Synthesis and Characterization of Aza Analogue Inhibitors of Squalene and Geranylgeranyl Diphosphate Synthases

Arthur Steiger, Hyung-Jung Pyun, and Robert M. Coates*

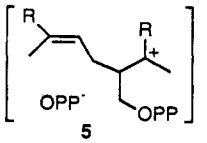
Department of Chemistry, University of Illinois, 1209 West California Street, Urbana, Illinois 61801

Received December 26, 1991 (Revised Manuscript Received March 20, 1992)

One-carbon homologation of geranyl and farnesyl chlorides to secondary *N*-methylamines (**12** and **17**) via α-lithioformamidine alkylations followed by N-alkylation with bromoacetate esters afforded α-(homogeranylamino)- and α-(homofarnesylamino)acetates **13** and **18**. After α-farnesylation of **13**, hydride reductions gave branched and straight-chain tertiary β-amino alcohols **15** and **19**. The diphosphate derivatives (**7** and **8**) of **15** and **19** prepared by S_N2 displacements may be regarded as "aza analogs" of plausible carbocation intermediates (**5** and **6**) in the biosynthesis of squalene and geranylgeranyl diphosphate since, in preliminary collaborative evaluations, they inhibit the respective synthase enzymes at micromolar concentrations.

Farnesyl diphosphate (FPP, **1**) is situated at an important branch point in the isoprenoid biosynthetic pathway (Scheme I).[1,2] Reductive head-to-head coupling of two molecules of FPP via presqualene diphosphate (**2**) leads to squalene (**3**), the precursor of triterpenes and sterols. Chain elongation by alkylation of FPP with isopentenyl diphosphate (IPP) produces geranylgeranyl diphosphate (GGPP, **4**), which is an intermediate in the biosynthesis of cyclic diterpenes, carotenoids, dolichol, ubiquinones, phytyl derivatives, and the recently discovered geranylgeranylated proteins.[3,4] The farnesylation of proteins represents a third branch in the pathways arising from FPP.[4] In view of the diverse biological activities and functions of these isoprenoid end products, we became interested in the design and synthesis of compounds which may act as specific inhibitors of the branch point enzymes, squalene,[5] and GGPP synthases.[6]

Although the exact mechanism by which squalene synthase effects cyclopropane ring formation (**1** → **2**) is presently unclear,[1c,7] tertiary carbocation **5** (or a bridged ion equivalent) is a plausible intermediate.[1c] The extensive mechanistic studies on FPP synthase[1a,8] lead to the expectation that carbocation **6** would be an intermediate at the active site of GGPP synthase. Since aza analogs[9,10]



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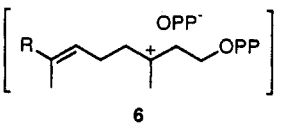

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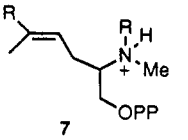

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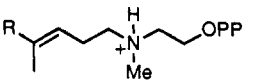

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of other presumed carbocation intermediates in isoprenoid biosynthetic reactions have proven to be effective inhibitors of the relevant enzymes,[9-13] it was logical to propose ammonio diphosphates **7** and **8** as potential branch point inhibitors of squalene and GGPP synthases.[14]

In this paper we report the synthesis and characterization of aza analogs **7** and **8** as well as some preliminary indications of their properties as enzyme inhibitors based on results of collaborative investigations.[15]

Synthesis of Aza Alcohol Precursors. The required β-amino alcohols **15** and **19** were synthesized by straightforward alkylations and reductions as shown in Schemes II and III. Alkylation of α-lithio formamidine **10** generated from *N*-cyclohexyl-*N'*,*N'*-dimethylformadine by the procedure of Meyers and Hoeve[16] with geranyl and farnesyl chlorides (THF, −78 °C → rt) afforded the homologated amidines (e.g., **11**) which were hydrolyzed (KOH, aq MeOH, reflux) to *N*-methylamines **12** and **17** (84%). Alkylation of the amines with ethyl or *tert*-butyl bromoacetate (THF, Et_3N, 0 °C) furnished β-amino esters **13a** (90%), **13b** (93%), **18a** (81%), and **18b** (91%).

The additional isoprenoid substituent of the branched chain aza analog **7** was attached by lithiation of **13a** and **13b** (LDA, THF, HMPA, −78 → 0 °C) and subsequent alkylation of the resulting lithium enolates with farnesyl

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(2) *Biosynthesis of Isoprenoid Compounds*; Porter, J. W., Spurgeon, S. L., Eds.; Wiley: New York, 1983; Vol. 2; (a) Spurgeon, S. L.; Porter, J. W., Chapter 1; (b) Threlfall, D. R., Chapter 4; (c) Hemming, F. W., Chapter 5; (d) Benedict, C. R., Chapter 6.

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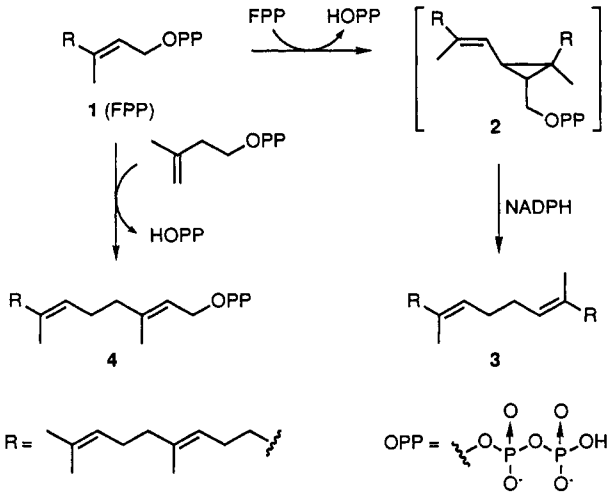
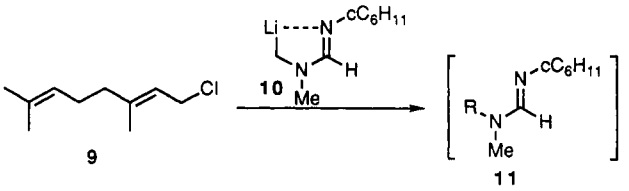
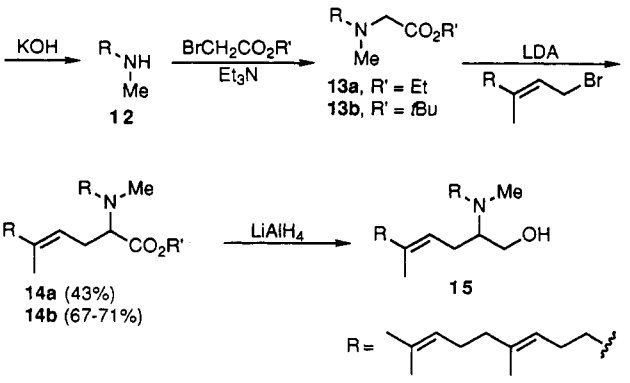
Scheme I

