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Synthesis and antibacterial activity of novel enolphosphate derivatives

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ABSTRACT

A new class of enolphosphates derivatives, the 1-alkenyldiphosphates, was designed and a rapid and efficient synthesis for these compounds was developed. These new molecules showed interesting in vitro antibacterial activities (MIC) against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative pathogens including *Pseudomonas aeruginosa* and *Escherichia coli*.

1. Introduction

The emergence of pathogenic bacteria's resistant strains is providing intense interest in the search of new antimicrobials. The efforts in this area have focused on the development of antibacterial drugs' novel classes with new modes of action.

Initial studies indicate that the inhibition of 3-deoxy-6-manno-2-octulosonic acid 8-phosphate synthase (KDO8PS) and 3-deoxy-D-arabino-2-heptulosonic acid 7-phosphate synthase (DAHP7PS) represents an attractive and promising target for the development of new anti-infectious agents devoid of side effects [1]. Both enzymes appear to proceed via a common mechanism involving the reaction of phosphoenolpyruvate (PEP) with arabinose-5-phosphate or erythrose-4-phosphate, to produce the corresponding ulosonic acids, KDO8P and DAH7P, respectively. In previous articles, we have presented biological and crystallographic investigations with several synthesized substrate or transition states analogs. The obtained results provide a better understanding of the chemical groups that must be incorporated in the design of a successful inhibitor and so antibacterial agent. The results suggest that the phosphoenolpyruvate moiety plays a crucial role in the binding of the substrates. For example, D-glucophosphoenolpyruvate, that mimics the KDO8PS's bisubstrate and close of the supposed transition state, is a competitive inhibitor with respect to PEP [1a,2]. The carboxylic moiety and the hydroxyl groups corresponding to the 4-OH and 5-OH of D-glucophosphoenolpyruvate are not essential for bonding and may be dispensable [1a]. However, these observations suggest that highly specific inhibitors of KDO8PS may be obtained by incorporating chemical groups with high affinity for the two phosphate binding pockets. That led us to consider that 1-alkenyldiphosphate derivatives, where the phosphorylated groups would be spaced exactly as they are in the active site of KDO8PS, would be compounds that fulfill the requirements. It may be possible to extend the design of KDO8PS's inhibitor into DAHP7S's inhibitor by shortening the spacer connecting the two phosphate groups by one carbon unit.

In continuation of these works and to validate our own observations, we herein report the synthesis and the biological evaluation of 1-alkenyldiphosphate derivatives as postulated future inhibitors of KDO8PS and DAH7PS and related alkyldiphosphates.

2. Results and discussion

The challenge in the synthesis of 1-alkenyldiphosphate derivatives is to construct the vinylphosphate moiety. If this last plays an important role in living cells, it is substituted with a carboxylic group [3]. The electron withdrawing substituent attached to the α-position of the vinylgroup stabilizes the structure. However, the preparation of single vinylphosphates is specific and difficult. Several methods for the chemical synthesis of enolphosphate derivatives have been reported. In most cited examples, the preparation of the enolphosphate group is based on the phosphorylation of enolates, that react at the *O*-position with phosphochloridate derivatives [4]. The method is subordinated to the preparation and the stability of the enolates and is totally inadequate for our compounds, derived from aldehyde enolates. The other common method to prepare enolphosphates involves the reaction of α-haloketones with trialkylphosphites (Perkow reaction) [5]. Its general use is limited by the competitive Arbuzov reaction and the availability of the halogenated carbonyl

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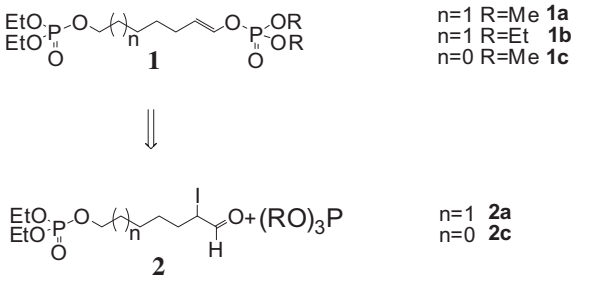


Scheme 1. Retrosynthetic scheme for the preparation of 1-alkenyldiphosphates **1.**

compounds. Thus, the study of this reaction is restricted to haloketones bearing a non-sensitive functionality. We recently reported a rapid and efficient synthesis of glucidic phosphoenolpyruvic acid derivatives based on a Perkow reaction using a suitable halogenated precursor, β-halogeno-α-ketoester or α-halogenoglycidic ester, and trimethylphosphite [2,6]. Prompted by these results, we decided to investigate the Perkow reaction between α-iodoaldehydes **2** and trialkylphosphite in the order to prepare the first 1-alkenyldiphosphates **1** (Scheme 1).

