Synthesis and Biological Evaluation of Prostaglandin-F Alkylphosphinic Acid Derivatives as Bone Anabolic Agents for the Treatment of Osteoporosis

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A series of novel C_1 alkylphosphinic acid analogues of the prostaglandin-F family have been evaluated at the eight human prostaglandin receptors for potential use in the treatment of osteoporosis. Using molecular modeling as a tool for structure-based drug design, we have discovered that the phosphinic acid moiety (P(O)(OH)R) behaves as an isostere for the C_1 carboxylic acid in the human prostaglandin FP binding assay in vitro and possesses enhanced hFP receptor selectivity when compared to the parent carboxylic acid. When evaluated in vivo, the methyl phosphinic acid analogue (**4b**) produced a bone anabolic response in rats, returning bone mineral density (BMD) to intact levels in the distal femur in the ovariectomized rat (OVX) model. These results suggest that prostaglandins of this class may be useful agents in the treatment of diseases associated with bone loss.

Introduction

Osteoporosis and related bone metabolic diseases have emerged as leading health care issues worldwide. In the United States alone, it is estimated that approximately 30% of postmenopausal women suffer from osteoporosis, as based on the World Health Organization definition using bone mineral density (BMD) *T*-score < −2.5,[1] with an estimated annual patient base growth at 2%. It is anticipated that the estimated cost of hip fracture alone in the United States will approach $250 billion dollars by year 2050.[2] Currently, major emphasis has been placed on the development of agents which slow the progression of bone loss with antiresorptive therapies which target inhibition of osteoclasts.[3] These therapies include bisphosphonates,[4] calcitonins,[5] estrogens,[6]and SERMs.[7] More recently, interest has turned to treatments which produce new bone growth via targeting the osteoblast as a strategy toward treatment of metabolic bone disease. These therapies include PTH (parathyroid hormone)[8] and fluoride.[9] Due to the inherent difficulties associated with these current anabolic treatments (dose route, narrow therapeutic index, bone quality), there remains considerable interest in small molecules which produce new bone growth as potential treatments for bone diseases including osteoporosis.

Prostaglandin E_2, $PGF_{2\alpha}$, and analogues within the prostaglandin-F (PGF) family have been reported to produce a bone anabolic response in animal models, including the ovariectomized rat model, and thus may be useful as potential treatments for bone diseases such as osteoporosis.[10] Although PGE_2 is a strong anabolic agent, it also possesses a considerable resorptive component and complex pharmacology, in part due to an inherent lack of receptor specificity of this ligand for the corresponding receptor family, EP_1-EP_4.[11] Within a program of identifying other receptor selective prostaglandins which produce a bone anabolic effect, we have evaluated a variety of prostaglandin-F-type compounds at the eight human prostaglandin receptors and have observed that potency and selectivity for the hFP receptor was greatly influenced by substitution at the C_{16}–C_{20} region of the prostaglandin skeleton.[12] To develop a more thorough understanding of the other key elements within the FP pharmacophore, we wished to explore substitution at the C_1 position in an attempt to modify both the intrinsic binding affinity of ligands for the FP receptor and the selectivity for this receptor relative to the other known prostaglandin receptors. We have recently evaluated sulfonamide substitution at C_1 and have found a significant effect of sulfonamide structure on the binding affinity of ligands for the FP receptor.[13] On the basis of the results of this work, we wished to evaluate replacement of the C_1 carboxylic acid with a functional group which would more precisely mimic the parent COOH moiety and would allow for the flexibility of substitution in order to probe the steric and electron requirements necessary for receptor binding affinity and selectivity for the prostaglandin FP receptor at the 1-position. Using molecular modeling as guide, we chose phosphorus as a suitable replacement at C_1 and subsequently designed a series of phosphinate analogues as potential surrogates for the carboxylic acid at the 1-position. We now wish to report our findings in this series; the result of which is the discovery of a novel carboxylic acid mimic, the methyl phosphinic acid, which possesses enhanced selectivity for the FP receptor in vitro and a bone anabolic effect in vivo in the ovariectomized rat model.

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Table 1. Sequence Alignments of Receptor Site Helices for the Prostaglandin Receptor Family

TM3		SXXXLXXXXXDRY	
hFP	105	NVLCSIFGICMVFSGLCPLLLGSVMAIERCIGV	137
EP1	107	GGACHFLGGCMVFFGLCPLLLGCGMAVERCVGV	139
EP2	106	SRACTYFAFAMTFFSLATMLMLFAMALERYLSI	138
EP3	127	GRLCTFFGLTMTVFGLSSLFIASAMAVERALAI	159
EP4	89	QPLCEYSTFILLFFSLSGLSIICAMSVERYLAI	121
TP	102	CRLCRFMGVVMIFFGLSPLLLGAAMASERYLGI	134
DP	102	NSLCQAFAFFMSFFGLSSTLQLLAMALECWLSL	134
IP	89	PALCDAFAFAMTFFGLASMLILFAMAVERCLAL	121
TM5		FXXPXXXXXXXY	
hFP	193	IKDWEDRFYLLLFSFLGLLALGVSLL	218
EP1	195	PGGWRQALLAGLFASLGLVALLAALV	220
EP2	197	FIRHGRTAYLQLYATLLLLLIVSVLA	213
EP3	222	SHNWGNLFFASAFAFLGLLALTVTFS	247
EP4	176	TNVTAHAAYSYMYAGFSSFLILATVL	201
TP	188	GAESGDVAFGLLFSMLGGLSVGLSFL	213
DP	191	EGSLSVLGYSVLYSSLMALLVLATVL	216
IP	176	WAQPGGAAFSLAYAGLVALLVAAIFL	201
TM6		FXXCWXP	
hFP	243	HHLEMVIQLLAIMCVSCICWSPFLVTMANIGI	274
EP1	291	HDVEMVGQLVGIMVVSCICWSPMLVLVALAVG	322
EP2	258	EETDHLILLAIMTITFAVCSLPFTIFAYMNET	289
EP3	276	IETETAIQLMGIMCVLSVCWSPLLIMMLKMIF	307
EP4	266	AEIQMVILLIATSLVVLICSIPLVVRVFVNQL	297
TP	239	SEVEMMAQLLGIMVVASVCWLPLLWFIAQTVL	270
DP	259	EELDHLLLLALMTVLFTMCSLPVIYRAYYGAF	290
IP	233	DEVDHLILLALMTVVMAVCSLPLTIRCFTQAV	264
TM7		LXXXXXXXNPXXY	
hFP	284	ETTLFALRMATWNQILDPWVY	304
EP1	331	RPLFLAVRLASWNQILDPWVY	351
EP2	295	KWDLQALRFLSINSIIDPWVF	305
EP3	326	NFFLIAVRLASLNQILDPWVY	346
EP4	309	NPDLQAIRIASVNPILDPWIY	329
TP	288	KELLIYLRVATWNQILDPWVY	308
DP	303	AEDLRALRFLSVISIVDPWIF	323
IP	272	MGDLLAFRFYAFNPILDPWVF	292

Molecular Modeling

We focused on the previously constructed homology model of the hFP receptor that maximized the hypothesized ionic, hydrogen bonding, and hydrophobic interactions with the $PGF_{2\alpha}$ prostaglandin.[12] Although X-ray coordinate information is currently unavailable for prostaglandin receptors, they are members of the G-protein-coupled receptor (GPCR) family type and therefore possess structural similarity to the Swiss Institute rhodopsin template, upon which this model is based.[14] The putative prostaglandin receptor site was composed of residues from the third, fifth, sixth, and seventh transmembrane helices. Sequence alignments of these four transmembrane helices within the prostaglandin receptor family are shown in Table 1. In previous work, we had focused on the C_{16}–C_{20} region of the ligands in order to identify the necessary contributions of the ligand/receptor interactions at the "tail" region that supported the high degree of specificity observed with the analogues described. In this work, we focused on the C_1 carboxylate region of the ligand, which forms an ionic interaction with a conserved arginine residue found across all prostaglandin receptors and which has been shown previously via site-directed mutagenesis studies to be a key component of the prostaglandin ligand/receptor binding.[15] The critical receptor residues hypothesized to be involved in prostaglandin binding and function are highlighted in Table 1.

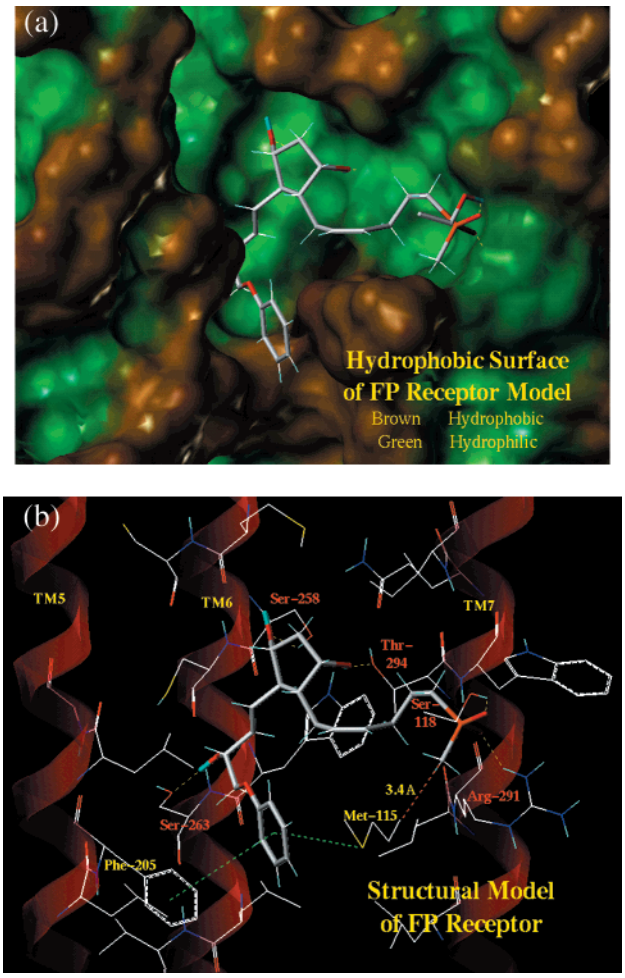
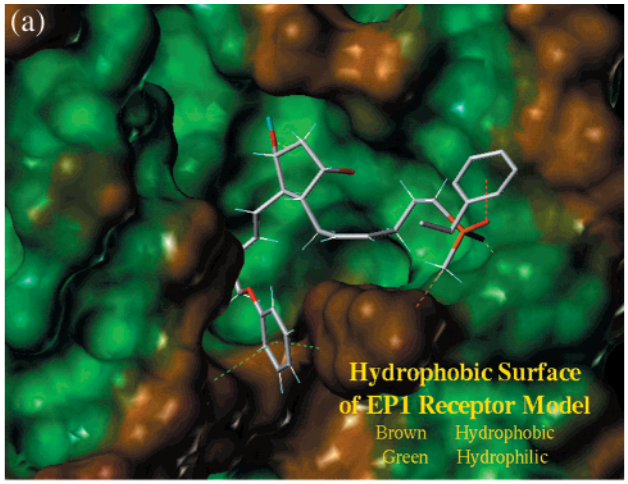


Figure 1. (a) Compound **4b** docked in the surface model of the hFP receptor. (b) Three-dimensional structural model of compound **4b**/hFP receptor binding site.

