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Chemical Conversion of Corticosteroids to $3\alpha,5\alpha$ -Tetrahydro Derivatives. Synthesis of Allotetrahydro-11-deoxycortisol Glucuronides¹⁾

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A method for the conversion of 11-deoxycortisol into 3α -hydroxy- 5α -compounds is described. The allotetrahydro-11-deoxycortisol monoacetates (**8**, **14**), which are key intermediates in the preparation of the 3- or 21-glucuronide of allotetrahydro-11-deoxycortisol, were the target compounds. The preparation of 5α -dihydro-11-deoxycortisol 21-acetate (**4**) was carried out by hydrogenation of the 3-ethoxy-3,5-diene (**2**), followed by acid hydrolysis. When the 21-tetrahydropyranyl ether (**6**) and 21-*tert*-butyldimethylsilyl ether (**7**) were treated with potassium tri-*sec*-butylborohydride in tetrahydrofuran under mild conditions, selective reduction of the carbonyl group at C-3 took place, yielding the 3α -alcohols (**9** and **10**, respectively). Allotetrahydro-11-deoxycortisol 3-glucuronide (**21**) and allotetrahydro-11-deoxycortisol 21-glucuronide (**23**) were then prepared.

Keywords—11-deoxycortisol metabolite; allotetrahydro-11-deoxycortisol; 5α -dihydro-11-deoxycortisol; allotetrahydro-11-deoxycortisol 3-acetate; allotetrahydro-11-deoxycortisol 21-acetate; potassium tri-*sec*-butylborohydride; allotetrahydro-11-deoxycortisol glucuronide

Cortisol metabolism in humans involves various transformations, for example, reduction of the α,β -unsaturated carbonyl system to form 3α -hydroxy products, oxidation at C-11, and reduction at C-20. Most of the metabolites, such as tetrahydrocortisol, cortol and allotetrahydrocortisol, are excreted in the urine as conjugates with glucuronic acid.²⁾ Tetrahydro-11-deoxycortisol (THS) and its 5α -isomer (allo-THS), which are metabolites of 11-deoxycortisol, have also been identified as glucuronides in the urine of normal subjects or certain patients.³⁾ Recently, radioimmunoassays for some of these steroids have been developed.⁴⁾ The assays have been done on urine samples treated with β -glucuronidase or unprocessed samples. Non-isotopic immunoassay is an attractive method, particularly if a direct assay procedure to measure the glucuronides in the biological fluids can be developed. We have previously prepared the glucuronides of the 5β -metabolites for use in metabolic studies and immunoassays of corticosteroids.⁵⁾ This paper deals with methods of synthesizing allotetrahydro derivatives, which may be useful as intermediates in the preparation of 3- or 21-monoglucuronides: model experiments were carried out with allo-THS derivatives.

In the present work, the corresponding Δ^4 -3-ketosteroid, that is, 11-deoxycortisol, was selected as a starting material, since this is commercially available, and if a route to allo-THS derivatives could be developed, it might also be applicable to the cases of cortisol and cortisone. The allo-THS monoacetates (**8**, **14**), key intermediates for the introduction of the glucuronyl residue at C-3 or C-21, were the target compounds. There are two general routes to 3α -hydroxy- 5α -compounds: a simultaneous reduction method yielding a 3-hydroxy- 5α -derivative and a route *via* a 5α -3-ketone. The former route utilizes lithium-ammonia reduction, which requires protection of the side chain as the bismethylenedioxy derivative,

and the resulting 3β -alcohol must epimerize into the desired 3α -alcohol⁶⁾; however, this route is not satisfactory with respect to yield. Thus, we examined the latter method.

