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15β-Hydroxysteroids (Part III).* Steroids of the human perinatal period: The synthesis of 3β, 15β, 17α-trihydroxy-5-pregnen-20-one. Application of *n*-butyl boronic acid protection of a 17,20-glycol

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*We report the synthesis of 3β,15β,17α-trihydroxy-5-pregnen-20-one (**1**) from 3β,15β-dihydroxy-5,16-pregnadien-20-one (**11**) in 7 steps using boronate derivatives as a means of protecting the 17,20-glycol side-chain of steroid intermediates. 16α,17α-Epoxy-3β,15β-dihydroxy-5-pregnen-20-one (**12**), an intermediate in the synthesis was prepared by epoxidation of **11** using a mixture of sodium hydroxide and hydrogen peroxide. Reduction of **12** with lithium aluminium hydride gave the two isomers of 5-pregnene-3β,15β,17α,20 (S+R)-tetrol (**13a** and **13b**) which on subsequent reaction with* n*-butyl boronic acid gave 5-pregnene-3β,15β,17α,20(S+R)-tetrol 17α,20-butyl boronate (**15a** and **15b**). Acetylation with acetic anhydride and pyridine yielded 3β,15β-diacetoxy-5-pregnene-17α,20(S+R)-diol 17α,20(S+R)-butyl boronate (**15c** and **15d**). Oxidative cleavage of the boronic ester using sodium hydroxide and hydrogen peroxide gave 3β,15β-diacetoxy-5-pregnene-17α,20(S+R)-diol (**13c** and **13d**). After isolation of these latter two products, dibromide protection of the C-5,6 olefin of **13d** and oxidation with* N*-bromosuccinimide gave 3β,15β-diacetoxy-17α-hydroxy-5-pregnen-20-one (**16**) which on deacetylation gave in good yield (35%) the desired product 3β,15β,17α-trihydroxy-5-pregnen-20-one (**1**) in an overall yield of 24% from **11**. (Steroids **61**:11–17, 1996)*

Keywords: adrenal, *n*-butyl boronate; 15β-hydroxysteroids; hyperplasia; neonate; synthesis

Introduction

An increasing number of 15β-hydroxylated steroids have been identified in human urine.[1,2] Of these, 3β,15β,17α-trihydroxy-5-pregnen-20-one (**1**) and 3α,15β,17α-trihydroxy-5β-pregnan-20-one (**2**) have emerged as the two most important urinary metabolites. The former is a normal metabolite of the human perinatal period and a probable precursor of all known 15β-hydroxylated steroids, while the latter is a metabolite pathognomonic of congenital adrenal hyperplasia (CAH) in newborn infants.[1] Other similar steroids identified more recently are those of 3α,15β,17α-trihydroxy-5α-pregnan-20-one (**3**), 3β,15β,17α-trihydroxy-5α-pregnan-20-one (**4**), 3β,15β,17α-trihydroxy-5β-pregnan-20-one (**5**), 5α-pregnane-3α,15β,17α,20*S*-tetrol (**6**), and 5β-pregnane-3α,15β,17α,20*S*-tetrol (**7**), found in the urine of a four-month-old girl affected with CAH due to 21-hydroxylase deficiency (Figure 1).[2]

In our investigation of 15β-hydroxylated steroids, the synthesis of **1** became the primary aim. To this end, the synthesis of 3β,15β-dihydroxy-5,16-pregnadien-20-one

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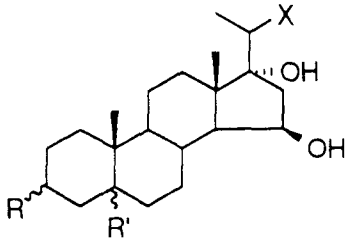


	R	R'	X
1	3β-OH	Δ^5	O
2	3α-OH	5β-H	O
3	3α-OH	5α-H	O
4	3β-OH	5α-H	O
5	3β-OH	5β-H	O
6	3α-OH	5α-H	(20S) OH,H
7	3α-OH	5β-H	(20S) OH,H
14	O	Δ^4	O

Figure 1 The chemical structures of compounds **1–7** and **14.**

(**11**) from 3β-hydroxy-5,15-androstadien-17-one (**8**), was achieved as a first-step objective (data submitted for publication) in a scheme where selective oxidation of isomeric 5-pregnen-3β,15β,17α,20(*S*+*R*)-tetrols (**13a** and **13b**) with *N*-bromosuccinimide would yield the desired product (Scheme 1).[3] In brief, reaction of **8** with 2-lithio-2-methyl-1,3-dithiane gave 20,20-trimethylenedithio-5,15-pregnadien-3β,17β-diol (**9**) which when treated with aqueous acid rearranged to give 20,20-trimethylenedithio-5,16-pregnadien-3β,15β-diol (**10**). Cleavage of the dithioacetal group with mercuric chloride gave **11** which on epoxidation using basic hydrogen peroxide afforded 16α,17α-epoxy-3β,15β-dihydroxy-5-pregnen-20-one (**12**). Reduction with lithium aluminium hydride gave the desired isomeric 5-pregnene-3β,15β,17α,20(*S*+*R*)-tetrols (**13a** and **13b**). However, oxidation with *N*-bromosuccinimide, after protection of the C-5,6 olefin as the dibromide, gave 15β,17α-dihydroxy-4-pregnen-3,20-dione (**14**) in only fair yield[3] with no evidence of the desired product **1.**

In this investigation we report the successful conversion of the isomeric tetrols (**13a** and **13b**) to **1.** This required the development of a protocol whereby the 3β- and 15β-hydroxy groups were protected prior to the oxidation of the 17α,20-glycol.