Various hypotheses for achieving receptor selectivity were considered, based on a model of the hFP receptor/$PGF_{2\alpha}$ complex and on sequence alignments of the prostaglandin family of receptors. It was observed that, adjacent to the common ionic interaction between the C_1 carboxylic acid and the arginine (amino acid 291 in hFP) residue on the seventh transmembrane helix, there appeared to be a unique serine residue in the third transmembrane helix of the hFP receptor which differentiated it structurally from the other prostaglandin receptors. The sequence difference at this site suggested that a larger C_1 carboxylic acid isostere may be better tolerated in the hFP receptor and provide improved selectivity for the FP receptor relative to the other receptors. Docking studies suggested a favorable dipole interaction between the unique serine 118 and a phosphinic acid replacement at the 1-position, thus offering differentiation between the FP receptor and the other prostaglandin receptors, which all possessed a phenylalanine at this location, and thus a potential for a less favorable interaction. As an example, the key interactions between analogue **4b** and the hFP receptor site are illustrated in the surface model Figure 1a and the three-dimensional structure model Figure 1b. In these models, the alkyl substitution on the phosphinic acid moiety points directly toward helix 3 (not shown except for serine 118 and methionine 115 for the hFP receptor) and in close proximity to the guanidinium group of the



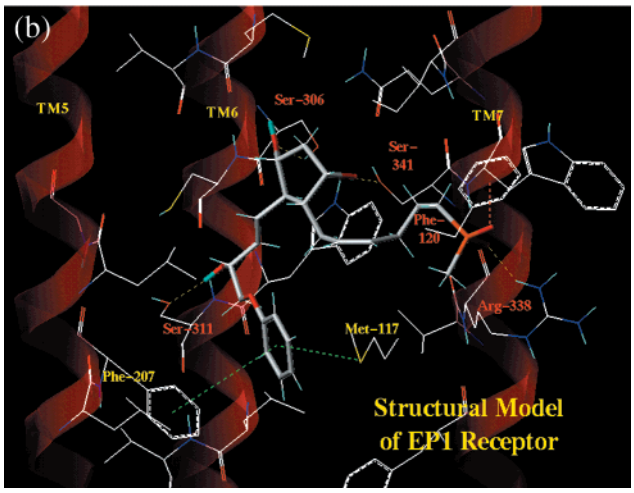
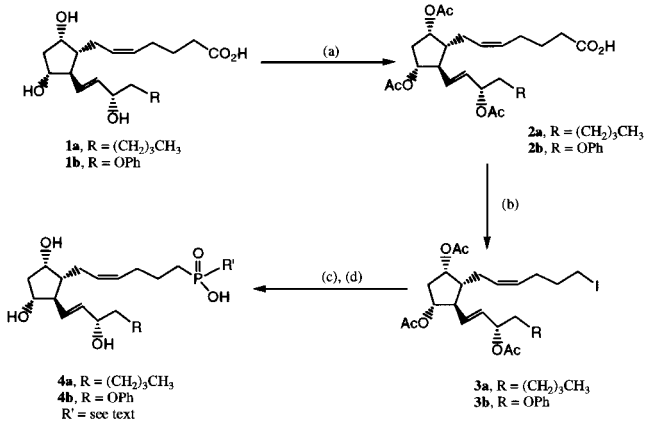


Figure 2. (a) Compound **4b** docked in the surface model of the EP1 receptor. (b) Three-dimensional structural model of compound **4b**/hEP1 receptor binding site.

conserved arginine 291, thus implicating a potential restriction of chain length to smaller substituents on phosphorus. In the case of the other receptors modeled, the positioning of the bulky phenylalanine (amino acid 120 in EP1) may result in an unfavorable interaction between the FP-selective compound (**4b**) and the receptor site (represented by the hEP1 in Figure 2a,b).

The other key interactions are also illustrated in Figures 1 and 2. On helix 7, one turn away from the arginine 291 in the hFP receptor, is a hydrogen bond specificity site in most receptors for the C-9 hydroxyl or carbonyl group of the prostaglandin ligand (Figure 1b). The structural difference between the serine (residue 341 on EP1, Figure 2b) and threonine (residue 294 on hFP, Figure 1b) at this site in the receptor is hypothesized to influence the ligand conformation of the α chain and the resulting orientation of the headgroup containing the C-9 hydrogen bonding site. Helix 6 contains two representative hydrogen bonding sites in the FP (serine 258, 263 in Figure 1b) and EP1 (serine 306, 311 in Figure 2b) receptors for the C-11 and C-15 hydrogen bonding sites of the prostaglandin. In addition, the other hypothesized hydrogen bonding locations/interactions on helix 6 are also included (Table 1). The important hydrophobic residues involved between the respective receptors and ligand **4b** are illustrated in Figures 1a and 2a, including the methionine residue

Scheme 1[a]



[a] Key: (a) 30 equiv of Ac_2O, Et_3N, DMAP, CH_2Cl_2, room temperature; then saturated aqueous Na_2CO_3, 99%. (b) $(COCl)_2$, DMF, CH_2Cl_2; then CF_3CH_2I, DMAP, sodium salt of *N*-hydroxypyridine-2-thione, CH_2Cl_2, *hν*, reflux, 65%. (c) $P(OEt)_2R'$, reflux, 50−85%. (d) NaOH, EtOH−H_2O, 25−70%.

from helix 3 with both the α and *ω* chain of the prostaglandin. Finally, the hypothesized sequence alignments of helix 5 residues suggest the importance of the phenylalanine/tyrosine hydrophobic interaction in both receptors with the "tail" of the prostaglandin, thus stabilizing the active conformation of the receptor for signal transduction (Figures 1a, 2a).

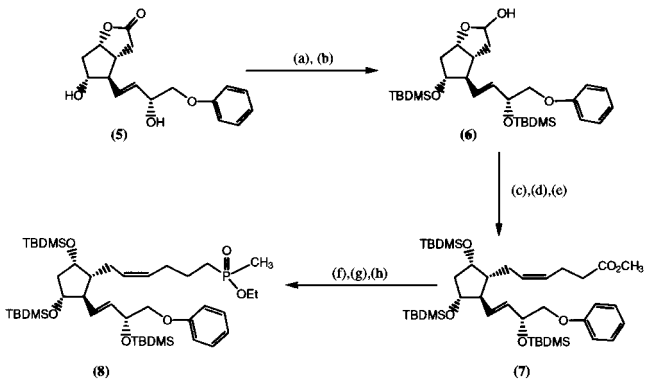
We subsequently designed a series of phosphinic acid isosteres at the 1-position to test the validity of the modeling hypothesis. We chose $PGF_{2\alpha}$ and the 16-phenoxy $PGF_{2\alpha}$ series, based on the commercial availability of the starting materials, as well as previous work supporting the selectivity and potency of the 16-phenoxy tail for the hFP receptor. [16]

Chemistry

Compounds **10**−**15** and **4b** were assembled in a straightforward fashion using methodology previously developed in conjunction with the synthesis of the phosphonic acid derivatives of prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$.[17] Accordingly, the commercially available prostaglandins (**1a, 1b**) were protected as the tris-acetates (**2a, 2b**), and then subjected to a decarboxylation/iodination protocol using a modified Barton−Hundsdieker reaction[18] to provide the primary iodides (**3a, 3b**). In a subsequent step, the iodide was treated with a variety of alkyl phosphinites via Arbuzov reaction[19] to provide the protected prostaglandin derivative, which was saponified to provide the appropriate C_1 phosphinic acids of **4a** or **4b** (Scheme 1).

For larger quantities of compound **4b** needed for in vivo studies, a more traditional approach involving Wittig chemistry for installation of the C_1 phosphinic acid side chain proved to be the most efficient (Scheme 2). Consequently, commercially available **5** [20] was protected with excess *tert*-butyldimethylsilyl trifluoromethanesulfonate[21] and reduced with Dibal-H[22] provided lactol (**6**). The lactol **6** subsequently underwent Wittig condensation with (3-carboxypropyl)triphenylphosphonium bromide[23] to provide, after esterification with trimethylsilyl diazomethane, the protected prostaglandin nucleus as an undetermined mixture of C_9–C_{15} and C_{11}–C_{15} bis-silyl ethers due to transfer of the *tert*-

Scheme 2[a]



[a] Key: (a) 2.2 equiv of TBDMSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C−rt; then 1 N HCl, 91%; (b) 2.2 equiv of Dibal-H, THF, −78 °C, 80%; (c) 4.4 equiv of NaHMDS, 2.2 equiv of $BrP(Ph)_3^+(CH_2)_3COOH$, THF, 88%; (d) 5 equiv of TBDMSOTf, 10 equiv of 2,6-lutidine, CH_2Cl_2, 0 °C−rt; then 1 N HCl, 92%; (e) $TMSCH_2N_2$, Et_2O, quant.; (f) $LiAlH_4$, Et_2O, 95%; (g) $Ph_3P{\cdot}Br_2$, CH_3CN, 95%; (h) $CH_3P(OEt)_2$, toluene, 98%.

butyldimethylsilyl protecting group under the reaction conditions. Only the $C_{5-}C_6$ (*Z*)-olefin geometry was observed of each silyl regioisomer. After exhaustive silylation of the product mixture to provide the ester **7**, reduction of the ester to the primary alcohol, bromination with triphenylphosphine−dibromide,[24] and treatment with diethylmethylphosphinite provided **8**. Final deprotection in sequential fashion using HF/pyridine and then NaOH/EtOH/H_2O provided the 16-phenoxy analogue **4b** in gram scale quantities.

Results and Discussion

Compounds were tested in vitro in the human FP receptor binding assay as described previously[12] and progressed for screening at the other prostaglandin receptors when binding affinity < 250 nM at the FP receptor was achieved. The results are shown in Table 1. As a direct comparison, $PGF_{2\alpha}$ **9** and 16-phenoxy tetranor $PGF_{2\alpha}$ **16** were tested as benchmarks for these studies. As seen from the compounds tested, the modification of substituents on phosphorus has a dramatic effect on the binding affinity of individual compounds for the FP receptor. For example, a comparison of **13** with either **12**, **14**, or **15** suggests that binding affinity *decreases* with increasing steric bulk on phosphorus, with the H−P substitution pattern being most potent (H−P > CH_3-P > Et−P > ^{n}Bu−P). In addition, the phosphinic acid series proved more potent than the phosphonic acid, as seen by comparison of **12** or **13** versus **10**. Most importantly, incorporation of the alkylphosphinic acid resulted in a dramatic *increase in selectivity for the FP receptor* when compared to binding affinity at the other human prostaglandin receptors tested. Thus, when comparing $PGF_{2\alpha}$ **9** and the phosphinic acid **13**, a marked increase in selectivity for the FP receptor was seen for the later compound, while $PGF_{2\alpha}$ also possessed binding affinity for the EP1, EP3, EP4, and DP receptors. This trend also was observed when comparing the 16-phenoxy tetranor analogues **16** vs **4b**, again with the latter compound being considerably more selective for the FP receptor.

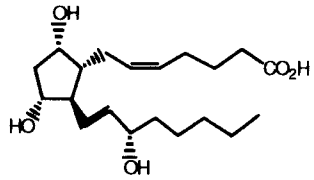
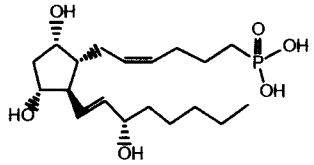
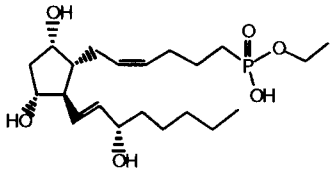
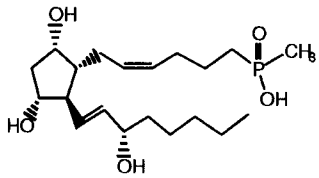
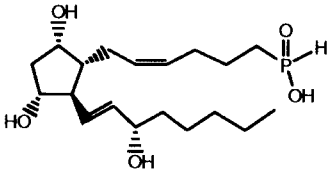
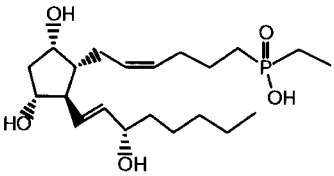
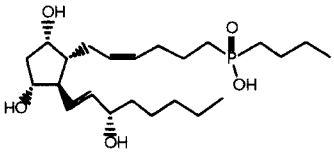
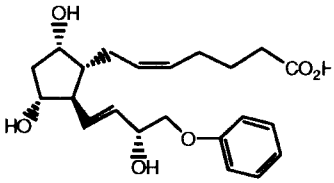
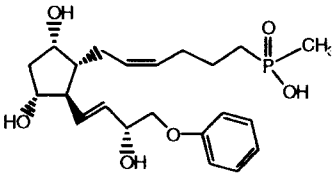
We attempted to correlate FP receptor binding affinity with ligand pK_a, as has been implicated previously in the evaluation of C_1 sulfonamides of the PGE family.[25] Based on the data obtained from these calculations,[26] there appeared to be no clear correlation between in vitro binding affinity and acidity at the P_1 position of the analogues tested (Table 2). This lack of correlation suggests that other factors, such as steric and dipole interactions originally proposed by modeling, may play a more significant role in the binding and selectivity of ligands of this type.

We chose to evaluate the methyl phosphinic acid analogue **4b** in vivo using the ovariectomized rat model (OVX).[27] Female Sprague−Dawley rats (Simonsen Labs, Gilroy, CA) were received at 3 months of age and allowed to age to 6 months. Rats were ovariectomized at 6 months and allowed to lose bone for 60 days before treatment with compound. Animals then treated for 60 days using doses of 10, 30, 100, 300, 1000, and 3000 μg/kg of compound **4b** in a once daily subcutaneous injection. The positive controls included two agents known to increase bone formation, volume, and density; the nonselective prostaglandin agonist PGE2, and the FP-selective agonist Cloprostenol. In addition, the bisphosphonate Alendronate[28] was used as a representative of an antiresorptive agent which maintains or increases bone density through decreasing the rates of both bone formation and resorption.

