DISTILLATION №3 · ALEMBIC LABS

RETATRUTIDE — ALA-2 → A-AMINOISOBUTYRIC ACID (AIB) SUBSTITUTION AT POSITION 2

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DISCARDED METABOLIC

ALA-2 → A-AMINOISOBUTYRIC ACID (AIB) SUBSTITUTION AT POSITION 2

GLUCAGON-LIKE PEPTIDE-1 RECEPTOR (GLP-1R), GIP RECEPTOR, GLUCAGON RECEPTOR

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
71.4%	0.623 / 0.142	DISCARDED
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Glucagon-like peptide-1 receptor (GLP-1R), GIP receptor, Glucagon receptor	P43220	—

TLDR

FOLD №13 explores an Aib-2 substitution in Retatrutide, the investigational triple agonist (GLP-1R/GIPR/GCGR), to predict whether α-aminoisobutyric acid at position 2 could enhance DPP-4 resistance while preserving balanced tri-receptor engagement. Structural prediction yielded low inter-chain confidence (ipTM 0.142), rendering the receptor-docked complex unreliable — a meaningful structural verdict was not achievable in this DISTILLATION. The modification rationale is chemically sound and well-precedented in the broader incretin field, but the critical question of whether DPP-4 degradation is even a rate-limiting liability for native Retatrutide — given its once-weekly clinical dosing — remains unanswered. This FOLD is flagged as structurally inconclusive; the chemical hypothesis merits wet-lab follow-up before any computational re-attempt.

EXECUTIVE SUMMARY

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DETAILED ANALYSIS

Retatrutide (LY3437943) represents the leading edge of polypharmacology in metabolic medicine: a 39-residue synthetic peptide designed to co-activate GLP-1R, GIPR, and GCGR simultaneously. Phase 2 data established dose-dependent weight reductions of up to 24.2% at 48 weeks, outcomes that exceed those of dual agonists and GLP-1 monotherapy by a clinically meaningful margin. The three-receptor engagement is not redundant — cardiac pharmacology studies demonstrate that antagonism at any single receptor measurably diminishes retatrutide's functional signature, underscoring the tight pharmacological integration of all three arms. Any structural modification to this scaffold therefore operates in a high-stakes context where subtle shifts in receptor selectivity ratios can have outsized functional consequences.

The modification under investigation — substitution of Ala at position 2 with α-aminoisobutyric acid (Aib) — draws on one of the most validated strategies in incretin peptide chemistry. DPP-4 cleaves after position 1 of GLP-1 family peptides when position 2 presents a small, accessible residue (Ala, Ser, or Pro in some contexts). The gem-dimethyl α-carbon of Aib introduces steric bulk that physically occludes the DPP-4 active site, a mechanism exploited in the design of exenatide, semaglutide, and tirzepatide. Aib is also a canonical helix nucleator — its constrained backbone (φ/ψ angles restricted to the helical region by the Thorpe-Ingold effect) can stabilize the N-terminal α-helix that is critical for receptor engagement across the glucagon superfamily. The hypothesis is therefore chemically coherent and mechanistically grounded in established SAR.

However, FOLD №13 encounters a critical empirical complication: Retatrutide as a clinical entity is already administered once weekly subcutaneously with apparent sufficient metabolic stability, implying that existing structural features — potentially including C-terminal modifications or backbone elements not fully disclosed in public sequences — may already mitigate DPP-4 susceptibility. If DPP-4 cleavage is not the rate-limiting degradation pathway for native Retatrutide in vivo, the Aib-2 substitution addresses a solved problem while introducing uncertain perturbation to the pharmacological balance. No published study characterizes DPP-4 cleavage kinetics for native Retatrutide, leaving this foundational question open.

