TIRZEPATIDE — ALA-2 → A-AMINOISOBUTYRIC ACID (AIB) SUBSTITUTION AT POSITION 2, REPLACING THE NATIVE ALA WITH THE CA,A-DISUBSTITUTED NON-CANONICAL RESIDUE WHILE RETAINING THE NATIVE C20 FATTY DIACID ON LYS-20

generated 2026-05-03T09:56:14.612686+00:00

PROMISING METABOLIC

ALA-2 → A-AMINOISOBUTYRIC ACID (AIB) SUBSTITUTION AT POSITION 2, REPLACING THE NATIVE ALA WITH THE CA,A-DISUBSTITUTED NON-CANONICAL RESIDUE WHILE RETAINING THE NATIVE C20 FATTY DIACID ON LYS-20

GLUCAGON-LIKE PEPTIDE 1 RECEPTOR

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
70.6%	0.751 / 0.128	PROMISING
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Glucagon-like peptide 1 receptor	P43220	—

TLDR

Fold №31 introduces an Aib-2 substitution at the canonical DPP-4 scissile bond of tirzepatide, predicting improved enzymatic stability orthogonal to its existing C20 fatty diacid half-life extension. Structural prediction returns a pLDDT of 0.71 and pTM of 0.75, consistent with a well-folded central α-helix and a preserved N-terminal pharmacophore geometry. The ipTM of 0.13 means receptor engagement cannot be confirmed in silico, leaving the dual GLP-1R/GIPR agonism hypothesis supported by literature precedent rather than this run's docking signal. The fold is rated

PROMISING: the conformational logic is sound, the stability rationale is well-grounded in incretin chemistry, but the marginal PK benefit over the native molecule's ~5-day half-life and the GIPR pocket tolerance for the Cα-methyl remain genuinely open questions.

EXECUTIVE SUMMARY

Tirzepatide Aib-2: pLDDT 0.71, pTM 0.75 — helical scaffold intact, N-terminal pharmacophore preserved. DPP-4 resistance predicted on chemical first principles; ipTM 0.13 leaves receptor engagement unconfirmed. PROMISING, but marginal PK gain above the C20 diacid baseline remains the key open question.

DETAILED ANALYSIS

Tirzepatide is a 39-residue dual GIP/GLP-1 receptor agonist with an approved once-weekly clinical regimen, achieving HbA1c reductions of up to 2.30 percentage points and ~20% body weight loss in the SURPASS and SURMOUNT trial series. Its pharmacokinetic durability is primarily conferred by a C20 fatty diacid conjugated to Lys-20, which drives albumin binding and extends the plasma half-life to approximately five days. Despite this lipidation-based stability, the N-terminal Tyr1-Ala2 dipeptide remains a canonical substrate for dipeptidyl peptidase-4 (DPP-4), the serine protease responsible for rapid inactivation of native GLP-1 and GIP. Cleavage at this bond removes the N-terminal pharmacophore (Tyr1, Ala2/Aib2, Glu3, Gly4) that is required for transmembrane bundle engagement at both receptors, meaning even a small fractional contribution from DPP-4 proteolysis could reduce the pool of biologically active peptide.

The modification hypothesis centers on replacing Ala-2 with α-aminoisobutyric acid (Aib), the Cα,α-disubstituted non-canonical amino acid whose gem-dimethyl geometry sterically occludes the DPP-4 active site and eliminates it as a substrate. This is not a novel chemical strategy in the incretin field — semaglutide and several investigational GLP-1/GIP analogues employ this or closely related approaches — but its application to the tirzepatide scaffold specifically is uncharacterized in the published literature. The rationale for this fold is thus an informed extrapolation from validated incretin chemistry rather than tirzepatide-specific SAR data, a distinction the literature agent correctly flags as a knowledge gap.

AlphaFold3 returned a pLDDT of 0.71 and pTM of 0.75 for the modified peptide in isolation, consistent with the researcher's predicted range of 0.70–0.78 and with a well-ordered central amphipathic α-helix. The N-terminal region encompassing Tyr1 through Gly4 is resolved within the helical fold, which is structurally consistent with the hypothesis that the Aib Cα-methyl caps the N-terminal turn without introducing backbone distortion. This mirrors the pLDDT of 0.71 observed in Fold №23 (tirzepatide Cys-24 → αMe-Cys), suggesting the tirzepatide scaffold tolerates Cα-

methylation at multiple positions without gross conformational disruption. The disordered C-terminal GPSSGAPPPS tail and Lys-20 lipidation anchor are present but not separately scored; their expected flexibility is consistent with prior tirzepatide structural models.

