washed thoroughly with water, and then pumped to dryness. Recrystallization from ethanol produced 5.6 mg (36%) of the clavicipitic acids as a 1:1 mixture of isomers: R_f 0.27 and 0.24 (silica gel, 75:25:1 $CHCl_3$/MeOH/concentrated NH_4OH); mp 239–244 °C dec; UV (EtOH) λ_{max} 221, 288 nm; IR (KBr) 3300, 1580, 1410 cm^{-1}; mass spectrum (15 eV), m/z 270, 269, 255, 225, 215, 196, 183, 182, 169, 167, 154.

Derivitization of the Clavicipitic Acids. The acids were treated with 1 mL of dry methanol containing 0.25 mL of acetic anhydride, and the resulting solution was stirred under a nitrogen atmosphere for 9 h at room temperature. The reaction mixture was concentrated and chromatographed on SilicAR CC-7 to yield the *N*-acetyl methyl ester derivative **28** of clavicipitic acid: mp 117–119 °C (CCl_4–hexanes); UV ($CHCl_3$) λ_{max} 285 nm; IR ($CHCl_3$) 3485, 1736, 1636 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.75 (s, 3 H), 1.91 (s, 3 H), 2.17 (s, 3 H), 3.37, 3.93 (AB portion of ABX, 2 H, J_{AB} = 16.0 Hz, J_{AX} = 6.05 Hz, J_{BX} = 4.03 Hz), 3.72 (s, 3 H), 4.44 (dd, 1 H, J = 6.05, 4.03 Hz), 5.23 (d, 1 H, J = 7 Hz), 5.86 (d, 1 H, J = 7 Hz), 6.85–7.29 (m, 4 H), 8.31 (br s, 1 H); mass spectrum (15 eV), m/z 326, 311, 283.

Acknowledgment. We are indebted to the National Institutes of Health (Grant No. HL-20579) and the Ciba-Geigy Corporation for support of this work. We thank Professor Heinz Floss for the authentic sample of the clavicipitic acids.

Registry No. 1 (isomer 1), 33062-26-9; 1 (isomer 2), 72690-85-8; **7**, 79681-04-2; **8**, 90150-60-0; **9**, 90150-61-1; **10a**, 90150-69-9; **10b**, 90150-62-2; **11**, 90150-63-3; **12**, 90150-64-4; **14**, 90150-65-5; **15**, 90150-66-6; **16**, 88246-05-3; **18**, 90150-67-7; **19**, 90150-68-8; (*E*)-**21**, 82958-16-5; (*Z*)-**21**, 82958-15-4; (*Z*)-**22**, 82958-20-1; (*E*)-**22**, 82958-19-8; (*Z*)-**23**, 82958-22-3; (*E*)-**23**, 82958-21-2; **24**, 84935-69-3; **26**, 84935-70-6; **27**, 90150-59-7; **28**, 90242-26-5; *cis*-4-(3-methyl-1-butenyl)-3-[(dimethylamino)methyl]indole, 82958-18-7; *trans*-4-(3-methyl-1-butenyl)-3-[(dimethylamino)methyl]indole, 82958-17-6; methyl *cis*-2-carbomethoxy-3-[4-(3-methyl-1-butenyl)-3-indolyl]propionate, 90150-57-5; methyl *trans*-2-carbomethoxy-3-[4-(3-methyl-1-butenyl)-3-indolyl]propionate, 90150-58-6; dimethyl 1-carbethoxy-3,4-dihydro-6-(1-bromo-2-methylpropyl)azepino[5,4,3-*cd*]indole-4,4-dicarboxylate, 84935-71-7; indole-4-carboxaldehyde, 1074-86-8; (2-methylpropyl)triphenylphosphonium bromide, 22884-29-3; dimethyl malonate, 108-59-8; ethyl chloroformate, 541-41-3; 2-methyl-1-propenyl bromide, 3017-69-4; phenylselenyl chloride, 5707-04-0; methyl glycinate, 616-34-2.

