in 5 mL of acetonitrile was added to a solution of 720 mg (5.4 mmol) of *N*-chlorosuccinimide and 1.03 g (6.1 mmol) of silver nitrate in 25 mL of 8:2 acetonitrile–water at −5 °C.[47] After being stirring for 0.5 h at −5 °C and 1 h at room temperature, the mixture was treated with 30 mL of ether and then 3 mL of a saturated aqueous solution of sodium sulfite. The crude product was isolated with ether and was purified by silica gel chromatography with 30% ether in pentane to yield 283 mg (72%) of **24b*** having IR and ^{1}H NMR spectra identical with those of the previously obtained racemic **24b**. Lactone **24b***: $[\alpha]^{20}_D$ −45° (*c* 5, dichloromethane).

Anal. Calcd for $C_{17}H_{24}O_4$: C, 69.83; H, 8.27. Found: C, 70.25; H, 8.40.

(1*R*,2*E*,6*S*,10*E*,11a*S*,13*S*,14a*R*)-1,6,7,8,9,11a,12,13,14,14a-Decahydro-1-hydroxy-13-methoxy-6-methyl-4*H*-cyclopent[*f*]oxacyclotridecin-4-one (24c*). A 253-mg (0.87 mmol) sample of **24b*** in 9 mL of methanol at −110 °C was treated with 40 mg (1.05 mmol) of sodium borohydride and then stirred for 45 min at −110 °C. Acetone (500 μL) was then added, and the reaction mixture was allowed to warm to room temperature. The crude product was isolated with ethyl acetate and was purified by medium-pressure liquid chromatography with 2% methanol in dichloromethane to give 243 mg (95%) of **24c***: mp 82–82.5 °C (ether–pentane); $[\alpha]^{20}_D$ +92° (*c* 3, dichloromethane). The IR (film) and ^{1}H NMR spectra of **24c*** were identical with those of the previously obtained racemic **24c**.

Anal. Calcd for $C_{17}H_{26}O_4$: C, 69.36; H, 8.90. Found: C, 69.27; H, 8.76.

(1*R*,2*E*,6*S*,10*E*,11a*S*,13*S*,14a*R*)-1,6,7,8,9,11a,12,13,14,14a-Decahydro-1,13-dihydroxy-6-methyl-4*H*-cyclopent[*f*]oxacyclotridecin-4-one [(+)-Brefeldin-A] (1*). A 30-mg (0.1 mmol) sample of methyl ether **24c*** in 1 mL of acetonitrile was treated with 250 μL of a 20% solution of (*N*-trimethylsilyl)diethylamine in pentane and then left for 15 h at room temperature.[49] Removal of volatiles under reduced pressure left the crude silyl ether of **24c***. This residue was dissolved in 1.5 mL of dry acetonitrile and was treated, protected from light at room temperature under argon, with 45 mg (0.3 mmol) of dry sodium iodide and 40 μL (0.3 mmol) of freshly distilled chlorotrimethylsilane.[50] After the reaction mixture was stirred for 1 h, an additional 30 mg (0.2 mmol) of sodium iodide and 26 μL (0.2 mmol) of chlorotrimethylsilane were added, and stirring was continued for 2.25 h. Water (1 mL) and ethyl acetate (5 mL) were added to the mixture, which was then extracted with ethyl acetate. The organic phase was washed with sodium thiosulfate and brine and dried over magnesium sulfate. Removal of the solvent and rapid filtration of the residue over a small amount of silica gel with 50% methanol in methylene chloride afforded a mixture of **24c*** and **1***. Separation of the mixture by HPLC (Whatman Partisil M9 10/50 ODS2, 80% methanol–20% water; retention times: **1***, 12 min; **24c***, 18 min at 3 mL/min) afforded 5.2 mg (17%) of starting material **24c*** and 16.1 mg (56%, or 68% based on consumed **24c***)[66] of (+)-brefeldin-A (**1***): mp 204–205 °C (ethyl acetate) (lit.[1c] mp 204–205 °C); mmp 204–205 °C; $[\alpha]^{20}_D$ +93° (*c* 2, methanol) [authentic sample: +93° (*c* 2, methanol)]. The IR (KBr) and ^{1}H NMR (CD_3OD) spectra were identical in all respects with those of an authentic sample. In addition, an admixture of the synthetically and naturally derived compounds was chromatographically (TLC, HPLC) inseparable in a number of different solvent systems.

