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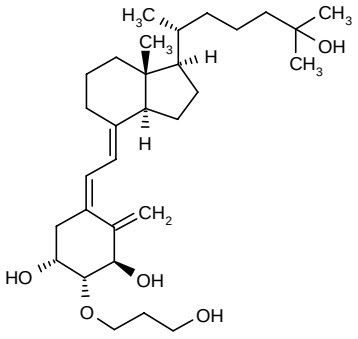
ED-71

Treatment of Osteoporosis
Vitamin D Analogue

2β-(3-Hydroxypropoxy)-1α,25-dihydroxyvitamin D_3

(1α,2β,3β,5*Z*,7E)-2-(3-Hydroxypropoxy)-9,10-secocholesta-5,7,10(19)-triene-1,3,25-triol



$C_{30}H_{50}O_5$
Mol wt: 490.715
CAS: 104121-92-8
EN: 158236

Abstract

Osteoporosis results from an imbalance in bone homeostasis and leads to bone fragility with an increased risk of fractures. It is, however, a preventable disease, with calcium and vitamin D supplementation both playing a part. The development of vitamin D analogues has led to improved treatment and prevention of osteoporosis, with benefits in terms of skeletal muscle function and balance, increased bone volume and inhibition of bone resorption. ED-71 is a second-generation vitamin D analogue with improved binding to vitamin D-binding protein (DBP) compared with calcitriol, the most potent metabolite of natural vitamin D. The preventive and therapeutic effects of ED-71 on bone mineral loss have been demonstrated in rat models of osteoporosis, where it significantly increased bone mineral density without inducing hypercalcemia. Bone histomorphometric studies showed that this effect resulted from stimulation of bone formation together with a reduction in bone resorption. Open, randomized, controlled studies conducted in subjects with osteoporosis have demonstrated a significant positive effect for ED-71 on bone mineral density following 6 and 12 months of treatment. Phase III trials are ongoing in Japan.

Synthesis

ED-71 can be prepared by several ways:

1) Starting from the known Diels-Alder adduct (I) of a cholestadiene derivative with 4-phenyl-1,2,4-triazolidinedione, epoxide ring opening in adduct (I) with 1,3-propanediol (II) in the presence of *p*-toluenesulfonic acid in THF at reflux gives the 1,3-dihydroxy-2-(hydroxypropoxy) derivative (III), which by subsequent retro-Diels-Alder reaction under reductive conditions with $LiAlH_4$ in THF furnishes the cholestadiene derivative (IV) (1). In a related sequence, adduct (I) is submitted to an initial retrocycloaddition reaction in hot dimethylimidazolidinone, followed by ring opening of the resultant epoxide (V) with 1,3-propanediol (II) to provide an alternative access to diene (IV) (2). Finally, cholestadiene (IV) is converted to ED-71 by irradiation using a high-pressure mercury lamp, followed by thermal isomerization in boiling THF (1, 2). Scheme 1.

2) Starting from 3,4:5,6-*O*-diisopropylidene-D-mannitol (VI), dehydration of sugar (VI) by means of dimethylformamide dimethylacetal and Ac_2O provides olefin (VII), which by selective hydrolysis of the terminal acetonide by means of 80% AcOH affords diol (VIII). Acylation of diol (VIII) with tosyl chloride and pyridine in $CHCl_3$ to the primary tosylate (IX), followed by treatment with Na_2CO_3 gives epoxide (X). Oxirane ring opening in (X) with NaCN in MeOH/H_2O furnishes the β-hydroxynitrile (XI), which is then protected as the unsymmetric acetal (XII) by acid-catalyzed addition of ethyl vinyl ether in CH_2Cl_2. Partial reduction of the nitrile function of (XII) with DIBAL in toluene at –78 °C results in aldehyde (XIII), which is further derivatized as the corresponding oxime (XIV) with hydroxylamine in pyridine. Treatment of oxime (XIV) with NaOCl and Et_3N generates a nitrile oxide, which undergoes a dipolar cycloaddition to the olefin, leads to the isoxazoline (XV). Hydrogenolysis of the isoxazoline ring of compound (XV) in the presence of Raney nickel and boric acid in MeOH/H_2O affords the (hydroxymethyl)-cyclohexanone (XVI). Protection of the hydroxyl group of compound (XVI) with TBDMS-Cl, DMAP and TEA in