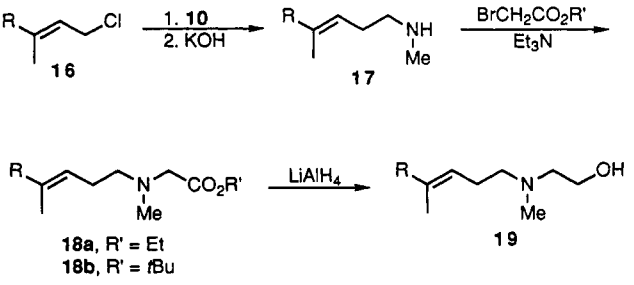


Scheme II





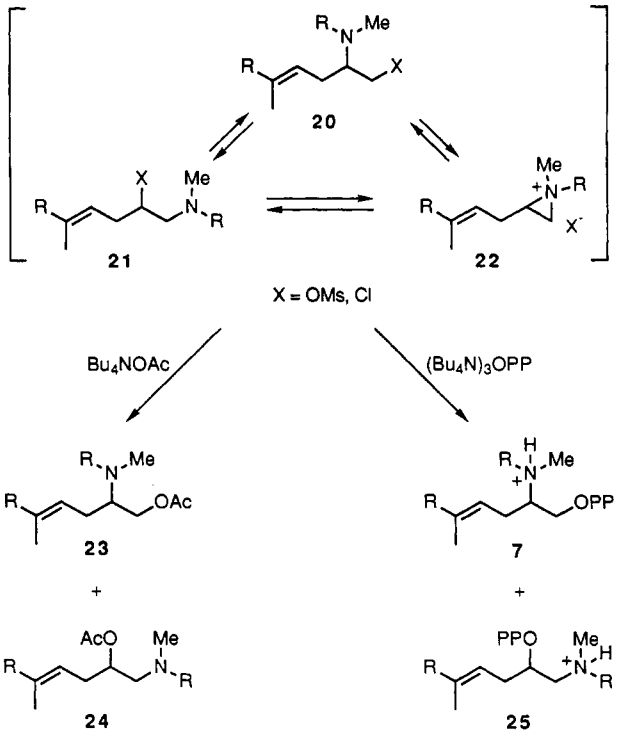
Scheme III



bromide[17] (−78, 0, 25 °C). The substantially higher yield of *tert*-butyl ester **14b** (67%) is presumably a consequence of the greater stability of the enolate intermediate during the slow alkylation reactions. Reduction of the amino esters ($LiAlH_4$, ether, rt) gave β-amino alcohols **15** and **19** in 90–96% yield.

Synthesis and Characterization of "Aza Analog" Diphosphates 7 and 8. The β-amino alcohols were converted to the corresponding diphosphates in two steps by

Scheme IV



activation with methanesulfonyl chloride (CH_3SO_2Cl, Et_3N, CH_2Cl_2, −15 to 0 °C, 30 min)[18] and S_N2 displacement with diphosphate anion [$(Bu_4N)_3OPP$, CH_3CN, rt, 5–24 h] according to the procedures developed by Poulter and co-workers.[19,20] Although satisfactory yields (ca. 40–50%) of water-soluble ammonium salts were obtained, it became clear that the reaction of the branched-chain amino alcohol **15** was accompanied by aziridinium ion formation which gave rise to a 3:1 mixture of primary and secondary isomers, **7** and **25** (Scheme IV). This was evident from the ^{31}P NMR spectrum of purified ammonium diphosphate which displays two pairs of characteristic signals for the diphosphate group in a 3:1 intensity ratio.

The occurrence of this well-known rearrangement[21] was verified by conducting a similar displacement reaction with acetate anion (nBu_4NOAc, CH_3CN, rt, 17 h). The product proved to be a mixture of primary and secondary acetates **23** and **24** (ca. 3:1 ratio, 59%) and secondary chloride (**21**, X = Cl; 16%), pure samples of which were obtained by column chromatography and identified by their ^1H NMR and IR spectra.

No evidence is available to indicate whether aziridinium ion formation occurs during reaction of the amino alcohol with methanesulfonyl chloride, during solvent extraction and exchange, and/or during the displacement with diphosphate anion. The structure(s) of the activated intermediate (primary/secondary, chloride/mesylate, amine/aziridinium ion) and the actual species which reacts

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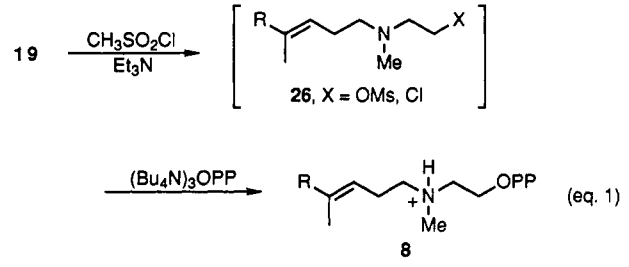
(20) Phosphorylation of **15** by the Cramer method [$(Et_3NH)_2HPO_4$, CCl_3CN, CH_3CN] was also performed to circumvent the aziridinium ion formation; see: (a) Cramer, F.; Rittersdorf, W.; Boehm, W. *Liebigs Ann. Chem.* **1962**, *654*, 180. (b) Cramer, F.; Rittersdorf, W. *Tetrahedron* **1967**, *23*, 3015. However, the low yields of diphosphate and the difficulty of separating it from the monophosphate lead us to abandon this approach.

(21) Deyrup, J. A. In *Heterocyclic Compounds*; Hassner, A., Ed.; J. Wiley: New York, 1983; Chapter 1, Vol. 42, Part 1.

with the nucleophiles are also unknown. Fortunately, the primary diphosphate 7 could be separated in a state of high purity (≥95% purity, 30% yield) by chromatography of the mixture on cellulose with an eluant consisting of 2-propanol/$CHCl_3$/CH_3CN/0.1 M aqueous NH_4OH (5:1:2:2 ratio).

The diphosphate derivative (8) of amino alcohol 19 was prepared and purified by similar procedures (eq 1). An



^1H NMR spectrum of the activated intermediate in CD_2Cl_2 exhibits peaks attributable to both the methanesulfonate and the chloride (26, X = OMs, Cl). Reaction of 26 with acetate anion (Bu_4NOAc, CH_3CN, rt, 1 h) gave a mixture of the corresponding acetate (26, X = OAc; 44%) and chloride (26, X = Cl; 18%). It is not certain whether aziridinium ion formation occurred in these reactions since the S_N2 displacements of 26 are substantially faster than those of 20 and the same products are formed with or without rearrangement.

