of 4 (125 mg, 0.15 mmol), sodium trifluoroacetate (160 mg, 1.1 mmol), CuI (110 mg, 0.5 mmol) and hexamethylphosphoramide (2.5 mL) was heated at 160 °C under N_2 for 6 h. The cooled mixture was poured into water and then extracted with Et_2O. The extract was washed with water, dried (Na_2SO_4), and concentrated to give a brown oil. This was purified by column chromatography (EtOAc/light petroleum ether). White cubic crystals of **10** were obtained (10 mg, 10%), mp 129–130 °C. ^1H NMR (CD_3COCD_3): δ 1.57 [d, 12 H, 2 $CH(CH_3)_2$], 3.94 (s, 6 H, 2 OCH_3), 4.02 [m, 2 H, 2 $CH(CH_3)_2$], 4.16 (s, 6 H, 2 OCH_3), 5.40 (s, 4 H, 2 OCH_2Ar), 7.77 (s, 2 H, ArH), 7.80 (s, 2 H, ArH). IR: 1593, 1036 cm^{-1}. MS: *m/e* 514 (100), 515 (34, M^+, $M^+ + 1$). Anal. Calcd for $C_{32}H_{34}O_6$: C, 74.71; H, 6.61. Found, C, 74.59; H, 6.73.

1,12-Dibromo-2,3,10,11-tetramethoxy-4,9-diisopropyl-6,7-(methanoxymethano)dinaptho[1,2-*b*:2′,1′-*d*]furan (11) and **1,12-Dibromo-2,3,10,11-tetramethoxy-4,9-diisopropyl-14-oxo-6,7-(methanoxymethano)dinaptho[1,2-*b*:2′,1′-*d*]furan (12). Method A.** AgF (0.78 g, 6 mmol) was heated at 200 °C in vacuo for 5 h and cooled to rt under N_2. Then a solution of **4** (0.5 g, 0.6 mmol) in glyme (10 mL) was added. The reaction mixture was stirred at 120 °C under N_2 for 2 h and at 130 °C for 8 h. The resultant black mixture was poured into water (50 mL) and extracted with Et_2O. The extract was washed with water and dried over Na_2SO_4. Concentration of the extract in vacuo provided a brown oil which was purified by column chromatography (1:20 ethyl acetate/light petroleum ether) to yield crystalline **11** (20 mg) and **12** as rhombic crystals (10 mg). **11**: mp 264–265 °C. ^1H NMR (CD_3COCD_3): δ 1.55 [d, 12 H, 2 $CH(CH_3)_2$], 3.95 (d, 12 H, 4 OCH_3), 4.00 [m, 2 H, 2 $CH(CH_3)_2$], 5.37 (s, 4 H, 2 OCH_2Ar), 8.00 (s, 2 H, ArH-4). IR: 1568, 1344, 1039 cm^{-1}. MS: *m/e* 670 (49), 672 (100), 674 (52, M^+). Anal. Calcd for $C_{32}H_{32}Br_2O_6$: C, 57.14; H, 4.76. Found: C, 56.98; H, 4.82. **12**: mp 210–212 °C. ^1H NMR (CD_3COCD_3): δ 1.56–1.65 [q, 12 H, 2 $CH(CH_3)_2$], 3.98 [m, 2 H, 2 $CH(CH_3)_2$], 4.01–4.06 (q, 12 H, 4 OCH_3), 5.78 (s, 2 H, $ArCH_2O$), 8.29 (s, 1 H, ArH), 9.11 (s, 1 H, ArH). IR: 1715, 1452, 1022 cm^{-1}. MS: *m/e* 684 (49), 686 (100), 688 (53, M^+), 640 (4, $M^+ - CO2$). Anal. Calcd for $C_{32}H_{30}O_7Br_2$: C, 55.98; H, 4.37. Found: C, 55.83; H, 4.46.

Method B. To a stirred solution of **4** (130 mg, 0.16 mmol) in HMPA (2 mL) under N_2 were added tetrakis(triphenylphosphine)palladium (30 mg) and tetrakis(pentafluorophenyl)tin (300 mg, 0.37 mmol). The resulting yellow solution was heated at 80 °C for 40 h. Water (5 mL) was added. The solid that separated from solution was collected by filtration and was extracted with Et_2O. The extract was added to the filtrate. The resulting two layers were separated and the organic layer was dried (Na_2SO_4) and concentrated. Purification of the residue by preparative TLC (EtOAc/light petroleum ether, 9:1) and extraction (Et_2O) of the single chromophoric band gave, upon concentration of the extract, **11** as light yellow crystals (30 mg, 30%).

Method C. A mixture of **4** (100 mg, 0.12 mmol), Na_2S (43 mg, 0.55 mmol), benzene (3 mL), *t*-BuOH (10 mL), and water (2 mL) was refluxed for 10 h. The mixture was cooled to rt and Et_2O (50 mL) was added. The two layers were separated. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/light petroleum ether) to yield **11** as light yellow crystals (41 mg, 50%).

Acknowledgment. We thank Prof. Yi-Wen Wang for performing the NOE experiments and Prof. Qing-Yun Chen for valuable discussions.

Unsaturated Phosphonates as Acyclic Nucleotide Analogues. Anomalous Michaelis–Arbuzov and Michaelis–Becker Reactions with Multiple Bond Systems[1]

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Reaction of adenallene (**4a**) with methanesulfonyl chloride in pyridine afforded 4′-chloro-4′-deoxyadenallene (**6a**). A similar reaction with toluene-4-sulfonyl chloride (NEt_3, CH_2Cl_2) led to elimination of the unsaturated moiety and formation of N^9-(4-toluenesulfonyl)adenine (**8a**). Michaelis–Arbuzov reaction of *E*- and *Z*-unsaturated chlorides **13a** and **16b** with triethyl phosphite afforded phosphonates **14a** and **17b**. Dealkylation of the latter products, coupled in case of **17b** with acid hydrolysis, led to phosphonic acids **15a** and **18**. By contrast, Michaelis–Arbuzov reaction with butynyl chlorides **19a** and **19b** led to elimination of unsaturated moiety and alkylation of the released heterocyclic bases to give N^9-ethyl derivatives **20a** and **20b**. In the presence of iodide ion, N^9-(2,3-butadien-1-yl)adenine (**30a**, from **19a**) and/or unsaturated diphosphonates **25a** and **25b** were obtained. The Michaelis–Arbuzov reaction of chloroallene **6a** led to 2′-phosphonate **33a** which, after dealkylation, afforded phosphonic acid **35a**. When iodide ion was present, both 2′- and 4′-phosphonates **33a** and **36a** were obtained. Compound **36a** was also prepared by Michaelis–Becker reaction of chloroallene **6a** with sodium diethyl phosphite in THF–HMPA. In DMSO, both phosphonates **33a** and **36a** were formed. Under similar conditions (DMF), chlorobutyne **19a** gave 4′-phosphonate **36a**. Dealkylation of **36a** furnished phosphonic acid **37a**. Adenallene (**4a**) and diethyl chlorophosphite in pyridine afforded phosphonate **33a** whereas butynol **39a** afforded only adenine (**10a**). The probable reaction course of these transformations and spectral properties of the reaction products will be discussed.

Unsaturated analogues of nucleosides, cyclic and acyclic, are a focus of much current attention as antiviral and antitumor agents (Chart I). Thus, compounds **1a–1c** are effective agents[3] against human immunodeficiency virus (HIV), a cause of acquired immunodeficiency syndrome (AIDS). Neplanocin A (**2a**), a naturally occurring antibiotic, and its cytosine analogue **2b** exhibit a broad spectrum of antiviral and antitumor activities.[4] In the acyclic series, alkenediols **3a–3c** were found to be only moderately

(1) Presented in part at the 9th International Round Table *Nucleosides, Nucleotides & Their Biological Applications*; July 30–Aug 3, 1990, Uppsala, Sweden, Abstract No. 46; *Nucleosides Nucleotides* **1991**, *10*, 275.

(2) Dedicated to my esteemed teacher Dr. Zdeněk Arnold on the occasion of his 70th birthday.

(3) Recent review: Mitsuya, H.; Yarchoan, R.; Broder, S. *Science* **1990**, *249*, 1533.

(4) Recent review: Marquez, V. E.; Lim, M.-I. *Med. Res. Rev.* (*London*) **1986**, *6*, 1.