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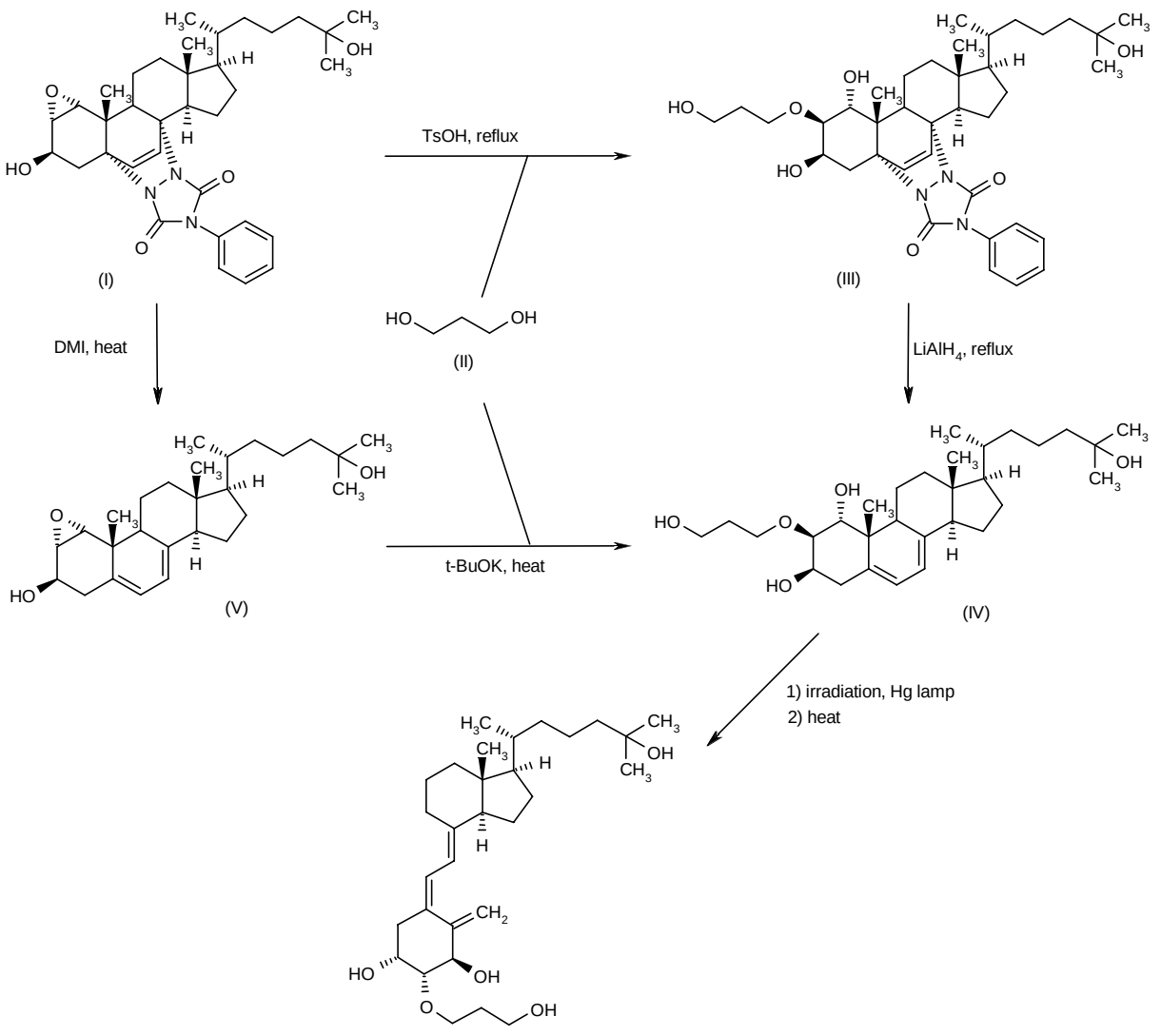
Scheme 1: Synthesis of ED-71



CH_2Cl_2 gives the silyl ether (XVII), which is submitted to a Peterson olefination of its ketone function with the lithium derivative of ethyl trimethylsilylacetate (XVIII) in THF to furnish the unsaturated ester (XIX). Desilylation of compound (XIX) with TBAF in THF gives the primary alcohol (XX), which is dehydrated to compound (XXI) by treatment with methanesulfonyl chloride and pyridine. Replacement of the 1-(ethoxy)ethoxy protecting group of (XXI) with a silyl group by acidic ketal hydrolysis with *p*-toluenesulfonic acid and pyridine in EtOH, followed by treatment with *tert*-butyldiphenylsilyl chloride and imidazole in CH_2Cl_2 affords the silyl ether (XXII). Subsequent acetonide hydrolysis of compound (XXII) in acid medium gives diol (XXIII), which was regioselectively monosilylated to provide intermediate (XXIV). Reduction of the ester function of compound (XXIV) with DIBAL in toluene affords alcohol (XXV), which is further protected at the primary hydroxy group by reaction with dihydropyran and TsOH in CH_2Cl_2 to yield the tetrahydropyranyl ether (XXVI). The free secondary hydroxyl of (XXVI) is then alkylated with allyl bromide (XXVII) and NaH to produce the allyl ether (XXVIII), which by selective olefin hydroboration at the allyl ether moiety with 9-BBN in THF, followed by oxidative work-up with H_2O_2 and NaOH results in the primary alcohol (XXIX). After silylation of (XXIX) with *tert*-butyldiphenylsilyl chloride and imidazole in CH_2Cl_2, the tetrahydropyranyl ether is hydrolyzed with ethanolic HCl providing the tris-*O*-silylated compound (XXX). Allyl alcohol (XXX) is converted to chloride (XXXI) by means of *N*-chlorosuccinimide and dimethylsulfide in CH_2Cl_2. Displacement of the chloride atom of (XXXI) with lithium diphenylphosphide, followed by H_2O_2 oxidation of

the resulting phosphine gives the phosphine oxide (XXXII). This compound is subjected to a Wittig condensation with the functionalized indanone (XXXIII) by means of butyl lithium in THF to produce the triene adduct (XXXIV). Finally, desilylation of adduct (XXXIV) is effected by treatment with tetrabutylammonium fluoride in THF (3). Scheme 2.

3) Using the *tert*-butyldimethylsilyl-protected analogue of intermediate (XXX), (XLIX), prepared as follows: Cleavage of the symmetrical epoxide (XXXV) with 1,3-propanediol (II) in the presence of potassium *tert*-butoxide gives the diol (XXXVI), which after protection of the primary alcohol as the pivalate ester (XXXVII), the benzyl ether groups are removed by hydrogenation over $Pd(OH)_2$ to provide compound (XXXVIII). Protection of the vicinal diol moiety of (XXXVIII) by treatment with 2,2-dimethoxypropane and TsOH affords the corresponding acetonide (XXXIX), which by subsequent Swern oxidation of the primary alcohol function gives aldehyde (XL). Addition of vinylmagnesium bromide to aldehyde (XL) affords the allylic alcohol (XLI) as a diastereomeric mixture. Esterification of alcohol (XLI) with pivaloyl chloride provides the dipivalate ester (XLII), which is submitted to an acidic acetonide hydrolysis, followed by cyclization of the resultant vicinal diol under Mitsunobu conditions to afford epoxide (XLIII). Addition of the *O*-protected lithium acetylide (XLIV) to epoxide (XLIII) furnished the acetylene adduct (XLV), which under basic hydrolysis of the pivalate esters, followed by protection with *tert*-butyldimethylsilyl chloride provides the trisilylated derivative (XLVI). The *p*-methoxybenzyl protecting group of (XLVI) is then removed by treatment with DDQ, yielding the propargyl alcohol (XLVII), which is reduced with Red-Al, followed by iodination to produce the vinyl iodide (XLVIII). Intramolecular cyclization of iodide (XLVIII) under Heck reaction conditions give rise to the key allyl alcohol intermediate (XLIX), along with its undesired diastereoisomer (L), which are separated by column chromatography (4). Scheme 3.

