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Synthesis and biological evaluation of α-hydroxyalkylphosphonates as new antimicrobial agents

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ABSTRACT

A set of α-quaternary 3-chloro-1-hydroxyalkylphosphonates, analogues of fosfomycin and fosfonochlorin, some of which are new compounds, was synthesized. The compounds were screened for bioactivity against several clinical and standard microbial isolates. Some were found to have moderate activity. The activity was higher with phenyl protection of the phosphoryl ester groups and α-phenyl substitution. Compound **11** was as effective or more potent than fosfomycin or chloramphenicol against several Gram-negative bacteria as well as against some Gram-positive ones.

Antibiotics are in great demand. Although many successes have been achieved in the fight against infectious diseases in the last century, the continuous development of resistance by bacteria to the current antibiotics, and even the development of multidrug resistant strains, has left us with a 'rather bare antibiotic cupboard to meet the challenges of new outbreaks'.[1] Bacterial infections are still a major cause of death in the developing world. Another problem is resistant bacteria from hospitals, which cause 'community-acquired' infections, difficult to treat, particularly in immuno-compromised patients. These resistant bacteria have also acquired toxins that make them more virulent. Examples are Gram-positive bacteria, like *Staphylococcus aureus* and *Enterococcus* spp., which frequently display multidrug resistance,[2] and Gram-negative *Stenotrophomonas maltophilia*. Drug resistance develops as a result of gene mutations, rearrangements and genetic transfer between different bacteria.[3] These are serious problems to populations and to public health systems due to the morbidity and mortality they cause, and the costs related to the implementation of effective control measures, and led the World Health Organization to select 'antimicrobial resistance' as the theme for World Health Day 2011.

The development of effective antimicrobial control measures involves both a search for novel compounds and finding new uses for older drugs. One revival is fosfomycin, a phosphonic acid derivative with a broad spectrum of activity, used mainly for the treatment of uncomplicated urinary tract and gastrointestinal infections.[4] Recent studies showed its potential for the treatment of mutidrug-resistant, including extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae infections[5] like those caused by *Escherichia coli* and *Klebsiella pneumoniae*, and other Gram-negative species such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.[6,7] In fact fosfomycin is one of the very few antibiotics presently available to treat Gram-negative infections,[8] besides colistin and the new tigecycline and doripenem.[4] Fosfomycin acts by inactivating the enzyme UDP-*N*-acetylglucosamine enolpyruvyl transferase (Mur A), thereby irreversibly blocking condensation of uridine diphosphate-*N*-acetylglucosamine with phosphoenolpyruvate, one of the first steps in the peptidoglycan bacterial cell wall synthesis. Normally it is used together with another antibiotic to prevent the development of resistance, and it is compatible to many.

We have been interested in the chemistry of phosphonic acid derivatives for the past few years.[9] Many compounds of this class are biologically active, due to their structural similarity to phosphates and carboxylates, competing with them for enzyme binding sites.[10] Examples of other potent phosphonate antibiotics are fosmidomycin, which is also a potent antimalarial, plumbemycin A and fosfonochlorin, an antibiotic with spheroplast-forming activity isolated from cultures of *Fusarium* sp., moderately active against some species of Gram-negative bacteria (Fig. 1).[11]

Inspired by the structure of fosfomycin, we decided to synthesize analogues that could have antimicrobial activity. It is known

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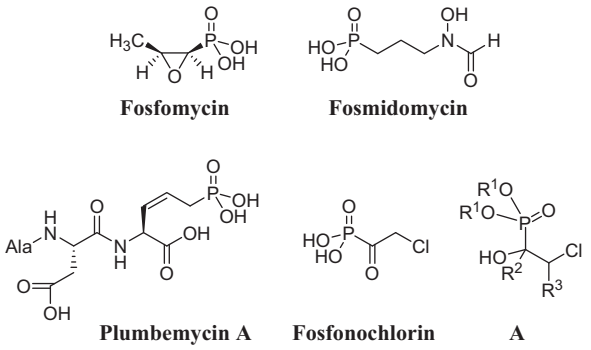


Figure 1. Phosphonate antibiotics.

