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Synthesis and Biological Evaluation of Nonionic Prenyl, Geranyl, and Farnesyl Diphosphate Surrogates

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Prenyl, geranyl, and farnesyl derivatives containing nonionic surrogates for the diphosphate moiety, including disulfones **4–6** and **7–9,** methylene disulfonamides **10–12,** and carbamyl sulfamides **13–15,** have been synthesized and evaluated biologically in an effort to find suitable nonlabile, neutral inhibitors for enzymatic reactions which use these isoprenoid diphosphates as substrates. Farnesyl derivatives **6, 9, 12,** and **15** were ineffective as squalene synthase inhibitors *in vitro.* Compounds **4–15** were screened in human skin fibroblasts for their effects on fatty acid, cholesterol, and DNA synthesis. In general, compounds **10–15** showed more inhibition than **4–9** and had a greater effect on DNA synthesis than on lipid synthesis, with the exception of **15.** © 1996 Academic Press, Inc.

This study was undertaken in an effort to find an appropriate surrogate for the diphosphate group that is nonlabile and neutral and that could be used in prospective inhibitors of enzymatic reactions which have substituted diphosphates as substrates. The diphosphate moiety itself is unsuitable as part of inhibitors because it is readily hydrolyzed by phosphatases *in vivo* and because, being ionic, it presumably would have difficulty crossing cell membranes (*1*). A number of modified diphosphates, e.g. (phosphinylmethyl)phosphonates (*2*), have been prepared in order to remove the hydrolytic lability, but these compounds are still ionic, and in general, with some recent exceptions (*3*), have not been effective inhibitors *in vivo*.

The specific type of diphosphate substrates for which nonionic surrogates are considered in the present work are the isoprenoid building blocks prenyl **(1),** geranyl **(2),** and farnesyl diphosphates **(3).** These mevalonate-derived intermediates are of central biochemical importance (*4–6*). They play a key role in the biosynthesis of lipids, including cholesterol, steroid hormones, bile acids, ubiquinone, and dolichols. In addition, these isoprenoids are intermediates in the biosynthesis of isoprenylated tRNAs, of protein conjugates involved in cell signaling, such as the RAS family, and of heme **a** of cytochrome oxidase. Thus, specific inhibitors of the formation or further metabolism of prenyl, geranyl, or farnesyl diphosphates have been widely

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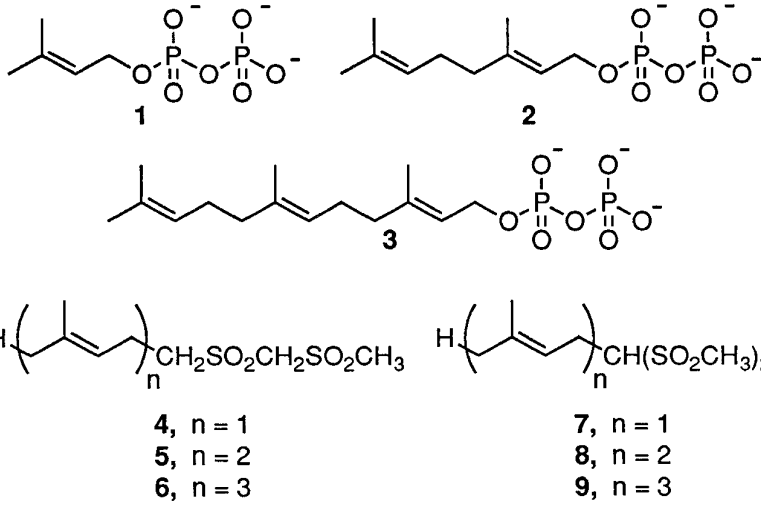


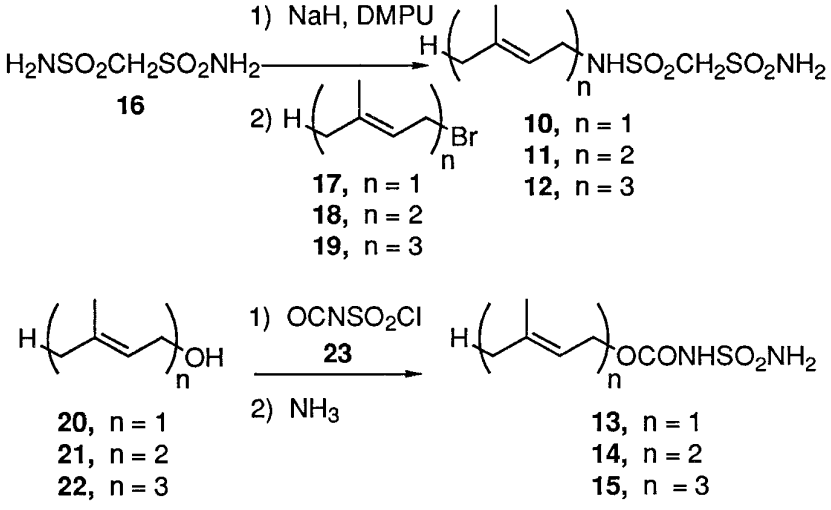
sought because they could have a profound impact on a variety of cellular processes and could serve as therapeutic agents for the prevention or treatment of atherosclerosis, malignancies, or other proliferative disorders.

