filtered. The residue was washed with acetone and then recrystallized twice from EtOAc to give acetate **1b** in 60% yield contaminated with ca. 4% of the 5β isomer. Acetate **1b**: mp 143–144 °C (lit.[1,2a] mp 148.5–150 °C; lit.[2e] mp 137–138 °C; lit.[2f] mp 143–144 °C; lit.[3c] mp 139–140 °C); $[\alpha]^{25}_D$ −58.2° (*c* 1, $CHCl_3$) [lit.[1] $[\alpha]_D$ −35.0°; lit.[2a] $[\alpha]_D$ −36.0°; lit.[2e,f] −50.0°]; IR 2960, 2880, 1760 cm^{-1}; ^1H NMR δ 5.4 (m, H-15, -22, -23, 3 H), 4.8 (m, H-3, 1 H), 2.5–0.8 (m, remaining H); mass spectrum, *m/e* 439 (M + H), 379, 313, 125.

Anal. Calcd for $C_{30}H_{46}O_2$; C, 82.14; H, 10.57. Found: C, 82.54; H, 10.45.

(3β,5α,17β)-Cholesta-8,14-dien-3-ol Benzoate (2a) and Acetate (2b). 7-Dehydrocholesterol (**4a**) (100 g, 0.2 mol) was isomerized with HCl in the same fashion as in the preparation of **1b** to furnish, following benzoyl chloride/pyridine workup, benzoate **2a** (63 g, 65%) as crystals (EtOAc): mp 146–147 °C (lit.[2d] mp 147–148 °C); $[\alpha]^{25}_D$ −5.5° (*c* 1, $CHCl_3$) [lit.[2d] $[\alpha]^{22}_D$ −6.9°]; IR 2940, 2880, 1720 cm^{-1}; ^1H NMR δ 8.10 and 7.5 (m, aromatic, 5 H); 5.4 (s, 1 H, H-15); 5.00 (m, 1 H, H-3); 2.5–0.8 (m, remaining H); mass spectrum, *m/e* 489 (M + H), 367, 123.

Anal. Calcd for $C_{34}H_{48}O_2$: C, 83.55; H, 9.90. Found: C, 83.53; H, 9.80.

Employing an acetic anhydride/pyridine workup afforded acetate **2b** (55 g, 65%) after two recrystallizations from Et_2O: mp 100–102 °C (lit.[2b] mp 99–100 °C; lit.[2d] mp 101–102 °C); $[\alpha]^{25}_D$ −27.1° (*c* 1, $CHCl_3$) [lit.[2b] $[\alpha]^{25}_D$ −21.0°; lit.[2d] $[\alpha]^{24}_D$ −22.9°]; IR 2940, 2880, 1760 cm^{-1}; ^1H NMR δ 4.8 (m, H-3, 1 H), 2.5–0.8 (m, remaining H); mass spectrum, *m/e* 427 (M + H), 405, 367.

Anal. Calcd for $C_{29}H_{46}O_2$: C, 81.63; H, 10.87. Found: C, 81.69; H, 10.89.

Registry No. **1a**, 53639-76-2; **1b**, 71242-49-4; **2a**, 74524-23-5; **2b**, 5226-33-5; **3a**, 57-87-4; **3b**, 5035-30-3; **4a**, 434-16-2; **6a**, 113089-06-8; **6b**, 113089-07-9.

Supplementary Material Available: Tables of fractional atomic coordinates, thermal parameters, and interatomic distances and angles for both **1a** and **6b** (45 pages). Ordering information is given on any current masthead page. Tables of observed and calculated structure factors are available from the authors upon request.

Designer Spin Traps with a Cyclic Nitrone Structure[1]

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The technique of spin trapping[2] is now being widely applied to probe for free radicals in biological systems.[3,4] For the method to work well, the traps must be efficient scavengers of transient free radicals and the trapping reaction must lead to persistent spin adducts whose hyperfine splittings point to the identity of the scavenged radical.

