Steroid Conjugates. VII. Preparation of *N*-Acetylglucosaminides of 17α- and 17β-Estradiol*

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ABSTRACT: Condensation of 17β-estradiol 3-benzyl ether (I) with 1α-bromo-*N*-trifluoroacetamido tri-*O*-acetylglucopyranoside (Xb) in the presence of cadmium carbonate gave the corresponding 17β-estradiol 17-(α- and β-)glycosides (IIb, III). Selective deacylation and reacetylation followed by debenzylation afforded the 17β-estradiol 17-(α- and β-)*N*-acetylglucosaminides. The same synthetic sequence perfomed on 17α-estradiol 3-benzyl ether (XII) gave 17α-estradiol 17-β-*N*-acetylglucosaminide (XVI), identical with the compound derived from a natural source. The physical characteristics of the anomeric estradiol *N*-acetylglucosaminides have been determined, and supported the assigned structures.

The isolation of 17α-estradiol 3-glucuronide 17β-acetylglucosaminide from rabbit urine by Layne and coworkers (1964) revealed a new conjugating pathway for steroids. In a subsequent series of papers, Layne and coworkers (Jirku and Layne, 1965; Layne *et al.*, 1965; Layne, 1965; Collins *et al.*, 1967, 1968) studied C-17-glucosaminide formation by the action of an *N*-acetylglucosaminyl-transfer enzyme system and showed that the stereochemical configuration of the 17α-hydroxyl group was a necessary structural feature for estrogens containing a C-3-glucuronide.

Recently, other double conjugates containing a *N*-acetylglucosaminide grouping have been isolated from human urine. These include pregn-5-ene-3β,20α-diol 3-sulfate 20-*N*-acetylglucosaminide (Arcos and Lieberman, 1967), and the 15-*N*-acetylglucosaminides of 15α-hydroxyestrone sulfate and 15α-hydroxyestradiol sulfate (Jirku and Levitz, 1969, 1970).

The first chemical synthesis of a steroidal glucosaminide, 11β,17α-dihydroxy-3,20-dioxopregna-1,4-dien-21-yl 2′-acetamido-2′-deoxy-β-D-glucopyranoside, was reported by Hirschmann and coworkers (1964). Subsequently, the synthesis of the α and β anomers of 3β-hydroxy-5α-pregnan-20-α-yl 2′-acetamido-2′-deoxy-D-glucopyranoside were described by Matsui and Fukushima (1969). As a result of these studies, the structure of the Arcos and Lieberman (1969) compound noted above was established as containing the *N*-acetylglucosaminide moiety as a β-glycoside.

The studies by Sauer and coworkers (1969) and Fukushima and Matsui (1969) on the physical characteristics of a series of synthetically prepared C-19-steroid *N*-acetylglucosaminides have contributed greatly to the clarification of the stereochemistry of steroidal glucosaminides.

We wish to report here synthetic work on estrogen C-17-*N*-acetylglucosaminides which has resulted in, among other things, establishing unequivocally the structure of 17α-estradiol 17-β-*N*-acetylglucosaminide previously derived from a biological source as described by Layne *et al.* (1964).

For preparing these compounds, a Koenigs–Knorr synthesis utilizing the amino sugar, 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (Xa, Baker and coworkers, 1954) was considered with the use of cadmium carbonate as condensing agent.[1]

The condensation of 1α-chloro-*N*-acetylglucosamine (Xa) (Scheme I) with 17β-estradiol 3-benzyl ether (I) in the presence of cadmium carbonate gave less than 10% of the blocked estradiol aminoglycoside (IIa). This low yield was attributed to the possibility that the 17β-hydroxyl group was sterically hindered, and under the conditions of the reaction, the chloro sugar, Xa, underwent decomposition and preferential internal condensation to an oxazoline or an oxazolidene (Micheel and coworkers, 1955, 1959).

Removal of the *O*-acetyl groups on the glycoside IIa gave the crystalline *N*-acetamidobenzyl ether, Vb. This in turn could be debenzylated to provide 17β-estradiol 17-β-*N*-acetylglucosaminide (VIa).

Subsequently, to avoid the undesirable internal ring closures resulting from the *N*-acetyl group as mentioned above, there was employed a differently substituted aminoglycoside, 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl bromide (Xb, Strachan and coworkers, 1966). When Xb was similarly reacted with 17β-estradiol 3-benzyl ether (I) the trifluoroacetamidobenzyl ether, IIb, was formed in a 55% yield, together with a small amount (6%) of the α anomer, III.[2]

An additional advantage in the use of trifluoroacetyl as an *N*-protective group in amino sugar synthesis has been its lability under basic conditions (Newman, 1965; Wolfrom and Bhat, 1966). For our purposes, by employing ammonia in methanol (overnight 0–5°)[3] the *O*-acetylglycoside groups