The effect of **4b** was measured at several sites in an attempt to fully understand the anabolic response of cancelous and cortical sites, and the tissue level mechanisms of the response.[29] Initially, bone mineral density (BMD) was measured in the distal femur using dual-energy X-ray absorptiometry (Figure 3). The methyl phosphinic acid (**4b**) produced a significant, dose-dependent increase in bone mineral density at the four highest doses (100−3000 μg/kg) as compared to final OVX. At the two highest doses, the increase was statistically significant when compared to the pretreatment group as well ($p < 0.005$). The ED_{50} for compound **4b** at this site was calculated to be 146 μg/kg (95% CI = 58−364 μg/kg). Although the effect was not as strong as with PGE_2, compound **4b** did return bone mineral density to levels greater than the bisphosphonate and similar to the intact vehicle treated group. In addition, μCT [30] (microscopic computerized tomography, Table 3) was used in the vertebrae to assess cancelous bone volume and architecture. The methylphosphinic acid induced a dose-dependent increase in bone volume (ED_{50} = 30 μg/kg, 95% CI = 17−53 μg/kg), with increases in trabecular thickness and number, but not connectivity density (a more accurate index of the number of trabeculae) compared to the final OVX group (Tables 4 and 5). At 100 μg/kg the methyl phosphinic acid (**4b**) returned bone volume to levels similar to the final intact group, and 300 and 3000 μg/kg doses increased bone volume above the starting values of the pretreatment OVX group ($p < 0.05$). The methyl phosphinic acid also increased cancelous bone volume in tibial metaphysis with increases in trabecular thickness and number. These increases were brought about by decreases in the rate of bone turnover (BFR/BV) compared to the high levels induced by ovariectomy.

These results indicate that compound **4b** produced an anabolic response in the aged, ovariectomized rat model and suggests that compounds of this class may be useful

Table 2. In Vitro Binding and pK_a Data Comparison of Prostaglandin F Derivatives and Phosphinic Acid Analogues[a]

		IC_{50} (nM)								
Compound	Structure	hFP	EP1	EP2	EP3	EP4	TP	IP	DP	pKa
9		3-12	220	$>10^4$	110 -322	4200	$>10^4$	$>10^4$	4500	4.77
10		1000	NT	NT	NT	NT	NT	NT	NT	2.38
11		$>10^4$	NT	NT	NT	NT	NT	NT	NT	2.36
12		311	NT	NT	NT	NT	NT	NT	NT	3.10
13		114	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	3.39
14		4100	NT	NT	NT	NT	NT	NT	NT	3.24
15		6700	NT	NT	NT	NT	NT	NT	NT	3.36
16		2.0	38*	NT	186*	NT	510*	NT	NT	4.77
4b		42	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	3.10

[a] NT = not tested. An asterisk marks values evaluated for functional activity in RAT-1 cells, transiently transfected with human receptor having a constitutively expressed β-galactosidase reporter gene stably transfected within. Result is reported as EC50 shown after timed hydrolysis of galactosidase pseudosubstrate. For details see: Messier, T.; Dorman, C. M.; Brauner, H.; Eubanks, D.; Brann, M.R. *Pharmacol. Toxicol.* **1995**, *76*, 308.

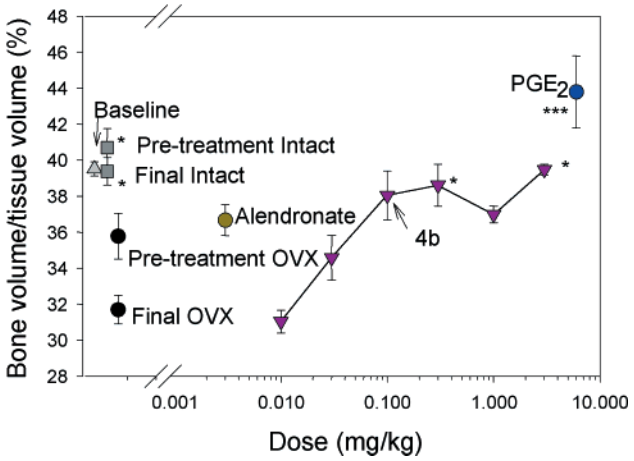


Figure 3. Vertebral cancelous bone volume for **4b**.

in the treatment of bone degenerative disease, including osteoporosis.

In conclusion, we have designed and prepared a series of alkyl phosphinic acid analogues of $PGF_{2\alpha}$ and have evaluated them in vitro in an hFP receptor binding assay and in vivo in the ovariectomized rat model. We have found that, in general, binding affinity for the hFP receptor decreases with increasing size of the alkyl substituent on phosphorus. We have also found that replacement at C_1 with a phosphinic acid moiety results in greater receptor selectivity for the FP receptor when compared to the C_1 carboxylic acid.[31] Finally, we have found that the methyl phosphinic acid (**4b**) produced a dose-dependent increase in bone mineral density when compared to both an antiresorptive agent and to the final intact rat group. We are continuing to explore the implications of these results within the realm of alternative treatments for bone degenerative diseases including osteoporosis, as well as other therapeutic areas where selective prostaglandin analogues may be desirable.

Experimental Section

General Methods. ^{1}H NMR spectra were recorded on a Varian Unity Plus 300 MHz spectrometer and are referenced to either the deuteriochlororform singlet at 7.27 ppm or deuteriomethanol singlet at 4.87 ppm. ^{13}C spectra were obtained on a Varian Unity Plus 300 MHz spectrometer and are referenced at either the center line of the deuteriochloroform triplet at 77.0 ppm or the deuteriomethanol heptet at 49.15 ppm. Infrared absorption spectra were obtained on a Perkin-Elmer Model 197 spectrophotometer and are referenced to polystyrene (1601 cm^{-1}). Mass spectra were obtained on either a Fison Platform-II Quadrupole Mass Spectrometer or a Fison Trio2000 Quadrupole Mass Spectrometer. High-Resolution mass spectra were obtained from the Procter & Gamble CRD Mass Spectrometry Lab (M. Lacey). Elemental analyses were obtained from the Procter & Gamble EA Lab in Norwich, NY. Melting points were determined in open Pyrex capillary tubes on a Thomas-Hover Unimelt apparatus. Melting points and boiling points are uncorrected. All solvents were purchased anhydrous (Aldrich Chemical) and used without further purification. All air-sensitive reactions were performed under an anhydrous nitrogen atmosphere. Compounds **9** and **16** were purchased from Cayman Chemical and used without further purification. Compounds **10** and **11** were synthesized previously.[17] Flash chromatography was performed on silica gel (70−230 mesh; Aldrich) or (230−400 mesh; Merck) as appropriate. Thin-layer chromatography analysis was performed on glass mounted silica gel plates (200−300 mesh; Baker) and visualized using UV, 5% phosphomolybdic acid in EtOH, or ammonium molybdate/cerric sulfate in 10% aqueous H_2SO_4.

Radioligand Binding Assay. COS-7 cells were transiently transfected with a hFP recombinant plasmid using LipofectAMINE Reagent. The transfected cells were washed 48 h later with Hank's Balanced Salt Solution (HBSS, without $CaCl_2$, $MgCl_2$, $MgSO_4$, or phenol red). The cells were detached with versene, and HBSS was added. The mixture was centrifuged at 200*g* for 10 min, at 4 °C to pellet the cells. The pellet was resuspended in phosphate-buffered saline−EDTA buffer (PBS; 1 mM EDTA; pH 7.4; 4 °C). The cells were disrupted by nitrogen cavitation (Parr Model 4639), at 800 psi, for 15 min at 4 °C. The mixture was centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was centrifuged at 100000*g* for 60 min at 4 °C. The pellet was resuspended to 1 mg of protein/mL of TME buffer (50 mM Tris; 10 mM $MgCl_2$; 1 mM EDTA; pH 6.0; 4 °C) based on protein levels measured using the Pierce BCA Protein Assay kit. The homogenate was mixed using a Kinematica Polytron for 10 s. The membrane preparations were then stored at −80 °C, until thawed for assay use.

The receptor competition binding assays were developed in a 96 well format. Each well ($n = 3$) contained 100 μg of hFP membrane, 5 nM [^{3} H] PGF2α, and the various competing compounds in a total volume of 200 μL. The plates were incubated at 23 °C for 1 h. The incubation was terminated by rapid filtration using the Packard Filtermate 196 harvester through Packard UniFilter GF/B filters that were prewetted with TME buffer. The filter was washed four times with TME buffer. Packard Microscint 20, a high-efficiency liquid scintillation cocktail, was added to the filter plate wells and the plates remained at room temperature for 3 h prior to counting. The plates were read on the Packard TopCount Microplate Scintillation counter.

The hEP1 receptor was transiently expressed in COS-7 cells and followed the above-mentioned transient transfection protocol. Five of the other receptors, hEP2, hEP3, hEP4, hTP, and hIP, were stably expressed in the CHO cell line. The hDP receptor was stably expressed in the HEK-293 cell line and required a TME buffer pH of 7.4. The CHO cells and HEK-293 cells expressing the receptors were collected 4−5 days after seeding, were washed with PBS and detached with versene. Cells were centrifuged at 100*g* for 10 min at 4 °C. Cells were resuspended in lysis buffer (10 mM Tris-HCl; 5 mM EDTA; 10 μg/mL leupeptin and benzamidine; 2μg/mL aprotinin) and the homogenate was centrifuged at 200*g* for 10 min at 4 °C. The supernatant was centrifuged at 100000*g* for 60 min at 4 °C. The pellet was resuspended to 1 mg of protein/mL of TME buffer based on measured protein levels. The homogenate was mixed using a Kinematica Polytron for 10 s. The membrane preparations were then stored at −80 °C, until thawed for assay use. The radiolabeled ligands used for the competition binding assays of the other seven receptors were as follows: the EP_1, EP_2, EP_3, and EP_4 assays used [^{3}H]PGE_2, the DP assay used [^{3}H]PGD_2, the IP assay used [^{3}H]Iloprost, and the TP assay used [^{3}H]SQ-29548. All of the radiolabeled ligands were run at 5 nM in the experiment and were purchased from DuPont NEN and Amersham LIFE SCIENCE.

2-Decarboxy-2-(P-methylphosphinico)prostaglandin $F_{2\alpha}$ *(12).* A mixture of 2-decarboxy-2-iodoprostaglandin $F_{2\alpha}$ triacetate (**3a**, 46.1 mg, 82 μmol), diethyl methylphosphonite (0.2 mL, 1.32 mmol), and toluene (0.41 mL) was stirred at 100 °C for 6 h. The solvents were evaporated, and the residue was purified by dry-flash column chromatography (SiO_2, 5−25% 2-propanol in 20% dichlormethane−hexane). Appropriate fractions were concentrated, diluted with water (15 mL), and extracted with ether (3 × 5 mL). The combined extracts were washed with water (5 mL), dried (brine, Na_2SO_4), and evaporated to give 2-decarboxy-2-(*O*-ethyl-*P*-methylphosphinico)-prostaglandin $F_{2\alpha}$ triacetate (38.1 mg, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.46−5.55 (m, 2 H), 5.27−5.38 (m, 2 H), 5.17−5.25 (m, 1 H), 5.07 (t, $J = 4.6$ Hz, 1 H), 4.87 (ddd, $J = 8.8$, 7.6, 4.4 Hz, 1H), 4.03 (dq, $J = 7.0$, 7.0 Hz, 2 H), 2.46−2.60 (m, 2

