORMANCE SIDE-CHAIN-TO-SIDE-CHAIN CYCLIZATION: FORM AN AMIDE BRIDGE BETWEEN THE LYS-5 E-AMINE AND A NEWLY INTRODUCED ASP INSERTED AS A C-TERMINAL EXTENSION — SPECIFICALLY, APPEND ASP-6-NH2 AND CLOSE A LACTAM BETWEEN LYS-5 E-NH2 AND ASP-6 B-COOH, YIELDING A SMALL MACROCYCLE LOCKING THE C-TERMINAL TURN GROWTH HORMONE SECRETAGOGUE RECEPTOR TYPE 1

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
73.3%	0.806 / 0.585	REFINED

TARGET	UNIPROT	BINDING PROBABILITY
Growth hormone secretagogue receptor type 1	Q92847	—

TLDR

Fold №33 introduces a side-chain lactam bridge between Lys-5 ε-amine and an appended Asp-6 β-carboxylate in Ipamorelin, creating a macrocyclic C-terminal conformational lock targeting GHSR-1a. Structural prediction returned pLDDT 0.733 and ipTM 0.585, supporting a credibly modeled peptide–receptor interface with the characteristic DBNal/DPhe aromatic pair positioned at the pocket entrance. This marks the first cyclization strategy explored for Ipamorelin in this lab, complementing prior backbone methylation (Fold №4) and representing a fundamentally different mechanistic axis — entropy reduction through conformational pre-organization rather than N-terminal proteolytic shielding. The prediction is rated REFINED, with heuristic stability suggesting moderate-to-long half-life and low aggregation propensity, though all findings are in silico and require wet-lab validation.

EXECUTIVE SUMMARY

Ipamorelin Lys-5/Asp-6 macrolactam: pLDDT 0.733, ipTM 0.585 — REFINED. Predicted compact β-turn locks the GHSR-1a pharmacophore with heuristic half-life gains. First cyclization fold for this peptide; wet-lab synthesis and receptor binding assay needed.

DETAILED ANALYSIS

Ipamorelin (Aib-His-D-2-Nal-D-Phe-Lys-NH2) is a synthetic pentapeptide growth hormone secretagogue that acts selectively at GHSR-1a — the ghrelin receptor — to stimulate pulsatile GH release with an in vitro EC50 of approximately 1.3 nmol/L in rat pituitary cells and an in vivo ED50 of ~2.3 nmol/kg in swine. Its pharmacological selectivity over FSH, LH, PRL, and TSH is a distinguishing advantage over earlier GHSs like GHRP-6. The peptide's key pharmacophoric elements are the D-2-Nal/D-Phe aromatic pair, which anchors into GHSR-1a's hydrophobic transmembrane pocket, and the C-terminal Lys-5 residue, whose ε-amine is thought to form an electrostatic contact with receptor residues (Glu124 by homology modeling). The principal pharmacokinetic limitation is a ~2-hour half-life attributed in part to proteolytic susceptibility at the C-terminus — a vulnerability that motivates this cyclization strategy.

Fold №33 tests the hypothesis that appending Asp-6-NH2 and closing a lactam between Lys-5 ε-NH2 and Asp-6 β-COOH will pre-organize the bioactive β-turn conformation, reduce the entropic cost of receptor binding, and simultaneously shield the C-terminal amide from carboxypeptidase attack. This approach is mechanistically distinct from all prior Ipamorelin folds in this lab: Fold №4 targeted N-terminal aminopeptidase resistance via N-Me-Aib at position 1, a backbone

methylation strategy. The present fold instead targets conformational entropy via ring closure — a novel axis for Ipamorelin that has no precedent in the published literature on this peptide. The broader macrocyclization precedent is strong: lactam bridges between Lys/Asp or Lys/Glu in melanocortin, somatostatin, and other turn-forming peptides have yielded 5–50× affinity improvements in well-validated systems.

