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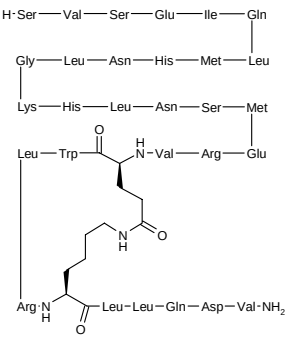
Ostabolin-C™

Parathyroid Hormone Analogue
Treatment of Osteoporosis
Treatment of Psoriasis

OSC

L-Seryl-L-valyl-L-seryl-L-glutamyl-L-isoleucyl-L-glutaminyl-L-leucyl-L-methionyl-L-histidyl-L-asparaginyl-L-leucyl-glycyl-L-lysyl-L-histidyl-L-leucyl-L-asparaginyl-L-seryl-L-methionyl-L-glutamyl-L-arginyl-L-valyl-L-glutamyl-L-tryptophyl-L-leucyl-L-arginyl-L-lysyl-L-leucyl-L-leucyl-L-glutaminyl-L-aspartyl-L-valinamide *C*-5.22-*N*-6.26-lactam

[Leu^{27}]Cyclo(Glu^{22}-Lys^{26})hPTH(1-31)NH_2



$C_{162}H_{267}N_{49}O_{45}S_2$
Mol wt: 3685.2886

CAS: 188899-65-2

EN: 352957

Abstract

Available treatment for osteoporosis includes agents that reduce bone resorption and those that increase bone formation, such as parathyroid hormone (PTH). The PTH approach is a particularly attractive strategy for reversing the course of osteoporosis in individuals who already have substantial bone loss. PTH, however, may be associated with adverse effects on radial bone density and it may act peripherally, inducing unwanted effects. Researchers have focused efforts on the synthesis of novel PTH analogues in an attempt to discover more effective and safer molecules. The smallest osteogenic PTH fragment synthesized was the linear hPTH(1-31)NH_2 (Ostabolin), which exerted stimulatory effects on trabecular bone similar to larger fragments but was less effective than hPTH(1-34) when given in suboptimal daily doses. Modifications were made on the peptide in an attempt to increase its anabolic efficacy and yielded the cyclic lactam [Leu^{27}]cyclo(Glu^{22}-Lys^{26})-hPTH(1-31)NH_2 (OSC, Ostabolin-C™). Ostabolin-C™ was more potent than hPTH(1-31)NH_2 in stimulating adenylyl cyclase and more effective in stimulating trabecular bone growth at suboptimal daily boluses. Ostabolin-C™ was chosen for further development as a treatment for osteoporosis.

Synthesis

Ostabolin-C™ is synthesized by solid-phase peptide synthesis on a Tentagel® amino resin. Coupling of *N*-Fmoc-L-valine (I) to the solid support is performed manually by using TBTU and HOBt to provide the resin-bound valine (II). Subsequent coupling steps with the appropriate amino acids utilizing an automated peptide synthesizer leads to the protected pentadecapeptide resin (III). This is then treated with $Pd(PPh_3)_4$ and NMM to remove the Glu^{22} allyl ester and the Lys^{26} Alloc group, yielding (IV), which is cyclized to the lactam (V) in the presence of HOAt and NMM. The peptide resin (V) is then subjected to further coupling steps in the automated peptide synthesizer to afford the fully protected peptide resin (VI). The title peptide is finally obtained by deprotection and simultaneous resin cleavage in the presence of reagent K (trifluoroacetic acid, thioanisole, phenol/water and ethaneditihiol) (1-3). Scheme 1.

Background

Osteoporosis is the most common type of metabolic bone disease and is characterized by low bone density and structural deterioration of bone microarchitecture, which leads to bone fragility and increased susceptibility to fractures. The International Osteoporosis Foundation (IOF) estimates that there are 75 million people with osteoporosis in the U.S., Europe and Japan combined. In 2004, the U.S. Surgeon General reported that there were 10 million individuals in the U.S. alone diagnosed with osteoporosis and another 33.6 million with low bone mass at high risk for the disease. Women are particularly affected by the disorder, with more than 200 million affected worldwide. However, between 1 and 2 million men in the U.S. also have the disease, with another 13 million exhibiting low bone mass (4, 5).