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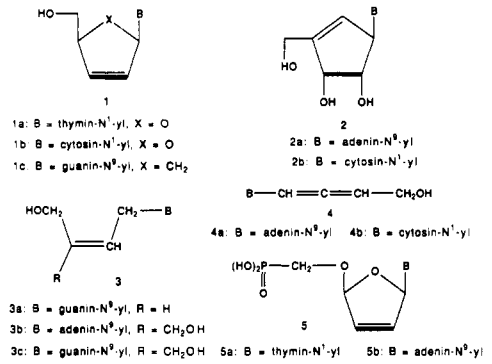
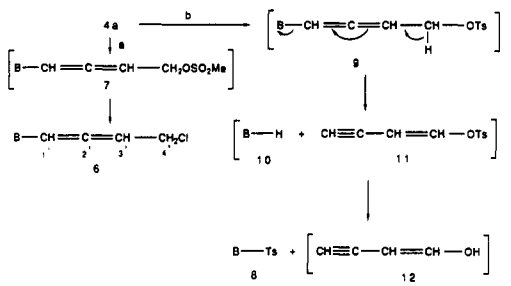
Chart I



Scheme I[a]



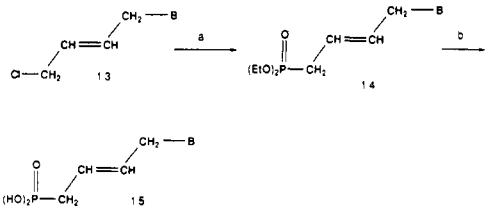
[a] In Schemes I–XI, series a denotes B = adenin-N^9-yl and series b B = 2-amino-6-chloropurin-N^9-yl. Key: (a) $MeSO_2Cl$, pyridine; (b) 4-$MeC_6H_4SO_2Cl$ (TsCl), NEt_3, CH_2Cl_2.

effective antiviral agents.[5,6] A group of allenic analogues,[7] particularly adenallene (**4a**) and cytallene (**4b**), are an exception because of their high and selective anti-HIV effect[3,8] in vitro. More recently, biologically active phosphonate analogues of nucleotides have attracted significant attention because of their metabolic stability and ability to penetrate cell membranes.[9] Examples in the area of unsaturated phosphonates include active antiretroviral agents[10] **5a** and **5b**. The former can be regarded as an analogue of the 5′-phosphate of **1a**.

The subject of our study is the investigation of routes applicable for synthesis of acyclic analogues of nucleotides containing multiple bond systems. Our attention has been focussed primarily on two procedures routinely used for synthesis of a variety of phosphonates—Michaelis–Arbuzov and Michaelis–Becker reactions.

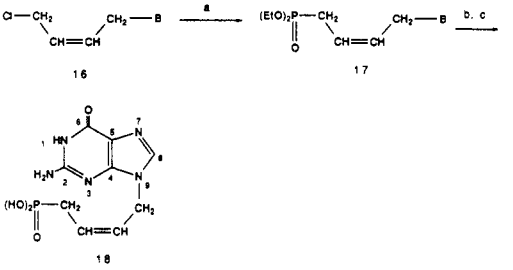
Starting Materials. Generally, alkyl halides are conveniently used as starting materials in both procedures mentioned above. Synthesis of a series of such derivatives containing a reactive chloro atom of allylic type and a nucleic acid base was described earlier.[7,11-13] Initially,[7] compound **6a** was prepared from adenallene (**4a**) by reaction with triphenylphosphine and CCl_4. An alternate

Scheme II[a]



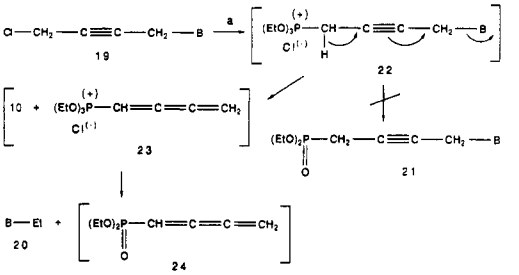
[a] Key: (a) $(EtO)_3P$, reflux; (b) Me_3SiI, $CHCl_3$.

Scheme III[a]



[a] Key: (a) $(EtO)_3P$, 110 °C; (b) Me_3SiI, $CHCl_3$; (c) 0.1 M HCl, reflux.

Scheme IV[a]



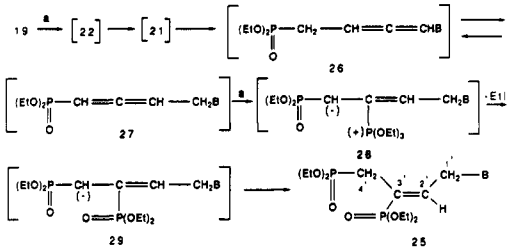
[a] Key: (a) $(EtO)_3P$, 110 °C.

procedure is the reaction of **4a** with methanesulfonyl chloride in pyridine. In this case, the intermediary methanesulfonate underwent an in situ displacement with chloride ion, and compound **6a** was obtained in 40% yield (Scheme I). More surprising was an attempted reaction of **4a** with 4-toluenesulfonyl chloride and NEt_3 in CH_2Cl_2. The only product obtained in 50% yield was N^9-(4-toluenesulfonyl)adenine[14] (**8a**). The presence of a strong base might have favored elimination over a simple displacement observed with methanesulfonyl chloride. This transformation gave the first indication that unsaturated moieties attached to heterocyclic bases and containing reactive groups might be prone to elimination. Thus, the formation of **8a** can be explained by toluenesulfonylation of allenol **4a** to give intermediary sulfonate ester **9a**. Elimination of the unsaturated moiety led to a release of adenine (**10a**) which in turn could react in situ with fragment **11** to give product **8a** and (presumed) 1-butyn-4-al (**12**).

Michaelis–Arbuzov[15] and Michaelis–Becker Reactions.[16] The Michaelis–Arbuzov reaction of (*E*)-chloroalkene[11] **13a** (refluxing in triethyl phosphite for 3.5 h) was routine and afforded the expected phosphonate **14a** in 61% yield (Scheme II). Dealkylation[17] of **14a** with io-

(5) Haines, D. R.; Tseng, C. K. H.; Marquez, V. E. *J. Med. Chem.* **1987**, *30*, 943.
(6) Larsson, A; Stenberg, B. G.; Ericson, A.-C.; Haglund, U.; Yisak, W.-A.; Johansson, N. G.; Oberg, B.; Datema, R. *Antimicrob. Agents Chemother.* **1986**, *30*, 598.
(7) Phadtare, S.; Zemlicka, J. *J. Am. Chem. Soc.* **1989**, *111*, 5925.
(8) Hayashi, S.; Phadtare, S.; Zemlicka, J.; Matsukura, M.; Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6127.
(9) *Nucleotide Analogues as Antiviral Agents*; Martin, J. C., Ed.; ACS Symposium Series, American Chemical Society: Washington, DC, 1989; Vol. 401, pp 17, 51, 72, and 88.
(10) Kim, C. U.; Luh, B. Y.; Martin, J. C. *J. Org. Chem.* **1991**, *56*, 2642.
(11) Phadtare, S.; Zemlicka, J. *J. Med. Chem.* **1987**, *30*, 437.
(12) Phadtare, S.; Zemlicka, J. *J. Org. Chem.* **1989**, *54*, 3675.
(13) Phadtare, S.; Kessel, D.; Corbett, T. H.; Renis, H. E.; Court, B. A.; Zemlicka, J. *J. Med. Chem.* **1991**, *34*, 421.
(14) Martirosyan, Z. A.; Gunar, V. I.; Zav'yalov, S. I. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1970**, 1841.
(15) Engel, R. *Synthesis of Carbon–Phosphorus Bonds*; CRC Press: Boca Raton, FL, 1988; p 21.
(16) Reference 15, p 7.

Scheme V[a]



[a] Key: (a) $(EtO)_3P$, NBu_4I, 80–110 °C.

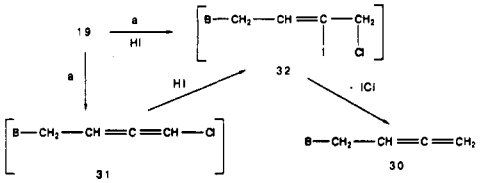
dotrimethylsilane in $CHCl_3$ furnished phosphonic acid **15a** (69%). Reaction of (*Z*)-chloroalkene[13] **16b** with triethyl phosphite (110 °C, 4 h) was also smooth and gave compound **17b** in 68% yield (Scheme III). Dealkylation and hydrolysis of the chloropurine residue (reflux in 0.1 M HCl) were performed in one pot, and phosphonic acid **18** was isolated using an adsorption–desorption procedure[18] on activated carbon in 65% yield.

In a stark contrast to the Michaelis–Arbuzov reaction observed with compounds **13a** and **16b**, chloroalkynes or -allenes behaved in an entirely different fashion. Thus, compounds[7] **19a** or **19b** when subjected to the conditions of Michaelis–Arbuzov reaction (triethyl phosphite, 110 °C, 3 h or reflux for 2 h) afforded N^9-ethyl derivatives of the corresponding heterocycles **20a** and **20b** in the yield of 39 and 27%, respectively (Scheme IV). None of the Michaelis–Arbuzov product **21a** or **21b** was obtained. It is likely that the initial complexes **22a** and **22b** were formed but they suffered elimination to give base **10a** or **10b** (see also Scheme I) and species **23** before they could have been transformed to "normal" products **21a** or **21b**. The released bases **10a** and **10b** could then be alkylated with **23** to give products **20a** and **20b** along with the presumed cumulene **24** which was not isolated.

