The Synthesis of Novel Agents Which Inhibit Tumour-Stimulated Bone Resorption

by

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To Mum and Dad

With Love

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ABSTRACT

The Synthesis of Novel Agents Which Inhibit Tumour-Stimulated Bone Resorption

Ian Mansfield

A range of conjugates which focused on bisphosphonates as delivery molecules and on oestrogens as resorption inhibitors were synthesised. Various moieties were coupled through an ester linkage to bisphosphonates which were then converted to the corresponding bisphosphonic acids. This ester linkage was considered to be readily hydrolysable under normal physiological conditions.

In vitro assays performed on the bis-oestrogenic bisphosphonic acid highlighted stability and high resistance to ester hydrolysis. Consequently, a number of less sterically hindered mono-oestrogenic conjugates, for example n-(oestra-1, 3, 5 (10)-trien-3-hydroxy-17β-yloxy-carbonyl) (methoxycarbonyl) propylene bisphosphonic acid and n-(oestra-1, 3, 5 (10)-trien-3-hydroxy-17β-yloxy-carbonyl) (ethoxycarbonyl) propylene bisphosphonic acid, were prepared.

Tetrabenzyl bisphosphonates were incorporated in to the oestrogenic conjugates, and subsequent hydrogenolysis revealed the bisphosphonic acids. This represented a more efficient and simplified route to bisphosphonic acids.

CONTENTS

Abbreviations

Ac	acetyl
aq.	aqueous
^{t}Bu	tertiary butyl
ca.	circa
DCC	dicyclohexylcarbodiiimide
DCU	dicyclohexylurea
DMAP	4-dimethylaminopyridine
DCM	dichloromethane
EDTA	ethylenediaminetetraacetic acid
eq.	equivalent
Et_2NH	diethylamine
g	gram(s)
IR	infra red
Me	methyl
mg	milligram(s)
MHz	megahertz
mL	millilitre(s)
mmol	millimole(s)
NMR	nuclear magnetic resonance
pTSA	para-toluene sulfonic acid
Pd	palladium
^{i}Pr	iso-propyl
RSA	radioimmuno assay
THF	tetrahydrofuran
tlc	thin layer chromatography
TMS	trimethylsilyl
TMSBr	bromotrimethylsilane

CHAPTER ONE

Introduction

1.1 Bone remodelling-coordination of bone resorption and formation

Bone resorption and formation are closely coordinated within each bone modeling unit.[1] The remodelling process begins with a chemical signal from resting osteoblasts. This paracrine signal stimulates recruitment and differentiation of osteoclast precursors and activation of the mature osteoclasts. These osteoclasts then resorb a segment of bone by literally tunnelling their way into the mineralised bone, after which macrophages "clean up" the residue of the resorption process. Osteoblasts are then recruited to the same site and fill in the newly created resorption cavity with new organic matrix and mineralise it. Thus, the first phase of the remodelling cycle is actually resorption, which lasts about 10 days. The second phase, formation, takes about 3 months to complete. Any imbalances in the bone remodelling can lead to bone diseases such as osteoporosis.

All aspects of the remodelling cycle are influenced by a large array of hormones and growth factors, as well as by cytokines from immune cells. These molecules can affect one or more of the various steps either positively or negatively, or even both, depending on the concentration of each regulatory factor and the duration of exposure.

Bone remodelling is ordinarily regulated to compensate for change. For example, if the primary effect of a hormone is to stimulate formation, this effect will be at least partially balanced by a secondary increase in resorption.

It has been known for some time that oestrogens play a role in the bone remodelling process, however, their exact mode of action remains uncertain. Recently, research has suggested that 17 β-oestradiol regulates the circuitry of cytokine action that controls the rate of the bone remodelling cycle.[2]

1.2 Osteoporosis

The most common bone disease found in developed countries are osteoporosis and osteopenia. Osteoporosis is characterised by low bone mass and can lead to the development of bone fractures. In its early stages when there is low bone mass but no risk of fracture, this disease is known as osteopenia.

Osteoporotic fractures may occur anywhere within the human body with the exception of the skull. Such fractures are commonly found in the distal forearm, thoracic, lumbar vertebrae, and proximal femur.[3] Certain social groups are at a greater risk than others in developing the disease. The incidence of bone fracture increases with age, is higher in whites than in blacks, and is higher in women than in men.[3] Furthermore, the risk for women is dramatically increased for caucasian women during menopause.

The two principal factors that effect the development of osteoporosis are the peak bone mass attained by an individual and the rate at which it is consequently lost.[4] Peak bone mass is pre-determined genetically, but calcium intake and lifestyle may also be contributing factors.[5] At the age of 35, most individuals have reached their peak bone mass and then age-related bone loss takes place. This bone loss is caused by an imbalance in the bone remodelling sequence, whereby more bone is resorbed (or dissolved) than is replaced by new bone within each cycle of remodelling. The result is a small net decrease in bone mass, typically in the region of 1% per year. The excessive bone loss that characterises osteoporosis can result from further abnormalities in the bone remodelling cycle. The condition known as high-turnover osteoporosis is one in which the net bone loss in each cycle remains constant but in which the total bone loss is increased due to a greater number of cycles. Low turnover osteoporosis describes a condition in which the activation of the remodelling cycle remains constant, but the net bone loss in each cycle increases. This is a result of reduced efficiency of osteoblast recruitment.

There are two types of cells which play important roles in bone remodelling, namely osteoclasts and osteoblasts. Osteoclasts are responsible for the removal or 'resorption' of bone, while osteoblasts are responsible for the formation of bone.

The marked increase in the rate of bone loss during the menopause has been extensively studied, and consequently attributed to the rapid falls in the levels of oestrogen , in the form of 17β-oestradiol, during this period. Once the oestrogen levels of oestrogen return to normal then the more gradual age-related bone loss process continues.

It has been known for some time that oestrogen plays a role in the bone remodelling process, however its precise mode of action remains uncertain. Recently, research has suggested that 17β-oestradiol regulates the circuitry of cytokine action that controls bone remodelling.[6]

Oestrogen replacement therapy, (or hormone replacement therapy, HRT), Ca^{2+} supplementation, and a regular weight-bearing excercise program have been the most common therapeutic approaches used to minimise or reverse bone loss. However, oestrogen therapy has been linked to an increased risk of breast cancer, and Ca^{2+} alone has not been as effective in halting bone thinning, as was once hoped. The Food and Drug Administration (FDA), has recently approved two new drugs for the treatment of osteoporosis, and several other potential osteoporosis drugs are in the experimental stage. The two approved drugs are alendronate and calcitonin in a nasal-spray form. Alendronate is the first nonhormonal osteoporosis drug. It appears to work by blocking osteoclasts' bone-destroying actions. Calcitonin, the thyroid C cell hormone that slows osteoclast activity, had been used in the past to treat advanced osteoporosis, but it had to be injected daily, a deterrent to patient compliance. Now calcitonin is available in a more patient-friendly nasal spray.

Nearing approval for use in treating osteoporosis is slow-release sodium fluoride, which promotes the rebuilding of bone by mechanisms that are as yet

unclear. Although sodium fluoride was known to promote bone deposition, it caused troubling side effects, such as making the bone more susceptible to fracture (the opposite to the desired effect) and causing peptic ulcers. The slow-release formula appears to avoid these side effects. Another osteoporosis drug available in other parts of the world but not approved in the United States is calcitriol, a form of vitamin D that helps the body absorb Ca^{2+}. A problematic side effect with this agent is increased risk of kidney stones. Eli Lilly, however, are currently marketing an antioestrogen drug (raloxifene) which appears to exhibit the positive effects of oestrogen on bone and the cardiovascular system, but without the negative effects on reproductive tissue.[7]

Despite advances in osteoporosis therapy, treatment is often less than satisfactory, and prevention is by far the best approach to managing this disease. Development of strong bones to begin with before menopause through a good Ca^{2+}-rich diet and adequate exercise appears to be the best preventative measure. A large reservoir of bone at midlife may delay the clinical manifestations of osteoporosis in later life. Continued physical activity throughout life appears to retard or prevent bone loss, even in the elderly.

It is well documented that osteoporosis can result from disuse - that is, from reduced mechanical loading of the skeleton.[3] Space travel has clearly shown that the lack of gravity results in a decrease in bone density. Study of athletes, on the other hand, demonstrates that physical activity increases bone density.[3] Within groups of athletes, bone density correlates directly with the load that the bone must bear. If one looks at athletes' femurs (thigh bones), the greatest bone density is found in weight lifters, followed in order by throwers, runners, football players, and finally swimmers. In fact, the bone density of swimmers does not differ from that of non-athletic controls, since swimming does not place any strain on bones. The bone density in the playing arm of male tennis players has been found to be 35% greater than in their other arm; female tennis players have been found to have 28% greater density in their playing arm than in their other arm. One study found that very mild activity in nursing home patients, whose average age was 82 years, not only slowed bone loss

but even resulted in bone buildup over a 36 month period.[3] Thus, exercise is a good defence against osteoporosis.

The exact mechanism by which exercise increases bone mass is unknown. According to one proposal, exercise places strain on bone, which causes changes in electrical potential that induce bone formation.

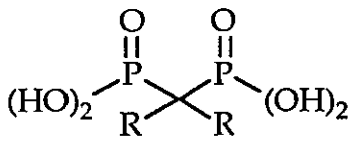
A gene has recently been identified that influences a person's risk of developing osteoporosis. Researchers have found that the gene that codes for vitamin D receptors comes in two varieties. One produces receptors that result in higher bone density than does the other. For those with the less efficient bone-building gene, a combination of adequate dietary Ca^{2+} intake and exercise is particularly important to promote development of strong bones and overcome the genetic predisposition for osteoporosis.

1.3 Bisphosphonates as anti-resorptive drugs

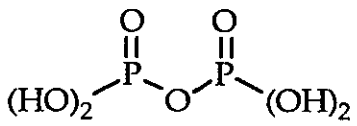
The therapeutic nature of inorganic pyrophosphate (a common by-product from most metabolic pathways eg. the biosynthesis of proteins, lipids, DNA and glycogen) has been realised for over 30 years. It is potentially useful in the treatment of various metabolic mineral disorders since its presence in low concentrations has been shown to inhibit the precipitation and aggregation of calcium salts from solution, and also impair the dissolution of calcium phosphate crystals (hydroxyapatite).[8] However, pyrophosphate is rapidly hyroylsed under normal physiological conditions and therefore rather ineffective if administered orally. Consequently, it was considered whether analogues of pyrophosphate which were resistant to hydrolysis would have the same therapeutic affects.

Geminal bisphosphonates are analogues of pyrophosphates in which the two phosphate moieties are bridged by a methylene group, P-C-P (Figure 1). In contrast to the P-O-P bond of pyrophosphates, the P-C-P bond of bisphosphonates is resistant to hydrolysis by most reagents and by heat, and

is completely resistant to enzymatic hydrolysis. A plethora of bisphosphonates may be synthesised by altering the structure of the two side chains (R) on the methylene group.



Bisphosphonic acid



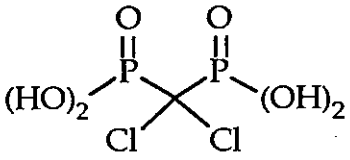
Pyrophosphoric acid

Figure 1

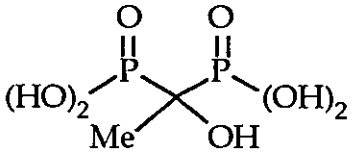
After numerous clinical trials it was concluded that the physiological effects of bisphosphonates closely resembled those of pyrophosphate. It was shown that bisphosphonates could displace phosphate and bind to the surface of hydroxyapatite by co-ordination with calcium ions, since bisphosphonates have a strong affinity for metal ions such as calcium, magnesium and iron.

Furthermore, *in vivo* studies on experimental animals highlighted the inhibition by bisphosphonates of bone resorption induced by parathyroid hormone. Bisphosphonates were also shown to prevent bone resorption in human diseases of the skeleton characterised by excessive bone remodelling, such as in Paget's disease.

Early research into the potential therapeutic uses of bisphosphonates focused on those with halogen or short alkyl side chains, since the effects of these compounds on hydroxyapatite had previously been studied. Dichloromethylene-1,1-bisphosphonic acid (Cl_2MBP or Clodronate) and 1-hydroxyethylidene-1,1-bisphosphonic acid (HEBP or Etidronate) were among the first generation of bisphosphonates (Figure 2). By using the tibia from young rats as a model of normal bone remodelling (involving the processes of both bone resorption and mineralisation) it was found that Cl_2MBP and HEBP impaired the remodelling of the metaphysis, and resorption at the periosteal bone surface.



Clodronate
(Cl_2MBP)

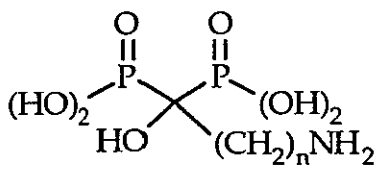


Etidronate
(HEBP)

Figure 2 First generation bisphosphonates

High doses of Etidronate, however, also impaired normal mineralisation of the growth of cartilage and of osteoid in the metaphysis and diaphysis. These effects were linked to the affinity of Etidronate for hydroxyapatite; the presence of a hydroxyl substituent on the central carbon atom of the methylene group is known to enhance this affinity. Clodronate, although having a lower affinity for bone mineral, appeared to be a more potent inhibitor of bone resorption.

Unfortunately, the effectiveness of these compounds was somewhat limited by their low potency when taken orally. A second generation of disubstituted bisphosphonates was therefore synthesised which contained a hydroxyl group at the central carbon atom (thus retaining a high affinity for hydroxyapatite), but were of higher potency as inhibitors of bone resorption and hence could be administered at lower doses (Figure 3). These bisphosphonates were further functionalised with an aliphatic side chain with a terminal primary amino group.



n = 2 Pamidronate (AHPrBP)

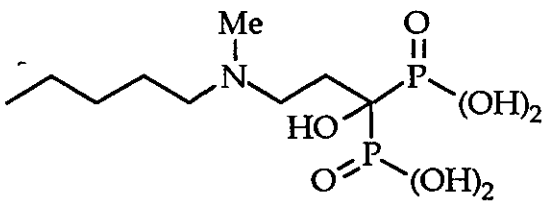
n = 3 Alendronate (AHBuBP)

n = 5 AHHexBP

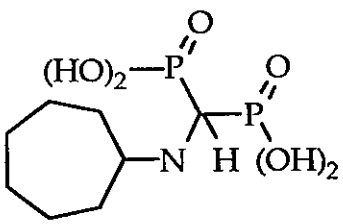
Figure 3 Second generation bisphosphonates

Potency was observed to increase as the aliphatic side chain increased up to C_9H_{19}, and examples with a terminal amine group proved to be even more potent as bone resorption inhibitors.

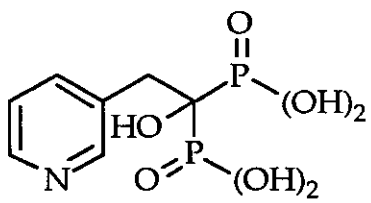
In an attempt to maximise the potency of these bisphosphonates, a further series (third generation) was synthesised in which the primary amine group was modified, or contained a nitrogen moiety within a heterocyclic ring (Figure 4). These compounds containing a cyclic substituent or a nitrogen proved to be extremely potent inhibitors of bone resorption.



MePentAHPrBP



YM175



Risedronate
2(3-PHEBP)

Figure 4 Third generation bisphosphonates

Despite the use of molecular modelling and the synthesis of a vast range of bisphosphonates, there still appears to be no clear structure-activity relationship of bisphosphonates as inhibitors of bone resorption, other than the presence of a hydroxyl moiety at the central carbon atom (which enhances affinity for bone mineral), and a basic nitrogen atom in an aliphatic, cyclic or heterocyclic environment is thought to be important.

1.4 Oestrogen-geminal bisphosphonate compounds

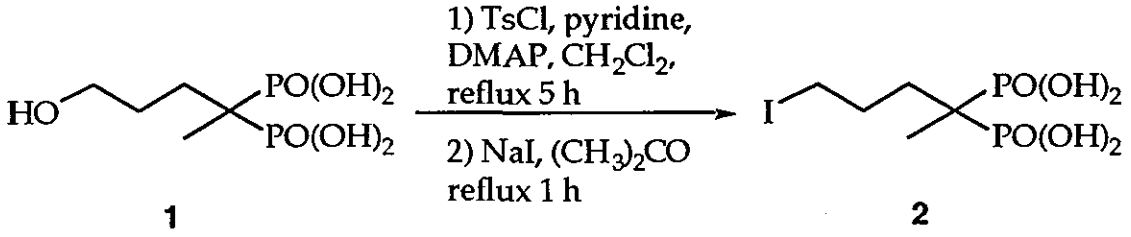
Geminal bisphosphonates have a high affinity for hydroxyapatite, the main constituent of mineralised bone. They are rapidly absorbed through the plasma on to bone thus avoiding undesirable side effects in other tissues. In view of this, bisphosphonates provide a distinct advantage over traditional HRT when used as a preventative treatment for osteoporosis. The geminal bisphosphonate group, if coupled through a covalent link to an oestrogen, has the potential of targeting the oestrogen to the bone surface where oestrogen receptor sites are known to exist.

Previously, there have been numerous patents published covering a host of compounds containing oestrogen coupled to bisphosphonates through various linkages. The rationale behind such work has been to combine the properties of both oestrogens and bisphosphonates in inhibiting bone resorption. It has been reasoned that the oestrogen constituent of these compounds can be targeted to the bone surface by exploiting the bisphosphonate's high affinity for mineralised bone. Subsequently, this would increase the concentration of oestrogen at the bone surface and potentially increase the inhibition of bone resorption. Targeting oestrogen in this way would hence avoid side effects in other tissues.

When considering this class of compound we find that the linkage used between the oestrogen and geminal bisphosphonate moieties is rather important. Indeed, the compound may be classed by the type of link chosen, and its relative stability *in vivo*, as either a drug or pro-drug. By the term drug it is understood that the whole compound stays intact until it has reacted with the targeted site. By the term pro-drug it is understood that a chemical transformation takes place releasing the drug before interaction with the targeted site. There are obviously advantages and disadvantages of following either approach. The drug approach relies on the whole compound being active and the whole compound reaching the targeted cells; cell membrane permeability is likely to be problematic due to the high polarity of the geminal bisphosphonic acid group. If we consider the pro-drug approach, the linkage may break before the compound reaches the bone surface resulting in no

targeting of the oestrogen constituent, and if the compound successfully reaches the bone surface the link must be capable of being broken. Furthermore, it is clearly important that the cleaved fragments must be non-toxic.

One example of a drug approach is described in a Japanese patent[9] in which an oestrogen-bisphosphonate compound has a linkage which is completely non-hydrolysable and which is attached directly to the steroidal carbon skeleton. The straight carbon linkage is synthesised by the generation of a carbanion at an acetylenic group of steroid **3**, followed by reaction with a halo-alkylidene geminal bisphosphonate **2**. Intermediate **2** was prepared from the corresponding hydroxylalkylidene geminal bisphosphonate **1**, via the tosylate by nucleophilic displacement with iodide (Scheme 1).

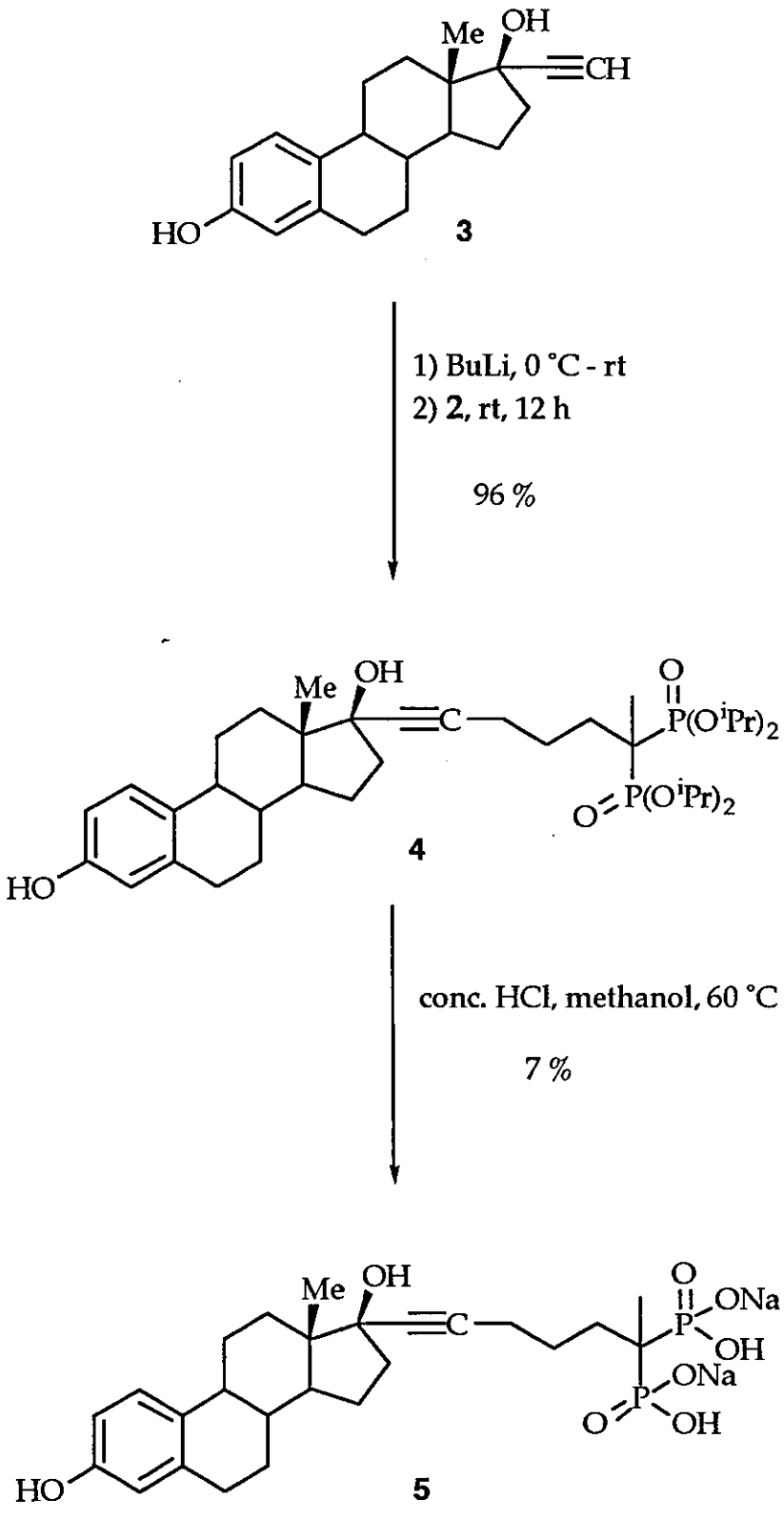


1 **2**

71 %

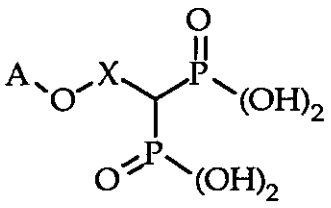
Scheme 1

17 α-Ethynyl-oestradiol was deprotonated using a solution of butyl lithium in hexane, and the resulting carbanion reacted with intermediate **2** to afford the oestrogen functionalised bisphosphonate **4** in virtually quantatative yield (96%). The subsequent phosphonate ester hydrolysis step with concentrated hydrochloric acid, however, provided bisphosphonic acid **5** in very low yield (7%) (Scheme 2).


Me
OH
≡CH
HO
3
1) BuLi, 0 °C - rt
2) 2, rt, 12 h
96 %
Me
OH
C
O
P(O^{i}Pr)$_2$
O
P(O^{i}Pr)$_2$
HO
4
conc. HCl, methanol, 60 °C
7 %
Me
OH
C
O
ONa
P
OH
ONa
P
O
OH
HO
5

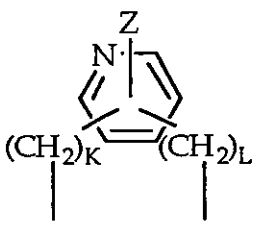
Scheme 2

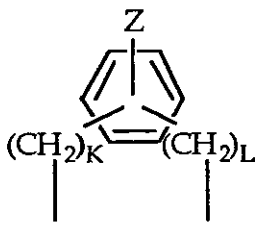
Hoechst Japan Ltd.[10] published a patent in which an ether linkage is employed in a drug approach, utilising the fact that ether bonds are not easily cleaved under normal physiological conditions. The patent lays claims to a range of compounds shown in Figure 5.

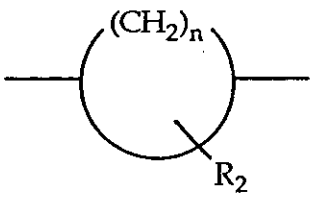


A = an oestrogenic compound

X = a single bond, C_1-C_{10} or a cyclic group of the formula;



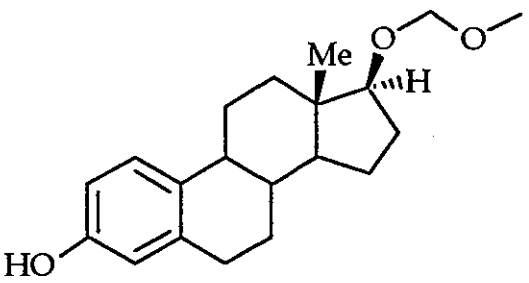




Where Z is a nitro or halogen group, and R_2 is H or a C_1-C_5 alkyl group.

Figure 5

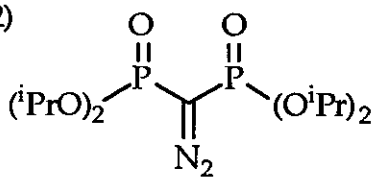
Two different syntheses are described within the patent relating to compounds with or without a spacer unit. Scheme 3 depicts how a compound lacking any spacer is constructed. 17 β-MOM protected oestradiol **6** was heated to reflux with copper (II) acetylacetone, and the resulting mixture was reacted with tetraisopropyl (diazomethylene) bisphosphonate **7** by slow addition of the bisphosphonate. After stirring for 1 h and purification by chromatography the oestogen functionalised bisphosphonate **8** was isolated in 46% yield. Treatment of **8** with trimethysilyl bromide displaced the MOM protecting group and the phosphonate esters; the addition of water hydrolysed the silyl esters



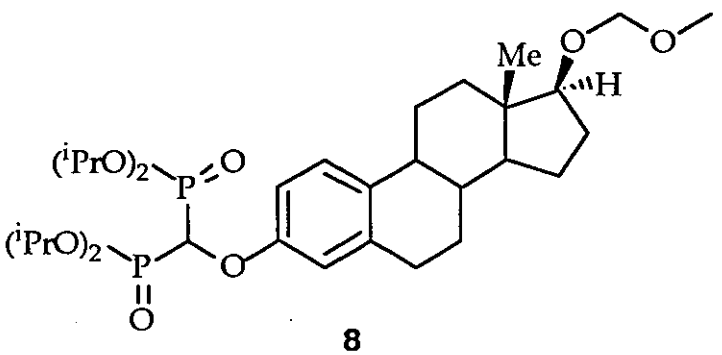
6

1) Cu^{II} acetylacetone
toluene, reflux

2)



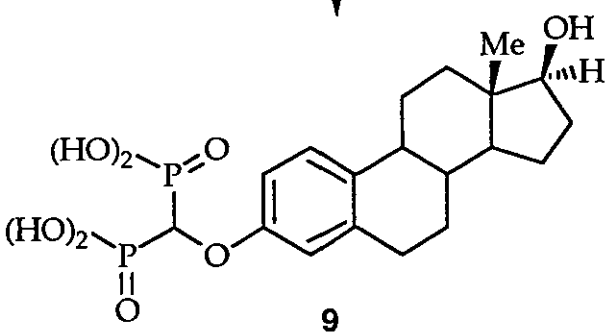
7



8

1) TMSBr, dry chloroform

2) H_2O



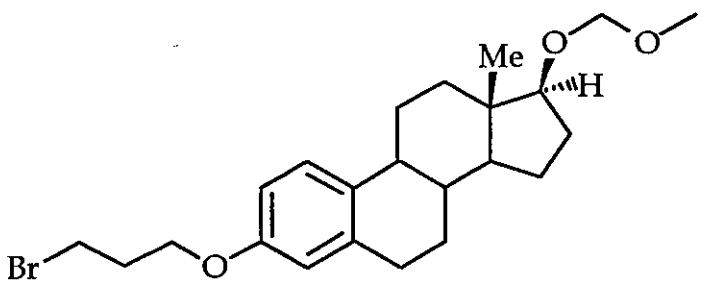
9

Scheme 3

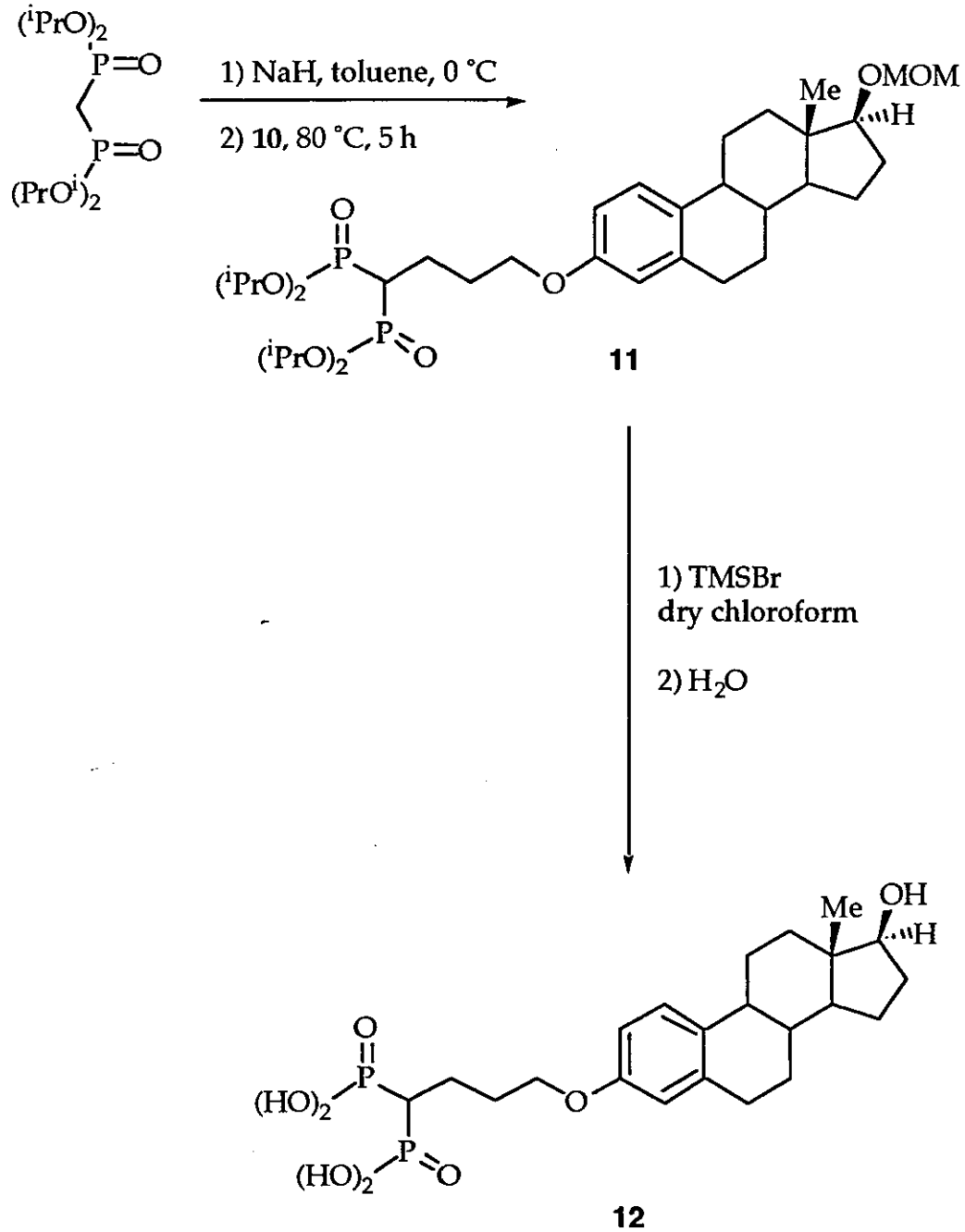
to reveal the bisphosphonic acid **9** in 68% yield. The overall yield for the conversion of **6** to **9** was 31%.

The second procedure describes the synthesis of a compound containing a spacer unit between the oestrogen and bisphosphonate moieties (Scheme 4). This spacer group could have an important role since the bulky geminal bisphosphonate group may interfere with the ability of the oestrogen to bind to an oestrogen receptor. Attaching further non-polar constituents to the bisphosphonate may also increase its lipophilicity, and therefore allow the compound to cross target cell membranes.

A solution of sodium hydride in tetrahydrofuran was employed to deprotonate 17 β-MOM protected oestradiol. 1,3-Dibromopropane (1.2 eq.) in dimethylformamide was added and the reaction mixture stirred at room temperature overnight. The resulting 3-bromopropane-functionalised oestrogen **10** was isolated in 62% yield. Tetraisopropyl methylene bisphosphonate was deprotonated with sodium hydride in toluene at 0 °C and reacted with the bromopropane intermediate **10** at 80 °C for 5 h. The resulting compound **11** was isolated in 36% yield after purification by chromatography. Subsequent deprotection with trimethylsilyl bromide / water (53% yield) revealed the desired bisphosphonic acid **12** in an overall yield of 12%.



10



Scheme 4

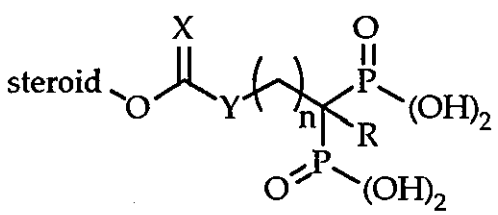
The results of several animal studies with these compounds highlighted a number of interesting points. The first experiment compared the effects of the two compounds and 17 β-oestradiol, suspended in olive oil, and olive oil alone, on uterine weights of three week old SD rats when subcutaneously administered. After 4 h, the results showed that the test compounds had little or no effect on the uterine weight compared to 17 β-oestradiol (which had a

large dose-dependent effect). This implies that the compounds were either targeted to the bone and therefore had no effect on the uterine weight, or that the compounds lost their oestrogenic nature.

The second experiment measured the effects of the test compounds on a hypercalcaemic model, induced by parathyroid hormone (PTH). The results showed that the Ca^{2+} levels were significantly increased by injection with PTH, indicating that hypercalcaemia was induced. Furthermore, both the test compounds were observed to reduce the effects of this PTH induced hypercalcaemia (the compound containing a spacer unit displaying a slightly greater effect).

The final experiment involved the study of the anti-osteoporotic effects of the compounds on ovariectemised mice for six consecutive weeks. After administration, the right femurs were removed and measurements taken of the outer width and the cortical width of the centre part of the femur shafts by soft x-ray. The results showed that both the compounds had a beneficial effect on the bone metabolic disorder induced by ovariectomy, with the spacer compound displaying greater activity than the non-spacer compound.

Another example of a pro-drug approach is described in a patent published by Merck and Co. Inc.[11], in which use is made of a number of different linkages including carbamate, thiocarbamate, carbonate and thiocarbonate. The patent lays claim to componds such as those highlighted in Figure 6.

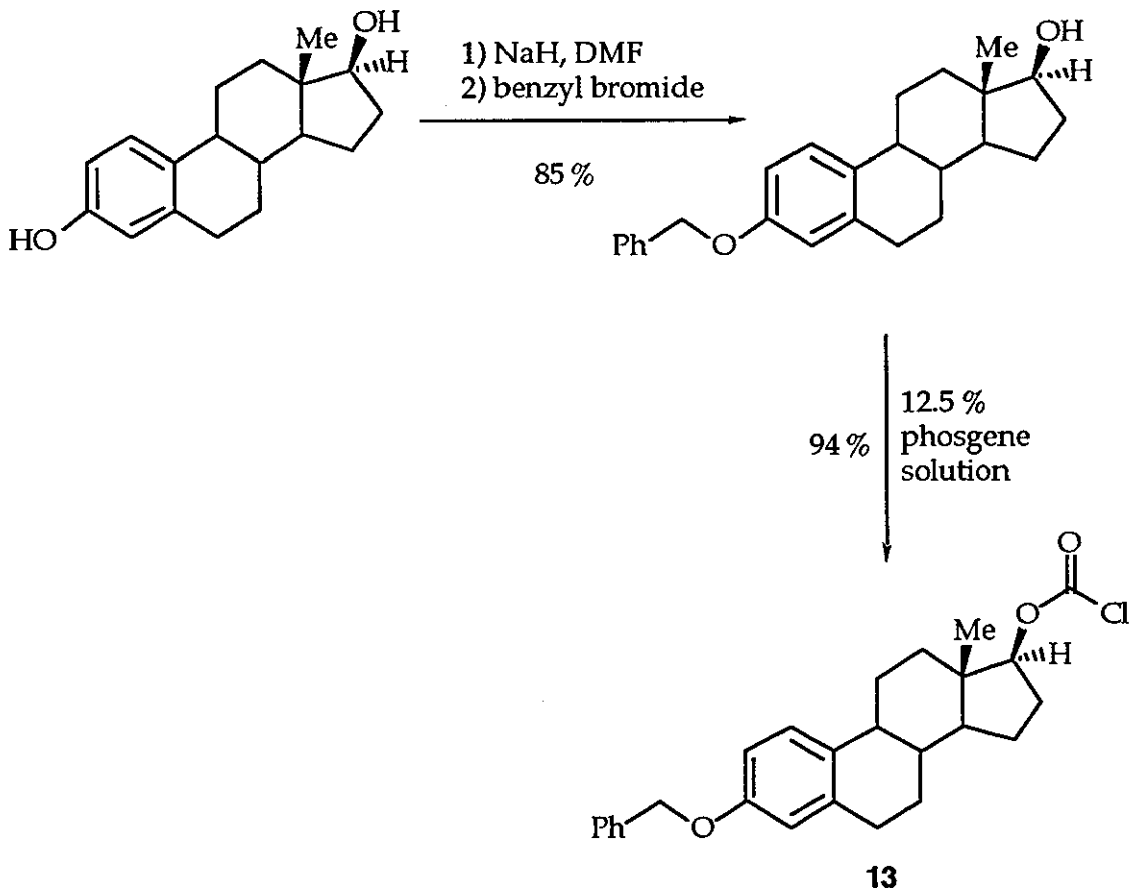


Where X = O or S, Y = O, NH or N-alkyl C_1-C_4
n = 1-4 and R = H or OH

Figure 6

Although no biological data were offered in this publication, it is of interest to consider the methodology employed in the synthesis of such compounds.

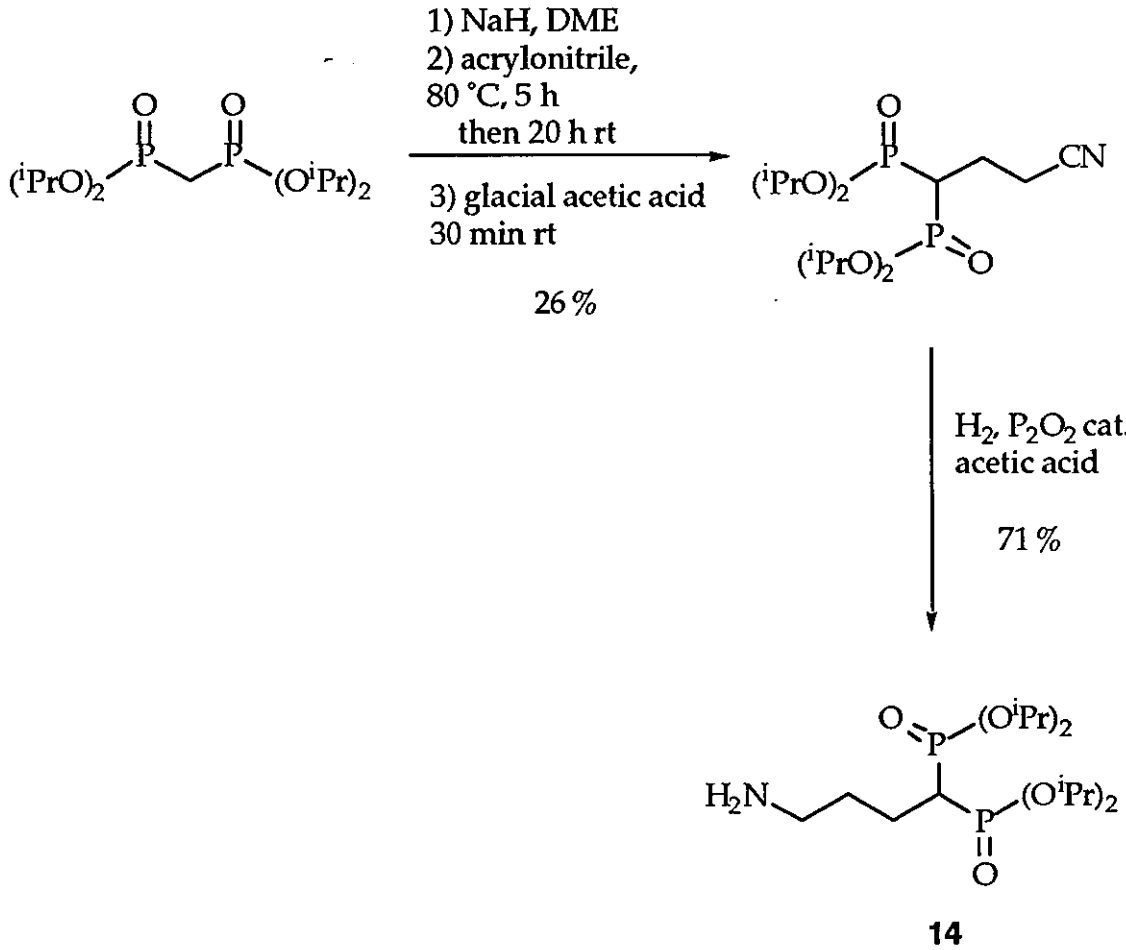
Initially, the oestrogen moiety was suitably protected prior to conjugation with the functionalised bisphosphonate (Scheme 5). Hence, 17 β-oestradiol was selectively benzylated by deprotonation of the more acidic phenolic proton, by stirring in a cooled solution of sodium hydride (1.6 eq.) in dimethyl formamide for 1 h. Benzyl bromide (1.6 eq.) was added and the reaction mixture stirred at room temperature for a further 20 h. 3-Benzyl 17 β-oestradiol was isolated (85% yield) and subsequently reacted with a 12.5% solution of phosgene in toluene. After stirring at room temperature for 20 h, the desired chloroformate **13** was isolated in 94% yield.



13

Scheme 5

The bisphosphonate constituent contained a primary amine group and was prepared from a simple methylene bisphosphonate (Scheme 6). First of all, tetraisopropyl methylene bisphosphonate was deprotonated by a solution of sodium hydride (1.2 eq.) in dimethoxyethane (room temperature for 20 mins.), and subsequently reacted with acrylonitrile (1.2 eq.) at 80 °C for 5 h. The anion formed from this Michael addition was quenched with glacial acetic acid (1.4 eq.), affording after chromatography the desired nitrile in 26% yield. Hydrogenation of the nitrile was achieved using PtO_2 catalyst in a Parr apparatus at a hydrogen pressure of 50 psi for 20 h. Thus, the aminopropylene bisphosphonate **14** was isolated in 71% yield.

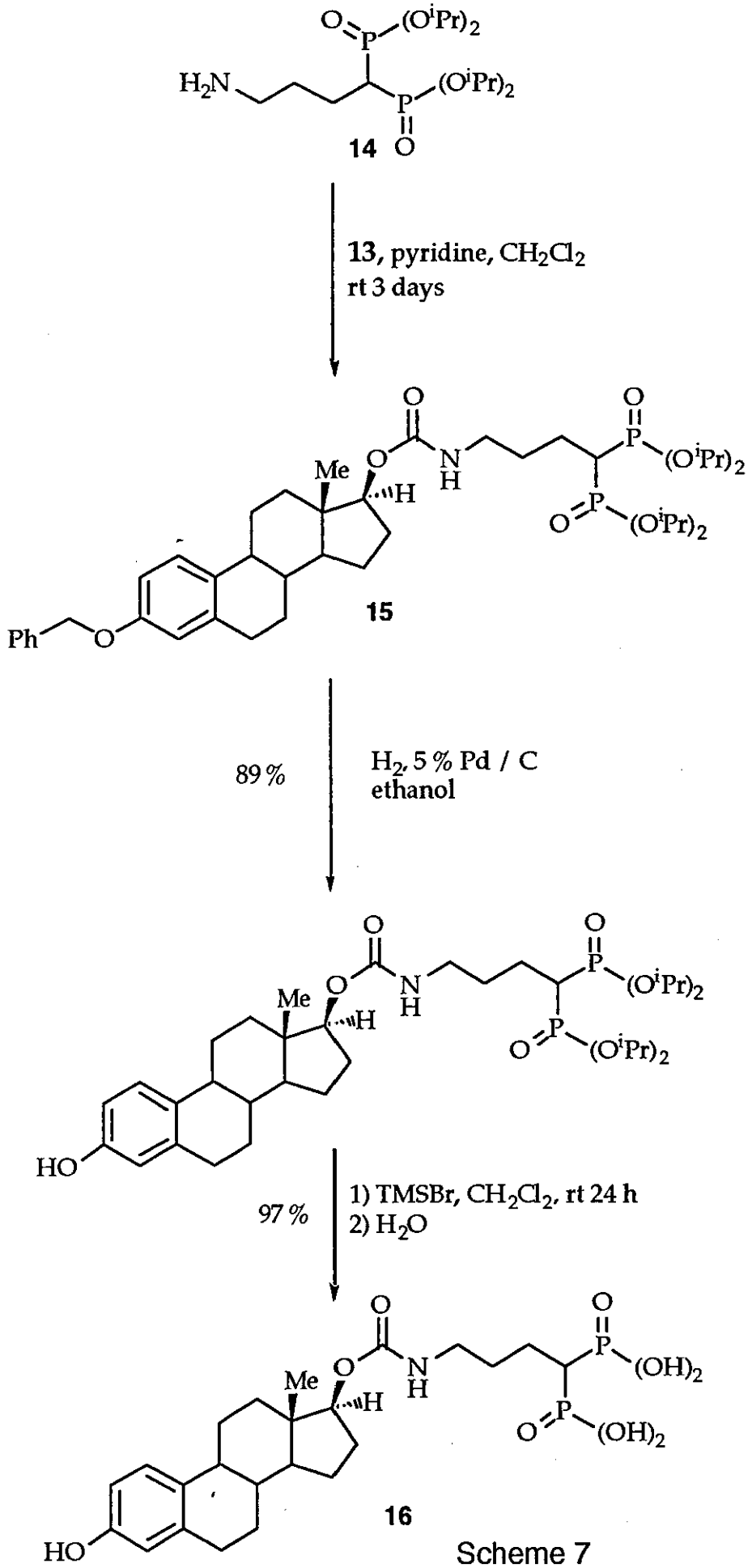


14

Scheme 6

Chloroformate **13** and bisphosphonate **14** were brought together by treatment with pyridine in dichloromethane and stirring at room temperature for 3 days (Scheme 7). This acylation of amine **14** afforded the amide linked

oestrogen-bisphosphonate compound **15** in 82% yield. Subsequent deprotection (H_2, Pd/C) and phosphonate ester hydrolysis (5 eq. TMSBr, H_2O) afforded the desired bisphosphonic acid **1 6** in 94% yield.


14
13, pyridine, CH2Cl2
rt 3 days
15
89 %
H2, 5 % Pd / C
ethanol
97 %
1) TMSBr, CH2Cl2, rt 24 h
2) H2O
16

Scheme 7

The Mitsubishi Kasei Corporation[12] described their work with compounds containing mixed ester and amide functionalities (Figure 7).

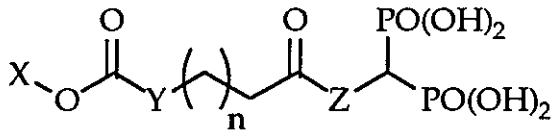
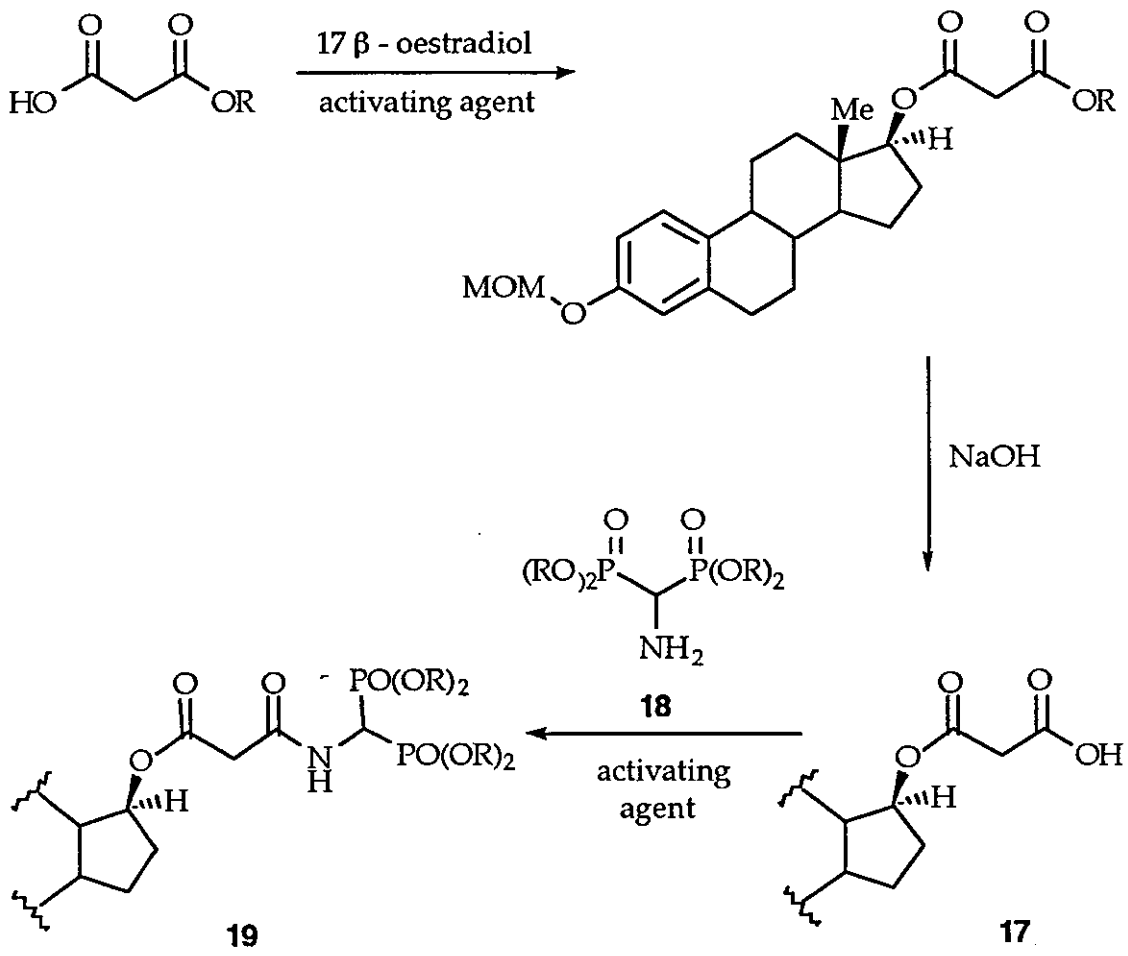


Figure 7

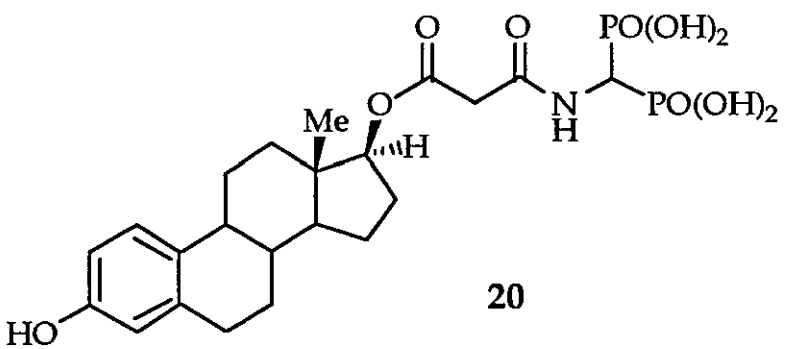
Where X = a steroid residue, eg. oestradiol, testosterone, androsterone or pregnolone; Y = NH or CH_2; Z = NH or CH_2; n = either 1-4 straight chain alkyl or cyclohexane/phenyl.

In particular, the patent describes the synthesis and clinical evaluation of **20** in which a nitrogen atom is positioned alpha to the geminal bisphosphonate moiety (Scheme 8). Malonic half-acids were coupled to the MOM-protected-steroid residue using carboxylic acid activators such as dicyclocarbodiimide, isobutyl chloroformate, *N,N'*-carbonyldiimidazole and thionyl chloride. The resulting diesters were selectively hydrolysed with sodium hydroxide to afford the carboxylic acid **17**. Tetraalkyl aminomethylene bisphosphonate **18** was added to the suitably activated carboxylic acid, yielding the fully functionalised bisphosphonate **19**.



Scheme 8

Subsequent reaction with a trimethylsilyl halide and water removed the methoxymethyl protecting group and hydrolysed the bisphosphonate esters to reveal the bisphosphonic acid **20** (Figure 8).



Scheme 16

The patent goes on to discuss the therapeutic potential of compound **20** with reference to two *in vivo* experiments. The first experiment assesses the ability of the bisphosphonic acid to reach the bones. Typically SD male rats subcutaneously received a vehicle (95% corn oil and 5% benzyl alcohol) [Group A], or 17β-oestradiol [Group B], or an equimolar amount of compound **20** [Group C]. The blood and tibia were collected after 2 h, 1 day and 2 days in each group. 17β-Oestradiol was measured directly by the RIA method. The tibia was pulverised in hydrochloric acid at room temperature, the resultant solution mixed with EDTA, water and sodium hydroxide and allowed to stand at room temperature for 30 mins. to isolate the 17β-oestradiol of compound **20**. The isolated 17β-oestradiol was extracted with isoamyl alcohol, and the extract was concentrated to dryness and dissolved in phosphate buffer (pH 7.4) for assaying by RIA. Table 1 shows a mean value of measurements for one group consisting of five rats with standard deviation.

Amount of 17β-oestradiol in Plasma and in Bone						
Compound administered	2 Hours		1 Day		2 Days	
	Plasma (pg/ml)	Bone (pg/100 mg)	Plasma (pg/ml)	Bone (pg/100 mg)	Plasma (pg/ml)	Bone (pg/100 mg)
Vehicle [A]	<20	<20	<20	<20	<20	<20
17β-oestradiol [B]	5187 ±846	<20	102 ± 30	<20	<20	<20
Compound **20** [C]	<20	51 ± 25	<20	359 ± 134	<20	376 ± 92

Table 1

The results show that, in Group B, 17 β-oestradiol was detected in plasma until the next day after administraton but it was subsequently below the detection limit in bone throughout the test period. Conversely, when compound **20** was administered, in Group C, 17β-oestradiol was below the

detection limit in plasma and it was detected in bone as early as 2 h after admistration. The amount of 17β-oestradiol in bone increased with time. Hence, it was concluded that bisphosphonic acid **20** had the ability to transport itself to bone

The second experiment was designed to assess the ability of compound **20** in inhibiting bone resorption in ovariectomised female rats. During this experiment SD female rats of 12 weeks of age which had undergone ovariectomy (OVX) received subcutaneously a vehicle (95% corn oil and 5% benzyl alcohol) [Group 2], or 17 β-oestradiol [Group 3], or an equimolar amount of compound **20** [Group 4] for 28 days since the next day of operation. The rats were subjected to an autopsy on the 29th day, and the weight of wet uterus and the amount of a bone volume in tibia (Cancellous bone volume / Tissue volume x 100) were measured. Table 2 shows a mean value of the measurements for one group consisting of 10 rats with standard error.

Bone resorption inhibitory effect of compound **20**

Group		Bone volume (BV / TV,%)
1	Administration of vehicle	26 ± 2
2	OVX : Administration of vehicle	18 ± 2
3	OVX : Administration of 17 β-oestradiol	30 ± 2
4	OVX : Administration of **20**	30 ± 3

Group 1 vs, Group 2; $P < 0.01$

Group 2 vs, Group 3; $P < 0.001$

Group 2 vs, Group 4; $P < 0.01$

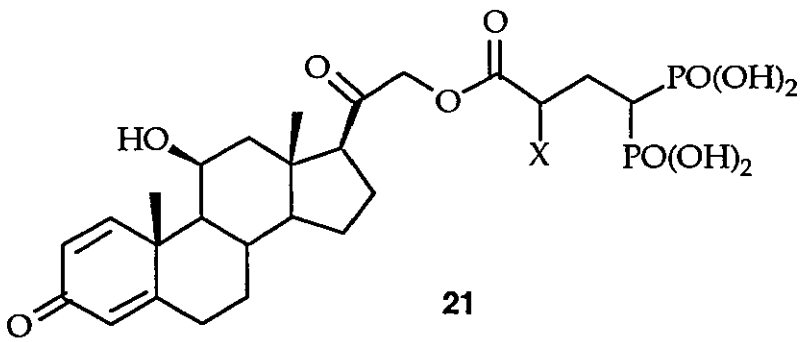
Table 2

With regard to the rats which received the vehicle (Group 1 and Group 2), it was observed that those which had been ovariectomised had a significantly lower bone volume than those whose uteri remained intact. In Group 3 and Group 4, the bone resorption was notably inhibited. However, the weight of

uterus increased in Group 3 up to the level of Group 1, but no effect was observed in Group 4 in this respect. Thus, from these observations it was concluded that compound **20** acts selectively on bones and significantly inhibits bone resorption without giving the uterus weight gain.