Experimental

The starting material, **8,** was obtained in our laboratory as an intermediary product of synthesis of **14.** The gas chromatography (GC), GC-mass spectroscopy (MS), elemental analysis, and NMR spectra of **14** were reported elsewhere.

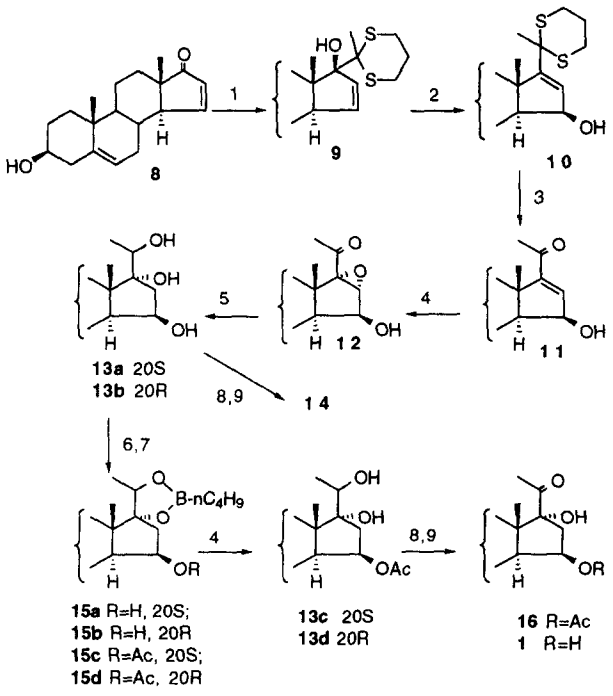
Solvents were laboratory grade or better. Melting points were determined on a Gallenkamp Melting Point Apparatus and are uncorrected. Ultraviolet spectra were determined on a Varian Techtron ultraviolet-visible spectrophotometer. ^{1}H NMR were recorded at 200 MHz using a Bruker AC-200F spectrometer using TMS as internal reference and deuterochloroform as the solvent unless otherwise noted. $W_{1/2}$ refers to the peak width at its half height. Gas chromatography was performed on a Hewlett Packard 5710A flame ionization gas chromatograph using a glass solid injector and interpreted using a Shimadzu CR-4A chromatopac utilizing Eurekasoft analytical software (Eurekanalytical, Camperdown, Australia), results are expressed as methylene units (MU).[5] A 30 m capillary column from Heliflex Capillaries (RSL-150 polydimethylsiloxane; ID 0.25 mm) was used with helium as the carrier gas (2.0 mL/min), temperature programming from 197–270°C at 1°C/min with the detector and injector block temperatures were 300°C and 250°C, respectively. Mass spectra were recorded on a Finnigan MAT TSQ-70 mass spectrometer scanning from 80 to 800 d at 70 eV. CIMS was obtained using methane ($CICH_4$) or methane/ammonia ($CINH_3$) as plasma and with a reagent gas pressure of 5–10 torr. Low resolution CIMS using isobutane and high resolution mass spectra were run on a VG Autospec. Silica gel H (Merck, type 60) was used for chromatography.[6] Steroid derivatization for GC and GC-MS analyses were either as the methyloxime-pertrimethylsilyl derivative (MOTMS) or as the pertrimethylsilylated derivative (TMS) and were carried out as reported earlier.[1]

16α,17α-Epoxy-3β,15β-dihydroxy-5-pregnen-20-one (12)

A solution of **11** (198 mg, 0.6 mmol) in methanol (13 mL) was cooled in an ice bath then aqueous sodium hydroxide (600 µl, 2 M) and aqueous hydrogen peroxide (500 µl, 30%) was added. The reaction mixture stood for 16 h at 4°C. After neutralization with acetic acid (70%) and evaporation to dryness, the residue was partitioned between ethyl acetate and water. The organic phase was washed with water, dried (Na_2SO_4), and evaported to dryness. Recrystallization using dichloromethane-light petroleum gave 160 mg (77%) of **12:** m.p. 172–174°C; ^{1}H NMR δ 5.34 (1 H, $W_{1/2}$ = 10 Hz, H-6), 4.20 (1 H, d J = 5 Hz, H-15), 3.71 (1 H, br s, H-16), 3.50 (1 H, $W_{1/2}$ = 26 Hz, H-3), 2.05 (3 H, s, H-21), 1.27 (3 H, s, H-18), 1.05 (3 H, s, H-19); SP-EIMS m/z (%) 346 (37) [M^+], 328 (26) [$M-H_2O$], 313 (11) [$M-H_2O$-Me], 310 (10) [$M-2\times H_2O$], 285 (10) [$M-H_2O$-MeCO], 267 (20), 253 (17), 220 (21), 205 (76), 175 (27), 171 (25), 169 (14), 161 (15), 159 (22), 157 (14), 148 (44), 147 (33), 145 (39), 143 (27), 133 (20), 127 (20), 119 (43), 91 (100). Found C 72.5%, H 8.4%; $C_{21}H_{30}O_4$ requires C 72.8%, H 8.7%. GC (as MOTMS) MU = 29.70; GC-EIMS m/z (%) 519 (10) [M^+], 504 (12) [M-Me], 488 (55) [M-OMe], 398 (20) [M-OMe-OTMS], 356 (20), 324 (16), 308 (23), 282 (17), 254 (26), 197 (19), 171 (58), 129 (100).