The purity and structural identity of ammonio diphosphates 7 and 8 (mono NH_4^+ salts) were established by ^1H, ^{13}C, and ^{31}P NMR spectra as well as low- and high-resolution mass spectral data.[22] The ^{31}P NMR spectra exhibit two doublets (δ_p −5.4 to −5.8, −9.4 to −10.0 ppm; J = 21–22 Hz) for the primary diphosphate groups. The absence of other signals shows that the excess diphosphate ion has been completely removed. Although 7 must exist as a pair of diastereomeric salts, no peak doubling is seen in any of its NMR spectra, presumably owing to rapid interconversion of the isomers. The elemental composition was verified by low-resolution, negative-ion FAB-MS ($M^{2-} + H^+$),[22] and low- and high-resolution, positive-ion FAB-MS ($M^{2-} + 3H^+$). The combustion analytical data (C, H, N, P) are reasonably consistent with the calculated compositions for predominantly monoammonium salts.[23]

Results of Preliminary Inhibition Evaluation. Preliminary evaluations of the potency of 7 and 8 as inhibitors of squalene and GGPP synthases were performed in collaborations with Poulter (University of Utah) and with Sagami and Ogura (Tohoku University, Sendai, Japan). Aza analog 7 inhibited squalene synthase from yeast[5b,24] with IC_{50} ca. 20–25 μM. This level of inhibition is similar to those of three cyclopropylammonium analogs of 2 (IC_{50} = 3–10 μM)[7] but considerably less than that of the *O*-methyl phosphinylmethanephosphonate derivative of farnesol.[25]

Aza analog 8 inhibited GGPP synthase activity in incubations with a highly purified protein fraction isolated from rat liver and with crude supernatant extracts from rat liver and brain.[15,26] The inhibition was highly selective for GGPP synthase in comparative assays with FPP and GGPP synthases. The larger inhibiting effect of 8 on GGPP synthase activity in the crude supernatant fractions compared to that of GGPP itself provides a further validation of the aza analog concept and a measure of support for the assumption of a carbocationic mechanism for this prenyl transferase reaction.

Experimental Section

General Procedures. Melting points were determined in open ended capillary tubes and are uncorrected. All δ values in ^{31}P NMR spectra are reported in ppm using 85% H_3PO_4 as an external standard. In FAB-MS analyses the maxtrix was "magic bullet" (MB): 90% 3:1 dithiothreitol–dithioerythritol/10% methanol. GC analyses were carried out on a 30-m DB-5 capillary column. Flash chromatography was performed on Woelm 32–64-μm silica gel, and TLC was conducted on Merck glass plates precoated with 0.25 mm of silica gel 60 F-254. Cellulose chromatography was performed on Whatman CF-11 fibrous cellulose which was prepared according to the literature procedure,[19c] and TLC was carried out on Merck glass plates precoated with 0.1 mm of cellulose. Dowex 50W-X8 cation-exchange resin (100–200 mesh, H form) was purchased from Bio-rad, and the ammonium form of the resin was generated by the literature procedure.[19c] All products except the ammonio diphosphates 7 and 8 were obtained as colorless oils.

***N*-Cyclohexyl-*N'*,*N'*-dimethylmethanamidine:**[16] yield 48.4 g (77%); bp 80–85 °C (3 mm) [lit.[16] bp 90–110 °C (12 mm)].

(*E*)-*N*-Methyl-4,8-dimethyl-3,7-nonadien-1-amine (12) was synthesized using a modified general procedure of Meyers and Hoeve.[16] A solution of 11.20 g (72.6 mol) of the preceding formamidine in 250 mL of dry THF was stirred and cooled at −78 °C under N_2 as 56.6 mL (83.5 mmol) of 1.65 M *tert*-butyllithium in pentane was added via syringe. The solution was stirred at −25 °C for 1 h and then recooled to −78 °C prior to the dropwise addition of 11.40 g (66.0 mmol) of geranyl chloride in 20 mL of dry THF. The solution was allowed to warm to rt. After 1 h, 60 mL of a 2:1 mixture of methanol–water was added and most of the organic solvent was evaporated under reduced pressure. The concentrate was dissolved in 200 mL of methanol, 50 mL of water containing 18.5 g of KOH was added, and the heterogeneous mixture was heated under reflux overnight. After concentration under reduced pressure to ca. 100 mL, another 150 mL of water was added and the aqueous solution was extracted with four 150-mL portions of CH_2Cl_2. The combined extracts were evaporated. Distillation of the resulting yellow oil through a 10-cm Vigreux column gave 8.53 g (71%) of amine 12: bp 61–63 °C (0.3 mm); IR (film) 3420, 2930, 2854, 2781, 1653, 1576, 1450, 1363, 1232, 1112, 1041, 908 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.1 (br, 1 H, NH), 1.58 and 1.61 (2 s, 6 H, $=C(CH_3)_2$), 1.66 (s, 3 H, CH_3), 2.01–2.23 (m, 6 H, $3CH_2$), 2.41 (s, 3 H, NCH_3), 2.56 (t, 2 H, J = 7 Hz, CH_2N), 5.05–5.15 (m, 2 H, 2 =CH); ^{13}C NMR ($CDCl_3$), 50 MHz) δ 15.97, 17.55, 25.58, 26.51, 28.38, 36.43, 39.68, 51.84, 121.86, 124.16, 131.22, 136.85; MS 181 (14), 69 (51), 68 (12), 67 (15), 52 (13), 44 (31), 43 (100), 42 (12), 40 (18). Anal. Calcd for $C_{12}H_{23}N$: C, 79.49; H, 12.79; N, 7.72. Found: C, 79.03; H, 12.58; N, 7.51.

Ethyl 2-[*N*-Methyl-*N*-((*E*)-4,8-dimethyl-3,7-nonadienyl)-amino]ethanoate (13a). A solution of 1.63 g (9.00 mmol) of amine 12 and 1.38 g (9.90 mmol) of triethylamine in 10 mL of

(22) Davisson, V. J.; Sharp, T. R.; Poulter, C. D. *Bioorg. Chem.* 1988, *16*, 111.

(23) Values for carbon content were low (2–4.6% of total C) and nitrogen values deviated in both directions (±15–24% of total N assuming monoammonium salt).

(24) Unpublished results by D. Zhang and C. D. Poulter. Squalene synthase activity was assayed in 50 mM MOPS buffer containing 3% between 80 and 50 μM [^3H]FPP at pH 7.2 in the presence and absence of 1 mM diphosphate ion. If the IC_{50} values of 20–25 μM observed for (±)-7 are assumed to be associated with the *R* enantiomer only, the IC_{50} value for (*R*)-7 is ca. 10–12 μM.

(25) Biller, S. A.; Sofia, M. J.; DeLange, B.; Forster, C.; Gordon, E. M.; Harrity, T.; Rich, L. C.; Ciosek, C. P., Jr. *J. Am. Chem. Soc.* 1991, *113*, 8522–8524.

