Novel Synthesis of Bis(phosphonic acid)−Steroid Conjugates

Philip C. B. Page,*,† Michael J. McKenzie,† and James A. Gallagher‡

Department of Chemistry, Loughborough University, Loughborough, Leicestershire LE11 3TU, England, and Department of Human Anatomy and Cell Biology, University of Liverpool, Liverpool L69 3GE, England

Received October 17, 2000

An efficient synthesis has been realized for several members of a new class of potential bone resorption inhibitors consisting of steroidal oestrogenic units linked at the 3 and 17 positions to a geminal bisphosphonate moiety through an ester linkage of variable length. The convergent synthesis utilizes benzyl bisphosphonates, transesterification, and Meldrum's acid chemistry and has the potential to allow many oestrogenic derivatives as well as other biologically active compounds to be coupled to the geminal bisphosphonate moeity.

Introduction

Geminal bisphosphonates are stable pyrophosphate analogues which bind efficiently to bone surface.[1] They have been used in the clinic to inhibit bone resorption, in most cases with few side effects.[2] Some simple examples, such as etidronate and alendronate, are already marketed for the treatment of osteoporosis and Paget's disease.[3] It has been suggested that bisphosphonates are general metabolic inhibitors.[4] The low level of side effects has been attributed to the rapid absorption of the geminal bisphosphonate to the bone surface and incorporation within the bone matrix, thereby preventing undesired effects within other organs.[2] Other clinically used treatments for osteoporosis, such as hormone replacement therapy, are effective, but can have effects on other tissues.[5] We are interested in using geminal bisphosphonates as bone-targeting moieties for the treatment of bone-related disease;[6] our aim is to use the bone tropism to deliver therapeutics at the desired site of action, thus reducing unwanted side effects in other tissues. We report here a new synthesis of geminal bisphosphonic acids functionalized with derivatives of oestradiol, an osteoclast inhibitor known to regulate the circuitry of cytokine action that controls bone remodeling.[7]

Discussion

We have recently reported the use of tetrabenzyl bisphosphonates as synthetically useful precursors of bisphosphonic acids, the conversion being experimentally simple, clean, and high yielding.[8] Bis(phosphonic acid)s are typically produced from tetraalkyl bisphosphonate esters either by acid hydrolysis[9] or through silylation−dealkylation using bromotrimethylsilane.[10] While acid hydrolysis is reliable it is often incompatible with other functionalities. Silylation−dealkylation is a milder method;

† Loughborough University.

‡ University of Liverpool.

(1) Geddes, A. D.; D'Souza, S. M.; Ebetino, F. H.; Ibbotson, K. *J. Bone Miner. Res.* **1994**, *9*, 265. Geddes, A. D.; Bevan, J. A.; Kasting, G. B.; Francis, M. D.; Mosekilde, L.; Hasling, C. *J. Bone Miner. Res.* **1992**, *7*, S328. Storm, T.; Thamsborg, G.; Steiniche, T.; Genant, H. K.; Sorensen, O. H. *New Engl. J. Med.* **1990**, *332*, 1265; Fleisch, H. *Bone* **1999**, *25*, 158. Vitte, C.; Fleisch, H.; Guenther, H. L. *Endocrinology* **1996**, *137*, 2324. Fleisch, H. *Osteoporosis Int.* **1996**, *6*, 166.

(2) Fleisch, H. *Br. J. Clin. Pract.* **1994**, *48*, 323. Body, J. J.; Bartl, R.; Buckhardt, P.; Delmers, P. D.; Fleisch, H.; Kanis, J. A.; Kyle, R. A.; Mundy, G. R.; Paterson, A. H. G.; Rubens, R. D. *J. Clin. Oncol.* **1998**, *16*, 3890. Guenther, H. L.; Fleisch, H.; Walz, A. *J. Bone Miner. Res.* **1997**, *12*, 111. Jowsey, J.; Hopkins, S. G. *J. Lab. Clin. Med.* **1970**, *76*, 126. Dodwell, D. J.; Howell, A.; Ford, J. *Br. J. Cancer* **1990**, *61*, 123. Sahni, M.; Guenther, H. L.; Fleisch, H.; Collin, P.; Martin, T. J. *J. Clin. Invest.* **1993**, *91*, 2004.

(3) Fleisch, H. *Int. J. Clin. Pract.*, **1999**, 2. Fleisch, H. *Scand. J. Rheumatol.* **1998**, 64. Russell, R. G. G.; Croucher, P. I.; Rogers, M. J. *Osteoporosis Int.* **1999**, *9*, 66. Li, Q. N.; Liang, N. C.; Huang, L. F.; Wu, T.; Hu, B.; Mo, L. E. *Acta Pharm. Sin.* **1998**, *19*, 160. Kyriakidou Himonas, M.; Aloia, J. F. *Adv. Therapy*, **1997**, *14*, 281. Reginster, J. Y. L.; Halkin, V.; Gosset, C.; Deroisy, R. *Drugs Today*, **1997**, *33*, 563. Khan, S. A.; Kanis, J. A.; Vasikaran, S.; Kline, W. F.; Matuszewski, B. K.; McCloskey, E. V.; Beneton, M. N. C.; Gertz, B. J.; Sciberras, D. G.; Holland, S. D.; Orgee, J.; Coombes, G. M.; Rogers, S. R.; Porras, A. G. *J. Bone Miner. Res.* **1997**, *12*, 1700; Fleisch, H. *Hormone Metab. Res.* **1997**, *29*, 145. Black, L. J.; Sato, M.; Rowley, E. R.; Magee, D. E.; Bekele, A.; Williams, D. C.; Cullinan, G. J.; Bendele, R.; Kauffman, R. F.; Bensch, W. R.; Frolik, C. A.; Termine, J. D.; Bryant, H. U. *J. Clin. Invest.* **1994**, *93*, 63; Heaney, R. P. *Am. J. Med.* **1991**, *91*, 238.