4) Using solid-phase methodology within a parallel synthesis protocol, the A-ring synthon (LiX) was synthesized as follows: Esterification of the previously reported alcohol intermediate (LI) with pivaloyl chloride, DMAP and pyridine gives the pivalate ester (LII). Acetonide hydrolysis of compound (LII), followed by selective mono-silylation of the resultant diol (LIII) with TBDMS-Cl and imidazole yields alcohol (LIV). Michael addition of ethyl acrylate (LV) to the free hydroxyl group of (LIV) in toluene furnishes ester (LVI), which by subsequent reduction of the methyl ester group and simultaneous reductive cleavage of the pivaloyl group in the presence of $LiAlH_4$ leads to diol (LVII). Chlorination of the allylic alcohol of (LVII) by means of *N*-chlorosuccinimide and dimethylsulfide, followed by silylation of the remaining hydroxyl group yields compound (LVIII). Conversion of chloride (LVIII) into the phosphine oxide (LIX) is then performed using lithium diphenylphosphide and butyl lithium, followed by H_2O_2 oxidation.

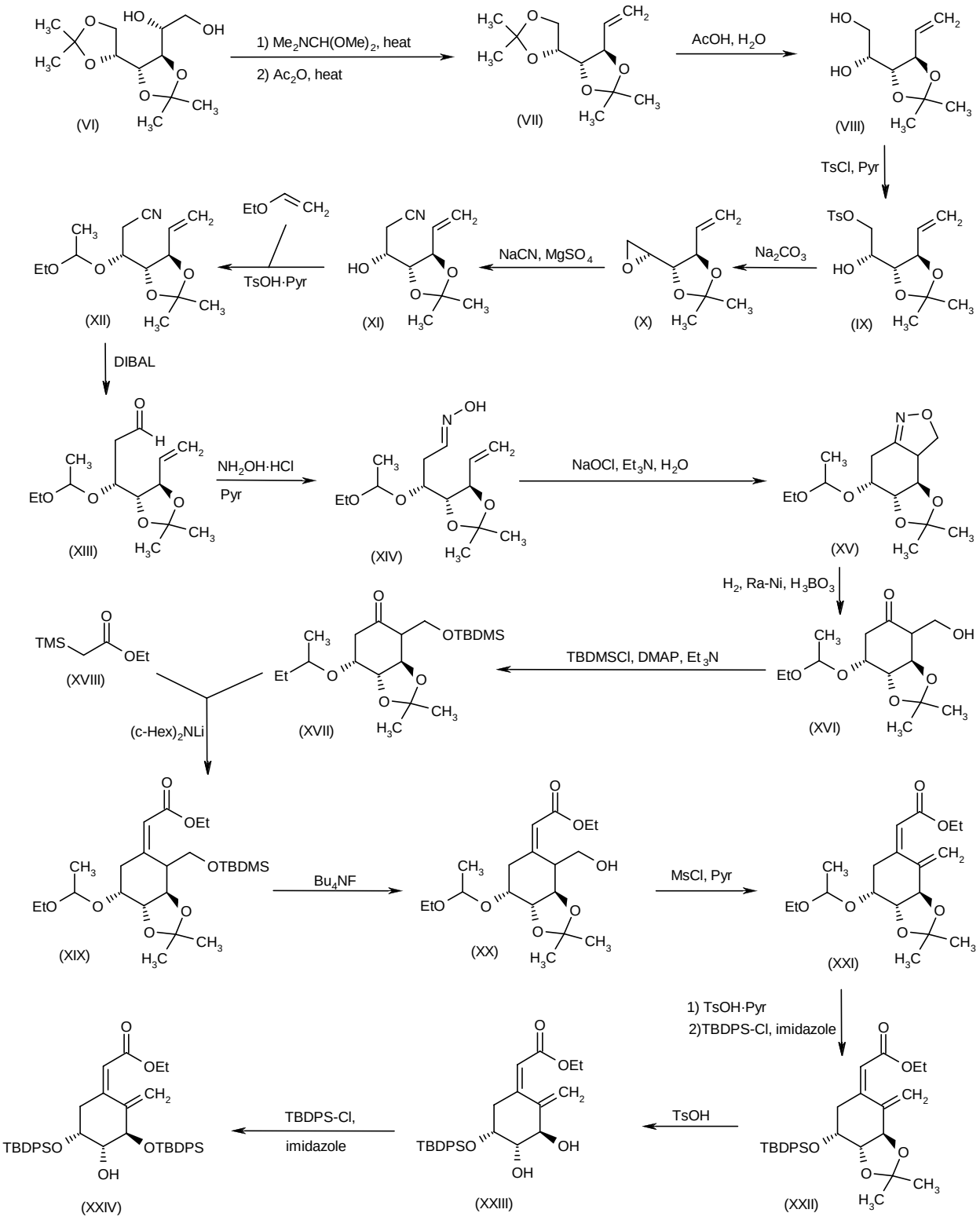
Attachment of the CD-ring synthon to the solid-phase support requires the introduction of an appropriate spacer group. Coupling between methyl 4-hydroxybenzenesulfonate (LX) and the tetrahydropyranyl-protected diol (LXI) under Mitsunobu conditions affords the alkoxy sulfonate (LXII), which is hydrolyzed with LiCl in refluxing acetone, and the resulting sulfonic acid is converted to the sulfonyl chloride (LXII) by treatment with PCl_5 in DMF. Coupling of the CD-ring alcohol (LXIV) with the sulfonyl chloride (LXIII), followed by acidic tetrahydropyranyl group cleavage gives the sulfonate linker-bound CD synthon (LXV), which is attached to a previously chlorinated diethylsilyl resin to furnish the resin-bound CD ring (LXVI). Alternatively, resin (LXVI) is prepared by a more general method consisting of the initial attachment of the sulfonate linker (LXIII) to the resin support, and then loading alcohol (LXIV) to the resultant sulfonyl chloride resin (LXVII). Horner-Wadsworth-Emmons condensation of the resin-bound ketone (LXVI) with the phosphine oxide (LIX) by means of butyl lithium in THF yields compound (LXVIII). Cleavage of the sulfonate resin and simultaneous introduction of the side-chain substitution is achieved by copper-catalyzed displacement of sulfonate resin (LXVIII) with the Grignard reagent (LXIX). Finally, the silyl protecting groups are removed by treatment with CSA in aqueous methanol (5). Scheme 4.

