BIOORGANIC CHEMISTRY **24,** 242–250 (1996)
ARTICLE NO. 0022

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

ALFREDO CASTRO,* SANDRA K. ERICKSON,† ISHAIAHU SHECHTER,†,[1] AND THOMAS A. SPENCER*,[2]

**Department of Chemistry, Dartmouth College, Hanover, New Hampshire 03755*; †*Department of Medicine, University of California, San Francisco and Veterans Administration Medical Center, San Francisco, California 94121*; *and* ‡*Eleanor Roosevelt Institute, Denver, Colorado 80206*

Received February 2, 1996

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

[1] Current address: Department of Biochemistry, Uniformed Services University School of Medicine, Bethesda, MD 20814.

[2] To whom correspondence should be addressed.

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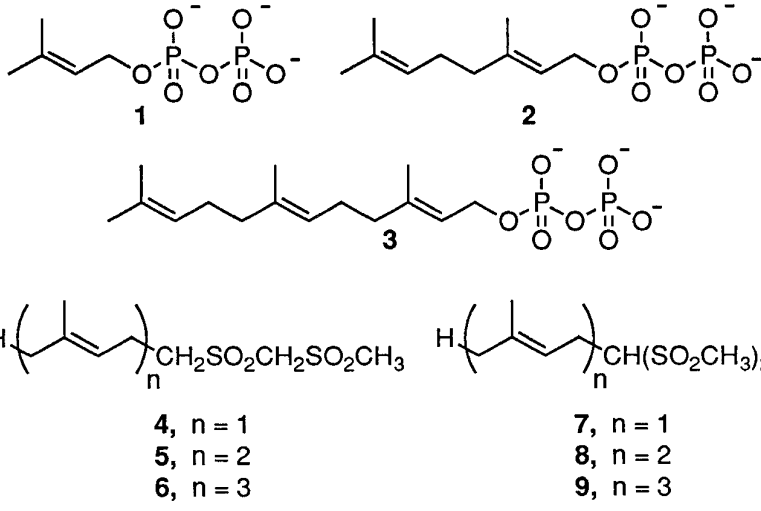


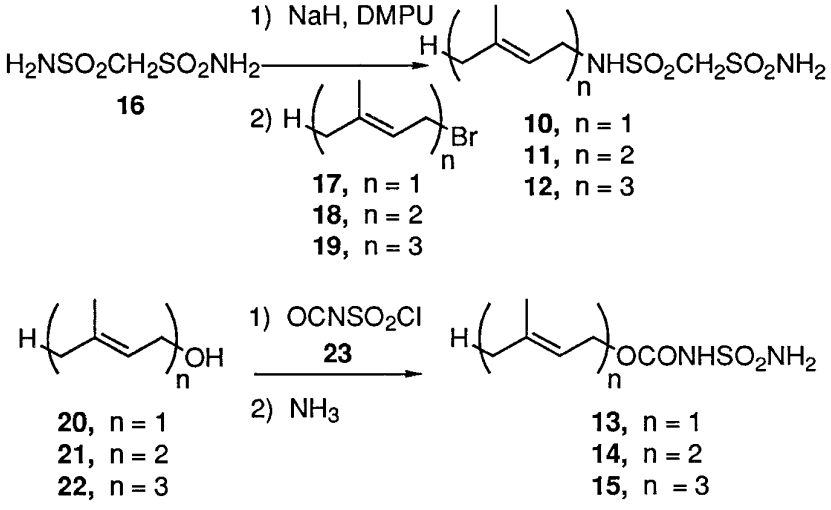
sought because they could have a profound impact on a variety of cellular processes and could serve as therapeutic agents for the prevention or treatment of atherosclerosis, malignancies, or other proliferative disorders.