Anal. Calcd for $C_{16}H_{24}O_4$: C, 68.54; H, 8.63. Found: C, 68.61; H, 8.56.

Acknowledgment. We thank Professors P. Crabbé and A. Rassat and Dr. J.-L. Luche for their interest in this work and Drs. H. P. Sigg and A. von Wartburg (Sandoz A. G., Basel) for samples of natural brefeldin-A. This work was supported by the CNRS.

Registry No. 1, 62989-97-3; **1***, 20350-15-6; **7**, 82679-38-7; **7***, 82729-87-1; **10**, 694-98-4; **10***, 16620-79-4; **11**, 76101-85-4; **11***, 82729-81-5; **14a**, 76101-83-2; **14c** (R = C_4H_9), 76101-84-3; **15**, 1193-18-6; **16**, 21889-89-4; **17**, 928-39-2; **18a**, 69177-44-2; **18a***, 65756-08-3; **18b**, 82679-36-5; **18b***, 82729-82-6; (*E*)-**19**, 82679-37-6; (*E*)-**19***, 82729-83-7; **20**, 76110-77-5; **20***, 82729-84-8; **21a**, 76101-86-5; **21a***, isomer 1, 82729-85-9; **21a***, isomer 2, 82729-86-0; **21b**, 76101-87-6; **21b***, 82729-88-2; **22a**, 76101-88-7; **22a***, 82729-89-3; **22b**, 76101-89-8; **22b***, 82729-90-6; **22c**, 76101-90-1; **22c***, 82729-91-7; **23a**, 76101-91-2; **23a***, 82729-92-8; **23b**, 76101-92-3; **23b**, 2-pyridinethiol ester, 82679-39-8; **23b***, 82729-93-9; **23b***, 2-pyridinethiol ester, 82729-94-0; **24a**, isomer 1, 76101-93-4; **24a**, isomer 2, 82729-77-9; **24a***, isomer 1, 82729-95-1; **24a***, isomer 2, 82730-74-3; **24b**, isomer 1, 76101-94-5; **24b**, isomer 2, 82729-78-0; **24b***, 82729-96-2; **24c**, 76101-95-6; **24c***, 82729-97-3; **25***, 1517-7-5; **26***, 15754-50-4; **27***, 82679-40-1; **28***, 82691-61-0; **29a***, isomer 1, 82679-41-2; **29a***, isomer 2, 82679-42-3; **29b***, isomer 1, 82729-79-1; **29b***, isomer 2, 82729-80-4; 2-chloroacrylonitrile, 920-37-6; cyclopentadiene, 542-92-7; 2-chloro-2-cyanobicyclo[2.2.1]hept-5-ene, 6945-87-5; pentynylcopper, 19093-51-7; methyl methanethiosulfonate, 2949-92-0; ethyl (trimethylsilyl)acetate, 4071-88-9; 2-thiopyridyl chloroformate, 73371-99-0; acryloyl chloride, 814-68-6; 1-iodobutane, 542-69-8.

(66) In another run (30.5 mg), the yield of (+)-brefeldin-A (**1***) was 77% (63% conversion).

Nucleotidophospholipids: Oligonucleotide Derivatives with Membrane-Recognition Groups

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Contribution from the Department of Chemistry, State University of New York at Stony Brook, Stony Brook, New York 11794. *Received January 11, 1982*

Abstract: The synthesis of nucleotidophospholipids, a new type of structure, is reported. In these compounds, the primary or secondary alcohol functions of a dinucleotide, thymidylyl(3′→5′)thymidine, are esterified by the optically active 1,2-diacylglycerophosphoric acid having the chirality of the phospholipids present in biological membranes. The ultraviolet spectrum, circular dichroism, and optical rotatory dispersion of the nucleotidophospholipids in chloroform/methanol solution are described.