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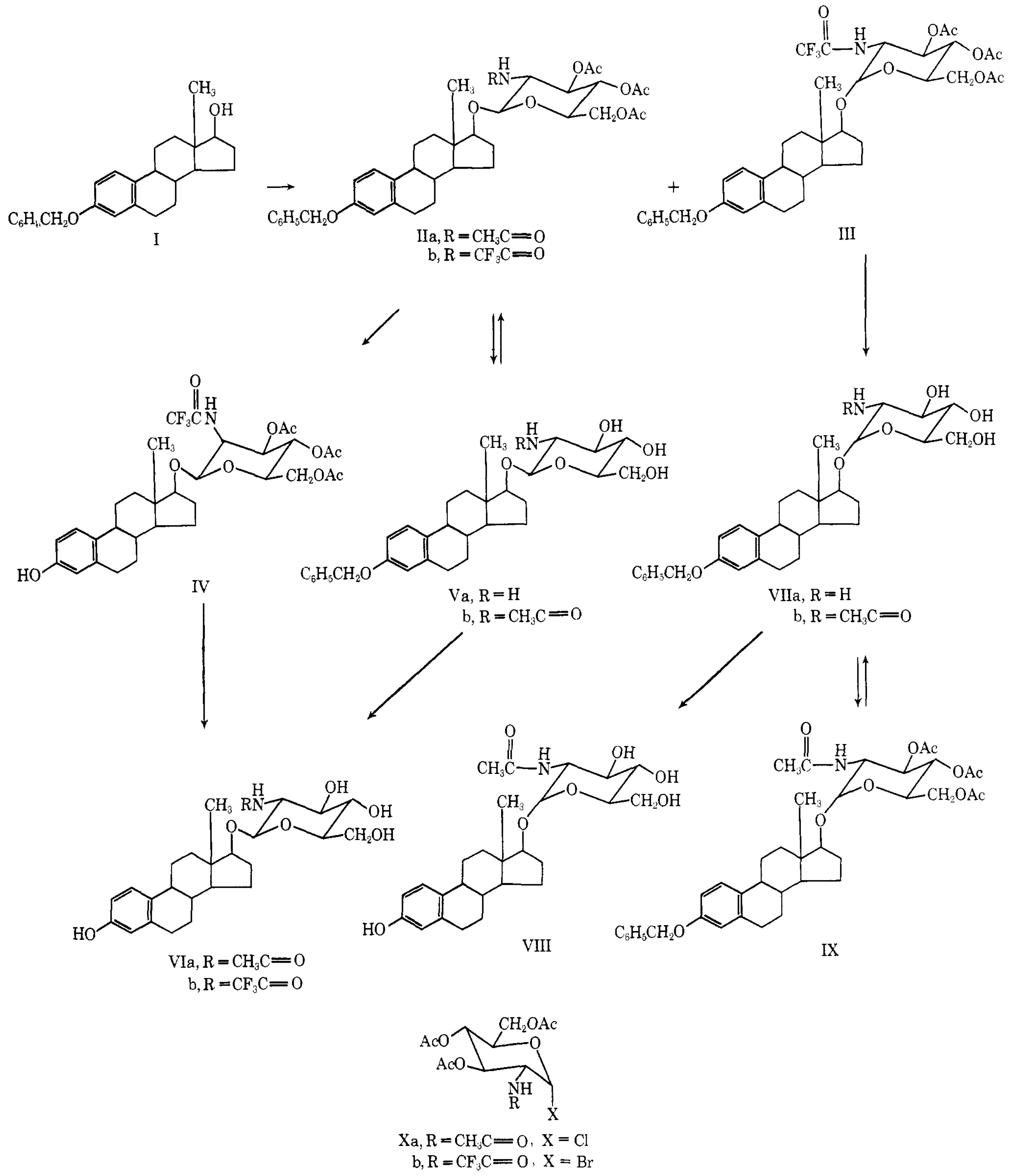
[1] When this work was initiated, extensive research had been completed in this laboratory on the use of cadmium carbonate in the Koenigs–Knorr reaction (Conrow and Bernstein, 1971). This reagent had been shown to be uniquely successful in improving the yields of estrogen C-3-glucuronidation from less than 10% to over 70%. Usually, Koenigs–Knorr (1901) condensations have been performed in the presence of silver carbonate, silver oxide, *etc.* In a recent publication by Sauer *et al.* (1969) salts such as mercuric chloride and mercuric cyanide have been used. For a discussion of these agents, see Chapter 1 by Bernstein *et al.* (1970).

[2] Meyer zu Rechendorf and coworkers (1970a,b) have reported that the trifluoroacetyl group gave them exclusively β glycosidation in the preparation of disaccharides.

[3] Wolfrom and Conigliaro (1969) have reported the complete removal of all protective groups for 6-benzamido-9-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α-(and β)-D-glucopyranosylpurine was achieved by treatment with methanolic ammonia at room temperature for 1 week.