2.1. Synthesis

The synthesis of required α-iodoaldehydes **2** is shown in Schemes 2 and 4.

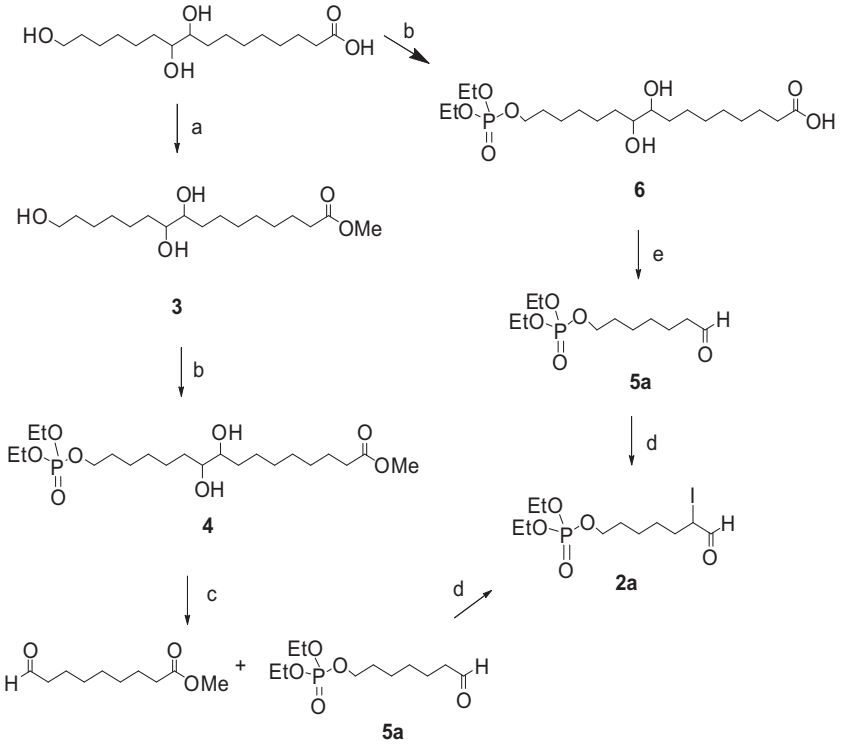
Treatment of commercially available 9,10,16-trihydroxyhexadecanoic acid with MeOH in the presence of catalytic of BF_3-Et_2O led to the formation of the corresponding methyl ester **3** [7]. Our direct route uses the diethylphosphate group as a protecting group for the synthesis. The selective phosphorylation of the primary OH-16 of triol **3** with one equivalent of diethyl chlorophosphate and 0.5 equivalent of DMAP in pyridine give **4** in high yield. The use of periodate oxidation under Malaprade conditions [8], 1.1 equivalent sodium periodate in H_2O/MeOH 1:3 at 20 °C for 18 h, afforded the target aldehyde **5a** in good yield.

This satisfactory result obtained, it was of interest to compare the behavior of 9,10,16-trihydroxyhexadecanoic acid in oxidative cleavage with that obtained for ester **3**. Selective phosphorylation was carried out directly on 9,10,16-trihydroxyhexadecanoic acid to give diethylphosphate **6** in excellent yield. **6** has been successfully treated with sodium periodate, taking care to maintain the pH to 9 with direct adding of solid sodium hydrogenocarbonate. The conditions give satisfactory yield of the corresponding aldehyde **5a**.

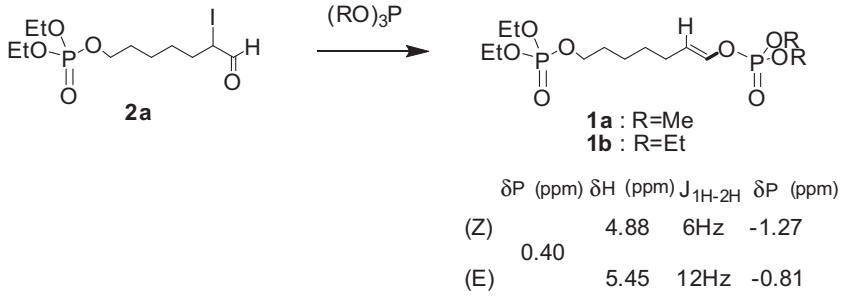
7-phosphate-2-iodohexanal **2a** was synthesized by direct iodination of **5a** using the system $HgCl_2/I_2$ in dichloromethane described by Barluenga with long chain aldehydes [9]. The mixture reaction was stirred vigorously for 2 h at room temperature. The solution was filtered and the filtrate was washed with an aqueous sodium thiosulfate solution. After standard work-up, 7-phosphate-2-iodoheptanal **2a** was cleanly obtained in an excellent yield. It should be noted the very good stability of the phosphate group in spite of acidic medium of the iodination conditions.

