in 900 mL of 30% hydrogen bromide in acetic acid, and the mixture was allowed to stir at room temperature. After 6 h the acetic acid was removed under aspirator pressure to $^1/_4$ of the original volume, and 1800 mL of ethylacetate was then added. A solid precipitated and was then filtered under an inert atmosphere. The product was recrystallized by dissolving it in a minimum amount of methanol at reflux. Cooling followed by the addition of 400 mL of isopropyl alcohol provided a white solid, which was filtered and dried under reduced pressure. The product weighed 48 g, which represents an 81% yield. Mp: 258–259 °C (coloration occurs at 234 °C). NMR (D_2O): δ 4.73 (m, 1 H), 4.62 (m, 1 H), 3.8–3.6 (m, 4 H), 3.08 (s, 3 H), 2.65 (m, 1 H), 2.35 (m, 1 H). Anal. Calcd for $C_6H_{12}N_2$·2HBr: C, 26.30; H, 5.15; N, 10.22; Br, 58.33. Found: C, 26.26; H, 5.15; N, 10.08; Br, 58.58. $[\alpha]_D$: +13.21° (c = 0.946, CH_3OH).

***allo*-4-*cis*-Hydroxy-D-proline Ethyl Ester Hydrochloride (15)** (Baker, D. L.; Fritschel, S. J.; Stille, J. R.; Stille, J. K. *J. Org. Chem.* **1981**, *46*, 2954–2960). 4-*cis*-Hydroxy-D-proline (80 g, 0.61 mol) was suspended in 500 mL of anhydrous ethanol, and anhydrous HCl gas was allowed to bubble through the mixture until the reaction mixture became homogeneous. The reaction was then heated to reflux for 5 h, and the volume of the solvent was reduced by half; 100 mL of diethyl ether was then added, and the mixture was kept in a freezer overnight. The resulting precipitate was filtered and washed with diethyl ether and dried under reduced pressure to yield 111 g of product (93% yield). Mp: 152–153 °C (lit. mp 157–158.4 °C).

***allo*-1-(4-Tolylsulfonyl)-4-((4-tolylsulfonyl)oxy)-D-proline Ethyl Ester (16).** To 110 g (562 mmol) of the *allo*-4-hydroxy-D-proline ethyl ester hydrochloride were added 1 L of pyridine and 79 mL of triethylamine at 0 °C. After the mixture was stirred for 10 min, 242.1 g (1.24 mol) of *p*-toluenesulfonyl chloride was added in small portions as to control the temperature between 0–5 °C, and the reaction mixture was allowed to stir at 0 °C over night. The next day the reaction was added to 750 mL of ice-cold water, and the slurry was left to stir at room temperature for 1 h. The solids were filtered and dried in a vacuum oven at 30 °C for 48 h to provide 243.9 g of product (92% yield). NMR ($CDCl_3$): δ 7.73 (d, 2 H), 7.68 (d, 2 H), 7.27 (m, 4 H), 4.93 (m, 1 H), 4.49 (dd, 1 H), 4.10 (m, 2 H), 3.60 (dd, 1 H), 3.40 (dd, 1 H), 2.40 (s, 3 H), 2.37 (s, 3 H), 2.33 (m, 1 H), 2.20 (m, 1 H), 1.16 (t, 3 H). Mp: 122–123 °C.

4-(Acetyloxy)-1-(4-tolylsulfonyl)-D-proline Ethyl Ester (17). To 218 g (466 mmol) of *allo*-1-(4-tolylsulfonyl)-4-((4-tolylsulfonyl)oxy)-D-proline ethyl ester in 1500 mL of toluene was added 81 g (606 mmol) of tetramethylammonium acetate, and the mixture was heated to reflux for 2 h. The reaction was cooled, washed with 2 × 500 mL of water, and dried over Na_2SO_4. Evaporation of the solvent and drying the resulting solids in a vacuum oven over night at 30 °C provided 120.6 g of product (72% yield). NMR ($CDCl_3$): δ 7.75 (d, 2 H), 7.30 (d, 2 H), 5.10 (m, 1 H), 4.28 (dd, 1 H), 4.18 (dq, 2 H), 3.69 (dd, 1 H), 3.50 (m, 1 H), 2.40 (s, 3 H), 2.30 (m, 1 H), 2.17 (m, 1 H), 1.65 (s, 3 H), 1.27 (t, 3 H). ^{13}C NMR ($CDCl_3$): δ 171.46, 169.75, 143.77, 134.71, 129.61, 127.75, 72.62, 61.64, 59.80, 53.99, 36.62, 21.46, 20.47, 14.05. Anal. Calcd for $C_{16}H_{21}NO_6S$: C, 54.07; H, 5.96; N, 3.94; S, 9.02. Found: C, 54.20; H, 6.05; N, 4.0; S, 9.0. Mp: 82–83 °C.