It was recognized that one of the reasons why elimination of the base from **22a** or **22b** prevailed over a more usual dealkylation process could have been a relatively weak nucleophilicity but stronger basicity[19] of chloride ion. Introduction of a less basic but more powerful nucleophile, such as iodide ion, into the reaction mixture might then change the reaction course in favor of dealkylation of the Michaelis–Arbuzov intermediates **22a** or **22b**. Additional benefit may be seem in a possible conversion of chlorobutynes **19a** or **19b** to more reactive iodides under the reaction conditions. Indeed, Michaelis–Arbuzov reaction of **19b** (triethyl phosphite, 110 °C, 2 h) in the presence of tetrabutylammonium iodide (NBu_4I) did not afford any base **20b**. Instead, compound **25b** was obtained in 58% yield (Scheme V) as >90% pure (*E*)-3′,4′-diphosphonate isomer. The latter result indicates a high degree of regio- and stereoselectivity of this "double" Michaelis–Arbuzov reaction. The skeleton of **25b** somewhat resembles a nucleoside 3′,5′-diphosphate.

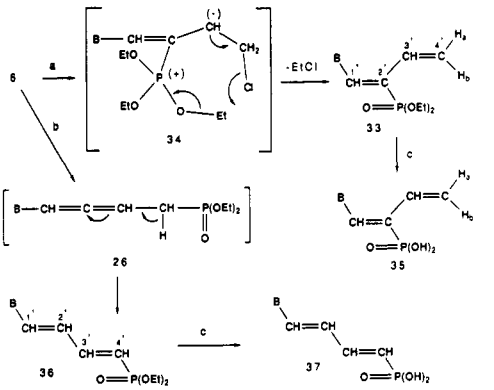
The reaction course can be explained as follows. The first step in sequence is probably the formation of phosphonate **21b** via complex **22b** (Scheme IV). The intermediate **21b** then undergoes isomerization to the respective allenic phosphonates **26b** and **27b**. The latter allene contains an activated double bond with a nucleophilic center at the sp-hybridized carbon 3′. It should be noted

Scheme VI[a]



[a] Key: (a) $(EtO)_3P$, NBu_4I, 120 °C.

Scheme VII[a]



[a] Key: (a) $(EtO)_3P$, reflux; (b) $(EtO)_2PONa$, THF–HMPA (7:5); (c) Me_3SiI, $CHCl_3$.

that triethyl or dialkyl phosphites are capable of reacting with double bonds of α,β-unsaturated carboxylic[20] acids or dialkyl allene phosphonates.[21] The interaction of **27b** with triethyl phosphite leads to formation of ylide **28b** which is then dealkylated to give carbanion **29b**. The protonation of **29b** is most likely accomplished by diethyl phoshite generated in situ by triethyl phosphite catalyzed elimination[22] of HI as shown by eqs 1 and 2:

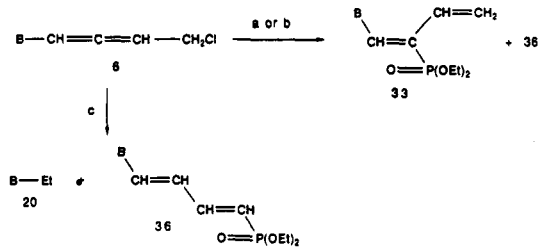
$$(C_2H_5O)_3P + C_2H_5I = /(C_2H_5O)_3PH/^+I^- + CH_2{=}CH_2 \quad (1)$$

$$/(C_2H_5O)_3PH/^+I^- = (C_2H_5O)_2POH + C_2H_5I \quad (2)$$

A similar transformation of chlorobutyne **19a** effected by triethyl phosphite (80 °C, 16 h), and NBu_4I furnished diphosphonate **25a** in 20% yield. In this case, an additional product, N^9-(2,3-butadien-1-yl)adenine (**30a**, 10%) was obtained. The latter product is an example of a hitherto undescribed type of heterocyclic allene. When the temperature was raised to 120 °C (reaction time 40 min) compound **30a** was obtained in 30% yield. Apparently, under those conditions, formation of **30a** competes more effectively with Michaelis–Arbuzov reaction. Because compound **30a** contains one more hydrogen atom than the starting material **19a** an addition–elimination mechanism of formation of **30a** utilizing HI generated according to the eqs 1 and 2 is likely (Scheme VI). It is also clear that diphosphonate **25a** cannot function as an intermediate in the formation of **30a**. Therefore, we propose that the first step in the reaction sequence is an allenic isomerization to intermediate **31a**. Addition of HI then leads to vicinal dihalide **32a** which then eliminates ICl spontaneously[23] or

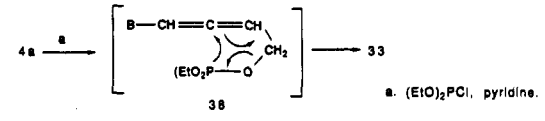
(17) Blackburn, G. M.; Ingleson, D. *J. Chem. Soc., Chem. Commun.* **1978**, 870.
(18) Hurlbert, R. A. In *Methods of Enzymology*; Colowick, S. P., Kaplan, N. O., Eds.; Academic Press: New York, 1957; Vol. III, p 785.
(19) March, J. *Advanced Organic Chemistry*; MacGraw Hill: New York, 1977; p 322.
(20) Kirby, A. J.; Warren, S. G. *The Organic Chemistry of Phosphorus*; Elsevier: New York, 1967; p 52.
(21) Pudovik, A. N.; Khusainova, N. G. *Zh. Obshch. Khim.* **1966**, *36*, 1236.
(22) Aksnes, G.; Aksnes, D. *Acta Chem. Scand.* **1965**, *19*, 898.
(23) Sonnet, P. E.; Oliver, J. E. *J. Org. Chem.* **1976**, *41*, 3284.

Scheme VIII[a]

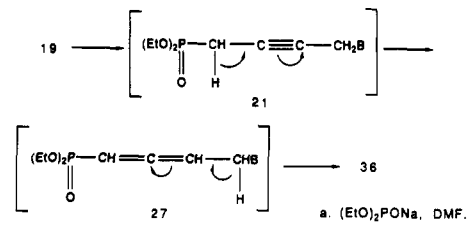


[a] Key: (a) $(EtO)_3P$, NBu_4I, 110 °C; (b) $(EtO)_2PONa$, DMSO; (c) $(EtO)_2PONa$, HMPA.

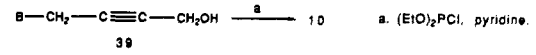
Scheme IX



Scheme X



Scheme XI



with an assistance of triethyl phosphite[24] to furnish allene **30a**.

The results mentioned above indicate a sharp difference between the course of Michaelis–Arbuzov reaction of compounds **13a** and **16b** containing a single olefinic bond on one hand and acetylenes **19a** and **19b** on the other. In a similar fashion, chlorobutyne[7] **19a** gave a different product than chloroallene[7] **6a**. Thus, reflux of **6a** in triethyl phosphite for 0.5 h afforded 2′-phosphonate **33a** in 30% yield (Scheme VII). Again, this reaction is of a remarkable regio- and stereoselectivity giving only the *E*-isomer of 2′-phosphonate **33a**. Obviously, formation of such a product is difficult to explain by invoking the Michaelis–Arbuzov reaction at the carbon 4′ and subsequent rearrangement. More likely, compound **6a** is attacked at the allenic 2′ carbon atom by triethyl phosphite to form intermediate **34a**. The latter then suffers dealkylation coupled with an elimination of chloride in an intramolecular fashion as described in Scheme VII or by an intermolecular mechanism (elimination is then preceding the dealkylation). A routine dealkylation[17] of **33a** using iodotrimethylsilane in $CHCl_3$ afforded phosphonic acid **35a** in 60% yield.

The Michaelis–Becker reaction of chloroallene **6a** with sodium diethyl phosphite in THF–HMPA mixture gave an *all-trans*-4′-phosphonate **36a** (26%), also in a high stereoselectivity (Scheme VII). Conventional dealkylation of **36a** furnished phosphonic acid **37a** in 68% yield. In this case, it is reasonable to postulate an initial formation of allenic phosphonate **26a** (see Scheme V). This is consistent with a conventional view[16] that Michaelis–Becker reaction proceeds with a direct attack of phosphorus atom at the nucleophilic center. Compound **26a** is in turn converted to diene **36a**. This facile isomerization reflects formation of a highly stable conjugated system of double bonds in **36a**. The composition of the reaction products is strongly solvent-dependent. Thus, in dimethyl sulfoxide (DMSO) both phosphonates **33a** and **36a** were formed (Scheme VIII). The reaction of adenallene (**4a**) with diethyl chlorophosphite in pyridine afforded **33a**, via intermediate **38a**, albeit in a low (12%) yield (Scheme IX). Similar transformations of (diphenylphosphinoxy)allenes were described[25] but diethoxyphosphinoxy derivatives did not react. In our case, compound **38a** was of limited stability to be isolated. In HMPA alone (Scheme VIII), N^9-ethyladenine (**20a**) was the major product (23%) accompanied by phosphonate **36a** (6%). The latter result indicates that elimination of the base residue (see Scheme IV) can compete with the formation of phosphonate even under relatively mild conditions of Michaelis–Becker reaction. Interestingly, both phosphonates **33a** and **36a** were also formed by Michaelis–Arbuzov reaction in the presence of iodide (Scheme VIII). Apparently, conversion of chloroallene **6a** to the corresponding iodide increased reactivity at the $C_{4'}$, and competition with a direct attack of phosphite at the allenic carbon $C_{2'}$ (see Scheme VII) became relevant.