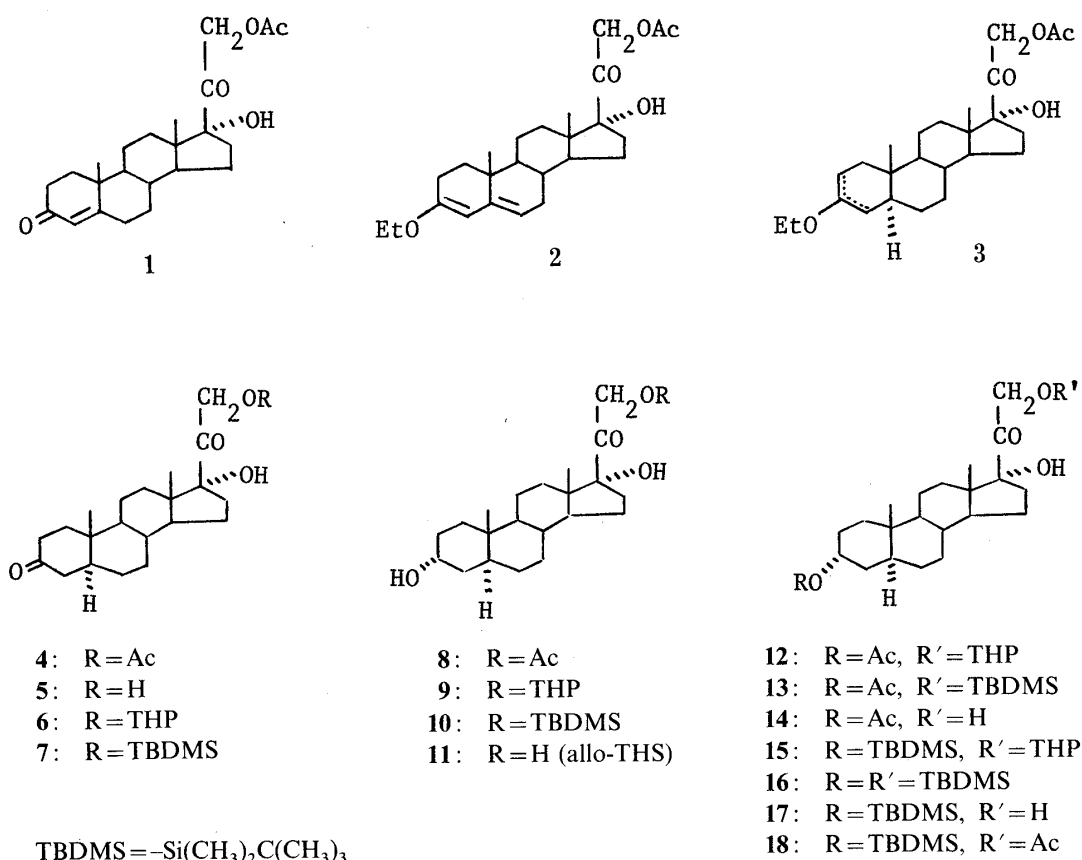


Chart 1

Preliminary experiments using various derivatives of 11-deoxycortisol, cortisol and cortisone showed that C-3- or C-5-isomers were not easily separated from each other by chromatography and fractional crystallization, and hence, selective reactions were required in order to obtain $3\alpha,5\alpha$ -derivatives. Harnik^{3a)} has reported on the hydrogenation of 11-deoxycortisol 21-acetate (1) in ethyl acetate in the presence of palladium-on-charcoal; this gives 5α -dihydro-11-deoxycortisol 21-acetate (4) and the 5β -isomer in the ratio of *ca.* 2:3. Such hydrogenations of the $4,5$ -double bond, including the method using homogeneous catalysts,⁷⁾ are not convenient for the preparation of the desired 4. Instead, we employed the route *via* the 3-ethoxy-3,5-diene (2).⁸⁾ Treatment of 1 with triethyl orthoformate in the presence of a catalytic amount of sulfuric acid in ethanol-dioxane gave 2 in good yield. The γ,δ -double bond in 2 was selectively reduced over palladium, yielding the enol ether (3), which, on treatment with hydrochloric acid, gave the desired 5α -compound (4). The overall yield from 1 was approximately 56%. The formation of the 5β -isomer was less than 5%, as judged by thin-layer chromatography.

Next, there are several methods for reduction of the carbonyl group at C-3 in 5α -steroids; these are the Raney nickel,^{3a)} metal hydride, the Urushibara⁹⁾ and the Henbest¹⁰⁾ reductions. The former two methods were examined in this work. For this purpose, 5α -dihydro-11-deoxycortisol (5) prepared from 4 was derivatized into the tetrahydropyranyl ether (6) and the *tert*-butyldimethylsilyl ether (7). It has been reported^{3a)} that the Raney nickel hydrogenation of 4 in dioxane gave the corresponding 3β -alcohol in 72% yield, leaving the carbonyl group at C-20 intact. In our hands, the hydrogenation of 4, 6 or 7 gave an inseparable mixture of the 3α -alcohol (8–10) and its 3β -epimer. The proton nuclear magnetic resonance ($^1\text{H-NMR}$)

spectra of these products showed that the $\alpha:\beta$ ratio was 1:2—2:3. The 3β -alcohols, without isolation, can be converted into their epimeric derivatives by known methods involving nucleophilic substitution of the 3β -tosylate by reagents such as acetate and *N,N*-dimethylformamide, but this route is not satisfactory with respect to yield. It should be mentioned that the use of the Mitsunobu reaction¹¹⁾ also gave unsatisfactory results. Bose *et al.*¹²⁾ have found, in the inversion reaction using triphenylphosphine, benzoic acid, and diethyl azodicarboxylate as reagents, a distinct difference in reactivity between 5α -cholestan- 3β -ol and 5α -cholestan- 3α -ol: under the usual conditions, the former was converted into 5α -cholestan- 3α -ol benzoate, whereas the 3α -alcohol did not react. However, when we applied this reaction to a 1:3 mixture of allotetrahydrocortisol 21-acetate and its 3β -epimer, the 3β -alcohol, but not the expected 3α -alcohol, was isolated from the unchanged fraction.