Table 3. Vertebral Bone Volume and Architecture Measured Using μCT[a]

group	n	bone vol/tissue vol (%)	derived trabecular thickness (um)	derived trabecular no. (mm^{-1})	directly measd thickness (um)	connectivity dens (mm^{-3})
baseline	6	39.52 ± 0.96[a]	73.62 ± 4.22	5.38 ± 0.24[a]	121.17 ± 5.46	92.32 ± 19.33[a]
pretreatment intact	6	40.70 ± 2.60[a]	78.29 ± 2.86[b]	5.20 ± 0.22[a]	127.50 ± 5.75[b]	75.56 ± 3.19[a]
final intact	8	39.38 ± 2.17[a]	78.09 ± 1.63[b]	5.04 ± 0.21[a]	127.63 ± 3.11[c]	70.75 ± 7.53[b]
pretreatment OVX	6	35.78 ± 3.12[a]	73.85 ± 3.03	4.84 ± 0.30[a]	121.33 ± 4.72	77.06 ± 8.24[a]
final OVX	5	31.70 ± 1.78	74.81 ± 1.81	4.24 ± 0.26	121.60 ± 3.91	60.21 ± 8.08
PGE2, 6000 μg/kg	3	43.79 ± 3.46[a]	95.14 ± 3.93[a]	4.60 ± 0.18	149.00 ± 6.56[a]	47.81 ± 2.69[b]
Alendronate, 3 μg/kg	6	36.67 ± 2.10[a]	76.62 ± 2.34	4.79 ± 0.31[a]	123.83 ± 2.40	68.78 ± 9.57
Cloprostenol, 250 μg/kg	6	42.04 ± 1.90[a]	85.81 ± 1.77[a]	4.90 ± 0.16[a]	137.60 ± 3.91[a]	61.13 ± 7.00
4b, 10 μg/kg	4	31.04 ± 1.26	73.01 ± 2.09	4.26 ± 0.17	118.50 ± 2.65	60.28 ± 8.57
4b, 30 μg/kg	4	34.58 ± 2.49[b]	77.77 ± 1.41	4.44 ± 0.27	125.50 ± 2.65	59.20 ± 5.21
4b, 100 μg/kg	4	38.04 ± 2.72[a]	78.71 ± 1.94[b]	4.83 ± 0.26[a]	127.25 ± 3.40[b]	68.10 ± 5.10
4b, 300 μg/kg	4	38.61 ± 2.33[a]	79.46 ± 3.07[a]	4.86 ± 0.25[a]	127.00 ± 4.08[b]	66.71 ± 7.59
4b, 1000 μg/kg	4	36.98 ± 0.94[a]	80.62 ± 1.45[a]	4.59 ± 0.19[b]	127.50 ± 1.73[b]	59.66 ± 5.95
4b, 3000 μg/kg	3	39.48 ± 0.52[a]	83.76 ± 1.44[a]	4.71 ± 0.12[b]	134.00 ± 2.00[a]	55.90 ± 5.49

[a] $p \leq 0.005$ vs final OVX. [b] $p \leq 0.05$ vs final OVX. [c] $p \leq 0.01$ vs final OVX.

Table 4. Bone Volume and Formation Rates in the Proximal Tibial Metaphysis Measured Histomorphometrically

group	n	bone vol/ tissue vol (%)	trabecular thickness (mm)	trabecular no. (mm^{-1})	mineralizing surface/bone surface (%)	mineral apposition rate (mm/day)	bone formation rate/tissue vol (%/year)	bone formation rate/bone vol (%/year)
baseline	6	12.35 ± 3.44[a]	43.99 ± 4.06[b]	2.77 ± 0.54[a]	27.19 ± 3.94[b]	0.93 ± 0.02[b]	42.67 ± 10.80[a]	351.66 ± 55.24[a]
pretreatment intact	6	11.71 ± 2.07[a]	43.92 ± 2.75	2.66 ± 0.40[a]	29.52 ± 3.72	0.91 ± 0.05[c]	43.74 ± 10.43[a]	371.09 ± 41.06[a]
final intact	8	11.15 ± 2.28[a]	45.10 ± 3.93[b]	2.46 ± 0.40[a]	25.81 ± 4.02[a]	0.81 ± 0.04[a]	31.74 ± 9.55[a]	283.23 ± 49.86[a]
pretreatment OVX	6	5.74 ± 1.21[c]	44.21 ± 5.60[b]	1.31 ± 0.29[a]	34.12 ± 3.51	1.09 ± 0.08	30.35 ± 5.99[a]	538.26 ± 88.19
final OVX	6	2.44 ± 0.73	36.73 ± 6.50	0.66 ± 0.18	31.65 ± 3.89	1.07 ± 0.11	13.68 ± 3.91	576.20 ± 126.60
PGE2, 6000 μg/kg	6	11.65 ± 2.30[a]	66.14 ± 3.17[a]	1.76 ± 0.29[a]	36.23 ± 2.32[b]	1.19 ± 0.05	45.62 ± 12.58[a]	394.70 ± 13.53[c]
Alendronate, 3 μg/kg	6	6.49 ± 3.16[a]	48.64 ± 7.60[c]	1.29 ± 0.51[a]	17.72 ± 2.46[a]	0.63 ± 0.09[a]	8.81 ± 3.62	144.92 ± 47.06[a]
Cloprostenol, 250 μg/kg	5	9.50 ± 1.63[a]	53.90 ± 5.95[a]	1.77 ± 0.30[a]	22.99 ± 3.57[a]	1.09 ± 0.10	26.44 ± 2.27[a]	286.86 ± 63.28[a]
4b, 10 μg/kg	4	2.23 ± 1.48	35.68 ± 8.12	0.58 ± 0.29	28.37 ± 2.45	0.99 ± 0.03	9.81 ± 5.12	498.01 ± 133.83
4b, 30 μg/kg	4	3.63 ± 1.25	41.23 ± 5.80	0.87 ± 0.27	30.59 ± 5.44	1.09 ± 0.22	17.55 ± 7.45	495.65 ± 139.75
4b, 100 μg/kg	4	5.84 ± 1.05[c]	45.48 ± 5.70[b]	1.28 ± 0.14[a]	27.11 ± 4.62[b]	0.87 ± 0.11[a]	18.23 ± 3.90	320.16 ± 84.37[a]
4b, 300 μg/kg	4	5.72 ± 2.28[b]	44.05 ± 3.77	1.27 ± 0.38[a]	23.57 ± 2.13[a]	0.98 ± 0.06	18.03 ± 6.25	322.61 ± 52.05[a]
4b, 1000 μg/kg	4	9.43 ± 3.33[a]	56.68 ± 7.90[a]	1.64 ± 0.39[a]	22.04 ± 2.36[a]	1.06 ± 0.22	23.44 ± 8.80[b]	251.82 ± 56.88[a]
4b, 3000 μg/kg	3	8.51 ± 1.69[a]	55.84 ± 4.39[a]	1.51 ± 0.22[a]	23.66 ± 3.66[a]	0.94 ± 0.03	20.13 ± 5.49	247.55 ± 28.77[a]

[a] $p \leq 0.005$ vs final OVX. [b] $p \leq 0.05$ vs final OVX. [c] $p \leq 0.01$ vs final OVX.

Table 5. Bone Volume and Formation Rates in the Tibial Diaphysis Measured Histomorphometrically

group	n	tissue area (mm)	marrow area (mm)	endosteal mineralizing surface (%)	endosteal eroded surface (%)	mineral apposition rate (mm/day)	periosteal mineralizing surface (%)	periosteal mineral apposition rate (mm/day)
baseline	6	4.61 ± 0.37[a]	0.76 ± 0.09[a]	19.09 ± 4.59[a]	2.62 ± 0.82[a]	0.20 ± 0.31[a]	50.82 ± 20.77[a]	0.99 ± 0.22[a]
pretreatment intact	6	4.91 ± 0.21[b]	0.87 ± 0.04[c]	21.86 ± 3.73[a]	2.84 ± 1.40[a]	0.43 ± 0.47[a]	31.12 ± 6.59	0.36 ± 0.28
final intact	8	5.06 ± 0.22	0.92 ± 0.09[b]	23.83 ± 4.59[c]	3.39 ± 1.70[a]	0.58 ± 0.37[b]	19.29 ± 15.74	0.25 ± 0.36
pretreatment OVX	6	4.96 ± 0.47[b]	0.95 ± 0.20	25.21 ± 8.74[b]	12.00 ± 7.47	0.81 ± 0.15	42.49 ± 18.74[a]	0.89 ± 0.05[a]
final OVX	6	5.30 ± 0.40	1.03 ± 0.14	36.87 ± 6.91	9.52 ± 3.66	0.98 ± 0.08	19.37 ± 4.77	0.28 ± 0.31
PGE2, 6000 μg/kg	6	5.22 ± 0.30	0.76 ± 0.08[a]	61.22 ± 9.24[a]	0.79 ± 0.71[a]	1.33 ± 0.26	58.37 ± 7.13[a]	1.14 ± 0.23[a]
Alendronate, 3 μg/kg	6	5.01 ± 0.44	0.82 ± 0.10[a]	29.40 ± 4.74	1.99 ± 1.36[a]	0.35 ± 0.39[a]	29.59 ± 12.38	0.46 ± 0.36
Cloprostenol, 250 μg/kg	5	4.78 ± 0.06[c]	0.70 ± 0.09[a]	55.18 ± 8.18[a]	2.22 ± 1.07[a]	1.60 ± 0.40[a]	9.91 ± 3.60	0.00 ± 0.00
4b, 10 μg/kg	4	5.09 ± 0.27	0.93 ± 0.07	46.62 ± 15.10	11.83 ± 5.81	0.71 ± 0.49	25.86 ± 10.11	0.49 ± 0.35
4b, 30 μg/kg	4	5.19 ± 0.11	0.87 ± 0.18[b]	28.72 ± 8.90	2.01 ± 0.98[a]	0.88 ± 0.07	26.23 ± 28.42	0.31 ± 0.62
4b, 100 μg/kg	4	4.95 ± 0.17	0.82 ± 0.05[a]	22.28 ± 14.84[c]	2.30 ± 1.21[a]	0.42 ± 0.49[a]	15.21 ± 7.91	0.38 ± 0.44
4b, 300 μg/kg	4	5.01 ± 0.29	0.89 ± 0.14[b]	29.02 ± 10.71	1.32 ± 0.49[a]	0.53 ± 0.63[b]	9.56 ± 4.93	0.19 ± 0.38
4b, 1000 μg/kg	4	4.92 ± 0.17[b]	0.86 ± 0.11[b]	35.29 ± 9.19	2.74 ± 1.22[a]	1.01 ± 0.13	10.35 ± 2.13	0.00 ± 0.00
4b, 3000 μg/kg	3	4.88 ± 0.31[b]	0.74 ± 0.10[a]	29.40 ± 4.74	2.20 ± 0.34[a]	1.03 ± 0.40	11.09 ± 6.03	0.00 ± 0.00

[a] $p \leq 0.005$ vs final OVX. [b] $p \leq 0.05$ vs final OVX. [c] $p \leq 0.01$ vs final OVX.

H), 1.98−2.13 (m, 4 H), 2.04, 2.03, 2.00 (3 s, 3 H each), 1.50−1.75 (m, 8 H), 1.42 (d, J = 13.5 Hz, 3 H), 1.30 (t, J = 7.0, 7.0 Hz, 3 H0, 1.20−1.30 (m, 6 H), 0.87 (t, J = 6.6 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 170.3, 170.2, 131.9, 131.5, 129.5, 128.2, 77.7, 74.2, 73.9, 59.9 (d, J = 6 Hz), 51.8, 47.4, 38.8, 34.2, 31.4, 29.2 (d, J = 94 Hz), 28.0 (d, J = 16 Hz), 24.9, 24.7, 22.4, 22.0 (br), 21.3, 21.2, 21.0, 16.5 (d, J = 6 Hz), 13.9, 13.5 (d, J = 91 Hz); ^{31}P NMR δ 55.3. m/z (FAB) 565 (M + Na, 100), 483 (95), 363 (55); Found (FAB) MNa^+ 565.2905, $C_{28}H_{47}O_8PNa$ requires MNa^+ 565.2905.

A mixture of 2-decarboxy-2-(*O*-ethyl-*P*-methylphosphinico)-prostaglandin $F_{2\alpha}$ triacetate (38.1 mg, 70 μmol), 95% ethanol (2.8 mL), and 2.5 M aqueous sodium hydroxide (2.8 mL, 7.0 mmol) was stirred at reflux for 3 h. The ethanol was evaporated and the residue was diluted with water (5 mL) and washed with ethyl acetate (2 × 3 mL). The combined washes were extracted with water (2 mL). The combined aqueous phases were acidified with 2.5 M aqueous hydrochloric acid and extracted with ethyl acetate (5 × 5 mL). The combined extracts were dried (brine, Na_2SO_4) and evaporated to give 2-decarboxy-2-(*P*-methylphosphinico)prostaglandin $F_{2\alpha}$ (**12**). (24.2 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.56 (dd, J = 15.2, 7.1 Hz, 1 H), 5.47 (dd, J = 15.2, 8.5 Hz, 1 H), 5.32−5.44 (m, 2 H), 4.11 (br t, 1 H), 4.05 (q, J = 6.7 Hz, 1 H), 3.94 (br s, 1 H), 3.73 (br s, 4 H), 2.30−2.42 (m, 3 H), 2.19−2.28 (m, 1 H), 1.99−2.06 (m, 2 H), 1.49−1.82 (m, 6 H), 1.45 (d, J = 13.2 Hz, 3 H), 1.23−1.41 (m, 6H), 0.89 (t, J = 6.7 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.5, 133.0, 129.8, 129.1, 77.5, 73.2, 72.1, 55.3, 49.9, 42.9, 36.9, 31.7, 27.9 (br d, J = 12 Hz), 25.4, 25.3, 22.6, 22.1 (br), 14.0 (signals for carbons adjacent to P not

observed); ^{31}P NMR δ 56.6; MS *m/z* 411 (M + Na, 100), 393 (5), 371 (10), 335 (10); FAB 433 (M − H + 2Na); Found (FAB) MNa^+ 411.2264, $C_{20}H_{37}O_5P + Na^+$ requires 411.2276.