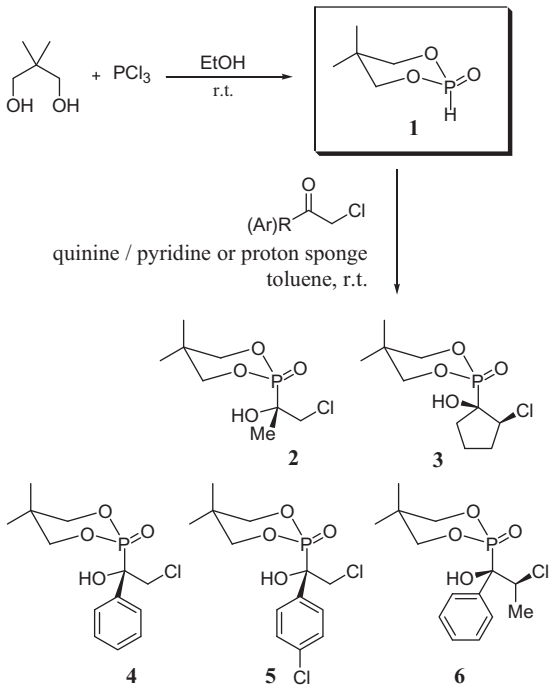
that the enzymes that cause resistance to fosfomycin, namely, Fos A, Fos B, and Fos X, function by nucleophilic attack at the carbon atom α to phosphorus.[12] If this centre were to be a quaternary carbon atom, reaction at this position would become more difficult, and interesting biological activity may result. We recently developed an organocatalyzed method for the synthesis of β-chloro-α-quaternary hydroxyphosphonates **A**, which are also structurally related to fosfonochlorin.[13] There are existing examples of biologically active α-hydroxyphosphonic acids which are enzymes inhibitors, but not of biologically active α-quaternary β-chloro-α-hydroxyphosphonates.[13] We produced a set of these compounds to screen for potential bioactivity and for structure-activity relationship studies. In this paper we report the synthesis and characterization of some new phosphonates, not previously described in the literature, and our results on the evaluation of the full set for potential antimicrobial activity.

The synthesis of the required α-hydroxyphosphonates was based on a method recently developed by us,[13] which involves an organocatalyzed regioselective modified Pudovik reaction between a dialkylphosphite and a α-haloketone.[14,15] This reaction may be carried out under mild conditions using catalytic amounts of quinine in the presence of a stoichiometric base such as proton-sponge or pyridine.[13] When these conditions are used, β-chloro-α-hydroxyphosphonates are obtained. The reaction is compatible with aliphatic, cyclic or aromatic α-haloketones. Since phosphonic acids are negatively charged at nearly all physiological pH values,[16] compounds bearing these groups are very polar. High polarity can be a set back in a drug, preventing efficient delivery to target organs, since highly ionized species do not pass readily across mucosal and cellular membranes. The volume of distribution and bioavailability will be relatively low. In drugs, phosphonates and phosphates are usually derivatized as neutral esters that can break down in the body to release the parent drug, since this modification alters membrane permeability and improves oral (GI permeability), brain, tumour and cellular delivery. A few studies have shown the influence of the nature of the phosphonate ester groups on drug delivery and bioavailability. For example, the acyclic nucleotide analogue 9-[2-(phosphonomethoxy)ethoxy]adenine, a potent and selective inhibitor of the human immunodeficiency virus-1 (HIV-1) and immunodeficiency virus-2 (HIV-2), has very low bioavailability.[17] Derivatization as bis[pivaloyloxy) methyl]ester or diphenyl ester provide good oral bioavailability.[17] A similar effect of the phenyl group was shown in Adefovir, 9-[2-(phosphonomethoxy)ethyl]adenine, a nucleotide analogue used clinically to treat chronic hepatitis B virus infection. It also enhanced the in vitro activity against HSV-2 (G-strain) when compared with the unprotected drug (IC_{50} = 77 μM vs 119 μM in the unprotected drug).[18] With the endopeptidase inhibitor CGS 25462, diphenyl ester derivatization provided a 200-fold increase of the active drug in the plasma above its IC_{50} value.[19]