Phosphonate **36a** was also formed in 23% yield from acetylene **19a** by a Michaelis–Becker reaction in *N,N*-dimethylformamide (DMF, Scheme X). In this case, the reaction course probably includes an intermediary formation of phosphonate **21a** (see Scheme IV) and subsequent isomerization to phosphonate **36a** via allene **27a** (see Scheme V). Because chloroalkyne **19a** is readily available,[7] this procedure is more advantageous than that starting from chloroallene **6a** (Scheme VII) although the yields are almost identical. Reaction of butynol[7] **39a** with diethyl chloro phosphite in pyridine (Scheme XI) led only to formation of adenine (**10a**). No allenic product was observed (see, e.g., refs 25, 26). Lack of N-alkylation is attributed to the presence of a weak base (pyridine) insufficient to effectively ionize the imidazole portion of the released **10a**.

Structure of the Reaction Products. The structures of all new products and, particularly, those resultant from an anomalous course of Michaelis–Arbuzov and Michaelis–Becker reactions (compounds **25a**, **25b**, **30a**, **33a**, and **36a**) were confirmed by NMR spectroscopy. The NMR spectra of these products, with the exception of allene **30a**, are quite complex because of extensive heteronuclear coupling. In spite of such complications, the structure of compound **25b** was deduced from the ^1H NMR spectrum. Thus, the broad doublet of doublet of doublets (signal width 41 Hz) at δ 6.69 was assigned to $H_{2'}$. The $J_{2',P\text{-}3'}$ (22 Hz) is within the region (13–25 Hz) characteristic for a cis arrangement of the $H_{2'}$ and phosphonyl moiety.[25,27] Furthermore, the $H_{4'}$ is coupled to both $P_{4'}$ and $P_{3'}$ atoms to give a doublet of doublets with coupling constants of 22.4 and 16.6 Hz, respectively. Irradiation at the $H_{1'}$ resulted in a collapse of $H_{2'}$ to a doublet of doublets with J = 22 ($J_{2',P\text{-}3'}$) and 6 Hz ($J_{2',P\text{-}4'}$) whereas the multiplicity of $H_{4'}$ was not changed. Conversely, decoupling at the $H_{2'}$ signal changed an overlapping doublet of triplets of $H_{1'}$ to poorly resolved triplet. Again, the $H_{4'}$ was preserved as

(24) Formation of olefins from vicinal dibromides and triphenylphosphine was described: Tung, C. C.; Speziale, A. J. *J. Org. Chem.* **1963**, *28*, 1521.

(25) Huché, M.; Cresson, P. *Bull. Soc. Chim. Fr.* **1975**, No. 3–4, 800.

(26) Reference 15, p 176.

(27) Williamson, M. P.; Castellano, S.; Griffin, C. E. *J. Phys. Chem.* **1968**, *72*, 175.

a doublet of doublets. These results are best explained in terms of a long-range interaction of $H_{2'}$ with $P_{4'}$ and $H_{1'}$ with both $P_{3'}$ and $P_{4'}$. Coupling of protons with phosphorus across four or five bonds was described.[28,29] In a NOE experiment, the strongest effect was noted, after irradiation at $H_{1'}$, on a vicinal $H_{2'}$ proton (5.7% enhancement) accompanied by weaker enhancements at the H_8 (3%) and $H_{4'}$ (2.5%), respectively. These data have established the structure of **25b** as the *E*-isomer of 3',4'-diphosphonate.

Further confirmation of structure came from the ^{31}P and ^{13}C spectra. Thus, the proton-undecoupled spectrum of **25b** exhibits two multiplets at δ 17.41 and 25.13, respectively, which after decoupling afforded two doublets of $^3J_{P,P}$ = 13.2 Hz. The phosphonate group-carrying $C_{3'}$ appears as a doublet of doublet and it has, as expected, the largest coupling constant found in **25b** ($J_{3',P\text{-}3'}$ = 189.5 Hz) whereas $J_{3',P\text{-}4'}$ is 12.1 Hz. The former value is similar to that found for the C_1 in diethyl vinylphosphonate[30] (182.1 Hz). In diphosphonate **25b** the $C_{2'}$ lines are overlapped with C_8 and the multiplicity of the signal is not clear. By contrast, the $C_{2'}$ of adenine derivative **25a** is well separated from the heterocyclic signals, and it forms an apparent triplet instead of the expected doublet of doublets. This is most likely the result of two overlapped doublets of almost equal coupling (J = ca. 10 Hz) to both $P_{3'}$ and $P_{4'}$. As expected, the $C_{1'}$ is coupled only to P_3($J_{1',P\text{-}3'}$ = 22.2 Hz). As in the $H_{4'}$ coupling, the $C_{4'}$ forms a doublet of doublets which is coupled to both $P_{4'}$($J_{4',P\text{-}4'}$ = 139.8 Hz) and $P_{3'}$-($J_{4',P\text{-}3'}$ = 7 Hz). The NMR spectra of diphosphonate **25a** are, except the signals associated with the adenine moiety, very similar to those of **25b**.

In contrast to adenallene[7] (**4a**) which has the system of cumulated double bonds attached directly to the heterocyclic moiety, the spectral properties of compound **30a** are more in line with simple alkylated or arylated allenes.[31,32] Thus, the IR spectrum exhibits a well-pronounced band at 1950 cm^{-1}, and the signal for $C_{3'}$ carbon in ^{13}C NMR spectrum is at δ 208.19. The DEPT experiment[33,34] showed the presence of three CH and two CH_2 groups. The UV maximum of **30a** corresponds to an N^9-alkyladenine.

The ^1H NMR was also instrumental for assignment of structures of phosphonates **33a** and **36a**. Thus, the signal of the $H_{1'}$ in the 2'-phosphonate **33a** appears at the lowest field of all protons of the diene portion as a doublet. Double resonance experiments showed no coupling with any other proton in the molecule. The observed doublet thus reflects an interaction with the phosphorus atom. The $J_{1',P}$ value of 16.5 Hz is in agreement with a cis arrangement of the $H_{1'}$ and phosphonate group.[27] The $H_{3'}$ forms a split doublet of doublet of doublets at δ 6.70 (signal width 54.3 Hz) which is coupled with phosphorus ($J_{3',P}$ = 25.5 Hz) as well as $H_{4'a}$ ($J_{3',4'a}$ = 11 Hz) and $H_{4'b}$ ($J_{3',4'b}$ = 17.7 Hz). As expected, the latter protons exhibit a pattern typical for a vinyl group. Overall, these findings have established the structure of **33a** as the *E*-isomer of 2'-phosphonate.

The ^{13}C spectra and, particularly, the DEPT experiment corroborated further the proposed structure. Thus, **33a** contains a single type of methyl group (ethoxy functions) and two different kinds of methylene moieties including $=CH_2$. In addition, from the four CH groups present two are coupled with phosphorus ($C_{1'}$ and $C_{3'}$). The fact that all carbons of the diene system are coupled to phosphorus is in accord with the structure of **33a**. The quaternary $C_{2'}$ which carries the phosphonate moiety has, as expected, the largest coupling constant $J_{2',P}$ = 178 Hz.

The lowest field doublet of all diene protons in the ^1H NMR spectrum of phosphonate **36a** was assigned to the $H_{1'}$. It is coupled to the $H_{2'}$ with $J_{1',2'}$ 13.8 Hz indicating a trans relationship of both hydrogens. Furthermore, irradiation at δ 7.74 ($H_{1'}$) led to a collapse of the triplet at δ 7.27 ($H_{2'}$) to a doublet of $J_{2',3'}$ = 10 Hz. The latter value falls within the range typical for an arrangement =CHCH= (9–13 Hz).[35] Neither the $H_{1'}$ nor $H_{2'}$ are coupled with the $H_{4'}$ or phosphorus which is in line with the proposed structure of **36a**. Irradiation at the $H_{2'}$ frequency converted $H_{1'}$ to a singlet and it also produced changes in the pattern of $H_{3'}$. The latter signal forms an apparent doublet of doublet of doublets at δ 7.08 (signal width 48 Hz) resembling the $H_{2'}$ pattern in diphosphonates **25a** and **25b**. The changes caused by irradiation of the $H_{3'}$ were less straightforward. Thus, the $H_{2'}$ was transformed to a split doublet, $H_{4'}$ gave a split doublet of doublets, and $H_{1'}$ was not influenced. The doublet of doublets of $H_{4'}$ at δ 5.94 arose from coupling with the trans $H_{3'}$ (J = 16.5 Hz) and phosphorus (J = 19.7 Hz). The latter value is in accordance with a magnitude of similar interaction in diethyl vinylphosphonate (21–22 Hz).[27]

The DEPT experiment indicated the presence of six CH groups and no $=CH_2$. None of the vinylic carbons is quaternary. Two of the CH's belong to adenine ring, and three ($C_{2'}$ through $C_{4'}$) from the remaining are coupled to phosphorus. All these data support the structural assignment of **36a** as the *all-trans*-4'-phosphonate.