The critical caveat is the ipTM of 0.13, which represents the inter-chain confidence score for the peptide-receptor complex. This score is too low to support any claim about preserved GLP-1R or GIPR transmembrane bundle engagement from this run alone. The dual agonism hypothesis — that Aib-2 is accommodated in both receptor binding pockets without potency loss — rests entirely on the mechanistic precedent from GLP-1R and GIP analogue literature, not on the structural output of this prediction. The GIPR binding pocket geometry at position 2 is less characterized than GLP-1R, and the literature agent appropriately identifies this as the higher-risk unknown: GIPR is noted to have a narrower orthosteric cavity, and the added Cα-methyl may impose a steric penalty that differentially reduces GIPR versus GLP-1R potency.

Comparing across the lab's running narrative, this fold revisits the Aib-2 concept previously explored in Fold №3 (Retatrutide, DISCARDED, pLDDT 0.71), where the triple-agonist scaffold's more complex N-terminal hydrogen-bonding network may have been less tolerant of the modification. The researcher's argument that tirzepatide's distinct GIPR-biased pharmacology and N-terminal geometry represent a meaningfully different structural context is plausible, but the structural output here does not resolve whether tirzepatide's Aib-2 variant performs better than Retatrutide's at the receptor interface — that question remains open pending a higher-quality complex run or wet-lab SAR data.

Heuristic property estimates (sequence-based, not wet-lab numbers) suggest low aggregation propensity (0.195), moderate stability (0.54), negligible BBB penetration (0.062), and a long half-life consistent with the C20 diacid — none of these values are materially altered by the single Aib-2 point change, as expected. The practical clinical question of whether Aib-2 provides meaningful additional in vivo stability on top of the existing ~5-day half-life is the central pharmacokinetic uncertainty: if DPP-4 proteolysis contributes only marginally to tirzepatide inactivation at weekly steady-state dosing, this modification may offer limited incremental PK benefit, however chemically elegant.

Overall, Fold №31 represents a scientifically credible and chemically grounded modification with a clear mechanistic rationale, supported by strong incretin field precedent and a structurally consistent prediction. The PROMISING verdict is warranted: the fold demonstrates a preserved helical scaffold and a testable stability hypothesis, but the receptor engagement confidence is insufficient for a REFINED classification, and the marginal PK benefit in the context of an already long-acting molecule tempers the headline claim. The path forward requires either a higher-quality receptor-complex prediction (ensembled or Boltz-2 affinity-enabled) or direct DPP-4 cleavage assay data on the tirzepatide Aib-2 variant.

RESEARCH BRIEF

FOLD №31 — TIRZEPATIDE AIB-2 SUBSTITUTION

Verdict: PROMISING | Class: METABOLIC | Modification: Non-canonical amino acid

---

MECHANISM OF ACTION

Tirzepatide is a 39-residue synthetic peptide dual agonist of the GIP receptor (GIPR) and glucagon-like peptide-1 receptor (GLP-1R), approved for type 2 diabetes and obesity management. Its N-terminal sequence (Tyr1-Ala2-Glu3-Gly4-Thr5...) is GIP-derived and engages the orthosteric transmembrane binding pockets of both GIPR and GLP-1R to activate Gαs-coupled cAMP signaling, driving insulin secretion, glucagon suppression, and CNS-mediated appetite reduction. The intact N-terminal pharmacophore — particularly Tyr1, Glu3, and Gly4 — is required for receptor activation at both targets. Metabolic stability is primarily conferred by a C20 fatty diacid conjugated to Lys-20, enabling albumin binding and a ~5-day plasma half-life that supports once-weekly dosing. However, the Tyr1-Ala2 bond remains the canonical DPP-4 scissile bond, and proteolytic removal of the N-terminal dipeptide eliminates receptor agonism regardless of lipidation status.

PERFORMANCE APPLICATIONS

Tirzepatide's clinical efficacy in the SURPASS and SURMOUNT trial series is well established: HbA1c reductions of up to 2.30 percentage points and body weight reductions approaching 20% at 15 mg weekly doses, with emerging data in MASH, sleep apnea, and cardiovascular outcomes. SURMOUNT-4 weight regain data following discontinuation underscore the dependency on continuous dosing and motivate exploration of variants with enhanced pharmacokinetic durability. A tirzepatide analogue with orthogonal DPP-4 resistance at Aib-2 could, in principle, maintain or extend the pharmacological profile while reducing the theoretical contribution of N-terminal proteolysis to dose-to-dose variability — though the marginal PK benefit on top of the existing C20 diacid half-life remains the central unresolved question.

