Synthesis of Oxazinyl Analogues of Fosmidomycin Using RCM Methodology

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Abstract: Fosmidomycin is a promising antimalarial compound with a novel mode of action, the inhibition of 1-deoxy-D-xylulose 5-phosphate reductoisomerase, a key enzyme in the biosynthesis of isoprenoids through the nonmevalonate pathway. This paper describes the synthesis of a series of analogues in which the hydroxamate moiety is incorporated in a ring structure, leaving the complexation with the enzyme up to the oxygen lone pairs instead of the free hydroxyl group. The antimalarial activities of the different analogues are currently under investigation.

Key words: fosmidomycin, oxazinyl analogues, 1-deoxy-D-xylulose 5-phosphate reductoisomerase, ring-closing metathesis, heterogeneous ruthenium catalysis

According to the WHO, between 300 million and 500 million clinical cases of malaria occur every year and the disease is estimated to kill one to three million people annually. Almost all of the fatal cases of malaria in humans are due to the parasite *Plasmodium falciparum*, the causative agent of *Malaria tropica*. The disease is still gaining ground as the parasite's resistance to drugs and the parasite-carrying mosquito's resistance to insecticides increase.[2] This highlights the urgent requirement for safe and effective antimalarials with new modes of action. Fosmidomycin and FR-900098 (Figure 1) were proven to show activity against the *P. falciparum* parasite, both in vitro and in clinical studies.[3,4] It was only in 1998, after extensive research, that the mechanism of action of this new class of antimalarial drugs was revealed. The compounds are selective inhibitors of 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), a key enzyme in the biosynthesis of isoprenoids through the mevalonate-independent 1-deoxy-D-xylulose 5-phosphate/2-*C*-methyl-D-erythritol 4-phosphate (DOXP/MEP) pathway.[5] This pathway is present in algae, higher plants, bacteria and the malaria parasite *P. falciparum*, but as it is absent in humans and mammalians, the involved enzymes are promising targets for new drugs.

Clinical trials have shown that fosmidomycin is efficient in the treatment of acute uncomplicated *Malaria tropica*. However, the oral bioavailability of both fosmidomycin and FR-900098 is only moderate, with a resorption rate of approximately 30%.[6] In addition, the short plasma half-life of fosmidomycin requires a repeated administration of relatively high dosages.[4,7] Various modifications were carried out to synthesize more bioavailable and more active derivatives. The nature of the investigations was also influenced by the elucidation of the crystallographic structure of DXR.[8] Important structural elements for antimalarial activity are the phosphonic acid moiety, the hydroxamic acid group and the three-carbon spacer between both functionalities. A lot of work concerned the modification of the phosphonate moiety, into ester prodrugs,[9–11] carboxylic acids[12] or phosphates.[13] The hydroxamate group has also been frequently altered into benzoxazolone, benzoxazolethione, oxalopyridinone, hydroxamic acid or other functionalities.[14–16] Variations of the propyl spacer are rather scarce. Different conformationally restricted derivatives were already investigated in order to gain new insights regarding the structure–activity relationships.[17–19] Furthermore, the synthesis and evaluation of some α-aryl-substituted analogues as superior inhibitors compared to fosmidomycin, revealed the probability of an extra cavity in the DXR enzyme at this α-position.[19,20] Another approach that has not yet been investigated is the incorporation of the hydroxamate moiety in a closed ring system. The oxygen atom is hereby no longer present as a free hydroxyl group, but still possesses two lone pairs to complex with the enzyme. Analysis of compounds of this kind could give additional information on the necessity of the free OH group for activity against malaria parasites. This paper deals with the synthesis of such analogues, employing the ring-closing metathesis (RCM) reaction as a key step in the reaction pathway. The use of RCM to synthesize heterocycles containing the 1,2-oxaza group has been given little attention. In the literature, only few reports have been published concerning the synthesis of 1,2-oxazines and 1,2-oxazepines by RCM.[21] And even fewer references mentioned the preparation of 3-oxo-1,2-oxaza compounds, which makes these products interesting research topics.