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.

tiation. In contrast, farnesyl carbamyl sulfamide **(15),** unlike any of the other compounds in this group, was very effective at inhibiting cellular cholesterol and fatty acid synthesis (IC_{50}'s in the nanomolar range) but had little effect on DNA synthesis in these growing cells. Because **15** had minimal effect on squalene synthase *in vitro*, its inhibition of cholesterol synthesis in intact cells is probably at another site. Compound **15** could prove useful as a lipid synthesis inhibitor with relatively little effect on cell growth. In contrast, geranyl carbamyl sulfamide **(14)** was effective at inhibiting *all* tested functions in growing cells, although its effect on DNA synthesis was 100-fold greater than that on lipid synthesis. The mechanism by which any of these effects in growing HSF takes place is unknown, and it is unclear whether any of the polar groups in **4–15** is functioning as a diphosphate surrogate, but compounds **10–15** certainly exhibit inhibitory properties deserving further exploration.

EXPERIMENTAL

General synthesis procedures. Melting points were determined in a Thomas-Hoover apparatus in unsealed capillaries. Infrared (IR) spectra were recorded on a Perkin–Elmer Model 599 spectrophotometer for neat liquids on NaCl plates or in KBr pellets for solids. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL-300 spectrometer in acetone-d6 unless otherwise noted. Elemental Analyses were performed by Atlantic Microlab Inc. (Norcross, GA). Dry flash chromatography was carried out by the method of Harwood (*14*) on Kieselgel 60 silica (230–400 mesh). Reaction progress was monitored by thin layer chromatography (TLC) using Kieselgel 60 F_{254} silica plates from EM Science. Visualization was accomplished by either exposure to iodine vapors or illumination with 254- and 365-nm UV light. All reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) and were used directly or purified according to procedures described below. Tetrahydrofuran (THF) was distilled from sodium metal with benzophenone indicator. Titration of *n*-butyllithium in hexanes (*n*-BuLi) with Ph_2CHCO_2H in THF was performed in duplicate prior to use. Methylene chloride (CH_2Cl_2) was distilled from calcium hydride. Diethyl ether (ether) was distilled from sodium metal. 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*h*)-pyrimidinone (DMPU) was distilled from calcium hydride and stored over molecular sieves (4 Å). Buten-1-ol, 3-methyl-2-buten-1-ol **(20),** *trans*-3,7-dimethyl-2,6-octadienol (geraniol, **21**), and *trans*,*trans*-3,7,11-trimethyl-2,6,10-dodecatrienol (farnesol, **22**) were dried over molecular sieves (4 Å) for at least 12 h prior to use. Sodium hydride as a 60% dispersion in mineral oil (60% NaH) was washed with hexane and dried by passing N_2 or Ar over the white powder prior to use. Brine refers to saturated aqueous NaCl. Anhydrous reactions were performed in glassware that had been flame-dried or heated in an oven overnight at 125°C and then cooled in a desiccator containing Ca_2SO_4 or P_2O_5. The term ''under N_2'' or ''under Ar'' refers to the maintenance of a positive pressure of that gas which had been passed through a column of anhydrous Ca_2SO_4.

(*E*)-*3,7-Dimethyl-2,6-octadienyl bromide* (*geranyl bromide,* ***18***). According to a procedure of Edstrom (*15*), to a solution of 2.0 ml (12 mmol) of **21** in 10 ml of hexane at 0°C in a flask wrapped in Al foil was added 0.36 ml (3.8 mmol) of PBr_3. The mixture was allowed to warm to 24°C, stirred under N_2 for 18 h, and 5.0 ml

of 5% aqueous $NaHCO_3$ solution was added. The aqueous layer was removed after gas evolution ceased. Evaporation of solvent and vacuum distillation gave 2.0 g (82%) of **113:** bp 72°C, 0.6 Torr (lit. (*16*) bp 110°C, 3 Torr); ^{1}H NMR δ 5.35 (t, J = 7.2 Hz, 1H), 5.08 (br s, 1H), 4.05 (d, J = 8.4 Hz, 2H), 2.20-1.95 (m, 4H), 1.72 (s, 3H), 1.66 (s, 3H), 1.59 (s, 3H) (lit. (*16*) ^{1}H NMR (CCl_4) δ 5.46 (br t, J = 8 Hz, 1H), 5.02 (br s, 1H), 3.95 (d, J = 8.0 Hz, 2H), 2.05 (m, 4H), 1.70 (s, 3H), 1.65 (s, 3H), 1.58 (s, 3H)).

