TIRZEPATIDE — C-TERMINAL EXTENSION BY ONE RESIDUE: APPEND LYS-40 BEARING A ΓGLU SPACER AND C20 FATTY DIACID (EICOSANEDIOIC ACID) ON ITS E-AMINE, YIELDING ...PPPS-K(ΓGLU-C20-DIACID)-OH. NATIVE LYS-20 LIPIDATION (ΓGLU-ΓGLU-C20 DIACID) AND ALL OTHER RESIDUES ARE PRESERVED.

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PROMISING METABOLIC

C-TERMINAL EXTENSION BY ONE RESIDUE: APPEND LYS-40 BEARING A ΓGLU SPACER AND C20 FATTY DIACID (EICOSANEDIOIC ACID) ON ITS E-AMINE, YIELDING ...PPPS-K(ΓGLU-C20-DIACID)-OH. NATIVE LYS-20 LIPIDATION (ΓGLU-ΓGLU-C20 DIACID) AND ALL OTHER RESIDUES ARE PRESERVED.

GLUCAGON-LIKE PEPTIDE 1 RECEPTOR

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
71.6%	0.660 / 0.096	PROMISING
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Glucagon-like peptide 1 receptor	P43220	—

TLDR

Fold №73 appends a C-terminal Lys-40 bearing a γGlu-C20 fatty diacid to Tirzepatide's native PPPS tail, hypothesizing that a second albumin anchor will

extend plasma half-life beyond the ~5-day native value through bivalent albumin engagement. The structural prediction returns a moderate overall confidence (pLDDT 0.72) consistent with retention of the central amphipathic helix, but the receptor interface is poorly resolved (ipTM 0.096), preventing confirmation of canonical GLP-1R TMD engagement. The heuristic profile suggests low aggregation propensity and a long half-life estimate, lending qualified support to the pharmacokinetic rationale. This fold is scored PROMISING — the signal is real but insufficient to confirm receptor-binding preservation without higher-resolution interface data.

EXECUTIVE SUMMARY

Tirzepatide Fold №73 adds a second C20 fatty diacid anchor at a new C-terminal Lys-40, targeting bivalent albumin binding for extended half-life. pLDDT 0.72 supports an intact helical core; ipTM 0.096 leaves the receptor interface unresolved. PROMISING — the PK rationale is sound, but albumin-binding and receptor assays are needed.

DETAILED ANALYSIS

Tirzepatide is a 39-residue dual GIP/GLP-1 receptor agonist approved for type 2 diabetes and obesity, distinguished from earlier incretin mimetics by its balanced dual-agonism and a C20 fatty diacid conjugated via a γGlu-γGlu spacer to Lys-20, which drives tight reversible albumin binding and yields a plasma half-life of approximately five days — sufficient for once-weekly subcutaneous dosing. The PPPS C-terminal tail (residues 36–39) is understood to be disordered in solution and in receptor-bound cryo-EM structures of homologous class B GPCRs, projecting away from both the extracellular domain and transmembrane domain interfaces that mediate GLP-1R and GIPR pharmacology. This structural context motivates the hypothesis that the C-terminus is a pharmacologically permissive site for additional lipid conjugation.

Fold №73 extends Tirzepatide by one residue — appending Lys-40 C-terminally — and decorates its ε-amine with a γGlu spacer and C20 fatty diacid (eicosanedioic acid), deliberately matching the chain length of the native Lys-20 lipidation for synthetic and physicochemical consistency. The design intent is bivalent albumin engagement: two fatty acid anchors on the same molecule could in principle increase the effective albumin-binding avidity, slowing renal clearance and proteolytic exposure and pushing the half-life meaningfully beyond five days — potentially enabling bi-weekly or monthly dosing formats that are not achievable with the native molecule. The γGlu (rather than γGlu-γGlu) spacer at Lys-40 is intentionally shorter than the native Lys-20 linker, providing spatial differentiation between the two anchors.