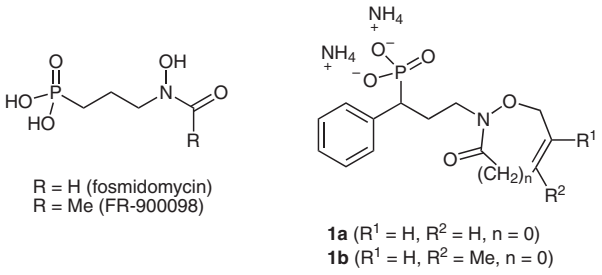

Figure 1 Structures of fosmidomycin, FR-900098 and target compounds **1a**,**b**

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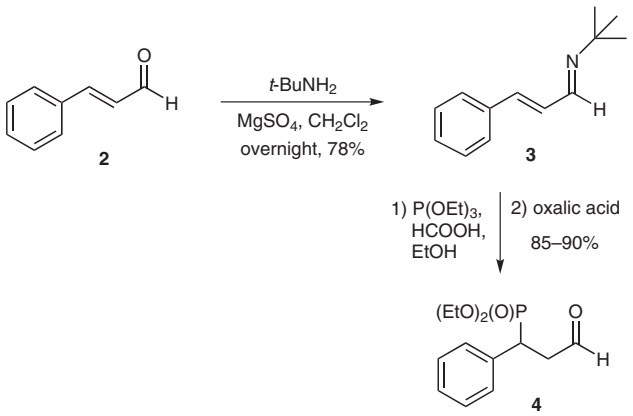
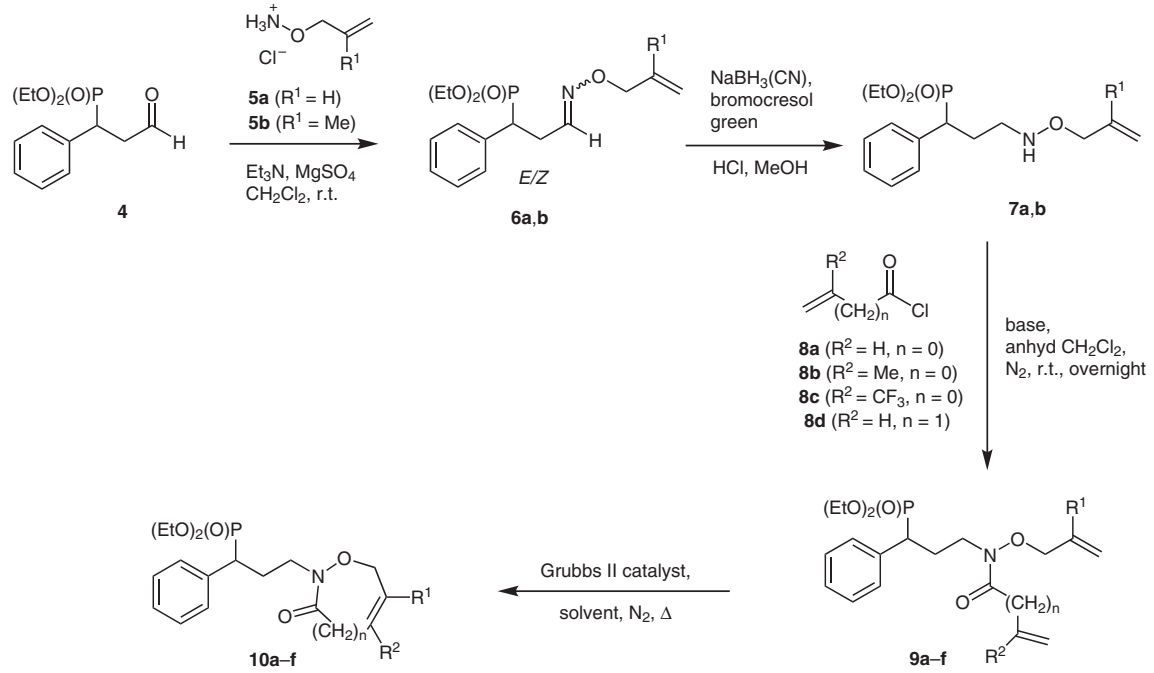
The preparation of the RCM precursors **9a–f** started with the formation of aldimine **3** from cinnamaldehyde (**2**), followed by a 1,4-addition of triethyl phosphite and subsequent hydrolysis of the imine function. These reactions were already optimized by other members of our research group,[22] based on a procedure reported by Teulade and Savignac.[23] As such, phosphonate **4** was prepared in good yield (Scheme 1).