The results from these two experiments show how a steroid compound linked to a bisphosphonate can act more selectively towards bone tissue than towards other organs, and that it is a useful therapeutic agent for the treatment of bone metabolism diseases such as osteoporosis, without producing responses in other organs due to their high selectivity to bone tissue.

Guervenou and Sturtz have extended the bisphosphonate delivery system to other steroids such as cortisone.[13] Their patent lays claim to compounds resembling **21**, in which an amino bisphosphonate moiety is coupled to a cortisone residue through a hydrolysable ester linkage (Figure 9).



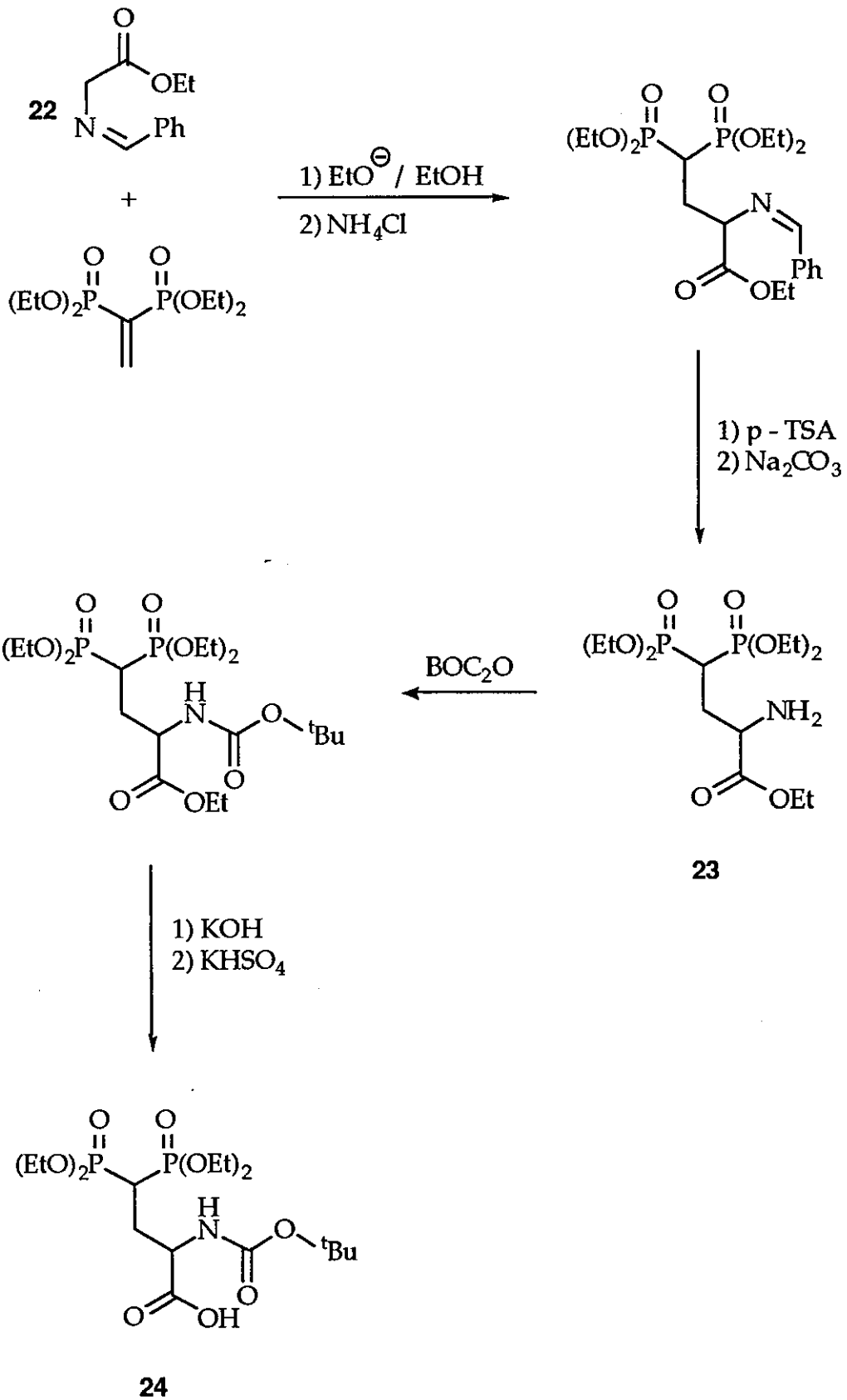
21

where X = H_2 or NH_2

Figure 9

The bisphosphonate component was prepared by a Michael addition of the *N*-protected[14] ethyl glycinate **22** with tetraethyl ethylidene bisphosphonate (Scheme 9). Deprotection of the amine with *p*-TSA revealed aminoester **23**. Re-protection of the amine using BOC anhydride prevented undesirable side reactions occurring during subsequent steps. Saponification of the ethyl

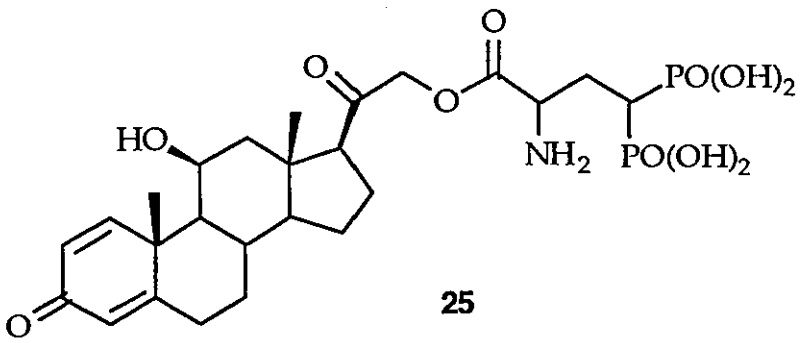
carboxylate afforded the corresponding N-protected amino-bis (diethylphosphono) butanoic acid **24**.


22
OEt
N
Ph
+
(EtO)2P
P(OEt)2
1) EtO⊖ / EtOH
2) NH4Cl
N
Ph
OEt
1) p - TSA
2) Na2CO3
NH2
23
BOC2O
H
N
O
tBu
1) KOH
2) KHSO4
OH
24

Scheme 9

Guervenou and Sturtz considered a number of carboxylic acid activating groups for the esterification of the steroids, namely isobutyl chloroformate, diethyl cyanophosphonate, diisopropylcarbodiimide and dicyclohexylcarbodiimide. Isobutyl chloroformate[15] was discarded due to its lack of regioselectivity towards the alcohol group. Diethyl cyanophosphonate is often used in peptide synthesis,[16] but may also be employed in esterification reactions.[17,18]. However, the authors experienced a number of side reactions resulting in the formation of phosphonate and phosphate derivatives, or in the attack on the carbonyl group of the steroid by the cyanide anion.

Reactions using DIC and DCC and a catalytic amount of DMAP proved to be much more efficient,[19] giving 30-70% and 57-95% yields respectively, and without the occurrence of side reactions. Dicyclohexylcarbodiimide was finally selected as the coupling agent of choice due to its efficiency and ease of use.



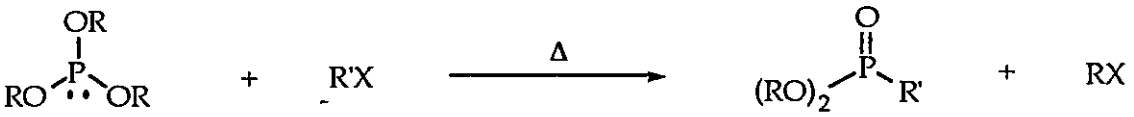
25

Figure 10

The cortisone-functionalised amino bisphosphonates were converted in to their silyl ester equivalents by treatment with trimethylsilyl bromide according to the method desribed by McKenna.[20,21] The *t*-butoxycarbonyl protecting group was simultaneously removed producing the silylated amine. All the silyl groups were removed on the addition of methanol to reveal the amino bisphosphonic acid **25** (Figure 10).

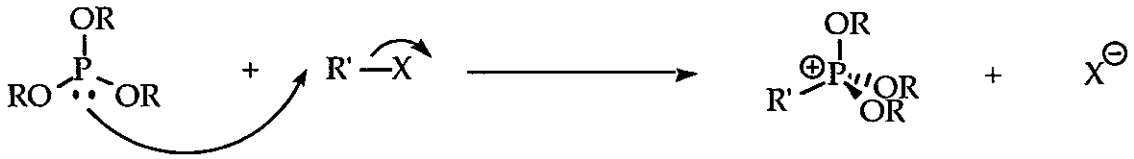
1.5 General Methods for the formation of carbon-phosphorus bonds

In considering carbon-phosphorus (V) bonds only, there are two main methods for their formation. One of the most commonly employed methods for synthesising phosphonates, phosphinic acid esters and phosphine oxides is the Michaelis-Arbuzov reaction.[22] It typically involves the interaction of a trialkylphosphite with an alkyl halide, forming a dialkylphosphonate and alkylhalide by-product (Scheme 10).



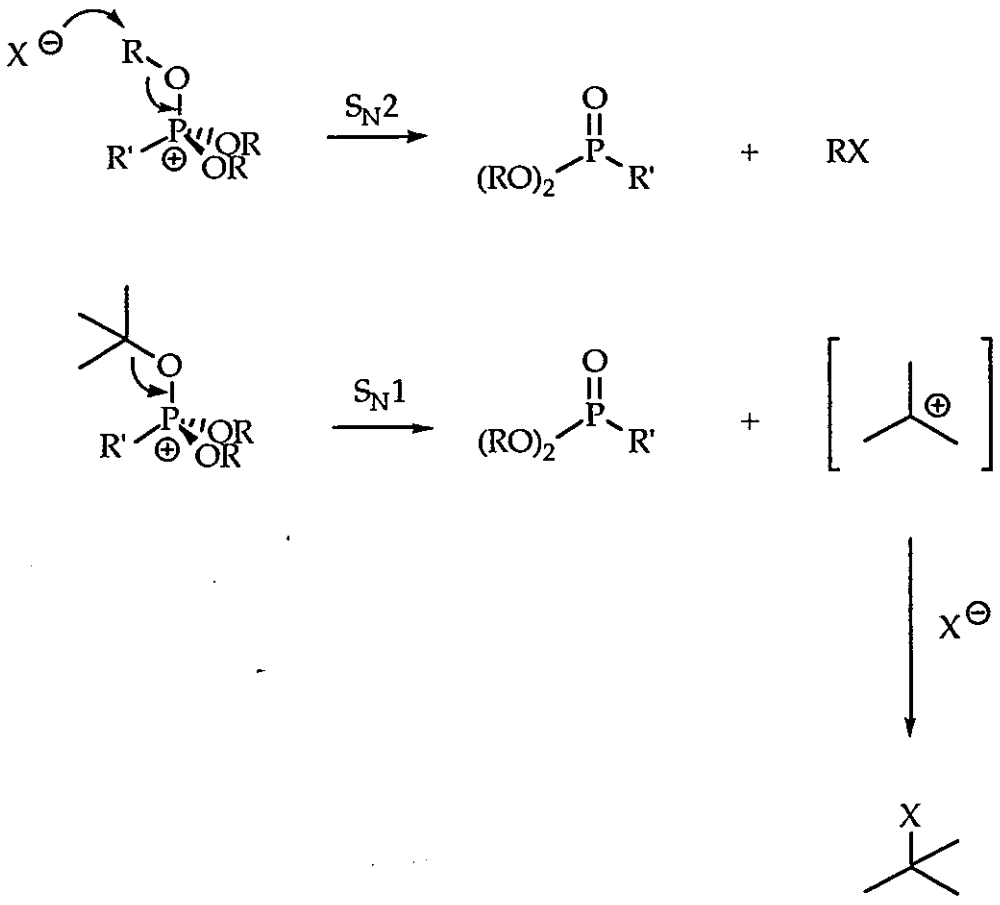
Scheme 10

The reaction mechanism proceeds with initial attack of the phosphite lone pair on the alkyl halide, displacing the halide to form a phosphonium halide intermediate (Scheme 11).[23]



Scheme 11

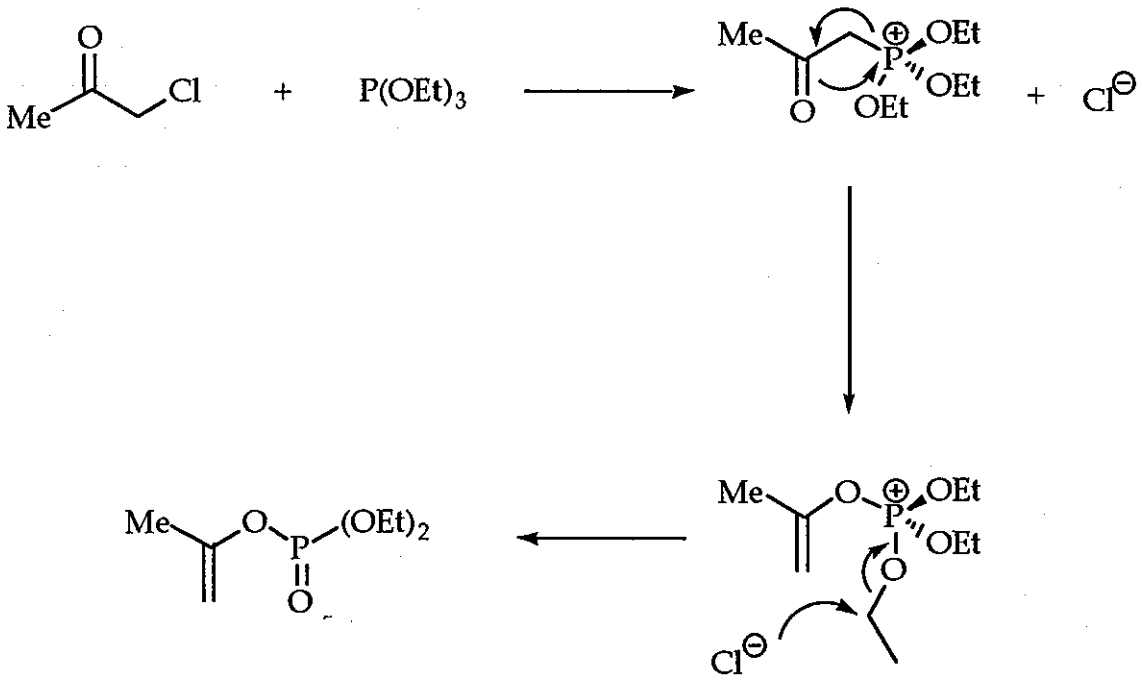
Subsequent dissociation of the alkyl ester group yields a phosphorus-oxygen double bond, this bond formation providing the driving force for the reaction. The displacement of the alkyl group usually proceeds through an S_N2 mechanism with the halide ion attacking the alkyl group.[24] If however the alkyl group is capable of stabilising a carbocation, then it is possible for an S_N1 mechanism to take place (Scheme 12).



Scheme 12

The reactivity of organic halides in the Michaelis-Arbuzov reaction follows the sequence; RCO-Hal > RCH_2-Hal > R_2CH-Hal >> R_3C-Hal; RI > RBr > RCl. There are only a few examples of secondary alkyl halides reacting to yield phosphonates. The role of the halide is one of both a leaving group and then a nucleophile in the dealkylation step.

Saturated α-chloro and α-bromo carbonyl compounds react with trialkylphosphites to give little phosphonate product. Instead, they react to form a phosphorus-oxygen bond with the carbonyl oxygen. The procedure known as the Perkow reaction affords a dialkyl vinyl phosphate.[25] The mechanism is considered to involve initial nucleophilic displacement of the halide by the phosphorus atom, followed by intramolecular rearrangement resulting in a new phosphorus-oxygen bond (Scheme 13). Finally, the displacement of oxygen yields the vinyl phosphate.



Scheme 13

α-Iodoketones react to give the Arbuzov phosphonate product. This highlights the greater nucleophilicity of iodide, which is able to displace the phosphonate to form the phosphorus-oxygen double bond before the phosphonium cation is attacked by the carbonyl oxygen.

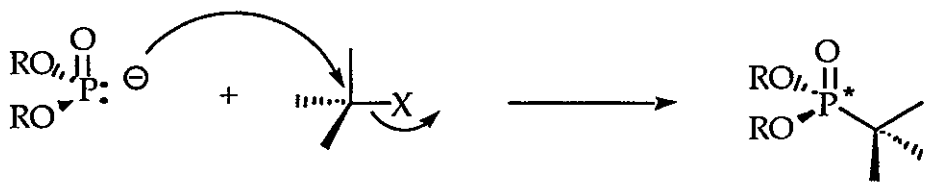
It is possible to influence the type of products formed when *p*-substituted benzyl α-bromo/α-chloro ketones react in this way by adjusting the type of substituents in order to stabilise or destabilise the phosphonium cation. By increasing the electron withdrawing effects of the substituents, one can destabilise the phosphonium cation rendering it more susceptible to attack by the carbonyl group, resulting in a Perkow phosphate product. Conversely, placing electron releasing groups in the para position increases the amount of Arbuzov phosphonate product.

The second important route to alkyl phosphonates is the Michaelis-Becker reaction. In this process, a dialkyl phosphite anion displaces an alkyl halide leading to the formation of a dialkyl phosphonate (Scheme 14).[26] The

mechanism has been shown to proceed via the initial attack by phosphorus (rather than oxygen) at the alkyl halide (Scheme15).

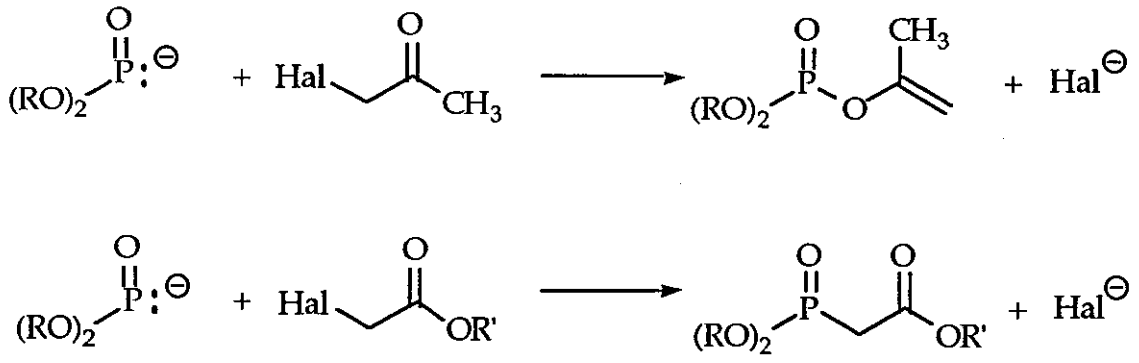
Scheme 14

Sodium hydride is typically employed to generate the anion, and on the addition of an alkyl halide, a chiral phosphorus atom is alkylated with net retention of configuration at its chiral centre. This implies that the anion retains significant stereochemical integrity in solution. Displacement of the halide occurs in an S_N2 fashion.[27]



Scheme 15

Tertiary amines have also been employed in an alternative method of generating the anion, usually when highly reactive substrates are used. Under Michaelis-Becker conditions, α-halocarbonyl compounds generally proceed to give Perkow type products,[28] whereas α-halocarboxylic esters[29] and α-halophosphonate esters[30] proceed with simple displacement (Scheme 16).



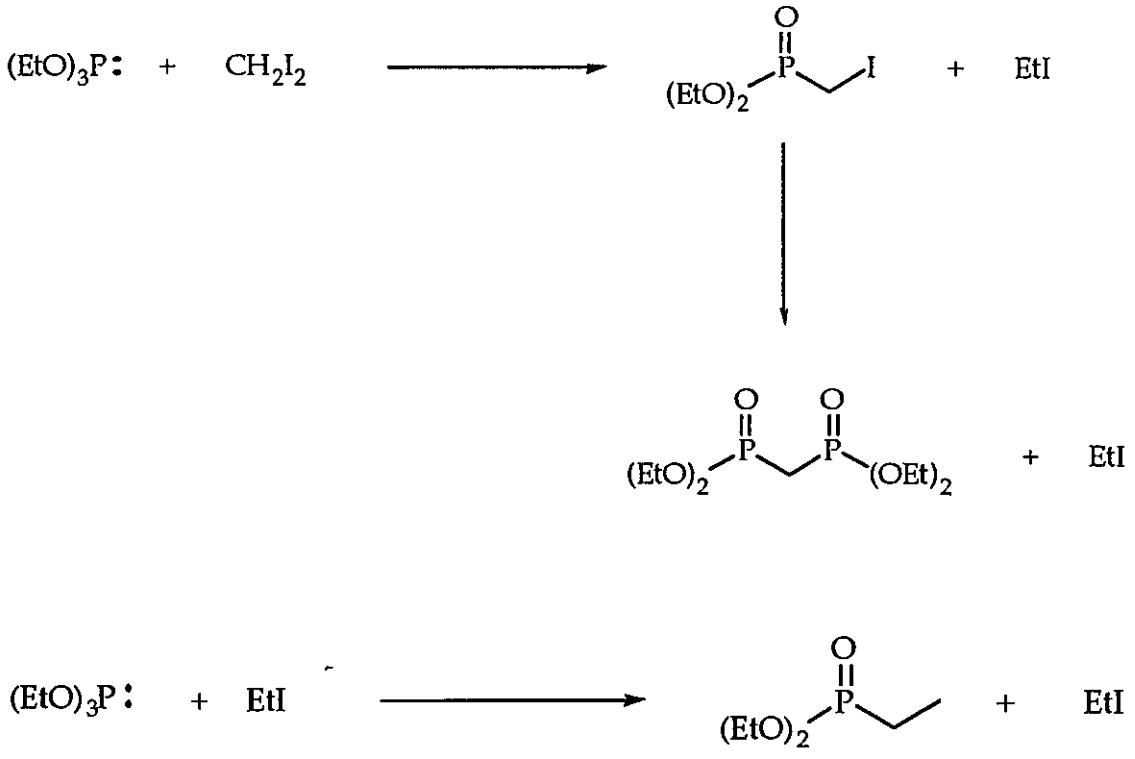
Scheme 16

Other leaving groups used in the Michaelis-Becker system include acetates and tertiary amines.[30] Epoxides and aziridines are also suitable substrates yielding β-hydroxyphosphonates and β-aminobisphosphonates respectively. Due to the low solubilities of the metal salts of the phosphorus acids in aprotic solvents, toluene and xylene are the most commonly employed solvents.

1.6 Synthesis of geminal bisphosphonates

The synthesis of the basic bisphosphonate unit has been achieved through several strategies. The two main approaches are a one-pot procedure in which both phosphonate moieties are introduced under the same reaction conditions, and a procedure where the phosphonate groups are introduced into the molecule in two different steps. The methods designed to synthesise the geminal bisphosphonate in a one-pot procedure involve either the Michaelis-Arbuzov reaction or the Michealis-Becker reaction.

Kosolapoff *et al* have developed a synthesis using diiodomethane and triethylphosphite.[32] The procedure requires a large excess of triethylphosphite to obtain an 18% yield. The low yield can be explained by the ethyl iodide by-product acting as a substrate for a further Arbuzov reaction, yielding more ethyl iodide (Scheme 17).

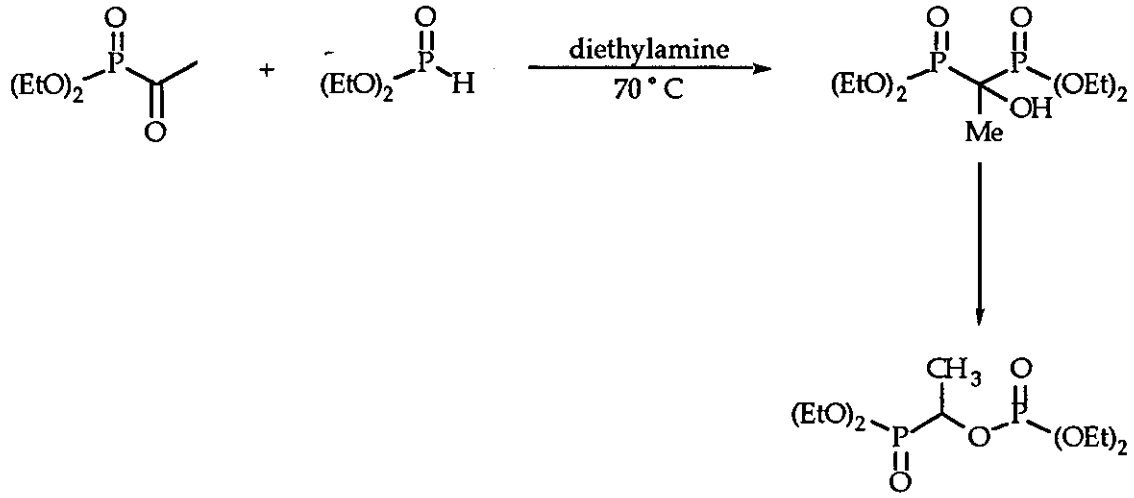


Scheme 17

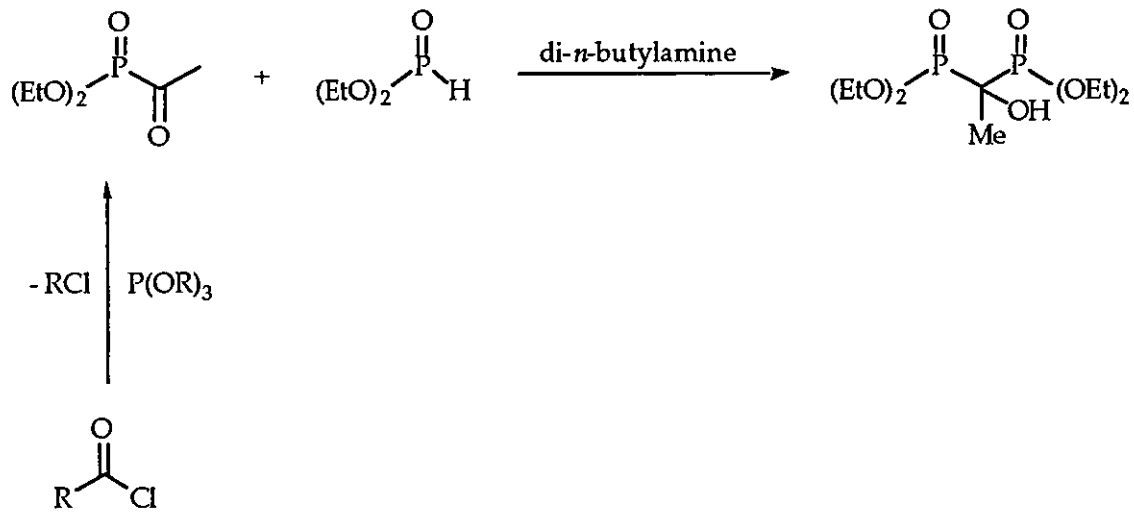
A Procter and Gamble patent covers the synthesis of tetra (secondary alkyl) methylene bisphosphonates using the Michaelis-Arbuzov reaction.[33] Tri-isopropyl phosphite and dibromomethane were gradually heated over 7 h to 185 °C, and the reaction temperature maintained for a further 2 h. The isopropyl bromide by-product was removed by distillation throughout the reaction and the desired tetra-isopropyl methylene bisphosphonate was isolated by distillation (93%), a considerable improvement on previous methods. The patent also lays claims to the preparation of tetra-sec-butyl methylene bisphosphonate in excellent yield (82%).

The first attempted synthesis of 1-hydroxy-methylene bisphosphonates was originally reported by Mconnell and Coover, using a base-catalysed addition of dialkyl phosphites to acylphosphonates.[34] However, under their reaction conditions they were unable to produce the desired hydroxy-methylene-bisphosphonate. Instead they isolated a phosphate-phosphonate compound, which was the product of a base-catalysed rearrangement (Scheme 18)

Nicholson employed a similar idea and reported conditions which gave good yields of the desired 1-hydroxy-methylene bisphosphonate when di-n-butylamine was used as a base.[35] The acylphosphonate was prepared by a Michaelis-Arbuzov reaction from the corresponding acyl chloride and the second phosphonate was introduced into the molecule by a Michaelis-Becker reaction (Scheme 19). The tetraethyl bisphosphonate esters were easily synthesised as these products were not soluble in the cold ether solvent, and the product simply precipitated out of solution during the reaction. The short contact time of the 1-hydroxy-methylene bisphosphonate with the basic medium may be a reason for little rearrangement of the product occurring.

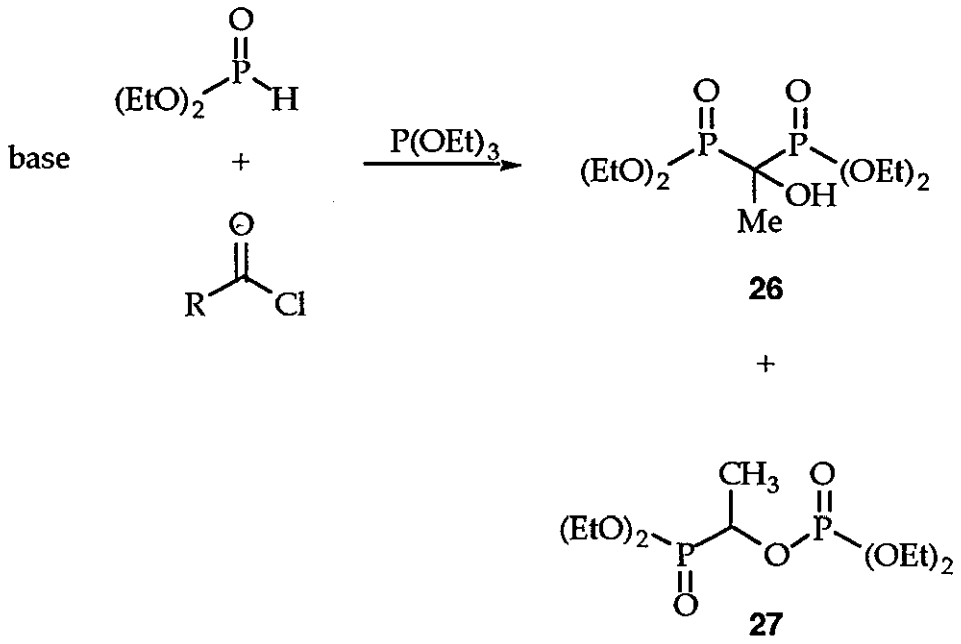


Scheme 18 McConnell and Coover



Scheme 19 Nicholson

Recently, a one-pot Michaelis-Becker procedure has been reported for the synthesis of 1-hydroxymethylene bisphosphonates.[36] An acyl chloride and trialkylphosphite are reacted through to an acylphosphonate, followed by a second Michaelis-Becker reaction to give the desired hydroxymethylene bisphosphonate, or phosphate-phosphonate rearranged product. The reaction conditions are chosen such that the second Michaelis-Becker reaction proceeds to limit the base-catalysed rearrangement (Scheme 20).



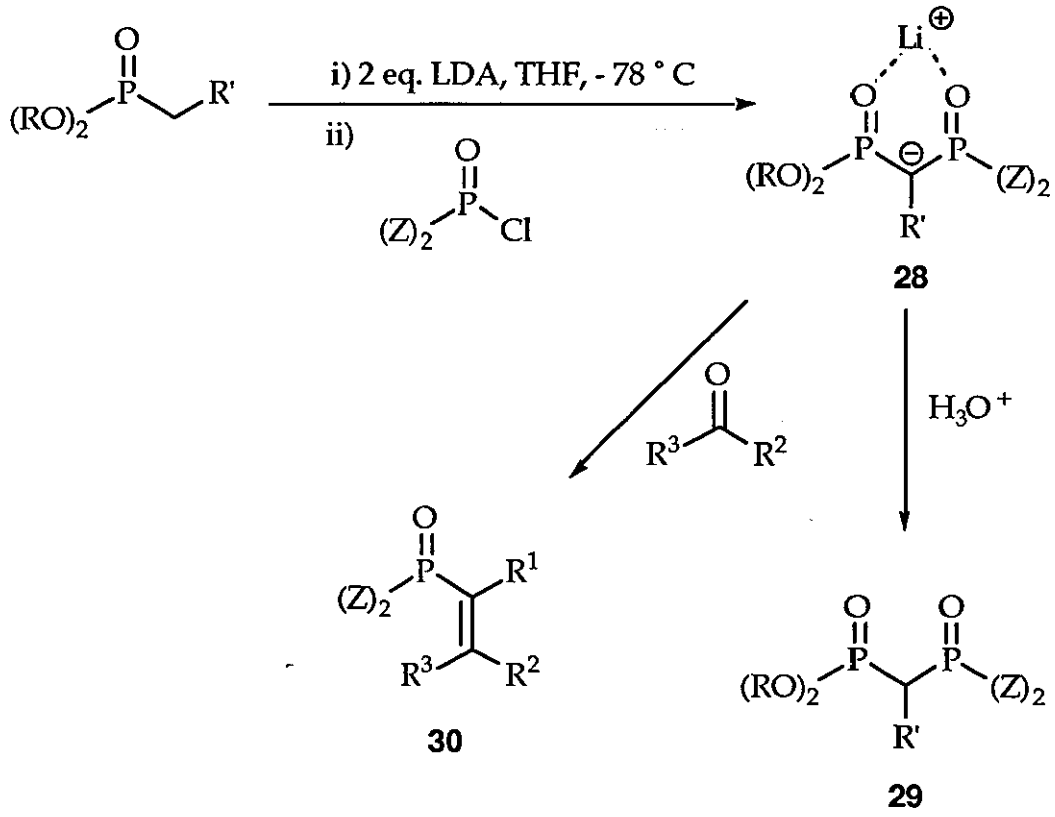
Scheme 20

Base	R	Rxn time (sec.)	Ratio **26:27**	Yield (%)
LHMDS	$PhCH_2CH_2$	10	1 : 25	85
KHMDS	$PhCH_2CH_2$	10	7 : 1	72
LHMDS	$PhCH_2$	10	nd : 1	92
KHMDS	$PhCH_2$	10	6 : 1	93
LHMDS	$CH_3(CH_2)_4$	10	1 : nd	72
LHMDS	$CH_3(CH_2)_4$	150	1 : 5	70
KHMDS	$CH_3(CH_2)_4$	150	6 : 1	68
NHMDS	$CH_3(CH_2)_4$	150	2 : 1	71

Table 3

The choice of counter-ion can control the rearrangement of **26** to **27**; eg., a potassium counter ion restricts the amount of **27** formed. Bulky groups in the α-position of the acid chloride and bulky dibenzylphosphites both favour the rearrangement product.

The introduction of a second geminal phosphonate group into simple phosphonates has been realised by phosphonylation of a phosphonate-stabilised anion, (Scheme 21).[37] The subsequent bisphosphonate anion **28** was either quenched with a proton source to give **29**, or reacted *in situ* with carbonyl groups in a Wadsworth-Emmons fashion for the synthesis of substituted vinyl phosphonates **30**.



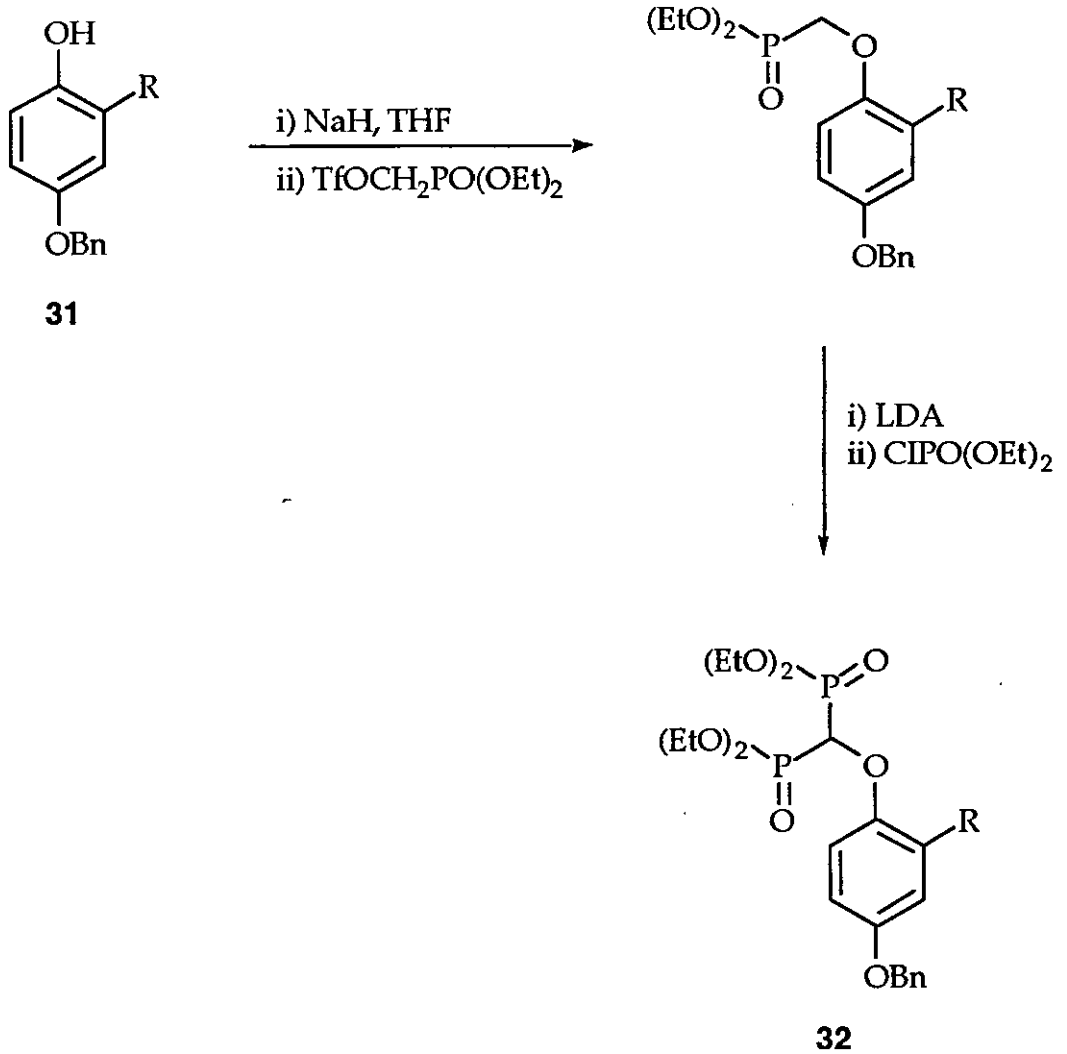
Scheme 21

Z	R'	Yield (%)
C_2H_5O	H	83
C_2H_5O	CH_3	81
C_2H_5O	C_2H_5	69
C_2H_5O	Cl	81
$(CH_3)_2N$	H	73
$(CH_3)_2N$	CH_3	82
C_6H_5	H	84

Table 4

This strategy has since been employed in the preparation of more complex geminal bisphosphonates.[38] The bisphosphonic acid derivatives **3 2** were

synthesised as potential inhibitors of myo-inositol monophosphatase (Scheme 22).



31

32

Scheme 22

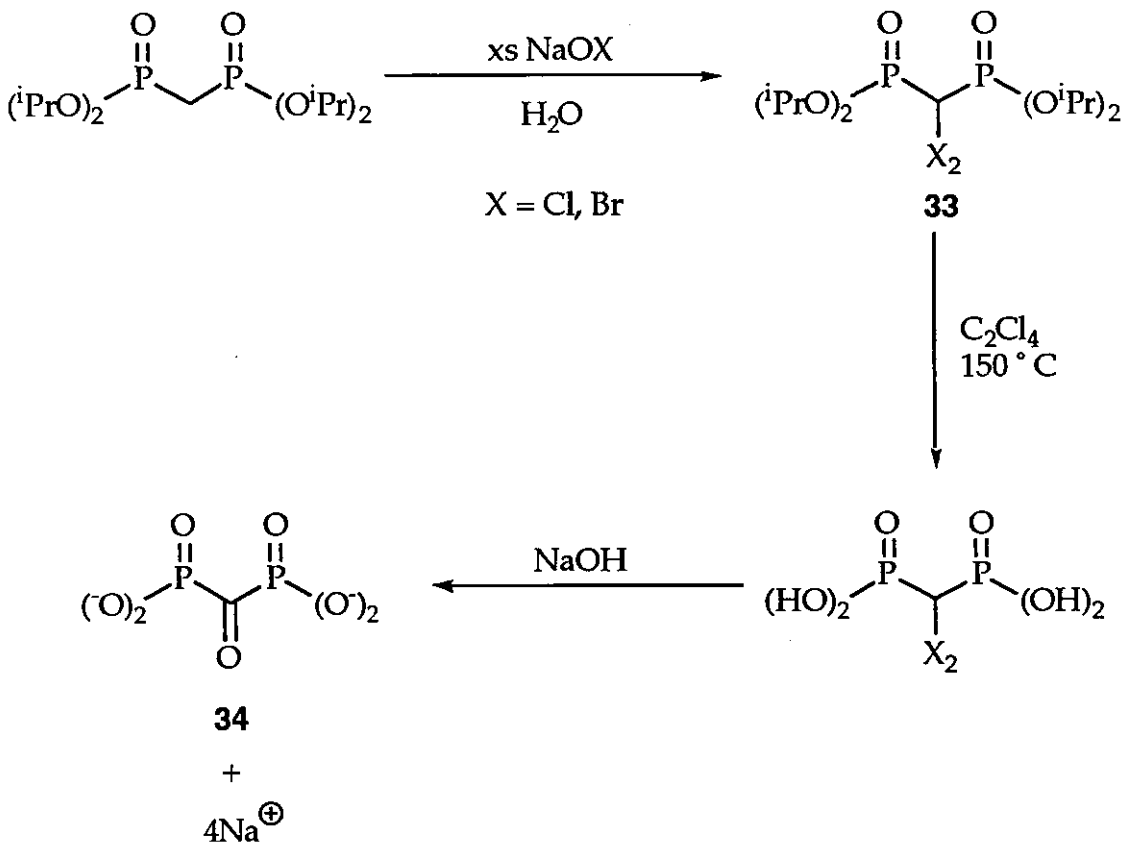
A phosphonate containing a triflate funtionality was used to introduce the phenolic group **31,** by the displacement of the triflate group. The second phosphonate was introduced by phosphonylation of a phosphonate-stabilised carbanion. It is interesting to note that neither of the phosphonate groups in this synthesis were introduced in the traditonal fashion, through Michaelis-Arbuzov or Michaelis-Becker reactions.

Geminal methylene bisphosphonates with larger or more complicated alkyl groups may be synthesised by the reaction of methylene bis(phosphonyl dichloride) with the appropriate alcohol in toluene and pyridine.[39]

1.7 Reactions of tetraalkyl methylene geminal bisphosphonates

The reactions of tetraalkyl methylene bisphosphonates can be characterised as involving either the reaction of the methylene carbon, or the reaction of the phosphonate esters.

An early attempted synthesis[40] of tetramethyl carbonylbisphosphonate through the Michaelis-Arbuzov reaction of phosgene and 2 equivalents of trimethyl phosphite proved unsuccessful.[41] Nicholson, however, reported a synthesis of tetramethyl carbonylbisphosphonate via the di-halogenated geminal bisphosphonate **3 3**.

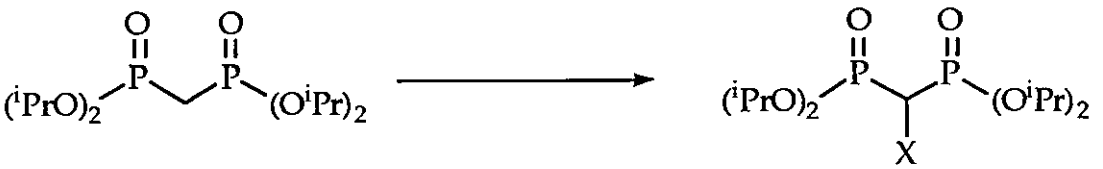


Scheme 23

Pyrolysis of **3 3** followed by the nucleophilic displacement of the halogen atoms with sodium hydroxide yielded the 1,1-dihydroxy methylene bisphosphonic acid. Subsequent basic rearrangement afforded the tetra sodium carbonylbisphosphonate **3 4**.

Reactions of the methylene carbon of the bisphosphonate typically proceed through deprotonation to form an anion which is stabilised by both phosphonate groups. In fact, the anion is stabilised to such an extent that heat is often required for any subsequent reaction with electrophiles.

A lot of research has been reported on the alkylation of the methylene carbon of tetraalkyl bisphosphonates. Such work has concentrated on the effect of altering the base, the alkyl groups on the phosphorus,[42,43] and the effects of the presence of a halide substituent at the methylene carbon, as well as the use of different alkylating agents.[42]



Scheme 24

X	% alkylated by ^{31}P nmr	% isolated yield
CH_3	60-70	20
n-C_4H_9	65-80	242
n-$CH_3(CH_2)_{13}$	80	39
$C_6H_5CH_2$	< 70	26
RO_2C	25-30	5
$C_2H_5OC(O)CH_2$	55-94	93
$(RO)_2P(O)CH_2$	22-28	12
$[(RO)_2P(O)]_2CH$	84	40
Cl_2	30-54	3

Table 5

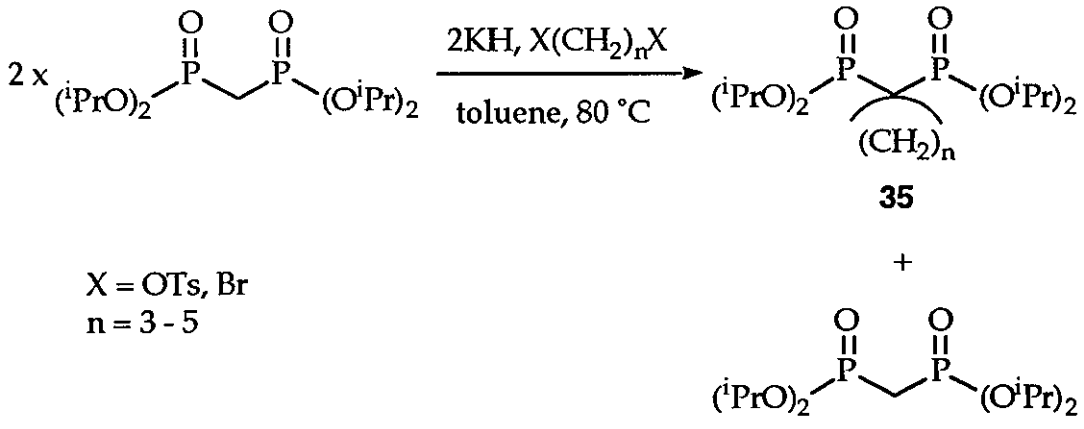
Mono and di-halogenation of tetraalkyl methylene bisphosphonate has been achieved using aqueous alkaline hypohalite solution at room temperature.[42] Control of the reaction to yield mainly a mono-halogenated product was achieved by means of a two phase reaction system. The aqueous phase contained a high concentration of electrolyte, (eg. K_2CO_3), which reduced the total bisphosphonate concentration in that phase. The mono-halogenated product was found to be less soluble in the aqueous phase than the tetraalkyl methylene bisphosphonate substrate. Thus, the aqueous phase contained mainly unreacted tetraalkyl methylene bisphosphonate and a small amount of mono-halogenated product, preventing over-halogenation occurring. Di-halogenated bisphosphonate can be obtained quantitatively by using greater than 2.2 equivalents of hypohalite.

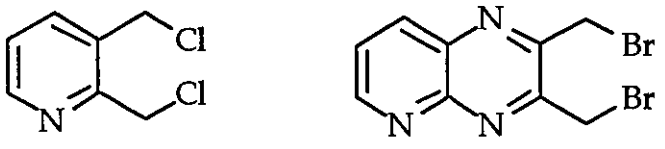
The mono-halogenated derivatives have been employed in subsequent alkylation reactions. The halogenated bisphosphonates have one acidic proton which may be easily removed, which simplifies the problem of over-alkylation. It also makes the study of the effects on alkylation, such as different counter cations and different alkyl ester groups, somewhat easier. Thallium appears to the counter-cation of choice,[43] side-reactions occurring with the use of the sodium and lithium species.[44]

The mono-chloronated derivative of tetraalkyl bisphosphonate was used to study the effects of different alkylester groups on alkylation.[43] It was found that alkylation was more rapid with primary alkyl halides when the alkyl ester groups were isopropyl. The author postulates that the two phosphoryl groups and the central carbon atom are unable to attain co-planarity due to the bulkiness of the four isopropyl groups. The anion formed by deprotonation cannot, therefore, delocalise over the phosphoryl groups, leading to increased activity of the anion over the tetramethyl and tetraethyl analogues. When secondary halides were used as alkylating reagents, it was found that the yields were dramatically lowered, presumably due to steric hindrance.

Certain bisphosphonates are believed to cause mineralisation defects in mammals. Bisphosphonates which remain bound to the bone surface for a long period of time are more likely to cause such defects. Those bisphosphonates which contain a methylene carbon atom in a cyclic system are known to have a lower affinity for bone compared to acyclic bisphosphonates. It was considered desirable by scientists to prepare bisphosphonates with a lower affinity for bone, so reducing mineralisation defects. Hence, if such a compound could maintain high antiresorptive potency then it could be a potentially useful drug.

In view of this, Ebitino reported the synthesis of bisphosphonates with a cyclic methylene carbon atom.[45] He employed the alkylation of a bisphosphonate anion with an alkyl di-halide or di-tosylate.



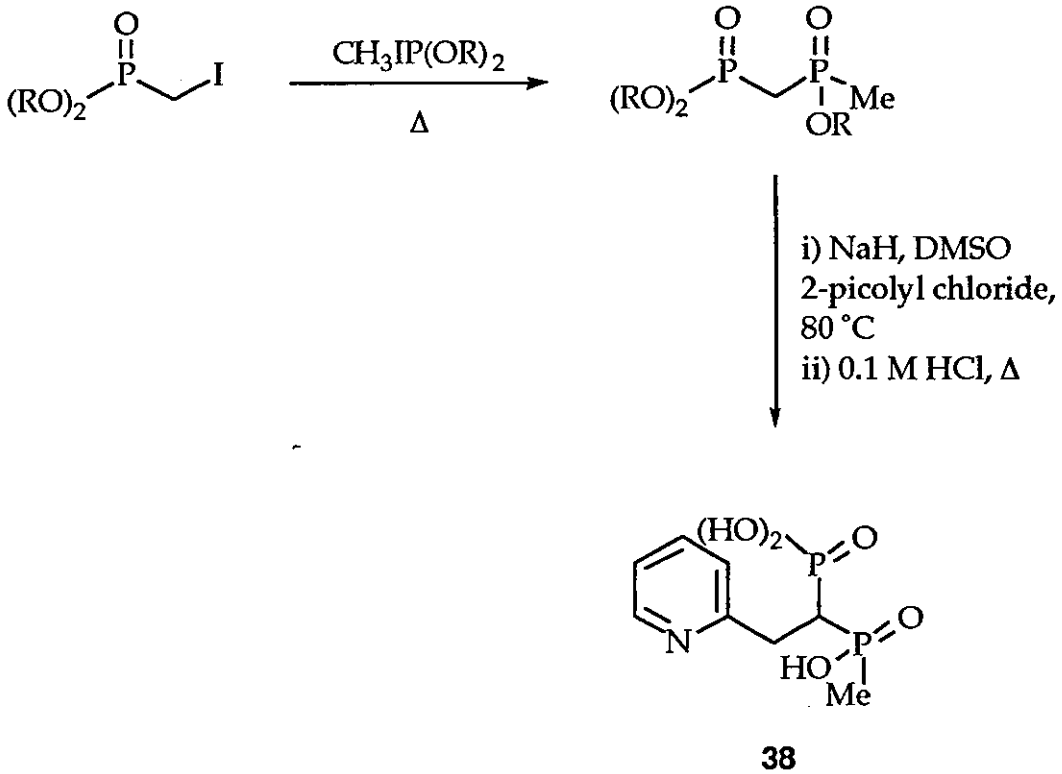


36 **37**

Scheme 25

The product **35** was isolated in good yield (70%) when 1,3-propanediol tosylate was used as the alkylating agent. The cyclo-alkylation reaction was also successful when the alkylating agent contained heterocycles such as **36** and **37** (Scheme 25).

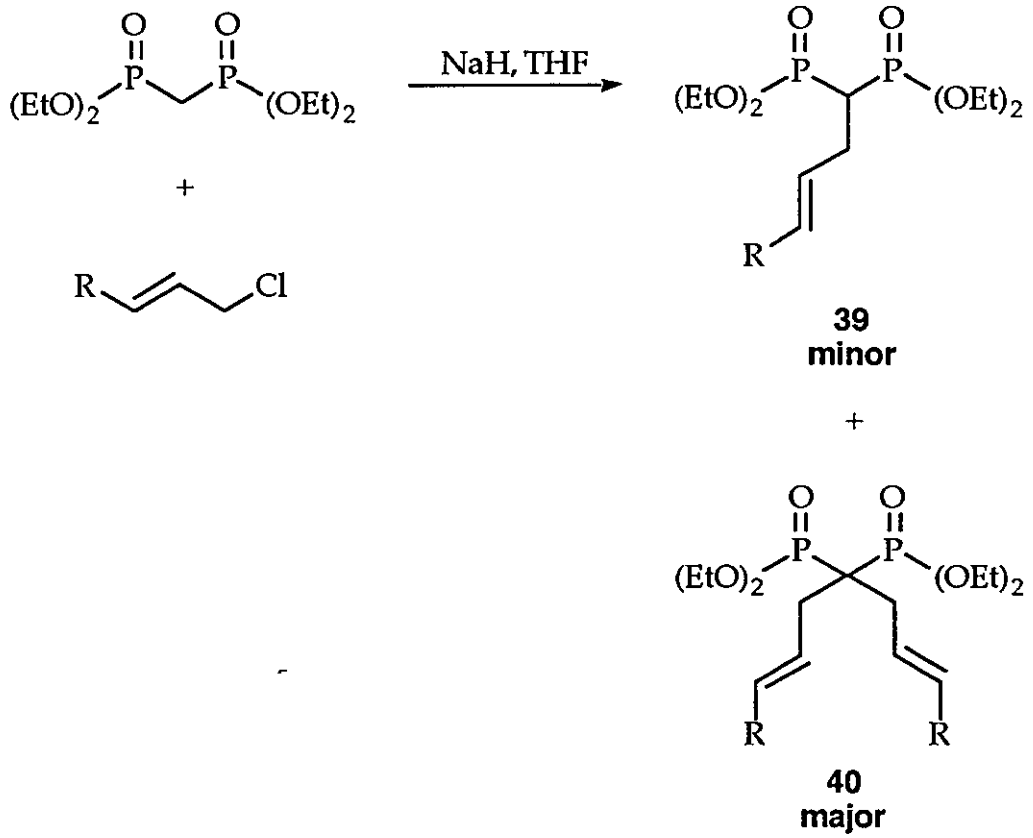
The synthesis of phosphonate-phosphinate derivatives **38** has equally been reported, in the search for compounds of reduced bone affinity to minimise mineralisation defects (Scheme 26).



38

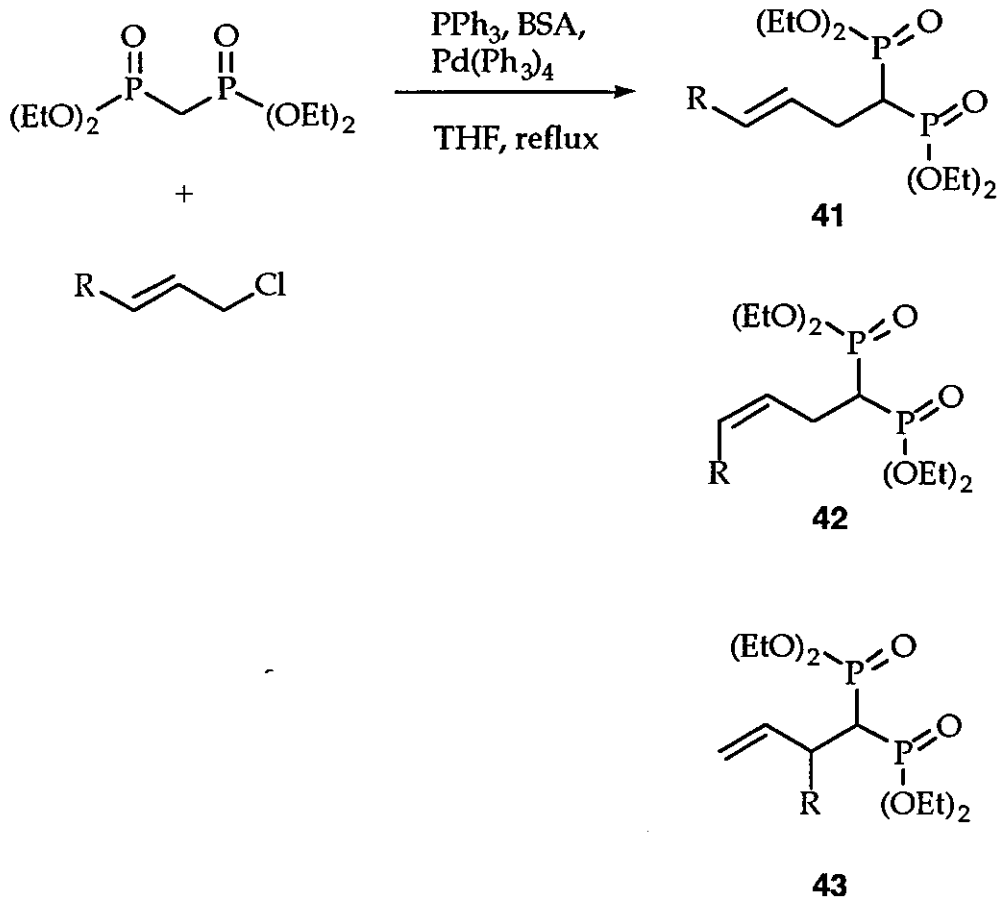
Scheme 26

Dialkylation predominates when allylic halides are used as electrophiles, even when an excess of bisphosphonate is used.[46] The ratio of mono-alkylated **39** to di-alkylated **40** for the reaction of a two-fold excess of tetramethyl methylene bisphosphonate using sodium hydride as a base, with cinnamyl chloride was 34:66 (Scheme 27).



