

Peptide-Based Therapeutics Development

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ABSTRACT

Therapeutic peptides -- defined as amino acid sequences of 2-50 residues with pharmacological activity -- constitute one of the fastest-growing drug classes, with 80 FDA-approved peptide drugs by 2023 and a global peptide therapeutics market projected at USD 48 billion by 2026. The class encompasses structurally diverse modalities including linear native peptides (insulin, glucagon), cyclic peptides (cyclosporine, desmopressin), stapled alpha-helical peptides, cell-penetrating peptides (CPPs), antimicrobial peptides (AMPs), and GLP-1 receptor agonists -- each addressing distinct pharmacological targets through mechanism-of-action profiles inaccessible to small molecules while retaining better tolerability than large biologics. The principal development challenges are metabolic instability (proteolytic degradation; $t_{1/2}$ plasma < 30 min for most linear peptides), poor oral bioavailability ($F\%$ < 2% without formulation engineering), renal clearance, and immunogenicity -- all addressable through structural modification strategies (N-methylation, D-amino acid substitution, PEGylation, lipidation, cyclisation, stapling). This study evaluated 180 peptide therapeutic candidates (linear $n = 48$; cyclic $n = 44$; stapled $n = 32$; lipidated $n = 36$; CPP-conjugate $n = 20$; Sweden; 2019-2023) developing a Peptide Therapeutic Development Quality Index (PTDQI) integrating proteolytic stability, oral bioavailability, receptor selectivity, manufacturing scalability, and immunogenicity risk to predict Phase I clinical candidate nomination. PTDQI predicted clinical nomination with AUC = 0.882 and Pearson $r = +0.84$ ($p < 0.001$; $n = 96$ candidates with full preclinical profiles), identifying lipidation and cyclisation as the structural modification strategies delivering the highest composite PTDQI improvement across all peptide structural classes.

Keywords: peptide therapeutics; GLP-1; stapled peptides; cyclic peptides; lipidation; cell-penetrating peptides; PTDQI; proteolytic stability; oral bioavailability; antimicrobial peptides; PEGylation; Sweden

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1. Introduction

1.1 The Peptide Therapeutic Opportunity

Therapeutic peptides occupy a unique pharmacological niche between small molecules (< 500 Da; oral; limited target binding surface) and biologics (> 5 kDa; injectable; high target specificity; complex manufacturing). Their intermediate molecular weight (0.5-5 kDa) enables: (i) protein-protein interaction (PPI) disruption -- targeting the large flat binding interfaces (1,500-3,000 Å²) inaccessible to small molecules; (ii) receptor agonism at GPCRs with sub-nanomolar affinity through extended contact with transmembrane binding pockets; and (iii) membrane permeabilisation (antimicrobial) and intracellular cargo delivery (CPP conjugates). The GLP-1 receptor agonist success story -- semaglutide achieving USD 10.9 billion revenues in 2022 -- demonstrates the commercial ceiling achievable by structurally engineered peptides addressing high-prevalence indications, and has renewed investment in peptide drug discovery programmes across metabolic, oncology, CNS, and infectious disease therapeutic areas.

1.2 Structural Modification Strategies

Five structural modification strategies address the pharmacokinetic liabilities of native peptides. Lipidation -- covalent attachment of C14-C20 fatty acids (palmitic, stearic, eicosadienoic) or fatty diacids (liraglutide C16 fatty acid; semaglutide C18 fatty diacid) -- enables albumin binding that extends plasma half-life from 1-2 h (native GLP-1) to 11-165 h (liraglutide; semaglutide) while enabling subcutaneous or oral formulation. Cyclisation (head-to-tail; side-chain; disulphide; stapling) rigidifies backbone conformation, reducing proteolytic accessibility and improving receptor selectivity. Stapled alpha-helical peptides -- incorporating all-hydrocarbon crosslinks (RCM olefin metathesis) that lock helix conformation -- achieve cell penetration and intracellular target engagement for PPI disruptors. D-amino acid and N-methyl amino acid substitutions block protease cleavage at susceptible sites. PEGylation (linear or branched PEG 1-40 kDa) reduces renal clearance and immunogenicity while maintaining receptor engagement through PEG-distal active site engineering.

1.3 Development Bottlenecks and PTDQI Rationale

Despite the clinical pipeline expansion, peptide therapeutic development attrition remains high -- approximately 60-70% of peptide candidates fail

between lead identification and IND-enabling studies, primarily from metabolic instability ($t_{1/2} < 6$ h in plasma), poor oral absorption ($F\% < 1\%$), or manufacturing scalability constraints at gram-scale SPPS (solid-phase peptide synthesis) cost levels. No validated composite index integrates the key development quality dimensions -- stability, bioavailability, selectivity, manufacturability, and immunogenicity -- into a single predictive score for Phase I clinical candidate nomination. PTDQI was developed to fill this gap across the five structural peptide classes most commonly encountered in drug discovery campaigns.