Introduction

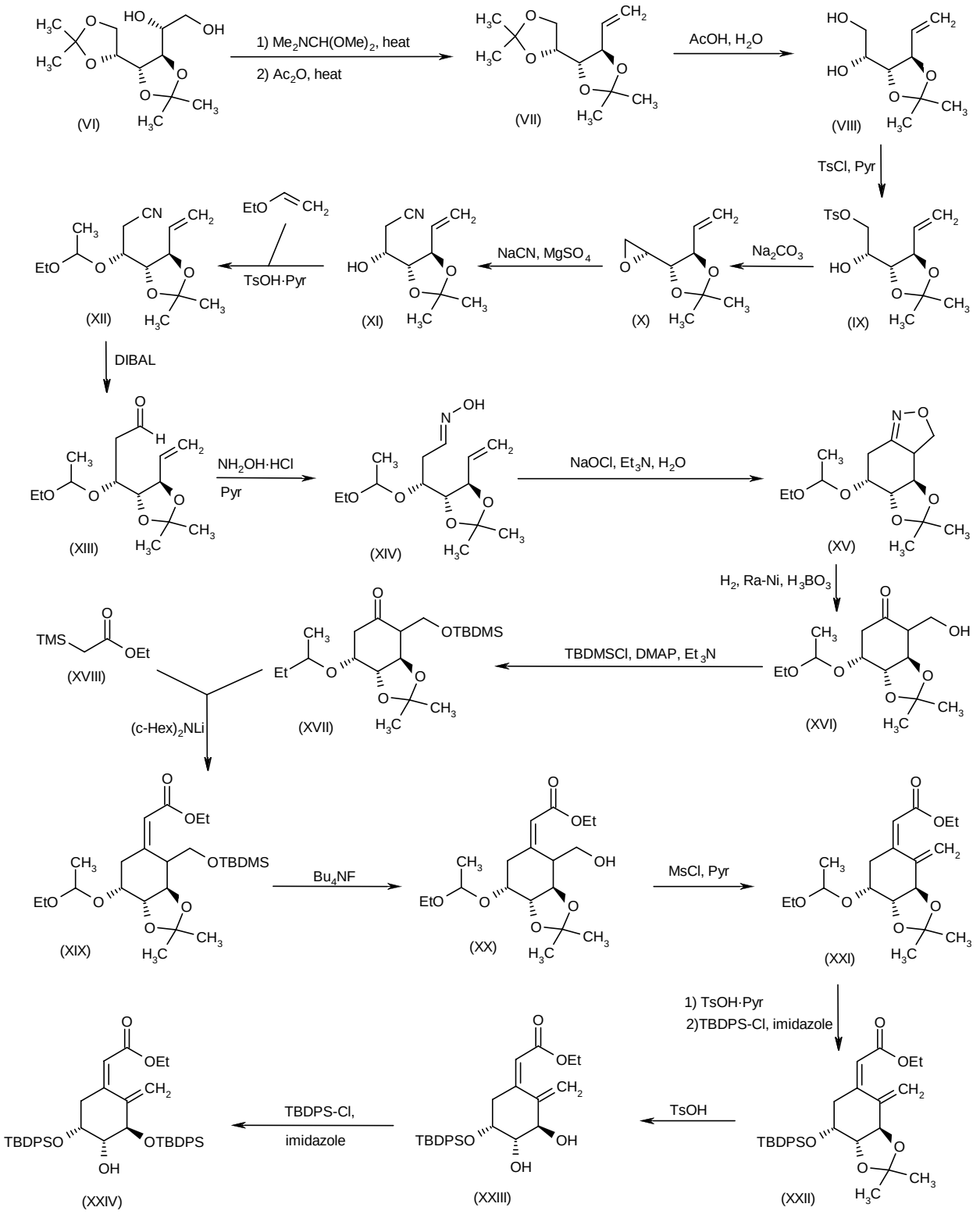
Osteoporosis is a metabolic bone disorder characterized by low bone mass and structural deterioration of bone tissue, which leads to bone fragility and an increased likelihood of fractures, even resulting from low impact. An imbalance in bone homeostasis whereby bone resorption occurs too quickly or bone formation occurs too slowly results in the development of the condition, which is particularly prevalent in postmenopausal women, but is also associated with normal aging in both sexes. Although not widely viewed as a severe or life-threatening disease, significant morbidity and mortality are associated with osteoporotic fractures, and up to one-quarter of patients suffering an osteoporotic hip fracture die within 1 year. However, osteoporosis is a preventable disease, with calcium and vitamin D supplementation, together with good nutritional status and weight-bearing exercise, all factors in its prevention. Calcium is essential to bone health both in childhood and in adulthood. Vitamin D increases calcium absorption by up to 80% and vitamin D supplementation in elderly populations may reduce the risk of falls by more than 20%. Vitamin D_3 analogues improve skeletal muscle function and balance, increase bone volume and inhibit bone resorption (6-8).

ED-71 is a second-generation vitamin D_3 analogue in phase III development for the treatment of osteoporosis (9).