Scheme 27

Magnin, however, developed a procedure to prepare mono-alkylated bisphosphonates exclusively, using allylic halides and a palladium (0) complex.[46] The synthesis involves the reaction between either primary or secondary acetates with an excess of tetramethyl methylene bisphosphonate, O, N-bis(trimethylsilyl) acetamide and 5 mol% tetrakis (tri-phenylphosphine) palladium. Carried out in tetrahydrofuran under reflux conditions, this method provides Z olefins stereoselectively when cinnamyl or, 2,4-dienylacetates are used. Non-conjugated allylic acetates produce mixtures of E **41** and Z **42** isomers (Scheme 28). The alkylation proceeds selectively at the primary carbon atom of the allyl system, rather than yielding the tertiary product **43**. This regioselectivity is due to the steric bulk of the bisphosphonate anion nucleophile.

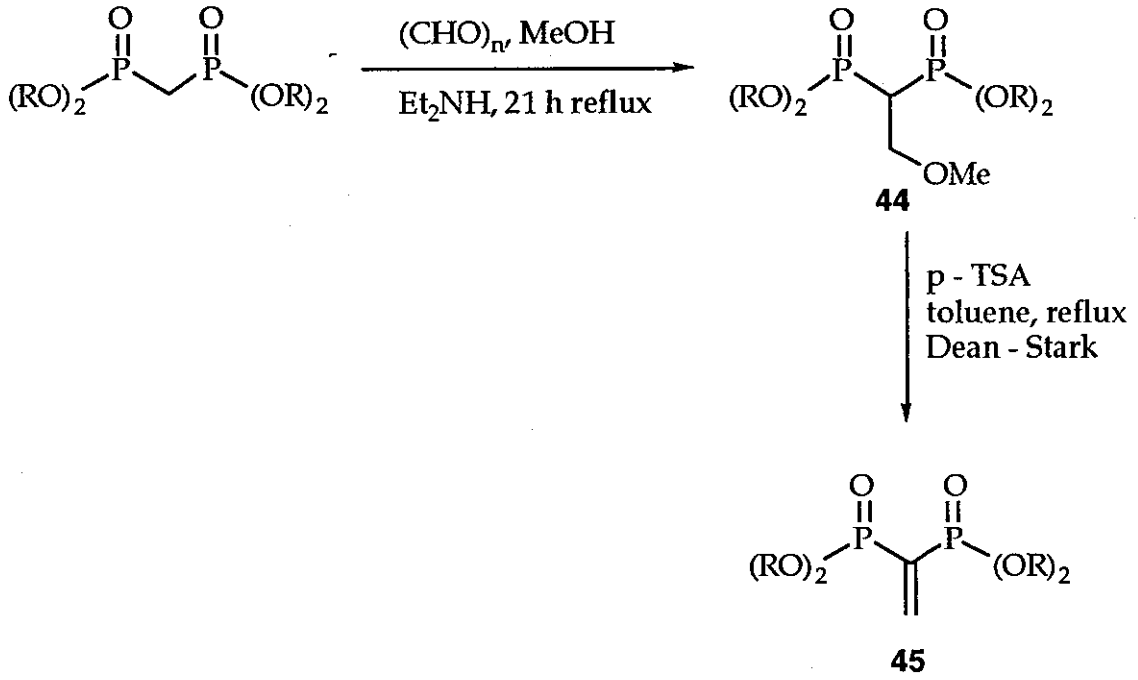


Scheme 28

When sodium hydride was used in place of O, N-bis(trimethylsilyl) acetamide in the palladium-catalysed reactions, there was a dramatic effect on the selectivity of the reaction, yielding mainly the dialklylated products. The role of O, N-bis(trimethylsilyl) acetamide in transition metal promoted allylic alkylations is not fully understood. However, Trost has suggested that O, N-bis(trimethylsilyl) acetamide serves as a weak equilibrium base in the allylic alkylation reaction. The expected low kinetic basicity of O, N-bis(trimethylsilyl) acetamide and the greater kinetic acidity of tetraethyl methylene bisphosphonate compared to mono-allylated product, may explain the lack of dialkylation. The greater kinetic basicity of sodium hydride allows the formation of the more reactive anion derived from **39**, and therefore **40** is the major product.

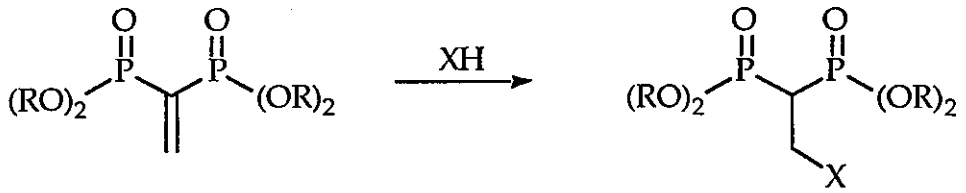
Tetraalkyl methylene bisphosphonates have also been reported to react with paraformaldehyde in an aldol-type reaction.[47] A subsequent acid-catalysed

elimination reaction yields tetraalkyl ethylidene bisphosphonate **45** (Scheme 29), a useful intermediate which has subsequently been used as a Michael acceptor. The reaction between tetraalkyl methylene bisphosphonate and paraformaldehyde typically proceeded to completion with 1 equivalent of diethylamine base and 5 equivalents of paraformaldehyde. The reaction mixture was heated in methanol under reflux conditions for 21 h. Removal of excess formaldehyde, diethylamine and solvent under reduced pressure afforded the methoxy ethylene bisphosphonate intermediate **44**. Elimination of methanol was catalysed by *p*-TSA under reflux conditions in toluene. The desired ethylidene bisphosphonate **45** was isolated in high yield (79%).



Scheme 29

Michael additions to tetraalkyl ethylidene bisphosphonates produce C-substituted methylene bisphosphonates (Scheme 30). Hutchinson reported various Michael additions using heteroatom nucleophiles with tetraethyl and tetraisopropyl ethylidene bisphosphonates.[48]

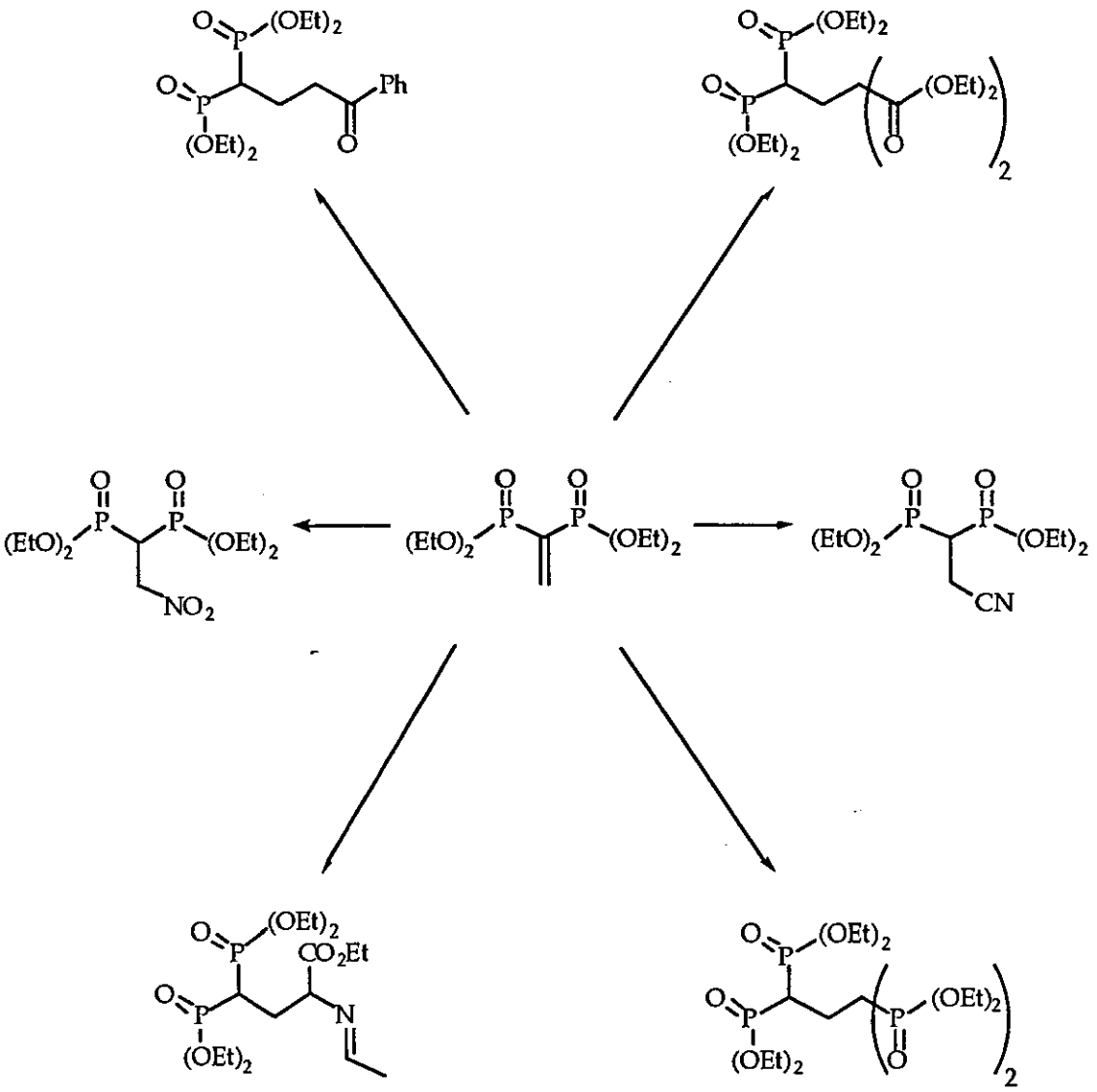


Scheme 30

The reaction using amines, thiols and phosphites all reached completion acording to ^{31}P NMR spectroscopy. Dialkylphosphites required 1 equivalent of diisopropylamine base for the reaction to take place, but in many cases the products from these reactions could not be isolated. The products from Michael additions of amines and phosphites were too unstable, and the reverse reaction took place, eliminating amine and phosphite when chromatography or distillation were attempted. Those products of Michael additions from sulfur nucleophiles, were however stable to chromatography on silica gel and were isolated as colourless oils.

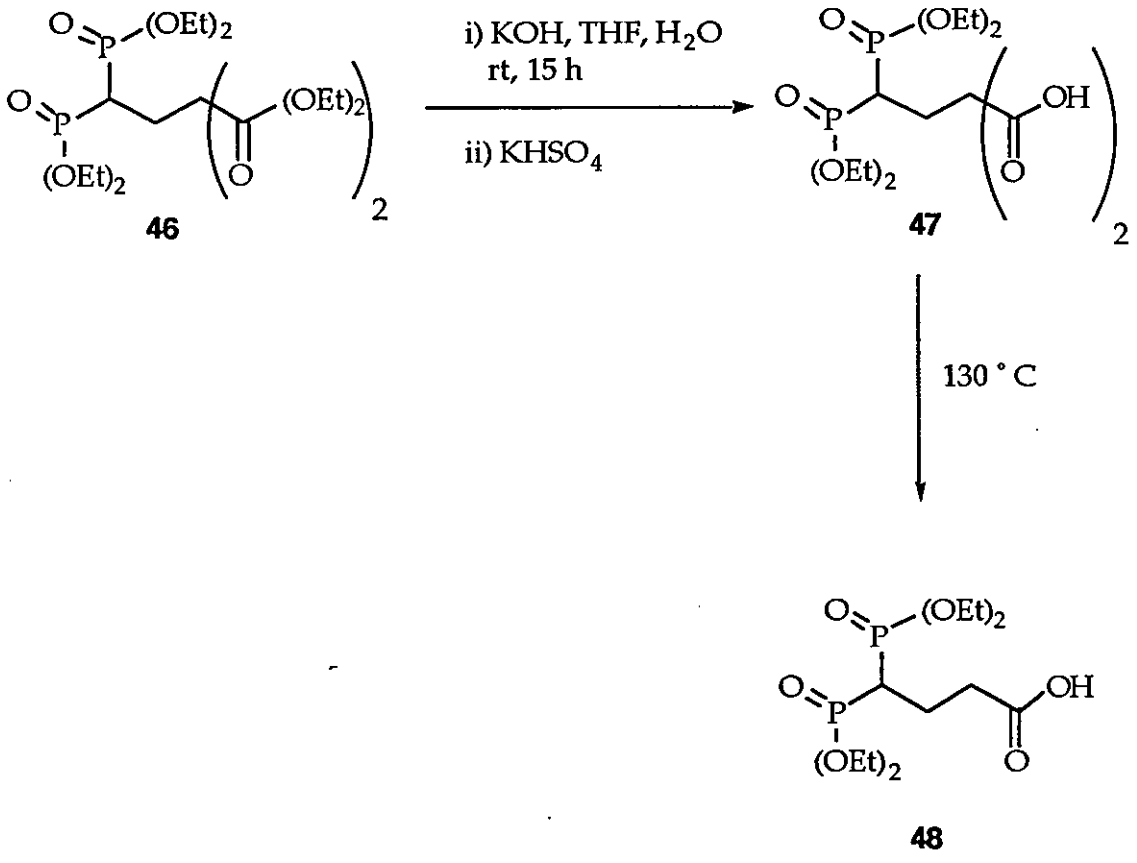
Attempted Michael additions using oxygen and carbon nucleophiles were unsuccessful - treatment of tetraethyl ethylidene bisphosphonate with alkoxide or hydroxide nucleophiles resulted in hydrolysis of the phosphonate esters before conjugate addition could be observed by ^{31}P NMR spectroscopy.

Stabilised carbanion nucleophiles have also been reported to react with tetraethyl ethylidene bisphosphonates, in Michael addition fashion[49] (Scheme 31). These reactions were typically carried out at room temperature, in the presence of a base, and generally reached completion in only 15 min. In most cases, good to excellent yields of addition products were isolated.



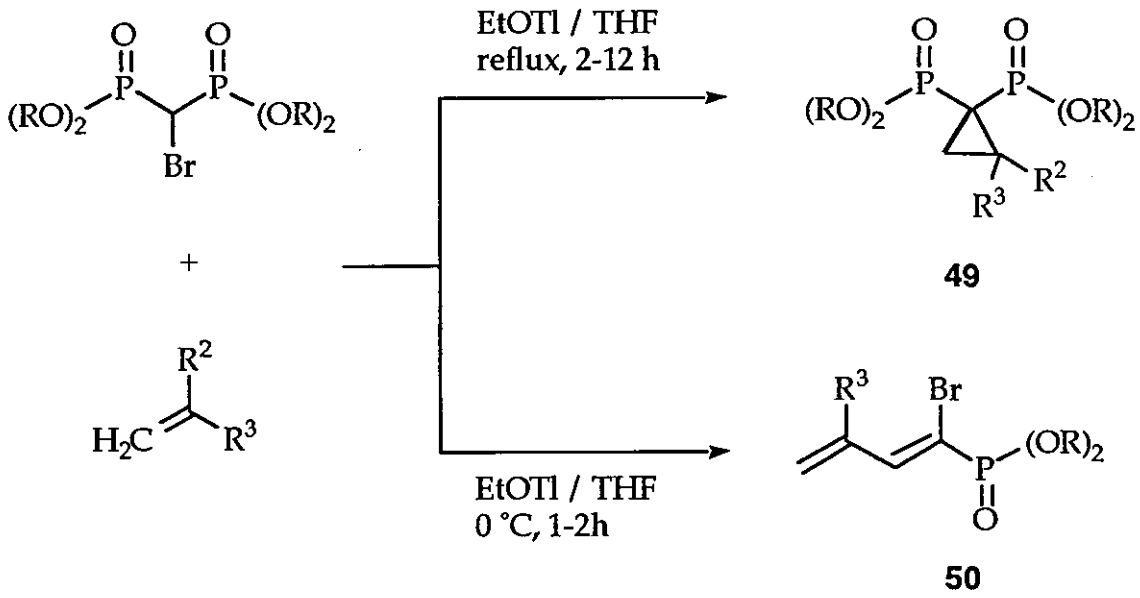
Scheme 31

The products of some of these Michael additions have been utilised in subsequent reactions, the products of which were capable of being funtionalised further. The di-carboxylic bisphosphonate **46** was selectively hydroysed (Scheme 32) to reveal the malonic 1,3-di-acid **47**. De-carboxylation to the mono-acid **48** was achieved by heating the bisphosphonate to 130 °C for approximatley 15 min.



Scheme 32

Yuan has reported the Michael addition of the tetraethyl bromo-methylene bisphosphonate to various electron-deficient double bonds, resulting in the displacement of the bromide anion to form a cyclopropane ring.[50] The use of sodium ethoxide as a base caused many side reactions, and only poor yields of the desired products were obtained. When thallium (I) ethoxide was used, however, the reaction took place smoothly and higher yields of the desired products were isolated (Scheme 33). Employing more reactive olefins also resulted in higher yields of **49**. When alkenes such as acrolein or methylacrolein were used, the carbonyl group reacted in preference to the carbon-carbon double bond, resulting in the formation of the Wadsworth-Emmons product dialkyl Z 1-bromo-1, 3-butadiene-1 phosphonate **50**.



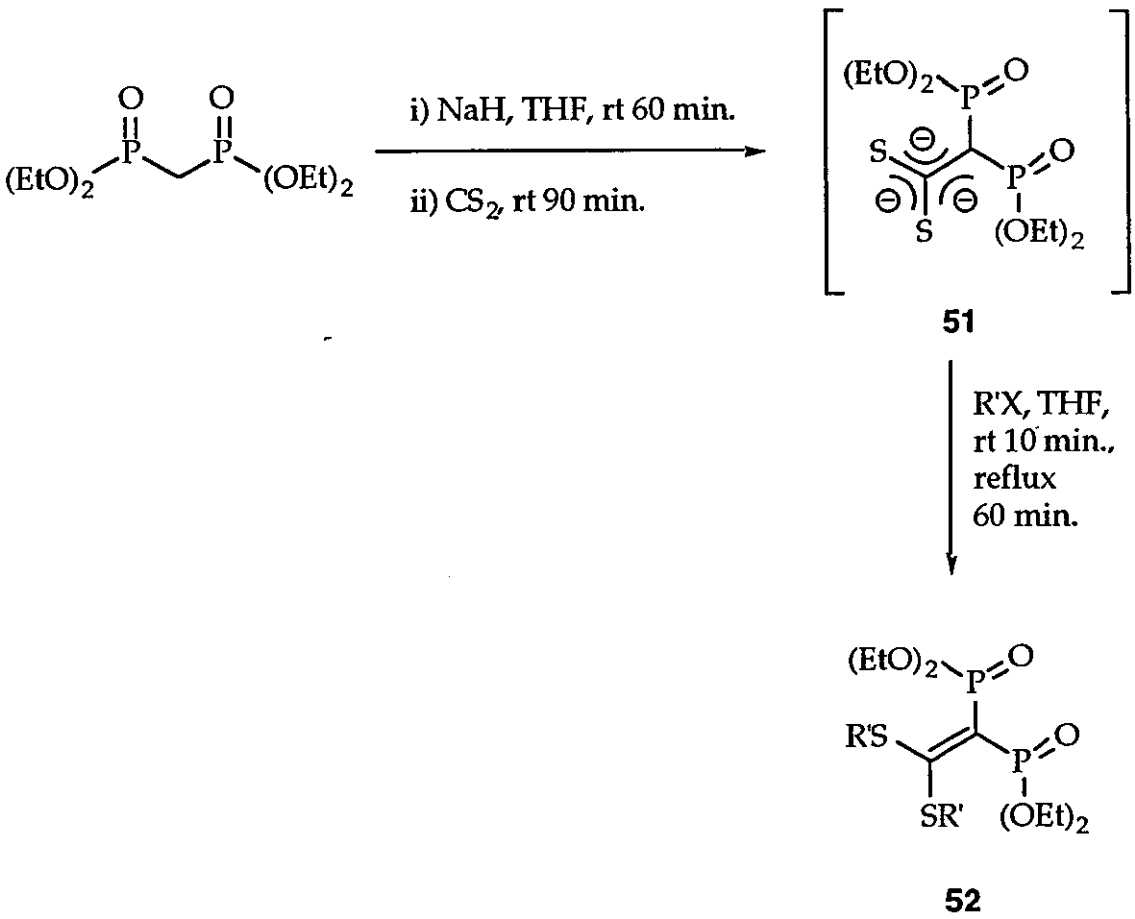
Scheme 33

Methyl vinyl ketone reacts with such bisphosphonate anions under reflux conditions to give both Wadsworth-Emmons and Michael addition products. At lower temperatures (20 °C) however, only the conjugate addition and subsequent cyclisation product was isolated. The table below shows a list of Michael acceptors and the conditions used.

R	R^2	R^3	Temp. / °C (time / h)	Yield /%
Et	CN	H	67 (6)	46
^{i}Pr	CN	H	67 (6)	59
Et	CO_2Et	H	67 (6)	40
^{i}Pr	CO_2Et	H	67 (6)	37
Et	CO_2Et	CN	67 (2)	63
^{i}Pir	CO_2Et	CN	67 (2)	57
Et	CO_2Me	Me	67 (10)	39
^{i}Pr	CO_2Me	Me	67 (10)	38
Et	H	C(O)Me	20 (12)	55
^{i}Pr	H	C(O)Me	20 (12)	63

Table 6

The anion of tetraethyl methylene bisphosphonate reacted with carbon disulfide at room temperature in tetrahyrofuran; further deprotonation of the product gave a dianionic complex **5 1**.[51] This dianion was alkylated with a range of alkylhalides in tetrahydrofuran to give 2,2-bis (alkylthio) ethylidene bisphosphonates, Scheme 34.

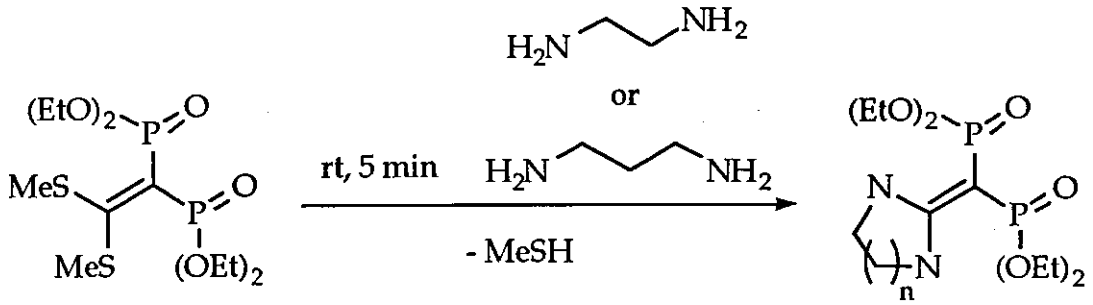


51

52

Scheme 34

The dimethylated product from this reaction was subsequently treated with diamines, Scheme 35. The double addition-elimination reaction was complete in 5 min and the tetraethyl (cyclic-2,2-diaminoethylidene)1,1-bisphosphonates **5 3** were isolated in practically quantitative yields.



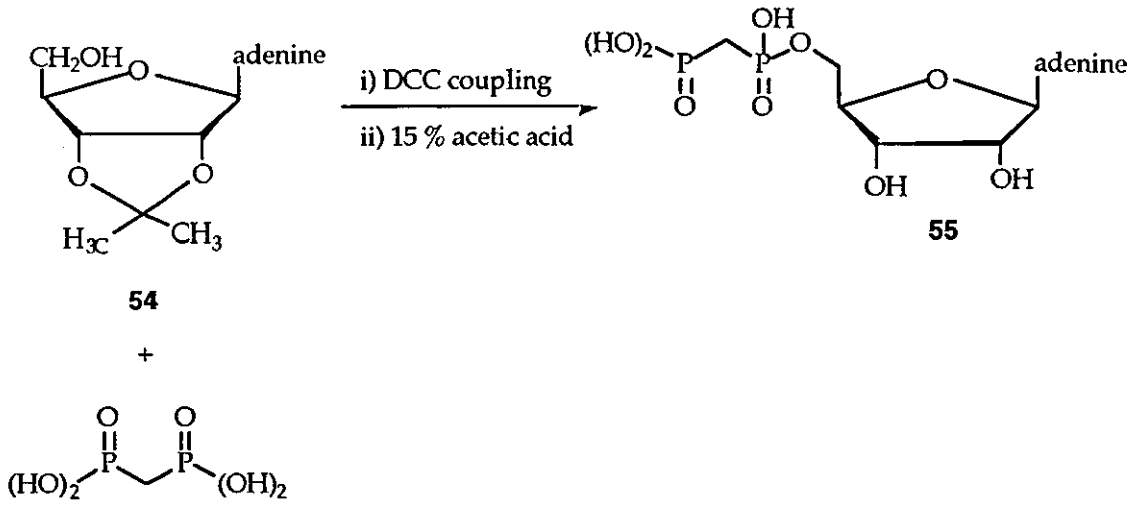
53

n = 1 - 2

Scheme 35

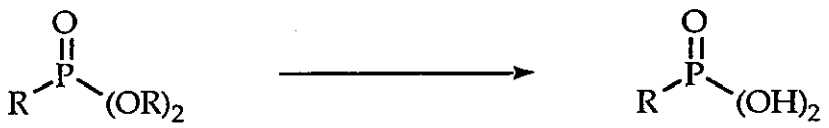
Reactions of methylene bisphosphonates considered above have all involved the interaction of the methylene carbon atom. Examples of reactions with the phosphonate ester groups, and the phosphonic acid groups of bisphosphonates have also been reported. These reactions are generally alkylations of methylene bisphosphonic acids or dealkylations of the tetraalkyl methylene bisphosphonate esters.

Myers has previously reported the synthesis of the bisphosphonic acid analogue **55** of adenosine-5'-diphosphonate.[52] This method couples methylene bisphosphonic acid with isopropylideneadenosine **54** by employing dicyclohexyl carbodiimide (Scheme 36). The reaction was carried out in the presence of excess tri-*n*-butylamine in pyridine, at 60 °C for 14 h. Hydrolysis of the isopropylidene group was achieved with 15% acetic acid and the crude product purified on an ion-exchange column to reveal the desired adenosine-functionalised bisphosphonic acid **55**.



Scheme 36

1.8 Methods of dealkylating phosphonate esters



56 **57**

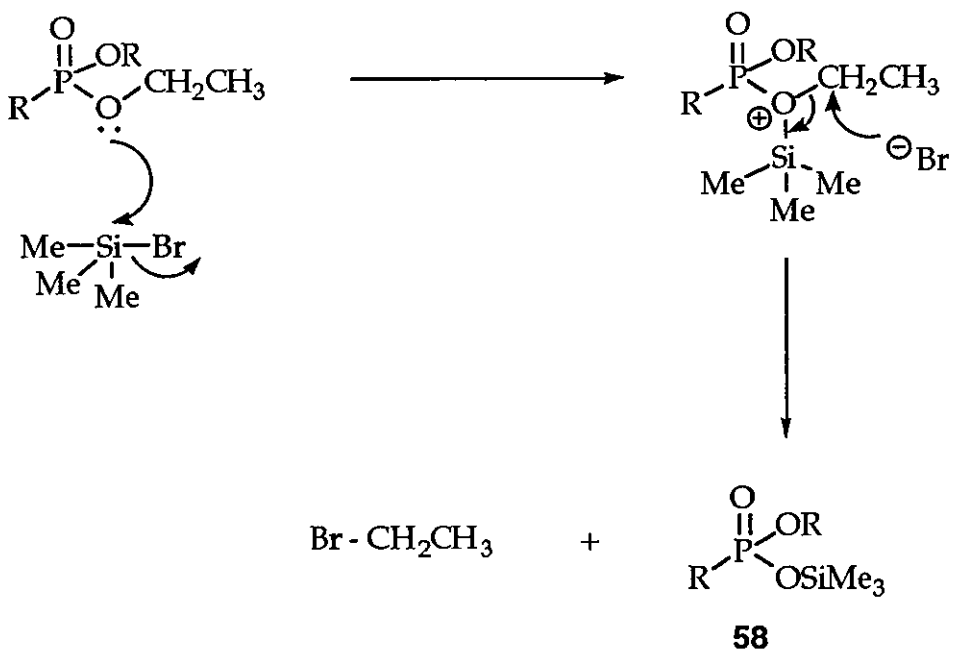
Scheme 37

Phosphonic acids **57** have been traditionally prepared by the dealkylation of their corresponding dialkyl phosphonate esters **56** (Scheme 37). Acid-catalysed hydrolytic dealkylation is effective for this purpose, the most common reagents being HCl[53,54,55], H_2SO_4[56] and HBr.[57,58] However, the harsh conditions required (heating to reflux for 3-10 h) renders this process unsuitable for phosphonates containing delicate functional groups, such as carboxylic esters. Alkaline hydrolysis of phosphonates usually gives only mono-dealkyllated product,[59] as does the use of alkali,[60] or alkaline earth[61] metal halides. Conversion of phosphonate esters to a suitable form susceptible to very mild hydrolysis offers a solution to this problem, provided

that the conversion step itself is facile and compatible with sensitive functional groups.

Over 30 years ago, Rabinowitz[62] showed how trimethylsilyl chloride could be used to prepare bis (trimethylsilyl) phosphonates which subsequently hydrolyse to phosphonic acids on contact with H_2O at room temperature. Although this presented a gentle hydrolysis step, the dealkylation required days, or even weeks, of reflux with excess silylating reagent. Later publications[63,64,65,66,67] have since reinforced the potential usefulness of this method as a reliable route to highly functionalised phosphonic acids.

If one considers the mechanism for the dealkylation of dialkyl phosphonates using trimethylsilyl chloride (Scheme 38), one notices the similarities with that of the Arbuzov reaction. Initial attack of the phosphoryl oxygen on silicon displaces the halide anion, which in turn displaces a phosphonate ester alkyl group from the same molecule, yielding a mixed alkyl-trimethylsilyl diester **58**. A second molecule of trimethylsilyl halide would obviously lead to the bis (trimethylsilyl) phosphonate.



58

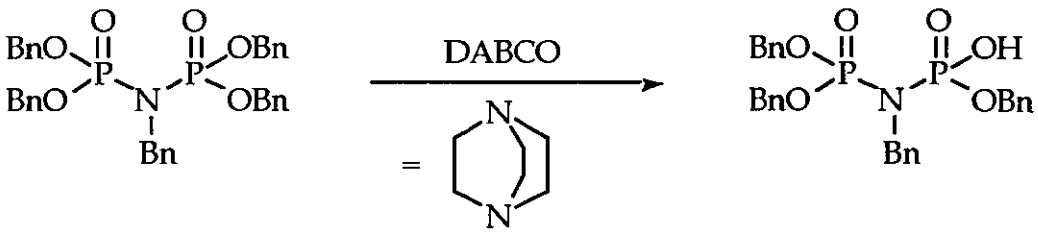
Scheme 38

In view of the fact that the halide acts as both a leaving group and a nucleophile in this mechanism, McKenna reasoned that the use of trimethylsilyl bromide should accelerate the reaction. Indeed, McKenna was able to convert a number of dialkyl phosphonates to their corresponding bis (trimethylsilyl) esters under extremely mild conditions, typically 1-2 h at 25 °C.[68] Furthermore, he reported the success of trimethylsilyl bromide in dealkylating geminal bisphosphonates, which were essentially unreactive with trimethylsilyl chloride under similar conditions. More recently, Roberts[69] and Classen[70] employed trimethylsilyl bromide with their fluorine functionalised bisphosphonates. Long chain alkylidene bisphosphonates have also been shown to be suitable substrates.[71,72]

One could also envisage employing trimethylsilyl iodide in dealkylation reactions since iodide is an even better leaving group than bromide. Indeed, Mimura has reported the hydrolysis of his amino bisphosphonates using trimethylsilyl iodide and water under mild conditions (0 °C, 30 mins. and rt, 1 h).[58] However, since trimethylsilyl iodide also reacts with carboxylic esters, its application is restricted to simple bisphosphonates.

An alternative route to phosphonic acids involves the hydrogenolysis of benzyl phosphonate esters.[73,74,75] The reaction is typically catalysed by 10% palladium on activated charcoal in a suitably polar solvent (usually methanol) at room temperature. This process is not dependent on the formation of silyl ester intermediates or a secondary hydrolysis step, instead all benzyl esters are removed smoothly in one step. Furthermore, it is also applicable to geminal bisphosphonate derivatives.[76,77,78]

The use of benzyl esters also allows a degree of synthetic flexibility. For example, Mioskowski has reported a procedure that allows for selective monodeprotection of various organophosphorus benzyl esters.[79]



Scheme 39

By reacting a multi-benzylated bisphosphonate with 1 eq. DABCO in refluxing toluene for 0.5-2 h, Mioskowski *et al* were able to remove regioselectively only one of the benzyl substituents (Scheme 39). Their work was directed towards the synthesis of analogues of biologically important polyphosphorylated compounds using a strategy in which monodeprotected organophosphorus building blocks are coupled the aglycons prior to final deprotection.

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CHAPTER TWO

Results and Discussion

2.1 Aims and background to project

The aims of this project were to synthesise novel compounds which inhibit tumour-stimulated bone resorption, such as osteoporosis. We hoped to use the high affinity of bisphosphonates for bone matrix to transport an oestrogenic compound to the oestrogen receptor in the bone cells. By targeting our oestrogenic compound in this way, we sought to avoid any unwanted side effects in other tissues. With the Regulatory Drug Authority imposing tighter guidelines on the pharmaceutical industry regarding the safety and efficacy of its products, a physiological rationale such as ours is becoming increasingly more important.

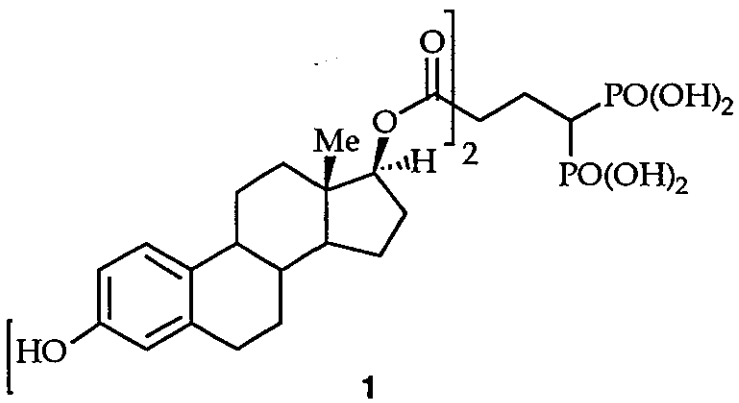
Traditional Hormone Replacement Therapy involves the patient taking a combination drug consisting of two human hormones, namely oestrogen and progestogen. The oestrogen content obviously has beneficial effects in relieving the unwelcome symptoms of menopausal and post-menopausal woman. However, continued intake of oestrogens can lead to proliferation of cell walls, especially in the uterus. Consequently, a small dose of progestogen is taken to help alleviate this problem. Unfortunately though, this progestogen invokes undesirable side effects in patients, ie. facial hair and a deepened voice. There is therefore a need for a treatment that can deliver the oestrogen moiety efficiently and one which causes little or no side effects.

It is important to maintain the bisphosphonic acid moiety for affinity to bone, and therefore the oestrogenic compound must be linked to the methylene carbon atom rather than being linked through oxygen directly to a phosphonic acid group.[1]

There are two main approaches that one can consider when designing such compounds, namely a drug or pro-drug philosophy. The drug approach relies on the whole compound remaining intact until it has interacted with its biologically active site (eg. an oestrogen receptor on the surface of bones), that is the linkage between the bisphosphonate and oestrogen moiety is not cleaved under normal physiological conditions. This approach however,

requires the whole compound to remain oestrogenic so that it can bind to the oestrogen receptors in bone cells. Small changes to steroids can alter their biological activity and the receptors for such compounds are rather specific to individual steroids. We reasoned that it would be unlikely that an oestrogenic compound linked to a bisphosphonate would maintain biological activity.
We therefore chose to adopt a pro-drug approach, in which the linkage between the oestrogenic moiety and the bisphosphonate could be cleaved under normal physiological conditions (pH = 7 , T = 25°C). In this way, the oestrogenic compound would be released before it interacted with the biologically active site, and thereby maintain biological activity.

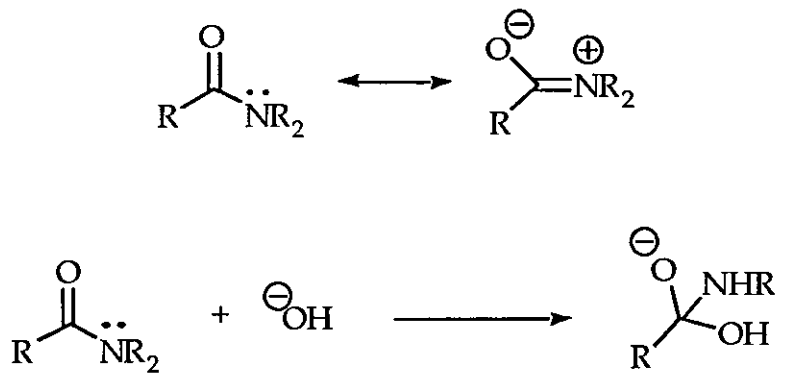
Our initial efforts concentrated on the synthesis of a bis-oestrogenic compound **1**, linked to a suitably functionalised geminal bisphosphonic acid.



1

Other linkages were considered, such as one incorporating an ether bond. However, the robust nature of this bond would render it unsuitable for a pro-drug approach. Amide and carbonate/carbamate are considered more lablie linkages, although conjugates comprising such bonds have been shown to be too stable to hydrolysis under normal physiological conditions.

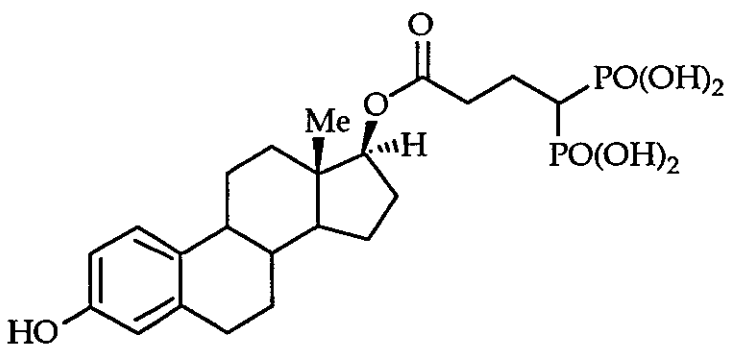
The hydrolysis of amides to carboxylic acids and amines requires considerably more vigorous conditions than those required for the hydrolysis of esters. The reason for this is that the electron releasing nitrogen substituent imparts a very significant stabilisation to the carbonyl group, which is then lost in the hydrolysis of the tetrahedral intermediate (Scheme 1).



Scheme 1

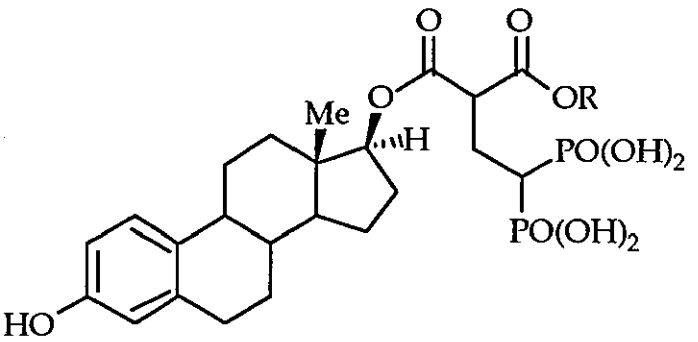
The principal difference lies in the poorer ability of amide ions to act as leaving groups, compared to alkoxides.

We also reasoned that, by reducing the steric bulk surrounding the ester linkage, we could increase the ease by which it was hydrolysed. By synthesising conjugates containing only a single oestrogen moiety such as compound **2**, we sought to enhance the biological activity.



2

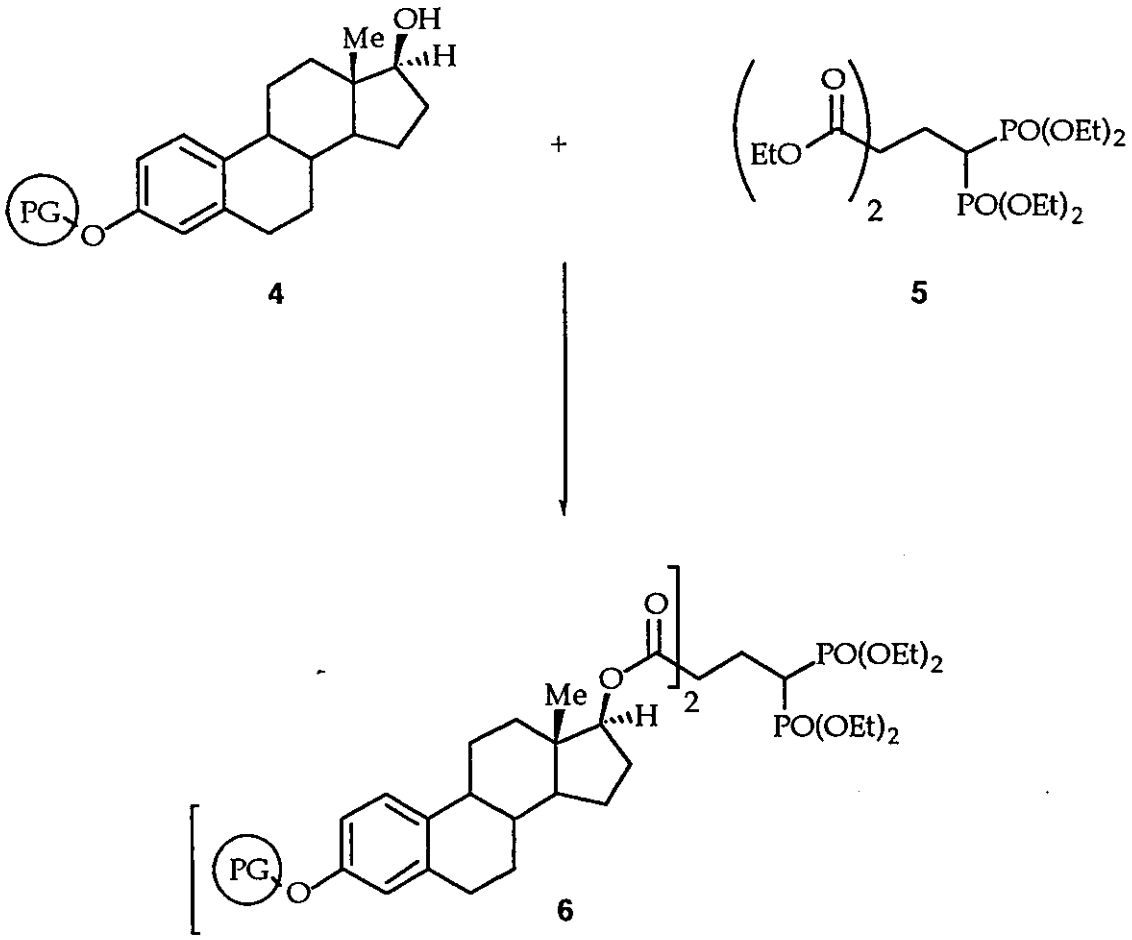
Furthermore, we envisaged preparing compounds such as **3** with a carboxylic ester group adjacent to the steroid, the size of which we could alter to vary the stability of the ester linkage. In this way, we hoped to be able to 'tune' the lability of the ester bond and so prepare numourous oestrogen-bisphosphonic acids with a range of biological activities.



3

2.1.1 Synthetic Strategy

We continued the strategy previously developed within the group for combining the oestrogen and bisphosphonate moieties. This involved coupling a suitable functionalised bisphosphonate **5** with C_3 benzyl- protected 17β-oestradiol **4** through a base catalysed transesterification reaction (Scheme 2). Conjugates joined at the C-17 position such as **6** are known to be more reactive than those joined at the C-3 position.



Scheme 2

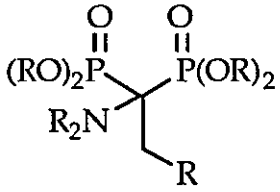
Subsequent deprotection and phosphonate ester hydrolysis would provide us with bisphosphonic acids for biological testing.

2.1.2. Synthesis of bisphosphonate moiety

There is a plethora of examples of functionalised bisphosphonates in the literature. For example, Sturtz et al[2] have prepared sulfur and sulfone derivatives resembling those depicted below (Scheme 3). A German patent by Thomae[3] lays claims to a number of nitrogen functionalised bisphosphonates, incorporating alkyl, aryl, acyl and bromide groups (Scheme 4).

R = alkyl, aryl, cyclic

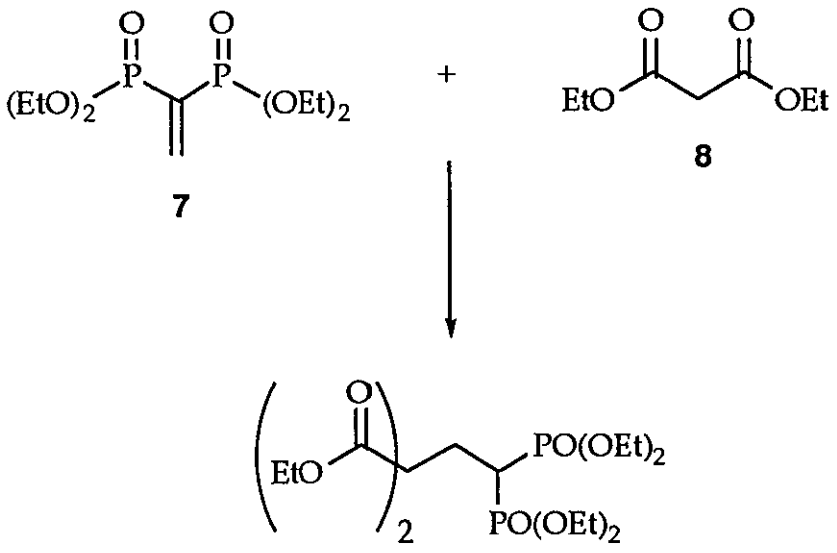
Scheme 3



R = alkyl, aryl, cyclic

Scheme 4

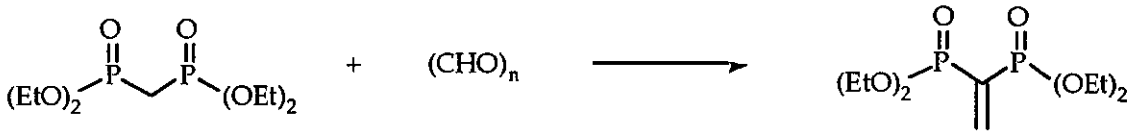
In particular, Sturtz[4] described an efficient route to a dicarboxylate bisphosphonate **5**, employing diethyl malonate **8** and an ethylidene bisphosphonate **7** in a Michael type reaction (Scheme 5).



7 **8** **5**

Scheme 5

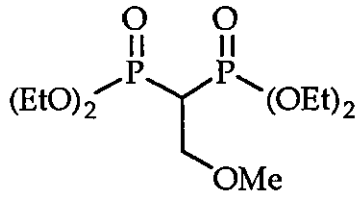
The tetraalkyl ethylidene bisphosphonate is easily accessible by condensing paraformaldehyde with a simple methylene bisphosphonate[5] (Scheme 6).



10 **7**

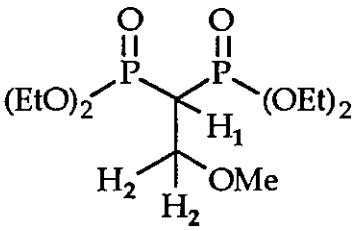
Scheme 6

We were able to prepare bisphosphonate intermediate **7** in large quantities (10 g) in a rather efficient manner (80%). One equivalent of diethylamine was added to five equivalents of paraformaldehyde in methanol, and the mixture warmed at 60°C to aid de-polymerisation. The addition of one equivalent of tetraethyl methylene bisphosphonate **10** produced the methoxy ethylene bisphosphonate intermediate **11** after 18 h. stirring at room temperature.

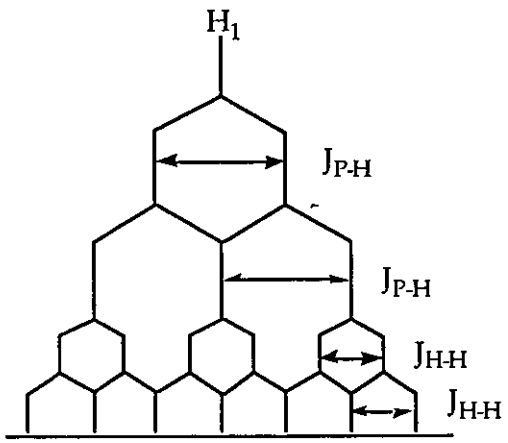


11

The course of the reaction was conveniently followed using ^{1}H NMR spectroscopy. The triplet at ~2.5ppm corresponding to the methylene group of the starting material is replaced by a triplet of triplets at 2.7ppm ($P_2C\underline{H}CH_2OMe$) and a triplet of doublets at 3.9ppm ($P_2CHC\underline{H}_2OMe$) (Scheme 7).



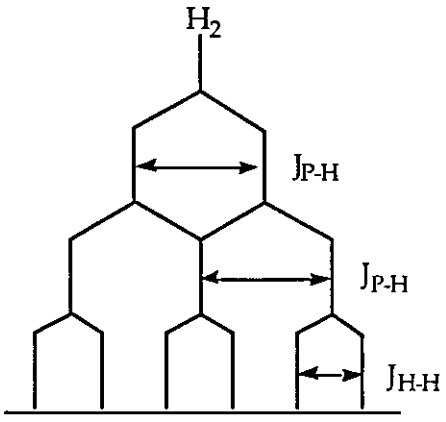
$\delta_P = 21$ ppm



$\delta_H = 3.9$ ppm

$J_{P-H} = 23.9$ Hz

$J_{H-H} = 5.5$ Hz



$\delta_H = 2.7$ ppm

$J_{P-H} = 16.2$ Hz

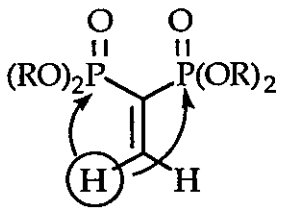
$J_{H-H} = 5.5$ Hz

Scheme 11

The methoxy methylene bisphosphonate intermediate **11** was isolated as a clear oil after any residual methanol had been removed azotropically with toluene (2 x 50 ml). The crude product was sufficiently pure to be taken through to the next stage without the need for chromatography. ^{31}P NMR spectroscopy proved to be a valuable tool for determining its purity (methylene bisphosphonate **10** δ_P = 20ppm, methoxy ethylene intermediate **11** δ_P = 21ppm). A single peak was observed in the ^{31}P spectrum of the product, indicating the presence of only one type of phosphorus atom.

A catalytic amount of p-TSA was added to a solution of bisphosphonate **11** in toluene and the mixture refluxed overnight. The reaction flask was fitted with a Dean and Stark apparatus to collect the methanol forced over by elimination.

It was convenient to follow this reaction by ^{1}H NMR spectroscopy; the complete disappearance of the triplet of triplets and triplet of doublets, and the emergence of a doublet of doublets (6.9ppm) (Scheme 8) indicated that the reaction had reached completion.



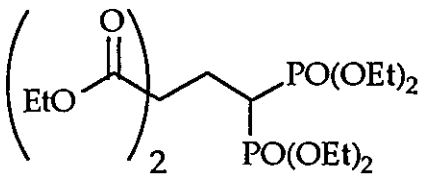
$J_{P\text{-}H}$ (cis) = 37.53 Hz

$J_{P\text{-}H}$ (trans) = 37.39 Hz

Scheme 8

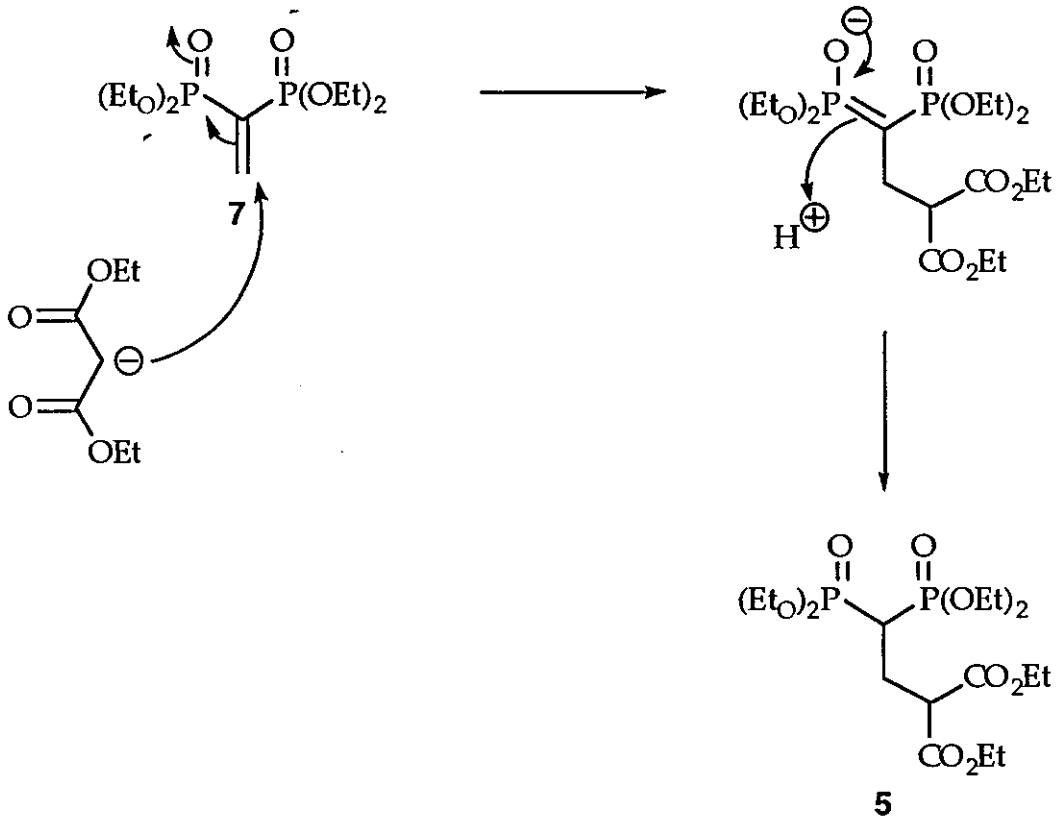
The elimination typically proceeded very cleanly and so the crude ethylidene bisphosphonate product required no purification by chromatography. Residual p-TSA was washed away with water (3 x 100 ml). The purity of the clear brown oil was checked by ^{31}P NMR spectroscopy; a single peak was observed at 11.61ppm.

Sturtz and Guervenou[4] described the highly efficient Michael addition of this ethylidene bisphosphonate with nucleophiles such as ethyl N-benzylidene glycinate, in their route to amine functionalised bisphosphonates. We realised the potential of preparing the dicarboxylate bisphosphonate **5** by coupling diethyl malonate and ethylidene bisphosphonate in a Michael type fashion (Scheme 9), using a catalytic amount of fresh sodium ethoxide as our base.



5

After approximately 30 mins. stirring at ambient temperature, the mixture was neutralised with NH_4Cl solution and the organic fraction extracted with EtOAc. Again, there was no need for further purification by chromatography. Like the previous bisphosphonate intermediates, final traces of solvent were removed after several hours under high vacuum.



5

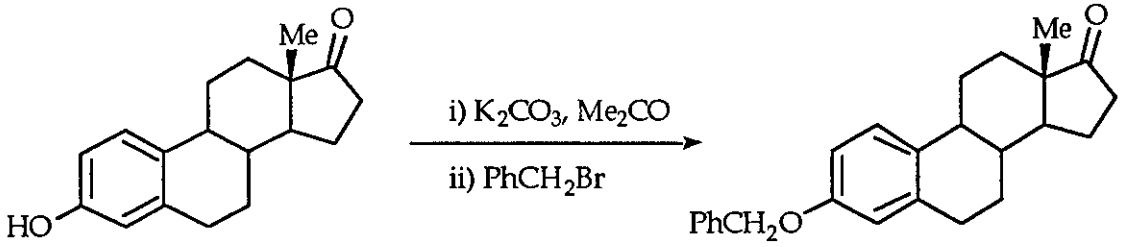
Scheme 9

2.1.3 Synthesis of oestrogenic moiety

To prevent any unwelcome interference in the key transesterification step, it was obviously necessary to protect the hydroxyl group at the C-3 position of 17β-oestradiol. A suitable protecting group should be one which can be easily attached, will be resistant to subsequent chemical reactions, and be conveniently removed at an appropriate stage in the synthesis. There are a number of potential methods of protecting a primary alcohol functional group. One could imagine converting the C-3 hydroxyl to an acetate group by the addition of acetic anhydride and pyridene, or alternatively, forming the silyl ether from TMS-Cl[6].