Synthesis of *P*-Thioadenylyl-(2′–5′)-adenosine and *P*-Thioadenylyl-(2′–5′)-*P*-thioadenylyl-(2′–5′)-adenosine[1]

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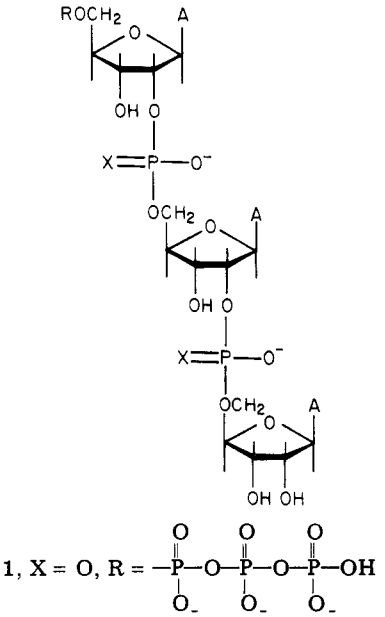
Received November 14, 1983

Dimer and trimer adenylates with 2′–5′ phosphorothioate linkages were synthesized via the phosphite triester approach in conjunction with sulfur oxidation. Both stability of the 2′–5′ phosphorothioate internucleotide bond and absolute configuration of the A2′p(s)5′A diastereomers (**6**) were investigated by snake venom phosphodiesterase degradation.

In recent years, analogues of the antiviral and antitumor agent pppA2′p5′A2′p5′A (**1**, commonly known as 2–5A) and its core (**2**) have been the subject of numerous syntheses.[2]



Only a few syntheses have involved the alteration of the internucleotide phosphodiester linkage, all of which removed the electrostatic charge of the phosphodiester resulting in nonionic derivatives.[2c,f] An analogue of 2–5A core, such as phosphorothioate **3**, which possesses a modified phosphodiester with retention of ionic character, would be a most interesting species. Along with the potential antiviral value of **3**, the added chirality of the two phosphorothioate groups, which results in four stereoisomers, may prove beneficial as mechanistic probes for the action of 2–5A.[3]

Since 2–5A is rapidly digested by a 2′-phosphodiesterase which shortens its duration of action, stability toward this enzyme is an essential element in the design of any 2–5A analogue.[4] It has been previously demonstrated that the

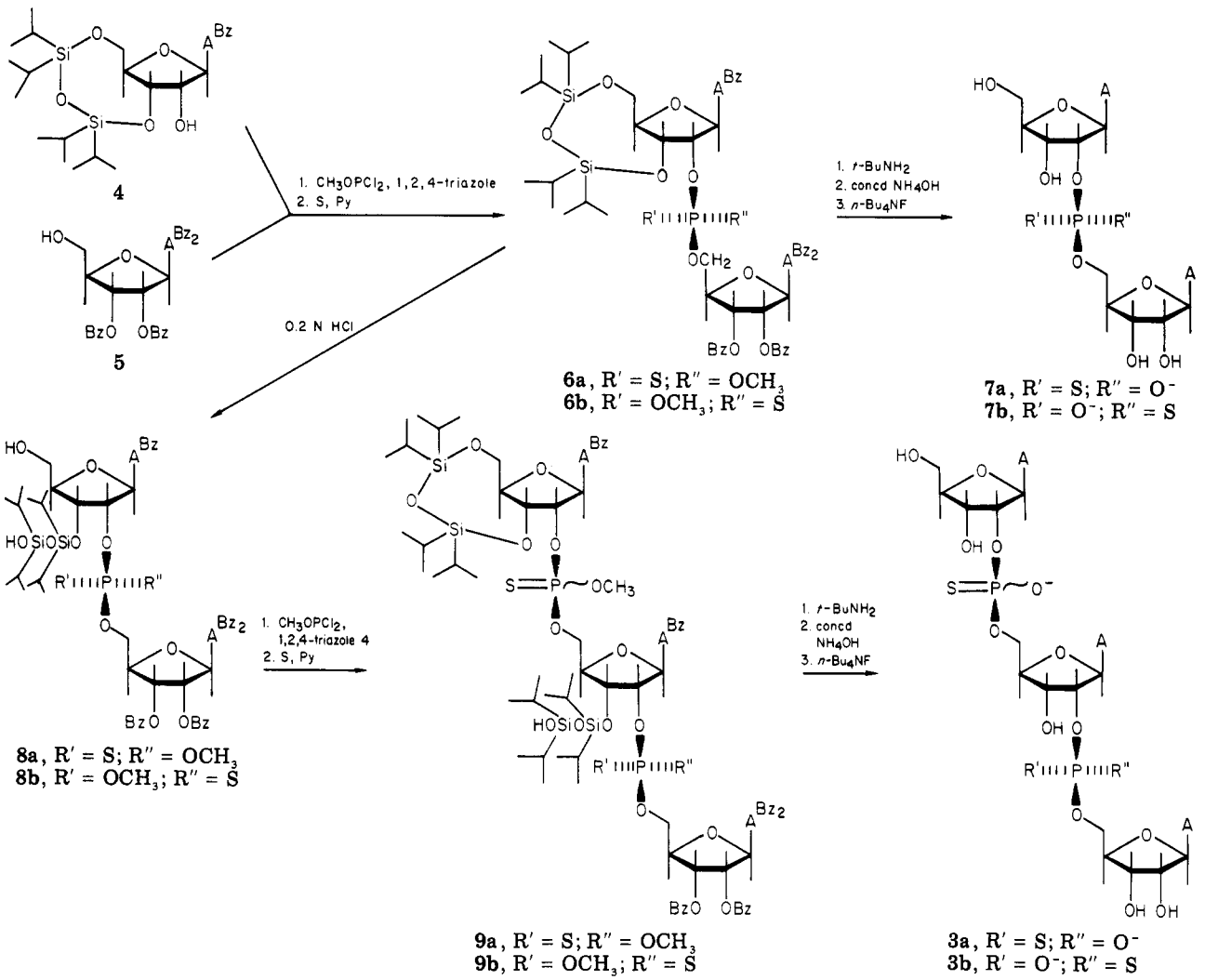
(1) This paper is contribution no. 176 from the Institute of Bio-Organic Chemistry, Syntex Research, Palo Alto, CA 94304.