(*E,E*)-*3,7,11-Trimethyl-2,6,10-dodecatrienyl bromide* (*farnesyl bromide,* ***19***). Exactly the same procedure used for **18** was employed with 1.0 ml (4.0 mmol) of **22** to afford 0.92 g (82%) of **114:** bp 80°C, 0.05 Torr (lit. (*17*) bp 100–110°C, 0.15 Torr); ^{1}H NMR δ 5.51 (t, J = 8.0 Hz, 1H), 5.09 (m, 2H), 3.99 (d, J = 9.0 Hz, 2H), 2.20-1.90 (m, 8H), 1.71 (s, 3H), 1.65 (s, 3H), 1.59 (s, 3H), 1.58 (s, 3H) (lit. (*17*) ^{1}H NMR δ 5.52 (t, J = 9 Hz, 1H), ~5.09 (br, s, 2H), 3.95 (d, J = 9 Hz, 2H), 2.2-2.0 (m, 8H), 1.69 (s), 1.61 (s) (12H)).

Methylene disulfonamide ***(16).*** According to the procedure of Fild and Rieck (*18*), to a mixture of 23 ml (0.25 mol) of $POCl_3$ and 16 ml (0.24 mol) of $ClSO_3H$ was added 7.5 ml (0.13 mol) of CH_3CO_2H. The resulting mixture was heated at 105°C for 1 h and then at 145°C for 8 h. The residue was distilled under vacuum to give 22 g (85%) of methylenedisulfonylchloride as a clear fuming oil: bp 85°C, 0.05 T (lit. (*18*) 93°C, 0.2 T); ^{1}H NMR ($CDCl_3$) δ 5.58 (s) (lit. (*18*) ^{1}H NMR (neat) δ 5.69). According to the procedure of Bauer and Jenkins (*10*), to a solution of 20.2 g (0.0950 mol) of methylenedisulfonylchloride in 100 ml of benzene was added a solution of 17.8 g (0.189 mol) of phenol and 30.5 ml (0.370 mol) of pyridine in 100 ml of benzene via cannula, under Ar, with both flasks at 0°C. The resulting mixture was allowed to warm to rt, stirred for 16 h, quenched with 25 g of ice, and extracted with 2 M HCl (5 × 50 ml), water (50 ml), and warm (~45°C) 2 M NaOH (8 × 50 ml). The combined basic extracts were acidified with 450 ml of 2 M HCl and cooled in an ice-water bath. The precipitate was collected and recrystallized from 4 : 1 ethanol-water to give 24.9 g (80%) of diphenyl methylenedisulfonate: mp 83–84°C (lit. (*10*) mp 82–83°C): ^{1}H NMR (benzene-d6) δ 7.10–7.20 (m, 4H), 6.75–6.90 (m, 6H), 4.03 (s, 2H); ^{13}C NMR (benzene-d6) δ 149.4, 130.2, 127.8, 122.3, 62.1. A solution of 10.95 g (0.0334 mol) of diphenyl methylenedisulfonate in 60 ml of benzene cooled in an ice bath was saturated with dry ammonia gas (from ~20 ml of liq NH_3 and sodium) in a bomb flask. The flask was sealed and placed in a pipe heater for 4 h at 145°C (external temperature). The resulting mixture was filtered to give 5.78 g (99%) of off-white **16.** Recrystallization from water gave 4.98 g (86%) of **16** as scaly white crystals: mp 237–239°C (lit. (*8*) 232–233°C): ^{1}H NMR (DMSO-d6) δ 7.24 (s, 4H), 4.66 (s, 2H); ^{13}C NMR (DMSO-d6) δ 72.2.