Reaction of 7-phosphate-2-iodoheptanal **2a**, without purification, with trialkylphosphite at room temperature and stirring for 12 h, led to the clean and quantitative formation of dimethyl and diethylenolphosphates **1a** and **1b** (Scheme 3).

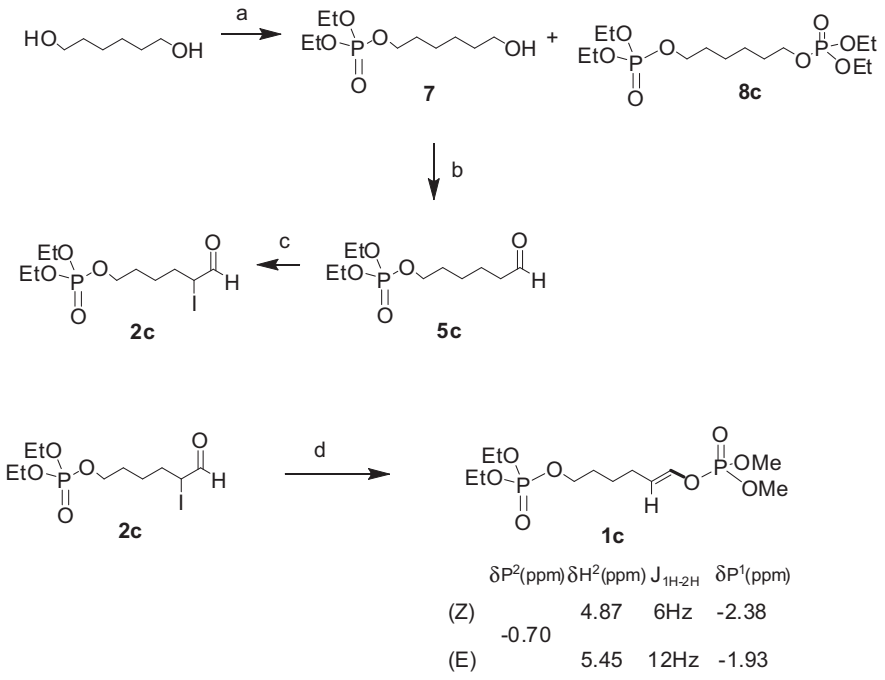
As clearly indicated, especially by the ^{31}P NMR spectra of the crude materials, the α-iodoaldehyde **2a** is completely consumed. No by-product can be detected; in contrast to the halogenoketones [5c] the reaction between 2-iodoheptanal **2a** and trialkylphosphite did not induce the formation of phosphonate compound; the competition between Perkow and Michaelis-Arbuzov reactions does not occur. After evaporation of residual trialkylphosphite, the purity and yield of 1-heptenyldiphosphates **1a** and **1b** were excellent. The NMR spectra were consistent with formation of a 1:1 mixture of *E* and *Z* geometrical isomers of enolphosphates **1a** and **1b** (Fig. 1).



Scheme 2. Synthesis of α-iodoaldehyde **2a**. Reagents and conditions: (a) BF_3-Et_2O cat., MeOH, 15 h, 35 °C, 75%; (b) 1 equiv $(EtO)_2P(O)Cl$, 0.5 equiv DMAP, pyridine, 12 h, 0 °C, 96%; (c) 1.1 equiv $NaIO_4$, H_2O/MeOH 1:3, 18 h, 20 °C, 69%; (d) 1 equiv $HgCl_2$, 1 equiv I_2, CH_2Cl_2, 2 h, 25 °C, 98%; (e) 1.1 equiv $NaIO_4$, $NaHCO_3$, H_2O/MeOH 1:3, 18 h, 20 °C, 59%.



Scheme 3. Reaction of 7-phosphate-2-iodoheptanal **2a** with trialkylphosphite. Conditions: 12 h, 20 °C, 100%.



Scheme 4. Preparation of α-iodoaldehyde **2c** and 1-hexenyldiphosphate **1c**. Reagents and conditions: (a) 1 equiv $(EtO)_2P(O)Cl$, 0.5 equiv DMAP, pyridine, 12 h, 0 °C, 70%; (b) 1.7 equiv ClCOCOCl, 2.2 equiv DMSO, CH_2Cl_2, N_2, −55 °C, 15 min, then 5 equiv Et_3N, adding to −30 °C, then 25 °C, 2 h, 60%; (c) 1 equiv $HgCl_2$, 1 equiv I_2, CH_2Cl_2, 2 h, 25 °C, 80%; (d) $(MeO)_3P$, 20 °C, 12 h, 100%.