The NMR spectra of phosphonates **35a** and **37a** (sodium salts at pH 7) followed the pattern found with parent compounds **33a** and **36a**. A comparison of the $^1J_{C,P}$ coupling constants of unsaturated phosphonates resultant from this study indicates a range of 178–190 Hz for esters **25a**, **25b**, **33a**, and **36a** as well as 157 and 169 Hz for acids **35a** and **37a**. The latter values are approximately between those of diesters **33a**, **35a** (or diethyl vinylphosphonate[30]) and dichloro vinylphosphonate.[30] Thus, even in complex-substituted vinylphoshonates such as **25a** and **25b** or in conjugated dienes such as **33a** and **35a** the $^1J_{C,P}$ values are very similar. This is in agreement with the previous postulate[30] that the Fermi term, reflecting a contribution of s-orbital component to the C(sp^2)–P bond, is of decisive importance for the magnitude of the $^1J_{C,P}$.

Biological Data. Among the phosphonic acids **15a**, **18**, and **37a**, only compound **18** inhibited the growth of murine leukemia L1210 at concentration <300 μM (IC_{50} 10 μM). Other biological testing is in progress.

Experimental Section

General Methods. See ref 7. The following solvents were used for thin-layer (TLC) and column chromatography: (S_1) CH_2Cl_2–THF (4:1), (S_2) CH_2Cl_2–MeOH (9:1), (S_3) 2-propanol–NH_4OH–H_2O (7:1:2), (S_4) CH_2Cl_2–ether (1:1), (S_5) CH_2Cl_2–MeOH (95:5), (S_6) CH_2Cl_2–MeOH (96:4), and (S_7) CH_2Cl_2–MeOH (97:3). The UV spectra were measured in ethanol unless specified otherwise. The ^1H NMR spectra were determined at 300 MHz in $(CD_3)_2SO$ unless stated otherwise. The ^{13}C and ^{31}P NMR were measured in the same solvent at 75.48 and 121.47 MHz, respectively. For ^{31}P NMR chemical shifts H_3PO_4 was used as a ref-

(28) Mavel, G. *Annu. Rep. NMR Spectrosc.* **1973**, *5B*, 1; loc. cit. 51.
(29) Siddall, T. H., III; Stewart, W. E. *Spectrochim. Acta* **1968**, *24A*, 81.
(30) Althof, W.; Fild, M.; Rieck, H.-P.; Schmutzler, R. *Chem. Ber.* **1978**, *111*, 1845.
(31) Runge, W. In *The Chemistry of Allenes*; Landor, S. R., Ed. Academic Press: New York, 1982; Vol. 3, p 777.
(32) Reference 31, p 832.
(33) Benn, R.; Günther, H. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 350.
(34) Doddrel, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Res.* **1982**, *48*, 323.
(35) Bible, R. H., Jr. *Interpretation of NMR Spectra*; Plenum Press: New York, 1965; p 38.

erence. Electron impact (EI-MS), chemical ionization (CI-MS), and fast-atom bombardment mass spectra (FAB-MS) were determined as described previously.[7] Paper electrophoresis was performed using a flat-bed instrument (Savant) on Whatman No. 1 paper at 100 V/cm for 1 h in 0.02 M Na_2HPO_4 (pH 7.5). Mobilities of compounds are based on adenosine 5'-phosphate (AMP, mobility = 1.00).

N^9-(Toluene-4-sulfonyl)adenine (8a). Adenallene[7] (**4a**, 406 mg, 2 mmol) was suspended in CH_2Cl_2 (200 mL), and triethylamine (202 mg, 2 mmol) was added followed by toluene-4-sulfonyl chloride (380 mg, 2 mmol). The mixture was stirred overnight at room temperature, it was evaporated, and the residue was chromatographed on a silica gel column using solvent S_1 as eluent to give compound **8a** (355 mg, 50%), mp 207–211 °C (sintering from 140 °C) after crystallization from cyclohexane–ethyl acetate (4:1) (lit.[14] mp 206–207 °C: UV max 240, 280 (shoulder); ^1H NMR δ 8.59 and 8.20 (2s, 2, H_2 and H_8), 8.07 and 7.47 (2d, 4, toluene-4-sulfonyl), 7.63 (s, 2, NH_2), 2.35 (s, 3, Me).

N^9-(4-Chlorobuta-1,2-dien-1-yl)adenine (6a). Methanesulfonyl chloride (78 μL, 1 mmol) was added to the solution of adenallene[7] (**4a**, 203 mg, 1 mmol) in pyridine (15 mL) with stirring which was continued for 1 h at room temperature. The solution was evaporated (oil pump), and the crude product was chromatographed on a silica gel column using solvent S_2 as an eluent to give compound **6a** (112 mg, 40%), mp 170 °C dec, identical with an authentic sample[7] (IR, mixed mp, and TLC, S_2) (lit.[7] 175 °C dec).

(*E*)-N^9-(4-(Diethylphosphono)-2-buten-1-yl)adenine (14a). Compound[11] **13a** (1.11 g, 5 mmol) was refluxed in triethyl phosphite (30 mL) under N_2 for 3.5 h. TLC (S_2) showed a complete disappearance of **13a**. After cooling, the solution was decanted from a brown syrup which was washed with triethyl phosphite (2 × 10 mL). Evaporation in vacuo (oil pump) gave product **14a** homogeneous on TLC (S_2), 0.99 g (61%), which was crystallized from cyclohexane–ethyl acetate (1:1), mp 115–116 °C: UV max 261 (15 400), 210 (18 600); ^1H NMR ($CDCl_3$) δ 8.34 and 7.80 (2s, 2, H_2 and H_8), 6.28 (s, 2, NH_2), 5.84 and 5.78 (2m, 2, $H_{2'}$ and $H_{3'}$, $J_{2',3'}$ = 15.4), 4.79 (t, 2, $H_{1'}$), 4.08 (dq, 4, CH_2 of EtO), 2.62 (dd, 2, $H_{4'}$, $J_{4',3'}$ = 7.1 Hz, $^2J_{4',P}$ = 21.8 Hz), 1.28 (dt, 6, Me); EI-MS 325 (M, 3.0), 188 (M – $PO(OEt)_2$, 64.2), 174 (M – $CH_2PO(OEt)_2$, 100.0), 135 (**10a**, 25.6). Anal. Calcd for $C_{13}H_{20}N_5O_3P$: C, 47.99; H, 6.19; N, 21.53; P, 9.52. Found: C, 48.11; H, 6.32; N, 21.71; P, 9.36.

(*E*)-N^9-(4-Phosphono-2-buten-1-yl)adenine (15a). A solution of phosphonate **14a** (0.65 g, 2 mmol) in chloroform (20 mL) was cooled to −40 °C, and iodotrimethylsilane (1.6 g, 8 mmol) was added dropwise under N_2 with magnetic stirring. The stirring was continued for 30 min and at room temperature for 2 h. The solvent was evaporated, and the residue was stirred in water (15 mL) for 15 min. After evaporation, a solid was obtained which was washed several times with acetone (45 mL total) to give 0.37 g (69%) of phosphonic acid **15a**, homogeneous on TLC (S_3). For analysis, the product was dissolved in boiling water (150 mL), and the solution was filtered and concentrated to 15–20 mL whereupon it deposited crystalline **15a**, mp 306–310 °C: electrophoretic mobility 0.79 of AMP; UV (pH 7) max 261 (13 700), 210 (16 700); ^1H NMR (D_2O, sodium salt) δ 7.92 and 7.88 (2s, 2, H_2 and H_8), 5.72 and 5.66 (2m, 2, $H_{2'}$ and $H_{3'}$, $J_{2',3'}$ = ca. 15 Hz), 4.57 (t, 2, $H_{1'}$), 2.44 (dd, 2, $H_{4'}$, $J_{4',3'}$ = 7.1 Hz $^2J_{4',P}$ = 20.9 Hz); ^{31}P NMR 35.06. Anal. Calcd for $C_{12}H_{12}N_5O_3P$: C, 40.15; H, 4.49; N, 26.01; P, 11.50. Found: C, 40.14; H, 4.59; N, 25.88; P, 11.28.