(26) Unpublished results by T. Korenaga, H. Sagami, and K. Ogura, Institute for Chemical Reaction Science, Tohoku University. The standard assay mixture contained 50 mM phosphate buffer (pH 7.0), 5 mM $MgCl_2$, 2 mM dithiothreitol, 20 μM FPP (or other allylic diphosphate substrates), 25 μM [1-^{14}C]IPP (56 Ci/mol), and a suitable amount of enzyme. Incubations were conducted at 37 °C for 2 h and terminated by heating at 55 °C for 5 min. The enzymatic products were hydrolyzed with alkaline phosphatase, and the liberated alcohols were extracted with hexane and separated by normal-phase and reversed-phase TLC. The radioactivity in the hexane extracts was measured by liquid scintillation counting, and that on TLC plates was determined with a radiochromatoscanner or by autoradiography.

dry THF was stirred and cooled in 0 °C under N_2 as 1.05 mL (9.45 mmol) of ethyl bromoacetate was added via syringe over 10 min. After 1 h at 0 °C, 50 mL of dry ether was added. The resulting precipitate of triethylammonium bromide was filtered, and the solvents were evaporated under reduced pressure. The remaining oil was filtered through 50 g of silica gel in 1:1 ether–pentane. The colorless oil obtained was dried under high vacuum for 2 h. Further purification by Kugelrohr distillation (150–170 °C oven temperature (0.4 mm)) gave 2.17 g (90%) of amino ester **13a**: R_f = 0.22 (ether/hexane (1:4)); IR (film) 2970, 2922, 1740, 1447, 1375, 1281, 1240, 1180, 1123, 1059, 1032 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.28 (t, 3 H, J = 7.1 Hz, CH_2CH_3), 1.60 and 1.61 (2 s, 6 H, $=C(CH_3)_2$), 1.68 (s, 3 $=C(CH_3)$), 2.01–2.21 (m, 6 H, $3CH_2$), 2.40 (s, 3 H, NCH_3), 2.49 (t, 2 H, J = 7.4 Hz, CH_2N), 3.27 (s, 2 H, CH_2CO), 4.19 (q, 2 H, J = 6.9 Hz, CH_2CH_3), 5.05–5.15 (m, 2 H, 2 =CH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.22, 14.29, 16.02, 17.65, 25.65, 26.16, 26.63, 39.67, 56.97, 58.51, 60.39, 121.41, 124.21, 131.29, 136.45, 170.96; MS 267 (10), 194 (39), 131 (50), 130 (100), 102 (41), 81 (18), 74 (20), 69 (97), 68 (10), 67 (13). Anal. Calcd for $C_{16}H_{29}NO_2$: C, 71.86; H, 10.93; N, 5.24. Found: C, 72.53; H, 10.72; N, 4.90.

1,1-Dimethylethyl 2-[*N*-Methyl-*N*-(4,8-dimethyl-3(*E*),7-nonadienyl)amino]ethanoate (13b). The preceding procedure for **13a** was followed using 2.11 g (11.65 mmol) of amine **12** and 1.97 mL (12.23 mmol) of *tert*-butyl bromoacetate. Kugelrohr distillation at 150–160 °C (0.5 mm) gave 3.20 g (93%) of amino ester **13b**: R_f = 0.51 (ether/hexane (1:1)); IR (film) 2976, 2928, 2860, 2797, 1749, 1736, 1452, 1367, 1290, 1252, 1215, 1152, 1060, 841 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.47 (s, 9 H, $C(CH_3)_3$), 1.60/1.61 (2 s, 6 H, $=C(CH_3)_2$), 1.67 (s, 3 H, $=C(CH_3)$—), 1.90–2.25 (m, 6 H, $3CH_2$), 2.39 (s, 3 H, NCH_3), 2.48 (t, 2 H, J = 5 Hz, CH_2N), 3.17 (s, 2 H, CH_2CO), 5.07–5.11 (m, 2 H, 2 =CH—); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 15.99, 17.63, 25.64, 26.52, 26.62, 28.11, 39.66, 42.19, 56.74, 59.11, 80.68, 121.57, 124.22, 131.20, 136.25, 170.21; MS 295 (9), 195 (15), 194 (100), 159 (38), 158 (100), 103 (69), 102 (100), 81 (24), 74 (39), 69 (100), 67 (21), 58 (21), 57 (95). Anal. Calcd for $C_{18}H_{33}NO_2$: C, 73.17; H, 11.26; N, 4.74. Found: C, 73.20; H, 11.23; N, 4.73.

Ethyl 2-[*N*-Methyl-*N*-(4,8-dimethyl-3(*E*),7-nonadienyl)amino]-5,9,13-trimethyl-4(*E*),8(*E*),12-tetradecatrienoate (14a). Alkylation of amino ester **13a** (306 mg, 1.153 mmol) with farnesyl bromide (395 mg, 1.394 mmol) in THF/HMPA by a procedure similar to that described below for **14b** afforded 235 mg (43%) of amino ester **14a**: R_f = 0.35 (ether/hexane (1:9)); IR (film) 2969, 2920, 2853, 1730, 1445, 1375, 1152, 1096, 1030; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.26 (t, 3 H, J = 7.1 Hz, CH_2CH_3), 1.60, 1.61, and 1.63 (3 s, 15 H, 5 CH_3), 1.68 (s, 6 H, 2 CH_3), 1.95–2.62 (m, 18 H, 9 CH_2), 2.35 (s, 3 H, NCH_3), 3.25 (dd, 1 H, J = 6.4, 8.7 Hz, CHCO), 4.15 (q, 2 H, J = 7.1 Hz, CH_2CH_3), 5.09 (m, 5 H, 5 =CH—); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.52, 15.97, 16.01, 16.20, 17.69, 25.71, 26.72, 28.59, 38.35, 39.72, 39.81, 54.30, 59.96, 66.62, 120.03, 121.76, 124.07, 124.30, 124.36, 131.25, 131.35, 135.00, 136.20, 137.38, 172.43.