SCHEME I

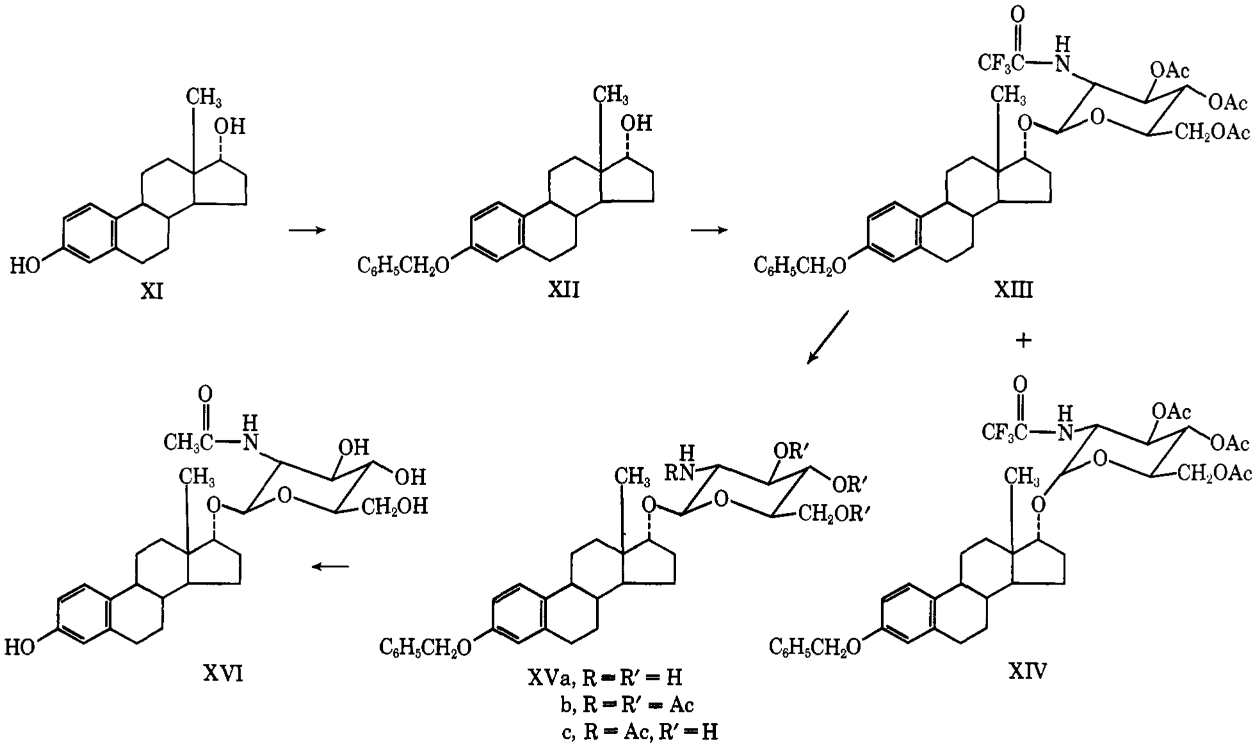


could be removed selectively, whereas stronger basic conditions (aqueous or methanolic alkaline hydroxide) gave complete O and N deacylation (Strachan and coworkers, 1966).

Thus the trifluoroacetamidoglycoside IIb after debenzylation was selectively de-O-acetylated in cold ammonia to give the *N*-trifluoroacetylglycoside, VIb. When complete deacylation of IIb occurred in 1 N methanolic potassium hydroxide, the completely deblocked aminoglycoside Va was readily obtained (the 3-benzyl ether group was retained here to permit organic solvent solubility). Acetylation of Va followed by de-O-acetylation and debenzylation through the sequence Va → IIa → VI → VIa, gave the previously mentioned VIa. Although this sequence was more indirect than the previously described route (I → IIa → VI → VIa) the improved yield of IIb over IIa made it a more practical pathway.

An additional advantage to the trifluoroacetyl route was

Scheme II



the formation of the α anomer III. The structural assignment for III was based on elemental analysis, rotation, and nuclear magnetic resonance data, which will be discussed below.

Using the same pathway as described for the β anomer, the α anomer III was completely deacylated to VIIa, acetylated (IX), de-O-acetylated (VIIb), and finally debenzylated to provide 17β-estradiol 17-α-*N*-acetylglucosaminide (VIII).

The above synthesis developed for 17β-estradiol served as a model for similar chemical sequences in the 17α-estradiol series. When 17α-estradiol 3-benzyl ether (XII) (Scheme II) was condensed with the amino sugar Xb, there was obtained, after chromatographic separation, a 43% yield of the β-glucosaminide XIII, and a 2% yield of the α anomer XIV. Unfortunately there was only sufficient material for characterization of α anomer XIV and further transformations were not possible.

In the usual manner deacylation of β anomer XIII gave the aminoglycoside XVa which was converted into the *N*-acetyl derivative, XVc, either by selective N acetylation, or, more conveniently, by O,N acetylation followed by O deacetylation (XIII → XVa → XVb → XVc). When XVc was debenzylated, 17α-estradiol 17-β-*N*-acetylglucosaminide (XVI) was formed, and was identical (infrared, ultraviolet, nuclear magnetic resonance, thin-layer chromatography, and $[\alpha]_D$) with a sample of material obtained from Dr. D. S. Layne (*op. cit.*).

While only three (VIa, VIII, XVI) of the four stereochemically possible estradiol *N*-acetylglucosaminides were prepared, all four (IIb, III, XIII, and XIV) were obtained as their *N*-trifluoroacetyltri-*O*-acetyl derivatives.

The structural assignment given to the glycoside anomers was based on rotational and nuclear magentic resonance data. Sauer *et al.* (1969) prepared a series of C-19 steroid α- and β-peracetylated glucosaminides, and calculated the molecular rotation differences in the anomeric pairs. The values of the estradiol anomers described herein are in agreement with their published data, the α anomer having a very positive rotation value, and the $[M]_D$ (α–β) having a range of +400 to +700 (see Table I). Certain nuclear magnetic resonance chemical shifts and coupling constant values for the $C_1'H$ which show a difference between the axial proton of the β anomer and the equatorial proton of the α anomer, are also given in this paper (Sauer *et al.*, 1969). Here also our results were in agreement with the published data (see Table II). The δ value for the β anomer was slightly lower than that for the α anomer, and the coupling constant, $J_{1,2}$, for the α anomer (equatorial relationship) was less than one-half that of its β counterpart as anticipated.

With regard to the nuclear magnetic resonance analysis, it is interesting to note that while integration revealed the same number of acetate protons in all fully blocked trifluoroacetyl anomers only two signals were observed for the β anomers (IIb and XIII), while the α anomers (III and XIV) exhibited three signals in the same region.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Optical rotations were determined at 25° on a Perkin-Elmer 141 polarimeter in the solvents stated. The ultraviolet spectra were determined in methanol in a Carey 11 spectrophotometer. The infrared spectra are for a pressed potassium bromide disk, and were determined on a Perkin-Elmer 21 spectrophotometer. Nuclear magnetic resonance data were obtained on a Varian A-60 or HA-100 instrument as indicated using tetramethylsilane as in internal indicator in the solvents noted. Mass spectroscopy data were made on an AE 1 MS-9 instrument. Celite is a diatomaceous earth supplied by Johns-Manville Co. Magnesol is a hydrous magnesium silicate supplied by Waverly Chemical Corp. Thin-layer chromatography was carried out on silica gel F

TABLE I: Molecular Rotation Differences of Estradiol *N*-Acylglucosaminides.