The structural prediction component of this DISTILLATION returned a low ipTM of 0.142, indicating that the inter-chain confidence for peptide-receptor complex modeling was below the threshold for reliable structural interpretation. The peptide-alone pLDDT of 0.714 is moderate, consistent with a partially disordered peptide in isolation (expected for a 39-mer with an unstructured C-terminal tail), but the complex modeling failed to generate a trustworthy binding pose. This means the primary structural hypothesis — that Aib-2 would preserve RMSD <1.5 Å at the receptor interface versus wild-type — cannot be assessed from this prediction run. The structural verdict is DISCARDED.

The peptide-alone physicochemical predictions offer some interpretable signal. An aggregation propensity of 0.144 is low-to-moderate and consistent with a relatively soluble peptide — the Aib substitution would not be expected to dramatically alter this given its modest steric footprint. The stability score of 0.677 is acceptable. BBB penetration is predicted at 0.05, essentially zero — appropriate and expected for a 39-residue peptide with no CNS targeting rationale. Half-life is predicted as long (>6 hours), consistent with the molecule class, though Retatrutide's actual once-weekly clinical half-life reflects subcutaneous depot kinetics and likely additional chemical stabilization beyond what sequence-level predictions capture.

The literature context raises a specific concern about differential receptor effects. GLP-1R, GIPR, and GCGR have distinct N-terminal binding pocket geometries, and the identity of position 2 influences receptor subtype selectivity ratios. Aib's steric bulk and backbone rigidification could differentially reduce binding affinity at GCGR or GIPR relative to GLP-1R — an outcome that would shift the compound from a balanced triple agonist toward a GLP-1R-biased entity, potentially blunting the incremental benefits that distinguish Retatrutide from semaglutide. Wang et al. (2025) demonstrate that receptor activation biases in triple agonist scaffolds alter metabolic outcomes, meaning this risk is not theoretical.

In summary, FOLD №13 presents a chemically well-motivated hypothesis with clear precedent in the incretin field, but faces three compounding challenges: structural prediction confidence was insufficient for complex modeling, the underlying problem (DPP-4 susceptibility) may not apply to the native clinical formulation, and the risk of disrupting balanced tri-receptor agonism is meaningful. This FOLD should be regarded as a hypothesis-generating exploration rather than a validated design lead. The next step is not computational — it is experimental characterization of native Retatrutide's DPP-4 cleavage kinetics in vitro.

RESEARCH BRIEF

FOLD №13 — RESEARCH BRIEF

RETATRUTIDE AIB-2 SUBSTITUTION: DPP-4 RESISTANCE ENGINEERING IN A TRIPLE AGONIST SCAFFOLD

MECHANISM OF ACTION

Retatrutide is a 39-residue synthetic peptide triple agonist that simultaneously activates three closely related class B GPCRs: the glucagon-like peptide-1 receptor

(GLP-1R), the glucose-dependent insulinotropic polypeptide receptor (GIPR), and the glucagon receptor (GCGR). All three receptors signal primarily through Gs-coupled cAMP elevation, but their tissue distributions and downstream effects differ in ways that make their combined activation pharmacologically superior to any single-receptor approach for metabolic disease.

GLP-1R activation drives glucose-dependent insulin secretion, suppresses glucagon release, delays gastric emptying, and reduces appetite through hypothalamic circuits. **GIPR activation** enhances insulin secretion in a glucose-dependent manner, promotes adipogenesis/lipolysis balance, and contributes to appetite suppression through mechanisms distinct from GLP-1R. **GCGR activation** increases hepatic glucose output (a liability in isolation) but in the context of combined GLP-1R/GIPR co-activation, this effect is metabolically tolerated while GCGR's contribution to energy expenditure, lipolysis, and potentially adipose browning provides net benefit.

The N-terminal helix of Retatrutide (approximately residues 1–12) is the pharmacophoric core for receptor engagement. Upon binding to any of the three receptors, this helix inserts into the orthosteric binding pocket of the receptor's extracellular domain and transmembrane bundle, stabilizing the active receptor conformation and initiating G-protein coupling. The exact binding pose and residue-level contacts at each of the three receptors are not publicly resolved by cryo-EM for Retatrutide specifically, but are inferred from extensive structural biology of related glucagon superfamily peptides.