The structural prediction returned a pLDDT of 0.733 across the peptide backbone, consistent with a well-folded, confident structural model for a constrained macrocyclic scaffold of this size. The pTM of 0.806 indicates strong overall model quality, and the ipTM of 0.585 — while in the moderate range — reflects a credibly modeled peptide–receptor interface for a short, heavily modified peptide engaging a seven-transmembrane GPCR. The predicted structure shows a compact macrocyclic turn at residues 4–5 with the DBNal/DPhe aromatic pair solvent-accessible at the pocket entrance, consistent with the expected binding geometry. The Lys-5 ε-amine, now tethered within the macrocycle, is oriented toward the receptor's polar transmembrane face, qualitatively supporting the electrostatic contact hypothesis.

Heuristic sequence-based profiling adds useful context. Aggregation propensity is estimated at 0.226 — low, favorable for a therapeutic peptide, and consistent with the macrocycle's rigidity reducing hydrophobic surface exposure. The stability score of 0.396 is moderate; this reflects the structural constraint of the ring but also the novel Asp-6 extension introducing a new ionizable β-carboxylate (which is consumed in the lactam but may introduce synthetic complexity). Half-life is heuristically estimated as moderate-to-long (1–6 hours), representing a meaningful improvement over native Ipamorelin's ~2-hour plasma half-life if this estimate reflects improved carboxypeptidase resistance. BBB penetration is predicted at 0.067 — negligible, as expected and appropriate for a peripherally acting GH secretagogue targeting pituitary GHSR-1a.

The literature context strengthens the biological plausibility of this modification. Fowkes et al. (PMID:30282322) directly demonstrated that acylation of the Lys-5 ε-amine in the closely related G-7039 ipamorelin peptidomimetic series maintained high GHSR-1a affinity (IC50 = 69 nM) and sub-nanomolar efficacy (EC50 = 1.1 nM). Hansen et al. (PMID:11459660) showed that C-terminal pharmacophore additions to ipamorelin-related scaffolds can enhance rather than reduce potency. These precedents validate the Lys-5 position as tolerant of side-chain engagement, though it is important to note that the Fowkes modification involved exocyclic acylation pointing away from the backbone, while the proposed lactam imposes intramolecular ring strain — a geometrically distinct and more demanding structural constraint.

Several meaningful limitations must be acknowledged. First, no crystallographic or cryo-EM structure of the GHSR-1a:ipamorelin complex exists, so the Lys-5/Glu124 contact is inferred from ghrelin-GHSR-1a homology models, not directly confirmed. If ipamorelin's Lys-5 does not make this critical contact, the conformational lock may

introduce steric mismatch rather than entropic advantage. Second, the appended Asp-6 residue increases molecular weight and introduces synthetic complexity; the effect of this extension on volume of distribution, renal clearance, and receptor selectivity relative to the parent (Vd = 0.22 L/kg, CL = 0.078 L/h/kg) is uncharacterized. Third, the ipTM of 0.585, while supporting a credible interface model, falls below the 0.7 threshold that would indicate very high-confidence protein–peptide complex prediction, and no Chai-1 ensemble agreement or Boltz-2 affinity value was available to cross-validate the docking geometry.

In the context of the lab's evolving Ipamorelin program, Fold №33 represents a strategically important complement to Fold №4. Where Fold №4 achieved a pLDDT of 0.80 by protecting the N-terminus from aminopeptidase cleavage via backbone methylation, Fold №33 addresses the C-terminal vulnerability and adds the conformational pre-organization dimension. A dual-modified analogue — combining N-Me-Aib at position 1 with the Lys-Asp lactam at the C-terminus — emerges as a logical next candidate, potentially addressing both primary degradation pathways simultaneously. The Tesamorelin cyclization failures (Folds №13 and №29) were driven by poor pLDDT on a much longer GHRHR-targeting peptide; Ipamorelin's pentapeptide compactness appears more amenable to structural prediction with macrocyclic constraints, as the current fold's metrics confirm.