(4) Fleisch, H.; Shinoda, H.; Adamek, G.; Felix, R.; Schenk, R.; Hagan, P. *Calcif. Tissue Int.* **1983**, *35*, 87.

(5) Beresford, S. A. A.; Weiss, N. S.; Voight, L. F.; McKnight, B. *Lancet* **1997**, *349*, 458. Colditz, G. A.; Hankinson, S. E.; Hunter, D. J.; Willet, W. C.; Manson, J. E.; Stampfer, M. J.; Hennekens, C.; Rosner, B.; Speizer, F. E. *New Engl. J. Med.* **1995**, *332*, 1589. Sturtz, G.; Guervenou, J. *Phosphorus Sulfur*, **1994**, *88*, 1. Sturtz, G.; Guervenou, J. Fr. Patent Appl 2683527 (1991). Hiroaki, U.; Kadowaki, S.; Kamizono, A.; Masahiko, M.; Akihisa, M. Eur. Patent Appl 0555845 (1993). Bosies, E.; Esswein, A.; Bauss, F. Int. Patent WO 92/05187 1992. Saari, W. S.; Rodan, G. A.; Fisher, T. E.; Anderson, P. S. Eur. Patent Appl 0496520 A (1992). Sugioka, T.; Inazu, M. Eur. Patent Appl 0548884 A (1992). Sturtz, G.; Guervenou, J. *Synthesis* **1991**, 661. Bosies, E.; Esswein, A.; Bauss, F. Eur. Patent 0549645 B1 (1991). Cust, M. P.; Gangar, K. F.; Whitehead, M. I. In *HRT and Osteoporosis*; Studd, J. W. W.; Drife, J. O., Eds.; Springer-Verlag: London, 1990; p 209.

(6) Gallagher, J. A.; Bowler, W.; Moore, J. P. G.; Page, P. C. B. U.K. Patent Appl 9613722.9, 1996.

(7) Horowitz, M. C. *Science* **1993**, *260*, 626; Pacifici, R.; Rifas, L.; Teitelbaum, S.; Slatopolski, E.; McCracken, R.; Bergfeld, M.; Lee, W.; Avioli, L. V.; Peck, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 4616. Oursler, M. J.; Keeting, P. E.; Wiegand, K. E.; Bonde, S. K.; Spielsberg, T. C.; Riggs, B. L. *J. Bone Min. Res.*, **1992**, *7*, S111. Oursler, M. J.; Pederson, L.; Pyffereon, J.; Osdoby, P.; FitzPatrick, L.; Spielsberg, T. C. *Endocrinology* **1993**, *132*, 1373. Oursler, M. J.; Osdoby, P.; Pyffereon, J.; Riggs, B. L.; Spielsberg, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6613.

(8) Page, P. C. B.; McKenzie, M. J.; Gallagher, J. A. *Synth. Commun.* **2001**, in press.

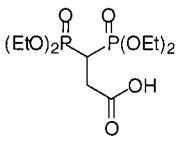
(9) For examples see: Mimura, M.; Hayashida, M.; Nomiyama, K.; Ikegami, S.; Iida, Y. *Chem. Pharm. Bull.*, **1993**, *41*, 1971. Dufau, C.; Sturtz, G. *Phosphorus Sulfur* **1992**, *69*, 93. den Hartog, J. A. J.; Wijnands, R. A.; van Boom, J. H.; Crea, R. *J. Org. Chem.* **1981**, *46*, 2242.

(10) Classen, R.; Haegele, G. *J. Fluorine Chem.* **1996**, *77*, 71. McKenna, C. E.; Schmidhauser, J. *J. Chem. Soc.*, **1979**, *35*, 739. McKenna, C. E.; Higa, M. T.; Cheng, N. H.; McKenna, M. C. *Tetrahedron Lett.* **1977**, 155.

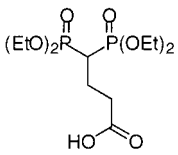
10.1021/jo001489h CCC: $20.00
Published on Web 05/05/2001

however, in our hands reactions were often incomplete, leading to mixtures of products. This prompted us to investigate the benzyl group as a potential protecting group for bis(phosphonic acid)s which might be easily and selectively removed.[11] Hydrogenolysis of benzyl phosphonates has been widely utilized;[12] however, to our knowledge geminal bis(phosphonic acid)s have rarely been synthesized in this way. Mioskowski has shown that simple benzyl bisphosphonate esters can be deprotected by the use of catalytic transfer hydrogenolysis[13] or with DABCO in boiling toluene,[14] or even converted into chlorophosphonates.[15] More recently, a 2-substituted geminal bis(phosphonic acid) has also been prepared by catalytic transfer hydrogenolysis.[16]

We wished to incorporate this chemistry in the synthesis of steroidal bis(phosphonic acid) conjugates, and we now report some of our results in this area. In the design of such compounds it is vital to consider the linkage between the geminal bisphosphonate moiety and the oestrogenic unit. A conjugate with a linker not readily cleaved by hydrolysis or by enzyme action may not retain oestrogenic activity, while a conjugate with a linker too readily cleaved would not achieve targeting. Oestrogen−bisphosphonate conjugates are known with nonhydrolyzable linkers,[17] and with ether,[18] carbonate,[19] and amide[20] linkers. Cortisone−bisphosphonate conjugates are known with ester linkers.[21] We reasoned that a carboxylic ester linkage might provide an appropriate balance between stability and ease of cleavage, and, further, that this balance might be adjusted by, for example, altering the steric environment around the ester unit. In the case of oestradiol, such an ester linkage might readily be positioned at either the 3- or 17- hydroxylated positions, and this design would in addition allow the incorporation of other hydroxyl functionalized biologically active compounds. Initially, methodology was established using the commercially available tetraethyl methylenebisphosphonate. Sturtz has reported the synthesis of the propanoic **1** and butanoic **2** acid derivatives for the synthesis of cortisone-derived conjugates, using standard peptide coupling techniques.[22] He derived bisphosphonate **2** from tetraethyl ethylidene-1,1-bisphosphonate **3** and diethyl malonate, followed by basic hydrolysis and decarboxylation.