(2) (a) Torrence, P. F.; Imai, J.; Lesiak, K.; Johnston, M. I.; Jacobsen, H.; Friedman, R. M.; Sawai, H.; Safer B. "Chemistry and Biology of Interferon: Relationship to therapeutics"; Merigan, T. C., Friedman, R. M.; Fox, C., Eds.; Academic Press: New York, 1982; Vol. XXV, pp 123–142. (b) Charubala, R.; Pfleiderer, W. *Tetrahedron Lett.* **1982**, *23*, 4789. (c) Eppstein, D. A.; Marsh, Y. V.; Schryver, B. B.; Larsen, M. A.; Barnet, J. W.; Verheyden, J. P. H.; Prisbe, E. J. *J. Biol. Chem.* **1982**, *257*, 13390. (d) Drocourt, J. L.; Dieffenbach, C. W.; Ts'o, P.O.P.; Justesen, J.; Thang, M. N. *Nucleic Acids Res.* **1982**, *10*, 2163. (e) Kwiatkowski, M.; Gioeli, C.; Chattopadhyaya, J. B. *Chemica Scripta* **1982**, *19*, 49. (f) Jager, A.; Engels, J. *Nucleic Acids Res.* **1981**, *Symp. No. 9*, 149. (g) Crea, R. *J. Org. Chem.* **1981**, *46*, 2242. (h) Gosselin, G.; Imbach, J. *Tetrahedron Lett.* **1981**, *22* 4699. (i) Charubala, R.; Pfleiderer, W. *Ibid.* **1980**, *21*, 4077. (j) Engels, J. *Ibid.* **1980**, *21*, 4339 and references therein.

(3) Brody, R. S.; Adler, S.; Modrich, P.; Leznikowski, Z. J.; Stec, W. J.; Frey, P. A. *Biochemistry* **1982**, *21*, 2570.

0022-3263/84/1949-2314$01.50/0

Scheme I



phosphorothioate group is more stable to hydrolysis by phosphodiesterases.[5] We felt that the introduction of such a group into the 2–5A molecule would also provide the desired stability. Reports of the chemical synthesis of nucleoside phosphorothioates are rare in the literature and to our knowledge involve only dimers having a 3′–5′ internucleotide linkage.[6] In this paper, we describe the synthesis of the novel 2–5A phosphorothioate analogue **3**.