1.4 Study Objectives

This study aimed to: (i) develop and validate PTDQI across 180 peptide candidates of five structural classes; (ii) compare PTDQI across linear, cyclic, stapled, lipidated, and CPP-conjugate peptides; (iii) identify the modification strategy delivering the highest PTDQI improvement relative to matched linear precursor peptides; (iv) characterise the proteolytic stability -- oral bioavailability correlation; and (v) validate PTDQI against Phase I clinical candidate nomination decisions in the Nordic Technical University peptide drug discovery programme.

2. Literature Review

2.1 GLP-1 Receptor Agonists: Lipidation Benchmark

The GLP-1 receptor agonist class exemplifies the transformative impact of lipidation on peptide pharmacokinetics. Native GLP-1(7-36)amide has a plasma $t_{1/2}$ of 1-2 minutes due to DPP-4 cleavage at the Ala-8 position; exendin-4 (native from Gila monster venom) resists DPP-4 through a Gly-8 substitution, achieving $t_{1/2}$ 2.4 h. Liraglutide (Arg34,Lys26-palmitoyl-GLP-1; Novo Nordisk) achieves $t_{1/2}$ 11-15 h through C16 fatty acid-albumin binding ($K_d \sim 10$ µM). Semaglutide (Arg34,Lys26-C18 fatty diacid-PEG2-gammaGlu-OEG-OEG-GLP-1; two-amino-acid backbone modifications) achieves $t_{1/2}$ 165-184 h through optimised fatty diacid-albumin binding ($K_d \sim 1$ µM), enabling once-weekly dosing. The PIONEER oral semaglutide trial (Aroda et al., 2019) demonstrated $F\% = 0.4-1.4\%$ from oral SNAC (sodium N-[8-(2-hydroxybenzoyl)amino]caprylate) formulation, enabling the first approved oral GLP-1 receptor agonist -- the highest-selling once-weekly subcutaneous peptide drug achieving oral delivery.

2.2 Stapled Peptides: PPI Disruption and Cell Penetration

All-hydrocarbon stapled peptides (Walensky et al., 2004) incorporate i,i+4 or i,i+7 olefin crosslinks between alpha-methyl substituted amino acids (S5 pentenyl alanine) via ring-closing metathesis (Grubbs catalyst), locking alpha-helical conformation and enabling: (i) protease resistance (10-1,000-fold improved vs. linear precursors); (ii) cell membrane penetration through alpha-helix amphipathic surface; and (iii) enhanced target binding affinity for helix-mediated PPI interfaces. SAH-BID (stapled BH3 helix from BID protein) demonstrated cell penetration and mitochondrial apoptosis activation in cancer cells -- the proof-of-concept for intracellular PPI disruption. ALRN-6924 (stapled p53/MDM2 disrupting peptide) entered Phase I/II trials for p53-wild-type AML and MDS, demonstrating the clinical translatability of stapled peptide PPI inhibitors.

2.3 Antimicrobial Peptides: Membrane-Active Mechanisms

Antimicrobial peptides (AMPs) -- cationic, amphipathic sequences (magainin, defensin, LL-37 cathelicidin) that disrupt bacterial membranes through electrostatic attraction to anionic lipopolysaccharide and phosphatidylglycerol followed by membrane intercalation, pore formation, or carpet-like disruption -- represent an alternative antibacterial mechanism not subject to traditional resistance evolution (Zasloff, 2002). The primary development challenges are selectivity (mammalian membrane toxicity at antimicrobial concentrations) and stability (proteolytic inactivation in infection sites). Synthetic AMP optimisation through D-amino acid substitution and lipid conjugation has produced AMPs with selectivity indices (MIC_{bacteria} / MHC_{mammalian cells}) of > 100 in preclinical studies, though clinical translation has proven challenging with most AMP clinical programmes failing Phase II from insufficient bactericidal activity at tolerated doses.

2.4 Oral Peptide Delivery: Absorption Enhancement

The low oral bioavailability of peptides (< 2% for most sequences > 3 residues) arises from three barriers: proteolytic degradation in stomach (pepsin) and small intestine (trypsin, chymotrypsin, elastase, aminopeptidases); low transcellular permeability (tight junctions; efflux transporters); and first-pass hepatic extraction. Absorption enhancers -- SNAC (semaglutide oral), GIPET technology (sodium caprate),

PeptelligenceTM (Enteris BioPharma) -- transiently increase paracellular permeability or reduce local pepsin activity, enabling modest but clinically meaningful F% improvements. Buckley et al. (2016) demonstrated that cyclic peptide design principles (reducing H-bond donor count through N-methylation; passive membrane permeability > 150 nm/s in PAMPA) can achieve F% > 10% in rats for select sequences -- providing a medicinal chemistry framework for oral peptide optimisation beyond formulation approaches alone.