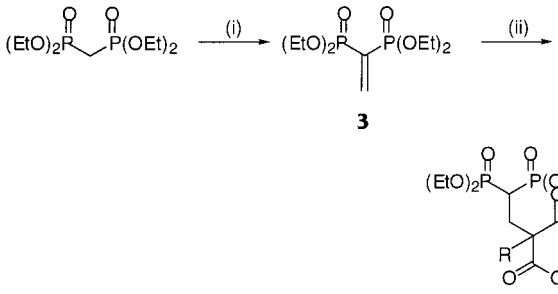
1



2

We reasoned that we could shorten this procedure considerably using the cyclic malonate 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) as a preactivated carboxylic acid equivalent.[23] Tetraethyl methylenebisphosphonate was converted into **3** in multigram quantities using the method of Degenhardt.[24] Michael addition of stabilized nucleophiles to **3** is known.[21,22] Conjugate addition of Meldrum's acid using mediated lithium bis(trimethylsilyl)amide in THF was successful, but yields of **4** varied considerably. When DBU was used as the basic mediator,[25] however, reactions were very reliable, giving essentially pure **4** (Scheme 1).

Scheme 1[a]



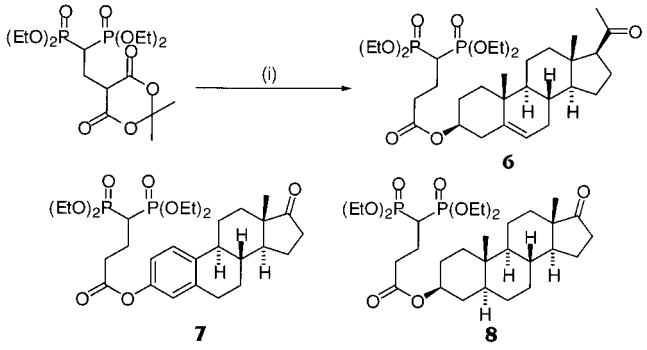
3

4 (R = H); 5 (R = Me)

[a] (i) $(HCHO)_n$, $HNEt_2$, MeOH; *p*TSA, Tol, Δ; (ii) Meldrum's acid derivative, DBU, THF, RT; **4**: 97%; **5**: 65%.

Preliminary attempts at coupling **4** with pregnenolone by heating in acetonitrile gave **6** as desired, but with only 75% conversion after 24 h at reflux. Gratifyingly, by switching to toluene the reaction was complete in 4 h. The transformation was also successful for other steroids such as oestrone and *trans*-androsterone, to give **7** and **8**, respectively (Scheme 2). Interestingly, compound **5**,

Scheme 2[a]



6

7

8

[a] (i) Steroid, toluene, Δ; **6**: 87%; **7**: 77%; **8**: 99%.

(11) Greene, T. W.; Wuts, P. G. M. *Protecting Groups in Organic Synthesis*, 2nd ed.; Wiley-Interscience: New York, 1991. Kocienski, P. J. *Protecting Groups*; Thieme: Stuttgart, 1994.

(12) Amann, F.; Schaub, C.; Mueller, B.; Schmidt, R. R. *Chem. Eur. J.* **1998**, *4*, 1106. Lampe, T. F. J.; Weitz-Schmidt, G.; Wong, C.-H. *Angew. Chem., Int. Ed.* **1998**, *37*, 1707. Cristau, H.-J.; Lambert, J.-M.; Pirat, J.-L. *Synthesis* **1998**, *8*, 1167. Hoffmann, M. *Synthesis* **1988**, 62. Alewood, P. F.; Johns, R. B.; Hoogenraad, N. J.; Sutherland, T. *Synthesis* **1984**, 403.

(13) Saady, M.; Lebeau, L.; Mioskowski, C. *Helv. Chim. Acta* **1995**, *78*, 670. Hoffmann, M.; Wasielewski, C. *Phosphorus Sulfur* **1990**, *53*, 69. Mikroyannidis, J. A.; Tsolis, A. K.; Gourghiotis, D. J. *Phosphorus Sulfur* **1982**, *13*, 279.

(14) Saady, M.; Lebeau, L.; Mioskowski, C. *J. Org. Chem.* **1995**, *60*, 2946.

(15) Mioskowski, C.; Saady, M.; Lebeau, L. *Tetrahedron Lett.* **1995**, *36*, 4785.

(16) Gil, L.; Han, Y.; Opas, E. E.; Redan, G. A.; Ruel, R. *Bioorg. Med. Chem.* **1999**, *7*, 901.

(17) Kirin, S. Japan Patent 6-100576.

(18) Sugioka, T.; Inza, M. Eur Patent 0548884 A1.

(19) Saari, W. S.; Rodan, G. A.; Fisher, T. A.; Anderson, P. S. Eur Patent 0496520 A1.

(20) Ueno, H.; Kadowaki, S.; Kamizono, A.; Morioka, M. Eur Patent 0555845 A2.

(21) Sturtz, G.; Guervenou, J. *Phosphorus Sulfur* **1994**, *88*, 1.

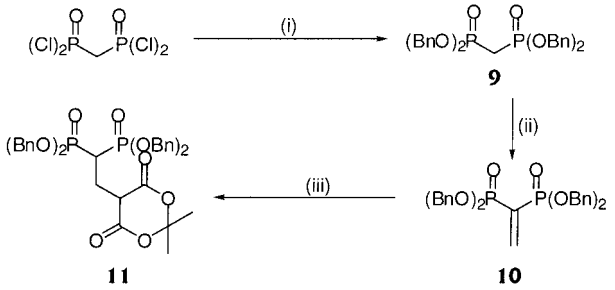
(22) Hutchinson, D. W.; Thorton, D. M. *J. Organomet. Chem.* **1988**, *346*, 341; Sturtz, G.; Guervenou, J. *Synthesis* **1991**, 661.