The appropriately protected mononucleoside building blocks **4** and **5** were prepared essentially by literature procedures[7,8] and condensations were accomplished by using a modification of the phosphite triester approach.[9] Succesive treatments of a solution of methyl phosphorodichloridite (1.2 equiv) and 1,2,4-triazole (4.8 equiv) in dry pyridine–tetrahydrofuran (1:4) with nucleoside blocks **4** (1.2 equiv) and **5** (1 equiv) at −78 °C followed by sulfur oxidation afforded the two diastereomers **6a** and **6b** in 52% isolated yield (Scheme I). The diastereomeric mixture of **6** was separated into high R_f (0.60) and low R_f (0.40) (TLC, ethylacetate–methylene chloride, 1:3) components in a respective ratio of 2:1 by silica gel column chromatography.

The diastereomers of **6** were deprotected in order to conduct enzymatic experiments. It was envisioned that snake venom phosphodiesterase degradation of the separate isomers of deprotected phosphorothioate diester **7** would not only establish the stability of the 2′–5′ phosphorothioate internucleotide bond but also hopefully determine the absolute configuration of the phosphorus atom. Separate diastereomers of phosphorothioate **7** were obtained by individually reacting the high R_f and low R_f diasteromers of **6** as follows: (1) gentle reflux in *tert*-butylamine for 1–3 h,[10] (2) treatment with concentrated ammonium hydroxide for 5–6 h at 55–60 °C, and (3) reaction with 0.3 M tetra-*n*-butylammonium fluoride at ambient temperature for 6 h. After final purification by Sephadex G-10 chromatography, the fully deprotected phosphorothioate dimers (**7**), in the form of tetra-*n*-butylammonium salts, were isolated in 65–84% yields. Each sample was found pure and different by HPLC and ^{31}P NMR analysis. Treatment of **7** derived from high R_f **6**, **7** derived from low R_f **6**, and natural dimer A2′p5′A with snake venom phosphodiesterase gave calculated half lives of 1 day, 40 days, and only 2.5 min, respectively. Not only did this confirm

(4) (a) Imai, J.; Johnston, M. I.; Torrence, P. F. *J. Biol. Chem.* **1982**, *257*, 12739. (b) Bagioni, C. B.; D'Alessandro, S. B.; Nilsen, T. W.; den Hartog, J. A. J.; Crea, R.; van Boom, J. H. *Ibid.* **1981**, *256*, 3253. (c) Schmidt, A.; Chernajovski, Y.; Shulman, L.; Federman, P.; Berissi, H.; Revel, M. *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 4788.

(5) For review of phosphorothioate analogues of nucleotides see: Eckstein, F. *Angew. Chem., Int. Ed. Eng.* **1983**, *22*, 423.

(6) (a) Uznanski, B.; Niewiarowski, W.; Stec, W. J. *Tetrahedron Lett.* **1982**, *23*, 4289. (b) Marlier, J. F.; Benkovic, S. J. *Ibid.* **1980**, *21*, 1121. (c) Burgers, P. M.; Eckstein, F. *Ibid.* **1978**, 3835. (d) Eckstein, F. *Ibid.* **1967**, 1157, 3495.

(7) Gioeli, C.; Kwiatkowski, M.; Oberg, B.; Chattopadhyaya, J. B. *Tetrahedron Lett.* **1981**, *22*, 1741 and references therein.

(8) Lohrmann, R.; Khorana, H. G. *J. Am. Chem. Soc.* **1964**, *86*, 4188.

(9) Letsinger, R. L.; Lunsford, W. B. *J. Am. Chem. Soc.* **1976**, *98*, 3655.

(10) Gray, M. D. M; Smith, D. J. H. *Tetrahedron Lett.* **1980**, *21*, 859.

the stability of the 2′–5′ phosphorothioate internucleotide linkage, but suggested that **7** derived from high R_f **6** and hence high R_f **6** both possessed the R_P configuration; i.e., **7a** and **6a**, respectively. The S_P configuration was assigned to low R_f **6** and its corresponding phosphorothioate **7** (**6b** and **7b**). This conclusion was based on ample precedent that phosphodiesterases selectively hydrolyze phosphorothioates of R_P configuration.[5,6a,b,11]

Selective 5′-hydroxyl deprotection of **6** with 0.2 M hydrogen chloride (dioxane)[2e,7] and direct condensation of the resulting phosphorothioate **8** with nucleoside **4** using the phosphite triester method as previously described followed by oxidation with elemental sulfur in pyridine afforded the trimer **9**. In order to keep defined chirality of one of the P atoms, coupling reactions were performed separately on both **6a** and **6b** to give diasteromeric pairs **9a** and **9b**, respectively in 15–30% yield after chromatographic purification. Lastly, deprotection of each diasteromeric pair (**9a** and **9b**) was accomplished by employing the same procedures used for the deprotection of dimer **6** and afforded the 2–5A core phosphorothioate analogues **3a** and **3b** isolated in 73–80% yield as diastereomeric pairs.