Successful results were obtained by the method using potassium tri-*sec*-butylborohydride¹³⁾ (K-Selectride, Aldrich) as a reducing agent. Contreras and Mendoza¹⁴⁾ have found that the reduction of 5α -cholestan-3-one with K-Selectride gave the corresponding 3α -alcohol and 3β -alcohol in the ratio of 92:8; this is in marked contrast to the results obtained with the conventional reagents, such as sodium borohydride and lithium aluminum hydride. Selective reductions with this reagent have also been investigated by other workers; for example, the reduction of 5α -pregnane-3,20-dione regioselectively gave 3α -hydroxy- 5α -pregnan-20-one, but the reported yield was not very high.¹⁵⁾ In the present work, the large bulk of the tetrahydropyranyl or *tert*-butyldimethylsilyl group in the 3,20-diketones **6** and **7** was expected to be advantageous for selective reduction of the carbonyl group at C-3, in addition to protection of the hydroxyl group at C-21. Reaction of **6** with 1.5 eq of K-Selectride in dry tetrahydrofuran at -78°C afforded the desired **9** in 74% yield. In a similar manner, the reduction of **7** was carried out to give **10**. The alkaline hydrogen peroxide work-up, which is generally employed, was found to be not always necessary. In the ^1H -NMR spectra of **9** and **10**, the C-3 proton signal was observed at 4.02 ppm as a multiplet with the half-band width of *ca.* 10 Hz, showing the equatorial nature of this proton; each 3β -epimer should exhibit a signal of $W_{1/2} = \text{ca. } 20 \text{ Hz}$ at *ca.* 3.6 ppm. Deprotection of **9** and **10** yielded allo-THS (**11**). On the other hand, acetylation of these compounds with acetic anhydride in pyridine gave the 3-acetates (**12**, **13**, respectively). Removal of the protecting groups at C-21 in **12** and **13** furnished the desired allo-THS 3-acetate (**14**).

The preparation of the 21-acetate (**8**) was then undertaken. The K-Selectride reduction was not applicable to **4** owing to the low solubility of this compound in tetrahydrofuran. Thus, the routes starting from **9** and **10** were investigated. Treatment of the 3α -alcohols (**9**, **10**) with *tert*-butyldimethylsilyl chloride and imidazole in dimethylformamide–pyridine gave the 3-silyl ethers (**15**, **16**). Selective removal of the protecting groups at C-21 in these compounds was effected by treatment with pyridinium *p*-toluenesulfonate in 90% acetone or ethanol, yielding the 3-monosilyl ether (**17**). Acetylation of **17** with acetic anhydride in pyridine, followed by desilylation with 1% hydrofluoric acid in acetonitrile, afforded the desired **8** (50% yield from **9**). This compound was also obtained from allo-THS (**11**) by selective acetylation.

Finally, the preparations of allo-THS 3-glucuronide (**21**) and allo-THS 21-glucuronide (**23**) were carried out. Introduction of the glucuronyl residue into **8** was achieved by using the Koenigs–Knorr reaction with methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy- α -D-glucopyranuronate in toluene in the presence of silver carbonate, yielding the glucuronide acetate-methyl ester (**19**) in 60% yield. Prior to saponification of **19**, the alkali-sensitive side chain at C-17 was protected by derivatization into the 20-semicarbazone (**20**). Sequential removal of the protecting groups in **20** was carried out by treatment with methanolic potassium hydroxide, and then with pyruvic acid–acetic acid to give **21** in a satisfactory yield. The Koenigs–Knorr reaction of **14**, followed by simultaneous removal of the protecting groups in both the steroid and sugar moieties by treatment with methanolic potassium

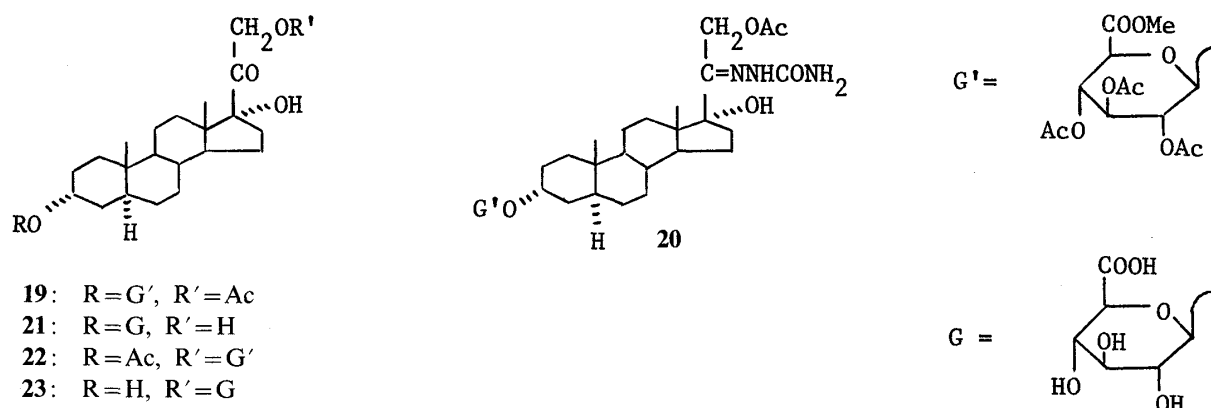


Chart 2

hydroxide, gave **23**. In the $^1\text{H-NMR}$ spectra of **21** and **23**, the signal due to the anomeric proton was observed as a doublet of $J=7\text{ Hz}$ at 4.36 ppm, showing β -configuration of the anomeric center.