(23) McNab, H. *Chem. Soc. Rev.* **1978**, *7*, 345.

(24) Degenhardt, C. R.; Burdsall, D. C. *J. Org. Chem.* **1986**, *51*, 3488.

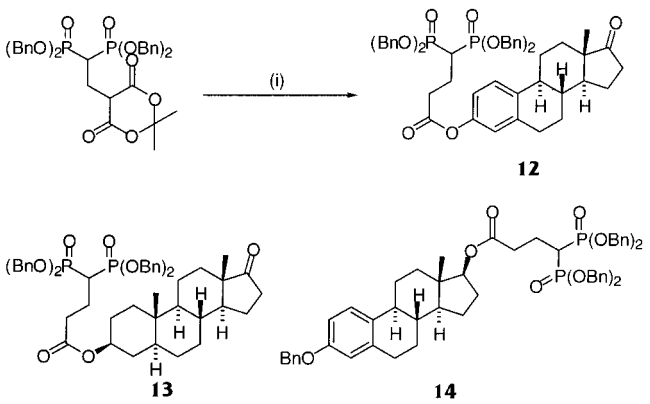
(25) Nugent, R. A.; Schlachter, M.; Murphy, M.; Dunn, C. J.; Staite, N. D.; Galinet, L. A.; Shields, S. K.; Wu, H.,; Aspar, D. G.; Richard, K. A. *J. Med. Chem.* **1994**, *37*, 4449.

Scheme 3[a]



[a] (i) BnOH, pyridine, toluene, 0 °C; 76% (ii) $(HCHO)_n$, $HNEt_2$, MeOH, RT; *p*TSA, Tol, Δ; 99%; (iii) Meldrum's acid, DBU, THF, 98%.

Scheme 4[a]



[a] (i) Steroid, toluene, Δ; **12**: 60%; **13**: 86%; **14**: 76%.

derived from 2,2,5-trimethyl-1,3-dioxane-4,6-dione and **3**, was unreactive toward the same steroids under these conditions.

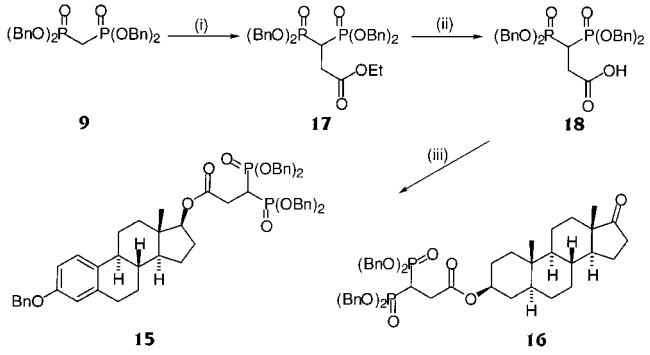
This synthesis of conjugates was short and convenient, the final step eliminating the need for any reagents. We next applied this methodology to tetrabenzyl methylenebisphosphonate conjugates. We have previously reported the benzyl bisphosphonates **9** and **10**, prepared as shown, for the synthesis of oxiranylidene-2,2-bis(phosphonic acid).[8] Reaction of **10** with Meldrum's acid in the presence of catalytic DBU in THF solution yielded **11** quantitatively (Scheme 3). Synthesis of **12** and **13** was readily achieved by heating **11** with oestrone and *trans*-androsterone respectively in toluene. The sterically congested 3-benzyl-17β-oestradiol also reacted successfully, giving **14** after overnight reflux (Scheme 4).[26]

To provide a range of steroidal bisphosphonate conjugates with a spectrum of lability toward hydrolysis in vivo, we also prepared the propanoic acid derivatives **15** and **16** as shown in Scheme 5. Alkylation of **9** with ethyl bromoacetate produced ethyl ester **17**, which was hydrolyzed to produce carboxylic acid **18**.[21,22] Standard DCC coupling with *trans*-androsterone and oestrone gave the corresponding conjugates **15** and **16**.

Conversion of **13**, **14**, **15**, and **16** into the corresponding bis(phosphonic acid)s was accomplished by hydrogenolysis over 10% palladium on charcoal, the reactions requiring only filtration and evaporation to provide isolated products (Scheme 6). Conjugate **12**, derived from

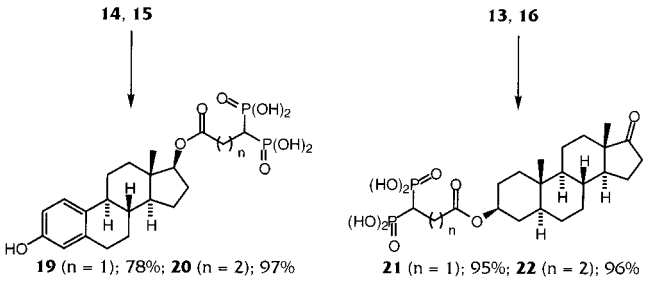
(26) 3-*O*-Benzyl-17β-oestradiol was synthesized from oestrone using standard procedures in two steps: Levitz, M. *J. Am. Chem. Soc.* **1953**, *75*, 5352.

Scheme 5[a]



[a] (i) NaH, THF, ethyl bromoacetate; 60%; (ii) KOH, $MeOH/H_2O$; 78%; (iii) steroid, DCC, DMAP, CH_2Cl_2; **15**: 86%; **16**: 75%.

Scheme 6



oestrone, proved less stable, with some degree of ester hydrolysis (up to 20%) always observed on hydrogenolysis in a range of solvents. We have also observed this phenolic ester hydrolysis using standard trimethylsilyl bromide-promoted cleavage of the corresponding tetraethyl conjugate **8**.