As expected, diastereomeric pair **3b**, which was derived from O-methyl phosphorothioate **6b** possessing Sp configuration, proved to be more stable to murine phosphodiesterase[12] hydrolysis than **3a** and 2–5A core itself. A detailed account of the biological evaluation of the isomers of **3** will be presented elsewhere.[13]

Experimental Section

The snake venom phosphodiesterase was purchased from Worthington Biochemical Corporation. Analtech Silica Gel GF (0.25 mm) plates were used for thin-layer chromatography and developed with a 20% perchloric acid spray or visualized by UV light. Column chromatography employed silica gel (70–230 mesh) supplied by E. Merck (Darmstadt) or Sephadex G-10 manufactured by Pharmacia Fine Chemicals, AB, Uppsala, Sweden. HPLC analyses were performed with an Altex Model 332 gradient liquid chromatograph. Melting points were observed by using a commercial Thomas Hoover apparatus. The ultraviolet spectra were obtained from a Hewlett-Packard 8450 UV/VIS or Cary-15 spectrometer. Proton magnetic resonance measurements were made with a Bruker WM-300 using either tetramethylsilane or sodium 2,3-dimethyl-2-silapentane-5-sulfonate (DSS, aqueous solutions as an internal standards). Phosphorus (^{31}P) magnetic resonance measurements were made with a Varian HA-100 using H_3PO_4 as an external standard. The mass spectra (FAB) were recorded by Dr. D. H. Williams (Cambridge University, England) on a Kratus MS-50 instrument using xenon as the primary beam and glycerol or thioglycerol as solvent. Fully protected compounds were analyzed in a positive ion mode whereas deprotected compounds were analyzed in a negative ion mode. Elemental analyses were determined by Syntex Analytical Research Division or Atlantic MicroLab, Atlanta, GA.

N^6-Benzoyl-3′,5′-*O*-TIPDSi-*P*-methyl-*P*-thioadenylyl-2′–5′)-N^6,N^6,O^2′,O^3′-tetrabenzoyladenosine (6).[14] To a stirred solution of 1,2,4-triazole (1.91 g, 27.6 mmol) in absolute pyridine (8 mL) and dry tetrahydrofuran was added methyl phosphorodichloridite (0.62 mL, 6.6 mmol) under a nitrogen atmosphere at −78 °C. The mixture was allowed to warm toward ambient temperature for 10 min and recooled to −78 °C, and N^6-benzoyl-3′,5′-*O*-TIPDSi-adenosine[7] (**4**, 4.24 g, 6.9 mmol) in dry tetrahydrofuran (30 mL) was slowly added. After warming toward room temperature for 15 min and recooling to −78 °C, N^6,N^6,O^2′,O^3′-tetrabenzoyladenosine[8] (**5**, 4.10 g, 6.0 mmol) in dry tetrahydrofuran (30 mL) was added and the mixture was stirred for 1 h (warming to room temperature). Elemental sulfur (2.21 g, 69 mmol) and absolute pyridine (30 mL) were added and the contents of the flask were stirred for 16 h at room temperature. The solvent was evaporated and the residue was coevaporated with toluene (3 × 50 mL). Purification and separation of the diasteromers (**6a** and **6b**) was accomplished by two successive chromatographies on silica gel (2.5 cm × 75 cm) with ethyl acetate-methylene chloride (1:5 (4 L) followed by 1:3 (2 L)) elution. The high R_f (2.84 g, TLC R_f 0.60, ethyl acetate–methylene chloride, 1:3) and low R_f (1.48 g, TLC R_f 0.40, ethyl acetate–methylene chloride, 1:3) diastereomers gave a combined yield of 4.32 g (52%) as amorphous white solids.

