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Organocatalytic Enantioselective Epoxidation of Some Aryl-Substituted Vinylidenebisphosphonate Esters: On the Way to Chiral Anti-Osteoporosis Drugs

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Abstract: The synthesis of a new class of epoxide derivatives from prochiral vinylidene bisphosphonate (VBP) precursors is reported using hydrogen peroxide as the terminal oxidant. The reaction is carried out using a series of possible organic activators having a basic character, with the best results being observed using quinine and sparteine. These activators not only provide from good to excellent epoxide yields with a large variety of VBPs, but also interesting enantioselectivities in the 67%–96% ee range, at least in the case of the Ph and *m*-MeO–Ph VBP derivatives, opening the way to a number of chiral anti-osteoporosis potentially active pharmaceutical ingredients.

Keywords: bisphosphonates; epoxidation; hydrogen peroxide; enantioselectivity; quinine; sparteine

1. Introduction

Osteoporosis is a skeleton disease characterized by loss of bone mass and deterioration of tissue micro-architecture that leads to bone fragility and an increase in fracture risk [1]. Nowadays, it affects hundreds of millions of elderly individuals worldwide (especially women) with a severe impact on their quality of life and significant social costs for medical treatments. The future perspectives, especially in the western world, are even worse because of the rapid increase of the elderly population [2,3].

Bisphosphonates (BPs) are currently the major class of drugs used for the treatment of both osteoporosis and other diseases characterized by increased bone resorption [4,5]. BPs are chemical analogs of biologically occurring pyrophosphates (major mineral constituents of bone) and they have a strong binding capacity to metal ions (e.g., calcium) as well as a very high affinity for the bone mineral (Figure 1).

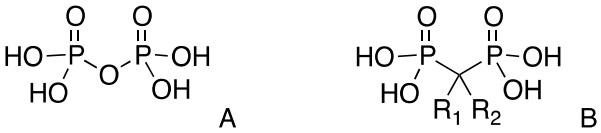


Figure 1. Pyropshosphoric acid (**A**) and a generic structure of a bisphosphonic acid (**B**).

BPs have been used as anti-osteoporosis drugs for the past 40 years and despite a relative increase in molecular complexity and potency over the years, the structures of the commercial drugs are still relatively simple. These molecules do not possess the complexity and structural variety necessary to cope with their therapeutic mechanism of action [6–9], to improve their poor bioavailability [10], or to tackle other related application fields where BPs promise interesting results such as, e.g., as anti-arthritis

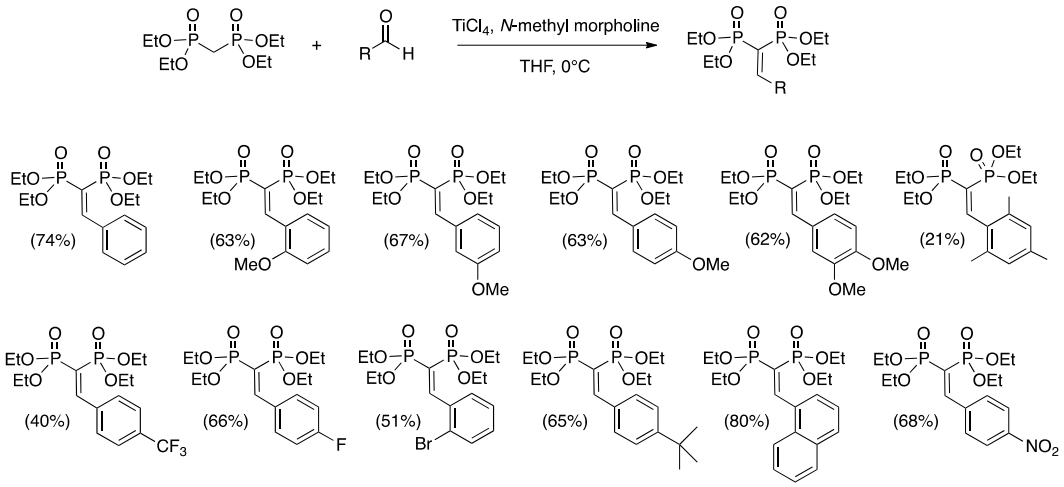
and anti-bone tumor drugs [4]. In this respect, the major limit seems to be the lack of reliable synthetic methods to prepare a wider range of complex molecules and tune the tridimensional neighborhood around the BP group. For example, even though the different biological response to molecules having different stereoisomerism is a very well-known concept, the lack of enantioselective synthetic methods has so far hampered the synthesis of chiral BPs where very few examples have been reported in the literature [11–13], with only one example of an enantio-enriched BP having been biologically tested, observing a 24-fold difference in activity between the enantiomers [14]. This situation reflects the problems of dealing synthetically with molecules having a bisphosphonate group which induces (i) a strong electrophilicity on the central C atom and eventually on adjacent moieties; (ii) a significant steric hindrance leading in many cases to limited accessibility and poor reactivity; and (iii) the capacity of strongly binding metal ions, thereby inactivating catalysts in transition metal–catalyzed reactions.

