Synthesis of 16-Fluoroestrogens by Unusually Facile Fluoride Ion Displacement Reactions: Prospects for the Preparation of Fluorine-18 Labeled Estrogens

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16α-Fluoroestradiol and 16β-fluoroestradiol can be prepared by the reaction of fluoride ion with the opposite corresponding epimeric [[(trifluoromethyl)sulfonyl]oxy]estrone precursors, followed by stereoselective reduction to the 17β-estradiols. The related 17β-ethynyl compounds can be prepared by ethynylation of the fluoroestrones. Stereochemical assignments, suggested by known reaction stereoselectivities, are supported by extensive ^1H and ^{19}F NMR analyses. The fluoride ion displacement reactions operate rapidly and efficiently at room temperature, even with low concentrations of fluoride ion. This rapid and convenient fluorination method is adaptable to the preparation of fluorine-18 labeled estrogens that may be useful as breast tumor imaging agents.

The introduction of fluorine into steroidal hormones and drugs is often associated with increased potency due to suppression of undesired metabolic reactions and reduced binding to serum proteins.[1] The preparation of such fluoropharmaceuticals involves reactions that are often quite slow and utilize an excess of fluorinating agent. The development of positron-emitting transaxial tomography (PETT) has resulted in an increased demand for pharmaceutical agents labeled with positron-emitting radionuclides, such as fluorine-18, in order to study local metabolic activities and receptor distributions.[2] However, the short half-life of fluorine-18 (110 min) and the need for high specific activity places interesting new constraints on synthetic approaches to these compounds—the preparative methods need to be rapid, convenient, and efficient in the source of fluorine, which is the limiting reagent.[3]

In connection with our interest in developing γ-emitting estrogens as imaging agents for estrogen receptor positive human breast tumors,[4] we have identified 16α- and 16β-fluoro-17β-estradiol as promising positron-emitting estrogens. In this paper, we describe the preparation of these 16-fluoroestradiols, as well as their 17α-ethynyl analogues, by fluoride ion displacements on reactive trifluoromethanesulfonate (triflate) derivatives. These displacements are rapid, efficient, and convenient and have been adapted elsewhere to the successful preparation of the 16-fluoroestradiols in fluorine-18 labeled form in reaction–purification sequences that take less than 1 half-life to complete.[5]

Results and Discussion

Chemical Syntheses. Preparation of 16β-Fluoroestradiol. An α-disposed triflate (**5**), prepared in four steps from estrone (**1**) [Scheme I], is the immediate precursor for fluoride displacement. Treatment of 16α-bromoestrone 3-acetate (**3**)[6] with 2.2 equiv of NaOH in aqueous pyridine[7] with careful monitoring of the progress of the reaction by TLC allows the isolation of 16α-hydroxyestrone (**4**). This product presumably arises via epimerization of **3** to the 16β-bromide, followed by hydroxide displacement[7] and concurrent acetate hydrolysis. Use of larger amounts of base results in partial or total isomerization of the desired product into 16-oxoestradiol. The 16α-alcohol is activated toward displacement by conversion to the triflate **5** with triflic anhydride and 2,6-lutidine in CH_2Cl_2; simultaneous triflation of the 3-hydroxyl group provides a convenient protection of the phenol that would, if free, be reactive towards the 16-triflate.

Upon treatment with 1 equiv or less of n-Bu_4NF in THF, the 16α-triflate **5** is converted into 16β-fluoroestrone 3-triflate (**6**) (to the exclusion (2–3%) of 16α-epimer **16**). Reduction of **6** with $LiAlH_4$ in THF provides 16β-fluoro-17β-estradiol (**7**) cleanly; because access to the β-face of the carbonyl group is unhindered in this molecule, the reduction is highly stereospecific. The reduction also results in the cleavage of the phenolic triflate by reduction to trifluoromethyl mercaptan.

If the bis-triflate **5** is treated with an excess of n-Bu_4NF in THF, an epimeric mixture of fluoroestrones with partial

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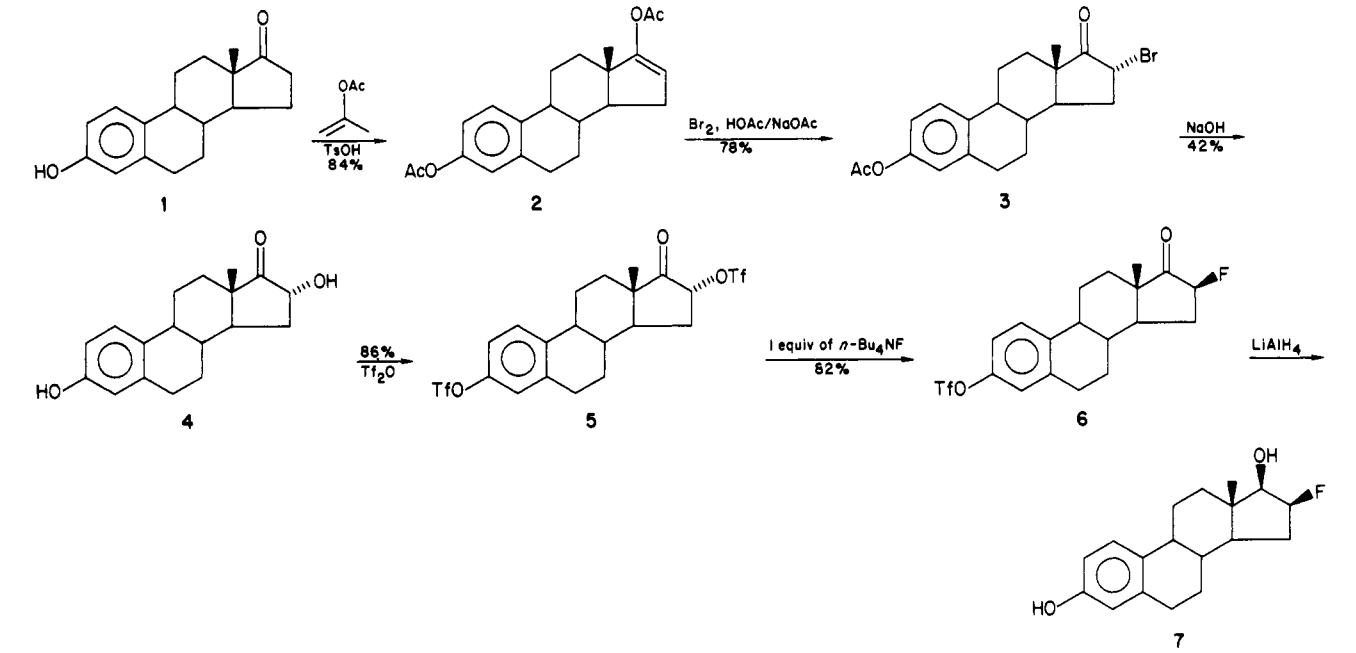
Scheme I