5-Pregnene-3β,15β,17α-20(S+R)-tetrol (13a and 13b)

To a solution of **12** (250 mg, 0.72 mmol) in anhydrous tetrahydrofuran (50 mL), lithium aluminium hydride (30 mg, 0.8 mmol) was added and the mixture was refluxed for 1 h. The excess reagent was destroyed by the addition of ethyl acetate and the mixture filtered through celite, dried (Na_2SO_4), and evaporated to dryness. Chromatography of the residue on silica, eluting with ethyl acetate/light petroleum (3:1, v/v) gave after recrystallization from acetone/hexane 180 mg (71%) of **13b:** m.p. 212–214°C; ^{1}H NMR δ 5.36 (1 H, $W_{1/2}$ = 10 Hz, H-6), 4.28 (1 H, $W_{1/2}$ = 12 Hz, H-15), 4.11 (1 H, q J = 6 Hz, H-20), 3.50 (1 H, $W_{1/2}$ = 26 Hz, H-3), 1.16 (3 H, d J = 6 Hz, H-21), 1.06 (3 H, s, H-18), 1.05 (3 H, s, H-19); SP-EIMS m/z (%) 350 (6) [M^+], 332 (7) [$M-H_2O$], 305



1) 2-methyl-1,3-dithiane, LDA, -78°C; 2) H^+, H_2O; 3) $HgCO_2$, $CaCO_3$; 4) NaOH, H_2O_2; 5) $LiAlH_4$, THF; 6) $n\text{-}C_4H_9B(OH)_2$; 7) Ac_2O, Pyr; 8) Pyridine.HBr.Br_2, NBS, NaI; 9) NaOH

Scheme 1 The synthesis of 5-pregnen-3β,15β,17α,20(*R+S*)-tetrol (**13a** and **13b**) and its conversion to 3β,15β,17α-trihydroxy-5-pregnene-20-one (**1**) and 15β,17α-dihydroxy-4-pregnen-3,20-dione (**14**).

(100) [M-CH_3CHOH], 287 (25) [M-CH_3CHOH-H_2O], 271 (16), 269 (20), 251 (11), 215 (20), 211 (16), 200 (15); SP-CIMS/CH_4 m/z (%) 349 (12) $[M-1]^+$, 333 (92) [MH-H_2O], 315 (100) [M-CH_3CHOH], 287 (37), 269 (13); Found: C 72.3%, H 9.8%; $C_{21}H_{34}O_4$ requires C 72.0% H 9.8%. GC (as TMS) MU = 29.71; GC-EIMS m/z (%) 638 (1) $[M^+]$, 521 (100) [M-CH_3C-OTMS], 431 (72) [M-117-OTMS], 391 (22), 333 (23), 251 (13), 191 (40), 147 (35), 117 (78).

Further elution gave after recrystallization from acetone/hexane 40 mg (17%) of **13a:** m.p. = 223–224°C; ^1H NMR ($CDCl_3$) δ p.p.m. 5.36 (1 H, m $W_{1/2}$ = 10 Hz, H-5), 4.28 (1 H, m $W_{1/2}$ = 15 Hz, H-15), 3.91 (1 H, q J = 6 Hz, H-20), 3.50 (1 H, m $W_{1/2}$ = 26 Hz, H-3), 1.19 (3 H, d J = 6 Hz, H-21), 1.05 (3 H, s, H-19), 0.99 (3 H, s, H-18); SP-EIMS m/z (%) 350 (not detected, ND), 305 (100) [M-CH_3CHOH], 287 (6) [M-45-H_2O], 269 (5), 211 (16); SP-CIMS/CH_4 m/z (%) 349 (13) $[M-1]^+$, 333 (100) [M-CH_3], 315 (83) [M-15-H_2O], 297 (14) [M-15-2 × H_2O], 271 (13) [M-15-3 × H_2O], 245 (3); Found: C 68.4%, H 9.9% $C_{21}H_{34}O_4 \cdot H_2O$ requires C 68.4%, H 9.8%. GC (as TMS) MU = 29.84; GC-EIMS m/z (%) 638 (1) $[M^+]$, 521 (100) [M-$CH_3CHOTMS$], 431 (72) [M-117-0TMS], 391 (22), 333 (23), 251 (13), 191 (40), 147 (35), 117 (78).

5-Pregnene-3β,15β,17α,20S-tetrol 17α,20S-butyl boronate (15a)

To a solution of **13a** (10 mg, 0.028 mmol) in ethyl acetate (5 mL), *n*-butylboronic acid (3 mg, 0.029 mmol) was added and after 5 min standing at room temperature, the solution was evaporated to dryness to give 11.9 mg of a mixture of **15a** and **13a** in a ratio of 74:26 (analysis by GC and GCMS): Compound **(15a):** ^1H NMR ($CDCl_3$) δ p.p.m. 5.34 (1 H, $W_{1/2}$ = 10 Hz, H-6), 4.3 (2 H, $W_{1/2}$ = 11 Hz, H-15 + 20), 3.48 (1 H, $W_{1/2}$ = 23 Hz, H-3), 1.26 (3 H, d J = 6 Hz, H-21), 1.05 (3 H, s, H-19), 0.99 (3 H, s, H-18); SP-CIMS/CH_4 m/z (%) 416 (8) $[M^+]$, 398 (24) [M-H_2O], 383 (10) [M-H_2O-Me], 315 (8) [M-H_2O-Me-nBuB], 297 (100), 287 (24), 269 (30), 253 (12), 215 (27), 183 (15), 145 (38); high resolution MS (SP-CIMS/CH_4) [as M^+] expected 416.3098; found = 416.3094. GC (as TMS) MU = 31.03; GC-EIMS m/z (%)560 (<0.5) $[M^+]$, 545 (3) [M-Me], 472 (12) [M-Me-Me_3Si], 470 (36) [M-Me_3SiOH], 455 (6) [M-Me_3SiOH-Me], 431 (5), 380 (12) [M-2 × Me_3SiOH], 365 (21), 353 (16), 297 (9), 278 (53), 263 (64), 251 (40), 238 (30), 224 (23), 209 (40), 184 (19), 171 (28), 157 (47), 145 (33), 129 (100), 118 (30), 105 (40); GC-CIMS/CH_4 m/z (%) 561 (25) $[MH^+]$, 559 (39), [M-1], 545 (100) [M-Me], 471 (54) [M+1-Me_3SiOH], 459 (17), 381 (59) [M+1-2 × Me_3SiOH], 369 (23), 297 (10), 279 (21), 255 (7), 129 (10), 91 (25).