Compound	$[\alpha]_D^{25}$ (deg)	Mol Rotation, $[M]_D$			$\Delta[M]_D$		
		Aglycone	α	β	α-Aglycone	β-Aglycone	α–β
17β-Estradiol 3-benzyl ether (I)	+59^a	+213					
III	+87^a		+648		+435		+529
IIb	+61^a			+119		−94	
17β-Estradiol							
VIII	+80^b	+217					
VIa	+150^c		+710		+493		+520
	+40^c			+190		−27	
17α-Estradiol β-benzyl ether (XII)	+40^a	+145					
XIV	+52^a		+387		+242		+566
XIII	−24^a			−179		−324	
17α-Estradiol (XI)	+55^b	+149					
XVI	−11^c			−52		−201	

^{a} Chloroform. ^{b} Dioxane. ^{c} Ethanol.

plates (E. Merck, Darmstadt), supplied by Brinkmann Instruments Co., coated 0.25 mm thick, unless otherwise noted. After development the plates were usually viewed under a Mineralite UVS lamp and/or detected with 10% methanolic phosphomolybdic acid spray. The development systems used were (A) benzene–acetone–water (2:1:2), upper phase; (B) acetone–benzene–water (2:1:2), upper phase; (C) cyclohexane–ethyl acetate (1:1); (D) cyclohexane–ethyl acetate (7:3); and (E) system A–methanol (7:3). Partition chromatography was carried out on acid-washed Celite columns using the solvent system heptane–methanol saturated.

3-Benzyloxyestra-1,3,5-(10)-trien-17β-yl 2′-Acetamido-3′,-4′,6′-tri-O-acetyl-3′-deoxy-β-D-glucopyranoside (IIa). A. *Via* CADMIUM CARBONATE CONDENSATION. A well-stirred mixture of 17β-estradiol 3-benzyl ether (543 mg, I) and cadmium carbonate (519 mg) in benzene (90 ml) was distilled until 15 ml of benzene was removed, when 1α-chloro-1-deoxy-2-acetamido-2-deoxy-3,4,6-glucopyranose (1.0 g, Xa) was added. The reaction was stirred under reflux with constant water removal for 18 hr. After filtration of the hot mixture, the filtrate was evaporated *in vacuo*, and the residue was acetylated in pyridine (2 ml) with acetic anhydride (1 ml) on a steam bath for 2 hr. Ice water was added, and the resulting gum was collected, dissolved in methylene chloride, and dried over magnesium sulfate. After filtration and evaporation there remained a gum which gave IIa, (111 mg), mp 207–208° (9.5%), on crystallization when triturated with anhydrous ether. The material was recrystallized from acetone–hexane to give 70 mg, mp 224–225°; $[\alpha]_D$ +16° (chloroform); λ_{max}^{MeOH} 288 nm; nuclear magnetic resonance (60 MHz, $CDCl_3$) 45, s [3]; 116, 120, 124, t [9] cps.

TABLE II: Nuclear Magnetic Resonance of $C_1{}'$ Proton of Estradiol *N*-Acylglucosaminides.

Compound	δ, $H_1{}'$ ppm	$J_{1,2}$ (Hz)
III, α anomer	4.92 (d)^a	3.9
IIb, β anomer	4.68 (d)	8.2
XIV, α anomer	4.92 (d)	3.4
XIII, β anomer	4.59 (d)	8.0

^{a} d = doublet.

Anal. Calcd for $C_{39}H_{49}NO_{10}$ (691.79); C, 67.71; H, 7.14; N, 2.03. Found: C, 67.87; H, 7.26; N, 2.11.

B. *Via* ACETYLATION OF THE AMINOGLYCOSIDE (Va). 3-Benzyloxyestra-1,3,5-(10)-trien-17β-yl 2′-deoxy-β-D-glucopyranoside (47 mg, Va) was dissolved in pyridine (0.3 ml) with acetic anhydride (0.15 ml) and allowed to remain at room temperature for 18 hr. After the addition of water there was obtained a white solid (44 mg) whose ir spectrum was identical with material obtained by procedure A.

3-Benzyloxyestra-1,3,5(10)-trien-17β-yl 2′-Acetamido-2′-deoxy-β-D-glucopyranoside (Vb). Crude 3-benzyloxyestra-1,3,5(10)-trien-17β-yl 2′-acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranoside (930 mg, IIa) was gradually dissolved in methanol (100 ml) saturated at 0–5° with ammonia gas and allowed to remain at 0–5° for 48 hr. The solution was evaporated *in vacuo* to give a white crystalline solid (624 mg, Vb), mp 223–225° dec. Recrystallization from methanol did not alter the melting point; $[\alpha]_D$ +31° (methanol); λ_{max}^{MeOH} 280–290 nm (ϵ 225); nuclear magentic resonance (60 MHz, d_6 Me_2SO) 41, s [3]; 108, s [3]; 261, m; 301, s [2]; 441, s [75] cps.

Anal. Calcd for $C_{33}H_{43}NO_7$ (565.68): C, 70.06; H, 7.66; N, 2.47. Found: C, 69.78; H, 7.42; N, 2.43.

The direct N acetylation of Va with acetic anhydride in ethanol gave a mixture of two products, the desired acetamido compound Vb, and O,N-acetylated product, noted by infrared and thin-layer chromatography (system E).