1-(4-Tolylsulfonyl)-4-hydroxy-D-proline (18). To 4-(acetyloxy)-1-(4-tolylsulfonyl)-D-proline ethyl ester (17) (127.9 g, 359.9 mmol) in 640 mL of THF was added KOH (100 g, 1.8 mol) dissolved in 640 mL of water at 0 °C. The mixture was warmed to room temperature and allowed to stir for 2 h. The organic solvents were removed in vacuo, and the pH of the resulting mixture was adjusted to neutral with concentrated HCl. A precipitate formed which was filtered and dried over night in a vacuum oven at 25 °C to provide 86.2 g of product (84% yield). NMR (CD_3OD): 7.73 (d, 2 H), 7.38 (d, 2 H), 4.95 (broad m, OH), 4.33 (m, 1 H), 4.23 (dd, 1 H), 3.58 (dd, 1 H), 3.28 (m, 1 H), 2.40 (s, 3 H), 2.09 (m, 2 H). Anal. Calcd for $C_{12}H_{15}NO_2S$: C, 50.52; H, 5.30; N, 4.91; S, 11.24. Found: C, 58.61; H, 5.30; N, 4.99; S, 11.35. Mp: 147–149 °C. $[\alpha]_D$: +100.07° (c = 1.1, CH_3OH).

(2*R*,4*S*)-2-(Hydroxymethyl)-4-hydroxy-1-(4-tolylsulfonyl)pyrrolidine (19). To 900 mL of THF was added sodium borohydride (21.75 g, 574.9 mmol), and the mixture was cooled to 10 °C before borontrifluoride etherate (97.92 mL, 776.2 mmol) was added dropwise over a period of 1 h. Then of *N*-(4-tolylsulfonyl)-4-hydroxy-D-proline (18) (82 g, 287.4 mmol) was added carefully in 330 mL of THF at 0 °C, and the mixture was warmed to room temperature and allowed to stir for 16 h. The reaction was then cooled 0 °C and quenched with methanol; 10% aqueous HCl solution was then added, and the mixture was gently heated to 60 °C for 1 h. The pH of the reaction was adjusted to neutral with 50% aqueous sodium hydroxide solution, and the volatiles were evaporated under reduced pressure. The product was then isolated via filtration, and the filter cake was washed with water. Drying under vacuum at 60 °C for 12 h yielded 78 g of the product as a white solid solid in 85% yield. Mp: 131–132 °C. NMR ($CDCl_3$): δ 7.75 (d, 2 H), 7.30 (d, 2 H), 4.3 (m, 1 H), 3.75–3.65 (m, 2 H), 3.6 (m, 2 H), 3.35 (m, 1 H), 3.1 (m, 1 H), 2.42 (s, 3 H), 1.9 (m, 2 H). Anal. Calcd for $C_{12}H_{17}NO_4S$: C, 53.14; H, 6.27; N, 5.17; S, 11.81. Found: C, 52.81; H, 6.36; N, 5.16; S, 11.80.

(2*R*,4*S*)-1-(4-Tolylsulfonyl)-2-(((4-tolylsulfonyl)oxy)-methyl)-4-((4-tolylsulfonyl)oxy)pyrrolidine (20). This compound was prepared in a similar fashion as described for compound 4. Mp: 125–130 °C. NMR ($CDCl_3$): δ 7.8–7.3 (m, 12 H), 4.78 (m, 1 H), 4.32 (m, 1 H), 4.1 (m, 1 H), 3.8 (m, 2 H), 2.45 (s, 6 H), 2.41 (s, 3 H), 2.04 (m, 2 H). $[\alpha]_D$: +55.9° (c = 1.168, acetone).

(2*R*,4*S*)-1-(4-Tolylsulfonyl)-2-(chloromethyl)-4-((4-tolylsulfonyl)oxy)pyrrolidine (21). This compound was prepared in a similar fashion as described for compound 12. Mp: 141–143 °C. NMR ($CDCl_3$): δ 7.70 (d, 2 H), 7.56 (d, 2 H), 7.30 (m, 4 H), 4.82 (m, 1 H), 3.75–4.0 (m, 2 H), 3.75 (m, 1 H), 3.60 (m, 2 H), 2.45 (s, 3 H), 2.42 (s, 3 H), 2.10 (m, 2 H). ^{13}C NMR ($CDCl_3$): δ 145.3, 144.3, 129.98, 129.86, 127.71, 127.67, 78.2, 58.8, 55.12, 47.67, 36.9, 21.68, 21.62. Anal. Calcd for $C_{19}H_{22}NClO_5S_2$: C, 51.4; H, 4.99; N, 3.15; S, 14.68. Found: C, 51.34; H, 5.01; N, 3.10; S, 14.77. $[\alpha]_D$: +34.0° (c = 1.0, acetone).