In conclusion, we have prepared a range of bis(phosphonic acid)−steroid conjugates for testing as novel potential bone targeting therapeutics. We have developed an expedient route to the butanoic acid derivatives using Meldrum's acid as a masked activated carboxylic acid, and we have shown the utility of tetrabenzyl bisphosphonates as bis(phosphonic acid) precursors.

Experimental Section

Tetraethyl 2-(2,2-Dimethyl-1,3-dioxane-4,6-dione)ethylene Bisphosphonate 4. DBU (0.051 g, 0.33 mmol) was added to a solution of tetraethyl ethylidene-1,1-bisphosphonate **3** (1.00 g, 3.33 mmol) and 2,2-dimethyl-1,3-dioxane-4,6-dione (0.480 g, 3.33 mmol) in dry THF (10 mL) at 20 °C. After 4 h the reaction was diluted with dichloromethane (30 mL) and washed with water (20 mL). Drying ($MgSO_4$) and concentration in vacuo gave **4** as a colorless oil (1.43 g, 97%): IR (liq) 972, 1251, 1748, 1785. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.36 (12H, t, J = 7.1 Hz), 1.76 (3H, s), 1.87 (3H, s), 2.50−2.68 (2H, m), 3.17 (1H, tt, J = 7.7, 23.2 Hz), 4.13−4.27 (8H, m), 5.13 (1H, t, J = 6.2 Hz). MS (EI) 444.1327, $C_{16}H_{30}P_2O_{10}$ requires 444.1314.

Tetraethyl 2-(2,2,5-Trimethyl-1,3-dioxane-4,6-dione)ethylene Bisphosphonate 5. LHMDS (0.33 mL, 0.016 mmol) was added to a solution of tetraethyl ethylidene-1,1-bis(phosphonate) **3** (0.50 g, 0.166 mmol) and 2,2,5-trimethyl-1,3-dioxane-4,6-dione (0.263 g, 0.168 mmol) in dry THF (20 mL) at 20 °C. After 16 h the reaction was concentrated in vacuo, diluted with dichloromethane (25 mL), and washed with water (3 mL). Drying (Na_2SO_4), concentration in vacuo, and flash column chromatography (2% MeOH/ CH_2Cl_2) gave **5** as a colorless oil (0.496 g, 65%): IR (liq) 974, 1256, 1736, 1771. ^{1}H

NMR ($CDCl_3$, 250 MHz) δ 1.35 (12H, t, J = 7.0 Hz), 1.65 (3H, s), 1.77 (3H, s), 1.87 (3H, s), 2.65 (2H, td, J = 7.1, 15.5 Hz), 2.97 (1H, tt, J = 6.9, 24.0 Hz), 4.07−4.23 (8H, m).

Tetraethyl (3-(3*β*-(5-Pregnenol-20-only)oxycarbonyl)-propylene) Bisphosphonate 6. A solution of **4** (0.250 g, 0.546 mmol) and pregnenolone (0.178 g, 0.563 mmol) in toluene (10 mL) was heated under reflux for 4 h. Concentration in vacuo and flash column chromatography (EtOAc then 10% EtOH/EtOAc) gave **6** as a colorless oil (0.323 g, 87%): IR (liq) 969, 1254, 1704, 1731. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.63 (3H, s), 1.02 (3H, s), 1.21−1.29 (3H, m), 1.35 (12H, t, J = 7.0 Hz), 1.45−1.73 (8H, m), 1.84−1.89 (2H, m), 1.97−2.07 (3H, m), 2.13 (3H, s), 2.15−2.33 (5H, m), 2.51 (1H, tt, J = 6.4, 22.1 Hz), 2.51−2.57 (1H, m), 2.64 (2H, t, J = 7.5 Hz), 4.15−4.23 (8H, m), 4,57−4.66 (1H, m), 5.37 (1H, br d, J = 4.9 Hz). MS (FAB) 659 ($M + H^+$), 681 ($M + Na^+$).

Tetraethyl (3-(3-(1,3,5-Oestratrien-17-onyl)oxycarbonyl)propylene) Bisphosphonate 7. A solution of **4** (1.280 g, 2.79 mmol) and oestrone (0.778 g, 2.88 mmol) in toluene (40 mL) was heated under reflux for 2.5 h. Concentration in vacuo and flash column chromatography (EtOAc then 10% EtOH/EtOAc) gave **7** as a colorless oil (1.314 g, 77%): IR (liq) 1738. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.91 (3H, s), 1.35 (12H, t, J = 7.0 Hz), 1.45−1.63 (7H, m), 1.94−2.55 (9H, m), 2.61 (1H, tt, J = 6.4, 23.4 Hz), 2.88−2.96 (3H, m), 4.14−4.26 (8H, m), 6.80−6.87 (2H, m), 7.28 (1H, d, J = 8.5 Hz). MS (FAB) 613 ($M + H^+$), 635 ($M + Na^+$).

Tetraethyl (3-(3*β*-(5α-Androster-17-onyl)oxycarbonyl)-propylene) Bisphosphonate 8. A solution of **4** (0.250 g, 0.546 mmol) and *trans*-androsterone (0.158 g, 0.546 mmol) in toluene (10 mL) was heated under reflux for 4 h. Concentration in vacuo gave **8** as a colorless oil (0.345 g, 99%): IR (liq) 1026, 1251, 1738. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.66−0.75 (1H, m), 0.85 (3H, s), 0.86 (3H, s), 0.93−1.07 (2H, m), 1.15−1.38 (7H, m), 1.35 (12H, t, J = 7.1 Hz), 1.46−1.57 (3H, m), 1.59−1.68 (2H, m), 1.72−1.83 (4H, m), 1.90−1.96 (1H, m), 2.02−2.12 (1H, m), 2.14−2.27 (2H, m), 2.41−2.48 (1H, m), 2.51 (1H, tt, J = 6.4, 23.8 Hz), 2.63 (2H, t, J = 7.5 Hz), 4.15−4.23 (8H, m), 4.65−4.73 (1H, m).). MS (FAB) 633 ($M + H^+$), 655 ($M + Na^+$). Anal. Calcd for $C_{31}H_{54}P_2O_9$: C, 57.55; H, 8.10. Found: C, 57.22; H, 8.31.