However, many of these would be unsuitable since their removal requires acidic or basic conditions, which might destroy our ester linkage. Benzyl ethers, on the other hand, are extremely resistant to both acidic and basic conditions and may be cleaved efficiently by simple hydrogenolysis techniques.[7,8]

The potential problem of selectively benzylating only the C-3 hydroxyl group of oestradiol was avoided by choosing oestrone as a starting material.



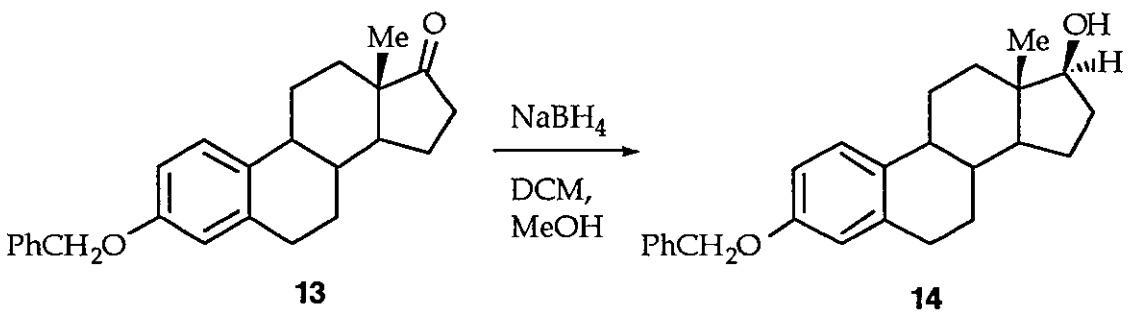
12 **13**

Scheme 10

A solution of potassium carbonate (5 eq.), oestrone and benzyl bromide (1.77 eq.) in acetone was stirred at room temperature for 8 h.. Benzyl bromide is hygroscopic and was stored under 4 A sieves prioir to use. The acidic phenol group was deprotonated under mildly basic conditions; the resulting

phenoxide ion displacing bromide by an S_N2 attack on benzyl bromide. The crude yellow product was purified by recrystallisation from methanol. Any residual benzyl bromide was washed repeatedly with cold methanol, yielding 3-benzyloestrone **13** as a crystalline white solid (60%).

The reducing agent of choice for the conversion of 3-benzyloestrone to oestradiol was the metal hydride, $NaBH_4$.[9] This reagent has two main advantages over other reducing agents. Firstly, it will not reduce carbon-carbon double (or triple) bonds, and secondly, it contains a lot of hydrogen in a small amount of reagent - $NaBH_4$ has four hydrogens usable for reduction.[10,11,12] Sodium borohydride was preferred to its more reactive partner, $LiAlH_4$, since it is more selective. Another advantage of $NaBH_4$ is that it can be conveniently used in water or alcoholic solvents, and so can reduce compounds that are not soluble in ethers.[13]

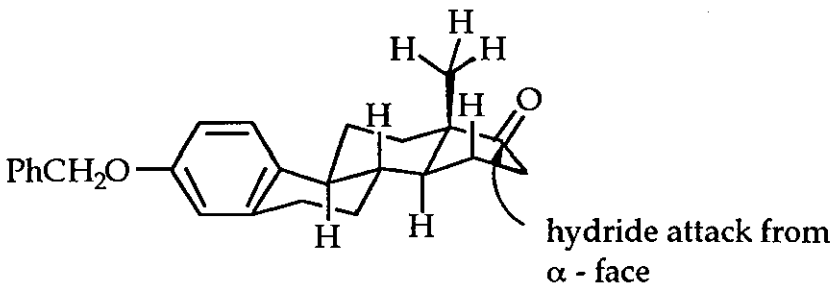


13 **14**

Scheme 11

3-Benzyl oestrone **13** (10 g) was typically dissolved in a dichloromethane/methanol (1:1) mixture, and $NaBH_4$ (3 eq.) was added slowly at 0 °C. After stirring for 3 h. under an atmosphere of nitrogen, the reaction mixture was allowed to cool to room temperature. The reaction mixture was then quenched slowly with cold water. Extracting with dichloromethane and washing with 1 M HCl, afforded an off-white solid which was purified by recrystalisation from methanol to give 3-benzyl 17 β-oestradiol **14** as white crystals. We were able to carry out this simple ketone reduction swiftly and successfully on a large scale (10 g, 69% yield).

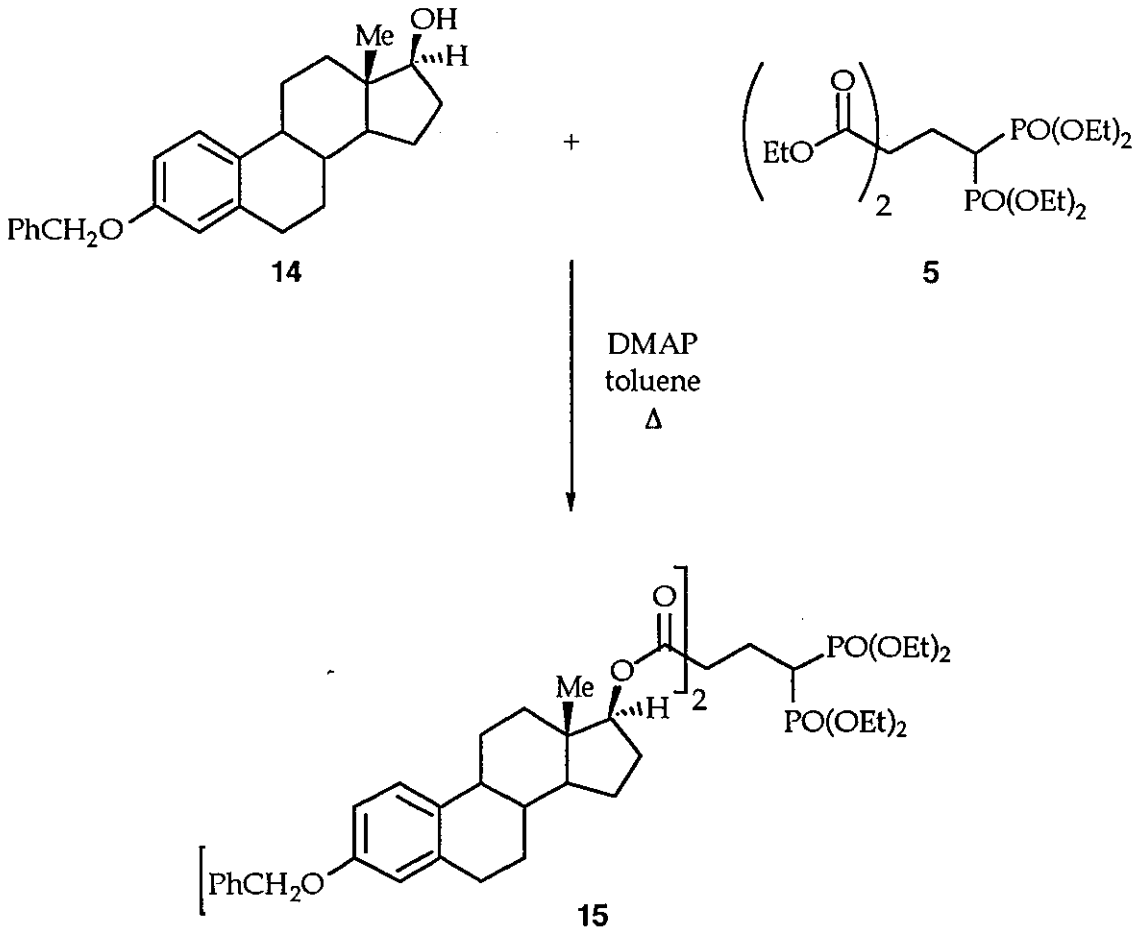
Predictably, we only isolated the 17β isomer since the methyl group at C-13 occupies the upper (β) face, and thus directs hydride attack from the lower (α) face.



Scheme 12

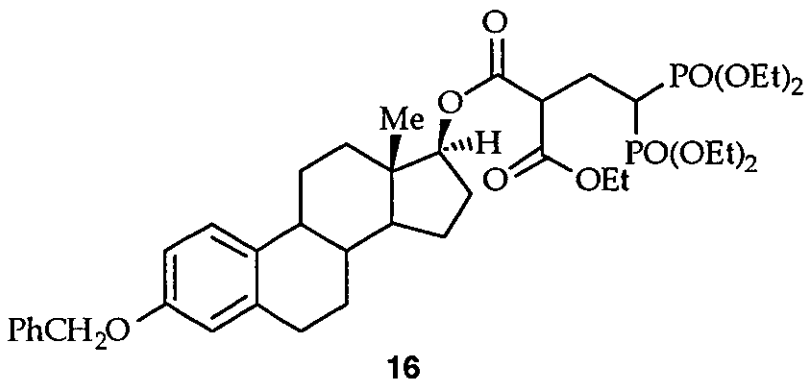
2.1.4 Linking of bisphosphonate and steroid moieties

With our dicarboxylate bisphosphonate **5** and protected oestradiol **14** in hand, we were poised for the key coupling reaction. We employed a traditional base catalysed transesterification reaction, promoted by 1, 4-(N, N) -dimethylaminopyridine.



Scheme 13

Forceful thermal conditions (reflux in toluene 11 days) were necessary in order to drive the reaction to completion. While monitoring the progress of the transesterification by tlc, we noticed that the mono-steroidal conjugate **16** was formed as well.



16

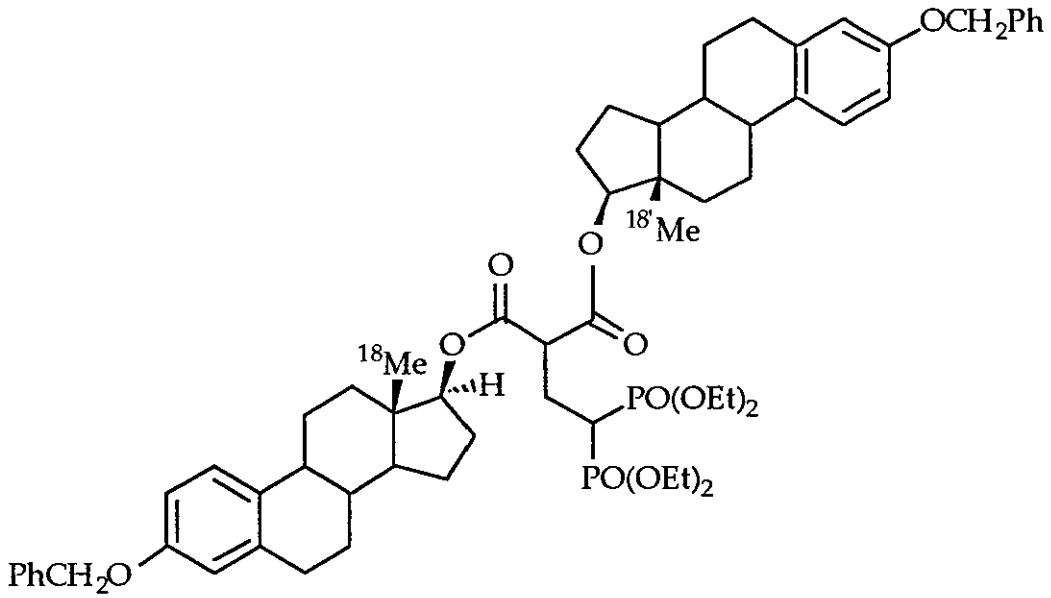
Attempts to synthesise the single oestrogen compound **16** in this way were unfruitful since an equal amount of the bis-steroidal conjugate was always present.

When the reaction was considered to be complete by tlc, the solvent was removed under reduced pressure leaving a dark brown oil. This crude material was purified using silica gel flash column chromatography, eluting with 1-3% methanol in dichloromethane. The desired product **15** was isolated as a viscous brown oil (64%).

^{1}H NMR spectroscopy confirmed the presence of the steroid, with a plethora of peaks at1.20-2.35ppm, and also of the bisphosphonate moiety with its ethyl ester patterns at 1.35ppm (12 H, t, J = 7 Hz, $POCH_2C\underline{H}_3$), and 4.1-4.8ppm (8 H, m, $POC\underline{H}_2CH_3$).

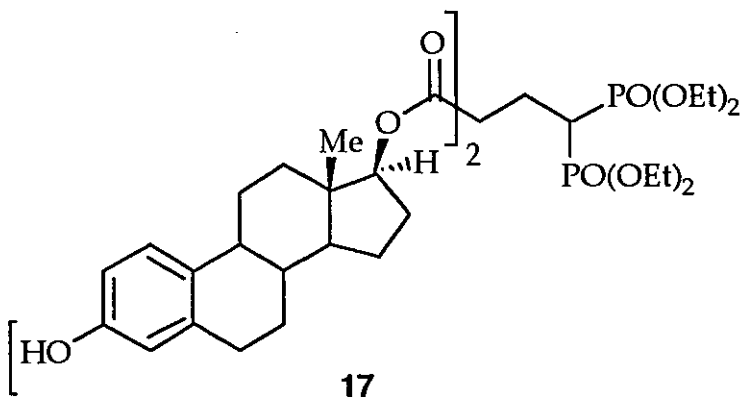
^{31}P NMR spectroscopy was a convenient tool for verifying the purity of our conjugate. It was also possible to conclude that the phosphonate esters had not undergone any transesterification themselves. The chemical shifts of phosphonate-steroid esters would be dramatically different in both ^{1}H and ^{31}P NMR spectra.

Interestingly, the ^{1}H NMR spectrum of the bis-steroidal conjugate **15** displays two singlet peaks for the C-18 methyl protons, indicating hindered rotation about the ester linkage (Scheme 14).



15

The removal of the benzyl protecting groups proved to be trivial. Both benzyl groups were removed by catalytic hydrogenolysis, using palladium upon activated charcoal as a heterogenous catalyst. After 14 h. under a hydrogen atmosphere (1 bar), the hydrogenolysis was complete. The catalyst was removed by filtration, and the crude product purified by silica gel flash column chromatography, eluting with 3-5% methanol in dichloromethane. Final traces of solvent were removed under high vacuum (30 mins.), yielding the debenzylated product **17** as a white foam (63% yield).

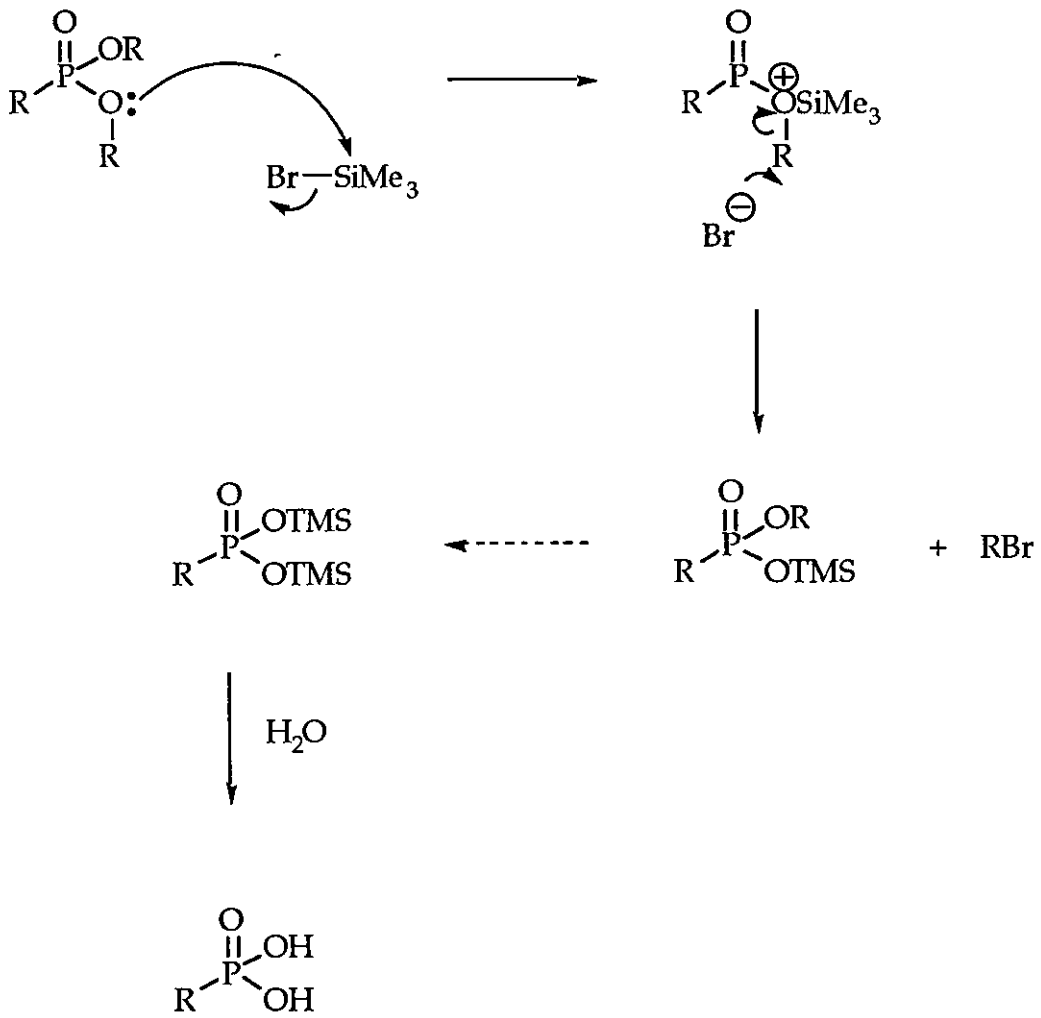


17

It is the bisphosphonic acid moiety, and its salts, that have a high affinity for bone, rather than the phosphonate alkyl ester. It is therefore necessary to hydrolyse the phosphonic ester groups to phosphonic acid groups for

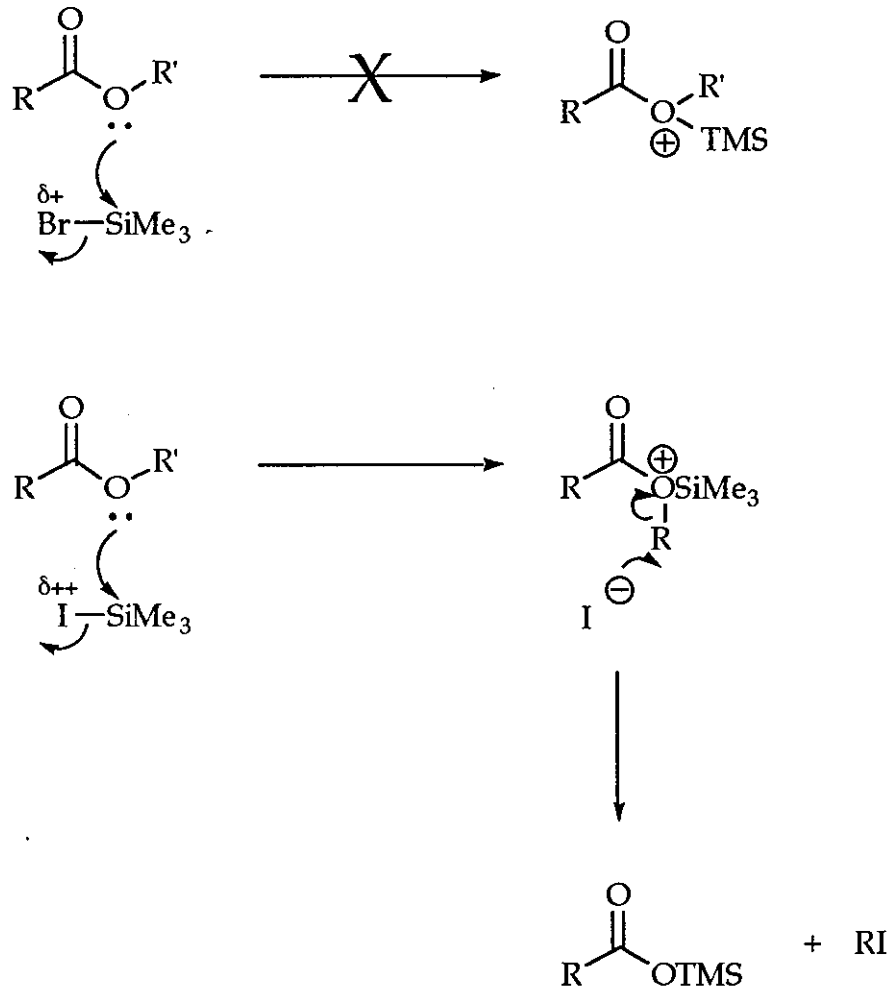
biological testing. This immediately poses the problem of selective hydrolysis of the phosphonate ester groups in the presence of carboxylic esters.

Phosphonate esters may be hydrolysed under acidic or basic conditions, but carboxylic esters may also be hydrolysed under similar conditions. Many of the patents which cover oestrogen-bisphosphonate conjugates use trimethylsilyl bromide[14,15,16], which reacts with the phosphonate alkyl ester to give trimethylsilyl esters. The phosphonate trimethylsilyl esters groups are readily hydrolysed on addition of water to yield the desired phosphonic acids (Scheme 14).



Scheme 14

Carboxylic esters do not normally react with trimethylsilyl bromide in the same fashion. Generally, a carboxylic ester is less nucleophilic at oxygen than a phosphonate ester, and therefore the nucleophilicity of the alkyl-oxygen atom is less than that of the phosphonate alkyl-oxygen atom. It is , however, possible to react carboxylic esters with trimethylsilyl iodide (a more electrophilic reagent) to form the trimethylsilyl esters, which can be subsequently hydrolysed upon addition of water (Scheme 15).

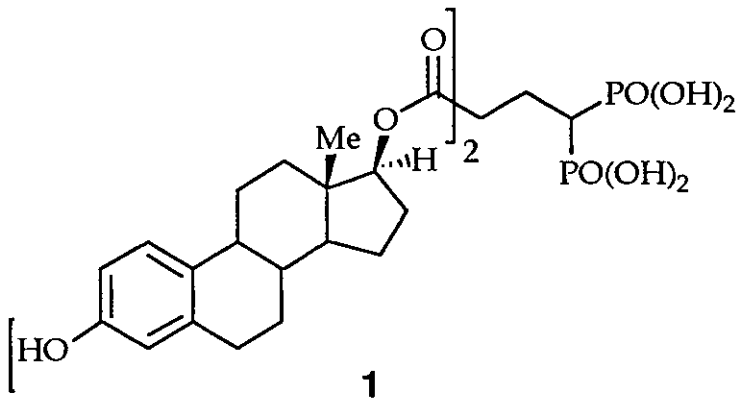


Scheme 15

Trimethylsilyl bromide was therefore used to hydrolyse selectively the phosphonate esters. To ensure complete hydrolysis of all four phosphonate groups, a large excess (35 eq.) of trimethylsilyl bromide was applied. The reaction mixture was stirred under an atmosphere of nitrogen for 2.5 days. Upon the addition of water an off-white precipitate formed which was

confirmed by ^{31}P NMR spectroscopy to contain only one type of phosphorus atom. ^{1}H NMR spectroscopy also indicated the lack of any ethyl groups. From our spectral data, we concluded that phosphonate ester hydrolysis had reached completion.

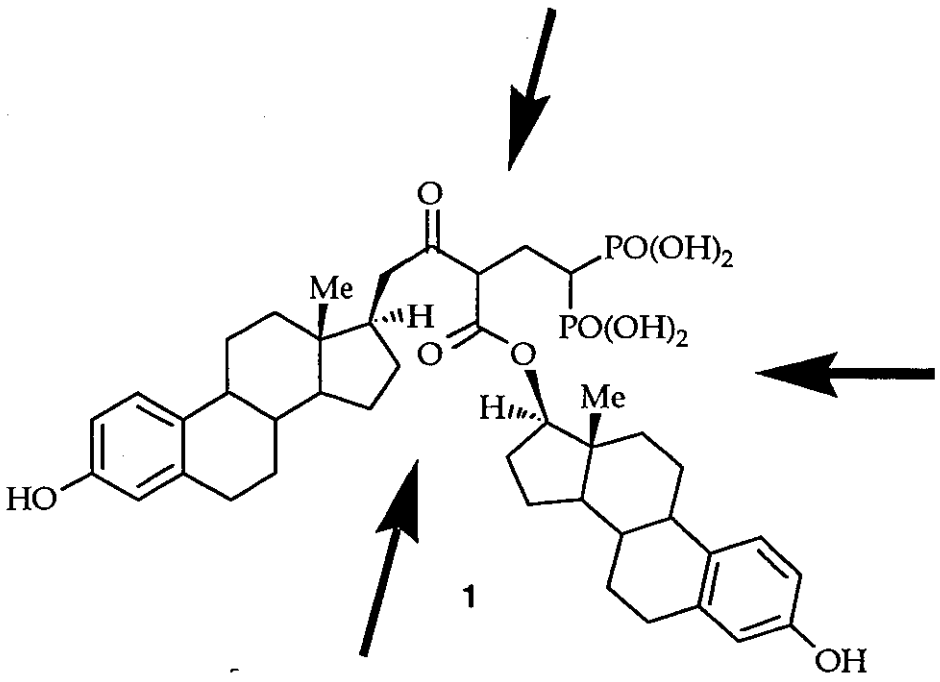
We were mindful that some hydrolysis of the ester linkage may have occurred concurrently, releasing free oestradiol. If this were the case, we would expect to see a singlet in the ^{1}H NMR spectrum at 0.77 ppm corresponding to the C-18 methyl hydrogen atoms of oestradiol. However, we only witnessed the singlets at 0.80ppm and 0.81ppm corresponding to the two C-18 methyl hydrogens in our bis-oestrogenic conjugate **1**. We were therefore confident that we had hydrolysed selectively the phosphonate esters.



1

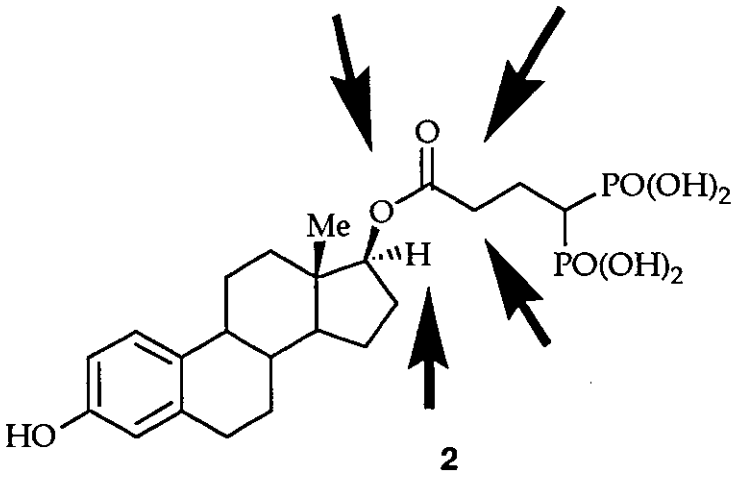
2.2 Mono-oestrogenic conjugates

The results from the assay studies performed on the bis-oestrogenic conjugate led us to propose a more hydrolysable bisphosphonic acid. We envisaged developing a compound with less steric bulk around the ester linkage, and thus lowering its resistance to hydrolysis. An obvious choice was a conjugate containing only one oestrogen moiety **2**.



1

Restricted route of attack

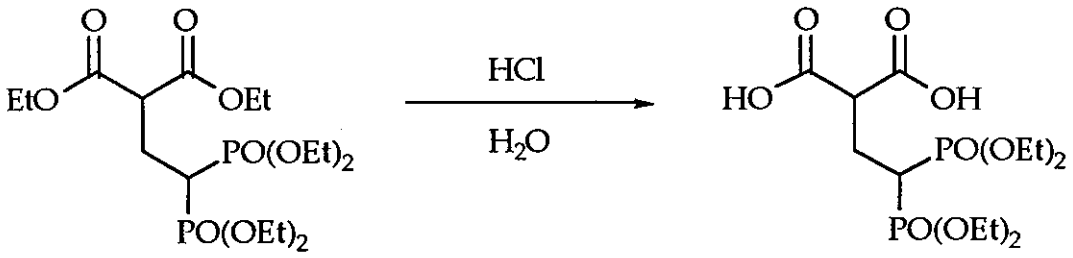


2

Open route of attack

Scheme16

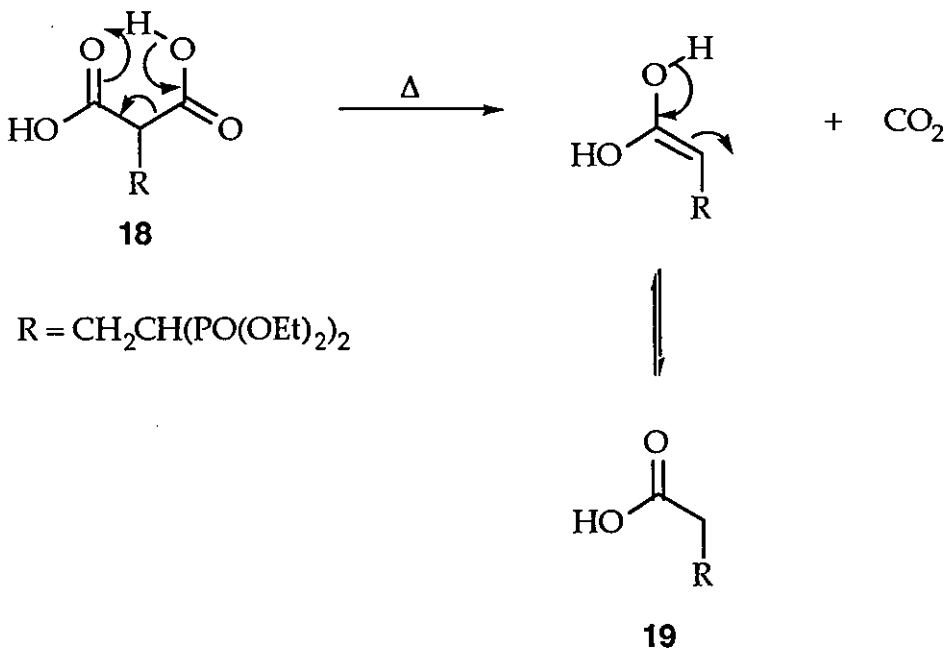
Our synthetic route began with transforming the dicarboxylate bisphosphonate intermediate **5** into its corresponding di-acid **18** (Scheme 17). This simple hydrolysis step is achieved by the addition of aqueous HCl.



5 **18**

Scheme 17

We thought it would then be possible to thermally decompose di-acid **18** to yield the key mono-acid intermediate **19**. Decarboxylation in this instance is a favourable reaction because an enol forms through a six-membered cyclic pathway (Scheme 18). Consequently, only mild heating should be required.



18

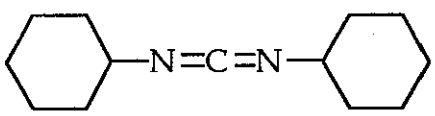
$R = CH_2CH(PO(OEt)_2)_2$

19

Scheme 18

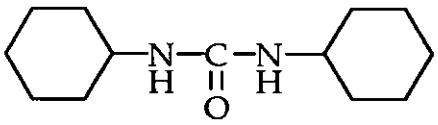
With our protected 17β-oestradiol and mono-acid intermediate in hand, our synthesis was conveniently set up for a classic esterification reaction utilising a dehydrating agent. There is much precedent in the the literature for esterifying a carboxylic acid by treating it with an alcohol in the presence of a

dehydrating agent,[17,18] such as dicyclohexylcarbodiimide (DCC) **20**. During this process the DCC is converted to dicyclohexylurea (DHU) **21**.



DCC

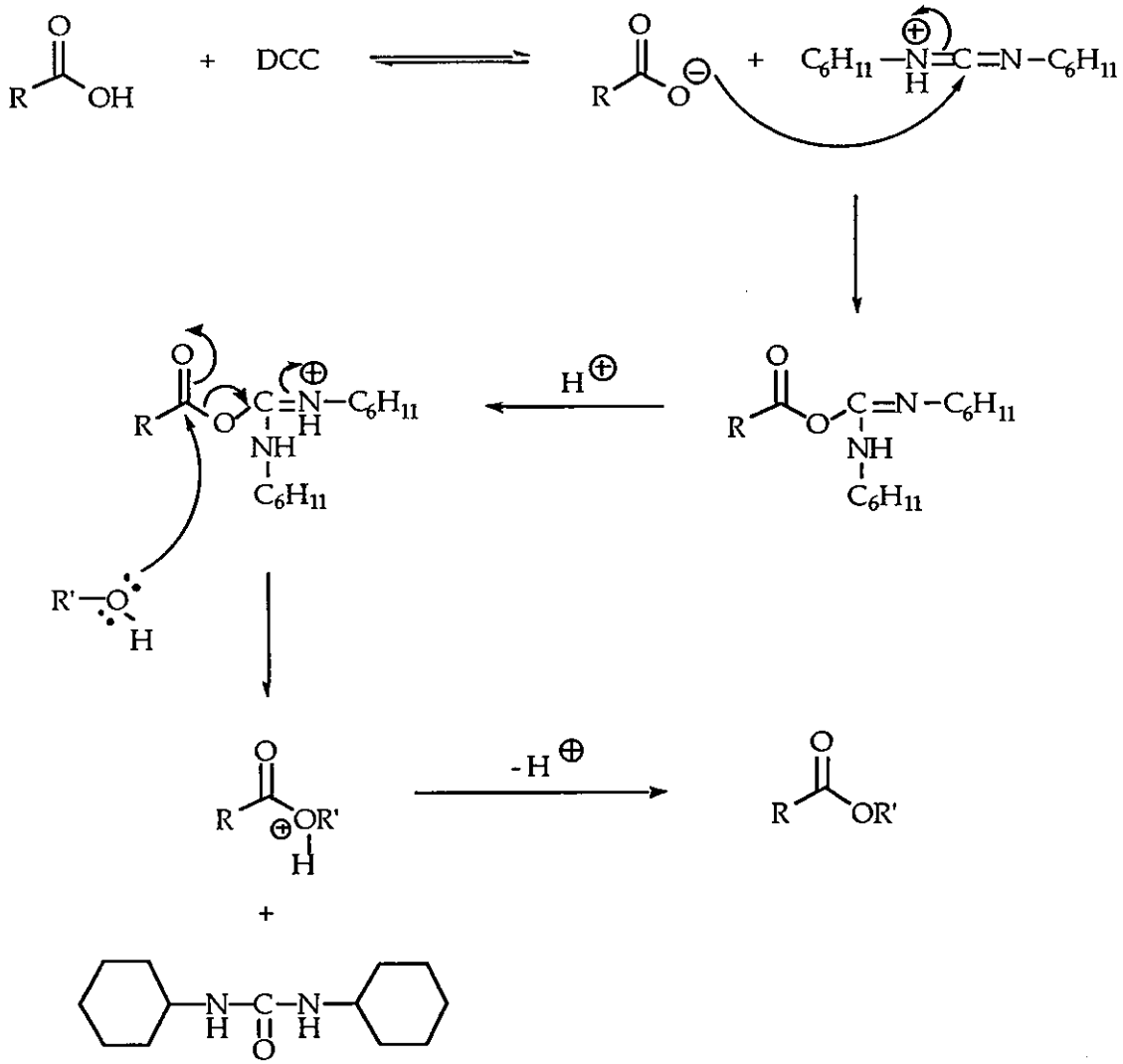
20



DHU

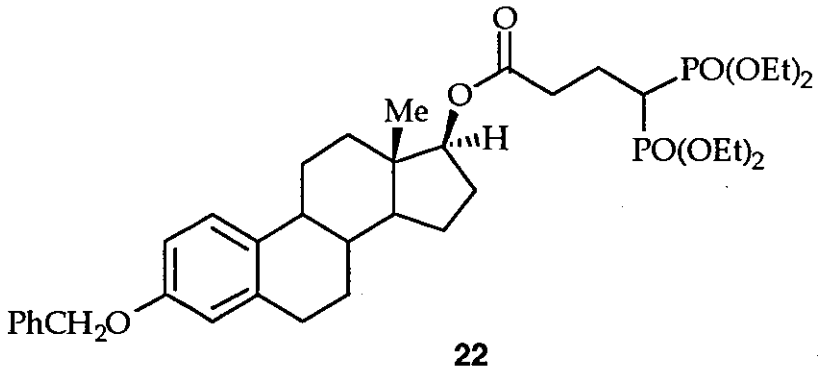
21

If the solvent is selected carefully, the DHU can be removed from the reaction mixture by precipitation as it is formed. The mechanism[19,20] is considered to be consistent with that outlined below (Scheme 19).



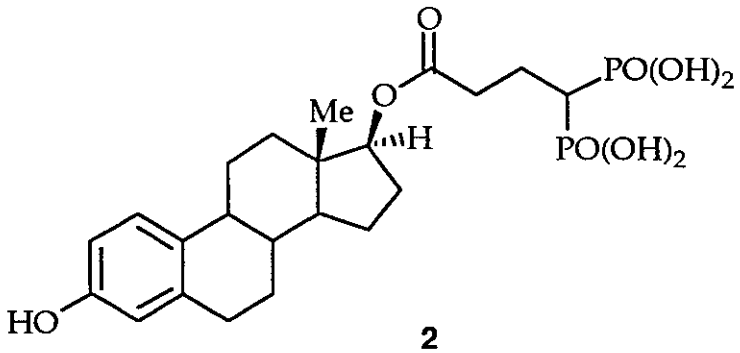
Scheme 19

This method of esterification differs from the one used previously for the construction of the bis-oestrogenic conjugate. The use of a dehydrating agent involves the activation of the acid to promote a chemical reaction at room temperature.



22

Once the oestrogen and bisphosphonate moieties have been successfully linked, deprotection and phosphonate ester hydrolysis would provide a mono-oestrogenic phosphonic acid for biological testing.

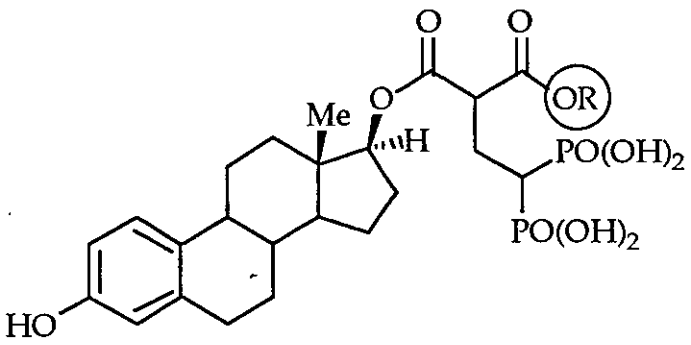


2

The synthetic route to this particular single oestrogen conjugate was performed within the group and later assigned to a contract chemical company for its synthesis on a multi gram scale.

2.3 Malonyl oestrogens

As outlined above, we sought to prepare conjugates resembling the one depicted in figure 1, and to alter the size of their alkoxyl group as a means to varying stability towards ester hydrolysis.

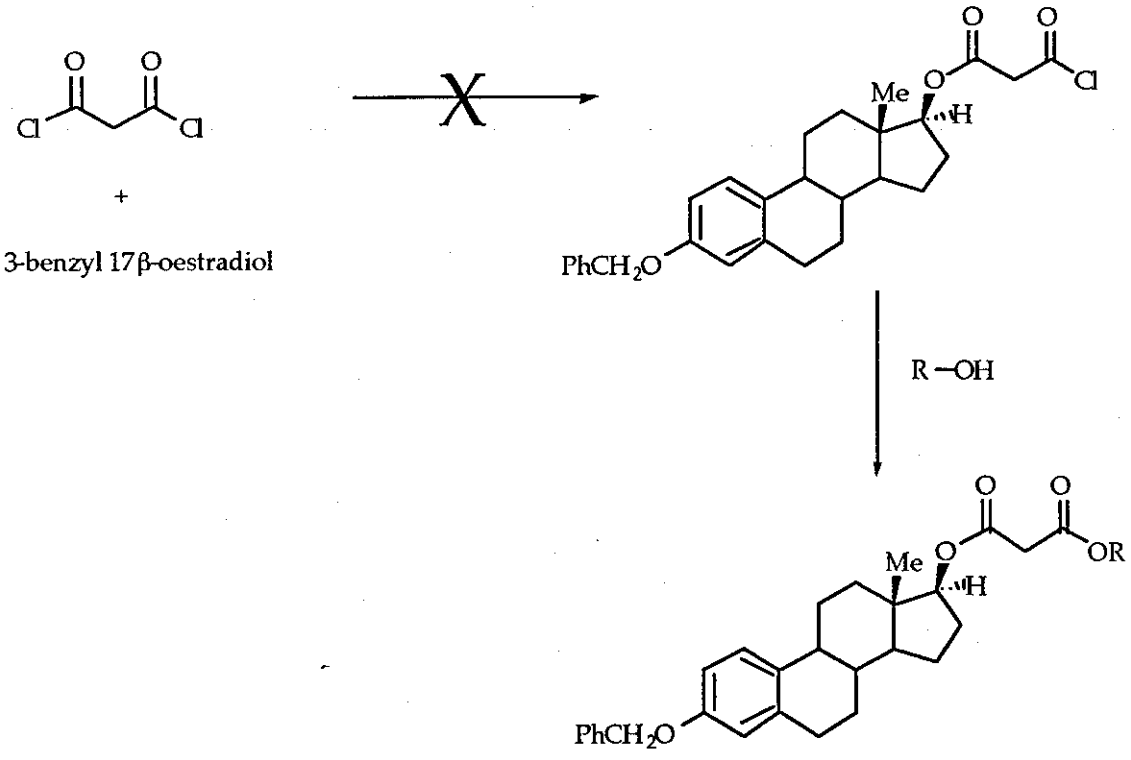


R = Me
Et
Pr
^{t}Bu

Figure 1

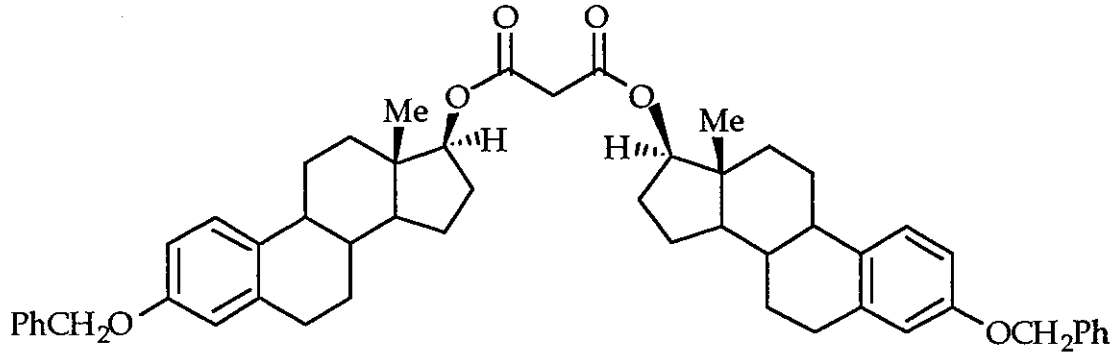
If the correct size alkoxyl group is chosen, this would result in a bisphosphonate conjugate that contained an ester linkage which could be enzymatically cleaved at such a rate to provide a useful pro-drug *in vivo*.

In order to achieve this goal, we hoped to combine our ethylidene bisphosphonate with a 17-malonyl oestrogen through a Michael-type addition. Obviously, this strategy relied upon the availability of malonyl functionalised oestrogens. Initially, we attempted to synthesise a conjugate derived from malonyl dichloride and 3-benzyl-17β-oestradiol (Scheme 20). The resulting chloro-malonyl oestrogen could then be reacted with virtually any alcohol providing the malonate of choice.



Scheme 20

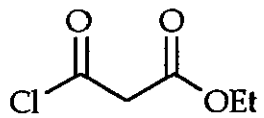
Unfortunately, the malonyl dichloride addition proved problematic. On stirring together molar equivalents of steroid and malonyl dichloride in acetone at room temperature, in the presence of DMAP for 1 h-5 days, we only isolated trace amounts of a new crystalline compound. ^{1}H NMR analysis displayed the malonyl methylene group as a singlet (2H, δ = 3.8 ppm), and the 17α-proton of the steroid (2H, t, J = 8 Hz). These data suggested that both the chlorine atoms of malonyl dichloride had been displaced by oestrogen atoms, resulting in the formation of a bis-steroid dimer **23**.



23

In an attempt to prevent the formation of this dimer, we repeated the reaction with an excess of malonyl dichloride. The reason for this was that, once one chlorine atom had been replaced, the resulting mixed-malonate might be less reactive to further displacement than the malonyl dichloride would be. However, our efforts proved fruitless, even after heating the reaction mixture in various solvents.

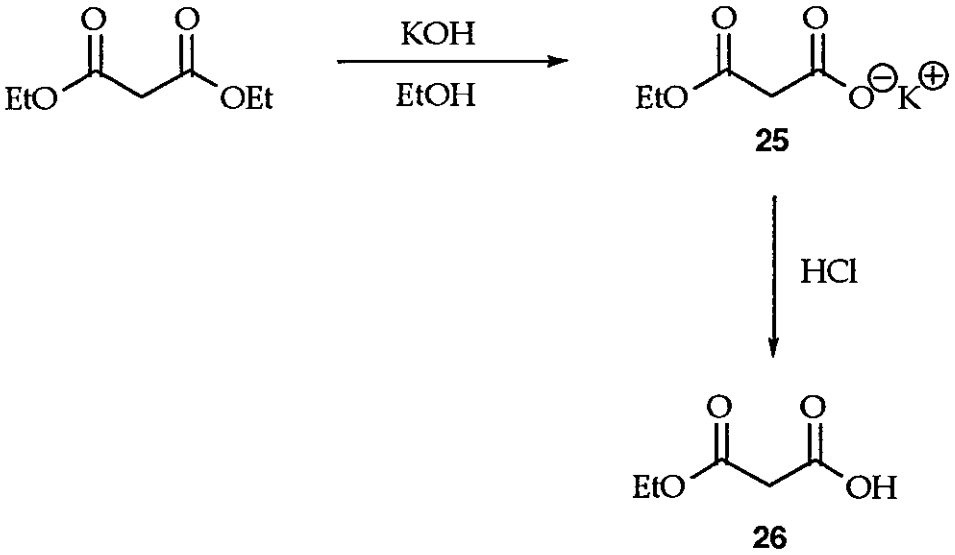
The availability of an alkyl malonyl chloride, such as ethyl malonyl chloride **24**, appeared to be a more attractive route to malonyl oestrogens since it would be considerably easier to control malonyl substitution. It was reasoned that the chlorine atom of ethyl malonyl chloride would be displaced in preference to the alkoxide group, or at least at a much faster rate. However, we witnessed no reaction when 3-benzyl 17 β-oestradiol and **24** were stirred together at ambient temperature. Instead, we were only able to isolate the starting materials.



24

2.4 Malonic half-acids

To avoid the selectivity problems encountered with the preparation of malonyl oestrogens, we decided upon another synthetic strategy. We envisaged a DCC coupling of the benzyl protected oestradiol with alkyl malonic half-acids. The synthesis of such acids appears to be a trivial one, although there is little precedent for them in the literature. Strube[21], however, described a simple efficient route to ethyl malonic half-acid on a multi gram scale (Scheme 21).



25

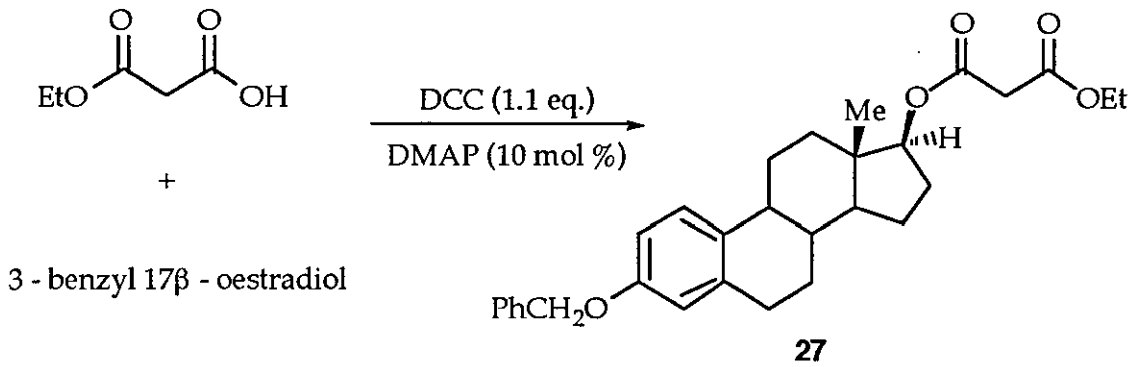
26

Scheme 21

Potassium hydroxide (0.16M) solution was added dropwise (over 1 h.) to diethyl malonate in absolute ethanol. A thick white precipitate formed quickly and a mechanical stirrer was required to agitate the mixture for 2 h. The precipitate was allowed to stand overnight and then purified by recrystallising from the mother liquor. The potassium salt **25** was isolated as colourless crystals (69% yield). This proved to be a rather messy procedure and consequently some material was lost throughout.

The potassium salt of ethyl malonic half-acid was hydrolysed by the slow addition of concentrated HCl over approximately 30 mins. After the reaction mixture had been filtered and the residue washed with a small amount of diethyl ether, the aqueous layer was extracted with further diethyl ether (3 x 10 ml). On drying the organic fraction and removing the solvent, the desired ethyl malonic half-acid **26** was isolated as a clear liquid (55% yield from potassium salt).

Although Strube and his co workers did not report their analyses, our I. R. and NMR data were consistent with the expected structures.



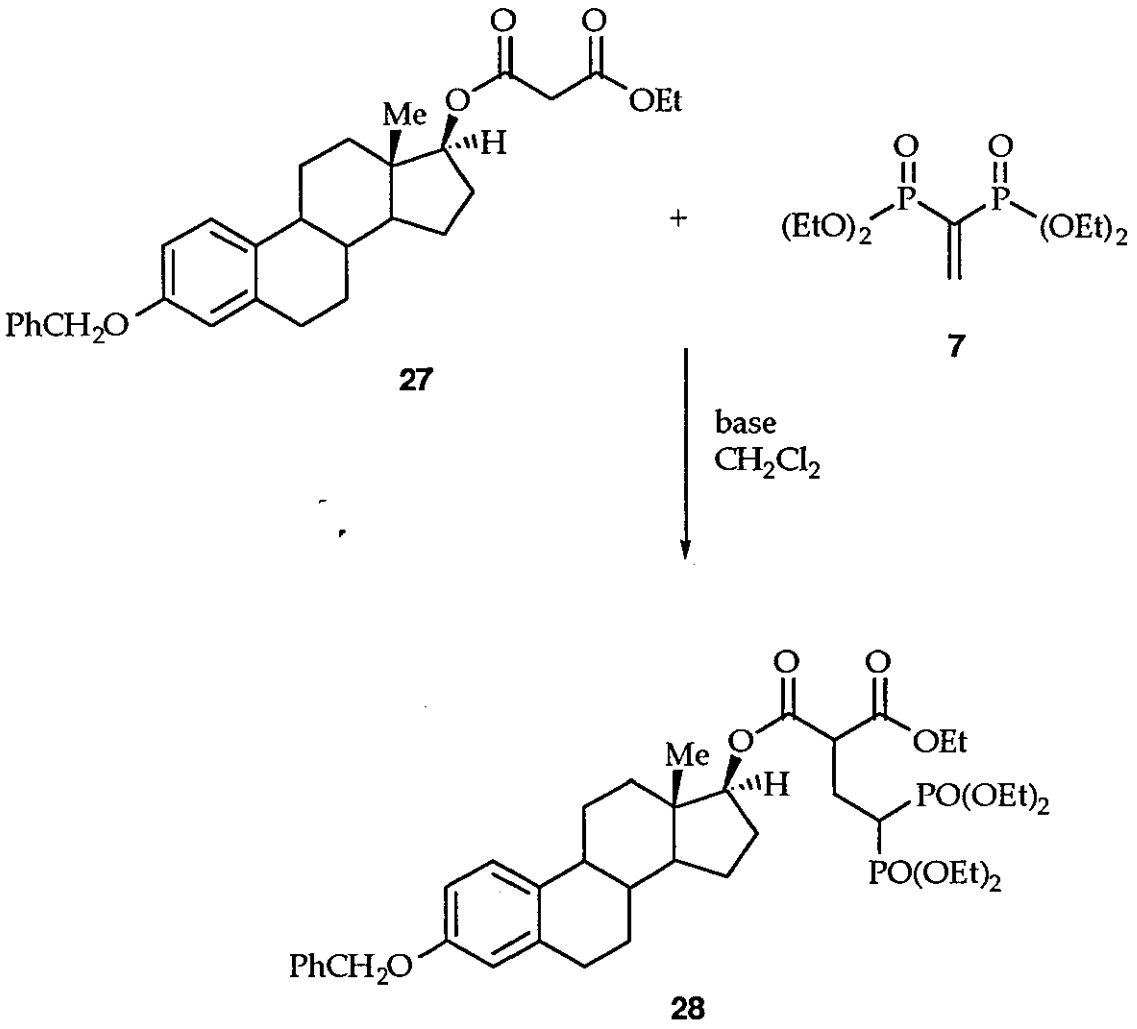
Solvent = Et_2O	46 % yield
CH_2Cl_2	58% yield

Scheme 22

Ethyl malonic half-acid was successfully coupled with 3-benzyl 17β-oestradiol through an esterification reaction using DCC as a dehydrating agent (Scheme 22).[17,18] The solvent selected originally was diethyl ether as the DHU by-product was insoluble in it, and hence precipitated out of solution as it was formed. The DHU could be conveniently filtered off and washed with a small amount of ether to extract any product present on the surface of the precipitate.

Although this was a reported system for DCC coupling reactions, we experienced modest yields (46%) and consequently changed the solvent to dichloromethane. DHU was slightly soluble in dichloromethane but this was not a problem since any lingering DHU impurity was removed during chromatography. By switching to our DCC/dichloromethane system we boosted the yield of ethyl malonyl oestrogen **27** to approximately 60% .

2.4.1 Addition of tetraethyl ethylidene bisphosphonate to ethyl malonyl oestrogen



Scheme 24

The key coupling step towards our newly functionalised bisphosphonates employed a traditional C-C bond forming reaction, namely a Michael addition (Scheme 24).[22,23] In this conjugate addition the base removes the acidic proton of the mixed malonate, generating the enolate which adds to the double bond of the substrate in a 1,4-fashion.

Our initial attempts at conjugation used 1.5 equivalents of triethylamine. Although a slight excess of base was used to ensure enolate formation, the

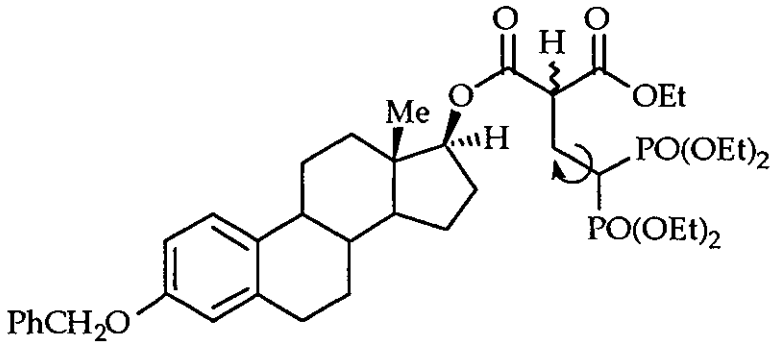
Michael addition proved to be rather sluggish and products were isolated in low yields (30%).

Switching to a stronger base system of 0.1 M NaOEt (generated *in situ* from sodium metal and ethanol), we hoped for an improvement in yield, although we were mindful that the nucleophilic ethoxide anion might attack the ester linkage and cleave the conjugate. Indeed, after only 15 mins. we witnessed the cleavage to 3-benzyl 17β-oestradiol. A similar result was obtained with sodium methoxide in methanol. In view of this, we investigated potassium *t*-butoxide as it is a stronger and less nucleophilic base. Although the ester linkage was unaffected, the malonate enolate did not form and consequently the reaction failed.

We eventually developed a successful system employing a strong (non-nucleophilic) lithium base in a non-protic solvent. Lithium hexamethyldisilylamine (LHMDS) was typically added (one molar equivalent), and the reaction mixture warmed at 60 °C in THF for 2 h. After purification by silica gel flash column chromatography, eluting with 1-2% methanol in dichloromethane, the desired functionalised bisphosphonate **28** was isolated as a colourless oil in a much improved yield (54%).

The spectral data for **28** highlighted several points of interest. The ^{31}P NMR spectrum contained two resonances of equal intensity and very similar chemical shifts (δ = 24.20 and 24.15 ppm), indicating that the sample contained phosphorus atoms in two different chemical environments.

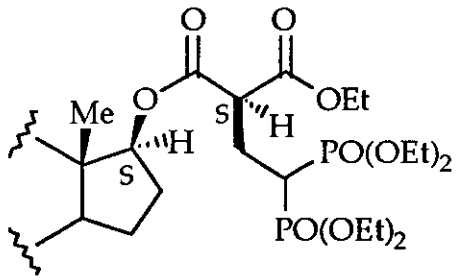
It is possible that the phosphonate moiety is restricted from rotating by the bulky steroid group (Scheme 25). The two phosphorous atoms would thus be in different chemical environments and hence display different chemical shifts in the ^{31}P NMR spectrum.