The synthesis of unsymmetrical phosphodiesters by means of cyclic enediol phosphoryl (CEP) derivatives involves three basic steps, which can be carried out in one or two laboratory operations depending on the complexity of the two alcohols that are to be joined by the phosphate bond.[1] In general, the first intermediate that is subjected to purification is the acyclic dialkyl 3-oxo-2-butyl phosphate.

Three CEP reagents have been developed to implement this synthetic strategy:[2] the crystalline pyrophosphate (CEP-O-CEP), the liquid phosphorochloridate (CEP-Cl), and the crystalline phosphoroimidazole (CEP-$N_2C_3H_3$). The pyrophosphate and phosphorochloridate are used in conjunction with triethylamine, which functions as Proton Sponge in the first step and as catalyst

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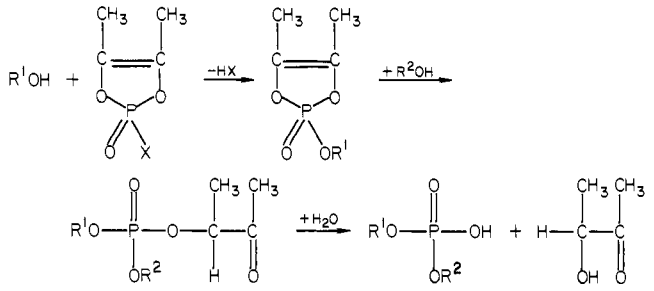
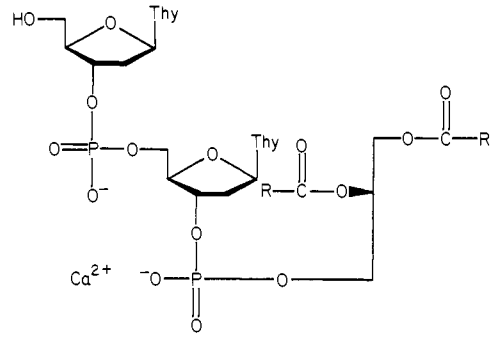
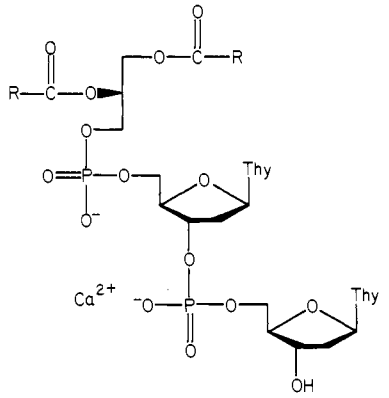


in the second step.[3] The byproduct, $(C_2H_5)_3NH^+X^-$, where X = the cyclic enediol phosphate anion or chloride ion, does not interfere in subsequent steps. The phosphoroimidazole generates imidazole (HX), which is also an efficient catalyst in the second step. The key deprotection of the phosphotriester depends on the rapid basic hydrolysis of neutral esters of phosphoacetoin.[4]

The CEP method for the synthesis of phosphodiesters has been applied to the preparation of deoxyribonucleotides[5] and phospholipids.[6] This paper describes the synthesis of compounds **1** and **2**, in which the secondary or the primary alcohol functions