(*1-*(*3-Methyl-2-butenyl*)*sulfamoyl*)*methanesulfonamide* ***(10).*** To 0.047 g (1.2 mmol) of 60% NaH was added a solution of 0.20 g (1.2 mmol) of **16** in 10 ml of DMPU and the resulting mixture was stirred at rt for 5 h. Then 0.13 ml (1.1 mmol) of **17** was added via syringe and the reaction mixture was stirred in the dark at rt under Ar for 16 h, quenched with 1.0 ml of 5% HCl, diluted to 50 ml with water, and extracted with ethyl acetate (4 × 25 ml). The combined organic layers were washed with water (2 × 50 ml) and brine (2 × 25 ml), dried over $MgSO_4$, filtered, and evaporated to give 0.18 g of oily residue, which was subjected to dry flash

chromatography (9:1 to 1:1 hexane-ethyl acetate) to yield 0.11 g (40%) of **10.** Recrystallization from acetone-hexane gave an analytical sample: mp 151.4–152.2°C; ^{1}H NMR (DMSO-d6) δ 7.45 (t, J = 5.3 Hz, 1H), 7.25 (s, 2H), 5.18 (t, J = 7.1 Hz, 1H), 4.72 (s, 2H), 3.62 (t, J = 6.2 Hz), 1.69 (s, 3H), 1.63 (s, 3H); ^{13}C NMR δ 137.2, 120.8, 69.6, 42.0, 25.7, 18.9. Anal. Calcd for $C_6H_{14}N_2O_4S_2$: C, 29.74; H, 5.82. Found: C, 29.82; H, 5.84.

(*1-(3,7-Dimethyl-2,6-octadienyl)sulfamoyl)methanesulfonamide* **(*11*).** To a mixture of 0.10 g (2.5 mmol) of 60% NaH and 0.44 g (2.5 mmol) of **16** was added 10.0 ml of DMPU and the resulting mixture was stirred at rt for 1 h. Then 0.57 g (2.6 mmol) of **18** was added via syringe and the reaction mixture was stirred in the dark at rt under Ar for 41 h, quenched with 3 ml of 5% HCl, diluted to 50 ml with water, and extracted with ethyl acetate (5 × 25 ml). The combined organic layers were washed with water (3 × 50 ml), brine (2 × 30 ml), dried over $MgSO_4$, filtered, and evaporated to give 0.536 g of oily solid residue, which was subjected to dry flash chromatography (9:1 to 1:1 hexane-ethyl acetate) to yield 0.26 g (34%) of **11.** Recrystallization from acetone-hexane gave an analytical sample: mp 127.5–128.3°C; ^{1}H NMR δ 6.53 (bs, 2H), 6.40 (bs, 1H), 5.32 (t, J = 7.0 Hz, 1H), 5.11 (t, J = 6.8 Hz, 1H), 3.81 (d, J = 6.8 Hz, 2H), 2.14–2.00 (m, 2H), 1.70 (s, 3H), 1.66 (s, 3H), 1.60 (s, 3H); ^{13}C NMR δ 140.7, 132.1, 124.7, 120.7, 69.6, 41.9, 40.2, 27.0, 25.8, 17.7, 16.3. Anal. Calcd for $C_{11}H_{22}N_2O_4S_2$: C, 42.56; H, 7.14. Found: C, 42.50; H, 7.13.