5-Pregnene-3β,15β,17α,20R-tetrol 17α,20R-butyl boronate (15b)

To a solution of **13b** (100 mg, 0.28 mmol) in ethyl acetate (27 mL), *n*-butyl boronic acid (29.6 mg, 0.29 mmol) was added and after 5 min standing at room temperature, the solution was evaporated to dryness to give 119 mg (102%) of **15b** and **13b** in a ratio of 97:3 (as analyzed by GC and GC-EIMS as the persilylated derivatives [M^+560] and M^+638], respectively). **15b:** ^1H NMR δ 5.3 (1 H, $W_{1/2}$ = 10 Hz, H-6), 4.35 (2 H, $W_{1/2}$ = 10 Hz, H-15 + 20), 3.47 (1 H, $W_{1/2}$ = 12 Hz, H-3), 1.29 (3 H, d J = 7 Hz, H-21), 1.07 (3 H, s, H-19), 0.93 (3 H, s, H-18); SP-CIMS/CH_4 m/z (%) 416 (11) [M+], 398 (37) [M-H_2O], 383 (11) [M-H_2O-Me], 315 (54) [M-H2O-Me-nBuB], 297 (100), 287 (29), 269 (61), 253 (33), 215 (14), 183 (17), 154 (16), 145 (13), 129 (21), 119 (11); high resolution MS expected (SP-CIMS/CH_4) [as M^+] 416.3098; found = 416.3099. GC (as TMS) MU = 30.70. GC-EIMS m/z (%) 560 (0.4) $[M^+]$, 545 (3) [M-Me], 470 (36) [M-Me_3SiOH], 455 (7%) [M-Me_3SiOH-Me], 431 (7), 380 (17), 365 (29), 353 (18), 297 (8), 281 (11), 278 (35), 263 (64), 251 (33), 238 (20), 224 (25), 209 (46), 184 (19), 171 (28), 157 (44), 147 (38), 129 (100), 105 (40). GC-CIMS/CH_4 m/z (%) 561 (28) $[MH]^+$, 559 (38), 545 (100), 470 (43), 459 (32), 381 (59), 369 (26), 297 (14), 279 (18).

3β,15β-Diacetoxy-5-pregnene-17α,20(S)-diol 17α,20(S)-butyl boronate (15c)

A solution of the crude boronate **15a** (15 mg, 0.036 mmol, 74% by GC) in pyridine (5 mL) and acetic anhydride (5 mL) stood for 48 h in the dark at room temperature. The mixture was then evaporated to dryness to give 18 mg of the **15c** as a gum [GC/MS analysis shows 63% pure with two other compounds 17% of **15a** and 20% of 3β,15β,20*S*-triacetoxy-5-pregnene-17α-ol (**13e**)]: Compound **(15c):** ^1H NMR ($CDCl_3$) δ p.p.m. 5.40 (1 H, m $W_{1/2}$ = 10 Hz, H-6), 5.15 (1 H, m $W_{1/2}$ = 16 Hz, H-15), 4.61 (1 H, m $W_{1/2}$ = 28 Hz, H-3), 4.32 (1 H, q J = 6.6 Hz, H-20), 2.04 (3 H, s, OAc), 2.01 (3 H, s, OAc), 1.32 (3 H, d J = 6.6 Hz, H-21), 1.04 (3 H, s, H-19), 0.88 (3 H, s, H-18); SP-CIMS/CH_4 m/z (%); 501 (3) $[MH^+]$, 441 (53) [M-CH_3CO_2H], 399 (10) [M-$C_4H_9B(OH)_2$], 381 (100) [M-2 × CH_3CO_2H], 339 (39), 313 (7), 297 (25), 279 (42); DCI probe-CI/Isobutane [as MH] m/z (%) 501 (0.5) $[MH^+]$, 441 (36) [M-CH_3CO_2H], 399 (19) [M-$nBuBO_2H_2$], 381 (100) [M-2 × CH_3CO_2H], high resolution MS (DCI probe-CI/Isobutane) expected 501. 3387; found = 501.3382. GC (not derivatized) MU = 32.84; GC-EIMS m/z (%); 500 (ND) $[M^+]$, 380 (69) [M-2 × CH_3CO_2H]$^+$, 365 (18), 343 (7), 279 (15), 263 (100), 251 (14), 236 (21), 220 (31), 209 (57), 193 (32), 170 (51), 155 (33), 145 (63), 129 (94), 105 (58).