MODIFICATION RATIONALE

Aib (α-aminoisobutyric acid) is a Cα,α-disubstituted non-proteinogenic amino acid with gem-dimethyl geometry at the α-carbon. This geometry sterically blocks

DPP-4's S1 pocket, which requires a planar Cα at the P1' position, rendering Aib-containing peptides non-substrates for DPP-4 cleavage. This is the same chemical logic that underpins the Aib-2 strategy used in semaglutide and validated across numerous GLP-1 and GIP analogue programs. Critically, Aib also constrains backbone dihedral angles to the α-helical region (φ ≈ −60°, ψ ≈ −30°), acting as a helix-capping residue that may pre-organize the N-terminal turn prior to receptor binding — a property structurally consistent with the requirement for an ordered helical N-terminus for incretin receptor engagement.

The Aib-2 and C20 diacid modifications are structurally orthogonal: the fatty diacid on Lys-20 operates via albumin-mediated half-life extension, while Aib-2 acts directly at the proteolytic cleavage site. They address different inactivation mechanisms and are not expected to sterically interfere with one another.

Cross-fold context: This fold revisits the Aib-2 concept previously tested in **Fold №3** (Retatrutide Aib-2, DISCARDED, pLDDT 0.71), where the triple-agonist scaffold's more complex N-terminal hydrogen-bonding network with GCGR appeared less tolerant of the modification, contributing to a weak structural signal. The present fold tests whether tirzepatide's distinct GIPR-biased pharmacology and simpler dual-receptor N-terminal geometry represents a more permissive context for this substitution. Separately, **Fold №23** demonstrated that tirzepatide tolerates Cα-methylation at position 24 (αMe-Cys, pLDDT 0.71, PROMISING), providing precedent that the tirzepatide scaffold is generally compatible with backbone-constraining non-canonical residue insertions without gross conformational disruption.

PREDICTED PROPERTIES (WHERE SIGNAL IS MODERATE)

Property	Native tirzepatide (reference)	Tirzepatide Aib-2 (predicted)	Confidence
pLDDT (fold quality)	~0.70–0.72 est.	**0.706**	Moderate
pTM (global topology)	~0.74 est.	**0.751**	Moderate
ipTM (receptor engagement)	N/A (isolated)	**0.128**	Low — not interpretable
N-terminal helix integrity	Resolved	Resolved (consistent)	Moderate
DPP-4 susceptibility	Yes (Ala-2 substrate)	Predicted abolished (Aib not a substrate)	High (chemical principle)
			Moderate

Property	Native tirzepatide (reference)	Tirzepatide Aib-2 (predicted)	Confidence
Half-life profile (heuristic)	Long (>6 hr, C20 diacid)	Long (>6 hr, unchanged)	
Aggregation propensity (heuristic)	~0.19	0.195	Low confidence
Stability score (heuristic)	~0.54	0.54	Low confidence
BBB penetration (heuristic)	~0.06	0.062	Not applicable

The pLDDT of 0.706 and pTM of 0.751 are consistent with a structurally plausible, well-folded helical peptide, matching the researcher's predicted range of 0.70–0.78. The N-terminal Tyr1-Aib2-Glu3-Gly4 pharmacophore is resolved within the helical fold, consistent with backbone geometry preservation. The ipTM of 0.128 is below the threshold for confident receptor engagement inference and should not be interpreted as evidence of reduced binding — it reflects the limitations of the single-run complex prediction, not a predicted potency loss.

The DPP-4 resistance prediction is made on chemical first principles (Cα,α-disubstitution abolishes DPP-4 substrate geometry) rather than the structural run; this is the highest-confidence claim in the fold. The practical magnitude of in vivo benefit is uncertain given tirzepatide's existing ~5-day half-life.

WHAT WOULD STRENGTHEN THIS SIGNAL

Additional in silico predictions: - **Ensemble prediction (≥5 AlphaFold3 seeds or Boltz-2 affinity module)** on the tirzepatide(Aib2)–GLP-1R and tirzepatide(Aib2)–GIPR complexes with full receptor structure included; ipTM from a single run is insufficient to assess dual agonism preservation. - **Chai-1 agreement run** (noted as None in this fold) — inter-model agreement on the complex would materially increase confidence in receptor interface geometry. - **Comparative docking** of native tirzepatide vs. Aib-2 variant against available GIPR and GLP-1R cryo-EM structures (PDB: 7DTX, 7MHZ, 7SK8) to assess steric accommodation of the Cα-methyl in both binding pockets. - **MD simulation** of the N-terminal turn (residues 1–8) comparing native Ala-2 vs. Aib-2 conformational sampling, to quantify the predicted helix pre-organization effect.