Sodium Salt of 2-Decarboxy-2-(P-ethylphosphinico)-prostaglandin $F_{2\alpha}$ (14). A mixture of 2-decarboxy-2-iodoprostaglandin $F_{2\alpha}$ triacetate (**3a**, 24.1 mg 0.43 mmol), diethyl ethylphosphonite (72 μL, 0.43 mmol), and toluene (0.21 mL) was stirred at 100 °C for 8 h. The solvents were evaporated, and the residue was purified by dry-flash column chromatography (SiO_2), 5−25% 2-propanol in 20% dichloromethane−hexane). Appropriate fractions were concentrated, diluted with water (15 mL), and extracted with ether (3 × 5 mL). The combined extracts were washed with water (5 mL), dried (brine, Na_2SO_4), and evaporated to give 2-decarboxy-2-(*O*−*P*-diethylphosphinico)prostaglandin $F_{2\alpha}$ triacetate (18.0 mg, 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.47−5.57 (m, 2 H), 5.29−5.38-(m, 2 H), 5.18−5.24 (m, 1 H), 5.07 (t, J = 4.6 Hz, 1 H, 4.87 (ddd, J = 8.9, 7.5, 4.4 Hz, 1 H), 4.04 (dq, J = 7.1, 7.1 Hz, 2 H), 2.48−2.59 (m, 2 H), 2.05−2.16 (m, 4 H), 2.05, 2.04, 2.01 (2 s, 2 H each), 1.50−1.77 (m, 10 H), 1.22−1.34 (m, 6 H), 1.30 (t, J = 7.0 Hz, 3 H), 1.13 (dt, J = 17.8, 7.7 Hz, 3 H), 0.86 (t, J = 6.7 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 170.3, 170.2, 132.0 131.5, 129.6, 128.2, 77.7, 64.2, 73,0, 59.5 (d, J = 6 Hz), 51.8, 47.4, 38.9, 34.3, 31.5, 28.1 (d, J = 15 Hz), 26.9 (d, J = 90 Hz), 24.9, 24.7, 22.5, 21.7 (br), 21.3, 21.2, 21.0, 20.9 (d, J = 91 Hz), 16.7 (d, J = 5 Hz), 13.9, 6.0 (d, J = 3 Hz); ^{31}P NMR δ 60.0; *m/z* (FAB) 579 (M + Na, 100%), 497, (45), 377 (45); Found (FAB) MNa^+ 579.3067, $C_{29}H_{49}O_8PNa$ requires MNa^+ 579.3063.

A mixture of 2-decarboxy-2-(*O, P*-diethylphosphinico)prostaglandin $F_{2\alpha}$ triacetate (17.8 mg, 32 μmol), 95% ethanol (1.3 mL), and 2.5 M aqueous sodium hydroxide (1.3 mL, 3.2 mmol) was stirred at reflux for 3 h. The ethanol was evaporated, and the residue was diluted with water (5 mL) and washed with ethyl acetate (2 × 3 mL). The combined washes were extracted with water (1 mL). The combined aqueous phases were acidified with 2.5 M aqueous hydrochloric acid and extracted with ethyl acetate (4 × 5 mL). The combined extracts were dried (brine, Na_2SO_4) and evaporated. The residue was taken up in methanol (3 mL) and stirred with Amberlite CG-50 (Na^+ form, 0.4 g) for 25 min. The resin was removed by filtration and the filtrate was concentrated to give the sodium salt of 2-decarboxy-2-(*P*-ethylphosphinico)prostaglandin $F_{2\alpha}$ (**14**, 13 mg, 100%). ^{1}H NMR (300 MHz, CD_3OD) δ 5.31−5.50 (m, 4 H), 4.09 (td, J = 16.4, 7.7 Hz, 3 H), 3.99 (q, J = 6.4 Hz, 1H), 3.82 (ddd, J = 8.4, 7.3, 5.3 Hz, 1H), 2.32 (ddd, J = 14.4, 8.5, 7.8 Hz, 1H), 2.30−2.03 (m, 3H), 1.62−1.26 (m, 16H), 1.06 (dt, J = 16.4, 7.7 Hz, 3H), 0.90 (t, J = 6.9 Hz, 3 H); ^{13}C NMR (75 MHz, CD_3OD) δ 136.5, 134.2, 130.8, 130.0, 77.9, 73.9, 72.1, 56.2, 50.0, 44.3, 38.4, 33.0, 30.6 (d, J = 91 Hz), 29.9 (d, J = 16 Hz), 26.4, 26.3, 24.2 (d, J = 3 Hz), 24.0 (d, J = 92 Hz), 23.8, 14.4, 7.4 (d, J = 5 Hz); ^{31}P NMR δ 45.0; *m/z* (negative ion FAB) 423 (M, 10%), 401 (M − Na, 100), 361 (10), 329 (10); Found (FAB) $(M - Na)^-$ 401.2457.

Sodium Salt of 2-Decarboxy-2-(P-butylphosphinico)-prostaglandin $F_{2\alpha}$ (15). A mixture of 2-decarboxy-2-iodoprostaglandin $F_{2\alpha}$ triacetate (**3a**, 30.1 mg, 55 μmol), diethyl butylphosphonite (0.11 mL, 0.58 mmol), and toluene (0.30 mL) was stirred at 100 °C for 7 h. The solvents were evaporated, and the residue was purified by dry-flash column chromatography (SiO_2, 5−25% 2-propanol−hexane). Appropriate fractions were concentrated, diluted with water (10 mL), and extracted with ether (3 × 5 mL). The combined extracts were washed with water (2 × 5 mL), dried (brine, Na_2SO_4), and evaporated. The residue was purified further by dry flash column chromatography (SiO_2, 5−45% tetrahydrofuran−dichloromethane) and preparative TLC (SiO_2 2% methanol−dichloromethane). Appropriate fractions were concentrated, taken up in 20% aqueous methanol (15 mL), and extracted with hexane (3 × 5 mL). The combined extracts were washed with water, dried (brine, Na_2SO_4), and evaporated to give 2-decarboxy-2-(*P*-butyl-*O*-ethylphosphinico)prostaglandin $F_{2\alpha}$ triacetate (11.7 mg, 36%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.47−5.57 (m, 2 H), 5.29−5.39 (m, 2 H), 5.18−5.25 (m, 1 H), 5.08 (t, J = 4.7 Hz, 1 H), 4.88 (ddd, J = 8.8, 7.5, 4.4 Hz, 1 H), 4.05 (dq, J = 7.1, 7.1 Hz, 2H), 2.47−2.60 (m, 2 H), 1.99−2.16 (m, 4 H), 2.06, 2.05, 2.02 (3 s, 3 H each), 1.49−1.75 (m, 12 H), 1.36−1.47 (m, 2 H), 1.19−1.35 (m, 6 H), 1.30 (t, J = 7.1 Hz, 3 H), 0.93 (t, J = 7.3 Hz, 3 H), 0.88 (t, J = 6.7 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 170.3, 170.2, 132.0, 131.5, 129.6, 128.22, 77.7, 74.3, 73.9, 59.9 (d, J = 6 Hz), 51.8, 47.4, 38.9, 34.3, 31.5, 28.1 (d, J = 16 Hz), 27.7 (d, J = 92 Hz), 27.6 (d, J = 91 Hz), 25.0, 24.8, 24.0 (d, J = 8 Hz), 23.9 (d, J = 4 Hz), 22.5, 21.3, 21.2, 21.0, 16.7 (d, J = 5 Hz), 14.0, 13.6; ^{31}P NMR δ 58.8; *m/z* (FAB) 607 (M + Na, 100%), 525 (80), 405 (35); Found (FAB) MNa^+ 607.3400, $C_{31}H_{53}O_8PNa$ requires MNa^+ 607.3376.

A mixture of 2-decarboxy-2-(*P*-butyl-*O*-ethylphosphinico)-prostaglandin $F_{2\alpha}$ triacetate (11.7 mg, 21 μmol), 95% ethanol (0.9 mL), and 2.5 M aqueous sodium hydroxide (0.9 mL, 2.3 mmol) was stirred at reflux for 2 h. The ethanol was evaporated, and the residue was diluted with water (7 mL) and washed with ethyl acetate (2 × 3 mL). The combined washes were extracted with water (1 mL). The combined aqueous phases were acidified with 2.5 M aqueous hydrochloric acid and extracted with ethyl acetate (4 × 5 mL). The combined extracts were dried (brine, Na_2SO_4) and evaporated. The residue was taken up in methanol (2 mL) and stirred with Amberilite CG-50 (Na^+ form, 0.45 g) for 30 min. The resin was removed by filration, and the filtrate was concentrated to give the sodium salt of 2-decarboxy-2-(*P*-butyphosphinico)prostaglandin $F_{2\alpha}$ (**15**, 7.0 mg, 74%). ^{1}H NMR (300 MHz, CD_3OD) δ 5.41−5.55 (m, 3 H), 5.31−5.39 (m, 1 H), 4.08 (td, J = 5.3, 2.2 Hz 1 H), 3.99 (q, J = 6.5 Hz, 1 H), 3.82 (ddd, J = 8.4, 7.4, 5.4 Hz, 1 H), 2.34 (ddd, J = 14.4, 8.6, 5.8 Hz, 1 H), 2.10−2.29 (m, 3 H), 2.10 (sept., J = 6.8 Hz, 2 H), 1.25−1.63 (m, 20 H), 0.92 (t, J = 7.1 Hz, 3 H), 0.91 (t, J = 7.2 Hz, 3 H); ^{13}C NMR (75 MHz, CD_3OD) δ 136.5, 134.3, 130.8, 130.0, 77.9, 74.0, 72.1, 56.2, 50.9, 44.3, 38.4, 33.0, 31.4 (d, J = 91 Hz), 31.3, (d, J = 91 Hz), 29.9 (d, J = 16 Hz), 26.4, 26.3 (d, J = 3 Hz), 26.2, 25.6 (d, J = 16 Hz), 24.2 (d, J = 3 Hz), 23.8, 14.4, 14.2; ^{31}P NMR δ 43.7; *m/z* (negative ion FAB) 429 (M − Na, 100), 329 (15); Found (FAB) (M+) 429.2749, $C_{23}H_{42}O_5P$ requires (M^+) 429.2770.

Sodium Salt of 2-Decarboxy-2-phosphinicoprostaglandin $F_{2\alpha}$ (13). A solution of 2-decarboxy-2-iodoprostaglandin $F_{2\alpha}$ triacetate (**3a**, 69.4 mg, 0.12 mmol) in dichloromethane (2.1 mL) was degassed through five freeze−pump−thaw cycles and was then placed under argon. Bis(trimethylsilyl) phosphonite (**Caution**! Pyrophoric) (2.1 mL, 2.3 mmol) was added, and the flask was sealed tightly and allowed to stand at room temperature for 25.5. h. The mixture was transferred, by means of a syringe, into saturated aqueous sodium hydrogen carbonate (15 mL) below the surface. The mixture was acidified with 2.5 M aqueous hydrochloric acid and stirred for 10 min, before being neutralyzed with saturated aqueous sodium hydrogen carbonate. The mixture was washed with ethyl acetate (4 × 7 mL). The combined washes were extracted with water (10 mL). The combined washed were dried (brine, Na_2SO_4) and evaporated to give unchanged starting material (40.2 mg, 58%). The combined aqueous phases were acidified with 2.5 M aqueous hydrochloric acid and extracted with ethyl acetate (5 × 10 mL). The combined extracts were dried (brine, Na_2SO_4) and evaporated to give 2-decarboxy-2-phosphinicoprostaglandin $F_{2\alpha}$ triacetate (14.5 mg, 23%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.89 (br s, 1 H), 7.15 (br d, J = 541.6 Hz, 1 H), 5.47−5.59 (m, 2 H), 5.29−5.41 (m, 2 H), 5.19−5.25 (m, 1 H), 5.09 (t, J = 4.6 Hz, 1 H), 4,89 (ddd, J = 8.9, 7.5, 4.4 Hz, 1 H), 2.49−2.61 (m, 2 H), 2.05−2.20 (m, 4 H), 2.06, 2.05, 2.02 (3 s 3 H each), 1.49−1.81 (m, 8 H), 1.23−1.34 (m, 6 H), 0.88 (t, J = 6.7 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 170.4, 170.3, 131.9, 131.6, 129.3, 128.5, 77.7, 74.3, 74.0, 51.8, 47.4, 38.9, 34.3, 31.5, 27.7 (br), 24.9, 24.8, 22.5 (br), 21.3, 21.2, 21.0, 20.4 (br), 14.0, signal for the carbon adjacent to P not observed; ^{31}P NMR δ 40.9; *m/z* (negative ion FAB) 499 (M − H, 100), 354 (5); Found (FAB) (M − H) 499.2484, $C_{25}H_{41}O_8P$ requires (M − H) 499.2461.

