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SYNTHESIS OF TRIPHOSPHONATE ANALOGUES OF FARNESYL PYROPHOSPHATE. INHIBITORS OF SQUALENE SYNTHASE AND PROTEIN:FARNESYL TRANSFERASE

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Abstract: Novel mono- and difarnesylated triphosphonates (i.e. **2** and **7**) and the corresponding cyclic derivatives (i.e. **3** and **8**) were synthesised and tested on inhibitory potential and specificity for the enzymes squalene synthase and protein:farnesyl transferase. The IC_{50} values of these new derivatives are in the range of 1-60 μM concentration. © 1997 Elsevier Science Ltd.

Squalene synthase (SS), the first pathway-specific enzyme in the biosynthesis of cholesterol, catalyses the reductive dimerization of two molecules of farnesylpyrophosphate to produce squalene.[1] Much attention has been directed to the development of inhibitors of SS with the aim to lower elevated blood plasma levels of cholesterol, one of the risk factors for cardiovascular diseases.[2] Apart from SS, other enzymes, such as protein:farnesyl transferase (PFT)[3], use farnesylpyrophosphate as a substrate. Farnesylation of the Ras precursor protein by PFT enables anchoring of the mature protein to the cell membrane, which is essential for the enzymatic function of Ras.[4] Mutated Ras proteins are involved in many human colon cancers and pancreatic carcinomas[5] and it is thought that inhibitors of PFT may serve as antitumor drugs.[6] A major class of potential SS and PFT inhibitors comprises farnesylpyrophosphate analogues having a modified pyrophosphate function.[6, 7] We[8f] and others[9a] earlier described the synthesis of the farnesylbisphosphonate **1** (Fig.1). The latter compound proved to be a potent and specific inhibitor of SS. The inhibitory action of **1** was explained by the fact that this compound may function, in agreement with the hypothesis of Biller *et al.*[10], as a mimic of the allyl cation-diphosphate tight ion pair.[9a] We reasoned that triphosphonate analogues of farnesylpyrophosphate (i.e. **2**-**3**) may resemble the Mg^{2+}-farnesylpyrophosphate complex (B in Fig. 1).

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In this paper we report the synthesis and biological activity of the novel farnesyltriphosphonate **2**, its corresponding cyclic derivative **3** and their difarnesylated counterparts **7** and **8**.

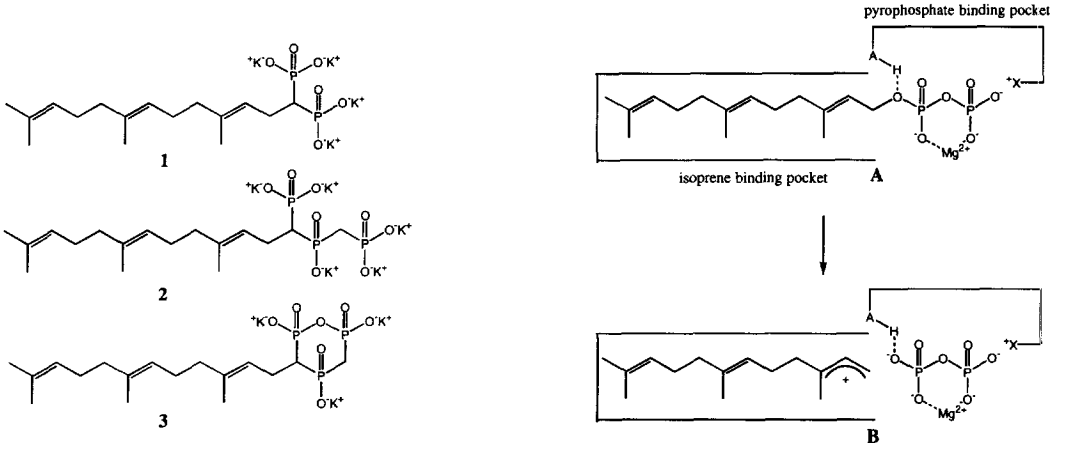
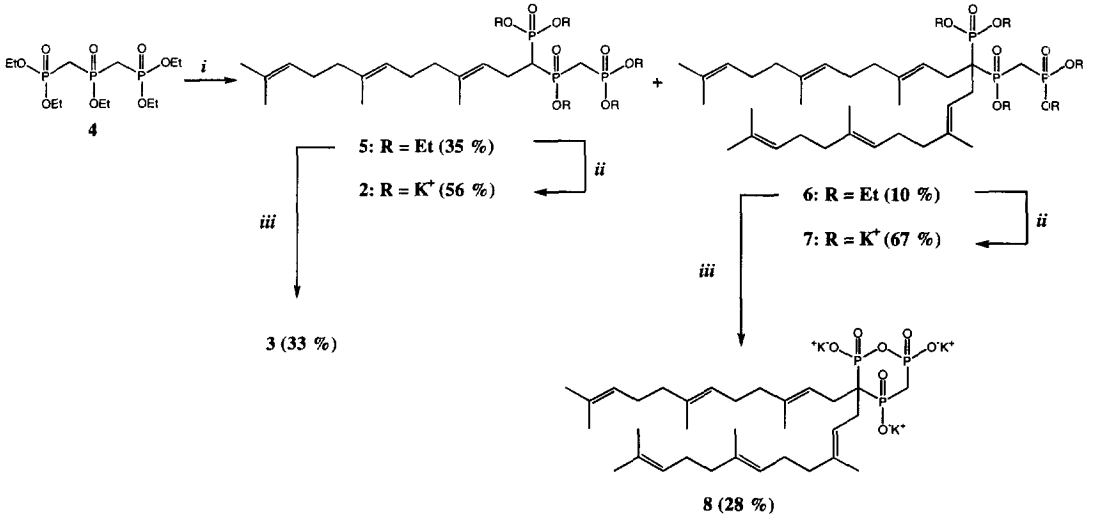