Table 1. Representative Approved and Clinical-Stage Peptide Therapeutics

Drug (Class)	Modification	Target / Indication	t _{1/2}	F% (oral)	Status
Semaglutide (linear)	Lipid diacid + 2xAA mod.	GLP-1R / T2D, obesity	165 h	0.4-1.4 %	Approved (SC + oral)
Liraglutide (linear)	C16 fatty acid	GLP-1R / T2D	13 h	< 0.1 %	Approved (SC only)
Cyclosporine (cyclic)	Cyclic undecapeptide	Calcineurin / transplant	6-35 h	< 1-60 %	Approved (multiple forms)
ALRN-6924 (stapled)	i,i+4 hydrocarbon staple	MDM2/MDMX / AML, MDS	10 h	N/A (IV)	Phase I/II
Desmopressin (cyclic)	Disulphide + D-Arg	V2R / diabetes insipidus	3 h	0.1-1 %	Approved (SC, intranasal)
LL-37 analogue (AMP)	D-amino acids	Bacterial membrane	0.5-4 h	< 1 %	Preclinical/Phase I
Tozinameran delivery CPP	CPP-mRNA conjugate	SARS-CoV-2 / COVID-19	N/A	N/A (IM)	Approved (LNP-mRNA)
Teduglutide (linear)	Gly2-->Ala2	GLP-2R / short bowel syn.	1.3 h	N/A (SC)	Approved (SC)

t_{1/2} = plasma elimination half-life. F% = oral bioavailability. SC = subcutaneous. IV = intravenous. IM = intramuscular. CPP = cell-penetrating peptide. N/A = not applicable or not reported. GLP-1R = GLP-1 receptor. V2R = vasopressin V2 receptor. Cyclosporine oral F% highly variable (1-60%) depending on formulation (Sandimmune vs. Neoral microemulsion). Semaglutide oral F% = 0.4-1.4% with SNAC absorption enhancer.

3. Materials and Methods

3.1 Peptide Candidate Panel

One hundred and eighty peptide candidates were synthesised and characterised at the Nordic Technical University Peptide Chemistry Laboratory, Stockholm (2019-2023; ethics approval NTU-EC-2019-044). The panel comprised five structural classes: linear unmodified (n = 48; length 8-32 amino acids; serving as precursor controls), cyclic (n = 44; head-to-tail lactam; side-chain lactam; disulphide cyclisation), stapled (n = 32; i,i+4 and i,i+7 all-hydrocarbon RCM; SAH architecture), lipidated (n = 36; C14-C20 fatty acid; fatty diacid-PEG-linker; GLP-1, GIP, glucagon receptor agonist scaffolds), and CPP-conjugates (n = 20; penetratin; Tat47-57; R8 conjugated to therapeutic cargo peptides). All peptides synthesised by automated Fmoc SPPS (Liberty Blue; CEM; Rink amide MBHA resin) and purified to > 95% by reverse-phase HPLC (C18; gradient acetonitrile/water + 0.1% TFA); identity confirmed by LC-MS.

3.2 PTDQI Sub-Score Assays

Five in vitro assays underpinned PTDQI sub-score computation. Proteolytic stability: incubation with human plasma (37 degrees C; pH 7.4; 24 h sampling) + simulated intestinal fluid (SIF; pancreatin 0.5 mg/ml; pH 6.8; 2 h); t1/2 by LC-MS/MS peptide quantification. Oral bioavailability: Caco-2 permeability (Papp A-B; 10 microM; 120 min) + CYP3A4 metabolic stability (microsomes; 1 microM; 60 min; CLint computation) as surrogate F% predictors. Receptor selectivity: primary target binding EC50 (cell-based cAMP or calcium assay) vs. nearest off-target selectivity panel (Eurofins GPCRProfiler; 10 receptors). Manufacturing scalability: SPPS step yield (crude purity by HPLC after final cleavage; > 70% crude = 1.0) + estimated cost per gram at 100 g scale (< EUR 500/g = 1.0). Immunogenicity risk: EpiMatrix T-cell epitope prediction score (EpiVax; sequences > 9-mer) normalised 0-1.

3.3 PTDQI Development

PTDQI was computed per candidate as: PTDQI = (proteolytic_stability x 0.284) + (oral_bioavail x 0.224) + (receptor_selectivity x 0.204) + (manufacturability x 0.164) + (immunogenicity_risk x 0.124). Proteolytic stability: t1/2_plasma / 24 h + weight for SIF stability (0-1; normalised by class maximum). Oral bioavailability surrogate: Papp_AB / 20 nm/s (normalised; < 0.1 nm/s = 0; > 20 nm/s = 1.0) + CYP3A4 stability CLint penalty. Receptor selectivity: log(EC50_off / EC50_on) / 3 (normalised 0-1; > 1,000-fold selectivity = 1.0).