The present methods for conversion of 11-deoxycortisol into allo-THS derivatives should be applicable to the cases of cortisol and cortisone. Hydrogenation of 3-methoxy-3,5-diene 17,21-acetonides, which are formed in one step from these Δ^4 -3-ketones, may also be suitable for preparing the corresponding 5α -compounds. Syntheses of the glucuronides of 11-oxygenated allotetrahydrocorticoids are under way in these laboratories. The allo-THS glucuronides obtained here should be useful in studies of metabolism in 11-hydroxylase deficiency and in the metyrapone test, an assessment of pituitary-adrenal reserve.

Experimental

All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were determined in CHCl_3 unless otherwise specified. $^1\text{H-NMR}$ spectra were measured with a JEOL FX-100 spectrometer at 100 MHz using tetramethylsilane as an internal standard.

3-Ethoxy-17 α ,21-dihydroxy-3,5-pregnadien-20-one 21-Acetate (2)—The preparation of **2** was carried out according to the method of Julian *et al.*¹⁶⁾ with slight modifications: a mixture of 11-deoxycortisol acetate (**1**) (1.0 g), triethyl orthoformate (1.1 ml) and conc. H_2SO_4 (14 μl) in EtOH (0.5 ml)–dioxane (12 ml) was stirred at room temperature for 1 h. After addition of pyridine (1 ml), the mixture was extracted with AcOEt. The organic layer was washed with 5% NaHCO_3 and H_2O , dried over anhydrous Na_2SO_4 , and evaporated down. Purification of the product by column chromatography on silica gel (25 g) with hexane–AcOEt (2:1) as an eluent gave **2** (*ca.* 1 g).

5 α -Dihydro-11-deoxycortisol 21-Acetate (4)—A solution of **2**, the whole obtained above, in AcOEt (6 ml)–EtOH (6 ml) was stirred under a hydrogen gas stream for 14 h at atmospheric pressure in the presence of 5% Pd–C (100 mg). After addition of AcOEt (40 ml) followed by removal of the catalyst by filtration, the filtrate, which contained the enol ether (**3**), was treated with 2N HCl (1.5 ml). The precipitated **4** was collected; 290 mg, mp 246–248 °C. The filtrate was washed with 5% NaHCO_3 and H_2O , dried over anhydrous Na_2SO_4 , and evaporated down. Recrystallization of the crude product from AcOEt gave an additional 270 mg of the same compound, mp 251–252 °C (lit. mp 249–251 °C).¹⁷⁾ These materials were free from 5 β -dihydro-11-deoxycortisol 21-acetate. Saponification of **4** with NaOH in MeOH–dioxane gave 5 α -dihydro-11-deoxycortisol (**5**).

5 α -Dihydro-11-deoxycortisol 21-Tetrahydropyranyl Ether (6)—A mixture of **5** (790 mg), 2,3-dihydropyran (1 ml), and pyridinium *p*-toluenesulfonate (250 mg) in CH_2Cl_2 (18 ml) was stirred at room temperature for 3 h. The resulting solution was concentrated to half of its initial volume under reduced pressure, diluted with AcOEt, washed with H_2O , dried over anhydrous Na_2SO_4 , and evaporated down. Recrystallization of the crude product from hexane– CH_2Cl_2 gave **6** (820 mg) as colorless plates. mp 171–172 °C. $[\alpha]_D^{15} + 35^\circ$ ($c=0.5$). Anal. Calcd for $\text{C}_{26}\text{H}_{40}\text{O}_5$: C, 72.19; H, 9.32. Found: C, 71.77; H, 9.31.

5 α -Dihydro-11-deoxycortisol 21-*tert*-butyldimethylsilyl Ether (7)—A solution of **5** (80 mg), imidazole (130 mg), and *tert*-butyldimethylsilyl chloride (65 mg) in pyridine (0.1 ml)–dimethylformamide (0.2 ml) was allowed to stand at room temperature for 1 h. The resulting solution was diluted with AcOEt, washed with H_2O , dried over anhydrous Na_2SO_4 , and evaporated down. Recrystallization of the crude product from MeOH gave **7** (90 mg) as colorless leaflets. mp 191–192 °C. $[\alpha]_D^{15} + 40^\circ$ ($c=0.5$). Anal. Calcd for $\text{C}_{27}\text{H}_{46}\text{O}_4\text{Si}$: C, 70.08; H, 10.02. Found: C, 69.93; H,

10.10.

The Raney Ni Reduction of Compounds 4, 6 and 7—A solution of the 5 α -dihydro compound (50 mg) in dioxane (6 ml) was hydrogenated for 20 h in the presence of Raney Ni (W-2) (*ca.* 100 mg). The filtrate was evaporated down under reduced pressure, yielding an inseparable mixture of the 3 α -alcohol (8, 9, 10) and the 3 β -epimer. The α : β ratio was estimated by comparisons of the C-19 and C-3 proton signals in the ^1H -NMR spectrum.