High R_f diastereomer **6a**: mp 115–125 °C dec; UV λ_{max} 277 (log ϵ 4.60, MeOH); ^{1}H NMR ($CDCl_3$) δ 3.84 (d, J = 14 Hz, 3 H, OMe), 8.38, 8.60, 8.66 and 8.71 (4 s, 4 H, Ad's); ^{31}P NMR ($CDCl_3$) δ 70.9; FAB mass spectrum, m/e 1389 (MH^+), 1390 (MH_2^+), 1411 (MNa^+).

Anal. Calcd for $C_{68}H_{73}N_{10}O_{15}PSSi_2$: C, 58.78; H, 5.30; N, 10.08; S, 2.31. Found: C, 58.52; H, 5.33; N, 9.99; S, 2.41.

Low R_f diastereomer **6b**: mp 112–115 °C dec; UV λ_{max} 277 (log ϵ 4.60, MeOH); ^{1}H NMR ($CDCl_3$) δ 3.94 (d, J = 14 Hz, 3 H, OMe), 8.19, 8.54, 8.60 and 8.66 (4 s, 4H, Ad's); ^{31}P NMR ($CDCl_3$) δ 71.0; FAB mass spectrum, m/e 1389 (MH^+), 1390 (MH_2^+), 1411 (MNa^+).

Anal. Calcd for $C_{68}H_{73}N_{10}O_{15}PSSi_2$: C, 58.78; H, 5.30; N, 10.08; S, 2.31. Found: C, 58.22; H, 5.27; N, 9.77; S, 2.49.

***P*-Thioadenylyl-(2′–5′)-adenosine (7).** Each respective protected phosphorothioate dimer (**6a** or **6b**, 100 mg) was dissolved in *tert*-butylamine (5 mL) and gently refluxed for 3 h. The solvent was evaporated and to the residue was added concentrated ammonium hydroxide (15 mL). After heating at 60 °C for 6 h, the solvent was evaporated and 0.2 M tetra-*n*-butylammonium fluoride (pyridine–tetrahydrofuran, 1:4, 2 mL) was added. The mixture was stirred for 6 h at room temperature, the solvent removed in vacuo, and the residue dissolved in water (7 mL). The aqueous phase was extracted with chloroform (8 × 10 mL) and evaporated to give a light yellow residue. Purification on a column of Sephadex G-10 (2.5 cm × 50 cm) afforded the desired phosphorothioates (**7a** or **7b**) in the form of tetra-*n*-butylammonium salts. The overall yields were 65% and 84% respectively from the corresponding precursors **6a** and **6b**.

Diastereomer **7a**: UV λ_{max} 258 (H_2O); ^{1}H NMR (D_2O) δ 7.75, 8.13, 8.19, and 8.20 (4 s, Ad's); ^{31}P NMR (D_2O) δ 55.47; FAB mass spectrum, m/e 611 (M − *n*-Bu_4N). HPLC analysis:[15] R_T 19.5 min [*A* = 0.3 M ammonium phosphate (Ph 7.0), B = methanol, 0–30% B, 20 min, hold 30% B].

Diastereomer **7b**: UV λ_{max} 258 (H_2O); ^{1}H NMR (D_2O) δ 7.80, 8.13, 8.16, and 8.22 (4 s, Ad's); ^{31}P NMR (D_2O) δ 54.80; FAB mass spectrum, m/e 611 (M − *n*-Bu_4N). HPLC analysis:[15] R_T 20.5 min [A = 0.3 M ammonium phosphate (pH 7.0), B = methanol, 0–30% B, 20 min, hold 30% B].

N^6-Benzoyl-3′,5′-*O*-TIPDSi-*P*-methyl-*P*-thioadenylyl-(2′–5′)-N^6-benzoyl-3′-*O*-TIPDSi(OH)-*P*-methyl-*P*-thioadenylyl-(2′–5′)-N^6,N^6,O^2′,O^3′-tetrabenzoyladenosine (9).[14] The protected phosphorothioate diastereomers **6a** and **6b** (100 mg) were separately treated with 0.2 M HCl in dioxane (14 mL/mmol) and stirred for 1 h at room temperature (homogenous on TLC, methanol–methylene chloride, 3:97).[2e,7] The solution was neutralized with 5% sodium bicarbonate and extracted twice with chloroform, and the organic extracts dried over sodium sulfate. After evaporation of the solvent and coevaporation of the white solid residue with absolute pyridine (3 times), the resulting 5′-hydroxyl deprotected methyl phosphorothioates, **8a** and **8b**, were individually condensed with protected nucleoside **4** in a fashion analogous to the procedure described for the preparation of **6** above. Purification on a column of silica gel employing ethyl acetate–methylene chloride (3:7) elution afforded

(11) Burgers, P. M.; Eckstein, F. *Proc. Natl. Acad. Sci. USA* **1978**, *75*, 4798.