The *E* geometry was assigned by ^1H NMR. The signals due to 1-H and 2-H showed J_{1H-2H} = 11.8 Hz. The corresponding signals in the *Z*-isomer showed J_{1H-2H} = 6.1 Hz. In addition, the 2-H signal (δ 5.45) in *E*-isomer appeared at considerably lower field compared with the corresponding 2-H signal in *Z*-enolphosphate (δ 4.88) due to deshielding by the ester phosphate group. Interestingly, the examination of the ^{31}P NMR spectra showed the presence of both *E*- and *Z*-enolphosphates (respectively δ_P −0.81 and −1.27 ppm). It could be noted the significant difference in δ_P values between saturated and unsaturated phosphate moiety (δ_P 0.40 for the saturated phosphate moiety).

This methodology was applicable for the synthesis of 1-hexenyldiphosphate **1c** with little modifications due to the commercial availability of appropriated diol (Scheme 4).

The synthesis started with a classical monophosphorylation of the 1,6-hexanediol, that gave a mixture of mono and diphosphate **7** and **8c**. Several attempts under different conditions (ratio $(EtO)_2P(O)Cl$/diol) were realized to reduce the amount of the undesired diphosphate compound **8c**; the mono and diphosphorylated products **7** and **8c** could be easily separated by flash chromatography.

6-phosphate hexanol **7** was oxidized into 6-phosphate hexanal **5c** by Swern reaction (60%). This moderate yield can be explained by partial cleavage of the phosphate group under the basic conditions used for the oxidation and the reaction treatment. A comparative attempt with Dess-Martin periodinane was realized, because it offers mild oxidation of alcohol to aldehyde. The oxidation of 1,6-hexanediol was performed in dichloromethane at room temperature, and was complete within 2 h. However the separation of the Dess-Martin iodo-compound byproduct and **5c** by basic work-up led to the same difficulty. Iodination of aldehyde **5c** with $HgCl_2/I_2$ afforded 6-phosphate-2-iodohexanal **2c** in a 100% yield. Finally, 1-hexenyldiphosphate **1c** was obtained in a quantitative yield by Perkow reaction.

In summary, we have shown that an extension of the Perkow reaction between α-iodoaldehydes and trialkylphosphites allows an easy access to vinyl phosphates. Theses derivatives can constitute an interesting alternative to the vinyl triflates in a number of transformations such cross-coupling reactions facilitated by Pd(0), Ni(0) or ring-closing metathesis [10].

In the course of our research program, directed toward the design of biologically active derivatives containing an enolphosphate unit,

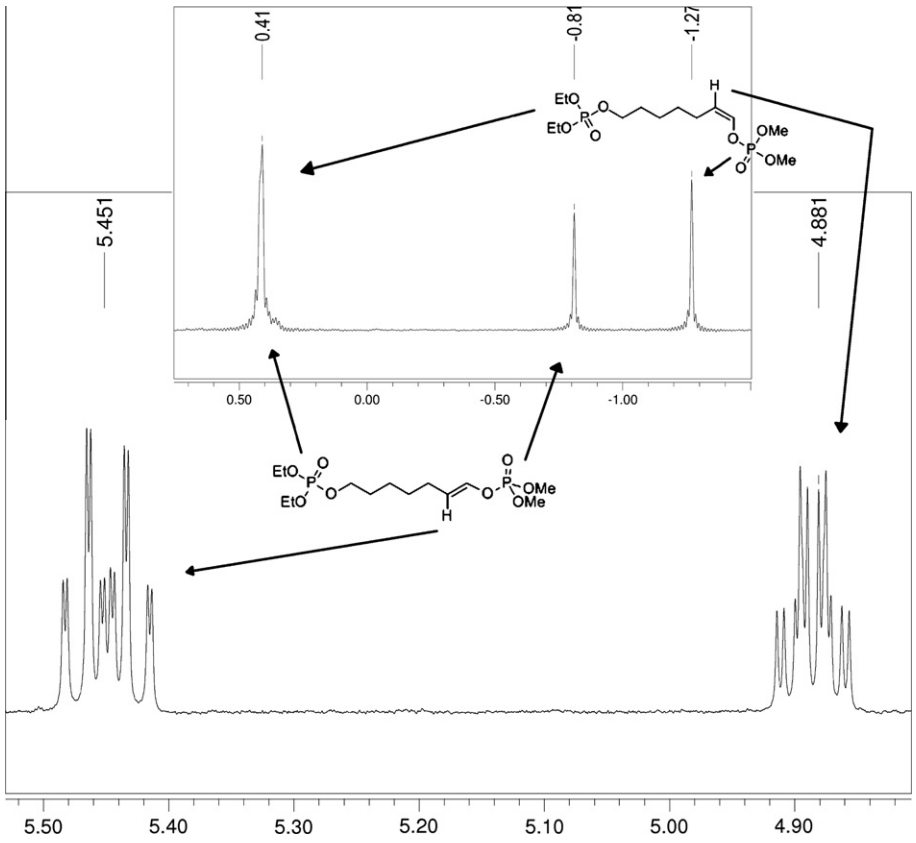


Fig. 1. Zoom in ^{1}H and ^{31}P NMR spectra of 1**a**–**b** ($CDCl_3$, 400 MHz).

we wished to demonstrate the importance of the carbon–carbon double bond. With respect to this point, we proposed the preparation of the saturated analogs of 1-alkenyldiphosphates **1**, the alkyldiphosphates **8**.