Scheme II

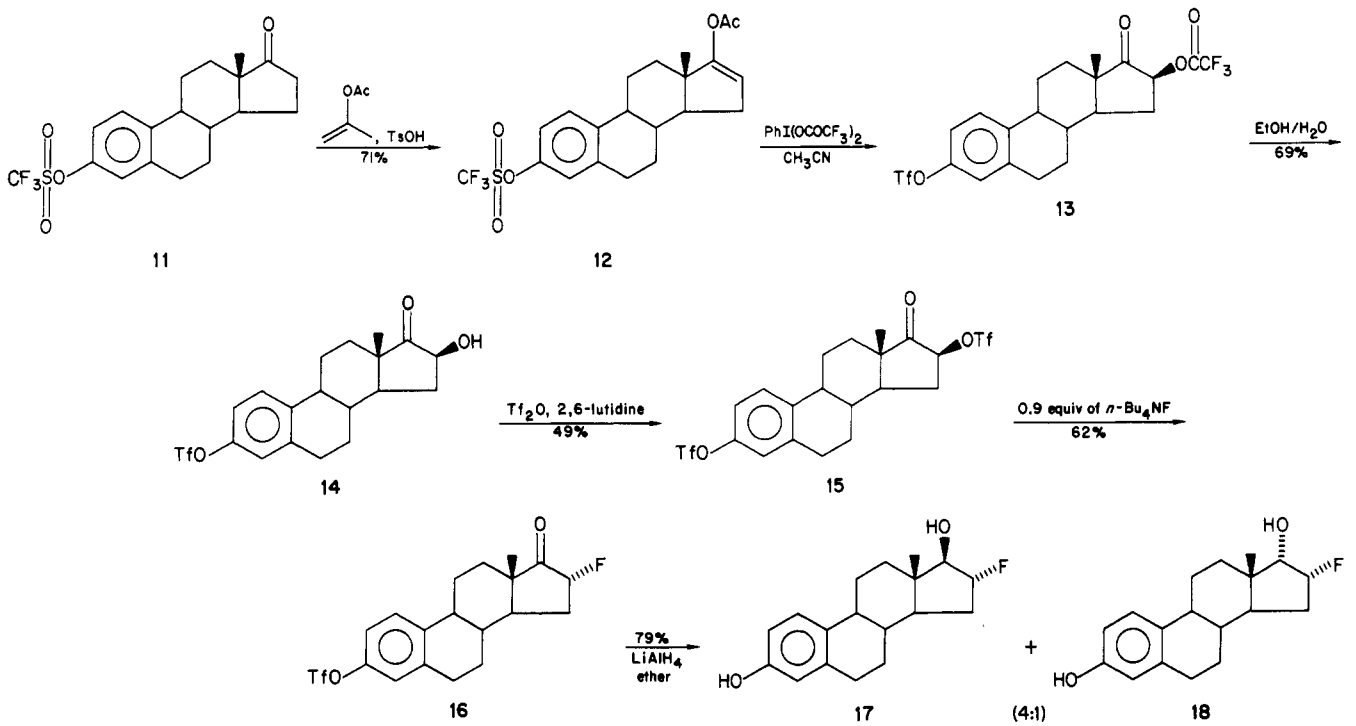


Table I. Stereoselectivity in the Hydride Reduction of 16

reagent[a]	solvent	time, min	temp.,[b] °C	ratio 17/18[c]
$LiAlH_4$	THF	15	25	1
$LiAlH_4$	THF	15	−78 (25)	1.4
$LiAlH_4$	ether	15	25	2.4
$LiAlH_4$	ether	15	−78 (25)	4.0
$NaBH_4$ (NaOH)[d]	ethanol	20	25	1.3

[a] $LiAlH_4$ reduction utilized 50 molar excess; $NaBH_4$ utilized 10 molar excess. [b] Warming to room temperature required for reductive cleavage of phenolic triflate. [c] Epimer ratio determined by HPLC. [d] NaOH is required to effect hydrolysis of phenolic triflate (MeOH, 60 °C).

cleavage of the phenolic triflate is obtained. Epimerization is thought to result from the enolization–reketonization of the fluoroestrone due to the base effect of excess fluoride ion on the 16β-fluoroestrone (rather than from inversion due to fluoride ion displacement on the 16β-fluoro ketone).

Preparation of 16α-Fluoroestradiols. Introduction of a 16β leaving group is required for preparation of 16α-fluoroestradiol. The preparation of 16β-hydroxyestrone **9** by the hydrolysis of 16β-acetoxyestrone (prepared as shown below) has been reported.[8] In our hands, however, this hydrolysis is invariably accompanied by partial or total isomerization of **9** to the more stable 16-oxoestradiol **10**. Attempts to isolate compound **9** from the mixture of **9** and **10** by chromatography resulted in even further isomerization to **10**. However, by expanding upon the work of Koser on iodoso compounds,[9] we were able to prepare the

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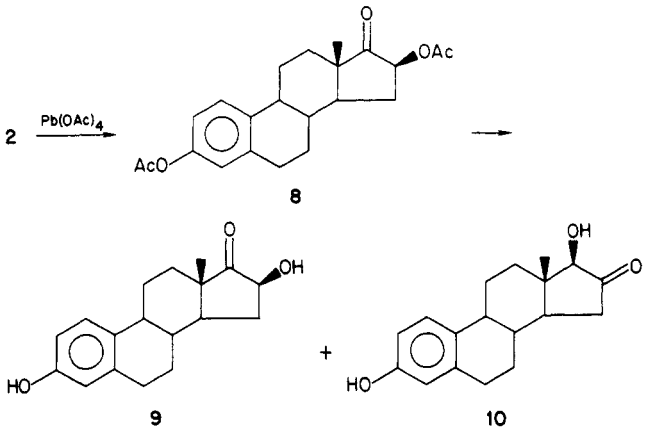
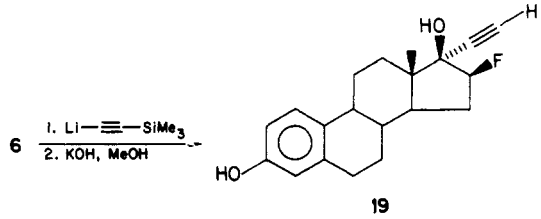


more hydrolytically labile 16β-trifluoroacetoxy compound **13**, as shown in Scheme II.