While 5,5-disubstituted 1-pyrroline *N*-oxides[5-7] are known to be good spin traps and are widely employed,[3,4,6,8-10] their 3,3,5,5-tetrasubstituted analogues[11,12]

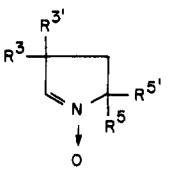
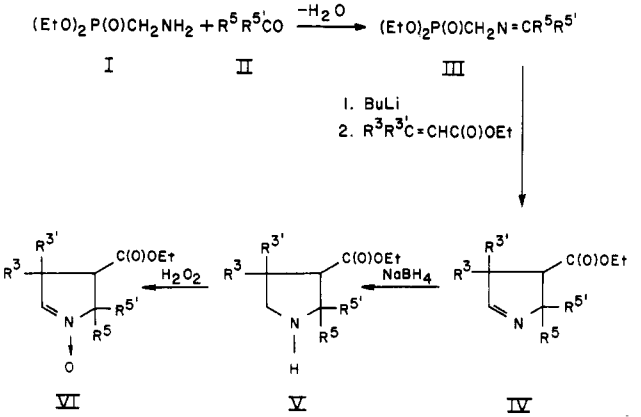


Figure 1.

Scheme I



	R^3	$R^{3'}$	R^5	$R^{5'}$
IVa–VIa	Me	Me	Me	Me
IVb–VIb	Me	Me	Ph	Me
IVc–VIc	Me	Me	$(CH_2)_{11}Me$	Me
IVd–VId	$(CH_2)_5$		$(CH_2)_5$	

Table I. Hyperfine Splittings for Spin Adducts VII Formed in the Reactions of Various Radicals with Spin Traps VI (eq 1)

	X·			
spin trap	*t*-BuO·[a] a^N, a^{H^2}, G	Ph·[b] a^N, a^{H^2}, G	$\dot{C}H_2OH$[c] a^N, a^{H^2}, G	$\dot{O}H$[d] a^N, a^{H^2}, G
VIa	12.9, 4.0	14.0, 21.3[e]	15.4, 18.0	15.5, 15.5
VIb	13.0, 10.5	13.2, 24.0	15.0 25.9	14.4, 19.1
VIc	13.3, 5.4	14.0, 23.5	15.0, 22.0[h]	*f*
VId	*g*	14.3, 25.0	15.0, 25.0[h]	14.7, 14.7

[a] Photolysis of di-*tert*-butyl peroxide in benzene. [b] Photolysis of phenylazotriphenylmethane in benzene. [c] Photolysis of H_2O_2 (1%) in phosphate buffer (pH 6) containing methanol (4%). [d] Photolysis of H_2O_2 (1%) in phosphate buffer (pH 6). [e] 10% of diastereometric spin adduct formed, c.f. ref 12. [f] Spin trap insoluble in buffer. [g] Spin adduct not detected. [h] In 60% methanol.

(Figure 1) are actually far superior since the presence of the additional substituents enhances the lifetimes of the spin adducts but has a minimal effect on the efficiency of the trapping reaction.[11] However, the synthesis of tetrasubstituted traps is not straightforward.[5,12] Three of the substituents have to be incorporated into an unsaturated ketone, which is one of the primary synthons, and the fourth has to be introduced in an alkylation procedure toward the end of the multistep synthesis.[5,12]

We have developed a simple method of making cyclic nitrone spin traps that allows substitutents of choice to be introduced at the critical 3,3- and 5,5-positions. In addition, the synthesis introduces a carbalkoxy group at