3-Hydroxyestra-1,3,5(10)-trien-17β-yl 2′-Acetamido-2′-deoxy-β-D-glucopyranoside (VIa). 3-Benzyl 17β-estradiol 17-glucosaminide (423 mg, Vb) was dissolved in absolute ethanol (100 ml), and 10% palladium on charcoal (150 mg) was added. The mixture was hydrogenated at 40 psig for 4 hr at room temperature, and the catalyst was removed by filtration through Celite. Removal of solvent gave a crystalline solid (331 mg, VIa), mp 268–270° dec. Recrystallization from absolute ethanol–hexane gave mp 305–309° dec with previous shrinking: $[\alpha]_D$ +40° (absolute ethanol); λ_{max}^{MeOH} 280–290 nm (ϵ 230); nuclear magnetic resonance (60 MHz, d_6-Me_2SO) 40, s [3]; 108, s [3]; 533, s [1] cps.

Anal. Calcd for $C_{26}H_{37}NO_7$ (475.57): C, 65.67; H, 7.84; N, 2.94. Found: C, 65.28; H, 8.10; N, 2.78.

3-Benzyloxyestra-1,3,5(10)-trien-17β-yl 3′,4′,6′-Tri-O-acetyl-2′-deoxy-2′-trifluoroacetamido-β-D-glucopyranoside (IIb) and 3-Benzyloxyestra-1,3,5(10)-trien-17β-yl 3′,4′,-6′-Tri-O-acetyl-2′-deoxy-2′-trifluoroacetamido-α-D-glucopyranoside (III). A. To a solution of 17β-estradiol 3-benzyl ether (I, 905 mg) in toluene (150 ml), cadmium carbonate [865 mg (2 mole/mole of steroid)] was added, and the mixture was stirred while toluene (50 ml) was removed by distillation. There was then added 1α-bromo-1-deoxy-2-trifluoroacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranose [Xb, 2.3 g (2 mole/mole of steroid)] and the mixture was refluxed for 18 hr. After filtration and evaporation *in vacuo*, the crude residue was acetylated in pyridine (5 ml) with acetic anhydride (2.5 ml) on a steam bath for 2 hr. Ice water was added, and the semisolid mass was collected by filtration, washed with water, and then dissolved in methylene chloride, and passed through a pad of Magnesol. This was followed by several washings with methylene chloride. On evaporation there was left a gelatinous solid which on thin-layer chromatography (system D) showed three principal spots, one corresponding to the product (most polar), one to acetylated starting material (least polar), and one slightly less polar than the product (the α anomer of the glycoside). Trituration of the residue with ether gave a white crystalline product (IIb, 766 mg) (41%), mp 217–218°, which gave only one spot IIb on thin-layer chromatography analysis (system D): λ_{max}^{MeOH} 287 nm (ε 930); nuclear magnetic resonance (60 MHz, $CDCl_3$) 44.5, s [3]; 122, 125, d [9] cps.

Anal. Calcd for $C_{39}H_{46}FNO_{10}$ (745.76): C, 62.81; H, 6.21; F, 7.64; N, 1.85. Found: C, 63.11; H, 6.27; F, 7.80; N, 1.81.

B. 17β-Estradiol 3-benzyl ether (I, 3.0 g) and cadmium carbonate (2.9 g) were added to 350 ml of chlorobenzene, and 70 ml of the solvent was distilled. With stirring a solution of 1α-bromo-1-deoxy-2-trifluoroacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranose (4.2 g, Xb) in dry chlorobenzene (50 ml) was added while the reaction was under reflux. After 2.5 hr an additional 4.2 g of the bromo sugar in chlorobenzene (50 ml) was added and the reaction was continued for 2 more hr.

After removal of the inorganic material by filtration, the solution was evaporated *in vacuo* and the residue was acetylated in pyridine (10 ml) with acetic anhydride (5 ml) for 4 hr at room temperature. This was to make the starting material (3-benzyloxyestradiol) less polar, and more easily separable from the products in subsequent chromatography. The acetylated material was added to water, and the gum which separated was collected, dried in methylene chloride solution over magnesium sulfate, and passed through a filter pad of Magnesol. The Magnesol was washed with methylene chloride and the combined wash and filtrate were evaporated *in vacuo* to give a gum which partially crystallized providing 2.3 g of the β isomer (IIb), mp 215–218°. This appeared to be homogeneous on thin-layer chromatography (system D).

The residue (7.5 g) from the crystallization was submitted for partition chromatography on Celite (heptane-methanol and 415 mg of noncrystalline α isomer (III) (6.7%) was obtained with an additional 1.1 g of β isomer; total yield of β isomer (IIb) was 55%.

Further purification of a sample of α isomer was accomplished by preparative thin-layer chromatography (system D): $[\alpha]_D$ +87° (chloroform); λ_{max}^{MeOH} 287 nm (ε 900), nuclear magnetic resonance (100 Hz, $CDCl_3$), 74, s [3]; 202, 206 d [12]; 478, d [1]; 502, s [2]; 736, m [>5]; mass spectrum (70 eV) $[M]^+$ 745.

Anal. Calcd for $C_{39}H_{46}F_3NO_{10}$ (745.76): C, 62.81; H, 6.21; F, 7.64; N, 1.85. Found: C, 62.86; H, 6.13; F, 7.54; N, 1.81.

The β isomer (IIb) showed: $[\alpha]_D$ +16° (chloroform); nuclear magnetic resonance (100 Hz, $CDCl_3$) 83, s [3]; 198, 202, 206, t [12]; 494, d [1]; 498, s [2]; 736, m [>5]. A thin-layer chromatography of the α and β isomers (system D) showed the α isomer to be slightly less polar than the β isomer.

