IPAMORELIN — REPLACE POSITION-1 AIB (2-AMINOISOBUTYRIC ACID) WITH N-METHYL-AIB (NA-METHYL-2-AMINOISOBUTYRIC ACID) TO ADD A BACKBONE METHYL ON THE FREE N-TERMINUS

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REFINED | PERFORMANCE

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GROWTH HORMONE SECRETAGOGUE RECEPTOR TYPE 1

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
80.4%	0.883 / 0.862	REFINED
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Growth hormone secretagogue receptor type 1	Q92847	—

TLDR

FOLD №15 explores N-methylation of the α-amine at position 1 of Ipamorelin, converting Aib to N-Me-Aib, as a strategy to block aminopeptidase-mediated N-terminal cleavage and extend the peptide's ~2-hour plasma half-life. Boltz-2 predicts a high-confidence complex with GHSR-1a (pLDDT 0.80, ipTM 0.86), with the bioactive D-2Nal/D-Phe/Lys pharmacophore cluster and the His2–D-2Nal3 β-turn both structurally preserved. The N-terminal methyl group projects outward from the binding cleft, consistent with the hypothesis that position 1 is sterically tolerated by the receptor. This is an in silico prediction only and requires wet-lab validation of both the metabolic stability gain and the preserved receptor agonism.

EXECUTIVE SUMMARY

N-Me-Aib1 Ipamorelin: pLDDT 0.80, ipTM 0.86 on GHSR-1a — high-confidence prediction with the D-2Nal/D-Phe/Lys pharmacophore intact and N-methyl projecting clear of the binding cleft. Aminopeptidase blockade hypothesis structurally supported; plasma stability assay is the critical next step.

DETAILED ANALYSIS

Ipamorelin (Aib-His-D-2Nal-D-Phe-Lys-NH2) is a selective pentapeptide growth hormone secretagogue receptor type 1a (GHSR-1a) agonist, first characterized by Raun et al. (1998) and distinguished by a clean selectivity profile that does not engage the cortisol, prolactin, or gonadotropin axes — a meaningful advantage over earlier GHRPs. Its pharmacophore is well-defined: the D-2Nal/D-Phe/Lys tripeptide cluster at positions 3–5 constitutes the primary receptor-engaging surface, while the N-terminal Aib residue functions as a conformational director, locking the backbone into restricted φ/ψ angles that stabilize a bioactive turn. The C-terminal lysine amide is already protected against carboxypeptidase activity, leaving the free N-terminus as the principal remaining site of exopeptidase vulnerability.

The modification under investigation in this fold is the conversion of position-1 Aib to N-methyl-Aib (Nα-methyl-2-aminoisobutyric acid) — adding a backbone N-methyl group to the free α-amine of the N-terminus. This is a well-established peptidomimetic strategy: Nα-methylation sterically occludes the exopeptidase binding groove, abolishing aminopeptidase recognition without introducing a chirality change or perturbing backbone φ/ψ geometry, because Aib's gem-dimethyl α-carbon already dictates the dihedral space accessible to the residue. The analogy to macimorelin's bulky N-cap and ghrelin's octanoyl-Ser1 — both tolerated by GHSR-1a — supports the premise that N-terminal steric additions at this receptor are unlikely to be catastrophic for binding.

The Boltz-2 structural prediction produced a confident result: pLDDT of 0.80, pTM of 0.88, and ipTM of 0.86. These metrics place this fold in the high-confidence tier — meaningfully above the 0.70 threshold typically used to consider a predicted complex structurally informative. The predicted complex shows the D-2Nal3/D-Phe4/Lys5 aromatic-cationic cluster engaging the canonical TM3/TM6 hydrophobic pocket of GHSR-1a, consistent with the established pharmacophore. Critically, the His2–D-2Nal3 β-turn appears structurally intact, and the N-terminal N-Me-Aib methyl group is predicted to project outward from the binding cleft rather than into the receptor interface — the most favorable possible geometric outcome for this modification.

The heuristic sequence-based property profile supports cautious optimism. Aggregation propensity is low (0.12), consistent with the short, conformationally constrained nature of ipamorelin and the absence of hydrophobic stretches that

typically drive aggregation. The heuristic half-life estimate of moderate-to-long (1–6 hours) represents an upward shift from the measured native half-life of ~2 hours in humans (Gobburu et al., 1999), though this is a sequence-level heuristic and not a pharmacokinetic measurement. BBB penetration is predicted to be very low (0.077), consistent with the molecular weight and polar surface area of a pentapeptide — CNS delivery is not the target application here, and peripheral GH secretagogue action does not require CNS access.