The 3-triflate-protected enol acetate **12**, prepared in 92% yield from estrone, reacts with [bis(trifluoroacetoxy)iodo]benzene[10] at room temperature to give the 16β-trifluoroacetate **13**. This material can be hydrolyzed to the protected 16-hydroxyestrone **14** under much milder conditions than those required for the hydrolysis of **8**. Best results are obtained if the trifluoroacetate is purified by flash chromatography prior to hydrolysis. While purification on conventional silica gel columns results in some hydrolysis and isomerization to 16-oxoestradiol, the rapid contact time in flash chromatography gives no isomerization. The bis-triflate **15** is prepared from the alcohol **14** by using triflic anhydride and 2,6-lutidine.

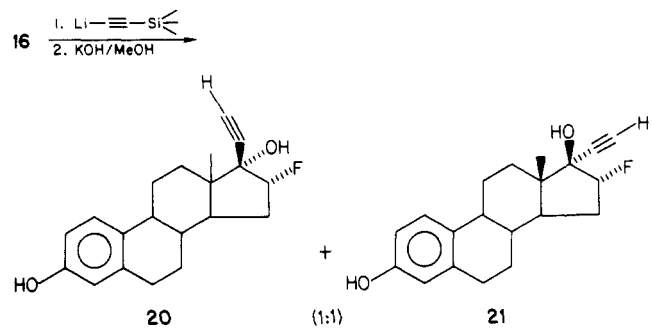
Treatment of **15** with 1 equiv or less of n-Bu_4NF provides 16α-fluoroestrone **16** with only a trace (2–3%) of epimer **6**. Hydride reduction of **16** provides two products epimeric at C-17 (**17** and **18**), which are easily separable by chromatography. The ratio of **17** to **18** varies with the conditions of reduction, as summarized in Table I. Since we desired to optimize the amount of 17β-epimer produced (**17**), as it displays more favorable biological properties, we used $LiAlH_4$ in ether at −78 °C for the reduction of **16**. Subsequent warming to 25 °C is required to effect reductive cleavage of the phenolic triflate.

Preparation of 16-Fluoro-17-ethynylestradiols. Fluoro ketone **6**, upon treatment with lithium (trimethylsilyl)acetylide and subsequent base hydrolysis, yields 17α-ethynyl-16β-fluoro-17β-estradiol (**19**). When



the same conditions are used, fluoro ketone **16** is converted into a 1:1 mixture of ethynyl estradiols **20** and **21**, which are separable by chromatography. Again, as was the case with the hydride reductions, ethynylation of the unhindered 16β-fluoroestrone is highly stereoselective, while 16α-fluoroestrone gives an epimeric mixture.

Confirmation of Stereochemical Assignments by NMR. The stereochemical assignments of epimers at the 16 and 17 position is based on well-established chemical



precedents for the stereochemistry of halogenation and ketone addition reactions in the D ring. The bromination of estrone enol acetate was shown by Fishman and Biggerstaff to provide only the 16α-epimer of bromoestrone, by chemical conversion via the 16β,17β-epoxide to estra-1,3,5(10)triene-3,16β-diol.[11] The predominance of α-attack by electrophilic reagents extends to the preparation of other 16-haloestrones, whose configurations have been correlated by optical rotary dispersion.[12] Hydride reductions of D-ring keto steroids are known to proceed with selective hydride delivery from the α-face.[13] The C-18 methyl group is the most important factor in obstructing β approach of hydride at C-17. However, the presence of a substituent in the 16α position reduces the selectivity, resulting in the production of some of the 17α-estradiols, though generally as the minor isomer.

We have confirmed these stereochemical assignments by extensive ^1H and ^{19}F NMR chemical shift and coupling studies. A full description of these studies is contained in the supplementary material section.

Conclusion

Many fluorine-substituted estrogens have been described. However, most methods for their preparation have involved either electrophilic fluorination (perchloryl fluoride, xenon difluoride)[14] or have required large excesses of fluoride ion and prolonged reaction times[12,14a,15] and thus are not suitable for the preparation of fluoroestrogens labeled at high specific activity with the short-lived positron-emitting radionuclide fluorine-18.[16]

In this report, we have described conditions for fluoride displacement that enable small quantities of fluoride ion to be introduced into the 16 position of estradiol, in reactions that are rapid, efficient, selective, and convenient. The stereochemistry of these displacement reactions and the ensuing further transformations have been verified by NMR spectroscopy. Utilizing these methods with cyclotron-produced [^{18}F]fluoride ion, we have been able to prepare 16α-[^{18}F]fluoro-17β-estradiol and 16β-[^{18}F]-fluoro-17β-estradiol in reaction–purification sequences that take less than 1 half-life of fluorine-18 ($t_{1/2}$ = 110 min) to complete. This has enabled us to produce sufficient

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quantities of these compounds for biological studies of their potential as imaging agents for estrogen receptor-positive human breast tumors.[5]

Experimental Section

Methods. Melting points were obtained on a Fisher-Johns hot stage melting point apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet 7199C FT-IR and are reported in units of frequency (cm^{-1}). ^1H NMR spectra were obtained on Varian EM-390, Varian HR-220, and Nicolet NT-360 spectrometers and are reported in parts per million downfield from internal tetramethylsilane. ^{19}F NMR spectra were obtained on Varian EM-390 and Nicolet NT-360 spectrometers at 84.6 MHz and 338.8 MHz, respectively, and are reported in parts per million from internal $CFCl_3$. Mass spectra were obtained on Varian MAT CH5 and MAT 731 spectrometers for low and high resolution spectra. Elemental analyses were performed by the Microanalytical Service, School of Chemical Sciences, University of Illinois.

