substrate, are susceptible to solvolytic degradation, this is not a significant problem when the pH of the buffer is maintained at 7 or above, even during prolonged incubations at 37 °C. We anticipate that methane- and difluoromethanediphosphonates will find increasing use as alternate substrates and inhibitors inert to hydrolytic activity and as mechanistic probes in cases where it is desirable to change the reactivity of the leaving group.

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## Novel Phosphonylphosphinyl (P-C-P-C-) Analogues of Biochemically Interesting Diphosphates. Syntheses and Properties of P-C-P-C- Analogues of Isopentenyl Diphosphate and Dimethylallyl Diphosphate

Ronald W. McClard* and Thomas S. Fujita

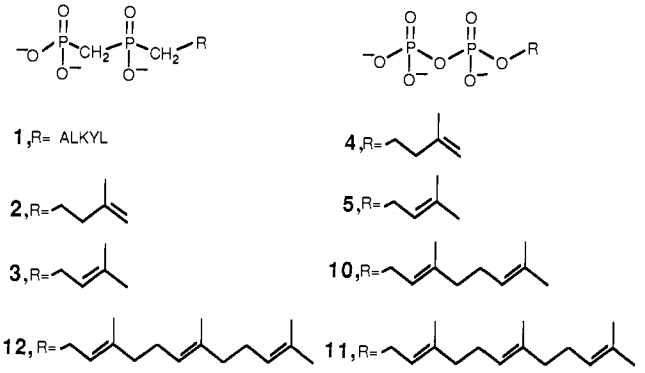
*Arthur F. Scott Laboratory of Chemistry*
*Reed College, Portland, Oregon 97202*

Kay E. Stremler and C. Dale Poulter*

*Department of Chemistry, University of Utah*
*Salt Lake City, Utah 84112*

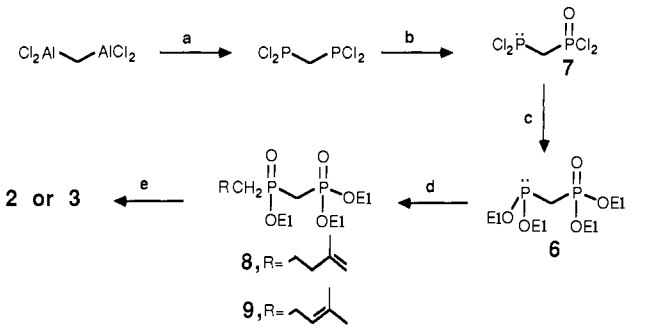
*Received March 23, 1987*

There are numerous reports of phosphonate analogues of biologically interesting compounds.[1] A related class of analogues whose biological properties have not been mentioned, except for an undocumented claim in the patent literature,[2] is the phosphonylphosphinyl system, a moiety in which the bridging oxygen between the two phosphorus atoms of the diphosphate unit and the bridging ester oxygen to the rest of the molecule are both replaced by methylene groups as shown below for **1**.[3,4] Attempts




by Gilmore and Huber[5] to prepare a vinyl derivative related to **1** by using the symmetric Horner-Emmons reagent ethyl bis-((diethoxyphosphinyl)methyl)phosphinate were unsuccessful. Some asymmetric derivatives related to **1** were recently reported by Novikova and co-workers,[6,7] but no derivatives of obvious biological relevance were prepared. We reasoned that application

**Scheme I**[a]




[a] (a) $PCl_3$, KCl, $P(O)Cl_3$;[7] (b) DMSO, 23 °C;[7] (c) 4 equiv EtOH + 4 equiv pyridine; (d) $RCH_2Br$, 100-200 °C; (e) TMSBr, 23 °C/MeOH, $H_2O/NH_3$.

of this chemistry could lead to a new class of diphosphate analogues and now report the syntheses of **2** and **3**, P-C-P-C-analogues of isopentenyl diphosphate (**4**) and dimethylallyl diphosphate (**5**), mandatory precursors of all isoprenoids.