3-Hydroxyestra-1,3,5(10)-trien-17β-yl 3′,4′,6′-Tri-O-acetyl-2′-deoxy-2′-trifluoroacetamido-β-D-glucopyranoside (IV). 3-Benzyloxyestra-1,3,5(10)-trien-17β-yl 2′-trifluoroacetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl-β-D-glucopyranoside (IIb, 3.0 g) was suspended in glacial acetic acid (100 ml), and 10% palladium on charcoal (1 g) was added. The mixture was hydrogenated at 40 psig for 3 hr at room temperature. After the catalyst was removed by filtration through Celite, the filtrate was evaporated *in vacuo* to afford a white solid (IV, 2.6 g), mp 204–206°. Recrystallization of a sample from acetone–hexane to constant melting point gave 213–215°: $[\alpha]_D$ +8.5° (chloroform); λ_{max}^{MeOH} 288 nm; nuclear magnetic resonance (60 MHz, $CDCl_3$ + d_6-Me_2SO) 44, s [3]; 120, 122, 125, t [8] cps.

Anal. Calcd for $C_{32}H_{40}NO_{10}F_3$ (655.65): C, 58.62; H, 6.15; N, 2.14; F, 8.69. Found: C, 58.59; H, 6.20; N, 2.09; F, 8.52.

A thin-layer chromatography of this material (system C) gave a single spot, more polar than the starting material.

3-Hydroxyestra-1,3,5(10)-trien-17β-yl 2′-Deoxy-2′-trifluoroacetamido-β-D-glucopyranoside (VIb). 3-Hydroxyestra-1,3,-5(10)-trien-17β-yl 2′,3′,4′-tri-*O*-acetyl-2′-deoxy-2′-trifluoroacetamido-β-D-glucopyranoside (IV, 0.5 g) was dissolved in methanol (20 ml) saturated at 0–5° with ammonia, and allowed to remain at 0–5° for 18 hr protected from atmospheric moisture. The solvent and excess ammonia were removed *in vacuo* leaving a glass which was triturated with water to remove any acetamide present. There remained 371 mg of glass of which 213 mg was chromatographed on two 20 × 20 cm plates coated with silica gel G (0.5 mm thick) and developin system B. There appeared to be only one major material which was eluted from the silica with acetone, and evaporation gave VIb as a glass (162 mg). This material showed one spot on thin-layer chromatography (system B) but did not crystallize. It was dissolved in acetone, filtered through Magnesol, evaporated, and submitted as a glass for analysis: $[\alpha]_D$ +41° (methanol); λ_{max}^{MeOH} 280–290 nm; nuclear magentic resonance (60 MHz, d_6-Me_2SO) 38, s [3]; 124, s; 532, s [1]; 545, s [1] (exchangeable with CD_3OD) cps.

Anal. Calcd for $C_{26}H_{34}F_3NO_7 \cdot CH_3COCH_3$ (587.62); C, 59.18; H, 6.86; F, 9.70; N, 2.38. Found: C, 58.80; H, 6.64; F, 9.69; N, 2.56.

3-Benzyloxyestra-1,3,5(10)-trien-17β-yl 2′-Amino-2′-deoxy-β-D-glucopyranoside (Va). 3-Benzyloxyestra-1,3,5(10)-trien-17β-yl 3′,4′,6′-tri-*O*-acetyl-2′-trifluoroacetamido-β-D-glucopyranoside (2.3 g, IIb) was suspended in methanol (10 ml), and 1 N potassium hydroxide in methanol solution (50 ml) and water (10 ml) was added. Solution took place and then a new solid separated in approximately 15 min. After 3 hr at room temperature the deblocked glycoside Va was collected, washed with cold methanol, and dried, 1.3 g (81%), mp 215–219°. A sample of the above material was recrystallized from methanol to constant mp 238–240°. A thin-layer chromatography of the recrystallized and crude materials in system E showed only one spot: $[\alpha]_D$ −2° (pyridine); nuclear magnetic resonance (60 Hz, d_6-Me_2SO) 45, s [3]; 301, s [2]; 442, s [5].

Anal. Calcd for $C_{31}H_{41}NO_6$: C, 71.11; H, 7.89; N, 2.67. Found: C, 71.01; H, 7.85; N, 2.53.

A sample of the crude Va when recrystallized from an acetone–methanol solution formed the *N*-isopropylidene derivative, 3-benzyloxyestra-1,3,5(10)-trien-17β-yl 2′-deoxy-2′-isopropylideneamino-β-D-glucopyranoside: mp 233–234°; $[\alpha]_D$ ±0° (pyridine); nuclear magnetic resonance (60 Hz, d_6-Me_2SO) 37, s [3]; 108, s [3]; 115, s [3]; 302, s [2] cps.

Anal. Calcd for $C_{34}H_{45}NO_6$ (563.71): C, 72.44; H, 8.05; N, 2.48. Found: C, 72.45; H, 7.93; N, 2.41.

3-Benzyloxy-17α-hydroxyestra-1,3,5(50)-triene (*XII*). To a solution of 17α-estradiol (XI, 750 mg) in absolute ethanol (40 ml), pulverized anhydrous potassium carbonate (1.7 g) and benzyl chloride (1.5 ml) were added, and the mixture was refluxed for 3 hr. After the addition of water the reaction was concentrated *in vacuo*, and ice was added. The residue partially solidified and was collected by filtration through Celite. The gummy solid was dissolved in methylene chloride, and dried over magnesium sulfate, filtered, and evaporated *in vacuo* to give a gum. Crystallization from acetone–hexane gave XII (675 mg), mp 86–88°. A sample was recrystallized from hexane: mp 88–89°; $[\alpha]_D$ +40° (chloroform); λ_{max}^{MeOH} 280–290 nm (ε 180); nuclear magnetic resonance (60 Hz, $CDCl_3$) 41, s [3]; 227, d [1]; 301, s [2]; 440, m [>5] cps.