Scheme 1

The results of the following reactions are summarized in Table 1. Reaction of the resulting aldehyde **4** with two different O-substituted hydroxylamine hydrochlorides gave oximes **6a** and **6b** in 93% and 74% yields, respectively (Scheme 2). Unfortunately, *O*-(2-methylallyl)hydroxylamine hydrochloride (**5b**) was not commercially available and had to be prepared starting from 3-bromo-2-methylpropene and hydroxylamine hydrochloride according to Albrecht's procedure.[24] Subsequent reduction of the oxime was carried out with sodium cyanoborohydride in methanol acidified with HCl, in the presence of a trace amount of the pH indicator bromocresol green as described by Eguchi.[25] Other reducing agents usually result in the cleavage of the N–O bond as a side-effect. Since the hydroxylamine moiety is an important feature for antimalarial activity, this cleavage must be avoided. With this method, reduction could be performed without side-effects and in good yields. To introduce the carbonyl function and a second double bond in the structure, a nucleophilic addition–substitution reaction of the hydroxylamine nitrogen to an acyl chloride was performed. In two cases, the acyl chloride (**8c** and **8d**) was not commercially available as such and had to be prepared in situ from the corresponding acid, using thionyl chloride (Table 1, entries 9 and 10). Purification of the resulting compound **9** was necessary for two analogues (**9d**,**e**) and could be performed by column chromatography. For the other derivatives, the crude reaction products were used in the following reaction without further purification. This reaction consisted of the closure of the N,O-substituted hydroxylamines **9a–f** and was carried out via a ring-closing metathesis (RCM) reaction.[26] Grubbs' second-generation catalyst (0.05 equiv) was added to a mixture of the hydroxylamine **9a–f** in refluxing solvent. In the case of compound **9e** (Table 1, entry 15), the presence of the fluorine atoms required a higher temperature to complete the reaction. Therefore, benzene (bp 80 °C) was used as solvent instead of dichloromethane (bp 40 °C), that was used for all the other derivatives. In all six cases the ring closure proceeded almost quantitatively, but the problem inherent to most metathesis reactions employing ruthenium carbenes was the effective removal of ruthenium impurities after completion of the reaction.



Scheme 2

Table 1 Summary of Reactions in Scheme 2 and Scheme 3

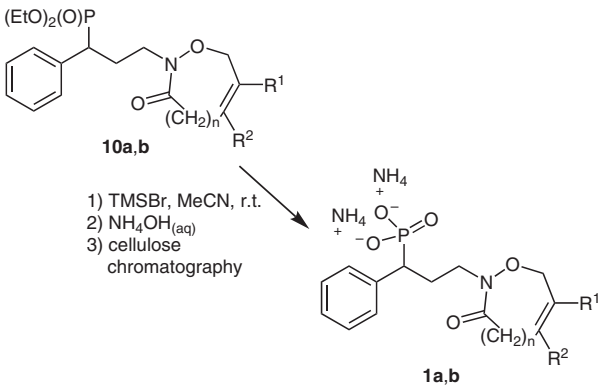
Entry	Starting materials	Product	R^1	R^2	n	Base	Solvent	Reaction time	Yield (%)
1	**4**	**6a**	H	–	–	Et_3N	CH_2Cl_2	15 h	93
2	**4**	**6b**	Me	–	–	Et_3N	CH_2Cl_2	15 h	74
3	**6a**	**7a**	H	–	–	–	MeOH	1 h	98
4	**6b**	**7b**	Me	–	–	–	MeOH	1 h	92
5	**7a**, **8a**	**9a**	H	H	0	Et_3N	anhyd CH_2Cl_2	24 h	90
6	**7a**, **8b**	**9b**	H	Me	0	Et_3N	anhyd CH_2Cl_2	24 h	99
7	**7b**, **8a**	**9c**	Me	H	0	Et_3N	anhyd CH_2Cl_2	24 h	85
8	**7b**, **8b**	**9d**	Me	Me	0	Et_3N	anhyd CH_2Cl_2	24 h	69[a]
9	**7a**, **8c**	**9e**	H	CF_3	0	pyridine	anhyd CH_2Cl_2	24 h	62[a]
10	**7a**, **8d**	**9f**	H	H	1	pyridine	anhyd CH_2Cl_2	24 h	83
11	**9a**	**10a**	H	H	0	–	anhyd CH_2Cl_2	6 h	71[a]
12	**9b**	**10b**	H	Me	0	–	anhyd CH_2Cl_2	6 h	75[a]
13	**9c**	**10c**	Me	H	0	–	anhyd CH_2Cl_2	6 h	68[a]
14	**9d**	**10d**	Me	Me	0	–	anhyd CH_2Cl_2	6 h	56[a]
15	**9e**	**10e**	H	CF_3	0	–	C_6H_6	5 h	97
16	**9f**	**10f**	H	H	1	–	anhyd CH_2Cl_2	8 h	43[a]
17	**10a**	**1a**	H	H	0	–	MeCN	24 h	62[a]
18	**10b**	**1b**	H	Me	0	–	MeCN	24 h	65[a]