Tetrabenzyl Methylene Bisphosphonate 9. A suspension of methylene bis(phosphonic dichloride) (5.00 g, 20.0 mmol) was stirred rapidly in dry toluene (10 mL) at 0 °C. A mixture of dry benzyl alcohol (8.66 mL, 83.0 mmol) and dry pyridine (6.15 mL, 76.1 mmol) was added over 80 min by syringe pump while the temperature was maintained at 0 °C. After the addition was complete, the reaction was allowed to reach 20 °C and stirred for a further 3 h. The solids were removed by filtration and washed twice with toluene (2 × 20 mL). The filtrate was washed twice with 2 M NaOH (2 × 15 mL) and water (15 mL), dried ($MgSO_4$), and concentrated in vacuo. Removal of benzyl alcohol impurity by distillation (120 °C, 1 mmHg) gave **9** as a colorless oil (8.11 g, 76%): IR (liq) 998, 1260. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.51 (2H, t, J = 21.2 Hz), 5.00 (4H, d, J = 2.2 Hz), 5.05 (4H, d, J = 2.2 Hz), 7.28 (20H, br s). MS (EI) 536.15082, $C_{29}H_{30}P_2O_6$ requires 536.1517.

Tetrabenzyl Ethylidene 1,1-Bisphosphonate 10. Paraformaldehyde (1.35 g, 45.0 mmol) and diethylamine (0.68 g, 9.33 mmol) were dissolved in dry methanol (30 mL) with warming. A solution of **9** (5.00 g, 9.33 mmol) in dry methanol (30 mL) was added at 20 °C and the reaction stirred for 5 d. The reaction was concentrated in vacuo, toluene (20 mL) added, and the solution concentrated again. This last step was repeated to remove all traces of methanol, yielding tetrabenzyl 1-methoxymethyl methylene 1,1-bisphosphonate as a colorless oil. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.86 (tt, J = 5.5, 23.9 Hz), 3.30 (s), 3.93 (td, J = 5.6, 16.3 Hz), 4.99−5.06 (8H, m), 7.28−7.33 (20H, m).

The tetrabenzyl 1-methoxymethyl methylene 1,1-bisphosphonate intermediate was added to *p*TSA (cat) and toluene (100 mL), and the mixture was heated under reflux through a Soxhlet apparatus containing 4 Å molecular sieve for 16 h. The mixture was allowed to cool to 20 °C and washed twice with water (2 × 20 mL). Drying ($MgSO_4$) and concentration in vacuo gave **10** as a colorless viscous oil (5.10 g, 99%): IR (liq) 996, 1248. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.95−5.05 (8H, m), 6.98 (2H, dd, J = 34.4, 38.9 Hz), 7.24−7.30 (20H, m). MS (EI) 548.1524, $C_{30}H_{30}P_2O_6$ requires 548.1517.

Tetrabenzyl 2-(2,2-Dimethyl-1,3-dioxane-4,6-dione)-ethylene Bisphosphonate 11. DBU (0.060 g, 0.37 mmol) was added to a solution of tetrabenzyl ethylidene 1,1-bisphosphonate **10** (2.00 g, 3.65 mmol) and 2,2-dimethyl-1,3-dioxane-4,6-dione (0.525 g, 3.65 mmol) in dry THF (20 mL) at 20 °C. After 4 h the reaction was diluted with dichloromethane (30 mL) and washed with water (20 mL). Drying ($MgSO_4$) and concentration in vacuo gave **11** as a colorless oil (2.48 g, 98%): IR (liq) 998, 1260, 1748, 1784. ^{1}H NMR ($CDCl_3$, 250 MHz) 1.55 (3H, s), 1.67 (3H, s), 2.61−2.73 (2H, m), 3.36 (1H, tt, J = 7.6, 23.2 Hz), 4.77 (1H, t, J = 6.0 Hz), 4.99−5.04 (8H, m), 7.22−7.34 (20H, m). MS (ES) 693.2016; $C_{36}H_{39}P_2O_{10}$ requires 693.2018 ($M + +H$).

Tetrabenzyl (3-(3-(1,3,5-Oestratrien-17-onyl)oxycarbonyl)propylene) Bisphosphonate 12. A solution of **11** (0.300 g, 0.434 mmol) and oestrone (0.120 g, 0.444 mmol) in toluene (15 mL) was heated under reflux for 4 h. Concentration in vacuo and flash column chromatography (40% EtOAc/petroleum ether) gave **12** as a colorless oil (0.283 g, 60%): IR (liq) 996, 1257, 1732, 1755. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.91 (3H, s), 1.39−1.67 (6H, m), 1.92−2.18 (5H, m), 2.25−2.43 (3H, m), 2.47−2.53 (1H, m), 2.70 (1H, tt, J = 6.4, 23.6 Hz), 2.82−2.89 (4H, m), 4.98−5.07 (8H, m), 6.70−6.76 (2H, m), 7.23 (1H, d, J = 9.2 Hz), 7.26−7.34 (20H, m). MS (ES) 861.3319; $C_{50}H_{53}P_2O_9$ requires 861.3321 ($M^+ - H$).