PERFORMANCE APPLICATIONS

Retatrutide's clinical target is obesity and type 2 diabetes mellitus, with Phase 2 data showing up to 24.2% body weight reduction at 48 weeks — among the highest ever recorded for a pharmacological intervention. The compound is not approved and is not available for use.

From a research and biohacking interest perspective, the triple-agonist mechanism engages several pathways relevant to metabolic performance:

- **Body composition**: Retatrutide produces substantial fat mass reduction with relative preservation of lean mass, mediated by appetite suppression, increased energy expenditure, and lipid mobilization across all three receptor arms.
- **Glycemic regulation**: Glucose-dependent insulin secretion enhancement and glucagon suppression (GLP-1R, GIPR) provide tight glycemic control without intrinsic hypoglycemia risk.
- **Hepatic fat reduction**: Clinical trials document significant liver fat content reduction, relevant to metabolic liver disease.

- **Cardiovascular metabolic risk**: Weight reduction, improved lipid profiles, and glycemic control collectively reduce cardiovascular risk factors, with cardiovascular outcome trial data pending.
- **Energy expenditure**: GCGR-mediated thermogenic and lipolytic effects may contribute to resting energy expenditure increases beyond what GLP-1R/GIPR agonism alone provides.

All applications listed above are derived from clinical and preclinical data on Retatrutide itself, not on the Aib-2 modified analog, which has not been synthesized or tested.

MODIFICATION RATIONALE

The Aib-2 substitution targets a canonical vulnerability of GLP-1 family peptides: rapid inactivation by dipeptidyl peptidase-4 (DPP-4). DPP-4 is a serine protease expressed on the surface of endothelial cells, lymphocytes, and circulating as a soluble enzyme. It cleaves after the penultimate N-terminal residue (position 2) when that residue is Ala, Ser, or Pro, generating truncated peptides with dramatically reduced or absent receptor activity.

Why Aib at position 2? α-Aminoisobutyric acid (Aib, 2-methylalanine) introduces a second methyl group at the α-carbon relative to Ala. This gem-dimethyl substitution has two distinct mechanistic consequences:

1. **Steric occlusion of DPP-4**: The DPP-4 active site accommodates Ala-2 in a sterically defined pocket. Aib's additional methyl group creates a steric clash with the enzyme active site geometry, dramatically reducing cleavage kinetics. This is the same rationale underlying semaglutide's Aib-2 substitution and is one of the most robustly validated modifications in incretin chemistry.
2. **Helix nucleation via Thorpe-Ingold effect**: The gem-dimethyl group restricts the backbone dihedral angles (φ, ψ) to the helical region of Ramachandran space, conferring conformational pre-organization. For a peptide whose N-terminal helix must adopt the correct geometry to engage three different receptor binding pockets, enhanced helical propensity at position 2 could reduce the entropic cost of binding — potentially maintaining or modestly enhancing binding affinity despite the steric change.

The complication: Retatrutide's native sequence begins with Tyr (not His, as in GLP-1), and the compound already achieves once-weekly subcutaneous dosing in clinical trials. This implies that the native molecule has sufficient metabolic stability for therapeutic use, possibly through fatty acid conjugation, backbone modifications, or other undisclosed chemical features. The incremental benefit of Aib-2 is therefore uncertain — it may address a degradation pathway that is not rate-limiting for Retatrutide in its clinical form.

The differential impact on GLP-1R vs. GIPR vs. GCGR binding is a further unknown. Position 2 identity influences receptor selectivity ratios across the glucagon peptide superfamily, and Aib's steric bulk could preferentially reduce GCGR or GIPR potency, shifting the compound's pharmacological profile in unpredictable ways.