(1*R*,4*R*)-2-(4-Tolylsulfonyl)-5-methyl-2,5-diazabicyclo-[2.2.1]heptane (22). This compound was prepared in a similar fashion as described for compound 13. Mp: 82–87 °C. NMR ($CDCl_3$): δ 7.70 (d, 2 H), 7.30 (d, 2 H), 4.23 (m, 1 H), 3.55 (dd, 1 H), 3.30 (m, 1 H), 3.0 (dd, 1 H), 2.85 (dd, 1 H), 2.63 (m, 1 H), 2.40 (s, 3 H), 2.33 (s, 3 H), 1.65 d, 1 H), 1.06 (d, 1 H); ^{13}C NMR ($CDCl_3$): δ 143.5, 135.4, 129.8, 127.4, 62.9, 61.1, 61.0, 49.9, 40.2, 34.9, 21.5. Anal. Calcd for $C_{13}H_{18}N_2O_2S$: C, 58.62; H, 6.81; N, 10.52; S, 12.04. Found: C, 58.75; H, 6.83; N, 10.55; S, 12.19. $[\alpha]_D$: −16.8° (c = 1.038, CH_3OH).

(1*S*,4*S*)-2-Methyl-2,5-diazabicyclo[2.2.1]heptane Dihydrobromide (23). This compound was prepared in a similar fashion as described for preparation of compound 2. Mp: 260–262 °C (coloration occurs at 240 °C). NMR (D_2O): δ 4.73 (m, 1 H), 4.62 (m, 1 H), 3.8–3.6 (m, 4 H), 3.08 (s, 3 H), 2.65 (m, 1 H), 2.35 (m, 1 H). Anal. Calcd for $C_6H_{12}N_2$·2HBr: C, 26.30; H, 5.11; N, 10.23; Br, 58.33. Found: C, 26.40; H, 5.10; N, 10.19; Br, 58.42. $[\alpha]_D$: −13.0° (c = 0.972, CH_3OH).

Reactions of Azines. 14. Preparation of 5*H*,7*H*-Pyrazolo[1,5-*d*][2,4]benzoxazepin-7-ones

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The previous papers in this series have shown that unusual heterocyclic compounds containing a pyrazole ring with one of its nitrogen atoms in a bridgehead position may be readily prepared from azine ylides 1 (see Scheme I). It has been shown that pyrazolo[5,1-*c*]-1,4-oxazines 4,[1,2] 4,5-dihydropyrazolo[1,5-*b*]isoquinolines 5,[1-4] and 4,5- and 6,7-dihydropyrazolo[1,5-*a*]pyridines (6 and 7)[5] are produced from ketenes. Isocyanates have given 4,9-dihydropyrazolo[5,1-*b*]quinazolines 8,[6,7] 2,3-dihydro-1*H*-imidazo-[1,2-*b*]pyrazol-2-ones 9,[4,6] and 4*H*-pyrazolo[1,5-*c*][1,3,5]-

† For the X-ray data.

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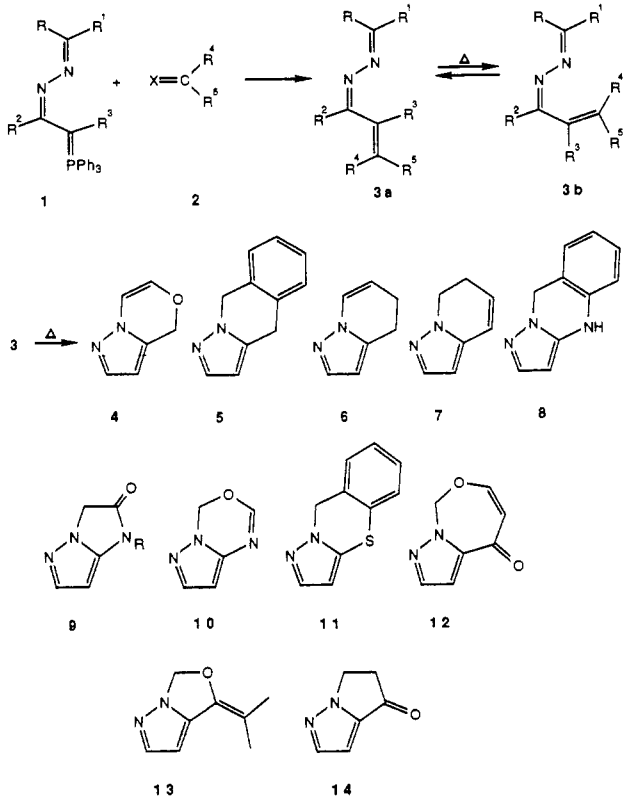
Scheme I