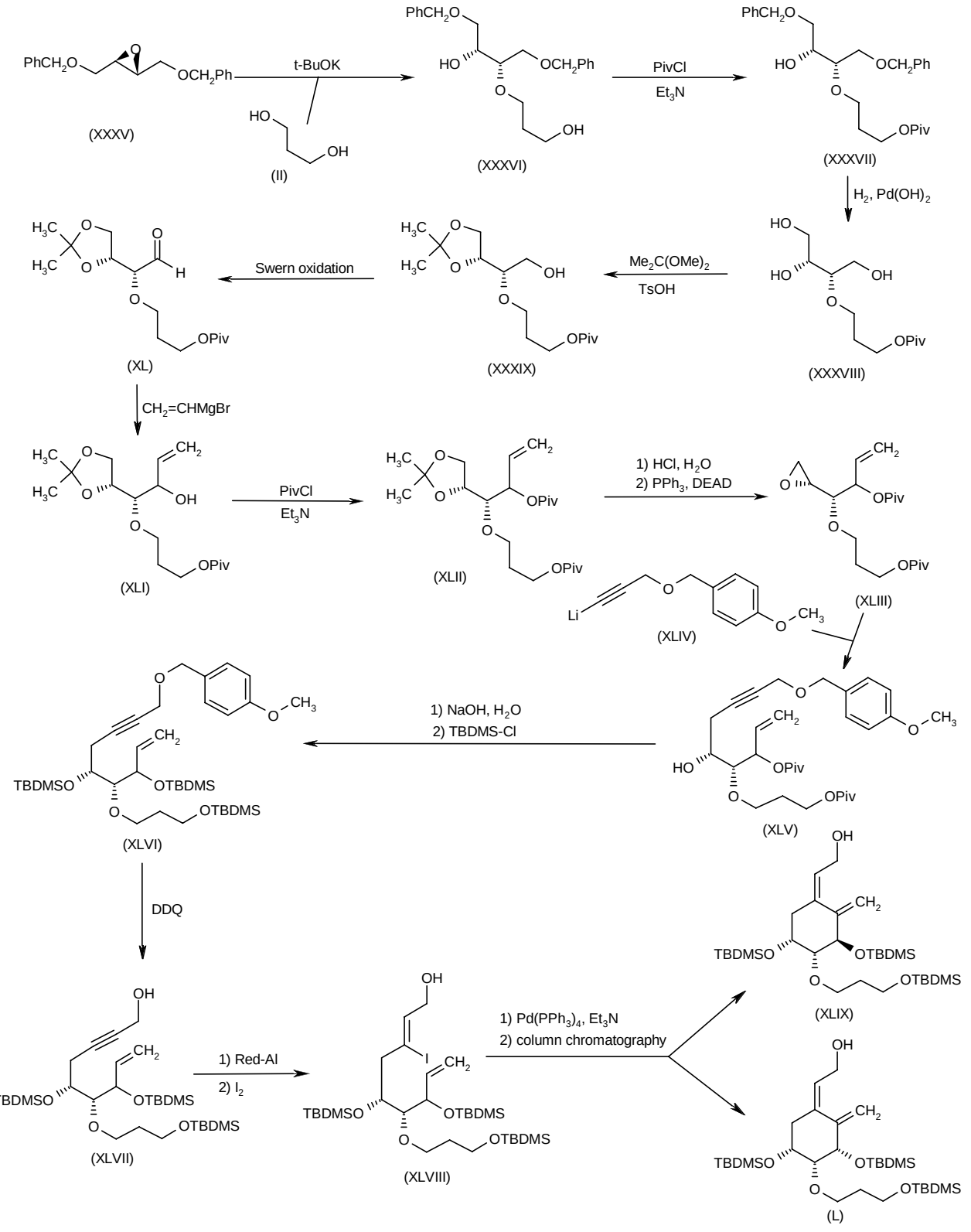
Scheme 2: Synthesis of ED-71



Scheme 2 (Cont.): Synthesis of ED-71



Scheme 3: Synthesis of Intermediate (XLIX)