(*Z*)-2-Amino-6-chloro-N^9-(4-(diethylphosphono)-2-buten-1-yl)purine (17b). Compound[7] **16b** (1.29 g, 5 mmol) was stirred in triethyl phosphite (30 mL) at 110 °C under N_2 for 4 h. The reaction mixture was cooled and evaporated (oil pump) to give syrup **17b** (1.7 g, 95%). TLC (S_2) showed only a trace of impurity at the origin. Crystallization from cyclohexane–ethyl acetate (4:1) afforded 1.22 g (68%) of pure **17b**, mp 138–141 °C, homogeneous on TLC (S_2). UV max 310 (7800), 247 (6000), 223 (21 100); ^1H NMR ($CDCl_3$) δ 7.86 (s, 1, H_8), 5.79 (m, 2, $H_{2'}$ and $H_{3'}$, $J_{2',3'}$ = ca. 9 Hz), 5.39 (s, 2, NH_2), 4.76 (t, 2, $H_{1'}$), 4.13 (dq, 8, CH_2 of Et), 2.85 (dd, 2, $H_{4'}$, $J_{4',3'}$ = 6.6 Hz, $^2J_{4',P}$ = 22.8 Hz), 1.33 (t, 6, Me); EI-MS 361, 359 (M, 3.8, 10.9), 210, 208 (M – $PO(OEt)_2$, 5.2, 14.1), 171, 169 (**10b**, 5.2, 15.5), 135 (**10b** – Cl, 18.6). Anal. Calcd for $C_{13}H_{19}ClN_5O_3P$: C, 43.40; H, 5.32; Cl, 9.86; N, 19.47; P, 8.61. Found: C, 43.52; H, 5.33; Cl, 9.92; N, 19.52; P, 8.37.

(*Z*)-N^9-(4-Phosphono-2-buten-1-yl)guanine (18). The procedure described for compound **15a** was followed on a 3-mmol scale in chloroform (30 mL). After evaporation, the crude product was refluxed in 0.1 M HCl (100 mL) for 14 h. The pH of a cooled (0–5 °C) reaction mixture was adjusted to 7.0 (pH meter) with 0.1 M LiOH, and the resultant solution (250 mL) was stirred with activated carbon (Norit A, 10 g) and Celite (10 g) for 2 h at room temperature. The mixture was filtered, and the solids were washed with water (3 × 25 mL). No UV absorption corresponding to guanine was detected in the filtrate. The elution was continued with 10 M NH_4OH until the UV absorption disappeared (200 mL). The eluate was evaporated, the resultant solid was dissolved in water (30 mL), the solution was filtered using a Celite pad, and the filtrate was evaporated to give **18** (ammonium salt, 0.58 g, 65%), homogeneous on TLC (S_3). For analysis, it was converted to sodium salt using NaI in acetone.[36] The resultant solid was free from iodide ($AgNO_3$): electrophoretic mobility 0.76 of AMP; UV (pH 7) max 252 (11 300), 204 (19 500), shoulder 271 (8200); ^1H NMR (D_2O) δ 7.70 (s, 1, H_8), 5.82, 5.60 (2m, 2, $H_{2'}$ and $H_{3'}$, $J_{2',3'}$ = 10 Hz), 4.60 (m, 2, $H_{1'}$), 2.56 (dd, 2, $H_{4'}$, $J_{4',3'}$ = 8 Hz, $^2J_{4',P}$ = 21 Hz); ^{31}P NMR δ 17.62. Anal. Calcd for $C_9H_{10}N_5Na_2O_4P \cdot 3H_2O$: C, 28.20; H, 4.20; N, 18.27; P, 8.08. Found: C, 28.25; H, 3.85; N, 17.95; P, 8.05.

Reaction of Compound 19b with Triethyl Phosphite. A. 2-Amino-6-chloro-N^9-ethylpurine (20b). Compound[7] **19b** (1.28 g, 5 mmol) was heated in triethyl phosphite (30 mL) at 110 °C for 3 h with stirring under N_2. TLC in S_2 showed a complete disappearance of **19b**. After cooling, the mixture was evaporated (oil pump). The crude product was chromatographed on a silica gel column using solvent S_4 to give 2-amino-6-chloro-N^9-ethylpurine[37] (**20b**, 0.39 g, 39%), homogeneous on TLC (S_1), mp 156–158 °C after crystallization from the same solvent: UV max 309 (7800), 247 (6000), and 223 (19 900); ^1H NMR ($CDCl_3$) δ 7.80 (s, 1, H_8), 5.17 (s, 2, NH_2), 4.16 (q, 2, CH_2), 1.52 (t, 3, Me); EI-MS 199, 197 (M, 32.6, 100.0), 171, 169 (**10b**, 17.0, 53.8), 162 (M – Cl, 46.8), 134 (57.5). Anal. Calcd for $C_7H_8ClN_5$: C, 42.53; H, 4.08; Cl, 17.93; N, 35.44. Found: C, 42.27; H, 4.28; Cl, 18.18; N, 35.16.

B. (*E*)-2-Amino-6-chloro-N^9-(3,4-bis(diethylphosphono)-2-buten-1-yl)purine (25b). The experiment was performed as in the preceding case with NBu_4I (1.84 g, 5 mmol) added to the mixture. The reaction time was 1 h. The work-up followed also the procedure described above. The column was eluted first with solvent S_1 to remove NBu_4I. Elution with solvent S_5 then afforded compound **25b** as a syrup (1.63 g, 58%), homogeneous on TLC (S_2): UV max 310 (5700), 221 (20 100), 205 (16 800); ^1H NMR ($CDCl_3$) δ 7.91 (s, 1, H_8), 6.69 (ddd, 1, $H_{2'}$, $^3J_{2',P-3'}$ = 22 Hz, $^4J_{2',P-4'}$ = 6 Hz), 5.50 (s, 2, NH_2), 5.02 (poorly resolved dt, 1, $H_{1'}$), 4.17 and 4.06 (2q, 8, CH_2O), 3.02 (dd, 2, $H_{4'}$, $^2J_{4',P-4'}$ = 22.4 Hz, $^3J_{4',P-3'}$ = 16.6 Hz), 1.34 and 1.29 (2t, 12, Me); ^{13}C NMR δ 159.18, 153.62, 151.32, 142.13, 125.04 (**10b**), 141.95 ($C_{2'}$, overlapped with C_8), 124.81 (dd, $C_{3'}$, $^1J_{3',P-3'}$ = 189.5 Hz, $^2J_{3',P-4'}$ = 12.1 Hz), 62.57, 62.48, 62.43, 62.33 (CH_2O), 42.18 (d, $C_{1'}$, $^3J_{1',P-3'}$ = 22.2), 25.69 (dd, $C_{4'}$, $^1J_{4',P-4'}$ = 139.8 Hz, $^2J_{4',P-3'}$ = 7 Hz), 16.42, 16.34, 16.28, 16.19 (Me); ^{31}P NMR δ 25.13, 17.41 ($^3J_{P,P}$ = 13.2 Hz); EI-MS 497, 495 (M, 1.2, 3.2), 360, 358 (M – $(EtO)_2PO$, 14.4, 41.8), 149 (100.0); CI-MS 498, 496 (M + H, 33.3, 94.9). Anal. Calcd for $C_{17}H_{28}ClN_5O_6P_2$: C, 41.18; H, 5.69; Cl, 7.15; N, 14.13; P, 12.49. Found: C, 41.20; H, 5.86; Cl, 7.31; N, 14.26; P, 12.23.

Reaction of Compound 19a with Triethyl Phosphite. A. N^9-Ethyladenine (20a). Compound[7] **19a** (0.2 g, 0.9 mmol) was refluxed in triethyl phosphite (10 mL) for 2 h. The mixture was evaporated, and the residue was chromatographed on a silica gel column in solvent S_5 to give N^9-ethyladenine (**20a**, 40 mg, 27%), mp 190–192 °C after crystallization from benzene, identical with an authentic sample, lit.[38] 191–193 °C.

B. N^9-(2,3-Butadien-1-yl)adenine (30a). A mixture of chlorobutyne[7] **19a** (200 mg, 0.9 mmol) and NBu_4I (333 mg, 0.9 mmol) was heated in triethyl phosphite (10 mL) at 120 °C for 40 min. After cooling, the solution was evaporated in vacuo and

(36) Moffatt, J. G. *Can. J. Chem.* **1964**, *42*, 599.

(37) This compound was recently reported without any characterization: Aswell, M.; Bleasdale, C.; Golding, B. T.; O'Neill, I. K. *J. Chem. Soc., Chem. Commun.* **1990**, 955.

(38) Fujii, T.; Wu, C. C.; Itaya, T. *Chem. Pharm. Bull. (Tokyo)* **1973**, *21*, 1835.

the residue was chromatographed on a silica gel column using first solvent S_1 to remove NBu_4I and then S_6 to afford allene **30a** (50 mg, 30%), mp 150–152 °C after crystallization from ethyl acetate–petroleum ether (1:3): UV max 261 (14 100), 210 (18 400); IR (KBr) 1950 cm^{-1} (C=C=C); ^1H NMR δ 8.10 and 8.09 (2s, 2, H_2 and H_8), 7.18 (s, 2, NH_2), 5.52 (m, 1, $H_{2'}$), 4.83 (m, 2, $H_{4'}$), 4.72 (m, 2, $H_{1'}$); ^{13}C NMR δ 208.19 ($C_{3'}$), 88.17 ($C_{2'}$), 78.62 ($C_{4'}$), 41.36 ($C_{1'}$); EI-MS 187 (94.3, M), 186 (100.0, M – H), 159 (26.9, M – HCN), 135 (33.5, **10a**), 108 (59.3, **10a** – HCN), 53 (51.7, CH_2=C=CHCH$_2$ or CH_2=CHCH=CH); CI-MS 188 (100.0, M + H), 159 (3.8, M – HCN), 135 (5.0, **10a**), 108 (7.5, **10a** – HCN). Anal. Calcd for $C_9H_9N_5$: C, 57.74; H, 4.84; N, 37.41. Found: C, 57.59; H, 5.03; N, 37.26.