Allo-THS 21-Acetate (8)—i) A solution of 18 (46 mg) and 47% HF (40 μl) in acetonitrile (1.8 ml) was allowed to stand at room temperature for 20 min. The resulting solution was diluted with AcOEt, washed with 5% NaHCO_3 and H_2O , dried over anhydrous Na_2SO_4 , and evaporated down. Recrystallization of the crude product from acetone gave 8 (35 mg) as colorless plates. mp 241–242 $^\circ\text{C}$ (lit. mp 231–234 $^\circ\text{C}$).^{3a)} $[\alpha]_D^{14} + 53^\circ$ ($c=0.7$). ^1H -NMR (CDCl_3) δ : 0.65 (3H, s, 18- CH_3), 0.77 (3H, s, 19- CH_3), 2.15 (3H, s, 21- OCOCH_3), 4.02 (1H, m, 3 β -H), 4.76 and 5.10 (each 1H, d, $J=18$ Hz, 21-H). *Anal.* Calcd for $\text{C}_{23}\text{H}_{36}\text{O}_5$: C, 70.37; H, 9.24. Found: C, 70.08; H, 9.10.

ii) A solution of 11 (33 mg) and acetic anhydride (20 μl) in pyridine (0.2 ml)–benzene (0.5 ml) was stirred at room temperature for 12 h. After addition of H_2O , the mixture was extracted with AcOEt. The organic layer was washed with H_2O , dried over anhydrous Na_2SO_4 , and evaporated down. Purification of the crude product by column chromatography on silica gel (9 g) with hexane–AcOEt (1:1) as an eluent, followed by recrystallization from acetone, gave 8 (25 mg). mp 237–238 $^\circ\text{C}$.

The Mitsunobu Reaction of Epimeric 3,11 β ,17 α ,21-Tetrahydroxy-5 α -pregnan-20-one 21-Acetates—Triphenyl phosphine (260 mg) and benzoic acid (130 mg) were added to a stirred solution of a 1:3 mixture (407 mg) of allotetrahydrocortisol 21-acetate and its 3 β -epimer, obtained from 5 α -dihydrocortisol 21-acetate by the Raney Ni reduction, in dry tetrahydrofuran (6 ml) at room temperature, and then diethyl azodicarboxylate (200 μl) was added. After 2 h, the resulting solution was diluted with AcOEt, washed with 5% NaHCO_3 and H_2O , dried over anhydrous Na_2SO_4 , and evaporated down. The residue obtained was subjected to column chromatography on silica gel (40 g) with hexane–AcOEt (1:1) as an eluent. The compound (80 mg) recovered as unreacted material was found to be the 3 β -epimer, not the 3 α -alcohol, by ^1H -NMR spectral analysis; this was an unexpected result.¹²⁾

Allo-THS 21-Tetrahydropyranyl Ether (9)—A solution of 6 (108 mg) and K-Selectride (1 M in tetrahydrofuran, 0.25 ml) in dry tetrahydrofuran (1 ml) was stirred for 1 h at -78°C under a nitrogen atmosphere, and then, additional K-Selectride (0.13 ml) was added. After 1.5 h, the resulting solution was diluted with AcOEt, washed with H_2O , dried over anhydrous Na_2SO_4 , and evaporated down. The residue obtained was chromatographed on silica gel (15 g). Elution with hexane–AcOEt (1:2) and recrystallization of the product from acetone–hexane gave 9 (80 mg) as colorless prisms. mp 180–181 $^\circ\text{C}$. $[\alpha]_D^{16} + 23^\circ$ ($c=0.5$). ^1H -NMR (CDCl_3) δ : 0.67 (3H, s, 18- CH_3), 0.77 (3H, s, 19- CH_3), 4.02 (1H, m, 3 β -H), 4.30 and 4.66 (each 1H, d, $J=18$ Hz, 21-H). *Anal.* Calcd for $\text{C}_{26}\text{H}_{42}\text{O}_5$: C, 71.85; H, 9.74. Found: C, 72.15; H, 9.77.

Allo-THS 21-*tert*-Butyldimethylsilyl Ether (10)—The K-Selectride reduction of 7 (260 mg) was carried out in the manner described for 9. The crude product obtained was chromatographed on silica gel (15 g). Elution with hexane–AcOEt (2:1) and recrystallization of the product from aqueous MeOH gave 10 (140 mg) as colorless leaflets. mp 175–177 $^\circ\text{C}$. $[\alpha]_D^{17} + 20^\circ$ ($c=0.6$). ^1H -NMR (CDCl_3) δ : 0.11 (6H, s, 21- $\text{OSi}(\text{CH}_3)_2$), 0.65 (3H, s, 18- CH_3), 0.77 (3H, s, 19- CH_3), 0.92 (9H, s, 21- OSi-tert-Bu), 4.02 (1H, m, 3 β -H), 4.40 and 4.52 (each 1H, d, $J=18$ Hz, 21-H). *Anal.* Calcd for $\text{C}_{27}\text{H}_{48}\text{O}_4\text{Si}$: C, 69.78; H, 10.41. Found: C, 69.54; H, 10.44.