Flash chromatography utilized Woelm 32–63-μm silica gel. HPLC was performed on a Varian 5060 HPLC utilizing a Varian Si-5 (4.6 mm × 30 cm) 5-μm silica gel column for analytical work and a Whatman M9 (1.3 cm × 50 cm) silica gel column for preparative work. Detection was accomplished with a Perkin-Elmer LC-75 variable wavelength detector. Quantitation utilized an external standardization technique and with the aid of a Hewlett-Packard HP 3390A reporting integrator.

Materials. Estrone was obtained as a generous gift from G. D. Searle, Co. [Bis(trifluoroacetoxy)iodo]benzene was prepared by the method of Spyroudis and Varvoglis.[10] Trifluoromethanesulfonic anhydride was prepared by the method of Burdon et al.[17] and redistilled periodically from P_2O_5. $LiAlH_4$ solutions in THF or ether were prepared as previously described.[4b] All other reagents were obtained from commercial sources and used as received.

Tetrahydrofuran (THF) and diethyl ether were distilled from sodium benzophenone ketyl immediately before use. Acetonitrile and 2,6-lutidine were distilled from barium oxide and stored under nitrogen.

3,17-Diacetoxyestra-1,3,5(1),16-tetrene (**2**) was prepared by the method of Leeds.[18] 16α-Bromo-3-acetoxyestra-1,3,5(10)-trien-17-one (**3**) was prepared by the method of Johnson and Johns.[6] Both compounds gave NMR spectra equivalent to that reported earlier.

3,16α-Dihydroxyestra-1,3,5(10)-trien-17-one (4). Bromoestrone **3** (500 mg, 1.28 mmol) was dissolved in 30 mL of 75% aqueous pyridine. Sodium hydroxide (2.8 mL of 1 N) was added, and the solution was stirred at room temperature for 11 h. The reaction was then poured into 100 mL 0.1 N HCl and extracted with three portions of ethyl acetate. The organic layers were washed with dilute copper sulfate, water, and brine and dried (Na_2SO_4). Removal of the solvent and recrystallization from ethyl acetate gave **4** (156 mg, 42%): mp 221–226 °C (lit.[8] mp 210–235 °C); NMR (90 MHz, Me_2SO-d_6) δ 0.9 (s, 3 H, 18-methyl), 1.5–3.0 (m, backbone), 4.2 (t, J = 6 Hz, 1 H, 16-H), 5.3 (d, J = 6 Hz, 1 H, OH), 6.4 s, 6.5 dd, 7.0 d (4, 2, 1-H, respectively); IR (KBr) 3430 (OH), 1740, 1620 cm^{-1}; mass spectrum (70 eV), m/z (relative intensity) 286 M^+ (100), 214 (92), 213 (47), 172 (58).

3,16α-Bis[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (5). 16α-Hydroxyestrone **4** (100 mg, 0.35 mmol) was dissolved in 300 μL of 2,6-lutidine and diluted with 2 mL of CH_2Cl_2. This solution was cooled in an ice bath and then treated with 1 mL of triflic anhydride. After 30 min, the reaction was quenched with water and extracted with ethyl acetate. The organic extracts were washed with water and aqueous $CuSO_4$ and dried (Na_2SO_4). Removal of the solvent and flash chromatography (20% ethyl acetate/hexane) yielded the bis-triflate **5** (166 mg, 86%); mp 139–141 °C; ^1H NMR (90 MHz, $CDCl_3$) δ 1.0 (s, 3 H, 18-methyl), 1.4–3.1 (m, backbone), 5.4 (m, 1 H, 16-H), 6.9 (s, 1 H, 4-H), 6.95 (dd, J = 9, 1 Hz, 1 H, 2-H), 7.3 (d, J = 9 Hz, 1 H, 1-H); ^{19}F NMR (84.6 MHz, $CDCl_3$) ϕ −73 (s, 3-triflate), −74.4 (s, 16-triflate); IR (KBr) 1750 (ketone), 1660, 1490, 1420, 1260 cm^{-1}; mass spectrum (70 eV), m/z (relative intensity) 550 M^+ (23), 346 (37), 345 (100), 213 (50). Anal. Calcd for $C_{20}H_{20}F_6O_7S$: C, 43.64; H, 3.64; F, 20.73; S, 11.64. Found: C, 43.81; H, 3.75; F, 20.99; S, 11.38. Anal. (exact mass, HR-EIMS) Calcd 550.0555, found 550.0538.

16β-Fluoro-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (6). The 16α-bis-triflate epimer **5** (153 mg, 0.278 mmol) was dissolved in dry THF (2 mL). Tetrabutylammonium fluoride (1 M in THF, 0.27 mmol) was added, and the light red reaction solution was stirred at room temperature for 10 min. The solution was filtered through silica gel and the solvent was removed in vacuo. The residue was subjected to flash chromatography (15 mm × 6 in SiO_2, 20% EtOAc in hexane) to provide **6** (96 mg, 82%). A 54-mg sample was recrystallized from acetone–hexane to obtain an analytical sample (39 mg): mp 162–164 °C; NMR (220 MHz, $CDCl_3$) δ 1.05 (s, 3 H, 13-CH_3), 1.30–2.10 (m, 7 H), 2.17–2.44 (m, 3 H), 2.55–2.67 (m, 1 H), 2.93–2.99 (m, 2 H, benzylic), 4.77 (dt, 1 H, J = 50.2, 8.3 Hz, 16α-H), 7.01 (br s, 1 H, 4-H), 7.04 (dd, 1 H, J = 8.9, 3 Hz, 2-H), 7.33 (d, 1 H, J = 8.4 Hz, 1-H); ^{19}F NMR (338 MHz, $CDCl_3$) ϕ −73.44 s, −185.4 (dd, J = 50.02, 22.22 Hz); IR ($CHCl_3$) 1755 (ketone), 1490, 1420, 1225 cm^{-1}; mass spectrum (70 eV), m/z (relative intensity) 420 M^+ (31.4), 346 (45.0), 213 (100). Anal. Calcd for $C_{19}H_{20}F_4O_4S$: C, 54.29; H, 4.76; F, 18.10; S, 7.62. Found: C, 54.28; H, 4.90; F, 18.96; S, 7.48. Anal. (exact mass, HR-EIMS) Calcd 420.1018, found 420.1013.