Tetrabenzyl (3-(3*β*-(5α-Andros-17-onyl)oxycarbonyl)-propylene) Bisphosphonate 13. A solution of **11** (0.200 g, 0.289 mmol) and *trans*-androsterone (0.084 g, 0.289 mmol) in toluene (8 mL) was heated under reflux for 4 h. Concentration in vacuo and flash column chromatography (50% EtOAc/petroleum ether) gave **13** as a colorless oil (0.219 g, 86%): IR (liq) 997, 1256, 1731, 1738. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.65−0.73 (1H, m), 0.82 (3H, s), 0.85 (3H, s), 0.94−1.03 (2H, m), 1.12−1.31 (6H, m), 1.40−1.58 (4H, m), 1.62−1.81 (5H, m) 1.89−1.97 (2H, m), 2.06 (1H, dt, J = 9.2, 19.6 Hz) 2.19−2.33 (2H, m), 2.39−2.46 (1H, m), 2.56 (2H, t, J = 7.4 Hz), 2.63 (1H, tt, J = 6.4, 24.0 Hz), 4.58−4.67 (1H, m), 4.98−5.05 (8H, m), 7.25−7.31 (20H, m). MS (ES) 881.3940; $C_{51}H_{63}P_2O_9$ requires 881.3947 ($M^+ + H$).

Tetrabenzyl (3-(17*β*-(3-*O*-Benzyloestra-1,3,5-trienyl)-oxycarbonyl)propylene) Bisphosphonate 14. A solution of **11** (0.200 g, 0.289 mmol) and 3-*O*-benzyl-17*β*-oestradiol (0.104 g, 0.289 mmol) in toluene (6 mL) was heated under reflux for 16 h. Concentration in vacuo and flash column chromatography (40% EtOAc/petroleum ether) gave **14** as a colorless oil (0.208 g, 76%): IR (liq) 996, 1254, 1731. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.74 (3H, s), 1.15−1.47 (8H, m), 1.65−1.72 (1H, m), 1.77−1.87 (2H, m), 2.12−2.33 (6H, m), 2.61 (2H, t, J = 8.0 Hz), 2.63 (1H, tt, J = 6.4, 24.4 Hz), 2.81−2.86 (2H, m), 4.63 (1H, dd, J = 8.0, 8.8 Hz), 4.99−5.06 (8H, m), 6.70−6.71 (1H, m), 6.76−6.79 (1H, m), 7.17−7.19 (1H, m), 7.25−7.42 (25H, m). MS (FAB) 953.4005; $C_{57}H_{63}P_2O_9$ requires 953.3947 ($M^+ + H$).

Ethyl 3,3-Bis(dibenzyloxyphosphoryl)propanoate 17. A solution of **9** (1.00 g, 1.87 mmol) in THF (10 mL) was added to a suspension of NaH (0.047 g, 1.96 mmol) in THF (20 mL) at 0 °C over 5 min. The solution was allowed to reach 20 °C, stirring continued until effervescence ceased (30 min approximately), and ethyl bromoacetate (0.22 mL, 1.98 mmol) added. After 16 h, normal workup, extraction with dichloromethane and flash column chromatography (70% EtOAc/petroleum ether) gave **17** as a colorless oil (0.694 g, 60%): IR (liq) 994, 1256, 1737. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.11 (3H, t, J = 7.1 Hz), 2.86 (2H, td, J = 6.3, 16.1 Hz), 3.30 (1H, tt, J = 6.3, 23.9 Hz), 3.93 (2H, q, J = 7.2 Hz), 4.99−5.04 (8H, m), 7.25−7.34 (20H, m). MS (EI) 622.1872; $C_{33}H_{36}P_2O_8$ requires 622.1885 (M^+).

3,3-Bis(dibenzyloxyphosphoryl)propanoic Acid 18. A solution of **17** (1.27 g, 2.04 mmol) in methanol (6 mL) was added to a solution of KOH (0.127 g, 2.26 mmol) in water (6 mL) and methanol (6 mL) at 0 °C. The reaction was allowed

to reach 20 °C over 1 h and stirred at this temperature for 16 h. Methanol was removed in vacuo and the remaining solution extracted with diethyl ether (20 mL). The aqueous layer was acidified to pH 2 with aqueous $KHSO_4$ and extracted with dichloromethane (3 × 20 mL) and EtOAc (1 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to give **18** as a colorless oil (0.944 g, 78%): IR (liq) 995, 1252, 1742. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.90 (2H, td, J = 6.1, 16.3 Hz), 3.23 (1H, tt, J = 5.9, 24.0 Hz), 4.95−5.01 (8H, m), 7.20−7.32 (20H, m).

Tetrabenzyl (3-(17*β*-(3-*O*-Benzyloestra-1,3,5-trienyl)-oxycarbonyl)ethylene) Bisphosphonate 15. Dicyclohexylcarbodiimide (0.175 g, 0.751 mmol) and DMAP (0.010 g, 0.082 mmol) were added to a solution of **18** (0.300 g, 0.505 mmol) and 3-*O*-benzyl-17*β*-oestradiol (0.184 g, 0.508 mmol) in dichloromethane (8 mL). The solution was stirred for 16 h and filtered, and the filtrate was concentrated in vacuo. Flash column chromatography (50% EtOAc/petroleum ether) gave **15** as a colorless oil (0.409 g, 86%): IR (liq) 1018, 1253, 1732. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.73 (3H, s), 1.10−1.45 (6H, m), 1.62−1.69 (2H, m), 1.73−1.76 (1H, m), 1.82−1.85 (1H, m), 2.02−2.20 (3H, m), 2.79−2.85 (2H, m), 2.91 (2H, td, J = 6.2, 16.2 Hz), 3.33 (1H, tt, J = 6.2, 23.9 Hz), 4.57 (1H, dd, J = 7.9, 8.8 Hz), 5.01−5.08 (10H, m), 6.69−6.71 (1H, m), 6.76−6.79 (1H, m), 7.15−7.18 (1H, m), 7.26−7.42 (25H, m). MS (FAB) 939 ($M + H^+$), 961 ($M + Na^+$).