oxadiazines **10**.[8] Carbon disulfide has given 9*H*-pyrazolo[5,1-*b*][1,3]benzothiazines **11**[4,9] as well as other unusual heterocycles. Furandiones have given 4*H*,8*H*-pyrazolo[1,5-*c*][1,3]oxazepin-4-ones **12** in high yields or mixtures of 4*H*,6*H*-pyrazolo[1,5-*c*]oxazole 4-ylidenes **13** and 4*H*-pyrrolo[1,2-*b*]pyrazol-4-ones **14** with (or without) **12**.[10]

We now wish to report the preparation of 5*H*,7*H*-pyrazolo[1,5-*d*][2,4]benzoxazepin-7-ones **18a** by the reaction of a variety of azine ylides **1** with phthalic anhydride (**15**).

A number of reports in the literature have shown that on reacting stabilized ylides with cyclic anhydrides olefination products occur readily with the ring closed species being the predominant result.[11] In the case of the reactions with phthalic anhydrides the ylidene phthalides, similar in structures to **16**, are produced.[11a,f,g] It is also

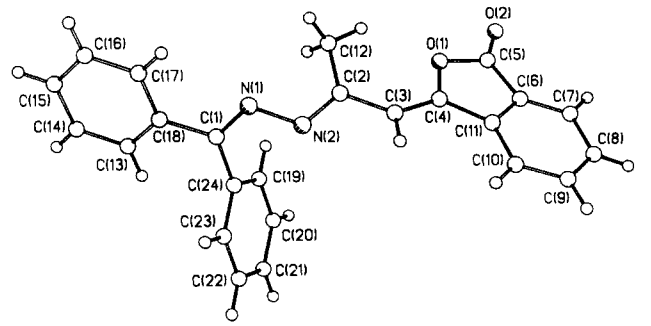
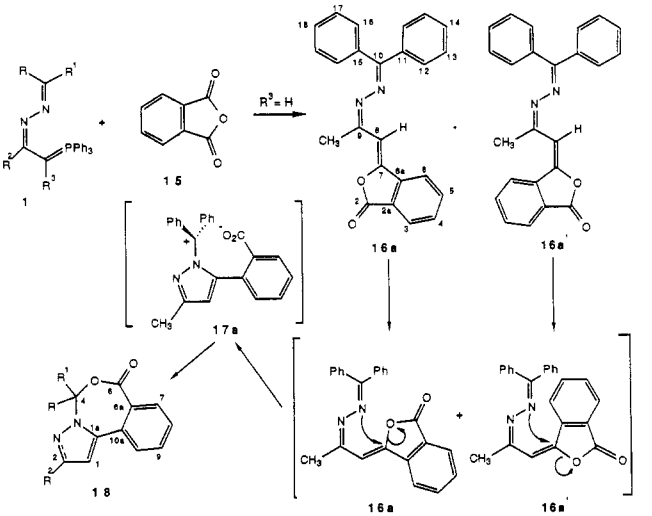


Figure 1. ORTEP diagram of **16a**.

Scheme II



reported that the *E* isomer, similar to **16a′**, is the predominant product. However, in one report, where the reaction between the anhydrides and the phosphoranes were undertaken in toluene under refluxing conditions, only the *Z* isomers were obtained.[11e] There is a report where isomerization of the *E* to the *Z* isomer was observed at ambient conditions.[11b]

A mechanism for the reaction of phthalic anhydride (**15**) with **1a** (R = R′ = Ph, R^2 = Me) is depicted in Scheme II. In order to show that the ylidene phthalide **16** was an intermediate in the reaction, the mixture of **15** and **1a** in toluene was allowed to react at room temperature for 20 h. The olefination products were found in 68% overall yield with a ratio of **16a** to **16a′** of 4.2 to 1. Our inability to characterize the major product as the *Z* (**16a**) or the *E* (**16a′**) form prompted us to undertake an X-ray analysis. A pure sample of the major product was obtained and found by X-ray to be the *Z* form **16a** (see Figure 1).

Heating **16a** (or the **16a** + **16a′** mixture) under reflux in dry toluene gave **18a** in 83% (or 70%) isolated yield.