A rapid method for the preparation of tetraethyl 1,7-heptane- and 1,6-hexanedioldiphosphoric acid ester, respectively **8b** and **8c**, is the phosphorylation of the two hydroxyl groups of 1,7-heptane- and 1,6-hexanediols (Scheme 5).

The formation of the completely phosphorylated compounds **8b–c** can easily be obtained by the reaction of diols with 4 equivalents of diethyl chlorophosphate, in the presence of DMAP catalyst, in pyridine. The reaction is quantitative.

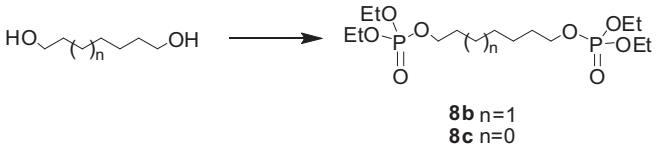
Our interest in the development of new antibacterial agents and the investigation of the diphosphorylated substrates' role about KDO8PS and DAH7PS's inhibition lead us to examine the selective cleavage of the ester phosphoric acid groups of the 1-alkene diphosphates **1**. The cleavage of two of the three O—P bonds of each phosphoric ester function is a particular tedious task. Different attempts using the temporary silylation of the phosphoric ethyl ester have been realized, but without success. The derivatives **1** were fragile and quickly degraded. This enol phosphate moiety's fragility does not allow the deprotection of the ester protecting groups thus the preparation of 1-alkenyldiphosphate tetraacid derivatives.

The development of a strategy based on the direct preparation of diacid phosphoric group is the best synthetic planning. The method of choice was alcoholysis of phosphorous trichloride oxide with 1,7-heptane- and 1,6-hexanediols, proceeding according to the convenient protocol of Modro [11] (Scheme 6).

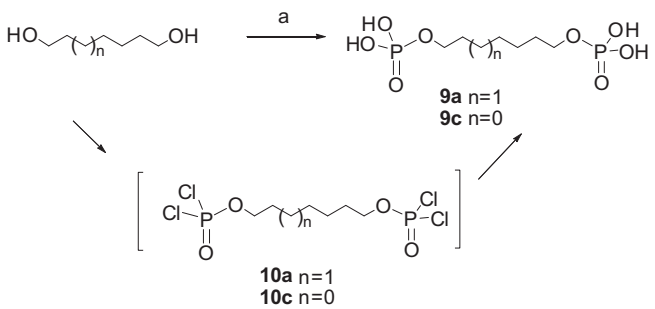
The reaction was easily monitored by ^{31}P NMR. Indeed, the chemical shifts of each phosphorylated species were characteristic (δ_P: $P(O)Cl_3$: 6 ppm; **10a** and **10c**: 4 ppm; **9a** and **9c**: −0.68 ppm).

Although nitrate silver is used as a catalyst, an excess is necessary to accomplish cleanly the alcoholysis of $P(O)Cl_3$. This resulted in the quantitative formation of desired phosphorylated tetraacid **9a** and **9c**.

In summary, this first synthesis of 1-alkenyldiphosphates was rapid, simple, efficient and can easily be scaled up. Chemical yields are excellent and the condition reactions of each step allow to



Scheme 5. Preparation of 1,7-heptane- and 1,6-hexanedioldiphosphates **8b**–**c**. Reagents and conditions: 4 equiv $(EtO)_2P(O)Cl$, 0.5 equiv DMAP, pyridine, 24 h, 20 °C, 100%.



Scheme 6. Preparation of diphosphate tetraacid derivatives **9a** and **9c**. Reagents and conditions: (a) 2 equiv $P(O)Cl_3$, 0 °C, 4 h then 4 equiv $AgNO_3$, 1:1H_2O/CH_3CN, 76%.

Table 1
MIC values in μg mL^{-1} obtained by broth microdilution method, according to CLSI guideline, of compounds **1a**, **1c**, **8b**, **8c** and **9a**, over two Gram negative (*E. coli* and *P. aeruginosa*) and three Gram positive (two *S. aureus* and *E. faecalis*) reference strains.