Tetrabenzyl (3-(3*β*-(5α-Androster-17-onyl)oxycarbonyl)ethylene) Bisphosphonate 16. Dicyclohexylcarbodiimide (0.078 g, 0.335 mmol) and DMAP (0.005 g, 0.041 mmol) were added to a solution of **18** (0.15 g, 0.253 mmol) and *trans*-androsterone (0.073 g, 0.251 mmol) in dichloromethane (15 mL). The solution was stirred for 16 h and filtered, and the filtrate was concentrated in vacuo. Flash column chromatography (50% EtOAc/petroleum ether) gave **16** as a colorless oil (0.164 g, 75%): IR 1015, 1249, 1730. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.62−0.70 (1H, m), 0.79 (3H, s), 0.85 (3H, s), 0.88−1.00 (2H, m), 1.01−1.67 (12H, m), 1.75−1.94 (5H, m), 2.06 (1H, dt, J = 8.8, 19.2 Hz), 2.42 (1H, dd, J = 8.8, 19.2 Hz), 2.85 (2H, td, J = 6.4, 16.4 Hz), 3.30 (1H, tt, J = 6.2, 23.6 Hz), 4.47−4.56 (1H, m), 4.97−5.04 (8H, m), 7.26−7.30 (20H, m). MS (ES) 867.3784; $C_{50}H_{61}P_2O_9$ requires 867.3791 ($M^+ + H$).

3-(17*β*-(Oestra-1,3,5-trienyl)oxycarbonyl)ethylidene Bis-(phosphonic acid) 19. A solution of **15** (0.200 g, 0.213 mmol) and 10% Pd(C) (0.020 g) in dry acetone (5 mL) was stirred under a balloon of hydrogen for 16 h. The Pd(C) was removed by filtration, the residue washed with acetone (2 × 20 mL), and the combined organic solution concentrated in vacuo to give **19** as a colorless powder (0.081 g, 78%). ^{1}H NMR ($(CD_3)_2CO$), 400 MHz) δ 0.85 (3H, s), 1.23−1.46 (6H, m), 1.55−1.63 (1H, m), 1.69−1.77 (1H, m), 1.83−1.93 (1H, m), 2.04−2.19 (2H, m), 2.24−2.29 (1H, m), 2.60−2.84 (6H, m), 4.66 (1H, t, J = 8.4 Hz), 6.49−6.50 (1H, m), 6.55−6.58 (1H, m), 7.08−7.11 (1H, m). MS (ES) 487.1287; $C_{21}H_{29}P_2O_9$ requires 487.1287 ($M^+ - H$).

3-(17*β*-(Oestra-1,3,5-trienyl)oxycarbonyl)propylidene Bis(phosphonic acid) 20. A solution of **14** (0.090 g, 0.095 mmol) and 10% Pd(C) (0.005 g) in methanol (2 mL) and dichloromethane (1 mL) was stirred under a balloon of hydrogen for 16 h. The Pd(C) was removed by filtration, the residue washed with methanol (5 mL), and the combined organic solution concentrated in vacuo to give **20** as a colorless powder (0.046 g, 97%). ^{1}H NMR (CD_3OD, 400 MHz) δ 0.86 (3H, s), 1.25−1.47 (6H, m), 1.53−1.62 (1H, m), 1.72−1.79 (1H, m), 1.85−1.89 (2H, m), 2.12−2.30 (5H, m), 2.36 (1H, tt, J = 6.4, 23.6 Hz), 2.67−2.82 (4H, m), 4.68 (1H, dd, J = 8.0, 8.8 Hz), 6.47−6.48 (1H, m), 6.52−6.58 (1H, m), 7.05−7.07 (1H, m). MS (ES) 501.1438; $C_{22}H_{31}P_2O_9$ requires 501.1443 ($M^+ - H$).

3-(3*β*-(5α-Androster-17-onyl)oxycarbonyl)ethylidene Bis(phosphonic acid) 21. A solution of **16** (0.045 g, 0.050 mmol) and 10% Pd(C) (0.005 g) in methanol (2 mL) was stirred under a balloon of hydrogen for 16 h. The Pd(C) was removed by filtration, the residue washed with methanol (5 mL), and the combined organic solution concentrated in vacuo to give **21** as a colorless powder (0.025 g, 95%). ^{1}H NMR (CD_3OD, 400 MHz) δ 0.74−0.80 (1H, m), 0.87 (3H, s), 0.90 (3H, s), 1.02−1.49 (10H, m), 1.53−2.10 (10H, m), 2.42 (1H, dd, J = 8.4, 18.8 Hz) 2.75−2.95 (3H, m), 4.64−4.77 (1H, m). MS (ES) 505.1758 ($M - H$), $C_{22}H_{35}P_2O_9$ requires 505.1756.

3-(3*β*-(5α-Androster-17-onyl)oxycarbonyl)propylidene Bis(phosphonic acid) 22. A solution of **13** (0.053 g, 0.060 mmol) and 10% Pd(C) (0.005 g) in methanol (1 mL) was stirred under a balloon of hydrogen for 16 h. The Pd(C) was removed by filtration, the residue washed with methanol (5 mL), and the combined organic solution concentrated in vacuo to give **22** as a colorless powder (0.030 g, 96%). ^{1}H NMR (CD_3OD, 400 MHz) δ 0.73−0.79 (1H, m), 0.87 (3H, s), 0.89 (3H, s), 1.00−1.09 (2H, m), 1.19−1.42 (6H, m), 1.48−1.70 (4H, m), 1.72−1.84 (4H, m) 1.91−1.99 (1H, m), 2.03 (1H, dt, J = 9.0, 19.2 Hz), 2.18−2.35 (3H, m), 2.39−2.46 (1H, m), 2.65 (2H, t, J = 7.6 Hz), 4.66−4.72 (1H, m). MS (ES) 519.1917; $C_{23}H_{37}P_2O_9$ requires 519.1913 ($M^+ - H$).

Acknowledgment. This investigation has enjoyed the support of Shire Pharmaceuticals Ltd.

Supporting Information Available: Additional analytical data; ^{1}H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

JO001489H