[a] Yield obtained after purification by column chromatography.

A variety of methods[27] have been used to scavenge ruthenium species, including the use of: water-soluble phosphines,[28a,b] oxidizing agents (such as triphenylphosphine oxide,[28c] DMSO,[28c] or lead tetraacetate[28d]), mesoporous silicates,[28e] polar isocyanides,[28f] and activated carbon.[28g] A few of these methods (ref. 28a, 28d and 28g) were applied on the analogues **10a–f** but in these cases, normal column chromatography gave the best results despite the inevitable losses of product.

Although some reports have been published on ester prodrugs of fosmidomycin analogues (vide infra), the biologically active compounds are the phosphonic acids. A commonly used method for the deprotection of a phosphonate group into a phosphonic acid function is the reaction with TMSBr in dichloromethane under a nitrogen atmosphere, followed by addition of water. This procedure was used for analogues **10a**, **10b** and **10f** with five equivalents of TMSBr, but it never led to the envisaged result. Finally, the phosphonate ester groups of **10a**,**b** could be cleaved according to the methodology reported by Devreux et al.[20] Addition of ten equivalents of TMSBr to a solution of phosphonate **10a**,**b** in anhydrous acetonitrile at ambient temperature was followed by the addition of a NH_4OH solution and lyophilization. The residual solids were purified by column chromatography on Whatman CF11 cellulose with a mixture of MeCN and NH_4OH (1 N) as solvent. The useful fractions were again lyophilized. The resulting bisammonium salts **1a**,**b**[29] of the ring-closed phosphonates (Scheme 3) proved to be hygroscopic solids.

Using the described procedures, six analogues of the phosphonate prodrugs have been synthesized and two of these analogues were deprotected to form their bisammonium salts. These two final compounds and two of the



Scheme 3

phosphonates were tested for inhibition of recombinant *Escherichia coli* DXR. The results will be disclosed in due course.

In conclusion, a synthetic procedure for the preparation of oxazinyl analogues of fosmidomycin was developed starting from cinnamaldehyde and the key reaction in this method consisted of an RCM reaction. Although the hydroxyl group is incorporated in the ring structure, the oxygen lone pairs still comprise the potential to perform the complexation with the enzyme.