Agents available for the treatment of osteoporosis include estrogens, bisphophonates and calcitonin, which

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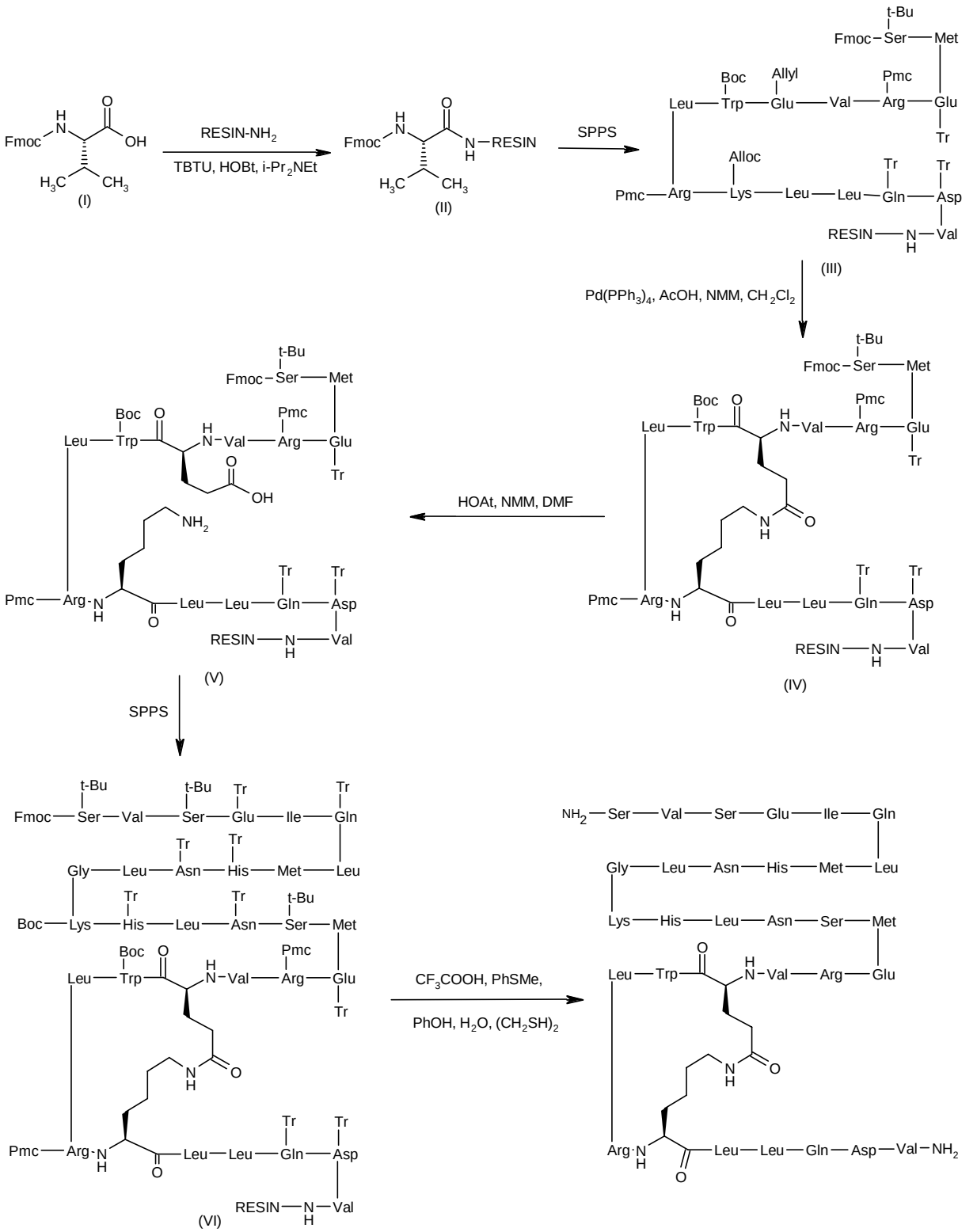
Scheme 1: Synthesis of Ostabolin-C™