A useful synthetic approach allowing the formation of a broad range of BPs is based on vinylidenebisphosphonate precursors (VBPs) as starting materials [15–18]. The application of a series of enantioselective catalytic reactions based on the use of a variety of nucleophilic reagents towards these precursors opens the way to the possibility of obtaining a wide range of structurally complex BPs, and in principle the opportunity to verify the different behaviors of the synthesized enantiomers. Among the different possibilities, enantioselective epoxidation represents a key technology because the oxirane ring can be easily opened by different types of reactants, allowing the preparation of chiral 1,2-disubstituted derivatives [19].

As a follow-up to our previous work in this area [15,20,21], in this paper we report the epoxidation of a series of monosubstituted prochiral VBPs using different possible oxidants and catalyzed by enantiopure organocatalysts, observing, in some specific cases, interesting levels of stereoselective induction. We also report some subsequent attempts to open the oxirane ring to lead to the formation of enantioenriched BPs having an -OH group between the two P atoms, a feature that improves their affinity with hydroxyapatite [22], i.e., the major mineral constituent of bone.

2. Results and Discussion

A series of monosubstituted VBP precursors has been synthesized following the method outlined by Lehnert and other groups [23–26] which consists of the addition of an aldehyde to methylene bisphosphonate tetraethyl ester (MBP) catalyzed by $TiCl_4$ in the presence of a base (Scheme 1) giving, in most cases, from good to excellent yields. As shown, the reaction proceeds well with aromatic aldehydes, whereas with aliphatic aldehydes or ketones it leads to only poor yields.



Scheme 1. Synthesis of VBP precursors. Isolated yields of the individual compounds are given in parentheses.

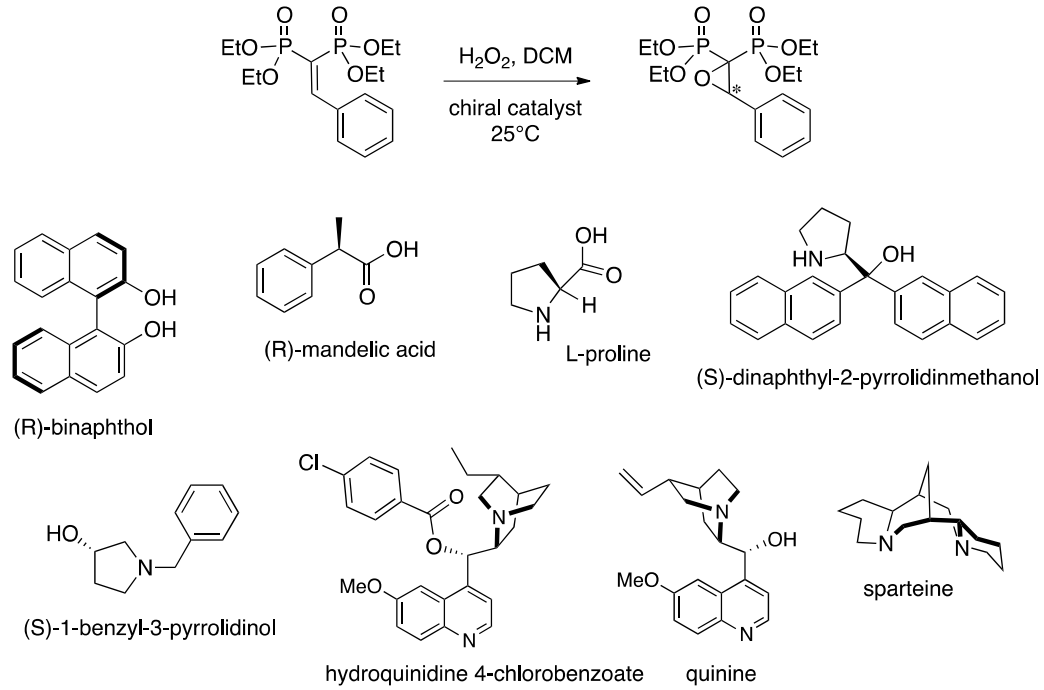
The epoxidation of the electron-poor C=C double bond [27,28] was carried out, adapting to the case of prochiral BPs the classical Weitz-Scheffer reaction for the epoxidation of α,β-unsaturated aldehydes, consisting of the use of hydrogen peroxide as the oxidant under basic conditions [29]. The use of H_2O_2 is recommended due to its highly environmentally acceptable character [30] and involves the nucleophilic attack of HOO^- generated in situ to the electron-poor C=C double bond. Initially the epoxides were synthesized in the racemic form from the VBPs shown in Scheme 1, using NaOH as the base to provide suitable amounts of material to perform the enantiomer separation, using chiral HPLC for the subsequent ee evaluation. The reaction was carried out in MeOH at room temperature and led to excellent conversions in most cases. All products were characterized by NMR and mass spectrometry.