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(2) Janzen, E. G. *Acc. Chem. Res.* **1971**, *4*, 31.
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(4) Rosen, G. M.; Finkelstein, E. *Adv. Free Radical Bio. Med.* **1985**, *1*, 345.
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(7) Improvements in nitrone synthesis devised by Huie and Cherry,[7a] have been applied to these compounds.[7b] (a) Huie, R.; Cherry, W. R. *J. Org. Chem.* **1985**, *50*, 1531. (b) Haire, D. L.; Hilborn, J. W.; Janzen, E. G. *J. Org. Chem.* **1986**, *51*, 4298.
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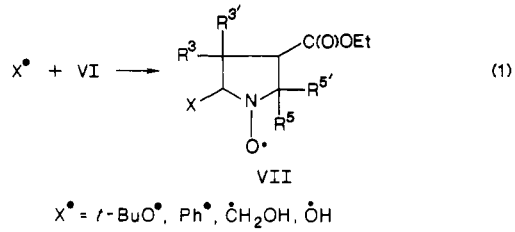
the 4-position, which can be elaborated during the synthesis as required.[13] The approach makes use of a 2 + 3 cycloaddition[14] in which the groups at the 5,5-position are derived from a ketone and those at the 3,3-position are introduced via an unsaturated ester (Scheme I). The synthesis of VIa that is described in the Experimental Section illustrates the method in detail.

A variety of nitrones VIa–d (Scheme I) were prepared by this method and all proved to be excellent spin traps (eq 1). Spin adducts VII derived in the trapping reaction



(1)

typically had half-lives of several hours at radical concentrations of ca. 10^{-4} M (Table I).

The synthesis described above achieves the flexibility of design advocated by Rosen[14] who argued in favor of structures that would allow site selective incorporation of spin traps into biological systems.

Experimental Section

The description of the synthesis of IVa that follows illustrates the synthetic method in detail. Diethyl (aminomethyl)-phosphonate[15] (I) was stirred for 48 h with acetone (10 mol excess) in the presence of molecular sieves. The solution was evaporated to give the corresponding imine IIIa. A tetrahydrofuran (THF) solution of IIIa (0.5 M) was then added under argon at −70 °C to a stirred solution of 1 equiv of butyllithium (0.5 M in THF). This was followed by the slow addition of 1 equiv of ethyl 3,3-dimethylacrylate in the same solvent at −70 °C. The mixture was then allowed to warm to 20 °C. After normal workup[14] the resulting pyrroline, IVa, was purified by distillation [bp 47 °C (0.1 mm); yield 47%].[16]

Pyrroline IVa (0.1 M, in ethanol) was reduced with a 2-fold excess of sodium borohydride at room temperature to give pyrrolidine Va. After isolation, the crude pyrrolidine (0.5 M, in methanol) was oxidized to the nitrone by using 3 equiv of hydrogen peroxide (30% in water) and sodium tungstate (4 mol %).[17] The inorganic salts were separated and the solvent was removed leaving VIa (yield 90%). The nitrone (mp 44–45 °C) was recrystallized from pentane before it was used in spin-trapping experiments. ^{1}H NMR ($CDCl_3$; standard Me_4Si) CH_3, m, δ 1.1–1.6 (15 H); H^4, s, δ 2.9 (1 H); OCH_2, q, δ 4.2 (2 H); H^2, s, δ 6.6 (1 H). ^{13}C NMR ($CDCl_3$; standard Me_4Si) CH_3, q, δ 14.2–28.5 (5 C); C^3, s, δ 41.0; C^4, d, δ 59.4; OCH_2, t, δ 60.7; C^5, s, δ 75.9; C^2, d, δ 139.0; C(O), s, δ 168.7.

In general, the compounds described in this work were new materials, with the exception of IVd, and they were characterized by elemental analysis [(calcd) found].

Pyrrolines obtained in the cycloaddition reactions were analyzed as picrates: IVa, $C_{17}H_{22}N_4O_9$, mp 138–139 °C, C (47.88), 47.60; H (5.20), 5.70; N (13.19), 13.01. IVb, $C_{22}H_{24}N_4O_9$, mp 167–168 °C, C (54.99), 54.82; H (4.95) 4.78; N (11.47), 11.37. IVc was used as a crude distillate. IVd, $C_{23}H_{30}N_4O_9$, mp 165 °C (lit.[5] mp 165 °C).

