Synthesis of 5β,17α-19-norpregn-20-yne-3β,17-diol and of 5β,17α-19-norpregn-20-yne-3α,17-diol, human metabolites of norethindrone

Stephen W. Curts, Douglas L. Wren, and Gary F. Cooper

Institute of Organic Chemistry, Syntex Research, Palo Alto, California, USA

A convenient synthesis of both 5β,17α-19-norpregn-20-yne-3β,17-diol **(1)** *and 5β,17α-19-norpregn-20-yne-3α,17-diol* **(2)** *in multigram quantities from estr-4-ene-3,17-dione is reported. Full characterization of these often-cited human metabolites of norethindrone is presented for the first time.* (*Steroids* **56**:8–11, 1991)

Keywords: steroids; norethindrone; metabolites; synthesis

Introduction

Partial and full reduction of the enone moiety in the A ring of norethindrone* constitute significant metabolic pathways for this important progestin.[1–3] Although the four tetrahydro metabolites 5β,17α-19-norpregn-20-yne-3β,17-diol **(1)**, 5β,17α-19-norpregn-20-yne-3α,17-diol **(2)**, 5α,17α-19-norpregn-20-yne-3β,17-diol **(3)**, and 5α,17α-19-norpregn-20-yne-3α,17-diol **(4)** (Figure 1) have been known for a long time and are often cited, only the two in the 5α series (**3** and **4**) have been prepared by a rational synthesis and characterized chemically.[4] A recent report describes the preparation of a mixture of all four isomers and their chromatographic separation.[5] The 5α isomers **3** and **4** were identified by comparison of melting points and specific rotations with literature values,[4] whereas the 5β isomers were only poorly characterized.†

We had need for the 5β isomers **1** and **2** in gram quantities. We describe a simple synthesis that gives both of these compounds and present their full characterizations.

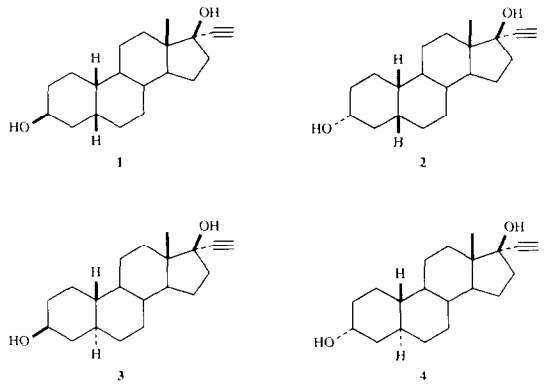


Figure 1 Tetrahydro metabolites of norethindrone.

Experimental

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were measured on a Nicolet 5PC FT-IR spectrometer using KBr pellets. Optical rotations were measured on a Perkin-Elmer 141 polarimeter in chloroform unless otherwise stated. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were measured on Bruker WM-300 or AM-500 spectrometers in deuteriochloroform. Chemical shifts are expressed in parts per million downfield from internal tetramethylsilane. Elemental analyses were performed by the Analytical and Environmental Research group, Syntex Research.

---

* Norethindrone (USAN; norethisterone [INN and BAN]) is 17-hydroxy-17α-19-norpregn-4-en-20-yn-3-one.

† In ref. 5, the melting point and specific rotation for compound **2** are compared with values given in *Steroidspekternatlas* (Neudert W and Röpke H, eds), Springer-Verlag, Berlin, compound no. 756. Only the melting point was given for compound **1**.

Address reprint requests to Dr. Gary F. Cooper, Institute of Organic Chemistry, Syntex Research, 3401 Hillview Avenue, Palo Alto, CA 94304, USA.

Received April 23, 1990; accepted August 21, 1990.

Estr-4-ene-3,17-dione was obtained from Syntex Corporation.