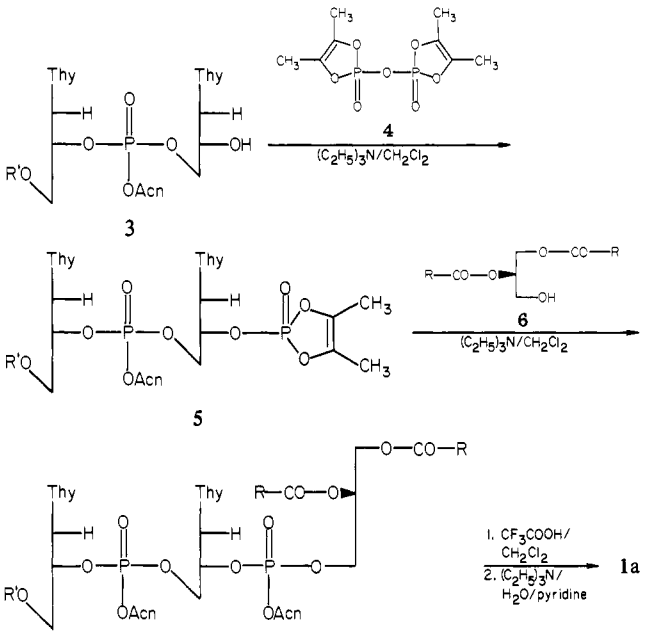
1, R = $C_{13}H_{27}$



2, R = $C_{13}H_{27}$

of a dinucleotide are esterified by the optically active 1,2-diacylglycerophosphoric acid having the chirality of the phospholipids present in biological membranes. The lipid portion of **1** and **2** confers lipophilicity to the oligonucleotide. Thus, **1** and **2** are prototypes of nucleotide derivatives that can, potentially, be bound to liposomes.[7] The nucleotidophospholipid–liposome complex can be viewed as a template for the complementary bases of other nucleosides or polynucleotides. To our knowledge, nucleotidophospholipids have not been reported. However, nucleotidopeptides, i.e., nucleotides covalently bonded to the second major component of biological membranes, are known.[8] An oligonucleotide derivative possessing a structural feature that enhances binding of the oligomer to complementary sequences in another polynucleotide has recently been prepared.[9] In that work,[9] the nucleotide modifier is covalently bonded at the internucleotidic bond as a phosphoramide.

Scheme I[a]



[a] R′ = $(p\text{-}CH_3O\cdot C_6H_4)(C_6H_5)_2C$–; Acn = $CH_3\cdot CO\cdot CH(CH_3)$–; R = $C_{13}H_{27}$.

Results and Discussion

Scheme I outlines the synthesis of a derivative of thymidylyl(3′→5′)thymidine in which the secondary alcohol function of the dinucleotide is esterified by the optically active 1,2-dimyristoylglycerophosphoric acid having the chirality of the phospholipids present in biological membranes. The dinucleotide **3**,[5] protected at the 5′-OH and phosphate groups, is phosphorylated by bis(1,2-dimethylethenylene) pyrophosphate (**4**).[2] The resulting cyclic enediol phosphate ester, **5**, is used directly, without purification, for the phosphorylation of 1,2-di-*O*-myristoyl-*sn*-glycerol[10] (**6**). This step establishes the second phosphate ester bond in the protected nucleotidophospholipid, **7**, without the need for extraneous activating reagents. After purification, intermediate **7** is treated with dilute trifluoroacetic acid at 0 °C, which removes the monomethoxytrityl protection and gives Tp(Acn)Tp(Acn)-DMG (**8** ≡ **7** with R′ = H). The final step is the removal of the acetoinyl protection from the two phosphotriester functions of the purified intermediate **8**. This step is performed in aqueous pyridine under triethylamine catalysis and does not affect the acyl esters of the phosphatidyl group. The nucleotidophospholipid is obtained as its bis(triethylammonium) salt, TpTp-DMG^{2-}[$(C_2H_5)_3NH^+$]$_2$, **1a**. This salt is purified and quantitatively converted into the desired calcium salt **1**, as described in the Experimental Section. The properties of the nucleotidophospholipids are summarized in Table I. The purified compounds **7**, **8**, and **1a** are obtained in 50, 70, and 45% yields, respectively, on a millimole scale.