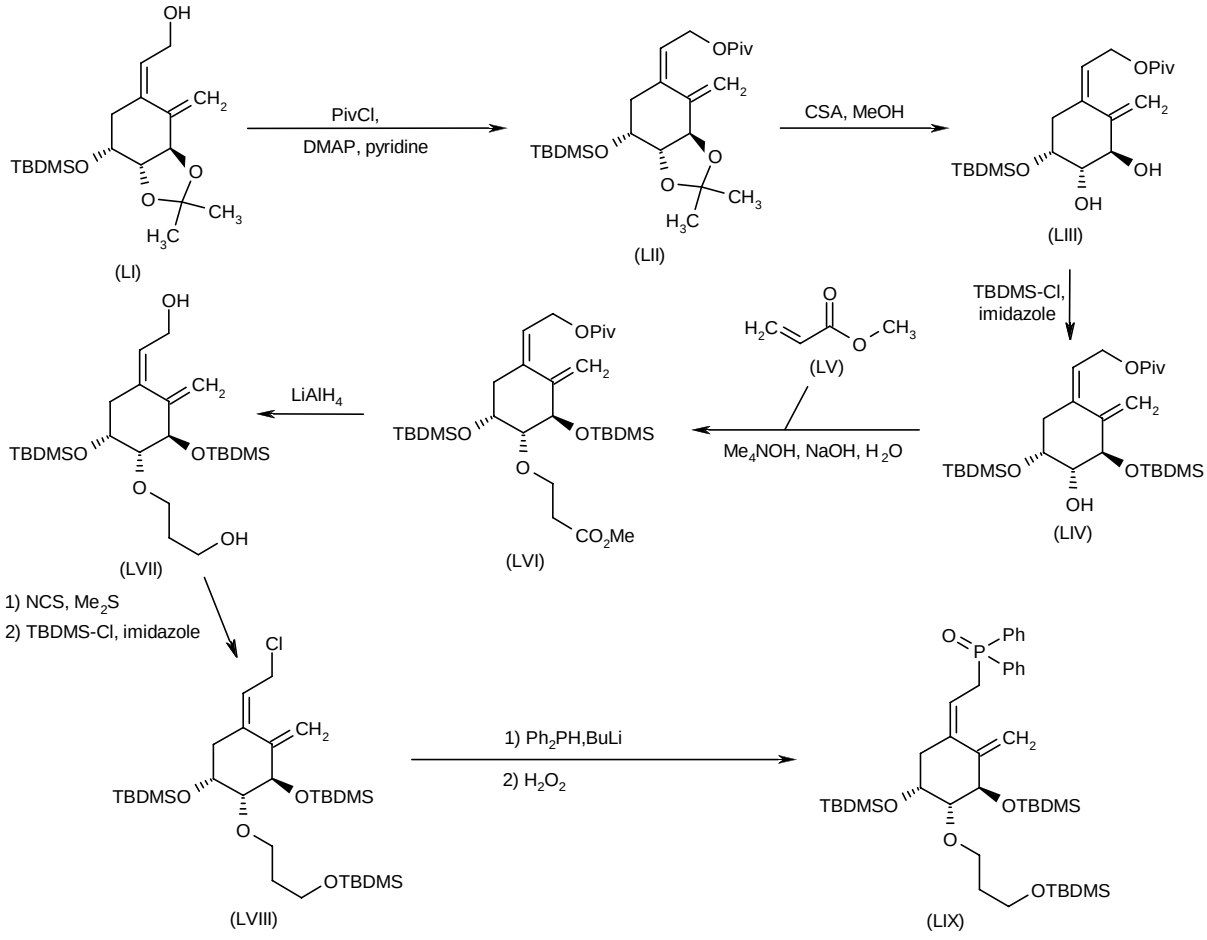
Pharmacological Actions

Calcitriol (1α,25-dihydroxyvitamin D_3) is the active form of vitamin D_3 and a potent steroid hormone. Its biological activities are expressed through binding to a nuclear receptor, VDR (vitamin D receptor). The biological activity of ED-71 was evaluated *in vitro* in terms of its binding affinity for this receptor and for rat plasma vitamin D-binding protein (DBP). It demonstrated an affinity for DBP about twice that of calcitriol, while its affinity for calf thymus cytosolic VDR was approximately similar to that of calcitriol. The binding affinity for chick embryonic intestinal VDR was approximately 8 times weaker than calcitriol (9-13).

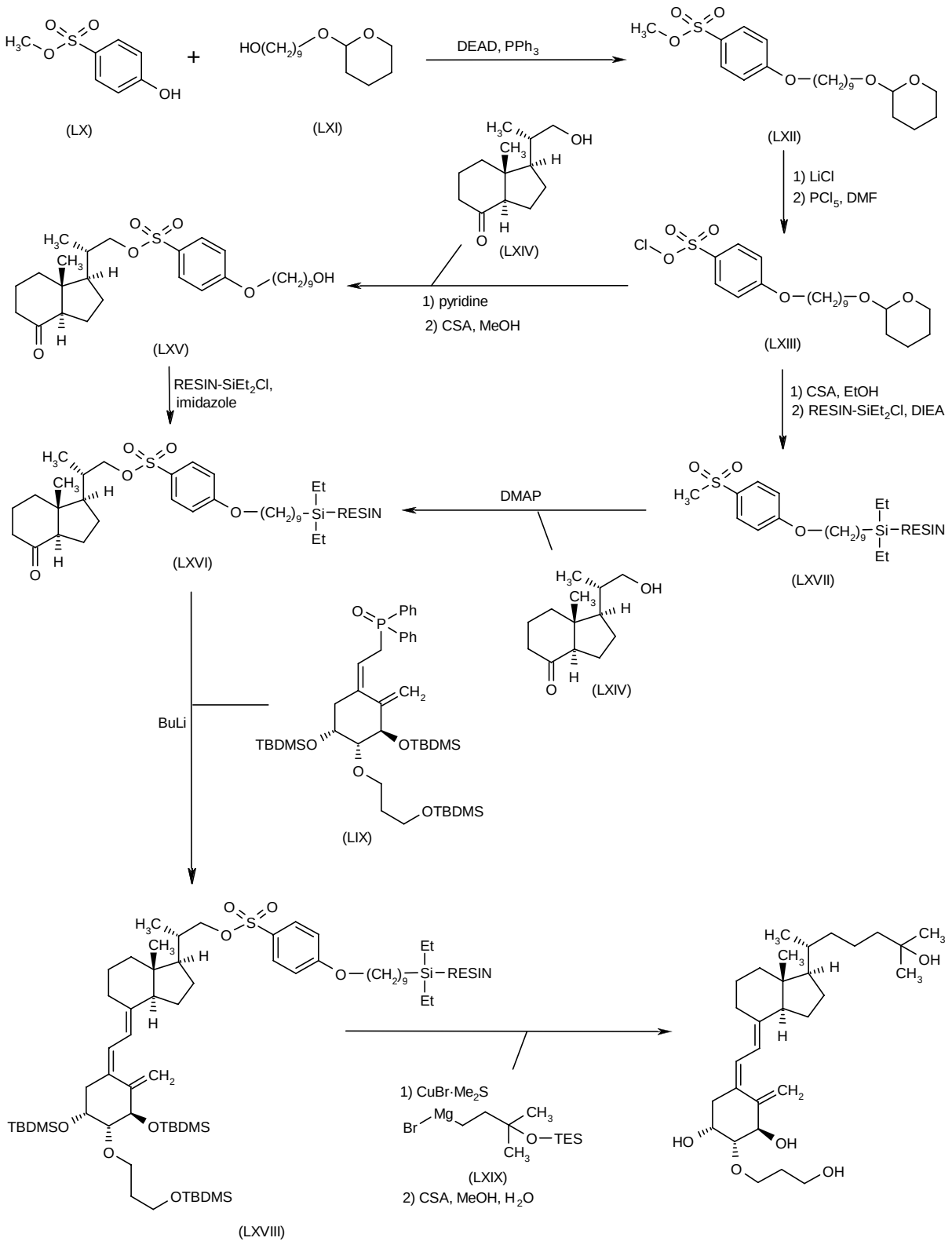
The effects of ED-71 on calcium metabolism were studied in rats fed a low-calcium, vitamin D-deficient diet and administered the drug s.c., i.v. or p.o. Plasma calcium levels increased almost to within the normal range. Following a single dose of ED-71 of 6.25 μg/kg s.c., increases in intestinal calcium absorption and bone mobilization were similar to those observed with calcitriol. In rats administered 6.25 μg/kg i.v., a biphasic action on intestinal calcium transport and bone mobilization was observed, and plasma parathyroid hormone (PTH) levels were significantly decreased compared with baseline at 24 and 48 h postdose; these effects were similar to those of calcitriol. Plasma calcium levels were also practically normalized in vitamin D-deficient rats on a low-calcium diet following oral administration of ED-71 (6.25 μg/kg/day x 5 days). Similarly, in mice administered a single i.v. injection of ED-71 of 10 μg/kg, mean ionized plasma calcium levels after 24 and 48 h were significantly increased compared to controls administered vehicle only (2.23 mmol/l *vs.* 1.31 mmol/l at 48 h). In normal rats administered 0.5 μg/kg ED-71 p.o., the half-life in plasma was twice that of calcitriol, its greater stability apparently being the result of stronger affinity for DBP. The potency of ED-71 in elevating plasma calcium levels also resulted from its long duration of action (2, 10-12, 14).