A mixture of 2-decarboxy-2-phosphinicoprostaglandin $F_{2\alpha}$ triacetate (14.5 mg, 29 μmol), methanol (1.2 mL), water (1.2 mL, and 1.08 M aqueous sodium hydroxide (0.2 mL, 0.17

mmol) was stirred at room temperature for 22 h. Amberilite CG-50 (H^+ form, 0.17 g) was added, and the mixture was stirred a further 1 h. The resin was removed by filtration, and the filrate was concentrated and reevaporated from acetonitrile (2 × 5 mL). The residue was taken up in methanol (3 mL) and stirred with Amberilite CG-50 (Na^+ form, 50 mg) for 30 min. The resin was removed by filtration, and the filtrate was concentrated to give the sodium salt of 2-decarboxy-2-phosphinicoprostaglandin $F_{2\alpha}$ (**9**, 12 mg, 100%). ^{1}H NMR (300 MHz, CD_3OD) δ 6.96 (dt, J = 487.1, 1.7 Hz), 5.42−5.55 (m, 3 H), 5.30−5.38 (m, 1 H), 4.08 (td, J = 5.3, 2.2 Hz 1 H), 3.99 (q, J = 6.4 Hz, 1 H), 3.83 (ddd, J = 8.5, 7.2, 5.3 Hz, 1 H), 2.03−2.37 (m, 6 H), 1.24−1.64 (m, 14 H), 0.90 (t, J = 6.9 Hz, 3 H); ^{13}C NMR (75 MHz, CD_3OD) δ 136.5, 134.2, 130.6, 130.2, 77.9, 73.9, 72.2, 56.2, 51.0, 44.3, 38.4, 33.4 (d, J = 91 Hz), 33.0, 29.4 (d, J = 17 Hz), 26.4, 26.3, 23.8, 23.2, 14.4; ^{31}P NMR δ 29.2; *m/z* (negative ion FAB) 373 (M − Na, 100%), 329 (25); Found (FAB) $(M + Na)^-$ 373.2142, $C_{19}H_{34}NaO_5P$ requires $(M + Na)^-$ 373.2144.

Sodium Salt of 2-Decarboxy-2-(P-methylphosphinico)-16-phenoxytetranorprostaglandin $F_{2\alpha}$ (4b). A mixture of 16-phenoxytetranorprostaglandin $F_{2\alpha}$ (**1b**, 81 mg, 208 μmol), DMAP (5 mg, 41 μmol), dichloromethane (1.9 mL), triethylamine (1.2 mL, 8.5 mmol), and acetic anhydride (0.70 mL, 7.3 mmol) was stirred at room temperature for 9.5 h and was then stored in a freezer overnight. The mixture was concentrated, and the residue was diluted with saturated aqueous sodium carbonate (12 mL) and stirred at room temperature for 3 h. The mixture was acidified with 1 M aqueous hydrochloric acid and was then extracted with ethyl acetate (5 × 10 mL). The combined extracts were dried (brine, Na_2SO_4) and evaporated to give 16-phenoxytetranorprostaglandin $F_{2\alpha}$ triacetate (**2b**, 95.6 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.0 (br s, 1 H), 7.28 (td, J = 8.6, 7.3 Hz, 2 H), 6.96 (t, J = 7.3 Hz, 1 H), 6.90 (d, J = 8.6 Hz, 2 H), 5.67−5.74 (m, 2 H), 5.59−5.65 (m, 1 H), 5.30−5.49 (m, 2 H), 5.11 (t, J = 4.5 Hz, 1 H), 4.92 (ddd, J = 8.8, 7.4, 4.3 Hz, 1 H), 4.08 (dd, J = 10.2, 6.0 Hz 1 H), 4.03 (dd, J = 10.2, 4.4 Hz, 1 H), 2.58−2.64 (m, 1 H), 2.54 (ddd, J = 15.9, 8.9, 5.7 Hz, 1 H), 2.31 (t, J = 7.3 Hz, 2 H), 2.11, 2.07, 2.02 (3 s, 3 H each), 1.96−2.16 (m, 4 H), 1.59−1.74 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.5, 170.6, 170.4, 170.2, 158.3, 134.1, 129.8, 129.4, 127.8, 127.4, 121.1, 114.6, 77.6, 74.2, 71.9, 68.9, 51.9, 47.3, 38.8, 31.1, 26.3, 24.7, 24.3, 21.1, 21.0, 20.9.

Oxalyl choride (80 μL, 0.92 mmol) was added to a solution of 16-phenoxytetranorprostaglandin $F_{2\alpha}$ triacetate (**12**, 50.6 mg, 98 μmol) and dimethylformamide (0.5 μL, 6.3 μmol) in dichloromethane (0.20 mL). The mixture was allowd to stand at room temperature for 30 min and was then concentrated. The residue was taken up in dichloromethane (0.4 mL). Meanwhile, a mixture of the sodium salt of *N*-hydroxypyridine-2-thione (16 mg, 0.11 mmol), DMAP (0.8 mg, 6.5 μmol), and dichloromethane (0.5 mL) under argon was brought to reflux by irradiation with a 250 W General Electric floodlamp. To this mixture was added 1,1,1-trifluoro-2-iodoethane (0.10 mL, 1.0 mmol) followed by the solution of acid chloride which was added over 15 min. Irradiation was continued for a further 45 min and the mixture was concentrated and purified by dry-flash column chromatography (SiO_2, 5−45% ethyl acetate−hexane) to give 2-decarboxy-2-iodo-16-phenoxytetranorprostaglandin $F_{2\alpha}$ triacetate (**3b**, 40.2 mg, 69%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (td, J = 8.6, 7.2 Hz, 2 H), 6.97 (t, J = 7.2 Hz, 1 H), 6.90 (d, J = 8.6 Hz, 2 H), 5.67−5.74 (m, 2 H), 5.57−5.64 (m, 1 H), 5.26−5.41 (m, 2 H), 5.11 (t, J = 4.6 Hz, 1 H), 4.94 (ddd, J = 8.6, 7.6, 4.3 Hz, 1 H), 4.10 (dd, J = 10.2, 6.1 Hz, 1 H), 4.04 (dd, J = 10.2, 4.7 Hz, 1 H), 3.15 (t, J = 6.7 Hz, 2 H), 2.59−2.67 (m, 1 H), 2.54 (ddd, J = 15.9, 8.9, 5.7 Hz, 1 H), 2.10, 2.09, 2.02 (3 s, 3 H each) 1.99−2.21 (m, 4 H), 1.64−1.87 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 170.4, 170.0, 158.4, 134.3, 129.5, 128.9, 128.5, 127.7, 121.2, 114.7, 77.6, 74.3, 72.0, 69.0, 52.0, 47.5, 38.9, 33.0, 27.8, 25.1, 21.3, 21.2, 21.0, 6.7; *m/z* (DEI) 598 (M, 5%), 581 (10), 538 (65), 522 (25), 505 (30), 478 (30); Found (DEI) $M^{+\cdot}$ 598.1425, $C_{27}H_{35}IO_7$ requires $M^{+\cdot}$ 598.1428.

A mixture of 2-decarboxy-2-iodo-16-phenoxytetranorprostaglandin $F_{2\alpha}$ triacetate (**3b**, 18.2 mg, 30 μmol), diethyl methylphosphonite (0.1 mL, 0.66 mmol), and toluene (0.2 mL) was stirred at 100° C for 7 h. The solvents were evaporated and the residue was purified by dry-flash column chromatography (SiO_2, 5−25% 2-propanol in 20% dichloromethane−hexane). Appropriate fractions were concentrated, diluted with water (15 mL), and extracted with ethyl acetate (3 × 5 mL). The combined extracts were washed with water (10 mL), dried (brine, Na_2SO_4), and evaporated to give 2-decarboxy-2-(*O*-ethyl-*P*-methylphosphinico)-16-phenoxytetranorprostaglandin $F_{2\alpha}$ triacetate (10.8 mg, 61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (td, J = 8.6, 7.3 Hz, 2 H), 6.97 (t, J = 7.3 Hz, 1 H), 6.90 (d, J = 8.6 Hz, 2 H), 5.65−5.73 (m, 2 H), 5.57−5.62 (m, 1 H), 5.29−5.49 (m, 2 H), 5.09 (t, J = 4.6 Hz, 1 H), 4.92 (ddd, J = 8.7, 7.5, 4.3 Hz, 1 H), 4.08 (dd, J = 10.2, 6.1 Hz, 1 H), 4.04 (dq, J = 7.1, 7.1 Hz, 2 H), 4.02 (dd, J = 10.2, 4.6 Hz, 1 H), 2.56−2.65 (m, 1 H), 2.53 (ddd, J = 15.8, 9.02, 5.7 Hz, 1 H), 2.10, 2.06, 2.01 (3 s, 3 H each), 1.99−2.21 (m, 4 H), 1.56−1.74 (m, 6 H), 1.42 (d, J = 13.4 Hz, 3 H), 1.11 (t, J = 7.0 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 170.3, 170.0, 158.4, 134.2, 129.6, 129.5, 128.2, 127.7, 121.2, 114.7, 77.6, 74.3, 71.9, 69.0, 59.9 (d, J = 6 Hz), 52.0, 47.4, 38.9, 29.3 (d, J = 93 Hz), 28.1 (d, J = 16 Hz), 25.0, 22.0 (br), 21.2, 21.1, 21.0, 16.6 (d, J = 6 Hz), 13.7 (d, J = 91 Hz); ^{31}P NMR δ 55.3; *mz* (FAB) 579 (M + H, 100), 519 (90), 399 (50); Found (FAB) MH^+ 579.2746, $C_{30}H_{43}O_9P$ requires MH^+ 579.2723.

A mixture of 2-decarboxy-2-(*O*-ethyl-*P*-methylphosphinico)-16-phenoxytetranorprostaglandin $F_{2\alpha}$ triacetate (9.9 mg, 17 μmol), 95% ethanol (0.7 mL), and 2.5 M aqueous sodium hydroxide (0.7 mL, 1.7 mmol) was stirred at reflex for 3 h. The mixture was diluted with water (6 mL) and washed with ethyl acetate (2 × 3 mL). The combined washes were extracted with water (2 mL). The combined aqueous phases were acidified with 1 M aqueous hydrochloric acid and extracted with ethyl acetate (4 × 5 mL). The combined extracts were dried (brine, Na_2SO_4) and evaporated. The residue was taken up in methanol (2 mL) and stirred with Amberilite CG-50 (Na^+ form, 0.3 g) for 15 min. The resin was removed by filtration, and the filtrate was concentrated to give the sodium salt of 2-decarboxy-2-(*P*-methylphosphinico)-16-phenoxytetranorprostaglandin $F_{2\alpha}$ (**4b**, 4.4 mg, 58%). ^{1}H NMR (300 MHz, CD_3OD) δ 7.22−7.28 (m, 2 H), 6.87−6.95 (m, 3 H), 5.63−5.72 (m, 2 H), 5.41−5.51 (m, 1 H), 5.29−5.37 (m, 1 H), 4.44 (ddd, J = 7.3, 3.7, 1.2 Hz, 1 H), 4.09 (td, J = 5.2, 2.0 Hz, 1 H), 3.95 (dd, J = 9.7, 4.7 Hz, 2 H), 3.92 (dd, J = 9.7, 6.6 Hz, 1 H), 3.86 (td, J = 7.9, 5.5 Hz, 1 H), 2.02−2.39 (m, 6 H), 1.34−1.63 (m, 6 H), 1.14 (d, J = 13.2 Hz, 3 H); ^{31}P NMR δ −43.4; *m/z* (ESI) 425.3 (M + H); Found (FAB) $(M + Na)^+$ 447.1927, $C_{22}H_{32}NaO_6P$ requires $(M + Na)^+$ 447.1912. Anal. Calcd for $C_{22}H_{33}O_6P\cdot H_2O$: C, 59.72; H, 7.97. Found: C, 59.83; H, 8.21.