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References and Notes

(1) Aspirant of the Fund for Scientific Research, Flanders (FWO, Vlaanderen), Belgium.
(2) Thayer, A. *Chem. Eng. News* **2005**, *83 (October 24)*, 69.
(3) Missinou, M.; Borrmann, S.; Schindler, A.; Issifou, S.; Adegnika, A.; Matsiegui, P.; Binder, R.; Lell, B.; Wiesner, J.; Baranek, T.; Jomaa, H.; Kremsner, P. *Lancet* **2002**, *360*, 1941.
(4) Lell, B.; Ruangweerayut, R.; Wiesner, J.; Missinou, M.; Schindler, A.; Baranek, T.; Hintz, M.; Hutchinson, D.; Jomaa, H.; Kremsner, P. *Antimicrob. Agents Chemother.* **2003**, *47*, 735.
(5) Wiesner, J.; Ortmann, R.; Jomaa, H.; Schlitzer, M. *Angew. Chem. Int. Ed.* **2003**, *42*, 5274.
(6) Tsuchiya, T.; Ishibashi, K.; Terakawa, M.; Nishiyama, M.; Itoh, N.; Noguchi, H. *Eur. J. Drug. Metab. Pharmacokinet.* **1982**, *7*, 59.
(7) (a) Kuemmerle, H. P.; Murakawa, T.; De Santis, F. *Chemioterapia* **1987**, *6*, 113. (b) Kuemmerle, H. P.; Murakawa, T.; Sakamoto, H.; Sato, N.; Konishi, T.; De Santis, F. *Int. J. Clin. Pharmacol. Ther. Toxicol.* **1985**, *23*, 521.
(8) Reuter, K.; Sanderbrand, S.; Jomaa, H.; Wiesner, J.; Steinbrecher, I.; Beck, E.; Hintz, M.; Klebe, G.; Stubbs, M. T. *J. Biol. Chem.* **2002**, *277*, 5378.
(9) Reichenberg, A.; Wiesner, J.; Weidemeyer, C.; Dreiseidler, E.; Sanderbrand, S.; Altincicek, B.; Beck, E.; Schlitzer, M.; Jomaa, H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 833.
(10) (a) Ortmann, R.; Wiesner, J.; Reichenberg, A.; Henschker, D.; Beck, E.; Jomaa, H.; Schlitzer, M. *Arch. Pharm. Chem. Life Sci.* **2005**, *338*, 305. (b) Ortmann, R.; Wiesner, J.; Reichenberg, A.; Henschker, D.; Beck, E.; Jomaa, H.; Schlitzer, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2163.
(11) Kurz, T.; Schlüter, K.; Kaula, U.; Bergmann, B.; Walter, R. D.; Geffken, D. *Bioorg. Med. Chem.* **2006**, *14*, 5121.
(12) Kurz, T.; Geffken, D.; Wackendorff, C. *Z. Naturforsch., B* **2003**, *58b*, 457.
(13) Woo, Y. H.; Fernandes, R. P. M.; Proteau, P. J. *Bioorg. Med. Chem.* **2006**, *14*, 2375.
(14) Courtois, M.; Mincheva, Z.; Andreu, F.; Rideau, M.; Viaud-Massuard, M. *J. Enz. Inh. Med. Chem.* **2004**, *19*, 559.
(15) Mincheva, Z.; Courtois, M.; Andreu, F.; Rideau, M.; Viaud-Massuard, M. C. *Phytochemistry* **2005**, *66*, 1797.
(16) Kuntz, L.; Tritsch, D.; Grosdemange-Billiard, C.; Hemmerlin, A.; Willem, A.; Bacht, T. J.; Rohmer, M. *Biochem. J.* **2005**, *386*, 127.
(17) Devreux, V.; Wiesner, J.; Goeman, J. L.; Van der Eycken, J.; Jomaa, H.; Van Calenbergh, S. *J. Med. Chem.* **2006**, *49*, 2656.
(18) Kurz, T.; Schlüter, K.; Pein, M.; Behrendt, C.; Bergmann, B.; Walter, R. D. *Arch. Pharm. Chem. Life Sci.* **2007**, *340*, 339.
(19) Haemers, T.; Wiesner, J.; Busson, R.; Jomaa, H.; Van Calenbergh, S. *Eur. J. Org. Chem.* **2006**, 3856.
(20) (a) Haemers, T.; Wiesner, J.; Van Poecke, S.; Goeman, J.; Henschker, D.; Beck, E.; Jomaa, H.; Van Calenbergh, S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1888. (b) Devreux, V.; Wiesner, J.; Jomaa, H.; Rozenski, J.; Van der Eycken, J.; Van Calenbergh, S. *J. Org. Chem.* **2007**, *72*, 3783.