Anal. Calcd for $C_{25}H_{30}O_2$ (362.49); C, 82.82; H, 8.34. Found: C, 82.53; H, 8.34.

3-Benzyloxyestra-1,3,5(10)-trien-17α-yl 3′,4′,6′-Tri-O-acetyl-2′-deoxy-2′-trifluoroacetamido-β-D-glucopyranoside (*XIII*), *and 3-Benzyloxyestra-1,3,5(10)-trien-17α-yl 3′,4′,6′-Tri-O-acetyl-2′-deoxy-2′-trifluoroacetamido-α-D-glucopyranoside* (*XIV*). 17α-Estradiol 3-benzyl ether (XII, 454 mg) was dissolved in dry benzene (30 ml), cadmium carbonate (430 mg) was added, and the mixture was stirred until 5 ml was distilled. To the reaction mixture under distillation conditions was added 1.2 g of 1α-bromo-1-deoxy-2-trifluoroacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranose (Xb, 1.2 g) in dry benzene (46 ml) over a period of 0.75 hr. The reaction was maintained under reflux with water removal and stirring for 6 hr. The solid material was removed by filtration, and washed with methylene chloride. The filtrate and wash were combined, evaporated *in vacuo*, and acetylated for 18 hr at room temperature in pyridine (2 ml) with acetic anhydride (1 ml). The crude acetylated product was isolated as a gum which was dissolved in methanol (5 ml), and added to methanol (200 ml) saturated with ammonia at 0–5°. After 18 hr at 5°, the methanol was removed *in vacuo* and the residue was dissolved in methylene chloride, washed with water, and dried over magnesium sulfate. After filtration and evaporation, the residual gum showed by thin-layer chromatography (system D) one polar and two much less polar materials, which were removed by trituration with refluxing hexane. The residue was reacetylated in pyridine (2 ml) with acetic anhydride (1 ml) for 18 hr at room temperature. After excess reagents were removed *in vacuo*, the residue was dissolved in methylene chloride, washed with water twice, and then dried over magnesium sulfate. There resulted a resinous solid which on trituration with ether gave XIII (200 mg) as white crystals: mp 188–189°; $[\alpha]_D$ −24° (chloroform); mass spectrum (70 eV) $[M]^+$ 745; λ_{max}^{MeOH} 275–285 nm (ε 225); nuclear magnetic resonance 100 MHz, $CDCl_3$) 70, s [3]; 202, 208, d [12]; 459, d [1]; 499, s [2]; 736, m [>5].

Anal. Calcd for $C_{39}H_{46}F_3NO_{10}$ (745.76): C, 62.81; H, 6.21; F, 7.64; N, 1.85. Found: C, 62.91; H, 6.04; F, 7.62; N, 1.76.

The residue from the ether trituration above, containing the α and some residual β anomer along with traces of nonpolar substances, was plated on two 20 × 20 cm silica gel plates (500 μ thickness). After development in system D a very nonpolar material was discarded, and the β anomer (181 mg) was separated from the less polar α anomer (56 mg). The total β anomer yield was 397 mg (43%). The α anomer, XIV, was purified again on silica gel plates to give 22 mg of a colorless glass (2.3%): $[\alpha]_D$ +52° (chloroform); λ_{max}^{MeOH} 280–290 nm (ε 225); nuclear magnetic resonance (60 MHz, $CDCl_3$) 44, s [3]; 122, 123, 126, t [12]; 303, s [2]; 441, m [>5].

Anal. Calcd for $C_{39}H_{46}F_3NO_{10}$ (745.76): C, 62.81; H, 6.30; N, 1.85. Found: C, 62.37; H, 6.05; N, 1.51.

3-Benzyloxyestra-1,3,5(10)-trien-17α-yl 2′-Amino-2′-deoxy-β-D-glucopyranoside (*XVa*). 3-Benzyloxyestra-1,3,5(10)-trien-17α-yl 3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-trifluoroacetamido-β-D-glucopyranoside (XIII, 197 mg) was dissolved in 1 N potassium hydroxide in methanol solution (10 ml), and allowed to remain at room temperature for 3 hr, and at 0–5° for 18 hr. Evaporation *in vacuo* gave an amorphous product (138 mg). Purification on two sequentially 20 × 20 cm silica gel plates (250 μ thickness) (system E) gave 20 mg of a solvated amorphous product (XVa): $[\alpha]_D$ −10° (pyridine); λ_{max}^{MeOH} 278–288 nm (ε 200); nuclear magnetic resonance (60 MHz, d_6-Me_2SO) 41, s [3]; 304, s [2]; 445, s [>5] cps.

Anal. Calcd for $C_{31}H_{41}NO_6 \cdot 2CH_3OH$ (587.73): C, 67.44; H, 8.40; N, 2.37. Found: C, 67.08; H, 8.05; N, 2.35.

Although after purification the yield obtained was low, the losses could be attributed to the ether elution process from the thin-layer plate since a thin-layer chromatography of original crude material showed primarily only one steroidal product.

3-Benzyloxyestra-1,3,5(10)-trien-17α-yl 2′-Acetamido-2′-deoxy-β-D-glucopyranoside (*XVc*). Crude 3-benzyloxyestra-1,-3,5(10)-trien-17α-yl 2′-amino-2′-deoxy-β-D-glucopyranoside (XVa, 434 mg) was dissolved in pyridine (4 ml) with acetic anhydride (2 ml), and allowed to remain at room temperature for 18 hr. Evaporation gave a residue which was dissolved in methylene chloride, and was washed with excess saturated sodium bicarbonate solution and water. The solvent was dried over magnesium sulfate, filtered, and evaporated leaving a white solid (537 mg, XVb) which gave essentially one spot on thin-layer chromatography (system D). Infrared analysis showed that both O and N acetylation had occurred.