Allo-THS (11)—i) A solution of 9 (10 mg) and 50% H_2SO_4 (10 μl) in acetone (1 ml) was allowed to stand at room temperature for 40 min. After addition of H_2O , the mixture was extracted with AcOEt. The organic layer was washed with 5% NaHCO_3 and H_2O , dried over anhydrous Na_2SO_4 , and evaporated down. Recrystallization of the crude product from AcOEt gave 11 (5 mg) as colorless leaflets. mp 225–226 $^\circ\text{C}$ (lit. mp 224–226 $^\circ\text{C}$).^{3a)}

ii) Hydrolysis of 10 (40 mg) was carried out in the manner described above, yielding 11 (32 mg).

3 α -Acetoxy-17 α ,21-dihydroxy-5 α -pregnan-20-one 21-Tetrahydropyranyl Ether (12)—A solution of 9 (590 mg) and acetic anhydride (2 ml) in pyridine (4 ml) was allowed to stand at room temperature for 20 h. After addition of H_2O , the mixture was extracted with AcOEt. The organic layer was washed with H_2O , dried over anhydrous Na_2SO_4 , and evaporated down. Recrystallization of the crude product from ether–hexane gave 12 (500 mg) as colorless leaflets. mp 154–155 $^\circ\text{C}$. $[\alpha]_D^{18} + 36^\circ$ ($c=0.5$). ^1H -NMR (CDCl_3) δ : 0.67 (3H, s, 18- CH_3), 0.78 (3H, s, 19- CH_3), 2.03 (3H, s, 3- OCOCH_3), 4.30 and 4.65 (each 1H, d, $J=18$ Hz, 21-H), 4.96 (1H, m, 3 β -H). *Anal.* Calcd for $\text{C}_{28}\text{H}_{44}\text{O}_6 \cdot 1/4\text{H}_2\text{O}$: C, 69.89; H, 9.32. Found: C, 70.13; H, 9.27.

3 α -Acetoxy-17 α ,21-dihydroxy-5 α -pregnan-20-one 21-*tert*-Butyldimethylsilyl Ether (13)—Acetylation of 10 (34 mg) was carried out in the manner described for 12. The crude product obtained was recrystallized from MeOH to give 13 (28 mg) as colorless needles. mp 179–180 $^\circ\text{C}$. $[\alpha]_D^{16} + 27^\circ$ ($c=0.4$). ^1H -NMR (CDCl_3) δ : 0.11 (6H, s, 21- $\text{OSi}(\text{CH}_3)_2$), 0.66 (3H, s, 18- CH_3), 0.79 (3H, s, 19- CH_3), 0.92 (9H, s, 21- OSi-tert-Bu), 2.04 (3H, s, 3- OCOCH_3), 4.40 and 4.49 (each 1H, d, $J=18$ Hz, 21-H), 4.96 (1H, m, 3 β -H). *Anal.* Calcd for $\text{C}_{29}\text{H}_{50}\text{O}_5\text{Si}$: C, 68.73; H, 9.95. Found: C, 68.46; H, 9.91.

Allo-THS 3-Acetate (14)—i) Hydrolysis of 12 (500 mg) was carried out in the manner described for 11. After usual work-up, the crude product obtained was recrystallized from aqueous MeOH to give 14 (350 mg) as colorless leaflets. mp 177–178 $^\circ\text{C}$. $[\alpha]_D^{15} + 38^\circ$ ($c=0.5$). ^1H -NMR (CDCl_3) δ : 0.65 (3H, s, 18- CH_3), 0.78 (3H, s, 19- CH_3), 2.04 (3H, s, 3- OCOCH_3), 4.27 and 4.64 (each 1H, d, $J=20$ Hz, 21-H), 4.96 (1H, m, 3 β -H). *Anal.* Calcd for $\text{C}_{23}\text{H}_{36}\text{O}_5$: C,

70.37; H, 9.24. Found: C, 70.30; H, 9.11.

ii) Desilylation of **13** (26 mg) was carried out in the manner described for **11**, yielding **14** (15 mg).

Allo-THS 3-tert-Butyldimethylsilyl Ether 21-Tetrahydropyranyl Ether (15)—Silylation of **9** (55 mg) was carried out in the manner described for **7**. After usual work-up, the crude product obtained was recrystallized from aqueous MeOH to give **15** (57 mg) as colorless leaflets. mp 146–147 °C. $[\alpha]_D^{19} + 20^\circ$ ($c=0.5$). $^1\text{H-NMR}$ (CDCl_3) δ : 0.02 (6H, s, 3-OSi(CH₃)₂), 0.68 (3H, s, 18-CH₃), 0.76 (3H, s, 19-CH₃), 0.88 (9H, s, 3-OSi-*tert*-Bu), 3.95 (1H, m, 3 β -H), 4.30 and 4.67 (each 1H, d, $J=18$ Hz, 21-H). *Anal.* Calcd for C₃₂H₅₆O₅Si · 1/2H₂O: C, 68.89; H, 10.30. Found: C, 69.09; H, 10.34.