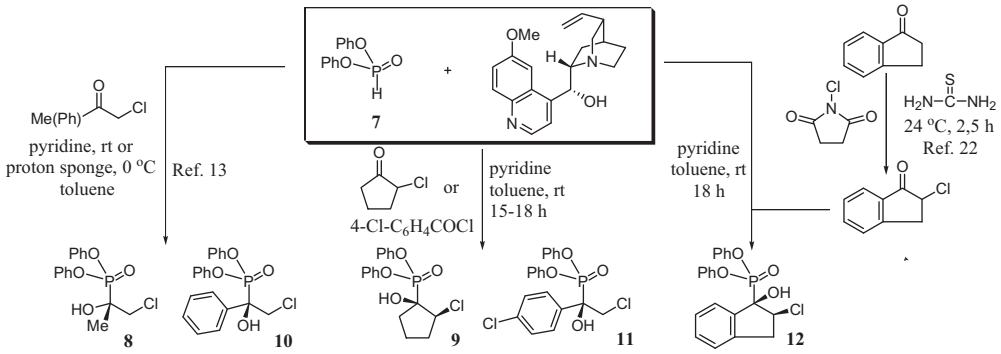
We chose two different types of ester protecting groups: cyclic phosphonate esters, which are usually more stable than the analogous alkyl esters, and phenyl phosphonate esters which are much more labile.[17,20] The two series of hydroxyphosphonates to be screened were prepared as shown in Schemes 1 and 2.

For the phosphonates shown in Scheme 1, the intermediate cyclic phosphite ester **1** needed was obtained via the reaction of 2,2-dimethyl-1,3-propanediol with phosphorus trichloride in the presence of ethanol, according to a previously published procedure.[21] Hydroxyphosphonates **2**–**6** were obtained after the modified Pudovik reaction (Scheme 1). Diphenyl phosphite is commercially available, and with it hydroxyphosphonates **8**–**12** could be synthesized (Scheme 2). 2-Chloroindanone, needed for the synthesis of **12**, was obtained via another organocatalyzed reaction, a thiourea-catalyzed α-chlorination, using *N*-chlorosuccinimide as the halogen source.[22] To the best of our knowledge, phosphonates **9**, **11** and **12** have not been previously described in the literature.[23]

In the synthesis of compounds **9**, **11** and **12**, the desired product was obtained as the major product, together with small amounts of enolphosphate, as determined by ^{31}P NMR spectroscopy. In each case there was also usually, in addition, an extra signal at δ = 128.5 ppm, due to unreacted starting material in the phosphite form (<5%).[24] Phosphonates **9** and **12** were obtained as mixtures of diastereoisomers in approximately a 90:10 ratio. The major diastereoisomers were isolated by chromatography on silica gel and subsequently recrystallized. Their structures were established by one- and two-dimensional NMR spectroscopic techniques and by infrared spectroscopy. Their composition was confirmed by elemental analysis. In the case of cyclopentanol derivatives **9** and **12**, NOESY experiments provided some information (included in the Supplementary data section). C-1 is quaternary and the hydroxyl proton did not appear as an independent signal in the spectrum showing coupling to the C-2 proton. It appears as a very broad singlet in the spectra of most of the compounds in these series. The nearest protons which could show cross peaks with H-2 and hence help to establish the stereochemistry at C-2 are the aromatic protons on the phosphorus ester substituents. They also showed no cross peaks with H-2, but this fact cannot be taken unambiguosly



Scheme 1. Synthesis of cyclic alkylphosphonate esters.