(*1-(3,7,11-Trimethyl-2,6,10-dodecatrienyl)sulfamoyl)methanesulfonamide* ***12***). To a mixture of 0.048 g (1.2 mmol) of 60% NaH and 0.20 g (1.2 mmol) of **16** was added 2.5 ml of DMPU and the resulting mixture was stirred at rt for 4 h. Then 0.31 g (1.1 mmol) of **19** was added via syringe and the reaction mixture was stirred in the dark at rt under Ar for 23.5 h, quenched with 1.5 ml of 5% HCl, diluted to 100 ml with water, and extracted with ethyl acetate (1 × 75 ml, 4 × 25 ml). The combined organic layers were washed with water (2 × 50 ml), brine (2 × 50 ml), dried over $MgSO_4$, filtered, and evaporated to give 0.31 g of oily solid residue, which was subjected to dry flash chromatography (9:1 to 1:1 hexane-ethyl acetate) to yield 0.13 g (32%) of **12.** Recrystallization from ethanol-water gave an analytical sample: mp 91–93°C; ^{1}H NMR δ 6.46 (bs, 2H), 6.32 (bs, 1H), 5.33 (t, J = 6.4 Hz, 1H), 5.19–5.01 (m, 2H), 4.77 (s, 2H), 3.81 (d, J = 6.4 Hz, 2H), 2.19–1.84 (m, 8H), 1.70 (s, 3H), 1.63 (s, 3H), 1.61 (s, 3H), 1.59 (s, 3H); ^{13}C NMR δ 140.7, 131.5, 130.8, 125.0, 124.6, 120.5, 69.5, 41.9, 40.3, 40.1, 27.3, 26.9, 25.8, 17.7, 16.3, 16.1. Anal. Calcd for $C_{16}H_{30}N_2O_4S_2$: C, 50.77; H, 7.99. Found: C, 50.85; H. 7.98.

3-Methyl-2-butenyl sulfamoylcarbamate **(*13*).** To a cold (−23°C) solution of 0.31 ml (3.0 mmol) of 99% **20** in 30 ml of CH_2Cl_2 was added 0.27 ml (3.1 mmol) of **23** all at once. The resulting mixture was stirred for 45 min, and 5 ml of ammonia was added dropwise from a CO_2-acetone-filled condenser over 45 min. The resulting mixture was allowed to warm to rt, stirred under Ar for 4 h, and evaporated, and the residue (0.86 g) was subjected to dry flash chromatography (4:1 to 1:9 hexane-ethyl acetate) to give 0.50 g (80%) of **13:** mp 113.0–114.0°C; ^{1}H NMR (DMSO-d6) δ 11.07 (s, 1H), 7.39 (s, 2H), 5.31 (t, J = 7.3 Hz, 1H), 4.56 (d, J = 7.3 Hz, 2H), 1.72 (s, 3H), 1.68 (s, 3H); ^{13}C NMR (DMSO-d6) δ 151.9, 138.6, 118.6, 61.6, 25.3, 17.7. Anal. Calcd for $C_6H_{12}N_2O_4S$: C, 34.61; H, 5.81. Found: C, 34.34; H, 5.83.

3,7-Dimethyl-2,6-nonadienyl sulfamoylcarbamate **(*14*).** To a cold (−23°C) solu-

tion of 0.53 ml (3.0 mmol) of 98% **21** in 30 ml of CH_2Cl_2 was added 0.27 ml (3.1 mmol) of **23** all at once. The resulting mixture was stirred for 1 h and 5 ml of ammonia was added dropwise from a CO_2-acetone-filled condenser over 1 h. The resulting mixture was allowed to warm to rt, stirred under Ar for 12 h, and evaporated, and the residue (1.2 g) was subjected to dry flash chromatography (9:1 to 1:9 hexane-ethyl acetate) to give 0.73 g (88%) of **14:** mp 99.8–101.3°C; ^{1}H NMR δ 9.88 (bs, 1H), 6.64 (s, 2H), 5.36 (t, J = 7.1 Hz, 1H), 5.11 (t, J = 6.8 Hz, 1H), 4.67 (d, J = 7.1 Hz, 2H), 2.16–1.94 (m, 4H), 1.73 (s, 3H), 1.66 (s, 3H), 1.60 (s, 3H); ^{13}C NMR δ 153.0, 143.2, 132.1, 124.5, 119.1, 63.1, 40.1, 26.9, 25.8, 17.7, 16.5. Anal. Calcd for $C_{11}H_{20}N_2O_4S$: C, 47.81; H, 7.29. Found: C, 48.25; H, 7.50.