The structural prediction (Boltz-2) returns a peptide-level pLDDT of 0.716, which falls into the moderate-confidence band and is consistent with a folded amphipathic helix being predicted for the pharmacophore core while the C-terminal extension remains locally disordered — the expected and acceptable outcome given the flexible PPPS-K tail. The pTM of 0.66 suggests that the overall topology of the full peptide is reasonably well-predicted, and the heuristic aggregation propensity of 0.222 is reassuringly low, which matters for a molecule carrying two large lipid-linker conjugates that could in principle drive self-association or peptide bridging of albumin molecules. The critical weakness of this prediction is the ipTM of 0.096, which is very low and reflects the known difficulty of Boltz-2 in resolving the complex interface between a short peptide and a large class B GPCR transmembrane bundle. This does not indicate a predicted failure of receptor engagement — it indicates that the tool cannot adjudicate the interface at this resolution for this target class.

The laboratory context strongly supports this fold. Retatrutide Fold №45 applied the same conceptual strategy — C-terminal Lys-40 with γGlu-C18 diacid on the PPPS tail — and returned PROMISING (pLDDT 0.70), establishing the template that the disordered C-terminus of PPPS-tail incretins tolerates lipid conjugation without distorting the helical pharmacophore. Fold №73 advances on that precedent by applying the strategy to Tirzepatide with a C20 (rather than C18) chain to match native chemistry, yielding a slightly higher pLDDT (0.716 vs 0.70) and consistent heuristic stability. Fold №63, which attempted an i,i+4 lactam staple in Tirzepatide's central helix, was DISCARDED — a failure mode explicitly avoided here by confining the modification to the disordered C-terminus. Folds №23 and №31 explored non-canonical residue substitutions in Tirzepatide's helix (αMe-Cys-24, Aib-2) and returned PROMISING results, confirming that the Tirzepatide helical core is structurally robust to perturbation; the current fold does not touch that core.

From a biological significance standpoint, the bivalent albumin-binding concept is well-motivated but not yet experimentally validated for incretin peptides. The GLP-1 analog precedent (liraglutide C16 ~13 h → semaglutide C18 diacid ~7 days → tirzepatide C20 diacid ~5 days) demonstrates that longer and more polar fatty acid chains correlate with extended half-life, but the mechanism is single-anchor albumin binding with varying affinity and off-rate. Whether a second anchor on the same molecule acts additively, synergistically, or instead drives inter-molecular albumin bridging and aggregation is an open experimental question. The clinical motivation is real but modest: tirzepatide's current half-life is already sufficient for once-weekly dosing with strong clinical outcomes, so further extension would serve niche use cases (adherence, bi-weekly convenience) rather than addressing a critical gap.

The most significant biological uncertainty is whether the C-terminal Lys-40 region, despite being distal to the GLP-1R TMD interface, could perturb GIPR engagement. Tirzepatide's C-terminal residues (approximately 30–39) are understood to participate in contacts with GIPR's extracellular loops and ECD in its dual-agonist binding mode, and the addition of a bulky lipid-linker at position 40 — even one

residue beyond the native C-terminus — could sterically compete with or alter the orientation of those C-terminal contacts. This is a genuine gap that in silico predictions cannot resolve.

In summary, Fold №73 presents a chemically logical, precedent-supported pharmacokinetic modification of Tirzepatide with a moderate structural confidence signal and a favorable heuristic property profile. The PROMISING verdict reflects a real but incomplete signal: the helical core appears intact, the C-terminal extension is appropriately disordered, and the heuristic aggregation risk is low — but the interface ipTM is too weak to confirm receptor engagement, and the bivalent albumin hypothesis requires dedicated experimental validation. This fold is a credible candidate for in vitro pharmacokinetic and receptor-binding follow-up, not a confirmed advance.

RESEARCH BRIEF

FOLD №73 — TIRZEPATIDE C-TERMINAL LYS-40 ΓGLU-C20 DIACID EXTENSION

Verdict: PROMISING | Class: METABOLIC | Target: GLP-1R (P43220) / GIPR

> **Mandatory disclaimer:** All findings are in silico predictions only. No wet-lab validation has been performed. Predicted properties do not constitute evidence of real-world biological activity. This is research, not medical advice.

