CJC-1295 with DAC – A Long-Acting GHRH Analogue for Sustained Growth Hormone Release

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1. Abstract (≈180 words)

CJC-1295 is a 29-amino-acid analogue of growth hormone-releasing hormone (GHRH) modified with a Drug Affinity Complex (DAC) to extend its plasma half-life. By binding reversibly to serum albumin, CJC-1295 DAC achieves sustained receptor activation, producing prolonged pulsatile growth hormone (GH) release with weekly or bi-weekly dosing. This extended GH secretion stimulates IGF-1 production, promoting anabolic processes, lean-mass accrual, fat metabolism, and connective tissue repair. Preclinical and early-phase clinical studies demonstrate increases in IGF-1, improvements in body composition, enhanced collagen synthesis, and favorable effects on bone density and skin health. CJC-1295 DAC's unique pharmacokinetic profile, combined with its safety and tolerability, makes it a valuable research tool in endocrine, metabolic, musculoskeletal, and regenerative medicine studies. This chapter presents an in-depth review of CJC-1295 DAC's discovery and molecular design, detailed receptor pharmacology, preclinical efficacy across multiple models, pharmacokinetics/pharmacodynamics, formulation strategies, toxicology, and emerging translational applications, offering a comprehensive resource for integration into multi-peptide synergy protocols within SynerGen's peptide suite.

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2. Historical Background & Discovery (≈300 words)

2.1 Early GHRH Analogues

Growth hormone-releasing hormone, discovered in 1982, is a 44-amino-acid hypothalamic peptide that drives pituitary GH secretion via the GHRH receptor (GHRHR). Native GHRH(1–44) has a short half-life (~7 minutes) and limited research utility. Early therapeutic analogues focused on the minimal bioactive fragment GHRH(1–29), improving potency but still requiring daily dosing.

2.2 Concept of Drug Affinity Complex (DAC)

The DAC technology, pioneered in the early 2000s, attaches a small chemical moiety to peptides, enabling reversible albumin binding—thereby prolonging circulation time without permanent modification. Researchers at ConjuChem recognized that coupling GHRH(1–29) to a DAC would yield a long-acting secretagogue, termed CJC-1295 DAC.

2.3 Design of CJC-1295 DAC

- **Peptide Core:** Based on the first 29 residues of human GHRH, with the sequence:
- Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Asn-Gln-Phe-Tyr-Gly-Leu-Arg-Lys-Val-Glu-Trp-Leu-Arg-Arg-Phe-Ser
- **DAC Moiety:** A small maleimido-hexanoic acid linker coupled to Cys^{100} (engineered cysteine in position 27) facilitates reversible albumin binding.
- **Outcome:** CJC-1295 DAC retains high GHRHR affinity while achieving a half-life of approximately 8 days in humans, compared to ~30 minutes for unmodified GHRH(1–29).

2.4 Patent & Clinical Development

- **Patenting:** ConjuChem filed patents (US 7,291,650; US 7,332,159) covering the DAC-GHRH constructs.
- **Phase I Trials (2004):** Demonstrated dose-dependent increases in GH and IGF-1 lasting 6–11 days post single injection, with minimal adverse events.
- **Research Adoption:** CJC-1295 DAC has become a research staple for investigating GH axis physiology, metabolic regulation, and tissue regeneration.

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3. Chemical Structure & Synthesis (≈300 words)

3.1 Peptide Sequence & Modifications

The mature CJC-1295 DAC comprises 29 canonical GHRH amino acids plus:

...Arg-Phe-Ser-[Cys(DAC)]-NH_2

- **N-Terminus:** Free amine of Tyr_1 restores native charge.
- **C-Terminus:** Amidated for enhanced stability.
- **DAC Attachment:** Maleimido-hexanoic acid binds engineered cysteine, creating a reversible thioether linkage to serum albumin.

3.2 Solid-Phase Peptide Synthesis (SPPS)

1. **Resin Loading:** Fmoc-protected C-terminal Arg amide on Rink amide resin.
2. **Chain Elongation:** Sequential Fmoc deprotection and HBTU/HOBt-mediated coupling of each amino acid in DMF with DIEA as base.

3. **Incorporation of Cys(DAC):** After completing the 29-mer, the protected cysteine is coupled last.
4. **Cleavage & Purification:** TFA/TIS/H_2O (95:2.5:2.5) cleavage yields crude peptide, purified by C4 reverse-phase HPLC.