(4*E*,8*E*)-1,1-Dimethylethyl 2-[*N*-Methyl-*N*-(4,8-dimethyl-3(*E*),7-nonadienyl)amino]-5,9,13-trimethyl-4,8,12-tetradecatrienoate (14b). A solution of 4.77 g (16.1 mmol) of **13b** in 28.5 mL of THF and 5.7 mL of HMPA was stirred at −78 °C as 13.6 mL (20.4 mmol) of 1.5 M LDA in cyclohexane was added over 20 min. After the addition, the solution was stirred at 0 °C for 30 min and cooled to −78 °C before 6.80 g (23.8 mmol) of farnesyl bromide[17] in 14 mL of THF was added over 30 min. The solution was stirred at 0 °C for 4 h and at rt for 14 h, and quenched by adding 28 mL of saturated NH_4Cl. The solution was diluted with water (150 mL) and extracted with ether (150 mL × 3). The combined extracts were washed with water (300 mL) and saturated NaCl (300 mL). After the organic fraction was dried ($MgSO_4$) and concentrated, purification of the residue by repeated flash chromatographies using 6% ether in hexane as eluent gave 5.42 g (67%) of **14b**: IR (film) ν_{max} 2973, 2923, 2843, 1727, 1449, 1368, 1146 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9 H, $C(CH_3)_3$), 1.60, 1.62, and 1.63 (3s, 15 H, $5CH_3$), 1.68 (s, 6 H, $2CH_3$), 1.98–2.7 (m, 18 H, $9CH_2$), 2.36 (s, 3 H, NCH_3), 3.15 (dd, 1 H, J = 8.9, 6.3 Hz, CHCO), 5.07–5.14 (br m, 5 H, 5CH=); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.92, 16.06, 16.25, 17.65, 25.66, 26.62, 26.70, 26.77, 28.24, 28.87, 38.27, 39.67, 39.77, 54.17, 67.03, 80.45, 120.21, 121.86, 124.09, 124.26, 124.32, 131.20, 134.90, 136.00, 136.96, 171.74; LRFABMS (MB) m/z 500.2; HRFABMS (MB) calcd for $C_{33}H_{58}NO_2$ 500.44673, found 500.44580. Anal. Calcd for $C_{33}H_{57}NO_2$: C, 79.30; H, 11.50; N, 2.80. Found: C, 77.30; H, 11.26; N, 2.73.

(4*E*,8*E*)-2-[*N*-Methyl-*N*-[4,8-dimethyl-3(*E*),7-nonadienyl]amino]-5,9,13-trimethyl-4,8,12-tetradecatrien-1-ol (15). A suspension of 1.85 g (46.3 mmol) of 95% $LiAlH_4$ in 125 mL of ether was stirred at 0 °C as a solution of 7.61 g (15.2 mmol) of **14b** in 65 mL of ether was added. The mixture was stirred at rt for 2 h and cooled to 0 °C. Water (1.75 mL), 15% aqueous NaOH (1.75 mL), and water (4.5 mL) were added in succession. The insoluble salts were removed by filtration, and the filtrate was dried ($MgSO_4$) and concentrated. Purification of the resulting oil by chromatography on 200 g of silica gel using ether–hexane (1:1 to 3:1) as eluent followed by drying under high vacuum at 50 °C for 2 h gave 6.23 g (95%) of **15**: IR (film) ν_{max} 3451, 2967, 2921, 2853, 1447, 1377, 1149 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.60 and 1.62 (2s, 15 H, $5CH_3$), 1.68 (s, 6 H, $2CH_3$), 1.76–2.19 (m, 17 H, $8CH_2$ and OH), 2.26 (s, 3 H, NCH_3), 2.35–2.43 and 2.54–2.60 (2m, 2 H, NCH_2), 2.67–2.74 (m, 1 H, NCH), 3.22 (t, 1 H, J = 10.4 Hz, CH_2O), 3.45 (dd, 1 H, J = 10.4, 5.0 Hz, CH_2O), 5.04–5.14 (br m, 5 H, 5CH=); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.90, 15.97, 16.05, 17.60, 23.47, 25.63, 26.42, 26.59, 26.64, 27.05, 36.06, 39.64, 39.71, 53.26, 60.77, 64.94, 120.96, 121.75, 123.92, 124.18, 124.27, 131.13, 131.23, 135.01, 136.57, 136.63; LRFABMS (MB) m/z 430.3; HRFABMS (MB) calcd for $C_{29}H_{52}NO$ 430.40487, found 430.40440. Anal. Calcd for $C_{29}H_{51}NO$; C, 81.05; H, 11.96; N, 3.26. Found: C, 80.39; H, 12.07; N, 3.19.

***N*-Methyl-4,8,12-trimethyl-3(*E*),7(*E*),11-tridecatrien-1-amine (17).** Amine **17** was prepared from **19c**, *N*-cyclohexyl-*N'*,*N'*-dimethylformamidine (2.57 g, 16.66 mmol), and farnesyl chloride (3.65 g, 14.99 mmol) in 50 mL of dry THF as described above for amine **12**. Distillation of the slightly yellow oil resulting from hydrolysis of the alkylated amidine through a 10-cm Vigreux column gave 3.12 g (84%) of **17**: bp 112–114 °C (0.2 mm); IR (film) 2964, 2923, 2788, 1685, 1444, 1376, 1106 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.15 (br, 1 H, NH), 1.60 (s, 6 H, $=C(CH_3)_2$), 1.63 and 1.68 (2s, 6 H, $2=C(CH_3)$), 2.02–2.22 (m, 10 H, $5CH_2$), 2.43 (s, 3 H, NCH_3), 2.58 (t, 2 H, J = 6.7 Hz, NCH_2), 5.05–5.2 (m, 3 H, 3 =CH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 16.01, 16.11, 17.67, 25.69, 26.52, 26.74, 28.47, 29.26, 36.50, 39.70, 51.93, 121.86, 124.08, 124.32, 131.26, 135.02, 137.04; MS 250 (9), 249 (44), 180 (22), 107 (11), 96 (11), 95 (17), 94 (10), 93 (27), 91 (15), 82 (12), 81 (49), 79 (22), 77 (15), 70 (18), 69 (100), 68 (63), 67 (58), 57 (14), 55 (34), 53 (43). Anal. Calcd for $C_{17}H_{31}N$: C, 81.86; H, 12.53; N, 5.61. Found: C, 81.59; H, 12.46; N, 5.40.

Ethyl 2-[*N*-Methyl-*N*-[(*E*,*E*)-4,8,12-trimethyl-3,7,11-tridecatrienyl]amino]ethanoate (18a). Alkylation of 3.11 g (12.48 mmol) amine **17** with 1.45 mL (13.10 mmol) of ethyl bromoacetate was carried out as described above for **13a**. Kugelrohr distillation at 160–180 °C (0.3 mm) gave 3.39 g (81%) of amino ester **18a**: R_f = 0.50 (ether/hexane (3:2)); IR (film) 2970, 2921, 2856, 1741, 1447, 1376, 1279, 1181, 1122, 1060, 1032 cm^{-1}; ^1H NMR ($CDCl_3$; 200 MHz) δ 1.28 (t, 3 H, J = 7.1 Hz, CH_2CH_3), 1.60 (s, 6 H, $=C(CH_3)_2$), 1.62 and 1.68 (2s, 6 H, 2 $=(CH_3)$), 2.0–2.25 (m, 10 H, $5CH_2$), 2.39 (s, 3 H, NCH_3), 2.50 (t, 2 H, J = 8.5 Hz, NCH_2), 3.26 (s, 2 H, CH_2CO), 4.19 (q, 2 H, J = 7.3 Hz, CH_2CH_3), 5.07–5.2 (m, 3 H, 3 =CH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.26, 15.98, 16.04, 17.66, 25.69, 26.17, 26.53, 26.71, 39.67, 42.39, 56.99, 58.51, 60.41, 121.40, 124.08, 124.34, 131.20, 134.94, 136.51, 170.96; MS 335 (10), 263 (11), 262 (53), 131 (97), 130 (100), 102 (53), 81 (31), 79 (11), 74 (52), 70 (13), 69 (100), 68 (33), 67 (33), 59 (50), 58 (70), 57 (100). Anal. Calcd for $C_{21}H_{37}NO_2$: C, 75.17; H, 11.12; N, 4.18. Found: C, 74.78; H, 10.87; N, 4.27.