Wet-lab experiments to validate: - **DPP-4 cleavage assay** (fluorogenic substrate competition or LC-MS peptide stability assay) comparing tirzepatide vs. tirzepatide(Aib2) in human plasma — this is the most direct test of the primary

hypothesis. - **cAMP accumulation assay** at GLP-1R and GIPR (HEK293 overexpression or primary cells) with full dose-response curves for tirzepatide vs. Aib-2 variant — essential to confirm that dual agonist potency is retained and to quantify any GIPR/GLP-1R selectivity shift introduced by the Cα-methyl. - **SPR or ITC binding affinity** measurements at purified GIPR and GLP-1R extracellular domains to isolate affinity effects from functional assay confounds. - **In vivo PK comparison** (rodent, sc dosing) of native tirzepatide vs. tirzepatide(Aib2) — specifically, plasma half-life and intact N-terminal peptide quantification by mass spectrometry to determine whether Aib-2 provides measurable additional stability above the C20 diacid baseline.

Key unknowns to resolve: 1. What fraction of tirzepatide inactivation in vivo is attributable to DPP-4 vs. renal/hepatic clearance? If DPP-4 contributes <10% to total clearance, the Aib-2 modification may be pharmacokinetically inert in practice. 2. Does the GIPR binding pocket at position 2 accommodate the Cα-methyl without potency loss? This is the highest-risk structural unknown and the primary differentiator from the GLP-1R precedent. 3. Does combining Aib-2 with the C20 diacid alter the balance of GLP-1R vs. GIPR agonism, and if so, in which direction?

SEQUENCES

NATIVE

```
YAEGTFTSDYSIYLDKQAAKEFVCWLLAGGPSSGAPPPS
```

MODIFIED

```
Y-Aib-EGTFTSDYSIYLDKQAAKEFVCWLLAGGPSSGAPPPS
```

CAVEATS

- In silico prediction only — requires wet lab validation
- Single-run prediction (not ensembled); ipTM 0.13 is insufficient to assess receptor engagement — ensemble complex runs required
- Predicted properties may not reflect real-world biological behavior
- This is research, not medical advice
- DPP-4 resistance prediction is made on chemical first principles (Cα,α-disubstitution), not from structural output — the actual fractional contribution of DPP-4 to tirzepatide inactivation in vivo is unknown
- GIPR binding pocket tolerance for Aib-2 Cα-methyl is uncharacterized; GIPR orthosteric cavity may be narrower than GLP-1R and penalize the added methyl group

- Heuristic property estimates (aggregation 0.195, stability 0.54, half-life, BBB) are sequence-based approximations, not experimental measurements
- Marginal PK benefit of Aib-2 above the existing C20 diacid (~5-day half-life) is uncertain and may be pharmacokinetically negligible at steady-state weekly dosing
- No tirzepatide-specific Aib-2 SAR data exist in the published literature; hypothesis rests on extrapolation from GLP-1 and GIP analogue precedents
- Chai-1 agreement not available for this fold; inter-model consensus on the complex is absent

CITATIONS

1. **PMID —** (2021) — — Tirzepatide versus Semaglutide Once Weekly in Patients with Type 2 Diabetes
2. **PMID —** (2021) — — Efficacy and safety of a novel dual GIP and GLP-1 receptor agonist tirzepatide in patients with type 2 diabetes (SURPASS-1)
3. **PMID —** (2024) — — Continued Treatment With Tirzepatide for Maintenance of Weight Reduction in Adults With Obesity: The SURMOUNT-4 Randomized Clinical Trial
4. **PMID —** (2025) — — Tirzepatide for Obesity Treatment and Diabetes Prevention
5. **PMID —** (2024) — — The impact of tirzepatide and glucagon-like peptide 1 receptor agonists on oral hormonal contraception
6. **PMID —** (2025) — — Discontinuing glucagon-like peptide-1 receptor agonists and body habitus: A systematic review and meta-analysis
7. **PMID —** (2025) — — Effect of glucagon-like peptide-1 receptor agonists and co-agonists on body composition: Systematic review and network meta-analysis
8. **PMID —** (2024) — — Tirzepatide for Metabolic Dysfunction-Associated Steatohepatitis with Liver Fibrosis

SOLANA SIGNATURE 4ZJcc33ZVwVayKnT7syzjSB7pYcnGxLhxmKcSuJaykciqrcs81Dmcuy5mnJReoAkHf2JYvC3pto5xcZMmG2kh4Kt
DATA SHA-256 3d1aecb61d740fb2057eb081e64f98bc7b8298933c874d60eecdf8ff43442dab
VERIFY https://solscan.io/tx/4ZJcc33ZVwVayKnT7syzjSB7pYcnGxLhxmKcSuJaykciqrcs81Dmcuy5mnJReoAkHf2JYvC3pto5xcZMmG2kh4Kt