The synthesis of the derivative of thymidylyl(3′→5′)thymidine, in which the primary alcohol function of the dinucleotide is esterified by the same 1,2-diacylglycerophosphoric acid, is shown in Scheme II. Now, the diglyceride, **6**, is phosphorylated by the

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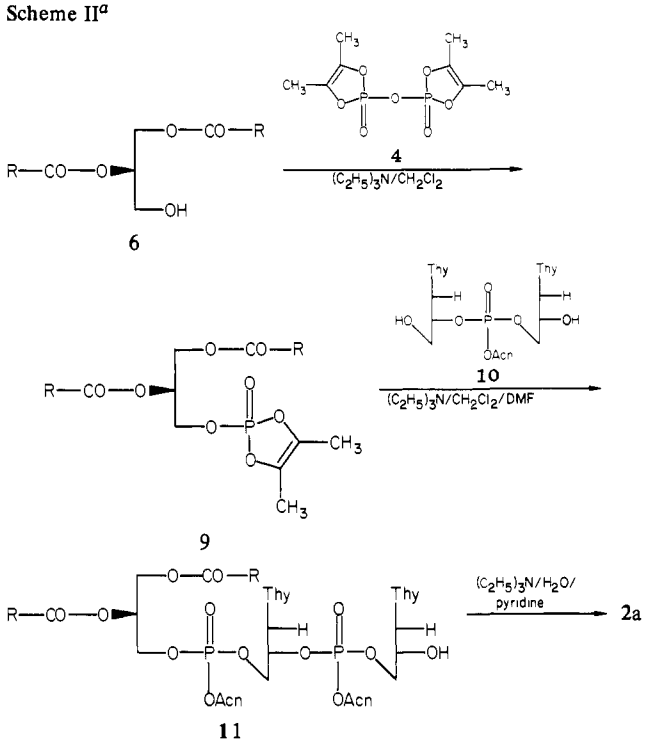
Table I. Properties of Nucleotidophospholipids

no.	compound[a]	molecular formula (M_r)	calcd C	calcd H	calcd Ca	found C	found H	found Ca	λ_{max},[b] nm	ϵ	R_f[c]
1	TpTp-DMG^{2-}Ca^{2+}[d]	$C_{51}H_{84}O_{19}N_4P_2Ca\cdot H_2O$ (1177.3)	52.03	7.36	3.40	51.92	7.38	3.27	268	17 325	0.28 (B)
2	DMG-pTpT^{2-}Ca^{2+}[e]	$C_{51}H_{84}O_{19}N_4P_2Ca\cdot 2H_2O$ (1195.3)	51.25	7.42	3.35	51.40	7.55	3.10	268	16 700	0.22 (B)
7	(mmtr)Tp(Acn)Tp(Acn)-DMG[f]	$C_{79}H_{114}O_{22}N_4P_2$ (1633.7)	61.86	7.49		61.68	7.49				0.72 (A)

[a] TpTp-DMG = 3′-*O*-(1,2-di-*O*-myristoyl-*sn*-glycero-3-phosphoryl)thymidylyl(3′→5′)thymidine; DMG-pTpT = 5′-*O*-(1,2-di-*O*-myristoyl-*sn*-glycero-3-phosphoryl)thymidylyl(3′→5′)thymidine; mmtr = monomethoxytrityl; Acn = acetoinyl (3-oxo-2-butyl). [b] All spectral data in 2:1 chloroform/methanol, v/v. Thymidine: λ_{max} 270 nm (ϵ 8230). [c] Silica gel plates (HPTLC 60F-254, Merck No. 5760). Solvent A: 7:3:1 ethyl acetate/acetone/water; solvent B: 2/1 chloroform/methanol. [d] TpTp-DMG^{2-}[$(C_2H_5)_3NH^+$]$_2$: mp 228–231 °C (sinters at 185–188 °C); λ_{max} 269 nm (ϵ 17 600). TpT-$(C_2H_5)_3NH^+$: λ_{max} 270 nm (ϵ 14 200) (solubility enhanced by $(n\text{-}C_4H_9)_4N^+OH^-$). [e] DMG-pTpT^{2-}-[$(C_2H_5)_3NH^+$]$_2$: mp 217–223 °C (sinters at 123–132 °C); λ_{max} 269 nm (ϵ 18 400). [f] Tp(Acn)Tp(Acn)-DMG (8): R_f 0.56 (A).