5β-Estrane-3,17-dione **(6)**

Estr-4-ene-3,17-dione **(5)**, 33.0 g, was hydrogenated at atmospheric pressure over 30.7 g 10% Pd on $CaCO_3$ in 1.5 L pyridine.[6] After 3.5 hours, hydrogen uptake ceased. The catalyst was filtered off and the solvent removed by rotary evaporation. Crystallization of the residue from ethyl acetate/hexanes gave 21.3 g (61.4%) of 5β-estrane-3,17-dione **(6)** as a tan solid: mp, 179 to 180 C; $[\alpha]_D$ 116.8° (c = 1.01) (lit.[7]: mp 181 to 181.5 C, $[\alpha]_D$ 114° [c = 1, $CHCl_3$]; lit.[8]: mp 182.5 to 185 C; $[\alpha]_D$ 113° [$CHCl_3$]); ν_{max} 1,737, 1,706 cm^{-1}; ^1H NMR δ 2.47 (dd, J = 18.8, 8.4, H-16α), 2.25 (dd, J = 13.9, 13.9, H-4α), 0.91 (s, 3H, H-18); ^{13}C NMR δ 220.78 (C-17), 212.30 (C-3), 50.40 (C-14), 47.86 (C-13), 42.76 (C-4), 40.88 (C-8), 39.68 (C-10), 38.50 (C-9), 38.14 (C-5), 36.27 (C-2), 35.77 (C-16), 31.50 (C-12), 30.34 (C-6), 27.59 (C-1), 25.10 (C-11), 24.30 (C-7), 21.58 (C-15), 13.77 (C-18).

Analysis calculated for $C_{18}H_{26}O_2$: C, 78.79; H, 9.55. Found: C, 78.68; H, 9.5.

Chromatography of the mother liquors on silica gel using a 5% to 12% gradient of ethyl acetate in hexanes gave an additional 1.3 g of product **6** after crystallization from ethyl acetate/hexanes and 2.25 g of 5α-estrane-3,17-dione: mp, 73 to 75 C (lit.[8]: mp, 73 to 75 C).

17-Oxo-5β-estrane-3-spiro-2'-(5',5'-diethyl-1',3'-dioxane) **(7)**

A solution of 21.9 g of 5β-estrane-3,17-dione **(6)** and 210.7 g of 2,2-diethyl-1,3-propanediol in 2 L of dimethoxyethane was treated with 0.15 g p-toluenesulfonic acid hydrate and stirred at room temperature.[9] After 4 hours 45 minutes, an additional 0.45 g p-toluenesulfonic acid hydrate was added. After 7 hours, 2 ml of triethylamine was added and the mixture was concentrated by rotary evaporation. Chromatography of the residue on silica gel eluting with a 5% to 8% gradient of ethyl acetate in hexanes gave 6.1 g of a mixture of product **7** and the corresponding bis-ketal, and 21.4 g (69.0%) of pure 17-oxo-5β-estrane-3-spiro-2'-(5',5'-diethyl-1',3'-dioxane) **(7)**: mp, 114 to 116 C; $[\alpha]_D$ 89.1° (c = 0.98); ν_{max} 1,737, 1,116 cm^{-1}; ^1H NMR δ 3.55 (s, dioxane OCH_2), 3.49 (s, dioxane OCH_2) 2.40 (dd, J = 19.1, 8.4, H-16α), 0.84 (s, 3H, H-18) 0.78 (t, J = 7.5, dioxane methyl), 0.76 (t, J = 7.5, dioxane methyl); ^{13}C NMR δ 220.99 (C-17), 98.71 (C-3), 66.84 (2C's, dioxane OCH_2), 50.54 (C-14), 47.84 (C-13), 41.08 (C-8), 40.26 (C-10), 38.04 (C-9), 35.77 (C-16), 34.93 (dioxane C-5'), 33.94 (C-4), 32.90 (C-5), 31.60 (C-12), 30.91 (C-6), 25.97 (C-2), 25.07 (C-11), 24.92 (C-7), 23.89 (C-1), 23.89 (dioxane CH_2), 23.58 (dioxane CH_2), 21.60 (C-15), 13.73 (C-18), 7.06 (dioxane methyl), 6.94 (dioxane methyl).

Analysis calculated for $C_{25}H_{40}O_3$: C, 77.27; H, 10.38. Found: C, 76.95; H, 10.01.