3β,15β-Diacetoxy-5-pregnene-17α,20(R)-diol 17α,20(R)-butyl boronate (15d)

To solution of the crude boronate **15b** (100 mg, 0.23 mmol, 97% by GC) in pyridine (5 mL), acetic anhydride (5 mL) was added and the mixture stood for 48 h in the dark at room temperature. The mixture was then evaporated to dryness to give 115 mg (95%) of **15d** as a gum [GC/MS analysis shows 97% pure with 3% of **13f**]. Compound **(15d):** ^{1}H NMR δ 5.40 (1 H, m $W_{1/2}$ = 11 Hz, H-6), 5.24 (1 H, m $W_{1/2}$ = 18 Hz, H-15), 4.65 (1 H, m $W_{1/2}$ = 28 Hz, H-3), 4.40 (1 H, q J = 6.6 Hz, H-20), 2.04 (3 H, s, OAc), 2.01 (3 H, s, OAc), 1.25 (3 H, d J = 6.6 Hz, H-21), 1.07 (3 H, s, H-19), 0.87 (3 H, s, H-18); SP-CIMS/CH_4 m/z (%) 500 (2) [M-1], 501 (ND) $[M+1]^+$, 441 (21) [M-CH_3CO_2H], 399 (3) [M-n$BuBO_2H_2$], 381 (100) [M-2 × CH_3CO_2H], 339 (28) [M-n$BuBO_2H_2$-CH_3CO_2H], 297 (13), 279 (11); DCI probe-CI/Isobutane m/z (%) 501 (0.5) $[MH^+]$, 441 (36) [M-CH_3CO_2H], 399 (19) [M-n$BuBO_2H_2$], 381 (100) [M-2 × CH_3CO_2H], high resolution MS (DCI probe-CI/isobutane) [as MH] expected 501.3387; found = 501.3379. GC (not derivatized) MU = 32.40; GC-EIMS m/z (%) 500 (ND) $[M^+]$, 440 (2) $[M\text{-}CH_3CO_2H]^+$, 398 (5) [M-n$BuBO_2H_2$], 380 (100) [M-2 × CH_3CO_2H], 365 (14), 278 (7), 263 (86), 251 (13), 209 (49), 183 (16), 155 (26), 129 (38); GC-CIMS/CH_4 m/z (%) 500 (1), 441 (11), 383 (30), 381 (100), 339 (18), 297 (22), 279 (69), 253 (8), 106 (20).

3β,15β-Diacetoxy-5-pregnene-17α,20(S)-diol (13c)

To a solution of the crude acetoxyboronate **15c** (20 mg, 0.025 mmol, 63% by GC) in THF (3 mL), sodium hydroxide (2 M, 100 µL) was added followed by hydrogen peroxide (30%, v/v; 100 µL). After 1 h at room temperature the solution was neutralized with hydrochloric acid (1 M, 200 µL), evaporated to low volume and extracted with ethyl acetate. The organic phase was washed with water, dried (Na_2SO_4) and evaporated to dryness. The residue was chromatographed on silica gel, eluting with ethyl acetate/light petroleum (1:2, v/v) to give 7 mg (40%) of **13c:** ^{1}H NMR δ 5.39 (1 H, $W_{1/2}$ = 10 Hz, H-6), 5.17 (1 H, $W_{1/2}$ = 16 Hz, H-15), 4.63 (1 H, $W_{1/2}$ = 28 Hz, H-3), 3.92 (1 H, q J = 6 Hz, H-20), 2.04 (3 H, s, OAc), 2.00 (3 H, s, OAc, 1.21 (3 H, d J = 6 Hz, H-21), 1.07 (3 H, s, H-19), 0.96 (3 H, 2, H-18). (d_6-acetone) δ p.p.m. 5.37 (1 H, $W_{1/2}$ = 9 Hz, H-6), 5.08 (1 H, $W_{1/2}$ = 16 Hz, H-15), 4.50 (1 H, $W_{1/2}$ = 32 Hz, H-3), 3.83 (1 H, q J = 6 Hz, H-20), 1.96 (3 H, s, OAc), 1.95 (3 H, s, OAc), 1.14 (3 H, d J = 6 Hz, H-21), 1.07 (3 H, s, H-19), 0.98 (3 H, s, H-18); SP-CIMS/Isobutane m/z (%); 417 (10) [M-H_2O], 399 (6) [M-2H_2O], 375 (100) [M-CH_3CO_2H], 357 (36) [M-H_2O-CH_3CO_2H], 339 (10) [M-2 × H_2O-CH_3CO_2H], 315 (59) [M-2 × CH_3CO_2H], 297 (61) [M-H_2O-2 × CH_3CO_2H], 279 (8), 253 (15); SP-CIMS/NH_3 m/z (%); 452 (100) $[MNH_4^+]$, 392 (18), 357 (4), 279 (7); high resolution MS (SP-CIMS/isobutane) [as MH-18, no MH present] expected = 417.2641; found = 417.2646. GC (as TMS): MU = 31.52; GC-EIMS m/z (%); 578 (ND) $[M^+]$, 518 (0.4) $[M\text{-}60]^+$, 401 (87), 368 (9), 341 (26), 268 (5), 251 (17), 209 (4), 195 (15), 169 (27), 157 (27), 147 (39), 117 (100).

3β,15β-Diacetoxy-5-pregnene-17α,20(R)-diol (13d)