Alternative Preparation of 2-Decarboxy-2-(P-methylphosphinico)-16-phenoxytetranorprostaglandin $F_{2\alpha}$ (4b). 5-(tert-Butyldimethylsilanyloxy)-4-[3-(tert-butyldimethylsilanyloxy)-4-phenoxybut-1-enyl]hexahydrocyclopenta[b]furan-2-ol (6). In a round-bottom flask at 0 °C was placed 3.5 g (11.5 mmol) of 16-phenoxy Corey lactone diol **(5)** in 80 mL of dichloromethane. To this was added 10.0 mL (86.4 mmol) of 2,6-lutidine and 10.0 mL (43.5 mmol) of TBDMS triflate; the reaction was allowed to warm to room temperature and then stirred overnight. The solution was added to 150 mL of dichloromethane and washed with 1 N HCl (2×) and brine. The organics were dried with $MgSO_4$ and reduced. The residue was chromatographed on SiO_2 gel using 5% EtOAc−hexane as the eluent. Appropriate fractions were combined and evaporated giving 5.58 g of the bis-silyl ether (91%) as a white solid. ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.31 (m, 2 H), 6.96 (t, J = 7.3 Hz, 1 H), 6.89 (m, 2 H), 5.65 (m, 2 H), 4.96 (dt, J = 2.5, 6.9 Hz, 1 H), 4.52 (m, 1H), 4.01 (q, J = 5.5 Hz, 1 H), 3.85 (d, J = 6.8 Hz, 2 H), 2.61−2.82 (m, 3 H), 2.46−2.53 (m, 2 H), 2.26−2.38 (m, 2 H), 2.00 (ddd, J = 2.6, 5.5, 14.7 Hz, 1 H), 0.89 (s, 9 H), 0.85 (s, 9 H), 0.08 (s, 3 H), 0.07 (s, 3 H), 0.03 (s, 3 H), 0.00 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 181.8, 163.5, 136.8, 135.7, 134.3, 125.6, 119.2, 88.0, 81.5, 76.9, 76.1, 61.6, 47.1, 45.5, 39.7,

30.7, 30.6, 23.2, 22.8, 1.9, 0.2, 0.1, 0.0; M/S *m/z* (ESI) 550.3 ($M + NH_4$); Found (FAB) MH^+ 533.3138, $C_{29}H_{49}O_5Si_2$ requires MH^+ 533.3119; Anal. Calcd for $C_{29}H_{48}O_5Si_2$: C, 65.37; H, 9.08. Found: C, 65.25; H, 9.09.

In a flame dried round-bottom flask at −78 °C under argon was placed 5.40 g of the bis-silyl ether (10.2 mmol) in 50 mL of anhydrous dichloromethane. To this was added 13.2 mL (13.2 mmol) of 1 M DiBAL in dichloromethane. The solution was stirred for 2 h and then quenched with $NaHCO_3$(aq). The organics were washed quickly with 1 N HCl (2 × 50 mL) and brine. By drying the organics over Na_2SO_4 and evaporating the solvent, the crude lactol (**6**) was used immediately. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (m, 2 H), 6.89−6.99 (m, 3 H), 5.64 (m, 2 H), 4.52 (m, 1 H), 4.02−4.28 (m, 1 H), 3.69−3.92 (m, 4 H), 1.62−2.37 (m, 7 H), 0.93−0.97 (m, 18 H), 0.05−0.18 (m, 12 H); *m/z* (ESI) 517.2 ($M + H - H_2O$).

6-{3,5-Bis(tert-butyldimethylsilanyloxy)-2-[3-(tert-butyldimethylsilanyloxy)-4-phenoxybut-1-enyl]cyclopentyl}-hex-4-enoic Ester (7). In a flame dried round-bottom flask, 9.57 g of (3-carboxypropyl)triphenylphosponium bromide (22.3 mmol) was dissolved in 80 mL of anhydrous THF under argon at −78 °C. A 44.7 mL aliquot of 1 M sodium bis(trimethylsilyl)-amide in THF (44.7 mmol) was added in one portion, and the solution was allowed to warm to 0 °C over 2 h. The solution is recooled to −78 °C, and lactol **6** in 10 mL of anhydrous THF is added over 10 min. The reaction is allowed to warm to room temperature and is stirred overnight. The reaction is quenched with water and stirred for 15 min. A 150 mL aliquot of EtOAc is added, and the solution is reduced to one-third the volume. The solution is taken up in 1:1 EtOAc−hexane (150 mL) and washed 3× with 1 N HCl (100 mL) and brine and then dried over $MgSO_4$. The solvent was removed, and the residue was taken up in methanol. The solution was treated with TMS diazamethane until complete esterification by TLC (10% EtOAc/hexane). The methanol was evaporated, and the residue was chromatographed on SiO_2 gel (10% EtOAc/hexane) A small portion of the C_9–C_{15} and C_{11}–C_{15} bis-silyl ether mixture was separated. The total combined yield was 5.58 g (88%) of colorless oil.

Less polar fraction: Rf 0.40 in 10% EtOAc/hexane. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (t, J = 7.4 Hz, 2 H), 6.96 (m, 1 H), 6.91 (m, 2 H), 5.68 (m, 2 H), 5.33−5.49 (m, 2 H), 4.52 (overlapping dt, J = 4.9, 5.5 Hz, 1 H), 4.28 (m, 1 H), 3.93 (m, 1 H), 3.89 (d, J = 5.9 Hz, 2 H), 3.69 (s, 3 H), 2.57 (d, J = 9.1 Hz, 1 H), 2.42 (m, 2 H), 2.36 (m, 3 H), 2.25 (t, J = 6.9 Hz, 2 H), 2.04 (m, 1 H), 1.82 (m, 1 H), 1.55 (m, 1 H), 0.95 (s, 9 H), 0.94 (s, 9 H), 0.14 (s, 3 H), 0.13 (s, 6 H), 0.11 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.8, 159.1, 133.8, 130.8, 130.2, 129.7, 128.3, 120.9, 114.7, 79.0, 74.9, 72.5, 72.0, 57.0, 51.8, 51.7, 43.6, 34.2, 26.4, 26.1, 23.1, 18.6, 18.2, −4.1, −4.3, −4.4, −4.8; M/S *m/z* 618 (M + H) 619.3.

More polar fraction: Rf 0.30 in 10% EtOAc−hexane. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (m, 2 H), 6.95 (m, 1 H), 6.91 (m, 2 H), 5.65 (m, 2 H), 5.34−5.53 (m, 2 H), 4.55 (m, 1 H), 4.14 (m, 1 H), 4.04 (m, 1 H), 3.87 (d, J = 5.9 Hz, 2 H), 3.67 (s, 3 H), 2.79 (d, J = 8.1 Hz, 1 H), 2.43 (m, 4 H) 2.39 (t, J = 5.9 Hz, 2 H), 2.18 (m, 1 H), 2.04 (m, 2 H), 1.82 (d, J = 4.3 Hz, 1 H), 1.53 (sept, J = 4.8 Hz, 1 H), 0.95 (s, 9 H), 0.91 (s, 9 H), 0.13 (s, 6 H), 0.08 (s, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9, 159.1, 133.4, 130.9, 130.2, 129.6, 128.5, 120.9, 114.7, 79.6, 73.9, 72.6, 71.8, 56.3, 51.7, 51.3, 43.5, 34.1, 26.5, 26.1, 22.9, 18.6, 18.2, −4.3, −4.4, −4.5; M/S *m/z* 618 (M + H) 619.2.

In a round-bottom flask at 0 °C was placed 5.5 g (8.88 mmol) of the mixture of silyl ethers in 60 mL of dichloromethane. To this was added 10.0 mL (86.4 mmol) of 2,6-lutidine and 10.0 mL (43.5 mmol) of TBDMS triflate; the reaction was allowed to warm to room temperature and then stirred overnight. The solution was added to 100 mL of dichloromethane and washed with 1 N HCl (2×) and brine. The organics were dried with $MgSO_4$ and reduced. The residue was chromatographed on SiO_2 gel using 5% EtOAc−hexane as the eluent. Appropriate fractions were combined and evaporated giving 5.97 g of **7** (92%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (m, 2 H), 6.96 (m, 1 H), 6.89 (m, 2 H), 5.66 (m, 2 H), 5.49 (m, 1 H), 5.33 (m, 1 H), 4.58 (m, 1 H), 4.14 (m, 1 H), 3.88 (m, 2 H), 3.68 (s, 3 H), 3.46 (m, 1 H), 2.35 (m, 3 H) 2.24 (m, 1 H), 2.18 (m, 2 H), 1.62 (ddd, J = 2.9, 5.2, 13.9 Hz, 1 H) 1.44 (m, 1 H), 0.95 (s, 9 H), 0.94 (s, 9 H), 0.90 (s, 9 H), 0.14 (s, 3 H), 0.14 (s, 3 H), 0.09 (s, 3 H), 0.09 (s, 3 H), 0.06 (s, 3 H), 0.04 (s, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.8, 159.1, 133.5, 131.6, 130.6, 129.6, 127.9, 120.8, 114.6, 77.54, 72.8, 72.0, 71.7, 55.2, 51.7, 49.9, 45.1, 34.3, 26.1, 25.2, 23.2, 18.6, 18.3, −3.9, −4.2, −4.2, −4.4, −4.7; *m/z* (ESI) 750.4 ($M + NH_4$); Found (FAB) $(M + Na)^+$ 755.4522, $C_{40}H_{72}NaO_6Si_3$ requires $(M + Na)^+$ 755.4534.

(6-{3,5-Bis(tert-butyldimethylsilanyloxy)-2-[3-(tert-butyldimethylsilanyloxy)-4-phenoxybut-1-enyl]cyclopentyl}-hex-4-enyl)methylphosphinic Acid Ethyl Ester (8). In a round-bottom flask under argon at −78 °C was placed 5.80 (7.92 mmol) of **7** in 80 mL of THF. A 10.3 mL aliquot of 1 M Lithium aluminum hydride (10.3 mmol) was added in one portion. The solution was stirred for 1 h at −78 °C and then quenched with water. Dichloromethane was added (100 mL), and the solution was acidified to pH = 1. The aqueous layer is extracted 3× with dichloromethane, and the combined organics were dried over $MgSO_4$. The solvent was removed, and the residue was chromatographed on SiO_2 gel using 5% EtOAc−hexane. Appropriate fractions were combined giving 5.29 g of the alcohol (95%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (m, 2 H), 6.99 (m, 1 H), 6.90 (m, 2 H), 5.65 (m, 2 H), 5.49 (m, 1 H), 5.40 (m, 1 H), 4.57 (m, 1 H), 4.14 (m, 1 H), 3.88 (m, 3 H), 3.64 (overlapping dt, J = 5.5, 6.2 Hz, 2 H), 2.45 (m, 1 H), 2.24 (m, 1 H), 2.15 (m, 3 H), 1.64 (m, 1 H), 1.64 (t, J = 6.9 Hz, 2H), 1.46 (m, 1H), 1.40 (t, J = 5.5 Hz, 1H), 0.95 (s, 9H), 0.94 (s, 9H), 0.90 (s, 9H), 0.15 (s, 6H), 0.09 (s, 3 H), 0.08 (s, 3 H), 0.05 (s, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.6, 131.5, 129.8, 129.7, 129.5, 77.6, 72.8, 72.0, 71.7, 62.8, 55.1, 49.9, 45.0, 32.8, 26.1, 25.3, 24.0, 18.3, −3.9, −4.2, −4.3; M/S *m/z* 704 (M + NH_4) 722.4; Found (FAB) $(M + Na)^+$ 727.4594, $C_{39}H_{72}NaO_5Si_3$ requires $(M + Na)^+$ 727.4585.