Scheme II[a]



[a] R = $C_{13}H_{27}$; Acn = $CH_3\cdot CO\cdot CH(CH_3)$–.

pyrophosphate, **4**. The resulting cyclic enediol phosphate ester **9** is used without purification for the phosphorylation of the dinucleotide[5] **10** protected only at the phosphate function. This step involves predominantly the primary alcohol function of **10** and yields the desired structural isomer DMG-p(Acn)Tp(Acn)T (**11**), which is obtained in 35% yield after purification. Small amounts (ca. 2%) of the isomer Tp(Acn)Tp(Acn)-DMG (**8**) are also isolated; this isomer is the result of phosphorylation at the secondary alcohol function of **10**. The final step is the removal of the two acetoinyl groups from intermediate **11**, as before, to give the nucleotidophospholipid DMG-pTpT^{2-}[$(C_2H_5)_3NH^+$]$_2$, **2a**. This salt is purified and converted into the corresponding calcium salt, **2** (45% yield based on **11**).

The calcium and the triethylammonium salts of the nucleotidophospholipids **1** and **2** are soluble in organic solvents, e.g., 2:1 chloroform/methanol mixtures. In this solvent, the molar extinction coefficients, ϵ, for **1**, **2**, and the corresponding unmodified dinucleotide, TpT, are very similar. Figure 1 displays the circular dichroism and optical rotatory dispersion curves[11] of **1** and **2**. There appears to be no significant differences in the spectra of the two modified nucleotides. The rotations of the solutions of

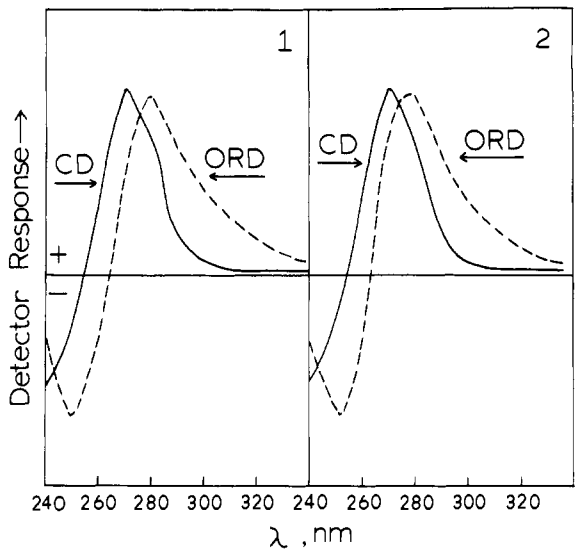


Figure 1. Circular dichroism (CD) and optical rotatory dispersion (ORD) curves of (1) TpTp-DMG^{2-}Ca^{2+} and (2) DMG-pTpT^{2-}Ca^{2+} in 2:1 chloroform/methanol (1.87×10^{-4} M) at 25 °C.

1 and **2** are barely measurable at 589 nm: $[\alpha]^{25}_D$ ~−0.6° and ~0° (*c*, 4.0), respectively.

Experimental Section

All reactions involving enediol cyclophosphoryl derivatives were carried out under anhydrous conditions. The nucleotides **3** and **10** were dehydrated by repeated evaporations from dry pyridine. Triethylamine and dichloromethane were distilled from sodium and phosphorus pentoxide, respectively. Purifications were carried out by preparative TLC on 20 × 20 cm precoated silica gel plates (2 mm thick, PLC 60F-254, Merck Cat. No. 5766). All evaporations were performed under vacuum. Samples were dried for 18 h at 20 °C (0.2 torr) prior to elemental analyses, which were performed by Galbraith Laboratories, Knoxville, TN. The properties of the new compounds are given in Table I.