17-Hydroxy-5β,17α-19-norpregn-20-yne-3-spiro-2'-(5',5'-diethyl-1',3'-dioxane) **(8)**

A solution of 27.23 g of lithium acetylide ethylene diamine complex in a mixture of 520 ml anhydrous tetrahydrofuran (THF) and 155 ml anhydrous dimethyl sulfoxide (DMSO) was stirred for 30 minutes at room temperature while a gentle stream of purified acetylene gas was passed through it. A solution of 21.4 g of 17-oxo-5β-estrane-3-spiro-2'-(5',5'-diethyl-1',3'-dioxane) **(7)** in a mixture of 150 ml anhydrous THF and 21.5 ml anhydrous DMSO was added dropwise. Stirring under acetylene purge was continued overnight. The mixture was slowly poured into half-saturated brine and extracted with three portions of ethyl acetate. The combined organic phases were washed to neutrality with half-saturated brine, dried over Na_2SO_4, filtered, and concentrated by rotary evaporation to give 17-hydroxy-5β,17α-19-norpregn-20-yne-3-spiro-2'-(5',5'-diethyl-1',3'-dioxane) **(8)** as a clear amber oil that still contained residual solvent. This was carried on to the next step without further purification. A 2-g sample of the crude product was purified by column chromatography on silica gel eluting with 10% ethyl acetate in hexanes. Purified **8** was obtained in 80% recovery after crystallization from refluxing hexanes: mp, 128 to 129 C; $[\alpha]_D$ −10.2° (c = 1.01); ν_{max} 3,416, 3,311, 2,120, 1,108 cm^{-1}; ^1H NMR δ 3.60 (s, OCH_2 of dioxane), 3.55 (s, OCH_2 of dioxane), 2.59 (s, H-21),2.28 (ddd, J = 13.9, 9.7, 5.5, H-16α), 0.85 (s, H-18), 0.82 (t, J = 7.6, dioxane methyl), 0.81 (t, J = 7.5, dioxane methyl); ^{13}C NMR δ 98.96 (C-3), 87.62 (C-20), 79.92 (C-17), 73.91 (C-21), 66.94 (dioxane OCH_2), 66.92 (dioxane OCH_2), 49.53 (C-14), 47.04 (C-13), 42.20 (C-8), 40.25 (C-10), 38.92 (C-16), 37.55 (C-9), 34.98 (dioxane C-5'), 33.74 (C-4), 33.04 (C-5), 32.72 (C-12), 31.08 (C-6), 26.30 (C-2), 25.64 (C-11), 25.51 (C-7), 23.88 (C-1), 23.73 (2 C's, dioxane CH_2), 22.92 (C-15), 12.71 (C-18), 7.12 (dioxane methyl), 7.05 (dioxane methyl).

Analysis calculated for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.05; H, 9.75.

17-Hydroxy-5β,17α-19-norpregn-20-yn-3-one **(9)**

A solution of 15.4 g of 17-hydroxy-5β,17α-19-norpregn-20-yne-3-spiro-2'-(5',5'-diethyl-1',3'-dioxane) **(8)** in 385 ml of THF was stirred at room temperature with 385 ml of 5% hydrochloric acid. After approximately 1.5 hours, the initially two-phase system became homogeneous. Stirring was continued overnight. The solvent was removed by rotary evaporation and the residue partitioned between half-saturated brine and ethyl acetate. The aqueous phase was extracted a further two times with ethyl acetate and the combined organic phases washed with aqueous sodium bicarbonate to pH 7–8, half-saturated brine, brine, and dried over Na_2SO_4. Filtration and concentration gave a mixture of the desired product **9** and 2,2-diethyl-1,3-propanediol. Chromatography on silica gel eluting with a 13.5% to 18% gradient of ethyl acetate in hexanes gave 9.8 g (87.8%) of 17-hydroxy-5β,17α-19-norpregn-20-yn-3-one **(9)**. Recrystallization of a sample from acetone/

hexanes gave an analytical sample: mp, 206 to 207 C; $[\alpha]_D$ −20.5° (c = 1.01) (lit.[5]: mp, 194 to 196 C; $[\alpha]_D$ 4.8° [c = 0.84, $CHCl_3$]); ν_{max}, 3,413, 3,272, 2,120, 1,701, 1,059 cm^{-1}; ^1H NMR δ 2.61 (t, J = 14.3, H-4α), 2.61 (s, H-21), 2.30 (ddd, J = 13.9, 9.5, 5.3, H-16α), 2.05 (s, OH), 0.89 (s, H-18); ^{13}C NMR δ 212.96 (C-3), 87.51 (C-20), 79.86 (C-17), 74.07 (C-21), 49.46 (C-14), 47.06 (C-13), 42.91 (C-4), 42.07 (C-8), 39.77 (C-10), 38.91 (C-16), 38.31 (C-5), 38.10 (C-9), 36.42 (C-2), 32.67 (C-12), 30.54 (C-6), 27.73 (C-1), 25.57 (C-11), 25.04 (C-7), 22.91 (C-15), 12.73 (C-18).

