DISTILLATION №70 · ALEMBIC LABS

IPAMORELIN — LYS-5 → ARG SINGLE SUBSTITUTION AT THE C-TERMINAL BASIC RESIDUE (YIELDING AIB-HIS-DBNAL-DPHE-ARG-NH2)

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REFINED | PERFORMANCE

LYS-5 → ARG SINGLE SUBSTITUTION AT THE C-TERMINAL BASIC RESIDUE (YIELDING AIB-HIS-DBNAL-DPHE-ARG-NH2)

GROWTH HORMONE SECRETAGOGUE RECEPTOR TYPE 1 (GHSR-1A)

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
79.5%	0.862 / 0.731	REFINED

TARGET	UNIPROT	BINDING PROBABILITY
Growth hormone secretagogue receptor type 1 (GHSR-1a)	Q92847	—

TLDR

Fold №70 tests a single Lys-5 → Arg substitution in Ipamorelin (yielding Aib-His-DBNal-DPhe-Arg-NH2) as a strategy to enhance GHSR-1a binding affinity through guanidinium-mediated salt-bridge contacts with acidic pocket residues. Boltz-2 predicted a well-resolved complex with pLDDT 0.79 and ipTM 0.73, supporting confident interface geometry and a stable docked pose. The Arg-5 guanidinium is positioned toward the TM3 acidic face, consistent with the bidentate salt-bridge hypothesis, while the DBNal-DPhe hydrophobic core is preserved. This fold advances the Ipamorelin lab narrative by being the first to directly probe affinity-driving electrostatics at the C-terminus, complementing prior stability and conformational folds.

EXECUTIVE SUMMARY

Ipamorelin Lys-5→Arg: pLDDT 0.79, ipTM 0.73 — confident GHSR-1a interface with Arg guanidinium predicted toward TM3 acidic face. First fold to probe C-terminal affinity electrostatics. In silico only.

DETAILED ANALYSIS

Ipamorelin (Aib-His-DBNal-DPhe-Lys-NH2) is a synthetic pentapeptide growth hormone secretagogue that selectively agonises GHSR-1a without the ACTH, cortisol, or prolactin side-effects typical of first-generation GHS compounds. Its minimalist five-residue scaffold encodes a precise pharmacophore: the N-terminal Aib provides proteolytic resistance and a helix-nucleating propensity; His-2 and DBNal-3 form a hydrophobic-aromatic cluster; DPhe-4 deepens engagement with the aromatic subpocket of GHSR-1a; and Lys-5-NH2 contributes a C-terminal basic charge proposed to interact with conserved acidic residues lining the orthosteric pocket. Fold №70 probes whether upgrading the primary ε-amine of Lys-5 to the geometrically richer guanidinium of Arg can tighten electrostatic engagement and thereby improve predicted binding affinity — a question the Ipamorelin program has not previously addressed.

The mechanistic rationale is grounded in established GHSR-1a structure-activity relationships. Cryo-EM and mutagenesis data for GHSR-1a identify Glu124 (TM3) and Asp99 (TM2/ECL1 boundary) as key acidic anchors for the C-terminal basic group of ghrelin and its synthetic analogs. Macimorelin, an orally active GHS approved for GH deficiency diagnosis, carries an Arg-like guanidinium motif and demonstrates that this contact geometry is tolerated in the GHSR-1a pocket. Unlike a lysine ε-amine (pKa ~10.5, monodentate), the arginine guanidinium (pKa ~12.5) is planar, resonance-stabilized, and capable of bidentate hydrogen bonding and ionic contacts — features that typically translate to tighter and more geometrically constrained salt bridges in receptor pockets lined by acidic residues.

Boltz-2 structural prediction produced a confident complex: pLDDT 0.79, pTM 0.86, ipTM 0.73. The ipTM value is particularly diagnostic — values above 0.7 are generally interpreted as indicative of a confident interface model rather than a loosely docked assembly. The predicted pose places the Arg-5 guanidinium toward the acidic TM3 face of GHSR-1a, consistent with the pre-specified hypothesis, while the DBNal-DPhe hydrophobic dyad occupies the aromatic subpocket in a geometry continuous with prior REFINED Ipamorelin folds. The heuristic stability profile (aggregation propensity 0.0, stability score 0.60, moderate half-life) suggests the Arg substitution does not introduce unfavourable biophysical liabilities.

Positioned within the broader Ipamorelin lab narrative, Fold №70 fills a meaningful gap. Fold №4 explored N-terminal backbone methylation for DPP-IV resistance (REFINED, pLDDT 0.80). Fold №33 introduced a Lys5–Asp6 side-chain lactam to lock

the C-terminal turn conformationally (REFINED, pLDDT 0.73). Fold №48 lipidated the Lys-5 ε-amine with a γGlu-palmitoyl chain to extend plasma half-life (REFINED, pLDDT 0.78). All three modifications treated Lys-5 as a handle for structural or pharmacokinetic engineering rather than as an affinity-driving pharmacophoric element. Fold №70 is the first to directly interrogate the electrostatic contribution of the C-terminal basic residue itself — posing the question: does the guanidinium geometry of Arg outperform the amine geometry of Lys at this position?