(12) Murine L cell S-10 extracts, a known source of 2–5A phophodiesterase, were prepared from cells grown in suspension culture in Joklik's minimal essential medium containing 8% fetal calf serum. For experimental detail see ref 2c.

(13) Schryver, B. B.; Marsh, Y. V.; Eppstein, D. A.; unpublished results.

(14) For the cyclic 1,1,3,3-tetrakis(1-methylethyl)-1,3-disiloxanediyl protection we have used the common abbreviation TIPDSi. We have also introduced the notation TIPDSi(OH) for the corresponding acyclic 3-hydroxy-1,1,3,3-tetrakis(1-methylethyl)disiloxanyl protection as an extension of this abbreviation.

(15) C_{18} bonded phase 10 μm ODS Spherisorb column (25 cm × 4.6 mm).

the respective pairs of diastereomers (**9a** and **9b**) in 15–30% yield as amphorous white solids.

Diastereomeric pair **9a**: UV λ_{max} 278 (log ϵ 4.77, MeOH); ^{1}H NMR ($CDCl_3$) δ 3.68, 3.69, 3.78, and 3.85 (4 d, J = 14 Hz, OMe's), 8.22, 8.24, 8.39, 8.43, 8.44, 8.46, 8.61 and 8.63 (8 s, Ad's), 8.68[16] (s, Ad's); ^{31}P NMR ($CDCl_3$) δ 69.47, 69.80, 70.41, 70.53; FAB mass spectra, m/e 2112 (MH^+), 2113 (MH_2^+), 2134 (MNa^+).

Anal. Calcd for $C_{98}H_{119}N_{15}O_{23}P_2S_2Si_4$: C, 55.69; H, 5.68; N, 9.94; S, 3.03. Found: C, 55.53; H, 5.72; N, 9.77; S, 3.05.

Diastereomeric pair **9b**: UV λ_{max} 278 (log ϵ 4.76, MeOH); ^{1}H NMR ($CDCl_3$) δ 3.38, 3.43, 3.81, and 3.88 (4 d, J = 14 Hz, OMe's), 8.21, 8.22, 8.34, 8.38, 8.44, 8.45, 8.660, 8.663, 8.67, 8.68, 8.737, and 8.744 (12 s, Ad's); ^{31}P NMR ($CDCl_3$) δ 70.20, 70.69, 70.98.[16]

Anal. Calcd for $C_{98}H_{119}N_{15}O_{23}P_2S_2Si_4 \cdot C_4H_8O_2$:[17] C, 55.65; H, 5.81; N, 9.54; S, 2.91. Found: C, 56.13; H, 5.89; N, 9.21; S, 2.77.

***P*-Thioadenylyl-(2′–5′)-*P*-thioadenylyl-(2′–5′)-adenosine (3).** The diastereomeric pairs **9a** and **9b** (100 mg) were separately deprotected by the same procedures used to prepare phosphorothioate dinucleotide **7** as described above to give corresponding diastereomeric pairs **3a** and **3b** in 80% and 73% yields, respectively.

Diastereomeric pair **3a**: UV λ_{max} 258 (H_2O); ^{1}H NMR (D_2O) δ 7.726, 7.730, 7.89, 7.94,[16] 8.05, 8.08, 8.12,[16] 8.18, 8.27, and 8.35 (10 s, Ad's); ^{31}P NMR (D_2O) δ 55.1,[16] 56.3;[16] FAB mass spectrum, m/e 1197 (M – n-Bu_4N). HPLC analysis:[15] R_T 21.3, 21.7 min [A = 0.3 M ammonium phosphate (pH 7.0), B = methanol, 0–30% B, 20 min, hold 30% B].