all act by reducing bone resorption, and fluorides and parathyroid hormone (PTH) and its analogues, which increase bone formation. PTH is a naturally occurring anabolic peptide that mediates bone metabolism endogenously and has been shown to exert osteogenic activity in dogs, rats and osteoporotic humans. Studies have demonstrated that intermittent administration of PTH results in anabolic effects, while continuous administration of high doses causes catabolic effects. The PTH approach for the treatment of osteoporosis is a particularly attractive strategy for reversing the course of the disease in individuals who already have substantial bone loss, have experienced a fracture or are at high risk for fracture. Analysis of results from several clinical studies in which subjects with postmenopausal or glucocorticoid-induced osteoporosis were administered PTH revealed that treatment reduced the incidence of vertebral fractures and increased spinal bone density. However, PTH may be associated with adverse effects on radial bone density and its effects on nonvertebral fractures are unknown. Moreover, in addition to regulating bone, kidney and intestinal calcium metabolism, high doses of PTH may act peripherally, inducing unwanted effects on vascular tone, cardiac muscle contraction, hematopoietic cell differentiation and nervous tissue function. Thus, researchers have focused efforts on the synthesis of novel PTH analogues in an attempt to discover more effective and safer therapies for osteoporosis (4-9).

Teriparatide (rhPTH[1-34], Forteo™; Lilly), introduced in 2002, was the first PTH analogue to be marketed and several others are currently under active development for the treatment of osteoporosis, as shown in Table I. Until recently, the smallest PTH fragment to exert osteogenic activity was hPTH(1-34). A smaller fragment, hPTH(1-31)NH_2 (Ostabolin), was then discovered and shown to have the same marked osteogenic effects on trabecular bone as the larger fragment when injected as daily boluses. However, when administered s.c. at suboptimal doses, hPTH(1-31)NH_2 was as effective as rhPTH(1-84) but less effective than hPTH(1-34) in stimulating femoral trabecular bone growth in ovariectomized (OVX) rats (10-14).

Consequently, an attempt to increase the anabolic efficacy of hPTH(1-31)NH_2 was made by incorporating two modifications. To increase the hydrophobicity of the receptor-binding hydrophobic face of the *C*-terminal amphiphilic α-helix, the polar Lys^{27} was replaced with the apolar Leu^{27}. A lactam linkage was then made between Glu^{22} and Lys^{26} to stabilize the molecule in an optimal receptor-binding configuration. Both modifications may also contribute to the molecule's increased resistance to proteases. The resulting novel fragment, [Leu^{27}]cyclo-(Glu^{22}-Lys^{26})hPTH(1-31)NH_2 (OSC, Ostabolin-C™), was more potent than hPTH(1-31)NH_2 in stimulating adenylyl cyclase in rat osteosarcoma (ROS) 17/2 osteoblast-like cells and more effective in stimulating trabecular bone growth in OVX rats when administered as suboptimal daily boluses. Ostabolin-C™ was chosen for further development as a treatment for osteoporosis (2, 15).

Preclinical Pharmacology

Ostabolin-C™ was 6 tmes more potent than hPTH(1-31)NH_2 in stimulating adenylyl cyclase activity in ROS 17/2 cells (EC_{50} = 3.3 ± 0.3 nM *vs.* 19.9 ± 3.9 nM) (2, 15). When administered to rats i.v. (0.8 nmol/100 g), both Ostabolin-C™ and hPTH(1-31)NH_2 transiently lowered blood pressure by about 30 mmHg within 1-2 min of dosing. However, when administered s.c., significantly less hypotensive activity was observed, although Ostabolin-C™ induced slightly greater hypotension than the parent peptide (2).

A study using OVX rats showed that preventive treatment with Ostabolin-C™ or hPTH(1-31)NH_2 (0.6 or 0.8 nmol/100 g s.c. once daily starting at the end of week 2 postovariectomy before evidence of significant trabecular bone loss and continuing for 6 more weeks) prevented the OVX-induced distal femoral trabecular volume loss of about 51% observed in controls. In fact, treatment with either agent significantly increased trabecular volume to supranormal levels (1.1 ± 0.1 and 1.2 ± 0.2 mm^3, respectively, *vs.* 0.34 ± 0.02 mm^3 in OVX controls and 0.72 ± 0.04 mm^3 in sham OVX controls). Another experiment was performed in which OVX rats were treated for 6 weeks with Ostabolin-C™ starting at the end of the ninth week postovariectomy when animals exhibit large distal femoral trabecular bone loss. At the end of the 6 weeks of this restorative treatment, control OVX animals displayed a significant 75% loss of trabecular volume (0.72 ± 0.05 to 0.18 ± 0.02 mm^3). In contrast, the trabecular volume of Ostabolin-C™-treated animals returned to normal values (0.74 ± 0.04 mm^3). Ostabolin-C™ was significantly more effective than hPTH(1-31)NH_2 in the restoration protocol and was also more potent than a bicyclic PTH analogue, indicating that the introduction of a second cyclic lactam linkage does not increase osteogenic activity. Estratriene-3-ol, an estrogen reported to potently stimulate bone growth in gonad-intact and OVX mice, was ineffective in