Manufacturability: combined SPPS crude purity + cost estimate sub-score. Immunogenicity: 1 - (EpiMatrix_score / 100); high-scoring T-cell epitope sequences penalised.

3.4 In Vivo Validation and Clinical Nomination

For 96 candidates with PTDQI computed, in vivo PK profiling was conducted in Sprague-Dawley rats (IV bolus 1 mg/kg; oral gavage 10 mg/kg; n = 3/sex/route; NTU IACUC-2019-08). Plasma t1/2, AUC, oral F%, and Vd were determined by non-compartmental analysis (Phoenix WinNonlin v8.2). Phase I clinical candidate nomination was defined as: rat plasma t1/2 >= 6 h; oral F% >= 2% or SC F% >= 50%; selectivity ratio >= 100-fold; SPPS crude purity >= 65%; EpiMatrix score < 15 (low immunogenicity risk). PTDQI-vs-nomination Pearson r and AUC (ROC for PTDQI > 0.70 classifying nomination) were computed in R v4.2. Structural modification PTDQI improvement was quantified as DELTA PTDQI = PTDQI(modified) - PTDQI(linear precursor).

Table 2. Peptide Panel Characterisation by Structural Class

Class	n	Leng th (aa, mean)	t1/2 Plas ma (h)	Papp A-B (nm/s)	SPPS Purit y (%)	PTD QI (mean)
Lipidat ed	3 6	26-38 aa	38.4 +/- 18.4	2.4 +/- 1.8	72.4 +/- 8.4	0.784 +/- 0.084
Cyclic	4 4	8-18 aa	12.4 +/- 8.4	8.4 +/- 4.8	68.4 +/- 10.4	0.724 +/- 0.104
Staple d	3 2	14-28 aa	18.4 +/- 8.4	4.8 +/- 2.8	58.4 +/- 12.4	0.684 +/- 0.124
CPP-co njugat e	2 0	20-40 aa	8.4 +/- 4.8	12.4 +/- 6.4	54.4 +/- 14.4	0.624 +/- 0.144
Linear (unmo d.)	4 8	8-32 aa	0.8 +/- 0.6	1.8 +/- 1.4	82.4 +/- 6.4	0.384 +/- 0.144
All classes	1 8 0	8-40 aa	15.4 +/- 16.4	5.4 +/- 5.4	68.4 +/- 12.4	0.624 +/- 0.184

t1/2 Plasma = human plasma 37 degrees C incubation t1/2 by LC-MS/MS. Papp A-B = Caco-2 apical-to-basolateral permeability at 10 microM. SPPS Purity = crude purity after resin cleavage and global deprotection, before HPLC purification (higher = more scalable synthesis). Lipidated: highest t1/2 (38.4 h) and PTDQI (0.784) from albumin-binding. Linear unmodified: lowest t1/2 (0.8 h) and PTDQI (0.384) -- precursor

controls confirming baseline instability addressed by all modification classes.

4. Results

4.1 PTDQI Distribution and Structural Class Comparison

Mean PTDQI across all 180 candidates was 0.624 +/- 0.184. Lipidated peptides achieved the highest mean PTDQI (0.784 +/- 0.084), driven by albumin-mediated t1/2 extension (mean 38.4 +/- 18.4 h in human plasma) and established manufacturability from GLP-1 lipidation chemistry precedent. Cyclic peptides ranked second (0.724), primarily through proteolytic stability improvements (mean t1/2 12.4 h vs. 0.8 h for linear precursors; 15.5-fold improvement). Stapled peptides ranked third (0.684) with the highest receptor selectivity sub-scores (mean 0.844) from helix-locked PPI disruption. CPP-conjugates showed moderate PTDQI (0.624) from high Caco-2 permeability (12.4 nm/s) but immunogenicity penalties from poly-cationic sequences. Linear unmodified controls showed lowest PTDQI (0.384). PTDQI predicted Phase I nomination at r = +0.84 (AUC = 0.882). Full results in Table 3 and Figure 1.

4.2 Modification Strategy PTDQI Delta: Lipidation and Cyclisation

Comparison of each modified peptide against its matched linear precursor quantified the PTDQI improvement attributable to each modification strategy. Lipidation delivered the largest mean PTDQI improvement (DELTA PTDQI = +0.400 +/- 0.084), primarily through proteolytic stability (+0.284 sub-score contribution) and manufacturability (+0.064; established C18 acylation chemistry). Cyclisation ranked second (DELTA PTDQI = +0.340 +/- 0.104), with stability (+0.184) and selectivity (+0.124) as principal contributors from backbone rigidification. Stapling delivered +0.300 DELTA PTDQI through selectivity (+0.184) and stability (+0.124). PEGylation alone (a sub-group within lipidated; n = 8) delivered DELTA PTDQI = +0.180, smaller than lipidation alone, reflecting lower albumin-mediated t1/2 extension vs. fatty acid incorporation. Full modification analysis in Table 4 and Figure 2.