Phosphonylphosphinyl analogues **2** and **3** were prepared from phosphonylphosphonite **6** and the appropriate alkyl halides via an Arbuzov reaction. Although **6** was previously reported by Novikova et al.,[6] we were unable to repeat their synthesis and instead used the route outlined in Scheme I to prepare the compound from **7** in 63% yield.[8] Phosphonite **6** was then allowed to react with 5-bromo-2-methyl-1-pentene[9] to give the triethyl ester **8** of **2**[10] in 26% yield or with 5-bromo-2-methyl-2-pentene[11] to give the triethyl ester **9** of **3**[12] in 66% yield. After deprotection with bromotrimethylsilane, the analogues were isolated as their crystalline ammonium salts.[10,12]

Phosphonylphosphinates **2** and **3** were evaluated as inhibitors of the 1'-4-condensation between isopentenyl diphosphate (**4**) and geranyl diphosphate (**10**) catalyzed by avian liver farnesyl diphosphate synthetase. Under standard assay conditions,[13] isopentenyl analogue **2** was a competitive inhibitor against **4**, $K_{i(2)} = 19 \pm 7$ $\mu$M, and diemethylallyl analogue **3** was a competitive inhibitor against **10**, $K_{i(3)} = 71 \pm 9$ $\mu$M. These values can be compared with $K_d = 2.4$ and $K_d = 2.5$ $\mu$M for the magnesium salts of **4** and **5**, respectively.[14] The reduced binding of these analogues, relative to the natural compounds, probably reflects

(1) Engel, R. *Chem. Rev.* **1977**, *77*, 349.
(2) Myers, T. C.: U.S. Patent 3 238 191 (March 1, 1966); *Chem. Abstr.* **1966**, *64*, 15972h.
(3) Kenyon and co-workers[4] made a di($P_\alpha$-$P_\beta$;$P_\beta$-$P_\gamma$)methylene analogue of ATP which is similar to **1**. The important difference is that our compounds possess a methylene group between $P_\alpha$ and the R group.
(4) (a) Trowbridge, D. B.; Kenyon, G. L. *J. Am. Chem. Soc.* **1970**, *92*, 2181. (b) Trowbridge, D. B.; Kamamoto, D. M.; Kenyon, G. L. *J. Am. Chem. Soc.* **1972**, *94*, 3816.
(5) Gilmore, W. F.; Huber, J. W., III. *J. Org. Chem.* **1973**, *38*, 1423. We have verified this observation in our (R.W.M.) laboratory.
(6) Novikova, Z. S.; Prishchenko, A. A.; Skorobogatova, S. Ya.; Martynov, V. I.; Lutsenko, I. F. *Zh. Obshch. Khim.* **1980**, *50*, 989.
(7) Novikova, Z. S.; Prishchenko, A. A.; Lutsenko, I. F. *Zh. Obshch. Khim.* **1977**, *47*, 775.