MECHANISM OF ACTION

Tirzepatide is a 39-residue dual GIP/GLP-1 receptor agonist that engages two class B GPCRs: GLP-1R, driving insulin secretion and satiety, and GIPR, which augments the GLP-1R signal and contributes to the superior weight-loss profile observed in clinical trials (SURPASS, SURMOUNT series). The pharmacophore is an amphipathic α-helix spanning roughly residues 1–29, with N-terminal Tyr-1 insertion into the GLP-1R TMD orthosteric pocket as the canonical activation mechanism. The native C20 fatty diacid conjugated via a γGlu-γGlu spacer to Lys-20 binds reversibly to human serum albumin, dramatically reducing renal clearance and proteolytic exposure to yield a plasma half-life of approximately five days and enabling once-weekly subcutaneous dosing.

The C-terminal PPPS tail (residues 36–39) is structurally disordered in solution and in cryo-EM structures of homologous class B GPCR incretin complexes, projecting away from both the ECD and TMD interfaces. This disorder is well-established for GLP-1 and semaglutide receptor complexes in the broader class B literature and is the structural premise underlying the C-terminal lipidation strategy.

PERFORMANCE APPLICATIONS

This modification targets **pharmacokinetic extension** rather than acute receptor potency. The primary application space is:

- **Extended dosing interval:** A substantially longer half-life (targeting >7–10 days vs. native ~5 days) could enable bi-weekly or potentially monthly subcutaneous injection formats, addressing adherence barriers in chronic metabolic disease management.
- **Obesity and MASH pharmacotherapy:** The clinical evidence base for tirzepatide in obesity (SURMOUNT) and MASH is strong; longer-acting formats could improve real-world adherence in these chronic indications where treatment discontinuation rates are high.
- **Comparator to semaglutide PK:** Semaglutide achieves ~7 days via a C18 fatty diacid/mini-PEG/γGlu linker; a bivalent-anchor tirzepatide analog could, in principle, meet or exceed semaglutide's PK profile while preserving the dual-agonist pharmacology that drives superior weight loss.

Note: Tirzepatide's current once-weekly dosing already satisfies clinical needs in approved indications. The incremental benefit of further half-life extension is real but targets convenience optimization rather than unmet medical need.

MODIFICATION RATIONALE

The modification appends a single Lys-40 residue C-terminally to the native PPPS tail, conjugating a γGlu spacer and C20 fatty diacid (eicosanedioic acid) to its ε-amine. Key design decisions:

- **C20 chain length:** Matches the native Lys-20 lipidation chemistry (also C20 diacid), minimizing synthetic novelty and maintaining physicochemical consistency. This contrasts with Retatrutide Fold №45, which used a C18 chain on the same conceptual scaffold — the C20 choice here is deliberate for Tirzepatide-specific synthetic integration.
- **γGlu (single) spacer at Lys-40:** The native Lys-20 uses a γGlu-γGlu (double) spacer; using a single γGlu at Lys-40 provides spatial differentiation between the two anchors, potentially reducing the risk of intramolecular steric conflict

between the two lipid chains while still providing sufficient linker flexibility for albumin engagement.

- **C-terminal positioning:** The PPPS tail is the most chemically permissive region of the peptide. Fold №63 (Tirzepatide, i,i+4 lactam in the central helix) was DISCARDED (pLDDT 0.69) due to tool-limit failure on helix-perturbing chemistry — the C-terminal strategy deliberately avoids that failure mode.
- **Retatrutide precedent:** Fold №45 applied the same conceptual framework to Retatrutide (C-terminal Lys-40, γGlu-C18 diacid) and returned PROMISING (pLDDT 0.70), validating that the PPPS tail of incretin peptides tolerates lipid conjugation without disrupting the helical pharmacophore in structural prediction.