C. (*E*)-*N*^{9}-(3,4-Bis(diethylphosphono)-2-buten-1-yl)adenine (25a). A mixture of chlorobutyne **19a** (180 mg, 0.8 mmol) and NBu_4I (300 mg, 0.8 mmol) was heated in triethyl phosphite (7 mL) at 80 °C for 16 h. After evaporation, the residue was chromatographed on a silica gel column as described in method B. Elution with solvent S_6 gave allene **30a** (15 mg, 10%, mp 150–152 °C, see method B) followed by compound **25a** as a syrup which solidified after prolonged standing at room temperature (72 mg, 20%), mp 141–143 °C: UV max 261 (14 000), 207 (22 700); ^1H NMR ($CDCl_3$) δ 8.22 and 7.91 (2s, 2 H_2 and H_8), 6.68 (ddd, 1, $H_{2'}$, $J_{2',P-3'}$ = 22.5 Hz), 6.38 (s, 2, NH_2), 5.09 (m, 2, $H_{1'}$), 4.11 and 4.05 (2m, 8, CH_2O), 3.04 (dd, 2, $H_{4'}$, $J_{4',P-4'}$ = 22.4 Hz, $J_{4',P-3'}$ = 16.6 Hz), 1.29 and 1.23 (2m, 12, Me); ^{13}C NMR δ 155.47, 152.83, 149.59, 140.16, 119.02 (**10a**), 142.72 (apparent t, $C_{2'}$, $^2J_{2',P-3'} = {}^3J_{2',P-4'}$ = 10 Hz), 123.92 (dd, $C_{3'}$, $^1J_{3',P-3'}$ = 184.5 Hz, $^2J_{3',P-4'}$ = 10.4 Hz), 62.48, 62.39, 62.34, 62.27 (CH_2O), 42.09 (d, $C_{1'}$, $^3J_{1',P-3'}$ = 22.2 Hz), 25.43 (dd, $C_{4'}$, $^1J_{4',P-4'}$ = 140.1 Hz, $^2J_{4',P-3'}$ = 10.2 Hz), 16.28, 16.20, 16.11, 16.03 (Me); ^{31}P NMR δ 25.3, 17.57 ($^3J_{P,P}$ = 13.2 Hz); EI-MS 461 (22.3, M), 324 (100.0, M – $(EtO)_2PO$), 135 (26.0, **10a**), exact mass calcd 461.1593, found 461.1597. Anal. Calcd for $C_{17}H_{29}N_5O_6P_2$: C, 44.25; H, 6.33; N, 15.17; P, 13.42. Found: C, 44.21; H, 6.58; N, 15.41; P, 13.23.

(*E*)-*N*^{9}-(2-(Diethylphosphono)-1,3-butadien-1-yl)adenine (33a). A. From Compound 6a and Triethyl Phosphite. Compound[7] **6a** (70 mg, 0.3 mmol) was refluxed in triethyl phosphite (5 mL) for 0.5 h. After cooling, the mixture was evaporated and the residue was chromatographed on a silica gel column using solvent S_5. Evaporation of the appropriate fractions afforded phosphonate **33a** (30 mg, 30%), mp 140–141 °C after crystallization from ethyl acetate–petroleum ether (1:3): UV max 257 (28 200), 206 (20 700), shoulder 298 (5900); ^1H NMR δ 8.38 and 8.19 (2s, 2, H_2 and H_8), 7.69 (d, 1, $H_{1'}$, $^3J_{1',P}$ = 16.5 Hz), 7.49 (s, 2, NH_2), 6.70 (split ddd, 1, $H_{3'}$, $J_{3',4'a}$ = 11 Hz, $J_{3',4'b}$ = 17.7 Hz, $^3J_{3',P}$ = 25.5 Hz), 5.77 (d, 1, $H_{4'b}$, $J_{4'b,3'}$ = 17.7 Hz), 5.50 (d, 1, $H_{4'a}$, $J_{4'a,3'}$ = 11 Hz), 4.12 (m, 4, CH_2), 1.25 (t, 6, Me); ^{13}C NMR δ 156.44, 154.18, 149.91, 139.89, 118.14 (**10a**), 130.69, 128.21, 123.17 (3d, $C_{1'}$, $C_{3'}$, $C_{4'}$, J = 22, 5, and 4.8 Hz), 119.50 (d, $C_{2'}$, $^1J_{2',P}$ = 178 Hz), 62.83, 62.90 (CH_2), 16.62, 16.54 (Me); ^{31}P NMR δ 17.20; FAB-MS 324 (100.0, M + H), 136 (51.9, **10a** + H). Anal. Calcd for $C_{13}H_{18}N_5O_3P$: C, 48.29; H, 5.61; N, 21.66; P, 9.58. Found: C, 48.40; H, 5.72; N, 21.73; P, 9.39.

B. From Adenallene (4a) and Diethyl Chlorophosphite. Diethyl chlorophosphite (0.34 mL, 2.36 mmol) was added to a stirred mixture of adenallene[7] (**4a**, 0.16 g, 0.78 mmol) and pyridine (7 mL) at –10 °C. The stirring was then continued at room temperature for 75 min. The solution was evaporated, and the crude product was chromatographed on a silica gel column using solvent S_6 to give phosphonate **33a** (30 mg, 12%, mp 140–141 °C) which was identical with the product obtained by method A. No adenallene (**4a**) was recovered.

(*E*)-*N*^{9}-(2-Phosphono-1,3-butadien-1-yl)adenine (35a). A solution of phosphonate **33a** (60 mg, 0.18 mmol) in $CHCl_3$ (10 mL) was cooled to –40 °C and iodotrimethylsilane (117 μL, 0.74 mmol, 90%) was added. The temperature was allowed to rise, and after 1 h at room temperature the solvent was evaporated. Water (5 mL) was added to the residue, and the mixture was stirred for 20 min, and then it was lyophilized. The residue was washed with acetone (10 mL), and it was crystallized from water to give free acid **35a** (30 mg, 60%), mp 266–268 °C: electrophoretic mobility 1.04 of AMP; UV max (pH 7) 252 (23 900), 206 (20 600); ^1H NMR (sodium salt, D_2O) δ 8.07 and 7.98 (2s, 2, H_2 and H_8), 7.16 (d, 1, $H_{1'}$, $^3J_{1',P}$ = 12.6 Hz), 6.32 (apparent dt, 1, $H_{3'}$), 5.84 (d, 1, $H_{4'b}$, $J_{4'b,3'}$ = 17.7), 5.37 (d, 1, $H_{4'a}$, $J_{4'a,3'}$ = 11.7 Hz); ^{13}C NMR δ 155.19, 152.55, 148.66, 141.88, 117.53 (**10a**), 137.08 (d, $C_{2'}$, $^1J_{2',P}$ = 156.5 Hz), 129.52, 123.03, 122.27 (3d, $C_{1'}$, $C_{3'}$, and $C_{4'}$ J = 18, 4.8, and 4.1 Hz); ^{31}P NMR δ 7.29. Anal. Calcd for $C_9H_{10}N_5O_3P\cdot H_2O$: C, 37.90; H, 4.24; N, 24.56; P, 10.86. Found: C, 38.02; H, 4.37; N, 24.40; P, 10.77.