1,1-Dimethylethyl 2-[*N*-Methyl-*N*-[(*E*,*E*)-4,8,12-trimethyl-3,7,11-tridecatrienyl]amino]ethanoate (18b). Alkylation of 4.96 g (19.88 mmol) of amine **17** with 3.37 mL (20.9 mmol) of *tert*-butyl bromoacetate was carried out as described above for **13b**. Filtration of the crude product with ether/pentane (1:2) through 100 g of silica gel gave after drying under high vacuum for 2 h 6.57 g (91%) of amino ester **18b**: R_f = 0.25 (ether/pentane (1:3)); IR (film) 2969, 1727, 1668, 1447, 1366, 1289, 1152, 1059 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.47 (s, 9 H, $C(CH_3)$), 1.95–2.10 (m, 8 H, $4CH_2$), 2.17–2.20 (m, 2 H, NCH_2CH_2), 2.39 (s, 3 H, NCH_3), 2.46–2.52 (m, 2 H, NCH_2CH_2), 3.17 (s, 2 H, CH_2CO), 5.05–5.15 (m, 3 H, 3 =CH); ^{13}C NMR ($CDCl_3$, 50 MHz)

δ 15.98, 16.03, 17.66, 25.68, 26.28, 26.54, 26.71, 28.12, 39.68, 42.22, 56.80, 59.15, 80.70, 121.57, 124.10, 124.35, 131.15, 134.89, 136.33, 170.25; FAB-MS (MB) *m/z* 364 (13), 306 (39), 262 (35), 158 (35), 124 (23), 102 (100). Anal. Calcd for $C_{23}H_{41}NO_2$: C, 75.98; H, 11.37; N, 3.85. Found: C, 75.84; H, 11.29; N, 3.84.

2-[*N*-Methyl-*N*-[(*E*,*E*)-4,8,12-trimethyl-3,7,11-tridecatrienyl]amino]ethan-1-ol (19). Reduction of 1.69 g (5.03 mmol) of **18a** with 400 mg (10.54 mmol) of $LiAlH_4$ as described above for **14b** followed by Kugelrohr distillation at 160–170 °C (0.2 mm) gave 1.41 g (96%) of amino alcohol **19**: IR (film) 3432, 2899, 1667, 1445, 1375, 1281, 1215, 1037, 875 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.60 (s, 6 H, $=C(CH_3)_2$), 1.62 and 1.68 (2s, 6 H, $=C(CH_3)$), 1.95–2.20 (m, 10 H, $5CH_2$), 2.27 (s, 3 H, NCH_3), 2.40–2.45 (m, 2 H, NCH_2), 2.53 (t, 2 H, *J* = 5.4 Hz, NCH_2CH_2OH), 2.96 (br, 1 H, OH), 3.57 (t, 2 H, *J* = 5.3 Hz, CH_2OH), 5.0–5.15 (m, 3 H, 3 =CH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 15.98, 16.09, 17.68, 25.69, 25.98, 26.56, 26.72, 39.69, 41.54, 57.49, 58.32, 58.55, 121.67, 125.07, 124.36, 131.20, 134.97, 136.49; MS 294 (6), 293 (7), 262 (28), 126 (12), 124 (12), 110 (11), 109 (11), 107 (12), 95 (22), 93 (22), 91 (13), 90 (15), 89 (100), 88 (100), 86 (28), 81 (65), 69 (100). Anal. Calcd for $C_{19}H_{35}NO$: C, 77.75; H, 12.02; N, 4.77. Found: C, 77.54; H, 11.97; N, 4.89.

(4*E*,8*E*)-2-[*N*-Methyl-*N*-(4,8-dimethyl-3(*E*),7-nonadienyl)ammonio]-5,9,13-trimethyl-4,8,12-tetradecatrien-1-yl diphosphate ammonium salt (7·NH_4) was prepared according to the procedure of Poulter et al.[19a] A solution of 332 mg (0.77 mmol) of alcohol **15** and 162 μL (1.16 mmol) of triethylamine in 8 mL of CH_2Cl_2 was stirred and cooled at −15 °C as 72 μL (0.93 mmol) of methanesulfonyl chloride was added. After 30 min, the solution was diluted with ice-cold CH_2Cl_2 (7 mL) and washed with ice-cold saturated $NaHCO_3$ (15 mL). The aqueous layer was extracted with CH_2Cl_2 (10 mL), and the combined extracts were washed with ice-cold saturated NaCl (10 mL). The solution was dried ($MgSO_4$), concentrated to ~8 mL, diluted with 15 mL of anhydrous acetonitrile, and concentrated at ≤35 °C to a volume of 8 mL.