To a solution of the crude diacetoxyboronate **15d** (100 mg, 0.2 mmol, 97% by GC) in tetrahydrofuran (10 mL), sodium hydroxide (2 M, 0.5 mL) was added followed by hydrogen peroxide (30%, v/v; 0.5 mL). After 1 h at room temperature the solution was neutralized with hydrochloric acid (1 M, 1 mL), evaporated to low volume and extracted with ethyl acetate (3×). The organic phase was washed with water, dried, (Na_2SO_4) and evaporated to dryness. The residue was chromatographed on silica gel, eluting with ethyl acetate/light petroleum (1:2, v/v) to give 60 mg (70%) of **13d:** ^{1}H NMR δ 5.36 (1 H, $W_{1/2}$ = 9 Hz, H-6), 5.12 (1 H, $W_{1/2}$ = 15 Hz, H-15), 4.6 (1 H, $W_{1/2}$ = 28 Hz, H-3), 4.1 (1 H, q J = 6 Hz, H-20), 2.03 (3 H, s, OAc), 1.99 (3 H, s, OAc), 1.16 (3 H, d J = 6 Hz, H-21), 1.07 (3 H, s, H-19), 1.02 (3 H, s, H-18). (d_6-acetone) 5.34 (1 H, $W_{1/2}$ = 8 Hz, H-6), 5.06 (1 H, $W_{1/2}$ = 12 Hz, H-15), 4.50 (1 H, $W_{1/2}$ = 32 Hz, H-3), 4.02 (1 H, q J = 5 Hz, H-20),1.96 (3 H, s, OAc), 1.94 (3 H, s, OAc), 1.09 (3 H, d J = 5 Hz, H-21), 1.07 (3 H, s, H-19), 1.05 (3 H, s, H-18); SP-CIMS/isobutane m/z (%); 417 (10) [M-H_2O], 399 (6)]M-2H_2O], 375 (100)]M-CH_3CO_2H], 357 (36)]M-H_2O-CH_3CO_2H], 339 (10) [M-2 × H_2O-CH_3CO_2H], 315 (59) [M-2 × CH_3CO_2H], 297 (61)]M-H_2O-2 × CH_3CO_2H], 279 (8), 253 (15); SP-CIMS/NH_3 m/z (%); 452 (100) $[MNH_4^+]$, 392 (12), 314 (3); high resolution MS (SP-CIMS/isobutane) [as MH-18, no MH present] expected = 417.2641; found = 417.2634. GC (as TMS) MU = 31.49; GC-EIMS m/z (%); 578 (ND) $[M^+]$, 518 (2) $[M\text{-}60]^+$, 502 (1), 457 (2.5), 401 (100), 368 (7), 341 (20), 195 (3), 169 (6), 157 (7), 131 (10), 117 (60).

3β,15β-Diacetoxy-5-pregnene-17α,20(S)-diol 17α,20S-n-butyl boronate (15c)

A solution of **13c** (2 mg, 0.0046 mmol) was dissolved in ethyl acetate (1 mL) and *n*-butyl boronic acid (0.6 mg) was added. After 5 min at room temperature GC analysis proved the mixture to consist of **13c** and **15c** in a ratio of 1:2.

3β,15β-Diacetoxy-17α-hydroxy-5-pregnen-20-one (16)

To a solution of **13d** (50 mg, 0.115 mmol) in methanol (5 mL, 0.115 mmol), pyridinium bromide perbromide (50 mg, 0.23 mmol) was added. After 10 min at room temperature, the solution was evaporated to dryness, extracted with dichloromethane, and washed with water. The organic layer was evaporated to dryness and the residue was dissolved in dioxan (1 mL), water (100 µL) was added, followed by *N*-bromosuccinimide (35 mg, 0.2 mmol). The solution was stirred in the dark for 24 h before being diluted with ethyl acetate. Solid sodium iodide (20 mg, 0.13 mmol) was added and the solution was stirred for 5 min then aqueous saturated sodium thiosulfate was added. The reaction mixture was stirred for a further 5 min before it was extracted three times with ethyl acetate. The combined organic phase was washed with sodium bicarbonate (1.2 M) then water, dried (Na_2SO_4), and evaported to dryness. The residue was chromatographed on silica gel eluting with dichloromethane/ethyl acetate to give after recrystallizing from dichloromethane/hexane 30 mg (60%) of **16:** m.p. 277–278°C; ^{1}H NMR δ 5.38 (1 H, $W_{1/2}$ = 9 Hz, H-6), 5.23 (1 H, $W_{1/2}$ = 17 Hz, H-15), 4.61 (1 H, $W_{1/2}$ = 34 Hz, H-3), 2.28 (3 H, s, H-21), 2.04 (6 H, s, OAc-3,15), 1.07 (3 H, s, H-19), 0.92 (3 H, s, H-18); SP-CIMS/CH_4 m/z (%); 433 (5) $[MH^+]$, 415 (5) [M-H_2O], 373 (65) [M-CH_3CO_2H], 355 (28) [M-H_2O-CH_3CO_2H], 313 (100) [M-2 × CH_3CO_2H], 295 (65); calculated for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.72; H, 8.33. GC (as the methyloxime) MU = 31.05; GC-CIMS/CH_4 m/z (%); 533 (30) $[MH^+]$, 502 (7), 473 (13) [M-CH_3CO_2H], 442 (100) [M-$(CH_3)_3SiOH$], 383 (9), 352 (34), 323 (18), 309 (12), 292 (55), 251 (10), 212 (11), 187 (29), 186 (38), 170 (49), 169 (34), 157 (26), 145 (35), 131 (21), 121 (23), 105 (24).

3β,15β,17α-Trihydroxy-5-pregnen-20-one (1)

To a solution of **16** (30 mg, 0.07 mmol) in ethanol (4 mL), sodium hydroxide (0.5 ml, 2 M) was added and the solution was heated at 40°C for 10 min. Hydrochloric acid (1 M, 1 mL) was added, the mixture was evaporated to low volume and diluted with ethyl acetate. The organic phase was washed with water, dried (Na_2SO_4), and evaporated to dryness to give after crystallization from acetone 22 mg (91%) of **1:** m.p. 258–260°C; ^{1}H NMR δ 5.37 (1 H, m $W_{1/2}$ = 10 Hz, H-6), 4.36 (1 H, m $W_{1/2}$ = 14 Hz, H-15), 3.50