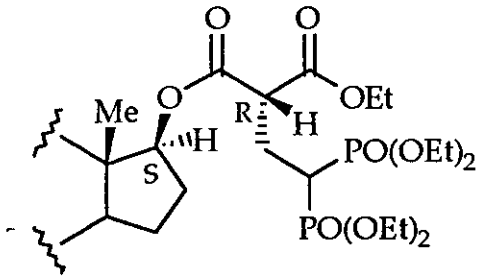
28

Scheme 25

More importantly, we noticed doubling-up of peaks in both the ^{1}H and ^{13}C NMR spectra corresponding to steroidal atoms. For example, the 18'CH_3 group displayed peaks at δ_H = 0.81, 0.82 ppm in the ^{1}H NMR spectrum, and δ_C = 12.44, 12.65 ppm in its ^{13}C NMR spectrum. Furthermore, we witnessed a pair of peaks for the quaternary 13'C atom (δ_C = 41.39, 41.60 ppm) and the 17'C atom (δ_C = 82.19, 82.22 ppm). This evidence would suggest that a 50:50 mixture of a single pair of diastereoisomers was formed as a result of the Michael addition, namely S,S and S,R diastereoisomers (Scheme 26).



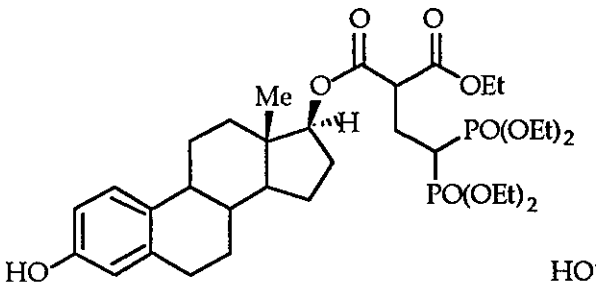
S,S-isomer



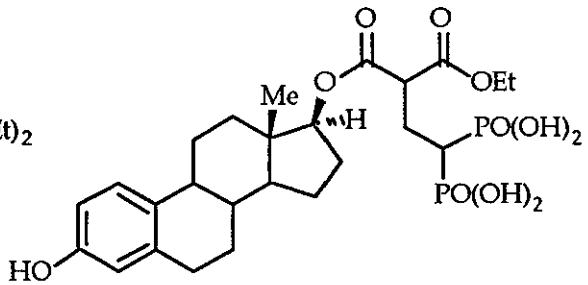
S,R-isomer

Scheme 26

Subsequent hydrogenation afforded the deprotected bisphosphonate **29**, and subsequent phosphonate ester hydrolysis provided us with our first novel malonyl-functionalised bisphosphonate **30** for biological testing.



29

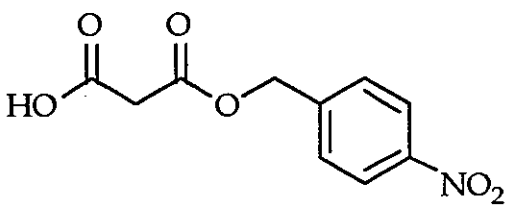


30

2.4.2 Meldrum's acid chemistry

We then sought to prepare a mini-library of compounds with a range of stabilities to hydrolysis. In order to achieve this, it was crucial to have at hand a practical route to further malonic half-acids, each with different sized alkyl ester groups. Due to the acute shortage of these acids, we required a reliable method to prepare them.

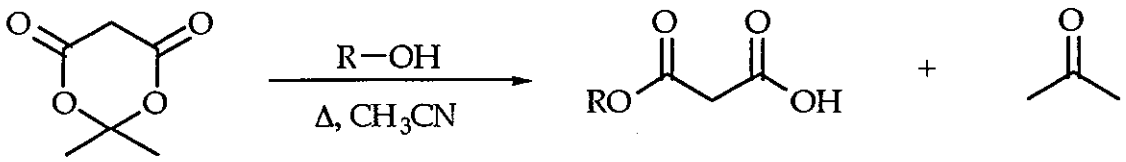
Equimolar amounts of Meldrum's acid and *p*-nitrobenzyl alcohol were refluxed together in acetonitrile for apporoximately 22 h.[24,25] The solvent was removed and the crude product dried under high vacuum for 30 mins., affording a yellow solid (97%). Recrystallisation from t-butanol/hexane (2:1) yielded the *p*-nitrobenzyl malonic half-acid **3 1** as a pale yellow solid (64%), mp. 95-98 °C. Both our samples were essentially pure by ^{1}H NMR spectroscopy, though we thought it prudent to work solely with the purified product.



31

The success of this reaction using a rather electron-defficient nucleophile such as *p*-nitrobenzyl alcohol was encouraging, and suggested that the preparation of malonic half-acids from virtuallly any alcohol would be possible. Indeed, it was possible to react 3-benzyl 17 β-oestradiol with Meldrum's acid in a similar fashion, although the product was formed in a lower yield (49%).

The reaction was extended to further nucleophiles, namely methanol, *iso*-propanol, *t*-butanol and phenol (Table 1).



R	Yield, %	Compound No.
p-NO_2Ph	64	**31**
Me	74	**32**
^{i}Pr	100 *	**33**
^{t}Bu	54	**34**
Ph	34	**35**
3-Benzyl 17β-oestradiol	49	**36**

* crude

Table 1

The resulting malonic half-acids were essentially pure by ^{1}H NMR spectroscopy, and the *iso*-propanol and *t*-butanol examples were used in their crude forms. However, the methyl and phenyl derivatives were purified by silica gel flash column chromatography, eluting with 5% methanol/dichloromethane and 20% ethyl acetate/light petroleum-100% ethyl acetate respectively.

Pure single crystals of *p*-nitrobenzyl malonic acid **31** and oestrogen malonic acid **36** were grown of sufficiently good quality that their structures could be determined by X-ray crystallography (Figures 2 and 3 respectively). Both sets of analyses displayed good agreement with their theoretical models; p-nitrobenzyl malonic half-acid (R = 0.035); 3-benzyl 17β-oestradiol malonic half-acid (R = 0.232).

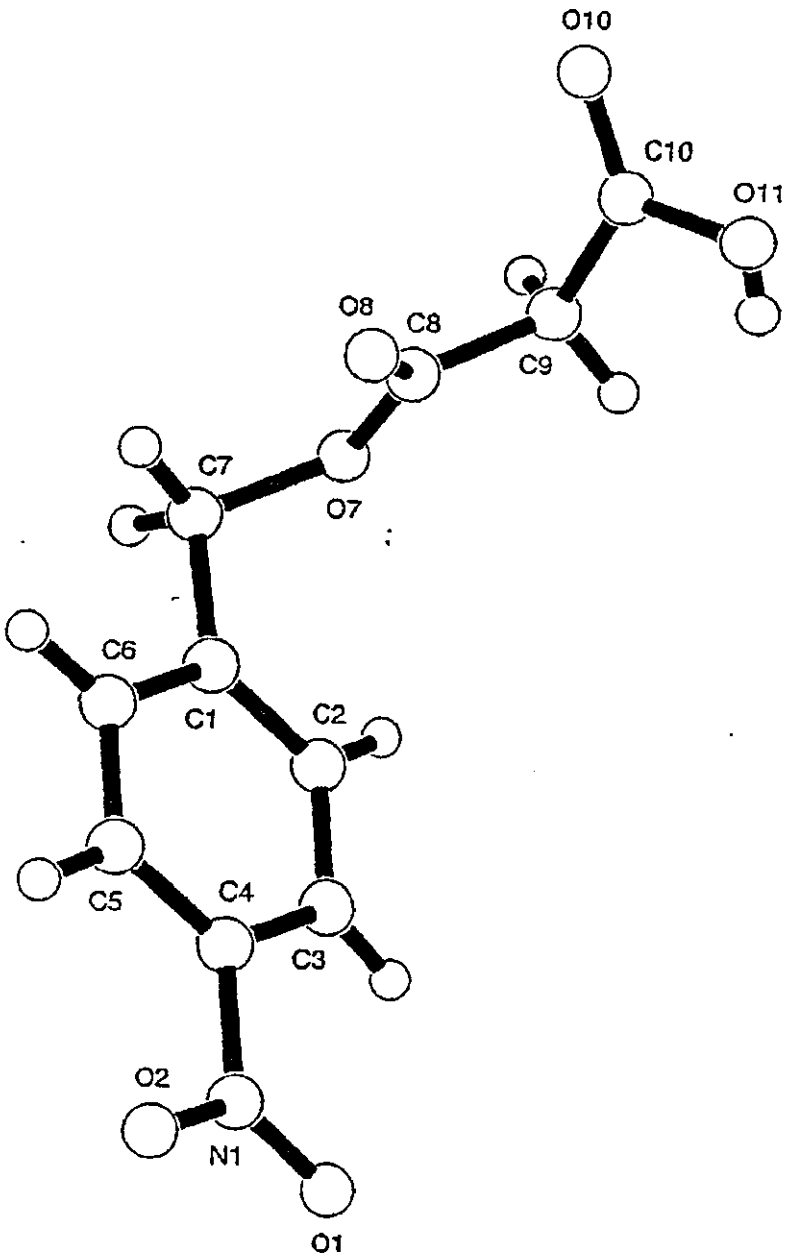


Figure 2

The X-ray structure of **31** (see Appendix)

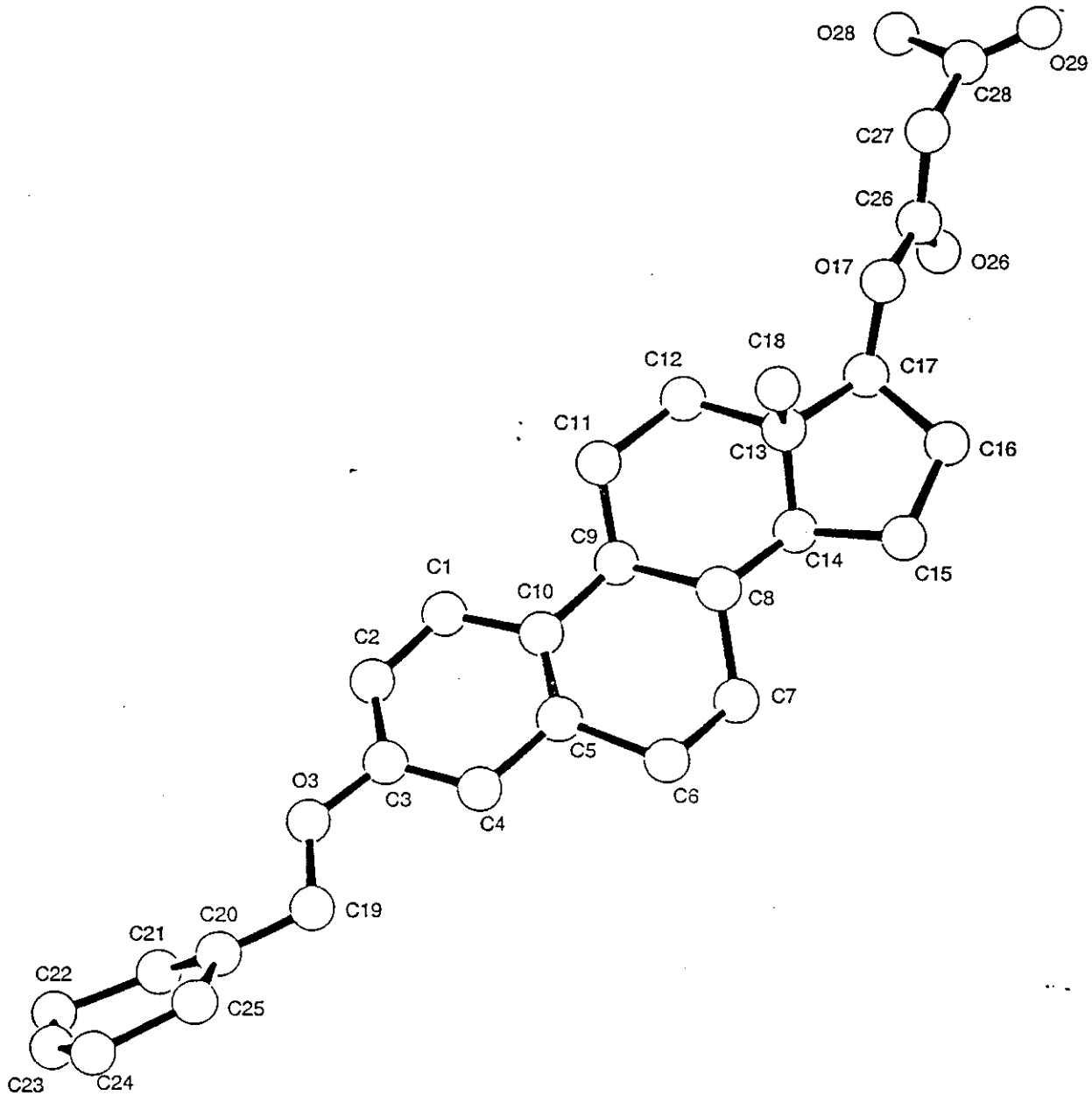
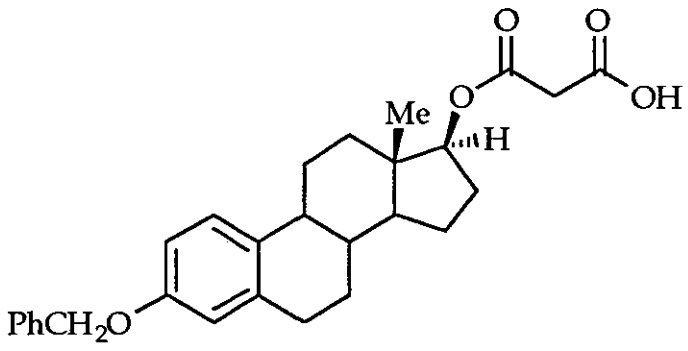


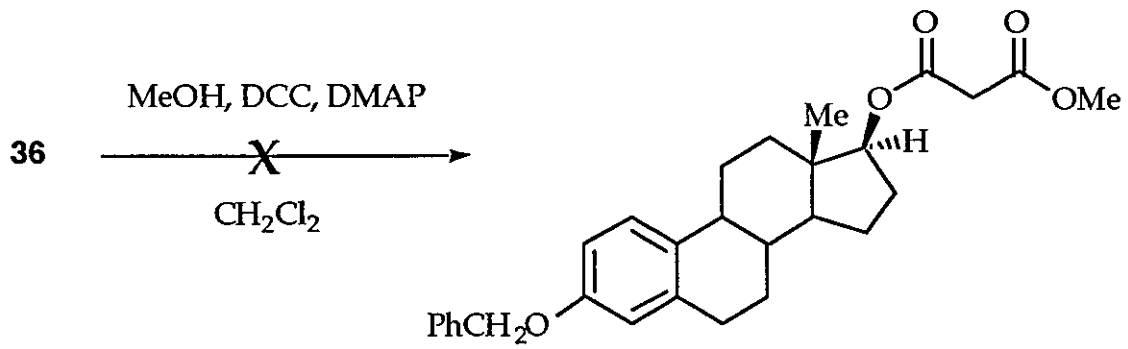
Figure 3

The X-ray structure of **3 6** (see Appendix)



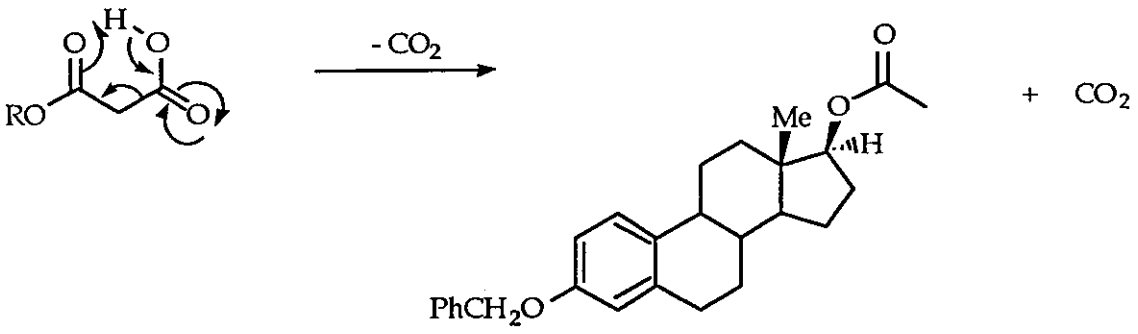
36

The synthetic utility of the oestrogen malonic half-acid **36** was somewhat limited since the presence of the carboxylic acid in the molecule at this early stage of the synthesis would prove to be problematic. Nonetheless, we saw the potential of reacting with methanol in the presence of DCC in an attempt to form the methyl ester. The reactants were stirred vigorously in a solution of dichloromethane and DMAP (10 mol%) for up to 6 days, under an atmosphere of nitrogen (Scheme 28).



Scheme 28

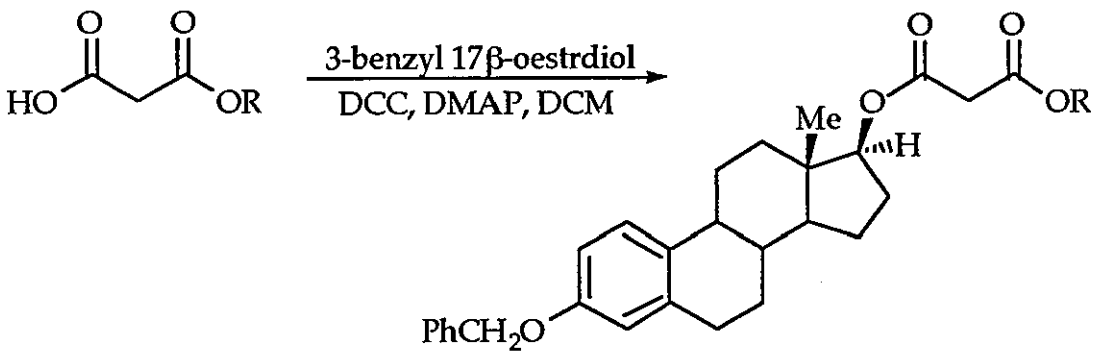
Thin layer chromatography [silica gel, ethyl acetate:light petroleum (1:4)] showed mainly starting materials and a small amount of a high-running contaminant, possibly the product of decarboxylation of **36** (Scheme 29).



Scheme 29

2.4.3 Oestrogen mixed-malonates

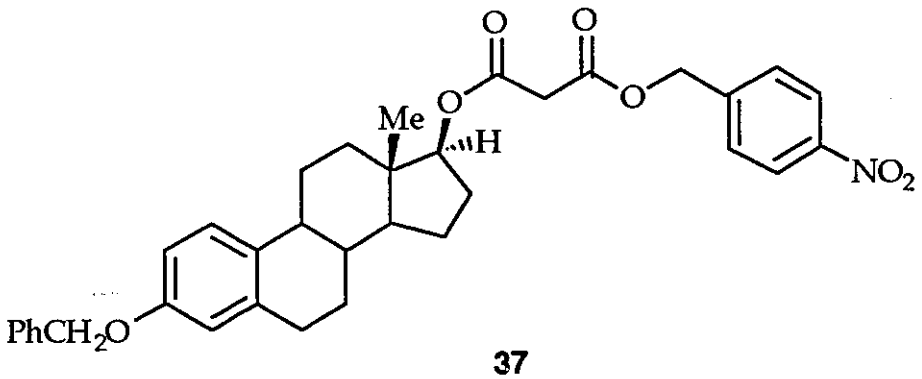
We reacted our malonic half-acids with 3-benzyl 17β-oestradiol through a DCC coupling, applying the conditions that we had developed previously. For the reactions involving *p*-nitrobenzyl, methyl and *iso*-propyl malonic half-acids, DCC was added in 1.2 equivalents. The coupling of 3-benzyl 17β-oestradiol with phenyl malonic half-acid proceeded rather sluggishly, and consequently a further 0.3 equivalents of DCC were added to the reaction mixture to help to take the reaction to completion (total number of equivalents used = 1.5). With the exception of the *t*-butanol example, the mixed-malonates were prepared in favourable yields (Table 2).



R	Yield, %	Compound No.
p-NO_2Ph	69	**37**
Me	68	**38**
^{i}Pr	69	**39**
Ph	92	**40**
^{t}Bu	15	**41**

Table 2

The *p*-nitrobenzyl mixed-malonate **37** was isolated as a clear yellow oil and consequently it was difficult to obtain an elemental analysis of sufficient quality. The methyl, *iso*-propyl and phenyl examples were collected as white crystals whose elemental analyses were of a satisfactory standard. It was also possible to grow a single crystal of the methyl derivative **38** which was suitable for structure elucidation by X-ray crystallography (R = 0.0532) (Figure 4).



37

The crude product of the reaction between *t*-butanol and 3-benzyl 17β-oestradiol was collected as a viscous blood-red oil which proved difficult to purify by silica gel flash column chromatography. Hence, the brick-red powder was isolated in a reduced yield.

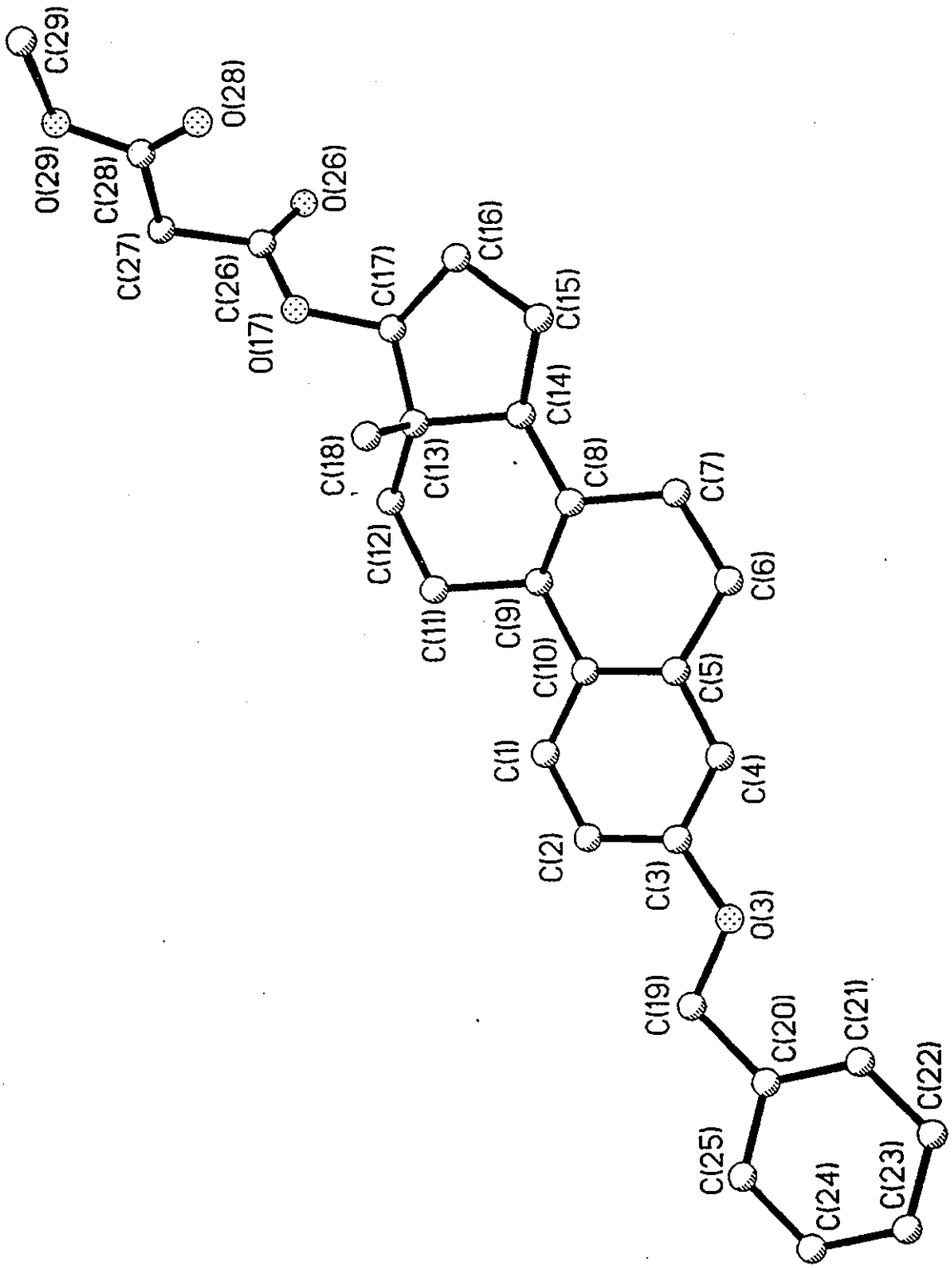
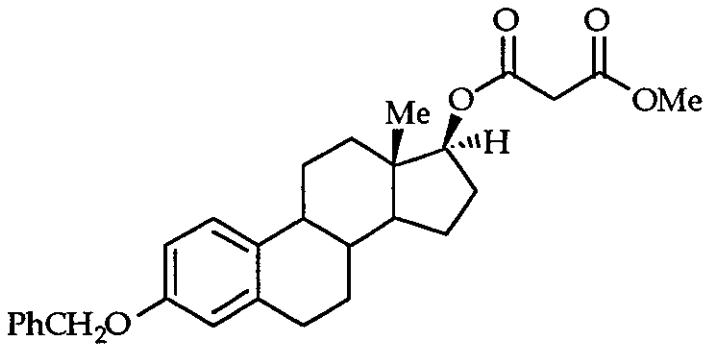


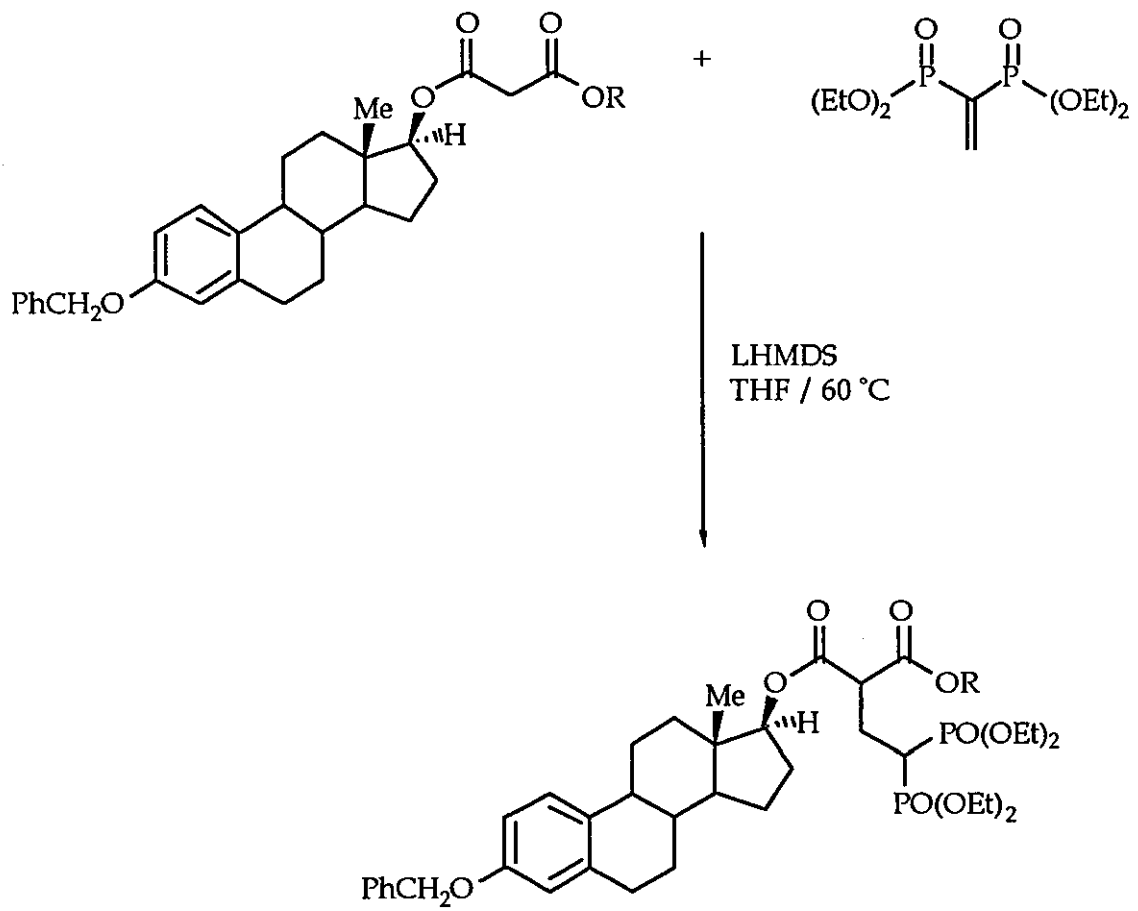
Figure 4

The X-ray structure of **3 8** (see Appendix)



38

2.4.4 Addition of tetraethyl ethyidene bisphosphonate to oestrogen mixed-malonates



R	Yield, %	Compound No.
p- NO_2Ph	24	**42**
Me	57	**43**
^{i}Pr	-	**44**
^{t}Bu	-	-

Table 3

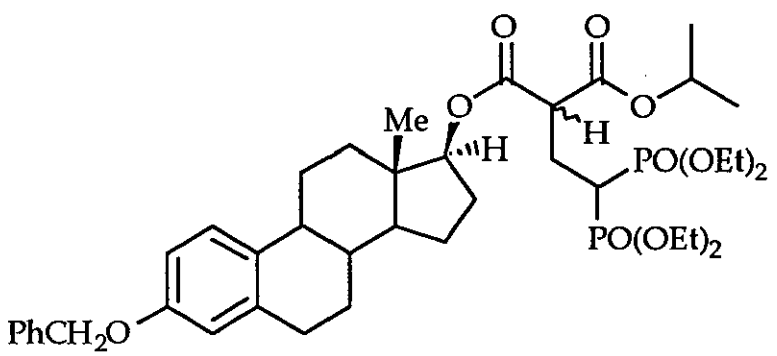
We initially attempted to functionalise tetraethyl ethylidene bisphosphonate through a conjugate addition with the p-nitrobenzyl-oestrogen malonate **37**. Although the following hydrogenation step would probably remove both the benzyl groups from the molecule revealing a problematic malonic acid, we considered it of synthetic interest to attempt the Michael reaction.

To ensure the complete formation of the ketone enolate, a solution of the mixed malonate and two molar equivalents of diethylamine in dichloromethane were stirred together for 10 minutes. The ethylidene bisphosphonate was added as a one molar equivalent and the reaction mixture stirred overnight under nitrogen. Thin layer chromatography (5% methanol/dichloromethane) indicated that no appreciable reaction had taken place, so the reaction mixture was heated to reflux. After approximately 24 h a new single spot was observed by tlc (R_f = 0.26) and all the starting material had disappeared. The crude dense brown oil was purified by silica gel flash column chromatography, eluting with 5% methanol/dichloromethane. The mixed malonate functionalised bisphosphonate was isolated as a clear brown oil (24%). There was no improvement in the yield of desired product when the base was changed to sodium ethoxide (1.1 eq.) in ethanol or sodium hydride (2 eq.) in dimethylformamide; 20% and 10% respectively. The product from the sodium system required continual washing with brine to remove a residual DMF impurity.

Further Michael additions were attempted on the methyl-oestrogen mixed malonate **38**. Initial reactions using the diethylamine/dicloromethane system proved to be unfruitful, with partial reaction after stirring for 24 h. The use of more traditional alkoxide bases, eg. NaOMe and NaOEt (1.5 eq.), resulted in the cleavage of the ester linkage after approximately 30 minutes, releasing 3-benzyl 17β-oestradiol.

We reasoned that a non-nucleophilic lithium base such as lithium hexamethyl disilylamide (LHMDS) in a non-protic solvent would be a more favourable system. Indeed, when a mixture of tetraethyl ethylidene bisphosphonate, methyl-oestrogen mixed malonate and LHMDS (1 eq.) was heated to 60 °C in frehly distilled THF for approximately 18 h, the desired conjugate was isolated as a brown oil (57%). A short (7 cm) silica column was used for purification to reduce the amount of time the bisphosphonate conjugate spent in contact with the silica gel, thus reducing the chance of it being decomposed by the column.

When the LHMDS/THF system was applied to the *iso*-propyl derivative, we witnessed no reacton with tetraethyl ethylidene bisphosphonate after heating for up to 18 h. However, the use of NaH (2eq.) in THF did provide some reaction, although stirring at room temperature for 48 h afforded a minimal amount of product **44**. The ^{31}P NMR spectrum displayed a number of peaks; a doublet at 24.1 ppm, J = 8 Hz (major) and two minor doublets at 24.2 ppm (J = 4 Hz) and 24.3 ppm (J = 8 Hz).

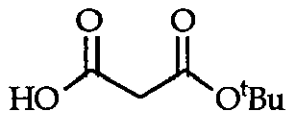


44

We believe that the major signal (24.1 ppm) corresponds to the pair of diastereoisomers, while the other two doublets could be an indication of

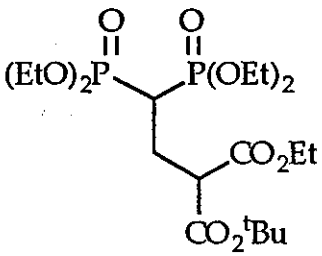
restricted rotation about the PCP bond, due to the steric bulk of the iso-propyl ester group.

The attempted conjugate addition of the t-butyl, oestrogen mixed malonate to tetraethyl ethylidene (LHMDS/THF, 60 °C, 48 h) resulted in the cleavage of the ester linkage and isolation of the t-butyl malonic half-acid **34**.



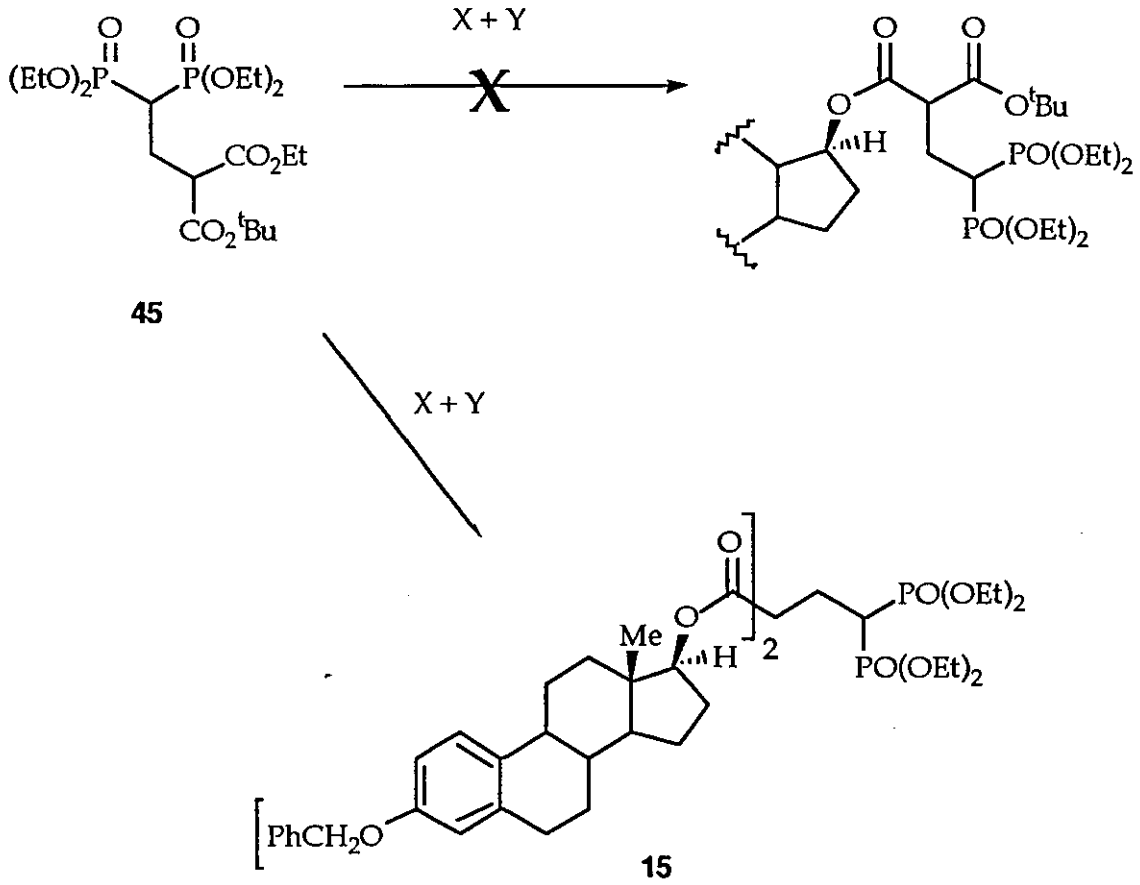
34

An alternative route to the t-butyl malonate conjugate became feasible when mixed bis (alkoxycarbonyl) tetraethyl propylene bisphosphonates **45** became available from another member of the group.



45

We envisaged a selective transesterification between 3-benzyl 17β-oestradiol and the mixed dicarboxylate functionalised bisphosphonate **45** (Scheme 30). We hoped that we could exploit the greater reactivity of the ethyl ester towards esterification, and that the t-butyl ester would not take part in the reaction. A higher dilution factor than previous transesterifications was employed to aid selectivity. After 18 h of heating to reflux, an aliquot was analysed by ^{1}H NMR spectroscopy. The spectrum lacked t-butyl functionality and the integration values were consistent with those of a bis-steroidal molecule. Indeed, the spectrum was identical to a previous bis-oestrogenic conjugate, **15**. It was therefore concluded that both the ethyl and t-butyl esters had been exchanged by oestrogen molecules (Scheme 30).



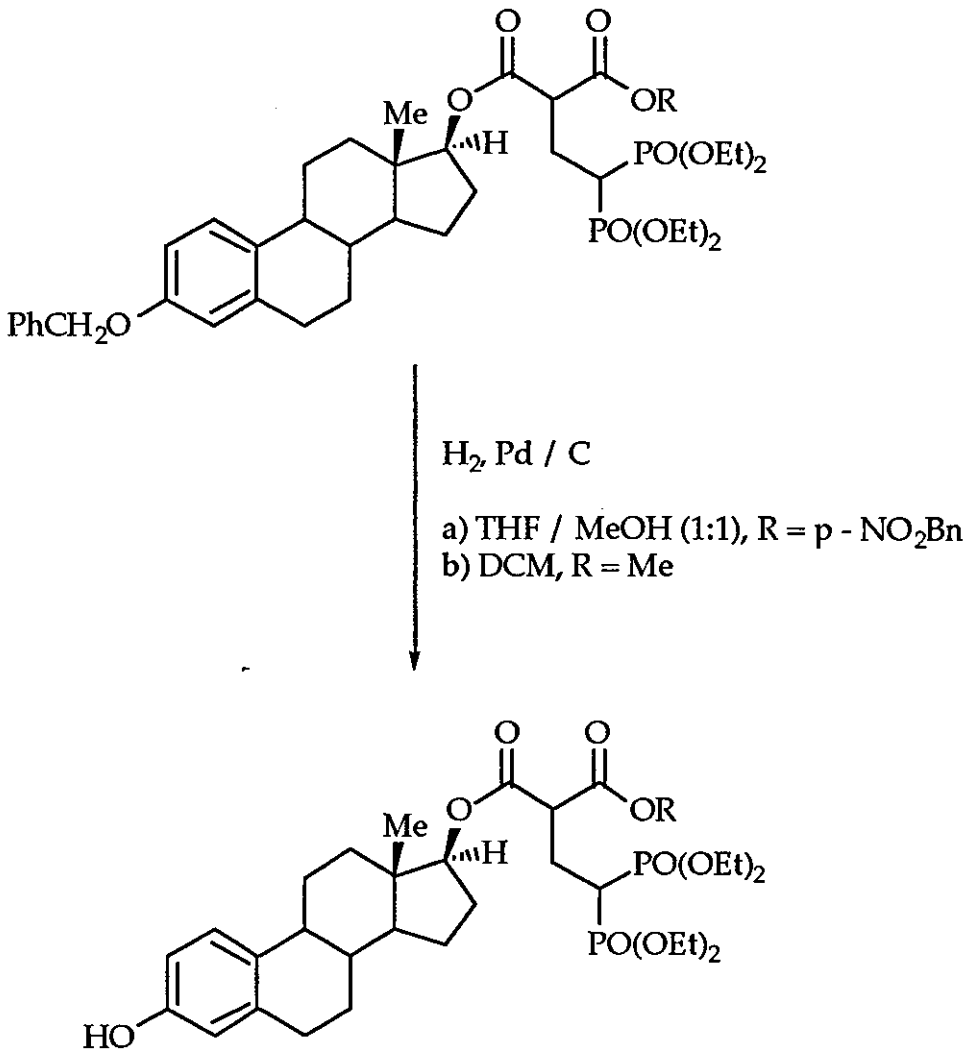
3-benzyl 17β-oestradiol = X

DMAP,toluene, Δ, 18h = Y

Scheme 30

2.4.5 Hydrogenation of mixed-malonate bisphosphonates

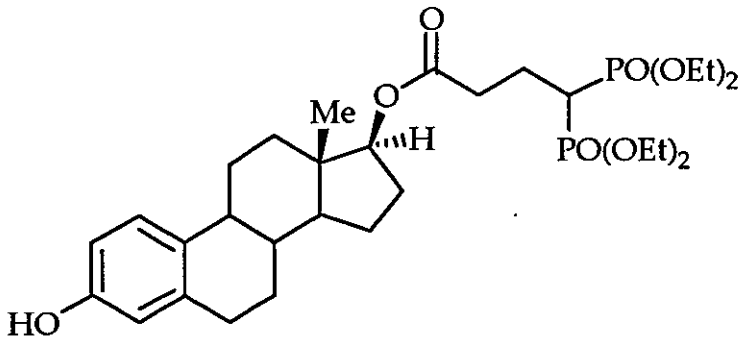
Once the oestrogen malonates had been functionalised with the bisphosphonate moiety, the next synthetic step was the removal of the benzyl protecting group to reveal the hydroxyl group at C-3 on ring A (Scheme 31).



Scheme 31

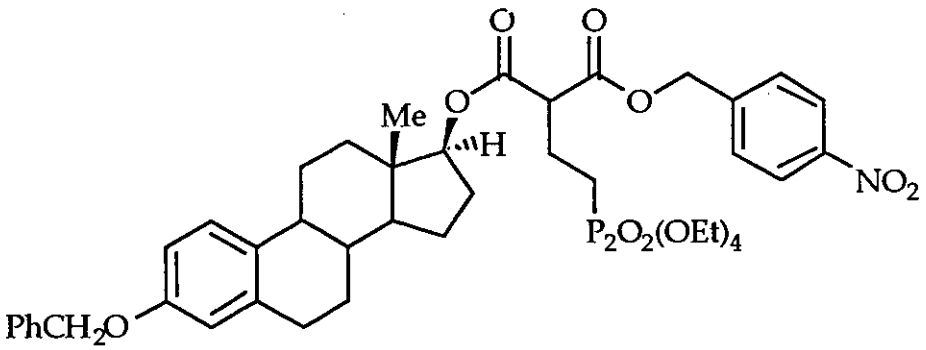
We were aware of the possibility that the p-nitrobenzyl conjugate could lose both its benzyl groups during the hydrogenolysis, resulting in the formation of a problematic malonic acid. Nonetheless, we attempted to selectively cleave the benzyl group at C-3. A catalytic amount of 10% palladium on activated charcoal was added to a solution of **46** in THF/MeOH (1:1). A hydrogen balloon (1 bar) was attached to the reaction flask and the mixture stirred vigorously. The reaction was monitored very closely by tlc which revealed the emergence of the fully deprotected conjugate after only 10 mins. Within 30 mins. we observed a high running contaminant; possibly a decarboxylation by-product **47**. After 1 h, the majority of the starting bisphosphonate material had been converted into products. The reaction was allowed to stir for 18 h,

after which time tlc analysis showed that the starting material was completely converted .



47

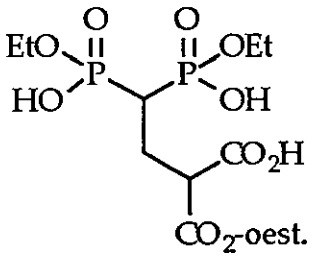
Purification of the two products was attempted using silica gel flash column chromatography, eluting with 10% MeOH/DCM. We were unable to remove the decarboxylation by-product, and witnessed further degradation of the major product while on the silica gel column.



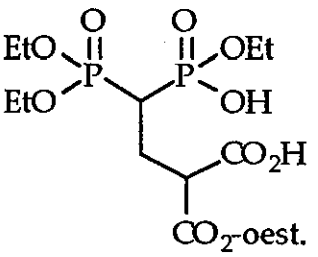
46

Subsequent hydrogenations on the p-nitrobenzyl conjugate employed a hydrogenator (approx 60 bar) for a duration of 18 h. By tlc the reaction appeared to have proceeded very cleanly to a single product. The crude product was purified by silica gel flash column chromatography, eluting with 5% MeOH/DCM on a small pad of silica. A white foam was isolated whose ^{31}P NMR spectrum revealed a number of signals. This information in conjunction with the disappearance of the ethyl phosphonate esters in the ^{1}H NMR spectrum, lead us to conclude that the phosphonate esters had somehow

begun to hydrolyse. Partial hydrolysis of the phosphonate esters would yield a conjugate containing 1-4 phosphonic acid groups (eg. **48** and **49**).

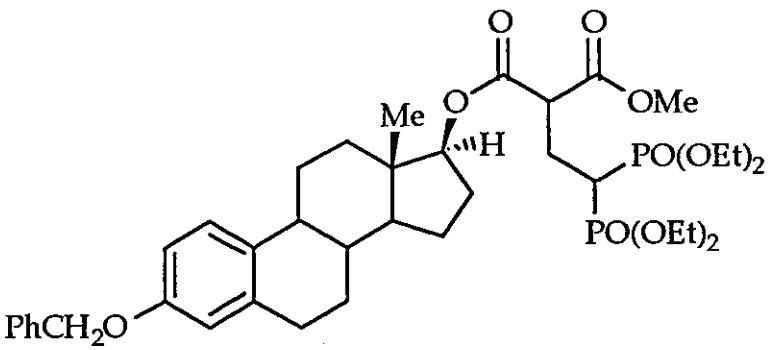


48

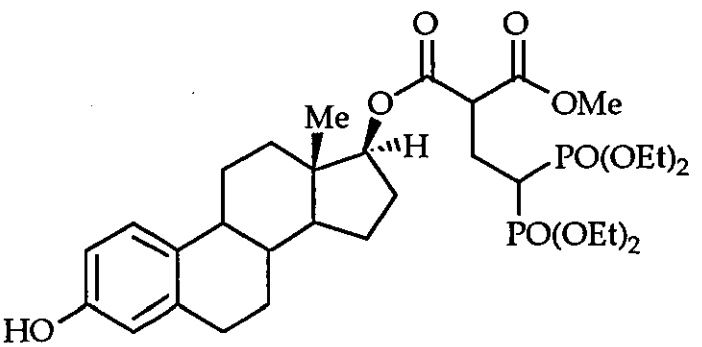


49

The hydrogenolysis of the methyl ester bisphosphonate **43** proceeded rather smoothly. A solution of **43** and 10% activated Pd on charcoal in dichloromethane was agitated in an hydrogenator for approximately 22 h. The solvent was removed by evaporation to leave a clear oil. Purification of the crude product by silica gel flash column chromatography afforded a clear oil, which collapsed under high vacuum to a white foam (79%) **50**.

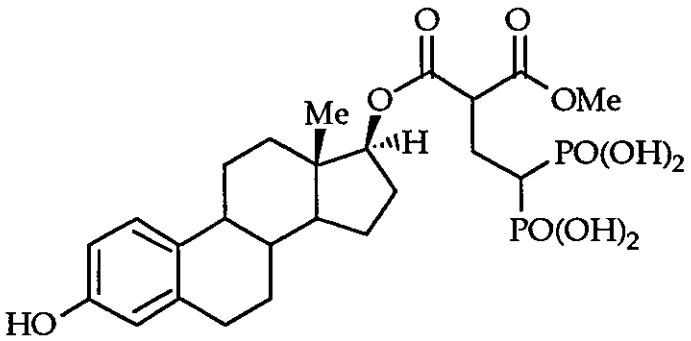


43



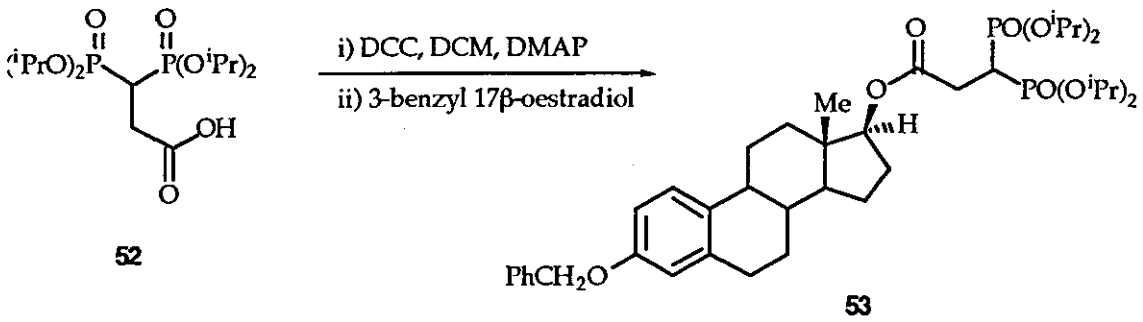
50

With the deprotected methyl ester conjugate in hand, the remaining step was the hydrolysis of the phosphonate esters. Eight equivalents of TMSBr (2 eq. per phosphonate ester) were added to a solution of **50** in dichloromethane and the mixture stirred at room temperature for approximately 2.5 days. The solvent was removed and the resulting concentrate was stirred in methanol for 30 mins. The solvent was removed under high vacuum, revealing the bisphosphonic acid **51** as an off-white foam (70%). Purification by chromatography was not attempted as we suspected that the highly polar bisphosphonic acid would stick to the column. There was no evidence of phosphonate esters in the ^{1}H NMR spectrum, and a single resonance was observed in the ^{31}P NMR spectrum (δ_P = 28.1 ppm). Thus we were confident that complete hydrolysis had occurred. Furthermore, there was no evidence of any free oestradiol impurity (δ_H = 0.7 ppm) which confirmed that the ester linkage had remained intact during the reaction. As with the bisphosphonic acids prepared early, we were not able to obtain mass spectrometric data or elemental analysis for **51**.



51

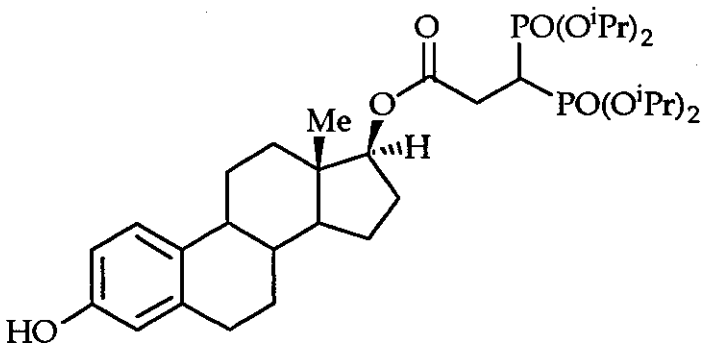
When the carboxylic acid funtionalised bisphosphonate **52** became available from a colleague in the group, we saw the opportunity of synthesising a novel oestrogen pro-drug, lacking a second alkyl ester funtionality. Our DCC coupling conditions (1.5 eq. DCC, DCM, DMAP, rt 18 h) were applied to **52** and 3-benzyl 17β-oestradiol to form the key ester linkage (Scheme 32). The crude product was purified by silica gel flash column chromatography, eluting with 5% methanol in dichloromethane. The desired conjugate was isolated as a brown oil (69%) **53**.



52 53

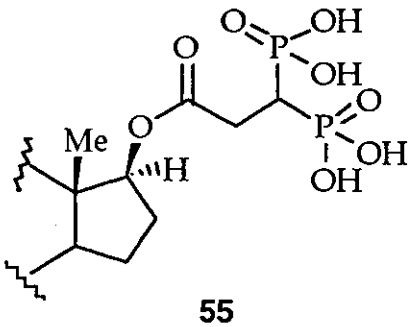
Scheme 32

Compound **53** was dissolved in dichloromethane and placed in a hydrogenator with 10% Pd on activated charcoal, and agitated for approximately 18 h. A small amount of a higher running contaminant was observed by tlc (10% MeOH/DCM) which we were unable to remove by chromatography (5% MeOH/DCM). The crude product was thus isolated as an off-white foam (49%). Although tlc analysis showed an impurity present, **54** was essentially pure by NMR spectroscopy (^{1}H, ^{31}P).

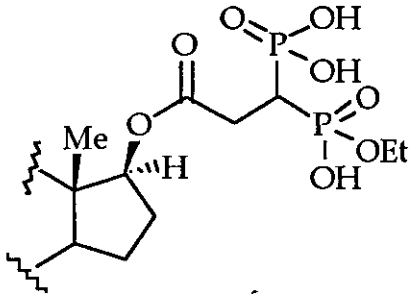


54

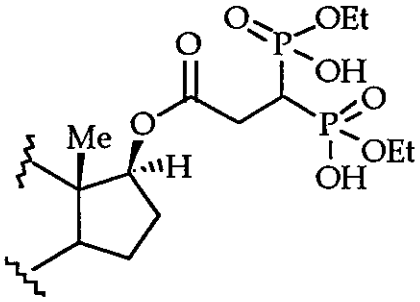
The hydrolysis of the phosphonate esters was attempted using TMSBr (8 eq.) in DCM and stirring for 3 days. The solvent was removed under vacuum and the concentrate was stirred for 30 mins. in methanol. After examining the spectroscpoic data we concluded that the isolated material was a mixture of the desired phosphonic acid **55** and a number of partially hydrolysed compounds, eg. **56** and **57**.



55



56



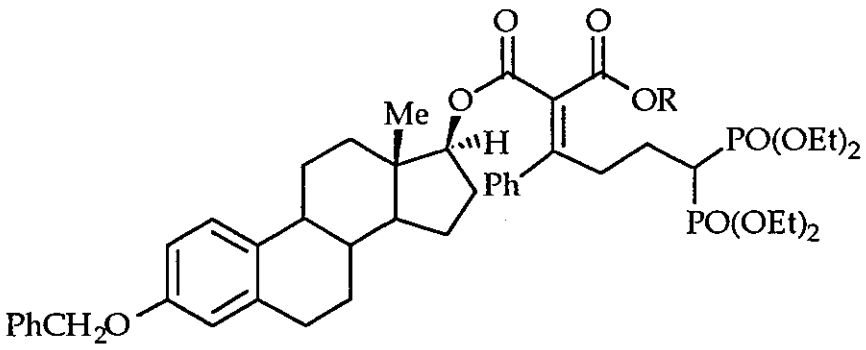
57

^{31}P NMR spectroscopy showed a major signal at 27.6 ppm, and two smaller signals at 28.3 and 27.0 ppm, which we tentatively assigned to structures **5 6**, **5 5** and **5 7** respectively.

2.5 Restricted rotation conjugates

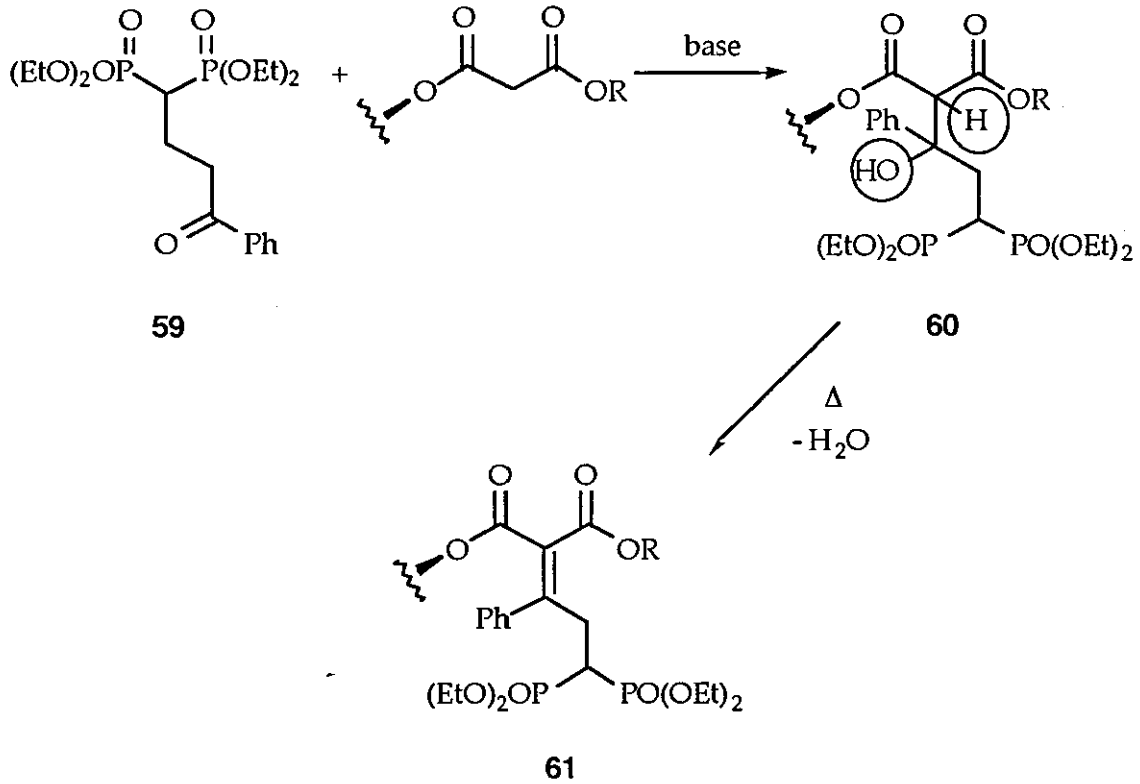
An interesting variable in the structural design of drug molecules is that of the relative flexibility or rigidity of the molecule as a whole, and of its component parts. Although rigidity may introduce stereospecificity in any interaction, it is often not a requirement for high activity.