Table I: PTH analogues under development for the treatment of osteoporosis (from Prous Science Integrity®)

Drug	Source	Phase
ALX1-11 (Preotact™, Preos®)	NPS Pharmaceuticals/Nycomed Pharma	L-2006
CHS-13340/SUN-E3001	Chugai Pharmaceutical/Daiichi Asubio	II
Ostabolin-C™	Zelos Therapeutics	II
PTH(1-34)	Nastech/Procter & Gamble	I
BA-058 (formerly BIM-44058)	Radius (formerly Nuvios)	I
PTH-131A (768974)	GlaxoSmithKline/Unigene Laboratories	I

this model when administered at doses replacing full estrogen functions (15-17).

Similar experiments using preventive and restorative treatment protocols (0.6 nmol/100 g s.c. once daily) showed that Ostabolin-C™ was significantly more effective than hPTH(1-31)NH_2 in preventing OVX-induced loss of femoral trabecular volume and in increasing trabecular thickness in OVX rats with severely depleted femoral trabecular bone. Higher doses of 2 nmol/100 g were also effective (18, 19).

Prolonged low-dose restorative infusion of Ostabolin-C™ (0.05, 0.1 and 0.5 nmol/100 g/day = 0.72, 1.48 and 7.36 μg/day, respectively, via Alzet minipumps starting at the end of the ninth week postovariectomy) for 6 weeks had no effect on femoral cancellous bone volume in OVX rats. Infusion of the agent also did not prevent OVX-induced reductions in femoral trabecular thickness or number. Results indicate that daily injections are required for the osteogenic activity of Ostabolin-C™ (20).

Ostabolin-C™ (1 or 2 nmol/100 g once daily for 6 weeks starting 2 weeks postovariectomy) was effective in stimulating vertebral and tibial bone growth in OVX rats. Treatment with the agent also prevented trabecular volume loss in the L5 vertebrae. hPTH(1-31)NH_2 was effective in stimulating vertebral bone growth at doses 10-25 times higher. Restorative Ostabolin-C™ treatment (starting 9 weeks postovariectomy) was also effective in increasing trabecular bone volume and thickness and bone mineral density (BMD) in the L5 vertebrae, and in increasing BMD of the L1-L4 vertebrae and tibiae. A minimal increase in pelvic BMD was also seen with treatment (21).

Ostabolin-C™ not only preserved bone structure after OVX but also inhibited bone resorption both *in vivo* and *in vitro*. Treatment of OVX rats with Ostabolin-C™ (6, 10 and 20 nmol/kg/day) increased femoral trabecular thickness and reduced marrow space. The agent also significantly increased mineralizing surfaces and bone formation rates and decreased osteoclast surface density, indicating both stimulation of osteoblast lineage cells and inhibition of mature osteoclasts. Experiments performed *in vitro* using rat calvaria organ and cell cultures revealed that intermittent pulsing with the agent significantly increased parietal bone thickness and the rate of bone nodule formation, while decreasing calvaria eroded space and TRAP stain intensity. In addition, treatment with Ostabolin-C™ increased osteoblast differentiation markers but decreased osteoclast markers. Ostabolin-C™ was more potent than hPTH(1-31)NH_2 or hPTH(1-34)NH_2 in these assays (22).

Ostabolin-C™ effectively increased bone formation and strength in gonad-intact rats. Treatment with doses of 2, 10 or 25 μg/kg s.c. for 26 weeks was well tolerated, with no evidence of antibody formation or hypercalcemia. Ostabolin-C™ increased levels of the bone formation marker osteocalcin; the bone resorption marker urinary deoxypyridinoline was unchanged, while C-telopeptide levels increased in the highest dose group. Moreover, all doses of the agent increased femoral and lumbar vertebral bone mass and significant increases in lumbar vertebral bone strength were also observed; femoral bone strength at the diaphysis and femoral neck also increased, although results did not reach significance. Tibial cancellous bone volume, mineralizing surfaces and surface referent bone formation rates increased with treatment. No effects were observed on bone resorption. The highest dose increased femoral diaphysis cortical area and cortical thickness in females (23).