The predicted outcome is positive. The combination of high pLDDT, confident ipTM, and pose consistency with the stated hypothesis meets the REFINED threshold. The heuristic profile adds modest supporting context: near-zero aggregation propensity is reassuring for a compound with an additional positive charge at physiological pH, and the stability score of 0.60 is comparable to the parent compound. BBB penetration (0.15) is low but expected for a charged pentapeptide administered parenterally.

Several limitations must be foregrounded. No Boltz-2 affinity delta (ΔΔG) value was returned, so the magnitude of predicted binding improvement over native Ipamorelin is not quantified — the REFINED verdict reflects structural confidence, not a measured affinity gain. The prediction is a single-run, non-ensembled model; Chai-1 agreement data were not available for this fold, removing one layer of cross-validator triangulation. The heuristic biophysical estimates (half-life, stability, BBB) are sequence-derived approximations, not experimental measurements. Perhaps most importantly, Arg at position 5 also removes the free ε-amine that Fold №48 used as a lipidation handle and Fold №33 used as a lactam partner — meaning this variant is not straightforwardly combinable with those modifications without redesign.

The biological significance, if validated, would be meaningful: a single conservative substitution that tightens receptor engagement without altering the non-natural residue pharmacophore (DBNal, DPhe, Aib) could serve as an improved affinity scaffold for next-generation GHS peptides. The result also raises a practical combinatorial question — whether Arg-5 could be incorporated into a palmitoylated or cyclized variant for simultaneous affinity and pharmacokinetic improvement. These are hypotheses for future folds, not conclusions of this one. All findings are in silico predictions requiring wet-lab validation before any biological claim can be substantiated.

RESEARCH BRIEF

FOLD №70 — IPAMORELIN LYS-5 → ARG: PROBING GHSR-1A AFFINITY VIA GUANIDINIUM SALT-BRIDGE ENGAGEMENT

Verdict: REFINED | pLDDT 0.79 | ipTM 0.73 | Single-run Boltz-2 prediction

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MECHANISM OF ACTION

Ipamorelin is a synthetic pentapeptide GHSR-1a agonist that mimics the GH-releasing action of endogenous ghrelin without stimulating ACTH or cortisol secretion. Its minimal scaffold (Aib-His-DBNal-DPhe-Lys-NH2) encodes a precise binding mode at the GHSR-1a orthosteric pocket: the N-terminal Aib nucleates a type-II β-turn; His-2 and DBNal-3 form a hydrophobic-aromatic cluster; DPhe-4 engages the aromatic subpocket; and Lys-5 contributes a C-terminal basic charge that interacts with conserved acidic residues (notably Glu124 in TM3) on the intracellular face of the extracellular-facing pocket. GHSR-1a activation stimulates somatotroph GH release, downstream IGF-1 elevation, and is associated with improvements in body composition, recovery, and metabolic signalling relevant to performance contexts.

Arginine's guanidinium group (pKa ~12.5) is planar, resonance-delocalized, and geometrically capable of bidentate H-bond and salt-bridge contacts that a primary lysine ε-amine (pKa ~10.5) cannot form. Replacing Lys-5 with Arg is hypothesized to tighten the C-terminal anchor at TM3/ECL2 acidic residues, translating to a more constrained and energetically favourable docked pose without perturbing the established DBNal-DPhe hydrophobic pharmacophore.

PERFORMANCE APPLICATIONS

As a GHSR-1a agonist, the Arg-5 variant of Ipamorelin shares the parent compound's performance-relevant activity profile: pulsatile GH secretion stimulation, IGF-1 elevation, and downstream anabolic and lipolytic signalling. If the predicted affinity improvement is validated experimentally, Arg-5 Ipamorelin could offer:

- **Enhanced receptor engagement at lower molar doses**, potentially reducing the peptide burden required for a GH pulse equivalent to native Ipamorelin.

- **A tighter, more selective binding mode** that leverages the same selectivity profile (no ACTH/cortisol co-stimulation) but with improved potency at GHSR-1a.
- **A base scaffold for combinatorial optimization** — pairing improved affinity with the pharmacokinetic gains explored in Folds №48 (palmitoylation) and №33 (lactam cyclization) in future design iterations.

These are predicted properties of an in silico model. No in vivo or clinical claims are made.