	1a	1c	8b	8c	9a
E. coli ATCC 25922	**256**	512	>16,384	>16,384	4096
S. aureus ATCC 25923	**256**	**256**	>16,384	>16,384	16,384
S. aureus ATCC 29213	**256**	**256**	16,384	16,384	4096
E. faecalis ATCC 29212	>16,384	>16,384	>16,384	>16,384	>16,384
P. aeruginosa ATCC 27853	4096	16,384	16,384	16,384	4096

Values are means of three experiments.

preserve functionality at phosphorus and to avoid difficult purifications of fragile products.

2.2. Antibacterial activity

Protected 1-alkenyldiphosphates **1** cannot be good indicators of the enzymatic affinity and so inhibition of enzymatic activity. Nevertheless, the biological activities of **1** have been evaluated by direct studies on the bacterial strains' growth.

The antibacterial activities (minimum inhibitory concentration, MIC) of five different enolphosphate derivatives, namely: 1-alkenyldiphosphates **1a** and **1c**, 1,7-heptanedioldiphosphate **8b**, 1,6-hexanedioldiphosphate **8c**, and 1,7-heptanedioldiphosphate tetraacid **9a** were evaluated in liquid phase against various Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and Gram-positive (*Staphylococcus aureus* ATCC 25923, and ATCC 29213, *Enterococcus faecalis* ATCC 29212) bacteria, according to CA-SFM [12] and NCCLS [13]. Experiments were carried out according to microdilution protocols performed in 96-well U shape microtiter plates described by NCCLS [13].

Influence of the enolphosphate moiety on the antibacterial activity was first examined. Interestingly, the results presented in Table 1 suggest that 1-alkenyldiphosphates **1a** and **1c** showed an antibacterial activity, while compounds **8b**, **8c** and **9a** demonstrated a lesser efficacy (MIC are much higher than 256 μg mL^{-1}). These results are very interesting, since they are underestimated because of the poor water solubility of **1a** and **1c,** compared to the one of **8b**, **8c** and **9a**. Consistent with our hypothesis, the presence of the enolphosphate group was necessary to obtain an interesting antibacterial activity. These results may reflect the inhibition of phosphoenolpyruvate lyase. The activity of **1a** and **1c** against *S. aureus* would be consistent with inhibition of DAH7PS, that is the sole phosphoenolpyruvate lyase in Gram-positive bacteria.

Moreover, these results seem to show that both 1-alkenyldiphosphates derivatives **1a** and **1c** did not present exactly the same antibacterial spectrum: **1a** was active on both *S. aureus* strains (MIC = 256 μg mL^{-1}), and also against *E. coli* (MIC = 256 μg mL^{-1}) and, to a lesser extent, *P. aeruginosa* (MIC = 4096 μg mL^{-1}) whereas **1c** who was active against both strains of *S. aureus* (MIC = 256 μg mL^{-1}) was less active against *E. coli* (MIC = 512 μg mL^{-1}) and showed no activity against *P. aeruginosa* (MIC = 16,384 μg mL^{-1}). These observations were interesting, because 1-heptenylphosphate **1a** bearing both phosphorylated groups spaced exactly as they are in the active site of KDO8PS, was efficient against the Gram-negative bacteria *E. coli*, that possesses KDO8PS. Inversely, **1c**, that did not require the structure to inhibit KDO8PS, showed less activity (two to four times less) against Gram-negative bacteria. We have already noticed similar results for the bis-amidino alkanes [14,15].

3. Conclusion

In conclusion, the results obtained in this preliminary study demonstrate that new 1-alkenyldiphosphates **1** are identified as possible antibacterial agents. They represent a significant departure for the discovery of new antibiotics. The improvement of antibacterial activity requires the development of parent compounds as well as enolphosphate derivatives that present best aqueous solubility characteristics. This is under current investigation.

4. Experimental

4.1. General

Reactions were monitored by TLC (Merck – 5535 – Kieselgel 60-F_{254}), detection being carried out by UV, by iodine vapor or by spraying solution of H_2SO_4 15% in ethanol followed by heating. NMR spectra were recorded on a Bruker DRX-250. Chemical shifts are expressed as parts per million downfield from the internal standard tetramethylsilane for ^{1}H and ^{31}C and from external standard phosphoric acid for ^{31}P. Multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (broadened singlet). IR spectra were recorded on Nicolet 210 FT-IR (film between NaCl pellets).

4.2. Preparation of 7-phosphate heptanal ***5a***

5a was easily prepared from **4** or **6** according to the experimental conditions described by Chattopadhyay et al. [8].