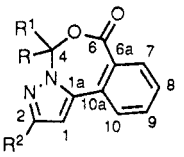
The general procedure employed in this work was to place a slight excess of phthalic anhydride into a slurry of the desired phosphorane **1** in dry toluene (see Scheme II). The mixture was heated under reflux for 15 to 48 h. Separation of the product from triphenylphosphine oxide was readily accomplished by column chromatography. The desired products **18**, when solid, were readily crystallized from diethyl ether.

Thus, it has been shown that good to excellent yields of the hitherto unknown 5*H*,7*H*-pyrazolo[1,5-*d*][2,4]-benzoxazepin-7-ones **18** may be produced readily from azine–phosphoranes **1** and phthalic anhydride (**15**).

Spectral Data. The proton NMR spectra showed characteristic peaks at 2.21–2.40 ppm for the C2-methyl

(1) Schweizer, E. E.; Evans, S. J. *J. Org. Chem.* **1978**, *43*, 4328.
(2) Schweizer, E. E.; Lee, K. J. *J. Org. Chem.* **1984**, *49*, 1959.
(3) Schweizer, E. E.; Hsueh, W.; Rheingold, A. L.; Durney, R. L. *J. Org. Chem.* **1983**, *48*, 3889.
(4) Schweizer, E. E.; Hayes, J. E.; Rheingold, A. L.; Wei, X. *J. Org. Chem.* **1987**, *52*, 1810.
(5) Schweizer, E. E.; Hayes, J. E.; Hirwe, S. N.; Rheingold, A. L. *J. Org. Chem.* **1987**, *52*, 1319.
(6) Schweizer, E. E.; Lee, K. J. *J. Org. Chem.* **1984**, *49*, 1964.
(7) Schweizer, E. E.; Hayes, J. E. *J. Org. Chem.* **1988**, *53*, 5562.
(8) Schweizer, E. E.; Lee, K. J. *J. Org. Chem.* **1987**, *52*, 3681.
(9) Lee, K. J. Unpublished results.
(10) Schweizer, E. E.; Hayes, J. E.; Lee, K. J.; Rheingold, A. L. *J. Org. Chem.* **1987**, *52*, 1324.
(11) (a) Chopard, P. A.; Hudson, R. F.; Searle, R. J. G. *Tetrahedron Lett.* **1965**, *2357*. (b) Knight, D. W.; Pattenden, G. *J. Chem. Soc., Perkin Trans. 1* **1979**, 62. (c) Massy-Westropp, R. A.; Price, M. F. *Aust. J. Chem.* **1980**, *33*, 333. (d) Marshall, P.; Mooney, B.; Prager, R.; Ward, A. D. *Synthesis* **1981**, 197. (e) Clemo, N. G.; Gedge, D. R.; Pattenden, G. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1448. (f) Abell, A. D.; Massy-Westropp, R. A. *Aust. J. Chem.* **1982**, *35*, 2077. (g) Abell, A. D.; Doyle, I. R.; Massy-Westropp, R. A. *Aust. J. Chem.* **1982**, *35*, 2277. (h) Allahdad, A.; Knight, D. W. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1855. (i) Ito, M.; Iwata, T.; Tsukida, K. *Chem. Pharm. Bull.* **1984**, *32*, 1709. (j) Mann, J.; Wong, L. T. F.; Beard, A. R. *Tetrahedron Lett.* **1985**, *26*, 167. (k) Tsuboi, S.; Fukumoto, H.; Wada, H.; Takeda, A.; Fukuyama, K. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 689.

Table I. Preparation of 5*H*,7*H*-Pyrazolo[1,5-*d*][2,4]benzoxazepin-7-ones 18 from Phthalic Anhydride (15) and Azine Ylides 1



no.	R	R^1	R^2	time, h	yield, %	mp, °C	exact mass calcd	exact mass found
a	Ph	Ph	Me	5	70	188–188.5	366.136	366.137
b	Ph	Ph	Ph	48	43	193–194	428.152	428.152
c	Ph	Me	Me	15	73	163–163.5	304.120	304.120
d	Ph	Et	Me	15	91	112–113	318.136	318.136
e	Ph	iPr	Me	15	85	150–151	332.152	332.152
f	Ph	PhCO	Me	10	66	212–213	394.132	394.131
g	Me	Me	Me	15	72	*a*	242.106	242.105
h	Me	$PhCH_2$	Me	15	75	*a*	318.136	318.136
i	$PhCH_2$	$PhCH_2$	Me	15	70	*a*	394.168	394.167
j	PhCH=CH	H	Me	30	68	*a*	316.121	316.120