Diastereomeric pair **3b**: UV λ_{max} 258 (H_2O); ^{1}H NMR (D_2O) δ 7.71, 7.81, 7.97, 8.00, 8.10,[16] 8.11, 8.14,[16] and 8.30 (8, s, Ad's); ^{31}P NMR (D_2O) δ 54.58, 55.03, 55.93, 56.13; FAB mass spectrum, m/e 1197 (M – n-Bu_4N). HPLC analysis:[15] R_T 21.8, 22.2 min [A = 0.3 M ammonium phosphate (pH 7.0), B = methanol, 0–30% B, 20 min, hold 30% B].

Snake Venom Phosphodiesterase Degradation. The enzymatic hydrolysis of dimers Rp-A2′p(s)5′A (**7a**), Sp-A2′p(s)5′A (**7b**), and A2′p5′A were performed at 37 °C in a reaction vessel containing 200 mM Tris·HCl (pH 8.75), 2 mM $Mg(OAc)_2$, 200 μM dimer, and 2 units/mL PDEase. Samples (50 μL) were removed at the appropriate times and incubated at 95 °C for 5 min, cooled to 4 °C, and centrifuged for 5 min. The supernatants were analyzed by HPLC[15] with a 300 mM ammonium phosphate (pH 7.0) phase, eluted with a 1% per min gradient of methanol, and detected by absorbance at 260 nm.

Acknowledgment. We thank Dr. John G. Moffatt for his helpful suggestions. We thank Brian Schryver for his assistance in the enzymatic degradation experiments. We would also like to extend our appreciation to Drs. Laszlo G. Tokes and Michael L. Maddox for their help in the spectral characterization of the above compounds.

Registry No. **3a**, 90108-24-0; **3b**, 90191-59-6; **4**, 79154-57-7; **5**, 58463-04-0; **6a**, 90108-20-6; **6b**, 90191-55-2; **7a**, 90108-21-7; **7b**, 90191-56-3; **8a**, 90108-22-8; **8b**, 90191-57-4; **9a**, 90108-23-9; **9b**, 90191-58-5.

(16) Nuclear magnetic resonances were superimposed and the corresponding integration was consistent.

(17) This sample was analyzed as a monosolvate after lyophilization from dioxane.

Studies on Terpenes. 8. Total Synthesis of (±)-Linderalactone, (±)-Isolinderalactone, and (±)-Neolinderalactone, Germacrane Furanosesquiterpenes

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Received December 23, 1983

The synthesis of linderalactone (**1**), isolinderalactone (**2**), and neolinderalactone (**3**) is described and exploits the unique ability of these fluxional systems to enter into Cope and abnormal Cope rearrangements. The symmetrical β-diketone **10** was converted into the vinyl furan **16** by standard reactions and subsequently alkylated with ethyl bromoacetate, followed by sodium borohydride reduction to give the lactones **18** and **18a**. Methylenation of **18** and **18a** gave isolinderalactone (**2**) and epiisolinderalactone (**5**), respectively. Heating **2** at 160 °C established an equilibrium (3:2) with linderalactone (**1**), and similar treatment of **5** gave neolinderalactone (**3**).

The germacrane furanosesquiterpenes, linderalactone (**1**),[2] isolinderalactone (**2**), and neolinderalactone (**3**)[3] were isolated by Takeda from the root of the shrub *Lindera strychnifolia* Vill. Their structures are based upon chemical degradation, and for linderalactone (**1**), an X-ray crystal structure is available.[4]

Linderalactone (**1**), on heating at 160 °C undergoes a reversible Cope rearrangement to isolinderalactone (**2**) (ratio 2:3).[5] It was determined that isolinderalactone (**2**) is antipodal to the elemane sesquiterpenes by chemical correlation with isofuranogermacrene(**4**), of known absolute configuration. Takeda has extensively examined the relationship between the conformation of cyclodeca-1,5-diene-type sesquiterpenes and the stereochemistry of their Cope rearrangement products (Scheme I). NOE studies have concluded that the stereochemical outcome of such rearrangements is dependent upon the confomation of the ten-membrered ring. with this technique neolinderalactone (**3**) was shown to exist as a 4:1 mixture of the conformers **3**/**3a**, at room temperature. Cope rearrangement of neolinderalactone (**3**) (300 °C for 1–2 min) gave isolin-

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0022-3263/84/1949-2317$01.50/0