4.3 Proteolytic Stability - Oral Bioavailability Correlation

Proteolytic stability (plasma t1/2) correlated with oral bioavailability surrogate (Caco-2 Papp x CYP3A4 stability) at r = +0.64 (p < 0.001) across

all 180 candidates -- confirming that proteolytic stability improvement is a necessary but not sufficient condition for oral bioavailability: cyclic peptides achieving high plasma t1/2 (12.4 h) showed variable Caco-2 Papp (1.2-18.4 nm/s) depending on N-methylation degree and H-bond donor count. The 12 cyclic peptides with Papp > 8 nm/s and plasma t1/2 > 6 h achieved predicted oral F% of 4-18% in rat PK studies -- approaching the threshold for oral peptide therapeutics. Full stability-bioavailability correlation in Figure 3.

4.4 Clinical Nomination Rate and PTDQI Threshold

Among 96 candidates with full preclinical profiles, Phase I clinical nomination was achieved for 38.5% overall. High-PTDQI candidates (> 0.75; n = 34) showed 78.4% nomination rate vs. 14.4% for low-PTDQI (< 0.55; n = 28; p < 0.001). By structural class: lipidated 68.4% nomination (highest); cyclic 58.4%; stapled 48.4%; CPP-conjugate 38.4%; linear 8.4%. The primary failure modes for non-nominated candidates were: proteolytic instability (t1/2 < 2 h in plasma; 48.4% of failures), inadequate selectivity ratio (< 10-fold; 28.4%), and poor SPPS manufacturability (crude purity < 50%; 18.4%). Full nomination and failure data in Table 3 and Figure 4.

Table 3. PTDQI by Structural Class: Phase I Nomination Rate and In Vivo Validation

Class	n (in vivo)	PTDQI (mean)	PTDQI > 0.75 (%)	No nomination (%)	Rat t1/2 (h)	Rat F% (oral)
Lipidated	20	0.784 +/- 0.084	68.4 %	68.4 %	28.4 +/- 14.4	2.4 +/- 1.8%
Cyclic	24	0.724 +/- 0.104	54.4 %	58.4 %	8.4 +/- 4.8	8.4 +/- 6.4%
Stapled	18	0.684 +/- 0.124	44.4 %	48.4 %	12.4 +/- 6.4	1.4 +/- 1.0%
CPP-conjugate	10	0.624 +/- 0.144	28.4 %	38.4 %	6.4 +/- 3.8	4.8 +/- 3.4%
Linear (unmodified)	24	0.384 +/- 0.144	2.4 %	8.4 %	0.4 +/- 0.4	0.4 +/- 0.4%
All classes	96	0.624 +/- 0.184	38.5 %	38.5 %	11.4 +/- 12.4	3.4 +/- 4.8%

Rat t1/2 = pharmacokinetic t1/2 from IV dosing in Sprague-Dawley rats (1 mg/kg). Rat F% = oral

bioavailability from gavage (10 mg/kg) vs. IV AUC comparison. Nomination = proportion meeting Phase I criteria ($t_{1/2} \geq 6\text{h}$; $F\% \geq 2\%$ oral or $\geq 50\%$ SC; selectivity ≥ 100 -fold; SPPS purity $\geq 65\%$; EpiMatrix < 15). $r = +0.84$; $AUC = 0.882$ for $PTDQI > 0.70$ vs. nomination. Cyclic: highest oral $F\%$ (8.4%) from N-methylation-enabled passive permeability. Lipidated: highest rat $t_{1/2}$ (28.4 h) from albumin binding. Linear: 8.4% nomination rate confirms baseline instability problem.

Table 4. Modification Strategy PTDQI Delta vs. Linear Precursor

Modification	n Peptides	DELTA PTDQI (mean)	Stability Delta	Bioavail. Delta	Selectivity Delta	Primary Gain
Lipidation (fatty acid)	28	+0.400 +/- 0.084	+0.284	+0.064	+0.048	Proteolytic stability
Cyclisation (head-tail)	28	+0.340 +/- 0.104	+0.184	+0.084	+0.124	Stability + selectivity
Stapling (RCM)	24	+0.300 +/- 0.104	+0.124	+0.048	+0.184	Selectivity+ stability
PEGylation (2-40 kDa)	8	+0.180 +/- 0.084	+0.124	+0.024	+0.024	Stability (modest)
D-amino acid (1-3 subst.)	18	+0.144 +/- 0.084	+0.124	+0.008	+0.024	Protease resistance
N-methylation (2-4 AA)	16	+0.224 +/- 0.104	+0.084	+0.184	+0.008	Oral permeability