[a] Sticky yellow liquid.

protons. The C1-proton on the pyrazole ring absorbed in the 6.43 to 7.07 ppm region. The aromatic multiplets fell in the range from 6.54 to 8.19 ppm. The methylene protons, in the ethyl group in **18d** and the benzyl group in **18h** and **18i**, are clearly separated due to restricted rotation. In **18d** there are two doublets of quartets at 2.68 and 2.80 ppm, with geminal and vicinal coupling of 14 Hz and 7 Hz, respectively. In **18h** doublets (J = 14 Hz) are found at 3.26 and 3.47 ppm whereas in **18i** doublets (J = 15 Hz) are found at 3.44 and 3.56 ppm.

The characteristic absorptions found in the ^{13}C NMR spectra appear as follows: 13.3–13.7 (C2-CH_3), 106.1–107.8 (C1) (104.3 for **18b**), 140.6–142.9 (C1a), 147.4–150.0 (C2), 90.9–96.9 (C4) (83.9 for **18j**), 165.7–167.5 (C6), the aromatic carbons ranged from 125.6 to 139.9 ppm.

The mass spectra of all of the 5*H*,7*H*-pyrazolo[1,5-*d*]-[2,4]benzoxazepin-7-ones **18** showed characteristic decomposition peaks at m/z = 184, 128, 104, 102. The proposed decomposition pathway is depicted in Scheme III. The exception was of course compound **18b**, which contained the C-2 phenyl, instead of the C-2 methyl, group on the pyrazole moiety, whose decomposition products could be rationalized when taking into account the C_6H_5 vs CH_3 mass (see Table IV, supplementary material).

Experimental Section

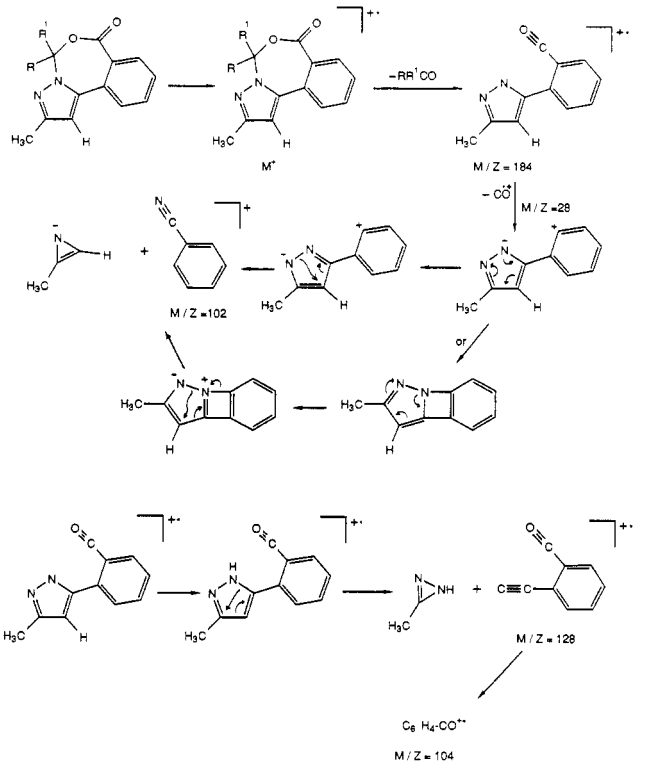
General. Dry nitrogen was routinely used as the reaction atmosphere in all reactions. All glassware was baked at 100–120 °C for a minimum of 2 h before being used. Melting points were obtained with a Mel-Temp capillary apparatus and were uncorrected.

The ^1H, ^{13}C, and ^{31}P NMR spectra of approximately 10% (w/v) solutions in $CDCl_3$ or Me_2SO-d_6 were obtained on a Burker Spectrospin Model WM 250 or AM 250 or on a Nicolet QE 300 instrument. Chemical shifts are reported in parts per million (δ scale), employing tetramethylsilane as an internal standard. In reporting the NMR data, we have employed the following abbreviations: coupling constant in hertz (J), singlet (s), double (d), doublet of doublet (dd), and multiplet (m). The numbering system used to depict the NMR is shown in Table I.

Precise mass spectra were recorded by using a Du Pont 21-492B instrument with a resolution of 3300 or 5000. All precise masses found were within 0.003 mass units of the calculated values.

Toluene was dried and distilled from sodium metal. Baker silica gel (60–200 mesh) and EM7747 silica gel for column chromatography were used throughout for product separation. Eastman Chromagram (silica gel with a fluorescent indicator on polyethylene) precoated sheets were employed in thin-layer chromatographic (TLC) operations.