A solution of 1.453 g (1.59 mmol) of $(Bu_4N)_3HP_2O_7$ in 30 mL of acetonitrile was concentrated to 8 mL and stirred at −15 °C as the mesylate solution above was added over 15 min. The mixture was stirred at −15 °C for 1 h and at rt for 14 h before concentrating under reduced pressure at ≤35 °C. The remaining oily residue was evacuated for 10 min, dissolved in 6 mL of 20% aqueous 2-propanol, and loaded on a Dowex 50W-X8 cation exchange column (NH_4^+ form, 100–200 mesh, 2-cm i.d. × 22 cm). The column was eluted with 25 mM aqueous NH_4HCO_3 (flow rate, 10 mL/20 min). The fractions containing iodine-active materials (4–9) were combined and lyophilized. Inorganic diphosphate was partially removed by three repeated extractions of the solid (749 mg) in 0.1 M aqueous NH_4HCO_3 (6 mL, 4.5 mL, and 3 mL in each extraction) with a mixture (1:1) of acetonitrile–2-propanol (20 mL, 15 mL, and 10 mL in each extraction). Although the third extract did not contain any iodine-active materials, all three supernatant extracts were combined, evaporated at 35 °C, and lyophilized. The residue (577 mg) was adsorbed onto cellulose by dissolving it in a small amount of water, lyophilized with a small amount of cellulose, and loaded onto a cellulose column (2.5-cm i.d. × 42 cm; Whatman CF11 medium-grade cellulose). The column was eluted (flow rate, 8–9 mL/15 min) with 2-propanol–acetonitrile–water–concd NH_4OH (5:3:1:1) as eluent (flow rate, 9 mL/6 min). Each fraction (~9 mL) was analyzed by silica gel TLC using 2-propanol–$CHCl_3$–acetonitrile–0.1 M aqueous NH_4HCO_3 (5:1:2:2) as developing solvent. Three fractions (41–52, 53–67, and 68–85) were collected and concentrated at 35 °C to ~5 mL each, and the remaining aqueous solutions were lyophilized. The first fraction (41–52, 102 mg) and second fraction (53–67, 221 mg) were mixtures of primary and secondary diphosphates as judged by ^1H NMR analysis. In addition, these fractions contained an unknown phosphorus-containing impurity, which was probably 1,2-dialkylated diphosphate, based on its ^{31}P NMR spectrum. The third fraction (68–85, 141 mg, 30%) was free of the unknown impurity, and the amount of **25** was estimated to be ≤5%. The physical data for **7** follow: ^1H NMR (300 MHz, CD_3OD) δ 1.60 (s, 9 H, $3CH_3$), 1.66 (s, 6 H, $2CH_3$), 1.69 (s, 6 H, $2CH_3$), 1.87–2.11 (m, 12 H, 6 CH_2), 2.28–2.59 (m, 4 H, $2CH_2$), 2.81 (s, 3 H, NCH_3), 2.97–3.12 (m, 2 H, NCH_2), 3.58 (br m, 1 H, NCH), 4.09–4.19 (br m, 2 H, CH_2O), 5.07–5.18 (m, 5 H, 5CH=); ^{13}C NMR (75 MHz, CD_3OD) δ 16.1, 16.4, 16.6, 17.8, 23.9, 24.5, 25.9, 27.6, 27.6, 27.8, 37.2, 40.8, 40.9, 54.7, 63.8, 63.9, 65.1, 65.2, 65.2, 119.2, 119.9, 125.1 (2 C), 125.4, 132.1, 132.4, 136.3, 140.3, 141.2; ^{31}P NMR (121.5 MHz, CD_3OD) δ −5.77 (br d, *J* = 20.6 Hz), −9.37 (br d, *J* = 20.6 Hz); negative-ion FABMS (glycerol) *m/z* 588.2; positive-ion LRFABMS (MB) *m/z* 590.3; HRFABMS (positive ion) calcd for $C_{29}H_{54}NO_7P_2$ 590.3375, found 590.3375. Anal. Calcd for $C_{29}H_{56}N_2O_7P_2$: C, 57.41; H, 9.30; N, 4.62; P, 10.21. Found: C, 54.81; H, 9.40; N, 5.71; P, 10.43.

In an earlier run 425 mg (0.989 mmol) of amino alcohol **15** was converted to the diphosphate as described above. Purification by chromatography on 10 g of QAE-Sephadex (HCO_3^- form) instead of cellulose using 2-propanol–acetonitrile–0.1 M aqueous NH_4HCO_3 (2:1:1) as eluant gave, after combining fractions and lyophilization, 131 mg (44%) of a 3:1 mixture of isomers **7** and **25** as a colorless, hygroscopic solid: R_f = 0.23 (2-propanol–$CHCl_3$–acetonitrile–0.1 M aqueous NH_4HCO_3 (5:1:2:2)); ^1H NMR (D_2O, 300 MHz) δ 1.52 (s, 9 H, $3CH_3$), 1.59 and 1.62 (2s, 12 H, $4CH_3$), 1.85–2.07 (m, 12 H, $6CH_2$), 2.20–2.50 (m, 4 H), 2.76 (s, 3 H, NCH_3), 2.95 (m, 1 H), 3.10 (m, 2 H), 3.60 and 4.05 (2m, 2 H), 4.95–5.15 (m, 5 H, 5=CH); ^{31}P NMR (D_2O, 121.5 MHz) δ −11.02 ($^1/_4P$, d, *J* = 20.9 Hz), −10.58 ($^3/_4P$, d, *J* = 20.0 Hz), −6.52 ($^3/_4P$, d, *J* = 20.9 Hz), −5.76 ($^1/_4P$, d, *J* = −21.4 Hz); negative ion FAB-MS (MB) *m/z* 588.3 ($M^2 + H^+$, 40), 238.9(24), 189.0(25), 177.0(32), 159.0(100).

Tetra-*n*-butylammonium acetate was prepared following a procedure of Kabalka.[27] The product was recrystallized under N_2 from a minimum amount of dry acetonitrile and filtered under N_2 to give 10.32 (58%) of the acetate salt in two crops of colorless needles (mp 114–116 °C (lit.[27] 114–115 °C)).

Amino Acetates 23 and 24. Reaction of amino alcohol **15** (243 mg, 0.565 mmol) and 53 μL (0.679 mmol) of methanesulfonyl chloride was carried out as described above for preparation of **7**. Extraction and solvent exchange gave a solution of the mesylate/chloride mixture (**20**, **21**, and/or **22**) in 4 mL of acetonitrile. This solution was transferred into a cooled flask containing 341 mg (1.131 mmol) of tetrabutylammonium acetate. The solution was stirred at 0 °C for 1 h and at rt for 17 h and then poured into 25 mL of 10% aqueous $NaHCO_3$. Extraction with two 25-mL portions of ether followed by drying ($MgSO_4$) and evaporation gave a yellow oil. Flash chromatography with ether/hexane (1:4) gave 78.2 mg (29%) of **23** and 8.2 mg (3%) of **24**. The mixed fractions were separated by another flash chromatography, and 29 mg (11%) of isomer **19** and 4.4 mg (1.7%) of isomer **20** and 36.3 mg (14%) of mixed fractions were obtained. In the first chromatography also 40 mg (16%) of chloride **21** (X = Cl) was isolated as a byproduct: R_f = 0.90 (ether/hexane (3:7)).

Data for **23**: R_f = 0.47 (ether/hexane (3:7)); IR (film) 2967, 2921, 2851, 1744, 1449, 1375, 1237, 1105, 1038, 980, 837 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.60 (s, 15 H, $5CH_3$), 1.68 (s, 6 H, $2CH_3$), 1.90–2.20 (m, 16 H, $8CH_2$), 2.05 (s, 3 H, $COCH_3$), 2.32 (s, 3 H, NCH_3), 2.50 (m, 2 H, NCH_2), 2.84, 3.99, and 4.15 (AMX-spin system, 3 H, J_{AM} = 11.5, J_{MX} = 7.1, J_{AX} = 4.9 Hz, $CHCH_2O$), 5.06–5.19 (m, 5 H, 5=CH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 15.97, 16.09, 17.66, 21.08, 25.68, 26.14, 26.52, 26.70, 26.72, 27.08, 37.58, 39.69, 39.73, 39.78, 54.13, 61.89, 63.88, 121.44, 121.95, 124.04, 124.29, 125.36, 131.16, 131.25, 134.99, 136.04, 136.58, 171.04.