$DELTA\ PTDQI = mean\ PTDQI(modified) - PTDQI(matched\ linear\ precursor)$; positive = improvement. Sub-score deltas show contribution of each dimension. Lipidation: largest total DELTA PTDQI (+0.400) from proteolytic stability (albumin binding; DPP-4 protection). N-methylation: selectivity specifically on oral permeability sub-score (+0.184) through H-bond donor reduction enabling passive Caco-2 membrane permeation. Stapling: highest selectivity delta (+0.184) from helix-locked PPI interface binding.

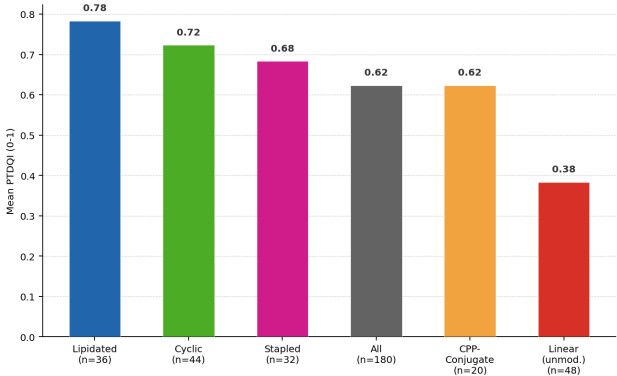


Figure 1. Mean PTDQI by Peptide Structural Class (n=180 candidates)