Analysis calculated for $C_{20}H_{28}O_2$: C, 79.95; H, 9.39. Found: C, 80.50; H, 9.04.

5β,17α-19-Norpregn-20-yne-3α,17-diol (2)

17-Hydroxy-5β,17α-19-norpregn-20-yn-3-one (9), 11.56 g, was dissolved in 690 ml methanol with gentle warming. To this solution was added 34.5 ml water and 11.5 g sodium borohydride. After 1 hour, thin-layer chromatography (silica gel, ethyl acetate/hexanes [2:3]) showed an approximately 90/10 mixture of the desired product **2** and the less polar 3β isomer **1**. Most of the excess sodium borohydride was consumed by the addition of 40 ml of acetone. After 10 minutes of stirring, glacial acetic acid was added dropwise until no effervescence was observed. Solvent was removed by rotary evaporation and the residue was partitioned between ethyl acetate and half-saturated brine. The aqueous phase was extracted with a further two portions of ethyl acetate and the combined organic phases washed with half-saturated brine, brine, and dried over Na_2SO_4. After filtration and concentration, the crude product was purified by chromagraphy on silica gel eluting with a 15% to 18.5% gradient of ethyl acetate in hexanes. Fractions containing pure **2** were pooled and concentrated by rotary evaporation to give 11.1 g of a clear, light yellow oil. Crystallization from hexanes gave 6.88 g (59%) 5β,17α-19-norpregn-20-yne-3α,17-diol **(2)** as a white solid: mp, 85 to 87 C; $[\alpha]_D$, −14.3° (c = 0.94) (lit.[5]: mp, 85 to 89 C; $[\alpha]_D$, −19.8 C [c = 0.74, $CHCl_3$]; lit.[†]: mp, 98 to 105 C; $[\alpha]_D$ −20°); ν_{max} 3,421, 3,309, 2,120, 1,062, 1,038 cm^{-1}; ^1H NMR δ 3.64 (m H-3β), 2.60 (s, H-21), 2.28 (ddd, J = 13.8, 9.6, 5.7, H-16α), 0.84 (s, H-18); ^{13}C NMR δ 87.61 (C-20), 79.76 (C-17), 73.89 (C-21), 71.54 (C-3), 49.47 (C-14), 46.99 (C-13), 42.35 (C-8), 39.86 (C-10), 38.84 (C-16), 38.14 (C-9), 36.18 (C-4), 35.64 (C-5), 32.70 (C-12), 31.39 (C-6), 29.51 (C-2), 25.99 (C-11), 25.74 (C-7), 25.28 (C-1), 22.92 (C-15), 12.70 (C-18).

Analysis calculated for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.68; H, 10.43.

5β,17α-19-Norpregn-20-yne-3β,17-diol (1)