(*E*,*E*)-*N*^{9}-(4-(Diethylphosphono)-1,3-butadien-1-yl)adenine (36a). A. From Compound 19a and Diethyl Phosphite in DMF. Diethyl phosphite (1.39 mL, 10.8 mmol) was added to a suspension of NaH (60% oil dispersion, 433 mg, 10.8 mmol) in DMF (30 mL) with stirring at room temperature. After 30 min, the mixture was cooled to 0 °C. A solution of compound[7] **19a** (0.8 g, 3.61 mmol) was then added. The mixture was brought to a room temperature, and the stirring was continued for 40 min. The reaction was quenched with 5% acetic acid (15 mL) at 0 °C, and the solvents were evaporated. The crude product was chromatographed on a silica gel column using solvent S_5 to give phosphonate **36a** (270 mg, 23%), mp 222–223 °C after recrystallization from petroleum ether–ethyl acetate (3:1): UV max 266 (27 600), 204 (12 000), shoulders 298 (15 100), 278 (23 000), 258 (26 800); ^1H NMR δ 8.42 and 8.21 (2s, 2, H_2 and H_8), 7.74 (d, 1, $H_{1'}$, $J_{1',2'}$ = 13.8 Hz), 7.44 (s, 2, NH_2), 7.27 (t, 1, $H_{2'}$, $J_{2',3'}$ = 10 Hz), 7.08 (ddd, 1, $H_{3'}$), 5.94 (dd, 1, $H_{4'}$, $J_{4',3'}$ = 16.5 Hz, $J_{4',P}$ = 19.7 Hz), 3.96 (m, 4, CH_2), 1.20 (t, 6, Me); ^{13}C NMR δ 156.53, 153.80, 149.19, 139.66, 119.63 (**10a**), 146.09, 117.90 (2d, $C_{2'}$ and $C_{3'}$, J = 11.2 and 27.9 Hz), 130.14 ($C_{1'}$), 118.04 (d, $C_{4'}$, $^1J_{4',P}$ = 187.9 Hz), 61.65, 61.59 (CH_2), 16.65, 16.59 (Me); ^{31}P NMR δ 18.82; FAB-MS 324 (M + H, 100), 136 (**10a** + H, 48.4). Anal. Calcd for $C_{13}H_{18}N_5O_3P$: C, 48.29; H, 5.61; N, 21.66; P, 9.58. Found: C, 48.12; H, 5.63; N, 21.46; P, 9.43.

B. From Allene 6a and Diethyl Phosphite in THF–HMPA. The reaction was performed as described in method A on a 0.54 mmol scale of allene[7] **6a** in THF (3.5 mL, generation of sodium salt of diethyl phosphite) and HMPA (2.5 mL, solvent for compound **6a**). The mixture was cooled to –20 °C before the addition of diethyl phosphite. A 26% yield of phosphonate **36a** was obtained.

In DMSO (0.5 mL, 45 μmol of **6a**, method A, room temperature, 50 min), both phosphonates **33a** and **36a** were present as shown by TLC in solvent S_2.

In HMPA (7 mL), method A was followed on a 0.8 mmol scale of **6a** for 3 h. Most of the solvent was removed in vacuo (oil pump, 60 °C). The crude product was chromatographed in solvent S_6 to give first the starting material **6a** (20 mg, 11%) followed by phosphonate **36a** (14 mg, 6%), and last, *N*^{9}-ethyladenine (**20a**, 30 mg, 23%).

Reaction of Compound 6a with Triethyl Phosphite in the Presence of NBu_4I. A mixture of compound **6a** (10 mg, 45 μmol), NBu_4I (17 mg, 45 μmol), and triethyl phosphite (0.5 mL) was heated at 110 °C with stirring. After 3.5 h, TLC and UV spectra of the spots eluted with ethanol showed the presence of phosphonate **36a** (major product) and **33a** (minor component).

(*E*,*E*)-*N*^{9}-(4-Phosphono-1,3-butadien-1-yl)adenine (37a). The procedure for phosphonic acid **35a** was followed with compound **36a** on a 0.14 mmol scale. The product was washed with acetone (2 × 3 mL), and it was dissolved in water (50 mL). The solution was concentrated to 5 mL to deposit compound **37a** (25 mg, 68%), mp 295–297 °C; electrophoretic mobility 1.09 of AMP; UV max (pH 7) 257 nm (22 300), 202 (9400), shoulder 294 (8300); ^1H NMR (sodium salt, D_2O) δ 7.95 and 7.86 (2s, 2, H_2 and H_8), 6.81 (m, 1, $H_{1'}$, $J_{1',2'}$ = 13.5 Hz), 6.64 (m, 2, $H_{2'}$ and $H_{3'}$), 6.08 (m, 1, $H_{4'}$, $J_{4',3'}$ = 14.7 Hz, $J_{4',P}$ = 14.9 Hz); ^{13}C NMR δ 154.81, 152.31, 146.73, 138.85, 117.76 (**10a**), 134.12, 122.43 (2d, $C_{2'}$, $C_{3'}$, J = 4.8 and 24.2 Hz), 133.39 (d, $C_{4'}$, $^1J_{4',P}$ = 168.9 Hz), 121.96 ($C_{1'}$); ^{31}P NMR δ 10.28. Anal. Calcd for $C_9H_{10}N_5O_3P\cdot{}^1/_2H_2O$: C, 39.13; H, 4.02; N, 25.36; P, 11.21. Found: C, 39.18; H, 3.99; N, 25.35; P, 11.39.

Reaction of Compound 39a with Diethyl Chlorophosphite. Compound[7] **39a** (10 mg, 49 μmol) was stirred in pyridine (0.5 mL) at 0 °C after addition of diethyl chlorophosphite (8.6 μL, 60 μmol). The stirring was then continued at room temperature for 16 h. TLC (S_2) indicated that all starting material disappeared and adenine (**10a**) was the major product.

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Characterization of the Macroscopic and Microscopic Acid–Base Chemistry of the Native Disulfide and Reduced Dithiol Forms of Oxytocin, Arginine-Vasopressin, and Related Peptides

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Acid–base properties of the native disulfide and reduced dithiol forms of oxytocin, arginine-vasopressin, tocinoic acid, pressinoic acid, and tocinamide were studied by 500-MHz ^{1}H NMR–pH titrations. Cysteine methyl ester and cysteinylglycine served as model compounds for the N-terminal cysteine-1 residue of the reduced oligopeptides and were studied by UV, ^{1}H NMR, and pHmetry. The resonances for ^{1}H nuclei in the various peptides and the model compounds undergo the expected downfield shift upon protonation of adjacent basic sites, with the exception of the resonances for the δ CH_2 protons of the proline-7 residues of oxytocin and arginine-vasopressin, which are shifted significantly upfield, presumably reflecting conformational changes. The interacting basicities of the amino and thiolate groups of the cysteine-1 residues of the reduced dithiol forms of the five peptides and the two model compounds are characterized in terms of protonation microconstants. All other basic groups of the peptides (tyrosine phenolate, cysteine-6 thiolate of the reduced peptides, cysteine-6 carboxylate of the reduced and disulfide forms of tocinoic acid and pressinoic acid, and cysteine-1 amino of the disulfide forms of the peptides) bind protons in isolated pH ranges or are separated by several bonds from other basic groups, and their basicities are characterized in terms of group constants. Analogous groups in the various peptides show somewhat different basicities, depending on the adjacent residues. However, the basicity of the cysteine-6 thiolate of the reduced peptides covers a remarkably wide range. Specifically, the protonation constant of the cysteine-6 thiolate of the reduced forms of tocinoic acid and pressinoic acid is about 1.8 log K units larger than that of the reduced forms of oxytocin and arginine-vasopressin, due to the significantly different electron-withdrawing effects of the adjacent carboxylate and peptide groups, while the protonation constant of the cysteine-6 thiolate group of the reduced form of tocinamide is 0.6 log K units larger than that of oxytocin, even though the covalent environments are the same up to four bonds removed. Using the microscopic and group protonation constants, the probabilities of both thiol groups being in the ionized form were calculated as a function of pD for the dithiol forms of the five peptides. The results show that, at physiological pH, intramolecular disulfide bond formation via thiolate anions is predicted to be more favorable for the nonapeptides oxytocin and arginine-vasopressin than for tocinamide, which in turn is more favorable than for tocinoic acid and pressinoic acid.

Introduction

The neurohypophyseal peptide hormones oxytocin (OXT) and arginine-vasopressin (AVP) have in common disulfide bonds between cysteine residues at positions 1 and 6 which are essential for their biological activity.[1]

Cys-Tyr-X-Gln-Asn-Cys-Y

oxytocin	X = Ile	Y = Pro-Leu-Gly-NH_2
arginine-vasopressin	X = Phe	Y = Pro-Arg-Gly-NH_2
tocinoic acid	X = Ile	Y = OH
pressinoic acid	X = Phe	Y = OH
tocinamide	X = Ile	Y = NH_2

Although the mechanism of formation of the disulfide bond in the biosynthesis of OXT and AVP is not known, it presumably takes place under conditions where the two thiol groups are aligned to favor intramolecular rather than intermolecular disulfide bond formation. However, it has been found that oxidation of the two thiol groups by ferricyanide in the chemical synthesis of neurohypophyseal peptide hormones also results predominantly, but not exclusively, in the formation of intramolecular disulfide bonds,[2] even though formation of the intramolecular disulfide bond requires closure of a 20-membered ring. This is not the case for the related hexapeptides, tocinamide and pressinamide, which comprise the first six amino acids of OXT and AVP, but lack the acyclic tripeptide tail. Oxidation of the dithiol forms of these hexapeptides results predominantly in the formation of dimers and higher polymers.[2]

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(1) Jost, K. In *CRC Handbook of Neurohypophyseal Hormone Analogs*; Jost, K., Lebl, M., Brtnik, F., Eds.; CRC Press: Boca Raton, FL, 1987; Vol. 1, Part 2, pp 144–155.

(2) Moore, G. *Biochem. J.* 1978, *173*, 403–409.

0022-3263/92/1957-2327$03.00/0