As a typical example, the ^{1}H NMR spectrum of **PPhO** (see Supplementary Material, Figure S2) shows a series of features, namely: (i) a typical triplet at 4.69 ppm ($^3J_{H\text{-}P}$ 5.0 Hz) attributed to the oxiranic proton coupling with two non-equivalent P atoms but with almost equivalent $^3J_{H\text{-}P}$ coupling constants; (ii) the signals attributed to the ester groups, i.e., the –CH_2– multiplet at 4.0 ppm and four different signals for –CH_3 at 1.41 ppm and 1.13 ppm due to the diastereotopic environments of the ester moieties derived by the presence of the stereocenter in the molecule; (iii) the $^{31}P\{^1H\}$ NMR spectrum showing two different doublets due to the diastereotopic environment of the P atoms. The mass spectrum (see Supplementary Materials, Figure S3) shows a very weak peak at 392 m/z corresponding to the molecular ion of the product, thus providing a further confirmation of the structure of the product.

The stereoselective version of the epoxidation reaction was first tested on the phenyl-substituted derivative (**PPh**) in the presence of a wide range of possible activators and oxidants. The enantioselective oxidation of α,β-unsaturated aldehydes has been the subject of numerous investigations in the past and was successfully accomplished both by organocatalysts by, e.g., Wynberg and coworkers using quininium salts [31] and by Julià and Colonna using poly-L-alanine [32] as well as by metal complexes, namely of Pt, reported by our group [33], or of Y, reported by Shibasaki and coworkers [34].

Initial tests with our original [(chiraphos)Pt(C_6F_5)(H_2O)](OTf) catalyst [33] were totally unsuccessful, probably because the bisphosphonate unit binds to the metal efficiently, preventing the coordination of the C=C double bond. So we reverted to a series of chiral organocatalysts (Scheme 2) able to activate either the substrate via hydrogen bonding ((*R*)-binaphthol, (*R*)-mandelic acid, L-proline) or hydrogen peroxide used as the oxidant increasing its nucleophilicity by deprotonation (L-proline, (*S*)-dinaphthyl-2-pyrrolidinmethanol, (*S*)-1-benzyl-3-pyrrolidnol, hydroquinidine 4-chlorobenzoate, quinine, sparteine). These were tested towards **PPh** under mild conditions (Scheme 2) and the results obtained are summarized in Table 1.