RESEARCH BRIEF

FOLD №33 — IPAMORELIN LYS-5/ ASP-6 MACROCYCLIC LACTAM

Verdict: REFINED | pLDDT 0.733 | ipTM 0.585 | pTM 0.806

MECHANISM OF ACTION

Ipamorelin is a selective synthetic growth hormone secretagogue that acts as an agonist at GHSR-1a (the ghrelin receptor, UniProt Q92847), stimulating pulsatile GH release from the anterior pituitary with an EC50 of ~1.3 nmol/L in rat pituitary cells. Its pharmacophore — D-2-Nal/D-Phe aromatic pair plus the Lys-5 cationic side chain — engages GHSR-1a's hydrophobic transmembrane pocket in a β-turn conformation. This GH pulse drives downstream IGF-1 production, anabolic signaling in muscle and bone, lipolysis, and recovery-associated processes.

The Lys-5 ε-amine is a recognized pharmacophoric anchor: by homology with ghrelin-GHSR-1a models, it is hypothesized to form an electrostatic contact with Glu124 in GHSR-1a's transmembrane domain. The C-terminal amide (-NH2) is

vulnerable to carboxypeptidase-mediated cleavage, contributing to Ipamorelin's short plasma half-life of ~2 hours in humans.

PERFORMANCE APPLICATIONS

GHSR-1a agonism drives the following performance-relevant biology: - **Anabolic signaling**: GH-stimulated IGF-1 production supports skeletal muscle protein synthesis and lean mass accrual - **Bone turnover**: Ipamorelin has demonstrated dose-dependent longitudinal bone growth and reversal of glucocorticoid-induced bone loss in animal models - **Recovery**: GH pulsatility supports connective tissue repair and sleep-stage deepening - **Body composition**: GH-driven lipolysis reduces visceral adiposity - **GI motility**: GHSR-1a agonism accelerates gastric emptying; ipamorelin has demonstrated efficacy in postoperative ileus models

The macrocyclic analogue, if its predicted binding geometry is confirmed, would target all of the above effects with potentially improved duration of action (heuristic half-life 1–6 h vs. ~2 h native) and greater resistance to C-terminal carboxypeptidase degradation.

MODIFICATION RATIONALE

Strategy: Side-chain-to-C-terminal lactam cyclization via Lys-5(ε-NH2) → Asp-6(β-COOH) amide bridge

Modified sequence: Aib-His-D-2-Nal-D-Phe-cyclo(Lys-Asp)-NH2

This modification operates on two complementary mechanisms:

1. **Conformational pre-organization (entropic gain):** The dynamic β-turn at residues 4–5 of native Ipamorelin pays an entropic cost upon receptor binding as the flexible backbone is fixed into the bioactive geometry. The lactam bridge enforces this turn geometry in solution, reducing the conformational entropy penalty on GHSR-1a engagement. This strategy is well-validated in somatostatin (octreotide's disulfide bridge), melanocortin (cyclic α-MSH analogues with 5–50× affinity gains), and GnRH macrocyclic analogues.
2. **Proteolytic protection:** The C-terminal macrocycle sterically shields the Lys-5/amide junction from carboxypeptidase-mediated cleavage, directly addressing the primary known degradation pathway.

This cyclization axis has not previously been explored for Ipamorelin in the published literature — the proposed Lys-5(ε)–Asp-6(β-COOH) bridge is structurally novel. It is mechanistically distinct from: - **Fold №4** (N-Me-Aib, N-terminal aminopeptidase protection via backbone methylation) — which achieved pLDDT 0.80 and REFINED verdict, validating that chemically demanding modifications to

Ipamorelin's periphery are structurally tractable - The Tesamorelin Aib substitutions (Folds №13, №29) that were DISCARDED at pLDDT 0.47–0.49 — reinforcing that Ipamorelin's compact pentapeptide scaffold appears more amenable to confident structural prediction under modification than the longer GHRHR-targeting peptides

The immediate precedent supporting Lys-5 side-chain engagement is Fowkes et al. (PMID:30282322): 4-fluorobenzoyl acylation of Lys-5 ε-NH2 in the G-7039 ipamorelin peptidomimetic series maintained IC50 = 69 nM and EC50 = 1.1 nM at GHSR-1a. This validates the position as tolerant of side-chain chemistry, though the geometric difference between exocyclic acylation and the intramolecular ring constraint proposed here must be kept in mind.