A solution of 8.4 g 5β,17α-19-norpregn-20-yne-3α,17 diol **(2)** in 150 ml anhydrous THF was treated under nitrogen with 9.3 g triphenylphosphine, 6.12 g diethyl azodicarboxylate (DEAD), and 3.97 g trifluoroacetic acid. After 10 minutes at room temperature, 5.3 g sodium benzoate was added and the mixture was allowed to stir overnight.[10] Thin-layer chromatography (silica gel, ethyl acetate/hexanes [2:3]) showed 5% to 10% residual **1**; therefore, 0.80 g triphenylphosphine, 0.53 g DEAD, 0.35 g trifluoroacetic acid, and 0.43 g sodium benzoate was added. After 4.5 hours, the mixture was concentrated by rotary evaporation to give an off-white opaque oil that was heated at reflux in 250 ml methanol overnight. After removal of solvent by rotary evaporation, the crude mixture was partitioned between methylene chloride and half-saturated brine. The organic phase, plus two further methylene chloride extractions of the aqueous phase, was washed with half-saturated brine, dilute aqueous sodium bicarbonate, half-saturated brine, brine, and dried over Na_2SO_4. After filtration and concentration, the crude product was purified by chromatography on silica gel eluting with a 16% to 18.5% gradient of ethyl acetate in hexanes. Fractions containing pure **1** were pooled and concentrated by rotary evaporation to give 5.8 g of a clear, light yellow oil. The product was crystallized by dissolving in hot diethyl ether (120 ml), adding hexanes at reflux to a total volume of 550 ml, boiling down to 450 ml (vapor temperature, 63 C), and allowing to cool overnight. Pure 5β,17α-19-norpregn-20-yne-3β,17-diol **(1)**, 4.43 g, was obtained on filtration and drying. An additional 0.55 g was obtained on concentration of the mother liquors. Total yield 4.98 g (59.3%) **1**: mp, 150 to 151.2 C (lit.[5]: mp, 165 to 170 C); $[\alpha]_D$, −28.8° (c = 0.66, methanol); ν_{max} 3,429, 3,260, 2,120, 1,062, 1,047, 1,024, 998 cm^{-1}; ^1H NMR δ 4.12 (m, H-3α), 2.59 (s, H-21), 2.28 (ddd, J = 13.9, 9.5, 5.7, H-16α), 0.85 (s, H-18); ^{13}C NMR δ 87.73 (C-20), 80.02 (C-17), 73.87 (C-21), 67.20 (C-3), 49.63 (C-14), 47.09 (C-13), 42.32 (C-8), 40.76 (C-10), 38.98 (C-16), 37.55 (C-9), 33.37 (C-4), 32.80 (C-12), 31.37 (C-6), 30.00 (C-5), 26.91 (C-2), 25.66 (C-11), 25.66 (C-7), 22.97 (C-15), 21.41 (C-1), 12.73 (C-18).

Analysis calculated for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.46; H, 9.99.

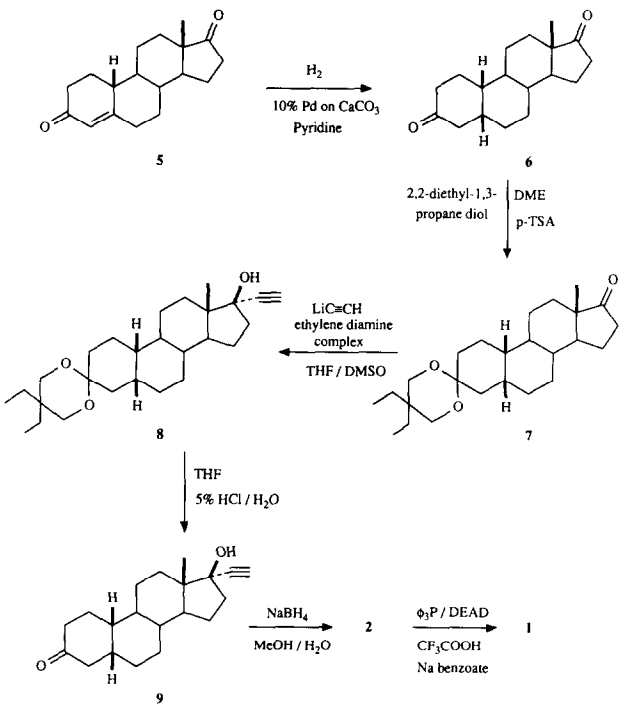
Results and discussion

Scheme 1 shows the straightforward synthetic route that produced both the 3β alcohol **1** and the 3α alcohol **2** from well-known estr-4-ene-3,17-dione **(5)**. Catalytic hydrogenation of enone **5** over Pd on $CaCO_3$ in pyridine[6] gave an approximately 18:1 mixture of the 5β and 5α diones. The desired 5β dione **6** was easily and cleanly isolated by crystallization.

Selective protection of the C-3 carbonyl group by the method of Smith and Newman[9] gave a 69% yield of the C-3 protected ketone **7** after chromatographic separation from the diprotected derivative. No monoprotection at C-17 was observed.

Ethynylation at C-17 with lithium acetylide ethylene diamine complex in THF/DMSO followed by acid-catalyzed deprotection gave the 5β C-3 ketone **9** ready for reduction to the desired C-3 alcohols.