Figure 1.

We previously reported that farnesylation of tertraethyl methylenediphosphonate using a slight deficiency of farnesyl bromide results in the formation of a major monofarnesylated and a minor difarnesylated product.[8f] Similarly, farnesylation of known[11] bis(dihydroxyphosphinyl)phosphinic pentaethyl ester **4** led to the desired monofarnesylated compound **5** as a 2:1 mixture of diastereoisomers (Scheme 1).[12] In addition, a mixture of difarnesylated products[13], one of which was characterized as compound **6**, was produced.



Scheme 1. *i*) NaH (0.98 equiv.), farnesyl bromide (0.7 equiv.) in THF, 16 h, 0 °C. *ii*) a. Me_3SiBr/2,4,6-collidine; b. 1N KOH. *iii*) a. Me_3SiBr/2,4,6-collidine; b. $MeOH/H_2O$; c. DCC, pyridine; d. Dowex-K^+.

Transesterfication of **5** with trimethylsilyl bromide (TMSBr) in the presence of *sym*-collidine and *in situ* basic hydrolysis of the intermediate trimethylsilyl esters led, after purification[14], to the isolation of racemic **2** (K^+-salt, Scheme 1), the spectroscopic data of which are in full accord with the proposed structure.[12] The difarnesylated derivative **6** was deprotected, in a similar sequence of reactions, to give homogeneous **7**.

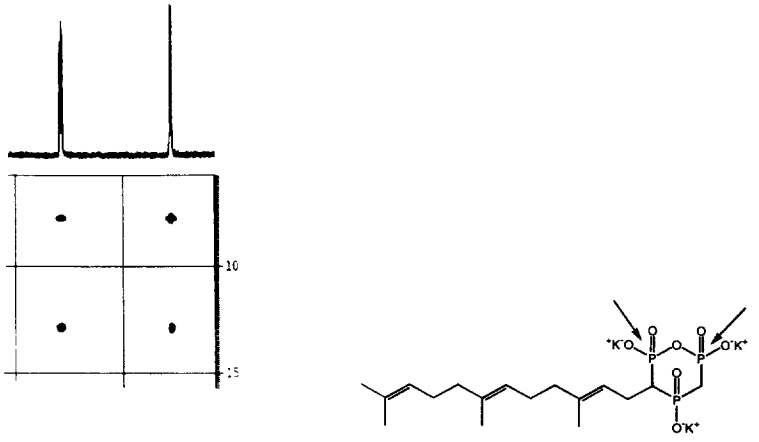


Figure 2. Part of the 600 MHz P-P COSY NMR spectrum of compound **3** showing the crosspeak, as indicated by arrows, between the two phosphorous atoms.