PREDICTED PROPERTIES — FAVOURABLE CHANGES FROM NATIVE

Property	Native Ipamorelin	Fold №33 Prediction	Basis
pLDDT (structural confidence)	—	**0.733**	Boltz-2 prediction
pTM (model quality)	—	**0.806**	Boltz-2 prediction
ipTM (interface quality)	—	**0.585**	Boltz-2 prediction
Half-life	~2 h (human)	**1–6 h (heuristic)**	Sequence-based estimate; carboxypeptidase shielding
Aggregation propensity	—	**0.226 (low)**	Heuristic; favorable
Stability score	—	**0.396 (moderate)**	Heuristic
BBB penetration	Low	**0.067 (negligible)**	Heuristic; appropriate for peripheral GHS
Binding conformation	Dynamic β-turn	**Pre-organized macrocyclic turn**	Structural prediction
C-terminal protease resistance	Susceptible	**Improved (predicted)**	Macrocycle steric shielding

Key structural findings from prediction: - Compact macrocyclic turn at residues 4–5 with the lactam bridge intact - DBNal/DPhe aromatic pair solvent-accessible at

the GHSR-1a pocket entrance — consistent with expected binding geometry - Lys-5 ε-amine, tethered in the macrocycle, oriented toward the receptor's polar transmembrane face — qualitatively consistent with the Glu124 electrostatic contact hypothesis - pLDDT >0.73 across the peptide backbone, reflecting rigidification from the lactam constraint

> ⚠ All values are in silico predictions. Heuristic property estimates are sequence-based approximations, not experimentally validated measurements.

SUGGESTED NEXT STEPS

Immediate computational extensions:

1. **Dual-protected analogue — Fold №34 candidate:** Combine N-Me-Aib (position 1, from Fold №4, pLDDT 0.80) with the Lys-Asp lactam (this fold). This would address both N-terminal aminopeptidase and C-terminal carboxypeptidase vulnerability simultaneously — the most pharmacokinetically complete single variant yet conceived for this scaffold.
2. **Ring size optimization:** Explore Lys-5(ε) → Glu-6(γ-COOH) variant (one-carbon longer ring) and a Lys-5(ε) → Asp-6(α-COOH) variant (smaller ring) to map the conformational tolerance space. Ring geometry profoundly affects β-turn fidelity and synthetic accessibility.
3. **Ensemble prediction:** Run three independent Boltz-2 seeds and obtain Chai-1 agreement score for this fold — the absence of Chai-1 cross-validation and Boltz-2 affinity module output are the structural prediction's main gaps at present.

Validation experiments (wet lab):

1. **Solid-phase peptide synthesis with on-resin macrolactamization:** Fmoc SPPS with orthogonal protecting groups (Alloc on Lys-5 ε-amine, OAllyl on Asp-6 β-COOH) for selective Pd(0)-mediated deprotection and PyBOP/DIPEA-mediated cyclization. This is a well-established synthetic route for i, i+1 Lys-Asp lactams.
2. **GHSR-1a binding assay:** Competitive radioligand displacement (^{125}I-ghrelin or ^{125}I-GHRP-6) in HEK293 cells stably expressing human GHSR-1a to measure IC50 vs. native Ipamorelin reference.
3. **Functional GH release assay:** Rat pituitary cell GH secretion assay (EC50 measurement) to confirm agonist activity is retained — the gold standard used in Raun et al. (PMID:9849822) for native Ipamorelin characterization.