Two organ culture systems – fetal rat long bones and neonatal mouse calvariae – were employed to examine the effect of ED-71 on bone metabolism. ED-71 was less

Scheme 4: Synthesis of ED-71



Scheme 4 (Cont.): Synthesis of ED-71



potent than calcitriol in inhibiting bone formation. The stimulating effect of ED-71 at a concentration of 10 nM on bone resorption was slightly greater than that of calcitriol, whereas it did not stimulate resorption at 0.1 nM (15).

The preventive and therapeutic effects of ED-71 on bone mineral loss have been evaluated in rat models of osteoporosis caused by ovariectomy. Rats were administered ED-71 (0.05, 0.1 or 0.2 μg/kg p.o. twice weekly for 3 months) either concomitantly with a reduced-calcium diet (preventive effect) or following 3 months of diet (therapeutic effect). In the first study, ED-71 dose-dependently and significantly increased the bone mineral density of the spine and tibia and femoral mechanical bone strength, without inducing hypercalcemia. The therapeutic effect was significant only at a dose of 0.2 μg/kg (16, 17), although in a previous therapeutic study, the total bone mineral content in the femur was restored at a dose of 0.05 μg/kg given twice weekly for 3 months (11). In the more recent study, its effects were superior to those of calcitriol.

In a further study in a rat osteoporosis model, ovariectomized animals were administered ED-71 orally at doses of 0.008 and 0.04 μg/kg 5 times per week for 6 weeks. Significant increases in bone mineral density were observed, with serum calcium values remaining within the normal range (18).

The effect of ED-71 on bone mass was assessed in another study in normal and estrogen-deficient rats treated twice weekly for 12 weeks. Normal rats were administered ED-71 at doses of 0.05, 0.1 or 0.2 μg/kg, and dose levels of 0.01, 0.05 and 0.1 μg/kg were administered to estrogen-deficient rats. In normal rats, vertebral cancellous bone volume increased significantly and dose-dependently from 34% in vehicle-treated rats to 41% in the lowest dose group and 54% in the highest dose group. Osteoid surface increased significantly in the two highest dose groups. There was a significant increase in the bone formation rate in the highest dose group. In estrogen-deficient rats, cancellous bone volume was significantly decreased by ovariectomy. Administration of ED-71 also increased bone volume and bone formation rates in this group. The study demonstrated the ability of ED-71 to increase bone mass by stimulating bone formation (19).

Further studies determined the mechanisms involved in the increase in bone mass observed following treatment with ED-71. In aged female rats administered oral ED-71 twice weekly for 3 months, spinal bone mineral density increased in a time-dependent manner. Bone morphometric studies indicated that ED-71 significantly and dose-dependently increased osteoid surface, osteoid volume and bone formation rate, with a stronger effect than calcitriol. In rat ovariectomy and orchidectomy models, doses of ED-71 above 0.05 μg/kg administered twice weekly for 12 weeks significantly increased bone mineral density by reducing high-turnover bone resorption and stimulating bone formation. In aged ovariectomized rats administered ED-71 0.08 μg/kg or human PTH 20 nmol/kg 5 times per week, lumbar spine bone mineral density and bone volume were restored to the levels observed in sham-operated animals after 4 weeks' treatment. However, whereas ED-71 appeared to stimulate bone formation and suppress bone resorption, PTH stimulated both formation and resorption (20-22).

The effect of ED-71 on bone mineral density and bone remodeling as a function of its effects upon calcium metabolism and PTH levels was also studied and compared to alfacalcidol in a rat ovariectomy model and in aged ovariectomized rats. Rats were administered ED-71 at oral doses of 0.05, 0.1 or 0.2 μg/kg twice weekly for 3 months. Increases in bone mineral density and bone formation rate were observed, with enhanced calcium absorption and significant decreases in serum PTH levels. ED-71 was more potent than alfacalcidol in suppressing markers of bone resorption and increasing bone mineral density, but had similar effects on calcium absorption and serum PTH. The studies indicated that inhibition of osteoclastic bone resorption and maintenance of osteoblastic function by ED-71 are independent of its effects upon calcium absorption and PTH secretion (23-25).

In addition to studies in normal and ovariectomized rats, the effect of ED-71 on bone mass and bone strength was evaluated in ovariectomized mice administered a dose of 0.4 μg/kg weekly for 6 weeks. ED-71 significantly increased bone mineral density, bone mineral content and bone strength and significantly inhibited bone turnover, similar to estradiol, but unlike the latter, it did not stimulate uterine proliferation (26). Ovariectomized cynomolgus monkeys were administered ED-71 at a dose of 0.3 μg/kg/day orally for 24 weeks. ED-71 increased cortical bone mineral density and cortical bone mineral content. Bone histomorphometric analyses showed that ED-71 decreased bone resorption parameters, as well as bone formation parameters, in cancellous bone, while not influencing cortical bone formation (27).