Scheme III



General Reaction of Phosphorane 1 with Phthalic Anhydride (15). Sublimed phthalic anhydride (**15**) (0.90 g, 6.0 mmol) was added, all at once, to a slurry of the phosphorane **1** (2.0 g, 3.8 mmol) in 60 mL of dry toluene. The mixture was heated with stirring under reflux from 5 to 48 h (see Table I). After cooling to room temperature, the solvent was removed by rotary evaporation. The residue was chromatographically separated with ethyl acetate–petroleum ether as eluent (beginning ratio 1:50, ending ratio 1:0). The product was obtained as white crystals, or an oil (see Table I). TLC with ethyl acetate–petroleum ether showed one spot. An analytically pure sample (recrystallized from diethyl ether) had melting point as reported in Table I. The ^1H and ^{13}C NMR spectral data are reported in Tables 4S and 5S, respectively. The mass spectral data are reported in Table 6S. (Tables 4S, 5S, and 6S are found in the supplementary material.)

Preparation of Intermediates 16a and 16a'. Sublimed phthalic anhydride (**15**) (0.80 g, 5.3 mmol) was added, all at once, to a slurry of phosphorane **1a** (2.50 g, 05.1 mmol) in 60 mL of dry toluene. The mixture was stirred at room temperature for 20 h.

Table II. Crystallographic Data for 16a

(a) Crystal Parameters

formula	$C_{24}H_{18}N_2O_2$	V, Å^3	1893 (1)
formula wt	366.38	Z	4
crystal system	monoclinic	D(calcd), g cm^{-3}	1.286
space group	$P2_1/c$	μ(Mo Kα), cm^{-1}	0.77
a, Å	14.802 (4)	color	yellow
b, Å	7.409 (2)	size, mm	0.35 × 0.35 × 0.40
c, Å	17.723 (5)	T, K	293
β, deg	103.12 (3)		

(b) Data Collection

diffractometer	Nicolet R3m/μ	data collected	3258
radiation	Mo Kα	indpdt data	2893
wavelength, Å	0.71073	R(int), %	3.5
monochromator	graphite	obs data ($5\sigma F_o$)	1761
2θ limits	$4 \le 2\theta \le 48$	decay, %	<1

(c) Refinement

$R(F)$, %	5.63	Δ/σ(firal)	0.05
$R(wF)$, %	6.27	$\Delta(\rho)$, e Å^{-3}	0.24
GOF	1.544	N_o/N_v	7.6

The solvent was evaporated on a rotary evaporator, using a bath temperature not greater than 27 °C. The residue was checked by ^{1}H NMR and was chromatographically separated (methanol–ethyl acetate–petroleum ether, 0:1:25 at the beginning and 1:3:7 at the end, using a 20 × 1.5 cm column with 50 g of silica gel G, F842). The solution was allowed to stand until **16a** and **16a′** crystallized from the eluant. The total yield of the intermediates was 68%, and the ratio of the isomers **16a** to **16a′** was 4.23:1. ^{1}H NMR ($CDCl_3$, TMS as internal standard) for mixture: δ 2.24 (s, CH_3, for **16a**), 2.56 (s, CH_3, for **16a′**), 6.27 (s, C8-H, for **16a**), 6.57 (s, C8-H, **16a′**), 7.13–7.82 (m, all Ar H for two isomers). A pure sample of **16a** was obtained by separating by chromatography (hexane–ethyl acetate/95–5), employing the method of Taber,[12] and recrystallizing from diethyl ether, mp 151–152 °C. ^{1}H NMR ($CDCl_3$) for **16a**: 2.39 (s, 3 H, CH_3), 6.28 (s, 1 H, C8-H), 7.21–7.95 (m, 14 H, Ar). ^{13}C NMR ($CDCl_3$) for **16a** (numbering system is shown in Scheme I): 17.40 (C9-CH_3), 107.82 (C8), 120.63, 125.58, 128.00, 128.21, 128.82, 128.96, 128.28, 130.00, 130.65, 134.62 (C3, C4, C5, C6, C12, C13, C14, C16, C17, C18), 123.63 (C9), 135.30 (C10), 138.02, 140.19 (C2a, C6a), 147.88 (C7), 158.44, 159,32 (C11, C15), 166.25 (C2). MS, m/z (rel intensity): 366 (M^+, 4), 351 (16), 185 (21), 184 (100), 183 (18), 128 (28), 127 (10), 105 (29), 102 (10), 77 (38), 51 (19). Exact mass calcd for 366.1368, found 366.1368. The X-ray result for **16a** is reported.