7-Phosphate heptanal (**5a**): IR (film) 2760 (C—H), 1740 (C=O); ^{1}H RMN ($CDCl_3$, 250 MHz) δ 1.34 (t, 3H, CH_3), 1.22–1.72 (m, 8H, CH_2), 2.44 (dt, 2H, CH_2), 4.03 (2H, m, CH_2—O), 4.11 (m, 4H, CH_2—O), 9.76 (t, 1H, CHO); ^{31}P RMN ($CDCl_3$, 101.6 MHz) δ 0.20 (s).

4.3. Preparation of α-iodoaldehydes ***2***

In a dark flask, to aldehyde (3 mmol) in 10 mL of CH_2Cl_2, $HgCl_2$ (1 equiv, 814 mg) and I_2 (1 equiv, 761 mg) were added. The resulting mixture was stirred virougously for 4 h at ambient temperature. The suspension was filtered and filtrate washed by aqueous solution of $Na_2S_2O_3$ (0.1 N) then saturated solution of KI. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. Product **2** was used directly in the Perkow reaction without any purification. Yields are quantitative.

The ^{1}H NMR of the crude product showed a complete iodination. The signal of the CHI appeared as a doublet of triplet at 4.5 ppm and the signal of the CHO as a doublet at 9.3 ppm. The absence of the triplet at 9.8 ppm confirmed the complete iodination of the starting aldehyde.

4.4. Preparation of 1-alkenyldiphosphates ***1***

Trialkylphosphite (1 equiv) was slowly added to the stirred neat α-iodoaldehyde (1 mmol.) at 0 °C. The reaction mixture was stirred overnight at ambient temperature before being concentrated under high vacuum. 1-Alkenyldiphosphates **1** were obtained pure and the yields were quantitative.

Dimethyl diethyl 1-heptenyldiphosphoric acid ester (**1a**): ratio E/Z = 52/48; IR (film) 1015 (P—O—C), 1271 (P=O), 1665 (C=C); ^{1}H RMN ($CDCl_3$, 250 MHz) δ 1.35 (t, 6H, CH_3), 1.30–2.50 (m, 8H, CH_2), 3.82 (6H, d, CH_3—O—P, J_{H-P} = 11.25 Hz, stereomers E and Z), 4.03 (dq, $J_{H-H} = J_{H-P}$ = 6.8 Hz, 2H, CH_2—O—P), 4.13 (dq, $J_{H-H} = J_{H-P}$ = 6.7 Hz, 4H, CH_2—O—P), 4.88 (td, J_{1H-2H} = 6.1 Hz, 0.48H, CH, stereomer Z), 5.45 (td, J_{1H-2H} = 11.8 Hz, 0.52H, CH, stereomer E), 6.39 (m, 1H, CH—O—P) ; ^{31}P RMN ($CDCl_3$, 101.6 MHz) δ −1.27 (s, enolphosphate Z), −0.81 (s, enolphosphate E), 0.40 (s, phosphate); ^{13}C RMN ($CDCl_3$, 62.9 MHz) δ 15.9 (CH_3), 23.0–30.1 (4 CH_2), 54.4 (CH_3—O), 62.7 (CH_2—O—P), 67.3 (CH_2—O—P), 116.1 (CH=), 134.8 (=CH—O); MS (FAB+) 375 ([M+H]+, 100%).

Tetraethyl 1-heptenyldiphosphoric acid ester (**1b**): Ratio E/Z = 52/48. IR (film) 1017 (P—O—C), 1272 (P═O), 1665 (C═C); ^{1}H RMN ($CDCl_3$, 250 MHz) δ 1.37 (t, 12H, CH_3), 1.30–2.50 (m, 8H, CH_2), 4.02 (dq, 2H, CH_2—O—P), 4.09–4.17 (m, 8H, CH_2—O—P), 4.88 (td, J_{1H-2H} = 6.1 Hz, 0.48H, CH, stereomer Z), 5.45 (td, J_{1H-2H} = 11.8 Hz, 0.52H, CH, stereomer E), 6.39 (m, 1H, CH—O—P) ; ^{31}P RMN ($CDCl_3$, 101.6 MHz) δ −1.26 (s, enolphosphate Z), −0.83 (s, enolphosphate E), 0.40 (s, phosphate); ^{13}C RMN ($CDCl_3$, 62.9 MHz) δ 15.9 (CH_3), 23.0–30.1 (4 CH_2), 62.7 (CH_2—O—P), 67.3 (CH_2—O—P), 116.1 (CH═), 134.8 (═CH—O); MS (FAB +) 403 ([M+H]+, 100%).