As expected, reduction with sodium borohydride in aqueous methanol gave predominantly the thermodynamically more stable equatorial 3α alcohol **2**. Confirmation of the stereochemical assignments at C-3 was



Scheme 1

obtained from the ^1H NMR chemical shifts and the half-band widths for the C-3 hydrogen resonances. The chemical shifts for the C-3 hydrogens in **1** and **2** are δ 4.12 ppm and 3.64 ppm, respectively, in excellent agreement with the values presented by Counsell[8] for the C-3 hydrogens in 3β-hydroxy-5β-estran-17-one and 3α-hydroxy-5β-estran-17-one, 4.11 ppm and 3.59 ppm, respectively. The C-3 hydrogen resonance in the 3α alcohol **2** had a half-band width of 21 Hz, whereas the C-3 hydrogen resonance in the 3β alcohol **1** had a half-band width of 9.8 Hz, as expected for axial and equatorial hydrogens, respectively.[11] In the ^{13}C NMR spectrum of **1**, the expected upfield shifts of the C-1 and C-5 resonances (relative to those in **2**), due to steric compression caused by the axial OH at C-3, are observed.[12]

Although a modest amount of 3β alcohol **1** was obtained directly from the reduction of ketone **9**, larger amounts were conveniently prepared by the method of Walker and co-workers.[10] Mitsunobu inversion of alcohol **2** using trifluoroacetic acid catalyzed by sodium benzoate gave the inverted trifluoroacetate, which was not isolated but, rather, was solvolyzed in situ with methanol, giving 3β alcohol **1** in good yield.

Acknowledgment

The expert assistance of Janis Nelson of the Analytical and Environmental Research group, Syntex Research, in obtaining and interpreting the NMR spectra is gratefully acknowledged.

References

1. Mahesh VB, Mills TM, Lin TJ, Ellegood JD, Braselton WE (1977). Metabolism, metabolic clearance rate, blood metabolites and blood half-life of norethindrone and mestranol. In: Garattine S, Berendes HW (eds), *Pharmacology of Steroid Contraceptive Drugs*. Raven, New York, pp. 117–130.
2. Stillwell WG, Horning EC, Horning RN, Stillwell RN, Zlatkis A (1972). Characterization of metabolites of steroid contraceptives by gas chromatography and mass spectrometry. *J Steroid Biochem* **3:**699–706.
3. Gerhards E, Hecker W, Hitze H, Nieuweboer B, Bellmann O (1971). Zum Stoffwechsel von Norethisteron (17α-Äthinyl-4-Östren-17β-ol-3-on) und DL-sowie D-norgestrel (18-Methyl-17α-Äthinyl-4-Östren-17β-ol-3-on) beim Menschen. *Acta Endocrinol (Copenh)* **68:**219–248.
4. Vilchis F, Chávez B, Pérez AE, García GA, Angeles A, Pérez-Palacios G (1986). Evidence that a non-aromatizable metabolite of norethisterone induces estrogen-dependent pituitary progestin receptors. *J Steroid Biochem* **24:**525–531.
5. Golubovskaya LE, Pivnitsky KK (1989). Simple synthesis of the complete set of the norethisterone unconjugated metabolites and their deuteroanalogues. *Bioorg Khim* **15:**411–416.
6. Combe MG, Henbest HB, Jackson WR (1967). Aspects of stereochemistry. Part XXI. Hydrogenation of 3-oxo-Δ^4-steroids over a palladium-calcium carbonate catalyst. *J Chem Soc* (C) 2467–2469.
7. Tsuji N, Suzuki J, Shiota M (1980). Highly stereoselective hydrogenation of 3-oxo-4-ene and 1,4-diene steroids to 5β compounds with palladium catalyst. *J Org Chem* **45:**2729–2731.
8. Counsell RE (1961). Isomeric estrane derivatives. *Tetrahedron* **15:**202–211.
9. Smith SW, Newman MS (1968). The *gem*-dialkyl effect. III. Kinetic and equilibrium studies of steroid cyclic ketal formation and hydrolysis. *J Am Chem Soc* **90:**1253–1257.
10. Varasi M, Walker KAM, Maddox ML (1987). A revised mechanism for the Mitsunobu reaction. *J Org Chem* **52:**4235–4238.
11. Bhacca NS, Williams DH (1964). *Applications of NMR Spectroscopy in Organic Chemistry*. Holden-Day, San Francisco, pp. 79–80.
12. Levy GC, Nelson GL (1972). *Carbon-13 Nuclear Magnetic Resonance for Organic Chemists*. Wiley-Interscience, New York, pp. 43–44.