(1 H, m $W_{1/2}$ = 24 Hz, H-3), 2.28 (3 H, s, H-21), 1.04 (3 H, s, H-19), 0.94 (3 H, s, H-18); SP-CIMS/CH_4 m/z (%); 349 (6) $[M+1]^+$, 347 (21), 332 (37), 331 (100), $[M-H_2O]$, 313 (93) $[M-2\times H_2O]$, 295 (57) $[M-3\times H_2O]$, 271 (9), 253 (5). SP-CIMS/NH_3 m/z (%); 366 (100) $[MNH_4^+]$, 348 (39), 331 (49), 313 (10), 295 (3); high resolution EIMS expected 348.2300; found = 348.231 GC (as MOTMS) MU = 29.11; GC-EIMS m/z (%); 593 (26) $[M^+]$, 562 (76), 503 (10), 472 (38), 362 (19), 258 (100), 231 (12), 188 (31), 129 (29). The GC and GC/MS data are identical to that reported earlier.[1]

Results and discussion

The synthesis of 3β,15β,17α-trihydroxy-5-pregnen-20-one **(1)** from 5-pregnene-3β,15β,17α,20(*S*+*R*)-tetrols **(13a** and **13b)** required the selective protection of the C-3β and C-15β hydroxy groups prior to the oxidation of the C-20 hydroxy functional group. In principle, this could be achieved by either the direct preparation of protected derivatives of **13a** and **13b** or by preparation of suitable derivatives of 3β,15β-dihydroxy-5,16-pregnadien-20-one **(11)** or 16α,17α-epoxy-3β,15β-dihydroxy-5-pregnen-20-one **(12)** followed by reduction to the protected **13a** and **13b.** Attempts to prepare derivatives of **11** and **12** followed by reduction in good yield included the formation and cleavage of the *t*-butyldimethylsilyl, tetrahydropyranyl, methoxymethylene ethers, and acetyl esters but were unsuccessful.

Since a high-yielding synthesis of protected forms of the C-3 and C-15 hydroxy was not found, the protection of the C-17,20 glycol was investigated prior to reaction of the C-3 and C-15 alcohols. The preparation of acetonide ketals was not successful due to the ease of dehydration of the C-15β alcohols. A scheme was devised using boronate derivatives to protect the glycol side chain prior to protection of the C-3 and C-15 hydroxy functional groups which after hydrolysis of the boronate ether would yield suitable derivatives of **13a** and **13b.** To this end a number of boronate derivatives were investigated.

Both *n*-alkylboronates and *n*-aryl boronates are known for the protection of diols or amino alcohols.[7–17] There are two methods employed to prepare the boronates. An ethyl acetate solution of the diol or amino alcohol is stirred with an *n*-alkyl boronic acid or *n*-aryl boronic acid[9,14,16] or the diol or amino alcohol is refluxed in the presence of the corresponding trialkyl boroxine, the anhydride of the corresponding boronic acid.[7,11,12] However, a side-product of this reaction is *n*-butyl boronic acid and, although only a weak acid, under these reaction conditions it was expected that catalytic dehydration of the C-15β hydroxy group could occur.

The quantitative conversion of 5β-pregnane-3α,17α,20(*S*+*R*)-triol **(18a** and **18b)** with *n*-butyl boronic acid to the C-20*R* and C-20*S* isomers of 5β-pregnane-3α,17α,20(*S*+*R*)-triol-17α,20-butyl boronate **19a** and **19b)** has been reported previously.[16] It was shown that the free alcohols of these compounds were easily converted to the trimethylsilyl ether or acetate derivatives. It is known that the benzene boronic esters tend to be resistant to hydrolysis when compared to the butyl boronic ester[14]; however, as the planned reaction sequence required the selective hydrolysis of the boronic ester in preference to the C-3β and C-15β acetates using a mixture of sodium hydroxide and hydrogen peroxide, the choice of boronic acid (e.g., methyl, *n*-butyl, *t*-butyl, or benzene boronic acids) was based upon the ease of formation and removal versus its stability to hydrolysis.

In this study, reaction of *n*-butyl boronic acid with 5-pregnene-3β,15β,17α,20*R*-tetrol **(13b)** resulted in a product which on analysis by GC and GC-MS, after reaction with bis-(trimethylsilyl)-trifluoroacetamide, proved to be a mixture of 97% 5-pregene-3β,15β,17α,20*R*-tetrol-17α,20*R*-butyl boronate **(15b)** and 3% **13b**. Acetylation of this mixture with acetic anhydride in pyridine gave 3β,15β-diacetoxy-5-pregnene-17α,20*R*-diol 17α,20*R*-butyl boronate **(15d)** with 3β,15β,20*R*-triacetoxy-5-pregnene-17α-ol **(13f)** as a minor component (3%). The boronte **(15d)** was rapidly cleaved on treatment with sodium hydroxide and hydrogen peroxide to give after chromatographic purification 3β,15β-diacetoxy-5-pregnene-17α,20*R*-diol **(13d)** in 70% overall yield (Scheme 1).

In contrast to the reactions observed with **13b,** reaction of the 20*S*-tetrol **(13a)** under identical conditions with *n*-butyl boronic acid in ethyl acetate gave a mixture of 74% of 5-pregnene-3β,15β,17α,20*S*-tetrol 17α,20*S*-butyl boronate **(15a)** and 26% of **13a.** Acetylation using acetic anhydride in pyridine could not be induced to go to completion and resulted in a mixture of 3β,15β-diacetoxy-5-pregnene-17α,20*S*-diol-17α,20*S*-butyl boronate **(15c), 15a** and 3β,15β,20*S*-triacetoxy-5-pregnene-17α-ol **(13e)** as identified by GC-MS. Hydrolysis of this mixture with sodium hydroxide and hydrogen peroxide gave after chromatography 3β,15β-diacetoxy-5-pregnene-17α,20*S*-diol **(13c)** in 40% overall yield.