16β-Fluoroestra-1,3,5(10)-triene-3,17β-diol (7). Fluoroestrone **6** (30 mg, 0.071 mmol) was dissolved in 5 mL of THF. Powdered $LiAlH_4$ (72 mg, 1.90 mmol) was added in a single portion, and after 20 min the reaction was quenched with EtOAc. The mixture was acidified with 6 N HCl, and then extracted with one portion of pentane and two portions of EtOAc. The combined organic layers were dried over Na_2SO_4; the solvent was removed and the residue crystallized from MeOH (16 mg, 78%). A small sample was purified by preparative HPLC (Whatman M9, 70% hexane, 28% CH_2Cl_2, 2% isopropyl alcohol, 5 mL/min, t_R = 12 min): mp 229–232 °C (lit.[14a] mp 220–223 °C); NMR (360 MHz, $CDCl_3$) δ 0.856 (s, 3 H, 18-CH_3), 1.00–2.40 (m, 12 H), 2.82–2.86 (m, 2 H, benzylic), 3.43–3.51 (ddd, 1 H, J = 20.4, 10.3, 6.3 Hz, 17α-H), 4.59 (br s, 1 H, phenol), 4.90–5.08 (dm, 1 H, J = 54 Hz, 16α-H), 6.56 (d, 1 H, J = 2.6 Hz, 4-H), 6.63 (dd, 1 H, J = 8.3, 2.7 Hz, 2-H), 7.15 (d, 1 H, J = 8.6 Hz, 1-H), ^{19}F NMR (338 MHz, Me_2SO) ϕ −180.8 (dddd, J = 54, 37, 23, 13 Hz); ($CDCl_3$) ϕ −186 m; IR (KBr) 3450 cm^{-1} (OH); mass spectrum (70 eV), m/z (relative intensity) 290 M^+ (8), 270 (100), 185 (45), 146 (50). Anal. (exact mass, HR-EIMS) Calcd for $C_{18}H_{23}FO_2$ 290.1682, found 290.1683.

3-[[(Trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (11). Estrone (1.5 g, 5.56 mmol) was dissolved in 3 mL of 2,6-lutidine and 30 mL of CH_2Cl_2 and cooled in an ice–H_2O bath. Triflic anhydride (1 mL, 5.59 mmol) was added via syringe, and the resulting red reaction mixture was stirred at 0 °C. After 30 min the reaction was quenched with water, the layers were separated, and the organic phase was washed with water and three portions of aqueous $CuSO_4$. Following a wash of the organic portion with brine and drying (Na_2SO_4), the organic phase was passed through a short silica gel pad. The solvent was removed and the residue subjected to flash chromatography (SiO_2, 30% EtOAc in hexane). Estrone triflate, **11**, was obtained in 91% yield (2.037 g) as a crystalline solid. Recrystallization was accomplished from methanol after seeding (1.7 g, 76%): mp 87–88 °C; NMR (90 MHz, $CDCl_3$) δ 0.90 (s, 3 H, 18-CH_3), 1.40–3.05 (m, 15 H), 6.95 (br s, 1 H, 4-H), 7.00 (dd, 1 H, J = 9, 2 Hz, 2-H), 7.32 (d, 1 H, J = 9 Hz, 1-H); ^{19}F NMR (84.6 MHz, $CDCl_3$) ϕ −73 s; IR (KBr) 1740 (ketone), 1490, 1420, 1250, 1210 cm^{-1}; mass spectrum (70 eV), m/z (relative intensity) 402 M^+ (100), 358 (48.1), 345 (36.8), 292 (18.1), 251 (34.8), 213 (64.7). Anal. Calcd for $C_{19}H_{21}F_3O_4S$: C, 56.72; H, 5.22; F, 14.18; S, 7.96. Found: C, 56.89; H, 5.03; F, 14.14; S, 7.73.

17-Acetoxy-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10),16-tetraene (12). Estrone triflate, **11** (1.061 g, 2.64 mmol), was dissolved in 20 mL of isopropenyl acetate. Toluenesulfonic acid (200 mg, catalytic) was added and the solution was heated to reflux. After 4 h, the solution was distilled to about one-third the original volume over 1 h and was then stirred at 25 °C for 7 h. The solution was then poured into H_2O and

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extracted with two portions of ether. The ether was washed the brine, dried (Na_2SO_4), and filtered through Al_2O_3. The solvent was removed and the residue was subjected to flash chromatography (SiO_2, 20% EtOAc in hexane) to provide the enol acetate **12** (828 mg, 70.6%) as an oil and starting ketone **11** (121 mg, 11.4%). **12**: NMR (90 MHz, $CDCl_3$) δ 0.90 (s, 3 H, 18-CH_3), 1.40–3.00 (m, 13 H), 5.47 (dd, 1 H, J = 3, 2 Hz, 16-H), 6.92 (br s, 1 H, 4-H), 6.96 (dd, 1 H, J = 9, 3 Hz, 2-H), 7.28 (d, 1 H, J = 9 Hz, 1-H); IR ($CHCl_3$) 1750 (C=O, ester), 1490, 1425, 1230, 1140 cm^{-1}; mass spectrum (70 eV), m/z (relative intensity), 444 M^+ (4.8), 402 (38.0), 387 (29.6), 83 (11.8), 43 (100). Anal. Calcd for $C_{21}H_{23}F_3O_5S$: C, 56.76; H, 5.18; F, 12.84; S, 7.20. Found: C, 57.17; H, 5.37; F, 13.03; S, 6.83.