PREDICTED PROPERTIES — WHERE THE SIGNAL IS MODERATE

Metric	Value	Interpretation
pLDDT (peptide)	0.716	Moderate confidence; consistent with intact helical core + disordered C-terminus
pTM	0.660	Moderate; overall peptide topology reasonably predicted
ipTM	0.096	Very low; GLP-1R interface not resolved by Boltz-2 at this target class
Chai-1 agreement	Not available	Single-model prediction; ensemble uncertainty unquantified
Aggregation propensity (heuristic)	0.222	Low; favorable for a dual-lipidated molecule
Stability score (heuristic)	0.627	Moderate-to-good
Half-life estimate (heuristic)	Long (>6 h, modification-dependent)	Consistent with extended PK rationale
BBB penetration (heuristic)	0.037	Negligible; appropriate for a peripheral metabolic agent

Where the PROMISING signal is genuine: - pLDDT of 0.716 is consistent with the helical pharmacophore being well-folded in the model, matching the predictions for Retatrutide Fold №45 (0.70) and Tirzepatide Folds №23 and №31 (both 0.71). - Low aggregation propensity (0.222) is encouraging given the dual-lipidation burden

— two C20 fatty acid chains on the same 40-residue peptide create real self-association risk that the heuristic does not flag. - The heuristic long half-life estimate is consistent with — though not quantitatively predictive of — the bivalent albumin hypothesis.

Where the signal is weak: - The ipTM of 0.096 means that GLP-1R TMD engagement, including the canonical Tyr-1 insertion, cannot be confirmed from this prediction. This is a known limitation of Boltz-2 for class B GPCR peptide-receptor complexes and reflects tool resolution rather than a predicted binding failure — but it leaves the receptor pharmacology hypothesis unvalidated. - Lipid conjugates are not explicitly modeled; both Lys-20 and Lys-40 sidechain positions appear solvent-exposed, which is consistent with the hypothesis, but the albumin-binding geometry of the dual-anchor configuration cannot be assessed in silico. - No Chai-1 ensemble data are available; single-model predictions carry higher uncertainty.

WHAT WOULD STRENGTHEN THIS SIGNAL

Additional computational work: - **Ensemble prediction (Chai-1 multi-seed):** Run 5+ seeds to assess structural convergence of the helical core and variability of the C-terminal tail disposition. Retatrutide Fold №45 would benefit from the same treatment as a direct comparator. - **Explicit albumin co-fold:** Model the dual-lipidated Tirzepatide in complex with human serum albumin (HSA, PDB 1AO6 fatty acid binding sites) using AlphaFold-Multimer or Boltz-2 multimer mode to assess whether both anchors simultaneously engage albumin or compete stereoelectronically. - **Free energy perturbation (FEP) or MM-GBSA:** Estimate relative albumin-binding affinity of single-anchor (native) vs. dual-anchor analog — this is the most direct computational test of the bivalent PK hypothesis. - **Molecular dynamics simulation:** Assess conformational stability of the PPPS-K(γGlu-C20) tail and its impact on receptor-proximal residues over nanosecond timescales.

Wet-lab validation experiments: - **SPR or ITC against human serum albumin:** Measure Kd and off-rate for native Tirzepatide vs. Fold №73 analog — direct test of whether dual lipidation increases albumin affinity. - **GLP-1R and GIPR cAMP assay (HEK293 or CHO overexpression):** Quantify EC50 for both receptors; critical to confirm that the Lys-40 C-terminal lipid does not perturb GIPR C-terminal engagement. - **In vitro DPP-4 stability assay:** Confirm that the C-terminal modification does not indirectly alter proteolytic susceptibility at the N-terminal scissile bond. - **Pharmacokinetic study in rodents or NHP:** The definitive test — plasma half-life measurement for dual-anchor vs. native Tirzepatide is the only way to confirm the bivalent albumin hypothesis experimentally. - **SEC or DLS aggregation assay:** Assess self-association of the dual-lipidated analog, particularly at concentrations relevant to subcutaneous injection formulation.

Key comparator experiment: Synthesize the Retatrutide Fold №45 analog (γGlu-C18 diacid at Lys-40) and the Tirzepatide Fold №73 analog (γGlu-C20 diacid at Lys-40) in parallel and run head-to-head albumin-binding and receptor-activation assays — this would validate the cross-peptide PPPS-tail lipidation strategy as a generalizable platform.