With this in mind, we sought to prepare a novel functionalised bisphosphonate with restricted rotation, and hence impart a degree of rigidity to the molecule.



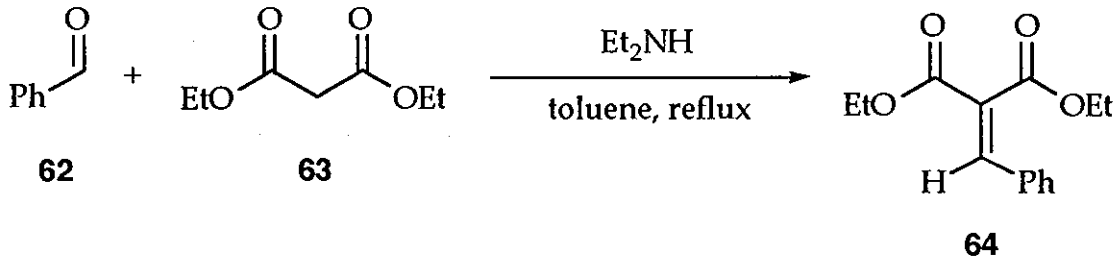
58

The introduction of a double bond into a molecule is an effective method of increasing its rigidity. We hoped to prepare compounds such as **58**, derived from a Knoevenagel condensation between a malonyl oestrogen and an acetophenyl functionalised bisphosphonate **59** (Scheme 33). Sturtz *et al* employed a Michael addition to functionalise tetraethyl ethylidene bisphosphonate with a number of nucleophiles, including acetophenone. [4] The following Knoevenagel condensation involves the initial addition of a malonyl-oestrogen enolate to the bisphosphonate in an aldol fashion, generating a hydroxyl intermediate **60**. The sequential elimination of a molecule of water produces the alkene **61** as *cis* and *trans* isomers. One would predict that the major product would be the *trans* isomer (steroid - bisphosphonate) on steric grounds.



Scheme 33

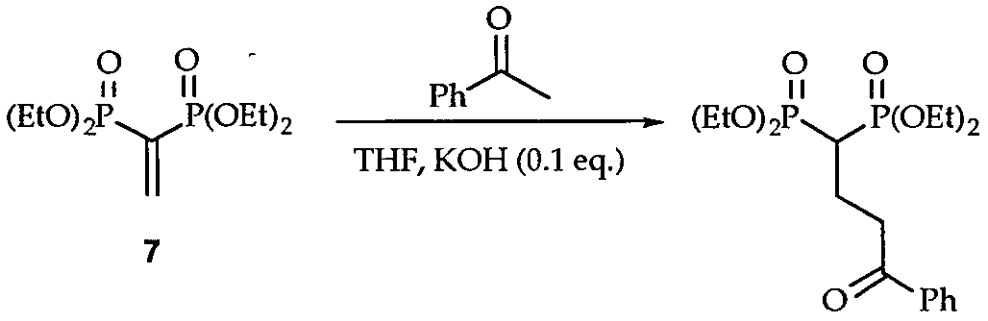
To confirm the feasibility of our synthetic route, and to avoid wasting precious intermediate compounds, a series of test reactions were carried out. First of all we needed to know if we could carry out the crucial Knoevenagel condensation on our malonates, and secondly if we could reproduce the Sturtz conjugate addition with tetraethyl ethylidene bisphosphonate. The Knoevenagel reaction was attempted using benzaldehyde, (a cheap, reactive substrate), and oestrogen-ethyl mixed malonate. Although a number of base/solvent systems were tried, eg. LHMDS/Et_2NH; THF/DCM; rt-60 °C, we found that no reaction took place - tlc showed only the presence of starting materials. In view of these results, we chose to study the reactivity of a simple malonate towards the Knoevenagel reaction. To this end we heated a solution of benzaldehyde **62** and diethyl malonate **63** in toluene under reflux in the presence of diethylamine for a duration of approximately 18 h. The ^1H NMR spectrum was consistent with the desired α-β unsaturated product **64**, although we were able to isolate it in only 10% yield after chromatography (10% MeOH/Petrol).



62 63 64

Scheme 34

Future work would apply these conditions to our oestrogen-alkyl mixed malonates.



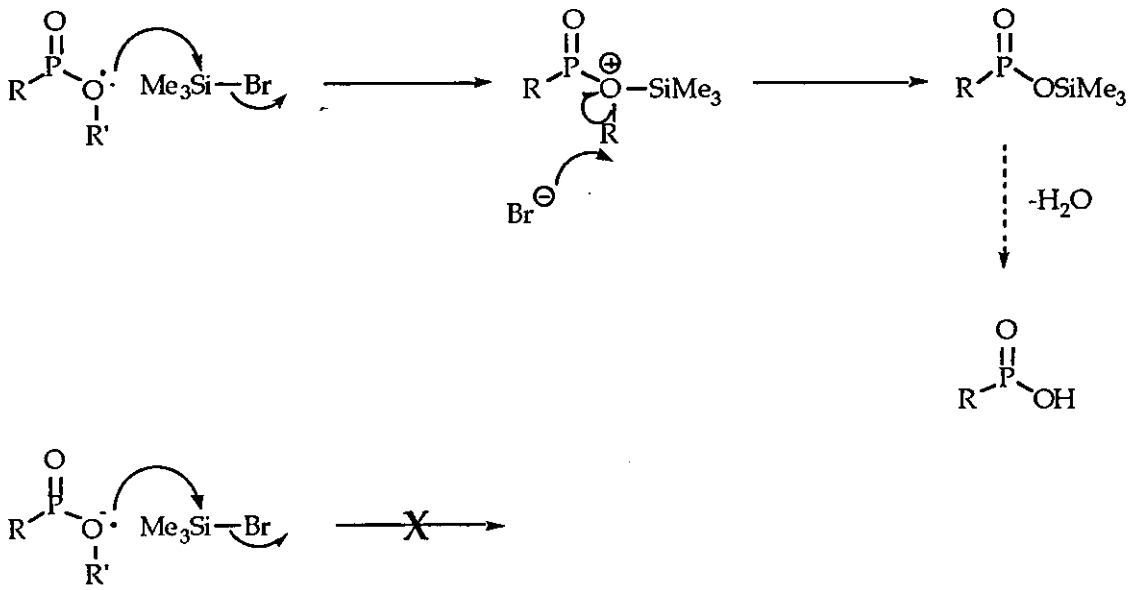
7 65

Scheme 35

We next carried out the Michael addition of benzophenone and tetraethyl ethylidene bisphosphonate, stirring the two reagents together in a solution of THF and KOH (0.1 eq.) at room temperature for several hours (Scheme 35). The crude mixture was purified by silica gel flash column chromatography, eluting with ethyl acetate/ethanol (1:1), affording the product as a clear oil. The ^{1}H NMR data, however, were rather inconclusive and implied possible contamination of the product with benzophenone starting material. The ^{31}P NMR spectrum displayed a sharp single peak, signifying the presence of only one type of phosphorus atom, but the chemical shift was not consistent with that of the expected product (**6 5** δ_P = 18.9 ppm, Sturtz δ_P = 23.0 ppm). Clearly this work needs to be repeated, and one ought to consider that Sturtz only quotes a yield of 32% at best for this Michael addition. It is feasible that it is a problematic reaction which may require further development.

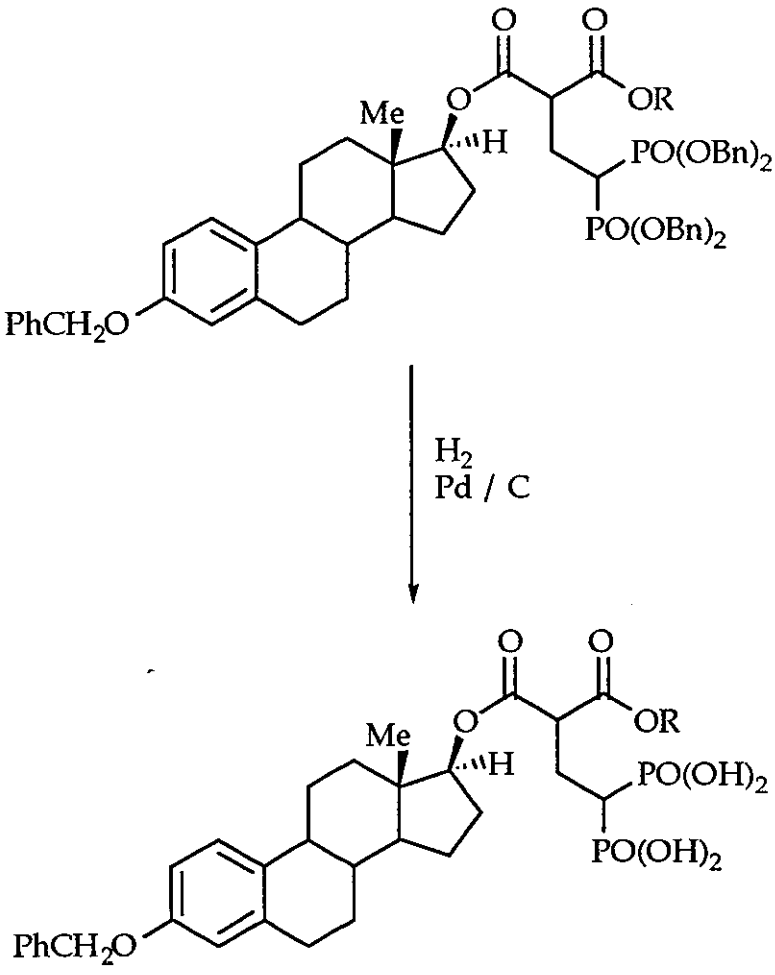
2.6 A simplified approach to bisphosphonic acids

Traditionally, the hydrolysis of phosphonates using bromotrimethylsilane (TMSBr) has been a convenient method of preparing phsphonic acids.[27,28,29,30] TMSBr is particularly selective towards phosphonate esters, and hence can be used in the presence of carboxylic esters. This can be explained by comparing the nucleophilicity of the two esters; in general, a carboxylic ester is less nucleophilic than a phosphonate ester. The addition of water to the silyl ethers releases the phosphonic acids (Scheme 36).



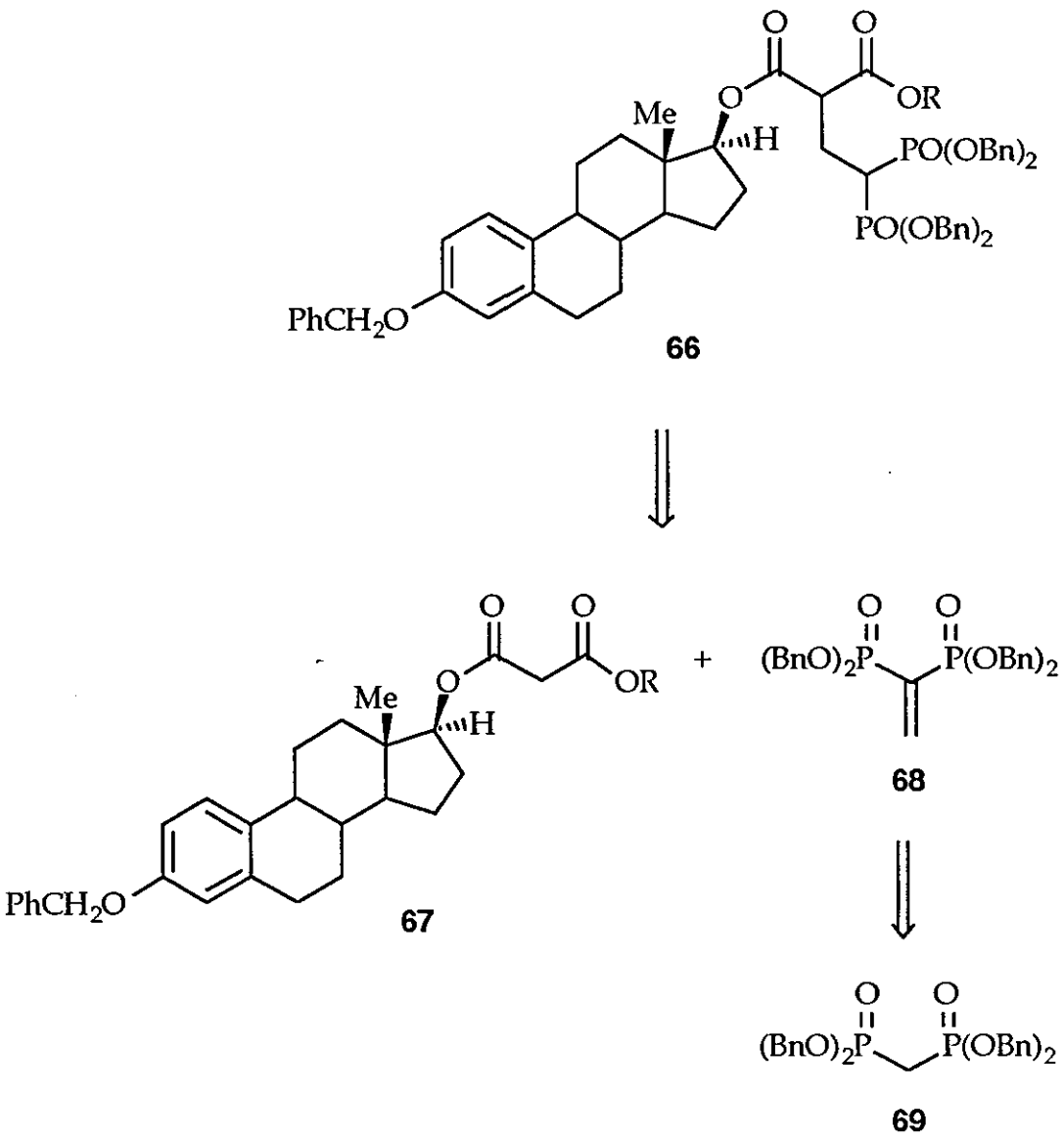
Scheme 36

Although this procedure works well with phosphonates and simple bisphosphonates, we found it to be somewhat capricious with our oestrogenic conjugates. The many reactions attempted in some cases produced partially hydroysed material rather sluggishly, but in some cases the desired bisphosphonic acids. We therefore sought a more reliable route to preparing our oestrogen funtionalised bisphosphonic acids.



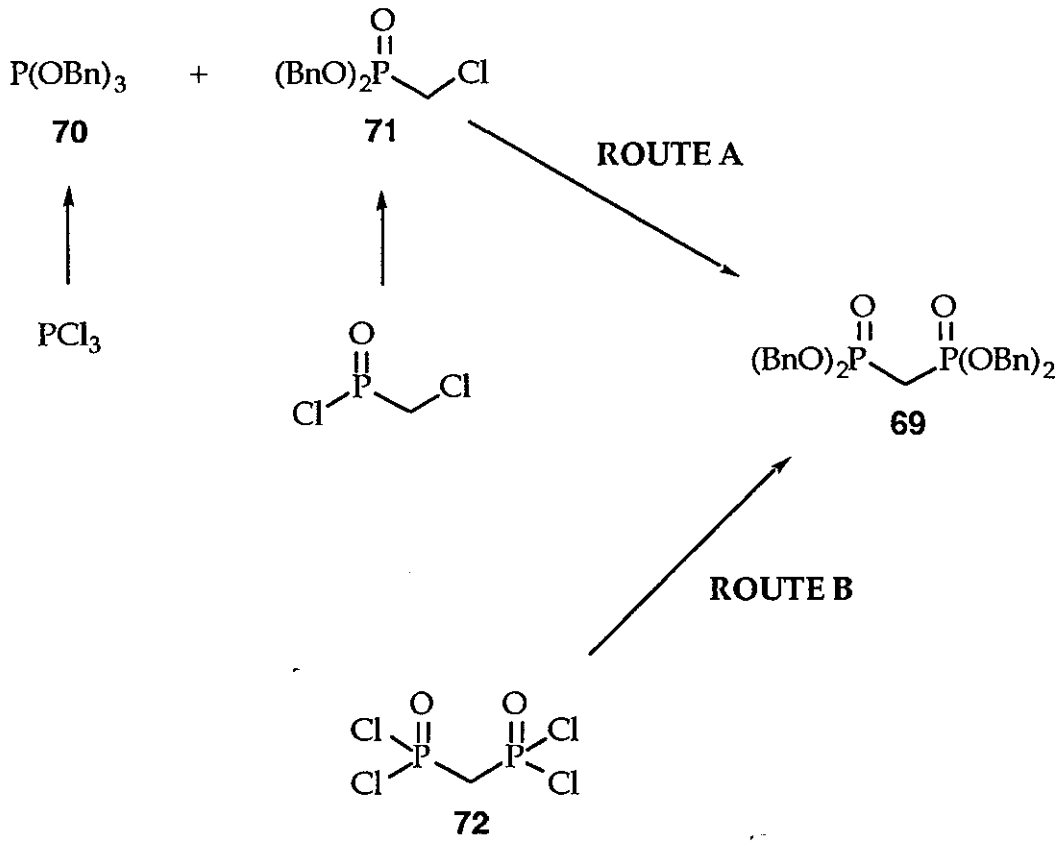
Scheme 37

We envisaged preparing tetrabenzyl ethylidene bisphosphonate **68** and incorporating this into one of our oestrogenic compounds. The subsequent hydrogenation step would then cleave all the benzyl ethers in one step to reveal both the hydroxyl group at C-3 and the bisphosphonic acid moiety (Scheme 37).



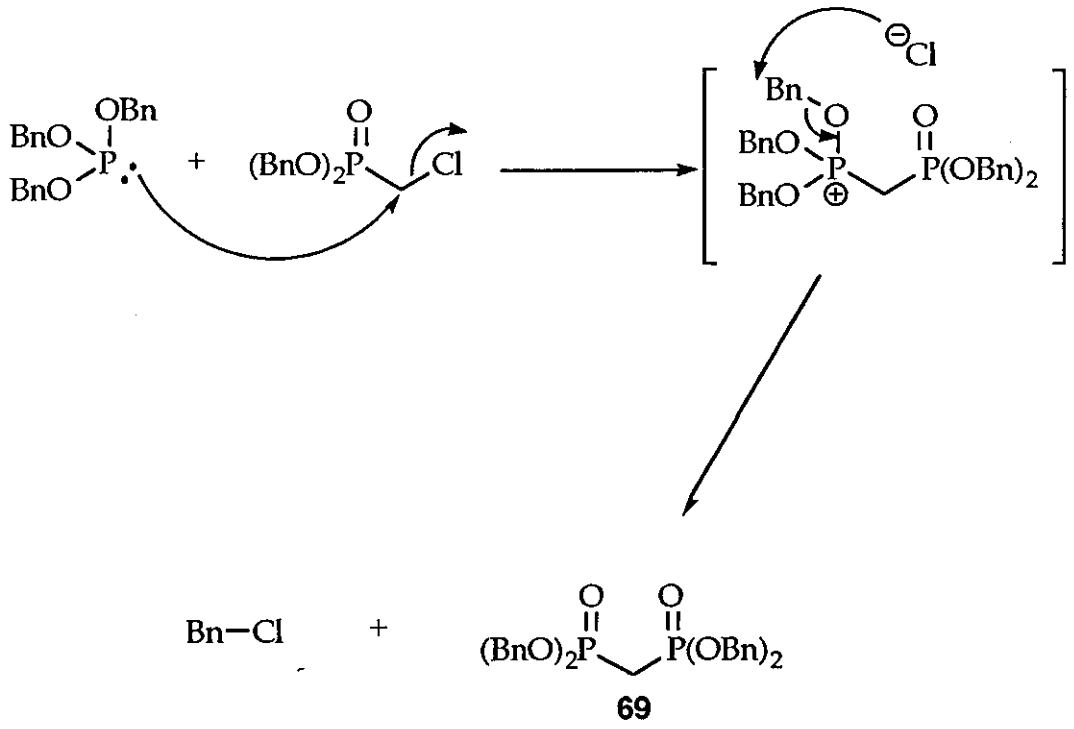
Scheme 38

Tetrabenzyl ethylidene bisphosphonate **68** was considered easily accessible through the addition-elimination reaction with paraformaldehyde. The preparation of the tetrabenzyl methylene bisphosphonate precursor **69** would be more difficult and as such we examined two possible syntheses (Scheme 39).



Scheme 39

Route A involves the reaction of tribenzyl phosphite **70** and dibenzyl (chloromethyl) phosphonate **71** in a Michaelis-Arbuzov reaction.[31] Benzyl chloride is generated during the reaction, and must be removed efficiently; this can be satisfactory achieved at high temperature (140 °C) and reduced pressure (4-20 Torr).



Scheme 40

Furthermore, a benzyl group is more sensitive than an alky group to nucleophilic attack by the chloride anion (Scheme 40), resulting in the formation of benzyl chloride and not cleavage of the intermediate to give the starting materials[18].

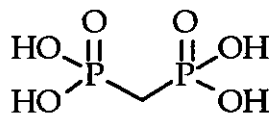
Tribenzyl phosphite was successfully prepared by the dropwise addition of anhydrous triethylamine (3.1 eq.) to trichlorophosphite in anhydrous diethyl ether at -78 °C, under nitrogen.[31] The reaction mixture was treated dropwise with benzyl alcohol (3 eq.) in anhydrous diethyl ether, stirred for 2 h at -78 °C and allowed to reach room temperature. After 24 h. the reaction was considered to have proceeded as far as was possible, although considerable starting material was still present. The crude mixture was filtered and the residue purified by silica gel flash column chromatography, eluting with 10% ethyl acetate in light petroleum, to afford the tribenzyl phosphite **70** as a clear oil (56%). The NMR data were consistent with that of the desired product; ^{1}H ($CDCl_3$) 7.4 (m, 15H), 4.9 (d, J = 8, 6H); ^{31}P ($CDCl_3$) 20.2.

The preparation of dibenzyl (chloromethyl) phosphonate **71** proved problematic. Anhydrous triethylamine (3 eq.) was added dropwise to (chloromethyl) phosphonic dichloride in anhydrous THF at 0 °C. Benzyl alcohol (2.2 eq.) in anhydrous THF was added dropwise and the mixture stirred at 0 °C for 1 h and then room temperature for 4 h. The reaction appeared to have reached completion by tlc, so the crude mixture was filtered. The filtrate was evaporated and the residue purified by silica gel flash column chromatography, eluting with 10-20% ethyl acetate in light petroleum. Unfortunately, only trace amounts of a clear oil were isolated whose ^{1}H NMR spectrum resembled benzyl alcohol. We reasoned that the dibenzyl (chloromethyl) phosphonate was lost by evaporation during the removal of the solvent. Further attempts to prepare such a phosphonate were unsuccessful, thus we could not attempt the final Michaelis-Arbuzov reaction with the aforementioned phosphonate and tribenzyl phosphite.

Route B consists of the alkylation of methylene bis (disphosphonic dichloride) **72** with benzyl alcohol using pyridine as a hydrogen chloride scavenger[31]. The disphosphonic dichloride was typically suspended in freshly distilled toulene under nitrogen and cooled to 0 °C. Dry benzyl alcohol (4.15 eq.) in dry pyridine was added using a syringe pump over 1.5 h at 0 °C under nitrogen. The ice bath was then removed and stirring continued at room temperature for a further 2.5 h. The reaction mixture was filtered and the solid material washed twice with toluene. Toluene was added to the filtrate, the mixture washed twice with sodium hydroxide (2M) and water, and dried over anhydrous $MgSO_4$. The crude oil was purified by silica gel flash column chromatography eluting with 80% ethyl acetate in light petroleum to remove any benzyl alcohol impurity, and then ethyl acetate. The tetrabenzyl methylene bisphosphonate **69** was isolated as a clear oil (47%). It was clear that the availability of such a compound in good yield and on a multigram scale was of the utmost importance to our synthetic strategy.

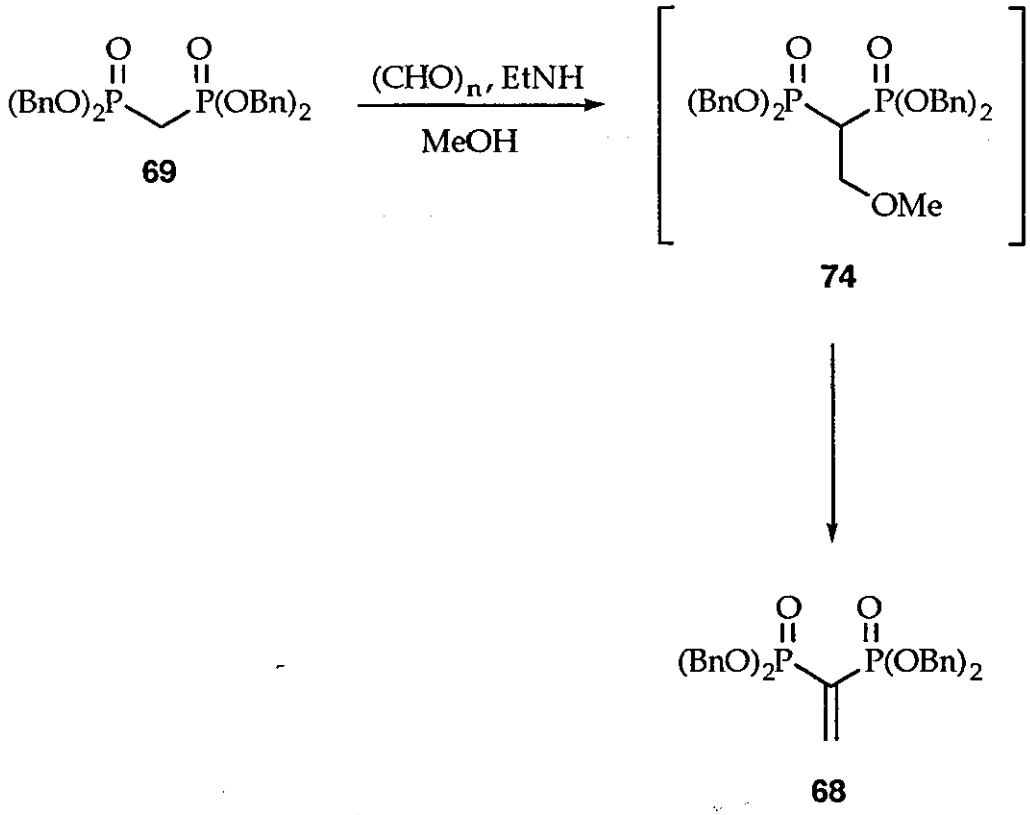
Before incorporating the tetrabenzyl methylene bisphosphonate into our oestrogen malonates, we wanted to verify that the benzyl esters could be removed by hydrogenolysis. 10% Pd on activated charcoal (20 mg) was

added to a solution of tetrabenzyl methylene bisphosphonate in ethyl acetate. The reaction flask was fitted with a rubber septum and a hydrogen balloon attached. The reaction was monitored by ^{1}H NMR spectroscopy and after approximately 18 h appeared to be incomplete. We reasoned that the sulfur in the rubber septum may have poisoned the palladium catalyst, and hence prevented the reaction. The hydrogenolysis was consequently repeated and the reaction flask fitted with a glass adaptor. As the bisphosphonic acid formed we noticed that the palladium coagulated on the surface of the solvent. After approximately 20 h, the reaction mixture was filtered through a pasteur pipetteand the solvent removed by evaporation. Methylene bisphosphonic acid **73** was isolated as a white solid (ca. 100%) which was essentially pure by ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy and like previous bisphosphonic acids, it proved to be unstable to mass spectrometry and elemental analysis.



73

Tetrabenzyl ethylidene bisphosphonate **68** was prepared in a similar fashion to its tetraethyl equivalent.[32] The formation of the methoxy ethylene bisphosphonate intermediate **74**, however, was somewhat more sluggish and required stirring for approximately 1 week (Scheme 41).

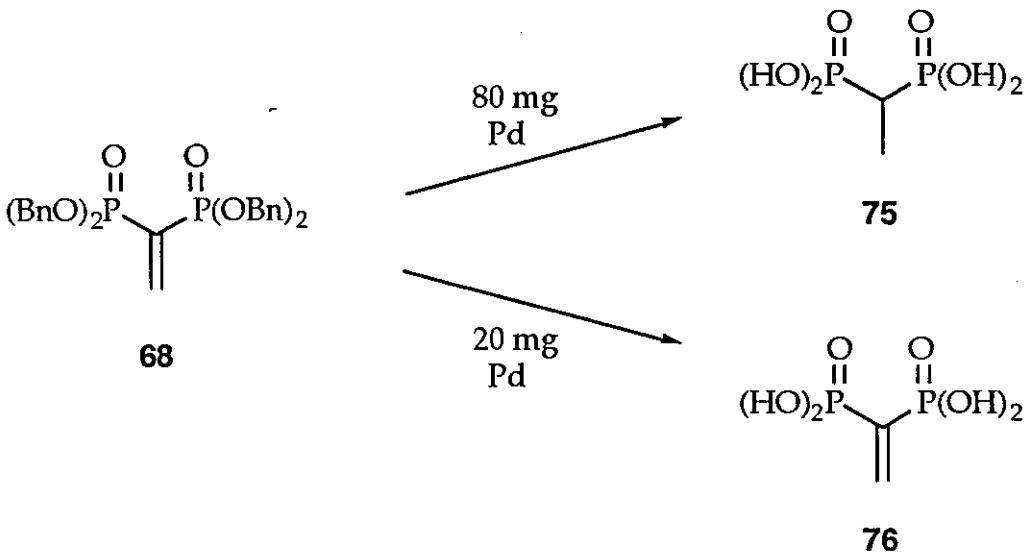


Scheme 41

For the efficient elimination of methanol, the methoxy ethylene intermediate **74** required heating to reflux in toluene in the presence of p-TSA using a Soxhlet apparatus. The product was extracted into dichloromethane and washed repeatedly with cold water. The aqueous layer was also extracted with dichloromethane to retrieve any of the highly water soluble bisphosphonate. The organic phases were combined, dried with anhydrous $MgSO_4$ and the solvent removed by evaporation. Tetrabenzyl ethylidene bisphosphonate **68** was isolated initially as a clear oil which formed brown crystals (98%) on elimination of the final traces of solvent under high vacuum. No chromatography was performed on the product since it was essentially pure by NMR spectroscopy (^{1}H, ^{13}C, ^{31}P).

Before we further functionalised the tetrabenzyl ethylidene bisphosphonate we thought it prudent to verify that the benzyl ethers could be removed by hydrogenolysis. During a series of hydrogenolysis experiments we made a

number of interesting observations. As indicated above, the use of rubber septa may have led to the poisoning of the palladium catalyst and consequently the use of glass stoppers was essential. The use of methanol to wash the surface of the palladium was also halted since we witnessed methanol addition across the double bond - ^{1}H NMR spectroscopy showed the presence of a methoxy ethylene intermediate **74**. Furthermore, we found that the amount of catalyst used was significant, eg. hydrogenolysis on 200 mg bisphosphonate using 80 mg palladium resulted in the removal of the double bond, while using only 20 mg palladium left the double bond intact.

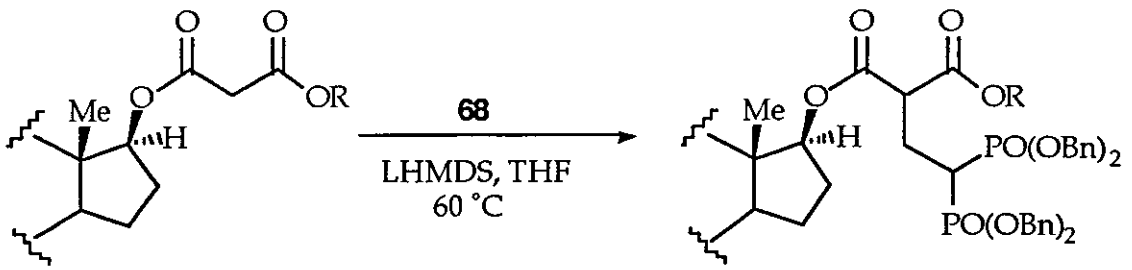


Scheme 42

Optimum conditions were hence developed which involved stirring tetrabenzyl ethylidene bisphosphonate **68** in dichloromethane with 20 mg palladium in a flask fitted with a glass adaptor and a hydrogen balloon for 6 h. The ethylidene bisphosphonic acid **76** was isolated as a white foam (ca 100%) which required no further purification by chromatography as it was essentially pure by NMR spectroscopy.

We continued to apply this bisphosphonate chemistry and attempted to incorporate tetrabenzyl ethylidene bisphosphonate **68** into our oestrogen malonates. Tetrabenzyl ethylidene bisphosphonate was added to a solution

of mixed malonate and LHMDS (1 eq.)in freshly distilled THF, and the mixture warmed at 60 °C (Scheme 43).

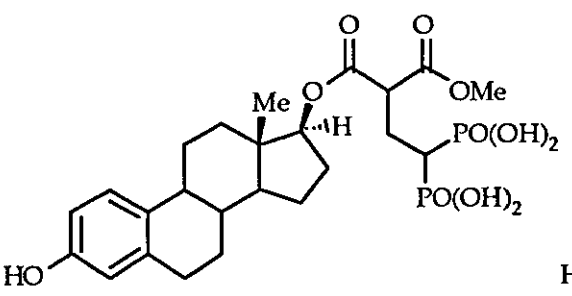


R = Me **77** t = 48 h, 47 %
Ph **78** t = 20 h, 28 %

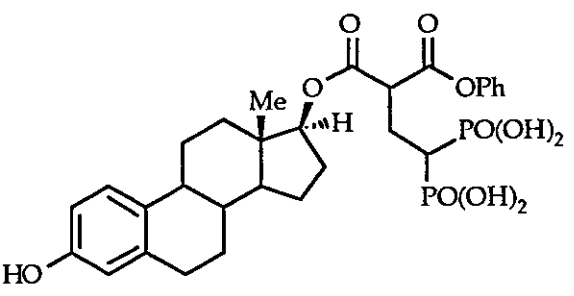
Scheme 43

The resulting funtionalised bisphosphonates were typically purified by silica gel flash column chromatography, eluting with 2% methanol in dichloromethane. Methyl **77** and phenyl **78** derivatives were isloated (as clear oils) in 47 and 28% yields respectively.

Predictably, the two new bisphosphonates were debenzylated by hydrogenolysis with 10% Pd on activated charcoal (20 mg). The solvent of choice was dichloromethane and the reactions were stirred under nitrogen for 18-40 h.



79



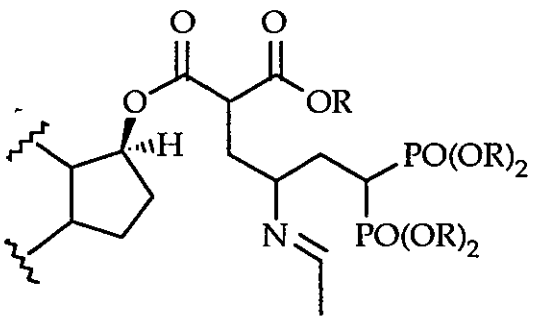
80

The methyl derivative **79** was collected as an off-white foam (89%) after 18 h stirring. Its NMR spectroscopy data were consistent with that of the desired structure; δ_P = 28.4, cf. **51** δ_P = 28.1. In common with our earlier bisphosphonic acids, **79** proved to be unstable to mass spectroscopy and elemental analysis. The formation of the **80** derivative was more sluggish and required stirring for approximately 40 h, and was finally collected as an off-white powder (> 99%). Analysis by ^{31}P NMR spectroscopy displayed a major peak for the bisphosphonic acid (δ_P = 28 ppm) but also a minor impurity (δ_P = 20 ppm), probably a partially hydrogenated bisphosphonate. In an attempt to purify the mixture the crude product was dissolved in methanol and added dropwise to a solution of sodium acetate in methanol. The resulting precipitate was filtered and washed with methanol and water to afford the sodium salt of the bisphosphonic acid. Unfortunately, we were unable to isolate sufficient sample for further analysis.

Hence, we have demonstrated the synthetic utility of benzyl bisphosphonates in the preparation of bisphosphonic acids. We have shown how this simple chemistry can be applied to more complicated systems, and the ease with which highly functonalised bisphosphonic acids may be obtained.

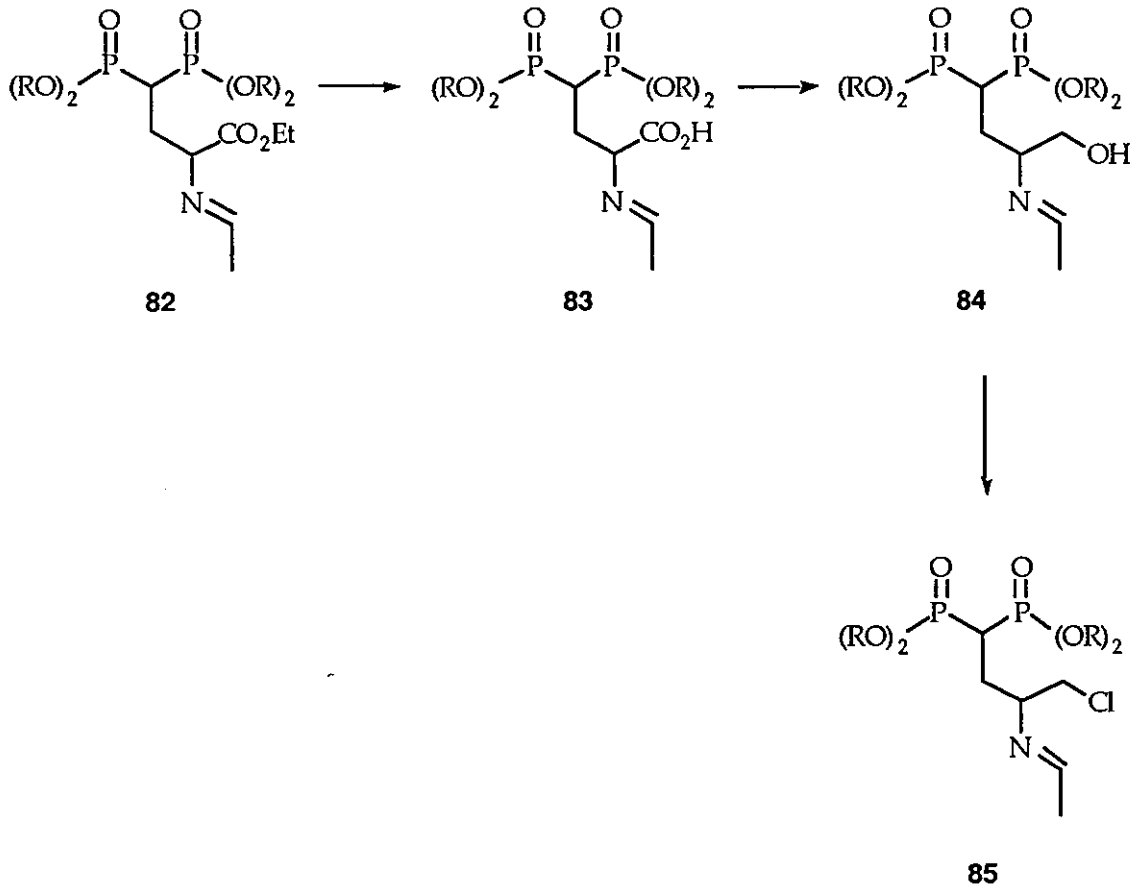
2.7 Future Work

It would be of both synthetic and therapeutic interest to incorporate further functionality into the oestrogen-bisphosphonate conjugates prepared in this chapter. The presence of a nitrogen atom within a molecule is believed to enhance binding to receptors. Furthermore, it is known that bisphosphonates bearing nitrogen functionality have increased potency against bone resorption. One could, therefore, consider the synthesis of compounds such as **81** derived from malonyl oestrogens and bisphosphonate imine **85**.

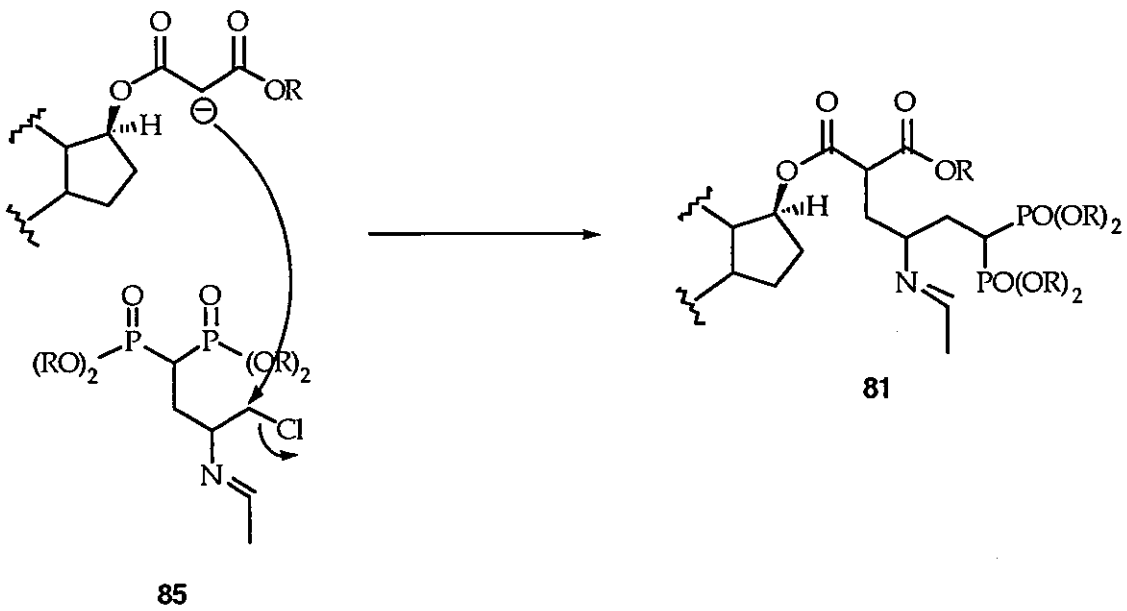


81

Sturtz[33] described the preparation of bisphosphonate **82** from a glycinate derivative and tetraethyl ethylidene bisphosphonate. Hydrolysis of the carboxylic ester with, e.g. NaOH, would yield the mono acid **83**. Subsequent $LiAlH_4$ reduction would reveal the primary alcohol **84**, and the addition of PCl_5 would produce the chlorinated bisphosphonate **85** (Scheme 44). The displacement of the chlorine atom by the oestrogen-malonyl anion would yield the target bisphosphonate conjugate **81** (Scheme 45).

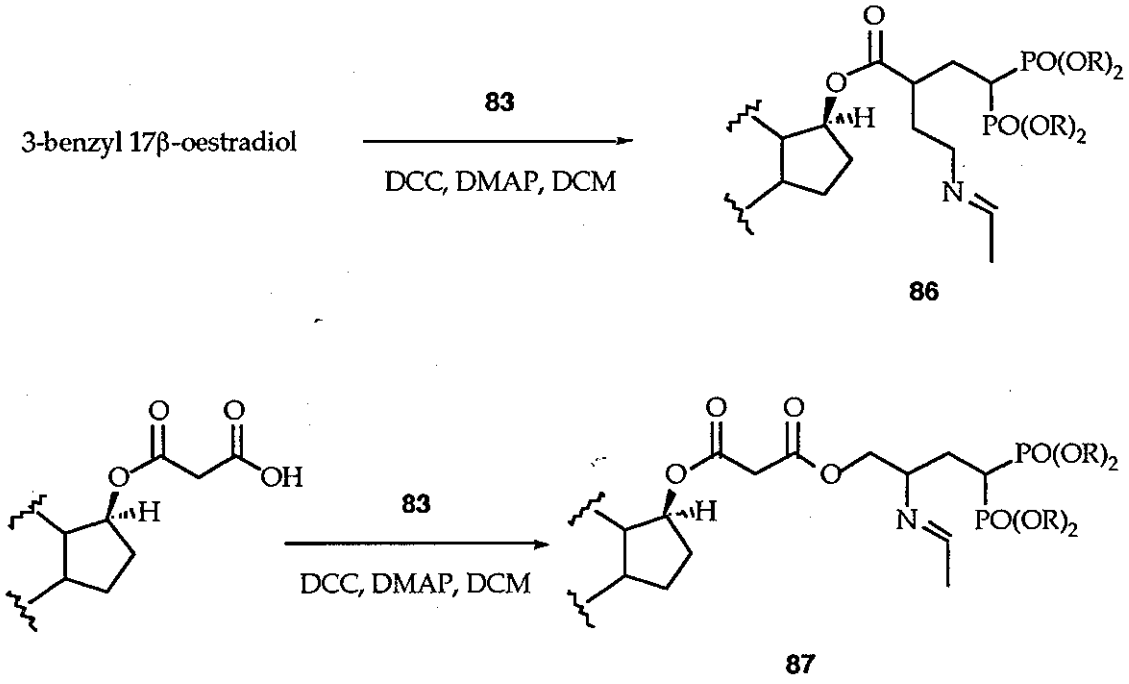


Scheme 44

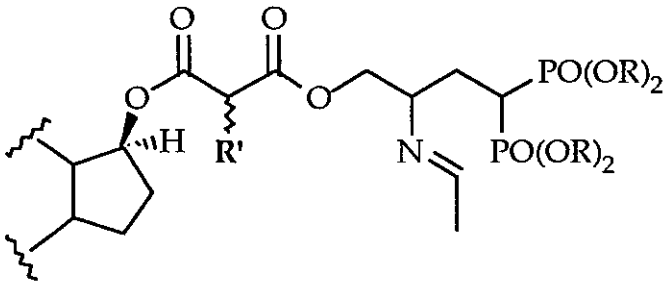


Scheme 45

Alternatively, one could esterify mono-acid **83** with 3-benzyl 17β-oestradiol, or oestrogen malonic half-acid **36**, using a dehydrating agent such as DCC to yield conjugates **86** and **87** respectively (Scheme 46). Conjugate **87** could be alkylated at the malonyl CH_2 position to provide further bisphosphonates **88**.



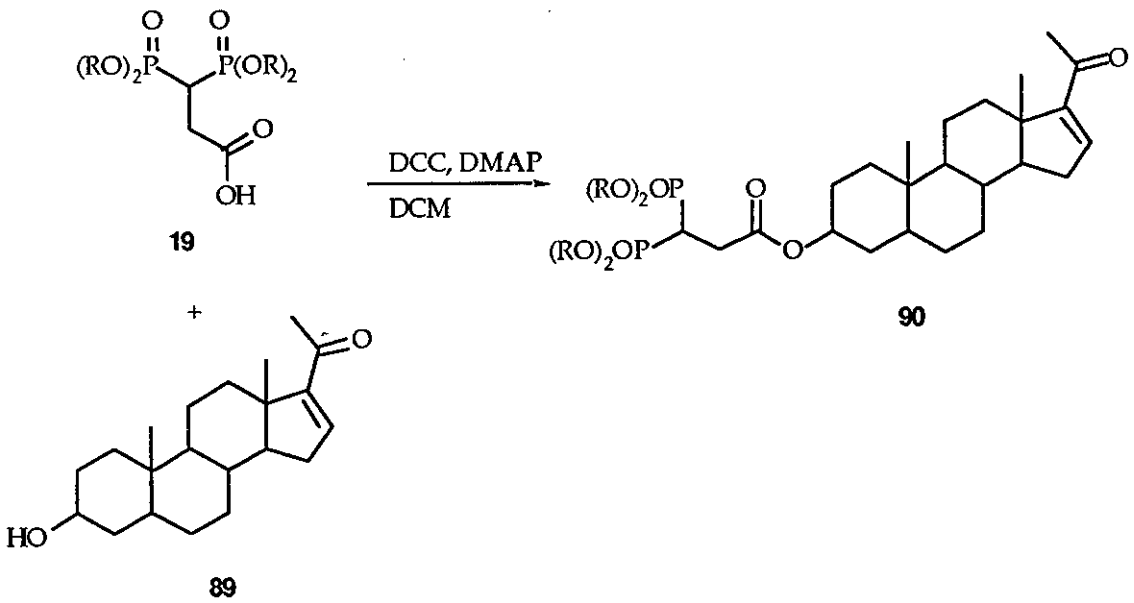
Scheme 46



88

One could also consider extending the use of our bisphosphonate delivery system to other steroids, such as those used in the treatment of asthma e.g. prednisolone. Patients on high-dose steroid therapy can develop a range of adverse effects, including metabolic problems, gastrointestinal symptoms and

osteoporosis. Coupling a prednislone derivative to one of our bisphosphonates could have the potential of relieving the symptoms of asthma, whilst protecting against the development of osteoporosis. A one-pot DCC coupling reaction could join bisphosphonate **19** at the 3'-OH of prednisolone derivative **89**, to yield conjugate **90** (Scheme 47).



Scheme 47

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CHAPTER THREE

Experimental

3.1 General Experimental Procedures

3.1.1 Purification of Solvents

Tetrahydrofuran was freshly distilled under nitrogen from the sodium/benzophenone ketyl radical, or purchased from the Aldrich Chemical Company in Sureseal® containers prior to use.

Petroleum ether (b.p. 40-60 °C) and **ethyl acetate** were distilled from calcium chloride prior to use.

Dichloromethane was distilled from phosphorous pentachloride prior to use.

Methanol, ethanol and **toluene** were distilled from calcium hydride prior to use.

3.1.2 Preparation of Glassware

All air and water sensitive reactions were carried out in round bottomed flasks which had been baked at 150 °C for a minimum of 4 h. The flasks were allowed to cool in a dessicator over self indicating gel. Air sensitive reactions were carried out under a slight positive pressure of nitrogen. Reagents and solvents were introduced *via* syringe or cannula through a septum cap.

3.1.3 Purification of Products

Thin layer chromatography was carried out on glass, plastic or aluminium backed plates coated with a 0.25 mm layer of 60 H silica gel containing flourescer. Compounds were visualised with U. V. Light (254 nm) or alkaline potassium manganate (1% w/v in water). For both chemical visualisations heating was usually required.

Flash column chromatography was carried out using Merck 9385 Kieselgel 60 (230-400 mesh). Pressure was applied with hand bellows.

3.1.4 Spectroscopy

^{1}H, ^{13}C and ^{31}P Nuclear magnetic resonance spectra were recorded using Bruker AC250 and Bruker DPX400 instruments. All spectra were recorded using deuteriochloroform or deuterioacetone, with tetramethylsilane ($^{1}H/^{13}C$) and phosphoric acid (^{31}P) as the internal references. The following symbols have been adopted in the description of NMR spectra;

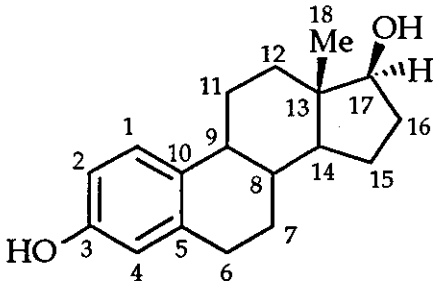
δ = chemical shift (ppm)
J = coupling constant (Hz)
s = singlet
d = doublet
t = triplet
q = quartet
m = multiplet
dd = doublet of doublets
dt = doublet of triplets

Mass spectra (chemical ionization (CI), fast atom bombardment (FAB) and electron impact (EI)) were obtained through the EPSRC National Mass Spectrscopy Service Centre, University of Wales, Swansea.

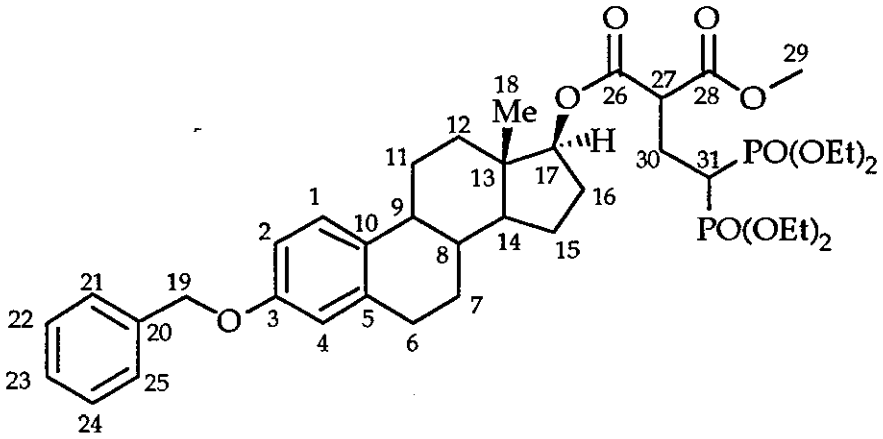
Infra red spectra were recorded in the range 4000 to 600 cm^{-1} using a Nicolet 205 FT-IR spectrophotometer, and were calibrated against the 1602 cm^{-1} absorption of polystyrene. Liquids were run as a thin film, solids as nujol mulls on sodium chloride plates.

Microanalyses was performed on a Perkin Elmer 2400 CHN elemental analyser.

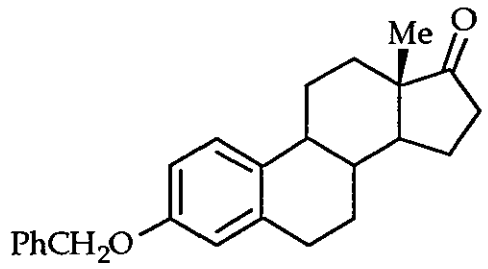
X-Ray reflections were recorded on a Rigaku AFC7S diffractometer.



Basic numbering system for oestradiol



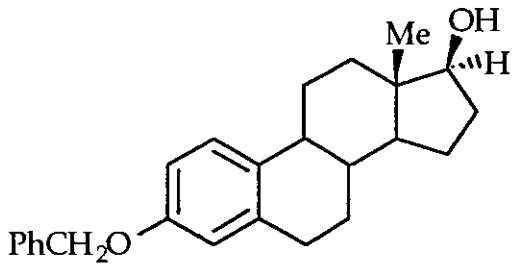
Extended numbering system for oestrogen conjugates



13

3-benzyl oestrone (13)

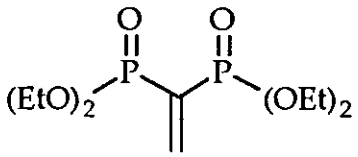
Oestrone (10.0 g, 37.0 mmol, 1 eq.) was stirred in acetone (150 mL) and anhydrous K_2CO_3 (25.6 g, 185 mmol, 5 eq.). Benzyl bromide (7.79 mL, 11.2 g, 65.4 mmol, 1.77 eq.) was added slowly and the reaction mixture was allowed to stir overnight at room temperature. The mixture was then filtered and taken up in CH_2Cl_2 (200 mL). The organic layer was washed with 1M HCl (3 x 100 mL) and dried over anhydrous $MgSO_4$. After the solvent had been removed, the product was recrystallised from methanol and washed repeatedly with cold methanol to remove any traces of benzyl bromide. The product was obtained as white crystals (6.00 g, 60%). I.R. ν_{max} 1729 (cyclic C=O), 1602, 1578 and 1497 (aromatic C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 0.90 (3H, s, 18'C$\underline{H}_3$), 1.45-1.70 (6H, m), 1.96-2.47 (7H, m), 2.87-2.89 (2H,m), 5.02 (2H,s, -OCH_2Ph), 6.72 (1H, s, 4'CH), 6.73-6.80 (1H, dd, J=8Hz, 2'CH), 7.18 (1H, d, J= 8Hz, 1'CH), 7.30-7.43 (5H, m, $\underline{Ph}$-CH_2O); δ_C (100 MHz, $CDCl_3$) 13.8 (18'CH_3), 21.6 (cyclic CH_2), 25.9 (cyclic CH_2), 26.5 (cyclic CH_2), 29.6 (cyclic CH_2), 31.7 (cyclic CH_2), 35.9 (cyclic CH_2), 38.3 (cyclic CH), 44.0 (cyclic CH), 48.0 (13'C), 50.4 (cyclic CH), 69.9 (-O$\underline{C}H_2$Ph), 112.4 (2'C), 114.9 (4'C), 126.4 (1'C), 127.4, 127.8 and 128.5 (phenyl CH's), 132.3 (10'C), 137.2 (quaternary phenyl), 137.8 (5'C), 156.8 (3'C), 221.0 (cyclic C=O) ; m/z (FAB) 361.21616 (M^+); $C_{25}H_{28}O_2$ requires 361.21676; mp=128-130°C.



14

3-benzyl 17β-oestradiol (14)

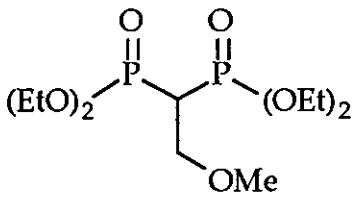
3-benzyl oestrone (9.50 g, 13.9 mmol, 1 eq.) was dissolved in CH_2Cl_2 (65.0 mL) and CH_3OH (65.0 mL), and $NaBH_4$ (1.58 g, 41.7 mmol, 3 eq.) was added slowly at 0 °C. The reaction mixture was stirred for 3 h. under an atmosphere of nitrogen and allowed to reach room temperature. After this time, the reaction was quenched slowly with cold water (150 mL). Dichloromethane (200 mL) was added to the reaction mixture and the organic layer was washed with 1M HCl (3x100 mL). The organic fraction was dried over $MgSO_4$, and the solvent removed under reduced pressure. The resulting off-white solid was recrystallised from methanol to afford white crystals (3.45 g, 69%). ν_{max} 3323 (broad, 2°alcohol), 1604 and 1499 (aromatic C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 0.77 (3H, s, 18'C$\underline{H}_3$), 1.45-1.70 (6H, m), 1.96-2.47 (7H, m), 2.80 (2H, m), 3.67 (1H, t, J=8.0 Hz, 17'C$\underline{H}$OH), 5.01 (2H, s, PhC$\underline{H}_2$O-), 6.70 (1H,s, 4'C$\underline{H}$), 6.74 (1H, dd, J=8.5 Hz, 8.6 Hz, 2'-C$\underline{H}$), 7.18 (1H, d, J = 8.5 Hz, 1'-C$\underline{H}$), 7.29-7.43 (5H, m, -OCH$_2\underline{Ph}$); δ_C (100 Mhz, $CDCl_3$) 11.05 (18'CH_3), 23.2 (cyclic CH_2), 26.3 (cyclic CH_2), 27.3 (cyclic CH_2), 29.8 (cyclic CH_2), 30.7 (cyclic CH_2), 36.8 (cyclic CH_2), 38.9 (cyclic CH), 43.3 (13'C), 44.0 (cyclic CH), 50.2 (cyclic CH), 70.1 (-O$\underline{C}H_2$Ph), 81.2 (17'C), 112.0 (2'C), 115.1 (4'C), 126.3 (1'C), 127.4, 127.8 and 128.5 (phenyl CH's), 133.0 (10'C), 137.5 (quaternary phenyl), 138.0 (5'C), 156.0 (3'C); m/z (FAB) 362.22399 (M^+); $C_{25}H_{30}O_2$ requires 362.22458.