In the current study, four types of nonionic diphosphate mimics were selected for attachment to these isoprenoid units. These include 1,3-disulfones with two substitution patterns, namely compounds **4–6** and **7–9.** Sulfones have previously been proposed as nonionic, nonhydrolyzable substitutes for biological phosphodiesters (*7*). In addition, methylene disulfonamide derivatives **10–12** and carbamyl sulfamide derivatives **13–15** were selected as more polar analogs than 1,3-disulfones **4–6.** The carbamyl sulfamide group has previously been used as an isosteric substitute for the diphosphate moiety in uridine diphosphate glucose (*8*).

To assess their potential as diphosphate surrogates, the farnesyl derivatives **6, 9, 12,** and **15** were tested for their ability to act as *in vitro* inhibitors of squalene synthase, the enzyme which couples two farnesyl diphosphates in a key step in cholesterol biosynthesis. All of the synthesized compounds, **4–15,** were evaluated for their effects on lipid and DNA synthesis in human skin fibroblasts in culture.

SYNTHESIS

The syntheses of the two classes of prenyl, geranyl, and farnesyl disulfones **4–6** and **7–9,** via alkylation of the trianion and dianion, respectively, of bis(methylsulfonyl)methane, have been described previously (*9*). Preparation of **10–12** was undertaken via analogous alkylation of methylene disulfonamide **(16)** (*10*). Since no alkylation reactions of **16** had been reported, treatment of **16** with $D_2O/D_3CSO_2CD_3$ was used to establish that the protons on N are much more readily exchanged than



SCHEME 1

those on C, suggesting that the desired N alkylation might be achieved by use of the monoanion of **16.** This proved to be correct, although alkylation was successful only when HMPA or DMPU was used as solvent, and then only in modest yield. Under the most efficient conditions discovered, consisting in treatment of **16** in DMPU with one equivalent of NaH and then with one equivalent of alkylating agent at room temperature, prenyl bromide **(17)** afforded 40% of **10,** geranyl bromide **(18)** gave 35% of **11,** and farnesyl bromide **(19)** gave 32% of **12** (Scheme 1).

Synthesis of carbamyl sulfamides **13–15** was achieved by a slight modification of the procedure of Graf (*11*). Treatment of the appropriate allylic alcohol **(20–22)** in CH_2Cl_2 at −23°C with one equivalent of chlorosulfonyl isocyanate **(23)** for 45 min followed by addition of a large excess of liquid NH_3 led to 80% of **13,** 88% of **14,** and 84% of **15,** respectively (Scheme 1).

BIOLOGICAL ACTIVITIES

The farnesyl derivatives **6, 9, 12,** and **15** were tested as substrate analog inhibitors of the conversion of farnesyl diphosphate **(3)** to squalene in a rat liver microsomal squalene synthase assay (*12*). None of these four compounds caused significant reduction of squalene synthase activity at concentrations up to 1 mM, with each exhibiting at most 5–10% inhibition at that highest concentration tested. These results suggest that anionic charge is an important component of any diphosphate surrogate for binding in the active site, consistent with earlier observations (*13*).

In addition, all twelve compounds **4–15** were screened for biological activity in

TABLE 1
Effects of Compounds **10–15** on [^{14}C]Acetate Incorporation into Fatty Acids and Cholesterol in Confluent Human Skin Fibroblast Cultures

Compound	FA (IC_{50}, mM)	CH (IC_{50}, mM)
10	10^{-3}	1
11[a]	$\sim 10^{-5}$	~0.1
12[a]	~0.05	~0.1
13	>1	>1
14	~0.1	~0.1
15[b]	~0.01	~0.1

[a] All cells dead at 1 mM.
[b] Many cells dead at 1 mM.

human skin fibroblasts (HSF). They were evaluated for effects on [^{3}H]thymidine incorporation into DNA in growing cultures, and for effects on [^{14}C]acetate incorporation into fatty acids and cholesterol in both growing and confluent cultures. Compounds **4–9** had little effect on fatty acid or cholesterol synthesis in either confluent or growing HSF, and **4–8** had no effect on DNA synthesis or cell viability in general. However, in growing HSF, farnesyl derivative **9** inhibited DNA synthesis by 50% at $\sim 10^{-4}$ mM and caused complete cell death at the highest concentration used, 1 mM.

Compounds **10–15** proved in general to be more interesting, although in confluent HSF only compound **11** showed a significant effect, selectively inhibiting fatty acid synthesis (Table 1), suggesting a degree of specificity. In the growing cell cultures, compounds **10–15** exhibited strikingly varied effects (Table 2). Farnesyl methylene disulfonamide **(12)** had little effect on either fatty acid or cholesterol synthesis, but was a potent inhibitor of DNA synthesis, with IC_{50} in the nanomolar range. This suggests that **12** might be useful in attenuating reactions involving nonsterol products of the mevalonate pathway that are known to be critical for cell growth and differen-

TABLE 2
Effects of Compounds **10–15** on [^{3}H]Thymidine Incorporation into DNA and [^{14}C]Acetate Incorporation into Fatty Acids and Cholesterol in Growing Human Skin Fibroblasts

Compound	DNA (IC_{50}, mM)	FA (IC_{50}, mM)	CH (IC_{50}, mM)
10	10^{-4}	10^{-4}	>1
11	$\sim 10^{-5}$	0.01	>1
12[a]	10^{-6}	>1	>1
13	10^{-5}	1	$\sim 10^{-3}$
14	5×10^{-6}	10^{-4}	$\sim 10^{-4}$
15	~0.1	10^{-6}	5×10^{-7}

[a] All cells dead at 1 mM.