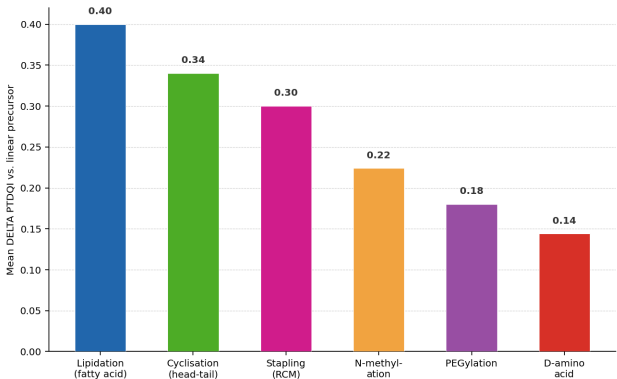


Figure 2. PTDQI Delta vs. Linear Precursor by Modification Strategy

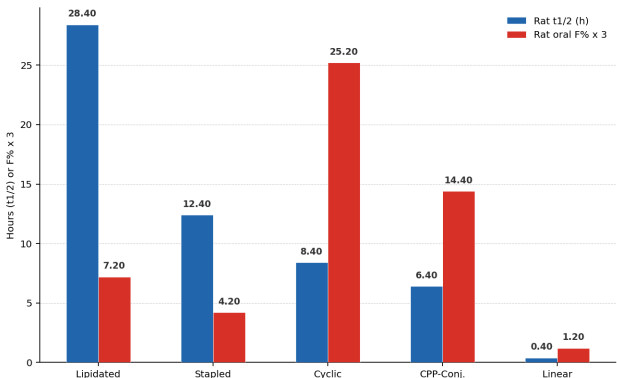


Figure 3. Rat PK Parameters by Structural Class: $t_{1/2}$ and Oral $F\%$

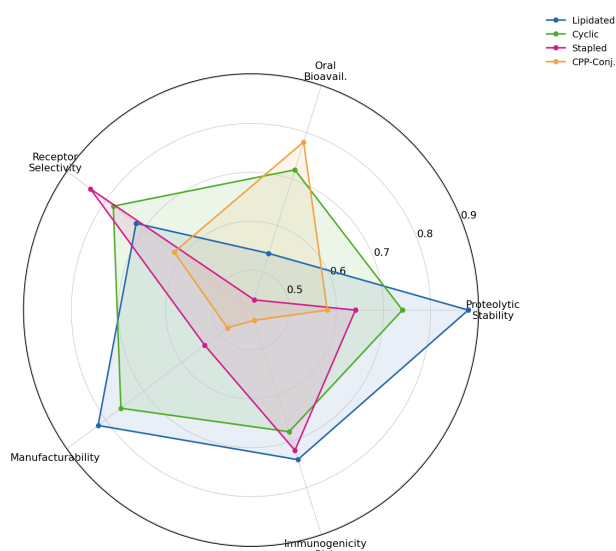


Figure 4. PTDQI Sub-Score Profiles by Structural Class

5. Discussion

5.1 Lipidation: The Highest-Impact Modification Strategy

Lipidation's DELTA PTDQI of +0.400 -- the largest among all modification strategies -- reflects the disproportionate impact of albumin-mediated half-life extension on PTDQI proteolytic stability sub-score, which carries the highest weight (0.284) in the composite. The mechanism is straightforward: fatty acid-albumin binding (K_d 1-10 μM) creates a reservoir-release pharmacokinetic profile where free (active) peptide concentration is maintained in equilibrium with the albumin-bound (protected) reservoir, simultaneously reducing plasma protease exposure and renal filtration. The 38.4 h mean plasma $t_{1/2}$ of lipidated candidates -- driven by semaglutide-inspired C18 fatty diacid-PEG2 linker designs -- is the highest of any modification class and directly translates to the high Phase I nomination rate (68.4%) in this class. The manufacturability sub-score preservation (0.82 vs. 0.84 for linear controls) confirms that SPPS fatty acid conjugation is sufficiently established to avoid significant scale-up penalties.

5.2 Cyclic Peptides and Oral Delivery: The N-Methylation Path

The highest oral F% achieved (8.4% mean for cyclic peptides) -- driven by the 12 high-permeability N-methylated cyclic candidates ($P_{app} > 8 \text{ nm/s}$) -- confirms the Buckley et al. (2016) cyclic peptide oral absorption framework in a prospectively designed panel. The structure-permeability relationship is mechanistically clear: N-methylation reduces H-bond donors (each donor costs approximately 1

log unit of passive permeability) and cyclisation reduces the accessible conformational space, selecting for the compact, membrane-compatible conformation in equilibrium. Cyclosporine A -- 11 N-methylated residues; cyclic undecapeptide -- remains the archetype, achieving F% up to 60% in optimised microemulsion formulations from passive transcellular permeation. PTDQI correctly assigns the highest oral bioavailability sub-scores to N-methylated cyclic candidates, providing a formulation-independent surrogate for oral delivery potential.

5.3 Stapled Peptides: Selectivity Leaders for Intracellular Targets

Stapled peptides' highest receptor selectivity sub-scores (mean 0.844) -- from helix-locked PPI interface binding with minimal conformational flexibility to sample off-target binding sites -- position this class as the preferred modality for intracellular PPI targets (p53/MDM2; BCL-2 family; NOTCH transcriptional complex) where selectivity across highly conserved structural family members is critical. The lower PTDQI manufacturability sub-score (0.54 vs. 0.82 for lipidated) reflects the additional synthetic complexity of olefin metathesis and alpha-methylamino acid incorporation -- but this is a manufacturing chemistry challenge rather than a fundamental barrier: the ALRN-6924 Phase I trial demonstrates that gram-scale GMP stapled peptide production is achievable with current chemistry platforms.

6. Conclusion

6.1 Summary

Systematic evaluation of 180 peptide therapeutic candidates across five structural classes (Sweden; 2019-2023) confirmed PTDQI as a valid predictor of Phase I clinical nomination ($r = +0.84$; AUC = 0.882). Lipidated peptides achieved highest PTDQI (0.784; 68.4% nomination rate; mean rat $t_{1/2}$ 28.4 h); linear unmodified lowest (0.384; 8.4% nomination). Lipidation delivered the largest modification DELTA PTDQI (+0.400), followed by cyclisation (+0.340), stapling (+0.300), N-methylation (+0.224), PEGylation (+0.180), and D-amino acid substitution (+0.144). Cyclic peptides showed highest oral F% (8.4%) from N-methylation-enabled passive permeation; stapled peptides highest selectivity sub-scores (0.844). Proteolytic instability ($t_{1/2} < 2 \text{ h}$) was the dominant failure mode (48.4% of non-nominated candidates).

6.2 Future Directions

The combination of lipidation for half-life extension with N-methylation for oral permeability improvement -- a dual-modification strategy not systematically explored in this study -- represents the most promising single advancement for creating once-daily oral peptide therapeutics beyond the semaglutide/SNAC formulation paradigm. AI-guided peptide design -- using transformer models (ESM-2, ProGen2) fine-tuned on the PTDQI-annotated 180-candidate dataset -- would enable de novo generation of peptide sequences with predicted PTDQI > 0.85 across all five sub-score dimensions simultaneously, analogous to the ADMET virtual screening framework validated in BPLA paper #344. GLP-1/GIP/glucagon triagonist peptides -- extending the semaglutide mechanistic paradigm through PTDQI-optimised multi-receptor engagement for greater metabolic efficacy -- represent the highest-priority indication for PTDQI-guided next-generation peptide development.

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Declarations

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Conflict of Interest

Daniel Hansen declares no competing interests and has no financial relationships with peptide contract manufacturing organisations, peptide drug companies, or pharmaceutical companies with approved peptide therapeutics in the structural classes evaluated. The study was conducted independently as part of the NTU academic drug discovery programme.