3-Benzyloxyestra-1,3,5(10)-trien-17α-yl 2′-acetamido-3′,-4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranoside (XVb, 535 mg) was dissolved in methanol (200 ml) saturated at 0° with ammonia, and allowed to remain at 0–5° for 18 hr. After evaporation *in vacuo*, the solid was triturated with water, and then with hexane to give the de-O-acetylated material (XVc, 366 mg), mp 122–126°. A sample was recrystallized from acetone–hexane: mp 120–130° dec; thin-layer chromatography (system E); $[\alpha]_D$ −8° (Me_2SO); λ_{max}^{MeOH} 278, 288 nm (ε 170); nuclear magnetic resonance (60 MHz, d_6-Me_2SO) 40, s [3]; 107, s [3]; 302, s [2]; 442, s [>5] cps.

Anal. Calcd for $C_{33}H_{43}NO_7$ (565.68): C, 70.06; H, 7.66; N, 2.47. Found: C, 69.72; H, 7.87; N, 2.39.

3-Hydroxyestra-1,3,5(10)-trien-17α-yl 2′-Acetamido-2′-deoxy-β-D-glucopyranoside (*XVI*). 3-Benzyloxyestra-1,3,5(10)-trien-17α-yl 2′-acetamido-2′-deoxy-β-D-glucopyranoside (XVc, 306 mg) was dissolved in absolute alcohol (100 ml) and 10% palladium on carbon (120 mg) was added. The mixture was hydrogenated at 40 psig for 3 hr. The catalyst was removed by filtration through Celite, and the filtrate was evaporated *in vacuo* leaving a glass which crystallized to give 266 mg of the 3-hydroxy compound, XVI, mp 224–226° dec. This was recrystallized from ethanol–water to give 173 mg of needles: mp 185–190° dec with previous shrinking; $[\alpha]_D$ −11° (ethanol); λ_{max}^{MeOH} 280–290° nm (ε 170); nuclear magnetic resonance (100 MHz, d_6-Me_2SO) 65, s [3]; 176, s [3]; 891, s [1] cps.

Anal. Calcd for $C_{26}H_{37}NO_7 \cdot H_2O$ (493.59): C, 63.26; H, 7.96; N, 2.83; H_2O, 3.7. Found: C, 63.56; H, 7.98; N, 2.83; H_2O, 3.7.

A sample of 17α-estradiol 17-β-*N*-acetylglucosaminide received from Dr. D. S. Layne had in our hands the following physical characteristics: mp 205–218° dec with previous shrinking; $[\alpha]_D$ −10.5° (ethanol); λ_{max}^{MeOH} 280–290° nm (ε 195). Both materials exhibited the same infrared and nuclear magnetic resonance and in thin-layer chromatography (system E) had the same mobility.

3-Benzyloxyestra-1,3,5(10)-trien-17β-yl 2′-Amino-2′-deoxy-α-D-glucopyranoside (VIIa). A solution of the blocked α-glycoside (III, 246 mg) in 1 N potassium hydroxide in methanol solution (4 ml) gave a white solid which was collected after 4 hr at room temperature, and was washed with water and dried. There was obtained 147 mg of VIIa, mp 198–209°, which was homogeneous on thin-layer chromatography (system E), and the infrared curve showed the absence of *O*- or *N*-acetyl groups.

3-Benzyloxyestra-1,3,5(10)-trien-17β-yl 2′-Acetamido-2′-deoxy-α-D-glucopyranoside (VIIb). The unblocked glycoside (VIIa, 180 mg) was dissolved in pyridine (2 ml) and acetic anhydride (1 ml) and left at room temperature for 3 days. When the solvent was removed, and ice water was added the crude tetraacetate (IX) as a yellow white solid was collected, and dried. This was dissolved in 1 ml of methylene chloride and added to a solution of ammonia gas in methanol (saturated at 0°). After 18 hr at 0–5°, the solvent was removed, and the acetamidoglycoside (VIIb, 154 mg) was obtained, mp 225–226° dec. This material showed an amide grouping on infrared analysis, and gave a homogeneous spot on thin-layer chromatography (system E).

3-Hydroxyestra-1,3,5(10)-trien-17β-yl 2′-Acetamido-2′-deoxy-α-D-glucopyranoside (VIII). The 3-benzyloxy 17β-estradiol glucosaminide (VIIb, 153 mg) was dissolved in absolute ethanol (100 ml) and 10% palladium on charcoal (60 mg) was added. The solution was hydrogenated at 40 psig for 4 hr, the catalyst was removed by filtration through Celite, and the filtrate was evaporated leaving (VIII, 128 mg) as a white solid. This material did not readily recrystallize, and was purified on four 20 × 20 cm silica gel G plates (system E) to give 81 mg of white crystals of VIII: mp 189–192° dec; $[\alpha]_D$ +150° (ethanol); λ_{max}^{MeOH} 280–290 nm (ε 190); nuclear magnetic resonance (100 MHz, d_6-Me_2SO) 76, s [3]; 181, s [3]; 465, d [1]; 892, m [1].

Anal. Calcd for $C_{26}H_{37}NO_7 \cdot 0.75H_2O$ (475.57): C, 63.85; H, 7.93; N, 2.86; H_2O, 2.8. Found: C, 63.70; H, 7.74; N, 2.88; H_2O, 2.8.

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