Allo-THS 3,21-Bis(tert-butyldimethylsilyl) Ether (16)—Silylation of **10** (55 mg) was carried out in the manner described for **7**. After usual work-up, the crude product obtained was recrystallized from aqueous MeOH to give **16** (60 mg) as colorless needles. mp 121–122 °C. $[\alpha]_D^{17} + 33^\circ$ ($c=0.6$). $^1\text{H-NMR}$ (CDCl_3) δ : 0.02 and 0.11 (each 6H, s, 3- and 21-OSi(CH₃)₂), 0.65 (3H, s, 18-CH₃), 0.75 (3H, s, 19-CH₃), 0.88 and 0.92 (each 9H, s, 3- and 21-OSi-*tert*-Bu), 3.92 (1H, m, 3 β -H), 4.39 and 4.51 (each 1H, d, $J=18$ Hz, 21-H). *Anal.* Calcd for C₃₃H₆₂O₄Si₂ · 1/2H₂O: C, 67.41; H, 10.80. Found: C, 67.61; H, 10.79.

Allo-THS 3-tert-Butyldimethylsilyl Ether (17)—i) A solution of **15** (44 mg) and pyridinium *p*-toluenesulfonate (20 mg) in 90% acetone was allowed to stand at 45 °C for 20 h. The resulting solution was diluted with AcOEt, washed with H₂O, dried over anhydrous Na₂SO₄, and evaporated down. The residue obtained was chromatographed on silica gel (2 g) with hexane–AcOEt (10:1) as an eluent. Recrystallization of the product from ether–hexane gave **17** (26 mg) as colorless leaflets. mp 176–177 °C. $[\alpha]_D^{15} + 34^\circ$ ($c=0.5$). $^1\text{H-NMR}$ (CDCl_3) δ : 0.02 (6H, s, 3-OSi(CH₃)₂), 0.66 (3H, s, 18-CH₃), 0.77 (3H, s, 19-CH₃), 0.90 (9H, s, 3-OSi-*tert*-Bu), 3.92 (1H, m, 3 β -H), 4.27 and 4.62 (1H, d, $J=20$ Hz, 21-H). *Anal.* Calcd for C₂₇H₄₈O₄Si: C, 69.78; H, 10.41. Found: C, 69.36; H, 9.77.

ii) A solution of **16** (35 mg) and pyridinium *p*-toluenesulfonate (12 mg) in EtOH was allowed to stand at room temperature for 6 h. The resulting solution was diluted with AcOEt, washed with 5% NaHCO₃ and H₂O, dried over anhydrous Na₂SO₄, and evaporated down. The crude product obtained was recrystallized from ether–hexane to give **17** (27 mg).

21-Acetoxy-3 α ,17 α -dihydroxy-5 α -pregnan-20-one 3-tert-Butyldimethylsilyl Ether (18)—Acetylation of **17** (45 mg) was carried out in the manner described for **12**. After usual work-up, the crude product obtained was recrystallized from hexane to give **18** (31 mg) as colorless leaflets. mp 167–168 °C. $[\alpha]_D^{16} + 55^\circ$ ($c=0.4$). $^1\text{H-NMR}$ (CDCl_3) δ : 0.02 (6H, s, 3-OSi(CH₃)₂), 0.67 (3H, s, 18-CH₃), 0.76 (3H, s, 19-CH₃), 0.88 (9H, s, 3-OSi-*tert*-Bu), 2.16 (3H, s, 21-OCOCH₃), 3.92 (1H, m, 3 β -H), 4.80 and 5.07 (each 1H, d, $J=18$ Hz, 21-H). *Anal.* Calcd for C₂₉H₅₀O₅Si: C, 68.73; H, 9.95. Found: C, 68.45; H, 9.80.

Methyl (21-Acetoxy-17 α -hydroxy-20-oxo-5 α -pregnan-3-yl-2',3',4'-tri-*O*-acetyl- β -D-glucopyranosid)uronate (19)—Freshly prepared Ag₂CO₃ (600 mg) and methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy- α -D-glucopyranuronate (900 mg) were added to a solution of **8** (170 mg) in toluene (10 ml), and the suspension was stirred at room temperature for 12 h. After addition of AcOEt, the resulting solution was passed through Florisil (10 g) on a sintered-glass funnel, and the filtrate was evaporated down. The residue was chromatographed on silica gel (20 g) with hexane–AcOEt (3:2) as an eluent. Repurification by chromatography using benzene–ether (5:1) and recrystallization of the product from acetone–hexane gave **19** (184 mg) as colorless leaflets. mp 231–232 °C. $[\alpha]_D^{15} + 10^\circ$ ($c=0.9$). $^1\text{H-NMR}$ (CDCl_3) δ : 0.65 (3H, s, 18-CH₃), 0.77 (3H, s, 19-CH₃), 2.01, 2.02, 2.04 and 2.15 (12H, –OCOCH₃), 3.73 (3H, s, –COOCH₃), 3.8–4.1 (2H, 3 β - and 5'-H), 4.57 (1H, d, $J=7$ Hz, 1'-H), 4.82 and 5.06 (each 1H, d, $J=18$ Hz, 21-H), 4.8–5.3 (3H, 2'-, 3'-, and 4'-H). *Anal.* Calcd for C₃₆H₅₂O₁₄: C, 61.00; H, 7.40. Found: C, 60.90; H, 7.37.