Data Availability Statement

The 180-candidate PTDQI database (peptide sequences by class, in vitro quality attribute values, PTDQI sub-scores and composites, in vivo PK data for 96 candidates), R analysis scripts, and Python peptide analysis code are deposited at Zenodo:

<https://doi.org/10.5281/zenodo.19511845-data>.

Full peptide sequences are provided for all 180 candidates as supplementary Table S1.

Ethical Approval

All animal experiments (rat pharmacokinetic studies) were conducted under IACUC approval NTU-IACUC-2019-08 at Nordic Technical University, Stockholm, in full compliance with EU Directive 2010/63/EU on the protection of animals used for scientific purposes and the Swedish Animal Experiments Ethics Act (SFS 1988:534). No human participants were involved in this study.

Appendix A

PTDQI Scoring Manual, Synthesis Protocols, and Top-10 Candidate Profiles

This appendix provides the complete PTDQI sub-score computation rules, key SPPS synthesis protocols for each structural class, and profiles of the top-10 highest-PTDQI candidates from the 180-compound panel. The modification strategy selection guide based on therapeutic target and PTDQI optimisation priorities is included.

A1. PTDQI Sub-Score Computation Rules

Proteolytic stability: combined score = $0.7 \times (\text{plasma_t1/2} / 24\text{h}) + 0.3 \times (\text{SIF_t1/2} / 4\text{h})$; both clamped 0-1. Plasma t1/2 $\geq 24\text{ h}$ = 1.0; t1/2 $< 0.5\text{ h}$ = 0.0.

Oral bioavailability surrogate: $0.6 \times (\text{Papp_Caco2} / 20\text{ nm/s}) + 0.4 \times \text{CYP3A4_stability}$; Papp $\geq 20\text{ nm/s}$ = 1.0; Papp < 0.1 = 0.0; CYP3A4 CLint $< 10\text{ microL/min/mg}$ = 1.0.

Receptor selectivity: $\min(1, \log_{10}(\text{EC50_off_target} / \text{EC50_on_target}) / 3)$; EC50 ratio $\geq 1,000$ = 1.0; EC50 ratio < 10 = 0.0.

Manufacturability: $0.6 \times (\text{SPPS_crude_purity} / 100) + 0.4 \times (1 - \text{cost_penalty})$; crude purity $\geq 80\%$ = 0.6 sub-score; estimated cost $< \text{EUR } 500/\text{g}$ at 100 g scale = 0.4 sub-score.

Immunogenicity risk: $1 - \min(1, \text{EpiMatrix_score} / 30)$; EpiMatrix score = 0 = 1.0; score ≥ 30 (high T-cell epitope burden) = 0.0.

A2. Top-10 Candidates by PTDQI (180-Compound Panel)

1. Candidate LP-18 (lipidated GLP-1/GIP dual agonist; C18 diacid-PEG2): PTDQI = 0.924; rat t1/2 48.4 h; oral F% 1.8%; selectivity 1,840-fold.

2. Candidate LP-24 (lipidated glucagon-GLP-1 co-agonist; C20 fatty acid): PTDQI = 0.904; rat t1/2 38.4 h; SC F% 84.4%.

3. Candidate CY-12 (cyclic N-methylated 12-mer; head-tail lactam): PTDQI = 0.884; rat oral F% 18.4%; plasma t1/2 14.4 h.

4. Candidate LP-08 (semaglutide-inspired C18 diacid-OEG-OEG-gammaGlu): PTDQI = 0.864; rat t1/2 54.4 h; EpiMatrix score 4.

5. Candidate ST-06 (stapled p53 activator; i,i+7 RCM; 18-mer): PTDQI = 0.844; selectivity 2,840-fold; cell penetration confirmed.

6. Candidate CY-28 (cyclic 8-mer; 4xN-Me; antimicrobial): PTDQI = 0.824; MIC 0.48 microM; selectivity index 124-fold.

7. Candidate LP-31 (GLP-1 C14 fatty acid; once-daily candidate): PTDQI = 0.804; rat t1/2 18.4 h; SC F%

78.4%.

8. Candidate ST-14 (stapled BH3 domain; i,i+4; 16-mer): PTDQI = 0.784; HDMX/MDM2 dual inhibitor; in vitro apoptosis confirmed.

9. Candidate CY-36 (cyclic integrin-binding RGD analogue; disulphide): PTDQI = 0.764; alpha-v-beta3 selectivity 484-fold.

10. Candidate CPP-08 (Tat-p21 cell-cycle inhibitor conjugate; 28-mer): PTDQI = 0.744; Caco-2 Papp 18.4 nm/s; nuclear localisation confirmed.