Dimethyl diethyl 1-hexenyldiphosphoric acid ester (**1c**): Ratio E/Z = 56/44; IR (film) 1015 (P—O—C), 1271 (P═O), 1665 (C═C); ^{1}H RMN ($CDCl_3$, 250 MHz) δ 1.35 (t, 6H, CH_3), 1.42–1.78 (m, 4H, CH_2), 2.01 (dt, 1.12H, CH_2, stereomer E), 2.19 (dt, 0.88H, CH_2, stereomer Z), 3.82 (3.36H, d, CH_3—O—P, J_{H-P} = 11.25 Hz, stereomer E), 3.83 (2.64H, d, CH_3—O—P, J_{H-P} = 11.25 Hz, stereomer Z), 4.05 (dq, J_{H-H} = J_{H-P} = 6.7 Hz, 2H, CH_2—O—P), 4.12 (dq, J_{H-H} = J_{H-P} = 6.7 Hz, 4H, CH_2—O—P), 4.87 (td, J_{1H-2H} = 6.1 Hz, 0.44H, CH, stereomer Z), 5.45 (td, J_{1H-2H} = 12.0 Hz, 0.56H, CH, stereomer E), 6.41 (m, 1H, CH—O—P); ^{31}P RMN ($CDCl_3$, 101.6 MHz) δ −2.38 (s, enolphosphate Z), −1.93 (s, enolphosphate E), −0.70 (s, phosphate); ^{13}C RMN ($CDCl_3$, 62.9 MHz) δ 16.1 (CH_3), 23.2 (s, CH_2, stereomer Z), 24.9 (s, CH_2), 25.3 (s, CH_2, stereomer E), 26.4 (s, CH_2), 54.7 (CH_3—O), 63.7 (d, CH_2—O—P), 67.2 (d, CH_2—O—P), 115.3 (CH═, stereomer Z), 116.7 (CH═, stereomer E), 135.1 (═CH—O, stereomer Z); 136.1 (═CH—O, stereomer E), MS (FAB+) 360 ([M+H]+, 100%).

4.5. Preparation of alkyldiphosphates **8**

In a typical procedure, alkanediol (8.47 mmol) and DMAP (0.5 equiv, 500 mg) were added under N_2 to pyridine (25 mL). Diethyl chlorophosphate (4 equiv, 4.4 mL) was added dropwise at 25 °C. The reaction mixture was stirred for 24 h. The solvent was evaporated under diminished pressure. The crude product was purified on a silica gel chromatographic column with acetone/EtOH 9:1 to give **8**.

Tetraethyl 1,7-heptanediol diphosphoric acid ester (**8b**): R_f (acetone/EtOH 9:1) 0.70; ^{1}H RMN ($CDCl_3$, 250 MHz) δ 1.38 (12H, m CH_3), 1.28–1.93 (10H, m, CH_2), 4.04 (4H, dq, CH_2—O); 4.10 (8H, dq, CH_2—O); ^{31}P RMN ($CDCl_3$, 101.6 MHz) δ − 0.68 (s).

Tetraethyl 1,7-hexanediol diphosphoric acid ester (**8c**): R_f (propanone/EtOH 9:1) 0.67; ^{1}H RMN ($CDCl_3$, 250 MHz) δ 1.38 (12H, m CH_3), 1.32–1.94 (8H, m, CH_2), 4.04 (4H, dq, CH_2—O); 4.11 (8H, dq, CH_2—O); ^{31}P RMN ($CDCl_3$, 101.6 MHz) δ −0.68 (s).

4.6. Preparation of alkyldiphosphoric tetraacids **9**

In a typical procedure, alkanediol (8.47 mol) was added under N_2, at 0 °C, to $P(O)Cl_3$ (2 equiv, 1.6 mL). The solution was stirred for 4 h at 0 °C, before it was slowly allowed to warm to 25 °C. Acetonitrile (4.2 mL) were added to the resulting mixture. Then, $AgNO_3$ (4 equiv, 6 g) in H_2O/acetonitrile (1:1) was added at 0 °C. After being stirred for 6 h, the mixture was kept at −18 °C. The so-obtained precipitate was filtered, filtrate concentrated and the residue was treated with EtOH (5 mL). The mixture was filtered once again. Evaporation of solvents provided the alkanediol diphosphate tetracids **9**.

1,7-heptanediol diphosphate tetraacid (**9a**): ^{1}H RMN ($CDCl_3$, 250 MHz) δ 1.25–1.95 (10H, m CH_2), 3.93–4.07 (4H, m, CH_2—O); ^{31}P RMN ($CDCl_3$, 101.6 MHz) δ 0.45 (bs).

1,6-hexanediol diphosphate tetraacid (**9c**): ^{1}H RMN ($CDCl_3$, 250 MHz) δ 1.32–1.93 (8H, m CH_2), 3.93–4.08 (4H, m, CH_2—O); ^{31}P RMN ($CDCl_3$, 101.6 MHz) δ 0.48 (bs).

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