Allo-THS 3-Glucuronide (21)—A mixture of **19** (330 mg), semicarbazide · HCl (1.3 g), and AcONa (830 mg) in MeOH (15 ml) was stirred at room temperature for 2 d. Upon addition of H₂O, a precipitate was formed, and this was collected by filtration and dried. The crude product was chromatographed on silica gel (20 g) with AcOEt as an eluent to give the 20-semicarbazone (**20**). This was dissolved in 2% methanolic KOH (9 ml) and allowed to stand at room temperature for 2 h. After addition of H₂O followed by neutralization with AcOH, the resulting solution was evaporated down under reduced pressure. The residue was dissolved in 80% pyruvic acid (5 ml)–AcOH (2 ml)–CHCl₃ (6 ml) and the solution was stirred at room temperature for 12 h. After removal of the organic solvent followed by addition of H₂O, the resulting solution was subjected to column chromatography on Amberlite XAD-2. Elution with EtOH gave the crude product, which was chromatographed on silica gel with CHCl₃–MeOH–H₂O–AcOH (100:10:2:0.1) as an eluent, and then on Amberlite XAD-2, yielding **21** (147 mg) as colorless crystals. mp 210 °C (dec.) (colorless leaflets from aqueous MeOH). $[\alpha]_D^{14} + 10^\circ$ ($c=0.4$, EtOH). $^1\text{H-NMR}$ (CD_3OD) δ : 0.59 (3H, s, 18-CH₃), 0.91 (3H, s, 19-CH₃), 3.93 (1H, m, 3 β -H), 4.25 (1H, d, $J=19$ Hz, one of 21-H), 4.36 (1H, d, $J=7$ Hz, 1'-H), 4.59 (1H, d, $J=19$ Hz, one of 21-H). *Anal.* Calcd for C₂₇H₄₂O₁₀: C, 61.58; H, 8.04. Found: C, 61.46; H, 8.63.

Methyl (3 α -Acetoxy-17 α -hydroxy-20-oxo-5 α -pregnan-21-yl-2',3',4'-tri-*O*-acetyl- β -D-glucopyranosid)uronate (22)—The Koenigs–Knorr reaction of **14** (445 mg) and purification by chromatography were carried out in the manner described for **19**. Recrystallization of the crude product from aqueous MeOH gave **22** (550 mg) as colorless leaflets. mp 145–146 °C. $[\alpha]_D^{15} - 4^\circ$ ($c=1.7$). $^1\text{H-NMR}$ (CD_3OD) δ : 0.61 (3H, s, 18-CH₃), 0.84 (3H, s, 19-CH₃), 1.98, 2.02 and 2.05 (12H, –OCOCH₃), 3.70 (3H, s, –COOCH₃), 4.21 (1H, d, $J=9.5$ Hz, 5'-H), 4.5–5.5 (7H, 3 β -, 21-, 1'-, 2'-, 3'-, 4'-H). *Anal.* Calcd for C₃₆H₅₂O₁₄ · 1/4H₂O: C, 60.62; H, 7.42. Found: C, 60.46; H, 7.21.

Allo-THS 21-Glucuronide (23)—A solution of **22** (470 mg) in 2% methanolic KOH (20 ml) was stirred at room temperature for 12 h. After addition of H₂O followed by neutralization with AcOH, the resulting solution was evaporated down under reduced pressure. The crude product was subjected to column chromatography on Amberlite XAD-2. Elution with EtOH gave the crude product, which was chromatographed on silica gel with CHCl₃–MeOH–H₂O–AcOH (100:20:2:0.1) as an eluent, and then on Amberlite XAD-2, yielding **23** (215 mg). mp 190°C (dec.) (colorless leaflets from aqueous MeOH). $[\alpha]_D^{19} + 1.8^\circ$ ($c=0.6$, MeOH). ¹H-NMR (CD₃OD) δ : 0.62 (3H, s, 18-CH₃), 0.81 (3H, s, 19-CH₃), 3.93 (1H, m, 3 β -H), 4.36 (1H, d, $J=7$ Hz, 1'-H), 4.46 and 4.94 (each 1H, d, $J=19$ Hz, 21-H). *Anal.* Calcd for C₂₇H₄₂O₁₀·1/4H₂O: C, 61.06; H, 8.07. Found: C, 60.88; H, 8.03.

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