Data for **24**: R_f = 0.42 (ether/hexane (3:7)); IR (film) 2967, 2918, 2851, 1738, 1449, 1374, 1239, 1177, 1107, 1030, 943, 895, 835 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.60 (s, 15 H, $5CH_3$), 1.68 (s, 6 H, $2CH_3$), 1.9–2.2 (m, 16 H, $8CH_2$), 2.03 (s, 3 H, $COCH_3$), 2.25–2.55 (m, 4 H, $2NCH_2$), 2.27 (s, 3 H, NCH_3), 5.0 (m, 1 H, CHO), 5.06–5.15 (m, 5 H, 5=CH).

2-[*N*-Methyl-*N*-[4,8,12-trimethyl-3(*E*),7(*E*),11-tridecatrienyl]ammonio]ethyl Diphosphate, Monoammonium Salt (8·NH_4). Amino alcohol **19** (294 mg, 1.0 mmol) was converted to the mesylate/chloride **26** (0 °C, 35 min), and the latter was displaced with $(nBu_4N)_3HP_2O_7$ in acetonitrile (rt, 5 h) as described above for **7**. Ion exchange and ammonium diphosphate precipitation were carried out according to procedures, similar to those presented above and in ref 19a. The diphosphate was purified by two chromatographies on cellulose eluting with 2-propanol–H_2O–concd NH_4OH (8:1:1) and 2-propanol–$CHCl_3$–aceto-

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nitrile/0.1 M aqueous NH_4HCO_3 (5.5:2:1:1.5), respectively. Lyophilization of appropriate fractions provided 177 mg (40%) of diphosphate 8 which was characterized by appropriate ^1H, ^{13}C, and ^{31}P NMR spectra; negative ion-LRFABMS; positive ion FABMS (LR and HR); and combustion analysis for C, H, N, P.[23] Selected data follow: ^1H NMR (300 MHz, D_2O–ND_4OD) δ 1.57 (s, 6 H, $=C(CCH_3)_2$), 1.64 (s, 6 H, $2CH_3$), 1.95–2.11 (m, 8 H, $4CH_2$), 2.43 (br m, 2 H, CH_2), 2.80 (s, 3 H, NCH_3), 3.04 (br, 2 H, NCH_2), 3.30 (br t, 2 H, NCH_2), 4.18 (br, 2 H, CH_2O), 5.13 (m, 3 H, 3CH=); ^{31}P NMR (121.5 MHz, D_2O–ND_4OD, pH 8~9) δ –5.55 (br), –10.03 (br); ^{31}P NMR (121.5 MHz, 1 w/v% EDTA in D_2O–ND_4OD, pH 8~9) δ –5.35 (d, 0.63 P, J = 22 Hz), –9.80 (d, 1 P, J = 22 Hz); calcd for $C_{19}H_{38}NO_7P_2$ 454.2123, found 454.2115. A complete procedure and all characterization data for 8 are provided in ref 15.

Acknowledgment. We wish to thank C. D. Poulter, H. Sagami, and K. Ogura for their collaboration on the enzyme evaluations and for their agreement to citation of the preliminary results in this manuscript. We are also grateful to the National Institutes of Health for financial support.

Supplementary Material Available: ^1H NMR spectra of compounds 7, 8, 14a, 23, and 24 (7 pages). Ordering information is given on any current masthead page.

Carriers for Liquid Membrane Transport of Nucleotide 5′-Triphosphates

Tingyu Li and François Diederich*

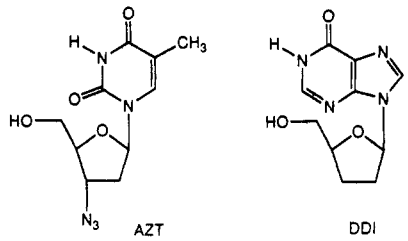
Department of Chemistry and Biochemistry, University of California at Los Angeles, Los Angeles, California 90024-1569

Received February 5, 1992

The transport of the nucleotide triphosphates adenosine 5′-triphosphate (ATP), cytidine 5′-triphosphate (CTP), 2′,3′-dideoxythymidine 5′-triphosphate (ddTTP), and 3′-azido-2′-deoxythymidine 5′-triphosphate (AZTTP) across a liquid organic membrane mediated by cationic DABCO-derived carriers was studied. With their branched aliphatic chains, these compounds, which bear two (2), three (3), or four (4) quaternary ammonium centers show excellent solubility in chloroform, the solvent chosen as liquid organic membrane. The bisquaternary mono(DABCO) derivative **2** was found to be the most efficient carrier for all nucleotides. Concentration-dependent extraction studies showed that **2** undergoes formation of a 2:1 carrier–nucleotide complex in the chloroform phase. This stoichiometry differs from the 1:1 stoichiometry previously suggested by Tabushi et al. for the association between nucleotide 5′-triphosphates and a bisquaternary DABCO derivative. The tricationic compound **3** is generally inferior to **2** in its carrier properties but shows an unexpectedly high selectivity for transporting ddTTP. The tetracationic bis(DABCO) derivative **4** shows poor carrier properties since it forms highly water-soluble associations which prefer distribution into the aqueous phase rather than into the liquid membrane.

Introduction

Recently, 2′,3′-dideoxynucleotide 5′-triphosphates have been the focus of attention because of their application in the treatment of AIDS.[1,2] Some of these compounds have been shown to act as potent chain-terminating inhibitors of HIV reverse transcriptase, a prime target in AIDS therapy.[2,3] However, due to their highly charged nature, the triphosphates cannot penetrate across cell membranes. Therefore, the corresponding nucleosides, instead of the nucleotide triphosphates, are administered to patients. In fact, two members of the family of dideoxynucleosides, namely 3′-azido-2′-deoxythymidine (AZT) and 2′,3′-dideoxyinosine (DDI), are now approved for AIDS therapy.[4]



To serve as inhibitors for the HIV reverse transcriptase, the nucleosides must be transformed into nucleotide triphosphates through the action of cellular nucleoside kinases. However, this transformation may not be very efficient, at least in the case of AZT.[5] It is imaginable that the development of an efficient transmembrane carrier could allow the dideoxynucleotide triphosphates to be directly administered to patients, therefore avoiding the need for high concentrations of the modified nucleosides which is problematic in view of their toxicity.[6]

The development of artificial carriers for nucleotide 5′-triphosphate transport has not received much attention in the past.[7,8] Among the early work is the report by

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