Synthesis of TpTp-DMG (1; Cf. Table I). A solution of the 5′-protected dinucleotide triester[5] (**3**; 0.444 g, 0.5 mmol) in dichloromethane (2 mL) was added to a stirred dichloromethane solution (1 mL) of bis(1,2-dimethylethenylene) pyrophosphate[2] (**4**; 0.141 g, 0.5 mmol) containing triethylamine (0.075 mL; ca. 0.5 mmol). After 3.5 h at 25° C, the solution was evaporated to give the cyclic phosphate **5** (this compound is quite sensitive to moisture). A solution of 1,2-di-*O*-myristoyl-*sn*-glycerol[10] (**6**; 0.256 g, 0.5 mmol) in dichloromethane (1 mL) at 0° C was added to a dichloromethane solution (2 mL) of the cyclic phosphate **5** containing triethylamine (0.14 mL, ca. 1 mmol), at 0° C. After 3.5 h at 0° C and 36 h at 25° C, the solution was concentrated under vacuum to a volume of ~1 mL and was applied to the preparative TLC plate. The plate was developed with 7:3:1 ethyl acetate/acetone/water. The band containing the desired product (detected under UV) was scraped, and the silica was extracted with 3:1 chloroform/methanol (5 × 75 mL). The solution was evaporated, the residue was redissolved in chloroform (3 mL), and the solution was filtered to remove traces of silica. The solution was evaporated, and the residue was dried under vacuum to give the 5′-protected nucleotidophospholipid triester **7** (0.386 g, 50% yield based on the 1,2-diacylglycerol).

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A solution of the 5'-protected triester (**7**, 0.230 g) in dichloromethane (25 mL) was cooled to 0 °C and was added to a stirred dichloromethane solution of trifluoroacetic acid (100 mL, 0.026 M) at 0 °C. After 25 min at 0 °C, the solution was treated with pyridine (2.7 mL) in dichloromethane (20 mL). The solution was evaporated, and the residue was dried under vacuum and dissolved in chloroform (1 mL) for application to the preparative TLC plates. The development of the plate and the isolation of the 5'-unprotected triester **8** (0.133 g, 70% yield) were carried out as described above.

The 5'-unprotected triester (**8**; 0.120 g) was mixed with pyridine (1.5 mL), water (1.5 mL), and triethylamine (0.14 mL) at 25 °C. The mixture was stirred for 36 h at 25 °C and was freeze-dried. The salt TpTp-DMG^{2-}[$(C_2H_5)_3NH^+$]$_2$ (**1a**; 0.057 g, 45% yield) was obtained in pure form by preparative TLC (two successive elutions by 2:1 chloroform/methanol in the same plate; extraction with the same solvent, 10 × 100 mL; precipitation as free-flowing powder from mininum of chloroform upon addition of acetone). The conversion of the triethylammonium salt **1a** into the calcium salt **1** was performed as follows. A solution of **1a** (0.116 g) in 2:1 chloroform/methanol (30 mL) was mixed with 3:48:47 chloroform/methanol/2 M aqueous calcium chloride (20 mL). The upper phase was discarded, and the procedure was repeated two additional times with the lower phase. The final lower phase was washed twice with 3:48:47 chloroform/methanol/water (20 mL) and evaporated. The residue was kept for 18 h (0.2 torr) to yield **1** (0.10 g).