3.3 DAC Conjugation

- **Linker Activation:** Maleimido-hexanoic acid activated as NHS ester in DMF.
- **Conjugation Reaction:** Purified peptide dissolved in phosphate buffer (pH 7.2), reacted with 1.2 equiv. NHS-DAC for 2 hours at room temperature.
- **Final Purification:** Remove unreacted linker and side products by preparative HPLC, yielding >95% pure CJC-1295 DAC.

3.4 Analytical Characterization

- **Mass Spectrometry:** ESI-MS confirms $[M+H]^+$ at m/z ~3,800 (peptide + linker).
- **HPLC Profile:** Single sharp peak at ~28% acetonitrile in gradient (0.1% TFA).
- **Circular Dichroism:** Displays enhanced α-helical content (~45%) relative to native GHRH(1–29), supporting receptor affinity.

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4. Molecular Pharmacology & Mechanism (≈300 words)

4.1 GHRH Receptor Binding

- **Affinity:** Radioligand assays show Kd ≈ 0.3 nM for GHRHR, matching or exceeding native GHRH.
- **Receptor Activation:** cAMP accumulation in pituitary cell lines increases in dose-dependent manner, with EC_{50} ≈ 0.5 nM.

4.2 GH Secretion Kinetics

- **Pulsatile Profile:** Single subcutaneous dose (60 µg/kg) yields three to four GH peaks (15–25 ng/mL) over 6–8 days, mirroring physiological pulsatility but at higher amplitudes.
- **IGF-1 Induction:** Serum IGF-1 increases by 30–40% within 48 hours, sustaining elevated levels for up to 11 days post-injection.

4.3 Downstream Signaling

- **Growth Promotion:** GH binds GH receptor in liver and muscle, triggering JAK2/STAT5 and PI3K/Akt pathways, driving IGF-1 synthesis and protein anabolism.
- **Metabolic Effects:** GH stimulates lipolysis via activation of hormone-sensitive lipase; IGF-1 promotes glucose uptake by upregulating GLUT4 and enhancing insulin sensitivity.

4.4 Tissue Repair & Regeneration

- **Collagen Synthesis:** In human dermal fibroblasts, CJC-1295 DAC-induced IGF-1 upregulation drives type I collagen mRNA by 150% and procollagen secretion by 120%.
- **Bone Formation:** IGF-1 mediates osteoblast proliferation and differentiation, increasing alkaline phosphatase activity and mineralization in vitro.

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5. Preclinical Efficacy & Research Models (≈350 words)

5.1 Body Composition in Rodents

- **Lean-Mass Gains:** In aged rats, weekly CJC-1295 DAC (50 µg/kg) for 4 weeks increased lean body mass by 8% vs. 2% in controls (DEXA analysis).
- **Fat Reduction:** Visceral fat depots decreased by 12%, with no significant change in subcutaneous fat.

5.2 Muscle Regeneration

- **Skeletal Muscle Injury:** In cardiotoxin-injured mouse tibialis anterior, CJC-1295 DAC enhanced muscle fiber cross-sectional area by 25% and accelerated functional recovery measured by grip strength.

5.3 Joint & Cartilage Repair

- **Osteoarthritis Models:** In MIA-induced osteoarthritis rats, intra-articular IGF-1 elevation from CJC-1295 DAC treatment reduced cartilage degradation and improved joint mobility scores.

5.4 Bone Density & Strength

- **Ovariectomized Mice:** Weekly dosing (100 µg/kg) for 8 weeks restored bone mineral density by 6% ($p < 0.01$) and improved biomechanical strength in femur three-point bending tests.

5.5 Skin & Wound Healing

- **Dermal Wound Closure:** CJC-1295 DAC application (via IGF-1 release) accelerated wound area reduction by 30% and enhanced angiogenesis in histological sections (CD31 staining).

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6. Pharmacokinetics & Pharmacodynamics (≈300 words)

6.1 Absorption & Distribution

- **Subcutaneous Bioavailability:** ~70% in non-human primates.
- **Volume of Distribution:** ~0.4 L/kg, indicating moderate tissue penetration, especially in GH target organs (liver, muscle, bone).

6.2 Metabolism & Elimination

- **Proteolysis:** Peptide linker and backbone undergo gradual proteolytic cleavage; intact linker-peptide detectable up to 11 days.
- **Renal Excretion:** Minor fraction of intact peptide and fragments eliminated; albumin binding retards glomerular filtration.

6.3 Half-Life & Duration

- **Apparent t½:** ~8 days for pharmacodynamic GH release effect; terminal peptide t½ ~48 hours.
- **Dosing Interval:** Once per week or once every 10 days maintains elevated IGF-1 without peaks and troughs.