MODIFICATION RATIONALE

The Lys-5 → Arg substitution was selected as the first Ipamorelin fold to directly interrogate **affinity-driving electrostatics at the C-terminus** — a dimension the prior Ipamorelin program has not explored. Previous folds addressed:

- **Fold №4**: N-Me-Aib at position 1 → backbone methylation for DPP-IV/ aminopeptidase resistance (REFINED, pLDDT 0.80)
- **Fold №33**: Lys5–Asp6 side-chain lactam → C-terminal conformational lock (REFINED, pLDDT 0.73)
- **Fold №48**: γGlu-C16 palmitoyl on Lys-5 ε-amine → albumin-binding half-life extension (REFINED, pLDDT 0.78)

In each prior fold, Lys-5 was treated as a **structural or pharmacokinetic handle** — its intrinsic pharmacophoric contribution to GHSR-1a binding was never independently tested. Fold №70 isolates that question: does swapping the ε-amine for a guanidinium strengthen receptor engagement? The rationale is supported by SAR precedent in related GHS scaffolds (macimorelin carries an Arg-derived guanidinium motif; ghrelin analogs with Arg at equivalent positions maintain or improve potency) and by the known preference of GHSR-1a acidic pocket residues for geometrically rich bidentate contacts.

PREDICTED PROPERTIES

Property	Native Ipamorelin	Arg-5 Variant	Change
pLDDT	~0.78–0.80 (Fold №48)	**0.79**	Comparable
ipTM	—	**0.73**	Confident interface
pTM	—	**0.86**	High overall fold quality

Property	Native Ipamorelin	Arg-5 Variant	Change
Guanidinium–Glu124 contact	Lys ε-amine (monodentate)	Arg guanidinium (bidentate)	↑ predicted contact richness
Aggregation propensity	—	**0.0**	Favourable
Stability score	—	**0.60**	Moderate
BBB penetration	Low (charged peptide)	**0.15**	As expected; parenteral route
Half-life estimate	~2 h (parent)	**Moderate (30 min–2 h)**	Comparable; no lipidation

> ⚠ Heuristic biophysical estimates are sequence-derived approximations, not experimental measurements. No quantitative ΔΔG value was returned by Boltz-2; the REFINED verdict reflects structural confidence, not a measured affinity delta.

The predicted docked pose preserves the DBNal-DPhe hydrophobic engagement seen consistently across all REFINED Ipamorelin folds, confirming the core pharmacophore is not disrupted by the C-terminal substitution.

SUGGESTED NEXT STEPS

Computational (in silico): 1. **Ensembled Boltz-2 run + Chai-1 cross-validation** — Chai-1 agreement data were unavailable for this fold; running both predictors in ensemble mode would strengthen confidence and flag any pose divergence. 2. **FEP/MM-GBSA rescoring** — Apply free-energy perturbation or molecular mechanics generalized Born surface area calculations on the Boltz-2 pose to obtain a quantitative ΔΔG estimate for Lys → Arg at position 5. 3. **Combinatorial fold: Arg-5 + γGlu-Palm (Fold №48 logic)** — Test whether a palmitoylated Arg-5 variant (replacing the ε-amine lipidation handle with a backbone-compatible linker at a different site, or N-terminal acylation) can combine improved affinity with half-life extension. 4. **Combinatorial fold: Arg-5 + N-Me-Aib-1 (Fold №4 logic)** — Pair the predicted affinity gain at position 5 with the established DPP-IV resistance at position 1 in a single variant.

Wet-lab validation: 1. **Solid-phase peptide synthesis** of Aib-His-DBNal-DPhe-Arg-NH2 using standard Fmoc chemistry; Arg is straightforwardly incorporated. 2. **Competitive radioligand binding assay** (^{3}H-ghrelin or ^{125}I-ghrelin displacement at GHSR-1a-expressing HEK293 cells) to obtain Ki and compare directly with native Ipamorelin. 3. **GH stimulation assay** (pituitary cell culture or in vivo rat model) to confirm functional agonism and potency ratio vs. parent. 4. **Plasma stability**

assay — compare half-life of Arg-5 variant vs. Lys-5 parent under standard plasma incubation conditions to confirm the substitution does not introduce unexpected proteolytic vulnerability.

SEQUENCES

NATIVE

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AibHisDBNalDPheLysNH2
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MODIFIED

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AibHisDBNalDPheArgNH2
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CAVEATS

- in silico prediction only — requires wet lab validation
- single-run prediction (not ensembled); Chai-1 cross-validator data unavailable for this fold
- predicted properties may not reflect real-world biological behavior
- this is research, not medical advice
- no quantitative ΔΔG affinity delta was returned by Boltz-2 — REFINED verdict reflects structural confidence, not a measured binding improvement
- heuristic biophysical estimates (aggregation propensity, stability score, BBB penetration, half-life) are sequence-derived approximations, not experimental measurements
- Arg-5 eliminates the Lys ε-amine used as a lipidation handle in Fold №48 and as the lactam partner in Fold №33 — combinatorial variants would require redesign
- GHSR-1a structural model used by Boltz-2 may not fully capture receptor flexibility or ECL2 dynamics relevant to guanidinium engagement

SOLANA SIGNATURE 3mwT5AwKqPzU5YxJpr3hxH3kivgkaQ58C3dTm555bfYarQeQUWReK9MS9BxxFQeg5rqWXrLgf82AsdhtxJgekVz4
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