Pyrrolidines were analyzed as their hydrochloride salts or as pure crystalline materials: Va (hydrochloride), $C_{11}H_{22}NO_2Cl$, mp 115–116 °C, C (56.04), 55.83; H (9.41), 9.26; N (5.94), 5.58; Cl (15.04), 14.51. Vb, $C_{16}H_{24}NO_2$, mp 142–143 °C, C (64.50), 64.70; H (8.12), 8.30; N (4.70), 4.52. Vc used as a crude oil. Vd, $C_{17}H_{29}NO_2$, mp 51–52 °C, C (73.07), 72.70; H (10.46), 10.37; N (5.01), 4.88.

Nitrones were generally analyzed as pure solids: VIa, $C_{11}H_{19}NO_3$, mp 44–45 °C, C (61.94), 61.69; H (8.98), 9.02; N (6.57), 6.50. VIb, $C_{16}H_{21}NO_3$, mp 60–61 °C, C (69.75), 69.95; H (7.69), 7.21; N (5.09), 4.73. VIc, $C_{22}H_{41}NO_3$, oil identified by GC/MS; ion (relative abundance), 367.4 (4) p^+, 278.4 (100). VId, $C_{17}H_{29}NO_3$, mp 130 °C, C (69.59), 68.79; H (9.28), 9.07; N (4.77), 4.42.

Registry No. I, 50917-72-1; IIIa, 113086-37-6; IVa, 113086-38-7; IVb, 113086-41-2; IVc, 113086-42-3; IVd, 75373-56-7; Va, 113086-39-8; Vb, 113086-43-4; Vc, 113086-44-5; Vd, 113086-45-6; VIa, 113086-40-1; VIb, 113086-46-7; VIc, 113086-47-8; VId, 113086-48-9; VIIa (x = t-BuO), 113086-49-0; VIIa (X = Ph), 113086-52-5; VIIa (X = $HOCH_2$), 113086-55-8; VIIa (X = OH), 113086-58-1; VIIb (X = t-BuO), 113086-50-3; VIIb (X = Ph), 113086-53-6; VIIb (X = $HOCH_2$), 113086-56-9; VIIb (X = OH), 113086-59-2; VIIc (X = t-BuO), 113086-51-4; VIIc (X = Ph), 113086-54-7; VIIc (X = $HOCH_2$), 113086-57-0; VIId (X = Ph), 113108-96-6; VIId (X = $HOCH_2$), 113108-97-7; VIId (X = OH), 113108-98-8; t-BuO•, 3141-58-0; Ph•, 2396-01-2; $HOCH_2$•, 2597-43-5; HO•, 3352-57-6; acetone, 67-64-1; ethyl 3,3-dimethylacrylate, 638-10-8.

(13) For example, base hydrolysis of pyrrolidine V leads to the free acid which can be elaborated before the final oxidation step to the nitrone.

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Enhanced and Reversed Enantioselectivity of Enzymatic Hydrolysis by Simple Substrate Modifications: The Case of 3-Hydroxyglutarate Diesters

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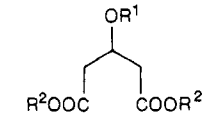
The enzymatic resolution of meso and prochiral compounds has been applied to several diesters in order to prepare chiral synthons of high enantiomeric excess (ee).[1,2] Among 3-substituted glutarate diesters, 3-hydroxyglutarates **1a** and **1b** have been used as substrates for the enzymatic hydrolysis catalyzed by pig liver esterase (PLE),[3] α-chymotrypsin (CHY),[3,4] and esterases from microorganisms.[5] From the results so far obtained, glutarates **1a**



1a: R^1 = H, R^2 = Me
b: R^1 = H, R^2 = Et
c: R^1 = Ac, R^2 = Me
d: R^1 = Ac, R^2 = Et

and **1b** appear to be poor substrates for PLE and CHY, since variable and low enantioselectivity has been found in the enzymatic hydrolyses.[2,6] On the other hand, methyl hydrogen (*R*)- and (*S*)-3-hydroxyglutarates (**2a**) could be excellent starting materials for the synthesis of several natural products such as pimaricin,[3] the lactone portion of mevinic acids,[7] L-carnitine, and (*R*)-4-amino-3-hydroxybutanoic acid (GABOB).[5]

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