As can be seen in Table 1, the use of catalysts being either Brønsted acidic or able to activate the substrate via hydrogen bonding (entries 1, 2) is completely ineffective, whereas the nucleophilic activation of the oxidant produces either no or very moderate effects when using secondary amines (entries 3, 4). Somehow, expectedly, higher yields were observed in the presence of tertiary amines such as quinine and sparteine. The absence of -OH groups capable of stabilizing the nucleophilic oxidant formed as in Figure 2 had a dramatic effect on the reaction yield. Compare, for example, the behavior of quinine (entry 7) which gives a 73% yield, with a very similar activator but modified with a *p*-Cl-benzoate ester moiety (entry 6) which results almost completely inactive. Interestingly, the absence of activity observed with benzyl-pyrrolidinol seems to indicate that basicity and the presence of an OH group is per se an insufficient condition because a critical distance between N and OH is necessary to favor the approach of the oxidant via hydrogen bonding (Figure 2). The drop in conversion observed with hydroquinidine 4-chlorobenzoate could also be the result of a higher steric hindrance in the catalyst. The sensitivity of this system to steric factors is demonstrated also by the poor but not null conversion observed with (*S*)-dinaphthyl-2-pyrrolidinmethanol, which is structurally similar to quinine, and also exploring the scope of the reaction (vide infra).



Scheme 2. Enantioselective epoxidation of **PPh** with hydrogen peroxide using a variety of possible organoactivators.

Table 1. Enantioselective epoxidation of **PPh** with hydrogen peroxide and other oxidants using different organocatalysts.

Entry	Organocatalyst	Catalyst amt (mol %)	Oxidant	Yield [a] (%)	e.e. [b] (%)
1	(*R*)-1,1′-2-naphthol	100	H_2O_2	3	=
2	(*R*)-mandelic acid	100	H_2O_2	0	=
3	L-proline	100	H_2O_2	0	=
4	(*S*)-dinaphthyl-2-pyrrolidinmethanol	100	H_2O_2	6	=
5	(*S*)-1-benzyl-3-pyrrolidinol	100	H_2O_2	0	=
6	hydroquinidine 4-chlorobenzoate	100	H_2O_2	2	=
7	quinine	100	H_2O_2	73	68
8	quinine	50	H_2O_2	42	67
9	quinine	10	H_2O_2	4	=
10	quinine	100	*t*-BuOOH	0	=
11	quinine	100	urea.H_2O_2	>99	20
12	sparteine	100	H_2O_2	90	94
13	sparteine	50	H_2O_2	43	96
14	sparteine	20	H_2O_2	36	94
15	sparteine	100	*t*-BuOOH	0	=
16	sparteine	100	urea·H_2O_2	>99	13

Experimental conditions: **PPh** 0.080 mmol, oxidant 0.096 mmol, CH_2Cl_2 2 mL, T 25 °C, reaction time 18 h. [a] Determined by GC analysis; [b] Determined by chiral HPLC.

With quinine as the catalyst, an interesting 68% ee was observed, while the yield was strongly dependent on the amount of catalyst loaded (entries 7–9). The same effect was observed with sparteine (entries 12–14), suggesting that a stoichiometric amount of the tertiary amine activator is necessary to speed the reaction up. Under these circumstances, the "catalysts" reported in Table 1 would be better termed "activators".

Other oxidants tested were either ineffective (*t*-BuOOH, entries 10, 15) or very effective such as the organosoluble urea·H_2O_2 adduct that led to slightly higher yields but, more importantly, with a

significant loss of enantioselectivity (entries 11, 16). A blank reaction under the same conditions but without sparteine to check whether urea alone could be a possible activator for the reaction yielded no products even after 18 h. Similarly, the use of urea·H_2O_2 (with urea as the H-bond acceptor) associated with (*S*)-1-benzyl-3-pyrrolidinol (as the base) gave no results in the epoxidation reaction, indicating that probably for steric reasons the basic character and the H-bond acceptor role must coexist in the same molecule.

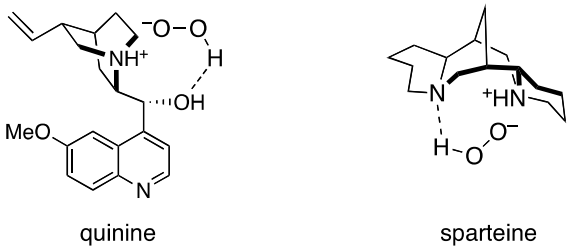


Figure 2. Activation of hydrogen peroxide by quinine and sparteine evidencing the ion pairing and hydrogen bonding stabilization of HOO^- by the activator.