7

Tetraethyl ethylidene bisphosphonate (7)

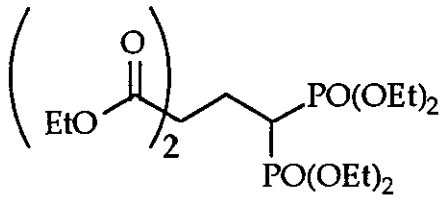
Paraformaldehyde (6.8 g, 228 mmol, 5 eq.) was dissolved in methanol (130 mL) and diethylamine (3.3 g, 45.6 mmol, 1 eq.), and heated at 60 °C until the mixture became colourless (approximately 1h). Tetraethyl methylene bisphosphonate (13.1 g, 45.6 mmol, 1 eq.) was added and the reaction mixture heated to reflux under an atmosphere of nitrogen overnight. The mixture was concentrated under reduced pressure and toluene (60 mL) added. The solvent was removed under reduced pressure, and the concentration-evaporation process repeated a second time. This procedure was performed in order to remove any residual methanol from the crude viscous intermediate. The methoxy ethylene bisphosphonate intermediate **11** was isolated as a clear oil; purification at this stage was not required and the crude product was used in its crude form. δ_H ($CDCl_3$) 1.35 (12H, t, $J_{H\text{-}H}$ = 7 Hz, $P_2(OCH_2C\underline{H}_3)_4$), 2.69 (1H, tt, $J_{H\text{-}H}$ = 5.5Hz, $J_{P\text{-}H}$ = 23.9Hz, PCHP) 3.9 (2H, td, $J_{H\text{-}H}$ = 5.5Hz, $J_{P\text{-}H}$ = 16.2Hz $P_2CHC\underline{H}_2OCH_3$) and 4.2 (8H, m, P-$OCH_2CH_3$).



11

p-TSA (0.04 g, cat.) was added to a solution of crude tetraethyl (2-methoxy) ethylene bisphosphonate **11** in toluene (65 mL) and the reaction mixture heated to reflux overnight under a nitrogen atmosphere. When the elimination was complete, the reaction mixture was washed with water (3x130 mL) and the organic layer dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to leave a clear brown oil (9.8 g, 71% for 2 steps), which was essentially pure by NMR and required no further purification. δ_H

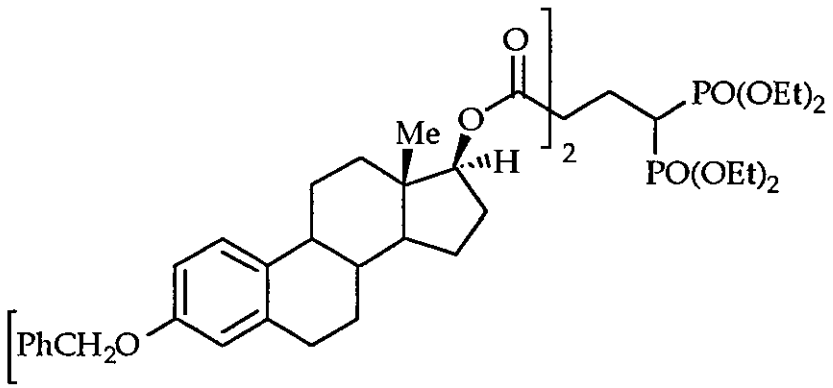
(250 MHz, $CDCl_3$) 1.30 (12H, t, J = 7Hz, P-$OCH_2\underline{CH_3}$), 4.0-4.2 (8H, m, P-$O\underline{CH_2}CH_3$), 6.9-7.1 (2H, dd, cis J_{P-H} = 37.53Hz, trans J_{P-H} = 37.39Hz, $P_2C{=}CH_2$); δ_P (100 MHz, $CDCl_3$) 11.6; m/z (CI) 301.09700 $(M+H)^+$; $C_{10}H_{22}O_6P_2$ requires 301.09699.



5

Bis(ethyloxycarbonyl)tetraethyl propylene bisphosphonate (5)

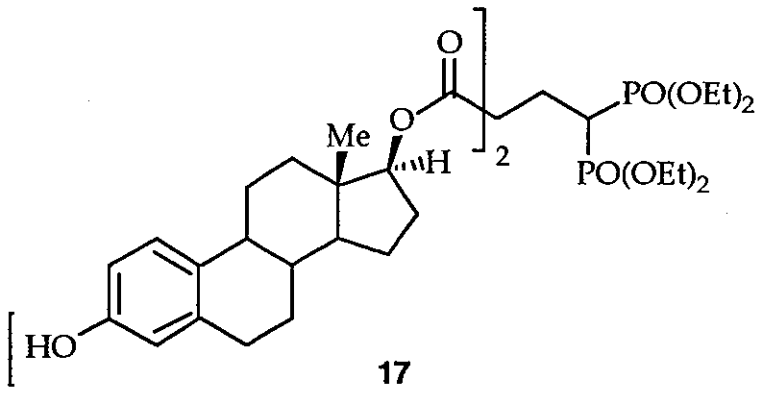
Sodium (0.08 g, 3.26 mmol, 0.1 eq.), was dissolved in ethanol (32.6 mL) at 0°C under an atmosphere of nitrogen. The mixture was allowed to reach ambient temperature and the sodium ethoxide formed transferred to a solution of tetraethyl ethylidene bisphosphonate (9.8 g, 32.6 mmol, 1 eq.) and diethyl malonate (5.2 g, 32.6 mmol, 1 eq.) in ethanol (50 mL). The reaction mixture was stirred for approximately 30 mins. under an atmosphere of nitrogen. The mixture was neutralised with NH_4Cl solution (16 mL), and the organic residue extracted with EtOAc (3x35 mL). The organic layer was dried with anhydrous $MgSO_4$ and the solvent removed under reduced pressure. The product was placed on a high vac. line for a further 5 h to remove final traces of solvent. The desired product was isloated as a brown oil (11.8 g, 80%). I.R. ν_{max} 1730 (C=O), 1254 (P=O), 1163 (P-O); δ_H (250 MHz $CDCl_3$) 1.28 (6H, t, J_{H-H} = 7.2Hz, $CO_2CH_2C\underline{H_3}$), 1.35 (12H, t, J = 7.7Hz, P-$O_2CH_2C\underline{H_3}$), 2.4-2.6 (3H, m), 3.98 (1H, t, J_{H-H} = 7.7Hz, -$C\underline{H}(CO_2Et)_2$), 4.0-4.2 (8H, m, P-$O\underline{CH_2}CH_3$), 4.25 (4H, q, J_{H-H} = 7.2Hz, -$0\underline{CH_2}CH_3$); δ_C (60 MHz, $CDCl_3$) 13.9 (2C, s, $CO(OCH_2\underline{C}H_3)$, 16.2 (4C, t, J_{P-C} = 6.1Hz, $PO(OCH_2C\underline{H_3})$, 24.7 (1C, t, J_{P-C} = 4.7Hz, $CH\underline{C}H_2CH$), 34.1 (1C, t, J_{P-C} = 133.0Hz, P$\underline{C}$P), 49.9 (1C, t, J_{P-C} = 7.7Hz, $CO_2Et\underline{C}HCH_2$), 61.4 (2C, s, $CO(O\underline{C}H_2CH_3)$, 62.7 (4C, t, J_{P-C} = 8Hz, $PO(O\underline{C}H_2CH_3)$, 169.3 (2C, s, $\underline{C}O_2Et$); δ_P (100 MHz, $CDCl_3$) 22.5; m/z (CI) 460.16317 (M^+); $C_{17}H_{34}O_{10}P_2$ requires 460.16272.



15

Bis(carbonyl oestrogen)tetraethyl propylene bisphosphonate (15)

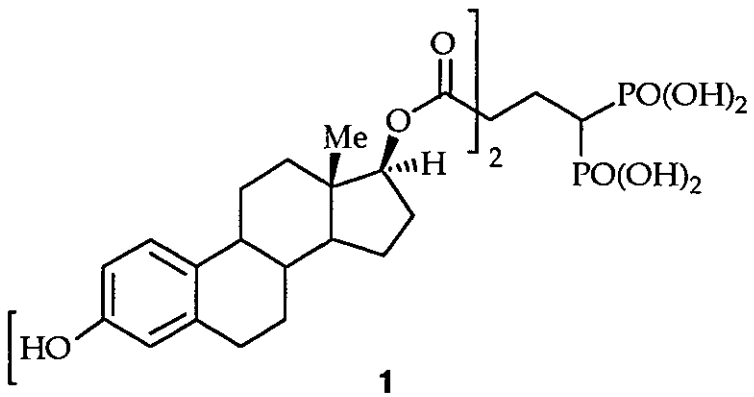
3-Benzyl 17β-oestradiol (0.52 g, 1.43 mmol, 2.2 eq.), and 1,4-(*N,N*-dimethylamino) pyridine (8.7 mg, 0.018 mmol, 0.1 eq.) were added to a solution of bis(ethyloxycarbonyl) tetraethyl propylene bisphosphonate 49 (0.32 g, 0.716 mmol, 1 eq.) in toluene (6 mL). The reaction mixture was heated to reflux under a nitrogen atmosphere for 11 days. The solvent was removed under reduced pressure and the crude material purified by silica gel flash chromatography. The eluent used was 1-3% methanol in CH_2Cl_2, and the desired product was isolated as a viscous brown oil (0.486 g, 64%). δ_H (400 MHz, $CDCl_3$) 0.85 (3H, s, 18'C$\underline{H}_3$), 0.88 (3H, s, 18'C$\underline{H}_3$), 1.20-1.95 (21H, m), 1.35 (12H, t, $J_{H\text{-}H}$ = 7 Hz, PO(OCH$_2$C$\underline{H}_3$)), 2.10-2.35 (13H, m), 4.10-4.30 (8H, m, P-OC$\underline{H}_2$CH$_3$), 4.69 (2H, t, 17'H), 5.05 (4H, s, -OC$\underline{H}_2$Ph), 6.71 (2H, s, 4'H), 6.8 (2H, d, $J_{H\text{-}H}$ = 8.5 Hz, 1'H), 7.19 (2H, d, $J_{H\text{-}H}$ = 8.7 Hz, 2'H), 7.29-7.43 (10H, m); δ_C (100 MHz, $CDCl_3$) 10.9 and 11.1 (18'CH$_3$), 15.3 (4C, t, $J_{P\text{-}C}$ = 4.85 Hz, PO(OCH$_2$CH$_3$), 22.2 (cyclic CH$_2$), 24.8 (1C, t, $J_{P\text{-}C}$ = 4.6Hz, CH$\underline{C}$H$_2$CH), 25.1 (cyclic CH$_2$), 26.1 (cyclic CH$_2$), 26.4 (cyclic CH$_2$), 28.7 (cyclic CH$_2$), 34.5 (1C, t, $J_{P\text{-}C}$ = 125 Hz, P$\underline{C}$P), 35.8 (cyclic CH$_2$), 37.5 (cyclic CH), 42.0 and 42.2 (13'C), 42.7 (cyclic CH), 48.7 (cyclic CH), 52.4 (CO$_2$R$\underline{C}$HCH$_2$CHP$_2$-), 61.7 (4C, t, $J_{P\text{-}C}$ = 9.7 Hz, PO(O$\underline{C}$H$_2$CH$_3$), 68.9 (-O$\underline{C}$H$_2$Ph), 82.9 (17'C), 111.2 (2'C), 113.8 (4'C), 125.3 (1'C), 126.4, 126.8 and 127.5 (phenyl CH's), 131.6 (10'C), 136.2 (quaternary phenyl), 136.8 (5'C), 155.7 (3'C), 166.7 and 167.8 (CO$_2$R); δ_P (100 MHz, $CDCl_3$) 21.3; m/z (FAB) 1092.52117 (M^+); $C_{63}H_{82}O_{12}P_2$ requires 1092.52816.



17

Tetraethyl {3,3-bis[oestra-1,3,5-triene-3-hydroxy-17β-yloxy-carbonyl]propylidene}bisphosphonate (17)

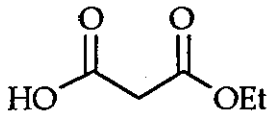
To a solution of **17** (330 mg, 0.30 mmol, 1 eq.) in tetrahydrofuran and methanol (1:1) (9.60 mL), was added palladium (10% activated on charcoal, 67 mg). The reaction mixture was stirred rigorously under a hydrogen atmosphere (1bar) for 14 h.. The mixture was filtered, taken up in CH_2Cl_2 and washed with sat. brine solution (3x10 mL). The combined organic fractions were dried over anhydrous $MgSO_4$ and the solvent removed *in vacuo*, affording an off-white foam (208 mg, 77%). The crude product was purified by silica gel flash chromatography using 3-5% methanol in CH_2Cl_2 as the eluant. The solvent was removed and after 10mins. under high vacuum a white foam formed (170 mg, 63%). δ_H (400 MHz, $CDCl_3$) 0.79 (3H, s, 12'C$\underline{H}_3$), 0.81 (3H, s, 12'C$\underline{H}_3$), 1.2-1.95 (21H, m), 1.35 (12H, t, J_{H-H} = 7 Hz, PO($OCH_2C\underline{H}_3$)), 2.10-2.35 (13H, m), 4.02 (1H, t, J_{H-H} = 7 Hz, -$CO_2CHCH_2PO(OCH_2CH_3)$), 4.16-4.24 (4H, m, -$PO(OCH_2CH_3)$), 4.68-4.74 (1H, m, 17'CH), 6.56 (1H, s, 4'H), 6.62 (1H, d, J_{H-H} = 8 Hz, 1'H), 7.07 (1H, d, J_{H-H} = 8 Hz, 2'H); δ_C (100 MHz, $CDCl_3$) 11.8 and 11.9 (18'CH_3), 16.1 (PO(OCH_2CH_3)), 23.1 (cyclic CH_2), 24.8 (1C, t, J_{P-C} = 4.6 Hz, CH$\underline{CH}_2$CH), 26.1 (cyclic CH_2), 27.1 (cyclic CH_2), 27.3 (cyclic CH_2), 29.4 (cyclic CH_2), 34.5 (1C, t, J_{P-C} = 125 Hz, P$\underline{C}$P), 36.7 (cyclic CH_2), 38.5 (cyclic CH), 42.9 and 43.0 (13'C), 43.6 (cyclic CH), 49.6 (cyclic CH), 52.4 ($CO_2R\underline{C}HCH_2CHP_2$-), 62.9 (PO($OCH_2CH_3$)), 83.9 and 84.0 (17'CH), 112.8 (2'C), 115.4 (4'C), 126.3 (1'C), 131.7 (10'C), 137.9 (5'C), 154.3 (3'C), 169.0 (CO_2R); δ_P (60 MHz, $CDCl_3$) 24.4; m/z (FAB) 912.47430 ($M+H^+$); $C_{49}H_{70}O_{12}P_2$ requires 912.43426.



1

Tetraethyl {3,3-Bis-(oestra-1,3,5-trien-3-hydroxy-17β–yloxy-carbonyl) propylidene} bis (phosphonic acid) (1)

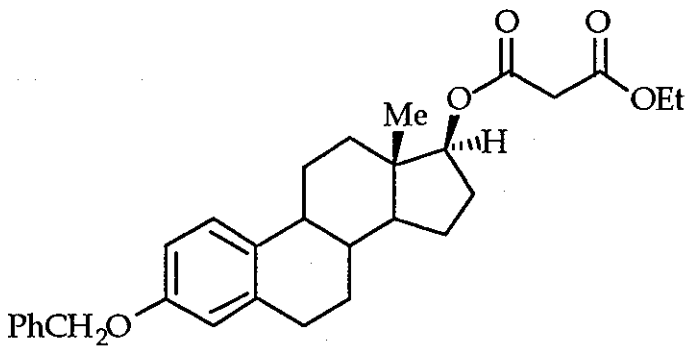
Trimethylsilyl bromide (0.170 mL, 0.199 g, 1.30 mmol, 35 eq.) was added to a solution of **1** (34.0 mg, 0.037 mmol, 1 eq.) in $CCl_4/CHCl_3$ 1:1 (0.4 mL) and the reaction mixture stirred under an atmosphere of nitrogen for 2.5 days. On the addition of water (0.7 mL) a white precipitate formed. The precipitate was filtered and washed with cold water and CH_2Cl_2. The product was then dried under high vacuum yielding an off-white powder (20 mg, 66%); δ_H (400 MHz, d^6-acetone) 0.80 (3H, s, 18'CH_3), 0.81 (3H, s, 18'CH_3), 1.23-2.73 (30H, m), 4.66 (2H, t, J_{H-H} = 8.3, 17'H), 6.46 (2H, s, 4'H), 6.51 (2H, d, J_{H-H} = 8.4, 1'H), 7.01 (2H, d, J_{H-H} = 8.4, 2'H); δ_C (100 MHz, d^6-acetone) 13.8 and 13.9 (18'CH_3), 22.1 (cyclic CH_2), 25.2 (cyclic CH_2), 28.3 (cyclic CH_2), 29.3 (cyclic CH_2), 29.5 (cyclic CH_2), 39.1 (cyclic CH_2), 41.0 (cyclic CH), 45.2 and 45 3 (13'C), 46.0 (cyclic CH), 51.8 (cyclic CH), 85.8 and 85.9 (17'C), 114.9 (2'C), 117.2 (4'C), 128.4 (1'C), 133.1 (10'C), 139.6 (5'C), 157.2 (3'C), 170.8 (CO_2R) ; δ_P (100 MHz, d_6-acetone) 22.7.



26

Ethyl malonic half-acid (26)

Diethyl malonate (10.0 g, 62.5 mmol, 1 eq.) was dissolved in absolute ethanol (40 mL), and a potassium hydroxide solution (3.5 g, KOH pellets in 40 mL ethanol) added dropwise over 1h. The reaction mixture was stirred using a mechanical stirrer for 2 h. and then left overnight. The crude intermediate was recrystallised from the mother liquor and washed with a small amount of ether to yield the potassium salt as a white crystalline solid (5.0 g, 50%); $C_5H_7KO_4$ (requires C = 35.28% , H = 4.15%) found C = 34.47% , H = 4.13%; mp = 194-196°C. The potassium salt (4.45 g, 26 mmol, 1 eq.) was dissolved in water (3 mL) and conc. HCl (2.5 mL) added slowly over 30 mins., taking care that the temperature did not rise above 10°C. The reaction mixture was filtered and the residue washed with diethyl ether (5 mL). The aqueous layer was extracted with diethyl ether (3x 10 mL) and the combined organic fractions dried over anhyd. $MgSO_4$. On removal of the solvent under reduced pressure, the desired ethyl malonic acid was isolated as a clear liquid (1.9 g, 55%). ν_{max} 3486 (C-OH), 1743 (CO_2Et) and 1735 (CO_2H); δ_H (250 MHz, $CDCl_3$) 1.29 (3H, t, $J_{H\text{-}H}$ = 7.1 Hz, -$OCH_2C\underline{H}_3$), 3.43 (2H, s, CO_2H-$C\underline{H}_2$-CO_2Et), 4.23 (2H, q, $J_{H\text{-}H}$ = 7.2 Hz, -$OC\underline{H}_2CH_3$), 8.21 (1H, broad, $CO_2\underline{H}$).

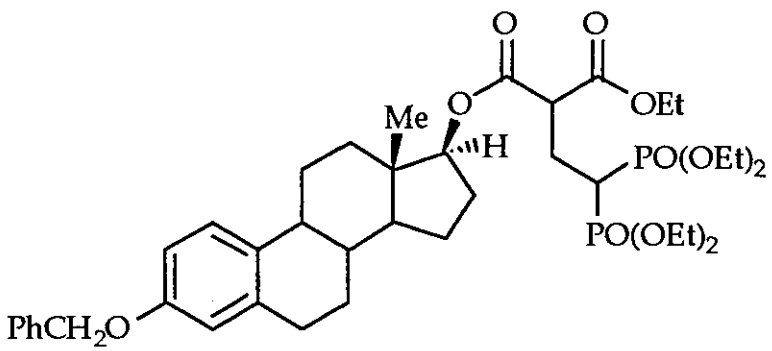


27

***n*-(oestra-1,3,5(10)-trien-3-benzyloxy-17β-yloxy-carbonyl) (ethyloxycarbonyl) (27)**

3-Benzyl 17β-oestradiol (1.0 g, 2.96 mmol, 1 eq.), ethyl malonic half-acid (391 mg, 2.96 mmol, 1 eq.) and dicyclohexylcarbodiimide (672 mg, 3.26 mmol, 1.1 eq.) were strirred in diethyl ether (35 mL). 1,4-(*N,N*-dimethyl amino) pyridine (10 mol% catalytic) was added and the reaction mixture was stirred rigorously under an atmosphere of nitrogen for 18 h. The mixture was filtered, the white solid washed with ether, and the solvent removed under reduced pressure. The crude product was purified using silica gel flash chromatography eluting with 10% ethyl acetate in petroleum ether. The solvent was removed *in vacuo* and placed under high vacuum for a further 30 mins., to yield a colourless crystalline solid (640 mg, 46 %). ν_{max} 1752 (CO_2Et), 1729 (CO_2R), 1605 and 1498 (aromatic C=C); δ_H (400 MHz, $CDCl_3$) 0.82 (3H, s, 18'CH_3), 1.28 (3H, t, J_{H-H} = 7.5 Hz, C-OCH_2CH_3), 1.39-1.57 (9H, m), 1.8-1.9 (2H, m), 2.3-2.4 (2H, m), 2.84 (2H, m), 3.38 (2H, s, 20'CH_2-malonate), 4.17 (2H, q, J_{H-H} = 7.5 Hz, -$COCH_2CH_3$), 4.76 (1H, t, J_{H-H} = 7.8 Hz, 17'H), 5.03 (2H, s, Ph$C\underline{H}_2$O-), 6.70 (1H, s, 4'H), 6.77 (1H, d, J_{H-H} = 8.0 Hz, 1'H), 7.23 (1H, d, J_{H-H} = 8.5 Hz, 2'H), 7.30-7.43 (6H, m, $\underline{Ph}CH_2O$-); δ_C (100 MHz, $CDCl_3$) 12.4 (18'CH_3), 14.4 (-O$\underline{C}H_2CH_3$), 23.6 (cyclic CH_2), 26.6 (cyclic CH_2), 27.6 (cyclic CH_2), 27.8 (cyclic CH_2), 30.1 (cyclic CH_2), 37.2 (cyclic CH_2), 39.0 (cyclic CH), 41.8 ($CO_2R\underline{C}H_2CO_2Et$), 43.6 (13'C), 44.2 (cyclic CH), 50.2 (cyclic CH), 61.9 (-O$\underline{C}H_2CH_3$), 70.4 (-O$\underline{C}H_2Ph$), 84.2 (17'C), 112.7 (2'C), 115.3 (4'C), 126.7 (1'C), 127.8, 128.2 and 128.9 (phenyl CH's), 133.2 (10'C), 137.8 (quaternary phenyl CH), 138.3 (5'C), 157.2

(3'C), 167.0 (-CO_2Et), 167.1 (CO_2R). $C_{30}H_{36}O_5$ (requires C = 76.93% , H = 7.06%) found C = 76.59% , H = 7.43%.

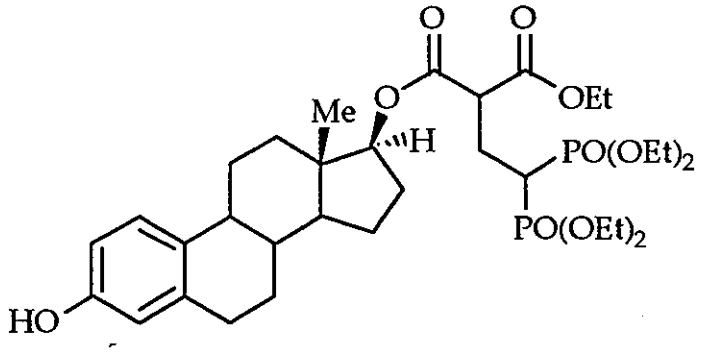


28

***n*-(Oestra-1,3,5(10)-trien-3-benzyloxy-17β–yloxy-carbonyl) (ethyloxycarbonyl) propylene tetraethyl bisphosphonate (28)**

Ethyl malonyl oestradiol (100 mg, 0.297 mmol, 1 eq.) was dissolved in CH_2Cl_2 (2 mL). Triethylamine (120 mg, 1.186 mmol, 4 eq.) and tetraethyl ethylidene bisphosphonate (89 mg, 0.297 mmol, 1 eq.) were added and the reaction mixture stirred at room temperature overnight under an atmosphere of nitrogen. The solvent was removed *in vacuo* and the crude product purified by silica gel flash chromatography, eluted with 1-2% methanol in CH_2Cl_2. The desired product was isolated as a colourless oil (70 mg, 30%). ν_{max} 1755 (CO_2Et), 1731 (CO_2R), 1608 and 1500 (aromatic C=C), 1254 (P=O), 1163 (P-O); δ_H (400 MHz, $CDCl_3$) 0.81 and 0.82 (3H, s, 18'C$\underline{H}_3$), 1.26-1.40 (15H, m, $PO_2CH_2C\underline{H}_3$ and $CO_2CH_2C\underline{H}_3$), 1.80-2.85 (16H, m, cyclic -CH's and -CH_2's, and PC$\underline{H}$P), 3.9-4.15 (1H, m, CO_2R-C$\underline{H}$-CO_2Et), 4.19-4.24 (12H, m, $PO_2C\underline{H}_2CH_3$, $CO_2C\underline{H}_2CH_3$, and P_2CH-C$\underline{H}_2$-CO_2Et), 4.75-4.80 (1H, m, 17'CH), 5.02 (2H, s, -OC$\underline{H}_2$Ph), 6.71 (1H, s, 4'H), 6.74 (1H, d, $J_{H\text{-}H}$ = 8.5 Hz, 2'H), 7.20 (1H, d, $J_{H\text{-}H}$ = 8.0 Hz, 1'H), 7.30-7.43 (5H, m, $\underline{Ph}CH_2O$-); δ_C (100 MHz, $CDCl_3$) 11.8 and 11.9 (18'CH_3), 14.1 ($CO_2CH_2C\underline{H}_3$), 16.3 (4C, t, $J_{P\text{-}C}$ = 6.3 Hz, $PO_2CH_2C\underline{H}_3$), 23.2 (cyclic CH_2), 24.7 (1C, t, $J_{P\text{-}C}$ = 4.6Hz, CH$\underline{C}H_2$CH), 26.1 (cyclic CH_2), 27.1 (cyclic CH_2), 27.2 (cyclic CH_2), 29.7 (cyclic CH_2), 34.2 (1C, t, $J_{P\text{-}C}$ = 138.4 Hz, P$\underline{C}$P), 36.7 (cyclic CH_2), 38.4 (cyclic CH), 41.4 and 41.6 (13'C), 42.2 ($CO_2\underline{C}HCO_2$), 44.4 (cyclic CH), 49.6 (cyclic CH), 61.8, 62.0 ($CO_2C\underline{H}_2CH_3$), 62.6 (4C, t, $J_{P\text{-}C}$ = 6.3 Hz, $PO_2C\underline{H}_2CH_3$), 69.9 (-O$\underline{C}H_2$Ph), 83.6 and 83.9 (17'C), 112.2

(2'C), 114.7 (4'C), 126.3 (1'C), 127.4, 127.8 and 128.5 (phenyl CH's), 132.4 (10'C), 137.0 (5'C), 137.8 (quaternary phenyl CH), 156.6 (3'C), 167.1 and 167.2 (CO_2Et), 167.3 and 167.3 (CO_2R); δ_P (60 MHz, $CDCl_3$) 24.15 and 24.20; m/z (FAB) 777.37968 (M^+); $C_{40}H_{58}O_{11}P_2$ requires 777.35327.

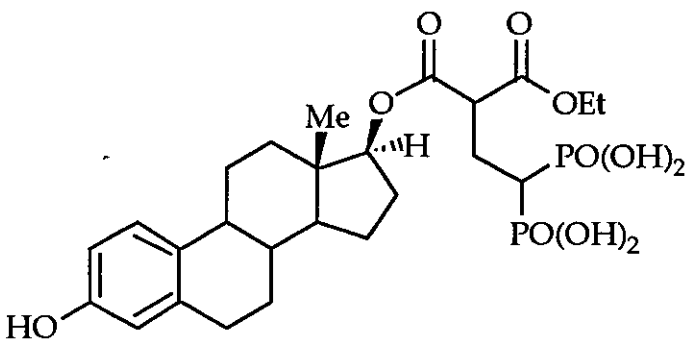


29

***n*-(Oestra-1,3,5(10)-trien-3-hydroxy-17β-yloxy-carbonyl) (ethyloxycarbonyl) propylene tetraethyl bisphosphonate (29)**

10% Palladium on activated charcoal (158 mg) was added to a solution of *n*-(oestra-1,3,5(10)-trien-3-benzyloxy-17β-yloxy-carbonyl) (ethyloxycarbonyl) propylene tetraethyl bisphosphonate (778 mg, 1 mmol, 1 eq) and dichloromethane (15 mL). The reaction mixture was stirred for 18 h. under a hydrogen atmosphere (60psi). The solvent was removed under reduced pressure and the crude material purified by silica gel flash chromatography, using 5% methanol in dichloromethane as the eluant. Removal of the solvent under reduced pressure yielded the unprotected compound as a white foam (550 mg, 80%). ν_{max} 3276 (C-OH), 2982 and 2930 (Ar-CH), 1745. (CO_2Et), 1731 (CO_2R), 1611 and 1503 (aromatic C=C), 1246 (P=O), 1164 (P-OEt) ; δ_H (400 MHz, $CDCl_3$) 0.79 (3H, s, 18'CH_3), 1.25 (18H, dt, J_{H-H} = 7 Hz, $PO_2CH_2C\underline{H}_3$ and $CO_2CH_2C\underline{H}_3$), 1.59-2.75 (16H, m, cyclic -CH's and -CH_2's, and PC$\underline{H}$P), 3.95-4.09 (1H, m, -C$\underline{H}_2$CHP), 4.20-4.25(10H, m, $PO_2C\underline{H}_2CH_3$ and $CO_2C\underline{H}_2CH_3$), 4.70-4.80 (1H, m, 17'CH), 6.62 (1H, s, 4'CH), 6.65 (1H, d, J_{H-H} = 8.5 Hz, 2'CH), 7.10 (1H, d, J_{H-H} = 8.7 Hz, 1'CH); δ_C (100 MHz, $CDCl_3$) 13.1, 13.2 (18'CH_3), 15.28, 15.31 ($CO_2CH_2\underline{C}H_3$), 17.5 (4C, t, J_{P-C} = 4.7 Hz, $PO_2CH_2\underline{C}H_3$),

24.7 (1C, t, J_{P-C} = 4.6Hz, CH<u>C</u>H_2CH), 25.6 (cyclic CH_2), 27.0 (1cyclic CH_2), 27.8 (cyclic CH_2), 28.8 (cyclic CH_2), 28.9 (cyclic CH_2), 35.8 (1C, t, J_{P-C} = 134.8 Hz, P<u>C</u>P), 38.5 (cyclic CH_2), 40.5 (cyclic CH), 42.1 (CO_2<u>C</u>HCO_2), 42.5, 42.6 (13'C), 45.4 (cyclic CH), 51.16, 51.18 (cyclic CH), 62.9, 63.1 (COO<u>C</u>H_2CH_3), 63.9, 64.2 (POO<u>C</u>H_2CH_3), 85.1, 85.3 (17'CH), 114.5 (2'CH), 116.8 (4'CH), 128.0 (1'CH), 133.9 (10'C), 139.1 (5'C), 156.9 (3'C), 170.19, 170.21 (CO_2Et), 170.3, 170.4 (CO_2R); δ_P (60 MHz, $CDCl_3$) 24.14 and 24.08; m/z (FAB) 687.30706 ($M+H^+$); $C_{33}H_{52}O_{11}P_2$ requires 687.30632.

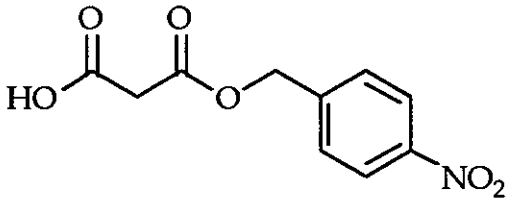


30

***n*-(Oestra-1,3,5(10)-trien-3-hydroxy-17β–yloxy-carbonyl) (ethyloxycarbonyl) propylene bisphosphonic acid (30)**

n-(Oestra-1,3,5(10)-trien-3-hydroxy-17β-yloxy-carbonyl) (ethyloxycarbonyl) propylene tetraethyl bisphosphonate (457 mg, 0,666 mmol, 1 eq.) was stirred with trimethylsilyl bromide (816 mg, 0.703 mL, 5.329 mmol, 8 eq.) in dichloromethane (10 mL). After two and a half days, the solvent was removed and the resulting concentrate was stirred with methanol (20 mL) for a further 30 mins. The solvent was removed *in vacuo* and the desired product was isolated as an orange foam (450 mg, 100%). ν_{max} 2683 (P-OH, broad), 1752 (CO_2Et), 1730 (CO_2R), 1182 (P=O); δ_H (400 MHz, d^6-acetone) 0.85 (3H, s, 18'CH_3), 1.11-2.83 (20H, m), 3.90-4.14 (1H, m, -C<u>H</u>$_2$CHP), 4.19-4.36 (2H, m, CO_2C<u>H</u>$_2CH_3$), 4.7 (1H, q, J_{H-H} = 8.5 Hz, 17'CH), 6.52 (1H, s, 4'CH), 6.59 (1H, d, J_{H-H} = 8.5 Hz, 2'H), 7.09 (1H, d, J_{H-H} = 8.5 Hz, 1'CH); δ_C (100 MHz, d^6-acetone) 11.5 (18'CH_3), 15.4 (CO_2CH_2<u>C</u>H_3), 24.9 (cyclic CH_2), 25.9 (1C, t, J_{P-C} = 4.6Hz, CH<u>C</u>H_2CH), 26.3 (cyclic CH_2), 27.2 (cyclic CH_2), 27.3 (cyclic CH_2) 29.8 (cyclic CH_2), 35.9 (PCP), 36.9 (cyclic CH_2), 39.7 (cyclic CH), 44.0 (13'C), 45.7 (cyclic

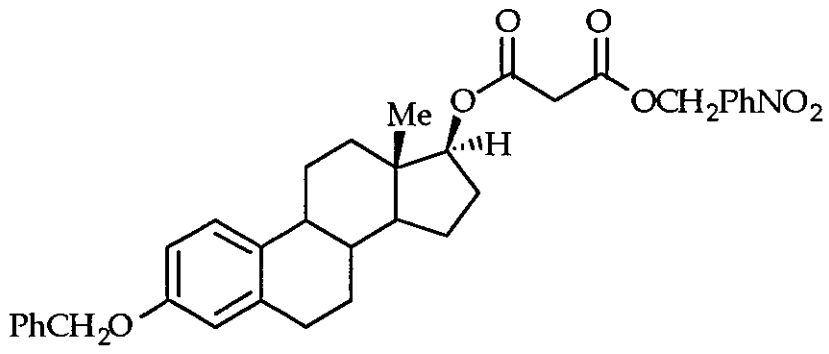
CH), 50.9 (cyclic CH), 49.8 ($CO_2R\underline{C}HCO_2Et$), 63.2 ($CO_2\underline{C}H_2CH_3$), 84.0 (17'C), 113.4 (2'C), 115.7 (4'C), 126.8 (1'C), 131.5 (10'C), 138.2 (5'C), 156.0 (3'C), 169.6 CO_2Et) 169.8 (CO_2R); δ_P (60 MHz, d^6-acetone) 28.16; m/z (FAB) 575 ($M+H^+$), 597 ($M+Na^+$).



31

***p*-Nitrobenzyl malonic half-acid (31)**

p-Nitrobenzyl alcohol (1.00 g, 6.53 mmol, 1 eq.) and Meldrum's acid (1.00 g, 6.94 mmol, 1.06 eq.) were refluxed in acetonitrile (3.5 mL) under an atmosphere of nitrogen for 22 h. The solvent was removed under reduced pressure to leave an orange/brown oil. After 30mins. under a high vacuum the oil formed a dense yellow solid (1.5 g, 97%). The crude product was recrystallised from ^{t}butanol/hexane (2:1) and afforded a pale yellow solid (1.00 g, 64%). ν_{max} 3500 (broad shoulder, -OH), 1747 (CO_2p-NO_2Ph), 1727 (CO_2H), 1605 (aromatic C=C), 1522 and 1335 (NO_2); δ_H (250 MHz, d^6-acetone) 3.5 (2H, s, $C(O)CH_2CO_2H$), 5.22 (2H, s, $-OCH_2PhNO_2$), 7.58 (2H, d, $J_{H\text{-}H}$ = 2.6, 6'H), 8.11 (2H, d, $J_{H\text{-}H}$ = 4 Hz, 7'H); δ_C (60 MHz, d^6-acetone) 40.1 ($CO_2R\underline{C}H_2CO_2H$), 64.6 ($-O\underline{C}H_2PhNO_2$), 122.8 (6'C), 128.1 (7'C), 166.8 (CO_2R), 205.2, (CO_2H); mp. 95-98°C.

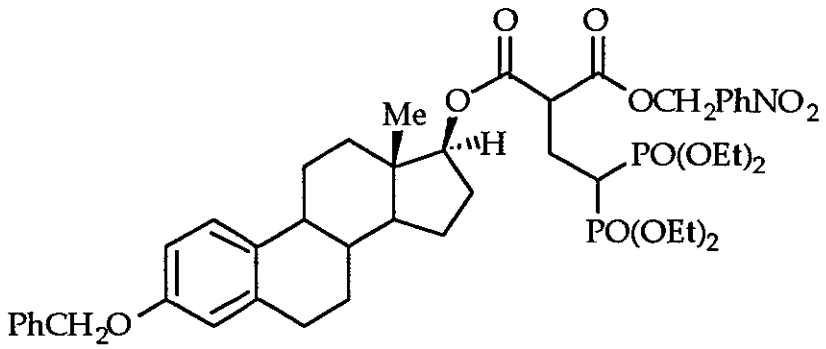


37

_n_-(Oestra-1,3,5(10)-trien-3-benzyloxy-17β-yloxy-carbonyl)(_p_-nitrobenzyloxycarbonyl) (37)

3-benzyl 17β-oestradiol (608 mg, 1.68 mmol, 1 eq.), *p*-nitrobenzyl malonic half-acid (400 mg, 1.68 mmol, 1 eq.), dicyclohexylcarbodiimide (382 mg, 84.8 mmol, 1.1 eq.) and 1,4-(*N,N*-dimethyl amino) pyridine (10 mol% catalytic) were stirred vigorously together in CH_2Cl_2 (15 mL) under an atmosphere of nitrogen for 4 days. The reaction mixture was filtered and the solvent removed *in vacuo* to afford a white foam, which collapsed to form a clear yellow oil under high vacuum (700 mg, 69%). The crude product was purified by silica gel flash chromatography eluted with 20% ethyl acetate in petroleum ether. The desired product was isolated as an off-white solid (700 mg, 69%). ν_{max} 1750 (CO_2*p*-NO_2Ph), 1731 (CO_2R), 1607 (aromatic C=C), 1519 and 1533 (NO_2); δ_H (400 MHz, $CDCl_3$) 0.69 (3H, s, 18'CH_3), 1.16-1.20 (6H, m), 1.26-1.37 (3H, m), 1.71-1.74 (3H, m), 2.01-2.16 (3H, m), 3.39 (2H, s, malonyl CH_2), 4.65 (1H, t, J_{H-H} = 8.5 Hz, 17'CH), 4.93 (2H, s, -OC$\underline{H_2}$Ph), 5.29 (2H, s, -OC$\underline{H_2}$PhNO_2), 6.6 (1H, s, 4'CH), 6.69 (1H, d, J_{H-H} = 6 Hz, 2'H), 7.09 (1H, d, J_{H-H} = 8 Hz, 1'H), 7.27-7.35 (5H, m, -OCH$_2\underline{Ph}$), 7.44 (2H, d, J_{H-H} = 8 Hz, phenyl CH's), 8.13 (2H, d, J_{H-H} = 8 Hz, phenyl CH's); δ_C (100 MHz, $CDCl_3$) 11.0 (18'CH_3), 22.2 (cyclic CH_2), 25.1 (cyclic CH_2), 26.3 (cyclic CH_2), 26.9 (cyclic CH_2), 28.7 (cyclic CH_2), 35.8 (cyclic CH_2), 37.5 (cyclic CH), 40.6 ($CO_2RCO_2\underline{C}H_2PhNO_2$), 42.1 (13'C), 42.7 (cyclic CH), 48.7 (cyclic CH), 64.5 (-O$\underline{C}H_2PhNO_2$), 68.9 (-O$\underline{C}H_2$Ph), 83.0 (17'C), 111.3 (2'C), 113.8 (4'C), 122.8 (phenyl CH), 125.3 (1'C), 126.4 (phenyl CH), 126.6 (phenyl CH), 126.8 (phenyl CH), 127.3 (phenyl CH), 131.6 (10'C), 136.3 (quaternary phenyl C), 141.5 (quaternary phenyl C), 146.8 (quaternary *p*-

nitrophenyl C), 155.8 (3'C), 165.2 ($\underline{C}O_2CH_2PhNO_2$), 165.2 ($CO_2R$); mp = 97-99°C.

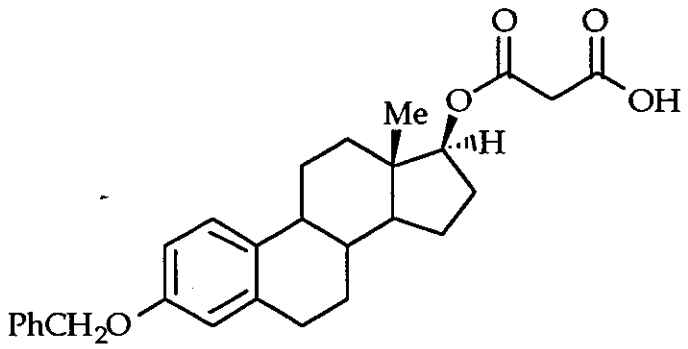


42

***n*-(Oestra-1,3,5(10)-trien-3-benzyloxy-17β–yloxy-carbonyl)(*p*-nitrobenzyloxycarbonyl) propylene tetraethyl bisphosphonate (42)**

n-(Oestra-1,3,5(10)-trien-3-benzyloxy-17β–yloxy-carbonyl)(*p*-nitrobenzyloxycarbonyl) (200 mg, 0.33 mmol, 1 eq.) was dissolved in CH_2Cl_2 (2 mL), diethylamine (49 mg, 70 μL, 0.66 mmol, 2 eq.) added,and the solution stirred for 10 mins. Tetraethyl ethylidene bisphosphonate (99 mg, 0.33 mmol, 1 eq.) was added and the reaction mixture was stirred at room temperature overnight under an atmosphere of nitrogen. The solvent was removed under reduced pressure and the crude product purified by silica gel flash chromatography, eltuting with 5% methanol in dichloromethane. The desired product was isolated as a brown oil (70 mg, 24%). ν_{max} 1749 (CO_2p-NO_2Ph), 1731 (CO_2R), 1608 (aromatic C=C), 1524 (NO_2), 1499 (aromatic C=C), 1347 (NO_2), 1252 (P=O), 1163 (P-O); δ_H (400 MHz, $CDCl_3$) 0.72, 0.75 (3H, s, 12'CH_3), 1.35 (12 H, t, $J_{H\text{-}H}$ = 7 Hz, P(O)$OCH_2C\underline{H}_3$), 1.74-2.85 (16H, m, cyclic -CH's, -CH_2's, and PCHP), 3.39 (0.24H, s, malonate H), 4.14-4.24 (8H, m, P(O)$OC\underline{H}_2CH_3$), 4.40 (2H, m, -$CHC\underline{H}_2CHP_2$), 5.03 (2H, s, -$OC\underline{H}_2Ph$), 5.27 (2H, td, -$CHCH_2CHP(O)(OEt)_2$), 5.30 (2H, s, -$OC\underline{H}_2PhNO_2$), 6.71 (1H, s, 4'H), 6.78 (1H, d, $J_{H\text{-}H}$ = 8 Hz, 1'H), 7.16 (1H, d, $J_{H\text{-}H}$ = 4 Hz, 2'H), 7.31-7.44 (5H, m), 7.55 (2H, d, $J_{H\text{-}H}$ = 8.8 Hz, p-$NO_2\underline{Ph}$), 8.22 (2H, d, $J_{H\text{-}H}$ = 8.7 Hz, p-NO_2Ph); δ_C (100 MHz, $CDCl_3$) 13.0, 13.1 (18'CH_3), 17.5 (4C, t, $J_{H\text{-}H}$ = 5.8 Hz, $PO(OCH_2CH_3)$), 24.4 (cyclic CH_2), 27.0 (cyclic CH_2), 28.2 (cyclic CH_2), 28.4 (cyclic CH_2), 30.8 (cyclic CH_2), 34.9 (1C, t, $J_{P\text{-}C}$ = 142.9Hz, P$\underline{C}$P), 37.9 (cyclic CH_2), 39.5 (cyclic

CH), 44.3 (13'C), 44.7 (cyclic CH), 50.7 (cyclic CH), 63.9 (1C, t, $J_{H\text{-}H}$ = 10.4 Hz, $P_2CHCH_2\underline{C}H(CO_2R)_2$), 66.7 (-O$\underline{C}H_2PhNO_2$), 71.0 (-O$\underline{C}H_2Ph$), 85.0 (17'$CH_3$), 113.4 (2'C), 115.87 (4'C), 124.9 (phenyl CH), 127.4 (1'C), 128.5, 128.9, 129.60, 129.61 and 129.7 (phenyl CH's), 133.6 (10'C), 138.3 (quaternary *p*-nitrophenyl C), 143.2 (quaternary phenyl C), 148.4 (quaternary *p*-nitrophenyl C), 157.8 (3'C), 169.5 ($\underline{C}O_2CH_2PhNO_2$), 169.7 ($CO_2R$); δ_P (60 MHz, $CDCl_3$) 23.49 and 23.51; $[\alpha]_D$ = 1.6.

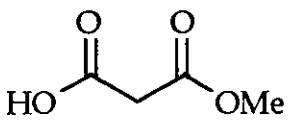


36

n-(Oestra-1,3,5(10)-trien-3-benzyloxy-17β–yloxy-carbonyl) malonic half-acid (36)

3-Benzyl 17β–oestradiol (200 mg, 0.552 mmol, 1 eq.) and Meldrum's acid (79.6 mg, 0.552 mmol, 1 eq.) were heated to reflux in acetonitrile (1.75 mL) for 22 h under an atmosphere of nitrogen. The solvent was removed under reduced pressure, and after a further 30mins. under high vacuum, the crude product was isolated as a pale yellow solid. Purification by silica gel flash column chromatography, eluting with 5% methanol in dichloromethane afforded the desired malonic half-acid as a white solid (120 mg, 49%). ν_{max} 3420 (broad, enolic -OH), 2361 (H-bonded -OH of dimer), 1749 (CO_2R), 1718 (CO_2H), 1654 and 1462 (aromatic C=C); δ_H (400 MHz, $CDCl_3$) 0.84 (3H, s, 12'CH_3), 1.25-2.26 (13H, m), 2.84-2.87 (2H, m), 3.45 (2H, s, 20'CH_2 malonate), 4.78 (1H, t, 17'CH), 5.03 (2H, s, -OC$\underline{H}_2$Ph), 6.70 (1H, s, 4'CH), 6.77 (1H, d, $J_{H\text{-}H}$ = 12 Hz, 1'CH), 7.18 (1H, d, $J_{H\text{-}H}$ = 8 Hz, 2'CH), 7.25-7.43 (5H, m, -OCH$_2\underline{Ph}$); δ_C (100 MHz, $CDCl_3$) 12.4 (18'CH_3), 23.6 (cyclic CH_2), 26.6 (cyclic CH_2), 27.6 (cyclic CH_2), 27.8 (cyclic CH_2), 30.1 (cyclic CH_2), 37.2 (cyclic CH_2), 38.9 (cyclic

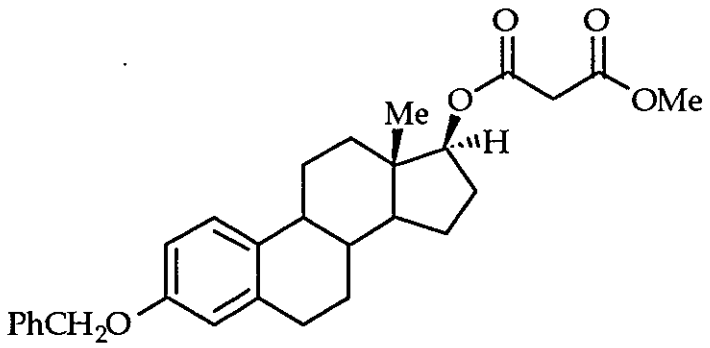
CH), 41.2 ($CO_2RCH_2CO_2H$, 43.6 (13'C), 44.2 (cyclic CH), 50.1 (cyclic CH), 70.4 (-OCH_2Ph), 84.8 (17'C), 112.7 (2'C), 115.3 (4'C), 126.8 (1'C), 127.9, 128.3 and 129.0 (phenyl CH's), 133.1 (10'C), 137.7 (phenyl CH), 138.3 (5'C), 157.2 (3'C), 167.7 (CO_2R), 170.9 (CO_2H); $C_{28}H_{32}O_5$ (requires C = 74.96% , H = 7.20%) found C = 73.65% , H = 7.20%; mp = 174-175°C .



32

Methyl malonic half-acid (32)

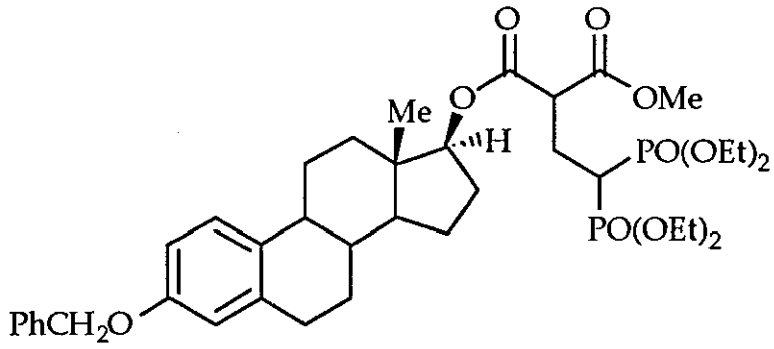
Meldrum's acid (1.00 g, 6.94 mmol, 1 eq.) and methanol (222 mg, 6.93 mmol, 1 eq.) were heated to reflux in acetonitrile (3.5 mL) for 20 h. under a nitrogen atmosphere. The solvent was removed under reduced pressure and the crude mixture purified using silica gel flash chromatography, eluting with 5% methanol in dichloromethane. The desired product was isolated as a clear oil (530 mg, 65%). ν_{max} 2600 (broad, H-bonded -OH of dimer,), 1736 (CO_2Me), 1728 (CO_2H); δ_H (250 MHz, $CDCl_3$) 3.48 (2H, s, $-CH_2$), 3.81 (3H, s, $-CO_2\underline{Me}$), 9.98 (1H, broad, $-CO_2\underline{H}$); δ_C (60 MHz, $CDCl_3$) 40.7 ($CO_2H\underline{C}H_2CO_2Me$), 52.7 ($CO_2\underline{Me}$), 167.1 ($\underline{C}O_2Me$), 171.3 ($\underline{C}O_2H$).



38

***n*-(Oestra-1,3,5(10)-trien-3-benzyloxy-17β-yloxy-carbonyl) methylene methyloxycarbonyl (38)**

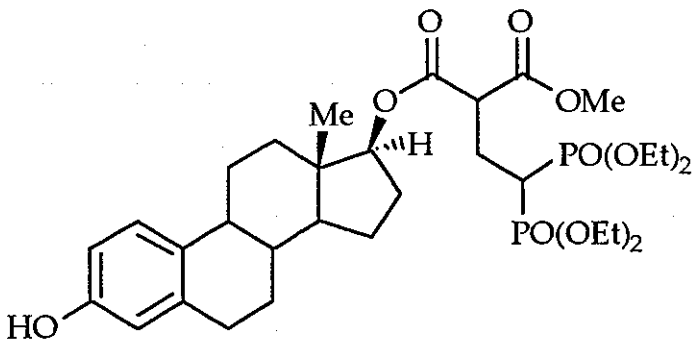
3-Benzyl 17β-oestradiol (1.00g, 2.76 mmol, 1 eq.), methyl malonic acid (323 mg, 2.76 mmol, 1 eq.), dicyclohexylcarbodiimide (627 mg, 3.04 mmol, 1.1 eq.) and 1,4-(*N,N*-dimethyl amino) pyridine (10 mol% catalytic) were stirred together in dichloromethane (25 mL) for 18 h. under a nitrogen atmosphere. The solvent was removed under reduced pressure and the crude mixture purified by silica gel flash chromatography using 20% ethyl acetate in light petroleum. The desired product was isolated as a colourless crystalline solid (846 mg, 68%). ν_{max} 1741 (CO_2Me), 1726 (CO_2R), 1461 and 1165 (aromatic C=C); δ_H (400 MHz, d^6-acetone) 0.83 (3H, s, 18'CH_3), 1.25-1.51 (6H, m), 1.55-1.65 (2H, m), 1.85-1.95 (2H, m), 2.18-2.22 (3H, m), 2.80-2.2.89 (2H, m), 3.40 (2H, s, -$CO_2RC\underline{H_2}CO_2Me$), 3.76 (3H, s, -$CO_2\underline{Me}$), 4.77 (1H, t, $J_{H\text{-}H}$ = 8.0 Hz, 17α-H), 5.04 (2H, s, -$OC\underline{H_2}Ph$), 6.72 (1H, s, 4'H), 6.78 (1H, d, $J_{H\text{-}H}$ = 8.0 Hz, 2'H), 7.20 (1H, d, $J_{H\text{-}H}$ = 8.0 Hz, 1'H), 7.30-7.44 (5H, m, -$OCH_2\underline{Ph}$); δ_C (100 MHz, $CDCl_3$) 11.9 (18'CH_3), 23.1 (cyclic CH_2) 26.1 (cyclic CH_2), 27.1 (cyclic CH_2), 27.3 (cyclic CH_2), 29.6 (cyclic CH_2), 36.7 (cyclic CH_2), 38.4 (cyclic CH), 42.5, 43.0 (13'C), 43.7 (cyclic CH), 49.6 (cyclic CH), 52.3 ($CO_2\underline{Me}$), 69.9 (-$O\underline{C}H_2Ph$), 83.8 (17'C), 112.2 (2'C), 114.7 (4'C), 126.3, (1'C), 127.3, 127.7 and 128.4 (phenyl CH's), 132.6 (10'C), 137.2 (5'C), 137.8 (20'C), 156.6 (3'C), 166.4 (CO_2Me), 167.7 (CO_2R); m/z (FAB) 462.24120 (M^+); $C_{29}H_{34}O_5$ requires 462.24063.