This fold is meaningfully contextualized by FOLD №12, which tested D-Ala substitution at position 2 of Sermorelin as a DPP-IV blocking strategy, yielding a DISCARDED verdict (pLDDT 0.49). That failure illustrated how stereochemical perturbation near the N-terminus of a helix-dependent peptide can destabilize the receptor-binding conformation and produce a structurally uninformative prediction. The Ipamorelin №15 approach deliberately avoids this failure mode: rather than a chirality flip, N-methylation adds steric bulk without altering backbone stereochemistry. The high pLDDT of 0.80 versus the 0.49 seen in Fold №12 is a structurally meaningful contrast and supports the conclusion that the N-Me-Aib strategy is better tolerated by this scaffold.

Important limitations must be acknowledged. First, the specific contribution of aminopeptidase activity versus renal filtration or endopeptidase cleavage to ipamorelin's 2-hour half-life has never been directly measured — if renal filtration dominates, N-terminal methylation will produce only marginal pharmacokinetic gains regardless of structural predictions. Second, N-methylation of an already quaternary α-carbon (Aib bears two methyl groups; adding Nα-methyl creates a highly congested junction) may complicate solid-phase synthesis, reduce coupling efficiency, and introduce cis/trans rotameric populations around the N-Me-Aib–His amide bond that could not be resolved in this single-run Boltz-2 prediction. Third, no structural data exists for the native ipamorelin–GHSR-1a complex, meaning the predicted binding pose cannot be compared against an experimental reference. Fourth, Chai-1 agreement data was unavailable for this fold, and the Boltz-2 affinity module did not return quantitative binding change values, limiting our ability to assess whether receptor affinity is preserved, enhanced, or subtly eroded.

Overall, FOLD №15 represents the strongest structural prediction in the current Ipamorelin series and provides a credible in silico rationale for synthetic exploration of N-Me-Aib1-Ipamorelin. The next productive step is synthesis and in vitro plasma stability profiling, followed by GHSR-1a functional assay to confirm that GH-releasing potency is preserved at this modified analogue.

RESEARCH BRIEF

FOLD №15 — N-ME-AIB1 IPAMORELIN

Verdict: REFINED | Target: GHSR-1a (UniProt Q92847) | Class: PERFORMANCE

MECHANISM OF ACTION

Ipamorelin is a selective pentapeptide GHSR-1a agonist. Upon binding to the growth hormone secretagogue receptor in pituitary somatotrophs, it triggers Gαq/11-coupled signaling, elevating intracellular calcium and stimulating pulsatile GH release. Uniquely among GHRPs, ipamorelin does not stimulate ACTH, cortisol, prolactin, FSH, or LH release at pharmacological doses, conferring a cleaner endocrine safety profile than earlier secretagogues. GH release drives downstream IGF-1 production in the liver, mediating anabolic, lipolytic, and tissue-repair effects relevant to performance and recovery applications.

The pharmacophore is centered on the D-2Nal3/D-Phe4/Lys5 tripeptide cluster, which engages the hydrophobic TM3/TM6 pocket of GHSR-1a via aromatic stacking and electrostatic interactions. The N-terminal Aib1 residue is a conformational director — it constrains backbone φ/ψ angles into the restricted space associated with a β-turn around His2–D-2Nal3 — but is not considered a primary receptor contact residue based on SAR studies (Raun 1998; Hansen 2001).

PERFORMANCE APPLICATIONS

GHSR-1a agonism via ipamorelin is principally pursued for: - **Pulsatile GH restoration**: mimicking physiological GH release patterns without suppressing the hypothalamic–pituitary axis (unlike exogenous GH) - **Lean body composition**: GH-driven lipolysis and IGF-1-mediated muscle protein synthesis - **Recovery and tissue repair**: accelerated post-exercise or post-injury tissue remodeling - **Sleep quality**: GH release is coupled to slow-wave sleep; secretagogue administration may amplify nocturnal pulses - **Bone mineral density**: validated in animal models of glucocorticoid-induced bone loss

The principal pharmacokinetic limitation of native ipamorelin is its ~2-hour plasma half-life (Gobburu et al., 1999), necessitating frequent administration. A half-life-extended analogue would reduce dosing frequency and potentially improve the GH pulse profile.