In a flame dried round-bottom flask under argon is placed 5.2 g (7.38 mmol) of the alcohol, 3.11 g (10.3 mmol) of dibromotriphenylphosphorane, and 0.60 mL (11.8 mmol) of pyridine in 40 mL of anhydrous acetonitrile. The solution was monitored by TLC until complete. The mixture was then poured with stirring into 400 mL of 5% EtOAc−hexane. A precipitate formed and was filtered through a 6 in. plug of SiO_2 gel with additional 5% EtOAc−hexane to wash. The solvent is removed, giving 5.39 g (95%) of the bromide as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 2 H), 6.97 (t, J = 7.4 Hz, 1 H), 6.90 (m, 2 H), 5.67 (m, 2 H), 5.51 (m, 1 H), 5.31 (m, 1 H), 4.58 (m, 1 H), 4.16 (overlapping dt, J = 2.2, 5.1 Hz, 1 H), 3.89 (m, 3 H), 3.40 (t, J = 6.6 Hz, 2 H), 2.47 (m, 1 H), 2.25 (m, 1 H), 2.19 (m, 2 H), 1.92 (qnt, 6.9 Hz, 2 H), 1.63 (ddd, J = 2.6, 5.1 14.3 Hz, 1 H), 1.45 (s, 1 H), 0.96 (s, 9 H), 0.95 (s, 9 H), 0.91 (s, 9 H), 0.15 (s, 6 H), 0.10 (s, 3 H), 0.08 (s, 3 H), 0.05 (s, 6 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.1, 133.5, 131.6, 130.8, 129.7, 127.9, 120.8, 114.6, 77.6, 72.8, 72.0, 71.7, 55.2, 49.9, 45.1, 335, 32.9, 26.2, 25.4, 18.6, 18.3, −3.9, −4.2, −4.3, −4.7; *m/z* (ESI) 767.3 ($M + H^+$); Found (FAB) $(M + H)^+$ 767.3955, $C_{39}H_{71}O_4BrSi_3$ requires $(M + H)^+$ 767.3922. Anal. Calcd for $C_{39}H_{71}O_4BrSi_3$: C, 60.98; H, 9.32. Found: C, 58.88; H, 8.37.

In a flame dried 200 mL high-pressure flask was added 30 mL of toluene and 20 mL (excess) of diethoxy methylphosphinite. The solution was purged with argon for 5 min, and 4.90 g (6.40 mmol) of the bromide was added. The flask was sealed and heated to 145 °C for 22 h. The mixture was evaporated to a thick oil, which was placed on a SiO_2 column and eluted with 1:20:0.1 MeOH/dichloromethane/acetic acid. The appropriate fractions were combined and reduced to give 4.99 g (98%) of compound **8** as a glassy oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (t, J = 7.6 Hz, 2 H), 6.94 (t, J = 7.3 Hz, 1 H), 6.87 (d, J = 8.1 Hz, 1 H), 5.64 (m, 2 H), 5.50 (m, 1 H), 5.29 (m, 1 H), 4.56 (bs, 1 H), 4.13 (m, 1 H), 3.86 (m, 3 H), 2.44 (m, 1 H), 2.20 (m, 2 H), 2.11 (m, 2 H), 1.73 (m, 2 H), 1.63 (m, 2 H), 1.46 (d, J = 13.5 Hz, 3 H), 1.32 (t, J = 7.0 Hz, 3 H), 1.27 (m, 1 H), 0.94 (s, 9 H), 0.93 (s, 9 H), 0.89 (s, 9 H), 0.13 (s, 6 H), 0.08 (s, 3 H), 0.05 (s, 3 H), 0.03 (s, 6 H); ^{13}C

NMR (75 MHz, $CDCl_3$) δ 175.0, 159.1, 133.5, 131.6, 130.6, 129.4, 128.4, 120.8, 114.6, 77.6, 72.7, 71.9, 71.6, 60.7, 60.6, 55.2, 48.9, 45.0, 29.8, 28.5, 28.3, 26.1, 25.3, 22.1, 21.2, 18.6, 18.3, 16.8, 16.8, 16.7, 14.2, 13.0, −3.9, −4.2, −4.2, −4.3, −4.7; MS *m/z* 795 (M + Na) 818.4.

2-Decarboxy-2-(P-methylphosphinico)-16-phenoxytetranorprostaglandin $F_{2\alpha}$ (4b). In a round-bottom flask under argon at 0 °C was added 4.82 g (6.06 mmol) of **8** and 18 mL of HF/pyridine in anhydrous acetonitrile. The solution was allowed to warm to room temperature and stirred for 5 h. The solvent was removed, and the residue was taken up in methanol and dichloromethane. The solution was placed on a SiO_2 column and eluted with 1:20:0.1 MeOH:dichloromethane: acetic acid. Appropriate fractions were collected and evaporated to give 2.31 g (84%) of the triol as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (m, 2 H), 6.98 (m, 1 H), 6.94 (m, 2 H), 5.73 (m, 2 H), 5.32−5.52 (m, 2 H), 4.54 (m, 1 H), 4.16 (m, 1 H), 4.04 (m, 2 H), 4.98 (m, 3 H), 3.14 (bs, 3 H), 2.35 (m, 3 H), 2.15 (m, 3 H), 1.82 (m, 1 H), 1.74 (m, 3 H), 1.53 (m, 1 H), 1.46 (d, J = 13.6 Hz, 3 H), 1.34 (t, J = 6.9 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.9, 135.1, 130.2, 129.8, 129.3, 121.3, 114.9, 78.2, 72.9, 72.1, 71.0, 60.5, 56.2, 50.8, 43.4, 29.6, 28.2, 25.8, 22.3, 16.8, 14.7, 13.5; MS *m/z* 452 (M + H) 453.3.

In a round-bottom flask was placed 1.7 g (3.76 mmol) of the triol and 20 mL of 2.5 N sodium hydroxide in 40 mL of 1:1 ethanol:water. The solution was refluxed for 3 h, and then solvent was removed. The residue was diluted with 20 mL of water. The aqueous layer was extracted 2× with 20 mL of EtOAc, and the organic layer was discarded. The aqueous layer was then acidified to pH = 1 and extracted 4× with EtOAc (30 mL). Organics were combined and quickly washed with brine (20 mL), followed by drying over Na_2SO_4. The solvent was evaporated and placed on high vacuum to dry. A total of 1.43 g (90%) of 2-decarboxy-2-(*P*-methylphosphinico)-16-phenoxytetranorprostaglandin $F_{2\alpha}$ (**4b**) was isolated as a colorless oil. ^{1}H NMR (300 MHz, CD_3OD) δ 7.22−7.28 (m, 2 H), 6.87−6.95 (m, 3 H), 5.63−5.72 (m 2 H), 5.41−5.51 (m, 1 H), 5.29−5.37 (m, 1 H), 4.46 (ddd, J = 7.3, 3.7, 1.2 Hz, 1 H), 4.09 (td, J = 5.2, 2.0 Hz, 1 H), 3.95 (dd, J = 9.7, 4.7 Hz, 1 H), 3.92 (dd, J = 9.7, 6.6 Hz, 1 H), 3.86 (td, J = 7.9, 5.5 Hz, 1 H), 2.02−2.39 (m, 6 H), 1.34−1.63 (m, 6 H), 1.14 (d, J = 13.2 Hz, 3 H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.2, 159.2, 134.7, 131.3, 129.8, 129.5, 128.8, 120.9, 114.6, 76.6, 71.9, 71.2, 70.9, 55.0, 49.5, 43.2, 30.2, 28.9, 28.1, 27.9, 25.2, 22.1, 22.0, 19.9, 14.3, 13.0; ^{31}P NMR δ −43.4; *m/z* (ESI) 425.3 (M + H); Found (FAB) $(M + Na)^+$ 447.1927, $C_{22}H_{32}NaO_6P$ requires $(M + Na)^+$ 447.1912. Anal. Calcd for $C_{22}H_{33}O_6P\cdot H_2O$: C, 59.72; H, 7.97. Found: C, 59.83; H, 8.21.

In Vivo Assays. Seventy-two female 3-month-old Sprague−Dawley rats were acclimated to local vivarium conditions (Simonsen Laboratories, Gilroy, GA). They were pair-fed in cages with the room temperature maintained at 72 °F and 12: 12 light/dark cycles. The rats were allowed free access to water and pelleted commercial natural diet (Teklad Rodent Laboratory Chow no. 8604, Harlan Teklad, Madison, WI) that contains 1.46% calcium, 0.99% phosphorus, and 4.96 IU/g of vitamin D_3. At 6 months of age, the rats were divided into 10 body-weight-matched groups with 6−8 rats per group. One group was killed as baseline control (Basal); the others were sham or bilateral ovariectomized. After 60 days of operation, pretreatment sham (60-d sham) or ovariectomized (60-d OVX) animals (6 per group) were euthanized as additional pretreatment controls. The remaining rats were treated daily with 0.01, 0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg methyl phosphinic acid analogue for 60 days or with vehicle of acetate buffers physiologic saline, methylcellulose, and polyoxyethylenesorbitan monooleate. A group was injected with 6 mg/kg/day of PGE2 (Cayman Chemicals, Ann Arbor, MI) to serve as positive control. All the rats received 90 mg/kg of Xylenol orange before treatments and 10 mg/kg of Calcein (Sigma Chemical Co., St. Louis, MO) 14 and 4 days before sacrifice. At necropsy the final sham (120-d sham) and ovariectomized (120-d OVX) vehicle-treated and methyl phosphinic acid analogue-treated rats were anesthetized by an intraperitoneal injection of Ketamine (50 mg/kg) and Xylazine (10 mg/kg) and sacrificed by cardiac puncture. Changes of bone mass were measured in the tibia by bone histomorphometry; in the femur by dual-energy X-ray absorbtiometer (DEXA, Hologic QDR-2000 plus bone densitometer. Hologic, Inc., Waltham, MA) and in the fifth lumbar vertebra by microcomputed tomography system (μCT 20, serial no. 96-2004, Scanco Medical, AG).

(a) Bone Histomorphometry. The proximal tibiae and the middle third of the right tibiae were stained with Villanueva bone stain, dehydrated in graded concentrations of ethanol, defatted in acetone, and embedded in methyl methacrylate (Fisher Scientific, Fairlawn, NJ). Longitudinal sections of proximal tibiae (PT) and cross-sections at the tibiofibular junction of the tibial shafts (TX) were cut to 230 μm thickness using a low-speed metallurgical saw and then ground to 20 μm (PT) and 30 μm (TX) for histomorphometric measurements. Histomorphometry was done using a semiautomatic image analysis system (OsteoMeasure, OsteoMetrics Inc., Decatur, GA) linked to a microscope equipped with transmission and fluorescent light.

The region of the proximal tibial metaphysis that was studied was from 1 to 4 mm distal to the growth plate−metaphyseal junction. Static measurements included total tissue area, bone area, and bone perimeter. Dynamic measurements included single and double-labeled perimeter, eroded perimeter, and interlabel width. These indices were used to calculate percent bone volume per tissue volume, trabecular number, trabecular thickness, percent eroded surface, mineral apposition rate, percent mineralizing surface, and bone formation rate per unit of either bone volume or total tissue volume.[32] Cortical bone measurements included total cross-sectional area, marrow area, eroded perimeter, single- and double-labeled perimeter, and interlabeled width. These parameters were used to calculate the percent mineralizing surface, eroded surface, and mineral apposition rate of the periosteal and endocortical bone surfaces.[33]

(b) Microcomputed Tomography. The fifth lumbar vertebral bodies were removed from all animals and were cleaned of soft tissue. The processes were removed and the vertebral bodies placed in 70% ethanol. Each lumbar vertebral body was imaged using a microcomputed tomography system (μCT 20, serial no. 96-2004, Scanco Medical AG) by stacking at least five vertebrae in a single holder. The caudal end of the vertebra was placed on the left side of the holder alignment line to aid in consistent positioning of the bone. A sponge material moistened with 70% ethanol, which acts to secure vertebra in position and keep the sample moist, separated the samples. Image acquisition parameters for the vertebra included standard resolution (300 projections), 26 μm slice increment, and 150 ms integration time. Approximately 186 slices were scanned per vertebra. Once acquisition was complete, the images were sent to a SGI Octane Workstation for all subsequent analyses. The image analysis involved the following: (a) setting threshold of the images to bone and background; (b) determining of the volume of interest (VOI); (c) separating of the cortical from the trabecular bone; and (d) measuring of structural parameters (5). Measurements made on the 3D datasets included trabecular bone volume, surface area, the derived trabecular thickness and trabecular number according to Parfitt using the plate model,[32] and the directly measured connectivity density and trabecular thickness.[29]

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