The effects of intermittent Ostabolin-C™ treatment on fracture healing, including tibial fracture strength, callus dimensions and callus tissue mechanical quality, were examined in rats after 8 and 16 weeks of healing. Animals were treated (15 nmol/kg/day s.c.) during the first 8 weeks of healing after fracture. During the first 8 weeks, fracture strength and callus volume increased with treatment such that ultimate load, ultimate stiffness and callus volume were enhanced 66%, 58% and 28%, respectively. Ostabolin-C™ had no significant effect on callus tissue mechanical quality. Treatment with hPTH(1-31)NH_2 or hPTH(1-34)NH_2 resulted in similar effects. After 16 weeks of healing and withdrawal of Ostabolin-C™ during the second 8 weeks of healing, fracture strength, callus volume and callus tissue mechanical quality were similar to in vehicle-treated animals; compared to at 8 weeks, Ostabolin-C™-treated animals showed further increases in fracture strength and callus tissue mechanical quality (ultimate load, ultimate stress and elastic modulus increased by 23%, 88% and 87%, respectively) (24).

One-year treatment with Ostabolin-C™ (2, 10 and 25 μg/kg/day s.c.) effectively uncoupled bone turnover in skeletally immature cynomolgus monkeys (30-40 months old). Treatment was well tolerated and no hypercalcemia was observed. Significant increases in osteocalcin and significant decreases in C-telopeptide were observed, but no changes in the bone resorption marker deoxypyridinoline. Lumbar spine, femoral and tibial bone mass increased with treatment and significant increases in bone strength were seen. Moreover, osseous accretion in cancellous and endocortical proximal tibial bone compartments increased and a significant increase in tibial cancellous bone volume of more than 50% was reported; increases in cortical width and relative cortical area and reductions in medullary area were also detected in the tibial mid-diaphysis. The Ostabolin-C™-induced increases in bone mass were suggested to be due not only to increases in bone formation but also to reductions in bone resorption, since significant decreases in osteoclast surface were observed (25).

Safety

It has been reported that treatment of rat enterocytes with PTH induces phosphatidylcholine 3-kinase (PI-3)/mitogen-activated protein kinase (MAPK)-mediated proliferation and that primary hyperparathyroidism in humans is associated with a high incidence of colon cancer. Results from a study examining the carcinogenic potential of Ostabolin-C™ in the rat colon showed that

both Ostabolin-C™ and hPTH(1-34)NH_2 stimulated femoral bone formation. However, neither agent caused the appearance of aberrant crypts in the colon, an indication of the initiation of colon carcinogenesis, nor did they increase the number of aberrant crypt foci induced by the colon carcinogen azoxymethane (26).

The safety, tolerability and pharmacokinetics of single doses of Ostabolin-C™ (10, 20, 40, 80 or 160 μg s.c.) were examined in a randomized, double-blind, placebo-controlled, escalating-dose phase I study in 40 healthy older adults (45-73 years). C_{max} values were dose-proportional and mean t_{max} and $t_{1/2}$ values were 0.25-0.75 h and 0.83-1.12 h, respectively. The agent was concluded to be safe and well tolerated after single s.c. doses up to 80 μg, but poor tolerability was noted in females receiving the highest dose. No significant alterations in ECGs were seen. However, dose-dependent increases in heart rate were observed. The most common adverse events were nausea, headache, vomiting, back pain and dizziness. Only 1 female subject treated with the highest dose exhibited a single total serum calcium value above the normal range (2.65 mmol/l). Biological activity of the agent was suggested by significant increases in urinary cAMP seen at all doses and significant increases in plasma cAMP detected at doses of 80 and 160 μg (27).

Clinical Studies

Three phase I trials of injectable Ostabolin-C™ have been completed in about 100 subjects and the product is currently undergoing phase II development for the treatment of osteoporosis. A phase II trial has enrolled 261 postmenopausal women with low BMD who will receive one of four Ostabolin-C™ doses or placebo for up to 1 year. A topical gel formulation of Ostabolin-C™ is also undergoing phase II testing for the treatment of psoriasis (28).

Source

Zelos Therapeutics, Inc. (US); collaboration with Nektar Therapeutics for a pulmonary formulation of Ostabolin-C™ for osteoporosis.

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