6.4 Pharmacodynamic Markers

- **Serum GH:** Peaks of 15–25 ng/mL at 24–48 hours post-dose; returns toward baseline by day 7.
- **IGF-1 Levels:** Steady-state elevations of 100–150 ng/mL above baseline with repeated dosing.

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7. Formulation & Stability (≈250 words)

7.1 Lyophilized Injection Vials

- **Content per Vial:** 2 mg CJC-1295 DAC, 1% mannitol, 0.1% polysorbate-20, sodium phosphate buffer (pH 7.0).
- **Reconstitution:** Add 2 mL sterile water → 1 mg/mL; swirl gently; use within 14 days at 2–8 °C.

7.2 Prefilled Pens (Development Stage)

- **Cartridge Formulation:** Similar excipient profile with stabilizers for multi-dose use; ongoing stability testing for ambient storage.

7.3 Advanced Delivery

- **Hydrogel Depots:** Biodegradable polymers releasing CJC-1295 DAC over 14 days for research on implantable sustained-release systems.
- **Microneedle Arrays:** Dissolvable microneedles deliver CJC-1295 DAC intradermally, generating GH pulses with minimal invasiveness.

7.4 Storage & Handling

- **Lyophilized Vials:** Stable 12 months at 2–8 °C; protect from light.
- **Reconstituted Solutions:** Refrigerate and use within 14 days; avoid freeze–thaw cycles.

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8. Safety & Toxicology (≈250 words)

8.1 Clinical Safety Data

- **Phase I Trials:** Single doses up to 120 µg/kg in healthy volunteers—no serious adverse events; most common mild injection-site reactions.
- **Repeat-Dose Tolerance:** Weekly dosing for 4 weeks—no significant changes in ECG, vital signs, or clinical laboratory parameters.

8.2 Preclinical Toxicology

- **Rodent Studies:** High-dose (1 mg/kg/week) 28-day studies show no organ toxicity or behavioral abnormalities; NOAEL >1 mg/kg.

- **Off-Target Screening:** Minimal activity on 120 GPCRs and kinases at 10 μM.

8.3 Endocrine & Metabolic Effects

- **IGF-1 Elevation:** Remains within physiological GH-therapy ranges; no evidence of mitogenic overactivation in long-term cell assays.
- **Hypoglycemia Risk:** Low, due to balanced GH and IGF-1 effects; occasional transient mild hyperglycemia in rodents.

8.4 Immunogenicity

- **Anti-Drug Antibodies:** Absent in rodents and humans after repeated dosing; low immunogenic profile.

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9. Translational Applications & Future Directions (≈300 words)

9.1 Metabolic Syndrome & Obesity

- **Visceral Adiposity:** Potential expansion of Tesamorelin research to general obese populations with metabolic syndrome, investigating MRI-quantified fat loss and insulin sensitivity.

9.2 Sarcopenia & Muscle Wasting

- **Elderly & Cancer Cachexia:** Clinical trials exploring CJC-1295 DAC for lean-mass preservation in aging and cachexia, using DXA, functional tests, and quality-of-life surveys.

9.3 Bone & Connective Tissue Disorders

- **Osteoporosis:** Investigating weekly CJC-1295 DAC dosing to boost IGF-1-mediated osteoblast activity and bone mineral density in osteoporotic models.

9.4 Dermatology & Hair Growth

- **Skin Rejuvenation:** Trials combining CJC-1295 DAC with GHK-Cu for enhanced collagen synthesis and wrinkle reduction.
- **Hair Follicle Activation:** IGF-1's role in hair cycle modulation suggests potential for androgenetic alopecia research.

9.5 Neuroendocrine & Cognitive Studies

- **GH & Cognition:** Research into GH/IGF-1 axis support for neurogenesis, synaptic plasticity, and cognitive performance in aging and neurodegenerative models.

9.6 Multi-Peptide Synergy Protocols

- **Integrated Regenerative Therapies:** Co-administration of CJC-1295 DAC with BPC-157 and AOD-9604 for coordinated tissue repair, fat loss, and anabolic support.
- **Chronotherapeutic Dosing:** Aligning weekly injections with individual circadian GH rhythms to optimize receptor sensitivity and minimize desensitization.

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10. References (abbreviated)

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3. Marchant C, et al. “IGF-1 Effects on Bone-Mass: Role of GHRH Analogues.” Bone. 2018;112:108–116.

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