16β-(Trifluoroacetoxy)-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (13). An oven-dried flask was placed under a dry N_2 atmosphere and charged with [bis(trifluoroacetoxy)iodo]benzene[10] (502 mg, 1.17 mmol). The solid was dissolved in 10 mL of dry CH_3CN (distilled from CaH_2 and stored under N_2 in an Aldrich Sure Seal bottle). Enol acetate **12** (496 mg, 1.12 mmol) dissolved in 3 mL of CH_2Cl_2 was added. The reaction mixture was stirred at room temperature until TLC indicated complete consumption of starting material (about 8–12 h). The solvent was removed in vacuo, and the residue was subjected to flash chromatography (20% EtOAc/hexane, 30-mm column, silica gel dried prior to use 120 °C, 380 T, 24 h) to yield **13** (373 mg, 64.8%) as a colorless oil which solidified on standing. A 39-mg sample was recrystallized from absolute ethanol to yield 30 mg of analytical material (seeding was required): mp 118–121 °C; ^1H NMR (90 MHz, $CDCl_3$) δ 1.08 (s, 3 H, 18-CH_3), 1.30–3.15 (m, 13 H), 5.15–5.37 (t, 1 H, J = 9 Hz, 16α-H), 7.0 (br s, 1 H, 4-H), 7.07 (dd, 1 H, J = 9, 3 Hz, 2-H), 7.35 (d, 1 H, J = 9 Hz, 1-H); ^{19}F NMR (84.6 MHz, $CDCl_3$) ϕ −73.5 s, −78.1 s; IR ($CHCl_3$) 1795 (CF_3CO_2), 1760 (ketone), 1490, 1422, 1250 cm^{-1}; mass spectrum (70 eV), m/z (relative intensity) 514 M^+ (20.5), 346 (43.5), 213 (100). Anal. Calcd for $C_{21}H_{20}F_6O_6S$: C, 49.03; H, 3.89; F, 22.18. Found: C, 48.72; H, 3.89; F. 22.42.

16β-Hydroxy-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (14). The chromatographed trifluoroacetate **13** (188 mg, 0.366 mmol) was dissolved in 6 mL of 10% aqueous ethanol and 3 mL of THF and stirred at room temperature for 11 h. The solvent was removed under reduced pressure, and the residue was subjected to flash chromatography (40% EtOAc/hexane) to yield the 16β-alcohol (105 mg, 69%). An analytical sample was crystallized from ethanol: mp 147–151 °C: NMR (220 MHz, $CDCl_3$) δ 1.00 (s, 3 H, 18-CH_3), 1.40–1.70 (m, 6 H), 2.01–2.09 (m, 2 H), 2.33–2.57 (m, 3 H), 2.67–2.68 (d, 1 H, J = 2.5 Hz), 2.93–2.99 (m, 2 H), 3.99–4.07 (dt, 1 H, J = 8.7, 2.5 Hz, 16-H), 7.00 (br s, 1 H, 4-H), 7.13 (br d, 1 H, J = 9 Hz, 2-H), 7.32 (d, 1 H, J = 8.8 Hz, 1-H); IR (KBr) 1740 (ketone), 1490, 1420, 1250, 1220, 1140 cm^{-1}; mass spectrum (70 eV), m/z (relative intensity) 418 M^+ (57.5), 345 (100), 213 (89.0). Anal. Calcd for $C_{19}H_{21}F_3O_5S$: C, 54.55; H, 5.02. Found: C, 54.71; H, 5.21. Anal. (exact mass, HR–EIMS) Calcd 418.1061, found 418.1069.

3,16β-Bis[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (15). Ketol **14** (68 mg, 0.163 mmol) was dissolved in CH_2Cl_2 (2 mL) and cooled in an ice/H_2O bath. Triflic anhydride (41 μL, 0.244 mmol) was added followed by 2,6-lutidine (28 μL, 0.247 mmol). After 15 min the reaction was passed through neutral alumina, the solvent removed in vacuo at room temperature, and the residue subjected to flash chromatography (15 mm × 6 in. SiO_2 30% EtOAc in hexane) to yield **15** as a white foam (54 mg, 60%). Crystallization was induced from EtOAc–hexane by seeding (44 mg, 49%): mp 129–130 °C; NMR (220 MHz, $CDCl_3$) δ 1.06 (s, 3 H, 18-CH_3), 1.45–1.75 (m, 6 H), 1.85–2.15 (m, 2 H), 2.25–2.55 (m, 2 H), 2.65–2.85 (m, 1 H), 2.90–3.00 (m, 2 H), 5.00 (t, 1 H, J = 8.6 Hz, 16α-H), 7.01 (br s, 1 H, 4-H), 7.04 (d, 1 H, J = 9 Hz, 2-H), 7.33 (d, 1 H, J = 9 Hz, 1-H); ^{19}F NMR (84.6 MHz, $CDCl_3$) ϕ −72.9 s, −74.2 s; IR ($CHCl_3$) 1765 (ketone), 1490, 1420 cm^{-1}; mass spectrum (70 eV), m/z (relative intensity) 550 M^+ (28.03), 345 (100), 213 (60.40). Anal. (exact mass, HR–EIMS) Calcd for $C_{20}H_{20}F_6O_7S_2$ 550.0555, found 550.0565.

16α-Fluoro-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (16). The 16β-bis-triflate epimer **15** (74 mg, 0.135 mmol) was dissolved in 200 μL of dry THF. n-Bu_4NF (1 M in THF, 125 μL, 0.125 mmol) was added, and the red reaction solution was stirred at room temperature for 30 min. The solvent was removed in vacuo and the residue subjected to flash chromatography (15 mm column, 20% EtOAc/hexane), yielding 53 mg (100%) of **6a** as an oil. Trituration with ether and recrystallization from acetone–hexane gave **16** as needles (32.4 mg, 62%): mp 172–175 °C; NMR (220 MHz, $CDCl_3$) δ 0.974 (s, 3 H, 18-CH_3), 1.46–2.47 (m, 11 H), 2.94–2.97 (m, 2 H), 5.15 (dd, 1 H, J = 51, 7 Hz, 16β-H), 7.00 (s, 1 H, 4-H), 7.03 (dd, 1 H, J = 9, 2 Hz, 2-H), 7.34 (d, 1 H, J = 9 Hz, 1-H); ^{19}F NMR (338 MHz, $CDCl_3$) ϕ −73.45 s, −193 (ddd, J = 56.4, 28.8, 27.3 Hz, 16α-F); IR ($CHCl_3$) 1760 (ketone), 1490, 1420 cm^{-1}; mass spectrum (70 eV), m/z (relative intensity) 420 M^+ (29.8), 346 (42.6), 213 (100). Anal. (exact mass, HR–EIMS) Calcd for $C_{19}H_{20}F_4O_4S$ 420.1018, found 420.1006.