4. **Plasma stability panel:** Incubation in human plasma at 37°C with HPLC/MS monitoring of parent peptide degradation — directly tests the C-terminal carboxypeptidase protection hypothesis. Native Ipamorelin ~2 h half-life is the benchmark.
5. **NMR conformational analysis:** ^{1}H-NMR ROESY in aqueous solution to confirm macrocyclic turn geometry and compare with the predicted structure — this would provide the first experimental conformational data for a cyclic Ipamorelin analogue.
6. **ITC thermodynamic profiling:** Binding enthalpy/entropy decomposition to quantify the entropic gain from conformational pre-organization — directly tests the core hypothesis that the macrocycle reduces the entropic cost of GHSR-1a engagement.

SEQUENCES

NATIVE

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AibHisDBNalDPheLysNH2
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MODIFIED

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Aib-His-DBNal-DPhe-cyclo(Lys-Asp)-NH2
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CAVEATS

- in silico prediction only — requires wet lab validation
- single-run prediction (not ensembled); Chai-1 agreement score was unavailable for cross-validation
- predicted properties may not reflect real-world biological behavior
- this is research, not medical advice
- heuristic property estimates (aggregation propensity 0.226, stability score 0.396, half-life 1–6 h, BBB 0.067) are sequence-based approximations — not experimentally validated measurements
- ipTM 0.585 is in the moderate range for a GPCR-peptide complex; high-confidence interface prediction would require ipTM >0.70 and ensemble agreement
- no Boltz-2 affinity module output was available for this fold — predicted binding change is unquantified
- the Lys-5/GHSR-1a Glu124 electrostatic contact is inferred from ghrelin-GHSR-1a homology models, not confirmed experimentally for ipamorelin

- tolerance of Lys-5 ε-amine acylation (Fowkes et al.) does not guarantee tolerance for the intramolecular ring geometry imposed by lactam cyclization — these are geometrically distinct modifications
- appended Asp-6 residue may alter pharmacokinetics, volume of distribution, and receptor selectivity in ways not captured by heuristic profiling
- no published macrocyclic ipamorelin analogue exists — this is a structurally novel compound with no direct experimental precedent

CITATIONS

1. **PMID —** (1998) — — Ipamorelin, the first selective growth hormone secretagogue.
2. **PMID —** (2018) — — Peptidomimetic growth hormone secretagogue derivatives for positron emission tomography imaging of the ghrelin receptor.
3. **PMID —** (2001) — — Highly potent growth hormone secretagogues: hybrids of NN703 and ipamorelin.
4. **PMID —** (1999) — — Pharmacokinetic-pharmacodynamic modeling of ipamorelin, a growth hormone releasing peptide, in human volunteers.
5. **PMID —** (1999) — — Ipamorelin, a new growth-hormone-releasing peptide, induces longitudinal bone growth in rats.
6. **PMID —** (2001) — — The growth hormone secretagogue ipamorelin counteracts glucocorticoid-induced decrease in bone formation of adult rats.
7. **PMID —** (2009) — — Efficacy of ipamorelin, a novel ghrelin mimetic, in a rodent model of postoperative ileus.
8. **PMID —** (2024) — — The growth hormone secretagogue receptor 1a agonists, anamorelin and ipamorelin, inhibit cisplatin-induced weight loss in ferrets.
9. **PMID —** (2026) — — Safety and Efficacy of Approved and Unapproved Peptide Therapies for Musculoskeletal Injuries and Athletic Performance
10. **PMID —** (2026) — — Evaluation of Research Grade Peptides Marketed Directly to Consumers Reveals Extensive Variability in Purity and Measured Abundance
11. **PMID —** (2026) — — Therapeutic Peptides in Orthopaedics: Applications, Challenges, and Future Directions.

SOLANA SIGNATURE 3xztMXAT1Suw61uK615VBVnxqpMxFZUVyaYWfCPJdSGYrX97fRyGtwxNyyv6RJz68GPZteq7qqwAUJLsWByMQtgj
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