MODIFICATION RATIONALE

The conversion of position-1 Aib → N-methyl-Aib (Nα-methyl-2-aminoisobutyric acid) adds a single methyl group to the free α-amine of the N-terminus. This modification is rationally motivated by three converging lines of evidence:

1. **Aminopeptidase blockade**: Free α-amines are the substrate recognition element for N-terminal exopeptidases (leucine aminopeptidase,

aminopeptidase N). Nα-methylation sterically occludes this recognition without inverting backbone chirality — the mechanism is purely steric, not conformational.

2. **Backbone geometry preservation**: Because Aib already enforces gem-dimethyl α-carbon geometry with restricted φ/ψ space, adding an Nα-methyl does not alter the torsional envelope accessible to position 1. The bioactive turn at His2–D-2Nal3 is predicted to be preserved, unlike the D-Ala2 substitution in Sermorelin (FOLD №12, DISCARDED, pLDDT 0.49) where stereochemical inversion destabilized the GHRHR-binding helix.
3. **Receptor tolerance at position 1**: SAR studies (Hansen 2001; Fowkes 2018) confirm that ipamorelin-related scaffolds tolerate significant chemical diversity at the N-terminus — including naphthylalanine substitution at position 1 in hybrid analogues — while retaining nanomolar binding affinity. Ghrelin's octanoyl-Ser1 and macimorelin's bulky N-cap provide precedent that GHSR-1a accommodates N-terminal steric additions. The C-terminal amide is already protected, making N-terminal methylation the logical remaining stability lever.

This approach is deliberately distinguished from the FOLD №12 failure mode: rather than introducing a stereochemical perturbation near a critical secondary structure element, N-methylation adds steric bulk without altering backbone φ/ψ or chirality.

PREDICTED PROPERTIES (FAVOURABLE CHANGES FROM NATIVE)

Property	Native Ipamorelin	N-Me-Aib1 Analogue (predicted)
pLDDT (Boltz-2)	—	**0.80** (high confidence)
pTM / ipTM	—	**0.88 / 0.86**
Bioactive conformation	β-turn His2–D-2Nal3	Preserved (structurally)
D-2Nal/D-Phe/Lys cluster orientation	TM3/TM6 pocket	Maintained
N-terminal methyl position	—	Projecting outward from binding cleft
Heuristic half-life	~2 h (measured)	Moderate-to-long, ~1–6 h (heuristic)
Aggregation propensity (heuristic)	—	Low (0.12)

Property	Native Ipamorelin	N-Me-Aib1 Analogue (predicted)
BBB penetration (heuristic)	—	Very low (0.077) — not a target requirement
Stability score (heuristic)	—	0.321

Key structural findings: The predicted complex shows the N-Me-Aib1 methyl group projecting outward from the receptor binding cleft — the geometrically optimal outcome for this modification. The His2–D-2Nal3 β-turn is intact, and the D-2Nal3/D-Phe4/Lys5 aromatic-cationic cluster maintains its orientation toward the canonical TM3/TM6 hydrophobic pocket. No steric clash at the receptor interface is predicted.

Note: All properties above marked "predicted" or "heuristic" are in silico estimates only and have not been experimentally validated.

SUGGESTED NEXT STEPS

SYNTHETIC AND ANALYTICAL

- **Synthesis**: Solid-phase peptide synthesis of (N-Me-Aib)His(D-2Nal)(D-Phe)Lys-NH2 using N-methylated Aib building block. Note that Nα-methylation at a quaternary α-carbon may reduce coupling efficiency — a potential manufacturing challenge flagged by the literature review.
- **Analytical characterization**: HPLC purity, LC-MS molecular weight confirmation, NMR rotameric analysis (cis/trans population around N-Me-Aib–His amide bond).

IN VITRO STABILITY

- **Plasma stability assay**: Incubation in human plasma with HPLC/MS monitoring of parent compound degradation — the critical experiment to test the aminopeptidase blockade hypothesis.
- **Aminopeptidase digestion assay**: Leucine aminopeptidase and aminopeptidase N panel to directly confirm N-terminal protection.
- **Endopeptidase profiling**: To determine whether renal/endopeptidase clearance is the dominant half-life determinant (which would limit the benefit of N-terminal methylation).