Scheme 2. Synthesis of diphenyl alkylphosphonate esters.

to imply that they occupy the region in space on the face opposite to H-2, because they could be simply too far away to be detected. With **12** a cross peak between one of the H-3 protons and the hydroxyl group suggests a correlation, which being absent with H-2, must imply that H-2 and the hydroxyl group occupy the space on opposite faces of the ring. In cyclopentanones the lowest energy conformation is the envelope, and when there is a substituent at the α-position two situations are possible: **B** and **C** (Fig. 2).[25] The reacting nucleophile can then approach from side a or b in each case. However, envelope **B**, with the chloro substituent pseudo-equatorial, should be lower in energy, and it is the conformation considered when a prediction of the stereochemical outcome of reactions is made. Approach of the nucleophile is expected, from empirical observations in other nucleophilic additions to carbonyl compounds, to take place at an angle of ~110° (the Bürgi–Dunitz trajectory), that is via path a. This approach gives rise to a product in which Cl and OH groups are *trans*. However, when there are with very bulky substituents at the α-position, or when the nucleophile is very bulky, which is the present case, unfavourable steric interactions between the α-substituent and the nucleophile will favour nucleophile approach along path b [23], giving rise to a *cis* product. Hence we predict that in our products **9** and **12** the stereochemistry is one in which the Cl and OH groups are *cis*. All the hydroxyphosphonates used in this study were crystalline compounds that could be obtained in a very pure state after chromatography and recrystallization.

The antimicrobial activity of the compounds prepared was evaluated *in vitro* against standard and clinically isolated strains of the Gram-negative bacteria *Escherichia coli* BW54, *E. coli* BW55, *Acinectobacter haemolyticus* BW62, *Stenotrophomonas maltophilia* D457R and *Pseudomonas aeruginosa* PAO1, and the Gram-positive *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 29213 and *S. aureus* BW61. For comparison, the activity of the standard drugs fosfomycin, ceftriaxone, ciprofloxacin and chloramphenicol was evaluated under the same conditions. A preliminary screening was carried out using the disc diffusion method, according to the Clinical Laboratory Standards Institute (CLSI) Guidelines,[26,27] and the results are presented in Table 1. All the hydroxyphosphonates tested, except compound **2**, exhibited some antimicrobial activity. The measurement of the zones of growth inhibition for 10 μg of each compound showed that the most active compounds were hydroxyphosphonates **8**, **10**, **11** and **12**, which inhibited the growth of Gram-negative as well as Gram-positive bacteria. These compounds were selected for further screening. They have in common the diphenyl ester-protecting group, which suggests that the presence of this group may be important for activity. Compound **9**, which has the same protecting group but was less active, was also selected, as well as **5**, a cyclic ester which was active against *E. coli* BW55, an ESBL-producing *E. coli*.

The minimum concentrations of each compound required to inhibit growth (MIC values) and the minimum bactericidal concentrations of each compound, that is the concentration required to kill each pathogen (MBC values) were then determined for the chosen compounds as well as for a set of standard drugs: fosfomycin, ceftriaxone, ciprofloxacin and chloramphenicol. They represent the epoxyphosphonate, the cephalosporin, and the fluoroquinolone classes of drugs, and chloramphenicol stands as a broad spectrum bacterial protein synthesis inhibitor. The strain *S. Aureus* ATCC 29213 was used for quality control. The results are presented in Tables 2–4.

MIC and MBC values were determined according to CLSI specifications.[26,27] The most active compound was **11**, with MIC values

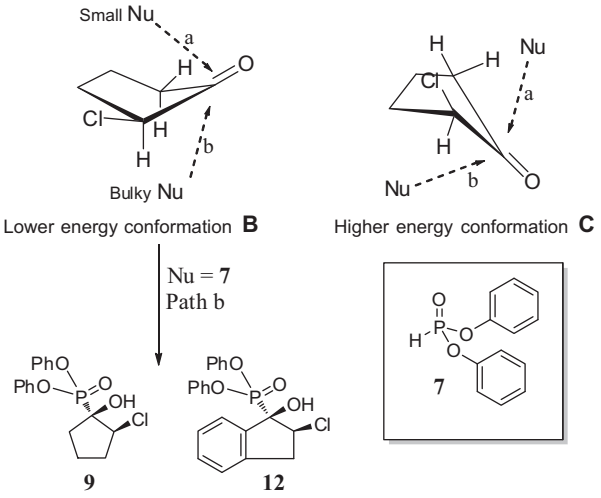


Figure 2. The lowest energy conformations of **9**.