(21) (a) Yang, Y.-K.; Tae, J. *Synlett* **2003**, 1043. (b) Miyabe, H.; Yoshida, K.; Matsumura, A.; Yamauchi, M.; Takemoto, Y. *Synlett* **2003**, 567. (c) Koide, K.; Finkelstein, J. M.; Ball, Z.; Verdine, G. L. *J. Am. Chem. Soc.* **2001**, *123*, 398. (d) Le Flohic, A.; Meyer, C.; Cossy, J.; Desmurs, J. R. *Tetrahedron Lett.* **2003**, *44*, 8577. (e) Shustov, G. V.; Chandler, M. K.; Wolfe, S. *Can. J. Chem.* **2005**, *83*, 93.
(22) Van Meenen, E.; Moonen, K.; Verwee, A.; Stevens, C. V. *J. Org. Chem.* **2006**, *71*, 7903.
(23) Teulade, M.; Savignac, P. *Synthesis* **1987**, 1037.
(24) Albrecht, S.; Defoin, A.; Tarnus, C. *Synthesis* **2006**, 1635.
(25) Eguchi, S.; Furukawa, Y.; Suzuki, T.; Kondo, K.; Sasaki, T.; Honda, M.; Katayama, C.; Tanaka, J. *J. Org. Chem.* **1985**, *50*, 1895.
(26) **Preparation of Diethyl 1-Phenyl-3-(4-methyl-3-oxo-3,6-dihydro-2*H*-1,2-oxazin-2-yl)propylphosphonate (10b)**: A solution of **9b** (1 g, 2.53 mmol) in anhyd CH_2Cl_2 (30 mL) was brought under a N_2 atmosphere. The mixture was stirred at reflux temperature and Grubbs' second-generation catalyst (0.1073 g, 0.126 mmol) was added. After 6 h of refluxing, the solvents were evaporated under vacuum to yield the product **10b** as a crude oil. Purification by column chromatography was necessary to remove the traces of the ruthenium catalyst, yielding **10b** (0.70 g, 1.90 mmol, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.08 (t, *J* = 7.0 Hz, 3 H, OEt), 1.29 (t, *J* = 7.0 Hz, 3 H, OEt), 1.88 (q, *J* = 1.7 Hz, 3 H, CMe), 2.22–2.35 (m, 1 H, CH_AH_BCHP), 2.40–2.54 (m, 1 H, CH_AH_BCHP), 3.11 (ddd, *J* = 3.1, 11.2, 23.1 Hz, 1 H, CHP), 3.46 (ddd, *J* = 6.7, 6.8, 14.0 Hz, 1 H, CH_AH_BN), 3.59 (ddd, *J* = 7.2, 7.2, 14.0 Hz, 1 H, CH_AH_BN), 3.66–4.11 (m, 4 H, 2 × OEt), 4.35 (ddq, *J* = 1.7, 3.5, 15.4 Hz, 1 H, $OCH_AH_BCH{=}C$), 4.44 (ddq, *J* = 1.7, 3.5, 15.4 Hz, 1 H, $OCH_AH_BCH{=}C$), 6.35 (tq, *J* = 1.7, 3.5 Hz, 1 H, $OCH_2CH{=}C$), 7.23–7.37 (m, 5 H, CH_{arom}). ^{13}C NMR (75 MHz, $CDCl_3$, ref. $CDCl_3$): δ = 15.70 (CMe), 16.27, 16.35, 16.45, 16.54 (2 × OEt), 27.78 (J_{C-P} = 2.3 Hz, CH_2CHP), 42.33 (J_{C-P} = 138.5 Hz, CHP), 45.14 (J_{C-P} = 18.5 Hz, CH_2N), 61.97 (J_{C-P} = 6.9 Hz, OEt), 62.81 (J_{C-P} = 6.9 Hz, OEt), 67.54 ($OCH_2CH{=}C$), 77.13 ($CDCl_3$), 127.44 (J_{C-P} = 2.3 Hz, CH_{arom}), 128.67 (J_{C-P} = 2.3 Hz, 2 × CH_{arom}), 129.40 (J_{C-P} = 6.9 Hz, 2 × CH_{arom}), 129.67 (CMe), 132.92 ($OCH_2CH{=}C$), 135.48 (J_{C-P} = 6.9 Hz, $C_{q,arom}$), 166.46 (CO). ^{31}P NMR (109 MHz, $CDCl_3$): δ = 28.92. IR (NaCl): 1674, 1635 (C=O, C=C), 1242 (P=O), 1058, 1028 (PO) cm^{-1}. MS (ESI, +ve mode, %): *m/z* = 368.3 (100) [M + H$^+$]. Chromatography (PE–EtOAc, 1:4): R_f = 0.18.
(27) For a summary of existing methods for removal of Ru species, see: Conrad, J. C.; Fogg, D. E. *Curr. Org. Chem.* **2006**, *10*, 185.
(28) (a) Maynard, H. D.; Grubbs, R. H. *Tetrahedron Lett.* **1999**, *40*, 4137. (b) Grubbs, R. H. *Org. React. (N.Y.)* **2004**, *22*, 123. (c) Ahn, Y. M.; Yang, K.; Georg, G. I. *Org. Lett.* **2001**, *3*, 1411. (d) Paquette, L. A.; Schloss, J. D.; Efremov, I.; Fabris, F.; Gallou, F.; Mendez-Andino, J.; Yang, J. *Org. Lett.* **2000**, *2*, 1259. (e) McEleney, K.; Allen, D. P.;