43

***n*-(Oestra-1,3,5(10)-trien-3-benzyloxy-17β-yloxy-carbonyl) (methyloxycarbonyl) propylene tetraethyl bisphosphonate (43)**

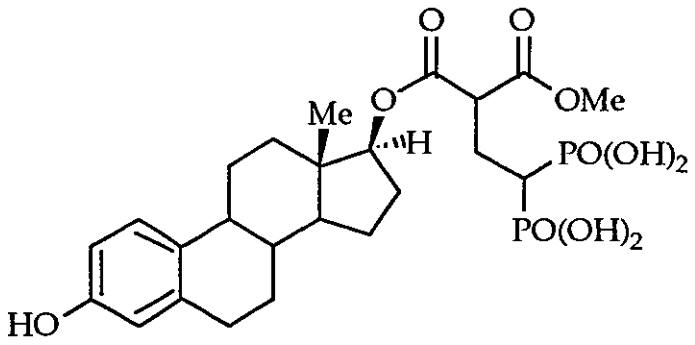
LHMDS (0.133 mL, 0.119 g, 0.711 mmol, 1.1 eq.) was added to *n*-(oestra-1,3,5(10)-trien-3-benzyloxy-17β-yloxycarbonyl) methylene methyloxycarbonyl (300 mg, 0.66 mmol, 1 eq.) and tetraethyl ethylidene bisphosphonate (200 mg, 0.664 mmol, 1 eq.) and heated in THF (4.5 mL) at 60°C for 18 h. The solvent was removed under reduced pressure and the crude product purified by silica gel flash chromatography, eluting with 1-2% methanol in dichloromethane. The desired compound was isolated as a brown oil (289 mg,57%). δ_H (400 MHz, $CDCl_3$) 0.80 (3H, s, 18'CH_3), 1.35 (12H, t, J_{H-H} = 7.0 Hz, -P(O)$OCH_2\underline{C}H_3$), 1.85-2.05 (6H, m), 2.21-2.35 (4H, m), 2.40-2.71 (3H, m), 2.80-2.97 (3H, m), 3.75 (3H, s, $CO_2\underline{Me}$), 3.94-4.10 (1H, m, CO_2R-C<u>H</u>-CO_2Et), 4.14-4.26 (10H, m, -P(O)$OC\underline{H}_2CH_3$, and P_2CH-$C\underline{H}_2$-CO_2Et), 4.67-4.82 (1H, m, 17'CH), 5.03 (2H, s, -$OC\underline{H}_2Ph$), 6.72 (1H, s, 4'H), 6.78 (1H, d, J_{H-H} = 8.0 Hz, 2'H), 7.20 (1H, d, J_{H-H} = 8.0 Hz, 1'H), 7.30-7.45 (5H, m, -OCH_2Ph); δ_C (100 MHz, $CDCl_3$) 11.9 (18'CH_3), 16.4 (4C, t, J = 6.0 Hz, PO($OCH_2\underline{C}H_3$), 23.3 (cyclic CH_2), 24.8 (1C, t, J_{P-C} = 5Hz, $CH\underline{C}H_2CHP_2$), 26.2 (cyclic CH_2), 27.2 (cyclic CH_2), 27.3 (cyclic CH_2), 29.8 (cyclic CH_2), 34.2 (1C, t, J_{C-P} = 134.1 Hz, P<u>C</u>HP), 36.9 (cyclic CH_2), 38.5 (cyclic CH), 43.1 (13'C), 43.9 (cyclic CH), 49.7 ($CO_2R\underline{C}HCO_2Me$), 50.1 (cyclic CH), 52.6 ($CO_2\underline{Me}$), 62.7 (4C, t, J_{C-P} = 7.0 Hz, PO($O\underline{C}H_2CH_3$), 69.9 (19'$\underline{C}H_2Ph$), 84.0 (17'C), 112.4 (2'C), 114.9 (4'C), 126.4 (1'C), 127.4, 127.8 and 128.5 (phenyl CH's), 131.0 (10'C), 137.2 (5'), 137.8 (Ph-quaternary), 156.7 (3'C), 168.5 (-$\underline{C}O_2Me$), 169.8 (-$\underline{C}O_2R$). δ_P (60 MHz, $CDCl_3$), 24.09, 24.01; m/z (FAB) 762.32857 (M^+); $C_{39}H_{56}O_{11}P_2$ requires 762.32979; $[\alpha]_D$ = 2.1.



50

***n*-(Oestra-1,3,5(10)-trien-3-hydroxy-17β-yloxy-carbonyl) (methyloxycarbonyl) propylene tetraethyl bisphosphonate (50)**

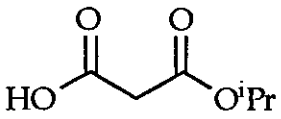
n-(Oestra-1,3,5(10)-trien-3-benzyloxy-17β-yloxy-carbonyl) (methyloxycarbonyl) propylene tetraethyl bisphosphonate (200 mg, 0.262 mmol, 1 eq.) was stirred with palladium on activated charcoal (41 mg) in dichloromethane (4 mL), under an atmosphere of hydrogen (60 atms.) for 22 h. The solvent was removed under reduced pressure to leave a clear oil. The crude product was purified by silica gel flash chromatography, using 5% methanol in dichloromethane as the eluant. After 1 h under high vacuum, the desired product was isolated as a white foam (140 mg, 79%). δ_H (400 MHz, $CDCl_3$) 0.79 (3H, s, 18'CH_3), 1.35 (12H, t, J_{H-H} = 7.5 Hz, PO($OCH_2\underline{C}H_3$), 1.50-1.98 (10H, m), 2.08-2.70 (4H, m), 2.77-2.86 (1H, m), 3.72 (3H, s, $CO_2\underline{Me}$), 3.96-4.09 (2H, m, $C\underline{H}_2CHP$), 4.10-4.33 (8H, m, $POC\underline{H}_2CH_3$), 4.65-4.82 (1H, m, 17'CH), 6.59 (1H, s, 4'CH), 6.65 (1H, d, J_{H-H} = 8.3 Hz, 2'CH), 7.10 (1H, d, J_{H-H} = 8.3 Hz, 1'CH); δ_C (100 MHz, $CDCl_3$) 11.9 and 12.1 (18'CH_3), 16.4 (4C, $POCH_2\underline{C}H_3$), 23.3 (cyclic CH_2), 24.8 (cyclic CH_2), 26.2 (cyclic CH_2), 27.3 (cyclic CH_2), 29.6 (cyclic CH_2), 34.1 (1C, t, J_{C-P} = 133.8 Hz, P$\underline{C}$P), 36.8 (cyclic CH_2), 38.6 (cyclic CH), 43.1 and 43.2 (13'C), 43.8 (cyclic CH), 49.9 (1C, t, J_{C-P} = 10.8 Hz, $CO_2R\underline{C}HCO_2Me$), 52.6 and 52.7 ($CO_2\underline{Me}$), 63.0 (4C, t, J_{P-C} = 7.3 Hz, $PO\underline{C}H_2CH_3$), 83.9 and 84.0 (17'CH), 112.87 (2'C), 115.4 (4'C), 126.3 (1'C), 131.2 (10'C), 137.8 (5'C), 154.7 (3'C), 168.8 ($\underline{C}O_2Me$), 169.5 ($\underline{C}O_2R$); δ_P (60 MHz, $CDCl_3$) 24.11 and 24.07; m/z (FAB) 673.29139 ($M+H^+$); $C_{32}H_{51}O_{11}P_2$ requires 673.29067; $C_{32}H_{50}P_2O_{11}$ (requires C = 57.12% , H = 7.50%) found C = 56.58% , H = 7.34%.



51

***n*-(Oestra-1,3,5(10)-trien-3-hydroxy-17β–yloxy-carbonyl) (methyloxycarbonyl) propylene bisphosphonic acid (51)**

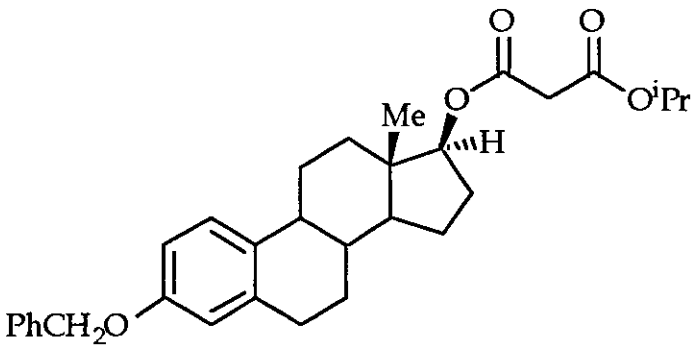
TMSBr (0.102 mL, 118 mg, 0.773 mmol, 8 eq.) was added slowly to a solution of *n*-(oestra-1,3,5(10)-trien-3-hydroxy-17β-yloxy-carbonyl)(methyloxycarbonyl) propylidene tetraethyl bisphosphonate (65 mg, 0.0967 mmol, 1 eq.) in dichloromethane (1.5 mL). The reaction mixture was stirred at ambient temperature for 2.5 days. The solvent was removed under reduced pressure, and the residue stirred in methanol (2 mL) for 30 mins. Removal of the solvent afforded the biphosphonic acid as a creamy brown foam (38 mg, 70%). δ_H (400 MHz, d^6 acetone) 0.84 (3H, s, 18'CH_3), 1.08-1.97 (10H, m), 2.36-2.82 (5H, m), 3.74 (3H, s, $CO_2\underline{Me}$), 3.87-4.06 (4H, m, PC$\underline{H}PO_2H$, -$C\underline{H}_2CHP_2$, and CO_2R-C$\underline{H}$-CO_2Me), 4.22-4.4.40 4.75 (1H, q, $J_{H\text{-}H}$ = 8.3 Hz, 17'CH), 6.53 (1H, s, 4'CH), 6.58 (1H, d, $J_{H\text{-}H}$ = 8.4 Hz, 2'CH), 7.07 (1H, d, $J_{H\text{-}H}$ = 8.4 Hz, 1'CH); δ_C (100 MHz, d^6 acetone) 16.6 and 16 7 (18'CH_3), 24.5 (cyclic CH_2), 25.7 (cyclic CH_2), 28.2 (cyclic CH_2), 29.9 (cyclic CH_2), 31.4 (cyclic CH_2), 32.4 (cyclic CH_2), 42.0 (P$\underline{C}$HP), 44.0 (cyclic CH), 48.2 and 48.4 (13'C), 49.0 (cyclic CH), 54.7 (cyclic CH), 55.4 ($CO_2R\underline{C}HCO_2Me$), 57.2 and 57.3 ($CO_2\underline{Me}$), 88.8 and 88.9 (17'CH), 117.9 (2'C), 120.2 (4'C), 131.4 (1'C), 136.1 (10'C), 142.7 (5'C), 160.2 (3'C), 173.5 and 173.6 ($\underline{C}O_2Me$), 174.3 and 174.4 ($\underline{C}O_2R$); δ_P (60 MHz, d^6 acetone) 28.13.



33

***iso*-Propyl malonic half-acid (33)**

Meldrum's acid (2.0 g, 13.9 mmol, 1 eq.) and isopropyl alcohol (834 mg, 13.89 mmol, 1 eq.) were refluxed together in acetonitrile (7.0 mL) for 22 h. The solvent was removed under reduced pressure and the desired malonic half-acid was collected as a colourlessr oil (2.03 g, 100%). The crude product was essentially pure by NMR so was not purified by chromatography. ν_{max} 1750 ($CO_2{}^iPr$), 1720 (CO_2H); δ_H (250 MHz, $CDCl_3$) 1.31 (6H, dd, J = 6.25 Hz, -$CH\underline{Me}_2$), 3.41 (2H, s, $CO_2C\underline{H}_2CO_2{}^iPr$), 5.10 (1H, hept, J_{H-H} = 6.23 Hz, $CO_2C\underline{H}Me_2$).

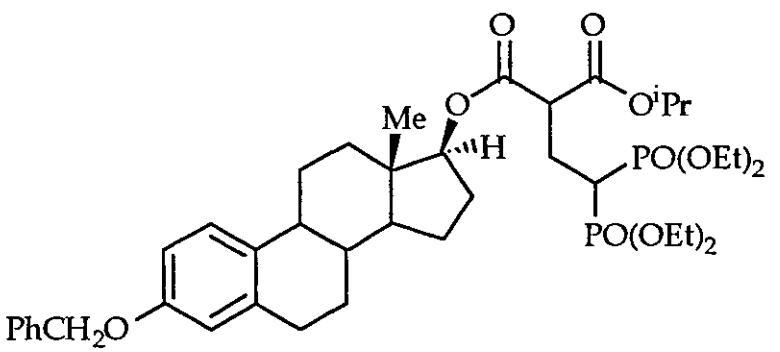


39

***n*-(Oestra-1,3,5(10)-trien-3-benzyloxy-17β-yloxy-carbonyl) methylene isopropyloxycarbonyl (39)**

Dicyclohexylcarbodiimide (308 mg, 1.5 mmol, 1.2 eq.) and 1,4-(*N,N*-dimethyl amino) pyridine (10 mol% catalytic) were added to a solution of 3-benzyl 17β-oestradiol (450 mg, 1.24 mmol, 1 eq.) and *i*-propyl malonic half-acid (182 mg, 1.24 mmol, 1 eq.) in acetonitrile (4 mL). The reaction mixture was stirred at ambient temperature for 18 h under a nitrogen rich atmosphere. The crude product was purified using silica gel flash chromatography, eluting with 5-10% ethyl acetate in light petroleum. The desired mixed malonate ester was isolated as a clear oil, which yielded a colourless crystalline solid when

exposed to a high vacuum for 1h (420 mg, 69%). ν_{max} 1747 ($CO_2{}^iPr$), 1498 and 1458 (aryl C=C), 1722 (CO_2R), 1376 ($CHMe_2$); δ_H (400 MHz, $CDCl_3$) 0.82 (3H, s, 13'C$\underline{H}_3$), 1.24 (6H, dd, J_{H-H} = 6.3 Hz, -CH$\underline{Me}_2$), 1.30-1.95 (10H, m), 2.14-2.34 (3H, m), 2.78-2.89 (2H, m), 3.35 (2H, s, $CO_2RC\underline{H}_2CO_2{}^iPr$), 4.76 (1H, t, J_{H-H} = 7.5 Hz, 17'CH), 5.02 (2H, s, -OC$\underline{H}_2$Ph), 5.06 (1H, hept, J_{H-H} = 6.25 Hz, -C$\underline{H}Me_2$), 6.70 (1H, s, 4'CH), 6.75 (1H, d, J_{H-H} = 8.55 Hz, 2'CH), 7.17 (1H. d. J_{H-H} = 8.55 Hz, 1'CH), 7.35 (5H, m, -OCH$_2\underline{Ph}$); δ_C (100MHz, $CDCl_3$) 12.0 (18'CH_3), 21.7 and 21.8 (-OCH$\underline{Me}_2$), 23.3 (cyclic CH_2), 26.2 (cyclic CH_2), 27.2 (cyclic CH_2), 27.4 (cyclic CH_2) 29.8 (cyclic CH_2), 36.9 (cyclic CH_2), 38.6 (cyclic CH), 42.3 ($CO_2RC\underline{H}_2CO_2{}^iPr$), 43.2 (13'C), 43.8 (cyclic CH), 49.8 (cyclic CH), 52.4 (-O$\underline{C}HMe_2$), 70.0 (-O$\underline{C}H_2$Ph), 83.8 (17'C), 112.3 (2'C), 114.9 (4'C), 126.4, 127.2 and 127.9 (phenyl CH's), 128.6 (1'C), 132.8 (10'C), 137.4 (5'C), 137.9 (quaternary phenyl), 156.8 (3'C), 166.3 ($CO_2{}^iPr$), 166.8 (CO_2R); m/z (FAB) 491.27910 ($M+H^+$); $C_{31}H_{39}O_5$ requires 491.27976; mp = 57-59°C.

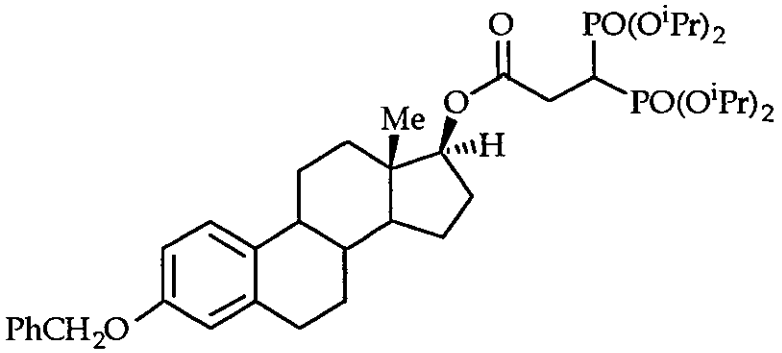


44

n-(Oestra-1,3,5(10)-trien-3-benzyloxy-17β-yloxy-carbonyl)(iso-propyloxycarbonyl)propylene tetraethyl bisphosphonate (44)

To a solution of *n*-(oestra-1,3,5(10)-trien-3-benzyloxy-17β-yloxy-carbonyl) methylene *i*-propyloxycarbonyl (100 mg, 0.2 mmol, 1 eq.) and sodium hydride (9.6 mg, 0.4 mmol, 2 eq.) in THF (1 mL) was added tetraethyl ethylidene bisphosphonate (60.2 mg, 0.2 mmol, 1 eq.) dissolved in THF (0.5 mL). The reaction mixture was allowed to stir gently at ambient temperature under a nitrogen atmosphere for 1h. The solvent was removed under reduced pressure and the crude product purified using silica gel flash column chromatography, eluting with 1-2% methanol in dichloromethane. The

desired compound was isolated as a brown oil (82 mg, 40%). δ_H (400 MHz, $CDCl_3$) 0.81 (3H, s, 18'CH_3), 1.25 (6H, d, $J_{H\text{-}H}$ = 6.13 Hz, -CH$\underline{Me_2}$), 1.33 (12H, t, $J_{H\text{-}H}$ = 7.6 Hz, PO(OCH$_2$C$\underline{H_3}$), 2.10-2.13 (13H, m, cyclic), 2.81-2.90 (2H, m, cyclic), 4.19 (8H, q, $J_{H\text{-}H}$ = 7.6 Hz, PO(OC$\underline{H_2}$CH$_3$), 5.03 (2H, s, -OC$\underline{H_2}$Ph), 6.71 (1H, s, 4'CH), 6.75 (1H, d, $J_{H\text{-}H}$ = 8.3 Hz, 2'CH), 7.17 (1H, d, $J_{H\text{-}H}$ = 8.3 Hz, 1'CH), 7.35 (4H, m, -OCH$_2$$\underline{Ph}$); δ_C (100 MHz, $CDCl_3$) 11.9 (18'CH_3), 16.2 (4C, t, $J_{C\text{-}P}$ = 6.0 Hz, PO(OCH$_2$$\underline{C}H_3$), 21.6 (-CH($\underline{C}H_3$)$_2$, 23.2 (cyclic CH$_2$), 24.8 (cyclic CH$_2$), 26.0 (cyclic CH$_2$), 27.1 (cyclic CH$_2$), 27.2 (cyclic CH$_2$), 29.7 (cyclic CH$_2$), 34.2 (1C, t, $J_{C\text{-}P}$ = 160.4 Hz, P$\underline{C}$HP), 38.4 (cyclic CH), 43.1 (13'C), 43.7 (cyclic CH), 49.6 (cyclic CH), 52.4 (-O$\underline{C}$HMe$_2$), 62.7 (4C, t, $J_{C\text{-}P}$ = 7.5 Hz, PO(O$\underline{C}$H$_2$CH$_3$)), 83.6 (17'CH), 112.8 (2'C), 115.3 (4'C), 126.8, 127.9 and 128.3 (phenyl CH's), 129.0 (1'C), 133.1 (10'C), 137.7 (5'C), 138.3 (quaternary phenyl), 157.2 (3'C), 168.4 ($\underline{C}$O$_2$^{i}Pr), 168.9 ($\underline{C}$O$_2$R); δ_P (60 MHz, $CDCl_3$) 24.10 and 24.25.

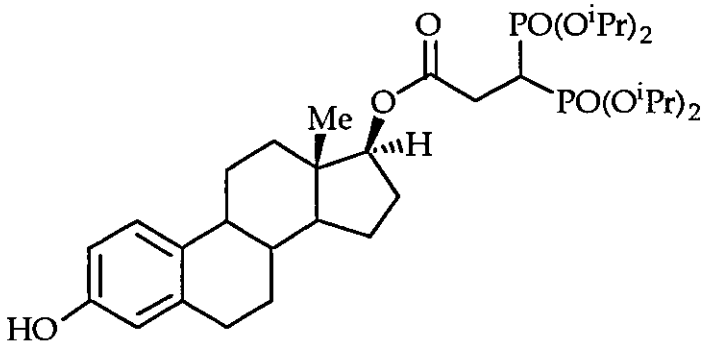


53

n-(Oestra-1,3,5(10)-trien-3-benzyloxy-17β–yloxy-carbonyl) ethylene tetraiso-propyl bisphosphonate (53)

Dicyclohexylcarbodiimide (384 mg, 1.86 mmol, 1.5 eq.) and 1,4-(*N,N*-dimethyl amino) pyridine (10 mol% catalytic) (22 mg, 0.18 mmol, 0.1 eq.) were added to a solution of 3-benzyl 17β-oestradiol (450 mg, 1.24 mmol, 1 eq.) and (hydroxycarbonyl) tetraiso-propyl ethylene bisphosphonate (500 mg, 1.24 mmol, 1 eq.) in dichloromethane (5 mL). The reaction was stirred rigorously for 24 h in a nitrogen atmosphere. The crude mixture was purified using silica gel flash column chromatography, eluting with 5% methanol in

dichloromethane. Subsequent removal of the solvent under reduced pressure afforded the desired compound as a clear oil (634 mg, 69%). ν_{max} 1738 (CO_2R), 1500 and 1454 (Aryl C=C), 1386 and 1375 ($CHMe_2$), 1252 (P=O), 990 (P-OR); δ_H (250 MHz, $CDCl_3$) 0.84 (3H, s, 18'CH_3), 1.27 (24H, dd, $J_{H\text{-}H}$ = 6.3Hz, -OCH$\underline{Me_2}$), 1.28-1.39 (10H, m), 2.15-2.31 (3H, m), 2.71-2.89 (2H, m), 3.00 (1H, tt, $J_{P\text{-}H}$ = 22.5Hz, $J_{H\text{-}H}$ = 5.3Hz, PC$\underline{H}$P), 4.65-4.86 (6H, m, PCHC$\underline{H_2}$P and OC$\underline{H}Me_2$), 5.03 (2H, s, -OC$\underline{H_2}$Ph), 6.71 (1H, s, 4'CH), 6.75 (1H, d, $J_{H\text{-}H}$ = 8.6Hz, 2'CH), 7.18 (1H, d, $J_{H\text{-}H}$ = 8.7Hz, 1'CH), 7.30-7.47 (5H, m, -OCH$_2\underline{Ph}$); δ_C (60 MHz, $CDCl_3$) 12.2 (18'CH_3), 23.2 (cyclic CH_2), 23.9 (4C, m, POCH$\underline{Me_2}$), 26.2 (cyclic CH_2), 27.2 (cyclic CH_2), 27.6 (cyclic CH_2), 29.8 (cyclic CH_2), 34.2 (1C, t, $J_{P\text{-}C}$ = 138.5Hz, PCHP), 37.0 (cyclic CH_2), 38.6 (cyclic CH), 43.0 (13'C), 43.8 (cyclic CH), 49.8 (cyclic CH), 70.0 (-OCH_2Ph), 71.3 (PO$\underline{C}HMe_2$), 83.6 (17'C), 112.3 (2'C), 114.8 (4'C), 126.4, 127.5 and 127.9 (phenyl's), 128.5 (1'C), 132.8 (10'C), 137.3 (5'C), 137.9 (quaternary phenyl), 156.8 (3'C), 170.5 ($\underline{C}O_2RCH_2$); δ_P (60 MHz, $CDCl_3$) 22.1

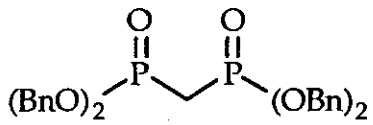


54

n-(Oestra-1,3,5(10)-trien-3-hydroxy-17β-yloxy-carbonyl)ethylene tetraiso-propyl bisphosphonate (54)

A solution of *n*-(oestra-1,3,5(10)-trien-3-benzyloxy-17β-yloxy-carbonyl) ethylene tetraiso-propyl bisphosphonate (594 mg, 0.8 mmol, 1 eq.) and 10% activated palladium on charcoal (122 mg) in dichloromethane (12 mL) were agitated in a hydogenator (60 psi) for 18 h. The crude material was purified using silica gel flash column chromatography, eluting with 5% methanol in dichloromethane. The debenzylated product was isolated as a white foam (256 mg, 49%). δ_H (250 MHz, $CDCl_3$) 0.82 (3H, s, 18'CH_3), 1.32 (24H, dd, $J_{H\text{-}H}$ =

6.5Hz, -OCH<u>Me$_2$</u>), 1.45 (10H), 2.05-2.25 (3H, m), 2.78-2.89 (2H, m), 3.01 (1H, tt, J_{P-H} = 24Hz, J_{H-H} = 5.6Hz, PC<u>H</u>P), 4.69-4.82 (6H, m, PCHC<u>H$_2$</u>P and -OC<u>H</u>Me$_2$), 6.60 (1H, s, 4'CH), 6.66 (1H, d, J_{H-H} = 8.4Hz, 2'CH), 7.10 (1H, d, J_{H-H} = 8.5Hz, 1'CH); δ_C (60 MHz, $CDCl_3$) 12.0 (18'CH_3), 23.2 (cyclic CH_2), 23.9 (4C, m, POCH<u>Me$_2$</u>), 26.2 (cyclic CH_2), 27.2 (cyclic CH_2), 27.5 (cyclic CH_2), 29.5 (cyclic CH_2), 34.0 (1C, t, J_{P-C} = 137.9Hz, PCHP), 36.9 (cyclic CH_2), 38.6 (cyclic CH), 49.7 (cyclic CH), 71.7 (PO<u>C</u>HMe$_2$), 83.7 (17'C), 112.8 (2'C), 115.3 (4'C), 126.1 (1'C), 131.3 (10'C) 137.7 (5'), 154.5 (3'C), 170.8 (<u>C</u>O$_2$RCH$_2$); δ_P (60 MHz, $CDCl_3$) 22.0 and 22.2.

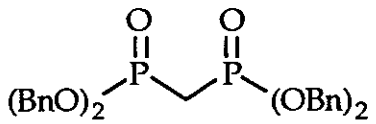


69

Tetrabenzyl methylene bisphosphonate (69)

Methylene bis(diphosphonic dichloride) (4.80 g, 19.2 mmol, 1 eq.) was suspended in freshly distilled toluene (10 mL) under a nitrogen atmosphere and cooled to 0 °C. Dry benzyl alcohol (8.3 mL, 79.7 mmol, 4.15 eq.) in dry pyridine (6.0 mL, 73.1 mmol) was added via a syringe pump over 90mins., with stirring at the same temperature. After stirring for a further 2.5 h. at ambient temperature, the solid matter was filtered off and washed twice with toluene (15 mL). The filtrate was taken up in toluene (25 mL) and washed with 2M NaOH and water. The residue was dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Purification of the crude material with silica gel flash column chromatography (80-100% ethyl acetate in light petroleum) afforded the desired tetrabenzyl bisphosphonate as a clear oil, (5 g, 47%). I.R. ν_{max} 1260 (P=O), 998 (P-OAr); δ_H (250 MHz, $CDCl_3$) 2.52 (2H, t, J_{P-H} = 21.2Hz, PCH_2P), 4.95-5.10 (8H, m, -OC<u>H$_2$</u>Ph), 7.27-7.41 (20H, m, -OCH$_2$<u>Ph</u>); δ_C (60 MHz, $CDCl_3$) 26.2 (1C, t, J_{P-C} = 136.8Hz, PCH_2P), 68.0 (-O<u>C</u>H$_2$Ph), 128.0.128.1, 128.4 and 128.5 (-OCH$_2$<u>Ph</u>); δ_P (60 MHz, $CDCl_3$) 21.8.

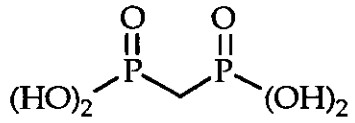
6.5Hz, -OCHMe$_2$), 1.45 (10H), 2.05-2.25 (3H, m), 2.78-2.89 (2H, m), 3.01 (1H, tt, J_{P-H} = 24Hz, J_{H-H} = 5.6Hz, PCHP), 4.69-4.82 (6H, m, PCHCH$_2$P and -OCHMe$_2$), 6.60 (1H, s, 4'CH), 6.66 (1H, d, J_{H-H} = 8.4Hz, 2'CH), 7.10 (1H, d, J_{H-H} = 8.5Hz, 1'CH); δ_C (60 MHz, $CDCl_3$) 12.0 (18'CH$_3$), 23.2 (cyclic CH$_2$), 23.9 (4C, m, POCHMe$_2$), 26.2 (cyclic CH$_2$), 27.2 (cyclic CH$_2$), 27.5 (cyclic CH$_2$), 29.5 (cyclic CH$_2$), 34.0 (1C, t, J_{P-C} = 137.9Hz, PCHP), 36.9 (cyclic CH$_2$), 38.6 (cyclic CH), 49.7 (cyclic CH), 71.7 (POCHMe$_2$), 83.7 (17'C), 112.8 (2'C), 115.3 (4'C), 126.1 (1'C), 131.3 (10'C) 137.7 (5'), 154.5 (3'C), 170.8 (CO_2RCH_2); δ_P (60 MHz, $CDCl_3$) 22.0 and 22.2.



69

Tetrabenzyl methylene bisphosphonate (69)

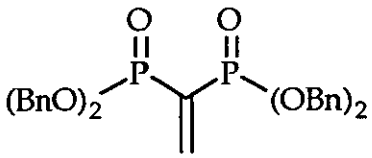
Methylene bis(diphosphonic dichloride) (4.80 g, 19.2 mmol, 1 eq.) was suspended in freshly distilled toluene (10 mL) under a nitrogen atmosphere and cooled to 0 °C. Dry benzyl alcohol (8.3 mL, 79.7 mmol, 4.15 eq.) in dry pyridine (6.0 mL, 73.1 mmol) was added via a syringe pump over 90mins., with stirring at the same temperature. After stirring for a further 2.5 h. at ambient temperature, the solid matter was filtered off and washed twice with toluene (15 mL). The filtrate was taken up in toluene (25 mL) and washed with 2M NaOH and water. The residue was dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Purification of the crude material with silica gel flash column chromatography (80-100% ethyl acetate in light petroleum) afforded the desired tetrabenzyl bisphosphonate as a clear oil, (5 g, 47%). I.R. ν_{max} 1260 (P=O), 998 (P-OAr); δ_H (250 MHz, $CDCl_3$) 2.52 (2H, t, J_{P-H} = 21.2Hz, PCH$_2$P), 4.95-5.10 (8H, m, -OCH$_2$Ph), 7.27-7.41 (20H, m, -OCH$_2$Ph); δ_C (60 MHz, $CDCl_3$) 26.2 (1C, t, J_{P-C} = 136.8Hz, PCH$_2$P), 68.0 (-OCH$_2$Ph), 128.0.128.1, 128.4 and 128.5 (-OCH$_2$Ph); δ_P (60 MHz, $CDCl_3$) 21.8.



73

Methylene bisphosphonic acid (73)

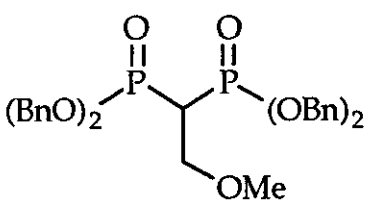
To a solution of tetrabenzyl methylene bisphosphonate (200 mg, 0.37 mmol) in ethyl acetate (4 mL) was added 10% palladium on activated charcoal (15 mg). A hydrogen balloon attached to a glass adapter was fitted to the reaction flask and the mixture was stirrred rigorously for 20 h. The crude mixture was filtered and the filtrate reduced *in vacuo* to yield the desired acid as a white solid (62 mg, 100%). δ_H (250 MHz, D_2O) 2.49 (2H, t, J_{P-H} = 21.9Hz, PCH_2P); δ_P (60 MHz, D_2O) 22.2.



68

Tetrabenzyl ethylidene bisphosphonate (68)

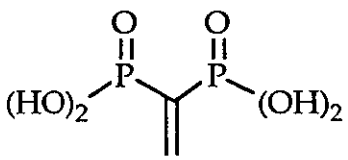
Paraformaldehyde (1.08 g, 37.3 mmol, 5 eq.) and diethylamine (546 mg, 0.772 mL, 7.46 mmol, 1 eq.) were warmed together in methanol (22 mL) until the solution went clear (approximately 30 mins.). Tetrabenzyl methylene bisphosphonate (4 g, 7.46 mmol,1 eq.) was added and the reaction mixture stirred at ambient temperature overnight under nitrogen. Methanol (22 mL) was then added and the solvent removed under reduced pressure. The concentrate was dissolved in toluene (10 mL) and the solvent removed. This concentration-evaporation process was repeated with further toluene (10 mL) to remove any residual methanol from the crude viscous intermediate.



74

δ_H (250 MHz, $CDCl_3$) 2.86 (1H, tt, J_{P-H} = 23.9Hz, J_{H-H} = 5.5Hz, PC<u>H</u>P), 3.29 (3H, s, -OC<u>H</u>$_3$), 3.93 (2H, td, J_{P-H} = 16.6Hz, J_{H-H} = 5.5Hz, $P_2CHC\underline{H}_2OCH_3$), 4.95-5.09 (8H, m, -OC<u>H</u>$_2$Ph), 7.26-7.32 (20H, m, -OCH$_2$<u>Ph</u>); δ_C (60 MHz, $CDCl_3$) 39.4 (1H, t, J_{P-C} = 132.9Hz, P<u>C</u>P), 58.7 (-OMe), 68.0 (-OCH_2Ph), 127.9, 128.0, 128.2, and 128.4 (-OCH$_2$<u>Ph</u>); δ_P (60 MHz, $CDCl_3$) 23.6.

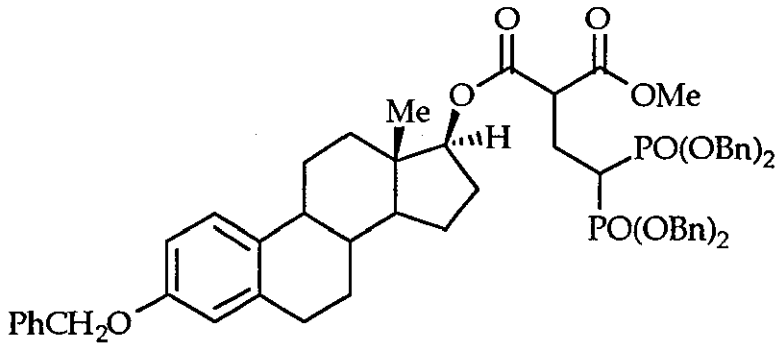
p-TSA (cat.) was added to a solution **74** in toluene (10 mL), and the mixture refluxed under nitrogen for 18 h using Soxhlet apparatus, loaded with 4Å sieves. The crude product was diluted with dichloromethane (30 mL) and washed with water (2x20 mL). The organic fracton was dried over anhydrous $MgSO_4$ and the solvent removed under reduced pressure, affording brown crystals (4 g, 98%). ν_{max} 1247.9 (P=O), 996 and 867 (P-OAr); δ_H (250 MHz, $CDCl_3$) 4.92-5.06 (8H, m, -OC<u>H</u>$_2$Ph), 6.98 (2H, dd, cis J_{P-H} = 38.48Hz, trans J_{P-H} = 38.45Hz, $P_2C=CH_2$), 7.20-7.40 (20H, m, -OCH$_2$<u>Ph</u>); δ_C (60 MHz, $CDCl_3$) 68.1 (-O<u>C</u>H$_2$Ph), 127.6, 128.0, 128.4, and 128.5 (-OCH_2Ph), 131.6 (1C, t, J_{P-C} = 105.5Hz, P<u>C</u>P), 135.8 (PC=<u>C</u>H$_2$); δ_P (60 MHz, $CDCl_3$) 15.3.



76

Ethylidene bisphosphonic acid (76)

A solution of tetrabenzyl ethylidene bisphosphonate (200 mg, 0.365 mmol) in dichloromethane (4 mL) was stirred with 10% palladium on activated charcoal (15 mg) for 6 h under an atmosphere of hydrogen (1 atm.). The palladium was removed by filtration and the solvent was removed under reduced pressure, affording the bisphosphonic acid as an off-white foam (62 mg, 100%). δ_H (250 MHz, D_2O) 6.72 (2H, dd, cis J_{P-H} = 36.1Hz, trans J_{P-H} = 35.8Hz, $P_2C=CH_2$); δ_P (60 MHz, D_2O) 15.5; m/z (ES)187 (M^+).

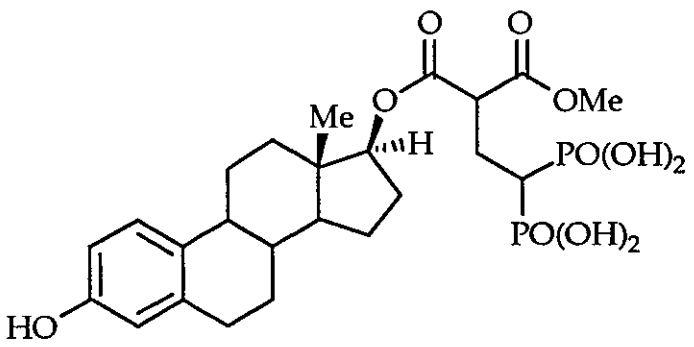


77

_n_-(Oestra-1,3,5(10)-trien-3-benzyloxy-17β-yloxy-carbonyl) (methyloxycarbonyl) propylene tetrabenzyl bisphosphonate (77)

LHMDS (0.133 mL, 0.708 mmol, 1 eq.) was added to a solution of _n_-(oestra-1,3,5(10)-trien-3-benzyloxy-17β-yloxy-carbonyl) methylene methyloxycarbonyl (320 mg, 0.71 mmol, 1 eq.) in freshly distilled THF (5 mL). Tetrabenzyl ethylidene bisphosphonate (390 mg, 0.71 mmol, 1 eq.) was added and the reaction mixture heated to 60 °C for 2 days. The solvent was removed under reduced pressure and the crude product purified using silica gel flash column chromatography, eluting with 2% methanol in dichloromethane. The desired compound was isolated as a clear oil (335 mg, 47%). ν_{max} 1748 (CO_2Me), 1732 (CO_2R), 1253 (P=O), 997 and 868 (P-OAr); δ_H (400 MHz, $CDCl_3$) 0.69 and 0.70 (3H, s, 18'CH_3), 1.34-1.45 (8H, m, cyclic), 2.00-2.20 (3H, m, cyclic), 2.49 (2H, h, cyclic), 2.61-2.81 (2H, m, cyclic), 2.85 (1H, tt, J_{H-H} = 7.9Hz, PC<u>H</u>P), 3.59 (3H, s, CO_2<u>Me</u>), 3.97 (1H, t, J_{H-H} = 7.9Hz, 17'CH), 4.64 (2H, td, J_{P-H} = 15.8Hz, J_{H-H} = 7.9Hz, CH_2CHP_2), 4.98-5.10 (10H, m, -OCH_2Ph), 6.68 (1H, s, 4'CH), 6.74 (1H, d, J_{H-H} = 8.2Hz, 2'CH), 7.03 (1H, d, J_{H-H} = 8.2Hz, 1'CH), 7.30 (25H, m, -OCH_2Ph); δ_C (100 MHz, $CDCl_3$) 11.87 and 11.94 (18'CH_3), 23.3 (cyclic CH_2), 26.2 (cyclic CH_2), 27.3 (cyclic CH_2), 27.4 (cyclic CH_2), 29.7 (cyclic CH_2), 35.1 (1C, t, J_{P-C} = 132Hz, P<u>C</u>P), 36.9 (cyclic CH_2), 38.6 (cyclic CH), 43.1 and 43.3 (13'C), 43.8 (cyclic CH), 49.9 and 50.0 (cyclic CH), 52.3 and 52.5 (CO_2<u>Me</u>), 68.2, 68.25, 68.32 and 68.4 (PO (OCH_2Ph)), 70 1 (-OCH_2Ph), 83.9 and 84.0 (17'C), 112.5 (2'C), 115.0 (4'C), 126.3 (1'C), 127.4, 127.8 and 127.9 (-OCH_2<u>Ph</u>), 128.05, 128.10, 128.2, 128.4 and 128.5 (PO(OCH_2<u>Ph</u>), 132.8 (10'C), 136.2 (quaternary phenyl), 137.5 (5'C), 137.9 (quaternary phenyl), 156.9 (3'C), 168.5 and 168.6 (<u>C</u>O_2Me), 169.2 and 169.3 (<u>C</u>O_2R); δ_P (60 MHz,

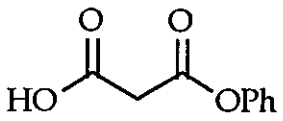
$CDCl_3$) 24.89; m/z (FAB) 1011.40028 ($M+H^+$); $C_{59}H_{65}O_{11}P_2$ requires 1011.40022.



79

***n*-(Oestra-1,3,5(10)-trien-3-yloxy-17β-yloxy-carbonyl) (methyloxycarbonyl) propylene bisphosphonic acid (79)**

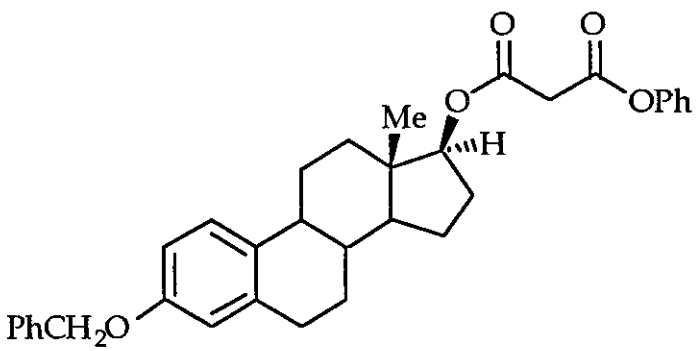
To a solution of *n*-(oestra-1,3,5(10)-trien-3-benzyloxy-17β-yloxy-carbonyl) (methyloxycarbonyl) propylene tetrabenzyl bisphosphonate (78 mg, 0.08 mmol, 1 eq.) in THF (2 mL), was added palladium (10% activated on charcoal, 20 mg). The reaction mixture was stirred under an atmosphere of hydrogen (1bar) for 18 h. The palladium was removed by filtration and the solvent was removed under reduced pressure to yield a white foam (40 mg, 0.071 mmol, 89%). δ_H (400 MHz, d^6-acetone) 0.85 and 0.86 (3H, s, 18'CH_3), 1.30-2.82 (15H, m), 3.76 (3H, s, $CO_2\underline{Me}$), 3.90-4.25 (4H, m, -$C\underline{H}_2CHP_2O_2(OH)_4$, PC$\underline{H}$P, and -$CH_2C\underline{H}P_2O_2$), 4.72-4.80 (1H, m, 17αH), 6.53 (1H, s, 4'CH), 6.61 (1H, d, $J_{H\text{-}H}$ = 8.4Hz, 2'CH), 7.10 (1H, d, $J_{H\text{-}H}$ = 8.4Hz, 1'CH); δ_C (100 MHz, d^6-acetone) 13.1 and 13.2 (18'CH_3), 24.6 (cyclic CH_2), 26.3 (cyclic CH_2), 27.8 (cyclic CH_2), 28.7 (cyclic CH_2), 28.8 (cyclic CH_2), 38.4 (cyclic CH_2), 40.4 (cyclic CH), 44.6 and 44.8 (13'C), 45.4 (cyclic CH), 51.1 (cyclic CH), 53.6 ($CO_2\underline{Me}$), 85.0 and 85.2 (17'C), 114.3 (2'C), 116.6 (4'C), 127.8 (1'C), 132.5 (10'C), 139.1 (5'C), 156.7 (3'C), 170.2 and 170.3 (CO_2Me), 171.1and 171.2 (CO_2R); δ_P (60 MHz, d^6-acetone) 28.41.



35

Phenyl malonic half-acid (35)

Meldrum's acid (1.84 g, 12.76 mmol, 1.2 eq.) and phenol (1 g, 10.63 mmol, 1 eq.) were heated to reflux in acetonitrile (3.5 mL) under a nitrogen atmosphere for 22 h. The solvent was removed under reduced pressure and the crude product purified using silica gel flash column chromatography, eluting with 20-100% ethyl acetate in light petroleum. The desired malonic acid was isolated as a clear oil which formed slightly coloured crystals (648 mg, 34%) after 1h under high vacuum. ν_{max} 1754 (CO_2Ph), 1698 (CO_2H); δ_H (250 MHz, $CDCl_3$) 3.68 (2H, s, $CO_2HC\underline{H}_2CO_2Ph$), 7.08-7.17 (2H, m, m-CH), 7.21-7.30 (1H, m, p-CH), 7.33-7.44 (2H, m, o-CH), 9.51 (1H, broad, $CO_2\underline{H}$); δ_C (60 MHz, $CDCl_3$) 41.1 ($CO_2HCH_2CO_2Ph$), 121.2 (o-CH), 126.3 (p-CH), 129.5 (m-CH), 150.4 (quaternary C), 164.9 ($\underline{C}O_2PH$), 171.5 ($\underline{C}O_2H$); $C_9H_8O_4$ (requires C = 59.99% , H = 4.48%) found C = 59.98% , H = 4.54% .

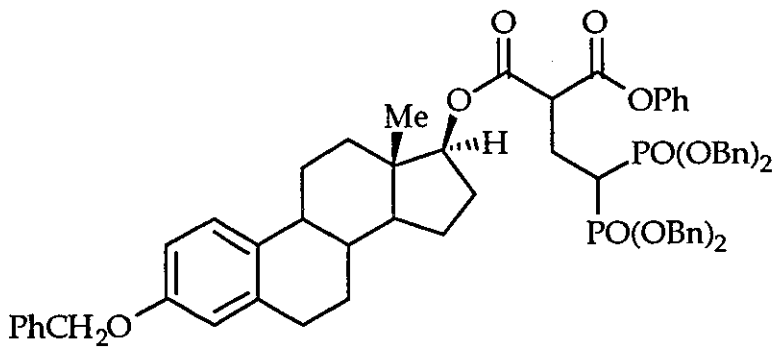


40

***n*-(Oestra-1,3,5(10)-trien-3-benzyloxy-17β-yloxy-carbonyl) methylene phenyloxycarbonyl (40)**

Dicyclohexylcarbodiimide (429 mg, 2.08 mmol, 1.5 eq.) and and 1,4-(*N,N*-dimethyl amino) pyridine (10 mol% catalytic) were added to a solution of 3-benzyl 17β-oestradiol (500 mg, 1.39 mmol, 1 eq.) and phenyl malonic half-acid (249 mg, 1.39 mmol, 1 eq.) in dichloromethane (5 mL). The reaction mixture was stirred for 18 h under a nitrogen atmosphere. The solvent was

removed under reduced pressure and the crude product purified using silica gel flash column chromatography, eluting with 10% ethyl acetate in light petroleum. The product was finally isolated as a white solid (670 mg, 92%). ν_{max} 1774 (CO_2Ph), 1723 (CO_2R); δ_H (400 MHz, $CDCl_3$) 0.85 (3H, s, 18'CH_3), 1.19-1.50 (6H, m, cyclic), 1.60-1.80 (4H, m, cyclic), 2.15-2.33 (3H, m, cyclic), 2.82-2.86 (2H, m, cyclic), 3.61 (2H, s, $CO_2RC\underline{H}_2CO_2Ph$), 4.82 (1H, t, $J_{H\text{-}H}$ = 8.5 Hz, 17'CH), 5.01 (2H, s, -$OC\underline{H}_2PH$), 6.70 (1H, s, 4'CH), 6.77 (1H, d, $J_{H\text{-}H}$ = 8.5 Hz, 2'CH), 7.11-7.42 (11H, m, phenyl's); δ_C (100 Mhz, $CDCl_3$) 12.4 (18'CH_3), 23.5 (cyclic CH_2), 26.5 (cyclic CH_2), 27.5 (cyclic CH_2), 27.7 (cyclic CH_2), 30.0 (cyclic CH_2), 37.1 (cyclic CH_2), 38.8 (cyclic CH), 42.2 ($CO_2R\underline{C}H_2CO_2Ph$), 43.5 (13'C), 44.1 (cyclic CH), 50.0 (cyclic CH), 70.2 (-$O\underline{C}H_2Ph$), 84.4 (17'C), 112.6 (2'CH), 115.1 (4'CH), 121.6, 126.5, 126.7, 127.7, and 128.1 (phenyl CH's), 128.8 (1'CH), and 129.8 (phenyl CH), 133.0 (10'C), 137.6 (5'C), 138.2 (quaternary phenyl, -$OCH_2\underline{C}Ar$), 150.7 (quaternary phenyl, -$O\underline{C}Ar$), 157.1 (3'C), 165.5 (CO_2Ph), 166.4 (CO_2R); m/z (FAB) 524.25626 (M^+); $C_{34}H_{36}O_5$ requires 524.25628.



78

***n*-(Oestra-1,3,5(10)-trien-3-benzyloxy-17β–yloxy-carbonyl) (phenyloxycarbonyl) propylene tetrabenzyl bisphosphonate (78)**

LHMDS (122 mg, 0.14 mL, 0.730 mmol, 1 eq.) was added to a solution of *n*-(oestra-1,3,5(10)-trien-3-benzyloxy-17β-yloxy-carbonyl) methylene phenyloxycarbonyl (383 mg, 0.730 mmol, 1.0 eq.) in freshly distilled THF (5 mL). Tetrabenzyl ethylidene bisphosphonate (400 mg, 0.730 mmol, 1.0 eq.) was added and the reaction mixture heated to 60 °C for 2 h. The solvent was removed under reduced pressure and the crude material purified using silica

gel flash column chromatography, eluting with 2% methanol in dichloromethane. The desired compound was isolated as a clear oil (220 mg, 28%). ν_{max} 1766 (CO_2Ph), 1732 (CO_2R), 1253 (P=O), 1163 (P-O), 997 and 870 (P-OAr; δ_H (400 MHz, $CDCl_3$) 0.75 (3H, s, 18'CH_3), 1.24-1.89 (8H, m, cyclic), 2.16-2.31 (3H, m, cyclic), 2.59-2.77 (2H, m, cyclic), 2.84-2.89 (2H, m, cyclic), 2.91 (1H, tt, J_{P-H} = 24.3 Hz, J_{H-H} = 8.5Hz, PC<u>H</u>P), 4.25 (1H, t, J_{H-H} = 8.5Hz, 17'CH), 4.73 (2H, m, C<u>H</u>$_2$CHP), 5.03 (10H, m, -OC<u>H</u>$_2$Ph), 6.72 (1H, s, 4'CH), 6.96 (1H, d, J_{H-H} = 8.6 Hz, 2'CH), 7.32 (31H, m, phenyl's); δ_C (100 MHz, $CDCl_3$) 12.0 and 12.1 (18'CH_3), 23.2 (cyclic CH_2), 25.0 (-CO_2RCH<u>C</u>H$_2$CHP), 26.1 (cyclic CH_2), 27.2 (cyclic CH_2), 27.4 (cyclic CH_2), 29.7 (cyclic CH_2), 34.8 (PCP), 36.8 (cyclic CH_2), 38.5 (cyclic CH_2), 43.0 and 43.2 (13'C), 43.7 (cyclic CH), 49.6 (CO_2R<u>C</u>HCO$_2$Ph), 50.1 and 50.2 (cyclic CH), 68.3 (m, P(O)O<u>C</u>H$_2$Ph), 69.2 and 69.3 (P(O)O<u>C</u>H$_2$Ph), 69.9 (-O<u>C</u>H$_2$Ph), 84.1 and 84.3 (17'C), 112.3 (2'CH), 114.8 (4'CH), 121.3, 126.1, 126.4, 127.5 (phenyl CH's), 127.9 (1C, d, J = 33.6 Hz, phosphonate phenyl CH), 128.0 (1C, d, J = 51.6 Hz, phosphonate phenyl CH), 128.1 (phenyl CH), 128.2 (1C, d, J = 34.6 Hz, phosphonate phenyl CH), 128.4 (1C, t, J = 11.2 Hz, phosphonate phenyl CH), 128.6 (1'CH), 129.4 (phenyl CH), 132.6 (10'C), 137.3 (5'C), 137.9 (quaternary phenyl, -OCH$_2$<u>C</u>Ar), 150.3 (quaternary phenyl, -O<u>C</u>Ar), 156.7 (3'C), 167.37 and 167.44 (CO_2Ph), 168.25 and 168.31 (CO_2R); δ_P (60 MHz, $CDCl_3$) 25.72 and 25.81. m/z (FAB) 1073.43186 ($M+H^+$); $C_{64}H_{67}O_{11}P_2$ requires 1073.41587

APPENDIX

Crystal data for **3 1** (fig. 2, p98)

A. Crystal Data

Empirical Formula	$C_{10}H_9NO_6$
Formula Weight	239.18
Crystal Color, Habit	colourlessblock, block
Crystal Dimensions	0.10 X 5.00 X 0.30 mm
Crystal System	orthorhombic
Lattice Type	Primitive
No. of Reflections Used for Unit Cell Determination (2θ range)	24 (63.2 - 74.8°)
Omega Scan Peak Width at Half-height	0.26°
Lattice Parameters	a = 22.399(1)Å b = 6.296(1) Å c = 7.360(2) Å V = 1038.0(4) Å^3
Space Group	$Pca2_1$ (#29)
Z value	4
D_{calc}	1.530 g/cm^3
F_{000}	496.00
μ(CuKα)	10.68 cm^{-1}

B. Intensity Measurements

Diffractometer	Rigaku AFC7S
Radiation	CuKα (λ = 1.54178 Å)

	graphite monochromated
Attenuator	Ni foil (factor = 9.42)
Take-off Angle	6.0°
Detector Aperture	9.0 mm horizontal 13.0 mm vertical
Crystal to Detector Distance	400 mm
Voltage, Current	0kV, 0mA
Temperature	20.0°C
Scan Type	ω
Scan Rate	16.0°/min (in ω) (up to 4 scans)
Scan Width	$(0.94 + 0.35 \tan \theta)°$
$2\theta_{max}$	120.2°
No. of Reflections Measured	Total: 952
Corrections	Lorentz-polarization Absorption (trans. factors: 0.7075 - 1.0000) Decay (0.33% increase) Secondary Extinction (coefficient: 4.33934e-05)

C. Structure Solution and Refinement

Structure Solution	Direct Methods (SIR92)
Refinement	Full-matrix least-squares
Function Minimized	$\Sigma w(\|Fo\| - \|Fc\|)^2$
Least Squares Weights	$w = \frac{1}{\sigma^2(Fo)} = [\sigma_c^2(Fo) + \frac{p^2}{4}Fo^2]^{-1}$
p-factor	0.0000
Anomalous Dispersion	All non-hydrogen atoms
No. Observations (I>3.00σ(I))	742
No. Variables	155
Reflection/Parameter Ratio	4.79

Residuals: R; Rw	0.035 ; 0.030
Goodness of Fit Indicator	2.87
Max Shift/Error in Final Cycle	0.07
Maximum peak in Final Diff. Map	0.16 $e^-/\AA^3$
Minimum peak in Final Diff. Map	-0.13 $e^-/\AA^3$

Crystal data for **3 6** (fig. 3, p99)

A. Crystal Data

Empirical Formula	$C_{28}H_{30}O_5$
Formula Weight	446.54
Crystal Color, Habit	clear, needle
Crystal Dimensions	0.05 X 0.05 X 0.15 mm
Crystal System	monoclinic
Lattice Type	Primitive
No. of Reflections Used for Unit Cell Determination (2θ range)	10 (19.9 - 31.2°)
Omega Scan Peak Width at Half-height	0.13°
Lattice Parameters	a = 10.104(3) Å b = 6.542(4) Å c = 18.678(5) Å β = 103.93(2)°
	V = 1198.2(7) Å^3
Space Group	$P2_1$ (#4)
Z value	2
D_{calc}	1.238 g/cm^3
F_{000}	476.00
μ(CuKα)	6.79 cm^{-1}

B. Intensity Measurements

Diffractometer	Rigaku AFC7S

Radiation	CuKα ($\lambda = 1.54178$ Å) graphite monochromated
Attenuator	Ni foil (factor = 9.42)
Take-off Angle	6.0°
Detector Aperture	9.0 mm horizontal 13.0 mm vertical
Crystal to Detector Distance	400 mm
Voltage, Current	0kV, 0mA
Temperature	20.0°C
Scan Type	ω
Scan Rate	16.0°/min (in ω) (up to 4 scans)
Scan Width	$(1.21 + 0.35 \tan \theta)°$
$2\theta_{max}$	120.1°
No. of Reflections Measured	Total: 2099 Unique: 1975 ($R_{int} = 0.453$)
Corrections	Lorentz-polarization Absorption (trans. factors: 0.1175 - 1.0000) Decay (0.33% decline)

C. Structure Solution and Refinement

Structure Solution	Direct Methods (SHELXS86)
Refinement	Full-matrix least-squares
Function Minimized	$\Sigma w(\|Fo\| - \|Fc\|)^2$
Least Squares Weights	$w = \frac{1}{\sigma^2(Fo)} = [\sigma_c^2(Fo) + \frac{p^2}{4} Fo^2]^{-1}$
p-factor	0.0020
Anomalous Dispersion	All non-hydrogen atoms
No. Observations (I>1.60σ(I))	670
No. Variables	133
Reflection/Parameter Ratio	5.04

Residuals: R; Rw	0.232 ; 0.203
Goodness of Fit Indicator	10.92
Max Shift/Error in Final Cycle	2.76
Maximum peak in Final Diff. Map	0.94 $e^-/Å^3$
Minimum peak in Final Diff. Map	-0.96 $e^-/Å^3$

Crystal data for **38** (fig. 4, p103)

Identification code	impp4
Empirical formula	$C_{29}H_{34}O_5$
Formula weight	462.56
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	a = 14.0516(5) Å alpha = 90° b = 9.5812(3) Å beta = 95.051(2)° c = 18.6022(5) Å gamma = 90°
Volume, Z	2494.71(14) $Å^3$, 4
Density (calculated)	1.232 Mg/m^3
Absorption coefficient	0.083 mm^{-1}
F(000)	992
Crystal size	.01 x .15 x .3 mm
θ range for data collection	1.45 to 23.23°
Limiting indices	$-15 \le h \le 15$, $-10 \le k \le 9$, $-20 \le l \le 14$
Reflections collected	10912
Independent reflections	6834 (R_{int} = 0.0313)
Absorption correction	Sadabs
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6784 / 9 / 646
Goodness-of-fit on F^2	0.968
Final R indices [I>2σ(I)]	R1 = 0.0532, wR2 = 0.1140
R indices (all data)	R1 = 0.1042, wR2 = 0.1545
Absolute structure parameter	-1.8(15)
Extinction coefficient	0.0043(7)
Largest diff. peak and hole	0.152 and -0.154 $eÅ^{-3}$