STABILITY ANALYSIS

Aggregation propensity (predicted): 0.144 — Low-to-moderate. Retatrutide's amphipathic helical segment and hydrophobic C-terminal region create some aggregation risk, but the overall score is acceptable. Aib substitution at position 2 is unlikely to materially alter this, given that the modification is conservative in terms of hydrophobicity and located at the solvent-exposed N-terminus.

Stability score (predicted): 0.677 — Moderate. This reflects peptide-level predicted stability and is consistent with a large, partially structured 39-mer. The Aib substitution would be expected to slightly increase resistance to proteolytic cleavage at the N-terminus without substantially altering global stability.

DPP-4 resistance (predicted, qualitative): Aib-2 would be predicted to confer substantial DPP-4 resistance based on the established steric mechanism — Aib at position 2 is the single most validated DPP-4 resistance modification in the incretin literature. However, as noted, whether DPP-4 cleavage is a relevant degradation pathway for native Retatrutide in vivo is uncharacterized.

Half-life estimate (predicted): Long (>6 hours) — Consistent with molecule class. Clinical Retatrutide has a half-life consistent with once-weekly dosing (approximately 6 days), which is attributable to the overall molecular architecture, likely including subcutaneous depot formation and potentially fatty acid conjugation. Sequence-level half-life predictions substantially underestimate the in vivo half-life of peptides with such modifications and should be interpreted with caution.

BBB penetration (predicted): 0.05 — Essentially zero, as expected for a 39-residue peptide. No CNS penetration is anticipated or desired for this metabolic application.

Comparison to wild-type Retatrutide: The Aib-2 modification is predicted to incrementally improve N-terminal protease resistance without degrading global stability or aggregation behavior. The structural impact on receptor binding — the critical unknown — could not be evaluated in this DISTILLATION due to insufficient complex modeling confidence (ipTM 0.142).

RESEARCH DIRECTIONS

The following experimental steps would be required to validate or refute the Aib-2 hypothesis for Retatrutide:

1. **DPP-4 cleavage kinetics of native Retatrutide (immediate priority)**: Before synthesizing the Aib-2 analog, determine whether native Retatrutide is a DPP-4 substrate under physiologically relevant conditions. HPLC-MS-based cleavage assay with recombinant DPP-4 at physiological enzyme concentrations and pH. If native Retatrutide is already DPP-4-resistant, the modification hypothesis loses its primary justification.

2. **Solid-phase peptide synthesis of Aib-2 Retatrutide**: Fmoc-SPPS incorporating Fmoc-Aib-OH at position 2. Purity verification by analytical HPLC and MALDI-TOF/ESI-MS. This is technically straightforward — Aib is a commercially available non-natural amino acid Fmoc building block, though its coupling can require extended reaction times due to steric hindrance.

3. **In vitro receptor potency panel**: cAMP accumulation assays (HTRF or BRET-based) at GLP-1R, GIPR, and GCGR expressed in HEK293 cells. Full concentration-response curves for both native Retatrutide and Aib-2 analog. EC50 and Emax at each receptor. The key outcome is whether the selectivity ratio is preserved — a shift in pEC50 at any single receptor of >1 log unit would be pharmacologically significant.

4. **DPP-4 resistance comparison**: Side-by-side HPLC-MS cleavage assay of native vs. Aib-2 Retatrutide with recombinant DPP-4. Rate constants and half-lives of cleavage. This directly tests the primary hypothesis.

5. **Structural characterization**: Circular dichroism (CD) spectroscopy to compare helical content in native vs. Aib-2 Retatrutide — Aib should increase α-helical signal intensity and thermal stability of the helix. If receptor-bound cryo-EM is feasible (requires significant resources), single-particle analysis of Aib-2 Retatrutide bound to each receptor in complex with Gs would be the definitive structural validation.

6. **In vitro plasma stability**: Incubation in human plasma with HPLC-MS monitoring to compare overall metabolic stability profiles, capturing proteolytic pathways beyond DPP-4.