Sparteine seems to be the best activator tested, capable of giving better yields, probably due to a better approach to the prochiral substrate because of a lower steric hindrance, as was further demonstrated by a full reaction profile showing that the reaction was completed in slightly more than 1 h (see Supplementary Material, Figure S1). Similarly, the higher ee values observed probably rely on its ability to create a more rigid and stable adduct with HOO^-, thus favoring the stereoselective recognition of either face of the C=C double bond in **PPh**. The drop in enantioselectivity observed with urea·H_2O_2 seems to suggest some loss of the intramolecular H-bond, broken by the presence of urea as an external H-bond acceptor.

The good results observed with sparteine led to the extension of the above experimental procedure to the VBP structures reported in Scheme 1. In Table 2 we report the results obtained in the enantioselective epoxidation of the different prochiral precursors with hydrogen peroxide promoted by sparteine.

Table 2. Enantioselective epoxidation of prochiral VBPs with hydrogen peroxide promoted by sparteine.

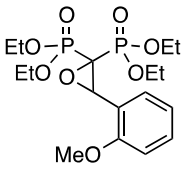
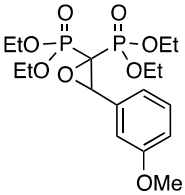
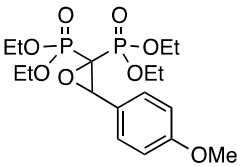
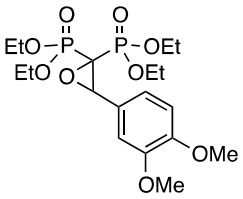
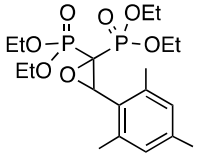
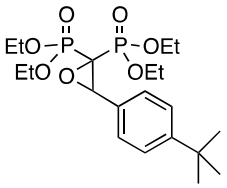
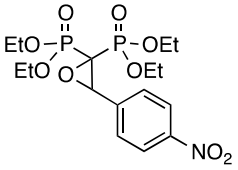
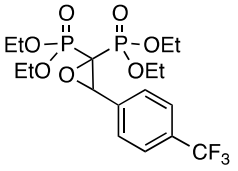
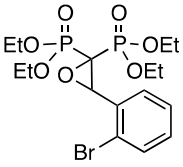
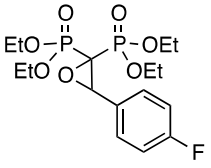
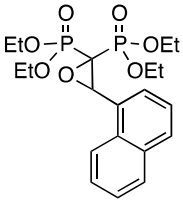
Entry	Epoxide	Acronym	Yield [a] (%)	Ee [b] (%)
1		**PoOMeO**	63	4
2		**PmOMeO**	74	81
3		**PpOMeO**	77	1

Table 2. *Cont.*

Entry	Epoxide	Acronym	Yield [a] (%)	Ee [b] (%)
4		**PVERO**	71	9
5		**PMESO**	10	= [c]
6		**PptBuO**	0	=
7		**PpNO_2O**	75	= [d]
8		**PpCF_3O**	95	17
9		**PoBrO**	35	10 (17 [e])
10		**PpFO**	71	5 (16 [e])
11		**P1NAPO**	65	3 (12 [e])

Experimental conditions: substrate 0.080 mmol, oxidant 0.096 mmol, sparteine 0.080 mmol, CH_2Cl_2 2 mL, T 25 °C, reaction time 18 h. [a] Determined by $^{31}P\{^1H\}$ NMR analysis; [b] Determined by chiral HPLC; [c] Not determined because of poor yield; [d] The enantiomers could not be separated; [e] Reaction carried out at 0 °C.