RECEPTOR PHARMACOLOGY

- **GHSR-1a binding assay**: Radioligand competition or TR-FRET binding assay to confirm IC50 is preserved relative to native ipamorelin.

- **GH-release functional assay**: cAMP or calcium flux assay in GHSR-1a-expressing cells, or ex vivo pituitary cell GH secretion assay.
- **Potency comparison**: Full dose-response curve versus native ipamorelin to detect any partial agonism or affinity shift.

COMPUTATIONAL NEXT STEPS

- **Ensemble prediction**: Run multiple Boltz-2 seeds and/or Chai-1 predictions to assess conformational reproducibility (Chai-1 agreement was unavailable for this fold).
- **Affinity module**: Obtain quantitative predicted binding affinity values when tooling permits.
- **MD simulation**: Short molecular dynamics run on the predicted complex to assess stability of the N-terminal methyl in the binding pocket over ns timescales.
- **Further N-terminal variants**: Consider acylated N-terminus (e.g., acetyl-Aib) as a comparator fold to benchmark N-methylation against acylation as alternative aminopeptidase-blocking strategies.

CROSS-FOLD CONTEXT

This fold complements FOLD №12 (Sermorelin D-Ala2, DISCARDED, pLDDT 0.49) by demonstrating that N-terminal metabolic stability strategies are not uniformly disruptive — the choice of mechanism matters. Where FOLD №12's stereochemical inversion destabilized a helix-dependent binding interface, FOLD №15's N-methylation preserves backbone geometry and yields a structurally confident prediction. Future ipamorelin folds might explore position-5 Lys modifications (informed by Fowkes 2018 PET work) or D-2Nal3 analogues as orthogonal pharmacophore optimization strategies.

> **Mandatory disclaimer**: All findings in this report are in silico predictions from computational structure prediction tools (Boltz-2). Heuristic property estimates (aggregation, stability, half-life, BBB) are sequence-based computational approximations and do not represent experimental measurements. This is exploratory research only and does not constitute medical advice. Wet-lab validation is required before any conclusions about biological activity, safety, or efficacy can be drawn.

SEQUENCES

NATIVE

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AibHisDBNalDPheLysNH2
```

MODIFIED

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(NMe-Aib)HisDBNalDPheLysNH2
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CAVEATS

- in silico prediction only — requires wet-lab validation
- single-run prediction (not ensembled); Chai-1 agreement data unavailable for this fold
- predicted properties may not reflect real-world biological behavior
- this is research, not medical advice
- heuristic half-life, aggregation, stability, and BBB estimates are sequence-based approximations only — not pharmacokinetic measurements
- no experimental structure of native ipamorelin–GHSR-1a complex exists; predicted binding pose cannot be benchmarked against a reference
- the relative contribution of aminopeptidase vs. renal filtration vs. endopeptidase activity to ipamorelin's 2-hour half-life is unknown — N-terminal methylation may yield modest pharmacokinetic benefit if aminopeptidase cleavage is not the dominant clearance mechanism
- N-methylation of a quaternary α-carbon (Aib → N-Me-Aib) may reduce synthetic coupling efficiency and introduce cis/trans rotameric complexity at the N-Me-Aib–His amide bond — not modeled by single-state structure prediction
- Boltz-2 affinity module did not return quantitative binding change values for this fold — receptor affinity preservation is inferred from structural geometry, not predicted numerically

CITATIONS

1. **PMID —** (1998) — — Ipamorelin, the first selective growth hormone secretagogue.
2. **PMID —** (1999) — — Pharmacokinetic-pharmacodynamic modeling of ipamorelin, a growth hormone releasing peptide, in human volunteers.
3. **PMID —** (2001) — — Highly potent growth hormone secretagogues: hybrids of NN703 and ipamorelin.
4. **PMID —** (2018) — — Peptidomimetic growth hormone secretagogue derivatives for positron emission tomography imaging of the ghrelin receptor.
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

SOLANA SIGNATURE BALz7UPfqrHPc51xwwcgsgzLD2pVtp8UD7u2F2Fe8mjUnRwPWr6Ghsh7svCwEAekR13RrFC3TCcZvv3grvC8L44
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