Holliday, A. E.; Crudden, C. M. *Org. Lett.* **2006**, *8*, 2663.
(f) Galan, B. R.; Kalbarczyk, K. P.; Szczepankiewicz, S.; Keister, J. B.; Diver, S. T. *Org. Lett.* **2007**, *9*, 1203.
(g) Cho, J. H.; Kim, B. M. *Org. Lett.* **2003**, *5*, 531.

(29) **Preparation of Diammonium 1-Phenyl-3-(4-methyl-3-oxo-3,6-dihydro-2*H*-1,2-oxazin-2-yl)propylphosphonate (1b)**: To a solution of **10b** (0.50 g, 1.36 mmol) in anhyd MeCN at r.t. was added dropwise TMSBr (13.6 mmol) and this mixture was stirred at r.t. for 24 h. The solvents and traces of TMSBr were removed under reduced pressure and under high vacuum. The residual oil was dissolved in H_2O and the pH was adjusted with a 5% NH_4OH solution to pH 8–9. Lyophilization of the solution resulted in a solid and purification was performed by column chromatography on Whatman CF11 cellulose. The solvent used was MeCN–aq 1 M NH_4OH (5:1) and the fractions were analyzed on cellulose TLC with the use of UV light to visualize the spots after dipping the plates in a pinacryptol yellow solution (0.1% in H_2O) and drying with hot air. The desired fractions were again lyophilized, yielding **1b** as a pale brown hygroscopic solid (0.31 g, 0.88 mmol, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.72 (d, *J* = 1.7 Hz, 3 H, CMe), 2.14–2.42 (m, 2 H, CH_2CHP), 2.93 (ddd, *J* = 3.0, 11.8, 22.0 Hz, 1 H, CHP), 3.50 (t, *J* = 6.5 Hz, 2 H, CH_2N), 4.31 (ddd, *J* = 1.9, 3.6, 15.7 Hz, 1 H, OCH_AH_BCH=C), 4.44 (ddq, *J* = 1.9, 3.4, 15.7 Hz, 1 H, OCH_AH_BCH=C), 4.79 (D_2O), 6.45 (tq, *J* = 1.7, 1.7 Hz, 1 H, OCH_2CH=C), 7.19–7.35 (m, 5 H, 5 × CH_{arom}). ^{13}C NMR (75 MHz, $CDCl_3$, ref. MeCN): δ = 1.47 (ref. MeCN), 15.20 (CMe), 28.13 ($J_{C–P}$ = 2.3 Hz, CH_2CHP), 44.79 ($J_{C–P}$ = 129.2 Hz, CHP), 45.94 ($J_{C–P}$ = 17.3 Hz, CH_2N), 68.24 (OCH_2CH=C), 119.69 (ref. MeCN), 127.17 ($J_{C–P}$ = 2.3 Hz, CH_{arom}), 128.36 (CMe), 129.00 ($J_{C–P}$ = 2.3 Hz, 2 × CH_{arom}), 129.69 ($J_{C–P}$ = 6.9 Hz, 2 × CH_{arom}), 135.26 (OCH_2CH=C), 139.14 ($J_{C–P}$ = 6.9 Hz, $C_{q,arom}$), 166.73 (CO). ^{31}P NMR (109 MHz, $CDCl_3$): δ = 22.91. IR (NaCl): 1668 (C=O, C=C), 1033 (PO) cm^{-1}. MS (ESI, –ve mode, %): *m*/*z* = 310.3 (100) [M – $^+NH_4$ – NH_3]. Chromatography (MeCN–1 M NH_4OH, 5:1): R_f = 0.16.