(8) $^1H$ NMR ($CDCl_3$) $\delta$ 1.21 and 1.26 (12 H, dt, -O-$CH_2$-$CH_3$, $J_{H,H}$ = 7), 2.14 (2 H, dd, P-$CH_2$-P, $J_{H}P$ = 4.8 Hz, $J_{H,P}'$ = 19.8 Hz), 3.60-4.27 ppm (8 H, m, -O-$CH_2$-$CH_3$); lit.[6] (benzene), P-$CH_2$-P, $\delta$ 2.16 (dd, $J_{H,P}$ = 20 Hz, $J_{HP}'$ = 4 Hz); $^{31}P$ NMR ($^1H$ decoupled, benzene/$CDCl_3$, internal reference $(CH_3O)_3P$ taken as +140.7) $\delta$ + 23.6 (d, phosphonyl), +164.9 (d, phosphonite), $J_{PP}'$ = 40 Hz; lit. 6 (neat) $\delta$ +25, +164, $J$ = 38 Hz.
(9) This material was synthesized from 4-methyl-4-penten-1-ol (Wiley Organics) according to a published procedure: van der Gen, A.; Wiedhaup, K.; Swoboda, J.; Dunathan, H. C.; Johnson, W. S. *J. Am. Chem. Soc.* **1979**, *95*, 2656.
(10) $^1H$ NMR ($D_2O$) $\delta$ 1.48 (4 H, m, -$CH_2$-s), 1.55 (3 H, s, -$CH_3$), 1.85 (2 H, dd, P-$CH_2$-P, $J_{H,P}$ = 17.1 Hz, $J_{H,P}'$ = 18.8 Hz), 1.94 (2 H, m, -$CH_2$-), the vinyl protons were obscured by the HOD peak at 4.55-4.75 ppm; $^{31}P$ NMR ($D_2O$, 85% $H_3PO_4$ reference, $^1H$-decoupled) $\delta$ +36.59 (d, phosphinyl), and +12.73 ppm (d, phosphonyl), $J_{P,P}'$ = 5 Hz. Anal. Calcd for the monoammonium salt, $C_7H_{19}NO_5P_2$: C, 32.44; H, 7.39; N, 5.40; P, 23.90. Found: C, 30.62; H, 7.21; N, 5.33; P, 23.81. Triethyl ester **8** gave appropriate $^1H$ NMR, $^{31}P$ NMR, and GCMS spectra.
(11) This compound is commercially available, but we made it by acidification of 5-bromo-2-methyl-1-pentene, which was synthesized as described above.[9] The boiling point and $^1H$ NMR were identical with those of the commerical product.
(12) $^1H$ NMR ($D_2O$) $\delta$ 1.47 (3 H, s, -$CH_3$), 1.52 (3 H, s, -$CH_3$), 1.55 (2 H, br m, -CH$CH_2$-P, under -$CH_3$ groups), 1.87 (2 H, dd, P-$CH_2$-P, $J_{H,P}$ = 16.5 Hz, $J_{H,P}'$ = 19.0 Hz), 2.03 (2 H, q, =CH-$CH_2$-$CH_2$, $J_{apparent}$ = 7.5 Hz), 5.08 (1 H, dt, = CH-, $J_{H,H}$ = 6 Hz, $J_{HCCP}$ = 1 Hz); $^{31}P$ NMR ($D_2O$, 85% $H_3PO_4$ reference, $^1H$-decoupled) $\delta$ +35.23 (d, phosphinyl) and +12.92 ppm (d, phosphonyl), $J_{P,P}'$ = 4.3 Hz. Anal. Calcd for the monoammonium salt, $C_7H_{19}NO_5P_2$: C, 32.44; H, 7.39; N, 5.40; P, 23.90. Found: C, 30.46; H, 7.23; N, 5.45; P, 23.73. Triethyl ester 9 gave appropriate $^1H$ NMR, $^{31}P$ NMR, and GCMS spectra.
(13) Laskovies, F. M.; Krafcik, J. M.; Poulter, C. D. *J. Biol. Chem.* **1979**, *254*, 9458.
(14) Rilling, H. C. *Pure Appl. Chem.* **1979**, *51*, 597.

0002-7863/87/1509-5544$01.50/0 

(1) the differences between the p$K_a$'s of the phosphonylphosphinyl analogue and the normal diphosphate substrates and (2) small geometric differences between C–P and O–P bonds.[1,15]

Analogue **2** has the unusual property of being a substrate for the 1'-4-condensation, thereby generating a phosphonylphosphinyl product that is a nonreactive inhibitor for subsequent reactions in the pathway. When a solution (4.5 mM, 2.7 μmol) in **2** was incubated at 37 °C with 11.9 mM (7.1 μmol) **10** and 0.14 mg (2.1 μmol min$^{-1}$ mg$^{-1}$) of avian liver farnesyl diphosphate synthetase,[16] the AB pattern at δ −13.2 and −10.2 ppm in the $^1$H-decoupled $^{31}$P NMR spectrum of the allylic substrate disappeared and was replaced by a singlet at −1.3 ppm, characteristic of inorganic pyrophosphate.[17] In a related experiment, **2** (40 μg, 0.14 μmol) was incubated at 37 °C with [1-$^3$H]**10** (4.7 μg, 0.013 μmol, 70 μCi/μmol). The sample was lyophilized, and the residue was analyzed by TLC[18] with use of authentic samples of **2**, **10**, and farnesyl diphosphate (**11**) as standards. A new radioactive component was identified, $R_f$ = 0.75 ($R_{f(10)}$ = 0.55, $R_{f(11)}$ = 0.70), whose mobility was consistent with the phosphonylphosphinyl analogue **12** of farnesyl diphosphate (**11**). Similar experiments using [1-$^3$H]**5** (23 μCi/μmol) as the allylic substrate gave a radioactive product, $R_f$ = 0.58, whose mobility was consistent with formation of a geranyl phosphonylphosphinyl derivative.