LAB CONTEXT & CROSS-FOLD CONNECTIONS

This fold is part of a developing Tirzepatide medicinal chemistry series at Alembic Labs:

- **Fold №45 (Retatrutide)** — the direct structural precedent: C-terminal Lys-40 γGlu-C18 diacid on the homologous PPPS tail, PROMISING (pLDDT 0.70). Fold №73 advances this to Tirzepatide with a C20 chain for chemistry consistency, returning an equivalent PROMISING verdict with slightly higher pLDDT (0.716).
- **Fold №63 (Tirzepatide, DISCARDED)** — the cautionary precedent: i,i+4 lactam staple in the central helix was DISCARDED (pLDDT 0.69) due to AlphaFold-blind crosslink chemistry. Fold №73 explicitly avoids helix-perturbing modifications.
- **Folds №23 and №31 (Tirzepatide, both PROMISING, pLDDT 0.71)** — non-canonical substitutions (αMe-Cys-24, Aib-2) in the helical core returned consistent moderate-confidence predictions, establishing the baseline pLDDT range for this peptide scaffold. Fold №73 falls within this established range, suggesting the C-terminal modification does not degrade the global structural prediction.
- **Fold №54 (Retatrutide, REFINED)** — Lys-17/Asp-21 lactam in the central helix returned REFINED (pLDDT 0.71), confirming that the incretin helix is a productive target for conformational pre-organization; however, this strategy targets potency rather than PK, and Fold №73 occupies the complementary pharmacokinetic space.

The emerging Alembic Labs narrative for Tirzepatide modifications is: the helical core is structurally robust to non-canonical substitutions at positions 2 and 24 (Folds №23, 31), the helix is not tolerant of crosslinking chemistry visible to AlphaFold (Fold №63), and the PPPS C-terminus is a permissive site for lipid conjugation (Fold №73, consistent with Fold №45 for Retatrutide). The next logical step in this series is a triple-combination analog combining Aib-2 (DPP-4 resistance, Fold №31) with C-terminal lipidation (Fold №73) to address both enzymatic stability and PK extension simultaneously.

SEQUENCES

NATIVE

```
YAEGTFTSDYSIYLDKQAAKEFVCWLLAGGPSSGAPPPS
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MODIFIED

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YAEGTFTSDYSIYLDKQAAKEFVCWLLAGGPSSGAPPPSK
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CAVEATS

- in silico prediction only — requires wet-lab validation
- single-run prediction (not ensembled); Chai-1 multi-seed data unavailable for this fold
- predicted properties may not reflect real-world biological behavior
- this is research, not medical advice
- ipTM of 0.096 means GLP-1R and GIPR interface geometry cannot be confirmed — receptor pharmacology is inferred from structural analogy, not predicted binding mode
- lipid conjugates (γGlu-C20 diacid on Lys-20 and Lys-40) are not explicitly modeled in the structural prediction; sidechain solvent exposure is inferred, not computed
- bivalent albumin engagement hypothesis has not been demonstrated experimentally for any incretin peptide; dual-anchor configuration could alternatively drive inter-molecular albumin bridging or peptide aggregation
- heuristic half-life estimate (>6 h) is sequence-based and does not model albumin binding kinetics, subcutaneous depot pharmacokinetics, or the specific contribution of the second lipid anchor
- potential perturbation of GIPR C-terminal ECD contacts by Lys-40 extension cannot be assessed from this prediction
- aggregation propensity score (0.222) is a heuristic estimate — empirical DLS/SEC validation is required for a dual-lipidated molecule at formulation-relevant concentrations

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 —** (2025) — — Discontinuing glucagon-like peptide-1 receptor agonists and body habitus: A systematic review and meta-analysis
6. **PMID —** (2024) — — The impact of tirzepatide and glucagon-like peptide 1 receptor agonists on oral hormonal contraception
7. **PMID —** (2024) — — Tirzepatide for Metabolic Dysfunction-Associated Steatohepatitis with Liver Fibrosis
8. **PMID —** (2026) — — Starvation ketosis following self-administered tirzepatide obtained via online services in a young woman later diagnosed with anorexia nervosa: a case report

SOLANA SIGNATURE EFr8mgd2PseuejUuWwrkUccKhFscmADHk4kUfR9839sD3r4kk8xq9ypjydn6FkU64jQTQweQZEjNuWL9FXWxEt2
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