Transformation of **5** into its cyclic counterpart **3** was effected by the following one-pot three-step procedure: Transesterfication, followed by hydrolysis and cyclisation[15] gave, after ion-exchange (Dowex-K^+) and purification[14], the cyclic farnesyltriphosphonate **3** (Scheme 1). The cyclic structure of compound **3** was firmly established[12] by 600 MHz ^{31}P-^{31}P COSY NMR. Thus, a crosspeak was observed between the two quartet signals at 7.8 and 12.9 ppm corresponding to the two phosphonate functionality's (Fig. 2). Moreover, the phosphorous resonance of the central P-atom (30.1 ppm) exhibited two crosspeaks with the two neighbouring phosphonates (not shown in Fig.2).[16] In a similar fashion the difarnesylated compound **6** was transformed into the cyclic derivative **8**.

The results of the inhibition of the enzymes SS and PFT by the novel compounds **2**, **3**, **7**, **8** and the previously reported bisphosphonate **1** are listed in Table 1.[17] It can be seen that the farnesyltriphosphonate **2** is 60 times less active in the SS assay than compound **1**. However, compound **2** is 8 times more effective in the inhibition of PFT than compound **1**. Unfortunately, compound **2** shows no enzyme specificity. Surprisingly, the cyclic farnesyltriphosphonate **3** proved to be a less effective inhibitor of both enzymes. The latter result may be explained by the conformational constrained negatively charged phosphorous functionalities in **3** which prevent a

favourable interaction with the enzymes. Difarnesylated derivative **7** is more specific and slightly less active than compound **2** in the inhibition of PFT. Furthermore, the corresponding cyclic derivative **8** is a poor inhibitor of both enzymes.

Table 1. IC_{50} values of compounds 1, 2, 3, 7 and 8 in squalene synthase and protein:farnesyl transferase assays.

Compound	Squalene Synthase IC_{50} (μM)	Protein:Farnesyl Transferase IC_{50} (μM)
1	0.018 ± 0.002	13.5 ± 1.1
2	1.1 ± 0.24	1.81 ± 0.38
3	46.4 ± 7.5	34.2 ± 3.7
7	23.3 ± 6.7	3.99 ± 0.84
8	>50	56.2 ± 5.0

Conclusion

Four novel triphosphonate analogues of farnesylpyrophosphate (**2**, **3**, **7**, **8**) were prepared and tested on their ability to inhibit the enzymes SS and PFT. The presence of the additional phosphonate group in these compounds reduces the inhibitory potency toward SS. In contrast, compounds **2** and **7** are better inhibitors of PFT in comparison with **1**. The presence of an additional farnesyl chain in compound **7** results in specific inhibition of PFT. Cyclisation has an unfavourable effect on the inhibitory activity. In order to improve both inhibitory potency and specificity, we are currently exploring other triphosphonate analogues of farnesylpyrophosphate.

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12. The compounds **2-8** were fully characterized. For example: ^{31}P NMR (300 MHz) of compound **2**: D_2O δ: 36.45 (q, $J_{p\text{-}p}$ 3.3 and 9.6 Hz), 19.62 (d, $J_{p\text{-}p}$ 3.3 Hz), 14.23 (d, $J_{p\text{-}p}$ 9.6 Hz). ^{31}P NMR (600 MHz) of compound **3**: D_2O δ: 30.1 (q, $J_{p\text{-}p}$ 6.3 and 30 Hz), 12.87 (q, $J_{p\text{-}p}$ 29.4 and 40.8 Hz), 7.76 (q, $J_{p\text{-}p}$ 6.0 and 40.8 Hz). ^{31}P NMR (300 MHz) of compound **5** (2:1 ratio of diastereoisomers): $CDCl_3$ δ: 43.06 (major) (q, $J_{p\text{-}p}$ 8.5 and 13.6 Hz), 42.47 (minor) (t, $J_{p\text{-}p}$ 2.3 Hz), 24.07 (minor) (d, $J_{p\text{-}p}$ 2.3 Hz), 23.85 (major) (d, $J_{p\text{-}p}$ 13.6 Hz), 20.96 (major) (d, $J_{p\text{-}p}$ 8.3 Hz), 20.92 (minor) (d, $J_{p\text{-}p}$ 2.3 Hz).
13. The other difarnesylated product proved to be a regioisomer of compound **6** which was obtained as mixture of 4 diastereoisomers in 10 % yield.
14. CHP20P column chromatography purification, using a linear gradient of acetonitrile and water, was applied to purify compounds **2**, **3**, **7** and **8**. For a purification procedure see: Biller, S.A.; Forster, C. *Tetrahedron* **1990**, *46*, 6645.
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16. The ^{31}P NMR spectra of compound **3** did not change upon standing for three weeks in D_2O at 0 °C.
17. The in vitro assays were performed according to ref. 8c.

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