First of all, it must be pointed out that some HPLC analytical problems have been found (e.g., entry 7). In this respect, some examples of successful and unsuccessful HPLC separations are reported in the Supplementary Materials. In general, sparteine promoting the epoxidation of

aromatic MBPs appears to produce (with some exceptions) from good to excellent chemical yields, while in terms of enantioselectivity it is much less rewarding. The reaction seems to be sensitive mostly to steric factors, for example the presence of a substituent group (both electron withdrawing and electron releasing) on the aromatic ring leads to a general decrease of the reaction yields, the latter being more pronounced when the substituent is in the *ortho* position (Table 2, entries 1, 5, 9). The reaction is similarly inhibited by very bulky substituents even if in the *para* position (entry 6), indicating a difficulty of approach between the substrate C=C double bond and the base-H_2O_2 adduct. With respect to the enantioselectivity, while with **PPh** the reaction showed excellent results (Table 1, entry 12), with the naphthyl derivative (Table 2 entry 11) the ee dropped dramatically. Decreasing the reaction temperature to 0 °C produced only modest improvements (Table 2, entries 9–11). Altogether, the use of sparteine as an enantioselective activator for the epoxidation of a variety of VBPs seems to be successfully applicable preferentially to precursors such as **PPh** (ee 94%) or to the corresponding *m*-MeO- derivative (**PmOMe**) (ee 81%). In all other cases tested, ee values were very low.

Since the synthesized epoxides have a significant electrophilic character at the C-2 position, they could, in principle, react with a wide range of nucleophiles [35,36]. Hence, the epoxides obtained in their racemic form were tested towards sulfur-containing nucleophiles and organometals. This should lead, in principle, to BPs having an extra OH group on the C atom between the two P atoms, a factor known to improve their affinity for hydroxyapatite [22]. The epoxide coming from **PPh** (**PPhO**) was reacted with a stoichiometric amount of organometals such as *n*-BuLi or PhMgBr, leading to the complete consumption of the starting **PPhO** epoxide and the appearance of a product whose $^{31}P\{^1H\}$ NMR spectrum showed two clearly different ^{31}P singlet resonances, one at 20.95 ppm and one at −1.51 ppm. The large up-field shift of one of the two P atoms is not compatible with the typical chemical shift for phosphonate units, but rather in agreement with the presence of a phosphate group [37].

The same **PPhO** epoxide was reacted with both thiophenol and the nucleophilically stronger potassium thiophenate without producing the ring-opening derivative and leaving a large amount of unreacted epoxide behind despite the long reaction time.

3. Materials and Methods

^1H NMR, $^{31}P\{^1H\}$ NMR spectra were run on: (i) a Bruker Avance 300 spectrometer (Bruker Italia, Milano, Italy) operating at 300, 122 MHz, respectively, at 298 K; (ii) a Bruker Avance 400 spectrometer (Bruker Italia, Milano, Italy) operating at 400 and 162 MHz, respectively, at 298 K; δ values in ppm are relative to $Si(CH_3)_4$ and 85% H_3PO_4.

GC-MS analyses were performed on a GC Trace GC 2000 (ThermoQuest Italia, Milano, Italy) coupled with a quadrupole MS Thermo Finnigan Trace MS with Full Scan method.

HPLC analyses were performed on a Hewlett Packard Series 1100 G1311A QuatPump (Agilent Technologies Italia, Cernusco sul Naviglio, Italy).

Flash chromatography was performed on 230-400 mesh silica and thin layer chromatography was carried out on 20 cm × 20 cm Alugram® Xtra SIL G/UV_{254} from Macherey-Nagel. The solvent used was ethyl acetate.

Diethyl phosphite, the aromatic aldehydes, thiophenol and potassium thiophenate, *n*-BuLi and PhMgBr used as reactants were commercial products (Sigma-Aldrich, Milano, Italy) and were used without any purification. All synthetic work was carried out without the exclusion of atmospheric oxygen. Solvents were dried and purified according to standard methods.

3.1. Synthesis of Methylenebisphosphonate Tetraethyl Ester (MBP) [38]

A sodium ethoxide solution was prepared by addition of metallic sodium (5 g, 220 mmol) in portions to ethanol (130 mL). Diethyl phosphite (220 mmol) was then added with stirring to the sodium ethoxide solution, the resulting mixture was stirred for 1 h at RT and finally concentrated on a rotary evaporator. The residue was dissolved in 10 mL of methylene chloride (156 mmol) and the mixture was stirred for two weeks at RT. The mixture was washed with brine, the methylene chloride phase

was dried with sodium sulphate and brought to dryness on a rotary evaporator. The residue was distilled in vacuo to give MBP in 54% yield.