Conversion of 16a (16a′) into 18a. Conversion of **16a** (**16a′**) into product **18a** was accomplished by 3 h of heating in dry toluene, employing the procedure described in General Reaction paragraph above. The recovered yield of **18a** was 83% (70%) and was identical with **18a** obtained employing the general reaction, given above.

Crystal Structure Determination for 16a. Crystallographic data are summarized in Table II. The ORTEP diagram is depicted in Figure 1. A well-formed specimen of **16a**, recrystallized from ether, was mounted on a glass fiber. Film data and unit-cell parameters uniquely determined the monoclinic space group, $P2_1/c$. No correction for absorption was required. The structure was solved by direct methods. To conserve data, the two terminal phenyl rings were constrained to rigid hexagons. All non-hydrogen atoms were refined with anisotropic thermal parameters. All hydrogen atoms, except for H(3) (numbering on Figure 1), were located to verify stereochemistry, were isotropically refined, and were treated as idealized, updata isotropic contributions. All computations used SHELXTL (5.1) software (G. Sheldrick, Nicolet XRD, Madison, WI). Bond distances and angles are given in Table II.

Supplementary Material Available: Atomic coordinates and isotropic thermal parameters (Table 1S), anisotropic thermal parameters (Table 2S), H-atom coordinates and isotropic thermal parameters (Table 3S), ^{1}H NMR (Table 4S), ^{13}C NMR (Table 5S), and mass spectral data (Table 6S) (6 pages). Ordering information is given on any current masthead page.

(12) Taber, D. F. *J. Org. Chem.* **1982**, *47*, 1351.

Preparative, Enzymatic Synthesis of Linoleic Acid (13*S*)-Hydroperoxide Using Soybean Lipoxygenase-1

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Lipoxygenase-1 (EC 1.13.11.12) is a non heme iron dioxygenase which catalyzes the incorporation of dioxygen into polyunsaturated fatty acids possessing a (1*Z*,4*Z*)-pentadienyl unsaturation to yield *E*,*Z* conjugated diene hydroperoxides[1] as depicted in Scheme I.

Plant lipoxygenases are one of the most available enzymes of this class and serve as models for the study of mammalian lipoxygenases, relevant to leukotriene biosynthesis and, to a lesser extent, to prostaglandin synthase.[1]

We describe herein an easy method for preparative scale production of the (13*S*)-hydroperoxide of linoleic acid, using commercial lipoxygenase-1 from soybean as catalyst.

Studies on lipoxygenases are generally done in dilute solutions of fatty acids in aqueous buffers because of their poor solubility in water. Fatty acid solutions up to 5 × 10^{-3} M are usually obtained with added agents such as surfactant (Tween 20, 80) and cosolvent (ethanol).[1] Only few examples describe the preparative synthesis of hydroperoxides by enzymes (continuous process, 10^{-4} M;[2] batch process, 2 L, 1 × 6 10^{-3} M[3]).

We have observed that oxidation of 0.1 M fatty acid emulsions (28 g/L) in 0.1 M (pH 9) sodium borate buffer in the presence of a commercial preparation of the enzyme (2 mg/mL) proceeds rapidly. Indeed, using a Schlenck tube under weak O_2 pressure (i.e. 4 atm) at 0–4 °C with vigorous magnetic stirring, we obtained a clear solution of linoleic acid hydroperoxides (yield 80%) after 1 h. Normal- and chiral-phase HPLC analyses showed that one major chiral component was obtained (96% isomer purity, 95% ee). This compound was identified as (9*Z*,11*E*,13*S*)-13-hydroperoxy-9,11-octadecadienoic acid according to the literature,[4,5] and the structure assignment is confirmed by NMR spectroscopy.

The selectivity of soybean lipoxygenase-1 is not affected by our conditions in terms of regio-, stereo-, and enantioselectivity.[1] The acid, as a monomer in aqueous solution, is known to be the enzyme substrate.[6] Under these conditions, this form is probably present in sufficient quantity to initiate catalysis. The fact that soybean lipoxygenase-1 has its optimum pH in the basic range is an advantage, since fatty acids are more soluble as salts in aqueous solution. The hydroperoxide formed solubilizes more linoleic acid as noted by Haining et al.,[7] allowing the reaction to progress. The reasons why the enzyme is not rapidly deactivated in this reaction are under investigation. However, the presence of great quantities of dissolved oxygen seems to prevent the total self-deactivation of the enzyme during catalysis, allowing high yields of hydroperoxide to be obtained.

In conclusion, this report describes a simple procedure by which soybean lipoxygenase-1 catalyzes formation of

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