16α-Fluoroestra-1,3,5(10)-triene-3,17β-diol (17) and 16α-Fluoroestra-1,3,5(10)-triene-3,17α-diol (18). The 16α-fluoro ketone **16** (40 mg, 95 μmol) was dissolved in dry ether (8 mL) and cooled in a CO_2/isopropyl alcohol bath under N_2. $LiAlH_4$ (1.2 M ether, 1200 μmol) was added and the mixture was stirred for 10 min and warmed to room temperature over 10 min. The reaction was then quenched with ethyl acetate, acidified with 6 N HCl, and extracted with two portions EtOAc. The organic phase was dried through a column of Na_2SO_4. The solvent was removed in vacuo, and the products were separated by preparative HPLC (Whatman M9,70% hexane, 28% CH_2Cl_2, 2% IPA; 5 mL/min; **16**:**17** by integration 3.5:1) to yield **17** (18 mg, 65% t_R = 20 min) and **18** (4 mg, 14%, t_R = 11 min).

17: mp 183–187 °C; NMR (360 MHz, $CDCl_3$) δ 0.795 (s, 3 H, 18-CH_3), 1.25–2.32 (m, 12 H), 2.80–2.83 (m, 2 H), 3.86 (br d, 1 H, J = 28.2 Hz, 17α-H), 4.74 (br s, 1 H, OH), 4.96 (dm, 1 H, J = 46 Hz, 16β-H), 6.56 (d, 1 H, J = 2.5 Hz, 4-H), 6.63 (dd, 1 H, J = 8.3, 2.6 Hz, 2-H), 7.14 (d, 1 H, J = 4 Hz, 1-H); ^{19}F NMR (338 MHz, $CDCl_3$) ϕ −180.35 (dq, J = 54, 28 Hz); IR (KBr) 3450 cm^{-1} (OH); mass spectrum (70 eV), m/z (relative intensity) 290 M^+ (100), 270 (8.3), 185 (6.8), 146 (30.8). Anal. Calcd for $C_{18}H_{23}FO_2$: C, 74.48; H, 7.93; F, 6.55. Found: C, 74.64; H, 8.06; F. 6.44. Anal. (exact mass, HR–EIMS) Calcd 290.1682, found 290.1683.

18: mp 214–221 °C; NMR (360 MHz, $CDCl_3$) δ 0.715 (s, 3 H, 18-CH_3), 1.25–2.37 (m, 12 H), 2.80–2.84 (m, 2 H), 3.85–3.87 (dd, 1 H, J = 4.9, 1.7 Hz, 17α-H), 4.52 (br s, 1 H, OH), 5.28 (dm, 1 H, J = 52 Hz, 16β-H), 6.56 (d, 1 H, J = 2.4 Hz, 4-H), 6.63 (dd, 1 H, J = 8.4, 2.6 Hz, 2-H), 7.16 (d, 1 H, J = 8.4 Hz, 1-H); ^{19}F NMR (338 MHz, Me_2SO) ϕ −194 (ddd, J = 51, 29, 20 Hz); ($CDCl_3$–D_2O) ϕ −195 (ddd, J = 53, 29, 24 Hz); IR (KBr) 3410 (OH), 2915, 1610, 1500, 1450 cm^{-1}; mass spectrum (70 eV), m/z (relative intensity) 290 M^+ (100), 262 (8), 213 (26), 172 (34), 157 (32), 146 (36). Anal. (exact mass, HR–EIMS) Calcd for $C_{19}H_{23}O_2F$ 290.1682, found 290.1684.

17α-Ethynyl-16β-fluoroestra-1,3,5(10)-triene-3,17β-diol (19). (Trimethylsilyl)acetylene (27.4 mg, 29 μL, 0.28 mmol) was dissolved in pentane (1 mL), placed under N_2, and cooled in an ice/H_2O bath. Butyllithium (1.9 M in hexane, 147 μL, 0.28 mmol) was added dropwise followed by 100 μL of dry THF to dissolve the white precipitate. 16β-Fluorestrone **6** (33 mg, 0.079 mmol) in 400 μL of THF was added to the cool lithium (trimethylsilyl)acetylide solution. The transfer syringe and vessel containing the substrate were rinsed with another 300-μL portion of THF and this also was added. After 5 min TLC analysis indicated consumption of starting material. The reaction was quenched with aqueous NH_4Cl and the mixture extracted with one portion of pentane and two portions of ether. The combined organics were washed with brine and dried over Na_2SO_4 and the solvent removed in vacuo to provide 41 mg (100%) of the intermediate silylacetylene.

This intermediate was dissolved in MeOH (600 μL). KOH (140 μL, 5 N) was added and the solution heated at 65 °C for 1 h. The reaction was quenched with NH_4Cl(aq) and extracted with EtOAc. After the extracts were dried (Na_2SO_4) and the solvent removed, the residue was purified by preparative TLC to yield 21 mg of **19**. Crystallization from acetone/hexane gave **19** as cubes (15 mg, 61%): mp 76–80 °C; NMR (220 MHz, $CDCl_3$) δ 0.92 (s, 3 H, 18-CH_3), 1.25–2.57 (m, 11 H), 2.61 (s, 1 H, ≡CH), 2.65 (d, 1 H, J = 7 Hz, 17-OH, disappears after D_2O exchange), 2.82–2.86 (m, 2 H), 4.56 (s, 1 H, 1-OH), 4.9 (ddd, 1 H, J = 53, 8, 2 Hz, 16α-H), 6.57 (br s, 1 H, 4-H), 6.64 (dd, 1 H, J = 9, 3 Hz, 2-H), 7.16 (d, 1 H, J = 8 Hz, 1-H); ^{19}F NMR (338 MHz, $CDCl_3$) ϕ −168.8 (dddd, J = 55.3, 41.3, 13.1, 7.1 Hz); IR ($CHCl_3$) 3590 (OH), 3300 (≡CH) cm^{-1}; mass spectrum (70 eV), m/z (relative intensity) 314 M^+

(46.8), 213 (100). Anal. (exact mass, HR-EIMS) Calcd for $C_{20}H_{23}FO_2$ 314.1682, found 314.1677.