^{1}H NMR (400 MHz, $CDCl_3$)δ 4.20–4.00 (m, 8H), 2.39 (t, *J* = 21.0 Hz, 2H), 1.29 (t, *J* = 7.1 Hz, 12H) ppm. ^{31}P {^{1}H}-NMR (162 MHz, $CDCl_3$) δ 19.38 (s, 2P) ppm. ^{13}C {1H}-NMR (101 MHz, $CDCl_3$) δ 62.62 (t, *J* = 3.0 Hz), 25.43 (t, *J* = 136.9 Hz), 16.36 (d, *J* = 3.2 Hz) ppm. GC-MS (70 eV) m/z: 288 [M^+], 261 [$M^+ - CH_2CH_3$], 233 [$M^+ - 2(CH_2CH_3)$], 205 [$M^+ - 3(CH_2CH_3)$].

3.2. General Procedure for the Synthesis of the Mono-Substituted Alkylidene Bisphosphonates (VBPs)

A flame-dried 50 mL round bottom flask equipped with a magnetic stirring bar was loaded with $TiCl_4$ (15 mmol) and 3.9 mL of CCl_4 at 0 °C. Then 30 mL of dry THF was added dropwise to the flask and a bright yellow precipitate formed. Then the aldehyde (5 mmol) and MBP (5 mmol) were added followed by a 2.3 mL solution of 4-Methylmorpholine (21 mmol) in 5.0 mL dry THF that was added dropwise to the stirred mixture over 1 h. The reaction was allowed to warm to RT and stirred overnight. The reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration under vacuum followed by column chromatography provided the corresponding alkylidene bisphosphonate.

The spectroscopic characterization of all compounds is reported in the Supplementary Material section.

3.3. Epoxidation of Prochiral VBPs

In a vial equipped with a small magnetic bar were introduced in the order: the prochiral VBP (0.080 mmol), CH_2Cl_2 as the solvent (2.5 mL), a 4.22 M aqueous solution of H_2O_2 (0.096 mmol) and 1 equivalent of KOH or one equivalent of chiral activator. The reaction vessel was closed and stirred overnight at RT. The mixture obtained was purified by TLC and characterized by NMR, mass spectrometry and chiral HPLC.

3.4. Attempts of Oxirane Ring Opening with Organometals

In a vial equipped with a small magnetic bar were introduced 30 mg PPhO in 1 mL methylene chloride followed by 1 eq. of either *n*-BuLi or PhMgBr at 0 °C allowing the reaction to react for 0.5 h then raising the temperature to RT for 1 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl and extracted with EtOAc. The reaction mixture obtained was concentrated under vacuum and analyzed by NMR. Attempted purification by TLC were unsuccessful because of the high affinity of the addition products for silica.

3.5. Oxirane Ring Opening with S-Nucleophiles

In a vial equipped with a small magnetic bar were introduced 30 mg PPhO in 1 mL methylene chloride followed by 1 eq. of either thiophenol or potassium thiophenate and the mixture was allowed to react overnight at RT. The reaction mixture obtained was concentrated under vacuum and analyzed by NMR.

4. Conclusions

In this paper we have demonstrated that epoxidation of aryl-substituted vinylidene bis-phosphonates accomplished with traditional methods is a viable functionalization strategy for the achievement of more elaborate molecular architectures capable of tackling the diverse facets involved in the clinical applications of this important class of drugs.

The enantioselective version of this reaction is possible using hydrogen peroxide associated with some chiral organic bases as activators (quinine, sparteine), resulting in high enantio-differentiation in just a few cases. Despite some interesting results, the general behavior of prochiral substrates reveals

a rather elusive stereoselective course, strongly affected by somehow unpredictable steric factors in the substrate.

The new (chiral) BPs obtained via the synthetic routes outlined here are currently under study in order to investigate their biological and toxicological properties as single enantiomers to determine their cytotoxicity and their ability to induce osteoclast inhibition and apoptosis.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4344/7/3/90/s1.

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