Phosphonylphosphinyl analogues **2** and **3** are both good inhibitors of the 1'-4-condensation reaction. In addition, homoallylic analogue **2** has the unusual property of functioning as a substrate for 1'-4-condensation and generating a product that can presumably inhibit the next step in the pathway. When dimethylallyl diphosphate (**5**) is the allylic substrate, the next step is the second prenyl transfer catalyzed by farnesyl diphosphate synthetase. When geranyl diphosphate (**10**) is the allylic substrate, the putative phosphonylphosphinyl farnesyl product (**12**) is also a potential inhibitor for all of the normal isoprenoid reactions that utilize farnesyl diphosphate as a substrate, including squalene synthetase (sterols), geranylgeranyl diphosphate synthetase (carotenoids), dehydrodolichol synthetase (dolichols), and decaprenyl diphosphate synthetase (ubiquinones). A related phosphonylphosphate metabolic block at the farnesyl stage was synthesized by Corey and Volante.[19] The major difference between their inhibitor and the phosphonylphosphinyl class is that the latter compounds cannot be hydrolyzed enzymatically or chemically to less potent phosphonate analogues. The accumulation of nonhydrolyzable allylic analogues should be particularly devastating to higher polyprenyl diphosphate synthetases which catalyze multiple 1'-4-condensations.

The synthetic approach described here can be applied to other systems to give P–C–P–C analogues of diphosphates or higher phosphate anhydrides. Work in that direction is continuing in our (R.W.M.) laboratory.

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**Supplementary Material Available:** Details of the syntheses of **2**, **3**, and **6** (2 pages). Ordering information is given on any current masthead page.

(15) (a) Blackburn, G. M.; England, D. A.; Kolkmann, F. *J. Chem. Soc., Chem. Commun.* **1981**, 930. (b) Blackburn, G. M. *Chem. Ind. (London)* **1981**, 134.

(16) Enzymatic experiments were done in a standard buffer containing 20 mM *endo*-bicyclo[2.2.1]heptane dicarboxylate, 1 mM magnesium chloride, pH 7.00.

(17) Spectra are referenced to external $H_3PO_4$.

(18) Mixtures were cospotted with authentic samples (cellulose TLC, tetrahydrofuran: 100 mM ammonium bicarbonate, 75:25, $R_{f(11)}$ = 0.70, $R_{f(10)}$ = 0.55, $R_{f(2)}$ = 0.26, $R_{f(6)}$ = 0.17).

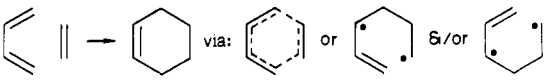
(19) Corey, E. J.; Volante, R. P. *J. Am. Chem. Soc.* **1976**, *98*, 1291.

## Transition State Structure Variation in the Diels–Alder Reaction from Secondary Deuterium Kinetic Isotope Effects: The Reaction of a Nearly Symmetrical Diene and Dienophile Is Nearly Synchronous

Joseph J. Gajewski,* Karen B. Peterson, and John R. Kagel

*Department of Chemistry, Indiana University*
*Bloomington, Indiana 47405*
*Received February 13, 1987*

Diels–Alder transition states are of concern.[1] There is little doubt but that the reaction of unsymmetrical dienophiles proceeds via an unsymmetrical transition state[2] (and is not synchronous[3]), but the question of concert still remains in these cases, and in the case of symmetrical addends the question of synchrony is paramount. Dewar has calculated that the reaction involves a highly unsymmetrical transition state if not a biradical.[3] Previous work with secondary deuterium kinetic isotope effects (KIEs),[4] the most appropriate probe for these questions, has been criticized for not distinguishing between the alternatives particularly with symmetrical addends.