7. **In vivo pharmacokinetic study (rodent)**: Subcutaneous administration of native vs. Aib-2 Retatrutide in lean and diet-induced obese mice, with serial blood sampling and PK modeling. This would reveal whether Aib-2 extends circulating half-life in the relevant in vivo milieu.

8. **Computational re-attempt**: With improved structural templates (if cryo-EM structures of Retatrutide-receptor complexes are published) or with ensemble docking approaches, computational modeling of the Aib-2 complex at each

receptor could provide better-grounded binding pose predictions. AlphaFold multimer modeling of peptide-GPCR complexes remains technically challenging and should be interpreted cautiously until experimental structural data is available.

SEQUENCES

NATIVE

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YAQGTFTSDYSIYLDKQAAKDFVQWLLAGGPSSGAPPPS
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MODIFIED

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Y[Aib]QGTFTSDYSIYLDKQAAKDFVQWLLAGGPSSGAPPPS
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CAVEATS

- In silico prediction only — requires wet lab validation before any interpretive weight can be assigned to structural or binding claims.
- Structural complex prediction was DISCARDED due to insufficient inter-chain confidence (ipTM 0.142); receptor binding pose analysis is not available for this FOLD.
- Single-run prediction (not ensembled) — structural predictions may not represent the lowest-energy conformational ensemble.
- Predicted physicochemical properties (aggregation, stability, BBB, half-life) are sequence-level estimates and do not account for undisclosed chemical modifications (e.g., fatty acid conjugation) likely present in the clinical Retatrutide formulation.
- The DPP-4 susceptibility of native Retatrutide has not been experimentally characterized — if the molecule is already DPP-4-resistant through existing structural features, the primary rationale for Aib-2 substitution is unvalidated.
- Differential impact of Aib-2 on potency at GLP-1R vs. GIPR vs. GCGR is unknown and could alter the balanced tri-receptor pharmacology that underlies Retatrutide's clinical superiority.
- Retatrutide is an investigational compound not approved for clinical use; this analysis pertains to a further-modified analog with no human data whatsoever.
- This is research exploration, not medical advice. No claims of therapeutic efficacy or safety are made.

CITATIONS

1. **PMID —** (2023) — — Triple-Hormone-Receptor Agonist Retatrutide for Obesity - A Phase 2 Trial
2. **PMID —** (2023) — — Retatrutide, a GIP, GLP-1 and glucagon receptor agonist, for people with type 2 diabetes: a randomised, double-blind, placebo and active-controlled, parallel-group, phase 2 trial conducted in the USA
3. **PMID —** (2025) — — Retatrutide-A Game Changer in Obesity Pharmacotherapy
4. **PMID —** (2024) — — The power of three: Retatrutide's role in modern obesity and diabetes therapy
5. **PMID —** (2024) — — Triple hormone receptor agonist retatrutide for metabolic dysfunction-associated steatotic liver disease: a randomized phase 2a trial
6. **PMID —** (2024) — — Effects of once-weekly subcutaneous retatrutide on weight and metabolic markers: A systematic review and meta-analysis of randomized controlled trials
7. **PMID —** (2026) — — Retatrutide for the treatment of obesity, obstructive sleep apnea and knee osteoarthritis: Rationale and design of the TRIUMPH registrational clinical trials
8. **PMID —** (2025) — — Effects of retatrutide on body composition in people with type 2 diabetes: a substudy of a phase 2, double-blind, parallel-group, placebo-controlled, randomised trial
9. **PMID —** (2025) — — Contractile effects of retatrutide in isolated mouse atrial preparations
10. **PMID —** (2026) — — Inotropic effects of retatrutide in isolated human atrial preparations
11. **PMID —** (2025) — — Strategic Design of Triple GLP-1R/GCGR/GIPR Agonists with Varied Receptor Potency: Achieving Comparable Glycemic and Weight Reduction Effects