Table 1
Zone of growth inhibition (mm) of the hydroxyalkylphosphonates against different Gram-negative and Gram-positive clinical and other isolates

Strain	Compound[a]									
	2	**3**	**4**	**5**	**6**	**8**	**9**	**10**	**11**	**12**
Gram-negative										
E. coli BW54	–	–	–	–	–	8	–	9	9	–
E. coli BW55	–	–	–	7	–	7	–	10	10	8
A. haemolyticus BW62	–	–	–	–	–	8	–	8	7	–
S. maltophilia D457R	–	6	–	–	7	–	–	7	11	–
P. aeruginosa PAO1	–	6	6	–	–	7	–	7	10	9
Gram-positive										
B. cereus ATCC 11778	–	–	7	–	–	12	–	7	9	–
S. aureus ATCC 29213	–	–	–	–	–	9	8	–	9	5

[a] 10 μg of each compound was used; –, no bioactivity observed.

Table 2
Minimum inhibitory concentration (μg/mL) of the hydroxyalkylphosphonates against different Gram-negative and Gram-positive clinical and reference isolates

Strain	Compound					
	5	**8**	**9**	**10**	**11**	**12**
Gram-negative						
E. coli BW54	64	128	128	64	64	128
E. coli BW55	64	64	128	64	32	128
A. haemolyticus BW62	64	64	128	64	64	128
S. maltophilia D457R	64	128	128	64	32	128
P. aeruginosa PAO1	128	64	256	64	128	128
Gram-positive						
B. cereus ATCC 11778	64	64	128	64	32	128
S. aureus ATCC 29213	64	128	256	64	32	256
S. aureus BW61	64	128	256	64	32	256

Table 3
Minimum bactericidal concentration (μg/mL) of the hydroxyalkylphosphonates against different Gram-negative and Gram-positive clinical and reference isolates

Strain	Compound					
	5	8	9	10	11	12
Gram-negative						
E. coli BW54	128	⩾256	256	64	64	256
E. coli BW55	128	256	256	64	64	256
A. haemolyticus BW62	128	⩾256	256	64	128	256
S. maltophilia D457R	256	⩾256	256	64	64	256
P. aeruginosa PAO1	128	256	256	64	128	256
Gram-positive						
B. cereus ATCC 11778	128	128	256	32	32	256
S. aureus ATCC 29213	128	⩾256	⩾256	64	64	256
S. aureus BW61	128	256	⩾256	64	64	256

Table 4
MIC (μg/mL)/MBC (μg/mL) of the class representative antimicrobial agents against different Gram-negative and Gram-positive clinical and reference isolates

Strain	Antimicrobial agent[a]			
	FOS	CFX	CIPX	CHLOR
Gram-negative				
E. coli BW54	32/32	⩽0.5/0.5	4/4	128/⩾256
E. coli BW55	32/128	32/64	2/1	8/128
A. haemolyticus BW62	128/128	4/4	4/4	128/⩾256
S. maltophilia D457R	256/256	64/128	4/256	128/⩾256
P. aeruginosa PAO1	64/64	128/128	4/4	⩾256/⩾256
Gram-positive				
B. cereus ATCC 11778	32/32	32/64	4/4	0.5/64
S. aureus ATCC 29213	8/64	4/4	4/4	8/64
S. aureus BW61	64/32	4/4	4/4	16/128

[a] FOS = fosfomycin; CFX = ceftriaxone; CIPX = ciproflaxin; CHLOR = chloramphenicol.

of 32 μg/mL (76 μM) against all Gram-positive pathogens tested, including methicillin-resistant *S. aureus* (MRSA) BW61. This very dangerous pathogen which can cause very serious infections in hospitals, is also resistant to many other antibiotics, ant it is appearing with increasing frequency in the communities outside.[28] For **11** MIC values were in the range of 32–64 μg/mL (76–152 μM) against the Gram-negative pathogens, except for *P. aeruginosa* PAO1 which was inhibited at 128 μg/mL (304 μM).