KIEs have now been determined with 4,4-dideuterio- and 1,1,4,4-tetradeuterioisoprene, *4,4-$d_2$* and *-$d_4$*, respectively, in their reaction with the acrylonitrile, fumaronitrile, vinylidene cyanide, and methyl *trans*-β-cyanoacrylate in benzene solvent (Table I). Isoprene is a diene of choice because its methyl group might not affect the symmetry of a near-synchronous path, but because the methyl strongly affects the regiochemistry with highly unsymmetrical dienophiles the methyl must strongly perturb the relative energies of the potential biradical pathways *even with symmetrical dienophiles*. The KIEs were determined by competition, reacting the dienophile with excess (>10-fold) of a mixture of $d_0$ and $d_n$ isoprene and observing the $d_0/d_n$ ratio in each adduct by either GCMS in CI mode[5] or by capillary GC.[6] The $d_0/d_n$ ratio in starting isoprene was found by using an excess of the dienophile.[5] KIEs from $d_4$ when divided by those from *4,4-$d_2$* give the KIEs for 1,1-dideuterioisoprene, *1,1-$d_2$*. Also listed in Table I are the maximum kinetic isotope effects expected for two deuteriums, which are derived from the equilibrium constants for fractionation of deuterium between exomethylene carbon and secondary saturated allylic carbon in degenerate thermal 1,3- and 3,3-shifts over nearly a 200 °C range.[7]

(1) For a review, see: Sauer, J.; Sustmann, R. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 779. For the most recent work on the stereochemistry of the reaction and a summary of theoretical work, see: Houk, K. N.; Lin, Y.-T.; Brown, F. K. *J. Am. Chem. Soc.* **1986**, *108*, 554.

(2) Woodward, R. B.; Katz, T. J. *Tetrahedron* **1959**, *5*, 70. Fleming, I. *Frontier Orbitals and Organic Chemical Reactions*; Wiley; London, 1976, Chapter 4.

(3) (a) Dewar, M. J. S.; Olivella, S.; Rzepa, H. J. *J. Am. Chem. Soc.* **1978**, *100*, 5650. (b) Dewar, M. J. S.; Pierini, A. B. *Ibid.* **1984**, *106*, 203. (c) Dewar, M. J. S.; Olivella, S.; Stewart, J. J. P. *Ibid.* **1986**, *108*, 5771.

(4) (a) van Sickle, D. W. *Tetrahedron Lett.* **1961**, *687*. van Sickle, D. W.; Rodin, J. O. *J. Am. Chem. Soc.* **1964**, *86*, 3091. (b) Seltzer, S. *Ibid.* **1963**, *85*, 1360; **1965**, *87*, 1534. (c) Brown, P.; Cookson, R. C. *Tetrahedron* **1965**, *21*, 1973. (d) Taagepera, M.; Thornton, E. R. *J. Am. Chem. Soc.* **1972**, *94*, 1168.

(5) GCMS in CI mode was performed on a Hewlett-Packard Model 5985 equipped with a 30-m DB-5 capillary column. GCMS analyses of the excess vinylidene cyanide reactions were irreproducible so the value of the $d_0/d_2$ ratio in the standard is that from the excess acrylonitrile runs.

(6) Capillary GC analyses were performed on a 100-m SPB-5 column with a 60-m SP2330 column connected in series. Under conditions of 3-h retention times, all four peaks for $d_0$ and $d_2$ (and $d_0$ and $d_4$) regioisomers from methyl *trans*-β-cyanoacrylate were separated sufficiently (valley 30% above base line in the worse case). Any inaccuracy in the absolute values of the ratios is offset by the cancellation of errors because of identical analytical techniques for the standard.

(7) Calculated from the equation log $K^D2/K^H$ = (291.6/2.303$RT$) −0.0818 ($r$ = 0.977). Conrad, N. D. Ph.D. Thesis, Indiana University, 1978. For a discussion of the use of this equation in other pericyclic reactions, see: Gajewski, J. J. In *Isotopes in Organic Chemistry*; Buncel, E., Lee, C. C., Eds.; Elsevier: Amsterdam, The Netherlands, Vol 7, Chapter 3, p 1987. Note that the equilibrium IEs reported in Appendix B Vol 7, should be inverted.

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