The effects of ED-71 on bone remodeling were also evaluated *in vivo* using a mouse long bone ablation model. Mice were administered ED-71 0.8 μg/kg i.p. either the following day or 8 days after the operation. Bone volume was increased by 50% compared with controls 7 days after surgery in the first group, whereas after 14 days bone volume was similar in the two groups. In the group administered ED-71 8 days postoperatively, however, bone volume was increased by 70% compared with the control group 14 days postoperatively. The studies indicated that ED-71 increased bone volume both in the formation phase and in the bone resorption phase, with the ability to both promote osteoblastic activity and suppress osteoclastic activity. ED-71 was similarly effective after oral administration of 0.2 μg/kg once daily for 6 days following surgery (28-30).

ED-71 was also tested for its effect on the process of bone remodeling in a rabbit leg lengthening model of distraction osteogenesis. The tibiae of rabbits were lengthened by 10 mm in 10 days. ED-71 was administered at a dose of 0.05 μg/kg s.c. twice weekly for 1, 3 and 8 weeks. The bone mineral content was significantly higher in the

Table I: Clinical studies of ED-71 (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
Osteoporosis	Randomized Open	ED-71, 0.25 μg/d p.o. x 6 mo ED-71, 0.5 μg/d p.o. x 6 mo ED-71, 0.75 μg/d p.o. x 6 mo ED-71, 1 μg/d p.o. x 6 mo	109	ED-71 was well tolerated and increased bone mass in osteoporotic patients	37
Osteoporosis	Randomized Double-blind	ED-71, 0.5 μg/d + Vitamin D_3, 200 or 400 IU/d x 12 mo ED-71, 0.75 μg/d + Vitamin D_3, 200 or 400 IU/d x 12 mo ED-71, 1.0 μg/d + Vitamin D_3, 200 or 400 IU/d x 12 mo Placebo + Vitamin D_3, 200 or 400 IU/d x 12 mo	218	ED-71 was well tolerated and dose-dependently increased lumbar and hip bone mineral density in osteoporotic patients	39

treated group than in untreated controls at all time points, and at 1 and 3 weeks the endosteal mineral apposition rate and endosteal bone formation rate were also significantly higher in the ED-71 group. The results indicated that ED-71 increased callus volume during the early period after completion of lengthening, and resulted in thick cortical bone formation. The acceleration of distraction osteogenesis was also demonstrated in rabbits administered ED-71 at doses of 0.1 and 0.2 μg/kg s.c. weekly for 5 weeks. Bone mineral density was significantly increased in treated animals, without evidence of hypercalcemia. These studies indicated the potential therapeutic use of ED-71 in accelerating the healing process following bone lengthening treatments, and allowing early release from external fixators (31, 32).

Experiments using a rat femoral fracture model assessed whether ED-71 disturbed the normal fracture healing process. Rats were administered ED-71 0.025 or 0.05 μg/kg orally 5 times per week for 4 weeks prior to femoral fracture. Treatment was continued until animals were sacrificed at 6 or 16 weeks postsurgery. ED-71 did not delay callus remodeling, nor disturb the restoration of the mechanical strength of the fracture, indicating that the fracture healing process was not disturbed (33).

Other studies have demonstrated the potential therapeutic effects of ED-71 in counteracting corticosteroid-induced osteoporosis and for the treatment of X-linked hypophosphatemic vitamin D-resistant rickets. Bone resorption was increased in prednisolone-treated rats, and in hypophosphatemic mice bone mineral density was increased without hypercalcemia (34, 35).

Clinical Studies

In an early study in humans, 40 healthy male subjects were administered oral ED-71 (0.1-1.0 μg/day) for 15 days. Dose-dependent increases in urinary calcium excretion were observed, but no subject showed sustained increases of > 400 mg/day, nor hypercalcemia > 10.4 mg/dl. Dose-dependent suppression of bone resorption markers such as urinary deoxypyridinoline was also observed, but no significant changes in bone formation markers such as serum bone-specific alkaline phosphatase. Based on these results, an open, randomized, controlled phase II study was conducted in 109 subjects with osteoporosis (mean age 65 years), 102 of whom were female. Subjects received ED-71 orally at doses of 0.25, 0.5, 0.75 or 1.0 μg daily for 6 months. The bone mineral density of the lumbar spine increased dose-dependently from 0.34% in the 0.25-μg group to 3.00% and 2.66% in the 0.75- and 1.0-μg groups, respectively. The percentage of patients demonstrating an increase in bone mineral density > 3% also increased dose-dependently, with approximately 50% of patients achieving such an increase in the two highest dose groups. The dose-dependent suppression of urinary deoxypyridinoline indicated that ED-71 was able to suppress bone resorption, whereas serum osteocalcin was not suppressed, indicating maintenance of bone formation. ED-71 was well tolerated, with no evidence of hypercalcemia or hypercalciuria (36-38) (see Table I).

The effect of ED-71 on bone mass was further evaluated in 218 subjects (mean age 67 years) with osteoporosis receiving concomitant vitamin D supplementation. In this randomized, placebo-controlled study, 75% of subjects had serum 25-hydroxyvitamin D levels of < 20 ng/ml at entry. These subjects were treated with 400 IU/day vitamin D_3, while all other subjects received 200 IU/day. Within each group, subjects received ED-71 at doses of 0.5, 0.75 or 1.0 μg/day orally for 12 months. Lumbar spine bone mineral density increased significantly and dose-dependently in all treated groups compared with placebo. Total hip bone mineral density also increased significantly in the two highest dose groups. ED-71 was well tolerated (39) (Table I).

Phase III clinical trials are in progress in Japan (40).

Source

Chugai Pharmaceutical Co., Ltd. (JP).

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