Compounds **10** and **5** were slightly less active than **11**, but showed MIC values of 64 μg/mL (165 and 189 μM, respectively), against all pathogens tested also with the exception of *P. aeruginosa*. These values are within the susceptibility breakpoint, which is usually regarded to be 64 μg/mL.[5] Hydroxyphosphonate **8** also met with this criterion for a few of the pathogens tested (Table 2). Compounds **5**, **10** and **11** have in common an aromatic substituent at the α-position.

Hydroxyphosphonate **10** displays bactericidal activity at lower concentrations against all pathogens tested (32–64 μg/mL, i.e., 83–165 μM), followed by **11** (32–128 μg/mL, i.e., 76–304 μM). In comparison with the standard drugs, **10** and **11** were either more or equally bactericidal than chloramphenicol, and in most cases better than fosfomycin. In fact, hydroxyphosphonate **11** showed better inhibitory activity than fosfomycin against several Gram-negative strains. Fosfomycin resistant *S. maltophilia* D457R has the multidrug efflux system SmeDEF over-expressed.[29] It is possible that **11** blocks the activity of the multidrug efflux system SmeDEF and/or it's activity is not impaired by SmeDEF overexpression, and if so, the use of this compound as an additive might restore the activity of other antimicrobial agents towards Gram-negative bacteria. Although at present there are no efflux inhibitors in clinical use, they are the subject of active research, either in clinical phases of development, or are being used to determine the efflux prevalence in clinical isolates. The possibility of **12** being an efflux inhibitor will be a subject of our future investigations.

In summary, a set of α-quaternary 3-chloro-2-hydroxyalkylphosphonates was prepared, some of which have not been described previously. The compounds were screened for antimicrobial activity and some were found to be moderate growth inhibitors and bactericides against a range of clinical and standard isolates of pathogenic bacteria. Compound **11** was more active than fosfomycin or chloramphenicol against several Gram-negative and also some Gram-positive isolates. Phenyl protection of the phosphoryl ester groups and aromatic substitution at the α-position was found to enhance bioactivity. To the best of our knowledge bioactivity of this class of compounds has not been reported before. Our studies in this area are continuing.

Acknowledgments

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Supplementary data

Supplementary data (Experimental protocols, full characterization data of compounds **9**, **11**, and **12** and their NMR and IR spectra as well as the description and source of the strains used in this study.) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.12.002.

References and notes

1. Walsh, C. T.; Wright, G. *Chem. Rev.* **2005**, *105*, 391.
2. Woodford, N.; Livermore, D. M. *J. Infect.* **2009**, *59*, S4.
3. Patrick, G. L. *An Introduction to Medicinal Chemistry*, 4th ed.; Oxford University Press: Oxford, 2009.
4. Falagas, M. E.; Kastoris, A. C.; Karageorgopoulos, D. E.; Rafailidis, P. I. *Int. J. Antimicrob. Agents* **2009**, *34*, 111.
5. Falagas, M. E.; Kastaris, A. C.; Kapaskelis, A. M.; Karageorgopoulos, D. E. *Lancet Infect. Dis.* **2010**, *10*, 43.
6. Falagas, M. E.; Kanellopoulou, M. D.; Karageorgopoulos, D. E.; Dimopoulos, G.; Rafailidis, P. I.; Skarmoutsou, N. D.; Papafrangas, E. A. *Eur. J. Clin. Microbiol. Infect. Dis.* **2008**, *27*, 439.
7. Falagas, M. E.; Marakid, S.; Karageorgopoulos, D. E.; Kastoris, A. C.; Mavromanolakise, E.; Samonis, G. *Int. J. Antimicrob. Agents* **2010**, *35*, 240.
8. Giamarellou, H.; Poulakou, G. *Drugs* **1879**, *2009*, 69.
9. (a) Faísca Phillips, A. M.; Barros, M. T. *Org. Biomol. Chem.* **2012**, *10*, 404; (b) Barros, M. T.; Faísca Phillips, A. M. *Tetrahedron: Asymmetry* **2010**, *21*, 2746; (c) Barros, M. T.; Faísca Phillips, A. M. *Eur. J. Org. Chem.* **2008**, 2525.
10. Metcalf, W. W.; van der Donk, W. A. *Annu. Rev. Biochem.* **2009**, *78*, 65.