The above results are for reactions of **13a** and **13b** with *n*-butyl boronic acid run at the same time and identical conditons; therefore, these results reflect the equilibrium position of these reactions. Numerous repetitions of these reactions under different conditions, i.e., changing reagent concentrations and so on gave similar results. Under identical reaction conditions to that used in the reactions of **13a** and **13b,** we were able to reproduce the reported results[16] that **18a** and **18b** react quantitatively with *n*-butyl boronic acid to give **19a** and **19b.** The rate of hydrolysis of the acetonides of **18a** and **18b** have been found to be vastly different. The acetonide of **18a** was hydrolyzed using 60–80% aqueous acetic acid at room temperature within 24 h. In stark contrast, the acetonide of **18b** was stable under these conditions, requiring refluxing 80% acetic acid to hydrolyze. These results were interpreted in terms of the ease of approach of the proton to the 20*S* acetonide and also due to the additional ring strain of the acetonide due to the steric interaction of the C-18 and C-21 methyl groups.[20]

Inspection of molecular models reveals that the 17α,20*S*-glycol is locked is a *cis* conformation and there exists a serious non-bonded interaction between the C-18 and the C-21 methyl groups (Figure 2). Addition of a 15β-hydroxy group as in **15a** adds another 1-3 diaxial non-bonded interaction between the C-15β hydroxy group and the C-18 methyl group, increasing the steric congestion of the C-18 methyl group, which results in destabilization of the C-17,20*S*-boronate. The difference in ease of acetylation of **15a** and **15b** is indicative of an increase in steric congestion of the C-15β-hydroxy group by the C-18 methyl group in **15a** as compared to **15b,** where the C-18/C-21 intense meth-

yl/methyl interaction is replaced with a minor methyl/hydrogen interaction. Further evidence supporting this premise was obtained by reaction of **13c,** a compound in which the C-15β hydroxy group has been acetylated to the C-15β acetate, thereby slightly increasing the 1,3-diaxial interaction of the C-15β group with the C-18 methyl groups, under identical conditions to those reactions reported above. The equilibrium position, as reflected by the ratio of products obtained **13c** (33%) and **15c** (67%), has been significantly shifted towards the unreacted diol. The relative stability of these molecules is complicated by the combination of a spiroannular biscyclopentane unit in which one of the cyclopentanes is part of a strained trans-8-methylhydrindane unit. An in-depth investigation using molecular dynamics would be needed to gain greater insight of the factors affecting the equilibra.

Although benzene boronic acid is known to yield esters of greater stability than the *n*-butyl boronic esters, reaction of **13a** with benzene boronic acid in ethyl acetate yielded 5-pregnene-3β,15β,17α,20(*S*)-tetrol 17α,20*S*-benzene boronate and the starting material **13a** in a similar ratio to that obtained with *n*-butyl boronic acid with no added advantage and therefore were not exploited in our studies.

N-Bromosuccinimide has been shown to be effective in glycol oxidation while at the same time avoiding glycol cleavage.[18,19] Neither chromium compounds nor silver carbonate were successful in avoiding the cleavage of the glycol.[3] Early attempts using *N*-bromosuccinimide gave numerous side products and low yields (10%); however, protection of the C-5 alkene as the dibromide resulted in a much cleaner reaction.[3] In this study the reaction of **13d** with pyridinium hydrobromide perbromide gave 3β,15β-diacetoxy-5,6-dibromopregnane-17α,20*R*-diol which without isolation was oxidized with *N*-bromosuccinimide to give after debromination with sodium iodide, 3β,15β-diacetoxy-17α-hydroxy-5-pregnen-20-one **(16)** in 60% yield (Scheme 1). Hydrolysis of **16** with sodium hydroxide in ethanol gave 3β,15β,17α-trihydroxy-5-pregnen-20-one **(1),** the desired product, in an overall yield of (35%) from **13b.** No attempts were made to obtain the same desired product from the corresponding isomer **13a.**

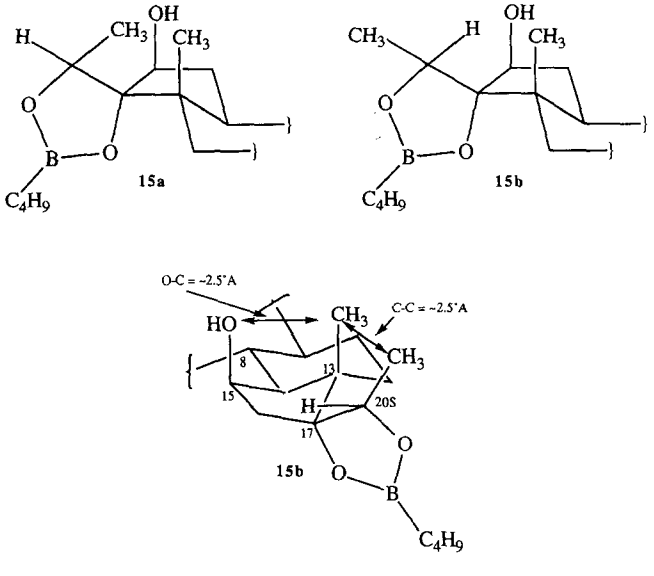


Figure 2 The stereochemical relationship of the C-21, C-18 methyls and the C-15β hydroxy groups.

This study shows that the application of boronate chemistry as a means of protecting the 17,20-glycol has been successful in assisting with the synthesis, of 15β-hydroxylated C-21 steroids. This overcame the major problem of protecting the 15β-hydroxy group which proved difficult to derivatize due to steric hindrance, a rate-limiting step with other competing functional groups such as those at C-3 and C-20, respectively. It is envisaged that this method can also be applied in the synthesis of isomeric C-21 steroids hydroxylated at C-15α.

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