3,7,11-Trimethyl-2,6,10-dodecatrienyl sulfamoylcarbamate **(*15*).** To a cold (−23°C) solution of 2.0 ml (7.8 mmol) of 98% **22** in 30 ml of CH_2Cl_2 was added 0.70 ml (7.9 mmol) of **23** all at once. The resulting mixture was stirred for 45 min and 5 ml of ammonia was added dropwise from a CO_2-acetone-filled condenser over 15 min. The resulting mixture was allowed to warm to rt, stirred under Ar for 20 h, and evaporated, and the residue (3.0 g) was subjected to flash chromatography (1:1 hexane-ethyl acetate) to give 2.2 g (84%) of **15:** mp 91.0–91.6°C; ^{1}H NMR δ 9.83 (bs, 1H), 6.63 (bs, 2H), 5.36 (t, J = 7.0 Hz, 1H), 5.20–5.01 (m, 2H), 4.66 (d, J = 7.0 Hz, 2H); 2.18–1.86 (m, 8H), 1.74 (s, 3H), 1.66 (s, 3H), 1.61 (s, 3H), 1.59 (s, 3H); ^{13}C NMR δ 153.0, 143.2, 135.9, 131.6, 125.1, 124.5, 119.1, 63.1, 40.4, 40.2, 27.4, 26.9, 25.8, 17.7, 16.5, 16.1. Anal. Calcd for $C_{16}H_{28}N_2O_4S$: C, 55.79; H, 8.19. Found: C, 55.87; H, 8.23.

Biochemical methods. The rat liver microsomal squalene synthase assays were conducted as previously described (*12*). The screening for effects in cell cultures was conducted as follows. Human skin fibroblasts (HSF) obtained from the UCSF Cell Culture Facility were grown in DME H21 medium containing 4.5 g glucose, 0.58 μg L-glutamine, and 3.7 g $NaHCO_3$/liter of 10% fetal calf serum, and penicillin/streptomycin. The cells were grown in six well plates to sparse density (growing cells) or to confluency. The medium was changed to fresh medium containing 0–1 mM of each compound and incubated further at 37°C overnight (~18 h). Five μCi of [^{3}H]thymidine (82 Ci/mmol) for determination of effects on DNA synthesis or 2 μCi of [14C]acetate (57 mCi/mmol) for determination of effects on cholesterol and fatty acid synthesis was added to each well and the cells were incubated for a further 2 h. The media were removed and the cells were washed twice with phosphate-buffered saline containing Ca^{2+} and Mg^{2+}. The cells were then taken up in 1 ml of 0.1 N NaOH/well. Aliquots were taken for determination of [^{3}H]incorporation into DNA, 100 μg of calf thymus DNA was added as carrier, and DNA was extracted (*19*) and analyzed. [^{14}C] Incorporation into cholesterol was assessed after addition of 100 μg of cholesterol as carrier to these aliquots, saponification, and extraction, followed by thin layer chromatography. [^{14}C] Incorporation into fatty acids was assessed after acidification of the remainder following extraction of the nonsaponifiable fraction at basic pH (*20*). Samples were counted in a liquid scintillation counter and the results expressed graphically as a percentage of control values (wells with no compound added). The IC_{50} (concentration required for 50% inhibition of function studied) was then estimated. Cells were also examined microscopically for gross morphological changes and changes in cell number and/or viability.

ACKNOWLEDGMENTS

Partial support for this research was provided by NIH Grant HL23083 (T.A.S.), by NIH Grant DK38553 and the Department of Veterans Affairs (S.K.E.), and by a grant from the Frost Foundation (I.S.). The authors are grateful to Drs. J. A. Watson and L. Pulliam for assistance in obtaining materials, including HSF cells, and to Steven R. Lear for technical assistance with some of the experiments.

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