11. Takeuchi, M.; Nakajima, M.; Ogita, T.; Inukai, M.; Kodama, K.; Furuya, K. *J. Antibiot.* **1989**, *42*, 198.
12. Rigsby, R. E.; Fillgrove, K. L.; Beihoffer, L. A.; Armstrong, R. N. *Methods Enzymol.* **2005**, *401*, 367.
13. Barros, M. T.; Faísca Phillips, A. M. *Eur. J. Org. Chem.*, IYC Special Issue: Women in, Chemistry **2011**, 4028.
14. For other organocatalyzed reactions leading to the synthesis of α-hydroxyphosphonates, but not to β-chloro-α-hydroxyphosphonates see: (a) Mandal, T.; Samanta, S.; Zhao, C.-G. *Org. Letts* **2007**, *9*, 943; (b) Kolodyazhnaya, A. O.; Kukhar, V. P.; Kolodyazhnyi, O. I. *Russ. J. Gen. Chem.* **2008**, *78*, 2043; (c) Liu, J.; Yang, Z.; Wang, Z.; Wang, F.; Chen, X.; Liu, X.; Feng, X.; Su, Z.; Hu, C. *J. Am. Chem. Soc.* **2008**, *130*, 5654; (d) Chen, X.; Wang, J.; Zhu, Y.; Shang, D.; Gao, B.; Liu, X.; Feng, X.; Su, Z.; Hu, C. A. *Chem. Eur. J.* **2008**, *14*, 10896; (e) Wang, F.; Liu, X.; Cui, X.; Xiong, Y.; Zhou, Y.; Feng, X. *Chem. Eur. J.* **2009**, *15*, 589; (f) Jiang, J.; Chen, X.; Wang, J.; Hui, Y.; Liu, X.; Lin, L.; Feng, X. *Org. Biomol. Chem.* **2009**, *7*, 4355; (g) Gondi, V. B.; Hagihara, K.; Rawal, V. H. *Angew. Chem. Int. Ed.* **2009**, *48*, 776; (h) Uraguchi, D.; Ito, T.; Nakamura, S.; Ooi, T. *Chem. Sci.* **2010**, *1*, 488; (i) Perera, S.; Naganaboina, V. K.; Wang, L.; Zhang, B.; Guo, Q.; Rout, L.; Zhao, C.-G. *Adv. Synth. Catal.* **2011**, *353*, 1729; (j) Lee, H. J.; Kim, J. H.; Kim, D. Y. *Bull. Korean Chem. Soc.* **2011**, *32*, 785.
15. For a recent review on organocatalysis for the synthesis of phosphonates see: Faísca Phillips, A. M. *Mini-Rev. Org. Chem.* **2013**, accepted for publication.
16. Krise, J. P.; Stella, V. J. *Adv. Drug Deliv. Rev.* **1996**, *19*, 287.
17. Serafinowska, H. T.; Ashton, R. J.; Bailey, S.; Harnden, M. R.; Jackson, S. M.; Sutton, D. *J. Med. Chem.* **1995**, *38*, 1372.
18. Starrett, J. E., Jr; Tortolani, D. R.; Russell, J. R.; Hitchcock, M. J. M.; Whiterock, V.; Martin, J. C.; Mansuri, M. M. *J. Med. Chem.* **1994**, *37*, 1857.
19. De Lombaert, S.; Erion, M. D.; Tan, J.; Blanchard, L.; El-Chehabi, L.; Ghai, R. D.; Sakane, Y.; Berry, C.; Trapani, A. J. *J. Med. Chem.* **1994**, *37*, 498.
20. Hecker, S. J.; Erion, M. D. *J. Med. Chem.* **2008**, *51*, 2328.
21. McConnell, R. L.; Coover, H. W., Jr *J. Org. Chem.* **1959**, *24*, 630.
22. Mei, Y.; Bentley, P. A.; Du, J. *Tetrahedron Lett.* **2008**, *49*, 3802.