Synthesis of DMG-pTpT (2; Cf Table I). A dichloromethane solution (1 mL) of 1,2-di-*O*-myristoyl-*sn*-glycerol (**6**; 0.256 g, 0.5 mmol) was added to a stirred dichloromethane solution (0.5 mL) of the pyrophosphate (**4**; 0.141 g, 0.5 mmol) containing triethylamine (0.07 mL) at 25 °C. After 2 h at 25 °C, the solution was evaporated to give the cyclic phosphate **9** (this compound is quite sensitive to moisture). A solution of the 5'-unprotected dinucleotide triester (**10**; 0.308 g, 0.5 mmol) in dimethylformamide (1 mL) was added to a solution containing the cyclic phosphate **9**, triethylamine (0.14 mL, ca. 1 mmol), and dichloromethane (2 mL) at 25 °C. After 36 h at 25 °C, the solution was evaporated, and the residue was dissolved in chloroform (1.5 mL) for application to preparative-TLC plates. The nucleotidophospholipid triester (**11**, 0.220 g, 35%) was isolated as described above (three successive elutions per plate utilizing 7:3:1 ethyl acetate/acetone/water, extraction with 2:1 chloroform/methanol, 5 × 50 mL).

The triester (**11**, 0.100 g) was mixed with pyridine (1.5 mL), water (1.5 mL), and triethylamine (0.14 mL). The mixture was stirred for 24 h at 25 °C and was freeze-dried. The salt, DMG-pTpT^{2-}[$(C_2H_5)_3NH^+$]$_2$ (**2a**, 0.050 g, 47%) was obtained in pure form by preparative TLC (two successive elutions per plate utilizing 2:1 chloroform/methanol as solvent; extraction with the same solvent, 10 × 100 mL; precipitation as free-flowing powder from chloroform upon addition of acetone). The conversion of the triethylammonium salt **2a** into the calcium salt **2** was performed as described above.

Acknowledgment. This research was supported by the National Institutes of Health (Grant GM 20672).

Registry No. 1, 82732-09-0; **1a**, 82769-18-4; **2**, 82740-47-4; **2a**, 82795-53-7; **3**, 62930-01-2; **4**, 55894-94-5; **5**, 62930-09-0; **6**, 1069-82-5; **7**, 82732-10-3; **8**, 82732-11-4; **9**, 82263-08-9; **10**, 62962-23-6; **11**, 82740-48-5.

Synthesis of (±)-Lineatin, an Aggregation Pheromone of *Trypodendron lineatum*

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Abstract: A regioselective synthesis of lineatin (**1**) was accomplished, starting from anhydromevalonolactone (**8**). Photochemical cycloaddition of **8** with acetylene gave the cyclobutene **9**, which was converted to lactone **13** upon methylation followed by oxidation. Hydroboration–oxidation of **13** afforded a mixture of **14** and **15**, from which tosylate **18** was obtained. Reduction of **18** gave the endo hemiacetal **19**, which underwent an intramolecular displacement to produce **1**. Isolineatin (**2**) was obtained by reduction of **13** to hemiacetal **20**, followed by intramolecular oxymercuration with mercuric pivalate and reduction with sodium borohydride.

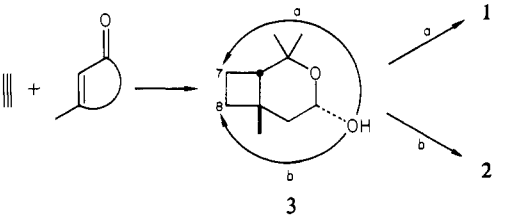
Lineatin (**1**), an aggregation pheromone from the frass of the female ambrosia beetle *Trypodendron lineatum* (Olivier),[1] has been shown to elicit powerful secondary attraction in laboratory[2] and field trials.[3] The extensive damage to fallen and sawn timber, especially Douglas fir, caused by *T. lineatum*[4] lends particular significance to this pheromone as a possible means for controlling this pest.[5] Originally formulated as either **1** or **2**,[1] lineatin was

1

2

subsequently shown to be 3,3,7-trimethyl-2,9-dioxatricyclo-[3.3.1.0^{4,7}]nonane (**1**) by unambiguous synthesis.[6] Recently, a synthesis of (+)-**1** was described that established the absolute configuration of this biologically active enantiomer as 1*R*,4*S*,5*R*,7*R*.[7]

Scheme I



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The need for substantial quantities of lineatin for entomological work, coupled with the fact that previous preparations have yielded

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