17β-Ethynyl-16α-fluoroestra-1,3,5(10)-triene-3,17α-diol (20) and 17α-Ethynyl-16α-fluoroestra-1,3,5,(10)-triene-3,17β-diol (21). Butyllithium (1.9 M in hexane, 180 μmol) was added to a solution of (trimethylsilyl)acetylene (25 μL, 180 μmol) in pentane at 0 °C. Tetrahydrofuran (100 μL) was added to bring the acetylide into solution. Fluoro ketone **16** (26 mg, 62 μmol) dissolved in THF (0.5 mL) was added, and after 5 min TLC analysis indicated consumption of starting material was complete. The reaction was quenched with dilute aqueous NH_4Cl and extracted once with pentane and once with EtOAc. The organic phase was dried over Na_2SO_4, the solvent removed in vacuo, and the residue dissolved in MeOH (0.5 mL). Three drops of 5 N KOH were added. The solution was heated at 60 °C for 30 min, quenched with aqueous NH_4Cl, and extracted with EtOAc. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure, and the residue was subjected to flash chromatography (15 mm × 6 in. SiO_2, 30% EtOAc in hexane). Two products were formed (R_f 0.34 and 0.17). Each fraction was further purified by preparative HPLC (Whatman M9, 5 mL/min, 90% hexane, 8.7% CH_2Cl_2, 1.3% *i*-PrOH) to yield **20** (5 mg, 26%, t_R = 16 min) and **21** (5 mg, 26%, t_R = 28 min).

20: mp (powder) 183-185 °C; NMR (220 MHz, CD_2Cl_2) δ 0.91 (s, 3 H, 18-CH_3), 1.26-2.37 (m, 11 H), 2.56 (s, 1 H, ≡CH), 2.77-2.80 (m, 3 H), 4.89-4.90 (br s, 1 H, OH), 5.26 (ddd, 1 H, J = 51.5, 8.4, 3.1 Hz, 16β-H), 6.53 (d, 1 H, J = 2.1 Hz, 4-H), 6.60 (dd, 1 H, J = 8.5, 2.6 Hz, 2-H), 7.13 (d, 1 H, J = 8.3 Hz, 1-H); ^{19}F NMR (338 MHz, CD_2Cl_2) φ -194.2 (dddd, J = 58.64, 29.08, 21.16, 7.53 Hz); mass spectrum (70 eV), m/z (relative intensity) 314 M^+ (54.6), 288 (38.5), 213 (100). Anal. (exact mass, HR-EIMS) Calcd for $C_{20}H_{23}FO_2$ 314.1682, found 314.1679.

21: mp (powder) 186-192 °C; NMR (220 MHz, CD_2Cl_2) δ 0.89 (s, 3 H, 18-CH_3), 1.35-2.13 (m, 12 H), 2.77 (s, 1 H, ≡CH), 2.78-2.81 (m, 2 H), 4.83 (br s, 1 H, OH), 5.10 (ddd, 1 H, J = 58.9, 32.3, 20.3 Hz, 16β-H), 6.54 (br s, 1 H, 4-H), 6.59 (dd, 1 H, J = 8.3, 2.8 Hz, 2-H), 7.13 (d, 1 H, J = 8.4 Hz, 1-H); ^{19}F NMR (338 MHz, CD_2Cl_2) φ -185.8 (ddd, J = 53.6, 29.0, 18.1 Hz); mass spectrum (70 eV), m/z (relative intensity) 314 M^+ (46.7), 288 (22.5), 213 (100). Anal. (exact mass, HR-EIMS) Calcd for $C_{20}H_{23}FO_2$ 314.1682, found 314.1679.

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Registry No. **1**, 53-16-7; **2**, 20592-42-1; **3**, 1239-35-6; **4**, 566-76-7; **5**, 92844-09-2; **6**, 92817-03-3; **6** (silylacetylene deriv), 92817-13-5; **7**, 84693-92-5; **11**, 92817-04-4; **12**, 92817-05-5; **13**, 92817-06-6; **14**, 92817-07-7; **15**, 92817-08-8; **16**, 92817-09-9; **17**, 92817-10-2; **18**, 92817-11-3; **19**, 92817-12-4; **20**, 92935-39-2; **21**, 92817-14-6; $(CH_3)_3SiC{\equiv}CH$, 1066-54-2; 16β-chloroestrone triflate, 92817-15-7; 16β-bromoestrone acetate, 65912-80-3; 16α-chloroestrone triflate, 92817-16-8; 16α-bromoestrone acetate, 1239-35-6; 16α-iodoestrone triflate, 92817-17-9.

Supplementary Material Available: NMR studies of 16-fluoroestrogen stereochemistry (10 pages). Ordering information is given on any current masthead page.

Improvements in Oligodeoxyribonucleotide Synthesis: Methyl *N,N*-Dialkylphosphoramidite Dimer Units for Solid Support Phosphite Methodology

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Two procedures for the synthesis of methyl *N,N*-dialkylphosphoramidite dinucleotides (dimer units) compatible with the current solid support phosphite methodology of oligodeoxynucleotide synthesis are described for the first time. In the first procedure a condensation is performed between the 3'-methyl *N,N*-dialkylphosphoramidite moiety of a fully protected nucleotide and the 5'-hydroxyl of a 3'-*O*-levulinyl base protected nucleoside. The phosphite triester internucleotide bond of the resulting dimer is oxidized to the phosphotriester, 3'-*O*-levulinyl is selectively cleaved, and the 3'-hydroxyl of the product is derivatized to methyl *N,N*-dialkylphosphoramidite to give a dimer unit usable in the conventional phosphite methodology of oligonucleotide synthesis. For synthesis of some of the dimer units, especially those containing a 3'-cytidine residue, 3'-*O*-*tert*-butyldimethylsilyl protection in place of the 3'-*O*-levulinyl group was found to be advantageous. Using these dimer phosphite units, the synthesis of several oligonucleotides ranging in size from 16 to 19 units is described. The efficiencies of condensation of the dinucleotide units (98-100%) are quite comparable to the monomer condensations.

Introduction

Chemical synthesis of oligonucleotides has undergone revolutionary developments during the last decade. Although fundamental studies in the synthesis of oligonucleotides were carried out by the classical diester methodology,[1] the development of the triester technique[2,3] in conjunction with the highly efficient and rapid high-performance liquid chromatographic (HPLC) techniques for the purification of oligomeric products added an element of speed to the method. Finally, the use of solid support in the triester method of synthesis[3] accelerated the speed of synthesis dramatically and it became practical

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