23. *Representative procedure for diphenyl [2-chloro-1-hydroxy-1-(4-chlorophenyl)ethyl]phosphonate* (***11***): To a solution of the phosphite (1.0 mmol) in dry toluene (3 mL) was added quinine (0.10–0.30 mmol) and the mixture was stirred under argon until the catalyst had dissolved. The base (proton sponge or pyridine) (1.0 mmol) was added and the mixture was stirred until clear. The solution was cooled in an ice bath and the α-chloroketone (1.5 mmol) was added. When the starting material was consumed, HCl (1 M) was added and the product was extracted with chloroform. The combined organic extracts were washed with water and the solution was filtered through anhydrous sodium sulphate. The solvent was removed on a rotary evaporator to give the crude product as a solid, purified by chromatography (silica gel, 3:1 hex/EtOAc) and recystallized from EtOAc/hexane. Compound **11** was obtained as white crystals, mp 138–139 °C. ^{1}H NMR ($CDCl_3$): δ 4.28 (t, 1 H, J_{HH} = J_{HP} 11.7 Hz), 4.41 (dd, 1 H, J_{HP} 4.8 Hz, J_{HH} 11.7 Hz), 6.86 (d, 2 H, J_{HH} 8.5 Hz), 7.05–7.25 (m, 6 H), 7.29 (t, 2 H, J_{HH} 7.7 Hz), 7.37 (d, 2 H, J_{HH} 8.4 Hz), 7.64 (dd, 2 H, J_{HH} 2.4, 8.8 Hz) ppm; ^{13}C NMR ($CDCl_3$): δ 49.89 (d, J_{CP} 14.9 Hz, CH_2), 75.33 (s, C_q), 120.2 (d, J_{CP} 4.3 Hz, CH), 120.5 (d, J_{CP} 4.1 Hz, CH), 125.4 (s, CH), 125.6 (s, CH), 128.0 (d, J_{CP} 4.4 Hz, CH), 128.7 (d, J_{CP} 2.7 Hz, CH), 129.6 (d, J_{CP} 0.7 Hz, CH), 129.8 (d, J_{CP} 0.8 Hz, CH), 134.5 (s), 134.8 (d, J_{CP} 3.6 Hz, C_q), 150.1 (d, J_{CP} 4.9 Hz, C_q), 150.2 (d, J_{CP} 4.9 Hz, C_q) ppm; ^{31}P NMR ($CDCl_3$): δ 11.37 ppm; IR (KBr): ν 3314, 3056, 2948, 1654, 1589, 1489, 1457, 1429, 1402, 1363, 1291, 1238, 1207, 1184, 1161, 1124, 1097, 1073, 1023, 1016, 1007, 991, 973, 948, 913, 825, 777, 761, 722, 686, 619 cm^{-1}. Anal. Calcd for $C_{20}H_{17}Cl_2O_4P$: C 56.76, H 4.05; found: C 57.18, H 4.08.
24. Abbaraju, S.; Bhanushali, M.; Zhao, C.-G. *Tetrahedron* **2011**, *67*, 7479.
25. Smith, M. B. *Organic Synthesis*, 3rd ed.; Academic Press: New York, 2011.
26. Clinical and Laboratory Standards Institute (CLSI), Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Approved Standards, CLSI Document M7–A7, 7th ed., 2006.
27. Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Susceptibility Testing, 17th Informational Supplement, CLSI Document M100–S17, 2007.
28. Garwood, J. *Lab Times* **2011**, *7*, 18.
29. Alonso, A.; Morales, G.; Escalante, R.; Campanario, E.; Sastre, L.; Martinez, J. L. *J. Antimicrob. Chemother.* **2004**, *53*, 432.